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ORGANIC SULFUR COMPOUNDS FROM MARINE ORGANISMS

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The present review deals with sulfur-containing organic natural products from marine organisms. It includes a survey of selected sulfur-containing primary metabolites and biochemical transformations believed to be of importance in the following discussions of sulfur-containing secondary metabolites of marine origin. The main text deals with about 90 naturally occurring sulfur compounds isolated from marine organisms. Spectroscopic details are reported for most of these compounds and data on their biological activity have been discussed if available. Synthetic approaches to these structures are dealt with. The origin of the metabolites has been critically evaluated. The bulk of structures treated contains reduced sulfur, but examples of compounds with sulfur in higher oxidation states are also given. For comparative purposes numerous natural products of terrestrial or freshwater origin have been included as have some non-sulfur compounds when appropriate. Special sections on life under extreme conditions and future perspectives on sulfur-containing marine natural products research serve to place the topic in a broader perspective. A total of 291 structures and 344 references from the current literature are recorded.

Key words: Amino acids, alkaloids, isothiocyanates, aliphatic sulfur compounds.

CONTENTS

1. INTRODUCTION

The subject of sulfur-containing natural products from aquatic organisms has never been reviewed before. There are presumably very good reasons for that. First of all, though excellent reviews of naturally occurring organic sulfur compounds exist, none of these treats the area in its entirety. Exactly the same is the case within marine natural products chemistry; the chemistry of the natural products of freshwater organisms has to our knowledge never been reviewed. The areas mentioned above are all in rapid development and have attracted much interest recently. As they all expand so rapidly, it is clearly impossible to extract the most recent information from the current literature simply because of its volume and also because of its diversity. Nevertheless, it is important to collect the existing knowledge within this area, even if this first attempt is likely to fail in extracting all the important and serious investigations. Also taking into account the diversity of the matter and the scattering of the papers in the literature, it is inevitable that a review of this type must to a great extent reflect the interest of the authors. We hope that we have overcome this at least to such an extent that others with a different focus of interest will be able to extract at least the leading references from this treatise.

A problem encountered by most authors in reviews of natural products chemistry is to decide whether a certain compound is a primary or a secondary metabolite. As the knowledge of metabolic patterns advances, these problems will undoubtedly prove meaningless, but for the time being there are still organizational benefits from the use of this concept. We have tried to solve the problem by a short introduction to the primary sulfur metabolites in order to present most of the types of known low-molecular weight compounds before we proceed to the discussion of others, more distant from the primary biochemical processes.

In order to make the treatise more valuable to the experimentally working investigator we have given spectroscopic details, when available, for the secondary sulfur compounds

secured from marine or freshwater organisms. In order to place the available structures in a broader perspective related material originating from terrestrial organisms has often been included. For compounds mentioned solely for illustration purposes no physical parameters have been given unless needed for the discussion. Whenever possible the discussions are based on reviews or monographs; in such cases the reader is referred to these for further references.

Within the chemistry of marine natural products the most exhaustive and up-to-date text is the series edited by Scheuer.[1-5] In the area of marine toxins the books by Hashimoto[6] and Halstead[7] are worth consulting, as is the book by Baslow on marine pharmacology.[8] The best guide to the early literature is still a book by von Fürth[9] and the now classical book by Scheuer.[10] Impressive compilations of marine natural products have been provided by Baker and Murphy.[11,12] Many reviews deal with various aspects of marine natural products and the chemistry of marine organisms. Most of these treatises mention sulfur compounds just in passing. This is the case with "Marine Natural Products Chemistry" edited by Faulkner and Fenical,[13] "Marine Natural Products",[14] "Antibiotics from Marine Organisms",[15] "Marine Natural Products: Metabolites of Marine Algae and Herbivorous Marine Molluscs" and "Metabolites of Marine Invertebrates"[16] by Faulkner, "Compounds from Microalgae —Their Influence on the Field of Marine Natural Products" by Shimizu,[17] "Natural Products Chemistry of the Marine Environment" by Faulkner and Anderson,[18] "The Varied and Fascinating Chemistry of Marine Molluscs", by Scheuer,[19] "Marine Pharmaceuticals" by Der Marderosian,[20] "Drugs from the Sea" by Ruggieri,[21] "Marine Drugs: Chemical and Pharmacological Aspects" by Youngken and Shimizu,[22] "The Ecology of Marine Antibiotics and Coral Reefs" by Burkholder,[23] "Biochromes. Occurrence, Distribution and Comparative Biochemistry of Prominent Natural Pigments in the Marine World" by Fox,[24] "Marine Natural Products" by Christophersen and Jacobsen,[25] "Marine Alkaloids" by Christophersen,[26] "Current Status of Marine Biotoxicology: An Overview" by Halstead,[27] and "Marine Toxins" by Southcott,[28] just to mention a few.

There are fewer accounts of naturally occurring sulfur compounds and most of them are scattered as chapters in various monographs, generally rather outdated, except for a few specialized treatises. Thus, the reader may wish to consult Jocelyn's book on the biochemistry of the SH group[29] or some of the specialized reviews in "Natural Sulfur Compounds",[30] the excellent reviews of "Low-Molecular Weight Sulfur-Containing Compounds in Nature" by Kjær,[31] "Sulfur Compounds in Plants" by Ettlinger and Kjær[32] or "Sulfur Compounds" by Richmond.[33]

Each volume of *Organic Compounds of Sulfur, Selenium, and Tellurium* covers a two-year period.[34] These *Specialist Periodical Reports* mention many of the structures determined during their periods of coverage. However, the series is published with a substantial delay and, worse, the editorial organization is such as to make the location of the natural products a very time-consuming task. Admittedly, a section on naturally occurring organosulfur compounds is included; this is often so brief (in Vol. 6, 13 lines of text), however, as to make it of little value.

The section on sulfur-containing primary metabolites and related compounds is mainly based on standard biochemistry texts such as the one by Metzler[35] combined with more specialized reviews.[36-38]

2. PRIMARY METABOLITES AND RELATED COMPOUNDS

Since the literature on the biochemistry of sulfur compounds is so vast, and most educational systems until recently did not place much emphasis on the main biochemical pathways, a basic account of some aspects of sulfur's biochemistry is included. The intention intention therefore is not to cover any area exhaustively or to treat any subject in detail, but merely to give an overview of some of the most basic metabolites of sulfur and some of the reactions in which they participate. This approach has the advantage that it provides even the uninitiated with a mental tool often allowing an apparent insight into the possible modes of formation and degradation of a specific secondary metabolite. This is a mentally very stimulating and satisfactory way of perceiving the wealth of information inherited in natural products chemistry. A serious drawback is, however, that very little information exists on the biochemical pathways operating in the sulfur metabolism of aquatic organisms. Accordingly, it will usually be impossible to confront the outcome of the speculation with facts. It is our hope that this situation may eventually cause so much irritation in some readers that it may trigger studies of some of the truly extraordinary chemical pathways that must be operative in some aquatic organisms.

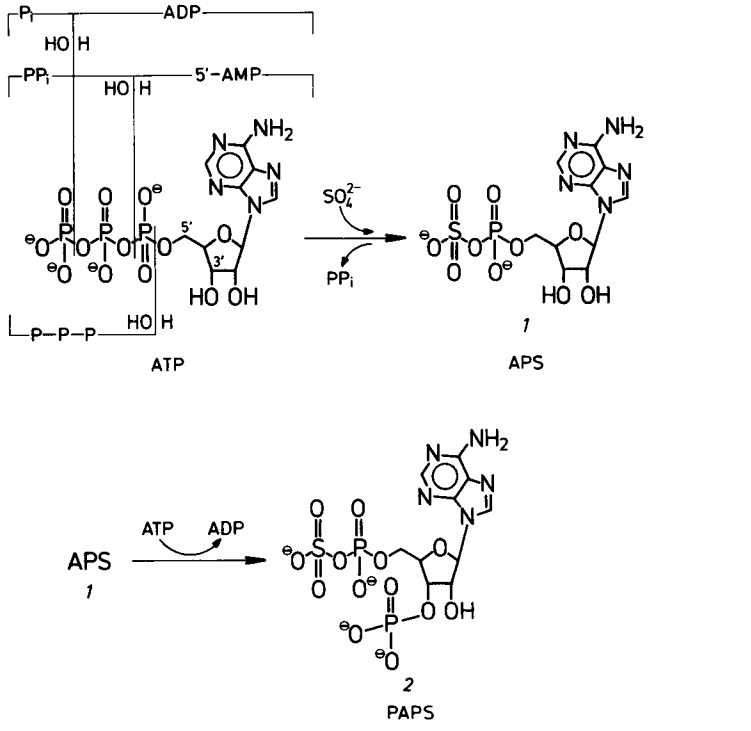
As already mentioned, only very little is known about the specific biochemical mechanisms operating in aquatic organisms; therefore the following account is based on the current knowledge of general biochemistry. Some of the processes mentioned here may very well eventually turn out to be modified in some aquatic species.

A. Reduced Sulfur from Sulfate Ion

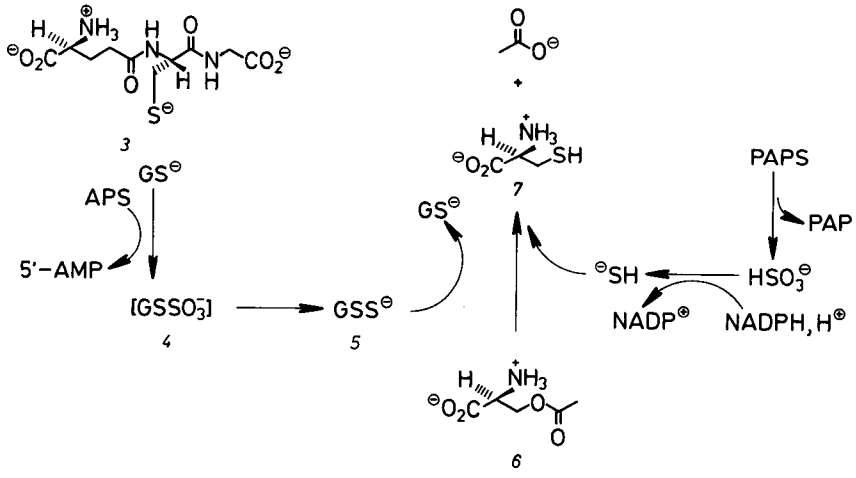
Plants and many microorganisms rely on their supply of sulfate as the sole source of sulfur. The first problem encountered by these organisms is to reduce the sulfur to the -2 oxidation state. The bulk of these organisms converts sulfate to a mixed organophosphate-sulfate anhydride, adenosine phosphosulfate (APS, **1**, adenylyl sulfate or adenosine 5′-phosphosulfate) formed by elimination of pyrophosphate (PP_i from adenosine triphosphate (ATP) by the action of sulfate ion and the enzyme ATP-sulfurylase. In the presence of a thiol carrier, mainly glutathione (GSH, γ-L-glutamyl-L-cysteinylglycine, protonated form of **3**), a Bunte salt (**4**) is formed with release of adenosine-5′-phosphate (5′-AMP). Ferredoxin-mediated reduction of the thiosulfate (**4**) produces the active reagent **5** ($GSS^{\ominus}$) which transfers the thiol group to *O*-acetyl-L-serine (**6**) forming L-cysteine (**7**) and acetate.

Another major route for reduction of sulfate is via adenosine 3′-phosphate 5′-phosphosulfate (PAPS, **2**), where the sulfate is released as sulfite. Reduction of the sulfite with NADPH forms sulfide ion and $NADP^{\oplus}$. The sulfide may react with *O*-acetyl-L-serine (**6**) to form L-cysteine (**7**), but other substrates are also known, *e.g.*, *O*-acetyl-, *O*-succinyl-, *O*-phospho-, *O*-malonyl-, and *O*-oxalyl-L-homoserine, all forming L-homocysteine.

These assimilatory reactions give rise to incorporation of reduced sulfur in the biomass. Dissimilatory reductions carried out by anaerobic bacteria produce the well known sulfide-containing anaerobic biotopes.



SCHEME 1 The main pathway for incorporation of inorganic sulfate.



SCHEME 2 Reduction of sulfate and formation of L-cysteine.

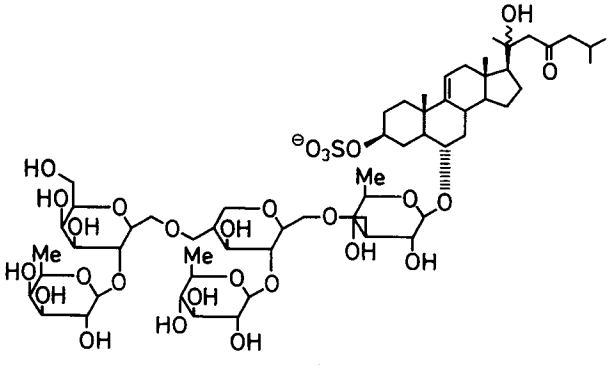
B. Sulfuric Acid Derivatives

Various sulfotransferases transfer sulfate from PAPS (**2**) to a variety of compounds. It is not the intention in this review to cover the multitude of sulfated compounds present in living organisms. However, in order to demonstrate the wide occurrence of these compounds, a summary treatment of selected examples is given below.

In terrestrial organisms both sulfate esters (termed *O*-sulfates) and sulfamic acid derivatives (termed *N*-sulfates) occur widely. Cells contain choline sulfate and ascorbic acid 2-sulfate. The latter may act as donor of sulfate radicals in transfer reactions. Sulfate esters of mucopolysaccharides, steroids, and phenols are of general occurrence. Dermatan, keratan, heparan, and chondroitin sulfates are examples.

Many red algae (*Rhodophyta*) contain polysaccharide sulfates, *e.g.*, the carrageenans from *Chondrus crispus* and *Gigartina stellata*. The sulfated polysaccharides in red and brown algae have been reviewed.[39]

All five classes of Echinodermata (echinoderms) contain steryl sulfates.[40] The classes Holothuroides (sea cucumbers) and Asteroidea (starfishes) have appreciable concentrations of saponins.[41] The asterosaponins have steroidal aglycones, while the holothurins have triterpenoid aglycones. Both classes are often present as sulfate esters. In the case of the asterosaponins the sulfate group is located in the aglycone as exemplified



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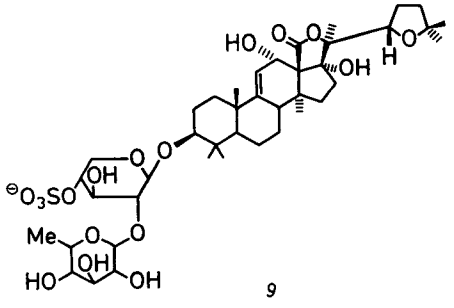
in thornasteroside A (**8**), the main saponin of the Crown-of-thorns starfish *Acanthaster planci*. Thornasteroside A (**8**) is composed of the sulfate of the aglycone thornasterol A and the sugars quinovose, fucose, xylose, and galactose in the ratio 2 : 1 : 1 : 1.[42] The holothurins bear the sulfate group in the saccharide part of the saponin as exemplified by



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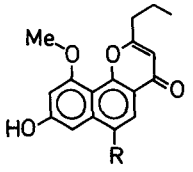
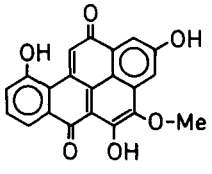
holothurin B (**9**) from *Holothuria leucospilota* and *H. lubrica*.[43,44] Crinoidea (feather stars) contain phenolic sulfates derived from naphthopyrones.[11,45] Comaparvin (**10**) and 6-methoxycomaparvin (**11**) from the feather star *Comanthus parvicirrus timorensis*[46]



10 R = H
11 R = OMe

occur at monosulfates. The position of the sulfate ester group is unknown. Carotenoid sulfates are presumably quite common in marine organisms.[47,48]

The common lugworm *Arenicola marina* L., when found in environments with low oxygen tension, contains a pigment, arenicochrome, which is a derivative of benzopyrene.[49] The native pigment is a disulfate, but the position of the sulfate groups was not



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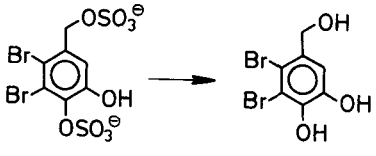
unambiguously determined. After hydrolysis the desulfated pigment, arenicochromine, was shown to have structure **12**.

The dipotassium salt of 2-hydroxy-4-sulfatomethyl-5,6-dibromophenyl sulfate (**13**) has been isolated in high yield from several red algae.[50] The sulfate itself is non-toxic

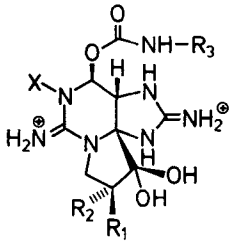


13 *14*

towards algae and bacteria, but the hydrolysis product lanosol (**14**) is highly toxic and thus may have important ecological implications. This question has been studied using the red alga *Rhodomela larix* (Turner) C. Agardh as a model.[51]

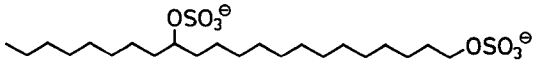
Dinoflagellate toxins of the saxitoxin group are often encountered as sulfate esters or amides.[16,52,53] Thus, gonyautoxin II (**15**) and gonyautoxin III (**16**), gonyautoxin I (**17**), and gonyautoxin IV (**18**) are the 11α- and 11β-*O*-sulfates of saxitoxin and neosaxitoxin, respectively, isolated from *Gonyaulax tamarensis* and *Gonyaulax excavata* (syn. *G. tamarensis* var. *excavata*). Upon hydrolysis gonyautoxin VIII (**19**) afforded **16**, a species of *Protogonyaulax* gave 11-*epi*-gonyautoxin VIII (**20**), gonyautoxin V (**21**), and gonyautoxin VI (**22**). The carbamoyl *N*-sulfates are less toxic than the free carbamates.

The precursors of Tyrian purple from the hypobranchial glands of a variety of molluscs are substituted indoxyl sulfates (see Section 4C).



15 X=H $R_1=R_3=H$ $R_2=OSO_3^{\ominus}$
16 X=H $R_1=OSO_3^{\ominus}$, $R_2=R_3=H$
17 X=OH $R_1=R_3=H$ $R_2=OSO_3^{\ominus}$
18 X=OH $R_1=OSO_3^{\ominus}$, $R_2=R_3=H$
19 X=H $R_1=OSO_3^{\ominus}$, $R_2=H$ $R_3=SO_3^{\ominus}$
20 X=H $R_1=H$ $R_2=OSO_3^{\ominus}$, $R_3=SO_3^{\ominus}$
21 X=H $R_1=R_2=H$ $R_3=SO_3^{\ominus}$
22 X=OH $R_1=R_2=H$ $R_3=SO_3^{\ominus}$

Several reviews discuss a series of chlorosulfatides isolated from the chrysophyte *Ochromonas danica*.[54] These compounds are based on either the 1,14-docosanediol disulfate (**23**) or the 1,15-tetracosanediol disulfate (**24**) skeleton. In the docosanediol disulfate series the parent compound (**23**) and compounds with 1, 2, 3, 4, 5, and 6 chlorine substituents are known, while the tetracosanediol disulfate (**24**) is found mixed with mono-, di-, penta-, and hexachloro derivatives. Another chrysophyte, *Poterioochromonas malhamensis* Peterfi (syn. *Poterioochromonas stipitata* Scherffel and *Ochromonas malhamensis* Pringsheim) has yielded mainly 1,14-tetracosanediol disulfate and the mono- and dichloro derivatives. These surfactants have been proposed to function as the antibiotic principle of the alga.[55]



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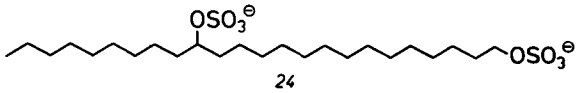

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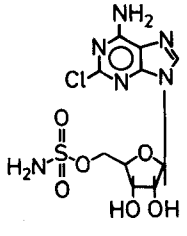
Many other microorganisms have yielded sulfated metabolites, *e.g.*, the antibiotic **25** from *Streptomyces rishiriensis*.[56]



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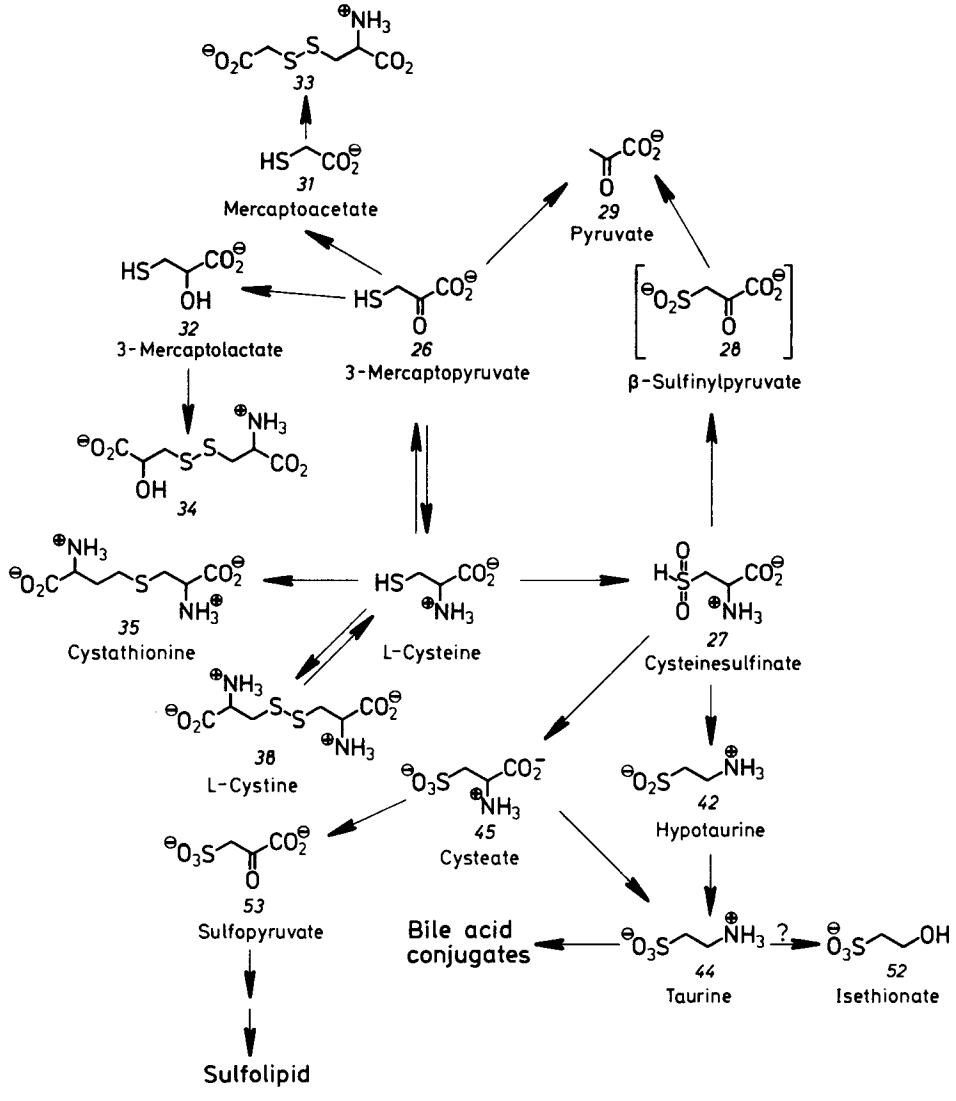
High levels of sulfuric acid have been reported from the cells of several marine organisms. A concentration as high as 1.8 N sulfuric acid was believed to be present in vanadocytes of ascidians. The vanadocytes are blood cells with very high concentration of V(IV) or V(III). It was recently shown that the internal pH of vanadocytes is nearly neutral and that the older results were due to liberation of sulfuric acid upon cell lysis.[57,58] The intracellular pH was measured by a ^{31}P nmr determination of the species of phosphate ions present in the intact cell. The ^{31}P nmr method is presumably the best available at present even if there are some problems, especially in the case of the vanadocytes, where the vanadium present acts as a shift reagent making the identification of the phosphorus shifts difficult. These complications should eventually be overcome by studies of model systems. Other pH determination techniques are less reliable in this case since for example microelectrodes damage the cells and make direct determination impossible and addition of pH indicators may give erroneous results due to the unusual redox properties of the cells and due to complexation with vanadium.

Another example, at least well known among algae collectors, is the acidity of brown alga of the genus *Desmarestia*. Some of these species cause serious decoloration of the tissue and of other species if mixed after collection due to liberation of sulfuric acid in the medium. Under natural circumstances sea urchins will avoid feeding on *Desmarestia*. If, however, they are forced to do so, *Strongylocentrotus franciscanus* feeding on *D. ligulata* showed reduced growth and had badly eroded teeth.[59] Recently *Desmarestia liguta* var. *liguta* (Lightf.) Lamour. and *D. viridis* (Mull.) Lamour. were found to irreversibly accumulate sulfuric acid to an average internal pH of 0.5–0.8 while the related *D. aculeata* (L.) Lamour. does not accumulate sulfuric acid.[60] The internal pH was calculated from the pH of a ground slurry of tissue. As discussed above this method is open to criticism since the acid may originate from hydrolytic reactions. It would be very interesting to see these systems reinvestigated by the ^{31}P nmr technique.

C. Cysteine and Derivatives

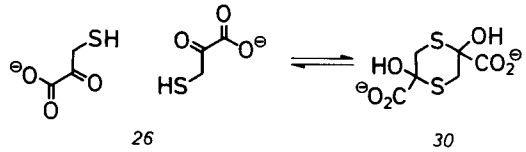
Animals are unable to incorporate sulfide directly to form cysteine and hence rely on dietary intake of this amino acid or on transformation of methionine from dietary sources into cysteine. Some microorganisms are able to convert L-serine to L-cysteine reversibly. Cysteine itself, apart from being an indispensable unit of numerous peptides and most proteins, participates in an overwhelming number of metabolic reactions, some of which are depicted in Scheme 3.

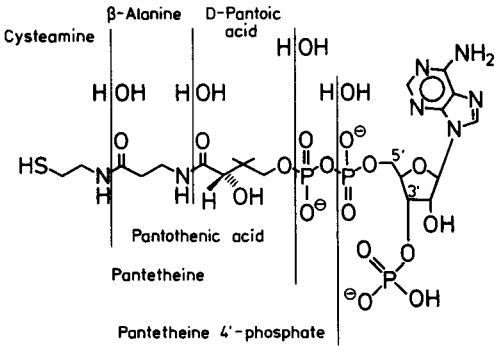
At least in mammals the major catabolic pathways of cysteine are the transamination pathway and the direct oxidation pathway. The former produces 3-mercaptopyruvate (**26**) and the latter cysteine sulfinate (**27**), which in turn may be transaminated to β-sulfinylpyruvate (**28**). The latter compound has never been isolated or synthesized; it seems to decompose to pyruvate (**29**) and sulfite ion spontaneously. Pyruvate may also arise from 3-mercaptopyruvate (**26**) by reductive processes such as elimination of sulfur, elimination of thiosulfonate on reaction with a sulfinate, elimination of thiocyanate ion on reaction with cyanide, or elimination of thiosulfate on reaction with sulfite. 3-Mercaptopyruvate (**26**) exists in equilibrium with 2,5-dihydroxy-1,4-dithiane-2,5-dicarboxylate (**30**) in solution.



SCHEME 3 The metabolic fate of L-cysteine.

3-Mercaptopyruvate (**26**) may be oxidatively decarboxylated to give mercaptoacetate (**31**) or reduced to give 3-mercaptolactate (**32**), which may be encountered as the mixed disulfides with L-cysteine, **33** and **34**, respectively. *O*-Succinylhomoserine and cysteine may form cystathionine (**35**), which, following β-elimination, gives homocysteine.





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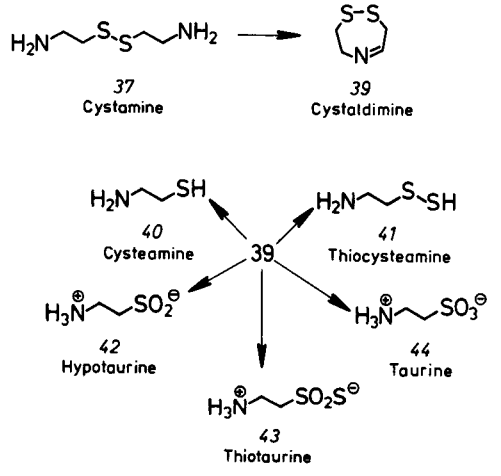
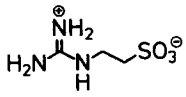
Coenzyme A (CoA-SH, **36**) is biogenetically derived from cysteine via 4′-phosphopantothenate forming 4′-phosphopantotheinyl cysteine which upon decarboxylation and reaction with ATP forms CoA-SH. The catabolism of CoA-SH may lead to cystamine (**37**), the amine corresponding to cystine (**38**). The enzyme diamine oxygenase transforms cystamine (**37**) into the cyclic intermediate cystaldimine (**39**), which in enzymatic reactions may give rise to cysteamine (**40**), thiocysteamine (**41**), hypotaurine (**42**), thiotaurine (**43**), and taurine (**44**). Thiocysteamine (**41**) is unstable and readily loses sulfur, which may then participate in transsulfuration reactions.



SCHEME 4 Cystaldimine, formation and transformations.

Taurine (**44**) may arise from hypotaurine (**42**), but also from cysteate (**45**) by decarboxylation. Many organisms are capable of producing taurine (**44**) from sulfite or sulfate without the intermediacy of cysteine, *e.g.*, cysteate (**45**) may originate from a reaction between aminoacrylate (from serine) and PAPS (**2**). Taurine (**44**) has subsequently been isolated from many marine organisms, while *N*-guanidinotaurine, taurocyamine, (**46**) was first discovered in a marine polychaete worm. Several other taurine derivatives are known, hypotaurocyamine (**47**), phosphotaurocyamine (**48**), and phosphohypotaurocyamine (**49**). An account of guanidine derivatives in 48 species of



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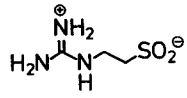

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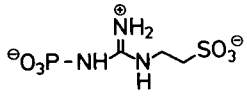

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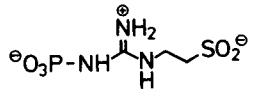

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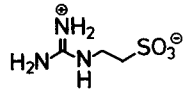
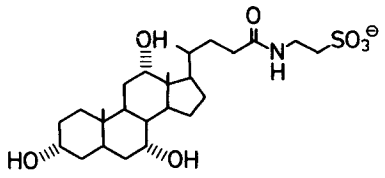


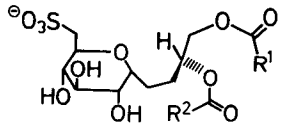
50

marine worms (Annelida, Echiuroidea, Nemertea, Phoronidea, and Sipunculidea) has been published.[61] Asterubin (**50**) has been reported from starfish. Taurocholic acid (**51**), formed from cholyl-CoA, is an example of a bile salt. It is uncertain whether isethionate (**52**), a constituent of nerve tissue, can be derived from taurine in higher organisms.



51

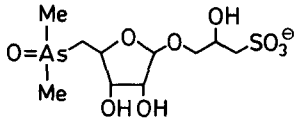
Sulfopyruvate (**53**) is the precursor for sulfolipids occurring in the chloroplast. Presumably via sulfolactaldehyde and dihydroxyacetone phosphate 6-sulfoquinovose is formed. This sugar is incorporated in chloroplast lipids of the general structure 6-sulfo-6-deoxy-α-D-glucopyranosyl diglyceride (**54**).[62] These glycerides are usually assumed to be intimately connected with photosynthesis. There are indications that they



54

have other functions as well. For example, they have been found in an Okinawan marine sponge, *Phyllospongia foliascens* (Pallas) with R^1, R^2 in **54** in a ratio of 1 : 2 of the radicals *cis*-8-hexadecenoyl and palmitoyl. These compounds exhibit resistant activity against a complement fixation reaction.[63] The sea urchin, *Anthocidaris crassispina* A. Agassizi contains the corresponding monoglycerides (R^2CO = H) with R^1 = myristoyl or palmitoyl in the ratio 4 : 96.[64] Sulfonoglycolipids are also known from the green alga *Ulva pertusa* Kjellman.[65] It cannot at present be excluded that the sponge and the sea urchin have acquired their content of **54** from the diet. However, a non-photosynthetic diatom also contains substantial amounts of **54** (see below).

The brown alga *Ecklonia radiata* concentrates arsenic in three arseno sugars, one of which is the interesting sulfonate **55**.[66,67]



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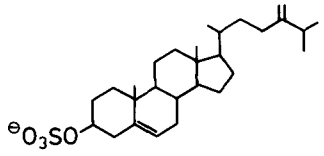
Aquatic organisms may still hold surprises in this area as exemplified by investigations of the non-photosynthetic marine diatom *Nitzschia alba*. The lipid composition of this species has been very thoroughly studied while many other species, in more superficial qualitative studies, have given hints that their lipid compositions are related to the highly unusual one treated below. In *N. alba* the neutral lipids show no spectacular deviation from the pattern expected for a diatom. A remarkable trait is that the triglycerides constitute more than 20% of the dry weight of the cells harvested in the late logarithmic phase. The polar lipids, however, show quite a unique composition since there are four different classes of sulfur-containing lipids in this fraction. More than 8% of the polar lipids consist of 24-methylenecholesterol sulfate (**56**) which is also present in the free form in the neutral lipids. Sulfoquinovosyl diglycerides (**54**) and lysosulfoquinovosyl diglycerides constitute 19%. Lyso derivatives are formed by cleavage of one of the ester



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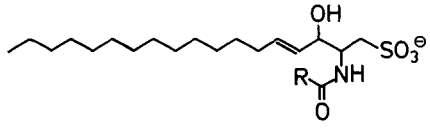
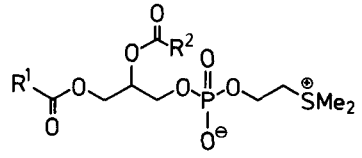


57

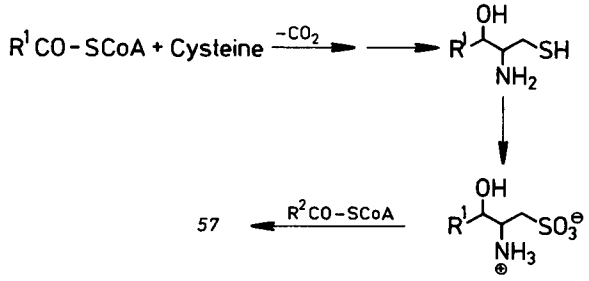
functions leading to a monoglyceride. The major fatty acid residues of the sulfoquinovosyl diglycerides are 14:0, 16:0, 18:1, 20:5, and 22:6, while the major residues in lysosulfoquinovosyl diglycerides are 22:6, 20:5, and 16:0. More than 5% of the polar lipid fraction is made up of a unique set of 1-deoxyceramide 1-sulfonates (**57**), where the major species is *N*-trans-3-hexadecenoyl-1-deoxyspingenine 1-sulfonate.[68,69] Most unusual and interesting, however, is the finding that more than 40% of the polar lipids consist of phosphatidylsulfocholines (**58**) and lysophosphatidylsulfocholines. The major fatty acid residues in **58** are 20:5, 14:0, and 18:1 while the lyso derivatives contain mostly 20:5, 18:1, and 18:2. The diatom was found to be totally devoid of the normal lecithins (phosphatidylcholines) and accordingly their role must have been taken over by the sulfonium analogs.[70]



58

The biochemical origin of the sulfur atoms was investigated by feeding cultures of *N. alba* L-[^{35}S]cysteine, L-[^{35}S]cystine, L-[^{35}S]methionine, and a mixture of L-[^{35}S]methionine and L-[Me-^{3}H]methionine.[71] The sulfoquinovosyl diglycerides (**54**), lysosulfoquinovosyl

diglycerides, and 24-methylenecholesterol sulfate did not incorporate any label from the above-mentioned precursors and consequently may derive their sulfur from inorganic sulfate. Cysteine and cystine are equally well incorporated in **57**, **58**, and lyso-**58** showing that cysteine plays an important role in the sulfolipid biosynthesis. In the case of 1-deoxyceramide 1-sulfonates (**57**) methionine was not incorporated meaning that methionine was not transformed to cysteine in any appreciable amount during the experiment. The biosynthesis of **57** may therefore be analogous to the biosynthesis of sphingosine, only that the usual serine in this case is being substituted with cysteine in the reaction with palmitoyl-CoA.

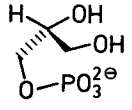


The phosphatidylsulfocholines (**58**) and lysophosphatidylsulfocholines were found to incorporate sulfur as well as methyl groups from methionine. In experiments with doubly labelled methionine the products were shown to have a considerably higher $^{3}H/^{35}S$ ratio than present in the substrate. This observation is consistent with a reaction mechanism where methionine supplies a methylthio group and also supplies the second methyl group, presumably via the participation of *S*-adenosylmethionine (SAM). The labelling derived from cysteine and cystine could originate from methionine formed via the cystathione pathway (see Scheme 3).

The *sn*-3-phosphatidylsulfocholines† are accessible synthetically either from condensation of *sn*-3-phosphatidic acid (dimyristoyl, dipalmitoyl,distearoyl, or dioleyl) with sulfocholine chloride or from reaction of 1,2-dipalmitoyl-*sn*-glycerol with phenylphosphoryldichloridate followed by reaction with sulfocholine and removal of the blocking group by hydrogenolysis.[73]

Synthetic phosphatidylsulfocholines (di-14:0, di-16:0, di-18:0, and di-18:1) have been the subject of a field desorption mass spectrometric study.[74]

The biosynthesis and metabolism of *S*-methylcysteine was reviewed in 1967.[75] It is very descriptive of the area of the biochemistry of sulfur compounds that another review on



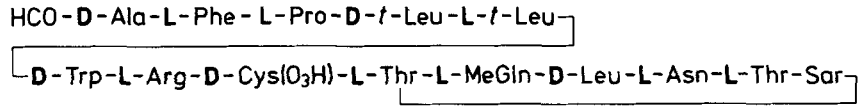
sn-3-glycerol phosphate

† According to a suggestion by Hirschmann[72] for a stereochemical numbering system in which the carbons are numbered beginning with the end of the chain that occupies the *pro-S*-position. Glycerol phosphate used in the *in vivo* phospholipid biosynthesis always bears the phosphate group at the hydroxymethyl group in the *pro-R* position and therefore should be named *sn*-3-glycerol phosphate.

the biochemistry of *S*-methylcysteine and its principal derivatives appeared in 1982—fifteen years later—and had 244 references.[76]

An account of the biochemistry and physiological properties of the tripeptide glutathione (γ-L-glutamyl-L-cysteinylglycine, GSH, protonated form of (**3**)) is likewise outside the scope of the present text. A recent review treating glutathione[38] lists 438 references selected among more than 2000. Suffice to mention here that glutathione has important functions in such diverse areas as the synthesis of proteins and DNA, in enzyme activity, transport processes, intermediary metabolism, drug metabolism, protection of cells, etc.

Unusual peptides with strong biological activity are known from molluscs and tunicates (see Section 4D). The first antimicrobial peptide from a marine sponge, *Discodermia kiiensis*, has recently been identified.[77] Discodermin A (**59**) is a formamide, has L- and D-*t*-Leu, besides several D-amino acids, and an oxidized cysteine moiety. Whether this interesting compound actually originates with the sponge or with some associated microflora, is at present unknown.



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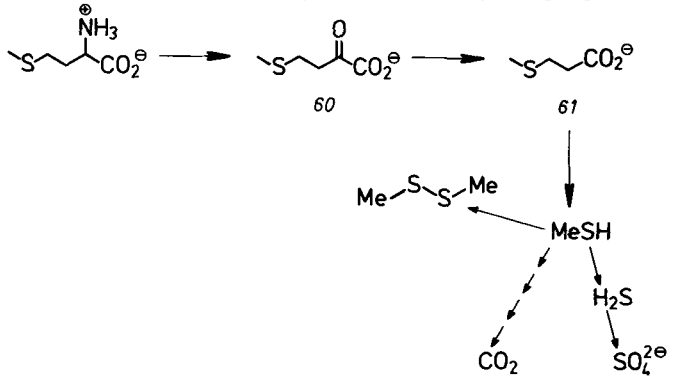
D. Methionine and Related Compounds

In vertebrates L-methionine is the precursor of homocysteine and cysteine sulfur. In microorganisms the enzyme β-cystathionase degrades cystathionine (**35**) formed from cysteine and *O*-succinylhomoserine, giving homocysteine which is then methylated to give methionine. Homocysteine may also originate from hydrogen sulfide and *O*-acetylhomoserine.

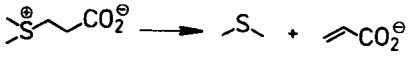
Methionine may be catabolized *via* a transamination pathway giving 4-methylthio-2-ketobutyrate (**60**) and, after decarboxylation, 3-methylthiopropionate (**61**). Many



60 *61*

bacteria transform methionine to methanethiol, dimethyl sulfide, and dimethyl disulfide.[78] Four marine macroalgae, *Ulva lactuca*, *Ascophyllum nodosum*, *Gracilaria tikvahia*, and *Hypnea musciformis* were found to have a content of 2.7, 19, 2000, and 79 μg dimethyl sulfide per g dry weight.[79] The freshwater microalga *Ochromonas danica*

Pringsheim, strain L 933-7 (Chrysophyceae) produces an unpleasant odor when cultivated. An investigation of the culture medium served to identify eight low-molecular weight sulfur compounds, dimethyl disulfide (1520 μg/l suspension), dimethyl trisulfide (250), dimethyl tetrasulfide (trace), 1,2,4-trithiolane (trace), methyl ethanethioate (1160), methyl propanethioate (130), methyl 2-methylpropanethioate (90), and methyl 3-methylbutanethioate (10).[80]



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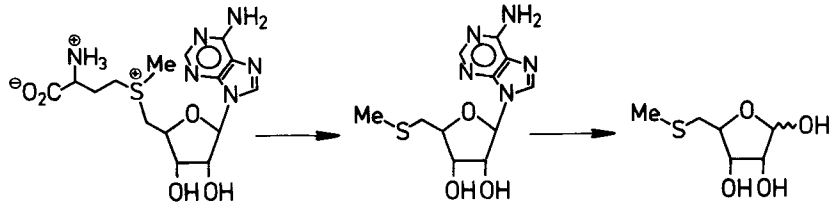
Usually dimethyl sulfide in algae is formed by elimination from dimethyl-β-propiothetin (**62**) which originates from methionine.[81] This has been demonstrated in many cases, *e.g.*, in brackish water phytoplankton where the dimethyl sulfide was believed to be transferred to the Baltic herring (*Clupea harengus membras*) conveying a flavor to the muscle tissue of that fish.[82] In accordance with these investigations, fourteen individual sulfur compounds have been identified in the head space of North Sea fish oils.[83] The skin of the flat-head (*Calliurichthys doryssus*) from Japanese waters was shown to contain as much as 1.2 μg/g of methanethiol and 5 μg/g of dimethyl disulfide while the viscera contained as much as 0.38 μg/g of dimethyl sulfide.[84] Cyanobacteria (blue–green algae) of the genus *Microcystis* have been characterized as two distinct chemotypes, one releasing isopropyl methyl disulfide, diisopropyl disulfide, and diisopropyl trisulfide, and the other excreting dimethyl disulfide and in some cases dimethyl trisulfide and dimethyl tetrasulfide.[85] The fetid odor of the hypobranchial glands of two marine muricid gastropod molluscs, *Reishia* (*Thais*) *clavigera* and *R.* (*T.*) *bronni* was shown to originate from methanethiol (~ 180 μg/g tissue) and dimethyl disulfide (~ 25 μg/g tissue). In some samples hydrogen sulfide and dimethyl sulfide were detected as well.[86] An investigation of dimethyl sulfide in a stratified coastal salt pond has been published.[87] An estimate of the contribution of biologically produced dimethyl sulfide to the global sulfur cycle has been published[88] as has an account of dimethyl sulfoxide in marine and fresh waters.[89]



S-Adenosylmethionine — 63 — SAM

5'-methylthioadenosine — 64 — MTA

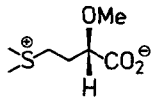
5-methylthioribose — 65 — MTR

Apart from the transaminative pathway methionine takes part in many other catabolic processes, one of the most important being the transsulfuration pathway. Methionine reacts with ATP under elimination of inorganic phosphate (γ-phosphate of ATP) and pyrophosphate (α,β-phosphate of ATP) to form (−)-*S*-adenosyl-L-methionine, SAM (**63**). At least the bulk of SAM produced has the *S* configuration at the sulfonium center. The current status of the knowledge of the enzymes methionine adenosyltransferase

(*S*-adenosylmethionine synthetase) and *S*-adenosylmethionine decarboxylase has recently been reviewed.[90] The methyl group of the sulfonium center may be transferred to a variety of substrates, *e.g.*, phospholipids, proteins, polysaccharides, and nucleic acids with the formation of methylated products and *S*-adenosylhomocysteine. The latter may form homocysteine and enter the main metabolic processes again. Although by far the most numerous reactions are the transfer of a methyl group from SAM, transfer of the adenosyl portion or the 3-amino-3-carboxypropyl group may also occur. The latter possibility is exemplified by the hydrolysis of SAM to homoserine lactone and 5′-methylthioadenosine (MTA), **64**. *Aerobacter aerogenes* degrades SAM to MTA and then to 5-methylthioribose (MTR), **65**. Instead of homoserine lactone, 1-aminocyclopropane-1-carboxylate may be formed. The latter compound forms ethylene upon oxidation. 5′-Methylthioadenosine sulfoxide has been isolated from human urine.[91]

Decarboxylated *S*-adenosylmethionine has been isolated from the tapetum lucidum of the eye of the sea catfish (*Arius felis*).[92] The tapetal pigment consists of oligomers of 5,6-dihydroxyindole-2-carboxylic acid. The two above-mentioned compounds together with a minor amount of thiamine form the reflecting pigment. Hydrolysis at pH 4 and 100 °C for 5 h was found to liberate small amounts of adenine and 3-methylthiopropylamine, while the parent compound was still the main constituent. This result is not in accordance with a published hydrolysis experiment carried out under the same conditions and giving 3-amino-1-propanol and 5′-methylthioadenine.[93] The authors ascribe this discrepancy to a possible difference in stereochemistry around the sulfonium ion center. *S*-Adenosylmethionine decarboxylase has been found in a variety of procaryotic and eucaryotic cells. This enzyme is an example of the pyruvoyl enzymes, i.e. it is dependent on a covalently bound pyruvoyl residue.[94]

The Mediterranean red alga *Rytiphloea tinctoria* (Clem.) C. Ag. yielded 0.1% of wet weight of (−)-(*S*)-4-dimethylsulfonio-2-methoxybutyrate (**66**).[95] The same compound was also shown to occur in *Halopitys incurvus* (Hyds.) Batt. and *Vidalia volubilis* (L.) J. Ag. belonging to the tribe Amansieae as does *R. tinctoria*. The structure was determined



66

66: $C_7H_{14}O_3S$, colorless syrup, $[\alpha]_D^{25}$ − 26.6° (H_2O), ir, 1590, 1400 cm^{-1}. ^1H nmr (270 MHz, D_2O plus CF_3CO_2D to pH 2) δ 2.16 (1H, four d, J = 8, 8, 8.1, 15, H_a C-3), 2.37 (1H, four d, J = 3.9, 8, 8, 15, H_b C-3), 2.85 (3H, s, S–Me), 2.86 (3H, s, S–Me), 3.36 (2H, m, H_2 C-4), 3.38 (3H, s, OMe), 4.06 (1H, dd, J = 3.9, 8.1, H C-2). ^{13}C nmr (20.1 MHz, D_2O) δ 23.43, 23.73 (SMe_2), 25.49 (t, C-3), 39.03 (t, C-4), 78.20 (d, C-2), 56.28 (q, OMe), 175.74 ($-CO_2$). Ms, M^+ m/z 178.

from the spectroscopic data and the fact that the natural product upon hydriodic acid treatment gave (−)-(*S*)-dimethylsulfonio-2-hydroxybutyrate, identical with a synthetic sample. This compound (**66**) is presumably related to methionine biogenetically. Formally it may result from 4-methylthio-2-ketobutyrate (**60**) by reduction and *O*,*S*-dimethylation. As *S*-methylmethionine seems to be widely distributed among higher plants,[96] this compound could be involved.

Incidentally, it may be noted that calcium D,L-α-hydroxy-γ-methylthiobutyrate is produced in bulk quantities as additive to livestock feed. Soybean protein is limiting in methionine and addition of methionine results in unappetizing flavors from bacterial degradation and is expensive. The α-hydroxy-γ-methylthiobutyrate is converted to α-keto-γ-methylthiobutyrate *in vivo*. The latter compound can now be transaminated to yield methionine.

E. Life Under Extreme Conditions

The major part of the marine ecosystem has a remarkably uniform composition with most physical parameters, except pressure, being nearly constant. From a quantitative biological point of view this is of little interest because only extremely limited organic production and activity is associated with these conditions.

The primary photosynthetic producers are confined to the euphotic zone (where gross primary production exceeds respiration) which is very rarely deeper than 120 m, often no more than 10–20 m. Within this zone chemical and physical parameters may vary considerably both geographically and locally. In freshwater environments the variations may be very large due to the limited buffering capacity of the smaller bulk of water.

While the main salinity of ocean water is 35‰ living organisms tolerate waters with salt content ranging from nearly saturated as in the Dead Sea, in certain brine springs in the Red Sea, and in some tide pools to nearly zero as in some Amazonian tributaries. The physiological problems encountered by organisms are very diverse in high- and low-salinity environments since in the former they have to eliminate excess salt against a gradient while in the latter they have to retain salts endergonically. The interested reader is referred to the excellent review by Maetz, describing some of the more fundamental aspects of the adaption of organisms to hypoosmotic and hyperosmotic environments.[97] In this context attention may be drawn to the well known fact that many species are able to cope with a nearly total desication of their environments. In a study of 15 species of Gastropoda, Pelecypoda, and Crustacea a correlation was found between the logarithm of the concentration of sulfated glycosaminoglycan in the organisms and the salinity of the habitat. All organisms investigated contained chondroitin sulfate, heparan sulfate, dermatan sulfate-like compounds, and unknown glycosaminoglycans.[98].

Since life is known even in the hadal zone (below 6–7000 m) some organisms must be able to tolerate these high hydrostatic pressures. Some species seem to be truly barophilic, *i.e.*, unable to survive and reproduce at lower pressures.[99]

A very wide range of temperatures is tolerated by living organisms. One end of the extreme is exemplified by organisms surviving below freezing temperatures.[100] These species have an anti-freeze component in their body fluids, *e.g.*, the intensively studied anti-freeze glycopeptides in antarctic fishes.[101,102] The other end of the extreme is illustrated with the report of bacteria cultured at 105 °C.[103] A report claiming growth of bacteria in "black smokers" at 250 °C[104] has later been refuted.[105] Apparently only procaryotic organisms will survive 75 °C. Some organisms are truly thermophilic, as, *e.g.*, a photosynthetic purple bacterium, isolated from a Yellowstone hot spring, that grows optimally at a temperature of about 50 °C, oxidizing sulfide to elemental sulfur.[106]

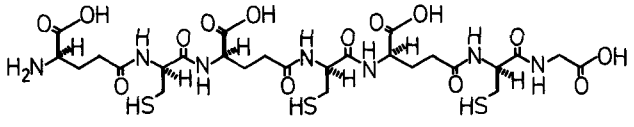
Many aerobic organisms have the ability to survive conditions of low oxygen tension or even anaerobic conditions for extended periods of time. The bivalve *Solemya velum* Say found in the reducing muds of eelgrass beds has intracellular sulfur-oxidizing chemoautotrophic bacteria.[107] There are several examples of these symbiotic relationships which may be of rather wide occurrence. The most intriguing and at present most intensively studied relationship of this kind is offered by the biological communities associated with hydrothermal vents. The deep-sea hydrothermal vent communities are beautiful and unique examples of life under extreme conditions.[108,109] Thermal vents may be divided into two classes: Galápagos-type warm water vents emitting water of a temperature of 5–22 °C and sulfide mound hot-water vents emitting water with temperatures in excess of 350 °C.[110] Both types emerge from the deep-sea floor at depths of approximately 2.5 km at an ambient water temperature of about 2 °C. Most of these vents emit hydrogen sulfide. For the low temperature type, values ranging from 0 to 160 μM were found[109] while the high temperature type give rise to values as high as 4300 μM.[111] Both types have dense communities associated with them, the most conspicuous members being giant clams, brachyuran crabs, and vestimentiferan worms. The vestimentiferan worm, *Riftia pachyptila* Jones (phylum Pogonophora) may reach a length of 3 m and an average diameter of 3.5–4 cm. The animal dwells in a white, flexible cylindrical tube and lacks mouth and gut. Interestingly enough the trophosome, a structure occupying part of the trunk cavity, contains great numbers of close-packed bacteria and usually crystals of elemental sulfur.[112] Stable carbon isotope abundance ($^{13}C/^{12}C$ expressed as $\delta\,^{13}C$ ‰) determinations revealed an unexpectedly high $\delta\,^{13}C$ value of about -11 ‰ as compared to the range -8 to -25 ‰ found for nonvent marine organisms.[113] High $\delta\,^{13}C$ values usually are taken as evidence of the utilization of near-shore C_4 plant material as a food source. Clearly the latter explanation is invalid in this case since the only food available, apart from the produce of the vent community, is deep-sea benthos. Accordingly the vestimentiferan worms, devoid of a way to ingest particulate food sources, must rely on organic carbon produced within their bodies. Two of the Calvin–Benson cycle enzymes, ribulose 1,5-biphosphate carboxylase (EC 4.11.39) and ribulose 5-phosphate kinase (EC 2.7.1.19), were detected with high activities in the trophosome, but not in muscle. In addition, high activities of rhodanese, APS reductase, and ATP sulfurylase were demonstrated in the trophosome, but, again, absent in muscle.[114] These observations strongly indicate that *R. pachyptila* is able to generate ATP and reducing power from the oxidation of sulfide and to reduce carbon dioxide. Sulfur isotopic ratio analyses ($^{34}S/^{32}S$) have convincingly shown that the sulfur incorporated in the biomass originates from the vent and not from seawater sulfate.[115] Furthermore, the existence of dense cultures of procaryotic cells in the trophosome strongly suggests that these organisms are responsible for the chemoautotrophic metabolism described above.[116] Studies of hemoglobin from the blood of these animals have shown that it has characteristics supportive of a chemoautotrophic animal in an unusually variable environment.[117,118]

Recently dense biological communities similar to the hydrothermal vent fauna have been described from the Florida Escarpment.[119] In this case the fauna apparently is nourished by sulfide-rich hypersaline water seeps at ambient temperature.

As mentioned before reducing environments are not at all uncommon. Often the reducing milieu is the result of the activity of sulfate reducing bacteria of the genera

Desulfovibrio or *Desulfotomaculum* excreting the excess sulfur as hydrogen sulfide. The sulfide may be oxidized chemically or microbiologically. The colorless sulfur bacteria will generate sulfate from sulfide via elemental sulfur using molecular oxygen. Green or purple sulfur bacteria and cyanobacteria (blue–green algae) may use hydrogen sulfide as the hydrogen donor in anoxygenic photosynthesis, thus producing elemental sulfur or sulfate.[120] It has recently been shown that methanogenic bacteria may reduce molecular sulfur forming hydrogen sulfide.[121] As discussed above certain organisms have evolved means to cope with these harsh environments. A further example of this adaption is offered by the lug worm, *Arenicola marina*, being capable of surviving 24 h in hydrogen sulfide-saturated sea water with no apparent after-effects. It was shown that the hemoglobin of this species is very well adapted to its way of life.[122]

One aspect of environmental toxicity is lacking under reducing conditions since most heavy metals are eliminated as insoluble sulfides. In many other ecosystems heavy metal ion concentrations are high. Undoubtedly organisms inhabiting these areas have evolved protective mechanisms comparable to the cadmium binding peptides cadystin A (**67**) and B (**68**) secured from the fission yeast *Schizosaccharomyces pompe*.[123] Other extreme



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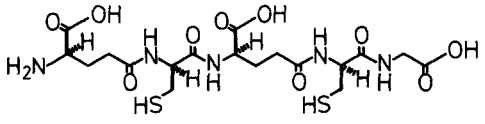
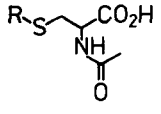


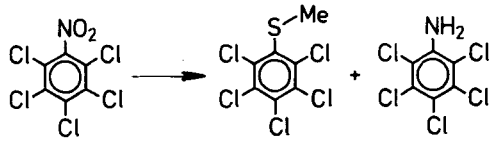
68

conditions may arise when toxic compounds, whether antropogenic or naturally occurring, gain access to an ecosystem. The most general and immediate method of detoxification, at least in mammals, is the excretion *via* mercapturic acids, *S*-substituted *N*-acetyl-L-cysteines (**69**).[29] Presumably the mercapturic acids are formed by enzyme-mediated reactions between the foreign compound and glutathione (GSH). Types of



69

substrates include aromatic hydrocarbons, aryl halides, halonitrobenzenes, alcohols, halides, esters, nitroalkanes, etc. When the fungicide pentachloronitrobenzene (**70**) was fed to the free-living protozoan *Tetrahymena thermophila*, pentachloro(methylthio)benzene (**71**) and pentachloroaniline (**72**) were the major metabolites.[124] Double



70 71 72

labelling experiments (^3H and ^{35}S) indicate that **70** is first transformed to *S*-(pentachlorophenyl)glutathione and subsequently, via *S*-(pentachlorophenyl)cysteine and pentachlorobenzenethiol, to **71**. The methyl group seems to originate from *S*-adenosylmethionine (**63**).[124] The metabolisms of most xenobiotics have not been studied in any detail at present.

The above-mentioned examples of life under extreme conditions are in no way exhaustive. For example the reader may look in vain for a discussion of the neuston, where the organisms experience extreme levels of radiation and other physical and chemical conditions. However, as most of the common ecological relationships are so little investigated it is perhaps predictable that the more specialized ones should be virtually unknown from a chemical point of view.

3. AMINO ACIDS

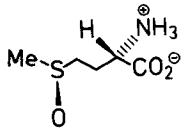
A. Amino Carboxylic Acids

Many investigations concerning amino acids and peptides in marine organisms are based on chromatography of crude extracts and identification of the constituents based on R_f values. In many studies the criterion of identity with an authentic sample is only the chromatographic parameter determined under one set of conditions. In most cases this presumably leads to the correct assignment, but the method gives no hints as to the composition of unknown compounds. Only two such investigations will be mentioned here as examples before we proceed to cases where amino acids have been identified by chemical and physical methods.

Using a combination of electrophoresis and chromatography the free amino acid composition of 20 sponges was investigated.[125] Another work using the same techniques investigated the free amino acid pattern of 67 species of sponges representing 50 genera.[126] Most species contained taurine (**44**), many species hypotaurine (**42**), and some taurocyamine (**46**). Of a total of 87 species of Demospongia, 26 had free methionine and 34 free cysteine. In the second investigation,[126] 11 species were found to contain free methionine sulfoxide and 12 methionine sulfone. Cysteic acid was present in 9 species. The amino acid patterns were used as a character in taxonomic discussions. Most interestly, 11 unknown amino acids or peptides were encountered during these investigations. Undoubtedly it would be fruitful to reinvestigate the amino acid composition of sponges using modern techniques. The work on sponge amino acids has been reviewed.[127] Methionine has been found in a variety of marine organisms including algae, gorgonians, clams, and fish. This is interesting because methionine displays lipotropic activity. Large amounts of methionine are toxic. Methionine derivatives are in demand as additive to soybean protein (see Section 2D).

While methionine sulfone seems never to have been positively identified by isolation and examination from marine materials, methionine sulfoxide is a verified constituent of algae. A review of amino acids from marine algae has appeared.[128]

L-Methionine sulfoxide



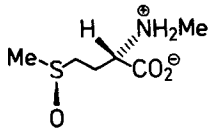
73

73: $C_5H_4NO_3S$, mp 234 °C (dec), $[\alpha]_D^{18}$ − 75° (H_2O), ir absorption between 1000 and 1100 (s) cm^{-1}.

Isolated in 0.03% yield from the red alga *Grateloupia turuturu* (Grateloupiacea),[129] the structure was inferred from the formation of L-methionine on reduction of the natural product with hydrochloric acid, hydriodic acid, or sodium hydrogen sulfite. The specific optical rotation is in good agreement with the one reported for the L-methionine sulfoxide with *R*-configuration at the asymmetric sulfur atom. Later **73** has been shown to exist in many red algae.[130]

A constituent of the red alga *Chondrus ocellatus* (Gigartinaceae),[131] $C_5H_{11}NO_3S$, mp 238–239 °C, $[\alpha]_D^9$ − 73° (H_2O), p*K*'s 2.0, 9.1, and 11.4, was believed to be *S*-hydroxymethylhomocysteine; it seems likely that the compound was actually L-methionine sulfoxide.

N-Methylmethionine sulfoxide

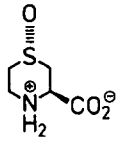


74

74: $C_6H_{13}NO_3S$, mp 226 °C (dec), $[\alpha]_D^{18}$ − 56° (H_2O), ir 1040 cm^{-1} (S=O), ^1H nmr (CF_3CO_2D) δ 2.89 (CH_2-3), 2.95 (S–Me), 3.1 (N–Me), 3.44 (CH_2-4), 4.42 (CH-2).

The sulfoxide **74** was isolated in a yield of 0.04% of fresh weight from *G. turuturu*[129] and in 0.05% of fresh weight from another red alga, *Centroceras clavulatum* (Ceramiaceae).[130] Reduction with hydriodic acid gave *N*-methyl-L-methionine. The specific rotation suggests *R*-configuration at the asymmetric sulfur center.

Chondrine, 1,4-thiazane-3-carboxylic acid 1-oxide

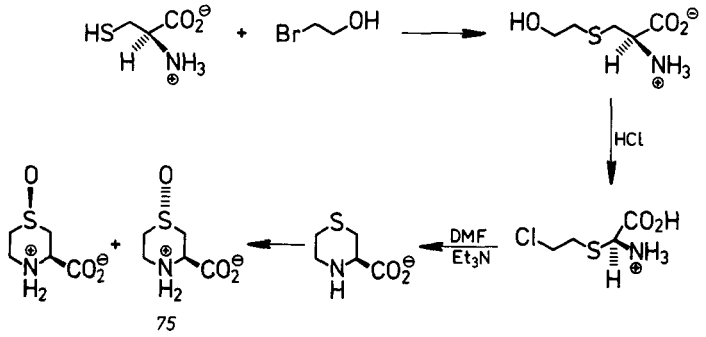


75

75: $C_5H_9NO_3S$, mp 255–257 °C (dec), $[\alpha]_D^{19}$ 20.91 ° (H_2O), $[\alpha]_D^{12}$ 30.15 ° (6N HCl).

Chondrine was isolated in a yield of about 0.03% from the red alga *Chondria crassicaulis* (Rhodomelaceae) simultaneously by two groups.[132–134] Reduction with hydriodic acid gave the sulfide which upon hydrogen peroxide oxidation gave the sulfoxide. Raney nickel reduction afforded *N*-ethyl-L-alanine. Permanganate oxidation gave taurine and cysteic acid.

The structure was confirmed by synthesis[135] (Scheme 5) from L-cysteine by reaction with 2-bromoethanol to give *S*-(2-hydroxyethyl)-L-cysteine. Hydrochloric acid treatment gave *S*-(2-chloroethyl)-L-cysteine hydrochloride, which cyclized in *N*,*N*-dimethylformamide-triethylamine to give thiomorpholine-3(*R*)-carboxylic acid. Oxidation of the latter compound yielded a mixture of diastereomers from which the dextrorotary isomer chondrine (**75**), could be secured by fractional recrystallization. The stereochemistry has later been firmly established by X-ray analysis.[136]

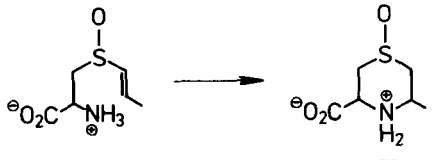


75

SCHEME 5 Synthesis of chondrine.

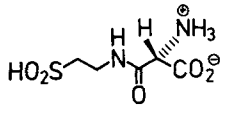
Chondrine has later been identified in other species of Rhodophyta, *Neodilsea yendoana*, *Chondrus ocellatus*, *Martensia elegans*, *Laurencia nipponica*, Phaeophyta, *Zonaria sinclairii*, *Zonaria turneriana*, *Desmarestia ligulata*, *Laminaria japonica*, *Ecklonia radiata*, *Undaria pinnatifida*, *Cystophora moniliformis*, *Sargassum confusum*, Chlorophyta, *Enteromorpha linza*, *Ulva pertusa*, and *Codium fragile*.[128]

Curiously enough, the only other 1,4-thiazane from natural sources, 3-methyl-1,4-thiazane-5-carboxylic acid 1-oxide (**77**) was encountered during a search for the precursor of the lachrymatory factor of onion (*Allium cepa*). The precursor, trans-(+)-*S*-(1-propenyl)-L-cysteine sulfoxide (**76**) under weakly basic conditions cyclizes to form cycloalliin (**77**).[137] Analogously the synthetic *S*-vinylcysteine sulfoxide gives chondrine.[138,139] *S*-Vinylcysteine sulfoxide has never been identified from natural sources.



76 *77*

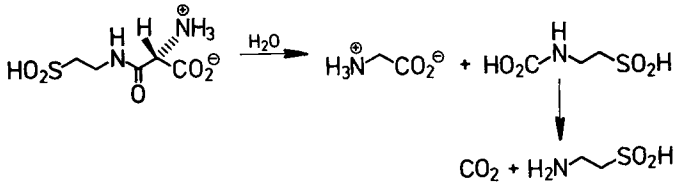
Arcamine



78

78: $C_6H_{10}N_2O_6S$, colorless syrup, ir 1639 (amide), 1580 (CO_2^-), 3300 (free NH) cm^{-1}, ^1H nmr (D_2O) δ3.1 (t, CH_2), 3.7 (t, CH_2) 4.8 (s, CH), ms (field descorption) m/z166 ($M^+ - CO_2$), 149 ($M^+ - H_2O$, CO_2H), 145 ($M^+ - SO_2H$), 127 ($M^+ - H_2O$, SO_2H).

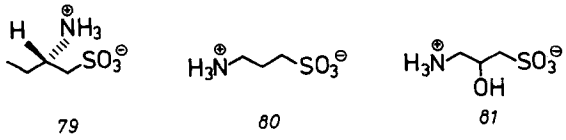
The unusual "dipeptide", arcamine (**78**), was isolated from the mussel *Arca zebra* (Bivalvia) as an unstable syrup.[140] Within minutes after dissolution in water arcamine produced hypotaurine and glycine. Hypotaurine is easily oxidized to taurine in air. The presence of 2-aminomalonic acid during the oxidative hydrolysis was tentatively suggested on the basis of TLC experiments.



Arcamine (**78**) elicits Exploratory Feeding Behavior in fish and is thus one of the very few attracting principles isolated. It is of interest that a compound with such an unusual structure possesses this activity, often believed to be associated with mixtures of common amino acids.

B. Amino Sulfonic Acids

Amino sulfonic acids of algae include taurine (**44**), *N*-methyltaurine, *N*,*N*-dimethyltaurine, *N*,*N*,*N*-trimethyltaurine, D-cysteinolic acid (**79**), 3-aminopropanesulfonic acid (**80**), 3-amino-2-hydroxypropanesulfonic acid (**81**), glyceryltaurine (**82**), and rhodoic acid (**83**).[128] Nothing is known about the ecological or physiological functions of these sulfonic acid derivatives.



79 80 81

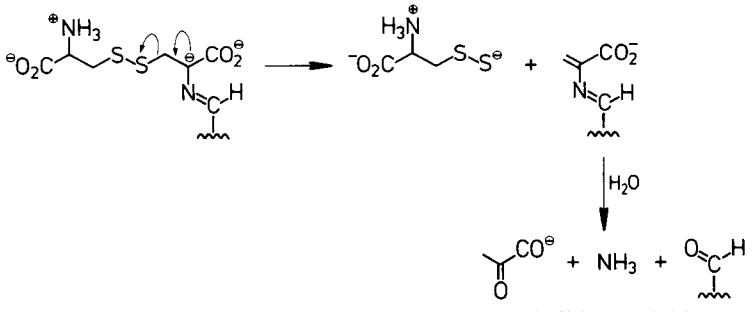
82 83

4. ALKALOIDS

A. Introduction

Alkaloids are nitrogen-containing secondary metabolites. Most often they are believed to be derived from amino acids. However, the latter may be rearranged or otherwise changed to a degree precluding identification of the primary precursor. Furthermore, the knowledge of the metabolic pathways operative in marine organisms is often so scanty that a given metabolite cannot at present be distinguished as primary or secondary. Obviously the above-mentioned dilemma leaves an author with a relatively free hand to include material of his choice. In the present context a rather extensive definition has been used resulting in inclusion of material which might with equal right be classified as amino acids, peptides, etc.

Some sulfur-containing marine alkaloids are clearly derived from the sulfur-containing amino acids of the intermediary metabolism. In other cases, however, this is far from certain and very often it is highly unlikely. As biogenetic studies are lacking in this area anybody is free to speculate as to the possible biosynthetic schemes responsible for the unusual structural features encountered. In this connection the complexity of intermediary metabolites should be borne in mind. Apart from the amino acids there are many other sulfur-containing primary and secondary metabolites and notably sulfur-transferring enzymes. Rhodanese (thiosulfate sulfurtransferase EC 2.8.1.1) and 3-mercaptopyruvate sulfurtransferase (EC 2.8.1.2) transfer sulfane sulfur directly via sulfur substituted enzymes while cystathionase (cystathionine γ-lyase, EC 4.4.1.1) and thiosulfate reductase (sulfane reductase) cleave carbon-sulfur bonds and produce sulfane sulfur.[141] Thiosulfate reductase is glutathione dependent and possibly cleaves the sulfur-sulfur bond of thiosulfate and transfers the sulfur to glutathione forming a persulfide which in turn may discharge sulfide by reaction with a second glutathione thiol group. Cystathionase functions by a pyridoxal phosphate dependent mechanism to produce a persulfide via the Schiff base of the disulfide substrate. Cystathionase will react with cystine to form the corresponding Schiff base with the pyridoxal phosphate prosthetic group. The apoenzyme directs the point of cleavage of the enzyme-stabilized carbanion intermediate of this Schiff base to the carbon-sulfur bond, resulting in cysteine



persulfide. Hydrolysis of the remaining aminoacrylate Schiff base yields pyruvate and ammonia. 3-Mercaptopyruvate sulfurtransferase requires no prosthetic group and presumably reacts with 3-mercaptopyruvate, which is the only known donor substrate, to form a sulfur-substituted enzyme releasing pyruvic acid in the process.

$$HSCH_2COCO_2^- + \text{enzyme} \rightleftharpoons CH_3COCO_2^- + \text{enzyme-S}$$

$$\text{enzyme-S} + \text{acceptor} \rightleftharpoons \text{S-acceptor} + \text{enzyme}$$

The sulfur enzyme is very reactive and will transfer the sulfur atom to a variety of substrates, including thiols, forming persulfides which may give rise to polysulfide accumulation or the discharge of either elemental sulfur or hydrogen sulfide. Rhodanese will react with thiosulfate releasing sulfite ion to form the sulfur-substituted enzyme.

$$SSO_3^{2-} + \text{enzyme} \rightleftharpoons SO_3^{2-} + \text{enzyme-S}$$

$$\text{enzyme-S} + \text{acceptor} \rightleftharpoons \text{S-acceptor} + \text{enzyme}$$

The latter is quite stable, but will react slowly with many active sulfur nucleophiles. 2-Mercaptoethanol, cysteine, and glutathione are inactive, but 2,3-dimercapto-1-propanol, reduced lipoate, reduced lipoamide, and cyanide ions (to form thiocyanate ion) will react at acceptable rates.[142] Generally, no elemental sulfur or sulfide ions are formed in these reactions.

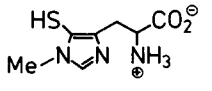
Whether any of these enzymes are involved in the synthesis of marine sulfur-containing alkaloids is at present unknown.

Another important question concerns the origin of the alkaloids. Several sponges, bryozoans, and tunicates are known to harbor dense populations of microorganisms. In most investigations it has not been possible to differentiate between the metabolites of the microorganisms and those of the host. It is therefore entirely possible that many of the natural products treated in the following Sections will eventually turn out to be microbial secondary metabolites.

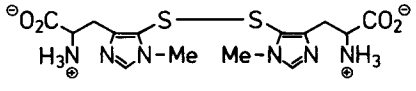
The chemistry, occurrence, and activity, including the possible role of microorganism associations, have been reviewed.[26]

B. Histidine-Derived Compounds

A simple derivative of L-histidine, 1-methyl-5-mercapto-L-histidine or 1-methyl-L-histidine-5-thiol, **84** has been detected in the unfertilized eggs of certain echinoderms.[143] The corresponding disulfide, **85**, was demonstrated also to be present in the same material.



84



85

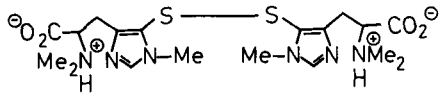
84: $C_7H_{11}N_3O_2S$; data not reported.

85: $C_{14}H_{20}N_6O_4S_2$; mp darkening at 202–205 °C; $[\alpha]_D^{20}$ + 76° (0.1 N HCl); uv (0.1 N HCl) 275br nm (log ε 3.83); ^{1}H nmr (D_2O) δ 3.44 (2Hx2, d, J = 7.5, β-CH_2), 3.94 (3Hx2, s, N-Me), 4.29 (1Hx2, t, J = 7.5, α-CH), 8.95 (1Hx2, s, C–2H); ^{13}C nmr (D_2O) δ 25.5, 54.5, 173.3 (CH_2–$CH(NH_2)CO_2H$), 34.0, 130.0, 134.8, 141.1 (N-methylated imidazole rings).

The total amount of the two compounds was determined from isolated **85** following oxidative work-up and ion-exchange chromatography. The structure was determined spectroscopically and the stereochemistry by comparison of the Raney nickel desulfurated product with authentic 1-methyl-L-histidine. The combined yield of **84** and

85 from the unfertilized eggs of the sea urchin *Paracentrotus lividus* was on the average 0.4 mg/g wet eggs.

Examination of the extract of the eggs of the echinoid *Sphaerechinus granularis*[144] led to the isolation of a related metabolite, **86**. The structure determination was carried out



86

86: $C_{18}H_{28}N_6O_4S_2$; $[\alpha]_D^{20}$ + 79.0°; uv (0.1 N HCl) 257 nm (log ε 3.83); ^{1}H nmr (D_2O) δ 3.05 (6Hx2, *N*-Me_2), 3.37 and 3.54 (1Hx2, dd, *J* = 10.3, 13.2, 1Hx2, dd, *J* = 4.4, 13.2, β-CH_2), 3.93 (3Hx2, s, *N*-Me), 4.08 (1Hx2, dd, *J* = 4.4, 10.3, α-CH), 8.06 (1Hx2, s, C–2H).

as described for **85**. Automatic amino acid analysis and HPLC were used to determine the amount of **85** and **86**, respectively, calculated as mg/g of protein from the unfertilized eggs of the following echinoderms:

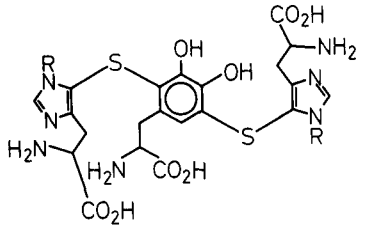
		85	**86**
Sea urchin	*Paracentrotus lividus*	8.0	0.9
Sea urchin	*Arbasia lixula*	2.8	0.7
Sea urchin	*Sphaerechinus granularis*	0.3	4.1
Starfish	*Marthasterias glacialis*	4.0	–
Starfish	*Astropecten aurantiacus*	4.8	–
Sea cucumber	*Holothuria tubulosa*	1.2	–

The function and biosynthesis of these sulfur-containing metabolites is not known.

The existence of a cyclic redox process involving thiol groups with a regulatory function in cell cleavage and differentiation in the sea urchin egg seems to be widely accepted.[145,146] During the fertilization and first cell division cycle no significant change in the intracellular levels of glutathione, glutathione disulfide, and protein glutathione disulfide occurs.[147] Usually glutathione is believed to be the chief non-protein thiol present in living cells.[148] In the eggs of *P. lividus*, however, the ratio of 1-methyl-L-histidine-5-thiol to glutathione is about 60 : 1.[144] It is thus possible that the histidinethiols in these echinoderms have replaced glutathione in at least some of the functions traditionally associated with the latter species. This is an interesting and important proposition which clearly merits further investigation.

The structural features of **84** are encountered in an interesting red–violet marine pigment, adenochrome, from the branchial heart of the common octopus, *Octopus vulgaris*. The structure of adenochrome has been one of the structural problems of long standing in marine natural products research.[149–152] The early structural investigations have been reviewed.[153]

Modern investigations have revealed that adenochrome is actually a mixture of iron(III)-binding peptides of approximate molecular weight 1300–1500 as determined by osmometry or Sephadex gel filtration.[154] The structural elucidation has been carried out on the colorless desferriadenochrome (DFA).[154–156] The branchial heart contains varying amounts of DFA. DFA showed uv absorption at 306 nm (pH 1) shifting to 320 nm (pH 10) indicative of an *ortho*-dihydric phenol chromophore. Hydrolysis of



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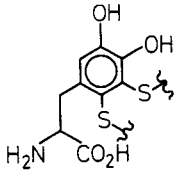

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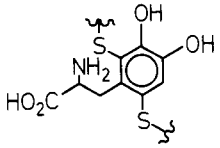


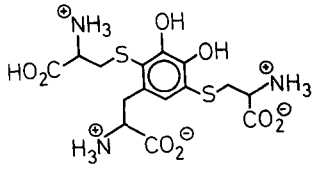
89

R=H or Me

DFA gave rise to complicated mixtures of amino acids, glycine, adenochromine A (**87**), B (**88**), and C (**89**). The structures were determined by extensive hydrolytic, hydrogenolytic, and spectroscopic studies. The stereochemistry of the building blocks was beyond doubt since the hydrolytic experiments yielded L-dopa, histidine-5-thiol, and 1-methylhistidine-5-thiol which on Raney nickel desulfuration gave L-histidine and L-1-methylhistidine, respectively. Adenochrome has a ratio of 3:1 of histidine and 1-methylhistidine homologues.

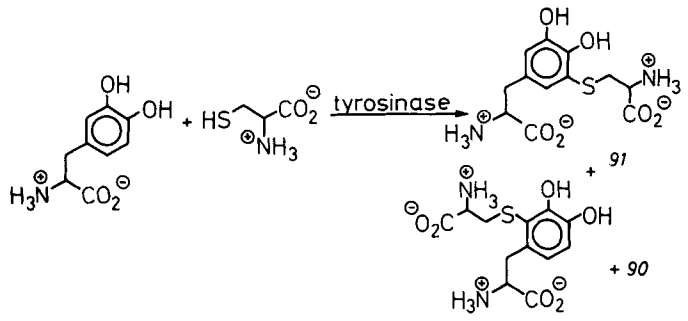
Adenochrome thus is a complex mixture of closely related peptides consisting of two moles of glycine and one mole of adenochromine with the isomers occurring in a 3:2:1 (adenochrome A, B, and C) ratio and with one fourth of the histidines methylated at the imidazole N-1. These units may form larger peptides. Adenochrome is not only present in the branchial heart, but also in other organs. While it was not found in the skin, kidney, and branchial gland, the following concentrations (mg adenochrome/g wet weight) were determined in the branchial heart 12, ovary 0.52, amoebocytes 0.24, and gills 0.14. The amoebocytes are the circulating cells of the blood. Furthermore the white bodies contain a possible precursor of adenochrome with the composition of adenochromines, glycine, and aspartic acid in the molar ratio 1:2:2. The two aspartic acid residues are released on mild hydrolysis generating adenochrome. This observation lends support to the suggestion that the white bodies are the site of origin of the amoebocytes,[157] which may then carry endochrome to the different organs.

Although the biosynthesis has not been investigated it has been shown that the dopaquinone generated from dopa by tyrosinase oxidation reacts with histidine-5-thiol to give the three adenochromines by way of the parent monoadducts, the secoadenochromines.[158] These reactions are remarkably analogous to the formation of phaeomelanins from dopaquinone and cysteine.[159,160] Furthermore, a related compound, 2,5-*S*,*S*-dicysteinyldopa, (**90**), has been isolated from the tapetum lucidum of the eye (0.5 mg/eye) of the freshwater fish *Lepisosteus spatula* (alligator gar),[161] see Section 2D, and from the urine of patients suffering from melanoma.[162]



90

SCHEME 6 Biomimetic synthesis of 5-*S*-cysteinyldopa and 2-*S*-cysteinyldopa.



A synthesis of **90** was effected by treating L-dopa with mushroom tyrosinase (EC 1.14.18.1) in the presence of L-cysteine (Scheme 6).[163] The reaction mixture afforded an 8.5% yield of **90** in addition to 68% 5-*S*-cysteinyldopa (**91**) and 5.8% 2-*S*-cysteinyldopa (**92**).

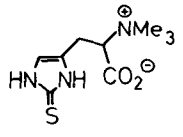
Since tyrosinase is a bifunctional enzyme capable of hydroxylating tyrosine to form dopa and of oxidizing dopa to dopaquinone, the above-mentioned reactions may well be of biochemical significance. Recently it has been demonstrated that tyrosinase will transform tyrosine in proteins to dopaquinone.[164] If the protein contains cysteine residues, 5-*S*-cysteinyldopa structural elements will be formed as in bovine serum albumin (19 tyrosine and 1 cysteine residues per molecule) and yeast alcohol dehydrogenase (14 tyrosine and 8 cysteine residues per molecule). Bovine insulin (4 tyrosine and no cysteine residues per molecule) only gave protein-bonded dopa on tyrosinase treatment. Occurrence of cysteinyldopa in proteins has been reported.[165,166] The marine mussel *Mytilus edulis* L. contains an adhesive protein with 11% dopa.[167]

The occurrence of an adenochrome-like pigment has been reported from the marine bryozoan *Bugula neritina* (L.).[168] From 30 g sea water washed bryozoan 21 mg of dry pigment was isolated by extraction with boiling distilled water followed by precipitation with potassium hydroxide (pH 11). The pigment forms a wine-red aqueous solution with pH 6.8. Although the pigment is water soluble and very soluble in alkaline solution it precipitates out from an approximately 0.04% solution at pH 11.1 and redissolves at pH 9.2. At pH 2.0 the pigment also precipitates from a solution of about the same concentration as the above-mentioned. The pigment is insoluble in common organic solvents and addition of acetone to the aqueous extract precipitates the pigment. Treatment of the aqueous solution with concentrated potassium hydroxide or sodium hydroxide gave small bubbles which reacted with Nessler's reagent. Decoloration was effected with zink in potassium hydroxide, sodium hydrosulfite, potassium permanganate, and hydrogen peroxide. The biuret and the xanthoproteic reaction were negative as were the murexide test for purines, the Salkowski test for indoles, Liebermann's test for phenols, and also reduced sulfur appeared to be absent. The ninhydrin test was positive. The compound is an acid-base indicator changing from blue-purple (pH 2.4) through purple, red-wine, red to purple-blue (pH 11.0) and exhibited a sharp absorption band at 545 nm (pH 6.8), seemingly with a shoulder around 500 nm.

In the above-mentioned paper from 1948 the author believed that no pigment had been reported from Bryozoa. However, chlorophyll was reported from *Flustra foliacea* as early as 1887[169] and, more important in this connection, "*Bugula* Purpur" (*Bugula* Purple) was reported from *Bugula neritana* (presumably a misspelling of *B. neritina*) at least as early as 1903.[170] The latter account describes the coloring matter as a water and glycerol-soluble component, insoluble in alcohol, ether, chloroform, carbon disulfide, etc. The decolorizing of the compound was effected by air, hydrogen sulfide, chlorine, and hydrogen peroxide. The color is stable in boiling water and the acid-base indicator properties were also described.

The structure elucidation of this interesting pigment will have to await future studies. However, very preliminary results indicate that the material is actually (a) sulfur containing compound(s).[171] A crude aqueous extract of defatted (hexane followed by methanol) bryozoans, collected around Okinawa, gave after lyophilization a colored material containing common salts. The elemental analysis showed C: 16.59%, H: 3.57%, N: 3.83%, S: 3.89% with 42.75% combustion residue containing 13.65% Cl. A total sulfur microanalysis with divanadium pentoxide added gave 8.86% sulfur. The ultraviolet spectrum (H_2O, pH 10) of the crude product exhibited absorption maxima at λ 215, 270, and 331 nm. The visible spectrum (H_2O) exhibited absorption maxima at 545 nm with a shoulder at 504 nm in agreement with the published spectrum. The infrared spectrum of the crude product in a KBr disk had strong absorption at 3410, 1640, 1425, 1140, and 615 cm^{-1}. The ^1H nmr (D_2O) was uninformative due to the high salt concentration. The problem is at present being investigated.[171]

There is at present no evidence that *Bugula* purple contains histidinethiol fragments making the histidine-5-thiols, and the adenochromes the only known natural products based on this unit. Ergothioneine, derived from 2-mercapto-L-histidine by quarternization of the α-amino group (**93**), has, however, been known for a long time (isolated 1909, structure elucidated 1911). It was initially isolated from ergot-infested rye, but has later been shown to have a very wide occurrence in plants and animals. Presumably **93** is taken up from the excretion products of the microorganisms who synthesize it and distributed through the food chains. Chemically, ergothioneine is quite different from



93

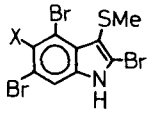
the 5-mercapto derivatives since **93** is mainly present in the thione tautomeric form as depicted. Ergothioneine (**93**) and the desulfurated analogue herzynine are present in the crab *Limulus polyphemus* L.[172,173] The origin of **93** in the crab is unknown.

C. Indole-Derived Compounds

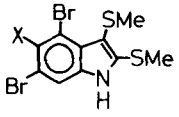
Although most compounds in this section presumably are derived from L-tryptophan or intermediates in the tryptophan metabolism, there is no proof for this hypothesis since biosynthetic studies are lacking in this area. In this connection it is also worthwhile to

remember that a different biochemical pathway to indoles could in principle exist, for example, the hypothetical formation of the indole ring via intramolecular cyclization of dopaquinone originating from dopa and hence from tyrosine. In the cases where the side chain of tryptophan is lacking, as is actually the case in most of the compounds dealt with in this section, there is no *a priori* reason to refer the structures to the tryptophan metabolic schemes.

The occurrence of four simple brominated methylthioindoles has been reported from a Taiwanese collection of the red alga *Laurencia brongniartii*.[174] The compounds were identified by a combination of spectroscopic studies and chemical investigations. Thus 2,4,6-tribromo-3-methylthioindole (**94**) and 2,4,5,6-tetrabromo-3-methylthioindole (**95**) on reductive debromination produced 3-methylthioindole while 4,6-dibromo-2,3-bis(methylthio)indole (**96**) and 4,5,6-tribromo-2,3-bis(methylthio)indole (**97**) gave 2,3-bis(methylthio)indole. Raney nickel desulfurization of **94** and **96** yielded 4,6-dibromoindole.



94 X = H
95 X = Br



96 X = H
97 X = Br

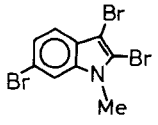
94: uv (EtOH) 233, 292 nm (log ε 4.53, 3.83); ir (CCl_4) 3430, 3250, 2910, 2830, 1600, 1465, 1400, 1370, 1310, 1080 cm^{-1}; ^1H nmr (270 MHz, $CDCl_3$) δ 2.34 (3H, s), 7.37 (1H, d, J = 1.5), 7.48 (1H, d, J = 1.5), 8.55 (1H, br s); ^{13}C nmr (CD_3COCD_3) 21.1 (q), 109.0 (s), 113.9 (s), 113.9 (s), 115.2 (s), 121.7 (s), 125.9 (s), 127.7 (d), 138 (s).

95: uv (EtOH) 238, 298 nm (log ε 4.64, 3.88); ir (CCl_4) 3440, 3200, 2960, 2900, 2850, 1600, 1470, 1390, 1290, 1205 cm^{-1}; ^1H nmr (270 MHz, $CDCl_3$) δ 232 (3H, s), 7.44 (1H, s), 8.66 (1H, br s), ^{13}C nmr (CD_3COCD_3) 21.1 (q), 114.5 (s), 115.8 (d), 116.5 (s), 117.8 (s), 120.5 (s), 123.2 (s), 128.1 (s), 136 (s).

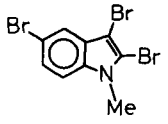
96: uv (EtOH) 237, 296, 313 nm (log ε 4.42, 3.89, 3.99); ir (CCl_4) 3440, 3300, 2980, 2920, 2850, 1600, 1460, 1400, 1370, 1320, 1260, 1180, 1080, 1040, 970, 940 cm^{-1}; ^1H nmr (270 MHz, $CDCl_3$) δ 2.37 (3H, s), 2.56 (3H, s), 7.39 (1H, d, J = 1.5), 7.46 (1H, d, J = 1.5), 8.38 (1H, br s); ^{13}C nmr ($CDCl_3$) 17.3 (q), 21.4 (q), 110.4 (s), 121.1 (s), 113.2 (s), 114.8 (s), 127.2 (d), 136.7 (s), 138.5 (s).

97: uv (EtOH) 239, 321 nm (log ε 4.51, 3.99); ir (CCl_4) 3440, 3300, 2970, 2900, 2850, 1600, 1470, 1400, 1350, 1300, 1220, 1980, 980 cm^{-1}; ^1H nmr (270 MHz, $CDCl_3$) δ 2.35 (3H, s), 2.58 (3H, s), 7.60 (1H, s), 8.46 (1H, br s).

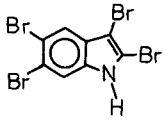
Interestingly, *L. brogniartii* had earlier yielded two tribromoindoles (**98** and **99**) and two tetrabromoindoles (**100** and **101**), but none of these possess the bromo substitution pattern of the methylthioindoles.[175]



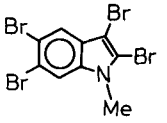
98



99



100

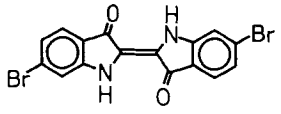


101

On the other hand demethyl-**98** might formally on exchange of the 3-bromo substituent for the methylthio group, followed by bromination at position 4, give **94** and on dibromination at position 4 and 5 give **95**. Analogously, **97** might formally originate from **100** by substitution of the methylthio group for the bromine atoms at position 2 and 3, followed by bromination at position 4. However, **96** could not be formed from any of the bromoindoles without a reductive debromination. Likewise a reductive debromination has to be involved in order to transform any of the sulfur compounds to the bromoindoles **98**, **99**, **100**, or **101**.

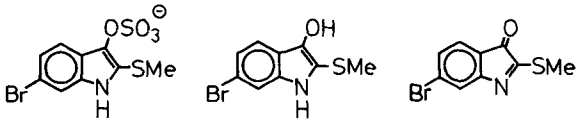
The same brominated methylthioindoles have been isolated from an Okinawan red alga identified as *Laurencia grevilleana* which may be synonymous with *Laurencia brongniartii* (Dr. T. Higa, personal communication).

Simple brominated methylthioindoles are encountered in the precursors of the pigment Tyrian purple (**102**), used since ancient times as a valuable coloring matter.[176] The precursor present in the hypobranchial gland of a number of species from the



102

molluscan families Muricidae and Thaisidae is 6-bromo-2-methylthioindoxyl 3-sulfate (**103**).[177] In the secretion of the gland or in the molested gland the sulfate ester is hydrolyzed by enzymatic action producing **104** and/or the corresponding keto tautomer. Oxidation of **104** may be responsible for **105**, isolated from extracts of the glands.[178]



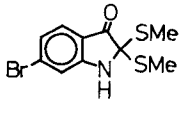
103 *104* *105*

103: Potassium salt ^{1}H nmr (H_2O) δ 2.42 (3H, s, S–CH_3), 9.76 (1H, s, NH), 7.14 (3H, ABC system, ArH).

104: Not isolated.

105: C_9H_6BrNOS mp 109.5 °C, uv (hexane) 217 (log ε 3.94), 242 (4.40), 247 (4.40), 278 (4.04), 318 (3.72), 333 (3.70), 335 (3.43), 426 (3.23), 447 nm (3.23), ir (Nujol) 1742, 1592, 1502, 1120, 1090, 1042, 880, 776 cm^{-1}. ms *m/z* 257 (56%), 225 (54), 242 (100), 240 (97), 133 (47), 75 (47), 74 (20), metastables 227.5 (257 → 242), 225.5 (255 → 240), 189.2 (242 → 215), 187.2 (240 → 212), 148 (229 → 184), 146 (227 → 182), 83 (214 → 133), (212 → 133). ^{1}H nmr (CCl_4) δ 2.60 (3H, s, SCH_3), 7.3 (3H, m, ArH).

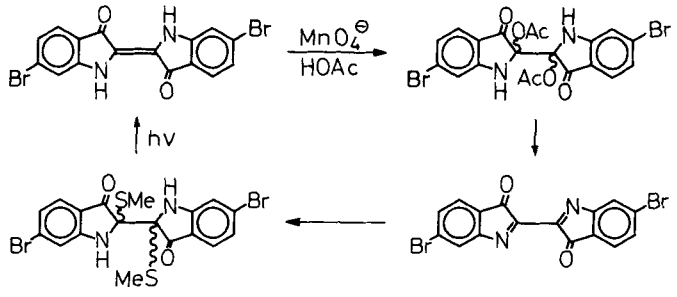
Methanethiol easily and reversibly transforms **105** to **106**.



106

106: $C_{10}H_{10}BrNOS_2$. Mp 177 °C (dec.). uv (EtOH) 225 sh (log ε 4.32), 248 (4.40), 270 sh (3.90), 355 sh (3.20), 402 nm (3.43). ir (Nujol) 3360, 3300, 1680, 1600, 1310, 1090, 1048, 900 cm^{-1}. ms *m/z* 305 (2.4%), 303 (2.4), 290 (0.6), 288 (0.6), 258 (97), 257 (64), 256 (95), 255 (52), 242 (100), 240 (98), 229 (6), 227 (6), 214 (9), 212 (9), 184 (13), 182 (19), 133 (38), 75 (39), 74 (21), 48 (19), 47 (26), 46 (9), 45 (17) metastables 227.5 (257 → 242), 225.5 (255 → 240), 189.2 (242 → 214), 187.2 (240 → 212), 148 (229 → 184), 146 (227 → 182), 83 (214 → 133), (212 → 133). ^{1}H nmr ($CDCl_3$) δ 2.20 (6H, s, SCH_3), 5.45 (1H, br, s, NH), 7.4 (3H, m, ArH).

Compound **106** was found in the extracts, but may be an artefact. Reaction between **104** and **105** now produces tyriverdin (**107**), which in a photochemical reaction forms Tyrian purple and dimethyl disulfide. The structure of tyriverdin was confirmed by synthesis[179] according to Scheme 7.



107

SCHEME 7 The photochemical transformation of tyriverdin to Tyrian purple.

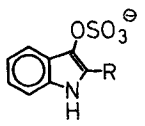
107[180]: $C_{18}H_{14}Br_2N_2O_2S_2$, uv (MeOH) 237 sh (log ε 4.37), 252 (4.51), 275 sh (4.08), 350 (3.41), 4.02 (3.46), 598 nm (2.86), ir (KBr) 3387, 2924, 1680, 1602, 1571, 1448, 1421, 1363, 1315, 1273, 1240, 1190, 1100, 1090, 1045, 1030, 959, 900, 851, 814, 771, 719, 641, 605, 565, 537 cm^{-1}. ^{1}H nmr (d_6-DMSO) δ 1.88 (s, Me), 6.97 (dd, *J* = 8, 1.8 Hz), 7.3 (d, *J* = 1.8 Hz), 7.48 (d, *J* = 8 Hz), 8.22 (br s) [(another isomer shows 1.92 (s, Me), 6.89 (dd, *J* = 8, 1.7 Hz), 7.08 (d, *J* = 1.7 Hz), 7.33 (d, *J* = 8 Hz), 8.03 (br, s)]. ^{13}C nmr (d_6-DMSO) δ 11.6 (SMe), 72.7 (C-2), 195.5 (C-3), 118.0 (C-3a), 125.6 (C-4), 121.5 (C-5), 131.8 (C-6), 114.5 (C-7), 159.9 (C-7a).

The stereochemistry of tyriverdin is not known, but some investigations[180] indicate that a mixture of the possible stereoisomers is formed. Other studies indicate that crystalline tyriverdin is the *meso* form.[181]

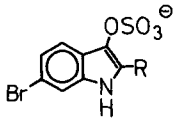
The photochemical reaction generating Tyrian purple from tyriverdin is extremely efficient[179] having a quantum yield of more than 5.

Other precursors (**108**, **109**, **110**, and **111**) have been reported from molluscs from the Mediterranean Sea.[182] No analytical data have appeared in support of these structures.

Tyrian purple has been isolated from an acorn worm *Ptychodera flava laysanica* Spengel, together with two other indigotin derivatives (**112** and **113**).[183,184] In this case the

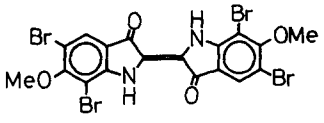


108 R=H
109 R=SMe

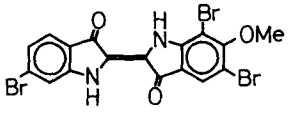


110 R=H
111 R=SO_2Me

compounds seem to be true metabolites. Nothing is known about the biosynthesis of the indigotin derivatives in the acorn worms, it is therefore not clear whether sulfur-containing precursors are present also in these animals.



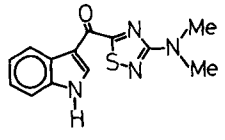
112



113

Dendrodoine The cytoxic sulfur-containing alkaloid dendrodoine (**114**) is unique in containing the first identified naturally occurring 1,2,4-thiadiazole ring system.[185] In addition this compound may equally well be classified as an indole alkaloid or guanidine alkaloid.

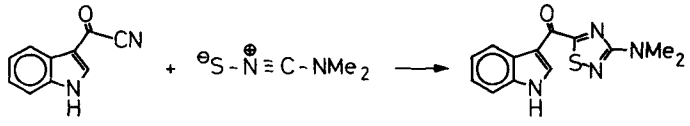
The structure was established by an X-ray structure determination carried out on a yellow crystal of the monoacetate.



114

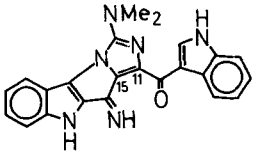
114: $C_{13}H_{12}N_4OS$, mp 280–285 °C, ir (KBr) 3225, 1630 cm^{-1}. ^1H nmr (acetone) δ 9.35 (d), 8.60 (1H, m, ArH), 7.73 (1H, m, ArH), 7.45 (2H + NH). ^{13}C nmr, δ 138.12 (d, C-2), 112.59 (s, C-3 and C-7), 121.40 (d, C-4), 123.63 (d, C-5), 122.66 (d, C-6), 112.59 (d, C-7), 136.57 (s, C-8), 126.21 (s, C-9), 187.81 (s, C-10), 172.20 (s, C-3 or C-5), 175.73 (s, C-5 or C-3), 38.39 (s, NMe_2); ms *m/z* 144 (100%), 116, 89.

Dendrodoine (**114**) originates from a tunicate *Dendrodoa grossularia* collected off North Brittany in the month of September. The yield based on lyophilized animals was 0.045%. Dendrodoine (**114**) has been synthesized by 1,3-dipolar addition reaction between 2-(indole-3-yl)oxoacetic acid nitrile (**115**), prepared from indole, oxalyl chloride, and copper(I) cyanide, and *N,N*-dimethylaminocarbamic acid nitrile *N*-sulfide



115 *116* *113*

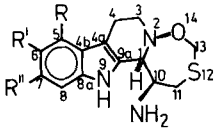
(**116**) obtained *in situ* by thermolysis of 5-(*N*,*N*-dimethylamino)-1,3,4-oxathiazol-2-one.[186] It is unfortunate that the latter paper christens the tunicate *Dendroda grossular* instead of *Dendrodoa grossularia*. Later, the same animal source has given rise to yet another alkaloid, grossularine.[187] Structure **117** proposed for the new alkaloid (the bond



117

C(11)—C(15) was shown as a single bond in the formula, however, according to the data given in the text, it should be a double bond). This structure is certainly highly unusual from a biogenetic point of view nor is the chemistry of the compound easily explained (*e.g.*, the proposed imino NH group could not be acetylated). Clearly a reinvestigation of this interesting species is warranted.

Eudistomins A series of bromoindoles containing a unique oxathiazepine structural element has been identified from the colonial Caribbean tunicate *Eudistoma olivaceum*.[188]



118 R = H, R' = OH, R'' = Br
119 R = Br, R' = OH, R'' = H
120 R = H, R' = H, R'' = Br
121 R = H, R' = Br, R'' = H

118: $C_{14}H_{17}BrN_3O_2S$, pale yellow oil, $[\alpha]_D^{25}$ − 52° (MeOH), uv (MeOH) 226 (log ε 4.37), 287 nm (3.90), ir 3250 cm^{-1}. ^1H nmr (360 MHz, CD_3CN) δ 4.00 (1H, s, br, H-1), 2.70 (2H, m, H-3), 3.01, 3.54 (2H, m, H-4), 6.93 (1H, s, H-5), 7.45 (1H, s, H-8), 8.97 (1H, s br, H-9), 3.54 (1H, m, H-10), 3.25 (1H, d, J = 14.6 Hz, H-11), 2.70 (1H, m, J = 14.6 Hz, H-11), 4.73, 4.87 (2H, d, J = 9.1 Hz, H-13).

119: $C_{14}H_{17}BrN_3O_2S$, pale yellow oil, $[\alpha]_D^{25}$ − 18° (MeOH). ^1H nmr (360 MHz, CD_3CN) δ 4.00 (1H, s br, H-1), 3.52, 2.99 (2H, m, H-3), 3.25, 3.52 (2H, m, H-4), 6.73 (1H, d, J = 8.5, H-7), 7.14 (1H, d, J = 8.5, H-8), 9.08 (1H, s br, H-9), 3.52 (1H, m, H-10), 3.25, 2.73 (2H, m, J = 14.6, H-11), 4.72, 4.86 (2H, d, J = 9.1, H-13).

120: $C_{14}H_{17}BrN_3OS$, $[\alpha]_D^{25}$ − 102° (MeOH). ^1H nmr (360 MHz, CD_3CN) δ 4.05 (1H, s br, H-1), 2.78 (2H, m, H-3), 3.05, 3.55 (2H, m, H-4), 7.36 (1H, d, J = 8.4, H-5), 7.16 (1H, dd, J = 8.4, 1.5, H-6), 7.52 (1H, d, J = 1.5, H-8), 9.23 (1H, s br, H-9), 3.55 (1H, m, H-10), 3.29, 2.78 (2H, m, H-11), 4.77, 4.91 (2H, d, J = 9.2, H-13).

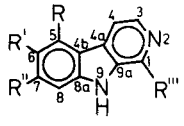
121: $C_{14}H_{17}BrN_3OS$, $[\alpha]_D^{25}$ − 77° (MeOH). ^1H nmr (360 MHz, CD_3CN) δ 4.06 (1H, s br, H-1), 2.77 (2H, m, H-3), 3.04, 3.54 (2H, m, H-4), 7.60 (1H, d, J = 1.3, H-5), 7.20 (1H, dd, J = 8.5, 1.3, H-7), 7.28 (1H, d, J = 8.5, H-8), 9.30 (1H, s br, H-9), 3.54 (1H, m, H-10), 3.29, 2.77 (2H, m, H-11), 4.77, 4.90 (2H, d, J = 9.1, H-13).

The structure determinations were carried out by extensive spectroscopic studies of the eudistomins and their acetyl derivatives. The stereochemistry of the oxathiazepine ring was tentatively assigned from chemical shifts, coupling constants, and CD measurements. Eudistomin C (**118**) and E (**119**) were isolated in yields of 0.0017% and 0.0015% of wet weight, respectively. Both compounds (**118** and **119**) show very strong inhibition of *Herpes simplex* virus, type 1 at 50 ng/12.5 mm disc. In the same assay eudistomin K (**120**) and L (**121**) inhibit growth at 250 ng and 100 ng/disc, respectively.

A fifth compound, eudistomin F, $C_{16}H_{18}BrN_3O_4S$, was isolated. The compound is formally derived from eudistomin C (**118**) by addition of a $C_2H_2O_2$ unit at C-10, C-11, or N-10.

The biosynthesis of the eudistomins has not been studied, but it seems likely that they are formed from tryptophan (N-2 to C-9a) and cysteine (C-1, C-10, C-11, and S-12).

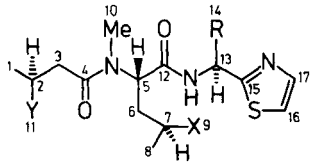
Substituted β-carbolines, eudistomins A (**122**), D (**123**), G (**124**), H (**125**), I (**126**), J (**127**), M (**128**), N (**129**), O (**130**), P (**131**), and Q (**132**) have been obtained from the same source.[189]



122	R = H	R' = OH	R'' = Br	R''' = 2-pyrrolyl
123	R = Br	R' = OH	R'' = H	R''' = H
124	R = H	R' = H	R'' = Br	R''' = 1-pyridin-2-yl
125	R = H	R' = Br	R'' = H	R''' = 1-pyridin-2-yl
126	R = H	R' = H	R'' = H	R''' = 1-pyridin-2-yl
127	R = H	R' = OH	R'' = Br	R''' = H
128	R = H	R' = OH	R'' = H	R''' = 2-pyrrolyl
129	R = H	R' = Br	R'' = H	R''' = H
130	R = H	R' = H	R'' = Br	R''' = H
131	R = H	R' = OH	R'' = Br	R''' = 1-pyridin-2-yl
132	R = H	R' = OH	R'' = H	R''' = 1-pyridin-2-yl

D. Thiazoles and Thiazolidinones

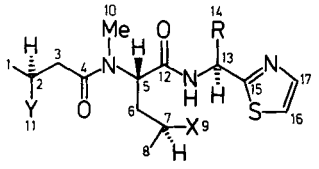
Dysidenins and isodysidenins Dysidenin (**133**) was isolated from a collection of the marine sponge *Dysidea herbacea* from the Great Barrier Reef. The structure was solved by a combination of chemical and spectroscopic studies.[190]



133 X=Y=CCl_3, R=H

136 X=Y=CCl_3, R=H

139 X=Y=Me, R=Me



134 X=Y=CCl_3, R=Me

135 X=Y=CCl_3, R=H

137 X=CCl_3, Y=$CHCl_2$, R=H

138 X=$CHCl_2$, Y=CCl_3, R=H

140 X=Y=Me, R=H

133: $C_{17}H_{23}Cl_6N_3O_2S$; mp 98–99 °C; $[\alpha]_D^{21}$ − 98° ($CHCl_3$); uv (MeOH), 240 nm (log ε 3.6); ir (KBr) 3270, 1680, 1620, 1540 cm^{-1}, ^1H nmr ($CDCl_3$) (also reported in C_6D_6 and $CDCl_3$/TFA-d_1) δ 1.36 (3H, d, *J* = 7, C1-H), 3.3 (1H, m, C2-H), 2.51 (1H, dd, *J* = 16.5, 8.5, C3-H), 5.27 (1H, dd, *J* = 4, 11, C5-H), 3.15 (1H, dd, *J* = 16.5, 3, C3-H), 1.94 (1H, m, C6-H), 2.64 (1H, ddd, *J* = 15, 11, 2, C6-H), 2.20 (1H, m, C7-H), 1.33 (3H, d, *J* = 7, C8-H), 3.04 (3H, S, C10-H), 5.2 (1H, dq, *J* = 7, 8, C13-H), 1.56 (3H, d, *J* = 7, C14-H), 7.60 (1H, d, *J* = 3.5, C16-H), 7.26 (H, d, *J* = 3.5, C17-H), 6.86 (1H, bd, *J* = 8, NH); ^{13}C nmr δ 171.9 (s), 171.2 (s), 168.2 (s), 142.3 (d, d, *J* = 185.5, 5.9), 118.9 (d, d, *J* = 189.4, 15.6), 105.5 (s), 105.1 (s), 54.0 (d), 51.9 (d), 51.4 (d), 47.3 (d), 37.4 (t), 31.0 (t), 30.8 (q), 21.8 (q), 17.3 (q); ms (HR) *m/z* 543 (0.2%, M^+), 508 (8, $M^+ - Cl$), 388 (19, $C_{11}H_{16}Cl_6NO$), 380 (7, $C_{12}H_{15}Cl_5NO_2$), 202 (100, $C_6H_{11}Cl_3N$), 155 (12, $C_6H_7N_2OS$), 112 (56, C_5H_6NS).

134: $C_{17}H_{23}Cl_6N_3O_2S$; $[\alpha]_D^{22}$ + 47° ($CHCl_3$); uv (MeOH), 238 nm (log ε 3.52); ir (film) 3320, 1688, 1630, 1530 cm^{-1}; ^1H nmr (270 MHz, $CDCl_3$) δ 1.36 (3H, d, *J* = 6, C1-H), 139 (3H, d, *J* = 6.5, C8-H), 1.49 (1H, m, C6-H), 1.65 (3H, d, *J* = 7, C14-H), 2.51 (1H, dd, *J* = 16, 9.5, C3-H), 2.68 (1H, m, C7-H), 2.94 (1H, m, C6-H), 2.98 (3H, s, C10-H), 3.08 (1H, dd, *J* = 16, 2, C3-H), 3.30 (1H, m, C2-H), 5.34 (1H, dd, *J* = 4.5, 9, C5-H), 5.43 (1H, dq, *J* = 8, 7, C13-H), 6.97 (1H, bd, *J* = 8, NH), 7.31 (1H, d, *J* = 3.5, C17-H), 7.70 (1H, d, *J* = 3.5, C16-H); ms identical to that of **133**.

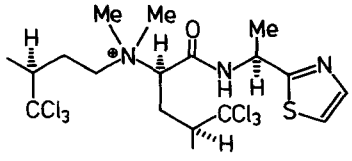
135: $C_{16}H_{21}Cl_6N_3O_2S$; colorless gum; $[\alpha]_D^{20}$ + 52° ($CHCl_3$); ir (film) 3330, 3000, 2950, 1685, 1630, 790, 765, 740 cm^{-1}; ^1H nmr ($CDCl_3$ + D_2O) δ 7.64 (d, *J* = 3.5, H-15), 7.30 (br t, *J* = 5, *N*H), 7.26 (d, *J* = 3.5, H-16), 5.32 (dd, *J* = 5, 9, H-5), 4.73 (doubled AB pattern, *J* = 16, 5, H-13), 3.3 (m, H-2), 3.1 (m, H-3b), 2.9 (m, H-6b), 3.01 (s, H-10), 2.71 (m, H-7), 2.46 (dd, *J* = 9, 16, H-3a), 1.5 (m, H-6a), 1.36 (d, *J* = 7, H-1 or H-8), 1.33 (d, *J* = 7, H-8 or H-1); ^{13}C nmr ($CDCl_3$ + D_2O) δ 17.3 (C-1), 51.6 (C-2), 37.5 (C-3), 171.5 (C-4), 54.4 (C-5), 31.6 (C76), 51.6 (C-7), 16.5 (C-8), 105.4 (C-9), 31.3 (C-10), 105.1 (C-11), 166.8 (C-12), 40.7 (C-13), 169.2 (C-14 or C-12), 142.4 (C-15), 119.4 (C-16); ms (EI) *m/z* 494 (M − Cl), 458, 412, 388, 380, 352, 202 (100%), 166, 141, 123, 113, 98, 57, 42; ms (CI) *m/z* 531. 9530 ($C_{16}H_{22}{}^{35}Cl_5{}^{37}ClN_3O_2S$, $M^+ + 1$), 533.9505 ($C_{16}H_{22}{}^{35}Cl_3{}^{37}Cl_3N_3O_2S$).

136: $C_{16}H_{21}Cl_6N_3O_2S$; colorless gum; $[\alpha]_D^{20}$ − 97° ($CHCl_3$); ^1H nmr ($CDCl_3$ + D_2O) δ 7.65 (d, *J* = 3.5, H-15), 7.24 (d, *J* = 3.5, H-16), 5.40 (dd, *J* = 4, 11, H-5), 4.73 (tripled AB pattern, *J* = 16, 3, H-13), 3.32 (m, H-2), 3.20 (m, H-3b), 3.01 (s, H-10), 2.6 (m, H-6b), 2.47 (dd, *J* = 9, 16, H-3a), 2.20 (m, H-7), 1.94 (m, H-6a), 1.37 (d, *J* = 7, H-1 + H-8); ^{13}C nmr ($CDCl_3$ + D_2O) δ 17.3 (C-1), 51.5 (C-2), 37.5 (C-3), 172.1 (C-4), 53.8 (C-5), 30.7 (C-6), 51.8 (C-7), 16.3 (C-8), 105.4 (C-9), 30.7 (C-10), 105.1 (C-11), 166.7 (C-12 or C-14), 40.6 (C-13), 169.4 (C-14 or C-12), 142.4 (C-15), 119.4 (C-16); ms (EI) major fragments identical to those of **135**; ms (CI) *m/z*, 531.9541 ($C_{16}H_{22}{}^{35}Cl_5{}^{35}ClN_3O_2S$, $M^+ + 1$), 533.9505 ($C_{16}H_{22}{}^{35}Cl_4{}^{37}Cl_2N_3O_2S$), 535.9496 ($C_{16}H_{22}{}^{35}Cl_3{}^{37}Cl_3N_3O_2S$).

137: $C_{16}H_{22}Cl_5N_3O_2S$; colorless gum; $[\alpha]_D^{20}$ + 85° ($CHCl_3$); ^1H nmr ($CDCl_3$ + D_2O) δ 7.64 (d, J = 3.5, H-15), 7.24 (d, J = 3.5, H-16), 5.98 (d, J = 3, H-11), 5.26 (dd, J = 5, 9, H-5), 4.71 (doubled AB pattern, J = 4, 16, H-13), 2.98 (s, H-10), 2.90 (m, H-6b), 2.3–2.8 (m, H-2 + H-3 + H-7), 1.50 (m, H-6a), 1.37 (d, J = 7, H-8), 1.17 (d, J = 7, H-1); ^{13}C nmr ($CDCl_3$ + D_2O) δ 15.4 (C-1), 40.5 (C-2), 36.3 (C-3), 172.1 (C-4), 54.3 (C-5), 31.6 (C-6), 51.7 (C-7), 16.6 (C-8), 105.4 (C-9), 31.3 (C-10), 77.9 (C-11), 166.9 (C-12 or C-14), 40.7 (C-13), 169.2 (C-14 or C-12), 142.4 (C-15), 119.4 (C-16); ms (EI) m/z 460 (M – Cl), 424, 354, 346, 202 (100%), 166, 98, 89, 84, 58, 49, 42; ms (CI) m/z 497.9921 ($C_{16}H_{23}{}^{35}Cl_4{}^{37}ClN_3O_2S$), 499.9896 ($C_{16}H_{23}{}^{35}Cl_3{}^{37}Cl_2N_2O_2S$), 501.9861 ($C_{16}H_{23}{}^{35}Cl_2{}^{37}Cl_3N_3O_2S$).

138: $C_{16}H_{22}Cl_5N_3O_2S$; colorless gum; $[\alpha]_D^{20}$ + 69° ($CDCl_3$); ^1H nmr $CDCl_3$ + D_2O) δ 7.65 (d, J = 3.5, H-15), 7.26 (d, J = 3.5, H-16), 5.86 (d, J = 3, H-9), 5.24 (t, J = 8, H-5), 4.73 (doubled AB pattern, J = 16, 5, H-13), 3.3 (m, H-2), 3.1 (m, H-3b), 2.98 (s, H-10), 2.45 (dd, J. = 9, 16, H-3a), 1.7–2.3 (m, H-6b and H-7), 1.60 (m, H-6a), 1.36 (d, J = 7, H-8); ^{13}C nmr ($CDCl_3$ + D_2O, quarternary carbons not observed) δ 17.3 (C-1), 51.3 (C-2), 37.3 (C-3), 53.4 (C-5), 30.9 (C-6), 40.3 (C-7), 14.2 (C-8), 77.8 (C-9), 31.3 (C-10), 40.7 (C-13); ms (EI) m/z 460 (M – Cl), 354, 346, 168 (100%), 124, 98, 59, 49, 42; ms (CI) m/z 497.9911 ($C_{16}H_{23}{}^{35}Cl_4{}^{37}ClN_3O_2S$), 499.9886 ($C_{16}H_{23}{}^{35}Cl_3{}^{37}Cl_2N_3O_2S$), 501.9861 ($C_{16}H_{23}{}^{35}Cl_2{}^{37}Cl_3N_3O_2S$).

The relative and absolute configuration was later determined by a chemical correlation between **133** and isodysidenin (**134**). Isodysidenin (**134**) was isolated in > 2% yield of dry weight from *D. herbacea* Keller collected at Laing Island (Papua-New Guinea). The structure and absolute configuration of isodysidenin (**134**) resulted from an X-ray structure determination of the derivative **141** prepared from **134** by reaction with the



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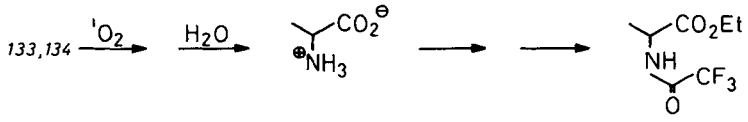
diborane-THF complex followed by methylation (MeI/MeCN).[191] Together with **134** small amounts of **133** were isolated as well. It has later been shown that the absolute stereochemical assignment from the X-ray analysis is in error and that the actual configuration is the opposite of the one published.[192] Accordingly structure **141** has been corrected to depict the true stereochemistry. The revised stereochemistry was obtained by treatment of **133** and **134** with singlet oxygen followed by hydrolysis. Singlet oxygen disrupts the aromaticity of the heterocyclic ring and upon hydrolysis alanine is released.



Analysis by chiral gas chromatography of the *N*-trifluoroacetylated ethyl ester served to identify the amino acid as L-alanine signifying that the thiazole fragment must have the *S* absolute configuration and not *R* as reported in the X-ray study. Hence dysidenin (**133**) and isodysidenin (**134**) have identical stereochemistry around the 2-(1′-aminoethyl)thiazole unit, but opposite to the one reported. By inference dysidenin (**133**) and

isodysidenin (**134**) are the enantiomers of the structures previously reported. Formulas **133** and **134** depict the correct configurations.†

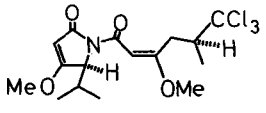
The chemical correlation between **133** and **134** is shown in Scheme 8. Hydrolysis of **133** and **134** produced the acid **143** and the thiazole **144** isolated as the *p*-bromophenacyl ester (**145**) and the 2,4-dinitrophenyl derivative **146**, respectively. The identity of these derivatives of **133** and **134** established the configuration of dysidenin (**133**) at C-2 and C-13 as *S* viz. the same as that of C-2 and C-13 of isodysidenin (**134**). The acid hydrolysis also generated the amino acid **147** characterized as the 2,4-dinitrophenyl derivative of the methyl ester of **148**.

As **148** generated from **134** is a mixture of diastereomers, partial epimerization of one of the asymmetric centers (at C-5 or C-7) must have occurred (note that epimerization of both centers will only produce a d,l pair). Epimerization during conversion of **147** to **148** does not occur and the starting material is homogeneous. The observations leave only the possibility that epimerization has occurred during the acid treatment. Isodysidenin (**134**) has the R5, S7 configuration, hence the diastereomers must be either R5, S7 and S5, S7 or R5, S7 and R5, R7. Dysidenin (**133**) analogously gives the same pair of diastereomers, which then must be either S5, S7 or R5, R7. The asymmetric centers at C-7 were eliminated by Zn—Cu reduction of the trichloromethyl groups of **133** and **134**, producing **139** and **140** having also lost the asymmetry associated with C-2. These two compounds (**139** and **140**) are diastereomeric and thus must have opposite configurations at C-5 since they are identical at C-13. Dysidenin (**133**) accordingly has the absolute configuration: C-2 (S), C-5 (S), C-7 (S), and C-13 (S).[196]

Another collection of *D. herbacea* from the Great Barrier Reef gave four new derivatives related to **133** and **134**. All compounds were isolated as colorless gums and identified mainly by spectroscopic methods. 13-Demethylisodysidenin (**135**),

† The assignment of erroneous absolute stereochemistry in X-ray investigations is not unprecedented and may serve as a warning against the dogmatic character X-ray structural determinations have in the minds of many scientists. X-Ray structural investigations are still usually the single most reliable proof of a structural assignment, but must be substantiated by chemical methods. A recent review treats some of the pitfalls open to the non-specialist crystallographer and presents good advice how to critically evaluate a published crystal structure.[193]

Another proof of the correctness of the newly revised stereochemical assignment of dysidenin (**133**) and isodysidenin (**134**) comes from a synthetic study of dysidin (**142**), another natural product from *D. herbacea*.[194] The absolute stereochemistry of (+)-dysidin (**142**) is known from X-ray crystallographic



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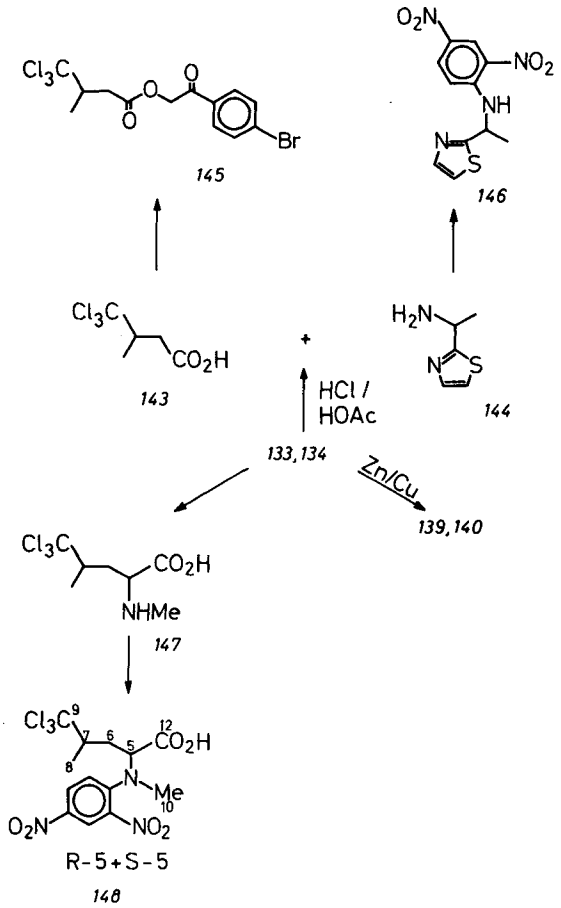
studies.[195] The synthesis of dysidin was carried out starting from (+)-3-(trichloromethyl)butanoic acid since this acid was isolated after degradation of dysidenin and isodysidenin (*vide infra*). The result of the synthesis was a mixture of (−)-dysidin and the diastereomeric (+)-5-epidysidin (epimeric at C(5) of the pyrrolinone ring). Since naturally occurring dysidin is (+)-dysidin (**142**), as shown by one of the X-ray crystallographic absolute stereochemical determinations, either the one of dysidin or the one of the isodysidenin must be in error. To determine which structure is correct the 3-(trichloromethyl)butanoic acid was resolved and the absolute configuration unambiguously determined as (+)-(*R*)-3-(trichloromethyl)butanoic acid by chemical transformation to a product with known absolute stereochemistry. These experiments proved beyond doubt that (+)-dysidin has structure **142** and that dysidenin accordingly must be **134**.

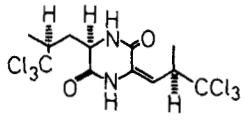


SCHEME 8 Chemical degradation of dysidenin and isodysidenin.

13-demethyldysidenin (**136**), 11-monodechloro-13-demethylisodysidenin (**137**), and 9-monodechloro-13-demethylisodysidenin (**138**), constituted 33%, 3%, 2%, and 0.6%, respectively, of a methylene chloride extract which yielded 2.1% based on dry sponge weight.[197]

An elegant demonstration of the C-5 epimeric relationship between the dysidenin and isodysidenin series was affected by reduction of 13-demethylisodysidenin (**135**) to 13-demethylhexadechloroisodysidenin (**140**), $[\alpha]_D^{25}$ + 112° ($CHCl_3$) with zinc in acetic acid. The same procedure transformed 13-demethyldysidenin (**136**) to the enantiomeric 13-demethylhexadechlorodysidenin (**139**) $[\alpha]_D^{20}$ − 118° ($CHCl_3$).[197] Since structures **135**, **136**, **137**, and **138** were correlated to the structures **133** and **134**, the absolute stereochemistry of the former has been corrected to conform to the revised stereochemistry of dysidenin and isodysidenin.

The biogenesis of these unusual compounds is unknown. As they all encompass an *N*-methyl-5,5,5-trichloroleucine moiety it seems fairly safe to predict that this unusual amino acid may be present somewhere along the biogenetic pathway. Incidentally another derivative of the latter amino acid is known from the same organism, namely



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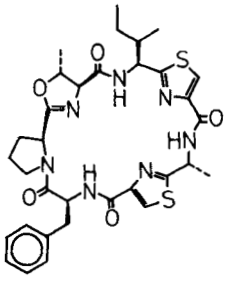
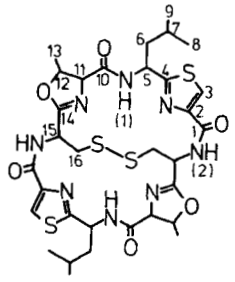
the diketopiperazine (**149**).[194,198] *D. herbacea* collected from various localities has yielded a wide variety of different compounds. In this context it should be borne in mind that this thin encrusting sponge harbors blue-green algae, constituting at times half the cellular weight of the material.[198]

Thiazoles and thiazolines in macrocyclic structures The tunicate *Lissoclinum patella* (class Ascidiacea, order Enterogona, family Didemnidae) collected from Palau, Western Caroline Islands, gave the first two examples of thiazole-containing macrocyclic peptides.[199] The compounds ulicyclamide (**150**) and ulithiacyclamide (**151**) were identified by a combination of spectroscopic and degradative studies. The initially proposed structure of ulicyclamide was later revised to structure **150** as a result of FAB (Fast Atom Bombardment) mass spectrometric studies.[200]



150

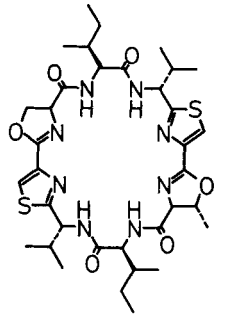


151

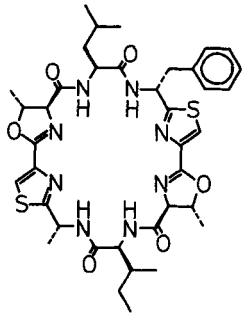
150: $C_{33}H_{39}N_7O_5S_2$; colorless oil; $[\alpha]_D^{25}$ + 35.6° (CH_2Cl_2); uv (MeOH) 248 nm (log ε 3.90); ir 3300, 1670, 1650 cm^{-1}; ^1H nmr (220 MHz, $CDCl_3$) δ 9.06 (d, J = 5); 8.67 (d, J = 7), 8.08 (s), 8.03 (s), 7.85 (d, J = 10), 7.30 (s, 5H), 5.38 (dq, J = 5, 7), 5.26 (dd, J = 10, 7), 4.89 (m), 4.82 (dq, J = 4, 7), 4.52 (t, J = 8), 4.26 (d, J = 4), 3.25 (m, 3H), 2.93 (dd, J = 14, 10), 2.60 (m), 2.1 (m, 2H), 1.9 (m, 2H), 1.71 (d, 3H, J = 7), 1.44 (d, 3H, J = 7), 1.30 (m, 1H), 1.20 (m, 2H), 0.85 (t, 3H, J = 7), 0.73 (d, 3H, J = 7); ^{13}C nmr ($CDCl_3$) δ 171.9 (3, s), 170.5 (s), 167 (s), 161.1 (s), 160.5 (s), 151.4 (s), 148.9 (s), 136.8 (s), 130.6 (2, d), 129.5 (2, d), 128.2 (d), 124.3 (d), 123.8 (d), 83.3 (d), 76.3 (d), 57.5 (d), 54.4 (2, dt), 49.6 (d), 48.1 (d), 41.8 (t), 39.0 (d), 29.8 (t), 26.0 (t), 25.9 (t), 25.4 (q), 22.9 (q), 16.2 (q), 10.9 (q); ms (EI) m/z 677 (M^+), 620 ($M^+ - C_4H_9$), 586 ($M^+ - C_7H_7$).

151: $C_{32}H_{42}N_8O_6S_4$; colorless oil; $[\alpha]_D^{25}$ + 62.4° (CH_2Cl_2); uv (MeOH) 247 nm (log ε 3.85); ir (CH_2Cl_2) 3300, 1670, 1650 cm^{-1}; ^1H nmr (C_6D_6) δ 7.72 (s, H-3), 5.24 (m, H-5), 1.35 (m, 2H, H-6), 1.66 (m, H-7), 0.78 (d, 3H, J = 7) and 0.90 (d, 3H, J = 7, H-8 and H-9), 4.05 (dd, J = 8, 2, H-11), 4.71 (m, H-12), 1.1 (d, 3H, J = 7, H-13), 5.36 (m, H-15), 3.22 (dd, J = 14, 6) and 3.02 (dd, J = 14, 4, H-16), 7.70 (d, J = 9, N(1)–H), 8.50 (d, J = 9, N(2)–H); ^{13}C nmr ($CDCl_3$) δ 170.5 (C-1), 160.1 (C-2), 124.1 (C-3), 149.2 (C-4), 48.5 (C-5 or C-15), 46.5 (C-6), 25.3 (C-7), 22.8, 22.7 (C-8 and C-9), 170.0 (C-10), 74.3 (C-11), 81.7 (C-12), 22.1 (C-13), 167.3 (C-14), 48.4 (C-15 or C-5), 46.5 (C-16).

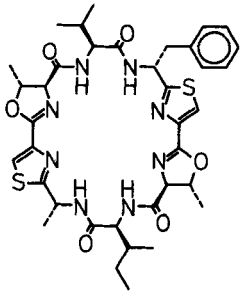
Didemnid ascidians are known to harbor photosynthetic unicellular algae.[201-204] *L. patella* is dark green, hence the name of the compound derived from Hawaiian *Uli*, a dark color as the green of vegetation. The synthetic ability of the symbionts is unexplored. Ulicyclamide (**150**), 0.05% of dry weight, is built up by six amino acids: L-proline, L-phenylamine, L-threonine, L-isoleucine, D-alanine, and cysteine.[200] The threonine moiety is masked by oxazoline formation and cysteine by thiazole formation. Ulithiacyclamide (**151**), 0.04% of dry weight, is made up of L-cysteine, L-threonine, and a thiazole amino acid, derived from D-leucine, meaning that the thiazoles in **151** have the *R* absolute configurations.[205] Again the threonine structure is masked by oxazoline formation. The latter molecule is symmetric as evidenced by the ^{13}C nmr spectrum which exhibited only half the number of signals expected from the number of carbon atoms. Later investigations of the same species, collected in the Western Carolines, served to identify three new compounds: patellamide A (**152**), patellamide B (**153**), and patellamide C (**154**).[206] Again the structures were determined by extensive spectroscopic and degradative studies. All compounds were effective against L 1210 murine leukemia cells, ulithiacyclamide (**151**) being the most active (IC_{50} of 0.35 μg/ml). Patellamide A (**152**) and ulithiacyclamide (**151**) inhibited the human ALL cell line (T cell acute leukemia) CEM with ID_{50} values of 0.028 and 0.010 μg/ml, respectively.[206]



152



153



154

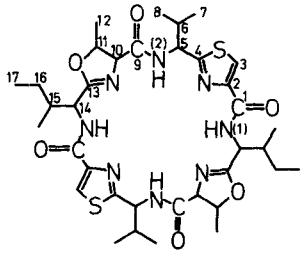
152: $C_{35}H_{50}N_8O_6S_2$; $[\alpha]_D$ + 113.9° (CH_2Cl_2); ir (CH_2Cl_3) 3395, 3130, 3055, 2695, 2940, 2885, 1675, 1655, 1535, 1510, 1489 cm^{-1}; ^1H nmr (270 MHz) δ 4.30 (dd, J = 8, 4, H-2), 4.80 (m, 2H, H-3), 7.83 (s, H-7), 5.22 (m, H-8), 2.32 (m, H-9), 1.13 (d, J = 7, H-10 or H-11 or H-28 or H-29), 1.08 (d, J = 7, H-11 or H-10 or H-28 or H-29), 4.65 (dd, J = 8, 6, H-13 or H-31), 1.96 (m, H-14), 0.75 (t, J = 7, H-16 or H-34), 0.81 (d, J = 7, H-17), 4.30 (d, J = 4, H-19), 4.89 (dq, J = 6, 4, H-20), 1.47 (d, J = 6, H-21), 7.83 (s, H-24), 5.22 (m, H-26), 2.32 (m, H-27), 1.13 (d, J = 7, H-28 or H-10 or H-11 or H-29), 1.08 (d, J = 7, H-29 or H-10 or H-11 or H-28), 4.56 (dd, J = 10, 8, H-31 or H-13), 1.96 (m, H-32), 0.73 (t, J = 7, H-34 or H-16), 0.81 (d, J = 7, H-35), 7.95 (m, N(1)–H), 7.41 (d, J = 10, N(2)–H), 7.95 (m, N(3)–H), 7.41 (d, J = 10, N(4)–H); ^{13}C nmr δ 169.5 (C-1 or C-12 or C-18 or C-30), 67.4 (C-2), 72.2 (C-3), 169.1 (C-4), 149.4 (C-5), 123.0 (C-6), 160.5 (C-7), 54.9 (C-8), 37.1 (C-9 or C-27), 19.2 (2, C-10 or C-28 or C-29), 171.5 (2, C-12 or C-1 or C-18 or C-30), 52.4 (C-13), 33.3 (C-14), 24.9 (C-15 or C-33), 11.1 (C-16 or C-34), 15.0 (C-17 or C-35), 171.8 (C-18 or C-1 or C-12 or C-30), 73.6 (C-19), 81.6 (C-20), 21.7 (C-21), 168.5 (C-22), 149.4 (C-23), 123.0

(C-24), 160.5 (C-25), 54.9 (C-26), 36.8 (C-27 or C-9), 17.9 (2, C-28 or C-29 or C-10 or C-11), 52.1 (C-31), 33.3 (C-32), 24.7 (C-33 or C-15), 10.6 (C-34 or C-16), 14.9 (C-35 or C-17); HRMS *m/z* 742.3280 (M^+).

153: $C_{38}H_{48}N_8O_6S_2$; $[\alpha]_D$ + 29.4° (CH_2Cl_2); ir (CH_2Cl_2) 3374, 3330, 1662, 1480 cm^{-1}; ^1H nmr, ^{13}C nmr, and ms comparable to the ones reported for **152** (see Ref. 205).

154: $C_{37}H_{46}N_8O_6S_2$; $[\alpha]$ + 19° (CH_2Cl_2); ir 3380, 1675, 1655, 1535, 1510 cm^{-1}; ^1H nmr, ^{13}C nmr, and ms comparable to the ones reported for **152** (see Ref. 205).

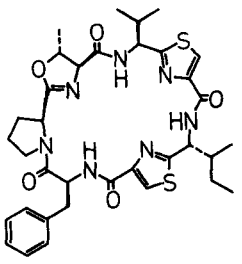
Ulithiacyclamide (**151**) has recently been identified together with ascidiacyclamide (**155**) from an unidentified ascidian collected from Rodda Reef, Queensland, Australia.[207] Ascidiacyclamide (**155**) has a strong effect on PV_4 cultured cells transformed with polyoma virus: T/C 100% was observed at 10 μg/ml. The structure which is symmetric, exhibiting only 18 ^{13}C nmr signals, gave on hydrolysis isoleucine, threonine and a third product.[207]



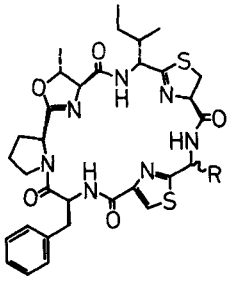
155

155: $C_{36}H_{51}N_8O_6S_2$; colorless prisms mp 139–139.5 °C (benzene); $[\alpha]_D^{25}$ + 164° ($CHCl_3$); uv (MeOH) 232 nm (log ε 4.32); ir (film) 3380, 3340, 1680, 1653 cm^{-1}; ^1H nmr (360 MHz, $CDCl_3$) δ 7.91 (1H, s, H-3), 5.22 (1H, dd, J = 6.3, 10.0, H-5), 2.31 (1H, dqq, J = 6.3, 6.1, 6.1, H-6), 1.07 (3H, d, J = 6.1, H-7), 1.13 (3H, d, J = 6.1, H-8), 4.27 (1H, dd, J = 6.3, 1.2, J-10), 4.86 (1H, dq, J = 6.3, 6.3, J-11), 1.49 (3H, d, J = 6.3, H-12), 4.83 (1H, ddd, J = 8.1, 6.1, 1.2, H-14), 1.95 (1H, m, H-15), 1.17 (1H, m, H-16), 1.27 (H, m, H-16), 0.72 (3H, dd, J = 6.8, 6.8, H-17), 0.80 (3H, d, J = 6.8, H-18), 8.01 (1H, d, J = 8.1, N(1)–H), 7.40 (1H, d, J = 10.0, N(2)–H); ^{13}C nmr (25 MHz, $CDCl_3$) 171.2 (C-1), 160.3 (C-2), 123.0 (C-3), 149.5 (C-4), 54.7 (C-5), 33.4 (C-6), 19.1 (C-7), 17.9 (C-8), 168.9 (C-9), 73.5 (C-10), 81.5 (C-11), 21.7 (C-12), 168.4 (C-13), 52.0 (C-14), 37.0 (C-15), 24.6 (C-16), 10.7 (C-19); ms *m/z* 756 (M^+), 713 ($M^+ - \dot{C}HMe_2$).

Extensive use of fast atom bombardment (FAB) mass spectrometry and other modern spectroscopic techniques in combination with chemical degradation experiments has allowed the identification of a further three cyclic peptides from a Western Caroline Islands collection of *L. patella*.[200] One of these peptides, **156**, on hydrolysis gave L-threonine, L-proline, L-phenylalanine, L-valine thiazole, and D-isoleucine thiazole in equimolar amounts. Again threonine was present as an oxazoline moiety. The yield of **156** was 3.4 × 10^{-3}% of dry weight.



156



157 R=----CH_3

158 R=——CH_3

156: $C_{35}H_{43}N_7O_5S_2$; ir (CH_2Cl_2) 3390, 3320, 2940, 2820, 1672, 1640, 1542, 1500 cm^{-1}; ^1H nmr, ^{13}C nmr, and ms comparable to the ones reported for **152** (see Ref. 200).

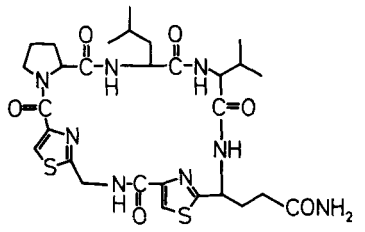
157: $C_{33}H_{41}N_7O_5S_2$; ir (CH_2Cl_2) 3380, 3315, 2985, 2935, 2860, 1674, 1530, 1440 cm^{-1}; ^1H nmr, ^{13}C nmr, and ms comparable to the ones reported for **152** (see Ref. 200).

158: $C_{33}H_{41}N_7O_5S_2$; ir (CH_2Cl_2) 3380, 3320, 3020, 2980, 2940, 1665, 1635, 1510, 1410 cm^{-1}; ^1H nmr, ^{13}C nmr, and ms comparable to the ones reported for **152** (see Ref. 200).

Two epimeric peptides (**157** and **158**) present in 0.096% and 0.048% of dry weight, respectively, represent the first thiazoline-containing peptides in the *Lissoclinum* series. Hydrolysis of these peptides proved the presence of L-threonine, L-proline, and L-isoleucine, together with alanine thiazole. The latter compound, after addition of singlet oxygen followed by acid hydrolysis, gave D-alanine in the case of **157** and L-alanine in the case of **158**. Ulicyclamide (**150**) is thus closely related to **157**, since identical amino acids are linked in the same sequence.

The peptides **156**, **157**, and **158** display borderline cytotoxicity in L 1210 tissue culture assay, exhibiting IC_{50} values greater than 10 μg/ml.

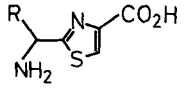
Another series of antineoplastic thiazole-containing cyclic peptides, the dolastatins, has been isolated from the Indian Ocean sea hare *Dolabella auricularia* (phylum Mollusca, order Aplysiomorpha, family Aplysiidae). Nine dolastatins have been isolated in yields of about 1 mg from 100 kg wet sea hare: Dolastatin 1, mp 105–110 °C; 2, 118–121 °C; 3, not reported; 4, 102–109 °C, 5, 52–56 °C; 6, 57–58 °C; 7, 142–145 °C; 8, 72–88 °C; and dolastatin 9, mp 149–152 °C.[208] Dolastatin 1 may represent the most potent anticancer agent known with a curative response of 33% at a dose of 11 μg/kg in the National Cancer Institute's murine B16 melanoma.[208] A structure of dolastatin 3, with $ED_{50} < 10^{-4}$–10^{-7} μg/ml in murine P 388 lymphocytic leukemia cell line, has been proposed.[209] This structure (**159**), based on studies carried out on approximately 1 mg of substance, was later abandoned as a consequence of information gathered in a synthetic study.[210] Hydrolysis of 25–100 μg dolastatin 3 and subsequent amino acid analysis indicated a 1 : 1 : 1 molar ratio of proline, valine, and leucine in addition to two unknown amino acid components. That dolastatin 3 is primarily dèrived from five amino acids was verified by hydrolysis, methylation, acetylation, and gas chromatographic mass spectral analysis of the products. Subtraction of ^1H and ^{13}C signals due to Leu, Pro, and Val from



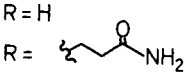
159

159: $C_{29}H_{40}N_8O_6S_2$; colorless amorphous solid mp 133–137°C (from methylene chloride–methanol or ethyl acetate–ethanol); $[\alpha]_D^{26}$ − 35.5° (MeOH); uv (MeOH) 206 (log ε 4.14, thiazole K band) and 238 nm (3.95, thiazole B band); ir (KBr) 3427, 3379, 3330 (NH), 3090 (NH), 1670 (—CONH—), 1629 (—CONH—), 1544 (—CONH—), 1501, 1494, 1445 (thiazole), 1390, 1370, 1310, 1240 (thiazole), 1065 (thiazole), 823, 760, 620 cm^{-1}; ^1H nmr ($CDCl_3$), δ 3.69 (1H, m, H-1), 3.85 (1H, m, H-1), 1.9–2.3 (4H, n, H-2 and H-3), 3.975 (1H, dd, J = 7.9, H-4), 3.85 (1H, m, H-6), 2.14 (1H, m, H-7), 1.53 (1H, m, H-8), 0.975 (3H, d, J = 6.59, H-9), 0.905 (3H, d, J = 6.59, H-10), 4.758 (1H, dd, J = 7.4, 9.2, H-12), 2.06 (1H, m, H-13), 1.048 (3H, d, J = 6.59, H-14), 1.161 (3H, d, J = 6.84, H-15), 5.542 (1H, dd, J = 9.0, 10.6, 4.2, H-17), 2.54 (2H, m, H-18), 2.30 (2H, m, H-19), 8.082 (1H, H-22), 5.249 (1H, dd, J = 7.3, 18.1, H-25), 4.661 (1H, dd, J = 2.2, 18.3, H-25), 8.070 (1H, H-27), 5.992 (1H, d, J = 6.8, N(1)–H), 8.318 (1H, d, J = 9.3, N(2)–H), 7.885 (1H, d, J = 90, N(3)–H), 8.775 (1H, dd, J = 5.4, 1–2, N(4)–H), 6.305 (1H, brs, N(5)–H), 5.442 (1H, br, s); ^{13}C nmr ($CDCl_3$) δ 48.3 (C-1), 25.5 (C-2), 29.7 (C-3), 62.6 (C-4), 169.5 (C-5), 48.6 (C-6), 41.0 (C-7), 25.5 (C-8), 23.3 (C-9), 21.2 (C-10, 171.9 (C-11), 55.7 (C-12), 31.8 (C-13), 18.6 (C-14), 19.6 (C-15), 171.1 (C-21), 124.4 (C-22), 161.0 (C-23), 174.8 (C-24), 37.7 (C-25), 148.3 (C-26), 123.8 (C-27), 160-2 (C-28), 171.2 (C-29); extensive HREI mass spectral data are reported.

the pertinent spectra of dolastatin 3 revealed the presence of two thiazole units. The structure of these units was inferred from spectral analyses as (gly)Thz (**160**) and (gln)Thz (**161**). The amino acid **160** is presumably formed from glycine + cysteine and therefore formed (gly)Thz analogously to glutamine + cysteine → **161**, (gln)Thz. The



160 R = H

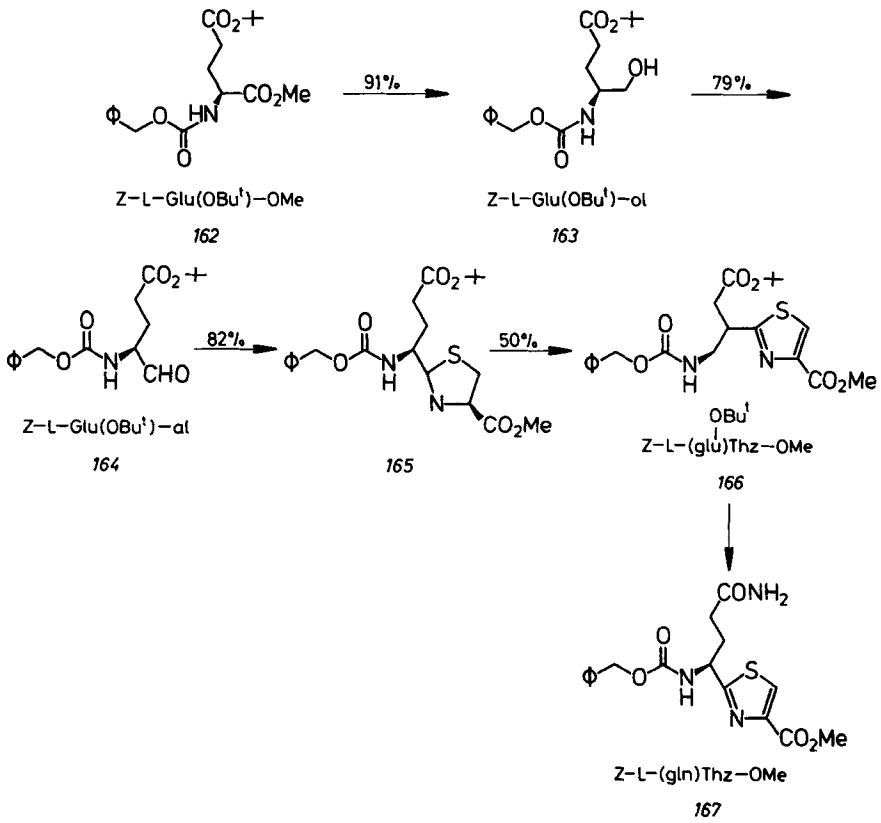
161 R = 

sequence was inferred from lack of reaction of dolastatin 3 with acetic anhydride indicating a cyclic arrangement and by detailed analysis of the electron-impact high-resolution mass spectrum of dolastatin 3. All evidence seems to be in accordance with the formulation *cyclo*[Pro-Leu-Val(Gln)Thz-(gly)Thz] (**159**) for dolastatin 3. Dolastatin 3 is very resistant to enzymatic cleavage, which may be an inherited characteristic of the cyclic structure, but may as well warrant a study of the stereochemistry of the amino acids involved.

Especially in the light of the findings of the unusual stereochemistry of some of the amino acids in the other macrocyclic thiazole derivatives it is highly likely that D-amino acids could be part of dolastin 3.

A compound with the proposed structure of dolastatin 3, *cyclo*[Pro-Leu-Val-(gly)Thz-(gln)Thz], **159** has been synthesized.[210] The synthetic product (all L-amino acids) was not identical with the natural product. All the combinations with one and with two D-amino acids were prepared as well (L-L-L-D, L-L-D-L, L-D-L-L, D-L-L-L, L-L-D-D, L-D-L-D, D-L-L-D). None of these were identical with dolastatin 3. The compounds with reverse order of bonding, *cyclo*[(gly)Thz-(gln)Thz-Val-Leu-Pro], were obtained by synthesis. None of the eight stereochemically different products (L-L-L-L, D-L-L-L, L-D-L-L, L-L-D-L, L-L-L-D, D-D-L-L, D-L-D-L, D-L-L-D) were identical with dolastatin 3. Furthermore none of the synthetic cyclic peptides had activity in the L 1210 murine leukemia cell system at concentrations of 250 μg/ml. Thus the structure of dolastatin 3 is in need of revision.

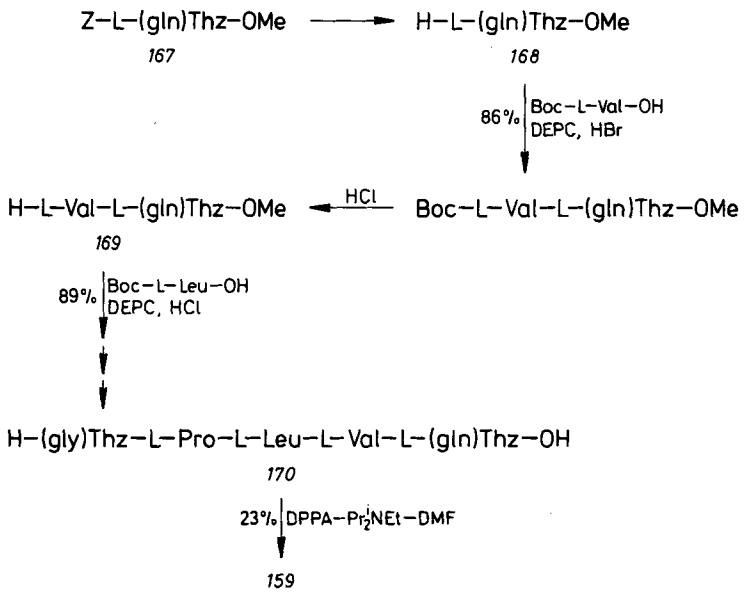
The thiazole amino acids necessary for the synthesis of the proposed structure of dolastatin 3 were prepared from the corresponding amino acids (Scheme 9). The protected L-Glu derivative **162** was selectively reduced ($NaBH_4$/LiCl) to the corresponding alcohol **163**. The aldehyde **164**, formed by oxidation ($PySO_3/Me_2SO$) of **163**, on reaction



SCHEME 9 Synthesis of the protected glutamine thiazole synthon.

with L-cysteine methyl ester gave a mixture of diastereomeric thiazolidines (**165**). The thiazole nucleus of **166** arose from oxidation (activated MnO_2) of **165**. The γ-amide function of **167** resulted from the acid cleavage (CF_3CO_2H) of the ester group of **166**, activation of the γ-carboxylic acid function ($ClCO_2Et$) and ammonolysis. The optical purity of **167** was determined to be 98.6%. The (gly)Thz derivative was prepared analogously.

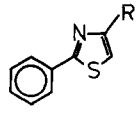
Starting from Z-L-(gln)Thz (**167**) the protected pentapeptide was synthesized stepwise in an overall yield of 70.5% (Scheme 10). Hydrolysis of the methyl ester **167** (HBr/AcOH) released the amine (**168**), which was coupled to Boc-L-Val-OH using diethyl phosphorocyanidate (DEPC, $(EtO)_2P(O)CN$). After removal of the protecting group the resulting dipeptide methyl ester (**169**) was subjected to a coupling procedure etc., finally resulting in the linear pentapeptide **170**. Cyclization of **170** was effected in 23% yield by treating a 1 mM solution of **170** in dimethyl formamide at pH 7.5 (*N,N*-diisopropylethylamine) with diphenyl phosphorazidate (DPPA, $(Ph_2O)_2P(O)N_3$).



SCHEME 10 Synthesis of the proposed dolastatin 3 structure.

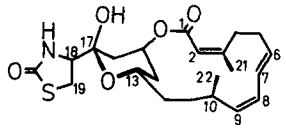
The remaining 15 cyclic peptides were prepared analogously.[210]

Simple thiazole derivatives are known from *Pseudomonas cepacia*.[211] The structures of 2-(2-hydroxyphenyl)thiazole (**171**), 2-(2-hydroxyphenyl)-4-thiazolecarbaldehyde (**172**), and 2-(2-hydroxyphenyl)-4-carboxythiazole (**173**) have been reported.

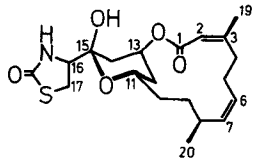


171 R = H
172 R = CHO
173 R = CO_2H

Latrunculins Strongly ichthyotoxic latrunculins are constituents of the red-colored marine sponge *Latrunculia magnifica* Keller, collected between 6–30 m in the Gulf of Eliat and the Gulf of Suez.[212–214] The structure of the hemiketal Latrunculin A (**174**) was determined by an X-ray structural determination of the crystalline methyl ketal.[213,214] As the ketalization of **174** presumably proceeds *via* an oxonium ion the asymmetry of the hemiketal carbon may be destroyed during the reaction. However, as the 2-thiazolidinone group of **175** is equatorial in the solid state and expected to be equatorial in solution as well and as the multiplicities of H-13 and H-15 are essentially the same in **174** and **175** the configuration of C-17 is presumably the same in **174** and **175**.



174

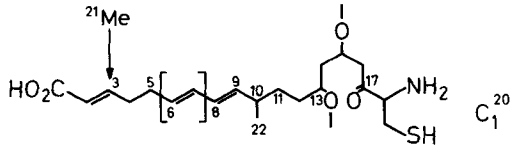


175

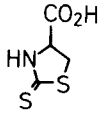
174: $C_{22}H_{31}NO_5S$; foam, $[\alpha]_D^{24}$ + 152° ($CHCl_3$); uv (MeOH) 218 (log ε 4.37) and 268 nm (sh); ir ($CHCl_3$) 3570, 3430, 2960, 2830, 2690 (strong), 1495, 1455, 1440, 1380, 1360, 1270, 1220, 1140, 1095, 1065, 990, 825 cm^{-1}; ^1H nmr (170 MHz, $CDCl_3$) δ 6.41 (dd, *J* = 15, 10.5, H-7), 5.98 (t, *J* = 10.5, H-8), 5.80 (brs, *N*H), 5.74 (dt, *J* = 15, 4.5, H-6), 5.69 (d, *J* = 1.3, H-2), 5.43 (br, t, *J* = 3, H-5), 5.02 (t, *J* = 10.5, H-9), 4.29 (m, H-13), 3.93 (s, *O*H), 3.87 (dd, *J* = 8.7, J-18), 3.51 (dd, *J* = 11.5, 7, H-19), 3.48 (dd, *J* = 11.5, 8, H-19), 3.00 (dt, *J* = 13, 8, H-4), 2.83 (m, H-10), 2.60 (dt, *J* = 13, H-4), 2.26 (1H, m, H-5), 1.92 (d, *J* = 1.3, H-21), 0.98 (d, *J* = 6.7, H-22); ^{13}C nmr (75.46 MHz, $CDCl_3$) δ 166.0 (C-1 or C-20), 117.6 (C-2), 158.3 (C-3), 32.7 (C-4), 30.6 (C-5), 131.8 (C-6), 126.3 (C-7), 127.3 (C-8), 136.5 (C-9), 29.2 (C-10), 31.8 (C-11 or C-12 or C-14 or C-16), 31.2 (C-12 or C-11 or C-14 or C-16), 62.3 (C-13), 35.1 (C-17 or C-11 or C-12 or C-14), 68.1 (C-15), 32.1 (C-16 or C-11 or C-12 or C-14), 96.9 (C-17), 62.1 (C-18), 18.7 (C-19), 175.5 (C-20 or C-1), 24.7 (C-21), 21.8 (C-22), coupling constants and T_1 values are reported, ms (14 eV) *m/z* 421 (20%), 403 (47), 385 (46), 301 (100), 149 (48), 135 (50), 102 (10), 85 (44).

175: $C_{20}H_{29}NO_5S$; $[\alpha]_D^{24}$ + 112° ($CHCl_3$); uv (MeOH) 212 (log ε 4.24) and 269 nm (sh), ir ($CHCl_3$) 3520, 3400, 2910, 1675, 1205, 1115, 1085, 1045, 987, 975, 960 cm^{-1}; ^1H nmr and ^{13}C nmr are very similar to the ones reported for **174**; ms (14 eV) *m/z* 377 (50%), 359 (27), 326 (16), 375 (40), 256 (16), 149 (52), 128 (63), 109 (68), 95 (65), 81 (87), 71 (72), 69 (86), 57 (100).

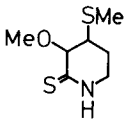
Latrunculin B (**177**) was identified by comparison with **174**.[213,214] The biogenesis of these 16- (**174** and latrunculin C) and 14-macrolides is believed to proceed through polyketides with cysteine as the starting acyl unit. The oxygen functions could then be reduced at C-5 and C-11 and eliminated from the positions at C-3, C-7, and C-9. C-9, C-10, and C-22 may originate from propionate while C-20 and C-21 are two C_1-units.[213,214] The absolute stereochemistry of the latrunculins has not yet been determined.



The latrunculins are the first natural products containing a 2-thiazolidinone unit, however, 2-thioxothiazolidine-4-carboxylic acid (**177**) was recently isolated from light-exposed seedlings of Sakurajima radish (*Raphanus sativus* var. *hortensis* f. *gigantissimus* Makino, Cruciferae).[215] This compound inhibits the growth of hypocotyl sections of etiolated Sukurajima radish seedlings and of etiolated lettuce.[215] 3-Methoxy-4-methylthio-2-piperidinethione (**178**) has been isolated from the same source.[216]



177



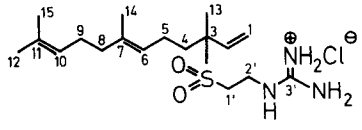
178

During the isolation of the latrunculins a problem, often met in marine natural product chemistry, was encountered, *viz.* that some collections were low in toxicity. The chemical content of many marine organisms changes with parameters not well understood, *e.g.*, at least three different *Latrunculia* collections with differing toxicities were made. Another serious problem in this branch of natural product chemistry is also illustrated by the latrunculin work, namely the question of identification and classification of marine material. Whether the different collections of *Latrunculia* represent variations within specimens of one species, a general variation in, *e.g.*, seasonal depth dependence, etc., or whether different species were actually collected is at present unknown. *Latrunculia corticata* Carter has been described from The Red Sea; this species may be a synonym for *Latrunculia magnifica* Keller.[213]

As mentioned earlier the latrunculins are ichthyotoxic. If the sponge is squeezed into an aquarium the toxins will cause excitation of the fish followed by hemorrhage, loss of balance, and death within 4–6 minutes.[217] Latrunculin A (**174**) and B (**176**) cause major reversible alterations in the organization of the microfilaments of cultured mouse neuroblastoma and fibroblast cells. The effect is comparable to the one exerted by the mold metabolites, 0.1 to 0.01 times lower than the cytochalasins and the mechanism of action, although unknown, is different.[218]

E. Miscellaneous Alkaloids

Agelasidine A A marine sponge, *Agelas* sp., collected around Okinawa, gave a 0.0034% yield of wet weight of antispasmodically active agelasidine A.[219] The structure was determined as **179** from chemical degradation experiments and spectroscopic studies.



179

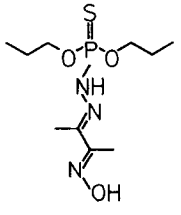
179: $C_{18}H_{34}ClN_3O_2S$; mp 108–108.5 °C; $[\alpha]_D^{25}$ + 19.1° (MeOH); uv (MeOH) < 200 nm; ir (KBr) 3350, 3160, 1678, 1648, 1640, 1380, 1295, 1135, 1005, 945, 830 cm^{-1}; ^1H nmr (CD_3OD) δ 5.49 (d, J = 17.5, J-1), 5.56 (d, J = 11, H-1), 5.98 (dd, J = 11, 17.5, H-2), 1.44 (s, H-13),

1.68–2.06 (m, H-4 and H-5), 5.11 (br t, J = 6.0, H-6), 1.50 (br s, H-14), 1.68–2.06 (m, H-8 and H-9), 5.05 (br, t, J = 7.0, H-10), 1.50 (br s, H-15), 1.58 (br s, H-12), 3.25, 3.28 (ABX_2, J = 14, 6.5, H-1′), 3.68 (t, J = 6.5, H-2′); ^{13}C nmr (CD_3OD) δ 121.7 (t, C-1), 134.7 (d, C-2), 68.3 (s, C-3), 16.0 (q, C-13), 22.8 (t, C-4), 31.6 (t, C-5), 124.1 (d, C-6 or C-10), 136.4 (s, C-7), 16.0 (q, C-14), 39.6 (t, C-8), 26.6 (t, C-9), 122.6 (d, C-10 or C-6), 131.3 (d, C-11), 17.6 (q, C-15), 25.6 (q, C-12), 46.0 (t, C-1′), 35.0 (t, C-2′), 157.5 (s, C-3′); Field desorption ms, m/z 356 (M + H).

The position of the double bonds was confirmed by ozonolysis followed by reduction and acetylation. The sesquiterpene skeleton afforded a diacetyl compound identical with an authentic sample prepared from nerolidol by the same procedure. The guanidine function formed the expected pyrimidine derivative on reaction with 2,4-pentanedione.

It is conceivable that this sesquiterpene taurocyamine derivative originates from farnesol and cysteine. The isolation was guided by monitoring the antispasmodic activity on isolated guinea pig ileum. Another *Agelas* sp. collected ar Argulpelu Reef, Palau, gave the same compound (**179**) in 0.16% yield of dry weight.[220] Agelasidine A inhibited *Candida albicans* at 5 μg/disc, *Bacillus subtilis* at 10 μg/disc, and *Staphylococcus aureus* at 25 μg/disc and was lethal to goldfish (*Carassius auratus*) at 25 μg/ml. Physical and spectroscopic data are reported for the free base.[220]

Gymnodium toxin, Gb-4 The red tide dinoflagellate *Gymnodium breve* (*Ptychodiscus brevis*, presumably *Phytodiscus brevis*) produces toxins of lipoidal nature. This is in contrast to other toxic red tide organisms (*Gonyaulax catenella* and *G. tamarensis*) which produce water soluble toxins of the saxitoxin class. One of the toxins of *G. breve*, identified by X-ray analysis, is a most unusual sulfur-containing phosphorus compound, *O,O*-dipropyl (*E*)-2-(1-methyl-2-oxopropylidene)phosphorohydrazidothioate (*E*)-oxime (**180**).[221]



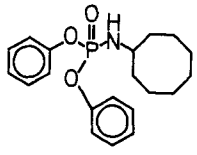
180

180: $C_{10}H_{22}N_3O_3PS$; colorless needles, mp 82–83 °C (benzene); ir (CCl_4) 3590, 3350, 2930 cm^{-1}; ^{1}H nmr (400 MHz, $CDCl_3$) δ 0.9393 (6H, t, J = 7.45), 1.6988 (4H, m, J = 7.06), 1.9415 (3H, s), 2.0483 (3H, s), 3.2127 (1H, br s), 3.9791 (2H, m, $\omega_{1/2}$ 12.62 Hz), 5.17 (1H, br s); ms, m/z 295.1 (44%), 278.1 (19), 253.0 (24.4), 211 (42), 178.9 (17), 278 (19), 96.9 (39), 42 (100).

The toxin, exhibiting ichthyotoxicity of 0.9 ppm against the common guppy *Lebistes reticulatus*, was isolated using a special extraction technique, from cultures of the fragile naked dinoflagellate *G. breve*. Control batches of medium without inoculum were allowed to incubate along with the dinoflagellate cultures. The controls were processed

exactly like the toxin cultures, but yielded no trace of toxins. These experiments strongly indicate that **180** is a true metabolite of *G. breve*.[221]

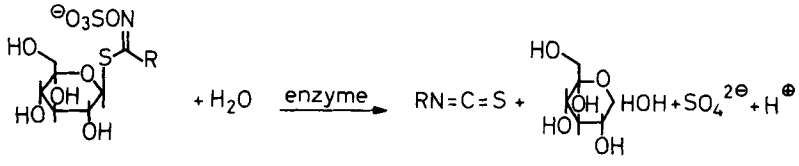
Another phosphorus toxin (**181**) was recently isolated in an amount of 1 μg together with 2.5 mg **180** from 1.2×10^9 cells (100-liter culture) of *Phytodiscus brevis*. Ichthyotoxicity was determined as LD_{100} (1 hr) 1 ppm towards *L. reticulatus*.[222]



181

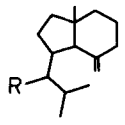
5. ISOTHIOCYANATES

Organic isothiocyanates are a well established group of natural products. In terrestrial plants they seem invariably to originate from glucosinolates.[31,32] The aglucone seems to be derived from amino acids.

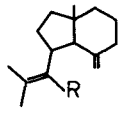


The few isothiocyanates isolated from marine organisms are terpenoids, often associated with the corresponding isocyanides and formamides. Although the biosynthesis of these compounds is totally unknown there is no indication that glucosinolates are involved. Moreover, the marine isothiocyanates may be directly extracted from the fresh material indicating that they occur in free or loosely bound form whereas the terrestrial counterparts are released from the glucosinolate.

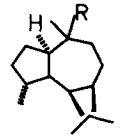
The first marine derivative to appear was axisothiocyanate 1 (**182**),[223] from the marine sponge *Axinella cannabina*. The structure was elucidated by a combination of chemical and spectroscopic methods and by comparison with axisonitrile 1 (**183**), another constituent of the sponge. The yield was 0.014%, based on dry weight after extraction. The correlation to **183** was inferred from the fact that both compounds on reduction with lithium aluminium hydride gave the same methylamine derivative and **182** was obtained from the reaction (120 °C) of **183** with elemental sulfur. The structure of **183** was assigned on the basis of extensive chemical and spectroscopic studies. Axisothiocyanate 1 (**182**) gave the expected thiourea derivative (**185**) on reaction with aniline. The sponge also contains the formamide derivative axamide 1 (**184**).[224] Another series originating from the same biological material consists of axisothiocyanate 4 (**186**), axisocyanide 4 (**187**), and axamide 4 (**188**).[225] Axisothiocyanate 4 (**186**) was not isolated in the pure state, but as the *N,N*-dimethylthiourea derivative **189** which was identical with the product formed from the synthetic thiourea resulting from the reaction (120 °C) between axisocyanide 4 (**187**), elemental sulfur, and dimethylamine. Analogously, axisothiocyanate 2 (**190**), axisocyanide 2 (**191**), and axamide 2 (**192**) were reported from



182 R= N=C=S
183 R= N=C
184 R= NHCHO
185 R= $NHCSC_6H_5$



186 R= N=C=S
187 R= N=C
188 R= NHCHO
189 R= $NHCSNMe_2$



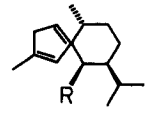
190 R= N=C=S
191 R= N=C
192 R= NHCHO
193 R= $NHCSNMe_2$

182: $C_{16}H_{25}NS$; oil; $n_D^{25°}$ 1.5394, $[\alpha]_D$ + 5.9° ($CHCl_3$); ir (CCl_4) 3050, 2120, 1650, 1385, 1375, 895 cm^{-1}, iv (CCl_4) 243 nm (log ε 3.40); ^1H nmr (100 MHz, CCl_4) δ 4.78 (2H, bm, H-15), 3.27 (1H, t, J = 5.5, H-10), 1.00 (3H, d, J = 7, H-12 or H-13), 0.98 (3H, s, H-14), 0.89 (3H, d, J = 7, H-12 or H-13); ms, m/z 263 (M^+), 230 ($M^+ - HS$), 204 ($M^+ - HNCS$).

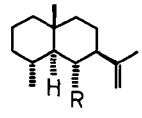
186: $C_{16}H_{23}NS$; Not isolated but transformed to the *N,N*-dimethylthiourea derivative **189**, amorphous solid, $[\alpha]_D$ + 54.7°, synthetic **186**; n_D 1.5493, $[\alpha]_D$ − 35.9° ($CHCl_3$); ir (CCl_4) 2096 cm^{-1}, ^1H nmr (CCl_4) δ 0.98 (3H, s), 1.60 (3H, s), 1.78 (3H, d), 4.61 (2H, m).

190: $C_{16}H_{25}NS$; Not isolated (ir (CCl_4) 2120 cm^{-1}), but transformed to the *N,N*-dimethylthiourea derivative **193**, amorphous solid, $[\alpha]_D$ + 12.5° ($CHCl_3$).

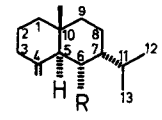
A. cannabina.[224] Again the isothiocyanate was isolated as its dimethylamine derivative **193**. The structure of **190** was determined by chemical and spectroscopic methods, in particular by noting the fact that it was formed from axisocyanide 2 (**191**)[226] by reaction (120 °C) with elemental sulfur. The spiro[4.5]decane skeleton of axisothiocyanate 3 (**194**)[227] was identified by comparison with axisonitrile 3 **195**. The structure of the latter was determined by X-ray crystallographic analysis. Axisothiocyanate 3 (**194**) was formed from **195** by reaction (120 °C) with elemental sulfur. The isothiocyanate (**194**) was isolated as the *N,N*-dimethylthiourea derivative **197**. Axamide 3 (**196**) was present in the sponge. Two additional series of sesquiterpene isothiocyanates, isocyanates, and formamides, **198–200** and **201–203**, have recently been isolated and identified from the



194 R= N=C=S
195 R= N=C
196 R= NHCHO
197 R= $NHCSNMe_2$



198 R= N=C=S
199 R= N=C
200 R= NHCHO



201 R= N=C=S
202 R= N=C
203 R= NHCHO

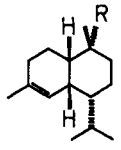
194: $C_{16}H_{25}NS$; synthetic **194**: $[\alpha]_D$ + 165.2° ($CHCl_3$); n_D 1.5415; ir (CCl_4) 2120 cm^{-1}; ^1H nmr (CCl_4) δ 5.08 (1H, bs, H-1), 3.65 (1H, bs, H-6), 2.54 (2H, m, H-3), 1.75 (3H, s, H-15), 0.95 (6H, d, J = 6, H-12 and H-13), 0.80 (3H, d, J = 6, H-14); ms, m/z 263 (M^+); *N,N*-dimethylthiourea derivative **197**; $[\alpha]_D$ − 61.9° ($CHCl_3$).

198: $C_{16}H_{25}NS$; $[\alpha]_D$ − 24.4° ($CHCl_3$); ir (CCl_4) 2100, 1640, 1375, 1365, 895 cm^{-1}; ^1H nmr ($CDCl_3$) δ 4.95 and 4.92 (1H each, m, vinyl protons), 3.46 (1H, t, J = 10.6, H-6), 1.77 (3H, bs, H-11), 1.14 (3H, d, J = 6.5, H-4), 0.86 (3H, s, H-10).

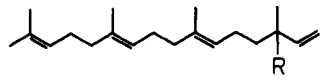
201: $C_{16}H_{25}NS$; $[\alpha]_D$ + 41.0° ($CHCl_3$), ir (CCl_4) 2100, 1640, 895 cm^{-1}, ^1H nmr ($CDCl_3$) δ 5.04 and 4.64 (1H each, m, vinyl protons), 2.33 (1H, bd, J = 11.5, H-5), 2.05 (1H, octet, J = 6.5, H-11), 1.86 (1H, m, H-7), 1.14 and 1.01 (3H each, ds, J = 6.5, H-12 and H-13), 0.80 (3H, s, H-10).

same biological material.[228] One of the isocyanides (**199**) has previously been isolated and identified from the marine sponge *Acanthella acuta*, belonging to the same family (Axenellida) as *A. cannabina*, and named acanthellin 1.[229] On reaction with elemental sulfur, acanthellin 1 (**199**) generated **198**, thus revealing the structure of the latter. Analogously, isocyanide **202** was transformed into **201** on reaction with sulfur. The structure of **202** was assigned on the basis of chemical and spectroscopic studies. Compounds **198** and **201** were recovered in 0.005% and 0.006% yield, respectively, based on dry weight after extraction.

Attempts to incorporate radioactivity into axisonitrile 1 (**183**) by feeding *A. cannabina* labelled axamide 1 (**184**) failed.[230] Although the findings are not conclusive, the concept that the isocyanides are precursors of the formamides and isothiocyanates is favored by the latter investigators.



204 R= N=C=S
205 R= N=C
206 R= NHCHO

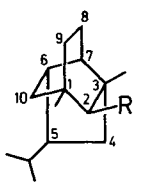


207 R= N=C=S
208 R= N=C
209 R= NHCHO
210 R= $NHCSNHC_6H_5$

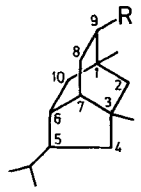
204: $C_{16}H_{25}NS$; oil; $[\alpha]_D$ − 63° (CCl_4); ir (CH_2Cl_2) 2250, 2100 cm^{-1}, uv (95% EtOH) 214 (log ε 3.26), 243 nm (2.97); ^1H nmr (100 MHz, CCl_4) δ 5.25 (bs), 1.70 (bs), 1.40 (s), 0.94 (d, J = 6), 0.88 (d, J = 6); ms, m/z 263 (M^+, 100%), 230 (M^+ − HS, 22), 203 (M^+ − HSCN, 51), 161 (M^+ − HSCN − isopropyl, 43).

207: $C_{21}H_{33}NS$; Only isolated as the *N*-phenylthiourea derivative **210**.

Degradative work and spectroscopic studies resulted in the structure elucidation of two isothiocyanates (**204** and **207**) from a marine sponge *Halichondria* sp., collected at 200 m depth. Both compounds were accompanied by the corresponding formamides (**206** and **209**) and isocyanides (**205** and **208**). One of the isothiocyanates, **204**, is derived from the 4-amorphene sesquiterpenoid skeleton,[231,232] while the other, **207**, is the isothiocyanato analogue of the diterpenoid geranyllinalool known from jasmine.[232,233] The two isothiocyanates could only be separated after treatment with aniline which left the unreactive **204** unaltered. The sesquiterpene compounds were isolated together with two sesquiterpene hydrocarbons (5%) in a ratio of 42% isocyanide (**205**), 10% isothiocyanate (**204**), and 43% formamide (**206**), based on a total yield of 2.3% of dry weight. Isothiocyanate **204** was formed from the isocyanide (**205**) and elemental sulfur.



211 R = NCS
212 R = NC
213 R = NHCHO



214 R = NC
215 R = NHCHO
216 R = $NHCSC_6H_5$

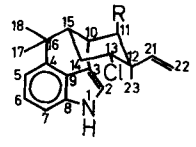
211: $C_{16}H_{25}NS$; data not published.

A marine sponge, *Hymeniacidon* sp., gave about 0.045% yield, based on wet weight, of the unusual sesquiterpene 2-isothiocyanatopupukeanane (**211**).[234] The isothiocyanate (**211**) was accompanied by the corresponding formamide (**213**) and the isocyanide (**212**). The structure of the latter was determined by an X-ray crystallographic analysis.[235] The structure of a concomitant isocyanide, 9-isocyanopupukeanane (**214**), was solved by X-ray crystallography of the corresponding phenylthiourea derivative, **216**, obtained from **214** by hydrolysis to the formamide, **215**, and lithium aluminium hydride reduction of this to the amine, followed by reaction with phenyl isothiocyanate.[236] The occurrence of the isothiocyanate corresponding to **214** has not yet been published.

The biosynthesis of the isocyano, formamido, and isothiocyanate functionalites was investigated by feeding the sponge carbon-13 labelled **211** (R = $N{=}^{13}C{=}S$), **212** (R = $N{=}^{13}C$), **213** (R = $NH{-}^{13}CHO$), or [^{13}C]formate. The results are in accordance with the findings of Sodano *et al.*[23] (*vide supra*), namely that formate is not utilized by the sponge for isocyano biosynthesis and that 2-isocyanopupukeanane (**212**) is the precursor of the formamide (**213**) and isothiocyanate (**211**) in *Hymeniacidon sp.*[234]

An interesting ecological relationship exists between this sponge and the predator, the nudibranch *Phyllida varicosa* Lamarch 1801.[19,25,235–238] The delicate, brightly colored mollusc lacking a shell feeds on the sponge and concentrates isocyanides for its own protection.

A remarkable alkaloid isothiocyanate has recently been isolated and identified from the cyanobacterium (blue-green alga) *Hapalosiphon fontinalis* (Ag.) Bornet (Stigonemataceae) strain number V-3-1.[239] The organism was isolated from a soil sample collected in the Marshall Islands by repeated subculture on solidified media. The material used for the isolation of hapalindole A (**218**) and hapalindole B (**217**) was obtained from large (25 liter) liquid cultures yielding 0.58% **218**, accompanied by smaller amounts of several



217 R= N=C=S
218 R= $\overset{19}{N}{=}\overset{20}{C}$

217: $C_{21}H_{23}ClN_2S$; oil; $[\alpha]_D^{25}$ − 194° (CH_2Cl_2), ir ($CHCl_3$) 2080, 2160 cm^{-1}, ^1H nmr (300 MHz, $CDCl_3$) δ 8.064 (br, *NH*), 7.197 (septet, J = 7.2, 0.6, H-5), 7.183 (m, J = 8.2, 7.2, H-6), 6.961 (m, J = 8.2, 0.6, H-7), 6.882 (t, J = 2.0, H-2), 6.018 (dd, J = 17.4, 10.9, H-21), 5.322 (dd, J = 10.9, 0.5, H-22 cis to H-21), 5.132 (dd, J = 17.4, 0.5, H-22 trans to H-21), 4.534 (d, J = 2.3, H-11), 4.391 (dd, J = 12.6, 3.9, H-13), 3.867 (br, m, H-10), 2.220 (ddd, J = 12.8, 4.6, 3.6, H-15), 2.149 (dtd, J = −13.3, 3.9, 3.6, 1.1, H-14 eq), 1.555 (s, H-17), 1.489 (q, J = −13.3, 12.8, 12.6, H-14 ax), 1.198 (s, H-18), 0.870 (s, H-23); ^{13}C nmr (75 MHz, $CDCl_3$) δ 143.46 (C-21), 137.78 (C-8), 133.33 (C-4), 132.54 (C-20), 123.83 (C-9), 123.39 (C-6), 118.65 (C-2), 115.74 (C-22), 113.84 (C-7), 110.65 (C-3), 108.45 (C-5), 66.91 (C-11), 63.65 (C-13), 46.04 (C-12), 45.23 (C-15), 38.05 (C-16), 37.49 (C-10), 31.89 (C-18), 31.11 (C-14), 24.31 (C-17), 19.23 (C-23); ms HR, EI, m/z 370.1243 (M^+).

related compounds, one of which was **217**. The structures were determined from extensive spectroscopic studies including two-dimensional spectra for determining the homonuclear ^1H (COSY/16) and heteronuclear ^1H–^{13}C connectivities (CSCM), and ^1H–^1H NOE experiments.

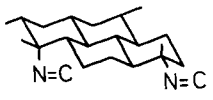
Hapalindole A (**218**) was responsible for most of the antialgal and antimycotic activity of *H. fontinalis*.

Strictly speaking **217** and **218** should not be included in this treatise at all since they are not of marine origin. However, the true biological origin of the marine metabolites has not been verified. Sponges are known in some instances to harbor a wealth of symbionts, *e.g.*, a *Verongia* sp. contains an extracellular blue-green alga (cyanobacterium) and intracellular bacteria.[240,241] These bacterial populations may grow so large that they constitute nearly 40% of the tissue volume.[242] In the case of the *Halichondria* terpenoids the depth of collection (200 m) excludes presence of photosynthetic activity associated with this sponge; however, other microorganisms may still significantly contribute to the metabolic pattern of this species. It is thought-provoking to note that in the only cases where isocyanides are encountered in nature they have been metabolites of molds, *e.g.*, the classic example of *Penicillium notatum*.[243]

Among the mold metabolites the existence of isothiocyanates has not been proven. The biosynthesis of the mold isocyanides is unknown. It is possible that the isocyano carbons in xanthocillin X dimethyl ether from *Aspergillus clavatus* may originate from glycine.[244]

Since the marine isothiocyanates seem to originate from the isocyanides it is perhaps worthwhile to note that sulfur-transferring enzymes exist (see Section 4A). Incidentally rhodanese as well as 3-mercaptopyruvate sulfurtransferase are able to transfer a sulfur atom to cyanide ion forming thiocyanate ion. The fate of the latter ion, in human saliva for example, is lactoperoxidase-catalyzed oxidation to *S*-hydroxythiocyanic acid and the corresponding anion and further oxidized derivatives.[245,246]

Other isocyanides have been reported from sponges. If the isocyanides are precursors of the isothiocyanates and formamides, the existence of isothiocyanates in these animals is possible and should be investigated. *Acanthella acuta* (Axinellida) contains several unidentified isocyanides.[229] The diterpenoid diisocyano derivative **219** comes from a sponge *Adocia* sp.,[247] and the diterpenoid diisocyanide **220** and formamide-isocyanide **221** from a Caribbean sponge *Hymeniacidon amphilecta*.[248] Recently the antimicrobial tricyclic diterpene Kalihinol A (**222**) was identified from an *Acanthella* sp., collected in

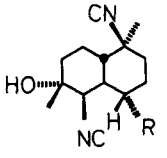


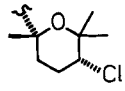
219

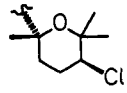
220 R= N=C

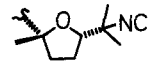
221 R= NH-CHO

Apra Harbor, Guam.[249] Later the C-14 epimer of **222**, Kalihinol E (**223**), Kalihinol F (**224**), Kalihinol B (**225**), and Kalihinol C (**226**) were identified.[250]

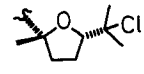


222 R= 

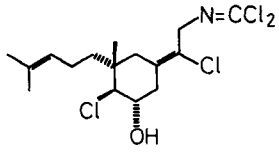
223 R= 

224 R= 

226 R= 

225 R= 

The possible biogenetic sequence of events indicated by the isopropenyl (**226**), chloroisopropyl (**225**), and isocyanoisopropyl (**224**) residues remains to be investigated.



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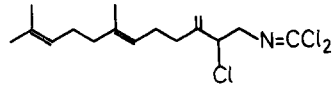

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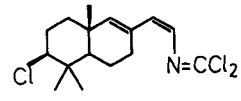

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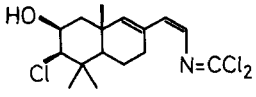

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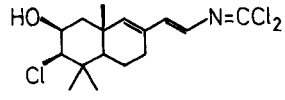

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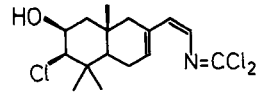


232

An interesting series of carbonimidic dichlorides (**227–232**) have been isolated from the Indo-Pacific sponge *Pseudoaxinyssa pitys*.[251–253] These compounds may conceivably be produced by a biological chlorination of an isocyanide precursor although none has ever been detected in *P. pitys*.

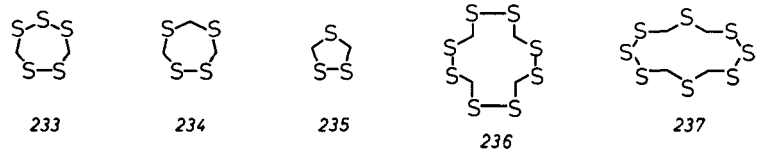
6. ALIPHATIC SULFUR COMPOUNDS

A. Introduction

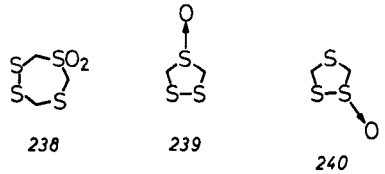
The naturally occurring marine aliphatic sulfur compounds constitute a most inhomogeneous collection of seemingly unrelated structures. They are at present few in number, but purely speculative biogenetic considerations and comparison with other naturally occurring sulfur compounds tend to leave the impression that they are only examples, often unique, of classes of compounds with potentially wide occurrence. The diversity of structures—cyclic methylene–sulfur compounds, C_{11}–sulfur derivatives, a 1,2-dithiolane, two sulfur-containing furanosesquiterpenes, a sulfur substituted polyether, and a sulfoxonium ion—are rivalled by the biological spectrum of organisms from which they have been isolated—a red alga, brown algae, a worm, two sponges, and a bryozoan, respectively. The geographical sites of collection of these specimens were Mexico, Hawaii, Japan, Australia, U.S.A., and The North Sea. The methylene–sulfur compounds exhibit antimicrobial activity, the C_{11}–sulfur compounds are intimately associated with the biosynthesis of C_{11}–hydrocarbons in some brown seaweeds and presumably hence with the sperm-attracting substances of these plants, nereistoxin is strongly insecticidal, acanthifolicin highly antineoplastic, and the sulfoxonium compound is a hapten, leaving only the furanosesquiterpenes with unknown function or activity.

B. Cyclic Methylene–Sulfur Compounds

A series of cyclic polysulfides have been identified from the red alga *Chondria californica*.[254] The compounds, responsible for antibiotic activity against *Vibrio anguillarium*, are characterized as cyclic structures where the sulfur atoms are connected through methylene groups. The specimen used for the isolation of sulfur compounds was collected at Isla San Jose in the Gulf of California from a depth of 2 m, but a sample from La Jolla seems to have the same metabolites suggesting that they occur throughout the range of the alga. Chloroform and ethyl acetate extraction gave a combined yield of 1.6% of dry weight. This extract contained 0.9% 1,2,3,5,6-pentathiepane (**233**), 0.06% of 1,2,4,6-tetrathiepane (**234**), 0.015% octasulfide **236** or **237**, 17.5% 4,4-dioxo-1,2,4,6-tetrathiepane (**238**), 1% 4-oxo-1,2,4-trithiolane (**239**), and 0.6% 1-oxotrithiolane (**240**).



233 234 235 236 237



238 239 240

233: $C_2H_4S_5$; mp 56–57 °C; ^{1}H nmr ($CDCl_3$) δ 4.33 (s, 4H).

234: $C_3H_6S_4$; mp 78–79 °C; ^{1}H nmr ($CDCl_3$) δ 4.22 (s, 2H), 4.26 (s, 4H).

236 or **237**: $C_4H_8S_8$; mp 177–178 °C; ^{1}H nmr ($CDCl_3$) δ 4.33 (s); ms, *m/z* 312 (3%), 156 (7), 142 (16), 124 (40), 110 (31), 78 (45), 64 (13), 46 (100); HR 311.8392 (M^+).

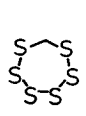
238: $C_3H_6O_2S_4$; mp 154–155 °C; ir ($CHCl_3$) 1330, 1125, 1120 cm^{-1}; ^{1}H nmr ($CDCl_3$) δ 4.18 (s, 2H), 4.43 (s, 2H), 4.56 (s, 2H); ^{13}C nmr (Me_2SO-d_6) δ 43.8, 54.1, 63.3; ms, *m/z* 202 (33%), 138 (16), 124 (8), 110 (9), 92 (20), 64 (30), 46 (100); HR 201.9257 (M^+), 109.9319 ($CH_2S_3^+$).

239: $C_2H_4OS_3$; mp 76–77 °C; ir ($CDCl_3$) 1105, 1043 cm^{-1}; ^{1}H nmr ($CDCl_3$) δ 3.97 (d, 2H, J = 12), 4.25 (d, 2H, J = 12); ms, *m/z* 140 (60%), 124 (5), 110 (42), 78 (66), 64 (12), 62 (50), 46 (96), 45 (100); HR 139.9422 (M^+).

240: $C_2H_4OS_3$; oil; CD (MeOH) $[\theta]_{258}$ − 100, $[\theta]_{300}$ + 20, $[\theta]_{341}$ − 130; ir ($CHCl_3$) 1120, 1087, 1065 cm^{-1}; v 335 (log ε 1.85), 210 nm (3.43); ^{1}H nmr ($CDCl_3$) δ 3.99 (d, 1H, J = 12), 4.40 (d, 1H, J = 10), 4.64 (d, 1H, J = 12), 4.73 (d, 1H, J = 10); ms, *m/z* 140 (44%), 124 (10), 110 (22), 94 (26), 78 (20), 60 (36), 46 (77), 45 (100), HR 139.9424 (M^+).

The melting point of **233** is lower than that reported for the synthetic material, namely 60–61 °C.[255] Combined GC–MS analysis showed the depression to be due to small amounts of two isomeric tetrathianes of unknown structure and 1,2,4-trithiolane (**235**), identified by coinjection of an authentic sample.

Lenthionine (**233**)[256] and 1,2,4,6-tetrathiepane (**234**)[255] were earlier isolated from an extract of the edible "Shiitake" mushroom *Lentinus edodes* (Berk.) Sing. After soaking of the dried material in water overnight small amounts of 1,2,3,4,5,6-hexathiepane (**241**) were also isolated. The compounds are not present as such in the dried mushroom, but are generated during treatment with water. In the original study a probable precursor, which itself was not present in the dried material, but appeared during soaking, was isolated. Based mainly on mass spectrometric evidence the tentative structure **242** was proposed.[255] Later a precursor, lentinic acid (**243**), was found.[257] The same material was also isolated from the mushrooms *Micromphale perforans* (Hofm. ex. Fr.) Sing. and *Collybia hariolum* (DC. ex Fr.) Quel. and its structure was determined as 2-(γ-glutamylamino)-4,6,8,10,10-pentaoxo-4,6,8,10-tetrathiaundecanoic acid (**243**).[258]



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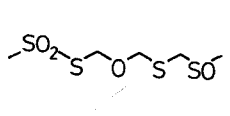

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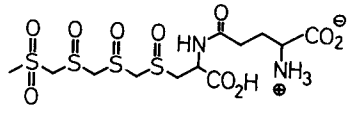

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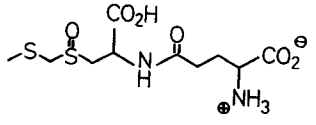
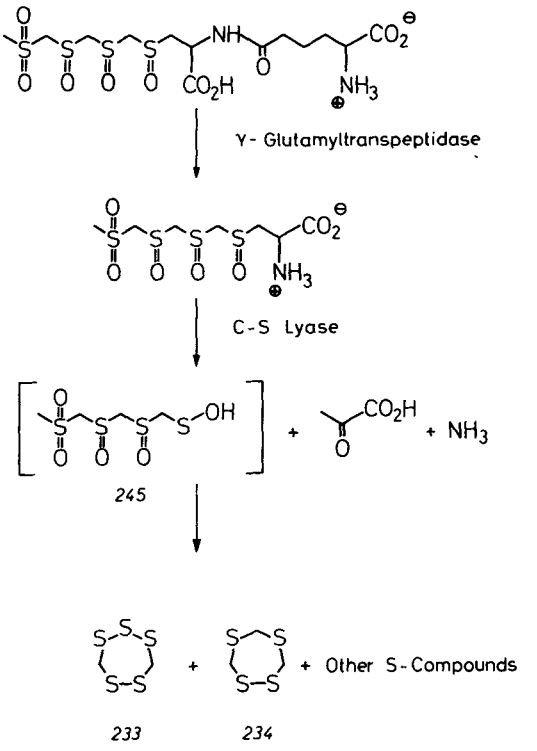


244

This precursor, together with a stereoisomer, epilentic acid, was isolated from *Micromphale foetidum* (Sow. ex. Fr.) Sing., *M. cauvetii* (Mre. u. Kühn. ex Hora), and *Collybia impudica* (Fr.) Sing. as well.[259] The related compound γ-glutamylmarasmin

(**244**) has been shown to be the precursor of the smell of the basidiomycetes *Marasmius alliaceus* (Jacq.), *M. prasiosmus* (Fr.), and *M. scorodonius* (Fr.) Quel.[260] In the case of lentinic acid and epilentinic acid the mechanism of formation of the odorous compounds was shown to involve cleavage of the peptide bond effected by the action of a γ-glutamyl-transpeptidase followed by the formation of an unstable intermediate (**245**) by the action of a C–S lyase on the cysteine sulfoxide formed (Scheme 11).



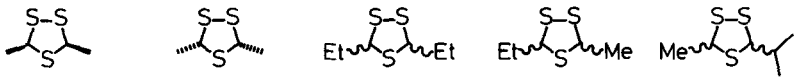
SCHEME 11 Proposed biogenesis of lentionine and 1,2,4,6-tetrathiepane.

The sulfenic acid (**245**) spontaneously gives rise to the flavor compounds lenthionine (**233**) and 1,2,4,6-tetrathiepane (**234**) and additional unidentified sulfur compounds.[259]

The odorous materials, especially lenthionine (**233**), show antibiotic activity against a number of microorganisms, including bacteria and fungi.[255]

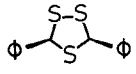
In spite of claims to the contrary[261] 1,2,4-trithiolane (**235**) does not seem to have been detected in the extract of *L. edodes*, but was isolated in the synthesis of **234** from methylene chloride and $Na_2S_{2.5}$ at pH 12.[255] This simple compound seems to be present in or formed during processing of a number of biological materials. It has been detected in head space fractions from North Sea fish oils,[262] as excretion product of axenically grown *Ochromonas danica*,[263] as an egg aroma volatile,[264] and from the roots of *Acacia pulchella* R. Br.[265]

In the light of these findings it is tempting to propose that 1,2,4-trithiolane (**235**) is an artifact formed from formaldehyde equivalents and some sulfur compounds. If this



246 247 248 249 250

hypothesis is correct we would expect also to find 3,5-disubstituted 1,2,4-trithiolanes in natural sources. Furthermore we would expect the derivatives to occur as optically inactive mixtures of stereoisomers. The diastereoisomeric 3,5-dimethyl-1,2,4-trithiolanes (**246** and **247**) have been identified in the mushroom *Boletus edulis*,[266] in dry red beans,[267] in potato oil flavor,[268] in pork flavor,[269] in roasted filberts,[270] in beef food flavor,[271-274] in cheese,[275] in hen manure,[276] and in several other materials. The optically inactive mixture of stereoisomers of 3,5-diethyl-1,2,4-trithiolane (**248**) was obtained by ether extraction of the steam distillate of *Allium cepa*,[277] *Allium grayi*, and *A. bakeri*;[278] *A. genus*, together with 3-methyl-5-ethyl-1,2,4-trithiolane (**249**);[279] *A. fistolosum* Linn. var. *caespitosum* Makino;[280] and from boiled Antarctic krill, again in mixture with **249**.[281] The occurrence of **249** in admixture with **250** has also been reported.[282] A study of the sensory properties of *cis–trans* isomers of 3,5-dialkyl-1,2,4-trithiolanes has appeared.[283] *Petiveria alliacea* gave rise to the isolation of trithiolaniacin (**251**).[284] The chloroform extract of the wet root also contained sulfur, *trans*-stilbene, benzaldehyde, and benzoic acid. The *cis* configuration was proposed due to lack of optical activity and the occurrence of an intense band at 725 cm^{-1}. Supposedly the analytical data refer to a sublimed sample and therefore contain no information on other stereoisomers in the extract.



251

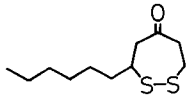
The octasulfide isolated from *C. californica* was assigned structure **236** in preference to structure **237** due to lack of the S_3^+ fragment (m/z 96) in the mass spectrum. Structure **238** was chosen in favor of the thiolsulfonate structure on the basis of the identification of the m/z 110 peak as the $CH_2S_3^+$ fragment which might result from simple cleavage of the sulfone **238**. Furthermore, no reaction could be demonstrated with thiols as would be expected for a thiolsulfonate.

The structures **239** and **240** were inferred from spectroscopic data. In the case of **240** reaction with triphenylphosphine smoothly produced **235** in quantitative yield whereas **239** under identical conditions gave less than 10% of **235**. A mixture of **239** (27% yield) and **240** (26% yield) was synthesized by sodium periodate oxidation of **235**. The absolute configuration of naturally occurring **240** is still unknown.

Interestingly enough *C. californica* is the only marine organism where this type of sulfur compound has been recorded. Even if the materials isolated should eventually turn out to be artifacts, one would expect to encounter the conditions necessary for their formation in other marine organisms, too. A variety of, *e.g.*, red algae have been investigated with respect to their brominated terpenoid metabolites without any recorded indication of sulfur compounds. Future research will hopefully shed more light on this intriguing problem.

C. C_{11}–*Sulfur Compounds*

Certain members of the brown algae genus *Dictyopteris* contain, in addition to C_{11} hydrocarbons, C_{11} organic sulfur compounds.[285] Thus, *Dictyopteris plagiogramma* yielded a chloroform-methanol extract containing (−)-3-hexyl-4,5-dithiacycloheptanone (**252**), identified spectroscopically.[286]



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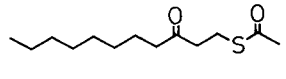

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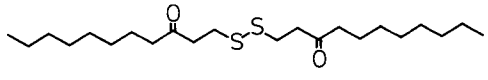


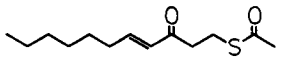
254

252: $C_{11}H_{20}OS_2$; oil; $[\alpha]_D$ − 65° (CCl_4); ir 1712 cm^{-1}; ^1H nmr (C_6D_6) δ 2.70 (1H, m), 1.0–1.4 (10H, m), ~2.45 (2H), 2.37 (4H, s), 0.86 (3H, t); ms, *m/z* 232 (M^+), 167, 120.

253: $C_{13}H_{24}O_2S$; pale yellow oil, bp 80–85° (5 × 10^{-4} mm Hg); ir (neat) 1690, 1715 cm^{-1}; ^1H nmr ($CDCl_3$) δ 3.05 (t, J = 7, 2H), 2.75 (t, J = 7, 2H), 2.38 (t, J = 7, 2H), 2.31 (s, 3H), 1.56 (quintet, J = 7 Hz, 2H), 1.28 (br, 10H), 0.88 (t, J = 7, 3H); ms, *m/z* 201, 169, 146, 141, 43 (100%).

254: $C_{22}H_{42}O_2S_2$; mp 67–67.5 °C; ir 1705 cm^{-1}; ^1H nmr (C_6D_6) δ 2.75 (t, J = 7, 4H), 2.40 (t, J = 7, 4H), 2.00 (t, J = 7, 4H), 1.48 (quintet, J = 7, 4H), 1.21 (br, 20H), 0.91 (t, J = 7, 6H); ms, *m/z* 402 (M^+), 304, 201, 200, 169, 141 (100%).

The same extract also yielded *S*-(3-oxoundecyl) thioacetate (**253**) and bis-(3-oxoundecyl) disulfide (**254**).[286] Acid hydrolysis of **253** gave 1-mercaptoundecan-3-one whereas basic hydrolysis produced bis-(3-oxoundecyl) sulfide. Air oxidation of 1-mercaptoundecan-3-one gave, as expected, **254**, which in turn could be converted to **253** by reduction and acetylation (Zn, HOAc, Ac_2O). A small amount of *S*-(*trans*-3-oxoundec-4-enyl) thioacetate (**255**) was also obtained from the extract. All fractions were contaminated with an unstable oil, undec-1-en-3-one (**256**), believed to be an artifact since the essential oil itself does not seem to contain this compound. Addition of thioacetic acid to **256** gave **253**.



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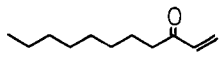
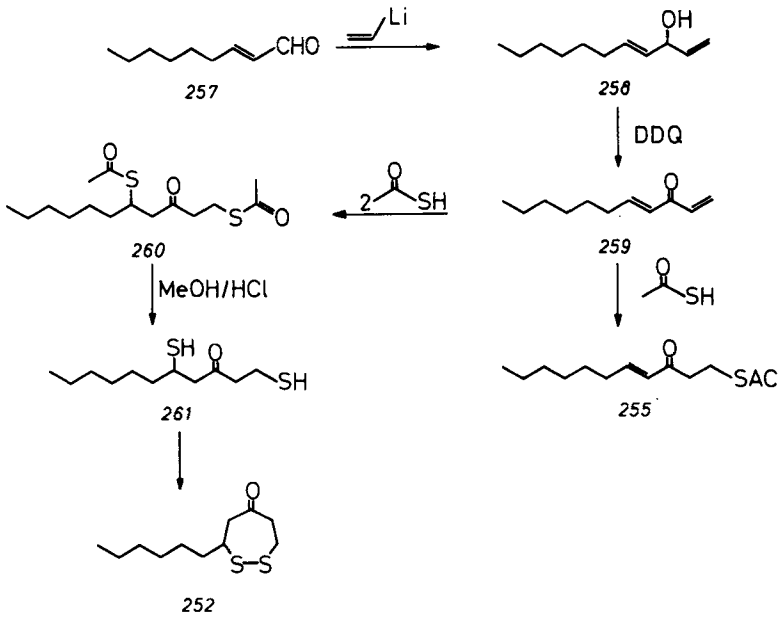


256

255: $C_{13}H_{22}O_2S$; oil; ^1H nmr (CCl_4) δ 5.98 (dt, J = 1.5, 16, H-4), 6.75 (dt, J = 7, 16, H-5), 2.26 (br, q, H-6), 2.85 (t, H-2), 3.11 (t, H-1), 2.31 (s, CH_3CO); ms, *m/z* 242 (M^+), 199, 167, 139.

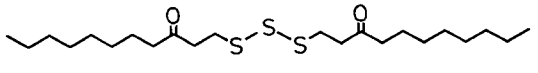


SCHEME 12 Synthesis of 3-hexyl-4,5-dithiacycloheptanone and *S*-(*trans*-3-oxoundec-4-enyl) thioacetate.

A synthetic route to **252** (Scheme 12)[287] starts out with a reaction between vinyllithium and 2-nonenal (**257**), yielding 1,4-undecadien-3-ol (**258**) which on DDQ oxidation gave the highly reactive 1,4-undecadien-3-one (**259**).

Reaction between **259** and two equivalents of thiolacetic acid produced 1,5-bis-acetylthio-3-undecanone (**260**) which on transesterification with methanol gave 1,5-dimercapto-3-undecanone (**261**). The racemic keto disulfide **252** was obtained by iodine oxidation in 66–70% yield. Reaction of **259** with one equivalent of thiolacetic acid smoothly gave **255**, identical in all respects with the naturally occurring compound.

The Hawaiian algae *Dictyopteris plagiogramma* and *D. australis* by extraction as described above gave rise to two polysulfides closely related to **254**, namely bis-(3-oxoundecyl) trisulfide (**262**) and bis-(3-oxoundecyl) tetrasulfide (**263**). The structures were determined by spectroscopic methods.[288] A solution of **263** in methanol generates **262** and sulfur. Photolysis of **262** in pentane in turn generates **254** as the major product. Inversely, a mixture of **262** and **263** could be synthesized by the triethylamine catalyzed reaction of **254** and sulfur in refluxing benzene.



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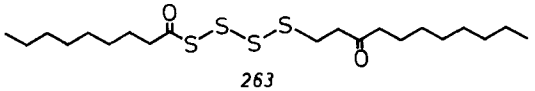
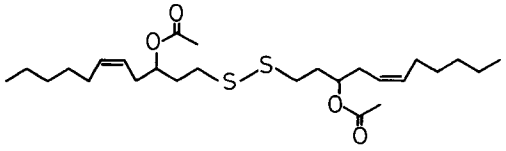


263

262: $C_{22}H_{42}O_2S_3$; mp 60.5–61 °C, uv 200 (log ε 3.76) 250 nm (br sh, 3.26); ^{1}H nmr (100 MHz, CCl_4) δ 3.02 (t, J = 7, H-2), 2.83 (t, J = 7, H-2), 2.37 (H-4), 1.55 (H-5), 0.89 (H-11); ms, m/z 434 (M^+), 402, 304, 201, 200, 169, 141 (100%).

263: $C_{22}H_{42}O_2S_4$; mp 32–33 °C; ^{1}H nmr (100 MHz, CCl_4) δ 3.10 (H-1), 2.85 (H-2), 2.38 (H-4), 1.56 (H-5), 0.89 (H-11); ms, m/z 466 (M^+).

The same material gave minor amounts of (−)-bis(3-acetoxyundec-5-enyl) disulfide (**264**) and *S*-(−)-3-acetoxyundec-5-enyl thiolacetate (**265**), characterized spectroscopically.[289] To separate **254** and **264**, the mixture was reductively acetylated (Zn, HOAc, and Ac_2O), generating **256** and **265**. After separation **264** was regenerated by treatment with neutral alumina in hexane. The *cis* configuration of the double bond of **265** was inferred from the coupling constant of a doublet of triplets at 5.31 ppm, which on irradiation at 2.35 ppm (C-4 methylene protons) collapsed to a doublet with J = 11 Hz.



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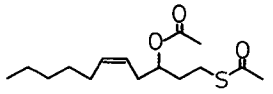


265

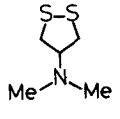
264: $C_{26}H_{46}O_4S_2$; low-melting, white solid, negative optical rotation; ^{1}H nmr, δ 2.64 (H-1), 2.0 (H-2, H-7 and CH_3CO), 4.89 (H-3), 2.28 (H-4), 5.31 (H-5), 5.50 (H-6), 1.3 (H-8, H-9, H-10), 0.90 (H-11).

265: $C_{15}H_{26}O_3S$; liquid; $[\alpha]_D$ − 25 ± 10 °C (CCl_4); ^{1}H nmr (CCl_4) δ 2.30 (CH_3COS and H-4), 2.0 (CH_3CO and H-7), 2.84 (H-1), 1.76 (H-2), 4.84 (H-3), 5.31 (H-5), 5.50 (H-6), 1.3 (H-8, H-9, H-10), 0.90 (H-11).

1-Undecen-3-ols have been suggested as precursors of the sulfur compounds.[285,290] These precursors have not as yet been detected in *Dictyopteris* or other seaweeds. The bis-(3-oxoundecyl) polysulfides may originate by reaction of elemental sulfur or dihydrogen disulfide with **254** or 3-oxoundecanethiol and may serve as precursors of 3-hexyl-4,5-dithiacycloheptanone (**252**).[288] The sulfur compounds may in turn generate a series of unsaturated C_{11}–hydrocarbons characteristic of these *Dictyopteris* spp.[289]

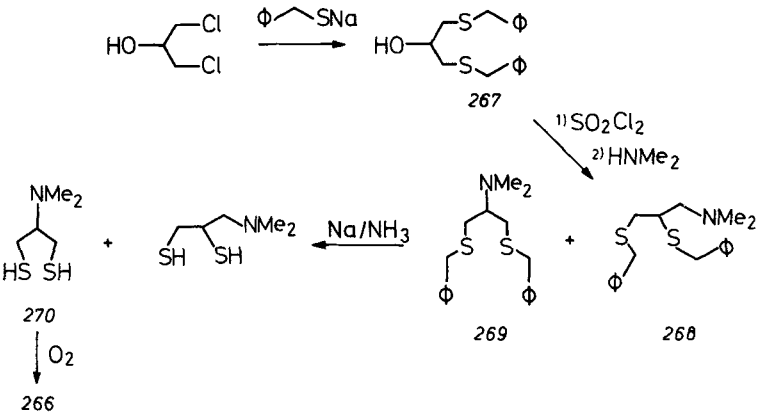
D. Nereistoxin

The 1,2-dithiolane ring system has been encountered several times in natural products. The only marine representative known so far, nereistoxin (**266**), originates from the marine annelids *Lumbriconereis heteropoda* and *Lumbrinereis brevicirra*.



266

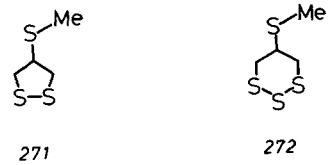
Nereistoxin (**266**) was first isolated by Nitta from *L. heteropoda*.[291] Reisolation of the toxin from *L. brevicirra* by Hashimoto and Okaichi[292] led to the assignment of structure **266**, which was later confirmed by synthesis[293] (Scheme 13). 1,3-Dichloro-2-propanol on reaction with benzylthiolate yielded 1,3-bis(benzylthio)-2-propanol (**267**) which, after treatment with thionyl chloride and subsequently dimethylamine, yielded a mixture of **268** and **269**. The thiol groups were liberated by reduction. Oxidation of **270**, during the work-up procedure generated nereistoxin (**266**). Other synthetic approaches have been described.[294,295]



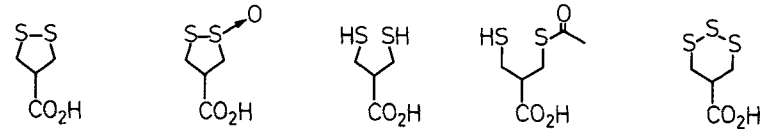
SCHEME 13 Synthesis of nereistoxin.

The pharmacological activities of nereistoxin have been studied extensively. The original interest in the annelid was awakened by the observation of a patient complaining of respiratory abnormality, headache, and vomiting after having handled the worms.[291] Another observation, namely that flies would die on contact with the worms started the research which culminated with the marketing of a derivative of nereistoxin as a new insecticide in 1967. For a detailed discussion of the pharmacology, the reader is referred to the extensive review by Hashimoto[6] and Ref. 296.

As mentioned above the 1,2-dithiolane ring is not unknown in other areas of natural products research. 4-Methylthio-1,2-dithiolane (**271**) was isolated and identified co-occurring with 5-methylthio-1,2,3-trithiane (**272**) in the freshwater green alga *Chara globularis* Thuillier 1799 (syn. *Chara fragilis* Gesvaux in Loiseleiur-Deslongchamps 1810).[297] Synthesis of these compounds met with difficulties as in the case of nereistoxin, but was accomplished eventually.[298] The trithiane **272** was effective in inhibiting photosynthesis while **271** was an effective insecticide.[299] Derivatives of **271** were later assayed as potential insecticides with good results.[300] Neurophysiological studies of these compounds have been described.[301]

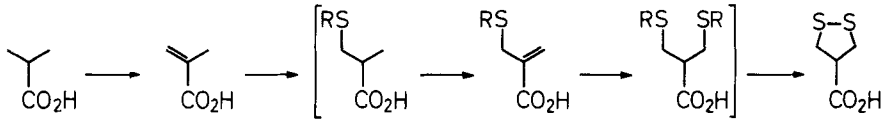


Another series of 1,2-dithiolanes was obtained from etiolated young *Asparagus officinalis* L. Asparagusic acid (**273**), the two sulfoxides (*syn*-**274** and *anti*-**274**), dihydroasparagusic acid (**275**), and *S*-acetyldihydroasparagusic acid (**276**); all inhibit growth in



273 274 275 276 277

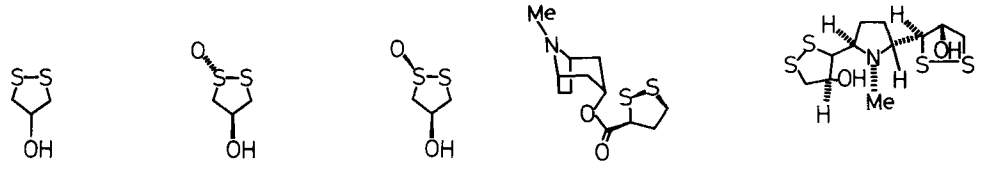
several test plants.[302,303] The 1,2,3-trithiane **277** was also found in the same material.[304] The biosynthesis of asparagusic acid has been investigated.[305] The results are in accordance with a biogenetic pathway where dehydrogenation of isobutyric acid, or isobutyryl-coenzyme A, to methacrylic acid is followed by a Michael-type addition of a sulfur



273

nucleophile. An analogous series of reactions could then create the intermediate which would ultimately yield asparagusic acid. The arguments leading to these conclusions have been discussed in detail.[306]

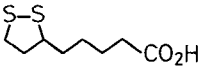
One other family of higher plants, the Rhizophoracea, have yielded 1,2-dithiolane derivatives.[307] *Bruguiera cylindrica* gave 4-hydroxy-1,2-dithiolane (**278**),[308] brugierol (**279**),[309] isobrugierol (**280**),[309] and brugine (**281**).[310,311] Brugierol and isobrugierol are



278 279 280 281 282

synthetically available.[312,313] Brugine (**281**)[314] and gerrardine (**282**)[315,316] were isolated from *Bruguiera sexangla* and *Cassipourea gerrardii*, respectively.

A vast body of literature exists on lipoic acid (**283**).[317] The lipoic acid-containing enzymes function by reversibility opening the dithiolane ring, *e.g.*, in the α-keto acid

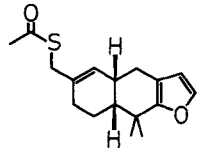


283

dehydrogenase complexes.[318] The disulfide is reductively acetylated in the latter enzyme complex and the position of the acyl group has been investigated.[319]

E. Thiofurodysin Acetate and Thiofurodysinin Acetate

Considering the plethora of novel terpenes that have originated from marine natural product research it is curious that only two sulfur-containing sesquiterpenes have been reported. They both originate from a sponge *Dysidea* sp. collected near Cronulla, New South Wales, Australia.[320] Thiofurodysin acetate (**284**) and thiofurodysinin acetate (**285**) were obtained in yields of 0.1% and 2%, respectively, based on dry weight. The structure determinations were carried out by chemical and spectroscopic methods and by comparison with the corresponding furanoterpenes which were also present in the sponge and were subjected to X-ray crystallographic analysis. The ratio of rearranged thiol acetate (**285**) to the regular terpenoid thiol acetate was 20 : 1 while the corresponding furanosesquiterpenes had a ratio of 3 : 1.



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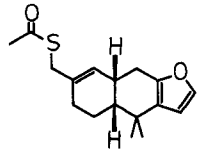
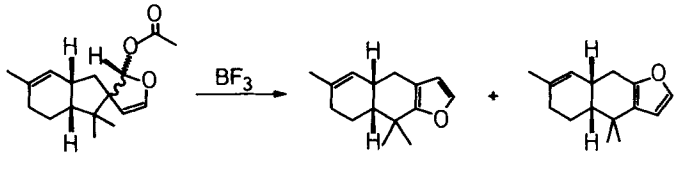


285

284: $C_{17}H_{22}O_2S$; oil; ^{1}H nmr (CCl_4) δ 6.98 (1H, d, J = 1.5), 5.86 (1H, d, J = 1.5), 5.77 (1H, bd, J = 6), 3.43 (2H, s), 2.70 (1H, m), 2.50 (1H, dd, J = 16, 6), 2.23 (3H, s), 1.22 (6H, s).

285: $C_{17}H_{22}O_2S$; oil; $[\alpha]_D$ + 52° ($CHCl_3$); ir 1695 cm^{-1}; ^{1}H nmr (CCl_4) δ 6.98 (1H, bs), 6.01 (1H, d, J = 1.5), 5.78 (1H, bd, J = 6), 3.39 (2H, s), 2.66 (1H, dd, J = 17.6), 2.60 (1H, m), 2.21 (3H, s), 1.13 (6H, s); ^{13}C nmr ($CDCl_3$) δ 195.4 (s), 146.9 (s), 140.6 (d), 132.8 (s), 130.0 (d), 124.7 (s), 108.2 (d), 44.3 (d), 35.7 (t), 32.9 (2c), 31.3, 30.5, 29.0, 27.3, 26.2, 19.1 (t); ms, m/z 275, 215, 199, 122.

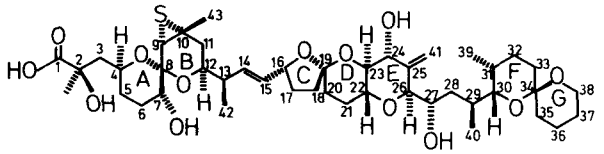
The rearranged terpenoid skeleton of (**285**) may arise by rearrangement of a precursor such as **286**, where alternative migration of the appropriate bond would produce the respective sesquiterpenes. It was found that BF_3-catalyzed rearrangement of **286**, isolated from the sponge, gave an equimolar mixture of the sesquiterpenes. Furanoid sesquiterpenes have been isolated from *Dysidea pullescens*, *Pleraplysilla spinifera*, and *Microciona toxistyla*.



286

F. Acanthifolicin

The highly cytotoxic polyether antibiotic, acanthifolicin (**287**), was isolated from the sponge *Pandaros acanthifolium*.[321] The structure was confirmed by X-ray structure



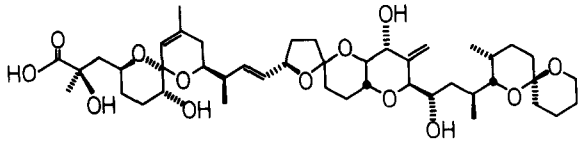
2R, 4S, 7R, 8S, 9S, 10R, 12S, 13R, 16R, 19S, 22R, 23S, 24R, 26S, 27S, 29S, 30S, 31R, 34S

287

287: $C_{44}H_{68}O_{13}S$; mp 167–169 °C; $[\alpha]_D$ + 25.3° ($CHCl_3$); ir 3400 (br), 1720 (br), 1080 (br) cm^{-1}; ^1H nmr (360 MHz, $CDCl_3$), δ 0.93 (d, J = 6.75), 0.97 (d, J = 6.75, H-42), 1.05 (d, J = 6.75), 1.38 (s, H-44), 1.72 (s, H-43), 3.24 (dd, J = 14.6, 2.25, H-11_{eq}), 3.17 (s, H-9), 3.28 (dd, J = 11.25, 2.25, H-7), 3.33 (br t, J = 9, H-12), 3.36 (t, J = 9, H-23), 3.48–3.70 (m, 3H), 3.94 (d, J = 9, H-26), 4.04 (m, 1H), 4.12 (d, J = 9, H-24), 4.55 (q, J = 6.75, H-16), 5.06 (br s, H-41), 5.39 (dd, J = 14.6, 8.55, H-15), 5.45 (br s, H-41), 5.60 (dd, J = 14.6, 9, H-14), 0.8–2.25 complex overlapping multiplets.

determination. Acanthifolicin (**287**) is unique in being the first polyether antibiotic not isolated from a bacterium. Also the episulfide function is unprecedented among polyether antibiotics as is the long backbone (C_{38}). One end of the molecule assumes a cyclic conformation forming a cavity with a diameter of 5–7 Å and kept in place by an intramolecular hydrogen bond between the carboxyl group and the hydroxyl group of ring E. Ten oxygen atoms cluster around this cavity with seven oxygen atoms within 4.0 Å from its central point suitable for complexation with a cation.

As microorganisms associated with the sponge are thought to be responsible for the synthesis of acanthifolicin, work is in progress to isolate such microorganisms. Acanthifolicin exhibits ED_{50}'s of 2.8×10^{-4}, 2.1×10^{-3}, and 3.9×10^{-3} μg/mL against cell cultures of lymphocytic leukemia (P388), human carcinoma of the nasopharynx (KB), and lymphoid leukemia (L1210), respectively. As active materials usually are defined as materials displaying $ED_{50} \leqq 20$ μg/mL the reported activity is very promising.

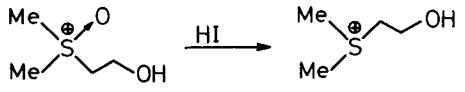


288

A related metabolite, okadaic acid (**288**), has been isolated from two sponges *Halichondria* (syn *Reniera*) *okadai* Kadota and *Halichondria melanodocia* but also from the dinoflagellate *Procentrum lima*.[322] Acanthifolicin is the episulfide of okadaic acid.

G. Dogger Bank Itch Hapten

Dogger Bank itch is the popular name of an eczematous allergic contact dermatitis provoked by repeated exposure to the marine bryozoan *Alcyonidium gelatinosum* (L.). Sensitized individuals frequently develop hypersensitivity. At that stage this severe occupational disease is totally disabling.[323–325]



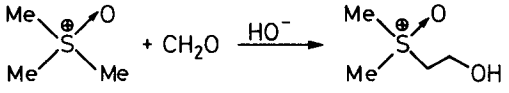
289 290

289: $C_4H_{11}ClO_2S$; ^1H nmr (270 MHz, D_2O) δ 3.9 (6H, br s), 4.3 (4H, br s); ^{13}C nmr (67.889 MHz, D_2O) δ 42.3, 56.0, 57.5; ms (HR–EI, 200 °C), *m/z* 78.0145 (C_2H_6OS), 62.9951 (MeOS), metastable at 50. 88 (78 → 63); ms (FD, 80 °C ion source, *m/z* 108 (25%), 90 (25), 78 (100); *m/s* (EI, 200 °C) mz 90 (3%), 78 (88), 63 (100), 61 (17), 50 (95)/52 (30). Collision induced decomposition (CID, He, p = 10^{-4} mm Hg) of *m/z* 90 produced a fragmentation pattern identical to the one observed for methyl vinyl sulfoxide.

The causative agent, present in the animal in about 5 ppm based on wet weight, was identified by extensive spectroscopic studies as (2-hydroxyethyl)dimethylsulfoxonium ion (**289**).[326]

On hydrogen iodide reduction the well known sulfocholine (**290**) was produced.

Compound **289** is the first example of a naturally occurring sulfoxonium ion, a class of compounds known only from a few synthetic studies. The synthesis of **289** was eventually accomplished by carefully controlled base-catalyzed addition of formaldehyde to trimethylsulfoxonium chloride.[326]



289

Because of the low molecular weight of **289** the compound is technically a hapten, which must react with some high-molecular weight compound, presumably a protein, from the skin or serum in order to produce the holoallergen.

Access to the pure hapten has removed the uncertainty in the determination of the diagnosis in affected subjects and furthermore has allowed the type of allergy to be identified as a type 4 reaction *viz.* delayed, cell-mediated hypersensitivity.[325]

Bryozoan metabolites, including **289**, have been reviewed.[327]

7. FUTURE PERSPECTIVES

At present research in the area of sulfur-containing marine natural products is faced with challenging fundamental problems. Evidently isolation and structure elucidation of the occasionally encountered sulfur compounds will still add to the basic knowledge in this area; however, a much more disciplined and dedicated effort is called for in order to establish a more comprehensive basis.

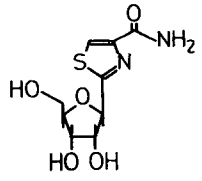
Although the global biogeochemical sulfur cycle is understood in principle with regard to the main fluxes betwen the most important reservoirs many details remain to be learned both with respect to quantity and quality.[328] Just to mention one example: sulfur is presumably added to the oceans at twice the pre-industrial rate and anthropogenic

sulfur may exceed natural emissions in the atmosphere by a factor of two or three.[328] On the other hand estimates of the magnitude of biogenic sulfur emissions are in the range $\sim 70\%$ of the total atmospheric burden.[329] Even the chemical nature of these emissions has not been clearly demonstrated, but, *e.g.*, carbon disulfide and carbonyl sulfide may contribute $\leqq 19\%$ to the sulfate aerosol burden of the stratosphere, but $\leqq 0.2\%$ only to the tropospheric sulfur burden.[329] Clearly such fundamental questions need to be studied much more intensively since they are connected with the immediate need of protection and managing of our global ecosystem (*e.g.*, "acid rain", etc.). Much of the surplus atmospheric sulfur is a result of the rather uninhibited use of fossil fuel. However, the origin of the sulfur content in coal and oil is still a matter of debate. Some must be accounted for by the remnants of sulfur metabolites of the organisms originally forming the coal or oil. However, through geological time these metabolites have been transformed to such an extent that it is now impossible to trace their origin. In coal, organic sulfur can represent more than 50% of the total sulfur and it has been shown that elemental sulfur is actually incorporated in the organic compounds even at the peat-forming stage.[330] Also hydrogen sulfide is easily incorporated into peat as demonstrated with modern progenitors of coal from the Okefenokee Swamp and $H_2{}^{35}S$.[331] The main target for the hydrogen sulfide is the humic acid fraction, even if this fraction represents less than 20% of the total organic matter in peat. Our knowledge of the detailed chemical structure of the humic acids is still far too limited to allow valid deductions about the extremely complex organosulfur compounds formed in these reactions. Clearly this lack of reliable data impedes the progress of any description of the fate of the organic sulfur in the coal-forming sequence from peat to lignite to bituminous coal to the stage of anthracite coal. It is of crucial importance that these problems be studied not only from an academic point of view, but also in recognition of the fact that peat may contain as much as 10% total sulfur (*e.g.*, the marine-derived mangrove peats in the Florida Everglades) with 30–50% organically bound sulfur. These figures represent an accumulation of sulfur sometimes an order of magnitude higher than that found in associated plants. Incidentally the organic sulfur component is the most difficult to remove to free coal of this serious contaminant.

Turning now to oil and gas deposits, the picture is as complex as the one encountered in the discussion of coal.[332,333] When the kerogen (insoluble material) is formed from sediments a complicated set of reactions transforms the organic content. During diagenesis, catagenesis and eventually metagenesis the organic matter is steadily rearranged and broken down into smaller aggregates thereby destroying more and more effectively the biological markers originally present. An additional complication in the analysis of oil deposits is related to the fact that the crude oil in question has always undergone a primary and secondary migration. During the migrations a chromatographic effect is operative resulting in the retention of polar constituents, *in casu* sulfur-containing molecules. This effect means that the sulfur content of a certain crude oil does not necessarily reflect the sulfur content of the parent kerogen. Reduced, nonpolar molecules have been used as biological markers. Future research will have to show if comparable sulfur compounds can be found and traced to the metabolites from which they originate. This approach has the added advantage of making information available about the migration process. That there is, at least, a theoretical possibility is evidenced by the identification of thiophenes in precambrian kerogens.[334] It is noteworthy that a

homologous series of bicyclic and tetracyclic terpenoid sulfides and sulfoxides have been identified in the Athabaska bitumen.[335] Whether the sulfoxide function was biologically introduced or not was undetermined. Owing to economical considerations, especially the enormous cost of deep-sea drilling, it is presumably safe to predict that the area of source rock analysis and correlation will experience a spectacular evolution in the near future, in spite of the inherent difficulties in handling the minute amounts of extraordinarily complex mixtures encountered. An intensified research effort within source rock investigations is bound to include also the sulfur compounds and to greatly expand our knowledge of the structure of kerogen,[336] the quantitatively most important form of organic carbon on earth.

There is at present no indication that research on aquatic sulfur-containing natural products should expand as such. However, as evidenced by this review the sulfur-containing metabolites obtained so far have a remarkably high ratio of biologically active representatives. Undoubtedly, this phenomenon to some extent reflects the use of bioassays to guide the isolation procedure. Nonetheless, the fact remains that many sulfur compounds must have far-reaching ecological implications. In contrast to pure natural product research, the latter areas: biological activity and ecological studies are areas with an accelerating research commitment. This is, of course, a consequence of the increasing need for new lead structures in drug research and is part of the effort of conscientious environmental management, including the search for new agricultural chemicals. As these studies progress the results will inevitably stimulate much needed research in biochemistry and biosynthesis. For example, the biochemical basis of the pronounced antineoplastic activity of some of the macrocyclic thiazoles of Section 4D is totally unexplored. Other synthetically available antitumor thiazoles are known, *e.g.*, thiazofurin (**291**)[337,338] with *in vitro* activity against both RNA and DNA viruses (type 1 *Herpes simplex* virus, type 3 parainfluenza virus and type 13 rhinovirus) and with inhibiting effect on guanine biosynthesis. *In vivo*, **291** was active in the mouse leukemias P388 (prolongs mean survival time, T/C 245%) and L1210 (T/C 230%) and was found essentially curative for the Lewis lung carcinoma in mice.



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As mentioned several times before one major obstacle remains to be removed before any deeper understanding of the ecological role of these metabolites can be acquired. This is the question of the true origin of the agents. There is ample circumstantial evidence that many marine natural products are actually microbial metabolites, but at present no conclusive evidence exists to settle the question. The impetus to solve this difficult problem may well come from medicinal chemical research once it is realized that microorganisms may be responsible for the production of economically attractive compounds. Very few higher marine organisms could be harvested in reliable amounts

for an industrial bulk production. Furthermore, many compounds are of a complexity which prevents a synthetic approach. On the other hand the technology of large-scale cultivation of microorganisms is so advanced that a sufficiently urgent and lasting demand for any microbial metabolite could presumably be met in an industrial production.

Several minor areas of marine natural product chemistry may conceivably render new information on sulfur-containing compounds. One is an investigation of the communities associated with the hydrothermal vents (Section 2E) of which new examples are being discovered.[339] Chemical investigations of the fossils described from fossil hydrothermal vents (350 Myr old)[340] might be rewarding. An area where chemical investigations are long overdue are the organisms associated with locations of low oxygen tensions. Perhaps the initially most yielding organisms would be the cyanobacteria (blue–green algae) capable of carrying out facultative anoxygenic photosynthesis.[341]

Finally we wish to draw attention to the fact that although naturally occurring selenium compounds have been known for a long time[342] only selenoamino acids and selenoproteins have been described.[343] It is thought-provoking that investigations of the unicellular red alga, *Porphyridium cruentum*, cultivated in sublethal concentrations of selenite, revealed that 80% of the cellular selenium content was concentrated in the lipids.[344] This observation could well signal the initiation of the study of a more extensive selenium biochemistry.

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