Starfish Saponins. Part 34.† Novel Steroidal Glycoside Sulphates from the Starfish *Asterias amurensis*

Raffaele Riccio, Maria Iorizzi, and Luigi Minale *
Dipartimento di Chimica delle Sostanze Naturali, Università di Napoli, Via L. Rodinò, 22—80138 Naples, Italy
Yasukatsu Oshima and Takeshi Yasumoto
Faculty of Agriculture, Tohoku University, Tsutsumidori-Amamiyamachi, 1-1, Sendai, Japan

A reinvestigation of the extracts from the starfish *Asterias amurensis* has led to the isolation of eight novel steroidal glycosides along with major amounts of the already reported glycoside B_2 (**1**), ovarian asterosaponin-1 (**3**), and ovarian asterosaponin-4 (**4**). Four novel compounds are 'asterosaponins' assigned as the asteroside A (**8**), B (**9**), C (**10**), and D (**11**), consisting of a $\Delta^{9(11)}$-3β,6α-dihydroxysteroidal moiety, a pentasaccharide portion attached at C-6, and a sulphate group at C-3. Asterosides A—C possess the same saccharide portion, β-D-quinovopyranosyl-(1 ⟶ 2)-β-D-galactopyranosyl-(1 ⟶ 4)-[β-D-quinovopyranosyl-(1 ⟶ 2)]-β-D-xylopyranosyl-(1 ⟶ 3)-β-D-quinovopyranosyl, already encountered in glycoside B-2, while the saccharide chain of asteroside D is identical with that of ovarian asterosaponin-4 (**4**) and differs from the previous one by the replacement of the galactose by glucose. Main differences among the novel 'asterosaponins' reside in the steroid side chains.

The second group of saponins consists of four new polyhydroxysteroidal xylopyranosides, designated amurensosides A—D (**12**)—(**15**). Three of them, A—C, are sulphated compounds.

Studies of the chemistry of physiologically active saponins from starfishes have shown that they have varied structures and can be divided into three major groups: sulphated steroidal penta- and hexa-glycosides ('asterosaponins'), steroidal cyclic glycosides, and glycosides of polyhydroxysteroids consisting of a polyhydroxysteroid with one or two sugar units.[1,2] The 'asterosaponins,' which have common structural features, including a $\Delta^{9(11)}$-3β,6α-dihydroxysteroidal moiety, an oligosaccharide chain made up from five or six sugar units attached at C-6, and a sulphate at C-3, are widely distributed and have been found in all examined species, except those of the *Echinaster* genus which contain exclusively the cyclic steroidal glycosides. The third group of saponins, the glycosides of polyhydroxysteroids, which are found in both sulphated and non-sulphated form and usually occur in minute amounts, appear to be as widespread as the 'asterosaponins' among starfishes.

The chemical and biological studies of the saponins of *Asterias amurensis*, a very common starfish in the Pacific Ocean, have been pursued for some time. In 1964 Yasumoto *et al.*[3] reported the isolation of two asterosaponins; in 1972 Ikegami *et al.*[4] isolated active substances, which inhibit spawning of mature starfish, from the ovaries of *Asterias amurensis*, and identified the compounds as asterosaponins A and B. In 1979 the complete structure of an ovarian asterosaponin, designed glycoside B_2 (**1**), was determined.[5] Its structure differs from that of thornasteroside A (**2**), first isolated from *Acanthaster planci*,[6] by the replacement of the terminal fucose by quinovose. More recently Ikegami's group described the separation of five ovarian asterosaponins from the starfish *Asterias amurensis*,[7] they designated the saponins as OA-1 (later named asterosaponin-1), OA-2 (the former glycoside B_2), OA-3, OA-4 (the former glycoside B_1; later named asterosaponin-4), and OA-5. In the subsequent paper they reported the structure determination of the major ovarian asterosaponin-1 (**3**), which contains the rare D-*xylo*-hexos-4-ulopyranosyl unit.[8] More recently the same group[9] determined the structure of ovarian asterosaponin-4 (**4**), which suppressed spontaneous oocyte maturation of starfish, and differs from glycoside B_2 in the steroid side chain and in the saccharide chain by replacement of galactose by glucose. Two new hexaglycosides, versicoside A (**5**) and B (**6**), along with the known thornasteroside A (**2**), its 24-methyl analogue, named versicoside C (**7**),‡ and ovarian asterosaponin-1 (**3**), were isolated from the fresh whole bodies of *Asterias amurensis* by Komori's group.[11,12] Figure 1 shows the structures of the reported asterosaponins from *A. amurensis*.

Continuing with our work on biologically active compounds from echinoderms[1,2] we also analysed the extractives from the whole bodies of *A. amurensis* and have now isolated four new 'asterosaponins', designated as asteroside A, B, C, and D (**8**)—(**11**) and four new xylosides of polyhydroxysteroids, named

Table 1. Steroidal oligoglycoside composition of the starfish *Asterias amurensis*

Glycoside	Amount[a] (mg)
Glycoside B_2 (**1**)	60
Asterosaponin-1 (**3**)	220
Asterosaponin-4 (**4**)	10.5
Asteroside A (**8**)	15
Asteroside B (**9**)	4
Asteroside C (**10**)	17
Asteroside D (**11**)	4.5
Amurensoside A (**12**)	3.5
Amurensoside B (**13**)	6.8
Amurensoside C (**14**)	3.5
Amurensoside D (**15**)	12
Steroid	
(**1a**)	12.5
(**4a**)	2
(**10a**)	2

[a] From frozen starfish (3 kg) collected from the Pacific coast of Hokkaido, Japan.

† Part 33. R. Riccio, L. Minale, S. Bano, N. Bano, and V. U. Ahmad, *Gazz. Chim. Ital.*, 1987, **117**, 755.

‡ The same compound was isolated by us from *Coscinasterias tenuispina*[10] and designated thornasteroside B, because its aglycone was originally named thornasterol B (I. Kitagawa, M. Kobayashi, and T. Suguwara, *Chem. Pharm. Bull.*, 1978, **26**, 1852).

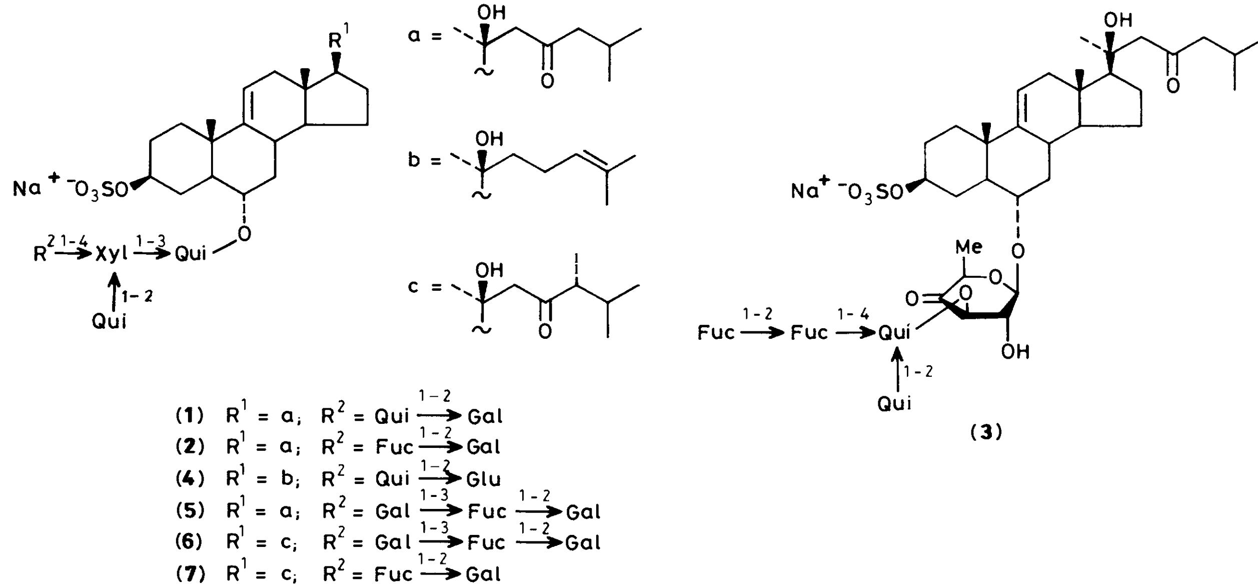


Figure 1. Reported structures of 'asterosaponins' from *Asterias amurensis*; Qui = quinovose, Xyl = xylose, Gal = galactose, Fuc = fucose, Glu = glucose; all sugars are in the pyranose form and the linkages are β

(8) R1 = a; R2 = Gal
(9) R1 = b; R2 = Gal
(10) R1 = c; R2 = Gal
(11) R1 = d; R2 = Glu

(12) R = H
(13) R = OH

(15)

(14)

Figure 2. Additional new glycosides from *Asterias amurensis*; Qui = quinovose, Xyl = xylose, Gal = galactose, Glu = glucose; all sugars are in the pyranose form and linkages are β

Table 2. Selected 250 MHz ^1H n.m.r. signals (δ_H) for the aglycone protons of 'asterosaponins.' J (Hz)-values are shown in parentheses[a]

Compound	18-H_3	19-H_3	21-H_3	26,27-H_3	28-$H_{3(2)}$	3-H	11-H	22-$H_{2(1)}$	23-$H_{2(1)}$	24-$H_{2(1)}$
(1), **(3)**	0.81	1.02	1.37	0.93 d, 0.94 d (6.5) (6.5)		4.22 m	5.37 br d (5.5)	2.62 ABq, 2 H (15.0)		2.40 d, 2 H (6.5)
(4)	0.82	1.03	1.31	1.65 1.70		4.22 m	5.37 br d (5.5)			5.13 t (6.5)
(8)	0.82	1.02	1.30	1.01 d, 6 H (7.0)		4.22 m	5.37 br d (5.5)	2.76 d (2.5)	2.94 dt (6, 2.5)	
(9)	0.83	1.03	1.30	0.98 d, 1.04 d (6.2) (6.2)		4.22 m	5.37 br d (5.5)	2.83 d (2.2)	2.76 dd (7.5, 2.2)	
(10)	0.82	1.02	1.37	0.88 d, 0.97 d (6.5) (7)	1.00 d (7.5)	4.22 m	5.37 br d (5.5)	2.62 d, 2.76 d (15) (15)		2.52 qui (7.5)
(11)	0.83	1.03	1.31	1.07 d, 6 H (7.0)	4.72 br s 4.78 br s	4.22 m	5.37 br d (5.5)			

[a] The spectra were run in CD_3OD. Signals for the aglycone protons of glycosides **(1)** and **(3)** were virtually identical. Here we report the data for **(3)**.

Table 3. ^{13}C N.m.r. shifts (δ_C) of aglycone carbon atoms of 'asterosaponins'[a]

Carbon	**(1)**, **(3)**	**(4)**	**(8)**	**(9)**	**(10)**	**(11)**
1	35.7	36.2	35.7	35.9	36.1	35.9
2	29.0	29.6	29.0	29.2	29.4	29.3
3	77.5	77.9	77.6	77.5	78.0	78.0
4	30.4	30.9	30.4	30.6	30.8	30.6
5	49.0	49.6	49.0	49.2	49.5	49.2
6	79.7	80.7	79.7	80.0	80.5	80.0
7	41.0	41.8	41.0	41.7	41.8	41.5
8	35.1	35.6	35.1	35.2	35.5	35.2
9	145.6	145.8	145.5	145.6	145.8	145.5
10	38.0	38.5	38.0	38.2	38.4	38.2
11	116.3	116.9	116.3	116.4	116.7	116.5
12	42.2	42.8	42.0	42.2	42.6	42.6
13	41.3	41.8	41.5	41.2	41.4	41.2
14	53.8	54.3	53.5	53.7	54.0	53.9
15	23.0	23.6	22.5	22.9	23.4	23.6
16	24.8	25.4	25.0	25.1	25.2	25.1
17	59.3	59.1	59.4	59.7	59.7	58.9
18	13.2	13.6	13.1	13.3	13.6	13.4
19	19.0	19.4	19.0	19.1	19.4	19.1
20	73.7	74.0	71.4	71.4	74.0	74.1
21	26.7	25.8	23.0	23.2	27.2	25.9
22	54.6	44.7	65.4	64.5	53.6	43.2
23	211.8	23.2	53.3	59.5	215.9	29.7
24	53.7	125.9	40.9		54.2	158.3
25	24.2	130.8	26.3	30.2	29.9	34.1
26	22.2	26.4	22.3	19.1	18.6	21.9
27	22.3	17.7	22.6	19.0	21.4	21.9
28					12.1	106.3

[a] Spectra were run in $[^2H_5]$pyridine at room temperature. Sometimes, to improve the solubility, a few drops of D_2O were added and this causes shifts ranging from 0.1—0.5 p.p.m. of the signals relative to the spectra run in dry pyridine. Assignments were aided by DEPT measurements. The signals for the aglycone carbon atoms of compounds **(1)** and **(3)** were virtually identical. Here we report the data for **(1)**.

amurensoside A, B, C, and D **(12)**—**(15)** (Figure 2),[13] along with major amounts of the known ovarian asterosaponin-1 **(3)** and -4 **(4)** and glycoside B_2 **(1)**. We have also isolated in small amounts the 3-*O*-sulphated steroids **(1a)**, **(4a)**, and **(10a)** (see Experimental section). The results of our analysis, shown in Table 1, are closer to those of Ikegami *et al.*[4,5,7,8] We note that the 5α-cholestane-3β,6α,15α,24-tetraol, now determined as the aglycone of **(12)**, was reported as a minor component of the hydroxylate of the crude saponins from *A. amurensis*.[14]

Separation and isolation of the individual compounds from the aqueous extracts of the animals followed the steps described previously.[10]

The 'Asterosaponins.' Known Compounds.—The major saponins, ovarian asterosaponin-1 **(3)** and glycoside B_2 **(1)**, after the usual steps of separation, including chromatography on Sephadex LH-60, and droplet counter-current chromatography (DCCC) using butan-1-ol–acetone–water (3:1:5) in both ascending and descending mode, gave a single peak in reverse-phase h.p.l.c. under different conditions. Separation of the two saponins was eventually achieved by DCCC in chloroform–methanol–water (40:42:18) in the ascending mode. The FAB (negative-ion mode) mass spectrum of **(3)** gave molecular-anion peaks at m/z 1 257 (hydrated form) and m/z 1 239 (keto form), and on SiO_2 t.l.c. [chloroform–methanol–water (10:5:1)] compound **(3)** gave two spots. These data are in agreement with the structure of ovarian asterosaponin-1, which includes a keto sugar easily converted into the hydrate. On solvolysis with pyridine and dioxane, compound **(3)** gave thornasterol A [*i.e.* (20*S*)-3β,6α,20-trihydroxy-5α-cholest-9(11)-en-23-one], because of the lability of glycopyranosiduloses in alkaline media, which results in release of the substituents[15] [*e.g.* at C-1 and C-3 in **(3)**]. Thus the keto sugar in compound **(3)** is attached to the aglycone. On acid methanolysis, this compound gave methyl fucoside and methyl quinovoside in the ratio 1:1, and the ^1H n.m.r. spectrum showed five 3 H doublets at δ_H 1.28, 1.29, 1.31, 1.41, and 1.49 for the methyl protons of quinovose (× 2), fucose (× 2) and 6-deoxyhexulose units. Based on these data we identified the major saponin as ovarian asterosaponin-1 **(3)**.[8,12] In confirmation we measured the ^{13}C n.m.r. spectrum. Signals for the aglycone moiety (Table 3) match those of the many thornasterol A 3β-sulphated saponins;[16] assignments for the carbohydrate chain are difficult.[12] We have assigned the signals for the quinovose and fucose residues (see Experimental section). The remaining signals [δ_C 201.1 (C), 104.8 (CH), 92.8 (C), 91.7 (CH), 87.7 (CH), 77.8 (CH), 71 (CH), 14.3 (CH_3), and 13.5 (CH_3)] are in agreement with the presence of the *xylo*-hexos-4-ulopyranosyl unit in both keto (major, δ_C 201.1) and hydrate (minor, δ_C 92.8) form.

The second major saponin has been identified as glycoside B_2 **(1)**.[5] The FAB (negative-ion mode) mass spectrum gave a molecular anion peak at m/z 1 243. On acid methanolysis it gave methyl xyloside, methyl quinovoside, and methyl galactoside in the proportions 1:3:1. In the ^{13}C n.m.r. spectrum (Tables 3 and 4) the carbon chemical shifts assigned to the sequence Gal–Xyl(Qui)–Qui–Aglycone match very closely those of thornasteroside A **(2)**.[16] The remaining sugar signals (five *CH* and one CH_3) in the spectrum of compound **(1)** were almost identical with those assigned to the terminal quinovose.

The third known asterosaponin was identified as the ovarian asterosaponin-4 **(4)**.[9] The structure of the aglycone was derived

Table 4. ^{13}C N.m.r. shifts (δ_C) of sugar carbon atoms of the 'asterosaponins'[a]

	(1), (8), (9), and (10)					(4) and (11)				
Sugar Carbon	Qui I	Xyl	Qui II	Gal	Qui III	Qui I	Xyl	Qui II	Glu	Qui III
1	104.3	104.0	105.2	101.9	106.0	104.6	105.1	104.3	101.6	106.1
2	73.9	82.5	75.4	82.7	75.2	74.0	82.7	75.5	84.6	76.1
3	89.7	75.9	76.5	74.5	77.3	89.7	75.5	76.6	76.6	77.4
4	74.1	78.5	76.2	69.2	76.1	74.3	79.1	76.3	70.9	76.9
5	71.7	64.2	73.5	76.8	73.0	71.8	64.2	73.3	78.1	73.7
6	17.7		18.1	61.7	18.1	17.8		18.3	62.2	18.3

[a] Spectra were run in [$^2H_5$]pyridine at room temperature. Signals for the sugar carbon atoms of compound (**1**), (**8**), (**9**), and (**10**) were virtually identical; similarly for compounds (**4**) and (**11**). Here we report the data for compounds (**1**) and (**11**).

from ^1H and ^{13}C n.m.r. spectroscopy (Tables 2 and 3) and comparison with the published data.[9] On acid methanolysis, compound (**4**) gave methyl xyloside, methyl quinovoside, and methyl glucoside in the proportions 1:3:1. The sequence and the interglycosidic linkages of the saccharide chain were determined both by FAB (negative-ion mode) mass spectrometry showing fragments at *m/z* 1 081, 919, 641, and 495, corresponding to the sequential loss of quinovose (= 146), glucose (= 162), xylose + quinovose (= 132 + 146 = 278), and quinovose (= 146) from the molecular anion at *m/z* 1 227, and by ^{13}C n.m.r. data (Table 4). The signals assigned to the sugar carbons were virtually identical with those of glycoside B_2 (**1**) except that the signals assigned in (**1**) to the galactose unit were replaced in (**4**) by those of a glucose unit.[17]

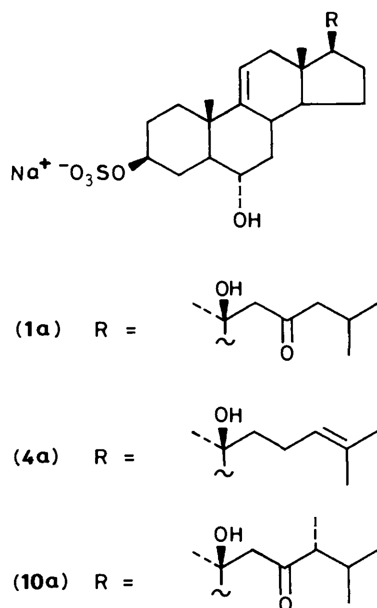
The 'Asterosaponins.' Novel Compounds.—Asteroside A (**8**). On acid methanolysis this compound liberated methyl xyloside, methyl quinovoside, and methyl galactoside in the proportions 1:3:1. The FAB (negative-ion mode) gave a molecular anion peak at *m/z* 1 243 and fragments at *m/z* 1 097, 935, 657, and 511, corresponding to the sequential loss of quinovose, galactose, quinovose + xylose, and quinovose, also present in the spectrum of glycoside B_2 (**1**). Thus asteroside A (**8**) is isomeric with glycoside B_2 (**1**). The ^1H n.m.r. spectrum of the intact saponin (Table 2) revealed signals due to the aglycone protons identical with those observed in the spectrum of tenuispinoside A, a saponin containing the (20*R*,22*R*,23*S*)-22,23-epoxycholest-9(11)-ene-3β,6α,20-triol 3β-sulphated aglycone, derived from the starfish *Coscinasterias tenuispina*.[10] Particularly characteristic are the signals for the epoxymethyne protons at δ_H 2.94 (dt, *J* 6, 2.5 Hz, 23-H) and 2.76 (d, *J* 2.5 Hz, 22-H). In the ^{13}C n.m.r. spectrum of compound (**8**) the aglycone carbon signals (Table 3) were superimposable on those of tenuispinoside A,[10] while the sugar carbon signals (Table 4) match very closely those of glycoside B_2 (**1**). Thus the novel asterosaponin asteroside A can be defined as (20*R*,22*R*,23*S*)-22,23-epoxy-20-hydroxy-6α-*O*-β-D-quinovopyranosyl-(1 ⟶ 2)-β-D-galactopyranosyl-(1 ⟶ 4)-[β-D-quinovopyranosyl-(1 ⟶ 2)]-β-D-xylopyranosyl(1 ⟶ 3)-β-D-quinovopyranosyl-5α-cholest-9(11)-en-3β-yl sodium sulphate (**8**).

Asteroside B (**9**). The FAB (negative-ion mode) mass spectrum of compound (**9**) showed the molecular anion peak (*m/z* 1 229) and the fragment ions corresponding to the sequential loss of the sugar units, shifted by fourteen mass units relative to asteroside A (**8**) (*m/z* 1 243). The ^1H n.m.r. spectrum of (**9**) (Table 2) showed a 1 H doublet (*J* 2.2 Hz) at δ_H 2.83 coupled to the 1 H double doublet (*J* 2.2, 7.5 Hz) at δ_H 2.67, indicative for an epoxide functionality, and the isopropyl methyl signals shifted to δ_H 0.98 and 1.04. The remaining signals for the aglycone protons (Table 2) were identical with those of asteroside A (**8**). Based on these data, the 22,23-epoxy-24-nor-5α-cholest-9(11)-ene-3β,6α,20-triol-3β-sulphated structure for the new aglycone has been assumed. The ^{13}C n.m.r. spectrum (Table 3) supports this assumption by showing, in addition to the signals for the steroidal tetracyclic nucleus observed in the spectrum of asteroside A (**8**), seven signals (one C, three CH and three CH_3; DEPT pulse sequence) due to the side-chain carbons. The 22*R*,23*S* stereochemistry was suggested based on ^{13}C n.m.r. data. The chemical shift of C-25 (δ_C 30.2 in [$^2H_5$]-pyridine) close to that of C-25 of a model compound containing the (*trans*)-epoxy-24-norcholestane side chain[18] (δ_C 31.6 in [$^2H_4$]methanol) is indicative of *trans*-stereochemistry. In a 22,23(*cis*)-epoxy-24-norsteroid structure we would expect for C-25 an upfield shift of *ca.* 5 p.p.m. because of the γ-gauche interaction C-20/C-25. The resonance of C-17 (δ_C 59.4), virtually identical with that of the same carbon atom in asteroside A (**8**) (δ_C 59.7), indicates the same 22*R*,23*S* stereochemistry. In the case of the (22*S*,23*R*)-isomer we would expect for C-17 an upfield shift of *ca.* 2 p.p.m. [*cf.* (22*R*,23*R*)- and (22*S*,23*S*)-22,23-epoxycholestan-3β-ols, C-17: δ_C 54.7 and 56.2, respectively;[10] note that the specification of configuration at C-22 changes on going from 22,23-epoxysterols to the 20-hydroxy-22,23-epoxysaponins (**8**) and (**9**), according to the Cahn–Ingold–Prelog convention]. The 20*R* (α)-configuration is suggested based on the chemical shift of the 21-methyl protons, δ_H 1.30 [*cf.* 20*S* (α)- and 20*R* (β)-20-hydroxycholesterols, δ_H (21-H_3) 1.28 and 1.13, respectively;[19] once again note the change of the specification of the configuration at C-20 on going from 20-hydroxycholesterol to (**8**) and (**9**)]. A detailed comparison of the ^{13}C n.m.r. data for compound (**9**) and asteroside A (**8**) showed that the saccharide chain is identical in both compounds (Table 4). Thus the novel asterosaponin asteroside B is determined to have structure (**9**).

C_{26}-Sterols with a 24-norcholestane (or 26,27-dinorergostane) skeleton are a widespread class of marine sterols of dietary origin and have been found as minor sterol components in every marine invertebrate phylum.[20] The isolation of asteroside B, which is the third occasion on which asterosaponins with a C_{26}-steroidal aglycone have been discovered,[16,18] suggests a dietary source for the steroidal portion of these compounds. Furthermore, the occurrence of 22,23-epoxysteroid glycosides is of biological interest because of their probable role in the biosynthesis of the 23-oxo function of the many aglycones of starfish saponins. In particular asteroside A (**8**) could be the biological precursor of the major glycoside B_2 (**1**). We note that 22,23-epoxysteroids have been earlier found in 'asterosaponins' from *Halityle regularis*[21] and *Coscinasterias tenuispina*[10] and in minor cyclic glycosides from *Echinaster sepositus*.[18]

Asteroside C (**10**). This compound proved to be the 24-methyl analogue of glycoside B_2 (**1**). The FAB (negative-ion mode) mass spectrum showed a molecular anion at *m/z* 1 257 shifted

by fourteen mass units relative to glycoside B_2 (**1**) (*m/z* 1 243), and a major fragment at *m/z* 1 143 [cleavage of the C(20)–C(22) bond and 1 H transfer] identical with that observed in the spectrum of glycoside B_2 (**1**), thus indicating the presence of an extra methyl group in the side chain of (**10**). In agreement with a 24-methylglycoside B_2 structure, the ^1H n.m.r. signals for the



aglycone protons (Table 2) were identical with those observed in the spectrum of thornasteroside B (**7**) (24-methylthornasteroside A),[10] and the signals in the sugar region were superimposable with those of glycoside B_2 (**1**). The ^{13}C n.m.r. spectrum (Tables 3 and 4) confirmed this assignment. The stereochemistry at C-24 is suggested to be the 24*R* based on the negative Cotton maximum ($[\theta]_{290}$ − 6 826) in the c.d. spectrum as compared with those of the diacetyl derivatives of (20*S*,24*R*)-thornasterol B and (20*S*,24*S*)-thornasterol B, which are reported as $(\theta]_{288}$ − 5 720 and $[\theta]_{277}$ − 631, respectively.[22]

Asteroside D (**11**). On acid methanolysis this compound liberated methyl xyloside, methyl quinovoside, and methyl glucoside in the proportions 1:3:1. In the FAB mass spectrum (negative-ion mode) of compound (**11**), a molecular anion species was observed at *m/z* 1 241. Next to the molecular anion the spectrum displayed fragments with *m/z* 1 095, 933, 655, and 509, which correspond to the sequential losses of quinovose (= 146), glucose (= 162), xylose + quinovose (= 278), and quinovose, thus indicating the same saccharide sequence already found in the ovarian asterosaponin-4 (**4**). Comparison of the spectral data in Tables 2 and 3 immediately suggested that compound (**11**) is related to (**4**) by having an *exo*-methylene group at C-24 in the steroid side chain. The ^1H n.m.r. spectrum included two one-proton olefinic signals at δ_H 4.72 (br s) and 4.78 (br s) and the signals for 26- and 27-H_3 shifted to δ_H 1.07 (6 H, d, *J* 7.5 Hz), and the ^{13}C n.m.r. spectrum included two sp^2 carbon signals at δ_C 106.3 (CH_2) and 158.3 (C). Again the stereochemistry at C-20 is suggested to be 20*S* based on the chemical shift of the 21-methyl protons (δ_C 1.31).[19] A detailed comparison of the ^{13}C n.m.r. data for asteroside D (**11**) and ovarian asterosaponin-4 (**4**) showed that the saccharide chain is identical in both compounds.

Monoglycosides of Polyhydroxysteroids.—Amurensoside A (**12**). The $[M^-]$ peak at *m/z* 647 present in the FAB mass spectrum (negative-ion mode) and the molecular-ion species at *m/z* 693 $[M_{Na} + Na]^+$ in the FAB m.s. (positive-ion mode) suggested a molecular weight of 670 for the intact sodium salt. Elimination of 120 mass units ($-NaHSO_4$) from $[M_{Na} + Na]^+$, *m/z* 573, suggested that compound (**12**) is a sodium sulphated molecule.

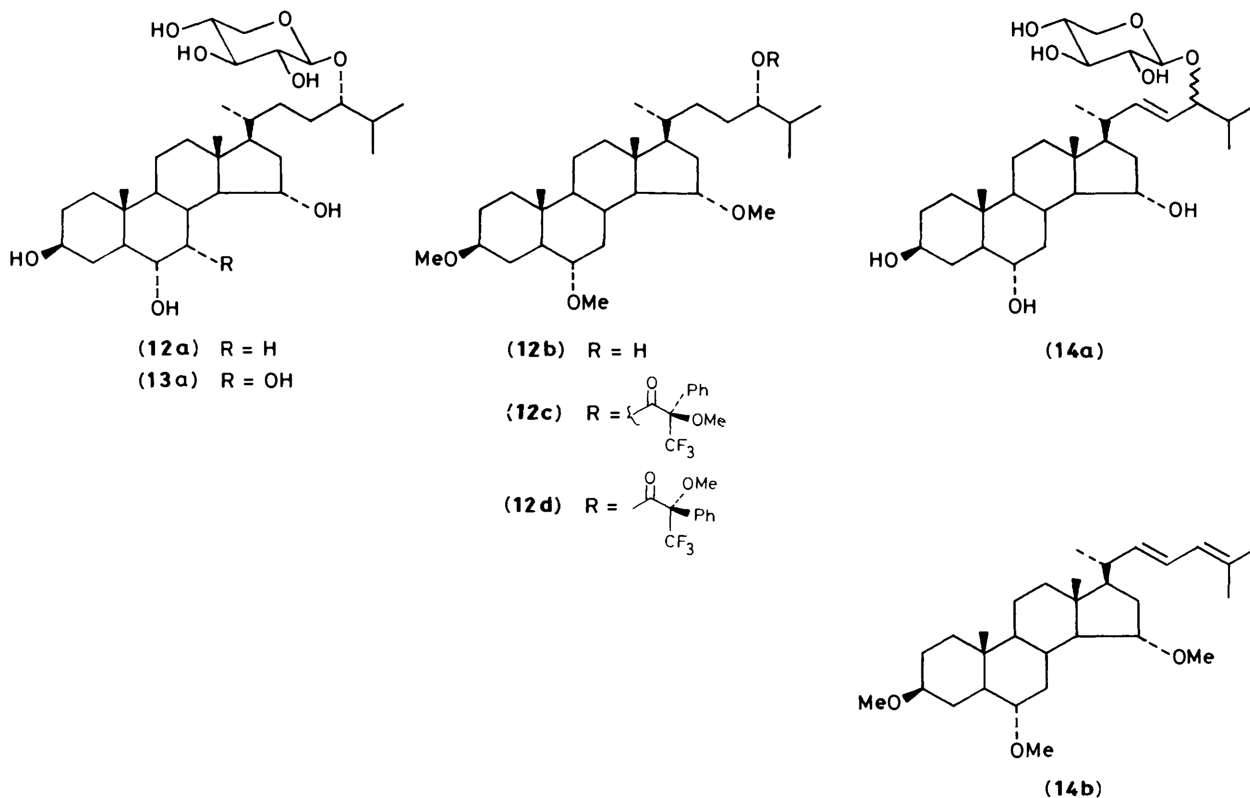


Table 5. Selected 250 MHz ^{1}H n.m.r. signals (δ_H) for amurensosides A—D (**12**)—(**15**). *J* (Hz)-values are shown in parentheses

H carbon	(12)	(13)	(14)	(15)
3	3.50 m	3.50 m	3.50 m	3.50 m
6	*a*	*a*	*a*	*a*
7eq	2.49 dt (4, 12.5)	4.21 t (2)	2.32 dt (4, 12.5)	4.05 t (2)
7ax	1.05 m			
15	4.50 dt (2.5, 10)	4.45 dt (2.5, 10)	3.91 dt (2.5, 10)	4.34 t (5)
18	0.77 s	0.78 s	0.77 s	0.97 s
19	0.88 s	0.89 s	0.88 s	0.91 s
21	0.96 d (7)	0.96 d (7)	1.06 d (6.5)	0.95 d (6.5)
24	*a*	*a*	3.72 dd (7, 5.5)	*a*
26	0.96 d (7)	0.96 d (7)	0.89 d (7)	0.98 d (6.5)
27	0.96 d (7)	0.96 d (7)	0.96 d (7)	0.98 d (6.5)
1′	4.25 d (7.5)	4.25 d (7.5)	4.26 d (7.5)	4.25 d (7.5)
2′	3.22 dd (7.5, 9.5)	3.22 dd (7.5, 9.5)	3.27 dd (7.5, 9.5)	3.22 dd (7.5, 9.5)
3′	3.30 t (9.5)	3.30 t (9.5)	3.51 t (9.5)	3.30 t (9.5)
4′	3.50 ddd (10, 9.5, 5)	3.50 ddd (10, 9.5, 5)	4.19 m	3.50 ddd (10, 9.5, 5)
5′eq	3.90 dd (10, 5)	3.90 dd (10, 5)	4.15 dd (10, 5)	3.90 dd (10, 5)
5′ax	3.16 t (10)	3.16 t (10)	3.30 t (10)	3.16 t (10)

[a] Signals overlapping with methanol signal.

Table 6. ^{13}C N.m.r. shifts (δ_C) of amurensosides A—D[a]

Carbon	(12)	(13)	(14)	(15)
1	38.6	38.3	38.8	38.5
2	31.9	32.0	32.0	32.0
3	72.0	72.0	72.0	72.1
4	33.0	32.9	33.1	32.9
5	52.7	44.4	52.9	44.6
6	70.2	72.0	70.3	72.5
7	41.4	71.3	41.3	70.6
8	35.1	39.9	35.4	35.8
9	55.3	46.5	55.5	46.4
10	37.2	37.2	37.4	37.3
11	22.1	22.2	22.2	22.0
12	42.0	41.6	42.0	42.3
13	43.8	43.0	44.8	43.3
14	61.5	56.0	63.9	55.8
15	81.7	82.0	74.0	71.8
16	38.7	38.6	42.9	42.4
17	55.1	55.3	54.9	57.8
18	13.7	13.5	13.9	15.0
19	13.9	12.8	13.9	12.8
20	36.6	36.8	40.9	37.0
21	19.0	19.0	21.3	19.3
22	32.7	32.7	140.8	32.9
23	29.0	28.9	128.4	28.8
24	86.3	86.3	89.0	86.4
25	32.0	31.9	33.9	32.0
26	18.2	18.3	19.2	18.3
27	18.5	18.5	18.4	18.4
1′	105.0	105.0	104.2	105.0
2′	75.4	75.4	75.4	75.4
3′	77.9	77.9	76.2	78.0
4′	71.4	71.4	77.6	71.4
5′	66.7	66.9	64.9	66.8

[a] Spectra were run in [$^2H_4$]methanol. Assignments were aided by DEPT measurements. Assignments of the sugar signals were made by comparison with those of methyl β-D-xylopyranoside.[17]

The presence of a sulphate group was confirmed by solvolysis of compound (**12**) in a dioxane–pyridine mixture,[23] affording the desulphated derivative, (**12a**), which gave in its FAB m.s. (negative-ion mode) a quasi-molecular ion at *m/z* 567 ($[M - H]^-$). Elimination of 132 mass units (= pentasaccharide unit) from $[M^-]$, *m/z* 575, in the FAB m.s. (negative-ion mode) of compound (**12**) suggested that the natural compound is a glycoside of a sulphated steroid aglycone. The ^{1}H n.m.r. spectrum (Table 5) assisted by the decoupling results, and the ^{13}C n.m.r. spectrum (Table 6), indicated that the molecule bears a β-xylopyranosyl moiety. This was confirmed by acid methanolysis of compound (**12**) which gave methyl xylosides. Thus, having established the presence of sodium sulphate and xylosyl residues in (**12**), the molecular weight of the aglycone is 436, corresponding to a cholestane structure with four hydroxy groups (all secondary from DEPT ^{13}C n.m.r.). The ^{1}H n.m.r. spectrum contained signals for five methyl groups [δ_H 0.77 (3 H, s), 0.88 (3 H, s), 0.96 (9 H, d, *J* 7 Hz)] and also two isolated signals for methine protons on oxygen-bearing carbons of the aglycone. A multiplet at δ_H 3.52 partially overlapped with a 4′-H signal had the complexity normally seen for a 3β-hydroxy group, and the double triplet (*J* 2.5, 10 Hz) at δ_H 4.50 shifted upfield to δ_H 3.90 in the spectrum of the desulphated derivative (**12a**), had the shape for a 15α-hydroxy group.[24] These data along with the ^{13}C n.m.r. frequencies of C-14, C-15, and C-16 in the spectrum of compound (**12**), which are shifted by −2.5, +7.7, and −1.6 p.p.m., respectively, relative to the model 15α-cholestanol,[25] established the location of the sulphate group at C-15. Support for the 15α-sulphoxy stereochemistry in compound (**12**) came from ^{1}H n.m.r. decoupling experiments, which showed that the small coupling (2.5 Hz) of the signal assigned to 15-H was due to the interaction with 16-H_α at δ_H 2.24 (br d, *J* 14 Hz), thus leaving one large coupling (10 Hz) due to the interaction with 14-H. In 15β-hydroxysteroids the coupling between 14-H and 15-H is *ca.* 5 Hz.[10] The remaining two hydroxymethine signals were obscured by the methanol signal at *ca.* δ_H 3.3. The ^{1}H n.m.r. spectrum of compound (**12**) also contained an isolated double triplet (*J* 12.5 and 4 Hz) at δ_H 2.44, which was transformed into a double doublet (*J* 12.5, 4 Hz) on irradiation at δ_H 3.3. Assignment of this signal to 7-H_β and the location of the third hydroxy group at C-6 of the steroid skeleton could account for these n.m.r. data. The chemical shifts of the 18- and 19-H_3 singlets at δ_H 0.75 and 0.88 in (**12a**), respectively, are in agreement with a 3β,6α,15α-trihydroxycholestane structure (calculated using Zürcher's substituent increment parameters,[26] 18-H_3: 0.73, 19-H_3: 0.85; the differences between our values and the calculated ones can be due to solvent effects, CD_3OD instead of $CDCl_3$). The ^{13}C n.m.r. spectrum (Table 6) of compound (**12**) and comparison with those of models 5α-cholestane-3β,6α-diol[27] and cholestan-15α-ol,[25] taking into account the sulphation shifts, definitively

established the 15α-sulphoxy-3β,6α-dihydroxy pattern of the steroid aglycone. The fourth hydroxy group was located in the side chain at C-24, which is also the site of glycosidation, from consideration of the ^{13}C n.m.r. data and comparison with those of the many 24-*O*-glycosidate steroids isolated from starfishes.[10] Thus the steroid aglycone of amurensoside A was identified as 5α-cholestane-3β,6α,15,24-tetraol, previously isolated by Ikegami and co-workers[14] from among the hydrolysates of the asterosaponins from *A. amurensis.* The configuration at C-24 remained unassigned. We have now determined the (24*S*) configuration based on the following data. Desulphated amurensoside A (**12a**) was methylated with CH_3I in dimethylformamide (DMF)NaH to afford a permethylated derivative, which, by hydrolysis with 2M-HCl–MeOH, gave 3β,6α,15α-trimethoxy-5α-cholestan-24-ol (**12b**). The ^{13}C n.m.r. shift values ($CDCl_3$) of the side-chain carbons of (**12b**) (Experimental section) compared better with those reported for (24*S*)-24-hydroxycholesterol than with those for the corresponding 24*R*-isomer.[28] Even so, the small differences observed between the spectra of the two epimeric model compounds[28] induced us to do further experiments to determine the configuration at C-24 in our compound. For this we took advantage of the generous gift by Professor N. Ikekawa of a sample of (22*E*,24*R*)-6β-methoxy-3α,5-cyclo-5α-cholest-22-en-24-ol (**16**),[29] which was easily converted into (24*S*)- and (24*R*)-6β-methoxy-3α,5-cyclo-5α-cholestan-24-ol (**18**) and (**19**) (Figure 3). Then we prepared the (+)-(*R*)-MTPA [MTPA = α-

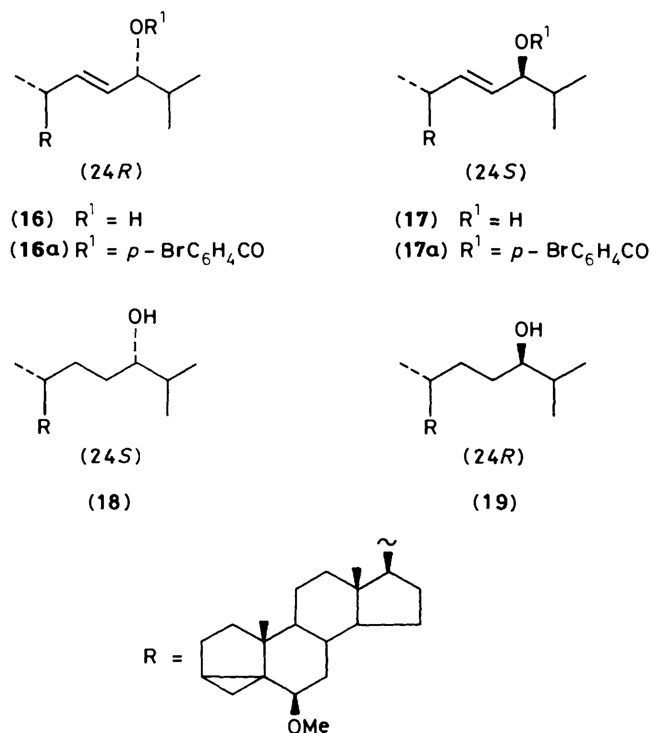


Figure 3. Model compounds

methoxy-α-(trifluoromethyl)phenylacetic acid]* esters of the 24-alcohol (**12b**), derived from the natural amurensoside A (**12**), and of the (24*S*) and (24*R*) model alcohols (**18**) and (**19**). The ^{1}H n.m.r. spectra of the model epimeric (+)-(*R*)-MTPA esters were significantly different, and the resonances of the isopropyl methyl protons appeared in the 500 MHz ^{1}H n.m.r. spectrum of the 24*S*-isomer (**18**) as two upfield doublets at δ_H 0.83 (*J* 6.5) and 0.85 (*J* 6.5 Hz), and in the spectrum of the 24*R*-isomer (**19**) as a 6-H doublet shifted downfield at δ_H 0.91, as expected on the basis of Mosher's models.[30] The shifts of the isopropyl methyl protons of the (+)-(*R*)-MTPA ester of (**12b**) [(**12c**)] (two doublets at δ_H 0.84 and 0.86) match those found in the (+)-(*R*)-MTPA ester of (24*S*)-6β-methoxy-3α,5-cyclo-5α-cholestan-24-ol (**18**). In confirmation we also prepared the (−)-(*S*)-MTPA ester of (**12b**) [(**12d**)] and in the ^{1}H n.m.r. spectrum the isopropyl methyl proton signals appeared shifted downfield to δ_H 0.89 and 0.91, close to the values found in the (+)-(*R*)-MTPA ester of (24*R*)-6β-methoxy-3α,5-cyclo-5α-cholestan-24-ol (**19**). We also noted differences in the chemical-shift values for 21- and 18-H_3 protons in the two spectra [(**12c**): δ_H 0.90 d, 0.66 s; (**12d**): 0.86 d, 0.64 s]. The same was observed in the spectra of the (+)-(*R*)-MTPA ester of the model epimeric 24*S*- and 24*R*-alcohol [24*S*: δ_H 0.91 d, 0.70; 24*R*; 0.88 d, 0.68 s]. Thus the structure of the amurensoside A has been determined as (24*S*)-24-*O*-β-D-xylopyranosyl-5α-cholestane-3β,6α,15α,24-tetraol 15-sulphate (**12**). The D-configuration of xylose was assumed, by comparison with the many D-xylosides isolated from starfishes.[2]

Amurensoside B (**13**) is related to amurensoside A (**12**) by the introduction of an extra hydroxy group at position 7 of the aglycone. The FAB m.s. (negative-ion mode) gave the anion peak at *m/z* 663, shifted by sixteen mass units relative to (**12**). In the ^{1}H n.m.r. spectrum of compound (**13**) one more hydroxymethine signal was observed, at δ_H 4.21 (apparent triplet, *J* 2.5 Hz), which replaced the signal at δ_H 2.44 assigned to 7-H_β in the spectrum of (**12**). Irradiation of overlapping signals under the methanol signal caused a sharpening of the signal at δ_H 4.21, thus confirming the presence of two vicinal hydroxy groups. The relationship between structures (**12**) and (**13**) was definitively confirmed by ^{13}C n.m.r. spectroscopy. Taking (**12**) as starting structure, the ^{13}C n.m.r. spectrum was calculated for the compound with an additional 7α-hydroxy group, using the substituent effects that have been published for hydroxysteroids,[25,31] and excellent agreement was obtained between the calculated [C-5: 44.3; C-6: 72.4; C-7: 71.0; C-8: 39.2; C-9: 46.8; C-14: 55.6] and the experimental (Table 6) spectra. The process was repeated with a 7β-hydroxy group and now the calculated [C-5: 50.1; C-6: 76.1; C-7: 80.7; C-8: 43.1; C-9: 53.5; C-14: 59.7] and the experimental (Table 6) spectra were significantly different. The chemical shifts for the side-chain carbons in compounds (**12**) and (**13**) were identical and on this basis we propose the same 24*S*-configuration in compound (**13**). We note that the introduction of a sugar moiety at C-24 should cause significant differences in the spectra of 24*S*- and 24*R*-epimers.[32]

Amurensoside C (**14**). Acid methanolysis of (**14**) gave methyl xylosides. The FAB m.s. (positive-ion mode) of (**14**) showed molecular-ion species at *m/z* 691 ($[M_{Na} + Na]^+$) and 669 ($[M_{Na} + H]^+$) and fragments at *m/z* 589 (loss of SO_3 from 669) and 571 (loss of $NaHSO_4$ from 691), all peaks shifted by two mass units relative to compound (**12**). The negative-ion FAB m.s. also showed the molecular-anion peak shifted by two mass units relative to (**12**), *m/z* 645. Upon solvolysis, compound (**14**) was desulphated to (**14a**). The negative-ion FAB m.s. of (**14a**) showed a quasi-molecular ion at *m/z* 565 ($[M - H]^-$) and a fragment at *m/z* 433 corresponding to the loss of a xylosyl residue. The 500 MHz ^{1}H n.m.r. of (**14**) and double-resonance experiments allowed the assignments of the sugar protons (Table 5). An upfield shift of 4′-H from δ_H 4.19 in (**14**) to 3.50 in the desulphated (**14a**) showed that C-4′ bears the sulphate. This was confirmed by ^{13}C n.m.r. spectroscopy which showed the C-4′ signal shifted by 6.2 p.p.m. to δ_C 77.6, whereas C-3′ and C-5′ are shifted upfield by 1.7 and 2.2 p.p.m., respectively relative to compound (**12**) (sulphation shift). In addition to the sugar protons, readily recognized from the n.m.r. data, are two tertiary methyls at δ_H 0.77 and 0.88 and two isolated hydroxymethine

* *Systematic name*: β,β,β-trifluoro-α-methoxy-α-phenylpropionic acid.

signals at δ_H 3.50 (m) and 3.91 (dt, J 2.5, 10 Hz), already observed in (**12a**), and assigned to 18-H_3, 19-H_3, 3-H, and 15-H of the steroid, the signal for 6-H being obscured by the methanol signal. Sequential decoupling from C-20 to C-27 [20-H: 2.12 m; 21-H_3: 1.06 d (6.5 Hz); 22-H: 5.47 dd (15, 6 Hz); 23-H: 5.37 dd (15, 7 Hz); 24-H: 3.72 dd (7, 5.5 Hz); 25-H: 1.87 m; 26- and 27-H_3: 0.89 d (7 Hz)—0.96 d (7 Hz)] allowed us to locate a double bond at C-22(*E*) and the remaining oxygenated function at C-24 in the side chain. Thus the steroid aglycone of amurensoside C is (22*E*)-5α-cholest-22-ene-3β,6α,15α,24-tetraol. The ^{13}C n.m.r. data (Table 6) and comparison with those of compound (**12**) confirmed this proposal and clarified that the 4-*O*-sulphated xylosyl residue is β-glycosidally linked at C-24. Compared with the models (22*E*)-6β-methoxy-3α,5-cyclo-5α-cholest-22-en-24-ols [C-24: δ_C 79.0 and 79.3, 24*R*- and 24*S*-isomer, respectively; C-23: 130.0; C-25: 35.3] the C-24 signal is shifted downfield by *ca.* 10 p.p.m. to δ_C 89.0 in compound (**14**), whereas C-23 and C-25 are shifted upfield by 1.6 and 1.4 p.p.m. (*cf.* glycosidation shifts[32,33]).

The stereochemistry at C-24 remained to be determined. Methylation of compound (**14a**) followed by hydrolysis with 2M-HCl–MeOH to remove the sugar portion only gave the (22*E*)-3β,6α,15α-trimethoxy-5α-cholesta-22,24-diene (**14b**). Scarcity of material did not allow further experiments directly to remove the sugar moiety without dehydration. Re-extraction of a large sample (6 kg) of *Asterias amurensis*, collected in March 1986 off Sendai, Japan, gave further amounts of amurensosides A (**12**) (30 mg), B (**13**) (20 mg), and D (**15**) (21 mg), and only traces of amurensoside C (**14**).

Amurensoside D (**15**). This compound is the only non-sulphated glycoside isolated from *A. amurensis*. The FAB m.s. (negative-ion mode) showed a quasi-molecular ion at *m/z* 583 ($[M - H]^-$) and a major fragment at *m/z* 451 corresponding to the loss of a pentasaccharide unit. Examination of its spectral properties (Tables 5, 6) indicated that compound (**15**) contains a β-xylopyranosyl unit, as confirmed by acid methanolysis affording methyl xylosides. Of the remaining signals (27 signals) present in the ^{13}C n.m.r. spectrum of compound (**15**) (Table 6) five (all CH by DEPT) are assigned to carbons bonded to oxygen. In addition to the sugar moiety, the ^1H n.m.r. spectrum showed signals for five methyl groups at δ_H 0.97 s (18-H_3), 0.91 s (19-H_3), 0.98 (6 H, d, J 6.5 Hz), and 0.95 (3 H, d, J 6.5 Hz), and signals for three hydroxymethine protons at δ_H 3.50 (3-H_α), 4.05 (apparent triplet, J 2.0 Hz, 7-J_β), and 4.34 (apparent triplet, J 5.0 Hz). Two hydroxymethine signals (6-H_β and 24-H) were obscured by the methanol signal. The signal at δ_H 4.34 had the shape for a 15β-hydroxy group[24] [J(14-H/15-H) 5 Hz] and the downfield shifts of 18-H_3 to δ_H 0.97 supported the 15β-hydroxy stereochemistry.

The ^{13}C n.m.r. data and comparison with those of the previous amurensosides and those published for hydroxysteroids[25,31] confirmed the formulation (**15**) for amurensoside D.

Experimental

The following instruments were used: n.m.r. spectra, Bruker WM-250 and WM-500; mass spectra, Kratos MS 50 mass spectrometer equipped with a Kratos FAB source and AEI MS-30 apparatus at 70 eV for low-resolution e.i. mass spectra; c.d., JASCO J 500 A spectropolarimeter; optical rotations, Perkin Elmer model 241 polarimeter; g.l.c., Carlo Erba fractovap 2900 for capillary column; h.p.l.c., 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 Büchi apparatus equipped with 300 tubes.

The FAB mass spectra were obtained by dissolving the sample in a glycerol or glycerol–thioglycerol (3:1) matrix and placing them on a copper tip prior to bombardment with Xe atoms of energy of 2—6 kV. The DEPT[34] experiments were made using polarization transfer pulses of 90° and 135°, respectively, obtaining in the first case only CH groups and in the other case positive signals for CH and CH_3 and negative ones for CH_2 groups. Polarization transfer delays were adjusted to an average CH coupling of 135 Hz.

Extraction and Isolation.—The animals, *Asterias amurensis versicolor* Sladen, were collected at the Pacific coast of Hokkaido, Japan, in August 1985 and frozen (3 kg). Frozen animals were then cut into small pieces and homogenized in one volume of water, using a Waring blender. The homogenates were centrifuged at 2 000 r.p.m. and the supernatant was passed through filter paper by suction. The aqueous extracts thus obtained (*ca.* 5 l) were passed through a column of Amberlite XAD-2 (bed volume 1 l). The column was washed with distilled water (1 l) and then eluted with methanol (2 l). The methanol eluate was taken to dryness to give a glassy material (4.2 g), which was then chromatographed on a column of Sephadex LH-60 (4 × 60 cm) with methanol–water (2:1) as eluant. Fractions (6.5 ml) were collected and analysed by t.l.c. on SiO_2 in butan-1-ol–acetic acid–water (60:15:25).

Fractions 55—75 (1.5 g) mainly contained the 'asterosaponins' and fractions 76—98 (0.97 g) mainly contained the amurensosides.

The crude 'asterosaponins' fraction was added to the more polar fractions (157 mg) obtained from DCCC separation of the 'amurensosides' fractions (see below) and further purified by DCCC with butan-1-ol–acetone–water (45:15:75) [descending mode; the upper phase was used as the stationary phase; fractions (6 ml) were collected] to give four main fractions: 50—58 (108 mg), 59—67 (330 mg), 68—78 (202 mg), and 79—98 (270 mg). These fractions were then separated by h.p.l.c. on a C_{18} μ-Bondapak column (30 cm × 8 mm i.d.) with methanol–water (45:55) as the eluant. The flow rate was 5 ml min^{-1}. Fractions 50—58 mainly contained the more polar 'asterone' saponins, the retro-aldol cleavage derivatives of the 'thornasterol A'-containing saponins (**1**), (**3**), and (**10**), probably artefacts produced during the extraction procedure,[35] and small amounts of asteroside B (**9**). The h.p.l.c. traces of the remaining fractions were dominated by a peak, eluted after *ca.* 20 min from the injection, consisting of a mixture (280 mg in total) of ovarian asterosaponin-1 (**3**) and glycoside B_2 (**1**). The fractions also contained small peaks corresponding to the epoxides asteroside B (**9**) (eluted after 16 min) and asteroside A (**8**) (eluted after 24 min) and the 24-methylsaponin asteroside C (**10**) (eluted after 28 min) in different proportions. The less polar DCCC fractions (79—98) also contained small amounts of the ovarian asterosaponin-4 (**4**). A mixture (120 mg) of the ovarian asterosaponin-1 (**3**) and glycoside B_2 (**1**) was eventually separated by DCCC in chloroform–methanol–water (40:42:18) [ascending mode; the lower phase was used as the stationary phase; fractions (4 ml) were collected] to give in the fractions 35—37 glycoside B_2 (13.9 mg) and in the fractions 41—46 the ovarian asterosaponins-1 (**3**) (58.0 mg). The fractions in between contained a mixture of both compounds. Fractions were analysed by t.l.c. on SiO_2 in chloroform–methanol–water (10:5:1) [(**1**) R_F 0.26; (**3**) R_F 0.32 and 0.34)].

The 'amurensosides' fractions (0.97 g) as eluted from the column of Sephadex LH-60 were submitted to DCCC with chloroform–methanol–water (7:13:8) [ascending mode; the lower phase was used as the stationary phase; fractions (4.5 ml) were collected] to give six main fractions: 10—19 (448 mg), 20—23 (157 mg), 24—28 (13 mg), 29—33 (21 mg), 34—39 (10.9 mg), and 51—59 (12 mg). Fractions 20—23 still contained a mixture of 'asterosaponins' and were combined with the 'asterosaponins'

fraction eluted from the column of Sephadex LH-60. Fractions 51—59 contained the less polar amurensoside D (**15**). The middle fractions 24—28, 29—33, and 34—39 were purified on a C_{18} μ-Bondapak column (30 cm × 8 mm i.d.) with methanol–water (1:1) to give (**4a**), (**1a**), and (**10a**), eluted in that order. The more polar fractions 10—19 were submitted again to DCCC in butan-1-ol–acetone–water (45:15:75) [ascending mode, the lower phase was used as the stationary phase; fractions (4.5 ml) were collected] to give three main fractions 179—199 (32 mg), 200—212 (27.6 mg), and 213—230 (50.3 mg). These fractions were separated by h.p.l.c. on a C_{18} μ-Bondapak column (30 cm × 8 mm i.d.) with methanol–water (53:47) to give pure compounds. The flow rate was 5 ml min^{-1}. Fractions 179—199 mainly gave amurensoside C (**14**) eluted after 19 min; fractions 200—212 gave amurensoside A (**12**) eluted after 18 min, and finally fractions 213—230 gave amurensoside B (**13**) eluted after 17 min, and the asterosaponins ovarian asterosaponin-4 (**4**), eluted after 16 min, and asteroside D (**11**), eluted after 21 min. The total amounts of each compound isolated from frozen *Asterias amurensis* (3 kg) are shown in Table 1. The ^1H and ^{13}C n.m.r. spectral data are in Tables 2—6.

Other Spectral Data.—Rotations were taken from solutions in MeOH (*c* ranging between 0.4—1.0).

Glycoside B_2 (**1**). $[\alpha]_D$ +4.1°; −ve ion FAB m.s., *m/z* 1 243 (M^-, 100%), 1 143 [$C_{20(22)}$ cleavage and 1 H transfer, 10], 1 097 (15), 935 (10), 657 (10), 511 (10), and 493 (10).

Ovarian asterosaponin-1 (**3**). $[\alpha]_D$ +19.3°; −ve ion FAB m.s., *m/z* 1 257 (M^-, hydrate form, 100%), 1 239 (M^-, keto form, 25), 1 157 [$C_{20(22)}$ cleavage and 1 H transfer, 8], 1 139 [$C_{20(22)}$ cleavage and 1 H transfer, 10], 1 111 (7), 1 093 (3), 965 (2), 947 (4), 701 (10), 673 (5), 665 (7), 539 (20), 511 (30), and 493 (25); δ_C ([$^2H_5$] pyridine) Qui I: 104.1, 83.1, 75.0, 85.8, 72.4, and 17.9; Qui II: 105, 75.0, 76.7, 76.3, 73.7, and 18.4; Fuc I: 102.4, 82.4, 73.8, 71.6, 71.6, and 16.7; Fuc II: 106.0, 71.8, 75.0, 73.2, 71.8, and 17.0; signals for the aglycone carbons are in Table 3; signals for the 6-deoxy-*xylo*-hexos-4-ulose moiety are in the text.

Ovarian asterosaponin-4 (**4**). $[\alpha]_D$ +3.7°; −ve ion FAB m.s., *m/z* 1 335 (M^+ thioglycerol, 50%), 1 227 (M^-, 100), 1 081 (10), 919 (10), 641 (5), and 495 (10).

Asteroside A (**8**). $[\alpha]_D$ +5.8°; −ve ion FAB m.s., *m/z* 1 243 (M^-, 100%), 1 097 (20), 935 (20), 657 (15), 511 (10), and 493 (10).

Asteroside B (**9**). $[\alpha]_D$ −9.2°; −ve ion FAB m.s., *m/z* 1 229 (M^-, 100%); the remaining fragments were very small.

Asteroside C (**10**). $[\alpha]_D$ −2.0°; −ve ion FAB m.s., *m/z* 1 257 (M^-, 100%), 1 143 [$C_{20(22)}$ cleavage and 1 H transfer, 10], 1 111 (5), 949 (3), 525 (aglycone, 20), and 507 (25); c.d. $[\theta]_{290}$ −6 826 (in water).

Asteroside D (**11**). $[\alpha]_D$ +5.0°; −ve ion FAB m.s., *m/z* 1 349 (M^- + thioglycerol, 100%), 1 241 (M^-, 50), 1 095 (5), 933 (5), 655 (5), and 509 (10).

Amurensoside A (**12**). $[\alpha]_D$ +16.7°; −ve ion FAB m.s., *m/z* 647 (M^-, 100%), 629 (25), and 497 (15); +ve ion FAB m.s., *m/z* 693 [$(M_{Na}$ + Na$)^+$, 60%], 687 [$(M_K$ + H$)^+$, 20], 573 (693 − $NaHSO_4$, 100), and 441 (10).

Amurensoside B (**13**). $[\alpha]_D$ +12.5°; −ve ion FAB m.s. *m/z* 663 (M^-, 100%) and 645 (30).

Amurensoside C (**14**). $[\alpha]_D$ +6.8°; −ve ion FAB m.s., *m/z* 645 (M^-, 100%) and 627 (20); +ve ion FAB m.s., *m/z* 691 [$(M_{Na}$ + Na$)^+$, 100%], 669 [$(M_{Na}$ + H$)^+$, 25], 633 (30), 589 (669 − SO_3, 50), and 571 (691 − $NaHSO_4$, 20).

Amurensoside D (**15**). $[\alpha]_D$ −4.5°; −ve ion FAB m.s., *m/z* 583 [$(M$ − H$)^-$, 100%] and 451 (20).

Steroid (**1a**). +ve ion FAB m.s., *m/z* 557 [$(M_{Na}$ + Na$)^+$] and 437 (557 − $NaHSO_4$); −ve ion FAB m.s., 511 (M^-, 80%) and 411 [$C_{20(22)}$ cleavage and 1 H transfer, 100%]; δ_H (CD_3OD) 3.54 (1 H, dt, *J* 4, 10 Hz, 6-H_β); remaining signals identical with those of (**1**) and (**3**) (Table 2).

Steroid (**4a**). −ve ion FAB m.s., *m/z* 509 (M^-, 100%) and 411 [$C_{20(22)}$ cleavage and 1 H transfer, 80]; δ_H(CD_3OD) 3.54 (1 H, dt, *J* 4, 10 Hz, 6-H_β); remaining signals identical with those of (**4**) (Table 2).

Steroid (**10a**). −ve ion FAB m.s. 525 (M^-, 100%) and 411 [$C_{20(22)}$ cleavage and 1 H transfer, 50]; δ_H(CD_3OD) 3.54 (1 H, dt, *J* 4, 10 Hz; 6-H_β); remaining signals identical with those of (**10**) (Table 2).

Methanolysis of Glycosides.—*Sugar analysis.* A solution of each glycoside (0.5—2 mg) in anhydrous 2M-HCl in MeOH (0.5 ml) was heated at 80 °C in a stoppered reaction vial for 8 h. After having 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. G.l.c. analysis (140 °C) using a 25 m column of SE-30 (hydrogen carrier flow 10 ml min^{-1}) gave peaks which co-eluted with those of the appropriate silylated standards.

Solvolysis of the Ovarian Asterosaponin-1 (**3**).—*Thornasterol A.* A solution of compound (**3**) (18.5 mg) in a mixture of dioxane (1 ml) and pyridine (1 ml) was heated at 120 °C 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 water (5 ml) and Bu^nOH (5 ml). The extraction was repeated three times and the combined extracts were washed with water, and evaporated to dryness under reduced pressure. The residue was purified by h.p.l.c. [C_{18} μ-Bondapack; methanol–water (65:35)] to give one major peak, eluted after 32 min, which was identified as (20*S*)-3β,6α,20-trihydroxy-5α-cholest-9(11)-en-23-one (thornasterol A) by ^1H n.m.r. and mass spectrometry.[36]

Solvolysis of the Amurensosides A–C (**12**)—(**14**).—A solution of each of the above compounds (2—4 mg) in a mixture of dioxane (0.5 ml) and pyridine (0.5 ml) was heated at 120 °C for 2 h in a stoppered reaction vial. Work-up as above and h.p.l.c. [C_{18} μ-Bondapack; methanol–water (75:25)] gave the desulphated compounds: compound (**12a**), −ve ion FAB m.s., *m/z* 567 [$(M$ − H$)^-$, 100%] and 435 (30); δ_H (CD_3OD) (aglycone) 0.75 (3 H, s, 18-H_3), 0.88 (3 H, s, 19-H_3), 0.95 and 0.97 (9 H, overlapping doublets with *J* 6.5 Hz, 21-, 26-, and 27-H_3), 2.32 (1 H, dt, *J* 12.5, 4 Hz, 7-H_β), 3.37 (partially overlapping with methanol signal, m, 6-H_β and 24-H), 3.50 (overlapping with 4′-H, 3-H_α), 3.90 (overlapping with 5′-H_{eq}, 15-H_β); δ_H(sugar) essentially unshifted with respect to compound (**12**) (see Table 5); compound (**13a**), −ve ion FAB m.s., *m/z* 573 [$(M$ − H$)^-$, 100%] and 451 (30); δ_H(CD_3OD) (aglycone) 0.76 (3 H, s, 18-H_3), 0.88 (3 H, s, 19-H_3), 0.97 (9 H, overlapping doublet, 21-, 26-, and 27-H_3), 3.38 (partially overlapping with methanol signal, m, 6-H_β and 24-H), 3.50 (m, 3-H_α), 3.87 (partially overlapping with 5′-H_{eq}, 15-H_β), and 4.02 (1 H, apparent triplet, *J* 2.5 Hz, 7-H_β); δ_H (sugar) essentially unshifted with respect to compound (**13**) (see Table 5); compound (**14a**), −ve ion FAB m.s., *m/z* 565 [$(M$ − H$)^-$, 100%] and 433 (40); δ_H(CD_3OD) (aglycone) virtually unshifted with respect to spectrum of (**14**); δ_H(sugar) 3.16 (1 H, t, *J* 10 Hz, 2′-H), 3.22 (1 H, dd, *J* 9.5, 7.5 Hz, 5′-H_{ax}), 3.30 (t, 9.5 Hz, 3′-H), 3.50 (m, partially overlapping with 3-H_α, 4′-H), 3.90 (dd, *J* 10, 5 Hz, 5′-H_{eq}), and 4.25 (1 H, d, *J* 7.5 Hz, 1′-H).

Methylation of Compound (**12a**) *and Subsequent Hydrolysis to Compound* (**12b**).—A solution of desulphated amurensoside A (**12a**) (20 mg) in DMF (5 ml) was slowly added under nitrogen to a stirred mixture of NaH (50 mg) in dry DMF (1 ml) cooled in an ice-bath. The mixture was stirred for 15 min, and then MeI (0.8 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 methanol and, after the addition of water, the mixture was extracted twice with chloroform. The organic layer was washed with water, dried (Na_2SO_4), and evaporated under reduced pressure. The residue was then methanolysed in anhydrous 2M-HCl–methanol (1 ml) at 80 °C in a stoppered reaction vial for 2 h. After cooling, the reaction mixture was neutralized with Ag_2CO_3, 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 silica gel in $CHCl_3$–methanol (99:1) to give the 3β,6α,15α-trimethoxy-5α-cholestan-24-ol (**12b**). (16 mg), EI m.s., m/z 478 (M^+) and 446, 414 (100%); $\delta_H(CDCl_3)$ 0.68 (3 H, s, 18-H_3), 0.82 (3 H, s, 19-H_3), 0.90 and 0.93 (9 H, overlapping doublets, J, 6.5 Hz, 21-, 26-, and 27-H_3), 3.26, 3.34, and 3.36 (each 3 H, s, OMe), and 3.40—3.62 (4 H, overlapping signals for >CHO protons); $\delta_C(CDCl_3)$ C-1: 36.2, C-2: 27.9, C-3: 79.9, C-4: 28.4, C-5: 50.2, C-6: 78.8, C-7: 37.5, C-8: 33.9, C-9: 53.8, C-10: 36.6, C-11: 21.2, C-12: 40.1, C-13: 43.3, C-14: 60.7, C-15: 82.6, C-16: 36.9, C-17: 53.9, C-18: 13.4, C-19: 13.6, C-20: 35.7, C-21: 18.7, C-22: 32.3, C-23: 31.0, C-24: 77.5, C-25: 33.4, C-26 and −27: 16.8 and 19.0.

Compound (**12c**), *the* (+)—(R)-MTPA *Ester of* (**12b**).—Compound (**12b**) (8 mg) was treated with freshly distilled (+)-methoxy(phenyl)trifluoromethylacetyl chloride* (40 μl) in dry pyridine (0.2 ml) for 4 h. After removal of solvent, the product was eluted through a Pasteur pipette, filled (5 cm) with a slurry of silica gel in $CHCl_3$, to give the ester (**12c**); $\delta_H(CDCl_3$; 500 MHz) 7.57, 7.43, and 7.37 (Ph), 4.95 (1 H, m, 24-H), 3.24, 3.34, 3.37, and 3.51 (each 3 H, s, OMe), 0.90 (3 H, d, J 6 Hz, 21-H_3), 0.86 (3 H, d, J 7 Hz, 26- or 27-H_3), 0.84 (3 H, d, J 6.5 Hz, 27-H_3 or 26-H_3), 0.80 (3 H, s, 19-H_3), and 0.66 (3 H, s, 18-H_3). Assignments of the secondary Me protons were aided by decoupling.

Compound (**12d**) (−)—(S)-MTPA *Ester of* (**12b**).—Compound (**12b**) (8 mg) was treated with freshly distilled (−)-methoxy(phenyl)trifluoromethylacetyl chloride* (40 μl) under the same conditions as above to give compound (**12d**); $\delta_H(CDCl_3$; 500 MHz) 7.37, 7.43, and 7.57 (Ph), 4.93 (1 H, m, 24-H), 3.27, 3.34, 3.39, and 3.52 (each 3 H, s, OMe), 0.90 (6 H, apparent t, J 6.5 Hz, 26- and 27-H_3), 0.86 (3 H, J 6 Hz, 21-H_3), 0.79 (3 H, s, 19-H_3), and 0.64 (3 H, s, 18-H_3). Assignments of the secondary Me protons were aided by decoupling.

Model Compound (24S)- *and* (24R)-6β-*Methoxy*-3α,5-*cyclo*-5α-*cholestan*-24-*ol* (**18**) *and* (**19**).—A sample of (22*E*,24*R*)-6β-methoxy-3α,5-cyclo-5α-cholest-22-en-24-ol (**16**)[29] (13 mg) given to us by Professor N. Ikekawa (Tokyo Institute of Technology) was dissolved in methylene dichloride (1 ml) and an excess of pyridine–chromic anhydride was added to the solution and the mixture was stirred for 1 h. Further methylene dichloride (2 ml) was added and the organic phase was evaporated to give an u.v.-active compound (Δ^{22}, 24-one) which, without further purification, was stirred with lithium aluminium hydride (2 mg) in dry tetrahydrofuran (4 ml) for 4 h. Excess of lithium aluminium hydride was destroyed by addition of ethyl acetate, then the reaction mixture was diluted with water, adjusted to pH 4, and extracted with ether. Evaporation of the extract afforded a residue, which was chromatographed on a silica gel column (9 cm × 0.5 cm) in hexane and increasing amounts of ether to give the less polar (22*E*,24*S*)-6β-methoxy-3α,5-cyclo-5α-cholest-22-en-24-ol (**17**) (5.6 mg) and the more polar (22*E*,24*R*)-6β-methoxy-3α,5-cyclo-5α-cholest-22-en-24-ol (**16**) (6.4 mg recovery). The absolute configurations of these two samples were then checked by preparing the corresponding *p*-bromobenzoates [*p*-bromobenzoyl chloride in pyridine and 4-(diethylamino)pyridine; 65 °C; 8 h; usual work-up and purification by preparative SiO_2 t.l.c. in hexane–ether (9:1)] and c.d. measurements[37]: less polar compound (**17a**), (24*S*-isomer), c.d. (hexane) $\Delta\varepsilon_{245}$ +8.5; more polar compound (**16a**) (24*R*-isomer), c.d. $\Delta\varepsilon_{245}$ −9.2.

A sample (2 mg) of (22*E*,24*R*)-6β-methoxy-3α,5-cyclo-5α-cholest-22-en-24-ol (**16**) dissolved in ethanol (1 ml) was hydrogenated over 10% Pd–C (2 mg) to yield (24*S*)-6β-methoxy-3α,5-cyclo-5α-cholestan-24-ol (**18**) (2 mg), $\delta_H(CDCl_3)$ 0.45 (1 H, dd, J 7.5, 5 Hz, cyclopropyl H), 0.68 (1 H, t, J 5 Hz, cyclopropyl H), 0.73 (3 H, s, 18-H_3), 0.91 and 0.94 (9 H, overlapping doublets, 21-, 26-, and 27-H_3), 1.03 (3 H, s, 19-H_3), 2.78 (1 H, t, J 2.5 Hz, 6-H), 3.33 (3 H, s, OMe).

A sample (2 mg) of (22*E*,24*S*)-6β-methoxy-3α,5-cyclo-5α-cholest-22-en-24-ol (**17**) dissolved in ethanol (1 ml) was similarly hydrogenated to give (24*R*)-6β-methoxy-3α,5-cyclo-5α-cholestan-24-ol (**19**) (2 mg), $\delta_H(CDCl_3)$ 0.92 (9 H, overlapping doublets, 21-, 26-, and 27-H_3); the remaining signals were identical with those of epimer (**18**).

Each sample of compounds (**18**) and (**19**) were then treated with freshly distilled (+)-methoxy(phenyl)trifluoromethylacetyl chloride as before to give the corresponding (+)-(*R*)-MTPA esters, characterized by their 500 MHz ^1H n.m.r. spectra: (**18**) (+)-(R)-MTPA ester, 0.71 (3 H, s, 18-H_3), 0.83 (3 H, d, J 6.5 Hz, 26- or 27-H_3), 0.85 (3 H, d, J 7 Hz, 27- or 26-H_3), 0.91 (3 H, d, J 6 Hz, 21-H_3), 1.03 (3 H, s, 19-H_3), and 4.95 (1 H, m, 24-H); (**19**) (+)-(*R*)-MTPA ester, 0.69 (3 H, s, 18-H_3), 0.86 (3 H, d, J 6 Hz, 21-H_3), 0.92 (6 H, d, J 7 Hz, 26- and 27-H_3), 1.03 (3 H, s, 19-H_3), and 4.93 (1 H, m, 24-H).

Methylation of Compound (**14a**) *followed by Acid Methanolysis: Formation of Compound* (**14b**).—Desulphated amurensoside C (**14a**) (3.5 mg) was methylated in the conditions described for compound (**12a**), and the permethylated derivative was then methanolysed as above in anhydrous 2M-HCl–methanol at 80 °C for 2 h. Preparative t.l.c. on SiO_2 in hexane–ethyl ether (1:1) of the reaction mixture gave (22*E*)-3β,6α,15α-trimethoxy-5α-cholesta-22,24-diene (**14b**), EI m.s. m/z 458 (M^+); $\delta_H(CDCl_3)$ 0.71 (3 H, s, 18-H_3), 0.82 (3 H, s, 19-H_3), 1.03 (3 H, d, J 7 Hz, 21-H_3), 1.74 and 1.76 (each 3 H, s, together 26- and 27-H_3), 2.90, 3.1, and 3.35 (each 1 H, m, C*H*OMe), 3.24, 3.34, and 3.37 (each 3 H, s, OMe), 5.40 (1 H, dd, J 14, 7.5 Hz, 22-H), 5.76 (1 H, br d, J 10 Hz, 24-H), and 6.14 (1 H, dd, J 14, 10 Hz, 23-H).

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