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STARFISH SAPONINS, 45.[1] NOVEL SULFATED STEROIDAL GLYCOSIDES FROM THE STARFISH *ASTROPECTEN SCOPARIUS*

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ABSTRACT.—Five sulfated steroidal glycosides have been isolated from the H_2O extracts of the whole bodies of the starfish *Astropecten scoparius*. One of them has been identified with indicoside B [**1**], a sulfated steroidal xylofuranoside previously isolated from *Astropecten indicus*, and the remaining four are new compounds. Three of them, scopariosides A [**2**], B [**3**], and C [**4**] have the same (24*S*)-5α-cholestane-3β,6α,8,15β,24-pentaol aglycone as indicoside B but differ in the sugar moieties. The aglycone is also present in the H_2O extracts as 24-*O*-sulfate derivative. The fourth new glycoside, scoparioside D, has been characterized as (24*R*)-24-*O*-β-xylopyranosyl-5α-cholest-22*E*-ene-3β,6α,8,15-tetraol 15-sulfate [**5**].

These glycosides are accompanied by four known polyhydroxysteroids **7–10** and one known asterosaponin, the hexaglycoside marthasteroside A_1.

Recently we have investigated the steroid glycoside constituents of the starfish *Astropecten indicus* and isolated three novel compounds, indicoside A, which contains a rare 5-*O*-methyl-β-galactofuranosyl moiety attached at C-28 of 24-methyl-5α-cholestane-3β,6α,7α,8,15β,24,28-heptaol aglycone (1), and indicosides B [**1**] and C (16β-hydroxy derivative of **1**) (2), which represent the first examples of steroidal glycosides from echinoderms in which a xylose moiety is in its furanose form.

Continuing with our work on biologically active compounds from echinoderms (3,4), we have analyzed the extracts from the whole bodies of a second *Astropecten* species, i.e. *Astropecten scoparius* Valenciennes (family Astropectinidae), collected at Okkirai Bay (Japan), and we have now isolated four new steroid glycoside sulfates, designated as scopariosides A [**2**], B [**3**], C [**4**], and D [**5**], along with the previously isolated indicoside B [**1**].

Scopariosides A–C have the same 5α-cholestane-3β,6α,8,15β,24-pentaol aglycone as indicoside B [**1**], and more interestingly, scoparioside B also contains the same rare xylofuranosyl moiety as the indicosides B and C from *A. indicus*.

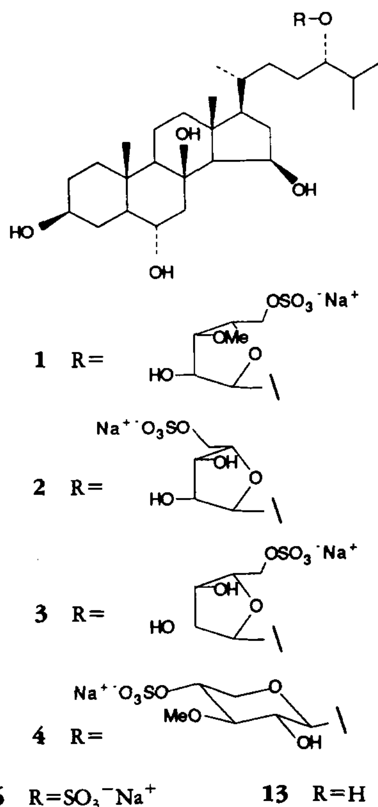
It is interesting for taxonomic purposes to note the presence of identical structural features in steroid glycosides from two species of the same genus (5). These glycosides co-occur with 5α-cholestane-3β,6α,8,15β,24-pentaol 24-sulfate [**6**], four known polyhydroxysteroids **7–10** (6–8), and the known asterosaponin marthasteroside A_1 (9).

In this paper we describe the isolation and structure elucidation of the new compounds.

RESULTS AND DISCUSSION

Separation and isolation of individual compound from the aqueous extracts of the animals followed the steps described previously (2). The results of our analysis are shown in Table 1. Identification of the known compounds has been achieved by direct comparison (fabms, ^1H nmr, hplc) with authentic samples.

[1]For Part 44, see Zollo *et al.*, *J. Nat. Prod.*, **53**, 1000 (1990).



Scoparioside A [**2**].—The fabms (negative ion mode) exhibited a molecular anion species at *m/z* 663 $[M]^-$, and no further fragmentation was observed. Upon solvolysis in a dioxane/pyridine mixture, **2** afforded a less polar desulfated derivative, which gave a quasi molecular ion at *m/z* 583 $[M-H]^-$ on fabms (negative ion mode), accompanied by a fragment at *m/z* 451 $[M-H-132]^-$ (loss of a pentose residue). On acid methanolysis it liberated methyl arabinosides. Those data suggested a steroid sulfated glycoside with the sulfate on the arabinosyl moiety. Examination of the ^{1}H-nmr spectral data (Table 2) of **2** immediately indicated the presence of the same 5α-cholestane-

TABLE 1. Steroidal Glycosides and Polyhydroxysteroids from the Starfish *Astropecten scoparius* (350 g fresh wt).

Compound	Amount (mg)	Rotations [α]	hplc[a] mobility (min)	Reference
Indicoside B [**1**]	1.5	−3.0	13.6	Riccio *et al.* (2)
Scoparioside A [**2**]	3.2	−8.5°	12.8	new
Scoparioside B [**3**]	2.0	0°	12.0	new
Scoparioside C [**4**]	7.0	−6.3	14.8	new
Scoparioside D [**5**]	1.5	+11.6°	12.6	new
Steroid **6**	6.0	+8.6°	10.0	new
Steroid **7**	12.0	+0.4°	15.6	Iorizzi *et al.* (6)
Steroid **8**	1.5	+8.2°	14.0	D'Auria *et al.* (7)
Steroid **9**	1.5	+16.0°	15.2	Iorizzi *et al.* (6)
Steroid **10**	1.7	0°	17.6	Iorizzi *et al.* (8)
Marthasteroside A_1	10.5	+3.1°	13.6	Bruno *et al.* (9)

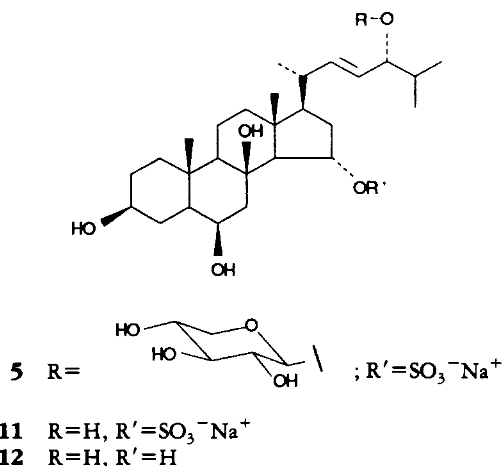
[a]C_{18} μ-Bondapak column (30 cm × 3.9 mm i.d.), MeOH-H_2O (1:1) for **1–6** and marthasteroside A_1, MeOH-H_2O (7:3) for **7–10**, flow rate 2.0 ml/min.

TABLE 2. Selected ^{1}H-nmr Signals (δ_H in CD_3OD) for Compounds **1**–**6**. J (Hz) Values are Shown in Parentheses.

Proton	Compound					
	1	**2**	**3**	**4**	**5**	**6**
H-3	3.55 m	3.52 m	3.52 m	3.52 m	3.50 m	3.52 m
H-6	3.72 ddd (10.5, 10.5, 4)	3.71 ddd (10.5, 10.5, 4)	3.71 ddd (10.5, 10.5, 4)	3.72 ddd (10.5, 10.5, 4)	3.89 br s	3.72 ddd (10.5, 10.5, 4)
H-7	2.42 dd (12.5, 4)	2.42 dd (12.5, 4)	2.42 dd (12, 3.5)	2.41 dd (12.5, 4)	2.56 dd (15, 3)	2.40 dd (12, 3.5)
H-15	4.48 m	4.48 m	4.46 m	4.44 t (5.5)	4.95 dt (10, 3)	4.44 m
H-16	2.45 m	2.45 m	2.46 m	2.41 m	—	2.44 m
H-18	1.30 s	1.30 s	1.30 s	1.30 s	1.02 s	1.30 s
H-19	1.01 s	1.01 s	1.01 s	1.02 s	1.20 s	1.02 s
H-21	0.95 d (6.8)	0.95 d (6.8)	0.96 d (6.8)	0.96 d (6.8)	1.04 d (7)	0.98 d (6.8)
H-22					5.49 dd (7.5, 15)	
H-23					5.36 dd (7.5, 15)	
H-24					3.75 t (7.5)	4.15 m
H-26	0.92 d (6.8)	0.92 d (6.8)	0.93 d (7)	0.96 d (6.8)	0.88 d (7)	0.95 d (7)
H-27	0.92 d (6.8)	0.92 d (6.8)	0.93 d (7)	0.96 d (6.8)	0.96 d (7)	0.94 dd (7)
	Xylose	Arabinose	Xylose	Xylose	Xylose	
H-1′	4.95 d (1.5)	4.94 d (1.5)	4.96 d (1.5)	4.30 d (7.5)	4.29 d (7.5)	
H-2′	4.10 t (1.5)	3.99 dd (1.5, 3.7)	4.04 dd (1.5, 3.7)	3.28 dd (7.5, 9)	3.15 dd (7.5, 9)	
H-3′	3.72 dd (1.5, 5)	3.90 dd (6, 3.7)	4.07 dd (3.7, 5)	3.35 t (9)	3.30 t (9)	
H-4′	4.48 m	4.15 m	4.42 m	4.28 m	3.50 m	
H-5′	4.34 dd (11, 5)	4.15 m	4.20 dd (12, 5)	4.25 dd (10.6, 5)	3.85 dd (10.5, 3.5)	
	4.16 dd (11, 7.5)	4.15 m	4.42 dd (12, 6.2)	3.22 t (10.6)	3.20 t (10.5)	
OMe	3.45 s			3.65 s		

[a]Signal under solvent signal.

3β,6α,8,15β,24-pentaol aglycone found in indicoside B [1] (2) and in the previously described attenuatoside A-I and halityloside E, 24-*O*-steroidal glycosides from *Hacelia attenuata* (10) and *Halityle regularis* (8), respectively. We note that 5α-cholestane-3β,6α,8,15β,24-pentaol was also isolated from *Gomophia watsoni* (11). In addition to the steroid moiety, the ^{1}H-nmr spectrum showed signals (Table 2) for a moiety equivalent to an α-arabinofuranoside determined by sequential decoupling. The upfield shift of H_2-5′ from δ 4.15 in **2** to 3.66 and 3.78 ppm in the desulfated derivative clarified that C-5 of the arabinofuranosyl moiety bears the sulfate. Analysis of the ^{13}C-nmr spectrum (Table 3) of **2** confirmed all the above structural data; furthermore the glycosidation shift observed for C-24 [85.4 ppm in **2** vs. 77.2 ppm in the aglycone (11)] established the location there of the α-arabinofuranosyl 5-sulfate moiety. The 24*S* configuration is proposed by analogy with **6**, for which the configuration at C-24 has been determined (see below); the L configuration for arabinose is suggested by analogy with the other steroid L-arabinosides from starfishes.



Scoparioside B [**3**].—This compound is isomeric with scoparioside A [**2**]. The fabms (negative ion mode) of **3** gave the anion peak at *m/z* 663, and fabms of its desulfated derivative (dioxane/pyridine, 120°) gave the pseudomolecular ion peak at *m/z* 583 accompanied by the strong peak at *m/z* 451 (loss of a pentose residue). On acid methanolysis, the desulfated derivative of **3** liberated methyl xylosides. Comparison of the ^{1}H-nmr spectra of **2** and **3** (Table 2) showed that the two compounds have the same aglycone and established that the β-xylosyl residue in **3** is in its furanose form. In particular we note that the major difference in the spectra of **2** and **3** is the chemical shift of H-4′, which in **3** is downfield-shifted to 4.42 ppm (δ 4.15 in **2**) because of the 1,3-syn interaction between H-4′/2′-OH in the xylofuranose structure as compared with the anti arrangement in the arabinofuranose structure. Upfield shifts of H_2-5′ from δ 4.42/4.20 in **3** to 3.90/3.79 ppm in the desulfated derivative confirmed the location of the sulfate group. The 24*S* configuration is proposed by analogy with **6**, while the D configuration for xylose is suggested by analogy with the other steroid D-xylosides from starfishes.

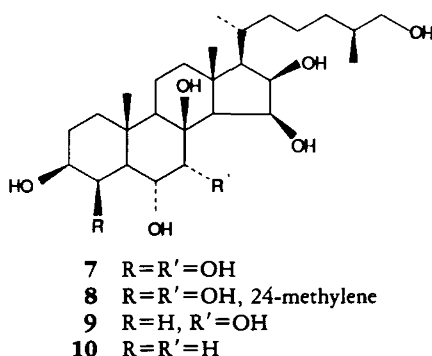
Scoparioside C [**4**].—The fabms (negative ion mode) of **4** gave an anion peak at *m/z* 677 $[M]^-$. Upon solvolysis it afforded a less polar desulfated derivative, which gave a quasi molecular ion peak at *m/z* 597 $[M-H]^-$ accompanied by a fragment at *m/z* 451 corresponding to the loss of a methoxylated pentose unit (=146 mass units) from $[M-H]^-$ peak.

TABLE 3. ^{13}C-nmr Shifts (62.9 MHz, CD_3OD) of Compounds **2**, **4**, and **6**.

Carbon	Compound		
	2	**4**	**6**
C-1	39.5	39.5	39.5
C-2	31.5	31.5	31.5
C-3	72.3	72.3	72.3
C-4	32.5	32.4	32.4
C-5	53.9	54.0	54.0
C-6	67.8	67.8	67.7
C-7	[a]	49.4	[a]
C-8	77.6	77.4	77.5
C-9	57.5	57.6	57.6
C-10	38.0	38.0	38.0
C-11	19.8	19.8	19.7
C-12	43.5	43.5	43.5
C-13	44.5	44.4	44.4
C-14	62.6	62.8	62.8
C-15	71.3	71.3	71.3
C-16	42.6	42.5	42.4
C-17	58.2	58.2	58.0
C-18	16.6	16.5	16.5
C-19	14.1	14.1	14.1
C-20	36.5	36.4	36.3
C-21	19.0	19.0	19.0
C-22	33.0	32.2	32.3
C-23	29.1	29.0	28.4
C-24	85.4	86.3	85.9
C-25	32.2	32.2	31.9
C-26	18.4	18.4	18.5
C-27	18.4	18.4	18.0
C-1′	109.6	104.7	
C-2′	83.9	74.6	
C-3′	79.4	85.1	
C-4′	82.8	77.6	
C-5′	68.8	64.7	
OMe		60.1	

[a]Under solvent signal.

Examination of ^{1}H- and ^{13}C-nmr spectra (Tables 2 and 3) of **4** indicated the presence of 5α-cholestane-3β,6α,8,15β,24-pentaol aglycone. In addition to the steroid moiety, the ^{1}H-nmr spectrum (Table 2) showed one methoxyl singlet at δ 3.65 and four methine protons at δ 4.30 (d, $J=7.5$ Hz), 3.28 (dd, $J=7.5$, 9 Hz), 3.35 (t, $J=9$ Hz), and 4.28 (m), which couple to their neighbors in this order. The last methine proton is further coupled to methylene protons at δ 3.22 (t, $J=10.6$ Hz) and 4.25 (dd, $J=5$, 10.6 Hz). The coupling constants indicated that the molecule bears a moiety equivalent to a β-xylopyranoside. The upfield shift of H-4′ from δ 4.28 in **4** to 3.50 ppm in the desulfated derivative established the location of the sulfate group at C-4 of the sugar. Desulfation was also accompanied by moderate upfield shifts of H-3′ (δ 3.04) and H_2-5′ (δ 3.18/3.83). The high field shift observed for H-3′ (δ 3.04) in the spectrum of the desulfated derivative, when compared with the shift observed in steroid xylopyranosides (δ 3.30, see, for example, **5** in Table 2) was indicative of a 3-*O*-methyl xylopyranosyl unit. The location of the sulfate at 4-OH and of the methyl group at 3-OH of the xylopyranosyl unit in **4** was confirmed by ^{13}C nmr, which also established



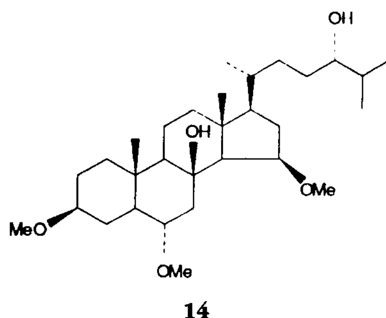
7 R=R'=OH
8 R=R'=OH, 24-methylene
9 R=H, R'=OH
10 R=R'=H

the sugar moiety to be linked at C-24 of the steroid. The 24*S* configuration is suggested by analogy with **6** and supported by the ^{13}C-nmr spectrum of **4**, which showed signals for the side chain carbons identical with those of previous (24*S*)-24-*O*-β-D-xylopyranosyl steroids isolated from starfishes (12). We prefer the D configuration for xylose by analogy with other steroid glycosides isolated from starfishes.

Scoparioside D [**5**].—The fabms (negative ion mode) showed a molecular anion peak at *m/z* 661 $[M]^-$ accompained by a fragment at *m/z* 529 corresponding to the loss of a pentose unit. On acid methanolysis it liberated methyl xylosides. In addition to the signals assigned to a β-xylopyranosyl moiety, the ^{1}H-nmr spectrum (Table 2) showed the presence of the 5α-cholest-22*E*-ene-3β,6β,8,15α,24-pentaol 15-sulfate aglycone [**11**], previously isolated from the starfish *Coscinasterias tenuispina* (13). In confirmation, enzymatic hydrolysis with a glycosidase mixture from *Charonia lampas* (Scikagaku Kogyo Co. Ltd, Tokyo, Japan) removed xylose to yield the sulfated polyhydroxysteroid **11**, identical in all respects (^{1}H nmr, hplc, tlc) with an authentic sample (13). The major difference between the spectra of the glycoside **5** and its aglycone **11** was observed for the signal assigned to H-24, which in **5** appeared as a dd at δ 3.75, and in **11** as a triplet at δ 3.68. On this basis we located the xylosyl residue at C-24. The 24*R* configuration is proposed on the basis of the chemical shifts of H-24 and H-22 signals of the desulfated derivative **12**, in comparison with the synthetic models (22*E*,24*R*) and (22*E*,24*S*)-6β-methoxy-3α,5-cyclo-5α-cholest-22-en-24-ol (12, 14), which showed signals at δ 3.71 (t, *J* = 7 Hz, H-24) and 5.52 (dd, *J* = 16, 8 Hz, H-22) and 3.68 (t, *J* = 7 Hz, H-24) and 5.46 (dd, *J* = 16, 8 Hz, H-22), respectively. In the spectrum of **12** the peaks observed at δ 3.71 and 5.50 compared well with those of 24*R* isomer.

Compound **6**.—This compound is the 24-*O*-sulfated derivative of the (24*S*)-5α-cholestane-3β,6α,8,15β,24-pentaol [**13**] previously isolated from the starfish *G. watsoni* (11). The fabms (negative ion mode) showed a molecular anion peak at *m/z* 531. The ^{1}H-nmr spectrum showed the signal assigned to H-24 shifted downfield to δ 4.15 (δ 3.24 in **13**) whereas the remaining signals were virtually identical in both spectra. The ^{13}C-nmr spectrum of **6** (Table 3) and comparison with that of **13** confirmed the location of the sulfate at C-24 [C-24: 85.9, C-23: 28.4, C-25: 31.9 ppm in **6**; C-24: 78.2, C-23: 31.8, C-25: 34.5 ppm in **13** (11)]. The 24*S* configuration was assigned to **13** isolated from *G. watsoni* based on the ^{13}C-nmr side chain carbon signals and comparison with the spectra of the model (24*S*)- and (24*R*)-24-hydroxycholesterol (11). The ^{13}C-nmr spectra of the epimeric model steroids show characteristic differences even if quite small. Therefore we have decided to determine the configuration at C-24 of **6** by using a different approach consisting of preparing the 24-(+)-(*R*)-α-methoxy-α-(trifluoromethyl)-phenyl acetate [MTPA, Mosher's reagent (15)] and comparing its ^{1}H-nmr spectrum with those of the (+)-(*R*)-MTPA ester of (24*S*)-24-OH and (24*R*)-24-

OH model steroids which are easily differentiated by the signals of the isopropyl methyl protons (i.e., two doublets at δ 0.84 and 0.86 ppm in the 24*S* isomer and one 6H doublet at δ 0.92 in 24*R* isomer) (12). Thus, **6** was methylated (CH_3I/NaH in DMF) and then solvolyzed (dioxane/pyridine) to yield the 3β,6α, 15β-trimethoxy-5α-cholestane-8,24-diol **14**, which was esterified with (+)-α-methoxy-α-(trifluoromethyl)-phenyl chloride in dry pyridine. The ^{1}H-nmr spectrum of the resulting (+)-(*R*)-MTPA ester showed signals for the isopropyl methyl protons at δ 0.84 d and 0.87 d, which compared well with the signals (δ 0.84 and 0.86 ppm) found in the spectrum of the (+)-(*R*)-MTPA ester of (24*S*)-24-OH model steroid.



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EXPERIMENTAL

GENERAL EXPERIMENTAL PROCEDURES.—Nmr spectra, Bruker WM-250 (^{1}H at 250 MHz, ^{13}C at 62.9 MHz), δ (ppm), *J* in Hz, spectra referred to CHD_2OD signal at 3.30 ppm and to central carbon CD_3OD signal at 49.0 ppm; mass spectra, VG ZAB mass spectrometer equipped with fab source [in glycerol or glycerol-thioglycerol (3:1) matrix; Xe atoms of 2–6 kV]; optical rotations, Perkin-Elmer model 241 polarimeter; glc, Carlo Erba Fractovap 2900 for capillary column (SE-30, 25 mt, 125°, He carrier flow 5 ml/min); reversed-phase hplc, C_{18} μ-Bondapak column (30 cm × 8 mm i.d., flow rate 5 ml/min) and C_{18}μ-Bondapak column (30 cm × 3.9 mm i.d., flow rate 5 ml/min), Waters Model 6000 A pump equipped with a U6K injector and a differential refractometer, model 401; dccc, DCC-A apparatus manufactured by Tokyo Rikakikai Co. equipped with 250 tubes and Buchi apparatus equipped with 300 tubes.

EXTRACTION AND ISOLATION.—The animals, *A. scoparius* (0.7 kg), were collected at Okkairi Bay, Iwate Predecture, Japan in May 1986 and identified at the School of Fisheries Sciences of the Kitasato University, Sanriku-Cho, Japan; a voucher specimen is preserved there. The animals were chopped and soaked in H_2O (2 × 1 liter); the aqueous extracts were centrifuged and passed through a column of Amberlite XAD-2 (1 kg). This column was washed with distilled H_2O (1 liter) and eluted with MeOH (2 liters). The MeOH eluate was taken dryness to give a glassy material (2.8 g), which was then chromatographed (1.4 g) on a column of Sephadex LH-60 (2.5 × 80 cm) with MeOH-H_2O (2:1) as eluent. Fractions (3 ml) were collected and analyzed by tlc on SiO_2 with *n*-BuOH–HOAc–H_2O (12:3:5).

Fractions 49–72 mainly contained the "asterosaponins" (sulfated steroidal penta- and hexa-glycosides), and the residue (175 mg in total) was subjected to droplet counter-current chromatography (dccc) using *n*-BuOH–Me_2CO–H_2O (3:1:5) in the descending mode (the upper phase was the stationary phase, flow rate 12 ml/h; 5-ml fractions were collected and monitored by tlc. Fractions 42–51 (25 mg) were evaporated, and the residue was subjected to reversed-phase hplc with MeOH-H_2O (1:1) to collect marthasteroside A_1 (10.5 mg) after 13.6 min. The identification was based on comparison (^{1}H nmr, fabms, hplc) with an authentic sample (9).

Fractions 73–92 from the column of Sephadex LH-60 mainly contained the sulfated steroid monoglycosides **1–5** and the sulfated polyhydroxysteroid **6**. The residue (154 mg) from these fractions was subjected to dccc using *n*-BuOH–Me_2CO–H_2O (3:1:5) in the ascending mode (the lower phase was the stationary phase; flow rate 10 ml/h; 3.5-ml fractions were collected and monitored by tlc (Table 4).

Each fraction was then submitted to hplc with MeOH-H_2O (1:1) on the C_{18} column (30 cm × 3.9 mm i.d.) to give pure compounds: indicoside B [**1**] (1.5 mg), scoparioside A [**2**] (3.2 mg), scoparioside B [**3**] (2.0 mg), scoparioside C [**4**] (7.0 mg), scoparioside D [**5**] (1.5 mg), and the sulfated steroid **6** (6 mg). Identification of indicoside B [**1**] was based on direct comparison (hplc, ^{1}H nmr, and fabms) with an authentic sample (2); the physical data of the new compounds are in Tables 1–3 and in the text.

TABLE 4. Dccc Fractionation of the Sulfated Steroidal Monoglycosides Fractions (fractions 73–92 from Sephadex LH-60).[a]

Fractions	Amount (mg)	Compound
78–85	6.1	**6**
86–90	9.7	**6**
91–95	6.3	**6+3+1+4**
96–102	7.2	**6+4+2**
103–116	10.7	**4+2**
145–158	6.3	**5**

[a]Solvent system n-BuOH–Me_2CO–H_2O (3:1:5), ascending mode, 250 tubes, 3.5-ml fractions collected.

Fractions 93–115 from the column of Sephadex LH-60 mainly contained the polyhydroxysteroids **7**–**10**, which were separated by dccc using $CHCl_3$-MeOH-H_2O (7:13:8) in the ascending mode (the lower phase was the stationary phase) to give the pure compounds in that order. Final purification was achieved by hplc on the C_{18} column (30 cm × 3.9 mm i.d.) with MeOH-H_2O (7:3), yielding compounds **7** (12 mg) (6), **8** (1.5 mg) (7), **9** (1.5 mg) (6), and **10** (1.7 mg) (8). Identification of each polyhydroxysteroid was achieved by direct comparison (^1H and ^{13}C nmr and fabms) with authentic samples.

Solvolysis of compounds **2**, **3**, **4**, *and* **6**.—A solution of each of the above compounds (1–2 mg) in a mixture of dioxane (0.5 ml) and pyridine (0.5 ml) was heated at 120° for 2 h in a stoppered reaction vial. After the solution had cooled, the solvents were removed under reduced pressure and the residue was partitioned between H_2O (2 ml) and n-BuOH (2 ml). The extraction was repeated three times, and the combined extracts were washed with H_2O and evaporated to dryness under reduced pressure. The residues were purified by hplc [C_{18} μ-Bondapak, 30 cm × 3.9 mm i.d.; MeOH-H_2O (75:25 or 80:20)] to give the desulfated compounds. Desulfated **2**: fabms (negative ion) m/z $[M-H]^-$ 583 (100%), $[M-H-132]^-$ 451 (15%); ^1H nmr δ_H (CD_3OD) (aglycone) essentially unshifted with respect to compound **2** (Table 2), δ_H (sugar) 4.94 (1H, d, $J=1.5$ Hz, H-1′), 4.00 (dd, $J=3.5$, 1.5 Hz, H-2′), 3.87 (1H, dd, $J=6.2$, 3.5 Hz, H-3′), 4.00 (m, H-4′), 3.78 and 3.66 (each 1H, dd, $J=11.5$, 4.8 Hz and $J=11.5$ and 3 Hz, H_2-5′). Desulfated **3** fabms (negative ion) m/z $[M-H]^-$ 583 (100%), $[M-H-132]^-$ 451 (10%); ^1H nmr δ_H (CD_3OD (aglycone) essentially unshifted with respect to compound **3**, δ_H (sugar) 4.97 (1H, d, $J=1.5$ Hz, H-1′), 4.05 (2H, overlapping signals, H-2′ and H-3′), 4.23 (1H, m, H-4′), 3.90 and 3.79 (each 1H, dd, $J=12.5$, 5.6 Hz and $J=12.5$, 6.2 Hz, H_2-5′). Desulfated **4**: fabms (negative ion) m/z $[M-H]^-$ 597 (100%), $[M-H-146]^-$ 451 (25%); ^1H nmr δ_H (CD_3OD) (aglycone) essentially unshifted with respect to compound **4**, δ_H (sugar) 4.27 (1H, d, $J=7.5$ Hz, H-1′), 3.25 (1H, dd, $J=9$, 7.5 Hz, H-2′), 3.04 (1H, t, $J=9$ Hz, H-3′), 3.50 (1H, m, H-4′), 3.83 (1H, dd, $J=11.2$, 5.2 Hz, Heq-5′), 3.18 (1H, t, $J=11.2$ Hz, Hax-5′), 3.65 (3H, s, OMe). Desulfated **6** (**13**), fabms (negative ion) $[M-H]^-$ 451 (100%), δ_H (CD_3OD) 0.92 and 0.94 (each 3H, d, $J=6.8$ Hz, H_3-26, -27), 3.24 (1H, m, H-24), the remaining signals essentially unshifted with respect to compound **6**.

METHANOLYSIS OF GLYCOSIDES.—*Sugar analysis.*—A solution of each glycoside (0.5 mg), the desulfated derivatives of **2** and **3**, and compound **5** in anhydrous 2 M HCl in MeOH (0.5 ml) was heated at 80° in a stoppered reaction vial for 8 h. After having been cooled the reaction mixture was neutralized with Ag_2CO_3 and centrifuged, and the supernatant was evaporated to dryness under N_2. The residue was trimethylsilylated with TRISIL Z (Pierce Chemical) for 15 min at room temperature. Glc analysis gave peaks which co-eluted with those of methyl arabinoside (from the desulfated derivative of **2**) and methyl xyloside (from the desulfated derivative of **3** and from compound **5**) standards.

Stereochemical assignments at C-24 of **6**.—A solution of the sulfated steroid **6** (3 mg) in DMF (2 ml) was slowly added under N_2 to a stirred mixture of NaH (10 mg) in dry DMF (0.5 ml) cooled in an ice bath. The mixture was stirred for 15 min, and MeI (0.1 ml) was added. The reaction mixture was kept for a further 4 h at room temperature. The excess of NaH was destroyed by a few drops of MeOH and, after addition of H_2O, the mixture was extracted twice with CH_2Cl_2. The organic layer was washed with H_2O and evaporated under reduced pressure. The residue was then solvolyzed in dioxane/pyridine as above, and the product was purified by passage through a Pasteur pipette filled with a slurry of Si gel in $CHCl_3$ to give (24*S*)-3β,6α,15β-trimethoxy-5α-cholestane-8,24-diol [**14**]: eims m/z $[M-H_2O]^+$ 476; ^1H nmr δ ($CDCl_3$) 0.91, 0.93, and 0.94 (9H, three doublets, $J=6.5$ Hz, H_3-27, -26, and -21), 0.99 (3H, s, H_3-19), 1.19 (3H, s, H_3-18), 2.34 (1H, dd, $J=12$, 3.5 Hz, H-7β), 3.26, 3.27, 3.39 (each 3H, s, OMe), 3.25–3.40 (3H, H-3, -6, -24), 3.92 (1H, t, $J=5.5$ Hz, H-15).

The trimethylated steroid **14** (2.5 mg) was treated with freshly distilled (+)-methoxy-(phenyl)-trifluoromethyl acetyl chloride (20 μl) in dry pyridine (0.1 ml) for 1 h. After removal of solvent, the product was eluted through a Pasteur pipette filled with a slurry of Si gel in $CHCl_3$ to give the 24-(+)-MTPA ester: ^{1}H nmr δ ($CDCl_3$) 7.56, 7.43 and 7.35 (Ph), 4.48 (1H, m, H-24), 3.40, 3.39, 3.22 (each 3H, s, OMe), 2.34 (1H, dd, J = 12, 3.5 Hz, H-7β), 1.17 (3H, s, H_3-18), 0.97 (3H, s, H_3-19), 0.90 (3H, d, J = 6.5 Hz, H_3-21), 0.87 (3H, d, J = 7 Hz, H_3-26, or H_3-27), 0.84 (3H, d, J = 7 Hz, H_3-27 or H_3-26).

Enzymatic hydrolysis of scoparioside D [**5**] *giving* **11**.—Glycoside **5** (2 mg) in citrate buffer (1 ml, pH 4.5) was incubated with a glycosidase mixture (2.5 mg) of *Charonia lampas* (Scikagaku Kogyo) at 37° for 3 days. The reaction mixture was passed through a Sep Pak C-18 cartridge, washed with H_2O, and eluted with MeOH. The eluate was evaporated to dryness and purified by hplc [C_{18} μ-Bondapak, 30 cm × 3.9 mm i.d.; MeOH-H_2O (1:1)] to give **11**, fabms (negative ion mode) m/z $[M]^-$ 529; ^{1}H nmr δ (CD_3OD) 0.88 and 0.94 (each 3H, d, J = 7 Hz, H_3-26, -27), 1.03 (3H, d, J = 7 Hz, H_3-21), 1.04 (3H, s, H_3-18), 1.19 (3H, s, H_3-19), 2.53 (1H, dd, J = 12, 3.5 Hz, H-7), 3.60 (1H, m, H-3α), 3.68 (1H, t, J = 7 Hz, H-24), 3.89 (1H, br s, H-6α), 4.90 (1H, td, J = 10, 2.5 Hz, H-15), 5.37 (1H, dd, J = 15, 7 Hz, H-23), 5.45 (1H, dd, J = 15, 7 Hz, H-22), indistinguishable from the authentic sample from *Co. tenuispina* (13).

Upon solvolysis in the conditions described above, **11** was desulfated to **12**: fabms (negative ion mode) m/z $[M-H]^-$ 449, ^{1}H nmr δ_H (CD_3OD) 5.27 and 5.50 (1H each, dd, J = 15, 7 Hz and 15, 8 Hz, H-22, -23), 4.34 (1H, ddd, J = 10, 10, 2 Hz, H-15), 3.71 (1H, t, H-24), 2.40 (1H, dd, J = 14, 2.5 Hz, H-7), 1.02 (3H, s, H_3-18), and others indistinguishable from the signals in the spectrum of **11**.

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