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STARFISH SAPONINS, PART 22.[1] ASTEROSAPONINS FROM THE STARFISH *HALITYLE REGULARIS*: A NOVEL 22,23-EPOXYSTEROIDAL GLYCOSIDE SULFATE[2]

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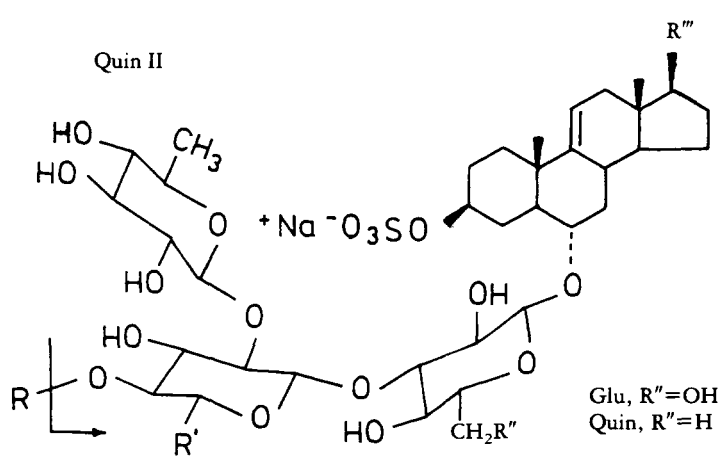
ABSTRACT.—A new sulfated asterosaponin, regularoside A (**1**), containing the unprecedented (20*R*,22*R*,23*S*,24*S*)-22,23-epoxy-24-methyl-5α-cholesta-9(11)-ene-3β,6α,20-triol aglycone, has been isolated from the Pacific starfish *Halityle regularis*. The sulfate residue is at C-3, and the oligosaccharide chain, β-D-fucopyranosyl (1↦2)-β-D-quinovopyranosyl (1↦4)[-β-D-quinovopyranosyl (1↦2)]-β-D-quinovopyranosyl (1↦3)-β-D-glucopyranosyl, is at C-6. Regularoside A (**1**) co-occurs with the common thornasteroside A (**2**) and the novel regularoside B (**3**). The latter also has thornasterol A as its aglycone and the oligosaccharide chain has been characterized as β-D-fucopyranosyl (1↦2)-β-D-fucopyranosyl (1↦4)[-β-D-quinovopyranosyl (1↦2)]-β-D-xylopyranosyl (1↦3)-β-D-quinovopyranosyl.

The number of reported structures of asterosaponins is rapidly growing (1), and all the described saponins contain several common structural features. All structures include a $\Delta^{9(11)}$, 3β,6α-dihydroxysteroidal moiety and the differences reside in the side-chains; the oligosaccharide is attached at C-6 and the sulfate is at C-3. In the saccharide portion the interglycosidic linkages are invariant also and the position of the branched unit is always located on the second monosaccharide unit (xylose or quinovose) starting from the aglycone. All sugars are in their pyranose forms with β-anomeric configurations (Figure 1).



1a

Quin I, R′=CH_3
Xyl, R′=H

[1]For Part 21, see R. Segura De Correa, R. Riccio, L. Minale, and C. Duque, *J. Nat. Prod.* (in press).

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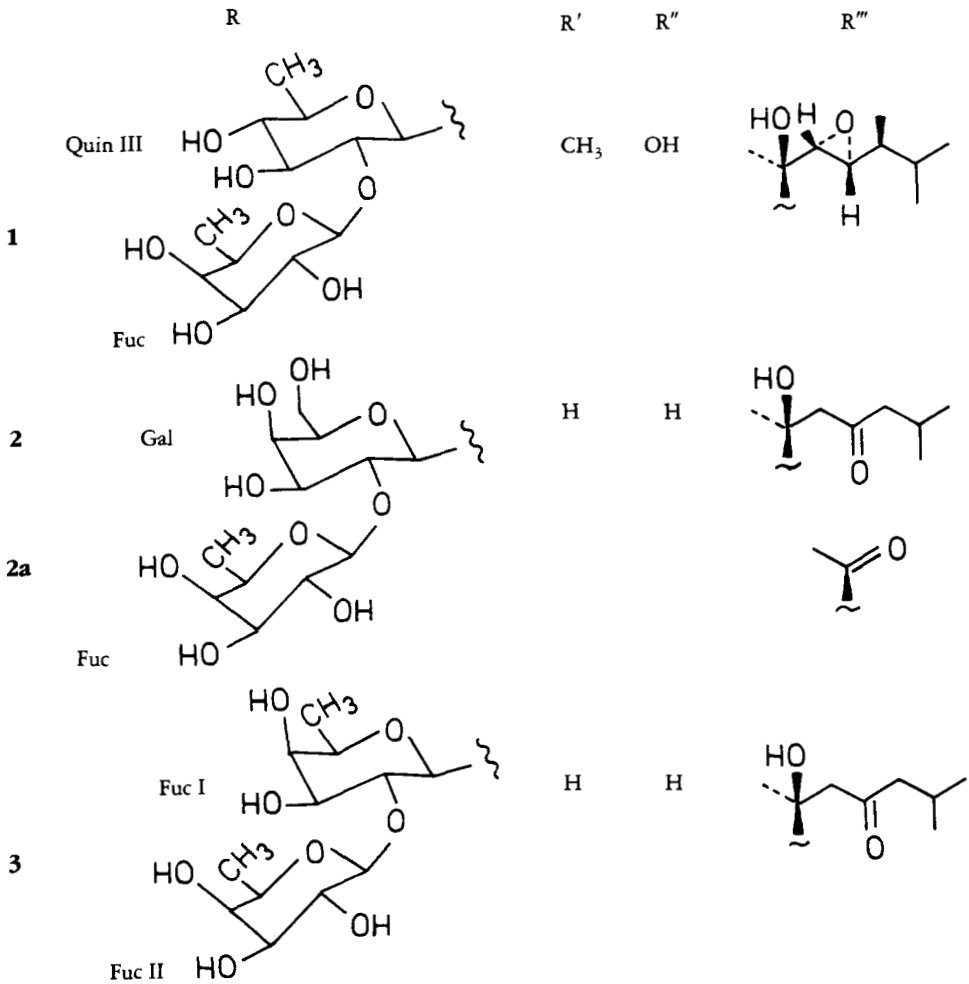

Continuing with our investigation of biologically active steroidal glycosides from echinoderms, we have examined the polar components of the Pacific starfish *Halityle regularis* Fisher and have isolated a new, minor, sulfated asterosaponin (**1**), which we named regularoside A. It has a novel aglycone, which differs from the common thornasterol A, i.e., 3β,6α,20-trihydroxy-5α-cholest-9(11)-en-23-one (2), only in the side chain by placement of an epoxide functionality at C-22 and C-23 instead of the carbonyl group at C-23, and of a methyl group at the biogenetically usual C-24 position. Once again the carbohydrate portion, β-D-fucopyranosyl (1→2)-β-D-quinovopyranosyl (1→4)[-β-D-quinovopyranosyl (1→2)]-β-D-quinovopyranosyl (1→3)-β-D-gluco pyranosyl, is very close to the carbohydrate moiety of the many described asterosaponins (1); it is attached at C-6 and the sulfate group is at C-3. Regularoside A co-occurs with major amounts of the common thornasteroside A (**2**) (1,3) and minor amounts of a new saponin, named regularoside B (**3**). The latter also has thornasterol A as its aglycone.

EXPERIMENTAL

INSTRUMENTAL.—The following instruments were used. For nmr: Brüker WM-250; for mass spectrometry: Kratos MS 902 mass spectrometer equipped with Kratos Fab source; for hplc: Waters Model 6000 A pump equipped with U6K injector and a differential refractometer, model 401 detector; for glc: Carlo Erba Fractovap 2900 capillary column; and for DCCC: DCCC apparatus manufactured by Büchi

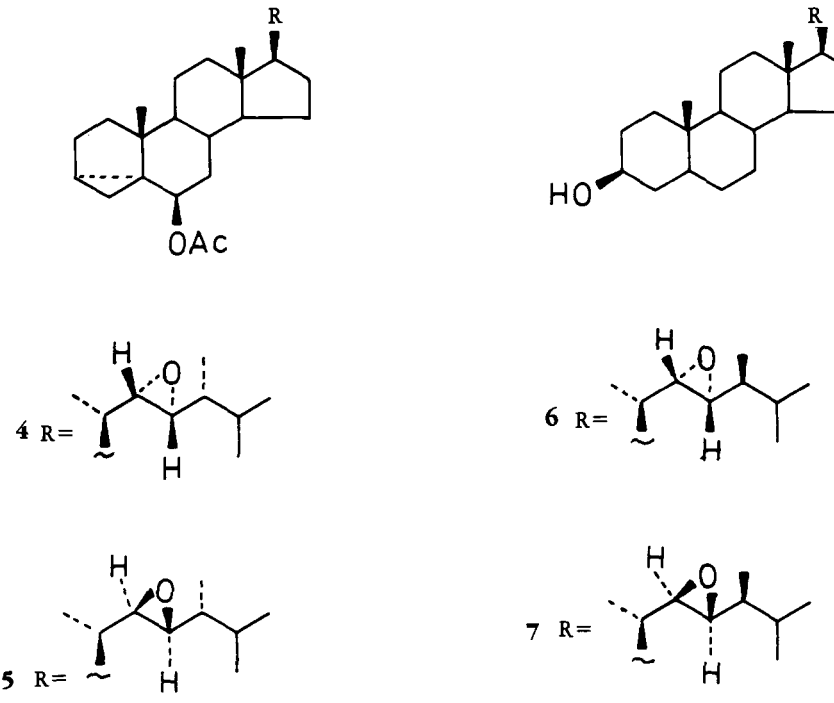

FIGURE 1. Structures of asterosaponins from *Halityle regularis*; compound **2a**, the retro-aldol cleavage derivative of thornasteroside A (**2**), is probably an artifact of extraction.

equipped with 300 tubes. The fab-mass spectra were obtained by dissolving the samples in a glycerol matrix and placing them on a copper probe tip prior to bombardment with argon atoms of energy 2-6 KV.

EXTRACTION, ISOLATION AND FRACTIONATION OF SAPONINS.—The animals (7.5 kg) were collected in October 1983, off Nouméa, New Caledonia, identified by the Zoologists of the Centre ORSTOM, (Office de la Recherche Scientifique et Tecnique Outre-Mer, Centre de Nouméa), where a reference specimen is deposited, and lyophilized (2.7 kg). The lyophilized material (2.0 kg) was extracted with a mixture of Me_2CO and increasing amounts of H_2O (from 4:1 to 1:1) several times. The combined extracts were concentrated, and the remaining aqueous solution was defatted with Et_2O and passed through a column of Amberlite XAD-2 (1 kg). This column was washed with H_2O (3 bed volumes) and then MeOH (2 bed volumes). The MeOH eluates were dried on a rotary evaporator to give 8.2 g of glassy material. The H_2O eluate from this column was then extracted with *n*-BuOH (1.5 liters, two times) to recover a further 8.5 g of crude polar extracts. The MeOH eluates and the *n*-BuOH extracts were separately chromatographed on a column of Sephadex LH-60 (4×80 cm; 100 g) using MeOH-H_2O, (2:1) as eluent. The flow rate was 10 ml/h. The eluents were collected in 7-ml fractions and monitored by tlc on silica precoated glass plates (Merck) with *n*-BuOH-HOAc-H_2O (12:3:5) with detection by ceric sulfate/H_2SO_4. Fractions 64-78 contained the crude saponins (3.8 g, total yield from both columns); the successive fractions contained a complex mixture of steroidal glycosides, which will be the subject of a future paper. Fractionation of the saponins was continued by droplet counter-current chromatography (dccc) with *n*-BuOH-Me_2CO-H_2O (3:1:5) (descending mode; the upper phase was used as stationary phase; flow 24 ml/h; 6-ml fractions were collected and analyzed by tlc on silica with *n*-BuOH-HOAc-H_2O, 12:3:5) to give five main fractions. Fractions 52-68 (126 mg) and fractions 69-76 (69 mg) contained mainly thornasteroside A (**2**); fractions 77-88 (55 mg) contained thornasteroside A and the novel regularoside B (**3**); fractions 89-100 (29 mg) contained a mixture of (**1**) and (**3**), and fractions 101-135 (51 mg) contained almost exclusively regularoside A (**1**).

All fractions were finally separated by hplc on a C_{18} μ-bondapack column (30 cm×7.8 mm i.d.) with MeOH-H_2O (45:55) (flow rate: 5 ml/min). The saponins were dissolved in H_2O (ca. 0.2 ml/100 mg), and the solution added to an equal volume of saturated aqueous NaCl. This solution was applied to the column (ca. 30 mg saponin mixture for each injection). The total yield of each saponin was: thornasteroside A (**2**), 34.3 mg (retention time: 16.8 min), regularoside B (**3**), 9.0 mg (retention time: 26 min), and regularoside A (**1**), 8.4 mg (retention time: 30.8 min). We note that the more polar dccc fractions 52-68 also contained small amounts of the retro-aldol cleavage derivative of thornasteroside A, **2a**, probably an artifact produced during the extraction procedure (4,5). In hplc it was eluted after 5.6 min; the ^1H nmr contained sig-

nals at δ 0.57 (3H, s, 18-H_3), 1.03 (3H, s, 19-H_3), 2.16 (3H, s, 21-H_3), and 5.45 (1H, broad d, J=5 Hz, 11-H) ppm, typical of the pregnane asterone (4,6); in the sugar-protons region, the spectrum was virtually superimposable on that of thornasteroside A (3,7).

PHYSICAL DATA OF SAPONINS-REGULAROSIDE A (**1**).—[α]D +12.3° (c, 0.5, MeOH); fabms *m/z* 1333 (M_{Na}+K), 1317 (M_{Na}+Na), 1213 (1333-$NaHSO_4$), 1197 (1317-$NaHSO_4$), 787, 785, 769 (pentasaccharide cation), 625, 623, 607 (tetrasaccharide cation), 477, 461, 445 (trisaccharide cation, branching point); ^{1}H nmr in CD_3OD, δ_H (aglycone) 0.83 (3H, s, 18-H_3), 0.93 (3H, d, J=6.9 Hz, 26- or 27-H_3), 0.97 (3H, d, J=6.8 Hz, 27- or 26-H_3), 1.02 (3H, d, J=6.6 Hz, 28-H_3), 1.03 (3H, s, 19-H_3), 1.29 (3H, s, 21-H_3), 2.75 (1H, d, J=2.4 Hz, 22-H), 2.78 (1H, dd, J=7.5 and 2.4 Hz, 23-H), 4.22 (1H, m, 3α-H), 5.38 (1H, broad d, J=5.5 Hz, 11-H); δ_H (sugars) 1.28, 1.30, 1.40, 1.47 (each 3H, d, J=5.5, 6.7, 6.3, and 5.5 Hz, 5-CH_3 of quinovose and fucose units), 4.47 (2H, d, J=7.5 Hz, anomeric-H's), 4.53, 4.58, 4.60 (1H each, d, J=7.7, 7.5 and 7.5, anomeric-H's), in pyridine-d_5, δ_H (aglycone) 0.86 and 0.87 (each 3H, J=6.7 and 6.8 Hz, 26- and 27-H_3), 0.91 and 0.93 (3H each, s, 18- and 19-H_3), 0.97 (3H, d, J=6.2 Hz, 28-H_3), 1.45 (3H, s, 21-H_3), 2.84 (1H, d, J=2.0 Hz, 22-H), 2.93 (1H, dd, J=7.5 and 2.0 Hz, 23-H); δ_H (sugar) 1.42, 1.46, 1.68 and 1.73 (each 3H, d, J=4.8, 5.1, 6.0, 6.2 Hz, 5-CH_3 of quinovose and fucose); ^{13}C nmr pyridine-d_5) δ_C (aglycone), C-1: 36.2, C-2: 29.5, C-3: 77.9, C-4: 31.0, C-5: 49.5, C-6: 80.9, C-7: 41.6, C-8: 35.5, C-9: 146.0, C-10: 38.5, C-11: 116.7, C-12: 42.5, C-13: 42.0, C-14: 54.0, C-15: 23.2, C-16: 25.3, C-17: 59.8, C-18: 13.5, C-19: 19.3, C-20: 71.4, C-21: 23.7, C-22: 64.3, C-23: 57.6, C-24: 42.0, C-25: 31.8, C-26 and C-27: 19.4 and 20.4, C-28: 13.0 ppm; δ_C (sugar) in Table 1; sugar analysis: glucose, quinovose (times 3), fucose.

TABLE 1. ^{13}C-nmr Shifts (pyridine-d_5, ppm) of Sugar Carbon Atoms in Regularoside A (**1**)

Carbon atoms	Glu	Quin I	Quin II	Quin III	Fuc
1	105.1	103.9	105.2	102.3	106.9
2	74.0	82.6	75.6	84.3	71.6
3	91.9	75.2	76.9	76.0	75.1
4	69.9	85.8	76.4	77.5	73.7
5	77.7	72.6	73.8	73.1	72.0
6	62.7	18.0	18.3	17.9	17.1

REGULAROSIDE B (**3**).—[α]D=+4.2° (c, 0.5 MeOH), fabms *m/z* 1289 (M_{Na}+K), 1273 (M_{Na}+Na), 1169 (1289 -$NaHSO_4$), 1153 (1273 -$NaHSO_4$), 1007 (1153 -146), 757-755-739 (pentasaccharide cation), 611-609-593 (tetrasaccharide cation), 463-447-431 (trisaccharide cation, branching point), 331-315 (disaccharide cation); ^{1}H nmr (CD_3OD), δ_H (aglycone, 0.81 (3H, s, 18-H_3), 0.94-0.945 (6H, two doublets, J=6.5, 6.5 Hz, 26- and 27-H_3), 1.02 (3H, s, 19-H_3), 1.37 (3H, s, 21-H_3), 2.41 (2H, d, J=7.5 Hz, 24-H_2), 2.60 (2H, ABq, J=15 Hz, 22-H_2), 4.22 (1H, 3α-H), 5.37 (1H, broad d, J=5.5 Hz, 11-H); δ_H (sugar) 1.30 and 1.31 (9H, two doublets, J=6.6 and 5.6 Hz; 5-CH_3 of quinovose and fucose), 1.40 (1H, d, J=6.5 Hz, 5-CH_3 of quinovose or fucose), 4.14 (1H, dd, J=12 and 4 Hz, 5-H_e of xylose), 4.42 (2H), 4.50 and 4.57 (2H) (each d, J=7.0, 7.5 and 7.5 Hz, anomeric-H's); ^{13}C nmr (pyridine-d_5), δ_C (aglycone), C-1: 36.0, C-2: 29.3, C-3: 77.8, C-4: 29.8, C-5: 49.3, C-6: 80.1, C-7: 41.6, C-8: 35.4, C-9: 145.6, C-10: 38.3, C-11: 116.5, C-12: 42.5, C-13: 41.4, C-14: 54.0, C-15: 23.3, C-16: 25.1, C-17: 59.6, C-18: 13.4, C-19: 19.2, C-20: 73.8, C-21: 27.0, C-22: 54.8, C-23: 211.9, C-24: 54.0, C-25: 24.4, C-26 and C-27: 22.5 ppm; δ_C (sugars) in Table 2; sugar analysis: quinovose (times 2), fucose (times 2), xylose.

TABLE 2. Comparison ^{13}C-nmr Shifts (pyridine-d_5, ppm) of Sugar Carbon Atoms in Regularoside B (**3**) and in Maculatoside[a] (*in parenthesis*)

Carbon atoms	Quin I	Xyl	Quin II	Fuc I	Fuc II
1	105.1 (105.8)	104.1 (104.1)	104.8 (105.0)	101.7	106.5
2	74.8 (74.1)	82.1 (82.0)	75.6 (75.5)	82.4	71.8
3	89.9 (90.0)	75.2 (75.3)	77.0 (76.7)	74.2	74.8
4	74.4 (74.5)	78.4 (78.5)	76.1 (76.1)	71.5	73.5
5	72.4 (72.5)	64.3 (64.2)	73.7 (73.8)	71.8	71.9
6	17.9 (17.9)		18.3 (18.1)	16.8	17.0

[a]Maculatoside was isolated from the starfish *Luidia maculata* and assigned the structure: 6α-*O*-{β-D-fucopyranosyl-(1→2)-β-D-quinovopyranosyl-(1→4)-[β-D-quinovopyranosyl (1→2)]-β-D-xylopyranosyl-(1→3)-β-D-quinovopyranosyl}20-hydroxy-23-oxo-5α-cholest-9(11)-en-3β-yl sodium sulfate (7).

THORNASTEROSIDE A (**2**).—^{1}H nmr is described by Kitagawa and Kobayashi (3) and Minale *et al.* (7), Fabms and ^{13}C nmr are described by Minale et al. (7).

METHANOLYSIS OF SAPONINS: SUGAR ANALYSIS.—Methanolysis of regularoside A (**1**) and B (**3**) (0.5-1 mg) and subsequent glc analysis of the silylated sugar compounds was carried out at 140° on a 25 m SE-30 capillary column as previously described (4). The identification was based on co-chromatography with standards.

METHYLATION OF SAPONINS FOLLOWED BY METHANOLYSIS: TERMINAL SUGARS.—Regularoside (**1**) (1 mg) in 0.2 ml dry DMF was added under N_2 to a stirred mixture of NaH (20 mg) in dry DMF (0.2 ml). The mixture was stirred for 2 h and then MeI (0.1 ml) was added. The reaction mixture was kept for 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 with $CHCl_3$. The organic layer was washed with H_2O and evaporated under vacuum. The residue in anhydrous 2 N HCl/MeOH (0.3 ml) was heated at 80° in stoppered reaction vial for 8 h. After cooling, the mixture was concentrated under a stream of N_2 and was used for glc analysis (SE-30, 25 m 87°, hydrogen carrier, flow 10 ml/min). Glc peaks co-eluted with those of methyl-2,3,4-tri-*O*-methyl fucoside and methyl-2,3,4-tri-*O*-methylquinovoside standards.

Regularoside B (**3**), when subjected to the same procedure gave the same methyl-2,3,4-tri-*O*-methyl-fucoside and methyl-2,3,4-tri-*O*-methylquinovoside.

ENZYMATIC HYDROLYSIS OF REGULAROSIDE A (**1**).—*Prosapogenol* (**1a**).—Saponin **1** (6 mg) in 1 ml of citrate buffer (pH 4.5) was incubated with 6 mg of glycosidase mixture of *Charonia lampas* (Scikagaku Kogyo) at 40° for 24 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-pack cartridge, which was eventually washed with 1 ml of H_2O and eluted with MeOH (3 ml). The MeOH eluate was evaporated to dryness under reduced pressure to give the trisaccharide **1a**. Fabms *m/z* 1025 (M_{Na}+Na); ^{1}H nmr (CD_3OD) δ_H (aglycone) 0.83 (3H, s, 18-H_3), 0.93 (3H, d, J=6.9 Hz, 26- or 27-H_3), 0.97 (3H, d, J=6.8 Hz, 27- or 26-H_3), 1.02 (3H, d, J=6.6 Hz, 28-H_3), 1.04 (3H, s, 19-H_3), 1.29 (3H, s, 21-H_3), 2.74 (1H, d, J=2.5 Hz, 22-H), 2.78 (dd, J=7.5, 2.5 Hz, 23-H), 4.22 (1H, m, 3α-H), 5.38 (1H, br d, J=5.5 Hz, 11-H); δ_H (sugar) 1.32 and 1.40 (3H each, d, J=6.1 and 6.2 Hz, 5-CH_3 of quinovose unit), 4.46 (1H, d, J=7.5 Hz, anomeric-H), 4.54 (1H, d, J=7.5 Hz, anomeric-H), and 4.56 (1H, d, J=7.5 Hz, anomeric-H) ppm; sugar analysis: glucose, quinovose (times 2).

MODEL EPOXIDES.—*(22S,23S,24R)-24-Methyl-22,23-epoxy-3α,5-cyclo-5α-cholestan-6β-yl acetate* (**4**) *and the 22R,23R-diastereoisomer* (**5**).—To a solution of (22*E*,24*R*)24-methyl-3α,5-cyclo-5α-cholest-22-en-6β-yl acetate (1 g), obtained from ergosterol as described by Anastasia et al. (8,9), in CH_2Cl_2 (30 ml), 70% *m*-chloroperbenzoic acid (475 mg) was added and the mixture was stirred at room temperature overnight. Usual work up followed by flash chromatography on silica gel with hexane-EtOAc (20:1) gave the epoxide **5** (552 mg), [α]D=+31.1° (c, 3; $CHCl_3$); eims *m/z* 456 (M^+, 1%), 396 (M^+ -AcOH, 100%), 326 (M^+ -AcOH-C_5H_{10}, 10%); ^{1}H nmr ($CDCl_3$) δ_H 0.39 (1H, dd, J=5, 7.5 Hz, 4-H), 0.48 (1H, t, J=5 Hz, 4-H), 0.83 (1H, m, 3-H), 0.68 (3H, s, 18-H_3), 0.88-0.89 (each 3H d, J=6.8 and 6.9 Hz, 26-, 27-H_3), 0.93 (3H, d, J=6.9 Hz, 28-H_3), 0.97 (3H, s, 19-H_3), 1.05 (3H, d, J=6.3 Hz, 21-H_3), 2.01 (3H, s, CH_3-CO), 2.33 (1H, dd, J=7.6, 2.2 Hz)-2.63 (1H, dd, J=8.4, 2.2 Hz) (22-, 23-H), 4.48 (1H, br s, 6-H); ^{13}C nmr ($CDCl_3$) C-1: 33.2, C-2: 24.9, C-3: 24.2, C-4: 12.2, C-5: 36.3, C-6: 76.1, C-7: 35.4, C-8: 30.7, C-9: 47.6, C-10: 43.0, C-11: 22.6, C-12: 40.0, C-13: 43.1, C-14: 56.0, C-15: 24.5, C-16: 27.9, C-17: 53.9, C-18: 12.2, C-19: 20.2, C-20: 39.4, C-21: 16.8, C-22: 63.5, C-23: 62.9, C-24: 42.4, C-25: 31.0, C-26, C-27: 18.6, 19.2, C-28: 12.5, CH_3CO: 21.3, 170.5 ppm; and the epoxide **4** (425 mg); [α]D=+6.7° (c, 2; $CHCl_3$), eims *m/z* 456 (M^+, <1%), 396 (M^+ -AcOH, 100%), 326 (M^+ -AcOH-C_5H_{10}, 10%). ^{1}H nmr ($CDCl_3$) δ_H 0.93-0.95-0.97 and 0.99 (each 3H, d, J=4.9, 5.3, 6.1, 5.3 Hz, sec Me's), 2.46 (1H, dd, J=7.6, 2.1 Hz)-2.59 (1H, dd, J=6.4, 2.1 Hz) (22-, 23-H); the remaining nuclear protons have chemical shifts identical to those of **5**; ^{13}C nmr ($CDCl_3$) C-1 to C-15 and C-18, C-19 have chemical shifts identical to those of **5**; C-16: 27.1, C-17: 56.1, C-20: 38.6, C-21: 16.0, C-22: 64.0, C-23: 60.3, C-24: 42.2, C-25: 31.1, C-26, C-27: 19.4, 20.1, C-28: 13.5 ppm. The spectral data of **4** and **5** recorded for solutions in CD_3OD are given in Tables 3 and 4.

(22S,23S,24S)-24-Methyl-22,23-epoxy-5α-cholestan-3β-ol (**6**) *and the 22R,23R-diastereoisomer* (**7**).—To a solution of (22*E*,24*S*)-24-methyl-5α-cholest-22-en-3β-ol (17 mg), isolated from *Euretaster insignis* (10), in $CHCl_3$ (1 ml), 70% *m*-cholorperbenzoic acid (15 mg) was added, and the mixture was stirred at room temperature for 10 h. The reaction mixture was then neutralized with aqueous 10% $NaHCO_3$, washed with H_2O, and evaporated in vacuo. The epoxide mixture was then fractionated by hplc using a Partisil M9 10/50 ODS-2 column and MeOH as eluent (flow: 5 ml/min) to give the epoxide **6** (7.7 mg) (retention time: 17.0 min), mp 130-132° (from MeOH), [α]D=−11.1° (c, 1; $CHCl_3$), eims *m/z* 416 (M^+, 20%), 346 (M^+ -C_5H_{10} 100%) and the epoxide **7** (5.8 mg) (retention time: 19 min), mp 85-87° (from

TABLE 3. ^{1}H-nmr Data (250 MHz, CD_3OD) for **1** and Model Epoxides[a] in δ (Hz)

Compounds	21-Me	26-, 27-Me	28-Me	22,23-H
1	1.30 s	0.93 d—0.97 d	1.02 d	2.75 d—2.78 dd
		(6.9) (6.8)	(6.6)	(2.4) (7.5,2.4)
4 (22*S*,23*S*,24*R*) . . .	1.05 d	0.98 d—1.00 d	1.01 d	2.52 dd—2.64 dd
	(6.3)	(6.7) (6.0)	(6.7)	(7.6,2.1)(6.4, 2.1)
5 (22*R*,23*R*,24*R*) . .	1.12 d	0.96 d—0.97 d	1.01 d	2.43 dd—2.73 dd
	(5.8)	(6.9) (7.0)	(6.9)	(7.9,2.1)(7.8, 2.1)
6 (22*S*,23*S*,24*S*) . . .	1.02 d	0.93 d—0.96 d	1.01 d	2.53 m
	(6.5)	(6.8) (6.8)	(6.9)	
7 (22*R*,23*R*,24*S*) . .	1.07 d	0.89 d—0.96 d	0.98 d	2.56 dd—2.79 dd
	(6.1)	(6.9) (6.8)	(6.7)	(7.3,2.1)(6.1,2.1)

[a]Other signals; in **4** and **5**: 0.46 (dd, 7.5, 5.0 Hz)—0.58 (t, 3 Hz) (cyclopropyl-H′$_2$s), 0.79 (s, 18-H_3), 0.83 (m, cyclopropyl-H), 1.09 (s, 19-H_3), 2.06 (s, MeCO), 4.55 (t, 3 Hz, 6-H); in **6** and **7**: 0.71 (s, 18-H_3), 0.87 (s, 19-H_3), 3.54 (m, 3α-H).

MeOH), $[\alpha]_D = -9.3°$ (c, 1, $CHCl_3$), eims *m/z* 416 (M^+, 20%), 346 ($M^+ - C_5H_{10}$, 100%), ^{1}H nmr and ^{13}C nmr are given in Tables 3 and 4.

CONVERSION OF THORNASTEROL A INTO 3β,6α,23ξ-TRIACETOXY-5α-CHOLEST-9(11)-EN-20-OL.—Treatment of thornasterol A, i.e., 3β,6α,20-trihydroxy-5α-cholest-9(11)-en-23-one (2) (5 mg) in EtOH (1 ml) with excess $NaBH_4$ at room temperature and work up in the usual way afforded the corresponding 23-hydroxy derivative which was purified by hplc on a C_{18}μ-bondapack column (MeOH-H_2O, 4:1); ^{1}H nmr ($CDCl_3$) δ_H 0.79 (3H, s, 18-H_3), 0.92 and 0.94 (each 3H, d, J=7.0 and 6.8 Hz, 26- and 27-H_3), 0.97 (3H, s, 19-H_3), 1.42 (3H, s, 21-H_3), 3.60 (2H, m, 3- and 6-H), 4.12 (1H, m, 23-H), 5.32 (1H, m, 11-H) ppm.

TABLE 4. ^{13}C-nmr Data (62.9 MHz, CD_3OD) for **1** and Model Epoxides[a]

Compounds	C-17	C-20	C-21	C-22	C-23	C-24	C-25	C-26,27	C-28
1[b]	60.5	72.5	23.7	65.0	58.8	42.9	32.6	19.6-20.6	13.0
4[c]	57.5	39.9	16.5	65.5	61.6	43.5	31.2	19.9-20.4	13.8
5[c]	55.2	40.6	17.4	64.9	64.3	43.8	32.1	19.2-19.9	13.1
6[d]	57.5	39.6	16.4	63.9	61.0	43.3	32.6	19.3-20.6	13.0
7[d]	55.3	40.0	16.7	64.8	64.3	42.3	31.9	18.9-21.1	11.6

[a]The multiplicities of each signal were determined by DEPT pulse sequence at 90° (only CH group) and 135° (positive signals for CH and CH_3 and negative ones for CH_2 groups).

[b]Remaining aglycone carbon resonances are reported in the experimental section in pyridine-d_5 and are similar (±0.1 ppm) to those described for many thornasterol A containing saponins (4,7).

[c]Remaining carbon resonances are reported in the experimental section.

[d]Remaining carbon resonances are almost identical to those described for 5α-cholestan-3β-ol (11).

Acetylation of the above 23-hydroxyderivative with Ac_2O (0.2 ml)-pyridine (0.2 ml) in the usual way at room temperature afforded a triacetate, i.e., 3β,6α,23ξ-triacetoxy-5α-cholest-9(11)-en-20-ol, eims *m/z* 500 (M^+ -AcOH<1%), 485 (M^+ -AcOH-Me, 15%), 297 (100%). ^{1}H nmr ($CDCl_3$) δ_H 0.78 (3H, s, 18-H_3), 0.92 (6H, d, J=6.2 Hz, 26-, 27-H_3), 1.02 (3H, s, 19-H_3), 1.29 (3H, s, 21-H_3), 4.63 (1H, m, 3-H), 4.84 (1H, m, 6-H), 5.12 (1H, m, 23-H), and 5.33 (1H, br d, J=5.0, 11-H) ppm.

RESULTS AND DISCUSSION

The extraction of *H. regularis* and separation of the polar compounds followed the steps described previously (4), and gave two novel asterosaponins, regularoside A (**1**) and regularoside B (**3**), along with major amounts of the common thornasteroside A (**2**) (1,3).

Regularoside A (**1**).—On acid methanolysis, regularoside A (**1**) liberated methyl glucoside, methyl quinovoside, and methyl fucoside in the ratio 1:3:1. The aglycone

was degraded to intractable material. Thus, the elucidation of its structure was pursued on the intact saponin.

Fabms of regularoside A gave molecular ion species at *m/z* 1333 (M_{Na}+K) and 1317 (M_{Na}+Na) from which the mw of 1294 dalton for the new saponin (sodium salts, **1**) could be derived. The glycosyl residue accounts for 747 m.u. (sodiated fragments at *m/z* 769, 785, and 787 in the spectrum) out of 1294 mw, leaving 547 m.u. for the aglycone. Assuming the presence of a SO_3Na residue, the mw of the free aglycone is 446. The ^{1}H nmr (Table 3) contained three methyl doublet signals and two one-proton signals at δ 2.75 (d, J=2.4 Hz) and 2.78 (dd, J=7.5, 2.4 Hz), assigned to epoxide methine protons. The ^{1}H nmr also contained signals at δ 0.83 (3H, s, 18-H_3), 1.03 (3H, s, 19-H_3), 1.30 (3H, s, 21-H_3), 4.22 (1H, m, 3α-H), and 5.38 (1H, broad d, J=5.5 Hz, 11-H) reminiscent of the spectra of asterosaponins containing a $\Delta^{9(11)}$-3β,6α,20-trihydroxysteroidal aglycone (3,4,7). Based on these data, the presence of a 20-hydroxy-22,23-epoxide-24-methyl side-chain in **1** has been assumed. This assumption was further substantiated by the ^{13}C-nmr spectrum of **1**, which showed, in addition to the signals for the tetracyclic nucleus carbons already observed in the spectra of the many asterosaponins (4,7), nine signals (one C, four CH and four CH_3, DEPT) due to the side-chain carbons (Table 4). The CH signals downfield shifted to 58.8 and 65.0 ppm were consistent with the presence of an epoxide functionality. The stereochemistry of the hydroxyl group at C-20 was suggested to be 20*R* (α) based on the chemical shift of the 21-methyl protons (δ 1.29). The 21-methyl protons of (20*S*) (α)- and (20*R*) (β)-20-hydroxycholesterol were reported to resonate at δ 1.28 and 1.13, respectively (12). Note that the specification of configuration at C-20 changes on going from the 20-hydroxycholesterol to the 20-hydroxy-22,23-epoxysteroid (**1**) according to the Cahn Ingold-Prelog convention.

In order to elucidate the configurations at C-22, C-23, and C-24 of regularoside A, we have compared the ^{1}H- and ^{13}C-nmr spectra of **1** with those of the four stereoisomers **4, 5, 6,** and **7,** all having the 22,23-epoxy-*trans*-stereochemistry. The epoxy-*cis*-stereochemistry could be eliminated because of the low field resonance of C-24 in the spectrum of **1**. In the model 22,23 (*cis*)-epoxycholesta-5,7-dien-3β-ol the resonances of C-20 and C-24 were reported shifted upfield by 5.8 and 4.5 ppm, respectively, relative to the *trans*-models (γ-gauche interaction) (13). Therefore, the signal at 42.9 ppm for C-24 in **1**, in the same range of the signals for C-24 observed in the spectra of the *trans*-models (Table 4), clearly indicated the *trans*-epoxide stereochemistry. The (24*R*)-models **4** and **5** have been prepared by treating (22*E*,24*R*)-24-methyl-3α-5-cyclo-5α-cholest-22-en-6β-yl acetate, obtained from ergosterol (8,9), with *m*-chloroperbenzoic acid. The stereochemistry 22*R*,23*R* has been assigned to the less polar isomer **5** based on ^{13}C-nmr shift of C-17, which in **5** is shifted upfield to 53.9 ppm relative to 5α-cholestan-3β-ol (56.1 ppm), while in **4** C-17 resonated at 56.1 ppm (13). The two (24*S*)-models **6** and **7** have been prepared by treatment of a sample of (22*E*,24*S*)-24-methyl-5α-cholesta-22-en-3β-ol, obtained from the starfish *Euretaster insignis* (10) with *m*-chloroperbenzoic acid. The stereochemical assignments at C-22 and C-23 of the two isomers were based on the same criteria used before (Table 4).

The resonance of C-17 (δ c 59.8 ppm in pyridine-d_5) in **1** is virtually unshifted with respect to the same signal in the thornasterol A-containing saponin, δ c 59.6 ppm (4), as is the resonance of C-17 in the model 22*S*,23*S*-isomers with respect to the same signal in 5α-cholestan-3β-ol. This suggested the 22*R*,23*S*-configuration for the natural saponin (**1**). Note that the specification of configuration at C-22 changes on going from the 22,23-epoxysterols to the 20-hydroxy-22,23-epoxysaponin **1**. This assignment was corroborated by the chemical shift values of the isopropyl methyl hydrogen signals, which also suggested the 24*S*-configuration in **1**. Our values of 0.93 and 0.97 ppm

compared well to those of the 24*S*-isomer (**6**), δ 0.93-0.96, which occur at higher field relative to those of the corresponding 24*R*-isomer (**4**), δ 0.98-1.00. In confirmation, the ^{13}C-nmr chemical shifts of the carbons from C-24 to C-28 in **1** compared well to those of **6**, while the shifts of the corresponding carbons in the isomers **4, 5,** and **7** are significantly far from our values (Table 4). Based on these data the stereochemistry 20*R*, 22*R*, 23*S*,24*S* in **1** has been suggested.

The sulfate group is assigned to C-3 by consideration of chemical shift (δ 4.22; 3-H) and coupling patterns ($W_{1/2}$=24 Hz) in comparison with ^{1}H-nmr data for related sulfated asterosaponins (4,7), and confirmed by ^{13}C-nmr data (see Experimental section) (7,14). ^{13}C nmr similarly established the oligosaccharide chain to be located at C-6.

Permethylation of regularoside A (**1**) and methanolysis of the methylated material gave permethylated methyl quinovoside and permethylated fucoside. This data placed one fucose and one quinovose as the terminal monosaccharides and indicated that the carbohydrate moiety contains one branching point. The shifts of the anomeric carbons by ^{13}C nmr (Table 1) and the coupling constants of the anomeric protons (see Experimental section) by ^{1}H nmr have suggested that all the linkages are β. The fragmentation pattern observed in the fabms (see Experimental section) is closely reminiscent of those observed in spectra of marthasteroside B and C, pentaglycosides with the β-D-fucopyranosyl (1↦2)-β-D-fucopyranosyl (1↦4)-[β-D-quinovopyranosyl (1↦2)]-β-D-quinovopyranosyl (1↦3)-β-D-glucopyranosyl oligosaccharide chain (14). The sugar fragments have identical masses in all spectra. The peaks at *m/z* 785-769 and *m/z* 623-607 (loss of 162 mass units: glucose) indicated that the glucose is directly linked to the aglycone in **1**, while the triplet at *m/z* 477, 461, 445 suggested that the branched sugar (quinovose) is located on the second monosaccharide starting from the aglycone (14). On enzymatic hydrolysis with *Charonia lampas* glycosidase mixture, compound **1** gave one major prosapogenol (**1a**). Acid hydrolysis of **1a** yielded quinovose and glucose in the ratio 2:1. The ^{1}H-nmr spectrum in the sugar protons region was virtually superimposable on those of 6α-*O*[-β-D-quinovopyranosyl(1↦2)-β-D-quinovopyranosyl-(1↦3)-β-D-glucopyranosyl]-23-oxo-5α-cholest-9(11)-en-3β-yl sodium sulfate, and its 24,25-didehydroderivative, prosapogenols obtained from marthasteroside C and B (14), respectively, by using the same glycosidase mixture. The location of the linkage (1↦3) to the glucose is confirmed by the appearance in the ^{13}C-nmr spectrum of **1** of a signal shifted downfield to 91.9 ppm. The same signal was observed in marthasterosides B and C and assigned to C-3 of glucose (14). The remaining monosaccharides, T-fucose-quinovose, in **1** are attached at C-4 of the branched quinovose, as indicated by the ^{13}C-nmr signals in **1** at 82.6 and 85.8 ppm for C-2 and C-4 of the branched quinovose unit (14) and confirmed by the downfield methyl doublet at δ 1.47 in **1** for the methyl protons at C-5 of a 4-substituted quinovose unit (14,15). The same signal was observed in the spectrum of **1a** at δ 1.40, indicating that the substitution at C-4 has been removed on passing from **1** to **1a**. Further, the appearance in the ^{13}C-nmr spectrum of **1** of one anomeric carbon signal at relatively highfield (102.3 ppm) and one glycosidated carbon at 84.3 ppm indicated the (1↦2) linkage T-fucose-quinovose. Thus the saccharide chain of regularoside A (**1**) can be defined as β-D-fucopyranosyl(1↦2)-β-D-quinovopyranosyl(1↦4)-[β-D-quinovopyranosyl (1↦2)]-β-D-quinovopyranosyl (1↦3)-β-D-glucopyranosyl, which is identical to that assigned to two asterosaponins recently described from *Luidia maculata* by Komori *et al.* (16). In **1** the configurations of the sugars have not been proved. We prefer the common D-configuration by analogy with the previous saponins. The occurrence of a 22,23-epoxysteroid glycoside in this starfish is of biological interest because of its probable role in the biosynthesis of the 23-oxo function of the many aglycones of starfish saponins. We

note that 22,23-epoxysteroids have been found before in minor cyclic glycosides from the starfish *Echinaster sepositus* (17).

Regularoside B (**3**).—On acid methanolysis, regularoside B (**3**) liberated methyl quinovosides, methyl fucosides, and methyl xylosides in the ratio 2:2:1. The aglycone of regularoside B is thornasteryl A 3-sulfated. The ^{1}H-nmr spectrum of the intact saponin showed signals for the aglycone protons (see Experimental section), which were virtually identical to the signals that we had previously seen for the thornasterol A-containing saponins (4,7). The ^{13}C-nmr spectrum (see Experimental section) substantiated the identification of the aglycone and confirmed the C-3 attachment of the sulfate and C-6 attachment of the oligosaccharide. Fabms gave molecular ion species at *m/z* 1289 (M_{Na}+K) and 1273 (M_{Na}+Na) corresponding to the expected molecular weight of 1250 for a pentaglycoside of thornasteryl A sodium sulfate. The fragmentation pattern (see Experimental section) is closely reminiscent of those observed in spectra of previously described asterosaponins, and the triplet at *m/z* 463, 447, and 431 suggests the presence of one branched sugar (xylose) located on the second monosaccharide starting from the aglycone (14,15). Permethylation of regularoside B and methanolysis of the methylated material gave permethylated methyl quinovoside and methyl fucoside These data placed one quinovose and one fucose as the terminal monosaccharides. The ^{13}C-nmr spectrum of **3** (Table 2) when compared with those reported for similar glycosides (7,14,15) and methyl β-fucopyranoside (18) suggested the carbohydrate structure as shown in **3**. The shifts of the anomeric carbons (Table 2) and the coupling constants of the anomeric protons at δ 4.42 (2H, J=7.0 Hz), 4.50 (1H, J=7.5 Hz), and 4.57 (2H, J=7.5 Hz) have indicated that all the linkages are β. In the ^{13}C-nmr spectrum one glycosidated carbon signal appeared shifted downfield to 89.9 ppm and was assigned to C-3 of quinovose directly attached to the aglycone (7,14,15). The branched xylose is 2,4-disubstituted, as indicated by the ^{13}C-nmr signals at 82.1, 78.4, and 64.3 ppm for C-2, C-4, and C-5 (14,15,19), and supported by the downfield signal at δ 4.14 (dd, J=12.0 and 4 Hz) for the equatorial proton at C-5 of a 4-*O*-substituted xylopyranose (14). Many described asterosaponins contain the β-D-quinovopyranosyl (1↦2)-β-D-xylopyranosyl (1↦3)-β-D-quinovopyranosyl moiety attached to the aglycone with further substitution at C-4 of xylose (1). We suggest that the same structural feature is present in regularoside B, since its ^{13}C-nmr spectrum contains the same signals observed in those of the previous saponins (7,14,15,18). From the ^{13}C-nmr signals we have subtracted those due to the above trisaccharide moiety (Table 2), and the remaining signals in **3** are only consistent with a β-fucopyranosyl (1↦2)-β-fucopyranosyl moiety. The location of the linkage (1↦2) to the fucose was based on the appearance in the spectrum of **3** of one anomeric carbon signal at relatively highfield (101.7 ppm, γ-effect) and one glycosidated carbon signal at 82.4 ppm, which is shifted downfield by 10.6 ppm (β-effect) relative to C-2 of the terminal fucose.

Thornasteroside A (**2**).—This was identified by comparison (fabms and ^{1}H nmr) with an authentic sample and sugar analysis. The spectral data of thornasteroside A are described by Kitagawa and Kobayashi (3) and Minale *et al.* (7). Even if thornasterol A is the most common aglycone among asterosaponins (1,5), the stereochemistry at C-20 was not assigned (2). We now suggest the 20*S* (α) configuration based on the chemical shift of the 21-methyl protons of thornasterol A itself (δ 1.31) (3), the 23-hydroxy-derivative (δ 1.41), and its triacetate (δ 1.29). The 21-methyl protons of (20*S* - and (20*R*)-20-hydroxycholesterol were reported to resonate at δ 1.28 and 1.13, respectively (12).

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