Role of sulfur chirality in the chemical processes of biology

Ronald Bentley

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Chiral structures profoundly influence chemical and biological processes. While chiral carbon biomolecules have received much attention, chirality is also possible in certain sulfur compounds; just as with carbon, there can be differences in the physiological behavior of chiral sulfur compounds. For instance, one drug enantiomer, Nexium® (esomeprazole, a chiral sulfoxide), is used for its superior clinical properties as a proton pump inhibitor over the racemic mixture, Prilosec® (Losec®, omeprazole). This *critical review* introduces sulfur stereochemistry and nomenclature, and provides a comprehensive approach to chiral sulfur compounds and their enzymatic reactions in general and secondary metabolism. The major structural types of biological interest are sulfonium salts, sulfoxides, and sulfoximines. (103 references)

1 Introduction

The chirality of biomolecules influences physiological events; enantiomers of the same compound may have different tastes or odors, and may behave differently as drugs, plant growth regulators, insect pheromones, and enzyme substrates. Such phenomena have been extensively investigated with chiral carbon compounds. Another bioelement with chiral behavior under appropriate conditions is sulfur. Sulfur accounts for about 1% of the dry weight of the human body—a little less than the potassium content, a little more than that of sodium. Many sulfur compounds participate in general metabolism; examples are the amino acids, cysteine and methionine, the peptides, glutathione, cystathionine, *etc*., sulfolipids, and cofactors such as biotin, coenzyme A, lipoic acid, and thioredoxin. Many more sulfur compounds occur as natural products, some, such as the penicillins and cephalosporins, being of considerable pharmaceutical importance; moreover, synthetic drugs frequently contain sulfur. A 1982 review on chiral organosulfur compounds deals almost exclusively with chemical topics.[1a] However, the important roles of chiral sulfur compounds in the chemical reactions of living organisms have received almost no attention in texts of biochemistry and molecular biology.

Ronald Bentley

Ronald Bentley, PhD 1945, London University (Imperial College, chemistry of penicillin), and earned DSc 1965, London University, was a Faculty member, Department of Biological Sciences, University of Pittsburgh for 40 years. His research concerned the biosynthesis of a wide variety of secondary metabolites and enzymes for microbial synthesis of ubiquinone and vitamin K_2 (menaquinone). His interest in stereochemical implications in biology led to the two-volume text, Molecular Asymmetry in Biology (1969, 1970). He retired at age 70 in 1992 and as Professor Emeritus he has continued with writing and was an editor for The Oxford Dictionary of Biochemistry and Molecular Biology (1997, revised edition, 2000). In 2002 he received the Waksman Outstanding Educator Award from the Society for Industrial Microbiology. He takes pride in being a member of the Royal Society of Chemistry for more than 60 years.

2 Sulfur as a stereogenic center

2.1 General considerations

More than a century ago, two sulfur compounds, $(CH_3)(C_2H_5)SR^1R^2$ (R^1 = $CH_2COC_6H_5$ or CH_2COOH, R^2 = halogen) were resolved into enantiomeric forms by diastereoisomer formation with camphorsulfonic acid or bromocamphorsulfonic acid.[1a] Sulfur was then regarded as tetracovalent, and the observed optical activity was considered similar to that of tetrahedral carbon compounds. However, these compounds proved to be sulfonium salts, $[R^1R^2R^3S]^+X^-$, X = halogen. The sulfonium ion is not planar; X-ray crystallography of such salts indicates a pyramidal geometry with a lone pair of electrons. A pyramidal structure can undergo an atomic inversion through a planar (or near planar) transition state forming an enantiomeric, mirror-image structure. For sulfonium ions, the inversion barrier is in the range 25–29 kcal mol^{-1} so that if $R^1 \neq R^2 \neq R^3$, stable enantiomers may be obtained. A pyramidal configuration is also found in sulfoxides, R^1R^2SO, and some similar compounds. For typical sulfoxides, the pyramidal inversion barrier is in the range 35–43 kcal mol^{-1}. Hence, stable sulfoxide enantiomers are possible if $R^1 \neq R^2$.

Since sulfoxides are of particular importance, a brief account of the bonding of sulfur to oxygen is necessary. Unlike carbon, sulfur does not form a typical π bond with oxygen. In a sulfoxide, oxygen contributes electrons (from unshared lone pairs) to a d orbital of sulfur. There is an

overlap of the sulfur d orbital and a p orbital of oxygen; the result is described as d_π–p_π bonding, or, more simply as d–π bonding. The sulfoxide bond is best represented as a partial double bond with two resonance structures:

$$R^1R^2S{=}O \leftrightarrow R^1R^2S^+{-}O^-$$

In this review, the sulfur–oxygen bond is generally written as S–O, the + and − charges, as in S^+–O^-, being implied. Where necessary the electron lone pair on the sulfur is indicated by *e*. The pyramidal geometry of R^1R^2SO is quite different from that of R^1R^2CO where the three atoms directly attached to carbon are planar.

In the sulfone structure, $R^1R^2SO_2$, two of the oxygen unshared pairs may overlap with d orbitals on the central sulfur atom leading to four resonance structures:

$$R^1R^2S(=O){=}O \leftrightarrow R^1R^2S^+(=O){-}O^- \leftrightarrow R^1R^2S^+(-O^-){=}O \leftrightarrow R^1R^2S^{2+}(-O^-){-}O^-$$

The sulfone geometry is that of a (nearly) regular tetrahedron (see later). The d_π–p_π bonding may cause confusion in drawing formulae. R^1R^2SO and $R^1R^2SO_2$ are often written as double bond structures; in such formulae, the sulfoxide sulfur has a shell with 10 electrons and in the sulfone, the shell has 12 electrons.

2.2 Chirality specification at pyramidal sulfur

Chirality at pyramidal centers can be specified as (*R*) or (*S*) by the usual Cahn–Ingold–Prelog (CIP) system. To do so, valence bond structures are defined. If these structures are not self-evident, four "conventions" are used to define an appropriate valence-bond approximation for unsaturated compounds. Convention (b) states that "*contributions by d orbitals to bonds of quadriligant atoms are neglected*".[1b] The sulfur–oxygen bond is, therefore, treated as a formal single bond, **:**S–O. The "absent" ligand, normally a lone electron pair, is assigned an atomic number of zero and in determining the sequence order of precedence has the lowest priority. Thus, for methyl propyl sulfoxide, the priority sequence is O > C_3H_7 > CH_3 > lone electron pair; the (−) enantiomer has the (*R*) configuration **1a–1c** (Fig. 1). Absolute configurations are known for many other sulfoxides[2,3] and for several amino acid sulfoxides (see later).

Configurational descriptors for sulfonium salts are derived in the same way as illustrated for the (+) enantiomer of ethyl methyl propyl sulfonium ion **2a–2c** (Fig. 1). If sulfonium salts and sulfoxides are drawn as modified Fischer projection formulae **1c** and **2c**, with the lowest priority group, the electron pair, at the bottom of the structure, the configurational assignment can be read directly as is done for carbon chirality.

For compounds with both carbon and sulfur chiral centers, the descriptors are modified by the use of subscript c for carbon and subscript s for sulfur. Care is always necessary not to confuse *S* referring to substitution at sulfur, with (*S*) referring to configuration. A compound with one chiral sulfur and one chiral carbon has four stereoisomers, usually described as "four diastereoisomers". This widely used terminology lacks precision since isomers are diastereoisomeric only if they are not in an object–mirror image relationship. Of

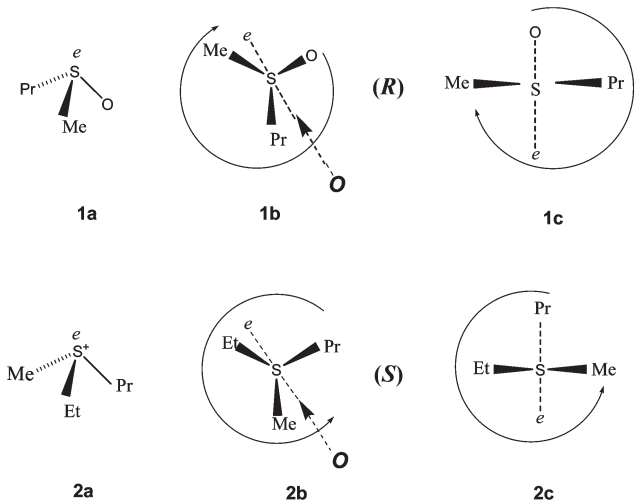


Fig. 1 Configurational assignments for sulfoxides and sulfonium salts. In this and subsequent figures, *e* indicates a lone electron pair. In **1b** and **2b**, the group of lowest priority, *e*, is positioned away from the observer, ***O***.

the four isomers of methionine *S*-oxide (methionine sulfoxide, see later), there are two pairs of enantiomers (compounds with object–mirror image relationship): $(R_cR_s)/(S_cS_s)$ and $(R_cS_s)/(S_cR_s)$. For each individual isomer, *e.g.*, (R_cR_s), there is one enantiomer, (S_cS_s), and two diastereoisomers, (R_cS_s, S_cR_s).

While the CIP system is unambiguous, the vagaries of the sequence rule often impinge when dealing with sulfur compounds; compounds showing structurally the same configuration at a chiral center may have opposed configurational descriptors. Thus, whereas most L-amino acids have (*S*) configuration (*e.g.*, L-serine), L-cysteine is (*R*). Similarly, the isomers of L-methionine sulfoxide **3** and L-*S*-methylcysteine sulfoxide **4** (Fig. 2) are structurally related but the stereochemical descriptors are different: for **3**, (S_cS_s), and for **4**, (R_cS_s). The α carbon priority sequence for **3** is NH_2 > COOH > $CH_2CH_2SOCH_3$ > H, and for **4**, NH_2 > CH_2SOCH_3 > COOH > H.

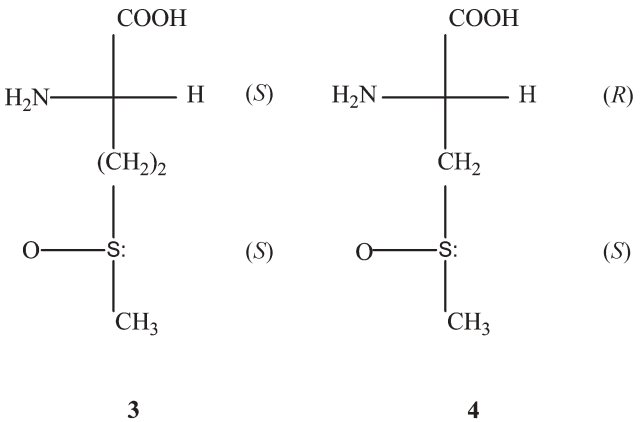


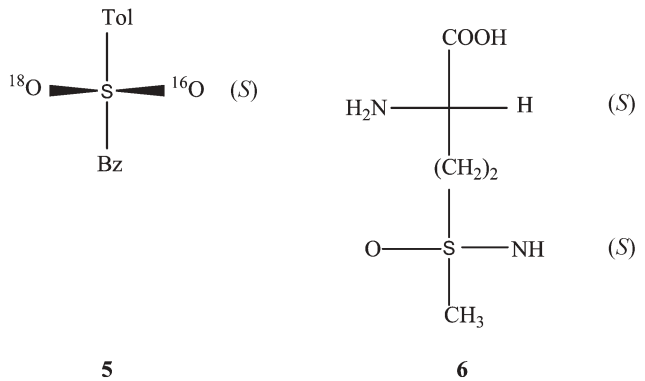
Fig. 2 Configurational descriptors for L-methionine sulfoxide **3** and L-*S*-methylcysteine sulfoxide **4**.

2.3 Compounds with a tetrahedral structure

Although sulfones, $R^1R^2SO_2$, have a tetrahedral geometry, enantiomers can only be produced by using two different oxygen isotopes. The first of several examples[1] was (*S*)-(−)-[$^{18}O^{16}O$]-benzyl *p*-tolyl sulfone **5** (Fig. 3). A similar tetrahedral geometry occurs in sulfoximines as exemplified by methionine sulfoximine **6**. This compound is discussed in detail later. Absolute configurations are known for some other sulfoximine structures.[2]

2.4 Planar chirality involving sulfur

Remarkable examples of sulfur chirality of a very different structural type are some naturally occurring pentathiepin heterocycles lacking a conventional chiral center. Veracin **7** (R = CH_3, Fig. 4) from *Lissoclinum vareau* and lissoclinotoxin A **7** (R = H) also from a *Lissoclinum* sp., have a substituted benzene ring, linked to a further ring of five sulfur atoms.[4–6] As a result of planar chirality there are two enantiomeric molecular arrangements. The inversion barrier



5 **6**

Fig. 3 Configuration of (*S*)-(−)-[$^{18}O^{16}O$]-benzyl *p*-tolyl sulfone, **5**, Bz = benzyl, Tol = *p*-tolyl, and L-(+)-methionine sulfoximine, **6**.

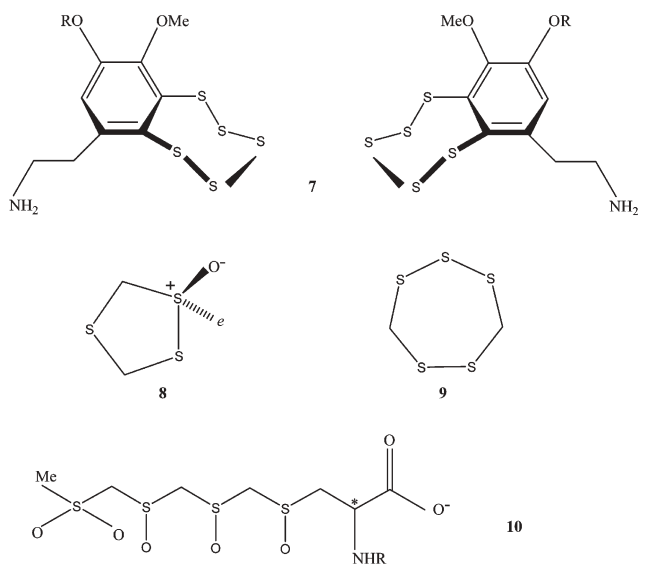


Fig. 4 Polysulfur compounds; note, planar chirality in **7**. In lentinic acid, **10**, R = γ-glutamyl.

(*ca.* 29 kcal mol^{-1}) is sufficiently high to allow, in principle, the biosynthesis of one or other enantiomer. However, isolated materials such as lissoclinotoxin A were racemic. A biosynthesized enantiomer may have racemized during isolation.[5] These compounds have potent antitumor, antifungal and antimicrobial properties.

Simpler naturally occurring cyclic polysulfides have the sulfoxide central chirality. The red alga, *Chondria californica*, contained 1-oxo-1,2,4-trithiolane **8** (Fig. 4) in which one of the sulfur atoms of the ring carries an oxygen; hence, two enantiomeric sulfoxides are possible. The achiral 1,2,3,5,6-pentathiepane **9** from *C. californica*, and as well from the shiitake mushroom, *Lentinus edodes*, is also named as lenthionine.[7] It is noted here because of the remarkable structure of its biosynthetic precursor, lentinic acid **10** (Fig. 4). Lentinic acid contains one sulfone moiety and three sulfoxide groups;[8] since it is also a complex glutamyl-cysteinyl dipeptide, there are two chiral carbons and three chiral sulfurs. The amino acids are presumably (*S*)-glutamic acid and (*R*)-cysteine but the sulfur chiralities are unknown. However, lentinic acid diastereoisomers apparently occur in various organisms.

2.5 Nomenclature for the two faces of a sulfide, R^1–S–R^2 ($R^1 \neq R^2$)

In terms of "paper chemistry" (but not by actual reaction mechanisms) chiral sulfoxides and sulfonium salts derive formally from a sulfide, R^1–S–R^2, $R^1 \neq R^2$, respectively by oxidation or by addition of a cation, *e.g.*, CH_3^+. For trigonal carbon compounds, two "faces" or "sides" of a molecular plane are defined; preferential reagent attack at one face generates stereoselectivity. In the same way, a sulfide molecular plane can be considered to have two faces and stereoselective reagent attack is possible.

Trigonal atom faces are named by adapting the CIP procedure. Multiple bonds are expanded with replicate atoms, and priorities are given to the three ligands by the sequence subrules. Replicate atoms on the central atom are ignored. If the sequence of three ligands, in order of priorities, follows a right-handed path the face is (*re*); if left-handed, the face is (*si*). The process was originally described in general terms[9] with the trigonal atom designated as Yghi. Clearly, it was not intended to limit the nomenclature to carbon. In applying it to R^1–S–R^2, $R^1 \neq R^2$, the two electron lone pairs are treated as a single sulfur to electron pair bond. For Me–S–Pr, the priority sequence is Pr > Me > *e* and the two faces of Me–S–Pr are (*re*) **11a** or (*si*) **11b** (Fig. 5). Such faces are "enantiotopic" and separate attack at each face leads to enantiomers. Oxygen attack on the (*re*) face of Me–S–Pr gives (*R*)-(−)-methyl propyl sulfoxide **1a**, and attack on the (*si*) face gives the (*S*) enantiomer **12** (Fig. 5).

If a sulfide has a further chiral center, usually at carbon, in one (or both) of the substituent R groups, the product formed by attack at the sulfur (*re*) face is a diastereoisomer of that produced by attack at the (*si*) face. The two faces in this situation are "diastereotopic". For example, oxygen attack on the (*re*) face of (*R*)-methyl phenethyl sulfide, C_6H_5–CH(CH_3)–S–CH_3, yields the (R_cR_s)-sulfoxide and on the (*si*) face the product is the (R_cS_s) diastereoisomer.

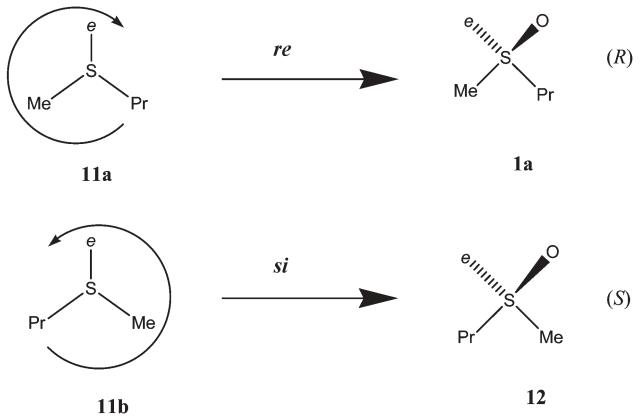


Fig. 5 The (*re*) and (*si*) faces of Me–S–Pr. The priority sequence is Pr > Me > *e*. The two faces are enantiotopic.

2.6 The sulfide, R^1–S–R^2 ($R^1 \neq R^2$), is prochiral

Since addition of a third atom or group to R^1–S–R^2, $R^1 \neq R^2$, can yield a chiral structure, this sulfide is prochiral; and there is an alternative means to consider the process just described. The two electron lone pairs on sulfur are regarded as enantiotopic (or diastereotopic) prochiral ligands and are defined as pro-*R* or pro-*S*. The pro-*R*/pro-*S* assignments are made by arbitrarily assigning one electron lone pair a higher priority than the other in the sequence subrules.[9] If, with this arbitrary sequence, a structure with (*R*) configuration results, the "promoted" lone pair is pro-*R*; if the structure has (*S*) configuration the promoted lone pair is pro-*S*. Thus for Me–S–Pr **1a** (Fig. 6), the lone pair electrons can be distinguished for convenience as *e*″ and *e*′ and the arbitrarily defined sequence becomes Pr > Me > *e*″ > *e*′. Since the sequence, Pr → Me → *e*″, is right-handed, the promoted electron pair, *e*″, is pro-*R*.

Replacement of the pro-*R* electron pair with O leads to (*R*)-(−)-methyl propyl sulfoxide **1a** (priority sequence, O > Pr > Me > *e*). Whether or not pro-*R* replacement yields (*R*) assignment by the CIP system depends on the nature of the substituents and their influence on the sequence subrule. If the pro-*R* lone pair electron of Me–S–Pr is replaced by Et, the priority sequence of the resulting sulfonium salt is Pr > Et > Me > *e*, leading to (*S*) specification for the ethyl methyl propyl sulfonium ion (see also **2a** and **2b**, Fig. 1). With a chiral element in one (or both) of the R groups of R^1–S–R^2, the lone pair electron groups are diastereotopic. Oxidation of Me–S–Pr to the sulfoxide can be treated in two ways; either as attack on the (*re*) or (*si*) face or as replacement of the pro-*R* or pro-*S* electron pair.

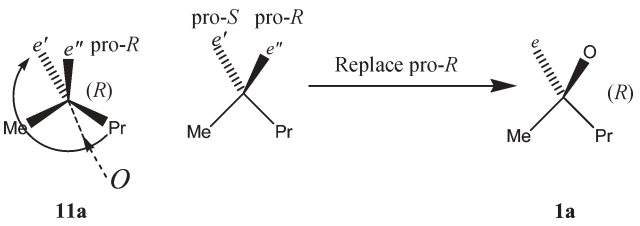


Fig. 6 Prochiral assignments for lone pair electrons of Me–S–Pr. The arbitrarily assigned priority sequence is Pr > Me > *e*″ > *e*′. In **11a**, the group of lowest priority, *e*′. is positioned away from the observer, ***O***.

3 Stereoselective oxidation/reduction of sulfides/sulfoxides

As already indicated, the sulfide to sulfoxide conversion can be either enantioselective or diastereoselective depending on the nature of the sulfide substituent groups. There has been much interest in the formation of chiral sulfoxides since they are used as "chiral auxiliaries" to influence the stereoselectivity of a reaction to produce a desired enantiomer or diastereoisomer. In fact, the chiral sulfinyl group is "*one of the most efficient and versatile chiral controllers in C–C and C–X bond formation*".[10] No attempt will be made here to describe the synthetic methods since there are very extensive reviews.[10,11]

In addition to chemical methods, biotransformations using intact organisms (often bacteria, fungi or yeasts) or isolated preparations of a variety of oxidase enzymes, have also been widely studied. There is little consistency in the observed stereoselectivities. To take one of many examples, the oxidation of C_6H_5–S–CH_3 by cyclohexanone monooxygenase from *Acinetobacter calcoaceticus* formed the (*R*) sulfoxide with high optical purity (99% ee) and good yield (88%). When the alkyl group was *n*-C_3H_7, the product was (*S*) sulfoxide with lower purity and yield (respectively, 68% ee and 54%).[12] Some empirical models that might predict the outcome of various biotransformations have been developed. They are complex and reviews should be consulted.[12,13] The transformation of 1,2 disulfides, R^1–S–S–R^2, to thiosulfinates, R^1–SO–S–R^2, is also carried out by some oxidases. All of these techniques have been extensively reviewed.[12–17]

Enantioselective reduction of methyl *p*-tolyl sulfoxide to the corresponding sulfide was observed with whole cells of *Rhodobacter capsulatus, Escherichia coli*, and *Proteus mirabilis*. *R. capsulatus* rapidly reduced the (*S*) enantiomer, but this specificity was reversed with *E. coli* and *P. mirabilis*. Enantioselective behavior was observed for some other sulfoxides (methylthiomethyl methyl, methoxymethyl phenyl, ethyl 2-pyridyl), with that for *E. coli* and *Proteus vulgaris* being the opposite of that for *R. capsulatus*. These sulfoxides had unknown configurations but enantiomers were characterized by different elution times on chiral HPLC.[18]

Stereoselective reduction of sulfoxides to sulfides has also been studied at the enzyme level. A purified dimethyl sulfoxide reductase from *R. capsulatus* (also termed DorA) rapidly reduced the (*S*) enantiomer from racemic methyl *p*-tolyl sulfoxide.[18] Optically pure (*R*) enantiomer was recovered unchanged (88%). The important methionine *S*-oxide reductases are discussed later.

4 Stereoselective binding of chiral sulfoxides

Some sulfoxides are uncompetitive inhibitors of alcohol dehydrogenases and bind preferentially to the enzyme–NADH complexes. With the horse liver enzyme, the (*S*) enantiomers of hexyl methyl sulfoxide and methyl phenyl sulfoxide bound more tightly (about 5–8-fold) than the (*R*) enantiomers. The observed stereospecificities varied with the

enzyme structure. In the horse liver enzyme, with the change Phe → Ala at position 93 of the protein amino acid sequence, the (*R*) enantiomer was the stronger inhibitor. In addition, human isoenzyme α (Thr at position 48 and Ala at position 93) had less selectivity for hexyl methyl sulfoxide and more for methyl phenyl sulfoxide than did the horse mutant (Phe93Ala). In general, Phe-93 and Ser-48 tended to produce (*S*) selectivity, whereas Ala-93 and Thr-48 tended to yield (*R*) selectivity.[19]

The four stereoisomers of 3-butylthiolane 1-oxide were separated by chiral phase chromatography and configurations were established by NMR and X-ray crystallography. The best inhibitor against horse liver alcohol dehydrogenase was the ($1S_s3R_c$) isomer (K_{ii} = 0.31 μM). Other values for K_{ii} (μM) were as follows: ($1S_s3S_c$), 0.72; ($1R_s3R_c$), 7.3; ($1R_s3S_c$), 37. As with the simpler sulfoxides, binding of (S_s) isomers was preferred.[20]

The (non-natural) dipeptide, (*R*)-phenylglycyl-(*R*)-phenylglycine, forms inclusion compounds with some sulfoxides. The host molecules self-assemble by intermolecular salt formation to form layer structures and the sulfoxide guests are accommodated between layers. Inclusion compound formation is enantioselective but a slight difference in the shape of the guest molecule induces a conformational change in the host structure, and leads to a change in enantioselectivity. For the isomers of tolyl methyl sulfoxide, the enantioselectivities were as follows: 2-tolyl, (*R*); 3-tolyl, (*S*); 4-tolyl, racemic. Similar results were obtained for isomers of chlorophenyl methyl sulfoxide.[21]

5 Chiral sulfur compounds in general metabolism

In the following are described important examples of chiral sulfonium salts and sulfoxides that are concerned in general metabolism. In both groups, chiral carbon atoms are often present as well. There are only a few sulfonium salts; however, the most prominent example, *S*-adenosyl methionine, AdoMet, is the second most widely used enzyme substrate after ATP.[22] Sulfoxide structures are common.

5.1 Sulfonium salts as metabolites

The first sulfonium natural product to be isolated was achiral dimethylsulfoniopropionate, DMSP, an important precursor for dimethyl sulfide, one of the so-called volatile organic sulfur compounds, VOSCs.[23] Later, AdoMet was identified and has assumed much importance. Since it contains a chiral sulfur atom, a chiral α-carbon in the L-methionine portion, and four more chiral carbons in the D-ribose component of adenosine, 2^6 stereoisomers are possible. The ribose carbons are usually assumed invariant and are not considered. Hence, with the chiral sulfur and chiral α-carbon, AdoMet has four stereoisomers. If the α-carbon of the methionine moiety has the usual L-(*S*) configuration, two diastereoisomers need to be considered.

The existence of these two stereoisomers was established as follows.[24]

A. AdoMet synthesized by action of rabbit liver methionine adenosyltransferase, EC 2.5.1.6, an enzyme specific for L-(*S*)-methionine, was 100% utilized by guanidinoacetate *N*-methyltransfer ase, EC 2.1.1.2. The latter enzyme transfers methyl from AdoMet to guanidinoacetate with formation of creatine.

B. AdoMet synthesized by chemical methylation of *S*-adenosyl-L-homocysteine, AdoHcy, was only 50% utilized under the same experimental conditions.

It was assumed that the enzymatic synthesis gave a single, biologically active diastereoisomer, now known to be **13** (Fig. 7), and the chemical synthesis a mixture of two diastereoisomers only one of which was biologically active. The products had significantly different optical rotations: enzymatic product, $[\alpha]_{589}$ = +48, chemical product, $[\alpha]_{589}$ = +52.

Sulfur configuration was indicated at first by the use of (+) or (−); enzymatically synthesized AdoMet was (−)-*S*-adenosyl-L-methionine, and chemically synthesized material was (±)-*S*-adenosyl-L-methionine. (+)-*S*-Adenosyl-L-methionine, the biologically inactive isomer, was prepared by a large-scale reaction of (±)-*S*-adenosyl-L-methionine with guanidinoacetate *N*-methyl transferase. The 50% (+)-AdoMet remaining at the end of the reaction was recovered and purified. Chemical methylation was used to prepare (±)-*S*-adenosyl-D-methionine. The optical rotations, $[\alpha]_{589}$, of the four samples were as follows: (−)-L, +48.5; (+)-L, +57.0; (±)-L, +52.2; (±)-D, +16.0.

The experimental approach to determine configuration at the sulfur atom of AdoMet is simple in concept, but the actual details were complex to make sure that configuration was retained at the sulfur atom during the manipulations.[25] The following description is much abbreviated. Since crystals of AdoMet, suitable for X-ray crystallography, were unavailable, enzymatically synthesized AdoMet was degraded to a diastereoisomer of the *S*-carboxymethyl derivative of L-(*S*)-methionine, termed Isomer A, and finally shown to be **15** (Fig. 7). The carboxymethyl group was derived from C-5 and C-4 of the D-ribose component. The two isomers A **15** and B **16** were also obtained as iodides by reaction of L-(*S*)-methionine **14** with iodoacetic acid. After some difficulty, they were isolated as trinitrobenzenesulfonates; only isomer B formed salt crystals suitable for X-ray crystallography. The X-ray results made possible a correlation between the sulfur atom and the known configuration of the

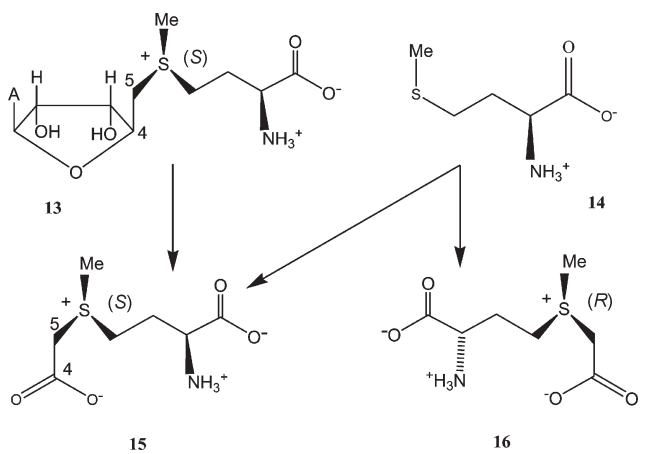


Fig. 7 Absolute configuration of enzymatically synthesized AdoMet, **13**, A = adenine. In all structures the α amino position has (*S*) configuration.

α-carbon of the L-(*S*)-methionine; isomer B had structure, **16**. By inference, isomer A was **15** and enzymatically active (−)-AdoMet was **13**. Both chiral centers in enzymatically synthesized AdoMet **13** are (*S*); thus, biologically active (−)-*S*-adenosyl-L-methionine is (S_cS_s)-*S*-adenosylmethionine.

The two AdoMet diastereoisomers, (S_cS_s) and (S_cR_s), were separated by cation-exchange HPLC and it was shown that the mixture obtained by chemical methylation of AdoHcy contained about 60% of the inactive (S_cR_s) isomer.[26] The chromatographic separation also made stability studies possible. At the "physiological" pH of 7.5, (S_cS_s)-AdoMet was unstable (48 h at 37 °C), being converted to 5′-methylthioadenosine and homoserine lactone (by cleavage) and to adenine and *S*-pentosylmethionine (by hydrolysis). In addition there was some racemization at sulfur to (S_cR_s)-AdoMet. At pH 1.5, racemization was the only significant reaction.

With the chromatographic separation of the diastereoisomers as an analytical method, the proportion of the two stereoisomers in commercial samples of AdoMet was determined. The percentage of AdoMet in these samples as the (S_cS_s) isomer ranged from 68.6 to 99.3. As a control, mouse liver AdoMet had 97% of the (S_cS_s) diastereoisomer. The commercial materials were frequently contaminated with 5′-methylthioadenosine, adenine, and AdoHcy. In the worst case the composition of 258 nm absorbing material as (S_cS_s):(S_cR_s):(impurity) was 46.2:13.4:40.4 and in the best, 81.8:18.2:0.0. *Caveat emptor*!

Several studies have verified that (S_cR_s)-AdoMet does not function as a methyltransferase substrate; for instance, (S_cR_s)-AdoMet was stable to a mouse liver extract that rapidly consumed 100% of the (S_cS_s) form.[26] (S_cR_s)-AdoMet is, in fact, a potent inhibitor for the *N*-methyltransferases for histamine, EC 2.1.1.8, and phenylethanolamine, EC 2.1.1.28, and the *O*-methyltransferases for catechol, EC 2.1.1.6, and hydroxyindole, EC 2.2.2.4. While these enzymes bound (S_cR_s)-AdoMet, they could not transfer its methyl group.[27]

The enzyme, thetin-homocysteine *S*-methyltransferase, EC 2.1.1.3, transfers methyl groups to homocysteine with the formation of methionine (the name, thetin, derives from an early nomenclature for sulfonium compounds). With horse liver enzyme, dimethylsulfonioacetate, $(CH_3)_2S^+–CH_2–COOH$, was the best methyl donor. Methylethylsulfonioacetate used as a "*racemic mixture of two isomers asymmetric around the sulfur atom*" was less effective, but more than 50% of the methyl groups were transferred. Hence, both enantiomers were apparently donors; *i.e.*, there was a lack of specificity.[28] The same enzyme from *Saccharomyces cerevisiae*, unlike that from horse liver, used AdoMet as methyl donor. Both diastereoisomers were methyl donors—an exception to the above generalization that methyl transferases are specific for (S_cS_s)-AdoMet.[29]

Although *S*-adenosylethionine has some metabolic roles, the stereochemistry at the chiral sulfur is unknown.

Decarboxylation of AdoMet yields dcAdoMet **17** (Fig. 8), a propylamino group donor for synthesis of polyamines such as spermidine and spermine. This action of *S*-adenosylmethionine decarboxylase, EC 4.1.1.50, commits AdoMet to polyamine synthesis. The decarboxylase enzyme, abbreviated as AdoMetDC (not to be confused with the decarboxylated

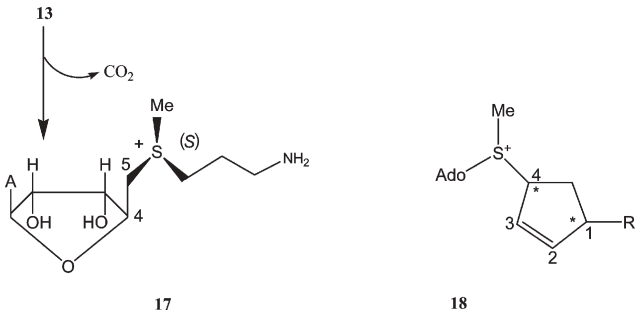


Fig. 8 Decarboxylation of AdoMet, **13**, and AdoMet decarboxylase inhibitors. A = adenine, Ado = adenosine.

product, dcAdoMet), is a pyruvate-dependent protein. Human AdoMetDC, expressed in high yield in *E. coli*, has been purified to electrophoretic homogeneity.[30] It is generally assumed that decarboxylation does not change the configuration at the sulfur atom; hence, enzymatically-synthesized dcAdoMet **17** has (*S*) configuration at the sulfur. A mixture of the dcAdoMet stereoisomers (ignoring ribose carbons) obtained by chemical synthesis, was separated by HPLC.[31] Assuming that spermidine synthase was specific for (*S*)-dcAdoMet, the ratio (*S*)-dcAdoMet:(*R*)-dcAdoMet was 48:52.

AdoMetDC is a target for mechanism-based irreversible inhibition leading to possible antitumor or antiparasitic pharmaceutical agents.[32] One inhibitor is the methyl ester of AdoMet, MeAdoMet, obtained by chemical synthesis as a mixture of diastereoisomers. AdoMetDC only binds the (S_s) isomer.[33] Other inhibitors[34,35] were isomers of AdoMac **18** (R = NH_2) and AdoMao **18** (R = ONH_2, Fig. 8) with two further chiral carbon atoms at positions 1 and 4 of the cyclopentene ring. The compounds were said to exist as "*four possible diastereoisomeric forms*".[36] Assuming that the ribose carbons are neglected, there are actually eight possible isomers on account of the sulfur. However, no information on sulfur chirality was provided.

AdoMet is the substrate for 1-aminocyclopropane-1-carboxylate synthase, EC 4.4.1.14, a pyridoxal phosphate-dependent enzyme using an α,γ-elimination of 5′-methylthioadenosine. The product, 1-aminocyclopropane-1-carboxylate (ACC), derives from carbons C-1 to C-4 of the methionine moiety of AdoMet. Tomato ACC synthase used (S_cS_s)-AdoMet as substrate; the (S_cR_s) isomer was a strong inhibitor.[37,38]

5.2 Methionine *S*-oxide

One of the many metabolic processes of methionine is a fairly easy oxidation to the *S*-oxide form, both as the free amino acid or when in peptide linkage. The formation of these derivatives of methionine in proteins is often accompanied by significant structural and functional changes.[39] The recommended name for a sulfoxide of methionine is methionine *S*-oxide, methionine oxide, since it is felt that methionine includes the sulfur and that methionine sulfoxide might imply two sulfurs in the molecule.[40] This recommendation is largely ignored! However, the recommended symbol, MetO, is one of several in use.

There are four possible stereoisomers of MetO. Oxidation of L-methionine with H_2O_2 yielded a diastereoisomer mixture and hence, by fractionation, a (less soluble) dextrorotatory MetO, $[\alpha]_D^{25} = +99$ ($c = 0.05$ M, H_2O) and a (more soluble) levorotatory MetO, $[\alpha]_D^{25} = -71.6$ ($c = 0.05$ M, H_2O).[41] These materials were originally designated, respectively, as L-methionine *d*-sulfoxide and L-methionine *l*-sulfoxide. Chemical oxidation of Met was, under certain conditions, stereoselective. Thus, reaction with I_2 at pH 7, with long standing for intermediate decomposition, gave a solution containing 66.2% of L-methionine *d*-sulfoxide; this diastereoisomer was isolated with 92.8% optical purity.

The first, naturally occurring, stereoisomer of MetO to be isolated was that from the blowfly, *Phormia regina*, identical to L-methionine *d*-sulfoxide, but renamed as L-(+)-methionine sulfoxide;[42] it was tentatively assigned (S_cS_s) configuration **3** (Fig. 9). This assignment was confirmed by decarboxylation to a compound of known absolute configuration **19**.[43] L-Methionine *d*-sulfoxide is formally (S_cS_s)-2-amino-4-(methylsulfinyl)butanoic acid.

All four MetO stereoisomers were prepared by diastereoselective oxidation of protected methionine derivatives by *Beauveria bassiana* (ATCC 7159) and *Beauveria caledonica* (ATCC 64970).[44] The best protecting group for fungal oxidation was *N*-phthaloyl; following the biocatalytic oxidation, the product was treated with hydrazine to obtain the sulfoxides. From the phthaloyl derivative of L-(S_c)-methionine, *B. caledonica* provided (S_cS_s)-*S*-oxide, mp > 260 °C, dec., $[\alpha]_D$, +96.2 ($c = 0.5$ H_2O), with a diastereoisomeric excess (de) of 90% and 98% yield. The yield of (R_cS_s)-*S*-oxide, mp 235–240 °C, dec., $[\alpha]_D$, +78 ($c = 0.5$ H_2O) from D-(R_c)-methionine was somewhat lower, but the de was higher (92%). The enantiomers of these materials had the same mps and identical but opposed optical rotations.

Methionine oxidation in proteins *in vivo* is carried out by reactive oxygen species (ROS) by processes that are not well understood. It is assumed that the two diastereoisomeric *S*-oxides are formed, although there is apparently no information regarding the ratio of the two forms in proteins. The process is unusual since the oxidation can be reversed by methionine *S*-oxide reductases: MetO + reduced thioredoxin → Met + oxidized thioredoxin. These enzymes thus function as *in vivo* antioxidants. Only a brief account can be given here; a comprehensive account of current research has recently been published.[45]

There are two reductase types. EC 1.8.4.6, protein-methionine-*S*-oxide reductase, uses both free and protein-bound MetO as substrate, and is often termed pMsr (or pMSR). EC 1.8.4.5, methionine-*S*-oxide reductase, does not oxidize the protein-bound form, and is often termed FMsr. Unhappily, terminology in this area is wildly inconsistent; in one instance, a diastereoisomer of MetO was referred to as an enantiomer. There are many abbreviations for MetO isomers; to eliminate ambiguity, configurational descriptors will be used here. The names and abbreviations for the reductase enzymes are further modified to indicate diastereoselective action for the MetO isomers. A recent proposal for new nomenclature is to term a reductase for both free and protein-bound (S_cS_s)-MetO as pSMsr and its counterpart for (S_cR_s)-MetO as pRMsr.[46] A reductase specific for free (S_cS_s)-MetO would be FSMsr, and its counterpart for (S_cR_s)-MetO, FRMsr. Letters, A, B, C *etc.*, would indicate isozymes. These changes may be difficult to implement since MsrA and MsrB are well established terms (see below), with numbers being used for isozymes.[47] Moreover, stereochemists would resist R and S as configurational descriptors rather than (*R*) and (*S*).

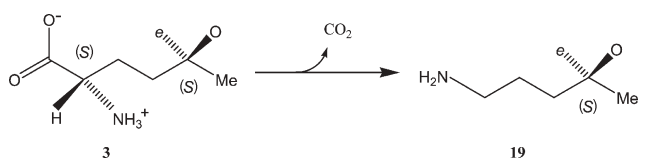


Fig. 9 Determination of the absolute configuration of L-(+)-methionine *S*-oxide, **3**, by decarboxylation.

The reductase, MsrA, initially isolated from *E. coli*, is ubiquitous.[48] It was specific for (S_cS_s)-MetO in both free and protein-bound forms.[49,50] Genes for the *E. coli* and bovine liver enzymes have been cloned and sequenced.[51]

Overexpression of the *msrA* gene in *Drosophila* markedly extended the life span of these flies. They retained normal food intake and body weight but were more physically active.[52] In an intriguing conclusion the authors stated: "*It will be of great interest to see whether overexpression of MSRA extends lifespan in mammals including humans*".

A reductase for (S_cR_s)-MetO was predicted since protein-bound MetO contained both (S_cS_s) and (S_cR_s) forms and since *E. coli* and yeast utilized either diastereoisomer for growth.[48,50] Alternatively, these organisms might contain an epimerase activity. Tentative evidence for such an epimerase has been claimed, but details have not yet been disclosed.[48] A second activity, MsrB, specific for (S_cR_s)-MetO is present in several organisms including bacteria, yeast, fruit fly and mammals.[47] An unusual feature is that a mammalian protein, selenoprotein R (SelR), with MsrB type activity contains selenocysteine, the 21st ribosomally incorporated amino acid.[53] In a mammalian homolog, CBS-1, this moiety is replaced with Cys. A *Neisseria gonorhoeae* protein, pilB, has tandem domains (MsrA and MsrB) that recognize the MetO diastereoisomers.[54]

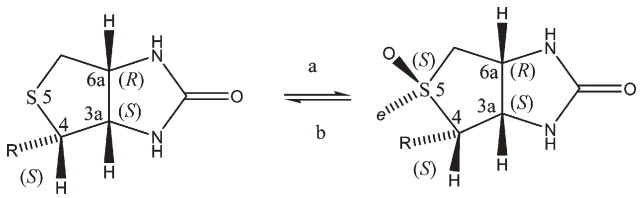
5.3 Ethionine *S*-oxide

Ethionine *S*-oxide isomers were obtained in the same way as those of MetO.[44]

N-Phthaloyl-L-ethionine *S*-oxide, $[\alpha]_D = -21.1$ ($c = 0.7$, ethanol) was shown to be (S_cS_s) by X-ray crystallography. Removal of the protecting group gave (S_cS_s)-ethionine *S*-oxide: mp 247–250 °C, dec., $[\alpha]_D = +50$ ($c = 1.04$, H_2O). (S_cR_s)-Ethionine *S*-oxide had mp 219–221 °C, dec., $[\alpha]_D = -13.2$ ($c = 0.7$, H_2O).

5.4 Biotin *S*-oxide

The water-soluble vitamin, biotin, has three chiral carbon atoms, hence eight possible stereoisomers.[55] From anomalous X-ray dispersion, the naturally occurring, biologically active and dextrorotatory form, (+)-biotin, was shown to have the following configurations: 3a*S*, 4*S*, 6a*R* **20** (Fig. 10).[56,57] Biotin is readily oxidized and *S*-oxides occur naturally. With a chiral



20 **21**

Fig. 10 Biotin, **20**, and biotin *S*-oxide, **21**, R = $(CH_2)_4COOH$. Chemical oxidation by H_2O_2 is represented by reaction a and action of biotin *S*-oxide reductase by b.

sulfur, there are sixteen stereoisomers of biotin *S*-oxide (biotin sulfoxide). Further oxidation leads to biotin sulfone. Peroxide oxidation of (+)-biotin gave (+) and (−) stereoisomers in a 4:1 ratio.[58] The (+)-biotin (+)-*S*-oxide, mp 200–203 °C, had $[\alpha]_D^{20}$ = +130 (*c* = 1.24 in 0.1 M NaOH); the (+)-biotin (−)-*S*-oxide, mp 238–241 °C (dec.), had $[\alpha]_D^{20}$ = −39.5 (*c* = 1.01 in 0.1 M NaOH). X-Ray crystallography indicated (*S*) chirality for sulfur in (+)-biotin (+)-*S*-oxide **21** (Fig. 10).[56,57] A correct but cumbersome description of (+)-biotin (+)-*S*-oxide is (S_{c3a},S_{c4},R_{c6a},S_s).

In early work, (+)-biotin (−)-*S*-oxide, a material isolated from *Aspergillus niger* as AN factor, was observed to substitute for the biotin requirement of *Neurospora crassa*.[59,60] In fact, the biotin *S*-oxides have growth promoting activities for several microorganisms; if the biotin activity is taken as 100, the activity ratios for (+)-*S*-oxide to (−)-*S*-oxide are as follows: *Lactobacillus arabinosus*, 100:5; *Lactobacillus casei*, 0:5; *Leuconostoc dextranicum*, 75:0; *Neurospora crassa*, 100:100; *Saccharomyces cerevisiae*, 100:0.002. Interestingly, *N. crassa*, the organism used in the isolation of AN factor, shows a 100% response to both isomers. Neither *S*-oxide cured biotin deficiency in rats, even with high doses. Reduction of (+)-biotin (+)-*S*-oxide to (+)-biotin is catalyzed by biotin *S*-oxide reductase, BSOR, a member of the DMSO reductase family. The enzyme occurs in *Rhodobacter sphaeroides* f. sp. *denitrificans* and to a lesser extent in *E. coli*.[61] The precise role of BSOR is somewhat unclear.

5.5 Lipoic acid *S*-oxides

The naturally occurring cofactor lipoic acid (α-lipoic acid) has the structure (*R*)-(+)-1,2-dithiolane-3-pentanoic acid. In amide linkage with a lysine residue, it forms lipoamide, a material involved in several enzymatic reactions, particularly of α-ketoacid dehydrogenase complexes. β-Lipoic acid, obtained by oxidation with various reagents, is a thiosulfinate structure, –S–SO–. Depending on which of the two sulfur atoms has undergone oxidation, there are two structural possibilities. The sulfur in these materials is chiral so there are two isomeric forms for each thiosulfinate (assuming that carbon chirality at position 3 is ignored). Four stereoisomers derived from the methyl ester of lipoic acid have been isolated by thin-layer chromatography and characterized by NMR chemical shift reagents.[62] However, precise structures and stereochemical relationships have, apparently, not been obtained. The two possible sulfones have been examined in the same way. Other dithiolane oxides are discussed later.

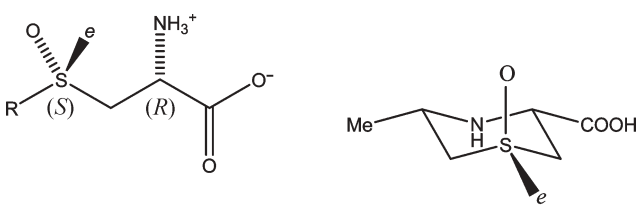
6 Chiral sulfur compounds in secondary metabolism

6.1 Chiral sulfur compounds in the genus *Allium* and some related materials

Members of the genus *Allium* (*e.g.*, onion, garlic, leek, ramp) have long been used as food and as medicines. The characteristic pungent odors and some medicinal properties are attributed to sulfur compounds, some of which are chiral sulfoxides. In intact garlic bulbs (*Allium sativum*) the major sulfur compound occurring at the level of 5–14 mg g^{-1} fresh weight is alliin. It has been identified as *S*-allylcysteine sulfoxide, 3-(2-propenylsulfinyl)-L-alanine, and it occurs in many other plants. With a chiral carbon and sulfur, there are four stereoisomers identified in terms of optical rotation, $[\alpha]_D^{20}$, H_2O, and carbon chirality as follows: (+)-L, +62.8; (−)-L, −60.7; (+)-D, +64.7; (−)-D, −59.2.[63,64]

While a good case can be made that, by analogy with methionine *S*-oxide, the cysteine sulfoxides are better described as cysteine *S*-oxides, it seems appropriate to retain sulfoxide in view of the very extensive literature in which this term is used. The naturally occurring (+)-L isomer of alliin is (R_cS_s)-(+)-*S*-allylcysteine sulfoxide **22** (R = –CH_2–CH=CH_2, Fig. 11). Small amounts (0.2–1.2 mg g^{-1} fresh weight) of the isomer, isoalliin, (R_cR_s)-(+)-*S*-*trans*-1-propenyl-L-cysteine sulfoxide **22** (R = –CH=CH–CH_3) are also present in garlic. (Note that the sulfur configurations in alliin and isoalliin are the same but the sequence rule provides opposite descriptors). A further isomer, cycloalliin, 3-methyl-1,4-thiazane-5-carboxylic acid-1-oxide **23**, derived formally by addition of the alliin amino group to the allyl unit, has two chiral carbon atoms in addition to chiral sulfur. Alliin was likely the first naturally occurring compound containing chiral sulfur and carbon to be isolated and synthesized, although this distinction has been claimed for sulforaphene (see later). Both of these compounds preceded the isolation of AdoMet by some 5 years.

Plants, usually crucifers, contain other amino acid sulfoxides, especially L-cysteine derivatives, termed alk(en)yl cysteine sulfoxides, ACSs. Prior to 2000, *S*-methyl-L-cysteine sulfoxide (methiin) **22**, R = Me, and propiin **22**, R = Pr (Fig. 11), had been well recognized. Subsequently, ethiin **22**, R = Et, and butiin **22**, R = Bu, were identified[65,66] in several *Allium* sp. *Petiveria alliacea*, a tropical herbaceous perennial plant, contains 6-hydroxy-ethiin in both diastereoisomeric forms (one is **22**, R = –CH_2CH_2OH).[67] This plant also contains the diastereoisomers of *S*-benzyl-L-cysteine sulfoxide,



22 **23**

Fig. 11 Sulfoxides related to cysteine.

named as petiveriin A (R_cR_s) **22**, R = $-CH_2C_6H_5$, and petiveriin B (R_cS_s).[68] It appears that the two isomers are synthesized as such, and not by epimerization, either during growth or on isolation. (The petiveriins are not technically ACSs since the substituent is aryl). The occurrence of both sulfoxide forms is unusual, especially in the same plant. The ACS composition varies from plant to plant. Garlic contains (*inter alia*) the methyl, propyl and allyl derivatives (and small amounts of the 1-propenyl compound), onion contains the methyl, propyl and 1-propenyl derivatives (but not alliin) and Chinese chive, *Allium tuberosum*, contains all of these ACSs.

Several volatile sulfur compounds with the characteristic garlic odor are thiosulfinates, R^1–SO–S–R^2. They are formed from alliin and some other ACSs by a complex process; it begins with the action of a C–S lyase enzyme, alliinase, EC 4.4.1.4, on alliin **22** (R = $-CH_2-CH=CH_2$). In this reaction, there is an elimination of aminoacrylic acid, and the formation of allylsulfenic acid **24** (Fig. 12). Two molecules of the latter combine to form allicin, diallyl thiosulfinate **26**, a compound with the characteristic garlic odor. The unstable aminoacrylate **25**, decomposes to pyruvate and ammonia. Alliinase from garlic shows the highest activity with the (R_cS_s) diastereoisomer of alliin. It has low activity with the (R_cR_s) form and isoalliin, and none at all if cysteine has the D-(*S*) configuration. Allicin is a rather unstable compound and apparently the configuration at the sulfoxide sulfur is not known. It is an antimicrobial agent and has many other biological properties including antithrombotic, antitumor, antiatherosclerotic actions, as well as hypoglycemic and hypolipemic effects.

Although alliinase is present in various plant species, garlic contains unusually high amounts, up to 10% of the total clove protein.[69] The amounts of alliin and alliinase are approximately equal. This fact may help to explain the very rapid formation of the garlic odor when the cloves are injured. Alliinase is located in vacuoles while alliin is cytosolic. Hence, the intensely pungent allicin is only formed when the slightly odoriferous garlic cloves are injured, *e.g.*, by crushing. Alliinase, a pyridoxal phosphate-dependent, homodimeric protein, has been purified from garlic and Chinese chive (*A. tuberosum*).[70]

Allicin is a prolific precursor for other materials[69] but only a few examples can be given. Many of them are sulfoxides, but as with allicin itself, configurations have apparently not been determined. One interesting compound is ajoene (from *ajo*, Spanish for garlic).[71] This sulfoxide **27** (Fig. 13), contains two further sulfur atoms in a disulfide linkage. While sulfur stereochemistry is apparently unknown, the central C=C double bond can exist in (*Z*) and (*E*) configurations; the *E* isomer is **27**. Ajoene has many physiological actions, including antiplatelet activity, modulation of membrane-dependent functions of immune cells, antitumor activity and inhibition of cholesterol biosynthesis. In antithrombotic assays, the (*Z*) isomer was more active than the (*E*) form.[71]

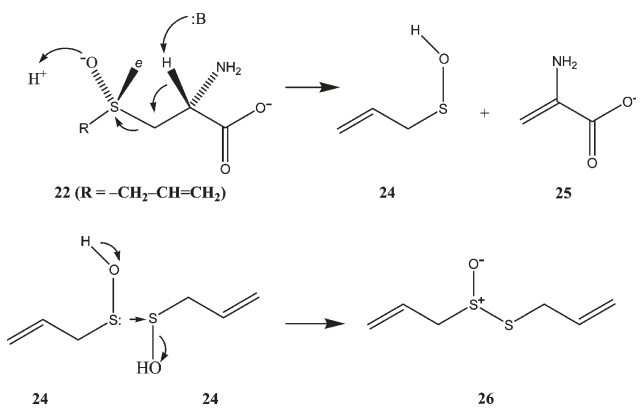


Fig. 12 Lyase action of alliinase and formation of allicin, **26**, from alliin, **22**.

In onion, the characteristic lachrymatory factor, propanethial-*S*-oxide, is prominent. While this compound is achiral, geometrical isomerism about the C=S bond is possible (for the *E* configuration see **28**, Fig. 13). The natural material contains the isomers in the ratio, *Z*:*E* = 95:5. The lachrymatory oxide is formed from isoalliin by action of both alliinase (elimination of aminoacrylate) and a newly described synthetase.[72] The alliinase action presumably forms propenylsulfenic acid, CH_3–CH=CH–S–OH, and synthetase rearrangement of the latter yields propanethial-*S*-oxide. It was suggested that down-regulation of the synthetase might lead to a non-lachrymatory, but still flavorful onion. This would be a major achievement for molecular biology!

The means for stereoselective sulfoxide production in *Allium* are not well understood; unspecified oxidases are often invoked. Alliin and isoalliin may be formed by oxidation of allyl- and *trans*-1-propenylcysteines, respectively.[69] If these materials are oxidase substrates, the oxidations must be stereoselective to account for the formation of alliin **22**, R = $-CH_2-CH=CH_2$ and isoalliin **22**, R = $-CH=CH-CH_3$. This would be possible, for instance for alliin formation, by attack on the (*si*) face of *S*-allyl-L-cysteine **29** (Fig. 14). In mammalian systems, diallyl disulfide and *S*-allyl-L-cysteine were oxidized, respectively, to allicin and alliin by flavin monooxygenases and cytochromes P450 with unknown stereochemistry.[73,74] Similar enzymes may be involved in plants.

6.2 γ-Glutamylcysteine sulfoxides and related compounds

Several cysteine sulfoxides occur as glutamyl dipeptides, possibly as major storage forms.[69] The glutamyl peptide containing isoalliin was isolated as two diastereoisomers from different sources. With two chiral carbons and one chiral sulfur there are eight possible stereoisomers. In both isolated materials, the amino acid residues have L configuration: L-(*S*)-glutamate and L-(*R*)-cysteine. Ignoring the glutamyl

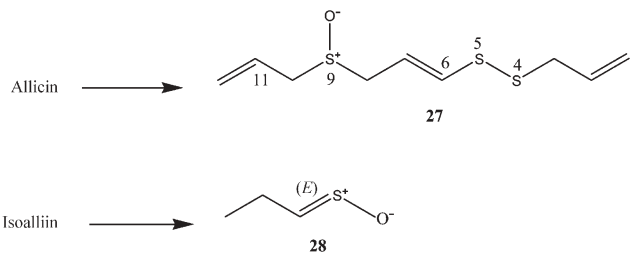


Fig. 13 Formation of ajoene, **27**, and lachrymatory factor, **28**.

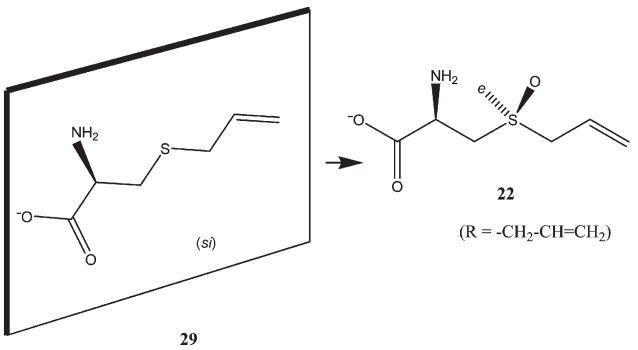


Fig. 14 Formation of alliin by oxygen attack on the (*si*) face of *S*-allyl-L-cysteine, **29**.

component, the diastereoisomer from *Sandalum album*, is (R_cR_s) **30** (Fig. 15); that from onion, *Allium cepa*, is (R_cS_s).[75]

Different sulfur configurations are also found in natural products containing marasmine, 3-[(methylthio)methylsulfinyl]-L-alanine **31** (R = H, Fig. 15) and a glutamyl peptide thereof. A dipeptide precursor of the mushroom garlic odor (in *Marasmius* sp.) was shown to be γ-glutamylmarasmine and the absolute configuration of the sulfur in this peptide **31** (R = glutamyl) was (*S*).[76] A diastereoisomer of marasmine itself, with (*R*) chirality at sulfur, occurs in fruit of the tree, *Scorodocarpus borneensis* ("wood garlic").[77]

Sparsomycin **32** from *Streptomyces sparsogenes* var. *sparsogenes* and *Streptomyces cuspidosporus*, is a derivative of (methylthio)methylcysteine sulfoxide, with (S_cR_s) configuration.[78] It has antitumor and antimicrobial activities and inhibits protein biosynthesis. The sulfur-containing unit probably derives from L-(*R*)-cysteine, by way of *S*-methyl-L-(*R*)-cysteine. The sulfoxide oxygen is introduced after the reduction of the cysteine COOH group to CH_2OH and epimerization to the (*S*) configuration apparently occurs at a late stage of biosynthesis.[79] Sparoxomycins A_1 and A_2 are related materials derived from *S. sparsogenes* SN-2325. Structures are similar to that of sparsomycin but with both sulfur atoms as sulfoxides. The sulfoxide on the sulfur marked * in **32** is either (*R*) in sparoxomycin A_1 or (*S*) in A_2.[80]

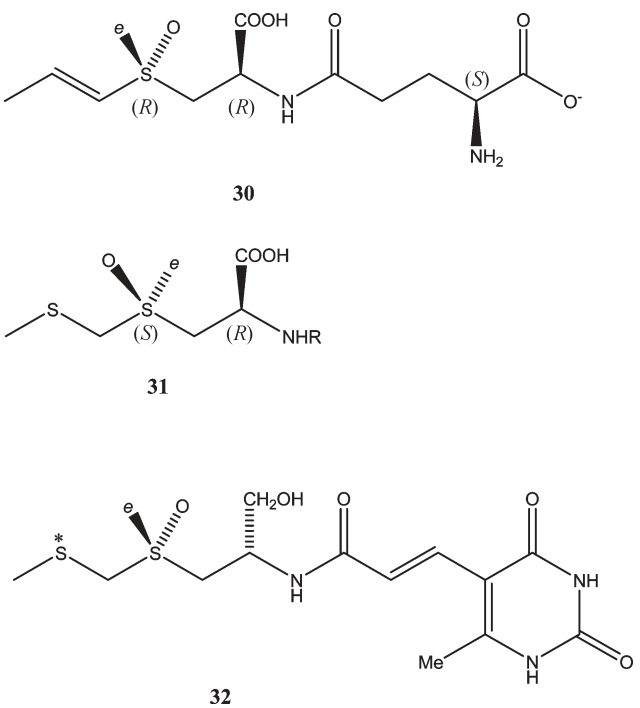


Fig. 15 Cysteine sulfoxide derivatives in various diastereomeric forms.

6.3 Penicillin and cephalosporin sulfoxides

The oxidation of penicillins to sulfoxides was discovered in the classic work to determine the β-lactam structure and many sulfoxides of various penicillins and cephalosporins are now known. Depending on the oxidizing agent and conditions, either the (*R*) or (*S*) configuration at sulfur can be obtained. Representative structures for (*R*) sulfoxides of a penicillin and a cephalosporin are respectively **33** and **34** (Fig. 16). Penicillin sulfoxides are important as intermediates since they can be rearranged into cephalosporin structures.

There is little information on the biological activity of penicillin sulfoxides. "*Pen G SO*", presumably the (*S*) sulfoxide of benzylpenicillin, was a competitive inhibitor of penicillin G acylase and also stabilized the acylase against thermal inactivation at alkaline pH and inactivation from multipoint attachment on aldehyde–agarose gels.[81] Crystal structures of wild type and inactive mutant acylases complexed with "*penicillin G sulphoxide*" [again, presumably the (*S*) sulfoxide of benzylpenicillin] have been obtained.[82] For antibacterial activity, the (*R*) sulfoxide of phenoxymethylpenicillin had fivefold more activity than the (*S*) sulfoxide.[83] Sulfoxides of the semi-synthetic antibiotic, hetacillin, were obtained in a 70:30 ratio on oxidation with *m*-chloroperbenzoic acid.[84] They had antibacterial activities that were less than those of hetacillin itself. The (*R*) isomer **35** was from four- to eightfold more active than the (*S*) diastereoisomer.

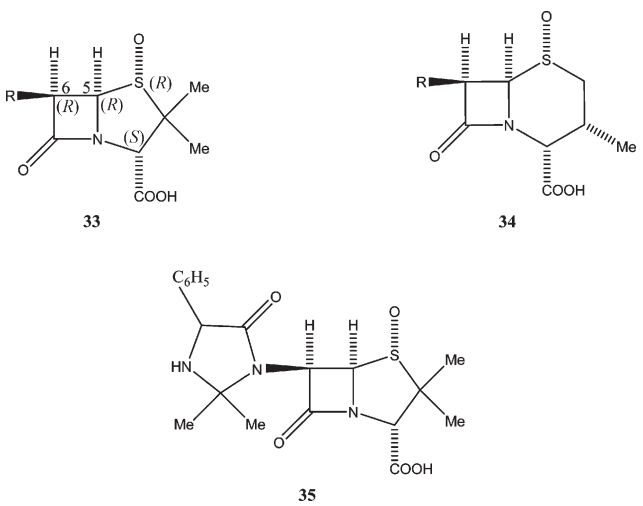


Fig. 16 Representative examples of (*R*) sulfoxides for penicillins and cephalosporins.

6.4 Mustard oils

Mustard oils, formed by enzymatic hydrolysis of glucosinolates, are alkyl isothiocyanates, R–N=C=S; the alkyl moiety often contains a methylsulfinyl group. The (*R*) configuration was assigned to iberin **36** (X = $[CH_2]_3$, Fig. 17) and related mustard oils.[2] Sulforaphene **36** (X = –CH=CH–CH_2–CH_2–) isolated from radish in 1948, has been claimed as the first natural product with optical activity due to sulfur.[85] However, alliin and sulforaphene were both isolated at about the same time; alliin probably has priority. The reduced form, (−)-sulforaphane, from broccoli, has (*R*) configuration.[86] It had previously been synthesized[85] as "L" (levorotatory) and "D" (dextrorotatory) forms with unknown configurations. Sulforaphane, found in other plants, induced phase II enzymes for xenobiotic metabolism (glutathione *S*-transferase, quinone reductase).[86] Sulfur chirality did not influence inducer potency since synthetic, racemic sulforaphane had closely similar activity to the broccoli product.

6.5 Dithiolane oxides

1,2-Dithiolanes and their 1-oxides are of considerable interest, both as secondary metabolites and as synthetic analogs. One such material, isolated from a *Streptomyces* species, is the antitumor antibiotic, leinamycin, **37** (Fig. 18). It contains a 1-oxo-1,2-dithiolane-3-one five-membered ring, spiro fused to a complex macrolactam. In a remarkable process, reaction of leinamycin with a thiol, followed by a profound rearrangement, leads to an episulfonium ion.[87] This ion efficiently alkylates the N7-position of guanosine residues in double-stranded DNA. The unstable adduct is depurinated by hydrolysis of the glycosidic bond between the alkylated base and the deoxyribose residue.

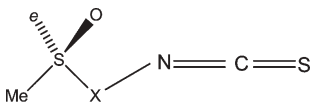


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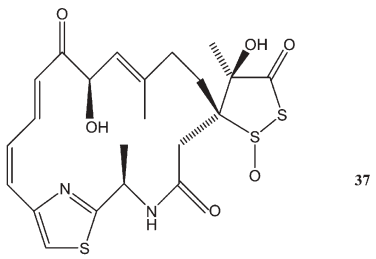
Fig. 17 Structure for mustard oils.



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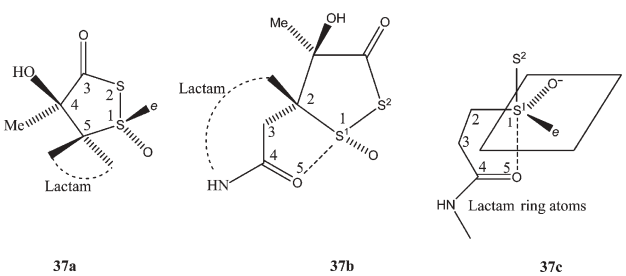


37a **37b** **37c**

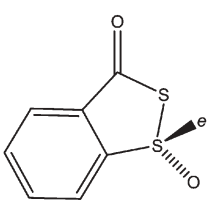
Fig. 18 Structures for leinamycin. Note that numbering in **37b** and **37c** describes the 1,5 sulfur–oxygen interaction and is not that of the usual nomenclature, **37a**.

Leinamycin has 4 chiral carbons as well as a chiral sulfur in the sulfoxide structure; the latter has (S_s) configuration. This stereochemistry is shown, using conventional dithiolane numbering, in the partial structure **37a**. An important structural feature is a 1,5 sulfur–oxygen non-bonded interaction between the sulfoxide sulfur, S^1, and an amide oxygen in the lactam ring **37b**; note that the numbering used in **37b** to describe the interaction does not correspond with that for nomenclature purposes.[87] This interaction alters the thiosulfinate ester conformation of leinamycin and stabilizes the sulfur containing ring. The geometry induced at S^1 is that of a distorted trigonal bipyramid **37c**.

The heterocyclic unit in leinamycin has been studied in simpler, model compounds. One example is 3*H*-1,2-benzodithiol-3-one 1-oxide **38** (Fig. 19). An X-ray crystal structure indicates that the sulfur has (*S*) configuration, as is also the case for leinamycin.[88] Whether this configuration is necessary for DNA cleavage is not known.

Several dithiolane oxides occur as secondary metabolites and in some cases exist with both (*R*) and (*S*) configurations at sulfur. Examples are brugierol and isobrugierol from *Brugeria conjugata* (respectively, R_s, and S_s, **39**, R^1 = OH, R^2 = R^3 = R^4 = H, Fig. 19), zeylanoxide A and *epi*-zeylanoxide A from *Sphenoclea zeylanica* (respectively, S_s, and R_s, **39**, R^1 = R^4 = H, R^2 = OH, R^3 = CH_2OH), and the two oxides, *anti* and *syn*, of the methyl ester of asparagusic acid from asparagus (respectively, S_s, and R_s, **39**, R^1 = R^3 = R^4 = H, R^2 = $COOCH_3$). The pair, zeylanoxide B and *epi*-zeylanoxide B, resemble the A series but in these compounds the oxygen is on the sulfur at position 2. The zeylanoxides inhibited root growth in rice seedlings and the germination of lettuce seeds.[89] They have also been synthesized in unnatural enantiomeric forms with chirality at the two carbons (positions 3 and 4) being reversed. There were no activity differences between all eight of the stereoisomers in these bioassays.

An interesting stereochemical situation is presented by the oxidation of 2-aryl-1,3-dithiolanes.[90] These substrates are achiral but the substituted 2 position is prochiral. Hence, one of the sulfur atoms is pro-*R* and the other is pro-*S*, **40** (Fig. 20, X = an aryl group such as phenyl- or a *para*-substituted phenyl). A substituent such as oxygen on the pro-*S* sulfur leads



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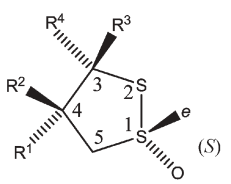

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Fig. 19 1,2-Dithiolane structures.

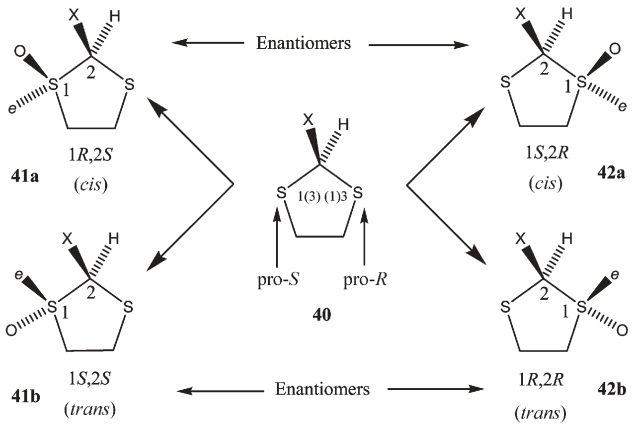

Fig. 20 1,3-Dithiolane structures. X = aryl group.

to (*S*) chirality at C-2, **41a**, **41b**, and at the pro-*R* sulfur to (*R*) chirality, **42a**, **42b**. Moreover, each separate sulfur possesses a pair of prochiral electron pairs in the usual way and becomes chiral by addition of a single oxygen. There are, therefore, four possible mono-oxygenated sulfoxide products. In two of the products, **41a**, **42a**, there is a *cis* relationship between the aryl group and oxygen substituent and in the other two products, **41b**, **42b**, this relationship is *trans*. The overall result of sulfoxide formation is diastereoselective, but the selection of each individual sulfur, and the formation of (*R*) or (*S*) sulfoxide at the sulfur is enantioselective. Note that sulfone formation at one or other sulfur atoms would lead to two enantiomeric products with chirality at the C-2 position.

The stereochemistry of hog liver flavin monooxygenase (FMO) and of rat or mouse liver cytochrome P450 monooxygenase (P450) was investigated with 2-phenyl-1,3-dithiolane and with related compounds with a *para* substituent (*e.g.*, OCH_3) on the phenyl group. The observed results depended on the substituent group. Thus, with 2-*p*-methoxyphenyl-1,3-dithiolane, the oxidation gave *trans* addition product in high yield; for FMO the product was (1*R*,2*R*) and for P450 enzymes it was (1*S*,2*S*). In other words, these two enzyme groups distinguished between the pro-*R* and pro-*S* sulfur atoms, with FMO yielding (*R*) chirality at carbon 2 and with P450 yielding (*S*) chirality. FMO was less specific with 2-phenyl-1,3-dithiolane, also forming some *cis* product with (*S*) chirality at C-2 (1*R*,2*S*). With this substrate, rat liver P450 produced almost exclusively *trans* addition product with (*S*) chirality at C-2 (1*S*,2*S*). The mouse liver P450 enzyme was much less specific both with respect to *cis*/*trans* addition and selection of pro-*R* or pro-*S* sulfur. The original paper[90] should be consulted for more information with other substrates.

6.6 Amatoxins

Toxins from *Amanita* species have been responsible for many cases of fatal mushroom poisoning, with much emphasis on *Amanita phalloides*, the death cap fungus. There are three groups of toxins, all of which are complex cyclic peptides; amatoxins, phallotoxins and virotoxins (*Amanita virosa*, *Amanita subalpiacea*). The best known are the amatoxins and the phallotoxins. The former prevent transcription by interacting with RNA polymerases (especially polymerase II) and the latter stimulate polymerization of G-actin and stabilize F-actin filaments. The first two groups are bicyclic. A tryptophan (or substituted tryptophan) is linked to a cysteine sulfur at the carbon next to the NH group of the pyrrole ring, forming the unit, $–CH_2–S–C(NH)=C$ in phallotoxins. In amatoxins, *e.g.*, α- and β-amanitin, the sulfur atom carries an oxygen, forming a sulfoxide structure. Hence, in these compounds, in addition to multiple carbon chiral centers (amino acid residues), sulfur chirality is also possible.

The two amanitins differ only in one amino acid residue: α-amanitin **43** (Fig. 21) contains asparagine and in β-amanitin this amino acid is replaced with aspartic acid. The abbreviations and stereochemistries for the amino acids shown in Fig. 21 are as follows: Asn, L-asparagine; Hyp, (2*S*,4*S*)-4-hydroxy-L-proline; γ,δ-$(OH)_2$-Ile, (2*S*,4*R*)-4,5-dihydroxy-L-isoleucine; Gly, glycine; Ile, L-isoleucine; the cysteine and 6-hydroxytryptophan units are both L. Complete stereoviews of amanitins are available[91] and structures of α-amanitin RNA polymerase II cocrystal are posted on the Web (http://www.rcsb.org, PDB code 1K83 or with the same designation from PubMed at http://www.ncbi.nlm.nih.gov). The highly toxic, naturally occurring materials are both (*R*) sulfoxides. To investigate the possible role of sulfur chirality in toxicity, the 6′-*O*-methyl derivative (methylation of the OH group on the tryptophan residue) was deoxygenated to the thioether (sulfide) form. Re-oxidation of the latter gave a mixture of (*R*) and (*S*) sulfoxide isomers, and as well, the sulfone structure. While the thioether, sulfone and (*R*)-sulfoxide were all highly toxic, the (*S*)-sulfoxide was at least 20 times less toxic. The LD_{50} values, in white mice, mg kg^{-1}, were 0.3 for (*R*)-sulfoxide and 25.0 for (*S*)-sulfoxide.[91] Similar differences between the two sulfoxide isomers were also observed in other amatoxins.[92] It was possible that these differences were due to different conformational arrangements in the two sulfoxide isomers. However, the three-dimensional structures were essentially identical, not only in the solid state, but also in dimethyl sulfoxide solution. There was one small difference: the oxygen of the (*R*)-sulfoxide was oriented towards the molecule's exterior, while for the (*S*)-sulfoxide it was oriented

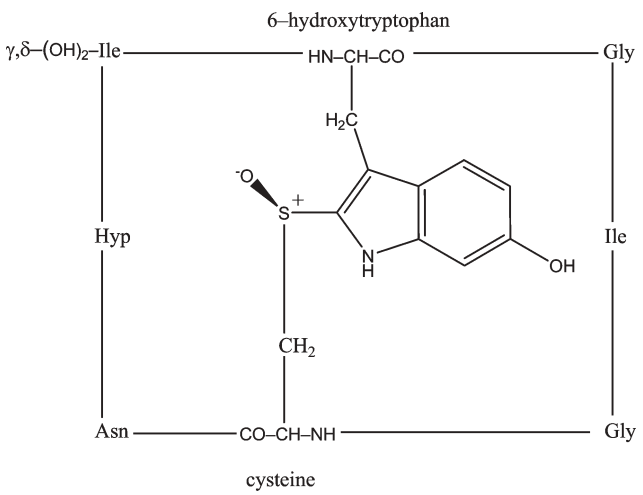

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Fig. 21 α-Amanitin.

to the interior of the polypeptide. Studies of the interaction between RNA polymerase and the amanitins did not reveal critical interactions involving the SO group and a precise connection between sulfur stereochemistry and toxicity was not revealed.

6.7 Sulfoximines

Flour from freshly milled wheat is unsuitable for baking but on aging by long storage the baking properties improve. Various agents have been used to improve flour, including nitrogen trichloride, ''agene''. In 1946, it was estimated that 90% of flour milled in England was agenized.[93] However, agenized flour, used as a substantial component of the diet of dogs gave rise to epileptiform fits and eventual death. Ferrets, but not rats or mice, were also very susceptible. Needless to say, the use of agene is now discontinued.

From agenized gluten, after enzymatic digestion (papain, trypsin) and acid hydrolysis, a highly active crystalline material was obtained by fractionation (3 mg from 17.7 kg of flour).[93] The same material was similarly isolated from zein (a corn protein).[94] It was analysed to have formula $C_5H_{12}O_3N_2S$ and was shown to be methionine sulfoximine. In a sulfoximine, the sulfur atom carries both a nitrogen and an oxygen function. As in a sulfone, $R^1R^2S^{16}O^{18}O$, $R^1 \neq R^2$, the sulfur is chiral with a tetrahedral arrangement and methionine sulfoximine forms four stereoisomers. The absolute configuration of the biologically active isomer (see below) **6** (Fig. 3) was found to be (S_cS_s). Methionine sulfoximine from agenized proteins was not a natural product since it was derived chemically from peptide-bound methionine. However, the same material has now been isolated from fresh seeds of *Cnestis palala*, a tropical woody plant of the family Connaraceae. The isolated material was toxic to beagles.[95]

(S_cS_s)-Methionine sulfoximine inhibits both glutamine synthetase and γ-glutamylcysteine synthetase; the latter enzyme is the first in the glutathione biosynthetic pathway. Glutamine synthetase using ATP adds a phosphate to the sulfoximine nitrogen atom; both methionine sulfoximine phosphate and ADP are tightly bound to the enzyme. Only the (S_cS_s) isomer is phosphorylated, but both (S_cS_s) and (S_cR_s) forms bind reversibly to a single subunit site. Methionine sulfoximine has found extensive use in studying intermediate structures in the reaction of glutamine synthetase.[96]

Methionine sulfoximine analogs, in which the CH_3 group is replaced by other alkyl units (ethyl, propyl, butyl), vary in their inhibitory actions on glutamine synthetase and γ-glutamylcysteine synthetase. Thus, buthionine sulfoximine, *S*-(*n*-butyl)homocysteine sulfoximine, inhibits γ-glutamylcysteine synthetase but not glutamine synthetase. It is the only analog for which stereochemical information is available.[97] The (S_cR_s) isomer was obtained by repeated crystallization of the (S_cS_s) and (S_cR_s) mixture. From the mother liquor of the initial crystallization, containing 66% (S_cS_s) and 34% (S_cR_s) forms, further crystallizations yielded the (S_cS_s) isomer. X-ray crystallography of the (S_cR_s) isomer provided the configuration at the sulfur atom using internal comparison with the known carbon configuration. The (S_cS_s) isomer was bound to and inhibited γ-glutamylcysteine synthetase as the sulfoximine phosphate. In mice, the (S_cS_s) isomer depleted glutathione in liver, kidney and pancreas, but the (S_cR_s) form did not do so in liver or pancreas.

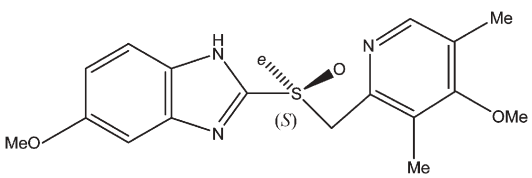
7 Drug molecules containing chiral sulfur atoms

7.1 Sulfoxides

Drug enantiomers often have different physiological actions. While much of this work centers on carbon chirality, sulfur has also been of concern. Many drugs and drug candidates containing sulfur have been synthesized or obtained as natural products (*e.g.*, the antibacterial ''sulfa'' drugs, penicillins and cephalosporins). Many drugs contain the $–SO_2–NH–$ grouping, but a significant number of sulfoxides and sulfones are used.

A very successful sulfoxide drug is the proton pump inhibitor, omeprazole. Investigated in the late 1970s it was marketed as Losec® (Europe, 1988) and as Prilosec® (USA, 1990). Sales quickly approached US $6 billion per annum and Prilosec® is now available ''over the counter'' in the USA. Omeprazole, marketed as a racemate, is a prodrug, the active agent being an achiral sulfenamide formed by biotransformation. Omeprazole interacts with a unique H^+-, K^+-ATPase target and successfully inhibits gastric acid secretion.[98] Indeed, it was acknowledged as the gold standard therapy for treatment of gastric acid-related problems such as erosive esophagitis and gastroesophageal reflux disease (GERD).

However, omeprazole had an inter-individual variability (pharmacokinetics and effect on acid secretion) and a significant number of patients needed higher or multiple doses for relief. Such patients, known as ''slow metabolizers'', lack a liver isozyme of the P450 family, responsible for metabolizing several drugs, including omeprazole. In a search for a drug with improved bioavailability the (S_s) enantiomer of omeprazole **44** (Fig. 22) was found to be clinically superior (bioavailability, more potent in reducing acid secretion) to other materials, including omeprazole.[98] The situation is



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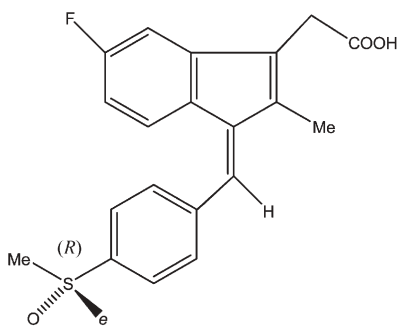

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Fig. 22 Sulfoxide drugs: esomeprazole (Nexium®), **44**, and sulindac, **45**.

reversed in the rat, where the (R_s) enantiomer is more active; in the dog, the enantiomers are equipotent.

The (S_s) enantiomer of omeprazole, esomeprazole, was marketed as Nexium® in 2000. By 2001, USA sales of Prilosec® had declined 2.3% as those of Nexium® grew rapidly. Nexium® became the number 1 best selling drug in the USA, 1999/2000, but has subsequently declined to 5th place for the 12 months ending September, 2003. In addition to treatment of GERD and other gastric-related problems, Nexium® is used in combination with two antibiotics to eliminate *Helicobacter pylori*, a leading cause of peptic ulcer.[98]

Enantioselective chemical oxidation of an achiral sulfide has been spectacularly successful for the industrial preparation of Nexium®. The reagent for oxidation included titanium tetraisopropoxide and (*S*,*S*)-(−)-diethyl tartrate as the chiral influence. An enantioselectivity of >94% was achieved and in plant operation, mass yields were >90%.[99] The use of the (*S*,*S*)-(−) tartrate enantiomer is unusual since in most applications of this system, the (*R*,*R*)-(+) enantiomer is used.

Sulindac, a sulfoxide used as an antiarthritic, is marketed as a racemate. It is a prodrug, being converted to the active, sulfide form. Re-oxidation of the latter to the sulfoxide is possible, as is the formation of the sulfone, an inactive metabolite, by continuing oxidation. While there are apparently no pharmacological studies with the sulindac enantiomers, the biochemistry of sulindac sulfide oxidation has been studied.[100] Sulindac sulfide was a good substrate for a purified FAD-containing monooxygenase, EC 1.14.13.8, from hog microsomes; the (*R*)-(+)-sulfoxide **45** (Fig. 22) was formed with high purity. With microsomal cytochrome P450 isozymes, the same chirality was observed, but sulindac sulfide was a poor substrate in these cases. In experiments with the simpler structure, 4-tolyl ethyl sulfide, the FAD-containing monooxygenase also produced the (*R*)-(+)-sulfoxide, whereas cytochrome P450 isozymes produced the (*S*)-(−) configuration. A similar result was obtained with a cyclohexanone monooxygenase from *Acinetobacter* sp.

More recently, sulindac reduction by *E. coli* preparations containing methionine *S*-oxide reductases has been studied.[101] There are at least six such reductases in *E. coli*. The MsrA activity (see earlier) reduced the (*S*) enantiomer of sulindac, whereas a membrane associated preparation [with activity to (S_cS_s)-MetO and (S_cR_s)-MetO in both free and protein-bound forms] reduced primarily the (*R*) enantiomer. It is proposed that MetO reductases in liver may be responsible for sulindac reduction in mammals.

Although investigations are less complete than for Prilosec® and Nexium®, other drugs showing enantiomeric differences include aprikalim, [RP52891, the (−)-(R_cR_s) enantiomer of racemic RP49356], and BOF 4272, a compound of possible use in treating hyperuricemia. The benzimidazole, albendazole, is a sulfide prodrug being converted to the active (+) sulfoxide, the major blood component. Two microsomal systems catalyze the oxidation: cytochromes P450 form predominantly (−) sulfoxide and flavin containing monooxygenases form the (+) enantiomer. Drugs showing more rapid clearance of one enantiomer, or promoting chiral inversion, include flosequinan, modafinil and pantoprazole.

7.2 Sulfonium salts as muscarinic agonists

In work relating to the much studied acetyl choline, the sulfur isosteres of 3-acetoxy-*N*-methylpiperidine were examined as muscarinic agonists.[102] The sulfur compound, 3-acetoxy-1-methylthiane, is a sulfonium salt; it has a higher barrier to pyramidal inversion than the piperidine so that *cis* and *trans* isomers with chiral sulfurs can exist. There are four stereoisomers: a pair of *cis* enantiomers (R_cS_s and S_cR_s) **46a** and **46b** (Fig. 23) and a pair of *trans* enantiomers (R_cR_s and S_cS_s) **47a** and **47b**. These ring structures (5 carbons, 1 sulfur) assume a variety of conformations (compare pyranoses, 5 carbons, 1 oxygen). Chair conformations are of most interest and can be "inverted" as in the $^4C_1 \leftrightarrow {}^1C_4$ pyranose case. Thus, the *cis* enantiomer (R_cS_s) can interconvert between a structure with both methyl and acetoxy groups equatorial **46a** and one with both substituents axial **46b**. The *trans* enantiomer (R_cR_s) can interconvert between a structure with methyl axial and acetoxy equatorial **47a** and one with methyl equatorial and acetoxy axial **47b**. The 3-acetoxy substituent prefers the axial position and this form predominates in solution; thus the ratio **46a**:**46b** = 29:71, and **47a**:**47b** = 14:86. For the four isomers of 3-acetoxy-1-methylthiane, the *trans* forms are the most potent with the (+)-(R_cR_s) form having a greater effect as a muscarinic agonist than the enantiomer, (−)-(S_cS_s). The absolute configuration of the latter was determined by X-ray crystallography.[103]

8 Conclusion

While not quantitatively comparable to chiral carbon, the chirality of sulfur compounds is a significant factor in biology. However, not all of the physiological responses observed with chiral carbon are duplicated with chiral sulfur. Thus, many flavoring and odoriferous materials are sulfur compounds, but there are apparently no cases of different tastes or odors for the enantiomers of a material with chirality dependent only on sulfur. New aroma compounds continue to be isolated; perhaps an aroma or taste difference for enantiomeric sulfur compounds awaits discovery. That there were odor differences between enantiomeric carbon compounds was long a contentious issue.

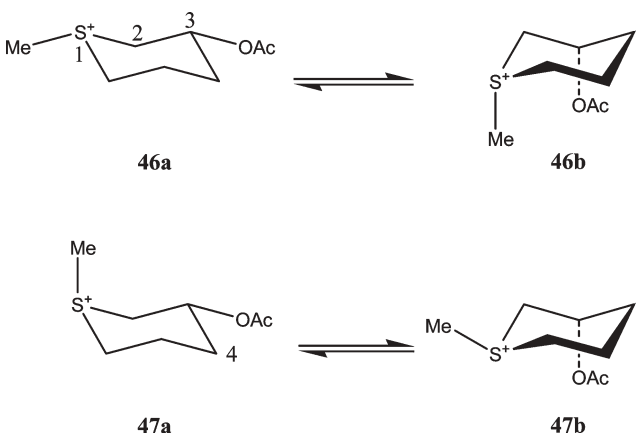

Fig. 23 Muscarinic agonists. Isomeric forms of 3-acetoxy-1-methylthiane.

Chiral sulfonium salts, sulfoxides and sulfoximines have stereoselective interactions with enzymes in general metabolism. For a compound with a single chiral sulfur, the interactions are enantioselective; for compounds with both chiral sulfur and carbon, the interactions are diastereoselective. The very important metabolite, AdoMet, is a chiral sulfonium salt. The sulfur chirality in this compound leads to countless examples of stereoselective behavior, yet, strangely, the stereochemistry of AdoMet has usually been ignored in textbooks of biochemistry and molecular biology. Moreover, many interesting examples of stereoselective behavior are found in the reactions of sulfides, both chemical and enzymatic, as well as with intact organisms.

Sulfur chirality can exemplify structure–activity relationships, for instance when dealing with drug enantiomers. As with chiral carbon drugs, enantiomers of sulfur-containing drugs are of increasing interest and, as noted earlier, a very successful ''racemic switch'' has been accomplished with the marketing of the sulfoxide, omeprazole, as the single (*S*) enantiomer. More examples may be expected. There is much of interest and value in considering physiological situations depending on the chirality of sulfur.

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Ronald Bentley
Department of Biological Sciences, University of Pittsburgh, Pittsburgh, PA 15260, USA. E-mail: rbentley@pitt.edu

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