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Full Papers

Starfish Saponins. 55.[1] Isolation, Structure Elucidation, and Biological Activity of the Steroid Oligoglycosides from an Antarctic Starfish of the Family *Asteriidae*[†]

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This paper reports an analysis of the chemical constituents from an Antarctic starfish of the family *Asteriidae*. Different steroid glycoside types are represented among its constituents, including the five hexaglycoside steroidal sulfates ("asterosaponins") asteriidosides A−E (**1**−**5**), differing in their oligosaccharide chain, and the two nonsulfated diglycosides asteriidoside F (**6**) and G (**7**), in which **6** is the 26-methyl analogue of **7**. Also present are the two sulfated diglycosides asteriidoside H (**8**) and I (**9**) and one sulfated monoglycoside asteriidoside L (**10**). Eight of the compounds were tested against human nonsmall-cell lung carcinoma cells (NSCLC-L16) and found to be moderately cytotoxic.

Steroid oligoglycosides are the predominant metabolites of starfishes and have been reported to exhibit cytotoxic, hemolytic, antiviral, antifungal, and antimicrobial activities.[2] It is known[3] that there are three types of steroid glycosides in the Asteroidea. The asterosaponins, which include a $\Delta^{9(11)}$-3β,6α-dioxygenated steroidal moiety, from five to six sugar units attached at C-6, and a sulfate at C-3 compose the first group, and the steroidal cyclic glycosides found in the genus *Echinaster*[4] form the second. The last group consists of steroid monoglycosides and diglycosides in both sulfated and nonsulfated forms, which show great structural variability and usually occur as complex mixtures, probably reflecting the complexity of sterols arising through the food chain.

Continuing our search for new biologically active metabolites from echinoderms, we have been working on the polar extracts of Antarctic starfish of the family *Asteriidae* and have isolated several novel steroidal glycosides. In the preceding paper[5] we described three novel sulfated steroids named asterasterols A−C, and we now report the isolation and structure elucidation of the remaining 10 new compounds. Five are asterosaponins (**1**−**5**), the highest molecular weight components, made up of a hexasaccharide chain linked at C-6 of a $\Delta^{9(11)}$-3β,6α-dihydroxysteroid-3-sulfated aglycon. The second group is made up of four new steroidal diglycosides (**6**−**9**) and a single sulfated monoglycoside (**10**). The two major glycosides **6** and **7** are characterized by a common 3β,6α,8,15β,16β,26-hexahydroxysteroid nucleus, while **8** and **9** differ in the hydroxylation of the steroid aglycon and possess the same saccharide moieties, linked at C-3 and C-24.

Results and Discussion

Specimens of the *Asteriidae* family (order Forcipulata) were collected at depth of 50−100 m in January 1990, from Thethis Bay, Antarctica, during the Italian−Antarctic

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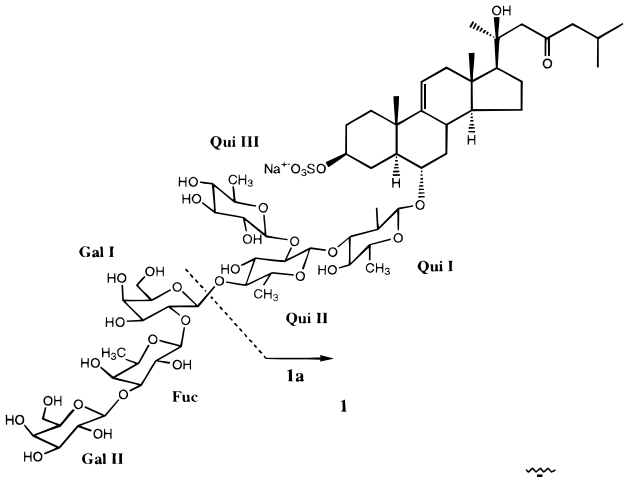

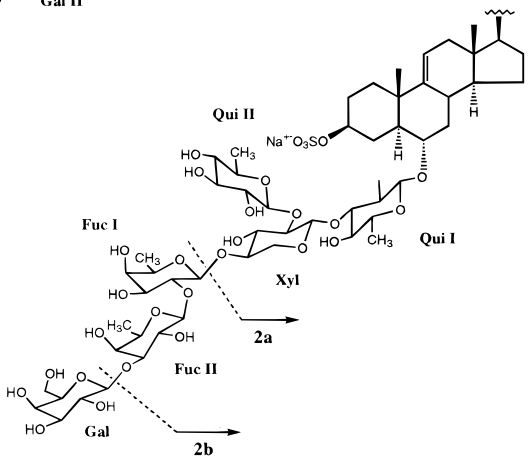

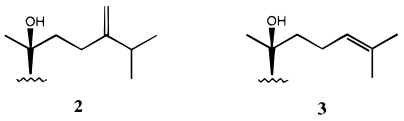

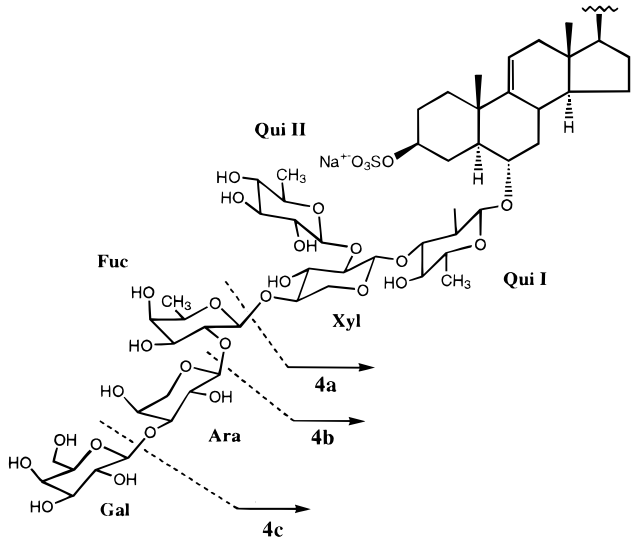

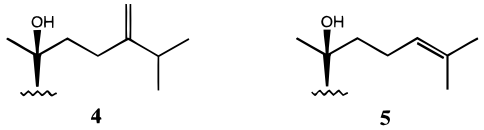

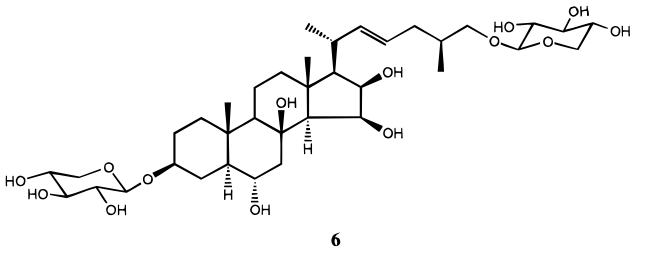

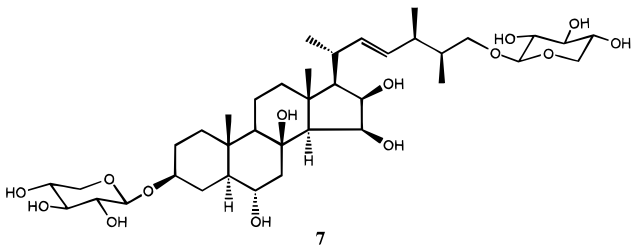

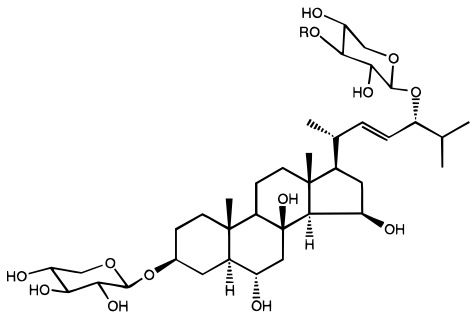

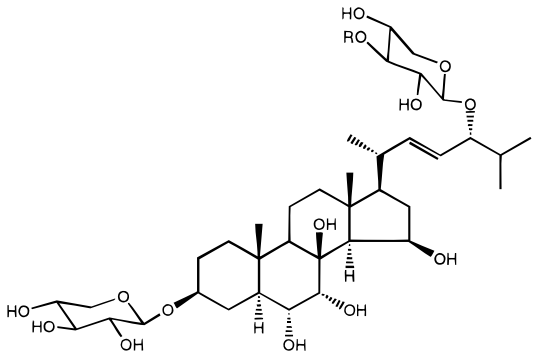

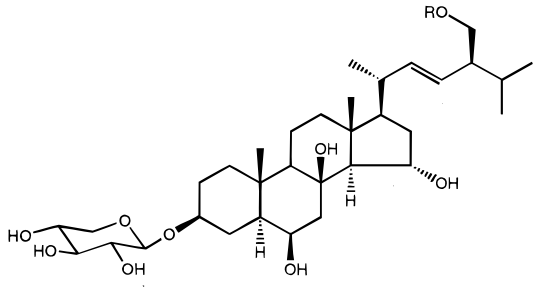

expedition. The freeze-dried material was extracted with H_2O and then with Me_2CO. The asterosaponins and sulfated and nonsulfated glycosides were recovered from the aqueous extract by passing it through a column of Amberlite XAD-2 resin, which was washed with distilled H_2O and then eluted with MeOH. The Me_2CO extract was partitioned between H_2O and Et_2O, and the aqueous fraction was then extracted with *n*-BuOH. The MeOH

eluate from the Amberlite column and the *n*-BuOH-soluble material from the Me_2CO extract were combined and fractionated by sequential application of gel permeation on Sephadex LH-60, droplet counter current chromatography (DCCC), and HPLC, to yield five sulfated steroidal pentasaccharides or hexasaccharides designated asteriidosides A−E (**1**−**5**), three steroidal diglycosides designated asteriidosides F−H (**6**−**9**), the sulfated diglycoside asteriidoside I (**9**), and the sulfated monoglycoside L (**10**).

On acid methanolysis, asteriidoside A (**1**) liberated methyl quinovoside, methyl fucoside, and methyl galactoside in the ratio 3:1:2. Its FABMS (negative ion mode) gave a molecular anion peak, $[MSO_3]^-$, at *m*/*z* 1419, with a major fragment at *m*/*z* 1319 corresponding to the C(20)−C(22) cleavage of the steroid side chain with 1H transfer (retro−aldol cleavage). Minor fragments are present at *m*/*z* 1257 $[MSO_3 - 162]^-$ and *m*/*z* 1111 $[1257 - 146]^-$, corresponding to the sequential losses of hexose and deoxyhexose units.

The ^1H and ^{13}C NMR spectra of the intact saponin revealed signals due to the common thornasterol A-3β-sulfated aglycon and also confirmed the attachment of the oligosaccharide chain at the C-6 position[6] (see Experimental Section). ^1H and ^{13}C NMR spectra also indicated that all the sugar residues are in their pyranose forms, and the glycosidic linkages are β-oriented. The common D-configuration for xylose, quinovose, galactose, and fucose and the L-configuration for arabinose were assigned by analogy with the sugar configurations of previous saponins. Permethylation and methanolysis of the methylated material gave permethylated methyl quinovoside and methyl galactoside. On enzymatic hydrolysis with *Charonia lampas* glycosidase mixture, asteriidoside A (**1**) gave only the trisaccharide **1a**, which gave a negative ion FABMS molecular ion at *m*/*z* 949. Acid methanolysis of this trisaccharide provided methyl quinovoside as the sole sugar component, and ^1H NMR spectroscopy showed shifts superimposable on those of 6-*O*-β-D-Qui-(1→2)-β-D-Qui-(1→3)-β-D-Qui of 3-*O*-sulfothornasterol A, a prosapogenol obtained from laevigatoside[7] and later from pectinioside G.[8] Having established quinovose as the terminal sugar in the trisaccharide portion, the galactose can be located as terminal in accordance with the above FABMS and permethylation data. The ^{13}C NMR data (Table 1) established the linkage to be T Gal-(1→3)-Fuc. The C-3 glycosidic linkage is evidenced by the downfield shift exhibited by C-3 of fucose (δ_C 84.1; δ_C 75.0 in β-D-methylfucose)[9] and the relatively high field of C-2 and C-4.

The sequence T (terminal) Gal-(1→3)-Fuc-(1→2)-Gal met the structure requirements of the terminal trisaccharide of versicoside A.[10] A detailed comparison of ^{13}C NMR data for **1** and versicoside A[11] indicated that all the signals in the two spectra were virtually superimposable. The structure of asteriidoside A (**1**) can thus be defined as sodium (20*S*)-6α-*O*-{β-D-galactopyranosyl-(1→3)-β-D-fucopyranosyl-(1→2)-β-D-galactopyranosyl-(1→4)-[β-D-quinovopyranosyl-(1→2)]-β-D-quinovopyranosyl-(1→3)-β-D-quinovopyranosyl}-20-hydroxy-23-oxo-5α-cholest-9(11)-en-3β-yl sulfate.

An examination of the ^1H and ^{13}C NMR spectra of the intact saponin asteriidoside B (**2**) revealed signals due to aglycon protons (see Experimental Section and Table 2) identical with those observed in asteroside D, containing the (20*S*)-5α-cholesta-9(11),24(28)-diene-3β,6α,20-triol-3β-sulfated aglycon, isolated from the starfish *Asterias amurensis*.[6] The FABMS (negative ion mode) spectrum of **2** showed a molecular anion peak at *m*/*z* 1387 $[MSO_3]^-$ and fragment ions at *m*/*z* 1225 $[MSO_3 - 162]^-$ and *m*/*z* 1079 $[1225 - 146]^-$, corresponding to sequential losses of hexose and deoxyhexose units. ^1H NMR showed six anomeric proton signals [δ 4.42 (1H), 4.48 (1H), 4.51 (2H), 4.56 (1H), 4.57 (1H)], each a doublet with *J* ranging from 7.0 to 7.5 Hz. A double doublet at 4.14 (J = 11.5, 4.5 Hz) due to 5-Heq of a xylose unit indicated the presence of substitution at C-4 of a xylopyranose. In the ^{13}C NMR spectrum (Table 1) the carbon chemical shifts assigned to the sequence aglycon-Qui-(Qui)-Xyl matched very closely those of thornasteroside A.[12]

Acid methanolysis afforded methyl fucoside, methyl xyloside, methyl quinovoside, and methyl galactoside in the ratio 2:1:2:1. This carbohydrate composition is identical to that of marthasteroside A_1;[13] however, permethylation followed by methanolysis of the methylated material gave permethylated methyl quinovoside and methyl galactoside, as in the case of saponin **1**. The galactose unit can thus be located as the terminal sugar of the hexasaccharide moiety. On enzymatic hydrolysis with *C. lampas* glycosidase mixture, asteriidoside B gave two prosapogenol sulfates, the tetrasaccharide **2b** and the trisaccharide **2a** (FABMS *m*/*z* 933, negative ion mode).

Table 1. ^{13}C NMR Shifts of Sugar Carbon Atoms of the Asterosaponins **1**, **2**, and **4**[a]

carbon	**1**	**2**[b]	**4**[c]
	Qui I	**Qui I**	**Qui I**
1	104.2	105.4	105.6
2	74.0	74.4	74.4
3	90.1	89.7	88.9
4	74.5	74.9	74.8
5	71.9	72.5	72.1
6	18.2	18.1	18.2
	Qui II	**Xyl**	**Xyl**
1	103.4	104.1	104.5
2	83.5	82.6	82.6
3	73.9	75.4	75.8
4	84.9	78.3	77.1
5	71.7	64.3	63.9
6	18.2		
	Qui III	**Qui II**	**Qui II**
1	106.0	104.7	105.5
2	75.1	75.7	75.1
3	76.9	77.0	77.0
4	75.7	76.2	76.2
5	74.7	73.8	73.9
6	18.5	18.5	18.5
	Gal I	**Fuc I**	**Fuc**
1	102.6	101.7	101.3
2	82.1	82.7	80.7
3	76.2	73.9	74.3
4	69.4	71.5	71.7
5	76.8	71.7	72.0
6	62.1	16.8	16.8
	Fuc	**Fuc II**	**Ara**
1	105.8	106.3	103.7
2	71.3	71.5	71.5
3	84.1	84.1	83.1
4	71.8	72.0	67.7
5	72.4	72.0	65.6
6	16.8	16.9	
	Gal II	**Gal**	**Gal**
1	106.4	106.4	106.3
2	72.8	73.0	72.8
3	74.9	75.0	75.0
4	69.9	70.0	70.0
5	76.9	77.1	77.1
6	62.0	62.2	62.1

[a] Spectra were run in [$^2H_5$]pyridine at room temperature + one drop of H_2O. [b] The data reported for **2** are identical with those of **3**. [c] The data reported for **4** are identical with those of **5**.

Table 2. ^{1}H and ^{13}C NMR of the Side Chains of Compounds **1**, **2**[a], **3**[b]

	1		**2**		**3**	
position	δ_H	δ_C	δ_H	δ_C	δ_H	δ_C
20		74.1		74.2		73.8
21	1.37 s	26.9	1.30 s	26.0	1.31 s	25.3
22	2.64 ABq (15.0)	55.3		43.3		44.1
23		212.8		29.8		22.6
24	2.42 d (7.5)	54.0		156.9	5.13 t (6.5)	125.3
25		24.5		34.2		130.2
26	0.93 d (6.5)	22.5	1.07 d (7.0)	22.0	1.64 s	17.1
27	0.94 d (6.5)	22.7	1.07 d (7.0)	22.0	1.70 s	26.0
28			4.72 br s	106.3		
			4.78 br s			

[a] The data reported for compound **2** are identical with those of compound **4**. [b] The data reported for compound **3** are identical with those of compound **5**.

The tetrasaccharide **2b** showed a molecular anion peak at *m/z* 1079 in the FABMS (negative ion mode) and a fragment at *m/z* 933, corresponding to the loss of a fucose unit. These data confirmed the structure of the trisaccharide moiety on the prosapogenol and also indicated that, in compound **2**, the remaining sugar chain, made up by T Gal-Fuc-Fuc, is attached at C-4 of the xylosyl residue as in many other asterosaponins.[14]

The ^{13}C NMR data of the tetrasaccharide portion of the intact asteriidoside B matched very closely those of the tetrasaccharide of regularoside B,[14] a pentaglycoside isolated from *Halityle regularis*. The ^{13}C NMR data of the remaining sugars are virtually identical with the data reported for **1**, confirming the linkages T Gal-(1→3)-Fuc-(1→2)-Fuc. Thus, we propose for asteriidoside B (**2**) the structure sodium (20*S*)-6α-*O*-{*β*-D-galactopyranosyl-(1→3)-*β*-D-fucopyranosyl-(1→2)-*β*-D-fucopyranosyl-(1→4)-[*β*-D-quinovopyranosyl-(1→2)]-*β*-D-xylopyranosyl-(1→3)-*β*-D-quinovopyranosyl}-20-hydroxy-5α-cholest-9(11),24(28)-dien-3*β*-yl sulfate.

Asteriidoside C (**3**) is related to asteriidoside B (**2**), and differences are only found in the steroidal side chain. The ^{1}H NMR spectrum of the intact saponin revealed signals due to an aglycon proton identical with those observed in ovarian asterosaponin-4, an asterosaponin containing the (20*S*)-5α-cholest-9(11)-24-diene-3*β*,6α,20-triol-3*β* sulfated aglycon, derived from the starfish *A. amurensis*[6] (see Experimental Section and Table 2). Particularly diagnostic for the 24(25) double bond are the signals at *δ* 5.13 (1H, t, $J = 6.5$ Hz) for the 24 hydrogen and those at *δ* 1.65 and 1.70 (each 3H, s) for the C-26 and C-27 methyl protons. The ^{1}H and ^{13}C NMR spectra, and the results of the sugar analysis of compound **3** agreed in all aspects with the data reported for asteriidoside B (**2**). The FABMS (negative ion mode) gave a molecular anion peak at *m/z* 1373 $[MSO_3]^-$ accompanied by a fragment at *m/z* 1211 (loss of 162 mass units from *m/z* 1373) and *m/z* 1065 and *m/z* 919, corresponding to the loss of two fucose units also observed in the spectrum of asteriidoside B (**2**).

The new asteriidoside D (**4**) is a hexaglycoside, and the ^{1}H and ^{13}C NMR data of the aglycon and trisaccharide portion are superimposable on those reported for asteriidoside B (**2**) (Tables 1 and 2). On acid methanolysis, **4** liberated methyl arabinoside, methyl fucoside, methyl xyloside, methyl quinovoside, and methyl galactoside in the ratio 1:1:1:2:1. The ^{1}H NMR spectrum showed signals for anomeric protons at *δ* 4.42 (1H), 4.49 (1H), 4.51 (1H), 4.56 (1H), 4.57 (1H), and 4.59 (1H), each a doublet, and the ^{13}C NMR spectrum showed signals for six anomeric carbon signals at *δ* 101.3, 103.7, 104.5, 105.5, 105.6, and 106.3. In the FABMS (negative ion mode) intact asteriidoside D (**4**) showed a molecular anion at *m/z* 1373 $[MSO_3]^-$ and two major fragments at *m/z* 1211 $[MSO_3 - 162]^-$ and *m/z* 1079 $[1211 - 132]^-$, corresponding to the sequential losses of hexose and arabinosyl (or xylosyl) residue. Permethylation followed by methanolysis of the methylated material gave permethylated methyl quinovoside and permethylated methyl galactoside, implying that the sugar moiety of **4** has one branch. On enzymatic hydrolysis with a *C. lampas* glycosidase mixture, asteriidoside D (**4**) gave the trisaccharide **4a**, the tetrasaccharide **4b**, and a small amount of the pentasaccharide **4c**. The trisaccharide contained quinovose and xylose in a ratio of 2:1 and showed FABMS (negative ion mode) *m/z* 933 as in the asteriidoside B (**2**).

The tetrasaccharide **4b** showed a molecular anion at *m/z* 1079 in the FABMS (negative ion mode), identical to tetrasaccharide **2b**, with a fragment at *m/z* 933. The pentasaccharide **4c** showed a molecular anion at *m/z* 1211 in the FABMS (negative ion mode) and two fragments at *m/z* 1079 and 933 due to sequential losses of arabinose and fucose units.

The ^{13}C NMR data were assigned to a terminal galactose in the identical manner as asteriidoside A (**1**) and B (**2**). The presence of a (1→3) linkage T Gal-arabinose was determined by ^{13}C NMR spectroscopy by comparison with the spectra of the appropriate methyl arabinoside[16] and assignments reported for similar glycosides.[17,18] The appearance in the ^{13}C NMR spectrum of **4** of a C-3′ carbon signal at low field (83.1 ppm) can be explained in terms of the substitution effect at C-3′ position of the arabinopyranose moiety; in addition the C-2′ and C-4′ of arabinose were observed shifted to relatively high field (71.5 and 67.7 ppm). The signal at relatively high field for the anomeric carbon (δ_C 101.3 ppm) is consistent with a substitution of the fucose residue at C-2 as reported in forbeside C, previously isolated from *Asterias forbesi*[15] and for asteriidoside B (**2**). Thus, the novel asterosaponin asteriidoside D (**4**) can be defined as sodium (20*S*)-6α-*O*-{*β*-D-galactopyranosyl-(1→3)-α-L-arabinopyranosyl-(1→2)-*β*-D-fucopyranosyl-(1→4)-[*β*-D-quinovopyranosyl-(1→2)]-*β*-D-xylopyranosyl-(1→3)-*β*-D-quinovopyranosyl}-20-hydroxy-5α-cholest-9(11),24(28)-dien-3*β*-yl sulfate.

Asteriidoside E (**5**) proved to be related to asteriidoside D (**4**). It gave molecular anion peaks at *m/z* 1359 $[MSO_3]^-$ and key fragments at *m/z* 1197, *m/z* 1065, and *m/z* 919 due to the sequential losses of galactose, arabinose, and fucose units, respectively. The ^{1}H NMR signals for the aglycon protons (Table 2 and Experimental) were identical with those observed in the spectrum of asteriidoside C (**3**), and the signals in the sugar region were superimposable with those of asteriidoside D (**4**). The ^{13}C NMR spectrum (Tables 1 and 2) confirmed this assignment.

Asteriidoside F (**6**) is a further example of those diglycosides in which the two monosaccharides are not linked to each other. The FABMS (negative ion mode) showed a

Table 3. ^{1}H and ^{13}C NMR Signals (δ_H) for the Aglycon Protons of Compounds **6**[a]−**10**

	6		**8**		**9**		**10**	
position	δ_H	δ_C	δ_H	δ_C	δ_H	δ_C	δ_H	δ_C
1		39.2		39.5		39.4		41.2
2		30.6		29.9		29.8		29.9
3	3.64 m	79.5	3.64 m	79.5	3.64 m	79.8	3.73 m	79.8
4	2.43, 1.32	29.7		29.0	2.22, 1.27	28.6	1.72, 1.86	32.8
5	1.05 m	53.4		53.7	1.55	44.1	1.25	49.0
6	3.75 dt (10.5, 3.9)	67.5	3.75 dt (10.5, 3.9)	67.8	3.84 brt[b]	69.5	3.89 brt (2.5)	74.2
7	2.43 dd (12.2, 4.2)	49.3	2.42 dd (12.2, 4.2)	49.4	3.88 brt[b]	76.2	2.40 dd (14.4, 2.5)	45.2
	1.38						1.63	
8		77.0		77.6		79.5		77.0
9		57.2		57.6		50.1		56.9
10		38.0		38.0		37.8		36.6
11		19.3		19.8		19.5		19.7
12		43.1		43.6		43.7		42.5
13		44.3		44.5		43.2		45.4
14	1.05	60.9	1.08	62.6	1.78	56.6	1.23 d (10.0)	66.4
15	4.39 t (6.5)	71.1	4.42 t (6.5)	71.1	4.55 t (3.5)	71.1	4.28 dt (9.6, 3.7)	69.7
16	4.16 t (6.5)	72.9	2.38 m	43.3	2.26, 1.46	42.9	1.93, 1.63	42.0
17	1.01	63.3		57.4	1.28	57.6	1.40	55.4
18	1.30 s	17.8	1.33 s	16.8	1.34 s	16.5	1.01 s	15.4
19	1.02 s	13.8	1.03 s	14.1	1.02 s	13.9	1.19 s	15.8

[a] The data reported for compound **6** are identical with those of compound **7**. [b] Overlapped each other.

Table 4. NMR Data of the Steroidal Side Chain of Compounds **6**−**10**

	6		**7**		**8**		**9**		**10**	
position	δ_H	δ_C	δ_H	δ_C	δ_H	δ_C	δ_H	δ_C	δ_H	δ_C
20	2.64 m	34.4	2.64 m	34.3	2.27 m	41.0	2.26	41.0	2.06 m	40.7
21	1.07 d (7.0)	20.0	1.07 d (6.8)	20.1	1.05 d (7.0)	21.0	1.05 d (7.0)	20.9	1.03 d (6.6)	21.1
22	5.60 dd (14.0, 7.0)	139.5	5.55 dd (15.6, 7.8)	137.8	5.44 dd (15.0, 7.5)	141.1	5.44 dd (15.0, 7.0)	141.5	5.33 dd (15.3, 8.5)	140.4
23	5.50 dt (14.0, 5.5)	127.0	5.43 dd (15.2, 7.7)	133.9	5.37 dd (15.0, 7.5)	128.2	5.36 dd (15.0, 9.0)	128.1	5.24 dd (15.3, 9.0)	126.7
24	2.20 m, 1.90 m	37.7	2.17 m	40.0	3.75[b]	89.2	3.74[b]	89.0	2.15 m	49.5
25	1.84 m	34.9	1.67 m	39.7		33.9	1.86	33.9	1.86	29.1
26	3.72 dd (10.5, 5.5)	74.8	3.85 dd (10.8, 6.5)	74.5	0.89 d (6.8)	19.3	0.89 d (7.0)	19.2	0.89 d (6.1)	18.6
	3.35 dd[a]		3.35 dd[a]							
27	0.96 d (6.8)	17.2	0.95 d (6.8)	17.2	0.97 d (6.8)	18.5	0.96 d (7.0)	18.4	0.95 d (6.8)	20.8
28			0.99 d (6.8)	14.6					3.99 d (6.0)	70.5

[a] Overlapped with solvent signal. [b] Overlapped with other signals.

Table 5. Assignments of the NMR Signals of the Sugar Residues of Compound **6**[a]

	Xyl_1		Xyl_2	
position	δ_H	δ_C	δ_H	δ_C
1	4.40 d (7.5)	105.3	4.21 d (7.5)	102.9
2	3.17 dd (8.5, 7.5)	75.6	3.19 dd (8.5, 7.5)	74.8
3	3.32[b]	77.8	3.32[b]	77.8
4	3.50 m	71.1	3.49 m	71.1
5	3.87 dd (11.5, 5.4)	66.7	3.87 dd (11.5, 5.4)	66.7
	3.20 t (11.5)		3.19 t (11.5)	

[a] The data reported for compound **6** are identical with those of compound **7**. [b] Overlapped with solvent signal.

molecular anion peak at *m*/*z* 729 [M − H]$^-$ and a fragment at *m*/*z* 597 due to the loss of 132 mass units. Examination of ^{1}H and ^{13}C NMR data (Table 5) indicated that **6** contains two *β*-xylopyranosyl units, as confirmed by acid methanolysis, which afforded methyl xyloside. The ^{13}C NMR shifts of the anomeric carbons at 105.2 and 102.9 ppm and the coupling constants of the anomeric protons at *δ* 4.40 (*J* = 7.0 Hz) and 4.21 (*J* = 7.0 Hz) suggested that the glycosidic linkages in **6** are *β* and that the xylosyl units are in their pyranose form. In addition to the sugar moiety, the ^{1}H NMR spectrum showed the presence of the 3*β*,6α,8,-15*β*,16*β*-pentahydroxy steroidal tetracyclic nucleus previously found in steroid glycosides from *Halityle regularis*[19] and *Culcita novaeguinea*[20] (Table 3). The ^{1}H NMR spectrum also showed signals for the side chain: two methyl doublets at *δ* 0.96 (*J* = 6.8 Hz) and 1.07 (*J* = 7.0 Hz), two olefinic protons at *δ* 5.60 dd (*J* = 14.0, 7.0 Hz) and 5.50 dt (*J* = 14.0, 5.5 Hz), and two double doublets at *δ* 3.72 (*J* = 10.5, 5.5 Hz) and 3.35 (partially overlapped with solvent signal) for CH_2OH group. These data indicated a $\Delta^{(22E)}$,-26-hydroxycholestane side chain, previously found in polyhydroxysteroids from the starfish *Dermasterias imbricata*.[21]

^{13}C NMR data also established the sugar moieties to be linked at C-3 (79.5 ppm) and C-26 (74.8 ppm) of the steroid; these signals appear at 72.3 ppm[20] and 68.6 ppm[21] in the free steroid. The 25*S* configuration was then assigned by comparison of the ^{1}H NMR spectrum of the free 26-hydroxysteroid **6a**, with those of (25*R*)- and (25*S*)-26-hydroxysteroid models[3] and natural (25*R*)- and (25*S*),$\Delta^{(22E)}$,-26-hydroxylated steroids.[22] It has been shown that the C-25 epimers can be differentiated by the ^{1}H NMR of their (+)-*R*- and (−)-(*S*)-α-(trifluoromethyl)phenylacetic acid esters.[23] We thus treated asteriidoside F (**6**) with 2M HCl−MeOH and obtained 5α-cholesta-8(9),22-dien-3*β*,6α,15*β*,-16*β*,26-pentol (**6a**); dehydration at C-8 occurred together with hydrolysis (see Experimental Section). Treatment of the steroid with (−)-α-methoxy-α-(trifluoromethyl)phenylacetyl chloride afforded the corresponding 3,26-di-O-(−)-MTPA ester. The 26-methylene proton signals appeared as a double doublet at *δ* 4.13 and 4.31 supporting the Δ^{22} 25*S* configuration. The 26-methylene protons signals, in the Δ^{22} 25*R* model, appeared closer at *δ* 4.17 and 4.23.[22] Thus **6** can be defined as (25*S*)-3-*O*-(*β*-D-xylopyranosyl)-26-*O*-(*β*-D-xylopyranosyl)-5α-cholest-(22*E*)-ene-3*β*,6α,8,15*β*,-16*β*,26-hexaol.

Asteriidoside G (**7**) is the 24-methyl derivative of **6**. In the FABMS (negative ion) it gave a quasi molecular ion peak at *m/z* 743 $[M - H]^-$ accompanied by a fragment at *m/z* 611 related to the loss of a pentose unit (132 mass units). Examination of ^1H and ^{13}C NMR spectra (Tables 3 and 5) indicated the presence of two *β*-D-xylopyranosyl units and a 3*β*,6α,8,15*β*,16*β*-pentahydroxy cholestane structure identical to those observed in the asteriidoside F (**6**). The structure of the side chain of **7** was elucidated by analysis of 2D NMR. The COSY spectrum allowed construction of the side chain as a Δ^{22},24-methyl-26-oxygenated unit, previously encountered in starfish-derived steroid constituents.[3,24] The ^{13}C NMR spectrum confirmed the location of the xylose unit at C-3 (79.5 ppm) and established the second xylose unit to be attached at C-26 (74.5 ppm). The ^{13}C NMR data confirmed the side chain structure except for the stereochemistry at C-24 and C-25, which was then assigned as 24*R*,25*S* as follows. Acid hydrolysis gave 24-methylcholest-8(9),22-dien-3*β*,6α,15*β*,16*β*,26-pentaol (**7a**) whose NMR data (δH_2-26: 3.31−3.61 dd, δH_3-27,28: 0.94 d, 1.00 d), were indicative for a *threo* stereochemistry at C-24,C-25,[3,25] the same signals being observed at *δ* 3.34 dd−3.53 dd, 0.87 d, and 1.02 d in the spectrum of the *erythro* model steroid.[3,25] Assignment of the *S*-absolute configuration at C-25, and hence *R* to C-24, was made by observing the shape of the C-26 methylene proton signals in the ^1H NMR spectrum of the 3,26-di-*O*-(−)-MTPA ester derivative, *δ* 4.08 dd−4.40 dd, close to that found in the spectrum of the 26-(−)-MTPA ester 24*R*,25*S threo* model steroid (*δ* 4.13 dd−4.38 dd) and different from that found in the 24*S*,25*R*-isomer (*δ* 4.19 dd−4.31 dd).[25]

The remaining glycosides asteriidoside H (**8**), I (**9**), and L (**10**) are sulfated compounds. The FABMS (negative ion) of asteriidoside H (**8**) exhibited a molecular anion species at *m/z* 793 $[MSO_3]^-$ and a fragment at *m/z* 661 relative to the loss of 132 mass units from $[MSO_3]^-$. Upon solvolysis in a dioxane−pyridine mixture, **8** afforded a less polar desulfated derivative, which gave a quasi molecular ion at *m/z* 713 $[M - H]^-$ and a fragment at *m/z* 581. On acid methanolysis, **8** liberated methyl xylosides. Examination of the ^1H and ^{13}C NMR spectral data (Table 3) indicated the presence of the same 5α-cholestane-3*β*,6α,8,15*β* nucleus previously found in the halytiloside E and scoparioside A from *H. regularis*[19] and *Astropecten scoparius*,[26] respectively. The ^1H NMR spectrum also showed signals for the side chain: three methyl doublets at *δ* 0.89 and 0.97 (CH_3-26 and CH_3-27) and *δ* 1.05 (CH_3-21), two olefinic protons at *δ* 5.44 dd (J = 15.0, 7.5 Hz) and 5.37 dd (J = 15.0, 7.5 Hz), and a triplet at *δ* 3.75 (J = 7.5 Hz). Those data indicated a $\Delta^{(22E)}$,24-hydroxycholestane side chain, previously found in glycosides of *A. scoparius*.[25] The signals relative to the aglycon and the side chain are virtually unshifted relative to the data reported in the literature. The structure of the saccharide portion was elucidated by analysis of 2D NMR data. A COSY spectrum revealed two spin systems; one of them could be attributed to the known *β*-xylopyranose. The second spin system showed four methine protons at *δ* 4.36 (d, J = 7.6 Hz), 3.41 (t, J = 8.5 Hz), 4.18 (t, J = 9.0 Hz), and 3.70 (m), which couple to their neighbors in this order. The last methine proton is further coupled to methylene protons at *δ* 3.24 (t, J = 11.5 Hz) and 3.91 (dd, J = 11.5, 5.4 Hz). The coupling constants indicated that the molecule bears a moiety equivalent to a *β*-xylopyranoside. The upfield shift of H-3′ from *δ* 4.18 in **8** to 3.30 ppm in the desulfated derivative **8a** established the location of the sulfate residue at C-3′ of the sugar. This placement was confirmed by ^{13}C NMR (C-3′: 85.3 ppm) and was supported by downfield shifts of C-2′ (73.7 ppm) and C-4′ (70.0 ppm) when compared with the shifts observed in xylopyranosides. ^{13}C NMR data also established the sugar moieties to be linked, as in the previous glycosides, at C-3 and C-24 of the steroidal nucleus (Tables 3 and 4). A NOE experiment showed correlation of H-24, at *δ* 3.75, to H-1′ of xylose-3-*O*-sulfate at *δ* 4.35 and confirmed the attachment of sulfated sugar at C-24. The 24*R* configuration is proposed by analogy with the previous 5α-cholest-(22*E*)-ene-3*β*,6*β*,8,15α,24-pentol,15-sulfate aglycon isolated from *Coscinasterias tenuispina*[27] and *A. scoparius*.[26]

Table 6. Assignments of the NMR Signals of the Sugar Residues of Compound **8**[a] and **9**[b]

	β-D-xylopyranose		*β*-D-xylopyranose-3-*O*-sulfated	
position	δ_H	δ_C	δ_H	δ_C
1	4.40 d (7.6)	103.8	4.36 d (7.6)	101.3
2	3.17 dd (8.5, 7.6)	75.0	3.41 dd (8.5, 7.6)	73.7
3	3.30[c]	77.9	4.18 t (9.0)	85.3
4	3.48 m	71.3	3.70[d]	70.0
5	3.85 dd (11.5, 5.4)	66.8	3.91 dd (11.5, 5.4)	66.3
	3.22 t (11.5)		3.24 t (11.5)	

[a] ^1H assignments were aided by COSY experiments. [b] Data extracted from **8**. [c] Overlapped with solvent signal. [d] Overlapped with other signals.

Asteriidoside I (**9**) is related to asteriidoside H (**8**) by introduction of an additional hydroxyl group at the 7α position of the steroidal aglycon. The structure was derived by FABMS, NMR data, and comparison with **8**. The FABMS (negative ion) gave a molecular anion peak at *m/z* 809 $[MSO_3]^-$, 16 mass units shifted relative to **8**, and a fragment at *m/z* 677 due to the loss of xylose unit. The ^1H NMR spectrum (Tables 4 and 6) was virtually identical to that of **8** for the signals relative to side chain, *β*-D-xylose, and xylose-3-O-sulfated and showed signals corresponding to the 3*β*,6α,7α,8,15*β*-pentahydroxycholestane structure. The presence of one more signal in the downfield region of the spectrum at *δ* 3.88 (broad triplet), overlapped with 6*β*-H, is assigned to 7*β*-H. This assignment was supported by ^{13}C NMR signals for C-7 shifted downfield to 76.2 ppm and for C-5 and C-9 shifted highfield to 44.1 (53.7 in **8**) and 50.1 (57.6 in **8**) ppm, respectively, and by comparison with other 6α,7α-dihydroxylated steroids from starfishes.[28,29]

Asteriidoside L (**10**) gave a molecular anion peak in the FABMS (negative ion) at *m/z* 675 $[MSO_3]^-$, with a fragment at *m/z* 543 corresponding to the loss of 132 mass units. Upon solvolysis with dioxane−pyridine, **10** was desulfated to a less polar glycoside (**10a**), and FABMS of this product showed a pseudomolecular ion at *m/z* 595 $[M - H]^-$. Analysis of the ^1H and ^{13}C NMR spectra of **10** (Table 3) provided evidence for the 3*β*,6α,7α,8,15α-pentahydroxycholestane nucleus with a *β*-D-xylopyranose unit at C-3 found in the previous known pisasteroside B from *Pisaster ochraceus*.[30] In addition to the steroidal nucleus, the ^1H NMR spectrum contained signals for three secondary methyl groups (δ_H 1.03 d, 0.95 d, and 0.89 d), two 1H olefinic signals at *δ* 5.24 dd (J = 15.3, 9.0 Hz) and 5.33 dd (J = 15.0, 8.5 Hz), and a 2H doublet signal at *δ* 3.99 (J = 6.0 Hz) for an oxygenated methylene group. These features can be arranged in a $\Delta^{(22E)}$,28-(oxygenated)-methylcholestane side chain identical to those observed in the $\Delta^{(22E)}$,-24-hydroxymethylcholestane 28-sulfate side chain found in a polyhydroxysteroid from *Styracaster caroli*.[31] In the ^1H NMR spectrum of the desulfated derivative (**10a**), the signal for the oxygenated methylene group was observed shifted upfield to *δ* 3.58 dd (J = 9.3, 6.5 Hz) and 3.53 dd (J = 9.3, 6.2 Hz), thus allowing the sulfate group to be

assigned to C-28. Assignment of the *S* configuration at C-24 was based on the ^{1}H NMR pattern of the 28-methylene proton signals in the 28-(−)-MTPA ester of the desulfated derivative. The resonances of the C-28 protons were observed as two overlapping doublet doublets at δ 4.30 (J = 10.5, 4.8 Hz) and 4.33 (J = 10.5, 6.0 Hz) closer than the corresponding signals in the 28-(+)-MTPA, in agreement with the 24*S* configuration in the natural products[31] and in the model compounds.[32]

A selection of eight asterosaponins and steroid glycosides were tested for cytotoxicity against human broncopulmonary nonsmall-cell lung carcinoma cells (NSCLC-L16). The results, determined for three different product concentrations with two measurements carried out for each concentration, show that the hexaglycosides (asterosaponins) asteriidoside C (**3**), D (**4**), and E (**5**) are more active, with an IC_{50} = 19.7 ± 0.1 μg/mL, 11.4 ± 0.3 μg/mL, and 18.1 ± 0.2 μg/mL, respectively. The remaining asterosaponins, asteriidosides A (**1**) and B (**2**), possess moderate cytotoxicity, with IC_{50} >30 μg/mL. Among the steroid glycosides, only the asteriidosides F (**6**), I (**9**), and L (**10**) were tested in the same cell line, and these are less active (IC_{50} >30 μg/mL) than the asterosaponins, paralleling previous investigation.[33]

Experimental Section

General Experimental Procedures. NMR spectra, Bruker AMX-500 (^{1}H at 500 MHz, ^{13}C at 125 MHz), δ (ppm), J in Hz, referred to CHD_2OD signal at 3.34 ppm and central carbon CD_3OD signal at 49.0 ppm; MS, VG AUTOSPEC instruments (Cs^+ ions bombardment) with FIB source [in glycerol or glycerol−thioglycerol (3:1) matrix]; optical rotation Perkin−Elmer 141 polarimeter; GLC, Carlo Erba Fractovap 2900 for capillary column (SPB-1, 25 m, 150°; helium carrier flow 10 mL min^{-1}); reversed-phase HPLC, C_{18} μ-Bondapak column (30 cm × 8 mm i.d.; flow rate 5 mL min^{-1}) and C_{18} μ-Bondapak column (30 cm × 3.9 mm i.d.; flow rate 2 mL min^{-1}), Waters model 6000 A pump equipped with U6K injector and a differential refractometer, model 401; DCCC, DCC-A apparatus manufactured by Tokyo Rikakikai Co., equipped with 250 tubes.

Determination of Biological Activity. NSCLC-N6-L-16 cells were maintained in a suspension culture of BME medium supplemented with 5% calf serum containing 1% of glutamine solution at 200 mM and 1% of a penicillin−streptomycin mixture (10 000 UI/mL). A 50-mL aliquot of the cell culture [NSCLC cells (1×10^4)] was mixed with a 50-mL aliquot of serial dilution of compounds **1**−**6**, **9**, and **10** (30, 10, and 3.3 μg/mL), and the mixture was incubated in a microtiter well plate (96-well Flacon 3072) for 72 h at 37 °C in a humified incubator containing 5% CO_2 in air. Cell proliferation was estimated by a colorimetric test: 10 mL of MTT [3-(4,5-dimethylthiazol-2-yl)-2,5-diphenyltetrazolium bromide] (Sigma) was added. After 4 h the dark blue crystals, formed in mitochondria of living cells during the reduction of MTT, were solubilized with 100 mL of isopropanoic acid. Microplates were read by ELISA using a multiskar Titertek multiscan MK2 with a 570-nm filter.

Animal Material. The animal (only one organism was collected) was collected at Tethys Bay in January 1990, in the course of the Italian−Antarctic expedition. A voucher specimen is kept at Istituto per la Chimica di Molecole di Interesse Biologico del CNR, Arco Felice, Napoli, under the reference number MOR 24; identification was made by Prof. Michel Jangoux, Université Livree de Bruxelles, Laboratoire de Biologie Marine.

Extraction and Isolation. The animal (500 g fresh) was cut into small pieces and soaked in H_2O for 5 h. The aqueous extracts were centrifuged and passed through a column of Amberlite XAD-2 (700 g). The column was washed with distilled H_2O (2.5 L) and then eluted with MeOH (4 L). The MeOH eluate was taken to dryness to give a glassy material (2.5 g). The remaining solid mass, after extraction with H_2O, was then reextracted with Me_2CO (2 L), and the Me_2CO extracts, were combined, evaporated under vacuum, and partitioned between H_2O and Et_2O. The aqueous residue was then extracted with *n*-BuOH. Evaporation of the *n*-BuOH extracts afforded 1.1 g of a glassy material that was combined with the above MeOH eluate from Amberlite XAD-2 column and chromatographed on a column of Sephadex LH-60 (4 × 80 cm) with MeOH−H_2O (2:1) as eluent. Fractions (9 mL each) were collected and analyzed by TLC on SiO_2 in *n*-BuOH−AcOH−H_2O (12:3:5) and $CHCl_3$−MeOH−H_2O (80:18:2).

Fractions 40−57 (700 mg) mainly contained the asterosaponins. The crude asterosaponins fraction was submitted to DCCC with *n*-BuOH−Me_2CO−H_2O (3:1:5) [descending mode; the upper phase was used as the stationary phase; flow rate 14 mL/h; fractions (7 mL) were collected] to give two main fractions: 51−82 (38 mg) and 83−110 (73 mg).The flow rate was 5 mL/min. Fractions 51−82 contained asteriidoside A (**1**). Fractions 83−110 contained a mixture of asteriidosides B (**2**), C (**3**), D (**4**), and E (**5**). These fractions were then purified by reversed-phase HPLC (C_{18} μ-Bondapak 30 cm × 7.8 mm i.d.) with MeOH−H_2O (45:55) as the eluent, to give pure saponins: **1** (11.0 mg), **2** (10.5 mg), **3** (4.5 mg), **4** (9.0 mg), and **5** (5.0 mg).

Fractions 58−71 (500 mg) contained steroidal glycoside sulfates, and fractions 72−95 (800 mg) contained the sulfated steroids. DCCC fractionation of the sulfated steroidal glycoside fractions was pursued using *n*-BuOH−Me_2CO−H_2O (3:1:5) [ascending mode; the lower phase was used as the stationary phase]. Fractions (6 mL each) were collected and monitored by TLC on SiO_2. Fractions 32−43 (50 mg) contained compound **10** (6.5 mg). Fractions 44−54 (30 mg) contained compounds **8** (4.9 mg) and **9** (7.4 mg). Each fraction was purified by HPLC on a C_{18} column with MeOH−H_2O (1:1) to give the pure compounds.

DCCC fractionation of the sulfated steroids was pursued using $CHCl_3$−MeOH−H_2O (7:13:8) in the ascending mode (the lower phase was the stationary phase). Fractions 12−18 were purified by HPLC with MeOH−H_2O (6:4) to give pure asterasterols A, B, and C.[5] Fractions 19−26 were purified by HPLC with MeOH−H_2O (7:3) to give pure **6** (5.0 mg), and fractions 27−29 were purified by HPLC with MeOH−H_2O (75:25) to give pure **7** (3.8 mg).

Asteriidoside A (1): 11.0 mg; FABMS (negative ion) *m*/*z* 1419 $[MSO_3]^-$; $[\alpha]_D$ +5.9° (MeOH, *c* 1); ^{1}H NMR δ_H (CD_3OD) (steroidal nucleus) 5.37 (1H, br d, J = 5.5 Hz, H-11), 4.22 (1H, m, H-3), 1.02 (3H, s, H_3-19), and 0.81 (3H, s, H_3-18); ^{13}C NMR δ_c (pyridine-d_5) (steroidal nucleus) 145.5 (C-9), 116.7 (C-11), 80.0 (C-6), 78.0 (C-3), 59.3 (C-17), 54.0 (C-14), 49.1 (C-5), 42.4 (C-12), 41.6 (C-13), 41.2 (C-7), 38.3 (C-10), 36.0 (C-1), 35.3 (C-8), 30.7 (C-4), 29.2 (C-2), 25.1 (C-16), 23.3 (C-15), 19.3 (C-19), and 13.6 (C-18); ^{1}H and ^{13}C NMR (side chain) in Table 2; ^{1}H NMR δ_H (CD_3OD) (saccharide portion): 4.56, 4.52 (× 2), 4.50, 4.44 (d, anomeric-H's), 1.48, 1.40, 1.30 (× 2) (each 3H, d, J = 6.8 Hz, H_3-5 quinovose and fucose); ^{13}C NMR δ_c (pyridine-d_5) (saccharide portion) in Table 1.

Asteriidoside B (2): 10.5 mg; FABMS (negative ion) *m*/*z* 1387 $[MSO_3]^-$; $[\alpha]_D$ +9.2° (MeOH, *c* 1); ^{1}H NMR δ_H (CD_3OD) (steroidal nucleus) 5.38 (1H, br d, J = 5.5 Hz, H-11), 4.22 (1H, m, H-3), 1.03 (3H, s, H_3-19), and 0.83 (3H, s, H_3-18); ^{13}C NMR δ_c (pyridine-d_5) (steroidal nucleus) data identical to those reported for **1**; ^{1}H and ^{13}C NMR (side chain) in Table 2; ^{1}H NMR δ_H (CD_3OD) (saccharide portion) 4.58, 4.51 (× 2), 4.50, 4.43 (d, anomeric-H's), 4.14 (1H, dd, He-5 of xilose), 1.40, 1.33, 1.31, 1.29 (each 3H, d, J = 6.8 Hz, H_3-5 quinovose and fucose); ^{13}C NMR δ_c (pyridine-d_5) (saccharide portion) in Table 1.

Asteriidoside C (3): 4.5 mg; FABMS (negative ion) *m*/*z* 1373 $[MSO_3]^-$; $[\alpha]_D$ +10.7° (MeOH, *c* 1); ^{1}H and ^{13}C NMR (steroidal nucleus) identical to those reported for **1**; ^{1}H and ^{13}C NMR δ_c (pyridine-d_5) (side chain) in Table 2; ^{1}H NMR δ_H (CD_3OD) (saccharide portion) identical to those reported for **2**; ^{13}C NMR δ_c (pyridine-d_5) (saccharide portion) in Table 1.

Asteriidoside D (4): 9.0 mg; FABMS (negative ion) *m*/*z* 1373 $[MSO_3]^-$; $[\alpha]_D$ +7.7° (MeOH, c 1); ^{1}H and ^{13}C NMR

(steroidal nucleus) identical to those reported for **2**; ^{1}H and ^{13}C NMR (side chain) in Table 2; ^{1}H NMR δ_H (CD_3OD) (saccharide portion) 4.59, 4.57, 4.56, 4.51, 4.49, 4.42 (d, anomeric-H's), 4.41 (1H, dd, He-5 of xylose), 1.40, 1.32, 1.30 (each 3H, d, J = 6.8 Hz, H_3-5 quinovose and fucose); ^{13}C NMR δ_c (pyridine-d_5) (saccharide portion) in Table 1.

Asteriidoside E (5): 5.0 mg; FABMS (negative ion) *m/z* 1359 $[MSO_3]^-$; $[\alpha]_D$ +8.5° (MeOH, *c* 1); ^{1}H and ^{13}C NMR (steroidal nucleus) identical to those reported for **1**; ^{1}H and ^{13}C NMR (side chain) in Table 2; ^{1}H NMR δ_H (CD_3OD) (saccharide portion) identical to those reported for **4**; ^{13}C NMR δ_c (pyridine-d_5) (saccharide portion) in Table 1.

Asteriidoside F (6): 5.0 mg; FABMS (negative ion) *m/z* 729 $[M - H]^-$; $[\alpha]_D$ −8.3° (MeOH, *c* 1); ^{1}H and ^{13}C NMR (steroidal nucleus) in Table 3; ^{1}H and ^{13}C NMR (side chain) in Table 4; ^{13}C NMR δ_c (pyridine-d_5) (saccharide portion) in Table 5.

Asteriidoside G (7): 3.8 mg; FABMS (negative ion) *m/z* 743 $[M - H]^-$; $[\alpha]_D$ −17.9° (MeOH, *c* 1); ^{1}H and ^{13}C NMR (steroidal nucleus) in Table 3; ^{1}H and ^{13}C NMR (side chain) in Table 4; ^{13}C NMR (saccharide portion) in Table 5.

Asteriidoside H (8): 4.9 mg; FABMS (negative ion) *m/z* 793 $[MSO_3]^-$; $[\alpha]_D$ −8.3° (MeOH, *c* 1); ^{1}H and ^{13}C NMR (steroidal nucleus) in Table 3; ^{1}H and ^{13}C NMR (side chain) in Table 4; ^{13}C NMR (saccharide portion) in Table 6.

Asteriidoside I (9): 7.4 mg; FABMS (negative ion) *m/z* 809 $[MSO_3]^-$; $[\alpha]_D$ −8.8° (MeOH, *c* 1); ^{1}H and ^{13}C NMR (steroidal nucleus) in Table 3; ^{1}H and ^{13}C NMR (side chain) in Table 4; ^{13}C NMR (saccharide portion) in Table 6.

Asteriidoside L (10): 6.5 mg; FABMS (negative ion) *m/z* 675 $[MSO_3]^-$; $[\alpha]_D$ +5.7° (MeOH, *c* 1); ^{1}H and ^{13}C NMR (steroidal nucleus) in Table 3; ^{1}H and ^{13}C NMR (side chain) in Table 4; ^{1}H NMR (saccharide portion): 4.39 (1H, d, J = 7.6 Hz, H-1), 3.85 (1H, dd, J = 11.5, 5.4 Hz, H-5′), 3.48 (1H, m, H-4′), 3.34 (1H, overlapped with solvent signal, H-3′), 3.22 (1H, t, J = 11.5 Hz, H-5″), 3.16 (1H, t, J = 8.1 Hz, H-2′); ^{13}C NMR (saccharide portion) 105.0 (C-1′), 77.7 (C-3′), 74.8 (C-2′), 71.1 (C-4′), 66.8 (C-5′).

Methanolysis of Asterosaponins 1−5. Sugar Analysis: A solution (0.5 mg each) of asterosaponins in anhydrous 2M HCl−MeOH (0.5 mL) was heated at 80 °C in a stoppered reaction vial for 8 h. Once 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 trisyl Z (Pierce Chemical Co.) for 15 min at room temperature. GLC analysis (25 m, SPB-1 capillary column; 152 °C; H_2 carrier flow 10 mL min^{-1}) gave peaks that coeluted with those of the appropriate methyl glycosides silylated standards. The ratio for **1** was quinovose−galactose−fucose 3:2:1, established by integration of the areas of the corresponding peaks. The ratio for **2**, **3** was quinovose−xylose−fucose−galactose 2:1:2:1 and for **4** and **5** was quinovose−xylose−fucose−arabinose−galactose 2:1:1:1:1.

Methylation of Asterosaponins (1, 2 and 4) Followed by Methanolysis. Terminal Sugar: Saponins **1**, **2**, and **4** (3.5 mg each) in dry DMF (0.5 mL) were slowly added under nitrogen to a stirred mixture of NaH (60 mg) in dry DMF (0.5 mL) cooled in an ice-bath. The mixtures were stirred for 15 min, and then MeI (0.25 mL) was added. The reaction mixtures were kept for a further 4 h at room temperature. The excess of NaH was destroyed by a few drops of MeOH and, after addition of H_2O, the mixtures were extracted twice with $CHCl_3$. The organic layer was washed with H_2O, dried (Na_2SO_4), and evaporated under reduced pressure. The residues were methanolyzed in anhydrous 2M HCl−MeOH (0.3 mL) at 80 °C in a stoppered reaction vial for 8 h. After cooling, the reaction was neutralized with Ag_2CO_3, centrifuged, and the supernatant evaporated to dryness under reduced pressure. The residues were separately analyzed by GLC (25 m, SPB-1, capillary column, 100 °C, hydrogen carrier flow 10 mL min^{-1}) and the GLC peaks coeluted with those of methyl 2,3,4,-tri-*O*-methylquinovoside and methyl 2,3,4,6-tetra-*O*-methylgalactoside.

Enzymatic Hydrolysis of Asterosaponins 1, 2, and 4. The saponin **1** (2 mg) in a citrate buffer (1 mL; pH 4.5) was incubated with a glycosidase mixture (5 mg) of *C. lampas* (Shikagaku Kogyo) at 37 °C. After reaction for 8 h, the TLC analysis (SiO_2 with *n*-BuOH−AcOH−H_2O 60:15:25) showed that the starting material (R_f = 0.14) has disappeared and displayed one major spot (R_f = 0.47). The trisaccharide **1a**. The mixture was passed through a C-18 SEP-PACK cartridge, washed with H_2O and eluted with MeOH. The MeOH eluate was evaporated to dryness, and the residue was submitted to HPLC [C_{18} μ-Bondapak column, 30 cm × 3.9 mm i.d.; MeOH−H_2O 52:48] to give the trisaccharide **1a**.

Compound 1a: ^{1}H NMR (aglycon) identical with that of natural **1**, δ_H (sugar) 4.56, 4.52, 4.44 (each 1H,d, J = 7.5 Hz, anomeric H's), 1.40, 1.32, 1.30 (each 3H, d, J = 6.5 Hz, 5-Me of quinovosyl residue) sugar analysis quinovose × 3.

The saponin **2** (3.5 mg) in 1 mL of citrate buffer (pH = 4.5) was incubated with 5.0 mg of glycosidase mixture of *C. lampas* at 37 °C for 3 h. The subsequent analysis was carried out in the condition previously described to give the trisaccharide **2a** and the tetrasaccharide **2b**.

Prosapogenol 2a. FABMS (negative ion) *m/z* 933 $[MSO_3]^-$; ^{1}H NMR (aglycon) δ_H (CD_3OD) identical to those reported for intact **2**; δ_H (sugars) 4.56, 4.54, 4.46 (1H, d, J = 7.5 Hz, anomeric-H's), 1.40, 1.32, 1.30 (each 3H, d, J = 6.8 Hz, H_3-5 of quinovose and fucose).

The saponin **4** (5.0 mg) in 1 mL di citrate buffer (pH = 4.5) was incubated with 6.0 mg of glycosidase mixture of *C. lampas* at 37 °C for 4 h. The analysis was carried out in the condition previously described to give the major trisaccharide **4a** and minor amount of tetrasaccharide **4b** and pentasaccharide **4c**.

Prosapogenol 4a. FABMS (negative ion) *m/z* 933 $[MSO_3]^-$; ^{1}H NMR (aglycon) δ_H (CD_3OD) virtually identical to those reported for intact **4**; δ_H (sugars) identical to those reported for prosapogenol **2a**.

Prosapogenol 4b. FABMS (negative ion) *m/z* 1079 $[MSO_3]^-$; ^{1}H NMR (aglycon) δ_H (CD_3OD) identical to those reported for intact **4**; δ_H (sugars) virtually identical to those reported for **2b**.

Prosapogenol 4c. FABMS (negative ion) *m/z* 1211 $[MSO_3]^-$; ^{1}H NMR (aglycon) δ_H (CD_3OD) identical to those reported for intact **4**; δ_H (sugars) 4.57, 4.56, 4.51, 4.49, 4.42 (1H, d, J = 7.5 Hz, anomeric-H's), 1.40, 1.31, 1.29 (each 3H, d, J = 6.8 Hz, H_3-5 of quinovose and fucose).

Solvolysis of Asteriidosides H (8), I (9), and L (10). A solution (2 mg each) of the glycosides in a mixture of pyridine (125 μL) and dioxane (125 μL) was heated at 150 °C for 3 h in a stoppered reaction vial. The residue was evaporated to dryness and purified by HPLC (C_{18}-μ Bondapak column 30 cm × 3.8 mm i.d.) with MeOH−H_2O (7:3) as eluent.

Acid Treatment of 6 to Give 5α-cholesta-8(9),22-dien-3*β*,6α,15*β*,16*β*,26-pentol (6a) and 7 to give 24-Methyl-5α-cholesta-8(9),22-dien-3*β*,6α,15*β*,16*β*,26-pentol (7a). A solution (2 mg each) of the glycosides in 2M HCl−MeOH (0.5 mL) was heated at 80° in a stoppered reaction vial. After 2 h, TLC analysis [SiO_2 with $CHCl_3$−MeOH−H_2O (80:18:2)] showed that the starting material had disappeared and was replaced by a UV-active spot. The reaction mixture was cooled, neutralized with Ag_2CO_3, and centrifuged, and the supernatant was taken to dryness under N_2. The residue was purified by HPLC [C_{18} μ-Bondapak (30 cm × 3.9 mm i.d.), MeOH−H_2O (7:3)]. ^{1}H NMR (CD_3OD) of **6a**: 5.60 (1H, dd, J = 14.0, 7.0 Hz, H-22), 5.50 (1H, dt, J = 14.0, 5.5 Hz, H-23), 4.30 (1H, t, J = 6.5 Hz, H-15), 4.20 (1H, t, J = 6.5 Hz, H-16), 3.75 (1H, dt, J = 10.5, 3.9 Hz, H-6), 3.55 (1H, m, H-3), 3.44 (1H, dd, J = 10.5, 5.5 Hz, H-26), 3.39 (1H, dd, partially overlapped with solvent signal, H-26), 3.08 (1H, dd, J = 12.2, 4.2 Hz, H-7), 2.64 (1H, m, H-20), 1.12 (3H, d, J = 7.0 Hz, H_3-21), 1.07 (3H, s, H_3-19), 0.98 (3H, s, H_3-18), 0.95 (3H, d, J = 6.8 Hz, H_3-27).

^{1}H NMR (CD_3OD) of **7a**: 5.55 (1H, dd, J = 15.6, 7.8 Hz, H-22), 5.43 (1H, dt, J = 15.2, 7.7 Hz, H-23), 4.30 (1H, t, J = 6.5 Hz, H-15), 4.20 (1H, t, J = 6.5 Hz, H-16), 3.74 (1H, dt, J = 10.5, 3.9 Hz, H-6), 3.55 (1H, m, H-3), 3.61 (1H, dd, J = 10.8, 6.5 Hz, H-26), 3.31 (1H, dd, partially overlapped with solvent signal, H-26), 3.09 (1H, dd, J = 12.2, 4.2 Hz, H-7), 2.63 (1H, m, H-20), 1.12 (3H, d, J = 6.8 Hz, H_3-21), 1.07 (3H, s, H_3-19),

1.00 (3H, d, $J = 6.8$, H_3-28), 0.98 (3H, s, H_3-18), 0.94 (3H, d, $J = 6.8$ Hz, H_3-27).

3,26 Di-(−)-MTPA Ester of 6a. The steroid **6a** (0.7 mg) was treated with freshly distilled

(−)-methoxy(trifluoromethyl)phenyl acetyl chloride (2 mL) prepared from (−)-(*S*)-MTPA acid, in dry pyridine (0.2 mL) for 1 h at room temperature. After removal of solvent the product was analyzed by ^{1}H NMR.

3,26 Di-(−)-MTPA Ester of 7a. The steroid **7a** (0.8 mg) was treated in identical manner as compound **6a** and the data analyzed by ^{1}H NMR.

28-(−)-MTPA Ester of 10a. The steroid **10a** (0.7 mg) was treated in identical manner as compounds **6a** and **7a** and the data analyzed by ^{1}H NMR.

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