Steroids of the Marine Sponge Cinachyra tarentina: Isolation of Cholest-4-ene-3,6-dione and (24R)-24-Ethylcholest-4-ene-3,6-dione

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STEROIDS OF THE MARINE SPONGE *CINACHYRA TARENTINA*: ISOLATION OF CHOLEST-4-ENE-3,6-DIONE AND (24*R*)-24-ETHYLCHOLEST-4-ENE-3,6-DIONE

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ABSTRACT.—The MeOH extract of the demospongia *Cinachyra tarentina* was shown to contain, in addition to three common 3β-hydroxysterols and three cholest-4-en-3-ones, two steroids: cholest-4-ene-3,6-dione [**1**] and (24*R*)-24-ethylcholest-4-ene-3,6-dione [**2**], which have not been previously found as naturally occurring compounds. The structures of **1** and **2** have been elucidated by spectroscopic analyses (^{1}H and ^{13}C-nmr, uv, ir) and confirmed via synthesis.

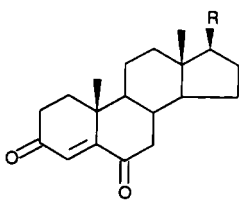
Marine sponges are a rich source of unusual sterols, including the cholest-4-en-3-ones that were first encountered in the sponge *Stelleta clarella* (1). These sterols have been isolated from sponges occurring in several parts of the world; sometimes the enone function is further conjugated with additional carbon-carbon and carbon-oxygen double bonds as in cholesta-4,7,22-triene-3,6-dione, 24-methylcholesta-4,7,22-triene-3,6-dione, and 24-ethylcholesta-4,7,22-triene-3,6-dione, which are present in the sponge *Raphidostila incisa* (2); 24-methylcholesta-4,24(28)-diene-3,6-dione and cholesta-4,22-diene-3,6-dione, present in the sponge *Anthoarcuata graceae* (3); and (22*E*)-cholesta-4,6,8(14),22-tetraen-3-one recently isolated from the sponge *Dictyonella incisa* (4).

As part of our continuing research on the metabolites from Mediterranean organisms, we examined the steroid fraction of the demospongia *Cinachyra tarentina* (Pulitzer-Finali) (Porifera). This sponge has a hemispherical shape more or less flattened with a wide base of attachment; the color in life is light yellowish brown, and the consistency, in spite of the strong radiating bundles of oxeas, is weak (5).

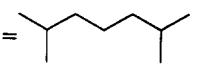
This study led to the identification of three common 3β-hydroxysterols [cholesterol, 24-methylcholesta-5-22-dien-3β-ol, and 24-methylcholesta-5-24(28)-dien-3β-ol], three cholest-4-en-3-ones [cholest-4-en-3-one, 24-methylcholesta-4,24(28)-dien-3-one, and 24-methylcholesta-4,22-dien-3-one], and two cholest-4-ene-3,6-diones, **1** and **2**. Compounds **1** and **2** until now have not been reported as naturally occurring compounds.

EXPERIMENTAL

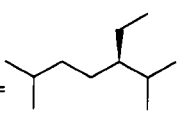
GENERAL EXPERIMENTAL PROCEDURES.—Mplc was performed on a Buchi 861 apparatus using a SiO_2 (230–400 mesh) column. Hplc separations were performed on a Varian HPLC Model 5000 with Hibar Si60 LiChrosorb 7 μm (7 × 250



1 R=



2 R=



mm) and Hibar SiO_2 LiChrospher super 100 columns, using a dual cell refractometer detector. Combined glc-ms analysis was performed on a Hewlett-Packard 5890 gas chromatograph with a mass selective detector MSD HP 5970 MS and a split/splitless injector for capillary columns, using a fused-silica column, 25 m × 0.20 mm HP-5 (cross linked 5% Ph Me silicone, 0.33 μm film thickness). Ms spectra were recorded on a Kratos MS80 mass spectrometer at 70 eV. Ft-ir spectra were recorded on a Bruker IFS-48 spectrophotometer in $CHCl_3$. Uv spectra were performed on a Beckman DU70 spectrometer in EtOH. Mps were measured on a Kofler apparatus and are uncorrected. ^{1}H-nmr spectra were recorded on a Bruker WM-400 spectrometer in $CDCl_3$ and C_6D_6, and the assignments were confirmed by spin-spin decoupling and ^{1}H-^{1}H COSY experiments (C_6D_6). ^{13}C-nmr spectra were taken on a Bruker WM-400 spectrometer in C_6D_6. The nature of each carbon signal was deduced through Distortless Enhancement by Polarization Transfer (DEPT) experiments performed by using polarization transfer pulses at 90° and 135°, respectively, obtaining in the first case only signals for CH groups and in the other case positive signals for CH and Me and negative ones for CH_2 groups. The shift correlation with polarization transfer via ^1J and via $^{2,3}J$ (COLOC) experiments were performed using Bruker microprograms on a 256 × 1024 data matrix, adjusting the fixed delays to give maximum polarization for J_{CH} = 135 Hz and J_{CH} = 6.25 Hz, respectively.

EXTRACTION AND ISOLATION OF STEROIDS.—*C. tarentina* was collected in the Mediterranean sea near Taranto (lagoon south of Porto Cesareo, depth 0.5–1 m) during spring 1989 and stored frozen at −18°. Reference specimens are deposited at the Istituto di Zoologia dell'Università di Genova.

The sponge (48 g dry wt after extraction) was homogenized and extracted at room temperature with MeOH (200 ml × 4); the combined MeOH solutions, after filtration, were concentrated in vacuo to an aqueous suspension which was extracted with Et_2O. The residue (1.1 g) from Et_2O evaporation was chromatographed by mplc on a Si gel column (Merck, 200 g) using an eluent solvent of increasing polarity from 40–70° petroleum ether to EtOAc through Et_2O, thus obtaining two steroid fractions, A and B. The more polar fraction B (307 mg), eluted with Et_2O-EtOAc (7:3), was acetylated with Ac_2O-pyridine (1:1) for 18 h at room temperature. The steryl acetates were filtered on a Si gel column which was eluted with 40–70° petroleum ether containing increasing amounts of Et_2O. The fractions eluted with Et_2O-40–70° petroleum ether (8:2) were further analyzed by glc-ms on a HP-5 capillary column (flow of N_2 1.5 ml/min, oven temperature 250°).

The identification of the steryl acetates [cholesterol (Rt = 1.00), 400 mg/100 g dry sponge; 24-methylcholesta-5,22-dien-3β-ol (Rt = 1.09), 116 mg/100 g dry sponge; 24-methylcholesta-5,24(28)-dien-3β-ol (Rt = 1.24), 106 mg/100 g dry sponge] was based upon their gc Rt and comparison of the gc-ms spectra with those of authentic specimens. The quantitation of the sterols was performed by a programmable integrator using 5α-cholestane as an internal standard.

The less polar fraction A (73 mg), eluted with Et_2O and rechromatographed by hplc on a Si gel column LiChrosorb Si60 (7 × 250 mm) using hexane-EtOAc (9:1) as eluent, afforded 9.2 mg of cholest-4-en-3-one, 5 mg of 24-methylcholesta-4,24(28)-dien-3-one, 4 mg of 24-methylcholesta-4,22-dien-3-one, and 6 mg of a crude mixture of **1** and **2**. Compounds **1** and **2** were separated through a further hplc chromatography on a LiChrospher Si60 super 100 column with hexane-EtOAc (9:1), thus obtaining pure **1** (2.5 mg) [mp 132–134°; uv λ max (EtOH) 249.5 nm (ε = 12500); ir ν max ($CHCl_3$) 2955, 2857, 1691, 1641 cm^{-1}] and **2** (1.5 mg) [mp 160–163°; uv λ max (EtOH) 249 nm (ε = 12500); ir ν max ($CHCl_3$) 2952, 2854, 1691, 1640 cm^{-1}]. ^{13}C- and ^{1}H-nmr assignments of **1** and **2** are in Table 1.

SYNTHESIS OF **1**.—Compound **1** was prepared according to Ross (6), and its chromatographic, physical, and spectroscopic properties are identical to those of natural **1**.

SYNTHESIS OF **2**.—Sitosterol (1 g) in warm HOAc (50 ml) solution was oxidized with Jones reagent (CrO_3 1 g, H_2O 0.7 ml, and HOAc 5 ml) at 23–25° by a method analogous to that used for the preparation of **1**, thus obtaining 300 mg of **2** identical to natural **2** on the basis of their chromatographic and spectroscopic properties.

RESULTS AND DISCUSSION

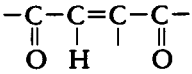
The Et_2O-soluble material from the MeOH extract of *C. tarentina* was fractionated by Si gel flash chromatography using a solvent system with increasing polarity, thus obtaining two steroid fractions, A and B. Fraction B, which emerged from the Si gel column with Et_2O-EtOAc (7:3), contained common 3β-hydroxysterols [cholesterol, 24-methylcholesta-5-22-dien-3β-ol, and 24-methylcholesta-5,24(28)-dien-3β-ol] which were identified by glc-ms of their acetates, obtained by treatment of fraction B with Ac_2O/pyridine.

As detailed in the Experimental section, five compounds have been isolated

by Si gel hplc of fraction A. From the physical and spectroscopic data and also by comparison with authentic samples, three of these compounds were found to be products previously reported as naturally occurring compounds, namely, cholest-4-en-3-one, 24-methylcholesta-4,24(28)-dien-3-one, and 24-methylcholesta-4,22-dien-3-one (1).

The remaining compounds, cholest-4-ene-3,6-dione [**1**] and 24-ethylcholest-4-ene-3,6-dione [**2**], whose structure determination is here briefly discussed, to our knowledge have not been found as naturally occurring compounds; however, compound **1** has been synthetized starting from cholesterol (6).

Compound **1** gave a parent ion in the ei hrms at *m/z* 398.3190 (calcd 398.3186) which was consistent with a molecular formula of $C_{27}H_{42}O_2$. Uv [λ max (EtOH) 249.5 nm, ε = 12500] (7), ir [ν max ($CHCl_3$) 1691 cm^{-1}], ^{1}H-nmr ($CDCl_3$, δ 6.17, s), and ^{13}C-nmr [C_6D_6, δ 197.5 (>C=O), 125.7 (-CH=), 160.1 (>C=) and 202.0 (>C=O)] data indicated the presence of the system:



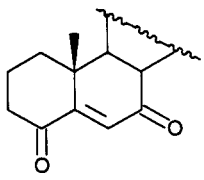
The remainder of the ^{1}H-nmr spectrum ($CDCl_3$) of **1** was typical of a steroid.

Resonances at δ 0.7 (3H, s), 1.16 (3H, s), 0.92 (3H, d, J = 7 Hz), and 0.86 (6H, d, J = 6.5 Hz) were assigned to the C-18, C-19, C-21, and C-26–C-27 methyl protons, respectively. On the basis of the above data only two structures, **a** and **b**, could accommodate the above enedione function in a steroid nucleus.

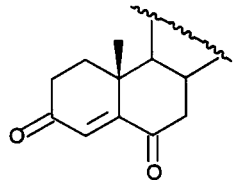
The structure **a** was ruled out on the basis of a comparison of properties of natural **1** with a synthetic sample obtained through a Jones oxidation of cholesterol as reported by Ross (6). On the synthetic **1** we have also performed a detailed spectral analysis that allowed us to assign all the proton and carbon atoms in the ^{1}H- and ^{13}C-nmr spectra (Table 1). Comparison of the ^{1}H-nmr spectrum of **1** in C_6D_6 versus $CDCl_3$ revealed that the proton resonances were more resolved in C_6D_6; hence, these values are reported in the assignments of **1**, which were based on ^{1}H-^{1}H COSY experiments and extensive spin-spin decoupling studies, which enumerated the ^{1}H spin-spin relationships in **1**, and on two-dimensional ^{13}C-^{1}H shift correlation spectroscopy via ^1J and $^{2,3}J$ (COLOC).

The molecular formula of compound **2**, deduced from hrms ($[M]^+$ *m/z* 426.3503, calcd 426.3500), corresponded to $C_{29}H_{46}O_2$. Comparison of uv and ir absorptions (see Experimental) and ^{1}H- and ^{13}C-nmr resonances (see Table 1) with those of **1** strongly suggested that they differ only in the nature of the side chain. Particularly, in the mass spectrum of both **1** and **2** an intense peak at *m/z* 285, derived from the molecular ions by loss of the side chains, is present.

The only significant difference in their ^{1}H-nmr spectra (see Table 1) was the presence in **2** of a 3H triplet at δ 0.97, which indicated the presence of an ethyl group located in the side chain, very probably at C-24. The structure **2** was definitively confirmed by its synthesis starting from sitosterol through an oxidation in the same experimental conditions described by Ross (6) for prepara-



a



b

TABLE 1. ^{13}C and ^{1}H-nmr Data for Compounds **1** and **2**.

Position	Compound			
	1		**2**	
	δC[a]	δH[b] (mult., *J*)	δC[a]	δH[b] (mult., *J*)
1	35.6	Hax 1.50[c] Heq 1.52[c]	35.7	Hax 1.51[c] Heq 1.52[c]
2	34.1	Hax 2.12 (ddd 17.3, 17, 5) Heq 2.23 (m)	34.1	Hax 2.12 (ddd 17.3, 17, 5) Heq 2.24 (m)
3	197.5		197.6	
4	125.7	6.58 (s)	125.8	6.58
5	160.1		160.2	
6	202.0		200.2	
7	46.6	Hax 1.59 (dd, 15, 13) Heq 2.51 (dd, 15, 3.2)	46.5	Hax 1.60 (dd, 15, 13) Heq 2.51 (dd, 15, 3.2)
8	34.1	1.45[f]	34.2	1.46[f]
9	50.9	0.85 (m)	50.9	0.86 (m)
10	39.4		39.4	
11	21.0	Hax 1.11[d] Heq 0.98[d]	21.0	Hax 1.11[d] Heq 0.97[d]
12	39.6	Hax 0.98[f] Heq 1.92 (m)	39.6	Hax 0.97[f] Heq 1.92 (m)
13	42.7		42.7	
14	56.6	0.99[e]	56.4	
15	24.3	Hax 1.30[e] Heq 1.32[e]	24.0	
16	28.2	Hax 1.79[g] Heq 1.29[g]	28.3	
17	56.5	0.70 (m)	56.6	
18	11.9	0.52 (s)	12.0	0.52 (s)
19	17.2	0.63 (s)	17.2	0.63 (s)
20	36.0	1.43[f]	34.5	
21	18.9	1.01 (d, 7.0)	19.0	1.04 (d, 7.0)
22	36.6		36.4	
23	24.0		22.0	
24	39.9		46.5	
25	28.3	1.33 (m)	29.8	
26	22.9	0.97 (d, 6.5)	19.3	0.96 (d, 6.5)
27	22.7	0.97 (d, 6.5)	20.0	0.94 (d, 6.5)
28			23.7	
29			12.2	0.97 (t, 6.5)

[a]δ values (C_6D_6) are in ppm from the residual solvent signal (δ 128).
[b]δ values (C_6D_6) are in ppm from the residual solvent signal (δ 7.19).
[c–e]Partially overlapped.
[f]Submerged by other signals.
[g]Values may be interchanged.

tion of **1**. The above synthesis also allowed us to assign the chirality at C-24 in natural **2**, which must be *R*.

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