STUDIES IN NATURAL PRODUCTS CHEMISTRY

Vol. 7
PT. 2

Atta-ur-Rahman (Ed.), *Studies in Natural Products Chemistry, Vol. 7*

Toxins of Echinoderms

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1. INTRODUCTION

Echinoderms are marine invertebrates possessing a characteristic, usually fivefold symmetry. Their skeleton consists of calcium carbonate lamina. The skin is firm and cast with spines, thorns or pedicellariae. The phylum Echinodermata comprises about 5,300 species distributed in all seas from the tropics to the arctic zones. It is divided into two subphyla:

1. Pelmatozoa
2. Eleutherozoa

Pelmatozoa (sealilies) have scarcely been investigated so far. The Eleutherozoa are subdivided into four classes:

1. Holothuroidea (sea cucumbers)
2. Echinoidea (sea urchins)
3. Asteroidea (starfishes)
4. Ophiuroidea (brittle stars)

All echinoderms have developed toxic substances in various parts of their body, which may be used either as protectives against predators or as active toxins for catching prey. It can, however, not be completely excluded that some of these compounds may have additional functions within the animals. Remarkable is the antibiotic activity of saponins (1, 2). Since the early papers of Friess et al. (3) or Hashimoto and Yasumoto (4) many papers have been published. Reviews considering various aspects have been published from time to time (5-17). In this review, therefore, we report on results of the last years only.

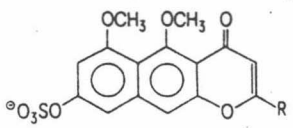
The characteristic chemical feature is that these toxins are sulfate esters or glycosides, the aglycones being sterols or triterpenoids. In the earlier works the main interest was put on those genins obtained by acid hydrolysis. Under these conditions some of the genins underwent chemical reactions so that the published structures represent artifacts rather than the original structures.

2. PELMATOZOA

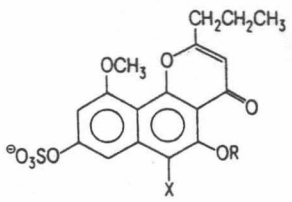
2.1 Crinoidea

Among the Pelmatozoa the brightly colored Crinoidea only have been investigated so far. They are distributed in the tropical and subtropical seas especially in the Indo-West-Pacific and Caribbean coral reefs. Some species which have been studied recently are Comantheria perplexa (18, 19, 20), C. briareus (20), Comanthus parvicirrus (18, 19, 20, 21), Comatula pectinata (20, 22), and Lamprometra palmata gyges (20, 23), resulting in the isolation of polyketides as well as sulfate esters of these compounds. Rideout et al. (20, 22) found that the polyketide sulfates of C. pectinata and C. perplexa tested at concentrations of 2% substance in jelly, were highly effective in reducing the palatability of otherwise attractive food to 5 marine fish species. Some polyketide sulfates are shown in figure 1.

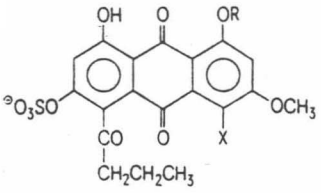
Elyakov et al. (24) succeeded in isolating sterol sulfates from Himerometra robustipinna. These compounds have also been found in all other classes of the phylum Echinodermata.



R = CH_3
or: R = CH_2-CH_2-CH_3



R = X = H
or: R = H, X = O-CH_3
or: R = CH_3, X = O-CH_3



R = X = H
or: R = CH_3, X = H
or: R = H, X = OH

Fig. 1. Some polyketide sulfates isolated from Crinoidea.

3. ELEUTHEROZOA

3.1 Holothuroidea

The holothurians or sea cucumbers possess an elongate body. Living on the bottom of the sea they feed on the organic material in the sand. Their toxins therefore are not used actively to catch prey but as self defense; they are produced in the skin and also in the Cuvier's tubules which may be found in many but not all species. In case of danger, they are ejected from the body cavity through the anus. Sticking to the aggressor the latter immediately becomes unconscious. Even in a dilution of 1:100,000 the crude toxin was found to kill fishes within a few minutes. Poisoning in man may occur after consumption of holothurians if during preparation of "Trepang" the venoms have not been completely removed by watering. In mild cases digestive problems will be observed; in the rare severe cases paralysis and death have occurred.

Chemically, these compounds are glycosides of tetracyclic triterpenes which are remarkable derivatives of lanosterol in so far as they are the first glycoside triterpenes derived from animals, as early as 1942 by Yamanouchi (25, 26). All of these compounds are derivatives of holostanol (27), differing in the side chain as well as the double bonds in the lanosterol skeleton. The sugar residue is attached to the oxygen at C-3. Yamanouchi obtained a crystalline toxin, named holothurin, from Holothuria leucospilota (= H. vagabunda). The toxin was surface active and strongly hemolytic. Nigrelli (28) isolated another "holothurin" from Actinopyga agassizi with strong ichthyotoxic activity not knowing the papers of Yamanouchi in 1952. Hydrolysis of the saponins yielded a mixture of aglycones; the main compound was called holothurinogenin. The 7:8,9:11-diene system (as may be found in many other aglycones) is an artifact due to the strong acidic conditions during hydrolysis. Much of the chemical work on the holothurins done during the 1960s and 1970s centered on the isolation and characterization of these aglycones. A complete listing of all triterpenoids from holothurians as of 1982 may be found in the paper of Burnell and ApSimon (5). In recent years the native saponins have been investigated, the structures being elucidated by means of modern NMR- and mass spectroscopic methods as well as by x-ray crystal structure analysis. Here, as well as in the case of saponins derived from starfishes (see chapter 3.3), this paper will give a review about the glycosidic natural

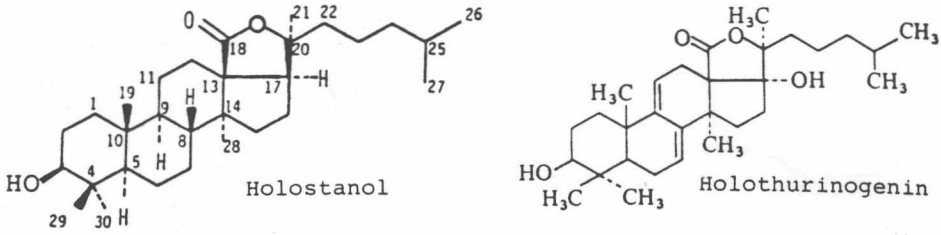


Fig. 2. Structures of Holostanol and Holothurinogenin.

compounds only.

Most of the sea cucumbers which have been investigated belong to the family Holothuriidae and the first complete structures reported were those of holothurins A and B, isolated from Holothuria leucospilota BRANDT (= H. vagabunda SELENKA) (29 - 33). To obtain these compounds, body walls and Cuvierian tubules separated from the sea cucumber were extracted with 70% ethanol, followed by partitioning between butanol-water, silica gel chromatography, and recrystallization. While holothurin A has been isolated as the major lanostane-type triterpene oligoglycoside from the Cuvierian tubules of this sea cucumber, holothurin B is contained mainly in the body walls. Both compounds have the same aglycone and one sulfate residue. They are only different in the sugar parts. Holothurin A is a tetraglycoside containing D-glucose, 3-O-methyl-D-glucose, D-quinovose, and D-xylose whereas holothurin B comprises D-quinovose and D-xylose only. Acid hydrolysis of both saponins with 3N hydrochloric acid resulted in an artificial aglycone, 22,25-oxidoholothurinogenin, as judged by the heteroannular 7:8,9:11-diene absorption maxima observed in its UV spectrum. On hydrolysis of holothurin B with snail enzyme, it gave two prosapogenols; both are monoglycosides containing xylose, and one of them lacked the sulfate residue. Solvolysis of holothurin B with dioxane-pyridine furnished a desulfated derivative. Methylation of this derivative and of the native glycoside, respectively, followed by methanolysis and identification of the (partly) methylated sugars permitted determination of the sequence and connectivity of the sugar residues as well as of the position of the sulfate group. On enzymatic hydrolysis, holothurin A gave holothurin B together with the two identical monoglycosidic prosapogenols mentioned above. Solvolysis of holothurin A followed by methylation and methanolysis clarified the structure of

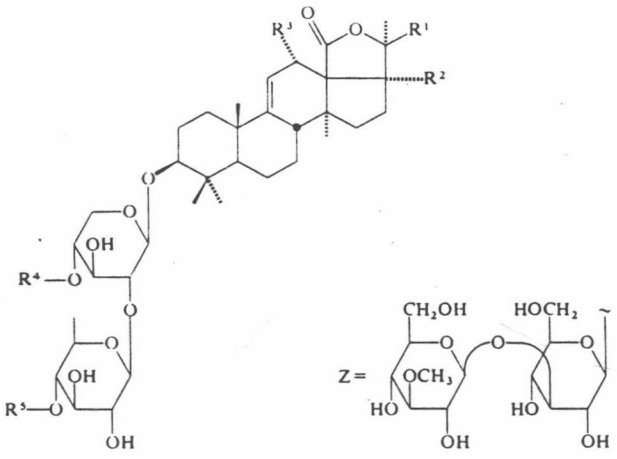
the sugar part. UV, IR, CD, ^{1}H-NMR, and ^{13}C-NMR spectra gave further evidence for the structures of holothurins A and B. Holothuria lubrica (29) and H. atra (34) also contain holothurin A and B and the compound first mentioned has been isolated from Actinopyga agassizi (35), Holothuria squamifera (36), and Bohadschia graeffei (37), all species belonging to the family Holothuriidae. Oleinikova and Kuznetsova (34) found that the glycosidic fraction of H. atra contains 2.1% of desulfated holothurin B besides 12.5% holothurin A, 84.2% holothurin B, and 1.08% holothurin B_1 (this saponin is described in the next paragraph). The last of these glycosides has not been isolated from natural sources before, but it was obtained by solvolysis of holothurin B as mentioned above.

A tetraglycoside similar to holothurin A, but differing in the nature of the aglycone side chain, has been isolated by Oleinikova et al. (38) from 2 species of holothurians, Holothuria floridana and H. grisea. They named it holothurin A_1. Echinosides A and B from Actinopyga echinites have the same carbohydrate moiety as holothurin A and B, respectively, and similarly differed from each other only in the side chain of the aglycones (39). Later, Kalinin and Stonik (40, 41), and Oleinikova et al. (42) described the isolation and structure elucidation of holothurin A_2 from Holothuria edulis, H. floridana, and Bohadschia graeffei while holothurin B_1 has been found in H. floridana (43 - 45) and H. atra (34). Holothurin A_2 and B_1 were identical with echinosides A and B, respectively, except that the cation associated with the sulfate residue in the glycoside from H. edulis was an ammonium ion. The structure of the native aglycone of holothurin A_1 has been established as holost-9(11)-ene-3ß,12α,17α,22ξ-tetraol, which was confirmed by the result of the acid hydrolysis furnishing holosta-7,9(11)-diene-3ß,17α,22ξ-triol (griseogenin). The aglycones of the other two saponins, echinosides A and B (or holothurins A_2 and B_1), are similar, only lacking the hydroxyl function at C-22. Kalinin and Stonik (40) reported that their isolated glycoside, holothurin A_2, during storage at room temperature, underwent spontaneous desulfation, which has not been observed in the case of other sulfated glycosides. The authors found that the counter-ion for the sulfate group is the ammonium ion, which is responsible for this reaction. Another tetraglycoside sulfate belonging to the same group of saponins is 24-dehydroechinoside A which, together with holothurin A, has

been isolated from Actinopyga agassizi in an approximate ratio of 2:1 (35). The authors reached the conclusion that "holothurin A" described by Chanley and Rossi (46) already in 1969 from the same species is not identical with holothurin A from H. leucospilota and other species as mentioned above, but was the same mixture as isolated here. This mixture, which showed a single spot on TLC, could be separated as desulfated derivatives by HPLC after treatment with dioxane-pyridine under reflux.

On the basis of chemical and physicochemical evidence, structures were assigned to four oligoglycosides lacking a sulfate group, bivittosides A to D from Bohadschia bivittata by Kitagawa et al. (47). The aglycones of these four compounds do not have a 17-hydroxyl group and bivittoside C even lacks the one at C-12. On acid hydrolysis, bivittoside A furnished the dienic artifact sapogenol seychellogenin and one mole each of D-xylose and D-quinovose. The unknown configuration at C-20 of seychellogenin has been defined S, based on ^{1}H-NMR analysis. Enzymatic hydrolysis of bivittoside B with crude hesperidinase afforded bivittoside A and that of bivittoside D with mixed glucosidase from Turbo cornutus gave bivittoside B. With the aid of the already mentioned reactions, methylation and methanolysis, of compounds A, B, and D, the structures of the sugar parts of the di-, tetra-, and hexaglycoside, respectively, could be determined. Bivittoside C has an identical sugar residue as bivittoside D but furnished on acidic hydrolysis another sapogenol which does not contain the hydroxyl group at C-12. The structure of bivittoside C genine has been confirmed by partial synthesis (48). The artifical triterpene aglycone of bivittosides A, B, and D, seychellogenin, has also been synthesized (49). The native aglycone, holost-9(11)-ene-3ß,12α-diol - these names being assigned in accordance with the convention proposed by Habermehl and Volkwein (27) already in 1971 -, could be obtained after Smith degradation of the glycosides from Bohadschia argus (50). Bivittoside B and C isolated from B. argus, B. marmorata, B. vitiensis, and B. tenuissima (51) on Smith reaction with potassium borohydride (52) gave two progenins by cleavage of the glycosidic bond between xylose and quinovose, thus leading to triglycosides. A second Smith degradation of these progenins gave the aglycones.

Sarma et al. (53) made an acidic hydrolysis of the methanol extracts of Holothuria atra and H. scabra using 2N sulfuric acid on a steam-bath for 1hr. Besides nine sapogenins they obtained



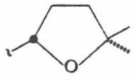
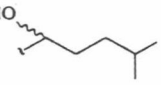
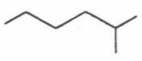
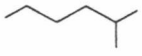
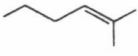
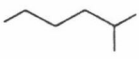
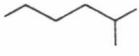
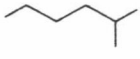
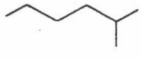
	R¹	R²	R³	R⁴	R⁵
Holothurin A		OH	OH	SO_3Na	Z
Holothurin B		OH	OH	SO_3Na	H
Holothurin A_1		OH	OH	SO_3Na	Z
Echinoside A		OH	OH	SO_3Na	Z
Echinoside B		OH	OH	SO_3Na	H
24-Dehydro-echinoside A		OH	OH	SO_3Na	Z
Bivittoside A		H	OH	H	H
Bivittoside B		H	OH	Z	H
Bivittoside C		H	H	Z	Z
Bivittoside D		H	OH	Z	Z

Fig. 3. Structures of glycosides from sea cucumbers of the family Holothuriidae.

two artificial glycosides. The first one was a monoglycoside composed of xylose and an aglycone containing the heteroannular diene moiety while the second was desulfated holothurin B.

From the saponins discussed above, obtained from members of the family Holothuriidae, we can see that the genuine aglycones all contain the 9(11)-double bond and, with the exception of bivittoside C, a hydroxyl function at C-12. All structures are summarized in figure 3.

Glycosides of four sea cucumbers of the family Stichopodidae have been investigated, Stichopus chloronotus, S. variegatus, Astichopus multifidus, and Thelenota ananas. S. chloronotus was examined simultaneously by a Japanese and a Russian group and provided eight saponins. Kitagawa et al. (54, 55) isolated from the body walls of this sea cucumber stichlorosides A_1, A_2, B_1, B_2, C_1, and C_2 which contained, like all other glycosides known from holothurians of this family so far, lanost-7-ene type aglycones and no sulfate residue. On enzymatic hydrolysis stichlorosides A_1 and A_2 liberated stichlorogenol and 25-dehydrostichlorogenol, respectively. Catalytic hydrogenation of the latter compound over Pd-C furnished stichlorogenol. The structure of stichlorogenol, including the C-9, C-20, and C-23 configurations, was clarified by X-ray analysis. During acidic hydrolysis of parent stichlorosides the genuine 7-ene moieties are converted to artifact 8-ene and 9(11)-ene moieties. All six stichlorosides are hexaglycosides, A_1, A_2, B_1, and B_2 having two moles each of xylose, glucose, and 3-O-methylglucose, while C_1 and C_2 contain two moles of xylose, two moles of 3-O-methylglucose and one mole each of glucose and quinovose. Alkaline treatment in 1/6N NaOMe-MeOH gave the desacetylated saponins which on enzymatic hydrolysis with crude naringinase yielded the aglycone besides a mixture of prosapogenins. Methylation of the desacetylated saponins and the prosapogenins followed by methanolysis led to the determination of the sugar sequences in all six natural products. Catalytic hydrogenation over 5% Pd-C of stichlorosides A_2, B_2, and C_2 gave quantitatively stichlorosides A_1, B_1, and C_1, respectively. The Russian group named the substances isolated by them stichoposides and assigned the structure of stichlorogenol to the aglycone derived from them (56). Stichoposides A and B were two new diglycosides (57), whereas the structures given for stichoposides C (58), D (59), and E (60) were identical with those of stichlorosides C_1, B_1, and A_1, respectively. Stichoposides A and B have

the same aglycone and one mole of xylose; they are only different in the terminal sugar which is D-quinovose in A and D-glucose in B. Stichoposide D has also been found in Stichopus variegatus (59). The genuine aglycone of astichoposide C from Astichopus multifidus (56) has the same structure as dehydrostichlorogenol and the saponin itself (58) was identical with stichloroside C_2.

Two thelothurins, A and B, from Thelenota ananas (61) have identical but unusual sugar chains containing an unidentified substituent not being sulfate. The genuine aglycones of both saponins, which differ only in the side chain, appear to be of a common type in most species in the family Stichopodidae. Later, Stonik et al. (62) isolated from the same sea cucumber thelenotosides A and B, two glycosides with structures similar to those from the other species of this family. The authors separated the total glycosides into a series of chromatographically individual fractions. Analysis of the ^{13}C-NMR spectra of each of them showed that they were two-component mixtures with similar structures of the carbohydrate chains and having as their native aglycones 23(S)-acetoxyholost-7-en-3ß-ol and 23(S)-acetoxyholosta-7,25-dien-3ß-ol. All fractions were hydrogenated over Adams catalyst, thus, individual glycosides resulted, having only the first mentioned aglycone. Besides stichoposides A and C the two thelenotosides could be isolated, which are both tetraglycosides and different only in one monosaccharide residue. The enzymatic cleavage of thelenotoside A with a cellulase led to the formation of a progenine which is identical with stichoposide A. Partial hydrolysis of thelenotoside B by 1N oxalic acid gave two progenines, a monoglycoside containing xylose and a triglycoside.

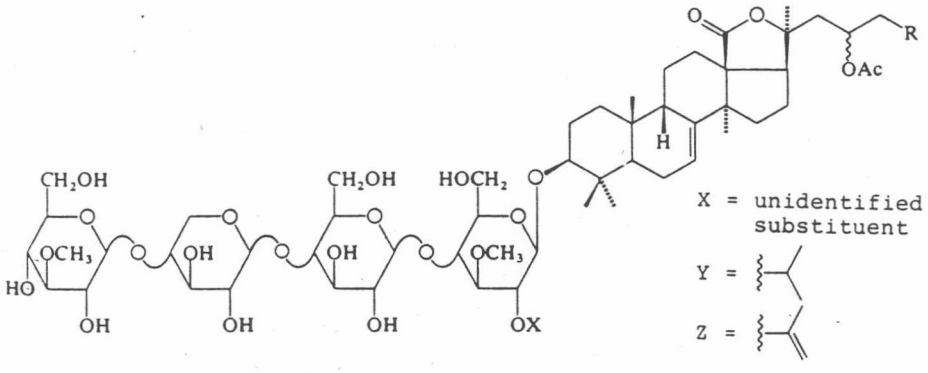
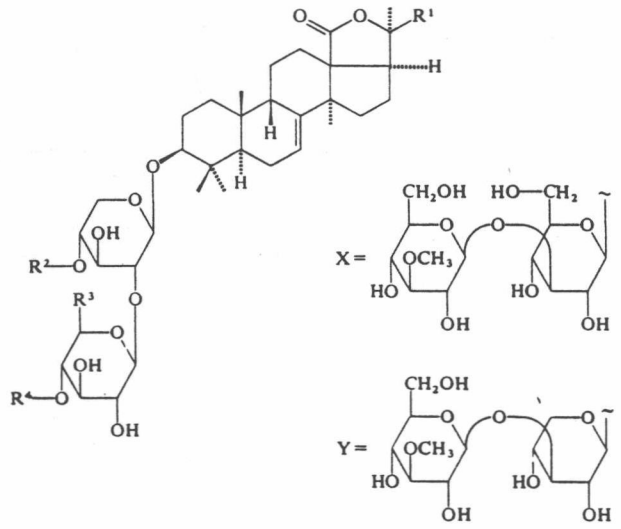


Fig. 4a. Structures of thelothurins A (R = Y) and B (R = Z).



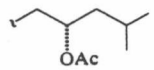
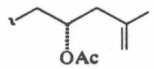
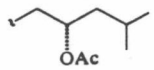
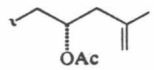
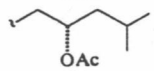
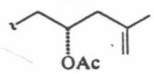
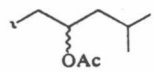
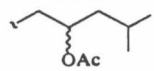
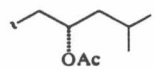
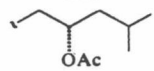
	R^1	R^2	R^3	R^4
Stichloroside A_1		X	H	X
Stichloroside A_2		X	H	X
Stichloroside B_1		X	CH_2OH	Y
Stichloroside B_2		X	CH_2OH	Y
Stichloroside C_1		X	CH_3	Y
Stichloroside C_2		X	CH_3	Y
Stichoposide A		H	CH_3	H
Stichoposide B		H	CH_2OH	H
Thelenotoside A		H	CH_3	Y
Thelenotoside B		H	CH_2OH	Y

Fig. 4b. Structures of glycosides from sea cucumbers of the family Stichopodidae.

From holothurians of the family Cucumariidae the species Eupentacta (= Cucumaria) fraudatrix has been investigated mostly. Afiyatullov et al. (63 - 65) isolated from this species three glycosides, cucumariosides G_1, C_1, and C_2. The aglycones of all these compounds have a 7-ene and a 16ß-acetoxy function. Acidic hydrolysis of cucumarioside G_1 gave the native aglycone, which could be determined as 16ß-acetoxyholosta-7,24-dien-3ß-ol (56, 66), and a mixture of D-xylose, D-quinovose, D-glucose, and 3-O-methyl-D-xylose in a ratio of 1:1:1:1. This was the first time that 3-O-methyl-D-xylose has been detected in hydrolysates of holothurian glycosides. Solvolysis of the glycoside gave a desulfated product which was then permethylated. The following methanolysis showed that the carbohydrate chain is unbranched. To define the sequence of the monosaccharides in the carbohydrate chain, an enzymatic cleavage of cucumarioside G_1 was performed, which gave a progenine containing xylose and quinovose. The position of the sulfate group at C-4 in the xylose residue was determined by comparing the ^{13}C-NMR spectra of the progenine and that of its desulfated derivative. The counter ion at the sulfate residue, which was first erroneously given as as ammonium ion (63), has later been established by atomic absorption spectroscopy as sodium (64).

The stuctures of cucumariosides C_1 and C_2 have been established by aid of ^{13}C-NMR and ^{1}H-NMR spectroscopy, partial acidic hydrolysis, periodate oxidation, and methylation. A comparison of the ^{13}C-NMR spectra of both saponins showed their considerable structural similarity. The difference consists only in the signals of the side chains of the aglycones. While cucumarioside C_1 has a 22,24-diene system with cis-configuration, that of C_2 is trans-configurated. On catalytic hydrogenation of each of the saponins the identical tetrahydro derivatives could be obtained. The methylation products indicated the presence of branching in the carbohydrate chains of the glycosides. Total hydrolysis gave 3-O-methyl-D-xylose, D-quinovose, D-xylose and D-glucose in a ratio of 1:1:2:1. Partial hydrolysis of the hydrogenation product performed with 2N sulfuric acid gave three progenines, two tetra- and one triglycoside, the latter by loss of the two terminal monosaccharide residues.

Cucumaria japonica furnished cucumarioside A_2-2 (67) with 3ß-hydroxyholosta-7,25-dien-16-one as the native aglycone (56, 68), and D-quinovose, D-xylose, 3-O-methyl-D-glucose, D-glucose

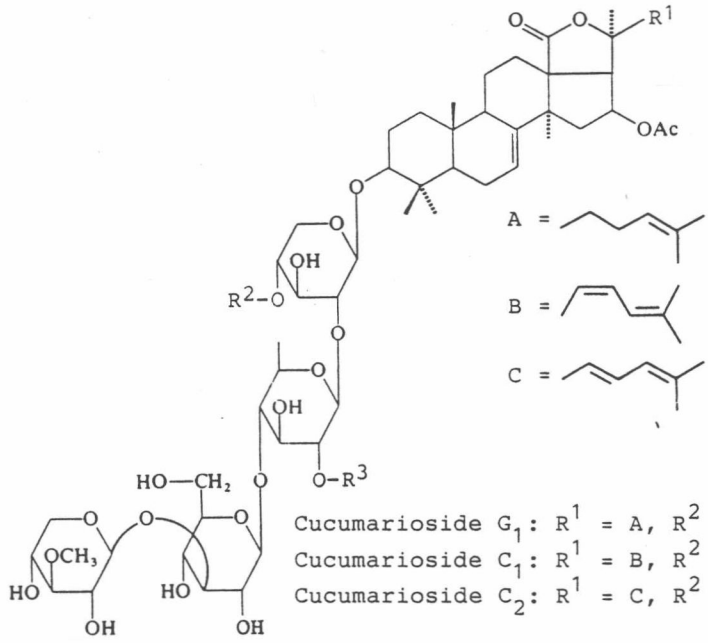


Fig. 5. Structures of glycosides from Cucumaria fraudatrix.

in a ratio of 1:2:1:1 as the attached sugars (thus being also a pentaglycoside), and one sulfate residue. Usual spectroscopic and chemical methods led to the structure of the glycoside.

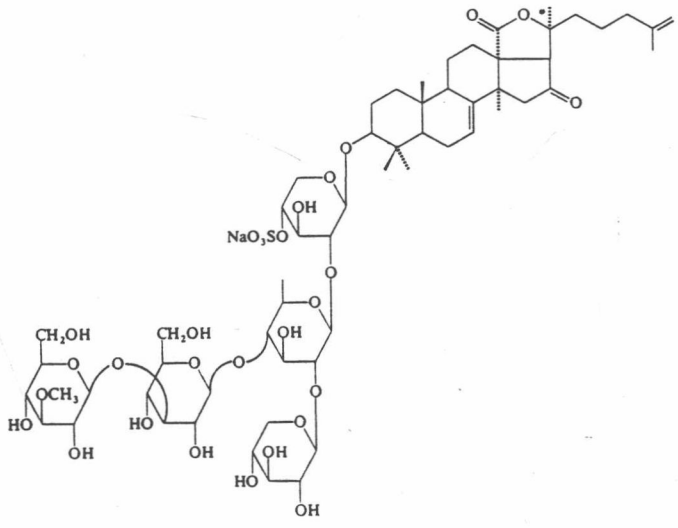


Fig. 6. Structure of cucumarioside A_2-2.

A further native genine has been isolated after mild hydrolysis of the corresponding glycosides from Cucumaria frondosa (69). The name frondogenin was suggested and the structure has been elucidated as 16α-acetoxy-holost-7-en-3ß-ol.

Parathyonosides R and T have been isolated from a methanolic extract of a holothurian, Parathyona sp. It has been shown that the native aglycone of the glycoside R is 16,23-epoxyholosta-7,24-dien-3ß-ol (70). Thus, like all glycosides of sea cucumbers of the family Cucumariidae mentioned above, this compound also contains a 7-ene moiety and an oxygen function at C-16. Qualitative analysis of the monosaccharides gave D-xylose, D-quinovose, and 3-O-methyl-D-glucose; a partial structure is shown in figure 7.

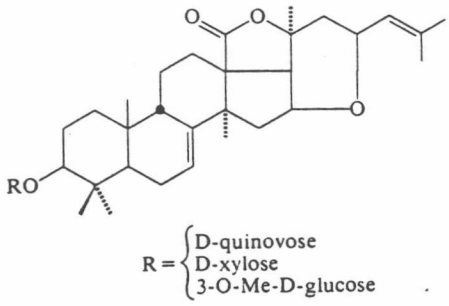


Fig. 7. Partial structure of patathyonoside R.

A further group of saponins from holothurians is characterized by a 9(11)-double bond and a 16-keto function. The 9(11)-ene structure has been found in several aglycones as a result of acidic hydrolysis which led to a rearrangement of the original 7-double bond. First reports of this structure in native glycosides dates from the 1970s. The structures of holotoxins A and B from Stichopus japonicus reported in 1976 (71, 72) have been revised, later (73). The identical structure of the native aglycones of both compounds, named holotoxigenol, was 3ß-hydroxyholosta-9(11),25-dien-16-one. The saponins are different in the sugar parts; while holotoxin A contains two 3-O-methyl-D-glucose units besides D-glucose (2x), D-xylose (1x), and D-quinovose (1x), in holotoxin B one 3-O-methyl-D-glucose is exchanged to D-glucose. Later, Maltsev et al. (74) investigated the same species but collected at another place. They found similar compounds, the holotoxins A_1 and B_1. Both saponins possess also holotoxigenol as aglycone moieties. Holotoxins A and B, however, differ from A_1

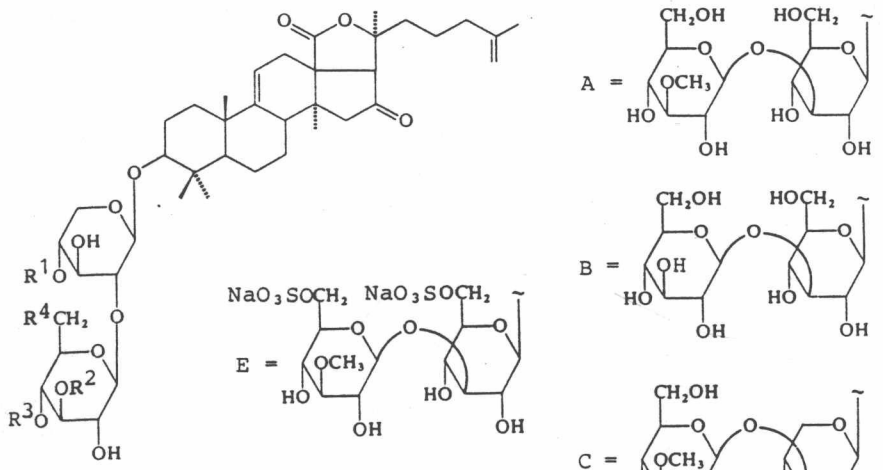
and B_1, respectively, in one sugar residue. One glucose unit is exchanged against xylose. Obviously, the carbohydrate ingredients in the holothurian oligoglycosides may be able to vary if the locality of the animal differs.

Psoluthurin A from Psolus fabricii also has holotoxigenol as its aglycone (75). It is an unusual compound containing two sulfate residues in the sugar chain which is formed of one mole each of xylose, glucose, quinovose, and 3-O-methylglucose. Because each monosaccharide residue has a different mass, the carbohydrate sequence could be deduced by FAB mass spectrometry of the complete saponin, which also showed the location of the sulfates. Kalinin et al. (76) isolated psolusoside A from the same species, a tetraglycoside, which after hydrolysis liberated 3ß-hydroxyholosta-9(11),25-dien-16-one, D-quinovose, D-xylose, D-glucose, and 3-O-methyl-D-glucose, but its structure is not identical with psoluthurin A (77). The given structures of both compounds are different in the carbohydrate residue; the sequence of sugars 2 and 3 is reverse. The ^{13}C-NMR spectra of psoluthurin A and its desulfated derivative agree with the corresponding characteristics for psolusoside A and the product of its desulfatation. That's why Kalinin et al. (77) supposed that there was an error in the interpretation of the mass spectra as a result of which the sequence of the monosaccharides in the carbohydrate chain of psoluthurin A was determined incorrectly.

Recently Zurita et al. (78) described the main component of the glycoside fraction isolated from the New Caledonian sea cucumber Neothyonidium magnum. Neothyonidioside contains the same aglycone as the saponins from Stichopus japonicus and Psolus fabricii mentioned before, 3-O-methyl-D-glucose, D-xylose, D-quinovose in a ratio of 1:2:1 and a sulfate residue. The authors also used FAB mass spectroscopy to determine the sequence of the sugar chain.

Paracaudina ransonetii furnished caudinoside A which on acid hydrolysis gave holotoxigenol in addition to D-quinovose, D-xylose, 3-O-methyl-D-glucose, and D-glucose (1:1:1:3). The complete structure of this hexaglycoside has not been elucidated so far (79).

A new triterpene genine, onekotanogenin, from the holothurian Psolus fabricii differ substantially in its structure from all other known aglycones from glycosides of sea cucumbers (80). The genin was obtained by hydrolysis with 2N sulfuric acid in the



	R^1	R^2	R^3	R^4
Holotoxin A	A	H	A	H
Holotoxin B	B	H	A	H
Holotoxin A_1	A	H	C	H
Holotoxin B_1	B	H	C	H
Psoluthurin A	H	D	H	OSO_3Na
Psolusoside A	H	H	E	H
Neothyonidioside	SO_3Na	H	C	H

Fig. 8. Holothurian glycosides containing a 9(11)-en-16-one moiety.

presence of butanol of the hydrogenated desulfated derivative of the glycoside named psolusoside B. Its structure has been determined as 20(S)-acetoxy-3ß,16-dihydroxylanost-7-en-18-oic acid-18,16-lactone. It has been shown that its 25,26-dehydro derivative is the native genine of psolusoside B. Another triterpene containing the 18,16-lactone ring could be obtained after treatment of 3ß-hydroxyholosta-7,25-dien-16-one with sodium tetrahydroborate in aqueous dioxane (68).

A further aglycone, not belonging to the holostane group, has been obtained after acid hydrolysis of the glycosides from the Far Eastern holothurian Duasmodactyla kurilensis (81). This compound which does not possess a lactone ring was named kurilogenin and its structure has been determined as 3ß-hydroxy-4,4,14-trimethylpregna-9(11),16-dien-20-one.

Poisoning by holothurians can reputedly result from inges-

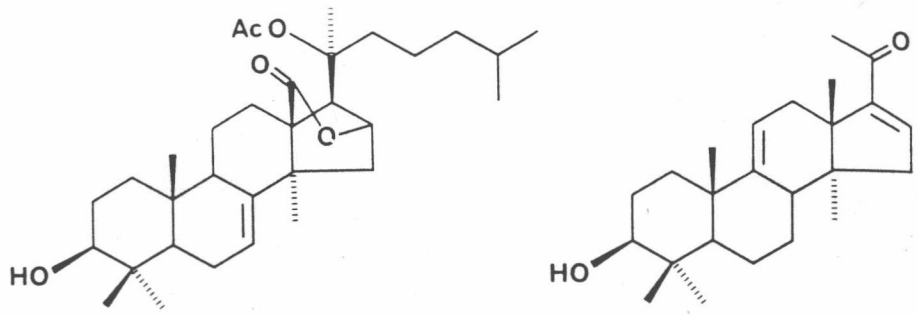


Fig. 9. Structures of onekotanogenin (left) and kurilogenin (right).

tion of toxic sea cucumbers. Liquid ejected from the visceral cavity of some species may result in contact dermatitis or blindness. Reef fishes reject pieces of sea cucumbers as food (82). The ichthyotoxicity of holothurians has been used traditionally by Pacific islanders as a means of capturing fishes in tide pools (83, 84). The toxicological and pharmacological activity of echinoderm saponins has been reviewed by Burnell and ApSimon (5), and by Halstead (7). Dilute holothurin solutions show toxicity to various animals, among these coelenterates, planarians, nematodes, annelides, mollusks, crustaceans, frogs, and protozoans. Concentrations of a few ppm are lethal to fish. Holothurins have shark repelling effects. A shark placed into a solution of 2 ppm of holothurin attempts to escape, vomites a few minutes later and dies within 50 minutes. The lethal dose of holothurin for mice is 400 mg/kg when orally administered, 70 mg/kg by subcutaneous injection and 0.75 mg/kg intravenously. Contrary, in other reports the LD_{50} of holothurin A by intravenous administration in mice is estimated at 9 mg/kg.

The antibacterial effect of holothurins is negligible, but they are active against a variety of fungi. Holothurin A and B are more active than holotoxin C - the structure of the latter is still unknown - and holothurin A is ten times less active than desulfated holothurin A. Species of the genus Bohadschia have a more powerfull effect against fungi than other members of Holothuriidae and Cucumariidae. Echinosides A and B (39), the bivittosides (47) and the stichlorosides (54) are all descibed to exhibit antifungal activities.

Holothurins produce hemolysis of mouse, rabbit, and human