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FOUR NEW BIOACTIVE POLYHYDROXYLATED STEROLS FROM THE BLACK CORAL *ANTIPATHES SUBPINNATA*

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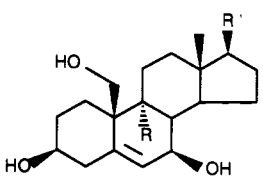
ABSTRACT.—Four new polyoxygenated sterols **2–5** were isolated from the marine black coral *Antipathes subpinnata*. The structures of these compounds, including stereochemical details, were deduced by ms, ^{1}H- and ^{13}C-nmr, ^{1}H-^{1}H COSY, and nOe difference spectroscopy. Compounds **2–5** are lethal to brine shrimp (*Artemia salina*).

We recently reported the isolation of five new highly oxygenated sterols [(20*S*,22*E*)-cholesta-1,4,22-triene-18,20-diol-3-one, (20*S*,22*E*)-24-methylcholesta-1,4,22-triene-18,20-diol-3-one, (20*S*)-cholest-4-ene-16β,18,20-triol-3-one, (20*S*, 22*E*)-cholesta-4,22-diene-16β,18,20-triol-3-one, and (20*S*,22*E*)-24-methylcholesta-4,22-diene-16β,18,20-triol-3-one] from the Anthozoan (order Antipatharia) *Antipathes subpinnata* (Ellis & Solander), commonly named black coral (1). In the course of examining slightly more polar fractions of the extract, we uncovered a series of sterols analogous to those disclosed earlier but distinguished by a different oxidation level of the skeleton. We report the isolation, structure elucidation, and biological activity of four new sterols.

RESULTS AND DISCUSSION

As was noted earlier (1), Si gel chromatography of the EtOH extract of *An. subpinnata*, collected in the Bay of Naples, yielded fractions possessing highly functionalized sterols. Further purification by repeated hplc gave pure compounds **1–5**. Compounds **2**, **3**, **4**, and **5** showed unexpected toxicity toward the brine shrimp (*Artemia salina*), with LC_{50} of 55.6, 30.7, 7.2, and 139.4 μg/ml respectively (2).

Comparison of the spectral data (Tables 1 and 2) of **1** with those reported in the literature showed it was a product previously isolated from *Litophyton viridis*, a marine soft coral (3). Compounds **2** ($[M]^+$ 418.3445, calcd 418.3449 for $C_{27}H_{46}O_3$) and **3** ($[M]^+$



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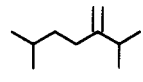

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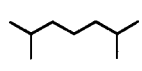

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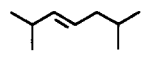
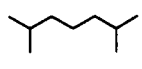
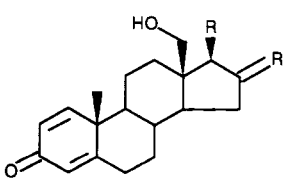
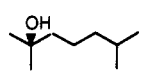


4 R=OH, R′=

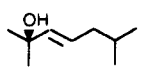




5 R=O, R′=



6 R=H_2, R′=



416.3288, calcd 416.3292 for $C_{27}H_{44}O_3$), which to our knowledge have not been previously reported, were identical to **1** in their nuclear structure, as evidenced by ir [**2** ν max ($CHCl_3$) 3460 cm^{-1}; **3** ν max ($CHCl_3$) 3450 cm^{-1}] and nmr data (see Tables 1 and 2) and differed only in the nature of the side chains. The ^{1}H-nmr spectrum of **3** con-

TABLE 1. ^{1}H-nmr Data[a] ($CDCl_3$) of Compounds **1–5**.

Proton	Compound				
	1	**2**	**3**	**4**	**5**
H-1	ax 1.07[b]	ax 1.09[b]	ax 1.09[b]	ax 2.05[b]	7.01, d (11)
	eq 2.05, ddd (12.5,4,4)	eq 2.05, ddd (12.5,4,4)	eq 2.05, ddd (12.5,4,4)	eq 1.58[b]	—
H-2	ax 1.65[b]	ax 1.65[b]	ax 1.65[b]	ax 1.98[b]	6.25, bd (11)
	eq 1.89[b]	eq 1.89[b]	eq 1.89[b]	eq 1.45[b]	—
H-3	3.63[b]	3.65[b]	3.65[b]	3.68[b]	—
H-4	ax 2.22, dd (14,12.5)	ax 2.21, dd (14,12.5)	ax 2.21, dd (14,12.5)	H_{ax} 2.22, dd (14,12.5)	6.09, bs
	eq 2.42, dd (14,4)	eq 2.43, dd (14,4)	eq 2.43, dd (14,4)	eq 2.49, dd (14,4)	—
H-6	5.66, bs	5.66, bs	5.65, bs	5.97, d (4)	—
H-7	3.83[b]	3.83[b]	3.83[b]	3.98, m	ax 2.49, dddd (13,13,13,5)
	—	—	—	—	eq 2.42, dddd (13,5,5,5)
H-8	1.82, ddd (12,11.5,4)	1.82, ddd (12,11.5,4)	1.82, ddd (12,11.5,4)	2.20, dd (12,4)	
H-9	1.02[b]	1.02[b]	1.02[b]	—	—
H-11	—	—	—	ax 1.99[b]	—
	—	—	—	eq 1.42[b]	—
H-12	—	—	—	ax 1.54[b]	—
	—	—	—	eq 1.87[b]	eq 2.00, ddd (13,3.5,3.5)
H-14	—	—	—	1.93[b]	—
H-15α	—	—	—	1.18[c]	—
H-15β	—	—	—	1.68[c]	—
H-16α	—	—	—	1.95[d]	—
H-16β	—	—	—	1.32[d]	—
H-17	—	—	—	1.28[b]	2.11, s
H-18	0.75, s	0.74, s	0.75, s	0.73, s	3.92, d (13.5)
	—	—	—	—	3.85, d (13.5)
H-19	3.84, d (12)	3.85, d (12)	3.83, d (12)	3.85, d (12)	1.26, s
	3.63, d (12)	3.63, d (12)	3.63, d (12)	3.68, d (12)	—
H-20	—	—	—	1.40[b]	—
H-20	—	—	—	1.40[b]	—
H-21	0.96, d (6.5)	0.93, d (6.5)	1.01, d (6.5)	0.93, d (6.5)	1.33, s
H-22	—	—	5.20, ddd (16,7,7)	—	—
H-23	—	—	5.30, dd (16,7)	—	—
H-25	—	—	—	1.52, m	—
H-26	1.01, d (6.5)	0.87, d (6.5)	0.85, d (6.5)	0.86, d (6.5)	0.86, d (7)
H-27	1.01, d (6.5)	0.87, d (6.5)	0.85, d (6.5)	0.86, d (6.5)	0.86, d (7)
H-28	4.68, d (16)	—	—	—	—

[a]δ values are in ppm from the residual solvent signal (δ 7.26); *J* values, reported in parentheses, are in Hz. ^{1}H assignments were based on spin-spin decoupling and COSY experiments.

[b]Submerged by other signals.

[c,d]The resonances with the same superscript may be reversed.

TABLE 2. ^{13}C-nmr Data[a] ($CDCl_3$) of Compounds **1–5**.

Carbon	Compound				
	1	**2**	**3**	**4**	**5**
C-1	34.0	33.8	34.0	24.8	154.9
C-2	31.9	31.9	31.8	31.5	127.8
C-3	71.1	71.0	71.1	70.2	186.0
C-4	41.8	41.8	41.8	42.2	124.2
C-5	138.2	138.2	137.9	137.6	168.0
C-6	130.5	130.6	130.5	128.0	33.6[f]
C-7	72.4	72.4	72.4	65.9	34.3[f]
C-8	42.1	42.2	42.1	40.9	35.5
C-9	48.8	48.7	48.8	75.2	52.1
C-10	43.2	43.1	43.0	47.5	43.4
C-11	21.8	21.7	21.8	27.7	22.1
C-12	39.9	39.9	39.9	35.1	32.5
C-13	41.6	41.4	41.4	42.4	48.7
C-14	56.8	56.8	56.8	45.2	49.7
C-15	26.0	26.0	26.2	24.1	39.2
C-16	28.8	28.5	28.5	28.2	216.9
C-17	55.6	55.5	55.8	55.6	68.0
C-18	12.1	12.1	12.1	11.2	62.1
C-19	62.8	62.6	62.9	63.3	18.7
C-20	36.1	35.7	36.9	35.7	74.2
C-21	18.9	18.7	22.3	18.7	24.8
C-22	34.8	36.2	137.3	36.1	42.7
C-23	31.3	23.8	127.1	23.7	22.4
C-24	156.8	39.5	41.9	39.5	39.1
C-25	36.1	27.9	28.5	28.0	27.9
C-26	21.9[b]	22.7[c]	21.2[d]	22.8[e]	22.6[f]
C-27	22.0[b]	22.5[c]	21.1[d]	22.5[e]	22.5[f]
C-28	106.2	—	—	—	—

[a]δ values are in ppm from the residual solvent signal (δ 77.0). ^{13}C assignments were based on DEPT and two-dimensional ^{1}H-^{13}C (one bond) correlation experiments, comparison with model compounds (1,10,11), and substituent parameters effects (7–9).

[b–f]The resonances with the same superscript may be reversed.

tained a further coupled AB system [H_a δ 5.20, ddd (J = 16, 7, 7 Hz); H_b δ 5.30, dd (J = 16, 7 Hz)] which could be assigned to Δ^{22} trans protons. The position of the double bond was confirmed by features in the methyl region; the doublet resonating at δ 0.91 in **2** was shifted to δ 1.03 in **3**, which was expected for the C-21 methyl group (20*R* configuration) in a saturated and Δ^{22} unsaturated side chain (4). Compound **2** was therefore established as cholest-5-ene-3β,7β,19-triol and compound **3** as (22*E*)-cholesta-5,22-diene-3β,7β19-triol.

In the hreims of compound **4** the highest peak at *m/z* 416.3288 (calcd 416.3292 for $C_{27}H_{44}O_3$) corresponded to loss of H_2O from the molecular formula $C_{27}H_{46}O_4$, which was determined by ^{13}C-nmr and DEPT measurements. Two successive losses of 18 mass units (*m/z* 398 and 380) suggested the presence of at least three hydroxyl groups. Several ions seem likewise to arise from the loss of a CH_2O unit (ions at *m/z* 386 $[M-H_2O-CH_2O]^+$, 368 $[M-2H_2O-CH_2O]^+$, 350 $[M-3H_2O-CH_2O]^+$). In the ^{1}H-nmr spectrum of **4** the signals due to the Me, CH_2OH, and CHOH groups had similar shapes and positions to those of compound **2** (see Table 1), apart from the resonance of H-7 which was shifted downfield from δ 3.83 to δ 3.98. An analogous shift was observed for H-6 (from δ 5.66 to δ 5.97). The introduction in **4** of an extra hy-

droxyl group on a tertiary carbon atom, suggested by the presence in the ^{13}C-nmr spectrum of a signal at δ 75.21 (s), could explain the differences in the ^{1}H-nmr spectra.

The following arguments allowed location of this functionality at C-9. H-8 resonates in **4** as a double doublet at δ 2.20 (J = 12, 4 Hz); the lack of additional coupling indicated the presence of an adjacent quaternary center. Consequently, the hydroxyl group could be linked at C-9 or C-14. The second possibility could be discarded on the basis of a further consideration of ^{1}H-nmr data including extensive ^{1}H decoupling studies and a ^{1}H-^{1}H COSY plot. This allowed us to extend the side chain up to C-5 through the protonated carbon atoms 17, 16, 15, 14, 8, 7, and 6, as reported in Table 1, which contains the assignments of all the hydrogen resonances of **4** except those of side-chain methylene groups. The reported data, when compared with those of **2**, further confirm that the compounds have identical functionality patterns apart from the additional OH group at C-9 in **4**. In comparing the chemical shifts of H-14 and H_{ax}-12 to those of the corresponding protons in previously reported 7β-Δ^5-hydroxysterols (5,6), a significant shift toward lower field in **4** was evident, which can be easily explained by the deshielding effect of the α-oriented 9-OH group. Table 2 contains the resonances of all the carbon atoms of **4**, which were assigned on the basis of two-dimensional ^{1}H-^{13}C (one bond) shift correlated spectroscopy. The observed chemical shift dissimilarities between the carbon resonances of **2** and **4** afforded an excellent confirmation of the presence of the additional hydroxyl group at position 9α in **4**, taking into account the substituent effects in cyclic systems (7–9).

Compound **5** was isolated as a uv-absorbing [λ max (EtOH) 243 nm (ε = 12.000)] amorphous powder. Its molecular formula, $C_{27}H_{40}O_4$, was deduced from its high resolution mass spectrum, which showed the highest peak at *m/z* 410.2827 (calcd 410.2822 for $C_{27}H_{38}O_3$ $[M-H_2O]^+$) and from ^{13}C-nmr data. In the infrared spectrum strong absorptions at 3450, 1731, 1654 cm^{-1} indicated the presence of hydroxyl, saturated, and α,β-unsaturated carbonyl functionalities.

A comparison of overall ^{1}H- and ^{13}C-nmr data revealed similarities between the sterol **6**, previously isolated from the same source (1), and compound **5**, and showed that the cross-conjugated enone system in the A ring and the hydroxylation at C-18 and C-20 were also present in the latter compound. However, there were several significant differences in their spectral data; the ^{1}H- and ^{13}C-nmr spectra of **5** lacked in the downfield region the signals due to a Δ^{22} double bond, indicating that it possesses a saturated side chain. The most striking difference between the two sterols was, however, the presence in **5** of an additional carbonyl group, indicated by the ir band at 1731 cm^{-1} and ^{13}C-nmr resonance at δ 216.9 (5).

The position of this functionality in the molecule was illustrated by the ir absorption frequency of the carbonyl group, characteristic of a ketone in a five-membered ring, and by the presence in the ^{1}H-nmr spectrum of a 1H singlet resonating at δ 2.11. This signal could be assigned to an H-17 proton lacking any coupling due to the presence of an OH group at C-20 and a C=O at C-16. This conclusion was amply substantiated by the mass spectrum, which showed the extremely favored loss of H_2O due to formation of a C-17–C-20 double bond conjugated to the C-16 carbonyl group, and, in addition, the C-17–C-20 and C-20–C-22 cleavages characteristic of 20-hydroxy-16-oxosterols (10,11) [*m/z* 269 (17–20 cleavage − CH_2O from M^+), *m/z* 343 (20–22 cleavage from M^+)]. Compound **5** possesses the same configuration at C-20 found in the 20-hydroxysterols so far isolated from *An. subpinnata* as indicated by nOe difference measurements; a strong enhancement (5%) was observed for H_{eq}-12 resonating at δ 2.00 upon irradiation of the Me-21 (δ 1.33). This significant nOe requires a preferential conformation having Me-21 and H_{eq}-12 spatially very close; as previously reported, this occurs when the 20-hydroxysterols possess the *S* configuration (1).

EXPERIMENTAL

GENERAL EXPERIMENTAL PROCEDURES.—Hreims of compounds **1–5** were obtained at 70 eV on a Kratos MS 50 mass spectrometer. Ft-ir of $CHCl_3$ solutions were recorded on a Bruker IFS-48 spectrophotometer. The uv spectrum of **5** (EtOH) was recorded on a Beckman DU-70 spectrometer. ^{1}H- and ^{13}C-nmr spectra were recorded in $CDCl_3$ on a Bruker AMX-500 spectrometer, and the solvent was used as an internal standard (^{1}H δ 7.26, ^{13}C δ 77.0). The assignments in the ^{1}H-nmr spectra were confirmed by spin-spin decoupling and ^{1}H-^{1}H COSY experiments ($CDCl_3$). The nature of each carbon resonance was deduced from Distortionless Enhancement by Polarization Transfer (DEPT) experiments performed using polarization transfer pulses of 90° and 135°. The nOe difference experiment on a degassed solution of **5** was performed on a Bruker WM-250 spectrometer in $CDCl_3$ with the aid of a Bruker microprogram. The heteronuclear chemical shifts correlation with polarization transfer was recorded by taking 64 blocks of 2048 points each, with interpulse delays optimized for a $^1J_{CH}$ of 125 Hz. Medium pressure liquid chromatography (mplc) was performed on a Buchi apparatus. Hplc was performed on a Varian hplc Model 5000 with a Hibar RP-18 LiChrospher super 100 column using a dual cell refractometer detector.

EXTRACTION AND ISOLATION OF STEROIDS **1–5**.—*An. subpinnata*, identified by Dr. M. Pansini, University of Genoa, was collected by hand using SCUBA gear in the Bay of Naples (depth 30 m). A voucher specimen is deposited in the Dipartimento di Chimica delle Sostanze Naturali, University of Naples.

Freshly collected material (360 g dry wt after extracton) was chopped, then homogenized with EtOH and extracted at room temperature (500 ml × 4). The combined EtOH extracts were concentrated in vacuo, thus obtaining an aqueous suspension which was extracted with Et_2O (300 ml × 3). Evaporation of Et_2O extracts gave an oil (6 g) which was fractionated by mplc on a Si gel column (230–400 mesh, Merck, 500 g) using gradient elution (40°–70° petroleum ether→Et_2O→EtOAc→MeOH). The portion eluted with MeOH was evaporated to give a crude residue (1.5 g) that was rechromatographed on a Si gel column (70–230 mesh, Merck, 200 g) eluting with a linear gradient of MeOH (2% to 50%) in $CHCl_3$. Fractions eluted with $CHCl_3$-MeOH (98:2) (fraction A) and $CHCl_3$-MeOH (9:1) (fraction B) were finally purified by hplc on a LiChrospher RP-18 super 100 column using MeOH-H_2O (95:5) as eluent, thus obtaining, from fraction A, compound **5** (3.5 mg, colorless oil) and, from fraction B, compounds **1** (2 mg, colorless oil), **2** (9.7 mg, mp 154–156°), **3** (5 mg, mp 150–152°), and **4** (4.5 mg, mp 165–167°).

BIOLOGICAL ASSAY.—Brine shrimp (*Ar. salina*) assays were performed in triplicate in DMSO (1% of final volume), using 10 animals suspended in artificial sea water, as reported by Meyer *et al.* (2). Briefly, for each dose tested, survivor shrimp were counted after 24 h and data were statistically analyzed by the Finney program (12), which yields LC_{50} values with 95% confidence levels.

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LITERATURE CITED

1. A. Aiello, E. Fattorusso, and M. Menna, *Steroids*, **56**, 513 (1991).
2. B.N. Meyer, N.R. Ferrigni, J.E. Putnam, L.B. Jacobsen, D.E. Nichols, and J.L. McLaughlin, *Planta Med.*, **45**, 31 (1982).
3. M. Bortolotto, J.C. Braekman, D. Dalorze, D. Losman, and B. Jursch, *Steroids*, **28**, 461 (1976).
4. D.J. Vanderah and C. Djerassi, *J. Org. Chem.*, **43**, 1442 (1978).
5. A. Aiello, E. Fattorusso, and S. Magno, *J. Nat. Prod.*, **50**, 191 (1987).
6. E. Fattorusso, V. Lanzotti, S. Magno, and E. Novellino, *J. Org. Chem.*, **50**, 2868 (1985).
7. H. Heggert, C.L. Van Antwerp, N.S. Bhacca, and C. Djerassi, *J. Org. Chem.*, **41**, 71 (1976).
8. J.W. Blunt and J.B. Stothers, *Org. Magn. Reson.*, **9**, 439 (1977).
9. E. Breitmaier and W. Voelter, "Carbon-13 NMR Spectroscopy," VCH. Weinhein-New York, 1990, pp. 209–213.
10. R. Benvegnù, G. Cimino, S. De Rosa, and S. De Stefano, *Experientia*, **38**, 1443 (1982).
11. G. Cimino, S. De Rosa, S. De Stefano, and G. Sodano, *Experientia*, **40**, 246 (1983).
12. D.J. Finney, "Probit Analysis," 3rd ed., Cambridge University Press, Cambridge, 1971.

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