A New Cacospongionolide Derivative from the Sponge *Fasciospongia cavernosa*

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Cacospongionolide F (**4a**), a new bioactive cacospongionolide-related sesterterpene, has been isolated from the Northern Adriatic sponge *Fasciospongia cavernosa*. The structure was proposed on the basis of spectroscopic data and chemical transformations. The absolute configuration was established using the modified Mosher's method. A molecular mechanics study of the dehydrodecalin ring explained the observed differences in dynamic behavior between cacospongionolide F and mamanuthaquinone, a related compound. Antimicrobial activity, brine shrimp and fish lethalities of this new compound are reported.

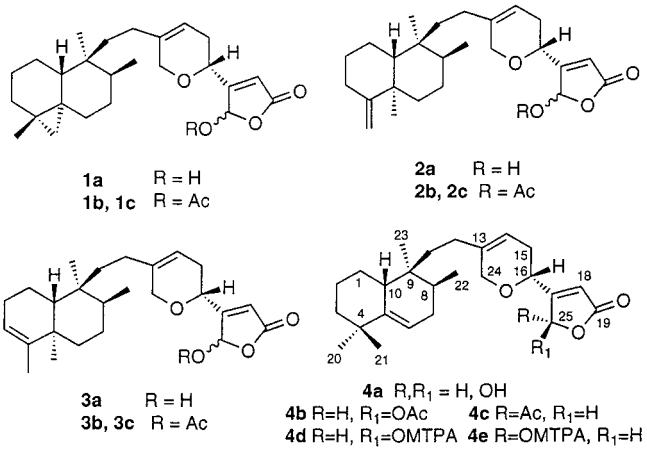
We reported previously on the isolation and structural elucidation of cacospongionolide (**1a**),[1,2] a highly cytotoxic metabolite from the sponge *Fasciospongia cavernosa* (erroneously classified as *Cacospongia mollior*).[3] Recently we isolated from some specimens of *F. cavernosa* Schmidt, (order Dictyoceratida; family Thorectidae) collected in the North Adriatic and Tyrrhenian seas, two new metabolites, cacospongionolide B (**2a**)[3] and cacospongionolide E (**3a**),[4] both compounds were characterized by cytotoxic and antimicrobial activity. Further pharmacological screening revealed **2a** and **3a** displayed antiinflammatory properties and inhibited phospholipase A_2.[4,5] We then undertook an extensive collection of *F. cavernosa* from the Mediterranean Sea to isolate sufficient cacospongionolides for exhaustive pharmacological evaluation. In this paper we report on the isolation, structure elucidation and biological activity of cacospongionolide F (**4a**).

The Et_2O-soluble fraction of the Me_2CO extract of *F. cavernosa* was chromatographed on Si gel to give some pure cacospongionolide B (**2a**) and a mixture that was separated by reversed-phase HPLC to give more **2a** and a new sesterterpene named cacospongionolide F (**4a**, 0.2% dry weight).

The spectral data of **2a** were in excellent agreement with those of cacospongionolide B.[3]

Cacospongionolide F (**4a**) had $[\alpha]_D$ −123.0 and a molecular formula $C_{25}H_{36}O_4$, as derived by HRMS. The ultraviolet absorption band at 224 nm (ϵ 4400) and the infrared bands at 3380, 1782, and 1764 cm^{-1} are characteristic of a γ-hydroxybutenolide moiety. The analysis of its ^1H and ^{13}C NMR spectra showed that **4a** is closely related to other cacospongionolides: **1a**,[1] **2a**,[3] and **3a**.[4] As already observed for **1a**−**3a**, the NMR spectra of **4a** are highly solvent dependent and the interpretation of the signals due to the polar moiety of the molecule is difficult.

The ^1H and ^{13}C NMR spectra of acetates **4b** and **4c**, obtained by treatment of **4a** with acetic anhydride in pyridine at room temperature, show a single set of sharp resonances for the polar moiety (the heterocyclic region) of cacospongionolide F. Their chemical shifts were in excellent agreement with those of the corresponding resonances observed for **2b** and **2c**,[3] defining the structure of this polar functionality as shown. The ^1H NMR spectrum of cacospongionolide F, apart from the signals due to the polar moiety, shows signals due to one secondary and three tertiary methyl groups at δ 0.77 (3H, d, J = 6.6 Hz), 0.81 (3H, s), 1.01 (3H, s), and 1.04 (3H, s) and a broad singlet corresponding to one olefinic methine at δ 5.32.



Taking into account the molecular formula and the data discussed thus far, the nonpolar region of **4a** must possess a carbobicyclic skeleton.

The COSY-45 spectrum indicated that the olefinic proton H-6 (δ 5.32) is strongly coupled to the nonequivalent methylene protons Hs-7 (δ 2.05, 1.70) and weakly, via allylic coupling, to the methine H-10 (δ 1.95). It was also possible to establish that the methylene protons (Hs-7) are coupled to a proton at δ 1.65 (H-8), which in turn is coupled to a methyl at δ 0.77 (H-22). From COSY-45 data it was also possible to define the spin system corresponding to the protons H-10/H-1/H-2/H-3 and H-11/H-12. HMBC correlations observed between the H-10 methine proton (δ 1.95) and the carbons observed at δ 26.7 (C-11) and 145.5 (C-5) defined the connection between the heterocyclic region and the dehydrodecalin (DhD) ring. Other HMBC correlations reported in Table 1, and the biosynthetic context of this family of compounds allowed us to build the structure **4a**, without stereochemical implications.

The relative stereochemistry of the DhD ring of **4a** was determined using a NOESY spectrum. NOEs indicated that the Me at C-9 (δ 0.81) and H-1_{ax} (δ 1.75) belong to the same side (α) of the molecule, while H-10_{ax} (δ 1.95) has the same orientation (β) as the Me at C-8 (δ 0.77). The axial orientation of H-10 (J = 11.5 and 2.1 Hz) and H-16 (J = 10.5 and 4.2 Hz) was deduced from the magnitude of their

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Table 1. NMR Spectral Data of **4a** in $CDCl_3$ Solution[a] at 300 K

C	^{13}C	^{1}H	HMBC (J_{C-H} = 10 Hz)
1	30.9 br t	1.75 m, 1.05 m	–
2	23.0 t	1.58 m, 1.45 m	1.75 (H-1), 1.42 (H-3)
3	41.9 br t	1.42 m, 1.20 m	1.75 (H-1), 1.01 (H-20), 1.04 (H-21)
4	36.7 s	–	5.32 (H-6), 1.01 (H-20), 1.04 (H-21), 1.42 (H-3)
5	145.5 br s	–	1.95 (H-10), 1.42 (H-3), 1.01 (H-20), 1.04 (H-21)
6	114.4 d	5.32 brs	–
7	28.6 br t	2.05 m, 1.70 m	–
8	32.7 d	1.65 m	5.32 (H-6), 0.81 (H-23), 0.77 (H-22)
9	35.7 s	–	0.81 (H-23), 0.77 (H-22)
10	40.6 d	1.95 dd (11.5, 2.1)	5.32 (H-6), 0.81 (H-23)
11	26.7 t	1.40 m, 1.30 m	1.95 (H-10)
12	26.7 t	1.80 m	5.54 (H-14)
13	138.3 s	–	4.16 (H-24), 1.80 (H-12)
14	115.9 d	5.54 br s	4.16 (H-24), 1.80 (H-12)
15	29.1 br t	2.27 m	–
16	69.5 br d	4.42 br m	4.16 (H-24)
17	168.4 br s	–	–
18	117.2 br d	6.04 brs	–
19	170.8 s	–	6.04 (H-18)
20	22.1 q	1.01 s	–
21	29.4 q	1.04 s	–
22	14.6 q	0.77 d (6.6)	1.65 (H-8)
23	22.1 q	0.81 s	–
24	68.3 t	4.16 ABq (15.8)	–
25	97.2 br d	6.15 br s	–

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

coupling constants in the ^{1}H NMR spectra of **4a** and **4b**, respectively.

The interpretation of the ^{13}C NMR spectrum of cacospongionolide F and its acetates, at room temperature, was quite difficult in the spectral region corresponding to the DhD moiety. Resonances corresponding to this moiety are broad. At 230 K, two sets of signals were clearly observable, i.e., there is an equilibrium between two different conformers, in intermediate exchange rate at room temperature. Lowering the temperature to 230 K shifted the equilibrium to the slow exchange rate case. The ^{13}C NMR spectrum of siphonodictyal D,[6] which contains the same carbo-bicyclic skeleton, exhibited the same broad signals, while mamanuthaquinone,[7] which contains a similar carbo-bicyclic skeleton but with different stereochemistry at C-8, exhibited a ^{13}C NMR spectrum with sharp signals.

A molecular mechanics study of the DhD ring provided an explanation of the observed differences in dynamic behavior between cacospongionolide F and mamanuthaquinone. The calculated energy of cacospongionolide F conformers exhibiting the polar moiety on C-9 and the methyl on C-8 in pseudoequatorial and pseudoaxial positions, respectively, differs only a few tenths of a kcal mol^{-1} (0.2 kcal mol^{-1} on the average, for conformers without interactions between the nonpolar ring and the polar region) from the energy of similar conformers with reversed positions of the two groups. On the contrary, an inversion of chirality at C-8 leads to a stability difference between pseudoequatorial−pseudoequatorial and pseudoaxial−pseudoaxial conformer families of several kcal mol^{-1} (7 kcal mol^{-1}, on average, for conformers without interactions between polar and nonpolar part of the molecule). The inclusion of the predicted compact structures, with direct interactions between the apolar and the polar rings, does not modify significantly the observed trends, but does complicate substantially the comparison, requiring the inclusion of a larger number of conformers.

To eliminate the effects due to the two diastereoisomers at C-25 we recorded the ^{13}C NMR spectrum of the major acetate **4b** at 300 and 230 K. We were surprised to observe that some carbons of dihydropyran ring, that are far from the DhD ring, gave two sets of signals at 230 K. On the basis of the ^{13}C NMR spectrum of the acetate of cacospongionolide B (**2b**), recorded at 230 K, where a single set of signals is present throughout, it seems fair to exclude the dihydropyran ring from the equilibrium phenomenon observed in the low temperature ^{13}C NMR spectrum of **4b**. Therefore, the solution conformation of cacospongionolide F and of its acetates should show a spatial interaction between the polar and the nonpolar moieties of the molecule. In fact, the conformational analysis of these molecules shows, for cacospongionolide F, that the spatial orientation of the polar part is strongly influenced by the conformation of the DhD ring, owing to the presence in some conformers of steric interactions between polar and apolar regions. The lack and/or the difficulty of extracting long-range NOEs from very crowded spectra prevented us from a detailed structural determination.

Recently, we determined the absolute configuration of cacospongionolides[4] by means of the modified Mosher's method.[8,9] Since the structure and the relative stereochemistry of **4a** are closely related to other cacospongionolides (**1a**−**3a**), we again used Mosher's method to determine the absolute stereochemistry, of this compound.

Cacospongionolide F (**4a**) was treated with an excess of (*R*)- and (*S*)-α-methoxy-α-(trifluoromethyl)phenylacetyl chloride (MTPA-Cl) to yield a pair of (*S*)- and (*R*)-MTPA esters (**4d** and **4e**), which were separated by silica gel chromatography. The S-configuration at C-25 of the MTPA esters **4d** and the R-configuration at C-25 of MTPA esters **4e**, respectively, were easily deduced from the $\Delta\delta$ values of their corresponding spectra. The stereostructure of cacospongionolide F is shown in **4a**: 8*S*, 9*S*, 10*R*, 16*R*.

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*,[10] *L. geometrica*,[11] and *Thorectandra excavatus*.[12]

Cacospongionolide F is highly active (LC_{50} = 0.17 ppm) in the *Artemia salina* bioassay,[13,14] moderately toxic (LC_{50} = 0.7 ppm) in a fish lethality assay[15] and highly active against the gram-positive bacteria, *Bacillus subtilis* (MIC = 0.78 μg/mL) and *Micrococcus luteus* (MIC = 0.78 μg/mL).

Experimental Section

General Experimental Procedures. General experimental procedures were as previously reported.[3]

Biological Material. *F. cavernosa* (order Dictyoceratida; family Thorectidae) was collected by dredging (−20 m) at Rovinj (Croatia) in September 1995 and frozen at −20 °C until extracted. A voucher specimen is maintained in the Arco Felice Institute collection (voucher no. S1CD/95).

Extraction and Isolation of Cacospongionolide F. Extraction of the sponge was carried out as described earlier.[3] The Et_2O extract (2.3 g) was applied to a column of Si gel. The column was eluted with a solvent gradient system from petroleum ether (40−70 °C) to Et_2O. From fractions eluted with petroleum ether−Et_2O (7:3) were recovered some pure cacospongionolide B and a mixture, that after reversed-phase HPLC purification (Spherisorb S5ODS2; acetonitrile; flow 3 mL/min) gave more cacospongionolide B, which crystallized from MeOH (220 mg, total amount), and cacospongionolide F as amorphous solid (100 mg).

Cacospongionolide B (2a): mp 116−118 °C; $[\alpha]_D$ +28.2° (c = 0.25, $CHCl_3$); UV; IR; MS; ^1H and ^{13}C NMR data are in agreement with published values.[3]

Cacospongionolide F (4a): amorphous solid; $[\alpha]_D$ −123.0° (c = 0.21, $CHCl_3$); UV λ_{max} (MeOH) 225 (ϵ 4600) nm; IR ν_{max} ($CHCl_3$) 3380 (br), 1785, 1764 cm^{-1}; NMR data, see Table 1; EIMS m/z (%) $[M]^+$ 400.2638 ($C_{25}H_{36}O_4$ requires 400.2635) (10), $[M - H_2O]^+$ 382 (11), 367 (5), 208 (20), 205 (9), 195 (21), 192 (80), 191 (95), 190 (25), 189 (100), 177 (10).

Acetylation of Cacospongionolide F (4a). A solution of cacospongionolide F (**4a**, 60 mg) in pyridine (3 mL) and acetic anhydride (0.5 mL) was kept at room temperature overnight. The excess reagents were removed in vacuo, and the residue was partitioned between H_2O and Et_2O. The ether extracts were dried over anhydrous sodium sulfate, and the solvent was evaporated to obtain a mixture of acetates **4b** and **4c** (57 mg). These were separated by a Si gel column using petroleum ether−Et_2O (4:1) as eluent, to obtain acetate **4b** (34 mg) and acetate **4c** (20 mg).

Acetate 4b: amorphous solid; UV λ_{max} (MeOH) 208 (ϵ 7800) nm; IR ν_{max} ($CHCl_3$) 1785, 1750 cm^{-1}; ^1H NMR ($CDCl_3$) δ 6.94 (1H, br s, H-25), 6.16 (1H, br s, H-18), 5.52 (1H, br d, J = 3.7 Hz, H-14), 5.31 (1H, br s, H-6), 4.33 (1H, ddd, J = 10.5, 4.2, 1.5 Hz, H-16), 4.17 (2H, ABq, J = 15.9, H-24), 2.17 (3H, s, $COCH_3$), 1.04 (3H, s, H-20), 1.01 (3H, s, H-21), 0.81 (3H, s, H-23), 0.77 (3H, d, J = 6.6 Hz, H-22); ^{13}C NMR ($CDCl_3$) δ 169.4 (s, $COCH_3$), 168.8 (s, C-19), 166.3 (s, C-17), 146.5 (brs, C-5), 138.6 (s, C-13), 117.9 (d, C-18), 115.7 (d, C-14), 114.4 (brd, C-6), 92.2 (d, C-25), 69.5 (d, C-16), 68.3 (t, C-24), 42.0 (brt, C-3), 40.7 (d, C-10), 36.7 (s, C-4), 35.8 (s, C-9), 32.8 (d, C-8), 31.0 (brt, C-1), 29.6 (t, C-15), 29.4 (q, C-21), 28.9 (brt, C-7), 26.8 (t, C-11 and C-12), 23.0 (t, C-2), 22.1 (q, C-20 and 23), 20.7 (q, $COCH_3$), 14.6 (q, C-22); EIMS m/z (%) $[M]^+$ 442 (8), $[M - HAc]^+$ 382 (14), 205 (18), 192 (100), 191 (90), 189 (80), 177 (20).

Acetate 4c: colorless oil; UV λ_{max} (MeOH) 206 (ϵ 7600) nm; IR ν_{max} ($CHCl_3$) 1785, 1750 cm^{-1}; ^1H NMR ($CDCl_3$) δ 7.01 (1H, brs), 6.09 (1H, brs), 5.54 (1H, brd, J = 3.8 Hz), 5.32 (1H, brs), 4.30 (1H, brdd, J = 8.9, 4.0 Hz), 4.11 (2H, ABq, J = 16.2), 2.17 (3H, s), 1.05 (3H, s), 1.01 (3H, s), 0.81 (3H, s), 0.77 (3H, d, J = 6.6 Hz); ^{13}C NMR ($CDCl_3$) δ 169.5 (s, $COCH_3$), 168.3 (s), 166.1 (s), 146.5 (brs), 138.3 (s), 118.5 (d), 115.5 (d), 114.0 (brd), 92.8 (d), 68.6 (d), 68.4 (t), 42.1 (brt), 40.8 (d), 36.6 (s), 35.9 (s), 32.6 (d), 31.1 (brt), 30.5 (t), 29.5 (q), 28.8 (brt), 26.5 (t), 23.0 (t), 22.1 (q), 20.7 (q, $COCH_3$), 14.7 (q); EIMS m/z (%) $[M]^+$ 442 (4), $[M - HAc]^+$ 382 (22), 205 (20), 192 (100), 191 (75), 189 (70), 177 (34).

Preparation of MTPA Esters. (*R*)- or (*S*)-MTPA chloride (Aldrich) (20 μL) was added to a solution of cacospongionolide F in dry pyridine (0.5 mL) and the resulting mixture was kept at room temperature for 2 h. After the removal of the solvent, under vacuum, the residue was chromatographed on a Si gel column and eluted with petroleum ether−ET_2O (3:2) to give the two C-25 diastereoisomeric Mosher esters, noting that the (*R*)-MTPA chloride gives (*S*)-MTPA ester and *vice versa*. Only chemical shifts of the hetereocyclic region are reported because all other protons are remote from the MTPA group, so that their $\Delta\delta$ values are zero.

(*R*)-MTPA Esters 4d and 4e. Cacospongionolide F (**4a**) (10 mg) was esterified according to the general procedure to give (*R*)-MTPA ester **4d** (5 mg) and (*R*)-MTPA ester **4e** (3 mg).

(*R*)-MTPA Ester 4d: ^1H NMR ($CDCl_3$) δ 7.10 (1H, br s, H-25), 6.15 (1H, br s, H-18), 5.55 (1H, br d, J = 3.8 Hz, H-14), 4.43 (1H, ddd, J = 10.5, 4.2, 1.5 Hz, H-16), 4.12 (2H, ABq, J = 15.7 Hz, H-24), 2.30 (2H, m, H-15).

(*R*)-MTPA Ester 4e: ^1H NMR ($CDCl_3$) δ 7.24 (1H, br s, H-25), 6.00 (1H, br s, H-18), 5.38 (1H, br d, J = 3.8 Hz, H-14), 3.95 (1H, br dd, J = 10.5, 4.2 Hz, H-16), 3.87 (2H, br s, H-24), 2.12 (2H, m, H-15).

(*S*)-MTPA Esters 4d and 4e. Cacospongionolide F (**4a**) (12 mg) was esterified according to the general procedure to give (*S*)-MTPA ester **4d** (7 mg) and (*S*)-MTPA ester **4e** (3 mg).

(*S*)-MTPA Ester 4d: ^1H NMR ($CDCl_3$) δ 7.10 (1H, br s, H-25), 6.15 (1H, br s, H-18), 5.38 (1H, br d, J = 3.8 Hz, H-14), 4.05 (1H, dd, J = 10.5, 4.2 Hz, H-16), 4.00 (2H, br s, H-24), 2.08 (2H, m, H-15).

(*S*)-MTPA Ester 4e: ^1H NMR ($CDCl_3$) δ 7.24 (1H, br s, H-25), 6.00 (1H, br s, H-18), 5.55 (1H, br d, J = 3.8 Hz, H-14), 4.30 (1H, br dd, J = 10.5, 4.2 Hz, H-16), 4.08 (2H, ABq, J = 15.7 Hz, H-24), 2.27 (2H, m, H-15).

Antimicrobial and Antifungal Activities. Gram-positive bacteria [*Bacillus subtilis* (DSM 347) and *Micrococcus luteus* (DSM 348), the gram-negative bacterium [*Escherichia coli* (DSM 1103)], and a fungus [*Candida albicans* (DSM 1665)] were used for the antimicrobial assays, as already described.[15] Cacospongionolide F (**4a**) showed activity only against gram-positive bacteria, with a minimum inhibitory concentration (MIC) of 0.78 μg/mL (*B. subtilis*) and 0.78 μg/mL (*M. luteus*).

Brine Shrimp Lethality. The brine shrimp (*Artemia salina)* lethality assay, performed as reported,[13,14] gave LC_{50} = 0.17 ppm (0.41/0.06, 95% confidence limits).

Fish Lethality. The fish (*Gambusia affinis*) lethality assay, performed as described earlier,[15] gave LC_{50} = 0.7 ppm (2.9/0.16, 95% confidence limits).

Molecular Mechanics Calculations. Models of cacospongionolide F and its derivatives were designed and refined with the SYBYL 6.3 package.[16] The Tripos force field was used for all the calculations, with a charge distribution obtained with the Gasteiger−Hückel method. The structures were obtained by simulated annealing with molecular dynamics. Each conformation underwent a 10 ps heating from 300 to 1000 K, followed by 50 ps at 1000 K and then a 50 ps cooling from 1000 to 200 K. Each structure was then energy minimized with a final gradient norm criterion of 10^{-3} kcal mol^{-1} $Å^{-1}$. For each set of molecules (different conformation of DhD ring, or different chirality at C-8) 100 conformers were obtained, and the analyses were performed on the 50 most stable structures, satisfying the desired structural requirements.

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