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STARFISH SAPONINS, PART 2.[1] STEROIDAL OLIGOGLYCOSIDES FROM THE STARFISH *COSMASTERIAS LURIDA*

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ABSTRACT.—Four new sulfated asterosaponins, designated as cosmasterosides A [**1**], B [**2**], C [**4**], and D [**5**], have been isolated from the Atlantic starfish *Cosmasterias lurida* along with large amounts of the pentaglycoside ophidianoside F [**3**] and the minor component forbeside H [**6**], previously isolated from *Ophidiaster ophidianus* and *Asterias forbesi*, respectively. Cosmasteroside A [**1**] contains the same oligosaccharide chain as ophidianoside F [**3**] and a (20*S*)-5α-cholesta-9(11),24-diene-3β,6α,20-triol-3β-sulfated aglycone. Cosmasterosides B [**2**], C [**4**], and D [**5**] are based on the known aglycone, thornasterol A, and differ in the number and type of monosaccharide components of the carbohydrate chain. Cosmasteroside B [**2**] contains a novel oligosaccharide chain that differs from that of ophidianoside F [**3**] by the replacement of the quinovose unit attached to C-6 of the steroidal aglycone by glucose. The tetraglycosides cosmasterosides C [**4**] and D [**5**] are structurally related to ophidianoside F [**3**] and cosmasteroside B [**2**], respectively, by the loss of a terminal fucose unit.

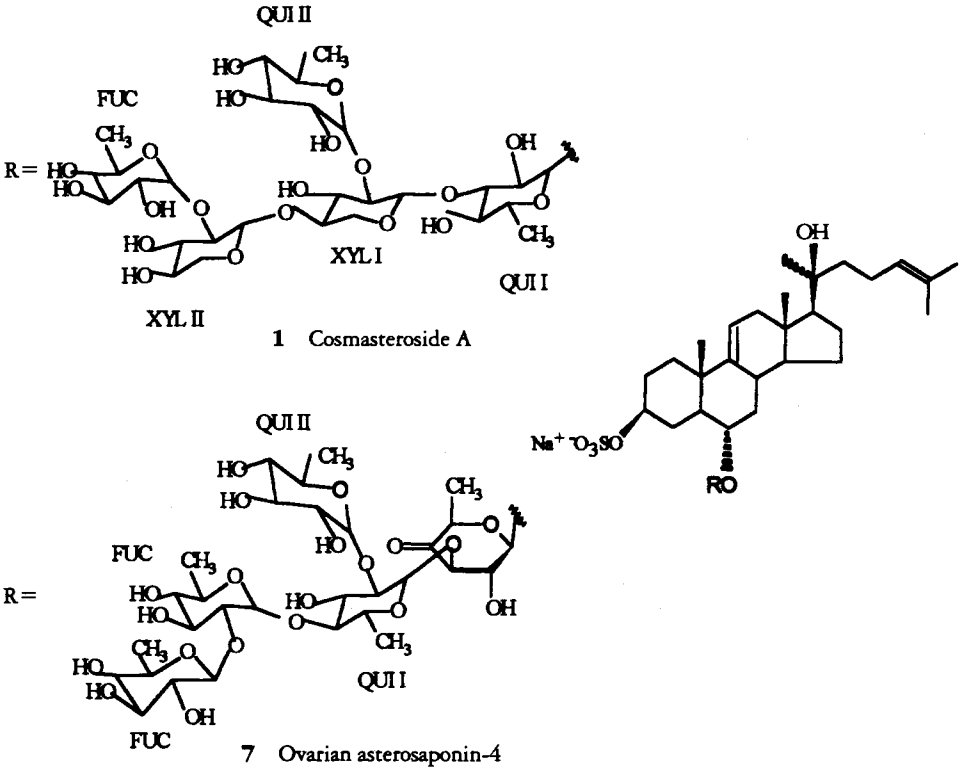
Steroidal glycosides are the predominant metabolites of starfishes and are responsible for their general toxicity. According to their chemical structures they have been subdivided into three main groups: the asterosaponins, which are sulfated glycosides (usually penta- and hexaglycosides) based on a $\Delta^{9(11)}$-3β,6α-dioxysteroidal aglycone with a sulfate group at C-3 and the oligosaccharide moiety at C-6; the cyclic steroidal glycosides, only found in two species of the genus *Echinaster*; and the glycosides of polyhydroxylated steroids, which consist of a polyhydroxylated steroidal aglycone linked to one, two, or three sugar units that can be found in both sulfated and non-sulfated forms (1,2). Recently a new class of saponins, in which the polyhydroxylated steroid has a 6α-*O*-phosphate grouping also linked to C-1′ of the β-glucosyl residue, was isolated from *Tremaster novaecaledoniae* (3).

Analysis of polar extracts of the starfish *Cosmasterias lurida* Philippi (family Asteridae, order Forcipulatida), a very common starfish collected in cold waters off the Patagonian coast of Argentina, has recently led us to report the structures of two novel polyhydroxylated xylosides sulfated at C-4′ of the xylosyl moiety (4). We now wish to report the isolation from the same source of four new asterosaponins, two pentaglycosides, cosmasterosides A [**1**] and B [**2**], and two tetraglycosides, cosmasterosides C [**4**] and D [**5**], together with two known saponins, the major pentaglycoside ophidianoside F [**3**], previously isolated from *Ophidiaster ophidianus* (5), *Linckia laevigata* (6), and *Thromidia catalai* (7) and small amounts of the triglycoside forbeside H [**6**], reported before from *Asterias forbesi* (8).

RESULTS AND DISCUSSION

The starfish *C. lurida* was homogenized and extracted with EtOH followed by

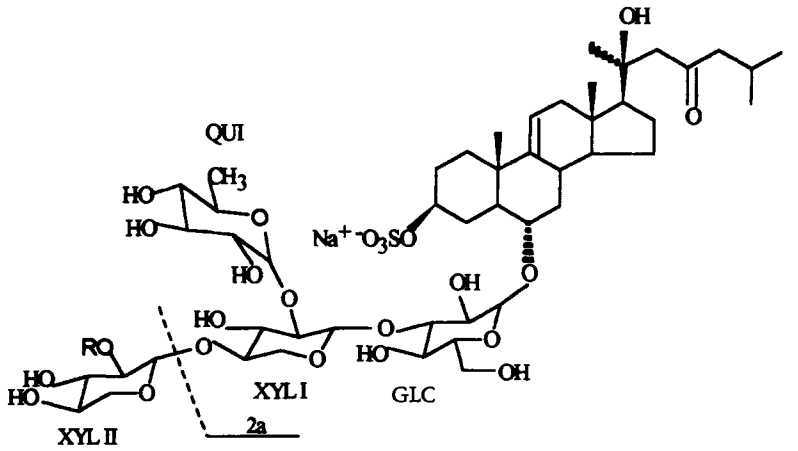
[1]For Part 1, see Maier *et al.* (4).

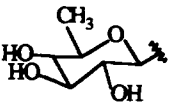


1 Cosmasteroside A

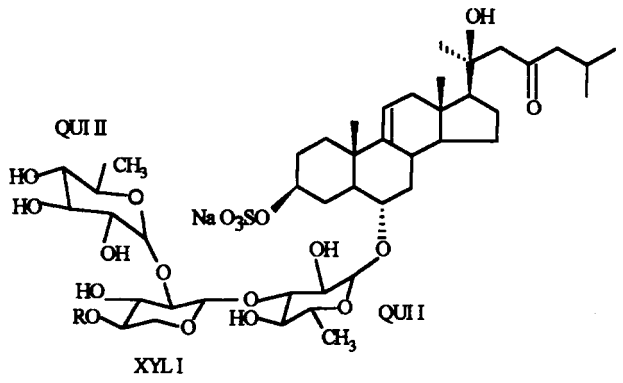
7 Ovarian asterosaponin-4

centrifugation and concentration. The aqueous extract thus obtained was passed through a column of Amberlite XAD-2 resin, which was washed with distilled H_2O and then eluted with MeOH in order to recover the saponins. Separation and isolation of the individual compounds from this residue was achieved by using the following successive

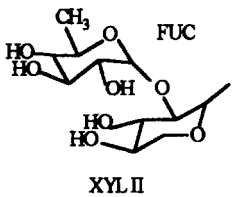


2 Cosmasteroside B R=  FUC

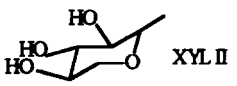
5 Cosmasteroside D R=H



3 Ophidianoside F R=



4 Cosmasteroside C R=



6 Forbeside H R=H

chromatographic steps: (a) chromatography of the MeOH eluate on a column of Sephadex LH-60 to separate the more polar sulfated "asterosaponins" from the less polar steroids, (b) dry-column flash chromatography on Davisil C_{18} reversed-phase, and (c) final purification by C_{18} reversed-phase hplc. The results of our analysis are shown in Table 1. Identification of the known ophidianoside F [**3**] and forbeside H [**6**] relied on interpretation of spectral data (^{1}H-nmr, ^{13}C-nmr, fabms).

An examination of the ^{1}H-nmr spectrum of the intact saponin **1** revealed signals due to aglycone protons (Table 2) identical with those observed in ovarian asterosaponin-4 [**7**], containing the (20*S*)-5α-cholesta-9(11),24-diene-3β,6α,20-triol-3β-sulfated aglycone, isolated from the starfish *Asterias amurensis* (9). The ^{13}C-nmr spectrum of **1** (cosmasteroside A) showed aglycone carbon signals (Table 3) that matched very closely those of ovarian asterosaponin-4, confirming that the oligosaccharide is attached at C-6 and the sulfate group is present at C-3 of the steroid, a general feature of the asterosaponins. In addition to the aglycone signals, the ^{1}H-nmr spectrum showed anomeric proton signals at δ 4.42 (d, J=6.3 Hz, xyl I and xyl II) and 4.57 (3H, d, J=7.1 Hz, qui I, qui II, and fuc), a doublet at δ 1.40 (J=6.3 Hz) due to the methyl group of D-quinovose and two doublets at δ 1.29–1.31 superimposed upon the signal of H-21, corresponding to the methyl groups of D-quinovose and D-fucose. The negative-ion fabms spectrum of **1** showed a molecular anion peak at *m/z* 1197 and fragment ions at *m/z* 1051 and 919, corresponding to the consecutive losses of fucosyl and xylosyl units. By anhydrous acid methanolysis, cosmasteroside A [**1**] liberated methyl fucoside, methyl xyloside, and methyl quinovoside in the ratio 1:2:2. This carbohydrate composition is identical with that of ophidianosides C and F, the major components of the

TABLE 1. Steroidal Glycosides Isolated from the Starfish *Cosmasterias lurida*.

Glycoside	Amount[a] (mg)	Reference	Rotation[b] $[\alpha]_D$
Ophidianoside F [3]	46.7	Riccio *et al.* (5–7)	+0.4° (MeOH)
Cosmasteroside A [1]	15.0		+5.3° (MeOH)
Cosmasteroside B [2]	19.2		+2.8° (MeOH)
Cosmasteroside C [4]	12.1		−7.0° (MeOH)
Cosmasteroside D [5]	2.8		+3.2° (H_2O)
Forbeside H [6]	4.3	Findlay *et al.* (8)	−4.7° (H_2O)

[a]From ca. 6 kg fresh animals.
[b]From solutions ranging from 0.17 to 0.69 g/100 ml.

saponin mixture of both *Ophidiaster ophidianus* and *Hacelia attenuata* (5). A detailed comparison of the ^{13}C-nmr data for the sugar moiety of compound **1** with those of ophidianoside F [**3**] (Table 4) showed that the saccharide chain is identical in both compounds. Thus, the novel cosmasteroside A can be defined as sodium (20*S*)-6α-*O*-{β-D-fucopyranosyl-(1→2)-β-D-xylopyranosyl-(1→4)-[β-D-quinovopyranosyl-(1→2)]-β-D-xylolopyranosyl-(1→3)-β-D-quinovopyranosyl}20-hydroxy-5α-cholesta-9(11),24-dien-3β-yl sulfate [**1**].

A second new saponin (cosmasteroside B, **2**) is a pentaglycoside based on the aglycone thornasterol A, that is, 3β,6α,20*S*-trihydroxy-5α-cholest-9(11)-en-23-one (B), as was established from the ^{1}H- and ^{13}C-nmr spectra (Tables 2 and 3) of the intact saponin. These spectra also allowed location of the sulfate group at C-3 and of the oligosaccharide chain at C-6 of the steroid. The fabms (negative-ion mode) spectrum of **2** showed a molecular anion peak at *m/z* 1229 and fragment ions at *m/z* 1129 ($[M]^+$ − side-chain) and at *m/z* 938 (1129−146), 851 (938−132), 573 (851−132−146) and 411 (573−162), corresponding to sequential losses of fucosyl, xylosyl, xylosyl and quinovosyl, and glucosyl units, respectively. The loss of 162 mass units (glucose) from the pentasaccharide fragments *m/z* 757 and 741, afforded peaks at *m/z* 595 and 579 respectively in the fabms (positive-ion mode) spectrum. These data indicated that glucose was the monosaccharide linked to the aglycone. Acid hydrolysis of compound **2** afforded methyl quinovoside, methyl fucoside, methyl xyloside, and methyl glucoside in the ratio 1:1:2:1. The ^{1}H-nmr spectrum of intact cosmasteroside B [**2**] showed four anomeric proton doublets (J=6.5 Hz), at δ 4.42 (xyl I and xyl II), δ 4.45 (fuc), δ 4.56 and δ 4.57 (qui and glc), two doublets at δ 1.31 (J=6.5 Hz), and δ 1.40 (J=6.5 Hz)

TABLE 2. Selected 500 MHz ^{1}H-Nmr (CD_3OD) Signals (δ_H) for the Aglycone Protons of Glycosides **1** (A) and **2–6** (B) (J values (Hz) are Shown in Parentheses).

Proton	A (Glycoside **1**)	B (Glycosides **2–6**)
3	4.22 m	4.22 m
11	5.38 br b	5.37 br b
18	0.82 s	0.81 s
19	1.02 s	1.02 s
21	1.31 s	1.37 s
22		2.62 AB q (15) 2.42 d (7.5)
24	5.13 t (6.5)	
26	1.64 s	0.93 d (7)
27	1.70 s	0.94 d (7)

TABLE 3. ^{13}C-Nmr Data (62.9 MHz, Pyridine-d_5) for the Steroidal Aglycones of Compounds **1** (A) and **2–6** (B) and Ovarian Asterosaponin-4 [7] (9) as a Reference Compound for Aglycone A.

Carbon	Steroidal Aglycones		
	A	B	Ref. Comp.
1	35.9	35.9	36.2
2	29.3	29.3	29.6
3	77.5	77.6	77.9
4	30.6	30.7	30.9
5	49.2	49.5	49.6
6	80.3	80.3	80.7
7	41.4	41.5	41.8
8	35.2	35.3	35.6
9	145.3	145.4	145.8
10	38.2	38.2	38.5
11	116.7	116.6	116.9
12	42.4	42.4	42.8
13	41.4	41.5	41.8
14	53.9	53.9	54.3
15	23.4	23.2	23.6
16	25.1	25.1	25.4
17	58.6	59.5	59.1
18	13.5	13.5	13.6
19	19.1	19.2	19.4
20	73.6	73.7	74.0
21	25.6	26.9	25.8
22	44.5	54.8	44.7
23	22.9	211.7	23.2
24	125.6	53.9	125.9
25	130.6	24.3	130.8
26	26.1	22.4	26.4
27	17.8	22.5	17.7

for the methyl protons of the fucosyl and quinovosyl units, respectively, and two double doublets at δ 4.13 (12 and 4 Hz) for 5-H_e of xylose I and at δ 3.94 (12 and 4 Hz) for 5-H_e of xylose II.

The interglycosidic linkages were deduced from ^{13}C-nmr data (Table 4) and comparison with those corresponding to cosmasteroside A [**1**] and ophidianoside F [**3**]. The oligosaccharide chain in these saponins differs only in the quinovose unit attached to C-6 of the steroid, which is replaced by a D-glucose unit (δ C-6′, 62.3 ppm) in cosmasteroside B [**2**]. Also, comparison with ^{13}C-nmr data of saponins containing D-glucose attached to C-6 of the steroid, such as luidiaglycosides C and D (9), i.e., fuc-(1→2)-qui-(1→4)-(qui$^{1-2}$>)qui$^{1-3}$>glc$^{1-6}$> aglycone, and marthasteroside B (10), i.e., fuc-(1→2)-qui-(1→4)-(qui$^{1-2}$>)qui$^{1-3}$>glc$^{1-6}$> aglycone, allowed us to establish that C-3′ of glucose is bound to the anomeric carbon of D-xylose I. On enzymatic hydrolysis with *Charonia lampas* glycosidase mixture, cosmasteroside B [**2**] gave, after 3 days, a prosapogenol sulfate, the trisaccharide **2a**. The fabms (positive-ion mode) spectrum showed a molecular ion peak at *m/z* 998 ([M]$^+$+H+Na) and fragments at *m/z* 877 ([M]$^+$+H−side-chain), 599 [877−146−132] corresponding to the sequential losses of quinovosyl and xylosyl units, and 463 [trisaccharide ion+Na]. Thus, cosmasteroside B can be defined as sodium (20*S*)-6α-*O*-{β-D-fucopyranosyl-(1→2)-β-D-xylopyranosyl-(1→4)-[β-D-quinovopyranosyl-(1→2)]-β-D-xylopyranosyl-(1→3)-β-D-glucopyranosyl}20-hydroxy-5α-cholest-9(11)-en-23-one-3β-yl sulfate [**2**].

TABLE 4. Carbon Chemical Shifts (62.9 MHz, Pyridine-*d*$_5$) of Saccharide Chains of Asterosaponins.

Carbon Number	Compound			
	1 and **3**	**2**	**4**	**6**
	Qui I	Glc	Qui I	Qui I
1	105.0	105.2	105.1	105.1
2	74.1	75.0	74.1	74.1
3	90.1	90.9	90.4	90.7
4	74.4	69.5	74.4	74.4
5	71.9	76.2	72.0	72.0
6	17.8	62.3	17.9	17.9
	Xyl I	Xyl I	Xyl I	Xyl
1	104.3	104.6	104.3	104.8
2	82.1	82.2	82.1	82.9
3	75.2	75.5	75.2	77.5
4	78.1	78.2	78.0	70.4
5	64.3	64.3	64.4	67.0
	Qui II	Qui	Qui II	Qui II
1	105.0	105.0	104.8	105.0
2	75.5	75.5	75.5	75.5
3	76.7	76.7	76.7	76.8
4	76.1	76.2	76.1	76.2
5	73.6	73.6	73.5	73.6
6	18.4	17.9	18.4	18.5
	Xyl II	Xyl II	Xyl II	
1	101.9	102.1	103.8	
2	84.2	84.4	74.0	
3	77.3	77.4	76.7	
4	70.3	70.4	70.8	
5	66.7	66.8	67.3	
	Fuc	Fuc		
1	106.8	106.9		
2	71.9	72.0		
3	74.8	75.0		
4	73.6	73.7		
5	72.5	72.5		
6	17.1	17.2		

A third new tetraglycoside (cosmasteroside C, **4**) is structurally related to ophidianoside F [**3**] by the loss of the terminal fucose. Elucidation of the tetrasaccharide moiety was carried out as follows. Acid methanolysis of compound **4** afforded methyl xyloside and methyl quinovoside in the ratio 1:1. Besides the usual thornasterol A signals, the ^{1}H-nmr spectrum of the intact saponin showed the presence of four anomeric proton doublets at δ 4.35 (1H), δ 4.42 (1H), δ 4.57 (1H) and δ 4.59 (1H), doublets with *J* values ranging from 6.6 to 7.3 Hz, and two doublets at δ 1.30 (5.7 Hz) and δ 1.39 (6.2 Hz) due to the methyl groups of D-quinovose I and II, respectively. In comparison, ophidianoside F presented an additional signal corresponding to the methyl group of D-fucose at δ 1.32 (6.2 Hz) and doublets at δ 4.42 (2H), and δ 4.57 (3H) for the anomeric protons.

The interglycosidic linkages were deduced from ^{13}C-nmr data (Table 4) and comparison with those corresponding to ophidianoside F [**3**] and methyl β-D-xylopyranoside (12). The negative-ion fabms spectrum of **4** showed a molecular anion peak at *m/z* 1067 and fragments ions at *m/z* 967 ($[M]^+$−side-chain) and *m/z* 835 (967−132) corresponding to the loss of a xylosyl unit. Enzymatic hydrolysis of ophidianoside F [**3**] with *Charonia lampas* glycosidase mixture gave cosmasteroside C [**4**]

after 3 days, as determined by spectral data (^{1}H-nmr, fabms). Thus, cosmasteroside C can be defined as sodium (20*S*)-6α-*O*-{β-D-xylopyranosyl-(1→4)-[β-D-quinovopyranosyl-(1→2)]-β-D-xylopyranosyl-(1→3)-β-D-quinovopyranosyl}20-hydroxy-5α-cholest-9(11)-en-23-one-3β-yl sulfate [**4**].

A fourth tetraglycoside (cosmasteroside D, **5**) is structurally related to cosmasteroside B [**2**] by the loss of the terminal fucose unit. The negative-ion fabms spectrum of **5** showed a molecular anion peak at *m/z* 1083 and fragments at *m/z* 951 (1083−132) and 673 (951−132−146), corresponding to the sequential losses of xylosyl as well as xylosyl and quinovosyl units, respectively. On acid hydrolysis, compound **5** liberated methyl quinovoside, methyl xyloside, and methyl glucoside in the ratio 1:2:1. The ^{1}H-nmr spectrum of the intact cosmasteroside D revealed signals due to thornasterol A aglycone (B) (Table 2), anomeric proton signals at δ 4.59 (1H), δ 4.57 (1H), δ 4.42 (1H) and δ 4.35 (1H), doublets with *J* values ranging from 7.0 to 7.5 Hz, as well as a doublet at δ 1.39 (5.7 Hz) due to the methyl group of D-quinovose and two double doublets at δ 4.08 (12 and 4 Hz) for 5-H$_e$ of xylose I and at δ 3.91 (12 and 4 Hz) for 5-H$_e$ of xylose II.

Cosmasterosides C [**4**] and D [**5**] are two of the very few examples of tetraglycosides isolated from starfishes, such as forbeside F from *Asterias forbesi* (8), myxodermoside A from *Myxoderma platyachanthum* (13), and santiagoside from *Nesmislaster georgianus* (14).

EXPERIMENTAL

GENERAL EXPERIMENTAL PROCEDURES.—^{1}H- and ^{13}C-nmr spectra were recorded on Bruker ACE-200 and Bruker AMX 500 instruments. Optical rotations were measured on a Perkin Elmer 141 polarimeter. Fabms were obtained on a VG-ZAB mass spectrometer equipped with FAB source [in glycerol matrix; Xe atoms of 2-6 kV]. Glc was performed on a Carlo Erba Fractovap 2900 instrument with a capillary column. Hplc was performed on a Waters Model 6000 A pump equipped with a U6K injector and a differential refractometer model 401 using a C$_{18}$ μ-Bondapack column (30 cm×7.8 mm i.d.) and a C$_{18}$ Bondclone 10 column (30 cm×7.8 mm i.d.); flow rate 2 ml/min. Tlc was performed on precoated Si gel F254 and C$_{18}$ reversed-phase plates.

EXTRACTION AND ISOLATION.—Specimens of *C. lurida* (6.0 kg) were collected in 1989 off the Golfo Nuevo near Puerto Madryn on the Argentine Patagonian coast and were identified by Dr. Alejandro Tablado of the Museo de Ciencias Naturales "Dr. Bernardino Rivadavia," where a voucher specimen (No. 31231) is preserved. The animals, frozen prior to storage, were homogenized in EtOH (6 liters) and centrifuged. The EtOH was evaporated and the aqueous extract thus obtained was purified through an Amberlite XAD-2 column (1 kg), eluted with distilled H$_2$O (until a negative reaction for chloride was observed), followed by MeOH. The MeOH eluate was evaporated to give a glassy material (8.1 g) that was chromatographed on a Sephadex LH 60 column (80 cm×4 cm i.d.; 100 g) with MeOH-H$_2$O (2:1) as eluent. Fractions (10 ml) were collected and analyzed by tlc on SiO$_2$ in *n*-BuOH-HOAc-H$_2$O (4:5:1) (upper layer) and detected by spraying with H$_2$SO$_4$. Fractions 25–45 and 46–65 contained the crude "asterosaponins." Each fraction was subjected to dry-column flash chromatography on Davisil C$_{18}$ (35–75 μ) using MeOH-H$_2$O (50:50 and 65:35), after which 50 ml fractions were collected and analyzed by C$_{18}$ reversed-phase tlc [MeOH-H$_2$O (65:35)]. Final isolation was accomplished by hplc on a C$_{18}$ μ-Bondapack column (30 cm×7.8 mm i.d.) with MeOH-H$_2$O (50:50) as eluent to give the pure compounds **1–6**.

METHANOLYSIS OF GLYCOSIDES AND SUGAR ANALYSIS.—A solution of each saponin (0.5–1.0 mg) in anhydrous 2M HCl in MeOH (0.5 ml) was heated at 80° in a stoppered reaction vial for 8 h. On cooling, each reaction mixture was neutralized with Ag$_2$CO$_3$, centrifuged, and the supernatant was evaporated to dryness under N$_2$ and then trimethylsilylated with TRISIL-Z® (5 μl) (Pierce Chemical Co.) for 15 min at room temperature. Glc analysis of the silylated sugar compounds was carried out at 158° on a 25-m OV-101 capillary column (He carrier, flow 10 ml/h). The identification was based on co-chromatography with standards.

ENZYMATIC HYDROLYSIS OF OPHIDIANOSIDE F [**3**] AND COSMASTEROSIDE B [**2**].—Saponins **3** and **2** (5.0 mg) in 1 ml of citrate buffer (pH 4.5) were incubated with a glycosidase mixture (6 mg) of *Charonia lampas* (Shikagaku Kogyo) at 37°. After reaction for 36 h, the tlc analysis [SiO$_2$ with *n*-BuOH-HOAc-H$_2$O (4:5:1) (upper layer)] showed that the starting material had disappeared. The reaction mixture was passed through a C$_{18}$ Sep-pak cartridge, washed with H$_2$O, and eluted with MeOH. The mixture was purified by hplc (C$_{18}$

Bondclone 10 column (30 cm×7.8 mm)) using MeOH-H_2O (50:50) as eluent to give cosmasteroside C [**4**] (2 mg) and the trisaccharide **2a** (1.8 mg).

Cosmasteroside C [**4**].—Fabms in the text. ^{1}H-Nmr data identical to those obtained for authentic cosmasteroside C isolated from *C. lurida*.

Prosapogenol **2a**.—Fabms in the text. ^{1}H-nmr data: δ_H (CD_3OD) (aglycone) identical to those reported for the intact cosmasteroside D [**2**]; δ_H (sugars) 1.40 (3H, d, J=6.5 Hz, H_3-5 quinovose), 4.42, 4.56, and 4.57 (each 1H, d, J=6.5 Hz, anomeric protons).

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