ISOLATION AND STRUCTURE OF NEW POLYHYDROXYLATED STEROLS FROM A DEEP-WATER STARFISH OF THE GENUS *Rosaster* (*) (**)

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Summary — Three novel polyhydroxylated steroids, (25S)-5α-cholestane-3β,5α,6β,15α,16β,26-hexol 15-sulphate (**1**), (25S)-5α-cholestane-3β,6β,7α,8,15α,16β,26-heptol (**4**) and (25S)-5α-cholestane-3β,4β,6β,7α,8,-15α,16β,26-octol (**5**), have been isolated from a Pacific deep-water starfish of the genus *Rosaster*. They co-occurr with two known polyhydroxysteroids (**2** and **3**). The novel compound **5** showed antifungal activity.

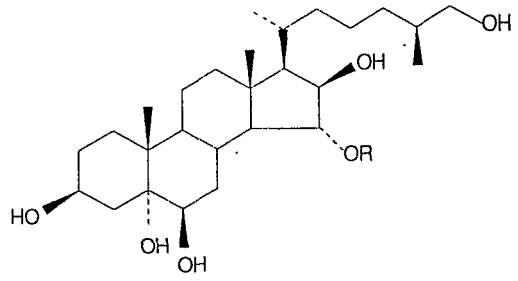
In the course of our continuing studies of oligoglycoside and polyhydroxy steroidal constituents of starfishes, we have been working on starfish species collected off Nouméa, New Caledonia, and in this Note we report three new polyhydroxylated sterols (**1**, **4** and **5**) isolated from a starfish of the genus *Rosaster*. The new compounds co-occur with two more known compounds (**2** and **3**) firstly isolated from *Protoreaster nodosus*[1] and then from other starfishes[2,3]. Of the isolated steroids the novel compound **5** inhibited growth of the patogenic fungus *Cladosporium cucumerinum* at a level of 5μg.

EXPERIMENTAL

Extraction and Isolation. - The fresh animals (1.5 kg), collected at a depth of 400-500 m off Nouméa, New Caledonia, in August 1987, were chopped and soaked in water for 4 h. The aqueous extracts were centrifuged and passed through a column of Amberlite XAD-2 (1 kg). This column was washed with water, then with methanol. The methanol eluate was taken to dryness to give 0.54 g of a glassy material which was chromatographed on a column of

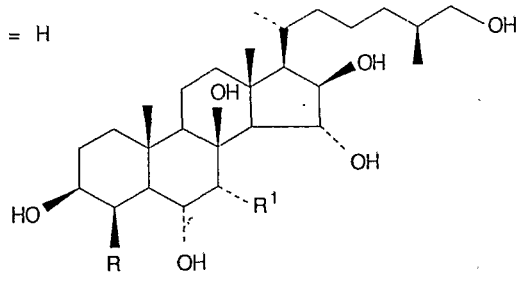
Each of the above fractions was then submitted to HPLC on a C_{18} μ-Bondapak column (30 cm x 7.8 mm i.d.) with 1:1 MeOH/H_2O (fr. 20-40) and 7:3 MeOH/H_2O (remaining fractions) to give single compounds (in parenthesis the amount of each compound after HPLC): **1**, $[\alpha]_D$ = +12.8° (*c*= 1, MeOH), FABMS (-ve ion), *m/z* 547 ($[M]^-$); steroid **2**, $[\alpha]_D$ = 13.8° (*c*= 2, MeOH), FABMS (-ve ion), *m/z* 467 ($[M-H]^-$), ^1H NMR identical with that of an authentic sample isolated from *Protoreaster nodosus* [1]; steroid **3**, $[\alpha]_D$ = +10° (*c*= 1, MeOH), FABMS (-ve ion) *m/z* 499 ($[M-H]^-$), ^1H NMR identical with that of an authentic sample isolated from *Protoreaster nodosus*[1]; steroid **4**, $[\alpha]_D$ = 5.5° (*c*= 1, MeOH), FABMS (-ve ion), *m/z* 483 ($[M-H]^-$); steroid **5** , $[\alpha]_D$ = 9° (*c*= 1, MeOH), FABMS (-ve ion), *m/z* 499 ($[M-H]^-$); the ^1H and ^{13}C NMR data of the new compounds are in the Tables 1 and 2.

Solvolysis of the steroid 1 giving 1a - A solution of **1** (2 mg) in dioxan (0.1 ml) and pyridine (0.1 ml) was heated in a stoppered reaction vial at 140 °C for 4 h. The residue, which was obtained by evaporation of the solvents to dryness under reduced pressure, was purified by HPLC (C_{18} μ-Bondapak column, 30 cm x 3.9 mm i.d., 65:35 MeOH/H_2O) to give (25S)-5α-cholestane-3β,5,6β,15α,16β,26-hexol (**1a**) by direct comparison (^1H NMR, TLC, HPLC)



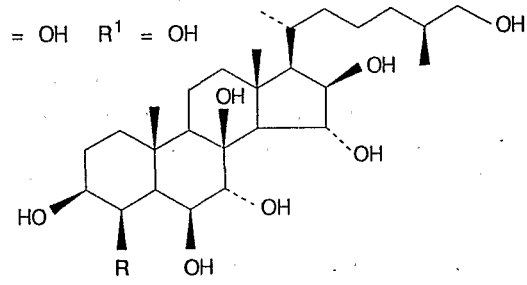
1 ; R = $SO_3^-Na^+$

1a ; R = H



2 ; R = H R^1 = H

3 ; R = OH R^1 = OH



4 ; R = H

5 ; R = OH

bombardment with Xe atoms of 2.6 kV. HPLC: Waters Model 6000A pump equipped with a U6K injector and a differential refractometer, Mod. 401. DCCC: DCCC-A apparatus manufactured by Tokyo Rikakikai, equipped with 300 tubes (2 mm i.d.)

RESULTS AND DISCUSSION

The steroid **1** , $[\alpha]_D$= 12.8°, is the 15-sulphated derivative of (25S)-5α-cholestane-3β,5α,6β,15α,16β,26-hexol, previously isolated from the starfish *Luidia maculata* [4]. The FAB mass spectrum (negative ion mode) showed a molecular anion peak at *m/z* 547, corresponding to a monosulphated derivative of a cholestane-hexol. On solvolysis in a dioxan-pyridine mixture it afforded a desulphated derivative, FABMS (negative ion mode), *m/z* 467 ([M-H]$^-$), identical (^{1}H NMR, TLC, HPLC) with (25S)-5α-cholestane-3β,5α,6,15α,16β,26-hexol [4] (**1a**). The location of the sulphate at C-15 in **1** was determined by comparison of ^{1}H and ^{13}C NMR spectra of **1** (Tables 1 and 2) with those of **1a** [4]. The ^{1}H NMR spectrum of **1** showed the 15β-H and 16α-H signals shifted downfield to δ 4.38 and 4.33 ppm as compared with the same signals (δ 3.76 and 4.02 ppm) in **1a** [4]; the ^{13}C NMR spectrum of **1** showed the signal for C-15 downfield shifted to 91.7 ppm (85.0 ppm in **1a**) and the signals for C-14 and C-16 upfield shifted to 58.5 ppm (61.2 ppm in **1a**) and 80.1 ppm (83.2 ppm in **1a**), respectively.

TABLE 1 - 250 MHz ^{1}H NMR (CD_3OD) SIGNALS (δ-H) FOR THE NOVEL STEROIDS **1, 4** AND **5**; COUPLING CONSTANTS (Hz) IN PARENTHESIS

Compounds	1	4	5
H at C			
3	4.08 m	3.60 m	3.50 m
4	=	=	4.10 br s
6	3.50 br s	3.72 dd (3.1, 2.9)	4.04 dd [c]
7	=	3.87 d (3.1)	3.85 d (3)
14	=	1.46 d (10)	1.46 d (10)
15	4.38 dd [a]	4.21 dd (10, 2.5)	4.21 dd (10, 2.5)
16	4.33 dd [a]	4.02 dd (7.5, 2.5)	4.02 dd [c]
18	1.0 s	1.15 s	1.15 s
19	1.20 s	1.15 s	1.43 s
21	0.98 d (7)	0.97 d (7)	0.97 d (7)
26	3.44 dd [b] (10.5, 6)	3.46 dd [b] (10.5, 6)	3.46 dd [b] (10.5, 6)
27	0.93 d (6)	0.94 d (6)	0.94 d (6)

([a]) - H-15 and H-16 partially overlap. ([b]) - The upfield part of the AB portion of the ABX system is hidden below the MeOD signal. ([c]) - H-6 and H-16 partially overlap.

The novel steroid **4**, $[\alpha]_D$= 5.5°, FABMS (-ve ion), *m/z* 483 ([M-H]$^-$) is isomeric with the previously isolated (25S)-5α-cholestane-3β,6α,7α,8,6β,15α,16β,26-heptol [1] and differs from it in the stereochemistry at C-6, which in **4** is 6β-OH. The ^{1}H NMR spectrum (Table 1) showed the 19-methyl singlet downfield shifted to δ 1.15 (1.00 in the 6α-isomer) and a dd (*J*= 3.1 and 2.9 Hz) at δ 3.72 (6α-H) ppm, characteristic of an equatorial proton, coupled with a doublet (*J*= 3.1 Hz) at δ 3.87 (7-H) ppm and with a signal confused in the high

TABLE 2 - ^{13}C NMR SHIFTS (δ ppm) OF THE NOVEL STEROIDS **1, 4** AND **5** IN CD_3OD SOLUTION MEASURED AT 62.9 MHz

Carbon	1	4	5
1	31.7	41.4	41.1
2	33.6	31.7	26.6
3	68.4	72.5	73.2
4	41.9	35.8	77.7
5	76.6	42.8	45.3
6	76.5	74.1	80.1
7	34.8	78.4	73.4
8	31.2	77.8	78.2
9	46.5	51.4	52.1
10	39.5	36.4	36.6
11	21.9	18.3	19.0
12	42.0	43.2	43.1
13	44.2	45.6	45.5
14	58.5	59.5	59.4
15	91.7	80.1	80.0
16	80.1	82.8	82.7
17	60.0	61.6	61.6
18	15.1	16.8	16.8
19	17.3	16.4	19.2
20	30.9	30.6	30.6
21	18.5	18.3	18.3
22	37.3	37.0	37.0
23	24.8	24.8	24.8
24	34.8	35.0	35.0
25	37.1	37.1	37.1
26	68.8	69.6	68.6
27	17.0	17.2	17.2

region of the spectrum. The remaining hydroxymethine signals in the spectrum at δ 3.60 (1H, m), 4.02 (1H, dd, *J*= 7.5, 2.5 Hz), 4.21 (1H, dd, *J*= 10, 2.5 Hz) and 3.46 (1H, dd, *J*= 10.5, 6 Hz) ppm were very close to those observed in the spectrum of the 6α-OH epimer[1] and assigned to 3α-H, 16α-H, 15β-H and 26-H (the remaining 26-H signal under CD_3OD), respectively. The ^{13}C NMR spectrum (Table 2) and comparison with model 24-methylene-5α-cholestane-3β,6β,8,15α,16β,26-hexol[6] and 5α-cholestane-3β,6α,7α,8,15α,16β, 26-heptol[1a] confirmed the proposed formulation **4** for the novel steroid. The most significant differences in the ^{13}C spectra of **4** and its 6α-OH epimer, which indicated the 6β-OH stereochemistry for **4** were the downfield shifts exhibited by C-6 (74.1 *vs* 68.9 ppm) and C-19 (17.3 *vs* 13.9 ppm in 6α-OH isomer), while the large upfield shifts observed for C-5 (-7.8 ppm), C-9 (-5.9 ppm) and C-10 (-4.5 ppm) (γ-gauche shifts)[7] in comparison with the model 24-methylene-5α-cholestane-3β,6β,8,15α,16β,26-hexol[6], supported the 7α-hydroxy stereochemistry in **4**. The addition of a 7β-hydroxy substituent to the model would result in much smaller shifts of the γ-carbons spanning in a range of +1.3 to-3.0 ppm (γ-*trans* shifts)[7]. The 25S-configuration is assumed by analogy with steroid **5**, for which the 25S-configuration has been determined (see below).

The new steroid **5**, $[\alpha]_D$ = +9°, FABMS (-ve ion), *m/z* 499 ($[M-H]^-$), is isomeric with **3**, now isolated from *Rosaster* sp. and previously from *Protoreaster nodosus*[1] and other starfishes[2,3], differing from it in the stereochemistry at C-6, which in **5** is 6β-OH and is related to **4** by introduction of an additional hydroxyl group at C-4β. The structure was derived from the analysis of the ^1H and ^{13}C NMR data (Tables 1 and 2) and comparison with those of the 6α-isomer **3** and the 4-deoxy derivative **4**. The stereochemistry at C-25 has been determined by using the MTPA method[1b]; the steroid **5** was treated with (+)-methoxy-trifluoromethylphenyl acetyl chloride (Mosher reagent[8]) in pyridine affording 26-(R)-(+)-methoxy-trifluoromethylphenyl acetate (MTPA). The ^1H NMR spectrum showed a signal at δ 4.21 (2H, d, *J*= 6 Hz) for 26-H_2, thus indicating the configuration to be 25S like other 26-hydroxysteroids from starfishes[9]. In the ^1H NMR spectrum of (R)-(+)-MTPA ester of (25R)-26-hydroxysteroids signals for 26-H_2 were found at δ 4.24 (dd, *J*= 11, 6 Hz) and 4.08 (dd, *J*= 11, 7 Hz)[10].

The steroid **5** showed moderate antifungal activity (inhibition growth of the fungus *Cladosporium cucumerinum*, active at 5 μg). The remaining isolated steroids (**1-4**) were inactive at a dose of 20 μg.

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REFERENCES

(1) a) R. Riccio, L. Minale, S. Pagonis, C. Pizza, F. Zollo, J. Pusset, *Tetrahedron, 38*, 3615 (1982).
b) M.V. D'Auria, L. Minale, C. Pizza, R. Riccio, F. Zollo, *Gazz. Chim. Itall 114*, 469 (1984).

(2) A.A. Kicha, A.I. Kalinovsky, E.V. Levina, V.A. Stonik, G.B. Elyakov, *Bioorg. Khim. 9*, 975 (1983) [*Chem. Abstr. , 99* P 1555 96 s (1983)].

(3) R. Higuchi, Y. Noguchi, T. Komori, T. Sasaki, *Liebigs Ann. Chem.*, 1185 (1988).

(4) L. Minale, C. Pizza, R. Riccio, O. Squillace Greco, F. Zollo, J. Pusset, J.L. Menou, *J. Nat. Prod. , 47*, 784 (1984).

(5) A.L. Homans, A. Fuchs, *J. Chromatogr. , 51*, 327 (1970).

(6) F. Zollo, E. Finamore, L. Minale, *J. Nat. Prod. , 50*, 794 (1987).

(7) H. Eggert, C.L. Van Antwerp, N.S. Bhacca, C. Djerassi, *J. Org. Chem ., 41*, 71 (1976).

(8) J.A. Dale, H.S. Mosher, *J. Amer. Chem. Soc. , 95*, 512 (1973).

(9) L. Minale, R. Riccio, C. Pizza, F. Zollo, in "Natural Products and Biological Activities", a NAITO Foundation Symposium; H. Imura, T. Goto, T. Murachi, T. Nakjima, Eds., University of Tokyo Press and Elsevier Science Publishers, 1986, pp. 59-73.

(10) K. Tachibana, M. Sakaitani, K. Nakanishi, *Tetrahedron, 41*, 1027 (1985).