Pure & Appl. Chem., Vol. 58, No. 3, pp. 423–436, 1986.
Printed in Great Britain.

Some terpenoid and steroid derivatives from echinoderms and sponges

Valentin A. Stonik

Pacific Institute of Bioorganic Chemistry, Far East Science Centre, Academy of Sciences, Vladivostok-22, USSR

Abstract - Some echinoderms and sponges produce cytotoxic terpenoid or steroid derivatives probably to defend themselves from fish and pathogenic microforms. Most of these compounds proved to be glycosides or steroid sulphates and may cause a disturbance in the permeability of biological membranes. The presence of such and other cytotoxins is likely to be correlated with the chemical composition of membranes. As a result, usual sterols such as cholesterol are absent or present in small amounts in these species. Structures, distribution and properties of the active compounds and sterols are described.

INTRODUCTION

Echinoderms and sponges are widespread marine invertebrates usually characterized by an abundance of protective secondary metabolites toxic to fish and microorganisms. Nigrelli and Yamanouchi (refs. 1, 2) were the first to discover the echinoderms, in particular sea cucumbers, a possible source of cytotoxic triterpene glycosides. Physiologically active steroid glycosides were found in starfishes by Yasumoto et al. (ref. 3), cytotoxic nucleosides were isolated from sponge extracts by Bergmann et al. (refs. 4, 5). Later, the studies on cytotoxic compounds from echinoderms and sponges were activated and resulted in the structural elucidation of some new natural products and in a series of review publications (refs. 6-9). From 1968 and especially in the past decade our laboratory has participated in this research.

The aim of the present work is to summarize some of our most significant efforts, giving attention to the structures, chemotaxonomic aspects and peculiarities of the biological action of isolated substances.

TRITERPENE GLYCOSIDES FROM SEA CUCUMBERS

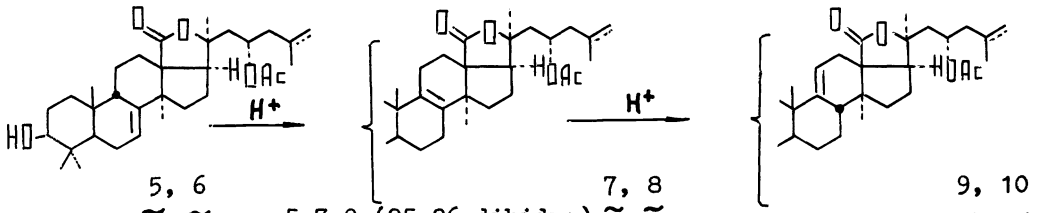
In the animal kingdom triterpene glycosides have been found in the exclusively marine phylum Echinodermata and particularly in species of the class Holothuroidea (sea cucumbers). At the very beginning of our research the following points still remained obscure : 1) the full chemical structures of these natural products, 2) the degree of variety of the glycosides from sea cucumbers, 3) the presence or absence of a relationship between the systematic positions of the animals and their glycoside content and 4) a relationship between their structures and activities. We have tried to provide answers to the above problems.

It was shown that all types of isolated glycosides have the related aglycones of the holostane skeleton (ref. 10), differing from each other by the structure of the side chain, the position of the endocyclic double bond and the presence of various functional groups. Triterpene glycosides are widely distributed in holothurians, and a correlation exists between the systematic position of the animals and the glycoside structures contained therein. There is a definite structural regularity in both the aglycones and carbohydrate moieties of different glycosides as well as resemblance (or identity) of such compounds from related species (refs. 11-14).

A group of so-called holothurins was isolated from Pacific, Indo-Pacific and Atlantic species belonging to the Holothuriidae family and particularly from species of Holothuria and Actinopyga genera (ref. 14). Their structures have been established by Kitagawa et al. (refs. 15-18) and by us (refs. 19-28). All these glycosides possess a 12-α-hydroxy-9(11)-ene fragment in the aglycone and a disaccharide or tetrasaccharide part with an O-sulphate group. From substances of the "holothurin B" and "holothurin A" series (general formulae 1 and 2), only triterpene glycosides with an hydroxyl group at C-17 have been isolated and purified (refs. 15-29). Their 17-desoxy-derivatives have not yet been obtained, although holothurinogenins which may be derived from these products, are well known (refs.11,30).

As an exception in the Holothuriidae family, of five Bohadschia species studied, four (Bohadschia argus, B. bivittata, B. marmorata and B. vitiensis) contain glycosides without an O-sulphate group and with holost-9(11)-en-12 α,3β -diol and holost-9(11)-en-3β -ol as genuine aglycones (ref. 31). Kitagawa et al. were the first to determine the full structures of these glycosides (for example, 3 and 4) named bivittosides after their isolation from B. bivittata (ref. 32). Since we have isolated the same products from three other representatives of Bohadschia genus we believe that the general name of bohadschiosides for these is more appropriate. Only B. graeffei yielded a set of holothurins typical of Holothuria and Actinopyga. It should be noted that B. graeffei differs morphologically from other animals of Bohadschia. There have been suggestions to assign it to a separate genus, Pearsonothuria (ref. 14).

In contrast to holothurins and bohadschiosides, the glycosides of another group, the so-called stichoposides, possess a double bond occupying the 7(8)-position (ref. 35). The genuine aglycones of stichoposides have been separated from other products of mild acid hydrolysis by chromatography on a column, using $AgNO_3$ -impregnated silica gel (ref. 36). Spectral data including X-ray analysis (ref. 37) showed the aglycones to be 23(S)-acetoxy-holost-7-en-3β -ol (5) and its 25(26)-dehydro derivative (6), respectively. Both aglycones and the corresponding glycosides were unstable under hydrolytic conditions, giving 23(S)-acetoxy-holost-8(9)-en-3β -ol (7), 23(S)-acetoxy-holost-9(11)-en-3β -ol (9) and their 25(26)-dehydro derivatives (8 and 10), as a result of double bond migration from the 7(8)-position to the 9(11)-position.



5, 6 7, 8 9, 10

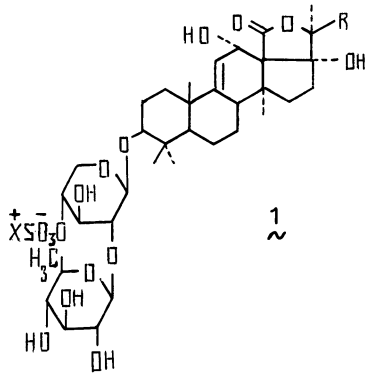
5,7,9 (25,26-dihidro)
6,8,10 (25,26-dehydro)

In fact, treatment of stichoposides or their peracetates with $CHCl_3$ saturated with HCl (20°C, 1 hr) completely transformed the $\Delta^{7,8}$-aglycone into the 8(9)-aglycone. This process was indicated by comparison of the corresponding ^{13}C-NMR spectra, which showed instead of the singlet at 145.6 ppm and the doublet at 120 ppm ($\Delta^{7,8}$) two singlet signals at 130.0 135.5 ppm. Under more strongly acid conditions the signals of $\Delta^{8,9}$ were substituted by a singlet at 151.0 and a doublet at 111.0 ppm ($\Delta^{9(11)}$) (refs. 36,38,39).

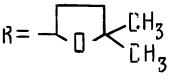
Stichoposides A and B from Stichopus chloronotus (ref. 40) turned out to have the simplest structures (11 and 12) in this series. Both compounds contain carbohydrate chains with a β-1,2-glycoside bond between monosaccharides, as in holothurin B(1).

The related tetrasaccharides, named thelenotosides A and B (13, 14), from Thelenota ananas closely resemble holothurin A(2) with respect to the structure of the carbohydrate moieties. However, 3-O-methyl-D-glucose in 13, 14 is attached to D-xylose while in 2 it is attached to D-glucose (ref. 41).

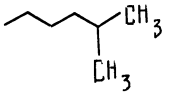
More complicated structures have been established for glycosides 15-20 isolated from several species of the Stichopodidae family (refs. 42-45).



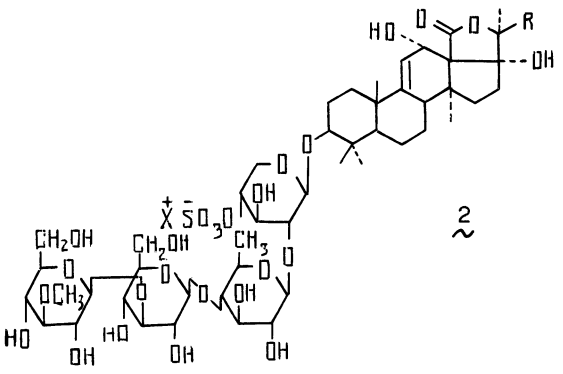
1

Holothurin B R= 

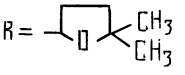
H. leucospilota (Ref. 15);
H. edulis (Ref. 21);
A. flammea (Ref. 29);
H. atra (Ref. 19)

Holothurin B_1 R= 

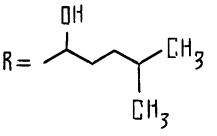
A. echinites (Ref. 16);
H. floridana (Refs. 22, 27)



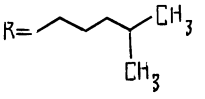
2

Holothurin A R= 

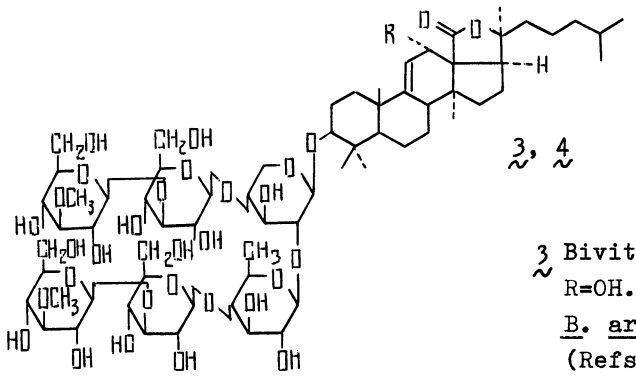
H. leucospilota (Ref. 17);
H. squamifera (Ref. 28);
A. agassizi (Ref. 18); A. flammea (Ref. 29)

Holothurin A_1 R= 

H. floridana (Refs. 20, 24);
H. grisea (Ref. 24)

Holothurin A_2 R= 

H. edulis (Ref. 23); H. floridana (Ref. 25); B. graeffei (Ref. 26);
A. echinites (Ref. 16)

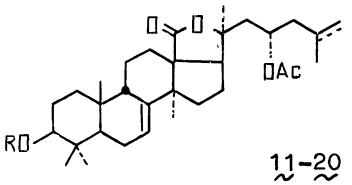


3, 4

3 Bivittoside C (Bohadschioside A), R=OH. B. bivittata (Ref. 32);
B. argus, B. marmorata, B. vitiensis (Refs. 14, 33, 34)

4 Bivittoside D (Bohadschioside A_1) R=H.
B. bivittata (Ref. 32);
B. argus, B. marmorata, B. vitiensis (Refs. 14, 33, 34)

Fig. 1 Structures of some glycosides from Holothuriidae family.



11-20

Stichoposide A(11) R=Qui-β-1,2-Xyl-;25(26)-dihydro (ref. 40)

Stichoposide B(12) R=Glc-β-1,2-Xyl-;25(26)-dihydro (ref. 40)

Thelenotoside A(13) R=3-OMe-Glc-β-1,3-Xyl-β-1,4-Qui-β-1,2-Xyl-; 25(26)-dihydro (ref. 41)

Thelenotoside B(14) R=3-OMe-Glc-β-1,3-Xyl-β-1,4-Glc-β-1,2-Xyl-; 25(26)-dihydro (ref. 41)

Stichoposide C or Stichloroside C_1(15) 25(26)-dihydro;
R= 3OMe-Glc-β-1,3-Glc-β-1,4-Xyl-
3-OMe-Glc-β-1,3-Xyl-β-1,4-Qui-β-1,2 (refs. 42, 43)

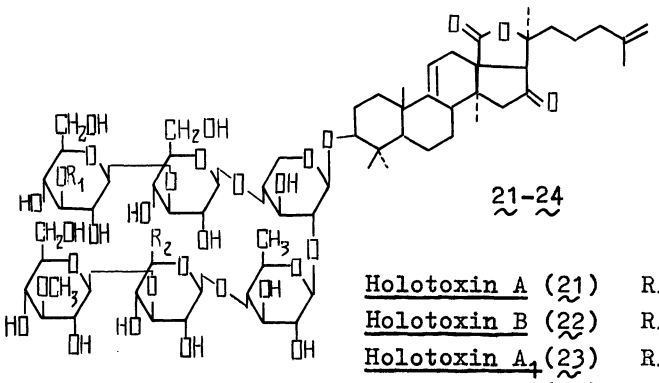
Astichoposide C or Stichloroside C_2(16) 25(26)-dehydro;
R=3-O-Me-Glc-β-1,3-Glc-β-1,4-Xyl-
3-OMe-Glc-β-1,3-Xyl-β-1,4-Qui-β-1,2 (refs. 42, 43)

Stichloroside B_1 or Stichoposide D(17) 25(26)-dihydro;
R=3-OMe-Glc-β-1,3-Glc-β-1,4-Xyl-
3-OMe-Glc-β-1,3-Xyl-β-1,4-Glc-β-1,2 (refs. 42, 13)

Stichloroside B2(18) 25(26)-dehydro;
R=3OMe-Glc-β-1,3-Glc-β-1,4-Xyl-
3-OMe-Glc-β-1,3-Xyl-β-1,4-Glc-β-1,2 (ref. 42)

Stichloroside A_1 or Stichoposide E(19) 25(26)-dihydro;
R=3-OMe-Glc-β-1,3-Glc-β-1,4-Xyl-
3-OMe-Glc-β 1,3-Glc-β-1,4-Xyl-β-1,2 (ref. 42, 45)

Stichloroside A_2(20) 25(26)-dihydro;
R=3-OMe-Glc-β-1,3-Glc-β-1,4-Xyl-
3-OMe-Glc-β-1,3-Glc-β-1,4-Xyl-β-1,2 (ref. 42)



21-24

Holotoxin A (21) $R_1=CH_3$; $R_2=CH_2OH$ (ref. 50)

Holotoxin B (22) $R_1=H$; $R_2=CH_2OH$ (ref. 50)

Holotoxin A_1(23) $R_1=CH_3$; $R_2=H$ (refs. 51, 52)

Holotoxin B_1(24) $R_1=H$; $R_2=H$ (ref. 52)

Fig.2. Structures of the reported glycosides from Stichopodidae family of sea cucumbers.

The compound 15 is probably one of the predominant components of glycoside fractions from these animals. However, the absence of 15 or related compounds in S. japonicus has been reported (refs. 46, 47). The glycosides from this holothurian and Parastichopus californicus (ref. 48) contain a recently isolated holosta-9(11),25-dien-3β-ol-16-one as a genuine aglycone (ref. 49). The structures of the two main glycosides, holotoxins A and B (21, 22), from S. japonicus collected near the Japanese coast have been proposed (ref. 50). We reported these structures with some differences in the carbohydrate moiety for holotoxins A_1 and B_1 (23, 24) from the same animals collected on the Soviet coast of the Sea of Japan (refs. 46, 47).

It was suggested earlier (ref. 50) that the carbohydrate components of sea cucumber glycosides may vary depending on the habitat of the animals. On the other hand, as we have noticed in our studies of fifty species of holothurians, the glycoside fractions from animals collected in different areas consist of the same compounds. The quantities of these natural products within each fraction could vary, but their chemical structures are invariable. It is thus doubtful that S. japonicus contains distinguishable glycosides depending on their place of origin. Further investigations will permit more precise conclusions regarding the non-regularity of carbohydrate chain structures of holotoxins isolated from different collections of S. japonicus. We also note that the carbohydrate moieties of holotoxin A_1 and B_1 more closely resemble those of related species of the Stichopodidae family than those of holotoxin A and B.

The appurtenance of glycosides to certain systematic groups of holothurians suggests that these animals may be able to perform biosynthesis of such natural products. Our experiments and the analogous studies carried out by Tursch et al. (refs. 51, 52) on injection of ^{14}C-acetate into a celomic cavity via the body wall of sea cucumbers have suggested this. The acetate was utilized for biosynthesis of aglycones, and the inclusion of radioactivity in the carbohydrate parts was low. On the other hand, the carbohydrate chains of Stichopodidae and Holothuriidae glycosides seem to be formed by the successive junction of definite bioside blocks. Thus, there are three bioside precursors: Qui-β-1,2-Xyl- , Glc-β-1,2-Xyl- and Xyl-β-1,2-Xyl- , which form a glycoside bond with the aglycone. Afterwards, blocks such as 3-O-Me-Glc-β-1,3-Xyl- , 3-O-Me-Glc-β-1,3-Glc and Glc-β-1,3-Glc are attached by β-1,4-glycoside bonds (Fig. 3).

From the several species of the Dendrochirota order studied we succeeded in the isolation and structural elucidation of triterpene glycosides from Far-eastern sea cucumbers Cucumaria japonica, C. fraudatrix and Psolus fabricii. Cucumariosides C_1 (25) (refs. 53, 54), C_1 and C_2 (26, 27) (ref. 55) from C. fraudatrix combine the features of stichoposides and holothurins. Thus, like holothurin A, 25 possesses a tetrasaccharide moiety with a sulphate group at the xylose residue. Like stichoposides, 26 and 27 include endocyclic 7(8)-double bonds in the aglycone parts. However, the presence of a 16β-acetoxy group and of the rare monosaccharide, 3-O-methyl-D-xylose, are characteristic of these substances. Moreover, 26 and 27 contain an odd number of monosaccharides and consequently their biosynthesis utilizes not only bioside carbohydrate precursors but monosaccharide ones.

C. japonica is the second most economically important species of holothurians after S. japonicus. This holothurian is utilized as food in Japan and other countries. The labile $\Delta^{7,8}$-aglycone isomeric to holotoxinogenin as well as a carbohydrate chain similar to those of 26 and 27 have been found in the cucumarioside A_2-2 (28) from this animal (ref. 56).

The more polar cytotoxin (29) has been isolated from P. fabricii by a Canadian group as well as by us (refs. 57-59). Both groups concluded that the genuine aglycone of 29 is holotoxinogenin, and that the carbohydrate chain contains two sulphate groups. However, the determination of the monosaccharide sequence yielded different results. Our Canadian colleagues used FAB-mass-spectrometry and established that there is a xylose-glucose-quinovose-3-O-methyl-glucose sequence. We applied partial acid hydrolysis followed by isolation of the progenins obtained and showed that this sequence in psolusoside is identical with the one in holothurin A as given in Fig. 4.

Therefore, holothurians contain a number of diverse cytotoxic triterpene glycosides, but only Aspidochirota has been attentively examined as a source of these natural products. The least studied group of sea cucumbers

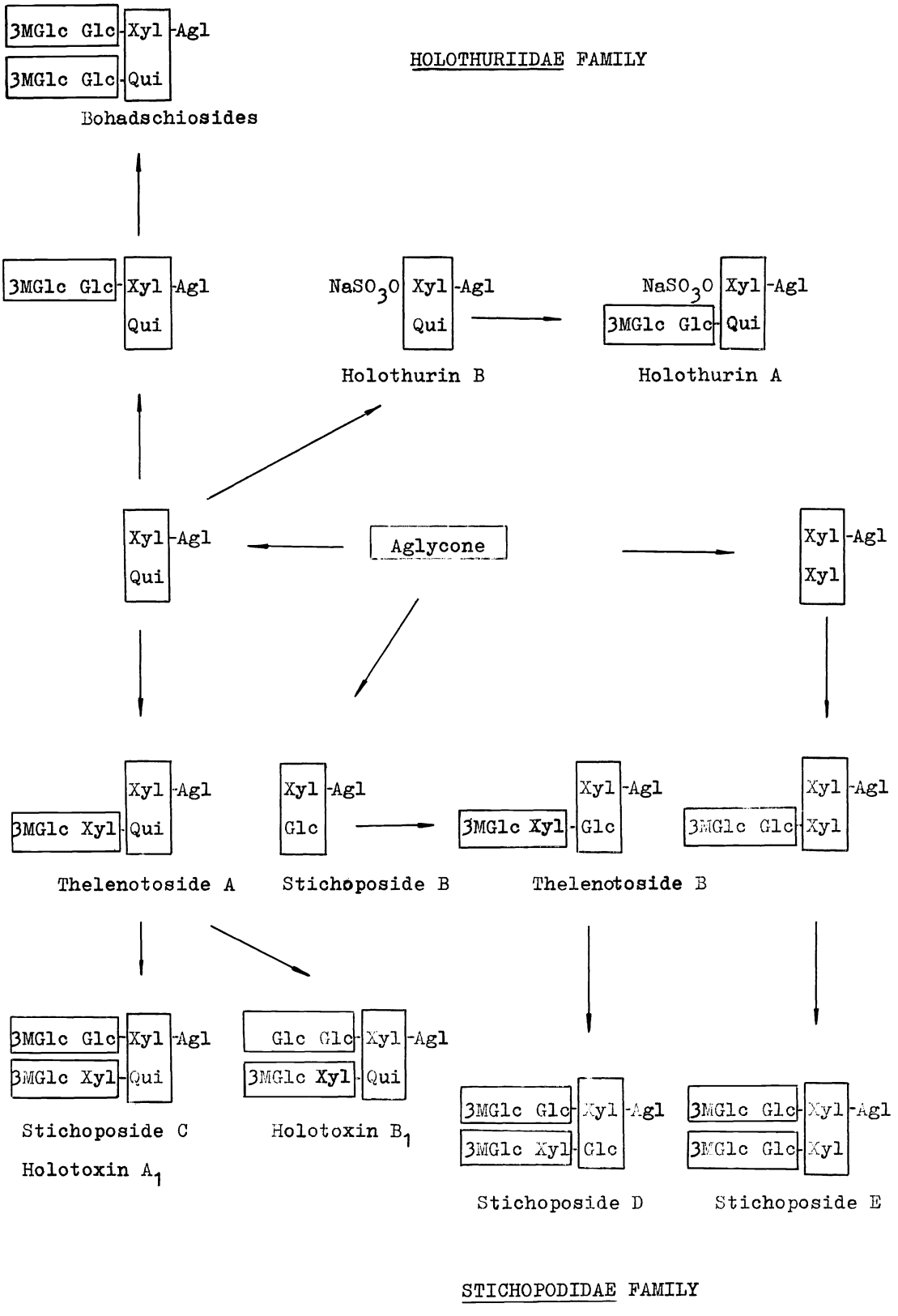


Fig. 3. Hypothetical scheme of biosynthesis of carbohydrate chains in Stichopodidae and Holothuriidae.

is that of Dendrochirota order. Some effort was made to study the glycosides from Apoda order (ref. 60). The remaining groups of sea cucumbers have not yet been investigated in view of isolating such compounds.

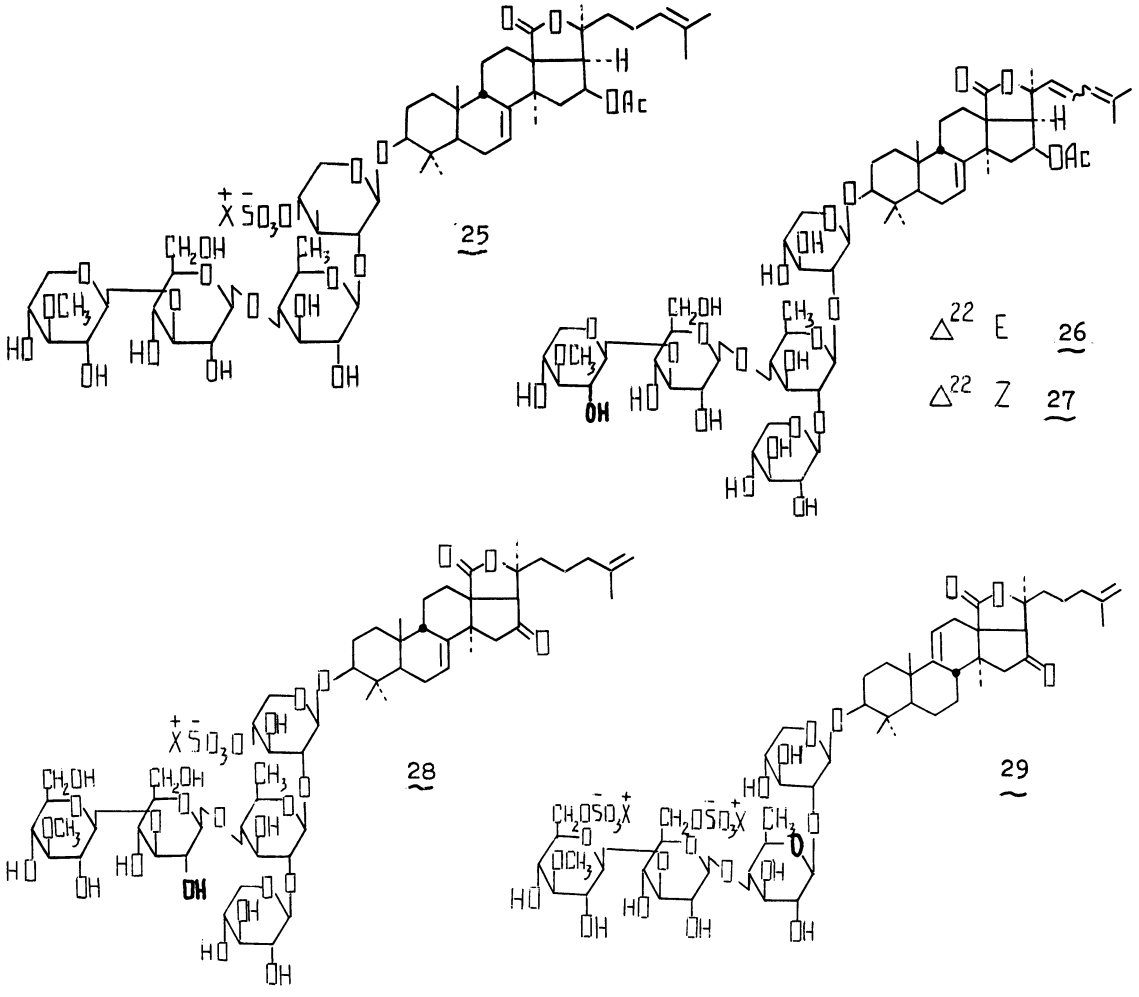
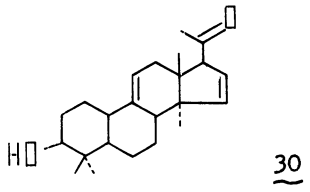


Fig. 4. Structures of the reported glycosides from sea cucumbers of Dendrochirota order.

It may be supposed that sea cucumbers possess new variants of triterpene cytotoxic compounds based on unholostane aglycones. Thus, after acid hydrolysis of Duasmodactyla kurilensis glycosides, we isolated the so-called kurilogenin (30) derived from an unknown glycoside type (ref. 61).



It is well known that holostane triterpene glycosides demonstrate physiological activity, including cytotoxic (ref. 63) and antifungal properties (ref. 62). Such substances from Far-eastern trepang S. japonicus were suggested for use as antifungal agents (ref. 62). However, structure-activity correlations have not been determined in this series. Recently we have established that activity depends on the structure of both the

aglycone and carbohydrate parts of the glycosides (refs. 64, 65). Thus, 25(26)-dihydroholotoxin A possesses strong antifungal action, but this property is absent in 3β-sulphoxy-25(26)-dihydroholotoxinogenin having similar polarity. Comparison of the activity of stichoposides having identical aglycones but different carbohydrate moieties showed: 1) the stronger action of tetrasaccharides than that of hexasaccharides and especially of disaccharides ; 2) the high activity of quinovose-containing glycosides in the series of analogous products (ref. 65). Physiological action of holothurins is a function of the side chain structure of their aglycones. The compounds with open side chains are often more active.

The antifungal properties and other aspects of the physiological activity of these metabolites of sea cucumbers are probably dependent on the glycoside's ability to form complexes with membrane sterols. As a consequence, these natural products may serve as instruments for studying the role of sterols in biological membranes (ref. 66). The ability of low concentrations of these substances to hinder the development of fertilized eggs of sea urchins shows the probable participation of sea cucumber glycosides in interspecies interactions.

STEROID GLYCOSIDES FROM ECHINODERMS

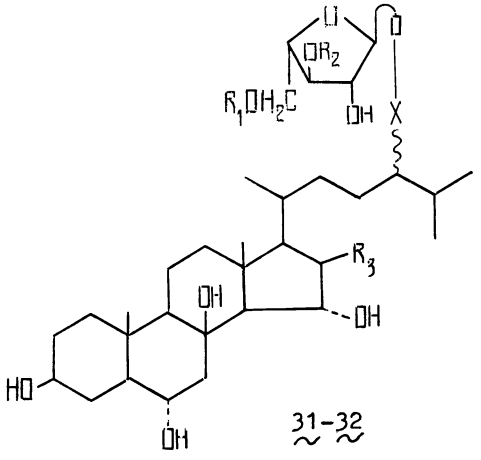
The other interesting group of physiologically active natural products is represented by steroid glycosides. Recently the modern investigations of asterosaponins and steroid glycosides from starfishes were discussed by Minale et al. (ref. 6). One such glycoside series includes glycosylated sterol polyols. These compounds have been recently discovered by Italian investigators (ref. 67) in two starfish species, the Pacific Protoreaster nodosus and the Mediterranean Hacelia attenuata. Several related natural products have been examined in our laboratory. A novel 24-O-glycosylated steroid designated P_1 has been isolated from the starfish Patiria pectinifera, and its structure determined as 5-O-sulphate-24-(α-3-O-methyl-arabinofuranosyl)-3β,6α,8β,15α,24ξ-pentahydroxy-5α-cholestane (31). The obtained glycoside was the first asterosaponin having the sulphate group attached to the carbohydrate moiety (ref. 68). Another steroid derivative (32) has been isolated and purified from the same starfish after mild desulphatation of the crude glycoside fraction (ref. 69). Analysis of the 250 MHz ^{1}H-NMR spectra and chemical transformations of 32 indicated the presence of the stigmastane polyhydroxylated aglycone with an additional hydroxyl group at C-16 and an α-L-arabinofuranosyl residue at C-28. Other structural features of 32 closely resemble those of asterosaponin P_1.

The main bioside component from the Indo-Pacific starfish Culcita novaguinea was named as culcitoside C, and its structure has been elucidated on the basis of chemical and physico-chemical data as 33 (ref. 70). Recently, a similar glycoside from H. attenuata has been described by Minale et al. (ref. 72), but in 33 the terminal monosaccharide was identified as 2,4-di-O-methyl-β-D-xylopyranose.

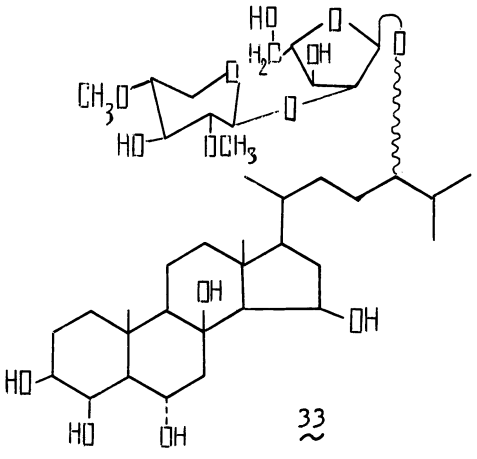
These glycosides are accompanied by related steroid polyols in the starfishes. Four such natural products, 34-37, have been isolated from extracts of P. pectinifera (ref. 71). Three of the four steroid polyols obtained have also been identified by Minale et al. (ref. 6) in P. nodosus and H. attenuata. Among similar natural products examined to date the steroids 34-37 are unique in being the most highly hydroxylated sterols isolated from natural sources.

In contrast to 3β-sulphated oligoglycosides from starfishes, 24-O- and 28-O-glycosylated steroid polyols show only a mild cytotoxic action irrespective of the presence of a sulphate group. As a rule, starfishes having these compounds also contain more active oligoglycosides.

The occurrence of steroid polyols and their derivatives in Asteroidae is of interest from both evolutionary and functional points of view. A participation of these compounds as digestive agents similar to bile acids in the utilization of food has been postulated by us on the basis of their structural similarity to bile alcohols of some fish as well as the presence of these compounds in the liver and pyloriccaeca of starfishes. If such a biological role can be confirmed, it may provide new insights into the chemical evolution of bile acids. The second possible biological value of these unique steroid derivatives obtained from starfishes involves their



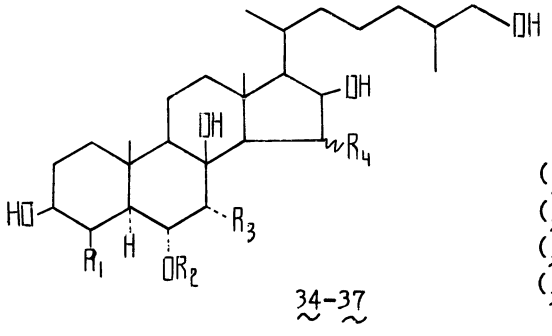
31-32



33

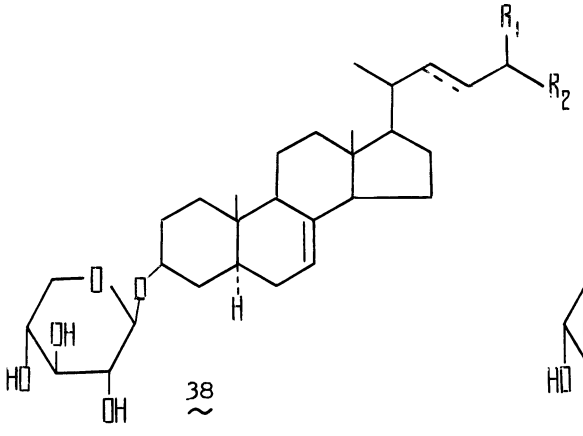
(31) R_1=SO_3Na, R_2=CH_3, R_3=H x=$(CH_2)_0$

(32) R_1=R_2=H, R_3=OH, x=$(CH_2)_2$

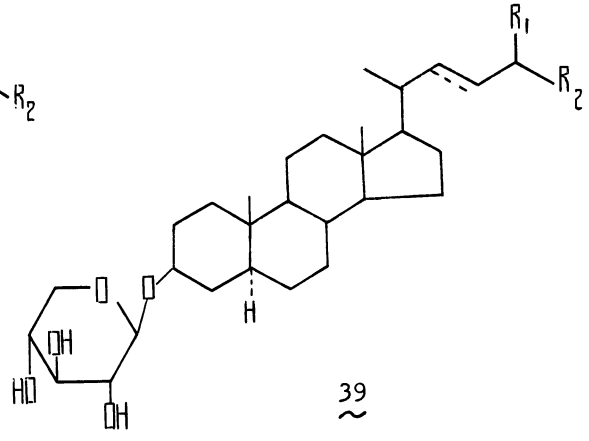


34-37

(34) R_1=R_2=R_3=H, R_4= α-OH
(35) R_1=R_2=H, R_3=OH, R_4= α-OH
(36) R_1=R_3=OH, R_2=H, R_4= α-OH
(37) R_1=R_3=OH, R_2=SO_3Na, R_4= β-OH



38



39

22(23)-dehydro, R_1=H, CH_3, CH_2, C_2H_5
R_2=$CH(CH_3)_2$

22(23)-dihydro, R_1=H, CH_3, CH_2, C_2H_5
R_2=$CH(CH_3)_2$

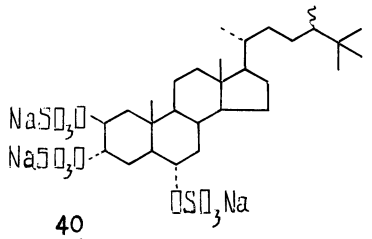
formation as products of sterol catabolism in these animals, necessary to expel any excess of sterols.

The diversity of steroid glycosides in echinoderms does not limit itself to asterosaponins. We have studied β-xylosides (general formulae 38 and 39) from sea cucumbers (refs. 73, 74). The aglycone compounds of these natural products turned out to be stanols, Δ^{7}, Δ^{22} and $\Delta^{7,22}$-sterols. However, β-xylosides as well as Δ^{5} sterol sulphates isolated from echinoderms (ref. 75) are not cytotoxins.

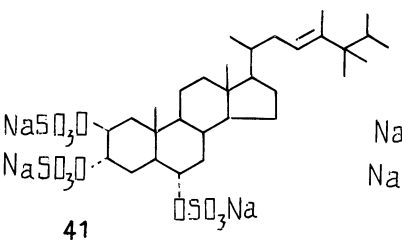
These natural compounds, being architecturally membrane constituents, are associated with the membrane phospholipid bilayers, and contribute to the stabilization of the bilayer phase (refs. 77, 76).

SULPHATED POLYHYDROXYLATED STEROIDS FROM SPONGES

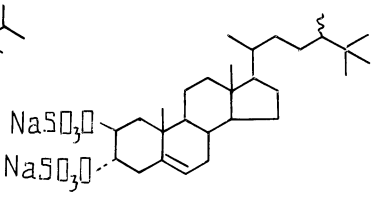
Steroids with two or three sulphated hydroxyl groups in the conventional C_{19} tetracyclic nucleus have been isolated from tropical species of Halichondriidae sponges in the last years. Halistanol sulphate (40) described by Japanese scientists (ref. 78), sokotrasterol sulphate (41) and the desulphated derivative (42) obtained by us (refs. 79,80) all possess cytotoxic action and induce strong foaming in aqueous solutions, similar to triterpene and steroid oligoglycosides from echinoderms.



40



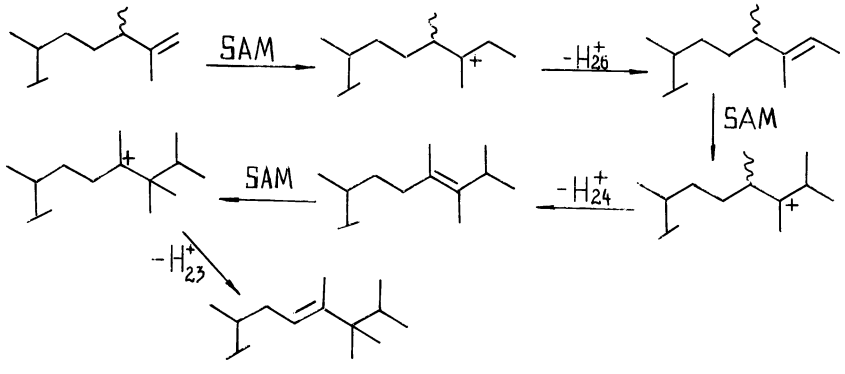
41



42

The physiological activity of the sulphated steroids from sponges as well as of the triterpene glycosides from sea cucumbers is associated with the formation of pores on the biological membranes. In contrast with holothurins, sponge metabolites do not decrease their membranolytic ability in the presence of cholesterol. At the same time, a diminished action on cell membranes was observed for halistanol sulphate or sokotrasterol sulphate when bovine serum albumin was added to the corresponding medium. It may be suggested that some cytotoxic effects of these substances arise from their interactions with protein membrane components.

The first identification of such unusual structural features as two additional methyl groups at C-26 and quaternary alkylation at C-25 raises some interesting questions concerning the biosynthesis of 41. There are several theoretical possibilities for massive bioalkylation of the side chain of sokotrasterol sulphate. Codisterol or epicodisterol could be its precursors in biosynthesis, as it was suggested by Djerassi et al.(ref. 81) for some C_{30} and C_{31} sterols of sponges. The following scheme consists of three successive S-adenosylmethionine (SAM) biomethylations followed by proton elimination from the resulting carbonium ions:



CORRELATION BETWEEN THE PRESENCE OF CYTOTOXINS AND THE STEROL COMPOSITION OF BIOMEMBRANES

It is known that the presence of cytotoxic agents in plants and animals is sometimes bound with biochemical alterations as compared with the related non-toxic species. Such alterations of sterol compositions of biomembranes may be seen in echinoderms and sponges containing either glycosides or polysulphated steroids.

Echinoderms and sponges are probably characterized by the most available set of sterols, even compared with all other terrestrial or marine invertebrates. Indeed, besides Δ^5 sterol, stanols, Δ^7, $\Delta^{7,22}$, Δ^{22}, $\Delta^{24(28)}$ sterols exist in sea cucumbers and starfishes as membrane constituents (ref. 82). In other echinoderms having no cytotoxic glycosides, cholesterol is the predominant sterol. Moreover, sea cucumbers show very low contents of free sterols and high contents of sterol sulphates and β-xylosides (ref. 77). It should be noted that such steroid derivatives as stanols, Δ^7-sterols, β-xylosides or sterol sulphates form complexes with cytotoxic glycosides from echinoderms with greater difficulty than cholesterol does or else they do not form complexes at all (ref. 77).

Therefore, the stability of echinoderm cells to the action of their own cytotoxins results from : 1) the low concentration of free sterols in biomembranes and 2) the absence of sterols being as sensitive to glycosides as are Δ^5-derivatives in these membranes.

Sea cucumbers C. japonica and C. fraudatrix represent an interesting case of replacing membrane cholesterol for other sterols. These animals contain 14 α-methyl-sterol (43) as the main component of their free sterol fractions (ref. 83).

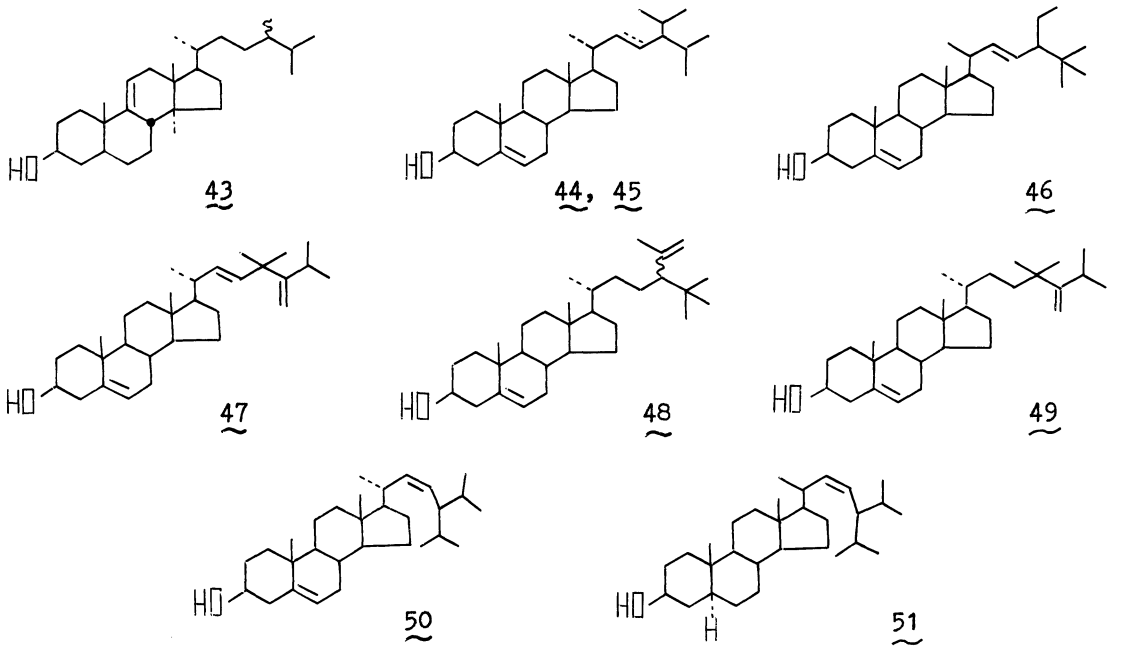


Fig. 6. Structures of some uncommon sterols isolated from echinoderms and sponges containing cytotoxins

Although the sulphated steroids from sponges form no complexes with membrane sterols, the sponge cells possess biomembranes of unusual chemical composition. Sterol and probably fatty acid content distinguish halistanol or sokotrasterol sulphate-containing species from other sponges. Tropical sponges of the Halichondriidae family have been shown to include only uncommon sterols with additional alkylation in the side chains in comparison with conventional sterols in Halichondria panicea from Far-eastern waters. The latter has no cytotoxin. 24-Isopropyl-5-cholesten-3β-ol (44) and 24-isopropyl-5,22-cholestadien-3β-ol (45) have been isolated from Halichondriidae gen. sp., the source of sokotrasterol sulphate (ref. 80). These rare sterols are predominant components of the free sterol fraction

from the sponge. Cholesterol and other conventional sterols have not been identified in this animal. Halichondria sp. contains halistanol sulphate and a novel uncommon sterol, 25-methyl-24-ethyl-5,22-cholestadien-3β-ol (46) as a sole sterol component. A series of unique sterols has been found in the extracts of Halichondria sp.[1] containing the same cytotoxin. All natural products mentioned (47-51) possess side chains with uncommon alkylation patterns as compared with conventional sterols (ref. 84, 85).

It is probable that biosynthesis of cytotoxic sulphated polyhydroxylated steroids and the presence of uncommon sterols are connected with specific microflora of the tropical Halichondriidae species.

Acknowledgement The author thanks Prof. G.B. Elyakov for fruitful discussions.

REFERENCES

1. R.F. Nigrelli, Zoologica, 37, 89-90 (1952).
2. T. Yamanouchi, Bull. Seto Marine Biol. Lab., 4, 184-202 (1952).
3. T. Yasumoto, T. Watanabe and Y. Hashimoto, Bull. Jap. Soc. Scient. Fish., 30, 357-364 (1964).
4. W. Bergmann and D.C. Burke, J. Org. Chem., 21, 226-228 (1956).
5. W. Bergmann and R.J. Feenky, J. Am. Chem. Soc., 20, 1501-1507 (1955).
6. L. Minale, C. Pizza, R. Riccio and F. Zollo, Pure and Appl. Chem., 54, 1935-1950 (1982).
7. Y. Hashimoto, Marine Toxins and Other Bioactive Marine Metabolites, pp. 266-288, Japan Scientific Societies Press, Tokyo (1979).
8. L. Minale, G. Cimino, S. De Stefano and G. Sodano, Fortschritte Chem. Org. Naturst., 33, 1-27 (1976).
9. P. J. Scheuer, Chemistry of Marine Natural Products, pp. 36-44, Academic Press, New York (1973).
10. G. Habermehl and G. Volkwein, Toxicon, 9, 319-326 (1971).
11. G.B. Elyakov, V.A. Stonik, E.V. Levina, V.P. Slanke, T.A. Kuznetsova and V.S. Levin, Comp. Biochem. Physiol., 44, 325-336 (1973).
12. G.B. Elyakov, T.A. Kuznetsova, V.A. Stonik, V.S. Levin and R. Albores, Comp. Biochem. Physiol., 52, 413-417 (1975).
13. V.A. Stonik, I.I. Maltsev, A.I. Kalinovsky and G.B. Elyakov, Khim. Prir. Soedin., 200-203 (1982).
14. V.S. Levin, V.I. Kalinin and V.A. Stonik, Biologia morya, 33-38 (1984).
15. I. Kitagawa, T. Nishino, T. Matsuno, H. Akatsu and Y. Kyogoku, Tetrahedron Lett., 985-988 (1978).
16. I. Kitagawa, T. Inamoto, M. Fushida, S. Okada, M. Kobayashi, T. Nishino and Y. Kyogoku, Chem. Pharm. Bull. (Tokyo), 28, 1651-1653 (1980).
17. I. Kitagawa, T. Nishino and Y. Kyogoku, Tetrahedron Lett., 1419-1422 (1979).
18. I. Kitagawa, M. Kobayashi and Y. Kyogoku, Chem. Pharm. Bull. (Tokyo), 30, 2045-2050 (1982).
19. V.A. Stonik, A.D. Chumak, V.V. Isakov, N.I. Belogortseva, V.Ia. Chirva and G.B. Elyakov, Khim. Prir. Soedin., 522-527 (1979).
20. G.K. Oleynikova, T.A. Kuznetsova, A.I. Kalinovsky, V.A. Stonik and G,B, Elyakov, Khim. Prir. Soedin., 101-103 (1981).
21. V.I. Kalinin, V.A. Stonik, S.A. Avilov and G.B. Elyakov, Khim. Prir. Soedin., 403-404 (1981).
22. G.B. Elyakov, N.I. Kalinovskaya, A.I. Kalinovsky, V.A. Stonik and T.A. Kuznetsova, Khim. Prir. Soedin., 323-327 (1982).
23. V.I. Kalinin and V.A. Stonik, Khim. Prir. Soedin., 215-219 (1982).
24. G.K. Oleynikova, T.A. Kuznetsova, N.S. Ivanova, A.I. Kalinovsky, N.V. Rovnih and G.B. Elyakov, Khim. Prir. Soedin., 464-469 (1982).
25. G.K. Oleynikova, T.A. Kuznetsova, N.V. Rovnih, A.I. Kalinovsky and G.B. Elyakov, Khim. Prir. Soedin., 527-528 (1982).
26. V.I. Kalinin and V.A. Stonik, Khim. Prir. Soedin., 789 (1982).
27. T.A. Kuznetsova, N.I. Kalinovskaya, A.I. Kalinovsky, G.K. Oleynikova, N.V. Rovnih and G.B. Elyakov, Khim. Prir. Soedin., 482-484 (1982).
28. N.S. Ivanova, O.F. Smetanina and T.A. Kuznetsova, Khim. Prir. Soedin., 448-451 (1984).
29. S. Bhatnagar, A. Ahond, B. Dudouet, C. Poupat and P. Potier, Fourth International Symposium on Marine Natural Products, Tenerife, Spain, P-10, (1982).

30. J.D. Chanley, T. Mezzetti and H. Sobotka, Tetrahedron, 22, 1857-1864 (1966).
31. V.A. Stonik, V.F. Sharypov, T.A. Kuznetsova, A.I. Kuznetsova and G.B. Elyakov, Khim. Prir. Soedin., 790 (1982).
32. I. Kitagawa, M. Kobayashi, M. Hori and Y. Kyogoku, Chem. Pharm. Bull. (Tokyo), 29, 282-285 (1981).
33. A.I. Kalinovsky, I.I. Maltsev, A.S. Antonov and V.A. Stonik, Bioorg. Khim., 10, 1655-1663 (1984).
34. A. Clastres, A. Ahond, C. Poupat and A. Intès, Experientia, 34, 973-974 (1978).
35. V.A. Stonik, V.F. Sharypov, A.I. Kalinovsky and G.B. Elyakov, Dokl. Akad. Nauk SSSR, 245, 1133-1134 (1979).
36. G.B. Elyakov, V.A. Stonik, Sh.Sh. Afiatullov, A.I. Kalinovsky, V.F. Sharypov and L.Ja. Korotkih, Dokl. Akad. Nauk SSSR., 259, 1367-1369 (1981).
37. S.G. Ilyin, V.F. Sharypov, V.A. Stonik, G.V. Malinovskaya, N.I. Uvarova and G.B. Elyakov, Structure and properties of polymetallic and metallorganic compounds, Abstracts, Chernogolovka, USSR, 145 (1981).
38. A.I. Kalinovsky, V.F. Sharypov, V.A. Stonik, A.K. Dzizenko and G.B. Elyakov, Bioorg. Khim., 6, 86-89 (1980).
39. A.I. Kalinovsky, V.F. Sharypov, Sh.Sh. Afiatullov, T.A. Kuznetsova, V.A. Stonik and G.B. Elyakov, Bioorg. Khim., 9, 1558-1563 (1983).
40. V.F. Sharypov, A.D. Chumak, V.A. Stonik and G.B. Elyakov, Khim. Prir. Soedin., 181-184 (1981).
41. V.A. Stonik, I.I. Maltsev and G.B. Elyakov, Khim. Prir. Soedin., 624-627 (1982).
42. I. Kitagawa, M. Kobayashi, T. Inamoto, T. Yasuzawa, Y. Kyogoku and M. Kido, Chem. Pharm. Bull. (Tokyo), 29, 1189-1192 (1981).
43. V.A. Stonik, I.I. Maltsev, A.I. Kalinovsky, K. Konde and G.B. Elyakov, Khim. Prir. Soedin., 194-199 (1982).
44. V.A. Stonik, I.I. Maltsev, A.I. Kalinovsky and G.B. Elyakov, First international conference on chemistry and biotechnology of biologically active natural products, 3, 326-329 (1981).
45. I.I. Maltsev, V.A. Stonik and A.I. Kalinovsky, Khim. Prir. Soedin., 308-312 (1983).
46. G.B. Elyakov, I.I. Maltsev, A.I. Kalinovsky and V.A. Stonik, Bioorg. Khim., 9, 280-281 (1983).
47. I.I. Maltsev, V.A. Stonik, A.I. Kalinovsky and G.B. Elyakov, Comp. Biochem. Physiol., 78B, 421-426 (1984).
48. Y.M. Sheikh, C. Djerassi, J. C. S. Chem. Comm., 1057-1058 (1976).
49. V.F. Sharypov, N.I. Kalinovskaya, V.A. Stonik and G.B. Elyakov, Khim. Prir. Soedin., 845-846 (1980).
50. I. Kitagawa, H. Yamanaka, M. Kobayashi, T. Nishino, I. Yosioka and T. Sugawara, Chem. Pharm. Bull. (Tokyo), 26, 3722-3731 (1978).
51. G.B. Elyakov, V.A. Stonik, E.V. Levina and V.S. Levin, Comp. Biochem. Physiol., 52B, 321-323 (1975).
52. A. Kelecom, D. Daloze and B. Tursch, Tetrahedron, 32, 2353-2358 (1976).
53. Sh.Sh. Afiatullov, V.A. Stonik and G.B. Elyakov, Khim. Prir. Soedin., 56-64 (1983).
54. Sh.Sh. Afiatullov, V.A. Stonik and G.B. Elyakov, Khim. Prir. Soedin., 59-64 (1983).
55. Sh.Sh. Afiatullov, A.I. Kalinovsky, V.A. Stonik and G.B. Elyakov, Khim. Prir. Soedin., (in press).
56. S.A. Avilov, L.Ja. Tischenko and V.A. Stonik, Khim. Prir. Soedin., 799-800 (1984).
57. F.-X. Garneau, J.-L. Simard, O. Harvey, J.W. Ap Simon and M. Girard, Can. J. Chem., 61, 1465-1471 (1983).
58. V.A. Kalinin, V.R. Stepanov and V.A. Stonik, Khim. Prir. Soedin., 789-790 (1983).
59. V.A. Kalinin, A.I. Kalinovsky and V.A. Stonik, Khim. Prir. Soedin., 212-217 (1985).
60. T.A. Kuznetsova, N.I. Kalinovskaya, A.I. Kalinovsky and G.B. Elyakov, Khim. Prir. Soedin., (in press).
61. A.I. Kalinovsky, S.A. Avilov, V.R. Stepanov and V.A. Stonik, Khim. Prir. Soedin., 724-727 (1983).
62. S. Shimada, Science, 163, 1462-1464 (1969).
63. M.M. Anisimov, N.G. Prokofieva, L.Ja. Korotkih, I.I. Kapustina and V.A. Stonik, Toxicon, 18, 221-223 (1980).
64. T.A. Kuznetsova, M.M. Anisimov, A.M. Popov, S.I. Baranova, Sh.Sh. Afiatullov, I.I. Kapustina, S.A. Antonov and G.B. Elyakov, Comp. Biochem. Physiol., 73C, 41-43 (1982).
65. I.I. Maltsev, S.I. Stechova, E.B. Shentsova, M.M. Anisimov and G.B. Elyakov, Khim. Pharm. Zhurn., 54-56 (1985).

66. M.M. Anisimov, E.B. Shentsova, V.V. Scheglov, Ju.N. Shumilov, V.A. Rasskazov, L.I. Strigina, N.S. Chetyrina and G.B. Elyakov, Toxicon, 16, 207-218 (1978).
67. R. Riccio, L. Minale, C. Pizza, F. Zollo and J. Pusset, Tetrahedron Lett., 23, 2899-2902 (1982).
68. A.A. Kicha, A.I. Kalinovsky, E.V. Levina, V.A. Stonik and G.B. Elyakov, Tetrahedron Lett., 24, 3893-3896 (1983).
69. A.A. Kicha, A.I. Kalinovsky, E.V. Levina, Ja.V. Raschkes, V.A. Stonik and G.B. Elyakov, Khim. Prir. Soedin., (in press).
70. A.A. Kicha, A.I. Kalinovsky, E.V. Levina, P.V. Andriyaschenko, Khim. Prir. Soedin., (in press).
71. A.A. Kicha, A.I. Kalinovsky, E.V. Levina, V.A. Stonik and G.B. Elyakov, Bioorg. Khim., 9, 975-977 (1983).
72. R. Riccio, L. Minale, S. Pagonis, C. Pizza and J. Pusset, Tetrahedron, 38, 3615-3622 (1982).
73. G.B. Elyakov, N.I. Kalinovskaya, V.A. Stonik and T.A. Kuznetsova, Comp. Biochem. Physiol., 65B, 309-314 (1979).
74. G.B. Elyakov, T.A. Kuznetsova, K. Konde, N.I. Kalinovskaya and O.F. Smetanina, Khim. Prir. Soedin., 799-802 (1979).
75. G.B. Elyakov, S.N. Fedorov, A.D. Chumak, V.V. Isakov and V.A. Stonik, Comp. Biochem. Physiol., 71B, 325-328 (1982).
76. A.M. Popov, N.I. Kalinovskaya, T.A. Kuznetsova, I.G. Agafonova and M.M. Anisimov, Antibiotiki, 656-659 (1983).
77. M.M. Anisimov, D.L. Aminin, Yu.G. Rovin, G.N. Lichatskaya, A.M. Popov, T.A. Kuznetsova, N.I. Kalinovskaya and G.B. Elyakov, Dokl. Akad. Nauk SSSR, 270, 991-993 (1983).
78. N. Fusetani, S. Matsunaga and S. Konosu, Tetrahedron Lett., 22, 1985-1988 (1981).
79. T.N. Makarieva, L.K. Shubina, A.I. Kalinovsky, V.A. Stonik and G.B. Elyakov, Steroids, 43, 267-292 (1983).
80. T.N. Makarieva, L.K. Shubina. A.I. Kalinovsky and V.A. Stonik, Khim. Prir. Soedin., 272-273 (1985).
81. C. Djerassi, Pure and Appl. Chem., 53, 873-890 (1981).
82. N.I. Kalinovskaya, T.A. Kuznetsova and G.B. Elyakov, Comp. Biochem. Physiol., 74B, 597-601 (1983).
83. N.I. Kalinovskaya, A.I. Kalinovsky, T.A. Kuznetsova, V.A. Stonik and G.B. Elyakov, Dokl. Akad. Nauk SSSR, 278, 630-634 (1984).
84. L.K. Shubina, T.N. Makarieva and V.A. Stonik, Khim. Prir. Soedin., 464-467 (1984).
85. L.K. Shubina, T.N. Makarieva, A.I. Kalinovsky and V.A. Stonik, Khim. Prir. Soedin., 232-239 (1985).