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Minor cacospongionolide derivatives from the sponge *Fasciospongia cavernosa*

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Abstract—Together with the known cacospongionolide, three new related sesterterpenes have been isolated from the sponge *Fasciospongia cavernosa*, collected from the Northern Adriatic Sea. The structures of the new compounds were proposed on the basis of spectroscopic data.

1. Introduction

Marine organisms have provided a large number of sesterterpenoids possessing novel carbon skeletons, which are different from those present in terrestrial species. Several sesterterpenoids isolated from marine organisms have shown biological activity.[1,2]

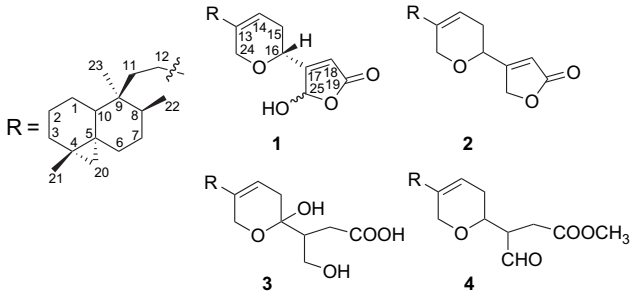
Marine sponges belonging to the family Thorectidae, which includes the genera *Cacospongia*, *Fasciospongia*, *Luffariella*, and *Thorecta*, are known to be a rich source of novel sesterterpenoids.[3,4] Some containing a γ-hydroxybutenolide moiety showed strong anti-inflammatory activity, e.g., manoalide,[5] the first sesterterpene to be reported from a *Luffariella* sp., has been extensively investigated as a potent inhibitor of phospholipase A2.[3,4]

The Mediterranean sponge *Fasciospongia cavernosa* is a rich source of a new class of sesterterpenoids, named cacospongionolides. Cacospongionolide (**1**) was the first sesterterpene isolated from the Adriatic Sea sponge *F. cavernosa*, which possesses antimicrobial and antitumor activities.[6] Subsequently, several related compounds were isolated from several specimens of *F. cavernosa* collected from the Mediterranean Sea. This class of marine metabolites is the inhibitor of phospholipase A2 with potent topical anti-inflammatory profile and they showed high antimicrobial activity against the Gram-positive bacteria *Bacillus subtilis* and *Micrococcus luteus*.[1]

Our group has investigated the chemistry of a number of specimens of *F. cavernosa* Schmidt (family Thorectidae) collected from the Mediterranean Sea, in order to provide sufficient cacospongionolides. We have reported the isolation of novel related metabolites,[1] including two luffarin derivatives.[7] From a sample of this sponge, collected from the Northern Adriatic Sea, together with cacospongionolide (**1**) we isolated three new derivatives (**2–4**). We report here the isolation and structure determination of the new metabolites.

2. Results and discussion

The Et_2O-soluble fraction of the acetone extract of *F. cavernosa* was chromatographed on silica gel, followed by reverse HPLC, to give, as the major component, the known cacospongionolide (**1**)[6] and three related compounds **2–4**.



The spectral data of cacospongionolide (**1**) were in excellent agreement with those reported in the literature,[6] and it was identified by comparison with an authentic sample.

Examination of NMR data of compounds **2–4** established that they were closely related to cacospongionolide (**1**). The chemical shifts of the carbotriclycic (C-1 to C-12)

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region in the ^{1}H and ^{13}C NMR spectra of compounds **2**–**4** were in excellent agreement with those of the corresponding resonances in the spectra of cacospongionolide (**1**).[6]

25-Deoxycacospongionolide (**2**) had $[\alpha]_D$ −9.2 and a molecular formula $C_{25}H_{36}O_3$, as derived by HRMS. The absence of the characteristic infrared band of the hydroxyl group (ν_{max} 3380 cm^{-1}), and the presence of bands at 1744 and 1650 cm^{-1} in the IR spectrum of **2** suggested the presence of a butenolide moiety in 25-deoxycacospongionolide, instead of a γ-hydroxybutenolide moiety shown in **1**. The presence of a methylene [δ 4.87 (ABq, *J*=17.7 Hz, H-25); 71.0 (t, C-25)], a methine [δ 5.95 (br s, H-18); 114.5 (d, C-18)], and two quaternary carbon atoms [δ 173.5 (C-19); 169.8 (C-17)] in the NMR spectra, confirms the β substituted butenolide moiety.

Fractions eluted with petroleum ether–AcOEt (3/2) were purified by preparative HPLC yielding compound **3** (2.1 mg) and an impure compound that showed an aldehydic group [δ 9.9 (d, *J*=1.8 Hz)], and only after methylation it was possible to isolate compound **4**, as methyl ester.

Compound **3** had $[\alpha]_D$ −9.4 and a molecular formula $C_{25}H_{40}O_5$, as derived by HRMS of the M^+-H_2O ion in conjunction with NMR data. The presence of bands at ν_{max} 3300–2820 (broad) and 1750 cm^{-1} (C=O stretching) in the IR spectrum of **3** suggested the presence of a carboxylic acid group. The ^{1}H NMR spectrum of **3** shows a broad AB quartet at δ 4.32 and 4.11 (*J*=15.8 Hz, H-24), long range coupled with an olefinic proton at δ 5.42 (br s, H-14), which, in turn, is coupled with a non-equivalent methylene as a broad AB quartet at δ 2.70 (d, *J*=17.8 Hz) and 2.21 (d, *J*=17.8 Hz) (H-15). The two signals at δ 4.32 and 4.11 show correlations (HMQC) with a carbon at δ 64.0 (t) and long-range correlation (HMBC) with the two carbons of the trisubstituted double bond at δ 136.2 (s, C-13) and 113.5 (d, C-14), and with a hemiketal carbon at δ 106.4 (C-16), indicating the presence of a dihydropyran ring with a hemiketal moiety in the molecule. Furthermore, the analysis of the NMR data established the presence of a methylene [^{1}H NMR, ABX system, δ 2.81 (1H, dd, *J*=16.5 and 10.2 Hz) and 2.54 (1H, dd, *J*=16.5 and 8.6 Hz), H-18; ^{13}C NMR δ 30.0 (t, C-18)], a methine [^{1}H NMR δ 2.48 (1H, m, H-17); ^{13}C NMR δ 44.9 (d, C-17)], and a hydroxymethylene group [^{1}H NMR, ABX system, δ 3.91 (1H, dd, *J*=12.0 and 2.8 Hz) and 3.71 (1H, dd, *J*=12.0 and 5.3 Hz), H-25; ^{13}C NMR δ 59.3 (t, C-25)]. The COSY spectrum indicated that both these methylenes were coupled to the methine at δ 2.48. The methine at δ 2.48 shows long-range correlation (HMBC) with the carboxyl carbon at δ 175.3 (C-19), suggesting the presence of a γ-hydroxy acid moiety in the molecule. Furthermore, the HMBC spectrum showed correlation between the protons of the α-carboxy methylene (δ 2.81 and 2.54) and the hemiketal carbon at δ 106.4, defining the conjunction between the last carbon and the C-17 methine, as depicted in the formula of **3**.

Compound **4** had $[\alpha]_D$ −8.4 and a molecular formula $C_{26}H_{40}O_4$, as derived by HRMS. The presence of bands at ν_{max} 2820 and 2720 (C–H stretching) and 1740 (C=O stretching) cm^{-1} in the IR spectrum of **4** suggested the presence of an aldehydic group. Further bands at ν_{max} 1725 (C=O stretching), 1250 and 1170 cm^{-1} (C–O stretching) suggested the presence of an ester group in compound **4**. The presence of a methyl [δ 3.68 (s, H-26), 53.0 (q, C-26)], a proton [δ 9.90 (d, *J*=1.8 Hz, H-25)], and two quaternary carbon atoms [δ 172.0 (C-19), 204.0 (C-25)] in the NMR spectra, confirms the aldehydic group and the methyl ester moiety. The ^{1}H NMR spectrum shows a two proton broad singlet at δ 4.08 (H-24), long range coupled with an olefinic proton at δ 5.50 (H-14), which, in turn, is coupled with a non-equivalent methylene at δ 2.25 (m) and 2.02 (m) (H-15). These latter are coupled with a proton at δ 3.80 (H-16). The two signals at δ 4.08 and 3.80 show correlations (HMQC) with carbons at δ 68.4 (t) and 73.0 (d) indicating the presence of a dihydropyran ring in the molecule. The COSY spectrum indicated that the oxymethine proton at δ 3.80 (H-16) was coupled to the methine proton at δ 2.98 (dddd, *J*=8.3, 5.3, 5.2, and 1.8 Hz, H-17), which, in turn, was coupled with a non-equivalent methylene at δ 2.80 (dd, *J*=16.8 and 8.3 Hz) and 2.51 (dd, *J*=16.8 and 5.3 Hz), and with the aldehydic proton at δ 9.90. These data, together with the HMBC correlations of the aldehydic proton at δ 9.90 (H-25) with the carbon at δ 53.0 (d, C-17), of the methine proton at δ 2.98 (H-17) with the carbonyl at δ 172.0 (C-19), and of one of the non-equivalent methylene proton at δ 2.80 (H-18) with the aldehydic carbonyl at δ 204.0 (C-25) allowed us to locate the aldehydic group in γ position of methyl ester group as indicated in the structure of compound **4**. The small coupling constant observed between the aldehydic proton and H-17 was justified by the presence of an intra-molecular interaction between the aldehydic and carboxylic groups that reduce the free rotation along the C-17 and C-25 bond, as a result of an dihedral angle of about 90° between the H-17 and H-25 protons.

Given the small quantity of compounds **2**–**4** that were available it was not possible to ascertain the absolute configuration about C-16 and C-17. A 4*S*, 5*S*, 8*S*, 9*S*, 10*R* absolute stereochemistry was ascribed to all new compounds on biosynthetic grounds.

The finding of these compounds and the related sesterterpene (**1**) may be the first step in the oxidative rupture of the γ-hydroxybutenolide ring to produce C_{21} terpenes, as previously hypothesized.[8,9]

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 was observed for the sponges *Luffariella geometrica*,[10] *Luffariella variabilis*,[11] and *Thorectandra excavatus*.[12]

3. Experimental

3.1. 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 FTIR spectrometer. Optical rotations were measured on a Jasco DIP 370 polarimeter, using a 10-cm microcell.

Mass spectra were recorded on an AEI MS-50 spectrometer. ^{1}H and ^{13}C NMR spectra were recorded at 400 and 100 MHz, respectively, on a Bruker Avance-400 spectrometer, using an inverse probe fitted with a gradient along the *Z*-axis, 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 pre-coated Merck F_{254} plates and Merck Kieselgel 60 powder.

3.2. Animal material

The sponge *F. cavernosa* (order Dictyoceratida; family Thorectidae) was collected by dredging (25 m) in May 2002 at Rovinj (Croatia). It was frozen at −20° C until extracted and identified by Prof. R. Pronzato of Dip.Te.Ris. dell'Università di Genova, Italy. A voucher specimen is maintained in the Pozzuoli Institute collection (voucher no. S6R/02).

3.3. Extraction and isolation

The frozen sponge (34 g dry wt after extraction) was extracted with acetone and, after elimination of the solvent under reduced pressure, the aqueous residue was extracted with diethyl ether. The ethereal extract was evaporated under reduced pressure to obtain brown oil (1.4 g), which was applied on a column of Si gel. The column was eluted with a solvent gradient system from petroleum ether (40–70) to AcOEt.

Fractions eluted with petroleum ether–AcOEt (4/1) were purified by preparative HPLC (Kromasil C18; MeOH, flow 3 ml/min) yielding 25-deoxycacospongionolide that crystallized from MeOH (**2**, 4.1 mg). From fractions eluted with petroleum ether–AcOEt (7/3), after crystallization from MeOH, was recovered cacospongionolide (**1**, 390 mg). Fractions eluted with petroleum ether–AcOEt (3/2) were purified by preparative HPLC (Kromasil C18; CH_3CN–H_2O, 94/6; flow 3 ml/min) yielding compound **3** (2.1 mg) and an impure compound after methylation with CH_2N_2 and purification by preparative HPLC (Kromasil C18; CH_3CN, flow 3 ml/min) was recovered compound **4** (3.5 mg).

3.3.1. Cacospongionolide (1). White crystals; mp 164–166 °C; $[\alpha]_D^{20}$ +27 (*c* 0.1, $CHCl_3$); UV, IR, MS, and ^{1}H, ^{13}C NMR data are in agreement with those of authentic sample.

3.3.2. 25-Deoxycacospongionolide (2). White crystals; mp 175–177 °C; $[\alpha]_D^{20}$ −9.2 (*c* 0.03, $CHCl_3$); λ_{max} (MeOH) (log ε) 222 (3.60) nm; ν_{max} ($CHCl_3$) 3380, 1744, 1650, 1115 cm^{-1}; δ_H (400 MHz, $CDCl_3$) 5.95 (1H, br s, H-18), 5.54 (1H, br s, H-14), 4.90 and 4.84 (2H, ABq, *J*=17.7 Hz, H-25), 4.42 (1H, ddd, *J*=10.4, 4.3, 1.4 Hz, H-16), 4.18 and 4.11 (2H, ABq, *J*=15.6 Hz, H-24), 2.23 (2H, m, H-15), 1.86 (3H, m, H-12 and H-6a), 1.75 (1H, m, H-7a), 1.57 (1H, m, H-8), 1.54 (1H, m, H-10), 1.52 (2H, m, H-3), 1.45 (1H, m, H-2a), 1.37 (2H, m, H-1a and H-11a), 1.28 (1H, m, H-6b), 1.17 (1H, m, H-11b), 1.12 (1H, m, H-2b), 0.99 (3H, s, H-21), 0.95 (1H, m, H-7b), 0.92 (3H, s, H-23), 0.91 (3H, d, *J*=7.2 Hz, H-22), 0.68 (1H, ddd, *J*=15.5, 12.5, 2.8 Hz, H-1b), 0.47 (1H, d, *J*=4.4 Hz, H-20a), 0.08 (1H, d, *J*=4.4 Hz, H-20b); δ_C (100.6 MHz, $CDCl_3$) 173.5 (s, C-19), 169.8 (s, C-17), 138.4 (s, C-13), 115.7 (d, C-14), 114.5 (d, C-18), 71.0 (t, C-25), 70.2 (d, C-16), 68.3 (t, C-24), 41.0 (d, C-10), 39.0 (s, C-9), 36.8 (t, C-11), 35.5 (d, C-8), 32.1 (t, C-3), 30.0 (t, C-15), 27.9 (t, C-6), 27.7 (t, C-7), 26.5 (t, C-12), 26.3 (s, C-5), 24.5 (t, C-20), 23.2 (t, C-2), 22.4 (q, C-21), 19.8 (t, C-1), 19.7 (q, C-23), 17.3 (s, C-4), 14.2 (q, C-22); *m/z* (EI, 70 eV) 384 (40, M^+), 369 (15), 205 (30), 193 (35), 191 (100), 189 (85), 177 (20%); HRMS (EI): M^+, found 384.2668. $C_{25}H_{36}O_3$ requires 384.2664.

3.3.3. Compound 3. Amorphous solid; $[\alpha]_D^{20}$ −9.4 (*c* 0.02, $CHCl_3$); ν_{max} ($CHCl_3$) 3300–2800, 1750 cm^{-1}; δ_H (400 MHz, $CDCl_3$) 5.42 (1H, br s, H-14), 4.32 and 4.11 (2H, ABq, *J*=15.8 Hz, H-24), 3.91 (1H, dd, *J*=12.0, 2.8 Hz, H-25a), 3.71 (1H, dd, *J*=12.0, 5.3 Hz, H-25b), 2.81 (1H, dd, *J*=16.5, 10.2 Hz, H-18a), 2.70 and 2.21 (2H, ABq, *J*=17.8 Hz, H-15), 2.54 (1H, dd, *J*=16.5, 8.6 Hz, H-18b), 2.48 (1H, m, H-17), 1.89 (2H, m, H-12), 1.85 (1H, m, H-6a), 1.75 (1H, m, H-7a), 1.57 (1H, m, H-8), 1.54 (1H, m, H-10), 1.52 (2H, m, H-3), 1.45 (1H, m, H-2a), 1.37 (2H, m, H-1a and H-11a), 1.28 (1H, m, H-6b), 1.17 (1H, m, H-11b), 1.12 (1H, m, H-2b), 0.99 (3H, s, H-21), 0.95 (1H, m, H-7b), 0.92 (3H, s, H-23), 0.91 (3H, d, *J*=7.2 Hz, H-22), 0.68 (1H, ddd, *J*=15.5, 12.5, 2.8 Hz, H-1b), 0.47 (1H, d, *J*=4.4 Hz, H-20a), 0.08 (1H, d, *J*=4.4 Hz, H-20b); δ_C (100.6 MHz, $CDCl_3$) 175.3 (s, C-19), 136.2 (s, C-13), 113.5 (d, C-14), 106.4 (s, C-16), 64.0 (t, C-24), 59.3 (t, C-25), 44.9 (d, C-17), 41.0 (d, C-10), 39.0 (s, C-9), 36.8 (t, C-11), 35.5 (d, C-8), 32.1 (t, C-3), 31.5 (t, C-15), 30.0 (t, C-18), 27.9 (t, C-6), 27.7 (t, C-7), 26.5 (t, C-12), 26.3 (s, C-5), 24.5 (t, C-20), 23.2 (t, C-2), 22.4 (q, C-21), 19.8 (t, C-1), 19.7 (q, C-23), 17.3 (s, C-4), 14.2 (q, C-22); *m/z* (EI, 70 eV) 402 (10, $M-H_2O^+$), 384 (5), 368 (15), 191 (85), 189 (100%); HRMS (EI): $M-H_2O^+$, found 402.2774. $C_{25}H_{38}O_4$ requires 402.2770.

3.3.4. Compound 4. Amorphous solid; $[\alpha]_D^{20}$ −8.4 (*c* 0.01, $CHCl_3$); ν_{max} ($CHCl_3$) 2820, 2720, 1740, 1725, 1250, 1170 cm^{-1}; δ_H (400 MHz, $CDCl_3$) 9.90 (1H, d, *J*=1.8 Hz, H-25), 5.50 (1H, br s, H-14), 4.08 (2H, br s, H-24), 3.80 (1H, m, H-16), 3.68 (3H, s, H-26), 2.98 (1H, dddd, *J*=8.3, 5.3, 5.2, 1.8 Hz, H-17), 2.80 (1H, dd, *J*=16.8, 8.3 Hz, H-18a), 2.51 (1H, dd, *J*=16.8, 5.3 Hz, H-18b), 2.25 (1H, m, H-15a), 2.02 (1H, m, H-15b), 1.80 (2H, m, H-12), 1.85 (1H, m, H-6a), 1.75 (1H, m, H-7a), 1.57 (1H, m, H-8), 1.54 (1H, m, H-10), 1.52 (2H, m, H-3), 1.45 (1H, m, H-2a), 1.37 (2H, m, H-1a and H-11a), 1.28 (1H, m, H-6b), 1.17 (1H, m, H-11b), 1.12 (1H, m, H-2b), 0.99 (3H, s, H-21), 0.95 (1H, m, H-7b), 0.92 (3H, s, H-23), 0.91 (3H, d, *J*=7.2 Hz, H-22), 0.68 (1H, ddd, *J*=15.5, 12.5, 2.8 Hz, H-1b), 0.47 (1H, d, *J*=4.4 Hz, H-20a), 0.08 (1H, d, *J*=4.4 Hz, H-20b); δ_C (100.6 MHz, $CDCl_3$) 204.0 (d, C-25), 172.0 (s, C-19), 133.0 (s, C-13), 115.8 (d, C-14), 73.0 (d, C-16), 68.4 (t, C-24), 53.2 (d, C-17), 53.0 (q, C-26), 41.0 (d, C-10), 39.0 (s, C-9), 36.8 (t, C-11), 35.5 (d, C-8), 32.1 (t, C-3), 31.0 (t, C-18), 27.9 (t, C-6), 27.8 (t, C-15), 27.7 (t, C-7), 26.0 (t, C-12), 26.3 (s, C-5), 24.5 (t, C-20), 23.2 (t, C-2), 22.4 (q, C-21), 19.8 (t, C-1), 19.7 (q, C-23), 17.3 (s, C-4), 14.2 (q, C-22); *m/z* (EI, 70 eV) 416 (20, M^+), 385 (8), 301 (28), 300 (35), 191 (85), 189 (100%);

HRMS (EI): M^+, found 416.2921. $C_{26}H_{40}O_4$ requires 416.2926.

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