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TETRAHEDRON

A Novel C_{21} Terpene Lactone from the Sponge *Fasciospongia Cavernosa*

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Abstract:

Together with the previously described cacospongionolide B (**1**) and cacospongionolide F (**2**) a new C_{21} terpene (**3**), related to cacospongionolide B has been isolated from the sponge *Fasciospongia cavernosa*, collected in the Aegean Sea. The structure and relative stereochemistry of the new compound, were proposed on the basis of spectroscopic data.

Keywords: Lactones; Natural products; Sponges; Terpenes and terpenoids.

The sponges belonging to the genus *Fasciospongia* Burton, are a rich source of novel terpenoids [1-14] many of which have shown a wide variety of biological activities. *Fasciospongia cavernosa* Schmidt (order Dictyoceratida; family Thorectidae) is characterized by a massive body with a fleshy consistency, dark brown-grey in colour. The surface is conulose, usually covered by epibionts, and the skeleton is constituted by stratified fibres that contain foreign material (exogenous spicules, sand grains etc.). The species is distributed in the Mediterranean Sea, Red Sea, Indian ocean and Australian region. From some specimens of *F. cavernosa* collected in the North Adriatic Sea, we have isolated two new related metabolites cacospongionolide B (**1**) [10] and cacospongionolide F (**2**) [14], characterized by cytotoxic and antimicrobial activity [10,14]. Further pharmacological screening revealed that this new class of compounds display anti-inflammatory properties and exhibits specific and potent inhibition of human synovial phospholipase A_2 [13]. We have then undertaken an extensive collection of *F. cavernosa* from the Mediterranean Sea, in order to isolate the required amount of cacospongionolides for exhaustive pharmacological evaluation. From a sample of this sponge, collected in the Aegean Sea, together with cacospongionolide B (**1**) and F (**2**) we isolated a new C-21 terpenoid (**3**). We report here the isolation and structure elucidation of the new metabolite.

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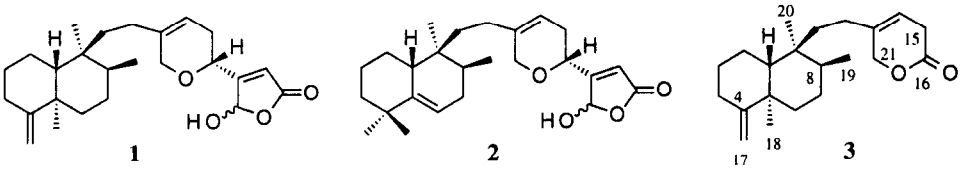
The Et_2O-soluble fraction of the Me_2CO extract of *F. cavernosa* was chromatographed on Silica gel to give, together with the well-known cacospongionolide B (**1**) and F (**2**) a new compound (**3**, 0.043% dry wt).

The spectral data of **1** and **2** were in excellent agreement with those of cacospongionolide B [10] and F [14], respectively. The identity of both compounds was assessed by comparison with authentic samples.

Compound **3** had $[\alpha]_D$ -33.3 ($CHCl_3$) and a molecular formula $C_{21}H_{32}O_2$, as derived by HRMS of the parent ion. The infrared bands at 1743, 1212 and 1073 cm^{-1} suggested the presence of an unconjugated ester. The ^{1}H-NMR spectrum of **3** shows a two-proton broad singlet at δ 4.77 (H-21), (COSY) long-range coupled with an olefinic proton at δ 5.52 (H-14), which, in turn, is coupled with a methylene at δ 3.04 (H-15). The hetereonuclear multi bond correlation experiment (HMBC) showed that both signals due to the carbinyl (δ 4.77) and the allylic (δ 3.04) protons are correlated with the carbonyl at δ 169.4, indicating the presence of a β,γ-unsaturated-δ-lactone ring. Examination of the ^{1}H- and ^{13}C-NMR spectra of this compound established that it was closely related to the cacospongionolide B (**1**)



The ^{1}H-NMR spectrum of **3** showed resonances due to one secondary and two tertiary methyl groups [δ 0.91 (3H, d, J = 7.0 Hz), 0.94 (3H, s) and 1.11 (3H, s)] and an exocyclic methylene [δ 4.49 (br s, 1H), 4.52 (br s, 1H)]. ^{13}C-NMR resonances observed at δ 102.2 (t) and 160.3 (s) confirmed the presence of a 1,1-disubstituted olefin.

Taking into account the molecular formula and the data quoted so far, the apolar region of **3** must possess a carbobicyclic skeleton. The HMBC showed correlations between the protons of the exomethylene and the carbon atoms at δ 32.9 (t), 40.4 (s) and 160.3 (s) which allowed for the identification of the allylic carbon atoms. In addition, correlations observed between the methyl protons of the two tertiary methyl groups (δ 0.94 and 1.11) and the surrounding carbon atoms allowed for their placement on C-9 and C-5 respectively (Table 1). The COSY-45 spectrum indicated that the methine proton observed at δ 1.58 (H-8) was coupled to the methyl doublet observed at δ 0.91 and with non-equivalent methylene protons observed at δ 1.50 and 1.35 (H-7). The latter two protons were in turn coupled to non-equivalent methylene protons observed at δ 1.81 and 1.30 (H-6). The remaining COSY-45 data allowed for the definition of the spin system delineated by H-10/H-1/H-2/H-3. HMBC correlations observed between the H-11 methylene protons (δ 1.45 and 1.10) and the carbons observed at δ 25.7 (C-12), 35.5 (C-8), 38.0 (C-9) and 135.6 (C-13) and the H-12 methylene protons (δ 1.97) and the carbons observed at δ 135.6 (C-13), 114.9 (C-14), 71.2 (C-21) and 37.5 (C-11) defined

the connection between the lactone ring and the carbobicyclic moiety, through an ethylene bridge. These considerations lead to structure **3** without stereochemical implication.

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

N.C	^{13}C	^{1}H	HMBC (J_{C-H} =10 Hz)
1	21.3 t	1.57 m, 1.40 m	2.10 (H-3), 1.17 (H-10)
2	28.7 t	1.88 m	2.28 (H-3), 1.57-1.40 (H-1)
3	32.9 t	2.28 m, 2.10 m	4.45-4.49 (H-17)
4	160.3 s	--	4.45-4.49 (H-17), 2.28-2.10 (H-3), 1.11 (H-18)
5	40.4 s	--	4.45-4.49 (H-17), 2.28-2.10 (H-3), 1.17 (H-10), 1.11 (H-18)
6	29.7 t	1.81 dd (), 1.30 m	1.17 (H-10), 1.11 (H-18)
7	25.3 t	1.50 m, 1.35 m	1.30 (H-6), 1.11 (H-18),0.91 (H-19),
8	35.5 d	1.58 m	1.50-1.35 (H-7), 1.45-1.10 (H-11), 0.94 (H-20)
9	38.0 s	--	1.45-1.10 (H-11), 1.17 (H-10), 0.94 (H-20),
10	47.8 d	1.17 dd (12.1, 2.6)	1.40 (H-1), 1.11(H-18), 0.94 (H-20)
11	37.5 t	1.45 m, 1.10 m	1.97 (H-12), 0.94 (H-20)
12	25.7 t	1.97 m	--
13	135.6 s	--	4.77 (H-21), 3.04 (H-15), 1.97 (H-12), 1.45-1.10 (H-11)
14	114.9 d	5.52 br s	4.77 (H-21), 3.04 (H-15), 1.97 (H-12)
15	30.1 t	3.04 br	--
16	169.4 s	4.42 br m	4.77 (H-21), 3.04 (H-15)
17	102.2 t	4.52 brs, 4.49 brs	2.28-2.10 (H-3)
18	21.3 q	1.11 s	1.81-1.30 (H-6), 1.17 (H-10)
19	14.8 q	0.91 d (7.0)	--
20	20.6 q	0.94 s	1.45-1.10 (H-11)
21	71.2 t	4.77 brs	1.97 (H-12)

[a] Chemical shifts are referred to TMS. Multiplicities are indicated by usual symbols. Coupling constants (Hz) are in parentheses.

The relative stereochemistry of the carbobicyclic part of **3** relies on a NOESY spectrum. NOEs indicate that the Me-5 (δ 1.11), Me-9 (δ 0.94), H_{ax}-3 (δ 2.28) and H_{ax}-1 (δ 1.57) are oriented on the same side (α) of the molecule, while the H_{ax}-10 (δ 1.17) has the same orientation (β) as Me-8 (δ 0.91). The axial position of H-10 (J=12.1 and 2.6 Hz) was deduced from the magnitude of its coupling constants.

The finding of this C_{21} terpene and the closely related sesterterpene (**1**) supports the biogenetic hypothesis [15] that the C_{21} terpenes are derived from sesterterpenes by loss of four C atoms, through an oxidative rupture of the γ-hydroxy-butenolide ring. The isolation of several related constituents from individual specimens of *F. cavernosa* confirms the peculiarity of the sponges belonging to the family Thorectidae. Similar variation of related metabolites were observed for the sponges *Luffariella variabilis* [16], *L. geometrica* [17] and *Thorectandra excavatus* [18].

EXPERIMENTAL [19]

Biological material: *Fasciospongia cavernosa* (order Dictyoceratida; family Thorectidae) was collected (-15 m) at Kalymnos (Greece) in March 1998, and frozen at -20° until

extracted. A voucher specimen is maintained in the Arco Felice Institute collection (voucher No. S6K/98).

Isolation of compounds: from fractions eluted with petroleum ether-Et_2O (7:3) after reversed-phase HPLC purification (Spherisorb S5ODS2; acetonitrile; flow 3 mL/min) were recovered cacospongionolide B that crystallized from MeOH (26 mg, 0.37% dry weight), cacospongionolide F as amorphous solid (12 mg, 0.17%) and compound **3** (3 mg, 0.043%).

Cacospongionolide B (**1**): mp 116-118°C; $[\alpha]_D$ +28.2 (c= 0.22, $CHCl_3$); UV; IR; MS; ^{1}H- and ^{13}C-NMR data are in agreement with those of authentic sample.

Cacospongionolide F (**2**): $[\alpha]_D$ -123.0 (c= 0.1, $CHCl_3$); UV; IR; MS; ^{1}H- and ^{13}C-NMR data are in agreement with those of authentic sample.

Compound 3: amorphous solid; $[\alpha]_D$ -33.3 (c= 0.03, $CHCl_3$); IR ν_{max} ($CHCl_3$) 1743, 1212, 1074 cm^{-1}; EIMS *m/z* (%) $[M]^+$ 316.2408 ($C_{21}H_{32}O_2$ requires 316.2402) (5), $[M\text{-}Me]^+$ 301 (8), 219 (5), 205 (8),192 (50), 190 (75), 150 (30), 137 (40), 123 (48), 96 (100); NMR data see Table 1. Cross peak were observed in a NOESY spectrum between the following signals : δ 1.11-0.94 (H_3-18; H_3-20), 1.11-2.28 (H_3-18; H-3α), 4.52-1.81 (H-17a; H-6α), 4.49-2.10 (H-17b; H-3β), 1.17-0.91 (H-10β; H_3-19).

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19. General experimental details and extraction of the sponge are the same as those described in the previous report [10].