Starfish Saponins. Part 17.[1] Steroidal Glycoside Sulphates from the Starfish *Ophidiaster ophidianus* (Lamarck) and *Hacelia attenuata* (Gray)†

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The major sulphated asterosaponins, ophidianosides B, C, E, and F, isolated from the Mediterranean starfish *Ophidiaster ophidianus*, have been completely characterized. The more polar compounds, ophidianosides B (**1**) and C(**2**), are pentaglycosides and contain a steroidal aglycone with 26 carbon atoms [3β,6α,20ξ-trihydroxy-24-nor-5α-cholest-9(11)-en-23-one (24-northornasterol A)] already found in saponins derived from *Hacelia attenuata*. The ophidianoside E was found to be thornasteroside A, first obtained from *Acanthaster planci*. Ophidianoside F (**4**) contains the same oligosaccharide moiety as ophidianoside C (**2**) and the usual C_{27} aglycone, thornasterol A [*i.e.* 3β,6α,20ξ-trihydroxy-5α-cholest-9(11)-en-23-one]. The two major saponins from the starfish *Hacelia attenuata*, which were reported to contain the 24-northornasterol A as the aglycone, have been shown to be identical with ophidianosides B (**1**) and C (**2**).

We have reported previously that the major sulphated asterosaponin from the Mediterranean starfish *Hacelia attenuata* (order Valvatida, family Ophidiasteridae) contains a novel C_{26} steroidal aglycone, 3β,6α,20-trihydroxy-24-nor-5α-cholest-9(11)-en-23-one; the sulphate group was shown to be linked at C-3, and the oligosaccharide moiety at C-6.[2] However, the samples were too small to permit the saccharide sequence to be established. Indeed in this starfish the sulphated asterosaponins are minor metabolites, the 24-*o*-glycosidated steroids being the major glycoside components.[3,4] We now report the complete structures of four sulphated asterosaponins derived from the Mediterranean starfish *Ophidiaster ophidianus* (order Valvatida, family Ophidiasteridae). The two major compounds, ophidianosides B (**1**) and C (**2**), are shown to contain the above novel C_{26} steroidal aglycone and are identical with the two major saponins of *H. attenuata*. The two minor compounds (**3**) and (**4**) contain the usual C_{27} aglycone, thornasterol A [3β,6α,20-trihydroxy-5α-cholest-9(11)-en-23-one[5]], and one is shown to be thornasteroside A (**3**), first isolated from *Acanthaster planci*.[6] The novel compounds each have the same invariant structural feature, Qui$^{1\rightarrow2}$Xyl$^{1\rightarrow3}$Qui$^{1\rightarrow6}$aglycone with the remaining sugars linked at C-4 of the branched xylose, as that present in thornasteroside A (**3**) and in other previously described asterosaponins,[1,6-11] apart from marthasterosides B and C, derived from *Marthasterias glacialis*. In these compounds, which contain aglycones lacking the 20-hydroxy function, the branched xylose is replaced by quinovose, also branched at C-2 and C-4, while the quinovose directly attached to the aglycone is replaced by glucose, again with the 3-hydroxy function involved in interglycosidic linkage.[12]

The saponin mixture was obtained from the aqueous extract of the fresh animals *via* successive column chromatography on Amberlite XAD-2 and Sephadex LH-60, and droplet counter-current chromatography (DCCC). Ophidianosides B, C, E, and F were then separated each from the others by semi-preparative reverse-phase h.p.l.c. (Figure 1).

Ophidianoside B (**1**) ([α]$_D$ +2.8°).—On acid methanolysis this compound, gave methyl fucoside, methyl xyloside, methyl quinovoside, and methyl galactoside in the ratio 1:1:2:1. The FAB mass spectrum showed a molecular ion species at *m/z* 1 275 (*M* + Na), fourteen mass units shifted relative to thornasteroside A (*m/z* 1 289) and sugar fragments with masses identical with those observed in the spectrum of thornasteroside A (**3**) (Table 1). At 250 MHz, the ^{1}H 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 the saponin containing the 24-northornasterol A aglycone, derived from *Hacelia attenuata*.[2] Particularly characteristic are the isopropyl methyl doublets, which occur at low field (δ 1.077 and 1.096) because of deshielding by the vicinal oxo group, and the broad singlet at δ 2.70 due to the methylene protons at C-22. The 3β-

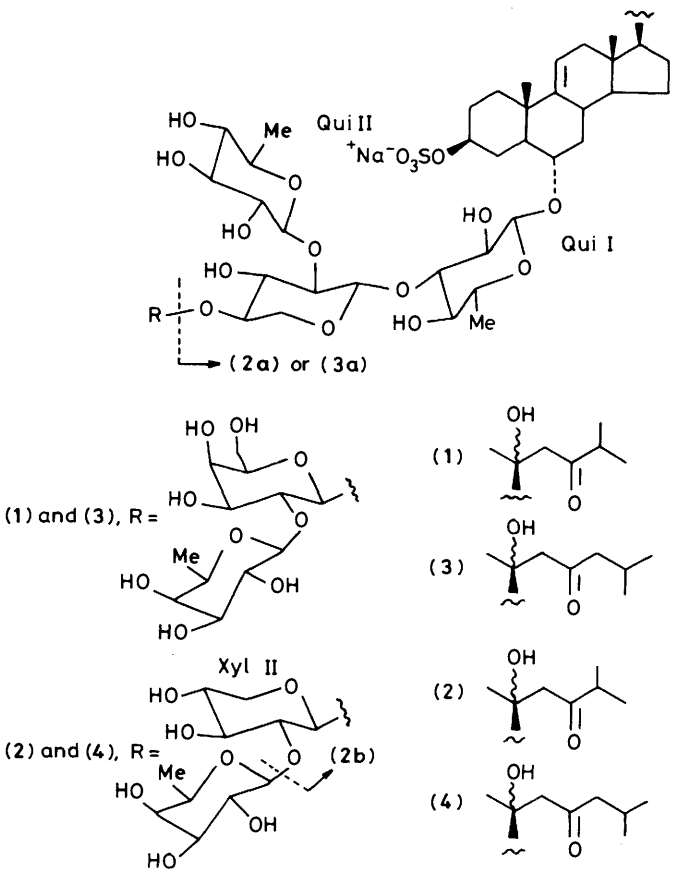

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Table 1. FAB Mass spectral data of compounds (**1**)—(**4**). The mass units lost correspond to the following fragments: 132, xylose, 146, quinovose; 278, xylose + quinovose.

	(1)	(3)	(2)	(4)
	Molecular ion species			
*M*K + Na				1 275
*M*Na + Na	1 275	1 289	1 245	1 259
*M*K + H	1 269			1 253
*M*Na + H	1 253			
	Carbohydrate fragments*			
Pentasaccharide cation	771	771	741	741
	755	755	725	725
Tetrasaccharide cation	625 (771 − 146)	625	595 (741 − 146)	595
	609 (755 − 146)	609	579 (725 − 146)	579
Trisaccharide cation	479 (625 − 146)	479	449 (595 − 146)	449
(branching point)	463 (609 − 146)	463	433 (579 − 146)	433
	447	447	417	417
Disaccharide cation		347		317
[Fuc-Gal in (**1**) and (**3**); Fuc-Xyl in (**2**) and (**4**)]		331		301

* All carbohydrate fragments are sodiated species.

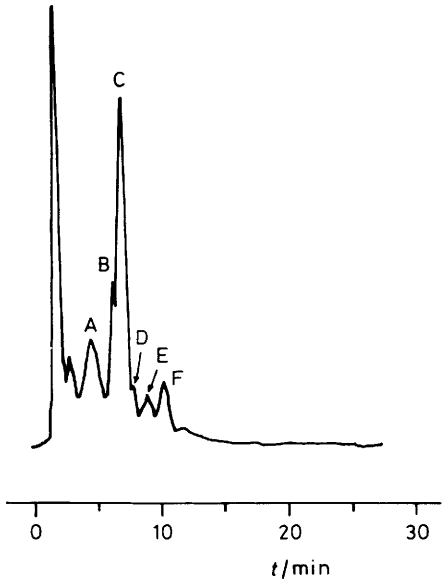

Figure 1. H.p.l.c. of the mixture of saponins from *Ophidiaster ophidianus* on a μ-Bondapack C_{18} (3.9 mm × 30 cm) column with methanol–0.1N-aqueous NaH_2PO_4 (64:36). B, Ophidianoside B (**1**); C, ophidianoside C (**2**); E thornasteroside A (**3**); F, ophidianoside F (**4**)

sulphated 24-northornasterol A [3β,6α,20-trihydroxy-24-nor-5α-cholest-9(11)-en-23-one] structure proposed for the aglycone of the new ophidianoside B (**1**) was supported by the ^{13}C n.m.r. spectrum of the intact saponin; this contained 26 aglycone carbons, of which 21 (C-1 to -21) have shifts within ±0.2 p.p.m. of those of the corresponding carbons of thornasteroside A (**3**) (Table 3).

The signal due to C-22, which in compound (**1**) is influenced by an additional γ-effect, was shifted upfield by −2.7 p.p.m. and the carbonyl carbon was shifted downfield by +4.1 p.p.m., as expected on the basis of the substituent parameter effects;[13] the remaining signals at δ 42.5, 17.9, and 18.2 were assigned to the isopropyl group. A detailed comparison of the ^{13}C n.m.r. data for compound (**1**) and thornasteroside A (**3**) showed that the saccharide chain is identical in both compounds (Table 4).

Ophidianoside C (**2**) ($[\alpha]_D$ −2.9°).—This compound is the major component of the saponin mixture. An examination of the ^1H n.m.r. spectrum immediately suggested the presence of 24-northornasterol A as the low-field isopropyl methyl doublets (δ 1.077 and 1.096) due to deshielding by the vicinal oxo function, and the presence of a broad singlet at δ 2.70 due to the protons at C-22. This was supported by the ^{13}C n.m.r. and FAB mass spectra. The FAB mass spectrum showed molecular ion species at *m/z* 1 245 (*M* + Na) and sodiated pentasaccharide fragments at *m/z* 741 and 725 (Table 1), which indicated the presence of a pentaglycoside with a sulphated steroidal aglycone corresponding to a northornasteryl A sulphate. The ^{13}C n.m.r. signals for the aglycone carbons (Table 3) were virtually identical with those of the ophidianoside B (**1**), and the ^{13}C n.m.r. spectrum also established that the oligosaccharide is attached at C-6 and the sulphate at C-3, a general feature of sulphated asterosaponins. The downfield shift of the 3α-H resonance, to δ 4.20 ($w_{\frac{1}{2}}$ 20 Hz), further confirmed the location of the sulphate at C-3.

The sugars obtained from the saponin hydrolysate were shown to be quinovose (×2), xylose (×2), and fucose. Permethylation and methanolysis of the methylated material gave permethylated methyl quinovoside and permethylated methyl fucoside, indicating that the saccharide chain contains one branching point. The fragmentation pattern observed in the FAB mass spectrum parallels that of thornasteroside A (**3**) (Table 1) and other asterosaponins,[8,11,12] and gives the probable location of the branching point on the xylose unit, the second monosaccharide starting from the aglycone (Figure 2). The ^{13}C n.m.r. shifts of the anomeric carbons (Table 4) and the ^1H n.m.r. coupling constants of the anomeric protons (Experimental section) suggested that all the linkages are β. On enzymatic hydrolysis with *Charonia lampas* glycosidase mixture, ophidianoside C gave two prosapogenol sulphates, the major one being the triglycoside (**2a**); *m/z* (FAB) 967 (*M* + Na) Acid methanolysis provided methyl xyloside and methyl quinovoside in the ratio 1:2. The ^1H n.m.r. spectrum showed three anomeric proton doublets at δ 4.56 (*J* 7.5 Hz), 4.54 (*J* 7.5 Hz), and 4.42 (*J* 7.5 Hz; xylose unit), two 3H doublets at δ 1.30 (*J*

Table 2. 270 MHz ^{1}H N.m.r. data (δ/p.p.m.) of compounds (**1**)—(**4**). *J* (Hz) Values are shown in parentheses*

Compound	18-H_3	19-H_3	21-H_3	26- and 27-H_3	3-H	11-H	22-H_2	24-H_2	25-H
(**1**), (**2**)	0.816	1.023	1.371	1.077d, 1.096d (6.8) (6.9)	4.21m	5.37br d (5.5)	2.70br s		2.73 m
(**3**), (**4**)	0.812	1.023	1.372	0.935d, 0.945d (6.6) (6.5)	4.21m	5.37br d (5.5)	2.62ABq (15.0)	2.420d (6.5)	

* m, multiplet; br d, broad doublet; br s, broad singlet; q, quartet. The spectra were run in CD_3OD. Signals for the aglycone protons of compounds (**1**) and (**2**), and of (**3**) and (**4**), are virtually identical. Here we report the data taken from the spectra of (**2**) and (**4**).

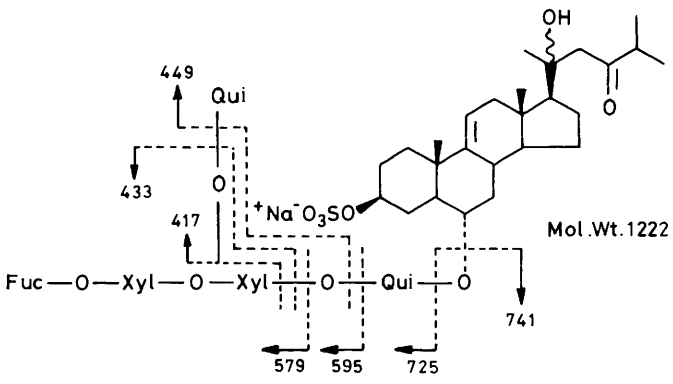

Figure 2. FAB Mass fragmentation of ophidianoside C (**2**); all fragments are sodiated species

5.8 Hz) and 1.40 (*J* 6.2 Hz) for the methyl protons of the internal and terminal quinovose units, respectively, and a double doublet (*J* 11.5 and 4.5 Hz) at δ 3.92 due to 5-H_{eq} of the xylose unit. These shifts were almost superimposable on those of the prosapogenol (**3a**), obtained from thornasteroside A[1] and marthasterosides A_1 and A_2[12] by similar procedures. The chemical shifts of the signals due to the aglycone protons in compound (**2a**) were superimposable on those of the starting material (**2**). A detailed comparison of the ^{13}C n.m.r. data of the intact saponin (**2**) with those of thornasteroside A (**3**) (Table 4) showed that the chemical shifts of the signals due to the trisaccharide sequence quinovose-xylose-quinovose-aglycone are virtually superimposable in both spectra. Taken together these data indicated the common β-D-quinovopyranosyl-(1→2)-β-D-xylopyranosyl-(1→3)-β-D-quinovopyanosyl structure for the saccharide chain of the prosapogenol (**2a**).

The minor prosapogenol sulphate was found to be the β-xylopyranoside of compound (**2a**); *m/z* (FAB) 1 099 (*M* + Na). Acid methanolysis provided methyl quinovoside and methyl xyloside in the ratio 1:1. Analysis of the ^{1}H n.m.r. spectrum established that the additional xylopyranose unit is located at C-4 of the original xylose. The ^{1}H n.m.r. spectra of compounds (**2a**) and (**2b**) are remarkably similar; in the sugar proton region the spectrum of compound (**2b**) includes another anomeric proton signal at δ 4.35 (*J* 6.3 Hz) and two double doublets at δ 4.09 (*J* 11.5 and 4.5 Hz) and 3.92 (*J* 10.5 and 5.0 Hz) due to the equatorial proton at C-5 of the xylopyranose units. The fact that one of these signals is found at relatively low field indicated the substitution at C-4.[12] The ^{13}C n.m.r. spectral data of the intact saponin confirmed the 4-*O*-substitution by the presence of the signal at δ 64.3 which is ascribable to C-5 of a 4-*O*-substituted xylose.[14] Thus, the structure of the minor prosapogenol sulphate obtained from ophidianoside B is (**2b**) and the sugar sequence in compound (**2**) must be fucose-xylose-(quinovose)-xylose-quinovose-24-northornasteryl 3β-sulphate. The linkage of the sequence T-fucose-xylose has been determined by ^{13}C n.m.r. spectroscopy. The appearance in the ^{13}C n.m.r. spectrum

Table 3. ^{13}C N.m.r. shifts (δ/p.p.m.) of aglycone carbon atoms*

Carbons	(**1**) and (**2**)	(**3**) and (**4**)
1	36.2	36.0
2	29.5	29.5
3	78.0	78.2
4	30.9	30.6
5	49.5	49.4
6	80.6	80.4
7	41.6	41.6
8	35.6	35.3
9	145.7	145.5
10	38.5	38.3
11	116.7	116.7
12	42.7	42.4
13	41.8	41.6
14	54.2	54.0
15	23.4	23.3
16	25.5	25.1
17	59.8	59.6
18	13.6	13.5
19	19.4	19.2
20	74.0	74.2
21	27.2	27.0
22	52.1	54.9
23	216.0	211.7
24		54.0
25	42.5	24.3
26	17.9	22.5
27	18.2	22.6

* Spectra were run in [$^2H_5$] pyridine (30 mg/0.4 ml) at room temperature. The signals for the aglycone carbon atoms of compounds (**1**) and (**2**), and those of (**3**) and (**4**), were virtually identical. Here we report the data taken from the spectra of (**2**) and (**4**).

of compound (**2**) (Table 4) of one anomeric carbon signal at relatively high field (δ_C 102.1) can be explained in terms of a substitution effect at the C-2 position of the xylopyranose II moiety; the C(2)-glycosidic linkage was also evidenced by the fact that the C-2 carbon of xylopyranose II is shifted downfield by *ca.* 10 p.p.m. (β-effect) to 84.0 p.p.m. Thus the novel asterosaponin ophidianoside C can be defined as 20-hydroxy-6α-*O*-β-D-fucopyranosyl-(1→2)-β-D-xylopyranosyl-(1→4)-[β-D-quinovopyranosyl-(1→2)]-β-D-xylopyranosyl-(1→3)-β-D-quinovopyranosyl-23-oxo-24-nor-5α-cholest-9(11)-en-3β-yl sodium sulphate (**2**). We prefer the D-configuration for all the monosaccharides on the basis of the rotation value of the intact saponin, which is in the same range as the asterosaponins described previously.[6–12]

Ophidianoside E (**3**) ([α]$_D$ −3.8°).—This has been identified as thornasteroside A, first isolated from *Acanthaster planci*,[6] by direct comparison (^{1}H and ^{13}C n.m.r., and FAB mass spectra, and h.p.l.c.) with an authentic sample isolated from the starfish *Luidia maculata*.[1]

Ophidianoside F (**4**) ([α]$_D$ +0.4°).—This is a homologue of ophidianoside C (**3**), with the same saccharide chain and the

Table 4. ^{13}C N.m.r. shifts (δ/p.p.m.) of sugar carbon atoms*

	(1) and (3)					(2) and (4)				
Sugar carbon atoms	Qui I	Xyl	Qui II	Gal	Fuc	Qui I	Xyl I	Qui II	Xyl II	Fuc
1	104.7	104.1	105.1	102.2	106.8	105.1	104.5	105.1	102.1	106.6
2	74.0	82.4	75.4	83.0	71.8	74.3	82.5	75.6	84.0	72.1
3	89.8	75.4	76.6	74.7	74.7	90.5	75.3	77.0	77.4	75.0
4	74.3	78.8	76.0	69.2	73.6	74.6	78.2	76.3	70.5	73.7
5	72.3	64.3	73.7	76.6	71.7	72.6	64.4	73.7	66.9	72.1
6	17.8		18.4	61.9	17.0	17.9		18.5		17.2

* Spectra were run in [$^2H_5$]pyridine (30 mg/0.4 ml) at room temperature. Signals for the sugar carbon atoms of compounds (**1**) and (**3**) and those of (**2**) and (**4**), were identical within experimental error (±0.1 p.p.m.). Here we report the data taken from the spectra of (**1**) and (**2**).

common moiety thornasterol A 3β-sulphated as the aglycone. The FAB mass spectrum exhibited molecular ion species at *m/z* 1 259 (*M* + Na), fourteen mass units shifted relative to those of compound (**2**) (*m/z* 1 245) and sugar fragments with masses identical with those observed in the spectrum of compound (**2**) (Table 1). In the ^{13}C n.m.r. spectrum of compound (**4**) (Table 3) the aglycone chemical shifts were superimposable on those of thornasteroside A (**3**), while the sugar carbon signals (Table 4) were superimposable on those of compound (**2**). Similarly the ^1H n.m.r. spectrum of compound (**4**) showed aglycone signals identical with those of compound (**3**) (Table 2), and signals due to sugar protons identical with those of (**2**) (Experimental section). On this basis, ophidianoside F is determined to have structure (**4**).

Identification of the Asterosaponins from Hacelia attenuata.—Following the same method as for the isolation of ophidianosides, we re-isolated the two major asterosaponins from *Hacelia attenuata*. One of them, already partially characterized,[2] now shown to be identical with ophidianoside C (**2**) (^1H and ^{13}C-n.m.r. and FAB mass spectra), while the second is identical with ophidianoside B (**1**) (h.p.l.c., t.l.c., ^1H n.m.r. and FAB mass spectra). The saponin mixture of *H. attenuata* also contains very minor oligoglycosides having the C_{27} aglycone thornasterol A; this was not characterized because of the lack of material.

C_{26} Sterols with a 24-norcholestane (or 26,27-bis-norergostane) skeleton are a widespread class of marine sterols of dietary origin and have been found as minor sterol components in every marine invertebrate phylum.[15] Recently they have been found, with additional nuclear and/or side-chain oxygenated functions, in starfishes.[16,17] The discovery of C_{26} steroidal glycosides in two starfishes of the same family (Ophidiasteridae) as the major components of the saponin mixture is noteworthy. The origin of the carbohydrate portion of the asterosaponins is still unknown. There have been a few preliminary studies involving the aglycone portion, which have left the *de novo* or dietary origin in doubt.[18] However, the skeleton of the steroids (**1**) and (**2**) certainly suggests a dietary source for these compounds.

Experimental

The following instruments were used: n.m.r. spectra, Bruker WM-250; mass spectra, Kratos MS 902 mass spectrometer equipped with a Kratos FAB source. The spectra were obtained by dissolving the samples in a glycerol matrix and placing them on a copper tip prior to bombardment with Xe atoms of energy 2—6 kV; h.p.l.c., Waters Model 6000 A pump equipped with a U6K injector and a differential refractometer, model 401; g.l.c., Carlo Erba Fractovap 2900 capillary column; droplet counter-current chromatography (DCCC), DCC-A apparatus manufactured by Tokyo Rikakikai Co. equipped with 250 tubes. The ^1H n.m.r. spectra were measured for solutions in CD_3OD; the ^{13}C n.m.r. spectra were measured for solutions in [$^2H_5$]-pyridine.

Extraction, Isolation, and Fractionation of Saponins from Ophidiaster ophidianus.—The animals (28 specimens, 1.5 kg fresh animals), collected in September 1983 in the bay of Naples, were chopped and soaked (1 h) in distilled water (2 l). The extraction was repeated five times. The aqueous extracts were passed through a column of Amberlite XAD-2 (500 g), eluting with water (1 bed vol) and then methanol (3 l). The water fractions were re-chromatographed on the same Amberlite XAD-2 column. The combined methanol fractions were dried on a rotary evaporator to give a glassy material (5.2 g), which was then chromatographed on a column of Sephadex LH-60 (4 × 60 cm) using methanol–water (2:1) as the eluant. 10-ml fractions were collected and analysed by t.l.c. on SiO_2 in n-butanol–acetic acid–water (60:15:25). Fractions 48—66 contained the crude asterosaponins (1.2 g), which were further purified by DCCC with n-butanol–acetone–water (45:15:75) (descending mode; the upper phase was the stationary phase) to give the unresolved saponins (0.42 g) in fractions 68—120 (6-ml fractions were collected). Fractionation was achieved by h.p.l.c. on a C_{18} μ-Bondapack column (30 cm × 8 mm i.d.) using methanol–water (45:55) as the eluant. The analytical h.p.l.c. chromatogram is shown in Figure 1. The total yield of each fraction was: peak A = a mixture of minor compounds, 35 mg; peak B = ophidianoside B (**1**), 54.5 mg; peak C = ophidianoside C (**2**), 103.8 mg; peak D = a mixture of minor compounds, 16.4 mg; peak E = thornasteroside A (**3**), 35.6 mg; peak F = ophidianoside F (**4**), 21.5 mg.

Physical Data of the Saponins.—The FAB (fast atom bombardment) mass spectral data are given in Table 1, the ^1H n.m.r. spectral data of the aglycone portions are given in Table 2, and the ^{13}C n.m.r. spectral data are in Tables 3 and 4. Optical rotations were measured for methanol solutions.

Ophidianoside B (**1**). $[\alpha]_D$ +2.8°, δ (sugars) 1.30 (d, 3 H, *J* 5.6 Hz, 5-Me of quinovose I), 1.322 (d, 3 H, *J* 6.0 Hz, 5-Me of fucose), 1.399 (d, 3 H, *J* 6.2 Hz, 5-Me of quinovose II), 4.17 (dd, *J* = 12.0 and 4.5 Hz, 5-H of xylose), 4.43 (d, 2 H, *J* 6.3 Hz, anomeric-H's), 4.53 (d, 1 H, *J* 7.5 Hz, anomeric-H), and 4.58 (d, 2 H, *J* 7.2 Hz, anomeric-H's); sugar analysis: quinovose (× 2), xylose, galactose, and fucose.

Ophidianoside C (**2**). $[\alpha]_D$ −2.9°; δ (sugars) 1.297 (d, 3 H, *J* 5.6 Hz, 5-Me of quinovose I), 1.318 (d, 3 H, *J* 6.1 Hz, 5-Me of fucose), 1.395 (d, 3 H, *J* 6.2 Hz, 5-Me of quinovose II), 3.95 (dd, 1 H, *J* 11.1 and 4.6 Hz, 5-H_{eq} of xylose II), 4.13 (dd, 1 H, *J* 11.5 and 4.6 Hz, 5-H_{eq} of branched xylose I), 4.43 (d, 2 H, *J* 6.3 Hz, anomeric-H's of xyloses), and 4.57 (d, 3 H, *J* 7.1 Hz, anomeric-H's); sugar analysis: quinovose (× 2), xylose (× 2), fucose.

Thornasteroside A (**3**) (*Ophidianoside* E). $[\alpha]_D$ +3.8°; δ (sugars) 1.297 (d, 3 H, *J* 5.6 Hz, 5-Me of quinovose I), 1.321 (d, 3 H, *J* 6.0 Hz, 5-Me of fucose), 1.399 (d, 3 H, *J* 6.2 Hz, 5-Me of quinovose II), 4.17 (dd, 1 H, *J* 12.0 and 4.5 Hz, 5-H_{eq} of xylose), 4.43 (d, 2 H, *J* 6.3 Hz, anomeric-H's), 4.53 (d, 1 H, *J* 7.5 Hz, anomeric-H), and 4.58 (d, 2 H, *J* 7.2 Hz, anomeric-H's); sugar analysis: quinovose (× 2), xylose, galactose, and fucose.

Ophidianoside F (**4**). $[\alpha]_D$ 0.4°; δ (sugars) 1.296 (d, 3 H, *J* 5.8 Hz, 5-Me of quinovose I), 1.317 (d, 3 H, *J* 6.2 Hz, 5-Me of fucose), 1.394 (d, 3 H, *J* 6.3 Hz, 5-Me of quinovose II), 3.95 (dd, 1 H, *J* 11.1 and 4.5 Hz, 5-H_{eq} of xylose II), 4.13 (dd, 1 H, *J* 11.5 and 4.6 Hz, 5-H_{eq} of branched xylose I), 4.43 (*J* 6.3 Hz, anomeric-H's of xyloses), and 4.57 (d, 3 H, *J* 7.1 Hz, anomeric-H's); sugar analysis: quinovose (× 2), xylose (× 2), and fucose.

Methanolysis of Saponins: Sugar Analysis.—Methanolysis of each glycoside (1—1.5 mg) and subsequent g.l.c. analysis of the sugar components were carried out as described previously.[19]

Methylation of Ophidianoside C (**2**) *followed by Methanolysis: Terminal Sugars.*—Ophidianoside C (**2**) (5 mg) in DMF (1 ml) was slowly added under nitrogen to a stirred mixture of NaH (60 mg) in dry DMF (0.6 ml) cooled in an ice-bath. The mixture was stirred for 15 min, and then MeI (0.3 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 analysed by t.l.c. on SiO_2 with chloroform–methanol (R_F 0.7) and was then methanolysed in anhydrous 2M-HCl–methanol (1 ml) at 80 °C in a stoppered reaction vial for 8 h. After cooling, the reaction mixture was neutralized with Ag_2CO_3, centrifuged, and the supernatant evaporated to dryness under reduced pressure. The residue was analysed by g.l.c. (SE-30 25-m column; 87 °C; hydrogen carrier, flow 10 ml min^{-1}) and the g.l.c. peaks co-eluted with those of methyl 2,3,4-tri-*O*-methylfucoside and methyl 2,3,4-tri-*O*-methylquinovoside.

Enzymatic Hydrolysis of Ophidianoside C (**2**): *the Prosapogenols* (**2a**) *and* (**2b**).—The saponin (20 mg) in citrate buffer (2.0 ml; pH 4.5) was incubated with a glycosidase mixture (10 mg) of *Charonia lampas* (Shikagaku Kogyo) at 37 °C. After reaction for 36 h, the t.l.c. analysis (SiO_2 with n-butanol–acetic acid–H_2O, 60:15:25) showed that the starting material had disappeared. The mixture was then extracted with n-butanol, evaporated, and the residue was fractionated by h.p.l.c. on a C-18 μ-bondapack column (30 cm × 8 mm i.d.) using methanol–water (1:1) as the eluant to give the prosapogenol (**2b**) (2 mg) and the prosapogenol (**2a**) (7 mg), eluted in that order.

Prosapogenol (**2a**), *m/z* (FAB) 967 (*M* + Na); δ (aglycone) 0.82 (s, 3 H, 18-H_3), 1.02 (s, 3 H, 19-H_3) 1.08 and 1.09 (each d, 6 H, *J* 6.8 and 6.9 Hz, 26- and 27-H_3), 1.37 (s, 3 H, 21-H_3), 2.70 (br s, 22-H_2), 2.74 (m, 25-H), 4.22 (m, 1 H, $w_{\frac{1}{2}}$ = 22 Hz, 3-H), 5.37 (br d, 1 H, *J* 5.5 Hz, 11-H); δ (sugars) 1.30 (d, 3 H, *J* 6.2 Hz, 5-Me of internal quinovose), 1.40 (d, 3 H, *J* 6.2 Hz, 5-Me of terminal quinovose), 3.92 (dd, 1 H, *J* 11.3 and 4.6 Hz, 5-H_{eq} of xylose), 4.42 (d, 1 H, *J* 7.1 Hz, anomeric-H of xylose), 4.54 and 4.56 (each d, 2 H, *J* 7.5 and 7.1 Hz, anomeric-H's); sugar analysis: quinovose (× 2), xylose.

Prosapogenol (**2b**), *m/z* (FAB) 1 099 (*M* + Na); δ (aglycone), identical with that of (**2a**); δ (sugars) 1.30 (d, 3 H, *J* 6.2 Hz, 5-Me of internal quinovose), 1.40 (d, 3 H, *J* 6.2 Hz, 5-Me of terminal quinovose), 3.92 (dd, 1 H, *J* 10.5 and 5.0 Hz, 5-H_{eq} of terminal xylose), 4.09 (dd, 1 H, *J* 11.5 and 4.5 Hz, 5-H_{eq} of internal xylose), 4.35 (d, 1 H, *J* 6.3 Hz, anomeric-H of terminal xylose), 4.42 (d, 1 H, *J* 5.5 Hz, anomeric-H of internal xylose), and 4.57 and 4.58 (each d, 2 H, *J* 7.5 and 7.5 Hz, anomeric-H's); sugar analysis: quinovose, xylose.

Extraction, Isolation, and Identification of Saponins from Hacelia attenuata.—Approximately 100 specimens of *H. attenuata* (820 g of fresh animals), collected in September 1983 in the Bay of Naples, were extracted with water as described before. The extracts were passed through a column of Amberlite XAD-2 (250 g) and the saponins were recovered by eluting with methanol. The crude material (0.7 g) was chromatographed on a column of Sephadex LH-60 (4 × 60 cm) using methanol–water (2:1) as the eluant. Fractions 45—48 contained the crude saponins (0.47 g), which were fractionated by DCCC with n-butanol–acetone–water (45:15:75) in the descending mode. 3-ml Fractions were collected. Fractions 91—109 mainly contained ophidianoside B (**1**) (46 mg), while fractions 110—138 mainly contained ophidianoside C (**2**) (36 mg). Purification was continued by h.p.l.c. on a C-18 μ-bondapack column under the same conditions as described before, to give pure ophidianoside B (**1**) (8.1 mg) and pure ophidianoside C (**2**) (10.1 mg). Identification was based on direct comparison (^{1}H n.m.r. and FAB mass spectra, t.l.c., and h.p.l.c.) with the samples obtained from *Ophidiaster ophidianus*. The ^{13}C n.m.r. spectrum of the relatively major compound was virtually identical with that of compound (**2**).

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