OXIDATION OF SOME BIOLOGICALLY ACTIVE AND RELATED SULFUR CONTAINING COMPOUNDS

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Abstract - Earlier works on oxidation of sulfides, thiols, and disulfides with both chemical and enzymic systems are reviewed. Enzymic oxidations of thiols and disulfides are compared with their chemical oxidations with N₂O₅ and H₂O₂ by the use of unsymmetrical disulfides, thiosulfimates and O¹⁸ tracer technique. In both enzymic oxidation and that with N₂O₅, oxidation of disulfides to thiosulfimates is more facile than that of thiosulfimates to thiosulfonates, which is presumed to be mainly the end by-products in the oxidation of either thiols or disulfides to sulfonic acids.

INTRODUCTION

Many years ago, when we initiated to study the mechanism of the Pummerer reaction of alkyl sulfoxides, we suggested that the rearrangement serves as a likely model pathway of enzymatic oxidative demethylation of methionine, in view of the facile hydrolysis of the resulting ester in the following reaction (Ref. 1).

\[
\begin{align*}
\text{CH}_3\text{CH}_3 & \xrightarrow{\text{CH}_3\text{O-PO}_3\text{Na}} \text{CH}_3\text{SCH}_2\text{OCCH}_3 \xrightarrow{\text{H}_2\text{O}} \text{CH}_3\text{SH} + \text{CH}_2\text{O} + \text{CH}_3\text{COOH} \\
\text{CH}_3\text{SCH}_2\text{OCCH}_3 & \xrightarrow{38^\circ\text{C}, \text{pH} 5, \text{1 day}} \text{CH}_3\text{SCH}_2\text{OCCH}_3
\end{align*}
\]

Methionine itself was found to give homocysteine similarly (Ref. 2).

\[
\begin{align*}
\text{CH}_2\text{S-CH}_3 & \xrightarrow{\text{Na}} \text{CH}_2\text{SCH}_2\text{OCCH}_3 \xrightarrow{\text{H}^+} \text{CH}_2\text{SH} + \text{COOH} \\
\text{CH}_2\text{SCH}_2\text{OCCH}_3 & \xrightarrow{\text{(CH}_3\text{C})_2\text{O}} \text{CH}_2\text{SCH}_2\text{OCCH}_3 \xrightarrow{\text{H}^+} \text{CH}_2\text{SH} + \text{COOH}
\end{align*}
\]

The formation of the sulfoxide in the metabolic oxidation was already noticed with 4-(phenylthioethyl)-1,2-diphenyl-3,5-pyrazolinedione (I) by Burns et al. (Ref. 3). Evidence, more concrete to support our hypothesis was found by Barnsley and Arnold group (Ref. 4) who identified following products (II)-(IV) among rat-urine after subcutaneous injection of S-methylcysteine into rats.
Biological oxidation of sulfides to sulfoxides is well-known (Ref.5), however it was Lee et al. who found a heavy concentration of sulfide oxidase in rat-liver microsome (Ref.6).

Meanwhile, the Pummerer reaction was found to take place with the following sulfonium ylides (Ref.7), suggesting another possibility of demethylation of methionine adenosyl sulfonium salts (V) via "ylide formation", though this would be of minor importance.

\[
\begin{align*}
\text{Ph-S-CH}_3 + \text{Ac}_2\text{O} & \rightarrow \text{Ph-S-CH}_2\text{OCOMe} + \text{MeCOCH(CO}_2\text{Me)}_2 \\
\end{align*}
\]

As compared to the clear-cut route of oxidation of sulfides to sulfoxides and to sulfones, those of thiols and disulfides are still practically unexplored and in jungle.

**OXIDATION OF THIOLS**

Anionic thiol oxidations, by air, to disulfides have been studied extensively and suggested to proceed in the following manner (Ref.8).

\[
\begin{align*}
\text{RSH} + \text{R'O}^- & \rightarrow \text{RS}^- + \text{R'OH} \\
\text{RS}^- + \cdot\text{O}_2 & \rightarrow \text{RS}^- + \cdot\text{O}_2^- \\
2 \text{RS}^- & \rightarrow \text{RSSR} \\
\cdot\text{O}_2^- + \text{RSH} & \rightarrow \text{RS}^- + \text{HO}_2^- \\
\text{HO}_2^- + \text{RSH} & \rightarrow \text{RS}^- + \text{HO}_2^- + \cdot\text{OH} \\
\end{align*}
\]

Similar reactions are known in many biological systems but will not be covered here.

There are direct transformations of thiols to the corresponding sulfinic acids both chemically and biochemically.

Autooxidation of thiols was studied earlier by Berger, and shown to involve the chain carrier, \(\text{RS}^-\) sulfenate ion, in the following sequence (Ref.9).

\[
\begin{align*}
1) \text{RS}^- + \cdot\text{O}_2 & \rightarrow \text{RSOO}^- \quad \text{(peroxysulfenate)} \\
2) \cdot\text{RSOO}^- + \text{RS}^- & \rightarrow 2 \text{RSO}^- \\
3) \text{RSO}^- + \cdot\text{O}_2 & \rightarrow \text{RSOO}^- \quad \text{(peroxysulfinate)} \\
4) \text{RSOO}^- + \text{RS}^- & \rightarrow \text{RSO}^- + \text{RSO}^- \quad \text{(sulfinate)} \\
5) \text{RSOO}^- + \text{RSO}^- & \rightarrow 2 \text{RSO}^- \\
6) \text{RSOO}^- + \text{RSO}_2^- & \rightarrow \text{RSO}^- + \text{RSO}_3^- \quad \text{(sulfonate)} \\
7) \text{RSOO}^- + \text{RSO}_2^- & \rightarrow \text{RSO}^- + \text{RSO}_3^- \\
\end{align*}
\]

According to this scheme it is obvious that both oxygen atoms in the resulting sulfinate should be originated from atmospheric oxygen instead of the medium, in this case, \(\text{t-butanol}\) and a small amount of water. However, our preliminary \(^{18}\text{O}\) tracer experiment showed that the resulting sulfinate contains \(^{18}\text{O}\) from the medium, a small amount of \(\text{H}_2^{18}\text{O}\). Therefore, the reaction is not as simple
as Berger suggested. In this autooxidation, acid is formed from mercaptide ion and disulfide is generated from unionized thiols. In other words, an equimolar amount of a strong base is necessary to accomplish the autooxidation of thiol to sulfinate.

Similarly, the following two paths (arrow headed a and b) appear to be the direct transformation of thiols to the corresponding sulfinic acids in biological systems.

Metabolic oxidation of cysteamine (VI) to hypotaurine (VII) and thiotaurine (VIII), (path a) was studied by Cavallini et al. (Ref.10,11 & 12) who revealed the oxidation to uptake molecular $^{18}$O, catalyzed by non-heme-iron-containing and sulfur- or disulfide-requiring dioxygenase enzyme (Ref.13 & 14).

Biological oxidation of cysteine (IX) to cysteine sulfinic acid (X), path b, requires molecular oxygen ($^{18}$O experiment), catalyzed by cysteine oxygenase which is a dioxygenase present in every mammalian liver. Cysteine oxidase has been carefully purified by Yamaguchi et al. and shown to contain Cu$^+$ in protein A subunit (Ref. 17). He also suggested the following scheme for the metabolic oxidation.

Oxidation of thiols by N$_2$O$_4$ is interesting. The reaction is extremely fast as compared to that of disulfides which also give nearly identical oxidation products. With an excess of N$_2$O$_4$, the main products are sulfonic anhydrides and sulfonic acids. However, when an equimolar amount of N$_2$O$_4$ was used, another peak appeared.
In order to examine the possible precursor of these oxidation products, the reaction was conducted at relative low temperatures. When the thiol is mixed with N₂O in ether at -50°C, immediately the color of the solution changes to reddish brown. However, upon removing N₂O with some nucleophiles in vacuo, disulfide was obtained. When the reaction mixture was treated at -20°C, then worked up as usual with liquid chromatography, a large unknown peak showed up, beside the usual oxidation products, i.e., sulfonic acid and sulfonic anhydride. The unknown intermediate, (tentatively called as A) contains (NO₂)ₓ functional group but is unstable.

The intermediate A, upon hydrolysis, gives the sulfonic acid, with alcohol to alkyl sulfinate and sulfonate, reacts with sec-amines to give sulfonamides and with thiols, form disulfides. The last reaction will become a useful synthetic procedure to prepare unsymmetrical disulfides, since the yields are generally over 90%.

The modes of the reactions seem to suggest that [A] is a sulfinate species containing (NOₓ)ⁿ group, while the formation of unsymmetrical disulfides proceeds via thiosulfinate which is eventually reduced by acid-catalyzed thiol reduction, similar to the facile reduction of the sulfinyl function by dithiophosphoric acid (Ref. 18).

OXIDATION OF DISULFIDES

Oxidation of disulfides leads ultimately to sulfonic acids while several intervening intermediates can actually be obtained. The general scheme is shown below (Ref. 19).

Cleavage takes place in the top sequence. Hydrolytic conditions favor the top sequence and anhydrous ones the bottom sequence. However, both sequences are more likely interplaying in actual reactions. One example for the top sequence is the one-step synthesis of sulfinate esters (Ref. 20 & 21). This reaction proceeds nicely with alkyl and diphenyl disulfides but is sluggish with diphenyl disulfides bearing electron-withdrawing substituents such as p,p'-dinitro or bulky ortho substituents.
Following mechanism was suggested.

\[
Pb(OAc)_4 \rightarrow Pb(OAc)_2 + AcO^+ + AcO^- + RSSR + AcO^+ \rightarrow RS(OAc)SR
\]

\[
RS(OAc)SR \rightarrow RS-SR + OAc + AcO^+ + CH_3OH \rightarrow AcOCH_3 + H^+
\]

\[
RS-OCH_3 + RSH
\]

Pryor also suggested the oxidation of disulfides with hydrogen peroxide to involve the formation of [RSOH] species (Ref. 22). Oxidation in alkaline condition was also suggested to involve the formation of sulfenic acid (Ref. 23). Since the mechanism of this reaction can be better understood by the \(^{18}O\) tracer experiment, it was carried out and the sulfonate formed was found to contain roughly 1/3 of \(^{18}O\) when Na\(^{18}OH\) was used in H\(_2\)O\(^{18}\). Namely the initial attack undoubtedly proceeds by hydroxide ion to form [RS\(^{18}OH\)] which then picks up \(O_2\) give the final sulfonate, while there is a possibility of disproportionation of the sulfenic acid to disulfide and sulfonate although of minor importance.

Following reaction between substituted diphenyl disulfides and pyridine N-oxide also appears to involve the prior cleavage of S-S bond (Ref. 24).

\[
\text{CH}_3O\text{-}(-S-S-(-NO}_2 + \text{N-oxide} \rightarrow \text{[RS-OCH}_3 + RSH}
\]

Based on the following kinetic data at 158°C, Hammett \(\rho\) value of -0.7 with \(\sigma\) values and a much smaller reactivity of more basic \(\sigma\)-methoxypyridine N-oxide (pKa=2.05) than unsubstituted pyridine N-oxide (pKa=0.79).

<table>
<thead>
<tr>
<th>Disulfide</th>
<th>(k_2 (1/mol \cdot sec))</th>
</tr>
</thead>
<tbody>
<tr>
<td>(\text{O}_2\text{N-S-S-NO}_2)</td>
<td>(6.27 \times 10^{-2})</td>
</tr>
<tr>
<td>(\text{CH}_3\text{O-S-S-NO}_2)</td>
<td>(5.75 \times 10^{-2})</td>
</tr>
<tr>
<td>(\text{Cl-S-S-NO}_2)</td>
<td>(3.10 \times 10^{-1})</td>
</tr>
<tr>
<td>(\text{CH}_3\text{O-S-S-NO}_2)</td>
<td>(1.18 \times 10^{-1})</td>
</tr>
</tbody>
</table>

The reaction was suggested to involve the prior formation of an intermediate (A) before cleavage of S-S bond.

\[
\text{CH}_3O\text{-}(-S-S-(-NO}_2 + \text{N-oxide} \rightarrow \text{[CH}_3\text{-}S-S\text{-S-N}_2\text{O}_2^+}
\]

Oxidation of disulfides with peracids or metallic oxides in acetic acid or hydrolytic media has been investigated extensively (Ref. 25) but not systematically. Thiosulfinate was obtained by direct oxidation of alkyl disulfide with a peracid in 1947 (Ref. 26) and the reaction was shown...
to follow a third-order kinetics, 1st order with disulfide and second order
\[
\text{RCO}_3\text{H} \xrightarrow{\text{peracid}} \text{RCOOH}
\]
with peracid respectively (Ref. 27). Allen was able to control oxidation of a symmetrical acyclic disulfide with 30% \( \text{H}_2\text{O}_2 \) to produce a monoxide in 80% yield, a dioxide in 77% yield and a tetraoxide in 16% yield (Ref. 28). However, the formation of dioxide may involve cleavage of \(-\text{S-S-}\) bond of thiol-sulfinate.

Disproportionation of thiolsulfinate was noticed earlier by us in the hydrolysis of \( \sigma^- \) or \( \rho^- \)-nitrophenylsulfenyl chloride (Ref. 29).

Thiolsulfinates are stable if the group \( R \) are long-chain but not if they are short chain or aryl. \( \sigma^-\)-Benzyolthiamine disulfide monoxide(A) is also quite stable, however, upon neutral hydrolysis, gives the sulfinic acid and disul-

Neutral oxidation of thiamine tetrahydrofurfuryl disulfide was studied by Suzuki's group carefully and shown to involve cleavage of \(-\text{S-S-}\) bond (Ref. 30 & 31). \( ^{18}\text{O} \) tracer experiment indicates the enzymic oxidation is catalyzed by a typical mono-oxygenase which requires NADPH (Ref. 32). However, no information is available as to the nature of \( \text{S-S} \) bond cleavage and the mode of the oxidation.
In order to shed light on these problems, we purified the enzymic protein taken from rabbit liver microsome and use it to oxidize 1,2-dithiane of which monoxide and dioxide are quite stable to be readily isolated (Ref. 34). The enzymic oxidation requires NADPH, O₂ and several minerals typical for monoxigenases and both mono- and dioxides were isolated as shown in the Table 1.

**TABLE 1. Requirement of enzyme system using Ms.**

<table>
<thead>
<tr>
<th>Expt.</th>
<th>System (stand. condn.)</th>
<th>Spec. activity¹</th>
<th>Rel. activity²</th>
</tr>
</thead>
<tbody>
<tr>
<td>I</td>
<td>complete</td>
<td>1.022</td>
<td>100</td>
</tr>
<tr>
<td></td>
<td>-NADPH</td>
<td>0.128</td>
<td>13</td>
</tr>
<tr>
<td></td>
<td>-G-6-P</td>
<td>0.140</td>
<td>14</td>
</tr>
<tr>
<td></td>
<td>-G-6-P-D</td>
<td>0.250</td>
<td>24</td>
</tr>
<tr>
<td></td>
<td>-MgCl₂</td>
<td>0.996</td>
<td>97</td>
</tr>
<tr>
<td></td>
<td>-O₂</td>
<td>0.051</td>
<td>5.0</td>
</tr>
<tr>
<td></td>
<td>-all co-factors</td>
<td>0.000</td>
<td>0.0</td>
</tr>
<tr>
<td></td>
<td>-Ms. + boiled Ms.</td>
<td>0.013</td>
<td>1.0</td>
</tr>
<tr>
<td></td>
<td>(100°C, 10min.)</td>
<td></td>
<td></td>
</tr>
<tr>
<td></td>
<td>blank expt.</td>
<td>0.000</td>
<td>0.0</td>
</tr>
<tr>
<td>II</td>
<td>complete minus NADPH-generating system</td>
<td>1.441</td>
<td>142</td>
</tr>
<tr>
<td></td>
<td>+NADPH-generating system</td>
<td>0.116</td>
<td>11</td>
</tr>
<tr>
<td></td>
<td>+NAD⁺</td>
<td>0.383</td>
<td>37</td>
</tr>
<tr>
<td></td>
<td>+NADH-generating system</td>
<td>0.165</td>
<td>16</td>
</tr>
</tbody>
</table>

¹ n mole/min/mg. protein  
² % of the complete system  
³ stand. condn.  
⁴ added 10μmol.  
⁵ consisted of 1.7mmol. EtOH, 10μmol. NAD⁺, and alcohol dehydrogenase (62 unit)
However, the step (a) proceeds much faster than the step (b) or direct formation of the dioxide from the disulfide.

The reaction of symmetrical acyclic disulfides with \( \text{N}_2\text{O}_4 \), gives the corresponding sulfonic anhydride in good yield (Ref. 36), like ozonolysis (Ref. 37).

Further, unsymmetrical acyclic disulfides, upon treatment with equimolar amounts of \( \text{N}_2\text{O}_4 \), at ice-cold temperature, gave all kinds of oxidized products obviously formed by the cleavage of S-S bond. All the oxidized products were identified by LLC, GLC, nmr and ir spectra.

Although no noticeable amount of the monoxide was obtained in the oxidation, the monoxide is also readily oxidized with \( \text{N}_2\text{O}_4 \), apparently forming the sulfonic acids besides the dioxide, which is obtainable mainly by direct oxidation and partly by disproportionation (Ref. 38). The reactivities of all these organosulfur species fall roughly in this order; PhSH) PhSSCH_3 > PhSSCH_3 > PhSSCH_3 > PhSSPh as shown at Table 2.

In the cases of thiols and disulfides, the initial formation of the unstable intermediate [A] was observed when \( \text{N}_2\text{O}_4 \) was used not much in excess at a low temperature. Apparently, the initial attack is electrophilic and takes place on the more nucleophilic sulfur atom, eventually cleaving S-S linkage in this oxidation, which somewhat contrasts the oxidation with hydrogen peroxide. Disproportionation of thiosulfinites to disulfides and thiosulfonates during the oxidation appears to be rather small in view of the scant formation of
TABLE 2. Oxidation of sulfur compounds with eq. N₂O₄ at 0°C for 2 hr.

<table>
<thead>
<tr>
<th>Substrate</th>
<th>Reaction condition</th>
<th>PhSO₂SPh</th>
<th>PhS⁺₂</th>
<th>PhSO₃H</th>
<th>MeSO₃H</th>
<th>MeS⁺₂</th>
<th>MeSO₂⁺₂O</th>
<th>PhSO₂⁺₂O</th>
</tr>
</thead>
<tbody>
<tr>
<td>PhSSMe</td>
<td>Air</td>
<td>40</td>
<td>37</td>
<td>25</td>
<td>49</td>
<td>small¹</td>
<td>11</td>
<td>1</td>
</tr>
<tr>
<td></td>
<td>O₂</td>
<td>29</td>
<td>17</td>
<td>40</td>
<td>45</td>
<td>1</td>
<td>40</td>
<td>45</td>
</tr>
<tr>
<td></td>
<td>N₂</td>
<td>20</td>
<td>20</td>
<td>40</td>
<td>39</td>
<td>17</td>
<td>&quot;</td>
<td>&quot;</td>
</tr>
<tr>
<td></td>
<td>N⁺⁺ Cs₂H (1/10² molar)</td>
<td>37</td>
<td>12</td>
<td>1</td>
<td>1</td>
<td>1</td>
<td>1</td>
<td>&quot;</td>
</tr>
<tr>
<td></td>
<td>N⁺⁺ Cs₂H (1/10 molar)</td>
<td>32</td>
<td>8</td>
<td>&quot;</td>
<td>&quot;</td>
<td>&quot;</td>
<td>&quot;</td>
<td>&quot;</td>
</tr>
<tr>
<td></td>
<td>Air+ Molecular Sieve 4</td>
<td>57</td>
<td>&quot;</td>
<td>&quot;</td>
<td>&quot;</td>
<td>&quot;</td>
<td>&quot;</td>
<td>&quot;</td>
</tr>
<tr>
<td>PhSSMe</td>
<td>Air</td>
<td>Ca.90</td>
<td>0</td>
<td>small³</td>
<td>50</td>
<td>0</td>
<td>26</td>
<td>1</td>
</tr>
<tr>
<td></td>
<td>O₂</td>
<td>77</td>
<td>0</td>
<td>small³</td>
<td>49</td>
<td>0</td>
<td>14</td>
<td>1</td>
</tr>
<tr>
<td>PhSH</td>
<td>Air⁶)</td>
<td>traceable</td>
<td>0</td>
<td>30</td>
<td>-</td>
<td>-</td>
<td>-</td>
<td>60</td>
</tr>
<tr>
<td>PhSSPh</td>
<td>N₂</td>
<td>0</td>
<td>30</td>
<td>-</td>
<td>-</td>
<td>-</td>
<td>50</td>
<td></td>
</tr>
</tbody>
</table>

¹) not determined 2) ca. 5% by nmr 3) 0-5% 4) ca. 5% 5) ? 6) only here, N₂O₄: excess cond. (eq.x5)

dimethylthiolsulfonate in the oxidation of PhSSCH₃ and the rather small amount of diphenylthiolsulfonates in that of PhSSOCH₃. The effects of small amount of thiol, molecular sieve or cellite on the product distribution are interesting but cannot be commented beyond speculation.

In the oxidations of unsymmetrical disulfides and monoxides with H₂O₂, disulfides are less reactive than the monoxides, while the attacking site appears to be mainly the sulfinyl sulfur rather than the sulfide sulfur atom, though undoubtedly the latter attack also takes place, where concurrent cleavage of S–S linkage and an interesting oxygen atom migration appears to take place. In this reaction most of the products appears to retain the original S–S linkage, while disproportionation of thiolsulfinate in this oxidation is also of minor significance.

In all these oxidations, i.e., enzymic and chemical, thiol sulfonates are quite stable and unreactive. Therefore it is quite likely that dioxides are side products in the oxidation to form sulfonic acids.

REFERENCES
32. T. Fujita and S. Suzuki, ibid, 74, 717 (1973), refs in this paper.
34. Y. H. Kim and D. Fukushima, from our Lab. Unpublished.