

BIOLOGICALLY ACTIVE COMPOUNDS OF SEMI-METALS

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Abstract: Semi-metals, viz. boron, silicon, arsenic, selenium, tellurium and astatine form organo-metal compounds, some of which are found in nature and have striking effects on the physiology of living organisms. Representatives of these compounds are, e.g., four boron-containing antibiotics (aplastomycin, borophycin, boromycin, and tatrolon). Silicon compounds, frequently present in "silicate" bacteria, which appear to be members or relatives of the genus *Bacillus*, are known to release silicon from aluminosilicates through the secretion of organic acids. Microbes and microbial enzymes have been used in the biotransformation of organosilicon compounds, and biodegradation of organosilicon under aerobic and/or anaerobic conditions has also been described. Arsenic can be incorporated into complex organic molecules such as arsenosugars and arsenobetaines by, e.g., marine algae and invertebrates, and fungi and bacteria can produce volatile methylated arsenic compounds. Arsenate is typically taken up by cells through phosphate transport systems, whereas arsenite is often acquired via aquaglyceroporins. Additionally, some prokaryotes can use arsenate as a terminal electron acceptor during anaerobic growth and others can utilize arsenite as an electron donor to generate energy. Organoarsenic compounds are also added to chicken feed as antibiotics. Selenium is incorporated into the amino acid selenocysteine that is found in some proteins. Selenide undergoes biomethylation to produce methylselenide and dimethylselenide. Selenium compounds are often used as analogues of sulfur compounds and have important pharmacological effects (e.g. selenium analogues of amino acids, antitumor, antibacterial, antifungal, antiviral, anti-infective drugs, and others). Tellurium is considered to be toxic to most organisms. Although genes correlated with tellurium resistance have been identified, the mechanisms of tellurium resistance are not completely understood. Tellurium-resistant fungi grown in the absence of sulfur and in the presence of tellurite ions incorporate tellurium into amino acids and proteins.

INTRODUCTION

Semi-metals or metalloids are a very small group of elements found in the periodic table of elements along the zig-zag line that distinguishes metals from non-metals and is drawn from between boron and aluminum to the border between polonium and astatine. Elements to the upper right of this line are nonmetals while metals are to the lower left. Together with metals and nonmetals, metalloids form one of the three categories of chemical elements as classified by ionization and bonding properties [1, 2].

Metalloids manifest some of the qualities of the metals and some of the qualities of the nonmetals. Because of their diverse nature, it is very difficult to establish a unique set of physical or chemical characteristics for these materials. There is no unique way of distinguishing a metalloid from a true metal but the most common is that metalloids are usually semiconductors rather than conductors. Also, unlike the metals which become better conductors at low temperatures, the metalloids become better conductors at higher temperatures.

Because of their unique electrical conductivity, they have received intensive attention from the computer and electronics industries. Their relative abundance in the environment is summarized below.

Table 1. Relative abundance of metalloids in the environment

Element	in Earth's crust mg kg ⁻¹	in sea water mg L ⁻¹
As	1.8 - 5.0	0.003
B	3 - 10	4.6
Se	0.05 - 0.09	0.000 09
Si	257 000 - 282 000	3
Te	0.001 - 0.005	0
At	0	0

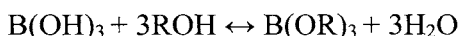
Semi-metallic behavior is not confined to the elements, but is also found in alloys and compounds. When involved in chemical bonding, the metalloids again exhibit intermediate qualities. They are capable of taking electrons from most metals and will readily lose electrons to most nonmetals. Their electronegativity values are also mid-range. Consequently, it is unlikely for them to be involved in ionic bonding; when found in compounds, they will usually establish covalent bonding.

The reactivity of the metalloids depends on the element with which they are reacting. For example, boron acts as a nonmetal when reacting with sodium yet as a metal when reacting with fluorine. In organic compounds, they can substitute other elements such as sulfur. Boron and arsenic form compounds with, e.g., lipids, sugars, phenols, organic acids and some polymers. Arsenic compounds, especially organic ones, are integral components of the food chains of many organisms including humans and may play potentially important biological roles in these organisms. Apart from its soluble inorganic forms, selenium is found in living organisms as protein-bound and free seleno-amino acids and volatile organoselenium compounds. With few exceptions, all small organic selenium compounds in bacteria, yeast, plants, or animals are isologues of sulfur amino acids or their derivatives. The interactions of silicon with living matter are the least understood, but they are vitally important for some organisms such as bacteria, which are known to release silicon from aluminosilicates through the secretion of organic acids, or for unicellular algae – diatoms for which it serves as the basic building block of their bodies. Unlike all the above elements, tellurium is highly toxic and is not thought to be required by biological systems; the highly radioactive astatine is so rare in nature that the existence of astatine compounds with any possible biological significance is highly improbable. All in all, many metalloid compounds, both organic and inorganic, have profound effects on living organisms, whether beneficial or adverse; these are the subject of this review.

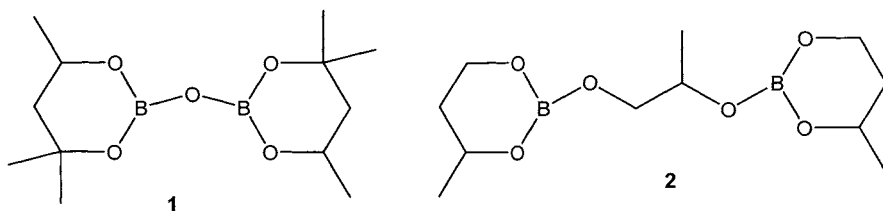
Boron

Boron is a ubiquitous element in rocks, soil, and water. Most of the Earth's soils have <10 ppm boron but large areas of the world are boron deficient. Boron concentrations in rocks range from 5 ppm in basalts to 100 ppm in shales, the average being 10 ppm in the Earth's crust overall. Soils have boron concentrations of 2 to 100 ppm. Seawater contains an average of 4.6 ppm boron, the range being from 0.5 to 9.6 ppm. Freshwaters normally range from 0.01 to 1.5 ppm, with higher concentrations in regions of high boron soil levels [3].

Simple alcohols react with boric acid to give esters. In aqueous solution, this equilibrium usually lies far to the left.



The partially esterified species $(\text{RO})_2\text{BOH}$ and ROB(OH)_2 are probably also involved [4]. Polyhydric alcohols form cyclic esters with boric acid. Both compounds (1) and (2) are mixed to give the jet fuel microbiocide Biobor JF. This additive prevents the growth of *Cladosporium resinae* and *Pseudomonas aeruginosa* in fuel tanks by distributing itself between the water and fuel phases. This hydrocarbon-metabolizing fungus and bacterium grow at the fuel-water interface and can cause corrosion and, e.g., foul fuel filters [5].



Tetrahedral borate or boronate complexes have been shown to be involved in enzyme inhibition. Serine proteases were proposed to be inhibited by boric acid [6], and simple borates have been patented as protease stabilizers in liquid detergent formulations [7, 8].

Reactions such as acylation and deacylation are catalyzed by enzymes through lowering of the energy of activation. Binding and stabilization of the near tetrahedral oxyanion transition state by the active site of the enzyme is how this is accomplished. Thus, a transition state analogue inhibitor is a species, which binds the active site in a way similar to that

of the substrate. Serine hydrolase enzymes react with various borates, boronates, and borinates by forming a tetrahedral complex between the serine hydroxyl group and the boron atom. Hydrogen bonding to the imidazole ring of an adjacent histidine adds further stabilization (Fig. 1).

X-ray structures have been worked out for the benzeneboronic and 2-phenyl-ethaneboronic acid (PEBA) complexes of subtilisin [9] and for the PEBA complex of α -chymotrypsin (α -CHT) dimer [10]. Further stabilization of the hydroxyls on boron is gained by hydrogen bonding to other amido groups lining the oxyanion hole.

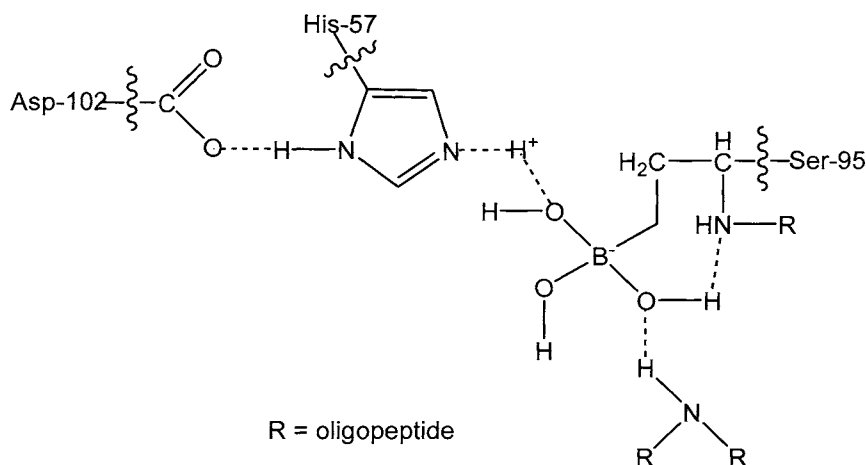
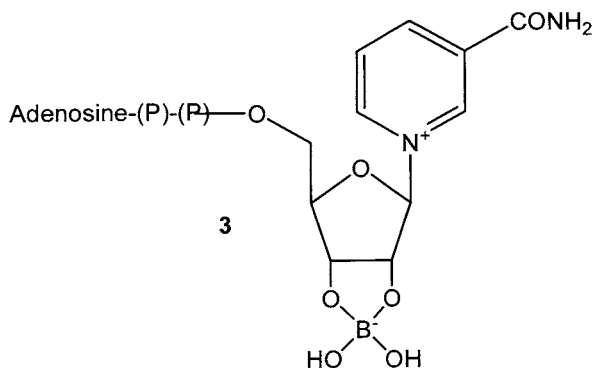


Fig. 1. Complex formation between a peptide and boron atom.

Subtilisin [11] and cholesterol esterase [12, 13] are also inhibited by a range of aliphatic and aromatic boronic acids. Work on inhibiting α -CHT and cell replication with a variety of boronic acids concluded that the α -CHT activity was associated with chromatin in normal and tumorous tissue of mice [14]. Certain boronic acids were demonstrated to inhibit protease activity in rat liver chromatin [15]. It was concluded that good boronate inhibitors of CHT, like PEBA, inhibit cell replication and that this effect is expected to be higher in rapidly proliferating cancer cells than in normal tissue [14].

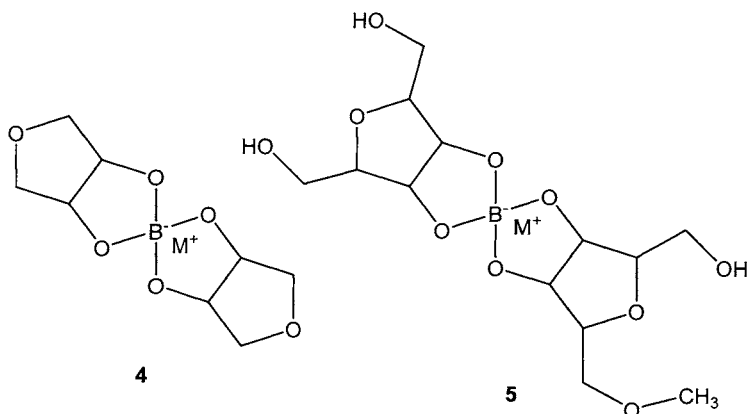
Complexing of the ribose group of nicotinamide adenine dinucleotide (NAD) (3) is preferred electrostatically over that of reduced nicotinamide adenine dinucleotide (NADH), leading to inhibition of this coenzyme system [16].



Compounds capable of complexing with boric acid include sugars or their derivatives, phenols, organic acids, and some polymers [17, 18]. Yamaguchi et al. [19] reported that boron-polysaccharide complexes were solubilized from cell walls of tomato leaves. Later these complexes were isolated from radish (*Raphanus sativus*) roots and characterized [20]. One of the complexes (BR-II) had a molecular weight of 7.5 kDa and contained boron (23%), uronic acid (52%) and neutral sugars (32%). According to ^{11}B -NMR analysis, it is suggested that the boron was present as a tetravalent 1:2 borate-diol complex [21]. ^{11}B -NMR was used to establish that BR-II boric acid links with two rhamnogalacturonan-II chains, which include apiose, aceric acid (3-*C*-carboxy-5-deoxy-*L*-xylose), 2-*O*-methyl-fructose, 3-deoxy-*D*-manno-2-octulosonic acid (Kdo), rhamnose, galactose, arabinose, 2-*O*-methyl-*L*-fucose, fucose, 2-*O*-methyl-*D*-xylose, galactouronic acid, and glucouronic acids residues [21]. Partially acid hydrolyzed BR-II complex from sugar beet (*Beta vulgaris*) was characterized and consisted of two disaccharide moieties: α -*L*-Rhap-(1 $\rightarrow$ 5)-*D*-Kdo and α -*L*-Araf-(1 $\rightarrow$ 5)-*D*ha (3-deoxy-*D*-*lyxo*-heptulosaric acid), an aceric acid-containing oligosaccharide [22]. The monosaccharide constituents of BR-II complexes are interconnected by at least 100 different glycosidic linkages [23]. Chemical fragmentation of the BR-II isolated from the walls of suspension-cultured sycamore (*Acer pseudoplatanus*) cells led to the isolation and structural characterization of two 3-deoxysugar containing disaccharides, i.e., α -*L*-Rhap-(1 $\rightarrow$ 5)-*D*-Kdo [24] and β -*L*-Araf-(1 $\rightarrow$ 5)-*D*-Dhap [25].

Benner and Klufers [26] used a combined X-ray and NMR method and reported for the first time two structures of furanoidic cis-1,2-diol borate

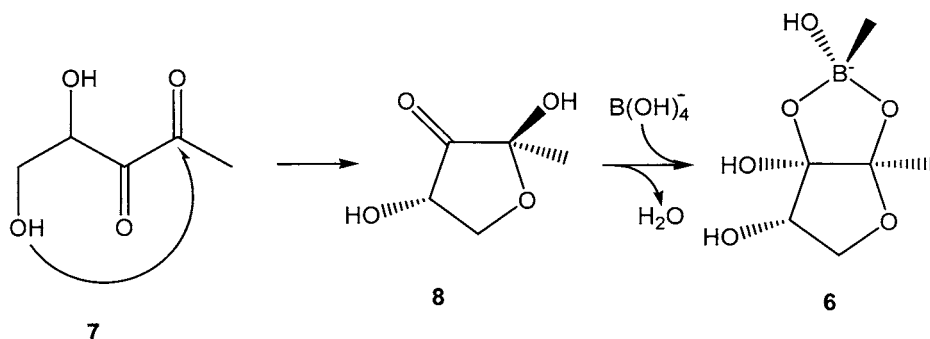
esters: 1,4-anhydroerythritol (4) and methyl β -D-ribofuranoside (5) boron-containing complexes.



The NMR spectroscopic analysis of autohydrolysate fragments obtained from the gel-forming water-soluble cell-wall sulphated polysaccharides from the green seaweed *Ulva* sp. allowed the identification of two major repeating units in ulvan. These were the aldobiuronic acids (1 $\rightarrow$ 4)- β -D-GlcpA-(1 $\rightarrow$ 4)- α -L-Rhap 3-sulphate and (1 $\rightarrow$ 4)- α -L-IdopA-(1 $\rightarrow$ 4)- α -L-Rhap 3-sulphate that were given the name ulvanobiuronic acid 3-sulphate A and B, respectively. The results indicate that the ring hydroxyl groups of the major repeating structures, which contain the iduronic acid rarely encountered in plant polysaccharides, are probably not involved in the boric acid fixation step during the gelation of ulvan [27]. Free and complex forms of accumulated borate in marine green (*Ulva fasciata* and *U. pertusa*), brown (*Laminaria japonica* and *Undaria pinnatifida*) and red algae (*Gloiopeltis tenax* and *Grateloupia turuturu*) were identified by ¹¹B-NMR [28]. Boron-containing complexes with compounds containing vicinal hydroxy groups, such as mannitol, laminarin, and alginic acid were also reported [28].

A novel AI-2 furanosyl borate diester complex (6) was originally identified in the bioluminescent marine bacterium *Vibrio harveyi* as one of two autoinducers that regulate light production in response to cell density [29, 30]. AI-2 is produced from S-adenosylmethionine in at least three enzymatic steps [31, 32]. In the final stage 4,5-dihydroxy-2,3-pentanedione (DPD) (7) is converted to the cyclic form of DPD, which is named pro-AI-2 (8), and can react with borate to form a cyclic borate

diester AI-2 (**6**). AI-2 has been proposed to serve as a ‘universal’ bacterial quorum-sensing boron-containing signal for inter-bacterial community communication [33].



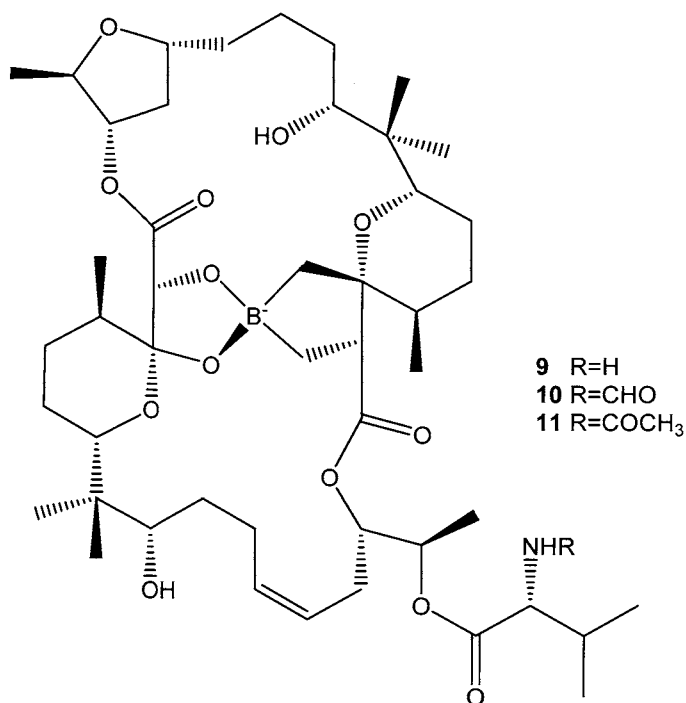
Boromycins

A new strain of *S. antibioticus* was isolated from the soil of Ivory Coast and it was observed that this species contained a novel antibiotic, boromycin, which appears to be the first well-defined boron containing compound to have been found in nature [34]. Boromycin (**9**) shows a growth impeding effect against Gram-positive bacteria, see Table 2. In the concentration 10 µg/ml it has no effect on the following microorganisms: *Escherichia coli* 2018, *Proteus vulgaris* ATCC 10145, *Salmonella typhimurium*, *Klebsiella pneumoniae*, *Aspergillus elegans*, *Trichophyton interdigitale*, and *T. mentagrophytes*.

Table 2. Biological activity of boromycin

Strain	MIC concentrations (in µg/mL)
<i>Staphylococcus aureus</i> SG 511	0.4
<i>Streptococcus mitis</i>	0.08
<i>Bacillus subtilis</i> ATCC 6633	0.3
<i>Bacillus megatherium</i>	0.3
<i>Candida albicans</i>	2
<i>Rhodotorula rubra</i>	1
<i>Paecilomyces varioti</i>	1
<i>Mycobacterium bovis</i>	10

The structure of boromycin was elucidated by X-ray analysis and NMR spectra [35]. The two minor products (**10**, **11**) (*N*-formyl of **9**, *N*-acetyl of **9**) of boromycin fermentation described by Lee et al. [36] differ in that acylation has occurred not on the 9-hydroxyl group but on the nitrogen of the valine moiety.



The strain *S. antibioticus* was cultivated at 28 °C on soybean flour and mannitol and its pH was adjusted to 7.8 [37]. The culture was stirred at ~800 rpm for 72 hours with aeration (volumetric ratio 1:20). The yield of boromycin was 0.167 g/L from a 3200 L fermentor. The test with *Botrytis cinerea* was used to test microorganisms for the presence of boromycin. The following Table 3 shows the various concentrations at which the boromycin inhibits different microorganisms in the plate diffusion test. The numbers give inhibition zones for different concentrations.

Table 3. The plate diffusion test of boromycin

Microorganism	Zone diameter in mm at an antibiotic concentration of		
	1 mg/mL	0.1 mg/mL	0.01 mg/mL
<i>Bacillus subtilis</i>	33	18	10
<i>Candida vulgaris</i>	10	-	-
<i>Saccharomyces cerevisiae</i>	10	-	-
<i>Paecilomyces varioti</i>	28	15	9
<i>Spicaria sp.</i>	21	18	11

The action against protozoa of the genera plasmodiae and babesiae has also been demonstrated [37]. The ED₅₀ of boromycin for *Plasmodium berghei* was 2.2 mg/kg in albino mice while the corresponding ED₅₀ for the routinely used drug [7-chloro-4-(4-diethylamino-1-methyl-butylamino)quinoline-diphosphate)] was 2 mg/kg. A similar test with chicks infected with *P. gallinaceum* causing avian malaria was performed and ED₅₀ was ~7 mg/kg. Another test was done again with albino mice infected with *Babesia rodhaini*; the ED₅₀ was 2.4 mg/kg and LD₅₀ was approximately 15 mg/kg.

A US patent [38] described the use of boromycin for the treatment and prevention of coccidiosis in susceptible poultry. Coccidiosis is a common and widespread bird disease caused by several species of protozoan parasites of the genus *Eimeria* (*E. tenella*, *necatrix*, *acervulina*, *maxima*, *hagani*, *brunette*, *meleagridis*, and *adenoides*). *Eimeria* is the causative agent of a severe and often fatal infection of the caeca, which is manifested by severe and extensive hemorrhage, and accumulation of blood in the caeca. When left untreated, severe forms of coccidiosis lead to poor weight gain, reduced feed efficiency and mortality of the poultry. Boromycin is usually used as a feed supplement in different materials such as corn or soybean meals. The compound is administered in an amount equal to about 0.006 to 0.0125% finished feed by weight. Higher amounts approximately up to about 0.05% by weight of the consumed feed are used for therapeutic treatment of an established coccidia infection.

Boromycin was found to influence the Ca²⁺ homeostasis in both excitable and non-excitable cells [39]. This could be explained by the indirect interaction of boromycin with both Ca²⁺ and Na⁺ transport systems via transmembrane ionic gradients of monovalent cations.

Differences in the effects of boromycin on “resting” transport of Ca^{2+} could potentially be used for determining whether the cells belong to excitable or non-excitable cells.

The DNA-damaging agent bleomycin arrests the cell cycle of Jurkat cells defective in the G1 checkpoint in the G2 phase, and microtubule-affecting colchicine arrests it in the M phase [40]. Boromycin showed no effect on the cell cycle status of Jurkat cells at least up to 340 nM but potentiated anti-tumor activity of bleomycin in SCID mice inoculated with Jurkat cells. These data suggest that boromycin disrupts the cell cycle at the G2 checkpoint of cancer cells selectively, leading to sensitization of cancer cells to anti-cancer reagents.

Boromycin was found [41] to strongly inhibit the replication of a clinically isolated HIV-1 (Table 4) strain as well as a cultured strain in *in vitro* laboratory experiments. The mechanism for the anti-HIV activity of boromycin is suggested to involve blocking the later stage of HIV infection, and probably the maturity step for replication of the HIV molecule.

Table 4. Anti-HIV activity and cytotoxicity of boromycin

Virus	Strain	Cell	Day of analysis	Anti-HIV activity		Cytotoxicity	SI
				IC ₅₀ (μM)	IC ₉₀ (μM)	CC ₅₀ (μM)	
HIV-1	LAV-1	MT-4	6	0.008	0.027	0.22	28
	RF	MT-4	5	0.11	0.18	0.17	15
	KK-1	MT-4	6	0.14	0.31	0.22	16
	KK-1	PBMC	10	<0.1	<0.1	4.3	>43
	KK-1	PBMC	10	1.8	4.8	4.3	2.4
HIV-2	LAV-2	MT-4	6	0.007	0.019	0.22	31

Boromycin at a concentration of 0.05 μg/mL inhibits the synthesis of protein, RNA and DNA in whole cells of *Bacillus subtilis* [42]. It is being antagonized by surface active compounds and is bound to lipoprotein. Binding of boromycin within the cell takes place especially at the cytoplasmic membrane.

The inhibitory effect on *Bacillus subtilis* is being reversed by high concentration of potassium salts (e.g. 0.2 M KCl). The reversion is specific for potassium salts. Addition of boromycin is followed by a discharge of potassium ions from the cells. The K^+ , Na^+ -activated ATPase of the cytoplasmic membrane is not influenced by boromycin. The degradation of boromycin through alkaline and acid hydrolysis leads to a loss of antibiotic activity, due to the boric acid splitting off from the molecule.

Aplasmomycins

A new boron containing antibiotic, aplasmomycin (**12**), was obtained from a strain of *Streptomyces griseus* isolated from shallow sea sediment in Sagami Bay, Japan [43, 44].

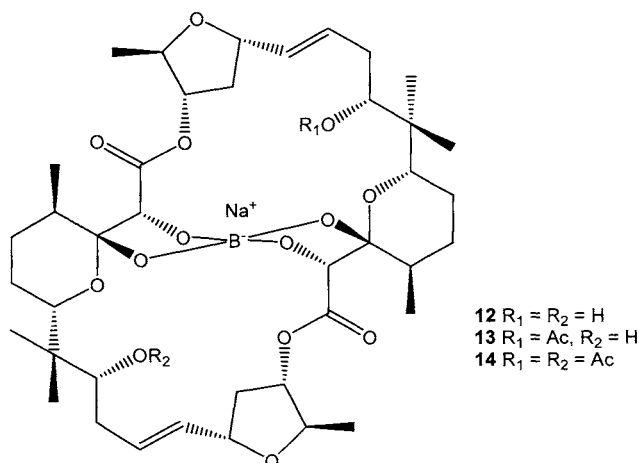
The strain was isolated from marine sediment which is poor in nutrients and fairly high in salt concentration; the effect of NaCl and nutrient concentrations on the production of aplasmomycin was studied in a basal medium containing yeast extract 0.4%, malt extract 1.0% and glucose 0.4%, at pH 7.4. The antibiotic was produced in a medium with diluted nutrients and high salt concentration [45]. The maximum yield (50 $\mu\text{g/mL}$) of aplasmomycin was obtained in medium diluted to 1/16 with deionized water supplemented with 3.0% NaCl and at 27 °C. The production of aplasmomycin in Kobu-Cha medium (with powdered *Laminaria* sp.) was also examined. This medium was used because the *Streptomyces* was isolated from sea mud containing organic residues arriving mostly from algae. Maximum production (125 $\mu\text{g/mL}$) was obtained on supplementation with 1.5% NaCl.

Aplasmomycin inhibits Gram-positive bacteria including mycobacteria *in vitro*, see also Table 5. Male mice were infected intraperitoneally by *Plasmodium berghei* and when aplasmomycin in peanut-oil was administered orally, the number of plasmodium-containing red cells decreased and all treated mice survived. It was on the basis of this potent antiplasmodium activity that aplasmomycin received its name. The acute toxicity in mice was 125 mg/kg by intraperitoneal injection.

Table 5. Biological activity of aplasmomycin

Organism	MIC ($\mu\text{g/mL}$)
<i>Staphylococcus aureus</i> FDA 209 P	1.56
<i>Staphylococcus aureus</i> Smith	1.56
<i>Staphylococcus aureus</i> 193	3.12
<i>Staphylococcus aureus</i> EMf	1.56
<i>Staphylococcus aureus</i> BBC	0.78
<i>Staphylococcus aureus</i> Terajima	3.12
<i>Staphylococcus aureus</i> MS 8800	3.12
<i>Micrococcus flavus</i> FDA 16	1.56
<i>Sarcina lutea</i> PCI 1001	1.56
<i>Bacillus anthracis</i>	0.78
<i>Bacillus subtilis</i> PCI 219	1.56
<i>Corynebacterium bovis</i> 1810	0.78
<i>Mycobacterium smegmatis</i> 607	6.25
<i>Mycobacterium phlei</i>	6.25
<i>Escherichia coli</i> K-12	>100
<i>Salmonella typhi</i> T-63	>100
<i>Candida albicans</i>	>100

The structure of the Ag-salt of aplasmomycin was solved by an X-ray crystallographic analysis [46] and the structure of the aplasmomycin was further proved by ^1H and ^{13}C NMR spectra [47]. It was found that the conformation of aplasmomycin in CDCl_3 solution is identical to that in the solid state and that removal of the boron atom from aplasmomycin results in only a slight conformational change of the molecule.



The strain *Streptomyces griseus* has produced two other minor components, aplasmomycins B and C, under the cultural conditions described previously [48]. Recently, an aplasmomycin C-producing actinomycete was isolated from a sandy sediment sample in California [45].

The three aplasmomycins (**12-14**) were compared in terms of antibacterial activity (Table 6), alkali metal ion selectivity (Table 7) and K^+ transport ability. The results summarized below show that the antibacterial activity of aplasmomycin B (**13**) was nearly equal to that of aplasmomycin, while aplasmomycin C (**14**) showed a weaker activity. It was also noted that the ability to form complexes with other metals does not directly correspond with antibacterial activity. Cation selectivity decreased in the order $Rb > K > Cs = Na > Li$. The three aplasmomycins did not show any affinity towards divalent cations.

Table 6. Biological activity of aplasmomycins

Compounds	<i>Bacillus subtilis</i> ^a	<i>Staphylococcus aureus</i>
12	21.5	22.0
13	22.0	22.0
14	11.0	10.5

^a Diameter of inhibition zone with **12-14** (500 $\mu\text{g/mL}$) determined by the disk diffusion method.

Table 7. Relative affinity of aplasmomycins for various cations

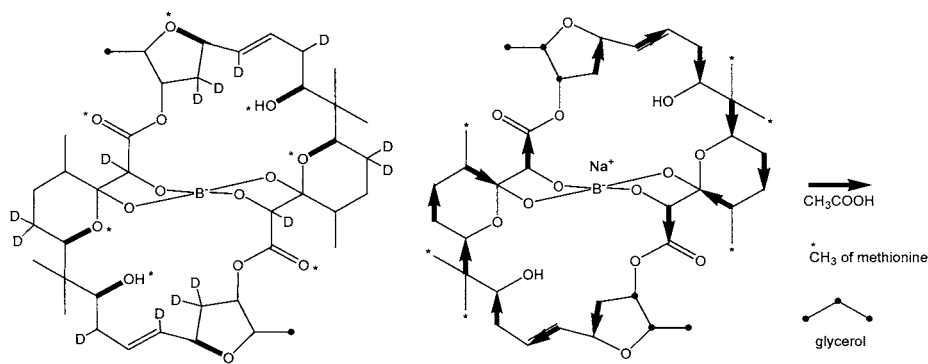
Cations	12	13	14
Na	0.27	0.40	0.45
K	1	1	1
Rb	1.1	1.4	1.5
Cs	0.9	0.6	0.8
Li	0.12	0.15	0.11
Mg	10^{-2}	$<10^{-2}$	$<10^{-2}$
Ca	10^{-2}	$<10^{-2}$	$<10^{-2}$

Labeled precursors, i.e. ($[1-^{13}\text{C}, 2-^{13}\text{C}, 1,2-^{13}\text{C}]$ acetates and L-[methyl- $^{13}\text{C}]$ methionine), were added to shaken cultures of *Streptomyces griseus* strain SS-20 and fermented. The antibiotics thus obtained were analyzed by ^{13}C NMR spectroscopy. The biosynthetic origin of aplasmomycin can therefore be summarized as shown in Fig. 2. Each half of the macrocyclic lactone ring is formed from one glycerol, seven acetate units, and three methyl groups of methionine. Surprisingly, propionate is not incorporated intact, but is converted, with decarboxylation, into acetate via symmetrical intermediates, i.e. succinate and the Krebs cycle. The starter unit of the polyketide thus does not originate from propionate [49].

At least two unusual features characterize this biosynthesis. One is the origin of the methyl branches in the polyketide chain from the methyl group of methionine. This is in contrast to the biosynthesis of most macrolide antibiotics in which chain branches are formed by utilization of the appropriate homologs in place of acetate chain extension units, i.e., propionate units. The second unusual feature of this biosynthesis is the mode of incorporation of glycerol and particularly the fact that it is a specific precursor of the three-carbon polyketide chain starter unit [50].

The following elucidation of aplasmomycin biosynthesis was done with isotopically (D, T, ^{13}C and ^{18}O) labeled precursors. In the biosynthesis of aplasmomycin by *Streptomyces griseus*, seven atoms of deuterium from C-2 of acetate and 4 atoms of oxygen from C-1 of acetate were incorporated into the molecule. The two hydrogens of the *pro-R*-hydroxymethyl group of glycerol are incorporated into carbon-17, giving rise to a chiral methyl group of *S* configuration when (1*R*, 2*R*)-[1-D, 1-T]glycerol is used as substrate. The three methionine-derived C-methyl groups per chain are transferred stereospecifically with inversion of

configuration, but racemization is observed during the formation of the methionine methyl group from stereospecifically labeled [3-D, 3-T]serine. The stereochemical and precursor feeding experiments point to phosphoglyceric acid or phosphoenolpyruvate as the glycerol-derived polyketide chain starter unit, ruling out serine, methylglyoxal, and pyruvate and compounds derived from them [51].



D = deuterium from 2-¹³C,₃D₃-acetate; O* ¹⁸O from 1-¹³C,¹⁸O₂-acetate. Heavy lines connect the ¹⁸O to the carbon from which it originated. '•' = deuterium from (2*R*)-1,1-D₂-glycerol

Fig. 2. Biosynthetic origin of aplasmomycin

A US patent [52] describes the use of aplasmomycins for modifying rumen metabolism in domestic ruminant animals (cattle, sheep, goats, and/or deer), by reducing the proportion of methane formed, and increasing the proportion of propionate at the expense of methane and/or acetate. This modification of rumen metabolism is believed to improve growth in ruminant animals. It describes compositions that may be used, an improved process for the production of aplasmomycin (which is called in this patent aplasmomycin A), and two novel analogues B and C together with processes for their manufacture.

A fermenter containing 30 L of sterilized medium (soya bean meal, Difco Bacto Casitone, glucose, calcium carbonate, and sodium nitrate) was stirred at 27 °C for 88 hours and aerated at a rate of 7.5 L per minute. The yield of aplasmomycin A was 4.7, B 8.1, and C 2.4 mg/L.

The ability of aplasmomycins to inhibit the production of methane in the rumen of ruminant animals, and to increase the proportion of

propionate (Ac/Pr) and butyrate (Ac/Bu) without depressing at the same time the overall digestive process is shown in Table 8.

Table 8. Effect of aplasmomycins on the methane production and the proportion of volatile acids in ruminants

aplasmomycins	concentration	% inhibition production of CH ₄ ^a	Ac/Pr ^a	Ac/Bu ^a
12	3ppm	69	-40	-49
13	3ppm	76	-45	-18
14	3ppm	44	-41	+22
12	1ppm	57	-41	-36
13	1ppm	63	-48	+21
14	1ppm	15	-33	+18

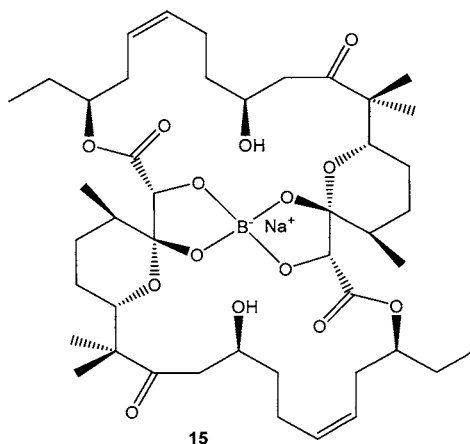
^a percentages of the values obtained in untreated controls

The animals were treated with an appropriate amount of aplasmomycins per day.

Borophycin

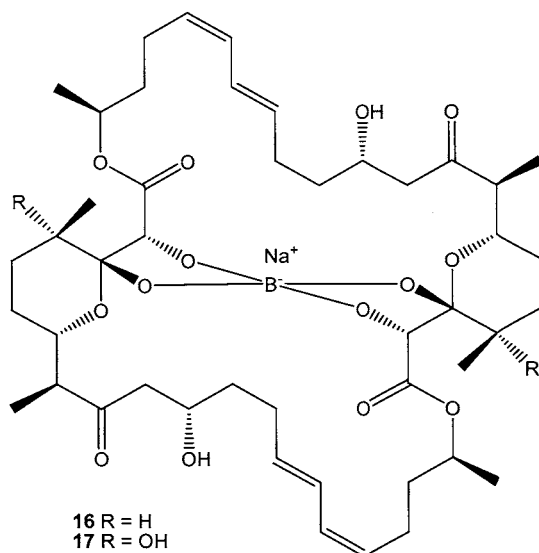
Borophycin (**15**) was found to display appreciable cytotoxicity against LoVo cells (MIC 0.066 µg/mL) and KB cells (MIC 3.3 µg/mL). It was isolated from the lipophilic extract of a marine strain of the blue-green alga *Nostoc linckia* [53]. Borophycin is made up of two identical halves with an overall structure reminiscent of other boron-containing antibiotics. The C3 starter unit for the biosynthesis of **15** is derived from acetate and methionine, but not propionate.

Borophycin and four new cyclic hexapeptides containing no boron, tenucyclamides A-D, were also isolated from the methanol extract of *Nostoc spongiaeforme* var. *tenu* collected in the Volcani Center, Israel. The yield was 0.1% on the basis of the dry weight of the blue-green alga [54].



Tartrolons

In a screening of myxobacteria for antibacterial metabolites, the *Sorangium cellulosum* strain So ce678 was selected for its activity against *Staphylococcus aureus* [55]. The active compounds were produced as two rather lipophilic active compounds, i.e. tartrolons B (**16**) and C (**17**). Tartrolon B was identified as a boric acid ester of tartrolon A3, whereas tartrolon A1 and tartrolon A2 are stereoisomers of tartrolon A3. All components could be chemically converted into each other.



A large difference was observed between the productions of the tartrolons in shaking flasks, with tartrolon B as the main product, or large-scale fermentation in steel fermenters with tartrolon B as the minor component. Table 9 shows a good correlation between the borate concentration in the medium and the production rate of tartrolon B. However, an increased boron supply by the addition of up to 0.5 g/L of sodium tetraborate showed no influence on the total amount of tartrolons formed.

Table 9. Production of tartrolons A1, A2, A3, and B in relation to the boron supply

Container material for sterilization ^a /incubation ^b	H ₃ BO ₃ [μmol/L] without inoculation	Tartrolon A1-A3 (μmol/L)	Tartrolon B (16) (μmol/L)
Glass/glass	55	0.6	13.8
Glass/polypropylene	40	0.8	5.7
Polypropylene/glass	25	3.0	11.9
Polypropylene/polypropylene	7	10.7	2.3

^a 121°C for 20 min; ^b 30°C for 7 days

A new member of the tartrolon series of macrodiolides, tartrolon C (17), was isolated from a *Streptomyces* species on the basis of its insecticidal activity [56]. The producing organism was isolated in 1990 from a soil sample collected near Braunschweig, Germany. Tartrolon C was active on the beet army worm and tobacco bud worm, with minimum emergent larvicide concentration of 125 ppm on both insects, approximately 40 x and 310 x less active than a standard of spinosyn A.

A partial 16S ribosomal-RNA sequence of CP1130 matched the genus *Streptomyces*, most closely the species *verticillus*. Therefore, this represents the first report of tartrolons from a *Streptomyces* species, since their only previously reported occurrence was in the myxobacterium *Sorangium cellulosum* [55, 57, 58].

Sorangium cellulosum was fermented in 65 liters at 30 °C with an aeration of 0.15 m³ air per hour and stirrer speed 150 rpm [4]. The yields of tartrolons A and B are given in Table 10. The main product was tartrolon A whereas in flask culture tartrolon B prevailed. Its proportion was increased on supplying the culture medium with borate.

Table 10. Influence of the fermentation vessel on the formation of tartrolons A and B

Fermentation vessel	Tartrolon (µg/mL)	
	A	B
Stainless steel bioreactor	42	2
Glass flask	5.6	38.7
Glass flask+0.005% Na ₂ B ₄ O ₇	4.7	41

The antibiotic spectrum of the tartrolons is given in Table 11. Both tartrolons acted against Gram-positive bacteria with similar MIC values. Gram-negative bacteria, yeasts, and fungi were insensitive, but mammalian cells were strongly inhibited, especially by tartrolon B.

Table 11. Antibiotic spectrum of tartrolons A and B

Test strain	Inhibition zone ^a (mm)		MIC (µg/mL)	
	A	B	A	B
<i>Arthrobacter simplex</i>	13	9	-	-
<i>Bacillus megaterium</i>	13	9	-	-
<i>Bacillus subtilis</i>	15	11	0.62	0.31
<i>Micrococcus luteus</i>	17	11	1.25	1.25
<i>Mycobacterium lacticola</i>	13	10	1.56	1.56
<i>Staphylococcus aureus</i>	16	11	0.62	0.62
<i>Nocardia corallina</i>	15	12	0.62	0.62
<i>Streptococcus faecalis</i>	12	11	>40	10
<i>Escherichia coli</i>	0	0	>40	>40
<i>Escherichia coli</i> tol C ^b	11	0	>40	>40
<i>Pseudomonas fluorescens</i>	0	0	-	-
<i>Salmonella typhimurium</i>	0	0	-	-
<i>Serratia marcescens</i>	0	0	-	-
<i>Candida albicans</i>	0	0	-	-
<i>Saccharomyces cerevisiae</i>	0	0	-	-
<i>Rhodotorula glutinis</i>	0	0	-	-
<i>Hansenula anomala</i>	0	0	-	-
<i>Schizosaccharomyces pombe</i>	0	0	-	-
<i>Mucor hiemalis</i>	0	0	-	-
<i>Aspergillus niger</i>	0	0	-	-
<i>Trichoderma harzianum</i>	0	0	-	-
Mouse fibroblast cells L929	-	-	0.46	0.017

^a With paper disks of 6 mm diameter and 10 µg tartrolon per disk.

^b Mutant with damaged outer membrane.

In *Staphylococcus aureus* the syntheses of several important cellular macromolecules were inhibited immediately after the addition of tartrolon B. Isolated RNA polymerase and DNA polymerase from *Escherichia coli* were not influenced by the antibiotic. This could mean that either tartrolon acts specifically on enzymes of Gram-positive bacteria or, more probably, that the antibiotic interferes with energy delivery or membrane integrity.

Experiments with feeding the production strain *Sorangium cellulosum* with sodium $[1-^{13}\text{C}]$ acetate, $[^{13}\text{CH}_3]$ methionine, and sodium $[1,2-^{13}\text{C}_2]$ acetate were performed to investigate the biosynthesis of the tartrolons [58] (Fig. 3). The ^{13}C -NMR spectrum of $[1-^{13}\text{C}]$ acetate-derived tartrolon B shows nine enhanced carbon signals. However, the three-carbon starter unit C-19 to C-21 was not derived from either acetate, propionate, or methionine. This result is in good agreement with boromycin and aplasmomycin where C-1 to C-14 are derived from seven acetate units and with borophycin (see above) where C-1 to C-16 are derived from eight acetate units. Whereas the three-carbon starter unit, C-15 to C-17 in boromycin and aplasmomycin, is derived from glycerol, C-17 to C-19 in borophycin are derived from acetate and methionine. Thus, regarding the biosynthesis, tartrolon is closely related to boromycin and aplasmomycin whereas borophycin with a propionyl starter unit derived from acetate and methionine exhibits a more distant relation.

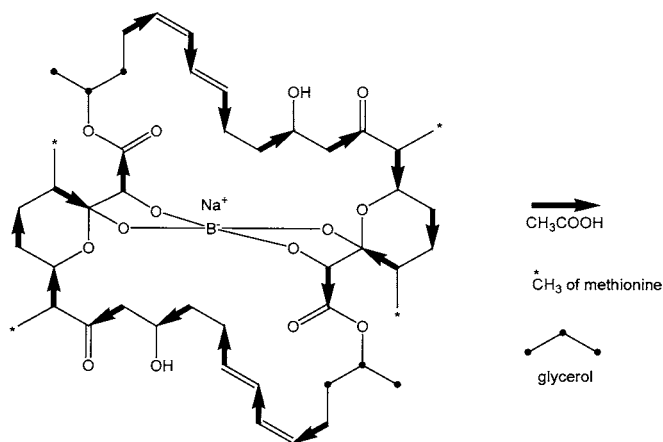


Fig. 3. Biosynthesis of tartrolon.

Tartrolon B and also previously isolated boromycin, aplasmomycin, and borophycin are polyketides and have the same boron-binding substructure C1-C7 in each half of the symmetric molecules (Fig. 4).

CONCLUSION

Boron is an essential trace element for plants and is beneficial for animals and humans. Dietary boron obviously plays a role in immune functions. Among the best-known natural boron-containing compounds are polyketide antibiotics such as boromycin, aplasmomycins, borophycin and tartrolons. Attempts are underway to incorporate boron into different biologically active molecules, particularly for medicinal application, e.g. for boron neutron capture therapy of brain tumors. Some boron-containing biomolecules may apparently act as signaling molecules that interact with cell surfaces.

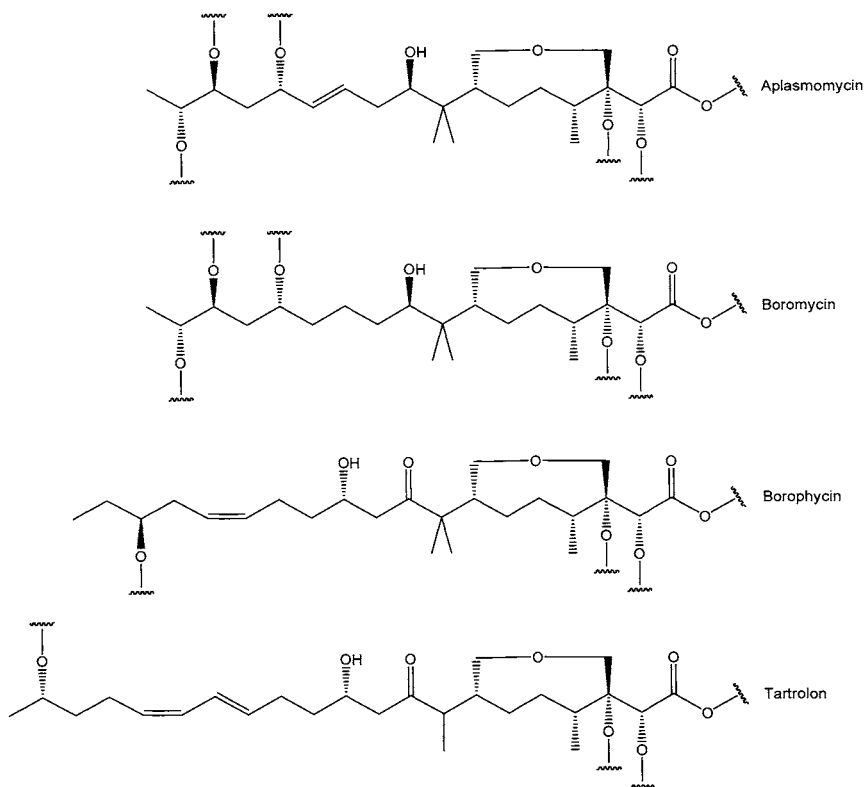


Fig. 4. The boron binding substructures of different boron-containing antibiotics.

Silicon

Silicon is a tetravalent metalloid and it is less reactive than its chemical analogue carbon. It is the second (after oxygen) most abundant element in the Earth's crust, making up 25.7% of it by weight. Elemental silicon is not found in nature. It occurs mainly in the form of silicon dioxide (also known as silica) and silicates (compounds containing silicon, oxygen, and metals). Amethyst, agate, quartz, rock crystal, flint, jasper, and opal are some of the forms in which the oxide appears. Granite, asbestos, feldspar, clay, hornblende, and mica are a few of the many silicate minerals. Its concentration in seawater is relatively low, only 3 mg/L [59].

Silicon has many similar chemical properties to carbon but it has a number of handicaps as a carbon analogue, however. Because silicon atoms are much bigger, they have difficulty forming double or triple bonds. Silanes (analogues of the alkanes) are highly reactive with water, and long-chain silanes spontaneously decompose. Molecules incorporating Si-O-Si bonds instead of Si-Si bonds are much more stable; ordinary sand is one such example [60].

Silicon is a biogenic element, although its content in the tissues of living organisms is not very high. The body of an adult human contains about 1 g silicon, mostly in bones, cartilages and tooth enamel; silicon is indispensable for normal growth and development of these tissues. In plant cells, increased silicon content can be found, e.g., in horse-tail or shave grass, and in stimuli of stinging nettles.

Some marine organisms have evolved the ability to biosynthesize exquisitely structured silicate shells. The "silicate" bacteria, which appear to be members or relatives of the genus *Bacillus*, are known to release silicon from aluminosilicates through the secretion of organic acids [61].

Silicon is extremely important as a building block of unicellular algae - diatoms. The main building material of the frustule (the finely sculptured protective surface layer of diatoms) is a float-stone, an opal-like water-containing polymer of silica: Diatoms are the only group of organisms whose development is totally dependent on the presence of soluble forms of silica in the environment. When silicon sources run out, DNA replication stops.

Microbes and microbial enzymes have been used in the biotransformation of organosilicon compounds. Biodegradation of

organosilicon under aerobic [62] and anaerobic [63] conditions has also been described.

Conceivably, some strange life forms might be built from silicon-containing substances were it not for an apparently fatal flaw in silicon's biological credentials. This is its powerful affinity for oxygen. When carbon is oxidized during the respiratory process of a terrestrial organism, it becomes the gas carbon dioxide, a waste material that is easy for a creature to remove from its body. The oxidation of silicon, however, yields a solid because, immediately upon formation, silicon dioxide organizes itself into a lattice in which each silicon atom is surrounded by four oxygens. Disposing of such a substance would pose a major respiratory challenge.

Life forms must also be able to collect, store, and utilize energy from their environment. In carbon-based biota, the basic energy storage compounds are carbohydrates and lipids in which the carbon atoms are linked in most cases by single bonds into a chain. Carbohydrates or lipids are oxidized to release energy and the waste products water and carbon dioxide in a series of controlled steps using enzymes, which catalyze specific reactions because of their shape and "handedness." A feature of carbon chemistry is that many of its compounds can take right and left forms, and it is this handedness, or chirality, that gives enzymes their ability to recognize and regulate a huge variety of processes in the body. Silicon's failure to give rise to many compounds that display handedness makes it hard to see how it could serve as the basis for the many interconnected chains of reactions needed to support life [64].

Even so, as has been pointed out, silicon may have had a part to play in the origin of life on Earth. A curious fact is that terrestrial life forms utilize exclusively right-handed carbohydrates and left-handed amino acids. One theory to account for this is that the first prebiotic carbon compounds formed in a pool of "primordial soup" on a silica surface having a certain handedness. This handedness of the silicon compound determined the preferred handedness of the carbon compounds now found in terrestrial life. An entirely different possibility is that of artificial life or intelligence with significant silicon content.

In the realm of science fiction there have occasionally been forms of life proposed that, while often highly speculative and unsupported by rigorous theoretical examination, are nevertheless interesting and in some

cases even somewhat plausible. One of the major sentient species in Terry Pratchett's Discworld universe is Trolls. Their being mineral-based has various interesting effects on their physiology and culture. Other silicon-based life forms are said to exist there but few appeared in the books. In the Star Wars universe, at least two life forms are based on silicon, and they live in space: the Mynocks and the Space slugs. A silicon-based life form, the Horta, appears also in the Star Trek.

As demonstrated in recent studies [65-68], organic molecules have a crucial role in the formation of biosilica owing to the specificity of interactions at the organic-inorganic interface. Biosilicification has been studied most extensively in diatoms and sponges. The proteins involved in biosilicification in these different marine species will be reviewed here, along with the mechanistic basis for their function.

As mentioned above, dissolved silicon in seawater occurs mostly as the undissociated orthosilicic acid, $\text{Si}(\text{OH})_4$, whose total content in the world's oceans amounts to about 9.6 teratons. A variety of marine organisms, such as diatoms, silicoflagellates, radiolarians, and sponges, contain silica skeletons ($\text{SiO}_2 \times n\text{H}_2\text{O}$) that are built up by taking up orthosilicic acid from seawater. The diatom cell wall (frustule) is made of nanostructured amorphous silica that is associated with polysaccharides and proteins. New cell walls are produced in a silica deposition vesicle (SDV); soluble silicon is taken up from the environment and concentrated in SDV, and here the insoluble silica is formed and subsequently secreted. Recently, the first data were reported that correlate distinct silica elements with specific proteins within the diatom cell wall. Three families of proteins have been identified in the organic matrix of the cell wall of the diatom *Cylindrotheca fusiformis*: frustulins, pleuralins, and silaffins. Frustulins were extracted from the cell wall with ethylenediaminetetraacetate (EDTA), and consisted of four (α , β , γ , δ) Ca^{2+} binding glycoproteins with molecular weights ranging from 75 to 200 kDa.

Three types of pleuralins, formerly known as HF-extractable proteins - pleuralin 1 (200 kDa), pleuralin 2 (180 kDa), and pleuralin 3 (150 kDa) were discovered. They are involved in the formation of new theca (one of the halves in the cell wall) when one parental diatom is divided into two daughter diatoms.

The silaffins induced and regulated silica precipitation at ambient temperature and pressure. Native silaffins (natSil) including natSil-1A (6.5 kDa), natSil-1B (10 kDa), and natSil-2 (40 kDa), were detected by treating the diatom cell wall with NH_4F . Native silaffins are highly post-translationally modified and some functional groups such as phosphate, sulfate, and carbohydrate are lost during the process of dissolving the cell wall with HF, resulting in lower molecular weight proteins: silaffin-1A (4 kDa), silaffin-1B (8 kDa) and silaffin-2 (17 kDa) proteins. A gene *sil 1* has been isolated from a *C. fusiformis* genomic library that encodes a polypeptide of 265 amino acids. Seven repeat sequences were identified in *sil 1* and termed R1 to R7. R1 and R2 peptides correspond to the precursors of silaffin-1B and silaffin-1A2, respectively, whereas R3 to R7 peptides correspond to the precursors of the silaffin-1A1.

The major component, 1-frustulin, is one of a number of isoforms of a 75 kDa protein that is immunologically related to proteins extracted from the cell walls of other diatom species, namely *Navicula pelliculosa*, *Nitzschia alba*, *N. angularis* and *Phaeodactylum tricornutum*.

Each frustulin contains at least three of five structural elements: 1) presequence domain, 2) acidic cysteine-rich domain with a highly repetitive structure, its common sequence being C-E/Q-G-D-C-D, 3) proline-rich domains, 4) polyglycine domains and 5) a tryptophan-rich domain.

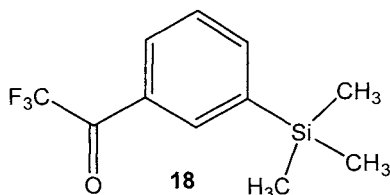
Diatoms were also investigated for the mechanism of silicon transport that is an integral part of the silicification process. As the environmental concentrations of dissolved silicon are rather low, diatoms must have an efficient transport system. Orthosilicic acid must not only be transported into the cell, but also transported intracellularly into the SDV where silica formation occurs. A protein of the diatom *C. fusiformis* was characterized that transports silicon from seawater into the cell. This discovery was accomplished by cloning and characterizing the DNA that codes for this protein. However, silicon transporter proteins of this particular type are not necessarily involved in intracellular transport.

A further major breakthrough in silicon biochemistry came from recent studies with the common marine sponge *Tethya aurantia*. After dissolution of the silica with buffered hydrofluoric acid, these filaments can be isolated, purified, and resolved into three very similar silicon-free subunits, named silicatein (silica protein) α , β , and γ . Analyses of the

amino acids demonstrated that the compositions of silicatein α (29 kDa), β (28 kDa), and γ (27 kDa) are highly similar, the relative proportions in the filaments being $\alpha:\beta:\gamma$ 12:6:1. Analysis of the DNA sequence for silicatein α (the principal subunit that comprises about 70% of the mass of the filaments) revealed that this protein is highly similar to members of the cathepsin L and papain family of proteases. These findings gave rise to the fascinating idea that the silicateins might possess an enzyme-like activity and might catalyze the hydrolysis of silicic acid esters. This result clearly indicates the catalytic function of the silicateins (in their native three-dimensional structure) in accelerating the formation of silica and silsesquioxanes. In summary, silicateins can catalyze the formation of silica and organyl-substituted silsesquioxanes from the corresponding silicic acid esters $\text{RSi}(\text{OEt})_3$ ($\text{R}=\text{OEt}$, Me, Ph) at neutral pH and ambient temperature, and can direct the structures of the resulting products. The complete amino acid sequence and the three-dimensional structure of silicatein α are highly homologous to those of cathepsin L. Thus, it has been proposed that silicatein α catalyzes the hydrolysis of $\text{Si}(\text{OEt})_4$ at neutral pH through the activity of the serine and histidine residues. Direct experimental evidence for the proposed role of the specific serine-26 and histidine-165 residues came from site-directed mutagenesis, in which the cloned recombinant DNA that codes for silicatein α was modified in a site-specific manner *in vitro*, and the mutant DNAs were then used as templates to direct the synthesis of the corresponding proteins in bacteria. Two structural variants of the silicatein α protein were produced, in which the serine-26 residue and histidine-165 residue were specifically replaced by an alanine moiety. Quantitative comparison of the catalytic activities of the resulting protein products with the activity of the original protein supports the suggestion that serine-26 and histidine-165 of silicatein α are indeed required for the efficient catalysis of silica formation from $\text{Si}(\text{OEt})_4$ at neutral pH. From these findings, a detailed mechanistic model was deduced for the silicatein α -mediated catalysis. Intra-silica proteinaceous materials have been extracted from the branches of primitive plants, *Equisetum telmateia* (great horsetail) and *E. arvense* (common horsetail), and from hairs found on the lemma of the grass *Phalaris canariensis*, where they comprise 0.015 - 0.03 wt % of the silica. The extracts contain protein and carbohydrate components enriched in xylose and glucose. In common with the earlier diatom studies, the amino acid compositions of the matrices contain around 25 mol % serine/threonine and 20 mol % glycine [69].

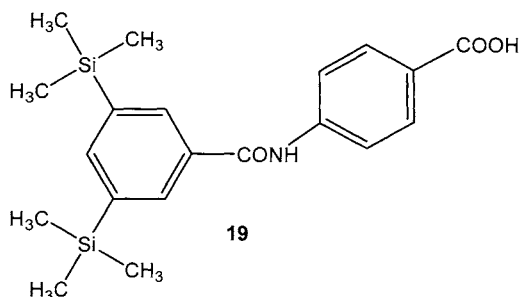
The bioorganic chemistry of silicon covers two diametrically different areas. One is the formation of silicon compounds in nature – see above – while the other is concerned with organosilicon compounds obtained by synthetic procedures. This latter group of compounds, though not of natural origin, deserves our attention since it can shed light on some of the mechanisms and interactions taking place in the former group. The highly varied, both structurally and pharmacologically, group of compounds is illustrated here on a few of them, in particular those that are in different stages of clinical trials or are already commercially produced [70, 71].

Zifrosilone (**18**) is a novel tight-binding inhibitor of acetylcholinesterase, which is in development as a potential therapeutic compound in the symptomatic treatment of Alzheimer's disease [72]. Pharmacokinetics and pharmacodynamics of the compound were studied in the dogs and rats after single intravenous and subcutaneous administrations. When evaluated in human healthy volunteers, the orally administered drug was well tolerated but displayed a strong dose-related inhibition of red blood cell acetylcholinesterase [73] and its development was consequently halted.



TAC-101 (**19**) is a synthetic retinoid with selective binding affinity for RAR- α (retinoic acid receptors) [74, 75]. RAR- α binding by TAC-101 has been shown to inhibit the activity of transcription factors, including activated protein-1, which activates the expression of metastasis-related genes, including urokinase-type plasminogen activator, matrix metalloproteinase-9, and vascular endothelial growth factor. TAC-101 has been demonstrated to inhibit liver metastases caused by intrasplenic or intraportal vein injection of tumor cell lines, including AZ-521 (human gastric adenocarcinoma), A549 (human lung adenocarcinoma), and colon 26-LF (metastatic variant of murine colon cancer). The pharmacokinetics and distribution of TAC-101 have been studied in mice, dogs, and monkeys. TAC 101 might be also of interest for the treatment of Alzheimer's disease and other age-related dementias.

Given the above preclinical observations, patients in a clinical study were monitored closely during treatment (including measurement of testosterone, luteinizing hormone, follicle-stimulating hormone levels, alkaline phosphatase, and serum vitamin A concentrations). This phase I clinical study of TAC-101 was conducted to determine the safety, toxicity, and pharmacokinetics of this agent in patients with advanced cancer. Currently, the drug is in a phase I/II clinical trial for advanced hepatocellular carcinoma.



Karenitecin (BNP1350) (**20**) is a derivative of camptothecins, a natural alkaloid of *Camptotheca acuminata*, a member of the family Nyssaceae native to China and Tibet [76, 77]. Derivatives of camptothecin are known to have broad anticancer activity, but their use has been limited in part due to common and serious side effects. One of these derivatives, karenitecin, has demonstrated very high potency and broad activity in preclinical studies against human cancers including prostate, pancreas, lung, breast, colon, ovary and head and neck [78]. A phase II trial for karenitecin with non-small cell lung cancer patients has been completed. Karenitecin is currently being evaluated in phase II trials in patients with primary brain tumors, melanoma, and advanced ovarian cancer. A phase I/II trial with orally administered karenitecin was initiated for patients with solid tumors/non-small cell lung cancer.

The silatecan (DB-67) (**21**) represents a new generation of camptothecin derivatives (see Table 12) that exhibits a potent *in vitro* DNA topoisomerase I (TOP1)-mediated DNA-damaging activity, improved blood stability, and holds significant promise for the treatment of human cancers [79]. This new agent was found to be 25-times more lipophilic than camptothecin; it incorporates readily into cellular and liposomal bilayers. In addition, its 10-hydroxy functionality enhances drug stability in the presence of human serum albumin. Thus, the net

lipophilicity and the 10-hydroxy moiety together function to promote the enhanced blood stability [80].

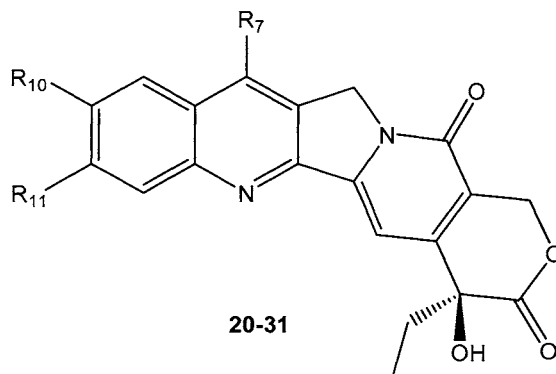
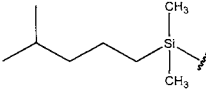
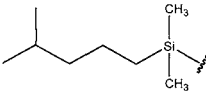
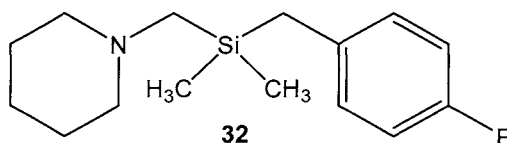


Table 12. Median effective concentrations for inhibition of the proliferation of five glioma cell lines by a variety of camptothecin derivatives

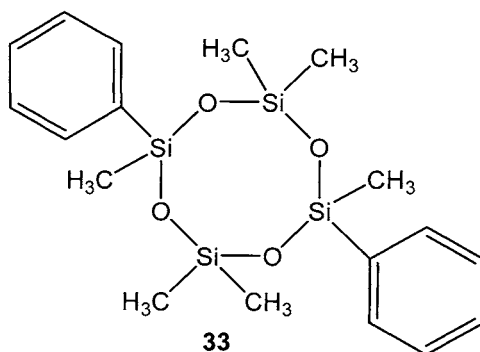
No	Name	R ₇	R ₁₀	R ₁₁	ED ₅₀ (ng/mL)				
					U87	A172	SG388	LN-Z308	T98G
20	BNP1350	(CH ₂) ₂ Si(CH ₃) ₃	H	H	-	-	-	-	-
21	DB67	t-Bu(CH ₃) ₂ Si	OH	H	2	30	3	40	6
22	CHJ439A	(CH ₃) ₃ Si	H	H	2	6	3	500	300
23	CHJ758A	(CH ₃) ₃ Si	OH	H	100	8	10	20	80
24	CHJ792A	(CH ₃) ₃ Si	NH ₂	H	10	20	2	20	30
25	CHJ800A	(CH ₃) ₃ Si	H	NH ₂	30	100	10	100	200
26	DB3		H	H	200	200	30	>1000	100
27	DB124		OH	H	300	600	300	500	>1000
28	DB148	Cl(CH ₂)(CH ₃) ₂ Si	H	H	30	30	30	60	60

29	DB202	t-Bu(CH ₃) ₂ Si	H	H	10	60	20	1000	60
30	DB204	t-Bu(CH ₃) ₂ Si	NH ₂	H	10	10	200	60	8
31	DB205A	t-Bu(CH ₃) ₂ Si	NH ₂	F	6	3	20	60	10

Silperisone (**32**) is a sodium channel blocker acting centrally as a muscle relaxant. The compound has been in preclinical animal studies [81, 82] and has been proposed recently to have potential as an antispastic drug in demyelinating diseases such as multiple sclerosis. *In vivo*, silperisone depressed ventral root reflexes and excitability of motoneurons. Whole-cell measurements in dorsal root ganglion cells revealed that silperisone depressed voltage gated sodium channel conductance at concentrations that inhibited spinal reflexes.

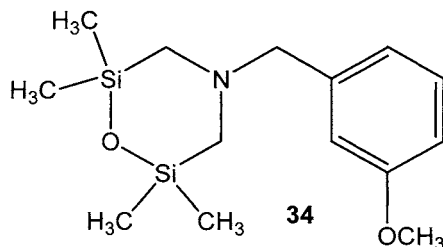


Cisobitan (**33**) has been the first molecule that was investigated as the silicon-containing drug [83]. This drug was shown to possess estrogenic, antigonadotropic [84], and antifertility activities in animals and was evaluated in a pilot and a prospective clinical study as an antiprstate cancer agent. In a limited trial, cisobitan was not adequate for tumour palliation. Cisobitan trials were discontinued because the compound did not provide any significant therapeutic advantage over estrogens [85].

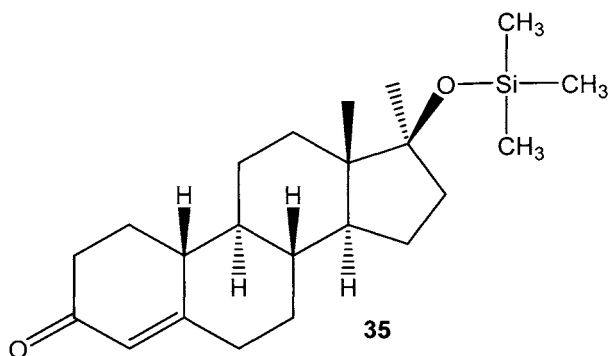


The pharmacokinetics of Sandoz compound 58-112 (**34**), a new chemical entity with a unique myotonolytic effect, was studied [86]. The

metabolic pathways of Sandoz compound 58-112 were evaluated in the rat, dog, and man after a single oral dose [87].

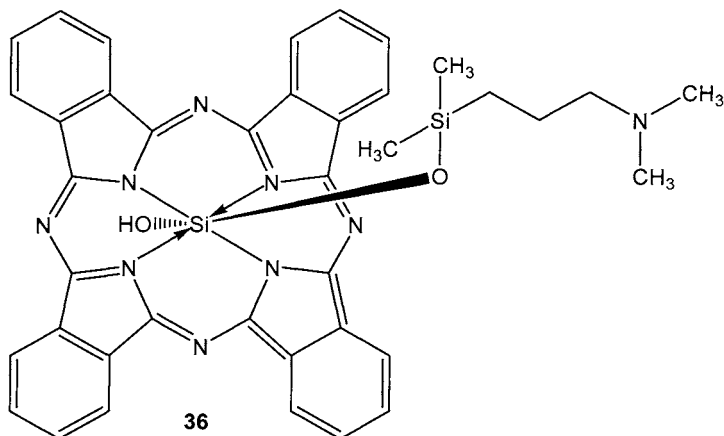


Silabolin (**35**) was used for the treatment of muscle wasting diseases in Russia and eastern Europe. It has often been abused as an anabolic [88].



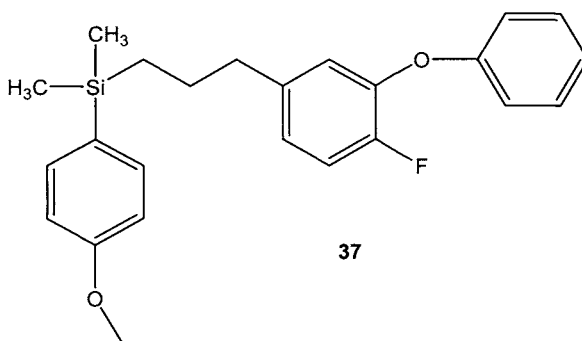
Photodynamic therapy is an innovative treatment for several types of cancer that was developed during the last 30 years. It is based on visible light excitation of a tumor-localized photosensitizer, which, after excitation, transfers energy to oxygen forming reactive oxygen species that further oxidize cellular targets. Pc4 (**36**) (silicon phthalocyanine 4), a synthetic photosensitizer agent containing a large macrocyclic ring chelated with silicon, has been extensively studied in cell models where it was shown to bind preferentially to the endoplasmic reticulum, Golgi complex and mitochondria and to induce rapid apoptosis after exposure to light through mitochondrial oxidative stress. Currently, phase I human photodynamic therapy clinical trials are being conducted with Pc4, for the treatment of respectively skin cancer, cutaneous T-cell lymphoma, actinic keratosis, squamous intraepidermal carcinoma (Bowen's disease), and adult solid tumors, breast cancer, head and neck cancer, hematopoietic and lymphoid cancers, and skin tumors [89, 90]. Pc4 localizes primarily

in mitochondrial membranes and, after photoexcitation, forms reactive oxygen species that induce apoptosis [91].

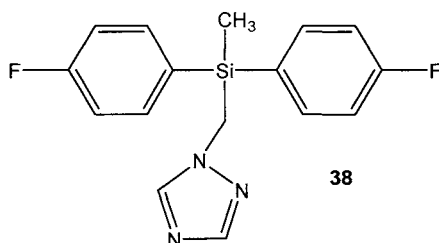


The tumor-promoting activities of different commercial compounds (e.g. the non-ester pyrethroid silafluofen, **37**) used as termiticides were measured by a cell-transformation assay employing Bhas 42 cells [92]. Their initiating activities were also measured by the microsuspension assay employing *S. typhimurium* TA98 and TA100 strains. The termiticidal performance of wood-based composites, hardwood plywood, softwood plywood, particleboard, and oriented strand board was evaluated following treatment with silafluofen, using supercritical carbon dioxide as a carrier solvent [93]. Degradation of termiticides by silafluofen and its effect on performance against Thailand's economically most important subterranean termite, *Coptotermes gestroi* (Isoptera: Rhinotermitidae) were described [94].

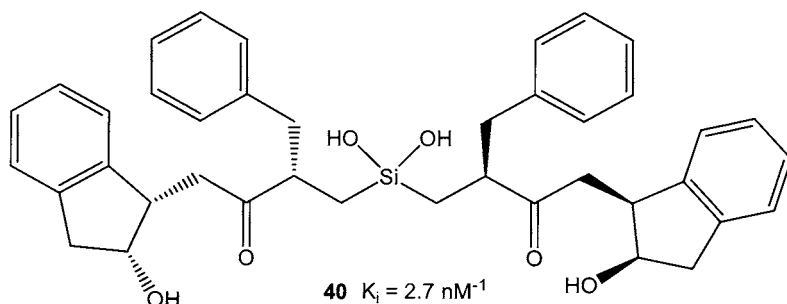
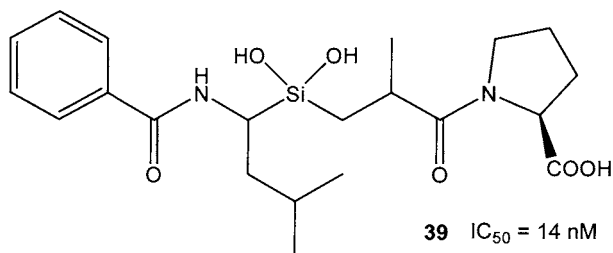
The effect of rice cleaning and cooking on the residues of silafluofen (**37**) was described in rice that had been sprayed in a paddy field with this pesticide [95]. Silafluofen was found to remain in the hull of the rice. The residual concentration of the pesticide in polished rice was higher than that in pre-washed rice processed ready for cooking. During the cooking procedure, the reduction of pesticides in polished rice was higher than that in brown rice.



Sensitivities (ED_{50}) of 200 monoconidial isolates of *Venturia inaequalis* to the sterol demethylation inhibitor flusilazole (**38**) were determined, based on the inhibitory effect on mycelial growth [96].



Proteases play essential roles in most biological processes and are important therapeutic targets for a multitude of diseases including cancer, viral, parasitic, fungal, and bacterial infections, inflammation, and cardiovascular disease. This is best exemplified by the HIV protease inhibitor drugs. The reaction mechanism of proteases is to convert the carbonyl group of the target peptide bond into an enzyme-stabilized tetrahedral diol transition-state intermediate by addition of water. This intermediate then collapses to yield two peptide products. The enzyme binds the tetrahedral diol intermediate, the specificity being provided by specific contacts for the amino acid side chains. A silicon diol structure, in which the $C(OH)_2NH$ group of the transition state is replaced by a $Si(OH)_2CH_2$ moiety, mimics the tetrahedral transition state and as silicon strongly favors sp^3 - over sp^2 -hybridisation, silicon diols ($R_2Si(OH)_2$) do not eliminate water to form silicon analogues ($R_2Si=O$) of ketones. It has been shown that incorporating such silicon diol moieties into peptide mimetics results in potent and selective inhibitors of proteases, represented e.g. by angiotensin-converting enzyme and HIV protease respectively (compounds **39** and **40**).



The decapeptides **41-43** were studied [97] *in vitro* in receptor binding and functional assays using recombinant cell lines expressing the human gonadotropin releasing hormone (GnRH) receptor. The C/Si/Ge analogues **41-43** are derivatives of the GnRH antagonist, which bears an (*S*)-tyrosine residue [instead of the (*S*)-Me₃C-Ala, (*R*)-Me₃Si-Ala, or (*R*)-Me₃Ge-Ala residue] in position 5 of its decapeptide backbone. All compounds behaved as potent GnRH antagonists (Table 13), the binding affinities and antagonistic potencies of the three C/Si/Ge analogues being quite similar. Compounds **41-43** were also studied for their *in vivo* activities in male rats after s.c. administration. They produced both a strong testosterone suppression (single-dose treatment, 1.5 mg/kg) and a strong LH (luteinizing hormone) suppression (castrated male rat; single-dose treatment, 0.05 mg/kg). For the silicon- and germanium-containing decapeptides **42** and **43** the testosterone and LH suppression lasted for a significantly longer period of time compared with the effects of the carbon analogue **41**.

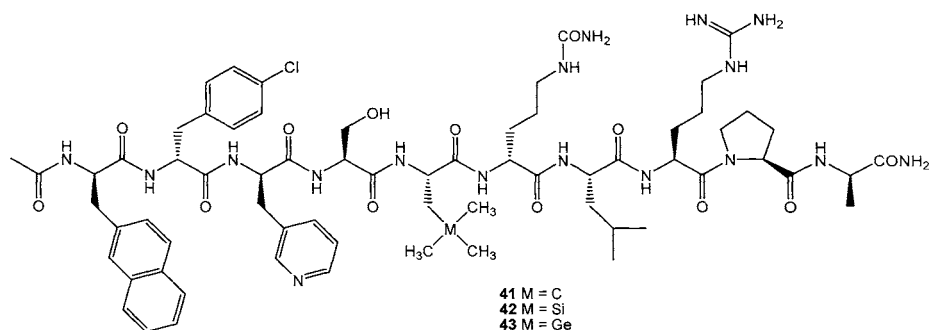


Table 13. Binding affinities (K_A values) and antagonistic potencies (IC_{50} values) of the decapeptides 41-43 and Cetrorelix^a at the human GnRH receptor

decapeptide	K_A [nM^{-1}]	IC_{50} [nM]
41	2.20	0.93
42	2.82	0.75
43	2.01	0.50
Cetrorelix ^a	5.94	0.25

^a Cetrorelix is a decapeptide with a sequence derived from luteinizing hormone releasing hormone and subsequently modified. Five of the ten amino acids present are in the D configuration. The peptide is protected from degradation by means of C- and N-terminal protective groups and was used as standard.

CONCLUSIONS

Silicon is essential for growth and biological function in a variety of plant, animal, and microbial systems, yet the molecular mechanisms of these interactions are still unknown. The *in vitro* studies of natural systems in the field of silica biosynthesis are complicated. Recently, studies have been conducted to test the ability of homologous enzymes to catalyze the formation and cleavage of siloxane bonds, in order to better understand the role of various proteins in the biosilicification process. Some enzymes, e.g. *Suberites domuncula* silicase, have been observed to hydrolyze an aqueous solution of amorphous silica during the formation of silicic acid. Genetic engineering, in conjunction with biotechnological

methods, offers the prospect of developing new, environmentally benign routes to the synthesis of organyl-substituted siloxanes. It can be expected that further studies of the proteins, genes, and molecular mechanisms controlling silicon metabolism in diatoms and sponges may also help to reveal the mechanisms involved in the essential requirement for silicon for optimal development and growth in many plants and animals.

The synthetic chemistry of organosilicon molecules offers the possibility to produce not only sila-substituted analogues of existing drug molecules, but also new drugs including silicon as a lipophilic bioisostere. Although the sila-substituted molecules have not been found to offer significant advantage in terms of affinity toward cell-membrane receptors relative to their original counterparts, they can provide improved receptor selectivity. The lipophilicity of organosilicon drugs is usually reflected in their easier cell penetration, which results in an improved intracellular activity as enzyme inhibitors or apoptotic inducers. The advantages of organosilicon drugs in terms of pharmacokinetics and pharmacodynamics are further documented by the fact that a number of these compounds are currently undergoing human clinical trials. The introduction of silicon bioisosteres in drugs is not a universally beneficial “miraculous” approach that medicinal chemists can rely on in every situation. In specific instances, however, silicon bioisosteres can lead to significant improvements in the performance of a given drug and they therefore deserve to be considered more often in structure-aided drug design and lead development.

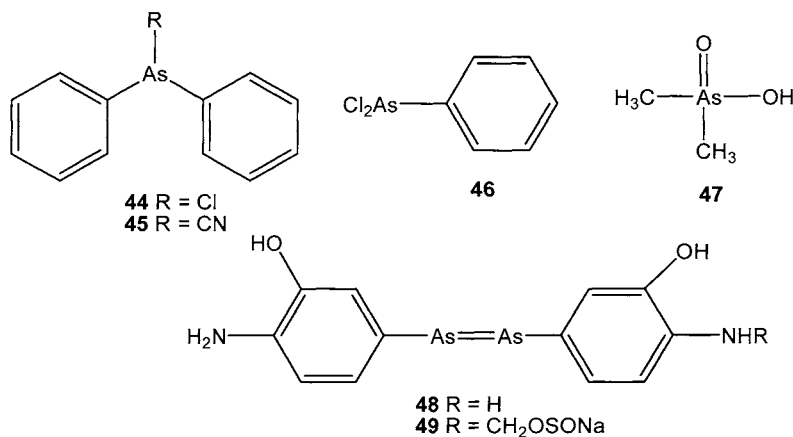
Arsenic

Arsenic is widely distributed in the environment. It ranks twentieth in the abundance of elements in the Earth's crust. In the environment, arsenic is mainly associated with sulfide minerals. Soils contain arsenic usually in the range of 0.5 to 35 mg/kg [98]. Arsenic levels in gold ore deposits in Zimbabwe attain 20 g/kg in soils [99].

Arsenic contents measured in sediments are between 0.1 and 490 mg/kg while levels up to 1.5 g/kg were found in coals (average: 13 mg/kg). The concentration of arsenic in seawater varies between 0.09 and 24 µg/L (average: 1.5 µg/L), and in freshwater between 0.15 and 0.45 µg/L (maximum: 1 mg/L). In mineral and thermal waters, arsenic was found in concentrations up to a factor of 300 higher than its mean concentration in groundwater [100]. The WHO recommends a maximum contaminant level for arsenic in drinking water 10 µg/L.

The smelting of Cu, Ni, Pb, and Zn ores is the most important anthropogenic arsenic source. Another anthropogenic source of arsenic in the environment is the burning of fossil fuels in households and power plants. The burning of coal leads to the emission of arsenic by volatilization of As_4O_6 which condenses in the flue system [101]. One important anthropogenic source of arsenic contamination of the environment was the use of arsenical fungicides, herbicides, and insecticides in agriculture and wood industry. In World War I organically bound arsenic (diphenylchloroarsine (44), diphenylcyanoarsine (45), phenyldichloroarsine (46)) was used as warfare. Dimethylarsinic acid (47) (agent blue, cacodylic acid) was used during the Vietnam War for defoliation for military purposes [102]. Until the discovery of antibiotics, arsenic compounds were widely used in medicine for the treatment of a variety of illnesses. A solution containing 1% of potassium arsenite (Fowler's solution) was used for the treatment of leukaemia and psoriasis or for fortifying. Donovan's solution (arsenic iodide) and Valagin's solution (arsenic trichloride) were recommended for the treatment of rheumatism, arthritis, asthma, malaria, trypanosomiasis, tuberculosis, and diabetes. Sodium arsenate was used for the treatment of chronic skin diseases, some parasitic diseases, and anaemia. An organically bound arsenic compound with 32 % arsenic (Salvarsan=Arsphenamin (48) and Neosalvarsan (49)) were used for the treatment of syphilis [102]. Today the reuse of arsenic is discussed for the treatment of cancer [103, 104].

Arsenic is considered to be an essential element but a lot of arsenic compounds are toxic [105]. Organic arsenic compounds are less toxic than inorganic ones. AsH_3 is the most toxic compound. The fatal dose is 250 mg/m^3 at an exposure time of 30 minutes [106]. The lethal dose (LD_{50}) for arsenic trioxide is 34.5 mg/kg , sodium arsenite 4.5 mg/kg , sodium arsenate 18 mg/kg , monomethylarsonic acid (**50**) 1800 mg/kg , dimethylarsinic acid (**47**) 1200 mg/kg , and trimethylarsine (**51**) 8000 mg/kg [107].



Arsenic can cause acute and chronic poisoning. Acute arsenic poisoning may cause vomiting, dryness of the mouth and throat, muscle cramps, colicky abdominal pain, tingling of the hands and feet, circulatory disorders, and nervous weakness. Cold and clammy skin, hallucinations, delirium, and diarrhea appear. If death does not occur within 24 h, irreversible organ disorders occur.

Chronic arsenic poisoning involves non-specific symptoms such as chronic weakness, loss of reflexes, weariness, gastritis, colitis, anorexia, weight loss, and hair loss. Long-term exposure through food or air results in hyperkeratosis, hyper pigmentation, cardiovascular diseases, disturbance in the peripheral vascular and nervous systems, circulatory disorders, brittle loose nails with transverse white bands across the nails called Mees lines, eczema, liver and kidney disorders.

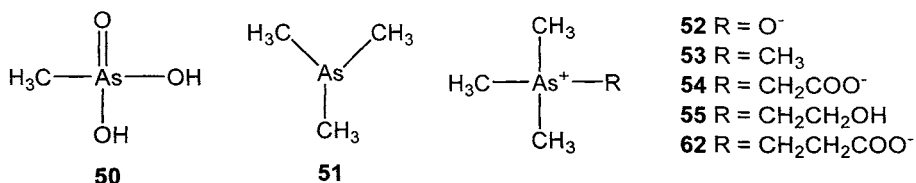
To a certain amount the human body can detoxify the inorganic arsenic compounds As(III) and As(V) by methylation, so that the affinity of arsenic for the tissue is reduced. The possibility of methylation of arsenic

is limited to an arsenic uptake of about 500 $\mu\text{g/day}$ [108]. Arsenic is genetically harmful because it inhibits the repair of DNA damage and is also carcinogenic. Uptake of arsenic by air causes lung cancer. Oral uptake causes bladder cancer, renal cancer, liver cancer, and skin cancer [109-112].

The arsenic compounds described in this chapter were arranged according to their source. This was motivated above all by the fact that nearly all organisms included in this review contain individual organic arsenic compounds. To organize the chapter according to the compounds would mean listing, with each compound, not only the names of the organisms but also citing all relevant literature sources; this would considerably detract from the readability of the text.

The term hyperaccumulators was first used by Brooks et al. [113] to describe the plants that take up and accumulate more than 1000 $\mu\text{moles As/g}$ dry weight. A report about an arsenic-hyperaccumulating fern species additionally discussed the phytoremediation potentials of such plants [114]. Recent investigation has shown that the arsenic compounds in terrestrial and aquatic plants, fungi, and lichen species are also interesting natural products [115, 116].

A study of plants and soil collected near an ore vein in Gasen (Austria) gave the concentration of total, inorganic, and organoarsenic species at this site [117]. The arsenic concentration in the soil ranged from 700 to 4000 mg/kg on dry weight, and arsenic concentrations in plant species ranged from 0.5 to 6 mg/kg on dry weight. The extracts of the plants, *Trifolium pratense*, *Dactylis glomerata*, and *Plantago lanceolata*, contain inorganic arsenic such as As(III), As(V), besides the simple methylated compounds **47**, **50**, and **52-55**. The main organoarsenic detected in *Dactylis glomerata* and *Plantago lanceolata* was **51**. Small amounts of **52-54** were present in all three species, whereas **55** was only detected in *Plantago lanceolata*.



Twelve green plants, namely *Achillea millefolium*, *Alnus incana*, *Asplenium viride*, *Dryopteris dilata*, *Deschampsia cespitosa*, *Equisetum pratense*, *Fragaria versa*, *Larix decidua*, *Picea abies*, *Rubus idaeus*, *Vaccinium myrtillis*, and *Vaccinium vitis idaea* as well as two lichen species, *Alectoria ochroleuca* and *Usnea articulata* have been examined for the presence of organoarsenic compounds [118]. All green plants and lichens contain **47**, **50**, and **54-56**. The maximum was determined in the lichen *A. ochroleuca*, i.e. 77 µg/kg dry weight. Acid **50** was found in *Agrostis scabra* (0.6%) and *Hordeum jubatum* (3.6%) from Yellowknife, Northwest Territories in Canada [118]. Terrestrial grasses, *Bidens cernua*, *Carex* sp., *Equisetum fluviatile* and *Typha latifolia*, as well as terrestrial plants, contain also compound **50**. Arsenosugars such as glycerol-ribose **56** and phosphate-ribose **57** as well as acid **50** were found in aquatic plants (submergents) such as *Lemna minor*, *Myriophyllum* sp. and *Sparganium angustifolium*.

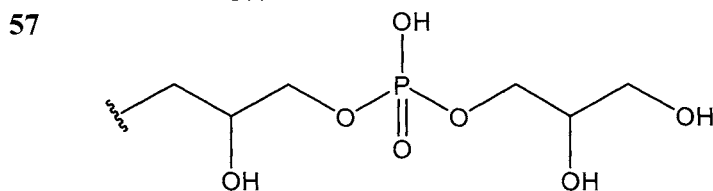
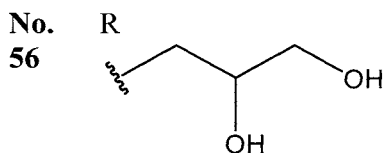
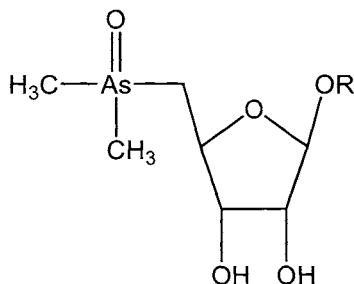
Levels of total arsenic and arsenic compounds were determined in terrestrial fungi, *Paxillus* sp., *Psathyrella* sp., *Leccinum* sp., *Coprinus* sp., *Lycoperdon* sp. (Giant Mine), and *Lycoperdon* sp. (Con Mine), from Yellowknife [120]. The mushroom *Lycoperdon* sp. from the Giant Mine tailings pond also contained a proportionally higher amount of arsenate, although the major arsenic species in both specimens of *Lycoperdon* sp. was arsenobetaine (**54**). Arsenobetaine (**54**) was also observed to be the major arsenic species extracted from *L. echinatum* (78%), *L. perlatum* (88%), and *L. pyriforme* (62%); minor components included As (III), As (V), **47** and **50** [121]. The arsenobetaine (88% of extracted arsenic) was determined as the water-soluble species in the mushroom *Coprinus comatus*. Minor components included **51**, arsenocholine (**55**) and an unknown arsenic compound [121]. Arsenic content of *Paxillus involutus* was unusual because a major proportion of extracted arsenic (36%) was in an unidentified form (unknown compound Y). An unknown peak possessing a retention time similar to that for unknown Y, in an almost identical chromatographic system, was observed in mussels [122].

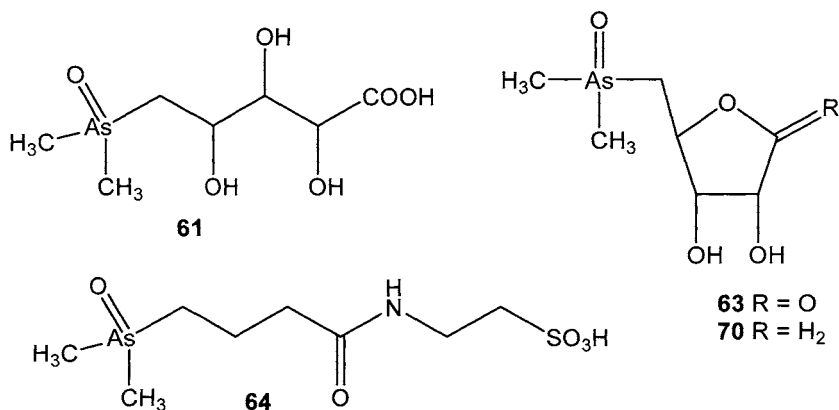
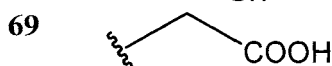
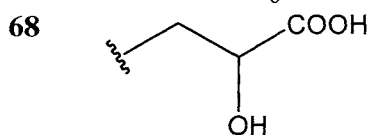
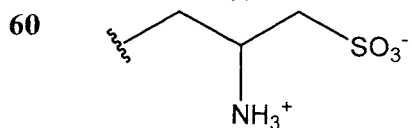
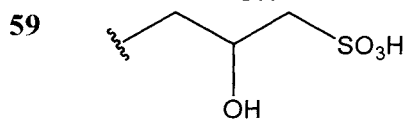
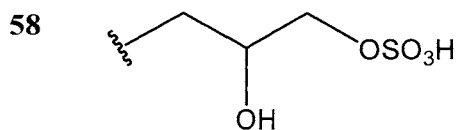
Arsenic compounds were determined in methanol extracts from 50 mushrooms. The main arsenic compound found in many mushrooms (Agaricales and Aphyllophorales) was **54**. Dimethylarsinic and methylarsonic acids were present in many mushrooms, but generally as minor components. *Collybia butyracea* contained mainly **54** (8.8 mg/kg) and dimethylarsinic acid (**47**) (1.9 mg/kg). In *Laccaria laccata*,

Leucocoprinus badhamii and *Volvariella volvacea*, **47** was the major metabolite. Arsenocholine (**55**) and the tetramethylarsonium ion (**53**) were present in a few species, generally in low concentrations. Arsenobetaine (**54**) was the main compound in *Sparassis crispa* [123].

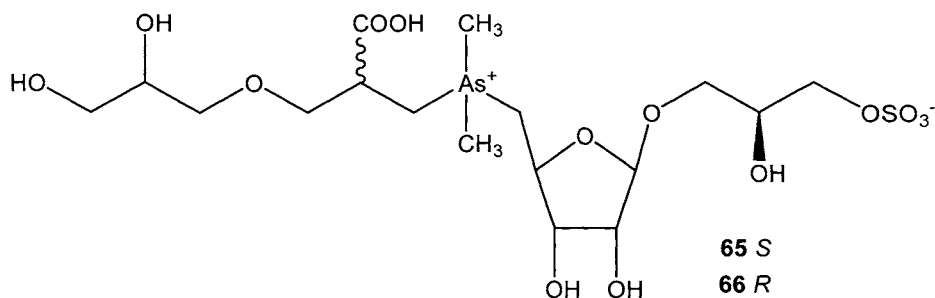
Arsenic species such as arsenic (III), arsenic (V), **47**, and **50** have also been found in many cultivated vegetables and fruits: garlic, onion, potato, carrot, beetroot, spinach, asparagus, cabbage, rice, radish, chard, corn, tomato, and beans, and the concentration of these arsenic species can be varying depending on the locations where the vegetables and fruits were collected [124-126].

Aqueous extracts of the marine brown alga *Hizikia fusiforme* (phylum Sargassaceae) contained methylarsonic acid **50** and dimethylarsinic acid **47** in addition to inorganic arsenic and other unknown organic compounds [127-129]. Further investigation of the *Hizikia* species showed that these algae contain also arsenosugars such as **56-60**. Studies of similar type directed at *Laminaria japonica* [130] and *Laminaria digitata* [131] indicated that arsenic species were present as **47**, **50**, and **54-59**, as well as some novel arsenolipids **61-63**.





Four organoarsenic species **56-59** have been isolated from *Laminaria* sp. [132]. Compound **64** and two diastereoisomers **65** and **66** were found as major compounds [133]. Arsenic-containing ribofuranosides **56-59** as well as inorganic compounds have been isolated from freshly collected samples of the Australian brown kelp *Ecklonia radiana* [134].



The distribution of arsenoribosides and inorganic arsenic compounds has been studied in three classes of marine algae: Phaeophyta (brown), *Echlonia radiata*, *Padina fraseri*, *Lobophora* sp., *Hormosira bankii*, *Sargassum* sp.; Rhodophyta (red), *Corallina officinalis*, *Amphiroa anceps*, *Laurencia* sp.; and Chlorophyta (green), *Cladophora subsimplex*, *Codium lucasii*, and *Ulva lactuca* collected from the coastline near Sydney (Australia) [135]. The authors isolated three major arsenoribosides **57-59** from all examined samples. Biotransformation of arsenic by the brown alga *Fucus serratus* was monitored by HPLC analysis, and arsenite, arsenate, **47**, **50**, and arsenosugars **56-59** were found [136].

Some studies attempted further characterization of the arsenic compounds in algae [137], which involved the growth of microalgae, *Tetraselmis chuii* [138], *Chaetoceros concavicornis* [139] in media containing radiolabeled arsenate.

Unicellular microalgae, *Chladomydomonas reihardtii* [140] and *Polyphysa peniculus* [141] as well as green algae *Chlorella* sp. [142], *Chlorella vulgaris* [143], and *Phormidium* sp. [144] can accumulate and biotransform inorganic arsenic to methylated species. Other marine microalgae, such as *Dunaliella salina*, *Chattonella antiqua*, *Heterosigma akashimo*, *Skeletonema costatum*, *Chaetoceros debile*, and *Thalassiosira weissflogii* [145], are tolerant to arsenate and accumulate As(III) and As(V) in high concentrations, and can transform arsenic (III) and arsenic (V) acids to other arsenic species such as **47**, **50**, **51**.

Thirty different types of Chinese edible seafood, including brown algae, red algae, fish, crab, shrimp, mussels, oysters, and clams, which are very popular foodstuffs in the Chinese cuisine, were examined for their total content of arsenic as well as its different compounds [146].

Arsenolipids such as **56-59** were detected in all of the extracted algae samples (1.5-33.8 $\mu\text{g/g}$ dry wt) and fish samples (0.018-0.78 $\mu\text{g/g}$ wet wt). Arsenobetaine **54** was detected in all of the extracted fish and shellfish samples (0.025-6.604 $\mu\text{g/g}$ wet wt). A major share of arsenic components in seafood was organic arsenic with a low toxicity [146].

The total arsenic concentrations of *Porphyra* collected from the China Sea ranged from 2.1 to 21.6 mg/kg [147]. The analysis report also showed that arsenosugars were the only arsenic species that could be detected in all of the extracts of samples. Arsenosugar PO_4 (**57**) was the major compound in most samples (up to 13.9 mg/kg of dry weight), followed by arsenosugar OH (**56**) (up to 6.2 mg/kg of dry weight). The arsenosugars were stable during a short-term heating at 100 °C. A substantial increase of dimethylarsinic acid (**47**) was detected in urine samples collected from six people. The results obtained indicated that arsenosugars had been metabolized to **47**, which is more toxic than arsenosugars.

The authors [148] describe arsenic binding to *Fucus vesiculosus* metallothionein (which is a metalloprotein having low molecular weight and high cysteine content, which binds a range of metals), characterized by electrospray ionization mass spectrometry. Five arsenic metallothioneins were detected with increasing As to protein ratios. These results provide important information about the metal-chelation behavior of this novel algal metallothionein, which is a putative model for arsenic binding to *F. vesiculosus in vivo*.

A study [149] examined arsenic compounds in raw and cooked edible seaweed and the bioaccessibility of arsenosugars (**56-59**). An *in vitro* digestion (pepsin, pH 2; pancreatin-bile extract, pH 7) was used to estimate arsenosugar bioaccessibility. Cooking of *Undaria pinnatifida* and *Porphyra* sp. did not alter the arsenic species present in the methanol-water extract, but it produced a substantial increase (2 and 5 times) in the As(V) extracted from *Hizikia fusiforme*. In all of the seaweeds analyzed, arsenosugar bioaccessibility was high (> 80%) and did not vary as a result of cooking. Arsenosugar degradation as a result of *in vitro* digestion was not observed.

Water-soluble arseno-compounds were detected in freeze-dried samples of the marine sponges *Halichondria permolis* (13 $\mu\text{g/g}$ dry wt), *H. japonica* (3.42 $\mu\text{g/g}$ dry wt), *H. okadai* (5.5 $\mu\text{g/g}$ dry wt) and *Haliclona* sp. (0.81 $\mu\text{g/g}$ dry wt), which were collected near Japan. Arsenobetaine **54**

and the two arsenosugars **56** and **57** were identified in all targeted samples, and these three compounds accounted for 74-96% of the water-soluble arsenic in all four sponge species. The most abundant arseno-compound in all four species of sponge was arsenosugar **57**, while arsenosugar **56** was only a minor component. The arsenobetaine **54** and arsenosugar **57** concentrations were considerably higher in the three sponges from Demospongiae (*Halichondria*) compared with the single species from Calcispongiae (*Haliclona*). According to [150], arsenosugar **57** has also been found in some marine sponges from Philippines.

Water-soluble fractions containing **54** were isolated from two species of jellyfish, *Aurelia aurita* and *Carybdea rastonii*. Besides arsenobetaine **54** (the major arsenic compound), the tetramethylarsonium ion **53** and arsenocholine **55** were also detected [151, 152]. Nine species of jellyfish, including the two species treated in the previous paper [151], were classified into arsenocholine-rich and arsenocholine-poor species.

The hydrothermal vent shrimp, *Rimicaris exoculata*, which is known to be a primary consumer of the chemolithoautotrophic bacteria, contained 13 µg/g of arsenic almost exclusively as arsenobetaine **54**. The major parts of the extractable arsenic species in the adductor muscle/mantle tissues and in the gill were present as riboside derivatives **56** and **57**, while **54** was present at 16% and 3.6%, respectively, in these tissues [153]. In spite of the absence of biosynthetically active algae, the pattern of arsenic species found in the shrimp species in the deep sea is similar to that found in their counterparts from the ocean surface.

The commercially important western rock lobster *Panulirus longipes cygnus* also contains arsenobetaine **55** as the main arsenic compound [154]. The arsenosugars **56-59** were present in a freshwater crayfish *Procambarus clarkii* collected in the area affected by a toxic mine-tailing spill at Seville, Spain [155]. Different concentrations of the arsenosugars **56** (0.18 µg/g dry mass), **57** (0.077 µg/g), **58** (0.08 µg/g), and **59** (0.14 µg/g) were determined. No significant differences were seen with respect to the total arsenic contents between the sexes but they were seen between the area affected by the spill and the area not affected.

Arsenobetaine **54** was present in the extracts of the earthworm, *Lumbricus terrestris*, as a minor constituent and traces of dimethylarsinic acid **47**, ribosides **56**, and **57** were also detected [156]. The major arsenic compound in the earthworm extracts was an arsenic-containing

dimethylarsinic acid **47**, methylarsonic acid **50**; carbohydrate **54** (55%), and glycerol arsenosugar **56** were also present as minor constituents [157]. Two species of earthworm, *Lumbricus rubellus* and *Dendrodrilus rubidus*, collected from an arsenic-contaminated mine spoil site and an uncontaminated site, were investigated for arsenic compounds [158]. Arsenobetaine **54** was the only organic arsenic species detected in both species of earthworms, with the remainder of the extractable arsenic being arsenate and arsenite.

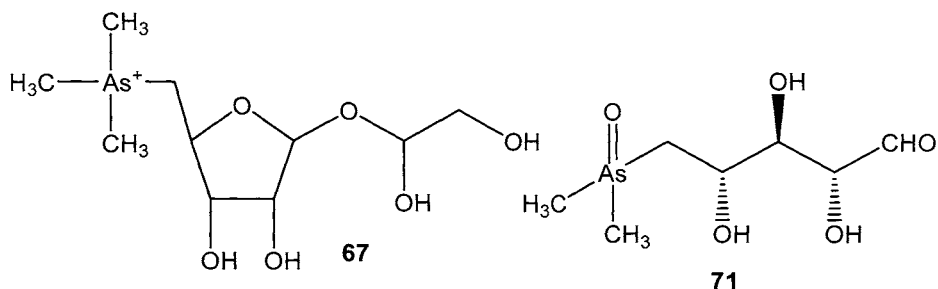
Arsenic compounds were determined in the marine lungworm *Arenicola marina* collected from Odensee Fjord, Denmark [159]. In contrast to most other marine animals, *A. marina* contained most water-soluble arsenic in inorganic forms, and arsenobetaine **54** was present as a minor constituent (6% only). Other arsenic compounds detected in *A. marina* were dimethylarsinate **47** (4%), tetramethylarsonium ion **53** (1.5%), arsenocholine **55** (<1%), and two arsenosugars (**56**, **57**, 1% and 3%, respectively). A new arsenobetaine, i.e. trimethylarsoniopropionate (**62**), previously only reported in fish, was also present in trace amounts (<1%).

Arsenic compounds were examined in three marine gastropods from Thailand, *Thais bitubercularis*, *T. distinguenda*, and *Manila musiva* [160], and the presence of arsenobetaine **54** (93-95% of total extractable arsenic), arsenocholine **55** (3.1-4.6%), tetramethylarsonium ion **53** (0.21-2.2%), a new natural product (**67**), one unknown arsenic compound (each approx. 0.1%), and an unresolved mixture of arsenic compounds (up to 1%) were observed.

The arsenobetaine **54** and riboside **56** as the major water-soluble arsenic compounds were characterized in the sea scallops (*Plactopecten magellanicus*) and Icelandic scallops (*Chlamys islandica*) collected near Newfoundland [161]. This result indicated that consumption of the scallops poses little human risk.

The tetramethylarsonium ion **53**, arsenobetaine **54**, and arsenocholine **55** were detected in the soft tissues of both the pearl-free and the pearl-containing pearl oysters *Pinctada fucata* [162]. More than 20 years ago, arsenic-containing sugars **56** and **58** have been isolated from the kidney of the giant clam, *Tridacna maxima* [163]. Water-soluble arsenic species were determined in the kidney of the *Tridacna derasa* [164]. A total of fifteen organoarsenic species were identified, and 13 of these possessed

the dimethylarsinoyl group, i.e. ribofuranosides **56-59**, **68**, **69**, acyclic compounds **61-62**, and dihydroxyfuran **70**. Arsenobetaine and dimethylarsinic acid were also detected. The major species (up to 50% water-soluble arsenic) was 5-dimethylarsinoyl-2,3,4-trihydroxypentanoic acid (**71**). Of the organoarsenic compounds found in *Tridacna derasa*, **56-59** are well known to be elaborated by marine algae [127-129], are presumably products of the zooxanthellae in the clam mantle, and have not been further metabolized by the clam. On the other hand, as has been recently observed, **68** has been previously isolated from the kidney of the related *T. maxima*, has not been found in algae, and might well be the result of metabolism by the clam of algal products [165]. The major arsenic compounds in the seaweed, *Hormosira banksii*, were arsenosugar **56** and an unidentified compound. Arsenobetaine **54** accounted for 95% of arsenic in the carnivorous gastropod *Morula marginalba*, the concentration of arsenocholine was higher and the concentrations of the minor arsenic compounds lower than in the herbivorous gastropod *Austrocochlea constricta* [166].



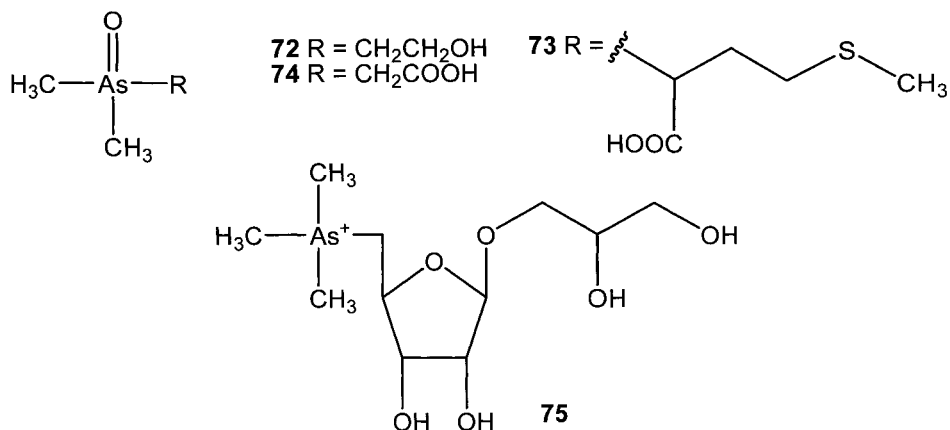
The presence of dimethylarsinate **47**, arsenobetaine **54**, arsenocholine **55**, and trimethylarsoniopropionate **62** was detected in kidney, liver, and lung of the sperm whale *Physeter catodon* cropped in the Andaman Sea [167].

A study [168] evaluated the intake, uptake, and excretion (urine and feces) of arsenic by sheep that live on the Orkney Islands and naturally consume large amounts of arsenosugars through their major food source - seaweed. The sheep eat a broad variety of seaweed species, *Laminaria digitata* and *L. hyperborea* having the highest arsenic content (~74 mg/kg dry mass). In a feeding trial, the average daily intake of arsenic by 12 ewes was ~35 mg (97% of water-extractable arsenic was present as

arsenosugars) gained from feeding on the two brown algae. To test the possible influence of microflora on the metabolism of arsenosugars, six of the sheep were adapted to feeding on grass for 5 months before the start of the trial, and the remaining six sheep were kept on their normal seaweed diet. No significant difference in seaweed/arsenic intake and arsenic excretion was found between the two groups of sheep. The arsenic excreted in the feces represented 13% of the total consumed, and the urinary excretion was estimated at about 86%. The main arsenic metabolite excreted in urine was dimethylarsinic acid (**47**) (60%), minor amounts being found of methylarsonic acid (**50**), tetramethylarsonium ion (**53**), dimethylarsinoylethanol (**72**), and arsenate (V) together with seven unknown arsenic compounds.

In a set of experiments, the alga *Laminaria* was ingested by healthy volunteers [169]. Total arsenic and speciation analysis revealed no marked increase in arsenic blood, serum, and packed cells levels up to 7 h after ingestion. Dimethylarsinic acid (**47**), methylarsonic acid (**50**), and dimethylarsinoylethanol (**72**) have been positively identified in urine sampled after algae intake. Another 5 compounds remained unknown. In simulated gastric fluid incubated with algae, the larger share of the arsenosugars degraded within a short time span into a compound with a mass of 254 Da.

The concentrations of the three arsenicals (**75-77**) were determined in 37 marine organisms comprising algae, crustaceans, bivalves, fish and mammals by high-performance liquid chromatography/inductively coupled plasma mass spectrometry (HPLC/ICPMS) [170]. All three organoarsenics, which occurred at $\mu\text{g/kg}$ concentrations, were detected in 25, 23 and 17 of the 37 samples analyzed, respectively. The limits of detection were 2-3 $\mu\text{g/kg}$ dry mass. The data illustrate that all three compounds are common minor constituents in practically all marine samples.



Dimethylarsinic acid (47), arsenobetaine (54), two arsenosugars (56, 59), and several unidentified arsenic species were detected in extracts of the haemolymph of the Dungeness crab, *Cancer magister*, by using HPLC-ICPMS [171]. The results demonstrate that arsenic compounds present in the diet of crabs are not fully metabolized in the gut. They are, at least partly, taken up into the haemolymph. The occurrence of arsenobetaine (54) and arsenosugars suggests that the use of repeated haemolymph sampling in crustaceans could facilitate investigations into the kinetics of the biotransformation pathways of arsenic compounds.

Analysis of 10 crude fish oils from various regions of the world for arsenolipids was performed by normal phase HPLC-ICPMS with various mixtures of organic solvents as mobile phases [172]. All ten fish oils appeared to contain the same 4 - 6 major arsenolipids, but in varying amounts depending on the origin of the fish. Further chromatography of some of the oils under both normal phase and reversed-phase conditions indicated the presence of many more minor arsenolipids. Unfortunately, the authors did not provide any data on the structures of the arsenolipids they described.

The pathway of formation of organoarsenics was examined in marine animals, e.g. fish (*Odax cyanomelas*), abalone (*Haliotis rubra*), and sea urchins (*Heliocidaris erythrogramma* and *Centrostephanus rodgersii*) that are directly exposed through their diets to dimethyl arsenoriboses in macroalgae (*Phyllospora comosa* and *Halopteris platycena*) [173]. The identification of dimethyl arsenoriboses (56, 57, 59) in both dominant

macroalgae species suggest that these arsenic species are to some degree accumulated directly from their diets without degradation or conversion. An unknown arsenic species in *H. rubra* intestinal tissue was identified as 2',3'-dihydroxypropyl 5-deoxy-5-trimethyl arsonioriboside (**75**) (5.0 µg/g dry mass) using tandem mass spectrometry. The presence of **75** in marine animal tissues may be due to microbial-mediated processes that promote the reduction and methylation of dimethyl arsonioriboses released during the breakdown of macroalgae in their diets. The identification of a large amount of trimethyl glycerol arsonioribose in *H. rubra* intestinal tissue suggests that this species is a main constituent in the pathway for arsenic metabolism in this marine animal.

CONCLUSION

Arsenic is a virtually ubiquitous element and arsenic compounds are present in a wide variety of environment components including living organisms, both terrestrial and aquatic. Because of the chemical properties of arsenic, it can be found as part of many types of organic compounds - arsenolipids, arsenosugars and many other types of compounds. Their known toxicity notwithstanding, arsenic compounds, especially organic ones, are integral components of the food chains of many organisms including humans and may play potentially important biological roles in these organisms. Their deeper and more detailed knowledge may contribute to their resurrection as medically or environmentally useful or beneficial agents.

Selenium

The relative proportion of selenium in the environment is very low. In the Earth's crust, selenium is present in a concentration of 0.05 – 0.09 mg/kg. Its level in sea water is usually 0.45 µg/kg, in stream water 0.2 µg/kg. In compounds, selenium is present as Se^{2-} , Se^{2+} , Se^{4+} , and Se^{6+} . In general, it is present in the environment in elemental form or in the form of selenide (Se^{2-}), selenate (SeO_4^{2-}), or selenite (SeO_3^{2-}). In soils, the identity and amounts of the various oxidation-state species depend enormously on the redox-potential conditions, with the lower oxidation states predominating in anaerobic conditions and acidic soils, while the higher oxidation states are favored in alkaline and aerobic conditions. Both selenites and selenates are taken up by plants and converted to protein-bound selenocysteine and selenomethionine, soluble inorganic forms, several free amino acids, and volatile organoselenium compounds. The elemental form of selenium, selenium dioxide, and volatile organoselenium compounds produced by industries and plants are incorporated in the environment. Selenium occurs naturally in water in trace amounts - see above - as a result of geochemical processes, such as weathering of rocks and erosion of soils, and is usually present there as selenate or selenite.

Although most selenium compounds are markedly toxic, considerable attention in the last years has been devoted to the effects of selenium shortage in the daily food intake. A permanently low selenium intake in foods has been found to have an adverse effect on the cardiovascular system and to increase the risk of myocardial infarction and vascular afflictions. The lack of selenium in the nutrition of pregnant women can negatively affect the development of the embryo. In the organism, selenium functions as an antioxidant that destroys free radicals and reduces thereby the risk of cancer. On the other hand, the daily intake of selenium should not exceed a certain limit. An optimum daily dose is currently thought to be 70 µg for men and 55 µg for women. On exceeding the physiological requirements of selenium for an adult man (70 µg/day), the threshold for toxicity may be as low as 700 µg/day.

One biological function for selenium is incorporation into the amino acid selenocysteine that is found in some proteins [174]. Microbial dissimilatory reduction of selenate to selenite and then to elemental

selenium has been described [175]. Selenide undergoes biomethylation to produce methylselenide and dimethylselenide [176].

Practically all small organic selenium compounds in plants, yeast, bacteria or animals are the isologues of corresponding sulfur compounds (Tables 14 and 15).

Table 14. Distribution of selenium compounds in plants, fungi, algae, etc

Compounds	Species
Selenocystathionine (76)	<i>Aspergillus fumigatus</i>
	<i>Aspergillus terreus</i>
	<i>Astragalus pectinatus</i>
	<i>Astragalus praeongus</i>
	<i>Brassica oleracea capitata</i>
	<i>Lecythis ollaria</i>
	<i>Morinda reticulata</i>
	<i>Neptunia amplexicaulis</i>
	<i>Penicillium chrysogenum</i>
	<i>Stanleya pinnata</i>
Se-Methylselenocysteine (77)	<i>Allium cepa</i>
	<i>Allium sativum</i>
	<i>Allium tricoccum</i>
	<i>Astragalus bisulcatus</i>
	<i>Astragalus crotalariae</i>
	<i>Astragalus praeongus</i>
	<i>Brassica oleracea botrytis</i>
	<i>Brassica oleracea capitata</i>
	<i>Dunaliella primolecta</i>
	<i>Melilotus indica</i>
	<i>Oenopsis condensata</i>
	<i>Phaseolus lunatus</i>
	<i>Phaseolus lunatus</i>
γ -Glutamyl-Se-methylselenocysteine (78)	<i>Allium cepa</i>
	<i>Allium sativum</i>
	<i>Aspergillus terreus</i>
	<i>Astragalus bisulcatus</i>

	<i>Penicillium chrysogenum</i>
	<i>Phaseolus lunatus</i>
Selenomethionine (79)	<i>Allium tricoccum</i>
	<i>Aspergillus fumigatus</i>
	<i>Aspergillus terreus</i>
	<i>Brassica juncea</i>
	<i>Brassica oleracea capitata</i>
	<i>Melilotus india</i>
Se-Methylselenocysteine Se-oxide (80)	<i>Brassica oleracea capitata</i>
Selenobiotin (82)	<i>Phycomyces blakesleeianus</i>
Selenocysteine (83)	<i>Fusarium</i> sp.
	<i>Vigna radiata</i>
Dimethyl selenide (84)	<i>Penicillium</i> sp.
γ -Glutamylselenocystathionine (858)	<i>Astragalus pectinatus</i>
γ -Glutamylselenomethionine (86)	<i>Allium sativum</i>
Se-Adenosylselenohomocysteine (87)	<i>Saccharomyces cerevisiae</i>
Selenocysteic acid (88)	<i>Dunaliella primolecta</i>
	<i>Fusarium</i> sp.
Se-Methylselenomethionine (89)	<i>Aspergillus fumigatus</i>
	<i>Dunaliella primolecta</i>
Selenolanthionine (90)	<i>Saccharomyces cerevisiae</i>
4-Selenouridine (91)	<i>Escherichia coli</i>
3-Butenyl isoselenocyanate (92)	<i>Stanleya pinnata</i>
Selenosinigrins (93)	<i>Armoracia lapathifolia</i>
	<i>Stanleya pinnata</i>
Selenosugars (94-96)	<i>Astragalus racemosus</i>

With few exceptions, they are isologues of sulfur amino acids or their derivatives. As a rule, their abundance in nature roughly parallels that of selenium in the environment or available feeds, respectively. This is not surprising since, in most cases, neither the initial steps of selenium assimilation nor the enzymes of the trans-sulfuration pathway discriminate between sulfur and selenium [177]. Usually the Michaelis constants (K_m) of these enzymes are slightly lower for the seleno

isologues, and this is balanced by smaller maximum velocities ($V_{\max}$). There are, however, exceptions to this rule. A highly specific selenate reductase has recently been purified from the proteobacterium *Thauera selenatis* [178]. Some cystathionine lyases reportedly prefer selenocystathionine (76) over cystathionine [179]. Also the key enzyme leading to *Se*-methylated selenocysteine (77) and derivatives thereof, the *S*-adenosyl methionine-dependent selenocysteine methyltransferase, works much less efficiently with, and has a lower affinity for, cysteine. Interestingly, this enzyme was discovered in *Astragalus bisulcatus*, a plant that can be considered as the prototype of the selenium accumulators (see below). The closely related species, chick-pea milkvetch *Astragalus cicer*, which is not a selenium accumulator, lacks selenocysteine methyltransferase under conventional culture conditions. *A. cicer* can, however, be slowly adapted to a selenium-rich substratum. The selenium tolerance thus achieved is accompanied by the appearance of selenocysteine methyltransferase. Evidently, the ability of specialized plants to methylate selenocysteine more efficiently and to accumulate selenium in the better tolerated *Se*-methylated forms is one of the prerequisites for surviving on seleniferous soils. In accordance with this assumption, 77 and derivatives such as γ -glutamyl-*Se*-methylselenocysteine (78) were predominantly detected in typical selenium accumulators of the genus *Astragalus* and other selenium-tolerant plants like *Brassica* and *Allium* species.

Table 15. Distribution of selenocompounds in various biological materials

Biological Materials	Selenate	Selenite	Percentage Distribution ¹			Other ²	References
			77, 78	79	83		
Rats injected with selenite				6-10	64-70	20-34	[180-182]
Rats injected with 79 (1 day)				63	22	15	[181, 182]
-“- (5-35 days)				14-25	46-57	18-40	[181, 182]
17 Fish	15-36						[183]
20 Vegetables	1-50						[184]
Wheat grain	12-19 ³		1-4	56-83	4-12	4-26	[185, 186]
Wheat straw	97 ⁴					3	[185]
Corn				61-64	15-16	20-24	[187]
Rice	1-3	5-13		68-81	6-10	19-31	[187]
Soybeans				>80			[188]
Grassland legume			10-13	51-70	19-39 ⁵		[189]
Phytoplankton ⁶ (15%)	1.0 (0.15)	83 (12)	12.8 (1.9)	3.2 (0.5)			[190]
<i>Astragalus praelongus</i> (95%)	1.4	9	52	37			[190]
Commercial preparation (95%)	0.6	98.7		0.7			[190]
Se-enriched yeast ⁶ (8.5%)		27 (2.3)	13 (1.1)	59 (5.0)		5 (0.4)	[190]
Se-enriched garlic	2-5	8	47-87	1-6	1-13	4-36	[191-193]
Se-enriched yeast	4		6-20	23-63	13-21	13-51	[190, 194-196]
Se-enriched onions			42-55	1-4	7-38	21-35	[192, 193]
Se-enriched broccoli florets			63	5	11	21	[192]
Se-enriched broccoli sprouts	20		45	12		3	[197]
Se-enriched wild leeks (bulbs)	12-25		35-50			1-3	[198]

¹ In most cases the percentage distributions were calculated from the areas under the curves of the chromatograms.

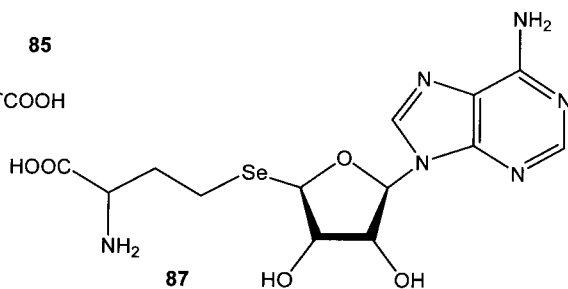
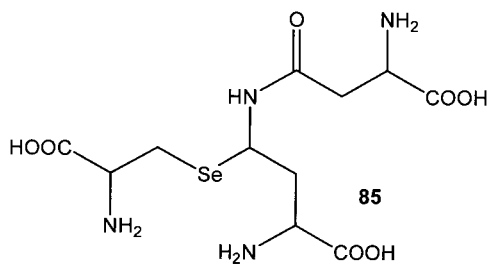
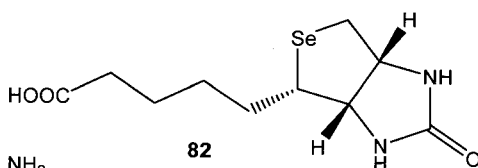
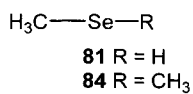
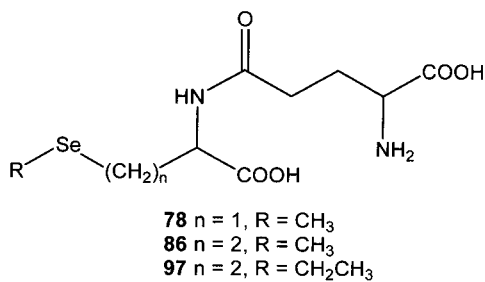
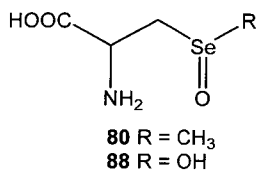
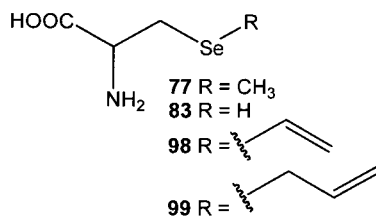
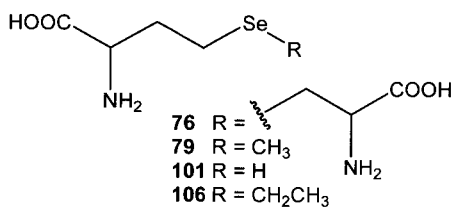
² Includes 76, 87, 90, 97, 98, 100, and unknown.

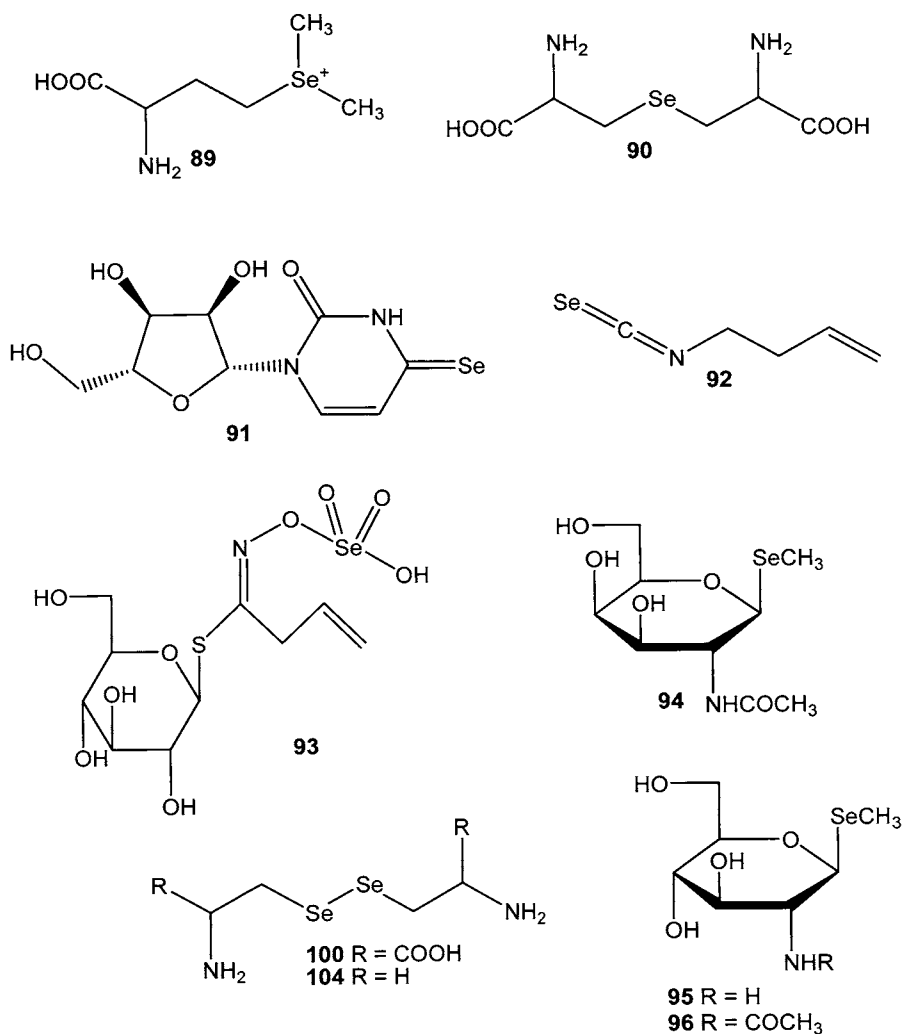
³ Combination of selenate, selenite and 88.

⁴ Mixture of selenate and 88.

⁵ 83 and 100.

⁶ Numbers in parentheses represent the percentage of selenium extracted by aqueous solution.





Amino acid **78** and its γ -glutamyl derivative are other components of the major pool of seleno compounds in accumulator plants, while selenomethionine (**79**) is the major compound in microorganisms like yeast, which is not specialized in selenium utilization. *Se*-Methylselenocysteine *Se*-oxide (**80**), which is found in marine algae, tends to spontaneously decompose with the formation of pyruvate and ammonia *via* aminoacrylic acid and methaneselenic acid (**81**). The latter reacts with sulfhydryls or selenols to selenodisulfides and diselenides,

respectively. Another group of seleno metabolites characteristic of some accumulator plants are the isoselenocyanates and their precursors, the selenosinigrins. Like their sulfur analogues they appear to be common in *Crucifera*. Their biosynthesis has been studied in horseradish and *Stanleya pinnata*. Selenobiotin (**82**) was detected in *Phycomyces* (*Zygomycetae*). Its biological role remains obscure. The inability of the trans-sulfuration pathway to discriminate between sulfur and selenium gives rise to the remaining selection of low molecular weight selenium compounds so far identified in nature.

Plants absorb Se from soil primarily as selenate and translocate it to the chloroplast, where it follows the sulfur assimilation pathway. Se is reduced (enzymatically and non-enzymatically) to selenide, which reacts with serine to form selenocysteine (**76**). It can be further metabolized to selenomethionine (**79**) and methylated to form products such as *Se*-methyl selenomethionine (**89**). Alternatively, selenocysteine-specific methyl transferase may form *Se*-methyl selenocysteine (**83**), allowing the plant to accumulate extraordinarily large amounts of Se.

Se-enriched plants may be divided into two broad groups [199], i.e. selenium accumulators and/or selenium non-accumulators. Selenium-accumulating plants can be divided into three subgroups: selenite-accumulators (broccoli and cucumber), selenomethionine accumulators (grains such as wheat, and mushrooms) and *Se*-methyl selenomethionine accumulators (garlic and onion) [200].

Most plants, which are considered to be non-accumulators, contain only moderate foliar Se concentrations, that rarely exceed 100 µg/g dry weight when growing on seleniferous soils [201]. Yet, a small number of plants, growing on naturally occurring soils containing only 2-10 ppm Se, can accumulate well over 1 mg/g dry weight Se, and these plants have been classified as Se hyperaccumulators [202]. The largest group of Se-hyperaccumulating plants belongs to the genus *Astragalus* (Fabaceae) [203]. Twenty-five species of *Astragalus* have been characterized as Se hyperaccumulators [204]. Some species can accumulate up to 0.6% of shoot dry weight as Se from soils with 2-10 µg/g dry weight. This is 100-1000 times more Se than found in adjacent non-accumulating plants including other *Astragalus* species [204-206].

There are limited reports about the chemical forms of Se in these plants, but the available information suggests that the Se is often in

methyated forms such as selenocystathione (76), *Se*-methyl selenocysteine (77), γ -glutamyl-*Se*-methyl selenocysteine (78), methyl selenol (81), γ -glutamyl selenocystathione (85), and selenohomocysteine (101). These forms of Se may be safely stored in membrane-bound structures within the plant; Se hyperaccumulators have a relatively small percentage of their total Se sequestered in the protein fractions of the plant. Certain species of *Astragalus* may accumulate in excess of 2 mg Se/g plant tissue, and that Se often is in forms such as 76-78, 81, 85, and 101. Selenocysteine-specific methyltransferase is the enzyme needed for production of many of these compounds, and insertion of the gene for this enzyme into an *Astragalus* species that does not accumulate Se converts the plant to a Se hyperaccumulator. Recently, this gene has been inserted into *Arabidopsis*, allowing accumulation of 77 and 85.

Se-enriched garlic contains Se primarily as γ -glutamyl-*Se*-methyl selenocysteine (78). Se from garlic does not accumulate in tissues to the same extent as Se from foods enriched in selenomethionine. Species of *Brassica* that are reported to accumulate Se include: broccoli (*Brassica oleracea*), Indian mustard (*Brassica juncea*), Brussels sprouts (*Brassica oleracea* L.), and canola (*Brassica napus*). *Brassica* spp., especially broccoli and canola, also have been used for phytoremediation, a process that uses Se hyperaccumulators to remove high (potentially toxic) concentrations of Se from soil and/or irrigation water. In a single growing season, broccoli may extract up to 20% of soluble Se from Se-laden drainage water.

Although not true plants, mushrooms are generally grouped with vegetables for dietary purposes. Some but not all mushrooms accumulate Se. A survey of 83 species of wild mushrooms reported Se concentrations ranging from 0.01 to 20 ppm. *Agaricus bisporus* can accumulate very high concentrations of Se. Other mushrooms that may accumulate Se include *Boletus edulis* and *B. macrolepiota*. A limited number of studies have reported Se from mushrooms to have low bioavailability, and it has also been reported to be present in low-molecular weight compounds that are not selenocysteine, selenomethionine, or selenite (Table 16).

A few investigators have studied the blue-green alga *Spirulina platensis* as a possible source of Se/iodide pharmaceuticals. Se in *Spirulina* has been reported to be in a high-molecular-weight form. Overall bioavailability of Se from *Spirulina* was reported to be low, but

an ultrafiltrable soluble fraction was highly bioavailable. Se-enriched kelp is commercially available as a dietary supplement.

Se-enriched yeast is the most abundant commercially available source of supplemental Se, with at least a dozen products. Selenomethionine is reported to be the primary chemical form of Se in yeast. Because manufacturers of Se-enriched yeast do not adhere to a single production and/or quality standard, the form of Se probably varies considerably from product to product.

Mutations in ATPS (sulfate adenylyl transferase, EC 2.7.7.4) in the yeast *Schizosaccharomyces pombe* resulted in increased selenate tolerance [207]. The selenate resistant phenotype of these mutants was correlated with low sulfate uptake capacity and low ATPS activity.

Table 16. Examples of plants that are Se accumulators or hyperaccumulators and are used as food

Plant	Concentration (mg/kg)	Compound	Reference
<i>Accumulators</i>			
Wheat	0.1–15	79	[208]
Brazil nuts	2.0–35 and more	79	[209]
Mushrooms	0.1–20	Unknown ¹	[210, 211]
Brussels sprouts	0.03–7.0	Unknown	[212]
<i>Hyperaccumulators</i>			
Garlic	>1200	77	[213]
	<300	78	[213]
Broccoli	~1000	77	[214]
Ramp	>500	79 +inorganic Se	[215]

¹ not seleno AA or selenite

Selenium-containing supplements are thought to be more effective when the selenium is ingested in an “organic” form, and many suppliers provide the selenium as selenized yeast, see above, which contains largely selenomethionine (**79**) bound in proteins in addition to many other unknown selenium species. Consumers of such products intent on improved health should be aware, however, of the toxicity of selenium and the possible toxic consequences of overindulgence. Because urine is a major excretory route for selenium, metabolic changes delineating the boundary between essential and toxic concentrations are likely to be

reflected in urinary selenium species. Indeed, all evidence indicates that selenium is methylated in biological systems after reduction of selenite/selenate to hydrogen selenide (excreted by respiration) and trimethylselenonium ion (**102**) (excreted in the urine). The general feeling that methylation processes are a form of detoxification seems to hold for selenium because dimethylselenide (**84**) has much lower acute toxicity (at least 200-fold) than do the inorganic selenium species selenite/selenate and selenoamino acids (Table 17). The rapid excretion of **84**, 71-79% by the rat in 6 h, is also suggestive of a metabolically inert detoxification product. Surprisingly, **102** is at least 20-fold more toxic than dimethylselenide, and thus the further methylation step to give **102** does not appear to benefit the organism in terms of detoxification.

HPLC/ICPMS is a powerful analytical technique and is now the most commonly used method for determining selenium urinary metabolites [215]. Over the last 10 years, most of the reports of selenium species in urine have used HPLC/ICPMS, sometimes together with molecular MS techniques. In this period, a total of 16 selenium species (see below) have been reported in urine, most of them novel human metabolites and some of them completely new compounds.

These 16 compounds identified in urine mostly by HPLC/ICPMS include the compound **102** plus 15 other selenium metabolites (Table 18). For many of these, the assignments have been made on very little evidence and require confirmation before the compounds can be accepted as typical urine metabolites [216, 217]. Some, such as methylselenol, have already been retracted by their discoverers. Selenosugar **95** is now firmly established as a major urinary metabolite when selenium is administered, and it is also a constituent of natural urine. There have also been reports of selenosugar **94** and selenosugar **96** as minor constituents. Selenite appears to be a common minor metabolite in normal urine.

Table 17. Acute toxicity of some selenium species to the rat

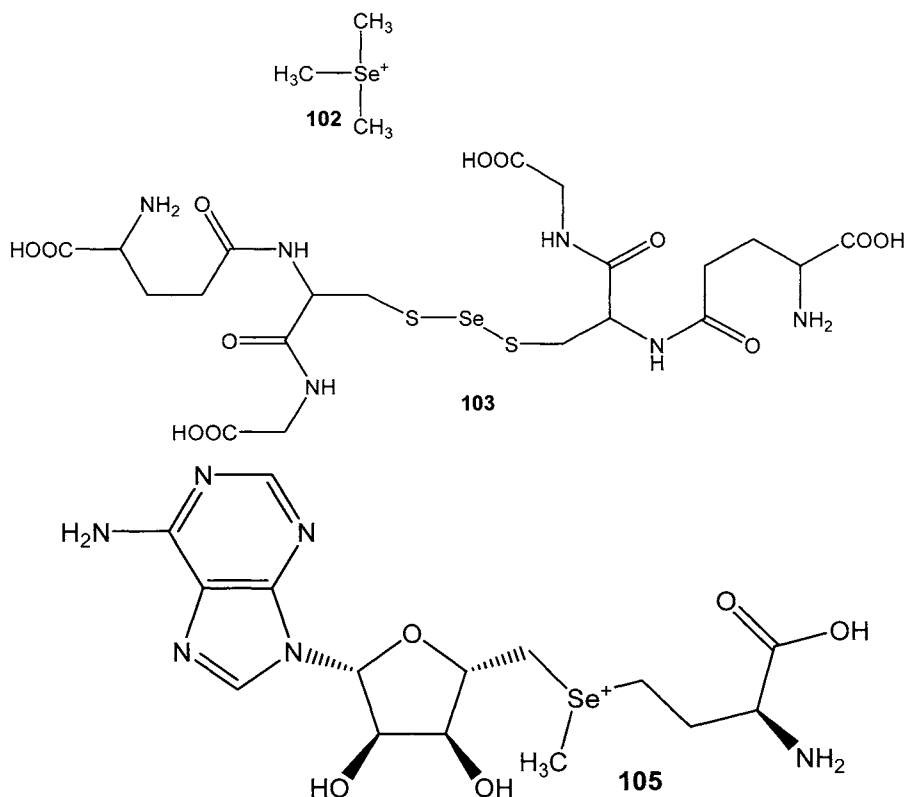
Compound	Se toxicity to rat
Sodium selenite	Oral; 10-day LD ₅₀ ^a = 3.2 mg/kg Se
	Intraperitoneal; 48-h LD ₇₅ = 3.5 mg/kg
Sodium selenate	Intraperitoneal; 48-h LD ₇₅ = 5.5 mg/kg
Selenocystine (100)	Intraperitoneal; 48-h LD ₇₅ = 4.0 mg/kg
Selenomethionine (79)	Intraperitoneal; 48-h LD ₇₅ = 4.25 mg/kg
Dimethylselenide (84)	Intraperitoneal; 24-h LD ₅₀ = 1600 mg/kg
Trimethylselenonium (102)	Intraperitoneal; 4-h LD ₅₀ = 49.4 mg/kg

^a LD₅₀ and LD₇₅, concentrations at which the dose of selenium is lethal in 50% and 75% of animals, respectively.

Table 18. Summary of reported selenium urinary metabolites: confirmed, unconfirmed, and retracted

Compound	No of reports	Accept as a normal urinary metabolite?
Trimethylselenonium ion (102)	46	Yes for now, but the early work urgently needs confirming with modern analytical methods
Selenite	16	Yes, but a minor one
Selenate	5	No
Selenodiglutathione (103)	1	No
Methylselenol (81)	3	No; the original discoverers have retracted this assignment
Selenocystine (100)	2	No
Selenocysteine (83)	1	No
Selenoethionine (106)	1	No
Se-methyl selenomethionine (89)	1	No; reported in the abstract but appears to have been retracted in the text of the same report
Selenomethionine (79)	4	Not just yet; several studies report it after ingestion of selenized yeast
Selenocystamine (104)	1	Not yet; the HPLC/ICPMS data do not match the MS/MS data
Se-adenosylmethionine (105)	1	Not yet; the species is reported as being very unstable
Selenosugar (94)	2	Yes, a minor constituent
Selenosugar (96)	4	Yes; firmly established as a major selenium metabolite after supplementation with selenite or selenized yeast; also data showing it is present in

		urine without selenium supplementation
Selenosugar (95)	1	Yes, a minor constituent
Methylselenite (106)	1	Thought to be formed in urine from oxidation of selenosugar 96



Recently, some organoselenium compounds have found pharmacological applications as therapeutic agents in the treatment of several diseases.

The first compounds demonstrating chemopreventive potential were found in plants such as broccoli and green tea. Today, chemoprevention refers to the use of both natural and synthetic compounds to inhibit carcinogenesis.

The basic idea that selenium is a cancer-protecting trace element began in the 1950s, when researchers tried to find substances that could prevent

tumor formation. The epidemiological literature on selenium and cancer has been reviewed [218-220], and it has been suggested that an increased risk for certain human diseases including cancer is related to insufficient intake of selenium; however, some inconsistency still remains [218].

It is clear that mammalian selenoproteins play critical roles in many vital cellular functions and are, therefore, essential for disease prevention [221]. There is an extensive amount of research into the use of organoselenium compounds for cancer prevention [222-224].

Different authors [225-229] found that the CH_3SeH -precursor and *Se*-methylselenocysteine (**77**) are anti-carcinogenic and somewhat more efficacious than the inorganic compound, selenite. Later work has shown that the CH_3SeH precursors methyl-selenocyanate (CH_3SeCN) and **77** each inhibit mammary cell growth, arresting cells in the G1 or early S phase and inducing apoptosis [230-232]. Various synthetic organoselenium compounds have been tested and found to be anti-carcinogenic.

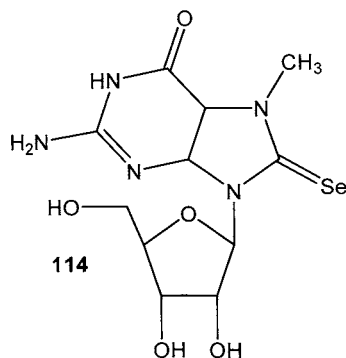
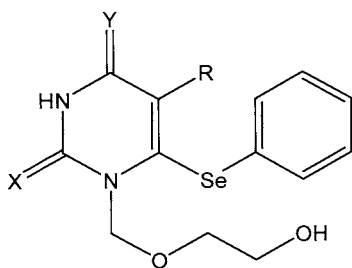
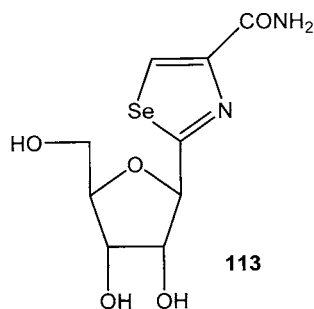
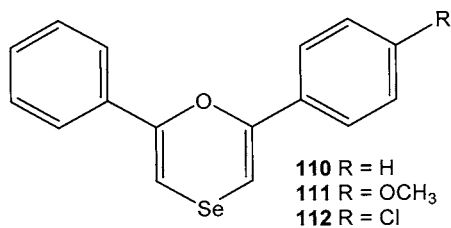
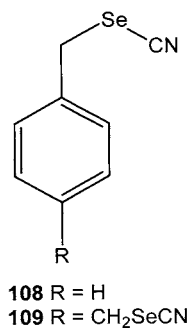
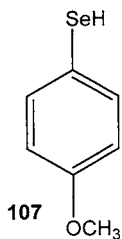
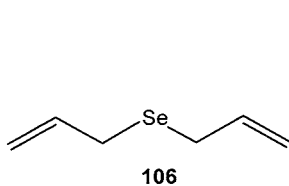
The same group showed that *Se*-allylselenocysteine (**98**), which is expected to yield allylselenol, a fairly hydrophobic metabolite, has more anti-carcinogenic power than **77** and propylselenocysteine (**99**) in rat methylnitrosourea model [233]. The diallyl selenide (**106**) has significant anti-carcinogenic activity using DMB-induced mammary tumor model and is 300 times more active than the diallyl sulfide, a flavor component of garlic [234].

Aromatic selenium compounds in cancer prevention were the first to be synthesized in the 1980s. The applicability of compounds **107-109** in tumor control has been demonstrated in some organs using animal models; they are effective in both the initiation and post initiation phase. These organoselenium compounds are less toxic and chemopreventive than inorganoseleniums and natural organoseleniums, and can be the candidates for excellent chemopreventive agents for human cancers.

γ -Glutamyl-*Se*-methyl selenocysteine **86** is the major selenium compound in natural and selenized garlic, and is an effective carcinogenic agent against mammary gland cancer in rats.

Novel organoselenium compounds like those shown below have been synthesized and used [235]. Oxaselenins, compounds **110-112**, have proliferation-inhibiting effects against human uterine cervical cancer

cells. Compound **112** showed the greatest growth inhibition with IC_{50} of 45.1. Compounds **110** and **111** exhibited only weak inhibitory activities, with IC_{50} of $> 100 \mu M$.



	R	X	Y
115	H	O	O

116	CH ₃	O	O
117	F	O	O
118	Cl	O	O
119	Br	O	O
120	CH ₃	S	O
121	H	O	H ₂
122	CH ₃	O	H ₂
123	F	O	H ₂
124	CH ₂ CH ₃	O	H ₂

Organoselenium compounds can be used as antiviral and antibacterial agents and this has been reviewed many times [222, 236-238].

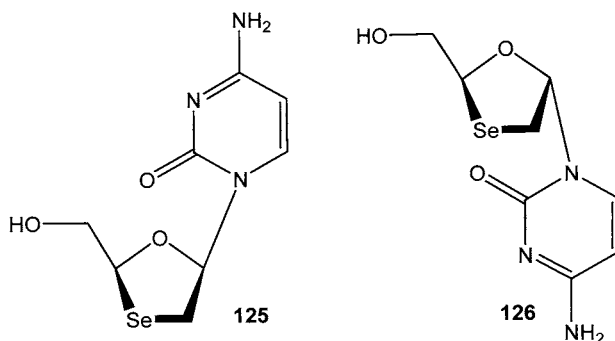
Antivirals

Several interesting organoselenium compounds exhibit antiviral activities. One such is selenazofurin **113**, effective against different types of viruses (herpes simplex, parainfluenza and rhinovirus). The purine analogue, 7-methyl-8-selenoguanosine **114**, has been tested *in vivo* for antiviral activity against Semliki Forest virus infection in a mouse model

Several selenium-substituted acyclouridine derivatives **115-120** were studied and their antiviral activity was evaluated in human peripheral blood mononuclear cells infected with HIV-1. Compounds **115** and **116** are the least and more effective compounds against HIV virus, respectively. Chemical modifications of the acyclic side chain produced compounds **121-124**. Compound **124** is the most potent antiviral against HIV-1. The pharmacokinetics and toxicity studies on compound **124** clearly show that this compound can act as an effective antiviral agent at low concentrations without exhibiting toxicity.

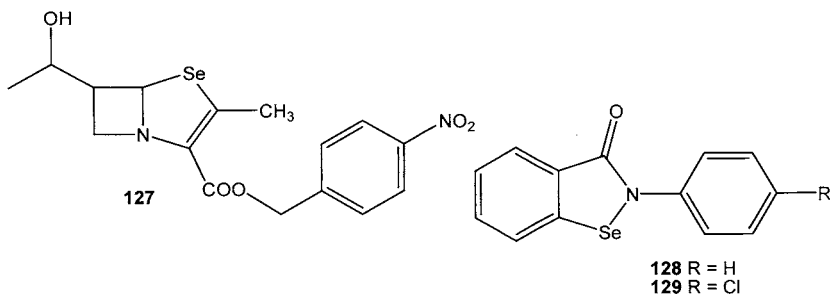
Some 3'-Se-substituted dideoxynucleosides in racemic forms, as well as the α - and β -anomers of oxaselenolane nucleosides have been synthesized and exhibited potent anti-HIV and anti-HBV activities. The synthesized racemic nucleosides (**125**, **126**) were evaluated for antiviral activity against HIV virus type-1 and hepatitis B virus in peripheral blood

mononuclear cells and 2.2.15 cells. The racemic ($\pm$)- β -cytosine (**125**, **126**) compounds exhibit the most potent anti-HIV activity (EC_{50} 2.69 and 5.55 μ M respectively) and anti-HBV activity (EC_{50} 1.2 μ M for both racemic compounds). The anti-HIV activity of resolved α - and β -enantiomers of (**125**, **126**) has been evaluated and the (-) enantiomers are more potent than their (+)-counterparts.



Antibacterials and Antifungals

Because of the comparability of organoselenium compounds and organosulfur compounds, many organoselenium compounds were synthesized and their antimicrobial activity was studied [238]. Early studies were centered at the skeletal modification of the naturally occurring β -lactam antibiotics. The optically active **127** has been synthesized and has antibacterial activity [239].

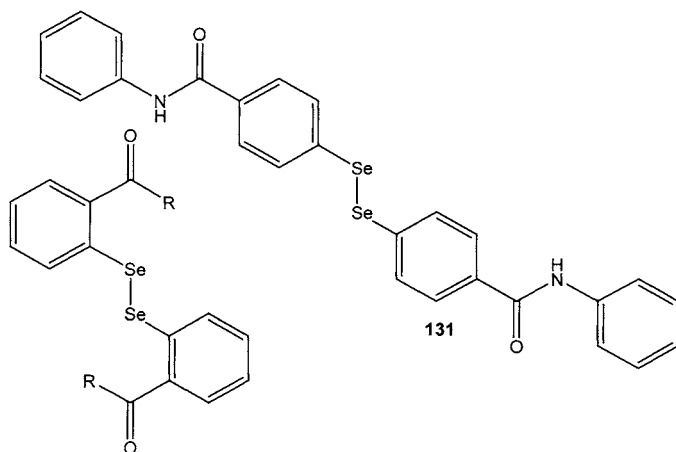


Ebselen (**128**) is a heterocyclic compound that possesses antiinflammatory, anti-atherosclerotic, cytoprotective, and antimicrobial activity against *Staphylococcus aureus*. Compound **128** and the *p*-chloro analogue **129** exhibited strong inhibitory activity against the growth of

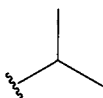
Saccharomyces cerevisiae Σ 127-8b strain. The latter compound also inhibited the growth of *Candida albicans* 258 strains.

On the other hand, diaryl diselenides, compounds (**130-133**), had no inhibitory effect on fungal growth. However, the benzoselenazolones show antibacterial activities against Gram-negative *E. coli* K-12 Row and Gram-positive *S. aureus* 209P bacterial strains [240].

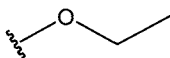
Since morpholine compounds are typically bactericidal and fungicidal, different selenomorpholines **134-136** were synthesized and their antimicrobial activity was tested against *Staphylococcus aureus*.



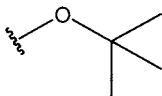
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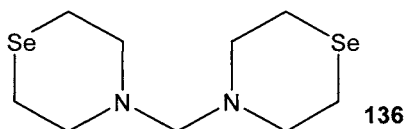
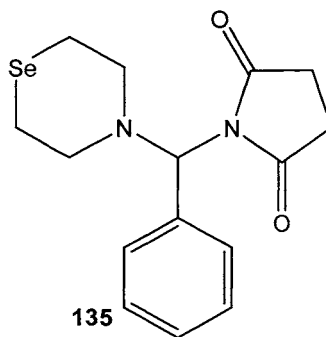
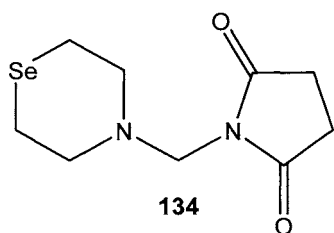


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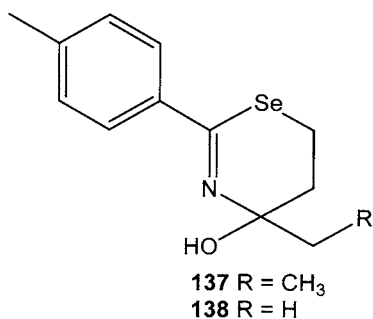




Organoselenium Compounds in Regulation of Apoptosis

Selenium compounds such as **77**, ebselen (**128**) or selenodiglutathione (**103**), are chemopreventive in animal models, inhibit cell growth and induce apoptosis *in vitro*. Compound **77** has been the most extensively investigated. It inhibits cell proliferation and induces apoptosis in several tumor cells. Ebselen (**128**) provides an efficient protection against mustard-induced cell death in normal and tumoral lymphocytes and might prove useful as an antidote against alkylating agents. Compound **103** is associated with induction of Fas ligand, a well-known mediator of apoptosis, and activation of so-called stress kinase signaling pathways, particularly in Jun NH₂-terminal kinase.

Compounds **137** and **138** inhibited HT-1080 proliferation through the induction of DNA fragmentation and were investigated for their inhibitory effect on the growth of eight human tumor cell lines, including stomach, lung, prostate and colon cancer cell lines *in vitro*. Both compounds exhibited the strongest cytotoxicity against a gastric adenocarcinoma and their IC₅₀ were 2.38 μ M and 2.78 μ M respectively. Furthermore, both compounds produce induction of apoptosis in human gastric adenocarcinoma cells.



CONCLUSION

Selenium, an essential micronutrient for plants, animals and microorganisms, has a direct impact on human health and environmental safety. Thus, Se supplementation of populations with low or deficient Se intakes may improve health, and supplementation of Se to populations with adequate intakes may reduce the risk of cancer. Selenium levels in the body are relevant for protection against chronic degenerative, neurological or neoplastic diseases.

The anticarcinogenic and chemopreventive properties of selenium compounds have been demonstrated in mammary, colon and prostate cancer prevention. Compounds **77**, **79**, and **83**, which participate in redox reactions, for instance in antioxidant enzymes, are important components of selenium-enriched yeast used as food supplement and therapeutic agent. Organoselenium compounds are promising also as agents reducing viral expression, combating antibacterial and antifungal infections and preventing heart disease and other cardiovascular and muscle disorders. They also exhibit considerable antioxidant activity.

Ongoing investigations into selenium metabolism include state-of-the-art methods such as HPLC/ICPMS in combination with MS/MS. Data on the profile of selenium metabolites will elucidate the element's essential and toxic roles and relate individual Se species with observed health effects.

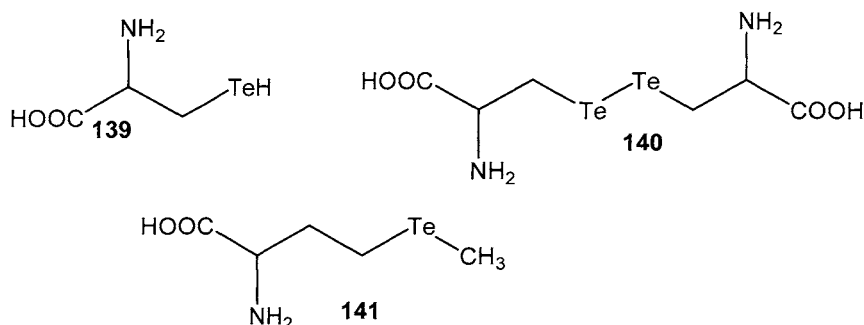
Tellurium

Tellurium is occasionally found as the free element, but is more often found as gold telluride (calaverite), and combined with other metals in the USA, Canada, Peru, and Japan. The principal source of tellurium is the anode sludges produced during the electrolytic refining of blister copper. It is also a component of dusts from blast furnace refining of lead. Tellurium is a relatively rare element, in the same chemical family as oxygen, sulfur, selenium, and polonium (the chalcogens). The relative proportion of tellurium in the Earth's crust is very low, and the content of this element is estimated to be in the range of 1 – 5 µg/kg. Its concentration in seawater is so low that it cannot be reliably determined by current analytical techniques.

Tellurium has no significant biological role. Tellurium and tellurium compounds should be considered to be toxic and need to be handled with care. Organic tellurides have been employed as initiators for living radical polymerization, and electron-rich mono- and di-tellurides possess antioxidant activity. Humans exposed to as little as 0.01 mg/m³ or less in air develop "tellurium breath", which has a garlic-like odor. This is due to formation of ethyl telluride within the body.

Tellurium, unlike the elements mentioned above, is not thought to be required by biological systems. However, tellurium-resistant fungi grown in the absence of sulfur and in the presence of tellurite ions incorporate tellurium into amino acids and proteins [241]. Tellurium is considered to be toxic to most organisms. Genes correlated with tellurium resistance have been identified, but the mechanisms of tellurium resistance are not completely understood [242]. Reduction of tellurite to insoluble elemental tellurium has been described in Gram-positive and Gram-negative bacteria and archaea, and is one of the mechanisms of microbial tellurium resistance [243]. *Aspergillus fumigatus*, *A. terreus*, and *Penicillium chrysogenum*, the tellurium tolerant fungi, are able to grow on sulfur free medium amended with 0.2% (w/v) tellurite. Tellurium was incorporated into several types of low and high molecular weight proteins. The newly detected telluro-proteins contained an extraordinary high level of tellurium, as well as telluro-cysteine (**139**), telluro-cystine (**140**), telluro-methionine (**141**), and serine [241]. Nitrate reductases [244] and terminal oxidases in the respiratory electron transport chain [245] have been implicated in tellurium reduction. Biomethylation of tellurite, tellurate,

and elemental tellurium to volatile dimethyltellurium has been observed in several species of bacteria and fungi, and may represent another mechanism of tellurium resistance [243]. Genes associated with tellurite resistance (TeR) are found in many pathogenic bacteria. Tellurite can be detoxified through interactions with cellular thiols, such as glutathione, or a methyltransferase-catalyzed reaction, although neither process appears to be involved in plasmid-mediated TeR [242].



Escherichia coli JM109 cells, expressing the genes localized in a 3.8-kb chromosomal DNA fragment from *Geobacillus stearothermophilus* V, produced volatile organotellurium compounds, which were released into the headspace in GC analysis [246]. In addition to dimethyl telluride and dimethyl ditelluride, two new organometalloidal compounds, methanetellurol (CH₃TeH) and dimethyl tellurenyl sulfide (CH₃STeCH₃), were detected.

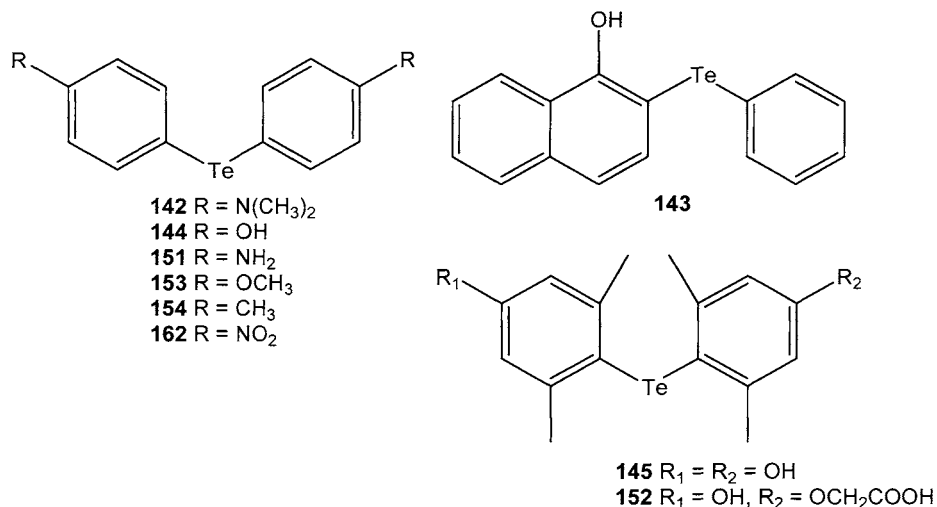
Inorganic and organic tellurium compounds are highly toxic to the central nervous system of mammals. Organotellurium compounds have been reported as potent inhibitors of squalene monooxygenase, causing a dramatic reduction in the rate of cholesterol biosynthesis and leading to degradation of the myelin sheath. The IC₅₀ value for these methyltellurium compounds was 100 nM and was 100-fold lower than that of sodium tellurite, indicating a role for hydrophobicity in the enzyme-inhibitor interaction. This study [235] also provides evidence that organotellurium compounds react with vicinal cysteine sulfhydryl groups on squalene monooxygenase. Dimethyltellurium dichloride was the most potent of these compounds, and its neuropathy resembled that caused by elemental tellurium. The data mentioned above are consistent with the hypothesis that demyelination induced by organotellurium compounds is a result of squalene monooxygenase inhibition, and suggest that a

dimethyltelluronium dichloride compound may be the neurotoxic species presented to Schwann cells *in vivo*.

The interaction of organoselenium and organotellurium compounds with δ -aminolevulinate dehydratase (δ -ALA-D) will be discussed together here, in view of their similar effects on enzyme activity. Organotellurium compounds can interact directly with low molecular thiols, oxidizing them to disulfides. Reduced cysteinyl residues from proteins can also react with these compounds, which may cause, in the case of enzymes, the loss of their catalytic activity. For instance, δ -ALA-D is a sulfhydryl-containing enzyme that is extremely sensitive to oxidizing agents. This enzyme catalyzes the asymmetrical condensation of two molecules of 5-aminolevulinic acid to form porphobilinogen, an intermediate in tetrapyrrol biosynthesis. Hence, this enzyme plays a fundamental role in most living aerobic and photosynthesizing organisms by participating in heme and chlorophyll biosynthesis. The mechanism of porphobilinogen synthesis is similar in animals and plants; however, the enzyme obtained from these sources exhibits subtle structural diversity.

Although the tellurium atom is generally regarded as a toxic metalloid, its role in biological systems has been reviewed [247]. Concerning organotellurium compounds little is known about their biological and pharmacological effects. As pointed out above, glutathione plays a central role in the endogenous antioxidant defense as a reducing agent and nucleophile as well as a substrate for glutathione peroxidases and transferases. Organotellurium compounds are readily oxidized from the divalent to the tetravalent state. Consequently, this property makes tellurides attractive as scavengers of reactive oxidizing agents such as hydrogen peroxide, hypochlorite, and peroxy radicals. These novel and potent antioxidants can play an important role in targeting therapeutic interventions in several diseases [235]. A number of diorganoyl tellurides were able to catalyze the reaction of hydrogen peroxide with thiols. The most active compound, **142** ($IC_{50} = 30$ nM), is among the most potent antioxidants in the microsomal system ever reported. This compound also caused a concentration-dependent delay of the onset of the linear phase of microsomal peroxidation stimulated by iron/ADP/ascorbate. Contrary to the conventional antioxidants examined, diaryl tellurides were found to inhibit peroxidation for a long time, seemingly with an autocatalytic mechanism. 2-Substituted-1-naphthols are the most potent 5-lipoxygenase inhibitors known. Based on this information, a series of 2-substituted-1-

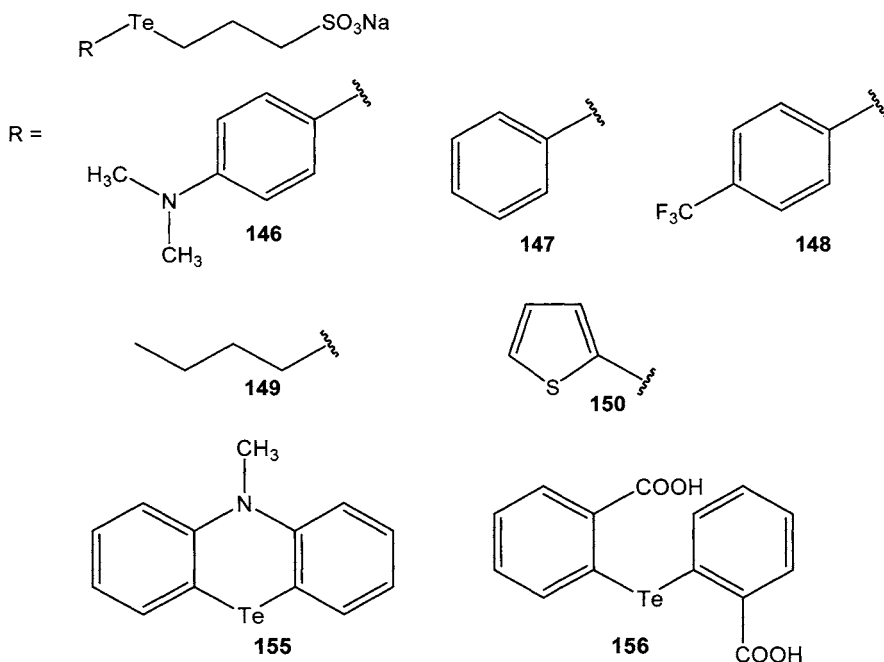
naphthol analogues with the benzylic group replaced by sulfur, selenium, and tellurium were prepared. Data obtained with 2-phenyl tellurenyl-1-naphthol, **143**, indicated that this compound inhibits stimulated leukotriene B₄ biosynthesis in human neutrophils. Besides, compounds **144** and **145** prevented leukocyte-mediated cell damage in Caco-2 cells and protected rat kidney tissue against oxidative damage caused by anoxia and reoxygenation.



In addition, a cyclodextrinyl ditelluride compound has been pointed out as an excellent glutathione peroxidase-mimic, as demonstrated by its high catalytic efficiency. Moreover, water-soluble organotellurium compounds **146-150** were screened as protectors against peroxynitrite. As a result, the compound **146** has been demonstrated to efficiently protect against peroxynitrite-induced oxidation in solution. Thus, it has been suggested that this molecule acts as a catalyst in scavenging ONOO⁻ in the presence of glutathione. Besides, peroxynitrites can cause tyrosine nitration of proteins and inactivate a variety of enzymes. Among the tested *para*-substituted diaryl tellurides, e.g. **151** offered the most efficient protection against peroxynitrite-mediated oxidation. This compound was 11 times more effective than the methylselenocysteine and approximately 3 times more active than **144** and **147** in this capacity. Compounds **152-154** showed activity similar to that of selenomethionine. Conversely, the heterocyclic organotellurium compounds **155** and **156**

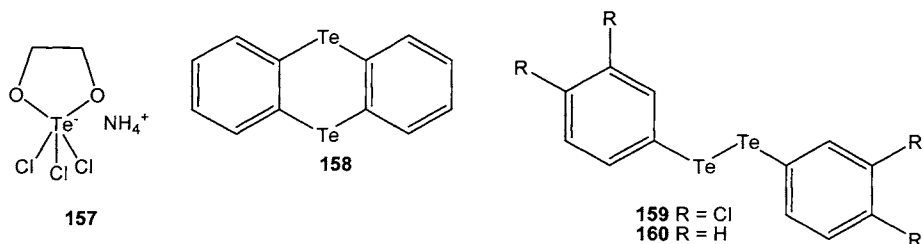
showed no significant activity. Diaryl tellurides act as scavengers of peroxynitrite by an oxygen transfer mechanism similar to that observed with hydrogen peroxide and hydroperoxides.

Organotellurium compounds have been investigated as chemopreventive agents. In 1987, the compound **157**, coded AS101, was demonstrated for the first time to present immunomodulating properties and, when administered to mice, to mediate antitumor effects. This compound has also been described to stimulate human lymphoid cells to proliferate and produce lymphokines. In fact, AS101 stimulates the production of IL-1, IL-2, colony-stimulating factor, tumor necrosis factor, and other cytokines *in vitro*.

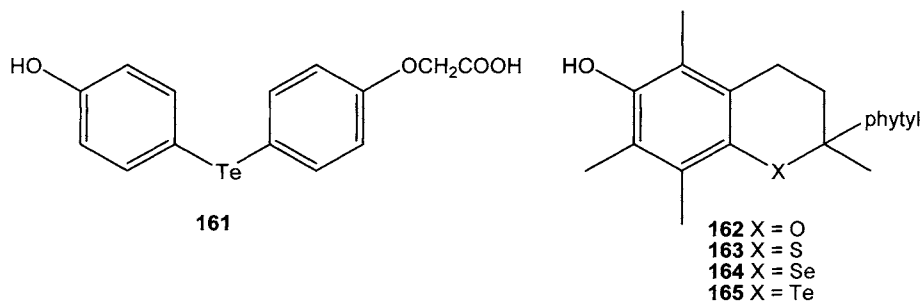


Telluranthrene **158** and compound **159** demonstrated bacterial cytotoxicity and a capacity to induce apoptotic cell death in eukaryotic HL-60 cells. The EC_{50} values for telluranthrene **158** and diphenyl ditelluride **160** were 196 and 33 μM , respectively. The series of organotellurium compounds **142**, **144**, **151**, and **161** function as selective inhibitors of thioredoxin reductase and have potential antitumor effects. They were good noncompetitive inhibitors of thioredoxin reductase,

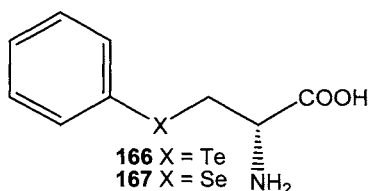
having IC_{50} values less than $10\ \mu M$. Compounds **144** and **150** were tested for antitumor activity against MCF-7 breast cancer; however, the antitumor activity was observed only at doses that produced lethality.



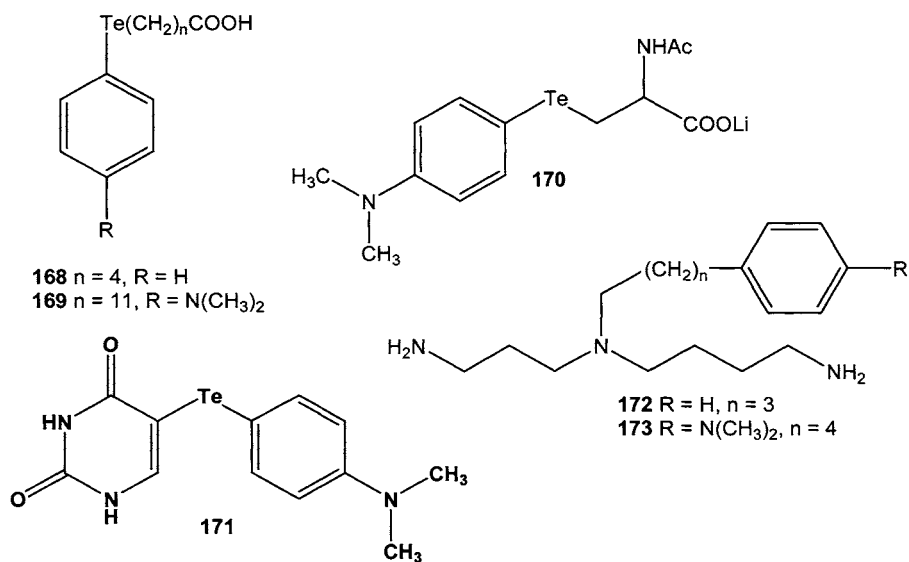
The diaryl telluride, alkyl aryl telluride, and dialkyl telluride carrying sulfopropyl groups were prepared and were found to be the most efficient tellurium based inhibitors of thioredoxin reductase ever tested. The results clearly showed that of the four cyclic aryl alkyl chalcogenides **162-165**, all primitive analogues of vitamin E, only the tellurium compound, **165**, showed interesting inhibition characteristics.



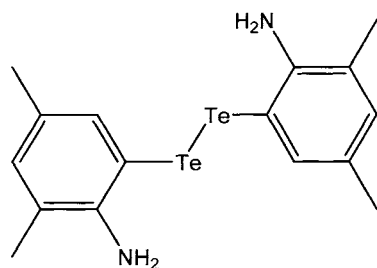
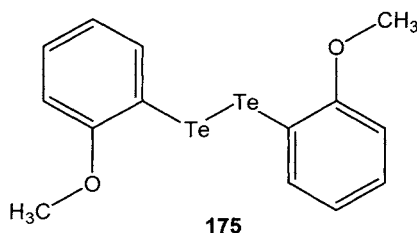
Further results demonstrated that phenyltelluriocysteine, **166**, is bioactivated to its corresponding tellurol analogously to what has been shown previously for phenylselenocysteine, **167**. Microsomal activation studies show that the three conjugates seem to show the trend $Te > Se > S$; phenyltellurocysteine was the strongest inhibitor of several human cytochrome P450 isoenzymes followed by phenylselenocysteine, while phenylthiocysteine was the weakest inhibitor. The results suggest that phenyltellurocysteine might be a significant novel class of prodrugs to generate biologically active tellurols.



The thioredoxins are small ubiquitous redox proteins with the conserved redox catalytic sequence Trp-Cys-Gly-Pro-Cys-Lys, where the Cys residues undergo reversible NADPH dependent reduction by selenocysteine containing flavoprotein thioredoxin reductases [248]. Thioredoxin expression is increased in several human primary cancers including lung, colon, cervix, liver, pancreatic, colorectal and squamous cell cancer. The thioredoxin/thioredoxin reductase pathway therefore provides an attractive target for cancer drug development. Organotellurium steroid, lipid, amino acid, nucleic base, and polyamine inhibitors were synthesized on the basis that they might be selectively or differentially incorporated into tumor cells. Some of the newly prepared classes of tellurium-based inhibitors (lipid-like compounds **168** and **169**, amino acid derivative **170**, nucleic base derivative **171**, and polyamine derivatives **172** and **173**) inhibited TrxR/Trx and cancer cell growth in culture with IC₅₀ values in the low micromolar range.



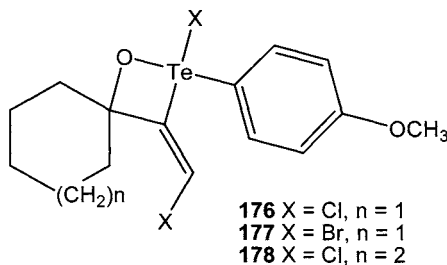
Two substituted aryl organotellurium compounds and a tellurium-free analogue of one of these were evaluated for *in vitro* cytotoxicity using human promyelocytic (HL-60) cells as an experimental system [249]. Both tellurium-containing ditellurides (**174**, **175**) were cytotoxic at concentrations as low as 5 μM . Data indicate that both compounds induce apoptosis in both a time and dose dependent manner; however, 2,2'-dimethoxybiphenyl, structurally comparable to the first of these but without the tellurium bridge, did not produce apoptosis under the concentrations and time course studied. Therefore, the telluride moiety was apparently an important factor in the apoptotic effect.

**174****175**

The effect of diphenyl ditelluride (**160**) on rat thymocytes was examined under *in vitro* conditions using a flow cytometer with fluorescent probes [250]. Incubation of thymocytes with **160** at 300 nM or more for 24 h significantly increased the populations of shrunken cells and of cells with hypodiploidal DNA. Benzyloxycarbonyl-Val-Ala-Asp(OMe) fluoromethylketone, a paninhibitor of caspases, greatly suppressed the increase in the hypodiploidal cell population, suggesting the involvement of caspase activation in **160** toxicity. Hence, it is possible that **160** would increase the population of thymocytes undergoing apoptosis if its blood concentration in mammals reached at least 300 nM or more.

New organotellurium compounds, e.g. **176-178**, with specific cysteine protease inhibitory activity were synthesized [251]. All compounds tested exhibited high specific second-order constant for cathepsin B inactivation. Compound **176** was the best inhibitor of the series, showing a second-order constant of $36,000 \text{ M}^{-1}\text{s}^{-1}$. This value is about 100-fold higher than the second-order rate for cysteine protease inactivation shown by the historic Te compound **157**. The inhibition was irreversible and time and

concentration dependent; no saturation kinetics were observed, suggesting a direct bimolecular reaction. The results described in this paper show that the new organotellurium compounds are powerful inhibitors of cathepsin B, constituting promising potential anti-metastatic agents.



A study [252] of the *in vitro* effect of diphenyl ditelluride (**160**), and ebselen (**128**) on Na^+ , K^+ -ATPase activity of rat brain demonstrated that all these compounds significantly inhibited (in the μM range) Na^+ , K^+ -ATPase activity. Diphenyl ditelluride, at low concentrations, provoked an increase in Na^+ , K^+ -ATPase activity. Dithiothreitol, at 3 mM, protected the enzyme against the inhibition caused by diphenyl ditelluride, diphenyl diselenide and ebselen. Post-incubation of diphenyl diselenide-treated homogenate with dithiothreitol completely recovered enzyme activity. Dithiothreitol was also able to abolish the enzyme inhibition induced by 20 μM diphenyl ditelluride and was partially able to alleviate the inhibition induced by high concentrations of this organotellurium compound; however, it did not alleviate ebselen-induced Na^+ , K^+ -ATPase inhibition. Cerebral Na^+ , K^+ -ATPase is a potential molecular target for the toxic effect of organochalcogens and the inhibition may occur through a change in the crucial thiol groups of this enzyme.

It was demonstrated that solutions of $(t\text{-Bu}_2\text{SnO})_3$ and $(p\text{-MeOC}_6\text{H}_4)_2\text{TeO}$ (Sn/Te ratio=1:1) rapidly absorb CO_2 to form an air-stable molecular tellurastannoxane [$\{(p\text{-MeOC}_6\text{H}_4)_2\text{TeOSn}(t\text{-Bu}_2)\text{CO}_3\}_2$] showing significant intramolecular $\text{Te}\cdots\text{O}$ interactions in both solution and the solid state [253]. The desorption of CO_2 occurs at rather low temperatures, which suggests applications of the complex for instance as a phase-transfer catalyst or as a precursor for the preparation of organic carbonates.

CONCLUSION

Tellurium is so rare in nature that it has no real significance for living organisms and is not thought to be required by biological systems. The highly toxic organotellurium compounds damage cells, e.g. by oxidizing sulfhydryl groups and depleting endogenous reduced glutathione in a variety of tissues. They cause damage to the central nervous system and block a number of other vital functions of the organism.

Astatine

The last element of the group of metalloids is astatine. It has been estimated that the whole Earth's crust contains less than 44 mg astatine and this element with the atomic number 85 can thus be considered one of the rarest naturally occurring elements on Earth. All isotopes of this radioactive element have short half-lives and are products of several radioactive decay series. ^{219}At ($t_{1/2} = 54$ s) occurs in one rare side branch of the ^{235}U decay series while ^{215}At , one of the products of a side branch of the ^{215}Po decay series, undergoes a very fast β decay ($t_{1/2} = 1 \times 10^{-4}$ s). ^{218}At ($t_{1/2} = 2$ s) is one of the products of the ^{238}U decay series, which was also recorded to contain trace amounts of ^{217}At ($t_{1/2} = 0.0323$ s) and ^{216}At ($t_{1/2} = 3 \times 10^{-4}$ s). Isotopes with the longest half-lives are ^{210}At and ^{211}At ($t_{1/2}$ approximately 1/3 days).

It has not as yet been possible to isolate macroscopic amounts of the element. Astatine chemistry can only be studied by trace methods in highly dilute solutions, and this study is understandably fraught with a high risk of experimental errors and faulty conclusions. Despite all these obstacles, increasingly more information is gradually being gathered about the nature of the element. The most suitable for these studies is the isotope ^{211}At ($t_{1/2} = 7.21$ hrs). Recently, the first data have appeared about the organic compounds of At; still, the obstacles in the study of inorganic At compounds including separation of the products, identification of their trace amounts and the danger of radioactive radiation make the acquisition of any information on organic At compounds very difficult. It has so far been possible to synthesize some compounds of the RAt, RAtCl₂, R₂AtCl and RAtO₂ types (R is phenyl or p-tolyl); all these syntheses were performed with iodine containing trace amounts of At.

Compared with radioactive iodine, astatine is a better tool for destroying anomalous thyroid gland tissues because it emits radiation with shorter range in tissues (70 μm) and higher energy (5.9 MeV), and

consequently has a better local affect than iodine, which emits a lower-energy β radiation with a range of up to 2 mm. However, the very low availability and high price of astatine-containing materials severely limit any practical applications [254, 255].

CONCLUSION

Because of the very low half-life of its isotopes, the radioactive astatine has no significance for living systems.

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