Starfish Saponins Part 40.[1] Structures of Two New 'Asterosaponins' from the Starfish *Patiria miniata*: Patirioside A and Patirioside B

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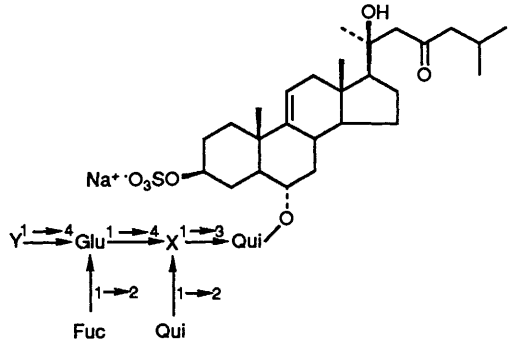
An investigation of the extracts from the starfish *Patiria miniata* has led to the isolation of four 'asterosaponins.' Two are new compounds designated as patirioside A (**1**) and patirioside B (**2**). They are hexaglycosides with two branches and have been characterized as sodium (20*R*,22*R*,23*S*,24*S*)-6α-*O*-{α-L-arabinopyranosyl-(1→2)-[(β-D-fucopyranosyl-(1→4)]-β-D-glucopyranosyl-(1→4)-[β-D-quinovopyranosyl-(1→2)]-β-D-xylopyranosyl-(1→3)-β-D-quinovopyranosyl}-22,23-epoxy-20-hydroxy-24-methyl-5α-cholest-9(11)-en-3β-yl sulphate (**1**) and sodium (20*S*)-6α-*O*-{β-D-fucopyranosyl-(1→2)-[β-D-galactopyranosyl-(1→4)]-β-D-glucopyranosyl-(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 sulphate (**2**). The spectral data of the two remaining asterosaponins agree with those of pectinioside G (**3**), an hexaglycoside recently described from *Asterina pectinifera*, and with those of acanthaglycoside C (**4**), a pentaglycoside first isolated from *Acanthaster planci*.

The saponins are responsible for the general toxicity of sea cucumbers and starfishes and have been reported to exhibit cytotoxic, haemolytic, ichthyotoxic, antiviral, antifungal, and antimicrobial activities.[2] The number of reported saponins from starfishes is rapidly growing and they have shown varied structures. We have suggested that they can be divided into three major groups: (a) the steroidal cyclic glycosides discovered in starfish of the genus *Echinaster*; (b) the steroidal mono- and di-glycosides which are found in both sulphated and non-sulphated forms; and (c) the 'asterosaponins' which are sulphated steroidal penta- and hexa-glycosides.[3–5] These latter are widely distributed and have many common structural features. The aglycone is always a $\Delta^{9(11)}$-3β,6α-dioxysterol, a sulphate residue is present at C-3, and the oligosaccharide is linked at C-6. Generalities also appear in the carbohydrate portion: sugars are in their pyranose forms with β-anomeric configuration (α for arabinose) and with a constant pattern of interglycosidic linkages, only varying in their position in the chain; a branch point is always located on the second monosaccharide (xylose or quinovose) starting from the aglycone. Recently a unique group of asterosaponins, wherein the hexasaccharide chain has two branches, has been isolated from the starfish *Asterina pectinifera*.[1,6–8]

Continuing with our work on biologically active compounds from echinoderms we have been working on the extractives from the starfish *Patiria miniata* (Brandt) collected off the gulf of California, and have now isolated two new 'asterosaponins' having oligosaccharide chains made from six sugar units with two branches, designated as patirioside A (**1**) and B (**2**), along with the already reported pectinioside G (**3**) and acanthaglycoside C (**4**). We have also isolated from extracts of *P. miniata* (*R*)-8-hydroxy-eicosa-(5*Z*,9*E*,11*Z*,14*Z*)-tetraenoic acid [(**8**)-*R*-HETE][9] and a series of polyhydroxysteroidal mono- and di-glycoside sulphates, which will be the subject of another paper.

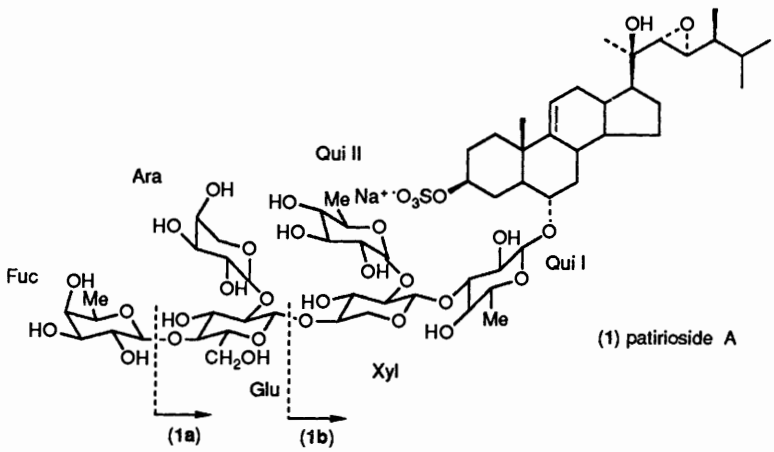
Separation and isolation of the individual compounds from the aqueous extracts of the animals followed the steps described previously.[10]

Structure Determination of Patirioside A (**1**) *and Patirioside B* (**2**).—On acid methanolysis Patirioside A (**1**), obtained in 10 mg amounts from fresh animals, (3.5 kg), liberated methyl arabinoside, methyl xyloside, methyl fucoside, methyl quinovoside, and methyl glucoside in the proportions 1:1:1:2:1. The FAB mass spectrum (negative-ion mode) gave a molecular anion peak at *m/z* 1 389 and fragments at *m/z* 1 257 ($[M - 132]^-$) and 1 243 ($[M - 146]^-$); corresponding to the losses of arabinosyl (or xylosyl) and fucosyl (or quinovosyl) residues from ($[M]^-$). The FAB mass spectrum (positive-ion mode) gave molecular ion species at *m/z* 1 435 ($[M + Na]^+$, where *M* is the molecular weight of the intact sodium salt) and 1 413 ($[M + H]^+$) accompanied by intense fragments at *m/z* 1 333 ($[M + H - SO_3]^+$) and 1 315 ($[M + Na - NaHSO_4]^+$), which indicated that compound (**1**) is a sodium sulphated compound with a molecular weight of 1 412 daltons.



(**3**) X = Qui, Y = Ara
(**4**) X = Xyl, Y = H

The ^{1}H NMR spectrum of the intact saponin revealed signals due to the aglycone protons, identical with those observed in regularoside A, an asterosaponin containing the (20*R*,22*R*,23*S*,24*S*)-22,23-epoxy-24-methyl-5α-cholest-9(11)-ene-3β,6α,20-triol 3β-sulphated aglycone, derived from the starfish *Halityle regularis*.[11] Particularly diagnostic for the 22,23-epoxy-24-methyl side chain are the signals at δ 2.76 (1 H, d, *J* 2.5 Hz) and 2.79 (1 H, dd, *J* 7.5 and 2.5 Hz) for the epoxymethine protons and those at δ 1.01 (3 H, d, *J* 6.6 Hz) for the C-28 methyl protons. In the ^{13}C NMR spectrum of compound (**1**) the



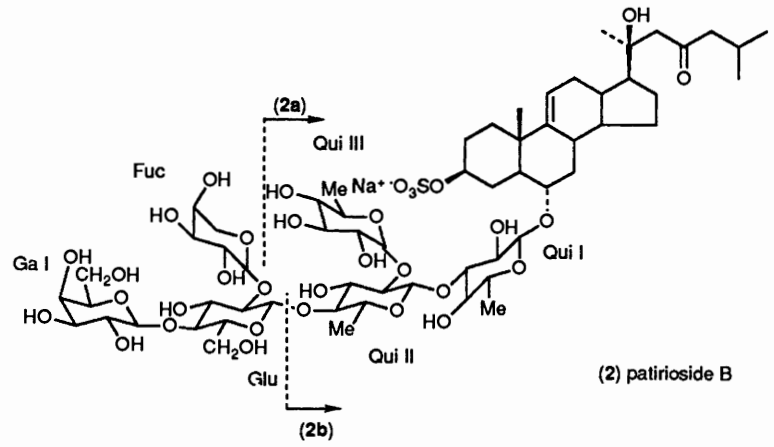


Table 1. ^{13}C NMR shifts (δ_C) of the aglycone carbon atoms of patiriosides A (**1**) and B (**2**) for solutions in [$^2H_5$]pyridine taken at 62.9 MHz.

Carbon	(1)	(2)
1	35.7	35.7
2	29.0	29.0
3	77.6	77.5
4	30.4	30.4
5	49.0	49.2
6	79.8	79.8
7	41.0	40.9
8	35.1	35.0
9	145.5	145.3
10	38.1	38.0
11	116.3	116.2
12	42.1	42.2
13	41.5	41.3
14	53.6	53.7
15	22.9	23.0
16	25.0	24.8
17	59.4	59.2
18	13.1	13.2
19	18.9	19.0
20	71.9	73.6
21	23.4	26.7
22	64.0	54.6
23	57.4	211.6
24	41.6	53.7
25	31.4	24.1
26	19.1	22.1
27	20.1	22.3
28	12.7	

aglycone carbon signals (Table 1) were superimposable with those of regularoside A,[11] thus also confirming that the oligosaccharide is attached at C-6 and the sulphate at C-3 of the steroid, a general feature of the asterosaponins.

^1H NMR [six anomeric proton signals, at δ 4.32 (1 H), 4.44 (1 H), 4.58 (2 H), and 4.60 (2 H), each a doublet with *J* ranging from 7.0 to 7.5 Hz)] and ^{13}C NMR (six anomeric carbon signals, at δ_C 106.3, 105.3, 104.9, 104.5, 103.9, and 101.3 ppm) spectroscopy also indicated that all sugar units are in their pyranose forms and that the glycoside linkages are β-oriented (α for arabinose). Elucidation of the structure of the hexasaccharide moiety was carried out as follows. Permethylation followed by methanolysis of the methylated material gave methyl 2,3,4-tri-*O*-methylarabinosides, methyl 2,3,4-tri-*O*-methylquinovosides, and methyl 2,3,4-tri-*O*-methylfucosides, implying that the sugar moiety of compound (**1**) has two branches. On enzymatic hydrolysis with *Charonia lampas* glycosidase mixture, patirioside A (**1**) gave two prosapogenol sulphates, the pentasaccharide (**1a**) and the trisaccharide (**1b**).

On acid methanolysis trisaccharide (**1b**) liberated methyl quinovosides and methyl xylosides in the ratio 2:1; the FAB mass spectrum (negative-ion mode) showed a molecular anion peak at *m/z* 949 and fragments with *m/z* 803, 671, and 525, which correspond to the sequential losses of quinovose (= 146), xylose (= 132), and quinovose. Thus the sequence of the trisaccharide moiety in compound (**1b**) is Qui-Xyl-Qui-steroid. The ^1H NMR spectrum showed three anomeric proton doublets, at δ 4.41 (*J* 7.5 Hz), 4.55 (*J* 7.5 Hz), and 4.56 (*J* 7.5 Hz), one double doublet (*J* 12 and 4.0 Hz) at δ 3.93 ascribable to 5-H_{eq} of the xylosyl residue, and two 3 H doublets, at δ 1.31 (*J* 6.5 Hz) and 1.40 (*J* 6.5 Hz) for the methyl protons of the quinovosyl units. These shifts, as well as the remaining shifts for the sugar protons,

were superimposable with those of a prosapogenin of marthasteroside A_1,[12] namely 6-*O*-β-D-Qui-(1→2)-β-D-xyl-(1→3)-β-D-Qui of 3-*O*-sulphothornasterol A. Moreover, a detailed comparison of the ^{13}C NMR data (Table 2) of the intact saponin (**1**) with those of marthasteroside A_1[12] showed that the shifts of the signals due to the trisaccharide sequence Qui-Xyl-Qui- are virtually superimposable in both spectra. This datum confirmed the structure of the trisaccharide moiety of the prosapogenol (**1b**), and also indicated that in compound (**1**) the remaining sugar chain made up by T Fuc–(T Arab)–Glu is attached at C-4 of the xylosyl residue, as in marthasteroside A_1[12] and in many other asterosaponins.[11]

An accurate analysis of the ^{13}C NMR data of the intact patirioside A (**1**) based upon comparison of appropriate methyl glycosides,[13] known glycosidation shifts,[14] and assignments reported for similar glycosides[12] established the glucose residue to be 2,4-disubstituted. From ^{13}C NMR signals we have subtracted those due to the terminal arabinose,[13] the terminal fucose,[13] and the sequence Qui–Xyl–Qui–aglycone; the remaining signals were consistent with only a 2,4-disubstituted glucose. The signal at relatively high field for the anomeric carbon (δ_C 101.3 ppm) is consistent with a substitution at C-2, whereas the C-4 glycosidic linkage is evidenced by the downfield shift exhibited by C-4 and the upfield shifts exhibited by C-3 and C-5 of the glucopyranose moiety in compound (**1**) (Table 2).

Analysis of the prosapogenol (**1a**) allowed us to prefer the sequence Fuc(1→4)-[Arab(1→2)]-Glu rather than Fuc(1→2)-[Arab(1→4)]-Glu in (**1**). The FAB mass spectrum (negative-ion mode) of compound (**1a**) [*m/z* 1 243 (M^-) and fragments at *m/z* 1 111 and 949 corresponding to the sequential loss of arabinosyl and glucosyl residues] and sugar analysis indicated that it is a pentaglycoside wherein the fucose moiety of (**1**) is missing. After more than 100 000 transients were collected using a DEPT[15] pulse sequence (polarization transfer pulse of 90°, CH groups), we obtained a quite poor ^{13}C spectrum of prosapogenol (**1a**) (*ca.* 2 mg) in which we could observe five CH signals, at δ_C 104.9, 104.2 (×2), 103.6, and 101.9 ppm, ascribable to the anomeric carbons. The appearance of the signal at relatively high field (δ_C 101.9) was interpreted as due to C-1 of a 2-substituted glucose. On this basis we propose the T Arab(1→2)-Glu sequence in (**1a**) and accordingly the structure of patirioside A can be defined as sodium (20*R*,22*R*,23*S*,24*S*)-6α-*O*-{α-L-arabinopyranosyl-(1→2)-[β-D-fucopyranosyl-(1→4)]-β-D-glucopyranosyl-(1→4)-[β-D-quinovopyranosyl-(1→2)]-β-D-xylopyranosyl-(1→3)-β-D-quinovopyranosyl}22,23-epoxy-20-hydroxy-24-methyl-5α-cholest-9(11)-en-3β-yl sulphate (**1**). We prefer the D-configuration for quinovose, fucose, xylose, and glucose by analogy with other asterosaponins,[4,6,12] the L-configuration of arabinose being preferred because L-arabinose occurs in nodososide, a steroidal glycoside from the starfish *Protoreaster nodosus*[16] and in luzonicoside, a steroidal cyclic glycoside, also originating from a starfish, *Echinaster luzonicus*.[17]

The structure (**2**) for patirioside B, which was obtained in 7 mg amounts from fresh animals (3.5 kg), was similarly derived. On acid methanolysis it liberated methyl quinovoside, methyl fucoside, methyl galactoside, and methyl glucoside in the proportion 3:1:1:1. The FAB mass spectrum (negative-ion mode) gave the molecular anion peak at *m/z* 1 419 and major fragments at *m/z* 1 319 ($[M - 100]^-$), corresponding to the C(20)–C(22) cleavage of the steroid side chain with 1 H transfer (retro-aldol cleavage) and *m/z* 1 257 ($[M - 162]^-$), corresponding to the loss of the galactosyl residue from ($[M]^-$). The FAB mass spectrum (positive-ion mode) gave molecular ion species at 1 465 ($[M + Na]^+$, where *M* is the molecular weight of the sodium salt) and 1 443 ($[M + H]^+$), accompanied by intense fragments at *m/z* 1 363 ($[M + H - SO_3]^+$) and 1 345 ($[M + Na - NaHSO_4]^+$), which indicated that compound (**2**) is a sodium sulphated compound with a molecular weight of 1 442 daltons.

The ^1H NMR spectrum of the intact saponin revealed signals due to the aglycone protons identical with those observed in the spectra of the many asterosaponins containing 3-*O*-sulphothornasterol A aglycone.[10] The thornasterol A structure for the aglycone of compound (**2**) was also supported by ^{13}C NMR spectroscopy (Table 1), which also confirmed that the oligosaccharide is attached at C-6 and the sulphate at C-3. ^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. Permethylation and methanolysis of the methylated material gave permethylated methyl quinovoside, methyl fucoside, and methyl galactoside, indicating that the saccharide chain of compound (**2**) also contains two branches. On enzymic hydrolysis with *Charonia lampas* glycosidase mixture, patirioside B (**2**) gave two prosapogenol sulphates, the pentaglycoside (**2a**) and the trisaccharide (**2b**).

Analysis of the trisaccharide, FAB MS (−ve ion, *m/z* 949), by acid methanolysis, which provided methyl quinovoside as the sole sugar component, and ^1H NMR spectroscopy, which showed shifts superimposable on those of 6-*O*-β-D-Qui-(1→2)-β-D-Qui-(1→3)-β-D-Qui of 3-*O*-sulphothornasterol A, a prosapogenol obtained from laevigatoside[18] and later from pectinioside G (**3**)[1] by similar procedures, established its structure as (**2b**). A detailed analysis of the ^{13}C NMR spectrum of the intact saponin (**2**) indicated that the remaining sugars, *i.e.* T Gal-(T Fuc)-Glu, are located at C-4 of the second quinovose residue (Qui II) and also established that the glucose residue is 2,4-disubstituted.

The FAB mass spectrum (negative-ion mode) of prosapogenol (**2a**) [*m/z* 1 273 (M^-) and fragments at *m/z* 1 111 corresponding to the loss of the galactosyl residue], and sugar analysis indicated that it is a pentaglycoside where the fucose of compound (**2**) is missing. The minute amounts of (**2a**) in our hands did not permit a ^{13}C NMR spectrum to be measured in order to get information on the interglycosidic linkage T Gal-Glu; even so we prefer the T Gal(1→4)-[T Fuc(1→2)]-Glu-instead of the T Gal(1→2)-[T Fuc(1→4)]-Glu-sequence, because the ^{13}C NMR shifts assigned in (**2**) to the T Gal-(T Fuc)-Glu-sequence are superimposable with those of the corresponding signals in pectinioside B, a hexasaccharide isolated from *Asterina pectinifera*, in which the T Gal(1→4)-[T Fuc(1→2)]-Glu-sequence was determined.[6] Thus we propose for patirioside B the structure sodium (20*S*)-6α-*O*-{β-D-fucopyranosyl-(1→2)-[β-D-galactopyranosyl-(1→4)]-β-D-glucopyranosyl-(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 sulphate (**2**).

The FAB mass spectra, the ^1H and ^{13}C NMR spectra, and the results of the sugar analysis of compounds (**3**) and (**4**) agreed in all aspects with the data reported for pectinioside G (**3**)[1] and acanthaglycoside C (**4**),[7] respectively, isolated from *Asterina pectinifera*. The occurrence of a characteristic set of asterosaponins in both *Patiria* and the closely related *Asterina*[19] species is of interest from a chemotaxonomic point of view and provide additional data which enhance the importance of chemical characteristics with respect to starfish taxonomy.[20]

Experimental

Instrumental.—For instruments used see Riccio *et al.*[10]

Extraction and Isolation.—The animals, *Patiria miniata* Brandt, were collected off the Gulf of California, USA, in November 1985 and frozen (3.5 kg). Frozen animals were then cut in small pieces and soaked in water (2 × 2 l). The aqueous extracts were centrifuged and passed through a column of

Table 2. ^{13}C NMR shifts (δ_C) of sugar carbon atoms of patiriosides A (**1**) and B (**2**) for solutions in [$^2H_5$]pyridine taken at 62.9 MHz.

Sugar carbon	(1)						(2)					
	Qui	Xyl	Qui II	Glu	Arab	Fuc	Qui I	Qui II	Qui III	Glu	Fuc	Gal
1	104.5	103.9	105.3	101.3	104.9	106.3	104.7	103.1	105.1	101.9	105.7	104.2
2	74.1	82.9	75.5	83.1	71.5	71.7	73.7	82.8	75.3	81.9	71.2	71.6
3	89.4	75.1	76.9	76.0	74.0	74.5	90.2	74.5	76.7	75.9	74.8	74.4
4	74.2	78.0	76.3	78.9	69.2	73.6	74.2	84.9	75.9	79.8	73.2	69.6
5	72.1	64.0	73.7	76.0	67.2	71.8	72.0	71.9	73.6	76.7	71.4	75.9
6	17.8		18.2	61.1		16.9	17.7	17.9	18.1	61.7	16.6	61.0

Table 3. Asterosaponin composition of *Patiria miniata* (3.5 kg frozen).

Asterosaponin	Amount (mg)	HPLC Ret. time (min)[a]	Rotations $[\alpha]_D$[b]
Patirioside A (**1**)	10	24	+5.8°
Patirioside B (**2**)	7	18	+6.0°
Pectinioside G (**3**)	18	19.2	+5.8°
Acanthaglycoside C (**4**)	39	14.9	−7.0°

[a] With methanol–water (45:55). [b] From solutions in MeOH (C ranging 0.4–1.0).

Amberlite XAD-2 (1 kg). This column was washed with distilled water (1 l) and then eluted with methanol (2 l). The methanol eluate was taken to dryness to give a glassy material (3 g), which was then chromatographed on a column of Sephadex LH-60 (4 × 80 cm) with methanol–water (2:1) as eluant. Fractions (8 ml) were collected and analysed by TLC on SiO_2 in butan-1-ol–acetic acid–water (60:15:25).

Fractions 56–79 (0.9 g) mainly contained the 'asterosaponins,' while fractions 85–96 (0.7 g) and 97–123 (0.4 g) mainly contained steroid mono- and di-glycosides and polyhydroxysteroids, respectively, which will be the subject of another paper.

The crude 'asterosaponins' fraction was submitted to droplet counter-current chromatography (DCCC) with butan-1-ol–acetone–water (3:1:5) [descending mode; the upper phase was used as stationary phase; flow rate 24 ml h^{-1}; fractions (6 ml) were collected] to give three main 'asterosaponin'-containing fractions, 71–79 (45 mg), 80–89 (47 mg), and 90–120 (62 mg). These fractions were then separated by HPLC on a $C_{18}\mu$-Bondapak column (30 cm × 8 mm i.d.) with methanol–water (45:55) as the eluant. Fractions 71–79 contained a mixture of the hexaglycosides (**1**), (**2**), and (**3**); fractions 80–89 contained a mixture of (**3**) and (**4**); fractions 90–120 contained the less polar pentaglycoside (**4**). The results of our analysis are summarized in Table 3.

^{13}C NMR spectra of the new compounds (**1**) and (**2**) are in Tables 1 and 2. Other spectral data:

Patirioside A (**1**), FAB MS (−ve, ion) *m/z* 1 389 (M^-, 100%), 1 257 (10), 1 243 (10), and 977 (<5); FAB MS (+ve ion) *m/z* 1 435 ($[M_{Na} + Na]^+$, 60%), 1 413 ($[M_{Na} + H]^+$, 100), 1 333 ($[M_{Na} + H - SO_3]^+$, 90), and 1 315 (90); $\delta_H(CD_3OD)$ (aglycone) 0.83 (3 H, s, 18-H_3), 0.93 and 0.97 (each 3 H, d, *J* 6.9 and 6.8 Hz, 26- and 27-H_3), 1.01 (3 H, d, *J* 6.6 Hz, 28-H_3), 1.03 (3 H, s, 19-H_3), 1.29 (3 H, s, 21-H_3), 2.76 (1 H, d, *J* 2.4 Hz, 22-H), 2.79 (1 H, dd, *J* 7.5, 2.5 Hz, 23-H), 4.22 (1 H, m, 3α-H), and 5.38 (1 H, br d, *J* 5.5 Hz, 11-H); δ_H(sugar) 1.30, 1.32, and 1.40 (each 3 H, d, *J* 5.7, 6.2, 6.2 Hz, 5-CH_3 of quinovose and fucose units), 4.15 (dd, *J* 12.0, 4.0 Hz, 5-H_e of the xylose unit), 4.32 (1 H, d, *J* 7.5 Hz, anomeric Hs), 4.44 (2 H, overlapping d, *J* 7.5 Hz, anomeric Hs), and 4.58 and 4.60 (2 H, overlapping d, *J* 7.5 Hz, anomeric Hs).

Patirioside B (**2**), FAB MS (−ve ion) *m/z* 1 419 (M^-, 100%), 1 319 (50), and 1 257 (25); FAB MS (+ve ion) *m/z* 1 465 ($[M_{Na} + Na]^+$, 60%), 1 443 ($[M_{Na} + H]^+$, 100), 1 363 ($[M_{Na} + H - SO_3]^+$, 70), and 1 345 (60); $\delta_H(CD_3OD)$ (aglycone) 0.81 (3 H, s, 18-H_3), 0.94 and 0.95 (6 H, two overlapping doublets, *J* 6.5 Hz, 26- and 27-H_3), 1.02 (3 H, s, 19-H_3), 1.37 (3 H, s, 21-H_3), 2.41 (2 H, d, *J* 7.5 Hz, 24-H_2), 2.61 (2 H, ABq, *J* 15 Hz, 22-H_2), 4.22 (1 H, m, 3α-H), and 5.37 (1 H, br s, *J* 5.5 Hz, 11-H); δ_H(sugar) 1.29, 1.30, 1.40, and 1.48 (all 3 H, d, *J* 6.0–6.5 Hz, 5-CH_3 of fucose and quinovose units), 4.39 (1 H, d, *J* 7.5 Hz, anomeric H), 4.44 (1 H, d, *J* 7.5 Hz, anomeric H), 4.18 (1 H, d, *J* 7.5 Hz, anomeric Hs), and 4.58 (3 H, two overlapping doublets, *J* 7.5 Hz, anomeric Hs).

Methanolysis of Glycosides.—Sugar analysis. A solution of each glycoside (0.5 mg) in anhydrous 2M HCl in MeOH (0.5 ml) was heated at 80 °C in a stoppered reaction vial for 8 h. After having cooled, the reaction mixture was neutralized with Ag_2CO_3 and centrifuged, and the supernatant was evaporated to dryness under N_2. The residue was trimethylsilylated with Trisil Z (Pierce Chemical Co.) for 15 min at room temperature. GLC analysis (127 °C) using a 25 m column of SE-30 (hydrogen carrier flow 10 ml min^{-1}) gave peaks which co-eluted with those of the appropriate silylated standards.

Methylation of Glycosides followed by Methanolysis: Terminal Sugars.—A solution of patirioside A (**1**) (2.5 mg) in dry dimethylformamide (DMF) (0.5 ml) was slowly added under nitrogen to a stirred mixture of NaH (30 mg) in dry DMF (0.5 mg) cooled in an ice-bath. The mixture was stirred for 15 min, and then MeI (0.2 ml) was added. The reaction mixture was kept for a further 4 h at room temperature. The excess of NaH was destroyed by a few drops of methanol and, after addition of water, the mixture was extracted twice with chloroform. The extract was washed with water, dried (Na_2SO_4), and evaporated under reduced pressure. The residue was methanolysed in anhydrous 2M HCl–MeOH (0.2 ml) at 80 °C in a stoppered reaction vial for 8 h. After cooling, the reaction mixture was neutralized with Ag_2CO_3, centrifuged, and the supernatant evaporated to dryness under reduced pressure. The residue was analysed by GLC (25 m, OV-101 capillary column; 100 °C; hydrogen carrier flow 10 ml min^{-1}) and the GLC peaks co-eluted with those of methyl 2,3,4-tri-*O*-methyl arabinoside, methyl 2,3,4-tri-*O*-methyl fucoside, and methyl 2,3,4-tri-*O*-methylquinovoside.

Terminal sugars analysis of patirioside B (**2**) was carried out in an identical manner and the GLC peaks co-eluted with those of methyl 2,3,4-tri-*O*-methyl fucoside, methyl 2,3,4-tri-*O*-methylquinovoside, and 2,3,4,6-tetra-*O*-methylgalactoside.

Enzymatic Hydrolysis of Patiriosides A (**1**) *and B* (**2**): *the Prosapogenols* (**1a**), (**1b**), *and* (**2a**), (**2b**).—Patirioside A (**1**) (5 mg) in citrate buffer (1 ml; pH 4.5) was incubated with a glycosidase mixture (3 mg) of *Charonia lampas* (Shikagaku Kogyo) at 37 °C. After 5 h reaction, TLC analysis [SiO_2 with butan-1-ol–acetic acid–water (60:15:25)] showed that the

starting material (R_f 0.27) had disappeared to be replaced by one major spot [R_f 0.30; pentasaccharide (**1a**)] and a minor one [R_f 0.42; trisaccharide (**1b**)]. The mixture was passed through a C-18 SEP-PAK cartridge, washed with water, and eluted with methanol. The eluate was evaporated to dryness and the residue was submitted to HPLC [$C_{18}\mu$-Bondapak column, 30 cm × 3.9 mm i.d.; MeOH–water (52:48); flow rate 2 ml min^{-1}] to give compound (**1a**) (t_R 10 min) (2.5 mg) and (**1b**) (t_R 12.4 min) (1.5 mg). Compound (**1a**); FAB MS (−ve ion) *m/z* 1 243 (M^-, 100%), 1 111 (loss of arabinose from M^-, 10), and 949 (loss of glucose from 1 111, 15); $\delta_H(CD_3OD)$ (aglycone) essentially unshifted with respect to those of compound (**1**); δ_H(sugars) 1.30 and 1.39 (each 3 H, d, *J* 6.2 Hz, 5-CH_3 of the quinovose units), 3.93 (1 H, dd, *J* 12, 4.0 Hz, 5-H_e of xylose), 4.30 (1 H, d, *J* 7.5 Hz, anomeric Hs), and 4.42 and 4.57 (each 2 H, d, *J* 7.5 Hz, anomeric Hs); δ_C([$^2H_5$]pyridine; DEPT with polarization transfer pulse of 90°; polarization transfer delay adjusted to an average coupling of 135 Hz): 104.9, 104.2 (×2), 103.6, and 101.9 ppm (anomeric Cs), other CH sugar signals are confused with the background, while the CH aglycone signals are more visible; sugar analysis: quinovose (×2), xylose, arabinose, and glucose.

Compound (**1b**), FAB MS (−ve ion) *m/z* 949 (M^-, 100%), 931 (20), 803 (loss of quinovose from M^-, 25), 671 (loss of xylose from *m/z* 803, 25), 525 (aglycone, 25), and 507 (10); $\delta_H(CD_3OD)$ (aglycone) essentially unshifted with respect to those of compound (**1**); δ_H(sugars) 1.30 and 1.39 (each 3 H, d, *J* 6.6 Hz, 5-CH_3 of the quinovosyl units), 3.93 (1 H, dd, *J* 12.0, 4.0 Hz; 5-H_e of the xylose units), 4.42, 4.55, and 4.56 (1 H each, d, *J* 7.5 Hz, anomeric Hs); sugar analysis: quinovose (×2) and xylose.

The saponin patirioside B (**2**) (2.5 mg) was similarly incubated with the glycosidase mixture from *Charonia lampas*. Work-up of the mixture gave the pentasaccharide (**2a**), FAB MS, *m/z* 1 273 (M^-, 60%) and 1 111 (loss of glucose from M^-, 100); $\delta_H(CD_3OD)$ (aglycone) essentially unshifted with respect to those of compound (**2**); δ_H(sugars) 1.30, 1.40, and 1.42 (each, d, *J* 6.0–6.5 Hz; 5-CH_3 of the quinovose units), and 4.39 (1 H), 4.43 (2 H), and 4.57 (2 H) (each doublet, *J* 7.5 Hz, anomeric Hs); sugar analysis: quinovose (×3), galactose, and glucose; and the trisaccharide (**2b**), FAB MS, *m/z* 949 (M^-, 100%) and 849 (20); $\delta_H(CD_3OD)$ (aglycone) essentially unshifted with respect to those of compound (**2**); δ_H(sugars) 1.30, 1.40, and 1.42 (each 3 H, d, *J* 6.0–6.5 Hz; 5-CH_3 of the quinovose units) and 4.43, 4.53, and 4.54 (each 1 H, d, *J* 7.5 Hz, anomeric Hs).

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References

1 Part 39, M. Iorizzi, L. Minale, and R. Riccio, *Gazz. Chim. It.*, in the press.
2 Y. Hashimoto, in 'Marine Toxins and Other Bioactive Marine Metabolites,' Japan Scientific Societies Press, Tokyo, 1979, p. 268.
3 L. Minale, C. Pizza, R. Riccio, and F. Zollo, *Pure Appl. Chem.*, 1982, **54**, 1935.
4 L. Minale, R. Riccio, C. Pizza, and F. Zollo, in 'Natural Products and Biological Activities,' a NAITO Foundation Symposium, eds. H. Imura, T. Goto, T. Murachi, and T. Nakajima, University of Tokyo Press, Elsevier Science, 1986, p. 59.
5 R. Riccio, M. Iorizzi, and L. Minale, in 'Biologically Active Natural Products,' Annual Proceedings of the Phytochemical Society of Europe, eds. K. Hostettmann and P. J. Lea, Claredon Press, Oxford, 1987, p. 153.
6 Y. Naguchi, R. Higuchi, N. Marubayashi, and T. Komori, *Liebigs Ann. Chem.*, 1987, 341.
7 M. A. Dubois, Y. Noguchi, R. Higuchi, and T. Komori, *Liebigs Ann. Chem.*, 1988, 495.
8 M. A. Dubois, R. Higuchi, T. Komori, and T. Sasaki, *Liebigs Ann. Chem.*, 1988, 845.
9 M. V. D'Auria, L. Minale, R. Riccio, and E. Uriarte, *Experientia*, 1988, **44**, 719.
10 See for example R. Riccio, M. Iorizzi, L. Minale, Y. Oshima, and T. Yasumoto, *J. Chem. Soc., Perkin Trans. 1*, 1988, 1337 and references cited therein.
11 R. Riccio, M. Iorizzi, O. Squillace Greco, L. Minale, M. Debray, and J. L. Menou, *J. Nat. Prod.*, 1985, **48**, 756.
12 I. Bruno, L. Minale, C. Pizza, F. Zollo, R. Riccio, and F. M. Mellon, *J. Chem. Soc., Perkin Trans. 1*, 1984, 1875.
13 P. A. Gorin and M. Mazurek, *Can. J. Chem.*, 1975, **53**, 1212.
14 K. Tori, S. Seo, Y. Yoshimura, H. Arita, and Y. Tomita, *Tetrahedron Lett.*, 1977, 175; S. Seo, Y. Tomita, K. Tori, and Y. Yoshima, *J. Am. Chem. Soc.*, 1978, **100**, 333; T. Kanisji, A. Toda, J. Shoji, R. Kasai, and O. Tanaka, *Chem. Pharm. Bull.*, 1978, **26**, 668.
15 M. R. Bendell, D. T. Pegg, D. M. Doddrell, and D. H. Williams, *J. Org. Chem.*, 1982, **47**, 3021; M. R. Bendell, D. M. Doddrell, D. T. Pegg, and W. E. Hull, 'DEPT Bruker Information Bulletin,' Bruker Analytishe Masstechnik, Karlsruhe, 1982.
16 R. Riccio, L. Minale, C. Pizza, F. Zollo, and J. Pusset, *Tetrahedron Lett.*, 1982, **23**, 2899.
17 R. Riccio, A. Dini, L. Minale, C. Pizza, F. Zollo, and T. Sevenet, *Experientia*, 1982, **38**, 68.
18 R. Riccio, O. Squillace Greco, L. Minale, J. Pusset, and J. L. Menou, *J. Nat. Prod.*, 1985, **48**, 97.
19 W. K. Fisher, 'Starfish of the Philippine Seas and Adjacent Areas,' Washington Government Printing Office, 1919, p. 410.
20 V. A. Stonik and G. B. Elyakov, in 'Bioorganic Marine Chemistry,' vol. 2, ed. P. J. Scheuer, Springer–Verlag, 1988, pp. 43–82.

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