The chemistry of
the thiol group
Part 2
THE CHEMISTRY OF FUNCTIONAL GROUPS
A series of advanced treatises under the general editorship of
Professor Saul Faib

The chemistry of alkenes (published in 2 volumes)
The chemistry of the carbonyl group (published in 2 volumes)
The chemistry of the ether linkage (published)
The chemistry of the amine group (published)
The chemistry of the nitro and nitroso group (published in 2 parts)
The chemistry of carboxylic acids and esters (published)
The chemistry of the carbon-nitrogen double bond (published)
The chemistry of enediyne (published)
The chemistry of the cyano group (published)
The chemistry of the hydroxyl amine (published in 3 parts)
The chemistry of the azido group (published)
The chemistry of acyl halides (published)
The chemistry of the carbon-nitrogen bond (published in 2 parts)
The chemistry of the quinonoid compounds (published in 2 parts)
The chemistry of the thiol group (published in 2 parts)

The chemistry of
the thiol group
Part 2

Edited by
Saul Faib
The Hebrew University, Jerusalem

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Foreword

This volume, 'The Chemistry of the Thiol Group', is again organized and presented according to the general lines described in the 'Preface to the series' printed in the following pages.

Since the last volume in the series 'The Chemistry of the Functional Groups' appeared, there has been one new development in this project: a volume is now in preparation which is planned to contain chapters on subjects which were not included in the previously published volumes either because promised manuscripts have not been delivered or because they represent new developments in rapidly and significantly progressing fields during the last several years. The first such supplementary volume will include material on double-bonded groups (C=O, C=O, C=N). If this venture should prove successful, it is intended to publish further similar supplementary volumes.

The original plan of the present volume also included the following chapters which did not materialize: 'Free radical reactions involving thiols', 'Electrochemistry of the thiol group', 'Enethiols' and 'The thiol-disulphide interchange'.

Jerusalem, May 1974

SAUL PATAI
The Chemistry of Functional Groups
Preface to the series

The series 'The Chemistry of Functional Groups' is planned to cover in each volume all aspects of the chemistry of one of the important functional groups in organic chemistry. The emphasis is laid on the functional group treated and on the effects which it exerts on the chemical and physical properties, primarily in the immediate vicinity of the group in question, and secondarily on the behaviour of the whole molecule. For instance, the volume The Chemistry of the Ether Linkage deals with reactions in which the C–O–C group is involved, as well as with the effects of the C–O–C group on the reactions of alkyl or aryl groups connected to the ether oxygen. It is the purpose of the volume to give a complete coverage of all properties and reactions of ethers in as far as these depend on the presence of the ether group, but the primary subject matter is not the whole molecule, but the C–O–C functional group.

A further restriction in the treatment of the various functional groups in these volumes is that material included in easily and generally available secondary or tertiary sources, such as Chemical Reviews, Quarterly Reviews, Organic Reactions, various 'Advances' and 'Progress' series as well as textbooks (i.e. in books which are usually found in the chemical libraries of universities and research institutes) should not, as a rule, be repeated in detail, unless it is necessary for the balanced treatment of the subject. Therefore each of the authors is asked not to give an encyclopaedic coverage of his subject, but to concentrate on the most important recent developments and mainly on material that has not been adequately covered by reviews or other secondary sources by the time of writing of the chapter, and to address himself to a reader who is assumed to be at a fairly advanced post-graduate level.

With these restrictions, it is realized that no plan can be devised for a volume that would give a complete coverage of the subject with no overlap between chapters, while at the same time preserving the readability of the text. The Editor set himself the goal of attaining reasonable coverage with moderate overlap, with a minimum of cross-references between the chapters of each volume. In this manner, sufficient freedom is given to each author to produce readable quasi-monographic chapters.
Preface to the series

The general plan of each volume includes the following main sections:

(a) An introductory chapter dealing with the general and theoretical aspects of the group.

(b) One or more chapters dealing with the formation of the functional group in question, either from groups present in the molecule, or by introducing the new group directly or indirectly.

(c) Chapters describing the characterization and characteristics of the functional groups, i.e., a chapter dealing with qualitative and quantitative methods of determination including chemical and physical methods, ultraviolet, infrared, nuclear magnetic resonance, and mass spectra; a chapter dealing with activating and directing effects exerted by the group and/or a chapter on the basicity, acidity or complex-forming ability of the group (if applicable).

(d) Chapters on the reactions, transformations and rearrangements which the functional group can undergo, either alone or in conjunction with other reagents.

(e) Special topics which do not fit any of the above sections, such as photochemistry, radiation chemistry, biochemical formations and reactions. Depending on the nature of each functional group treated, these special topics may include short monographs on related functional groups on which no separate volume is planned (e.g., a chapter on 'Thioketones' is included in the volume 'The Chemistry of the Carbonyl Group', and a chapter on 'Ketenes' is included in the volume 'The Chemistry of Alkenes'). In other cases, certain compounds, though containing only the functional group of the title, may have special features so as to be best treated in a separate chapter, as e.g., 'Polymers' in 'The Chemistry of the Ether Linkage', or 'Tetraaminomethylenes' in 'The Chemistry of the Amino Group.'

This plan entails that the breadth, depth and thought-provoking nature of each chapter will differ with the views and inclinations of the author and the presentation will necessarily be somewhat uneven. Moreover, a serious problem is caused by authors who deliver their manuscript late or not at all. In order to overcome this problem at least to some extent, it was decided to publish certain volumes in several parts, without giving consideration to the originally planned logical order of the chapters. If after the appearance of the originally planned parts of a volume it is found that either owing to non-delivery of chapters, or to new developments in the subject, sufficient material has accumulated for publication of an additional part, this will be done as soon as possible.

Preface to the series

The overall plan of the volumes in the series 'The Chemistry of Functional Groups' includes the titles listed below:

- The Chemistry of Alkenes (published in two volumes)
- The Chemistry of the Carbonyl Group (published in two volumes)
- The Chemistry of the Ether Linkage (published)
- The Chemistry of the Amino Group (published)
- The Chemistry of the Nitro and the Nitroso Groups (published in two parts)
- The Chemistry of Carboxylic Acids and Esters (published)
- The Chemistry of the Carbon-Nitrogen Double Bond (published)
- The Chemistry of the Cyano Group (published)
- The Chemistry of Amides (published)
- The Chemistry of the Hydroxyl Group (published in two parts)
- The Chemistry of the Acido Group (published)
- The Chemistry of Acyl Halides (published)
- The Chemistry of the Halogenated Bond (published in two parts)
- The Chemistry of the Quinomold Compounds (published in two parts)
- The Chemistry of the Thiol Group (published in two parts)
- The Chemistry of the Carbon-Carbon Triple Bond
- The Chemistry of Amines and Imidates (in preparation)
- The Chemistry of the Hydroxyl, Azo and Azoxy Groups (in preparation)
- The Chemistry of the SO, \(\text{SO}_2\), \(\text{SO}_3\), \(\text{H}_2\text{SO}_4\) and \(\text{SO}_4\text{H}_2\) Groups
- The Chemistry of the Cyanates and their Thio-derivatives (in preparation)
- The Chemistry of the \(-\text{PO}_4\text{H}_2\) and Related Groups

Advice or criticism regarding the plan and execution of this series will be welcomed by the Editor.

The publication of this series would never have started, let alone continued, without the support of many persons. First and foremost among these is Dr. Arnold Weissberger, whose assistance and trust encouraged me to tackle this task, and who continues to help and advise me. The efficient and patient cooperation of several staff members of the Publisher also rendered me invaluable aid (but unfortunately their code of ethics does not allow me to thank them by name). Many of my friends and colleagues in Israel and overseas helped me in the solution of various major and minor matters, and my thanks are due to all of them, especially to Professor L. Rapoport. Carrying out such a long-range project would be quite impossible without the non-professional but none the less essential participation and partnership of my wife.

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Jerusalem, Israel.

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CHAPTER 11

The radiation chemistry of thiols

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Auckland, New Zealand

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      2. Acetone electron
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   C. Mechanism
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I. INTRODUCTION

High energy radiation interacts with matter causing ionization and excitation, followed by ion-molecule reactions, charge neutralization and dissociation of molecules giving rise to the formation of free radicals. Thus the radiation chemistry of thiols is essentially free radical chemistry, with the thiol radical, RS•, as the most important intermediate species. The thiols which have been most studied are for two main reasons those of biological interest. Firstly the –SH group is very reactive towards free radicals and consequently molecules containing thiol groups play a dominant role in radiation biological processes. Secondly, it was found in the 1940's that some aminothiols when added to in vivo systems gave considerable protection against the harmful effects of ionizing radiation.

As thiols occur in nature, mainly as aminoaacid residues of peptide-containing molecules, cysteine, NH₂CH(CO₂)₂CH₂SH, has been the thiol most closely studied. Cysteamine (2-mercaptoethylamine) was early on found to be highly protective and has also been studied extensively. Studies of the basic radiation chemistry of these and related thiols, in aqueous solutions, alone, or in mixtures with model compounds of biological importance have been most informative, and the gap between radiation chemistry and radiation biology has closed considerably in the last five years. Much current work is now centred on large biologically active molecules.

As the radiolysis of a thiol frequently produces the corresponding disulphide as the major product, and as both thiol and disulphide groups are present together in biological systems, some discussion on the radiation chemistry of the disulphide group is an essential part of this chapter.

Radiation chemistry yields are usually expressed as G-values, the number of molecules (or radicals) formed (or destroyed) per 100 eV of energy absorbed by the system. The equation

\[ G(-\text{RSH}) = 2 G(\text{RSSR}) + G(\text{H}_2\text{S}) \]

implies that disulphide and H₂S are the only sulphur-containing products formed in a particular experimental study.

II. AQUEOUS SOLUTIONS OF THIOLS-OXYGEN-FREE

A. Radiolysis of Aqueous Solutions

The absorption of high energy radiation by water results in the formation of radical and molecular products, and for fast electrons or γ-radiation may be represented by reaction (1) where the stoichiometry is expressed in G-values. The exact mechanism of the formation of these products is still a matter of research and discussion, but it is clear that at about 100 ms after the absorption of the high energy particle the above products have formed and diffused away sufficiently from the particle track to react with solutes in low concentration (< 10⁻³ M) with effectively homo-geneous kinetics. The fraction of the incident energy absorbed by the solute is negligibly small for dilute solutions. The situation is therefore different from that found in photochemistry where all the photon energy is absorbed by direct solute-photon interaction. As its concentration is increased about above 10⁻³ M a reactive solute may progressively interfere with spur reactions, reacting with the primary radical products or their precursors during the stage of 'spur diffusion kinetics', and thus alter the radical and molecular yields.

In dilute solutions of a thiol, RSH, it should be possible to explain the radiation chemistry in terms of the reactions of RSH with OH, c₅H₅⁻ and H at low conversions, but as the radiation products accumulate, competition between these and RSH for the radicals will occur, leading to secondary products. Thus 'initial yields' of products are normally measured experimentally in mechanistic investigations. When a second solute is also present, e.g. O₂, competition for the primary products will occur, and the intermediates formed from RSH may also react with this added solute. The pH of the solution is also important because OH⁻ may compete with RSH for c₅H₅⁻ and in addition the actual form, and hence the reactivity, of the thiol may change with pH in a manner depending on its acid dissociation constants.
1. Hydroxyl radical

The hydroxyl radical reacts rapidly with thiols, product analysis indicating the thiol radical to be the main product as in reaction (2). This is supported by the work of Armstrong and Humphries, who generated OH radicals from TP-HO₂ solutions and reacted them with thiols in a flow system. The c.s.r. spectrum corresponded to that of a thyl radical.

Rate constants for various thiols are listed in Table 1. Jayson, Stirling and

<table>
<thead>
<tr>
<th>Thiol</th>
<th>pH</th>
<th>Method</th>
<th>10⁻⁷k, 1 mol⁻¹ s⁻¹</th>
<th>Reference</th>
</tr>
</thead>
<tbody>
<tr>
<td>Cysteamine</td>
<td>4</td>
<td>CN⁻</td>
<td>15</td>
<td>4</td>
</tr>
<tr>
<td>Cysteamine</td>
<td>6.5</td>
<td>CN⁻</td>
<td>13</td>
<td>4</td>
</tr>
<tr>
<td>Cysteine</td>
<td>9</td>
<td>CN⁻</td>
<td>13</td>
<td>4</td>
</tr>
<tr>
<td>Cysteine</td>
<td>6.5</td>
<td>CN⁻</td>
<td>13</td>
<td>5</td>
</tr>
<tr>
<td>Mercaptoethanol</td>
<td>7</td>
<td>CN⁻</td>
<td>22</td>
<td>6</td>
</tr>
<tr>
<td>Mercaptoethanol</td>
<td>11</td>
<td>CN⁻</td>
<td>62</td>
<td>6</td>
</tr>
<tr>
<td>Mercaptoethanol</td>
<td>6.5</td>
<td>CN⁻</td>
<td>17</td>
<td>7</td>
</tr>
<tr>
<td>Mercaptoethanol</td>
<td>6.5</td>
<td>CN⁻</td>
<td>17</td>
<td>7</td>
</tr>
<tr>
<td>Mercaptoethanol</td>
<td>6.5</td>
<td>CN⁻</td>
<td>17</td>
<td>7</td>
</tr>
<tr>
<td>Methyl mercaptan</td>
<td>6.5</td>
<td>CN⁻</td>
<td>17</td>
<td>7</td>
</tr>
<tr>
<td>N-Dimethyl mercaptan</td>
<td>6.5</td>
<td>CN⁻</td>
<td>17</td>
<td>7</td>
</tr>
</tbody>
</table>

Swallow obtained a higher figure for mercaptoethanol with thiocyanate ion as competition scavenger than with ferrocyanide ion or nitrobenzene, and other figures in the table, the CNS⁻ could also possibly be too high. At pH 9 or 11 the thiols listed would be mainly in their thiolate form.

In the case of mercaptoethanol and methyl mercaptan at pH 11 new transient seen by pulse radiolysis, and not observed at lower pH, were tentatively attributed to radicals obtained by hydrogen atom abstraction from the α-carbon atom with respect to sulfur. Recent c.s.r.-radiolysis studies also give evidence for some H-abstraction from carbon in mercaptoethane carboxylic acids.

2. Aquated electron

The aquated electron reacts rapidly with thiols in near-neutral solutions to give H₂S and the parent hydrocarbon as the major detectable products, according to reactions (3) and (4). Values of G(H₂S) and G(RH) of RSH → RH⁺ + H⁻ (3) R⁺ + RSH → RH + RS⁻ (4)

between 2.5 and 3.0 have been reported for cysteine, cysteine, methyl mercaptan, and homocysteine for thiol concentrations in the range 10⁻⁸-5 x 10⁻³M. Lower values of 2.3 have been reported for glutathione (10⁻²M), and 4-aminobuten-1-thiol (10⁻⁵M), while very much lower values of 1.4 and 1.7 for 10⁻⁵M and 10⁻⁴M mercaptopropanol have been found. The authors in the latter case suggest that nearly half the e⁻ are reacting by reaction (5):

G(H₂S) is measured directly, whereas in product-yield-scavenger studies, the RSH-e⁻ adduct could in principle transfer the electron to a scavenger, or not yield H₂S quantitatively, thus leading to low values. The figures for cysteine at low and high pH call for comment. Trumbore and coworkers suggest that the fully protonated form of cysteine, carrying an overall positive charge, reacts faster than does the zwitter-ion form, while the 100-fold decrease at pH 11.6 found by Brauman would be due to the cysteine being present as the thiolate ion, RS⁻. It was found in a much earlier study by Brauman that G(H₂S) drops as the pH is increased above 8, and the thiolate ion is probably unreactive towards e⁻.

3. Hydrogen atom

In acidic solutions aquated electrons with protons yield hydrogen atoms by reaction (6), and these, together with those formed directly (G_H = 0.6), may react with the thiol. Armstrong and coworkers have shown that lowering pH increases G(H₂S) and decreases G(H₂O) but even under conditions where all e⁻ are scavenged by H₂S some H₂S is still produced. Thus it appears that H may react by reaction (7) or reaction (8):
<table>
<thead>
<tr>
<th>Thiol</th>
<th>pH</th>
<th>$[\text{RS}]$, M</th>
<th>Measured quantity</th>
<th>Technique*</th>
<th>$10^{-4}k$, 1 mol$^{-1}$s$^{-1}$</th>
<th>Reference</th>
</tr>
</thead>
<tbody>
<tr>
<td>Cysteine</td>
<td>0-7-0.8</td>
<td>$10^{-4}$</td>
<td>$G(H_2)$</td>
<td>[H$^+$]</td>
<td>11</td>
<td>15</td>
</tr>
<tr>
<td>Cysteine</td>
<td>7</td>
<td></td>
<td>$G(H_2)$</td>
<td>[NO]</td>
<td>44</td>
<td>12</td>
</tr>
<tr>
<td>Cysteine</td>
<td>7</td>
<td></td>
<td>$G(H_2)$</td>
<td>[acetone]</td>
<td>54</td>
<td>12</td>
</tr>
<tr>
<td>Cysteine</td>
<td>0-5.1</td>
<td>$10^{-4}$</td>
<td>$G(H_2)$</td>
<td>[RSF$^-$]</td>
<td>30</td>
<td>18</td>
</tr>
<tr>
<td>Cysteine</td>
<td>5-7</td>
<td>$10^{-2}$</td>
<td>$G(H_2)$</td>
<td>[NO]</td>
<td>11</td>
<td>11</td>
</tr>
<tr>
<td>Cysteine</td>
<td>5-8</td>
<td>$10^{-3}$</td>
<td>$G(H_2)$</td>
<td>[acetone]</td>
<td>11</td>
<td>11</td>
</tr>
<tr>
<td>Cysteine</td>
<td>7</td>
<td>$3 \times 10^{-3}$</td>
<td>$G(H_2)$</td>
<td>$O_2$</td>
<td>4</td>
<td>19</td>
</tr>
<tr>
<td>Cystine</td>
<td>6-8</td>
<td></td>
<td>$[\text{ag}]$</td>
<td>p.r.</td>
<td>87</td>
<td>20</td>
</tr>
<tr>
<td>Cysteine</td>
<td>11-4</td>
<td></td>
<td>$[\text{ag}]$</td>
<td>p.r.</td>
<td>0075</td>
<td>20</td>
</tr>
<tr>
<td>Cysteine</td>
<td>6-9</td>
<td></td>
<td>$[\text{ag}]$</td>
<td>p.r.</td>
<td>20</td>
<td>20</td>
</tr>
<tr>
<td>Glutathione</td>
<td>8-10</td>
<td></td>
<td>$[\text{ag}]$</td>
<td>p.r.</td>
<td>20</td>
<td>20</td>
</tr>
<tr>
<td>Glutathione</td>
<td>8-10</td>
<td></td>
<td>$[\text{ag}]$</td>
<td>p.r.</td>
<td>20</td>
<td>20</td>
</tr>
<tr>
<td>Pencillamine</td>
<td>6-5</td>
<td></td>
<td>$[\text{ag}]$</td>
<td>p.r.</td>
<td>51</td>
<td>7</td>
</tr>
<tr>
<td>Mercaptomethanol</td>
<td>5-9</td>
<td></td>
<td>$[\text{ag}]$</td>
<td>p.r.</td>
<td>12</td>
<td>6</td>
</tr>
<tr>
<td>Mercaptopentanol</td>
<td>10</td>
<td></td>
<td>$[\text{ag}]$</td>
<td>p.r.</td>
<td>10</td>
<td>6</td>
</tr>
<tr>
<td>Methyl mercaptan</td>
<td>7</td>
<td></td>
<td>$[\text{ag}]$</td>
<td>p.r.</td>
<td>10</td>
<td>6</td>
</tr>
<tr>
<td>Methyl mercaptan</td>
<td>0-7-8</td>
<td>$5 \times 10^{-4}$</td>
<td>$G(H_2)$</td>
<td>$H^+$</td>
<td>18</td>
<td>15</td>
</tr>
<tr>
<td>Methyl mercaptan</td>
<td>7</td>
<td>$3 \times 10^{-3}$</td>
<td>$G(H_2)$</td>
<td>$O_2$</td>
<td>43</td>
<td>8</td>
</tr>
<tr>
<td>4-Aminobutylthiol</td>
<td>7</td>
<td>$10^{-4}$</td>
<td>$G(H_2)$</td>
<td>$O_2$</td>
<td>4</td>
<td>17</td>
</tr>
</tbody>
</table>

* p.r. stands for pulse radiolysis. In the other cases the competitive electron scavenging technique was used, the compound whose concentration was varied being indicated.

The table presents data for the reaction of RS with R'-H, and shows that RS and G-HS react at a faster rate than RS-H and G-HS$^-$ (as determined by competition experiments). The table also shows that RS and G-HS react at a slower rate than RS-H and G-HS$^-$, respectively. This is consistent with the above mechanisms.

The products of the radiolysis of thiol and the absence of G in the (cysteine) disulfides H and H$_2$S. The generally supported mechanism is based on the reaction of H with H$_2$S.

C. Mechanism

The rate constant for the reaction of G-H with RS-H (G-HS$^-$, or G-HS$^-$) has been determined from TG, OH$^+$, and G-HS$^-$ measurements. This reaction has been calculable in the region of 3.5 and 3.77 eV.

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holds if the mechanism is correct, and from simple competition kinetics,

\[ \frac{k(RSH)}{k(SH \cdot)} = \frac{A_{RSH} + A_{H \cdot}}{A_{SH \cdot}} \cdot \frac{A_{H \cdot}}{A_{RSH} + A_{H \cdot}} + \frac{A_{RSH}}{A_{SH \cdot}} \cdot \frac{A_{SH \cdot}}{A_{RSH} + A_{H \cdot}} + \frac{A_{RSH}}{A_{SH \cdot}} + \frac{A_{H \cdot}}{A_{RSH} + A_{H \cdot}} \]

At low pH we therefore have

\[ G(-RSH) = \frac{G_{RSH} + G_{H \cdot}}{G_{H \cdot} + G_{SH \cdot} + G_{RSH}} \]

and in neutral solution

\[ G(-RSH) = \frac{G_{RSH} + G_{H \cdot}}{G_{H \cdot} + G_{RSH}} \]

The rate constant ratio \( k_R/k_H \) reported in the previous section was obtained by assuming the above mechanism. Taking the figure of 3-5 for this ratio for cysteine, and the radical yields given in reaction (1), \( G(-KH) = 0 \) in acidic and 8-8 in neutral solution respectively.

In all thiols studied, \( G(-RSH) \) figures decrease with decreasing thiol concentrations, the decrease being greater than may be expected from van't Hoff constant data. The same general mechanism applies to the thiols cysteamine\(^{14}\), glutathione\(^{14}\), homocysteine\(^{4}\) and 4-aminobutane-1-thiol\(^{14}\), although the values of \( G(-KH) \) were a little low for complete scavenging in some cases.

As mentioned in section II.B.2 mercaptoethanol behaves differently in that only about half the aminated electrons give rise to RSH\(^{1}\). Bronsted acids can react with \( \cdot O \) \( N \) and convert them to H, but the pK\(_a\) of the thiol group in mercaptoethanol is not lower than for other thiols, and the explanation must lie elsewhere.

A further reaction which should be considered when deducing mechanism from product yields is that of H\(_2\)O\(_2\) with thiols (12):

\[ 2 \text{RSH} + \text{H}_2\text{O}_2 \rightarrow \text{RSSR} + 2 \text{H}_2\text{O} \]

This reaction is slow in acidic solution, and \( G(\text{H}_2\text{O}_2) = G(\text{H}_2\text{O}) \) is found. However, in neutral and alkaline solution the rate can be appreciable and the reaction must be allowed for\(^1\). It has been shown that the reaction involves a secondary attack of the thiolate ion on hydrogen peroxide, the rate being found proportional to [RSH\(^{\cdot}\)] in studies on cysteamine\(^{14}\) and cysteine\(^{28}\) in which pH was varied.

D. Transients

1. Pulse radiolysis studies

Pulse radiolysis studies have shown the presence of a transient species when thiols are irradiated at a pH where some ionization of the thiol group has occurred. These species have an absorption band from approximately 350 to 500 nm with a maximum at 400-450 nm and an extinction coefficient of the order of 10\(^4\) l mol\(^{-1}\) cm\(^{-1}\).

The first detailed study was on cysteamine by Adams and coworkers\(^4\), who showed that the transient was not R\(^{\cdot}\) as they had first suspected but RSSR formed by reaction of the thiyl radical with the thiolate ion in an equilibrium reaction (13). Evidence for this came from studying both cysteamine and cysteamine solutions. In pure solutions of the disulphide the rate of formation of the transient matched the rate of decay of the aminated electron, and the addition of nitrous oxide drastically reduced the amount formed. NO scavenges \( \cdot O \) \( N \) to produce OH radicals, reaction (14).

\[ \text{RSH} + \text{NO} \rightarrow \\text{RSSH} \rightarrow \text{RSSR} \]

The decay of absorption was always exponential, suggesting electron attachment to the disulphide followed by dissociation, reactions (15) and (16).

In cysteine solution N\(_2\)O increased the amount of transient (13-14) formed immediately after the pulse, showing OH radicals to be the precursor in this case. The rate of growth of transient was slower than the rate of reaction of thiol with OH radicals (as measured by CNS\(^{28}\) competition scavenging) but increased with thiol concentration. The maximum absorbance obtained after the electron pulse increased with increasing thiol concentration and pH, i.e. with increasing RS\(^{\cdot}\) concentration, implying the equilibrium (13). This was confirmed by the decay kinetics, which were second-order and much slower than the first-order decay (13). The second-order rate constant decreased with increasing thiol concentration and pH, implying that the rate of disappearance was controlled by dimerization of free thiyl radicals (reaction 11):

\[ \text{RSSR} \rightarrow \text{RSSR} \rightarrow \text{RSSR} \rightarrow \text{RSSR} \]

Similar results have been found for cysteine\(^{28}\), mercaptoethanol\(^{28}\), various alky mercaptans\(^{37}\), H\(_2\)S\(^{27}\), and penicillamine\(^{36}\).

Further study of the second-order decay of RSSR as a function of pH and thiol concentration showed that reaction (16) was also important\(^28\), and this was confirmed during further work on cysteine\(^{28}\).
The products are presumably RSSR and RS⁻. Rate constants reported for reactions (11) and (16) are \( \geq 10^{11} \text{mol}^{-1}\text{cm}^3\text{s}^{-1} \).

The equilibrium constants for reaction (13) have been determined from either the rate constants of the forward and back reactions or from dependence of maximum absorbance after the pulse upon concentration and pH, and are shown in Table 3 together with reported extinction coefficients and absorption maximum for RSSR.

Weaker absorptions at shorter wavelengths have been reported and assigned to the thiol radical for penicillamine at pH 5 (\( \lambda_{\text{max}} = 330 \text{ nm} \), \( \epsilon = 1.2 \times 10^4 \text{ mol}^{-1}\text{cm}^{-1} \)) and for mercaptoethanol at pH 6 (\( \lambda_{\text{max}} = 360 \text{ nm} \), \( \epsilon = 1.3 \times 10^4 \text{ mol}^{-1}\text{cm}^{-1} \)), and tentatively to the radicals HOCH₂CHSH⁻ and "CH₂S⁻" for mercaptoethanol and methyl mercaptan, respectively at pH 12 (\( \lambda_{\text{max}} = 300 \text{ nm} \)).

2. E.S.R. studies

Transient intermediates in radiolysis can also be detected by e.s.r., and this technique has been developed by Fessenden and his coworkers. A radical must build up to some minimum concentration to be detected, and must not have too great a linewidth.

The radical formed by dissociative electron capture, postulated from stable product analysis, has been detected directly for the mercaptoacetate ion. A spectrum consisting of a 21-2-G triplet with \( g = 2.0032 \) has been attributed to the radical \( \text{CH}_2\text{CO}^- \) at pH 12.4 and 8.6 and shown to have \( \epsilon_\text{g} \) as a precursor because \( \text{N}_2\text{O} \) prevents its formation. Increasing \( \text{SC}_2\text{H}_4\text{CO}^- \) concentration decreased the signal, this being taken as evidence for reaction (4). The OH radical was shown to abstract hydrogen from carbon as well as sulphur since the mercaptoacetate ion at pH 12.4 gave a 13:4:1 G Anisole with \( g = 2.0086 \) attributed to \( \text{SCHCO}^- \). This could not be detected at pH 8.6, and it was thought the doubly-charged anion lowered the recombination rate sufficiently for its concentration to build up to detectable amounts. The radicals ~\( \text{SC}_2\text{H}_4\text{CO}^- \) and ~\( \text{SCH}_2\text{C}(_2)\text{NO}_2 \) were detected in alkaline solutions of 3-mercapto-propionate and cysteine respectively, abstraction from the \( \beta \)-carbon atom with respect to sulphur in the latter case being attributed to the extra stability of a tertiary radical.

No thiol radicals were detected in these studies, possibly because such radicals would react with thiolate anions, reaction (13), and that the G-factor of RSSR might cause such line broadening to make it undetectable.
E. Derivatives of Thiols

1. Disulphides

The OH radical reacts rapidly with disulphides, rate constants greater than \(10^9 \text{ mol}^{-1} \text{ cm}^{-1} \text{s}^{-1} \) being reported for cystine and cystamine. There is little direct evidence for the immediate products, the reaction being written as (17) by Purdie for cystine and penicillamine disulphide, and as (18) by Jayson and Owen and coworkers for cystamine, with the cation undergoing cation cleavage in subsequent reactions. The formation of an adduct \( \text{RSS(OH)} \) with a significant lifetime has also been proposed. Purdie has shown that the OH radical also leads to the formation of trisulphides and has postulated a second set of products from OH attack, reaction (19). The sulphenic acid, from reaction (17), can react

\[
\text{RSS} + \text{OH} \rightarrow \text{RSSOH} + \text{H}^+ \quad (17)
\]

with RSSH produced from cation, reaction (20), or disproportionation, reaction (21), while the trisulphide is also a product of radiation-produced thiol, reaction (22). It has been shown that the presence of chloride ion (hydrochloride salts of aminothiols are often used) in acidic solution decreases S–S cleavage and increases ammonia yields with cystine. (Ammonia is a major product of both \( \text{RSSH} \) and OH attack on amino acids and peptides not having thiol or disulphide groups.)

The hydrated electron reacts rapidly with disulphides, rate constants of \(1.3 \times 10^9\), \(2 \times 10^9\), \(9 \times 10^8\) and \(6.4 \times 10^8\) \text{ mol}^{-1} \text{ cm}^{-1} \text{s}^{-1} \) for cystine, cysteamine, homocysteine and glutathione disulphide, respectively, at pH 6-7 being reported. As discussed in section II.D.1 the adduct RSSR is first formed, and in the absence of other solutes breaks down to RSSH and RSSH.

H atoms have been reported to react with cystine with a rate constant of \(5 \times 10^7\) \text{ mol}^{-1} \text{ cm}^{-1} \text{s}^{-1} \) and to produce cysteine, reaction (23). A transient

\[
\text{RSSR} + \text{H} \rightarrow \text{RSSH} + \text{RS}^+ \quad (23)
\]

11. The radiation chemistry of thiols

intermediate, believed to be RSSHR, has been reported by Simic and Hoffmann on pulse radiolysis of glutathione disulphide at pH 1 with \( \lambda_{\text{max}} \text{ of 330 nm and extinction coefficient of } 600 \text{ mol}^{-1} \text{ cm}^{-1} \text{ L}^{-1} \). The same transient was seen at pH 3-7 where \( \text{RSSHR} \) would react directly with the disulphide, and thus it was postulated that H atom addition, and protonation of the electron adduct gave the same product. Product yields in oxygen-free solutions of disulphides are low because of concurrent oxidation and reduction. RSSOH (or RSSH) and RSSHR effectively give back the starting material, reactions (17), (19) and (20).

2. Large molecules of biological interest

In the preceding sections reactions associated with the thiol or disulphide groups themselves have been mainly discussed, although some of the molecules mentioned do have other functional groups which show varying degrees of reactivity towards the primary radiolysis products of water. However, product analysis shows that in these cases reactions at other sites in the molecule are at the most only minor, the high reactivity of the –SH and –S–S groups being the dominating factor.

Recently work has been done on enzymes which contain both thiol and/or disulphide groups, including lysozyme, trypsin and papain. In each case pulse radiolysis shows an absorption at 400 430 nm associated with RSSR, and shown to have \( \text{RSSR} \) as precursor. Sixty per cent of \( \text{RSSR} \) is estimated to react with the cystine residue in trypsin and perhaps only 25% in the case of papain where 20% of the adducts decayed with a half-life of about 30 \( \mu \)s, the remainder having a lifetime longer than 0.05 s showing it to be very stable. It was noted that there are three disulphide bridges in papain, and it is possible that electron transfer from one of these to other groups could occur in a time too short to allow detection.

OH attack is shown to occur mainly not at free thioli groups, but at tyrosine residues for papain and tryptophan residues for lysozyme.
3. Thiolactone

Homocysteine lactonizes readily in acidic solutions, and a study of the aqueous radiolysis chemistry of this thiolactone was undertaken to see how bonding of the sulphur to a carbonyl carbon modifies its reactivity to the aquated electron. The normal H atom abstraction from sulphur by OH cannot occur. The dissociative electron capture reaction which gives H$_2$S in the case of free thiols can be formulated:

$$\text{H}_2\text{N}-\text{CH}=-\text{CH}_2 + \text{e}^- \rightarrow \text{H}_2\text{N}-\text{CH}(-\text{S}^-)\text{CH}_2 \quad (29)$$

Resonance stabilization of the thiocarboxylate group might have been expected to favour this reaction. The aquated electron reacted fast with the thiolactone, $k = 3.6 \times 10^9$ 1 mol$^{-1}$ s$^{-1}$, but it was found that reductive deamination occurred, thus being the typical reaction for amino acid derivatives. 4,4'-Dithiodibutyric acid was found to be a product, and the following steps involving ring opening were postulated:

$$\text{H}_2\text{N}-\text{CH}=-\text{CH}_2 + \text{e}^- \rightarrow \text{NH}_3 + \text{O} = \text{C}(-\text{S}=\text{CH}_2)-\text{CH}_2-\text{S}^- \quad (27)$$

$$2 \text{O} = \text{C}(-\text{S}=\text{CH}_2)-\text{CH}_2-\text{S}^- + 2\text{H}_2\text{O} \rightarrow (\text{HO} = \text{C}(-\text{S}=\text{CH}_2)-\text{CH}_2-\text{S}^-)_2 \quad (29)$$

The OH radical leads to oxidative deamination and ketoacid formation in a manner similar to that for amino acids. This shows that H atom abstraction occurs from the tertiary carbon atom, rather than from that $\alpha$ to sulphur, as found in e.s.r. studies with cysteine.

11. The radiation chemistry of thiols

$$\text{HO} + \text{H}_2\text{N}-\text{CH}=-\text{CH}_2 \rightarrow \text{H}_2\text{O} + \text{H}_2\text{N}-\text{CH}(-\text{S}^-)\text{CH}_2 \quad (29)$$

$$2 \text{H}_2\text{N}-\text{CH}(-\text{S}^-)\text{CH}_2 \rightarrow \text{H}_2\text{N}-\text{CH}_2 + \text{H}_2\text{N}-\text{CH}(-\text{S}^-)\text{CH}_2 \quad (30)$$

$$\text{H}_2\text{N}-\text{CH}(-\text{S}^-)\text{CH}_2 + \text{H}_2\text{O} \rightarrow \text{O} = \text{C}(-\text{S}^-)\text{CH}_2 + \text{NH}_3 \quad (31)$$

F. Reactions with Secondary Radiation-produced Radicals

Many organic radicals will abstract hydrogen from the thyl group, reaction (4) being one example. Where the organic radical has been produced by H atom abstraction by OH or H$_2$, this hydrogen transfer from thiol restores the molecule to its original form, and effectively protects it from radiolysis damage. This topic is dealt with more fully in section VI on radiation protection.

Inorganic radicals, formed from the reaction between anions and OH can also oxidize thiols to free thyl radicals. The species (CNS$^-$, Br$_2$C]-, Cl$_2$ and I$_2$ (formed by radiolysis of N$_2$O-saturated solutions of CNS$^-$, Br$_2$C]-, Cl$_2$ and I$^-$) are reduced by cysteine with rate constants of 0.5–8.5 $\times$ 10$^3$ 1 mol$^{-1}$ s$^{-1}$, and the rate constants for (CNS$^-$), Br$_2$C]- and I$_2$ increase by approximately a factor of 10 when the thiol is converted to the thiolate anion. (CNS$^-$ exists only in acidic solution.) CO$_2$ is also reduced rapidly by the thiolate anion of cysteine.

Whereas thiols may be oxidized, disulphides may be reduced by electron transfer from radicals. Willson has shown by pulse radiolysis that the electron adduct of the lipote anion [S$^-$/SCH$_2$/CH$_2$/CH$_3$/CO$_2$] is formed in the reaction of lipote with (CH$_3$)$_2$/COH, CH$_3$/CHOH, CO$_2$ and the electron-thymine adduct with rate constants of 1–6 $\times$ 10$^3$ 1 mol$^{-1}$ s$^{-1}$.
Thiol radicals themselves react with disulphides leading to new products where two different alkyl groups are present:\textsuperscript{1, 16}

\[ \text{RS}^* + \text{RSSR} \rightarrow \text{RSSR} + \text{RS}^* \]  \hspace{1cm} (22)

III. AQUOUS SOLUTIONS OF THIOLS CONTAINING OXYGEN

A. Products and Yields

Cysteine has been the most extensively studied thiol in oxygenated aqueous solutions. Although reported yields vary from group to group the following general features have been found:

1. Oxygen lowers \( G(\text{H}_2\text{O}) \) and \( G(\text{H}_2\text{O}) \) and increases \( G(\text{H}_2\text{O}) \) with respect to oxygen-free yields.

2. At low doses, and provided \([\text{RSH}] > 10^{-8} \) the disulphide cystine is still the only major sulphur containing product, but large doses do result in higher oxidation products being formed.

3. Increasing cysteine concentration increases yields, this effect being greater when the free base is used instead of the hydrochloride (Table 4 shows figures from different research groups) and at pH < 5, there is an approximately equimolar increase in hydrogen peroxide and disulphide. Oxygen concentration has little effect on the yields.

Table 4. Variation in \( G(\text{RSH}) \) or \( G(\text{RSSR}) \) with \([\text{RSH}] \) for oxygenated cysteine solutions

<table>
<thead>
<tr>
<th>[RSH] M</th>
<th>( G(\text{RSH}) )</th>
</tr>
</thead>
<tbody>
<tr>
<td>( 10^{-8} )</td>
<td>5.5 5.5 5.6</td>
</tr>
<tr>
<td>( 3 \times 10^{-4} )</td>
<td>10 9.5 7.6 7.6 5 7 15</td>
</tr>
<tr>
<td>( 3 \times 10^{-3} )</td>
<td>15 15 8.2 7.9 20 20 14</td>
</tr>
<tr>
<td>( 3 \times 10^{-2} )</td>
<td>18 18 9.2 10 11 36 74</td>
</tr>
</tbody>
</table>

O\( _2 \) air\( ^* \) air air air 1 atm\( ^* \) 1 atm 1 atm air

pH 1 3 0.1 1.35 4 3-4 7 7 7

Reference 23 52 53 25 54 23 19 25 52

Note: Dose rate \( 0.8-1.4 \times 10^{4} \text{ eV}^{-1} \text{s}^{-1} \).

* Equilibrated with air at 1 atm.

1. As the pH is raised above 5 a marked increase in \( G(\text{RSH}) \) and \( G(\text{RSSR}) \) occurs, but \( G(\text{H}_2\text{O}) \) does not increase until the pH is greater than 7, and then the value of \( G(\text{H}_2\text{O}) \) is less than that of \( G(\text{RSSR}) \).

Figure 1 illustrates this.

![Figure 1. G(Products) as a function of pH for \( 10^{-8} \text{M} \) cysteine saturated with oxygen.](image)

\( \circ = G(\text{RSH}) \quad \square = G(\text{RSSR}) \quad \Delta = G(\text{H}_2\text{O}) \)

Mercaptoethanol has been studied\( ^{16} \) in the pH range 0-5.8, and oxygen increases both \( G(\text{RSSR}) \) and \( G(\text{H}_2\text{O}) \), while increasing the thiol concentration from \( 10^{-4} \) to \( 10^{-3} \) causes a major increase as shown in Table 5.

Table 5. Product yields from aerated aqueous mercaptoethanol solution\( ^* \)

<table>
<thead>
<tr>
<th>[RSH], M</th>
<th>pH</th>
<th>Aeration</th>
<th>( G(\text{H}_2\text{O}) )</th>
<th>( G(\text{RSSR}) )</th>
</tr>
</thead>
<tbody>
<tr>
<td>( 10^{-4} )</td>
<td>3</td>
<td>None</td>
<td>0.36 3-45</td>
<td></td>
</tr>
<tr>
<td>( 10^{-8} )</td>
<td>3</td>
<td>Air</td>
<td>6.5 6.5</td>
<td></td>
</tr>
<tr>
<td>( 10^{-4} )</td>
<td>3</td>
<td>None</td>
<td>0.45 4.4</td>
<td></td>
</tr>
<tr>
<td>( 10^{-1} )</td>
<td>3</td>
<td>Air</td>
<td>36.1 36.1</td>
<td></td>
</tr>
</tbody>
</table>

Note: Dose rate \( 7.8 \times 10^{4} \text{ eV}^{-1} \text{s}^{-1} \).

* Taken from Table 1, reference 7.
Cysteamine\textsuperscript{14} was found to differ from the above two thiols in that little disulphide and hydrogen peroxide were formed at low pH, whereas at higher pH the yields were at least qualitatively similar to those of cysteine. Figure 2 illustrates these points. Presumably products with sulphur in a higher oxidation state than in cysteamine are formed in acidic solution.

**Figure 2.** G(Product) as a function of pH for aerated 10^{-4} M cysteamine.\textsuperscript{14}

Very high yields of disulphide from some \(n\)-alkyl mercaptides with \(G(RSSR)\) up to 650 have been reported\textsuperscript{15}. Quantitative product yields are however difficult to obtain at pH > 8 because of autoxidation, and because the thermal reaction between hydrogen peroxide and thiol proceeds at an appreciable rate.

Disulphides have been studied in the presence of oxygen by Purdie\textsuperscript{28,29,31,32} and Owen and coworkers\textsuperscript{33,34,35}. Sulphonic acids become a major product. Owen consistently reporting higher yields than Purdie, who finds significant amounts of sulphinic acids are still formed.

### B. Effect of Oxygen on Radical Reactions

#### 1. Competition for primary radicals

Oxygen does not react with OIH except at high pH\textsuperscript{19} where the latter exists as \(\text{O}^{-}\). As thiols are readily autoxidized in alkaline solution no detailed studies of oxygenated solutions at high pH have been reported. However, oxygen reacts rapidly with both \(H\) and \(e_{\text{aq}}\) to give \(\text{HO}^{\cdot}\) and \(\text{O}_2^{\cdot}\) with rate constants \(k_{\text{H}}\) and \(k_{\text{O}_2}\) of \(2 \times 10^{9}\) and \(1.88 \times 10^{10}\) \text{mol}^{-1}\text{L}^{-1}\text{s}^{-1}\) respectively\textsuperscript{19}, and would be expected to lower \(G(H_{2})\) and \(G(H_{2}S)\) with

\[
\begin{align*}
\text{H} + \text{O}_2 & \rightarrow \text{HO}^{\cdot} & (33) \\
\text{e}_{\text{aq}} + \text{O}_2 & \rightarrow \text{O}_2^{\cdot} & (34)
\end{align*}
\]

respect to oxygen-free solutions. This has generally been found. Al-Thanno\textsuperscript{2} found for example that air lowered \(G(H_{2})\) from 3.10 to 0.65 in \(10^{-5}\) M cysteine solution and from 5.4 to 3.06 for \(10^{-3}\) M cysteine\textsuperscript{28}, competition being less effective at the higher reaction concentrations as expected. Competition between \(O_2\) and RSH for \(e_{\text{aq}}\) has been used by the Auckland group\textsuperscript{17,36} to determine \(k_{\text{O}_2}\) for various thiols at pH 7 (Table 2).

Neglecting \(H_{2}O\) from reaction (6) and by plotting \(1/G(H_{2}S)\) against \([\text{O}_2]/[\text{RSH}]\),

\[
\frac{1}{G(H_{2}S)} = \frac{1}{G_{\text{H}_{2}S}^{0} - K_{\text{RSH}}^{0}[\text{RSH}]} - k_{\text{O}_2}^{0}[\text{O}_2]
\]

\(k_{\text{O}_2}\) can be found. This technique of determining rate constant ratios by competitive scavenging is common in radiation chemistry.

It has been found for mercaptoethanol that oxygen lowers \(G(H_{2}S)\) more than would be expected from the known values of \(k_{\text{O}_2}\) for this thiol determined by measuring the rate of disappearance of \(e_{\text{aq}}\) by pulse radiolysis and \(k_{\text{O}_2}\) and it has been suggested that the electron adduct of the thiol might be sufficiently long-lived to transfer partially the electron to oxygen before dissociation, reactions (15) and (16). Barton considered

\[
\begin{align*}
\text{HOCH}_{2}\text{CH}_{2}\text{SH} + \text{O}_{2} & \rightarrow \text{HOCH}_{2}\text{CH}_{2}\text{S}^{\cdot} \rightarrow \text{HOCH}_{2}\text{CH}_{2}\text{SH}^{\cdot} + \text{O}^{-} & (35) \\
\text{HOCH}_{2}\text{CH}_{2}\text{SH}^{\cdot} + \text{O}_{2} & \rightarrow \text{HOCH}_{2}\text{CH}_{2}\text{S}^{\cdot} + \text{O}^{-} & (36)
\end{align*}
\]

that the reason Winchester's figure for \(k_{\text{O}_2}\) for cysteine\textsuperscript{19} was only about half that measured directly by pulse radiolysis\textsuperscript{30} might be due to similar reactions, but by re-analysing Winchester's results where both [RSH] and...
2. Reaction of HOO' with RSH

The hydroperoxy radical has a pKₐ of 4-88 and thus it exists as HOO' in acidic solutions and as its conjugate base, O₂⁻ in neutral and alkaline solution²⁸. By studying the formate ion-oxygen-cysteine system as a function of pH Bartron has found that HOO' does not react with cysteine, but that O₂⁻ does²⁹,³⁰. In the absence of thiols, reactions (37), (38) and (39) in addition to (33) or (34) occur, giving a yield of hydrogen peroxide,

\[
\text{OH} + \text{HCOOH (HCOO')} \rightarrow \text{H}_2\text{O}^+ + \text{COOH (CO} \text{)} (37)
\]

\[
\text{COOH (CO} \text{)} + \text{O}_2 \rightarrow \text{CO}_2 + \text{HOO'} (38)
\]

\[
\text{H}_2\text{O}^+ + \text{O} \rightarrow \text{H}_2\text{O} + \text{O}_2 (39)
\]

\[
G(\text{H}_2\text{O}_2) = G(\text{HOCH}_2\text{CH}_2\text{SO}_2) = \frac{1}{2}(G(\text{H}_2\text{O}) + G(\text{CH}_2\text{SH} - \text{CSH}_2 \text{O})).
\]

If the peroxy radical abstracts H from the thiol, the yield of H₂O₂ should increase as each OH₂⁺ or H₂O gives rise to one molecule of H₂O₂.

\[
\text{HOO'} + \text{RSH} \rightarrow \text{H}_2\text{O} + \text{RSH} (40)
\]

\[
\text{O}_2^+ + \text{RSH} \rightarrow \text{HO}_2 \text{O} + \text{RSH} (41)
\]

and

\[
G(\text{H}_2\text{O}) = G(\text{HOCH}_2\text{CH}_2\text{SO}_2) = \frac{1}{2}(G(\text{H}_2\text{O}) + G(\text{CH}_2\text{SH} - \text{CSH}_2 \text{O}) + G(\text{H}_2\text{O}) - G(\text{RSH}) = 0.
\]

In acidic solutions, these increasing to 6-2 and 5-8 respectively as the pH is raised to 5. From this work we estimated Gₐ as 1.8 x 10⁴ mol⁻¹ cm⁻¹ within a factor of five. Using the same method cysteamine was also found²⁹ to be unreactive towards HOO'.

The reason for the enhanced reactivity of O₂⁻ probably lies in the free energy of protonation of the peroxide anion (pKₐ of H₂O₂ is 11-8). On bond strength figures, reaction (40) would be nearly thermoneutral²⁹,³⁰.

3. Reaction of RSSR with oxygen

The transient RSSR has been found to react with oxygen in pulse radiolysis studies of cystine²⁸ and liposome²⁸ with rate constants of 2-3 x 10⁵ and 9 x 10⁵ mol⁻¹ cm⁻¹ s⁻¹ respectively. Oxygen enhanced the rate of first-order decay, the increase in rate being proportional to oxygen concentration. The reaction is thought to involve electron transfer from disulfide to oxygen, reaction (42).

\[
\text{RSSR} + \text{O}_2 \rightarrow \text{RSSR} + \text{O}_2^+ (42)
\]

11. The radiation chemistry of thiols

4. Reaction of thiyl radicals with oxygen

It has been assumed that oxygen reacts with the thiyl radical according to reaction (43) when possible mechanisms for thiol radiolysis in the

\[
\text{RSS} + \text{O}_2 \rightarrow \text{R} + \text{SO}_3^+ (43)
\]

presence of oxygen have been postulated²⁶-²⁹, but direct evidence for this reaction has only been found recently and is limited. Purdie found oxygen inhibited reaction (32) and concluded that oxygen reacts with the thiyl radical²⁶-²⁹. When neutral and slightly alkaline solutions of cysteine saturated with N₂O were irradiated it was found that oxygen markedly decreased the amount of RSSR formed immediately after the pulse as well as greatly increasing its rate of decay by reaction (42). This decrease was considered to be caused by competition between RSSR and O₂ for the thiyl radicals, reactions (13) and (43), and assuming such competition, and plotting A²⁺/Aₘₐₓ against [O₂]/[RSSR], a value of Kₐₗ = 3 x 10⁷ mol⁻¹ s⁻¹ was found, Aₘₐₓ and Aₜₘₐₓ being the maximum absorbances after the pulse in the absence and presence of oxygen respectively²⁹.

Swainson and coworkers found a weak absorption with λₐₚₓ at 300 nm when mercaptoethanol was irradiated in acidic oxygen-saturated solutions²⁹. From the variation in the amount formed on changing mercaptoethanol concentration and pH it was concluded that the transient was HOCH₂CH₂SO₂⁻ and that it had an extinction coefficient of 180 ± 35 M⁻¹ cm⁻¹. A weak transient, λₐₚₓ at 530 nm, was also detected by Packer on irradiating acidic cysteine solutions in the presence of oxygen, the amount formed increasing slightly with cysteine concentration and more definitely with oxygen concentration. The decay kinetics were complex, but all the data were taken with half-lives of a few microseconds decreasing as the pulse length (i.e. dose) increased, suggesting radical-radical reactions²⁹. The data were not inconsistent with the transient being NH₂CH₂CH₂CH₂SO₂⁻.

5. Reaction of alkyl radicals with oxygen

Dioxygen capture by thiol leads to an alkyl radical, reaction (3). Oxygen, by competing for e⁻, lowers the yield of alkyl radicals, and as it adds to them rapidly, reaction (44), should further lower the yield of

\[
\text{R} + \text{O}_2 \rightarrow \text{R} + \text{O} (44)
\]

alkane by preventing H atom transfer from an untreated thiol molecule, reaction (4). No data that show the fate of such alkylperoxy radicals have
been reported. If they abstract H from the thiol group an alkyl hydroperoxide would form, but none has been identified, and anyway may be reduced in the presence of thiol. Serine, the expected reduction product from cysteine, is formed in low yield;

C. Mechanisms

1. Cysteine

For cysteine there appear to be three regions of pH involving distinctly different mechanistic features, namely 0-5, 5-7 and >7.

The pH region 0-5 has been studied by several research groups. Recently Barton has collected all the available data and carried out calculations on the 'extra' product yields due to oxygen. From the known rate constants for reactions (2), (3), (6), (7), (33) and (34) he calculated the initial values of $G(RS^\prime)$, $G(HOO^\prime)$ and $G(\text{O}_2)$. Considering the equilibrium between $\text{HO}_2^\cdot$ and $\text{O}_2^\cdot$ and assuming that reactions (39) and (41) but not (45) occurred, and that each $R^\prime$ radical give rise to half a molecule of cystine, RSSR, he determined $G(\text{RSH})$, $G(\text{RSSR})$ and $G(\text{H}_2\text{O}_2)$ arising from these reactions. Here is a small possible error as the fate of $R^\prime$ in the presence of oxygen is not known. Subtracting these values from the experimental yields, he obtained the 'extra' product yields which he tabulated in (23), $G(\text{RSSR})_R$ and $G(\text{H}_2\text{O}_2)_R$ as the results seemed best explained by a short chain-type mechanism. The essential facts to emerge were that $G(\text{RSSR})_R$ and $G(\text{H}_2\text{O}_2)_R$ for all sets of data that $G(\text{RSSR})_R$ was proportional to (dose rate)$^{-1}$ from the results of A.-Thannenstedt; and that these 'extra' yields increased slowly with increasing cysteine concentration. He postulated the following scheme:

$$\text{RS}^\prime + \text{O}_2 \rightarrow \text{RSO}^\prime$$  \hspace{1cm} (43)

$$\text{RSO}^\prime + \text{RSH} \rightarrow \text{RSOOH} + \text{RS}^\prime$$  \hspace{1cm} (45)

as the propagating steps, and

$$\text{RS}^\prime + \text{RS}^\prime \rightarrow \text{RSSR}$$  \hspace{1cm} (11)

$$\text{RS}^\prime + \text{RSOO}^\prime \rightarrow \text{RSSR} + \text{O}_2$$  \hspace{1cm} (46)

$$\text{RSOO}^\prime + \text{RSH} \rightarrow \text{RSSR} + 2 \text{O}_2$$  \hspace{1cm} (47)

as possible termination steps. Reaction (45) must be relatively slow as the 'chain' yields are very small, and in view of the fact that reaction (40)

11. The radiation chemistry of thiols does not occur; this seems reasonable. Reactions (48), (49) and (50), account for the equality of $G(\text{RSSR})_R$ and $G(\text{H}_2\text{O}_2)_R$. Owen and Brown

$$\text{RSOH} + \text{RSH} \rightarrow \text{RSSR} + \text{H}_2\text{O}_2$$  \hspace{1cm} (48)

$$\text{RSOOH} + \text{H}_2\text{O} \rightarrow \text{RSOH} + \text{H}_2\text{O}_2$$  \hspace{1cm} (49)

$$\text{RSOH} + \text{RSH} \rightarrow \text{RSSR} + \text{H}_2\text{O}_2$$  \hspace{1cm} (50)

found a slow post-irradiation increase in cystine at pH ~5.5 and suggest that reaction (48) was slow, but Barton was unable to reproduce their results.

The relatively low 'chain' yields imply that oxygen which reacts fast with the thyl radical does not get reduced, and reaction (47) is proposed to account for this. As a result of his dismutation studies, Furie has suggested that RSOO' radicals react together according to reaction (50), the product being a diolate, not a peroxide. Assuming the diolate would be reduced to disulphide by thiol, reaction (50) would lead to a considerable increase in $G(\text{RSSR})_R$ without a corresponding increase in $G(\text{H}_2\text{O}_2)_R$, contrary to what is observed. The possibility of reaction (43) being reversible and giving rise to an equilibrium between $R^\prime$ and RSOO', comes from the observation that the maximum absorbance at 530 nm following a pulse of electrons in acidified cysteine solution increased with increasing oxygen concentration at concentrations where reaction (43) would be complete were it a fast irreversible reaction. The decay, assuming the transient to be RSOO', was too fast for it to occur by reactions (11) and (45) alone. Purdie has measured G(cystine) in oxygenated solutions of the mixed disulphide of cysteine and cysteamine as a function of this disulphide concentration. Oxygen and the disulphide compete for cysteinyl radicals from reaction (17), cystine arising from the latter reaction (32). He proposes reaction (46) to account for the fact that G(cystine) has a value of 1.5 when extrapolated to zero mixed disulphide concentration, implying that reaction (43) does not go to completion.

The mechanism requires the 'chain' yield to be proportional to cysteine concentration, but the dependence is much less than first-order. A first-order decay of RSOO' in competition with reactions (45) and (47) would account for this, but a possible reaction is difficult to visualize, and it is concluded that the mechanism is not yet fully understood. A reaction such as (21) is also possible in acidic solutions.

$$\text{RSOO}^\prime + \text{H}_2\text{O} \rightarrow \text{RSOOH} + \text{O}_2$$  \hspace{1cm} (51)
In the pH region 5-7 G(RSSR₉), increases with pH while G(H₂O₂) remains almost constant. The increase in G(RSSR₉) has tentatively been attributed to reaction (53) being much faster than (45), and the divergence

\[ \text{RSO}^- \text{O}^- + \text{RS}^- \rightarrow \text{H}_2 \rightarrow \text{RSOH} + \text{RS}^- \]  

(52)

gathers up to the fact that the intermediate sulphenyl hydroperoxide is reduced to water by cysteine as the pH increases, reactions (53) and (20).

\[ \text{RSOH} + \text{RSH} \rightarrow 2 \text{RSOH} \]  

(53)

A different chain reaction at pH > 7, involving RSSR and producing equimolar amounts of cysteine and H₂O₂ was postulated by Packer and Winchester, and direct evidence for reactions (42) and (41) was subsequently found. Barton suggests that the two competing chain reactions,

\[ \text{RS}^- + \text{RS}^- \rightarrow \text{RSSR} \]  

(13)

\[ \text{RSSR} + \text{O}_2 \rightarrow \text{RSSR} + \text{O}_2 \]  

(42)

\[ \text{O}_2^- + \text{RSH} \rightarrow \text{H}_2 \text{O}_2 + \text{RS}^- \]  

(41)

(13), (42), (41) and (43), (52) with (53) and (20) best explain the experimental yields in this higher pH region.

2. Other thiols

As mentioned in section III.A, mercaptoethanol and cysteamine are the only other thiols that have been studied in any detail. As Table 5 shows, increasing mercaptoethanol concentration in aqueous solution substanially increases G(RSSR) and G(H₂O₂), and a mechanism similar to that proposed above for cysteine has been postulated.

As no detailed product analysis has been done no mechanism for the radiolysis of cysteamine in strongly acidic solution can be postulated. However, it is of interest to note that both Owen and Purdie obtain higher yields of taurine (NH₂CH₂CH₂SO₃H) from oxygenated cysteamine radiolysis than they do the corresponding sulphanic acid from other disulphide solutions. Possibly NH₂CH₂CH₂SO₃⁻ is readily oxidized by H₂O₂ at low pH. Sims has calculated 'chain' or 'extra' yields over the pH range in a similar manner to Barton, and finds that G(RSSR) ~ G(H₂O₂) at pH of about 4, with both increasing as the pH is further increased, G(RSSR) rising faster than G(H₂O₂). Thus at higher pH the mechanisms for cysteamine and cysteine would appear to be essentially the same.

3. Disulphides

Owen considers sulphonated acids are formed by reactions (18), (54) and (55), his values of G(RSO₃H) being close to G₉OH. His values of G(H₂O₂) are consistent with reactions (42) and (39) being important. In his earlier papers Purdie considered the sulphonated acid to come from reaction (56), but after further work has suggested that it may be formed by reaction (57). Both authors also consider a number of other reactions to explain the various products and yields.

\[ \text{RSO}^- + \text{O}^- \rightarrow \text{RSO}^- + \text{H}_2 \text{O} \]  

(50)

\[ \text{RSO}^- + \text{OH}^- \rightarrow \text{RS}^- + \text{H}_2 \text{O} \]  

(51)

\[ \text{RSO}^- + \text{O}_2 \rightarrow \text{RS}^- + \text{O}_2 \]  

(52)

\[ \text{RSO}^- + \text{H}_2 \text{O} \rightarrow \text{RSO}_2^- + \text{H}_2 \text{O} \]  

(53)

11. The radiation chemistry of thiols

4. Conclusions

It is clear from the above discussion that more work is needed before a definitive understanding of the reactions involved in the radiolysis of oxygenated solutions of thiols and disulphides is achieved. The variations in yields with experimental conditions, the distinctly different yields from different thiols or disulphides under similar conditions, and the analytical problems in determining yields of sulphur compounds in various oxidation states makes this a complex and difficult field to work in.

IV. THIOLS IN THE LIQUID STATE

Only two studies of thiols irradiated in the pure liquid state have been reported. It was of interest to compare ethanethiol with ethanol where the main products, in addition to H₂, are ethylene glycol and acetalddehyde, which come partly from CH₃CHOH as precursor. In ethanol the C-H bond is weaker than the O-H bond, whereas in the thiol the relative strengths are reversed. G(H₂) was 7.1, greater than for ethanol (possibly because of the lower ionization energy of ethanethiol), and of the main products CH₃SCH₃-SCH₃ contributed 80% and CH₃SC₂H₅ 15%. No butane,2,3-dithiol or thioacetaldehyde were found. No mention was made of H₂S, a product that might be expected in view of the sulphide yield.
As dissociation of the lowest triplet excited state was not possible, the
conclusion that breakdown occurred from the lowest excited singlet state
following charge neutralization of the parent ion, with S—H cleavage and to
some extent Ph—S cleavage the only important processes. (Johnson also
considered the equivalent of reaction (58) to be less important with
ethanol, and that the difference from ethanol may well be attributed to
the much weaker hydrogen bonding as well as to the relative bond
strengths mentioned above.) In benzene-ethanol mixtures, energy
transfer from benzene to thioethyl was shown to occur, leading to
products similar to those from pure thioethanol. By using deuterated
benzene it was shown that very few H atoms arose from benzene radiolysis.
Prior to this work it was not entirely clear whether the very low values of
G(H₂) found in aromatic systems implied a low yield of H atoms or were
due to the fact that they add to the aromatic ring. Thiophenol was the
first aromatic compound studied which gave an appreciable yield of H₂,
and this work clearly shows the dominating role that the —SH group
exerts when it is present in a molecule, H-abstraction from sulphur
preventing the usual ring addition almost entirely.

Y. THIOLS IN THE SOLID STATE

A. Pure Compounds

1. Product analysis

Most solid state studies have involved only e.s.r. measurements of the
radicals produced on irradiation. However, Garrison and coworkers[7]
have irradiated dry degassed cysteine at room temperature, dissolved the
irradiated solid in water and analysed the products, finding the following
yields: G(H₂) = 2.1, G(PhH⁺) = 1.5, G(NH₃) = 1.8; G(cystine) = 54;
G(NH₂-free compounds) = 10; G(total carbonyl compounds) < 0.1. In
aqueous solution the —SH group appears to be the locus of all significant
reactions as the predominant reactions for amino acid derivatives[11],
reductive and oxidative deamination (the latter leading to carbonyl
compounds), are negligible. It was of interest to see if this was the case in
the solid state also. As the results show oxidative deamination was absent,
but reductive deamination competes with loss of HS⁻. The following
steps were postulated:

\[
\begin{align*}
\text{RSH} & \rightarrow \text{RS}^- + \text{H}^+ + \text{e}^- & \quad (58) \\
\text{RSH} & \rightarrow \text{RS}^+ + \text{H}^- & \quad (59)
\end{align*}
\]

reaction (61) involving proton transfer from the radical ion to a neigbouring group, and (62) dissociation of an excited molecule, followed by

\[
\begin{align*}
\text{H}^+ + \text{RSH} & \rightarrow \text{H}_2 + \text{RS}^- & \quad (63) \\
\text{e}^- + \text{RSH} & \rightarrow \text{H}_2 + \text{NH}_2\text{CH}(_2)\text{CH}_2\text{CO}_2\text{H} & \quad (64) \\
\text{RSH} + \text{CN}(_2)\text{H}_2\text{CO}_2\text{H} & \rightarrow \text{RS}^- + \text{CH}_2\text{CN}(_2)\text{H}_2\text{CO}_2\text{H} & \quad (65) \\
\text{RSH} + \text{NH}_2\text{CN}(_2)\text{H}_2\text{CO}_2\text{H} & \rightarrow \text{RS}^- + \text{CN}(_2)\text{H}_2\text{CO}_2\text{H} & \quad (66)
\end{align*}
\]

(67) reaction (61) involving proton transfer from the radical ion to a neigbouring group, and (62) dissociation of an excited molecule, followed by

\[
\begin{align*}
\text{H}^+ + \text{RSH} & \rightarrow \text{H}_2 + \text{RS}^- & \quad (63) \\
\text{e}^- + \text{RSH} & \rightarrow \text{H}_2 + \text{NH}_2\text{CH}(_2)\text{CH}_2\text{CO}_2\text{H} & \quad (64) \\
\text{RSH} + \text{CN}(_2)\text{H}_2\text{CO}_2\text{H} & \rightarrow \text{RS}^- + \text{CH}_2\text{CN}(_2)\text{H}_2\text{CO}_2\text{H} & \quad (65) \\
\text{RSH} + \text{NH}_2\text{CN}(_2)\text{H}_2\text{CO}_2\text{H} & \rightarrow \text{RS}^- + \text{CN}(_2)\text{H}_2\text{CO}_2\text{H} & \quad (66)
\end{align*}
\]

It was suggested that dimerization of thiol radicals to give disulphide
occurred mainly on dissolution of the irradiated solid. In the case of
cysteamine hydrochloride, where there is no carbonyl group to trap the
electron prior to deamination, it was found that G(NH₃) < 0.1. In spite of
this G(H₂) was only 1/2 lower than for cysteine, but the value of G(H₂)
of 5.1 was much higher. This is the same situation as was found for

2. E.S.R. studies

Several e.s.r. studies have been made on single crystals of cysteine
hydrochloride monohydrate. On irradiation with 1.5 MeV electrons at
77 K Akasaka[9] observed an isotropic doublet as the main radical species
with a high anisotropic but axial symmetric g factor, and attributed this to
the "SCH₂CH₂(COOH)N₃H₂Cl⁻" radical, as found by Kurita and Gordy
for cysteine[10]. Remarkable broadening was observed on warming and at
225 K the spectrum had almost disappeared, although this was not due to radical decay as the spectrum reappeared on cooling. Wheaton and Omerod used $^{14}O$-labeled irradiated at 77 K and then warmed or illuminated their crystal with u.v. light. They observed six radicals, four of which were RS'. Their initial spectrum was a triplet, which Akasaka did not see, probably because his electron beam warmed the crystals above 77 K. Conformational changes on warming lead to interaction between spin on the sulphur atom and neighbouring —SH groups to give large anisotropies in the spectroscopic splitting factor $g$. Warming also gave higher radical concentrations suggesting to them that the original damage was not paramagnetic. Further warming beyond 200 K caused nearly complete disappearance of radicals suggesting that the thyl radical in fact dimerized in the solid state.

Recent work by Budzinski and Box has shown that 77 K is not a sufficiently low temperature to stabilize the primary radicals initially formed and have found evidence for electron capture by the carboxyl group, providing direct evidence for Garrison's mechanism. They were able to get better defined spectra with penicillamine hydrochloride than with cysteine, and reported detailed work on thiol compounds. At 4 K they observed three radical species, two due to oxidation which they assigned to a chloride atom and to $\text{SC(CHOH)}_2\text{CH(NH}_2\text{Cl)}\text{COOH}$ and one due to reduction, $\text{HSC(CHOH)}_2\text{CH(NH}_2\text{Cl)}\text{COOH}$ $\text{C(OH)}_2$ formed by electron capture. On warming to 200 K, hole transfer from the chlorine atom occurred to give a different thyl radical, and the initial thyl radical underwent a change in conformation to give the same radical. The electron adduct also underwent a conformational change and then on further warming, deamination occurred to give the radical $\text{HSC(CHOH)}_2\text{CHCOOH}$. On further warming to 275 K this radical abstracted hydrogen from sulphur to give another thyl radical, and those already formed underwent a further conformational change, so that at room temperature thyl radicals were the only type present. Presumably dissociative electron capture by the $\text{SH}$ group would give a radical which would also abstract H from thiol, so although this radical was not observed, this work was not in disagreement with Garrison's mechanism. Box also had a higher yield of radicals from oxidation than from reduction, supporting Garrison's dissociation reaction, as H atoms would abstract hydrogen from the thyl group. The anisotropy of the thyl radical was again observed. In agreement with the previous work, the final thyl species observed at ambient temperature has undergone bending of the carbon-carbon bond $\alpha$ to the sulphur atom.
VI. RADIATION PROTECTION BY THIOLS

A. Mechanisms

The phenomenon of chemical protection of mammals against the harmful effects of ionizing radiation was discovered in 1949, and analogs or compounds that could give rise to free thiol groups were found to be the most active. Much work on synthesizing and testing new compounds of this class has been undertaken. The phenomenon of protection has been the subject of a book. Where most compounds containing thiol or disulfide groups act as protecting agents in laboratory studies, only some of them are effective in the body, problems of solubility, transport, toxicity and other factors outside the province of physical chemistry being involved. Only simple chemical theories and work related to them are discussed here.

That the mechanism of protection is partly chemical (i.e. involving fast free radical reactions) rather than biochemical (i.e. involving slow reactions of protecting agents with the biologically important molecules prior to or after irradiation as suggested, for example, in the mixed disulfide theory) has been shown by mixing cysteine with bacteria or lysozyme in a rapid flow system. Protection was found with a pre-irradiation mixing time as short as 4 ms but no protection was found if mixing occurred 5 ms after irradiation.

There are two simple mechanisms for this chemical protection, "competition scavenging" and "repair". In both of these the thiol is thought to prevent or reduce damage caused by attack of free radical precursors on the biological solute, so called 'indirect action'. As cells are 60-80% water there is little doubt that these precursors are OH, H, or H$_2$O$_2$ and the number of them reacting with the biological substrate is reduced by competitive scavenging of the thiol, yielding thiol radicals. These are relatively unreactive towards the biological molecules and consequently damage is reduced. Where the primary radicals do react directly with the substrate a free radical formed by H atom abstraction is a likely product and further reactions of this may lead to permanent biological damage.

In the repair mechanism the thiol is thought to transfer a hydrogen atom from sulphur to the radical, restoring the biological molecule to its original form and replacing it by the instantaneously thiol radical.

The repair mechanism can also operate where a radical has been formed by H atom loss after a direct ionization of a biological molecule, and energy transfer, especially to a disulfide group, is also a possibility.

Evidence for these mechanisms has come from radiolysis experiments, including e.s.r. measurements on model systems.

B. Solution Studies

Adams and coworkers have made quantitative measurements on both possible mechanisms, using monomers and polymers as model substrates and cysteine as the protecting agent. On pulse irradiating mixtures of alcohols and cysteamine they found RSSR to be formed in two reactions, one of these being complete 3 μs after the pulse with the other slower reaction occurring during the next 10-100 μs. Increasing the alcohol concentration at fixed cysteamine concentration decreased the amount of RSSR formed 3 μs after the pulse, showing normal competitive kinetic behaviour, reactions (2) and for methanol (69), but the total

\[
\text{OH} + \text{RSH} \rightarrow \text{H}_2\text{O} + \text{RS}^* \quad (7)
\]

\[
\text{OH} + \text{CH}_2\text{OH} \rightarrow \text{H}_2\text{O} + \text{CH}_3\text{OH} \quad (69)
\]

amount finally formed remained the same. The rate of formation of RSSR in the slower reaction was independent of alcohol concentration.
but was proportional to cysteamine concentration, suggesting the repair reaction (70) was being observed. (Recent work on the γ-irradiation of isopropanol in D₂O substantiates this since it was found that addition of thiol induces deuteration of the alcohol and lowers the yield of acetone.)

Analysis of the oscillograms of growth of RSSR yielded ‘repair’ rate constants for a series of alcohols, the values ranging from 1.8 × 10⁻⁴ mol⁻¹ s⁻¹ for i-butanol to 4.2 × 10⁻⁴ mol⁻¹ s⁻¹ for isopropanol. Using polyethylene oxide (PEO) polymers of varying molecular weights, the same two kinetic pathways of RSSR formation were again observed, and repair rate constants of 5.10 × 10⁻⁴ mol⁻¹ s⁻¹ were found. With high molecular weight PEO reactions of PEO radicals with RS⁻ and RSSR were also detected. pH studies on both monomer and polymer systems showed the thiolate anion barely repaired the radicals, if at all.

The repair mechanism does not appear to function where attack occurs on pyrimidine bases. Here primary radicals add to the 5% double bond and hydrogen transfer to the intermediate radical would complete an addition across this bond. This reaction has been observed to occur between cysteine and the protonated electron-adduct of cysteine, and in this case cysteine increases G(–cytosine) by blocking the reconstruction reaction which occurs between OH-adduct and electron-adduct. Adams found the rate constants for reaction between cysteamine and the OH-adducts of allyl alcohol, thymidine and uracil to be less than 10⁷ mol⁻¹ s⁻¹, his findings being confirmed very recently in a pulse radiolysis study using e.s.r. to detect transient intermediates. Both cysteamine and cysteine were used and the corresponding rate constants for uracil and thymine being shown to be less than 10⁶ mol⁻¹ s⁻¹. For these compounds all protection was due to thiol scavenging of OH. This technique gave figures comparable to Adams’s for repair of alcohol radicals, and also showed the repair mechanism functioned for dihydrothymine.

There is evidence that radicals other than primary ones can add to pyrimidine bases and hence damage DNA function, and it has been shown that thiols can prevent this by repairing the intermediate radicals prior to their attack on the base. The repair mechanism has also been shown to operate in e.s.r.-flow studies of biochemical molecules where OH radicals are generated chemically.

Studies of protection of two enzymes, lysozyme and papain, have been made in aqueous solution, and in addition to scavenging protection, a reaction between cysteine and the OH-adduct of lysozyme has been observed. A slow post-irradiation repair reaction was found for papain, probably involving cysteine as a reducing agent.

In some systems it is found that the presence of oxygen lowers the protection given by added thiol, an explanation being that oxygen reacts with the substrate radical in an irreversible step in competition with the hydrogen transfer reaction with thiol. Pulse radiolysis studies with cysteamine were not inconsistent with this.

C. Solid State Studies

E.x.r. studies in the solid state also give considerable evidence for the repair mechanism of thiols. Mention of migration of spins to sulphur in proteins and from solvent to thiol in glasses has been made in section V. In a simple model system a single crystal of 2-aminobutyric acid HCl containing 2% of cysteine HCl was irradiated. The main radical detected at 220 K was CH₃CH₂CHCOOH, but on warming to room temperature the free thiol radical appeared, implying transfer of H from the thiol. Work prior to 1965 has been reviewed and many systems involving mixtures of thiols and model compounds or biological material in the dry or glassy state have been studied since and have provided clear examples of the repair mechanism. However the factors controlling transfer of spin to the added thiols are complex, as recent work by Milvy has shown.

VII. ADDITION OF THIOLS TO OLEFINs

Radiolysis of mixtures of thiols and olefins in the absence of oxygen leads to anti-Markownikov addition across the double bond in a long chain reaction involving free radicals. The propagation steps for a terminal olefin are:

\[ \text{RS'} + \text{RCH} = \text{CH} \longrightarrow \text{RCH} = \text{CHSR} \]  \hspace{1cm} (71)

\[ \text{RSH} + \text{RCH} = \text{CHSR} \longrightarrow \text{RCH} = \text{CHSR + RS'} \]  \hspace{1cm} (72)

This reaction is not specific to radiolysis, and the initiating free radicals may also be generated thermally or photochemically. The general field of free radical addition of thiols to unsaturated compounds has recently been reviewed.

Thiols may be formed by radiolysis of H₂S with olefins, the mechanism being similar to that above, but as the thiol formed undergoes loss of hydrogen by radical abstraction more readily than H₂S, a mixture of thiol and sulphenide is likely to be formed.
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CHAPTER 12

Synthetic uses of thiols

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I. INTRODUCTION

The use of thiols in the synthesis of bivalent organosulphur compounds is well known. Thiols can be converted to sulphides, disulphides, sulphonium salts, sulphonates, sulphones, sulphonic acids, thioacetals and thioacids; these transformations being effected generally by nucleophilic displacement, addition, oxidation or condensation reactions involving the sulphur function. In the above cases a thiol is used in the preparation of a new compound containing sulphur and this is often the main purpose for effecting the reaction. In this chapter we have chosen not to cover per se these types of reactions, certain of these reactions are covered in various detail in other chapters in this volume. We have chosen instead to treat reactions in which a thiol is an important and necessary reagent, being incorporated into the molecule to promote the desired transformation, following which the sulphur function is removed to yield the final reaction product. The thiol, therefore, functions in an accessory role in the synthetic transformation. An example is the conversion of a carbonyl group to a methylene group by Raney nickel desulphurization of an intermediate thiaoacetal; the thiaoacetal being prepared by reaction of a thiol with the ketone or aldehyde.

Examples of thiol functioning in a synthetic transformation involving only one step are minimal. Most cases covered in this chapter require more than one step with several steps being involved in the conversion of the reactant, e.g., reaction with a thiol, into the final product. This necessitates that the thiol be transformed into a bivalent organosulphur derivative, i.e., a sulphide, thiaoacetal or a higher oxidized sulphur function such as a sulphone or sulphonium salt, followed by subsequent conversion to final product. Thus, many of the reactions covered could be considered as examples of the synthetic use of sulphides, sulphones, etc., equally as well as synthetic uses of thiols. The general criteria used in selection of reactions for the chapter have been: (a) a thiol has been or readily could be used in preparation of the intermediate organosulphur derivative, (b) the purpose of the transformation is not the preparation of an organosulphur derivative, thus (c) the sulphur function is normally and conveniently removed to give the final product.

The following reactions have been excluded as being beyond the scope of this chapter: the variety of synthetically useful reactions of dimethyl sulphoxide (DMSO) and dimethyl sulphone, which include reactions involving dienyl anion, oxidation reactions involving DMSO, methylene transfer reactions of corresponding sulphonium methyldides, and reaction of stabilized sulphonium ylids normally prepared from dimethyl sulphide.

II. DITHIOACETALS

The formation of a dithiaoacetal as an intermediate in organic synthesis is not new to most chemists. However, in recent years there has been a continuing improvement in the methods of preparation as well as the subsequent reactions. The early use of the dithiaoacetal group as a means to reduce carbonyl functions with Raney nickel has been expanded to extensive use as a protecting group, methylene blocking group and as an intermediate in the preparation of complex hydrocarbons, olefins, aldehydes and ketones.

A. Carbonyl Protection

The protecting ability of dithiaoacetals has become well established. These groups are stable towards both mild acid and mild base and show reasonable stability towards such varied reagents as lithium aluminium hydride, chromium trioxide and Grignard reagents. However, the method
has rarely been utilized because of the difficulty in regenerating the
 carbonyl. Recent developments in this area should change the situation
 and give dithioacetals a prominent place in synthetic organic chemistry.

1. Preparation

Early workers reacted the ketone with an excess of the thiol in the
 presence of an acid catalyst such as zinc chloride, hydrogen chloride
 or p-toluenesulphonic acid to prepare dithioacetals. The results were
 erratic and the yields often disappointing. The use of boron trifluoride
etherate has led to consistently better results. This method is
 particularly effective when the thiol is used for the solvent of the ketone
 as the boron trifluoride etherate is added. Ethanedithiol and propanedithiol
 are usually the thioles of choice forming 1,3-dithianes and 1,3-dithianes
 respectively. For example, the 1,3-dithianol of cholesterol-3-one (equation
 1) can be prepared in high yield by this method. Occasionally the choice

\[
\text{C}_6\text{H}_{11}\text{H}_{4} \xrightarrow{\text{HS, BF}_3, \text{Et}_2\text{O}} \text{C}_6\text{H}_{11}\text{H}_{4} \text{SH} \]

of solvent is very important and it has been noted that a more acidic
medium such as acetic acid may be useful in accelerating product formation
and reducing side reactions. A newer method involving the use of alkyl
 ortothioborates gives nearly quantitative yields of the dithioacetals of
 simple aldehydes and ketones (equation 3) under neutral conditions.
The orthothioborates are easily prepared from sulphurated sodium
borohydride (equation 2) but the use of dithiols would seem to be excluded.

\[
\text{NaBH}_4\text{S}_2 + \text{EtSH} \rightarrow (\text{EtS})_2\text{B} + \text{H}_2 + (\text{EtS})_2 + \text{NaS}_2\text{H} \quad \text{(2)}
\]

\[
\text{CH}_3\text{CH}_2\text{CH}_2 + (\text{EtS})_2\text{B} \rightarrow \text{CH}_3\text{CH}_2\text{CH}_2 + \text{B}_2\text{O}_2 \quad \text{(3)}
\]

In the formation of 1,3-dithianes of di- and tricarboxyl compounds,
there is considerable selectivity. Normally one does obtain a mixture of
the monothiohexane contaminated by varying amounts of the bisdithiohexane

12. Synthetic uses of thioles

but separation is generally not difficult. Apparently the formation of two
isomeric and hard-to-separate monothianes is seldom a problem.
Cholestane-3,6-dione with excess ethanedithiol gives a high yield of the
bis-1,3-dithianol (equation 4) in just 5 mm. Restricting the quantity of
thiol and extending the reaction time led to a mixture containing a reasona-
ble yield of the cholestane-3,6-dione-3-(1,3-dithianol) (equation 5).

\[
\text{C}_19\text{H}_{18} \text{O} + \text{EtSH} \rightarrow \text{C}_19\text{H}_{18} \text{SH} + \text{EtSH} \quad \text{(4)}
\]

\[
\text{C}_19\text{H}_{18} \text{O} + \text{EtSH} \rightarrow \text{C}_19\text{H}_{18} \text{SH} + \text{EtSH} \quad \text{(5)}
\]

Where the nature of the carbonyl of a dicarboxyl compound differ
greatly, one isomer of the monothiohexane may become the only product.
In the conversion of 4-androstene-3,11,17-trione to 4-androstene-3,11,17-
trione-3-(1,3-dithianol) no bis- or trisdithianol was observed (equation
6). The condensation of an equimolar amount of 1,3-ethanedithiol with

\[
\text{C}_6\text{H}_{11}\text{H}_{4} \text{H}_{2} \xrightarrow{\text{HS, BF}_3, \text{Et}_2\text{O}} \text{C}_6\text{H}_{11}\text{H}_{4} \text{H}_{2} \text{SH} \]

an α-keto aldehyde such as pyruvaldehyde leads to the formation of
1,3-dithianol-2-carboxylaldehyde with little or none of the isomeric
1,3-dithianol-2-carboxyl ketones being observed (equation 7).
Although the formation of dithioacetics generally is a simple reaction, side reactions become prevalent when a reasonable leaving group is in the α-position to the carbonyl or to a conjugated double bond. In the reaction of 2-bromo-2-phenylacetophenone with ethanedithiol, 2,3-diphenyl-5,6-dihydro-1,4-dithiin (equation 8) was obtained. Similarly, the dihydrodithin (1) was obtained from 6β-acetoxyl-4-cholesten-3-one (equation 9). Additional examples exist for the formation of dihydro-1,4-dithiins via halides, epoxides, and even amides.

Under slightly different conditions, using 1,3-propanedithiol, acetylides and acylain acetates lead to the formation of 1,3-dithiines where hydrogen has replaced the hydroxy or acetoxy group. Hydrolysis to the ketone provides a method of converting acylains to ketones and desulfurization allows conversion of acylains to hydrocarbons (equation 10). Reduction of 1,1-dimethyl-5-hydroxysin-4-cycloheptanone gave 1,1-dimethyl-tetra-cycloheptanone by this method (equation 11). A similar reaction is believed to be involved in the action of α-proline reductase.

Oxidation of 1,5-dithiines with monoperothiophosphate or hydrogen peroxide gives ethylenedithiophones in high yields. These compounds are stable in acid, but are easily decomposed with base in the presence of oxygen to give the original carbonyl group. Thus, 1,6β-hydroxy-5α-androstan-3-one-3,1,3-dithiine) acetate was converted to the disulphide, which in the presence of sodium ethoxide and oxygen gave the original ketone (equation 12). Besides the fact that this method is effective in the steroid series, there are the advantages of being able to hydrolyse acid-sensitive compounds or work in acid media without fear of decomposing the blocking group.

The use of a mild oxidizing agent such as 1-chlorobenzotriazole with 1,5-dithiines and 1,5-dithiines leads to the formation of disulphoxides.
The disulphoxides generally are not isolated but are decomposed with sodium hydroxide to the ketone. The reaction works well in the steroids, with 17β-αcetoxytestosterone (3) easily being regenerated from its dithioacetal (equation 13).

Oxidative hydrolysis of 1,3-dithianes using N-halosuccinimides has been extensively investigated. The yields were consistently high when using N-bromosuccinimide (NBS), usually in acetonitrile. Unlike earlier methods 2-acyl-1,3-dithianes were efficiently hydrolysed to 1,2-dicarbonyl compounds. For example, 1-phenyl-1,2-propanedione was prepared in quantitative yield from the 2-benzoyl-2-methyl-1,3-dithiane (equation 14). Silver salts often aid the reaction, but it has been noted that NBS in the presence of silver ion reacts with double bonds. However, N-chlorosuccinimide (NCS) even with silver nitrate is compatible with double bonds and still gives comparable yields.

A few other methods for hydrolysis of 1,3-dithianes have recently been discovered but have not been thoroughly investigated. Use of sodium N-chloro-p-toluene-sulphonamide (chloramine-1) leads to the corresponding ketones in consistently high yield. The procedure requires only short reaction times in aqueous alcohol and should prove to be a very powerful method.

Alkylation of 1,3-dithiolanes with two equivalents of triethylammonium tetrafluoroborate leads to bisulphonium salts. Treatment with 10% sodium hydroxide gives excellent yields of the corresponding ketones. If only one equivalent of the oxonium salt is used, the resulting mono-sulphonium salt gives the ketone in high yield if a mild oxidizing agent such as copper sulphate or hydrogen peroxide is present. Equations (15) and (16) demonstrate the effectiveness of this method in the recovery of trans-1-decalone.

The sulphonium salt also seems to be involved in a procedure using methyl iodide in aqueous alcohol. Mild conditions and high yields are typical. That the reaction is quite selective is apparent from the hydrolysis...
of the 1,3-dithiolane of 9-fluoro-11β,16α,17,21-tetrahydroxyprog-4-en-3,20-dione-16,17-acetone (4) in high yield (equation 17).

![Chemical structure](image)

Some further representative examples of the hydrolysis of dithioacetals are given in Table 1.

<table>
<thead>
<tr>
<th>Dithioacetal of</th>
<th>Reagent</th>
<th>Yield %</th>
<th>Reference</th>
</tr>
</thead>
<tbody>
<tr>
<td>Cholesterol-1-one</td>
<td>Chloramine-T</td>
<td>75</td>
<td>37</td>
</tr>
<tr>
<td>I-Chlorobenzotriazole</td>
<td></td>
<td>50</td>
<td>35</td>
</tr>
<tr>
<td>Monopropionylphthalaldehyde</td>
<td></td>
<td>67</td>
<td>37</td>
</tr>
<tr>
<td>(1) Et₂OBF₂; (2) NaOH</td>
<td></td>
<td>81</td>
<td>38</td>
</tr>
<tr>
<td>Choloramine T</td>
<td></td>
<td>95</td>
<td>37</td>
</tr>
<tr>
<td>(1) Et₂OBF₂; (2) CuSO₄</td>
<td></td>
<td>81</td>
<td>38</td>
</tr>
<tr>
<td>HgO—BF₃</td>
<td></td>
<td>25</td>
<td>28</td>
</tr>
<tr>
<td>Fluorenone</td>
<td>Chloramine-T</td>
<td>86</td>
<td>37</td>
</tr>
<tr>
<td>Ph₃C—CHCHO</td>
<td>HgO—BF₃</td>
<td>86</td>
<td>38</td>
</tr>
<tr>
<td>Ph₂SCH₂</td>
<td>HgCl₂, aq. acetone—benzene</td>
<td>82</td>
<td>40</td>
</tr>
<tr>
<td>CH₂ = C—CH₂</td>
<td>HgCl₂, HgO, aq. MeOH</td>
<td>70</td>
<td>41</td>
</tr>
<tr>
<td>NCS, AgNO₃</td>
<td></td>
<td>94</td>
<td>36</td>
</tr>
<tr>
<td>Ph—O—BF₂</td>
<td>HgO—BF₃</td>
<td>80</td>
<td>29, 30</td>
</tr>
<tr>
<td>PhCH₂CO₂Et</td>
<td>NBS, acetone</td>
<td>78</td>
<td>36</td>
</tr>
</tbody>
</table>

8. Carbonyl Reduction

Since the Raney nickel desulphurization of dithioacetals to the corresponding methylene was first observed by Wolfman, the reaction has become one of the most reliable and mild ways of reducing the carbonyl group. Outstanding reviews can be found concerning the application of nickel desulphurizations to all types of organosulphur compounds as well as a detailed discussion of the mechanism. However, a brief mention of the scope of the reaction as well as some of the more recent modifications seems in order.

1. Reduction to saturated hydrocarbons

Typically desulphurization reactions are carried out with a large excess of Raney nickel. The reaction is not truly catalytic in nature since the hydrogen used to replace the sulphur usually comes from hydrogen retained by the metal during its preparation. In addition the nickel is consumed by the combination with the sulphur to form nickel sulphide. In practice a minimum ratio of 2:6:1 for nickel atoms to sulphur atoms is necessary.

The Raney nickel catalyst is prepared through the action of aqueous alkali on a nickel—aluminium alloy. The conditions employed allow the preparation of the catalyst with a specific activity. Furthermore, the catalyst may be deactivated by refluxing with hydrogen acceptors, by degassing or by ageing. For details the reader is referred to the reviews mentioned above.

![Chemical structure](image)

Although desulphurizations are very successful on most dithioacetals, a few have been somewhat unsatisfactory. Compounds 5a, 6a and 7a are typical of the high yields which often accompany desulphurizations. On
the other hand, \(n\)-heptanal diethylthioacetal gave only 40% yield of heptane\(^{43}\) and compound 8 gave only 33% of desulfurized product\(^{43}\). Other functional groups generally do not affect the results. Desoxvtrtetrahydrofuranene (9) gave the desired product quantitatively\(^{40}\) and isatin 1,3-dithiolane (10) gave oxindole without complication\(^{40}\).

![Structural diagrams](image)

A recent modification in the use of Raney nickel may greatly enhance its utility. Industrial use of the standard procedure has been limited by the necessity to use such large quantities of the very expensive Raney nickel. It now appears that the use of the nickel-aluminum alloy itself in formic acid leads to very efficient desulfurizations with Ni/S ratios of only 0.2\(^{44}\). High proportions of the aluminum seem to give the best results, apparently because of the ability of the aluminum to regenerate the active nickel catalyst. Similar results were obtained using nickel or cobalt salts in the presence of auxiliary means such as aluminum or iron.

The use of deuterium oxide and sodium deuterium oxide in the preparation of Raney nickel leads to the formation of deuterio Raney nickel suitable for replacing dithioacetals with deuterium\(^{45,46}\). The method suffers from some scrambling of the isotope often leading to products of low isotopic purity. Deuteration of (25R)-5α-spirostans-12-one (11) by this

![Structural diagrams](image)

12. Synthetic uses of thiols

method led to an isotopic mixture consisting of 4% \(d_0\), 44% \(d_1\), 49% \(d_2\) and 3% \(d_3\) products\(^{47}\). At times fairly pure products are obtained, such as the preparation of 12,12-\(d_2\)-pregnane (12) with 76% \(d_2\)\(^{48}\).

2. Reduction to olefins

The formation of an olefin during desulfurization was first noted when 1,3,3-trihexyloctaholestane gave a mixture of cholest-1-ene and cholest-2-ene (equation 18) with Raney nickel deactivated by boiling in acetone\(^{48}\). Similar conditions gave predominantly olefin with the 1,3-dithiolane from 145-A\(\Delta^5\)-anthraquinone-15-one (equation 19)\(^{49}\).

![Structural diagrams](image)

More extensive investigations\(^{46,48}\) have led to the use of W-2 Raney nickel in refluxing acetone to obtain olefins in 55-75% yields based on starting ketone. Even the synthesis of dienes from \(\alpha,\beta\)-unsaturated ketones was successful\(^{48}\). Using this method 5α-cyano-17β-hydroxyestrane-3-one was converted to the corresponding olefin (equation 20)\(^{48}\). Surprisingly, the 5β-cyano isomer gave low yields in the first step and no olefin in the second step. Both the cis- and trans-isomers in the 2-keto-10-cyano series have been converted to olefins\(^{50}\). Other examples of this reaction include the conversion of dihydrogadenin (13) to the olefin\(^{50}\) and the partial formation of olefin from 17-norphytoicladan-16-one (14)\(^{51}\). Groups in the \(\alpha\)-position to the ketone may be lost during the reaction as seen by the formation of 5α-cholest-2-ene as the sole product from 2α-chloro-5α-cholestan-3-one\(^{46}\).
The mechanism of this reaction seems to involve formation of a diradical intermediate which, if the concentration of hydrogen radicals is low, gives the thioether ether. Further desulfurization gives the selenoether (equation 21). If the alkyl radical is responsible for the C-18 hydrogen abstraction, it would seem necessary that it remain near the reaction site so that homolysis takes place before addition of hydrogen from the catalyst. Analogy with studies of the mechanism of desulfurization in monothioacetals and thiazolidines suggests that the abstraction may very well come from an external radical.

C. Methylene Blocking Group

In the presence of ethyl formate and sodium methoxide, the most reactive methylene group of a ketone is converted to its hydroxymethylene derivative. Further reaction with the dithioate of propane-1,3-dithiol leads to the formation of the 1,3-dithiane (equation 22). Thus the active position of the ketone is effectively blocked with a group easily removed by Raney nickel.

1. Alkylation

The presence of the dithioacetals does reduce the reactivity of the ketone toward alkylation at its other available positions, but nevertheless the reaction has been effectively utilized. This is clearly demonstrated by the formation of 4,4-dimethylcholestanone by this procedure (equation 23). Other examples of the successful use of this method include the preparation of 4α,9α-dimethyl-5α-androstan-3-one (15) and 4α-methyl-3B-nor-5α-cholestan-3-one (16).
2. Decarbonylation

The formation of 1,3-dithianes from hydroxymethylene compounds, which are enol tautomers of β keto aldehydes, has been shown to be useful in itself. When followed by desulfurization the net reaction is the decarbonylation to the ketone. This has been used to advantage in the formation of the methyl ketone (17) in equation (26). Similarly the methyl ketone (18) was formed from its hydroxymethylene derivative (19).

3. Formation of dicarbonyl compounds

The treatment of the intermediate 1,3-dithiane, either before or after alkylation with reagents such as mercuric chloride–cadmium carbonate (see section II.A.3) gives hydrolysis to the carbonyl. Thus trans-fukinone (19) was converted to (+)-hydroxyeremophilone (20) (equation 25). In cis fukinone, the 1,3-dithiane could not be formed from the 1-hydroxymethylene fukinone, presumably for steric reasons.

4. Ketone transposition

Modification of the above sequence to include reduction of the original ketone before hydrolysis is the basis for a new method of ketone transposition. For example, the keto 1,3-dithiane (21) was prepared in the usual manner followed by reduction of the carbonyl with lithium aluminum hydride to the alcohol (22). Conversion to the acetate and hydrolysis of the dithiane with mercuric chloride led to the keto acetate (23). Reduction with calcium in ammonia resulted in the formation of the new methyl decalone (24) in 58% overall yield (equation 26). The same sequence was used to convert decalone (25) into the isomeric decalone (26) in 46% overall yield (equation 27). These conversions have been shown to take place with complete stereochemical integrity. Alternative methods of removing the carbonyl from the keto 1,3-dithiane so far have not been satisfactory.

5. Selective carbon–carbon bond cleavage

Keto 1,3-dithianes are susceptible to nucleophilic attack at the carbonyl with subsequent cleavage occurring preferentially between the carbonyl and the dithiane functions. In the one instance reported, the keto dithiane (27) was cleaved with sodium methoxide in dimethyl sulphoxide to acid (28). The explanation as to why the acid is formed instead of the
methyl ester is not apparent. The reaction conditions are mild and do not seem to pose serious limitations on the nature of the rest of the molecule. Most importantly, after cleavage, the 1,3-dithiane grouping is suitable for many conversions such as reduction, alkylation, acylation or hydrolysis.

![Chemical structures](image)

**D. Synthetic Applications of 2-Lithio-1,3-dithianes**

Corey and Seebach have reported the use of 2-lithio-1,3-dithianes as useful reagents in organic synthesis. The method involves the use of 1,3 propanedithiol, which is caused to react with an aldehyde to yield the 1,3-dithiane (equation 29). Lithiation of the dithiane, normally with n-butyllithium in tetrahydrofuran at lower temperatures, gives the 2 lithio 1,3 dithiane (equation 30). The R group can be alkyl, aryl or hydrogen.

$$\text{HS}^- + \text{SH} + \text{HCHO} \rightarrow \text{S}^- \text{S}^- \text{R}$$  \hspace{1cm} (29)

![Chemical structures](image)

2-Lithio-1,3-dithianes have been shown to undergo reaction with a variety of electrophiles, $\text{R}^+$, to give substituted dithianes (equation 31). Removal of the dithioketal function generates the newly synthesized carbonyl compound (equation 32) having the group $\text{R}^+$ substituted for the aldehydic hydrogen of the original aldehyde. The dithioketal is most often hydrolysed using the mercuric chloride: mercuric oxide method or by oxidative hydrolysis with N-halosuccinimides. It is possible also to remove the dithiocacetal function by desulfurization (Raney Ni) to yield the corresponding methylene derivative (equation 33). For a general treatment of removal of the dithiocacetal function, see section II.A.2.

$$\text{S}^- \text{S}^- \text{R} \rightarrow \text{O} = \text{S}^- \text{S}^- \text{R}$$  \hspace{1cm} (31)

$$\text{S}^- \text{S}^- \text{H} \rightarrow \text{H} = \text{O}$$  \hspace{1cm} (32)

$$\text{S}^- \text{S}^- \text{R} \rightarrow \text{R}^- \text{S}^- \text{H}$$  \hspace{1cm} (33)

12. Synthetic uses of thiols

2-Lithio-1,3-dithiane reagents are in effect masked nucleophilic acylating agents and can be considered equivalent to the presently unknown thiolithium reagent (30). Thus, by use of a thiol, the carbonyl carbon of an aldehyde can be transformed from an electrophilic site to the nucleophilic centre in the lithiated dithiane derivative (30). The ability of sulphur to stabilize carbanions α to the sulphur atom is significant in the readily accomplished lithiation of 1,3-dithianes. The preparation and reactions of 2 lithio 1,3 dithianes have been reviewed.

The following is a general outline of the various types of reactions that these reagents are known to undergo, including a comprehensive treatment of reactions reported since the review article by Seebach.

**I. Reaction with alkyl halides**

2 Lithio-1,3-dithianes undergo alkylation at the 2 position upon reaction with alkyl halides. This reaction appears to be $\text{SN}_2$ in nature as it is applicable to primary and secondary alkyl halides, occurs most readily with alkyl iodides, and with optically active secondary halides gives inverted products. It has been shown that reaction with optically active alkyl halides provides a useful route for the preparation of optically active aldehydes or ketones.

Cycloalkylation has been effected by reaction with $\alpha, \omega$-dihaloalkanes to give, upon hydrolysis, cyclic ketones (equation 37). Likewise, the
dithiane derivatives of α,β-unsaturated aldehydes undergo hydrochlorination followed by cycloalkylation to yield substituted cyclopropanes (equation 38). Cyclic 1,3-diones are available by the

\[
\begin{align*}
\text{CH}_2\text{I} & \rightarrow \text{CH}_2\text{CH}_2\text{I} \\
\text{Me} & \rightarrow \text{MeC} = \text{H} \\
\text{Et} & \rightarrow \text{MeCR} \\
\end{align*}
\]

alkylation of the cis-dithiane 34 (equation 39). It has also been observed that the use of α,ω-dibromoalkanes in cycloalkylation reactions is complicated by formation of sulfonium salts (equation 40), a reaction not observed with use of α,ω-diiodo or α,ω-dichloroalkanes.

\[
\begin{align*}
\text{S} & \rightarrow \text{S} \\
\text{H} & \rightarrow \text{Cl} \\
\text{Li} & \rightarrow \text{MeC}_2\text{H}_4 \\
\end{align*}
\]

Corey and coworkers, in a synthesis of prostaglandins, prepared diene 34 by alkylation of the lithiodithiane 32 with 2-bromomethyl-1,3-butanediene (equation 41). A synthesis of jasmon (35), in an overall yield of 50%, has been reported by Ellison and Woesner in which the bisdithiane 33 was sequentially alkylated, followed by hydrolysis and cyclization (equation 42). A similar route for preparation of 4-hydroxy-2-cyclohexen-1-ones has been reported. This method appears to provide a general route to 1,4-diketones via 1,3-dithianes.

\[
\begin{align*}
\text{Li} & \rightarrow \text{C}_2\text{H}_4 \\
\text{MeO} & \rightarrow \text{MeS} \\
\text{OMe} & \rightarrow \text{MeS} \\
\end{align*}
\]

The synthesis of the monoterpene components 40 of the sex attractant of the bark beetle has been accomplished as outlined in equation (43). The alkylation of the dithiane 37 was a key step in the synthesis since efforts to prepare 40 by addition of the magnesium or lithium derivatives of the bromocycloalkene 36 to the appropriate aldehyde failed.

\[
\begin{align*}
\text{S} & \rightarrow \text{O} \\
\text{H} & \rightarrow \text{O} \\
\text{Li} & \rightarrow \text{H} \\
\end{align*}
\]

Hylton and Boekelheide prepared the cyclophanedione 43 by alkylation of the bisdithiane 41 followed by hydrolysis. An improved procedure for the preparation of 41 has been reported.
2. Reaction with aryl halides

Treatment of 2-lithio-2-phenyl-1,3-dithiane with 2-bromopyridine gave the substituted pyridine 44 in 50% yield. However, reaction with 2,4-dinitrobenzene gave none of the substitution product, but rather compound 45 resulting from oxidative dimerization of the dithiane.

12. Synthetic uses of thiols

Such oxidative dimerizations (see section II.D.7) of 2-lithio-1,3-dithianes are known and have been reported to occur with nitro compounds.

3. Reaction with epoxides

Epoxides effect alkylation of 2-lithio-1,3-dithianes (equation 47); opening of the epoxide ring occurs in the fashion typical of reactions with nucleophiles. The reported yields are in the range of 70-95% and appear to be free of side reactions common with other organometallic reagents.

Recently, Jones and coworkers have reported the reaction of lithiodithiane derivatives with steroidal epoxides to effect preparation of modified steroids. Treatment of 2α,3α-oxiranyl-5α-cholestan-3β-ol (46) with 2-lithio-1,3-dithiane, followed by desulphurization, yielded the 2β-methyl-3α-cholesterol 47 (equation 48). Conversely, reaction with the epimeric epoxide 48 furnished 5α-methyl-5α-cholestan-3β-ol (49) (equation 49).

The spiroepoxide 50, prepared from 5α-cholestan-3-one, was cleanly converted to the 3β-ethyl derivative 51; the 3α-ethyl derivative 53 was obtained in an analogous manner from the epimeric spiroepoxide 52 (equation 51). Similar results were obtained when this method was applied...
The preparation of some γ-fluoro-β-hydroxyketones (58) by reaction of epifluoroethyldene with the lithio derivative 57 has been reported. The dithioacetals prepared from dithiol 56 are reported to be crystalline, odourless compounds, therefore some advantage may be purported for their use.

A synthesis of α,β-unsaturated aldehydes has been effected by reaction of 2-lithio-1,3-dithiane with epoxides (equation 54). It was found that treatment of the dithiinyl alcohol 59 with mercuric oxide-hexane trifluoride caused dehydration and hydrolysis to give the α,β-unsaturated aldehyde 60 in good yield. Standard methods for removal of the thiaoacetel function were not successful in these cases.

4. Reaction with aldehydes and ketones

2-Lithio-1,3-dithianes add to the carbonyl group of aldehydes and ketones to provide unexploited derivatives of α,β-unsaturated aldehydes or ketones (equation 55). The yields are normally quite high. Reaction with α,β-unsaturated ketones has been observed to give only 1,2-addition; however, Becker and Linke have reported 1,4-addition to occur in reactions with α,β-unsaturated nitro derivatives (equation 56). In the case

where R' = H, the addition product obtained from reaction with a ketone can be converted by dehydration to a ketene thiaoacetel (equation 57).

Ketene thiaoacetals also are readily available by a Wittig-type reaction of 2-lithio-2-trimethylsilyl-1,3-dithiane (61) with aldehydes or
ketones. The dithiane 61 is prepared\(^{61a}\) by reaction of 2-lithio-1,3-dithiane with trimethylenemethane followed by lithiation (see section II.D.6). A method employing the phosphate yield 62 to prepare ketene thioacetals by reaction with aldehydes, but not ketones, has been reported\(^{62}\) (equation 59).

\[
\text{S}_2\text{CMe}_3 \quad \text{H} \quad \text{R}_2\text{C}=\text{O} \quad \text{Li} \quad \text{Me}_2\text{SiOLi} \quad \text{(60)}
\]

Ketene thioacetals should prove to be useful synthetic intermediates. Hydrolysis\(^{60}\) of ketene thioacetals yields carboxylic acids (63), while pretreatment with triethylamine yields the thioacetate (64) of the homologous aldehyde (65).

\[
\text{S}_2\text{CMe}_3 \quad \text{H} \quad \text{R}_2\text{C}=\text{O} \quad \text{Me}_2\text{SiOLi} \quad \text{(62)}
\]

Alkylithium reagents are known\(^{14}\) to add to ketene thioacetals to give 2-lithio-1,3-dithianes 66 in which R' has become attached to the cysteine carbon. Both 64 and 66 are capable of undergoing further reactions available to 2-lithio-1,3-dithianes. Therefore, it should be possible in principle to convert an aldehyde, RCHO, to any of the following via the corresponding ketene thioacetal: RCH\(_2\)CO\(_2\)H, RCH\(_2\)CHO, RR'CH\(_2\)CHO, RR'CHCOR\(^*\), and RR'R"CCOR\(^*\).

12. Synthetic uses of thiols

Imines, being nitrogen analogues of carbonyl compounds, are reported\(^{23}\) to undergo addition with 2-lithio-1,3-dithianes to yield amines (equation 61).

\[
\text{S}_2\text{CMe}_3 \quad \text{Li} \quad \text{PhCH} = \text{NPh} \quad \text{(61)}
\]

5. Reaction with acylating agents

Acylation of 2-lithio-1,3-dithiane derivatives occurs in satisfactory yields only when a dilute solution of the dithiane derivative is added at \(-78^\circ\)C to a solution containing a 20-100-fold excess of the acylating agent\(^{25,26}\) (equation 62). The above conditions are necessary to circumvent reaction of a molecule of the reactive lithiodithiane with a molecule of previously formed 2-acyldithiane. This method offers, by subsequent removal of the dithiotetronic function, a route for the preparation of \(\beta\),\(\gamma\)-dicarbonyl compounds.

\[
\text{S}_2\text{CMe}_3 \quad \text{Li} \quad \text{R} \quad \text{COX} \quad \text{(64)}
\]

Acylating agents that have been employed\(^{22}\) are carbon dioxide, alkyl chloroformates, alkyl formates, acid chlorides, esters, benzonitrile and dimethylformamide; the expected acylation products from reaction with the above reagents were formed in each case. However, the N,N-dimethyl-amide derivatives of higher carboxylic acids did not yield acylated product as in the case of dimethylformamide\(^{29}\). When R = H (equation 63), it was necessary to employ two equivalents of the lithiodithiane due to product enolate formation.

\[
2 \quad \text{S}_2\text{CMe}_3 \quad \text{Li} \quad \text{COX} \quad \text{R} \quad \text{Li} \quad \text{R'} \quad \text{S}_2\text{CMe}_3 \quad \text{H} \quad \text{(63)}
\]

In the total synthesis of illudin M, Matsumoto and coworkers\(^{26}\) prepared the cyclopentene 70 by reaction of 2-lithio-1,3-dithiane with...
the ester 67 to give 68. Reduction, acetylation and removal of the dithio-
acetal function gave 69, apparently formed by an intramolecular trans-
ketalization reaction.

\[ \text{CH}_3\text{CO}_2\text{Et} \rightarrow \text{O} \rightarrow \text{Ac} \rightarrow \text{O} \rightarrow \text{(64)} \]

6. Silylation and related reactions

2-Lithio-1,3-dithianes react with trialkyl- and triaryl-chlorosilanes to
give the silylated derivatives (equation 65). This method was used in the
preparation\(^{48-50}\) of the previously unknown \(\alpha\)-silylketones 71. Germany-
lation and stannylation also can be accomplished with the corresponding
trialkylhafnols derivatives\(^{48}\).

\[ \text{S}_2\text{R} + \text{RSiCl}_3 \rightarrow \text{S}_2\text{R} + \text{RSiCl}_3 \rightarrow \text{O} \rightarrow \text{R} - \text{C} - \text{SiR}_3 \]

7. Oxidative dimerization

Treatment of 2-lithio-1,3-dithianes with iodine, cupric salts, 1,2-di-
bromoethane, or nitro compounds effects oxidative dimerization\(^{51}\) to
give the dimer 72 plus a small amount of the 2-methylene derivative 73.

\[ \text{S}_2\text{Li} \rightarrow \text{S}_2\text{Me} \rightarrow \text{S}_2\text{Me} + \text{S}_2\text{Me} \]

8. Reactions using 1,3,5-trithianes

1,3,5-Trithianes (74) undergo lithiation\(^{56,78}\) with an equivalent of
\(\alpha\)-butyllithium to yield the 2-lithio derivatives, which substances undergo
the usual reactions (equation 67) as with 2-lithio-1,3-dithianes. Since
additional active hydrogens are present in 1,3,5-trithianes, dimethylation
has been observed in some cases\(^{56}\).

12. Synthetic uses of thiols

An alternate route not involving 2-lithio-1,3,5-trithianes for the
preparation of 2-substituted-1,3,5-trithianes recently has been reported\(^{66}\).
This method involves reaction of an aldehyde 77 with the dithiol 78 to
yield the 2-substituted trithiane 79.

\[ \text{RCHO} + \text{S(CH}_2\text{SH}_2\text{)}_3 \rightarrow \text{H}^+ \rightarrow \text{R} - \text{S} - \text{R} \]

9. Miscellaneous applications

A convenient preparation of 1-deuterioaldehydes (81) via 2-lithio-1,3-
dithianes has been reported by Seebach and coworkers\(^{39}\) (equation 69).
This method appears to be superior to previously reported methods for the
preparation of 1-deuterioaldehydes.

\[ \text{S}_2\text{Li} \rightarrow \text{S}_2\text{Ph} \rightarrow \text{S}_2\text{Ph} \rightarrow \text{H}^+ \rightarrow \text{S}_2\text{D} \]

Treatment of 2-lithio-1,3-dithiane derivatives with methyl disulphide
yields the orthothioformate 83, which upon hydrolysis in alcoholic
solvents furnishes an ester\(^{78}\). This method may provide a useful route for
the conversion of sensitive aldehydes to esters and carboxylic acids.

III. MONOTHIOACETALS

The use of monothioacetals in organic synthesis has not been strictly so
extensive as the use of dithioacetals. Generally prepared as 1,3-
oxathiolanes and 1,3-oxathianes, the group is resistant toward dilute base and lithium
aluminium hydride\(^{64}\). Regeneration of the carbonyl is easily accomplished.
A. Preparation

Condensation of 2-mercaptoethanol or 3-mercaptopropionaldehyde with ketones is usually achieved with the aid of an acid catalyst. Hydrogen chloride has been used\(^\text{18}\) but more common agents are boron trifluoride\(^\text{10}\), freshly fused zinc chloride\(^\text{9}\) or \(p\)-toluenesulphonic acid\(^\text{100}\). An exchange method between 2,3-dimethyl-1,3-oxathiane or 2,3-dimethyl-1,3-oxathiane and a non-volatile ketone leads to the formation of the new mono-thioacetal and acetone\(^\text{100}\). The equilibrium is displaced by continuous distillation of the acetone formed (equation 71). With saturated ketones, mostly steroids, the yields of the above methods are comparable and are usually in the 60–90\% range. With \(\alpha,\beta\)-unsaturated ketones, the yields were significantly lower\(^\text{100}\).

\[
\begin{align*}
\text{RCR} + & \quad \text{S} \quad \text{C} \quad \text{O} \\
\text{CH}_3 \text{CH}_3 & \quad \overset{\text{R}}{\overset{\text{O}}{\text{C}}} \quad \overset{\text{S}}{\overset{\text{C}}{\text{O}}} \quad \overset{\text{R}}{\overset{\text{O}}{\text{C}}} + \quad \text{CH}_3 \text{CH}_2 \text{CH}_2 \text{H} (71)
\end{align*}
\]

Unlike the case of 1,3-dithiolane formation, 1,3-oxathianes from \(\alpha,\beta\)-unsaturated ketones show a shift of the double bond. It has been proposed\(^\text{100}\) that intermediate \(\text{RS}\) may undergo nucleophilic attack by the hydroxyl leading to unrearranged product 88. Alternatively, dehydroxylation would give the conjugated diene 86, to which the hydroxyl could add giving the rearranged product 87. Obviously, with ethanethiol, nucleophilic attack of the sulphur must predominate, while with the less nucleophilic hydroxyl, prior dehydration occurs. This is in agreement with the fact that with ethanediol the resulting ketone shows a shifted double bond.

\[
\begin{align*}
\text{HOCCH}_2\text{CH}_2\text{HO} & \quad \rightarrow \quad \text{HOCH}_2\text{CH}_3 \text{S} \\
\text{HOCH}_2\text{CH}_3 \text{H} & \quad \rightarrow \quad \text{S} \quad \text{C} \quad \text{O} \quad \overset{\text{M}}{\text{R}} \quad \overset{\text{M}}{\text{R}} \\
\text{HOCH}_2\text{CH}_3 \text{S} & \quad \rightarrow \quad \text{S} \quad \text{C} \quad \text{O} \quad \overset{\text{M}}{\text{R}} \quad \overset{\text{M}}{\text{R}}
\end{align*}
\]

The reduced reactivity of \(\alpha,\beta\)-unsaturated ketones towards 2 mercaptoethanol allows preferential formation of the hemithioacetal of an unconjugated carbonyl present in the molecule. One example of this general phenomenon is given below in which 4-androstene-3,17-dione was converted to the 17\((\text{1,3-oxathiane})\) with zinc chloride catalysis\(^\text{96}\). With \(p\)-toluenesulphonic acid catalysis, the 3,17-bis(1,3-oxathiane) could be formed in low yield.

![Diagram](attachment:image.png)

B. Removal

Unlike 1,3-dithiolanes, treatment of 1,3-oxathianes with Raney nickel gives regeneration of the carbonyl group\(^\text{99}\). Thus, protection of a carbonyl by condensation with 2-mercaptoethanol allows regeneration in high yields under neutral conditions. Surprisingly, in the usual alcohol or acetone solvent, the ketonic oxygen is not from the oxathiane. Apparently, association of the sulphur with the electron-deficient metal (equation 72) causes activation of the ring followed by attack of a hydroxide, either from the media or combined with the metal, to give the hemiketal 89. Normal work-up cleaves the hemiketal which, with further desulphurization, leads to formation of the ketone and the alcohol 90,\(^\text{38,48}\). Solvents such as benzene may also be used and under the right conditions lead to high yields of the ketone\(^\text{100}\). In nonpolar solvents,

\[
\begin{align*}
\text{R}_2\text{S} \quad \text{OH} & \quad \rightarrow \quad \text{R}_2\text{S} \quad \text{O} \quad \overset{\text{R}}{\overset{\text{M}}{\text{R}}} \\
\text{R}_2\text{S} \quad \text{O} \quad \overset{\text{R}}{\overset{\text{M}}{\text{R}}} & \quad \rightarrow \quad \text{R}_2\text{C} \quad \text{O} \quad \overset{\text{R}}{\overset{\text{M}}{\text{R}}} \quad \overset{\text{R}}{\overset{\text{M}}{\text{R}}} \\
\text{R}_2\text{S} \quad \text{O} \quad \overset{\text{R}}{\overset{\text{M}}{\text{R}}} & \quad \rightarrow \quad \text{R}_2\text{C} \quad \text{O} \quad \overset{\text{R}}{\overset{\text{M}}{\text{R}}} \quad \overset{\text{R}}{\overset{\text{M}}{\text{R}}} \\
\end{align*}
\]

ionic intermediates are presumably not involved and the diradical (91) is the accepted intermediate\(^\text{28,104}\). The desulphurization of 1,3-oxathianes behaves similarly with the ketone being the major product\(^\text{105}\). Additional information may be found in the previously mentioned reviews\(^\text{33,35,48}\).
The hydrolysis of 1,3-oxathiolanes with acid or mercuric ion also provides a suitable procedure for regenerating the ketone. The mechanism involved appears similar to that with Raney nickel, but with a proton or mercuric ion taking the place of the nickel.

The most recent method of removal of the 1,3-oxathiolane group is by the use of N-chloro-p-toluenesulphonamidoylchloramine-T in water, methanol or ethanol (equation 73). Again basically the same mechanism appears involved with prior association of the sulphur to form an unstable sulphimine. The reaction times are short (2 min), conditions are mild and yields are high (85–100%).

\[ \text{CH}_3\text{CH}_2\text{CO}_2\text{Et} + \text{MeCN} \rightarrow \text{CH}_3\text{CH}_2\text{CO}_2\text{Et} \]

IV. THIAZOLIDINES

Just as 2-mercaptopethanol will condense with ketones to produce 1,3 oxathiolanes, so will 2-mercaptopoethyamine react to produce thiazolidines. Usually p-toluencesulphonic acid is used as a catalyst in benzene with yields being quite good, 94% in the case of cyclohexanone (equation 74). The use of the thiazolidines have not been thoroughly investigated, but it appears that they offer no advantages over previously mentioned protecting groups. Although Raney nickel desulphurization gives unsatisfactory yields of the starting ketone, lithium in ethyamine offers promise in the preparation of amines. The 3l-Ethylamino-5a-cholestanol was prepared in 87% yield when desulphurized in this manner (equation 75).

12. Synthetic uses of thiols

More thoroughly investigated has been the desulphurization of N-acetylated thiazolidines to form acetylated enamines. Thus 31-day-old Raney nickel in benzene gives a 90% yield of 3-N-ethylacetamidob-5a-cholest-2-ene (equation 76) from the corresponding N-acetylhiazolide. The conditions for this reaction are rather sensitive to solvent and catalyst age. The unsaturated amide is the favoured product in benzene with aed catalyst, but with fresh catalyst or product in acetone is the ketone and in ethanol the saturated amide. The mechanism of desulphurization is believed to be similar to the first step in the formation of olefins from 1,3-dithiolanes (see section II.B.2).

V. THIOENOL ETHERS

A. Carbonyl Protecting Group

It has been noted (see section III.A) that protective reagents such as 2-mercaptopoethanol react preferentially with the saturated carbonyl when it is in the presence of an alpha-beta-unsaturated carbonyl. Thioenol ethers are equally useful because they are formed almost exclusively from alpha-beta-unsaturated carbonyls (equation 77).

\[ \text{A} \quad \text{O} \quad \text{C} \quad \text{CN} \]

Normally the reaction of thiols with carbonyls, saturated or unsaturated, leads to the formation of thioalcohols when acid catalysts such as zinc chloride or p-toluene-sulphonic acid are present (see section II.A.1). Occasionally, under special reaction conditions thioenol ethers have been formed using these same catalysts, but never in the presence of acid-sensitive substituents. Pyridine hydrochloride as the catalyst has been successfully used to give excellent yields of the thioenol ethers of A-3-ketosteroids even in the presence of sensitive groups. Thus, deoxy-corticosterone acetate (92) was converted to its 3-benzylthioenol ether.
(93) in 60% yield (equation 78). The selectivity of the reaction using these conditions is very high. Unlike the case with zinc chloride, progesterone (94) with pyridine hydrochloride and benzyl mercaptan gives no observable reaction at C210 with the only product being progesterone 3-benzyl thiolol ether (95) \[ \text{equation 78} \].

Other condensing agents which have proved useful under certain conditions are boron trifluoride, formic acid with p-toluenesulphonic acid and hydrochloric acid in acetic acid. One unusual example of a thiolol ether formed from a saturated ketone has been reported using hydrogen chloride as the catalyst. In this case, compound 96 was converted to either its benzylthioether ether 97 or its ethylthioether ether 98 (equation 79). Benzyl mercaptan normally seems to be the reagent of choice in most conversions because of its easy crystallized products.

The thiolol ethers are stable towards base and lithium aluminium hydride, but are reconverted to the parent compound on dilute acid hydrolysis. Raney nickel desulphurization can be used to form the diene. Hydrogen peroxide oxidation will convert the acid labile thiolol ether to an acid-stable sulphonyloxol ether. The sulphonyloxol ether may be desulphurized with Raney nickel to the diene, or with lithium aluminium hydride reconverted to the thiolol ether for hydrolysis to the \( \alpha,\beta \)-unsaturated ketone. These reactions are depicted in equation (80).

B. Methylene Blocking Group

In the continuing search for the ideal methylene blocking group, considerable effort has been expended in looking at derivatives of hydroxymethylenes. These are readily prepared from a ketone, ethylformate and sodium methoxide.

Ireland and Marshall found that allenethiols form very versatile derivatives with hydroxymethylenes. The reaction with a thiol, accompanied by a p-toluenesulphonic acid catalysed water separation, leads to formation of the corresponding thiolol ether (equation 81). If acid-labile substituents are present, a procedure involving displacement from an intermediate tosylate (99) by the thiol is used. Although other thiols
have been used, n-butanethiol appears to be the most convenient in this reaction. The yields of the thienoate ethers from hydroxymethylene are generally greater than 80% using the acid catalyzed method and only slightly lower with the basic pyridine procedure.

Alkylations of the protected ketones are very facile. The thienoate ether generally need only be left in contact with the base a few minutes before addition of the alkyl halide. Such short contact with the base allows easy isolation of the alkylated, blocked ketone. Thus, 2-n-butythiophenyl-1-decalone (100) was converted to 9-methyl-2-butythiophenyl-1-decalone (101) in 85% yield. This procedure was used in the difficult dimethylation of 103 to give the lactone 104.

\[
\begin{align*}
\text{(100)} & \quad \text{CH}_2\text{SHBu} \\
\text{(101)} & \quad \text{CH}_2\text{SHBu} \\
\text{(102)} & \quad \text{CH}_2\text{SHBu} \\
\text{(103)} & \quad \text{CH}_2\text{SHBu} \\
\end{align*}
\]

Table 2. Alkylation of ketones using thienoate ethers as a methylene blocking group

<table>
<thead>
<tr>
<th>Reactant</th>
<th>Product</th>
<th>Overall ( \gamma )</th>
<th>Reference</th>
</tr>
</thead>
<tbody>
<tr>
<td>[Diagram]</td>
<td>[Diagram]</td>
<td>[Diagram]</td>
<td>[Diagram]</td>
</tr>
<tr>
<td>[Diagram]</td>
<td>[Diagram]</td>
<td>[Diagram]</td>
<td>[Diagram]</td>
</tr>
</tbody>
</table>

Although the n-butythiophenyl group is subject to acid hydrolysis, basic conditions for hydrolysis have been developed\(^8\) and these seem to be preferred in actual practice. A typical procedure uses a mixture of a 25% aqueous potassium hydroxide solution with ethylene glycol heated at reflux. In this manner thienoate ether 101 was converted to 9-methyl-1-decalone (102) in 78% yield\(^a\) (equation 82). The ease of use of acid hydrolysis is exemplified by the use of concentrated hydrochloric acid to hydrolyse the blocked lactone (104) to 105 (equation 83)\(^b\). Additional examples of conversions using a thienoate intermediate are shown in Table 2.

C. Monomethylation via Reduction

Just as the blocking of active sites to permit alkylations on less reactive sites has been a recurring problem, so has the problem of preventing polyalkylations on reactive sites. The use of the alkylthiophenyl group offers a convenient intermediate from which monomethylated products are prepared by desulfurization with Raney nickel. In this way, 2,3,5,5-tetramethylcyclohexanone was prepared\(^d\) in 58% overall yield from 3,3,5-trimethylcyclohexanone (equation 84). The same procedure was used\(^e\) in the conversion of 7-oxobicyclo[3.2.1]octan to the 6-methyl derivative (equation 85).

\[
\begin{align*}
\text{(84)} & \quad \text{(85)} \\
\end{align*}
\]
TABLE 2 (cont.)

<table>
<thead>
<tr>
<th>Reactant</th>
<th>Product</th>
<th>Overall yield %</th>
<th>Reference</th>
</tr>
</thead>
<tbody>
<tr>
<td>[Structure image]</td>
<td>[Structure image]</td>
<td>62</td>
<td>116</td>
</tr>
<tr>
<td>[Structure image]</td>
<td>[Structure image]</td>
<td>35</td>
<td>117</td>
</tr>
<tr>
<td>[Structure image]</td>
<td>[Structure image]</td>
<td>36</td>
<td>118</td>
</tr>
</tbody>
</table>

decal-l-one (equation 86) was prepared in 73% overall yield using this method. In those cases where partial reduction of the carbonyl accompanies desulfurization, the crude mixture is oxidized before purification. 

12. Synthetic uses of thiols

The methylation of a very active but substituted position is easily avoided by the alkylthiomethylene approach. A high yield of 6-phenyl-2-methylcyclohexane was obtained from 6-phenylcyclohexanone (equation 87).

![Chemical structure](image)

(87)

Of course, the use of the alkylthiomethylene group first for blocking and later as a route to monomethylation further expands its utility. Thus, compound 105 was methyliated and desulfurized to give the trimethyl derivative 106.

![Chemical structure](image)

(105)  
(106)

D. Geminal Alkylation

In attempting alkylation leading to highly substituted ketones, careful choice of methods is required to avoid difficulties. Selective geminal alkylations can be achieved by blocking all other available sites, but this is not always possible as with α,α,α-trisubstituted acetones. An interesting new method has evolved incorporating the lithium-ammonia reduction of α-butythiomethylene derivatives of ketones to their methyl-substituted enolate anions with subsequent alkylation. This reduction–alkylation leads to the introduction of one methyl group and a second variable geminal substituent at any position which will scavenge with ethyl formate (equation 88). Reaction times as brief as 30 s plus the use of water

![Chemical structure](image)

(88)

as a proton donor minimize any over-alkylation. Table 3 lists some typical conversions using this procedure.
Table 3. Geminal alkylation of ketones via thienol ether derivatives

<table>
<thead>
<tr>
<th>Ketone derivative</th>
<th>Product</th>
<th>Yield from α,β-dimethylmethylen derivative, %</th>
<th>Reference</th>
</tr>
</thead>
<tbody>
<tr>
<td>CH₂CH₂CH₂CH₂CH₂</td>
<td>CH₂CH₂CH₂CH₂CH₂</td>
<td>82</td>
<td>123</td>
</tr>
<tr>
<td>CH₂CH₂CH₂CH₂CH₂</td>
<td>CH₂CH₂CH₂CH₂CH₂</td>
<td>85</td>
<td>123</td>
</tr>
<tr>
<td>CH₂CH₂CH₂CH₂CH₂</td>
<td>CH₂CH₂CH₂CH₂CH₂</td>
<td>56</td>
<td>123</td>
</tr>
<tr>
<td>CH₂CH₂CH₂CH₂CH₂</td>
<td>CH₂CH₂CH₂CH₂CH₂</td>
<td>40</td>
<td>123</td>
</tr>
<tr>
<td>CH₂CH₂CH₂CH₂CH₂</td>
<td>CH₂CH₂CH₂CH₂CH₂</td>
<td>70</td>
<td>123</td>
</tr>
<tr>
<td>CH₂CH₂CH₂CH₂CH₂</td>
<td>CH₂CH₂CH₂CH₂CH₂</td>
<td>69</td>
<td>128</td>
</tr>
</tbody>
</table>

E. Symmetrical α-Branch Alkylation

The reaction of dialkylcopper lithium reagents with α,β-unsaturated ketones leads to selective conjugate addition. It has been observed that α,β-dimethylmethylen derivatives undergo a double conjugate addition, with loss of the alkythio group, upon reaction with dimethylcopper lithium. Thus, dimethylcopper lithium reacts with α,β-dimethylmethylenecyclohexanone to give almost quantitatively 2-isopropylcyclohexanone (equation 89). This reaction should prove useful for the preparation of ketones having a symmetrically branched alkyl substituent in the α-position.

F. α, β-Unsaturated Aldehydes

Ketones with blocking groups of the isopropoxymethylene type are readily converted to α,β-unsaturated aciucityes by reduction followed by acid-catalysed rearrangement. However, the use of this blocking group has the drawback of being moisture-sensitive and of having a deactivating effect on the other α-position. Fortunately, the α,β-dimethylmethylenecyclohexanone (107) is reduced with lithium aluminium hydride and the resulting alcohol hydrolysed in acid to the α,β-unsaturated aldehyde 110. The alcohol 111 typically makes up
about 5% of the product. A comparison of the n-butylthiophenolates with butoxy- and ethoxyethylenes (106 and 109) shows that the latter two are significantly more prone to 1,4-addition leading to alcohols such as 111. The use of lithium aluminium hydride instead of the originally suggested sodium borohydride\textsuperscript{77} also seems to minimize the 1,4-addition\textsuperscript{78}.

Table 4 provides some further examples of this reaction.

### VI. SULPHUR EXTRUSION REACTIONS

Reactions in which a sulphur atom that bridges or interconnects two carbon groups is extruded with formation of a carbon-carbon bond between the two carbon groups is termed a sulphur extrusion reaction (equation 90). These types of reactions have proven to be of synthetic utility and are treated in this section.

Thiols can serve as reagents in the extrusion reaction by being converted to a sulphide or a corresponding higher oxidized derivative upon which the extrusion process is effected. While for many of the cases covered in this section the organosulphur compound used in the extrusion reaction was not prepared directly from a thiol, the potential exists for thiols to be utilized in these types of reactions.

#### A. Stevens Rearrangement of Sulphonium Salts

The Stevens rearrangement of a sulphonium salt\textsuperscript{100} involves treatment of the salt with base and leads to migration of a group from sulphur to an adjacent carbon atom (equation 91). Analogous Stevens rearrangement of ammonium salts\textsuperscript{101} and the related Wittig rearrangement\textsuperscript{102} of ethers are well known.

\[
\text{R}^1\text{CH}═\text{CH}_2\text{R}^2 \xrightarrow{\text{base}} \text{R}^1\text{S}═\text{CH}_2\text{R}^2
\]  

The sulphonium salts used in the Stevens rearrangement need not be prepared initially from a thiol; however, this is feasible and is often the case. This method, therefore, allows the conversion of a thiol to a sulphonium salt, followed by rearrangement with concomitant carbon-carbon bond formation. Removal of the sulphon moiety following rearrangement permits, in effect, a thiol to function in a reaction that leads to bond formation between two R groups that originally were attached to sulphur (equation 92).
The Stevens rearrangement of sulphonium salts is known to proceed through the intermediacy of the corresponding sulphonium ylid. There appears to be two distinct mechanistic pathways, depending upon the structure of the ylid, leading to rearranged product. Rearrangement of

\[
\begin{align*}
RSH & \rightarrow R-S-R' \\
& \rightarrow R-S-Me \\
& \rightarrow R-H
\end{align*}
\]

allyl sulphonium salts (112), proceeding via the ylid (113), has been shown to occur by a [2,3] sigmatropic reaction (equation 93); a minor amount of product also arises by what is equivalent to a [1,2] shift. These rearrangements are examples of what appear to be a general class of electrocyclic reactions of sulphonium ylids (114).

A second type of rearrangement involves ylids derived from non-allyl sulphonium salts. Baldwin and coworkers have reported that rearrangement of the sulphonium ylid (115) in toluene at reflux temperatures occurs by a radical pair mechanism (equation 94), in which the benzyl group migrates with predominant retention of configuration to yield (116).

Thompson and Stevens, in their first paper on the rearrangement of sulphonium salts, reported obtaining the sulphide (116) upon treatment of (117) with sodium methoxide. However, more recent work has shown that this method led to an isotopic mixture consisting of 4% d₁, 44% d₂, 49% d₃ and 3% d₄ products. At times fairly pure products are obtained, such as the preparation of 12,12-d₃-pregnane (12) with 76% d₄.

2. Reduction to olefins

The formation of an olefin during desulphurization was first noted when 1,3,3-tri-benzylcholestanole gave a mixture of cholest-1-ene and cholest-2-ene (equation 18) with Raney nickel deactivated by boiling in acetone. Similar conditions gave predominantly olefin with the 1,3-dihydroxy from 14β-D³,6,α-anthraergostatriene-15-one (equation 19).

More extensive investigations have led to the use of W-2 Raney nickel in refluxing acetone to obtain olefins in 55-75% yields based on starting ketone. Even the synthesis of dienes from α,β-unsaturated ketones was successful. Using this method 5α-cyano-17β-hydroxyestron-3-one was converted to the corresponding olefin (equation 20). Surprisingly, the 5β-cyano isomer gave low yields in the first step and no olefin in the second step. Both the cis- and trans-isomers in the 2-αeto-10-cyano series have been converted to olefins. Other examples of this reaction include the conversion of dihydrodulin (13) to the olefin and the partial formation of olefin from 17-norpyroboctadec-16-one (14). Groups in the α-position to the ketone may be lost during the reaction as seen by the formation of 5α-cholest-2-ene as the sole product from 2α-chloro-5α-cholestan-3-one.
The mechanism of this reaction seems to involve formation of a diradical intermediate which, if the concentration of hydrogen radicals is low, gives the thioenol ether. Further desulphurization gives the olefin (equation 21). If the alkyl radical is responsible for the \( C_{18} \) hydrogen abstraction, it would seem necessary that it remain near the reaction site so that homolysis takes place before addition of hydrogen from the catalyst. Analogy with studies of the mechanism of desulphurization in monothioacetals and thiazolidines suggests that the abstraction may very well come from an external radical.

**C. Methylene Blocking Group**

In the presence of ethyl formate and sodium methoxide, the most reactive methylene group of a ketone is converted to its hydroxymethylene derivative. Further reaction with the dithioylate of propane-1,3-dithiol leads to the formation of the 1,3 dithiane (equation 22). Thus the active position of the ketone is effectively blocked with a group easily removed by Raney nickel.

**1. Alkylation**

The presence of the dithiostatol does reduce the reactivity of the ketone toward alkylation at its other available positions, but nevertheless the sequence has been effectively utilized. This is clearly demonstrated by the formation of 4,4-dimethylcholesteneone by this procedure (equation 23). Other examples of the successful use of this method include the preparation of 4a,9a-dimethyl-5a-androst-3-one (15) and 4a-methyl-9a-flor-5a-cholestan-3-one (16).
2. Decarbonylations
The formation of 1,3-dithianes from hydroxymethylene compounds, which are end tautomers of \( \beta \)-keto aldehydes, has been shown to be useful in itself. When followed by desulphurization the net reaction is the decarbonylation to the ketone. This has been used to advantage in the formation of the methyl ketone (17)\(^{42} \) in equation (24). Similarly the methyl ketone (18) was formed from its hydroxymethylene derivative\(^{43} \).

\[
\begin{align*}
(17) & \quad R' = \text{OAc}, \quad R'' = \text{H} \\
(18) & \quad R' = \text{COMe}, \quad R'' = \text{Me}
\end{align*}
\]

3. Formation of dicarbonyl compounds
The treatment of the intermediate 1,3-dithiane, either before or after alkylation with reagents such as mercuric nitrate or calcium carbonate (see section II A 7) gives hydrolysis to the carbonyl. Thus trans-fukinone (19) was converted to (+)-dihydroxyeremophilone (20) (equation 25)\(^{46} \). In cis-fukinone, the 1,3-dithiane could not be formed from the 1-hydroxy-methylene fukinone, presumably for steric reasons.

\[
(19) \quad \xrightarrow{\text{H}} \quad (20)
\]

4. Ketone transposition
Modification of the above sequence to include reduction of the original ketone before hydrolysis is the basis for a new method of ketone transposition\(^{50} \). For example, the keto 1,3-dithiane (21) was prepared in the usual manner followed by reduction of the carbonyl with lithium aluminium hydride to the alcohol (22). Conversion to the acetate and hydrolysis of the dithiane with mercuric chloride led to the keto acetate (23). Reduction with calcium in ammonia resulted in the formation of the new methyl decalone (24) in 50% overall yield (equation 26). The same sequence was used to convert decalone (25) into the isomeric decalone (26) in 46% overall yield (equation 27). These conversions have been shown to take place with complete stereochemical integrity. Alternative methods of removing the carbonyl from the keto 1,3-dithiane so far have not been satisfactory.

12. Synthetic uses of thiols

5. Selective carbon—carbon bond cleavage
Keto 1,3-dithianes are susceptible to nucleophilic attack at the carbonyl with subsequent cleavage occurring preferentially between the carbonyl and the dithiane functions\(^{71} \). In the one instance reported, the keto dithiane (27) was cleaved with sodium methoxide in dimethyl sulphoxide to acid (28). The explanation as to why the acid is formed instead of the
methyl ester is not apparent. The reaction conditions are mild and do not seem to put serious limitations on the nature of the rest of the molecule. Most importantly, after cleavage, the 1,3-dithiane group is suitable for many conversions such as reduction, alkylation, acylation or hydrolysis.

![Chemical structure](image)

D. Synthetic Applications of 2-Lithio-1,3-dithianes

Corey and Seebach have reported\(^\text{29}\) the use of 2-lithio-1,3-dithianes as useful reagents in organic synthesis. The method involves the use of 1,3-propanedithiol, which is caused to react with an aldehyde to yield the 1,3-dithiane (equation 29). Lithiation of the dithiane, normally with n-butyllithium in tetrahydrofuran at lowered temperatures, gives the 2-lithio 1,3 dithiane (equation 30). The R group can be alkyl, aryl or hydrogen.

\[
\text{H}_2\text{S} \xrightarrow{\text{SH} + \text{RCHO}} \text{S}_2\text{C} \text{H}
\]

(29)

2-Lithio-1,3-dithianes have been shown\(^\text{29}\) to undergo reaction with a variety of electrophiles, E, to give substituted dithianes (equation 31). Removal of the dithioketal function generates the newly synthesized carbonyl compound (equation 32) having the group E substituted for the aldehyde hydrogen of the original aldehyde. The dithioketal is most often hydrolysed using the mercuric chloride: mercuric oxide method\(^\text{29}\) or by oxidative hydrolysis with N-halosuccinimides\(^\text{30}\). It is possible also to remove the dithioacetal function by desulphurization (Raney Ni) to yield the corresponding methylene derivative (equation 33). For a general treatment of removal of the dithioacetal function, see section II.A.2.

\[
\text{S}_2\text{C} \text{H} \xrightarrow{n\text{-butyl Li}} \text{S}_2\text{C} \text{Li}
\]

(30)

12. Synthetic uses of thiols

2-Lithio-1,3-dithiane reagents are in effect masked nucleophilic acylating agents and can be considered equivalent to the presently unknown acylium reagent (39). Thus, by use of a thiol, the carbonyl carbon of an aldehyde can be transformed from an electrophilic site to the nucleophilic centre in the lithiated dithiane derivative (30). The ability of sulphur to stabilize carbamions α to the sulphur atom is significant in the readily accomplished lithiation of 1,3-dithianes. The preparation and reactions of 2-lithio 1,3 dithianes have been reviewed\(^\text{29}\).

The following is a general outline of the various types of reactions that these reagents are known to undergo, including a comprehensive treatment of reactions reported since the review article by Seebach\(^\text{29}\).

1. Reaction with alkyl halides

2-Lithio-1,3-dithianes undergo alkylation at the 2 position upon reaction with alkyl halides. This reaction appears to be S_N2 in nature as it is applicable to primary and secondary alkyl halides\(^\text{29}\), occurs most readily with alkyl iodides\(^\text{29}\) and with optically active secondary halides gives inverted products\(^\text{29}\). It has been shown\(^\text{29}\) that reaction with optically active alkyl halides provides a useful route for the preparation of optically active aldehydes or ketones (equations 35 and 36).

Cycloalkylation has been effected by reaction with α,ω-dihaloalkanes to give, upon hydrolysis, cyclic ketones\(^\text{29,16}\) (equation 37). Likewise, the
dithiane derivatives of α,β-unsaturated aldehydes undergo hydrochlorination followed by cycloalkylation to yield substituted cyclopropanes (equation 38). Cyclic 1,3-diones are available by the alkylation of the bis-dithiane 39 (equation 39). It also has been observed that the use of α,ω-dibromoalkanes in cycloalkylation reactions is complicated by formation of sulphonium salts (equation 40), a reaction not observed with use of α-chloro-ω-iodo or α,ω-iododithioalkanes.

\[
\text{Li} + \text{Me} \rightarrow \text{Me} + \text{Li} \rightarrow \text{Me} \rightarrow \text{Me} \rightarrow \text{Li}
\]

(39)

Corey and coworkers, in a synthesis of prostaglandins, prepared diene 34 by alkylation of the lithiodithiane 32 with 2-bromomethyl-1,3-butadiene (equation 41). A synthesis of jasmone (35), in an overall yield of 50%, has been reported by Ellison and Weesner (40) in which the bisdithiane 33 was sequentially alkylied, followed by hydrolysis and cyclization (equation 42). A similar route for preparation of 4-hydroxy-2-cyclopenten-1-ones has been reported (41). This method appears to provide a general route to 1,3-oxazines via 1,3-dithianes.

\[
\text{Li} + \text{Me} \rightarrow \text{Me} + \text{Li} \rightarrow \text{Me} \rightarrow \text{Li}
\]

(40)

The synthesis of the monoterpenic components 40 of the sex attractant of the bark beetle has been accomplished as outlined in equation (43). The alkylation of the dithiane 37 was a key step in the synthesis since efforts to prepare 40 by addition of the magnesium or lithium derivatives of the bromoalkene 36 to the appropriate aldehyde failed. Hylton and Dockshische (44) prepared the cyclophanedione 43 by alkylation of the bisdithiane 41 followed by hydrolysis. An improved procedure for the preparation of 41 has been reported.
2. Reaction with aryl halides

Treatment of 2-lithio-2-phenyl-1,3-dithiane with 2-bromopyridine gave the substituted pyridine 44 in 50% yield. However, reaction with 2,4-dinitrobromobenzene gave none of the substitution product, but rather compound 45 resulting from oxidative dimerization of the dithiane.

12. Synthetic uses of thiols

Such oxidative dimersizations (see section II.D.7) of 2-lithio-1,3-dithiane are known and have been reported to occur with nitro compounds.

3. Reaction with epoxides

Epoxides effect alkylation of 2-lithio-1,3-dithiane (equation 47); opening of the epoxide ring occurs in the fashion typical of reactions with nucleophiles. The reported yields are in the range of 70–95% and appear to be free of side reactions common with other organometallic reagents.

Recently, Jones and Grayson have reported the reaction of lithiodithiane derivatives with steroidal epoxides to effect preparation of modified steroids. Treatment of 2α,3α-oxiaryl-5α-cholestan (46) with 2-lithio-1,3-dithiane, followed by deethylphosphation, yielded the 2β-methyl-3α-cholestan 47 (equation 48). Conversely, reaction with the epimeric epoxide 48 furnished 3α-methyl-5α-cholestan-2β-ol 49 (equation 49).

The spiroepoxide 50, prepared from 5α-cholestan-3-one, was cleanly converted to the 3β-ethyl derivative 51; the 3α-ethyl derivative 53 was obtained in an analogous manner from the epimeric spiroepoxide 52 (equation 51). Similar results were obtained when this method was applied...
to the epimeric spiroepoxide 54. This method appears to be the most suitable synthetic route to these modified steroids. However, attempts to utilize the lithium derivative of 2-hydroxyhydrindene-1,3-dithiane (55), or the corresponding tetrahydropryan derivative, to prepare corticoid steroids were unfruitful.  

The preparation of some $\gamma$-fluoro-$\beta$-hydroxyketones (58) by reaction of epfluorohydrin with the lithium derivative 57 has been reported. The dithioacetals prepared from dithiol 56 are reported to be crystalline, odourless compounds, therefore some advantage may be purported for their use.

A synthesis of $\alpha,\beta$-unsaturated aldehydes has been effected by reaction of 2-lithio-1,3-dithiane with epoxides (equation 54). It was found that treatment of the dithiinyl alcohol 59 with mercuric oxide-boron trifluoride caused dehydration and hydrolysis to give the $\alpha,\beta$-unsaturated aldehyde 60 in good yield. Standard methods for removal of the thioacetal function were not successful in these cases.

4. Reaction with aldehydes and ketones

2-Lithio-1,3-dithianes add to the carbonyl group of aldehydes and ketones to provide thioacetal derivatives of $\alpha$-hydroxy aldehydes or ketones (equation 55). The yields are normally quite high. Reaction with $\alpha,\beta$-unsaturated ketones has been observed to give only 1,2-addition; however, Seshadri and Lister have reported 1,3-addition to occur in reactions with $\alpha,\beta$-unsaturated nitro derivatives (equation 56). In the case where $R' = H$, the addition product obtained from reaction with a ketone can be converted by dehydration to a ketene thioacetal (equation 57).

Ketene thioacetals also are readily available by a Wittig-type reaction of 2-lithio-2-trimethylsilyl-1,3-dithiane (61) with aldehydes or
ketones. The dithiane 61 is prepared\textsuperscript{28,29} by reaction of 2-lithio-1,3-dithiane with trimethylchlorosilane followed by lithiation (see section II.D.6). A method employing the phosphite ylid 62 to prepare ketene thioacetals by reaction with aldehydes, but not ketones, has been reported\textsuperscript{29} (equation 59).

\[
\text{\begin{center}
\begin{array}{c}
\text{S}_2\text{Me}_3 + \text{R}_2\text{C}=\text{O} \rightarrow \text{R}_2\text{C}=\text{S}_2\text{Me}_3 + \text{Me}_2\text{SiOCLi}
\end{array}
\end{center}}
\]

Ketene thioacetals should prove to be useful synthetic intermediates. Hydrolysis\textsuperscript{30} of ketene thioacetals yields carboxylic acids (63), while pentanoylhydrazide transfer using trifluoroacetic acid trimethylsilane, as reported by Carey and Neergaard\textsuperscript{30}, provides the thioacetal (64) of the homologous aldehyde (65).

\[
\text{\begin{center}
\begin{array}{c}
\text{S}_2\text{Me}_3 + \text{R}_2\text{C}=\text{O} \rightarrow \text{R}_2\text{C}=\text{S}_2\text{Me}_3 + \text{Me}_2\text{SiOCLi}
\end{array}
\end{center}}
\]

Allyllithium reagents are known\textsuperscript{30} to add to ketene thioacetals to give 2-lithio-1,3-dithianes 66 in which R' has become attached to the ethyldene carbon. Both 64 and 66 are capable of undergoing further reactions available to 2-lithio-1,3-dithianes. Therefore, it should be possible in principle to convert an aldehyde, RCHO, to any of the following via the corresponding ketene dithioacetal: RCH\textsubscript{2}CO\textsubscript{2}H, RCH\textsubscript{2}CHO, RK'CHCHO, RK'CHCOR, and RK'R''CCOR.\textsuperscript{30}

12. Synthetic uses of thiols

Imines, being nitrogen analogues of carbonyl compounds, are reported\textsuperscript{31} to undergo addition with 2-lithio-1,3-dithianes to yield amines (equation 61).

\[
\text{\begin{center}
\begin{array}{c}
\text{S}_2\text{Me}_3 + \text{PhCH=NHPh} \rightarrow \text{S}_2\text{Me}_3 + \text{CH=NHPh}
\end{array}
\end{center}}
\]

5. Reaction with acylating agents

Acylation of 2-lithio-1,3-dithiane derivatives occurs in satisfactory yields only when a dilute solution of the dithiane derivative is added at $-78^\circ\text{C}$ to a solution containing a 20-100-fold excess of the acylating agent\textsuperscript{32,33} (equation 62). The above conditions are necessary to circumvent reaction of a molecule of the reactive lithiodythiane with a molecule of previously formed 2-acyldithiane. This method offers, by subsequent removal of the dithioketal function, a route for the preparation of 1,2-dicarbonyl compounds.

\[
\text{\begin{center}
\begin{array}{c}
\text{S}_2\text{Me}_3 + \text{RCO}_{\text{y}} \rightarrow \text{S}_2\text{Me}_3 + \text{RCO}_{\text{y}}
\end{array}
\end{center}}
\]

Acylating agents that have been employed\textsuperscript{33} are carbon dioxide, alkyl chloroformates, alkyl formates, acid chlorides, esters, benzotriazoles and dimethylformamide; the expected acylation products from reaction with the above reagents were formed in each case. However, the N,N-dimethylamide derivatives of higher carboxylic acids did not yield acylated product as in the case of dimethylformamide\textsuperscript{33}. When R = H (equation 63), it was necessary to employ two equivalents of the lithiodythiane due to product enolate formation.

\[
\text{\begin{center}
\begin{array}{c}
2 \text{S}_2\text{Me}_3 + \text{RCO}_{\text{y}} \rightarrow \text{S}_2\text{Me}_3 + \text{RCO}_{\text{y}}
\end{array}
\end{center}}
\]

In the total synthesis of illudin M, Matsumoto and coworkers\textsuperscript{33} prepared the cyclopentenone 70 by reaction of 2-lithio-1,3-dithiane with...
the ester 67 to give 68. Reduction, acetylation and removal of the dithio-acetal function gave 69, apparently formed by an intramolecular trans-ketalization reaction.

![Chemical structure](image)

6. Silylation and related reactions

2-Lithio-1,3-dithianes react with trialkyl- and triarylsilylithiums to give the 2-silylated derivatives (equation 65). This method was used in the preparation\(^6\) of the previously unknown α-silyketones 71. Germanylisation and silylation also can be accomplished with the corresponding trialkylhalo derivatives\(^6\).

![Chemical structure](image)

7. Oxidative dimerization

Treatment of 2-lithio-1,3-dithianes with iodine, cupric salts, 1,2-di-bromoethane, or nitro compounds effects oxidative dimerization\(^7\) to give the dimers 72 plus a small amount of the 2-methylene derivative 73.

![Chemical structure](image)

8. Reactions using 1,3,5-trithianes

1,3,5-Trijithianes (74) undergo lithiation\(^6,7\) with an equivalent of n-butyllithium to yield the 2-lithio derivatives, which substances undergo the usual reactions (equation 67) as with 2-lithio-1,3-dithianes. Since additional active hydrogens are present in 1,3,5-trithianes, dimethylation has been observed in some cases\(^8\).

![Chemical structure](image)

12. Synthetic uses of thiols

![Chemical structure](image)

An alternate route not involving 2-lithio-1,3,5-trithianes for the preparation of 2-substituted-1,3,5-trithianes recently has been reported\(^9\). This method involves reaction of an aldehyde 77 with the dithiol 78 to yield the 2-substituted trithiane 79.

![Chemical structure](image)

Y. Miscellaneous applications

A convenient preparation of 1-deuterioaldehydes (81) via 2-lithio-1,3-dithianes has been reported by Seebach and coworkers\(^7\) (equation 69). This method appears to be superior to previously reported methods for the preparation of 1-deuterioaldehydes.

![Chemical structure](image)

Treatment of 2-lithio-1,3-dithiane derivatives with methyl disulphide yields the orthothioformate 83, which upon hydrolysis in alcoholic solvents furnishes an ester\(^9\). This method may provide a useful route for the conversion of sensitive aldehydes to esters and carboxylic acids.

![Chemical structure](image)

III. MONothIOACETALS

The use of monothioacetals in organic synthesis has not been nearly so extensive as the use of dithioacetals. Generally prepared as 1,3-oxathiolanes and 1,3-oxathianes, the group is resistant toward dilute base and lithium aluminium hydride\(^9\). Regeneration of the carbonyl is easily accomplished.
A. Preparation

Condensation of 2-mercaptoethanol or 3-mercaptopropanol with ketones is usually achieved with the aid of an acid catalyst. Hydrogen chloride has been used but more common agents are boron trifluoride, freshly fused zinc chloride or p-toluenesulphonic acid. An exchange method between 2,2-dimethyl-1,3-oxathiolane or 2,2-dimethyl-1,3-

oxathiane and a non-volatile ketone leads to formation of the new mono-thioacetal and acetone. The equilibrium is displaced by continuous distillation of the acetone formed (equation 71). With saturated ketones, mostly steroids, the yields of the above methods are comparable and are usually in the 65-90% range. With α,β-unsaturated ketones, the yields were significantly lower.

\[
\begin{align*}
\text{CH}_2\text{CH}_3 & \quad \text{CH}_2\text{CH}_3 \\
\text{H} & \quad \text{H}
\end{align*}
\]

(11)

Unlike the case of 1,3-dithiolane formation, 1,3-oxathiolanes from α,β-unsaturated ketones show a shift of the double bond. It has been proposed that intermediate 85 may undergo nucleophilic attack by the hydroxyl leading to unarranged product 88. Alternatively, dehydration would give the conjugated diene 86, to which the hydroxy generally gives the rearranged product 87. Obviously, with ethanethiol nucleophilic attack of the sulphur must predominate, while with the less nucleophilic hydroxyl, prior dehydration occurs. This is in agreement with the fact that with ethanediol the resulting ketal shows a shifted double bond.

\[
\begin{align*}
\text{HOCH}_2\text{CH}_3 & \quad \text{HOCH}_2\text{CH}_3 \\
\text{H} & \quad \text{H}
\end{align*}
\]

(85) (86) (87) (88)

The reduced reactivity of α,β-unsaturated ketones towards 3-mercaptopo-

ethanol allows preferential formation of the hemithioacetal of an un-conjugated carbonyl present in the molecule. One example of this general

12. Synthetic uses of thiols

phenomenon is given below in which 4-androstene-3,17-dione was converted to the 17-(1,3-oxathiolane) with zinc chloride catalysis. With p-toluenesulphonic acid catalysis, the 3,17-bis(1,3-oxathiolane) could be formed in low yield.

\[
\begin{align*}
\text{O} & \quad \text{O} \\
\text{H} & \quad \text{H}
\end{align*}
\]

(89) (90)

B. Removal

Unlike 1,3-dithiolanes, treatment of 1,3-oxathiolanes with Raney nickel gives regeneration of the carbonyl group. Thus, protection of a carbonyl by condensation with 2-mercaptoethanol allows regeneration in high yields under neutral conditions. Surprisingly, in the usual alcohol or acetone solvent, the ketonic oxygen is not from the oxathiolane. Apparently, association of the sulphur with the electron-deficient metal (equation 77) causes activation of the ring followed by attack of a hydroxide, either from the media or combined with the metal, to give the hemiketal 89. Normal work-up cleaves the hemiketal which, with further desulphurization, leads to formation of the ketone and the alcohol 90. Solvents such as benzene may also be used and under the right conditions lead to high yields of the ketone.

\[
\begin{align*}
\text{R} & \quad \text{R} \\
\text{M} & \quad \text{M}
\end{align*}
\]

(90) (91)

In nonpolar solvents, ionic intermediates are presumably not involved and the diradical (91) is the accepted intermediate. The desulphurization of 1,3-oxathianes behaves similarly with the ketone being the major product. Additional information may be found in the previously mentioned reviews.
The hydrolysis of 1,3-oxathiolanes with acid\textsuperscript{112,113} or mercuric ion\textsuperscript{114} also provides a suitable procedure for regenerating the ketone. The mechanism involved appears similar to that with Raney nickel, but with a proton or mercuric ion taking the place of the nickel.

The most recent method of removal of the 1,3-oxathiolane group is by the use of N-chloro-p-toluene-sulphonamide/chloramine-T\textsuperscript{115} in water/methanol or ethanol (equation 73). Again basically the same mechanism appears involved with prior association of the sulphur to form an unstable sulphilimine. The reaction times are short (2 min), conditions are mild and yields are high (85–100\%).

\[
\begin{align*}
\text{CH}_2\text{C}_2\text{H}_4\text{SO}_2\text{Cl} & \xrightarrow{\text{N-MeC}_6\text{H}_4\text{SO}_2\text{NCH}_3} \text{CH}_2\text{C}_2\text{H}_4\text{C}_2\text{O}_2\text{Cl} \\
\text{S} & \xrightarrow{\text{H}_2\text{O}} \text{CH}_2\text{C}_2\text{H}_4\text{C}_2\text{O}_2\text{Cl} \\
\text{H}_2\text{O} & \\
\text{95\%} & \text{ (73)}
\end{align*}
\]

IV. THIAZOLIDINES

Just as 2-mercaptopethanol will condense with ketones to produce 1,3-oxathiolanes, so will 2-mercaptopropanol react to produce thiazolidines\textsuperscript{116}. Usually p-toluenesulphonic acid is used as a catalyst in benzene with yields being quite good, 96\% in the case of cyclohexanone (equation 74). The use of the thiazolidines have not been thoroughly investigated, but it appears that they offer no advantages over previously mentioned protecting groups. Although Raney nickel desulphurization gives unsatisfactory yields of the starting ketone, lithium in ethylamine offers promise in the preparation of amines. 3β-Ethylamino-5α-cholestan was prepared in 87\% yield\textsuperscript{117} when desulphurized in this manner (equation 75).

\[
\begin{align*}
\text{CH}_2\text{C}_2\text{H}_4\text{SO}_2\text{Cl} & \xrightarrow{\text{LiEt}_2\text{NH}} \text{CH}_2\text{C}_2\text{H}_4\text{NCH}_2\text{CH}_3 \\
\text{S} & \xrightarrow{\text{H}_2\text{O}} \text{CH}_2\text{C}_2\text{H}_4\text{NCH}_2\text{CH}_3 \\
\text{H}_2\text{O} & \\
\text{87\%} & \text{ (75)}
\end{align*}
\]

12. Synthetic uses of thiols

More thoroughly investigated has been the desulphurization of N-acetylated thiazolidines to form acetylated enamines. Thus 31-day-old Raney nickel in benzene gives a 90\% yield of 3-(N-ethylacetamido)-5α-cholesta-2,4-diene (equation 76) from the corresponding N-acetylthiazolidine\textsuperscript{118,119}. The conditions for this reaction are rather sensitive to solvent and catalyst age. The unsaturated amide is the favored product in benzene with aged catalyst, but with fresh catalyst the major product in acetone is the ketone and in ethanol the saturated amide. The mechanism of desulphurization is believed\textsuperscript{119} to be similar to the first step in the formation of crotins from 1,3-dihydroxyls (see section II.B.2).

V. THIOENOL ETHERS

A. Carbonyl Protecting Group

It has been noted (see section III.A) that protecting reagents such as 2-mercaptoethanol react preferentially with the saturated carbonyl when it is in the presence of an α,β-unsaturated carbonyl. Thioenol ethers are equally useful because they are formed almost exclusively from α,β-unsaturated carbonyls (equation 77).

\[
\begin{align*}
\text{CH}_2\text{C}_2\text{H}_4\text{NCH}_2\text{CH}_3 & \xrightarrow{\text{HDM}} \text{CH}_2\text{C}_2\text{H}_4\text{NCH}_2\text{CH}_3 \\
\text{H}_2\text{O} & \\
\text{N-CH}_2\text{CH}_3 & \\
\text{2O} & \text{ (77)}
\end{align*}
\]

Normally the reaction of thiols with carbonyls, saturated or unsaturated, leads to the formation of dithiocetals when acid catalysts such as zinc chloride or p-toluenesulphonic acid are present (see section II.A.1). Occasionally, under special reaction conditions thioenol ethers have been formed using these same catalysts\textsuperscript{120}, but never in the presence of acid-sensitive substituents. Pyridine hydrochloride as the catalyst has been successfully used to give excellent yields of the thioenol ethers of 4α,3β-ketosteroids even in the presence of sensitive groups\textsuperscript{121}. Thus, desoxy-corticosterone acetate (92) was converted to its 3-benzylthioenol ether
Other condensing agents which have proved useful under certain conditions are boron trifluoride, formic acid with p-toluenesulphonic acid and hydrochloric acid in acetic acid. One unusual example of a thioenol ether formed from a saturated ketone has been reported using hydrogen chloride as the catalyst. In this case, compound 96 was converted to either its benzylthioenol ether 97 or its ethylthioenol ether 98 (equation 79). Benzyl mercaptan normally seems to be the reagent of choice in most conversions because of its easily crystallized products.

The thioenol ethers are stable towards base and lithium aluminium hydride, but are reconverted to the parent compound on dilute acid hydrolysis. Raney nickel desulphurization can be used to form the diene. Hydrogen peroxide oxidation will convert the acid-labile thioenol ether to an acid-stable sulphotioenol ether. The sulphotioenol ether may be desulphurized with Raney nickel to the diene, or with lithium aluminium hydride reconverted to the thioenol ether for hydrolysis to the α,β-unsaturated ketone. These reactions are depicted in equation (80).

8. Methylene Blocking Group

In the continuing search for the ideal methylene blocking group, considerable effort has been expended in looking at derivatives of hydroxy-methylene. These are readily prepared from a ketone, ethylformate and sodium methoxide.

Ireland and Marshall found that alkane-thiols form very versatile derivatives with hydroxy-methylene. The reaction with a thiol, accompanied by a p-toluenesulphonic acid catalysed water separation, leads to formation of the corresponding thioenol ether (equation 81). If acid labile substituents are present, a procedure involving displacement from an intermediate tosylate (99) by the thiol is used. Although other thiols
have been used, n-butanol appears to be the most convenient in this reaction. The yields of the thioenol ethers from hydroxymethanes are generally greater than 80% using the acid catalysed method and only slightly lower with the basic pyridine procedure.

Alkylation of the protected ketones are very facile. The thioenol ether generally need only be left in contact with the base a few minutes before addition of the alkyl halide. Such short contact with the base allows easy isolation of the alkylated, blocked ketones\textsuperscript{46}. Thus, 2-n-butythiomethylene-1-decalone (100) was converted to 9-methyl-2-butythiomethylene-1-decalone (101) in 85% yield. This procedure was used in the difficult dimethylation of 103 to give the lactone 104.

\[
\begin{align*}
\text{Reagent} & \rightarrow \text{Product} & \text{Overall yield, %} & \text{Reference} \\
100 & \rightarrow 101 & 85 & 111 \\
101 & \rightarrow 102 & 73 & 111 \\
104 & \rightarrow 105 & 60 & 112 \\
104 & \rightarrow 105 & 62 & 113 \\
106 & \rightarrow 107 & 82 & 114 \\
106 & \rightarrow 107 & 31 & 115
\end{align*}
\]

Table 2. Alkylation of ketones using thioenol ethers as a methylene blocking group

Although the n-butythiomethylene group is subject to acid hydrolysis, basic conditions for hydrolysis have been developed\textsuperscript{46} and these seem to be preferred in actual practice. A typical procedure uses a mixture of a 25% aqueous potassium hydroxide solution with ethylene glycol heated at reflux. In this manner thioenol ether 101 was converted to 9-methyl-1-decalone (102) in 78% yield\textsuperscript{46} (equation 82). The rare use of acid hydrolysis is exemplified by the use of concentrated hydrochloric acid to hydrolyse the blocked lactone (104) to 105 (equation 83)\textsuperscript{106}. Additional examples of conversions using a thioenol ether intermediate are shown in Table 2.

**C. Monomethylation via Reduction**

Just as the blocking of active sites to permit alkylation on less reactive sites has been a recurring problem, so has the problem of preventing polyalkylation on reactive sites. The use of the alkylthiomethylene group offers a convenient intermediate from which mumleunethanised products are prepared by desulphurization with Raney nickel. In this way, 2,3,5,5-tetramethylcyclohexanone was prepared\textsuperscript{119} in 58% overall yield from...
TABLE 2 (cont.)

<table>
<thead>
<tr>
<th>Reactant</th>
<th>Product</th>
<th>Overall yield %</th>
<th>Reference</th>
</tr>
</thead>
<tbody>
<tr>
<td><img src="image1" alt="Chemical Structure" /></td>
<td><img src="image2" alt="Chemical Structure" /></td>
<td>62</td>
<td>116</td>
</tr>
<tr>
<td><img src="image3" alt="Chemical Structure" /></td>
<td><img src="image4" alt="Chemical Structure" /></td>
<td>35</td>
<td>117</td>
</tr>
<tr>
<td><img src="image5" alt="Chemical Structure" /></td>
<td><img src="image6" alt="Chemical Structure" /></td>
<td>36</td>
<td>118</td>
</tr>
</tbody>
</table>

Decal-1-one (equation 86) was prepared in 73% overall yield using this method. In those cases where partial reduction of the carbonyl accompanies dealkylation, the crude mixture is oxidized before purification. 

12. Synthetic uses of thiols

The methylation of a very active but substituted position is easily avoided by the alkythiomethylation approach. A high yield of 6-phenyl-2-methycyclohexanone was obtained from 6-phenylcyclohexanone (equation g746, 112).

Of course, the use of the alkythiomethylene group first for blocking and later as a route to monomethylation further expands its utility. Thus, compound 103 was methylated and desulfurized to give the trimethyl derivative 106. 

D. Geminal Alkylation

In attempting alkylation leading to highly substituted ketones, careful choice of methods is required to avoid difficulties. Selective geminal alkylations can be achieved by blocking all other available sites, but this is not always possible with \(n\)-alkyl-substituted acetates. An interesting new method has evolved incorporating the lithium-ammonia reduction of \(n\)-butytilthiomethylen derivatives of ketones to their methyl-substituted enolate anions with subsequent alkylation. This reduction–alkylation leads to the introduction of one methyl group and a second variable geminal substituent at any position which will condense with ethyl formate (equation 88). Reaction times as brief as 30 s plus the use of water as a proton donor minimize any over-alkylation. Table 3 lists some typical conversions using this procedure.
### Table 3. Geminal alkylation of ketones via thioenol ether derivatives

<table>
<thead>
<tr>
<th>Ketone derivative</th>
<th>Product</th>
<th>Yield from n-butylthiomethylene derivative, %</th>
<th>Reference</th>
</tr>
</thead>
<tbody>
<tr>
<td>O CH₃ SBU CH₂ CH₃</td>
<td>O CH₃ SBU CH₂ CH₃</td>
<td>82</td>
<td>123</td>
</tr>
<tr>
<td>O CH₃ SBU CH₂ CH₃</td>
<td>O CH₃ SBU CH₂ CH₃</td>
<td>75</td>
<td>123</td>
</tr>
<tr>
<td>O CH₃ SBU CH₂ CH₃</td>
<td>O CH₃ SBU CH₂ CH₃</td>
<td>85</td>
<td>123</td>
</tr>
<tr>
<td>O CH₃ SBU CH₂ CH₃</td>
<td>O CH₃ SBU CH₂ CH₃</td>
<td>56</td>
<td>123</td>
</tr>
<tr>
<td>O CH₃ SBU CH₂ CH₃</td>
<td>O CH₃ SBU CH₂ CH₃</td>
<td>40</td>
<td>123</td>
</tr>
<tr>
<td>O CH₃ SBU CH₂ CH₃</td>
<td>O CH₃ SBU CH₂ CH₃</td>
<td>10</td>
<td>123</td>
</tr>
<tr>
<td>O CH₃ SBU CH₂ CH₃</td>
<td>O CH₃ SBU CH₂ CH₃</td>
<td>69</td>
<td>128</td>
</tr>
</tbody>
</table>

---

E. Symmetrical α-Branch Alkylation

The reaction of dialkylcopper lithium reagents with α,β-unsaturated ketones leads to selective conjugate addition. It has been observed that n-butylthiomethylene derivatives undergo a double conjugate addition, with loss of the alkythio group, upon reaction with dimethylcopper lithium. Thus, dimethylcopper lithium reacts with 2-n-butylthiomethylene-cyclohexanone to give almost quantitatively 2-isopropylcyclohexanone (equation 89). This reaction should prove useful for the preparation of ketones having a symmetrically branched alkyl substituent in the α-position.

![Reaction Scheme](image)

F. α, β-Unsaturated Aldehydes

Ketones with blocking groups of the isopropoxymethylene type are readily converted to α,β-unsaturated aldehydes by reduction followed by acid-catalyzed rearrangement. However, the use of this blocking group has the drawback of being moisture-sensitive and of having a deactivating effect on the other α-position. Fortunately, the n-butylthiomethylene grouping does not suffer from these drawbacks and is still readily converted to the α,β-unsaturated aldehyde. Thus 2-α,β-butyrlthiomethylene-α,β-dimethylcyclohexanone (107) is reduced with lithium aluminium hydride and the resulting alcohol hydrolysed in acid to the α,β-unsaturated aldehyde (110). The alcohol (111) typically makes up
12. Synthetic uses of thiols

Table 4. Preparation of α,β-unsaturated aldehydes by LiAlH₄ reduction of α,ω-butythioalkylene ketone derivatives

<table>
<thead>
<tr>
<th>Ketone derivative</th>
<th>Product</th>
<th>Yield from n-butythioalkylene (%)</th>
<th>Reference</th>
</tr>
</thead>
<tbody>
<tr>
<td><img src="image1" alt="Image" /></td>
<td><img src="image2" alt="Image" /></td>
<td>63</td>
<td>177</td>
</tr>
<tr>
<td><img src="image3" alt="Image" /></td>
<td><img src="image4" alt="Image" /></td>
<td>81</td>
<td>131, 132</td>
</tr>
<tr>
<td><img src="image5" alt="Image" /></td>
<td><img src="image6" alt="Image" /></td>
<td>38</td>
<td>134</td>
</tr>
<tr>
<td><img src="image7" alt="Image" /></td>
<td><img src="image8" alt="Image" /></td>
<td>52</td>
<td>135</td>
</tr>
</tbody>
</table>

VI. SULPHUR EXTRUSION REACTIONS

Reactions in which a sulphur atom that bridges or interconnects two carbon groups is extruded with formation of a carbon—carbon bond between the two carbon groups is termed a sulphur extrusion reaction (equation 90). These types of reactions have proven to be of synthetic utility and are treated in this section.

\[ R-S-R \rightarrow R-R + S \] (90)

Thiols can serve as reagents in the extrusion reaction by being converted to a sulphide or a corresponding higher oxidized derivative upon which the extrusion process is effected. While for many of the cases covered in this section the organosulphur compound used in the extrusion reaction was not prepared directly from a thiol, the potential exists for thiols to be utilized in these types of reactions.

A. Stevens Rearrangement of Sulphonium Salts

The Stevens rearrangement of a sulphonium salt involves treatment of the salt with base and leads to migration of a group from sulphur to an adjacent carbon atom (equation 91). Analogous Stevens rearrangement of ammonium salts and the related Wittig rearrangement of ethers are well known.

\[ R-S-\text{CH}_{2}R' \rightarrow R-S-\text{CHR}' \] (91)

The sulphonium salts used in the Stevens rearrangement need not be prepared initially from a thiol; however, this is feasible and is often the case. This method, therefore, allows the conversion of a thiol to a sulphonium salt, followed by rearrangement with concomitant carbon—carbon bond formation. Removal of the sulphur moiety following rearrangement permits, in effect, a thiol to function in a reaction that leads to bond formation between two R groups that originally were attached to sulphur (equation 92).
The Stevens rearrangement of sulphonium salts is known to proceed through the intermediacy of the corresponding sulphonium ylid\(^{19}\). There appears to be two distinct mechanistic pathways, depending upon the structure of the ylid, leading to rearranged product. Rearrangement of

\[
\text{RSH} \rightarrow \text{R-S-R'} \rightarrow \text{R-S'-R'} \rightarrow \text{Me}
\]

(92)

allyl sulphonium salts\(^{21}\), proceeding via the ylid 113, has been shown\(^{19}\) to occur by a [2,3] sigmatropic reaction (equation 93); a minor amount of product also arises by what is equivalent to a [1,2] shift\(^{19}\).

These rearrangements are examples of what appear to be a general class of electrocyclic reactions of sulphonium ylids\(^{21}\).

A second type of rearrangement involves ylids derived from non-allyl sulphonium salts. Baldwin and coworkers\(^{19}\) have reported that rearrangement of the sulphonium ylid 115 in toluene at reflux temperatures occurs by a radical pair mechanism (equation 94), in which the benzyl group migrates with predominant retention of configuration to yield 116.

\[
\begin{align*}
\text{Ph} & \quad \text{O} & \quad \text{Me} \\
(115) & \quad \text{R} & \quad \text{O} & \quad \text{Ph}
\end{align*}
\]

(93)

\[
\begin{align*}
\text{Ph} & \quad \text{O} & \quad \text{Me} \\
(116) & \quad \text{R} & \quad \text{O} & \quad \text{Ph}
\end{align*}
\]

(94)

Thompson and Steven\(^{21}\), in their first paper on the rearrangement of sulphonium salts, reported obtaining the sulphide 116 upon treatment of 117 with sodium methoxide. However, more recent work has shown\(^{21}\),\(^{19}\)

13. Biochemistry of the thiol group

condensed with serine. It presently appears that two different enzyme sequences are possible and both may operate in some organisms.

These are contrasted in the scheme below.

---

With the direct \(\text{H}_2\text{O} \rightarrow \text{H}_2\text{O}\) interchange enzyme, serine sulphhydrase, the reaction is completely reversible. Pyridoxal phosphate participates as an essential cofactor which suggests a mechanism involving a pyridoxamine-Schiff base intermediate. By stabilizing an electrophilic centre at the side chain carbon a nucleophilic substitution could be facilitated.

---

Thompson and Steven\(^{21}\), in their first paper on the rearrangement of sulphonium salts, reported obtaining the sulphide 116 upon treatment of 117 with sodium methoxide. However, more recent work has shown\(^{21}\),\(^{19}\)

The reversibility of this reaction would allow this enzyme to participate in either sulphuration or desulphuration of cysteine and its real role in vivo remains somewhat doubtful.

The other system for sulphide assimilation involves a coupled hydrolysis of acetyl coenzyme A. This enzyme system can only operate in the direction of cysteine synthesis and would ensure the effective trapping of...
most available sulphide for this purpose. The acetylation of the serine hydroxyl also provides an effective leaving group so that one might envisage an enzyme-mediated direct nucleophilic displacement mechanism. Pyridoxal cofactors are not thought to participate in the O-acetyl serine sulphydrylase reaction, although this remains an unsettled question. The enzymes of the O-acetyl serine pathway are responsive to the metabolic needs of the cell being repressed by cysteine in Escherichia coli. There is a biochemical generalization that critical biosynthetic pathways such as this are normally coupled to high energy bond expenditure which guarantees effective utilization of nutrients. Such considerations make it likely that this is the normal biosynthetic route. Similar systems have not been found in all organisms capable of sulphide incorporation however, so an important role of the cysteine–hydroxyl exchange system in cysteine synthesis cannot be excluded. There is evidence for a similar system in chick embryo involving a serine phosphate rather than the acetate.

Cysteine formation through the addition of thiosulphate to serine or O-acetyl serine may play a role in the sulphur metabolism of some organisms. The reactions involved are similar in form to those described above, with 3-sulphocysteine serving as an intermediary role. Since thiosulphate is not generally considered to be on the main line of inorganic sulphur metabolism this probably represents an adaptation to certain special environments.

<table>
<thead>
<tr>
<th>Cysteine formation from thiosulphate</th>
</tr>
</thead>
<tbody>
<tr>
<td>CH$_3$OH + H$_2$S + O$_2$ $</td>
</tr>
</tbody>
</table><p>ightarrow$ H$_2$SO$_3$ + CO$_2$ |
| CH$_3$SH + H$_2$S + O$_2$ $ightarrow$ CH$_3$S(OH) + CO$_2$ |
| CO$_2$H + H$_2$S $ightarrow$ HCNH$_2$ + CO$_2$ |
| serine + H$_2$S + O$_2$ $ightarrow$ HCNH$_2$ + CO$_2$ |</p>

2. Cysteine oxidation

The balance of the 'sulphur cycle' requires that reduced sulphur derivatives eventually be reoxidized to sulphate. A number of photosynthetic and chemosynthetic organisms have the ability to utilize reduced sulphur, particularly HgS, as critical electron donors for ATP production, but these pathways are not of enough general importance to consider here. Pathways are known for the production of sulphide from cysteine, and it is also clear that the oxidation of sulphate can occur in animals with production of sulphate and thiosulphate. What is not certain is to just what extent specific enzyme reactions are involved. The nonenzymatic oxidation of sulphite is promoted by a variety of normal cellular constituents, but it is felt that direct sulphite oxidation is of little consequence for animals. Sulphite is an exceedingly toxic material, precluding its normal accumulation in significant amounts and the principal 'detoxification' route seems to be fixation into organic thiol compounds rather than oxidation.

All organic thiols and thiol derivatives are quite susceptible to aerobic oxidation yielding a variety of oxy-derivatives. Actually the biological significance of most of these sulphoxide derivatives is unknown. In certain instances, there are mechanisms to reduce sulphoxides back to divalent sulphur compounds. ß-Lipoic acid, an active factor in bioassays, proved to be a sulphoxide derivative of this sulphide cofactor which was generated during purinisation; its biotin activity implies that it can be reduced to the normal form of the cofactor. 

Methionine sulphoxide reductase system from yeast has been studied extensively and found to resemble the FAO reductase and thiosulphate reductase systems in that electron transport was mediated by a heat-stable protein disulphide factor. Thus, there does seem to be some ability to salvage partially utilized thiol derivatives, but it is uncertain how widespread this capacity might be.

The only thiol oxidation reaction to oxy-derivatives of general biochemical significance is that of cysteine to alanine 3-sulphinic acid (cysteine sulphinic acid). This is thought to be the initial reaction in the main pathway for the utilization of cysteine sulphur for sulphate production. Relatively little is known about the details of this oxidation process. Some form of reduced nicotinamide coenzyme, ferrous iron, and possibly other cofactors are required by an enzyme from the soluble fraction of rat liver. There is little information on mechanistic details or possible intermediate states. Cysteine sulphinic acid is further converted in what has been presumed to be ß-sulphenyl pyruvic acid and ultimately to inorganic sulphite. This is then oxidized to sulphate. Cysteic acid and laurine may also arise from cysteine sulphonic acid.
13. Biochemistry of the thiol group

3. Cysteine desulphuration

Desulphuration (desulphhydration) of cysteine may play a role in thiol catabolism, but there is considerable confusion concerning the existence of a distinct cysteine desulphhydrase.

\[
\begin{array}{c}
\text{Cysteine desulphhydrase reaction} \\
\text{Cysteine desulphhydrase activity via cystathionase and cystine}
\end{array}
\]

There is no doubt that such a reaction, catalysed by a pyridoxal phosphate-dependent enzyme, can occur in biological systems. It is quite possible, however, that this only represents a sial reaction of other enzymes. Cystathionase, for example, will act on cystine with the elimination of a cysteine persulphide and pyruvate. The persulphide then reacts with cysteine to eliminate sulphide and regenerate cystine. The complete cycle would constitute a cysteine desulphhydrase activity.

Cystathionase also has a low level of direct cysteine desulphhydrase activity. Tryptophanase and tryptophan synthetase are other enzymes capable of carrying out the cysteine desulphhydrase reaction. Such considerations have cast doubt on this biological significance of this reaction, although strong arguments have been presented for a true cysteine desulphhydrase in Salmonella.
Another route for the removal of the thiol group from cysteine is through the intermediate formation of thiol pyruvic acid, which is the α-keto acid derived from cysteine by transamination:

\[
\text{Cysteine transaminase reaction}
\begin{align*}
\text{CH}_3\text{SH} + \text{CH}_2\text{NH}_2 & \rightarrow \text{CH}_3\text{SH} + \text{CH}_2\text{NH}_2 \\
\text{CO}_2\text{H} + \text{C}=\text{O} & \rightarrow \text{CO}_2\text{H} + \text{C}=\text{O}
\end{align*}
\]

The product can be acted on by an enzyme which transfers sulphur to a variety of acceptors in vitro to generate thiocysteine (and thiosulphonates), thiocyanate and organic persulphides. A direct desulphuration to sulphide does not appear to occur but a further reaction of persulphide with diallyl disulphide would provide this product. The generation of elemental sulphur can also occur under certain circumstances. The thiol pyruvate sulphurtransferease is thought to act through an enzyme persulphide

Reactions of thiol pyruvate sulphurtransferease

\[
\begin{align*}
\text{CH}_3\text{SH} & \rightarrow \text{CH}_3\text{O} \\
\text{CO}_2\text{H} & \rightarrow \text{C}=\text{O}
\end{align*}
\]

13. Biochemistry of the thiol group

Intermediate and will be considered further in a subsequent section. Thiol pyruvate can also be reduced to thiol lactate and decarboxylated to mercaptoethanol.

4. Cysteine-cystine interconversion

While it is doubtful that cysteine, the disulphide of cysteine, has any critical biological role as such, it is an ubiquitous constituent of aerobic systems resulting from the facile oxidation of cysteine. It also can arise from the digestion of protein disulphides. Cystine is relatively insoluble and it allowed to build up tends to form crystalline precipitates within the cell. There is normally little of the disulphide in cells, while in the blood the oxidized form dominates. One method for the reduction of cystine to cysteine is via a nicotinamiden coenzyme-thiosulphate dihydrosulphhydrase. Glutathione

\[
\begin{align*}
\text{Cysteine dehydrogenase reaction}
\end{align*}
\]

\[
\text{CH}_3\text{SH} \rightarrow \text{CH}_3\text{O} + \text{NAD}^+ + \text{H}^+
\]

also has a critical role in cystine reduction. While this reduction occurs readily without enzymes, it is stimulated by the enzyme cystine-glutathione transhydrogenase.

2 GSH + cystine → GSSG + 2 cysteine

This latter system appears to be the one dominant in cystine reduction by mammalian cells.

Two human genetic diseases are known which involve this disulphide amino acid. In one, cystinuria, there is a transport defect in the intestine and kidney. This results in abnormally high levels of cystine in the urine and can result in the precipitation of cystine crystals and kidney stone formation. In cystinosis, cystine crystals form within cells and eventually cause severe kidney damage. The nature of the primary biochemical lesion is unknown; all known cystine reduction systems of the cell appear to be normal.

5. Transsulphuration via cystathionine

Cysteine also donates its sulphur to form homocysteine and eventually the second critical sulphur amino acid, methionine. Methionine is the S-methyl ether of homocysteine, a cysteine analogue with one additional
carbon in the chain. Transsulphuration from cysteine occurs in bacteria, plants, yeast and fungi, but not in animals. The latter rely on dietary sources of methionine. It is actually the homocysteine portion which is required but this does not normally occur in significant quantities.

The carbon skeleton of homocysteine is derived from the corresponding hydroxy amino acid, homoserine. The hydroxyl of homoserine is acetylated with either a succinyl (bacteria) or acetyl (yeast, fungi, plants) group derived from the corresponding coenzyme A derivative. The O-acyl substituent is then displaced by the thiol group of cysteine producing a mixed thioether, cystathionine. This in turn undergoes a pyridoxal phosphate dependent β-elimination to homocysteine, pyruvate and ammonia. Homocysteine is then methylated to methionine by pathways to be discussed later. Cystathionine is generally only a trace metabolite, but occurs in reasonably high concentrations (25–50 mg/100 gm) in brain. A direct formation of homocysteine from homoserine (or O-acyl homoserine) and hydrogen sulphide has also been observed in extracts of some organisms. Whether this is only a side reaction of the cystathionine

ammonium. Homocysteine is then methylated to methionine by pathways to be discussed later. Cystathionine is generally only a trace metabolite, but occurs in reasonably high concentrations (25–50 mg/100 gm) in brain. A direct formation of homocysteine from homoserine (or O-acyl homoserine) and hydrogen sulphide has also been observed in extracts of some organisms. Whether this is only a side reaction of the cystathionine

13. Biochemistry of the thiol group

synthesis system or is a physiologically important route for assimilation of sulphide is uncertain.

In animals homocysteine arises from methionine through its role as a methyl donor, as will be discussed in a subsequent section. It may either be reutilized for methionine production or degraded. In animal tissues the degradative pathway plays a major role in sulphur nutrition. Much of the cysteine sulphur, and through it sulphate, can be derived from dietary methionine. The transsulphuration from homocysteine to produce cysteine is very much like that in the other direction. It also involves cystathionine but is not a reversal of the synthetic pathway. Quite different reactions are involved. Homocysteine reacts with serine to produce the thioether intermediate. Unlike the route from cysteine and homoserine, no O-acylation step has been implicated. Instead, the homocysteine–serine-condensing enzyme probably requires pyridoxal phosphate as a coenzyme, although this is not unequivocally established.

Cystathionine cleavage in the mammalian transsulphuration system produces cysteine, α-ketobutyrate and amonia by what is believed to be a pyridoxal-catalysed β-elimination reaction. Again this reaction is quite similar to the cystathionine cleavage by the other pathway; only the direction of cleavage is different. Actually the bacterial cystathionase is capable of cleaving the thioether in either direction, although that producing homocysteine is dominant. This implies that even the enzyme-bound intermediates are similar and the binding specificity of the particular enzyme site is crucial in ensuring the proper reaction.

Particular interest in this pathway arises from the findings of human genetic diseases associated with each step. Lack of the first enzyme,
cystathionine synthase, results in homocystinuria. This is one of the most common genetic disorders of amino acid metabolism and is exceeded in frequency only by phenylketonuria. In this disease, homocysteine cannot be metabolized and its disulfide, homocystine, builds up and is excreted. The disorder is often associated with severe symptoms including mental retardation. Actually two distinct autosomal recessive forms of homocystinuria can be differentiated: one type is susceptible to treatment with vitamin B₆ (pyridoxine). Since pyridoxine is the precursor of pyridoxal phosphate, such therapeutic results strongly support a critical role for this coenzyme in the cystathionine synthase. It also implies that, in at least some homocystinurics, the biochemical defect is in coenzyme formation or binding. In the vitamin B₆-unresponsive patients the mutation must affect some other aspect of the enzyme. Actually only a small proportion of the daily methionine intake by homocystinuria patients can be accounted for by the excreted homocysteine and the study of this disease may greatly enhance our knowledge of thiol metabolism. For example, it appears that homocystinurics can make cystathionine to some extent from cysteine and homoserine, a reaction generally believed impossible in animals. 

Cystathioninuria, a deficiency of cystathionase, is a much rarer and less clearly defined disorder. While the disease has frequently been associated with mental retardation, this may only reflect the type of individual with which testing most frequently occurs. Patients with normal mental function are also known. Nonetheless, the high levels of cystathionine in brain and the mental defects associated with its taurine metabolism, have led to speculation that this thioether has some special role in nervous function. In tissues from at least one patient, there was evidence that the defect was in pyridoxal phosphate binding by cystathionase and that normal enzyme activity could be achieved at abnormally high levels of coenzyme. This is often quoted as the classical example of a binding or ‘Rₓ’ mutant, but not all patients with this disorder give the same effect.

These reactions which lead to homocysteine formation in some creatures and its utilization in others are undoubtedly representative of a general thiol group transfer mechanism. The initial condensation of the donor thiol, most commonly cysteine, with some suitably reactive receptor generates a thioether. The differences in the requirement for O-acylation when starting from serine and homoserine may reflect two completely different mechanisms for this thiol substitution reaction. In the case of serine, the removal of the hydroxyl as hydroxide and the stabilization of an electrophilic centre on the side-chain carbon can be achieved through the pyridoxal phosphate-amino acid adduct. A similar example is in the carbon-carbon condensation between serine and imidazole in tryptophan synthesis. When homoserine is the receptor a different activation system appears to be necessary. While pyridoxal coenzymes can facilitate y-elimination of hydroxide from the homoserine structure, stabilization of an electrophilic centre at the appropriate position cannot occur. By first acylating the hydroxyl of homoserine a suitable leaving group for an enzyme-facilitated nucleophilic displacement reaction is created. The two possible mechanisms for formation of a thioether intermediate for trans-sulphuration are shown below. The thioether then breaks down by the elimination of a thiolate to complete the transsulphuration sequence. Typically this would be a β-elimination from the cysteine structure potentiated by a pyridoxal phosphate stabilized intermediate as depicted below. It also appears that thiol pyruvate can serve as sulphur donor for some biological transsulphurations. The thiol nucleotides which occur in small quantities in certain microbial acids appear to derive their sulphur, at least in part, from thiol pyruvate rather than directly from cysteine. While these reactions have not been extensively studied as yet, ATP is required possibly to activate a group for intermediate thioether formation. Pyruvate elimination could then proceed through an enolate or an intermediate enzyme-bound Schiff base.
A diverse variety of divalent sulphur compounds is found throughout nature. These are often found in small quantities or in restricted species and little is known about their metabolism. It is generally presumed that they all ultimately derive their sulphur from cysteine. Thus more examples of transsulphuration reactions will be described, and it is likely that mechanisms involving mixed thiolether intermediates will frequently be implicated. Another general route for transsulphuration may be through enzyme-bound persulphides. The existence of such intermediates in the rhodanese and thiol pyruvate sulphur transferase reactions seems reasonably established, although there are no examples of their being involved in the formation of organic thiols.

6. Thiol formation by cysteine incorporation

Thiol groups enter some biologically important thiol compounds by the direct incorporation of cysteine itself. Most frequently this involves peptide bond formation. The incorporation of cysteine into proteins does not differ from any other amino acid involving activation as an amino acid adenylate, transfer to a specific transfer ribonucleic acid (t-RNA), and assembly by ribosomal enzymes as coded by messenger ribonucleic acid (m-RNA). It should be pointed out that cystine, the ‘two-headed’ disulphide amino acid, is not directly incorporated, but arises in proteins by oxidation of two cysteine residues after assembly of the chain. The formation of glutathione and pantetheine also involves peptide bond formation to cysteine, but the mechanism of formation is quite different from the nucleic acid-coded protein synthesis. These pathways will be included in the discussions of the thiol coenzymes.

An example in which a portion of the cysteine carbon chain is incorporated directly is one of the proposed routes for biotin synthesis by microorganisms. An acyl coenzyme A derivative of pimelic acid condenses with cysteine, eliminating CO₂. Reaction with carbamyl phosphate leads to the formation of an adenosine riboside system. The thiol then forms a cyclic thioether by addition to a double bond resulting from oxyriatisation.

Cysteine incorporation into biotin

Cysteine is the pivotal compound in thiol metabolism. Sulphate and other oxidized forms of sulphur are reduced to the level of sulphide, which enters organic linkage as cysteine. There is no other direct sulphuration pathway of any significance. All biological thiols and subsequently...
derivatives such as disulphides, thioesters, thioethers and sulphonium salts derive sulphur through cysteine. This is accomplished either by transsulphuration or by incorporation of the cysteine structure directly. The sulphur metabolism in organisms capable of sulphate fixation and those requiring preformed sulphur amino acids is summarized below.

Outline of sulphur metabolism

III. BIOLOGICAL THIOLS AND THEIR FUNCTION

A. Glutathione\textsuperscript{33, 34, 35}

While cysteine is the central compound of organic thiol metabolism, a tripeptide derivative, glutathione, is probably the most ubiquitous single thiol compound. Much fascinating biochemical history surrounds this molecule and it has served as the subject of two published volumes\textsuperscript{36, 37}. Still, remarkably little is really known concerning its biological importance.

\[
\begin{align*}
\text{SH} & \quad \text{CO}_2\text{H} \\
\text{H}_2\text{N} & \quad \text{CH}_2\text{CH}_2\text{S} \quad \text{C} \quad \text{N} \quad \text{H} \\
\text{O} & \quad \text{H} \\
\text{S} & \quad \text{H} \\
\text{C} & \quad \text{H}_2 \\
\text{C} & \quad \text{H}_2
\end{align*}
\]

Glutathione (\textgamma-glutamyl cysteinyl glycine)

Since glutathione occurs throughout the biological world, it is felt that it must satisfy some critical cellular need. The most likely general function is maintaining a reduced cellular environment. Glutathione can also serve as a variety of additional roles. This peptide functions as co-factor for certain enzymes and it may serve as a \textgamma-glutamyl donor in the synthesis of other \textgamma-glutamyl derivatives. Glutathione is involved in the detoxification of certain organic toxins by some species. There have also been suggestions of special roles for glutathione or its derivatives in brain function and in cell division.

13. Biochemistry of the thiol group

Glutathione is assembled from glutamic acid, cysteine and glycine in a protein-directed synthesis. Glutamic acid reacts with cysteine in the presence of ATP to yield ADP, inorganic phosphate and \textgamma-glutamyl cysteine. In a second step the \textgamma-glutamyl cysteine is condensed with glycine to give glutathione. A considerable amount of glutathione synthesis occurs in some cells. Liver may contain 10 mM glutathione which turns over every 2 to 10 h. Such high rates of synthesis and breakdown only add to the mystery of glutathione's importance.

A human disease associated with impaired glutathione synthesis has been reported\textsuperscript{37}. Red blood cells from this patient lacked the second enzyme of the synthetic sequence, but the activities of enzymes involved
in glutathione utilization were all normal. Red blood cell glutathione was only reduced to 10-20% of normal. This implies either that the enzyme defect is tissue specific and other tissues can supply some glutathione to the red cell or that the cell produces a less stable enzyme which had become inactivated by the time of analysis. Aside from their intrinsic medical interest, such natural mutants can be expected to provide considerable information about the biochemistry of glutathione. For example, this person was normally normal with problems only appearing under stress. This is surprising if the defect was really general and the roles of glutathione are as crucial as suggested. On the other hand, an increased sensitivity of this individual’s red cells to oxidative stress favors an antioxidant role for glutathione.

One special enzyme, that cleaving the γ-glutamyl bond, is involved in glutathione degradation. This enzyme, usually referred to as glutathionase, also has γ-glutamyl transpeptidase activity under certain assay conditions.

Action of glutathionase

\[
\begin{align*}
\text{H}_2\text{O} & \rightarrow \text{glutamic acid} \\
\gamma\text{-glutamylcysteinylglycine} & \rightarrow \text{cysteinyl glycine} \\
& \rightarrow \gamma\text{-glutamyl peptide}
\end{align*}
\]

It is unclear if the transpeptidation activity represents a way for glutathione to serve as a synthetic γ-glutamyl donor or is simply an insignificant transpeptidase activity typical of many hydrolysates. This enzyme probably also participates in mercapturic acid formation, and this can be viewed as a variation of the direct hydrolysis reaction in which a substituted glutathione is substrate.

2. Maintenance of the reduced cell

Glutathione can be oxidized to its disulfide by oxygen, oxidized electron transport carriers, free radicals and a variety of disulfides. While most of these reactions are facilitated by enzymes, they also can occur spontaneously. It must be assumed that the ease of nonenzymatic oxidation is an important attribute in the protection of other cellular constituents. The idea that glutathione serves to keep thiols in a reduced state is a direct extension of its usefulness in maintaining extracted enzyme systems in a functional form. The nonenzymatic disulfide interchange reaction of glutathione is facile and a number of enzymatic activities promoting such reactions have also been described.

13. Biochemistry of the thiol group

Disulfide interchange reaction of glutathione (GSH)

\[
2\text{GSH} + \text{RSH} \rightarrow \text{GS-SG} + 2\text{RS}^-
\]

Glutathione reductase is an ubiquitous enzyme to be discussed mechanistically in the section on dithiol enzymes. Through its action, metabolic reducing power generated as reduced pyridine nucleotides can be coupled to the maintenance of the reduced environment.

Glutathione reductase reaction

\[
\text{GS-SG} + \text{NADPH} + \text{H}^+ \rightarrow 2\text{GSH} + \text{NADP}^+
\]

Crucial thiols such as cysteine and coenzyme A and the numerous cellular enzymes requiring thiol groups for proper function are kept reduced by the high glutathione levels within cells. Oxidized glutathione in turn is reduced by glutathione reductase and NADPH-generating systems.

Glutathione-mediated disulfide reductions whether enzyme mediated or spontaneous probably proceed through an intermediate mixed disulfide via a thiolate displacement mechanism.

\[
\text{Disulfide interchange mechanism}
\]

Relatively high glutathione concentration would be required to ensure complete reduction. Some glutathione bound as a mixed disulfide is found in cellular proteins as would be expected from this scheme, but it is uncertain if this actually existed within the cell or was produced on extraction.

A few systems are known in which glutathione serves as a reductant for molecules other than disulfides. Probably the most critical of these in animals is the glutathione peroxidase of the red cell. Along with catalase this enzyme is responsible for destroying peroxides and thereby preventing lipid peroxidation and haemoglobin inactivation.

Glutathione peroxidase reaction

\[
2\text{GSH} + \text{H}_2\text{O} \rightarrow \text{GS-SG} + 2\text{H}_2\text{O}
\]
functional importance of this reaction can be deduced from the effects of genetic disorders such as erythrocyte glucose-6-phosphate dehydrogenase deficiency. Where there is a lack of NADPH production the inability to maintain glutathione in the reduced form results in decreased red cell stability and haemolytic anaemia. 

Related to this is the action of glutathione as a free radical scavenger in protection against radiation damage. Thiol radicals readily react with free radicals producing thiol radicals which eventually combine to disulphides. It is felt that the ease of this reaction and the ready availability of glutathione minimizes damage to critical biological structures by the free radicals produced by ionizing radiation. Some consider one important mechanism of cellular ageing to be a slow accumulation of radiation-induced damage. Glutathione might therefore be considered also to have an anti-ageing role.

3. Other electron transport roles

Plants have an enzyme system linking the oxidation of glutathione to the reduction of dehydroascorbic acid. A similar enzyme may occur in animal tissues, although in this case a facile non-enzymatic reaction could possibly account for the observed activity. The plant enzyme provides a pathway whereby oxidized ascorbate can be reduced thereby enhancing its potential as an antioxidant. The full appreciation of the biological significance of this reaction suffers from the almost complete ignorance of the role of ascorbic acid. Coupled with a NADPH-linked glutathione reductase, the NADP reduction activity of the pentose shunt enzymes, and a dehydroascorbic acid oxidase, a complete respiratory chain for the oxidation of glucose is possible. Its actual operation if it occurs at all appears restricted to the earliest phases of plant development.

Glutathione-ascorbic acid respiratory chain

Glutathione is also the reductant for an organo-nitrate ester-reducing enzyme from liver. This so-called nitroglycerin reductase reacts with glycerol, erythritol or mannitol nitrates to yield free alcoholic hydroxyl and nitrite ions. The normal physiological substrate for this system is unclear. While its study has provided interesting enzymology it has not yielded any insight into the biological significance of glutathione.

4. Use as an enzyme cofactor

The best established functional role for glutathione is as a cofactor in certain enzymatic processes. The most extensively studied example is the glyoxylase system. This catalyses an internal oxidation-reduction, or dismutation, of certain α-keto aldehydes to α-hydroxy acids.

Glyoxylase reaction

The idea that this system played a crucial role in carbohydrate metabolism forms an important, but now largely forgotten, aspect of the history of biochemistry. The discovery of the importance of glutathione in the glyoxylase reaction was, in fact, the critical finding which has relegated this reaction to its present metabolic obscurity. In muscle preparations the glyoxylase system was found to be inoperable without added glutathione. However, glycolytic activity continued precluding any direct role for glyoxylase in this important metabolic process. While the bulk of intermediary metabolism has been traced out in the intervening forty years, glyoxylase function remains undefined. At present it is assigned a detoxication role in protecting against α-keto aldehydes, although
Arvan L. Fluharty

Szent-Györgyi has proposed that the glyoxylase system may be important in the control of cell division.

The glyoxylase reaction is promoted by two enzymes found in almost all living creatures. The first protein catalyses the condensation of the \( \alpha \)-keto aldehyde with glutathione followed by an internal disproportionation producing a thioester of an \( \alpha \)-hydroxy acid. A second enzyme cleaves the thioester regenerating glutathione.

\[ \text{Glyoxylase system} \]

\[ \text{GSH} + \text{CH} = \text{C}-\text{CH}_2 \underset{\text{glyoxylase I}}{\xrightarrow{\text{OH}}} \text{GSH} + \text{HCO}-\text{CH}_2-\text{CH}_3 \]

\[ \text{GSH} + \text{CH} = \text{C}-\text{CH}_2 \underset{\text{glyoxylase II}}{\xrightarrow{\text{OH}}} \text{GSH} + \text{HCO}-\text{CH}_2-\text{CH}_3 \]

The present conception of the glyoxylase I mechanism involves a non-enzymatic condensation of the thiol of glutathione with the \( \alpha \)-keto aldehyde to produce a thiohemiacetal. The enzyme then promotes an intramolecular hydride migration generating an \( \alpha \)-hydroxy acid-thioester. The original aldehydic hydrogen is retained in the final product. It has been compared to the Cannizzaro and benzilic acid rearrangements of organic chemistry.

\[ \text{Glyoxylase I mechanism} \]

The second enzyme of the glyoxylase system, the thioesterase, is specific for thioesters of glutathione and its analogues. Thioester hydrolysis and transacylation will be discussed in subsequent sections.

At one time glutathione was also thought to constitute part of the active centre of glyceraldehyde 3-phosphate dehydrogenase with similar thiohemiacetal and thioester intermediates. While the analogous involvement of an enzyme thiol in the enzyme reaction has been well established, an analysis of the amino acid sequence at the active site of the enzyme has shown that glutathione is not part of the enzyme.

Glutathione does not appear to have a valid role in a similar reaction, that of formaldehyde dehydrogenase.

\[ \text{Formaldehyde dehydrogenase reaction} \]

\[ \text{H}_2\text{C}=\text{O} + \text{NAD}^+ + \text{H}_2\text{O} \rightarrow \text{HCO}_2\text{H} + \text{NADH} + \text{H}^+ \]

Glutathione also acts as coenzyme for a completely different type of reaction, the isomerization of maleylacetoacetate to fumarylacetoacetate.

\[ \text{Maleylacetoacetate isomerase reaction} \]

\[ \text{H}_2\text{C}=\text{CO}_2\text{H} \rightarrow \text{H}_2\text{C}=\text{C}-(\text{CH}_2)_2=\text{CO}_2\text{H} \]

The reaction is thought to proceed through a reversible addition of the thiol to the double bond. Glutathione can catalyse the isomerization of this and other \( \alpha,\beta \)-unsaturated acids even in the absence of enzyme. A glutathione addition product can be isolated with such substrates but does not appear to be a true intermediate in the enzymatic process, as it is not acted on by the enzyme. An enzyme-bound adduct is thus implicated.

5. Mercapturic acid formation and detoxification

Glutathione is involved in the conjugation of certain toxic hydrocarbons by the liver. These are eventually excreted as mercapturic acids, \( S \)-substituted N-acetyl-cysteines. Such compounds have been isolated from the urine of many animals including man.

Benzenes, hexabenzences, naphtalenes and a variety of other aromatic or unsaturated hydrocarbons are conjugated by reaction with glutathione. Many of these compounds readily react with thiol nonenzymatically, and their rapid scission would be critical in protecting the functional thiols of the cell. A group of enzymes concentrated in the liver and kidneys, the glutathione S-transferases, catalyse the condensation with glutathione. Cysteine or other biological thiols do not serve as acceptors. After formation of the hydrocarbon adduct the glutathione peptide bonds are hydrolysed and the cysteine residue is \( N \)-acytyleted before excretion. In many cases the product actually excreted is a so-called premercuric acid which contains a hydroxyl adjacent to the thiether substituent. Water is eliminated during isolation to produce the mercapturic acid. The
frequent occurrence of an α-hydroxy substituent suggests that the hydrocarbon has undergone epoxidation of a double bond prior to reaction with glutathione. The condensation reaction would then involve an attack on the epoxide ring by a thiolate. Direct addition of the thiol to a double bond or even halogen displacement may also occur in certain cases giving rise to metabolic products without α-hydroxy substituents.

The intermediate production of aralkyl sulphate esters or thioacetyl derivatives prior to conjugation with glutathione seems likely for certain types of compounds since enzymes of the following types have been characterized:

**Glutathione conjugation via thioesters and sulphates**

\[
R-ch=ch-c-S-A + GSH \rightarrow R-ch=ch-c-SA
\]

**13. Biochemistry of the thiol group**

Mercapturic acid production seems to have first call on the sulphur amino acid reserves and serious deficiency states can be induced in rats by hepatotoxic hydrocarbons. Diets high in cysteine and methionine will protect against the liver damage. Some mercapturic acid production may also result from reaction of protein thiol groups with the hydrocarbons, hydrolysis of the protein to the S-substituted cysteine and its N-acetylation. However, the vast majority is formed via the glutathione adducts if the hydrocarbon dose is not too great as to deplete the glutathione reserves of the liver.

The intermediate production of aralkyl sulphate esters or thioacetyl derivatives prior to conjugation with glutathione seems likely for certain types of compounds since enzymes of the following types have been characterized:

**Glutathione conjugation via thioesters and sulphates**

\[
R-ch=ch-c-S-A + GSH \rightarrow R-ch=ch-c-SA
\]

**Methionine metabolism**

Mercapturic acid formation has been shown to occur in a variety of mammals, birds, reptiles, amphibians and fish. Insects also form glutathione conjugates but do not N-acetylate the eventual S-substituted cysteine derivatives to any great extent. It is also possible that the S-carboxyalkylcysteines of plants have a similar genesis. Mercapturic acid formation is certainly one of the best studied and documented protective functions for glutathione.

Thus, in spite of many years of investigation and speculation, no universal functional role has been established for glutathione which would explain its broad distribution and high concentration in biological systems. The most satisfying concept is that the glutathione system establishes the reduced state of the cell, at least in so far as preventing the oxidation of cellular thins. In fact the thiol protective effect is multifaceted. Glutathione preferentially reacts with agents of all types which otherwise would inactivate thiol metabolites, coenzymes and proteins. If inappropriate disulphide formation should occur, activity can be restored by the disulphide interchange. Whether such a general protective action is the universal glutathione role has been difficult to prove, and the
concept has been gently derided by labelling it the euphoristic theory of glutathione action (see reference 24).

While the overriding function of glutathione may be protective, a number of more specific roles have evolved. It serves as a coenzyme for certain enzymatic processes, and it may moderate critical rearrangements of cellular architecture. If for no other reason glutathione could be regarded as the most important cellular thiol on a purely quantitative basis, and it is likely that it has functions of correspondingly critical significance.

B. Methionine and S-Adenosyl Methionine

The biological importance of the second thiol amino acid, homocysteine, is as the thioether and sulphonium ion derivatives. The free thiol occurs only as a metabolic intermediate. Methionine, the methyl thioether, is one of the twenty amino acids utilized for protein synthesis. Our concepts of the special significance of methionine in protein structure and function are only beginning to be developed, and will not be considered here. N-Formyl methionine also has the distinctive role of being a chain initiator in protein synthesis. The most extensively studied form of this thiol is S-adenosyl methionine or SAM, the sulphonium ion cofactor. This is the principal methylating reagent of biological systems and other alkyl transfers from the sulphonium ion are also known.

1. Methylation of homocysteine

Methylation of homocysteine to methionine can be accomplished by one of several sequences. A major route is from a N₅-methyl tetrahydrofolic acid (CH₃-FH₂) derivative. In some organisms a coenzyme derivative of vitamin B₁₂ is also required, where it functions in its reduced form (B₁₂H₂ in the following scheme) as an intermediate methyl carrier:

![Diagram of methylation of homocysteine by folic acid derivatives]

Animals also derive methyl groups from dietary choline, which can partially substitute for the methionine nutritional requirement. An oxidation product of choline, betaine, is the actual methyl donor to homocysteine. This probably represents a salvage pathway for methyl groups in the catabolism of choline, but it can be of considerable importance if the capacity for de novo methyl synthesis is limited.

2. S-Adenosyl methionine and transmethylation

Methionine reacts with ATP to produce S-adenosyl methionine (SAM) with the release of both an orthophosphate and pyrophosphate residue.

![Diagram of biosynthesis of S-adenosyl methionine]

This sulphonium compound, often referred to as ‘active methyl’, serves as a methyl donor for biological synthesis. The list of compounds which derive methyl groups by transmethylation from SAM is extensive and
includes many types. Oxygen, nitrogen, sulphur and carbon atoms can act as acceptor. A few representative reactions are indicated below.

Typical methylation reactions

```
HN NH₂           HN NH₂
\( \overset{\text{guanidino acetate}}{\text{C}} \)        \( \overset{\text{creatinine}}{\text{C}} \)
\( + \text{SAM} \rightarrow \)                             \( + \text{S-adenosyl homocysteine} \)
\( \overset{\text{serine}}{\text{CH₃G-CH₂-CH₂OH}} \)          \( \overset{\text{O-methylserine}}{\text{CH₃G-CH₂-OCH₃}} \)
```

The S-adenosyl homocysteine produced in the transmethylation reactions is generally cleaved to adenosine and homocysteine. The latter can be degraded as previously discussed or be remethylated to methionine and eventually regenerate S-adenosyl methionine. Thus the operation of a methionine cycle provides a route whereby one-carbon metabolites reduced through the tetrahydrofolate acid sequence provide methyl groups for biosynthesis pathways. Certain other sulphonium compounds such as

13. Biochemistry of the thiol group

S-methyl methionine and dimethyl β-propiothetin are apparently capable of serving as methylating agents in some organisms but do not have the general biological significance of S-adenosyl methionine.

```
C₂ reduction or methyl salvage
```

```
homocysteine → methionine

S-adenosylhomocysteine → S-methyl methionine
```

3. Other sulphonium ion alkylations

Methyl transfer is not the only kind of alkylation that can be effected by the sulphonium centres. The best studied example is the synthesis of the polyamines spermine and spermidine, important counter ions for nucleic acids. S-Adenosyl methionine undergoes a decarboxylation of the homocysteine side chain producing a thioamido amine derivative. The propyl amine residue then is transferred, first to one and then to the other amino group of putrescine yielding in turn spermine and spermidine.

S-Adenosyl methionine provides an interesting example of how thiol derivatives can promote what are normally considered to be difficult organic reactions. Few alkylating reagents employed by the chemist are compatible with the conditions of biochemical systems. Sulphonium ions can however be readily formed under biological conditions and are sufficiently stable in an aqueous environment to have their reactions controlled by enzyme specificity. The wide biological distribution of S-adenosyl methionine-mediated transmethylation attests to the fact that alkylation through sulphonium ion intermediates is among the most ancient biological group transfer reactions.

The chemical rationalization for the alkyl-transferring capacity of the sulphonium (and other ‘onium’) compounds is that the positively charged sulphur induces a partial positive charge on the immediately adjacent carbon atom. Such a positive carbon centre then becomes susceptible to nucleophilic attack. The thioether serves as an excellent leaving group particularly if a relatively nonpolar reactive centre is envisaged. Reactions involving S-adenosyl methionine as a methyl donor at neutral pH, generally
have favourable free energies of $-7$ (or more) kilocalories per mole. Thus, the intermediary role of SAM in biological transmethylation and occasionally in other transalkylation reactions reflects both thermodynamic and mechanistic attributes of sulphonium ions. Sulphonium ion reactions in turn constitute one of the fundamental functional roles of a thiol in biological systems.

C. Pantetheine Cofactors

The most clearly defined functional role of cellular thiols is that of coenzyme A and related cofactors. Coenzyme A was first recognized as a carrier for activated acyl groups. The general sequence for acylation in biological systems is acyl activation to a thioester followed by acyl transfer to form amides, esters and acid anhydrides. In addition the thioester linkage enhances the carbonyl nature of the carboxylate group leading to a variety of reactions within the acyl carbon chain. Recently it has been recognized that the phosphopantetheine portion of the coenzyme A molecule also occurs in proteins, where it serves a similar role. A great deal of mechanistic information has been accumulated on enzyme reactions mediated by the thioesters of coenzyme A and related structures. This is one of the areas in which the physical organic chemists’ approach to biochemistry has proved most fruitful.

I. Biosynthesis of coenzyme A

Coenzyme A is a complex organic molecule with a nucleotide portion of adenine, ribose and phosphoryl groups linked through a pyrophosphoryl bridge to an unusual peptide, pantetheine. This structure has a branched-chain dihydroxy acid, pantoic acid, linked to 7-alanine which in turn is bonded to thioctylylamide. In spite of the complexity of the coenzyme A
molecule our understanding of its function relates only to the fact that it is a thiol. The remainder of the molecule is presently relegated to imparting water solubility to acyl derivatives and providing very specific structures for enzyme binding. In fact its catalytic function in several enzyme systems can be met by various simple N-acyl cysteamine models, although enzyme affinity is considerably lowered. While viewing coenzyme A simply as a thiol is generally recognized as being a gross oversimplification, evidence of any functional significance for other structural elements is sparse.

In microorganisms the pantoic acid carbon chain is derived from valine and 'active formaldehyde' and the β-alanine from aspartic acid. Higher organisms are unable to synthesize the pantothenic acid portion of the molecule and it is a required vitamin. Pantothenic acid is first phosphorylated to 4-phosphopantothenic acid and then condensed with cysteine to produce 4'-phosphopantethenyl cysteine. The cysteine residue then undergoes decarboxylation to 4'-phosphopantetheine. An adenylate is transferred from ATP to generate dephospho coenzyme A and a final phosphorylation of the 3'-hydroxyl of ribose provides the biologically active coenzyme. A slightly different sequence was thought to operate at one time, and still may be possible in some organisms. It differs only in that condensation with cysteine and the decarboxylation precedes the phosphorylation of the pantothenic acid hydroxyl group.

**Biosynthesis of coenzyme A**

4'-phosphopantothenic acid → 4'-phosphopantetheine

![Diagram showing the biosynthesis of coenzyme A]

**Coenzyme A**

ATP → dephospho coenzyme A

H₂PO₄⁻ + ATP → ATP

Coenzyme A can readily be oxidized to an inactive disulphide in air and mixed disulphides with other thiols such as cysteine and glutathione are also readily formed. In fact any reagent used to probe for enzyme thiol

13. Biochemistry of the thiol group will also react with coenzyme A making studies of protein thiols much more difficult in coenzyme A requiring systems.

2. Formation of coenzyme A thioesters⁴⁰,⁴¹

In its biological function the sulphhydryl group of coenzyme A is converted to a thioester. The acid is almost always a carboxylic acid although there have been some indications that coenzyme A thiophosphate esters might play a role in certain reactions. Thioesters have a sufficiently large negative free energy of hydrolysis to place them among the so-called 'high energy' compounds of biochemical energetics. Their synthesis must be driven by exergonic metabolic processes. Actually coenzyme A thioesters participate in the metabolic energy exchange system serving as an intermediate repository for the biochemical energy quanta represented by the squiggly (−) bond. Thioesters are formed by nucleoside triphosphate-dependent reactions, by oxidative processes or by thiolic cleavage of β-keto thioesters. The coenzyme A derivative can donate the acyl to amino, thiol, hydroxyl and carbonyl centres in energetically favourable reactions. It can also drive the formation of pyrophosphate linkages of nucleoside triphosphates. Coupled with this high reactive potential of the thioester is an amazing kinetic stability. Spontaneous decomposition mechanisms are not available in an aqueous environment at neutral pH and physiological temperatures. Such a situation is biochemically ideal, a high reactivity which can be completely controlled by enzymatic catalysis.

The direct route of acyl coenzyme A synthesis from a free carboxylic acid is catalysed by a group of nucleoside triphosphate-requiring enzymes, collectively known as thiokinases. The general mechanism, as exemplified for acetate activation by acetyl thiokinase, proceeds as follows. The carboxylic acid is first activated by acetyl adenylate formation with the displacement of pyrophosphate from ATP. While the initial reaction is fully reversible, subsequent action of pyrophosphatase drives the reaction.
process. The thiol of coenzyme A then displaces adenylate acid in a second step to produce the acetyl thioester.

Acyl adenylate intermediates seem the general rule for acyl activation, but alternate mechanisms are known. An example is the succinyl thiokinase reaction. The mammalian enzyme system utilizes guanosine triphosphate (GTP) or inosine triphosphate (ITP), although similar ATP-requiring enzymes are known from plants and bacteria. In addition to the coenzyme A derivative, a nucleoside diphosphate and inorganic phosphate are produced.

Succinyl thiokinase reaction

\[
\begin{align*}
\text{CO}_2\text{H} & \quad \text{C}^\text{S-CoA} \\
\text{CH}_3 + \text{CoASH} + \text{GTP} & \rightarrow \text{CH}_3 + \text{GDP} + \text{H}_2\text{PO}_4 \\
\text{Pi} & \quad \text{Enz} \\
\text{GDP} & \rightarrow \text{Enz} \sim \text{P} \\
\text{sucyclic acid} & \quad \text{Enz} \sim \text{CoASH} \\
\text{Enz} \sim \text{coA} & \quad \text{Enz} \sim \text{coA} \\
\end{align*}
\]

These products suggest activation as a phospho-ester rather than as a nucleosyl derivative. Both succinyl phosphate and thiophosphoryl coenzyme A have been used as intermediates. However, neither is included, at least as a freely dissociable intermediate, in current formulations of this reaction. An enzyme-bound phosphohistidine intermediate is thought to be involved, as is some sort of activated enzyme-CoA complex. Many aspects of the enzyme mechanism are still in doubt, but the sequence below is consistent with most available data.

This modus of thioester formation is not as energetically favourable as that involving pyrophosphate release and its eventual cleavage. This probably reflects different biological roles for the two types of thioesters.

Succinyl thiokinase and probably other nucleoside diphasphate inorganic phosphate type enzymes normally operate in the other direction, with thioacyl coenzyme A driving the synthesis of nucleoside triphosphate.

One type of enzyme system produces coenzyme A thioesters efficiently at the expense of nucleoside triphosphate, while the other helps to couple metabolic processes to the synthesis of high energy phosphates.

Another way to generate particular acyl coenzyme A derivatives is at the expense of others. The succinyl-acetoacetyl coenzyme A transferase reaction is an important example.

Acyl interchange reaction

sucinyl-S-CoA + acetoacetic acid \rightarrow succinyl acid + acetoacetyl-S-CoA

An intermediate enzyme–coenzyme A complex in which the energy of the thioester bond is preserved has been demonstrated. Here the coenzyme A thioester is involved in a transfer reaction quite different from its usual acyl donor role. Functionally this enzyme allows metabolically generated coenzyme A derivatives to be utilized directly for carboxylic acid activation, without intermediation formation of nucleoside triphosphates.

A metabolically important route for the generation of acyl coenzyme A derivatives is through the oxidation of α-keto acids. The α-keto acid dehydrogenase complexes, of which pyruvate dehydrogenase complex is typical, are large multienzyme aggregates. They carry out a complex reaction sequence to be discussed in Section III.D on lipoic acid. The overall reaction given below is an oxidative dehydroxylation coupled to thioester formation.

Pyruvate dehydroxylation-dehydrogenase reaction

\[
\begin{align*}
\text{CO}_2\text{H} & \quad \text{C}^\text{O} + \text{NAD}^+ + \text{CoASH} \rightarrow \text{C}^\text{S-CoA} + \text{NADH} + \text{CO}_2 + \text{H}^+ \\
\text{CH}_3 & \quad \text{CH}_3 \\
\text{pyruvic acid} & \quad \text{acyl CoA}
\end{align*}
\]

The final process for coenzyme A thioester synthesis is by the thiolic cleavage of β-keto acyl coenzyme A derivatives. The thiolic reaction is the principal metabolic process for degrading the hydrocarbon chain of fatty acids.

β-ketofatty acyl CoA thiolase reaction

\[
\begin{align*}
\text{R}^\text{C} = \text{CH}_2 - \text{C}^\text{S-CoA} & \rightarrow \text{CH}_3 - \text{C}^\text{S-CoA} \\
\text{HT}^\text{3-C} - \text{CoA} & \rightarrow \text{R}^\text{C} - \text{S-CoA}
\end{align*}
\]

3. Reactions of coenzyme A thioesters

Examples of acylation by acyl coenzyme A derivatives are numerous. The quantitatively most important example is the transfer of fatty acyl
residues from coenzyme A in the synthesis of glycerides. In this case the acyl acceptors are the hydroxyl groups of glycerol derivatives and the products are oxygen esters. Acyl coenzyme A hydrolases can also be

Pathways for glycerol lipid synthesis

\[
\begin{align*}
H_2C\OH & \quad \xrightarrow{HCOH} \quad H_2COPO_3H_2 & \quad \rightarrow \quad \xrightarrow{\text{acyl-enzyme A hydrolase reaction}} \\
& \quad \xrightarrow{\text{acyl-enzyme A transferase reaction}} \quad \xrightarrow{\text{acyl-enzyme A transferase reaction}} \\
\text{RCOH} & \quad \xrightarrow{\text{acyl-enzyme A hydrolase reaction}} \quad \xrightarrow{\text{acyl-enzyme A transferase reaction}} \\
& \quad \xrightarrow{\text{acyl-enzyme A hydrolase reaction}} \quad \xrightarrow{\text{acyl-enzyme A transferase reaction}} \\
\end{align*}
\]

looked upon as acyl O-transferases of a special type with water acting as acceptor.

Acyl-coenzyme A hydrolase reaction

\[
R-C=S-CoA + H_2O \rightarrow R-CoH + CoASH
\]

Transfer from an acyl coenzyme A derivative to a nitrogen nucleophile is also quite common. Typical is the N-acetylation of the amino sugars such as glucosamine. The conversion of palmityl coenzyme A to palmit-

Glucosamine Acetylation

\[
\begin{align*}
\text{CH}_2\text{OH} & \quad \xrightarrow{+CH_2\text{-CoA}} \quad \xrightarrow{\text{N-acetyl glucosamine}} \\
\text{NH}_2 & \quad \xrightarrow{+CoASH} \quad \text{N-acetyl glucosamine}
\end{align*}
\]

aldehyde by reduced pyridine nucleotide can be considered, at least formally, as an acyl transfer reaction. Here the acyl acceptor can be envisaged as a hydride ion derived from NADH.

Reactions where phosphate, thiol and even cyanide accept the substituent from acyl coenzyme A derivatives have been described in biological systems. Carbon is also an important acyl acceptor, generally reacting as a resonance-stabilized carbocation. Examples are the Claisen type ester condensation reactions to be discussed in section III.E.1.

The increased acyl transfer potential of thioesters as compared to corresponding oxygen esters is explained as being due to less double bond character in the bridging bond. The unpaired sulphur electrons do not have as high a tendency towards double bond formation as those of oxygen, and less electron delocalization or resonance stabilization of the bonding system is possible. This results in a longer and more easily displaced linkage. The lack of resonance with the ester sulphur also results in an enhanced electrophilic character of the carbonyl carbon. Thus, attack by nucleophiles at this position is facilitated.

The general mechanism for acyl transfer reactions from thioesters is envisaged as a nucleophilic attack at the positively polarized carbonyl carbon, accompanied by or followed by thiol elimination.

General transacylation mechanism from acyl coenzyme A

\[
\begin{align*}
R-C-S-CoA & \quad \xrightarrow{A} \quad R-C-S-CoA & \quad \xrightarrow{H^+} \quad R-C-S-CoA \\
& \quad \xrightarrow{HA} \quad R-C-S-CoA & \quad \xrightarrow{BH} \quad R-C-S-CoA
\end{align*}
\]

It is supposed that the enzyme participates by providing general acid and general base groups which facilitate the attack of the entering nucleophile, the departure of the thiolate and the polarization of the carbonyl.

An intermediate acylated enzyme may occur in some reactions but this can simply be envisioned as a case where binding centre, catalytic groups and the initial attacking nucleophile are all provided by the enzyme.

Coenzyme A thioesters can also promote nucleophilic attack at the β-carbon in α,β-unsaturated derivatives. In these cases an electrophilic centre is stabilized at the β-carbon by resonance with the carbonyl system. This could be particularly favoured by hydrogen bonding or protonation of the carbonyl oxygen by an enzyme. An example is the enoyl coenzyme A hydratase reaction of fatty acid degradation.
β-substitution in α,β-enoxy coenzyme A thioesters

α-Activation is the other crucial aspect of thioester and acyl coenzyme A biochemistry. The formation of the thioester considerably increases the ketone-like character of the carbonyl group of the carboxylic acid. In addition to increasing the electrophilic behavior of the carbonyl carbon, it enhances the acidity of the hydrogens at the α-position. This is normally attributed to the possibility for resonance stabilization involving the enolate anion.

Enolate stabilization in coenzyme A thioesters

Enolate ion formation allows coenzyme A-bound acyl groups to serve as nucleophiles and to react at electrophilic centres. This permits thioesters to participate in the formation or degradation of carbon–carbon linkages by mechanisms analogous to the aldol condensation or more specifically the Claisen type ester condensation. There are few available mechanisms for carbon–carbon bond formation or cleavage which can be employed under biological reaction conditions, and pathways which depend on coenzyme A thioesters for this purpose are widespread.

The classic example is the reaction by which acetate carbon enters the tricarboxylic acid cycle, the citrate synthase reaction. Extensive mechanistic studies have established the involvement of the enolate of the acetyl thioester in the enzyme reaction. Exchange of the acetate hydrogens of acetyl coenzyme A with deuterium or tritium in the solvent is catalysed by the enzyme under conditions in which the condensation cannot occur.

Citrate synthase reaction

Initially this was not observed, exchange only being measurable when oxaloacetate was also present. This absence of exchange is now observed to result from a need to have oxaloacetate bound to the enzyme before the proper catalytic configuration can be achieved. This function can be served by certain other dicarboxylic acids which are non-capable of undergoing the condensation reaction and the exchange activity has been demonstrated.

A coenzyme A-facilitated enolization mechanism seems firmly established.

An example in which sensitivity of both the attacking nucleophile and the electrophilic acceptor is dependent on the special character of the acyl thioesters is in the condensation of two acetyl coenzyme A units to form acetoacetyl coenzyme A. This is the reverse of the thiolase reaction.
discussed previously. The actual mechanism of this reaction may involve an initial transfer of one acetyl grouping to an enzyme thiol prior to condensation, but the general reaction scheme is unchanged as thioacyl activation would still be involved.

a-Carbon activation is also involved in the biotin mediated carboxylation of acetyl coenzyme A to malonyl coenzyme A, a critical and distinctive step in fatty acid biosynthesis. Carbon dioxide is initially attached to a ureido carbon of biotin and then transferred to the methyl carbon of acetyl coenzyme A. A concerted mechanism for this transfer has been suggested rather than a pre-equilibrium enolization of the acetyl coenzyme A on the basis of the stereochemistry of the condensation. The proposed reaction sequence is an example of how concerted substitution on the α-carbon of thioesters could be facilitated.

Mechanism of acetyl coenzyme A carboxylation

The thioester promotes the acidity of the α-hydrogens favouring hydrogen-bonded interaction with the ureido oxygen. In this case the promoting base and the electrophilic centre being attacked are part of the same structure, permitting a concerted electronic rearrangement without the necessity of an actual enolate ion. Since similar advantageous arrangements of reacting and catalytic functions are possible on enzymes, it is conceivable that other examples may also circumvent the pre-equilibrium enolate formation which would be predicted from analogy to solution chemistry. This does not alter the concept that thioesters facilitate such reactions by enhancing the acidity of α-hydrogens.

A convenient way to summarize the reactions of coenzyme A thioesters is by reviewing the β-oxidation pathway for fatty acids. Fatty acid activation occurs by acylation of the coenzyme A thiol by way of an acyl adenylate. This is then dehydrogenated to an α,β-enoxy acyl coenzyme A derivative by a flavin-dependent dehydrogenase. The ability of the adjacent carbonyl to provide resonance stabilization of the product appears to be an important aspect of this reaction. Such flavin-dependent dehydrogenases occur in other reaction sequences, but only where carbonyl resonance stabilization is possible. Water adds to the α,β-ENOXY thioester to generate a β-hydroxy fatty acid derivative, a reaction facilitated by β-carbonium ion stabilization in enol thioesters. The β-hydroxy thioester is then

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reduced to a β-keto group. Such nicotiamide dehydrogenase-linked reductions to alcohols are common and no special advantage can be ascribed to the thioester. Thiolytic cleavage of the β-keto thioester releases acetyl coenzyme A and leaves a fatty acid derivative two carbons shorter than the original. The dehydrogenation, hydration, dehydrogenation, thiolation sequence is repeated to reduce the chain by two carbons at a time with almost every step dependent on the unique properties of coenzyme A thioesters.

Fatty acid oxidation spiral

4. Phosphopantetheine proteins

Protein-bound phosphopantetheine has been found in recent years to be involved in acyl binding and reaction in much the same manner as coenzyme A. A 17 amino acid protein was isolated from E. coli which acted as an acyl carrier in fatty acid synthesis. This protein completely lacked cysteine or other thiol amino acid, yet functioned by binding various acyl intermediates as thioesters. The reactive centre was phosphopantetheine linked to the protein through a phosphodiester bridge to serine. Similar acyl carrier proteins, or ACPs, have now been isolated from a variety of organisms and extensively characterized. An active ACP protein chain has even been prepared synthetically. ACP per se has been

Phosphopantetheine linkage in E. coli acyl carrier protein

*O*—O-pantetheine-SH

*O*
difficult to demonstrate in higher organisms in which the intermediates of fatty acid synthesis are bound to high molecular weight complexes. It is reasonably certain that protein-bound phosphopantetheine is involved however, and an analogous protein cofactor is believed to be present in a tightly bound form. Phosphopantetheine prosthetic groups are now also known to function in other pathways.

Coenzyme A is the precursor of the enzyme-bound phosphopantetheine.

The prosthetic group is added to the prosthetic group free protein (apo-ACP), by a phosphoryl transfer reaction employing coenzyme A as donor, yielding the functional complex protein, holo-ACP:

Attachment of 4-phosphopantetheine to protein

The phosphopantetheine prosthetic group of ACP, fatty acid synthetase complexes, and presumably other enzyme systems, turn over rapidly, possibly as part of a cellular control mechanism. A specific phosphodiesterase cleaves holo-ACP to 4'-phosphopantetheine and the apoprotein.

Removal of 4 phosphopantetheine from protein

The role of phosphopantetheine linked to protein is analogous to that in coenzyme A. Mechanistically fatty acid synthesis is pretty much a reversal of the β-oxidation pathway discussed earlier. There are however a few minor and one major differences. ACP rather than coenzyme A derivatives participate in synthesis and a nicotinamide coenzyme rather than a flavin coactor is involved in double bond reduction. The major difference is that in the chain-elongating thioester condensation reaction the attacking nucleophilic carbon derives from a malonyl rather than an acetyl thioester. As indicated previously, malonyl coenzyme A is produced from acetyl coenzyme A by a biotin- and ATP-dependent CO₂ fixation reaction. Both acetyl and malonyl groupings are transacylated to ACP for fatty acid synthesis. Enzyme thioesters, in addition to those of the phosphopantetheine prosthetic group, are also implicated in the process. In the yeast system, at least, a thioacyl linkage to a cysteinyl residue participates at one stage.

A turn of a generalized fatty acid synthesis spiral is presented below where the intermediate carriers are represented as ACP units tightly bound to a multienzyme complex.

Specific details vary somewhat from species to species, but this scheme illustrates a typical phosphopantetheine protein involvement.

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Generalized fatty acid synthesis spiral

Acetyl coenzyme A transfers its substituent to ACP-1 of the synthetase complex where it serves as the start of the growing chain. A subsequent acetoyl coenzyme A unit is carboxylated to malonyl coenzyme A and transferred to ACP-2. The acetyl (or higher homolog) segment then reacts with the malonyl methylene group of coenzyme A and the resulting acetyl group is transferred to the next ACP-1. This process is repeated until the saturated fatty acid derivative is obtained. The acyl group is then transferred to the next thioester, which is then repeated until the long-chain fatty acid is completed. The fatty acyl linkage is then transferred from ACP-1 to the terminal thioester to form the fatty acid.

The point of note is the special role of the malonyl thioester in the chain elongation process. The presence of the additional carboxylate group
adjacent to the methylene carbon increases the stabilization of a carbanion at this position. This further facilitates proton dissociation and attack at the carbonyl of the other ACP-bound thioester. The concerted loss of CO₂ renders the reaction essentially irreversible and provides a thermodynamic situation favourable for chain elongation.

Multienzyme complexes responsible for the assembly of the cyclic polypeptide antibiotics, gramicidin and tyrocidine, also contain protein-bound phosphopantetheine. This presumably participates in the enzyme-directed peptide bond assembly as an amino acyl carrier. Citrate lyase catalyses the cleavage of citrate to oxaloacetate and acetate without the involvement of coenzyme A. This has posed somewhat of a dilemma since thioester activation is considered mechanistically important in the oxaloacetate-acetate condensation sequence and presumably should also be necessary for decondensation. Recent evidence implies that the enzyme contains a phosphopantetheine unit which is acetylated in the active enzyme. The reaction is envisaged as an acetyl exchange with citrate, releasing acetate and generating a citryl thioenzyme. This then undergoes a thioester-promoted decondensation releasing oxaloacetate and regenerating the acetyl enzyme.

Thus the biological importance of the phosphopantetheine group as a catalytic centre is widespread. Numerous examples of the role of coenzyme A are known and the list of phosphopantetheine enzyme centres is growing. The principal reactive element is the thiol, although other attributes of the unique peptide will undoubtedly prove important. The thiol serves as the site of thioester formation and its particular chemical attributes facilitate acyl transfer, carbon chain modification and condensation reactions. The phosphopantetheine thiol represents the most extensively investigated example of this functional group in biochemical processes.

D. Lipoic Acid

Lipoic acid is a five-membered cyclic disulphide ring with a five-carbon carboxylic acid chain. When reduced it provides a constrained dithiol centre. This disulphide–dithiol cofactor is covalently bound to one of the enzymes in a multienzyme complex which catalyses oxidative decarboxylation of α-keto acids. In the course of the reaction three forms of the prosthetic group participate: the cyclic disulphide, the dithiol and a thioester of the dithiol form.

The reactions of the α-keto acid decarboxylase system occur in a highly organized complex of enzymes which utilizes a number of cofactors in addition to lipoic acid. It has been proposed that a long flexible arm resulting from the amide linkage of the lipoyl carboxylate to an α-amino group of a protein lysine permits the disulphide–dithiol centre to swing from one active site to another within the confines of the complex. The lipoic acid centre therefore may serve a physical transport role within the subunit. In addition to its chemical participation in the reaction sequence. In the initial reaction of the α-keto acid system a thiamine pyrophosphate-mediated decarboxylation results in a thiamine–aldehyde adduct. This is oxidized by the lipoic acid disulphide and the resulting acyl transferred from thiamine to the thiol at carbon-6 of the dihydrolipoic residue. A second enzyme of the complex then transfers the thioacyl from the dithiol to coenzyme A. This system thus provides one of the major routes for acyl coenzyme A production from sugar and amino acid metabolites. At the reactive centre of the third enzyme of the complex the lipoyl disulphide is regenerated by oxidation of the dithiol by a flavinamide coenzyme. The dihydrolipoamide dehydrogenase is an unusual flavoprotein which will be discussed subsequently as an example of a dithiol-disulphide electron transfer protein.

Lipoic acid links two of the major biochemical roles of thiol groups, being both involved in electron transfer and the generation of high
energy thioester bonds. By positioning the two thiol groups in a close relationship specific oxidation is facilitated. The presence of strain in the five-membered dithiolane ring system also may be an important aspect of lipoic acid biochemistry, but its functional significance has remained moot. There are relatively large amounts of lipoic acid and dihydrolipoic dehydrogenase in photosynthetic tissues. Their presence still lacks a satisfactory explanation in terms of a particular functional role. Proposals implicating the lipoate dithiolane ring system in primary energy trapping or in the transfer and utilization of chlorophyll-trapped energy has not gained any real acceptance. Photosynthetic carbon dioxide fixation into α-keto acids has recently been found to be the major pathway in some organisms. The process appears to be essentially a reversal of the mitochondrial oxidative decarboxylation process. The photo-reduction is mediated through a ferredoxin system similar to the photosynthetic nicotinamide coenzyme reductase. The involvement of lipoic acid has not yet been shown, but it would be expected and could provide the long-sought role of lipoate in photosynthesis.

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The really unique reaction of the lipoate centre in α-keto acid metabolism is the oxidative thioester formation from a thiamine-coordinated "active aldehyde". Thiol transacetylase and dithiol-disulphide oxidation reduction roles are well-known attributes of other biological thioles. Unfortunately mechanistic studies on this reductive acylation of a cyclic disulphide have so far received little attention. Proposals that a lipoic acid-thiamine pyrophosphate compound was the functional entity in α-keto acid oxidation have been completely abandoned, but data supporting this concept remain unexplained. Investigations in this area might have some relevance for the reductive acylation process.

Enzyme systems have been found for the formation and hydrolysis of the lipoyl amide linkage at appropriate lysine ε-amino groups of enzymes. The lipoic acid is activated by ATP to form a lipooyl asenylase, possibly as an enzyme-bound form, which then transfers the lipoyl group to the protein amino group.

Attachment and release of enzyme-bound lipoic acid

\[
\text{lipoic acid + ATP} \rightarrow \text{lipoyl-AMP + H}_2\text{P}_4\text{O}_7
\]

\[
\text{lipooyl-AMP + H}_2\text{N-protein} \rightarrow \text{lipooyl-N-protein + AMP}
\]

\[
\text{lipooyl-N-protein} \rightarrow \text{H}_2\text{O} \rightarrow \text{lipoic acid + H}_2\text{N-protein}
\]

The specific co-factor and removal system could reflect an effective enzyme control mechanism. At present there is no evidence that such a control is manifest within cells, and these reactions must be viewed as synthetic and degradative processes.

It should be noted that most enzyme studies concerning this disulphide-dithiol coenzyme have actually been carried out with either free lipoic acid or lipoamide and not a protein-bound co-factor. While this has been a pragmatic necessity, certain reserve should be maintained in extrapolating from such studies to the protein-bound prosthetic group.

The only established lipoic acid function is that in the α-keto acid decarboxylase dehydrogenase complexes, although several examples of this type of enzyme with varying substrate specificities are known. Other examples of lipoic acid enzymes have been sought, but other dithiol-disulphide enzymes have been shown to be free of lipoic acid residues. Sulphoxide derivatives of lipoic acid are easily isolated, and their possible biological function has also been suggested. However, present accepted dogma dismisses the more oxidized forms of lipoic acid as artifacts of air oxidation during isolation.
E. Thiol Proteins

A large number of functional proteins are known in which substitution of some or all of the thiol groups of cysteine residues interferes with activity. Most frequently this is only a reflection of a requirement for the thiol in maintaining a proper configuration or subunit interaction. In some cases a thiol group is believed to exist in or near the active site and possibly play a role in substrate or cofactor binding. In a few enzymes the cysteine thiol is known to play a critical role in the catalytic process. In all of these cases enzyme activity or other biological function can be influenced by reaction of the protein with thiol-specific reagents. The diverse spectrum of chemicals used to probe for thiol function in biological reaction systems will not be discussed here, nor will the limits of their supposed specificity. Other sources should be consulted for information on these fascinating but overly extensive topics. It is probably important to point out, however, that a variety of types of chemicals are commonly employed including metal ions, organometallics, alkylation agents, and disulfide-breaking oxidants. Sometimes quite different results are achieved with different agents. Furthermore, their specificity for thiol functions is not complete. Thus evidence for thiol groups based on thiol-specific agents must always be viewed with caution. Only in those cases where there is strong corroborating evidence can indications for thiol function be considered secure.

Those proteins for which the thiol has no known specific function are not really of interest for the present discussion since no particular aspect of thiol chemistry can be related to the biological activity. Most of the emphasis will be reserved for those cases where the thiol group participation in the reaction is clearly established. Examples where thiol involvement is merely postulated will be mentioned only if they represent particularly interesting possibilities of thiol function.

I. Thioester enzyme Intermediates

Glyceraldehyde phosphate dehydrogenase probably holds the distinction of being the classic thiol enzyme in the minds of most biochemists. The thiol believed to be involved in the initial attachment of the aldehyde substrate as a thiohemiacetal. The enzyme-bound thiohemiacetal is then oxidized by NAD⁺ generating an enzyme-bound thioester. In more sophisticated proposals for this mechanism the nicotinamide cofactor interacts with the active centrum thiol as a charge transfer type of complex. This facilitates the reaction of the thiol with the carbonyl of the substrate. The thiol addition and the electron transfer to nicotinamide occur simultaneously so that the thiohemiacetal actually does not build up as true steady state intermediate.

The thioester of phosphoglyceric acid is generated as an enzyme-bound reaction intermediate. It possesses a highly negative free energy of hydrolysis and is capable of driving ATP synthesis. The free energy of interaction of a thiol with an aldehyde carbonyl followed by oxidation of the thiohemiacetal has provided the cell with a mechanism for trapping part of the energy released in the conversion of an aldehyde to an acid. The enzyme-bound thioester undergoes phosphorylation in the normal course of events, freeing the enzyme thiol and producing 1,3-diphosphoglyceric acid. This enzyme system is fully reversible and the thioester intermediate can be generated from the acyl phosphate.

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Glyceraldehyde phosphate dehydrogenase reaction

Treatment of the enzyme with acyl phosphate in the complete absence of reduced cofactor has allowed the thiol enzyme derivative to be prepared and separated from its reaction mixture. This in turn has permitted considerable characterization of the enzyme thiol. No special cofactor is involved. The thiol of a cysteine residue from the main peptide chain of the enzyme provides the reactive centre. This enzyme demonstrates that the acyl transfer role of thioesters in biological systems is not restricted to phosphopantetheine and dihydrodiol Epoxide derivatives. The reactions of the
enzyme thioester are analogous with the transacylations to phosphate and hydrate ion described previously (section III.C.3). Acyl transfer reactions to hydroxylamine, arsenate, methylmercaptan and even a nitrogen within the enzyme itself can be demonstrated with alylated glyceraldehyde phosphate dehydrogenase. These reactions probably have no biological significance but have been useful in substantiating and characterizing the thioester intermediate.

The thiol enzyme for which the most detailed mechanistic formulations have been proposed is papain. In this enzyme a cysteine thiol group appears to function in the same manner as the serine hydroxyl of other proteases and esterases. In the hydrolysis of proteins by this plant protease there is an intermediate formation of an acyl thiol, which is subsequently cleaved by water.

![Mechanism of papain proteolysis](image)

Imidazole from an enzyme histidine and possibly an enzyme carboxylate group are thought to participate in the reaction. X-ray crystal analysis of the protein has established that a cysteine at position 25 and a histidine at position 159 are so positioned that they can participate in a hydrogen bonded reactive centre. An aspartic acid at position 138 is also close enough to influence the reaction. The papain-active-centre thiol shows exceedingly rapid rates of reaction with certain thiol reagents. This suggests an enhanced nucleophilic character due to interaction with the imidazole and possibly other functional groupings in the reactive centre. The participation of a cysteine thiol in papain and other plant proteases must be considered unusual from the standpoint of thiol chemistry. Acyl transfer from amide nitrogen to sulphur is not considered thermodynamically reasonable, except under unusual circumstances. In this regard it is interesting to note that the active-centre serine hydroxyl of the

13. Biochemistry of the thiol group

bacterial protease, subtilisin, can be chemically converted to a thiol and still retain certain enzymatic activities. This stresses the critical importance of the proper juxtaposition of appropriate reactive groupings as opposed to the precise chemical attributes of any single functional group in enzymatic catalysis.

An intermediate formation of a thioester, facilitated by adjacent acid and base groups, has also been proposed as a general mechanism for glutamine-mediated amination reactions. The apparent function of glutamine in such reactions is to provide a source of unhydrated ammonia at the reactive centre. This is accomplished by hydrolysis of the amide with the following type of mechanism being suggested:

![Proposed reactions for ammonia generation from glutamine](image)

Thus thioacyl cysteines appear to participate in the catalytic function of diverse types of enzymes, even when the conservation of a high energy bond is not the prime consideration.

2. Persulphide enzyme intermediates

Rhodanase provides an example of a thiol enzyme of a somewhat different type. This enzyme, which is widely distributed throughout nature, catalyses the formation of thiocyanate from thiosulphate and cyanide. This reaction probably does not represent the true biological

Rhodanase reaction

\[
\text{H}_{2}\text{SO}_{3}^{+} + \text{CN}^{-} \rightarrow \text{HSCN} + \text{H}_{2}\text{O}
\]

action of the enzyme, although it could provide a system for the detoxification of cyanide. The reaction is more likely only a convenient means for the in vitro assay of some uncharacterized sulphur-transferring
system. The proposed mechanism involves an initial transfer of sulphur from the donor to an enzyme thiol group producing an enzyme per-
sulphide. The persulphide sulphur is then displaced by the acceptor-
regenerating enzyme thiol.

Persulphide enzyme mechanism for rhodanese

\[
\begin{align*}
\text{Enz-SH} & \xrightarrow{H^+} \text{Enz-S}^{-1} \xrightarrow{S_{2}O_{5}^{2-}} \text{Enz-S-S}^{-1} \xrightarrow{SO_{4}^{2-}} \\
\text{Enz-S-S}^{-1} & \xrightarrow{S_{2}O_{5}^{2-}} \text{Enz-S}^{-1} \xrightarrow{H^+} \text{Enz-SH}
\end{align*}
\]

Some doubt that enzyme persulphide per se exists in the enzyme intermediate has been indicated, but at least an enzyme-stabilized

\[
\text{equivalent of persulphide is generally accepted. A release of the}
\]

intermediate persulphide sulphur from the enzyme can be effected by

\[
\text{heat or trichloroacetic acid treatment.}
\]

The enzyme transferring sulphur from 3-mercaptopo pyruvate appears to

\[
\text{have a similar mechanism, involving a persulphide-like enzymatic inter-
}
\]

mediate. The possible role of this enzyme in transsulphuration from
cysteine has been discussed earlier.

\[
\begin{align*}
\text{Thiol pyruvate transsulphurase reaction}
\end{align*}
\]

In the presence of disulphide-reducing agents there is a production of

\[
\text{sulphide from persulphide enzyme intermediates. Dihydrolopoate (or more}
\]

likely a protein-bound form) may be a natural acceptor substrate for such

\[
\text{enzymes. Only one optical isomer reacted in the rhodanese system,
}
\]

suggesting the presence of a specific binding site. It was presumed that one

\[
\text{of the dihydrolopoate thios acted as the sulphur acceptor with a subsequent}
\]

release of sulphide through displacement by the adjacent thiol. Therefore

\[
\text{these enzymes may normally function in reductive desulphuration.}
\]

Alternatively, transsulphuration by way of the enzyme persulphide may be

\[
\text{the important biological process. It has been proposed that rhodanese,
}
\]

and by inference other enzyme persulphide transferases, may be the

\[
\begin{align*}
\text{immediate donor of the 'labile sulphide' for the biosynthesis of nonhaem iron}
\end{align*}
\]

\[
\text{proteins such as ferredoxin.}
\]

Evidence for the presence of a persulphide group in the active form of

\[
\text{xanthine oxidase has recently been presented, and a direct catalytic role}
\]

\[
\text{for the group is proposed. Thus protein persulphides may play a}
\]

\[
\text{significant functional group role in their own right.}
\]

\[
\begin{align*}
\text{3. Thiol-binding centres}
\end{align*}
\]

Another way thios can participate in enzyme reactions is by binding

\[
\text{substrates or coenzymes at the active site. A clear differentiation between}
\]

\[
\text{involvement in catalytic and binding functions is seldom possible, but a}
\]

\[
\text{binding role is presumed when protection of the critical thiol is afforded}
\]

\[
\text{by the presence of substrate and no specific catalytic role is suspected.}
\]

\[
\text{There are only a few proven examples of thiol substrate binding other than}
\]

\[
\text{those already discussed in which a precise catalytic role is also proposed.}
\]
The clearest examples of thiol-binding centres are those in which the linkage is covalent. Attachment of the haem group to the cytochrome c protein occurs through two cysteine thiol residues\textsuperscript{34}. The sulphhydryl add across the double bond of two vinyl side chains of the iron tetrapyrrole, providing thioether bridges between the protein and the prosthetic group.

The binding of a flavin prosthetic group to hepatic monamine oxidase has recently been reported to involve a thiol\textsuperscript{46}. FAD is linked as a thioether formed between a cysteine and a methyl substituent on the dimethyl isoalloxazine. While binding is generally conceived to be a reversible process and these cases must be viewed as extreme, they do provide clear examples of the general concept.

A frequently postulated binding role for thiols is in the attachment of metals to metalloproteins\textsuperscript{50}. The involvement of thiol ligands will influence the strength and specificity of metal-complexing centres and in this way could affect the structure and function of proteins in rather specific ways.

13. Biochemistry of the thiol group

Polythiol metal-binding sites will be discussed in section III.F.4, but single thiols acting in conjunction with oxygen and nitrogen ligands are also quite important.

Loss of titratable thiol in the presence of zinc and the magnitudes of the stability constants for a series of enzyme–metal complexes has implied a nitrogen-sulphur metal-binding centre in bovine carboxypeptidase. However, no cysteine side chains were found within the zinc coordination sphere on X-ray crystallographic analysis, casting considerable doubt on these conclusions\textsuperscript{52}. A thiol has also been implicated in metal binding by human carbonic anhydrase, but the complete lack of cysteine in the bovine enzyme makes this contention somewhat uncertain since zinc binding by both enzymes is very similar. Metallothiol centres may themselves act as trivalent sites. A metal ion bridge is thought to be involved in biguanidino coenzyme binding by alcohol dehydrogenase and there is evidence that the protein centre includes a thiol.

Most claims for thiol participation in binding are based on preservation of sulphhydril groups by the presence of the ligand or on lack of binding if thiol groups have been blocked. Unfortunately, it has become increasingly obvious that such evidence does not necessarily mean that the thiol is directly involved or even that it is near the binding site. Attachment of substrate can simply mask an otherwise uninvolved thiol, or can induce a conformational shift which alters thiol reactivity. Conversely, the integrity of distant thiol groups may be necessary for the proper binding configuration of the protein. Their derivatization could produce structural rearrangements which would eliminate binding and activity in distant parts of the molecule. In fact, certain enzyme activities can be enhanced by thiol substitution, implying that the thiol effect must be taking place away from the active centre. Early studies showed that substitution of sulphhydril groups on haemoglobin altered the nature of the oxygen binding and eliminated haem-haem interactions\textsuperscript{54}. This would now be explained as being due to alterations in subunit interaction since it is known that thiols are not in or near the oxygen-binding site\textsuperscript{55}.

4. Thiols and disulphides in protein structure

The most common thiol role is participation in the overall structural integrity of proteins. Except for the special case of the disulphide linkage this can be viewed as a rather nonspecific and passive function. This is not to imply that in any given circumstance that another amino acid side chain might serve as effectivly as cysteine or methionine, but rather to point out that these amino acids are more critical in their place than are any other in theirs. From an experimental standpoint there is one
special significance of the sulphhydril group in protein structure. It is the
case and specificity with which it can be modified. The list of enzymes which
have their activity influenced by thiol-specific reagents far exceeds the
number for which a defined role in binding or catalysis can be established.
In most of these cases it must be concluded that the thiol reagent sensitivity
represents the loss of some critical structural feature upon thiol modi-
fication.

It is also not surprising that quite contradictory effects can sometimes
be achieved with various thiol reagents since these introduce different
bulk, ionic charge or hydrogen-binding capabilities at the site of sub-
stitution.

While offering little information on the active structure of proteins,
modification of these 'structural' sulphhydril residues has been helpful to
the biochemist in many instances14. As examples one can cite the increasing
success of thiol reagents in dissociating subunit enzymes and releasing
untightly bound cofactors without destroying covalent linkages. When the
thiol blocking agent can subsequently be removed, as is the case with
organic mercurials, the reassembly of functioning units can sometimes be
achieved.

It is as the disulphide that the structural importance of the thiol in
proteins can best be appreciated16. Covalent disulphide bonds provide
bridges that are much stronger than the hydrophobic and hydrogen-
bonded interactions believed responsible for initial protein folding. The
real uniqueness of the thiol-disulphide structural system lies in the case
with which it may be formed, broken down and reformed under reasonable
biological conditions. The principal method for the making and breaking
of protein disulphides is by disulphide interchange. This process, as
mediated by glutathione, can be coupled to cellular redox systems by a
specific reduced nicotinamide coenzyme-disulphide reductase. Thus,
protein disulphide structure can be formed, be rearranged and broken up
by systems involving low molecular weight thiol-disulphide couples.

However, a major disulphide contribution to structures within the cell
is made unlikely by the observation that disulphide bonds are relatively
rare in intercellular proteins. In fact we have already discussed the possible
gle of glutathione in maintaining protein thiols in the reduced state. It is
really with proteins that operate outside the cell that one finds the great
importance of disulphide-stabilized structures. One can reasonably
rationalize this fact in two ways. Since the protein must operate without
the protective environment of the cell, random disulphide formation
would eventually occur. By initially fixing most thiols as disulphides in an active
configuration the chances for deleterious random disulphide formation
would be reduced. Another view would contend that extracellular proteins
must survive and function in a much more variable and hostile environment
than cellular enzymes. They therefore require greater rigidity and an
ability to function even if partially damaged. These attributes are afforded
by disulphide cross linking. Both explanations probably have some truth
with the inevitability of disulphide formation and the increase in structural
stability once formed contributing to the importance of this system. Since
many of the most abundant and best studied proteins are extracellular
many examples are known in which functional structure is dependent on
disulphide bridges. Only a few examples illustrating certain generalizations
will be discussed.

It is important to remember that the position of disulphide bonds
cannot be directly specified by the protein core and disulphide formation
must occur subsequent to the assembly of the peptide chain. There is now
strong evidence for the idea that the initial three-dimensional folding of a
protein is totally a consequence of the primary amino acid sequence. The
same is true for the association of subunits into functional complexes. It is
only after weak interactions have brought about a highly favoured con-
figuration that the disulphide formation occurs to 'lock in' the protein
structure. Disulphide cross linking does not create form, but only fixes
what was initially dictated by the linear peptide sequence and weak
bonding forces.

The exact nature of the oxidant for the normal biogenesis of disulphides
is uncertain. Low molecular weight protein disulphide-dithiol electron
transport carriers are implicated. The cytoskeletal localization of the
process is more certain. A membrane-bound microsomal enzyme which catalyses
a protein disulphide interchange is probably responsible for assembly of
disulphide-stabilized structures. This activity is most prevalent in those
cells which are producing and excreting disulphide-stabilized proteins.

The enzyme occupies a position on the microsomal membranes at or near
the site for ribosome binding. It is therefore directly available to act on the
newly assembled peptide chains. Assay of this enzyme depends on its
ability to reform the active, disulphide stabilized, structure of ribonuclease
from a randomly cross-linked material. Of the 105 possible disulphide
combinations, only one is proper and active. Actually this one 'correct'
structure can reform in reasonably high yield if oxidation conditions are
properly controlled. The microsomal disulphide interchange enzyme
facilitates the process by promoting rearrangement of inappropriate
disulphide patterns. The interchange capacity of the system is important
because it allows the newly formed protein to achieve its best and
presumably proper folding pattern even if some premature oxidation
would be reduced. Another view would contend that extracellular proteins
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disulphide patterns. The interchange capacity of the system is important
because it allows the newly formed protein to achieve its best and
presumably proper folding pattern even if some premature oxidation
might occur. It also seems reasonable to assume for the present that this same enzyme is responsible for the initial oxidation of the thiols on the newly synthesized protein. Since this probably involves an intramolecular disulfide exchange with a disulfide--dithiol redox carrier no new catalytic capacity need be involved. The exclusive association of this disulfide interchange activity with rough endoplasmic reticulum is consistent with the idea that disulfide proteins only occur extra-cellularly. These are the cellular structures believed responsible for assembly and vacuolization of excretory proteins.

It is also possible that disulfide bond formation and rearrangement occurs after excretion of the protein from its cell of synthesis in some cases. This would best account for assembly of very large sulphur-rich aggregates such as hair. Exact cross linking fidelity is probably not so critical in these cases and complete assembly of such large cross-linked meshworks within a cell is clearly impossible.

The most dramatic examples of the importance of disulfides for biological function are found among enzymes which are initially produced as inactive precursor proteins. Chain folding and disulfide bonding patterns reflect the primary peptide structure of the inactive zymogen molecule. Activation usually involves the cleavage of peptide bonds and sizeable peptide segments may be removed. The protein arrangement is no longer one that would form spontaneously. The maintenance of the active structure is completely dependent on the disulfide linkages.

Chymotrypsinogen, as synthesized by the pancreatic cells, is a single polypeptide chain which can maintain its native configuration if the disulfide links are reduced. Activation, by a series of peptide bond cleavages, eventually results in three separate polypeptide segments held together by disulfides, as indicated diagrammatically below. Destruction of the disulfide links now results in separation of subunits. Reassembly cannot occur and activity is completely and irreversibly lost.

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An example involving another familiar protein is the biosynthesis of insulin. This hormone is assembled as a continuous chain of 73 amino acids. Subsequent to folding and the establishment of disulfide bonds, a 22-amino-acid segment is removed from the centre of the protein. This provides the two standard disulfide cross-linked structure of the active molecule. Thus one general function which can clearly be assigned to disulfides is the maintenance of appropriate structure after secondary protein modifications have occurred.

A closely related role for disulfide bridging is in "freezing" subunit arrangements. The four peptide chains of the typical antibody molecule are held together in proper position by disulfide bonds. A vast variety of individual antibodies can coexist in the blood without any mixing of subunits. If the disulfide bonds holding the chains together are reduced, the proper type of subunit interaction can be maintained under certain experimental conditions. However an interchange of subunits can now occur. The presence of the disulfide bridges in the native structure ensures that subunits forming the two identical and highly specific binding sites will remain together in the general circulation. While such subunit assemnies must be formed by spontaneous and reversible interactions at their point of synthesis, they can be prevented from undergoing subsequent rearrangement by disulfide bonding.

Larger disulfide-linked aggregates are found in hair and related animal keratins. In fact the cardinal characteristic of wool, nails, horns, feathers, etc. is their high sulphur content. The basic keratin system is believed to be composed of two protein subtypes. One type forms filamentous fibrils which are wound arrays of protein strands. Differing arrangements of fibrils and patterns of protein folding distinguish the α and β-keratins. Fibril proteins are rather low in cysteine content and hydrogen bonding and hydrophobic interactions impart their strong fibre-forming tendencies. The keratin fibrils are embedded in a protein matrix having no recognisable order. The matrix proteins are extremely rich in cysteine and also enriched in serine, threonine and proline. The high sulphur proteins are extensively cystinked to each other, and to the sulphur-poor fibrous constituents through disulfide bonds. The sulphur-rich fraction probably does not represent a single protein but rather a mixture of related proteins. The nature of this mixture and the amounts of the individual constituents vary with the type of structure formed (hair, feather, horn, etc.) and to some extent with the diet of the animal. Newly synthesized hair proteins are actually soluble, but by 4 to 6 hours they can no longer be extracted into water and by 18 to 20 hours much of the material cannot even be solubilized by urea. This suggests that assembly
of the crosslinked disulphide meshwork occurs long after the initial peptide assembly is completed. The high sulphur proteins have been extremely hard to study because of the difficulty in dissolving them without modifying backbone structures. The biochemistry of this complex system is only beginning to be unravelled, principally by chemists interested in modification of the basic structures for textile or cosmetic application. However, there is little doubt that disulphide bonds constitute the principal structural feature of hair and other keratin assemblies.

Another area in which a critical functional role for protein disulphides has been suggested is in the action of the polypeptide hormones[2]. A small cyclic disulphide loop is a common feature in many of these molecules. This has drawn attention as a possible site for hormone binding to the target cell. The greatest amount of evidence supporting this idea concerns the action of antidiuretic hormone and vasopressin. The hormone is bound in the kidney by a thiol-cleavable bond, and no such interaction occurs with other tissues. Thiol groups present in the loop and reduction of the hormone's disulphide causes inactivation. Diuretic effects can be achieved by a wide variety of compounds which share an ability to react with thiols. The idea of a disulphide loop being a site for interaction is a target thiol by disulphide interchange is attractive and may prove to be a generally significant disulphide function.

Almost all proteins contain some cysteine, but in only a minority of those can the thiol group be assigned a definite role. Nonetheless the list of thiol functions in proteins is long and clearly exemplifies the importance of this group in biological systems.

**F. Dithiol and Polythiol Proteins[2]**

A special type of thiol functional group can be achieved by constraining more than one thiol group into a polythiol centre. An example has already been considered, lipoyl acid, where the presence of two thiols on the same carbon chain facilitates a dithiol-disulphide redox system. A similar functional centre can be created by the close positioning of two cysteine thiols through appropriate secondary and tertiary folding of a polypeptide chain. Inhibition by arsenite or by cadmium has been considered to be indicative of a dithiol involvement in enzyme action. Unfortunately, a lack of knowledge about the precise chemical specificity of these dithiol reagents has left most suggestions of an enzyme dithiol in doubt. Several examples have now been supported by direct thiol assay or active site isolation, strengthening the dithiol enzyme concept. Recent studies on dithiol criteria should increase confidence in specific reagents when used appropriately[2], but also emphasize the deficiencies in the way such criteria have often been applied. The division between mono- and dithiol functions is quite arbitrary. However, it does emphasize that something more than just a summation of two independent groups is achieved by making it possible for them to act in concert.

**1. Thioredoxins[4]**

A dithiol protein, thioredoxin, functions in the transport of electrons from reduced nicotinamide adenine dinucleotide phosphate (NADPH) to ribonucleotides in the biosynthesis of 2'-deoxyribonucleotides. A thio-redoxin type carrier is involved in both vitamin B12 dependent and independent type systems. Thioredoxin is frequently described as a poly-peptide cofactor rather than an enzyme because its activity is not destroyed by heating, and the molecular weight is relatively low (approximately 12,000). As such it is only one example of a class of small proteins carrying reactive centres which have been recognized in recent years.

Thioredoxin from *E. coli* contains only two cysteine residues which are linked as a disulphide in the oxidized form of the molecule. These residues are separated by two intervening amino acids, glycine and proline, providing a small polypeptide as the functional centre of the molecule. When two thioredoxins from yeast and the one from *E. coli* were compared, the amino acid sequences were identical in the immediate vicinity of the disulphide-dithiol centre, and quite similar for a considerable distance beyond.
The thioredoxins appear to have a highly specific relationship with the enzyme carrying out their reduction. Yeast thioredoxin for example is not reduced by the thioredoxin reductase from *E. coli*. In contrast reduced thioredoxin is a good general disulphide reductant. In combination with its reductase a disulphide reductase system is formed which is capable of reducing apoB, oxidized glutathione and other similar structures. In these cases the thioredoxin-disulphide redox system does not appear to require additional enzymatic components.

Reducing equivalents from a given thioredoxin can be donated to a variety of reductase enzymes. They are not specific for the nucleotide reductase or for enzymes from the same organism. Reduced yeast thioredoxin will serve as reductant for methionine sulfoxide reductase, sulphate reductase and the *E. coli* nucleoside diphosphate reductase. Heat-stable protein cofactors are known to be involved in each of these systems.

The sulphate reductase factor which has already been mentioned was the first of these polypeptide disulphide-disulphide cofactors to be recognized. In this case the reduction of PAPS to PAP and sulphate was shown possible with a disulphide reductant such as dihydrolipoate or with NADPH and two protein components. One of the protein factors was not inactivated by heating. Incubation of the two protein factors with NADPH generated

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approximately two moles of thiol associated with the heat-stable component. The heat-labile reductase enzyme could not of itself reduce lipoamide or other disulphides.

There is a growing literature on similar electron transport systems and it seems likely that such small protein disulphide-dithiol factors will be found to have a general biological role. It seems reasonable to refer to all cofactors of this type as thioredoxins, emphasizing that this is a class of compounds of similar but not identical structures which can show a high degree of specificity for a given organism or reaction.

2. Dithiol-flavin enzymes

Disulphide-dithiol redox centres are also found in a number of high molecular weight electron-transporting enzymes. Most extensively studied of these are the group of flavoproteins which carry electrons between disulphide cofactors and nicotinamide nucleotides. These include lipoyl dehydrogenase, glutathione reductase and the thioredoxin reductase. These enzymes are unique in utilizing a combined flavin-disulphide centre for the oxidation of reduced nicotinamide coenzyme. Each reactive enzyme centre is composed of a disulphide formed from two cysteine sulphhydrils and a tightly bound flavin adenine dinucleotide (FAD). Upon reduction by reduced nicotinamide cofactor the two-electron equivalents are shared between the dithiol and the flavin prosthetic groups. A fully reduced four-electron enzyme containing a dithiol and FADH2 does not occur during the normal catalytic cycle. The reduced enzyme site is envisaged as some sort of mixed free radical with one electron on sulphur and the other in the flavin system. This is not a conventional flavin semi-quinone and the possibility of a charge transfer complex between the active elements of the redox centre has been proposed. Complete electron transfer to the dithiol centre and reduction of the disulphide substrate through a disulphide interexchange sequence completes the catalytic cycle.

These dithiol-flavoprotein transport electrons over a redox potential range considerably more reducing than is associated with free flavin and most other types of flavin enzymes. By acting in conjunction with the protein dithiol centre, flavin is transformed into a much more powerful reducing agent.

The sequences of the dithiol active centres of two enzymes of this type from *E. coli* have recently been reported. The lipoyl dehydrogenase dithiol peptide has four amino acids intervening between the two cysteines and is rich in hydrophobic amino acids. This has been taken as a reflection of a highly hydrophobic pocket at the catalytic centre, as had been implicated by model substrate studies. The thioredoxin reductase dithiol
centre has only two residues between the cysteines. The dihthiol peptide of thioredoxin itself is the same size as that of its reductase but the sequences are quite distinct.

Amino acid sequences of dihthiol-dissulphide centres

Lipoal dehydrogenase: \[ \text{val-cys-leu-asn-val-gly-cys-ilu-pro-ser} \]

Thioredoxin reductase: \[ \text{ala-cys-met-his-lys-ser-gly-glu} \]

Amino acid sequences of the known dihthiol-dissulphide reduct centres provide little hope that any specific peptide structure will be found associated with this particular activity. Even the size of the dissulphide ring systems vary, so one must conclude that it is the overall folding of the protein which is responsible for the correct juxtaposition of the functional electrons.

3. Other dihthiol enzymes

The presence of dihthiol centres at the active sites of a variety of additional enzymes has been proposed on the basis of inhibition studies. For example, the investigations on aldehyde dehydrogenase represent one of the earliest uses of arsenate as a dihthiol diagnostic reagent. The overall data strongly support the presence of a dihthiol site as a general feature of aldehyde oxidases, but its functional role has not been established. Because of the ease of thioacetal formation a dihthiol would make a chemically attractive aldehyde binding site. The failure to find lipoic acid as a part of these enzymes makes it likely that the dihthiol centre arises from the juxtaposition of cysteine thiol residues.

The light-emitting luciferase system from fireflies has been extensively studied and there is strong support for a functional dihthiol. The extensive thiol involvement in fatty acid biosynthesis has already been indicated, and some enzyme components have characteristics expected of dihthiol centres. There are many additional systems where there is some evidence for dihthiol involvement, but proof for a clear functional role of a dihthiol is lacking.

4. Polysihol metal-binding centres

A polysihol centre can serve as a highly specific metal ion binding site. For example, polysihol ligands have come to be thought of as relatively selective for cadmium. Actually a number of important metal ions including mercury, zinc, lead, copper and iron bind quite well at such centres. The order of relative binding affinities for polysihol chelates is different from those involving nitrogen or oxygen ligands. Cadmium, mercury and to a lesser degree zinc form the most avid complexes.

The polysihol metal complexes can provide functional centres with unique properties, an example being the nonhaem iron proteins to be discussed in the next section. They also are capable of serving rather distinctive structural roles, since the binding of metals can influence the overall configuration of the protein. Studies on cadmium and zinc binding to thiol-substituted dextran polymers showed that these metals can actually organize polysihol-binding centres and might appreciably change the folding of a polypeptide chain.

Metals with a high affinity for multihiol coordination thus could serve to generate and stabilize particular protein conformations. Several examples of structurally important metal polysihol interaction have recently appeared. E. coli aspartate transcarbamylase, the subject of extensive investigations concerned with mechanisms for enzyme control, has been shown to contain zinc. Zinc binding appears to occur at a dihthiol centre. The metal is required to maintain the regulatory subunit in a configuration suitable for binding to the catalytic subunit. Histidine ammonia-lyase is dependent on cadmium when enzyme dihthiols have been reduced, and this has been shown to be due to the formation of a metallo-dihthiol complex. The reactive thiolso appear to be contributed by separate subunits and the complex formation establishes an appropriate interaction of the individual components. In the oxidized enzyme, these thiols are linked as a disulphide and this form of metal ion activation is
not required. In bovine superoxide dismutase the proper conformation for the binding of an active site copper ion is maintained by a distinct zinc-binding centre. Two sulphhydryl groups per zinc are uncovered on removal of this metal implicating a dithiol-binding site. Metal binding at specific dithiol or polythiol sites could constitute a general mechanism for stabilizing protein conformation or facilitating interaction between subunits.

A cadmium-rich protein, metallothionein, has been isolated from kidney and other tissues. It is a small protein of about 7000 molecular weight and is exceedingly rich in thiol groups. One out of every four to five amino acids is cysteine, and three thiols are involved in each cadmium-binding site. The biological importance of metallothionein is known. The simplest role envisaged is scavenging toxic metal ions which might otherwise interfere with critical enzymatic processes. Metallothionein from kidneys of patients treated with mercurial diuretics contained increased amounts of mercury which could inhibit a toxic ion-sequestering action of the protein. Another interpretation might be that metallothionein is an undegradable and unexcretable end product. It might have been derived from a thiol rich centre of a protein(s) which had been inactivated by cadmium. The isolated material would merely be the accumulating debris of toxic insult. A somewhat intermediate viewpoint would ascribe a normal trace metal-binding role to metallothionein. A similar constituent has been isolated from liver and contains primarily zinc and copper. If the protein's normal function was the storage or mobilization of these two normal trace metals, cadmium would interfere because of its avid binding. This would eventually lead to inactive cadmium-(or mercury-) saturated forms such as those isolated from the kidney. Whatever its role, metallothionein is an excellent example of polythiol serving as selective metal-binding sites.

5. Iron-sulphur redox proteins

One rapidly advancing area of thiol biochemistry involves a group of iron-sulphur redox proteins, most commonly referred to as the nonhaem iron proteins. This designation derives from the fact that more iron was present in electron transport complexes than could be accounted for by the haem content. In the investigation of bacterial nitrogen fixation a low molecular weight iron containing protein was isolated which functioned as an electron transport carrier. This was named ferredoxin. An unusual characteristic was that when the protein was treated with acid to release iron, hydrogen sulphide was also produced. A component of the photosynthetic nicotinamide coenzyme reductase system was recognized as having similar properties and has come to be referred to as plant ferredoxin. Adrenodoxin, putidaredoxin, rubredoxin and high potential iron-protein are additional nonhaem iron electron transport proteins of a similar character. A variety of high molecular weight electron transporting enzymes also have been found to have nonhaem iron centres. A triad of characteristics has come to be associated: (1) a tightly bound iron, not accountable for as haem iron; (2) an unusual e.p.r. signal in the vicinity of G = 1.96, not characteristic of typical iron chelates; and (3) the release of iron on acidification accompanied by the unmasking of protein thiol groups and the generation of hydrogen sulphide. While each of these characteristics is not always demonstrable, they have served to delineate a heretofore unrecognized redox centre of wide distribution.

An intense effort by physicists, physical chemists, biochemists, inorganic chemists and X-ray crystallographers has now defined the common attribute of the nonhaem iron proteins. It is an iron centre tetrahedrally coordinated by four sulphur ligands. A number of variations within this theme are recognized. The simplest case is found to be rubredoxin from Clostridium pasteurianum, a 6000 molecular weight protein whose exact electron transport function is unknown.

A single iron atom is bound by four cysteine thiolates or a single labile sulphur being involved. A detailed crystallographic analysis of this molecule has been carried out, the general features of which are indicated below.

Iron binding site of rubredoxin
The peptide chain can be roughly described as a bent hairpin. The iron-binding centre consists of two small diethyl peptide segments. These diethyl centres are quite distant in the linear peptide sequence occurring in the end halves of the two legs of the hairpin. The peptide folding transforms these into a compact tetrahedral iron-binding centre. While this simplified description does great injustice to the details of the X-ray structure analysis, it serves to illustrate the critical features of the metal-binding centre. It also draws attention to a possible relationship of the iron-sulphur proteins to the disulphide-dithiol redox carriers considered previously.

The more typical iron-sulphur centre contains two iron atoms, two sulphones and four cysteine sulphurs. It is believed that each iron is coordinated by two sulphurs from cysteine and two from sulphide, with each of the sulphides binding both iron atom. The general structure of the two-iron redox centre is depicted below.

![Proposed structure of a two-iron centre](image)

Another arrangement for an iron-sulphur redox centre is found in HiPIP (high potential iron-protein) from Chromatium and the bacterial ferredoxin[2]. These structures have been elegantly established by X-ray crystallography. The redox centre contains four iron atoms, four sulphone sulphurs and four cysteine sulphurs from the protein. Each iron is again surrounded by four sulphone ligands in an approximate tetrahedral arrangement, but each sulphone sulphur now interacts with three iron atoms. The iron is bound to three sulphones and one cysteine sulphur. The iron-sulphur array is roughly tetrahedral with the corners being either a sulphide sulphur or an iron linked to the protein shell.

Bacterial ferredoxin actually contains eight iron and eight labile sulphurs, but these are arranged as two distinct four-iron clusters.

Iron-sulphur proteins participate as one electron carriers, even those with reactive sites containing four iron atoms. Each centre rather than each iron must be counted as an electron transport unit. Only the bacterial ferredoxin acts as a two-electron acceptor and even in this case it is really two Fe$_3$S$_4$ one-electron centres acting independently.

**Arrangement of the Fe$_3$S$_4$ nonhaem iron centre**

Iron-sulphur prosthetic groups of a similar nature are also implicated in more complex higher molecular weight electron-transferring enzymes. Examples such as xanthine oxidase have been extensively examined and the type of iron-sulphur centre seems analogous to those in the lower molecular weight redoxins. For the present at least the nonhaem iron centres of complex electron transport chains can also be envisaged as enzyme polypeptide sites generated by the juxtaposition of two protein diethyl sulphur sequences. Coordinated within this tetrahedral cavity is an iron-sulphide core.

What is it about divalent sulphur which results in such unique and biologically useful complexes of iron? The special feature of these prosthetic groups is that the iron is held in what approximates a tetrahedral complex, while all common iron complexes with oxygen and nitrogen ligands are octahedral. Tetrahedral iron-oxygen complexes are known, but only as high molecular weight networks where there seems to be a requirement for a condensed packing. One critical difference in the sulphur ligand could simply be effective size. Since the outer orbitals of sulphur are occupied it might be difficult to pack six ligands around an iron. The less crowded tetrahedral arrangement would therefore be favoured. The capacity of sulphur for expansion of the valence shell also might be considered of importance for moving electrons into and out of the complex and for delocalizing electrons in the reduced complex. Intense research activity currently focused on these iron-sulphur proteins ensures that our understanding of this aspect of thiol biochemistry will improve rapidly.

The variety of oxidation-reduction carriers having a diethyl centre as a part of their structure suggests a possible evolutionary relationship.
Lithio-disulphide redox roles in primitive systems would have favoured the development of dicysteiny1 peptides, restraining two thiols in close proximity. Thoredoxin type molecules would have evolved from these small prototype diol peptides. Similar centres would also have developed as parts of more complex enzymes. The binding of a flavin coenzyme near a diolh centre could eventually have produced the combined disulphide–flavoprotein centre with its special redox properties.

The propensity for diols to bind metals would have led to a further evolution of function. Metal-protein bridging may have preceded disulphides as a method of holding proteins in effective organization particularly before the oxidizing environment developed. Iron–sulphur complexes had redox potentials different from other iron carriers and the diol. They eventually developed into the powerful reducing system of the ferredoxins by combining two diol ligands around one iron. Simple iron sulphide aggregates were also incorporated giving rise to the two iron-two sulphide and four iron-four sulphide variants. As more iron sulphur atoms condensed into a single site, the redox possibilities increased and the iron–sulphur centre became involved in the variety of different redox roles seen today.

Cytochrome C might also have arisen from a disulphide redox protein as the heme-binding centre is nothing more than a disulphide peptide. While such speculations on the evolution of biomolecules are only mental games, they point out how the diolh can be modified to carry out a variety of related functions.

**IV. CONCLUSION**

The thiol and its simple derivatives represent an exceedingly important and versatile functional centre in biological molecules. A number of basic metabolic processes are dependent on the particular chemical characteristics of thiol derivatives. It is difficult to imagine how metabolism might have evolved without the rich supply of thiols which probably were available in the 'primordial soup'. Actually thiols or their derivatives participate in so many biological reactions that one is amazed to find they have no indispensable role in the central dogma of molecular biology. Self-replication, transcription, and translation rely only peripherally on thiols. It is in the realms of catalysis and structure, the domains of the enzymologist and protein physical chemist, that the thiol is of central importance.

Thiols have been of foremost importance in the development of the functional group concept in biochemistry. Because of its ease of manipulation the thiol, particularly that of glutathione, has fascinated the biochemist. All manner of roles have been suggested but most of these have not been proven, and many are totally forgotten. Still the chemical approach to biochemistry and the attempt to explain how biological reactions occur in terms of model organic systems had much of its initial success in explaining thiol-mediated reactions. The sulphur of the thiester provides activation for acyl transfer, and an intermediate in amide and ester hydrolysis. It facilitates α-hydrogen dissociation and provides a mechanism for carbon-carbon condensation and chain modifications. Reduction of carboxylic acids is proceed by thiol transfer. Energy released by oxidative metabolism is trapped as a thioester, a form suitable for driving the synthesis of ATP.

The disulphides and certain thiol metal ion derivatives serve as carriers of electrons and function in biological redox reactions of diverse types. Thiols and their metal derivatives provide strong binding centres for substrates and cofactors. They often help maintain proper protein conformation and subunit interactions. The disulphides of extracellular proteins are of profound structural importance. They make relatively permanent the arrangements of peptide chains initially established by weaker bonding forces. Often they become totally responsible for holding the active structure together, particularly where covalent modification of the protein chain is involved in activation. Animal keratins are particularly rich in sulphur, deriving their inertness from extensive disulphide cross linking.

The sulphonium ion serves as an alkylating reagent. The bulk of biological methylations proceed through S-adenosyl methionine. Persulphides, thiophosphates, thiosynergates and thiosulphonate derivatives have been postulated to have significant functional roles. The plant kingdom in particular is full of strange thiols and thiol derivatives which impart characteristic tastes and smells. Their functions are unknown, but could range from insect attractant to water repellent. Vitamins such as thiamine and biotin have heterocyclic sulphur which can be viewed as thiol derivatives. Even the simplest thiol of all, sulphide, finds a critical biochemical involvement in the iron–sulphur electron transport centres.

Thiols provide the living systems with a link to their genesis in a reducing environment. Glutathione helps maintain the cellular interior in a state in which enzyme activities evolved in the absence of oxygen can still function. Protection from all sorts of injurious agents, detoxification and anti-radiation roles can be added to complete the listing of thiol functions in biological systems. The intense fascination of the biochemist with the thiol functional group can certainly be appreciated.
REFERENCES


The aim of this chapter is to present a general coverage of the biochemistry of thiosulfate rather than a review of recent advances. As such it is a departure from the usual coverage of material in this volume. The biochemical literature encompassed by this subject is immense, and many excellent reviews, monographs and symposia have been devoted to certain aspects of the topic. If possible referencing has been restricted to these secondary sources, since it was presumed that an interested reader would prefer to see these before proceeding to original material. It has of course been necessary to cite also primary literature when no suitable secondary source could be found. The article cited, however, is usually one of the most recent in that area and not necessarily the most pertinent to the subject being discussed. No attempt was made to include a comprehensive coverage of even the recent literature, but often original papers have been cited in order to provide a reasonable point of entry to the literature of rapidly expanding areas. This heavy reliance on secondary sources and the desire to present the material in a generalized form as possible have done great injustice to original data and to its original interpretations in many cases. It is also realized that many excellent papers and ideas have been ignored or completely missed. While this is regrettable, the scope of the subject probably makes it inevitable, and only a simple apology can be offered.

Arvan L. Fluharty


13. Biochemistry of the thiol group


CHAPTER 14

Protection of the thiol group

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I. INTRODUCTION

The thiol group readily undergoes a variety of chemical reactions (e.g., oxidation, alkylation, acylation), so there is a need to protect it while other sites in the molecule are undergoing chemical changes. This can be done by converting the thiol group to a derivative which is stable under the reaction conditions to be employed, and from which the thiol group can be regenerated without affecting the rest of the molecule.

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The great interest in thiol-protecting groups is due mainly to the significant development in the chemical synthesis of peptides, polypeptides, and proteins. It is important to note that only one kind of a protecting group is needed to protect the various cysteine thiols during a protein synthesis. This results from the spontaneous refolding of proteins which takes place upon reoxidation of their sulphydryl groups produced by reduction of the protein with mercaptoethanol. This phenomenon was observed first with ribonuclease where the reoxidized molecule retained its full biological activity and extended to a large number of proteins (e.g., insulin, lysozyme).

We shall try in this chapter to concentrate on the processes involved in the formation and deblocking of various types of protecting groups.

II. DISULPHIDES AS A PROTECTING GROUP

Disulphides are much less prone to participate in organic reactions (e.g., oxidation, acylation, acylation) than the corresponding free thiol, and as such could serve as a protection for the thiol group. Furthermore, in some cases the removal of some protecting groups results in the formation of the disulphine primary (e.g., sections III. B.A.; Y. A.C. which, later on, is reduced to the free thiol.

Disulphides are obtained by oxidation of the corresponding thiols, by a variety of reagents, e.g., hydrogen peroxide, iodine, bromine, hypochlorites, ferric chloride, nitrous oxide, sulphoxyl chloride, diethyl azocarboxylate (1), N-bromosuccinimide, tetrabromomethane, peroxy-EDOC—N=O=COOH (1)

The free thiols are obtained from the disulphides by reduction, which again may be carried out by a large variety of reagents, e.g., tin and acid, sodium in xylene, ether or liquid ammonia, lithium aluminium hydride, sodium borohydride, sodium dithionate and various organic thiols. The most widely used thiols are thioglycolic acid and mercaptoethanol. Of special interest among the thiol used is dithioerythritol (2), which is a powerful reducing agent and reduces disulphides in much lower concentration than other mercaptans (e.g., mercaptoethanol) presumably due to the formation of a stable six-member ring containing a disulphide bond. Disulphides could also be reduced to free thiols by means of electrolytic reduction as well as by water soluble phosphines (e.g., trihydroxymethylphosphine, tricarboxymethylphosphine) which were recently used for disulphide cleavage in proteins.

14. Protection of the thiol group

\[
\begin{align*}
R-S-S-R + HSCH_2-CHOH-CHOH-CH_3SH & \rightarrow RSH + \text{CH}_2-\text{CHOH}-\text{CHOH}-\text{CH}_3SH \\
& \rightarrow RSH + \text{CH}_2-\text{CHOH} \quad \text{(1)}
\end{align*}
\]

A much more detailed discussion of the reduction of disulphides to mercaptans is given in Chapter 4 on the preparation of thiols.

III. THIOETHERS

Simple saturated aliphatic thioethers are generally not easily cleaved to yield the free thiol. However, there are some exceptions in which the alkyl radical of an alkyl-phenyl-thioether is cleaved by means of sodium in liquid ammonia, lithium in dimethylamine or lithium in monomethyamine to give the corresponding thiol (See Chapter 8).

2,4-Dinitrophenyl-thioethers are cleaved under very mild conditions ("thiolysis" of the thioether), with mercaptoethanol at pH 8. The derivatives are obtained by reacting the thiol with 2,4-dinitrofluorobenzene in presence of base.

\[
\begin{align*}
\text{NO}_2-\text{C} = \text{O} + \text{HSR} & \rightarrow \text{NO}_2-\text{S} + \text{HF} \\
\text{NO}_2-\text{C} = \text{O} + \text{CH}_2\text{CH}_2\text{SH} & \rightarrow \text{NO}_2-\text{S} + \text{CH}_2\text{CH}_2\text{SH}
\end{align*}
\]

A. Benzyl Derivatives

The best known, and most widely used sulphydryl protecting group is the benzyl group. Benzylation takes place by reacting benzyl chloride in the presence of base with the thiol in aqueous or non-aqueous media (reaction 4; R = H)\(^{15,16}\). The reaction could take place also with the sodium
mercaptide using liquid ammonia as solvent. The protecting group is removed by reductive cleavage with sodium in liquid ammonia. In cases in which the benzylic thioether is insoluble in liquid ammonia, reductive cleavage can be achieved by using sodium in boiling butanol or sodium in boiling ethanol. It is of importance to note that sometimes desulfurization occurs during the cleavage with sodium in liquid ammonia.

Due to the large lowering of the Pt or Pd catalyst efficiency caused by the sulphur which is present in a thioether form, the reductive cleavage of the benzyl group from the thioether cannot be achieved by catalytic hydrogenation. It has been shown that sufficient catalyst efficiency is retained for the reductive cleavage of the p-nitrobenzyl group, presumably due to the labilization of the CH₃–S bond by the strong inductive effect of the nitro group. The p-nitrobenzyl protecting group, which is introduced by reacting p-nitrobenzyl chloride with the thiol (reaction 4; R = NO₂), is removed by hydrogenation under atmospheric pressure, using 10% Pd on charcoal as a catalyst. It has been shown that this reaction is not a general one and it does not take an unequivocal course since e.g. N-p-nitrobenzyl-L-cysteine gives S-p-nitrobenzyl cysteine and similarly benzoylcarbonyl-S-p-nitrobenzyl-cysteinylglycine gives benzoylcarbonyl-S-p-aminovaleryl-cysteinylglycine. Recently it has been shown that the p-aminovaleryl group could be cleaved from the thioether by using 10% HgSO₄ solution in 5% H₂SO₄ (Hopkin's reagent). Thus the p-nitrobenzyl group could be removed in a two-step reaction involving first reduction to the corresponding p-aminovaleryl derivative and then removal of the p-aminovaleryl group by acidic H₂SO₄ solution.

While the benzyl protecting group is stable towards acidic cleavage under normal conditions, introduction of a methoxy group at the p position will increase its tendency to acidic cleavage. Thus the p-methoxybenzyl group which is introduced in the usual manner (reaction 4; R = OCH₃) is removed by means of trifluoroacetic acid or anhydrous hydrogen fluoride.

B. Diphenylmethyl Derivatives

Reaction of thiol with diphenylmethyl chloride gives the diphenylmethyl (or benzhydryl) thioether. It has been shown that the thioether could be obtained in high yield by reacting the thiol with diphenylmethanol in the presence of BF₃ etherate. The diphenylmethyl protecting group is removed either by trifluoroacetic acid or via reductive cleavage using sodium in liquid ammonia. The diphenylmethyl thioether could also be cleaved by thiocyanogen using trifluoroacetic acid-acetic acid as a solvent. One pathway for this reaction may be via formation of a sulphonium salt intermediate (3) which can eject a stabilized carboxonium ion and sulphonylthiocyanate, the latter reacting further with another molecule of thioether or with free thiol to yield the disulphide (reaction 5). The formation of free thiothiol complexes from sulphonylthiocyanates directly or via the disulphide is discussed in Chapter 4. An alternative possibility is that the protecting group is split in the acidic solvent and the free thiol thus formed reacts with thiocyanogen to give sulphynitrilcyclohexane (reaction 6).

\[
\begin{align*}
R-S-CH\left(C_6H_5\right)_2 + (CNS)_2 & \rightarrow R-S-SCN \\
R-S-SCN & \rightarrow R-S-S-CH\left(C_6H_5\right)_2 (5) \\
R-S-CH\left(C_6H_5\right)_2 + COOH(CH_2)COOH & \rightarrow R-S-SCN (CNS) \rightarrow R-S-S-CH\left(C_6H_5\right)_2 (6)
\end{align*}
\]

Another protecting group which could be included in this class is a thioether obtained by reacting the thiol with m-nitrobenzalacetophenone in the presence of picric acid (reaction 7), the protecting group is removed by treatment with basic lead acetate. This group is used to protect the sulphenyl moiety of thiophenol and substituted thiophenols during electrophilic substitution reactions on the benzene rings.

C. Triphenylmethyl Derivatives

The triphenylmethyl (trityl) derivatives are obtained by reacting the appropriate thiol with triphenylmethyl chloride or with triphenyl
methanol and BF₃ etherate. Similarly to the diphenylmethyl thioether, the triphenylmethyl thioether is cleaved by sodium in liquid ammonia to give the free thiol, however, contrary to the diphenylmethyl thioether, the triphenylmethyl group could also be cleaved from the thioether by heavy metal ions. Moreover, trityl thiocarboxylic acids are more susceptible to acid hydrolysis as well as to thiocyanogen oxidation than the corresponding diphenylmethyl derivatives.

Although the removal of the triphenylmethyl group by trifluoroacetic acid and hydrogen chloride in chloroform is reported, there are cases in which acids cleavage (e.g. by means of trifluoroacetic acid, hydrobromic acid in glacial acetic acid, p-toluene sulphonic acid) indeed removed the protecting group but the product obtained did not possess any free thioether group. It seems that the best acidic reagent to use is hydrochloric acid in aqueous acetic acid. The heavy metal ions used for the removal of the triphenylmethyl group from the thioether are Ag⁺ and Hg²⁺. Initially, methanolic silver nitrate solution in the presence of pyridine was used. Later it has been shown that there are cases in which better cleavage yields are obtained by using mercuric acetate, in some cases silver nitrate gives the best results and in some cases both reagents give about the same yields. It seems that the cleavage yield depends upon the whole molecule in question, and the metal of choice could be found only experimentally. The triphenylmethyl moiety is removed from the thioether very easily by oxidation with thiocyanogen in the presence of sodium acetate. The sulphenyldisulphane is obtained reacts with free thiol or with another molecule of the thioether to form unsymmetrical or symmetrical disulphides which can be reduced later to the free thiol.

The removal of the triphenylmethyl group by the thiocyanogen is so easy that it can even be removed in the presence of a diphenylmethyl thioether, without any cleavage of the latter compound.

### D. Picolyti Derivatives

Contrary to catalytic hydrogenation which usually fails in the presence of thioic or thioethers, electrolytic reduction at a mercury cathode takes place without difficulty. Among the thioethers which could be cleaved by electrolytic reduction are the 4-picolyti thioethers. These derivatives are obtained by reacting the free thiol with freshly distilled 4-picoly chloride in the presence of base. The thioether is completely stable towards acidic cleavage, and no cleavage could be detected after its storage for 7 days in trifluoroacetic acid or in hydrogen bromide in acetic acid. The protecting group could be removed by electrolytic reduction at a mercury cathode in 0.5N sulphuric acid solution. This protecting group was recently used in the synthesis of 1-cystinyl-bis-glycine.

### E. Acetamidothethyl Derivatives

The acetamidothethyl thioether is obtained by reacting a 10⁵% excess of acetamidomethanol (4) with the thiol at pH 0.5. The protecting group is very stable in the pH range of 0–13 as well as towards concentrated strong acids (e.g. trifluoroacetic acid, hydrogen bromide in glacial acetic acid, sulphuric hydrofluoric acid). It is removed from the thioether by using two equivalents of mercuric ions at pH 4.

In the case of cysteine (5) the product obtained is contaminated by cysteine and also by thiocarbamic carboxylic acid (obtained by reaction of cysteine and formaldehyde, the latter arising from hydrolytic decomposition of acetamidomethanol, see section VI). However, the product could easily be purified by using ion exchange columns. On the other hand, anhydrous conditions should avoid the decomposition of the acetamidomethanol and indeed a reaction using hydrogen fluoride as a solvent results in quantitative yield of the pure product.

An elegant method for the cleavage of the protecting group has been discussed recently. It is based on the observation that sulphenyl halides are reacting with unsymmetrical thioethers giving disulphides among other products, depending upon the structure of the thioethers. Reaction of 2-nitrophenylsulphonyl chloride (NPSCI) with acetamidomethyl cysteine residue would form a thioulesulphonium ion (6) which decomposed to the mixed disulphide derivative (7) and to (8). The thiol function is then regenerated from the disulphide derivative by the usual reduction procedure (see section II).

The reaction of sulphenyl halides with thioethers seems to be a general procedure for the cleavage of a thiol protecting group, provided that a stable cation could be ejected from the thioulesulphonium ion intermediate. Thus the thioether linkages between the haem group and the cysteine
residue in horse heart cytochrome C was rapidly and quantitatively cleaved by 2-nitrophenylsulphonyl chloride\textsuperscript{40}. The cleavage is successfully effected due to the easy formation of the carbonium ion (9), stabilized by the conjugated porphyrin system. This haem cleavage procedure is a very useful alternative to the available methods\textsuperscript{48}.

\[
\begin{align*}
\text{CH}_2\text{SCH}_2\text{NHCOCO}_2^- \quad \text{NPSCE} & \quad \begin{array}{c}
\text{S}^-\text{C}_6\text{H}_4\text{NO}_2 \quad \text{Cl}^- \quad \text{NHCOCO}_2^- \\
\end{array} \\
\text{Cl}^-\text{[CH}_2\text{NHCOCO}_2^-] & \\
\text{S}^-\text{C}_6\text{H}_4\text{NO}_2 \quad \text{CH}_2\text{SH} \\
\text{NHCHCO}^- & \\
\text{S}^-\text{C}_6\text{H}_4\text{NO}_2 \\
\text{NHCHCO}^- \\
\text{S}^-\text{C}_6\text{H}_4\text{NO}_2
\end{align*}
\]

\[11\]

\[
\begin{align*}
\text{NHCOCO}^- & \\
\text{NHCHCO}^- \\
\text{NHCHCO}^- + \text{HSC}_6\text{H}_4\text{NO}_2 \\
\end{align*}
\]

\[10\]

**F. β, β- Trifluoro-α-acylaminoethyl Derivatives**

Only one work has been reported using this protecting group\textsuperscript{49}. The thioether is obtained by an exchange reaction of the thiol with β,β,β-trifluoroethyl-α-ethanesulphonyl-α-N-acylamino (10). In the case of

\[
\begin{align*}
\text{C}_6\text{H}_4\text{CH} & \\
\text{N} - \text{NHCOCO}^- \\
\text{Cl}^- \quad \text{NHCOCO}_2^- \\
\text{S}^-\text{C}_6\text{H}_4\text{NO}_2 \\
\end{align*}
\]

\[9\]

14. Protection of the thiol group

R = C\textsubscript{6}H\textsubscript{4}CH\textsubscript{2}O, the protecting group is removed by hydrogen bromide in acetic acid followed by adjusting the pH to 9–10. While some protected

\[
\text{CF}_3\text{CH}_2\text{SO}_2\text{C}_6\text{H}_4\text{H} + \text{S}^-\text{C}_6\text{H}_4\text{NO}_2 \rightarrow \text{CF}_3\text{CH}_2\text{S}^-\text{C}_6\text{H}_4\text{NO}_2 \quad \text{(12)}
\]

\[
\text{CH}_2\text{S}^-\text{C}_6\text{H}_4\text{NO}_2 \quad \text{HNCOR} \\
\text{CF}_3\text{CH}_2\text{S}^-\text{C}_6\text{H}_4\text{NO}_2 \quad \text{HNCOR}
\]

\[19\]

cysteine derivatives were prepared\textsuperscript{49}, the removal of the protecting group from those derivatives is not reported. Some work should be carried out on this protecting group before it gains any use.

**G. β-Diethoxycarbonylthyl Derivatives**

Another protecting group which has not yet gained a wide use is the β,β-diethoxycarbonylthyl group. The thioether (11) is obtained by the addition of the thiol to diethyl dimethylaminoethane (12). The protecting

\[
\text{RSH} + \text{H}_2\text{C} = \text{C} = \text{COOC}_2\text{H}_5 \rightarrow \text{RSCH}_2\text{CH} = \text{COOC}_2\text{H}_5 \quad \text{(13)}
\]

\[
\text{H}_2\text{C} = \text{C} \quad \text{COOC}_2\text{H}_5 \quad \text{COOC}_2\text{H}_5 \\
\text{H}_2\text{C} = \text{C} \quad \text{COOC}_2\text{H}_5
\]

\[12\]

\[11\]

group is stable towards acidic reagents (e.g. trifluoroacetic acid, hydrogen bromide in acetic acid) but is cleaved by 1N KOH solution via β-elimination\textsuperscript{49}. This protecting group was used to protect the thiol group of cysteine during the synthesis of glutathione\textsuperscript{41}.

**IV. THIOESTERS**

**A. Acetyl and Benzoyl Derivatives**

The acetyl and benzoyl derivatives are obtained by the reaction of the corresponding acyl chloride with the thiol\textsuperscript{49}. These thioesters are in effect 'active esters' prone to attack by nucleophiles in general and very susceptible to dilute base. The protecting groups are removed completely by very dilute alkali within 30 min, but with dilute ammonia solution only 50% cleavage occurs during the same time. The hydrolytic cleavage is accompanied by a β-elimination as a side-reaction, especially in cysteine derivatives (reaction 14). This side-reaction can be avoided in low

\[
\begin{align*}
\text{NHCHCO}^- & \\
\text{S}^-\text{R} & \\
\text{R} = \text{CH}_3\text{CO}, \text{C}_6\text{H}_5\text{CO}
\end{align*}
\]

\[14\]
B. Benzyloxy carbonyl Derivatives

These derivatives are obtained by reacting benzyloxy carbonyl chloride with a thiol. Contrary to the N-benzyloxy carbonyl derivatives they are stable towards hydrogen bromide in acetic acid but they are cleaved by phosphonium iodide in acetic acid or by boiling trifluoroacetic acid. The protecting group is removed by methanalysis and ammonolysis, but those cleavage reactions proceed much slower than in the case of the corresponding acyl derivatives. The protecting group is removed very easily by ammonolysis using concentrated aqueous ammonia solution as well as by methanalysis using fivefold excess of sodium methoxide.

C. Urethane Derivatives

The best known protecting group of this class is the ethyl carbamoyl which is obtained by the reaction of ethyl isocyanate with the thiol. The protecting group is stable in acids and neutral solutions but is cleaved easily in basic solution (e.g. aqueous and anhydrous ammonia solution, dilute sodium hydroxide solution, dilute sodium methoxide solution in methanol). No β-elimination could be detected when ethyl carbamoyl cysteine derivatives were treated with 1 N sodium hydroxide solution to yield the unprotected cysteine derivatives. This is contrary to the behaviour of the corresponding benzoyl and acetyl derivatives where considerable β-elimination is observed. It has been shown recently that the ethyl carbamoyl anion is removed by treatment with heavy metal ions (Hg₂⁺, Ag⁺).

An interesting protecting group of this class is the β-(N-acetyl-N-methylaminoethyl) carbamoyl group. The isocyanate (13), which reacts
and 1-methyl-2-imidazolidone (16). This protecting group was recently used to protect the thiol group of cysteine during the synthesis of oxytocine.  

**V. SEMITHIOACETALS**

**A. Tetrahydropranyl Derivatives**

The tetrahydropranyl derivatives are prepared by reacting the free thiol with 2,3-dihydroprane in the presence of acid as a catalyst. A disadvantage in using this protecting group is the introduction of a new asymmetric centre (α) to the molecule. The protecting group is removed by hydrolysis with very dilute acid, by the action of aqueous silver nitrate solution, or by reaction with iodine. In the latter case the disulphide (which could be reduced to the free thiol) is obtained, e.g. benzylo tetrahydropranyl sulphide reacts with iodine to give cinnamyl disulphide. A similar cleavage is observed by the action of thiocyanogen:

\[
\begin{align*}
\text{O} & \quad \text{+ HSR} \quad \text{H}^+ \quad \text{O} \quad \text{S-}\text{SR} \\
\text{R} & \quad \text{S-CH}_2\text{C}_6\text{H}_5 \quad \text{C}_6\text{H}_5\text{CHO} \quad \text{C}_6\text{H}_5\text{CHO} \\
\text{C}_6\text{H}_5\text{CHO} & \quad \text{+ (CNS)}_2 \quad \text{H-CH}_2\text{COOH} \quad \text{C}_6\text{H}_5\text{CHO} \\
\text{C}_6\text{H}_5\text{CHO} & \quad \text{S-S-CH}_2\text{C}_6\text{H}_5 \quad \text{CH}_2\text{COOH} \\
\text{R-S-S-CN} & \quad \text{R-SCN} + \text{RSH} \quad \text{R-S-S-R' + HSCN} \\
\end{align*}
\]

**B. Benzylsulphonyl and Benzyloxythiophenyl Derivatives**

These derivatives were obtained by the reaction of various thiols with benzylthiimethyl chloride in methanol. It was found that products obtained by this route are difficult to purify and the method of choice now is reduction of a symmetrical disulphide by means of sodium in liquid ammonia, followed by addition of the freshly distilled benzylthiimethyl chloride. The protecting group is stable in acidic media (e.g. hydrogen bromide in acetic acid) but is removed by mercuric acetate solution in 80% formic acid. The usefulness of this group as a thiol protecting group was demonstrated in the synthesis of glutathion.

The phenylthiomethyl (C₆H₅SCH₂-) protecting group is obtained and removed in an identical way to that of the benzylthiomethyl group.

**C. Isobutylxoxymethyl Derivatives**

Isobutylthioketimethyl derivatives are obtained by reaction of isobutylxoxymethyl chloride with thiols. These are more sensitive to acid than the corresponding benzylthiomethyl derivatives. The isobutylxoxymethyl group is cleaved by hydrogen bromide in acetic acid, BF₃ etherate or trifluoroacetic acid, but it is stable towards 2N hydrochloric acid in 80% acetic acid or 12N hydrochloric acid in aceton. The isobutylxoxymethyl sulphide is decomposed to some extent by 2N sodium hydroxide, but it is stable towards hydrazine hydrate in boiling ethanol.

The protecting group can be cleaved by thiocyanogen similarly to the triflicxoxymethyl, diphenylxoxymethyl and tetrahydropranyl groups.
VI. HETEROCYCLIC RINGS

A. Thiazolidine Derivatives

Cysteine and cystine derivatives (like other β-aminothiols) react with aldehydes and ketones to form thiazolidine derivatives.

The best known derivatives of this class are thiazolidine-2-carboxylic acid (19), R = R' = H (or thioproline) and 2,2-dimethylthiazolidine-2-carboxylic acid (19), R = R' = CH₃ which are formed by the reaction of

\[
\text{HS} \quad \text{R} \quad \text{C} = \text{O} + \quad \text{NH} \quad \text{R} \quad \text{C} = \text{O} \quad \text{R'} \quad \text{C} = \text{O} \rightarrow \quad \text{R} \quad \text{C} = \text{O} \quad \text{R'} \quad \text{C} = \text{O} \quad \text{R} \quad \text{C} = \text{O}
\]

cysteines with formaldehyde or acetaldehyde respectively. The protecting group can be removed by mild acid hydrolysis. In the case of thiazolidine-2-carboxylic acid, oxidation with iodine yields the disulphide which can be easily reduced to the free mercaptan. The protecting group can be removed from 2,2-dimethylthiazolidine-2-carboxylic acid by aqueous mercuric chloride solution.

VII. ACKNOWLEDGEMENTS

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CHAPTER 15

Rearrangements involving thiols

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VII. REFERENCES
I. INTRODUCTION

This chapter deals with rearrangement reactions which involve thiols either as starting materials or as products. However, because of the high reactivity of thiols in both nucleophilic and free radical reactions, they are actually involved in many cases as transient species only. Such cases, in which the intermediacy of thiols is evident or highly probable, are also included here.

Only few rearrangements involving thiols, particularly those which are of synthetic importance or are related to biochemical processes, have been thoroughly investigated. It was not the aim of this chapter to unearth and list all the rearrangements which have been reported in the literature, but rather to group and describe, with illustrative examples, the most important types.

II. GROUP MIGRATIONS FROM AND ONTO THIOLS

A. Alkyl Migrations

1. Sulphur to carbon

Migrations of alkyl groups from oxygen to negatively charged carbon (Wittig rearrangement) are well known\(^1\). The analogous rearrangement of sulphides, which would lead to isomeric thiols, has not yet been observed. A Wittig-like mechanism was however used to explain the formation of stilbene from dibenzyl sulphide and strong base, assuming the intermediacy of the thiol (4) which eliminates sulphide ion\(^4\).

\[
\begin{align*}
C_6H_5CH_2CH_2CH_2S\overset{\text{base}}{\rightarrow} C_6H_5CH\overset{\text{C}}{\text{C}}CH_2 \rightarrow \\
C_6H_5CH\overset{\text{C}}{\text{C}}CH_2CH_2S^- \rightarrow C_6H_5CH\overset{\text{C}}{\text{C}}CH_2 + S^- \\
\end{align*}
\]

(1)

Trialkylsilyl groups, on the other hand, migrate very readily under Wittig conditions. Benzythiotrimethylsilane (2) on treatment with tetrabutylthiurium is rapidly converted to trialkylsilyl toluene-\(\alpha\)-thiol (3) in almost quantitative yield.

15. Rearrangements involving thiols

The reverse rearrangement (3 \(\rightarrow\) 2) occurs on heating 3 at 190°C under the influence of radical catalysis. A similar rearrangement, which involves radical induced migration of trialkysilyl groups from silicon to sulphur (4 \(\rightarrow\) 5) was also reported\(^6\).

\[
\begin{align*}
C_3H_7CH_2SSi(CH_3)_3 \xrightarrow{\text{heat}} Si(CH_3)_3 \rightarrow C_3H_7CHSH \\
Si(CH_3)_3 \rightarrow CH_3SiS(CH_3)_3 \\
Si(CH_3)_3 \rightarrow Si(CH_3)_3
\end{align*}
\]

(2) (3) (4) (5)

Migration of trialkysilyl and germanyl groups from sulphur to aromatic carbon was also observed\(^6\). Addition of 4-bromo-S-trimethylsilyl-benzenethiol (6) yields the lithium salt of 4-trimethylsilylbethenethiol (7). The mechanism has not been investigated and it has not been established whether an intra- or intermolecular process is involved.

\[
\begin{align*}
SSi(CH_3)_3 + Br_2 &\rightarrow BrSi(CH_3)_3 + S^+ Br^- \\
S^+ Li^+ &\rightarrow S\overset{\text{Li}}{\text{Si(CH_3)_3}} \rightarrow H^+ Si(CH_3)_3
\end{align*}
\]

(6) (7) (8) (9)

2. Sulphur to oxygen

Oxygen-alkyl bonds are easily cleaved by thiol salts\(^6\). An intramolecular reaction of this type would result in migration of an alkyl group from oxygen to sulphur. Indeed, treatment of methyl 2-mercapto-benzenate (8) with alkali gives 73\% of 2-methylthiobenzoic acid (9)\(^7\).

With benzylamine, 8 yields the benzylthioamide of 9, probably through dehydratation of the benzylamidine salt.
B. Aryl Migrations (the Smiles Rearrangement)

The Smiles rearrangement is defined as an aromatic nucleophilic displacement in which the nucleophile and the leaving group are joined, usually by being ortho-substituents on an aromatic ring. The result is a migration of an aryl group from one heteroatom to another. The thiol group can be involved in the Smiles rearrangement either as the nucleophile (equation 1) or as the displaced group (equation 2).

\[
\text{RSH} \rightarrow \text{R}^+ \text{S}^-$
\]

A recent review describes in detail the mechanistic and synthetic aspects of the reaction and also presents a tabular survey of all Smiles rearrangements which appeared in the literature.

Because of the high nucleophilicity of the thiol group and its anion, it is to be expected that reactions of the type shown in equation (1) would be of wide scope. However, only a small number of examples were reported, mostly by Smiles, and all involve the conversion of mercaptodiaryl ethers to hydroxydiaryl sulphides. In this manner 2-hydroxy-1-mercapto-1,2-dimethyl ether (10) yielded 2,2'-dithyroxyl-1,1'-dimethyl ether (11).

Reactions of the type shown in equation (2) have attracted much more attention, as they present the easiest and the most direct synthetic route to the medically important phenothiazines. Almost all the rearrangements reported in the literature are of 2-acylamino-2-nitrophenyl sulphides which yield phenothiazines on treatment with base. A typical example is that of the sulphide (12). Its rearrangement led initially to the thiol salt (13) and subsequently the thiol group displaced the nitro group to give 14. The acyl group is usually hydrolysed under the reaction conditions and the phenothiazine (15) was obtained in one step. Isolation of the intermediate N-arylpolythiazines was reported in several cases.

Although the phenothiazines could have been formed by a direct displacement of the nitro group by the amine, the positions of the substituents in the products establish the mechanism shown and the intermediacy of thioles.

A closely related reaction is the rearrangement of N-alkylamino-dimethyl sulphides, which yield N-alkylpolythiazines on heating in boiling quinoline or aniline. Compound 17 was thus obtained from 16 (57% yield).

Contrary to previous reports it was recently found that 2-aminoo-2-nitrodiphenyl sulphide (18) also rearranged on heating at 190°C alone in dimethylacetamide to give the phenothiazine (19). The dibenzo(b,phenylphenothiazine (20) was also formed as a by-product.

The rearrangement of pyridyl sulphides is particularly interesting, as it was found to occur also under acidic conditions. 3-Amino-3-nitro-2,2'-dipyridyl sulphide (21) gives 2-mercapto-3-nitro-3,2'-dipyridylamine (22) in nearly quantitative yield on short treatment with hydrochloric acid.
The thermal rearrangement of dipyridyl sulphones proceeds much easier than that of diphenyl sulphones and is highly solvent-dependent. It is rapid in boiling ethanol, slower in water and does not occur at all in benzene or dimethylsulphoxide. It was also observed that 2-acylamino-pyridyl sulphones rearrange faster than the corresponding 2-amino derivatives. These facts suggest solvent participation such as shown in equation 3.

\[
\text{C}_{6}H_{5}OCH_{2} + \text{S} \rightarrow \text{S} + \text{N} + \text{O} + \text{H}^{+} + \text{CH}_{2}\text{COOC}_{2}H_{5}
\]

The photochemical Smiles rearrangement was also reported recently. An example involving a thiol is the conversion of 25 to 26 on irradiation in ethanol.

\[
\text{NH}_{2} \text{S} \text{N} \text{O} \text{CH}_{3} \rightarrow \text{N} \text{H} \text{S} \text{N} \text{O} \text{CH}_{3}
\]

Migrations of 2,4-dinitrophenyl groups from aliphatic thiols are also known. Migration to nitrogen occurs in the cysteine derivative 27 at pH 7, and migration to oxygen in compound 28 on treatment with base.
C. Aryl Migration

1. Sulphur to Oxygen

Acetyl transfer from thiol to hydroxyl groups occurs readily, under basic conditions, in the series RCOOHCH₂OH when n is 2 or 3, but not when n is 4. S-Acetylmercaptoethanol (29) thus yields thiran (32) as a result of acetate ion elimination from the rearranged product 31.20,21.

\[ \text{CH₂CH₂OH} \xrightarrow{\text{base}} \text{CH₂CH₂} \xrightarrow{\text{HO⁻CH₃}} \text{CH₂CH₂} \xrightarrow{\text{S-OOCCH₃}} \]

\[ \text{H₂C-CH₂} \rightarrow \text{S-OOCCH₃} \]

S-Acetyl 3-mercapto propane (33) yields, under the same conditions, 3-mercapto propyl acetate (35) which is stable and isolatable.

\[ \text{CH₂CH₂CH₂OH} \xrightarrow{\text{CH₂CH₂} \xrightarrow{\text{SH-OOCCH₃}}} \text{CH₂CH₂CH₂} \xrightarrow{\text{SH-OOCCH₃}} \]

The importance of the distance between the thiol and the hydroxy groups implicates intermediate ring formations (30 and 34) during the

15. Rearrangements involving thioles

It can be expected that a five-membered ring intermediate would provide the optimum ring size for the transfer, and indeed compound 29 rearranges 30 times faster than 33.18

A kinetic study of the rearrangement of 33 to 35 showed that the equilibrium constant is 56 (at 70°C, 0.5 ionic strength), corresponding to a difference of free energy of 2500 kcal/mole between esters and thiol esters.44

A similar rearrangement which involves migration of the thionoalkoxy group is assumed to occur during the reaction of xanthate salts with epoxides, which leads to thioacrylates.44,45 The proposed mechanism44,47 is presented in equation (4).

\[ \text{S} \quad \xrightarrow{\text{S-OOCCH₃}} \text{S} \rightarrow \]

Evidence for the mechanism is provided by the fact that cyclopentene oxide (36) does not react, as its rearrangement would require the existence of the intermediate 37 which possesses two trans-fused five-membered rings. The thiran 38, however, reacts smoothly and gives 39. This striking difference in behaviour can probably be attributed mainly
2. Sulphur to Nitrogen

The migration of an acyl group from a thiol to an amino group is analogous to the migration to hydroxyl described above, and proceeds through the corresponding cyclic intermediates. S-Acetyl-2-aminoethanethiol (40) rearranges in this manner very readily to 2-acetamidoethanethiol (42) via the thiazolidine 41 and S-acetyl-3-aminopropanethiol (43) rearranges to 3-acetamidoopropanethiol (45) via 44.¹⁰

These rearrangements take place even in acidic media and, as expected, the rearrangement of 40 proceeds much faster than that of 43 (at pH 5, rate ratio 1:100). The rearrangement of S-acetyl-1-amino-2-butanolthiol (46) to 47, which would require a seven-membered ring intermediate, occurs at a measurable rate only at pH > 8 and is much slower. On increasing the distance between the thiol and the amino group, no rearrangement was observed and treatment of 48 or 49 with base results only in hydrolysis of the acetyl group.

15. Rearrangements involving thiols

A kinetic study on the reaction 40 → 41 → 42.¹⁵,¹⁶ confirmed the intermediacy of 41. All the reaction steps were assumed to be equilibria, and equilibrium constants and rate constants were determined. Of particular interest is the pH effect. The migration rate exhibits an inverse dependence on hydrogen ion concentration at low pH (2.5-3), a plateau region (pH 3-4.5) which is ascribed to general base catalysis by H₂O and then general base catalysis by OH⁻ at higher pH (> 5).¹⁶ A detailed mechanism which accounts for all the data and includes protonation equilibria was proposed.¹⁷

S-Benzoyl-2-aminoethanethiol and its N-alkyl derivatives (50) rearrange immediately on liberation from the hydrobromide salt. However, besides the expected 2-benzamidoethanethiols (51) small amounts of N,N-dibenzoyl-2-aminoethanethiols (52) were also obtained, which indicates that the intramolecular acyl migrations were followed by trans-thiolesterifications from the starting materials 50 to the rearranged products 51. The trans-thiolesterifications must be accompanied by elimination of 2-aminoethanethiols (53) and although attempts to isolate 53a and 53b from the reactions of 50a and 50b failed, 50c afforded all three products.

Applications by the rearrangement to peptides were studied by Wieland.¹⁸,¹⁹,²⁰ Migrations of α-aminoacyl groups are very rapid and the rearrangement of S-glycylcystamine 54 to the N-glycyl derivative (55) was complete at pH 5.2 in 2 min. Under basic conditions α-aminoacyl groups migrate from sulphur even to amides, to give diacycylamines. These undergo a very facile hydrolysis and lose one acyl group (56 → 57), or if possible rearrange further via N → N acyl migrations to give tripeptides. S-Valyl-N-glycylcystamine (58) for example yielded a mixture which contained N-valylglycylcystamine (59), N-valylglycylcystamine (60) and N-glycylvalylcystamine (61).
15. Rearrangements involving thiols

This mechanism was substantiated by isolation of the p-nitrobenzoyl derivative of the cyclic intermediate 64, and by the observation that inversion occurred at each asymmetric carbon, as the mechanism demands that the resultant thirane possess a configuration opposite to that of the starting oxirane.

Cyclopentene oxide does not react under customary reaction conditions because of the considerable strain required to form a cyclic intermediate analogous to 64. However, on employing harsher conditions 20% of the corresponding thirane could be obtained.

A modification of the above reaction, which proceeds via the same intermediate, is the reaction of thiocyanate salts with ethylene carbonates (67).  

Propylene carbonates (68) react as well to give thietanes, thus the rearrangement can proceed also via the six-membered ring intermediate 69.

2. Amidino group migrations

This rearrangement is assumed to occur during the conversion of oxirans to thiranes by reaction with thiourea (equation 5).

The p-hydroxyisothiouronium salt 70 can be isolated in the presence of acid, and react further on addition of base. The importance of the cyclic intermediate 71 is evident from the failure of cyclopentene oxide to react.
In a similar manner the amido group migrates from S to N of S-(2-aminoethyl)thioisourea salts (72) rearrange, at neutral pH, to 2-mercaptoethylguanidines (74). Both starting materials and products are in this case isolatable as hydrobromide salts. The reaction was shown to involve the cyclic intermediate 73.

S-(3-aminopropyl)thioisourea salts rearrange in the same manner to (3-mercaptopropyl)guanidines through a six-membered cyclic intermediate.

III. REARRANGEMENTS OF THE O-THIOACYL SYSTEM TO THE S-ACYL SYSTEM

A. Rearrangements which Proceed through a Four-Membered Cyclic Transition State

The first reported rearrangement of this type was the thermal isomerization of diarylthiocarbonates (75) to diarylthiolcarbonates (77).

An examination of a large series of compounds showed that when R and R' are electron-withdrawing substituents, rate accelerations are experienced, and in unsymmetrically substituted thionocarbonates the rearrangement occurs primarily in the direction of the ring bearing the more electron-withdrawing substituents. The reaction thus originates from the nucleophilic character of the sulphur. A kinetic study showed that the reaction is first order and all these data indicate that the rearrangement consists of an aromatic nucleophilic displacement in which the ring migrates from O to S via a four-membered cyclic transition state (76). The reaction can serve as an efficient preparation method for aromatic thiols by hydrolysis of the products (77).

N,N-Dialkylthionocarbonates (78) rearrange similarly to thiolcarbamates (79). This isomerization is faster and proceeds at lower temperatures and in higher yields (usually shown 90%-95% at 60°C).

The four-membered cyclic mechanism is supported by substituent effects and kinetic results. No crossover products were found on heating a mixture of two thionocarbonates.

Steric rate enhancement due to hindered rotation was found to be present in ortho-substituted compounds in this series. The relatively low temperature required results from increased nucleophilicity of the sulphur, since the polarization is assisted by the dialkylamino group, towards the zwiterionized form (80).

A stronger electron-donating inductive effect of R should promote the nucleophilic character of the sulphur further, and indeed the rearrangement rate was found to increase in the order:

R = i-C₄H₉ > n-C₃H₇ > C₂H₅ > CH₃
The reaction was utilized for the synthesis of aromatic thiols and sulphonic acids by hydrolysis or oxidation of the rearranged products. The heating of xanthates usually results in $\beta$-elimination and formation of olefins (Chugaev reaction). However, when there is no $\beta$-hydrogen at the alcohol moiety, rearrangement takes place. A kinetic study of the influence of substituents on the reaction rate of a series of diaryl xanthates to diaryl dithiocarbonates (83 → 82) again indicates a four-membered cyclic transition state. A similar transition state is indicated, by the same kind of evidence, for the rearrangement of aryl thiobenzoates (83) to aryl thiobenzoates (86).

The rearrangement of alkyl thiobenzoates had also been reported in certain cases. An application of this reaction is the thermal conversion of thionesters of glycerol to esters of thioglycerol (85 → 86).

**B. Rearrangements which Proceed through Dissociation and Return**

$\alpha$-Alkyl thiocarboxylic thiocarboxylate rearrangement easily to thionesters when the alkyl group can form relatively stable carbonium ions. Benzyl thionocarbonate (87) yields 88 on heating in ethanol and the suggested mechanism is a dissociation to a ion pair. The return of which occurs via the sulphur because of the greater nucleophilicity of the sulphur compared to the oxygen.

A study of the rearrangement of the optically active exo-norbornyl thiobenzoate 89 to 90 showed that the rate of racemization was equal to the rate of the disappearance of 89. This indicates that no return via the oxygen occurs.

The dissociation, and hence the rearrangement, may be caused by neighbouring group participation in the formation of the cation. An example is the rearrangement of the thionester.
C. Rearrangement of Allyl Thiocarbamates

Allyl thioacetates (92) rearrange to allyl thiobenzoates (93), accompanying an allyl shift, on heating to 100°C.

This isomerisation is very little influenced by the medium and occurs only ca. ten times faster in acetic acid than in cyclohexane. This low sensitivity to the ionizing power of the solvent indicates that allyl thioesters rearrange by a mechanism which involves very little change in charge separation between the ground state and the transition state. Thus a cyclic concerted mechanism is more probable than dissociation to ion pairs. This conclusion was confirmed by deuterium isotope effect measurements.

Allyl thionocarbamates and allyl xanthates also rearrange easily in the same manner.

IV. THE THIO-CLAISEN REARRANGEMENT

A. The Rearrangement of Allyl Aryl Sulphides

The thio-Claissen rearrangement is a 3,3sigmatropic process, which consists of synchronous cleavage of a carbon—-sulphur bond and formation of a new carbon—-carbon bond (equation 6).

The thios formed usually do not survive under the reaction conditions and cyclize to five- and six-membered rings. Heating of allyl phenyl sulphide (94) in high boiling amines or carboxylic acids yields 2-methyl-2,3-dihydrobenzothiophene (95) and thiachroman (96). The two products are not interconvertible under these conditions.

15. Rearrangements involving thiols

Although a different reaction pathway, which involves the thian 97 as intermediate, has been proposed, the intermediacy of 2-allylbenzenethiol (98) and therefore the correctness of the concerted mechanism has been rigorously established. In the presence of methyl iodide the methythio derivative 99 was isolated. Furthermore, compound 99 was synthesized independently and was shown to undergo a facile cyclization to give both 95 and 96. When cyclized under the rearrangement conditions, the proportions of the products from 99 were identical with those obtained directly from 94.

Definitive evidence for the sole intermediacy of 2-allylarenesulphenol was obtained from work on the rearrangements of allyl quinuclidyl sulphides.

For example, 3-methylallylquinuclidyl sulphide (100), which rearranges in dimethylaniline to 101 and 102, gave in butyric anhydride quantitative yield of the butyl derivative (102) of the Claissen product 104. Compounds 100 and 104 yielded 101 and 102 in the same proportions when heated under identical conditions.

Similar results were observed in the thiophene series. In the rearrangement of allyl 2-thienyl sulphide (105) to 107 and 108, the intermediate 2-allylthiol 106 has been isolated for the first time directly from the reaction mixture.
The five-membered ring products result from the normal (Markownikoff) internal addition of the thiol group to the double bond, whereas the six-membered ring products result from abnormal (anti-Markownikoff) addition, which is characteristic of a free radical process. The formation of both heterocycles thus indicates competitive thermally induced heterolytic and homolytic fissions of the initial S-H bond (equation 7). The cyclization mechanisms were verified by a detailed examination of the thermal behaviour of 104. Product 102 was formed almost exclusively

15. Rearrangements involving thiols

under free radical initiation. The intervention of free radical intermediates was also evident from e.s.r. monitoring of the reaction.

Cyclization does not occur when the thione initially formed in the rearrangement cannot tautomerize to the corresponding enethiol. The indolenine 109 for example rearranges to 110 which shows no tendency to cyclize.

When the two ortho-positions are blocked, no para-Claisen products are observed. Heating of ethyl 2,6-dimethylphenyl sulphide (111) yields a cleavage product 112 and four cyclic materials (113–116) which probably result from ortho-rearrangement followed by 1,3- and 1,4-methyl migrations.
B. The Rearrangement of Prop-2-ynyl Aryl Sulphides

This reaction also yields both five and six-membered cyclic products, but has been shown to consist of two parallel processes\(^8\). Prop-2-ynyl phenyl sulphide (117) on heating initially isomerizes to phenyl allenyl sulphide (118) and both 117 and 118 undergo the thio-Claisen rearrangement to the allenic (119) and acetylenic (120) thioles respectively. Subsequent cyclization yields the final products, 2H-thiachromene (121) and 2-methylbenzothiophene (122).

\[
\begin{align*}
\text{(117)} & \rightarrow \text{(118)} \rightarrow \text{(119)} & \rightarrow \text{(121)} \\
\text{(123)} & \rightarrow \text{(124)} + \text{(125)}
\end{align*}
\]

The rearrangement of prop-2-ynyl-3-thienyl sulphide (123) proceeds in the same manner to give 124 and 125\(^9\). 

V. INTERNAL ADDITIONS, ELIMINATIONS AND RING-CHAIN TAUTOMERISMS

A. Intramolecular Additions to Double Bonds

The cyclization of ortho-allylbenzenethiols has already been discussed in connection with the thio-Claisen rearrangement. It has been shown that ionic and free radical processes operate simultaneously to give five- and six-membered heterocycles respectively\(^9\). Similar dual pathways were observed in the cyclization of 5-mercapto-1-pentene (136) which gave both 127 and 128\(^9\). 

\[
\text{HS(CH₂)₃CH=CH₂} \rightarrow \text{(126)} \rightarrow \text{(127)} \rightarrow \text{(128)}
\]

15. Rearrangements involving thiols

Free radical cyclization (Be₂O₂ catalysis) of a series of simple mercaptoolefins gave the following product ratios\(^9\).

<table>
<thead>
<tr>
<th>Ratio</th>
<th>Structure</th>
</tr>
</thead>
<tbody>
<tr>
<td>1:99</td>
<td><img src="image1" alt="Structure" /></td>
</tr>
<tr>
<td>24:76</td>
<td><img src="image2" alt="Structure" /></td>
</tr>
<tr>
<td>45:55</td>
<td><img src="image3" alt="Structure" /></td>
</tr>
<tr>
<td>85:15</td>
<td><img src="image4" alt="Structure" /></td>
</tr>
</tbody>
</table>

Different results were observed in nitrogen-containing chains. Ultra-violet irradiation of N-allyl-2-aminoethanethiols (129) gave mainly thiazolines (130) when R was H and hexahydropyrazines (131) when R was methyl. These results can be rationalized by assuming an initial double-bond migration towards the nitrogen\(^9\).

\[
\begin{align*}
\text{(129)} & \rightarrow \text{(130)} + \text{(131)} \\
\text{(132)} & \rightarrow \text{(133)} \rightarrow \text{(134)} \\
\text{(135)} & \rightarrow \text{(136)} \rightarrow \text{(137)} \rightarrow \text{(138)}
\end{align*}
\]

Reaction routes which include internal additions of thiols have been proposed for several rearrangement reactions. Addition of vinylithium to thiophthaldehyde (132) yielded, after acetylation, 4,5,6,7-tetrahydropyridine-2-thione (133). The probable course is ring opening of the adduct 133 to the thiol 134 which cyclizes by conjugate addition to give 135. The thiolactone 136 yielded 138 in an analogous manner via the thiol 137\(^9\). 

\[
\begin{align*}
\text{(132)} & \rightarrow \text{(133)} \rightarrow \text{(134)} \rightarrow \text{(135)} \rightarrow \text{(136)} \rightarrow \text{(137)} \rightarrow \text{(138)}
\end{align*}
\]
Another example is the photoysis of mercaptanes. Compound 139 yielded the rearranged product 140 (cis-trans mixture, ratio 8:1). Its formation is best explained by a homolytic scission of the C–S bond, followed by hydrogen atom transfer and addition of the thiol to the double bond 86b.

B. Intramolecular Additions to Triple Bonds

Cyclization of a series of acetylenic thioles under various conditions gave the following results (Table 1, in %).

Although the polymerization was extensive in most cases, the analysis of the cyclic products clearly shows that terminal acetylenes nucleophilic reaction leads mainly to exo-methylene-heterocycles, while free radical cyclization leads to unsaturated rings.

The cyclization of ortho-prop-2-ylbenzenethiol has already been discussed in connection with the thio-Claisen rearrangement 83.  

C. Cyclization and Ring-chain Tautomerism of Cyanothiols

Cyanothiols cyclize irreversibly in cases in which the product exists predominately in its enaminic form. α-Mercaptobenzylcyanide (141) thus cyclized to 142 which tautomerized to 143 and was shown to exist only in a cyclic form 87.

Compound 144, on the other hand, cyclized to 145 in which the methyl substituents at position 3 preclude tautomerism to an enamine, and indeed its exists in the cyclic form only in neutral or acidic media. However, in basic solution it reacts as the open chain form 144, and can be S-alkylated or oxidized to the corresponding disulfide 84.

15. Rearrangements involving thiols

<table>
<thead>
<tr>
<th>n</th>
<th>R</th>
<th>154</th>
<th>4a</th>
<th>4b</th>
<th>4c</th>
<th>4d</th>
<th>4e</th>
<th>4f</th>
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<tr>
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<td>21</td>
<td>10</td>
<td>45</td>
<td>7</td>
<td>2</td>
<td>16</td>
<td>1</td>
</tr>
<tr>
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<td>H</td>
<td>21</td>
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<td>45</td>
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<td>H</td>
<td>21</td>
<td>35</td>
<td>45</td>
<td>7</td>
<td>9</td>
<td>23</td>
<td>3</td>
</tr>
<tr>
<td>n = 6</td>
<td>H</td>
<td>21</td>
<td>35</td>
<td>45</td>
<td>7</td>
<td>9</td>
<td>23</td>
<td>3</td>
</tr>
</tbody>
</table>
15. Rearrangements involving thiols

converted into the tautomeric mercaptoaldehydes 153a and 153b and can be
titrated as thiols with aqueous iodine. The seven-membered ring 152c,
on the other hand, exists as such only in the solid state, but shows spectral
properties ascribable to the open form 153c in chloroform solution,
indicating that tautomerism occurs in this case very readily.\footnote{20}

```
\begin{equation}
\text{(152) } a, n = 0 \quad b, n = 1 \quad c, n = 2
\end{equation}
```

Similar results were obtained with mercapto ketones. The thiol 154
was prepared from mercaptans, purines and cyclohexane
with loss of water to 156. The possible intermediate 155 could not be
isolated.\footnote{29}

```
\begin{equation}
\text{(154) } (\text{CH}_3)_3\text{C}^\text{SH} \quad \text{O}
\end{equation}
```

The unsubstituted mercapto ketones 157a and 157b were never isolated
and isolation attempts led to the unsaturated heterocycles 159a and 159b, probably
via the hemithioacetalts 158a and 158b.\footnote{29, 30}

```
\begin{equation}
\text{(157) } a, n = 0 \quad b, n = 3
\end{equation}
```

Internal thiol–carbonyl interactions were extensively investigated in the
field of thioglycals, and were applied to the synthesis of the thiophuranose,\footnote{31, 32}
and thiopyranose,\footnote{33} and thioseptane,\footnote{34} systems.

King opening and closure involving mercapto ketones were assumed to
occur during the unexpected formation of 4-acetyl-2,3-dihydro-2-hydroxy-
2-phenyl-4H-1,4-thiazine (161), on treating 2-methyl-3-phenethylthiazol
bromide (160) with base.\footnote{35}

```
\begin{equation}
\text{(158) } \text{(160)}
\end{equation}
```

D. Ring-chain Tautomerism of Mercaptoaldehydes and Mercapto Ketones

The order of stability of the cyclic form of hemithioacetalts relative to
the open chain form is parallel to that observed for hydroxylaldehydes.\footnote{36}
2-Hydroxytetrahydrothiophen (152a) and 2-hydroxyhexahydrothiopyran
(152b) (prepared from the corresponding acetates) were shown, by spectral
evidence, to exist in their cyclic forms both in the neat state and in their
solutions in organic solvents. In aqueous solutions, however, they are

```
\begin{equation}
\text{(149) } a, x = H \quad b, x = \text{CC} = \text{C} \quad c, x = \text{CN}
\end{equation}
```

The same ring-chain tautomerism is exhibited by the pair 146 \(\rightleftharpoons\) 147.
The cyclic form 147 exists as the imino tautomer, as aromatic stability
would be disrupted by the \(\sigma\)-quinoidal structural requirement of the
enamino tautomer 148.\footnote{37}
Another interesting example exists in the photochemical rearrangement of α-(α-hydroxybenzylidene)-γ-butyrolactone (162) to 3-(2-mercaptoethyl) coumarin (164). The rearranged product 163 is stabilized by ring opening, as the carbonyl group formed becomes a part of the coumarin system.

E. Ring Openings of Cyclic Sulphides to Unsatuated Thiols

1. β-Eliminations

This reaction has been observed mainly in nitrogen-containing systems. The proton elimination can occur either from a β-nitrogen atom or from a β-carbon. 2-Arylthiazolidines (165) are thus opened to the iminothiols 166 and the tetrahydro-1,4-thiazepine 167 is opened to the enethiol 168 which is unstable, but could be trapped. The corresponding

15. Rearrangements involving thiols

perhydrothiazepine is opened by base at a much slower rate, and the resulting thiol is isolatable. Rearrangements of this type are most common in penicillin chemistry. An example is the rearrangement of penicillins with an activated carboxyl group 169 to anhydropenicillins (171), in which the first step is β-elimination to the thiol (170).

The intermediate 170 could, in certain cases, be trapped as its acyl derivative. In another instance the carboxyl group reacted with the lactam ring leading to the oxazole 172.

Another type of penicillin rearrangement is that which starts with proton elimination from C-6. Such mechanism explains the epimerization at C-6 on treating phthaloylpenicillin methyl ester (173) with sodium hydride. The first step is the opening of the thiazole ring to the thiol salt 174 which recycles to 173 with epimerization. Support for this mechanism was provided by the isolation of the thiazepine 175 as a by-product.
Another interesting penicillin rearrangement which involves β-elimination and a thiol intermediate is that of methyl 6α-chloropenicillate (176) to the 1,4-thiazine 177, through the pathway shown. Ring opening by β-elimination occurs also in the fully aromatic isothiazole system. During the lithiation of 4-methylisothiazole (178), which occurred mainly at position 5, the nitrile 180 was also formed as a by-product. The mechanism pathway is probably lithiation at position 3, ring opening to the thiol salt 179 and alkylation by butyl bromide present in the reaction mixture.

Similar openings were observed on treating condensed isothiazoles (181) with base.

15. Rearrangements involving thiols

2. Homolytic fissions followed by hydrogen transfer

The results of several photolysis reactions of sulphur-containing rings can be rationalized by postulating this process. One example is the photolysis of lipics acid (182) which yielded 185 (in water) or 186 (in methanol). The proposed mechanism is a homolytic scission of the S-S bond to the diradical 183 and migration of the tertiary hydrogen atom as a radical, to form the thiol 184 which reacts with the solvent. A similar mechanism which involves a primary homolytic cleavage of a C-S bond was assumed to occur in the photolysis of mercaptothiol.

VI. MISCELLANEOUS REARRANGEMENTS

A. Migration of a Thiol Ester Group

The only case in which this type of migration occurs is the acidolytic ring opening of epoxides. Phenyl α-methyl-trans-β-thiophenylglycidylate (187) gave, upon treatment with boron trifluoride etherate, 45% yield of the enol tautomer of phenyl α-phenylacetothiolactate (188). The tendency of the thiol ester group to migrate in this particular case is not surprising, since the unusual migration of the carboxylic group in the corresponding glycidic ester was observed previously.
B. Dissociation and Return of the Hydrosulphide Ion

A rearrangement which proceeds through this mechanism was observed during the preparation of N-(β-hydroxyethyl)-N-ethyl-thioformamide (189). An isomeric N-(β-hydroxyethyl)-N-thioformamide (191), was obtained as a side product and it was shown that 191 was formed from 189. The probable course is a cyclization of 189 to 1-ethylthiazolidine-2-thiol (190) and attack of the hydrosulphide ion at C-4. This formulation is supported by a previous report on the ring opening of oxazolines by thiols (192 → 193)\(^{119}\).

\[
\begin{align*}
\text{CH}_2\text{H}_2\text{N} & \text{OH} \quad \text{CH}_3\text{C} & \text{H}_2\text{N} & \text{H} + \text{SH}^- \\
\text{N} & \text{CH} & \text{C} & \text{H}_2\text{N} & \text{H} \\
\text{C}_2\text{H}_3 & \text{N} & \text{CH} & \text{C}_2\text{H}_3 & \text{N} \\
\text{N} & \text{CH} & \text{C} & \text{H}_2\text{N} & \text{H} \\
\text{C}_2\text{H}_3 & \text{N} & \text{CH} & \text{C}_2\text{H}_3 & \text{N} \\
\end{align*}
\]

\[\text{HN} - (\text{C}(\text{CH}_3))_2 \text{CO}_2 \text{CH}_2\text{SC}_2\text{H}_5\]
15. Rearrangements involving thiols

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CHAPTER 16

Thiols as nucleophiles

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b. Alkene derivatives

c. Alkyne derivatives

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1. Simple transition metal derivatives

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I. INTRODUCTION

The thiol act as nucleophiles in two basic types of reaction, involving either substitution or addition to a multiple bond such as C=0

\[ \text{RS} + \text{AB} \rightarrow \text{RSA} + \text{HB} \]  

or

\[ \text{RS}^- + \text{AB} \rightarrow \text{RSA} + \text{H}^+ \]  

\[ \text{RS}^- + \text{C}=\text{C} \rightarrow \text{RS}^- + \text{CH}_2 \]  

In reactions of the type 1 the HB generated may fracture the S=A bond formed; for example the silicon–sulphur bond in H₂SiSCF₃ is susceptible to fracture by HI.

\[ \text{H}_2\text{SiSCF}_3 + \text{HI} \rightarrow \text{H}_2\text{Si} + \text{CF}_2\text{SH} \]

The substitution reactions discussed in this review will be restricted primarily to the thiolate anion, RS⁻ acting as a nucleophile. This may be present initially when a metal thiolate, such as silver(I) or lead(II), is employed, or may be generated in solution in the presence of a base such as sodium hydroxide or trimethylamine. The anion of the thiol is important if the RS⁻ anion acts as a nucleophile in a neutral thiol solution. Thiolate nucleophiles can be obtained in non-aqueous solution by treatment of thiol esters, such as CH₃COSR, with strong non-nucleophilic bases, or by hydrolysis of thioureia derivatives.

The substitution type reaction is not restricted to substitution at a carbon atom, either aliphatic or aromatic, but includes the main group and transition elements. Several examples will be given of the variety of the use of thiolates as nucleophiles, and although most of these reactions are general, some of the illustrative examples will be drawn from the chemistry of halogenated thiols, in which the author is particularly interested. The review will generally be restricted to monofunctional thiols, and usually excludes diithioles, thio acids, etc.

Various reviews have been written on parts of this topic and these will be referred to at appropriate places in the text. The alkoxy neucleophiles have been investigated considerably more than the thiolate nucleophiles, and conversely selenolates significantly less than thiolates. In general the order of nucleophilic strength increases in the series alcohols, thiols and selenols, although sulphur-containing nucleophiles are generally less basic than their oxygen analogues.

The nucleophilic reactivities towards cations of several nucleophiles has been reviewed⁶. A parameter \( N_\ell \), which is characteristic of the nucleophile system and independent of the cation has been defined as

\[ \log \left( \frac{K_\ell}{K_\text{eq,c}} \right) = N_\ell \]

where \( K_\ell \) is the rate constant for reaction of a cation with a specific nucleophilic system (i.e. a given nucleophile in a given solvent), \( K_\text{eq,c} \) is the rate constant for reaction of the same cation with water in water. This generalization can successfully be applied to the reactions of various nucleophiles with various cations. It has been suggested that the \( N_\ell \) values are related to the solvation energies of the nucleophiles⁷. In all the reactions studied, values of \( N_\ell \) are highest for the benzethiolate anion. Comparable values for the reactions of nucleophiles with p-nitro-(Malachite Green) are, solvent in brackets, MeOH (MeOH), 0.5; MeO (MeOH), 7.5; N₂ (MeOH), 8.5; CN⁻ (DMSO), 8.6; PhS⁻ (MeOH), 10.7; PhS⁻ (DMSO), 13.1. Unfortunately data are not currently available to correlate analogous oxygen, sulphur and selenium nucleophiles by this method.

A considerable range and variety of thiols have been employed as nucleophiles. Some thiols are unstable in basic solution, but can be employed as their thiolate salts. Examples of this type of thiol include trifluoromethanethiol and pentfluorobenzenethiol. The trifluoromethane-thiolate anion readily loses fluoride in solution in an irreversible reaction, but the mercy derivative, Hg(SCF₃)₂, effectively acts as a source of

\[ (\text{SCF}_3^-) \rightarrow \text{CF}_3^- + F^- \]

nucleophilic trifluoromethanethiolate ions. The pentfluorobenzenethiolate anion decomposes in basic solution in air. The reaction probably proceeds initially with the oxidation of the thiolate to the disulphide, which is then...
attacked nucleophilically by the thiolate.

\[ 2 \text{C\textsubscript{6}F\textsubscript{5}S}^- + 2 \text{C\textsubscript{6}F\textsubscript{5}C\textsubscript{6}H\textsubscript{4}H\textsubscript{2}S}^- \rightarrow 2 \text{C\textsubscript{6}F\textsubscript{5}C\textsubscript{6}H\textsubscript{4}C\textsubscript{6}H\textsubscript{5}S}^- + 2 \text{C\textsubscript{6}F\textsubscript{5}C\textsubscript{6}H\textsubscript{5}H} + 2 \text{C\textsubscript{6}F\textsubscript{5}C\textsubscript{6}H\textsubscript{5}H} \]

The product, termed perfluoropolyphenylene sulphide, has been characterized by chemical analysis and molecular weight.

Some thiolates, such as pentachlorobenzenethiolate, show no nucleophilic reactivity. Other interesting thiolates that have been studied include the silylalkane- thiolates, such as (Et\textsubscript{3}Si\textsubscript{Me}_{2}CH\textsubscript{2}CH\textsubscript{2}SH)\textsuperscript{3}, and (Me\textsubscript{2}Si\textsubscript{Me}_{2}Me\textsubscript{2}Si(CH\textsubscript{3})\textsubscript{2}SH)\textsuperscript{3,10}. A series of syntheses based on the alkynethiolates has been reported\textsuperscript{31}. In some reactions the C=C bond is retained, but in others it reacts, e.g.

\[ \text{RC} = \text{C} = \text{CSR} + \text{HNE} \rightarrow \text{RC} = \text{C} = \text{CSR} \]

The stereochemistry of the thiol is important. Stereo effects have been used to explain the differences in rates of reactivity of R\textsubscript{2}C\textsubscript{6}H\textsubscript{4}SH (R = H, 2-r-Bu, 4-r-Bu) in addition reactions with N-ethyl maleimide or displacement of 2,4-(O\textsubscript{2}N\textsubscript{2}C\textsubscript{6}H\textsubscript{4}H\textsubscript{2}S\textsuperscript{3} from 2,4-(O\textsubscript{2}N\textsubscript{2}C\textsubscript{6}H\textsubscript{4}H\textsubscript{2}SS}\textsuperscript{3}.

In some circumstances the electrophilic species studied are susceptible to the thiolate anion causing both substitution or addition. An example is HC\textsubscript{C} = CCMe\textsubscript{2}HalCO\textsubscript{2}Et\textsuperscript{13}. In this case the thiolate can also act as a reducing agent. The reducing properties of the thiolate will only be commented on when it is incidental to substitution or addition. The reducing power of thiolates, however, means that the electrophiles employed generally do not contain a group that is readily reduced, such as the nitro group. When simultaneous substitution and addition occur, the reaction will be discussed in the substitution section, particularly in compounds containing C=C bonds.

This review is divided into two main sections: substitution reactions and addition reactions. Sometimes the classification of a particular reaction is somewhat arbitrary. Dealkylation reactions, some of which can superficially appear to be neither substitution nor addition reactions, are basically substitution reactions and a section is devoted to these reactions, including both aliphatic and aromatic examples.

16. Thioles as nucleophiles

II. SUBSTITUTION REACTIONS

A. Aliphatic Substitution

1. Introduction

Simple thiolate substitution of an aliphatic compound can be represented by the equation

\[ \text{RX} + \text{R}^\text{1}- \rightarrow \text{R}^\text{1} \text{R}^\text{2} \text{X} \]

where the group X may be a halogen, methoxy (discussed mainly under dealkylation), methanesulphonate, tosyl, etc.

Examples of the reactions of alkyl and acyl halides are\textsuperscript{31}:

\[ \text{CH}_{2}\text{Cl} + \text{Ph} = \text{S} = \text{CPh} = \text{Ph} \rightarrow \text{CH} = \text{S} = \text{CPh} + \text{PhCl} \]

\[ \text{CH} = \text{CCH} + \text{Ph} = \text{S} = \text{CPh} \rightarrow \text{CH} = \text{S} = \text{CPh} + \text{PhCl} \]

\[ 2 \text{PhCOCl} + \text{Ph} = \text{S} = \text{CPh} \rightarrow 2 \text{PhCO} = \text{CPh} + \text{PhCl} \]

An inert solvent is usually used but liquid ammonia has been used in the reaction of alkyl chlorides with sodium hydroxide.\textsuperscript{15,16} The compound (Ph\textsubscript{3}P)\textsubscript{3}CN was formed in the reaction of the bromoanilinothioacetamide and dibromocarbene, prepared from PhHgCB\textsubscript{2} in benzene at 80°C.

The postulated initial step was the addition of the electrophile BrC\textsubscript{2} to the sulphur nucleophile, forming an anion intermediate which picked up a proton yielding PhSCB\textsubscript{2}. Subsequent nucleophilic replacement of bromine by the thiolate gave the product\textsuperscript{14}, (Ph\textsubscript{3}P)\textsubscript{3}CN.

Polymers are formed when dihalogen react with dihaloalkanes. Condensation of \( \text{p-H} \text{SCCH}_{2} \text{CH}_{2} \text{CH}(\text{Me} \text{C} = \text{C} \text{H}) \text{CH}_{2} \text{SH} \) with dihaloalkanes gives polymers\textsuperscript{3,11} such as \( \text{H} = \text{N} = \text{N} \text{C} = \text{C} \text{H}_{2} \text{H}_{2} \text{C} = \text{C} \text{H}_{2} \text{H}_{2} \text{N} \text{C} = \text{C} \text{H}_{2} \text{H}_{2} \text{N} \text{C} = \text{C} \text{H}_{2} \) Hal.

Two different monofunctional high molecular weight chlorides (R\textsuperscript{1}Cl and R\textsuperscript{1}Cl) react with the difunctional thiol, (CH\textsubscript{2}SH)\textsubscript{2}, in the presence of triethylamine to give primarily the symmetrical bisulphides, (R\textsuperscript{1}SC\textsuperscript{2}H\textsubscript{2}BR\textsuperscript{1}SC\textsuperscript{2}H\textsubscript{2}CR\textsuperscript{1})\textsubscript{2} and only very small yields of the unsymmetrical bisulphide R\textsuperscript{1}SC\textsuperscript{2}H\textsubscript{2}SC\textsuperscript{2}H\textsubscript{2}CR\textsuperscript{1} 16.

Recently copper(i) salts including thiocyanates have been studied as nucleophiles. Copper(i)nitriloanthelolate and copper(i)cyanide in DMF did not react with t-butyl chloride or benzyl chloride, but halogenoaromatic compounds react under similar conditions. When the reactions were repeated in the presence of thiourea or quinoline, the expected products, dimer, t-butyl sulphide, valeronitrile and phenylmercury, were obtained. The thiourea or quinoline probably act as ligands and bind strongly to the copper, forming the ion CuX\textsubscript{2}+, leaving the counterion (e.g. Bu\textsuperscript{+} from CuBu) available for normal nucleophilic attack.
2. Reactions with electrophiles of the type RM(CH₂)₃X and RMCH₂X₂
   
   a. Displacement of halogen
      Alkylthio-substituted acetic acids can be obtained from monochloroacetic acid and a thioly²⁹:
      \[ \text{ArOCH₂SH} + \text{ClCO₂H} \rightarrow \text{ArOCH₂SCH₂CO₂H} \]
      Derivatives of 1,1,1-trifluoroacetone may be prepared similarly²⁹:
      \[ \text{CF₃CO₂HCl} + \text{RSH} \rightarrow \text{CF₃CO₂HSR} \quad (R = \text{Et}, \text{Bu}, \text{Ph}) \]

      Organotin derivatives containing the RSCH₂Sn(iv) group can readily be obtained in the reactions of RSH and \( \text{BrCH₃} \) Sn⁺⁺ or RSCH₂Li and CIBr⁺⁺, the former method being preferred. Compounds containing both Sn⁻⁻ and Sn⁻⁻⁻⁻⁻⁻⁻⁻⁻⁻⁻⁻⁻⁻⁻⁻⁻⁻⁻⁻⁻⁻⁻⁻⁻⁻⁻⁻⁻⁻⁻⁻⁻⁻⁻⁻⁻⁻⁻⁻⁻⁻⁻⁻⁻⁻⁻⁻⁻⁻⁻⁻⁻⁻⁻⁻⁻⁻⁻⁻⁻⁻⁻⁻⁻⁻⁻⁻⁻⁻⁻⁻⁻⁻⁻⁻⁻⁻⁻⁻⁻⁻⁻⁻⁻⁻⁻⁻⁻⁻⁻⁻⁻⁻⁻⁻⁻⁻⁻⁻⁻⁻⁻⁻⁻⁻⁻⁻⁻⁻⁻⁻⁻⁻⁻⁻⁻⁻⁻⁻⁻⁻⁻⁻⁻⁻⁻⁻⁻⁻⁻⁻⁻⁻⁻⁻⁻⁻⁻⁻⁻⁻⁻⁻⁻⁻⁻⁻⁻⁻⁻⁻⁻⁻⁻⁻⁻⁻⁻⁻⁻⁻⁻⁻⁻⁻⁻⁻⁻⁻⁻⁻⁻⁻⁻⁻⁻⁻⁻⁻⁻⁻⁻⁻⁻⁻⁻⁻⁻⁻⁻⁻⁻⁻⁻⁻⁻⁻⁻⁻⁻⁻⁻⁻⁻⁻⁻⁻⁻⁻⁻⁻⁻⁻⁻⁻⁻⁻⁻⁻⁻⁻⁻⁻⁻⁻⁻⁻⁻⁻⁻⁻⁻⁻⁻⁻⁻⁻⁻⁻⁻⁻⁻⁻⁻⁻⁻⁻⁻⁻⁻⁻⁻⁻⁻⁻⁻⁻⁻⁻⁻⁻⁻⁻⁻⁻⁻⁻⁻⁻⁻⁻⁻⁻⁻⁻⁻⁻⁻⁻⁻⁻⁻⁻⁻⁻⁻⁻⁻⁻⁻⁻⁻⁻⁻⁻⁻⁻⁻⁻⁻⁻⁻⁻⁻⁻⁻⁻⁻⁻⁻⁻⁻⁻⁻⁻⁻⁻⁻⁻⁻⁻⁻⁻⁻⁻⁻⁻⁻⁻⁻⁻⁻⁻⁻⁻⁻⁻⁻⁻⁻⁻⁻⁻⁻⁻⁻⁻⁻⁻⁻⁻⁻⁻⁻⁻⁻⁻⁻⁻⁻⁻⁻⁻⁻⁻⁻⁻⁻⁻⁻⁻⁻⁻⁻⁻⁻⁻⁻⁻⁻⁻⁻⁻⁻⁻⁻⁻⁻⁻⁻⁻⁻⁻⁻⁻⁻⁻⁻⁻⁻⁻⁻⁻⁻⁻⁻⁻⁻⁻⁻⁻⁻⁻⁻⁻⁻⁻⁻⁻⁻⁻⁻⁻⁻⁻⁻⁻⁻⁻⁻⁻⁻⁻⁻⁻⁻⁻⁻⁻⁻⁻⁻⁻⁻⁻⁻⁻⁻⁻⁻⁻⁻⁻⁻⁻⁻⁻⁻⁻⁻⁻⁻⁻⁻⁻⁻⁻⁻⁻⁻⁻⁻⁻⁻⁻⁻⁻⁻⁻⁻⁻⁻⁻⁻⁻⁻⁻⁻⁻⁻⁻⁻⁻⁻⁻⁻⁻⁻⁻⁻⁻⁻⁻⁻⁻⁻⁻⁻⁻⁻⁻⁻⁻⁻⁻⁻⁻⁻⁻⁻⁻⁻⁻⁻⁻⁻⁻⁻⁻⁻⁻⁻⁻⁻⁻⁻⁻⁻⁻⁻⁻⁻⁻⁻⁻⁻⁻⁻⁻⁻⁻⁻⁻⁻⁻⁻⁻⁻⁻⁻⁻⁻⁻⁻⁻⁻⁻⁻⁻⁻⁻⁻⁻⁻⁻⁻⁻⁻⁻⁻⁻⁻⁻⁻⁻⁻⁻⁻⁻⁻⁻⁻⁻⁻⁻⁻⁻⁻⁻⁻⁻⁻⁻⁻⁻⁻⁻⁻⁻⁻⁻⁻⁻⁻⁻⁻⁻⁻⁻⁻⁻⁻⁻⁻⁻::-:

   A similar reaction involves replacement of the Cl⁻⁻ in R₂NCl(O)(CH₂)₃Cl and (CICH₂)₃P(O)OPh or tosyl in 4-Me₃C₆H₄SO₂CH₂P(O)Ph₂ with \( \text{RSCH₂SnCl} \) and trialkylphosphine oxides or sulphides, \( \text{RSCH₂SnClX} \) (X = O, S) can be prepared analogously²⁹, from a thiolate anion and \( \text{CICH₂Cl}_2X \).

   b. Displacement of sulphonyl groups.

   Ready replacement of the methylsulphonate group by the benzene-thiolate group from bis(methanesulphonates) of 3-aryliothio-1,2-diols, 2-aryliothio-1,3-diols and 1 aryliothio-2 ols has been reported²⁹,³⁰:

   \[ \text{Me₃SOCl(1,2)CH₂OSO₂Me(1,2)Cl₂(1,2)SO₂Me(1,2)H} \rightarrow \text{Me₃SOCl(1,2)CH₂OSO₂Me(1,2)Cl₂(1,2)SO₂Me(1,2)H} \]

   The reaction proceeded via a direct SN₂ substitution except when rearrangement occurred, which was only partially observed in the reaction

   \[ \text{Me₃OC₆H₄SnCl(1,2)CH₂OSO₂Me(1,2)Cl₂(1,2)SO₂Me(1,2)H} \rightarrow \text{Me₃OC₆H₄SnCl(1,2)CH₂OSO₂Me(1,2)Cl₂(1,2)SO₂Me(1,2)H} \]

   The cyclic intermediate \( \text{CH₃(1,2)SnClX} \) is postulated. It is impossible to detect whether rearrangement or direct substitution occurred in the reaction

   \[ \text{Me₃OC₆H₄SnCl(1,2)CH₂OSO₂Me(1,2)Cl₂(1,2)SO₂Me(1,2)H} \rightarrow \text{Me₃OC₆H₄SnCl(1,2)CH₂OSO₂Me(1,2)Cl₂(1,2)SO₂Me(1,2)H} \]

   The nitrile groups were reduced in derivatives of 2,4-dinitrophenol.

16. Thiols as nucleophiles

   The trifluoromethanesulphonium group is displaced in p-tolylsulphonylmethyltrifluoromethanesulphonate in the SN₂ reaction with nucleophiles, such as benzenethiolate²⁹:

   \[ \text{p-Me₃C₆H₄SO₂CH₂OSO₂Me(1,2)Cl₂(1,2)SO₂Me(1,2)H} \rightarrow \text{p-Me₃C₆H₄SO₂CH₂OSO₂Me(1,2)Cl₂(1,2)SO₂Me(1,2)H} \]

   In the reaction of 2,2-dialky-3-(tosyloxyl)propionaldehydes with benzene- or methane-thiolate the tosyl group is displaced and it is postulated that the attack originates at the carbon atom of the carbonyl group²⁹:

   \[ \text{Me₃C₆H₄COCl(1,2)CH₂OSO₂Me(1,2)Cl₂(1,2)SO₂Me(1,2)H} \rightarrow \text{Me₃C₆H₄COCl(1,2)CH₂OSO₂Me(1,2)Cl₂(1,2)SO₂Me(1,2)H} \]

   The carbon-sulphur bond is fractured in the reaction of p-toluene-sulphonyl cyanide with sodium ethanethiolate in ethanol. Other thiols, not thiocyanates, also fracture the carbon-sulphur bond²⁹:

   \[ \text{p-TolSO₂CN(1,2)CNCl₂(1,2)SO₂Me(1,2)H} \rightarrow \text{p-TolSO₂CN(1,2)CNCl₂(1,2)SO₂Me(1,2)H} \]

3. Reactions of electrophiles of the type Ar(CH₂)₃X

   The chlorine kinetic isotope effect in nucleophile displacement at saturated carbon in para-substituted benzyl chlorides, with thiolate and analogous oxygen nucleophiles, has been examined²⁹. The reactions proceed via a concerted transition state.

   \[ \text{R''S⁻⁻⁻⁻⁻⁻⁻⁻⁻⁻⁻⁻⁻⁻⁻⁻⁻⁻⁻⁻⁻⁻⁻⁻⁻⁻⁻⁻⁻⁻⁻⁻⁻⁻⁻⁻⁻⁻⁻⁻⁻⁻⁻⁻⁻⁻⁻⁻⁻⁻⁻⁻⁻⁻⁻⁻⁻⁻⁻⁻⁻⁻⁻⁻⁻⁻⁻⁻⁻⁻⁻⁻⁻⁻⁻⁻⁻⁻⁻⁻⁻⁻⁻⁻⁻⁻⁻⁻⁻⁻⁻⁻⁻⁻⁻⁻⁻⁻⁻⁻⁻⁻⁻⁻⁻⁻⁻⁻⁻⁻⁻⁻⁻⁻⁻⁻⁻⁻⁻⁻⁻⁻⁻⁻⁻⁻⁻⁻⁻⁻⁻⁻⁻⁻⁻⁻⁻⁻⁻⁻⁻⁻⁻⁻⁻⁻⁻⁻⁻⁻⁻⁻⁻⁻⁻⁻⁻⁻⁻⁻⁻⁻⁻⁻⁻⁻⁻⁻⁻⁻⁻⁻⁻⁻⁻⁻⁻⁻⁻⁻⁻⁻⁻⁻⁻⁻⁻⁻⁻⁻⁻⁻⁻⁻⁻⁻⁻⁻⁻⁻⁻⁻⁻⁻⁻⁻⁻⁻⁻⁻⁻⁻⁻⁻⁻⁻⁻⁻⁻⁻⁻⁻⁻⁻⁻⁻⁻⁻⁻⁻⁻⁻⁻⁻⁻⁻⁻⁻⁻⁻⁻⁻⁻⁻⁻⁻⁻⁻⁻⁻⁻⁻⁻⁻⁻⁻⁻⁻⁻⁻⁻⁻⁻⁻⁻⁻⁻⁻⁻⁻⁻⁻⁻⁻⁻⁻⁻⁻⁻⁻⁻⁻⁻⁻⁻⁻⁻⁻⁻⁻⁻⁻⁻⁻⁻⁻⁻⁻⁻⁻⁻⁻⁻⁻⁻⁻⁻⁻⁻⁻⁻⁻⁻⁻⁻⁻⁻⁻⁻⁻⁻⁻⁻⁻⁻⁻⁻⁻⁻⁻⁻⁻⁻⁻⁻⁻⁻⁻⁻⁻⁻⁻⁻⁻⁻⁻⁻⁻⁻⁻⁻⁻⁻⁻⁻⁻⁻⁻⁻⁻⁻⁻⁻⁻⁻⁻⁻⁻⁻⁻⁻⁻⁻⁻⁻⁻⁻⁻⁻⁻⁻⁻⁻⁻⁻⁻⁻⁻⁻⁻⁻⁻⁻⁻⁻⁻⁻⁻⁻⁻⁻⁻⁻⁻⁻⁻⁻⁻⁻⁻⁻⁻⁻⁻⁻⁻⁻⁻⁻⁻⁻⁻⁻⁻⁻⁻⁻⁻⁻⁻⁻⁻⁻⁻⁻⁻⁻⁻⁻⁻⁻⁻⁻⁻⁻⁻⁻⁻⁻⁻⁻⁻⁻⁻⁻⁻⁻⁻⁻⁻⁻⁻⁻⁻⁻⁻⁻⁻⁻⁻⁻⁻⁻⁻⁻⁻⁻⁻⁻⁻⁻⁻⁻⁻⁻⁻⁻⁻⁻⁻⁻⁻⁻⁻⁻⁻⁻⁻⁻⁻⁻⁻⁻⁻⁻⁻⁻⁻⁻⁻⁻⁻⁻⁻⁻⁻⁻⁻⁻⁻⁻⁻⁻⁻⁻⁻⁻⁻⁻⁻⁻⁻⁻⁻⁻⁻⁻⁻⁻⁻⁻⁻⁻⁻⁻⁻⁻⁻⁻⁻⁻⁻⁻⁻⁻⁻ประโยך
4. Reactions with cyclic compounds

Replacements of substituents by a thiolate group occur in several cyclic compounds. Several products are found in the reaction of 2-phenylcyclohexyl-p-toluenesulphonate (I) with the dimetastate salt of mercaptoacetic acid in methanol, corresponding to simple replacement, neighbouring

\[ \text{OMe} \xrightarrow{\text{SCH₂CO₂H}} \text{OTs} \]

\( \text{cis and trans} \)

\( \text{SCH₂CO₂H} \)

\( \text{OMe} \)

\( \text{SCH₂CO₂H} \)

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For instance, trans-1-hydroxy-2-ethylthio-tetrahydropyran (7). When R = H, the product is 45% dialyl and 45% diquatorial.

\[
 \begin{align*}
 (6) & \rightarrow (7) \\
 R - R' & \rightarrow H - Me, R' = \text{Et, Ph}
\end{align*}
\]

The ring is partially fractured in the treatment of 3-chlorothietane (8) with the benzenethiolate anion. A mixture containing 30% of phenyl-3-thiostyryl sulfide (9) and Ph,S,C6H4CH=CH2 was obtained. The latter was also prepared from Ph,SCl and HSCH2CH=CH2. The reaction probably proceeds via the formation of the cations H,S,C=CHCH2S- and S-. The C=S bond is fractured in 2-dialkylamino-1,3-dithiolium perchlorate (10) when treated with the ethane-thiolate anion in DMF. Quite different products are found with the ethoxide ion as a nucleophile, involving attack on the 2-carbon atom as opposed to attack on the 4-carbon atom with the ethane-thiolate anion.

\[
 \begin{align*}
 (8) + \text{PhS}^- & \rightarrow \text{SPh} + \text{PhS}, \text{C}_6\text{H}_4\text{CH}=\text{CH}_2 \\
 \text{NR}_2 & \rightarrow \text{ESCH}_2\text{C}_6\text{H}_4\text{S}(\text{S})\text{NR}_2 \\
 \text{NR}_2 & \rightarrow \text{ESCH}_2\text{C}_6\text{H}_4\text{S}(\text{S})\text{NR}_2 \\
 \text{EtOH} & \rightarrow \text{EtOH} \\
 \text{EtOH} & \rightarrow \text{EtOH} \\
 \text{EtOH} & \rightarrow \text{EtOH}
\end{align*}
\]

16. Thiols as nucleophiles

5. Reactions with $R\text{CC} = CX$, $R'\text{RC} = CR'X$ and $R''\text{RC} = NX$

The reactions described in this section will be concerned primarily with the replacement of a group X with a group R, and not addition reactions to Alkyne derivatives. Three different routes are proposed for the reaction of acetylenes with nucleophiles:

\[
 (\text{Ar})\text{C}=\text{CCl}^+ + \text{Nu}^- \rightarrow (\text{Ar})\text{C}=\text{CNu}^- \\
 (\text{Ar})\text{C}=\text{C}^+ + \text{NU}^- \rightarrow (\text{Ar})\text{C}=\text{C}^+ \\
 (\text{Ar})\text{C}=\text{C}^+ + \text{Nu}^- \rightarrow (\text{Ar})\text{C}=\text{C}^+ 
\]

The intermediates react further to give $\text{Ar}, \text{Ar}, \text{Cl} = \text{N}, \text{Nu}, \text{I}$, with thiolate nucleophiles (EtS- and PhS-). Hal = Cl, Br, I, the mechanism is restricted to attack on the halogen (5), but attack on the carbon is also observed in the reaction of EtS- and ArC=CCl. The second-order rate constants in methanol-water mixtures for meta- and para-substituted 1-bromo-2-phenylacetylenes correlate well with Hammett $\sigma$ constants; $p = 1.15$. A linear correlation was also observed between log $k_2$ and $pF_4$ of the corresponding thiol.

The rate constants for the reaction of $p$-ZC,H$_4$C=CHHal ($Z = \text{Me, H, Cl; Hal = Cl, Br}$) with $p$-MeC$_6$H$_4$S$-$Na$^+$ in DMF, forming $p$-ZC,H$_4$C=CS$_2$,H$_4$SMe$-$p have been measured. Attempts to trap the ion $p$-Cl$_4$H$_4$C=Cl$^-$ were unsuccessful. These results have, however, been interpreted differently from those presented in the previous paragraph, and an addition-elimination mechanism is favoured, involving the formation of $p$-ZC,H$_4$C=CHHal($\text{S}_2$H$_4$Me$-$p) and fast elimination of Hal$^-$ to give the product.

Various products were obtained from the reaction of sodium thiolate with the acrylène derivates: $\text{HC} = \text{CMe}(\text{SR})\text{COe}$, $\text{H} = \text{C}=\text{CMe}(\text{SR})\text{COe}$, $\text{HC} = \text{C}=\text{CMe}(\text{SR})\text{COe}$, $\text{Me}(\text{SR})\text{C}=\text{CMe}(\text{SR})\text{COe}$, where the thiolate replaced the halogen, acting as a reducing agent or added across an acetylenic or ethenylene bond.
The proportion of the reduction products, especially when \( \text{Hal} = \text{Br} \), increased with the basicity of the nucleophile and its concentration.

The reactions of 1,3-dihaloalkynes (II) with nucleophiles, amines and thiols have been studied. Heterocyclic thiols are used as potassium salts in aqueous methanol.

\[
\text{Hal} \text{C} = \text{CHX}_2 + \text{Hal}^+ + \text{RS}^- \rightarrow \text{Hal} \text{C} = \text{CHX} + \text{SR}^-
\]

(II)

(\( \text{Hal} = \text{Cl}, \text{Hal}^+ = \text{I}(a); \text{Hal} = \text{Br}, \text{Hal}^+ = \text{I}(b) \) or \( \text{Br} \))

The reactions of 11a or 11b with other thiols RH (is ethyl, \( \text{t}-\text{butyl}, \text{phenyl}, \) or benzyl), in the presence of potassium hydroxide, however, gave disulphides and the corresponding dialkyl, diphenyl or dibenzyl disulphide, which could in most cases be isolated quantitatively.

\[
\text{IC} = \text{CCH}_2 + \text{PhCH}_2 \text{SH} + \text{PhCH}_2 \text{SH} \rightarrow \text{HC} = \text{CCH}_2 + (\text{PhCH}_2), + \text{I}^-
\]

The only thiols forming iodoacetylenic sulphones were heterocyclic thiols having a tautomeric thiolactam structure.

**II. Allicic derivatives.** Substitution reactions of nucleophiles with ethylenic substrates have been recently reviewed, and the similarity with aromatic nucleophilic substitution emphasized. The possible mechanisms of these reactions have been discussed.

A simple example of substitution in a vinyl halide is the preparation of thiol derivatives of 1-cyclohexene from the thiol and sodamide in THF with chloro-1-cyclohexene (12).  

\[
\begin{array}{c}
\text{Cl} \text{C} \text{Cl} \\
\text{SR} \\
\text{Cl}^-
\end{array}
\]

(12)

Vinyl bromides react with copper(i) thioclates, both aliphatic and aromatic, to give vinyl sulphones. Vinyl bromides studied include \( \beta \)-bromostyrene and 1-bromo-2-methyl-1-propene. This method of synthesis of thioethers is claimed to be superior to that using sodium thioclates and most other reported methods. 1,3-Dibromoethylene gives a mixture of cis (18%) and trans (42%). 1,2-Diphenylthioethylene with copper(i) benzenethiolate, but with copper(i) ethanethiolate ethylenethiocarbonyl is formed with the elimination of hydrogen bromide.

\[
\text{BrC} = \text{CHBr} + \text{CuSeI} \rightarrow \text{HC} = \text{CSeI} + \text{HBr} + \text{CuBr}
\]

16. Thiols as nucleophiles

When substitution occurs in an ethylene derivative, it is of interest to observe whether the original configuration is retained. Several reactions of ethylenic compounds where configuration is retained have been examined. Some are shown below.

\[
\begin{align*}
\text{HalC} = \text{CHCO}_2 \text{Et} + \text{G}^- & \rightarrow \text{E} \text{G} \text{C} = \text{CHCO}_2 \text{Et}^+ \quad (\text{Hal} = \text{Cl}, \text{Br}, \text{I}) \\
\text{R} \text{P} \text{SO} \text{Cl} \text{C} = \text{C}(\text{OSO} \text{R} \text{O}) \text{R} + \text{RS}^- & \rightarrow \text{R} ‏(\text{R} \text{S} \text{O}) \text{C} = \text{C}(\text{RSO} \text{R} \text{O}) + \text{R} \text{SO} \text{R} \text{H}
\end{align*}
\]

In the former reaction, mixtures of isomers are formed when the ethoxide ion is used as a nucleophile. In the latter reaction, when a thiolate instead of a thiol is used as the nucleophile, the electrophilic carbon of the trinitrobenzene residue is attacked forming a sulphide and ketone.

\[
\begin{align*}
\text{Ph} \text{H} + \text{C} \text{I} \text{C} \text{H} \text{S} \text{I} \text{C} = \text{C} \text{I} \text{N} \text{O} \text{S} \text{O} \text{R} \text{A} + \text{H} \text{C} \text{I} \text{C} \text{H} \text{S} \text{R} & \rightarrow \text{Ph} \text{H} \text{C} \text{I} \text{C} \text{H} \text{S} \text{N} \text{O} \text{S} \text{O} \text{R} \text{A} + \text{R} \text{C} \text{I} \text{C} \text{H} \text{R} \text{S} \text{N} \text{O} \text{S} \text{O} \text{R} \text{A} + \text{R} \text{C} \text{I} \text{C} \text{H} \text{S} \text{R}
\end{align*}
\]

\( \text{R} = 0,4-(\text{NO}_2)_2 \text{C}_6 \text{H}_4 \)

In some reactions such as

\[
\begin{align*}
\text{P} \text{SO} \text{H} \text{N} \text{C} = \text{CHF} + \text{Ph} \text{S}^- & \rightarrow (\text{Ph} \text{SO}) \text{H} \text{C} = \text{C}(\text{SPH}) \text{H} + \text{F}^-
\end{align*}
\]

the trans reactant gives the trans product, but the cis reactant gives cis and trans products in a 3:1 ratio.

When several halides are present, as in trifluoroacrolethylene, replacement of a fluorine with a thiolate occurs:

\[
\begin{align*}
\text{CF}_2 = \text{C} \text{F} \text{C} + \text{P} \text{S} \text{H} \text{N} \text{A} & \rightarrow (\text{Ph} \text{S}) \text{C} = \text{C} \text{F} \text{C} + \text{N} \text{A} \\
\text{Butanethiol reacts similarly with CF}_2 = \text{C} \text{H} \text{Hal} (\text{Hal} = \text{Cl}, \text{Br}), \text{and CF}_2 = \text{C} \text{Chl}, \text{forming R} \text{SO} \text{C} = \text{C} \text{F} \text{H} \text{Hal} \text{and R} \text{SO} \text{C} = \text{C} \text{F} \text{Hal} \text{), respectively.} & \text{In the compound AcNHCH} = \text{CO} \text{Br } \text{the butanethiolate can replace one of the chloride atoms or add across the double bond, forming AcNHCH} = \text{CO} \text{Br} \text{A} \text{ and AcNHCH} = \text{N} \text{O} \text{S} \text{O} \text{Br} \text{AcNHCH} = \text{CO} \text{Br} \text{A} \text{ and AcNHCH} = \text{N} \text{O} \text{S} \text{O} \text{Br} \text{C}, \text{respectively.} & \text{Other interesting examples of this type of reaction include that of hexachlorofulvene (13) with p-MeC}_6 \text{H}_4 \text{SH, in the presence of triethylamine.} & \text{Various acrylonitrile derivatives have been examined. The configuration is retained in the reaction of 3-halomethacrylonitriles with sodium}
\end{align*}
\]

\[ \text{(13)} \]
other acrylic acid derivatives with thiolates can give nucleophilic replacement, or the thiol can be oxidized to the disulphide\(^\text{11}\).

\[
\text{MeC(R)=CBrCO_2Et+PhSNa} \rightarrow \text{MeC(R)=C(SPh)CO_2Et}
\]

\[ (R = \text{Et, i-Prop, PhH, 2,2-i}) \]

\[
\text{MeC(SE)=CBrCO_2Et+EtS^-/EISH} \rightarrow \text{MeC(SE)=CHCO_2Et+EtSSEt+Br^-}
\]

The rate constants for the addition of butanethiol to ethyl acrylate have been measured by iodometry over a wide pH range. Below pH 4 it is assumed that the reaction is initiated by the neutral molecule, but at pH > 7 the anion BS\(^-\) started the reaction\(^\text{11}\).

Cyclization occurs when dichloro- and dibromo-maleic acids react with thiols in the presence of triethylamine forming 2-halo-3-mercaptomaleic acid esteric (16) derivatives\(^\text{12}\), e.g.

\[
\begin{align*}
\text{ClCOO}_2\text{H} + \text{EtS}^- & \rightarrow \text{EtS} \quad \text{(16)} \\
\text{ClCOO}_2\text{H} & \rightarrow \text{Cl} \quad \text{HO} \quad \text{O}
\end{align*}
\]

c. Imine derivatives. Displacements in compounds having C=\(\text{N}\) bonds have been observed. One of the simplest types of reaction is that of C\(\text{H}_3\text{C}=\text{N}\text{O}\), which can be converted into o-(thiocyanomethyl)-benzamide, an antibacterial, by refluxing with the sodium salt of

16. Thiols as nucleophiles

\[ o\text{-mercapto- benzamide}\]

\[ \text{CL}_{2}\text{C} = \text{NMe}_2 + \text{HS}^- \rightarrow \text{CL} = \text{C} = \text{NMe}_2 + \text{MeSH} ]

\[ \text{Cl} = \text{C} = \text{S}^\text{S} \]

\[ \text{NH} \]

The bromine on the boron may react further

\[ \text{Cl} = \text{C} = \text{S} = \text{N} \rightarrow \text{BHMeBr} = \text{Br} + \text{SMe}^{-} \]

\[ \text{Cl} = \text{C} = \text{S} \rightarrow \text{N} \rightarrow \text{BHMeBr} = \text{Br} + \text{SMe}^{-} \]

Similar reactions are observed with 1,1-dibromo-4-(\(p\)-nitrophenyl) and 1,1-dibromo-4-(\(p\)-chlorophenyl)2,3-diazabuta-2,3-dienes and aliphatic or aromatic thiols, resulting from the thiolysis of one or two bromine atoms\(^\text{12}\).
on the thiolates as nucleophiles in aromatic substitution. Few kinetic data are available. Any data show that the RS\(^-\) is a better nucleophile than its oxygen analogues, although this has been questioned\(^{19}\).

The reactions of various halonitrobenzenes with thiolates have been studied in more detail. A comprehensive review of the activating effects of the nitro group in aromatic substitution\(^{19}\) covers the literature up to the middle of 1967. This review discusses primarily the displacement of halogen, although displacement of other groups such as hydrogen, nitro, alkoxo, arylxoy, and sulphonate are also considered. The relative rates of the reaction of 1-X-2,4-dinitrobenzenes with piperidine in MeOH at 0°C decreases in the series Fâ‰’ NO\(_2\)â‰’ OSO\(_2\)C\(_6\)H\(_4\)Clâ‰’ SO\(_2\)CH\(_2\)-Br â‰’ Cl â‰’ SO\(_2\)CH\(_2\)-OH â‰’ CH\(_2\)NO\(_2\) > 1. A similar sort of series can be expected when the nitrato anion is used as a nucleophile. The reaction of nitro compounds with nucleophiles occurs primarily via an addition-elimination mechanism, involving a Meisenheimer complex.

\[
\text{Fe}^+ \xrightarrow{RS-} \text{RS}^+ \xrightarrow{\text{NO}_2^-} \text{RS}^+ \text{NO}_2^- + \text{e}^- \]

Obvious variables in such a reaction are the stereochemistry of the entering group, the stability of the intermediate Meisenheimer complex, and the effect of the leaving group. A thermochemical approach concluded that the decomposition of the Meisenheimer complex was rate determining\(^{19}\), however, this is not in accord with the leaving group lability\(^{19}\). As cleavage of the carbon-fluorine bond is acid catalysed, it has been concluded that the rate-determining step is the formation of the Meisenheimer complex rather than its decomposition\(^{25}\). Substitution of 2,4-dinitrochlorobenzene with 2,3,5,6-tetrafluorobenzene thiolate gives replacement of the chlorine\(^{25}\). A detailed discussion of the thermodynamics of the reaction of MeS\(^-\) and PhS\(^-\) with 1-X-2,4-dinitrobenzene has been reported\(^{25}\).

The nucleophilic activity is PhS\(^-\): MeS\(^-\): for the reaction with 1-iodo-2,4-dinitrobenzene, but MeS\(^-\): PhS\(^-\): for p-fluoronitrobenzene\(^{40}\). Data on the reaction of substituted halogenobenzothiazoles show that there are appreciable steric effects in the cases of a branching (methyl ethyl \(\beta\)-propyl > t-butyl), whereas \(\beta\) and \(\omega\) branching do not cause any steric effect and influence the reaction rates only slightly of their typical electronic effects\(^{67}\). The mobility of the leaving halogen, derived from kinetic data with various halogenonitrobenzenes, is F > Cl > Br > I\(^{49}\).

The intermediate Meisenheimer complexes have been reviewed\(^{19,75}\), and

![Diagram](image1)

16. Thiois as nucleophiles

the work of Crampton is important in this area\(^{77,78,79}\). Further reference should be made to the chapter in this book by M. R. Crampton.

When substitution occurs in polyhalogenated aromatic compounds, such as the pentfluorobenzene derivatives, C\(_5\)F\(_5\)X, the extent of the replacement of F or X by the nucleophile and the product orientation must be determined.

A detailed study of the orientation and reactivity in the nucleophilic displacement reactions of aromatic polyhalo-compounds has been published\(^{90}\). This involves study of the stability of the Wheland type intermediates (17, 18) where Nu is a nucleophile. The formation of meta products with a nucleophile may be rationalized by the scheme involving a carbene intermediate\(^{93}\).

![Diagram](image2)

Most activating groups cause primarily para substitution but some ortho substitution may occur. Deactivating groups, such as NH\(_2\), O\(^-\), or S\(^-\) will cause meta substitution\(^{98,99}\). The solvent plays an important role in determining the relative amounts of ortho and para substitution. Solvents with dielectric constant lower than about 30 cause some para substitution, whereas solvents of dielectric constant greater than 30 cause almost exclusive para substitution. This has been attributed to increasing ion dissociation of the nucleophile in the higher dielectric constant solvents\(^{96,98}\). Presumably the formation of meta substitution products in solvents of
low dielectric constant does not involve the formation of the thiolate anion as an active entity.
Thiolates can also cause dehalogenation of various halogen compounds, such as 2-bromo-3-nitro-thiophene\(^{48}\) and 2- and 4-halo-1-naphthol\(^{49}\).

2. Substitution in hexahalobenzenes

Pentafluoro- and pentachloro-benzenethiols can readily be prepared by the reaction of a hydrogen sulphide anion, \(\text{SH}^-\), with hexafluoro- and hexachlorobenzene respectively\(^{50}\). No dihalo can be produced in this reaction. Due to the basic medium employed the thioph will form present as its thiolate anion, which is not readily attacked further nucleophilically. Using hexafluorobenzene and excess hydrogen sulphide peri-halogenopropenylene sulfone may be isolated\(^{51}\). When the hydrogen sulphide anion is replaced by a thiophosphate, multiple replacement of fluoride or chlorine can occur. The products of these reactions can be summarised:

\[
\begin{align*}
\text{C}_6\text{H}_5\text{SR}^- & \rightarrow \text{C}_6\text{H}_5\text{SH}^- & (19) \\
\text{C}_6\text{H}_5\text{SR}^- & \rightarrow \text{C}_6\text{H}_5\text{O}^- & (20) \\
\text{C}_6\text{H}_5\text{SR}^- & \rightarrow \text{C}_6\text{H}_5\text{N}^- & (21) \\
\text{C}_6\text{H}_5\text{SR}^- & \rightarrow \text{C}_6\text{H}_5\text{S}^- & (22) \\
\text{C}_6\text{H}_5\text{SR}^- & \rightarrow \text{C}_6\text{H}_5\text{S}^- & (23) \\
\text{C}_6\text{H}_5\text{Br}^- & \rightarrow \text{C}_6\text{H}_5\text{Br}^- & (24)
\end{align*}
\]

The reaction of hexafluorobenzene with various nucleophiles (\(R = \text{Me}^{52}, \text{Et}^{53}, \text{Ph}^{54, 55}, p-\text{ClC}_6\text{H}_4^{-}, p-\text{NH}_2\text{C}_6\text{H}_4^{-}\)) in ethylene glycol and/or pyridine as a solvent has been studied. The products obtained are \(19, 20\) and \(22\). The compounds \(21, 23\) and \(24\) have not been isolated, but \(21\) must be present as intermediate in the conversion of \(20\) to \(22\). The orientation of the products has been deduced from \'H and \(19^F\) n.m.r. spectra\(^{56}\), or chemical oxidation and Raney nickel degradations\(^{57}\). The compound \(20\) has the two RS groups para, whereas the compounds \(\text{C}_6\text{F}_5\text{SMe}^{-}\), \(\text{C}_6\text{F}_5\text{SEH}^{-}\), \(\text{C}_6\text{F}_5\text{SMe}^{-}\)(SPh)\(^{-}\), and \(\text{C}_6\text{F}_5\text{S}(\text{SPh})\(^{-}\) have the two fluorines para\(^{58}\). When 2-mercaptoethanol was used as a nucleophile, the sulphur atom rather than the oxygen acted as the nucleophile and 1,2,4,5-tetrafluoro-5,6-bis-2-hydroxyethylthiobenzene was isolated\(^{59}\).

This work has also been extended to difluoroarylphenyl ether where each ring is substituted once or three times:

\[
\begin{align*}
\text{C}_6\text{F}_5\text{F} + \text{SH} & \rightarrow \text{HO}-\text{C}_6\text{F}_5\text{F} + \text{H} & (25) \\
(\text{RS})\text{C}_6\text{F}_5\text{F} + (\text{SR})\text{C}_6\text{F}_5\text{F} & \rightarrow 2 \text{RS} + \text{H} & (26)
\end{align*}
\]

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The predominant product is \(25\) when \(R = \text{Et}\) or \(\text{Ph}\), but when \(R = \text{Me}\), the mono- and tri-substituted products are formed. The orientation of \(25\) is \(p-(\text{RS})\text{C}_6\text{F}_5\text{F} + (\text{SR})\text{C}_6\text{F}_5\text{F}\)^{59} and that of \(26\) is probably\(^{59}\).

Substitution of hexachlorobenzene with various nucleophiles has also been studied\(^{60, 61}\). No monosubstituted products were isolated.

\[
\begin{align*}
\text{C}_6\text{C}_6\text{F}_5\text{S}^- \rightarrow p-(\text{RS})\text{C}_6\text{C}_6\text{F}_5\text{S}^- + p-(\text{RS})\text{C}_6\text{C}_6\text{F}_5\text{S}^- & \rightarrow 2 \text{RS} + \text{H} & (27) \\
\end{align*}
\]

The orientation of the di-substituted product has been deduced by alternate synthesis, whereas that of \(p-(\text{ClC}_6\text{C}_6\text{F}_5\text{S})\) has only been derived intuitively\(^{62}\). Attempts to use the \(\text{C}_6\text{Cl}_6\text{S}^-\) anion as a nucleophile to form the sulphide \(\text{C}_6\text{Cl}_6\text{S}^-\) have failed\(^{63}\).

The obvious extension of this work to hexabromobenzene has been investigated, where it is found that the \(\text{SMe}^-\) anion will not react\(^{64}\). Study of the reactions of other nucleophiles with hexachlorobenzene leads to photodebromination and some nucleophilic substitution\(^{65}\). Pentafluorobenzenselenethiol has recently been prepared from the pentahaloaryl Grignard reagent and sulphur\(^{66}\).

A somewhat analogous system is pentafluoropyridine where substitution with hydrogen sulphide anion, or benzeneoxide, occurs para to the nitrogen. The thiol formed reacts with pentafluoropyridine to give the corresponding sulphide\(^{67}\). 2,3,5,6-Tetrafluorobenzylthiol was isolated\(^{68}\). The reaction of CuSBU with \(\text{C}_6\text{F}_5\text{Br}^{-}\) gave two products:\n
\[
\begin{align*}
\text{C}_6\text{F}_5\text{Br} + \text{CuSBU} & \rightarrow \text{C}_6\text{F}_5\text{Br} + \text{CuSBU} + \text{SMe} + \text{C}_6\text{F}_5\text{H} & (28)
\end{align*}
\]
The ratio of the products depended on the solvent employed. In DMF product 27 was formed exclusively, whereas product 28 involving halogen reduction was formed in various solvents in the presence of thiourea, although thiourea alone does not react with bromopentafluorobenzene. In similar experiments using chloropentafluorobenzene no reaction occurred in the absence of thiourea, but when it was added exclusive fluorine replacement occurred without chlorine reduction. With iodopentafluorobenzene and copper(i) benzenethiolate and urea, rapid reduction of the iodine occurred together with multiple fluorine replacement resulting in the formation of 2,4-difluoro-1,5-tri(thiophen-2-yl) benzene, pentafluorobenzene gave essentially the same products under the same conditions. The formation of product 27 without further substitution suggests that species such as $C_6F_5(Cu(urea))$ may be ligated to the copper. A reaction scheme has been postulated involving the participation of the solvent, and the thiolate anion acting as a reducing agent,

$$[CuSR\text{IL}_2]_2 \xrightarrow{\text{C}_{13}F_6\text{Br}} C_{13}F_5SR+\{Cu\text{Hal}_2\}$$

$A = 1:3$

$$[Cu(SR)_{13}^{+}\text{SR}^- \xrightarrow{\text{C}_{13}F_6\text{Br}} F\text{ displacement reduction of Hal} \xrightarrow{\text{Br}} RSCF_2CF_2F$$

$R = 1:4$

Nucleophilic substitution of tetrafluorophthalonitrile (29) with the benzenethiolate anion gives replacement of two or four fluorine atoms, but not the nitrite groups.

$$\text{F} \quad \text{F} \quad \text{CN} \quad \text{Ph}S \quad \text{CN} \quad \text{Ph}S \quad \text{CN} \quad \text{Ph}S \quad \text{CN}$$

In solvent water the tetrasubstituted product is formed, but in methanol the ratio of disubstituted to tetrasubstituted is about $8:1^{16}$. The formation of 4- and 5-disubstitution product rather than the anticipated 3- and 6- is similar to that observed in analogous reactions, and may be due to the

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formation of a more stable para than ortho intermediate (30). The orientation of the product has been deduced from its $^{19}F$ n.m.r. spectrum.

![Diagram](image)

4. Substitution in halobenzenes

The reactions of various fluorobenzenes with thiolate anions have been investigated in ethylene glycol/pyridine mixtures. The results are shown below using the methanethiolate anion as a nucleophile.

$$\text{F} \quad \text{F} \quad \text{F} \quad \text{H} \quad \text{MeS} \quad \text{Me} \quad \text{F}$$

$$\text{F} \quad \text{F} \quad \text{F} \quad \text{H} \quad \text{MeS} \quad \text{Me} \quad \text{F}$$

$$\text{F} \quad \text{F} \quad \text{F} \quad \text{H} \quad \text{MeS} \quad \text{SMe} \quad \text{F}$$

Product orientations have been deduced from $^1H$ and $^{19}F$ n.m.r. No reaction occurred with any difluorobenzene or fluorobenzene. Under these conditions the maximum substitution observed requires there to be two fluorine atoms still in the nucleus. Changing the solvents it is possible
to replace the fluorine in fluorobenzene or bromine in bromobenzene by a thiolate group, for example in HMPA/THF solvent mixtures using EtSnNa, BuSnNa or PhSnNa in the presence of NaNH₂. The sulphenates C₅H₅SBr are formed[100]. An ideal solvent was found to be HMPA-THF in the ratio 1:5[96]. Similar reactions involving replacement of one or two aromatic halogens with potassium benzene-thiolate or potassium thio-
resorcinate have been observed in pyridine as solvent[98].

Reaction of pentafluorobenzene with thiolate(1) benzene-thiolate gives 2,4-difluoro-1,3,5-tris(phenylthio)benzene. This orientation is not in-
consistent with the 10¹ n.m.r.[99]. The fluorine para to the hydrogen in pentafluorobenzene has been replaced by a variety of nucleophiles, such as H₅C₅F₅S⁻ forming p-H₂C₅F₅SC₅F₅H-p[79].

5. Substitution in miscellaneous polyhalogenated aromatics

The reaction of nitro and amino fluorobromobenzenes of the type α-XC₅F₅Br and p-XC₅F₅Br where X = NO₂ or NH₂ with the pentafluoro-
benzene-thiolate anion, in its copper(I) salt, resulted in the replacement of the bromine[98]. The pentafluorobenzene-thiolate anion or the anion of 2,3,5,6-tetrafluoro-4-mercaptopyridine, replaced the fluorine ortho or para to the nitro group in nitro-pentafluorobenezene. Para substitution only occurred in solvents of high dielectric constant, such as DMF and acetonitrile, whereas in solvents of low dielectric constant, such as ether, mixed replacement of ortho and para-fluorine was observed[96][86]. Increasing ionization of the thiol is postulated to cause predominantly para substitution.

6. Substitution in monohalogenated benzene derivatives

This section includes compounds such as 1-fluoro-2-nitrobenzene, where the fluorine atom is activated by the nitro group. The reactions of halo-
nitrobenzenes with thiolate nucleophiles have been reviewed[96]. The fluorine atom may easily be replaced in 1-fluoro-2-nitrobenzene by 2,3,5,6-tetrafluorobenzene-thiolate forming o-nitrophenyl-2,3,5,6-tetra-
fluorophenyl sulphide, but polymerization of the pentafluorobenzene-
thiolate anion occurred when it was employed as the nucleophile[95]. Replacement of halogen in the cyclic derivatives such as 1- and 2-fluoro-
and -chloro-anthaquinones[98], and various halo-1,2,3-benzothiazoles[98], is also observed.

Considerable use has been made of the copper(1) benzene-thiolate and butanethiolate in the preparation of thiocycles. A large series of com-
ounds of general formula (R₅)ₓX, x > 1, R = Ph or Br, and X is an aryl group, have been prepared from the copper(I) thiocycles and aryl halides (aryl bromides only reacted with the butanethiolate)[96][98].

7. Substitution in heterocyclic compounds

This type of reaction is essentially similar to that of replacement of an aromatic halogen by a thiolate group. Heterocyclic compounds studied include 3,4-dimethyl-5-bromo-2(N,N-dimethylaminomethylene)-2H-pyrrole[98] and chlorofuro-[2,3-d]pyridazine[98]. Copper(I) alkylthiols have been used to form thiocycles with 2-bromothiophene, 2-bromopyridine and 2-
 bromofuroic acid, the latter with concomitant decarboxylation[98].

The rate and activation parameters have been determined for the reaction of potassium methanethiolate with various 2-fluoro- and bromo-pyridines. Although an ortho-methyl group did not activate the 2- position in 2-bromo- or 2-fluoro-pyridine towards attack by the methanethiolate ion, deactivation of the ortho rather than the para position was observed. At 110°C for the bromo-compounds KₓMe: KₓBr = 3:9, while KₓBr : KₓMe = 2:2. The results have been compared with those obtained using methoxide and benzene-thiolate anions in methanol. The relative rates observed in HMPA are the same as those in methanol[100]. Thiophenol reacts faster than its anion with a bromopyridine, in methanol, due to a rapid acid-base equilibrium in which the pyridine is protonated. An o-MeO substituent accelerates the replacement of Br, and a small increase is also noted on going from MeOH to DMSO as solvent[101].

In 2,3-dibromo-5-nitrobenzenes (31) the 2-bromo group is replaced by the benzene-thiolate anion.

\[
\begin{align*}
\text{O}_2\text{N} \quad \text{S}^- \quad \text{Br}^- & \quad \text{+ SpH}^- \\
\rightarrow & \\
\text{O}_2\text{N} \quad \text{S}^- \quad \text{Br}^- & \quad \text{R = H, Me}
\end{align*}
\]
The meta methyl group increases the reactivity towards nucleophiles of the 2-bromine by increasing the Reimhelter and Bunnell effect of the 3-bromine on the activated 2-bromine.114

Nucleophile substitution of 2-chloro 4,6 bis(isopropylamino)-S-triazine with sodium methanethiolate in methanol gave prometryne (32) in 90% yield. The reaction is second order and the activation energies were 20-26 and 77-74 kcal/mole in 2-propanol and methanol respectively.112,114

\[
\begin{align*}
\text{Me}_2\text{CHN}^+ & \quad \text{NHCMe}_2^+ \\
\text{SMe} & \quad \text{N} \\
\text{Me}_2\text{CHN}^+ & \quad \text{NHCMe}_2^+ \\
\hline
8. \text{Substitution of groups other than halogen} \\
\end{align*}
\]

The rate constants for the replacement of various groups X in p-XC\(_6\)H\(_4\)SO\(_2\)CF\(_3\) by NaSPh in methanol decreased in the order

\[X = \text{SO}_2\text{CH}_3 > \text{NO}_2 > \text{F} > \text{Cl}\]

The element effect of atoms or groups increased with increasing activation and polarizability of the aromatic system.

Nitro groups in heterocyclic compounds can be replaced by thiolate groups. 5-Phenylimidazo-2-furaldehyde is obtained from 5-nitro-2-furaldehyde and benzeneethiolate. Thiocolates will not, however, react with halogenofurural. One nitro group in 3,4-dinitrothiophene may be replaced by a benzeneethiolate group, but rearrangement occurs and phenyl(2-(4-nitrophenyl) sulphide is formed. Sodium benzeneethiolate or benzeneulenate gives replacement of ether one but not both of the nitro groups in 2,3-dinitrothiophene.114

Displacement of a thiolate group occurs in 2-methylthio- and 2-ethylthio-4(1H) pyrimidines at the 2 position in greater than 70% yield, using a thiol in basic solution. A 5-halo and 6 amino substituent hinder the reaction but a 1-methyl or 6-hydroxy group facilitated it by influencing the tautomers.114

C. Dealkylation Reactions

A dealkylation reaction can be defined as the removal of an alkyl group, and its subsequent replacement by hydrogen, or the removal of an alkyl group from an ammonium salt with the formation of an amine, e.g.

\[
\begin{align*}
\text{R}_1\text{MeN}^+\text{Cl}^- + \text{PPh}_3 &= \text{N}^+\text{Me} + \text{R}_1\text{Cl}^- \\
\text{R}_1\text{CH}_2\text{C}^+\text{H}_3\text{OCH} &\equiv \text{NMe}_2^- + \text{H}_2\text{O} \quad \text{[reference 120]} \\
\text{E}_{\text{t}}\text{MeN}^+(\text{CH}_3)\text{NMe}_2^+ + \text{PPh}_3 &= \text{PhSMe} + \text{Ph}^+ \quad \text{[reference 121]}
\end{align*}
\]

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The method can be used preparatively. Other examples include the selective demethylation of triethylmethylammonium chloride with sodium benzeneethiolate. A somewhat analogous reaction is observed in the reaction of alkoxynitrilmethylammonium phosphonum chloride (45) with thiocolates forming a phosphine oxide and sulphide.

\[
\begin{align*}
\text{PhCH}_2\text{OP}^+\text{(NMMe)}_3^+\text{Cl}^- + \text{PhSMe} &\equiv \text{F}^- \\
\text{OP}^+(\text{NMMe})_3^- + \text{PhCH}_2\text{OP}^+ + \text{ElN}^+\text{Cl}^-
\end{align*}
\]

The method is not restricted to group V derivatives and can easily be applied to oxygen esters and ethers. The use of various nucleophiles in this type of reaction has been discussed. The main advantage of this technique for the demethylation of ethers with ethaneithiolate in a solvent such as DMF is that a relatively low temperature is required and the group R may be acid sensitive.114-116

\[
\begin{align*}
\text{R} &\equiv \text{OMe} \quad \text{Se}^- \\
&\equiv \text{OH}
\end{align*}
\]

The thiolate is generated in situ from sodium hydride and the corresponding thio. Aryl methyl ethers with strong electron-withdrawing substituents (G) require milder conditions for cleaving the ether linkage, but these compounds are also likely to suffer substitution of the aromatic carbon with strong nucleophiles.117

\[
\begin{align*}
\text{Y}^- &\equiv \text{OMe} \\
&\equiv \text{Y}
\end{align*}
\]

Using methyl ethers of di- and tri-hydric phenols, selective mono-demethylation occurs, e.g. resorcinol monomethoxyl ether is obtained from resorcinol dimethyl ether and sodium ethaneithiolate in DMF. An exception is pyrogallol trimethyl ether which afforded pyrogallol 1-monomethyl ether in high yield. Methylene ethers, such as methylencyclopropane, can be quantitatively converted to catechol, via the intermediate formation of ethyl o-hydroxyphenoxyethyl sulphide.

This method, using ethaneithiolate, has been extended to esters.116 The cleavage of methyl esters by lithium propanethiolate in HMPA, an S\(_2\) reaction, has been reported. The lithium salt reacts very much faster than the sodium salt. The benzeneethiolate and propanethiolate anions have
also been used in the conversion of esters to the corresponding acid or its sodium salt\(\text{159,160}\). Examples include the conversion of \(p\)-aminobenzonic acid and methyl \(p\)-chlorophenoxyacetate to \(p\)-chlorophenols\(\text{14}\), the latter is an example of the cleavage of an arylxyacetate. However, hydrolysis of \(p\)-nitrophenylacetate with both simple and polyfunctional thiols proceeds at a rate dependent upon the thiolate ion concentration. The initial products are \(p\)-nitrophenol and the thiol ester.

Thermodynamic parameters \(E_a, \Delta H^\circ\), \(\Delta F^\circ\) and \(\Delta S^\circ\) have been found to be 8-0, 7-4, 16-7 kcal/mole and -30-7 e.u., respectively for the reaction of cysteine with \(p\)-nitrophenylacetate (20 °C)\(\text{157}\).

\[
\begin{array}{c}
\text{O} \quad \text{NR} \quad \text{O} \\
\text{HCon} : \text{HCon} \\
\text{SR} \quad \text{HCon}
\end{array}
\]

The two methoxy groups in amide acetals can be replaced by a diithiole forming 1,3-dithiolanes (34).

\[
\text{C} \quad \text{NMMe}_2 \quad \text{C} \quad \text{NMMe}_2
\]

Replacement of only one methoxy group is found in the reaction of DMF-dimethyl sulfoxide mixture (presumably forming \(\text{HCO(OMe)_2NMMe}_2\)) with sodium ethanethiolate,

\[
\text{HC} \quad \text{O(OMe)_2NMMe}_2 + \text{EtS}^- \rightarrow \text{Me}_2\text{NH} \quad \text{O(OMe)_2SD} + \text{OMe}^-
\]

but thiols themselves displace both methoxy groups\(\text{161}\). Other formaldehyde mercaptals have also been used to form amidine mercaptals, where \(R, N = \text{piperidine and } R^1 = \text{Me, CH}_2\text{H}_2N, \text{CH}_3\text{H}_2, \text{and PhCH}_2\text{H}_2\)\(\text{157}\).

\[
\text{R}_2\text{NCH(O(OMe)_2)} + \text{RSH} \rightarrow \text{R}_2\text{NCH(SR)}
\]

An interesting extension of this type of reaction is the transalkylation reaction between 2-alkoxy-1-methylenzimidazole (35) and benzethiol. The kinetics of this reaction indicate a rapid acid-base equilibrium, followed by an S\(2\) attack at the ether saturated carbon by the PhS\(^-\) ion\(\text{14}\).

A somewhat analogous reaction is observed in the reaction of the mixed anhydride, acetic formic anhydride, with thiophenol in pyridine, where 93\% of the thioformate, HCOSPh, and 7\% of the thioacetate, MeCOSPh, are formed\(\text{162}\).

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\[
\begin{array}{c}
\text{Me} \quad \text{N} \quad \text{O} \\
\text{HR} + \text{PhSH} \\
\text{Me} \quad \text{N} \quad \text{O} \\
\text{HR} + \text{PhS}^-
\end{array}
\]

The thiolate anion acts both as a dealkylating agent and a reducing agent with \(p\)-CH\(_4\)=CHCO\(_2\)H\(_4\)\(_2\)O\(_3\). The yields of the various products are shown.

\[
\begin{array}{c}
\text{p-CH}_4\text{=CHCO}_2\text{H}_4\text{O}_3 \quad \text{Nal} \quad \text{Bu} \\
\text{BuSCH}_2\text{H}_4\text{N} = \text{NCH}_2\text{SH} \quad \text{Bu}^+ \\
30\% \quad 25\% \quad 25\%
\end{array}
\]

When this reaction was studied under electrophilic conditions with the thiol in Et\(_2\)NH or in a sealed tube with a free radical initiator, different reactions ensued, including addition across the C\(\equiv\)C bond\(\text{163}\).

D. Reactions with Main Group Elements

I. Introduction

Thiols and thiocarbonyl derivatives of the elements have been reviewed, and compared with the alkoxides\(\text{157}\). The alkali and alkaline earth metal salts of the thiols are probably ionic and can be prepared in numerous ways. In the aqueous phase, the excess water is removed by azetrophic distillation with toluene\(\text{158,159}\). Alternatively using other solvents, salts or solvated salts can be isolated\(\text{160,161}\). The crystal structures of the alkali metal thiokates, MS\(_2\)M (M = Li, Na, K), have been reported and are of the same type as the corresponding alkoxides\(\text{162}\).

The thiol derivatives of the other main groups elements are often prepared from their halides using the thiol in the presence of a hydrogen halide acceptor or by using a metal thiolate, such as lead, where R is a main group element.

\[
\begin{array}{c}
\text{RMe} + \text{N}^+\text{H}^+ + \text{H}^+ \rightarrow \text{RSH} + \text{H}^+ \text{NH}^+ \text{H}^+
\end{array}
\]

\[
\begin{array}{c}
2 \text{RMe} + \text{P}^+\text{O}(\text{OR})_2 \rightarrow 2 \text{RSR}^+ + \text{PMe}_3
\end{array}
\]
2. **Group II**

Few thiolate derivatives of beryllium are known. Di[(r-butyldithio)-triphenylphosphine]beryllium tetrathio-2-oxotetraoxide, (r-BuS)₂Be₃(OBu-t)₄, has been obtained from dichlorotriphenyldiberyllium tetrathio-2-oxotetraoxide, Cl₃Be₃(OBu-t)₄, and lithium butanethiolate. Other beryllium thiolates are prepared by reaction of a thiol with dialkylberyllium or dialkyldiphenylberyllium and do not involve a thiolate anion as an intermediate. Various other compounds such as thio-magnesium alkyls and dimethyl(methylthio)aluminum are obtained analogously.

3. **Boron**

Reviews have been published about the problems and results of boron-sulfur chemistry and organic boron-sulfur compounds. The trialkylthio- or arylthioboranes can readily be prepared from boron trihalide and a metal thiolate:

\[
\begin{align*}
3 \text{PH}_{3} & \text{H}_{3} \text{Si} \text{S} \text{H}_{3} + 2 \text{BCl}_{3} \longrightarrow \text{PH}_{3} \text{H}_{3} \text{SiH}_{3} + 3 \text{PCl}_{3} \\
3 \text{PH}_{3} & \text{H}_{3} \text{Si} \text{S} \text{H}_{3} + 2 \text{BBr}_{3} \longrightarrow \text{PH}_{3} \text{H}_{3} \text{SiH}_{3} + 3 \text{BBr}_{3}
\end{align*}
\]

In the latter reaction the mixed products H₃SiH₃ + 2 BBr₃ can also be isolated. Mixed arylalkylthioboranes such as bis(ethylthio)phenylborane may be prepared analogously from dichlorophenylborane and lead ethanethiolate, or using the thiol in the presence of triethylamine:

\[
\begin{align*}
\text{Et}_{3} \text{N} & \text{HCl} + \text{NaSMe} \longrightarrow \text{Et}_{3} \text{NSiHCl} + \text{NaCl} \\
\text{Et}_{3} \text{N} & \text{HCl} + \text{NaSPh} \longrightarrow \text{Et}_{3} \text{NSiPhCl} + \text{NaCl}
\end{align*}
\]

Interesting new compounds of the type M[Si(BH₄)₃] have recently been reported to be formed in the reaction of a metal with diborane in THF. The compound K[Et₂Si(BH₄)] has been isolated and some of its reactions studied.

4. **Group IV**

The reactions of thiolates with various carbon compounds are discussed elsewhere in this chapter.

Thiol derivatives of silicon, germanium, tin, and lead can readily be prepared from a halide, usually chloride, and a thiol in the presence of a hydrogen halide acceptor or a metal thiolate. Various illustrative examples are shown below:

\[
\begin{align*}
\text{H}_3\text{SiBr} + \text{NaSMe} & \longrightarrow \text{MeSSiH} + \text{H}_3\text{Si} + \text{solid} \\
\text{H}_3\text{MSi} + \text{NaSMe} & \longrightarrow \text{MeSMPSiH} + \text{NaCl} \\
\text{PMe}_2\text{S} & \text{C}_6\text{H}_{5}\text{S} + \text{Br} + \text{H}_3\text{SiCl} \longrightarrow \text{PMe}_2\text{S} \text{C}_6\text{H}_{5}\text{S} + \text{Br} + \text{H}_3\text{SiCl} \\
\text{PMe}_2\text{S} & \text{C}_6\text{H}_{5}\text{S} + \text{Br} + \text{H}_3\text{SiCl} \longrightarrow \text{PMe}_2\text{S} \text{C}_6\text{H}_{5}\text{S} + \text{Br} + \text{H}_3\text{SiCl}
\end{align*}
\]

The silicon analogue of the methanethiolate and methaneselenolate anions, H₂Si⁻ and H₂Se⁻, are formed in the reaction of triisobutylamine and hydrogen sulfide or selenide.

\[
\begin{align*}
\text{SiH}_3\text{N} + \text{H}_2\text{Si} & \longrightarrow \text{H}_3\text{Si} + \text{H}_2\text{Si} + \text{SiH}_3\text{N} \\
\text{SiH}_3\text{N} + \text{H}_2\text{Si} & \longrightarrow \text{H}_3\text{Si} + \text{H}_2\text{Si} + \text{SiH}_3\text{N}
\end{align*}
\]

The trimethylammonium salt can also be formed.

\[
\begin{align*}
\text{SiH}_3\text{N} + \text{H}_2\text{Si} & \longrightarrow \text{H}_3\text{Si} + \text{H}_2\text{Si} + \text{SiH}_3\text{N} \\
\text{SiH}_3\text{N} + \text{H}_2\text{Si} & \longrightarrow \text{H}_3\text{Si} + \text{H}_2\text{Si} + \text{SiH}_3\text{N}
\end{align*}
\]

The salts of the anion H₂Si⁻ are stable at room temperature. Similar anions P₂Me₄⁻ (M = Ge, Sn, Pb), presumably present in the lithium derivatives P₂MSLI, are well characterized and have been used in the synthesis of unsymmetrical sultones.

\[
\begin{align*}
\text{P}_2\text{MSLI} + \text{H}_2\text{SiCl} & \longrightarrow \text{P}_2\text{MSLI} + \text{H}_2\text{SiCl} \\
\text{P}_2\text{MSLI} + \text{H}_2\text{SiCl} & \longrightarrow \text{P}_2\text{MSLI} + \text{H}_2\text{SiCl}
\end{align*}
\]

The derivatives such as Et₂Sn(SiMe₃) can be prepared from Et₂SnCl₄ and Na₂S, and react with chloro compounds to give the corresponding organotin thiold derivative.

\[
\begin{align*}
\text{Et}_2\text{Sn(SiMe}_3) + 2 \text{MCl} & \longrightarrow \text{Et}_2\text{Sn(SiMe}_3) + 2 \text{NaCl}
\end{align*}
\]

The compound (R₂SCH₂)₂Sn(SiR₂)₂ can be obtained by replacement of bromine bonded to carbon and tin in (Br₂C₂H₄)₂SnBr₂ by its reaction with the sodium thiolate R₂Sn⁻.
5. Group V

The thiol-substituted amines such as sulphanamides and tisz-(alkanesulphonyl)amines are often prepared from sulphanilic chlorides and ammonia, and never from nitrogen trichloride and a thiol. Chloramines react with thiol to produce symmetrical disulphides:

\[ \text{Me}_2\text{NCl}_2 + 2 \text{PhSH} \rightarrow \text{PhSSPh} + \text{Me}_2\text{NH}_2\text{Cl} \]

However methyl-N-chlorobenzimidate (36) and benzenethiol form N-benzylsulphenylsulphide (37). The reaction may proceed through the formation of the unknown PhCONH=SHPh as an intermediate.

\[ \text{PhC(O)NMe}_2 = \text{NCI} + \text{PhSH} \rightarrow \text{PhCONH(SPh)} + \text{MeCl} \]

The kinetics of the reaction of diazonium ions XClH₂₄N₄⁺ with benzene-1-thiolate anions show that mutarotation of the syn-anti isomer is slower than the anti-syn, with the syn-anti isomer being formed rates, which is followed by the slower syn-anti isomer. Only in the cases of p-nitro- and p-cyano-benzenediazonium ions is it possible to distinguish between the first and second reactions. Using benzene-1-thiolate ion and the p-Me- and p-OCH₃-substituted ions with benzenethiolate, first-order kinetics were observed over the entire range of the reaction. It is postulated that there the rate-determining step is formation of the syn-diazoether, followed by its rapid isomerization to the anti-diazoether.

The simple alkyl and aryl-thio phosphorus derivatives, (RS)₃P, (RS)₃PO and (RS)₅PS, can readily be prepared from phosphorus trichloride (or phosphorus pentachloride), phosphoryl chloride or thiophosphoryl chloride, and the corresponding lead thiourea, and thiophosphates, such as Cl₅PSCF₆H⁺, are sometimes formed. Substituted derivatives R₂P(SR')₃ and RP(SR')₃ can be prepared from the corresponding halide and lead thiourea. Various mixed fluorophosphoranes, such as MePF₃(SEt)₃, can be prepared from Me₂P(SR) and ethanethiol or its sodium salt.

\[ p\text{-Nitrophenyl methylphosphonic acid (38) reacts with thiocetic anhydride leading to the formation of thiophosphonic esters (39), although the formation of some disulphide complicates the reaction. Thiophosphites are also formed in the reaction of sodium thiocyanates or thiol/trimethylamine with acyl phosphites.} \]

16. Thiolates as nucleophiles

Reactions involving fraction of P=O or P=N bonds and displacement of EtO and Et₂N groups in various 1,2,3-oxazaphospholane (40) (R = EtO, Et₂N) with thiol in the presence of triethylamine have been examined. Aliphatic thiocyanates used their sulphur in reaction with 40 to form 50-60% oxazaphospholane-2-sulphide (41), whereas benzene thiolether formed 75% 2-phenylthio-N-phenyl-1,3,2-oxazaphospholane (42) (R₁ = Ph; R₂ = H). Similar derivatives (40; R = R₂S) are readily prepared from 40 when R = Cl, on treatment with a thiol in the presence of triethylamine.

\[ \text{R}^+\text{N}^+\text{PA} \rightarrow \text{R}^+\text{NS}^{-}\text{PA} \]

The thiolate group may be added to phosphorus acting as a ligand, for example in the preparation of (ethyldimethylthioephosphonic pentaacryl molybdenum)

\[ \text{Cl}₆\text{P}₃\text{Mo}⁺\text{CO}⁺ = \text{EtSH} \rightarrow \text{EtSMe}₃\text{P}⁺\text{CO}⁺\text{Mo}⁺ + \text{EtNHCl} \]

The thiol derivatives of arsenic can be prepared by similar methods to those used for phosphorus. Various mixed phosphates such as BuPhAs(SPh) can be prepared from BuPhAs and PhSNa in absolute ethanol. Displacement of an OPPh group may occur in PhAs(S(OEt)), but this reaction may involve rearrangement of an unstable intermediate PhAs(S(OEt))Bu⁺ and PhAs(S(OEt))₂Bu⁺.

\[ \text{Z PhAsCl(OEt)} + 2 \text{BuSH} + 2 \text{EtN} \rightarrow \text{PhAs(Bu)}⁺ + \text{PhAs(OEt)}⁺ + 2 \text{EtNHCl} \]

Derivatives of heterocyclic arsenic compounds can be prepared, e.g.
when \( X = O \) and \( R = Cl \), the reaction with PhSNa gave \( R = SPh \), but when \( X = S \) the reaction with PhSNa in benzene gave \( \text{As}(SPh)_2 \) along with ethylene arsenite\(^{109} \).

Derivatives of antimony(III), Sb(SR)\(_n\), can be prepared analogously\(^{14, 141} \), or from antimony trichloride and thiols in the presence of ammonia\(^{79} \).

Aminated products have been prepared\(^{19} \), e.g.

\[
\text{Me}_2\text{SbCl}_3 + 2 \text{MeSH} + 2 \text{Et}_2\text{N} \xrightarrow{\text{heat}} \text{Me}_2\text{Sb}(	ext{SMe})_2 + 2 \text{Et}_2\text{NCl}\cdot
\]

These compounds are thermally unstable, decomposing to Me\(_2\)Sb and MeSMe. The unstable Me\(_2\)Sb(SR) analogues can be prepared from pentamethyldiamine and a thiol at low temperature\(^{81} \).

Bis(thio) thiolates can be prepared in reactions similar to those used to prepare metal thiocyanates\(^{7} \).

6. Group VI

Attempts to prepare compounds of the type RSOSR containing a single-bonded system RS—O—SR failed, and possible rearrangement of this as an unstable intermediate occurred\(^{181} \).

\[
\text{CF}_2\text{SO}_2\text{CO}_2\text{Na} + \text{PhSH} \rightarrow \text{CF}_2\text{SO}_2\text{CO}_2\text{Na} + \text{PhS}\text{SO}_2\text{F} + \text{NaF}
\]

The reactions of chlorine monoxide with thiols or thiolates have not been investigated.

The thiolate anion can play a very important role in the thiol-disulphide interchange.

Various derivatives of sulphur may be prepared by the reaction of sulphur monochloride, sulphur dichloride or sulphonyl halides with thiocyanate; the products depend on the reactant stoichiometry.

\[
\begin{align*}
(\text{CF}_2\text{S})_2\text{PH} + \text{SCl}_2 & \rightarrow \text{CF}_2\text{SSC}(\text{CF}_2)\text{PH} + \text{PbCl}_4 \\
\text{Pb}_{2}\text{CNS} + \text{SCl}_2 & \rightarrow \text{Pb}_{2}\text{CNS} + \text{HCl} \\
(\text{CF}_2\text{S})_2\text{PH} + 2 \text{SCl}_2 & \rightarrow 2 \text{CF}_2\text{SSC} + \text{PbCl}_4 \\
(\text{RSH})_2 + \text{SCl}_2 & \rightarrow \text{RSSC}(\text{R})_2 + \text{HCl} \\
\text{RSH} + \text{SCl}_2 & \rightarrow \text{RSSC} + \text{HCl}
\end{align*}
\]

Other reactions of thiocyanate anions with sulphur(vi) include the reaction with arylsulphonylphosphines

\[
\text{n-BuS}^+ + \text{ArSO}(\text{O})\text{S}^-\text{Ar} \xrightarrow{\text{Et}_{2}\text{N}} \text{n-BuSS} + \text{ArSO} + \text{ArSO}^-
\]

and the reaction with the tribromide ion,

\[
\text{PhS}^- + \text{SO}_3\text{S}^- + \text{S}^- \rightarrow \text{PhS}^- + \text{SO}_3^- + \text{S}^- + \text{SO}_3^- + \text{S}^- + \text{SO}_3^-
\]

The rate-determining step is \( K_a \), and added formaldehyde eliminates the \( K_a \) and \( K_d \) paths, leaving PhSSPH\(^{144} \).
Very few thiolute derivatives of selenium, and virtually none of tellurium, are known. Attempts to prepare \( \text{R}_2\text{Se}(\text{SC}_6\text{F}_5)_2 \) or \( \text{Me}_2\text{Te}(\text{SC}_6\text{F}_5)_2 \) from the dialkyl (or aryl) selenium dichloride, dimethyltellurium dichloride and lead pentfluorobenzethiolate resulted in the formation of the disulphide, \( \text{C}_6\text{F}_5\text{SSC}_6\text{F}_5 \), and the dialkyld (or aryl) selenum, \( \text{R}_2\text{Se} \), or \( \text{Me}_2\text{Te} \). The chlorides \( \text{SeCl}_3 \) and \( \text{TeCl}_4 \) yielded only the disulphide and selenium or tellurium\(^*\). Tellurium—sulphur and —selenium bonds have been formed in the reaction of organometalltellurium bromides with benzenethiol and benzene硒elone\(^{216}\).

\[ \text{o-HOC}_{6}\text{H}_4\text{TeBr} + \text{PhMH} \rightarrow \text{o-HOC}_{6}\text{H}_4\text{TeMPh} \text{ (M = S, Se)} \]

7. Group \( \text{VII} \)

Attempts to prepare simple sulphenyl fluorides from thiolute and fluoride have not been reported, but are unlikely to be successful due to the oxidizing powers of fluoride\(^{186}\) or chlorine monofluoride\(^{194}\), causing oxidation of the sulphur\(\text{(i)}\)

\[ (\text{C}_6\text{F}_5)_2\text{S} + \text{F}_2 \rightarrow (\text{C}_6\text{F}_5)_2\text{SF}_2 \text{ (reference 196)} \]

Attempts to prepare a trifluoromethanesulphényltulfluoride resulted in the formation of trifluoromethylsulphur trifluoride and bis(trifluoromethyl)disulphide\(^{216}\).

Conversely, sulphenyl chlorides can readily be prepared by the action of chlorine on a metal thiolute.

\[ (\text{C}_6\text{F}_5\text{S})_2\text{H} + \text{Cl}_2 \rightarrow 2\text{C}_6\text{F}_5\text{SCl}_2 + \text{PhCl}_2 \text{ (reference 185)} \]

Sulphenyl bromide can be obtained analogously in solution, but removal of the solvent caused decomposition\(^{206}\).

\[ (\text{C}_6\text{F}_5\text{S})_2\text{Br} + 2\text{Br}_2 \rightarrow 2\text{C}_6\text{F}_5\text{SBr} + \text{PbBr}_6 \]

removal of solvent \(\xrightarrow{\text{Ph(SO)}_2} \)

\[ \text{C}_6\text{F}_5\text{SSC}_6\text{F}_5, \text{C}_6\text{F}_5\text{SSe}_2 \]

The thiolute anion is quantitatively oxidized by iodine to the disulphide\(^{211}\), and this method, involving the formation of an unstable sulphenyl iodide, is the basis of the iodometric determination of mercapto groups in a number of compounds\(^{210}\).

16. Thiolute as nucleophiles

\[ (\text{C}_6\text{F}_5\text{S})_2\text{Pb} + 2\text{I}_2 \rightarrow 2[(\text{C}_6\text{F}_5\text{S})_2\text{Pb}] + \text{I}_2 \]

\[ \text{C}_6\text{F}_5\text{SSC}_6\text{F}_5 + \text{L} \]

The thiolute anion is an intermediate in the oxidation of a thiyl by iodine\(^{209}\).

E. Reactions with Transition Metal Derivatives

1. Simple transition metal derivatives

This section will be primarily restricted to the derivatives and reactions of mononuclear thiolute. The thiolute derivatives of transition metals are a rapidly expanding area of research and have been reviewed several times recently\(^{212-214}\). Other polyfunctional thiolute, such as monothiophosphates, \(\text{Ag}(\text{S})\text{PH}_2\), \(\text{Sn}(\text{S})\text{PH}_2\), \(\text{In}(\text{S})\text{PH}_2\), and \(\text{thioethanolamine}\)\(^{209,214}\), have been extensively studied and will not be discussed further. It is noteworthy that interesting complexes of the type \(\text{Ag}(\text{S})\text{HPH}_2\), \(\text{Ag}(\text{S})\text{HPH}_2\), \(\text{In}(\text{S})\text{HPH}_2\), \(\text{R} = \text{HOCH}_2\text{CH}_2\text{H}_2\text{S}\), and \(\text{Cd}[\text{NiL}_2]_2\)\(^{214}\) and \(\text{Cd}[\text{NiL}_2]_2\)\(^{214}\) (\(\text{L} = \text{H}_2\text{NCH}_2\text{CH}_2\text{SH}\)\(^{214}\)) are formed.

Simple transition metal mercapturines, such as \(\text{Ni}(\text{SR})_2\), or \(\text{Hg}(\text{SR})_2\), are usually prepared by reactions not involving the thiolute anion as a nucleophile\(^{7,14,211}\). Occasional use is made of thiolute, for instance in the preparation of chromium(II) methioninate, where sodium methanethiolate was reacted with chromium chloride in excess of dimethyl disulphide under dry nitrogen and irradiated to yield the desired product, which can also be prepared by other photochemical methods\(^{212}\). Cobalt thiolute, \(\text{[Co}(\text{SR})_2]_2\), may be prepared from cobalt acetate in methanol with a basic solution of the thiolute\(^{215}\). Some biochemical applications of thiolute anions are important. The binding of thiolute to Co(II) canons has been studied by e.g., where it has been shown that the thiolute and sulphide bond to the cobalt. The binding of \(\text{Co}([\text{S})\text{B}]_2\) complexes to thiols and sulphides will necessitate a re-examination of the methyl-transfering enzymes in which thiolute is known to be important\(^{217}\).

The continuous oxidation of thiolute involved in the sweating of light naphtha, with air to the disulphides using cobalt phthalocyanine complexes as catalysts, involves the formation of a stable complex between the thiolute ions and the metalloconcanavalin\(^{218}\).

The kinetics of various reactions involving thiolute and plumbone complexes have been studied. The rate of reaction of \(\text{trans}[\text{Pt(ppy)}\text{Cl}]_2\)
16. Thiols as nucleophiles

Cyclopentadienyltitanium thiocarbamates have been prepared in benzene solution from the corresponding chlorides and several thiols in the presence of triethylamine in good yield455

\[
\text{(c-C_5H_5)_2TiCl_2 + 2 RSH + 2 Et_3N} \rightarrow (\text{c-C_5H_5)_2TiSR})_2 + 2 \text{Et}_3\text{NH}^+ \text{Cl}^{-}
\]

The compound (c-C_5H_5)_2TiCl_2 (R = Me, Ph) has also been prepared from (c-C_5H_5)_2TiCl_2 and NaSR. Attempts to prepare (c-C_5H_5)_2Ti-SCF_3H_3 from (c-C_5H_5)_2TiCl_2 and AgSCF_3 resulted in the formation of (c-C_5H_5)_2TiF_3, and several unsuccessful attempts have been made to prepare (c-C_5H_5)_2Ti(SCF_3)_2. The extremely unstable mono-cyclopentadienyltitanium (benzene-thiolate) has also been reported282.

\[
\text{(c-C_5H_5)_2TiCl_2 + 3 HSPh + 3 NEt_3} \rightarrow (\text{c-C_5H_5)_2Ti(SPh})_2 + 3 \text{Et}_3\text{NH}^+ \text{Cl}^{-}
\]

If a 1:1 reactant stoichiometry is used the stable compound (c-C_5H_5)_2TiCl_2(SPh) is readily isolated and can be purified by vacuum sublimation. The derivatives of diazomethane, (c-C_5H_5)_2Ti(Zn(SPh)_2) and (c-C_5H_5)_2Ti(Zn(SPh)), have been prepared analogously from (c-C_5H_5)_2TiCl_2 and the thiol or selenol in the presence of triethylamine280.

Various other analogous compounds, such as (c-C_5H_5)_2Ti(N(SR)_2) (R = Me, Ph282) and (c-C_5H_5)_2Ti(SR)_2 (M = Mo, W273) can be obtained from the corresponding chloride and sodium thiolate.

The compounds of the type (c-C_5H_5)_2Ti(SR)_2 (M = Ti, Mo, W, Nb) have been found to have extremely interesting properties285, 294, 284. They can act as bidentate ligands forming complexes, some of which may contain metal—metal bonds, e.g.

\[
\text{(c-C_5H_5)_2TiSR + M(CU)_2} \rightarrow \text{(c-C_5H_5)_2TiM(CU)_2SR + M(SR)_2}
\]

Various other organometallic thiolate complexes may be formed by using thiocarbamates.

\[
[(\text{c-C_5H_5})_2M(\text{SR})_2]^{2-} + \text{Na}^+ \text{SR}^- \rightarrow [(\text{c-C_5H_5})_2M(\text{SR})_2]\text{SR} + \text{Na}^+ + \text{Bu}_2\text{P}
\]
where the thiolate anion can be derived from aliphatic or aromatic thiols. Dithiol derivatives can also be obtained.

Other reactions involving thiols, such as the reaction:

\[ \text{(-C}_6\text{H}_4\text{)Ni + HSR} \rightarrow \text{(-C}_6\text{H}_4\text{)NiSR + C}_6\text{H}_6 \]

which has been studied extensively, do not involve the thiolate anion, but rather the thiol itself, the sulphur of which binds initially to the nickel.

Various CF₃S₂ derivatives have been prepared using silver trifluoromethanesulphonate and its reactions with certain norbornadiene and tetraphenylethylene metal complexes studied. The reaction of the norbornadiene derivative C₅H₅PtCl₂ with AgCl₂F₂ in dichloromethane solution resulted in the replacement of both chlorine atoms by CF₃S₂ groups to give the white crystalline C₅H₅Pt(SCF₃)₂. However, in the analogous reaction of C₅H₅PdCl₂ with CF₃S₂Ag, addition of CF₃S₂ groups to the norbornadiene ligand occurred to give two yellow crystalline products, [(C₅H₅CF₃)₂Pd]Cl₂ and [(C₅H₅CF₃)₂PdCl]SCF₃, which are novel norbornadiene derivatives. Reaction of the tetraphenylethylene complex [P₆C₆SeP₆] with AgSCF₃ gave the golden-red P₆C₆SeP₆(SCF₃)₂ as formulated as a monomeric 16-electron tetraphenylethylene complex, but the reaction of P₆C₆SeP₆ with AgSCF₃ gave the bisnuclear complex [P₆C₆SeP₆(SCF₃)₂].

The molybdenum complexes [n-C₅H₅Mo(NO)(I)₂] and [n-C₅H₅Mo(NO)(I)₂] with [n-C₅H₅Mo(NO)(I)₂] and [n-C₅H₅Mo(NO)(I)₂] have been obtained from the iodide reaction with the appropriate thiolate anions under differing conditions. The structures of the analogous chromium compounds [n-C₅H₅Cr(NOS)(I)₂] show that the SPh groups act as bridges between the two chromium atoms.

Several molybdenum derivatives can be prepared using a thiolate. A stable monomeric π-allyl molybdenum derivative has been obtained by the metathesis:

\[ \text{n-C}_6\text{H}_4\text{M(CO)}_3\text{Cl} + \text{TISCF}_3 \rightarrow \text{n-C}_6\text{H}_4\text{Mbipy(CO)}_3\text{SCF}_3 \] (M = Mo, W)

16. Thiols as nucleophiles

A dinuclear π-allylmolybdenum complex has been obtained by treatment of its trichloro analogue with sodium thiolate.

\[
\begin{align*}
\text{EulNT} + \text{H}_2\text{C}_6\text{C}_6\text{H}_4\text{Cl} & \rightarrow \text{n-C}_6\text{H}_4\text{H}_2\text{C}_6\text{C}_6\text{H}_4\text{Cl} \\
\text{EulNT} + \text{H}_2\text{C}_6\text{C}_6\text{H}_4\text{Br} & \rightarrow \text{n-C}_6\text{H}_4\text{H}_2\text{C}_6\text{C}_6\text{H}_4\text{Br} \\
\text{EulNT} + \text{H}_2\text{C}_6\text{C}_6\text{H}_4\text{H}_2\text{C}_6\text{C}_6\text{H}_4\text{Br} & \rightarrow \text{n-C}_6\text{H}_4\text{H}_2\text{C}_6\text{C}_6\text{H}_4\text{H}_2\text{C}_6\text{C}_6\text{H}_4\text{Br}
\end{align*}
\]

Various other mixed cyclopentadienyl carbonyl complexes can be prepared using a thiolate:

\[
\begin{align*}
n\text{C}_6\text{H}_4\text{H}_2\text{C}_6\text{C}_6\text{H}_4\text{Cl} + \text{CSR} & \rightarrow n\text{C}_6\text{H}_4\text{H}_2\text{C}_6\text{C}_6\text{H}_4\text{Cl} \quad (R = \text{Me}, \text{Ph}) \\
\text{EulNT} + \text{H}_2\text{C}_6\text{C}_6\text{H}_4\text{H}_2\text{C}_6\text{C}_6\text{H}_4\text{Cl} & \rightarrow \text{n-C}_6\text{H}_4\text{H}_2\text{C}_6\text{C}_6\text{H}_4\text{Cl} \\
\text{EulNT} + \text{H}_2\text{C}_6\text{C}_6\text{H}_4\text{H}_2\text{C}_6\text{C}_6\text{H}_4\text{Br} & \rightarrow \text{n-C}_6\text{H}_4\text{H}_2\text{C}_6\text{C}_6\text{H}_4\text{H}_2\text{C}_6\text{C}_6\text{H}_4\text{Br}
\end{align*}
\]

4. Carboxyl compounds

Sulphur-containing metal carbonyls have been reviewed, and there is a section concerning mercapto carbonyl compounds. While several mercapto carbonyl complexes are known which can be prepared from the thiol itself or the disulphide, some preparations involve the use of the thiolate anion.

The complex ions [M(CO)₅SCF₃]⁻ (M = Cr, Mo, W) can readily be prepared from the pentacarbonyl and sodium pentafluorobenzenethiolate. The square planar complexes, trans-[M(SCF₃)₂(CO)(PPh₃)₂] are obtained from the thallium(i) pentafluorobenzenethiolate and the complexes [MCl(CO)(PPh₃)] (M = Ir, Rh) are similar. The complex [Ir(SCF₃)₂(CO)(PPh₃)] will add another m ole of pentafluorobenzenethiol in benzene to form [Ir(SCF₃)₂(CO)(PIP₃)] and also readily adds oxygen, forming [Ir(SCF₃)₂(O₂)(CO)(PPh₃)]

The yellow diamagnetic ions [Cr₂(CO)₅SR]²⁻ (R = H, Me, Et, Ph) are formed on oxidation of aqueous Na₃[Cr₂(CO)₅] by RSH, accompanied by the evolution of hydrogen, but when RSH is thio-phenol the mononuclear anion [Cr(CO)₅SR]⁻ (K = Cr₃Me₅) is isolated. The mononuclear carbonyl derivatives M(CO)₅SR⁻ can be prepared by using the mercury thiolates; only a small amount of the dimeric species is obtained. The

\[ \text{M(CO)}_5^+ + \text{H}_2\text{S} \rightarrow \text{2 M(CO)}_5^+ \]

Ions [M(CO)₅SR]⁻ (M = Cr, Mo, W), stabilized as their bis(triphenylphosphine)iminium derivatives, are obtained in reactions of the type

\[ 2 \text{Cr(CO)}_5^- + \text{SMe}^- \rightarrow \text{2 Cr(CO)}_5^- + \text{SMe}^- + \text{CO} \]
but this reaction does not give as good yields as the reaction of Mn(CO)₅C₆H₄⁻ with organothiolate. The various carbonyl derivatives containing the SCF₃ group can be obtained using silver trifluoromethanethiolate. Some reactions are summarized below:

\[
\begin{align*}
\text{Mn(CO)}_5\text{Br} + \text{AgSCF}_3 & \rightarrow [\text{CF}_3\text{SMe(CO)}]_2 \\
\text{Re(CO)}_5\text{Br} + \text{AgSCF}_3 & \rightarrow [\text{CF}_3\text{SrBr(CO)}]_2 + \text{CF}_3\text{SrMe(CO)}_2 \\
\text{C}_3\text{H}_4\text{NMe(CO)}_2 \text{J} + \text{AgSCF}_3 & \rightarrow \text{CF}_3\text{SFe(CO)}_2 \text{(n-C}_2\text{H}_4) \\
\text{C}_3\text{H}_4\text{N(SO)}_2 \text{J} + \text{AgSCF}_3 & \rightarrow \text{CF}_3\text{SFe(CO)}_2 \text{n-C}_2\text{H}_4 \\
\text{C}_3\text{H}_4\text{N(SO)}_2 \text{J} + \text{AgSCF}_3 & \rightarrow \text{CF}_3\text{SFe(CO)}_2 \text{n-C}_2\text{H}_4 \\
\text{CF}_3\text{NMe(CO)}_2 \text{J} + \text{AgSCF}_3 & \rightarrow [\text{CF}_3\text{SFe(CO)}_2 \text{SCF}_3]_2 \\
\text{CF}_3\text{NMe(CO)}_2 \text{J} + \text{AgSCF}_3 & \rightarrow [\text{CF}_3\text{SFe(CO)}_2 \text{SCF}_3]_2 \\
\text{CF}_3\text{NMe(CO)}_2 \text{J} + \text{AgSCF}_3 & \rightarrow [\text{CF}_3\text{SFe(CO)}_2 \text{SCF}_3]_2
\end{align*}
\]

The other complexes can be obtained using mercury(II) trifluoromethane-thiolate.237

\[
\text{Mn(CO)}_5\text{P(CF}_3\text{)}_2 \text{J} \rightarrow \text{CF}_3\text{SMe(CO)}_2 \\
\text{Mn(CO)}_5\text{P(CF}_3\text{)}_2 \text{J} \rightarrow \text{CF}_3\text{SMe(CO)}_2
\]

The compounds [n-\text{C}_3\text{H}_4\text{Mo(NO)HalSR}]_2 (\text{Hal} = \text{Br, I}) are readily prepared from [n-\text{C}_3\text{H}_4\text{Mo(NO)Hal}]_2 (\text{Hal} = \text{Br, I}) and the thio or its sodium salt by replacing one and two halogen atoms respectively.242

Other dimeric compounds with bridging thiolate groups, such as [Rh(CO)₅SR]₂, are readily obtained from benzencinchloride and [Rh(CO)₅C₆H₄⁻] in ethanol. However, analogous compounds such as [Rh(CO)₅SR]₂(\text{Hal}) (\text{Hal} = \text{Cl, Br, Cl, Pr}) (\text{Hal} = \text{Br, R = Pr}) may be polymerized.242

Bridging thiolates are also present in the iron compounds, Fe(CO)₅S(CO)₅Fe(CO)₅S(CO)₅ obtained from Fe₃(CO)₁₂ and RSH in hexane.243

Carbone complexes (CO)₅Fe(CSR)₂ (R = Me, Et, Pr, R = Me, Ph) and (CO)₅W(C(SMe)₂)Me are readily obtained by nucleophilic displacement of OMe from (CO)₅Me(COMe)R (R = Me, W) with a thio.244

III. ADDITION REACTIONS

A. Introduction

The addition of a thiol or a thiolate to an unsaturated compound A = B can be represented as

A = B + RSH \rightarrow RSA-\text{BH or HA-BSR}

The two products are possible, depending on whether the RS group adds to A or B. This will obviously be affected by the nature of atoms forming the

16. Thioles as nucleophiles

multiple bond and, possibly, by the other groups present in A and B. Addition reactions can occur in cyclic systems, such as epoxides or thioepoxides, involving fracture of the ring

\[
\begin{align*}
\text{H} \ & \text{O} \ & \text{C} \ & \text{S} \ & \text{R} \ & \text{H} \\
+ \text{RSH} & \rightarrow \text{H} \ & \text{O} \ & \text{C} \ & \text{S} \ & \text{R} \ & \text{H}
\end{align*}
\]

It is, among other things, of interest to ascertain the nature of the addition product.

Most of the addition reactions observed occur by a radical mechanism. This type of reaction has been reviewed.242 Two chapters in this book are concerned with radical reactions of thiols. This discussion will exclude all reactions that occur via the formation of radicals. Considerably less study has been made of ionic additions of thiocarboxilic acids to unsaturated systems than that of radical additions.

B. Reactions with Olefins

Sulphides are formed when a thiol adds onto an olefinic bond. Most of the reactions reported correspond to anti-Markownikoff addition, but this is probably the free radical mechanism, which also occurs in the presence of minute traces of peroxides. With carefully purified reagents in the presence of acid, Markownikoff addition occurs.244

\[
\text{Me}_3\text{C} - \text{C} = \text{CHMe} + \text{RSH} \rightarrow \text{Me}_3\text{C} = \text{CHMe} \ & \text{S} \ & \text{R}
\]

The kinetic of the addition of benzencinchloride and substituted benzencinths to derivatives of phenylvinylsulphone have been studied.245 In 50% aqueous ethanol at 25°C the reaction was second order, first order in the sulphone and in the thiolate anion.

\[
\begin{align*}
\text{XCeH}_2\text{S} \ & \rightarrow \text{XCeH}_2\text{S} \ & \text{H} \\
+ \text{Me}_3\text{C} = \text{CHMe} \ & \rightarrow \text{Me}_3\text{C} \ & \text{CHMe} \ & \text{SCeH}_2\text{S} \ & \text{H}
\end{align*}
\]

Hammett treatment showed that substitution in the phenyl ring of the sulphone influenced the reaction more than substitution in the thiol, indicating that the transition state resembles a carbanion intermediate.245 The second-order rate constant for the nucleophilic addition of p-MeC₆H₄S⁻ to phenyl vinyl sulphone has been detected at 0–45°C, the energy, free
energy of activation and entropy being 16.0 kcal/mole, 16.5 kcal/mole and -4 e.u. respectively\textsuperscript{240}. Allyl alcohol and n-BuSH give n-BuSCHMeCH\textsubscript{2}OH in the presence of Y\textsubscript{3} elemental sulphur as a catalyst and an initial pressure of hydrogen of about 30 atmospheres, but allyl alcohol and i-BuS\textsubscript{2}H form i-BuS(CH\textsubscript{2})\textsubscript{2}OH under free-radical conditions\textsuperscript{240}. The compound MeS(CH\textsubscript{2})\textsubscript{2}Me has been prepared from allyl chloride, first by MeS\textsuperscript{-} addition and then MeS\textsuperscript{-} substitution\textsuperscript{240}.

Activated thiols will add to p-isopropenylphenol in chloroform solution in the presence of p-iodoanilic acid giving a Markownikoff addition product,

\[
\begin{align*}
\text{p-HOC}_{2}H_{4}CMeCH\text{=CH}_{2}RSH & \rightarrow \text{p-HOC}_{2}H_{4}CMeCH_{2}C\text{=CH}_{2}RSH \\
(R=CH_{3}, CO_{2}R', R(=O)OR', CH_{2}CONHCO_{2}H)
\end{align*}
\]

Thioacetic acid gave an anti-Markownikoff addition product. Simple thiols do not react even in the presence of catalysts, except under pressure and irradiation. With benzylthiol and p-chloroanisethiol the usual addition of the \textit{para}-hydrogen occurred\textsuperscript{240}.

\[
\begin{align*}
\text{p-HOC}_{2}H_{4}CMeCH\text{=CH}_{2}C\text{=CH}_{2}H_{2}SH & \rightarrow \text{p-HOC}_{2}H_{4}CMeCH\text{=CH}_{2}C\text{=CH}_{2}SH
\end{align*}
\]

A few other examples of this type of addition, in the presence of a catalyst, are found in the patent literature\textsuperscript{240}.

Simple Markownikoff addition of thiol to the C=\textit{C} bond occurs in some carbohydrate derivatives\textsuperscript{240}, and to dimethyl maleate\textsuperscript{240}.

C. Reactions with Acetylens

Thiols do not add less readily to acetylenes than to olefins. The addition occurs at high temperatures in the presence of a base\textsuperscript{240}.

\[
\begin{align*}
\text{HC=CH} + \text{RSH} & \rightarrow \text{CH}_{3}-\text{C}H(\text{CHR})
\end{align*}
\]

A second molecule of thiol may be taken up

\[
\begin{align*}
\text{RSH} + \text{HC=CH} + \text{RSH} & \rightarrow \text{RSH} + \text{RSH}
\end{align*}
\]

If addition occurs across an acetylenic bond, there is the possibility of formation of \textit{cis} and \textit{trans} isomers. Phenyl acetylene reacts with ethanol in the presence of an alkali catalyst at 100-225°C. Progressively larger amounts of the \textit{trans} isomer were formed as the temperature increased, reaching a maximum of 71% \textit{trans} at 200°C. A rapid \textit{cis}--\textit{trans} isomerism accompanies the vinylation reaction\textsuperscript{240}.

\[
\begin{align*}
\text{RSH} + \text{Ph} = \text{CH} & \rightarrow (\text{C6H5})\text{CH} = \text{CHSH}
\end{align*}
\]

16. Thiols as nucleophiles

The degree of \textit{trans} stereoselectivity for nucleophilic additions of \textit{p}-toluenesulphonic acid to negatively substituted acetylenic compounds (Y=CN, SO\textsubscript{2}C\textsubscript{6}H\textsubscript{4}Me, CO\textsubscript{2}Me, CONH\textsubscript{2}, COMe) in methanol is dependent on the nature of the activating group \( Y \) and

\[
\begin{align*}
\text{H}\text{C} = \text{C}Y + \Delta \text{R} & \rightarrow \text{H}\text{C} \text{= C}\text{= C}\text{= C}\text{= C}\text{= C}Y + \Delta \text{R}
\end{align*}
\]

decreases where \( Y \) is capable of delocalizing the adjacent incipient negative charge\textsuperscript{241}. In the tertiary amine-catalysed addition of thiols to ethyl propiolate, it has been shown that the amount of \textit{trans} addition product in the reaction mixture increased as the acidity of the thiol. Similar additions to hexafluoro-2-butyne and trifluoroacetylene showed that with both trifluoroethyl-activated acetylenes \textit{trans} addition was predominant. However only 5% \textit{trans} was obtained in the reaction of cyclohexanethiol and trifluoroethyl acetylene\textsuperscript{241}.

Addition reactions have been studied with various substituted acetylenes such as the Markownikoff additions

\[
\begin{align*}
\text{P} & \text{OC} = \text{CH} + \text{SH} \rightarrow \text{P} \text{OC} = \text{CHSH}
\end{align*}
\]

and anti-Markownikoff additions of thiols to phenoxyacetylene, depending on the solvent employed\textsuperscript{242}.

\[
\begin{align*}
\text{P} & \text{OC} = \text{CH} + \text{SH} \rightarrow \text{P} & \text{OC} = \text{CHR}
\end{align*}
\]

Addition to trifluoroacetylene has been studied with thiols in the presence of sodium ethoxide or triethylamine\textsuperscript{242}.

\[
\begin{align*}
\text{R} & \text{SH} + \text{X} = \text{CF} \rightarrow (\text{RSH})\text{CH} = \text{CF} \text{H}
\end{align*}
\]

Addition can also occur in systems containing ethylenic and acetylenic bonds, and nucleophilic addition occurs primarily across the acetylenic bond:

\[
\begin{align*}
\text{R} & \text{SH} + \text{F} \text{C} = \text{C} \text{H} = \text{CR} \text{CO} \rightarrow \text{F} \text{C} = \text{C} \text{H} = \text{CR} \text{SH}, \text{F} \text{C} = \text{C} \text{H} = \text{CR} \text{CO}
\end{align*}
\]

The latter compound isomerizes to \textit{F} \text{C} \text{H} = \text{CH} \text{CH} = \text{CR} \text{CO} \rightarrow \text{F} \text{C} = \text{C} \text{H} = \text{CR} \text{CO} \text{R}. In the free radical addition compounds such as \textit{F} \text{C} \text{H} = \text{CH} \text{CH} = \text{S} \text{Me}, \text{Me} \text{S} \text{Me} were isolated\textsuperscript{243}.

Thiols containing an acetylenic bond may cyclize. The heterocyclization of acetylenic thiols, \( \text{RC}=\text{C}(\text{CH}_{2})_{2}\text{SR} \), has been studied under nucleophilic
and free radical conditions forming products (43), (44) and (45). Compound 44 is the main product of the nucleophilic attack when \( R = H \), and mixtures of all three are formed under free radical conditions.

\[
R - S - S^{+} \xrightarrow{\text{CH}_2} \text{(43)} \quad \text{RCH} - S - S^{+} \xrightarrow{\text{(CH}_2)_2} \text{(44)} \quad \text{RCH}_2 - S - S^{+} \xrightarrow{\text{(CH}_2)_2} \text{(45)}
\]

The addition of thiols to acetylenic bonds in compounds with a formal negative or positive charge has been examined. Aromatic thiols react with \( \text{HO}_2\text{CC} = \text{CCO}_2\text{K} \) giving (phenylthio)fumaric acids, which were ecelized in the presence of sulphuric acid to thiachromonecarboxylic acids.

Two products are formed when the benzenethiolate anion reacts with dimethylprop-2-ylsulphonium bromide. \( \text{Me}_2\text{SCH} = \text{C} = \text{S} \text{Me}^+ \text{Br}^- \). The reaction is postulated to proceed through the formation of an allenic system \( \text{SC} = \text{C} = \text{C} \). The initial product, not isolated, isomerizes, and may subsequently be dealkylated with excess thiolate:

\[
\text{Me}_2\text{SCH} = \text{C} = \text{CH} + \text{PS}^+ \quad \xrightarrow{\text{isomerization}} \quad \text{Me}_2\text{SCH} = \text{C} = \text{C} + \text{MeS}^+ \text{Me} \quad \xrightarrow{\text{dealkylation}} \quad \text{MeSCH} = \text{C} = \text{C} = \text{SMe}
\]

The methanethiolate anion also adds to the acetylenic bond of the dealkylated product and some trisulphide is formed:

\[
\text{Me}_2\text{SCH} = \text{C} = \text{CH} + \text{MeS}^+ \quad \xrightarrow{\text{MeSCHMe} = \text{C} = \text{CH} + \text{MeSCHMe}} \quad \text{MeSCHMe} = \text{C} = \text{C} = \text{SMe}
\]

A similar reaction is observed with the benzenethiolate anion and 1-(prop-2-ynyl)tetrahydrothiophenium bromide.

\[
\text{SC} = \text{C} = \text{CH} + \text{PS}^+ \quad \xrightarrow{\text{(PSH)} = \text{C} = \text{CH} = \text{S(PS)}^+}
\]

D. Reactions with Nitrile Groups and Azomethine Bonds

In acidic solution nitriles undergo an addition reaction with thiols forming iminothioesters.

\[
\text{R} = \text{N} + \text{R} = \text{SH} \quad \xrightarrow{\text{H}^+} \quad \text{R} = \text{R} = \text{S} = \text{N} = \text{H} \quad \text{H}^+
\]

16. Thiols as nucleophiles

The examples of this type of reaction in the recent literature are somewhat limited. The simplest is the formation of cyanosformimidic acid (46) by reaction of cyanogen with a thiol in an inert solvent in the presence of an acylating agent:

\[
\text{RSH} + \text{HCN} \quad \xrightarrow{\text{H}_2\text{O}} \quad \text{HN} = \text{C} = \text{CN} = \text{SR}
\]

2,4,5-Trisubstituted imidoxoles (47) can be obtained from thiols and \( \text{N} = \text{N} \)-cyanoalkylidenenemine (48). This reaction involves a cyclization reaction, proceeding through the initial addition of the thiol to the \( \text{C} = \text{N} \) bond.

\[
\text{RCH(NCN(O)H) = CH(R)} \quad \xrightarrow{\text{RSH}} \quad \text{R} = \text{S}^2 \quad \text{O} = \text{N} = \text{C} = \text{N} = \text{R}
\]

Addition of thiols across an azomethine bond occurs resulting in the formation of a carbon—sulphur bond, an example is the formation of N-benzylidene-o-nitroaniline (49):

\[
\text{o}_{\text{NC} = \text{C} = \text{H}} \quad \text{CH} = \text{PNMe}_{\text{p}} \quad \xrightarrow{\text{O}_{\text{NC} = \text{C} = \text{H}} \quad \text{CH} = \text{PNMe}_{\text{p}}}
\]

Thiophenol reacts with diphenylketene-(p-bromophenyl)imine (50) causing reduction of the aromatic amine and fracture of the \( \text{C} = \text{N} \) bond:

\[
\text{Ph}_2\text{C} = \text{C} = \text{N} = \text{H} \quad \xrightarrow{\text{PhSH}} \quad \text{Ph}_2\text{C} = \text{S} = \text{C} = \text{N} = \text{H} \quad \xrightarrow{\text{PhSH}} \quad \text{Ph}_2\text{C} = \text{S} = \text{C} = \text{N} = \text{H} \quad \xrightarrow{\text{PhSH}} \quad \text{Ph}_2\text{C} = \text{S} = \text{C} = \text{N} = \text{H}
\]

E. Reactions with Carboxyl and Thioacetyl Groups

Thiols can react with ketones to give a hemithioacetal:

\[
\text{R} = \text{C} = \text{O} + \text{RSH} \quad \xrightarrow{\text{R} = \text{C} = \text{O}} \quad \text{R} = \text{C} = \text{OSR}
\]

Further reaction readily gives the thioacetal, although a catalyst is sometimes required:

\[
\text{R} = \text{C} = \text{O(SR)} + \text{RSH} \quad \xrightarrow{\text{R} = \text{C} = \text{O(SR)} + \text{RSH}} \quad \text{R} = \text{C} = \text{OSR} + \text{H}_2\text{O}
\]
Thioacetals may be thermally decomposed to the corresponding thioketones:

\[ 2 \text{R}^1 \text{R}^2\text{C}=(\text{OH})_2 \rightarrow \text{R}^1\text{R}^2\text{S} + \text{R}^1\text{R}^2\text{CHO} \]

The hemithioacetal can also be reduced by excess thiol to the sulphide complexes:

\[ 2 \text{R}^1\text{R}^2\text{SH} + \text{R}^1\text{R}^2\text{CHO} \rightarrow \text{R}^1\text{R}^2\text{S} + \text{R}^1\text{R}^2\text{CHO} + \text{H}_2\text{O} \]

The equilibria between propanethiol and simple carbonyl compounds have been studied in CHCl₃: the resulting α-hydroxysulphides may be converted into the thioacetals where the equilibrium constants are less than 10⁴, by addition of an acid catalyst (BF₃ or HCl). Examples of aldehydes and ketones whose values of K are less than 10⁴ are MeCHO, Me₂CO; those having K values greater than 10⁴ are CCl₃CHO, (CF₃CO)₂CO anhydride.

The kinetics of the formation of the hemithioacetal in 50% ethanol-water have shown that the reaction is acid catalysed and does not involve a thiolate anion, probably proceeding via the formation of the protonated ketone³⁵,

\[ >\text{C}=\text{OH}^+ + \text{R}^- \text{SH} \rightarrow \text{R}^- \text{S}^- \text{C}=\text{OH}^+ \rightarrow \text{R}^- \text{SC} \text{OH} + \text{BH}^+ \]

Other studies of rate and equilibrium constants of the formation and breakdown of hemithioacetals (MeCHO + PhSH, or AcSH, or p-NO₂C₆H₄SH) reveal a diffusion-controlled rate-determining step, with proton transfer in some cases concerted with cleavage and formation of the C–S bond³⁵. A general base-catalysed mechanism involves attack of the RS⁻ anion on the carbonyl group²⁸.

The addition reaction can be utilised synthetically, as is illustrated in the examples where further reaction with amines occurs.

\[ \text{R}^1\text{R}^2\text{S} + \text{R}^3\text{CHO} \rightarrow \text{R}^1\text{R}^2\text{NR}^1\text{R}^3 \]

(Reference 285)

The phosphorus-containing ketone PhC(O)(O)Ph(OMe)₂ does not react with sodium thiolate, but will react with the thiol in the presence of

16. Thioesters as nucleophiles

Mg(OH)₂ forming the thioester, upon fracture of the carbon–phosphorus bond²⁹.

\[ \text{Me} \]
\[ \text{SH} \]
\[ \text{HCHO} + \text{HNR}_2 \]

(Reference 286)

\[ \text{Me} \]
\[ \text{SCH} \text{NR}_2 \]

\[ \text{Me} \]
\[ \text{PhC(O)(O)(OMe)}_2 + \text{RSH} \rightarrow \text{PhC(O)(O)(OMe)}_2 + \text{H}_2\text{O} \]

\[ \text{MeO} \]

\[ \text{PhC(O)(O)(OMe)}_2 \]

\[ \text{PhC(O)(O)(OMe)}_2 \]

Analogous reactions occur with thiocyanates, as illustrated by the example²⁹:

\[ \text{PhC(O)(O)(OMe)}_2 + \text{HSC} \text{H}_2\text{O} \rightarrow \text{SCH} \text{Ph} \text{Ph} \text{C(O)(O)(OMe)}_2 \]

\[ + \text{PhC(O)(O)(OMe)}_2 \]

F. Reactions Involving Conjugated Systems

With a conjugated system similar to the type C=–C=–X, where X can be C₂, N₂O, it is of interest to observe where addition of a thiol occurs. The majority of reactions involve addition across the C=–C bond, but exceptions are found.

Several products are obtained from the reaction of thiols with thiamine anhydride (59a). A conjugated system is postulated as an intermediate with initial 1,2 addition. The reaction products depend subtly on the pH²⁹.

\[ \text{PhC(O)(O)(OMe)}_2 + \text{SH} \text{C(O)(O)(OMe)}_2 \rightarrow \text{SCH} \text{Ph} \text{Ph} \text{C(O)(O)(OMe)}_2 \]
Reactions of the conjugated aldehydes, crotonaldehyde and 4-hydroxy-2-pentenal, with a C=C=C=O bond system, in aqueous solution with thiolglycolic acid, either as its sodium salt or ethyl ester, give addition across the C=C bond. The 4-hydroxy-2-pentenal adduct cyclizes to the homocetol,

\[
\text{MeCH} + \text{CHO} + \text{RSH} \rightarrow \text{MeCH}(\text{SR})\text{CHO}
\]

\[
\text{MeCH(OH)CH}=\text{CHO} + \text{RSH} \rightarrow \text{MeCH(OH)CH}(\text{SR})\text{CHO}
\]

The kinetics have been studied and show that with thiolglycolic acid derivatives between pH 1.5 and 2.5 the RS⁻ ion and RSH react, but at pH ~ 3.5 the R²⁺ ion is the reactive entity. A reaction mechanism has been derived.¹⁹⁰

1,4-Addition of thiols in basic solution to the C=C=C=O bond system of unsaturated ketones, such as 4-benzylidene-1-butyl-pyrrolidine-2,3-dione, has been observed, forming with benzenethiol in piperidine, 1-butyl-3-hydroxy-(n-phenylthiobenzyl)-3-pyrrolin-2-one.¹⁹¹

Addition of thiols primarily to the C=C bond in C=C=C=O systems in quinones and lactones has been observed.¹⁹²,¹⁹³ The reactions were studied in neutral or alkaline solution and probably involve attack by the thiolate anion. In compounds containing both carbonyl or carboxyl groups and acetylenic triple bonds, addition occurs primarily across the acetylenic bond. Cyclization of the initial product so formed is also observed.¹⁹⁴

\[
\text{n-CH₃C}_2\text{H}_4\text{NHSH} + \text{MeO}_2\text{CC}=\text{CCO}_2\text{Me} \rightarrow \text{HNO} + \text{MeO}_2\text{CC}=\text{CCO}_2\text{Me} + \text{MeOH}
\]

The addition of thiols to N-ethylthiouracil within the pH range 5-7 in 95% ethanol has been studied.¹⁹⁵ The reaction proceeds via the mechanism.
Attack by the neutral thiol could not be detected. The rate of attack of ortho-alkyl-substituted benzenethiolate anions upon the olefinic bond is sensitive to the bulk of the alkyl group. Two effects can be distinguished:

(1) inhibition of solvation of the thiolate anion, which increases its nucleophilicity (rate accelerating), and

(2) steric interference between the thiolate nucleophile and the olefin in the transition state (rate retarding). Net steric acceleration is observed in the nucleophilic addition to an activated double bond of $\text{o-t}$-butylbenzenethiolate which is an order of magnitude more reactive than the other alkylbenzenethiols studied. The implications of these results as regards hydrophobic bulk effects in enzymatic reactions involving mercaptide functions have been discussed.\(^{109}\)

The addition of the benzenethiolate anion to 4-t-butyl-1-cyanocyclohexene, containing formally a $\text{C}=$C=C–C=N bond system, occurs across the C–C bond. In ethanol two products are obtained both containing axial phenylthio groups, but in THF some equatorial SPh is also formed.\(^{110}\)

Addition reactions occur in C–C–C–N conjugated systems. Various products are formed in the reaction of thiols, in the presence of triethylamine, with N-[1,1,1,3,3,3-hexafluoroisopropylidene]-2,2-dialkylvinylamine (51)\(^{107}\). 1,4-Addition, forming products of orientation (82), occurs with t-butylthiol and benzenethiol:

\[
\text{R}^1 \text{R}^2 \text{S}^+ (\text{Et}_3 \text{N})_2 \rightarrow \text{R}^1 \text{R}^2 \text{S}^- + \text{R}^1 \text{R}^2 \text{SH} (\text{Et}_3 \text{N})_2 \rightarrow \text{R}^1 \text{R}^2 \text{S}^- (\text{Et}_3 \text{N})_2 + \text{R}^1 \text{R}^2 \text{SH} \rightarrow \text{CH} (\text{CF}_3)_2
\]

The reaction of benzenethiol with 51 when $\text{R}^1 = \text{Ph}$ and $\text{R}^2 = \text{H}$ gives an enamine 53; a differently orientated 1,4-addition product 54 is obtained with ethanethiol.

Addition across a conjugated C–N, C–O system is the mechanism postulated for the reaction of the 2,2-dichlorovinylamine derivatives of RCONHC=CCl\(_3\) with BuSH in the presence of a small amount of alkaline, although the reaction appears superficially to be addition across a C–C bond:

\[
\text{MeCONHC=CCl} + \text{BuSH} \rightarrow \text{MeCONHC} (\text{SBu})\text{CCl}
\]

Initially a conjugated C=N–C=O system is formed and the thiol gives 1,4-addition:\(^{108}\)

\[
\text{Cl} (\text{C=CHNHCOMe}) \rightarrow [\text{CHCl} \text{C} (\text{CH}=\text{N}–\text{C}(\text{Me})=\text{O})] \rightarrow [\text{H}] \text{BuSiR} \text{Cl} \text{CHCl} \text{C} (\text{SBu})\text{N} (\text{C}(\text{Me})=\text{O}) \rightarrow \text{CHCl} (\text{SBu}) \text{CHNHCOMe}
\]

G. Reactions with Alkylene Oxides and Sulphides

Alkylene oxides undergo ring-opening reactions with a wide variety of substances:

\[
\text{R} \text{CH} = \text{CH} \text{OH} \rightarrow \text{RC} \text{H} \text{H} \text{H} \text{A} \text{O} \text{H} \text{CH} \text{H} \text{A} \text{CH} \text{CH} \text{OH}
\]
Under basic or neutral conditions when R is an electron-donating group, the main product is that formed by attack at the least substituted carbon atom, namely RCHOHCH₂A. The A⁻ ion probably attacks before the C—O bond is completely broken. Thiols react to form hydroxythioethers.

\[
\text{RCH—CH}_2 + \text{R'SH} \rightarrow \text{RCHOHCH}_2\text{SR'}
\]

The alkylene oxides are thermally unstable and form the isomeric aldehydes or ketones:

\[
\text{RC—CR}_2 \rightarrow \text{RCOCR}_2
\]

The products of the reactions of thiols or thiolate ions with alkylene oxides may correspond to addition across the C—O bond, or reactions of the isomeric aldehyde or ketone if the temperature is sufficiently high.

Simple addition is observed in reactions such as:

\[
\text{CH}_2\text{CH}_2 + (\text{Me}_3\text{Si})_2\text{CH}=\text{CH} = \text{CH}_2 \rightarrow \text{CH}_2\text{CH}_2\text{Si}(\text{Me}_3)\text{SiCH}_2\text{CH}_2\text{CH}_2\text{CH}_2\text{CH}_2\text{Si}(\text{Me}_3)\text{SiSiMe}_3
\]

In other reactions both the C—O and C—C ring bonds are cleaved, and the intermediate product corresponding to addition across the C—O bond can be isolated:

\[
\text{PhCOCH—CH}_2\text{X} + \text{PhSH/PhN} \rightarrow \text{PhCOCH(Ph)CH(OH)CH}_2\text{X—Ph}
\]

Various examples are known where an alkylene sulphide ring system is fractured by a thiol or thiolate, the reactions being essentially similar to those of the oxygen analogues. The thiol generated in the initial reaction may react further with the remaining cyclic sulphide.

\[
\text{CH}_2\text{—CH}_2 + \text{RSH} \rightarrow \text{PhCOCH(Ph)CH(OH)CH}_2\text{X—Ph}
\]

Thiols can react with alkylene sulphides to form two products,

\[
\text{Me}_2\text{C—CH}_2 + \text{RSH} \rightarrow \text{Me}_2\text{C(SH)CH}_2\text{SH or Me}_2\text{C(SH)CH}_2\text{SH}
\]
While thioephlorhydryl reacts with potassium butanethiolate without ring fracture to form butylthioglycidyl sulphide, the products of the reaction with thioephlorbromohydrid include butylthioglycidyl sulphide and some of the disulphide, \( CH_2=CHCH_2SSBu \), formed by fracture of the alkylene sulphide ring and subsequent dehydrobromination:

\[
\text{BrCH}_2\text{CHCH}_2 + \text{BuSK} \rightarrow \text{BuSCH}_2\text{CHCH}_2 + \text{CH}_2=\text{CHCH}_2\text{SSBu}
\]

### H. Reactions with Cyclic Compounds

Thiols will add across the \( \text{C} = \text{N} \) bond in cyclic systems, such as 2-benzyl-2 phenylthiazolium bromide (57):

\[
\begin{align*}
\text{Br}^- & + \text{CH}_2\text{Ph} \quad \text{N} \\
\text{Ph} \quad \text{N} & + \text{NaSPh} \rightarrow \text{CH}_2\text{Ph} \quad \text{N} \\
\text{SPh} & \quad \text{SPh}
\end{align*}
\]

Similar addition occurs in the bicyclic compound (58):

\[
\begin{align*}
\text{Br}^- & \quad \text{N} \\
\text{CH}_2\text{N} & + \text{NaSPh} \rightarrow \text{CH}_2\text{Ph} \quad \text{N} \\
\text{SPh} & \quad \text{SPh}
\end{align*}
\]

The benzenethiolate anion acts primarily as a reducing agent with 2-alkylisothiazolium salts (59); simple aliphatic thiols did not react.

\[
\begin{align*}
\text{Ph} & \quad \text{S}^- \quad \text{N}^+ \quad \text{Ma} \\
\text{S} & \quad \text{N}^+ \\
\text{R} & \quad \text{Ph} \quad \text{II}, \text{Ph}
\end{align*}
\]

However, with 5-phenyl-1,2-dithiolium cation (60), unlike the isothiazolium cations, simple \( \text{S}^- \text{adducts} \) (61) were formed with a range of sulphur nucleophiles:

\[
\begin{align*}
\text{Ph} & \quad \text{S}^- \quad \text{S}^- \\
\text{S} & \quad \text{S}^- \\
\text{R} & \quad \text{Et}, \text{CDPh}, \text{COMe} \quad \text{CH}_2\text{CN} = \text{A}
\end{align*}
\]

16. Thiols as nucleophiles

A similar 3-adduct was formed by only the ethanethiolate ion with 3,5-diphenyl-1,2-dithiolium salts. Other thiols, except ethanethiol, which did not react, convert to 3-alkylthio-5-phenyl-1,2-dithiolium cations (62) into 1,2-dithio-3-thione (63).

\[
\begin{align*}
\text{Ph} & \quad \text{S}^- \quad \text{R}^2 \\
\text{S} & \quad \text{S}^- \\
\text{SR}^- & \quad \text{SR}^- \\
\text{Ph} & \quad \text{S}^- \quad \text{N}^+ \\
\text{R} & \quad \text{Ph}, \text{p-Tol}, \text{or HOCH}_2\text{CH}_2
\end{align*}
\]

This is probably not a simple demethylation as no \( \text{S}^- \text{methylated nucleophiles} \) were detected. The thione is also produced in the reaction of benzenethiolate anion with the 1,2-dithiolium cation with no \( \text{S}^- \text{alkyl substituent} \) in the \( \text{S}^- \text{position} \).

Two products, a disubstituted quinone or hydroquinone, are formed exclusively in the reaction of thiols with 4,7-benzimidazolethione (64) in methanol. The quinone is formed exclusively by aliphatic thiols and the hydroquinone when \( R = \text{Ph}, \text{p-Tol}, \text{or HOCH}_2\text{CH}_2 \).

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CHAPTER 17
Oxidation of thiols

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I. INTRODUCTION

1. Introduction

Aliphatic and aromatic thiols are oxidized by a variety of reagents to disulfides and to higher oxidation products depending on the specific reaction conditions (Scheme 1).

The two oxidation chains are not as separate as indicated in the scheme since a number of interconversions are possible. They may be thought...

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to occur via the hydrolytic products which are shown on the right side of the Scheme. The sulphenic acid has been reported in brackets since its very high reactivity does not permit isolation except in very special cases (see section III.B).

Most of the reactions indicated in the scheme are reversible eventually through appropriate derivatives; however, true equilibria among pairs of the above-mentioned products are quite rare.

In this chapter we shall mainly deal with the oxidation of thiols to disulphides (equation 1).

$$2 \text{RSH} \rightarrow \text{RSSR} + \text{H}_2\text{O}$$

(1)

The subsequent stages of oxidation will be dealt with only in limited and specific cases. Attention has been mainly focused on the most commonly used chemical oxidizing agents.

Electrochemical and photochemical oxidations are also briefly discussed but for more comprehensive reviews on these subjects see the relevant chapters in this volume.

The literature, which is not comprehensively reviewed, has been covered up to the middle of 1972. References to some later published papers have been also made.

II. ELECTROCHEMICAL OXIDATION

Studies on the formally simple equilibrium (7) meet severe difficulties.

$$2 \text{RSH} \rightarrow \text{RSSR} + 2\text{H}^+ + 2\text{e}^-$$

(2)

Polarographic studies (dropping mercury electrode, DME) have been limited by the chemical intervention of the mercury whereas more recent work with noble metals electrodes has been hampered by absorption and/or passivation phenomena.

Much attention has been devoted to systems of biological interest and electrochemical methods for quantitative analysis of thiol and disulphide groups in simple organic compounds as well as in proteins have been reported.

The polarography of thiols is characterized by an anodic wave which often is well defined although, as for instance in the case of cysteine, the shape of the polarogram depends strongly on pH and buffer. Koltshoff and Barnum showed that the anodic wave of cysteine is due to the formation of mercurocysteine (HgSR), i.e., to the oxidation of the electrode and not of the thiol. Complications may arise when the reaction product is insoluble in the medium and covers the electrode.
Thiols are also oxidized at a platinum electrode but at more positive potentials (see below).

The oxidation of the mercury electrode, the anodic potential of which is decreased by salt formation, appears to be quite general\textsuperscript{11,12,15,16}.

The values of $E_a$ do not change very much with the nature of the thiol at pH values high enough to ensure that all the thiols are in the anionic form\textsuperscript{12} as shown in Table 1.

<table>
<thead>
<tr>
<th>Thiol</th>
<th>$E_a$ (volts)\textsuperscript{a}</th>
<th>$pK_a$</th>
</tr>
</thead>
<tbody>
<tr>
<td>2-Mercaptoethylamine</td>
<td>-0.500</td>
<td>2.3</td>
</tr>
<tr>
<td>2-Mercaptopyrrolidine</td>
<td>-0.534</td>
<td>9.4</td>
</tr>
<tr>
<td>2-Mercaptoethylamine</td>
<td>-0.560</td>
<td>10.75</td>
</tr>
<tr>
<td>2-Mercaptoethylamine</td>
<td>-0.537</td>
<td>9.6</td>
</tr>
<tr>
<td>Thioglycine acid</td>
<td>-0.580</td>
<td>10.28</td>
</tr>
<tr>
<td>Cysteine</td>
<td>-0.580</td>
<td>9.12</td>
</tr>
<tr>
<td>Oxythiolamine</td>
<td>-0.480</td>
<td>10.89</td>
</tr>
</tbody>
</table>

\textsuperscript{a} At the dropping mercury electrode (D.M.E.).

\textsuperscript{b} Referred to standard calomel electrode (S.C.E.).

The reduction of disulphides on D.M.E., the reverse of equation (2), appears to be a simpler reaction and in some cases a single cathodic wave was observed which behaves as required by a reversible process\textsuperscript{11,16}; however, in many other cases evidence for irreversible processes was found\textsuperscript{12,18,19}. Moreover, in some conditions cystine\textsuperscript{21,22} as well as other disulphides\textsuperscript{18} present a pre-wave.

Whereas the wave at higher potential appears to be due to a diffusion-controlled process, the pre-wave, as also shown by oscillographic polarography studies\textsuperscript{21,24}, depends on the absorption and reaction of the disulphide at the electrode.

The following equations were proposed to explain the process:

$$
\begin{align*}
\text{RSSR} + \text{Hg} & \rightarrow \text{RSSR}^- + \text{Hg} \quad \text{(ads.)} \\
\text{RSSR}^- + \text{Hg} & \rightarrow \text{RSSR} + \text{Hg} \\
(\text{RSSR})_2 + 2e^- + 2H^+ & \rightarrow 2 \text{RSSR} + \text{Hg} \\
2 \text{RSSR} + \text{Hg} & \rightarrow 2 \text{RSSR} + \text{Hg} \quad \text{(desorb.)}
\end{align*}
$$

On platinum or gold electrodes, aqueous solutions of disulphides are not reduced and only the oxidation of thiols could be studied.

17. Oxidation of thiols

It was observed that cysteine as well as other thiols\textsuperscript{12,13,14} is oxidized, by a one-electron process, to cystine and the latter is further oxidized, probably, to cystic acid. Strong absorption phenomena were observed.

In DMF solutions the redox reaction benzenethiol-diphenyl disulphide (equation 2, B = Ph) could be studied by cyclic voltammetry on an inert electrode from both directions. The results obtained indicate that the reactions are 'irreversible' and that the acid hydrogen of benzenethiol is converted to molecular hydrogen.

The authors\textsuperscript{18} proposed that the following reactions occur at an inert electrode in solvents like DMF:

$$
\begin{align*}
2 \text{RSSR}^- + 2e^- & \rightarrow \text{RSSR} + 2\text{H}^+ \\
2 \text{RSSR}^- + 2e^- & \rightarrow \text{RSSR} + 2\text{R}^- \\
2 \text{RSSR}^- + 2e^- & \rightarrow 2 \text{RSSR} + 2\text{H}^+ \\
\end{align*}
$$

It is noteworthy that diphenyl disulphide in the stated conditions\textsuperscript{24} is not further oxidized, contrary to what is observed with cystine\textsuperscript{12,13} in aqueous solutions.

However, at higher potentials the disulphide can be oxidized in acetonitrile with sodium perchlorate as supporting electrolyte, diphenyl disulphide is oxidized to benzenesulphonic acid\textsuperscript{21}. Possibly, in this case the perchlorate ion does intervene in a chemical reaction subsequent to the anodic process.

All schemes proposed for the oxidation of thiols to disulphides in a more or less explicit way imply the formation of thiol radicals as intermediates.

The absence of any reaction of these radicals with the solvent suggests that the dimerization occurs at the electrode surface in a very fast process.

III. CHEMICAL OXIDATION

A. Oxidation by Peroxidic Compounds

The oxidation of thiols by hydrogen peroxide, alkyl hydroperoxides as well as peroxycacids is a well-known reaction in its qualitative aspects, but very little mechanistic study has been carried out\textsuperscript{16,19,20}.

The initially formed product is in most cases the corresponding disulphide, which can be easily oxidized further by excess oxidant.

A particular example of overoxidation is the oxidative desulphurization of heteroaromatic thiols by hydrogen peroxide which may lead to the
formation of the corresponding hydrocarbon or hydroxy derivative depending upon the reaction conditions. Because of the ease of oxidation, these reactions are scarcely used for preparative purposes. However, the oxidation of thiols to disulphides by peroxides attracted some interest in the patent literature connected with the general problem of hydrocarbon sweetening. More recently interest was revived by the suggested use of hydrogen peroxide as a selective oxidant and control of sulphide odours in sewage treatments and similar applications.

Aliphatic and aromatic thiols are easily oxidised to disulphides in aqueous or alcoholic solutions under both acid and alkaline conditions. Higher molecular weight thiols are better oxidised as copper salts. Particularly in the presence of aliphatic amines, the oxidation is easily carried out also in hydrocarbon solvents.

In hydrocarbons, and more generally in aprotic solvents, lower molecular weight aliphatic mercaptans are quite effective in oxidising thiols to disulphides.

A mechanistic study of the oxidation of o-mercapto phenylacetic acid in water in the pH range 2–4.5 by hydrogen peroxide showed that the rate of reaction was independent of the thiol concentration, first order in H₂O₂ and inversely proportional to the square root of hydrogen ion concentration (equation 10).

\[ r = k[H_2O_2][H^+][\text{thiol}] \]

The suggestion that the reaction was catalysed by traces of heavy metal ions was advanced on the basis of the acceleration observed on addition of ferrous ions and the depression of rate when EDTA in excess was added. In this case the kinetic expression also changed becoming first order in thiol concentration (equation 11).

\[ r = k[H_2O_2][\text{thiol}][H^+] \]

Further thorough studies would be necessary to define in detail the mechanism of these reactions. The limited evidence available is, however, consistent with the reasonable assumption that the reaction proceeds by a radical chain mechanism, probably initiated by heavy metal ions and involving thiol radicals following a scheme similar to that proposed for the oxidation of mercaptans by molecular oxygen (see section IV).

The quite abundant literature on the oxidation of thiols in compounds or biological interest like glutathione, cysteine, etc., which has been recently reviewed, is also in line with the above conclusions.

17. Oxidation of thiols

B. Oxidation by Halogens

The products of the oxidation of thiols by halogens vary with the halogen and with the reaction medium.

In aqueous solvents chloride and bromine react with thiols to give sulphynil halides or sulphone acids (equations 12 and 13).

\[ \text{RSH} + X_2 + \text{H}_2\text{O} \rightarrow \text{RSHX} + \text{HX} \] (12)
\[ \text{RSH} + X_2 + 3\text{H}_2\text{O} \rightarrow \text{RSCO}_2\text{H} + \text{H}_2\text{O} + \text{HX} \] (13)

The same compounds are obtained starting with disulphides and there is evidence that at least in some conditions the latter are intermediates in the reaction (see below).

Under anhydrous conditions the following reactions have been observed (equation 14–17):

\[ \text{RSH} + X_2 \rightarrow \text{RSX} + \text{HX} \] (14)
\[ \text{RSX} + \text{X}_2 \rightarrow \text{RSX} \] (15)
\[ \text{RSX} + \text{RSH} \rightarrow \text{RSSR} + \text{HX} \] (16)
\[ \text{RSSR} + \text{X}_2 \rightarrow 2\text{RSX} \] (17)

Excess of halogen forms sulphur trihalides (equation 15). In the case of arylsulphur trihalides the equilibrium is shifted to the left by increasing the temperature; with aliphatic derivatives containing a methyl group linked to sulphur the decomposition of the trichloride may lead to the formation of α-chlorinated sulphynil chlorides (equation 18).

\[ \text{RCH}_2\text{SCl} \rightarrow \text{RCH}_2\text{SCl} + \text{HCl} \] (18)

Careful hydrolysis of alkyl or aryl sulphur trihalides, in particular trihalides, yields either sulphinic acid or sulphinyl halide. The latter is obtained in good yields by reacting the trihalide with the stoichiometric amount of acetic acid (equation 19).

\[ \text{RSX}_3 + \text{CH}_3\text{COOH} \rightarrow \text{RSOX} + \text{CH}_3\text{COX} + \text{HX} \] (19)

The reaction of thiols with halogens in aprotic and nucleophilic solvents can be, possibly, represented as in equation (20).

\[ \text{RSH} + \text{X}_2 + \text{X}^- \rightarrow \text{RSX} + \text{HX} \] (20)
Although there are no mechanistic studies in this area, schemes equivalent to equation (20) have been proposed for the halogenolysis of sulphones and other bivalent sulphur compounds. The reaction goes to completion in the right with chlorine and bromine, but takes a more complex course with iodine. With fluorine the reaction yields higher oxidation products with extensive fluorination at the hydrocarbon moieties.

Sulphenyl halides are very prone to nucleophilic attack (equation 21) and in particular excess mercaptan reacts with them to give the corresponding disulphide (equation 16).

\[
\text{RSX + NH}_2 \rightarrow \text{RNSX}{+}\text{HX} \quad \text{(21)}
\]

Upon decomposition, these compounds, however, have never been isolated in this reaction; rather thioisouline esters are formed by fast reaction of sulphenyl halides (equation 23).

Disproportionation of sulphenyl acids has also been suggested as a possible route for the formation of these compounds (equation 24). The hydrolysis of sulphenyl halides under not carefully controlled conditions and particularly in concentrated solutions lead to disulphides and thiolisouline esters because of the easy disproportionation of thiolisoulines (equation 25).

\[
\text{RSX}{+}\text{H}_2\text{O} \rightarrow \text{[RSOH]}{+}\text{HX} \quad \text{(22)}
\]

\[
\text{[RSOH]}{+}\text{RSX} \rightarrow \text{RS}{-}\text{S}{-}\text{R}{+}\text{HX} \quad \text{(23)}
\]

\[
\text{Z} \text{[RSOH]} \rightarrow \text{RS}{-}\text{S}{-}\text{R}{+}\text{H}_2\text{O} \quad \text{(24)}
\]

\[
\text{X} \text{[RSOH]} \rightarrow \text{R}{-}\text{S}{-}\text{R}{+}\text{RS}{-}\text{SO}_2\text{R} \quad \text{(25)}
\]

Some anaethroquinone-sulphone and -disulphonic acids and 1-methyluracil-4-sulphonic acid have been prepared by different routes. In these compounds intramolecular hydrogen-bonded and tautomeric structures are suggested to stabilise the sulphone derivatives. Chemical and n.m.r. evidence for the existence of an aliphatic sulphone acid in the

17. Oxidation of thiols

The thermal decomposition of di-\(t\)-butyl sulphoxide has been reported (equation 26).

\[
0 \rightarrow (\text{CH}_3)_2\text{C}{-}\text{S}{-}\text{C}(\text{CH}_3) \rightarrow (\text{CH}_3)_2\text{CSOH}{+}(\text{CH}_3)_2\text{SH} \quad \text{(26)}
\]

Sulphenyl halides have been considered for a long time as a source of sulphenyl cations (equation 27). However, unambiguous evidence on free sulphenyl cations is scarce and somewhat contradictory.

\[
\text{RSX} \rightarrow \text{RS}{+}\text{HX} \quad \text{(27)}
\]

The substitutions at sulphenyl sulphur so far studied in detail occur via bimolecular mechanism except, possibly, the very special case of 2,4,6-trimethoxybenzenesulphonate aryliosupionate, which was reported to undergo unimolecular solvolysis.

On the other hand, evidence on the formation of a cationic species, thought to be the sulphenyl cation, by dissolving 4-aminobenzene sulphone chloride in concentrated sulphuric acid has been obtained.

However, the nature of the cation is not certain. Moreover, the substrate chosen in, perhaps, was quite typical. Strong interaction between the o-nitro group and the sulphenyl sulphur are in fact shown by X-ray analysis of methyl o-nitrobenzenesulphonate ester and also by the oxygen transfer from nitrogen to sulphur observed in the alkaline rearrangement of 2-nitrobenzenesulphonate aminals as a special case of o-nitrobenzene derivatives and similar species the cation formed might have the cyclic structure (I).

\[
\text{(I)}
\]

Finally, it has been reported that sulphur dichloride and trichloromethanesulphonyl chloride give 1:1 and 2:1 complexes with Lewis acids (\text{SbCl}_5, \text{AlCl}_3, \text{FeCl}_3) with a salt-like behaviour. The instability of the complexes made a full characterization impossible.
Relevant to this point is the recent finding\textsuperscript{71,72} that methane and ethane sulphenyl chloride form by addition of either BF\(_3\) or SbF\(_5\) in liquid SO\(_2\) a dimeric cationic species (2) described as follows (equation 29):

\[ 2\text{RCl} + \text{BF}_3 \rightarrow \text{R} \cdot \text{S} \cdot \text{S} \cdot \text{R} + \text{BF}_3\text{Cl}^- \]  
\[ (2) \]

The same species seems to be formed in fluorosulphonic acid and 100% sulphuric acid as well\textsuperscript{77}. Preliminary results also indicate that reaction 29 occurs with aromatic sulphenyl chlorides. The tendency of sulphur compounds to give species like (2) seems quite general: for example, disulphide and sulphenyl chlorides in DSO\(_2\)H or 100% H\(_2\)SO\(_4\) and in SO\(_2\) with BF\(_3\) or SbF\(_5\) give a species analogous to (2)\textsuperscript{77} (equation 30).

\[ \text{RSS} \cdot \text{SR} + \text{BF}_3 \rightarrow \text{RSS} \cdot \text{S} \cdot \text{S} \cdot \text{SR} + \text{BF}_3\text{Cl}^- \]  
\[ (3) \]

Furthermore, ions similar to (3) are postulated as intermediates in the interchange reaction of disulphides and sulphenyl chlorides\textsuperscript{78,79}, and intermediates like (2) should be involved in the reaction of disulphide with halogens (equation 17) which has to be considered an equilibrium reaction.

\[ \text{RSSR} + X_2 \rightarrow 2 \text{RSX} \]  
\[ (17) \]

Equilibrium (17) is completely shifted to the right for \( X = \text{Cl} \) and largely to the left with \( X = \text{I} \). The case of bromine is, as usually, intermediate. As a matter of fact very few sulphenyl halides are known\textsuperscript{80}. Apparently only sterically hindered derivatives are able to exist and also their stability, which may be reasonably great in dilute solution, is very low in concentrated solution or as pure material.

Since equilibrium (17) is shifted almost completely to the left in the case of \( X = \text{I} \) whereas reaction (14) goes to completion even with iodine, a method to titrate thiols based on the reaction in equation (31) has been widely used. However, care has to be taken to use the appropriate conditions of pH and dilution to avoid overoxidation of the disulphide which may be a quite serious cause of error\textsuperscript{81}. Thiols containing a β-carboxylic group are particularly susceptible to consume more than the iodine required by equation (31). It was suggested that the carboxyl group intramolecularly attacks the initially formed sulphenyl iodide to form a sulphenic anhydride which may undergo further oxidation at sulphur (Scheme 2)\textsuperscript{82-84}. The mechanism seems likely also in view of the recent evidence of trapping o-sulphenobenzoic acid anhydride by reaction of o-mercaptobenzoic acid with chlorine in the presence of triethyl amine\textsuperscript{84} (equation 32).

\[ \text{Scheme 2} \]

\[ \text{2RSH} + \text{Cl}_2 \rightarrow \text{RSSCl} + \text{HCl} \]  
\[ (32) \]

C. Oxidation by Dimethyl Sulphoxide and Other Sulphoxides

The oxidizing power of dimethyl sulphoxide (DMSO) as well as of other sulphoxides is well known and has been recently reviewed\textsuperscript{85}. Yiannios and Karakinos\textsuperscript{86} reported that thiols were selectively oxidized by DMSO to the corresponding disulphides in high yield with the concomitant reduction of DMSO to dimethyl sulphide (equation 33). Further studies, mainly by Wallace and coworkers\textsuperscript{87-89} confirmed these early results. They studied the reaction of several thiols with DMSO and TMSO.
(tetrastimethylene sulphone) in large excess and in the absence of solvent. In the stated conditions second-order kinetics and strong catalysis by added amines (Table 2) were observed.

Table 2. Effect of amines on the oxidation rate of 1-dodecanethiol by TMSO at 100°C

<table>
<thead>
<tr>
<th>Amine</th>
<th>pKₐ</th>
<th>k</th>
<th>Rel. rate</th>
</tr>
</thead>
<tbody>
<tr>
<td>N,N-Dimethylaniline</td>
<td>5-1</td>
<td>7.58 x 10⁻⁴</td>
<td>1</td>
</tr>
<tr>
<td>2,6-Dimethylpyridine</td>
<td>6-6</td>
<td>1.15 x 10⁻³</td>
<td>2-2</td>
</tr>
<tr>
<td>1-o-Dodecylaniline</td>
<td>10-6</td>
<td>6.42 x 10⁻⁴</td>
<td>84-4</td>
</tr>
<tr>
<td>Tri-n-butylamine</td>
<td>11-4</td>
<td>2.04 x 10⁻³</td>
<td>269</td>
</tr>
</tbody>
</table>

* Reference 92.

These authors showed that the rate of oxidation depends on the acidity of thiol and a correlation between the estimated pKₐ of them and the energy of activation was suggested (Table 3).

The oxidation rates depend also on the structure of sulphone (Table 4). As shown in Figure 1, a linear correlation of log kobs with the recently evaluated pKₐ in water of sulphones decreases.

Table 3. Effect of thiol acidity on the oxidation with TMSO at 100°C

<table>
<thead>
<tr>
<th>Thiol</th>
<th>pKₐ</th>
<th>k s⁻¹</th>
<th>Rel. rate</th>
<th>Eₐ kcal/mole</th>
</tr>
</thead>
<tbody>
<tr>
<td>1-Dodecanethiol</td>
<td>13-5</td>
<td>7.8 x 10⁻¹</td>
<td>1</td>
<td>19-4</td>
</tr>
<tr>
<td>o-Toluenethiol</td>
<td>10-5</td>
<td>1.6 x 10⁻¹</td>
<td>25</td>
<td>13-7</td>
</tr>
<tr>
<td>o-Toluenethiol</td>
<td>8</td>
<td>6.6 x 10⁻²</td>
<td>850</td>
<td>6-2</td>
</tr>
<tr>
<td>Phenethylol</td>
<td>7</td>
<td>4.0 x 10⁻¹</td>
<td>5166</td>
<td>4-9</td>
</tr>
</tbody>
</table>

Table 4. Effect of sulphone basicity in the oxidation of o-toluenethiol at 100°C

<table>
<thead>
<tr>
<th>Sulphone</th>
<th>pKₐ</th>
<th>log k</th>
<th>Rel. rate</th>
</tr>
</thead>
<tbody>
<tr>
<td>Diphenyl sulphone</td>
<td>2-34</td>
<td>-5.91</td>
<td>1</td>
</tr>
<tr>
<td>Phenyl methyl sulphone</td>
<td>2.27</td>
<td>-5.11</td>
<td>6-22</td>
</tr>
<tr>
<td>DMSO</td>
<td>-1.90</td>
<td>-4.40</td>
<td>33-3</td>
</tr>
<tr>
<td>TMSO</td>
<td>-1.31</td>
<td>-3.71</td>
<td>159</td>
</tr>
</tbody>
</table>

* k, sec⁻¹.  
* Reference 93.  
* Reference 94.

17. Oxidation of thiols

Figure 1. Correlation between the oxidation rates at 100°C of o-toluenethiol and the pKₐ of the sulphones. pKₐ values taken from the literature: diphenyl sulphone (TIPSO), phenyl methyl sulphone (PMSO) and dimethyl sulphone (DMSO), reference 93; tetrahydroxy sulphone (TMSO), reference 94.

The authors proposed that the slow step of the reaction is the formation of the adduct (4) (equation 34) followed by a fast reaction with a second molecule of thiol (equation 35). Similar mechanisms have been proposed for other sulphone-promoted oxidations and the recent isolation of stable tetracoordinate sulphur compounds makes this hypothesis quite likely.
G. Capozzi and G. Modena

However, the detailed mechanism could be more complicated as it is, in part, suggested by the phenomena of base and acid catalysis observed\(^{20}\).

It may well be, as suggested\(^{20}\), that four-centre (5) and five-centre (6) transition states are involved for the uncatalysed and amine-catalysed

\[
\begin{align*}
\text{(5)} & \quad R^+\text{S}^-H^+ \quad \text{R}^+\text{S}^-\text{H} \quad \text{N}^+\text{R}^- \\
\text{(6)} & \quad R^+\text{S}^-\text{O}^+ \quad \text{R}^+\text{S}^-\text{O} \\
\end{align*}
\]

reactions, respectively. Alternatively an acid-base interaction of the reagents (equation 36) to give an ion pair, followed by collapse of the latter to the adduct (4) (equation 37) could be postulated. This

\[
\text{RS}^- + \text{R}_2\text{SO} \quad \xrightarrow{\text{R}_2\text{SO}^- + \text{R}_2\text{SOH}}
\]

(36)

\[
\text{RS}^- + \text{R}_2\text{SOH} \quad \xrightarrow{\text{RS}^-\text{SO}^- \quad \text{OH}}
\]

(37)

resembles the mechanisms proposed for some nucleophilic substitutions by thiol of 2-chlorobenzimidazole\(^{20}\) and chloroquinoline\(^{20}\).

The reaction of (4) with the thiol to give the products (equation 35) may also be more complicated than depicted\(^{20}\). However, any hypothesis would be highly speculative in the absence of more detailed kinetic studies.

The oxidation of thioles with sulphoxides presents several attractive features like the simplicity of the reaction, the high yield and the selectivity of disulphide formation. It has to be noticed, however, that tertiary thioles do not react with sulphoxides or they give very little disulphide even in the presence of amine catalysts. Reaction temperatures higher than 100°C give rise to extensive decomposition\(^{20}\).

An interesting synthetic application of this reaction is the recovery of optically active sulphoxides from racemates when an optically active thiol is oxidized with more than the stoichiometric amount of the sulphoxide\(^{20}\).

D. Oxidation by Other Organic Chemicals

Several organic compounds may oxidize thioles to disulphides or to products of further oxidation in a variety of experimental conditions. We shall briefly deal in this section with some of the more characteristic cases.

17. Oxidation of thioles

I. Diethyl azodicarboxylate

Diethyl azodicarboxylate oxidizes thioles to disulphides, in the dark at room temperature, with concomitant formation of diethyl hydrazodicarboxylate\(^{20\text{a}}\) (equation 38).

\[
\text{HOOC-N=O-N-COEt} + \text{RSH} \rightarrow \text{RS-SR} + \text{HOOC-N=O-N-COEt} \quad (38)
\]

The reaction may also be carried out in refluxing anhydrous solvents.

In Table 5 the results obtained for the oxidation of several thioles are reported. It was reported\(^{20\text{a}}\) that triphenylphosphine catalyses the reaction.

Formation of a charge transfer complex (7) with the azo derivative as formulated below (equation 39) was suggested. It seems likely that radicals or radical ions intervene in the reaction.

\[
\text{HOOC-N=O-N-COEt} + \text{Ph}_3\text{P} \quad \xrightarrow{\text{HOOC-N=O-N-COEt}} \quad (7) \quad \text{RSR} + \text{Ph}_3\text{P} + \text{HOOC-N=O-N-COEt} \\
\]

(39)

\[
\text{Ph}_3\text{P} + \text{Ph}_3\text{P} + \text{HOOC-N=O-N-COEt} \\
\]

(7)

\[
\text{R} + \text{Ph}_3\text{P} + \text{HOOC-N=O-N-COEt} \\
\]

(7)

\[
\text{RSR} + \text{Ph}_3\text{P} + \text{HOOC-N=O-N-COEt}
\]

(7)

\[
\text{R} + \text{Ph}_3\text{P} + \text{HOOC-N=O-N-COEt} \\
\]

(7)

\[
\text{RSR} + \text{Ph}_3\text{P} + \text{HOOC-N=O-N-COEt}
\]

(7)

\[
\text{R} + \text{Ph}_3\text{P} + \text{HOOC-N=O-N-COEt} \\
\]

(7)

\[
\text{RSR} + \text{Ph}_3\text{P} + \text{HOOC-N=O-N-COEt}
\]

(7)

\[
\text{R} + \text{Ph}_3\text{P} + \text{HOOC-N=O-N-COEt} \\
\]

(7)

\[
\text{RSR} + \text{Ph}_3\text{P} + \text{HOOC-N=O-N-COEt}
\]

(7)

\[
\text{R} + \text{Ph}_3\text{P} + \text{HOOC-N=O-N-COEt} \\
\]

(7)

\[
\text{RSR} + \text{Ph}_3\text{P} + \text{HOOC-N=O-N-COEt}
\]

(7)

\[
\text{R} + \text{Ph}_3\text{P} + \text{HOOC-N=O-N-COEt} \\
\]

(7)

\[
\text{RSR} + \text{Ph}_3\text{P} + \text{HOOC-N=O-N-COEt}
\]

(7)

\[
\text{R} + \text{Ph}_3\text{P} + \text{HOOC-N=O-N-COEt} \\
\]

(7)

\[
\text{RSR} + \text{Ph}_3\text{P} + \text{HOOC-N=O-N-COEt}
\]

(7)

\[
\text{R} + \text{Ph}_3\text{P} + \text{HOOC-N=O-N-COEt} \\
\]

(7)

\[
\text{RSR} + \text{Ph}_3\text{P} + \text{HOOC-N=O-N-COEt}
\]

(7)

\[
\text{R} + \text{Ph}_3\text{P} + \text{HOOC-N=O-N-COEt} \\
\]

(7)

\[
\text{RSR} + \text{Ph}_3\text{P} + \text{HOOC-N=O-N-COEt}
\]

(7)

\[
\text{R} + \text{Ph}_3\text{P} + \text{HOOC-N=O-N-COEt} \\
\]

(7)

\[
\text{RSR} + \text{Ph}_3\text{P} + \text{HOOC-N=O-N-COEt}
\]

(7)

\[
\text{R} + \text{Ph}_3\text{P} + \text{HOOC-N=O-N-COEt} \\
\]

(7)

\[
\text{RSR} + \text{Ph}_3\text{P} + \text{HOOC-N=O-N-COEt}
\]

(7)
2. Nitroso and nitro compounds

In basic medium thios are oxidized to disulphides by nitrosobenzene or nitrosobenzene trimers which are reduced mainly to azoxy and azobenzene.

Equations indicate the presence of stable radical anions derived by electron transfer from the thiol anion to the nitro or nitroso group (equation 40).

\[
\text{RS}^- + \text{NO}_2^- \quad \xrightarrow{\text{or}} \quad \text{RS}^- + \text{NO}_2^- \quad \text{or} \quad \text{NO}_3^- 
\]

(40)

Other species may oxidize thios to disulphides following a similar route. Among them azonicaronamide, maleic anhydride and 4-nitropyridine N-oxide seem to be the most reactive ones.

3. Iodosobenzene

In refluxing dioxane, iodosobenzene and benzenethiol give rise to the formation of diphenyl disulphide in fairly good yield (70%) (equation 41).

\[
\text{C}_6\text{H}_5\text{IO} + 2 \text{PhSH} \quad \xrightarrow{\text{reflux}} \quad \text{C}_6\text{H}_5\text{H} + \text{PhSSPh} + \text{H}_2\text{O} 
\]

(41)

Although extensive studies have not been made on this reaction, it may represent a general and convenient method for thiol oxidation.

4. Trimethylsulphonium iodide

When benzylsulphyl reacts with trimethylsulphonium iodide in dimethylformamide at 100°C, phenyl methyl sulphide, diphenyl disulphide and dimethyl sulphide are formed.

The reaction seems to be quite complex. Formation of a double adduct between the oxonium salt and the thiol is suggested (equation 42).

\[
\text{O} \quad \overset{\text{PhSH}}{\xrightarrow{\text{CH}_3\text{S}^-}} \quad \text{O} 
\]

(42)

Decomposition of this intermediate would lead to dimethyl sulphoxide and phenyl methyl sulphide (equation 43). Diphenyl disulphide should arise either from the reaction of the thiol with dimethyl sulphoxide (see section III.C) or from oxidation by iodine (see section III.B) generated in the reduction of dimethyl sulphoxide by hydrogen iodide (equations 44 and 45).

\[
\begin{align*}
\text{(CH}_3\text{S})_2\text{O} + 2 \text{HI} & \quad \xrightarrow{\text{oxidation}} \quad \text{(CH}_3\text{S})_2\text{S} + \text{H}_2\text{O} + \text{I}_2 \\
2 \text{PhSH} + \text{I}_2 & \quad \xrightarrow{\text{oxidation}} \quad \text{PhSSPh} + 2 \text{HI}
\end{align*}
\]

(44) (45)

5. Halogen transfer agents

Several 'positive halogen' compounds, ZHal, like N-halo-succinimide, N-chlorosuccinimide, dichloroiodobenzene, etc., react with thios to give sulphenyl halides or disulphides depending on the relative ratios of the reagents (equations 46 and 47).

\[
\begin{align*}
\text{ZHal} + \text{RSH} & \quad \xrightarrow{\text{oxidation}} \quad \text{ZH} + \text{RSHHal} \\
\text{ZHal} + 2 \text{RSH} & \quad \xrightarrow{\text{oxidation}} \quad \text{ZHalRSHHal} \quad \text{or} \quad \text{ZH} + 2 \text{RSHHal}
\end{align*}
\]

(46) (47)

Among these compounds, 2,4,4,6-tetramethylcyclohexa-2,5-dienone has been reported to be particularly selective.

6. Oxidation by Metal Ions and Oxides

Ions and oxides of transition metals which may exist in different valence states have been shown to oxidize thios. Most of the studies so far available on this topic deal with the oxidation by ferric ions; careful investigations with many other metals have been carried out as well. The catalytic effect of these metal ions on the auto-oxidation of thios has been pointed out (see section IV). The intervention of metals in a number of redox enzymes in which the metal is bound to a thiol group at the active site of the enzyme has been also suggested.

1. Ferric ion

Complexes of Fe(III) as Fe(CN)₆³⁻ and ferric octanoate, [Fe(Ooct)]⁺ quantitatively oxidize thios to disulphides in the absence of oxygen (equation 48). This reaction has been largely employed in the synthesis of synthetic rubber.

\[
\text{2 RSH} + 2 \text{Fe}^{3+} \quad \xrightarrow{\text{oxidation}} \quad \text{RSSR} + 2 \text{Fe}^{2+} + 2 \text{H}^+ 
\]

(48)

Oxidation of thios by Fe(Ooct)₃ has been carried out in acetonitrile and xylene. Kinetic studies indicate that the reaction follows a second-order rate law. It is suggested that disulphide arises from dimethylation of thiol radicals which are formed in the rate-determining reaction of thiol with Fe(Ooct)₃ (equations 49, 50).

\[
\begin{align*}
\text{2 RSH} + 2 \text{Fe(Ooct)}_3 & \quad \xrightarrow{\text{oxidation}} \quad 2 \text{RSSR} + 2 \text{Fe(Ooct)}_3 \\
2 \text{RSH} & \quad \xrightarrow{\text{oxidation}} \quad 2 \text{RSSR}
\end{align*}
\]

(49) (50)
The intermedialy of such radicals is explained by the reaction in the presence of alkynes. In this case formation of sulphide, probably arising from a chain reaction, is observed (equations 49, 51, 52). At constant

\[
\begin{align*}
RS + \text{Fe(OH)}_3 & \rightarrow RS + \text{Fe(OH)}_4 + \text{OCH} \\
RS + \text{RCH} = \text{CHR} & \rightarrow \text{RSCH} - \text{CHR} \quad (51) \\
\text{RSCH} - \text{CHR} + \text{RS} & \rightarrow \text{RSCHCHR} + \text{RS} \quad (52)
\end{align*}
\]

alkene and mercaptan concentrations the ratio of disulphide to sulphide formation decreases with decreasing metal ion concentration (Table 6).

<table>
<thead>
<tr>
<th>1-n-Dodecene</th>
<th>1-n-Dodecanethiol</th>
<th>Dodecyl disulphide</th>
<th>Diocetyl sulphide</th>
</tr>
</thead>
<tbody>
<tr>
<td>10</td>
<td>1</td>
<td>21</td>
<td></td>
</tr>
<tr>
<td>10</td>
<td>2</td>
<td>5-4</td>
<td></td>
</tr>
<tr>
<td>10</td>
<td>4</td>
<td>1-4</td>
<td></td>
</tr>
<tr>
<td>10</td>
<td>20</td>
<td>0-07</td>
<td></td>
</tr>
</tbody>
</table>

*1-n-Dodecanethiol 0.2 M in xylene at 1 W C.*

It is suggested that this is related to the increased rate of formation and consequently the greater steady concentration of thiyl radicals at higher metal concentration which makes the dimerization reaction faster than the sequence of reactions leading to the sulphide.

Oxidation of thiols by Fe(CN)₆³⁻ in alkaline and acid medium has been studied³⁸⁻⁴⁰. In both cases disulphide is the oxidation product; however, the reaction mechanism markedly differs. In the pH range 7 to 8 the rate of oxidation of n-octanethiol is pH dependent and exhibits a first-order dependence on Fe(CN)₆³⁻, thiyl and OH⁻.³⁸⁻⁴⁰

Cyanoide ion depresses the rate but at higher cyanoide concentration the rate of oxidation is practically independent from it.

Owing to the observed orders in OH⁻ and since the rate increases with the pH, thiol anion is believed to be the reactive species.

Different mechanisms are proposed for this reaction depending upon the presence of added cyanide. A mechanism similar to that outlined in equations (49) and (50) is suggested for the oxidation in the presence of added cyanide, i.e. slow formation of thiyl radicals and fast formation of disulphide via dimerization of the radicals or further oxidation of them to give a cationic species (equation 53) which is neutralized by thiolate anion (equation 54).

\[
\begin{align*}
\text{RS} + \text{Fe(CN)}_6^{3-} & \rightarrow \text{RS}^+ + \text{Fe(CN)}_6^{4-} \\
\text{RS}^+ + \text{RS}^- & \rightarrow \text{RSKR}
\end{align*}
\]

In the absence of added cyanide ion, a reversible substitution of a CN⁻ by an RS⁻ residue in the ferric complex has been postulated to be rate determining (equation 55).

Rapid decomposition of the sulphur-containing complex generates thiyl radical and pentacoordinate Fe²⁺ complex which reacts with the CN⁻ to give the ferrocyanide complex (equations 56 and 57).

Disulphide is then formed according to equation (50) or (53) and (54). Kinetic studies³⁸⁻⁴⁰ of acid oxidation of thiols by ferricyanide, suggest

\[
\begin{align*}
\text{Fe(CN)}_6^{3-} + \text{RS}^- & \rightarrow \text{Fe(CN)_5} \text{RS}^+ + \text{CN}^- \\
[\text{Fe(CN)_5} \text{RS}^+] & \rightarrow \text{Fe(CN}_6^{4-} + \text{RS} \\
\text{Fe(CN)}_6^{4-} + \text{CN}^- & \rightarrow \text{Fe(CN}_6^{3-} + \text{CN}_2
\end{align*}
\]

that the reaction mechanism is quite complex. The rate law shows a second-order dependence on the Fe(CN)₆³⁻ concentration and first on that of the thiol³⁸⁻⁴⁰. Inhibition by small amounts and catalysis by higher concentration of Fe(CN)₆³⁻ is observed; the rate of oxidation is also dependent on the initial ferricyanide concentration and on the pH.

Several mechanisms³⁸⁻⁴⁰ have been proposed for the acid oxidation of thiols by ferricyanide ions but since they are not fully established, we will not report them in detail.

2. Other metal ions

Like ferric ions, other heavy metal ions in their higher oxidation states react with thiols to give the corresponding disulphides. Quite frequently complexation of thiols with the metal occurs followed by a one-electron transfer to give thiyl radicals which dimerize to disulphide. This is the case, for example, with Ce⁴⁺, Co³⁺ and V⁵⁺ ions in acid solution³⁸⁻⁴⁰.

The homogeneous nature of such reactions was confirmed by an e.r.r. study of the Ce⁴⁺ oxidation of several thiols which showed the presence of thiyl radicals among other radical species. Thus primary thiols give a 1:2:1 triplet signal, secondary a 1:1:1 doublet and tertiary a single absorption line³⁸⁻⁴⁰.

The nature and the stability of the complex formed depends upon the metal³⁸⁻⁴⁰. In the V⁵⁺ oxidation for instance, kinetic evidence and
formation of more than one mole of base suggest the intervention of two
different complexes both leading to the disulfide but following separate
paths\textsuperscript{38} (Scheme 3).

\[
\text{VO}^+ + \text{RS}^- \xrightarrow{\text{H}} \left[ \text{VO}^- + \text{S}^- \right]^+ \xrightarrow{\text{H}^+} \text{V}^+ + \text{RS}^- + \text{OH}^-
\]

\[
2 \text{ RS}^- \xrightarrow{} \text{RSSR}
\]

\[
\left[ \text{VO}^- + \text{S}^- \right]^+ + \text{RS}^- \xrightarrow{} \left[ \text{RS}^- + \text{VO}^- + \text{SR}^- \right]^+
\]

\[
\left[ \text{RS}^- + \text{VO}^- + \text{SR}^- \right]^+ \xrightarrow{\text{H}^+} \text{V}^+ + \text{RSSR} + 2 \text{ OH}^-
\]

\[
\text{V}^+ + \text{VO}^+ \xrightarrow{} 2 \text{ VO}^2+
\]

\textbf{Scheme 3}

The importance of the nature and stability of the complexes between
metal ions and thiols is clearly indicated in the case of the oxidation with
\text{Mn}^{3+} and \text{Mn}^{4+} of thiglycolic acid, cysteine and glutathione\textsuperscript{137,138}.

A detailed study shows that the kinetic equation may change with \text{pH}
and with metal concentration as well as with the particular thiol. Indeed
the mechanism of the reaction is not unique although some of the
differences of the reaction features could be explained on the basis of
different stability and nature of the complexes formed in the early stages
of the reaction.

Other reaction paths are available at least in some special cases. For
instance in the oxidation with manganic acetylacetonate\textsuperscript{39}, disulfide is
believed to arise from reaction of a sulphhenium ion with the thiol
(equation 38) which implies that further oxidation of thiol radicals to

\[
\text{RS}^- + \text{RS}^- \xrightarrow{} \text{RSSR} + \text{H}^+
\]

sulphhenium ion is faster than dimerization. The intervention of thiol
radicals has been ruled out by the absence of addition products when the
reaction is carried out in the presence of alkenes.

The difference in mechanism between the \text{Fe}^{3+} and the \text{Mn}^{3+} oxidation
of thiols is probably due to the powerful ability of the latter in oxidizing
the radical first formed\textsuperscript{100}.

17. Oxidation of thiols

Oxidation by cupric complexes in non-polar media is a more complex
reaction, as shown by the formation of sulphone together with
disulphide\textsuperscript{137}. The former may arise from cupric thioclate (equation 59)
or via desulphurization of the disulphide by copper ions.

\[
\text{Cu}^{2+} + \text{SR}^- \xrightarrow{} \text{CuS} + \text{RS}^- \quad (59)
\]

Lead tetraacetate is also able to oxidize thiols at low temperature to
disulphides\textsuperscript{138-139}.

High yield of disulphide is obtained when one mole of lead tetra-
acetate is allowed to react with two moles of thiol\textsuperscript{140} (equation 60).

\[
2 \text{ RS}^- + \text{Pb}^{4+} \xrightarrow{} \text{RSSR} + 2 \text{ AcOH} \quad (60)
\]

When the lead salt-thiol ratio is \text{V:2}, lead mercaptide is formed together
with disulphide and acetic acid\textsuperscript{140} (equation 61).

\[
2 \text{ RS}^- + \text{Pb}^{4+} \xrightarrow{} \text{RSSR} + \text{Pb}^{2+} + 2 \text{ AcOH} \quad (61)
\]

Higher temperature and the presence of alcohols would cause further
oxidation of the disulphide and formation of sulphonic esters\textsuperscript{139}.

3. Metal oxides

A large variety of metal oxides like \text{MnO}_{2}, \text{FeO}_{2}, \text{CuO}_{2}, \text{Fe}_{2}O_{3}, \text{Cu}_{2}O_{3}
\text{CuO}_{2}\textsuperscript{134-138} oxidize thiols to disulphides at low temperature in chloroform
or xylene solution.

In the oxidation by lead dioxyde, formation of an intermediate by
addition of two molecules of thiol to the metal oxide has been suggested\textsuperscript{140}.
It may give the disulphide by decomposition (equation 62), or generate
an intermediate lead tetracarboxylate which decomposes giving disulphide
(equations 63 and 64).

\[
2 \text{ RS}^- + \text{Pb}^{4+} \xrightarrow{} [\text{RS}^- + \text{OH}^-]_{\text{Pb}^{4+}} \xrightarrow{} \text{Pb}^{4+} + \text{RSSR} + \text{H}^+ \quad (62)
\]

\[
[\text{RS}^- + \text{Pb}^{4+}]_{\text{Pb}^{4+}} \xrightarrow{} \text{Pb}^{4+} + \text{RSSR} + 2 \text{ H}^+ \quad (63)
\]

\[
[\text{Pb}^{4+} + \text{SR}^-]_{\text{Pb}^{4+}} \xrightarrow{} \text{RSSR} + \text{Pb}^{4+} \quad (64)
\]

Manganese dioxide is the most effective oxidizing agent among the
above-mentioned oxides.

The nature of such reactions has been checked for \text{MnO}_{2}, \text{FeO}_{2}\text{.}
\text{CuO}_{2}\text{.} by carrying out the oxidation in the presence of an alkene.
Formation of large amounts of thiol addition products to the double bond
suggests intermediacy of thiol radicals.
IV. OXIDATION BY MOLECULAR OXYGEN

The easy oxidation of thiols on exposure to air is well known as is the sensitivity of this reaction to catalysts like metal ions, u.v. light and other initiators of radical reactions. It is also known that autooxidation of thiols is accelerated by bases.

The interest in this reaction from the industrial (sweetening of crude petroleum) and biological points of view notwithstanding, the mechanism of the autooxidation of thiols is not as yet satisfactorily understood.

We shall attempt in this section to review critically the more significant contributions, with the interpretations of the phenomena as offered by the authors.

A Catalyst by Strong Bases

Cullis and coworkers studied the oxidation of ethanethiol in aqueous alkaline solution under constant pressure of oxygen. They observed low reproducibility of the oxygen uptake rates even when careful precautions were taken to avoid the presence of adventitious impurities. Under their conditions (ESH 0.3-0.5 M; NaOH 0.5-2 M; the base always in excess) the stoichiometry of the reaction was found, in agreement with other authors129,130,131, to be:

\[
4 \text{RSH} + O_2 \rightarrow 2 \text{RSSR} + 2 \text{H}_2\text{O}
\]

Dependence on the first power of both the oxygen pressure and the base concentration was also observed. The order in thiols was found to be about one at the beginning, decreasing to zero as the reaction progressed. The oxygen uptake rates were faster at the beginning and reached a stationary value after 20-30\% reaction. Apparently the change in order with respect to thiol as well as the change in rate depends on the disulphide formed. Indeed, disulphide added at zero time suppresses the typical features of the initial reaction (Table 7). It is not clear which is the effect of disulphide.

It is insoluble in water and hence a two-phase system results as soon as minor amounts of this product is formed. Partition of thiol between the two phases may be important and, possibly, be involved in the observed order in the base. With the minimum of base added, however, the thiol should be already fully in the anionic form and hence an excess of base should not affect the rates.

The authors emphasize the point that they cannot exclude even in their conditions that trace metal catalysis may still be active. Indeed the addition of sequestering agents like EDTA (ethylenediaminetetra-acetic acid) and EN (ethylenediamine) causes contradictory results. Furthermore, added cyanide ion gives slower rates of oxygen uptake, and the reaction no longer yields disulphide but products of more profound oxidation (Table 7).

The sequence does not appear simple. Steric effects could, perhaps, be responsible for the low reactivity of t-BuSH and electronic effects for that of hexamethyldithiatriacontane. However, the authors’ suspicions that the sequence could be partially determined by different amounts of adventitious catalytic impurities deserves careful attention.

The first-order dependence of the initial rate on thiol concentration as well as the base catalysis would indicate that thiolate ions play a particular role in the reaction.
Wallace and coworkers\textsuperscript{104-107} had reached similar conclusions by studying the oxidation rates of several thiols. They also observed that the solvent has a quite large effect, which, in a general way, may be explained on the same basis. As shown in Table 8, the rate increases quite steadily on passing from alcoholic to non-protonic and to dipolar aprotic solvents.

<table>
<thead>
<tr>
<th>Solvent</th>
<th>( k, s^{-1} )</th>
<th>Rel. rate</th>
</tr>
</thead>
<tbody>
<tr>
<td>Methanol</td>
<td>5.4 x 10^{-4}</td>
<td>1</td>
</tr>
<tr>
<td>Tetrahydrofuran</td>
<td>193 x 10^{-4}</td>
<td>36</td>
</tr>
<tr>
<td>Dioxane</td>
<td>482 x 10^{-4}</td>
<td>89</td>
</tr>
<tr>
<td>Dioxan</td>
<td>1.3 x 10^{-4}</td>
<td>&lt;0.01</td>
</tr>
<tr>
<td>Dimethylacetamide</td>
<td>1560 x 10^{-4}</td>
<td>289</td>
</tr>
<tr>
<td>Dimethylformamide</td>
<td>1795 x 10^{-4}</td>
<td>332</td>
</tr>
</tbody>
</table>

* \( \text{pH} \) constant, oxygen pressure 1 atm.

Table 9. Oxidation of \( n \)-butanethiol in alcoholic solvents at 23.5°C by molecular oxygen (1 atm)\textsuperscript{144}

<table>
<thead>
<tr>
<th>Alcohol</th>
<th>Basic*</th>
<th>( \nu )A</th>
<th>( k, s^{-1} )</th>
</tr>
</thead>
<tbody>
<tr>
<td>Methanol</td>
<td>NaOMe</td>
<td>15 x 10^{-5}</td>
<td>5.4 x 10^{-4}</td>
</tr>
<tr>
<td>Methanol</td>
<td>KOMe</td>
<td>52 x 10^{-5}</td>
<td>&lt;0.01</td>
</tr>
<tr>
<td>Ethanol</td>
<td>NaOEt</td>
<td>19 x 10^{-5}</td>
<td>9.6 x 10^{-4}</td>
</tr>
<tr>
<td>( \beta )-Butanol</td>
<td>NaOBu-t</td>
<td>57 x 10^{-5}</td>
<td>57.6 x 10^{-4}</td>
</tr>
<tr>
<td>( \beta )-Butanol</td>
<td>KOBu-t</td>
<td>321 x 10^{-4}</td>
<td>321 x 10^{-4}</td>
</tr>
<tr>
<td>( \beta )-Butanol</td>
<td>ROBu-t</td>
<td>&lt;0.01</td>
<td>&lt;0.01</td>
</tr>
<tr>
<td>( \beta )-Butanol</td>
<td>CuBu-t</td>
<td>321 x 10^{-4}</td>
<td>321 x 10^{-4}</td>
</tr>
</tbody>
</table>

* Two-fold excess in respect to \( n \)-butanethiol.

All these facts are interconnected in the sense that both the size of the cation and the cation solvating power of dipolar aprotic solvents have the effect of disrupting ion pairs and hence rendering the thiolate ion more basic. The protic solvents, on the other hand, by hydrogen-bonding thiolate ions behave in the opposite way.

This latter point is illustrated\textsuperscript{158} by the effect of added methanol on the oxidation rates of \( n \)-butanethiol in dimethylformamide (DMF) and di-(2-methoxyethyl)ether (diglyme) (Table 10).

Table 10. Effect of added methanol on the oxidation of \( n \)-butanethiol in DMF and diglyme at 23.5°C by molecular oxygen (1 atm)\textsuperscript{144}

<table>
<thead>
<tr>
<th>Methanol, %</th>
<th>DMF, %</th>
<th>Diglyme, %</th>
<th>( k, s^{-1} )</th>
<th>Rel. rate</th>
</tr>
</thead>
<tbody>
<tr>
<td>0</td>
<td>100</td>
<td>100</td>
<td>1.8 x 10^{-4}</td>
<td>314</td>
</tr>
<tr>
<td>25</td>
<td>75</td>
<td>100</td>
<td>6.1 x 10^{-4}</td>
<td>114</td>
</tr>
<tr>
<td>50</td>
<td>40</td>
<td>100</td>
<td>1.5 x 10^{-4}</td>
<td>2.8</td>
</tr>
<tr>
<td>75</td>
<td>25</td>
<td>100</td>
<td>1.5 x 10^{-4}</td>
<td>1.4</td>
</tr>
<tr>
<td>100</td>
<td>25</td>
<td>100</td>
<td>1.0 x 10^{-4}</td>
<td>1.9</td>
</tr>
</tbody>
</table>

* Sodium methoxide as base.

The above results lead the authors\textsuperscript{140-142} to propose the following scheme (Scheme 4) for the overall reaction:

\[
\begin{align*}
\text{RSH} + \text{B}^- & \rightarrow \text{RS}^- + \text{BH} \\
\text{RS}^- + \text{O}_2 & \rightarrow \text{RS}^- + \text{O}_2^- \\
\text{RS}^- + \text{O}_2^- & \rightarrow \text{RS} + \text{O}_2^- \\
\text{RS}^- + \text{H}_2 & \rightarrow \text{RSH} \\
\text{O}_2^- + \text{H}_2 & \rightarrow \text{O}_2 + \text{H}_2 \text{O} \\
\text{Scheme 4}
\end{align*}
\]

This scheme gives rise to some doubts which will be discussed further below. However, we wish to point out that reaction (70) is not essential in its present form since the protonation of \( \text{O}_2^- \) would give \( \text{H}_2\text{O}_2 \). It, in turn, will be quickly destroyed by excess of mercaptan.

Large excess of base\textsuperscript{144} and/or prolonged reaction times causes oxidation beyond the disulfide level in aqueous solutions. This phenomenon is more pronounced in dipolar aprotic solvents\textsuperscript{140, 137} where sulphonic acids...
G. Capozzi and G. Modena

are produced together with 

Table 11. Effect of solvent, base and temperature on the oxidation of

<table>
<thead>
<tr>
<th>Solvent</th>
<th>Base</th>
<th>Temperature, °C</th>
<th>Conversion of thiol, mole % (time, h)</th>
<th>Sulphonic acid, mole % in product</th>
<th>Disulphide, mole % in product</th>
</tr>
</thead>
<tbody>
<tr>
<td>HMPA</td>
<td>KOH</td>
<td>23.5</td>
<td>97 (24.5)</td>
<td>95</td>
<td>3</td>
</tr>
<tr>
<td>HMPA</td>
<td>KOH</td>
<td>23.5</td>
<td>95 (21.5)</td>
<td>95</td>
<td>1</td>
</tr>
<tr>
<td>HMPA</td>
<td>KOH</td>
<td>80</td>
<td>96 (26)</td>
<td>96</td>
<td>1</td>
</tr>
<tr>
<td>HMPA</td>
<td>NaOH</td>
<td>23.5</td>
<td>97 (24)</td>
<td>90</td>
<td>8</td>
</tr>
<tr>
<td>HMPA</td>
<td>NaOH</td>
<td>80</td>
<td>90 (18.5)</td>
<td>92</td>
<td>1</td>
</tr>
<tr>
<td>DMF</td>
<td>HCl</td>
<td>23.5</td>
<td>98 (1.5)</td>
<td>88</td>
<td>9</td>
</tr>
<tr>
<td>DMF</td>
<td>NaOH</td>
<td>23.5</td>
<td>94 (18)</td>
<td>67</td>
<td>74</td>
</tr>
<tr>
<td>THF</td>
<td>KOH</td>
<td>23.5</td>
<td>93 (23)</td>
<td>64</td>
<td>28</td>
</tr>
</tbody>
</table>

* Ratio base/thiol = 4.
5 HMPA = hexamethylphosphoramide, DMF = dimethylformamide.

Table 12. Effect of added water on the product distribution in the oxidation of n-butanol in HMPA at 23.5 °C

<table>
<thead>
<tr>
<th>H₂O, vol. %</th>
<th>Thiol conversion, %</th>
<th>Sulphonic acid, mole % in product</th>
<th>Disulphide, mole % in product</th>
</tr>
</thead>
<tbody>
<tr>
<td>10</td>
<td>96</td>
<td>54</td>
<td>41</td>
</tr>
<tr>
<td>20</td>
<td>99</td>
<td>48</td>
<td>52</td>
</tr>
</tbody>
</table>

* HMPA = hexamethylphosphoramide; constant oxygen pressure 1 atm.; ratio KOH/thiol = 4; reaction time 5 h.

reaction since the systems were always heterogeneous, but based on the rate of oxygen uptake for several mercaptans (Figure 2), the order of reactivity seems to be n-butyl > phenyl > 2,2-di-n-pentyl-1-hexyl. This parallels the order of reactivity found for the oxidation in hydroxyl solvents[138, 142, 143]. It was suggested[141, 142] that sulphonic acids derive from disproportionalisation of sulphenate ions formed by nucleophilic displacement at the S–S bond of the disulphide[140] (Scheme 5). This mechanism is supported by the fact that disulphide may undergo base-catalysed oxidation in the same solvent system (Table 13) and that increasing amounts of water added to the aprotic solvent markedly favour the formation of disulphide (Table 12 and Figure 3). The protic component of the solvent, decreasing the activity of the base, would inhibit the

Figure 2. Effects of temperature and thiol structure on the oxygen uptake in HMPA (hexamethylphosphoramide)[141]: ○ 1-C₆H₅SH in KOH/HMPA (23-5); □ CH₃CH₂SH in KOH/HMPA (23-5); ◇ 1-C₆H₅CH₂SH in KOH/HMPA (23-5); ◆ 1-C₆H₅CH₃SH in KOH/HMPA (80°). Reproduced by permission of the author and editor from Tetrahedron, 21, 221 (1965).
Table 13. Base-catalysed oxidation of disulphides in HMPA

<table>
<thead>
<tr>
<th>Disulphide</th>
<th>Temperature, °C</th>
<th>Disulphide conversion, %</th>
<th>Time, h</th>
<th>Sulphonic acid, mole %</th>
<th>Thiol, mole %</th>
</tr>
</thead>
<tbody>
<tr>
<td>Di-α-butyl disulphide</td>
<td>23.5</td>
<td>98</td>
<td>41</td>
<td>92</td>
<td>3</td>
</tr>
<tr>
<td>Di-α-butyldisulphide</td>
<td>80</td>
<td>96</td>
<td>45</td>
<td>97</td>
<td>3</td>
</tr>
<tr>
<td>Diferyl disulphide</td>
<td>23.5</td>
<td>98</td>
<td>22</td>
<td>88</td>
<td>9</td>
</tr>
<tr>
<td>Diferyl disulphide</td>
<td>80</td>
<td>98</td>
<td>22.5</td>
<td>99</td>
<td>9</td>
</tr>
<tr>
<td>Di-α-tolyl disulphide</td>
<td>80</td>
<td>98</td>
<td>23</td>
<td>98</td>
<td>9</td>
</tr>
</tbody>
</table>

*a HMPA = hexamethylphosphoramide, ratio KOH/disulphide = 8.*

Figure 3. Effects of added water on thiol conversion and molar product distribution in the oxidation of α-butylidithiol in HMPA at 25°C. Ratio KOH/RSH = 4, reaction time 5 h. Reproduced by permission of the author and editor from *Tetrahedron*, 21, 227/2 (1965).

The nucleophilic displacement at the disulphide linkage which is responsible for the further oxidation to sulphonic acid.

There is not, however, general agreement with this explanation. Indeed, direct oxidation of mercaptide ion to sulphonic acid was proposed by Berger who considers the formation of disulphide as a side reaction.

Figure 4. Oxidation of n-octanethiol in t-butanol at 25°C. Dependence of product distribution at complete conversion on potassium t-butoxide concentration**. α-Octanethiol 0.25 M (3.3 mmol in 12 ml of t-BuOH); the products formed and oxygen uptake are referred to the mercaptan as (mmoles product/mmoles KSH) x 100; 'acids' refer to the sum of RSH2 and RSO3. Reproduced by permission from *Rec. Trav. Chim.*, 82, 773 (1963).

Most of the work dealt with the oxidation of n-octanethiol but a few other thiols were briefly studied. The reactions were carried out in t-butanol with potassium t-butoxide as base under the assumption that in this solvent trace metal contaminations are less likely.

The oxidation under 1 atm pressure of oxygen gave sulphonic and sulphonic acids together with variable amounts of disulphide depending on the concentration of the base (Figure 4). Increasing amounts of base decrease the percentage of disulphide in the final products, thus suggesting a dependence of the distribution of products upon the extent of ionized mercaptan. Formation of disulphide and higher oxidation products are indeed processes which progress at different rates. Oxidation of α-octane thiol in the presence of insufficient base shows that in the earlier reaction stages formation of disulphide occurs almost quantitatively. This is even more evident for the oxidation of hexanethiol in which diphenyl disulphide is the only oxidation product up to 20–25% of reaction (Figure 3).
Catalytic effects on the oxidation of benzethiol by anthraquinone-1-sulphenic acid, tert-butyl hydroperoxide and phenyl benzethiosulphinate have been observed. It was taken as evidence that sulphenate ion is a key intermediate in the reaction chain leading to the oxidized products. Indeed the above reagents may give rise to the sulphenate ion by ionization or by oxidation (equation 74) or by nucleophilic displacement\(^4\) (equation 75).

\[
\begin{align*}
\text{RS}^- + \text{R'O}_2\text{OH} & \rightarrow \text{RSO}^- + \text{R'O}_2\text{H} \quad (74) \\
\text{RSSR} + \text{RS}^- & \rightarrow \text{RSO}^- + \text{RSSR} \quad (75)
\end{align*}
\]

The overall reaction was rationalized\(^4\) on the basis of Scheme 6. The results reported above and other observations including an analysis of the reaction kinetics lead the author\(^4\) to suggest that the first step is the formation of a peroxy sulphenate ion in the triplet state (equation 76) which may react with undissociated thiol, when present, to give, ultimately, disulphide (equation 77).

\[
\begin{align*}
\text{RS}^- + \text{O}_2 & \leftrightarrow [\text{RSO}^-]^- \quad (76) \\
[\text{RSO}^-]^- + \text{RSH} & \rightarrow \text{RS}^- + 2\text{H}_2\text{O} + \text{RSSR} \quad (77)
\end{align*}
\]

The above outlined scheme leads to the conclusion that completely ionized thiols would give exclusively sulphinic and sulphanic acids; nevertheless, the experimental results indicate formation of ca. 5% of disulphide in the oxidation of potassium benzethiolate even with base in large excess. Since formation of disulphide would require the presence of undissociated thiol, other mechanisms must be operative. Again it is possible that the intervention of trace metal catalysts in the oxidation reaction has to be taken into account. Cullis, Hopton and Trim\(^{28}\) reported that copper ions in concentrations as low as 10\(^{-3}\) M are still active as catalysts and indeed it is very hard to detect metal ions at such low concentrations and to exclude adventitious impurities of this order of magnitude.

Another puzzling point of the mechanisms proposed to explain the autoxidation of thiols in basic solutions (in particular see Scheme 4) is the assumption that mercapto radicals dimerize almost quantitatively without interacting with the solvents in which the reaction was studied.
Although the dimerization of thiol radicals has been found to be very fast (10^9-10^10 M^-1·sec^-1) the very low concentration of such species could still make the search for an alternative path to disulphide formation rewarding. It may be worth mentioning that Caspari and Granova observed that mercapto radicals generated by flash photolysis in aqueous solutions give rise to a radical ion, possibly by interaction with an ionized thiol molecule (equation 85).

\[ \text{RS} + \text{RS}^+ \rightarrow \text{RS}^{-} + \text{R} \]  
(85)

Similar radical anions have been observed as transient species in the reaction of various disulphides with hydrated electrons (equation 86) which eventually decay to give thiol radicals and mercapto ions (equation 85 from right to left).

\[ \text{RSSR}^+ + e^- \rightarrow \text{RS}^{-} + \text{R} \]  
(86)

A related observation was reported by Zweig and Hoffmann who observed a one-electron reduction of naphthalene 1,8-disulphide, contrary to the more usual two-electron reduction of disulphides (see Section II) and also that the radical anion generated from this disulphide with sodium in 1,2-dimethoxyethane has an ESR spectrum characterized by a single line with 1.04 gauss separation from peak to peak, \( g = 2.010 \). The electrochemical generation of the same radical partially resolves the line into an overlapped 1 : 2 : 1 triplet, \( a_H = 0.4 \) gauss. The lack of coupling of the unpaired electron with the aromatic \( \pi \) system indicates that the electron is localized on sulphur. This, in turn, suggests that disulphide radical ions may be a relatively long-living species and hence reaction intermediates.

Indeed, under special experimental conditions or with special geometrical constrictions they live long enough to be physically detected.

### B. Catalysis by Aliphatic Amines

Thiols and in particular aromatic thiols are acids strong enough to be partially transformed into their conjugate base by amines. It follows that the oxidation of thiols by molecular oxygen, which is much faster on the anion than on the undisassociated thiol (see Section IIIIA), may be catalysed by aliphatic amines acting simply as base (see, however, section IIIID).

These catalysts have been used in the oxidation of thiols in hydrocarbon solvents in which amines, but not the more basic alkali hydroxides, are soluble.

### 17. Oxidation of thiols

The hypothesis that the amine-catalysed oxidation of thiols is a particular case of the more general reaction of oxidation by molecular oxygen of thiolate ions is confirmed by the finding that arene-thiols, which are more acidic and hence more dissociated, are oxidized faster than arylalkane- and alkane-thiols in the presence of amines. A special case of combination of amine catalysis and solvent effect is given by the easy oxidation of aliphatic and aromatic thiols in tetramethylguanidine which acts both as base and as a dipolar aprotic solvent (see Table 14).

<table>
<thead>
<tr>
<th>Thiol</th>
<th>Disulphide yield, %</th>
<th>Reaction time, h</th>
</tr>
</thead>
<tbody>
<tr>
<td>n-Butanethiol</td>
<td>82</td>
<td>19</td>
</tr>
<tr>
<td>i-Propanethiol</td>
<td>82</td>
<td>19</td>
</tr>
<tr>
<td>n-Pentanethiol</td>
<td>82</td>
<td>19</td>
</tr>
<tr>
<td>Cyclohexanethiol</td>
<td>72</td>
<td>19</td>
</tr>
<tr>
<td>n-Tetradecanethiol</td>
<td>12</td>
<td>43</td>
</tr>
<tr>
<td>Benzenethiol</td>
<td>80</td>
<td>19</td>
</tr>
</tbody>
</table>

* Constant oxygen pressure 1 atm.

### C. Catalysis by Metal Ions

The addition of heavy metal salts to the basic aqueous solution of thiols increases the rate of oxygen uptake as shown in Table 15. It may be easily realized that the catalytic activity varies with the metal ion. The oxidation gives, except for very special cases (see below), only disulphide without any contamination by products of further oxidation (Table 16). The stoichiometric relation of one mole of oxygen for four moles of thiol has always been observed (equation 65).

The results reported in Table 15 have to be considered to be only qualitative; indeed many of the metal ions listed give in the reaction medium slightly soluble oxides and hence formation of precipitates is observed. The addition of these to these non-homogeneous solutions causes changes in the amount, colour and possibly nature of the insoluble material. In some cases the nature of the precipitate formed was investigated; in particular \( \text{Co(SC}_2\text{H}_4)_3 \), \( \text{Pt(SC}_2\text{H}_4)_3 \), \( \text{TiSC}_2\text{H}_4 \), \( \text{Ni(SC}_2\text{H}_4)_3 \), and \( \text{(C}_2\text{H}_5\text{S}_2\text{N})_2\text{Ni(OH)} \) were identified in the oxidation of \( \text{C}_2\text{H}_5\text{SH} \) catalysed by \( \text{Co}^{3+} \), \( \text{Pt}^{4+} \), \( \text{Ti}^{3+} \) and \( \text{Ni}^{3+} \) respectively.
TABLE 15. Effect of metal ions on the oxidation of ethanethiol.* 1-14

<table>
<thead>
<tr>
<th>Metal ion</th>
<th>Salt</th>
<th>Thiol conversion, %, after 1.5 h</th>
<th>$d[O_2]/d[\text{mol}]$</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td></td>
<td></td>
<td>1.7 × 10^{-4}</td>
</tr>
<tr>
<td>Cr(III)</td>
<td>(NH_4)_2Cr(NO_3)_4</td>
<td>12.8</td>
<td>3.1 × 10^{-4}</td>
</tr>
<tr>
<td>UO_2^{2+}</td>
<td>UO_2(NO_3)_2·6H_2O</td>
<td>11.8</td>
<td>2.9 × 10^{-4}</td>
</tr>
<tr>
<td>VO_2^{+}</td>
<td>VOSO_4·aq</td>
<td>11.5</td>
<td>2.6 × 10^{-4}</td>
</tr>
<tr>
<td>Cr(III)</td>
<td>Cr_2(SO_4)_3·K_2SO_4·24H_2O</td>
<td>6.4</td>
<td>2.1 × 10^{-4}</td>
</tr>
<tr>
<td>Mo(III)</td>
<td>(NH_4)_6Mo_7O_24·4H_2O</td>
<td>13.9</td>
<td>3.2 × 10^{-4}</td>
</tr>
<tr>
<td>W(III)</td>
<td>W_2(SO_4)_3·7H_2O</td>
<td>14.1</td>
<td>3.4 × 10^{-4}</td>
</tr>
<tr>
<td>Mo(III)</td>
<td>MoSO_4·4H_2O</td>
<td>11.4</td>
<td>4.6 × 10^{-4}</td>
</tr>
<tr>
<td>Fe(III)</td>
<td>FeSO_4·7H_2O</td>
<td>11.4</td>
<td>3.6 × 10^{-4}</td>
</tr>
<tr>
<td>Fe(II)</td>
<td>Haemin (Fe = 1.5 × 10^{-3} M)</td>
<td>90.0</td>
<td>26.8 × 10^{-4}</td>
</tr>
<tr>
<td>Cu^{2+}</td>
<td>CuCl_2·7H_2O</td>
<td>55.7</td>
<td>1.2 × 10^{-3}</td>
</tr>
<tr>
<td>Ni(II)</td>
<td>NiSO_4·6H_2O</td>
<td>45.7</td>
<td>1.9 × 10^{-4}</td>
</tr>
<tr>
<td>Fe(II)</td>
<td>FeC_2O_4·2H_2O</td>
<td>48</td>
<td>1.5 × 10^{-4}</td>
</tr>
<tr>
<td>Pt(II)</td>
<td>PtCl_2</td>
<td>12.2</td>
<td>2.6 × 10^{-4}</td>
</tr>
<tr>
<td>Ag(II)</td>
<td>AgNO_3·3H_2O</td>
<td>99.7</td>
<td>26.8 × 10^{-4}</td>
</tr>
<tr>
<td>Zn(II)</td>
<td>ZnSO_4·7H_2O</td>
<td>5</td>
<td>1.7 × 10^{-4}</td>
</tr>
<tr>
<td>Cd(II)</td>
<td>CdSO_4·8H_2O</td>
<td>13.9</td>
<td>3.9 × 10^{-4}</td>
</tr>
<tr>
<td>Hg(II)</td>
<td>HgCl_2</td>
<td>6.1</td>
<td>2.0 × 10^{-4}</td>
</tr>
<tr>
<td>Al(III)</td>
<td>Al_2(SO_4)_3·K_2SO_4·72H_2O</td>
<td>11.8</td>
<td>3.0 × 10^{-4}</td>
</tr>
<tr>
<td>Ti(III)</td>
<td>TiSO_4·7H_2O</td>
<td>10.7</td>
<td>2.4 × 10^{-4}</td>
</tr>
<tr>
<td>Se(IV)</td>
<td>SeCl_2·2H_2O</td>
<td>18.4</td>
<td>2.2 × 10^{-4}</td>
</tr>
</tbody>
</table>

* Metal ion = 1 × 10^{-3} M unless otherwise stated; ethanethiol = 0.5 M; NaOH = 2 M; constant oxygen pressure, 700 mm Hg at 30°C.

1 Rate of oxygen uptake.

17. Oxidation of thiols

It was suggested* 15, 16 that a contribution to the catalysis could be given by undissolved metal complexes. However, a careful study on the effect of these insoluble materials in the case of copper, cobalt, and nickel salts did not confirm this hypothesis* 17, 18.

In Table 17 the rates of oxygen uptake of solutions containing the precipitates are reported together with those obtained from solutions filtered before and after addition of the thiol.

TABLE 17. Effect of actual dissolved metal on the oxidation of ethanethiol** 14, 19

<table>
<thead>
<tr>
<th>Metal ion</th>
<th>Initial rate of oxygen uptake, mol·L^{-1}·s^{-1}</th>
<th>Conditions</th>
<th>Metal concentration in solution, M</th>
</tr>
</thead>
<tbody>
<tr>
<td>Cu</td>
<td>13.2 × 10^{-6} A</td>
<td>B</td>
<td>10^{-3}</td>
</tr>
<tr>
<td></td>
<td>13.2 × 10^{-6} B</td>
<td>C</td>
<td>10^{-3}</td>
</tr>
<tr>
<td></td>
<td>13.2 × 10^{-6} C</td>
<td>D</td>
<td>10^{-3}</td>
</tr>
<tr>
<td>Co</td>
<td>10.3 × 10^{-4} A</td>
<td>A</td>
<td>10^{-3}</td>
</tr>
<tr>
<td></td>
<td>7.4 × 10^{-4} B</td>
<td>B</td>
<td>10^{-3}</td>
</tr>
<tr>
<td></td>
<td>9.9 × 10^{-4} C</td>
<td>C</td>
<td>6.4 × 10^{-4}</td>
</tr>
<tr>
<td>Ni</td>
<td>15.2 × 10^{-4} A</td>
<td>A</td>
<td>10^{-3}</td>
</tr>
<tr>
<td></td>
<td>2.4 × 10^{-4} B</td>
<td>B</td>
<td>1.2 × 10^{-3}</td>
</tr>
<tr>
<td></td>
<td>14.6 × 10^{-4} C</td>
<td>C</td>
<td>5.3 × 10^{-4}</td>
</tr>
<tr>
<td></td>
<td>14.8 × 10^{-6} D</td>
<td>D</td>
<td>10^{-3}</td>
</tr>
</tbody>
</table>

* Reaction conditions as in Table 15.

1 A: no filtration; B: filtration before addition of ethanethiol; C: filtration after addition of thiol; D: metal added as thiol complex.

2 Concentration of metal ion added.

It is quite clear that precipitates, in this system, do not play any role.

The oxygen uptake rates of solutions not filtered (A) and those of solutions filtered before (B) or after addition of the thiol (C) are almost the same within experimental errors. The lower rates observed when the filtration is carried out before addition of thiol (B) could be due to a lower solubility of hydrosulfides in respect to that of metal mercaptates. This is further confirmed by the fact that addition of metals as thiol complexes gives again the same rate of oxidation (D).

An evaluation of the rate efficiency of the metals listed in Table 15 as catalysts is hindered by several factors. First of all the concentration of the metal ions in solution is not known, except in a few cases (see Table 17); for example, the different rates observed with FeSO_4 and haemin complex

28
increasing concentrations of the metals do not increase in the expected way the rates of oxygen uptake; possibly because of saturation effects. A typical feature often observed is that initial rates differ, and are frequently higher than final steady rates \(^{144}\) (Figure 6, Table 19).

The authors suggested that this change in rate is linked to the formation of disulphide which could compete with the thiol in coordination to the metal. Indeed the addition of disulphide at the beginning of the reaction depresses the initial rates but does not affect the final steady rate. Since the

---

**Table 18. Dependence on shake rate of the oxidation of ethanethiol**

<table>
<thead>
<tr>
<th>([\text{Cu}^{2+}]_0), M</th>
<th>Shake rate, cycles per minute</th>
<th>Rate of oxygen uptake (\text{mole} \cdot \text{s}^{-1} \cdot \text{g}^{-1})</th>
</tr>
</thead>
<tbody>
<tr>
<td>(10^{-3})</td>
<td>360</td>
<td>0.80</td>
</tr>
<tr>
<td>(10^{-2})</td>
<td>350</td>
<td>1.20</td>
</tr>
<tr>
<td>(10^{-1})</td>
<td>340</td>
<td>0.74</td>
</tr>
<tr>
<td>(10^{-0})</td>
<td>360</td>
<td>0.84</td>
</tr>
<tr>
<td>(10^{+1})</td>
<td>400</td>
<td>1.57</td>
</tr>
<tr>
<td>(10^{+2})</td>
<td>310</td>
<td>0.60</td>
</tr>
<tr>
<td></td>
<td>400</td>
<td>0.60</td>
</tr>
</tbody>
</table>

\(^a\) Reaction conditions as in Table 15.

\(^b\) Initial rates, expressed as percentage or min uptake/min.

---

**Table 19. Effect of metal concentration on the oxidation of ethanethiol**

<table>
<thead>
<tr>
<th>Metal ion</th>
<th>SALT</th>
<th>Concentration of metal added, M</th>
<th>(-\Delta [\text{O}_{2}] / \text{dt}) (\text{mole} \cdot \text{l}^{-1} \cdot \text{s}^{-1} \cdot \text{g}^{-1})</th>
</tr>
</thead>
<tbody>
<tr>
<td>Fe(^{3+})</td>
<td>FeSO(_4) (\cdot 7) H(_2)O</td>
<td>0</td>
<td>(2.2 \times 10^{-4})</td>
</tr>
<tr>
<td></td>
<td>10(^{-3})</td>
<td>(2.2 \times 10^{-4})</td>
<td></td>
</tr>
<tr>
<td></td>
<td>10(^{-2})</td>
<td>(2.2 \times 10^{-4})</td>
<td></td>
</tr>
<tr>
<td></td>
<td>10(^{-1})</td>
<td>(2.2 \times 10^{-4})</td>
<td></td>
</tr>
<tr>
<td></td>
<td>10(^{+1})</td>
<td>(2.2 \times 10^{-4})</td>
<td></td>
</tr>
<tr>
<td></td>
<td>10(^{+2})</td>
<td>(2.2 \times 10^{-4})</td>
<td></td>
</tr>
<tr>
<td>Mn(^{2+})</td>
<td>Haemin</td>
<td>1.5 (\times 10^{-3})</td>
<td>(2.6 \times 10^{-4})</td>
</tr>
<tr>
<td></td>
<td>6 (\times 10^{-3})</td>
<td>(1.5 \times 10^{-4})</td>
<td></td>
</tr>
<tr>
<td>Cr(^{3+})</td>
<td>Chrome alum</td>
<td>10(^{-6})</td>
<td>(4.8 \times 10^{-4} )</td>
</tr>
<tr>
<td></td>
<td>10(^{-6})</td>
<td>(2.5 \times 10^{-4} )</td>
<td></td>
</tr>
</tbody>
</table>

\(^a\) Reaction conditions as in Table 15.

\(^b\) Rate of oxygen uptake. I = initial rate; F = final steady rate.
Take 10% of the oxidation rates of ethylbenzene of several batches of Mo/Co. On the basis of the data, it is evident that the addition of cobalt to molybdenum enhances the oxidation rate. The results are consistent with previous observations, which indicate that the presence of cobalt increases the catalytic activity of the molybdenum oxide surface. The data also suggest that the optimal ratio of cobalt to molybdenum is approximately 1:1, which maximizes the rate of ethylbenzene oxidation.

The results of these experiments support the hypothesis that the interaction between cobalt and molybdenum is responsible for the enhanced oxidation activity. Further studies are needed to understand the underlying mechanisms and to optimize the catalytic performance for practical applications.
G. Capozzi and G. Modena

TABLE 21. Oxidation of ethanol and catalysed by metal complexes

<table>
<thead>
<tr>
<th>Metal</th>
<th>Metal complex</th>
<th>Concentration, M</th>
<th>Rate of oxygen uptake, A (mol/l × s × 10⁻⁶)</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td></td>
<td></td>
<td>No KCN</td>
</tr>
<tr>
<td>Co</td>
<td>CoSO₄·7 H₂O</td>
<td>10⁻³</td>
<td>10.2</td>
</tr>
<tr>
<td></td>
<td>Phthalocyanine</td>
<td>3.5 × 10⁻³</td>
<td>47.6</td>
</tr>
<tr>
<td>Ni</td>
<td>NiSO₄ + aqu.</td>
<td>10⁻³</td>
<td>80.4</td>
</tr>
<tr>
<td>Cu</td>
<td>CuSO₄·5 H₂O</td>
<td>10⁻³</td>
<td>15.1</td>
</tr>
<tr>
<td></td>
<td>Phthalocyanine</td>
<td>3.5 × 10⁻³</td>
<td>1.3</td>
</tr>
<tr>
<td>Fe</td>
<td>FeSO₄·7 H₂O</td>
<td>10⁻³</td>
<td>5.4</td>
</tr>
<tr>
<td></td>
<td>Phthalocyanine</td>
<td>3.5 × 10⁻³</td>
<td>4.0</td>
</tr>
<tr>
<td></td>
<td>Rhodanine</td>
<td>10⁻³</td>
<td>14.7</td>
</tr>
<tr>
<td></td>
<td></td>
<td></td>
<td>1.0</td>
</tr>
</tbody>
</table>

* Reaction conditions as in Table 15.

Indeed when, as in the case of cyanide complexes, it is assumed that the oxidation of thiols occurs by an outer-sphere process and hence thiol radicals are formed as free particles in the solution, disulphide is at the most a minor reaction product and the thiol is oxidized to sulphinic or sulphonic acids.

Most proposed schemes assume that hydrogen peroxide is a by product and that it is consumed in a subsequent probably metal-catalysed fast reaction. Although this cannot be ruled out, it could also be that when the oxygen enters into the co-ordination sphere of the metal it is reduced in successive steps to water rather than released at an intermediate stage of reduction.

D. Catalysis by Organic Redox Systems

Hydroquinone (QH₂) and p-phenylenediamine derivatives in basic medium as well as other easily oxidizable species like the reduced forms of several dyes may act as catalysts in the autooxidation of thiols to disulphides.

The rate of oxygen uptake for the oxidation of n-hexanethiol in the presence of hydroquinone is characterized by an initial slow rate which increases up to a maximum and then decreases at longer reaction times.

The maximum rate at constant oxygen pressure is dependent upon the first power of base and of catalyst concentration (equation 87)

\[
\frac{\text{d}Q\text{H}^{+}}{\text{d}t} = k[Q\text{H}][\text{OH}^{+}] 
\]

The first step of the reaction is assumed to be the oxidation of the hydroquinone anion (QH⁻) by the oxygen to generate the semiquinone (QH) (equations 88 and 89).

\[QH^- + \text{OH}^- \rightarrow QH^+ + \text{H}_2\text{O} \quad (88)\]

\[QH^+ + \text{O}_2 \rightarrow QH^+ + \text{O}_2^2^- \quad (89)\]

The semiquinone then reacts with the thiol to give the corresponding thyl radical (equation 90) which yields disulphide by dimerization.

\[QH^+ + \text{RS}^- \rightarrow QH^- + \text{RS}^- \quad (90)\]

The oxidation rates depend on the hydroquinone used as catalyst, but the catalytic power is not directly related to the oxidation rate of the catalyst. However, the two sets of data are obtained in different conditions and in particular at largely different pH, and this could justify the discrepancies observed. Alternatively it is possible that the quinone is...
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first transformed into its mercapto derivative (equation 91) and that the
substituted quinone is the true oxidizing species.188

![Chemical reaction diagram](image)

Studies of the oxidation of thiols with tetra-substituted quinones not
susceptible to further addition would shed light on this problem.
Unfortunately data of this kind are not available in the literature.
An identical mechanism has been proposed for the oxidation of thiols
catalysed by phenylethylenediamine derivatives.186,188

Flavine derivatives oxidize thiols to disulphides in the absence of
oxygen with formation of dihydroflavine (equation 92)

![Chemical reaction diagram](image)

The reduced form of this dye may be reoxidized by molecular oxygen
with regeneration of the oxidant and formation of hydrogen peroxide
which is itself an oxidizing agent toward mercaptans (see section III.A)
equation 93).

![Chemical reaction diagram](image)

Other organic redox systems are good catalysts for the oxidation of
thiols by molecular oxygen and probably act by similar mechanisms.94

17. Oxidation of thiols

E. Co-oxidation

The autooxidation of thiols in the presence of alkenes takes a quite
different course.148,156 They are in fact oxidized by oxygen to give, possibly
by a chain reaction, β-thiobiperoxides which eventually rearrange to
β-sulphinyl alcohols (equation 94). Acetylene derivatives give under the
same conditions a similar reaction which may be represented by
equation 95.

![Chemical reaction diagram](image)

These reactions are usually called co-oxidation of thiols since an
alkene (or an acetylene) is oxidized together with a thiol molecule. It has
been reported that the rate of co-oxidation depends on the alkene and on
the thiol, with aromatic derivatives reacting faster than the aliphatic ones.
Catalysis by typical radical initiators has also been observed.97

Kharash and coworkers first proposed a hydroperoxysulphide inter-
mediate in the formation of β-sulphinyl alcohols in the co-oxidation of
thiols with olefins. This was later confirmed by detection of peroxy
compounds in the reaction mixture. Further studies led to the isolation of
several hydroperoxysulphides when aromatic thiols were oxidized at
low temperatures.150,157

An example of this class of compounds is the 2-(2-naphthylmercapto)-1-
indanyli hydroperoxide (10) obtained as a solid, melting at 70°C, by
co-oxidation of 2-naphthalenethiol and indene (equation 96).

![Chemical reaction diagram](image)
When the hydroperoxide intermediate formed in the co-oxidation of 2-naphthalenemethiol and indene is allowed to decompose in the presence of 2-(4-chlorophenylmercapto)-1-indanol, none of the latter was oxidized. This would suggest an intramolecular transfer of the peroxydic oxygen at the sulphide sulphur.

Further evidence on the intramolecular character of the oxygen transfer as well as on the stereochemistry of the co-oxidation process stems from a careful investigation by Szmalt and Riganò[172] on the reaction of benzene with indene.

They isolated from the reaction mixture and fully characterized three of the four possible diastereoisomers 2-phenylsulphynyl-1-indanols and prepared the missing isomer by oxidation of cis-2-phenylmercapto indanol with hydrogen peroxide or with m-chloroperoxybenzoic acid.

The four stereoisomers (only one enantiomer is reported) are listed below with the relative yields obtained in the co-oxidation in benzene solution.

(11) m.p. 101° (53.3%)  (12) m.p. 158° (34.5%)

(13) m.p. 158° (15.6%)  (14) m.p. 166° (8.0%)

These results indicate that a 5:4:1 trans/cis mixture of hydroperoxides is formed and hence that in this system the co-oxidation is stereoselective rather than stereospecific as it was earlier suggested.[174, 175, 176]

The formation of only three of the four possible sulphinyl isomers and the ratio in which they are formed appears to be clear evidence of the intramolecular character of the oxidation step.

In fact the molecular models of the cis and trans phenylmercapto indene hydroperoxides, precursors of compounds 11-14, show that the trans

17. Oxidation of thiols

isomer may suffer intramolecular attack at sulphur from both sides through conformations of similar estimated energy and hence compounds 11 and 12 are formed in similar amounts. On the contrary in the case of cis hydroperoxide the conformation which would lead to compound 14 by direct oxygen transfer is not accessible requiring that the phenyl be above the indene ring. This may explain why only the cis isomer (13) is formed.

For the formation of the intermediate hydroperoxide the following mechanism based on a radical chain reaction may be formulated (equations 97-100).

\[ \text{RSH} \xrightarrow{\text{O}_2, \text{R}_{2} \text{C} = \text{C} = \text{O} \text{H}_2} \text{RS} \text{O} \]\n
(97)

\[ \text{RS}^+ + \text{C} = \text{C} = \text{O} \xrightarrow{+ \text{O}_2} \text{RS} - \text{C} = \text{C} = \text{O} \text{O} \text{H} \]

(98)

\[ \text{RS} - \text{C} = \text{C} = \text{O} - + \text{O}_2 \xrightarrow{+ \text{RSH}} \text{RS} - \text{C} = \text{C} = \text{O} \text{H} + \text{RS}^+ \]

(99)

When thiols and olefins are co-oxidized in the presence of an amine, the end-products are 2-mercaptoethanols, disulphides and water[174, 175] (equation 101).

\[ 3 \text{RSH} + \text{C} = \text{C} = \text{O} \xrightarrow{+ \text{O}_2} \text{R} \text{S} = \text{C} = \text{C} = \text{O} \text{H} + \text{RS-SR} \]

(100)

This reaction may be explained in terms of an amine catalysed oxidation of the thiol[172] by the 2-mercaptoethylhydroperoxy intermediate.

This was confirmed by the observation[172] that the complex of 10 with triethylamine oxidizes quantitatively benzene with to disulphide.

Olefins containing isolated double bonds with different reactivity towards thiol radicals are selectively co-oxidized at the more reactive unsaturation centre[175]. This is the case of co-oxidation of endo and exo cyclo pentadienes with 4-chlorobenzenethiol (equations 102, 103).

(101)

(T)he (b)rack (e)nt (a)ct (i)v (e)g (r) h (s) the (s)te (r)h (e)ch (e)m (e)try (i)s (u)n (n)known.)

Co-oxidation of thiols with 1,3-butadiene, the simplest conjugated diene, has been studied in the presence of tert-butylamine[174]. Products derived from 1,2- and 1,4-addition were observed in the reaction with methane- and ethane-thiols, predominant 1,2-co-oxidation products were formed when benzene or p-toluene thiol was used (equation 104).
The scheme suggested for these reactions is similar to that proposed for the co-oxidation of simple alkenes. The thiol radical attacks one of the terminal carbons to give an allyl radical followed by attack of oxygen at the 2 or 4 carbon depending on the relative stability of the two formal radicals (Scheme 8).

17. Oxidation of thiols

\[
\begin{align*}
RSH + R_{1}\text{C}=C=CR_{2}^{'} & \xrightarrow{O_2} RS \text{-} O \text{-} CH_{2} \text{-} CH_{2} \text{-} OH \\
RSH + CH_{2}=CH=CH_{2} & \xrightarrow{O_2} RS \text{-} CH_{2} \text{-} CH=CH_{2} + \text{H}_{2} \text{O}
\end{align*}
\]

\[
\text{RS-CH=CH=CH-OH}
\]

Co-oxidation of thiols and phenylacetylene with oxygen produces phenylglyoxal hemithioacetal\(^{279}\) (equation 107).

\[
\begin{align*}
RSH + \text{Ph} \equiv \text{CH} + O_2 & \xrightarrow{} \text{Ph} \equiv \text{CH} \equiv \text{SR} \\
\text{RSH} + \text{Ph} \equiv \text{CH} + O_2 & \xrightarrow{} \text{Ph} \equiv \text{CHO} + \text{RS} \text{-} \text{H}
\end{align*}
\]

The scheme suggested for these reactions is similar to that proposed for the co-oxidation of simple alkenes. The thiol radical attacks one of the terminal carbons to give an allyl radical followed by attack of oxygen at the 2 or 4 carbon depending on the relative stability of the two formal radicals (Scheme 8).
quite unpleasant smell into odourless and relatively stable compounds. It may also be a cheap method of synthesis of disulphides although care should be taken to avoid overoxidation. Furthermore, some thioles and their corresponding products of oxidation undergo easy base-promoted α-elimination leading to desulphurized compounds.\(^{96-98}\)

V. PHOTOOXIDATION

Thiols undergo an easy photolytic reaction (see chapter 10 on photochemistry) which is in fact an oxidation of mercaptans to disulphides (equation 109).

\[
2 \text{RSH} \xrightarrow{\text{hv}} \text{RSSR} + \text{H}_2 \tag{109}
\]

The instability of thiols to light has been known for a long time\(^{140}\) and there is a likely interest in the photolytic and radiolytic reactions with high energy radiations of thiols and sulphur compounds in general also because of the problem of biological effects of radiations.\(^{102,104,105}\)

Recent detailed work in the gas phase by Stee and Knight\(^{106,107}\), largely confirming earlier results\(^{99,104,105,106}\), showed that the primary photolytic process by irradiation at ca. 2500 Å for methane and ethane-thiols is the homolysis of the S—H bond (equation 110) to give thyl radicals and hydrogen atoms. The principal products of the reaction are molecular hydrogen and disulphides. The simple Scheme 9 was proposed for this reaction.

\[
\begin{align*}
\text{RSH} & \xrightarrow{\text{hv}} \text{RS} + \text{H}_2 & \tag{110} \\
\text{RSH} + \text{H} & \rightarrow \text{RSSH} & \tag{112}
\end{align*}
\]

Minor amounts of methane and hydrogen sulphide in the methane-thiol and of ethane, ethylene and hydrogen sulphide in the ethane-thiol reaction and of ethane, ethylene and hydrogen sulphide in the ethane-thiol reaction were also formed. The authors\(^{106,107}\) propose that these products are not formed in a primary process, but they derive from reaction of the thiol with a disulphide molecule which has not yet transferred the excess of energy which it contains at the act of formation (Scheme 10).

\[
\begin{align*}
2 \text{CH}_2\text{SH} & \rightarrow \text{CH}_2\text{SSCH}_2 \tag{113} \\
\text{CH}_2\text{SSCH}_2 + \text{CH}_2\text{SH} & \rightarrow \text{CH}_2\text{SSCH}_2 + \text{CH}_2\text{S} + \text{H}_2 \tag{114} \\
\text{CH}_3 + \text{CH}_2\text{SH} & \rightarrow \text{CH}_3 + \text{CH}_2\text{S} \tag{115} \\
\text{HS} + \text{CH}_2\text{SH} & \rightarrow \text{H}_2\text{S} + \text{CH}_2\text{S} \tag{116}
\end{align*}
\]

Scheme 10

In the case of ethanethiol in addition to the processes corresponding to reactions (114)—(116), equation (117) was proposed to explain the formation of ethylene. Reaction (117), because of the larger rearrangement involved, should be slower than the equivalent of reaction (114),

\[
\text{C}_2\text{H}_6\text{SSCH}_2\text{H} + \text{C}_2\text{H}_5\text{SH} \rightarrow \text{C}_2\text{H}_6\text{SSCH}_2\text{H} + \text{C}_2\text{H}_5 + \text{H}_2\text{S} \tag{117}
\]

as is in fact observed. Among the evidence presented by the authors\(^{99,107}\), in favour of the mechanism of formation of hydrogen sulphide and hydrocarbons the decrease of the yields of these products with the pressure of added inert gas is especially convincing.

As far as the primary process (Scheme 9) is concerned the supporting evidence is overwhelming: addition of ethylene, for instance, decreases the yields of hydrogen and disulphide with concomitant formation of ethyl sulphide via addition of the thyl radical to ethylene.

Flash photolysis studies\(^{108}\) allowed the direct detection of thyl radicals; these species were also detected by uv and nmr when the photolysis was carried out in solid matrices.\(^{107,109}\)

Quite similar processes occur also in aqueous solutions, as well as in other solvents.\(^{102,104,106}\) Sometimes complicated, however, by interaction of the radical initially formed (equation 110) with other species present. Indeed the photolysis of thiols has been used as a source of hydrogen atoms to study their reactions with several compounds.\(^{109}\)

Higher molecular weight thiols, particularly secondary and tertiary alkane-thiols, may undergo other primary photolytic processes, in particular breaking of the carbon-sulphur bond.\(^{106,107}\) In the majority of cases, however, the main path seems to be the sulphur-hydrogen bond breaking leading to the formation of thyl radicals which may undergo in appropriate experimental conditions several reactions besides dimerization to disulphides (section IV). Carbon-sulphur bond fission may also occur when shorter wavelength light is used. Under these conditions more complex phenomena due to the production of particles with excess energy content have also been observed.\(^{106,107}\)

VI. REFERENCES

17. Oxidation of thiol}s

CHAPTER 18

The synthesis and uses of isotopically labelled thiols

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I. INTRODUCTION

Isotopic labelling of thiol has been used in research disciplines ranging from atomic physics to forestry in the study of practically every atomic, molecular and biological process that thiols are known to undergo. In this review we will consider the changes that substitution of deuterium for the
hydrogen of the thiol group introduces into the translational, rotational and vibrational processes of thiols both in the ground and transition states. These perturbations have helped to elucidate some of the most fundamental structural and chemical properties of thiols. The low energy β-rays emitted by the thiol group when it is substituted with tritium or sulphur-35 allow the thiol group and its constituent atoms to be located in complex reaction mixtures. In this review we will consider the tracer applications of radio-isotope labelling in mechanistic studies of thiol reactions. However, we will also consider the use of tritiated and sulphur-35 labelled thiols in the optimization of industrial processes, as well as to trace the path that thiols follow in the body. We extend this review into these two areas of research which are usually considered to be beyond the research interests of the organic chemist for two reasons. First, the physicochemical phenomena which underlie these processes are the same as those encountered in the reaction vessel by the organic chemist. The same radical transfer reactions of thiols take place in the phantom chemical reaction vessel, synthetic rubber polymerization chambers, and within the body of an animal exposed to ionizing radiation. The relative liquid as compared to water-solubility of a thiol determines not only the best procedure for its extraction from a reaction mixture but also whether the thiol will penetrate the lipid-brain barrier. Second, we have included these industrial and biological studies for the sake of the chemist who may want to extend his research on thiols to more industrially or biologically significant problems. In total, we will cover processes as delicate as the passage of a thiol over a transition state or as intricate as the wearing down of steel. We will trace the flow of a thiol down the axon of a neuron and through the ecosystem of a forest.

II. MOTIONAL PROCESSES

The most fundamental chemical questions concerning the molecular weight, atomic coordinates and bond strengths of thiols have been answered in the most precise way by careful physical measurements of the translational, rotational and vibrational motions of thiols. Since in any one measurement the number of physical variables usually exceeds the observable parameters, meaningful physical parameters could not have been obtained if measurements had not been made on a series of isotopically substituted molecules. It is now common practice in molecular spectrometry to site a motional process from several isotopically labelled positions in a molecule. In the following sections, we will briefly describe the physical origin of the isotope effect in mass spectrometry, microwave and infrared spectroscopy and review how it has been used to answer fundamental chemical questions concerning thiols.

18. Synthesis and use of isotopically labelled thiols

A. Translation

Mass spectrometry is a relatively accurate and convenient method for the determination of the molecular weight of a molecule. Moreover, in the course of the measurement, the molecule often fragments to smaller molecular ions, whose molecular weights are also measured. Later the pattern of molecular fragments can be pieced together in a way that will reveal the structure of the thiol. However, very often fragments originating from different parts of a molecule will have the same mass and will not be distinguishable from each other. As we will see, isotopic labelling readily overcomes this problem and precisely traces the origin of molecule ion fragments.

When thiols enter the mass spectrometer, they are first ionized and partially broken into fragments. From the molecular parent ion and the fragment ion carry a charge, e. by virtue of which they can be accelerated through a potential, V. When the ions emerge from the accelerating chamber they all possess the same kinetic energy, \( \frac{1}{2}mv^2 \), and potential energy, eV (where \( m \) is the mass and \( V \) the velocity of the ion). When this process is applied to a mixture of normal and heavier isotopically labelled thiols, both the light and heavy ions will emerge with the same energy, but the light molecules will be travelling faster than the heavy molecules. The accelerated ions next enter the magnetic sector of the spectrometer, where the magnetic field, \( H \), exerts a centripetal force, \( eHV \), on the ions which is exactly balanced by a centrifugal force, \( m\omega^2r \), i.e., \( eHV = m\omega^2r \) (where \( r \) is the radius of the ions trajectory through the magnetic field). The heavier, normal ions travel with a greater velocity \( v \) and experience a greater centripetal force, and an even greater centrifugal force, than the heavier isotopically labelled thiols. Accordingly, the path of the lighter ions will have a smaller radius. The difference in paths of the light and heavy ions facilitates their separation and analysis.

The two most labile bonds in a thiol, \( \text{R - CH}_3, \text{SH} \), are the \( S - II \) and \( C - H \) bonds. However, removal of a hydrogen from the \( \text{CH}_3 \) or the SH group yields fragments with the same mass. Amos and coworkers have used isotopic labelling to show that \( \text{CD}_3 \text{SH} \) fragments to \( [\text{CD}_3 - \text{SH}]^+ \) and \( [\text{CD}_3^2\text{S}]^+ \) in the ratio 2:1, while the ratio in \( \text{CH}_3\text{CD}_2\text{SH} \) is approximately unity. Upon ionization, benzethionyl-S-5-d has been shown by Lautenst, Madden and Schlopp to lose equal amounts of mercapto-deuterium and ring hydrogen. In a later section, we will show how separation of ion fragments using isotope labelling has made possible a
number of mass spectrometric studies of the bond energies of thiols and the thermodynamics of their bond cleavages.

B. Rotation

Microwave spectroscopy has proved to be a powerful technique, providing data on the structure and bonding of gaseous molecules. The interaction of the dipole moment of the molecule with a microwave field induces transitions between the rotational energy levels of the gaseous molecule. The microwave frequencies, at which the transitions occur, depend entirely on the moments of inertia of the molecule about its principal rotational axes. The moment of inertia is determined by the atomic masses and bond lengths and angles of the molecule. Usually the determination of one set of moments of inertia is not sufficient to give a unique set of molecular parameters. To obtain such a unique set of molecular parameters, measurements must be made on a series of molecules, in which isotopic substitution has been used to create a series of changes in atomic mass along the molecule. As microwave measurements are quite sensitive, thiols containing $^{13}C$, $^{18}O$, and $^{34}S$ at natural abundance can be observed and used to provide a series of naturally occurring isotopically substituted molecules.

In the first application of microwave spectroscopy to a thiol, Solimene and Dailey$^4$ measured the $0_{00}-1_{00}$ transition in several isotopically substituted methane thiols, including $^{13}CH_2SH$, $^{13}CH_3SH$, $^{13}CD_2SH$, $^{13}CD_3SH$, $^{13}CH_2SH$, $^{13}CH_3SH$, and $^{13}CH_3SH$. From these data they derived the moments of inertia and corresponding structural parameters of methane-thiol. Kadziar, Ahbason and Imane$^4$ determined the structure of ethanethiol using $^{13}CH_2CH_2SH$ and $^{13}CH_2CH_2SH$. A more comprehensive set of molecular parameters for ethanethiol has been obtained by Hayashi and coworkers$^5$ from the spectra of the trans and gauche isomers of $^{13}CH_2CH_2SH$, $^{13}CH_2CH_2SH$ (syn and anti), $^{13}CH_2CD_2SH$, $^{13}CH_2CH_2SH$, and $^{13}CH_2CH_2SH$.

In addition to rotating with the molecule as a whole, the methyl group of methane-thiol can rotate against the thiol group along the C–S bond. The resulting modes of hindered rotation (i.e. torsional vibration) create an additional series of spectral lines. Solimene and Dailey$^5$, by measuring the intensity of the lowest-lying excited torsional states relative to the ground state in CH$_2$SH and CD$_2$SH, determined that the potential barrier for hindered rotation is sinusoidal with a height of 100 kcal/mole. Later, Kojima$^6$, measuring the $\Delta J = 1$, $\Delta K = 0$ lines in the ground state and the $\Delta J = 0$ lines in the first excited state of CH$_2$SH and CH$_3$SH, determined the potential barrier of methanethiol to be $444 \pm 10$ cm$^{-1}$.

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In similar measurements of CH$_2$DSH and CD$_2$DSH, Knopp, Daniel and Quad$^7$ showed that the staggered conformation for the methyl and thiol group corresponds to a threefold minima in the potential energy function for hindered rotation. Reddington$^8$ has found that the height of the potential barrier of CF$_3$SH and CF$_3$SD is quite close to that of CH$_3$SH. The fact that substitution of CF$_3$ for CH$_3$ has little effect on the height of the barrier rules out repulsion between non-bonded atoms as the source of the potential barrier. Measurements like these can be expected to continue to provide insight into the nature of the interaction between two internally rotating groups.

It is interesting to note that one of the first measurements of the electric nuclear quadrupole moment of $^{34}S$ was made by Bird and Townes$^9$ on a close examination of Solimene and Dailey’s microwave spectrum of methanethiol noticed a group of three very weak doublets. They assigned the doublets to the interaction of the electric quadrupole moment of natural abundance $^{34}S$ with the electric field of the molecule as a whole.

C. Vibration

Infrared spectroscopy can be used not only in a qualitative way to identify functional groups in a molecule, but also to provide precise data on the bond strengths. Before such calculations can be made, however, every observed spectral band must be assigned to one of the vibrational modes of the molecule. Such assignments can often be ambiguous. Replacing an atom in a molecule with one of its isotopes does not, to a high order of approximation, change the electronic structure of the molecule, and therefore does not alter the potential functions governing the vibrations of the atoms. However, the frequency of the vibration will be affected and will reveal itself in a shift of the vibrational band. The shift will be small, when the isotopically substituted atoms move very little in a particular vibrational mode; but when the atom has a large amplitude of vibration in a mode, the shift will be large$^{10}$. Plant, Tarbell and Whiteman$^{11}$ reported the first isotope shift observed in the vibrational spectrum of a thiol. They found that in benzothiol and n-hexanethiol deuteration of the thiol groups shifted the bands at 2600 cm$^{-1}$ to 1839 and 1870 cm$^{-1}$, respectively. Since then isotope shifts have helped elucidate the infrared spectra of several thiols. For example, CF$_3$SH displays a band at 906 cm$^{-1}$, which shifts to 699 cm$^{-1}$ in CF$_3$SD. This large spectral shift has allowed the band to be assigned to the CSH bending mode; whereas a series of bands near 500 cm$^{-1}$ shift very little upon isotopic substitution, verifying their assignment to the CF$_3$ deformation modes$^{12}$.
Takeoka\textsuperscript{4} has used the isotope shifts observed in the infrared spectrum of cyclohexane-thiol-\textsuperscript{3}D\textsubscript{4} to assign the observed bands to the proper vibrational modes. In addition, bands belonging to the axial and equatorial conformations of cyclohexane-thiol could be distinguished. Furthermore, the changes in the relative concentration of the two conformers on going from the liquid to the plastic to the hard crystalline phases could be followed.

Once the vibrational bands of a molecule have been assigned to their proper modes, calculations can be made of the interatomic forces that bond atoms together to form a molecule. The strength of these interatomic forces is measured in terms of a force constant for a particular vibrational mode. When the atomic co-ordinates and masses of a molecule are known, a complete set of force constants can be used in a normal co-ordinate analysis using the Wilson FG matrix method\textsuperscript{15}, to obtain a set of calculated vibrational bands. The set of force constants is then adjusted so as to obtain the best fit between observed and calculated frequencies. As occurs in other spectroscopic measurements, the number of force constants often exceeds the number of observed frequencies in any one spectrum. Since the force field is independent of isotopic substitutions, the spectra of isotopically substituted molecules can be used to provide additional frequencies. A particularly good check of a force field is its ability to predict the spectra of isotopically substituted molecules. May and Pace\textsuperscript{16,17} have obtained a force field for methanethiol based on the frequencies of CH\textsubscript{3}SH and CH\textsubscript{3}SD and microwave structural parameters. Their force field accurately predicts all the observed frequencies of the normal and isotopically labelled molecules. Hayashi and coworkers\textsuperscript{18} have obtained a reliable set of force constants for ethanedi-thiol from the frequencies of HSC\textsubscript{2}H\textsubscript{2}CH\textsubscript{2}SH and HSC\textsubscript{2}H\textsubscript{2}CH\textsubscript{2}SD. Furthermore, they have shown that when \textit{trans}, \textit{trans}, \textit{trans} and \textit{trans}, \textit{gauche} conformations are assumed, the force field satisfactorily predicts the observed frequencies of \textit{a}-propanethiol, \textit{b}-thiame-thylethane thiol, \textit{b}-halogenomethane thiol, and 1,2-dithio-diethylethane.

III. CLEAVAGE OF THE S–H BOND

A. The Primary Hydrogen Isotope Effect and the Nature of the Transition State

In the previous section we saw how isotope labelling has played an indispensable role in the elucidation of the motional processes and structure determinations of thiols. In this section we turn to the dynamics of the rupture of the S–H bond. The chemical phenomenon of the

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S–H bond cleavage is indeed only another motional process, in which the thiol hydrogen moves independently of the rest of the thiol molecule in a sort of extended S–H stretching mode. As we have seen, substitution of deuterium for the thiol hydrogen has a pronounced effect on the motion of a thiol, particularly the S–H bond stretching vibration. We might expect that deuterium substitution will greatly affect the dynamics of the S–H bond cleavage. In this section, after having reviewed the theoretical basis for primary hydrogen isotope effects\textsuperscript{19–21}, we will construct several transition state models for S–H bond cleavage\textsuperscript{22}, predict the isotope effect for each model, and compare these to the measured values. Finally, we will turn to the use of isotope labelling to trace the fate of the thiol hydrogen after it has been abstracted from a thiol.

For the purpose of theoretical discussion, we consider that the thiol lies on a surface of potential energy, whose co-ordinates are the bond lengths and angles of the thiol molecule in the horizontal direction and potential energy in the vertical direction. The exact topography of the surface is determined by the electronic structure of the molecule. During the processes of S–H bond cleavage, the thiol can be thought of as traveling across the surface along a path of lowest energy, which will correspond to the S–H stretching mode. The highest point along this pathway of lowest energy is called the transition state. The rate at which S–H bond cleavage will occur depends primarily on the probability of a thiol reaching the transition state, RSH\textsuperscript{*}. If we consider that ground state and transition state molecules are in equilibrium, then the process can be characterized by an equilibrium constant \( K^* \) (eqns. 1 and 2).

\[
\begin{array}{ccl}
\text{RSH} & \rightleftharpoons & \text{RSH}^* \\
K^* = \frac{[\text{RSH}^*]}{[\text{RSH}]} = \frac{\Pi \mathbb{P}_{\text{reactants}}}{{\Pi \mathbb{P}_{\text{products}}}} \exp(-\Delta E/RT)
\end{array}
\]

Equilibrium constants can be expressed in terms of the motional processes of a molecule, i.e. in terms of the partition function of the reactant and the product, which in this case is the transition state, as seen in equation (4). The partition function, \( \mathbb{P} \) (s for unit volume of an ordinary molecule), denotes the probability of a molecule existing in any one particular motional state, summed over all the possible translational, rotational and vibrational states available to the molecule. The energies of the motional states are calculated taking the lowest classical state, as having zero energy. The exponential term in equation (2) corrects for the difference in energy between the reactant and transition states.

Having written \( K^* \) in terms of motional states of the molecule, we are now prepared to ask how substitution of deuterium for the thiol hydrogen
will affect the probability of the thiol reaching the transition state RSH+. Experimentally the question is posed in the ratio of the rate of the S—H bond cleavage over rate of S—D cleavage. These rates are largely determined by the equilibria in equations (3) and (4). As seen in equation (5) the hydrogen isotope effect can be written in terms of the partition functions for the light and heavy thiol.

A major advance in the theory of primary hydrogen isotope effects came when the approximation was made that substitution of deuterium for hydrogen does not greatly affect the classical properties of the molecule, such as the mass or moments of inertia and consequently neither the translational nor rotational partition function. This left only the quantum mechanical vibrational partition function as a source of the isotope effect. Writing the deuterium isotope effect in terms of the complete vibrational partition function, equation (6) is obtained, where $u_i = h
u_i/kT$, $\nu_i$ is the frequency of the ith vibrational mode and N is the number of atoms in the molecule. The products and summations are

$$\supscript{\cdot H}{\text{RSH}} \xrightarrow{\text{K_H}} \supscript{\cdot D}{\text{RSD}}$$

(3)

$$\frac{K_D}{K_H} = \frac{\prod Q_i^{\cdot D}}{\prod Q_i^{\cdot H}}$$

(4)

$$\frac{k_H}{k_D} = \prod_{i=1}^{N-6} \left( \frac{1 - \exp(-u_i^{\cdot H})}{1 - \exp(-u_i^{\cdot D})} \right) \times \exp\left[ -\frac{1}{2} \sum_i (u_i^{\cdot H} - u_i^{\cdot D}) \right]$$

(5)

$$\frac{K_D}{K_H} = \left( \frac{m_H}{m_D} \right)^{1/2} \exp\left[ \frac{hc}{2kT} \left( \dot{\nu}_{\text{stretch}}^{\cdot H} - \dot{\nu}_{\text{stretch}}^{\cdot D} \right) + \dot{\nu}_{\text{H bend}}^{\cdot D} - \dot{\nu}_{\text{H bend}}^{\cdot H} \right]$$

(6)

taken over the 3N—7 vibrational modes of the ground state and over 3N—6 vibrational modes of the transition state, in which the vibrational mode corresponding to the reaction pathway (in our case the S—H stretch) is omitted. As seen in equation (6), an isotope effect will occur only when the deuterium participates in a vibrational mode, whose frequency changes on going from the ground to the transition state. We are now ready to characterize various transition states precisely in terms of what vibrational modes have changed, which is another way of locating the transition state on the potential surface.

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The simplest model that can be chosen for the transition state is one in which the only vibrational mode that has changed is the S—H stretching mode. Since this vibrational mode is the reaction coordinate itself, it does not contribute to the isotope effect in the transition state. Molecular vibrations involving hydrogen generally have vibrational bands above 700 cm⁻¹, for which exp(−hν/kT) is 0-03 at 300 K and products involving this term will be close to unity. Equation (6) therefore reduces simply to

$$\frac{k_D}{k_H} = \exp\left[ \frac{hc}{2kT} \left( \dot{\nu}_{\text{stretch}}^{\cdot H} - \dot{\nu}_{\text{stretch}}^{\cdot D} \right) \right]$$

(7)

where $\dot{\nu}$ is the wave number of the thiol stretching mode in the ground state. Using the literature value² for the thiol stretching mode of methanethiol, 2605 cm⁻¹ and 1893 cm⁻¹ for CH₃SH and CH₄SD respectively, a value of 5-5 is obtained for $k_D/k_H$. Using equation (8), this corresponds to a value of $k_D/k_H = 1.11 \left( \frac{m_D}{m_H} \right)^{1/2} \times 1.44$

(8)

or 11.29 for $k_D/k_H$, the primary tritium isotope effect.

Weakening the S—H bond in the transition state must certainly reduce the frequency of the C—S—H bending mode. If we consider the extreme case in which the frequency has gone to zero, the product term $[1 \exp(-u_i^{\cdot H})]/[1 \exp(-u_i^{\cdot D})]$ of equation (6) approaches $u_i^{\cdot D}/u_i^{\cdot H}$, which can be approximated by $(m_H/m_D)^{1/2}$, where m refers to mass. Equation (6) now reduces to

$$\frac{k_D}{k_H} = \left( \frac{m_H}{m_D} \right)^{1/2} \exp\left[ \frac{hc}{2kT} \left( \dot{\nu}_{\text{stretch}}^{\cdot H} - \dot{\nu}_{\text{stretch}}^{\cdot D} \right) \right]$$

(9)

Using values of 802 cm⁻¹ and 673 cm⁻¹ for the bending modes of CH₃SH and CH₄SD,¹² k_D/k_H increases to a value of 5.9 and k_D/k_H to 11.42. We may then expect that weakening the C—S—H bonding mode will tend to increase slightly the isotope effect.

In addition to unimolecular dissociation of the S—H bond, thiol bonds are often ruptured when an acceptor molecule (usually a free radical) abstracts hydrogen from the thiol. In this case, the transition state will contain the three-centre linear system S—H—A, where A is the acceptor atom. The stretching and bending modes of the C—S—H group of the ground-state thiol will make the same contribution to the isotope effect as they did in the unimolecular dissociation, and the S—H stretch will
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remain the reaction co-ordinate. However, in the transition state a new linear stretching mode associated with the S—H—A system will have to be introduced. If S—H—A is asymmetric, i.e., A does not resemble sulphur, then the stretching mode shown in Figure (1a) will tend to weaken the isotope effect for either of two reasons: (i) for large $u$ and $w_{12}^{0}>w_{12}^{p}$, the transition state vibration will detract from the contribution made by the ground state molecules or (2) for small $u$, $[1-\exp(w_{12}^{0})]/[1-\exp(w_{12}^{p})]$ will introduce the term $n_{12}^{0}n_{12}^{p}$. On the other hand, when S—H—A is symmetric, the linear vibration introduced, Figure (1b), in which H does not move, will not contribute to the isotope effect.

\[ \begin{aligned} \text{S} & \quad \cdots \quad \text{H} & \quad \cdots \quad \text{A} & \quad \cdots \quad \text{S} \\ \text{a} & \quad \cdots \quad \text{b} & \quad \cdots \quad \text{a} \end{aligned} \]

**Figure 1. Stretching modes of the S—H—A system.**

We might conceive of a reaction in which S—H bond cleavage occurs long before the thiol reaches the transition state, such as in the base-catalysed addition of RSH to an olefin, equations (10) and (11).

\[ \text{RSH} + \text{B} \rightarrow [\text{RS}^\cdot \text{HB}^+] \quad (10) \]

\[ \text{H} \quad \text{C} = \text{C} \quad \rightarrow \quad \text{RS}^\cdot \text{C} = \text{C} \quad \rightarrow \quad \text{RS} + \text{C} = \text{C} + \text{B} \quad \left(11\right) \]

Here isotope substitution exerts its effect on the rate of the reaction, via the pre-reaction equilibrium, equation (10). Rather than calculating the kinetic isotope effect for the reaction, we will want to obtain an expression for the ratio of the equilibrium reactions.

\[ \text{RS} + \text{B} \rightarrow \text{RS}^\cdot \text{HB} \quad \left(12\right) \]

\[ \text{RS} + \text{R} \rightarrow \text{RS} + \text{RB} \quad \left(13\right) \]

The ratio of equilibrium constants, $K_{1}/K_{2}$, for equations (12) and (13) is equivalent to the equilibrium constant $K_{1/D}$ for the isotope exchange equilibrium.

\[ \text{RSH} + [\text{RS}^\cdot \text{DB}] \rightarrow \frac{K_{1}}{K_{D}} \quad \text{RS} + [\text{RS}^\cdot \text{HB}] \quad \left(14\right) \]

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Expressing $K_{1/D}$ in terms of the vibrational partition functions we obtain

\[ K_{1/D} = \frac{Q_{\text{RSH}}}{Q_{\text{RSH}}} \frac{Q_{\text{DB}}}{Q_{\text{DB}}} \quad \left(15\right) \]

Equation (15) is simply the individual partition ratio function of isotope substituted RSH $Q_{\text{RSH}}/Q_{\text{RSH}}$, divided by $Q_{\text{RSH}}/Q_{\text{RSH}}$.

\[ \frac{Q_{\text{RSH}}}{Q_{\text{RSH}}} = \prod_{u} \frac{n_{\text{RSH}}^{u}}{n_{\text{RSH}}^{u}} \times \exp\left(\frac{\sum_{u} w_{\text{RSH}}^{u} n_{\text{RSH}}^{u}}{2} \times \Pi_{1} \frac{1-\exp(-w_{\text{RSH}}^{u})}{1-\exp(-n_{\text{RSH}}^{u})} \right) \quad \left(16\right) \]

\[ \frac{Q_{\text{RSH}}}{Q_{\text{RSH}}} = \prod_{u} \frac{n_{\text{RSH}}^{u}}{n_{\text{RSH}}^{u}} \times \exp\left(\frac{\sum_{u} w_{\text{RSH}}^{u} n_{\text{RSH}}^{u}}{2} \times \Pi_{1} \frac{1-\exp(-w_{\text{RSH}}^{u})}{1-\exp(-n_{\text{RSH}}^{u})} \right) \quad \left(17\right) \]

Just as in the case of the kinetic isotope effect, deuterium substitution is felt only in those vibrational modes that change on going from reactants to products.

The rate at which a particular reaction takes place is only partially accounted for by $E_{a}$. The rate of passage of a thiol over the potential barrier at the transition state is given by $v_{T}^{n}[\text{RSH}]$, in which $v_{T}^{n}$ is the frequency of the vibration that carries the thiol over the potential barrier and leaves the S—H bond apart. The magnitude of $v_{T}^{n}$ is determined by the curvature of the potential surface near the transition state and since the curvature is concave downwards the frequency is imaginary, but has the same absolute value as if the surface were concave upwards, with a real vibrational frequency. The rate is influenced by two other parameters, which intimately depend on the topography of the potential surface. These are the transmission coefficient, i.e., the fraction of molecules passing over the barrier in the forward direction, and the percentage of tunnelling of the molecules under the potential barrier. These parameters are generally ignored or considered to introduce no isotope effect; however, in cases where large deviations from the predicted isotope effects are found, they have to be considered. The way in which these phenomena are affected by isotope substitution is an active field of theoretical study.

The observation of a large kinetic isotope effect indicates that isotopically substituted thiol hydrogen participates directly in a vibrational mode, whose frequency changes on going to the transition state, i.e., that S—H bond cleavage is an integral part of the transition state. The fact that a value for $k_{a}k_{b}$ of 2:80 was obtained for the addition of benzenthiole-S-D$_{4}$ to nickelocene, led Elgen and Gregory to propose the mechanism below for the reaction. Although the authors did not comment.

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A new value of $k_{\text{H}}/k_{\text{D}}$ would seem to indicate that the zero point energy lost on cleavage of the $S-H$ bond is partially offset by the formation of the cyclopentadienyl hydrogen bond. The abstraction of thiol hydrogen by the triphenylmethyl radical proceeds with an abnormal large value for $k_{\text{H}}/k_{\text{D}}$ of 14.9, which was attributed by Lewis and Butler to tunnelling through the potential barrier, which occurs when a barrier is symmetrical.

Dmuchowsky, Vineyard and Zienty observed a quite unusual inverse isotope effect for $k_{\text{H}}/k_{\text{D}}$ of 0.65 for the base catalysed addition of $n$-butanethiol-$S-d_4$ to maleic anhydride. While inconsistent with any model of a transition state involving $S-H$ bond cleavage, the inverse isotope effect could be accounted for by postulating a pre-reaction equilibrium between butanethiol and triethylamine, much like the one in equations (10) and (11). In fact, substitution into equations (16) and (17) of 2566 and 1890 cm$^{-1}$ for the $S-H$ and $S-D$ stretching frequency, respectively, and 3253 and 2380 cm$^{-1}$ for the $N-H$ and $N-D$ stretches of the amine-thiol complex, yields an equilibrium isotope effect of 0.68.

Isotope equilibrium exchange constants for a number of thiol-water systems have been measured and the value $K_{\text{H,D}}$ is usually referred to as the equilibrium isotope separation factor, $\alpha$. Halil and Bieńkowska have measured a for HSCN/CH$_3$SD as a function of temperature and obtained $ln \alpha = 262/T - 0.1162$, which corresponds to a $\Delta H$ of $-520$ cal/mole.

Sakodynskii, Babkov and Zhavoronkov found that changing the structure and composition of a thiol had very little effect on $\alpha$, which indicates that, during hydrogen exchange with water, changes in vibrational frequencies are restricted to the $C=S-H$ bonds.

The measurement of kinetic isotope effects have provided insight into economically important industrial processes. Early in the course of the synthetic rubber programme it was found that the molecular weight of a polymer such as $G R - S$, could be quantitatively regulated by the addition of thiol to the polymerization system. Normally the polymerization occurs as in equation (18); however, a growing polymer can abstract a hydrogen atom from thiol, thereby transferring the radical to the thiol and inactivating the polymer chain, equation (19).

$$M_n + M \overset{k_D}{\longrightarrow} M_nH + RS$$

The chain length of the polymer formed is proportional to the transfer constant $k_D/k_{\text{H}}$, which is the ratio of the specific rate of radical transfer to the specific rate of chain propagation. Wall and Brown measured the isotope effect $k_{\text{H,D}}$ of the chain transfer step in the butanethiol-$S-d_4$ mediated polymerization of styrene. A value of 4, somewhat less than the predicted value of about 6, was obtained. The low kinetic isotope effect indicated that either the loss of zero point energy of the $S-H$ bond had been compensated by the formation of unusually strong bonds or that the reaction was complicated by the abstraction of butyl hydrogens as well as thiol hydrogen. Data such as these can often aid in the search for more efficient transfer agents.

B. Tracers of Atoms and Free Radicals during S $>$ H Bond Cleavage

In addition to its use in probing the nature of transition states, labelling with heavy hydrogen is an indispensable aid in following the fate of thiol hydrogen in the reaction mixture. It distinguishes thiol hydrogen not only from the hydrogens of the reaction mixture as a whole, but also from other hydrogen atoms of the thiol, which may have been dissociated under the reaction conditions that led to the dissociation of the $S-H$ bond.

Greig and Tynan have measured the relative rates at which methyl radicals abstract hydrogen and deuterium from CD$_3$SH. The hydrogen of the SH bond was abstracted 120 times faster than the methyl deuterium.

Riesz and Burr have measured the relative amounts of $D_2$ and $HD$ produced by the reaction of deuterium atoms with styrene-$S-d_4$, and $n$-butanethiol-$S-d_4$. The yields of $D_2$ were 80 and 83%, respectively, indicating that atom abstraction occurred primarily from the $\text{S-H}$ group.

Volman, Wolstenholme and Hadley irradiated CH$_3$SD at 77 K with 2537 Å light and detected e.s.r. signals originating from $D$- but not from $H$. This indicated, that if CH$_3$SH radicals were observed in the irradiated sample, they could only have been formed by a secondary radical abstraction reaction. Keys and Harrison were able to study the two major pathways of thios at the ion chamber of the mass spectrometer. Unlabelled CH$_3$SH yields fragments which cannot be separated, but CD$_3$SH, equations (20) and (21), yield [CD$_3$S$^-$] and [CD$_2$SH$^-$] ions, whose heat of formation were found to be 214 and
Labelling of the thiol group with heavy hydrogen can provide information concerning the nature of the hydrogen acceptor as well. The phenylethyl radical can exist as two isomers which can be inter-converted by a 1,2 hydrogen migration (equation 22). Slaight by

$$\text{PhCH}_2\text{CH}_2\text{H} \xrightarrow{\text{PAST}} \text{PhCHCH}_2\text{H}$$

allowing the radical to abstract hydrogen from benzene-thiol-S-d4 could be used to mark the site of the radical with tritium. Methanethiol S-d4 adds across the double bonds of cis- and trans-2-butene to form identical mixtures of erythro- and threo-3-deutero-2-(methylthio)butane. Skell and Allen found that the radical reaction takes place in two steps, the

18. Synthesis and uses of isotopically labelled thiols

addition of a methylthio radical to butene followed by the abstraction of deuterium from a molecule of CH3SD by the 3-methylthio-2-butyl radical (equation 23). The fact that with deuterium labelling a mixture of three

and erythro methylthiobutanes is obtained indicates that abstraction of thiol hydrogen is slower than the rate of rotation about the 2,3 carbon—carbon bond of the radical.

There are many reaction exchanges that can be detected only with the use of isotopic labelling. One such reaction is hydrogen exchange between a thiol and a protic solvent. For example, Deniss, Kazakova and Ryb with studied mixtures of MeSH (or iso-BuSH): MeOD and iso-BuSD: HOAc (or MeOH) to determine the relationship between the rate of hydrogen exchange and proton donor and acceptor properties. Sulphur-35 labelling was used by Dixon, Kornberg and Luns in a study of the enzyme, malate synthetase, to determine whether the enzyme had a catalytic effect on exchange between coenzyme A-S and acetyl coenzyme A (equation 24)

$$\text{CoA} - \text{acetyl} - \text{S-CoA} \xrightarrow{\text{acetyl}} \text{acetyl} - \text{S-CoA} + \text{CoA} - \text{S-H}$$

In the photolysis of the S—H bond it is possible to introduce into the thiol more than enough energy for the cleavage of the S—H bond. The very subtle question of whether upon bond cleavage this excess energy is channelled into the vibrational modes of the radical or into the translational energy of the dissociated hydrogen atom has been answered by White and coworkers by a clever use of isotope labelling. Translationally excited hydrogen atoms displace deuterium from D2 to form HD (equation 25) to an extent that is proportional to the energy of the hydrogen atom. By photolysing CH3SH in the presence of D2 and measuring the amount of HD produced, they found that the excess energy resided chiefly in the
translational mode of the hydrogen atom. Furthermore, hydrogen atoms formed at 2282 Å appeared to have an average significantly more energy than those produced at 2537 Å.

\[
\begin{align*}
\text{RSH} & \xrightarrow{\text{H}^+} \text{RS} + \text{H}^+ \\
\text{H}^+ + \text{D}_2 & \rightarrow \text{HD} + \text{D}^+ \\
\end{align*}
\]

IV. TRACING \textsuperscript{35}S-LABELLED THIOLS IN BIOLOGICAL SYSTEMS

In the previous section we have seen how isotopic labelling has been used to trace the fate of thiol sulphur and hydrogen atoms in the course of chemical reactions. However, by far the greatest application of isotopic labelling in tracer studies of thiols has been in biochemical, biological and clinical studies which have sought to map out the path followed by various thiols in the body from the time of their administration to their excretion. While many of these studies have been performed by scientists other than chemists, the phenomena they probe are essentially physical-chemical in nature. For this reason we have taken the liberty to extend the scope of this review to the biological applications of isotopic labelling of thiols. We have done this in the hope that it will familiarize the chemist working in an interdisciplinary group with the nature of a biological system from the point of view of tracer studies, for which he may be asked to design a chemical probe.

A. Macromolecular Systems

Before turning to body tracer studies, we might consider the application of \textsuperscript{35}S-tracing to a few isolated biochemical systems. The only place thiopurines and thiopyrimidines occur in nature are in the tRNA’s (transfer ribonucleic acid). The question that was immediately posed after their discovery was whether whole thiopurines and thiopyrimidines are incorporated in tRNA at the time of chain assembly or whether at some later time sulphur is exchanged for oxygen at particular sites in assembled tRNA chains that are deficient in sulphur. Sulphur-35 labelling has played an indispensable role in the discovery of the cysteine tRNA sulphur-transferase enzymes, that were found to substitute the sulphur-35 of labelled cysteine for the oxygen in the 4-position of uridine\textsuperscript{33}, in tRNA chains deficient in thiol sulphur. Sulphur-35 labelling also revealed that in some cases \(\beta\)-mercaptothiopuruvate could also serve as a donor of sulphur\textsuperscript{34}.

Sulphur-35 labelling of the cysteine residues in a protein has often been used as a convenient way of tagging a particular protein in the study of a macromolecular phenomenon. For example, the macromolecular machinery used in the bacterial cell for the synthesis of proteins initially consists of (1) a chain of mRNAs (messenger ribonucleic acid), (2) around which is clamped a 30S and a 50S ribosomal particle, which together form an active 70S ribosome complex, (3) to which is bound a fMet-tRNA\textsubscript{fMet} (N-formyl-methionyl transfer ribonucleic acid) molecule, that will supply the first amino acid to be incorporated. It was believed that upon completion of the synthesis of the polypeptide chain, the 70S ribosome is released in a form that cannot be immediately re-used and that it must first be dissociated back into 30S and 50S subunits. A protein known as initiation factor F\textsubscript{e} was later found to be essential for the formation of the initiation complex, and lately its function has been revealed in a study that has employed \textsuperscript{35}S-labelled F\textsubscript{e}. \textsuperscript{35}S-F\textsubscript{e} was shown to bind readily to 30S particles, but to neither 30S particles nor the 70S complex. When the \textsuperscript{35}S-F\textsubscript{e} charged 30S subunit is induced to a 50S subunit by increasing the Mg\textsuperscript{2+} concentration of the medium, \textsuperscript{35}S-F\textsubscript{e} is released. This suggested that when an initiation complex is formed from 30S and F\textsubscript{e}30S subunits, F\textsubscript{e} is released and is free to dissociate other used inactive 70S complexes into subunits that can subsequently reform active 70S complexes.

\textsuperscript{35}S-Labelling has also been used in a quantitative fashion to obtain data on the number of binding sites available to a labelled molecule in a particular macromolecular complex. For example, the 30S particle was found to have one site available for \textsuperscript{35}S-F\textsubscript{e}\textsuperscript{34}. Arabinomixyl-6-mercaptopurine-\textsuperscript{35}S (ara-M1\textsuperscript{35}S), a non-toxic suppressor of the homograft response, was found to bind the surface red blood cells with a minimum of \(6.7 \times 10^6\) sites on B red blood cells and 1.2 \(\times 10^6\) sites on tanned sheep blood cells\textsuperscript{35}.

Turning to a very simple biological system, \textsuperscript{35}S-labelling has proved to be quite efficient in visualizing the behaviour of viruses. Virus particles usually consist of a strand of nucleic acid contained in a sheath of coat protein. Upon infection of a cell at 37°C, the nucleic acid enters the cell leaving its coat protein bound to the cell surface, whereas at 4°C the nucleic acid prefers to remain on the cell surface with its coat on. This phenomenon has been visualized with Sendai virus, whose coat proteins have been labelled with cysteine-\textsuperscript{35}S\textsuperscript{36}. Ten minutes after infection of human amnion cell culture, faint uniformly distributed grains appear in the autoradiographs of the infected cell, reaching a maximum after 60 min. The uniform distribution of grains suggested that the labelled viral component was absorbed onto, but had not penetrated into, the cell. This was supported by the fact that identical grain counts were obtained at 37°C and 4°C. Mechanical shearing is often sufficient to
knock coat proteins off the cell surface. This technique together with 35S-labelling can be used to distinguish between viral components injected into and absorbed onto cells. MS-2 RNA coliphages contain two species of proteins, a coat protein and a maturation protein. The latter is required for both phage absorption to the F-pili of the host Escherichia coli cell and for the reconstitution of the infectious phage. 35S-labelled MS-2 phage was used to determine whether the maturation protein enters the cell together with RNA. After infection at 37°C and shaking, 400 cpm/10^6 cells remained associated with the cell, whereas at 4°C only 20 cpm/10^6 cells were obtained. This implied that during infection the maturation protein had penetrated beyond the F-pili of the E. coli cell.

B. Whole Body Systems

In the remaining part of this section, we will consider the fascinating use of 35S-labelling to follow the path taken by various thiols in the body. After ingestion or intravenous or intraperitoneal injection, thiols rapidly cross the gastro-intestinal barrier and enter the vascular system of the organism, where they are swept by the blood flow past the membranes, lipidal structures that insulate the organs and cells from the blood stream. At this point the thiol is evenly distributed in the vascular system of all the organs of the animal and its fate from here on will be determined largely by its physicochemical properties.

If the thiol is relatively soluble in lipids, it will be able to penetrate the lipid membranes, and will freely pass in and out of cellular structures. For example, thiopental, a rapidly acting anaesthetic, has a high solubility in lipids, and this allows it readily to penetrate the lipid membranes of the brain. A combination of 35S-labelled and autoradiography has shown that the distribution of thiopental-35S in the brain itself is not uniform. Once inside the brain the distribution of the thiol depends not so much on its lipid solubility, but on the pattern of blood flow in the cortex, geniculates, colliculi and white matter of the cat brain. In fact, thiopental-35S autoradiography has been used as a means of studying the physiologic territory of supply of cerebral blood vessels. While thiopental is freely passing in and out of the brain, its concentration in other organs is rapidly equilibrating in accord with the lipid solubility of the thiol. Ocular tissues, like the blood-brain barrier, behave as a lipid membrane and are thiopental-35S, with its high lipid solubility, experiences no delay in penetrating the uveal tissue. This is in contrast to more ionizable drugs, like phenobarbitone, which slowly penetrate the uveal tissue, but once inside bind to pigmented molecules. Thiopental-35S forms no such complexes and is rapidly swept out of the tissue by the blood flow. In 18. Synthesis and uses of isotopically labelled thiols

vital organs, such as the brain, lung and liver, 35S-activity reaches its maximum level within 15 s after injection and decreases to a plateau by 2 min. The liver then commences thiopental uptake again, obtaining a peak after 5 min, while depot fat takes up thiol at a constant rate. By the time the animal awakes, most of the thiol is concentrated in the liver and depot fat. It is interesting to observe that the lipid solubility of thiopental that allowed it to penetrate the brain so rapidly has led to the termination of its anaesthetic action. With time thiopental will gradually accumulate in the kidneys and will be excreted.

A rough idea of the path that a thiol follows in the body can be obtained by measuring its rate of excretion via urine, faeces and respiratory air. 35S-Labelling has allowed the following kind of data to be obtained: 70% of glutathione-35S subcutaneously injected in a mouse is excreted in the urine within 18 h; the radioactivity of 35S-thiobarbiturates are excreted 70-90% in the faeces and up to 1% by respiration; SKF 525-A (2,5-diphenylxanthen-9-one,2,2-diphenylvalerate) prolongs the thiopental induced sleeping time in mice by delaying the urinary excretion of injected 35S-labelled thiopental.

Often in the course of a thiol's travels through the body, it will encounter a compound with which it will form a complex. In contrast to thiopental, penicillamine-35S rapidly enters the plasma after oral administration where it is bound to the serum albumin. In this bound state, penicillamine is no longer able to pass through the semi-permeable membrane of the kidneys, which retards its excretion in the urine. Penicillamine-35S subsequently becomes evenly distributed in the body fluids, affording the drug an opportunity to scavenge copper efficiently from the body fluids. The resulting widespread and long-lasting action of the thiol makes it the drug of choice in the treatment of Wilson's Disease.

Inside a cell, a thiol might form a stable complex with a particular cellular constituent. Cystamine-35S does not seem to form any particularly marked complexes with the cell nuclei, mitochondria and microsomes of liver and spleen, while cysteamine-35S forms a very tight complex with the dinucleoprotein, which cannot be disrupted by repeated water shock and extraction.

In addition to forming a complex with a particular cellular substance, the thiol may encounter an enzyme that will alter its chemical composition. A change in the structure of the thiol can profoundly alter its distribution within the body. One of the most striking examples of this phenomenon is the accumulation of 6-methyl-thiopurine ribonucleotide-35S (6-MMPR) by erythrocytes. The ratio of radioactivity in the erythrocyte as compared to plasma is 80 : 1, whereas in the case of 6-mercaptopurine-35S the ratio is 1 : 100, representing a 4000-fold difference between...
the two compounds. The selective accumulation of 6-MMP-RS in erythrocytes has been attributed to its intracellular phosphorylation to the more ionizable and hence less difficult ribonucleotide. The fact that the behaviour of a thiol within an organism is largely determined by physical properties such as lipid as opposed to water solubility suggested that more efficient drugs might be designed on the basis of their solubility properties. An interesting experiment along this line was the conversion of the water-soluble, carcinostatic drug 9-(β-D-xyloturanosyl)-9H-purine 6-thiol (xyl-6-MP) to its triacetyl derivative (xyl-6-MP−TAC). It was hoped that the derivative, which is relatively insoluble in water, would be retained in the body longer than xyl-6-MP. Surprisingly, xyl-6-MP−TAC-RS was excreted in the form of xyl-6-MP-RS and sulphate-RS even more rapidly than xyl-6-MP-RS itself.29

If the thiol does not bind tightly to a cellular constituent or encounters an enzyme into whose binding site it can fit, it will eventually be excreted in an unaltered form. In one of the earliest applications of RS-labelling of thiols in a biological tracer experiment, mercaptotuzidine RS was administered to rats and boar to test whether a metabolic pathway exists for the conversion of mercaptotuzidine to its betaine derivative, the naturally occurring ergothioneine. Ergothioneine did not take up radioactivity and 90% of the administered 2-mercaptopurine RS was excreted in the urine by the twenty-first day.30

Tracer studies such as those just described have found a particularly important application in the design of drugs that retard the growth of tumours and increase the survival times of afflicted animals, including man. One of the basic strategies that underlie the search for effective carcinostatic drugs is the design of a drug that has a high toxicity for tumour cells, while relatively non-toxic for the host animal. The fast turnover rate of tumour cells, and the demands that this places on the synthesis of purines and pyrimidines and their incorporation into DNA have proved to be the Achilles heel of the tumour cell.

One group of compounds that have proved to be particularly effective in interfering with DNA synthesis of tumour cells are the mercaptopurines and pyrimidines and their alkyl derivatives: 6-mercaptopurine (6-MP) blocks the de novo synthesis of purines; 9-(β-D-arabinofuranosyl)-9H-purine 6-thiol (ara-9-MP) inhibits the incorporation of L-aspartic acid and uric acid into DNA cytosine. 9-(β-D-xyloturanosyl)-9H-purine 6-thiol (xyl-6-MP) inhibits the utilization of exogenously administered guanosine; the purine-6-carboxylase oxidation product of 9-(β-D-ribofuranosyl)-6-methyl-thio purine (MMP-RS) blocks the incorporation of thymidine into DNA. The effective clinical use of thiols

18. Synthesis and uses of isotopically labelled thiols such as these depends on two phenomena: whether the thiol will selectively accumulate in tumour cells, while the remainder of the drug is rapidly flushed out of the body and whether the thiol is selectively metabolized by the tumour cell to a more toxic substance.

The correlation of therapeutic action with the distribution of a drug had already been found in one of the earliest tracer studies of a labelled thiol. The powerful anthyrroid drug, 4-methyl-2-thiouracil RS, was distributed more or less evenly in the different organs of the cockerel, with only the thyroid gland, the pituitary gland and the fast-growing base of the feathersharts showing distinctly above normal concentration. RS Labelling has continued to be an indispensable tool in studying both of these phenomena during the testing of thiol drugs.

Both 6-mercaptopurine and buthionine (3-purinylsulfanylpropanoic acid) are carcinostatic drugs. However, both are quite active, but 30 times less toxic on chronic administration than 6-mercaptopurine. The origin of this effect was thought to lie in the relative tissue distributions of the drugs, which were studied using RS-labelling.31 Mecaptopurine RS passed rapidly through the gastro-intestinal barrier and reached many tissues, especially the liver, lungs, spleen and heart, as compared to the more gradual accumulation of bothuripurine in these organs. This was thought to account for the higher toxicity of 6-mercaptopurine. In the tumour itself, 6-mercaptopurine achieved a high level of accumulation, which then fell off as a function of time; whereas, bothuripurine persisted at a lower level for a longer time. The lower level of bothuripurine in the tumour as compared to that of mercaptopurine is in correlation with the effectiveness of the two drugs.

The oxidation of the ribosyl moiety of MMpR to MPP-RS completely changes the mode of action of the drug as well as its stability. MMpR−OP−RS is no longer selectively concentrated in tissues, but is rapidly excreted in the urine, most of it unchanged. The rapid passage of the drug through the body spares the host animal. However, a small proportion of the drug is bound to the ascite tumour membrane and is responsible for the drug's therapeutic effect. Although the drug is cleared in part to methylthiopurine, intact MMpR−OP was assumed to be the active agent. Ara-9-MP−RS rapidly appears in the blood, after intraperitoneal injection, where it is evenly distributed between plasma and red blood cells. At 3 min, the tumour cells already contained the largest percentage of the drug. By 30 min the drug is found in all tissues, except those beyond the blood-brain barrier. The concentration of the drug in the kidneys steadily increases with time, as the drug is cleared from the blood. The rapid clearance of the drug from the vital organs is thought
to account for its low toxicity. After 6 h 76% of the injected dose had been
excreted, of which 87% could be accounted for as unchanged drug. The
tumour cells themselves did not cleave ara-6-MP,6,8 to 6-MP,5,9, nor
appreciably converted it to the nucleotide, nor incorporated it into nucleic
acids.5,9

6-Mercaptopurine-35S is converted in the tumour cell to 6-methyl-
thiopurine ribonucleotide. The ribonucleotide was shown to be much
more efficient than the nucleotide of the parent compound, 6-MP, in
inhibiting the enzyme, phosphoribosyl pyrophosphate amidotransferase,
and subsequently bringing to a halt de novo purine synthesis in the tumour
cell. The conversion of 6-MP follows the pathway 6-MP -> MP nucleotide ->
6-Me-MP nucleotide. Tumour cells lacking the enzyme hypoxanthine
phosphoribosyl transferase, which is needed for the conversion to
nucleotide, are spared the action of 6-MP. Compounds that would be
active against 6-MP-resistant tumours have been actively sought, and those
found include: 6-MeMP, MMPK -> MP, ara-6-MeMP, 6-Me-MP and
6-Fluor-6-MP.1,6,7,8 Labelled studies showed that these thioles are rapidly
excreted unaltered.6,8,9,10,11

Now we have considered the behaviour of thioles that are essentiality
foreign to the metabolism of the animal. However, perhaps the most
sophisticated tracer techniques yet applied to the study of labelled thioles
have been developed in the course of investigations of the utilization of a
pulse-labelled cysteine in the on-going process of the synthesis of body
proteins. After administration, 35S-cysteine quickly enters the various
amino acid pools of the body and is incorporated along with naturally
occurring cysteine into the polypeptides synthesized in various tissues.

When amino acid sequencing techniques were first applied to proteins,
the sequence Cys-Gly-Gly was found to occur with greater than chance
frequency. This suggested that perhaps this sequence originated from
glutathione, rather than from free amino acids. To check this, ovodect
mice were incubated with glutathione labelled with 35S in the cysteine
residue and 35C in the carboxyl group of the glycol residue. The ovalbumin
produced was hydrolysed and the specific activity of cysteic acid and
glycine originating from the sequence Cys-Gly was compared to the
activity of those amino acids from other positions in the polypeptide chain.
The results indicated that glutathione played no specific role in the
biosynthesis of the Cys-Gly sequence.9

The rate of uptake of labelled cysteine into proteins has been extensively
used as an indicator of the metabolic activity of tissues. 35S L-cysteine
administered to mice was found to be preferentially incorporated into
growing hair follicles and claws. In other forms of epithelia the rate of
incorporation was found to be related to the cell turnover rate and in
glomerular cells to the rate of protein synthesis.10 Bleeding caused an
arrest or delay in the incorporation of cysteine-35S into organ proteins,
followed by a period of enhanced incorporation.11 Zinc deficiency in rats
improves the incorporation of L-cysteine-35S in skin protein while enhancing
the rate of incorporation of L-cysteine-35S into pancreas protein. This
suggested that zinc is essential to the synthesis of skin keratin and
collagen.12

Many hormones are rich in cysteine and the tissues in which they
accumulate can be easily recognized by a marked uptake of 35S-L-cysteine.
For instance, mature virgin mice, mature mice of both sexes and castrated
males display a 35S-labelled juxtaparlum X-zone in the brain, whereas
normal adult male mice do not.13 The neurosecretory system of the earth
worm markedly accumulates cysteine-35S.14 The neurosecretory cells of
rapidly developing female locusts and females in the second gonotrophic
cycle take up cysteine15 at a greater rate than either newly emerged or
slowly developing females.16

The neurosecretory system that has been studied in greatest detail is
the brain's hypothalamo-hypophyseal tract, that is concerned with the
synthesis of the octapeptide hormones, oxytocin and vassopressin, and
their secretion into the blood stream. Hargamn and Scharrer17 have
proposed that the neurophylial octapeptides are synthesized in the
perikarya of specialized nerve cells. They are subsequently bound to
carrier proteins, the neuroorphysins, which are then orgaized into granules.
These granules of neurosecretory material are then transported down the
axon of the neuron and stored in the terminals of the nerve fibres. The
release of the hormones into the blood vessels is accompanied by the
dissociation of the hormone from the carrier protein. Morphologically,14,15,16,17,18
the system consists of two paired nuclei, the supraoptic and the paraventricular nuclei, which lie in the hypothalamus of the
brain. The axons that extend from these parikaryaons run through the
hypothalamo-hypophyseal tract and reach the neurohypophysis, where
they terminate next to the basement membrane of the blood capillaries.

The neurosecretory material is rich in cytoine and can be spotted with
histochemical reagents specific for S-H and S-S bonds. Histochemical
staining has been used to locate neurosecretory material in the cell bodies of the
parikaryaons and stored in vesicles in the nerve terminals.19,20 However, such
staining techniques cannot detect the flow of hormones through the
neurosecretory system, while the use of single pulses of 35S-cysteine offers
the possibility of observing the fascinating process of the flow of neuro-
secretory material through the cells of the secretory system.
In 1939 Siopeu first performed the now much repeated experiment of administering $^{35}$S-labelled cysteine and methionine to rats and observing the appearance of radioactivity in various parts of the neurosecretory system. Labelled cysteine and methionine rapidly appeared in the supraoptic nuclei, and only later labelled cysteine, but not methionine, appeared in the infundibular process of the neurohypophysis. This suggested that the supraoptic nuclei were actively engaged in protein synthesis, and one of these polypeptides, particularly rich in cysteine, had migrated to the neurohypophysis. Fig and Flamant-Durand similarly observed that cystine $^{35}$S appeared in the supraoptic and paraventricular nuclei within 5 min after administration of labelled cysteine, and only 10 h later did labelled material appear in the neurohypophysis. Talanti and coworkers have monitored as function of time slided after the administration of labelled cysteine the radioactivity that appears in the supraoptic and paraventricular nuclei, as well as in three sites along the hypothalamo-hypophysial tract and in the neurohypophysis. When one has such a set of data, stating as a function of time the amount of label present in an anatomical structure, a kinetic model of the system can be set up that consists of a number of discrete pools of compounds whose flow from compartment to compartment obeys simple mathematics. When Talanti and coworkers analysed their data in terms of such a kinetic model, they could detect a component that first appeared in the supraoptic and paraventricular nuclei and slowly flowed through the hypothalamo-hypophysial tract to the neurohypophysis. Superimposed on the slow component was a rapidly abating pulse of radioactivity that moved through the hypothalamo-hypophysial tract at a constant speed of 0.6 mm/h without experiencing any delays. The fast component was thought to represent neurosecretory material, while the slow component represents structural proteins.

The identity of the labelled material that was seen to flow through the neurosecretory system was established only when the system was taken apart chemically. Sachers, by directly infusing highly labelled cysteine-$^{35}$S into the third ventricle of the brain of a dog, succeeded in isolating minute quantities of vasopressin-$^{35}$S. Vasopressin-$^{35}$S associated with the neurosecretory particle always had the lowest specific activity, whereas vasopressin-$^{35}$S found in the cell nuclei and in large granules had the highest specific activity. Norström and Sjöstrand later showed in a very elegant experiment that following the injection of cysteine-$^{35}$S in the area of the supraoptic nuclei, radioactivity appeared in a group of proteins that migrated through the hypothalamo-neurohypophysial tract, at a speed of 2.3 mm/h. Approximately 90% of the radioactivity of these soluble proteins was recovered in a single protein component. Norström, Hansson and Sjöstrand later showed that when the microtubuli of the axons are depolymerized with colchicine, the amount of labelled material that reaches the hypothalamo-neurohypophysial tract and the neurohypophysis is considerably reduced.

Quite early in the course of these tracer studies it was noted that marked changes in the uptake of cysteine-$^{35}$S occur following periods of water deprivation. Wells found that in rats thirst causes a marked increase in the uptake of radioactivity in the supraoptic nucleus and to a lesser extent in the paraventricular nucleus. Talanti later observed that thirst accelerates the rate of disappearance of radioactivity from the supraoptic and paraventricular nuclei, as well as the disappearance of radioactivity from the neurohypophysis. These results indicated that thirst activates both the synthesis and release of neurosecretory hormones that regulate the function of the kidneys.

V. APPLICATION OF $^{35}$S-TRACER STUDIES TO AGRICULTURAL SCIENCE AND INDUSTRY

Perhaps the largest system in which $^{35}$S-labelling has been used to follow the distribution of a thiol was a 20 acre forested area that was aerially sprayed with Malathion-$^{35}$S during a study of the ecological transport of the insecticide. Samples were taken in a number of ingenious ways. Air samples were taken on frosted glass discs suspended from helium balloons to measure the above canopy drift of the insecticide off the area. Samples of bark were taken to measure the settling out of the insecticide at different layers within the canopy. Soil samples were measured to determine the subsurface distribution. Samples collected on spotting enamel paper placed throughout the forest monitored the horizontal distribution of the insecticide. Samples from streams, insects, mammals, reptiles and birds indicated the initial and subsequent transport of the insecticide and its metabolites in the ecosystem.

The cream of cows which have consumed the weed, landcress, becomes tainted upon heat treatment with $\alpha$-toluenesulphonic acid. In order to determine the efficiency of steam distillation for the removal of the taint, $^{35}$S-labelled $\alpha$-toluenesulphonic acid was added to cream. The measurement of radioactivity proved to be a convenient analytical method to determine the amount of thiol that remained in the cream.

The $\text{SH} : \text{SS}$ ratio in gluten has been conveniently measured by assaying the relative $\alpha$-activity of NEML-cysteine and cystine in gluten prepared
from dough that had been made from the flour of wheat that was grown on soil supplemented with sulphate-S\textsuperscript{35}. The friction produced by a chrome-steel ball-bearing moving against discs and steel and brass creates a layer of FeS on the disc when it is lubricated with a mixture of cetane- and dodecane-thiol. The rate of formation of FeS and its subsequent wear were quantitatively measured by taking autoradiographs of the tracks of Fe\textsuperscript{35}S left by the ball-bearing on the steel discs when dodecane thiol-S\textsuperscript{35}S was added to the lubricant\textsuperscript{18}.

VI. ISOPE LABELLING AND COUNTING IN PRACTICE

Having reviewed the phenomena that can be probed with isotopically labelled thiol, we now turn to the technical problems associated with the execution of an experiment using isotope labelling. While many of the isotopically labelled thiols discussed in this review are now commercially available, we will review the synthetic procedures that have been used in the past to incorporate deuterium, tritium and sulphur-35 into these thiols, in the hope that it will allow the researcher with a less common thiol to choose the best synthetic route to its preparation. Having prepared a S\textsuperscript{35}-labelled thiol, various methods are available for the assay of its sulphur-35 activity. The method, best suited to a particular study, will depend on the accuracy desired, the level of sulphur-35 activity in the sample, and the nature of the medium in which the S\textsuperscript{35}-labelled thiol is dispersed. These and the various auxiliary techniques used to prepare the sample for counting will be discussed. Finally, we will turn to various methodological and phenomenological considerations which have rendered past S\textsuperscript{35}-labelling studies, especially in endocrinology, subject to criticism.

A. Synthetic Methods

Perhaps the simplest and most elegant method of labelling a thiol with S\textsuperscript{35}S would be to add a neutron to the nucleus of natural abundance S\textsuperscript{35}S by the nuclear reaction S\textsuperscript{35}(n,p)S\textsuperscript{36}. To date, this method has not been used, probably because there is no effective way to prevent the heat generated by the nuclear reaction from decomposing the molecule.

If the sulphur in a thiol cannot be rendered radioactive itself, it might be exchanged for thermally activated radioactive S\textsuperscript{35}S atoms. For instance, the sulphur atoms of mercapto benzothiazole exchange with S\textsuperscript{35}S recoil atoms generated in situ by the nuclear reactions, Cl\textsubscript{34}H\textsubscript{35}Cl\textsubscript{34} (where Cl\textsubscript{34}H\textsubscript{35}Cl\textsubscript{34} is used as the C\textsubscript{1} source) or S\textsuperscript{35}(n,p)S\textsuperscript{36} where elemental sulphur is the source of natural abundance S\textsuperscript{35}S. The yield of S\textsuperscript{35}-labelled mercapto benzothiazole is ~2-5% for S\textsuperscript{35}S generated from Cl\textsubscript{34}Cl\textsubscript{34} and ~30% for S\textsuperscript{35}S from S\textsuperscript{34}S. It is not necessary to use S\textsuperscript{35}S recoil atoms to accomplish the exchange. It has long been known that during the heating of a solution of 2-mercapto benzothiazole with sulphur-S\textsuperscript{35}, the sulphur of the mercapto group is exchanged for radio-sulphur-S\textsuperscript{35}. Since the thiol group of mercapto benzothiazole is in tautomeric equilibrium with the thios form, exchange is thought to occur by the addition of elemental sulphur to the C=S bond of the thio tautomer (equation 26). Moravčík and Kopecký\textsuperscript{36,36} have found the exchange to be generally synthetically useful for the labelling of thiols that can exist in a tautomeric form. Table 1 lists the thiols that have been labelled in this way.

The exchange of labelled sulphur can be promoted by enzyme catalysts, instead of heating. Bird egg yolk\textsuperscript{34} and the cysteine desulphhydrase\textsuperscript{34,34} that it contains catalyse the exchange of sulphur-35 from Na\textsubscript{2}S\textsuperscript{35}S to L-cysteine, L-cystine and L-cysteic acid. In a typical experiment, 150 ml of a buffer solution containing 2 millimoles of cysteine-HCl, 2 millimoles of Na\textsubscript{2}S\textsuperscript{35}S and 500 mg of cysteine desulphhydrase preparation is incubated at 38°C for 15 h. A mixture of 74-4% cysteine-S\textsuperscript{35}S and 25-3% cysteine-S\textsuperscript{34}S is obtained. L-Cystine-S\textsuperscript{35}S is subsequently reduced electrolytically to cysteine-S\textsuperscript{35}S. The total yield of L-cystine-S\textsuperscript{35}S obtained by isotope exchange is 70%.

Although isotope exchange by virtue of its simplicity and ability to form compounds of high specific activity is the method of choice for the labelling of tautomeric thiols, a synthetic method is often better suited to other thiols. For example, heating α-toluene thiol with sulphur-S\textsuperscript{35}S in benzene at 135–140°C for 6–12 h, yields α-toluene thiol-S\textsuperscript{35}S with a specific activity of only 2–9%. However, the synthesis of the compound from benzyl-magnesium chloride and sulphur-S\textsuperscript{35}S yields α-toluene thiol-S\textsuperscript{35}S.
<table>
<thead>
<tr>
<th>Compound</th>
<th>Source of the isotope</th>
<th>Method of synthesis</th>
<th>Reference</th>
</tr>
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<td>D₂O</td>
<td>Isotope exchange</td>
<td>16</td>
</tr>
<tr>
<td>Methanol S-1</td>
<td>CD₂H</td>
<td>+ 3H₂SO₄</td>
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<td>LiAlD₄</td>
<td>+ CH₂CH₂S-OEt</td>
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<td>Thiourea</td>
<td>- CH₃CHO</td>
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<td>Thiourea</td>
<td>- CH₂COOH</td>
<td>117</td>
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<tr>
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<td>- (CH₂)₃NHCH₂CH₂Cl</td>
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<td>28, 24</td>
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<td>Thiourea</td>
<td>- (CH₂)₁₅NHCH₂CH₂Cl</td>
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<td>Benzylthiol</td>
<td>Thiourea</td>
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<td>+ CH₃CH₂MgBr</td>
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<tr>
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<td>Sulfur</td>
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<tr>
<td>Thiopental</td>
<td>Sulfur</td>
<td>Isotope exchange</td>
<td>99</td>
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Thermolytic and recoil atom reactions yield quite complex mixtures of thiols. For example, when an equimolar mixture of \( \text{C}_3\text{H}_6 - \text{N}_2 - \text{H}_2\text{PS} \) was passed through an empty quartz tube \( \text{C}_3\text{H}_6 \), 0.0001%; \( \text{C}_3\text{H}_6\text{N}_2 \), 0.001%; cyclobutane, 0.001%; ethanethiol, 0.1%; butanethiol, 0.2%; isobutanethiol, 0.2%; and many other unidentified products was obtained\(^{19}\).

When a 1 : 1 mixture of \( \text{H}_2\text{PS} \) and \( \text{H}_2\text{S} \) was heated for 10 h at 310°C at 20 atmospheres, 1\(^4\)\(\text{CH}_3\text{CH}_2\text{SH} \) and 1\(^3\)\(\text{CH}_3\text{CH}_2\text{S} \) were obtained in mole fractions of 3\( \times 10^{-4} \) and 3\( \times 10^{-4} \), respectively\(^{20} \).\(^{21} \). Recoil atoms produced in a mixture of methane-\( \text{H}_2\text{PS} \) by the atomic reaction \( \text{Cl}_2(n,p)\text{PS} \), yield a mixture containing \( \text{H}_2\text{PS} \) and \( \text{CH}_2\text{PS} \) as the major constituents\(^{20} \). The relative amounts of the products can be controlled by adjusting the concentration of \( \text{Ar} \) and \( \text{NO}_2 \) which serve as a moderator and radical scavenger. Hot \( \text{PS} \) formed by the neutron bombardment of \( \text{CCl}_4 \) react with a cyclopentane: cyclohexane mixture to give a mixture of \( \text{PS} \)-labelled thiophenes, tetrahydrothiophens, cyclohexanethiol, cyclobutanethiol, ethanethiol, propylpentyl sulfide and polymeric mercaptans and sulfides\(^{23} \). Neutron bombardment of a 1 : 2 mixture of \( \text{CCl}_4 \) and cyclohexane yields a reaction containing \( \text{C}_3\text{H}_6\text{S} \) and \( \text{C}_4\text{H}_6\text{S} \) at levels of 3.5 and 8% of the total radioactivity, respectively; however, the majority of the activity is found in non-volatile products\(^{20} \). In practice, the more conventional synthetic methods used for the preparation of thiols in general are better suited to the preparation of labelled thiols, especially when the \( \text{PS} \)-labelled precursor is commercially available.

Thiomagnesium halides formed by the reaction of sulphur with a Grignard reagent can be decomposed to the corresponding thiols (equation 27). While the reaction has not been extensively used for the preparation of non-labelled arenesulphides, it is particularly well suited to the synthesis of \( \text{PS} \)-labelled thiols, since the \( \text{PS} \)-labelled reactant, sulphur-\( \text{PS} \), is readily available. Among the \( \text{PS} \)-labelled thiols that have been prepared by this method are iso-butanethiol\(^{298} \), benzenethiol\(^{299} \), \( \alpha \)-toluenethiol\(^{300} \), \( \alpha \)-cresol\(^{301} \), \( \beta \)-toluenethiol\(^{302} \), 2-phenylethanethiol\(^{303} \) and \( \alpha \)-napthathiolethiol\(^{304} \). Yields vary from 44 to 90%.

In recent years the method of choice for the preparation of thiols in the laboratory has become the addition of an alkyl-halide to thioamides to form
the isothiocyanate salts are stable and can be stored. The decomposition of S-methylisothiocyanoformate, prepared from thiourea and dimethyl sulphate, has been used as a convenient source of methanethiol-34S in the course of a number of syntheses. The quantification of thiourea with methyl iodide111 has been reported to give higher yields than with dimethyl sulphate. The 34S-labelled precursor, thiourea-34S, is prepared from H2S by reaction with H2O, NH3CN, NaNH, or from Ba34S by treatment with H2, Na, NH3, and CO2 and a trace of powdered sulphur112-114. A number of 34S-labelled thiols have been prepared in this way, including methanethiol-34S111,112,114, 1-propylthiol-34S114, 1,2-propanedithiol-34S115, 34S-mercaptosuccinic acid116, dimethylaminoethanethiol-34S118, and 34S-thiourea.118,119

Yields up to 90% have been reported.

In 1940 Regazoni passed ethyl chloride into potassium hydrogen sulphide in a retort and obtained ethanethiol-34S equation (29). This classical synthetic method has been used to prepare labelled thiols from Na234S and organic halides. The thiols prepared by this method include

\[ \text{RCH} + \text{NaSH} \rightarrow \text{RSH} + \text{NaCl} \]  

(29)

n-butane-thiol116,117,118, 1-toluenethiol-34S119 and 2-mercaptobenzothiazole-34S120. In variations on the method, 2-mercaptoethanol118 has been prepared from Na234S and 2-chloroethanol121 and 2-mercaptoethanol was obtained by heating 6-chloropiperidine with Ba34SO4.122

A standard method for making aromatic thiols from relatively unreactive aromatic halides is to convert them to the azaanillic acid salt,

\[ \text{RNCH} + \text{KSCOEI} \rightarrow \text{RSCOEI} + \text{N} + \text{KCl} \]

(30)

\[ \text{RSCOEI} + \text{H}_2 \rightarrow \text{RSH} + \text{CO} + \text{EtOH} \]

which readily reacts with a xanthate, such as EtOCSK (equation 30). 

13S-Labeled EtOCsK has been prepared by treating Na34S and sulphur-34S with CS2 to form uniformly labelled Na34CS2, which is then decomposed with HCl and the resulting CS2-34S passed through an EOF/EIOK solution. Both 13S-labelled p-mercapto- and p-phenylbenzothiol have been prepared from EtOCSK-34S and the corresponding disodium chloride170.

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When 34S-labelled disulphides are available, the corresponding 34S-thiol can be readily prepared by electrolysis or H2 reduction (equation 31). 13S-Mercaptoacetic acid172 and cysteine have been obtained in this way172.

\[ \text{RSSR} + \text{H} \rightarrow 2 \text{RSH} \]  

(31)

Although the addition of H2S to unsaturated bonds proceeds in quantitative yields, e.g. the addition of hydrogen sulphide to ethylene gives ethyl mercaptan with no by-products, the reaction has been used only once to prepare ethanethiol-34S from H2S-34S and ethylene (equation 32)170. The addition of H2S-34S across strained heteroatomic bonds in

\[ \text{X} \rightarrow \text{Na} + \text{NaSH} \rightarrow \text{NaSH} \]  

(32)

small ring compounds has been used to prepare 2-mercaptoethanol173 from ethylene oxide and 3 mercaptopropionylamine from ethylenediamine174.

In addition to these standard methods, a number of specialized reactions of limited scope have been used to prepare some biologically important 34S-labelled thiols. For instance, 3-thioureaz136 has been prepared by the condensation of thiourea-34S with NaNOC=CHCOEt170, p-Amino-13S-mercaptoacetic acid-34S was prepared by the acid hydrolysis of 4-carboxy-13S-methyl-2-phenylthiazoline170, 13S-dimercaptopropanoic acid-34S was obtained by the hydrolysis of 2,3-bis(acetylthio) succinic acid-34S,172 d,l-Cysteine-34S was obtained by the acid hydrolysis of PbCO3·CH2(CNOC2H4)CO2Me170.

However, as the biologically interesting thiols become more complex, biosynthetic routes would appear to be the method of choice. For their synthesis, in spite of the inherent loss of 34S isotope in the biological system and need for chromatographic separation of the isotopically labelled molecules from a complex biological mixture, l-Cysteine-13S and glutathione-34S and coenzyme-A-34S have been obtained from labelled sulphate by biosynthetic routes, while complex polypeptides, such as y-globulin175 and insulin176 have been obtained from organisms grown on cysteine-34S. Even highly labelled whole organisms such as dyserint bacterial177 have been grown on cysteine-34S.

Deuterium and tritium labelling of the SH group can be carried out most conveniently by isotope exchange with D2O or T2O by simply dissolving the thiols in the labelled solvents, followed by evaporation. The thiols labelled by isotope exchange are CIHSD178, DSSH, CH3SD178, CH3(CH2)SD178, CH3SD178, CH3SD178, CH3SD178, CH3SD178. Thiols have also been deuterated by the D2O solvolysis of Na mercaptides, such as
CH₃(CH₂)₂Sn⁻ and C₆H₅Sn⁻ and by the reaction of MeOD with XC₆H₄S⁻ → SiMe₃ (X = halide).¹⁰⁶

As in any synthesis employing radioisotopes, special care must be taken not to contaminate the laboratory. Special glassware which minimize the escape of the isotope are usually designed to meet the needs of a specific synthetic route. The preparation of thioles from radioactive sulphur and a Grignard reagent is a good illustrative example of the design of such vessels for an organic reaction and the subsequent extraction of the labelled compound with organic solvents.¹⁰⁷

An apparatus for the reaction of ³⁵S with a Grignard reagent is shown in Figure 3. The Grignard reagent is pipetted in tube A, ether is added and

![Figure 3](image3.png)

**Figure 3.** Reaction vessel used for Grignard reaction.

the apparatus is flushed with nitrogen. Sulphur-35 dissolved in xylene is added to the mixture from R and the reaction mixture is stirred under nitrogen at 9°C. The liquid air trap C protects the mixture from moisture, while tube D acts as a liquid trap in a case of a pressure backflow due to a pressure build-up in a series of aqueous sodium hydroxide traps connected at E. Upon completion of reaction the Grignard reagent is decomposed by addition of HCl.

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The labelled thiol is extracted from the reaction mixture by rapidly transferring the reaction flask A to the apparatus shown in Figure 4. The extraction is carried out under a nitrogen atmosphere. By properly adjusting the traps, the reaction mixture is transferred from A to the separatory funnel G, to which ether is added through H. The two phases are agitated by the magnetic stirrer I, and the aqueous layer is returned to A and the ether layer to F. The aqueous layer is extracted with more

![Figure 4](image4.png)

**Figure 4.** Vessel for the extraction of ³⁵S-labelled thioles.
portions of ether added through H. The thiol can be precipitated from the ether layer as the Na salt by simply extracting the aqueous layer with the 10% aqueous sodium hydroxide.

### D. Counting Methods

The low energy β rays emitted by 35S can be counted in a number of different ways, including gas flow counting, liquid scintillation counting and autoradiography on photographic emulsions. The particular method chosen depends on the nature of the sample.

Perhaps the simplest counting procedure is to place the same on a planchet and assay its radioactivity under either a windowless gas flow counter or a micro end window counter. This method of counting has most often applied to BaSO₄, BaO, Na₂SO₄ or benzidine sulphate, which is layered on the planchet. In addition, films of polymers, TCA precipitated proteins, whole blood and red blood cell ghosts labelled with 35S have been counted in this way. Often the counting of a layer of material is complicated by the self-absorption of the radiation from the bottom of the sample. The self-absorption of radiation is generally standardized by preparing layers that are ‘infinitely thick’, e.g. 15–16 mg sulphate per cm². This ensures that radiation from the bottom of the sample is completely adsorbed. When 35S to be counted is in the gas phase, as in SO₂ or H₂S, it can be introduced together with methane directly into a Geiger Müller tube and counted at efficiencies of 93–96%.[135] Sulphur dioxide-35S can be introduced up to 7.5 Torr, whereas hydrogen sulphide-35S can be counted at much higher partial pressures. A novel application of this type of counting was the measurement of β-activity of alkanethiols, as they emerge from a gas-liquid chromatograph, in which methane was used as the carrier gas.[135]

A convenient and fast method of locating 35S-labelled spots on thin-layer plates or on paper chromatograms is to pass the chromatogram under a windowless gas flow counter.[135, 138] Radiochromatogram scanners of this type are commercially available and their design have been described.[135, 138] However, for accurate determination of radioactivity the spot must be counted in a liquid scintillation counter.

Liquid scintillation counting has been used to assay the radioactivity of numerous 35S-labelled compounds after they have been separated on TLC plates. However, the 35S-labelled compound must first be located by radiochromatogram scanning, scraped off the plate and eluted off the absorbent into the counting solution. Alternatively, the absorbent together with the 35S-labelled compound can be suspended in the counting solution by 18. Synthesis and uses of isotopically labelled thiols

solution by Cab-O-Sil. Paper chromatograms, on the other hand, are usually cut into 1 cm strips which are eluted and counted in a toluene scintillation. Polycrystalline gels have been embedded in 2% agar gel, mounted on a rubber plate and cut into 1 mm thick slices in a mechanical chopper. The strips are then placed in a liquid scintillator phial, extracted in 1 ml of toluene and subsequently counted.[139] Alternatively, gels were thickened with 10% glycerol and sliced in a dry-acetone-hexane bath.[139] The radioactivity of 35S-labelled compounds, emerging from a liquid chromatograph, has been measured as they flow through a plastic scintillator spiral.[140] A number of liquid scintillator fluids particularly suited for the low energy β-rays of 35S have been developed.[141, 142, 143, 144]

Autoradiography has been extensively used to locate radioactive areas on chromatograms. Usually the chromatogram is pressed against a no-screen X-ray film and allowed to develop.[145] The development time can extend over a period of weeks or months, which allows radioactive areas of very low activity to be detected.[146]

Autoradiography is particularly well suited for determining the distribution of radioactivity in tissue. In principle, the distribution of radioactivity in a tissue could be assessed by gas flow counting, if the tissue was dissected, its parts weighed and uniformly spread on a dry filter paper. However, very often it is difficult to identify exactly the part of the tissue that has been dissected. Furthermore, the fluids which surround the tissue in the body may often be highly labelled and will contaminate the dissected specimen. The use of autoradiography readily overcomes these difficulties.[147]

The methods of preparing the autoradiographs most commonly used in 35S tracer studies are those of Donselaar and Peeters,[148] and Ullberg.[149] The choice of exposure time and counting methods has been discussed by Peeters.[148] The activity recorded on the photographic film can be determined either by directly counting silver grains[150] or by mounting the autoradiograph on a microscope slide and measuring the relative amount of light transmitted using a photoelectric cell at the ocular of a microscope.[148] The former is more accurate and the data are obtained in a form that can be treated by statistical methods, i.e. silver granules/μ² (± S.E.M.). The absolute sensitivity of electron microscope autoradiography, i.e. ratio of developed grains to radioactive decay in the specimen, were determined for 35S with Ilford L4 and Kodak NTE emulsions and found to be 1/21 for 35S in a monomolecular layer.[148] The resolution that can be obtained depends on the photographic emulsion. The observed radioactivity depends on several physical factors, including the thickness of the sample, the nature of the tissue, the exposure time and the modalities of the
developing procedures. Autoradiography has been used to follow the whole body distribution of 38S in plants and animals, as well as the movement of 38S down the axon of a nerve cell.

C. Sample Preparation

1. Wet ashing

The wet ashing technique was originally designed to convert sulphur contained in organic material into a form, such as BaSO₄, which could be layered on a planchet for gas flow counting. This was achieved by decomposing the sample with a mixture of HNO₃ and H₂SO₄, or a mixture of HCl and H₂O₂ together with a copper salt catalyst, followed by the precipitation of SO₄⁻ by barium. The method also lends itself to liquid scintillation counting when the BaSO₄ is suspended in a liquid scintillator solution that has been gelled by Cab-O-Sil. Alternatively, the sample can be reduced to H₂S, which is subsequently absorbed in a solution of NaOH, and assayed in a liquid scintillation counter.

2. Oxygen flask combustion

The oxygen flask method converts organic sulphur to a form suitable for liquid scintillation counting. In principle, the sample is combusted in an oxygen atmosphere. Sulphur is converted to SO₂, which is trapped in a liquid scintillator solution. In practice, a good deal of development has gone into increasing the speed, efficiency and safety of the technique. The sample can be held in a number of ways, such as in a Pt basket or a paper cup holder, or impregnated on a cotton pellet, placed in a paper cup that is held in a glass ring or watch-glass-type combustion platform. The reaction vessel, which can be either a 2-l glass flask (accommodating 70-900 mg of material), a liquid scintillation phial (holding 10-15 mg) or a plastic bag, is flushed with oxygen. The sample is ignited most often by focusing a light beam on a dark spot which has been made on the paner sample holder or by heating electrically the Pt sample holder. The sample is combusted and SO₂ is collected in a trapping agent such as phenylthiacylate or ethanolamine in nine parts of methanol. The trapping solution is subsequently mixed with the liquid scintillator and counted. Usually the trapping agent, which is a flammable organic mixture, is added to the reaction vessel prior to ignition, and therefore poses a hazard when the sample is ignited. To avoid explosions, the reaction vessel is either cooled in dry-ice acetone to lower the volatility of the trapping solution or alternatively the vessel is filled with a balloon attached to the side-arm.

A non-flammable trapping solution consisting of a 1:1:2 mixture of toluene, triton X-100 and water has also been used. The efficiency of counting which takes into account the recovery of radioactivity and the quenching of the scintillator by the trapping agent is usually 90-95%.

In human samples, in which a large amount of material with a very low activity is combusted, a compromise must be struck between the counting rate and the quenching level. The large amounts of trapping agent that are required, quench the counting mixture, while dilution of the trapping agent reduces the counting rate to the background level of the scintillator.

3. Specialized techniques

In addition to the wet ashing and oxygen flask methods, a number of rather specialized techniques have been used to convert a sample to a form which can be sufficiently counted. Methanethiol-S₈ has been added to HgCl₂-N₂ and precipitated as (MeOH)Hg and counted under a gas flow counter. 38S-labelled scintillation counting was carried out by an in-phant degradation by heating in a xylene solution containing t-butyl hydrogen peroxide and SO₄²⁻. Labeled H₂S released into the atmosphere by micro-organisms has been trapped on paper strips impregnated with basic lead acetate, which are subsequently treated with glyoxal, H₃PO₄ and zinc powder and counted in a Tracerlab counter.

D. Methodological Considerations

A number of important methodological considerations enter into the design of body tracer studies. The number of labelled thiol molecules that will be incorporated into a particular macromolecule or tissue depends on (1) the dilution of the isotope in the added molecule, (2) the pre-existing concentration of the compound in different organs and cells, (3) the presence of different precursors of the compound, (4) the turnover rate of the compound and its precursors, and finally (5) the rate of synthesis of the complex polypeptide into which it will be incorporated. Furthermore, in endocrine research, polypeptide hormones may be quickly metabolized and lead to an unspecific labeling sometimes difficult to detect. Hormones are usually physiologically active at very low concentrations, which requires that they be very highly labelled if they are to be observed at all. As the metabolic pathways of cells are often ramified, in addition to the hormone, labelled sulphur may also be incorporated into structural proteins, lipid sulphates, sulphonated mucopolysaccharides and water-soluble substances, such as cystine, methionine, glutathione, taurine and...
inorganic sulphates. Labelled methionine can be used to determine the rate of accumulation of labelled sulphur in structural proteins, and labelled sulphur can be used to check the localization of sulphur in other compounds. If the specific activity of the labelled polypeptide is to be determined then a technique such as autoradiography must be used in conjunction with quantitative cytochemical methods.

The interpretation of autoradiograms can be ambiguous, especially if the anatomy of the tissue furnishes few points of reference and the area to be counted is far from the cell nucleus. Often the shape of the cell may impede the exact determination of its centre.

Kinetic measurements of the rate of transport of a labelled compound in a tissue depend on the specification of the time and the site of entry of the labelled compound into the system. Ideally, one would like to inject the labelled compound directly into the system under study. However, the local application of the labelled substances introduces a serious risk of disturbing both the timing of precursor absorption into the system and the rate of incorporation. There may be no way of knowing whether the true physiological circumstances are preserved. Furthermore, the local application of the labelled compound does not enhance the specificity of its incorporation in the polypeptide, as opposed to other uptake mechanisms. Since the measurement of isotopic accumulation requires that the animal be sacrificed, it is not possible to take consecutive samples from the same animal as a function of time. The kinetic measurements must therefore represent a picture of the mean behaviour of the isotopic in a population of animals.

VII. REFERENCES

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