Starfish Saponins, Part 51. Steroidal Oligoglycosides from the Starfish Distolasterias nipon

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STARFISH SAPONINS, PART 51[1]. STEROIDAL OLIGOGLYCOSIDES FROM THE STARFISH *DISTOLASTERIAS NIPON*

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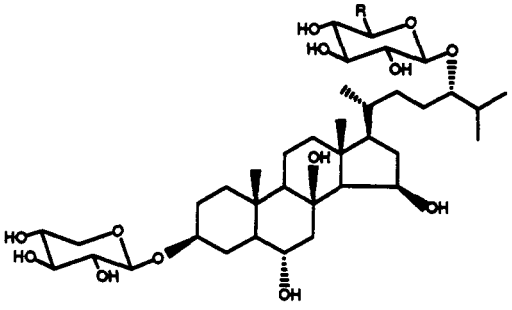
ABSTRACT.—A reinvestigation of the extracts from the starfish *Distolasterias nipon*, collected at Mutsu Bay, Japan, has led to the isolation of six glycosides of polyhydroxysteroids and six asterosaponins. Four steroidal glycosides have been identified as distolasterosides D_1 [**1**] and D_2 [**2**] (previously isolated from the same organism), and pycnopodioside C [**5**] and pisasteroside A [**6**], previously found in the related species *Pycnopodia helianthoides* and *Pisaster ochraceus* (family Asteridae), respectively. Two asterosaponins have been identified as the common versicoside A [**7**] and thornasteroside A [**7a**]. The two remaining glycosides of polyhydroxysteroids, named distolasterosides D_4 [**3**] and D_5 [**4**], and four asterosaponins designated nipoglycosides A [**8**], B [**9**], C [**10**], and D [**11**] are new compounds, and their structures have been elucidated mainly by interpretation of spectral data and comparison with known compounds.

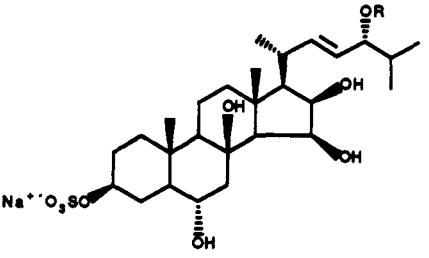
Steroidal glycosides from the starfishes have been subdivided into three main groups according to their chemical structures: the asterosaponins, which are sulfated steroidal penta- and hexaglycosides; the cyclic glycosides, so far only found in two species of the genus *Echinaster*; and glycosides of polyhydroxysteroids, which are as widespread as asterosaponins among starfishes (1). These compounds, which usually occur in minute amounts and in complex mixtures, consist of a polyhydroxylated steroidal aglycone linked to one or two sugar units and can be found in both sulfated and non-sulfated forms. We have recently isolated three cytotoxic triglycosides (2) from the New Caledonian species *Fromia monilis*; these constitute the only example of triglycosides among more than one hundred different mono- and diglycosides of polyhydroxysteroids isolated so far. Analysis of the polar extracts of the starfish *Tremaster novaecaledonia* has recently led to the discovery of a new class of steroidal glycosides in which the polyhydroxylated steroidal aglycones are conjugated to phosphate to which the sugars are glycosidally attached (3).

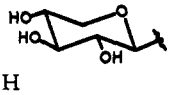
As part of our continuing study of biologically active compounds from echinoderms, we have analyzed the polar extracts of the starfish *Distolasterias nipon* Müller and Troschel (family Asteridae, order Forcipulata) collected at Mutsu Bay, Japan, and we now report the isolation of six glycosides of polyhydroxysteroids and six asterosaponins, which are further examples of the structural variety of steroidal glycosides co-occurring in the same organism. Four glycosides of polyhydroxysteroids have been identified as the known compounds: distolasterosides D_1 [**1**], and D_2 [**2**], previously described from the same organism (4,5), and pycnopodioside C [**5**] and pisasteroside A [**6**], already isolated from the related species *Pycnopodia helianthoides* (6) and *Pisaster ochraceus* and *Pisaster brevispinus* (7), respectively. Two new glycosides have been designated distolasterosides D_4 [**3**] and

[1]For Part 50, see I. Bruno, L. Minale, R. Riccio, L. Cariello, T. Higa, and J. Tanaka, *J. Nat. Prod.*, **56**, 1057 (1993).

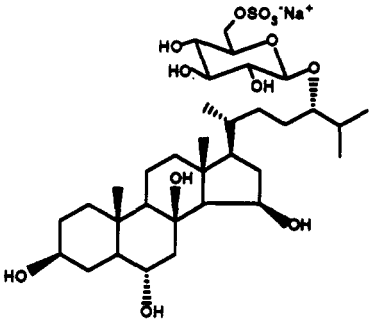


1 R=H distolasteroside D_1
2 Δ^{22}, R=H distolasteroside D_2
3 $R=CH_2OSO_3^-Na^+$ distolasteroside D_4

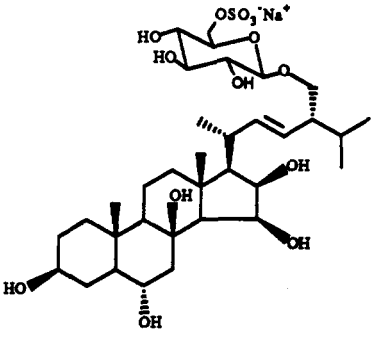


4 R=  distolasteroside D_5

4a R=H



5 pycnopodioside C



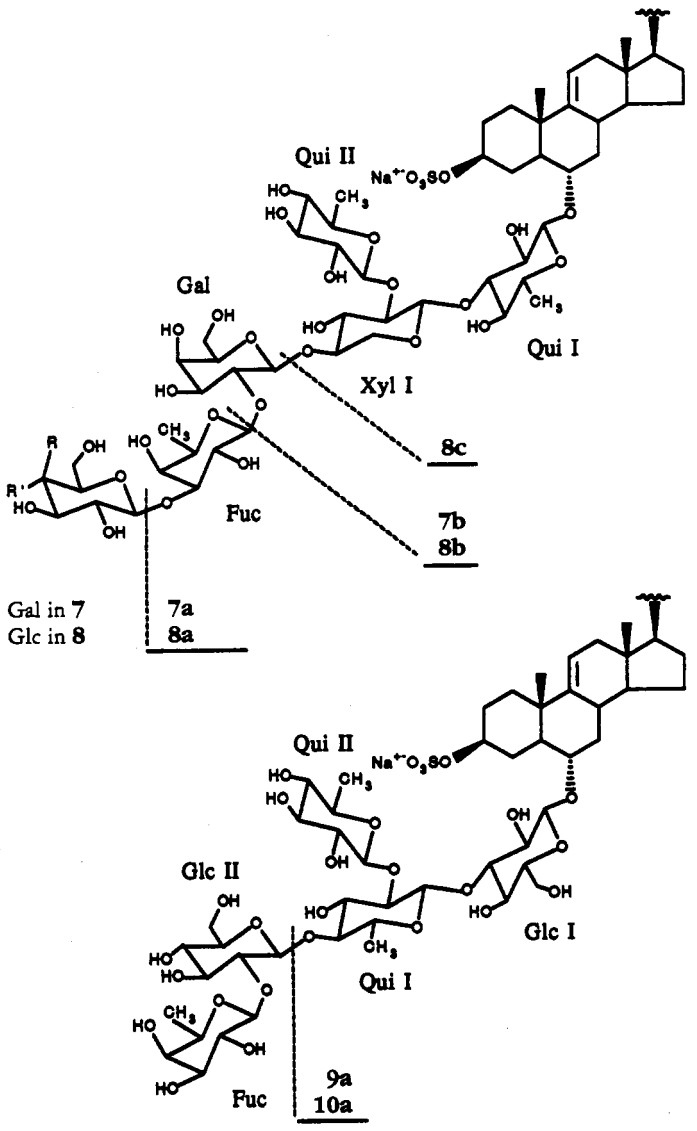
6 pisasteroside A

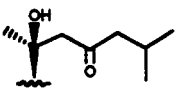
D_5 [**4**]. Distolasteroside D_3, which is the desulfated distolasteroside D_4, has been reported by Stonik and Elyakov (4) and by Kapustina *et al.* (5), but we failed to isolate it as individual compound from our sample. Four asterosaponins designated nipoglycosides A [**8**], B [**9**], C [**10**], and D [**11**] are new compounds, while two more asterosaponins have been identified as the common versicoside A [**7**] (8) and thornasteroside A [**7a**] (1,9). The 3-*O*-sulfated steroids **12** and **13** have also been isolated (see Experimental).

RESULTS AND DISCUSSION

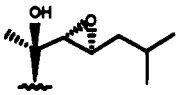
Separation and isolation of the individual compounds from the aqueous extracts of animals followed the steps described previously (10). In brief, saponins and polar steroids were recovered from the aqueous extracts by passing the solution through a column of Amberlite XAD-2 resin, washing salts with distilled H_2O, and eluting the absorbed material with MeOH. The residue of this elution was then subjected to chromatography on Sephadex LH-60, followed by dccc and finally purification by reversed-phase hplc. For discussion on the strategy for separation of saponins and polar steroids from starfishes see Minale *et al.* (1). The results of our analysis are shown in Table 1.

GLYCOSIDES OF POLYHYDROXYSTEROIDS: KNOWN COMPOUNDS.—Identification of the known pycnopodioside C [**5**] and pisasteroside A [**6**] was achieved by direct comparison (fabms, ^1H nmr, and hplc) with authentic samples. Identification of the known distolasterosides D_1 [**1**] and D_2 [**2**] (5) relied on interpretation of spectral data, which are summarized below.

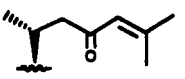




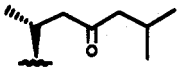
R=OH; R′=H
7 versicoside A



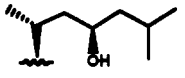
R=H; R′=OH
8 nipoglycoside A



9 nipoglycoside B

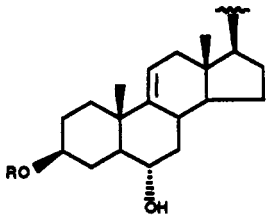


10 nipoglycoside C



11 nipoglycoside D

The fabms (negative ion mode) of compound **1** gave a quasi molecular ion at *m/z* 715 $[M-H]^-$ and a major fragment at *m/z* 583, corresponding to the loss of a pentose unit. Acid methanolysis yielded methyl xylosides (gc). The ^{1}H-nmr spectrum (Experimental)



12 R=$SO_3^-Na^+$

13 R=$SO_3^-Na^+$

14 R=H

TABLE 1. Steroidal Glycosides and Polar Steroids in the Composition of the Starfish *Distolasterias nipon*.

Compound	Amount[a] (mg)	Rotation[b] $[\alpha]_D$	References
Glycosides of polyhydroxysteroids			
Distolasteroside D_1 [**1**]	0.5		Stonik and Elyakov (4), Kapustina *et al.* (5)
Distolasteroside D_2 [**2**]	0.7		Stonik and Elyakov (4), Kapustina *et al.* (5)
Distolasteroside D_4 [**3**]	1.5	−4.6	
Distolasteroside D_5 [**4**]	1.2	+2	
Pycnopodioside C [**5**]	1.0	+4.5	Bruno *et al.* (6)
Pisasteroside A [**6**]	2.0	+3.8	Zollo *et al.* (7)
Asterosaponins			
Versicoside A [**7**]	10.6	+5.0	Itakura *et al.* (8)
Thornasteroside A [**7a**]	14.0	+5.3	Kitagawa and Kobayashi (9)
Nipoglycoside A [**8**]	3.7	+4.5	
Nipoglycoside B [**9**]	15.0	−1.5	
Nipoglycoside C [**10**]	5.6	0	
Nipoglycoside D [**11**]	2.0	+3.6	
Steroids			
12	1.0	−2	
13	1.5	+7.2	

[a]From 1.2 g of MeOH eluate as obtained from chromatography column of Amberlite XAD-2 of the aqueous extracts of ca. 5 kg fresh animals.

[b]From solution in MeOH ranging from 0.1 to 1.0.

indicated the presence of a 5α-cholestane-3β,6α,8,15β,24-pentaol aglycone found in many steroid glycosides from starfishes (1, 11–13). Thus compound **1** is a dixyloside of the 5α-cholestane-3β,6α,8,15β,24-pentaol aglycone. The shift of one of the two anomeric proton signals at δ 4.39 (d, J=7 Hz) was indicative of the location of one xylopyranosyl unit at C-3, whereas the doublet (J=7 Hz) shifted upfield to δ 4.26 for the second anomeric proton was of diagnostic value for the location of the second xylopyranosyl unit at C-24 (1). All these data are in agreement with the structure requirements of the reported distolasteroside D_1 (4,5). Analogously, the fabms (negative ion) of compound **2**, *m/z* 713 $[M-H]^-$, 581, and the analysis of the ^1H nmr (Experimental), indicated for it the structure of distolasteroside D_2: i.e., Δ^{22E} distolasteroside D_1. The 24*S* configuration assigned to **1** and **2** is suggested by analogy with the many steroid (24*S*)- 24-*O*-glycosides isolated from starfishes and especially by analogy with distolasteroside D_5, co-occurring in the same organism, for which the 24*S* configuration has been determined (see below).

Distolasteroside D_4 [**3**].—Examination of the spectral data (^1H nmr; Experimental) of distolasteroside D_4, fabms (negative ion), *m/z* 825 $[MSO_3]^-$ and 693 (loss of the xylosyl unit), indicated that **3** contained the same 5α-cholestane-3β,6α,8,15β,24-pentaol aglycone found in distolasterosides D_1 and D_2 and in many other starfish glycosides (1, 11–13). A detailed ^1H-nmr analysis with sequential decoupling indicated the presence of two monosaccharide moieties as β-xylopyranosyl and β-glucopyranosyl 6-*O*-sulfate units (Table 2). The ^{13}C-nmr spectrum, which, because of the very small amounts of sample available, was taken with a DEPT pulse sequence using polarization transfer of 90°, thus identifying only CH groups, confirmed the presence of the two sugar units attached to C-3 [δ_C 79.5 ppm; glycosidation shift of 7.3 ppm; in 5α-cholestane-

TABLE 2. ^{1}H-nmr and ^{13}C-nmr (DEPT using polarization transfer of 90°; only CH signals) Data of the Saccharide Units of **3**.

Position	β-xylopyranosyl		β-glucopyranosyl 6-*O*-sulfate	
	^{1}H (mult., *J* in Hz)	^{13}C	^{1}H	^{13}C
1	4.40 (d, 7.5)	102.8	4.31 (d, 7.5)	103.9
2	3.17 (dd, 7.5, 9)	75.6	3.20 (dd)	75.6
3	3.30 (m)[a]	77.6	3.38 (m)[a]	77.6
4	3.50 (m)[a]	71.4	3.48 (m)[a]	72.0
5	3.21 (t, 10.5) 3.85 (dd, 10.5, 3.5)	—	3.50 (m)[a]	76.2
6	—	—	4.18 (dd, 11.2, 3) 4.33 (dd)	—

[a]Signals partially overlapping with other signals.

3β,6α,8,15β,24-pentaol C-3, 72.2 ppm (13)] and at C-24 [δ_C 86.0 ppm, glycosidation shift of 7.8 ppm; in 5α-cholestane-3β,6α,8,15β,24-pentaol C-24, 78.2 ppm (13)] of the aglycone. Solvolysis in a dioxane/pyridine mixture afforded a desulfated derivative, fabms (negative ion) *m/z* 745 $[M-H]^-$, δ CH_2-OH of the glucosyl unit upfield shifted to δ 3.70 dd (*J*=11.5, 5 Hz)–3.90 dd (*J*=11.5, 2.5 Hz). On acid methanolysis, this liberated methyl xyloside and methyl glucoside identified by gc comparison with standards. The xylosyl moiety is assumed to be located at C-3 on the basis of the chemical shift of the anomeric proton at δ 4.40, and accordingly the glucopyranosyl unit is attached at C-24 in agreement with the chemical shift of its anomeric proton at δ_H 4.31 (1). We have systematically observed the anomeric proton signal of the xylopyranosyl unit appearing at δ 4.40–4.44 ppm in the ^{1}H-nmr spectra of steroidal 3-*O*-xylopyranosides, whereas in the spectra of the steroidal 24-*O*-xylopyranosides the same signal is seen at δ 4.24–4.26 ppm (1).

Thus, distolasteroside D_4 can be defined as (24*S*)-3-*O*-xylopyranosyl-24-*O*-glucopyranosyl-6-*O*-sulfate-5α-cholestane-3β,6α,8,15β,24-pentaol [**3**]. Once again, the 24*S* configuration is suggested only by analogy with distolasteroside D_5, for which the 24*S* configuration has been determined (see below).

Distolasteroside D_5 [**4**].—The fabms (negative ion) showed a molecular anion peak at *m/z* 677 $[MSO_3]^-$. Examination of ^{1}H-nmr data (Table 3) indicated that compound **4** contains a β-xylopyranosyl unit, as confirmed by acid methanolysis, which afforded methyl xyloside. In addition to the sugar moiety, the ^{1}H-nmr spectrum showed signals for the aglycone protons reminiscent of those observed in the spectrum of indicoside C (14) and in other starfish glycosides with a 3β,6α,8,15β,16β-pentahydroxycholestane tetracyclic nucleus (1), namely one double triplet at δ 3.78 (H-6β, *J*=5 and 10.5 Hz), one triplet at δ 4.16 (H-16α, *J*=6.5 Hz), and one double doublet at δ 4.40 (H-15α, *J*=6.5, 5.5 Hz), these two latter coupled to each other. The downfield chemical shift to δ 4.27 m and two olefinic double doublets observed at δ 5.50 (*J*=15, 7 Hz) and 5.65 (*J*=15, 7 Hz), suggested a sulfate group at C-3 and a *trans* double bond at C-22 and C-23. The downfield shifts of both the Me-21 protons (δ 1.08 d) and the H-24 (δ 3.71; δ 3.34 in saturated 24-hydroxysteroids) are in agreement with the Δ^{22E}-double bond. The chemical shifts of the remaining methyl signals, δ 0.91 d, 0.99 d, 1.04 s, and 1.32 s are close to those observed in indicoside C (14) and assigned to H_3-26, -27, -19, and -18, respectively. In addition, we would note that the shape of the H-7α signal, dd (*J*=12, 3.5 Hz) at δ 2.42, is consistent with the presence of an hydroxyl group at C-8. Thus distolasteroside D_5 is assumed to be a xylopyranoside of (22*E*)-5α-cholest-22-ene-3β,6α,8,15β,16β,24-hexaol-3-sodium sulfate.

TABLE 3. ^{1}H-nmr Data for **4** and its Steroidal Aglycone **4a**.

Position	Compound	
	4	**4a**
3	4.27 m	4.27 m
6	3.78 ddd (10.5, 10.5, 3.5)	3.78 ddd
7	2.42 dd (12, 3.5)	2.41 dd
15	4.40 dd (5.5, 6.5)	4.40 dd
16	4.16 t (6.5)	4.15 t
18	1.32 s	1.32 s
19	1.04 s	1.04 s
21	1.08 d (6.8)	1.09 d (6.8)
22	5.65 dd (15, 7)	5.74 dd (15, 7)
23	5.50 dd (15, 7)	5.48 dd (15, 7)
24	3.71 dd (7, 2.5)	3.71 t
26	0.91 d (7.0)	0.89 d (7.0)
27	0.99 d (7.0)	0.94 d (7.0)

[a]Xylose: H-1′ 4.33 d (7.5), H-2′ 3.18 dd (7.5, 9), H-3′ 3.30 (under solvent signal), H-4′ 3.50 m, H-5′ 3.20 t (10.5), H-5″ 3.81 dd (10.5, 3.5).

Removal of the xylose unit by enzymatic hydrolysis with the glycosidase mixture from *Charonia lampas* yielded the 5α-cholest-22-ene-3β-6α,8,15β,16β,24-hexaol-3-sodium sulfate [**4a**], fabms (negative ion), *m/z* 545 $[MSO_3]^-$, whose ^{1}H-nmr spectrum, when compared with that of the natural glycoside, showed small but significant differences of the chemical shifts of the side chain protons (Table 3) whereas the nuclear proton signals were virtually superimposable in both spectra. This led to the conclusion that in **4** the β-xylopyranosyl unit is attached at C-24 of the side chain. Once again we note the upfield shift of the anomeric proton at δ 4.33, as expected for a 24-*O*-xylopyranoside (1). The 24*R* configuration was assigned by using the exciton chirality method of the allylic benzoates described by Gonnella and Nakanishi (15). The cd curve ($\Delta\epsilon_{245}$ −8.2) of the 24-*O*-*p*-bromobenzoate of **4a** was in full agreement with the data reported for 24-allylic *p*-bromobenzoate of 24*R* Δ^{22}-24-hydroxysteroids (1). Thus, distolasteroside D_5 can be defined as (22*E*,24*R*)-24-*O*-β-xylopyranosyl-5α-cholest-22-ene-3β,6α,8,15β,16β,24-hexaol-3-sodium sulfate [**4**].

ASTEROSAPONINS: KNOWN COMPOUNDS.—*Thornasteroside A* [**7a**] *and versicoside A* [**7**].—Identification of the known thornasteroside A [**7a**] was achieved by direct comparison (^{1}H nmr, ^{13}C nmr, fabms, hplc) with an authentic sample.

The fabms (negative ion) of the astersaponin **7** gave a molecular anion peak at *m/z* 1405 $[MSO_3]^-$. The ^{1}H- and ^{13}C-nmr spectra of the intact saponin revealed signals due to the common thornasteryl-3-*O*-sulfate aglycone, and these data met the structure requirements of versicoside A [**7**], the first example of a hexaglycoside isolated from starfishes (1,8). A detailed comparison of the ^{13}C-nmr data (Table 4) for **7** and versicoside A (8,16) indicated that all the signals were virtually superimposable in the two spectra. In confirmation, the asterosaponin **7** was subjected to permethylation followed by methanolysis to give methyl 2,3,4-tri-*O*-methylquinovoside and methyl 2,3,4,6-tetra-*O*-methylgalactoside, thus implying that the sugar moiety has one branch with terminal quinovose and galactose units. Enzymatic hydrolysis with *Ch. lampas* glycosidase mixture gave an inseparable mixture of a major tetrasaccharide [**7b**], fabms (negative ion) *m/z* 1097 (100%) $[MSO_3]^-$, and a minor pentasaccharide **7a**, fabms (negative ion) 1243 (20%), $[MSO_3]^-$. Fabms on the mixture in the positive ion mode showed cationized molecular ion species at *m/z* 1289 $[MSO_3Na+Na]^+$ and 1267 $[MSO_3Na+H]^+$ corre-

sponding to the pentasaccharide **7a** and at *m/z* 1143 $[MSO_3Na+Na]^+$ and 1121 $[MSO_3Na+H]^+$ corresponding to the tetrasaccharide **7b**. An accurate analysis of the ^{13}C-nmr spectrum of the mixture (Table 4) confirmed the presence of the major tetrasaccharide **7b**. The spectrum also contained eight additional small signals assigned to the terminal fucosyl unit and to C-2 and C-3 of a 2-linked galactosyl moiety in the minor pentasaccharide **7a** (Table 4). Thus, the T Gal$^{1-3}\mapsto$Fuc$^{1-2}\mapsto$Gal sequence linked to the common trisaccharide $^{1-4}\mapsto$Xyl ($^{1-2}\mapsto$Qui) $^{1-3}\mapsto$Qui-aglycone was established, and the structure of the asterosaponin **7** definitively identified as versicoside A (8,16). We note that the pentasaccharide **7a** obtained by enzymic cleavage corresponds to the common thornasteroside A, as confirmed by the detailed comparison of their ^{13}C-nmr spectral data.

Nipoglycoside A [**8**].—This new saponin is isomeric with versicoside A [**7**]; the saccharide chain differs from **7** in that it has a terminal glucose instead of a terminal galactose unit and steroidal side chain has a 22,23-epoxy function instead of the common carbonyl group at C-23 in **7**. The fabms (negative ion) gave a molecular anion peak at *m/z* 1405 and a fragment at *m/z* 1243 $[MSO_3-162]^-$, corresponding to the loss of glucosyl (or galactosyl) residue. Acid methanolysis liberated methyl xyloside, methyl fucoside, methyl quinovoside, methyl galactoside, and methyl glucoside in the ratio 1:1:2:1:1. The ^{1}H-nmr spectrum of the intact nipoglycoside A [**8**] revealed signals due to the aglycone protons identical with those observed in tenuispinosides A and B isolated from *Coscinasterias tenuispina* (10) and in the asteroside A derived from *Asterias amurensis* (17), asterosaponins containing the (20*R*,22*R*,23*S*)-22,23-epoxycholest-9(11)-ene-3β,6α,20-triol-3β-sulfated aglycone. Particularly diagnostic for the 22,23-epoxy side chain were the two signals at δ 2.94 (td, J=6.0, 2.5 Hz, H-23) and 2.76 (d, J=2.5 Hz, H-22). In the ^{13}C-nmr spectrum of **8**, the aglycone carbon signals (Experimental) were superimposable with those of tenuispinoside A (10) and asteroside A (17), thus also confirming that the oligosaccharide is attached at C-6 and the sulfate at C-3 of the steroid, a general feature of asterosaponins. The sugar carbon signals (Table 4) were easily assigned, assuming a saccharide chain like that of versicoside A [**7**] with the only difference being the terminal galactose replaced by glucose. This was confirmed by permethylation followed by acid methanolysis, affording methyl 2,3,4-tri-*O*-methylquinovoside and methyl 2,3,4,6-tetra-*O*-methylglucoside, thus implying quinovose and glucose as terminal sugar units. Enzymic hydrolysis with a glycosidase mixture from *Ch. lampas*, gave the trisaccharide **8c**, fabms *m/z* 935 $[MSO_3]^-$, made up of xylose and quinovose (1:2), and a mixture of the tetrasaccharide **8b**, fabms (negative ion) *m/z* 1097, and the pentasaccharide **8a**, fabms (negative ion) *m/z* 1243, in a ratio ca. 1:1, resistant to attempts at separation. On acid methanolysis this mixture liberated methyl xyloside, methyl fucoside, methyl quinovoside, and methyl galactoside in a ratio ca. 1:0.5:2:1, and in the ^{13}C-nmr spectrum the sugar carbon signals match very closely those of the mixture of prosapogenols **7a** and **7b** obtained by enzymic hydrolysis of versicoside **7**.

Thus the novel nipoglycoside A can be defined as sodium (20*R*,22*R*,23*S*)-6α-*O*-{β-D-glucopyranosyl-(1↦3)-β-D-fucopyranosyl-(1↦2)-β-D-galactopyranosyl-(1↦4)-[β-D-quinovopyranosyl-(1↦2)]-β-D-xylopyranosyl-(1↦3)-β-D-quinovopyranosyl}-22,23-epoxy-20-hydroxy-5α-cholest-9(11)-en-3β-yl sulfate [**8**].

Nipoglycoside B [**9**], *C* [**10**], *and D* [**11**].—The saccharide chain is identical in the three compounds, and differences are only found in the steroidal side chains. Compounds **9** and **10** contain the previously known Δ^{24},23-keto and 23-keto functionalities, respectively, whereas the aglycone of **11** has a 23-hydroxy group not previously encountered among steroids of starfish asterosaponins.

TABLE 4. Carbon Chemical Shifts (pyridine-d_5, ppm) of Saccharide Chains of Asterosaponins.

Sugar carbon	Compound					
	7	**7b**[a]	**7a**[a]	**8**	**9**	**11**
	Qui I	Qui I	Qui I	Qui I	Glc I	Glc I
1	103.6	104.9		104.2	104.5	104.5
2	73.6	73.8		73.8	74.8	74.8
3	88.8	88.2		89.0	91.1	91.1
4	73.4	73.5		73.5	69.4	69.4
5	71.7	71.2		72.0	76.1	76.1
6	17.7	17.4		17.5	62.1	62.1
	Xyl	Xyl	Xyl	Xyl	Qui I	Qui I
1	103.4	103.4		103.5	103.4	103.4
2	81.9	82.1		81.9	83.0	83.0
3	74.7	74.9		75.0	75.1	75.1
4	77.6	77.7		77.6	85.5	85.5
5	63.6	62.7		63.7	73.6	73.6
6					17.8	17.8
	Qui II	Qui II	Qui II	Qui II	Qui II	Qui II
1	105.0	104.9		104.7	105.3	105.3
2	75.1	75.2		75.1	75.4	75.4
3	76.4	76.2		76.4	77.6	77.6
4	75.5	76.1		75.6	76.7	76.7
5	73.2	73.4		73.2	73.5	73.5
6	17.8	17.8		17.9	18.1	18.1
	Gal I	Gal	Gal	Gal	Glc II	Glc II
1	101.2	102.7		101.4	102.4	102.4
2	82.6	71.1	82.5	82.7	83.7	83.7
3	74.1	74.3	73.6	74.3	77.2	77.2
4	68.7	69.2		68.6	71.1	71.1
5	76.1	76.1		76.4	77.9	77.9
6	61.5	61.5		61.6	62.9	62.9
	Fuc		Fuc	Fuc	Fuc	Fuc
1	105.4		105.5	105.9	106.5	106.5
2	70.8		71.1	71.0	71.5	71.5
3	83.3		74.3	83.5	74.8	74.8
4	71.1		71.6	71.1	72.3	72.3
5	71.3		71.2	71.4	71.7	71.7
6	16.3		16.3	16.4	62.2	16.8
	Gal II			Glc		
1	105.3			105.8		
2	72.3			74.4		
3	73.7			76.4		
4	69.3			72.4		
5	76.1			76.9		
6	61.4			61.6		

[a]^{13}C nmr spectrum has been taken on a 8:2 mixture of the tetrasaccharide **7b** and pentasaccharide **7a**.

Structure determination of the saccharide chain was carried out mostly on compound **9**: fabms (negative ion) *m/z* 1255 $[MSO_3]^-$ and fragments at *m/z* 1109 $[MSO_3-146]^-$ and 947 $[1109-162]^-$, corresponding to the sequential losses of deoxyhexose and hexose units. The Δ^{24},23-keto structure of the aglycone was indicated by the olefinic singlet at δ_H 6.20, s (1H), the olefinic methyl protons at 1.93 and 2.15 and the methyl doublet at 0.94, d in the ^{1}H-nmr spectrum; the same signals were observed in the spectrum of marthasteroside B (18), an asterosaponin containing the

3β,6α-dihydroxycholesta-9(11),24-diene-24-one-3β-sulfated aglycone (18,19). The ^{13}C-nmr spectrum (Experimental) supported the presence of marthasterone B 3β-sulfated aglycone (18).

Elucidation of the pentasaccharide moiety was carried out as follows. On acid methanolysis **9** liberated methyl fucoside, methyl quinovoside, and methyl glucoside in the ratio 1:2:2. ^{1}H-nmr [anomeric proton signals at δ 4.47 (1H), 4.56 (2H), and 4.57 (2H), doublets with *J* ranging from 7.0 to 7.5 Hz] and ^{13}C-nmr [five anomeric carbon signals at 102.4, 103.4, 104.5, 105.3, and 106.5 ppm] spectroscopy indicated that the glycosidic linkages were β-oriented. Permethylation followed by methanolysis of the methylated material gave methyl 2,3,4-tri-*O*-methylquinovoside and methyl 2,3,4-tri-*O*-methylfucoside, implying that the sugar moiety of **9** has one branch. On enzymatic hydrolysis with *Ch. lampas* glycosidase mixture, nipoglycoside B [**9**] gave, after 8 h reaction, the trisaccharide **9a** made up from quinovose and glucose in a 1:2 ratio: fabms *m/z* 947 $[MSO_3]^-$. The ^{1}H-nmr signals of this shorter glycoside matched very closely those observed in the β-D-quinovopyranosyl-(1↦2)-β-D-quinovopyranosyl-(1↦3)-β-D-glucopyranosyl(1↦6)-sulfomarthasterone B, obtained in an analogous manner (enzymic hydrolysis) from marthasteroside B (19). Thus the sequence Fuc↦Glc↦(Qui↦)Qui↦Glc↦aglycone for the saccharide chain of **9** followed. The interglycosidic linkages were deduced from ^{13}C-nmr data (Table 4) and comparison with the many asterosaponins isolated from starfishes, especially marthasteroside B (19), and pectinioside A (20). Thus, nipoglycoside B can be defined as sodium 6α-*O*-{β-D-fucopyranosyl-(1↦2)-β-D-glucopyranosyl-(1↦4)-[β-D-quinovopyranosyl-(1↦2)]-β-D-quinovopyranosyl-(1↦3)-β-D-glucopyranosyl}-cholesta-9(11),24-dien-23-one-3β-yl sulfate [**9**].

The structure of nipoglycoside C [**10**], fabms (negative ion) *m/z* 1257 $[MSO_3]^-$ 1111 (loss of fucosyl unit) and 949 (losses of deoxyhexose and hexose units), both with two mass units shifted relative to **9**, followed from the same steps described above.

The structure of the saccharide chain of nipoglycoside D [**11**] was determined only by ^{13}C-nmr spectroscopy (Table 4) and comparison with that of nipoglycoside B [**9**]. For the aglycone portion, the presence of an hydroxylated side chain was first suggested by ^{13}C nmr, in which the usual carbonyl signal at δ_C 211.6 in **7** was replaced by an hydroxymethine carbon at δ_C 67.2 ppm. The fabms (negative ion) of **11** showed a molecular anion peak at *m/z* 1259 $[MSO_3]^-$, two mass units shifted relative to the 23-oxo-steroidal nipoglycoside C [**10**] *m/z* 1257. Analysis of the ^{13}C-nmr spectral data and the known substitution effects located the hydroxy group in the side chain at the C-23 position. The carbon signals assigned to the tetracyclic nucleus of **11** are virtually superimposable with the corresponding ones of **7**, **8**, and **9** and the many asterosaponins with the $\Delta^{9(11)}$-3β-6α-dioxysteroidal aglycone bearing a sulfate at C-3 and the oligosaccharide moiety at C-6. On acid hydrolysis, nipoglycoside D [**11**] yielded the steroid aglycone **14**, fabms (negative ion) *m/z* 417 $[M-H]^-$, which was characterized by ^{1}H nmr (Experimental). The 23*R* configuration was suggested by the downfield shift of the Me-18 signal (δ 1.06 vs. 0.95 in **14**) in the ^{1}H nmr of the benzoate derivative. In the case of the 23*S* configuration, we would expect a significant upfield shift (ca. δ_H 0.95) of the same signal (21). Thus nipoglycoside D can be defined as sodium (23*R*)-6α-*O*-{β-D-fucopyranosyl-(1↦2)-β-D-glucopyranosyl-(1↦4)-[β-D-quinovopyranosyl-(1↦2)]-β-D-quinovopyranosyl-(1↦3)-β-D-glucopyranosyl}-cholest-9(11)-en-23-ol-3β-yl sulfate [**11**].

EXPERIMENTAL

GENERAL EXPERIMENTAL PROCEDURES.—Nmr spectra, Bruker WM-250 (^{1}H at 250 MHz, ^{13}C at 62.9 MHz), δ (ppm), *J* in Hz, spectra referred to CHD_2OD signal at 3.34 ppm and to CD_3OD central carbon

signal at 49.0 ppm; mass spectra, VG ZAB mass spectrometer equipped with fab source [in glycerol or glycerol-thioglycerol (3:1) matrix; Xe atoms of 2-6 kV]; optical rotations Perkin-Elmer model 241 polarimeter; gc, Carlo Erba Fractovap 2900 for capillary column (SE-30, 25 mt, 150°, helium carrier flow 2 ml·min^{-1}); reversed-phase hplc, C_{18} μ-Bondapak column (30 cm×3.9 mm i.d.; flow rate 2 ml·min^{-1}), Waters Model 6000 A or 510 pump equipped with a U6K injector and a differential refractometer, model 401; dccc, DCC-A apparatus manufactered by Tokyo Rikakikai Co., equipped with 250 tubes and Buchi apparatus equipped with 300 tubes.; cd, JASCO J500A spectropolarimeter.

EXTRACTION AND ISOLATION.—The animals, 5 kg, *D. nipon*, were collected at Mutsu Bay, Japan; a voucher specimen is preserved at the Faculty of Agriculture, Tohoku University, Sendei. The animals were chopped and soaked in H_2O: the aqueous extracts were decanted and eluted through a column of Amberlite XAD-2. This column was washed with distilled H_2O and eluted with MeOH to give, after removal of the solvent, a glassy material (1.2 g). The MeOH eluate was chromatographed on Sephadex LH-60 column (2.5 cm×80 cm) with MeOH-H_2O (2:1) as eluent. Fractions (4 ml) were collected and monitored by tlc on SiO_2 with *n*-BuOH–HOAc–H_2O (12:3:5) and $CHCl_3$-MeOH-H_2O (80:18:2).

Fractions 71–91 (416 mg) contained crude asterosaponins, fractions 92–99 (190 mg) were a mixture of sulfated compounds, and fractions 100–120 contained the crude glycosides and polyhydroxysteroids (149 mg).

Fractions 100–120 (149 mg) were submitted to dccc using the solvent system $CHCl_3$-MeOH-H_2O (7:13:8) in the ascending mode (the lower phase was the stationary phase). Fractions (5 ml) were collected, monitored by tlc on SiO_2 with $CHCl_3$-MeOH-H_2O (80:18:2), and accordingly combined. Each of the above fractions was then submitted to hplc with MeOH-H_2O (7:3) on a C_{18}μ- Bondapak column (30 mm×3.9 mm i.d.), to give pure distolasterosides D_1 [**1**] and D_2 [**2**] and steroids **12** and **13**. Rotation data are in Table 1.

Fractions 92–99 (190 mg) eluted from LH-60, containing sulfated compounds, were submitted to dccc using *n*-BuOH–Me_2CO–H_2O (3:1:5) in the ascending mode (the lower phase was stationary phase; flow rate 12 ml/h); 6-ml fractions were collected and monitored by tlc. Fractions 27–88 contained pisasteroside A [**6**], which was further purified by hplc on the C_{18} column with MeOH-H_2O (55:45); fractions 89–157 contained distolasteroside D_5 [**4**], which was purified with MeOH-H_2O (1:1); fractions 158–210 contained distolasteroside D_4 [**3**], pycnopodioside C [**5**], and pisasteroside A [**6**], which were purified with MeOH-H_2O (45:55).

Fractions 71–91 (416 mg) from LH-60, containing asterosaponins, were submitted to dccc in *n*-BuOH–Me_2CO–H_2O (3:1:5) in descending mode (the upper phase was the stationary phase; flow rate 10 ml/h); 5-ml fractions were collected and combined according to tlc. Final purification affording pure asterosaponins **7**–**11** was achieved by reversed-phase hplc (30 mm×8 mm i.d. column) with MeOH-H_2O (45:55).

SPECTRAL DATA.—*Distolasteroside D_1* [**1**].—Negative ion fabms *m/z* [M−H]$^-$ 715 (100%), [(M−H)−132]$^-$ 583 (10); ^{1}H nmr (CD_3OD) δ_H (aglycone) 0.95 (3H, d, J=7 Hz, H_3-26 or H_3-27), 0.97 (3H, d, J=6.8 Hz, H_3-21), 1.02 (3H, s, H_3-19), 1.29 (3H, s, H_3-18), 2.40 (1H, dd, J=12, 3.5 Hz, H-7), 2.40 (1H, m, H-16), 3.55 (1H, m, H-3α), 3.73 (1H, ddd, J=10.5, 10.5, 3.5 Hz, H-6), 4.45 (1H, bt, J=5.5, H-15); δ_H (sugars) 3.18, 3.16 (each 1H, t, H-5′ and H-5″), 3.22 and 3.31 (each 1H, dd, H-2′ and H-2″), 3.50 (under solvent signals, H-3′ and H-3″, H-4′ and H-4″), 3.85 (each 1H, dd, J=10, 5, H-5′ and H-5″), 4.26 (1H, d, J=7 Hz, H-1′), 4.39 (1H, d, J=7 Hz, H-1″).

Distolasteroside D_2 [**2**].—Negative ion fabms *m/z* [M−H]$^-$ 713 (100%), [(M−H)−132]$^-$ 581 (25); ^{1}H nmr (CD_3OD) δ_H (aglycone) 0.90 (3H, d, J=7 Hz, H_3-26 or H_3-27), 0.97 (3H, d, J=7 Hz, H_3-27 or H_3-26), 1.02 (3H, s, H_3-19), 1.05 (3H, d, J=6.8 Hz, H_3-21), 1.32 (3H, s, H_3-18), 2.40 (2H, m, H-16 and H-7), 3.55 (1H, m, H-3α), 3.73 (1H, ddd, J=10.5, 10.5, 3.5 Hz, H-6), 4.47 (1H, bt, J=5.5 Hz, H-15), 5.41 (2H, t, J=6.3 Hz, H-22 and H-23); δ_H (sugars) identical to those of **1** except for H-1′ at δ 4.24 (1H, d, J=7 Hz).

Distolasteroside D_4 [**3**].—^{1}H nmr (CD_3OD) δ_H (aglycone) 0.96 (9H, d, J=7 Hz, H_3-21, H_3-26, H_3-27), 1.01 (3H, s, H_3-19), 1.29 (3H, s, H_3-18), 2.44 (1H, m, H-16), 2.23 (1H, dd, J=12, 3.5 Hz, H-7), 3.72 (1H, ddd, partially overlapped with other signals, H-6), 3.60 (1H, m, overlapped signal, H-3α), 4.47 (1H, m, H-15); ^{1}H and ^{13}C nmr shifts of the saccharide units see Table 2.

Distolasteroside D_5 [**4**].—^{1}H nmr see Table 3.

Pycnopodioside C [**5**].—Negative ion fabms *m/z* [M]$^-$ 693 (100%); ^{1}H nmr (CD_3OD) δ_H (aglycone) 0.96 (9H, d, J=7 Hz, H_3-21, H_3-26, H_3-27), 1.01 (3H, s, H_3-19), 1.29 (3H, s, H_3-18), 2.41 (1H, m, H-16), 2.42 (1H, dd, J=12, 3.5 Hz, H-7), 3.55 (1H, m, H-3α), 3.71 (1H, ddd, J=10.5, 10.5, 3.5 Hz, H-6), 4.45 (1H, bt, J=5.5 Hz, H-15); δ_H (sugars) 3.20 (1H, dd, J=7.5, 9.5, Hz, H-2′), 3.37 (1H, t, J=9.5 Hz, H-3′), 3.50 (1H, m, H-5′), 4.17 (1H, dd, J=5, 11.5 Hz, H-6′), 4.31 (1H, d, J=7.5 Hz, H-1′), 4.33 (1H, dd, J=2.5, 11.5 Hz, H-6′).

Pisasteroside A [**6**].—Negative ion fabms *m/z* $[M]^-$ 721 (100%); ^{1}H nmr (CD_3OD) δ_H (aglycone) 0.88 (3H, d, J=6.5 Hz, H_3-26 or H_3-27), 0.94 (3H, d, J=6.5 Hz, H_3-27 or H_3-26), 1.02 (3H, s, H_3-19), 1.07 (3H, d, J=6.5 Hz, H_3-21), 1.31 (3H, s, H_3-18), 2.43 (1H, dd, J=5, 12 Hz, H-7), 2.65 (1H, m, H-20), 3.50 (1H, m, H-3α), 3.54 (1H, t, J=10 Hz, H-28), 3.74 (1H, dd, J=5, 15 Hz, H-6), 3.99 (1H, dd, J=5, 10 Hz, H-28), 4.25 (1H, t, J=6.5 Hz, H-16), 4.43 (1H, dd, J=5.6, 6.7 Hz, H-15), 5.33 (1H, dd, J=9, 15 Hz, H-23), 5.48 (1H, dd, J=9, 15 Hz, H-22); δ_H (sugars) 3.21 (1H, dd, J=7, 9 Hz, H-2′), 3.35 (1H, under solvent signal, H-3′), 3.49 (2H, m, H-4′ and H-5′), 4.16 (1H, dd, J=2.5, 12 Hz, H-6′), 4.31 (1H, d, J=7 Hz, H-1′), 4.33 (1H, dd, J=5, 12 Hz, H-6′).

Versicoside A [**7**].—^{1}H nmr (CD_3OD) δ_H (aglycone) 0.81 (3H, s, H_3-18), 0.94 and 0.95 (each 3H, two overlapping doublets, J=6.8 Hz, H_3-26 and H_3-27), 1.02 (3H, s, H_3-19), 1.37 (3H, s, H_3-21), 2.41 (2H, d, J=7.5, H_2-24), 2.61 (2H, ABq, J=15, H_2-22), 4.22 (1H, m, H-3α), 5.37 (1H, br d, J=5, H-11); δ_H (sugars) 1.30, 1.32, 1.40 (each 3H, d, J=6.8 Hz, H_3-5 quinovose and fucose), 4.14 (1H, dd, H_e-5 of xylose), 4.42, 4.48, 4.50, and 4.57 (d, anomeric-H_x); ^{13}C nmr (pyridine-d_5) δ_C (aglycone) 35.3 (C-1), 28.6 (C-2), 77.3 (C-3), 30.0 (C-4), 48.5 (C-5), 79.5 (C-6), 40.5 (C-7), 34.7 (C-8), 144.9 (C-9), 37.7 (C-10), 116.0 (C-11), 41.8 (C-12), 40.9 (C-13), 53.3 (C-14), 23.8 (C-15), 24.4 (C-16), 58.8 (C-17), 12.9 (C-18), 18.6 (C-19), 73.2 (C-20), 26.3 (C-21), 54.4 (C-22), 211.9 (C-23), 53.3 (C-24), 23.8 (C-25), 21.8 (C-26), 22.0 (C-27); δ_C (sugars) see Table 4.

Nipoglycoside A [**8**].—^{1}H nmr (CD_3OD) δ_H (aglycone) 0.82 (3H, s, H_3-18), 1.01 (6H, d, J=7 Hz, H_3-26 and H_3-27), 1.02 (3H, s, H_3-19), 2.76 (1H, d, J=2.5, H-22), 2.94 (1H, dt, J=2.5, 6, H-23), 4.22 (1H, m, H-3α), 5.37 (1H, br d, J=5.5, H-11), δ_H (sugars) 1.30, 1.32, 1.40 (each 3H, d, J=6.8 Hz, H_3-5 quinovose and fucose), 4.15 (1H, dd, H_e-5 of xylose), 4.42, 4.50, 4.57 (d, anomeric-H_x); ^{13}C nmr (pyridine-d_5) δ_C (aglycone) 35.4 (C-1), 28.8 (C-2), 78.0 (C-3), 30.1 (C-4), 48.6 (C-5), 79.1 (C-6), 41.6 (C-7), 34.6 (C-8), 145.2 (C-9), 37.6 (C-10), 116.0 (C-11), 41.8 (C-12), 41.1 (C-13), 52.8 (C-14), 22.7 (C-15), 24.6 (C-16), 58.9 (C-17), 12.9 (C-18), 18.6 (C-19), 69.5 (C-20), 22.8 (C-21), 65.2 (C-22), 53.0 (C-23), 41.1 (C-24), 26.1 (C-25), 22.6 (C-26), 22.0 (C-27), δ_C (sugars) see Table 4.

Nipoglycoside B [**9**].—^{1}H nmr (CD_3OD) δ_H (aglycone) 0.70 (3H, s, H_3-18), 0.94 (3H, d, J=6.8 Hz, H_3-21), 1.03 (3H, s, H_3-19), 1.93 and 2.15 (each 3H, s, H_3-26 and H_3-27), 4.22 (1H, m, H-3α), 5.36 (1H, br d, J=5, H-11), 6.20 (1H, s, H-24), δ_H (sugars) 1.28, 1.40, 1.47 (each 3H, d, J=6.8 Hz, H_3-5 quinovose and fucose), 4.47, 4.56, 4.57 (anomeric-H_x); ^{13}C nmr (pyridine-d_5) δ_C (aglycone) 35.9 (C-1), 29.2 (C-2), 77.9 (C-3), 30.6 (C-4), 49.1 (C-5), 80.1 (C-6), 41.1 (C-7), 35.5 (C-8), 145.7 (C-9), 38.2 (C-10), 116.3 (C-11), 41.7 (C-12), 41.1 (C-13), 53.7 (C-14), 25.1 (C-15), 28.5 (C-16), 56.4 (C-17), 11.5 (C-18), 19.1 (C-19), 32.9 (C-20), 19.5 (C-21), 51.4 (C-22), 200.6 (C-23), 153.8 (C-24), 154.4 (C-25), 27.0 (C-26), 20.3 (C-27), δ_C (sugars) see Table 4.

Nipoglycoside C [**10**].—^{1}H nmr (CD_3OD) δ_H (aglycone) 0.70 (3H, s, H_3-18), 0.92 (3H, d, J=7 Hz, H_3-21), 0.93 (6H, d, J=7 Hz, H_3-26 and H_3-27), 5.36 (1H, br d, J=5 Hz, H-11), δ_H (sugars) signals identical to those reported for **9**.

Nipoglycoside D [**11**].—^{1}H nmr (CD_3OD) δ_H (aglycone) 0.68 (3H, s, H_3-18), 0.93 and 0.96 (each 3H, d, J=7 Hz, H_3-26 and H_3-27), 1.01 (3H, d, J=7 Hz, H_3-21), 1.03 (3H, s, H_3-19), 5.37 (1H, br d, J=5 Hz, H-11), δ_H (sugars) signals identical to those reported for **9**; ^{13}C nmr (pyridine-d_5) δ_C (aglycone) 35.4 (C-1), 28.8 (C-2) 77.5 (C-3), 30.3 (C-4), 48.6 (C-5), 79.6 (C-6), 40.6 (C-7), 35.0 (C-8), 145.3 (C-9), 37.7 (C-10), 116.0 (C-11), 41.4 (C-12), 40.9 (C-13), 53.2 (C-14), 24.2 (C-15), 28.3 (C-16), 56.9 (C-17), 11.1 (C-18), 18.8 (C-19), 33.6 (C-20), 18.8 (C-21), 45.3 (C-22), 67.2 (C-23), 46.5 (C-24), 24.9 (C-25), 21.4 (C-26), 23.6 (C-27), δ_C (sugars) see Table 4.

Steroid **12**.—Negative ion fabms *m/z* $[M]^-$ 493 (100%); ^{1}H nmr (CD_3OD) δ_H 0.71 (3H, s, H_3-18), 0.94 (3H, d, J=6.8 Hz, H_3-21), 1.01 (3H, s, H_3-19), 1.94 (3H, s, H_3-26 or H_3-27), 2.15 (3H, s, H_3-27 or H_3-26), 3.54 (1H, dt, J=12, 3 Hz, H-6), 4.24 (1H, m, H-3α), 5.37 (1H, bd, J=5 Hz, H-11), 6.20 (1H, s, H-24).

Steroid **13**.—Negative ion fabms *m/z* $[M]^-$ 495 (100%); ^{1}H nmr (CD_3OD) δ_H 0.70 (3H, s, H_3-18), 0.93 (3H, d, J=6.8 Hz, H_3-21), 0.94 (6H, d, H_3-26 and H_3-27), 3.54 (1H, dt, J=12, 3 Hz, H-6), 4.24 (1H, m, H-3α), 5.37 (1H, bd, J=5 Hz, H-11).

SOLVOLYSIS OF DISTOLASTEROSIDE D_4 [**3**].—A solution of **3** (1 mg) in pyridine (0.1 ml) and dioxane (0.1 ml) was heated at 130° for 2 h in a stoppered reaction vial. The residue was purified by hplc (C_{18} μ-Bondapak column) with MeOH-H_2O (7:3) to give the desulfated material: fabms (negative ion) *m/z* $[M-H]^-$ 745; ^{1}H nmr (δ_H in CD_3OD) for aglycone and xylose is superimposable to ^{1}H-nmr spectrum of **3**; δ_H (glucose) 4.32 (1H, d, J=7.5 Hz, H-1′), 3.20 (1H, dd, J=2.5, 7.5, H-2′), 3.38 (1H, under solvent signal, H-3′), 3.48 (1H, under solvent signal, H-4′), 3.50 (1H, m, H-5′), 3.90 (1H, dd, J=11.5, 2.5 Hz, H-6′), 3.70 (1H, dd, J=11.5, 5 Hz, H-6′).

METHANOLYSIS OF GLYCOSIDES: SUGAR ANALYSIS.—A solution of **3** desulfated in anhydrous 2M HCl in MeOH (0.5 ml) was heated at 80° in a stoppered reaction vial for 8 h. After having cooled, the reaction mixture was neutralized with Ag_2CO_3 and centrifuged, and the supernatant was evaporated to dryness under N_2 and then trimethylsilylated with TRISIL-Z (5 μl) (Pierce Chemical Co.) for 15 min at room temperature. Gc analysis (SE-30, 25 m capillary column, 125°) gave peaks which coeluted with those of the methylxylosides and methylglucosides.

ENZYMATIC HYDROLYSIS OF DISTOLASTEROSIDE D_5 [**4**].—Glycoside **4** (1 mg) in 1 ml of citrate buffer (pH 4.5) was incubated with 2.5 mg of glycosidase mixture of *Ch. lampas* (Scikagaku Kogyo) at 37° for 3 days. After the disappearance of the starting material, the reaction mixture was passed through a C_{18} Sep-pak cartridge, washed with H_2O, eluted with MeOH, and evaporated to dryness to give the corresponding hexaol **4a**: fabms *m/z* $[M]^-$ 545 (100%); ^1H nmr see Table 3.

The absolute configuration was checked by preparing the corresponding *p*-bromobenzoate [*p*-bromobenzoyl chloride in dry pyridine (1 ml) and 4-(dimethylamino)pyridine]. The mixture was stirred overnight at 60° under N_2, chilled H_2O was added to the solution, and after 30 min the solution was extracted with $CHCl_3$. The extract was washed successively with saturated aqueous $NaHCO_3$ and H_2O. The residue was evaporated and then purified by hplc (C_{18} μ-Bondapak column) with MeOH-H_2O (75:25) as eluent with a uv detector (UVLOG-5III, OYO-BUNKO KIKI Co.) at 259 nm. Cd measurement: (hexane) $\Delta\epsilon_{245}$ −8.2.

METHANOLYSIS OF SAPONINS: SUGAR ANALYSIS.—Methanolysis of versicoside A [**7**] and nipoglycosides A [**8**], B [**9**], C [**10**], and D [**11**] (0.5–1 mg) and subsequent gc analysis of the silylated sugar compounds (150° on a 25 m SE-30 capillary column) were carried out as previously described. The identification was based on co-chromatography with standards.

METHYLATION OF ASTEROSAPONINS FOLLOWED BY METHANOLYSIS: TERMINAL SUGARS.—A solution of **7** (5 mg) in 0.5 ml of dry DMF was slowly added under N_2 to a stirred mixture of NaH (60 mg) in dry DMF (0.5 ml) cooled in ice bath. The mixture was stirred for 15 min, and MeI (0.25 ml) was added. The reaction mixture was kept for a further 4 h at room temperature. The excess of NaH was destroyed by a few drops of MeOH and, after addition of H_2O, the mixture was extracted twice with $CHCl_3$. The organic layer was washed with H_2O and evaporated under vacuum. The residue in anhydrous 2 M HCl MeOH (0.2 ml) was heated at 80° in a stoppered reaction vial for 8 h. After cooling, the mixture was concentrated under a steam of N_2 and was used for gc analysis (Se-30, 25 m capillary column; 95°, helium carrier, flow rate 10 ml·min^{-1}). Gc peaks co-eluted with those of methyl 2,3,4-tri-*O*-methylquinovoside and methyl 2,3,4,6-tetra-*O*-methylgalactoside standards. In a analogous manner the saponins **8** and **9** were methylated followed by methanolysis to give from **8** methyl 2,3,4- tri-*O*-methylquinovoside and methyl 2,3,4,6-tetra-*O*-methylglucoside, and from **9** 2,3,4-tri-*O*-methylfucoside and 2,3,4-tri-*O*-methylquinovoside.

ENZYMATIC HYDROLYSIS OF VERSICOSIDE A [**7**].—Saponin **7** (5 mg) in 1 ml of citrate buffer (pH 4.5) was incubated with 6 mg of glycosidase mixture of *Ch. lampas* at 37° for 1 h. The reaction was followed by tlc on SiO_2 in *n*-BuOH–HOAc–H_2O (12:3:5). After the disappearance of the starting material, the reaction mixture was passed through a C_{18} Sep-pak cartridge, washed with H_2O, and eluted with MeOH. The mixture was purified by hplc (C_{18} μ-Bondapak column) with MeOH-H_2O (52:48) to give one peak containing both the pentasaccharide **7a** and the tetrasaccharide **7b**, in ratio ca. 8:2. The ^1H- and ^{13}C-nmr data were extracted from the spectrum of the mixture. (CD_3OD): δ_H (aglycone) 0.81 (1H, s, H_3-18), 0.94 (6H, d, J=6.8 Hz, H_3-26 and H_3-27), 1.02 (3H, s, H_3-19), 1.37 (3H, s, H_3-21), 2.40 (2H, d, J=7.0 Hz, H_2-24), 2.61 (2H, ABq, J=16.1 Hz, H_2-22), 4.23 (1H, m, H-3α), 5.37 (1H, br d, J=5.5 Hz, H-11); δ_H (sugar) 1.40, 1.30, 1.32 (small) H_3-5 quinovose and fucose), 4.14, (1H, dd, H_e-5 of xylose in **7a**), 4.10 (small) (1H, dd, H_e-5 of xylose in **7b**) 4.37, 4.42, 4.57, 4.59 (each 1H, d, J=7 Hz, anomeric H_a); ^{13}C nmr see Table 4.

ENZYMATIC HYDROLYSIS OF NIPOGLYCOSIDE A [**8**].—Saponin **8** (3.7 mg) in 1 ml of citrate buffer (pH 4.5) was incubated with 5 mg of glycosidase mixture of *Ch. lampas* at 37° for 4 h. The subsequent analysis was carried out in the condition previously described to give the trisaccharide **8c** and a mixture resistant to separation of the pentasaccharide **8a** and the tetrasaccharide **8b**.

Prosapogenols **8a** *and* **8b**.—Fabms (negative ion) see text; ^1H nmr δ_H (CD_3OD) (aglycone) identical to those reported for the intact **8**; δ_H (sugars) signals superimposable to data for **7a** and **7b**.

Prosapogenol **8c**.—Fabms (negative ion) see text; ^1H nmr (aglycone) identical to that of **8**; (sugars) 1.32, 1.40 (each 3H, d, J=6.8 Hz, H_3-5 of quinovose), 4.46, 4.54, 4.56 (1H, d, J=7.5 Hz, anomeric H_a).

ENZYMATIC HYDROLYSIS OF NIPOGLYCOSIDES B [**9**] AND C [**10**].—Saponins **9** (5.0 mg) and **10** (3.0 mg) in 1 ml of citrate buffer (pH 4.5) were incubated with 6 mg and 4 mg, respectively, of glycosidase mixture of *Ch. lampas* at 37° for 8 h. The analysis was carried out in the condition previously described to give the trisaccharides **9a** and **10a**: fabms (negative ion) see text; ^1H nmr δ_H (CD_3OD) (aglycone) identical

to those reported for the natural **9** and **10**; δ_H (sugars) 1.32, 1.40 (each 3H, d, J=7 Hz, H_3-5 quinovose), 4.45, 4.54, 4.56 (each 1H, d, J=7.5 Hz, anomeric H_x).

METHANOLYSIS OF NIPOGLYCOSIDE D [**11**].—A solution of **11** (1.8 mg) in anhydrous 2 M HCl MeOH (0.5 ml) was heated at 80° in a stoppered reaction vial for 30 min. After having cooled, the reaction mixture was neutralized with Ag_2CO_3 and centrifuged, and the supernatant was evaporated to dryness under N_2. The residue was purified by hplc (C_{18} μ-Bondapak column) with MeOH-H_2O (85:15).

Fabms (negative ion) *m/z* $[M-H]^-$ 417; ^{1}H nmr (CD_3OD) δ_H 0.68 (3H, s, H_3-18), 0.94 and 0.97 (each 3H, d, J=7 Hz, H_3-26 and H_3-27), 1.00 (3H, s, H_3-19), 1.01 (3H, d, J=7 Hz, H_3-21), 3.50 (1H, m, H-3α), 3.56 (1H, dt, J=12, 3 Hz, H-6), 3.73 (1H, m, H-23), 5.37 (1H, br d, J=5 Hz, H-11); ^{1}H nmr ($CDCl_3$) δ_H 0.62 (3H, s, H_3-18), 0.92 and 0.93 (each 3H, d, J=6.8 Hz, H_3-26 and H_3-27), 0.96 (3H, s, H_3-19), 0.95 (3H, d, J=7 Hz, H_3-21), 3.50 and 3.60 (overlapped signals, H-3α and H-6), 3.68 (1H, m, H-23), 5.31 (1H, br d, J=5 Hz, H-11).

The triol was benzoylated with benzoyl chloride (50 μl) in dry pyridine (100 μl) at room temperature for 3 h. After removal of the solvent the residue was eluted through a Pasteur pipette filled with a slurry of SiO_2 in $CHCl_3$ to give the 3,6,23-tribenzoate derivative: ^{1}H nmr ($CDCl_3$) δ_H 0.66 (3H, s, H_3-18), 0.95 and 0.96 (each 3H, d, J=7 Hz, H_3-26 and H_3-27), 1.06 (3H, d, J=7 Hz, H_3-21), 1.15 (3H, s, H_3-19), 5.00 (1H, m, H-3α), 5.17 (1H, dt, J=12, 3 Hz, H-6), 5.31 (1H, m, H-23α), 5.40 (1H, br d, J=5 Hz, H-11), 7.50, 7.65 and 8.15 (aromatic-H_x).

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