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STARFISH SAPONINS, 48.[1] ISOLATION OF FIFTEEN STEROL CONSTITUENTS (SIX GLYCOSIDES AND NINE POLYHYDROXYSTEROIDS) FROM THE STARFISH *SOLASTER BOREALIS*

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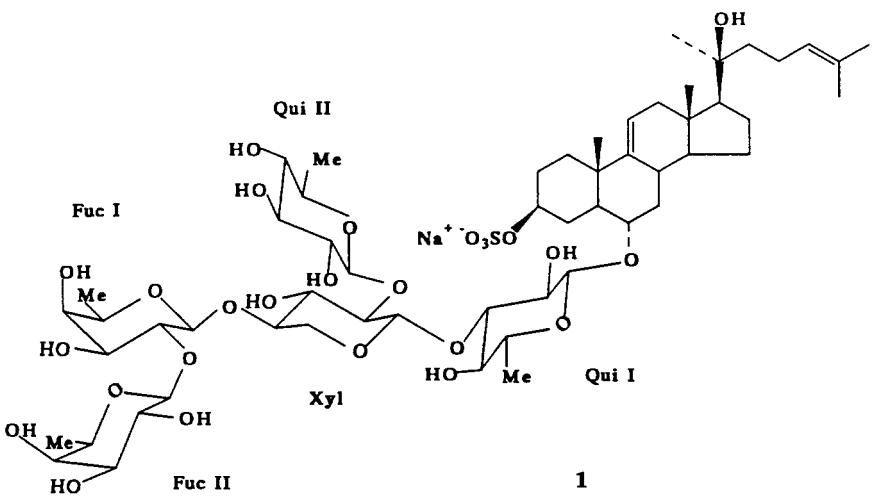
ABSTRACT.—This paper reports a complete steroid glycoside and polyhydroxysteroid analysis of the starfish *Solaster borealis*, collected at Mutsu Bay, Japan. The glycosides include a new pentaglycoside steroid sulfate ("asterosaponin"), designated solasteroside A [**1**], two new sulfated 24-*O*-diglycosides, both with the common 5α-cholesta-3β,6α,8,15α,24-pentaol aglycone, borealosides A [**2**] and B [**3**], two new 24-*O*-(3-*O*-methyl)xylosides, borealosides C [**4**] and D [**5**], having the same aglycone with an additional hydroxy group at 4β-position in **5**, and the known amurensoside B, previously isolated from *Asterias amurensis*. Among the polyhydroxysteroid constituents, four (**7–10**) are new, and five (**11–15**) have previously been isolated from starfishes.

Continuing our work on biologically active compounds from echinoderms, we have analyzed the extracts from the whole bodies of the starfish *Solaster borealis* Fisher (family Solasteridae) collected at Mutsu Bay, Japan, and have isolated one new pentaglycoside steroid sulfate "asterosaponin," designated as solasteroside A [**1**], two new sulfated diglycosides both with the common (24*R*)-5α-cholesta-3β,6α,8,15α,24-pentaol aglycone, named borealosides A [**2**] and B [**3**], and two new 3-*O*-methyl xylosides of the



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[1]For Part 46, see M. Iorizzi, L. Minale, R. Riccio, T. Higa, and J. Tanaka, *J. Nat. Prod.*, **54**, 1254 (1991). For Part 47, see E. Finamore, F. Zollo, L. Minale, and T. Yasumoto, *J. Nat. Prod.*, **55**, 767 (1992).

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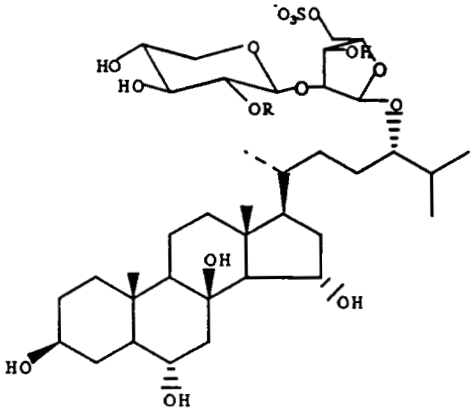
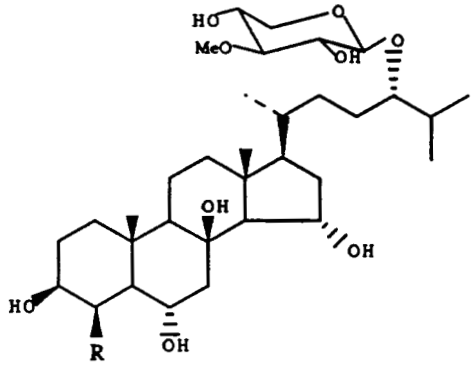
above steroid with an additional hydroxy group at 4β-position in **5**, named borealosides C [**4**] and D [**5**], and the known amurensoside B [**6**] (3). We have also isolated nine polyhydroxysteroids, four of which are new compounds **7–10** and five of which are known compounds **11–15**. Separation and isolation of the individual compounds from the aqueous extracts of the animals were carried out as previously described (1).

RESULTS AND DISCUSSION

The results of our analyses are shown in Table 1, demonstrating the complexity and variety of the steroid metabolites co-occurring in the same organism among the starfishes (1–4). Identification of known compounds was achieved by direct comparison (fabms, ^{1}H-nmr, hplc) with authentic samples.



2 R=Me
3 R=H



4 R=H
5 R=OH

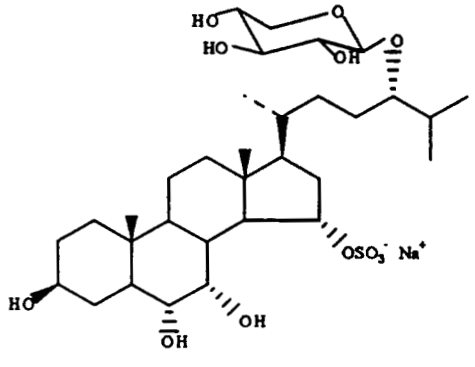


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TABLE 1. Steroid Composition of the Starfish *Solaster borealis*.

Glycoside	Amount[a] (mg)	$[\alpha]_D$[b]	Hplc Rt (min.)
Solasteroside A [**1**]	8.0	+1.1°	20.8[c]
Borealoside A [**2**]	7.1	0°	11.6[c]
Borealoside B [**3**]	3.1	+1.4°	10.0[c]
Borealoside C [**4**]	11.2	+6.7°	9.6[d]
Borealoside D [**5**]	15.8	+15.5°	9.2[d]
Amurensoside B [**6**][e]	4.2		16.5[c]
Steroid **7**	2.3	0°	14.4[d]
Steroid **8**	27.0	+10°	14.8[d]
Steroid **9**	1.3	−4.7°	12.0[d]
Steroid **10**	2.0	0°	17.6[d]
Steroid **11**[f]	27.0		11.2[d]
Steroid **12**[g]	2.3		8.8[d]
Steroid **13**[h]	6.2		10.8[i]
Steroid **14**[j]	8.3		9.2[d]
Steroid **15**[k]	10.0		12.2[i]

[a]From frozen starfish (7 kg) collected at Mutsu Bay, Japan.

[b]From solution in MeOH (*c* ranging from 0.1 to 1.0).

[c]C_{18} μ-Bondapak column (30 cm × 3.9 mm i.d.); MeOH-H_2O (50%) as mobile phase, flow rate 2 ml/min.

[d]C_{18} μ-Bondapak column (30 cm × 7.8 mm i.d.); MeOH-H_2O (75:25) as mobile phase, flow rate 5 ml/min.

[e]Data are from Riccio *et al.* (3).

[f]Data are from Iorizzi *et al.* (5).

[g]Data are from D'Auria *et al.* (6).

[h]Data are from Minale *et al.* (7).

[i]C_{18} μ-Bondapak column (30 cm × 7.8 mm i.d.); MeOH-H_2O (70:30), as mobile phase, flow rate 5 ml/min.

[j]Data are from Riccio *et al.* (8).

[k]Data are from Minale *et al.* (9).

STRUCTURE ELUCIDATION OF SOLASTEROSIDE A [**1**].—An examination of the ^{1}H-nmr spectrum (Table 2) revealed signals due to aglycone protons identical with those observed in the ovarian asterosaponin 4, containing the (20*S*)-5α-cholesta-9(11),24-diene-3β,6α,20-triol-3β-sulfated aglycone, derived from the starfish *Asterias amurensis* (3,10). In the ^{13}C-nmr spectrum of **1** (Table 3) the aglycone carbon signals were superimposable with those of ovarian asterosaponin 4 (3,10), thus also confirming that the oligosaccharide is attached at C-6 and the sulfate at C-3 of the steroid, a general feature of the asterosaponins. The negative ion fabms spectrum of **1** showed a molecular anion peak at *m/z* 1211 and fragment ions at *m/z* 1065 and 919, corresponding to the consecutive losses of the two 6-deoxyhexose units (146 mass units). On anhydrous acid methanolysis, solasteroside A [**1**] liberated methyl xylosides, methyl fucosides, and methyl quinovosides in the ratio 1:2:2. This carbohydrate composition has been found in regularoside B and tenuispinoside B, isolated from *Halityle regularis* (11) and *Coscinasterias tenuispina* (1), respectively. A detailed comparison of the ^{13}C-nmr data for the sugar moiety of compound **1** (Table 4) with those of regularoside B (11) and tenuispinoside B (1) showed that the saccharide chain is identical in all three compounds.

STRUCTURE ELUCIDATION OF BOREALOSIDES A [**2**], B [**3**], C [**4**], AND D [**5**].—Examination of the spectral data (^{1}H and ^{13}C nmr, Tables 2, 3 and 5) of borealoside A [**2**] immediately indicated that it contains a 5α-cholesta-3β,6α,8,15α,24-pentaol

TABLE 2. Selected 250 MHz ^{1}H-nmr (CD_3OD) Signals (δ_H) for the Aglycone Protons of the Glycosides **1–5** and for Protons of the Polyhydroxysteroids **7–10**. *J* (Hz) are shown in parentheses.

Proton	Compound						
	1[a]	**2–4**[b]	**5**	**7**	**8**	**9**	**10**
H-3	4.22 m	3.55 m	3.55 m	3.60 m	3.55 m	3.55 m	3.55 m
H-4			4.28 br s		4.08 br s	4.08 br s	4.08 br s
H-6		3.65 dt (10, 3.5)	4.11 ddd (11.2, 11.2, 4)	3.60 dd (3.0, 2.9)	4.00 dd[c]	3.99 dd[c]	4.00 dd[c]
H-7β[d]		2.42 dd (12.5, 3.5)	2.46 dd (12.0, 4)	4.03 d (3.0)	4.02 d (3.0)	4.02 d (3.0)	4.02 d (3.0)
H-15		4.23 dt (3.5, 10)	4.24 dt (10, 3.5)	4.50 dd (5, 6.2)	4.50 dd (5.2, 6.5)	4.49 dd (5.0, 6.2)	4.50 dd (5.0, 6.2)
H-16				4.25 t (6.2)	4.23 t (6.2)	4.13 t (6.2)	4.23 t (6.2)
H-18	0.81 s	1.00 s	0.98 s	1.30 s	1.30 s	1.32 s	1.30 s
H-19	1.01 s	1.05 s	1.21 s	1.18 s	1.45 s	1.46 s	1.45 s
H-21	1.30 s	0.97 d (7)	0.97 d (7)	0.99 d (7)	0.99 d (7)	1.09 d (7)	0.99 d (7)
H-22						5.61 dd (8, 14.8)	
H-23						5.50 dt (14.8, 7)	
H-26	1.64 s	0.93 d (6.8)	0.94 d (6.8)	3.46 dd (10.5, 6.2)	3.46 dd (10.5, 6.2)	3.46 dd (10.5, 6.2)	3.38 dd (10.5, 7)
						3.55 dd (10.5, 6.2)	3.52 dd (10.5, 5.2)
H-27	1.69 s	0.93 (6.8)	0.93 d (6.8)	0.94 d (7)	0.94 d (7)	0.93 d (7)	0.85 d (7)
H-28							0.84 d (7)

[a]H-11 δ 5.37 (br d, $J = 5.5$ Hz), H-24 δ 5.12 (t, $J = 6.5$); anomeric H's δ 4.57, 4.50, 4.42 (each d, $J = 7.5$ Hz).

[b]Data extracted from the spectrum of **2**.

[c]Signal partially overlapping with H-7 and H-4.

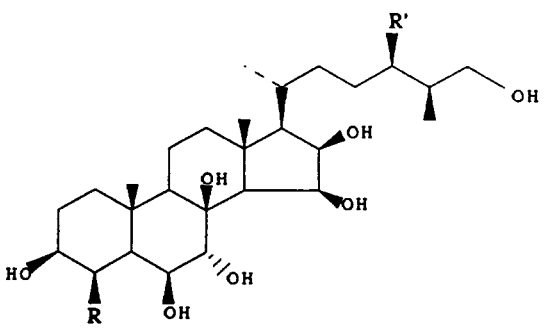
[d]The remaining H-7α signals are confused in the region of δ 1.5–1.7 ppm.

TABLE 3. ^{13}C-nmr Shifts (62.9 MHz) of Sterol Carbons in Compounds **1**, **2**, **4**, **5**, **8**, and **10** (in ppm).

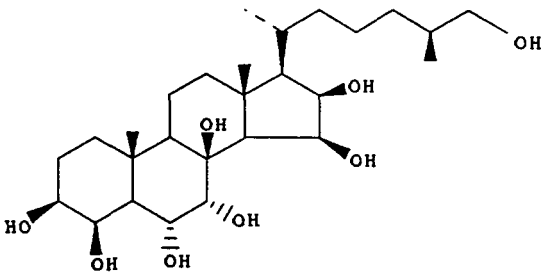
Carbon	Compound					
	1[a]	**2**[b]	**4**[b]	**5**[b]	**8**[b]	**10**[b]
C-1	35.5	39.3	39.6	39.9	41.0	40.7
C-2	28.8	31.2	31.5	26.2	26.7	26.4
C-3	77.2	71.9	72.2	73.6	73.0	72.7
C-4	30.1	32.1	32.4	69.1	77.7	77.4
C-5	48.6	53.3	53.7	57.0	45.2	44.8
C-6	79.5	67.4	67.7	64.7	81.2	80.9
C-7	40.8	50.0	49.7	50.6	73.4	72.7
C-8	34.8	76.0	76.1	77.4	78.0	77.8
C-9	145.0	57.0	57.4	58.4	50.8	50.6
C-10	37.8	37.5	37.8	38.1	36.6	36.9
C-11	116.2	19.4	19.6	19.1	18.6	18.2
C-12	42.0	42.6	42.9	42.8	43.0	42.7
C-13	41.3	45.4	45.5	44.7	44.9	44.5
C-14	53.5	66.7	67.2	67.3	54.8	54.5
C-15	23.0	69.6	69.9	69.9	71.2	70.8
C-16	24.6	41.5	41.7	41.7	72.6	72.3
C-17	58.2	55.7	55.9	55.9	63.2	62.8
C-18	13.1	15.2	15.4	15.4	17.8	18.2
C-19	18.8	13.9	14.2	17.1	19.1	18.9
C-20	73.3	36.1	36.2	36.2	31.0	34.8
C-21	25.1	18.7	18.9	18.9	18.5	17.5
C-22	43.9	32.8	32.8	32.8	37.1	34.3
C-23	22.5	28.4	28.8	28.7	24.8	32.5
C-24	125.1	84.5	86.2	86.2	35.0	34.8
C-25	130.0	31.6	31.9	31.9	37.0	40.9
C-26	25.7	18.1	18.3	18.3	68.5	66.7
C-27	16.9	17.8	18.3	18.3	17.3	14.5
C-28						11.8

[a]Spectrum run in pyridine-d_5.
[b]Spectra run in MeOH-d_4.

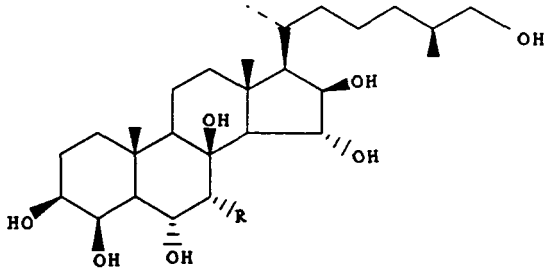
aglycone, already found in several other glycosides (12–15), and also indicated the presence of a 2-*O*-methyl-β-xylopyranosyl unit, which is a common sugar component among the glycosides isolated from the starfishes (16). The ^{13}C-nmr spectrum also indicated the location of the sugar residue at C-24. In addition to the signals for the aglycone and the 2-*O*-methyl xylosyl residue, the spectrum contained signals assigned to an α-arabinofuranosyl moiety. The large downfield shift observed for H_2-5′ (δ_H 4.15 m vs. 3.66–3.88 in α-arabinofuranosides) and for C-5′ (δ_C 68.1 vs. 62.4 ppm in α-



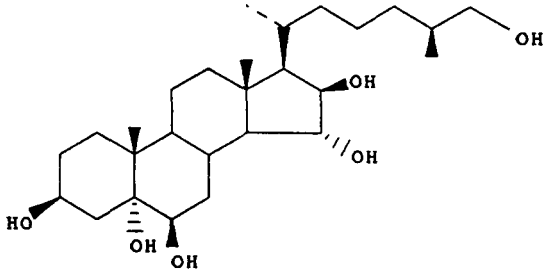
7 R=R′=H
8 R=OH, R′=H
9 R=OH, R′=H, Δ^{22E}
10 R=OH, R′=Me



11
12 24-methylene



13 R=H
14 R=OH



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arabinofuranosides) suggested the presence of a sulfate group at C-5′. The fabms (negative ion mode) showed the molecular anion peak at *m/z* 809 $[M]^-$ and a fragment ion peak at *m/z* 663, corresponding to the loss of the 2-*O*-methyl xylosyl residue (146 mass units), thus indicating the sequence 2-*O*-Me-xylose-arabinose (5-*O*-sulfate). On solvolysis in dioxane/pyridine, **2** afforded a desulfated derivative, fabms (negative ion mode) *m/z* 729 $[M-H]^-$ and 583 $[(M\text{-}H)-146]^-$. In the spectrum of the desulfated molecule, the arabinosyl hydroxymethylene proton signals were shifted upfield to δ 3.66 (dd, $J=12.5$, 5.0 Hz) and 3.85 (dd, $J=12.5$, 3.0 Hz) ppm in agreement with the location of the sulfate at C-5′ of the arabinofuranose unit in **2**. Finally, an analysis of the ^{13}C-nmr data of **2**, based upon comparison with methyl arabinofuranosides (17), the known glycosidation (18) and sulfation shifts (19), and assignments reported for

TABLE 4. Assignments of ^{13}C-nmr Signals (pyridine-d_5) of the Sugar Moiety of Solasteroside A [**1**].

Sugar carbon	Qui I	Xyl	Qui II	Fuc I	Fuc II
1	105.2	103.5	103.8	101.1	106.1
2	74.3	81.9	75.2	82.8	71.2
3	88.8	74.8	76.5	73.9	74.2
4	73.7	77.5	75.7	71.0	73.5
5	71.9	63.7	73.1	71.3	71.5
6	17.7		18.0	16.4	16.6

TABLE 5. Assignments of Nmr Signals (CD_3OD) to the Carbohydrate Moiety of **2**.

Position	Arabinose (5-*O*-sulfate)		2-*O*-Me xylose	
	^1H	^{13}C	^1H	^{13}C
1	5.10 d (1.4 Hz)	107.6	4.45 d (7.5 Hz)	105.1
2	4.06 m	92.0	2.89 dd (9.5, 7.5)	84.5
3	4.06 m	78.1	3.34 t (9.5)	77.1
4	4.13 m	81.3	3.50 m	70.9
5	4.15 m	68.1	3.17 t (10) 3.83 dd (10, 5.2)	66.6
OMe			3.62 s	60.9

similar glycosides (16), established the 2-*O*-methyl-β-xylopyranosyl residue to be attached at C-2 of the (5-*O*-sulfated)-α-arabinofuranosyl unit and confirmed the structure of borealoside A as (24*S*)-24-*O*-[2-*O*-methyl-β-D-xylopyranosyl-(1↦2)-5-*O*-sulfate-α-L-arabinofuranosyl]-5α-cholesta-3β,6α,8,15α,24-pentaol [**2**]. The 24*S* configuration is proposed, because the chemical shifts of the signals assigned to the side chain carbons for **2** are virtually identical to those of the corresponding signals for the (24*S*)-24-*O*-α-L-arabinofuranosyl steroids isolated from starfishes (20), and also by analogy with **4**, for which the configuration at C-24 has been determined (see below). The D configuration for the xylose and L configuration for arabinose are suggested by analogy with the other steroids, D-xylosides and L-arabinosides, from starfishes.

The fabms (negative ion mode) of the minor borealoside B [**3**] showed a molecular anion peak at *m/z* 795 $[M]^-$, shifted fourteen mass units relative to **2** (*m/z* 809), and a fragment ion peak at *m/z* 663, corresponding to the loss of a pentose residue (132 mass units) from $[M]^-$. The ^1H-nmr spectrum (Tables 2 and 6) of **3** showed close similarity to that of **2**, except that it lacks the methoxyl singlet at δ 3.62 and the signal assigned to H-2 of the xylosyl unit was shifted downfield to δ 3.17 (δ 2.89 in **2**). The remaining signals were virtually identical in both spectra. Based on these data, the structure **3** is proposed for borealoside B.

Examination of ^1H- and ^{13}C-nmr spectra of borealoside C [**4**] indicated that it contains the same 5α-cholesta-3β,6α,8,15α,24-pentaol aglycone as **2** and **3**. The fabms (negative ion mode) of **4** exhibited a quasi molecular ion peak at *m/z* 597 $[M-H]^-$ accompanied by a fragment ion peak at *m/z* 451, corresponding to the aglycone, arising from $[M-H]^-$ by loss of a methoxylated pentose unit (=146 mass units). In addition to the steroid moiety, the ^1H-nmr spectrum (Table 7) showed a methoxyl singlet at δ 3.65 and four methine protons at δ 4.26 (d, $J=7.5$ Hz), 3.24 (dd, $J=9.5$, 7.5 Hz), 3.03 (t, $J=9.5$ Hz), and 3.50 (m), which coupled with their neighbors in this order.

TABLE 6. Assignment of ^1H-nmr (CD_3OD) Signals to the Carbohydrate Moiety of **3**.

Position	Arabinose (5-*O*-sulfate)	Xylose
1	5.10 d	4.38 d (7.5)
2	4.06 m	3.17 dd (9.5, 7.5)
3	4.06 m	3.30 t (9.5)
4	4.13 m	3.50 m
5	4.15 m	3.20 t (10) 3.85 dd (10, 5)

TABLE 7. Assignments of Signals (CD_3OD) to the 3-*O*-Methyl-β-xylopyranosyl Moiety of Compounds **4** and **5**.[a]

Position	^1H	^{13}C
1	4.26 d (7.5)	104.9
2	3.24 dd (9.5, 7.5)	75.0
3	3.03 t (9.5)	87.6
4	3.50 m	71.0
5	3.17 t (10)	66.7
	3.83 dd (10, 5)	
OMe	3.65 s	60.8

[a]Data extracted from the steroid **4**.

The last methine proton is further coupled to methylene protons at δ 3.17 (t, $J = 10$ Hz) and 3.83 (dd, $J = 10$, 5 Hz). The coupling constants indicated the presence of a moiety equivalent to a β-xylopyranoside. The high field shift observed for H-3 (δ 3.03) when compared with that observed in steroid xylopyranosides [δ 3.30; see, for example, Riccio *et al.* (3)] was indicative of a 3-*O*-methyl xylopyranosyl unit. The location of the methyl group at 3-OH of the xylopyranosyl unit was confirmed by ^{13}C nmr, which also established the sugar moiety to be linked at C-24 of the steroid. The 24*S* configuration was suggested from the ^{13}C-nmr spectrum of **4**, which showed signals for the side chain carbons virtually identical with those of the previous (24*S*)-24-*O*-β-D-xylopyranosyl steroids isolated from starfishes (3), and it was confirmed by the following data. Borealoside C [**4**] was methylated with MeI in DMF/NaH to afford a permethylated derivative which, on hydrolysis with 2 M HCl/MeOH, gave the known 3β,6α,15α-trimethoxycholest-8-en-24-ol (a dehydration having also occurred) (5). This was converted into its (+)-MTPA [MTPA = α-methoxy-α-(trifluoromethyl)phenylacetic acid] ester; the term (+)- or (−)-MTPA ester refers to an ester obtained using the acid chloride prepared from (*R*)-(+) or (*S*)-(−) acid, respectively. In the ^1H-nmr spectrum, the resonances of the isopropyl methyl protons appeared as two upfield doublets at δ_H 0.84 and 0.86 in agreement with the shift observed in the spectrum of the (+)-MTPA ester of a 24*S* model alcohol (δ 0.83 d and 0.85 d) and far away from those observed in the spectrum of the (+)-MTPA ester of the corresponding 24*R* model alcohol (6H doublet at δ 0.91) (3). Thus borealoside C was assigned the (24*S*)-24-*O*-(3-*O*-methyl-β-D-xylopyranosyl)-5α-cholesta-3β,6α,8,15α,24-pentaol [**4**] structure. The D configuration of xylose was assumed by analogy with the many D-xylosides isolated from starfishes.

Borealoside D [**5**] is the 4β-hydroxyderivative of borealoside C [**4**]. The structure was derived from the ^1H- and ^{13}C-nmr spectra (Tables 2, 3 and 7) and comparison with **4**. The introduction of a further hydroxyl group at C-4β in **5** resulted in a downfield shift of the protons at position 6β (δ 4.11 vs. 3.65) and at position 19 (δ 1.21 vs. 1.05) relative to **4** and, of course, in the appearance of a narrowing signal for an equatorial proton at δ 4.28 (H-4α). The ^{13}C-nmr frequencies of C-1–C-10 and C-19 (rings A and B) in **5** are shifted as expected upon introduction of a 4β-hydroxy group in **4** based on the substituent effects that have been published for the hydroxysteroids (21,22). The fabms (negative ion mode) gave the quasi molecular ion peak at *m/z* 613 $[M - H]^-$ and a fragment at *m/z* 467 (loss of 3-*O*-methylxylose), shifted sixteen mass units relative to **4**, thus confirming the location of the additional hydroxy group on the aglycone.

STRUCTURE ELUCIDATION OF THE POLYHYDROXYSTEROIDS **7–10**.—The novel steroid **7**, fabms (negative ion mode) *m/z* 483 $[M - H]^-$, is isomeric with the pre-

viously isolated (24*S*)-5α-cholesta-3β,6α,7α,8,15β,16β,26-heptaol (23,5) and differs from it in the stereochemistry at C-6, which in **7** is 6β-OH. The ^{1}H-nmr spectrum (Table 2) showed the 19-methyl singlet shifted downfield to δ 1.18 (δ 1.02 in the 6α-isomer) and a dd (J = 3.0 and 2.9 Hz) at δ 3.62 (H-6α), characteristic for an equatorial proton, coupled with a doublet (J = 3.0 Hz) at δ 4.03 (H-7). The remaining hydroxymethine signals in the spectrum at δ 3.60 (m), 4.50 (dd, J = 6.2, 5 Hz) and 4.25 (t, J = 6.2 Hz) were very close to those observed in the spectrum of the 6α-OH epimer and assigned to H-3α, H-15α, and H-16α, respectively. The hydroxymethylene protons were observed at δ 3.46 (dd, J = 10.5 and 6.2 Hz) and at δ 3.30 obscured by the CHD_2OD signal. We note that the 3β,6β,7α,8-hydroxylation pattern has been previously found in a steroid isolated from a species of the genus *Rosaster* (24), which differs from **7** by having the 15α,16β-dihydroxy moiety instead of the 15β,16β-dihydroxy stereochemistry. A comparison of the appropriate signals of their ^{1}H-nmr spectra further supported the structure **7** for the novel steroid.

The steroid **8**, fabms (negative ion mode) *m/z* 499 $[M-H]^-$, is isomeric with **11**, now isolated from *S. borealis* and previously from *Asterina pectinifera* (5). Compound **8** differs from **11** in the stereochemistry at C-6, which in **8** is 6β-OH, and is related to **7** by introduction of an additional hydroxy group at C-4β. The structure was derived from the analysis of ^{1}H- and ^{13}C-nmr data (Tables 2 and 3) and comparison with those of the 6α-isomer **11** and the 4-deoxy derivative **7**. The ^{13}C-nmr spectrum of **8** featured signals at 77.7, 81.2, and 19.1 ppm assigned to C-4, C-6, and C-19, respectively, which are downfield-shifted relative to the 6α-isomer (**11** δ_C 69.6, 66.7, and 16.8 ppm), thus indicating the 6β-OH stereochemistry in **8**. The stereochemistry at C-25 of both **7** and **8** has been determined by using the MTPA method (25). The steroids were treated with α-methoxy-α-(trifluoromethyl)phenyl acetic chloride obtained from the (+)-(*R*) acid in pyridine affording the corresponding 3,26-di(+)-MTPA esters. The ^{1}H-nmr spectra of both esters showed a signal at δ 4.21 (2H, d, J = 6 Hz) for H_2-26, thus indicating the configuration to be 25*S*, like the many other 26-hydroxysteroids from starfishes. In the ^{1}H-nmr spectrum of the (+)-MTPA ester of (25*R*)-26-hydroxysteroids, signals for H_2-26 are observed as separated double doublets (26,27).

The third minor steroid **9**, fabms (positive ion mode) *m/z* 499 $[M+H]^+$, is the Δ^{22} dehydro derivative of **8**. The double bond was placed at the common 22 position with the *E* configuration on the basis of the ^{1}H-nmr olefinic signals observed at δ_H 5.50 (dt, J = 14.8 and 7 Hz, H-23) and 5.61 (dd, J = 14.8 and 8. Hz, H-22). The 25*S* configuration is assumed by analogy with the steroids **7** and **8** and confirmed by the conversion of **9** into the corresponding 3,26-di-(+)-MTPA ester, which in the ^{1}H-nmr spectrum showed a 2H br doublet at δ 4.21 for the 26-methylene protons.

The fabms spectrum (negative ion mode) of the steroid **10** exhibited a pseudomolecular ion peak at *m/z* 513 $[M-H]^-$, shifted fourteen mass units relative to **8** (*m/z* 499). Examination of ^{1}H- and ^{13}C-nmr data (Tables 2 and 3) immediately indicated that **10** is the 24-methyl derivative of **8**. The stereochemistry at C-24 and C-25 was assigned by comparison of ^{1}H- and ^{13}C-nmr data with those of stereospecifically synthesized model compounds (28), which identified the threo stereochemistry in **10**, followed by the comparison of the ^{1}H-nmr spectrum of the derived 26-(+)-MTPA ester of **10** with those of the 26-(+)-MTPA esters of the threo pair model compounds, (i.e., 24*R*,25*S*- and 24*S*,25*R*-isomers) which identified the absolute 25*S* configuration in **10**, and allowed the 24*R*,25*S* configuration to be assigned to the natural steroid. In particular the chemical shifts of H-27 and H-28 and those of C-27 and C-28 in **10** (Tables 2 and 3) are very close to those reported for the threo model compounds (δ_H 0.83 d, 0.81 d and δ_C 14.8 and 12.0 in the 24*R*,25*S*-isomer and δ_H 0.81 d, 0.81 d and δ_C 15.1 and 11.6 in the 24*S*,25*R*-isomer) and far away from those of the erythro model com-

pounds (24*S*,25*S*-isomer δ_H 0.93 d, 0.92 d and δ_C 17.6, 14.4 ppm; 24*R*,25*R*-isomer δ_H 0.91 d, 0.91 d and δ_C 17.4 and 14.1 ppm). In the ^{1}H-nmr spectrum of the derived 3,26-di(+)-MTPA ester of **10**, the 26-methylene protons appeared as a doublet at δ 4.25 very close to the 26-(+)-MTPA ester of the 24*R*,25*S*-isomer (δ_H 4.23, br d) and far from the 26-(+)-MTPA ester of the 24*S*,25*R*-isomer (δ_H 4.14, dd and 4.34 dd).

EXPERIMENTAL

GENERAL EXPERIMENTAL PROCEDURES.—For the instruments used see Riccio *et al.* (3).

EXTRACTION AND ISOLATION.—The animals *S. borealis* (7 kg) were collected at Mutsu Bay, Aomori Prefecture, Japan in 1986, and identified at the Faculty of Agriculture of the Tohoku University, Sendai, Japan; a voucher specimen is preserved there. The animals were chopped and soaked in H_2O (10 liters), and the aqueous extracts were centrifuged and passed through a column of Amberlite XAD-2 (1.1 kg). This column was washed with distilled H_2O (2.5 liters) and eluted with MeOH. The MeOH eluate was taken to dryness to give a glassy material (4 g), which was then chromatographed on a column of Sephadex LH-60 (2.5 × 80 cm) with MeOH-H_2O (2:1) as eluent. Fractions of 4 ml were collected and analyzed by tlc on SiO_2 (Merck) with *n*-BuOH–HOAc–H_2O (12:3:5) and $CHCl_3$-MeOH-H_2O (80:18:2).

Fractions 32–54 mainly contained the "asterosaponins" (sulfated steroidal penta- and hexaglycosides), and the residue (870 mg) was subjected to 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; 6-ml fractions were collected and monitored by tlc). Fractions 84–99 (48 mg) were evaporated, and the residue was subjected to reversed-phase hplc on a C_{18} μ-Bondapak column (30 cm × 7.8 mm i.d.) with MeOH-H_2O (1:1) to collect solasteroside A. Smaller amounts of solasteroside A were also collected from the preceding (72–88) and subsequent (100–119) dccc fractions to give 8 mg of pure solasteroside A [**1**]: fabms see text; ^{1}H nmr see Table 2; ^{13}C-nmr see Tables 3 and 4; other physical data see Table 1.

Fractions 55–63 from the Sephadex LH-60 column (580 mg in total) mainly contained the sulfated steroidal diglycosides **2**, **3**, and **6** along with nucleosides and tryptophan. Borealosides A [**2**] and B [**3**] and the sulfated glycoside amurensoside B [**6**] were separated by dccc using *n*-BuOH–Me_2CO–H_2O (3:1:5) in the ascending mode (the lower phase was the stationary phase; flow rate 12 ml/h; 4-ml fractions were collected). Fractions 160–195 contained small amounts of the pentaglycoside **1** along with the known amurensoside B [**6**] (3); fractions 196–205 contained borealoside A [**2**], and fractions 206–250 contained the more polar borealoside B [**3**]. Hplc of these fractions on a C_{18} μ-Bondapak column (30 cm × 3.9 mm i.d.) with MeOH-H_2O (1:1) as eluent gave pure compounds: amurensoside B [**6**] (4.2 mg), borealoside A [**2**] (7.1 mg), and borealoside B [**3**] (3.1 mg). For fabms of the new compounds see the text; ^{1}H and ^{13}C nmr of **2** see Tables 2, 3, and 5; ^{1}H-nmr of **3** see Tables 2 and 6; rotations see Table 1.

Fractions 64–120 from the Sephadex LH-60 column mainly contained the monoglycosides

TABLE 8. Dccc Fractionation of the Monoglycosides **4** and **5** and the Polyhydroxysteroids **7–15**[a] (fractions 64–120 from Sephadex LH-60).

Fractions	Amounts (mg)	Compounds
35–50	82.0	**14** + **15**
51–64	37.7	**13** + **4**
65–82	49.3	**4** + **11**
83–90	10.8	**4**
91–101 . . .	20.6	**5**
102–116 . . .	24.0	**5** + **12**
117–166 . . .	19.0	**7**
167–203 . . .	45.0	**8**
204–220 . . .	21.9	**9**
221–238 . . .	9.5	**10**

[a]Solvent system $CHCl_3$-MeOH-H_2O (7:13:8), ascending mode, 300 tubes, 4-ml fractions collected.

borealosides C [**4**] and D [**5**] and the polyhydroxysteroids **7–15**, 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) (Table 8). Final purification was achieved by hplc on the C_{18} column (30 cm × 7.8 mm i.d.) with MeOH-H_2O (75:25). Identificaiton of the known polyhydroxysteroids **11–15** was achieved by direct comparison (^{1}H-nmr, fabms, and hplc) with authentic samples (for the references see Table 1). For fabms of the new compounds see the text; ^{1}H and ^{13}C nmr of **4** and **5** see Tables 2, 3, and 7; ^{1}H and ^{13}C nmr of **7–10** see Tables 2 and 3; rotations and hplc Rt see Table 1.

METHANOLYSIS OF SOLASTEROSIDE A [**1**].—A solution of the glycoside **1** (1 mg) 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 Co.) for 15 min at room temperature. Glc analysis (SE-30 capillary column, 25 m, 150°, Helium carrier, flow 2 ml·min^{-1}) gave peaks that co-eluted with those of the methyl xylosides, fucosides, and quinovosides standards in the ratio 1:2:2.

DESULFATION OF BOREALOSIDE A [**2**].—A solution of borealoside A [**2**] (2 mg) in a mixture of dioxane (0.5 ml) and pyridine (0.5 ml) was heated at 130° 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 and *n*-BuOH. The *n*-BuOH extracts were evaporated to dryness, and the desulfated glycoside was purified by hplc on a C_{18} μ-Bondapak column (30 cm × 3.9 mm i.d.) with MeOH-H_2O (2:3). The fabms (negative ion mode) showed a pseudomolecular ion peak corresponding to the desulfated glycoside at *m/z* 729 (100%) $[M-H]^-$, accompanied by the fragment ions at *m/z* 583 $[(M-H)-146]^-$ (40%) and 451 $[(M-H)-146-132]^-$ (25%); ^{1}H nmr (CD_3OD), δ_H (arabinosyl), 5.11 (1H, d, J = 1.0 Hz, H-1), 4.08 (1H, dd, J = 3.7, 1.0 Hz, H-2), 4.00 (1H, t, J = 3.7 Hz, H-3), 3.97 (1H, m, H-4), 3.85 (1H, dd, J = 12, 3.0 Hz, H-5), 3.66 (1H, dd, J = 12, 5.4 Hz, H-5); δ_H (xylosyl) 4.45 (1H, d, J = 7.0 Hz, H-1), 2.88 (1H, dd, J = 9.0, 7.0 Hz), 3.34 (partially overlapping with CHD_2OD signal, H-3), 3.50 (1H, m, H-4), 3.17 (1H, t, J = 10.5 Hz, H_{ax}-5), 3.88 (1H, dd, J = 10.5, 3.5 Hz, H_{eq}-5), 3.61 (3H, s, OMe); δ_H (aglycone) identical signals with those of **2** (Table 2).

STEREOCHEMICAL ASSIGNMENT AT C-24 OF BOREALOSIDE C [**4**].—*Methylation of borealoside C* [**4**] *and subsequent hydrolysis to (24S)-3β,6α,15α-trimethoxy-5α-cholest-8-en-24-ol.*—A solution of **4** (8 mg) in dry DMF (2 ml) was slowly added under N_2 to a stirred mixture of NaH (25 mg) in dry DMF (0.5 ml) cooled in an ice-bath. The mixture was stirred for 15 min, and MeI (0.2 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 with CH_2Cl_2. The organic layer was washed with H_2O, dried (Na_2SO_4), and evaporated under reduced pressure. The residue was then methanolyzed in anhydrous 2 M HCl methanol (1 ml) at 80° in a stoppered reaction vial. After cooling, the reaction mixture was neutralized with Ag_2CO_3 and centrifuged, and the supernatant was evaporated to dryness under reduced pressure. The residue was purified by passage through a Pasteur pipette filled with a slurry of Si gel in $CHCl_3$-MeOH (99:1) to give 3β,6α,15α-trimethoxy-5α-cholest-8-en-24-ol: eims *m/z* [M − MeOH] 444 (100%), 429 (80%), 412 (50%); ^{1}H-nmr δ_H ($CDCl_3$) 0.63 (3H, s, H_3-18), 0.91 and 0.94 (together 9H, overlapping doublets, J = 6.8 Hz, H_3-21, -26, and -27), 0.97 (3H, s, H_3-19), 3.30, 3.36, and 3.37 (each 3H, s, OMe), 3.15 (1H, m, >CH-O), 3.28–3.40 (2H, overlapping signals for >CHO- protons), 3.55 (1H, m, >CHO-).

The trimethoxy-5α-cholest-8-en-24-ol was then treated with freshly distilled (+)-methoxy(phenyl)-trifluoromethylacetyl chloride [prepared from the corresponding (*R*)-(+)-acid] (25 μl) in dry pyridine (0.15 ml) for 1 h at room temperature. Removal of the solvent left the residue, which was partitioned between H_2O and *n*-hexane. The organic layer was evaporated to dryness to give the 3β,6α,15α-trimethoxy-5α-cholest-8-en-24-yl (+)-MTPA ester: ^{1}H-nmr δ_H ($CDCl_3$) 0.63 (3H, s, H_3-18), 0.84 and 0.87 (each 3H, d, J = 6.8 Hz, H_3-26, -27), 0.90 (3H, d, J = 6.8 Hz, H_3-21), 0.98 (3H, s, H_3-19), 3.29, 3.36, and 3.37 (each 3H, s, OMe), 3.14–3.50 (overlapping signals for -CHOMe); 4.94 (1H, m, H-24), 7.37, 7.45, 7.56 (Ph).

3,26-DI-(+)-MTPA ESTERS OF **7–10**.—Compound **7** (15 mg) was treated with freshly distilled (+)-methoxy(trifluoromethyl)phenylacetyl chloride (10 μl) in dry pyridine (0.1 ml) for 1 h at room temperature. After removal of the solvent, the product was analyzed: fabms (negative ion mode) *m/z* $[M-H]^-$ 915 (50%), 699 (100); ^{1}H nmr, δ_H (CD_3OD) 0.94 and 0.96 (6H, overlapping doublets, J = 6.8 Hz, H_3-21, -27), 1.18 (3H, s, H_3-19), 1.30 (3H, s, H_3-18), 3.60 (dd, H-6α), 4.03 (1H, d, J = 3 Hz, H-7β), 4.21 (2H, br d, J = 6.2 Hz, H_2-26), 4.25 (1H, t, J = 6.2 Hz, H-16α), 4.50 (1H, dd, J = 6.2, 5.0 Hz, H-15α), 4.90 (1H, m, H-3α).

Similarly, steroids **8**, **9**, and **10** were treated with (+)-methoxy(trifluoromethyl)phenylacetyl

chloride and the corresponding 3,26-di-(+)-MTPA esters analyzed by ^{1}H nmr. (+)-MTPA ester of **8**: δ_H (CD_3OD) 0.96 (6H, d, J = 7.0 Hz, H_3-21, -27), 1.29 (3H, s, H_3-18), 1.48 (3H, s, H_3-19), 4.00–4.01 (2H, overlapping signals, H-6, -7), 4.21 (3H, overlapping signals, H_2-26 and H-16), 4.30 (1H, br s, H-4α), 4.50 (1H, dd, J = 5.0, 6.2 Hz, H-15α), 4.90 (1H, m, H-3α. (+)-MTPA ester of **9**: δ_H (CD_3OD) 0.96 (3H, d, J = 7.0 Hz, H_3-27), 1.06 (3H, d, J = 7.0 Hz, H_3-21), 1.32 (3H, s, H_3-18), 1.45 (3H, s, H_3-19), 4.02 (2H, overlapping signals, H-6 and -7), 4.08 (1H, br s, H-4α), 4.13 (1H, t, J = 6.5 Hz, H-16α), 4.22 (2H, br d, J = 6 Hz, H_2-26), 4.49 (1H, dd, J = 5.0, 6.5 Hz, H-15α), 4.90 (1H, m, H-3α), 5.47 (2H, m, H-22 and -23). (+)-MTPA ester of **10**: δ_H (CD_3OD) 0.85 and 0.88 (each 3H, d, J = 6.8 Hz, H_3-27 and -28), 0.95 (3H, d, J = 6.8 Hz, H_3-21), 1.29 (3H, s, H_3-18), 1.45 (3H, s, H_3-19), 4.02 (2H, overlapping signals, H-6 and -7), 4.08 (1H, br s, H-4α), 4.13 (1H, t, J = 6.5 Hz, H-16α), 4.25 (2H, br d, H_2-26), 4.50 (1H, dd, J = 6.5, 5.0 Hz, H-15α), 4.90 (1H, m, H-3α).

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