Marine Natural Products. XIX.[1] Pervicosides A, B, and C, Lanostane-Type Triterpene-Oligoglycoside Sulfates from the Sea Cucumber *Holothuria pervicax*

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Triterpene-oligoglycoside constituents, contained in the body walls and the Cuvierian tubules of the sea cucumber *Holothuria pervicax* (torafu-namako in Japanese), were investigated and purified in their desulfated forms. These desulfated lanostane-type triterpene tetraglycosides, designated DS-pervicoside A (7), DS-pervicoside B (8), and DS-pervicoside C (9), have antifungal activity, and their structures were determined on the basis of chemical and physicochemical evidence. The parent oligoglycosides of these desulfated tetraglycosides, named pervicosides A (10), B (11), and C (12), were shown to be the 4′-sulfates of DS-pervicosides A (7), B (8), and C (9), respectively.

Keywords sea cucumber; *Holothuria pervicax*; oligoglycoside antifungal; lanostane-type triterpene oligoglycoside; oligoglycoside ^{13}C-NMR; pervicoside A; pervicoside B; pervicoside C

During the course of our investigations on biologically active constituents in marine organisms,[2] we have searched for antifungal substances from sea cucumber.[1] We have found that several lanostane-type triterpene oligoglycosides in sea cucumber exhibit antifungal activity and we have elucidated their structures. Those sea cucumber saponins were holotoxins A and B from *Stichopus japonicus* (namako in Japanese),[3a] holothurins A[3c] and B[3b] from *Holothuria leucospilota* (nise-kuro-namako), echinosides A and B from *Actinopyga echinites* (kutsu-namako),[3d] holothurin A and 24-dehydroechinoside A from the Bahamean sea cucumber *Actinopyga agassizi*,[3e] and bivittosides A, B, C, and D from *Bohadschia bivittata* (futasuji-namako).[1,3f] In this paper, we present a full account of our investigation on the saponin constituents of the sea cucumber *Holothuria pervicax* (torafu-namako in Japanese), which is a closely related species to *H. leucospilota*[3b,c] and inhabits the Indo-Pacific coasts. The body walls contained three triterpene oligoglycosides, named pervicoside A (**10**), pervicoside B (**11**), and pervicoside C (**12**), while the Cuvierian tubules contained pervicoside A as the major oligoglycoside. The isolations of pure products were effected as the desulfated forms, respectively designated DS-pervicoside A (**7**), DS-pervicoside B (**8**), and DS-pervicoside C (**9**).[4]

The body walls of the sea cucumber, which were collected in August at Kushimoto in Wakayama Prefecture, were extracted with methanol and the extract was partitioned into a 1-butanol–water mixture. Chromatographic separation of the 1-butanol-soluble portion (the 1-butanol extract) provided an oligoglycoside mixture, containing

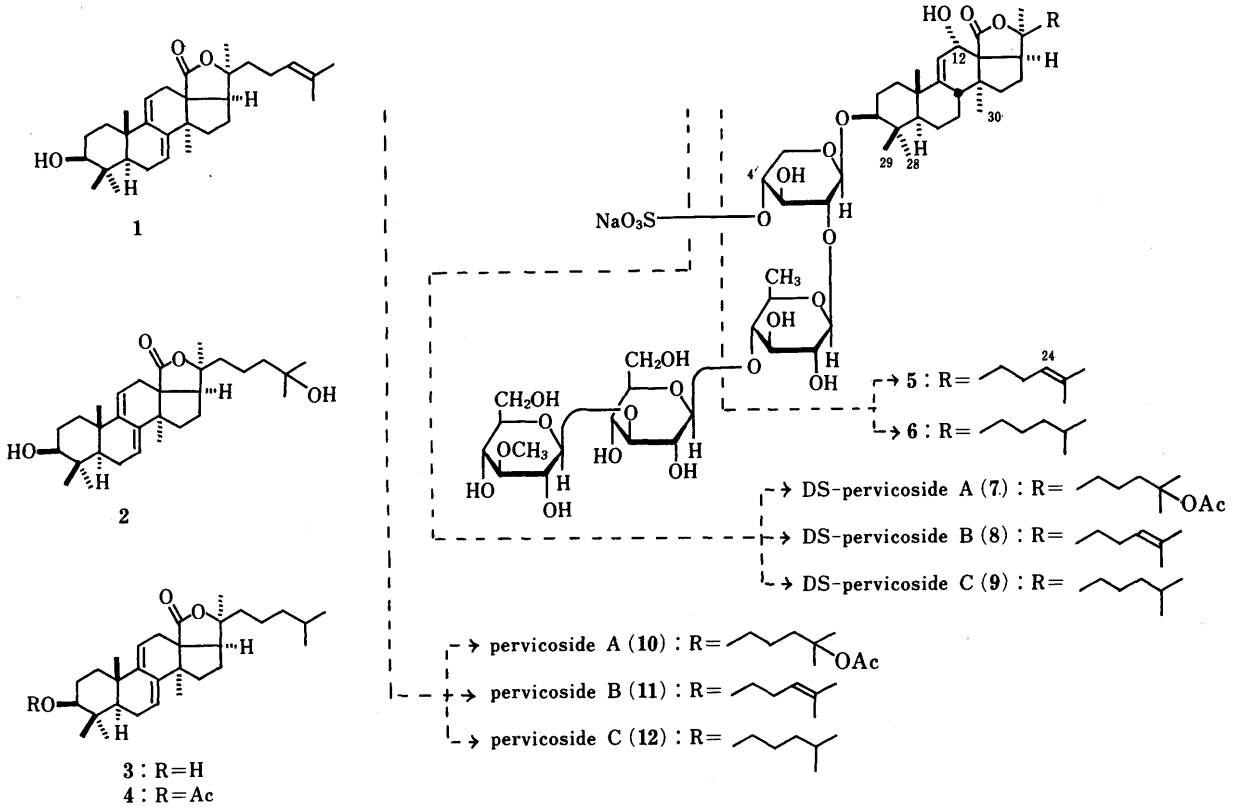

Chart 1

pervicosides A (**10**), B (**11**), and C (**12**), which showed a single spot on thin layer chromatographic (TLC) examination. The oligoglycoside mixture was shown to contain sulfate group(s), based on the positive potassium rhodizonate test.[5] Solvolytic removal of the sulfate group in the oligoglycoside mixture by treatment with a dioxane–pyridine mixture,[6] afforded three desulfated derivatives, which were separated by high-performance liquid chromatography (HPLC) to furnish DS-pervicoside A (**7**), DS-pervicoside B (**8**), and DS-pervicoside C (**9**) in 4, 21, and 3% yields, respectively, from the 1-butanol extract of the body walls. Purification of the methanolic extract of the Cuvierian tubules followed by solvolytic degradation in the same manner as described for the body wall extract, furnished as the major DS-pervicoside A (**7**) in 7% yield from the 1-butanol extract of the Cuvierian tubules.

The major oligoglycoside, DS-pervicoside B (**8**), was obtained as colorless needles of mp 212—214 °C, which comprised one mol each of xylose, quinovose, 3-*O*-methylglucose, and glucose. It lacked an ultraviolet (UV) absorption maximum at above 210 nm, while it showed in its infrared (IR) spectrum an absorption band due to a γ-lactone moiety (1732 cm^{-1}) and strong broad absorptions [3400, 1070 (br) cm^{-1}] consistent with glycosidic structure. The circular dichroism (CD) spectrum of **8** showed a negative maximum ($[\theta]_{222}$ −7800) attributable to the n→π^* transition of its γ-lactone moiety.

Acidic hydrolysis of DS-pervicoside B (**8**) provided two secondarily formed sapogenols **1** and **2**. The IR spectrum of **1** showed an absorption band due to a hydroxyl group (3300 cm^{-1}) and a γ-lactone moiety (1748 cm^{-1}), whereas the proton nuclear magnetic resonance (^{1}H-NMR) spectrum of **1** showed signals ascribable to the 7,9 (11)-heteroannular diene protons (δ 5.22, 5.53, both 1H, m), one olefinic proton (δ 5.08, 1H, m, 24-H), and two olefinic methyls [δ 1.62, 1.71, both 3H, s, 25-$(CH_3)_2$]. Reduction of **1** with Raney Ni (W-2) and subsequent acetylation of the product gave a dihydro derivative, which was shown to be identical with seychellogenin acetate (**4**), previously obtained by acetylation of the acidic hydrolysate of bivittoside A, a triterpene-oligoglycoside from the Okinawan sea cucumber *Bohadschia bivittata* MITSUKURI.[1] The ^{1}H-NMR spectrum of another sapogenol **2** showed signals assignable to the 7,9 (11)-heteroannular diene protons (δ5.22, 5.53, both 1H, m) and two methyls (δ 1.20, 6H, s) attached to a quaternary carbon [δ_C 69.5, s, in the carbon-13 (^{13}C) NMR) spectrum] bearing a hydroxyl group. Chemical correlation of both sapogenols was demonstrated by the fact that treatment of either **1** or **2** with boiling aqueous 2N HCl–acetone furnished a *ca.* 1 : 1 mixture of **1** and **2**. Thus, the structure of **2** has been shown to be the 24,25-hydrated derivative of **1**, *i.e.* 25-hydroxyseychellogenin.

To elucidate the structure of the genuine sapogenol of DS-pervicoside B (**8**), the following examinations were undertaken. Enzymatic hydrolysis of DS-pervicoside B (**8**) with a crude hesperidinase furnished a partial hydrolysate **5** in quantitative yield. Reduction of **5** with Raney Ni (W-2) yielded the dihydro derivative **6**, which was shown to be identical with bivittoside A.[1] Thus, the structure of the genuine sapogenol of DS-pervicoside B (**8**) was proved to be 3β,12α,20(*S*)-trihydroxylanost-9(11),24-dien-18,20-olide.

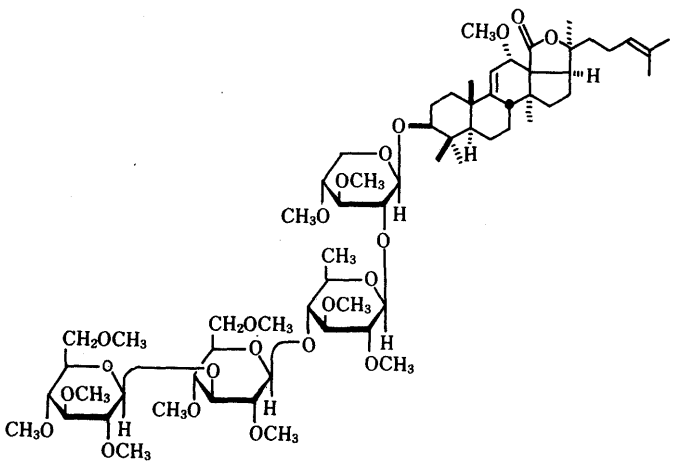

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Chart 2

Next, in order to clarify the sequence of the carbohydrate moiety, DS-pervicoside B (**8**) was subjected to complete methylation to afford the dodeca-*O*-methyl derivative (**13**). The ^{1}H-NMR spectrum of **13** showed four one-proton doublets [δ 4.30 (1H, J=7 Hz), 4.35 (1H, J=8 Hz), 4.65 (2H, J=7 Hz)] ascribable to four β-anomeric protons. Methanolysis of **13** liberated methyl 2,3,4,6-tetra-*O*-methylglucopyranoside, methyl 2,4,6-tri-*O*-methylglucopyranoside, methyl 2,3-di-*O*-methylquinovopyranoside, and methyl 3,4-di-*O*-methylxylopyranoside. Based on the above-mentioned evidence, the structure of DS-pervicoside B has been determined as **8**.

DS-pervicoside C (**9**), mp 215—217 °C, showed similar spectroscopic properties to DS-pervicoside B (**8**) and contained one mol each of xylose, quinovose, glucose, and 3-*O*-methylglucose in its structure. Acidic hydrolysis of DS-pervicoside C (**9**) provided an artifact sapogenol, seychellogenin (**3**).[1,7] The ^{13}C-NMR spectrum of DS-pervicoside C (**9**) was almost superimposable on that of DS-pervicoside B (**8**) except for signals due to several carbons in the terminal parts of sapogenol side chains, and the carbon signals of DS-pervicoside C (**9**) were assigned as given in Table I. Thus, DS-pervicoside C (**9**) was presumed to be the 24,25-dihydro derivative of DS-pervicoside B (**8**). In order to verify this, DS-pervicoside B (**8**) was subjected to reduction with Raney Ni (W-2) to afford the dihydro derivative, which was found to be identical with DS-pervicoside C (**9**). Thus, the structure of DS-pervicoside C (**9**) has been determined to be as shown.

DS-pervicoside A (**7**) is a tetraglycoside of mp 210—212 °C containing one mol each of xylose, quinovose, glucose, and 3-*O*-methyl-glucose in its structure, and it showed similar spectroscopic properties to DS-pervicoside B (**8**). The ^{13}C-NMR spectrum of DS-pervicoside A (**7**) showed signals assignable to one acetoxyl group [δ_C 170.5 (s), 22.3 (q)] and a carbon [δ_C 82.1 (s, C-25)] bearing an acetoxyl group. Acidic hydrolysis of DS-pervicoside A (**7**) yielded two artifact sapogenols **1** and **2**, as in the case of DS-pervicoside B (**8**). Thus, the structure of the genuine sapogenol in DS-pervicoside A (**7**) was presumed to be 3β,12α,20(*S*)-trihydroxy-25-acetoxylanost-9(11)-en-18,20-olide. The carbon signals in the ^{13}C-NMR spectrum of DS-pervicoside A (**7**) were assigned as given in Table I and the

TABLE Ia. ^{13}C-NMR Data for Aglycones of DS-Pervicosides A (7), B (8), and C (9)[a,b]

Carbon	DS-pervicoside A (7)	DS-pervicoside B (8)	DS-pervicoside C (9)
1	36.8 (t)[c]	36.8 (t)[c]	36.7 (t)[c]
2	27.3 (t)[d]	27.3 (t)[d]	27.3 (t)[d]
3	89.2 (d)	89.1 (d)	89.2 (d)
4	40.2 (s)	40.2 (s)	40.2 (s)
5	53.2 (d)	53.2 (d)	53.1 (d)
6	21.4 (t)	21.4 (t)	21.4 (t)
7	28.9 (t)[d]	29.0 (t)[d]	29.0 (t)[d]
8	40.3 (d)	40.4 (d)	40.3 (d)
9	153.7 (s)	153.6 (s)	153.6 (s)
10	39.8 (s)	39.8 (s)	39.6 (s)
11	116.4 (d)	116.4 (d)	116.5 (d)
12	68.5 (d)	68.5 (d)	68.5 (d)
13	64.3 (s)	64.4 (s)	64.3 (s)
14	46.8 (s)	46.8 (s)	46.8 (s)
15	24.5 (t)	24.3 (t)	24.3 (t)
16	37.3 (t)[c]	37.3 (t)[c]	37.3 (t)[c]
17	47.3 (d)	47.2 (d)	47.2 (d)
18	177.5 (s)	177.5 (s)	177.6 (s)
19	18.3 (q)	18.3 (q)	18.3 (q)
20	84.4 (s)	84.7 (s)	84.5 (s)
21	26.4 (q)	26.4 (q)	26.4 (q)
22	40.3 (t)	39.6 (t)	39.8 (t)
23	19.2 (t)	23.4 (t)	22.7 (t)
24	41.4 (t)	124.5 (d)	39.6 (t)
25	82.1 (s)	132.1 (s)	28.1 (d)
26	26.2 (q)	25.7 (q)	22.7 (q)
27	26.2 (q)	17.7 (q)	22.7 (q)
28	28.3 (q)	28.3 (q)	28.3 (q)
29	16.8 (q)	16.8 (q)	16.8 (q)
30	22.1 (q)	22.1 (q)	22.1 (q)
OAc	170.5 (s)		
	22.3 (q)		

a) Measured at 30 °C. *b*) Abbreviations given in parentheses denote signals observed in the off-resonance experiments. *c, d*) Assignments may be interchangeable in each column.

signals due to the sugar moiety carbons were found to be superimposable on those of DS-pervicoside B (**8**). Based on these findings, the structure of DS-pervicoside A has been determined to be **7**.

The parent oligoglycosides [pervicosides A (**10**), B (**11**), and C (**12**), respectively] of DS-pervicosides A (**7**), B (**8**), and C (**9**) gave a single spot of the same *Rf* value on TLC. The composition was clarified only after solvolytic removal of the sulfate group to provide three DS-pervicosides as mentioned above. Although the isolation of individual parent oligoglycosides was not effected, the location of each sulfate group in each parent pervicoside was determined by detailed ^{13}C-NMR spectral examination. In the ^{13}C-NMR spectrum of the parent oligoglycoside mixture containing the three pervicosides, carbon signals of sugar moieties were observed as in the case of a single compound (Table I). The signal assignable to C-4′ was observed at δ_C 75.1 (d), which was shifted lower due to sulfation[3b,8] as compared with the signal of the C-4′ of DS-pervicoside B (**8**) (δ_C 71.0). Thus, the location of the sulfate group in the parent oligoglycosides has been shown to be C-4′ in each pervicoside and the structures of pervicoside A, pervicoside B, and pervicoside C have been determined as **10**, **11**, and **12**, respectively.

The antifungal activities of DS-pervicosides A (**7**), B (**8**), and C (**9**) were as shown in Table II. As can be seen from

TABLE Ib. ^{13}C-NMR Data for Sugar Moieties of DS-Pervicosides A (7), B (8), C (9), and the Parent Oligoglycoside Mixture[a,b]

Carbon	DS-pervicoside A (7)	DS-pervicoside B (8)	DS-pervicoside C (9)	Oligoglycoside mixture
1′	105.1 (d)	105.1 (d)	105.2 (d)	105.0 (d)
2′	84.4 (d)	84.4 (d)	84.5 (d)	83.5 (d)
3′	78.3 (d)[c]	78.2 (d)[c]	78.3 (d)[c]	76.3 (d)
4′	71.0 (d)[d]	71.0 (d)[d]	71.0 (d)[d]	75.1 (d)
5′	66.8 (t)	66.8 (t)	66.9 (t)	64.3 (t)
1″	105.9 (d)	105.9 (d)	105.9 (d)	105.3 (d)[c]
2″	76.6 (d)	76.6 (d)	76.6 (d)	75.4 (d)[d]
3″	76.2 (d)	76.2 (d)	76.2 (d)	75.6 (d)[d]
4″	87.6 (d)	87.6 (d)	87.7 (d)	86.8 (d)
5″	71.9 (d)	71.9 (d)	71.9 (d)	72.0 (d)
6″	18.3 (q)	18.3 (q)	18.3 (q)	18.1 (q)
1‴	105.9 (d)	105.9 (d)	105.9 (d)	105.5 (d)[c]
2‴	73.9 (d)	73.9 (d)	73.9 (d)	74.1 (d)
3‴	88.5 (d)	88.5 (d)	88.5 (d)	88.1 (d)
4‴	70.1 (d)	70.1 (d)	70.1 (d)	69.8 (d)
5‴	78.1 (d)[c]	78.0 (d)[c]	78.1 (d)[c]	77.6 (d)
6‴	62.5 (t)	62.5 (t)	62.5 (t)	61.9 (t)
1⁗	105.9 (d)	105.9 (d)	105.9 (d)	106.0 (d)
2⁗	75.2 (d)	75.2 (d)	75.2 (d)	75.1 (d)
3⁗	88.1 (d)	88.1 (d)	88.2 (d)	88.1 (d)
4⁗	70.9 (d)[d]	70.9 (d)[d]	70.9 (d)[d]	70.8 (d)
5⁗	78.5 (d)[c]	78.4 (d)[c]	78.5 (d)[c]	78.3 (d)
6⁗	62.5 (t)	62.5 (t)	62.5 (t)	62.3 (t)
3⁗-OMe	60.8 (q)	60.8 (q)	60.8 (q)	60.8 (q)

a) Measured at 30 °C. *b*) Abbreviations given in parentheses denote signals observed in the off-resonance experiments. *c, d*) Assignments may be interchangeable in each column.

TABLE II. Minimum Growth Inhibitory Concentration (μg/ml)

	DS-pervicoside A (7)	DS-pervicoside B (8)	DS-pervicoside C (9)
Aspergillus niger	12.5	1.56	6.25
Aspergillus oryzae	12.5	3.12	6.25
Penicillium chrysogenum	6.25	1.56	3.12
Penicillium citrinum	6.25	1.56	6.25
Mucor spinescence	12.5	6.25	12.5
Cladosporium herbarum	50	6.25	12.5
Rhodotorula rubra	100	3.12	6.25
Trichophyton mentagrophytes	12.5	1.56	6.25
Trichophyton rubrum	12.5	1.56	6.25
Candida albicans	100	6.25	12.5
Candida utilis	100	6.25	12.5

the minimum growth inhibitory concentrations, DS-pervicosides A (**7**), B (**8**), and C (**9**) exhibited distinct antifungal activities against various pathogenic microorganisms, and DS-pervicoside B (**8**) was the most active.

Experimental

The instruments used to obtain physical data and the experimental conditions for chromatography were the same as described in our previous paper.[9]

Isolation of DS-Pervicosides A (7), B (8), and C (9) 1) The fresh body walls (1.3 kg) of the sea cucumber *Holothuria pervicax* (collected in August at Kushimoto in Wakayama Prefecture) were extracted with MeOH (5 l each) under reflux 3 times for 4 h each. The MeOH extract (50 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 (7.5 g). The 1-butanol-soluble portion (7.5 g) was then purified by column chromatography [Silica gel 60, 60—230 mesh (Merck), 300 g, $CHCl_3$–MeOH–H_2O=7:3:1 (lower phase)] to give the oligoglycoside mixture [containing pervicosides A (**10**), B (**11**), and C (**12**)] (3 g). A solution of the

oligoglycoside mixture (1.4 g) in dioxane (10 ml)–pyridine (20 ml) was heated under reflux for 3 h, then cooled. The residue, obtained after evaporation of the organic solvent under reduced pressure, was partitioned into a 1-butanol–water (300 ml each) mixture to give the 1-butanol-soluble portion (1.1 g). The 1-butanol-soluble portion (1.1 g) was then purified by HPLC (μ-Bondapak C_{18}, CH_3CN–MeOH–H_2O = 1 : 1 : 1) to give DS-pervicoside A (**7**) (150 mg), DS-pervicoside B (**8**) (730 mg), and DS-pervicoside C (**9**) (90 mg).

2) The combined Cuvierian tubules (80 g) of the same sea cucumber were extracted with MeOH (500 ml each) under reflux 3 times for 4 h each. The MeOH extract (3 g) thus obtained was partitioned into a 1-butanol–water (500 ml each) mixture to give the 1-butanol-soluble portion (500 mg). The 1-butanol extract (500 mg) was then subjected to silica gel column chromatography as above to give the oligoglycoside mixture (99 mg). A solution of the oligoglycoside mixture (90 mg) in dioxane (1 ml)–pyridine (4 ml) was heated under reflux for 3 h. Work-up of the reaction mixture as described above gave a product which was purified by HPLC to furnish DS-pervicoside A (**7**) (37 mg).

DS-Pervicoside A (**7**): Colorless needles, mp 210—212 °C (MeOH), $[\alpha]_D^{20}$ +0.3° (c=0.8, pyridine). *Anal.* Calcd for $C_{56}H_{90}O_{24}\cdot H_2O$: C, 57.70; H, 7.96. Found: C, 57.41; H, 7.95. IR ν_{max}^{KBr} cm^{-1}: 3400, 1750, 1730, 1065. UV λ_{max}^{MeOH}: transparent above 210 nm. CD ($c=6.6\times10^{-2}$, MeOH): $[\theta]_{244}$ 0, $[\theta]_{222}$ −7500 (neg. max.), $[\theta]_{215}$ 0. ^{13}C-NMR (50 MHz, pyridine-d_5) δ_C: as given in Table I.

DS-Pervicoside B (**8**): Colorless needles, mp 212—214 °C (MeOH), $[\alpha]_D^{20}$ +4° (c=1.4, pyridine). *Anal.* Calcd for $C_{54}H_{86}O_{22}\cdot H_2O$: C, 58.68; H, 8.03. Found: C, 58.40; H, 8.05. IR ν_{max}^{KBr} cm^{-1}: 3400, 1740, 1630, 1070 (br). UV λ_{max}^{MeOH}: transparent above 210 nm. CD ($c=2.9\times10^{-2}$, MeOH): $[\theta]_{240}$ 0, $[\theta]_{222}$ −7800 (neg. max.), $[\theta]_{214}$ 0, $[\theta]_{208}$ +18000. ^{13}C-NMR (50 MHz, pyridine-d_5) δ_C: as given in Table I.

DS-Pervicoside C (**9**): Colorless needles, mp 215—217 °C (MeOH), $[\alpha]_D^{20}$ +2° (c=0.8, pyridine). *Anal.* Calcd for $C_{54}H_{88}O_{22}\cdot 2H_2O$: C, 57.63; H, 8.24. Found: C, 57.43; H, 7.97. IR ν_{max}^{KBr} cm^{-1}: 3400, 1742, 1065 (br). UV λ_{max}^{MeOH}: transparent above 210 nm. CD ($c=8.3\times10^{-2}$, MeOH): $[\theta]_{240}$ 0, $[\theta]_{222}$ −8000 (neg. max.), $[\theta]_{214}$ 0. ^{13}C-NMR (50 MHz, pyridine-d_5) δ_C: as given in Table I.

Carbohydrate Compositions of DS-Pervicosides A (7), B (8), and C (9) A solution of DS-pervicoside A (**7**), DS-pervicoside B (**8**), or DS-pervicoside C (**9**) (10 mg each) in 2.5 N AcCl–MeOH (1.0 ml) was heated under reflux for 2 h, then cooled. The reaction mixture was neutralized with Ag_2CO_3 (powder) and filtered. Removal of the solvent from the filtrate under reduced pressure gave a residue, which was dissolved in pyridine (0.1 ml) and treated with *N,O*-bis(trimethylsilyl) trifluoroacetamide (BSTFA) (0.2 ml) at room temperature for 5 min. The reaction mixture was subjected to gas-liquid chromatographic (GLC) analysis [column of 5% SE-52 on Chromosorb WAW DMCS (80—100 mesh), 3 mm ×2 m; column temperature 170 °C; N_2 flow rate 30 ml/min] to show that each of DS-pervicoside A (**7**), DS-pervicoside B (**8**), and DS-pervicoside C (**9**) contained the same carbohydrate composition, comprising one mol each of xylose (t_R=6 min 35 s, 7 min 20 s), quinovose (t_R=8 min 05 s), 3-*O*-methylglucose (t_R=9 min 25 s), and glucose (t_R=17 min 50 s, 19 min 30 s). All component monosaccharides were deduced to be D, since these sugars hitherto obtained from the hydrolysates of sea cucumber saponins were D, as proved by their $[\alpha]_D$ values.[2]

Acidic Hydrolysis of DS-Pervicoside B (8) A mixture of DS-perivicoside B (**8**) (510 mg) and aqueous 2 N HCl (60 ml) was heated for 1.5 h on a boiling water-bath. After dilution with water, the reaction mixture was extracted with AcOEt. The AcOEt extract was washed with saturated aqueous $NaHCO_3$ and brine, then dried over $MgSO_4$. Removal of the solvent under reduced pressure from the AcOEt extract gave a product (210 mg), which was purified by column chromatography (SiO_2 20 g, benzene–acetone = 20 : 1) to furnish **1** (80 mg) and **2** (90 mg).

1: Colorless needles, mp 145—147 °C (MeOH), $[\alpha]_D^{18}$ −16° (c=0.6, $CHCl_3$). IR ν_{max}^{KBr} cm^{-1}: 3300, 1748. UV λ_{max}^{MeOH} nm (ε): 245 (12000), 238 (sh) (11000). CD ($c=1.8\times10^{-2}$, MeOH): $[\theta]_{265}$ 0, $[\theta]_{248}$ −42000 (neg. max.), $[\theta]_{236}$ 0, $[\theta]_{222}$ +49000 (pos. max.). ^{1}H-NMR (90 MHz, $CDCl_3$) δ: 0.91 (3H, s), 1.01 (6H, s), 1.12 (3H, s), 1.42 (3H, s, 20-CH_3), 1.62, 1.71 (both 3H, s, 25-$(CH_3)_2$), 3.25 (1H, t-like, 3α-H), 5.08 (1H, t-like, 24-H), 5.22, 5.53 (both 1H, m, 7, 11-H). ^{13}C-NMR (50 MHz, pyridine-d_5) δ_C: 177.1 (s, 18-C), 148.6 (s, 9-C), 141.1 (s, 8-C), 132.1 (s, 25-C), 124.5 (d, 24-C), 121.3 (d, 7-C), 112.8 (d, 11-C). MS m/z (%): 452 (M^+, 75), 365 ($M^+ - C_5H_9 - H_2O$, 66), 69 ($C_5H_9^+$, 100). High-MS Found: 452.330. Calcd for $C_{30}H_{44}O_3$: 452.329.

2: Colorless needles, mp 214—216 °C (acetone), $[\alpha]_D^{20}$ −7° (c=0.9, $CHCl_3$). IR ν_{max}^{KBr} cm^{-1}: 3500, 3430, 1750. UV λ_{max}^{MeOH} nm (ε): 243 (17000), 239 (sh) (16000). CD ($c=4.2\times10^{-3}$, MeOH): $[\theta]_{265}$ 0, $[\theta]_{248}$ −37000 (neg. max.), $[\theta]_{235}$ 0, $[\theta]_{222}$ +49000 (pos. max.), $[\theta]_{206}$ 0. ^{1}H-NMR (90 MHz, $CDCl_3$ + CD_3OD) δ: 0.89 (3H, s), 1.01 (6H, s), 1.09 (3H, s), 1.20 (6H, s, 25-$(CH_3)_2$), 1.42 (3H, s, 20-CH_3), 3.18 (1H, t-like, 3α-H), 5.22, 5.53 (both 1H, m, 7, 11-H). ^{13}C-NMR (50 MHz, pyridine-d_5) δ_C: 177.2 (s, 18-C), 148.6 (s, 9-C), 141.1 (s, 8-C), 121.3 (d, 7-C), 112.8 (d, 11-C), 69.5 (s, 25-C). MS m/z (%): 470 (M^+, 12), 365 ($M^+ - 2H_2O - C_5H_9$, 68), 69 ($C_5H_9^+$, 100). High-MS Found: 470.339. Calcd for $C_{30}H_{46}O_4$: 470.339.

Acidic Treatment of 1 or 2 A mixture of **1** (5 mg) or **2** (5 mg) and aqueous 2 N HCl (1 ml)–acetone (1 ml) was heated under reflux for 2 h. After dilution with water, the reaction mixture was extracted with AcOEt. The AcOEt extract was washed with saturated aqueous $NaHCO_3$ and brine, then dried over $MgSO_4$. Removal of the solvent under reduced pressure from the AcOEt extract gave a product, which was examined by thin-layer chromatography (TLC) [Silica gel 60 F_{254} (Merck), benzene–acetone = 4 : 1] and by HPLC (μPorasil, *n*-hexane–AcOEt = 3 : 1) to identify **1** and **2** (in *ca.* 1 : 1 ratio).

Acetylation of 1 Followed by Reduction A solution of **1** (55 mg) in pyridine (1 ml) was treated with Ac_2O (0.5 ml) and the whole mixture was left standing at room temperature (15 °C) for 6 h. The reaction mixture was poured into ice-water and the whole was extracted with AcOEt. Work-up of the AcOEt extract in the usual manner gave a product (54 mg), which was purified by HPLC (μPorasil, *n*-hexane–AcOEt = 15 : 1) to furnish the acetate (35 mg). A mixture of the acetate (25 mg) and a Raney Ni (W-2)–EtOH suspension (5 ml) was heated under reflux for 30 min. After removal of the insolubles by filtration, the filtrate was evaporated under reduced pressure to give seychellogenin acetate (**4**) (19 mg). **4**: $[\alpha]_D^{18}$ +19° (c=0.8, $CHCl_3$). [**4**[1]) from bivittoside A: $[\alpha]_D^{20}$ +20° (c=0.7, $CHCl_3$)]. IR ν_{max}^{KBr} cm^{-1}: 1755, 1730. UV λ_{max}^{MeOH} nm (ε): 238 (sh) (13000), 243 (14000). ^{1}H-NMR (90 MHz, $CDCl_3$) δ: 0.86, 0.92, 1.02, 1.14, 1.40 (each 3H, s), 0.95 (6H, d, J=7 Hz), 2.04 (3H, s), 4.51 (1H, t-like, J=7 Hz), 5.22 (1H, m), 5.51 (1H, m).

Enzymatic Hydrolysis of DS-Pervicoside B (8) Followed by Reduction A suspension of DS-pervicoside B (**8**) (302 mg) in an AcONa–AcOH buffer solution (pH 5.2, 100 ml) was treated with crude hesperidinase (620 mg, Lot No. 680930, provided by Tanabe Pharma Co.) and the whole mixture was stirred at 35 °C for 7 h. The reaction mixture was then treated with 1-butanol (50 ml) and heated at 50 °C for 10 min and filtered with Celite 535. The filtrate was extracted with 1-butanol and the 1-butanol extract was washed with water and concentrated under reduced pressure to give a product (300 mg). Purification of the product (300 mg) by column chromatography [SiO_2 20 g, $CHCl_3$–MeOH–H_2O = 7 : 3 : 1 (lower phase)] furnished a partial hydrolysate **5** (152 mg). The mixture of **5** (152 mg) and a Raney Ni (W-2)–EtOH suspension (15 ml) was heated for 5 h. After filtration, the solvent was evaporated off under reduced pressure to furnish bivittoside A (**6**) (93 mg), which was shown to be identical with an authentic sample[1,3f)] by HPLC and ^{13}C-NMR comparisons.

Methylation of DS-Pervicoside B (8) Followed by Methanolysis 1) A solution of DS-pervicoside B (**8**) (96 mg) in dimethyl sulfoxide (DMSO) (5 ml) was treated with a dimsyl carbanion solution (10 ml) [prepared from NaH (2 g), which was washed with dry *n*-hexane before use, and DMSO (35 ml) by stirring at 70 °C for 1 h under an N_2 atmosphere] and the whole mixture was stirred at room temperature (25 °C) for 1 h under an N_2 atmosphere. The reaction mixture was treated with CH_3I (3.5 ml) under ice-cooling and stirred at room temperature for a further 1 h in the dark. The reaction mixture was then poured into ice-water and the whole was extracted with AcOEt. The AcOEt extract was washed with water, then dried over $MgSO_4$. Removal of the solvent from the AcOEt extract under reduced pressure gave the dodeca-*O*-methyl derivative (**13**) (102 mg).

13: $[\alpha]_D^{18}$ +11° (c=0.7, $CHCl_3$). IR $\nu_{max}^{CCl_4}$ cm^{-1}: 1750. ^{1}H-NMR (90 MHz, $CDCl_3$) δ: 3.40—3.62 (OMe ×12), 4.30 (1H, d, J=7 Hz), 4.35 (1H, d, J=8 Hz), 4.65 (2H, d, J=7 Hz), 5.53 (1H, m).

2) A solution of **13** (10 mg) in 2.5 N AcCl–MeOH (2 ml) was heated under reflux for 1 h and neutralized with Ag_2CO_3 (powder). The whole mixture was filtered and the filtrate was subjected to GLC analysis [1) 5% butane-1,4-diol succinate (BDS) on Uniport B (80—100 mesh); column 3 mm × 2 m; column temperature 135 °C, 175 °C; N_2 flow rate 35 ml/min; 2) 15% neopentylglycol succinate (NPGS) on Chromosorb WAW (80—100 mesh); column 3 mm × 2 m; column temperature 180 °C; N_2 flow rate 35 ml/min] and TLC (benzene–acetone = 2 : 1) to identify methyl 2,3,4,6-tetra-*O*-methyl-glucopyranoside [1) BDS (135 °C), t_R=10 min 30 s; 15 min 55 s; 2) NPGS, t_R=5 min 30 s, 7 min 30 s; Rf=0.75], methyl 2,4,6-tri-*O*-methylglucopyranoside [1) BDS (175 °C), t_R =6 min 50 s, 9 min 55 s; 2) NPGS, t_R=14 min 45 s, 20 min 50 s; Rf=0.20, 0.35], methyl 2,3-di-*O*-methylquinovopyranoside [1) BDS (135 °C), t_R=10 min 00 s, 13 min 50 s;

2) NPGS, t_R=5 min 00 s, 6 min 20 s; Rf=0.45, 0.65], methyl 3,4-di-*O*-methylxylopyranoside [1) BDS (135 °C), t_R=12 min 50 s, 15 min 55 s; 2) NPGS, t_R=6 min 20 s, 7 min 30 s; Rf=0.20, 0.25].

Acidic Hydrolysis of DS-Pervicoside C (9) A mixture of DS-pervicoside C (**9**) (7 mg) and aqueous 2 N HCl (1 ml) was heated for 4 h on a boiling water-bath. After dilution with water, the reaction mixture was extracted with AcOEt. The AcOEt extract was washed with saturated aqueous $NaHCO_3$ and brine, then dried over $MgSO_4$. Removal of the solvent under reduced pressure from the AcOEt extract gave a product (7 mg), which was examined by TLC (benzene–acetone=4:1) to identify seychellogenin (**3**)[1,3f] as a single product.

Reduction of DS-Pervicoside B (8) A mixture of DS-pervicoside B (**8**) (120 mg) and a Raney Ni (W-2)–EtOH suspension (60 ml) was heated under reflux for 8 h. After removal of the insolubles by filtration, the filtrate was evaporated under reduced pressure to give a product, which was partitioned into a 1-butanol–water mixture. The 1-butanol-soluble portion (122 mg) was purified by HPLC (μBondapak C_{18}, CH_3CN–MeOH–H_2O=2:1:1) to furnish DS-pervicoside C (**9**) (41 mg) and **8** (43 mg, recovered). **9** was shown to be identical with an authentic sample by HPLC and ^{13}C-NMR comparisons.

Acidic Hydrolysis of DS-Pervicoside A (7) A mixture of DS-pervicoside A (**7**) (20 mg) and aqueous 2 N HCl was heated for 3 h on a boiling water-bath. Work-up of the reaction mixture as described above gave an AcOEt extract (15 mg), which was purified by column chromatography (SiO_2 10 g, benzene–acetone=20:1) to furnish **1** (3 mg) and **2** (5 mg). **1** and **2** were shown to be identical with authentic samples by TLC and HPLC comparisons.

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