Cavernosolide, a New Sesterterpene from a Tyrrhenian Sponge†

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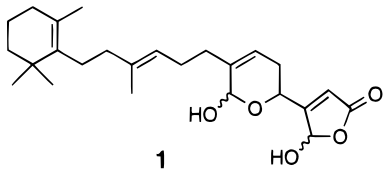
Cavernosolide (**3a**), a new sesterterpene, has been isolated from the Tyrrhenian sponge *Fasciospongia cavernosa*. The structure was proposed on the basis of spectroscopic data and by chemical transformations. Cavernosolide showed potent activity (LC_{50} = 0.37 μg/mL) in the *Artemia salina* bioassay and a moderate toxicity (LC_{50} = 0.75 μg/mL) in a fish (*Gambusia affinis*) lethality assay.

Marine sponges belonging to the family Thorectidae, which includes the genera *Cacospongia*, *Fasciospongia*, *Luffariella*, and *Thorecta*, are known[2] to be a rich source of novel sesterterpenoids, several of which have shown a wide variety of biological activities. Some containing a γ-hydroxybutenolide moiety showed strong antiinflammatory activity, for example, manoalide (**1**),[3] the first sesterterpene to be reported from a *Luffariella* sp., which has been extensively investigated as a potent inhibitor of phospholipase A_2.[4] Subsequently, many related metabolites from *Luffariella* sp. were reported.[2] Structurally related metabolites have been reported from the sponges *Thorectandra excavatus*,[5] *Hyrtios* sp.[6] and *Fasciospongia* sp.[7−9] Among the manoalide congeners, particularly interesting is cacospongionolide B (**2**), which exhibits specific inhibition of human phospholipase A_2[10] and is more stable than manoalide.

Our group has recently investigated the chemistry of a number of specimens of *Fasciospongia cavernosa* Schmidt (family Thorectidae) collected in the northern Adriatic, in order to provide sufficient cacospongionolide B (**2**), and has reported the isolation of novel related metabolites.[11] We then undertook an extensive collection of *F. cavernosa* from the Tyrrhenian Sea. We were surprised to find that a small number of samples, collected in the bay of Naples, contained a new metabolite, cavernosolide (**3a**), together with cacospongionolide B. The structure determination and some biological activities of this compound are reported in this paper.

Cavernosolide (**3a**) was obtained as an amorphous solid and had a molecular formula $C_{27}H_{40}O_6$ by FABMS and ^{13}C-NMR data. The UV absorption at 223 nm and IR bands at 3340, 1775, and 1760 cm^{-1} were characteristic of a γ-hydroxybutenolide moiety. Further absorptions at 1745 and 1240 cm^{-1} in the IR spectrum suggested the presence of an acetate group. The ^{13}C-NMR spectrum of **3a** shows resonances for two carbonyl groups [δ 170.4 (CH_3COO−) and 170.2 (C-19)], two hemiacetals [δ 98.1 (C-24) and 97.3 (C-25)] and two sp^2 [166.8 (C-17) and 117.5 (C-18)] carbons in addition to the signal of the CH_3COO group (δ 21.1), confirming the γ-hydroxybutenolide moiety and the acetate group, showed an additional hemiacetal carbon atom. A methyl singlet at δ 2.12 and two broad singlets, long-range coupled (COSY), at δ 6.10 and 6.02, assigned to H-25 and H-18, respectively, in the ^{1}H-NMR spectrum, afforded an additional proof for the proposed moieties, showing a β-substitution of the γ-hydroxybutenolide moiety.



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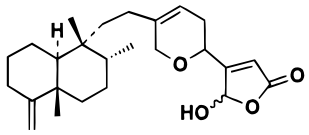

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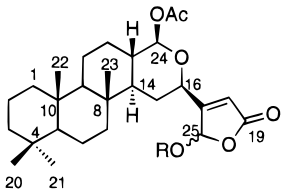

3a R = H
3b, 3c R = Ac

Treatment of **3a** with Ac_2O in pyridine at room temperature gave a mixture of two diastereomeric acetates (**3b** and **3c**) that were separated by Si gel chromatography. The ^{1}H- and ^{13}C-NMR spectra of both acetates showed a single set of sharp resonances disclosing the structure of the polar moiety of cavernosolide, the relative stereochemistry at C-25 remained undetermined.

Examination of the spectral data of the major acetate (**3c**) established that it was closely related to the sesterterpenoid luffolide[12] and the lintenolides.[13] The chemical shifts of the perhydrophenanthrene moiety in the ^{1}H- and ^{13}C-NMR spectra of both cavernosolide and its acetates were in agreement with those of the corresponding resonances in the spectra of lintenolides.[13] Differences were observed for the heterocyclic region. The ^{1}H-NMR spectrum of the major acetate (**3c**) shows a hemiacetal proton doublet at δ 5.33 (H-24), coupled (COSY) with a proton at δ 1.52 (H-13), which, in turn, is coupled with a nonequivalent methylene at δ 1.80 and 0.90 (H-12) and with a methine at δ 1.20 (H-14). This latter is coupled with a methylene at δ 1.25 (H-15),

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Table 1. NMR Spectral Data of **3a** in $CDCl_3$ Solution[a]

carbon	^{13}C	^1H	HMBC (J_{C-H} =10 Hz)
1	39.8 t	1.65 m, 0.77 m	1.32 (H-3), 1.12 (H-3), 0.83 (H-22)
2	18.4 t	1.55 m	1.65 (H-1), 1.32 (H-3)
3	42.0 t	1.32 m, 1.12 m	1.65 (H-1), 0.85 (H-21), 0.80 (H-20)
4	33.4 s		1.55 (H-2), 1.38 (H-6)
5	56.5 d	0.80 dd (12.3, 1.8)	1.65 (H-1), 1.32 (H-3), 0.85 (H-21), 0.83 (H-22)
6	18.5 t	1.38 m	
7	40.4 t	1.70 m, 1.05 m	1.38 (H-6)
8	37.5 s		1.62 (H-11), 1.38 (H-6)
9	59.4 d	0.86 dd (12.2, 1.9)	1.80 (H-12), 1.25 (H-11), 0.90 (H-12), 0.83 (H-22)
10	36.6 s		1.55 (H-2)
11	19.1 t	1.62 m, 1.25 m	1.80 (H-12), 0.90 (H-12)
12	27.6 t	1.80 m, 0.90 m	5.30 (H-24)
13	38.6 d	1.52 m	5.30 (H-24), 1.80 (H-12), 0.90 (H-12)
14	52.0 d	1.20 m	5.30 (H-24), 1.80 (H-12), 1.52 (H-13), 0.90 (H-12), 0.85 (H-23)
15	28.7[b] t	1.25[b] m	
16	71.9[b] d	4.45[b]	6.02 (H-18)
17	166.8[b] s		
18	117.5[b] d	6.02[b]	4.45 (H-16)
19	170.2 s		6.02 (H-18)
20	21.4 q	0.80 s	1.32 (H-3), 1.12 (H-3), 0.85 (H-21), 0.80 (H-5)
21	33.3 q	0.85 s	1.32 (H-3), 1.12 (H-3), 0.80 (H-5 and H-20)
22	16.3 q	0.83 s	1.65 (H-1)
23	15.0 q	0.85 s	1.20 (H-14), 1.05 (H-7)
24	98.1 d	5.30 d (8.8)	1.52 (H-13), 0.90 (H-12)
25	97.3[b] d	6.10[b]	6.02 (H-18)
26	170.4 s		5.30 (H-24), 2.12 (CH_3COO)
27	21.1 q	2.12 s	

[a] Chemical shifts are referred to TMS. Multiplicities are indicated by the usual symbols. Coupling constants (Hz) are in parentheses. [b] Broad signals due to the presence of a mixture of epimers at C-25.

which, in turn, is coupled with a proton at δ 4.35 (H-16), long-range coupled with the two broad singlets of the γ-acetoxybutenolide moiety (H-18 and H-25). The two signals at δ 5.33 and 4.35 show correlations (HETCOR) with carbons at δ 97.6 (d) and 71.6 (d) leading us to propose the presence in the molecule of a further six-membered heterocyclic structure.

The allylic coupling in the ^{1}H-NMR spectrum of **3c** between H-16 and H-18, along with $^{13}C-^1H$ couplings via ^3J correlating C-16 with H-18 and C-18 with H-16 in the HMBC experiment showed that the two heterocyclic rings were linked through the C-16, C-17 bond. An HMBC correlation observed between the H-24 proton (δ 5.30) and the carbonyl of the acetate group at δ 170.4 located the acetoxy group at C-24. Other HMBC correlations, reported in Table 1, allowed us to propose the structure **3a**, without stereochemical implications.

The NOESY spectrum of **3c** exhibited the presence of NOEs indicating that the H-7ax, H-12ax, H-14, H-16, and H-24 are oriented on the same side (α) of the molecule, while H-13 has the same orientation (β) of H_3-23. The axial position of H-24 (J = 8.8 Hz) and H-16 (J = 11.2, 1.9 Hz) was deduced from the magnitude of their coupling constants in the ^{1}H-NMR spectrum of **3a** and **3c**, respectively. These data supported the *trans* stereochemistry of the junction of the rings C and D.

Cavernosolide showed high cytotoxicity (LC_{50} 0.37 μg/mL) in the *Artemia salina* bioassay[14,15] and a moderate toxicity (LC_{50} 0.75 μg/mL) in the fish lethality assay.[16]

Experimental Section

General Experimental Procedures. Melting points were measured on a Kofler apparatus and are uncorrected. UV spectra were obtained on a Varian DMS 90 spectrophotometer. IR spectra were recorded on a Bio-Rad FTS-7 FT-IR spectrometer. Optical rotations were measured on a JASCO DIP 370 polarimeter, using a 10-cm microcell. FABMS were obtained on a VG-ZAB instrument equipped with a FAB source. MS were recorded on an AEI MS-50 spectrometer. ^{1}H-NMR and ^{13}C-NMR spectra were recorded at 500 and 125 MHz, respectively, on a Bruker AMX-500 spectrometer in $CDCl_3$, using the solvent signal as an internal standard. The 2D NMR spectra were obtained using Bruker's microprograms. Si gel chromatography was performed using precoated Merck F_{254} plates and Merck Kieselgel 60 powder.

Biological Material. *F. cavernosa* (order Dictyoceratida; family Thorectidae), collected in April 1996, in the bay of Naples (Italy) at a depth of 10 m, was frozen at −20 °C until extracted and identified by Prof. R. Pronzato of the Istituto di Zoologia dell'Università di Genova, Italy. A voucher specimen is maintained in the Arco Felice institute collection (voucher no. S6C/96).

Extraction and Isolation of Cavernosolide. The frozen sponge (50 g dry wt after extraction) was extracted with Me_2CO and, after elimination of the solvent *in vacuo*, the aqueous residue was extracted with Et_2O and then with *n*-BuOH. The Et_2O extract was evaporated *in vacuo* to obtain a brown oil (2.4 g), which was applied on a column of Si gel. The column was eluted with a solvent gradient system from petroleum ether (40−70 °C) to Et_2O. Cavernosolide (**3a**, 78 mg) was recovered from fractions eluted with petroleum ether−Et_2O (1:1), after crystallization from MeOH.

Cavernosolide (3a): mp 119−121 °C (MeOH); $[\alpha]_D$ +28.7° (*c* 0.3, $CHCl_3$); UV (MeOH) λ_{max} (log ϵ) 223 (3.63) nm; IR ($CHCl_3$) ν_{max} 3340 (br), 1775, 1760, 1745, 1240 cm^{-1}; FABMS *m/z* $[M + H]^+$ 461; EIMS (70 eV) *m/z* (%) $[M - AcOH]^+$ 400 (8), 385 (4), 382 (3), 367 (3), 205 (9), 191 (25), 177 (15), 149 (20), 123 (25), 109 (35), 83 (100); ^{1}H and ^{13}C-NMR ($CDCl_3$), Table 1.

Acetylation of Cavernosolide (3a). A solution of cavernosolide (**3a**, 25 mg) in pyridine (3 mL) and Ac_2O (0.5 mL) was kept at room temperature overnight. The excess reagents were removed *in vacuo*, and the residue was partioned between H_2O and Et_2O. The Et_2O extracts were dried over anhydrous Na_2SO_4, and the solvent was evaporated to obtain a mixture of acetates **3b** and **3c** (24 mg), which were separated by Si gel column, petroleum ether−Et_2O (3:2) as eluent, to obtain acetate **3b** (7 mg) and acetate **3c** (15 mg).

Acetate 3b: UV(MeOH) λ_{max} (log ϵ) 208 (3.84) nm; IR ($CHCl_3$) ν_{max} 1785, 1750 cm^{-1}; ^{1}H-NMR ($CDCl_3$) δ 7.02 (1H, br s, H-25), 6.03 (1H, br s, H-18), 5.24 (1H, d, J = 8.8 Hz, H-24), 4.36 (1H, dd, J = 11.2, 1.9 Hz, H-16), 2.14 (3H, s, $COCH_3$), 2.09 (3H, s, $COCH_3$), 0.87 (3H, s, Me-23), 0.86 (3H, s, Me-21), 0.84 (3H, s, Me-22), 0.81 (3H, s, Me-20); ^{13}C-NMR ($CDCl_3$) d 169.5 (s, $COCH_3$), 169.2 (s, $COCH_3$), 168.5 (s, C-19), 165.3 (s, C-17), 118.2 (d, C-18), 97.8 (d, C-24), 92.6 (d, C-25), 71.0 (d, C-16), 59.6 (d, C-9), 56.5 (d, C-5), 51.9 (d, C-14), 42.0 (t, C-3), 40.5 (t, C-7), 39.8 (t, C-1), 38.3 (d, C-13), 37.5 (s, C-8), 36.4 (s, C-10), 33.4 (s, C-4), 33.3 (q, C-21), 27.9 (t, C-15), 27.6 (t, C-12), 21.4 (q, C-20), 20.8 (q, $COCH_3$), 20.5 (q,

$COCH_3$), 19.1 (t, C-11), 18.5 (t, C-6), 18.4 (t, C-2), 16.3 (q, C-22), 15.0 (q, C-23); EIMS (70 eV) m/z $[M - HAc]^+$ 442 (1), 400 (4), 382 (11), 205 (25), 192 (30), 191 (60), 177 (32), 149 (42), 123 (100).

Acetate 3c: UV(MeOH) λ_{max} (log ϵ) 208 (3.86) nm; IR ($CHCl_3$) ν_{max} 1785, 1750 cm^{-1}; ^{1}H-NMR ($CDCl_3$) δ 6.92 (1H, br s, H-25), 6.14 (1H, br s, H-18), 5.33 (1H, d, J = 8.8 Hz, H-24), 4.35 (1H, d, J = 11.2, 1.9 Hz, H-16), 2.17 (3H, s, $COCH_3$), 2.13 (3H, s, $COCH_3$), 0.85 (6H, s, Me-21 and Me-23), 0.83 (3H, s, Me-22), 0.80 (3H, s, Me-20); ^{13}C-NMR ($CDCl_3$) δ 169.6 (s, $COCH_3$), 169.0 (s, $COCH_3$), 168.9 (s, C-19), 165.1 (s, C-17), 118.3 (d, C-18), 97.6 (d, C-24), 92.2 (d, C-25), 71.6 (d, C-16), 59.5 (d, C-9), 56.5 (d, C-5), 52.1 (d, C-14), 42.0 (t, C-3), 40.4 (t, C-7), 39.9 (t, C-1), 38.6 (d, C-13), 37.6 (s, C-8), 36.6 (s, C-10), 33.4 (s, C-4), 33.3 (q, C-21), 29.1 (t, C-15), 27.8 (t, C-12), 21.4 (q, C-20), 21.0 (q, $COCH_3$), 20.8 (q, $COCH_3$), 19.2 (t, C-11), 18.6 (t, C-6), 18.4 (t, C-2), 16.3 (q, C-22), 15.0 (q, C-23); EIMS (70 eV) m/z $[M - HAc]^+$ 442 (6), 400 (10), 382 (25), 367 (8), 269 (8), 257 (8), 191 (10), 177 (8), 109 (25), 81 (100). Cross peaks were observed in a NOESY spectrum between the following signals: δ 5.33−4.35 (H-24, H-16), 5.33−1.20 (H-24, H-14), 5.33−0.90 (H-24, H-12α), 4.35−1.20 (H-16, H-14), 1.20−1.05 (H-14, H-7α), 1.52−0.85 (H-13, H_3-23), and 1.25−0.83 (H-11β, H_3-22). The ^{1}H NOESY spectrum was recorded at 500 MHz; only cross peaks not sensitive to strong filtering are reported.

Biological Assays. A brine shrimp (*Artemia salina)* lethality assay performed as previously described,[14,15] gave LC_{50} 0.37 μg/mL (0.64/0.20, 95% confidence limits). A fish lethality assay using *Gambusia affinis* performed as already described,[16] gave LC_{50} 0.75 μg/mL (3.91/0.04, 95% confidence limits).

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