

## STARFISH SAPONINS, PART 36.<sup>1</sup> STEROIDAL OLIGOGLYCOSIDES FROM THE PACIFIC STARFISH *THROMIDIA CATALAI*<sup>2</sup>

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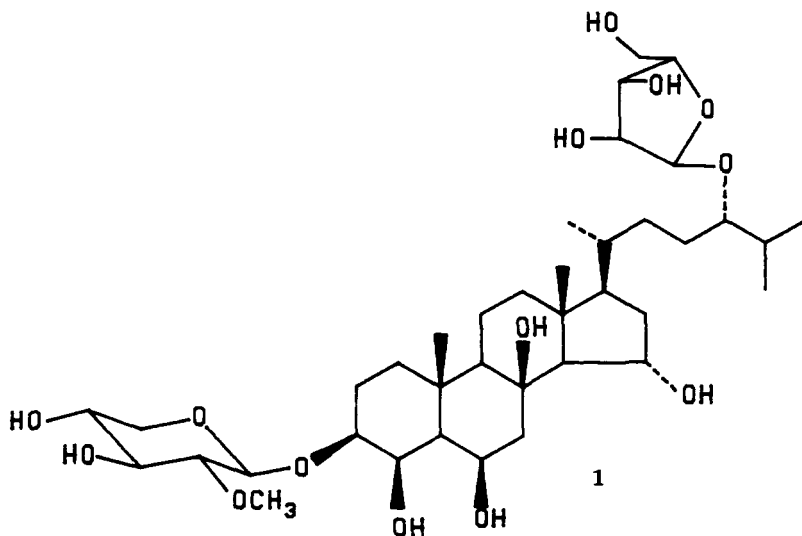
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Continuing our work on biologically active compounds from starfish (1), we have been working on the glycosides of the Pacific starfish *Thromidia catalai* Pope et Rowe (Mithzodiidae) (2) and have isolated three known sulfated "asterosaponins," thornasteroside A (3), ophidianoside F (4), and regularoside B (5); one known steroidal diglycoside, granuloside A {(24S)-3-O-[2-O-methyl- $\beta$ -D-xylopyranosyl]-24-O- $\alpha$ -L-arabinofuranosyl-5 $\alpha$ -cholestane-3 $\beta$ ,4 $\beta$ ,6 $\beta$ ,8,15 $\alpha$ ,24-hexol} [1], previously isolated from *Choriaster granulatus* (6); and one new minor steroidal monoglycoside, thromidioside [2].

Thornasteroside A [(20S)-6-O-  $\beta$ -D-

fucopyranosyl-(1 $\rightarrow$ 2)- $\beta$ -D-galactopyranosyl-(1 $\rightarrow$ 4)-[ $\beta$ -D-quinovopyranosyl-(1 $\rightarrow$ 2)]- $\beta$ -D-xylopyranosyl-(1 $\rightarrow$ 3)- $\beta$ -D-quinovopyranosyl]-20 $\alpha$ -hydroxy-23-oxo-5 $\alpha$ -cholest-9(11)-en-3 $\beta$ -yl sodium sulfate] (3) is widely distributed among starfish (1), while ophidianoside F and regularoside B, which are similar to thornasteroside A except that galactose is replaced by xylose in ophidianoside F and by fucose in regularoside B, have been rarely isolated [from *Ophidiaster ophidianus* (4) and *Halytile regularis* (5), respectively].

Thromidioside [2], [ $\alpha$ ]<sub>D</sub> + 10°, is related to the xyloside 3, previously isolated from the starfish *Poraster superbus*



<sup>1</sup>For Part 35, see R. Riccio, O. Squillace Greco, and L. Minale, *J. Nat. Prod.*, **51**, 989 (1988).

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(7), by introduction of an "extra" hydroxyl group at the 4 $\beta$  position of the aglycone. The fab/MS (negative ion mode) gave a pseudomolecular ion peak at  $m/z$  641 [ $M - H$ ]<sup>-</sup> and a major frag-

ment at  $m/z$  495  $[M - H - 146]^-$ , 16 mass units shifted relative to **2**.

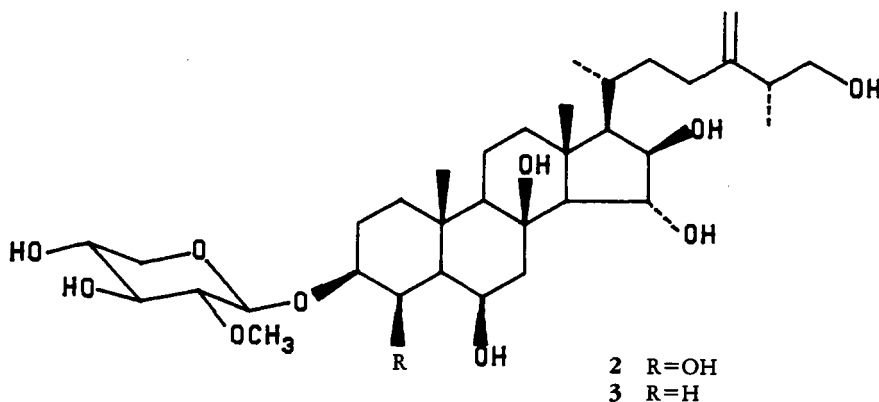
The differences in the  $^1\text{H}$ -nmr spectra of thromidioside [**2**] and **3**, which indicated the location of the extra hydroxyl group at C-4 $\beta$  in **2**, were the presence of one more narrow hydroxymethine signal at  $\delta$  4.30 and the down-field shift exhibited by the signals for 19-H<sub>3</sub> ( $\delta$  1.48 s in **2** vs.  $\delta$  1.20 s in **3**) and 6 $\alpha$ -H ( $\delta$  4.30, narrow signal overlapping with 4 $\alpha$ -H in **2** vs.  $\delta$  3.91 q,  $J = 3$  Hz, in **3**). The  $^{13}\text{C}$ -nmr spectrum (see Experimental) and comparison with reference compound **3**

derivative (**8**). The shifts of the side chain carbons in the  $^{13}\text{C}$ -nmr spectrum of thromidioside [**2**] were identical to those of the model compound. On this basis we suggest the same 25*R* stereochemistry for the new glycoside **2** as well as for the known **3**.

## EXPERIMENTAL

**INSTRUMENTAL.**—For instruments used, see Pizza *et al.* (6).

**EXTRACTION AND ISOLATION.**—The animals (0.5 kg) were collected in January 1986, off Nouméa and identified by Dr. Michael Jangoux



added conclusive support to this assignment. The differences in the spectra of the two related xylosides were the replacement of the signal at 38.3 ppm assigned to C-4 in **3** by a signal at 74.6 ppm in **2**, the up-field shifts exhibited by C-2 (25.2 vs. 30.3 ppm) and C-11 (19.1 vs. 19.5 ppm) and the down-field shifts exhibited by C-5 (50.5 vs. 49.0 ppm), C-6 (76.2 vs. 74.7 ppm), and C-19 (18.6 vs. 15.7 ppm) in **2** relative to **3**. Indeed, these shifts were also observed in the spectrum of granulatoside A [**1**], having the same 3 $\beta$ ,4 $\beta$ ,6 $\beta$ ,8-hydroxylation pattern with the 2-*O*-methyl- $\beta$ -xylopyranosyl residue located at C-3 (6).

More recently we isolated from the starfish *Sphaerodiscus placenta* (25*R*)-24-methyl-5 $\alpha$ -cholest-24(28)-en-3 $\beta$ ,6 $\beta$ ,15 $\alpha$ ,16 $\beta$ ,26-pentol to which the 25*R* stereochemistry was assigned on the basis of the positive cd curve of a 24-keto

of the Université Libre de Bruxelles; a voucher specimen is preserved at the Centre ORSTOM, Nouméa. The animals were chopped and soaked in H<sub>2</sub>O for 4 h. The aqueous extracts were centrifuged and passed through a column of Amberlite XAD-2 (0.5 kg). This column was washed with H<sub>2</sub>O and then with MeOH. The MeOH eluates were taken to dryness to give 0.71 g of glassy material, which was submitted to dccc [*n*-BuOH-Me<sub>2</sub>CO-H<sub>2</sub>O (45:15:75)] in the ascending mode at a flow rate of 20 ml/h; fractions of 7 ml were collected and monitored by tlc on silica in CHCl<sub>3</sub>-MeOH-H<sub>2</sub>O (80:18:2) and in *n*-BuOH-AcOH-H<sub>2</sub>O (60:15:25).

Fractions 21–30 contained granulatoside A [**1**] (6) and thromidioside [**2**]; the successive fractions 31–40 contained a mixture of two nonsteroidal components, while the asterosaponins, thornasteroside A (3), ophidianside F (4), and regularoside B (5), were dispersed between fractions 47 and 140, which were combined according to tlc results.

Granulatoside A and thromidioside [**2**] were then separated by hplc on a C<sub>18</sub>  $\mu$ -Bondapak column (30 cm  $\times$  7.8 mm i.d.) with MeOH-H<sub>2</sub>O (7:3) to obtain pure granulatoside A (12 mg) and thromidioside [**2**] (2.7 mg). The "asterosaponin"

fractions were also separated by hplc on a  $C_{18}$   $\mu$ -Bondapak column with MeOH-H<sub>2</sub>O (1:1). The total yield of each asterosaponin was thornasteroside A 25 mg, ophidianoside F 23 mg, and regularoside B 4 mg. The known compounds were identified by 250-MHz <sup>1</sup>H nmr, fabms, and authentic sample comparison by hplc and SiO<sub>2</sub> tlc.

*Thrombidioside* [2].—[ $\alpha$ ]<sub>D</sub>+10° ( $c$ =0.2, MeOH); fabms (negative ion)  $m/z$  [M-H]<sup>-</sup> 641 (100%) and aglycone fragment at  $m/z$  [M-H-146]<sup>-</sup> 495 (20); <sup>1</sup>H nmr (CD<sub>3</sub>OD, 250 MHz) aglycone,  $\delta$  H 0.98 (3H, d,  $J$ =7.0 Hz, 21-H<sub>3</sub>), 1.08 (3H, d,  $J$ =7.0 Hz, 27-H<sub>3</sub>), 1.15 (3H, s, 18-H<sub>3</sub>), 1.48 (3H, s, 19-H<sub>3</sub>), 1.62 (1H, dd,  $J$ =15.0, 2.5 Hz, 7 $\alpha$ -H), 2.33 (1H, m, 25-H), 2.48 (1H, dd,  $J$ =15.0, 2 Hz, 7 $\beta$ -H), 3.58 and 3.65 (each 1H, dd,  $J$ =5, 12 Hz and  $J$ =6.5, 12 Hz, 26-H<sub>2</sub>), 3.67 (1H, m, 3 $\alpha$ -H), 4.02 (1H, dd,  $J$ =8.0, 2.5 Hz, 16 $\alpha$ -H), 4.17 (1H, dd,  $J$ =11.0, 2.5 Hz, 15 $\beta$ -H), 4.30 (2H, m, 4 $\alpha$ - and 6 $\alpha$ -H), 4.77 and 4.85 (each 1H, br s, 28-H<sub>2</sub>); sugars,  $\delta$  H, 2.93 (1H, dd,  $J$ =9.0, 7.5 Hz, 2'-H), 3.20 (1H, dd,  $J$ =11.5, 11.5 Hz, 5'-Hax), 3.34 (overlapping with solvent signal, 3'-H), 3.50 (1H, m, 4'-H), 3.85 (1H, dd,  $J$ =11.5, 4.5 Hz, 5'-Heq), 4.48 (1H, d,  $J$ =7.5 Hz, 1'-H); <sup>13</sup>C nmr (CD<sub>3</sub>OD, 62.9 MHz) C-1 41.3, C-2 25.2, C-3 80.5, C-4 74.6, C-5 50.5, C-6 76.2, C-7 45.2, C-8 76.5, C-9 57.7, C-10 36.9, C-11 19.1, C-12 43.1, C-13 45.2, C-14 63.9, C-15 80.9, C-16 83.0, C-17 60.6, C-18 16.7, C-19 18.6, C-20 30.7, C-21 18.6, C-22 35.6, C-23 32.9, C-24 154.0, C-25 43.5, C-26 67.4, C-27 17.3, C-28 109.1, C-1' 102.4, C-2' 84.7, C-3' 77.6, C-4' 71.3, C-5' 66.8, OMe 61.0 ppm.

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#### LITERATURE CITED

1. L. Minale, R. Riccio, C. Pizza, and F. Zollo, in: "Natural Products and Biological Activities." Ed. by H. Imura, T. Goto, T. Murachi, and T. Nakajima, University of Tokyo Press/Elsevier Science Publishers, Amsterdam 1986, pp. 59-73, and references cited therein.
2. M. Jangoux, A. Guille, P. Laboute, and T.L. Menou, in: "Guide des étoiles de mer, oursins et autres échinoderms du lagon de Nouvelle-Caledonie." Editions de l'ORSTOM, Paris, 1986, p. 150.
3. I. Kitagawa and M. Kobayashi, *Chem. Pharm. Bull.*, **26**, 1864 (1978).
4. R. Riccio, C. Pizza, O. Squillace Greco, and L. Minale, *J. Chem. Soc., Perkin Trans. 1*, 655 (1985).
5. R. Riccio, M. Iorizzi, O. Squillace Greco, L. Minale, M. Debray, and J.L. Menou, *J. Nat. Prod.*, **48**, 756 (1985).
6. C. Pizza, L. Minale, D. Laurent, and J.L. Menou, *Gazz. Chim. Ital.*, **115**, 585 (1985).
7. R. Riccio, M. Iorizzi, O. Squillace Greco, L. Minale, D. Laurent, and Y. Barbin, *Gazz. Chim. Ital.*, **115**, 505 (1985).
8. F. Zollo, E. Finamore, and L. Minale, *J. Nat. Prod.*, **50**, 794 (1987).
9. C.L. VanAntwerp, H. Eggert, G.D. Meakins, J.O. Miners, and C. Djerassi, *J. Org. Chem.*, **42**, 789 (1977).

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