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Sea cucumbers triterpene glycosides, the recent progress in structural elucidation and chemotaxonomy

Vladimir I. Kalinin[1,*], Alexandra S. Silchenko[1], Sergey A. Avilov[1], Valentin A. Stonik[1] & Alexey V. Smirnov[2]

[1]*Pacific Institute of Bioorganic Chemistry, Far East Division of the Russian Academy of Sciences, Pr. 100-letya Vladivostoka 159, 690022, Vladivostok, Russia;* [2]*Zoological Institute of the Russian Academy of Sciences, 199164, Saint Petersburg, Russia; *Author for correspondence (Tel: +7-4232-31-11-68; Fax: +7-4232-31-40-50; E-mail: kalininv@piboc.dvo.ru)*

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Abstract

Triterpene glycosides are characteristic metabolites of sea cucumbers (Holothurioidea, Echinodermata). Majority of the glycosides belong to holostane type (lanostane derivatives with 18(20)-lactone). Carbohydrate chains of these glycosides contain xylose, glucose, quinovose, 3-*O*-methylglucose and 3-*O*-methylsylose. During the last 5 years, main investigations were focused on holothurians belonging to the order Dendrochirotida collected in the North Pacific, North Atlantic, Antarctic and in subtropical waters. The glycosides of holothurians belonging to the order Aspidochirotida have also been studied. The most uncommon structural features of carbohydrate chains of new glycosides were: (1) the presence of quinovose as fifth terminal monosaccharide unit and the presence of two quinovose residues; (2) the presence of glucose instead of common xylose as fifth terminal monosaccharide unit; (3) trisaccharide carbohydrate chain; (4) the presence of two 3-*O*-methylxylose terminal monosaccharide units; (5) the presence of sulfate group at C-3 of quinovose residue. New glycosides without lactone or with 18(16)-lactone and having shortened side chains have also been isolated. The presence of 17α and 12α-hydroxyls, which are characteristic for glycosides from holothurians belonging to the family Holothuriidae (Aspidochirotida) in glycosides of dendrochirotids confirms parallel and relatively independent character of evolution of glycosides. All three families belonging to the order Aspidochirotida: Holothuriidae, Stichopodidae and Synallactidae have similar and parallel trends in evolution of the glycosides carbohydrate chains, namely from non-sulfated hexaosides to sulfated tetraosides. Sets of aglycones in glycosides from holothurians belonging to the genus *Cucumaria* (Cucumariidae, Dendrochirotida) are specific for each species. The carbohydrate chains are similar in all representatives of the genus *Cucumaria*.

Introduction

Triterpene glycosides are very common for high plants but their presence in animals is very rare. The presence of triterpene glycosides is characteristic for most of sea cucumbers (Holothurioidea), the class of marine invertebrates belonging to the phylum Echinodermata. The glycosides were also found in some marine sponges. Sea cucumber glycosides are lanostane derivatives and majority of them belong to so called holostane type i.e. having 18(20)-lactone (Figure 1) (Habermehl and Volkvein, 1971; Stonik et al., 1999). During last 10 years several glycosides without such lactone have also been found.

The carbohydrate chains of the sea cucumber glycosides include from two to six sugars and first monosaccharide unit is always xylose. The

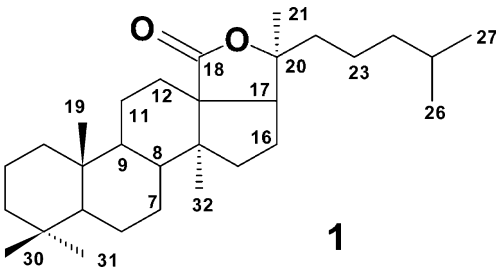


Figure 1. Holostane (**1**).

glycosides contain xylose, glucose, quinovose, 3-*O*-methylglucose and, rarely, 3-*O*-methylxylose. The monosaccharides having 3-*O*-methyl groups are always terminal onces. The glycoside with an *O*-acetyl group at C-6 of a terminal glucose has also been found. Many glycosides have sulfate groups at C-4 of first xylose and at C-6 of glucose and 3-*O*-methylglucose residues (Stonik et al., 1999).

The ability of the glycosides to form complexes with 5,6-unsaturated sterols of target cell membranes determines a wide spectrum of their biological activity including ichthyotoxic action that may effectively protect sea cucumbers against fish predation. Another biological role of the glycosides as reproduction regulators is caused by their ability to increase microviscosity of the oocyte membranes inhibiting Ca^{2+} transport into the cells and thus early oocyte maturation with subsequent synchronization of sea cucumber oogenesis (Kalinin et al., 1996).

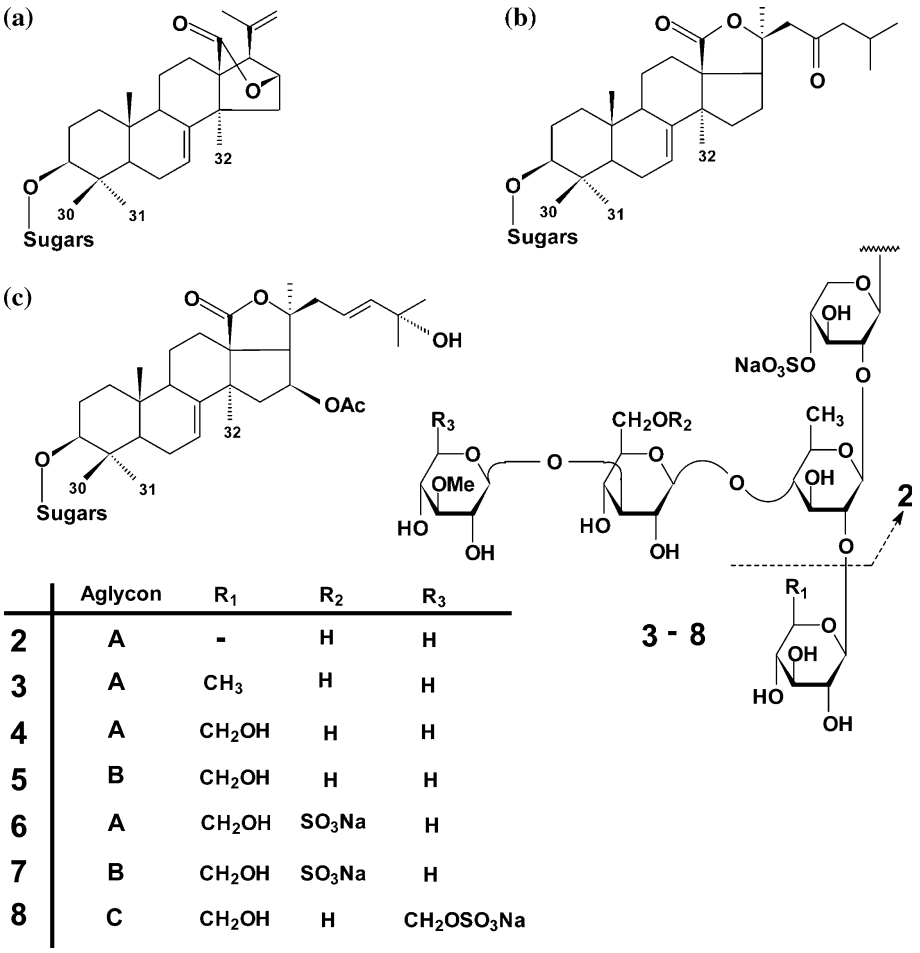
Triterpene glycosides have taxonomic specificity for different species and genera of the sea cucumbers and even for taxa of super-genus level. The glycosides have quite complicated structures and may be distinguished by many relatively independent characters: composition and number of monosaccharide units in the carbohydrate chain, number and positions of sulfate groups attached to monosaccharide units, position of double bond in the cyclic system of an aglycone, number and position of double bonds in the side chain of aglycone and number and different position of hydroxy, epoxy, acetyl and oxo groups in aglycone etc. This circumstance decreases a probability of full coincidence of the glycoside structures for distantly related taxa of sea cucumbers. However it does not exclude such coincidence absolutely due to parallel evolution (Kalinin et al., 1990; Stonik et al., 1999). Information about structures of triterpene glycosides has been used for resolving taxonomic problems in the class Holothurioidea (Levin et al., 1985). For example, taxonomic status of the sea cucumber *Bohadschia graeffei* was revised followed by isolation of this holothurian to a new established genus *Pearsonothuria* using the data concerning the structures of triterpene glycosides (Levin et al., 1984). Taxonomical separation of two North Pacific stichopodids *Apostichopus japonicus* and *Parastichopus californicus* was carried out on the base of chemical data, confirming the necessity of isolating them from the genus *Stichopus* and placing them in one genus (Levin et al., 1986).

This paper reviews the progress in structural elucidation of sea cucumber triterpene glycosides and in its application in chemotaxonomy during last 5 years.

Structures of the glycosides

Glycosides from representatives of the order Dendrochirotida

Family Phyllophoridae. The most extensive chemical studies of triterpene glycosides were carried out on sea cucumbers belonging to the order Dendrochirotida. For example, a series of very interesting from the point of view of their structures relationship mono- and disulfated glycosides was isolated from sea cucumber *Pentamera calcigera* (the subfamily Thyoninae, the family Phyllophoridae) collected in the Sea of Japan (Figure 2). Monosulfated glycosides were presented by cucumarioside G_2 (**2**), earlier isolated from *Eupentacta fraudatrix* (Avilov et al., 1994) and three new glycosides, calcigerosides B (**3**), C_1 (**4**) and C_2 (**5**) (Avilov et al., 2000a). New glycosides contain branched pentasaccharide carbohydrate chains with 3-*O*-methylxylose as a terminal residue that is a rare feature for sea cucumber glycosides. Calcigeroside B (**3**) has quinovose as terminal residue attached to another quinovose. The presence of two quinovose residues in a carbohydrate moiety is unique for sea cucumber glycosides. Calcigerosides C_1 (**4**) and C_2 (**5**) contain terminal glucose attached to quinovose residue, so far this feature was never found in sea cucumber glycosides. Disulfated glycosides were presented by three new glycosides, calcigerosides D_1 (**6**), D_2 (**7**) and E (**8**) (Avilov et al., 2000b). All these three glycosides have a terminal glucose attached to quinovose residue, glycosides **6**



	Aglycon	R_1	R_2	R_3
2	A	-	H	H
3	A	CH_3	H	H
4	A	CH_2OH	H	H
5	B	CH_2OH	H	H
6	A	CH_2OH	SO_3Na	H
7	B	CH_2OH	SO_3Na	H
8	C	CH_2OH	H	CH_2OSO_3Na

Figure 2. Glycosides from *Pentamera calcigera* (**2**–**8**).

and **7** in addition have a terminal 3-*O*-methylxylose, while glycoside **8** has a common terminal 3-*O*-methylglucose sulfated at C-6. It is interesting that glycosides **2**, **3**, **4** and **6** contain an aglycone with an 18(16)-lactone instead of a common 18(20)-lactone and shortened side chain.

Another glycoside containing a rare terminal 3-*O*-methylxylose residue, thyonoside A (**9**) (Figure 3) was isolated from another representative of the family Phyllophoridae, namely *Thyone aurea* collected in Namibia near the Atlantic Shore of South Africa (Bonnard and Rinehart, 2004). It is a disulfated tetraoside.

Family Psolidae. Patagonicoside A (**10**), a disulfated tetraoside with an uncommon aglycone was isolated from *Psolus patagonicus*, representative of the family Psolidae collected in the South Atlantic near the shore of Argentina (Figure 4) (Murray et al., 2001). The aglycone has two hydroxyl groups at 12α and 17α positions that is characteristic for aspidochirotid sea cucumber (the family Holothuriidae). Glycosides having 12α-hydroxy group were isolated earlier from the dendrochirotida sea cucumber *Neothyone gibbosa* (family Sclerodactilidae) (Encarnacion et al., 1989, 1996) but the glycosides with 17α-hydroxyl were never isolated from any dendrochirotid sea cucumbers. The simultaneous presence of 12α- and 17α-hydroxy groups and 7(8)-double bond is reported for the first time.

Family Cucumariidae. The sea cucumbers belonging to the family Cucumariidae were most extensively studied during last years. Four new glycosides, cucumariosides A_2-5 (**11**), A_3-2 (**12**),

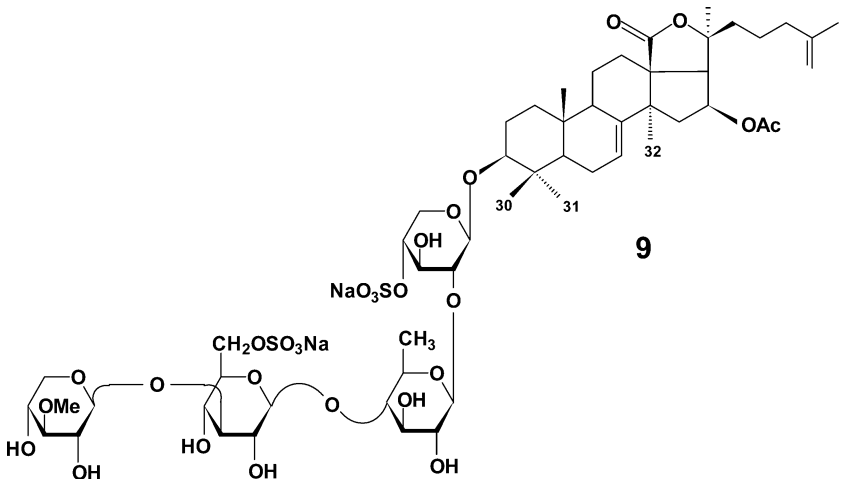


Figure 3. Glycoside from *Thyone aurea* (**9**).

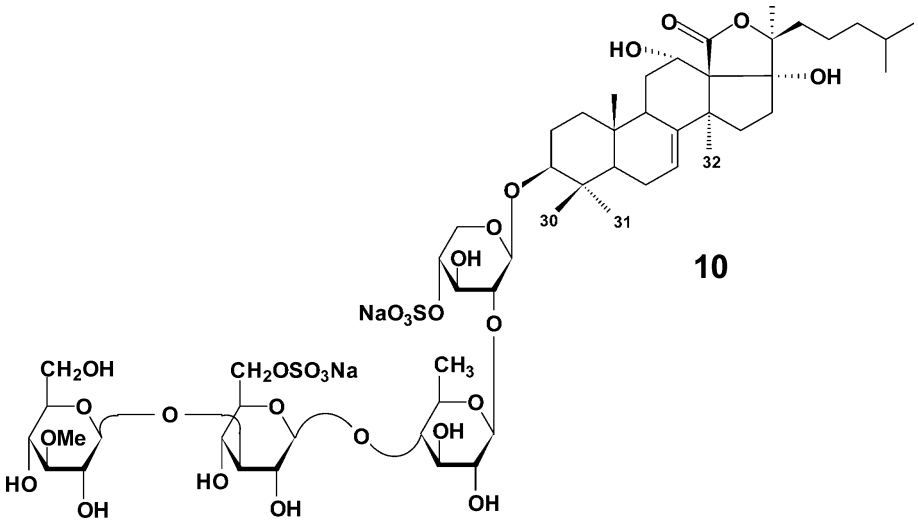


Figure 4. Glycoside from *Psolus patagonicus* (**10**).

A_3-3 (**13**) and isokoreoside A (**14**) with earlier known from *Cucumaria koraiensis* koreoside A (**15**) (Figure 5) were isolated from *Cucumaria conicospermium* collected in the Northern part of the Sea of Japan (Avilov et al., 1997, 2003). The glycosides contain mono-, di- and trisulfated branched pentasaccharide carbohydrate chains, quite common for glycosides of sea cucumbers belonging to the genus *Cucumaria* (Stonik et al., 1999). However glycosides **13**, **14** and **15** have uncommon aglycones without a lactone and with shortened side chain. Moreover, trisulfated glycosides **14** and **15** are isomers differing each other only for the position of a double bond in the aglycone nucleui, having 9(11) for glycoside **14** and 7(8) one for glycoside **15**. Simultaneous presence in one holothurian species of two glycosides distinguished from each other only by this feature is an unique case.

Two trisulfated tetraosides, liouvillosides A (**16**) and B (**17**) (Figure 6) were isolated from the Antarctic sea cucumber *Staurocucumis liouvillei* (Maier et al., 2001). These glycosides differ from each other only by the presence or absence of 24(25)-double bond in the side chain of aglycone.

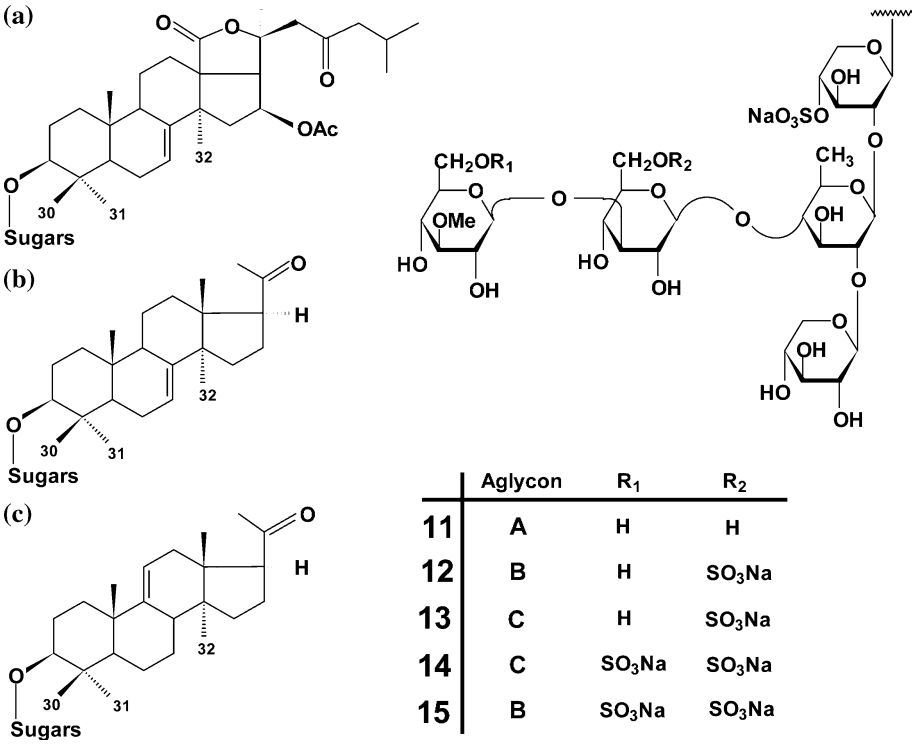


Figure 5. Glycosides from *Cucumaria conicospermium* (**11**–**15**).

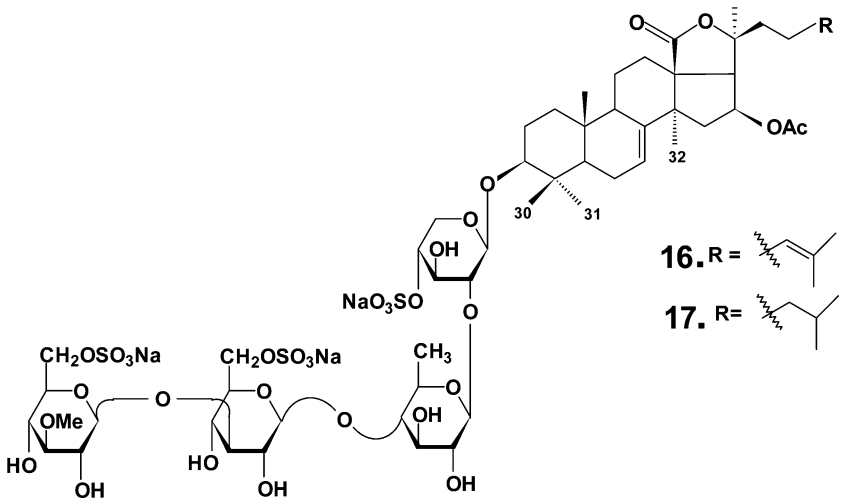


Figure 6. Glycosides from *Staurocucumis liouvillei* (**16**, **17**).

Disulfated glycoside hemoiedemoside A (**18**) and trisulfated glycoside hemoidemoside B (**19**) (Figure 7) were isolated from *Hemioedema spectabilis* collected in the South Atlantic near the Patagonian shore of Argentina (Chludil et al., 2002). The glycosides have the same aglycone and linear tetrasaccharide carbohydrate chain.

Three sulfated tetraosides, intercedensides A (**20**), B (**21**) and C (**22**) (Figure 8) were isolated from *Mensamaria intercedens* collected in the

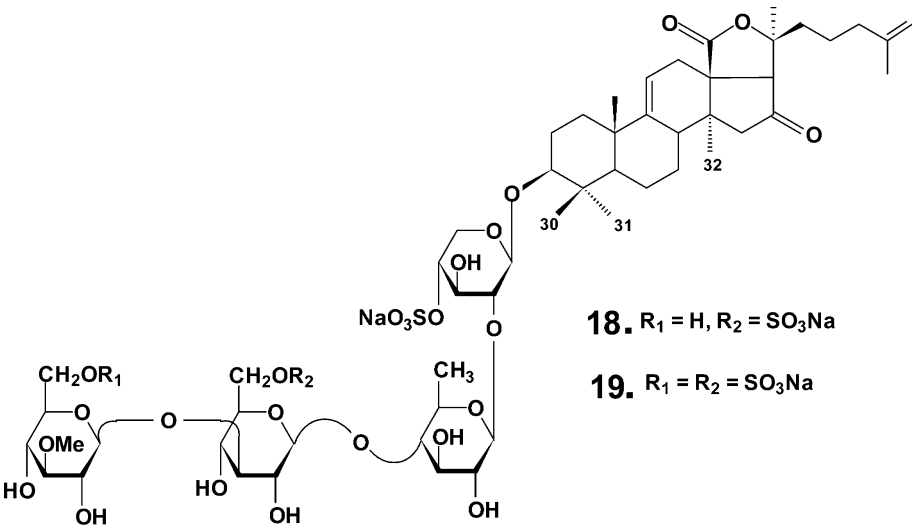


Figure 7. Glycosides from *Hemiodema spectabilis* (**18**, **19**).

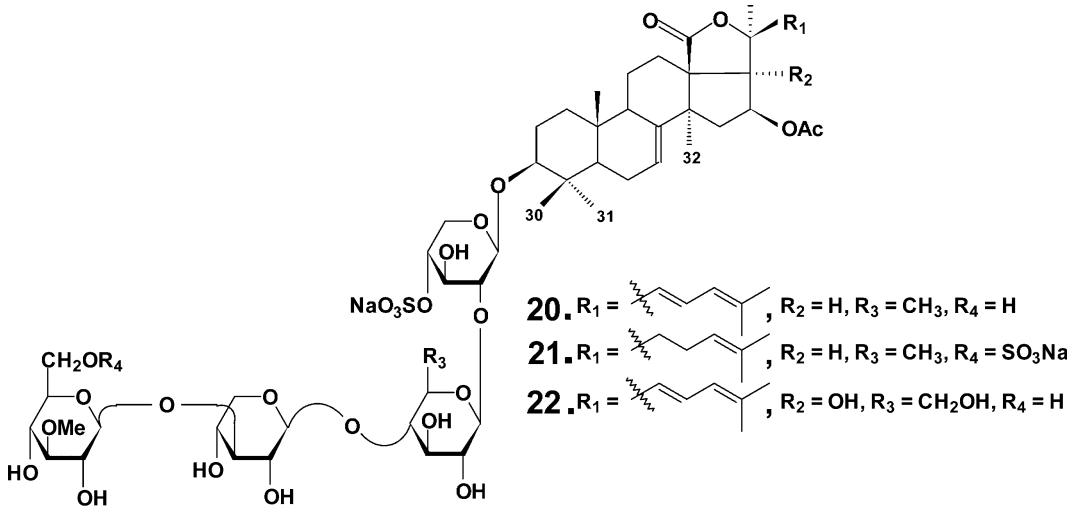


Figure 8. Glycosides from *Mensamaria intercedens* (**20**–**22**).

South Chinese Sea near Chinese shore (Zou et al., 2003a, b). Glycoside **21** has two sulfate groups while glycosides **20** and **22** are monosulfated ones. Intercedensides A (**20**) and C (**22**) have diene fragment in the side chain of the aglycone that is very rare in the sea cucumber glycosides. Analogous structural fragments were found earlier only in the glycosides of *Eupentacta fraudatrix* and *E. pseudoquinquiesemita* (family Sclerodactilidae) (Afiyatullov et al., 1987; Kalinin et al., 1988, 1992). Moreover intercedenside C (22) has 17α-hydroxy group that is not characteristic for dendrochirotid sea cucumber. It is only the second finding of this structural feature in representatives of the order Dendrochirotida.

Glycosides from representatives of the order Aspidochirotida

Family Synallactidae. Glycosides from only three representatives of the order Aspidochirotida were studied during last 5 years. Five non-sulfated glycosides, synallactosides A_1 (**23**), A_2 (**24**), B_1 (**25**) and B_2 (**26**) and C (**27**) (Figure 9) were isolated from the deep water North-Pacific sea cucumber

Synallactes nozawai collected in the Southern part of the Sea of Japan (Silchenko et al., 2002). The glycosides show a significant similarity with glycosides from sea cucumbers belonging to the family Stichopodidae. Moreover, glycoside **23** is identical to 25,26-dehydro derivative of thelenotoside A isolated earlier from *Thelenota ananas* as a mixture with thelenotoside A (Stonik et al., 1982). The presence of 3-*O*-methylxylose in the glycosides **24**, **25** and **26** is a rare structural feature noncharacteristic for glycosides from representatives of the family Stichopodidiae. Compound **24** is also the first glycoside with two terminal 3-*O*-methylxylose residues.

A new disulfated tetraoside pseudostichoposide B (**28**) (Figure 10) was isolated from another deep water North-Pacific synallactid *Pseudostichopus trachus* collected in the Pacific Ocean near Onekotan Island (Kurile Islands) (Silchenko et al., 2004). The glycoside contains a sulfate group at C-4 of the first xylose residue that is quite common and another sulfate group at C-3 of quinovose residue that is an unique case for sea cucumber triterpene glycosides.

Family Stichopodidae. Only one new representative of the family Stichopodidae, *Stichopus mollis*, collected near New Zealand shore, was studied during last 5 years (Moraes et al., 2004). The major component of its glycoside fraction was identified as neothyonidioside C (**29**) (Figure 11) known earlier from sea cucumber *Neothyonidium nagnum* (family Phyllophoridae, order Dendrochirotida) (Zurita et al., 1986). The glycoside **29** has holotoxinogenin as aglycone and carbohydrate chain with xylose as a third monosaccharide unit. However the presence of sulfate group at C-4 of the first xylose residue is an absolutely uncommon feature for glycosides from holothurians belonging to the family Stichopodidae (Kobayashi et al., 1991; Stonik et al., 1999).

Glycosides with doubtful structures or structures requiring more strong confirmation and sea cucumbers with wrong taxonomic identification

Two glycosides named by authors as glycosides 1 (**30**) and 2 (**31**) (Figure 12) were isolated from a sea cucumber identified as "*Telenata ananas* sp." collected in tropical zone of Indian Ocean near Andaman and Nicobar Islands (Hedge et al., 2002). Three member identificator "*Telenata ananas* sp." seems to be quite senseless from the taxonomic point of view. Moreover the genus "*Telenata*" has never been described for sea cucumbers and we may only assume that it means *Thelenota* and relates to the sea cucumber *Thelenota ananas* belonging to the family Stichopodidae, a common species for shallow waters of tropical zone of

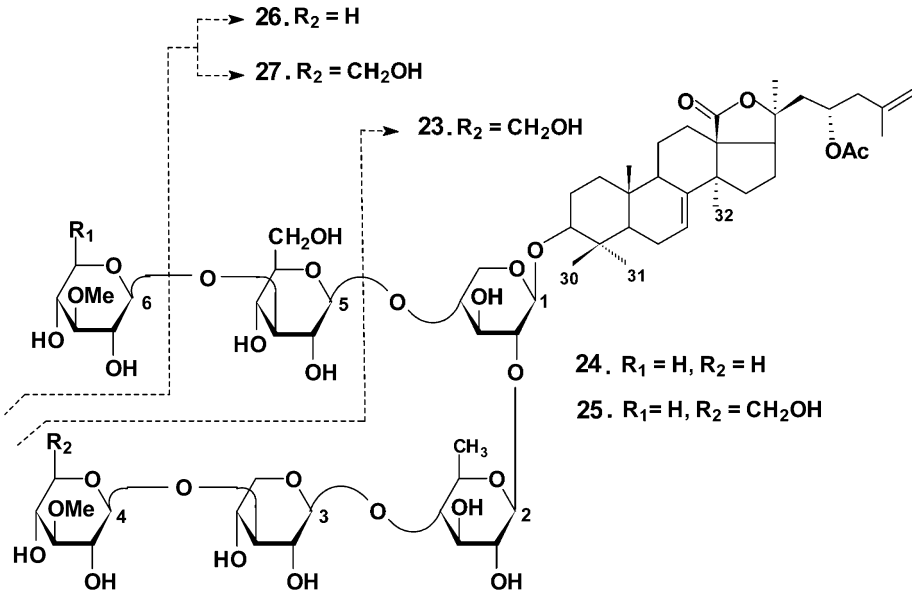


Figure 9. Glycosides from *Synallactes nozawai* (**24**–**27**)..

Pacific and Indian Oceans. Glycosides from this species were studied in details by two independent groups of researchers (Stonik et al., 1982; Kobayashi et al., 1991). Glycoside **31** described by the authors as a "new one" really is known as bivittoside D widespread in sea cucumbers belonging to the genus *Bohadschia* (the family Holothuriidae) (Stonik et al., 1999; Kobayashi et al., 1991). Glycoside **30** is really a new one and contains very uncommon trisaccharide carbohydrate chain. The aglycone of glycoside **30** has 12α-hydroxy-9(11)-ene fragment, 17α-hydroxyl and a 22(25)-epoxy group that is very common for representatives of the family Holothuriidae but not Stichopodidae. Similar glycosides and bivittoside D were never found from *Thelenota ananas*. Most probably those authors has collected a non-identified species belonging to the genus *Bohadschia* or even collected a mixture of two different species belonging to the family Holothuriidae because the region of collection was too wide.

Taxonomic identification of *Cucumaria frondosa* (Cucumariidae, Dendrochirotida) collected near Atlantic Shore of Canada seems to be quite

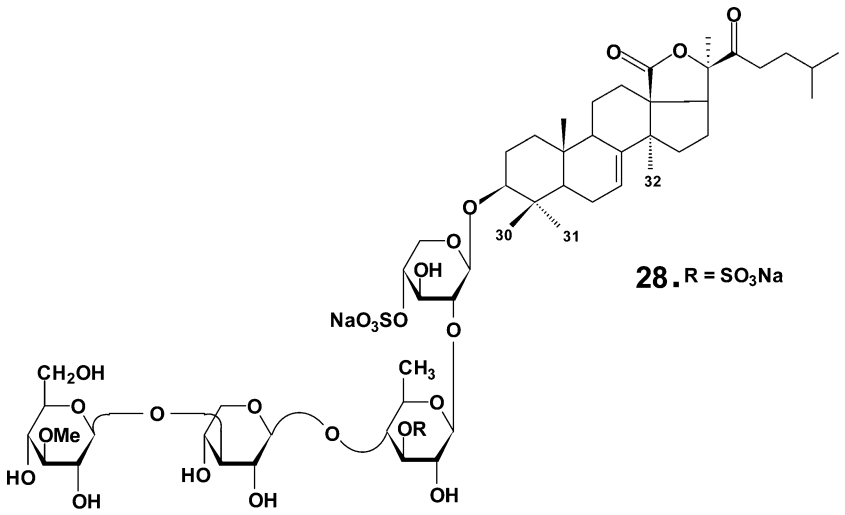


Figure 10. Glycoside from *Pseudostichopus trachus* (**28**).

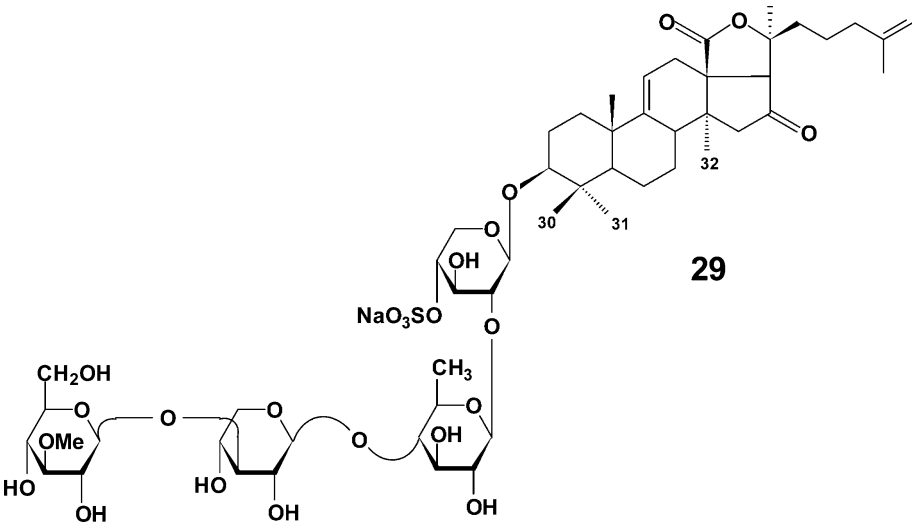


Figure 11. Glycoside from *Stichopus mollis* (**29**).

good however the structures of several glycosides isolated from this sea cucumber (Figure 13) seems to be doubtful (Findlay et al., 1992; Yayli, 2001). It has been described structural elucidation of two monosulfated pentaosides, frondosides E_1 (**32**) and E_2 (**33**) (Yayli, 2001). These glycosides have identical carbohydrate chains and similar aglycones differing from each other by positions of double bonds in nucleus and side chain. The structures were established by analysis of ^{13}C NMR spectra of the mixture of these two substances. It was found that glycoside **32** has 7(8) and 25(26) double bonds and identical to the known cucumarioside A_2-2 from *Cucumaria japonica* (Avilov et al., 1990) while glycoside **33** has 9(11) and 24(25)-double bonds. However, taking into attention the relation between the components of the mixture which was approximately 1:1.2, the reliable identification of the structures by ^{13}C NMR is impossible. The possibility for opposite combinations of double bond positions is absolutely equal. Recently we have found and isolated from *C. frondosa* two similar glycosides; one with 7(8) and 24(25)-double bonds and another with 9(11) and 25(26)-double bonds, correspondingly (Silchenko, 2005). Hence, structures suggested by Yayli (2001) seem to be doubtful.

The structure of one trisulfated pentaosides, frondoside F (**34**) isolated from *C. frondosa* also seems to be doubtful (Yayli, 2001). Glycoside **34** has 18(22)-lactone instead of 18(20)-lactone and a saturated polycyclic nucleus in the aglycone moiety. These structural features are unprecedent ones in sea cucumber glycosides. Although the author mentioned the use of most modern

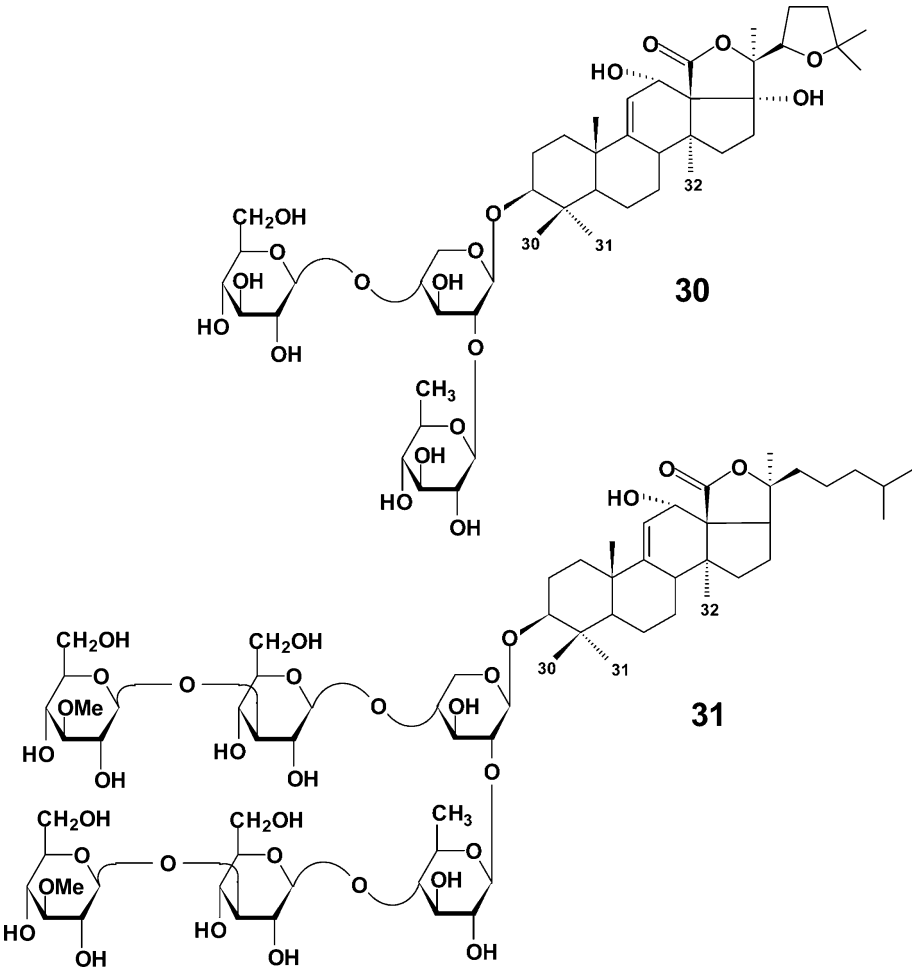


Figure 12. Glycosides from "*Telenata ananas* sp." (**30**, **31**).

physicochemical methods of structural elucidation, the experimental data raise many questions. Indeed, there was not a quasi-molecular ion in the FAB mass spectrum. There was not a correlation between protons at C-8 and C-9 in $^{1}H-^{1}H$-COSY spectrum, and the interpretation of NOESY data was not clear. A simple explanation could be that author was working with a very complicated mixture of glycosides having both 7(8) and 9(11)-double bonds whose signals were not accumulated in the ^{13}C NMR spectra. Earlier this group has described the structural elucidation of so called "frondecaside" (**35**), hexasulfated decaoside (Findlay et al., 1992). However, peculiarities of isolation of this compound, namely its adsorption on hydrophobic adsorbent Amberlit XAD-2 followed by elution with methanol seems to be strange for the proposed structure. The authors did not present any decisive spectral data which confirmed the proposed structure, namely molecular peak in a mass spectrum. Our own experience in studying trisulfated pentaosides of *C. frondosa* (Avilov et al., 1998) suggests that frondecaside'' may be a very complicated mixture of glycosides having the same carbohydrate chains and different aglycones. It may give an accumulation of signals of the carbohydrate chains in the ^{13}C NMR spectrum but not corresponding signals of aglycones due to low concentrations of each glycoside constituents in the mixture.

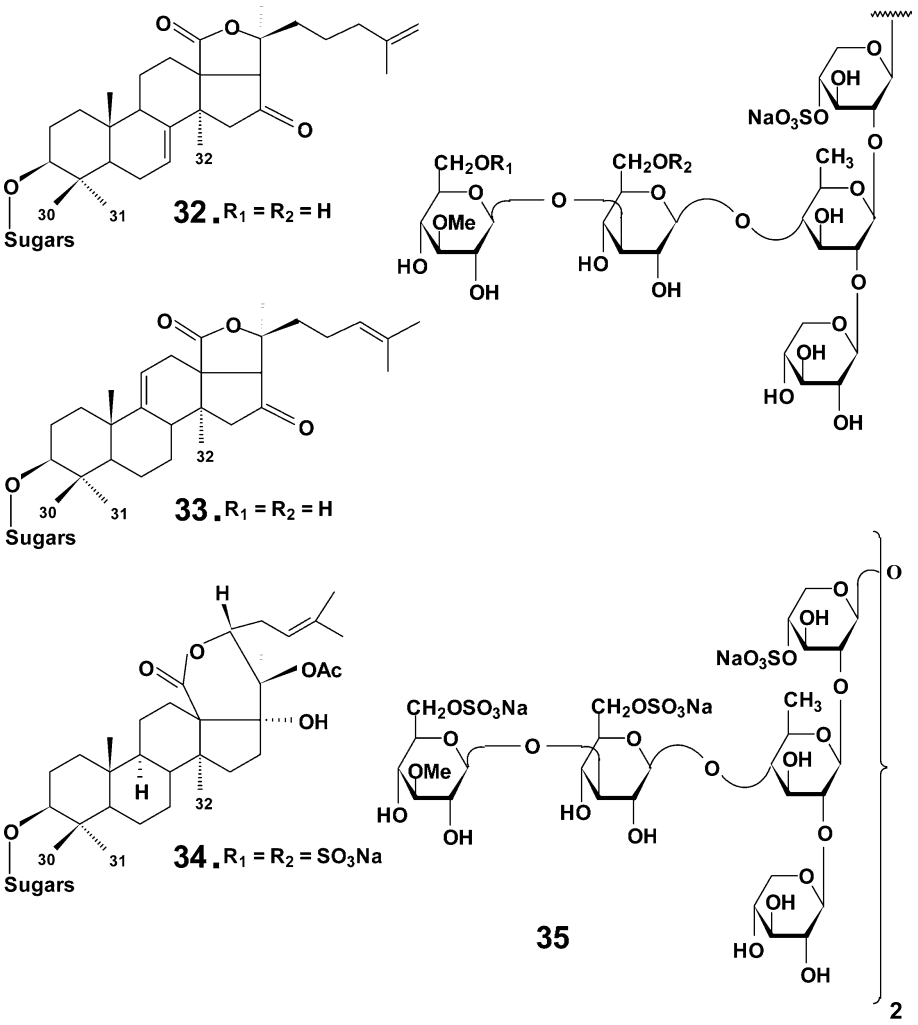


Figure 13. Doubtful structures of some triterpene glycosides (**32**–**34**) and "frondecaside" (**35**) from *Cucumaria frondosa*.

The structure of thyonoside B (**36**) isolated from *Thyone aurea* (Phylophoridae, Dendrochirotida) (Figure 14) also seems to require additional confirmation (Bonnard and Rinehardt, 2004). In this glycoside, terminal 3-*O*-methylxylose is attached to C-4 of third monosaccharide unit (xylose) instead of C-3, as it was found in all previously described sea cucumber glycosides without exception. The evidence is only poor by interpreted by 2D NMR data. Namely, the interpretation of the signals of a third monosaccharide unit (xylose) in ^{13}C NMR is not correct and the scheme of NOE and HMBC correlations is also absent. We believe that the proposed structure requires additional confirmation by simple periodate oxidation followed by monosaccharide analysis or methylation.

The structures of intercedensides A–C (**20**–**22**) from *Mensamaria intercedens* already discussed above (Figure 8) seem to be correct but in the original work (Zou et al., 2003a) all of them were published with very strange error, namely with an excessive CH_2 group inserted between C-4 of first xylose residue and a sulfate group. Fortunately, the structural elucidation of intercedenside B (**21**) was already published earlier (Zou et al., 2003a) although this glycoside was named as "compound 1". In this work the structure of carbohydrate chain was published absolutely correctly however C-21 methyl group in the aglycone was shifted to C-22 position. Simple combination of the structures from both these articles and analysis of systematic names of corresponding compounds allows us to understand and present the corrected structures in figure 8.

Recent achievements in taxonomic use of sea cucumber triterpene glycosides

Order Dendrochirotida

Family Cucumariidae. It was found that species belonging to the genus *Cucumaria* (Cucumariidae, Dendrochirotida) may be clearly distinguished by the species specific set of aglycones in the glycosides (Avilov et al., 2004). Structures of carbohydrate chains branched at the quinovose residue, sulfated at the first xylose residue and containing, as a rule, five monosaccharide units and having xylose, 3-*O*-methylglucose, glucose and 6-*O*-acetylglucose as terminal monosaccharide units are quite similar for all the glycosides isolated from species of this genus. Such set of carbohydrate chains is characteristic for all the studied species of this genus. Glycosides with trisulfated pentaoside carbohydrate chains were isolated from all the *Cucumaria* spices studied (see, for example, Figure 5). Hence the presence of this type carbohydrate chain may serve as a reliable taxonomic character of the genus.

Structures of the carbohydrate chains of glycosides from other genera of the family

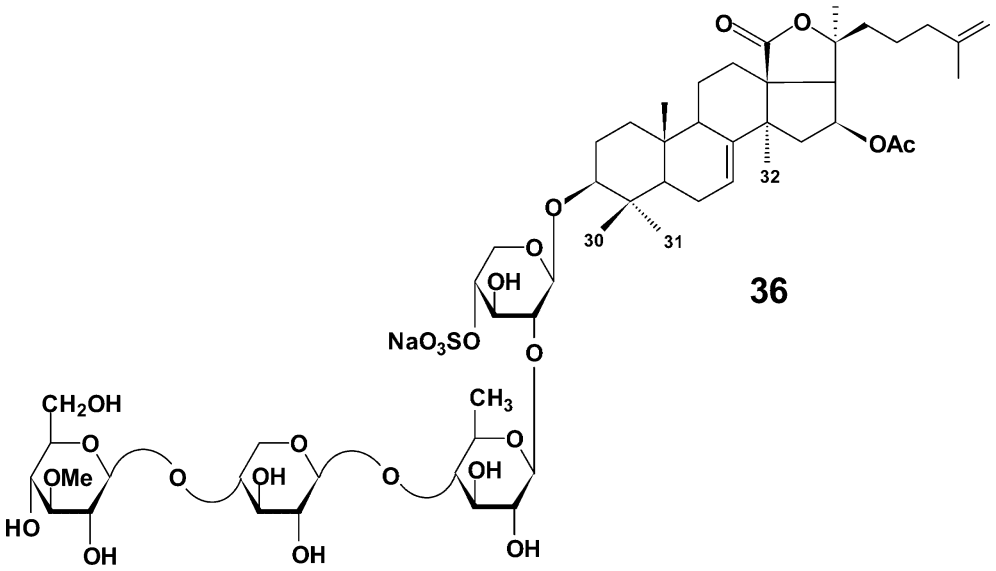


Figure 14. Doubtful glycoside form *Thyone aurea* (**36**).

Cucumariidae differ from those of species belonging to the genus *Cucumaria*. This validates their earlier separation from the genus *Cucumaria* made by taxonomists earlier.

Due to difference in aglycone moieties, sets of glycosides of the species belonging to the genus *Cucumaria* are species specific for each studied species. This is significantly different from the situation concerning glycosides of species belonging to the order Aspidochirotida, where the structures are specific for genera or even taxa above the genus level.

Order Aspidochirotida

Family Stichopodidae. The most significant recent achievement in sea cucumber chemotaxonomy is the use of triterpene glycosides in reclassification of New Zealand sea cucumber *Stichopus mollis* (the family Stichopodidae, the order Aspidochirotida) (Moraes et al., 2004). All the holotoxins, thelenotosides and stichoposides, triterpene glycosides isolated earlier from sea cucumbers belonging to the family Stichopodidae, lack a sulfate group. By the recent time the major part of representatives of the family Stichopodidae characteristically have hexaosides as major components in the glycoside fractions. Based on their glycoside composition all the studied representatives of the family Stichopodidae were divided into two groups. The first group comprised species containing stichoposides and thelenotosides and included *Stichopus chloronotus*, *Stichopus hermanni*, *Astichopus multifidus*, *Thelenota ananas* and *Thelenota anax*. The aglycones of thelenotosides and stichoposides are holostane derivatives having 7(8)-double bond and 23-*O*-acetyl group. The second group comprised species containing holotoxins and includes *Apostichopus japonicus* and *Parastichopus californicus*. Holotoxins are holostane derivatives having 9(11)-double bond and 16-oxo group.

Surprisingly, the major component of the glycoside fraction from *Stichopus mollis*, neothyonidioside C (**29**) having four sugars and sulfate group, is significantly different from the triterpene glycosides from *Stichopus chloronotus*, typical species of the genus, and from all other representatives of the family Stichopodidae studied so far (Figure 11). Because *S. mollis* has also significant morphological difference from representatives of the genus *Stichopus* and other stichopodid sea cucumbers it was separated into a new genus *Australostichopus* (Moraes et al., 2004).

The presence of glycoside **29** previously isolated from *Neothyonidium magnum*, representative of the order Dendrochirotida, in *Australostichopus mollis*, a representative of the order Aspidochirotida, is only second instance of structurally identical glycosides isolated from representatives of different orders of sea cucumbers. In the first case, a glycoside named pervicoside A isolated from *Holothuria pervicax* (Kitagawa et al., 1989) (the family Holothuriidae, the order Aspidochirotida) was found in *Neothyone gibbosa* (Encarnancion et al., 1989) and mamed as neothyoside A (the family Sclerodactilidae, the order Dendrochirotida).

Family Synallactidae. Glycosides of *Synallactes nozawai* (Figure 9) have significant similarities to those isolated from sea cucumbers belonging to family Stichopodidae (Kobayashi et al., 1991; Stonik et al., 1999). Indeed, the aglycone of synallactosides is identical to that of glycosides isolated from members of genera *Astichopus*, *Stichopus* and *Thelenota*. Several structural features of the carbohydrate chain of the glycosides isolated from *S. nozawai* were also found in glycosides from the family Stichopodidae; for example, the presence of a hexasaccharide branched carbohydrate chain as in glycosides **24** and **25**, the presence of a xylose residue as the third monosaccharide unit, and the absence of sulfate groups. As we have noted above, glycoside **23** had been previously found in *Thelenota ananas*. Taking into consideration that there is a relationship between the structure of glycosides and systematic position of the corresponding sea cucumbers (Stonik et al., 1999), these similarities undoubtly indicate phylogenetic closeness between families Synallactidae and Stichopodidae. However, synallactosides can be distinguished from stichopodid glycosides by the presence of 3-*O*-methylxylose terminal residues. Furthermore, the odd number of monosaccharide units present in glycoside **26** and **27** has not been previously found in glycosides from Stichopodidae. In our opinion, the structural peculiarities of glycosides of Stichopodidae and Synallactidae possibly arose in result of parallel and independent evolution of these closely related taxonomical groups of sea cucumbers.

Traditionaly, three families Synallactidae, Stichopodidae and Holothuriidae are included in the order Aspidochirotida. Some morphological characteristics of the family Synallactidae indicate that this family may be regarded as the most primitive in the same order. Some morphological characteristics also suggest the existence of a closer phylogenetic relationship between Synallactidae and Stichopodidae than between Synallactidae and Holothuriidae (Silchenko et al., 2002). The structural similarity of the glycosides from these two families confirms its close relationship. Finally, the presence of 3-*O*-methylxylose residues in the glycosides in synallactids which were reported only from some species of the more ancient order Dendrochirotida, confirms that Synallactidae is the most primitive family of the Aspidochirotida.

Another studied representative of the family Synallactydae is *Pseudostichopus trachus* (Silchenko et al., 2004) (Figure 10). Pseudostichoposide B (**28**) isolated from this species and discussed above has two sulfate groups including an uncommon sulfate at C-3 of quinovose residue. Another component of the glycoside fraction isolated earlier is pseudostichoposide A differing from glycoside **28** only by the absence of a sulfate group at quinovose (Kalinin et al., 1989).

The taxonomic position of the genus *Pseudostichopus* was discussed in different publications. Some authors included *Pseudostichopus* in the family Gephyrothuriidae, the others in the family Synallactidae of the order Aspidochirotida (O'Loughlin, 1998). We refer *Pseudostichopus* to the family Synallactidae following Deichmann (1940), Pawson (1982) and O'Loughlin (1998).

Indeed, the obtained chemical data may speak about the phylogenetic closeness of *P. trachus* and other representatives of the family Stichopodidae. Some chemical characters of pseudostichoposide B and A, namely the presence of tetrasaccharide linear sugar moiety with xylose residue as the third sugar, incidental for glycosides from *Synallactes nozawai* may confirm the similarity between *Pseudostichopus* and *Synallactes*, the type genus of the family Synallactidae. Nevertheless, the presence of sulfate groups in the glycosides of *Pseudostichopus trachus* shows the possible apartness of the genus *Pseudostichopus* within the family.

Hence we can see that all the families of Aspidochirotida have similar trends in variability of carbohydrate chains. There are two main groups of carbohydrate chains in this order: (1) non-sulfated hexasaccharide branched ones characteristic for sea cucumbers belonging to genera *Bohadschia* (family Holothuriidae), *Stichopus*, *Astichopus*, *Thelenota*, *Parastichopus* and *Apostichopus* (family Stichopodidae) and *Synallactes* (family Synallactidae) and (2) sulfated linear tetrasaccharide ones characteristic for sea cucumbers belonging to the genera *Holothuria*, *Actinopyga*, *Pearsonothuria* (Holothuriidae), *Australostichopus* (Stichopodidae) and *Pseudostichopus* (Synallactidae). Such taxonomic distribution of glycosides reflects parallel evolution of carbohydrate chains in all the families of Aspidochirotida.

Recent achievements in studying of biological activities of sea cucumber triterpene glycosides

The main data concerning biological activities and biological role of the sea cucumber triterpene glycosides were described in the review we already cited (Kalinin et al., 1996). During last 5 years many articles concerning elucidation of chemical structure cited above included the information about antitumor, antifungal and other kinds of cytotoxic activity, quite common for this class of natural products. It is very interesting finding that trioside **30** and hexaoside (**31**) (bivittoside D) isolated from not correctly identified sea cucumber (Hegde et al., 2003) exhibited inhibitory activity (K_i) of 30 and 5μM, respectively, in a chemokine receptor subtype 5 (CCR 5) assay because CCR 5 receptor is most commonly used receptor by HIV-1 strains and is thought to be important in viral transmission. Saponin **30** having only three monosaccharide residues is significanly lesser active than glycoside **31** possessing linear tetrasaccharide fragment in the carbohydrate chain. Such the data correlate with dependence of common cytytoxic action of sea cucumber triterpen glycosides and their structures (Kalinin, 2000). The inhibitory doses are also comparable with cytotoxic ones. Hence these very interesting data may not have a practical significance.

A series of individual saponins from *Cucumaria japonica* showed good immunostimulant activity (Aminin et al., 2001). Intraperitoneal injection of some saponins in subtoxic doses (0.2–20 ηg per mouse) induced macrophage lysosomal activity in a dose dependent manner (up to 250% of control). It was also showed that the most effective immu-

nostimulants are monosulfated glycosides but di- and tri-sulfated are immunodepressants. Hence it is very significant to have a pure standard glycoside preparation in order to have a guaranteed and stable immunostimulant effect. Thus some sea cucumber triterpene glycosides may be used as effective immunostimulants.

Conclusions

A series of successes in chemotaxonomic use of triterpene glycosides in sea cucumbers was achieved during recent 5 years. It was found that different species belonging to the genus *Cucumaria* have species specific set of aglycones, but the structures of carbohydrate chains are more conservative and quite characteristic for all the *Cucumaria*. On the other hand, carbohydrate chain structures are different in glycosides of sea cucumbers belonging to other genera of Cucumariidae. It was found that New Zealand sea cucumber *Stichopus mollis* contains a sulfated tetraoside as a major component of the glycoside fraction. That is not characteristic for all other representatives of Stichopodidae. Based on chemical and morphological characteristics this species was separated into a new genus *Australostichopus*.

Similarity in glycoside structures of families Synallactidae and Stichopodidae was found. This correlates with morphological closeness of these families. And, at last, it was showed that all the families of Aspidochirotida contain glycosides with both hexasaccharide non-sulfated carbohydrate chains and sulfated tetrasaccharide carbohydrate chains that confirms parallel character of the carbohydrate chains evolution in Synallactidae, Stichopodidae and Holothuriidae.

During these years many glycosides with uncommon structural features were found. Among such structural features there are: (1) the presence of quinovose as fifth terminal monosaccharide unit and the presence of two quinovose residues; (2) the presence of glucose instead of common xylose as fifth terminal monosaccharide unit; (3) trisaccharide carbohydrate chain; (4) the presence of two 3-*O*-methylxylose terminal monosaccharide units; (5) the presence of sulfate group at C-3 of quinovose residue. New non-holostane glycosides i.e. without lactone or with a 18(16)-lactone and having shortened side chains also were isolated. The presence of 17α- and 12α-hydroxyls, which are characteristic for glycosides from sea cucumbers belonging to the family Holothuriidae (order Aspidoschirotida), in some aglycones of glycosides from representatives of the order Dendrochirotida is very interesting and confirm parallel and relatively independent character of evolution of glycosides.

The last findings clearly demonstrate that sea cucumber triterpene glycosides remain as very interesting field for search of new structural variants of the glycosides despite of more than 40 years history of their chemical investigation.

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