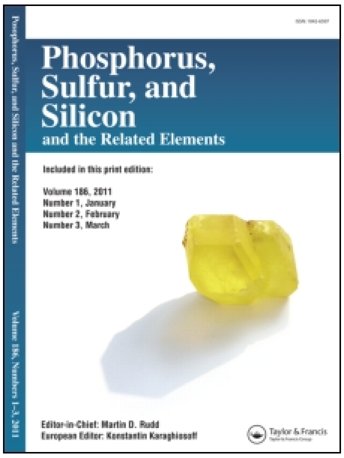

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CHEMISTRY OF BIOLOGICALLY ACTIVE SULFUR COMPOUNDS

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CHEMISTRY OF BIOLOGICALLY ACTIVE SULFUR COMPOUNDS

by

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I. INTRODUCTION

All living organisms require sulfur for various metabolic processes. Sulfur occurs in enzymes, in structural proteins of cells, and in a wide variety of naturally occurring compounds which often play key roles in metabolism. The chemistry and biochemistry of sulfur compounds has been reviewed.[1-3].

Most plants and microorganisms are capable of growing on inorganic sulfur compounds, such as sulfides and sulfates, which can be obtained directly from the environment and subsequently transformed into organic forms. Animals, on the other hand, obtain only small amounts of inorganic sulfur directly from the environment, and the major part of their requirement must be provided in the form of amino acids and vitamins. The "organic sulfur" is partly reconverted into sulfate by catabolic activities of animals, but the major part of it is degraded to hydrogen sulfide as a result of host bacterial action on plants and animals after their death.

The purpose of the present chapter is to familiarize the reader with some essential aspects of sulfur chemistry pertinent to the understanding of the bio-

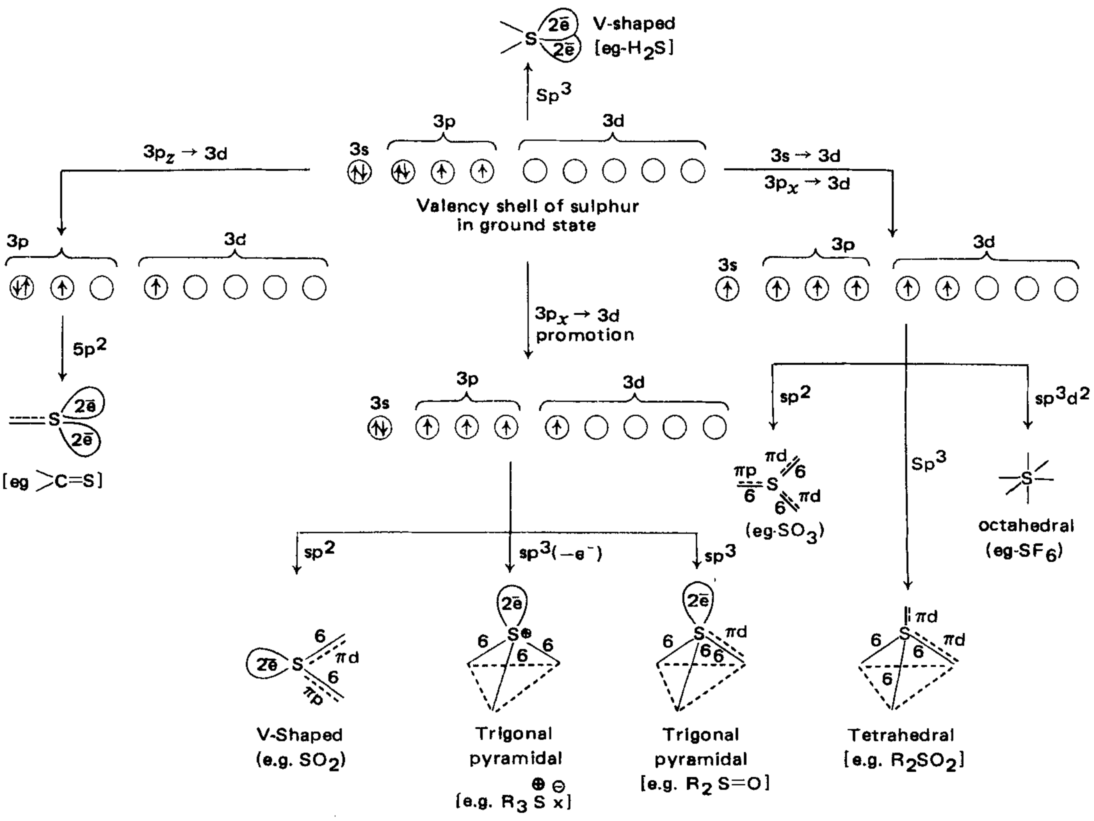

FIGURE I

Bonding and Stereochemistry of Sulfur-Containing Molecules

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chemical processes involving sulfur compounds. The aim is not to give a textbook account, but rather to present the basic principles briefly, supported by examples drawn from biochemical studies.

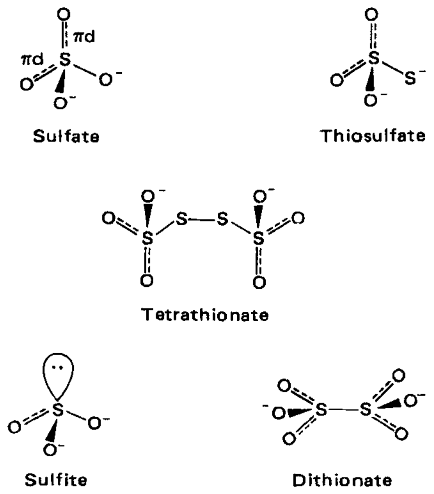
A. Bonding and Stereochemistry of Sulfur Compounds

It will be helpful to start with a discussion of the nature of bonding and stereochemistry of sulfur compounds. An understanding of these aspects is needed to appreciate the biological processes.

The sulfur atom (At. No. 32) with the ground state configuration - $1s^2, 2s^2, 2p^6, 3s^2, 3p^4$ - has vacant 3d orbitals, which permits several possible modes of bonding involving these orbitals. The principal modes of hybridizations and resulting stereochemical features of this atom in various states are summarized in Figure I,[4] wherein electron excitations before hybridization are indicated by arrows, (*e.g.*, 3pz → 3d).

From Figure I, the structures of some inorganic species of biological interest can be derived as shown in Figure II.

The nonequivalence of the two sulfur atoms in thiosulfate has been fully demonstrated and considerable X-ray, spectral, and other physical data are well accommodated by the structures shown.


Sulfate

Thiosulfate

Tetrathionate

Sulfite

Dithionate

FIGURE II

Dotted lines indicate πd bonds

II. INORGANIC SULFUR COMPOUNDS

A. Chemistry of Some Inorganic Sulfur Functions

A variety of reactions of the sulfur-containing functional groups involve cleavages of S–S bonds. Such cleavage can, in principle, occur in a heterolytic manner, (eq 1) or in a homolytic fashion, (2).[5-8] In the former case it can be an electrophilic, (1a) or a nucleophilic, (1b) process, and in the latter the fission can occur by a unimolecular, (2a) or a bimolecular, (2b) mechanism.

$$\mathrm{RS{-}SR} \longrightarrow \mathrm{RS{:}^{-} + \overset{+}{S}R} \qquad (1)$$

$$\mathrm{RS{-}SR + {:}\,\bar{N}u} \longrightarrow \mathrm{RS{:}^{-} + Nu{-}SR} \qquad (1a)$$

$$\mathrm{RS{-}SR + {}^{+}E} \longrightarrow \mathrm{RS^{+} + E{-}SR} \qquad (1b)$$

$$\mathrm{RS{-}SR} \longrightarrow \mathrm{2RS\cdot} \qquad (2)$$

$$\mathrm{RS{-}SR} \xrightarrow{h\nu} \mathrm{2RS\cdot} \qquad (2a)$$

$$\mathrm{RS{-}SR + R'S\cdot} \longrightarrow \mathrm{RS{-}SR' + RS\cdot} \qquad (2b)$$

Most of the important reactions of inorganic sulfur compounds, occurring at moderate temperatures and pH values, are bimolecular nucleophilic displacements, S_N2, and reactions of this type have often been used to account for enzymatic modifications of sulfur compounds. The nucleophilicity of a number of nucleophiles towards S–S bonds has been investigated.[5-7]

Disulfide exchange reactions[9] and biological methylations involving methyl carbonium ions[10] have been associated with electrophilic cleavage of S–S bonds.

Homolytic S–S bond cleavage has biological significance in relation to the effect of radiation on organisms.[11] Possible relations to photosynthetic mechanisms have also been suggested.

1. Elemental Sulfur

The most notable reactions of elemental sulfur involve nucleophilic cleavage of the S_8 ring by nucleophiles, particularly the cleavages with OH^-,[6] (3); ^-CN[12,13] (4); SO_3^{2-},[12,14] (5); ^-SH,[15] (6); and RSH,[16] (7).

$$\mathrm{S_8 + 8\,\bar{O}H} \longrightarrow \mathrm{[\bar{S}{-}S_6{-}S{-}OH]} \longrightarrow$$
$$\mathrm{2\,S_2O_3^{2-} + 4\,\bar{S}H + 2\,H_2O} \qquad (3)$$

$$\mathrm{S_8 + 8{:}\bar{C}N} \longrightarrow \mathrm{[\bar{S}{-}S_5{-}\overset{\bar{:}CN}{S}{-}SCN]} \xrightarrow{\text{etc.}} \mathrm{8\,\bar{S}CN} \qquad (4)$$

$$\mathrm{S_8 + 8\,SO_3^{2-}} \longrightarrow \mathrm{[{}^{-}\overset{SO_3^{2-}}{S}{-}S_6{-}SSO_3^{-}]} \xrightarrow{\text{etc.}} \mathrm{8\,S_2O_3^{2-}} \qquad (5)$$

$$\mathrm{S_8 + \bar{S}H} \longrightarrow \mathrm{S}_n\mathrm{{-}SH} \qquad (6)$$
$$(n = 2, 3 \cdots)$$

$$\mathrm{S_8 + 8\,RSH} \longrightarrow \mathrm{8\,RS{-}SH} \qquad (7)$$

Although alkaline hydrolysis of sulfur requires relatively drastic conditions, the possibility of some hydrolysis of this type under physiological conditions cannot be ruled out. Reactions of cycanide[13] and sulfite[17] with elemental sulfur are very rapid, in organic solvents at room temperature. This is also the case with the hydrosulfide group, where a second-order rate constant of greater than 1000 mol^{-1} sec^{-1} at 25°, has been recorded for the degradation of sulfur by triethylammonium hydrosulfide, in benzene solution.[15] Thiols also react with elemental sulfur, with rates comparable to those with hydrosulfides.[16]

2. Sulfides

The most notable reaction of sulfide, S^{2-}, is its reaction with organic disulfides.[18] Under alkaline conditions, the reaction proceeds with formation of disulfanes, (8), which react further, (9), in presence of excess sulfide.

$$\mathrm{RS{-}SR + S^{2-}} \longrightarrow \mathrm{RS{-}S^{-} + RS^{-}} \qquad (8)$$

$$\mathrm{RS{-}S^{-} + S^{2-}} \longrightarrow \mathrm{RS^{-} + S_2^{2-}} \qquad (9)$$

The overall reaction, (10), is thus a two-electron reduction of the disulfide, forming two moles of mercaptide.

$$\mathrm{RS{-}SR + 2e^{-}} \longrightarrow \mathrm{2RS^{-}} \qquad (10)$$

Under acidic conditions a similar reduction occurs, but only one mole of sulfide is needed per mole of disulfide; in this case, sulfur and not the persulfide is the product of the oxidation, (11).

$$\mathrm{RS{-}SR + S^{2-}} \xrightarrow{H^{+}} \mathrm{2RS^{-} + S} \qquad (11)$$

The reactions can have direct biochemical implication due to the abundance of disulfide compounds, *e.g.*, cystine, in organisms.

3. Sulfites

The important reactions involving sulfite are nucleophilic cleavage of disulfides, (12)[5,19] and the rapid autoxidation of sulfite to sulfate under physiological conditions.[20] The autoxidation is subject to rapid catalysis by traces of metal ions, such as Cu^{++}, Zn^{++}, Mn^{++}, Fe^{++}, and Fe^{+++}.

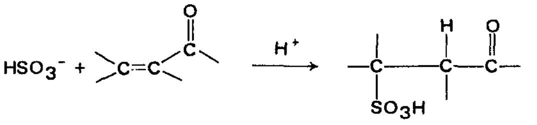
$$\mathrm{RS{-}SR + SO_3^{2-}} \longrightarrow \mathrm{RS{-}SO_3^{-} + RS^{-}} \qquad (12)$$

$$\mathrm{RS{-}SR + HSO_3^{-}} \longrightarrow \mathrm{RS{-}SO_3H + RS^{-}} \qquad (12a)$$

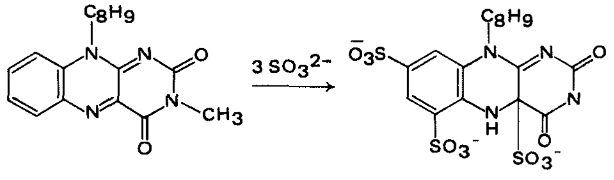
The second-order rate constant, at 25° and pH 6.9, for the reaction between cystine and sulfite, has been recorded to be 400 lit mol^{-1} min^{-1}.[21]

It has been shown currently that substantial amounts of inhaled sulfur dioxide is absorbed into the blood and there converted quantitatively into thiosulfonates, by reaction of bisulfite with the disulfide linkages of proteins and smaller molecules.[22,23]

The nucleophilic addition of sulfites and bisulfites to activated double bonds, *e.g.*, (13), has drawn considerable recent interest.[24-48]


(13)

Addition of sulfite to iso alloxazines, a basic structural unit of flavines and flavoenzymes, was shown to form the adduct 1,[29] (14), contrary to the structure (2), previously suggested.[30-32]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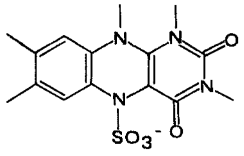
(14)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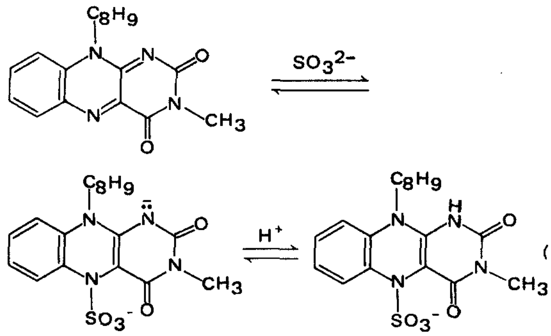
The total kinetic analysis of this reaction indicated a mechanism shown by (15)-(18).[29]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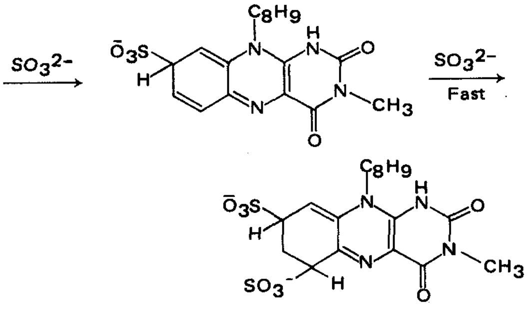
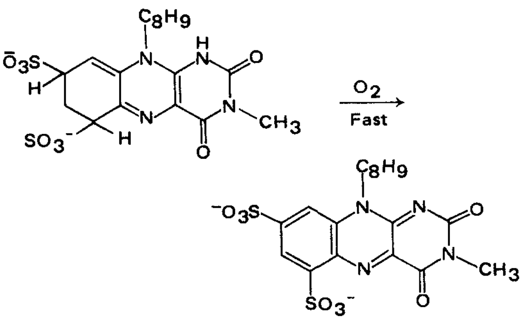
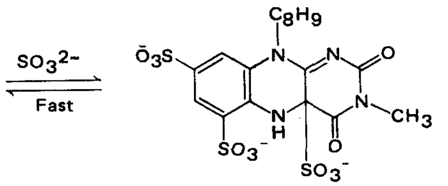
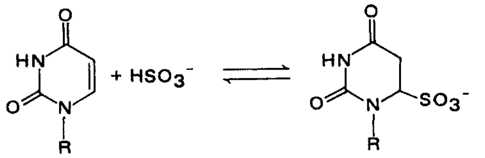
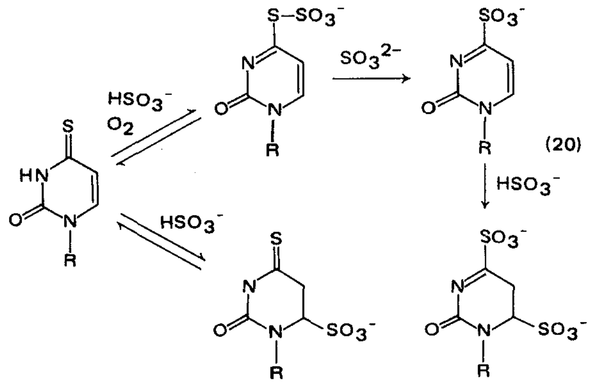

(16)


(17)


(18)

Analogous additions of sulfite and bisulfite have been investigated with uracils, (19),[36-37] and thiouracils, (20),[45] both of which are found in the nucleic acids of *E. coli.*[49]


(19)

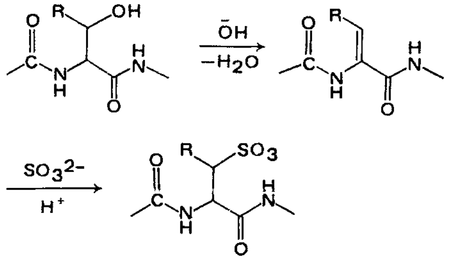

(20)

In the case of uracils it was demonstrated that partial saturation of polyuridylic acid with bisulfite reduced markedly its ability to form a helical complex with polyadenylic acid resulting in the diminished capability of polyuridylic acid to code the incorporation of phenylalanine in the *E. coli* cell-free protein synthesis system. Thus, inactivation of messenger RNA seemed to be one pathway by which bisulfite can inflict biological damage.

In the case of addition to the thiouracil systems, evidence was presented for a free radical mechanism of addition.[45]

Another area of sulfite addition to the activated double bonds is the biological application of such additions in the localization and quantitative determination of α-amino-β-hydroxy acid residues, *e.g.*, serine and threonine, in the polypeptide chains.[28]

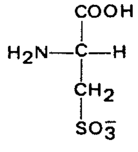
The reaction involves a base-catalyzed β-elimination from the seryl or threonyl residue followed by the nucleophilic addition of the sulfite to the resulting double bond, (21-22).



(21)

(22)

Using $^{35}SO_3^{2-}$ the seryl and threonyl residues can be labelled by radioactive sulfur, and on cleavage of the peptide the serine residue can be recovered as cysteic acid, (**3**), and the threonine residue can be recovered as α-amino-β-sulfonylbutyric acid, (**4**).



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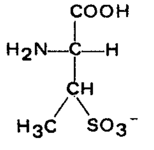


4

As a model for the interaction of sulfur dioxide with the mammalian organism, the interaction of sodium bisulfite with vitamin K_3 and nicotinamide-adenine dinucleotide, has also been currently investigated, and doubt cast on the hypothesis that sulfur dioxide may be a mutogen or carcinogen in mammalian organisms.[46]

4. Thiosulfates

Thiosulfates are stable only around neutral pH. In alkaline solution they degrade into sulfide and sulfate,[59] (23) whereas a complex degradation occurs in acidic media, (24-28),[14, 15, 51] the end product depending on the pH.

$$\bar{S}{-}SO_3^- + \bar{O}H \longrightarrow \bar{S}H + SO_4^{2-} \quad (23)$$

$$\bar{S}{-}SO_3^- + H^+ \longrightarrow H{-}S{-}SO_3^- \quad (24)$$

$$HS{-}SO_3^- + \bar{S}{-}SO_3^- \longrightarrow HS{-}S{-}SO_3^- + SO_3^{2-} \quad (25)$$

$$HS{-}S{-}SO_3^- + \bar{S}{-}SO_3^- \longrightarrow HS{-}S{-}S{-}SO_3^- + SO_3^{2-} \quad (26)$$

And so on

$$HS{-}S_7{-}SO_3^- \longrightarrow S_8 + HSO_3^- \quad (27)$$

$$HS{-}SO_3^- + HS{-}S{-}SO_3^- \longrightarrow HS^- + \bar{O}_3S{-}S{-}S{-}SO_3H \quad (28)$$

The product of degradation could be, therefore, elemental sulfur, sulfite, sulfide, and polythionates. The above mechanism of thiosulfate degradation has been subject to criticism.[52]

A more significant reaction of thiosulfates, from the biological view point, is their ability to oxidize thiols, *e.g.*, cysteine and glutathione, to corresponding disulfides.[53-55] The reaction at pH 7 involves organic thiosulfates as intermediates.[54] (eqs 29-30).

$$RSH + {}^-S{-}SO_3^- \longrightarrow RS{-}SO_3^- + \bar{S}H \quad (29)$$

$$RS{-}SO_3^- + RSH \longrightarrow RS{-}SR + HSO_3^- \quad (30)$$

In acidic media the following mechanism, eqs (31-32) has been proposed for the above oxidation.[55]

$$\bar{S}{-}SO_3^- \longrightarrow S + SO_3^{2-} \quad (31)$$

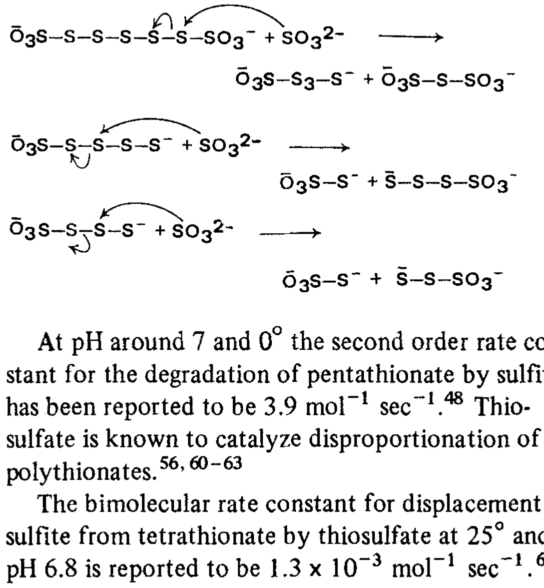
$$S + 2RSH \longrightarrow RS{-}SR + H_2S \quad (32)$$

Thiosulfates, at higher temperatures, *e.g.*, 50-70°, can also cleave disulfides, (eq 33).[54]

$$RS{-}SR + \bar{S}{-}SO_3^- \longrightarrow RS{-}S{-}SO_3^- + RS^- \quad (33)$$

5. Polythionates

Due to the presence of highly labile sulfane monosulfonate groups, $-S_n{-}SO_3$, polythionates are readily attacked by nucleophiles. Excess sulfite converts tetra- and higher polythionates completely into thiosulfate and trithionate,[56-59] *via* the mechanism[14] indicated by (eqs 34-36).



$$\bar{O}_3S{-}S{-}S{-}S{-}S{-}S{-}SO_3^- + SO_3^{2-} \longrightarrow \bar{O}_3S{-}S_3{-}S^- + \bar{O}_3S{-}S{-}SO_3^- \quad (34)$$

$$\bar{O}_3S{-}S{-}S{-}S{-}S^- + SO_3^{2-} \longrightarrow \bar{O}_3S{-}S^- + \bar{S}{-}S{-}S{-}SO_3^- \quad (35)$$

$$\bar{O}_3S{-}S{-}S{-}S^- + \bar{S}O_3^{2-} \longrightarrow \bar{O}_3S{-}S^- + \bar{S}{-}S{-}SO_3^- \quad (36)$$

At pH around 7 and 0° the second order rate constant for the degradation of pentathionate by sulfite has been reported to be 3.9 mol^{-1} sec^{-1}.[48] Thiosulfate is known to catalyze disproportionation of polythionates.[56, 60-63]

The bimolecular rate constant for displacement of sulfite from tetrathionate by thiosulfate at 25° and pH 6.8 is reported to be 1.3×10^{-3} mol^{-1} sec^{-1}.[64]

Although polythionates up to hexathionate are stable in neutral and in moderately acidic solutions,[65] under alkaline conditions they decompose forming various products depending upon the conditions.[50] The reaction involves nucleophilic displacement by the hydroxyl ion,[14, 66] *e.g.*, (37)-(39).

$$H\bar{O} + \bar{O}_3S{-}S{-}S{-}S{-}S{-}SO_3^- \longrightarrow \bar{O}_3S{-}S{-}OH + \bar{S}{-}S_2{-}S\bar{O}_3 \quad (37)$$

$$\bar{S}{-}S{-}S{-}SO_3^- \longrightarrow 2S + \bar{S}{-}SO_3^- \quad (38)$$

$$\bar{S}{-}SO_3^- + HO{-}S{-}SO_3^- \longrightarrow \bar{O}_3S{-}S{-}S{-}SO_3^- + \bar{O}H \quad (39)$$

Sulfide rapidly and quantitatively degrades polythionates to thiosulfate and elemental sulfur.[67]

Thiols such as cysteine and glutathione are spontaneously oxidized by polythionates to corresponding disulfides.[68-68] Tetrathionate is reduced to thiosulfate *via* a sulfodisulfane intermediate,[54] whereas trithionate is reduced to sulfite and thiosulfate.

From the above discussion it is evident that bimolecular nucleophilic cleavages of sulfur compounds (*cf.*, Sec. II. A). The relative nucleophilicities of a few nucleophiles of biological significance are as follows.[5-7]

$$HS^- > RS^- > CN^- > SO_3^{2-} > OH^- > S_2O_3^{2-} > RSO_2S^- > SCN^- > Cl^-$$

III. THIOLS, DISULFIDES, THIOETHERS, AND SULFONIUM COMPOUNDS

Most of the organic metabolic chemistry of sulfur in animals, plants, and microorganisms, centers around transformations involving the compounds of these subgroups. The chemistry of these classes of compounds, therefore, warrants a more detailed treatment.

A. Chemistry of the Thiol Function

1. Physical Properties

The larger atomic volume and the consequent low electronegativity of the sulfur atom are what determine, to a great extent, the reactivity of thiols and disulfides compared to their oxygen counterparts. The S–H bond length of about 1.33 Å compared to 0.96 Å of the O–H bond, is mainly responsible for increased acidity of thiols and the ease of their oxidation to disulfides.[2] Similarly the longer S–S bond (1.8-2.1 Å) in disulfides facilitates the homolytic cleavage to form free radicals and also the environmentally induced polarization of electrons, facilitating the heterolytic cleavage.[2]

The low polarity of S–H bond makes the intermolecular S–H····S bonding virtually non-existent,[70-71] however, there is evidence for S–H····N, S····H–N, S–H····O, and S····H–O bonds in several nonbiological molecules.[72-75] Hydrogen bonding of –SH group to a nearby basic atom in the enzyme alcohol-dehydrogenase has been suggested to account for pH independence of the reactivity of the group.[74]

A possible role of –SH groups in forming van der Waals type electrostatic bonds in protein and related complex molecules has also been postulated.[75-77]

Evidence has recently been reported that sulfhydryl groups play a role in the hydrophobic bonding of proteins to cellular membranes,[78] and that many of the previously observed effects of sulfhydryl binding agents on membranes[79-84] may be related to the nonspecific structural changes in the membrane.

Sulfhydryl groups may also play a role in the association of subunits of complex enzyme molecules[85]

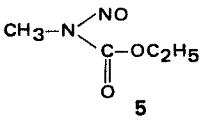
2. Nucleophilic Displacements

Thiols undergo a variety of chemical reactions which are of biological interest. Due to the high nucleophilicity of the sulfur of the –SH group, a majority of the reactions are simply nucleophilic displacements or additions involving the thiol groups as the nucleophile, in such cases the rate would be pH-dependent since the concentration of the more nucleophilic RS⁻ form is pH-dependent.[86-87]

a. Alkylations: The most typical nucleophilic displacement is the alkylation of the –SH group by a suitable alkylating agent, (40).

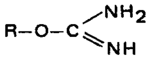
$$RSH + R'{-}X \longrightarrow RSR' + HX \quad (40)$$

X in R'–X can represent a variety of groups such as halide,[86-90] *p*-nitrobenzenesulfonate,[91] and miscellaneous other leaving groups as exemplified by compounds 5[92] and 6[93] below.



$CH_3{-}N(NO){-}C(=O){-}OC_2H_5$

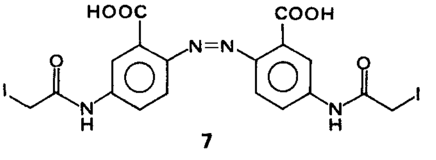
5



$R{-}O{-}C(=NH){-}NH_2$

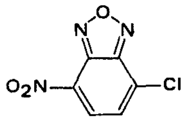
6

R' in R'–X may be an alkyl or aryl group. The bifunctional derivative 7 of iodoacetamide has been used for cross linking the –SH groups in proteins,[94] and the class of compounds represented by 8 has been used as drugs and enzyme inhibitors.[90]

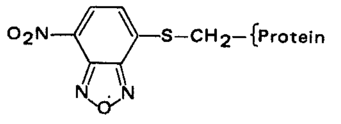


7

Compound 8 reacts specifically with sulfhydryl groups in a protein or enzyme, and the product (9) is fluorescent. This property has been used for the

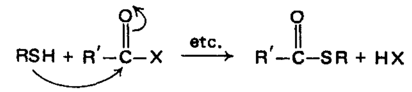

8

fluorescent labeling of sulfhydryl sites in enzymes and proteins for the study of the conformational changes, because the fluorescence is environmentally sensitive.[95]

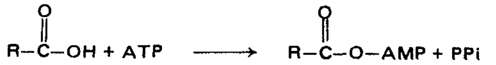

9

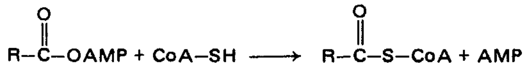
Specificities in alkylation of –SH group have been demonstrated.[96]

b. Acylations: Acylation of thiol groups to yield energy-rich thioesters,[97] (41), is another example of nucleophilic displacement at the carbonyl carbon, *via* a possible addition-elimination mechanism.

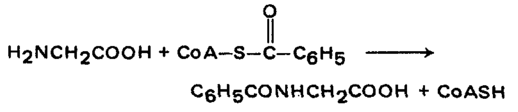

$$\mathrm{RSH} + \mathrm{R'}{-}\overset{\overset{\mathrm{O}}{\|}}{\mathrm{C}}{-}\mathrm{X} \xrightarrow{\text{etc.}} \mathrm{R'}{-}\overset{\overset{\mathrm{O}}{\|}}{\mathrm{C}}{-}\mathrm{SR} + \mathrm{HX} \qquad (41)$$

Thioesters are good acylating agents. A typical example is acyl-coenzyme A, which can be formed from coenzyme A and a carbonylic acid in an enzymatic process supported by ATP as the energy source (42-43).[98]


$$\mathrm{R}{-}\overset{\overset{\mathrm{O}}{\|}}{\mathrm{C}}{-}\mathrm{OH} + \mathrm{ATP} \longrightarrow \mathrm{R}{-}\overset{\overset{\mathrm{O}}{\|}}{\mathrm{C}}{-}\mathrm{O}{-}\mathrm{AMP} + \mathrm{PPi} \qquad (42)$$


$$\mathrm{R}{-}\overset{\overset{\mathrm{O}}{\|}}{\mathrm{C}}{-}\mathrm{OAMP} + \mathrm{CoA}{-}\mathrm{SH} \longrightarrow \mathrm{R}{-}\overset{\overset{\mathrm{O}}{\|}}{\mathrm{C}}{-}\mathrm{S}{-}\mathrm{CoA} + \mathrm{AMP} \qquad (43)$$

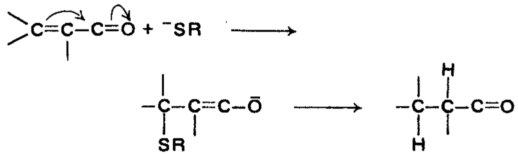
The acyl-coenzyme A reaction is responsible for the acylation of hydroxyl and amino groups in numerous enzyme-catalyzed reactions.[99] Benzoylation of glycine to N-benzoyl glycine by benzoyl-coenzyme A is an example, (eq 44).[100]


$$\mathrm{H_2NCH_2COOH} + \mathrm{CoA}{-}\mathrm{S}{-}\overset{\overset{\mathrm{O}}{\|}}{\mathrm{C}}{-}\mathrm{C_6H_5} \longrightarrow \mathrm{C_6H_5CONHCH_2COOH} + \mathrm{CoASH} \qquad (44)$$

Cyanylation reactions of sulfhydryl group have been recently used for labeling peptides and proteins at –SH sites.[101–103]

3. Nucleophilic Additions

a. Additions to activated olefinic bonds: Nucleophilic additions of thiols can occur across activated carbon-carbon bonds, *e.g.*, groups such as >C=C–C=O, >C=C–C=N–, >C=C–N=O, etc., where the carbon-carbon double bond is activated by conjugation to an electron-withdrawing function thereby rendering the β-carbon atom prone to nucleophilic attack, (45).[104]

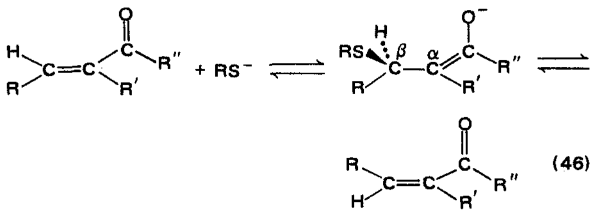

(45)

Additions of thiols to α-β-unsaturated lactones, ketones, aldehydes, and nitriles have been recorded.[88, 105–108]

Examples which might have some biological relevance are: addition of thiol to an indolenine, which may be a model for the addition of thiols to tryptophan in enzymatic reactions; and adducts of glutathione with malic acid,[110] and of cysteine with aconitate and crotonate,[111] which occur naturally.

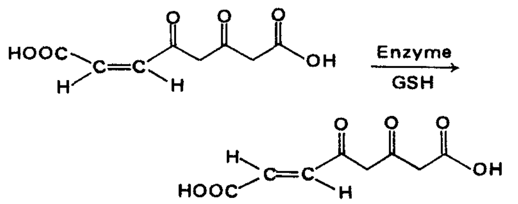
The addition of glutathione to 2-alkylnaphthaquinone, *i.e.*, a vitamin K derivative,[112] might have some biological significance.

Cis-trans isomerization occurs during addition of thiols to pure *cis* or *trans* isomers of olefins with activated double bonds due to rotation about C^{α}–C^{β} bond in the intermediate anion, (46).

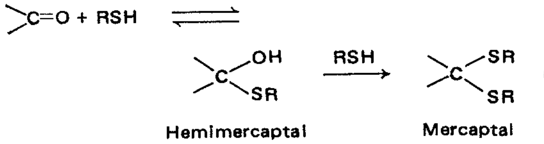

(46)

This reaction has biochemical relevance since some enzyme-catalyzed isomerizations of carbon-carbon double bonds require GSH for activity.[113–114]

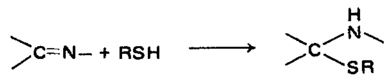
For example, maleyl-acetoacetate is isomerized to fumarylacetoacetate, (47) by the enzyme system, maleyl-acetoacetate-isomerase, in the presence of GSH,[113]


(47)

b. Addition to >C=O and >C=N– groups: Nucleophilic additions to >C=O group, (48) and to >C=N– group, (49), also have some biological relevance.



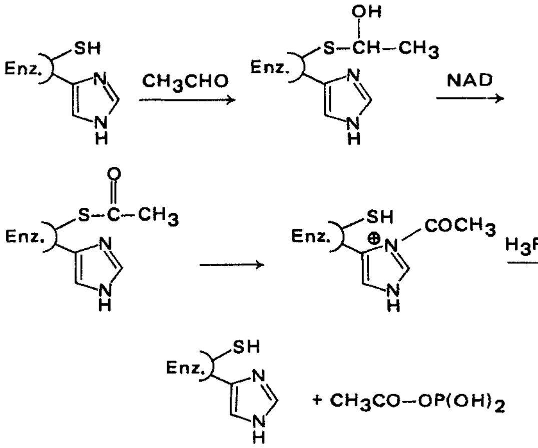
(48)



(49)

Mercaptals cannot usually be formed in aqueous solutions, but hemimercaptals are easily formed and are moderately stable at physiological pH and temperature.

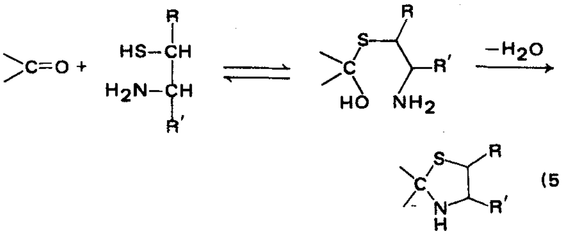
In formation of acetyl phosphate from acetaldehyde, catalyzed by glyceraldehyde phosphate dehydrogenase, indications are that an –SH group is involved in the active site, (50).[115]



(50)

Similarly oxidation of formaldehyde to formic acid is catalyzed by formaldehyde-dehydrogenase,[115-118] which requires GSH as the coenzyme.

Reaction of β-aminothiols with the carbonyl group $>$C=O, presents a biochemically important variation of hemimercaptal formation. The product here is a more stable thiazolidine derivative, (51).[119]



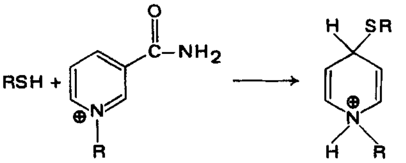
(51)

Thiazolidine

The toxic effect of L-penicillamine has been explained in terms of the inhibition of an important coenzyme, pyridoxal phosphate, presumably *via* formation of a thiazolidine derivative.[120-122]

It is interesting that D-penicillamine is not toxic.[123] This is an example of the well-known stereospecificity in enzymatic processes.

Addition of –SH groups of certain enzymes, across the nicotinamide ring of NAD, *e.g.*, (52),[124] has been postulated[74, 125] to account for their catalytic activity



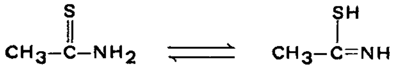
(52)

c. Nucleophilicity of Thiol Group: Before closing the section on the nucleophilic reactions of thiols, a comment on the nucleophilicity of the thiol group seems very appropriate, particularly in light of some current investigations.[126-134] These investigations indicate that the unionized thiol group of simple low molecular weight thiols, *e.g.*, cysteine, is not nucleophilic. However, in enzymes like papain and bromelin, the cysteine thiol group at the active site acquires nucleophilic character under the influence of a suitably placed imadazole group of a histidine residue, probably *via* hydrogen bonding.[128-133] The inhibition of these enzymes by certain aryl disulfides is explained by a mechanism involving an acid catalysis by the carboxylic group of the asparagine residue present in the enzyme. In the thiols where the –SH group is attached to an electron-withdrawing group, *e.g.*, compounds **10-14**, the unionized form is also nucleophilic.[126]

10

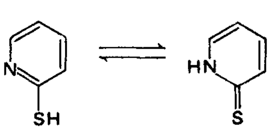
$$H_2N-\overset{\overset{S}{\|}}{C}-NH_2 \rightleftharpoons H_2N-\overset{\overset{SH}{|}}{C}=NH$$

11

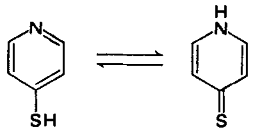


$$CH_3-\overset{\overset{S}{\|}}{C}-NH_2 \rightleftharpoons CH_3-\overset{\overset{SH}{|}}{C}=NH$$

12



13



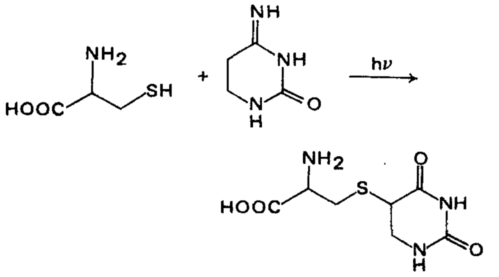
14

L-Ergothionine, which occurs very widely in nature, is another example.

The above observations contribute to the understanding a number of enzymatic phenomena, where the thiol group seems to play the role of nucleophile.[135-142] A number of such reactions have already been mentioned above.

4. Homolytic Displacements and Additions

Alkylation of thiols can proceed, in certain cases, by a free radical process involving RS-radicals. An example is shown by eq 53.[143]



(53)

The specific biological significance of such processes is not clear.

Free radical addition of thiols can occur to conjugated as well as non-conjugated carbon-carbon double bonds;[144-146] a radical initiator such as uv radiation, oxygen, or a peroxide is generally needed.

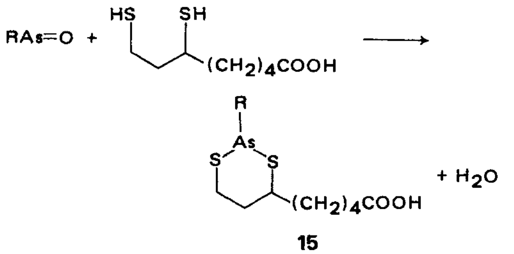
5. Metal binding by Thiols

Thiols form complexes with a variety of biological and nonbiological metal ions.[147-149] The nonbiological ions include mercury, silver, and arsenic which form stable complexes. Biological ions such as copper, iron, molybdenum, cobalt and zinc form relatively less stable complexes.

The most stable complexes are those of mercury, *e.g.*, $(RS)_2Hg$, $(RS)_2Hg_2$ and $(RS)_3Hg_3$.[150] Some diuretic drugs, which are univalent mercurials, *e.g.*, ($R{-}Hg^+$) may act by combining with –SH groups in kidney enzymes [51] and are partly excreted as their cysteine-complexes.

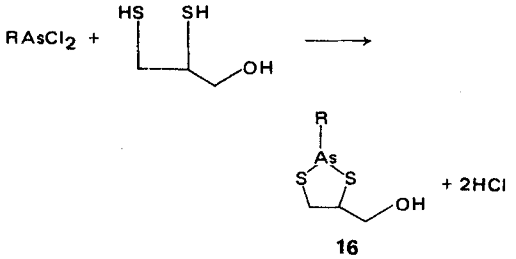
Arsenic poisoning has been long known, arsenicals, which have been extensively used as antiprotozoal agents, have been considered to act by combining with the –SH groups in microorganisms.[152]

Under physiological conditions, stable arsenic complexes are formed only by dithiols, especially by 1:2 and 1:3 dithiols, since more stable 5- and 6-membered chelate rings can be formed from these. Thus, dihydrolipoic acid and 2,3-dimercaptopropanol form stable complexes **15** and **16**, respectively, with RAsO or $RAsCl_2$ compounds, (eqs 54-55).[153-154]



(54)

15



(55)

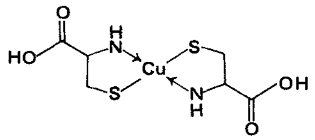
16

As a model for the binding of arsenite (AsO_2^-, $H_2AsO_3^-$) and Cd^{2+} by dithiol enzymes, the complexes of these ions with high molecular weight, N-dihydrolipoyl-aminoethoxy-dextran have been investigated.[155]

The binding constants for cadmium complexes, $(-SH)_2Cd^{2+}$ and $(-SH)_3Cd^{2+}$ were respectively, 2.7×10^{14} M^{-1} sec^{-1} and 7.7×10^{13} M^{-1} sec.$^{-1}$, while arsenite formed a weak complex ($K = 85$ M^{-1} sec^{-1}).

Complexes of monothiols are unstable at pH 7,[155] and can only form in acidic medium.[156,157]

The complexes **15** and **16** mentioned above have biological significance. Several oxidase enzyme systems, responsible for oxidative decarboxylation of α-keto acids,[158,159] need dihydrolipoic acid as a co-factor and are severely inhibited by arsenicals due to inactivation of dihydrolipoic acid by complex formation. Since such oxidative decarboxylations are vital to carbohydrate metabolisms in bacteria and all higher organisms, arsenicals can prove to be fatal to life. 2,3-Dimercaptopropanol, on the other hand, is the most effective antidote known against arsenic poisoning, since it can displace dihydrolipoic acid from the complex **15** to form a more stable 5-membered ring complex.[153-154]



17

Cuprous ion can form stable complexes with thiols;[160] however, cupric complexes can exist only in presence of oxygen since they are otherwise reduced by excess thiols.[161] The –SH groups are possibly responsible for binding Cu to protein in plastocyanine of plant chloroplasts.[162] Involvement of cupric complexes, *e.g.*, **17**, in copper ion catalyzed autoxidation of thiols, has also been suggested.[161]

Current investigations,[163-164] have given definitive evidence in this regard. Thus, reduction of Cu^{2+} is an obligatory step in the ability of thiols to mimic insulin action on fat cell glucose oxidation and lypolsis. The H_2O_2 produced by the reaction of thiols Cu^{2+} and

O_2 (56)–(57) mediates the effect of these reagents on fat cell glucose utilization.

$$2RSH + 2\,Cu^{2+} \longrightarrow RS{-}SR + 2\,Cu^{+} + 2H^{+} \tag{56}$$

$$2\,Cu^{+} + 2H^{+} + O_2 \longrightarrow 2\,Cu^{2+} + H_2O_2 \tag{57}$$

Both ferrous,[165-167] and ferric,[168] ion complex of thiols are known. Ferric ion in hemin chloride can bind a thiol and evidence suggests[169] that the thiol is involved in redox reactions, $Fe^{3+} \rightleftharpoons Fe^{2+}$, as indicated by (58)–(59).

$$\text{Hem-Fe(III)} + RS^{-} \longrightarrow \text{Hem-Fe(II)}{-}SR \tag{58}$$

$$2\,\text{Hem-Fe(II)}{-}SR \longrightarrow 2\,\text{Hem-Fe(II)} + RS{-}SR \tag{59}$$

Cytochrome P450, believed to be involved[170] in electron transport during biological hydroxylations,[171-174] is also suggested to be interacting as above, with an –SH group present in its own apoprotein moiety.[169]

A variety of metalloproteins, such as the respiratory pigment hemoerythrin,[175] ferritin,[176] ferridoxins,[177] adrenodoxin,[178] etc., contain nonhem iron bound through –SH groups. Ferridoxins, due to their role as electron carrier in a variety of redox reactions, have been most widely studied, and differ from other proteins in that, in addition to bound sulfur from cysteine moieties, there is present additional acid labile sulfur believed to be involved in bridges between neighboring iron atoms, (Figure III).

Several model systems representing the possible active sites in iron-sulfur proteins, have been synthesized and their properties investigated.[179-187]

Among other metals that can bound by –SH group are, Zn^{2+};[148,188-189] Co^{2+} and Co^{3+};[188,190-193] Mo;[194-195] Cd;[148,196-197] Mn;[148,197] and Ca and Mg[148,198]

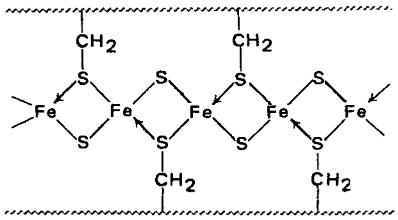


FIGURE III

Models of Co^{+}-enzyme complexes have been recently investigated.[199]

6. Oxidation of Thiols

The most important characteristic of thiols, from biological view point, is the readiness with which they can be oxidized. A variety of oxidation products are possible, with oxidation states of sulfur ranging from –1 to +6, (Figure IV).

Under mild conditions thiols are oxidized chiefly to disulfides which are highly resistant to further oxidation.

Disulfide can be produced from thiols in a number of ways such as: by oxidation with other disulfides, *i.e.*, the so called sulfide exchange reactions; by oxidation with oxygen and peroxides; by photochemical oxidations; by oxidation with biological oxidants, and by oxidation with nonbiological oxidants.

a. Thiol-disulfide redox systems: The thiol-disulfide exchange reactions, (60)–(61), are of great biological interest and several definitive roles of such redox reactions in biological systems are known.[200]

$$RSH + R'S{-}SR' \rightleftharpoons R'S{-}SR + R'SH \tag{60}$$

$$RSH + R'S{-}SR \rightleftharpoons R'SH + RS{-}SR \tag{61}$$

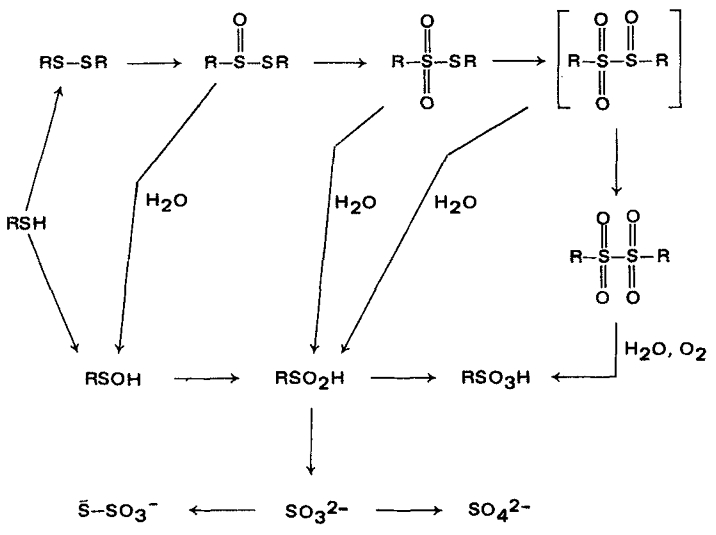


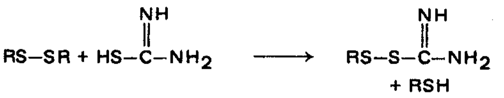
FIGURE IV

A detailed kinetic analysis of the exchange reaction, (62)-(63), between cystine and glutathione (GSH) as a function of pH and temperature has been carried out.[201–204]

$$\text{CyS–SCy + GSH} \rightleftharpoons \text{GS–SCy + CySH} \quad (62)$$

$$\text{GSH + GS–SCy} \rightleftharpoons \text{GS–SG + CySH} \quad (63)$$

Mixed disulfides can be obtained from the equilibrium mixture if an excess of disulfide is initially present.[201] In certain cases, *e.g.*, (64), the mixed disulfide may be the only product[205–206] (see also refs 126-132).

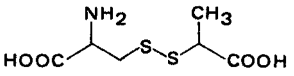


$$\text{RS–SR + HS–}\overset{\overset{\text{NH}}{\|}}{\text{C}}\text{–NH}_2 \longrightarrow \text{RS–S–}\overset{\overset{\text{NH}}{\|}}{\text{C}}\text{–NH}_2 + \text{RSH} \quad (64)$$

Many mixed disulfides, *e.g.*, **18**,[207] and **19**,[208] have a biological origin. The precise mechanism of thiol-disulfide exchange is not yet established.

CoA–S–SG

18



19

b. Autoxidations: Thiols can undergo autoxidations under the catalytic influence of free metals, *e.g.*, Hg, Pt, Au,[209] trace metal ions,[147] or selenites.[210]

The autoxidation under metal catalysis has been extensively investigated,[211] and the following mechanism, (65)-(69), is suggested for Fe^{2+} catalyzed oxidations[212–213] which also fits the kinetic data from other metals, *e.g.*, Cu^{2+}.[161]

$$(\text{RS})_2\text{Fe(II)} + \text{O}_2 \longrightarrow (\text{RS})_2\text{Fe(III)} + \cdot\text{O}_2^- \quad (65)$$

$$\text{RSH} + \cdot\text{O}_2^- \longrightarrow \text{RS}\cdot + \text{HO}_2^- \quad (66)$$

$$(\text{RS})_2\text{Fe(III)} + \text{RSH} \longrightarrow (\text{RS})_2\text{Fe(II)} + \text{RS}\cdot + \text{H}^+ \quad (67)$$

$$\text{RS}\cdot + \text{RS}\cdot \longrightarrow \text{RS–SR} \quad (68)$$

$$\text{overall } 2\text{RSH} + \text{O}_2 \longrightarrow \text{RS–SR} + \text{H}_2\text{O}_2 \quad (69)$$

Formation of peroxides has long been known in such reactions.[214] The hydrogen peroxide formed in certain cases contributes to oxidation of an additional two moles of thiol, (70),[215] or may decompose to oxygen and water.

$$2\text{RSH} + \text{H}_2\text{O}_2 \longrightarrow \text{RS–SR} + 2\text{H}_2\text{O} \quad (70)$$

In vitro oxidation of thiols by hydrogen peroxide also is well known, and the reaction is catalyzed by metal ions such as Cu^{2+} and Fe^{2+}.[216–218]

c. Photochemical Oxidations: The wellknown radioprotective action of thiols in animals[219–221] has been attributed by some workers to the ability of thiols to act as good free-radical scavengers. Thus they can react with $\cdot$OH, $\cdot$OH$_2$ and many organic radicals produced directly or indirectly during radiolysis, (71)-(74), and hence prevent damage to proteins and enzymes.

$$\text{RSH} + \cdot\text{OH} \longrightarrow \text{RS}\cdot + \text{H}_2\text{O} \quad (71)$$

$$\text{RSH} + \cdot\text{OH}_2 \longrightarrow \text{RS}\cdot + \text{H}_2\text{O}_2 \quad (72)$$

$$\text{RSH} + \text{R}'\cdot \longrightarrow \text{RS}\cdot + \text{R}'\text{H} \quad (73)$$

$$2\text{RS}\cdot \longrightarrow \text{RS–SR} \quad (74)$$

However, the objection to this hypothesis is that a very high concentration of the scavenger is needed for such a phenomenon to be effective and due to the toxicity of thiols, their effective concentration, in model systems, reaches only about one tenth of the required level for significant scavenging.[223]

Of the several other theories put forward to explain the radioprotective phenomenon, the biochemical shock hypothesis deserves mention.[223]

The thiol, most notably cystamine, within minutes after its introduction into tissues, is engaged in thiol-disulfide exchange reactions with the –SH and disulfide groups, *e.g.*, proteins in membranes and cells. The reaction triggers a series of physiological disturbances which include decreased carbohydrate metabolism, temporary inhibition of RNA and DNA synthesis causing mitotic delay; cardiovascular troubles, etc. The mitotic delay that results, allows more time for the repair of the radiation damaged nucleic acids.

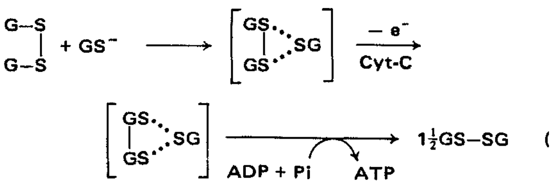
Evidence to support this hypothesis has been presented.[223]

d. Biological Oxidations: Biological oxidants such as flavins,[222] cytochromes,[224–226] and dehydroascorbic acid,[227–230] are known to oxidize thiols to disulfides.

Oxidation of glutathione by cytochrome-C (75) has been extensively investigated. (cf. Sec. IIIA. 1).

$$2\,\text{Cyto-Fe(III)} + 2\text{GSH} \longrightarrow \text{GS–SG} + 2\,\text{Cyto-Fe(II)} + 2\text{H}^+ \quad (75)$$

It is interesting that the above oxidation, when carried out in presence of ADP, inorganic phosphate and oxidized glutathione, G–S–S–G, brings about oxidative phosphorylation, *i.e.*, formation of ATP. The proposed mechanism is indicated in eq 76.[225–226]



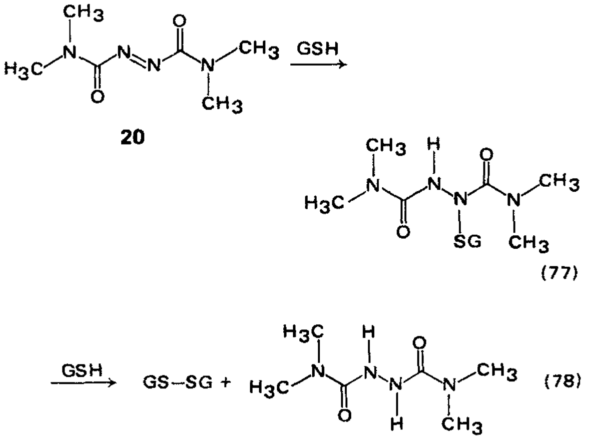
(76)

Hemin is also known to bring about analogous oxidative phosphorylations.[231]

It was shown recently that certain selenium compounds effectively catalyze oxidation of glutathione by cytochrome-C.[232] Current research[233-241] indicates that a relationship might exist between oxidative phosphorylation and the sulfhydryl groups in mitochondrial proteins.

e. Nonbiological oxidations: In addition to those discussed above, a variety of nonbiological oxidants can bring about oxidation of thiols. These include, iodine,[242] redox-dyes,[222,243-248] nitrate esters,[249-250] nitro compounds,[251-252] diazo compounds,[253-256] and sulfoxides.[257-260]

Several thiol-oxidizing agents have been introduced as the agents for perturbing the thiol-disulfide balance within the cells and in other biological preparations.[253,255,261] The diazo compound (**20**), called diamide, oxidizes glutathione preferably compared to other oxidizable compounds like NADH, CoASH and lipoic acid often bound to proteins and enzymes. A two-step reaction (eqs 77-78) was proposed.[255,261]



20

(77)

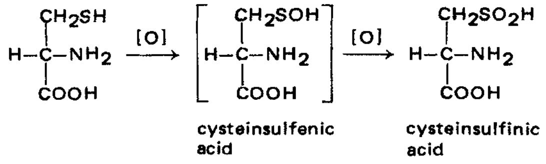
(78)

This claim of specificity, however, has been refuted.[262]

The effect of ozone, one of the photochemical oxidants present in smog, on the sulfhydryl groups present in enzymes has been investigated.[213-265] It was shown that the disintegration of chloroplast ribosomes in bean leaves by ozone involved the sulfhydryl groups.[263]

f. Oxidations Beyond Disulfide Stage: Whereas the reversible oxidation of thiols to disulfides is vital to a variety of biochemical processes (Secs. III.A. 6a-III.A. 6e), the major end product of the metabolism of cysteine in animals, plants and the microorganisms is sulfate.

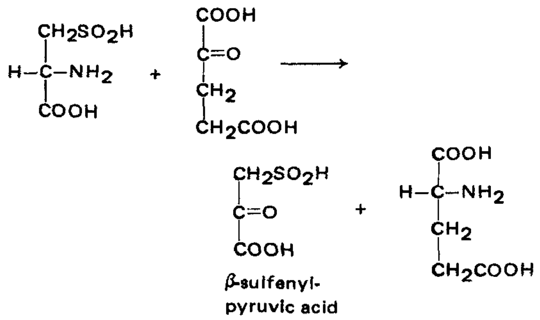
The most accepted pathway of thiol oxidation to sulfate is indicated by (79)-(82).[266-275]



$$\begin{array}{c}CH_2SH\\ |\\ H-C-NH_2\\ |\\ COOH\end{array} \xrightarrow{[O]} \left[\begin{array}{c}CH_2SOH\\ |\\ H-C-NH_2\\ |\\ COOH\end{array}\right] \xrightarrow{[O]} \begin{array}{c}CH_2SO_2H\\ |\\ H-C-NH_2\\ |\\ COOH\end{array} \qquad (79)$$

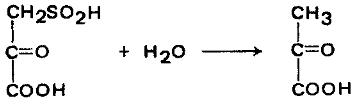
cysteinsulfenic acid

cysteinsulfinic acid



$$\begin{array}{c}CH_2SO_2H\\ |\\ H-C-NH_2\\ |\\ COOH\end{array} + \begin{array}{c}COOH\\ |\\ C=O\\ |\\ CH_2\\ |\\ CH_2COOH\end{array} \longrightarrow \begin{array}{c}CH_2SO_2H\\ |\\ C=O\\ |\\ COOH\end{array} + \begin{array}{c}COOH\\ |\\ H-C-NH_2\\ |\\ CH_2\\ |\\ CH_2COOH\end{array} \qquad (80)$$

β-sulfenyl-pyruvic acid



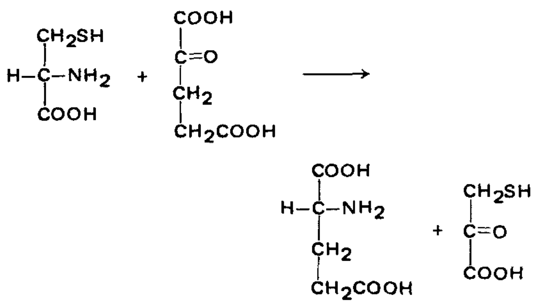
$$\begin{array}{c}CH_2SO_2H\\ |\\ C=O\\ |\\ COOH\end{array} + H_2O \longrightarrow \begin{array}{c}CH_3\\ |\\ C=O\\ |\\ COOH\end{array} + H_2SO_3 \qquad (81)$$

$$H_2SO_3 \xrightarrow{[O]} H_2SO_4 \qquad (82)$$

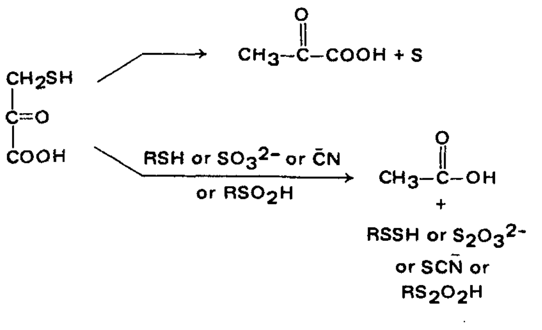
An alternative, but less likely pathway is that the sulfur is removed from cysteine before oxidation. This may occur by the processes indicated by (83)-(85).[276-278]

$$\begin{array}{c}CH_2SH\\ |\\ H-C-NH_2\\ |\\ COOH\end{array} + H_2O \longrightarrow \begin{array}{c}CH_3\\ |\\ C=O\\ |\\ COOH\end{array} + H_2S + NH_3 \qquad (83)$$

or



$$\begin{array}{c}CH_2SH\\ |\\ H-C-NH_2\\ |\\ COOH\end{array} + \begin{array}{c}COOH\\ |\\ C=O\\ |\\ CH_2\\ |\\ CH_2COOH\end{array} \longrightarrow \begin{array}{c}COOH\\ |\\ H-C-NH_2\\ |\\ CH_2\\ |\\ CH_2COOH\end{array} + \begin{array}{c}CH_2SH\\ |\\ C=O\\ |\\ COOH\end{array} \qquad (84)$$



$$\begin{array}{c}CH_2SH\\ |\\ C=O\\ |\\ COOH\end{array} \begin{array}{l}\nearrow CH_3-\overset{O}{\overset{\|}{C}}-COOH + S\\ \\ \xrightarrow[\text{or } RSO_2H]{RSH \text{ or } SO_3^{2-} \text{ or } \bar{C}N} CH_3-\overset{O}{\overset{\|}{C}}-OH + RSSH \text{ or } S_2O_3^{2-} \text{ or } \bar{SCN} \text{ or } RS_2O_2H\end{array}$$

There is evidence that oxidation of thiols may terminate with the formation of sulfonic acids, rather than sulfate. The sulfinic acid intermediate formed as a result of initial oxidation, can be further oxidized to sulfonic acid. This has been established in the case of the oxidation of cysteine, which is shown to be the precursor of the widely occuring natural sulfonic acid, taurine (2-aminoethanesulfonic acid).[279-280]

The transformation of cysteine sulfinic acid to taurine can follow two different pathways,[279-281] and the enzymes responsible for the decarboxylations in the above pathway have been purified and studied.[281-283]

B. Chemistry of Disulfides

As already mentioned in Sec. III.A. 1, the longer S–S bond in disulfides makes the homolytic as well as heterolytic cleavage of this bond relatively easy. Homolysis would result in the formation of free radicals, (86), whereas heterolytic cleavage could result in an electrophilic sulfur species, (87), or a nucleophilic sulfur species, (88), depending upon whether the heterolysis occurs under the effect of an electrophilic agent or a nucleophilic agent.

$$\mathrm{RS{-}SR} \xrightarrow{h\nu} 2\mathrm{RS}\cdot \qquad (86)$$

$$\mathrm{RS{-}SR} + {}^{+}\mathrm{E} \longrightarrow \mathrm{RS}^{+} + \mathrm{E{-}SR} \qquad (87)$$

$$\mathrm{RS{-}SR} + :\bar{\mathrm{N}}\mathrm{u} \longrightarrow \mathrm{R}\bar{\mathrm{S}}: + \mathrm{RS{-}Nu} \qquad (88)$$

1. Homolytic Reactions

Disulfides form free radicals when their aqueous solution is irradiated. The main product from the irradiation of an acidic solution of cystine is cysteine, (89)-(92), along with several minor products from side reactions.[284-286]

$$\mathrm{H{-}OH} \xrightarrow{h\nu} \mathrm{H}\cdot + \cdot\mathrm{OH} \qquad (89)$$

$$\mathrm{H}\cdot + \mathrm{CyS{-}SCy} \longrightarrow \mathrm{CySH} + \mathrm{CyS}\cdot \qquad (90)$$

$$\mathrm{CyS}\cdot + \mathrm{H_2O} \longrightarrow \mathrm{CySH} + \cdot\mathrm{OH} \qquad (91)$$

$$4\cdot\mathrm{OH} \longrightarrow \mathrm{H_2O} + \mathrm{O_2} \qquad (92)$$

Disulfides can also form free radicals under the catalytic influence of Fe^{2+}, (93).[287-288]

$$\mathrm{Fe^{2+}} + \mathrm{RS{-}SR} \longrightarrow \mathrm{RS^{-}} + \mathrm{RS}\cdot + \mathrm{Fe^{3+}} \qquad (93)$$

Aromatic disulfides form free radicals with particular ease due to delocalizability of the unpaired electrons over the aromatic nucleus. They can add to the olefins on irradiation, a property not shown by aliphatic disulfides.[289] Two different aromatic disulfides irradiated together also show some disulfide exchange.[290]

Biological significance of homolytic disulfide cleavage is not known. Although the disulfide groups are abundant in the extracellular proteins in organisms, they make only a minor contribution to the cellular structure. This, at least in part, could be attributed to the vulnerability of these groups to a variety of physical and chemical factors.

2. Electrophilic Reactions

The electrophilic cleavage of disulfides can be brought about under the influence of electrophilic species such as, Ag^+, Hg^{++}, and H^+. The resulting electrophilic sulfur species, RS^+, usually reacts with water to form unstable sulfenic acid which can disproportionate to a sulfinic acid and thiol.

The reaction with Ag^+,[291] is illustrated by the following equations.

$$\mathrm{RS{-}SR} + \mathrm{Ag^+} \longrightarrow \mathrm{RSAg} + \mathrm{RS^+} \qquad (94)$$

$$\mathrm{RS^+} + \mathrm{H_2O} \longrightarrow \mathrm{RSOH} + \mathrm{H^+} \qquad (95)$$

$$2\mathrm{RSOH} \longrightarrow \mathrm{RSO_2H} + \mathrm{RSH} \qquad (96)$$

$$\mathrm{RSH} + \mathrm{Ag^+} \longrightarrow \mathrm{RSAg} + \mathrm{H^+} \qquad (97)$$

overall

$$3\,\mathrm{Ag^+} + 2\mathrm{RS{-}SR} + 2\mathrm{H_2O} \longrightarrow \mathrm{RSO_2H} + 3\mathrm{RSAg} + 3\mathrm{H^+} \qquad (98)$$

Hg^{++} can bring about the cleavage in an analogous manner.

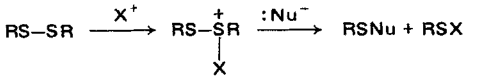
Disulfide exchange can occur when two disulfides are mixed in strongly acidic solution, as follows.[292]

$$\mathrm{RS{-}SR} + \mathrm{H^+} \rightleftharpoons \mathrm{RSH} + \mathrm{RS^+} \qquad (99)$$

$$\mathrm{R'S{-}SR'} + \mathrm{RS^+} \rightleftharpoons \mathrm{RS{-}SR'} + \mathrm{R'S^+} \qquad (100)$$

$$\mathrm{R'S^+} + \mathrm{RS{-}SR} \rightleftharpoons \mathrm{R'S{-}SR} + \mathrm{RS^+} \qquad (101)$$

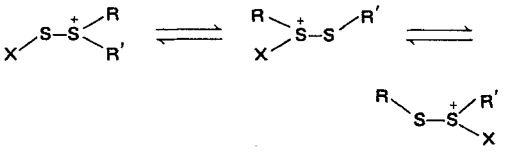
Electrophile-assisted cleavage reactions of S–S bonds (eq 102) are the subject of current investigations.[293-299]



$$\mathrm{RS{-}SR} \xrightarrow{\mathrm{X^+}} \mathrm{RS{-}}\underset{\mathrm{X}}{\overset{+}{\mathrm{S}}}\mathrm{R} \xrightarrow{:\mathrm{Nu^-}} \mathrm{RSNu} + \mathrm{RSX} \qquad (102)$$

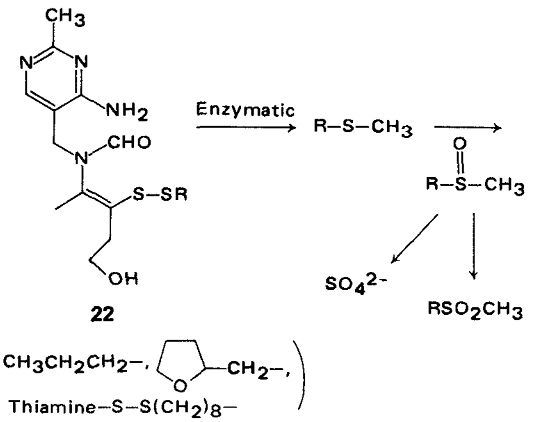
21

Stable sulfonium intermediates of the type **21** are known, and in a recent communication,[299] these were shown to undergo degenerate rearrangement of the type indicated by (103).



(103)

The possibility of an electrophile-assisted cleavage of disulfides in biological systems is an attractive idea to be examined. In fact, recent work on the metabolism of several thiamine alkyl disulfides, 22, in mammals,[300-310] indicated that such a cleavage might be the initial step in the process, resulting in the removal of the alkyl sulfide moiety as an S-methyl derivative which is subsequently metabolized by further oxidation to sulfate, or sulfoxides and sulfones.



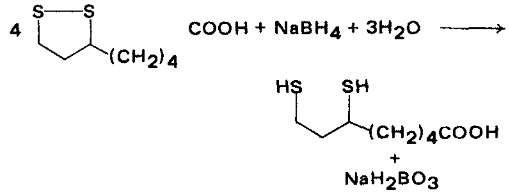
22

3. Nucleophilic Reactions

Nucleophilic cleavage of the disulfide group constitutes by far the most important class of reactions of this group. (cf Sec. II.A.) The biologically significant thiol-disulfide exchange reactions have already been discussed in Sec. III.A. 5, and the cleavage of disulfides by various sulfur nucleophiles has been discussed in Sec. II.A. A brief mention of other important reactions of this category should be made here.

a. Hydride reductions: Hydrides smoothly reduce disulfides to thiols. The examples in eqs 104,[311] and 105,[312] are illustrative.

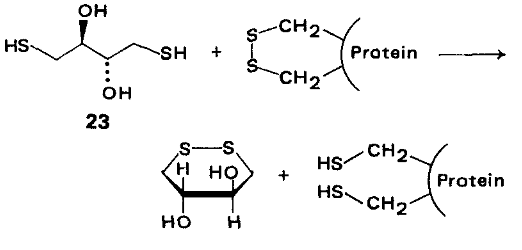
$$RS{-}SR + 2NaH \xrightarrow[2.\ H_2O]{1.\ DMSO} 2RSH + 2NaOH \qquad (104)$$



(105)

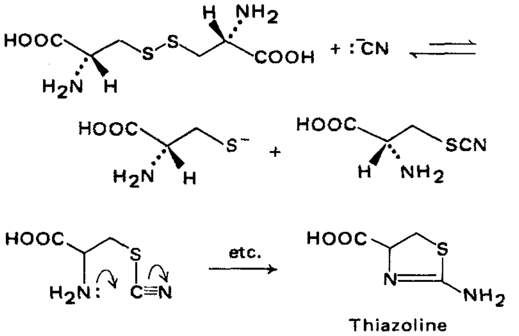
Protein S–S groups have been successfully reduced by $NaBH_4$, however, in certain cases they are quite resistant.[313-314]

A better reagent, recently introduced for the quantitative and rapid reduction of protein disulfide groups, is dithiothreitol (23) in liquid ammonia.[315] The reaction (106) represents another example of the thiol-disulfide exchange phenomenon mentioned earlier. The thiols resulting from disulfide cleavage can be quantitatively alkylated by addition of alkyl halides into the liquid ammonia solution.



(106)

b. Cyanide cleavage: The nucleophilic cleavage of the disulfide group by cyanide is only important with α-amino disulfides, *e.g.*, CyS–SCy, since the initially formed thiocyanate, (107)-(108), is removed from the site by cyclization to a thiazoline, thus shifting the otherwise unfavorable equilibrium[316] to the right.[317]



(107)

(108)

The tendency of this ring closure is so great that the cyanide has been used to cleave certain peptides at the cystine residue.[318]

The rapid antidote action of cystine against cyanide poisoning in rats may also be attributed to analogous reactions.[319-320]

In some peptides such as wool, the thiocyanate intermediate formed as a result of S–S bond cleavage, eliminates thiocyanate and forms a lanthionine, Cy–S–Cy, derivative.[291, 321]

The S–S bond cleavage of thiolsulfate by cyanide (eq 109) is of biological interest.[322-323]

$$\bar{S}{-}SO_3^{-} + \bar{C}N \longrightarrow SCN^{-} + SO_3^{2-} + SO_3^{2-} \qquad (109)$$

c. Miscellaneous nucleophilic cleavages: The other nucleophiles that can cleave the S–S bond, and are of biological interest include arsenites, *e.g.*, (110);[324-325] phosphorothioate, *e.g.*, (111);[326-327] and substituted phosphines, *e.g.*, (112).[328]

$$RS{-}SR + R'AsO + H_2O \longrightarrow R'AsO_2 + 2RSH \qquad (110)$$

$$RS{-}SR + SPO_3^{3-} \longrightarrow RS{-}SPO_3^{2-} + RS^{-} + RS^{-} \qquad (111)$$

$$RS{-}SR + (HOCH_2)_3P \longrightarrow RS{-}\overset{+}{P}(CH_2OH)_3 + R\bar{S} \qquad (112)$$

Inhibition of enzymes by arsenicals might involve a reaction analogous to (110).

C. Chemistry of Thioethers

The abundance of thioethers in nature is indicative of their biological significance. Table I shows some of the biologically important thioethers.

TABLE I

Some Naturally Occuring Thioethers

Compound	Name	Occurrence	Ref.
$CyS{-}CH_2{-}S{-}Cy$	Djencolic Acid	Djenkol Beans	329
$CyS{-}\overset{CH_3}{\underset{CH_3}{C}}{-}CH_2{-}CH_2OH$	Felinine	Cat Urine	330
CySCy	Lanthionine	Wool hydrosylate,	291, 321
		Certain peptide antibiotics	331–332
$CyCH_2SCH_2Cy$	Homolanthionine	E. Coli	333
$CyCH_2{-}S{-}Cy$	Cystathionine	Metabolic *intermediate*	334–335
$CyS{-}CH_3$	S-Methylcysteine	Plants	336–337
$CyS{-}CH_2CH_2CH_3$	S-*n*-Propylcysteine	Plants	336, 337
$CyS{-}CH_2{-}CH{=}CH_2$	S-Allylcysteine	Plants	336, 337
$CyS{-}CH{=}CH{-}CH_3$	S-Propen-1-ylcystein	Plants	336, 337
$CyS{-}CH_2CH_2COOH$	S-2-Carboxyethylcysteine	Plants	336, 337

Interestingly, formation of such thioethers in biology can be correlated with the known *in vitro* chemistry of these compounds.

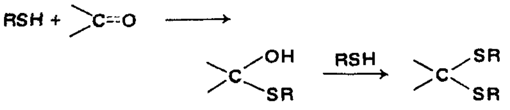
1. Formation of Thioethers

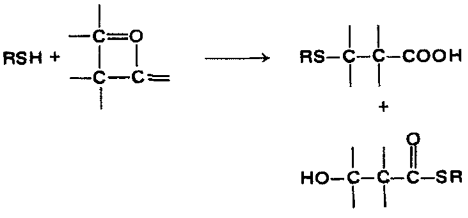
There are several ways thioethers can be prepared in the laboratory.

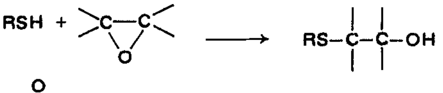
The reactions that have some biochemical analogy are shown below.

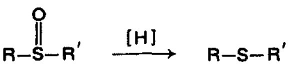
$$RS^- + R'X \longrightarrow R{-}S{-}R' + X^- \qquad (113)$$


(114)


(115)


(116)

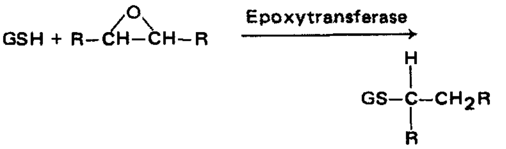

(117)


(118)

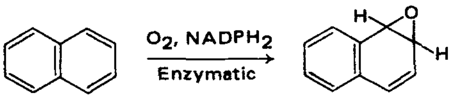
$$R{-}\overset{\overset{O}{\|}}{S}{-}R' \xrightarrow{[H]} R{-}S{-}R'$$

The biological significance of reactions (113)–(115), has been briefly discussed in connection with the reactions of thiols (*cf.* Secs. III.A.2 and III.B.3b).

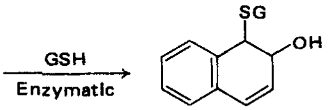
a. Addition of thiols to epoxides: The addition of thiols to epoxides has an interesting biochemical analogy. The enzyme systems S-epoxytransferases from mammalian liver catalyze the addition of thiols to epoxides, (119).^{328}



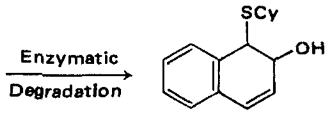
(119)



(120)



(121)



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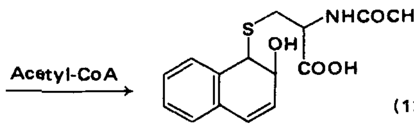
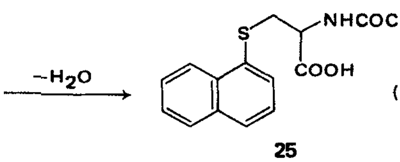


(123)

24



(124)

25

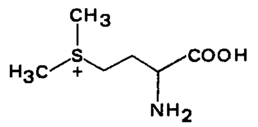
Aromatic hydrocarbons and certain halogenobenzenes, when fed to animals, are excreted as mercapturic acids, *e.g.*, compound **25** from naphthalene.[338–341] The reaction sequence shown by (120)–(124) is believed to be involved.

Compounds of the **24** type have been isolated in certain cases, from the urine.

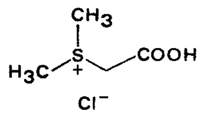
b. Biological alkylations: By far the most important reactions responsible for *in vivo* synthesis of thioethers, seem to be enzymatic alkylations of thiols. A number of methyl sulfonium compounds have been recognized as the methyl donors in biological methylations, occurring in plants, animals and micro-organisms. Methionine, $CH_3S{-}CH_2CH_2CH(NH_2)COOH$, can donate its methyl group to a number of acceptors including certain thiols. The active methyl donor in these reactions is the sulfonium compound, S-adenosylmethionine, SAM, formed from methionine and ATP.[342–343]

Microsomes from various mammalian tissues catalyze the methyl transfer from SAM to thiols like CH_3SH, CH_3CH_2SH, $HS{-}CH_2CH_2CH_2{-}SH$, H_2S and $HS{-}CH_2{-}COOH$; however, biological thiols, *e.g.*, cysteine and glutathione are not methylated[343] (see, however, ref. 344).

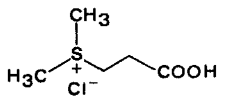
Some other biological sulfonium methyl donors are S-methylmethionine, (**26**);[345] dimethylthetin, (**27**) and dimethyl-β-propiothetin (**28**);[346–347] and trimethylsulfonium salts, **29**.[346–347]



26



27



28

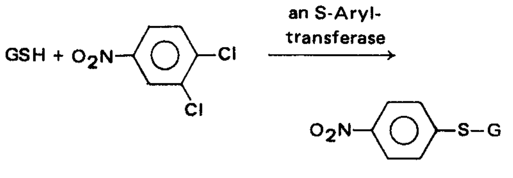
$(CH_3)_3\overset{+}{S}\,X^-$

29

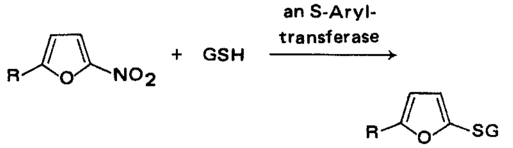
In addition to the formation of mercapturic acids from aromatic hydrocarbons, a variety of other compounds, such as alkyl halides, aryl halides, alcohols, halogenonitro aromatics, esters, nitroalkanes, sulfates, sulfonamides, etc., are also transformed into mercapturates in the animal body.[339–341]

The initial step in all these reactions is believed to be alkylation of glutathione which is further degraded to a mercapturate (*cf.* eqs 120–124). A number of enzyme systems have been isolated from rat liver, which catalyze such alkylations of GSH *in vitro*; (125)–(130) are illustrative.[348–352]

$$GSH + CH_3CH_2CH_2Br \xrightarrow{\text{an S-Alkyl-transferase}} GS{-}CH_2CH_2CH_3 \quad (125)$$



(126)



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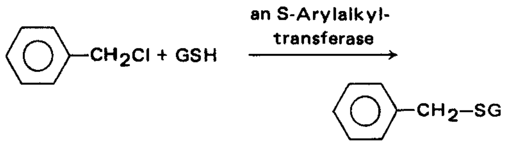


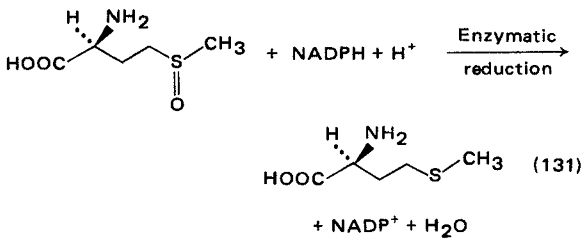
(128)

$$R{-}OSO_3H + GSH \xrightarrow{\text{an S-Alkyl-transferase}} R{-}S{-}G \quad (129)$$

$$R{-}O{-}SO_2NH_2 + GSH \xrightarrow{\text{an S-Alkyl transferase}} R{-}S{-}G \quad (130)$$

Enzymes catalyzing each of the above transformations have been characterized.[348–351]

c. Reduction of sulfoxides: Thioethers are also produced *in vivo* by reduction of sulfoxides.[353] An enzyme system isolated from yeast can cause the reduction of L-methionine-sulfoxide to L-methionine, with NADPH as the hydrogen donor, (131).[354]

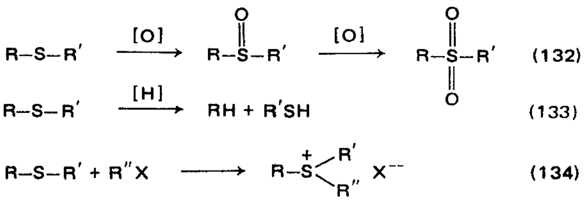


(131)

S-methylcysteine sulfoxide is similarly metabolized in plants and so is dimethyl sulfoxide, DMSO.[353]

2. Reactions of Thioethers

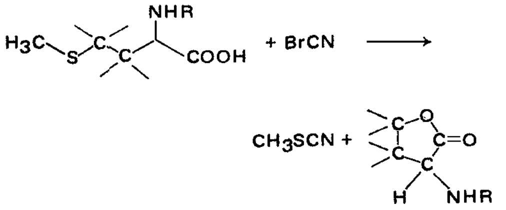
Reactions of thioethers that may have some biochemical significance are indicated by (132)–(136).



$$R{-}S{-}R' \xrightarrow{[O]} R{-}\overset{O}{\overset{\|}{S}}{-}R' \xrightarrow{[O]} R{-}\underset{\underset{O}{\|}}{\overset{\overset{O}{\|}}{S}}{-}R' \quad (132)$$

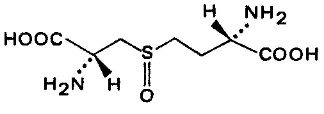
$$R{-}S{-}R' \xrightarrow{[H]} RH + R'SH \quad (133)$$

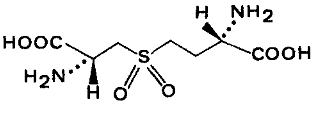
$$R{-}S{-}R' + R''X \longrightarrow R{-}\overset{+}{S}\begin{matrix}R'\\R''\end{matrix}\;X^- \quad (134)$$

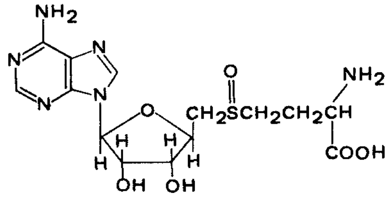

(135)

$$R{-}S{-}R' + H_2O \longrightarrow ROH + RSH \qquad (136)$$

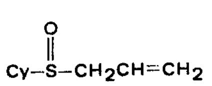
a. Oxidation of thioethers: *In vivo* oxidation of thioethers to sulfoxides can occur in animals and plants. Thus the enzymatic oxidation of S-methylcysteine to the corresponding sulfone can occur in plant tissues.[355] Similarly, compounds **30–32** have been shown to be among the metabolic products of methionine in animals.[356]


30

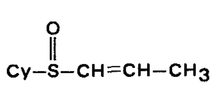

31


32

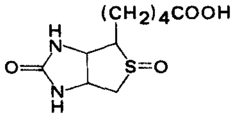
Naturally occuring sulfoxides, such as **33–36**, might also result from the enzymatic oxidation of corresponding sulfides.

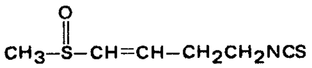

$Cy{-}\overset{O}{\overset{\|}{S}}{-}CH_2CH{=}CH_2$

33


$Cy{-}\overset{O}{\overset{\|}{S}}{-}CH{=}CH{-}CH_3$

34

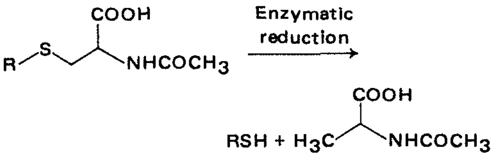

35


$CH_3{-}\overset{O}{\overset{\|}{S}}{-}CH{=}CH{-}CH_2CH_2NCS$

36

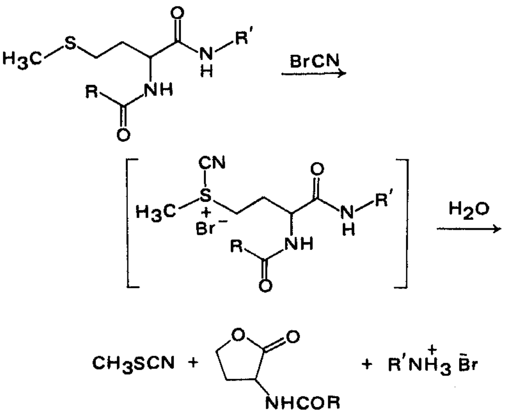
As mentioned previously, thiols are metabolized in certain mammals by initial S-methylation, followed by sulfoxidation of the resulting thioether. The sulfoxides are then further oxidized.[308-310]

b. Reductive cleavage: In biochemical systems not many analogies of the reductive cleavage of thioethers are known. However, there is some evidence that certain mercapturates (Sec. III.C.1) are further metabolized by reductive cleavage forming thiols, *e.g.*, (137).[352] The thiols may undergo further oxidative degradation.


(137)

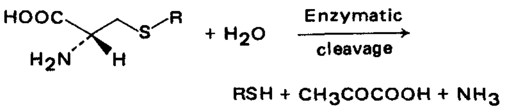
c. Sulfonium salt formation: *In vivo* formation of sulfonium salts from sulfides and their significance as methylating agents has already been discussed in Sec. III.C.1. Some further aspects of this are taken up later in the section covering the sulfonium compounds.

d. Cyanogen bromide cleavage: Cyanogen bromide has been used to cleave proteins and peptides at the methionine residue.[357] The methylthio group is cleaved as methyl isothiocyanate, and the methionine residue, even if bonded to a protein by its carboxylic group, is liberated as a γ-lactone (138).


(138)

Analogous cleavage of sulfonium compounds in biological systems is known to occur (Sec. III.D.).

e. Hydrolytic cleavage: Hydrolysis of a thioether into a thiol and an alcohol does not seem to have a direct analogy in biochemistry. Enzyme systems, called alkylcysteinases have been characterized, which hydrolytically cleave S-alkyl-cysteine into pyruvic acid, ammonia, and the corresponding thiol, (139).[358-360]

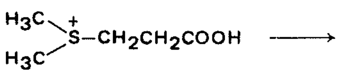

(139)

D. Chemistry of Sulfonium Compounds

The occurrence and formation of the sulfonium salts and their role as the biological alkylating agents has already been briefly discussed in Sec. III.C.1. S-Adenosylmethionine can act as a methyl donor not only to thiols but also to a variety of other types of substrates such as amines, alcohols, and phenols.[267,345,361-369] Biological alkylation of olefinic double bonds by sulfonium alkyl donors is believed to be involved in biosynthesis of steroids and terpenes.[370-387] *In vitro* dealkylation of sulfonium salts, under the influence of biological nucleophiles, has been intensively studied.[388-393]

1. β-Elimination from Sulfonium Salts

A number of S-dialkylthetins are known to break down in algae and fungi to give volatile dialkyl sulfides. In marine algae, for example, dimethyl-β-propiothetin (37) rapidly breaks down to dimethyl sulfide and acrylic acid, (140).[394-395]



37

$$(CH_3)_2S + H_2C{=}CH{-}COOH + H^+ \quad (140)$$

The mechanism of such transformations is not clearly understood.

IV. SULFOXIDES, SULFONES, SULFENATES, SULFINATES, AND SULFONATES

Compounds of this category have often been mentioned in previous sections as intermediates in the metabolic pathways. A brief discussion of the chemistry relevant to their biochemical role follows.

A. Sulfoxides and Sulfones

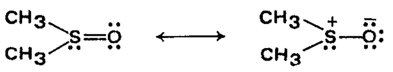
Sulfoxides and sulfones have recently gained in biological and medicinal importance.[397-401] The discovery of the exceptional biological properties of DMSO,[397] has aroused world wide interest in its chemistry. Both sulfoxides and sulfones occur naturally. A few known members of this family are listed in Table II.

The role of methionine sulfoxide in maintaining the redox balance in biological systems has been suggested,[404,411-412] and the possibility of the involvement of sulfide-sulfoxide redox reactions in oxidative phosphorylation has also been indicated.[413]

1. Physical Aspects of Sulfoxides and Sulfones

The sulfoxides are chemically unique in many ways. DMSO, for example, can function as a base,[414] as a weak acid,[415] as a liquid in metal complexes,[416-419] as an electrophile, a nucleophile, an oxidizing agent, and as a reducing agent.[420]

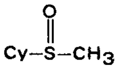
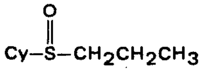
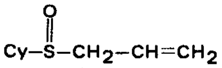
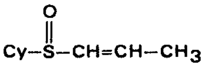
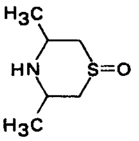
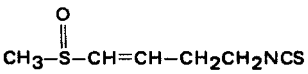
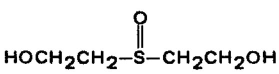
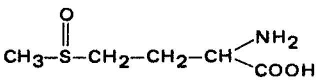
The unique character of sulfoxides is manifested in the structure of the sulfoxide group, which can be regarded as the hybrid of the contributing structures 38a and 38b. (*cf.* Sec. I.A.).



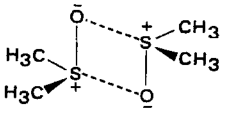
38a 38b

Structure 38b, which seems to make a greater contribution to the total structure of sulfoxide,[421] accounts for most of the observed properties, including the excellent solvent power,[422-423] and a high dipole moment of greater than 4D,[424-425]. Like water, DMSO is highly associated in both the liquid and solid phase. Existence of cyclic dimers such as 39 has

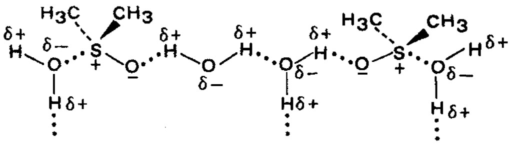
TABLE II
Some Naturally Occurring Sulfoxides and Sulfones

Compound	Occurrence	Ref.
 $Cy{-}S(O){-}CH_3$	Plants	336, 337
 $Cy{-}S(O){-}CH_2CH_2CH_3$	Plants	336, 337
 $Cy{-}S(O){-}CH_2{-}CH{=}CH_2$ (Alliin)	Plants	336, 337
 $Cy{-}S(O){-}CH{=}CH{-}CH_3$	Plants	336, 337
 (Cycloalliin)	Plants	336, 337
 $CH_3{-}S(O){-}CH{=}CH{-}CH_2CH_2NCS$	Radish Seeds	402
 $HOCH_2CH_2{-}S(O){-}CH_2CH_2OH$	Beef-Adrenals	403
 $CH_3{-}S(O){-}CH_2{-}CH_2{-}CH(NH_2)COOH$	Cockroach	404
$CH_3SO_2CH_3$	Animals	405–408
$CH_3SO_2CH_2CH_2CH_2NCS$	Wallflower	409
$CH_3SO_2CH_2CH_2CH_3CH_2NCS$	Wallflower	410

been suggested in benzene solution of DMSO at a certain concentration.[424] The dipole moment is lowest at this concentration. DMSO is miscible with water in all proportions and a 2:1 hydration complex of probable structure **40** has been suggested.[426]



39

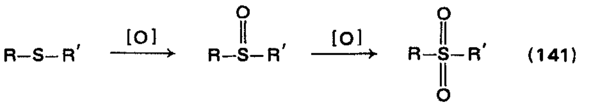


40

The tremendous capacity of DMSO to form hydrogen bonds may be, to a great extent, responsible for its biological activity. DMSO, not only can bind water but can also strongly effect other hydrogen-bonded structures such as proteins, sugars, and nucleic acids. It can effectively alter the configuration of proteins reversibly, due to its ability to replace or bind water, and effect the other hydrogen-bonded structures.[427-430] Conformation of nucleosides is also altered by DMSO.[431] The ability of DMSO to cross dermal barrier rapidly and in high concentration can be attributed to this property.[428-432] Incubation of cells with DMSO can greatly affect the normal physiological functioning of the cells.[433-434] Sulfones are relatively less polar and much less reactive than sulfoxides.

2. Formation of Sulfoxides and Sulfones

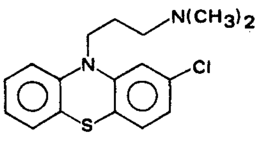
The sulfoxides and sulfones can be prepared in several ways in the laboratory,[435-449] however, the only reaction that has some biological importance is the oxidation of sulfides to sulfoxides and sulfones (141).



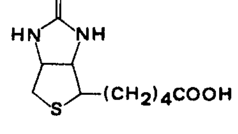
$$R{-}S{-}R' \xrightarrow{[O]} R{-}\overset{\overset{O}{\|}}{S}{-}R' \xrightarrow{[O]} R{-}\underset{\underset{O}{\|}}{\overset{\overset{O}{\|}}{S}}{-}R' \quad (141)$$

A variety of oxidizing agents can be used for the *in vitro* oxidation of sulfides to sulfoxides and sulfones.[435-449] *In vivo* oxidation of sulfides to sulfoxides is known to occur in biological systems.[450-458] Chlorpromazine, (**41**), is metabolized to sulfoxides in mammals;[450] biotin (**42**) has the same fate in bacteria,[453] and enzymatic oxidation of S-methyl cysteine (**43**) to the corresponding sulfoxides occurs in leaves.[454]

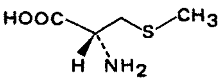
Microsomes of rat liver need NADPH for oxidation of S-*n*-propyl-L-cysteine to the corresponding sulfoxide. [58]



41

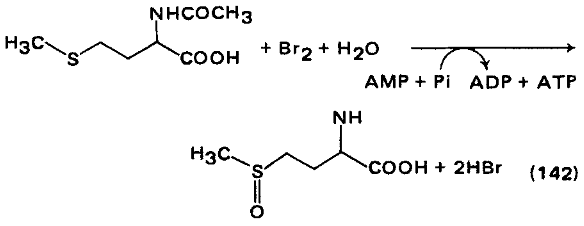


42

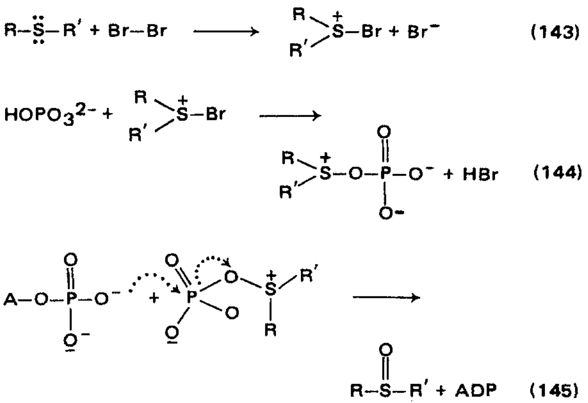


43

In vitro oxidation of sulfides to sulfoxides can be coupled to oxidative phosphorylation.[413] Thus oxidation of N-acetyl-DL-methionine by bromine in anhydrous pyridine, (142), in the presence of AMP and P_i, convert AMP to ATP along with many other phosphorylated products.[413]



Taking into consideration the pronounced catalytic effect of phosphate on oxidation the authors suggest the following mechanism, (143)-(145), for the phosphorylation:

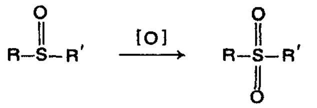


Based on these experiments and on similar observations of others,[459-463] the possibility of such a process in the oxidative phosphorylation in living systems was suggested.

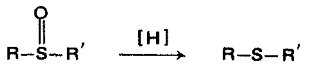
Oxidation of sulfoxides to sulfones in animals has been described.[464]

3. Reactions of Sulfoxides

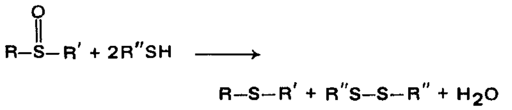
Out of a large number of reactions known for sulfoxides[420-436] the ones that might be of some biochemical interest, are shown by (146)-149).



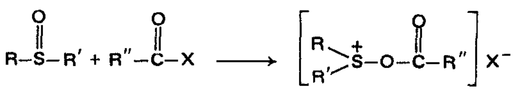
$$R-\overset{O}{\overset{\|}{S}}-R' \xrightarrow{[O]} R-\underset{\underset{O}{\|}}{\overset{\overset{O}{\|}}{S}}-R' \quad (146)$$



$$R-\overset{O}{\overset{\|}{S}}-R' \xrightarrow{[H]} R-S-R' \quad (147)$$



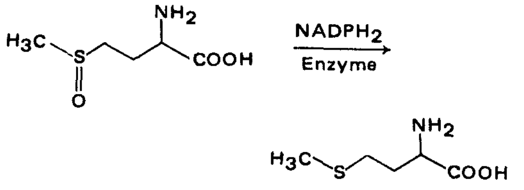
$$R-\overset{O}{\overset{\|}{S}}-R' + 2R''SH \longrightarrow R-S-R' + R''S-S-R'' + H_2O \quad (148)$$



(149)

As already seen, oxidation of sulfoxides into sulfones is known to occur in biological systems. *In vitro*, such oxidations can be brought about by a variety of oxidizing agents such as, permanganate;[465] dichromate;[466] ozone;[467] chlorine in acidic media;[468] and peroxides.[469-470]

Reduction of sulfoxides to sulfides is also known to occur in biological systems. Thus, two enzyme systems could be isolated from yeast, each of which could specifically reduce a stereoisomer of methionine sulfoxide, (150).[354]



(150)

Similarly, d-biotin-d-sulfoxide could be specifically utilized by *E. coli* mutants deficient in biotin biosynthesis.[471]

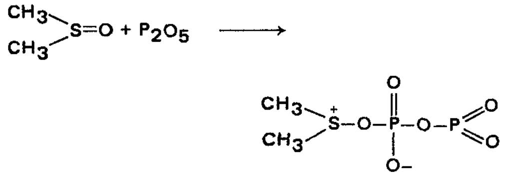
Reduction of sulfoxides by thiols[257-260, 472-473] could be more significant, due to the abundance of thiol groups in proteins and enzymes and might represent one way the sulfoxides can interact in biological systems. Thus, a possible role of methionine sulfoxide in maintaining the redox reactions in biological systems has been suggested. The reaction of sulfoxides represented by (149), might have some significance in relation to their metabolism.[474]

Metabolic studies in animals and man have shown that DMSO can be oxidized as well as reduced *in vivo*.[464, 475-479] Experiments with ^{14}C–DMSO have shown that in acetobacter aerogenes, grown on this compound as the only sulfur source, 80% of the radioactivity appeared as $^{14}CO_2$.[480] There is also evidence that the carbon structure of DMSO is partly metabolized in mammals.[481]

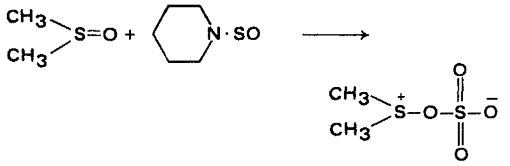
The scheme shown in Figure V has been suggested to explain the possible mode of metabolism of DMSO.

Reactions analogous to (149) have been postulated as the activation step in the metabolism of DMSO.[474]

Besides acylation, several other similar reactions can be used to activate sulfoxides. Two such reactions that could have some biological significance are shown by (150) and (151).[482-483]

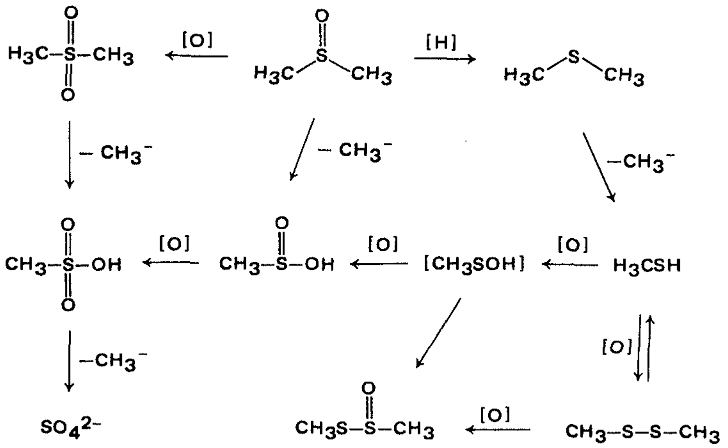


(150)



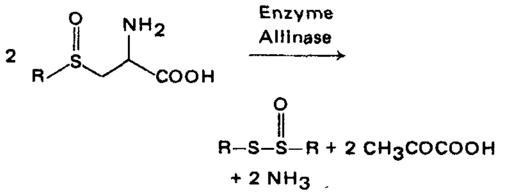
(151)

Another interesting reaction of sulfoxides that is known to occur *in vivo* is shown in (152).[484-486]



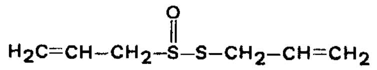
44

FIGURE V



(152)

The best known example of thiosulfinates so formed is allicin (**45**) which is the odoriferous component of garlic.



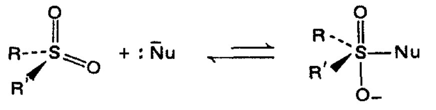
$H_2C{=}CH{-}CH_2{-}\overset{\overset{O}{\|}}{S}{-}S{-}CH_2{-}CH{=}CH_2$

45

Some individuals, who have had DMSO touched to their skin, get an annoying oyster or garlic odor to their breath.[487] It has been suggested that the odor-causing substance could be the methyl analog of allicin, resulting from metabolism of DMSO,[480] (Figure V, compound **44**).

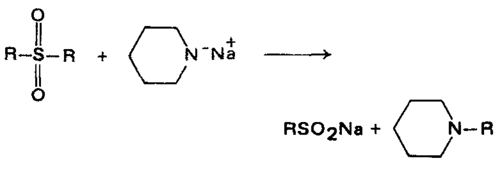
4. Reactions of Sulfones

Sulfones are characterized by their great stability and very low reactivity.[437] The chemical inertness can be attributed to the fact that all the valence electrons of sulfur in sulfones are used up in forming stable bonds and in the resulting tetrahedral sulfone molecule, (*cf.* Sec. I.A.), the sulfur atom is not easily approachable by an attacking nucleophile, which, if added to a S=O double bond, would result in a sterically unfavorable penta-coordinate intermediate, *e.g.*, (153).



(153)

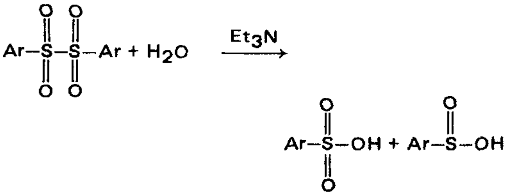
Nucleophilic cleavage of sulfones can, however, occur with highly basic nucleophiles[488] *e.g.*, sodiopiperidine (154), where sulfones are split with loss of an alkyl group to sulfinates.[489]



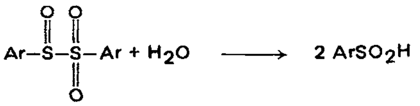
(154)

Analogous splitting of sulfones may be found in the metabolism of sulfones to sulfinic acids as indicated earlier, (see Figure V).

α-Disulfones, (155)[490] and α-sulfinylsulfones (156)[491] are relatively easily cleaved by nucleophiles.

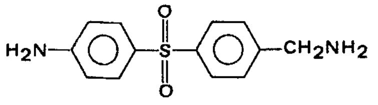


(155)



(156)

The metabolic fate of sulfones has not been established; however, a number of sulfone drugs are known.



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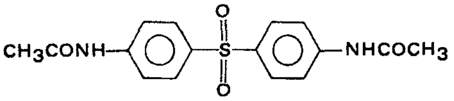

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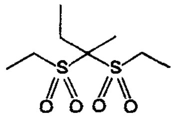

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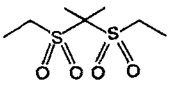

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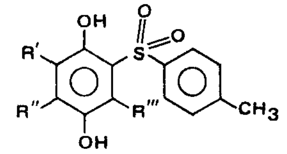


50

For example, compounds **46** and **47** are antimicrobial agents; compounds **48** and **49** are well known hypnotics; compounds of the type **50** were recently shown[492] to have hemostatic and hypolipermic activities in rats. The role of the sulfone group in their biological activity is, however, not known.

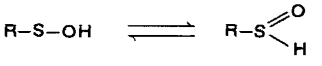
Sulfones are extremely resistant to *in vitro* reduction, and there is no evidence for their biological reduction.

B. Sulfenic, Sulfinic, and Sulfonic Compounds

Sulfenic, sulfinic, and sulfonic acids have already been mentioned as the products of successive oxidation of thiols (*cf.* Sec. III.A.5). *In vivo* presence of sulfinic acids and sulfonic acids has been demonstrated;[266,396,493-494] however, the natural occurrence of sulfenyl compounds has not been definitely established to date. Their involvement, as intermediates, however, has been suggested frequently. The biological interest in the sulfenyl compounds indicated by Kharasch,[495] has been supported by recent findings.

1. Sulfenic Acids and Related Compounds

Sulfenic acids are generally unstable, and only a few are definitely known:[496-500] however, their formation as intermediates has often been postulated.[501-512] The chemistry of sulfenic acid derivatives has been extensively investigated.[506, 513-516] There is evidence that sulfenic acids are tautomeric mixtures of two forms, **51a** and **51b**.[507]



$$R{-}S{-}OH \rightleftharpoons R{-}S(=O){-}H$$

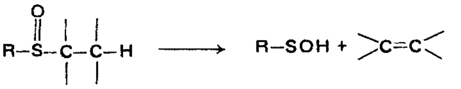
51a **51b**

The principal ways the sulfenic acids can be formed, in stable or unstable form, are indicated by (157)-(160).

$$RSH \xrightarrow{[O]} R{-}SOH \qquad (157)$$

$$RSX + H_2O \longrightarrow R{-}SOH + HX \qquad (158)$$

$$RS{-}SR + {}^{-}OH \longrightarrow R{-}SOH + RS^{-} \qquad (159)$$



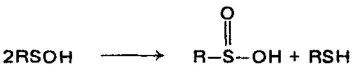
$$R{-}\overset{O}{\overset{\|}{S}}{-}\overset{|}{\underset{|}{C}}{-}\overset{|}{\underset{|}{C}}{-}H \longrightarrow R{-}SOH + {>}C{=}C{<} \qquad (160)$$

Sulfenic acids are also formed in the thermolysis of alkylthiosulfinates.[517]

Alylic sulfoxides are known to rearrange to sulfenate esters (161).[518-520]

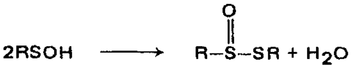
(161)

Sulfenic acids formed as unstable intermediates can disproportionate by one of the several ways shown by (162)-(164).



$$2RSOH \longrightarrow R{-}\overset{O}{\overset{\|}{S}}{-}OH + RSH \qquad (162)$$

Sulfenic Acid



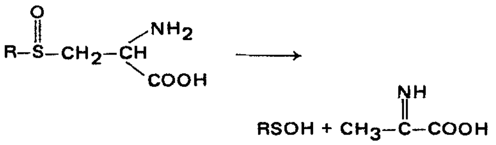
$$2RSOH \longrightarrow R{-}\overset{O}{\overset{\|}{S}}{-}SR + H_2O \qquad (163)$$

Thiosulfinate

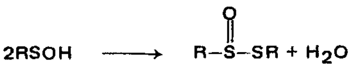
$$3RSOH \longrightarrow RSO_3H + 2RSH \qquad (164)$$

The sulfenate esters can be desulfurized to ethers,[521] and deoxygenated to thioethers.[522]

The transformation of S-alkylcysteine sulfoxides, into thiosulfinates, under the catalytic influence of enzyme allinase, (165)-(166), corresponds, respectively to reactions (160) and (163), indicated above (*cf.* Sec. IV.A.3).



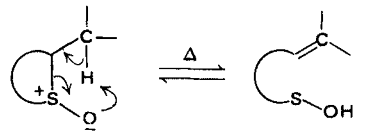
$$R{-}\overset{O}{\overset{\|}{S}}{-}CH_2{-}CH(NH_2)COOH \longrightarrow RSOH + CH_3{-}\overset{NH}{\overset{\|}{C}}{-}COOH \qquad (165)$$



$$2RSOH \longrightarrow R{-}\overset{O}{\overset{\|}{S}}{-}SR + H_2O \qquad (166)$$

The α-imino acid in (165) is further degraded into ammonia and pyruvic acid.

β-Eliminative rearrangements of sulfoxides to sulfenic acids, corresponding to (160), have been reported, *e.g.*, (167).



(167)

Oxidation of thiols by iodine proceeds *via* sulfenyl intermediates (168)-(172),[523-525]

$$RSH + I_2 \longrightarrow RSI + HI \qquad (168)$$

$$RSI + RSH \longrightarrow RSSR + HI \qquad (169)$$

$$RSI + H_2O \longrightarrow RSOH + HI \qquad (170)$$

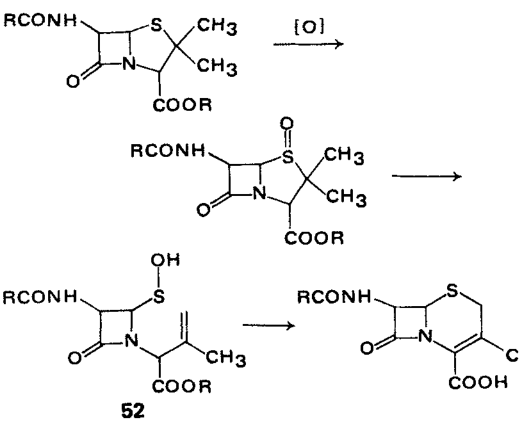
$$2RSOH \longrightarrow RSO_2H + RSH \qquad (171)$$

$$3RSOH \longrightarrow RSO_3H + 2RSH \qquad (172)$$

In the *in vitro* iodination of tyrosine, by H_2O_2 and iodide, catalyzed by several peroxidases,[526-531] the reactive electrophilic species had been suggested to be a sulfenyl iodide,[532-535] or a sulfenyl periodide, RSI_3.[536] Evidence concerning the involvement of a sulfenyl iodide has been presented by several investigators[533, 537-541] and recently confirmed by spectroscopic means.[542] An *in vivo* occurrence of similar mechanisms, however, is not established.

Oxidation of certain thiols by iodine or bromine, in anhydrous pyridine, can be coupled to oxidative phosphorylation of ADP to ATP in the presence of inorganic phosphate.[543]

The sulfenic acid **52** derived from penicillin is of considerable interest. The compound is formed as an intermediate during the transformation of penicillins into cephalosporins (173)[544-550] and has been isolated in pure crystalline form.[499]

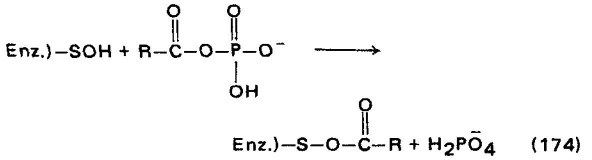


52

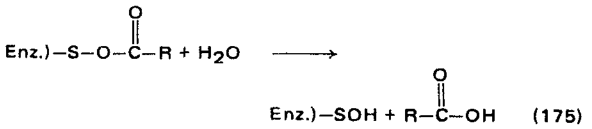
(173)

The question of the involvement of the common precursors in the biosynthesis of penicillins and cephalosporins has been of considerable current interest.[544–556] Whether a sulfenium compound such as **52** can possibly be involved *in vivo* is of interest. It may be noted that formation of **52** from the sulfoxide to the reaction in (160).

Evidence has been recently presented, that sulfenic acids are involved in the active site of certain enzyme systems.[557–563] For example, the oxidation of the sulfhydryl group at the active site of glyceraldehyde-3-phosphate-dehydrogenase in presence of ADP, to a sulfenic group, converts the enzyme into an acyl phosphatase.[557–558] The sulfenic acid moiety of the enzyme is directly involved in the hydrolysis of acyl phosphates, (174)-(175).[558–559]



$$\text{Enz.)–SOH} + \text{R–}\overset{\text{O}}{\overset{\|}{\text{C}}}\text{–O–}\overset{\text{O}}{\overset{\|}{\text{P}}}\underset{\text{OH}}{\text{}}\text{–O}^- \longrightarrow \text{Enz.)–S–O–}\overset{\text{O}}{\overset{\|}{\text{C}}}\text{–R} + H_2PO_4^- \quad (174)$$

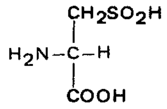


$$\text{Enz.)–S–O–}\overset{\text{O}}{\overset{\|}{\text{C}}}\text{–R} + H_2O \longrightarrow \text{Enz.)–SOH} + \text{R–}\overset{\text{O}}{\overset{\|}{\text{C}}}\text{–OH} \quad (175)$$

Nucleophilic species such as cyanamide, thiosulfate, thiols, semicarbazide, bisulfite, benzylamine, etc., which can interact with the sulfenic acids, deactivate the acyl-phosphatase activity of oxidized GDP.[558–560, 564]

2. Sulfinic Acids and Related Compounds

Sulfinic acids and their derivatives are relatively stable, and their *in vitro* chemistry has been very extensively investigated.[515, 565–568] Many sulfinic acids occur naturally, the most notable being hypotaunine, (**53**),[569] and alanine-3-sulfinic acid, (**54**)[570] both present in animals.



$$H_2N\text{–}\underset{\text{COOH}}{\overset{CH_2SO_2H}{C}}\text{–H}$$

54

$H_2N\text{–}CH_2CH_2SO_2H$

53

CH_3SO_2H

55

Methanesulfinic acid (**55**) has recently been identified in cauliflower.[571] Sulfinic acids can be produced *in vitro* by a variety of methods, however, the reactions that can have some biochemical significance, are shown by (176)-(183).[572–589]

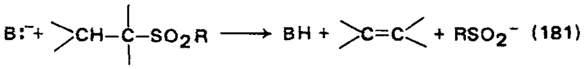
$$RSH + O_2 \longrightarrow RSO_2H \quad (176)$$

$$2RS\text{–}SR + 3OH^- \longrightarrow 3RS^- + RSO_2H + H_2O \quad (177)$$

$$2RS\text{–}SR + 3Ag^+ + 3OH^- \longrightarrow 3RSAg + RSO_2H + H_2O \quad (178)$$

$$RSO_2SR' + R''S^- \longrightarrow R'S\text{–}SR'' + RSO_2^- \quad (179)$$

$$RSO_2SR' + {}^-CN \longrightarrow R'SCN + RSO_2^- \quad (180)$$



$$B\!:^- + \gt CH\text{–}\overset{|}{\underset{|}{C}}\text{–}SO_2R \longrightarrow BH + \gt C{=}C\lt + RSO_2^- \quad (181)$$

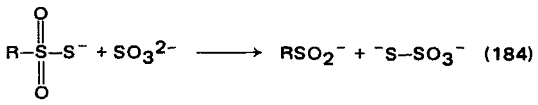
$$3RSOSR + H_2O \longrightarrow RSO_2H + 2RS\text{–}SR \quad (182)$$

$$R\text{–}M + SO_2 \longrightarrow RSO_2^-M^+ \quad (183)$$

Cysteamine is oxidized to hypotaurine under the catalytic influence of a widely distributed enzyme cystamine-oxygenase.[573–575]

Similar *in vivo* oxidation of cysteine to cysteine-sulfinic acid has been characterized.[590–592]

The nucleophilic S–S bond cleavage of thiosulfonates, (179)-(180), could have some analogy in biology. (*cf.* Sec. II.C). The cleavage of ethanethiosulfonate or toluenethiosulfonate (184) by sulfite is for example, catalyzed by the enzyme rhodanese from mammalian liver.[593]

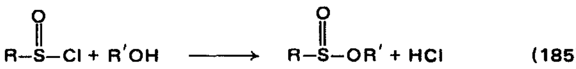


$$R\text{–}\underset{\underset{O}{\|}}{\overset{\overset{O}{\|}}{S}}\text{–}S^- + SO_3^{2-} \longrightarrow RSO_2^- + {}^-S\text{–}SO_3^- \quad (184)$$

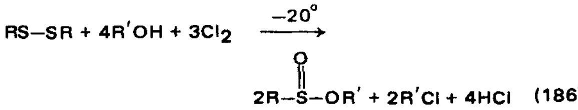
$(R = C_2H_5,\ p\text{-}CH_3\text{–}C_6H_4\text{–})$

Eliminative decomposition of sulfones (181) could be important in metabolism of sulfones, (*cf.* Sec. IV.A.4). Finally the interaction between SO_2 and C-metal bonds could be one reason for the strong toxic action of sulfur dioxide in man and other animals.

Sulfinic esters are prepared by direct interaction between alcohols and sulfenyl chlorides[594–595] (185), or by chlorination of a mixture of a disulfide and alcohol at low temperatures[596] (186).



$$R\text{–}\overset{\overset{O}{\|}}{S}\text{–}Cl + R'OH \longrightarrow R\text{–}\overset{\overset{O}{\|}}{S}\text{–}OR' + HCl \quad (185)$$



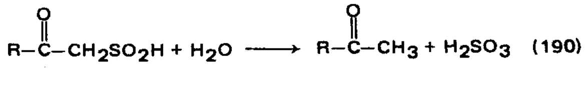
$$RS\text{–}SR + 4R'OH + 3Cl_2 \xrightarrow{-20^\circ} 2R\text{–}\overset{\overset{O}{\|}}{S}\text{–}OR' + 2R'Cl + 4HCl \quad (186)$$

Out of the numerous known reactions of sulfinic acids, those having some biological relevance are shown by (187)-(190).

$$RSO_2H \xrightarrow{[O]} RSO_3H \quad (187)$$

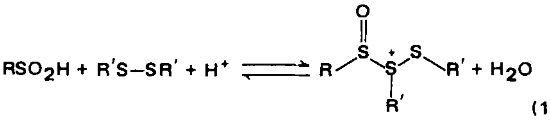
$$4RSO_2H + R'S\text{–}SR' \longrightarrow 3RSO_2SR' + RS\text{–}SR' + 2H_2O \quad (188)$$

$$RSO_2H \longrightarrow RH + SO_2 \quad (189)$$



$$R\text{–}\overset{\overset{O}{\|}}{C}\text{–}CH_2SO_2H + H_2O \longrightarrow R\text{–}\overset{\overset{O}{\|}}{C}\text{–}CH_3 + H_2SO_3 \quad (190)$$

Sulfinic acids are known to be involved as intermediates in the *in vivo* oxidation of thiols, *e.g.*, cysteine, to sulfonic acids.[99, 260, 493] Reaction of sulfinates with disulfides (188)[597–598] is of interest since disulfide groups often constitute the active sites in enzymes. The mechanism proposed for the reaction involves a sulfenium ion intermediate (191)–(196).



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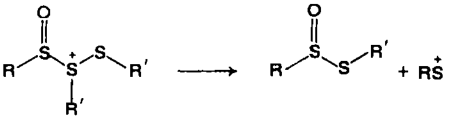

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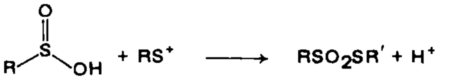

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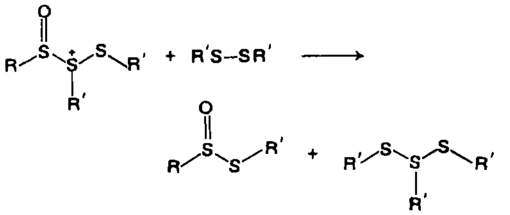

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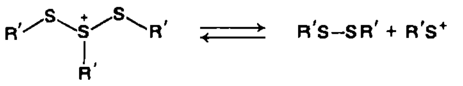

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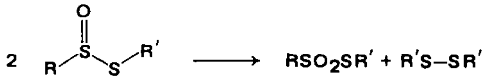
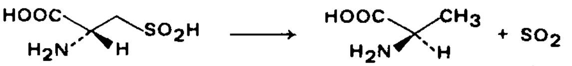


(196)

Desulfination of sulfenic acids is a fairly general reaction and occurs more readily with compounds where R in RSO_2H is an electron-withdrawing group, *i.e.*, can stabilize a negative charge.[599–600]

Alanine-3-sulfinic acid was shown to undergo enzymatic desulfination on incubation with rabbit liver slices under anaerobic conditions (197).[601]



(197)

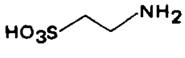
The acid however, was, decarbonylated *in vivo.*[602]

Desulfination occurs spontaneously with β-keto or similarly activated sulfinic acids (190). For example, β-sulfinylpyruvic acid, a key intermediate in the oxidation of cysteine, is spontaneously hydrolyzed with the cleavage of sulfite.

Thiosulfinates, which already have been mentioned in the preceding sections, constitute an important group of naturally occuring derivatives of sulfinic acid (*cf.* Sec. IV.A.3).

3. Sulfonic Acids

Sulfonic acids occur naturally; examples are taurine (56),[272] isothionic acid (57), found in animal tissues,[280, 603] and methane sulfonic acid (58), found in plants.[604]



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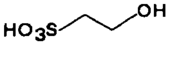
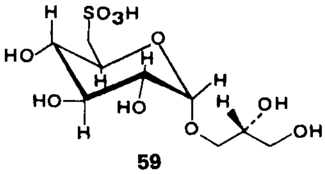


57

CH_3SO_3H

58



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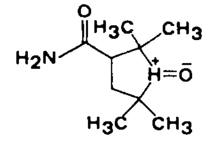


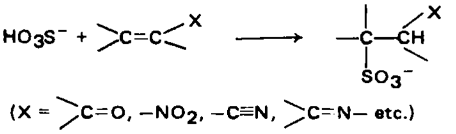
60

The sugar sulfonic acid (59) is a deacylation product of the plant sulfolipid[605–606] and occurs free in plants. The plant sulfolipid is widely distributed in plants and photosynthetic algae.

Sulfonic acids can be prepared *in vitro* by oxidation of thiols or disulfides with strong oxidizing agents, such as, performic acid,[607–608] DMSO in hot 6 *N* HCl,[609] and certain nitroxides, *e.g.*, 60.[610] In addition, sulfonic acids are also formed by oxidation of sulfinic acids and disproportionation of sulfenic acids, (164) (see also ref 611).

In vivo sulfonic acids are believed to be formed as the end products of the oxidation of thiols with intermediate formation of sulfenic and sulfinic acids, (*cf.* Sec. III.A.6f).

Formation of sulfonates as a result of conjugate addition of sulfite to an olefinic bond, (198) has been previously mentioned, and the biological importance of such reactions was pointed out.



(198)

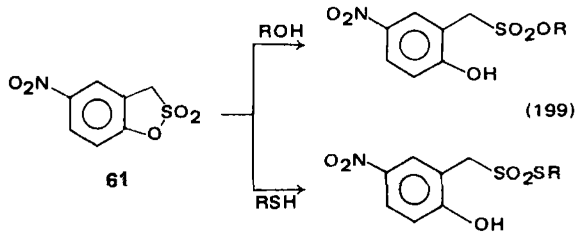
The biological role of sulfonic acids is not known. There is evidence that the sulfonic acid group in the compound 59 is derived from sulfate[612] and also degraded to sulfate[613] in biological systems.

Sulfoacetate, sulfolactate, and sulfoacetaldehyde have been recently identified as products of metabolism of 59 in plants.[614–615]

Alkylbenzenesulfonates are a group of compounds present in common domestic and industrial detergents. These compounds are biodegradable, and their metabolism by bacteria has been studied.[616–619]

A recent study[619] with certain bacteria indicated that the sulfonate group initially is cleaved as sulfite, which is subsequently oxidized to sulfate and reduced to sulfide.

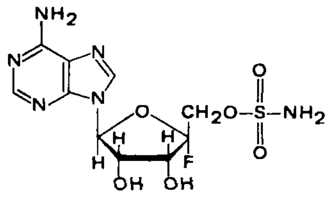
Cyclic sulfonate esters, or sulfones, *e.g.*, 61, have evoked some current biological interest[620–621] due to their high reactivity towards thiols and alcohols (199).



61 (199)

Sulfone **61** was used to modify the active sites of the enzymes α-chymotrypsin[620] and papain.[621]

A variety of sulfonic acid derivatives have been studied for their biological activity. Sulfonamide drugs are too well known to be discussed here. Considering the very high toxicity of the nucleoside antibiotic, **62**,[622–623] the biochemical properties of analogous nucleosides, **63**, have been investigated.[624–626]



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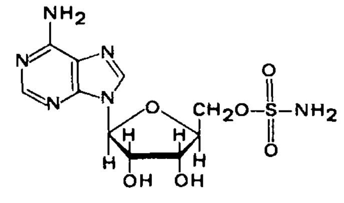


63

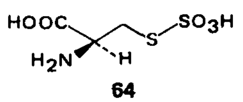
V. THIOSULFATES, THIOSULFINATES, AND THIOSULFONATES

Thiosulfates, thiosulfinates, and thiosulfonates all have a common characteristic in having a S–S bond with one of the sulfur atoms in a higher oxidation state, –SO– or $-SO_2-$. Thiosulfates, however, contain the most labile S–S bond since a strongly electronegative group, $-SO_3H$, is bonded to a sulfenyl sulfur.

Thiosulfinates and thiosulfonates have already been mentioned in connection with the biochemistry of sulfoxides (Sec. IV.A.3) and sulfinic acids, (Sec. IV.B.2) respectively. This section is devoted mainly to organic thiosulfates.

A. Organic Thiosulfates

The two most common thiosulfate esters occuring in nature are S-sulfocysteine, (**64**), and S-sulfoglutathione, (**65**).[627–630] The inorganic sulfate is shown to be the precursor of the S–SO_3 group in these compounds.



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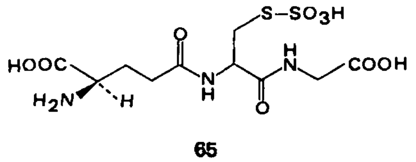


65

S-Sulfocysteine is suggested to be the key intermediate in the biosynthesis of cysteine from thiosulfate and serine.[631–634]

1. Formation of Thiosulfates

Of the various methods known for the *in vitro* synthesis of thiosulfate esters,[635–637] those indicated by (200)–(205) are of some biochemical interest.

$$RS{-}SR + SO_3^{2-} \longrightarrow RS{-}SO_3^- + RS^- \qquad (200)$$

$$RS{-}SR + HSO_3^- \longrightarrow RS{-}SO_3^- + RSH \qquad (201)$$

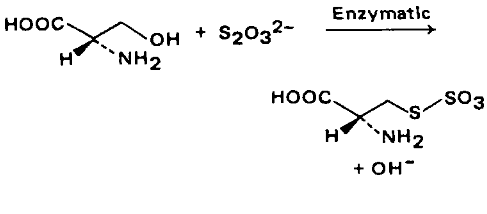
$$RS{-}SH + SO_3^{2-} \longrightarrow RS{-}SO_3^- + SH^- \qquad (202)$$

$$RSH + SO_2 \longrightarrow RS{-}SO_3H \qquad (203)$$

$$RSH + ClSO_3H \longrightarrow RS{-}SO_3H + HCl \qquad (204)$$

$$RSH + S_4O_6^{2-} \xrightarrow{O_2} RS{-}SO_3H + SO_2 + S_2O_3^{2-} \qquad (205)$$

The thiosulfate esters of the type R–S–SO_3^- are called Bunte salts. Numerous examples of the preparation of organic thiosulfates from halides are available.[638–640] The *in vivo* formation of S-sulfocysteine from thiosulfate and serine (206),[631–634, 641] represents an analogous reaction.



(206)

It is quite possible that the –OH group is activated by the enzyme converting it into a better leaving group, *e.g.*, –OCOR, and then the displacement by thiosulfate occurs. Such activation of serine is known to occur in the biosynthesis of cysteine from serine and sulfide.[642–643]

Cleavage of disulfides by sulfite is one of the main methods for producing thiosulfate *in vitro.*[644–650]

The reaction is favored by the presence of metal ions like Hg^{++} or Ag^+ which can remove the RS^- ion by complexing with the latter, and is pH dependent.[651] The reaction can also be promoted by concurrent oxidation of the resulting thiol by peroxide[652] or by cupric ion,[653] and thus two moles of thiosulfate are produced per mole of the disulfide, *e.g.*, (207)-(209).

$$RS{-}SR + SO_3^{2-} \longrightarrow RS^- + RS{-}SO_3^- \qquad (207)$$

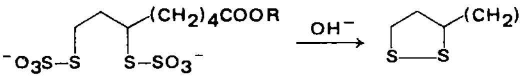
$$RS^- + SO_3^{2-} + H_2O_2 \longrightarrow RS{-}SO_3^- + 2OH^- \qquad (208)$$

$$RS^- + SO_3^{2-} + 2Cu^{2+} \longrightarrow RS{-}SO_3^- + 2Cu^+ \qquad (209)$$

Addition of catalytic amounts of cysteine in the presence of oxygen has been used for complete sulfitolysis of disulfide groups in proteins.[654]

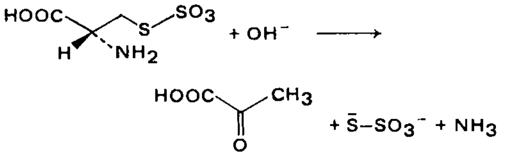
Reaction of thiols with tetrathionate normally gives a mixture of products arising from various S–S bond cleavages, *e.g.*, (210)-(212).[655]

Bifunction thiosulfates, with S-sulfo groups suitably placed with respect to each other, have been used to prepare 5 and 6-membered cyclic disulfides,[667,668] e.g. lipoic acid in (218).



(218)

S-sulfocysteine degrades, however, in alkali in the presence of traces of cupric chloride to give equimolar quantities of thiosulfate, pyruvic acid, and ammonia (219).[669]



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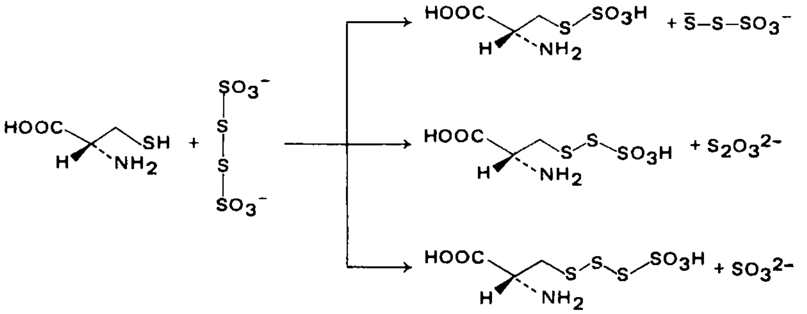


(210)

(211)

(212)

However, it was shown recently that in presence of oxygen the S-sulfothiols are formed quantitatively.[656]

2. Reactions of Thiosulfates

Reaction of organic thiosulfates have been subjected to intensive investigation.[657] Equations 213-216 indicate the reactions that could have some biochemical significance.

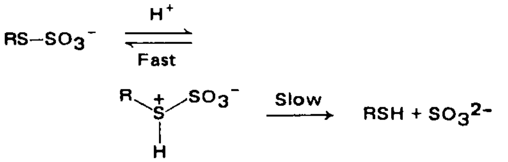
$$RS{-}SO_3^- + H_3O^+ \longrightarrow RSH + H_2SO_3 \qquad (213)$$

$$3RS{-}SO_3^- + 4OH^- \longrightarrow RS{-}SR + RSO_2^- + 2SO_3^{2-} + 2H_2O \qquad (214)$$

$$2RS{-}SO_3^- + O_2 \longrightarrow RS{-}SR + 3SO_3^{2-} \qquad (215)$$

$$RS{-}SO_3^- + {:}Nu \longrightarrow RS{-}Nu + SO_3^{2-} \qquad (216)$$

Thiosulfates are readily cleaved under acidic conditions to thiols.[658-662] An A-1 mechanism has been suggested, (217).[660-662]



$$RS{-}SO_3^- \underset{Fast}{\overset{H^+}{\rightleftharpoons}} R\overset{+}{\underset{H}{S}}{-}SO_3^- \xrightarrow{Slow} RSH + SO_3^{2-} \qquad (217)$$

Alkaline hydrolysis of thiosulfates also has been studied.[662-666]

Cystine, the corresponding disulfide, may be an intermediate in this reaction since alkaline degradation of cystine gives similar products.[670]

Disulfides can also be synthesized from thiosulfates by oxidation[671-677] The common oxidizing agents have been iodine, hydrogen peroxide, and thiourea in hydrochloric acid.

The nucleophilic cleavage of thiosulfates has been studied with a variety of nucleophiles.[678-689] The reactions of biochemical interest are those with the thiols and cyanide, (220)-(222).

$$RS{-}SO_3^- + R'SH \longrightarrow RS{-}SR' + HSO_3^- \qquad (220)$$

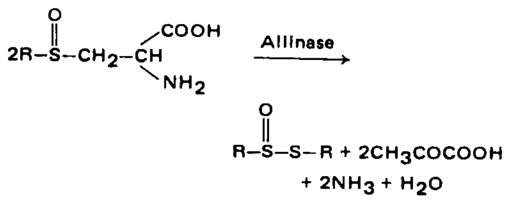
$$RS{-}SO_3^- + R'S^- \longrightarrow RS{-}SR' + SO_3^{2-} \qquad (221)$$

$$RS{-}SO_3^- + {}^-CN \longrightarrow RS{-}CN + SO_3^{2-} \qquad (222)$$

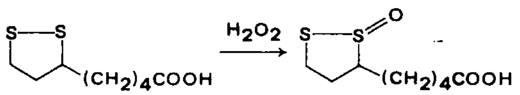
Reactions with thiols might represent one mode of *in vivo* interaction of thiosulfates with protein and enzyme thiol groups. The reaction with cyanide has some analogy to the proposed reactions involved in rhodanese-catalyzed formation of thiocyanate from cyanide and thiosulfate.[690]

B. Thiosulfinates

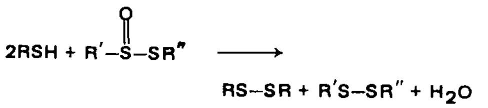
As has already been mentioned, many thiosulfinates occur in plants and are derived from S-alkylcysteine sulfoxides in a reaction catalyzed by the enzyme allinase (223).[484–486] (*Cf.* Sec. IV.A.3).


(223)

Lipoic acid thiosulfinate is made directly by the action of an acid peroxide on lipoic acid (224).[691]


(224)

A biochemically important feature of thiosulfinates is their ability to oxidize thiols to disulfide (225).[692]


(225)

$$2RSH + R'-\overset{O}{\overset{\|}{S}}-SR'' \longrightarrow RS-SR + R'S-SR'' + H_2O \qquad (225)$$

This might account for the inhibitory effect of thiosulfinates on enzymes that require thiol groups for activity.[692] In aqueous solution thiosulfinates are slowly hydrolyzed to free sulfinic acid and disulfide.[693]

C. Thiosulfonates

Thiosulfonates, RSO_2SR, are less stable than thiosulfinates and can be obtained by oxidation of disulfides only under anhydrous conditions.[694–696]

The S–S bond of thiosulfonates is cleaved by thiols forming a sulfinic acid and a disulfude (226).[697–699]

$$RSO_2-SR + R'SH \longrightarrow RSO_2H + R'S-SR \qquad (226)$$

In aqueous media thiosulfonates disproportionate into sulfinic acids and thiols (227).[697–699]

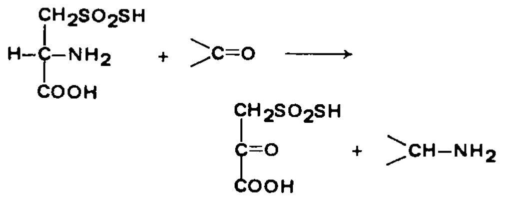
$$2RSO_2-SR + H_2O \longrightarrow 3RSO_2H + RSH \qquad (227)$$

There has been controversy about the structure of thiolsulfonate esters, and whether the structure is RSO_2SR or RSOSOR is uncertain.[693, 700–701]

Thiosulfonic acids are biologically important compounds. Alanine-3-thiosulfonic acid, for example, can be formed in rat tissues by sulfur transfer from mercaptopyruvate to alanine-3-sulfinic acid, in a reaction catalyzed by the enzyme 3-mercaptopyruvate sulfur transferase.[702–705]

A similar sulfur transfer from thiosulfate to alanine-3-sulfinic acid can occur in human erythrocytes.[706]

Alanine-3-thiosulfonic acid has been shown to be an efficient precursor of urinary thiosulfate.[707] The thiosulfate probably arises in a transamination reaction involving alanine-3-thiosulfonic acid. (228) and (229).[708]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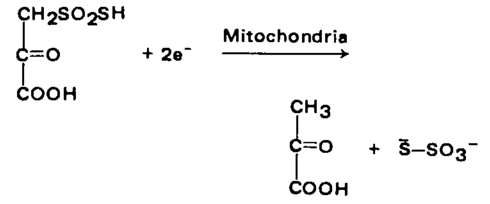

(229)

The S—S bond in thiosulfonic acids can be cleaved by nucleophiles, *e.g.*, CN^-, (230).[709]

$$RSO_2-S^- + CN^- \longrightarrow RSO_2^- + SCN^- \qquad (230)$$

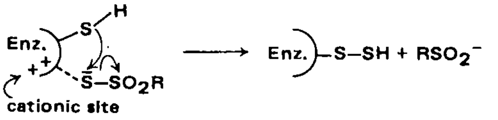
A similar reaction occurs with sulfite ions with the release of thiosulfate (231).[710]

$$RSO_2-S^- + SO_3^{2-} \longrightarrow RSO_2^- + {}^-S-SO_3^- \qquad (231)$$

The same reaction occurs *in vivo* under influence of the liver enzyme rhodanese.[711]

Thiosulfonates have been used as sulfur donor substrate for rhodanese-catalyzed sulfur transfer to nucleophilic species like cyanide, sulfite, and sulfinates.[711–712] The mechanism of these reactions has been elucidated by several workers.[713–718]

A thiol group at the active site of the enzyme is believed to be involved in nucleophilic displacement, at the sulfenyl sulfur of thiosulfonate. The resulting persulfide, which might have only a transient existence, subsequently donates the sulfur atom to the nucleophile, (232)-(233).



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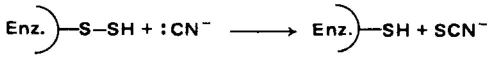

(233)

$$Enz.-S-SH + :CN^- \longrightarrow Enz.-SH + SCN^- \qquad (233)$$

Thiols were shown to catalyze *in vitro* transfer of sulfur from thiosulfonates to cyanide *via* persulfide intermediates.[718]

VI. PHOSPHOSULFATES AND SULFATE ESTERS

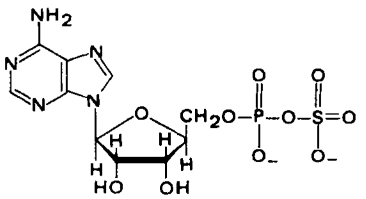
Phosphosulfates and sulfate esters are being considered together, since the biochemical formation of many sulfate esters is dependent on sulfate transfer from phosphosulfates.[719-721]

Phosphosulfates are mixed anhydrides of phosphoric acid, and sulfuric acid and sulfate esters usually are the mono ester of sulfuric acid with a hydroxyl compound.

Phosphosulfates are highly acid labile,[722] and so are the sulfate esters.[723] However, in alkali the phosphosulfates are relatively stable,[722-724] and sulfate esters are quite stable and hydrolyzed only under rather vigorous conditions.[725]

A. Phosphosulfates

Two most important naturally occuring members of this class are adenosine-5′-phosphosulfate (APS) (66) and 3′ phosphoadenosine-5′-phosphosulfate (PAPS) (67).[726-727]



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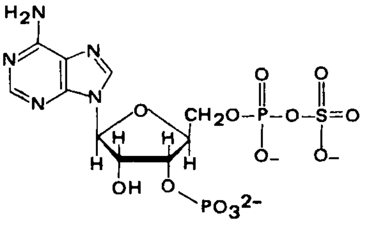

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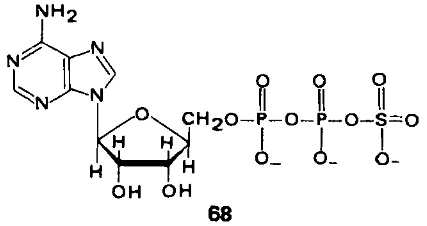
The related compounds, 68 and 69, have been described[728-729] as the potential analogues of ATP.



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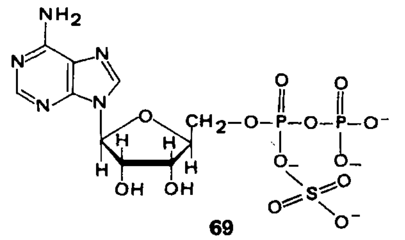

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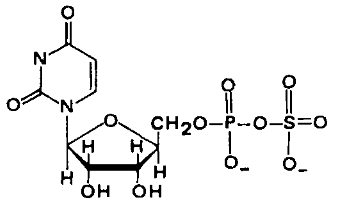
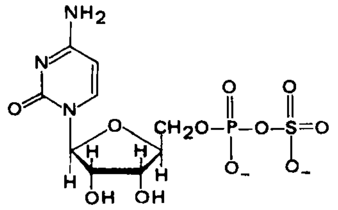
Phosphosulfates derived from other nucleosides, *e.g.*, compounds 70–72, have also been prepared.[730-731]



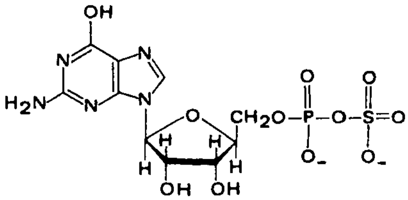
Uridine-5′-phosphosulfate

70



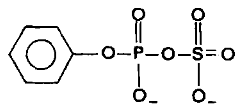
Cytidine-5′-phosphosulfate

71



Guanosine-5′-phosphosulfate

72



Phenylphosphosulfate

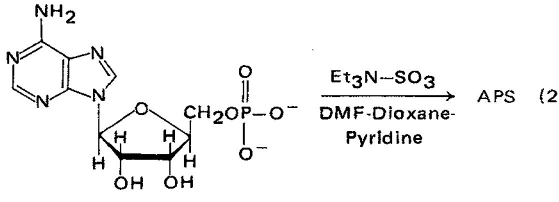
73

Phenyl phosphosulfate (73) was recently synthesized as a model for PAPS, and its hydrolytic behavior has been investigated.[732]

1. Preparation of Phosphosulfates

The general chemical approach for the preparation of phosphosulfates involves sulfation of the corresponding phosphoric acid. The various sulfating agents that have been used include, pyridine-SO_3 complex,[732-734] sulfuric acid-carbodiimide in pyridine,[735] triethylamine-SO_3 in DMF-dioxane-pyridine,[736] and anion exchange.[731]

The highest yields have been obtained in the case of triethylamine-SO_3 complex, and this seems to be the chemical method of choice. APS and PAPS were synthesized in yields up to 75% and 65%, respectively, by this procedure, (235)-(236).[736]



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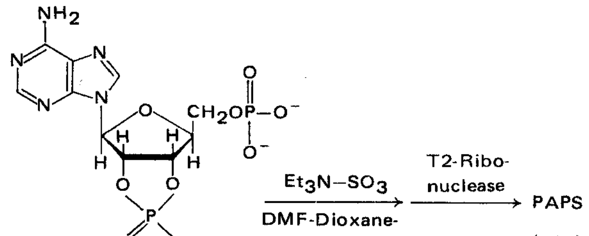


(236)

Enzymatic methods for the *in vitro* preparation of APS and PAPS using sulfate-activating systems have been described.[137-739] ^{35}S-labelled PAPS can be made using $^{35}SO_4$ in such procedures;[737] ATP is the substrate in such reactions.

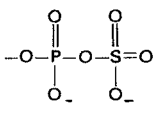
In vivo APS and PAPS are believed to be formed either by oxidative enzymatic coupling of AMP and sulfite,[740-741] or from ATP and sulfate in a reaction catalyzed by a sulfate-activating enzyme.

Such enzymes, called ATP-sulfurylases, have been purified from several sources.[742-744] Bivalent metal ions, such as Mn^{++}, Mg^{++}, Zn^{++}, Cu^{++}, and Co^{++} were found, in certain cases, to increase the enzyme activity whereas Ni^{++}, Ca^{++} and Ba^{++} inhibited the enzyme.[743]

It would be interesting to try *in vitro* preparation of APS from AMP and SO_3^{--} under the influence of an oxidizing agent. Metal ions such as Cu^{++} and Fe^{+++} could be of interest.

2. Reactions of Phosphosulfates

The obvious biological role of phosphosulfates, as already indicated above, seems to be at least threefold, namely: 1. the involvement in active sulfate transfer, 2. involvement in sulfate reduction, and 3. involvement in sulfite oxidation. The reason nature selected a phosphosulfate and not another sulfate derivative, (*e.g.* an acyl sulfate), for this purpose, may be the same uniqueness of the group, **74** compared to other activated sulfate derivatives, such as **75** or **76**.



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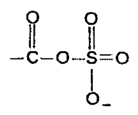

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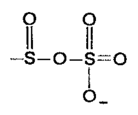

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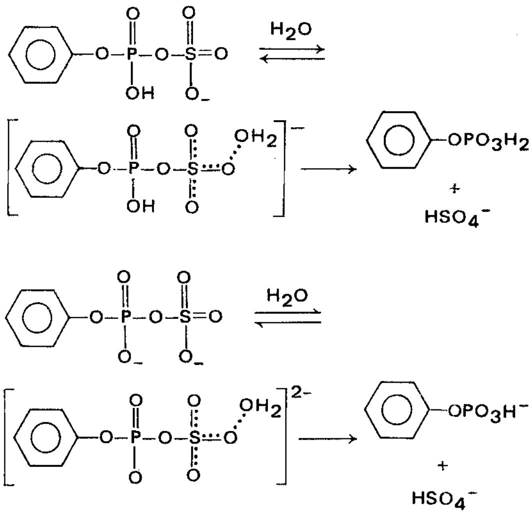
Attempts to study the chemical behavior of phosphosulfates in light of these facts, seem to have some current interest among investigators, *e.g.*, ref 732.

As already mentioned, phosphosulfates are highly acid labile, (*e.g.* halflife in 0.1 *N* HCl at 37° is 6 min.[724] However, 0.1 *N* NaOH at 100° effects only partial hydrolysis after two hours).

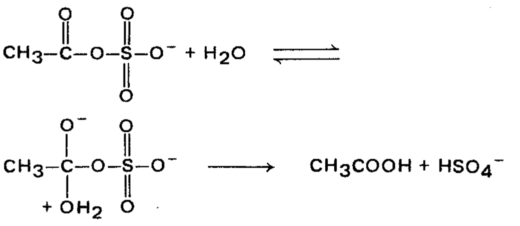
The kinetics of solvolysis of phenyl phosphosulfate was recently investigated,[732] and both monoanions and dianions were shown to be hydrolytically labile. Labeling experiments with H_2O^{18} indicated that the unimolecular elimination of solvated sulfur trioxide occurred mainly by cleavage of the S–O bond, (237)-(238).



(237)

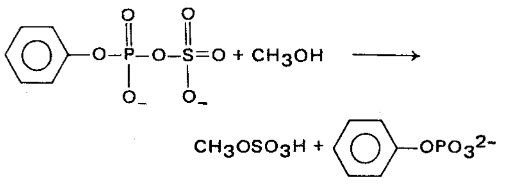
(238)

This was in contrast to the exclusive C–O bond cleavage in the corresponding reaction (239) with acetyl sulfate where the electrophilic carbonyl group promotes a bimolecular addition elimination mechanism.



(239)

Thus, whereas phenyl phosphosulfate can bring about sulfation of methanol (240), acetyl sulfate cannot (241).



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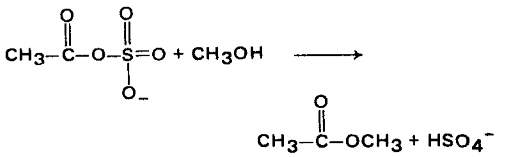
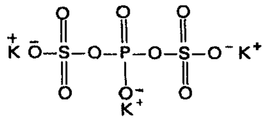


$$CH_3-C(=O)-O-S(=O)(-O^-)=O + CH_3OH \longrightarrow CH_3-C(=O)-OCH_3 + HSO_4^- \qquad (241)$$

The sulfating ability of phenyl phosphosulfate is solely due to the reduced reactivity of the phosphate group towards nucleophilic attack relative to the carbonyl group.

Another unique characteristic of phosphosulfates is their reducibility, not observed in other biological anhydrides. Thus, although sulfate and hydrogensulfate are not affected by hydrogen iodide, the phosphosulfate, 77, is readily reduced.[745]



$$\overset{+}{K}\,\bar{O}-S(=O)_2-O-P(=O)(O^-K^+)-O-S(=O)_2-O^-K^+$$

77

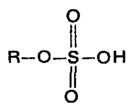
This reducibility may be at least in part responsible for the biological involvement of PAPS in the reduction of sulfate to sulfite.

However, it should be realized that the process of enzymatic activation plays the most important role in biological processes, and the choice of a chemical structure for a certain biological process might be dictated mainly by this.

B. Sulfate Esters

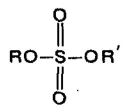
A wide variety of sulfates occur in nature[746-801] however relatively little is known about their biological significance.

Sulfuric acid can form a monosulfate, 78, or a disulfate, 79, depending upon whether one or both of the –OH groups are esterified.



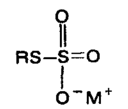
$$R-O-S(=O)_2-OH$$

78



$$RO-S(=O)_2-OR'$$

79



$$RS(=O)(=O)-O^-M^+$$

80

(M⁺ = Metal, R_4N)

Nearly all naturally occuring sulfate esters are monosulfates; they normally exist and are isolated in the form of the salt, (80).

1. Formation of Sulfate Esters

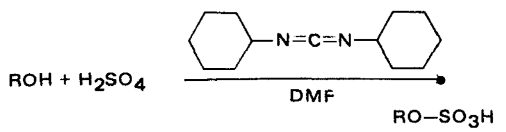
A variety of methods are available for the *in vitro* preparation of sulfate esters.[802-820] Most methods have the same basic approach–namely sulfation of the corresponding hydroxy compounds. Several reagents have been employed for this purpose, and the use of typical ones is illustrated (242)-(247).

$$ROH + ClSO_3H \xrightarrow{\text{Base}} RO-SO_3H \qquad (242)$$

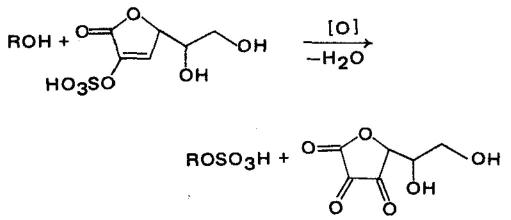
$$ROH + Py\cdot SO_3 \xrightarrow{\text{Pyridine}} ROSO_3H \qquad (243)$$

$$ROH + Et_3N-SO_3 \xrightarrow{\text{Pyridine}} RO-SO_3H \qquad (244)$$

$$ROH + H_2SO_4 \longrightarrow ROSO_3H + H_2O \qquad (245)$$



(246)



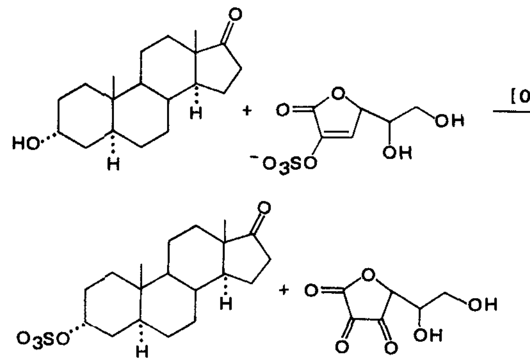
(247)

Preparation of carbohydrate sulfates[748-753] by these methods may present difficulties in the purification of the desired product,[821-824] since in addition to the desired hydroxyl group other hydroxyl groups may also be sulfated. Suitably protected carbohydrates are to be used in such cases.[748, 753, 825-830] The sulfur trioxide adduct of DMSO has been used for sulfation of cellulose.[828]

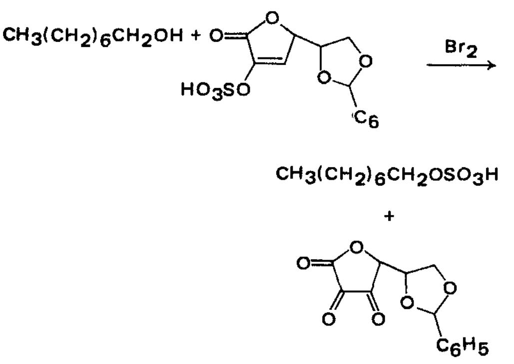
During the biological formation of sulfates, it generally is accepted that sulfate esters are formed by the transfer of active sulfate from PAPS to a suitable acceptor (alcohol or phenol) under the catalytic influence of a sulfotransferase enzyme system.[831]

Model systems for sulfate transfer recently have been discussed[832] and several sulfotransferases have been characterized.[833-838]

Thus, although this seems to be the main pathway of sulfate formation, the alternative routes have not been excluded,[839] and attention recently has been directed to the possible involvement of L-ascorbic acid-2-O-sulfate as an active sulfate carrier[780, 811, 840] in a reaction analogous to (247). Relationships between sulfation and L-ascorbic acid have been adequately demonstrated,[841-848] and *in vitro* sulfate transfer from L-ascorbic-2-O-sulfate to sterols, (242),[780] and alcohols, (243)[811, 849-851] has been demonstrated.



(242)



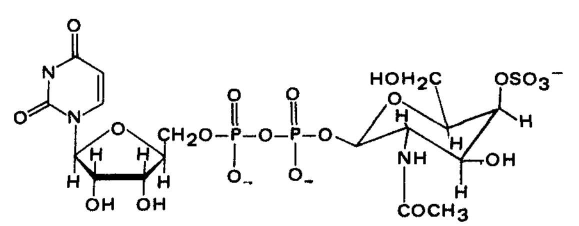
(243)

Whether such a reaction has some biological significance or not still is not known. Activity of ascorbic acid sulphate in conjunction with PAPS and its intermediate formation by sulfate transfer from PAPS may be interesting to investigate.

Formation of L-ascorbic acid-2-O-sulfate in the spleen and liver of rats, injected subcutaneously with $^{35}SO_4^{-}$ and (1-^{14}C) ascorbic acid, was recently demonstrated;[752] ascorbic acid-2-sulfate was also isolated from human urine.[753]

It should be mentioned here that ascorbic acid-2-O-sulfate was thought to be 3-O-sulfate in initial publications, however recent crystallographic studies have shown it to be the 2-O-sulfate.

In the sulfation of sugars, although once again the PAPS seems to be the direct sulfate donor, the involvement of UDP-acetylglactosamine-4-sulfate (**81**) as an alternative activated sugar sulfate carrier has been suggested.[752]

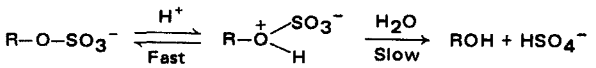


81

Biosynthesis of naturally occurring sulfates has been a matter of considerable current interest.[854-860]

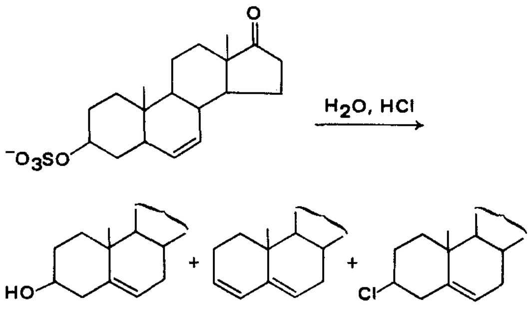
2. Reactions of Sulfate Esters

The most important chemical property of esters is their hydrolytic behavior. Most sulfate esters are readily cleaved in acid medium, and the ease of cleavage depends upon the electronegativity of the group, R, in the formula $R{-}O{-}SO_3H$.[723,803,861,862] Thus, aryl sulfates in general hydrolyze much faster than alkyl sulfates, and in each case the S–O bond undergoes the cleavage.[725,863,865] An A-1 type mechanism (244) has been suggested for such reactions.[723,864]



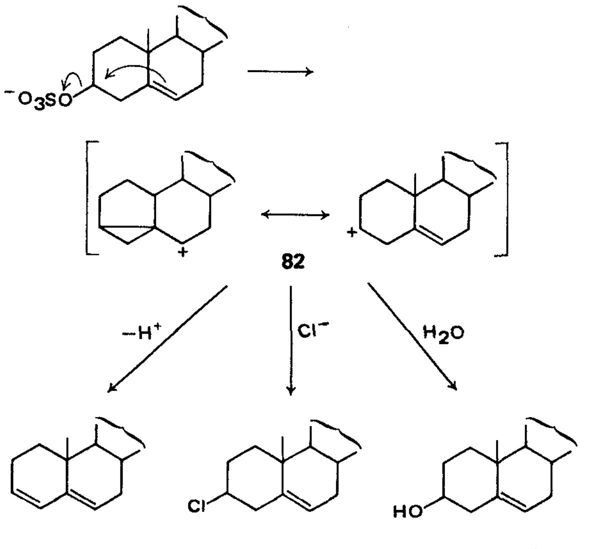
$$R{-}O{-}SO_3^- \underset{\text{Fast}}{\overset{H^+}{\rightleftharpoons}} R{-}\overset{+}{O}(SO_3^-)(H) \xrightarrow[\text{Slow}]{H_2O} ROH + HSO_4^- \qquad (244)$$

Acid hydrolysis of sulfates can proceed in certain cases, also via C–O bond cleavage. The sulfate group is displaced from the carbon atoms, and more than one product may result. Of special interest, in this regard, is the case of the steroid sulfates, as in eq 245.[866]



(245)

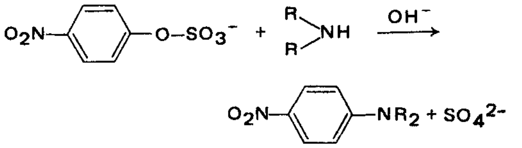
In this case, probably the neighboring double bond participates in displacement of the sulfate group giving an intermediate of the type **82** which then reacts further to give various products, (246).



(246)

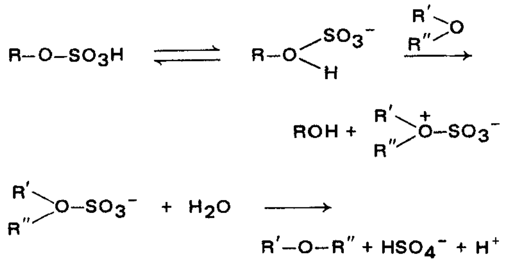
Alkyl and aryl sulfates are quite stable in alkali. For example, the half life for the hydrolysis of S-butylsulfate in 5 *N* NaOH at 100° is 20 hrs, compared to only 1 hr in 0.5 *N* H_2SO_4.[725] The hydrolysis in alkaline medium proceeds *via* a C–O bond cleavage and hence constitutes a nucleophilic displacement of the sulfate from the carbon atom, and the independence of the rate of hydrolysis of the OH^- ion concentration[725] indicates an SN1 type process involved.

Nitroarylsulfates have been used, in alkaline medium, for arylating secondary amines,[867-868] *e.g.*, eq (247).



(247)

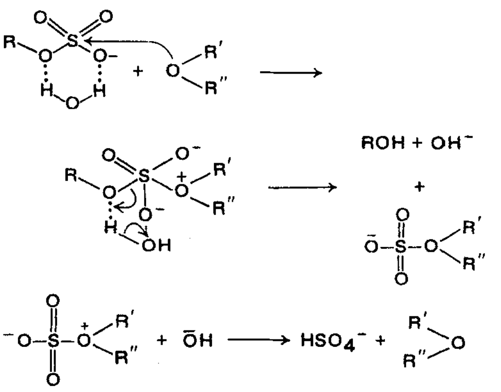
An interesting property of sulfate esters is that they are very readily solvolyzed in relatively less polar solvents such as ethers and the rate of solvolysis is greatly retarded by an increase in the polarity of the medium.[868-876] Thus the hydrolysis of methyl and ethyl sulfates is 10^7 times faster in moist dioxane than in pure water.[871] The participation of ethereal solvent is believed to be in removing the sulfur trioxide from the protonated sulfate intermediate, (248)-(249).[870-876]



(248)

(249)

In an alternative proposal,[877-878] water is assigned a prominent role in hydrating the sulfate core, (250)-(251).



(250)

(251)

A recent finding[879] however, has established that the solvolysis requires initiation by trace impurities, which behave as electrophiles in assisting the S–O bond cleavage.

Another recent report indicated that hydrolysis of sulfates, in benzene solution, is markedly enhanced by alkylammonium carboxylate salts.[880]

The ease of cleavage of the sulfates, under the conditions mentioned above, might be important in understanding their physiological behavior.

The hydrolysis of cyclic sulfates is attracting considerable attention.[881-882]

A large number of enzymes, called sulfatases, catalyze hydrolysis of sulfate esters in animals, plants, and microorganisms. The real physiological role of these enzymes is not clearly understood.

β-Cyclodextrin-catalyzed hydrolysis of aryl sulfates has been studied as a model for the enzymatic catalysis through binding.[884]

Sulfatases have been covered in detail in Section V.

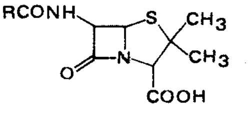
3. Biological Significance of Sulfate Esters

The physiological role of sulfate esters is not fully understood. The role of the sulfate group in determining biological activity may depend more on its physical characteristics than on chemical reactivity. Metabolism of sulfates has been investigated.[885-892] A detailed account of biological behavior of sulfate esters is elsewhere in this review.

VII. HETEROCYCLIC SULFUR COMPOUNDS

Sulfur heterocycles present a very diverse and large area of biochemically active compounds, and detailed consideration is out of the scope of the present paper. The biological role of biotin[893-897] and thiamine has been documented well, and these compounds are also covered in other sections of this review.

Penicillins, **83**, and cephalosporins, **84**, are known for their antibiotic activities and have been adequately documented in literature, including refs 898-907.



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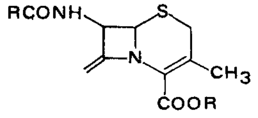
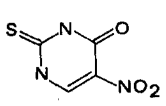


84

The continuing interest in the chemistry and biochemistry of these compounds is indicated by extensive research, particularly in the area of structural modification for better biological activity.[908-935] A variety of thiopyrimidine derivatives, *e.g.*, **85**, have therapeutic value and literature on these has been reviewed earlier.[936]



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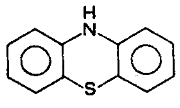
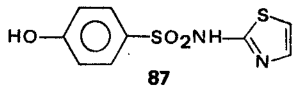


86

Phenothiozines, *e.g.*, **86**, present another group of widely known compounds of therapeutic value.[937-942]

Thiazole derivatives, *e.g.* **87-88**, have also found application in chemotherapy.



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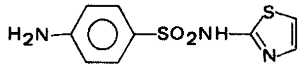

88

Biological significance of thiophene derivatives has been extensively investigated.[943-946]

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