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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)-5 α -cholestane-3 β ,5 α ,6 β ,15 α ,16 β ,26-hexol 15-sulphate (1), (25S)-5 α -cholestane-3 β ,6 β ,7 α ,8,15 α ,16 β ,26-heptol (4) and (25S)-5 α -cholestane-3 β ,4 β ,6 β ,7 α ,8,15 α ,16 β ,26-octol (5), have been isolated from a Pacific deep-water starfish of the genus *Rosaster*. They co-occur 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 pathogenic fungus *Cladosporium cucumerinum* at a level of 5 μ g.

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 LH-60 (4 x 60 cm) using MeOH/H₂O as eluent. Fractions of 7 ml were collected and checked by TLC on SiO₂ with CHCl₃/MeOH/H₂O (80:18:2). Fractions 38-56 contained mixed polyhydroxysteroids and other compounds (0.27 g) which were submitted to droplet counter-current chromatography (DCCC; with CHCl₃/MeOH/H₂O (7:14:8) in the ascending mode, the lower layer was the stationary phase, at 10.5 ml/h⁻¹; 5 ml fractions were collected) to give the following fractions:

Fractions No.	Amount, mg	Compounds
20-40	40	1 (6 mg)
41-70	20	2 (6 mg) + 3 (5 mg)
71-110	17	4 (5 mg)
111-150	26	5 (10 mg)

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Each of the above fractions was then submitted to HPLC on a C₁₈ μ -Bondapak column (30 cm x 7.8 mm i.d.) with 1:1 MeOH/H₂O (fr. 20-40) and 7:3 MeOH/H₂O (remaining fractions) to give single compounds (in parenthesis the amount of each compound after HPLC): 1, [α]_D = +12.8° (c=1, MeOH), FABMS (-ve ion), *m/z* 547 ([M]⁺); steroid 2, [α]_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, [α]_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, [α]_D = 5.5° (c=1, MeOH), FABMS (-ve ion), *m/z* 483 ([M-H]⁻); steroid 5, [α]_D = 9° (c=1, MeOH), FABMS (-ve ion), *m/z* 499 ([M-H]⁻); the ¹H and ¹³C 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 (C₁₈ μ -Bondapak column, 30 cm x 3.9 mm i.d., 65:35 MeOH/H₂O) to give (25S)-5 α -cholestane-3 β ,5,6 β ,15 α ,16 β ,26-hexol (1a) by direct comparison (¹H NMR, TLC, HPLC) with an authentic sample isolated from *Luidia maculata*⁴; the 25S-configuration has been determined by the MTPA method^{5b} (see below).

(+)-MTPA Esters of compound 5. - The steroid 5 (2.5 mg) was treated with freshly distilled (+)-methoxytrifluoromethylphenyl acetyl chloride (5 μ l) in dry pyridine (0.15 ml) for 1 h at room temperature. After removal of solvent the product was analysed by ¹H NMR, δ (CD₃OD) 0.94 (3H, d, *J* = 7 Hz, *sec*-Me), 0.96 (3H, d, *J* = 7 Hz, *sec*-Me) and 4.22 (2H, d, *J* = 6 Hz, 26-H₂) ppm, the remaining signals were virtually superimposable with those of the genuine 5, thus establishing that esterification had occurred at C-26 and that the stereochemistry at C-25 is 25S^{5b}.

Bioassay. - The steroids were tested for antifungal activity by using the direct bioautography on tic method⁵. *Cladosporium cucumerinum* was used as test organism.

INSTRUMENTS.

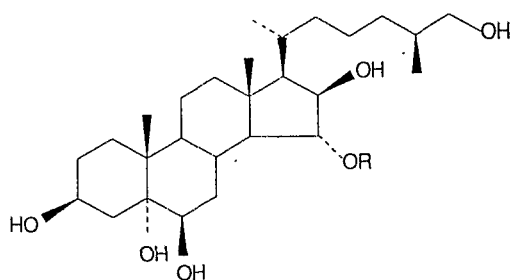
The following instruments were used. NMR: Bruker WM-250; a signal at 3.34 ppm for CHD₂OD was used as the standard for ¹H measurements in CD₃OD; a signal for ¹³CD₃OD was used as standard at 49.0 ppm for ¹³C measurements in CD₃OD. MS: Kratos MS 50 mass spectrometer equipped with a FAB source; mass spectra were obtained by dissolving the samples in a glycerol/thioglycerol 3:1 matrix and placing them on a copper probe prior to

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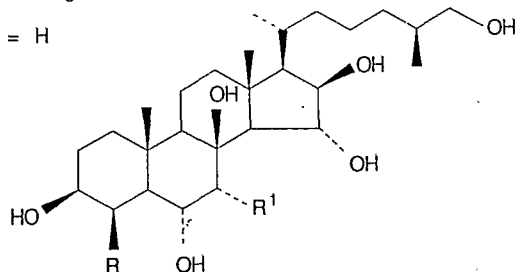
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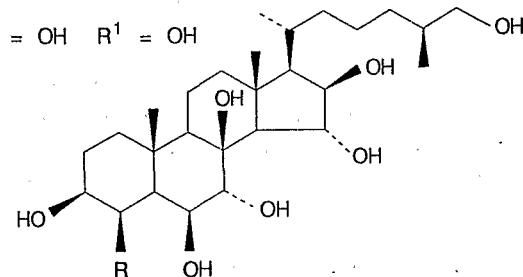
1; R = SO₃⁻Na⁺

1a; R = H



2; R = H R' = H

3; R = OH R' = 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**, [α]_D²⁵ = 12.8°, is the 15-sulphated derivative of (25S)-5 α -cholestane-3 β ,5 α ,6 β ,15 α ,16 β ,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 (¹H NMR, TLC, HPLC) with (25S)-5 α -cholestane-3 β ,5 α ,6,15 α ,16 β ,26-hexol⁴ (**1a**). The location of the sulphate at C-15 in **1** was determined by comparison of ¹H and ¹³C NMR spectra of **1** (Tables 1 and 2) with those of **1a**⁴. The ¹H NMR spectrum of **1** showed the 15 β -H and 16 α -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 ¹³C 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 ¹H NMR (CD₃OD) 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**, [α]_D²⁵ = 5.5°, FABMS (-ve ion), *m/z* 483 ([M-H]⁻) is isomeric with the previously isolated (25S)-5 α -cholestane-3 β ,6 α ,7 α ,8,6 β ,15 α ,16 β ,26-heptol⁴ and differs from it in the stereochemistry at C-6, which in **4** is 6 β -OH. The ¹H NMR spectrum (Table 1) showed the 19-methyl singlet downfield shifted to δ 1.15 (1.00 in the 6 α -isomer) and a dd (J = 3.1 and 2.9 Hz) at δ 3.72 (6 α -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 - ¹³C NMR SHIFTS (δ ppm) OF THE NOVEL STEROIDS **1**, **4** AND **5** IN CD₃OD 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 6 α -OH epimer¹ and assigned to 3 α -H, 16 α -H, 15 β -H and 26-H (the remaining 26-H signal under CD₃OD), respectively. The ¹³C NMR spectrum (Table 2) and comparison with model 24-methylene-5 α -cholestane-3 β ,6 β ,8,15 α ,16 β ,26-hexol⁶ and 5 α -cholestane-3 β ,6 α ,7 α ,8,15 α ,16 β ,26-heptol¹⁰ confirmed the proposed formulation 4 for the novel steroid. The most significant differences in the ¹³C spectra of 4 and its 6 α -OH epimer, which indicated the 6 β -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 6 α -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-5 α -cholestane-3 β ,6 β ,8,15 α ,16 β ,26-hexol⁶, supported the 7 α -hydroxy stereochemistry in 4. The addition of a 7 β -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, $[\alpha]_D^{25} = +9^\circ$, FABMS (-ve ion), m/z 499 ($[M-H]^+$), is isomeric with 3, now isolated from *Rosaster* sp. and previously from *Protorea-ster nodosus*¹ and other starfishes^{2,3}, differing from it in the stereochemistry at C-6, which in 5 is 6 β -OH and is related to 4 by introduction of an additional hydroxyl group at C-4 β . The structure was derived from the analysis of the ¹H and ¹³C NMR data (Tables 1 and 2) and comparison with those of the 6 α -isomer 3 and the 4-deoxy derivative 4. The stereochemistry at C-25 has been determined by using the MTPA method⁸; the steroid 5 was treated with (+)-methoxy-trifluoromethylphenyl acetyl chloride (Mosher reagent⁸) 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-H₂, thus indicating the configuration to be 25S like other 26-hydroxysteroids from starfishes⁹. In the ¹H NMR spectrum of (R)-(+)-MTPA ester of (25R)-26-hydroxysteroids signals for 26-H₂ were found at δ 4.24 (dd, $J=11, 6$ Hz) and 4.08 (dd, $J=11, 7$ Hz)⁹.

The steroid 5 showed moderate antifungal activity (inhibition growth of

the fungus *Cladosporium cucumerinum*, active at 5 μ g). The remaining isolated steroids (1-4) were inactive at a dose of 20 μ g.

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