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# STARFISH SAPONINS, 38.[1] STEROIDAL GLYCOSIDES FROM THE STARFISH *PYCNOPODIA HELIANTHOIDES*

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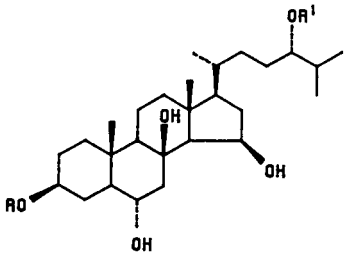
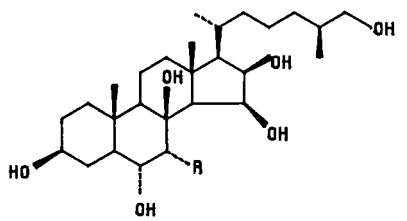
ABSTRACT.—Three novel steroidal monoglycosides, pycnopodiosides A [**1**], B [**2**], and C [**3**], together with one novel polyhydroxysteroid [**5**], have been isolated from the starfish *Pycnopodia helianthoides* collected off the Gulf of California. These compounds co-occur with the common asterosaponin thornasteroside A, one known steroidal monoglycoside, coscinasteroside B, and two known polyhydroxysteroids **6** and **7**. The structures of the new metabolites were determined from spectral data and comparison with those of related steroids.

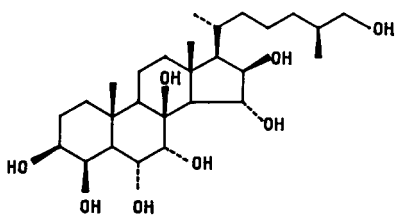
In the course of our continuing studies of oligoglycosides and polyhydroxysteroidal constituents of starfishes we have been working on starfish species collected off the Gulf of California. We now report on four new metabolites, the related steroidal glycosides pycnopodiosides A [**1**], B [**2**], and C [**3**] and the polyhydroxysteroid **5**, isolated from *Pycnopodia helianthoides* Brandt (family Asteriidae, order Forcipulata). The extracts also contained glycoside constituents, thornasteroside A [i.e., (20*S*)-20-hydroxy-6α-*O*-β-D-fucopyranosyl-(1↦2)-β-D-galactopyranosyl-(1↦4)-[β-D-quinovopyranosyl-(1↦2)]-β-D-xylopyranosyl-(1↦3)-β-D-quinovopyranosyl-23-oxo-5α-cholest-9(11)-en-3β-yl sodium sulfate, a widely distributed "asterosaponin" first isolated from *Acanthaster planci* (1)], and coscinasteroside B [i.e., (24*S*)-24-*O*-β-D-xylopyranosyl-5α-cholestane-3β,6β,8,15α,24-pentol 15-sulfate, recently reported from the starfish *Coscinasterias tenuispina* (2)], and two polyhydroxysteroid constituents **6** and **7**, previously isolated from *Halityle regularis* (3) and *Protoreaster nodosus* (4), respectively.




**1** R=H, R′=β-xylopyranosyl
**2** R=$SO_3^-Na^+$, R′=β-xylopyranosyl
**3** R=H, R′=β-glucopyranosyl-6′-*O*-sulfate
**4** R=H, R′=β-glucopyranosyl




**5** R=OH
**6** R=H




**7**

[1]For Part 37, see F. Zollo, E. Finamore, R. Riccio and L. Minale, *J. Nat. Prod.*, **52**, 693 (1989).

*Pycnopodioside A* [**1**].—$[\alpha]_D$ +2°. The fabms (negative ion mode) showed a quasi-molecular ion at *m/z* 583 $[M-H]^-$ and a major fragment at *m/z* 451 corresponding to the loss of a pentasaccharide unit. Examination of its spectral properties (Tables 1 and 2) indicated that compound **1** contains a β-xylopyranosyl unit, as confirmed by acid methanolysis affording methyl xylosides. In addition to the sugar moiety, the $^1$H-nmr spectrum showed signals for the aglycone protons almost superimposable with those observed in the spectrum of (24*S*)-5α-cholestane-3β,6α,8,15β,24-pentol, isolated as free sterol from *Gomophia watsoni* (5), except for the signals of the isopropyl methyl protons, which in **1** appeared as a doublet (6H) at δ 0.95 , whereas in the spectrum of the free sterol they appeared as separate doublets at δ 0.92 and 0.94 ppm. This suggested the sugar unit was located at C-24. The $^{13}$C-nmr data (Table 2) and comparison with those of the steroid aglycone (5) definitively established the location of the β-xylopyranosyl residue at C-24 and confirmed the formulation **1** for pycnopodioside A. The D configuration of xylose is assumed by comparison with the many D-xylosides isolated from the starfishes (6). The 24*S* configuration is suggested because all the signals assigned to the side chain carbon atoms are virtually identical with those assigned to the corresponding carbons of amurensoside A, (24*S*)-24-*O*-β-D-xylopyranosyl-5α-cholestane-3β,6α,15α,24-tetraol, isolated from *Asterias amurensis* (7). We note that the introduction of a sugar moiety at C-24 should cause significant differences in the spectra of 24*S*- and 24*R*-epimers (8).

*Pycnopodioside B* [**2**].—Pycnopodioside B [**2**], $[\alpha]_D$ +1.8°, fabms 663 $[M]^-$, is the 3-*O*-sulfated derivative of pycnopodioside A. The presence of a sulfate group was confirmed by solvolysis in a dioxane-pyridine mixture (9) affording a desulfated derivative, fabms 583 $[M-H]^-$, identical with **1** ($^1$H-nmr spectra superimposable).

The $^1$H- and $^{13}$C-nmr spectra (Tables 1 and 2) of compound **2** and comparison with those of **1**, taking into account the sulfation shifts, established the location of the sulfate group at C-3.

*Pycnopodioside C* [**3**].—Pycnopodioside C [**3**], $[\alpha]_D$ +4.2°, is the 24-*O*-β-glucopyranoside-6′-sulfate of the (24*S*)-5α-cholestane-3β,6α,8,15β-24-pentol. Acid

TABLE 1. $^1$H-nmr Data (250 MHz, $CD_3OD$) for Compounds **1–4** in δ (Hz).

| Proton | Compound | | | | |
|---|---|---|---|---|---|
| | **1** | **2** | **3** | **4** | **5** |
| H-3 | 3.55 m | 4.25 m | 3.55 m | | 3.53 m |
| H-6 | 3.73 ddd (10.5,10.5,3.5) | 3.71 ddd (10.5,10.5,5) | 3.71 ddd (10.5,10.5,3.5) | | 3.87 dd (2.5,12.5) |
| H-7 | 2.40 dd[a] (12,3.5) | 2.40 dd (12,3.5) | 2.40 dd[a] (12,3.5) | | 3.90 d (2.5) |
| H-14 | 1.05 d (5.5) | 1.05 d (5.5) | 1.06 d (5.5) | | 1.43 d (5.5) |
| H-15 | 4.44 bt (5.5) | 4.44 bt (5.5) | 4.44 bt (5.5) | | 4.50 dd (6,5,5.5) |
| H-16 | 2.40 m[a] | 2.40 m[a] | 2.40 m[a] | | 4.25 t (6.5) |
| H-18 | 1.29 s | 1.29 s | 1.29 s | | 1.28 s |
| H-19 | 1.02 s | 1.02 s | 1.01 s | | 1.02 s |
| H-21 | 0.97 d (6.3) | 0.98 d (6.5) | 0.96 d (7) | | 0.99 d (6.5) |
| H-26 | 0.95 d (7) | 0.94 d (7) | 0.96 d (7) | | 3.47 dd[c] (10,5.5) |
| H-27 | 0.95 d (7) | 0.94 d (7) | 0.96 d (7) | | 0.94 d (7) |
| H-1′ | 4.27 d (7.5) | 4.27 d (7.5) | 4.32 d (7.5) | 4.33 d (7.5) | |
| H-2′ | 3.22 dd (7.5,9.5) | 3.22 dd (7.5,9.5) | 3.21 dd (7.5,9.5) | 3.21 dd (2.5,7.5) | |
| H-3′ | [b] | [b] | 3.38 t (9.5) | 3.38 t (9.5) | |
| H-4′ | 3.50 ddd (10,9.5,5) | 3.50 ddd (10,9.5,5) | [b] | [b] | |
| H-5′ | 3.85 dd (10,5) 3.18 t (10) | 3.90 dd (10,5) 3.16 t (10) | 3.5 m | 3.5 m | |
| H-6′ | | | 4.34 dd (2.5,11.5) 4.18 dd (5,11.5) | 3.90 dd (2.5,11.5) 3.72 dd (5,11.5) | |

[a]The other 7- and 16-proton are confused in the high field region of the spectrum.
[b]Signal under solvent signal.
[c]The other 26-proton resonates under the solvent signal.

TABLE 2. $^{13}C$ nmr Shifts (62.9 MHz, $CD_3OD$) of Compounds **1–3**, **5** in ppm.

| Carbon | Compound | | | | | |
|---|---|---|---|---|---|---|
| | **1** | **2** | **3** | **5** | Reference steroid[a] | Reference steroid[b] |
| C-1 | 39.5 | 39.5 | 39.5 | 39.4 | 39.6 | |
| C-2 | 31.5 | 29.1 | 31.5 | 31.5 | 31.5 | |
| C-3 | 72.2 | 80.0 | 72.3 | 72.3 | 72.3 | |
| C-4 | 32.4 | 30.2 | 32.4 | 32.2 | 32.3 | |
| C-5 | 54.0 | 54.1 | 54.0 | 44.5 | 44.5 | |
| C-6 | 67.7 | 67.7 | 67.8 | 69.5 | 68.9 | |
| C-7 | 50.0 | 50.0 | 49.5 | 76.8 | 76.5 | |
| C-8 | 77.5 | 77.5 | 77.6 | 79.2 | 77.7 | |
| C-9 | 56.7 | 57.6 | 57.5 | 50.3 | 51.2 | |
| C-10 | 38.0 | 37.9 | 38.0 | 37.6 | 37.8 | |
| C-11 | 19.8 | 19.7 | 19.8 | 19.2 | 19.3 | |
| C-12 | 43.5 | 43.5 | 43.5 | 43.3 | 43.2 | |
| C-13 | 44.5 | 44.5 | 44.4 | 44.5 | 45.5 | |
| C-14 | 62.8 | 62.8 | 62.7 | 55.4 | | 61.4 |
| C-15 | 71.2 | 71.3 | 71.3 | 71.3 | | 71.3 |
| C-16 | 42.4 | 42.4 | 42.4 | 72.8 | | 72.8 |
| C-17 | 58.1 | 58.1 | 58.2 | 63.2 | | 63.2 |
| C-18 | 16.5 | 16.5 | 16.6 | 17.8 | | 17.9 |
| C-19 | 14.0 | 14.0 | 14.1 | 13.9 | 13.9 | |
| C-20 | 36.3 | 36.3 | 36.2 | 31.0 | | 31.0 |
| C-21 | 19.0 | 19.0 | 19.0 | 18.4 | | 18.4 |
| C-22 | 32.8 | 32.8 | 32.9 | 37.1 | | 37.1 |
| C-23 | 29.1 | 29.1 | 28.9 | 24.8 | | 24.8 |
| C-24 | 86.5 | 86.5 | 86.5 | 35.0 | | 35.0 |
| C-25 | 32.0 | 32.0 | 32.4 | 37.0 | | 37.0 |
| C-26 | 18.3 | 18.3 | 18.3 | 68.6 | | 68.6 |
| C-27 | 18.4 | 18.4 | 18.5 | 17.2 | | 17.3 |
| C-1′ | 104.9 | 104.9 | 104.3 | | | |
| C-2′ | 75.4 | 75.4 | 75.7 | | | |
| C-3′ | 78.0 | 78.0 | 78.0 | | | |
| C-4′ | 71.5 | 71.5 | 71.4 | | | |
| C-5′ | 66.7 | 66.7 | 76.0 | | | |
| C-6′ | | | 68.5 | | | |

[a](25*S*)-5α-cholestane-3β,6α,7α,8,15α,16β,26-heptol (4).
[b](25*S*)-5α-cholestane-3β,4β,6α,8,15β,16β,26-heptol (3).

methanolysis gave methyl glucosides. The fabms (negative ion mode) showed the molecular anion peak at *m/z* 693; upon solvolysis using dioxane/pyridine, compound **3** yielded the desulfated glucoside **4**, fabms (negative ion mode) *m/z* 613 $[M-H]^-$.

The 250 MHz $^1H$-nmr of **3** and double resonance experiments showed the signals for the methylene protons due to -$CH_2$-O- of glucose downfield shifted to δ 4.18 (dd, $J=5$, 12 Hz) and 4.34 (dd, $J=2$, 12 Hz) (δ 3.72 and 3.90 ppm in the desulfated analogue **4**). The remaining sugar signals (see Table 1) were consistent with a β-glucopyranosyl moiety. The $^{13}C$ nmr assisted by DEPT experiments showed the signal of C-6′ of glucose downfield shifted to 68.5 ppm [methyl -β-glucopyranoside: δ C-6, 61.9 ppm (10)]. These data clarified that C-6′ bears the sulfate in pycnopodioside C [**3**]. In addition to the sugar moiety, readily recognized from nmr data (Tables 1 and 2) were the signals for the 5α-cholestane-3β,6α,8,15β-24-pentol structure. As before, the comparison of the $^{13}C$-nmr spectrum of **3** with that of its aglycone (5) identified the site of glycosidation to be at C-24.

We assume the 24*S* configuration by analogy with **1** and **2**. Small variations in the chemical shifts for the side-chain carbons of the steroid in compounds **1** and **3** can be attributed to the different sugar structure linked at C-24 rather than to any change to the stereochemistry at C-24.

*(25S)-5α-Cholestane-3β,6α,7α,8,15β,16β,26-heptol* [**5**].—Compound **5**, $[\alpha]_D$ +16°, fabms (positive ion mode) *m/z* 485 $[M+H]^+$, is related to the known **6** (3) by introduction of an "extra" hydroxyl group at the 7α position.

The $^1$H-nmr spectrum of **5** contained several features already observed in the spectrum of **6**, namely two double doublets at δ 4.25 ($J$ = 6.5 and 6.5 Hz) and 4.50 ($J$ = 6.5 and 5.5 Hz), a broad multiplet at δ 3.53, and a double doublet at δ 3.47 ($J$ = 10 and 5.5 Hz; A part of an ABX system; B part under solvent), assigned to H-16α, H-15α, H-3α, and H-26, respectively. The chemical shifts of the methyl signals (Table 1) were also close to the values observed in the spectrum of the hexol **6**. In addition to the above signals, the spectrum of the new heptol **5** contained two overlapping hydroxymethine signals, a double doublet centered at δ 3.87 with one large coupling constant (12.5 Hz) and a small one (2.5 Hz), and a doublet at δ 3.90 with small coupling constant (2.5 Hz). These signals are assigned to H-6 and H-7, and the $J$ values were suggestive for the equatorial orientation of the hydroxyl group at C-6 (6α-OH) and the axial orientation of the hydroxyl group at C-7 (7α-OH). The 6α-OH stereochemistry is supported by the chemical shift value of the Me-19 protons, δ 1.02 ppm, virtually identical with that of the same signal in the hexol **6** [in 6β,8β-dihydroxylated steroids the signal for the Me-19 protons is found at ca. δ 1.20 ppm (2,3)], whereas the 7α-OH stereochemistry was also indicated by the downfield shifts observed for H-14 (δ 1.43 d, $J$ = 5.5 Hz) and H-15 (δ 4.50 dd, $J$ = 6.5 and 5.5 Hz) in **5** relative to **6** (δ 1.05 and 4.40 ppm, respectively). The $^{13}$C-nmr data (Table 2) and comparison with related steroids confirmed the proposed formulation **5** for the new compound. We note that the chemical shift values of the signals assigned from C-1 to C-13 and C-19 are close to those of the corresponding signals in 3β,6α,7α,8-hydroxylated steroids isolated from starfishes [e.g., (25*S*)-5α-cholestane-3β,6α,7α,8,15α,16β,26-heptol (4)], whereas the shifts of the signals from C-15 to C-27 are almost identical with those of corresponding signals observed in 8β,15β,16β,26-hydroxylated steroids isolated from starfishes (e.g., 5α-cholestane-3β,4β,6α,8,15β,16β,26-heptol (3)]. As expected the signal for C-14 in **5** is upfield shifted to 55.4 ppm relative to the previous 8β,15β,16β-hydroxylated steroids (61.4 ppm) because of the introduction of the hydroxyl group at the 7α-position (axial).

The 25*S* configuration has been determined for several 26-hydroxysteroids isolated from starfishes (2,11,12). We assume the same 25*S* configuration for this sterol because the shifts of the signals assigned to side chain carbons by $^{13}$C nmr in **5** are identical with those of the above 26-hydroxysteroids (Table 2).

## EXPERIMENTAL

INSTRUMENTAL.—For instruments used, see Riccio *et al.* (7).

EXTRACTION AND ISOLATION.—*P. helianthoides* (2 specimens, ca. 2.0 kg each) were collected off the Gulf of California, and identified by zoologists of the Scripps Institution of Oceanography, La Jolla, California; a voucher specimen is preserved at the Dipartimento di Chimica delle Sostanze Naturali, Università di Napoli. The animals were chopped and extracted with distilled $H_2O$ (5 liters) for 3 h at room temperature, and the extraction was repeated three times. The aqueous extracts were centrifuged and passed through a column of Amberlite XAD-2 (1 kg). This column was washed with $H_2O$ and then with MeOH. The MeOH eluates were taken to dryness to give 4.0 g of glassy material, which was chromatographed on a column of Sephadex LH-60 (80 cm × 4 cm i.d.; 100 g) using MeOH-$H_2O$ (2:1) as eluent. The eluents were collected in 8-ml fractions and monitored by tlc with *n*-BuOH–HOAc–$H_2O$ (12:3:5), detection with ceric sulfate/$H_2SO_4$. Fractions 40–61 (fraction A, 663 mg) contained the crude "asterosaponins."

Fractions 62–80 (fraction B, 600 mg) mainly contained the sulfated steroidal monoglycosides along with tryptophan, nucleosides, and other polar compounds, while fractions 81–134 (fraction C, 300 mg) mainly contained the polyhydroxysteroids along with a mixture of fatty acids. Each of the above fractions was separately submitted to droplet counter-current chromatography (dccc).

Fraction A was purified by using *n*-BuOH–$Me_2CO$–$H_2O$ (45:15:75) (descending mode; the upper phase was the stationary phase, flow rate 15 ml/h) to give mainly thornasteroside A (1), which was finally purified by hplc on a $C_{18}$ μ-Bondapak column (30 cm × 7.8 mm i.d.) with MeOH-$H_2O$ (45:55) to give 40 mg of pure compound.

Fraction B was purified by using $CHCl_3$-MeOH-$H_2O$ (7:13:8) (ascending mode, the lower phase as the stationary phase; flow rate 10 ml/h; 5-ml fractions were collected and monitored by tlc). The more polar fractions 10–16 (131 mg) were then submitted to hplc on a $C_{18}$ μ-Bondapak column (30 cm × 7.8 mm i.d.) with MeOH-$H_2O$ (1:1) to give pure compounds, pycnopodioside B [**2**] (24 mg), pycnopodioside C [**3**] (20 mg), and the known coscinasteroside B (7 mg) (2), eluted in that order; the less polar fractions 57–86 (29 mg) were also submitted to hplc with MeOH-$H_2O$ (7:3) and gave the known steroid **7** (6 mg) (4) and the new pycnopodioside A [**1**] (3 mg), eluted in that order.

Fraction C was submitted to dccc under the same conditions as fraction B and gave further amounts of pycnopodioside A [**1**] (fractions 41–63; 5 mg after hplc with MeOH-$H_2O$ (7:3)], the new steroid **5** (fractions 64–96; 4 mg after hplc), and the known steroid **6** (3) (fractions 97–117; 8 mg after hplc). $^1$H- and $^{13}$C-nmr data of the new compounds are in Tables 1 and 2; fabms data are in the text. All known compounds were identified by comparison of their spectral data with those of authentic specimens.

METHANOLYSIS OF PYCNOPODIOSIDES A [**1**] AND C [**3**].—*Sugar analysis.*—A solution of **1** (2 mg) in anhydrous 2 M HCl in MeOH (0.5 ml) was heated at 80° in a stoppered reaction vial for 10 h. After being 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.), 15 min at room temperature. Glc analysis at 140° using a 25-m column of SE-30 (hydrogen carrier, flow 10 ml/min) gave gc peaks which co-eluted with those of silylated methyl xylosides. Pycnopodioside C [**3**] (2 mg) after methanolysis and silylation gave gc peaks co-eluted with those of silylated methyl glucosides.

SOLVOLYSIS OF PYCNOPODIOSIDE B [**2**] AND PYCNOPODIOSIDE C [**3**].—A solution of **2** (5 mg) in pyridine (0.2 ml) and dioxane (0.2 ml) was heated at 140° for 2 h in a stoppered reaction vial. After the solution was cooled, $H_2O$ (2 ml) was added, and the solution was extracted with *n*-BuOH (3 × 1 ml). The combined extracts were washed with $H_2O$ and evaporated to dryness under reduced pressure. The residue was purified by hplc [$C_{18}$ μ-Bondapak, MeOH-$H_2O$ (70:30)] to give 3.1 mg of **1**, fabms *m/z* 583 $[M-H]^-$; $^1$H nmr see Table 1. A solution of **3** (5 mg) was similarly solvolyzed, affording **4** (2.9 mg after hplc): fabms *m/z* 613 $[M-H]^-$; $^1$H nmr see Table 1.

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## LITERATURE CITED

1. I. Kitagawa and M. Kobayashi, *Chem. Pharm. Bull.*, **26**, 1864 (1978).
2. R. Riccio, M. Iorizzi, and L. Minale, *Bull. Soc. Chim. Belg.*, **95**, 869 (1986).
3. M. Iorizzi, L. Minale, R. Riccio, M. Debray, and J.L. Menou, *J. Nat. Prod.*, **49**, 67 (1986).
4. R. Riccio, L. Minale, S. Pagonis, C. Pizza, F. Zollo, and J. Pusset, *Tetrahedron*, **38**, 3615 (1982).
5. R. Riccio, M.V. D'Auria, M. Iorizzi, L. Minale, D. Laurent, and D. Duhet, *Gazz. Chim. Ital.*, **115**, 405 (1985).
6. L. Minale, R. Riccio, C. Pizza, and F. Zollo, in: "Natural Products and Biological Activities," a NAITO Foundation Symposium. Ed. by H. Himura, T. Goto, T. Murachi, and T. Nakajima, University of Tokyo Press, Elsevier Science, Tokyo, 1986, p. 59.
7. R. Riccio, M. Iorizzi, L. Minale, Y. Oshima, and T. Yasumoto, *J. Chem. Soc., Perkin Trans. 1*, 1337 (1988).
8. S. Seo, Y. Tomita, K. Tori, and Y. Yoshimura, *J. Am. Chem. Soc.*, **100**, 3331 (1978).
9. J. McKenna and J.K. Norymberski, *J. Chem. Soc.*, 3889 (1957).
10. P.A. Gorin and M. Mazurek, *Can. J. Chem.*, **53**, 1212 (1975).
11. M.V. D'Auria, L. Minale, C. Pizza, R. Riccio, and F. Zollo, *Gazz. Chim. Ital.*, **114**, 469 (1984).
12. F. Zollo, E. Finamore, and L. Minale, *J. Nat. Prod.*, **50**, 794 (1987).

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