Marine Natural Products. XXVII.¹⁾ Distribution of Lanostane-Type Triterpene Oligoglycosides in Ten Kinds of Okinawan Sea Cucumbers

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Triterpene-oligoglycoside constituents in ten sea cucumber species inhabiting Okinawan coral reefs were investigated. Several antifungal oligoglycosides were isolated from six species [stichlorosides A₁ (15), A₂ (16), B₁ (17), B₂ (18), C₁ (19), and C₂ (20) from *Thelonota ananas* (baika-namako in Japanese) and *Stichopus hermanni*; 15, 17, and 19 from *Thelonota anax*; bivittosides C (13) and D (14) from *Bohadschia argus* (janome-namako); holothurin A (6) from *Holothuria edulis* (akamishikiri); and 6 and echinoside A (10) from *Bohadschia graeffei* (kurote-namako)]. Furthermore, several oligoglycosides were isolated in their desulfated forms from four species [6 and 10 from *Holothuria axiologa*; holothurin B (4), 6, echinoside B (8), and 10 from *Holothuria atra* (kuro-namako); 6, 10, and 24-dehydroechinoside A (24) from *Holothuria scabra* (haneji-namako); and 8, 10, 24-dehydroechinoside B (22), and 24 from *Actinopyga mauritiana* (kuriiro-namako)]. 24-Dehydroechinoside B (22) is a new lanostane-type triterpene oligoglycoside.

Keywords sea cucumber; lanostane-type triterpene oligoglycoside; saponin; holotoxin; holothurin; echinoside; bivittoside; stichloroside; 24-dehydroechinoside

In 1973, Elyakov *et al.* surveyed the chemical constituents of thirtyfour Pacific sea cucumber species collected in the islands of the West Pacific Ocean and discovered that triterpene saponins were widely distributed in Holothuroidea.²⁾ In 1974, we isolated two antifungal saponins named holotoxins A (1) and B (2), which exhibited significant antifungal activities, from the sea cucumber *Stichopus japonicus* (ma-namako in Japanese) and in 1978 determined the full structures of these lanostane-type triterpene oligoglycosides, in what was the first elucidation of the full structure of a sea cucumber saponin.³⁾

Since then, we have searched for new antifungal saponins from various sea cucumbers.^{4–10)} We have so far isolated nineteen then-new lanostane-type triterpene oligoglycosides from various sea cucumbers and elucidated their chemical structures: holothurins A (6)^{4a,b)} and B (4)^{5a,b)} from *Holothuria leucospilota* BRANDT (nisekuro-namako), echinosides A (10) and B (8)^{6a,b)} from *Actinopyga echinites* JAEGER, bivittosides A (11), B (12), C (13), and D (14)^{7a,b)} from *Bohadschia bivittata* MITSUKURI (futasuji-namako), and stichlorosides A₁ (15), A₂ (16), B₁ (17), B₂ (18), C₁ (19), and C₂ (20)^{8a,b)} from *Stichopus chloronotus* (BRANDT) (shikaku-namako). We have also isolated several new saponins in their desulfated forms and elucidated their chemical structures [holothurin A (6) and 24-dehydroechinoside A (24)⁹⁾ from the Bahamean sea cucumber *Actinopyga agassizi* SELENKA and pervicosides A (25), B (26), and C (27)¹⁰⁾ from *Holothuria pervicax* SELENKA (torafu-namako)].

In our continuing search for new bioactive marine natural products,¹¹⁾ we have surveyed the distribution of oligoglycosidic constituents in ten more species of sea cucumbers collected in Okinawan coral reefs. In this paper, we present the details of our investigations on the saponin constituents of those sea cucumbers.¹²⁾

In 1976, Kelecom *et al.* reported studies on the saponin constituent of the sea cucumber *Thelonota ananas*, which was collected at the coastal Indo-Pacific Ocean, and proposed partial structures of two saponins named thelothurins A and B.¹³⁾ On the other hand, we investigated the

saponin constituent of the Okinawan sea cucumber of the same species (baika-namako in Japanese) which was collected at Zamami-jima, Okinawa Prefecture. The methanolic extract of the body walls of *T. ananas* was partitioned into a 1-butanol–water mixture and then the 1-butanol-soluble portion (=1-butanol extract) was subjected successively to centrifugal chromatography on silica gel and reversed-phase high-performance liquid chromatography (HPLC) to furnish stichlorosides A₁ (15), A₂ (16), B₁ (17), B₂ (18), C₁ (19), and C₂ (20), which were previously isolated from the sea cucumber *Stichopus chloronotus* by us,^{8a,b)} in 4, 2, 15, 8, 14, and 10% yields, respectively, from the 1-butanol extract.^{12b)}

In order to clarify the structural correlation of thelothurins A and B and our stichlorosides A₁, A₂, B₁, B₂, C₁, and C₂, we reinvestigated thelothurins A and B, the mixture of which was generously provided by Dr. Daloze (Université Libre de Bruxelles). It was found that the thelothurin mixture gave three spots on a silica gel thin-layer chromatogram (TLC) running with the same *R_f* values as those of stichlorosides A₁ (and A₂), B₁ (and B₂), and C₁ (and C₂).^{8a,b)} Thus, the thelothurin mixture was subjected successively to centrifugal chromatography on silica gel and reversed-phase HPLC to furnish six saponin constituents, which were found to be identical with authentic stichlorosides A₁ (15), A₂ (16), B₁ (17), B₂ (18), C₁ (19), and C₂ (20).^{8a,b)} Consequently, it has been concluded that the structures proposed for thelothurins A and B must be revised and the thelothurin mixture¹⁴⁾ comprises stichlorosides A₁ (15), A₂ (16), B₁ (17), B₂ (18), C₁ (19), and C₂ (20).

Further, two more sea cucumber species were shown to produce stichlorosides. Thus, stichlorosides A₁ (15), A₂ (16), B₁ (17), B₂ (18), C₁ (19), and C₂ (20) were isolated from the body wall of the sea cucumber *Stichopus hermanni* SEMPER, which was collected at Zamami-jima, Okinawa, in 0.6, 0.3, 1.7, 1.8, 8.6, and 3.5% yields, respectively from the 1-butanol-soluble portion of the methanolic extract of the body wall.^{12b)} In the case of the sea cucumber *Thelonota anax* H. L. CLARK, which was also collected at

Chart 1

Chart 2

Zamami-jima, stichlorosides A₁ (15), B₁ (17), and C₁ (19) were obtained from the body wall in 7, 15, and 15% yields, respectively, from the 1-butanol-soluble portion.^{12b)}

Several kinds of sea cucumbers belonging to *Bohadschia*

sp., *Holothuria* sp., and *Actinopyga* sp. are equipped with Cuvierian tubules, defensive glands which extend from the anus when the sea cucumbers are attacked by other marine organisms. Cuvierian tubules contain a large amount of saponins which are piscicidal and seem to work as a chemical defence, especially against fish. It is noteworthy that the sea cucumber *Bohadschia graeffei* (SEMPER) (kurote-namako in Japanese) was found to contain echinoside A (10)^{6b)} at an extremely high level in the body wall: it was isolated in 47% yield from the 1-butanol-soluble portion of the methanolic extract of the body wall, i.e. 0.9 g of echinoside A (10) from the body wall of one sea cucumber.^{12b)} On the other hand, from the Cuvierian tubules of the same sea cucumber, holothurin A (6)^{4b)} was isolated in 33% yield as a sole saponin from the 1-butanol-soluble portion of the methanolic extract of the Cuvierian tubules.^{12b)} It is interesting that the Cuvierian tubules of this species contain a different kind of saponin from the saponin accumulated in the body wall.

In the case of the sea cucumber *Bohadschia argus* (JAEGER) (janome-namako in Japanese) which was collected at Kudaka-jima, Okinawa, bivittoside D (**14**)^{7b} was obtained in 21% yield from the 1-butanol-soluble portion of the methanolic extract of the body wall, whereas bivittosides C (**13**)^{7b} and D (**14**) were isolated from the 1-butanol-soluble portion of the Cuvierian tubules of the same sea cucumber in 7 and 37% yields, respectively.

Next, we investigated the saponin constituents of sea cucumbers belonging to *Holothuria* sp. and *Actinopyga* sp. The sea cucumber *Holothuria edulis* (LESSON) (akamishikiri in Japanese), collected at Okinawa Island, was shown to produce holothurin A (**6**)^{4b} which was isolated in 21% yield from the 1-butanol-soluble portion of the body wall.^{12a}

Silica gel column chromatography of the 1-butanol-soluble portion of the methanolic extract of the body wall of the sea cucumber *Holothuria scabra* JAEGER (haneji-namako) furnished an oligoglycoside mixture which showed a single spot on ordinary TLC. However, solvolysis¹⁴ of this oligoglycoside mixture provided a mixture of three desulfated derivatives which were separated by reversed-phase HPLC to furnish desulfated holothurin A (**5**)^{4b} desulfated echinoside A (**9**)^{6b} and desulfated 24-dehydroechinoside A (**23**)⁹ in 2, 10, and 3% yields, respectively from the 1-butanol-soluble portion. Thus, it has been concluded that the sea cucumber *H. scabra* produces holothurin A (**6**)^{4b} echinoside A (**10**)^{6b} and 24-dehydroechinoside A (**24**)⁹ in an approximate 2:10:3 ratio.^{12b}

From the sea cucumber *Actinopyga mauritiana* (QUOY & GAIMARD) (kuriiro-namako), which was collected at Kudaka-jima, Okinawa, two oligoglycosidic fractions, each giving a single spot on TLC, were obtained by silica gel column chromatography. Solvolytic degradation¹⁴ of the polar oligoglycosidic fraction afforded a desulfated product which was separated by reversed-phase HPLC to furnish desulfated echinoside A (**9**)^{6b} and desulfated 24-dehydroechinoside A (**23**)⁹ in 6 and 2% yields, respectively from the 1-butanol-soluble portion. Similar solvolytic degradation of the less-polar oligoglycosidic fraction also yielded a desulfated product, which was separated by HPLC to furnish desulfated echinoside B (**7**)^{6b} and a new desulfated oligoglycoside **21** in 1 and 1.5% yields, respectively, from the 1-butanol-soluble portion. The carbon-13 nuclear magnetic resonance (¹³C-NMR) spectrum of **21** showed two olefinic carbon signals [δ_C 124.8 (d), 131.6 (s)] attributable to the tri-substituted olefin at C-24 and C-25 and $\Delta^{9(11)}$ olefinic carbon signals which were comparable to those signals of desulfated echinoside B (**7**). Reduction of **21** with Raney Ni (W-4) furnished desulfated echinoside B (**7**) quantitatively, and thus the new desulfated oligoglycoside **21** has been confirmed to be a 24-dehydro analog of desulfated echinoside B (**7**). In conclusion, it has been clarified that the sea cucumber *A. mauritiana* produces echinosides A (**10**) and B (**8**), and 24-dehydroechinosides A (**24**) and B (**22**), in an approximate 6:1:2:1.5 ratio.^{12a}

Then, we investigated the oligoglycoside constituents of *Holothuria axiologa* and *H. atra* using the same method as described above. The following results were obtained. *Holothuria axiologa* H. L. CLARK produces holothurin A (**6**) and echinoside A (**10**) in 12:7 ratio^{12b} and *Holothuria*

Chart 3

atra (JAEGER) (kuro-namako in Japanese) produces holothurins A (**6**) and B (**4**), and echinosides A (**10**) and B (**8**) in 7:5:7:5 ratio.^{12a}

The location of the sulfate group in the oligoglycosidic constituent, which was produced by the sea cucumber *Holothuria scabra*, *Actinopyga mauritiana*, *H. axiologa*, or *H. atra* and furnished the respective desulfated oligoglycoside (e.g. **3**, **5**, **7**, **9**, **21**, or **23**) by solvolytic degradation, was determined by ¹³C-NMR analysis of the sugar moiety to be at the 4-OH of the xylose moiety in each case.^{4b,5b,9} Thus, both echinoside A (**10**) and 24-dehydroechinoside A (**24**) were shown to possess the same tetrasaccharide moiety including the location of the sulfate group (attached to the 4-OH of the xylose moiety) as that of holothurin A (**6**): the ¹³C-NMR spectra of the sugar moieties in these oligoglycosides (**6**, **10**, **24**) were superimposable. Both echinoside B (**8**) and 24-dehydroechinoside B (**22**) were also shown to possess the same disaccharide moiety with the sulfate group attached to the 4-OH of the xylose moiety as in holothurin B (**4**), since here again the ¹³C-NMR signals due to the sugar parts in these oligoglycosides (**4**, **8**, **22**)

TABLE I. Oligoglycoside (Lacking a Sulfate Group) Compositions of Sea Cucumbers

Sea cucumber		Oligoglycoside											
		A	Bivittoside			Holotoxin		A ₁	A ₂	Stichloroside		C ₁	C ₂
			B	C	D	A	B			B ₁	B ₂		
<i>Bohadschia</i>	Cuvierian tubules	+	+	+	+++								
<i>bivittata</i>	Body wall	+	+	+	+++								
<i>Bohadschia</i>	Cuvierian tubules			+	+++								
<i>argus</i>	Body wall				+++								
<i>Stichopus japonicus</i>						+	+						
<i>Stichopus chloronotus</i>								+	+	++	++	++	++
<i>Stichopus hermanni</i>								+	+	+	+	++	+
<i>Thelonota ananas</i>								+	+	++	++	++	++
<i>Thelonota anax</i>								+		++		++	

TABLE II. Oligoglycoside (Having a Sulfate Group) Compositions of Sea Cucumbers

Sea cucumber		Oligoglycoside									
		Holothurin		Echinoid		Δ^{24} -Echinoid		Pervicoside			
		A	B	A	B	A	B	A	B	C	
<i>Holothuria</i>	Cuvierian tubules	+++	+								
<i>leucospilota</i>	Body wall		+++								
<i>Holothuria atra</i>		+++	+	+++	+						
<i>Holothuria scabra</i>		+		++		+					
<i>Holothuria edulis</i>		+++									
<i>Holothuria axiologa</i>		++		+							
<i>Holothuria</i>	Cuvierian tubules								+		
<i>pervicax</i>	Body wall								+	++	+
<i>Actinopyga echinites</i>				+++	+						
<i>Actinopyga mauritiana</i>				+++	+	++	+				
<i>Actinopyga</i>	(Cuvierian tubules)	+				+++					
<i>agassizi</i>											
<i>Bohadschia</i>	Cuvierian tubules	+++									
<i>graeffei</i>	Body wall			++++ ^{a)}							

a) Yield: 0.9 g from one fresh sea cucumber.

were superimposable. Furthermore, the ^{13}C -NMR signals due to the sugar moieties in components of the oligoglycoside mixture were observed as if they were the ^{13}C -NMR signals of a single compound, i.e. they were superimposable on those of holothurin A (6) or holothurin B (4).

The results obtained above and in our previous studies are summarized in Tables I and II. The sea cucumbers investigated by us are classified into two groups: the one comprises sea cucumbers of *Bohadschia* sp., *Stichopus* sp., and *Thelonota* sp., which produce triterpenic oligoglycosides lacking a sulfate group in their sugar moieties and the other contains sea cucumbers of *Holothuria* sp., *Actinopyga* sp., and *B. graeffei* which produce triterpenic oligoglycosides having a sulfate group attached to the 4-OH of the xylose moiety. Sea cucumbers of *Bohadschia* sp. were shown to contain both types.

Experimental

The instruments used to obtain physical data and the experimental conditions for chromatography were the same as described in our previous paper.¹⁵⁾

Oligoglycoside Constituents of the Sea Cucumber *Thelonota ananas*
The fresh body walls (cut, 4.5 kg) of the sea cucumber *Thelonota ananas* (collected in August at Zamami-jima, Okinawa Prefecture) were extracted with MeOH (5 l each) under reflux 3 times for 4 h each. The MeOH extract (290 g), obtained after removal of the solvent under reduced pressure, was partitioned into a 1-butanol-water (4 l each) mixture to, give the

1-butanol-soluble portion (49 g). The 1-butanol-soluble portion (2 g) was then purified by centrifugal chromatography [KT gel 2061 (Fuji-gel), $\text{CHCl}_3:\text{MeOH}:\text{H}_2\text{O}=7:3:1$ (lower phase)] to give three oligoglycoside fractions (fr. 1, 149 mg; fr. 2, 556 mg; fr. 3, 568 mg), which showed a single spot on TLC (silica gel, $\text{CHCl}_3:\text{MeOH}:\text{H}_2\text{O}=65:35:10$, lower phase). These three oligoglycoside fractions were subjected to separation by HPLC ($\mu\text{Bondapak C}_{18}$, $\text{CH}_3\text{CN}:\text{MeOH}:\text{H}_2\text{O}=1:1:1$) to furnish stichlorosides A₁ (15) (76 mg) and A₂ (16) (42 mg) from fr. 1, stichlorosides B₁ (17) (306 mg) and B₂ (18) (167 mg) from fr. 2, and stichlorosides C₁ (19) (284 mg) and C₂ (20) (191 mg) from fr. 3. Stichloroside A₁ (15), mp 213–215°C (MeOH), $[\alpha]_D -44^\circ$ (pyridine, 19°C). Stichloroside A₂ (16), mp 205–207°C (MeOH), $[\alpha]_D -30^\circ$ (pyridine, 19°C). Stichloroside B₁ (17), mp 270–272°C (MeOH), $[\alpha]_D -44^\circ$ (pyridine, 19°C). Stichloroside B₂ (18), mp 264–266°C (MeOH), $[\alpha]_D -38^\circ$ (pyridine, 19°C). Stichloroside C₁ (19), mp 251–253°C (MeOH), $[\alpha]_D -47^\circ$ (pyridine, 19°C). Stichloroside C₂ (20), mp 249–251°C (MeOH), $[\alpha]_D -45^\circ$ (pyridine, 19°C). These oligoglycosides were identified by mixed melting point determination, and $[\alpha]_D$ and ^{13}C -NMR spectral comparisons with authentic samples.^{8a,b)}

Purification of Thelothurin Mixture The thelothurin mixture (40 mg) provided by Dr. Daloze was purified by centrifugal chromatography [KT gel 2061 (Fuji-gel), $\text{CHCl}_3:\text{MeOH}:\text{H}_2\text{O}=7:3:1$ (lower phase)] to give three oligoglycoside fractions (fr. 1, 5 mg; fr. 2, 15 mg; fr. 3, 17 mg). Fr. 1, fr. 2, and fr. 3 were respectively shown to be mixtures of stichlorosides A₁ (15) and A₂ (16), stichlorosides B₁ (17) and B₂ (18), and stichlorosides C₁ (19) and C₂ (20) by HPLC comparison with authentic samples^{8a,b)} ($\mu\text{Bondapak C}_{18}$, $\text{CH}_3\text{CN}:\text{MeOH}:\text{H}_2\text{O}=1:1:1$) and also by TLC comparison [Silica gel 60 F₂₅₄ (Merck), $\text{CHCl}_3:\text{MeOH}:\text{H}_2\text{O}=65:35:10$ (lower phase)]. Furthermore, stichloroside C₁ (19) (mp 251–253°C), which was isolated from fr. 3 by HPLC, was shown to be identical with an authentic sample by mixed melting point determination.

Oligoglycoside Constituents of the Sea Cucumber *Thelonota anax* The

fresh body walls (cut, 3.5 kg) of the sea cucumber *Thelonota anax* (collected in August at Zamami-jima, Okinawa Prefecture) were extracted with MeOH (3 l each) under reflux 3 times for 4 h each. The MeOH extract (138 g), obtained after removal of the solvent under reduced pressure, was partitioned into a 1-butanol–water (2 l each) mixture to give the 1-butanol-soluble portion (15.7 g). The 1-butanol-soluble portion (500 mg) was then purified successively by centrifugal chromatography and HPLC as described above to furnish stichlorosides A₁ (**15**) (34 mg), mp 211–212 °C, $[\alpha]_D -44^\circ$ (pyridine, 23 °C), B₁ (**17**) (77 mg), mp 269–271 °C, $[\alpha]_D -42^\circ$ (pyridine, 23 °C), and C₁ (**19**) (77 mg), mp 255–256 °C, $[\alpha]_D -50^\circ$ (pyridine, 23 °C). These oligoglycosides were identified by HPLC, mixed melting point determination, and $[\alpha]_D$ and ^{13}C -NMR spectral comparisons with authentic samples.^{8a,b)}

Oligoglycoside Constituents of the Sea Cucumber *Stichopus hermanni* The fresh body walls (cut, 3.5 kg) of the sea cucumber *Stichopus hermanni* (collected in August at Zamami-jima, Okinawa Prefecture) were extracted with MeOH (4 l each) under reflux 3 times for 4 h each. The MeOH extract (45 g), obtained after removal of the solvent under reduced pressure, was partitioned into a 1-butanol–water mixture to give the 1-butanol-soluble portion (7.5 g). The 1-butanol-soluble portion (5.6 g) was purified successively by column chromatography [Silica gel 60, 60–230 mesh (Merck), 400 g, CHCl_3 :MeOH:H₂O=7:3:1 (lower phase)] and HPLC ($\mu\text{Bondapak C}_{18}$, CH_3CN :MeOH:H₂O=1:1:1) to furnish stichlorosides A₁ (**15**) [mp 211–213 °C, $[\alpha]_D -45^\circ$ (pyridine, 19 °C)], 32 mg; A₂ (**16**) [mp 203–205 °C, $[\alpha]_D -34^\circ$ (pyridine, 19 °C)], 18 mg; B₁ (**17**) [mp 268–269 °C, $[\alpha]_D -44^\circ$ (pyridine, 19 °C)], 96 mg; B₂ (**18**) [mp 263–264 °C, $[\alpha]_D -37^\circ$ (pyridine, 18 °C)], 104 mg; C₁ (**19**) [mp 250–252 °C, $[\alpha]_D -49^\circ$ (pyridine, 20 °C)], 485 mg; and C₂ (**20**) [mp 248–250 °C, $[\alpha]_D -43^\circ$ (pyridine, 18 °C)], 199 mg. These oligoglycosides were identified by HPLC, mixed melting point determination, and $[\alpha]_D$ and ^{13}C -NMR spectral comparisons with authentic samples.^{8a,b)}

Oligoglycoside Constituents of the Sea Cucumber *Bohadschia graeffei* The fresh body walls (cut, 14 kg) of the sea cucumber *Bohadschia graeffei* (collected in August at Zamami-jima, Okinawa Prefecture) were extracted with MeOH (20 l each) under reflux 3 times for 4 h each. The MeOH extract (700 g), obtained after removal of the solvent under reduced pressure, was partitioned into a 1-butanol–water mixture to give the 1-butanol-soluble portion (230 g). The 1-butanol-soluble portion (230 g) was treated with a CHCl_3 (400 ml)–MeOH (1500 ml)–H₂O (100 ml) mixture and the whole mixture was thoroughly stirred at room temperature (20 °C) for 24 h to yield a suspension. The insoluble portion (130 g) was then collected by filtration. Recrystallization of the insoluble portion (6 g) from CHCl_3 :MeOH:H₂O furnished echinoside A (**10**) (5 g), which was identified by mixed melting point determination, and $[\alpha]_D$ and ^{13}C -NMR spectral comparisons with an authentic sample.^{6b)} The fresh Cuvierian tubules (0.9 kg) of the same sea cucumber were extracted with 95% aqueous MeOH (3 l) under reflux 3 times for 4 h each. The MeOH extract (69 g), obtained after removal of the solvent under reduced pressure, was partitioned into a 1-butanol–water mixture to give the 1-butanol-soluble portion (25 g). The 1-butanol-soluble portion (25 g) was treated with a CHCl_3 (100 ml)–MeOH (600 ml)–H₂O (150 ml) mixture and the whole mixture was stirred at room temperature (25 °C) for 12 h. The insoluble portion (14 g) was collected by filtration. Recrystallization of the insoluble portion (3 g) from CHCl_3 :MeOH:H₂O furnished holothurin A (**6**) (1.8 g), which was identified by mixed melting point determination, and $[\alpha]_D$ and ^{13}C -NMR spectral comparisons with an authentic sample.^{4b)}

Oligoglycoside Constituents of the Sea Cucumber *Bohadschia argus* The fresh body walls (cut, 27 kg) of the sea cucumber *Bohadschia argus* (collected in August at Kudaka-jima, Okinawa Prefecture) were extracted with MeOH (30 l each) under reflux 3 times for 4 h each. The MeOH extract, obtained after removal of the solvent under reduced pressure, was partitioned into a 1-butanol–water mixture to give the 1-butanol-soluble portion (120 g). The 1-butanol-soluble portion (10 g) was then purified by column chromatography [SiO_2 300 g, CHCl_3 :MeOH:H₂O=7:3:1 (lower phase)] to give bivittoside D (**14**) (2.1 g). Bivittoside D (**14**), mp 219–221 °C, $[\alpha]_D -7^\circ$ (pyridine, 20 °C) was identified by mixed melting point determination, and $[\alpha]_D$ and ^{13}C -NMR spectral comparisons with an authentic sample.^{7b)}

The fresh Cuvierian tubules (1.4 kg) of the same sea cucumber were extracted with MeOH (2 l each) under reflux 5 times for 4 h each. The MeOH extract (130 g), obtained after removal of the solvent under reduced pressure, was partitioned into a 1-butanol–water mixture to give the 1-butanol-soluble portion (65 g). The 1-butanol-soluble portion (32 g) was purified by column chromatography as described above to give bivittosides C (**13**), 2.3 g, mp 216–218 °C, $[\alpha]_D -31^\circ$ (pyridine, 28 °C) and D (**14**),

12 g, mp 219–221 °C, $[\alpha]_D -7^\circ$ (pyridine, 20 °C). These oligoglycosides were identified by mixed melting point determination, and $[\alpha]_D$ and ^{13}C -NMR spectral comparisons with authentic samples.^{7b)}

Oligoglycoside Constituents of the Sea Cucumber *Holothuria edulis* The fresh body walls (cut, 1.5 kg) of the sea cucumber *Holothuria edulis* (collected in August at Okinawa Island) were extracted with MeOH (1.5 l each) under reflux 3 times for 4 h each. The MeOH extract (31 g), obtained after removal of the solvent under reduced pressure, was partitioned into a 1-butanol–water mixture to give the 1-butanol-soluble portion (5.7 g). The 1-butanol-soluble portion (5.7 g) was treated with CHCl_3 :MeOH:H₂O (65:35:10, lower phase, 100 ml) and the whole mixture was stirred well at room temperature (20 °C) for 12 h. The insoluble portion (2.6 g) was collected by filtration and recrystallized from CHCl_3 –MeOH–H₂O to furnish holothurin A (**6**) (1.2 g). Holothurin A (**6**) thus obtained [mp 228.5–230 °C, $[\alpha]_D -9^\circ$ (pyridine, 24 °C)] was identified by mixed melting point determination, and $[\alpha]_D$ and ^{13}C -NMR spectral comparisons with an authentic sample.^{4b)}

Oligoglycoside Constituents of the Sea Cucumber *Holothuria scabra* The fresh body walls (cut, 4.3 kg) of the sea cucumber *Holothuria scabra* (collected in August at Okinawa Island) were extracted with MeOH (5 l each) under reflux 3 times for 4 h each. The MeOH extract (155 g), obtained after removal of the solvent under reduced pressure, was partitioned into a 1-butanol–water mixture to give the 1-butanol-soluble portion (11 g). The 1-butanol-soluble portion (11 g) was then purified by SiO_2 column chromatography (CHCl_3 :MeOH:H₂O=7:3:1, lower phase) to give an oligoglycoside mixture (2.1 g). A solution of the oligoglycoside mixture (1.5 g) in dioxane (10 ml)–pyridine (20 ml) was heated under reflux for 1 h, then cooled. The residue, obtained after evaporation of the organic solvent under reduced pressure, was purified by SiO_2 column chromatography (CHCl_3 :MeOH:H₂O=7:3:1, lower phase) to give a mixture of desulfated oligoglycosides (907 mg). The mixture (907 mg) was then subjected to HPLC separation ($\mu\text{Bondapak C}_{18}$, CH_3CN :MeOH:H₂O=1:1:1) to give desulfated holothurin A (**5**) (120 mg), mp 230–232 °C, $[\alpha]_D -3^\circ$ (MeOH, 19 °C), desulfated 24-dehydroechinoside A (**23**) (185 mg), mp 242–244 °C, $[\alpha]_D -6^\circ$ (pyridine, 20 °C), and desulfated echinoside A (**9**) (594 mg), mp 237–239 °C, $[\alpha]_D -1^\circ$ (pyridine, 20 °C). The desulfated oligoglycosides thus obtained were identified by mixed melting point determination, and $[\alpha]_D$ and ^{13}C -NMR spectral comparisons with authentic samples.^{4b,6b,9)} The oligoglycoside mixture: ^{13}C -NMR (50 MHz, pyridine-*d*₅, δ_c): 105.6 (d, C-1'), 83.4 (d, C-2'), 77.6 (d, C-3'), 69.5 (d, C-4'), 64.1 (t, C-5').

Oligoglycoside Constituents of the Sea Cucumber *Actinopyga mauritiana* The fresh body walls (cut, 13 kg) of the sea cucumber *Actinopyga mauritiana* (collected in August at Kudaka-jima) were extracted with MeOH (10 l each) under reflux 3 times for 4 h each. The MeOH extract (564 g), obtained after removal of the solvent under reduced pressure, was partitioned into a 1-butanol–water mixture to give the 1-butanol-soluble portion (76 g). The 1-butanol-soluble portion (76 g) was treated with MeOH (1.3 l) and the whole mixture was stirred at room temperature (25 °C) for 12 h. The resulting suspension was then filtered to obtain the soluble portion (29 g) and the insoluble portion (45 g). The soluble portion (29 g) was purified by SiO_2 column chromatography (CHCl_3 :MeOH:H₂O=7:3:1, lower phase) to give the oligoglycoside fraction I (3.8 g), which showed a single spot on TLC. The insoluble portion (45 g) was recrystallized from CHCl_3 :MeOH:H₂O to give the oligoglycoside fraction II (12.1 g), which also showed a single spot on TLC.

A solution of the oligoglycoside fraction I (500 mg) in dioxane (8 ml)–pyridine (16 ml) was heated under reflux for 1.5 h, then cooled. The residue, obtained after evaporation of the organic solvent under reduced pressure, was purified by SiO_2 column chromatography (CHCl_3 :MeOH:H₂O=10:3:1, lower phase) to give a mixture of desulfated oligoglycosides (240 mg). The combined desulfated oligoglycoside mixture (350 mg) was purified by HPLC ($\mu\text{Bondapak C}_{18}$, CH_3CN :MeOH:H₂O=2:2:1) to furnish desulfated 24-dehydroechinoside B (**21**) (139 mg) and desulfated echinoside B (**7**) (100 mg). Desulfated 24-dehydroechinoside B (**21**), colorless needles, mp 239–240 °C, $[\alpha]_D +2^\circ$ (pyridine, 18 °C). *Anal.* Calcd for $\text{C}_{41}\text{H}_{64}\text{O}_{13} \cdot 1/2\text{H}_2\text{O}$: C, 63.66; H, 8.41. Found: C, 63.89; H, 8.69. ^{13}C -NMR (25 MHz, pyridine-*d*₅, δ_c): 131.6 (s, C-25), 124.8 (d, C-24). Desulfated echinoside B (**7**), mp 245–246 °C, $[\alpha]_D +0.5^\circ$ (pyridine, 24 °C) thus obtained was identified by mixed melting point determination, and $[\alpha]_D$ and ^{13}C -NMR spectral comparisons with an authentic sample.^{6b)}

A solution of the oligoglycoside fraction II (10 g) in dioxane (80 ml)–pyridine (160 ml) was heated under reflux for 1.5 h, then cooled. The residue, obtained after evaporation of the organic solvent under reduced pressure, was purified by SiO_2 column chromatography

[CHCl₃:MeOH:H₂O=10:3:1 (lower phase)] to give a mixture of desulfated oligoglycosides (6.5 g). The mixture of desulfated oligoglycosides (800 mg) was then subjected to HPLC purification (μ Bondapak C₁₈, CH₃CN:MeOH:H₂O=1:1:1) to furnish desulfated 24-dehydroechinoside A (**23**) (152 mg), mp 243–244 °C, [α]_D –5° (pyridine, 18 °C) and desulfated echinoside A (**9**) (450 mg), mp 240–241 °C, [α]_D –1° (MeOH, 18 °C). These desulfated oligoglycosides were identified by mixed melting point determination, and [α]_D and ¹³C-NMR spectral comparisons with authentic samples.^{6b,9)}

Oligoglycoside fraction I: ¹³C-NMR (25 MHz, pyridine-*d*₅, δ_c): 105.4 (d, C-1'), 83.0 (d, C-2'), 76.5 (d, C-3'), 75.0 (d, C-4'), 63.8 (t, C-5'). Oligoglycoside fraction II: ¹³C-NMR (25 MHz, pyridine-*d*₅, δ_c): 105.7 (d, C-1'), 83.1 (d, C-2'), 76.1 (d, C-3'), 74.9 (d, C-4'), 64.0 (t, C-5').

Reduction of Desulfated 24-Dehydroechinoside B (21**)** A solution of **21** (240 mg) in dry EtOH (70 ml) was treated with a suspension (40 ml) of Raney Ni (W-4)–EtOH and the whole mixture was heated under reflux for 14 h. After removal of the catalysts by filtration, the filtrate was evaporated under reduced pressure to give a product, which was purified by SiO₂ column chromatography (CHCl₃:MeOH:H₂O=10:3:1, lower phase) to furnish a reduction product (154 mg). The product thus obtained above was identified by mixed melting point determination, and [α]_D and ¹³C-NMR spectral comparisons with authentic desulfated echinoside B (**7**).^{6b)}

Oligoglycoside Constituents of the Sea Cucumber *Holothuria axiologa* The fresh body walls (cut, 1.3 kg) of the sea cucumber *Holothuria axiologa* (collected in August at Zamami-jima, Okinawa Prefecture) were extracted with MeOH (3 l each) under reflux 3 times for 4 h each. The MeOH extract (65 g), obtained after removal of the solvent under reduced pressure, was partitioned into a 1-butanol–water mixture to give the 1-butanol-soluble portion (18 g). The 1-butanol-soluble portion (16 g) was treated with MeOH (500 ml) and the whole mixture was stirred well at room temperature (20 °C) for 24 h. Filtration of the suspension gave the insoluble portion (6.1 g), which showed a single spot on TLC. The insoluble portion thus obtained (1 g) was treated with dioxane (20 ml)–pyridine (40 ml) and then the whole was heated under reflux for 1 h. The residue, obtained after evaporation of the organic solvent under reduced pressure, was partitioned into a 1-butanol–water mixture to give the 1-butanol-soluble portion (890 mg). The 1-butanol-soluble portion (394 mg) was subjected to HPLC purification (μ Bondapak C₁₈, CH₃CN:MeOH:H₂O=1:1:1) to furnish desulfated holothurin A (**5**) (146 mg), mp 233–235 °C, [α]_D –2° (MeOH, 23 °C) and desulfated echinoside A (**9**) (79 mg), mp 235–237 °C, [α]_D –1° (pyridine, 23 °C). These desulfated oligoglycosides were identified by mixed melting point determination, and [α]_D and ¹³C-NMR spectral comparisons with authentic samples.^{4b,6b)} On the other hand, the above-mentioned MeOH-insoluble portion (1 g) was further purified by SiO₂ column chromatography (CHCl₃:MeOH:H₂O=7:3:1, lower phase) to give an oligoglycoside fraction (800 mg). Oligoglycoside fraction: ¹³C-NMR (25 MHz, pyridine-*d*₅, δ_c): 105.5 (d, C-1'), 83.4 (d, C-2'), 77.6 (d, C-3'), 69.5 (d, C-4'), 64.1 (t, C-5').

Oligoglycoside Constituents of the Sea Cucumber *Holothuria atra* The fresh body walls (cut, 1.8 kg) of the sea cucumber *Holothuria atra* (collected in August at Okinawa Island) were extracted with 95% aqueous MeOH (1.5 l each) under reflux 4 times for 4 h each. The MeOH extract (78 g), obtained after removal of the solvent under reduced pressure, was partitioned into a 1-butanol–water mixture to give the 1-butanol-soluble portion (17 g). The 1-butanol-soluble portion was then treated with MeOH (340 ml) and the whole mixture was stirred well at room temperature (25 °C) for 12 h then filtered to give the MeOH-soluble portion (10 g) and the MeOH-insoluble portion (6.2 g). The MeOH-soluble portion was purified by SiO₂ column chromatography (CHCl₃:MeOH:H₂O=7:3:1, lower phase) to give the oligoglycoside fraction I (2.1 g), which showed a single spot on TLC (CHCl₃:MeOH:H₂O=65:35:10, lower phase). The MeOH-insoluble portion was also subjected to SiO₂ column chromatography (CHCl₃:MeOH:H₂O=65:35:10, lower phase) to give the oligoglycoside fraction II (4.0 g), which showed a single spot on TLC (CHCl₃:MeOH:H₂O=65:35:10, lower phase).

A solution of oligoglycoside fraction I (980 mg) in dioxane (32 ml)–pyridine (16 ml) was heated under reflux for 1 h, then cooled. The residue, obtained after evaporation of the organic solvent under reduced pressure, was partitioned into a 1-butanol–water mixture to give the 1-butanol-soluble portion (800 mg). The 1-butanol-soluble portion (130 mg) was then purified by HPLC (μ Bondapak C₁₈, CH₃CN:MeOH:

H₂O=2:2:1) to furnish desulfated holothurin B (**3**) (58 mg), mp 275–278 °C, [α]_D –12° (MeOH, 19 °C) and desulfated echinoside B (**7**) (56 mg), mp 258–260 °C, [α]_D +0.7° (pyridine, 19 °C). These desulfated oligoglycosides were identified by HPLC, mixed melting point determination, and [α]_D and ¹³C-NMR spectral comparisons with authentic samples.^{5b,6b)}

A solution of oligoglycoside fraction II (800 mg) in dioxane (30 ml)–pyridine (15 ml) was heated under reflux for 1 h, then cooled. The residue obtained as above was partitioned into a 1-butanol–water mixture to give the 1-butanol-soluble portion (630 mg). The 1-butanol-soluble portion (112 mg) was subjected to HPLC purification (μ Bondapak C₁₈, CH₃CN:MeOH:H₂O=1:1:1) to furnish desulfated holothurin A (**5**) (39 mg), mp 231–233 °C, [α]_D –3° (MeOH, 19 °C) and desulfated echinoside A (**9**) (40 mg), mp 237–239 °C, [α]_D –1.2° (MeOH, 19 °C). These desulfated oligoglycosides were identified by HPLC, mixed melting point determination, and [α]_D and ¹³C-NMR spectral comparisons with authentic samples.^{4b,6b)} Oligoglycoside fraction I: ¹³C-NMR (25 MHz, pyridine-*d*₅, δ_c): 105.5 (d, C-1'), 82.9 (d, C-2'), 76.4 (d, C-3'), 74.8 (d, C-4'), 63.6 (t, C-5'). Oligoglycoside fraction II: ¹³C-NMR (25 MHz, pyridine-*d*₅, δ_c): 105.7 (d, C-1'), 83.2 (d, C-2'), 76.2 (d, C-3'), 74.9 (d, C-4'), 64.0 (t, C-5').

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