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3β,5α,6β-TRIHYDROXYSTEROLS FROM THE MEDITERRANEAN BRYOZOAN *MYRIAPORA TRUNCATA*

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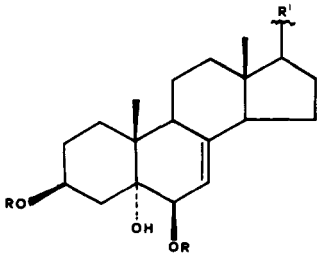
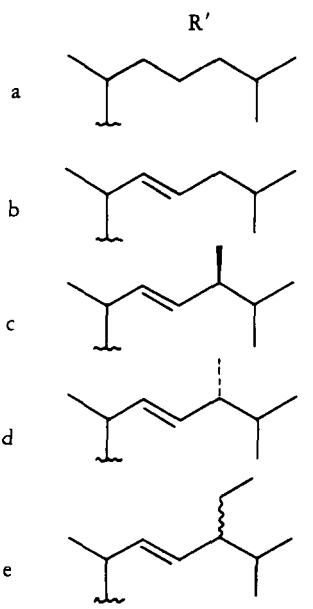
ABSTRACT.—The $CHCl_3$ extract of the Mediteranean Bryozoan *Myriapora truncata* was shown to contain, in addition to the known cholest-7-en-3β,5α,6β-triol (**1**) and (22*E*,24*S*)-24-methylcholesta-7,22-dien-3β,5α,6β-triol (**3**), three new sterols: (22*E*)-cholesta-7,22-dien-3β,5α,6β-triol (**2**), (22*E*,24*R*)-24-methylcholesta-7,22-dien-3β,5α,6β-triol (**4**), and (22*E*,24ξ)-24-ethylcholesta-7,22-dien-3β,5α,6β-triol (**5**), whose structures were elucidated on the basis of physico-chemical evidence. All the above compounds have been isolated as 3,6-diacetylderivatives.

Bryozoa are minute, sessile, colonial animals, commonly known as sea-mats, or false corals. Although they are among the most common marine invertebrates, only a limited number of chemical investigations on their metabolites have been reported. Recently, *Flustra foliacea* (1), *Zoobotryon verticillatum* (2), *Sessibugula translucens* (3), and *Philodopora pacifica* (4) were shown to contain several heteroaromatic compounds, while Carlé and Christophersen (5) found that the allergen of *Alcyonidium gelatinosum* was the (2-hydroxyethyl)-dimethylsulfonium ion. Finally, bryostatins, very interesting macrolides possessing antineoplastic activity, were isolated from *Bugula neritina* (6-9). We wish to report here that the bryozoan *Myriapora truncata* Pallas, rather common on rocky shores along the Mediterranean coasts, contains five 3β,5α,6β-trihydroxysterols (**1-5**) that have been isolated as diacetylderivatives (**6-10**).

Compound **3**, (22*E*,24*S*)-24-methylcholesta-7,22-dien-3β,5α,6β-triol, a minor yeast and ergot sterol, was reported in 1954 by Alt and Barton (10), while **1**, cholest-7-





1	R=H	R'=a
2	R=H	R'=b
3	R=H	R'=c
4	R=H	R'=d
5	R=H	R'=e
6	R=Ac	R'=a
7	R=Ac	R'=b
8	R=Ac	R'=c
9	R=Ac	R'=d
10	R=Ac	R'=e

en-3β,5α,6β-triol, has not been found as a naturally occurring compound but has been synthesized (11). The structure determination of the remaining compounds, (22*E*)-cholesta-7,22-dien-3β,5α,6β-triol (**2**), (22*E*,24*R*)-24-methylcholesta-7,22-dien-3β,5α,6β-triol (**4**), and (22*E*,24ξ)-24-ethylcholesta-7,22-dien-3β,5α,6β-triol (**5**), is described.

A $CHCl_3$ extract of freeze-dried *M. truncata* specimens collected in the Bay of Naples was chromatographed on silica gel, using a solvent gradient of increasing polarity from $CHCl_3$ to MeOH. Selected fractions, which on the basis of the spectral data (nmr and ms) were found to be complex mixtures of polyhydroxysterols, were acetylated, and the material was rechromatographed on reverse-phase hplc to obtain the diacetylderivatives **6-10**.

Compounds **6** and **8** were identified on the basis of their physical and spectral properties when compared with those of authentic samples synthesized according to literature procedures (11,10), starting from 7-dehydrocholesterol and ergosterol, respectively.

The crystalline compound **7** has the molecular formula $C_{31}H_{48}O_5$, deduced from hrms on the first fragment ion at *m/z* 440 (M^+-AcOH). The mass spectrum shows, in addition to the ion at *m/z* 440, intense peaks at *m/z* 422 (M^+-AcOH-H_2O), 380 (M^+-2AcOH), and 362 (M^+-2AcOH-H_2O), suggesting that this compound was a di-unsaturated C_{27} sterol having two acetyl and one hydroxyl groups. The location of these functionalities, together with the position of the nuclear double bond, was deduced from the ^{1}H-nmr spectrum (see Table 1) in which the chemical shifts and the multiplicities of H-3, H-6, H-7, H_3-18, and H_3-19 are almost identical to those of the corresponding protons in **6** and **8**. The second double bond, which on the basis of the mass spectrum (intense ion at *m/z* 251, deriving from M^+ by loss of 2 AcOH, H_2O and side chain) must be located in the side chain, was positioned at C-22 considering the chemical shifts of H_3-21, H_3-26, and H_3-27, reported in Table 1 (12). Further evidence was gained by double resonance experiments: irradiation at δ 1.98 (tentatively the frequency of H-20) simplified the multiplet at δ 5.16 (H-22) and collapsed the doublet at δ 1.01 (H_3-21) to a singlet. What remained to be established was the stereochemistry of the C-22 double bond, which was assigned as *E*, since in the *Z*-configuration, H_3-18 and H-22 and H-23 are reported to resonate at lower field and H_3-21 at higher field ($\Delta\delta$'s=0.03, 0.2, 0.2, and >0.03, respectively) (13-16).

Sterol **9** showed a molecular formula $C_{32}H_{50}O_5$, as indicated from hrms on the fragment ion at *m/z* 454 (M^+-AcOH). The close similarity of its mass (Experimental) and ^{1}H-nmr spectra (Table 1) with those of **8** suggested that the two compounds must be C-24 epimers. This was supported by the difference between the resonances of the H_3-21 doublets ($\Delta\delta$=0.02), since it has been reported that the signal of the 21-methyl of (24*R*,22*E*)-24-methyl-Δ^{22} sterols appears at lower field than their (24*S*)-analogues (12,17).

The molecular formula of **10**, deduced from hrms analysis, corresponds to $C_{33}H_{52}O_5$. The ^{1}H-nmr spectrum indicated a strong similarity between **10** and the sterols **6-9** (see Table 1), the only significant difference being the presence in **10** of a 3H triplet at δ 0.81, diagnostic for an ethyl group, which on the basis of mass spectral evidence (ions at *m/z* 269 and 251) must be located on the side-chain. Further information on the structure of the sterol **10** and, in particular, data to define the side-chain, was gained by spin decoupling experiments. Irradiation at δ 1.87 (tentatively the frequency of H-20) collapsed the double doublet at δ 5.05 (H-22) to a doublet (J=14 Hz) and the doublet at δ 1.02 to a singlet; further irradiation at δ 1.50 (tentatively the frequency of H-25) simplified the 3H doublets at δ 0.79 and 0.84 (H-26 and H-27) into two sharp singlets. These data established the part structure C_{20}-C_{23} and the presence of an isop-

TABLE 1. Selected 250 MHz ^{1}H-nmr Data for Compounds **6-10**

H	δ (m, Hz)				
	6	**7**	**8**	**9**	**10**
3	5.13 (m)	5.11 (m)	5.13 (m)	5.13 (m)	5.13 (m)
6	4.82 (bd, 5)	4.82 (bd, 5)	4.83 (bd, 5)	4.82 (bd, 5)	4.82 (bd, 5)
7	5.26 (bd, 5)	5.25 (bd, 5)	5.26 (bd, 5)	5.25 (bd, 5)	5.25 (bd, 5)
18	0.58 (s)	0.58 (s)	0.59 (s)	0.58 (s)	0.58 (s)
19	1.06 (s)	1.07 (s)	1.07 (s)	1.06 (s)	1.06 (s)
21	0.93 (d, 7)	1.01 (d, 7)	1.02 (d, 7)	1.00 (d, 7)	1.02 (d, 7)
22		5.16 (m)	5.21 (m)	5.16 (m)	5.05 (dd, 14,8)
23		5.16 (m)	5.21 (m)	5.16 (m)	5.12 (dd, 14,8)
26	0.87 (d, 7)	0.84 (d, 7)	0.83 (d, 7)	0.82 (d, 7)	0.79 (d, 7)
27	0.87 (d, 7)	0.86 (d, 7)	0.85 (d, 7)	0.84 (d, 7)	0.84 (d, 7)
28			0.92 (d, 7)	0.91 (d, 7)	
29					0.81 (t, 7)
C(3)-O-COCH$_3$	2.04 (s)	2.03 (s)	2.04 (s)	2.04 (s)	2.03 (s)
C(6)-O-COCH$_3$	2.07 (s)	2.07 (s)	2.07 (s)	2.06 (s)	2.06 (s)

ropyl group; as a consequence, the ethyl must be located at C-24. The stereochemistry of the C-22 double bond was assigned as *E* on the basis of the observed vicinal coupling (H-22/H-23) of 14 Hz.

EXPERIMENTAL

GENERAL EXPERIMENTAL PROCEDURES.—*Mass spectra were taken on a AEI MS-902 instrument.* Optical rotations were measured on a Perkin-Elmer 192 polarimeter, with a 10-cm microcell in $CHCl_3$. ^{1}H-nmr spectra were determined on a Brüker WM 250 spectrometer in $CDCl_3$, using TMS as internal reference (δ=0). Preparative hplc was carried out on a Varian 5010 instrument, using a dual cell refractometer detector.

ISOLATION OF **6-10**.—*M. truncata* was collected by hand using SCUBA in the Bay of Naples near Nisida (Italy) during the spring of 1984. A reference specimen is on file at our laboratories. Freshly collected specimens (6 kg, wet material) were freeze-dried, powdered, and extracted exhaustively with $CHCl_3$. After removal of the solvent in vacuo, the residue (12 g) was partitioned by Si gel column, using as eluent increasing amounts of MeOH in $CHCl_3$. Fractions eluted with $CHCl_3$-MeOH (9:1) afforded a product (25 mg) which, on the basis of its spectral data (nmr and ms), was shown to be a complex mixture of polyhydroxysterols (Fraction A). It was fractionated after acetylation (see below). The acetylated material was chromatographed by reverse-phase hplc on an RP-18 column (Merck, 1×25 cm), using MeCN as eluent to give crystalline **6** (2 mg), **7** (3 mg), **8** (3 mg), **9** (2 mg), and **10** (2 mg) which, on account of the small quantities available, could not be recrystallized.

Compound **6**.—[α]D=−131° (c=0.15, $CHCl_3$); eims (rel. int., assignment) *m/z* 442 (2%, M^+-AcOH), 424 (7, M^+-AcOH-H_2O), 382 (18, M^+-2AcOH), 364 (100, M^+-2AcOH-H_2O), 251 (87, M^+-2AcOH-H_2O-C_8H_{17}); ^{1}H nmr δ 5.26 (1H, bd, *J*=5 Hz, 7-H), 5.13 (1H, m, 3-H), 4.82 (1H, bd, *J*=5 Hz, 6-H), 2.07 (3H, s, 6-OCOCH$_3$), 2.04 (3H, s, 3-OCOCH$_3$), 1.06 (3H, s, 19-H$_3$), 0.93 (3H, d, *J*=7 Hz, 21-H$_3$), 0.87 (6H, d, *J*=7 Hz, 26-H$_3$ and 27-H$_3$), and 0.58 (3H, s, 18-H$_3$). Physical and spectral properties of **6** were identical to those of an authentic sample synthesized according to Rufer *et al.* (11).

Compound **7**.—[α]D=−111° (c=0.1, $CHCl_3$); eims (rel. int., assignment) *m/z* 440 (9%, M^+-AcOH), 422 (50, M^+-AcOH-H_2O), 407 (3, M^+-AcOH-H_2O -CH_3) 380 (80, M^+-2AcOH), 362 (100, M^+-2AcOH-H_2O), 347 (9, M^+-2AcOH-H_2O-CH_3), 251 (60, M^+-H_2O-2AcOH-C_8H_{15}); hrms, 440.3284 ($C_{29}H_{44}O_3$ requires 440.3290); ^{1}H nmr δ 5.25 (1H, bd, *J*=5 Hz, 7-H), 5.16 (2H, m, 22-H and 23-H), 5.11 (1H, m, 3-H), 4.82 (1H, bd, *J*=5 Hz, 6-H), 2.07 (3H, s, 6-OCOCH$_3$), 2.03 (3H, s, 3-OCOCH$_3$), 1.07 (3H, s, 19-H$_3$), 1.01 (3H, d, *J*=7 Hz, 21-H$_3$), 0.86 (3H, d, *J*=7 Hz, 27-H$_3$), 0.84 (3H, d, 7 Hz, 26-H$_3$), and 0.58 (3H, s, 18-H$_3$).

Compound **8**.—[α]D=−141° (c=0.1, $CHCl_3$); eims (rel. int., assignment) *m/z* 454 (1%, M^+-AcOH), 436 (3, M^+-AcOH-H_2O), 421 (1, M^+-AcOH-H_2O-CH_3), 376 (75, M^+-2AcOH-H_2O), 361 (25, M^+-2AcOH-H_2O-CH_3), 251 (100, M^+-2AcOH-H_2O-C_9H_{17}); ^{1}H nmr δ 5.26 (1H, bd, *J*=5 Hz, 7-H), 5.21 (2H, m, 22-H and 23-H), 5.13 (1H, m, 3-H), 4.83 (1H, bd, *J*=5 Hz, 6-H), 2.07 (3H, s, 6-OCOCH$_3$), 2.04 (3H, s, 3-OCOCH$_3$), 1.07 (3H, s, 19-H$_3$), 1.02 (3H, d, *J*=7 Hz, 21-H$_3$), 0.92 (3H, d,

J=7 Hz, 28-H_3), 0.85 (3H, d, J=7 Hz, 27-H_3), 0.83 (3H, d, J=7 Hz, 26-H_3), and 0.59 (3H, s, 18-H_3). Physical and spectral properties of **8** were identical to those of an authentic sample synthesized according to Alt and Barton (10).

Compound **9**.—$[\alpha]_D = -150°$ (c=0.2, $CHCl_3$); eims (rel. int., assignment) *m/z* 454 (1%, M^+-AcOH), 436 (3, M^+-AcOH-H_2O), 421 (1, M^+-AcOH-H_2O-CH_3), 376 (75, M^+-2AcOH-H_2O), 361 (25, M^+-2AcOH-H_2O-CH_3), 251 (100, M^+-2AcOH-H_2O-C_9H_{17}); hrms 454.3450 ($C_{30}H_{46}O_3$ requires 454.3447); ^{1}H nmr δ 5.25 (1H, bd, J=5 Hz, 7-H), 5.16 (2H, m, 22-H and 23-H), 5.13 (1H, m, 3-H), 4.82 (1H, bd, J=5 Hz, 6-H), 2.06 (3H, s, 6-$OCOCH_3$), 2.04 (3H, s, 3-$OCOCH_3$), 1.06 (3H, s, 19-H_3), 1.00 (3H, d, J=7 Hz, 21-H_3), 0.91 (3H, d, J=7 Hz, 28-H_3), 0.84 (3H, d, J=7 Hz, 27-H_3), 0.82 (3H, d, J=7 Hz, 26-H_3), and 0.58 (3H, s, 18-H_3).

Compound **10**.—$[\alpha]_D = -140°$ (c=0.1, $CHCl_3$); eims (rel. int., assignment) *m/z* 468 (10%, M^+-AcOH), 450 (40, M^+-AcOH-H_2O), 435 (4, M^+-AcOH-H_2O-CH_3), 408 (90, M^+-2AcOH), 390 (100, M^+-2AcOH-H_2O), 269 (15, M^+-2AcOH-$C_{10}H_{19}$), 251 (50, M^+-2AcOH-H_2O-$C_{10}H_{19}$); hrms, 468.3597 $C_{31}H_{48}O_3$ requires 468.3603); ^{1}H nmr δ 5.25 (1H, bd, J=5 Hz, 7-H), 5.13 (1H, m, 3-H), 5.12 (1H, dd, J=14 Hz and 8 Hz, 23-H), 5.05 (1H, dd, J=14 Hz and 8 Hz, 22-H), 4.82 (1H, bd, J=5 Hz, 6-H), 2.06 (3H, s, 6-$OCOCH_3$), 2.03 (3H, s, 3-$OCOCH_3$), 1.06 (3H, s, 19-H_3), 1.02 (3H, d, J=7 Hz, 21-H_3), 0.84 (3H, d, J=7 Hz, 27-H_3), 0.81 (3H, t, J=7 Hz, 29-H_3), 0.79 (3H, d, J=7 Hz, 26-H_3), and 0.58 (3H, s, 18-H_3).

ACETYLATION OF FRACTION A.—Fraction A (15 mg) was dissolved in a mixture of Ac_2O (0.5 ml) and dry pyridine (0.5 ml) and kept at room temperature overnight. Usual workup afforded 25 mg of a crude acetylated material which was fractionated to give **6-10** as described above.

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