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OXIDATION OF SOME BIOLOGIALLY ACTIVE AND RELATED SULFUR CONTAINING COMPOUNDS

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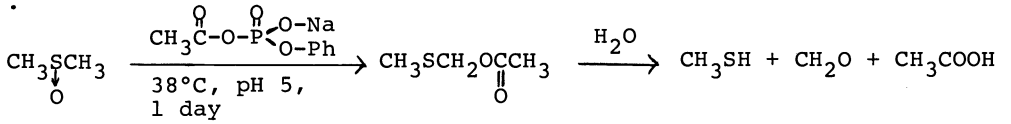
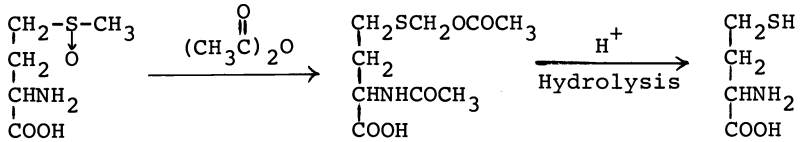
Abstract - Earlier works on oxidation of sulfides, thiols, and disulfides with both chemical and enzymic systems are reviewed. Enzymic oxidations of thiols and disulfides are compared with their chemical oxidations with N_2O_4 and H_2O_2 by the use of unsymmetrical disulfides, thiolsulfinates and O^{18}-tracer technique. In both enzymic oxidation and that with N_2O_4, oxidation of disulfides to thiolsulfinates is more facile than that of thiolsulfinates to thiolsulfonates, which is presumed to be mainly the end by-products in the oxidation of either thiols or disulfides to sulfonic acids.

INTRODUCTION

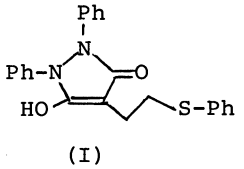
Many years ago, when we initiated to study the mechanism of the Pummerer reaction of alkyl sulfoxides, we suggested that the rearrangement serves as a likely model pathway of enzymatic oxidative demethylation of methionine, in view of the facile hydrolysis of the resulting ester in the following reaction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



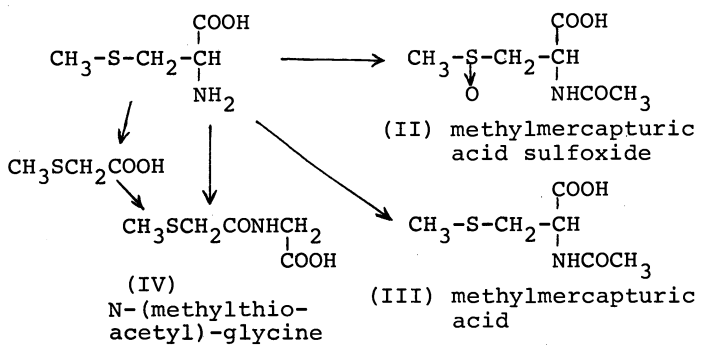
Methionine itself was found to give homocysteine similarly (Ref.2).



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.



(I)



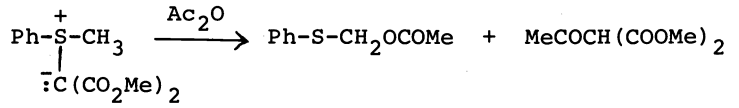
(II) methylmercapturic acid sulfoxide

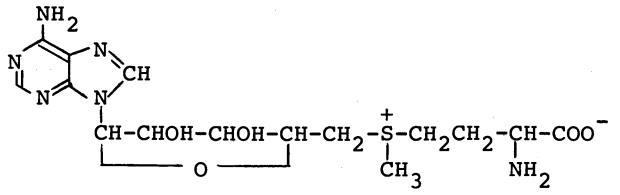
(IV) N-(methylthio-acetyl)-glycine

(III) methylmercapturic acid

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.



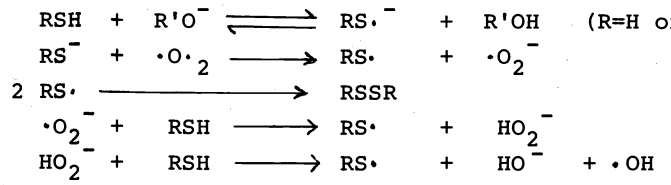


(V)

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

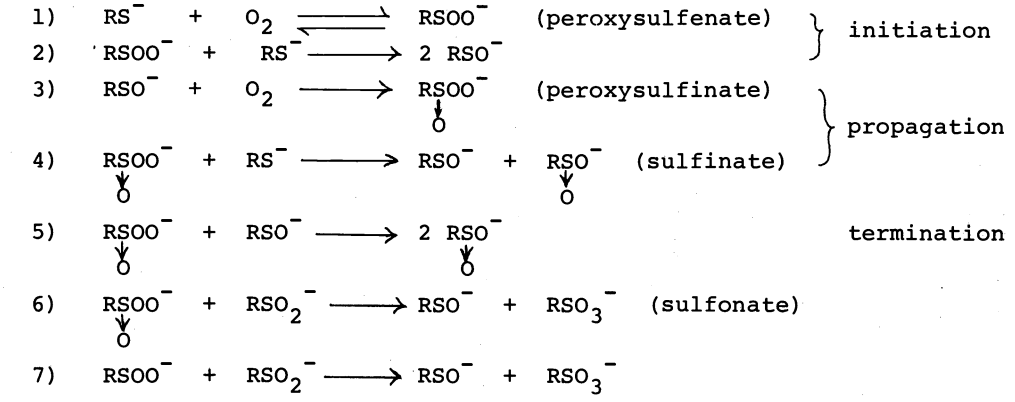
Anionic thiol oxidations, by air, to disulfides have been studied extensively and suggested to proceed in the following manner (Ref.8).



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

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

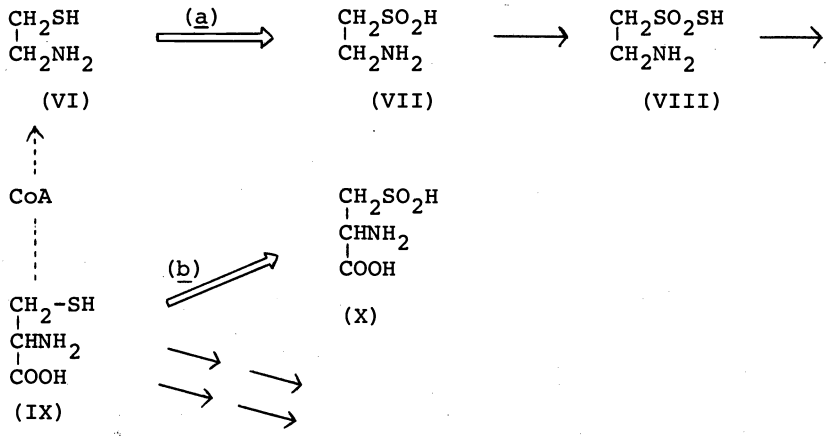
Autooxidation of thiols was studied earlier by Berger, and shown to involve the chain carrier, RSO^- sulfenate ion, in the following sequence (Ref.9).



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, t-butanol and a small amount of water. However our preliminary ^{18}O tracer experiment showed that the resulting sulfinate contains ^{18}O from the medium, a small amount of $H_2{}^{18}O$. Therefore, the reaction is not as simple

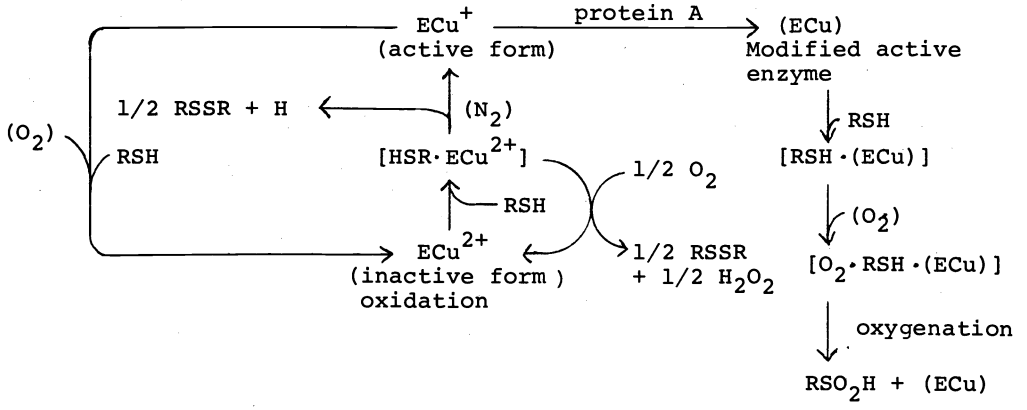
as Berger suggested. In this autooxidation, acid is formed from mercaptide ion and disulfide is generated from unionized thiols. In other words, an equimolar amount of a strong base is necessary to accomplish the autooxidation of thiol to sulfinate.

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.

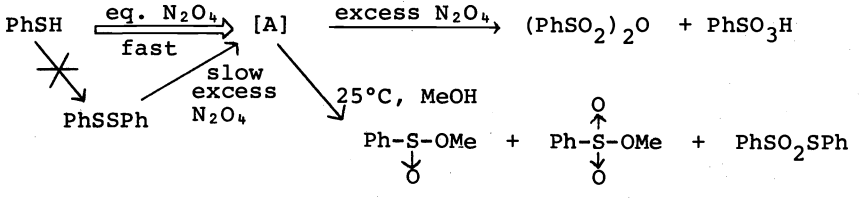


Metabolic oxidation of cysteamine (VI) to hypotaurine (VII) and thiotaurine (VIII), (path a) was studied by Cavallini et al. (Ref.10,11 & 12) who revealed the oxidation to uptake molecular ^{18}O, catalyzed by non-heme-iron-containing and sulfur- or disulfide-requiring dioxygenase enzyme (Ref.13 & 14).

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 manmalin liver. Cysteine oxydase 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.

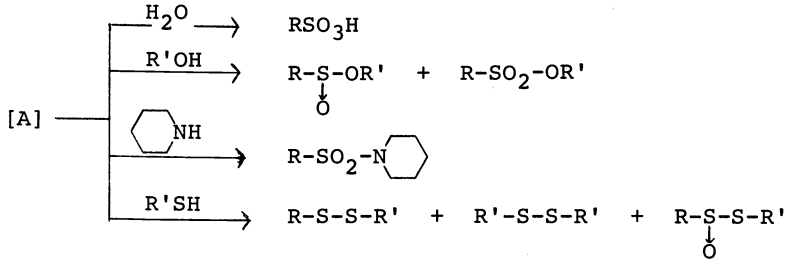


Oxidation of thiols by N_2O_4 is interesting. The reaction is extremely fast as compared to that of disulfides which also give nearly identical oxidation products. With an excess of N_2O_4, the main products are sulfonic anhydrides and sulfonic acids. However, when an equimolar amount of N_2O_4 was used, another peak appeared.

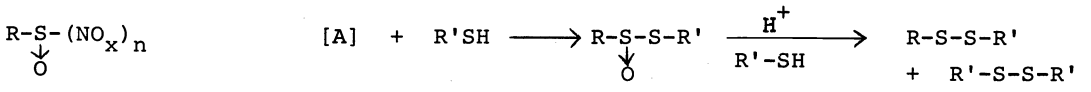


In order to examine the possible precursor of these oxidation products, the reaction was conducted at relative low temperatures. When the thiol is mixed with N_2O_4 in ether at -50°C, immediately the color of the solution changes to reddish brown. However, upon removing N_2O_4 with some nucleophiles 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 $(NO_2)_n$ 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 $(NO_x)_n$ group, while the formation of unsymmetrical disulfides

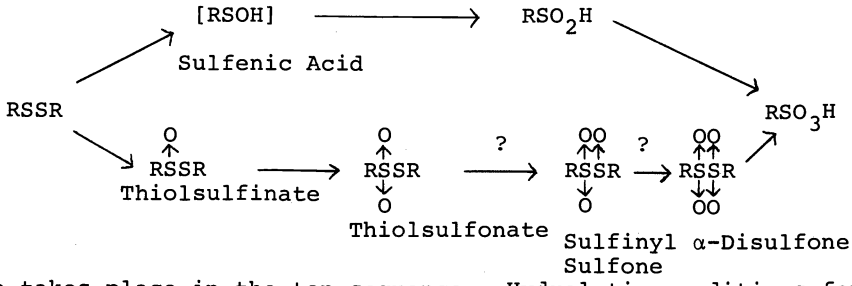


[A] ?

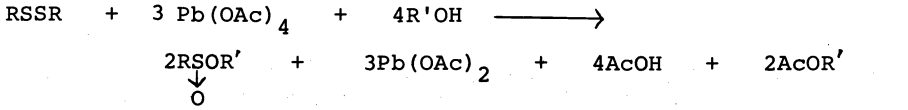
proceeds via thiolsulfinate which is eventually reduced by acid-catalyzed thiol reduction, similar to the facile reduction of the sulfinyl function by dithiophosphoric acid (Ref. 18).

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).

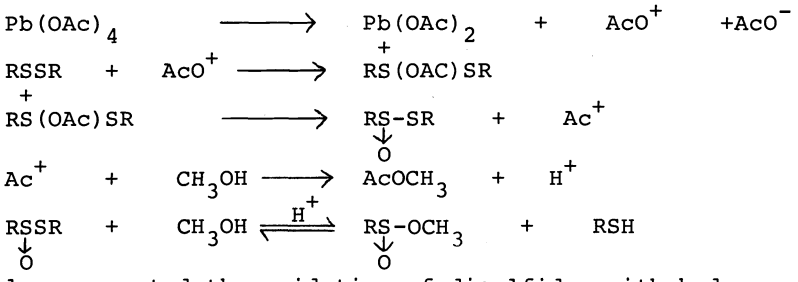


Cleavage takes place in the top sequence. Hydrolytic conditions favor the top sequence and anhydrous ones the bottom sequence. However, both sequences are more likely interplaying in actual reactions. One example for the top sequence is the one-step synthesis of sulfinate esters (Ref. 20 & 21). This reaction proceeds nicely with alkyl and diphenyl disulfides

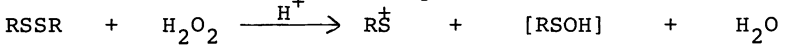


but is sluggish with diphenyl disulfides bearing electron-withdrowing substituents such as p,p'-dinitro or bulky ortho substituents.

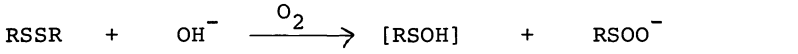
Following mechanism was suggested.



Pryor also suggested the oxidation of disulfides with hydrogen peroxide to involve the formation of [RSOH] species (Ref. 22). Oxidation in alkaline

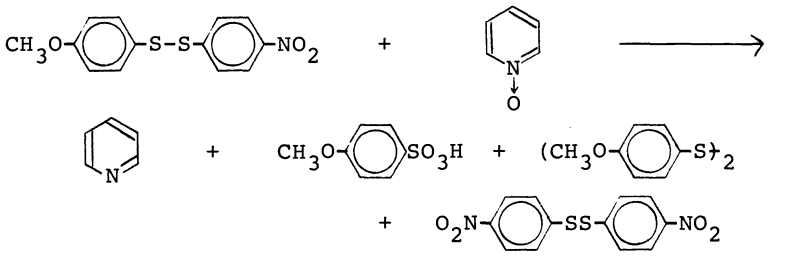


condition was also suggested to involve the formation of sulfenic acid (Ref. 23). Since the mechanism of this reaction can be better understood

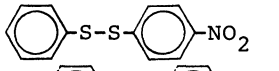
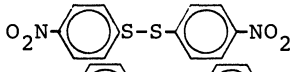
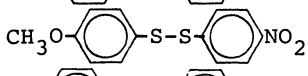
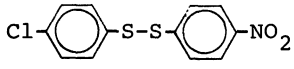


by the ^{18}O tracer experiment, it was carried out and the sulfonate formed was found to contain roughly 1/3 of ^{18}O when $Na^{18}OH$ was used in H_2O^{18}. Namely the initial attack undoubtedly proceeds by hydoxide ion to form $[RS^{18}OH]$ which then picks up O_2 give the final sulfonate, while there is a possibility of disproportionation of the sulfenic acid to disulfide and sulfonate although of minor importance.

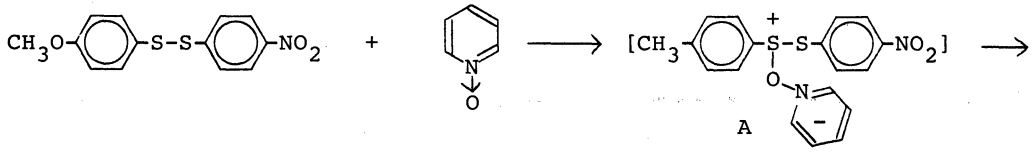
Following reaction between substituted diphenyl disulfides and pyridine N-oxide also appears to involve the prior cleavage of S-S bond (Ref. 24).



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

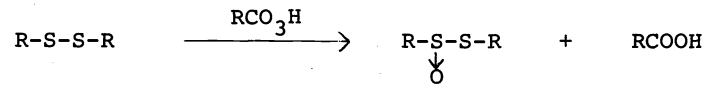
Disulfide	k_2(l/mol·sec)
	6.27×10^{-2}
	5.75×10^{-2}
	3.10×10^{-1}
	1.18×10^{-1}

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

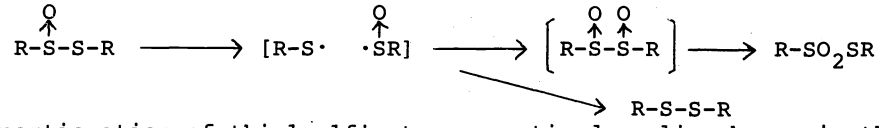


Oxidation of disulfides with peracids or metallic oxides in acetic acid or hydrolytic media has been investigated extensively (Ref. 25) but not systematically. Thiolsulfinate was obtained by direct oxidation of alkyl disulfide with a peracid in 1947 (Ref. 26) and the reaction was shown

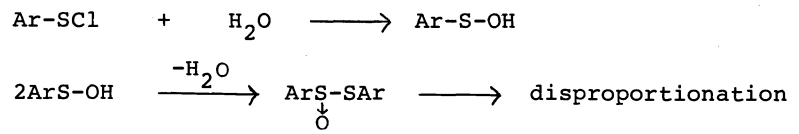
to follow a third-order kinetics, 1st order with disulfide and second order



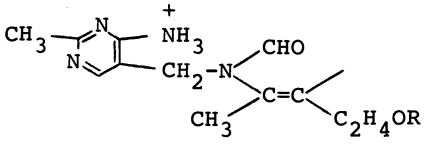
with peracid respectively (Ref. 27). Allen was able to control oxidation of a symmetrical acyclic disulfide with 30% H_2O_2 to produce a monoxide in 80% yield, a dioxide in 77% yield and a tetraoxide in 16% yield (Ref. 28). However, the formation of dioxide may involve cleavage of -S-S- bond of thiolsulfinate.



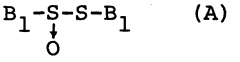
Disproportionation of thiolsulfinate was noticed earlier by us in the hydrolysis of o- or p-nitrophenylsulfenyl chloride (Ref. 29).



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

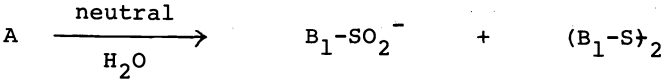


: B_1-(a moity of Vitamin B_1)

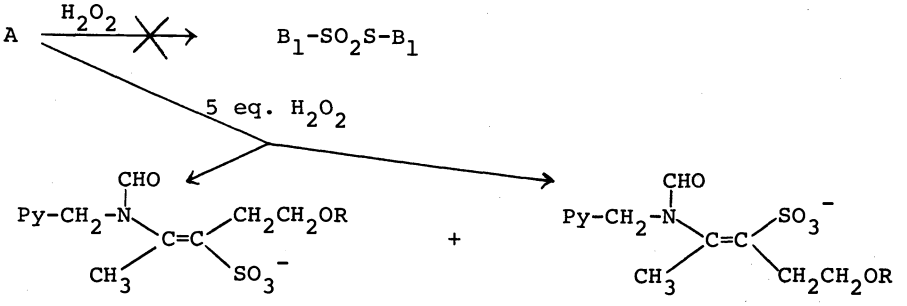


(A)

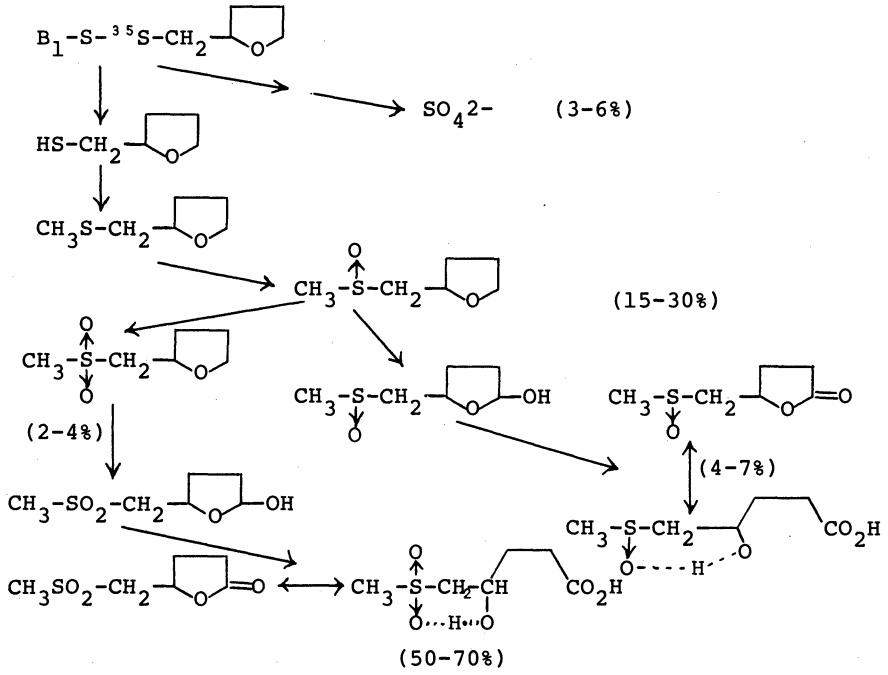
fide (Ref. 30). The monoxide(A) can be obtained by oxidation of the disulfide



with hydrogen peroxide but resists further oxidation to the dioxide(B) and only upon treatment with 5 eq. mol. amount of H_2O_2 gave the sulfonic acid.

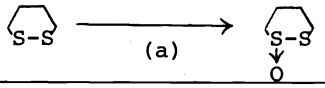


Metabolic oxidation of thiamine tetrahydrofurfuryl disulfide was studied by Suzuoki's group carefully and shown to involve cleavage of -S-S- bond (Ref. 30 & 31). ^{18}O tracer experiment indicates the enzymic oxidation is catalyzed by a typical mono-oxygenase which requires NADPH (Ref. 32).
However, no information is available as to the nature of S-S bond cleavage and the mode of the oxidation.



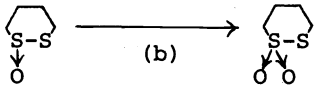
In order to shed light on these problems, we purified the enzymic protein taken from rabbit liver microsome and use it to oxidize 1,2-dithiane of which monoxide and dioxide are quite stable to be readily isolated (Ref. 34). The enzymic oxidation requires NADPH, O_2 and several minerals typical for monoxigenases and both mono- and dioxides were isolated as shown in the Table 1.

TABLE 1. Requirement of enzyme system using Ms.



Expt.	System(stand. condn.)	Spec. activity[1]	Rel. activity[2]
I	complete	1.022	100
	-NADP$^+$	0.128	13
	-G-6-P	0.140	14
	-G-6-P-D	0.250	24
	-$MgCl_2$	0.996	97
	-O_2	0.051	5.0
	-all co-factors	0.000	0.0
	-Ms. + boiled Ms. (100°C, 10min.)	0.013	1.0
	blank expt.	0.000	0.0
II	complete minus NADPH-generating system		
	+NADPH-generating system[3]	1.441	142
	+NAD$^+$[4]	0.116	11
	+NADH[4]	0.383	37
	+NADH-generating system[5]	0.165	16

1) n mole/min/mg. protein 2) % of the complete system
3) stand. condn. x 2 4) added 10μmol. 5) consisted of 1.7mmol. EtOH, 10μmol. NAD$^+$, and alcohol dehydrogenase (62 unit)



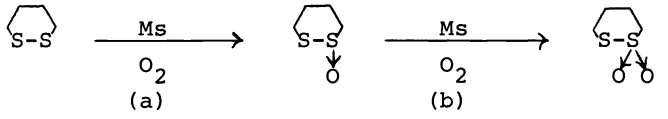
System	Spec. activity[1]	Rel. activity[2]
complete	0.323	100

-continued-

-all co-factors	0.010	3.0
-Ms.	0.000	0.0
blank expt.(buffer only)	0.000	0.0

1) n mol./min/mg. protein 2) % of the complete system

However, the step(a) proceeds much faster than the step(b) or direct formation



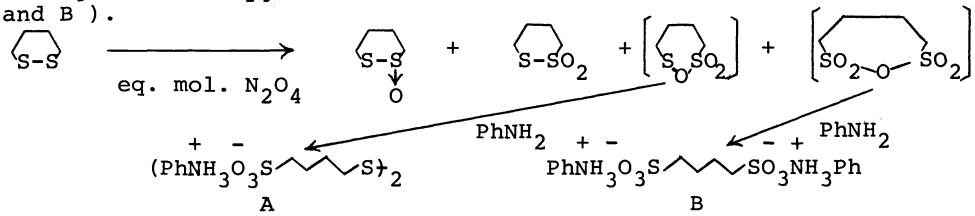
of the dioxide from the disulfide.
Formation of the dioxide, i.e., thiolsulfonate in any oxidation system is still unclear. In the absence of steric affects, oxidation of disulfide by peracid occurs at sulfur atom more distant from an electron-withdrawing group (Ref. 35). However, it is not clear whether or not the oxidation is stepwise.

$$R^1SSR^2 + 2R^3CO_3H \longrightarrow R^1SO_2SR^2 + 2R^3CO_2H$$

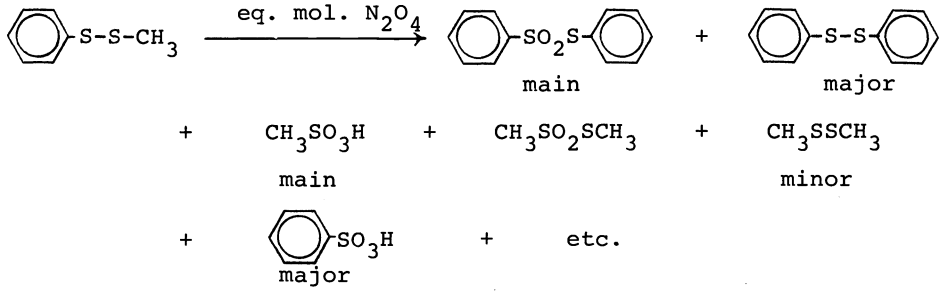
The reaction of symmetrical acyclic disulfides with N_2O_4, gives the corresponding sulfonic anhydride in good yield (Ref. 36), like ozonolysis (Ref. 37).

$$R\text{-}S\text{-}S\text{-}R \xrightarrow[N_2O_4]{} RSO_2OSO_2R$$

1,2-Dithiane, however, gives 1,1-dioxides with the same reagent (Ref. 38). Careful treatment of 1,2-dithiane with an equimolar amount of N_2O_4 gave the monoxide, together with the dioxide (Ref. 38) and other oxidation products containing 3 and 6 oxygen atoms, which were isolated as anilinium sulfonates (A and B).



Further, unsymmetrical acyclic disulfides, upon treatment with equimolar amounts of N_2O_4 at ice-cold temperature, gave all kinds of oxidised 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 noticable amount of the monoxide was obtained in the oxidation, the monoxide is also readily oxidized with N_2O_4, apparently forming the sulfonic acids besides the dioxide, which is obtainable mainly by direct oxidation and partialy by disproportionation (Ref. 38).
The reactivities of all these organosulfur species fall roughly in this order; PhSH $\gg$ PhSSCH$_3$ (O) $>$ PhSSCH$_3$ $>$ PhSSCH$_3$ (O) $>$ PhSSPh as shown at Table 2.
In the cases of thiols and disulfides, the initial formation of the unstable intermediate [A] was observed when N_2O_4 was used not much in excess at a low temperature. Apparently, the initail 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 thiolsulfinates to disulfides and thiolsulfonates during the oxidation appears to be rather small in view of the scant formation of

TABLE 2. Oxidation of sulfur compounds with eq. N_2O_4 at 0°C for 2 hr.

Substrate	Reaction condition	Products (%) $PhSO_2SPh$	$PhS\rangle_2$	$PhSO_3H$	$MeSO_3H$	$MeS\rangle_2$	$MeSO_2\rangle_2O$	$PhSO_2\rangle_2O$
PhSSMe	Air	40	37	25	49	small[1]	11	1)
	O_2	29	17	40	45		1)	"
	N_2	40	20	40	39		17	"
	N_2+ C_8SH(1/10^2molar)	37	12	1)	1)	1)	1)	"
	N_2+ C_8SH(1/10^4molar)	32	8	"	"	"	"	"
	Air+ Molecular Sieve	4	57	"	"	"	"	"
PhSSMe O	Air	Ca.90	0	small[3]	50	0	26	1)
	N_2	77	0	small	49	0	14	1)
PhSSMe O	Air	small[4]	small[5]	85	45	small[2]	11	1)
	N_2			50-87	39		1)	1)
PhSH	Air[6]	traceable	0	30	-	-	-	60
PhSSPh	N_2		0	30	-	-	-	50

1) not determined 2) ca. 5% by nmr 3) 0-5% 4) ca. 5% 5) ?
6) only here, N_2O_4: excess cond. (eq.x5)

dimethylthiolsulfonate in the oxidation of $PhS\overset{O}{\overset{\|}{S}}CH_3$ and the rather small amount of diphenylthiolsulfonates in that of $PhSSOCH_3$. The effects of small amounts 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 H_2O_2, 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, thiolsulfonates are quite stable and unreactive. Therefore it is quite likely that dioxides are side products in the oxidation to form sulfonic acids.

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