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The Toxicity of Sulfonium Compounds

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THE TOXICITY OF SULFONIUM COMPOUNDS

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Sulfonium compounds and their related salts are one class of charged sulfur compounds which play important rôles within biology. Those naturally occurring sulfonium compounds which are essential for biochemical processes within living systems have been summarised. Synthetic compounds, some of which are analogues of established pharmacological agents, possess a variety of biological activities and have been investigated for many potential therapeutic applications including antitumour and neurological properties. Recent developments within this field have been reviewed together with the adverse toxicological implications arising from the biological generation of reactive intermediates and subsequent enzyme interaction.

Key words: *S*-Adenosyl-L-methionine, dimethyl-β-propiothetin, *S*-methyl-L-methionine, sulfocholine, sulfonium compounds, toxicity

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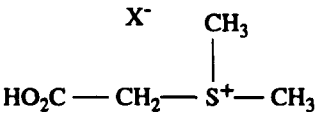
1. INTRODUCTION

The first synthetic sulfonium compounds prepared over a century ago[1,2] possessed quite simple chemical structures based on (carboxymethyl)dimethylsulfonium salts **1**, making them comparable to betaine, [(carboxymethyl)trimethylammonium hydroxide (inner salt)] **2**, one of the quaternary ammonium compounds found in plants. Knowledge of the remarkable biological properties, both pharmacological and toxicological, of these charged ammonium derivatives undoubtedly provided the incentive for the synthesis and examination of analogous sulfur compounds.[3,4] Astutely aware of this relationship, the researchers called their new class of sulfur-containing compounds thetines, as they explained, "*We have given the substances the name thetine to recall its relation to betaine and the fact that it contains sulphur*".[1,2]



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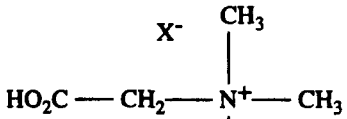

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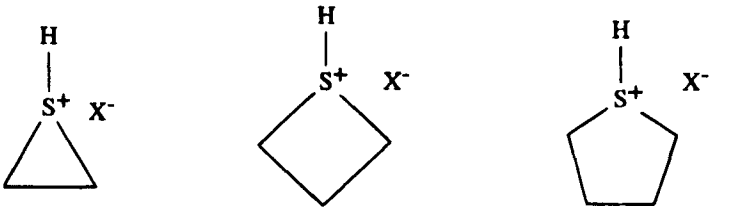
2. STRUCTURAL CHARACTERISTICS AND CHEMISTRY

The parent compound of this group is the simple sulfonium ion H_3S^+ which is structurally analogous to the hydroxonium (hydronium; oxonium) ion H_3O^+. Substitution of all the hydrogen atoms with organic groups such as methyl or ethyl results in the formation of the respective sulfonium derivatives, trimethylsulfonium and triethylsulfonium. The sulfur atom in all of these compounds is formally trivalent bearing an overall positive charge, and the outer electronic cover is best considered as having five electrons which reside within three bonds and one lone pair. Most simple sulfonium compounds are pyramidal, but introduction of bulky substituents (eg. phenyl) distorts the conformation as a consequence of steric repulsion.[5] Sulfonium compounds may also exist as cyclic structures, the simplest being the three-membered ring associated with the thiiranium or episulfonium ion **3**. Other ring systems which have been identified and studied include the four-membered thietanium ion **4** and the five-membered thiolanium ion **5**.



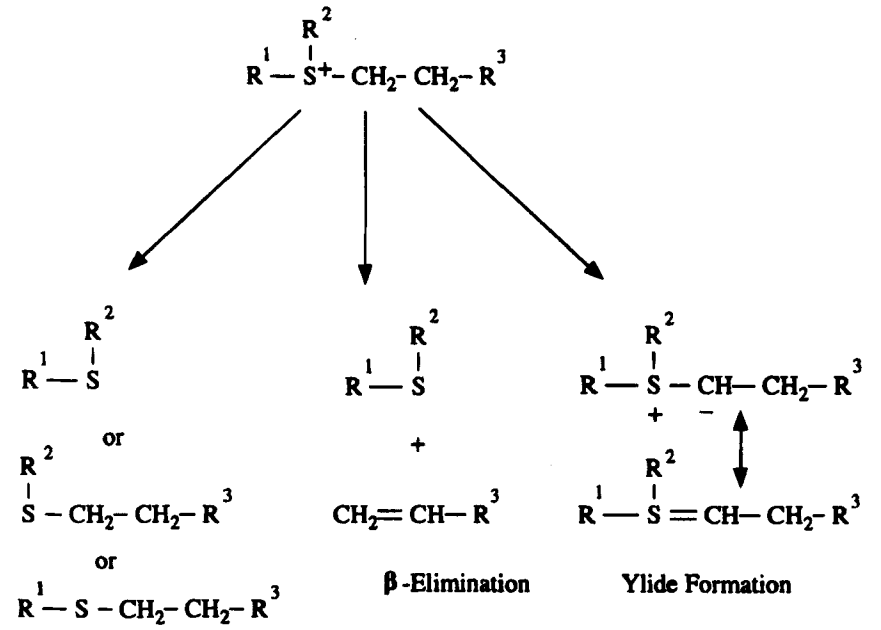
3 4 5

The sulfonium compounds were the first by which the potential optical activity of sulfur derivatives was established. Optical resolution of the camphorsulfonate and bromocamphorsulfonate of ethylmethylthetine[6,7] into their enantiomeric forms at the turn of the century were landmark discoveries in sulfur chemistry. It was realised that these enantiomers only had three groups attached to the active centre, the forth being a true ion, the sulfonium pole. The discovery of optically active sulfoxides[8,9] made it certain that a tricovalent sulfur atom could form an asymmetric molecule, raising the importance of the lone pair of electrons. A large number of optically pure sulfonium salts have been prepared, either by resolution or by stereospecific synthesis.[10]

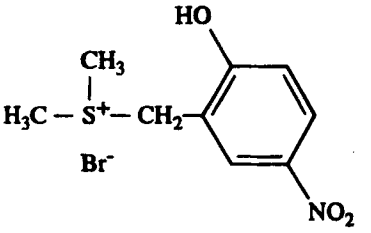
The chemical properties of sulfonium salts are well understood and have been more than adequately reviewed in the literature.[11–15] The most important reactions applicable to the biological field are (*i*) nucleophilic displacement in which there is C-S^+ bond fission, (*ii*) β-elimination which yields an olefin and a thioether, and (*iii*) α-deprotonisation resulting in ylide formation in which the sulfur atom stabilises the adjacent carbanion[16] (Scheme 1). One of the most important features of sulfonium salts is their relative instability in solution, particularly in alkaline environments. Complex mixtures of decomposition products are formed, presumably as a result of the sulfonium salt undergoing one or more of the above mentioned reactions.

This in turn has contributed to numerous difficulties associated with the isolation, characterisation and quantitation of these compounds. By their very nature sulfonium salts are polar, water-soluble, ionic compounds; factors which do not assist their analysis. A variety of techniques have been employed with limited success such as gravimetric analysis, titrimetric and colorimetric methods, thin-layer and paper chromatography, polarography and ultraviolet spectroscopy.[17] However, recent advances in high-pressure liquid chromatography have made possible the separation and quantitation of sulfonium compounds with diverse chemical structures in different types of biological and chemical matrices. Of particular importance has been the development and application of ion chromatography using conductivity detection[18–21] as well as conventional reverse phase or cation-exchange chromatography employing ultraviolet detection.[22–26]

The chemical reactivity of the sulfonium group has been found in certain instances to be advantageous when specifically incorporated into a derivatising reagent. Such compounds have found numerous applications not only in analytical chemistry but also in protein and



Scheme 1



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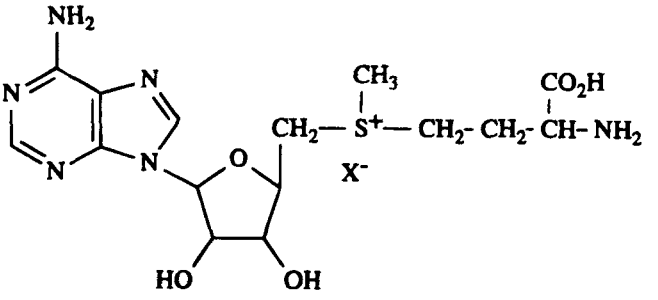
enzyme biochemistry. One such reagent, dimethyl (2-hydroxy-5-nitrobenzyl)sulfonium bromide **6** was found to react selectively with tryptophanyl residues in proteins under mild conditions.[27] This compound has proved to be a useful tool by which the role of this amino acid moiety can be studied in relation to the functional activity of an enzyme. Some enzyme systems which have been studied in this way include human plasminogen,[28,29] human thrombin,[30] human antithrombin,[31-33] rabbit muscle creatine kinase,[34] rabbit skeletal muscle myosin subfragment (S-1),[35,36] bovine pancreatic carboxypeptidase A,[37] yeast lactate dehydrogenase[38] and yeast glyceraldehyde-3-phosphate dehydrogenase.[39] The strong alkylating

properties of trimethylsulfonium and triethylsulfonium hydroxide have been used to derivatise theophylline,[40] pentachloro- and tetrachlorophenols[41] as well as bacterial fatty acids[42,43] prior to their analysis by gas-liquid chromatography.

3. SOURCES OF SULFONIUM COMPOUNDS

3.1. *Naturally Occurring Compounds*

3.1.1. *S-Adenosyl-L-methionine (S-(5′-deoxyadenosyl-(5′)-L-methionine)* S-Adenosyl-L-methionine **7** and its demethylated derivative [S-adenosyl-(5′)-3-methylthiopropylamine] appear to be widely distributed throughout nature, virtually all living tissues contain these compounds.[44] Within intermediary metabolism S-adenosyl-L-methionine is involved in so many of the cellular metabolic reactions that its function and status in cellular terms has been compared to that of adenosine triphosphate. It is a compound for which new roles are being discovered continually and the subject of many excellent books and reviews.[44–51]



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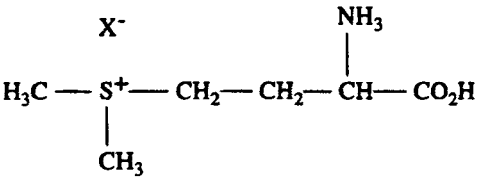
S-Adenosyl-L-methionine is known to interact with the catecholamine (β-adrenergic, dopaminergic) and related serotonin (5-hydroxytryptamine) neurotransmitter pathways within the central nervous system, leading to elevated serotonin and 5-hydroxyindoleacetic acid levels in some regions of the rat brain.[50] The continual search for compounds which alter catecholamine and indoleamine metabolism in the brain, deviations in which have been proposed as a biochemical basis for depression, led S-adenosyl-L-methionine to undergo clinical evaluation as an antidepressant agent[52] where it was found to be equally effective in treating the disorder when compared to standard regimens employing tricyclic antidepressants. S-Adenosyl-L-methionine has also been proffered to treat malnourished patients, especially with liver disease and cholestasis[53] as well as having anti-inflammatory and analgesic activities.[49,50]

3.1.2. *S-Methyl-L-methionine (methionine methylsulfonium)* This compound **8** has been found exclusively within the plant kingdom, being isolated from many vegetables such as cabbage, kohlrabi (turnip-cabbage) and related crops where it may account for up to 0.2% of the tissue dry weight[54] as well as fruit,[55] potato[56] and green tea.[57] Evidence suggests that it plays a significant role as a methyl donor during the biosynthesis of methionine and that it may also serve as a source of ethylene during the ripening of fruit and the development of flowers. Its presence in cow's milk probably results via direct transferance from the herbivore's diet.[58]



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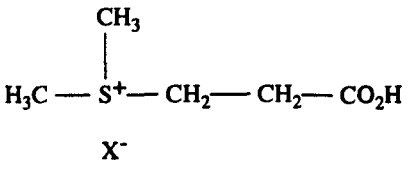
S- Methyl-L-methionine was originally established as having anti-ulcer properties when extracts of cabbage leaves were shown to prevent the development of experimentally induced gastric ulcers in guinea-pigs. The active factor extracted from fresh cabbage juice was termed vitamin U (anti-gizzard erosion factor; ulcer-preventative factor) and for a time it was fashionable to drink cabbage-water in the hope of enouraging the healing of peptic ulcers. Attempts to establish the effectiveness of *S*-methyl-L-methionine in the treatment of ulcers in man on a rational clinical basis has led to many studies[59–62] especially in Russia and Japan where other related clinical applications such as the stimulation of skin regeneration and wound healing[63] and anti-inflammatory properties are also being investigated.[64,65]

3.1.3. *Dimethyl-β-propiothetin (2-carboxyethyldimethylsulfonium)* Dimethyl-β-propiothetin **9** appears to be restricted to aquatic life and has been extracted from many different forms of algae, plankton and marine invertebrates.[66–69] The fact that these minute creatures are the principal foods of many fish and filter-feeders has presumably led the detection of dimethyl-β-propiothetin in molluscs, crustacea and fish.[70,71] It was the first sulfonium compound to be isolated in pure form from a living organism; the marine algae *Polysiphonia fastigiata* and *Polysiphonia nigrescens*.[68,72] A related tertiary sulfonium compound, 4-dimethylsulfonio-2-methoxybutanoate, has also been identified in marine algae.[73] Both of these compounds, when present, occur in very high concentrations, dimethyl-β-propiothetin comprising up to 2–9% of the wet weight of some algae,[66,74] suggesting that they have important biological functions, such as overcoming salt or water stress within these organisms.[75]



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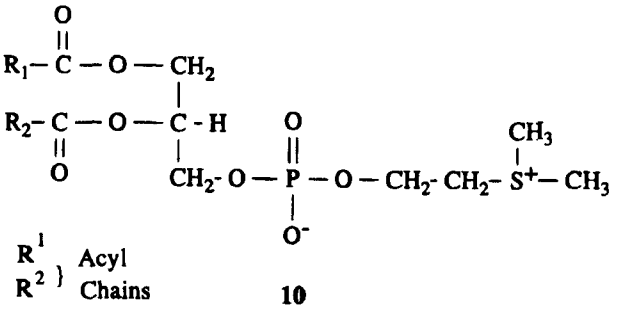
Owing to structural similarities with *S*-methyl-L-methionine, dimethyl-β-propiothetin has also been examined as an anti-ulcer agent and results suggest that it is more effective than the latter compound.[76,77] The methyl ester of dimethyl-β-propiothetin also stimulates the parasympathetic nervous system.

3.1.4. *Sulfocholine (2-hydroxyethydimethylsulfonium salt) derivatives* The novel sulfolipid, phosphatidyl sulfocholine **10**, has been found in a variety of marine diatoms, *Cylindrotheca fusiformis, Navicula pelliculosa, Nitzschia alba, Phaeodactylum tricornutum*[78–80] and appears to replace the function of the ubiquitous phospholipid, phosphatidyl choline, in cell membranes.[81] The absence of nitrogen-containing phospholipids (eg. phosphatidyl choline, phosphatidyl ethanolamine, phosphatidyl serine) encountered in other living organisms suggests an evolved intermediary metabolic system where the charged sulfur moiety replaces the more usual quaternary ammonium arrangement. The sulfocholine fragment probably arises from methionine via dimethyl-β-propiothetin, which, as previously mentioned, is present in many marine organisms.[82] Perhaps these single-celled algae (*Bacillariophyta*) have retained a closer link to the common sulfur-metabolising progenitor of life; an interesting curiosity.

2-Hydroxyethydimethylsulfoxonium salt **11**, which is the sulfoxide of sulfocholine, occurs in the marine bryozoan *Alcyonidium gelatinosum*. These small creatures live in aggregate colonies but maintain separate identities, each having an identifiable alimentary canal. The sulfonium salt is thought to act as a hapten; on repeat exposure it initiates a hypersensitivity reaction and is responsible for causing a form of eczema and contact dermatitis (colloquially known as the 'Dogger Bank itch') which is prevalent amongst North Sea fishermen.[83,84]

3.2. *Synthetic Sulfonium Compounds*

3.2.1. *Analogues of known pharmaceutical compounds* Exploring the mechanisms of action of pharmacological agents amidst the search for more potent derivatives with lower incidences of side-effects, has led to the development of several sulfonium analogues of current therapeutic agents, where the sulfur moiety becomes the important heteroatom at the functional site.



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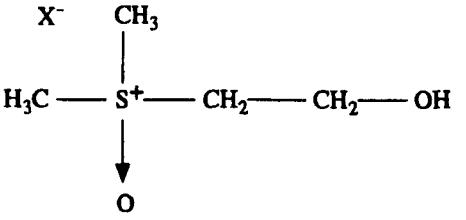

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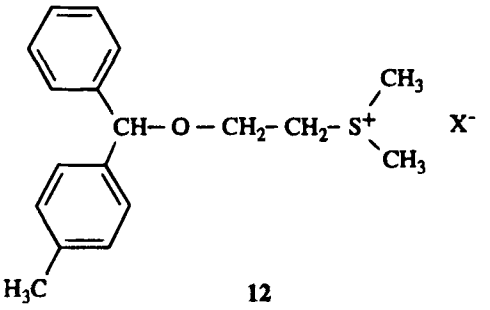
Sulfonium analogues of diphenhydramine hydrochloride, a widely employed antihistamine used for the symptomatic relief of allergy, also exhibited antihistamine activity with one compound **12** being five times more effective than the parent nitrogen-containing molecule.[85,86] A sulfonium analogue of theophylline, the iodide salt of 7-(β-methylthioethyl)-theophylline **13**, has been shown to have the same but weaker pharmacological properties as theophylline,[87] and sulfonium derivatives of 1-aroyl-4-oxo-4-aryl-2-butenylides **14** posses antimicrobial activity.[88] The sulfonium **15** and 2-benzylsulfonium **16** analogues of platelet activating factor (PAF) display a high degree of cytotoxicity towards HL60 cells whilst exhibiting weak hypotensive and platelet aggregating activity.[89]

3.2.2. *Antitumour activities* The structure-activity relationships and drug design of dimethylsulfonium compounds as biological response modifiers and as antitumour agents is a subject of contemporary interest. In particular, tris(2-chloroethyl)sulfonium halides and alkylbis(2-chloroethyl)sulfonium halides, which have highly reactive alkylating functional groups, exhibited a growth suppressing effect on two tumour cell lines, the Yoshida ascites sarcoma of rats and the Ehrlich carcinoma in mice.[90,91] A recently developed dimethylsulfonium compound, 'Suplatast Tosilate®' **17**, designed to treat allergy disorders, acts as a biological response modifier and suppresses IgE synthesis in both mice and humans.[92-94] Other sulfonium compounds reported to have antitumour activity in experimental systems include a number of substituted phenylacyl sulfonium bromides[95] and the monovalent



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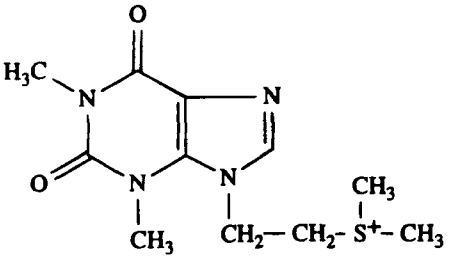

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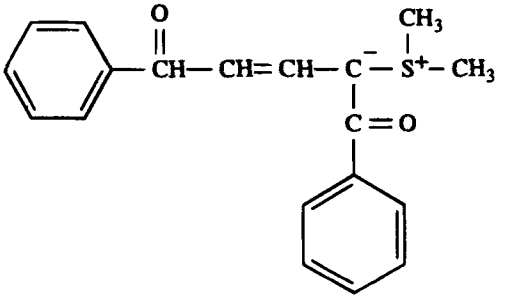

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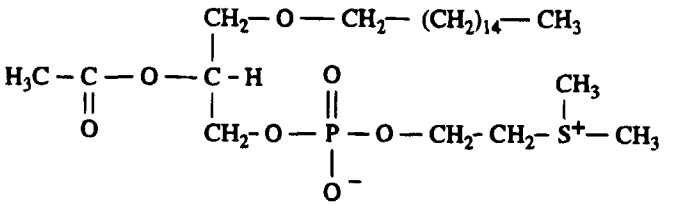
lipophilic cation 2,6-bis(4-aminophenyl)-4-[4-(dimethylamino)phenyl]thiopyrylium chloride **18**.[96] Clinical activity appears to be restricted to the major component of the antitumour drug 'Bleomycin', bleomycin A_2 **19**, which has within its complex molecular structure a sulfonium group. This compound is an important clinical agent used to treat head and neck tumours, testicular cancer and Hodgkin's disease.[97,98]

3.2.3. *Analogues of neuroactive compounds* In neurological studies, where sulfonium derivatives have been examined for their action within neural tissue, it has generally been found that the sulfonium analogues exhibit the same effect as their nitrogen-containing counterparts but to a lesser degree.[99]

This can be exemplified by the sulfonium analogue of choline, sulfocholine, which can be substituted for choline in phospholipid biosynthesis[81,100] and serve as a substrate for choline acetyltransferase to yield acetylsulfocholine,[101] the latter compound possessing pharmacological properties resembling acetylcholine but being less potent.[102] In addition, the sulfonium analogues of polymethylenebis(trialkylammonium) salts and succinylcholine, all neuroactive compounds, have been synthesised and evaluated as neuromuscular blocking agents. Amonst these were the salts of hexamethylene-1,6-bis(dimethylsulfonium) **20**,



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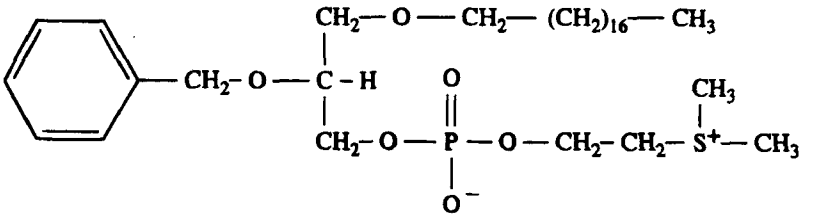

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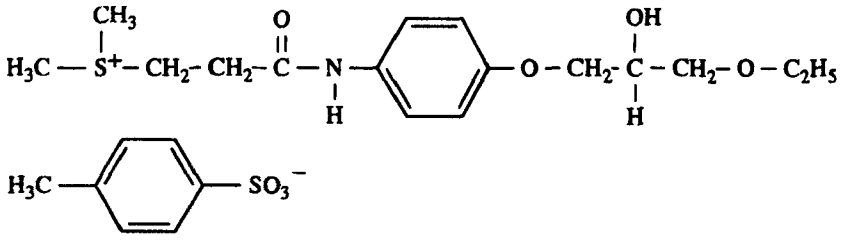

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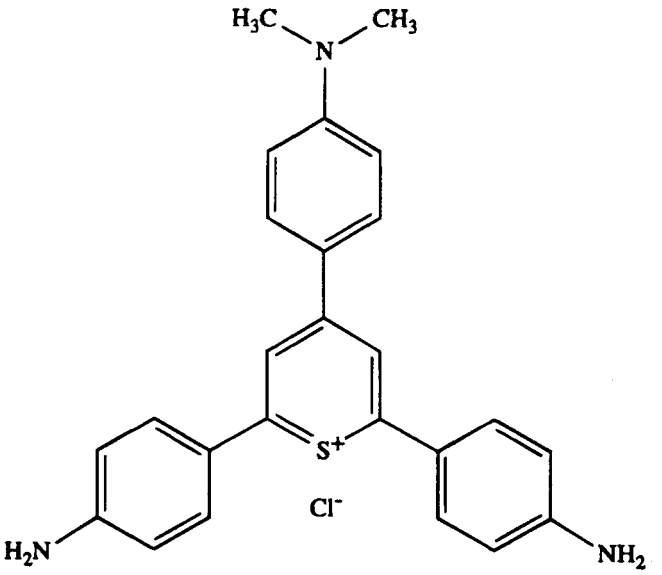
[103] decamethylene-1,10-(dimethylsulfonium) **21** [104] and succinylbis(sulfocholine) **22** [105] which, like their more potent nitrogen counterparts, were found to possess the ability to block neuromuscular transmission.

4. TOXICOLOGICAL INTERACTIONS

4.1. *Enzyme Interaction*

A number of sulfonium salts have been found to be specific cellular enzyme inhibitors. It has been shown that both triphenylsulfonium chloride and the monovalent lipophilic cation 2,6-bis(4-aminophenyl)-4-[4-(dimethylamino)phenyl]thiopyrylium chloride **18** are



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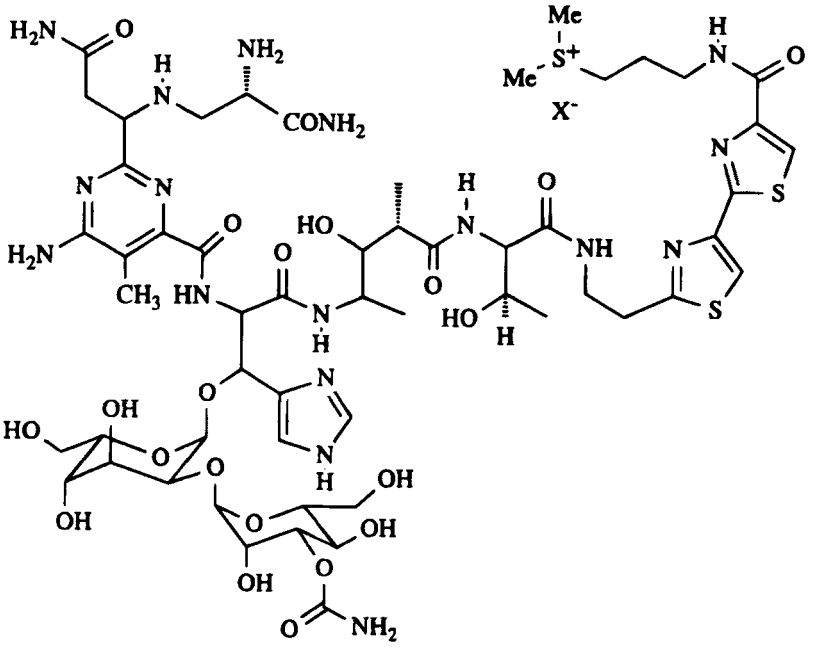

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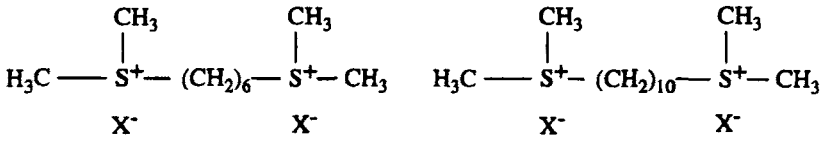

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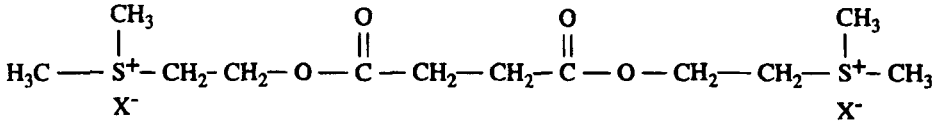

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selectively taken up by the mitochondria of cells and there inhibit oxidative phophorylation and adenosine triphosphatase activity as well as the electron-transfer system in the NAD-cytochrome b regions of the respiratory chain.[96,106] These interactions have been postulated to account for the tumoricidal properties of 2,6-bis(4-aminophenyl)-4-[4-(dimethylamino)phenyl]thiopyrylium chloride.

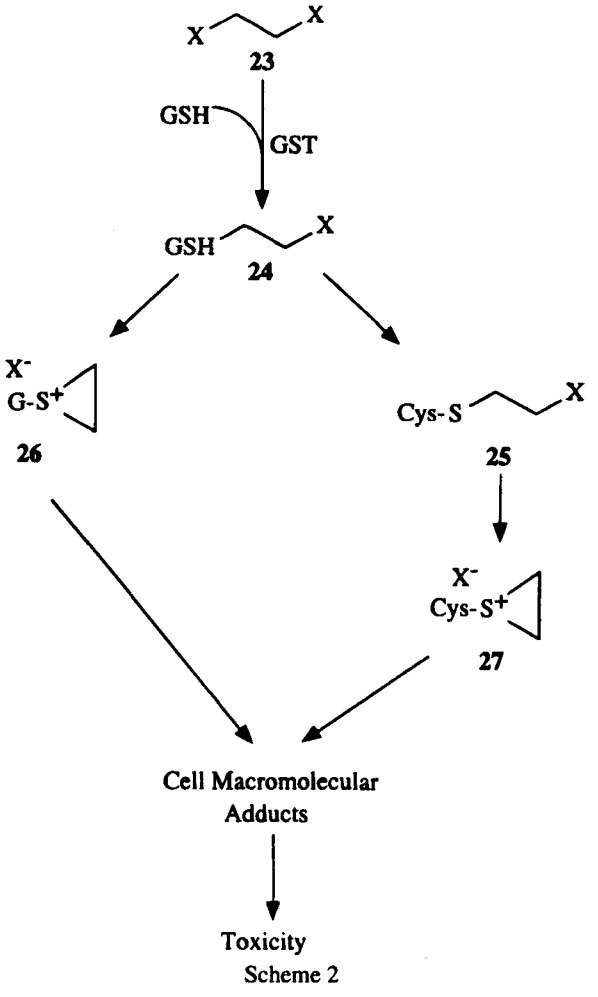
Compounds such as decamethylenebis(dimethylsulfonium) bromide and *n*-octadecyldimethylsulfonium bromide are potent inhibitors of phospholipase C, as are several other alkysulfonium and alkyldisulfonium salts.[107,10] As mentioned previously, sulfonium compounds are also capable of interfering with neuronal activity. Thus bis- and poly-onium molecules which contain sulfonium moieties, as well as monoalkylsulfonium compounds such as trimethylsulfonium iodide, display anticholinesterase activity as well as neuromuscular blocking activity.[109,110] Such compounds must be considered as potentially toxic and treated with due care.

4.2. *Formation of Reactive Intermediates*

Whilst few direct toxicological studies have been undertaken on sulfonium compounds the formation of sulfonium intermediates and their interaction with cellular processes have featured in a number of toxicological mechanisms.

It has been proposed that thiiranium or episulfonium ions are the ultimate toxic species associated with the toxicity of 1,2-dihaloethanes **23**,[51,111,112] a group of compounds once commonly used as soil fumigants, gasoline additives, solvents and synthetic intermediates. These compounds are now known to be mutagenic and carcinogenic and to be responsible for the production of tumours at multiple sites including the adrenal glands, kidney, liver, lungs, mammary glands, skin and stomach.[113–116]

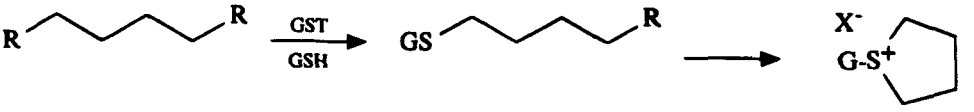
The 1,2-dihaloethanes are probably metabolised to the corresponding *S*-(2-haloethyl)glutathione conjugate **24** via glutathione S-transferase catalysed displacement of a halogen (Scheme 2). As expected, these tripeptide conjugates are degraded to the cysteine conjugate **25** by metabolic pathways associated with mercapturic acid formation. Both the glutathione and cysteine conjugates may be considered as biosynthetic sulfur half-mustards which have strong alkylating and electrophilic properties attributable to neighbouring group interactions in nucleophilic displacement reactions.[117,118] Consequently, highly reactive episulfonium ions **26,27** are formed through internal displacement of the second halogen atom by the sulfur atom. It is postulated that these intermediate species react with biological macromolecules forming covalent adducts which



Scheme 2

in turn give rise to toxicity.[119,120] Other haloalkyl compounds such as 1,2-dichloropropane[121] and 1,2-dibromo-3-chloropropane[122] are assumed to elicit toxicity by forming similar reactive episulfonium intermediates.

However, compounds such as 1,4-dihaloalkanes, for example the chemotherapeutic agent, 1,4-butanediol dimethanesulfonate[123–127] and the known mutagens 1,4-dibromobutane and 1,4-diiodobutane[128–130] are also mainly metabolised by glutathione conjugation, forming an unstable conjugate which rapidly cyclises to a five-membered thiolanium ion glutathione conjugate (Scheme 3). In comparison to the episulfonium ion, the thiolanium ion is chemically a more stable entity and therefore unlikely to react with celular macromolecules. The mechanism of toxicity of these particular compounds must lie elsewhere.

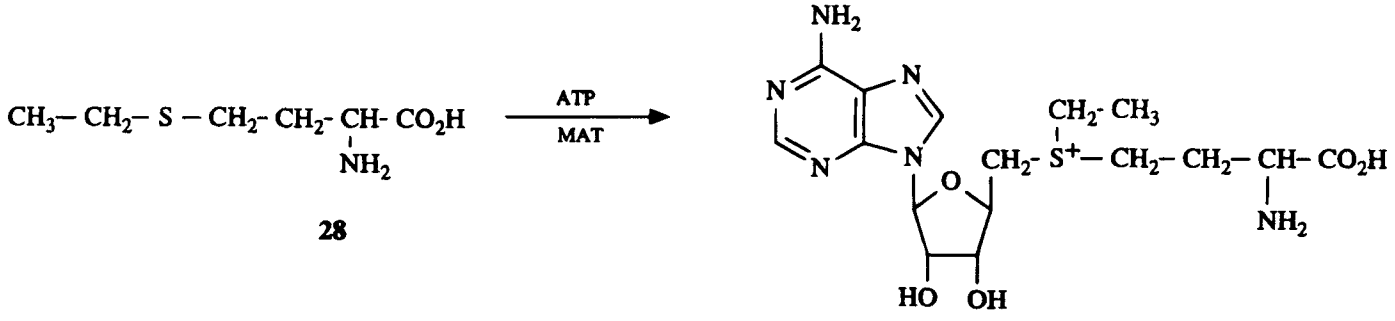


Scheme 3

4.3. *Sulfonium Compounds in the Metabolism Process*

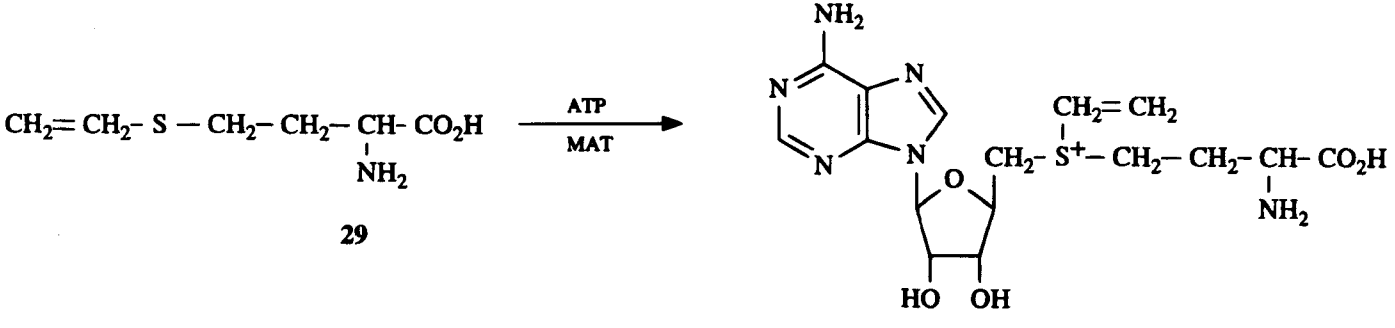
Bioactivation has also been evoked in the hepatocarcinogenicity of ethionine **28** and vinthionine **29**. The metabolic formation of their corresponding *S*-adenosylated derivatives **30** and **31** is thought to lead to mimicking of endogenous *S*-adenosyl-L-methionine thereby permitting enzymatic transfer of ethyl or vinyl groups to macromolecules such as DNA, with carcinogenic sequelae (Scheme 4). *S*-Adenosylvinthionine would be expected to be much more reactive towards cellular nucleophiles than either vinthionine or *S*-adenosylethionine because of the inductive and electron-sharing stabilisation of the carbanion intermediate formed during nucleophilic addition at the β-carbon atom of the vinyl group.[51,131]

A role for sulfonium intermediates in the metabolism of epoxides has also been proposed. It is suggested that methionine (or other methylthio cellular components) react with the epoxide to form methylsulfonium ions which undergo elimination reactions to yield methylthio metabolites.[51,132] Other evidence suggests that *S*-adenosyl-L-methionine may be directly involved in biotransformation. An *S*-adenosyl-L-methionine dependent thioether transferase has been identified in mice which can metabolise several alkyl sulfides including 2-chloroethyl ethyl sulfide[133,134] and a non-specific *S*-adenosyl-L-methionine dependent *N*-methyltransferase has been identified in rabbit liver which can metabolise a wide range of amines.[51]



28

30



29

31

Scheme 4

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