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ISOLATION OF TWO NOVEL 5α,6α-EPOXY-7-KETOSTEROLS FROM THE ENCRUSTING DEMOSPONGIA *OSCARELLA LOBULARIS*

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ABSTRACT.—The MeOH extract of the Mediterranean encrusting sponge *Oscarella lobularis* yielded small amounts of two new polyoxygenated sterols **1** and **2** containing the unique 5α,6α-epoxy-7-keto function. The stereostructures of the two new metabolites were established on the basis of spectral data including 2D nmr experiments.

Oscarella lobularis Schmidt (Oscarellidae) is a fleshy encrusting marine sponge belonging to the class Demospongiae; it is usually yellow-brown, occasionally red, green, or blue, and grows in the Mediterranean sea on the stones and rocks in shallow water in well-illuminated places.

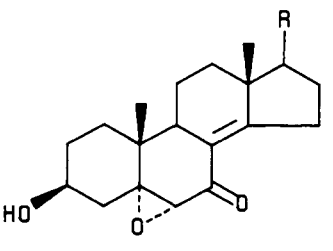
In 1975 Cimino *et al.* (1) reported the isolation from this organism of 3-alkylpyrrole-2-carboxaldehyde and 3-alkylpyrrole-2-carboxylic acid. As a part of our continuing search for biologically active compounds from marine invertebrates, we have re-investigated this sponge and have isolated two new polyoxygenated sterols, 5α,6α-epoxycholest-9(14)-en-3β-ol-7-one [**1**] and 5α,6α-epoxy-24-methylcholesta-9(14),22*E*-dien-3β-ol-7-one [**2**], containing the unique 5α,6α-epoxy-7-keto function.

In spite of the large number of sterols from marine organisms showing a wide variety of oxygenation patterns (2), relatively few epoxy compounds have been described so far, but most of these possess promising cytotoxic activities (3). Furthermore, the position of this function in compoounds **1** and **2** could be of some interest in consideration of its possible role in the biosynthesis of 5α,6β-dihydroxysterols recently isolated from several marine species (4).

The sponge was collected in the Bay of Naples, near Procida. Compounds **1** and **2** were isolated from the Et_2O solubles of the MeOH extract of the sponge by Si gel chromatography. Final purification was achieved by hplc.

The molecular formula of compound **1** ($C_{27}H_{42}O_3$) deduced from its high resolution mass spectrum and from ^{13}C-nmr data (Table 1) implied seven degrees of unsaturation. The ^1H-nmr spectrum, 400 MHz in $CDCl_3$, contained signals for the five methyl groups H_3-18 and H_3-19 (δ 0.92, 6H, s), H_3-21 (δ 0.95, 3H, d, $J=6.5$ Hz), and H_3-26 and H_3-27 (δ 0.88, 6H, d, $J\approx7$ Hz) characteristic of a sterol, although the C-18 methyl generally resonates at higher fields.

The presence of a typical 3β-hydroxyl group (ν max 3400 cm^{-1}) was indicated by a broad methine multiplet at δ 3.9, the higher-than-normal chemical shift being consistent with deshielding from a 5α oxygen. Ir (ν max 1675 cm^{-1}) and



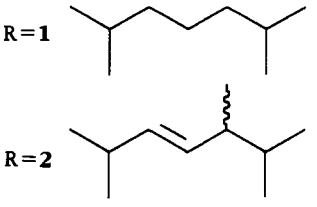


TABLE 1. ^{13}C- and ^{1}H-nmr Data for Compound **1**.

Position	δC[a]	δH[b] (mult., *J*)
1	37.1	2.99 (m)
2		Hax 1.28[c]
		Heq 1.72[h] (m)
3	63.9	3.68 (m)
4	40.4	Hax 1.90 (dd, 13, 12.5)
		Heq 1.18 (ddd, 13, 3, 1.2)
5	74.7	
6	69.2	3.28 (s)
7	188.9	
8	125.4[d]	
9	56.8	1.31[c]
10	36.8[e]	
11		2.91 (m)
12		Hax 1.24[c]
		Heq 1.86[h] (bddd, 14, 3.5, 3.5)
13	47.6[e]	
14	134.7[d]	
15	20.2	
16	27.9	
17	56.2	
18	16.5	0.79 (s)
19	19.4[f]	0.68 (s)
20	35.8	
21	19.2[f]	0.94 (d, 6.5)
22	36.5	
23	24.9	
24	40.0	
25	29.2	1.59 (m)
26	23.0[g]	0.97 (d, 7.0)
27	23.4[g]	0.97 (d, 7.0)

[a]δ values (CD_3OD) are in ppm from the residual solvent signal (δ 49.0).
[b]δ values (C_6D_6) are in ppm from the residual solvent signal (δ 7.19).
[c]Submerged by other signals.
[d–g]Values with identical superscripts may be interchanged.
[h]Partially overlapped.

^{13}C-nmr (δ 188.9) absorptions indicated the presence of an α,β-unsaturated ketone which was corroborated by uv data (λ max 266 nm, ε = 8600).

The above functionalities account for six of the seven degrees of unsaturation present in the new sterol **1**. Analysis of the ^{13}C-nmr spectrum, which shows only three signals in the sp^2 carbon region (δ 125.4, 134.7, and 188.9), suggested that the last unsaturation must be due to a ring. This was proved to be a trisubstituted epoxide by ^{1}H-nmr (δ 3.16, 1H, s, H-6) and ^{13}C-nmr data (δ 74.7, C-5; δ 69.2, C-6) and also by a peak in the mass spectrum at *m/z* 398.3191 corresponding to loss of an oxygen atom from the molecular ion.

Information gleaned from an accurate analysis of the ^{1}H-nmr spectrum led to incorporation of the above functionalities in the sterol nucleus. 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; these values are recorded in Table 1.

The multiplicity of H_2-4 signals [H_{ax} δ 1.90 (dd, *J* = 13.0, 12.5 Hz), H_{eq} δ 1.18 (ddd, *J* = 13.0, 3, 1.2 Hz), the further splitting being due to the long-range coupling with H_{eq}-2] clearly indicated the absence of any proton at C-5.

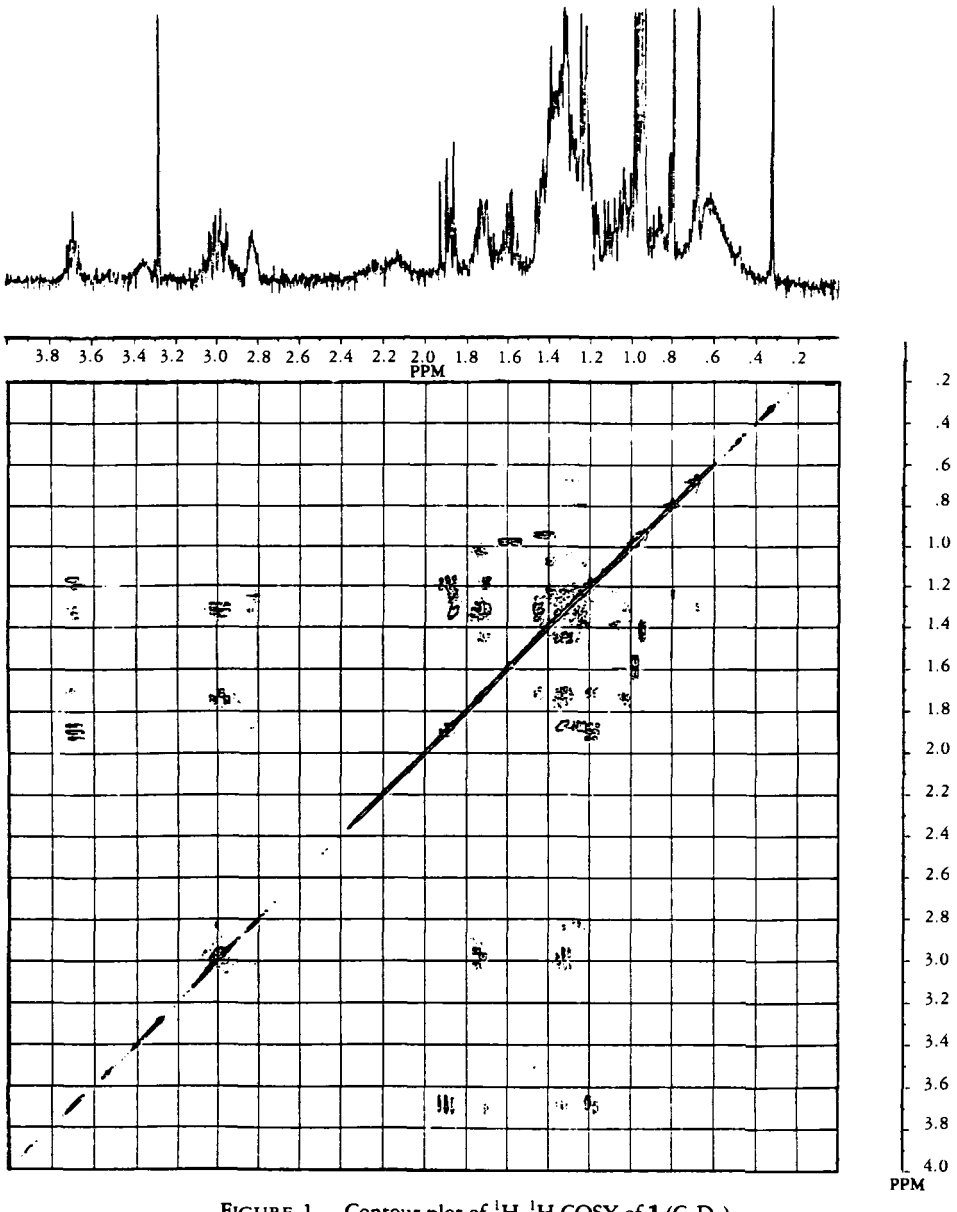


FIGURE 1. Contour plot of ^1H-^1H COSY of **1** (C_6D_6).

This led us to position the oxirane ring at C-5 and C-6 with the α configuration based on the above-mentioned downfield shift of H-3 resonance. On the other hand, the absence of any scalar coupling of the oxirane proton at C-6 (δ 3.28, s) was consistent with the presence of an unprotonated carbon at C-7 which, on consideration of the functionalities present in the molecule, must be identified as the carbonyl group. Because this group was proved to be α,β-unsaturated, the conjugated double bond could be located at C-8/C-9 or C-8/C-14. The former possibility was ruled out on the basis of the λ max and ε values of the uv absorption, which are consistent only with an enone in the cisoid form (5).

These conclusions were further supported by a ^1H-^1H COSY map of **1** in C_6D_6 (Figure 1) which allowed the resonances arising from the protons of rings

A, B, and C to be confidently assigned (Table 1).

Unequivocal assignment of the 3H singlets at δ 0.79 and δ 0.68 to Me-18 and Me-19, respectively, was based on nOe difference experiments, chemical shift arguments being inconclusive in this regard. Particularly, a positive nOe for H_{ax}-4 (δ 1.90) was observed by irradiation at δ 0.68.

The α configuration for the 5,6-epoxide of **1** was indicated by the chemical shift of the H-3 as reported above and was further supported by the following arguments. A ^1H-^1H COSY long range experiment showed a correlation of H_3-19 with H-9. By an accurate inspection of molecular models (Dreiding) it was evident that the planar zig-zag arrangement, responsible for the observed coupling through four σ bonds, fitted the α orientation of the oxygen atom much better than the opposite one.

In addition to compound **1**, the sponge also elaborates sterol **2**, isolated in smaller amounts and assigned the molecular formula ($C_{28}H_{42}O_3$) on the basis of hrms data.

Comparison of spectral features of **2** with those of **1** revealed that the two compounds were closely related, the only differences being in the side chain which in **2** contains a double bond at C-22 and an additional methyl group at C-24. The ^1H-nmr ($CDCl_3$) spectrum of **2** suggested the presence of the same 5α,6α-epoxide-7-keto,9(14)-ene functionalities as found in **1** [δ 3.91 (m, H-3), 3.16 (s, H-6), 0.92 (s, 6H, H_3-18 and H_3-19)]. This was corroborated by uv, ir and mass spectra (see Experimental).

The nature of the side chain was established by the ^1H-nmr data of **2**: δ 1.03 (d, $J=6.5$ Hz, 3H, H_3-21), 5.20 and 5.25 (AB system with further coupling, 2H, $J_{22\text{-}23}=14$ Hz, $J_{20\text{-}22}=J_{23\text{-}24}=7$ Hz, H-22 and H-23 or vice versa), 1.84 (m, 1H, H-24), 1.28 (m, 1H, H-25), 0.83 and 0.85 (d, $J=7$ Hz, 3H each, H_3-26 and H_3-27), 0.91 (d, $J=7$ Hz, 3H, H_3-28). Assignments were made with the aid of extensive decoupling experiments and confirmed by comparison with literature data (6). The stereochemistry of the side chain 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.—Eims of **1** and **2** were obtained at 70 eV on a Kratos MS80 mass spectrometer. Ft-ir spectra were recorded on a Bruker IFS-48 spectrophotometer in $CHCl_3$ solutions. Uv spectra were performed on a Beckman DU70 spectrometer in MeOH solutions. ^1H-nmr spectra were determined on a Bruker WM-400 spectrometer in $CDCl_3$ and C_6D_6, and the assignments were confirmed by decoupling, decoupling-difference, and ^1H-^1H COSY experiments. The ^{13}C-nmr spectrum was taken on a Bruker WM-400 in CD_3OD. Chemical shift values are in ppm with respect to the residual solvent signals.

The nOe's were determined on a Bruker WM-250 spectrometer in C_6D_6 with the aid of a Bruker microprogram. The sample used for nOe measurements was previously degassed by bubbling Ar through the solution for 40 min.

Medium pressure liquid chromatography (mplc) was performed on a Buchi 861 apparatus using an SiO_2 (230–400 mesh) column. Hplc separations were performed on a Varian HPLC Model 5000 with Hibar Si60 LiChrosorb 7 μm and Hibar RP-18 LiChrospher super 100 columns using a dual-cell refractomer detector.

EXTRACTION AND ISOLATION OF COMPOUNDS **1** AND **2**.—*O. lobularis* identified by Dr. Pansini, University of Genova, was collected by hand using scuba gear in the Bay of Naples near Procida (Grotte di Vivara) during spring 1988. It was frozen when still alive at −18° and dispatched to the laboratory. A voucher specimen is deposited in the Dipartimento di Chimica delle Sostanze Naturali, University of Naples. Freshly collected material (dry wt after extraction 34 g) was extracted at room temperature with MeOH (200 ml × 4). MeOH extracts were concentrated in vacuo to give an aqueous phase which was extracted with Et_2O. Evaporation of the combined Et_2O extracts afforded 1.5 g of a brown residue which was chromatographed by mplc on a Si gel column (Merck, 200 g) using the solvent gradient system petroleum ether→Et_2O→$CHCl_3$. The fractions eluted with $CHCl_3$-Et_2O (1:1) were combined. This polar sterol fraction contained compounds **1** and **2** and was rechromatographed by hplc on a Si gel column LiChrosorb Si60 (7 × 250 mm) using $CHCl_3$-EtOAc (6:4) to give

a mixture of **1** and **2**. Final separation was achieved by hplc on a LiChrospher RP-18 super 100 column using MeOH-H_2O (98:2) as eluent.

Compound **1**.—Yield 2 mg; ^{1}H- and ^{13}C-nmr spectra see Table 1; hrms (70 eV) *m/z* $[M]^+$ 414.3181, calcd for $C_{27}H_{42}O_3$, 414.3123; Ft-ir ($CHCl_3$) ν max 3400, 1675, 1601, 1460 cm^{-1}; uv (MeOH) λ max 266 nm (ε = 8600).

Compound **2**.—Yield 1 mg; ^{1}H-nmr ($CDCl_3$) δ 3.91 (m, 1H, H-3), 2.24 (dd, J = 13, J = 12.5 Hz, 1H, H_{ax}-4), 3.16 (s, 1H, H-6), 0.92 (s, 3H, H_3-18), 0.92 (s, 3H, H_3-19), 1.03 (d, J = 6.5 Hz, 3H, H_3-21), 5.20 and 5.25 (AB system with further coupling, 2H, $J_{22\text{-}23}$ = 14 Hz, $J_{20\text{-}22}$ = $J_{23\text{-}24}$ = 7 Hz, H-22 and H-23 or vice versa), 1.84 (m, 1H, H-24), 1.28 (m, 1H, H-25), 0.85 and 0.83 (d, J = 7 Hz, 3H each, H_3-26/H_3-27), 0.91 (d, J = 7 Hz, H_3-28); hrms (70 eV) *m/z* $[M]^+$ 426.3121, calcd for $C_{28}H_{42}O_3$, 426.3123; Ft-ir ($CHCl_3$) ν max 3400, 1675, 1601, 1460 cm^{-1}; uv (MeOH) λ max 266 nm (ε = 8300).

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