Journal of Natural Products
Vol. 58, No. 11, pp. 1776-1780, November 1995

CACOSPONGIONOLIDE B, A NEW SESTERTERPENE FROM THE SPONGE *FASCIOSPONGIA CAVERNOSA*

SALVATORE DE ROSA,* ANTONIO CRISPINO, ALFONSO DE GIULIO, CARMINE IODICE,

Istituto per la Chimica di Molecole di Interesse Biologico CNR, Via Toiano 6, 80072, Arco Felice, Napoli, Italy

ROBERTO PRONZATO,

Istituto di Zoologia, Università di Genova, Italy

and NEVENKA ZAVODNIK

Center for Marine Research, "R. Boskovic" Institute, Rovinj, Croatia

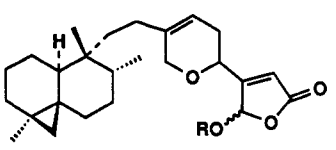
ABSTRACT.—Cacospongionolide B [**2a**], a new cacospongionolide-related sesterterpene, has been isolated from the Adriatic sponge *Fasciospongia cavernosa*. The structure was elucidated by spectral and chemical means. The antimicrobial activity and brine shrimp and fish lethalities of **2a** are reported.

In the course of our search for biologically active marine natural products, we reported previously a new sesterterpene, cacospongionolide [**1a**] (1,2), which showed strong cytotoxic activity. Cacospongionolide was isolated from a sponge originally identified as *Cacospongia mollior*, a horny sponge belonging to the family Thorectidae. In order to find related compounds, we have subsequently investigated the chemistry of other Mediterranean horny sponges belonging to the same family.

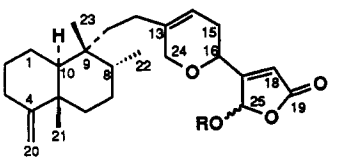
The taxonomic status of horny sponges is still under investigation, and is the subject of constant modification. In particular, the family Thorectidae, which includes the genera *Cacospongia, Fasciospongia, Luffariella,* and *Thorecta,* has been subjected to significant revision. Our group has investigated the chemistry of a number of specimens of *F. cavernosa* Schmidt collected in the northern Adriatic. Extracts of these sponges showed cytotoxic activity (LC_{50} value of 18 ppm) in the *Artemia salina* bioassay (3,4). Unfortunately, one specimen (voucher No. S6R/86), our original source of cacospongionolide [**1a**], was erroneously classified as *C. mollior* instead of *F. cavernosa*. The genera *Cacospongia* and *Fasciospongia* are closely related and have often been confused, so much so that the original description of *F. cavernosa* includes this species in the genus *Cacospongia*.

A continued investigation on recently collected specimens of *F. cavernosa* has yielded a new sesterterpene which we have named cacospongionolide B [**2a**]. Cacospongionolide B was responsible for the biological activity observed for the crude extract, and is closely related to the known **1a**. The isolation, structure determination, and biological activities of **2a** are reported in this paper.

Chromatography of the Et_2O-soluble fraction of the Me_2CO extract of *F. cavernosa* yielded cacospongionolide B (**2a**,



1a R=H
1b,**1c** R=Ac



2a R=H
2b,**2c** R=Ac

0.45% dry wt). A molecular formula of $C_{25}H_{36}O_4$ was suggested from the hrms of the parent ion. The uv absorption at 223 nm (ϵ 4500) and ir bands at 3380, 1785, and 1762 cm^{-1} were characteristic of a γ-hydroxybutenolide moiety. Examination of the ^{1}H- and ^{13}C-nmr spectra of this compound established that it was closely related to **1a** (1). As observed for **1a**, the nmr spectra of **2a** were highly solvent-dependent and interpretation of the signals of the polar moiety was difficult. In C_6D_6, two sets of resonances were observed for the polar region, while in $CDCl_3$, a single set of broad resonances was observed.

Treatment of **2a** with Ac_2O in pyridine at room temperature gave a mixture of two diastereoisomeric acetates [**2b**, **2c**] which were separated by Si gel cc. The ^{1}H- and ^{13}C-nmr spectra of both acetates showed a single set of sharp resonances for the polar moiety (C-12 to C-19 region) of cacospongionolide B. The chemical shifts were in excellent agreement with those of the corresponding resonances observed for cacospongionolide acetates, **1b** and **1c** (1), defining the structure of the C-12 to C-19 polar functionality as shown. The remaining resonances in the ^{1}H- and ^{13}C-nmr spectra of **2b** and **2c** were identical to those of **2a** and the spectra of **2a** were therefore used to define the remaining portions of the structure.

The ^{1}H-nmr spectrum of **2a** showed resonances due to one secondary and two tertiary methyl groups [δ 0.89 (3H, d, J=7.0 Hz), 0.92 (3H, s) and 1.09 (3H, s)] and an exocyclic methylene [δ 4.48 (1H, br s), 4.51 (1H, br s)]. ^{13}C-Nmr resonances observed at δ 102.04 (t) and 160.49 (s) confirmed the presence of a 1,1-disubstituted olefin.

Taking into account the molecular formula and the data discussed thus far, the non-polar region of **2a** must possess a carbobicyclic skeleton. A HMBC nmr experiment showed correlations between the exomethylene protons and carbon atoms at δ 32.87 (t), 40.35 (s), and 160.49 (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 0.92 and 1.09) and the surrounding carbon atoms allowed for their placement at C-9 and C-5, respectively (Table 1). The COSY-45 spectrum indicated that the methine proton observed at δ 1.57 (H-8) was coupled to the methyl doublet observed at δ 0.89 and with non-equivalent methylene protons that appeared at δ 1.60 and 1.35 (H-7). The latter two protons were in turn coupled to non-equivalent methylene protons observed at δ 1.98 and 1.80 (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 26.01 (C-12), 35.35 (C-8), and 37.94 (C-9) defined the connection between the polar moiety and the rigid part of molecule. These considerations led to structure **2a**, without stereochemical implications.

The relative stereochemistry of the rigid part of **2a** was deduced by a NOESY nmr spectrum, which exhibited the presence of nOes (Table 2) indicating that Me-5, Me-9, H-3ax and H-1ax are oriented on the same side (β) of the molecule, while H-10ax has the same orientation (α) as Me-8. The axial position of H-10 (J=12.0 and 2.3 Hz) and H-16 (J=10.5 and 4.2 Hz) was deduced from the magnitude of their coupling constants in the ^{1}H-nmr spectrum of **2a** and **2b**, respectively.

Cacospongionolide B [**2a**] is closely related structurally to **1a**, isolated previously from a sponge specimen identified as *C. mollior* (voucher No. S6R/86). This relationship led us to propose that the identification of specimen S6R/86 was incorrect. A comparative morphological analysis of the arrangement of spongine fibers in the skeleton of a recently col-

TABLE 1. Nmr Spectral Data of **2a** in $CDCl_3$ Solution.[a]

Position	^{13}C	^{1}H	HMBC (J_{C-H}=10 Hz)
1	21.24 t	1.57 m, 1.35 m	1.15 (H-10)
2	30.10 t	1.88 m, 1.20 m	—
3	32.87 t	2.30 ddd (13.6, 13.6, 5.1), 2.10 ddd (13.6, 2.8, 2.7)	4.51 (H-20a)
4	160.49 s	—	4.51–4.48 (H-20) 2.30 (H-3ax), 1.09 (H-21)
5	40.35 s	—	4.51–4.48 (H-20), 1.80 (H-6eq), 1.09 (H-21)
6	25.49 t	1.98 ddd (14.0, 13.8, 2.7), 1.80 m	1.09 (H-21)
7	28.62 t	1.60 m, 1.35 m	—
8	35.35 d	1.57 m	1.45–1.10 (H-11), 0.92 (H-23)
9	37.94 s	—	1.45–1.10 (H-11), 0.92 (H-23)
10	47.78 d	1.15 dd (12.0, 2.3)	1.09 (H-21), 0.92 (H-23)
11	37.78 t	1.45 m, 1.10 m	
12	26.01 t	1.83 m	1.45–1.10 (H-11)
13	138.11 s	—	4.18 (H-24), 1.83 (H-12)
14	115.90 d	5.53 br s	4.18 (H-24), 1.83 (H-12)
15	29.10[b] t	2.26[b] m	—
16	69.52[b] d	4.40[b]	4.18 (H-24)
17	168.04[b] s	—	—
18	117.51[b] d	6.04[b]	6.16 (H-25)
19	171.13 s	—	—
20	102.04 t	4.51 br s, 4.48 br s	—
21	21.24 q	1.09 s	—
22	14.75 q	0.89 d (7.0)	—
23	20.51 q	0.92 s	—
24	68.38 t	4.18 ABq (15.7)	—
25	97.98[b] d	6.16[b]	6.04 (H-18)

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

[b]Broad signal due to the presence of a mixture of epimers at C-25.

lected specimen (S1CD/94) and the preserved specimen (S6R/86) confirmed that they were both *F. cavernosa*. Moreover comparison of the structures of metabolites reported from *C. mollior* (1, 5–7), *Luffariella variabilis* (8), and *Luffariella* sp. (9) suggest a close similarity between the genera *Fasciospongia* and *Luffariella*, while the genus *Cacospongia* seems to be less closely related. In contrast, morphological observations (10) suggest that the genus *Luffariella* is closely related to the genus *Cacospongia*. A multidisciplinary approach which includes the study of sponge metabolism can be beneficial in reaching a correct taxonomic classification, as traditional taxonomy of sponges is based primarily on the study of skeletal morphology, in particular mineral spicules. Horny sponges which lack mineral spicules are classified based on the arrangement of spongine fibers in the skeleton and are inherently difficult to identify at the species level.

TABLE 2. Magnetization Exchange by Cross Relaxation (NOe) for **2a** in $CDCl_3$ as Observed from NOESY.[a]

Cross-peak Coordinates Below the Diagonal δ_x-δ_y	Protons Correlated
4.51–1.80	H-20a, H-6β
4.48–2.10	H-20b, H-3α
2.30–1.09	H-3β, Me-5
1.57–0.92	H-1β, Me-9
1.15–0.89	H-10α, Me-8

[a]The ^{1}H NOESY spectrum was recorded at 500 MHz. Only the cross-peaks not sensitive to strong filtering are reported.

Cacospongionolide B showed potent activity (LC_{50} value of 0.25 ppm) in the *Artemia salina* bioassay (3,4) a moderate toxicity (LC_{50} value of 1.05 ppm) in a fish lethality assay (11), and a high antibacterial activity against the Gram-positive bacteria, *Bacillus subtilis* (MIC value of

0.78 μg/ml) and *Micrococcus luteus* (MIC value of 0.78 μg/ml).

EXPERIMENTAL

GENERAL EXPERIMENTAL PROCEDURES.—Mps 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. Lrms and hrms were recorded on an AEI MS-50 spectrometer. ^{1}H- and ^{13}C-nmr spectra were recorded at 500 and 125 MHz, respectively, with TMS as internal standard, on a Bruker AM 500 instrument, under Aspect X32 control. 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. Microorganisms were obtained from DSM, Germany.

ANIMAL MATERIAL.—*Fasciospongia cavernosa* (order Dictyoceratida, family Thorectidae) was collected by dredging (at a depth of 20 meters) near Rovinj, Croatia, in November 1994, and frozen at −20° until extracted. The sponge is massive, and shows a dark-brown color. The external surface is hispid, owing to the presence of large conules. The consistency is firm and cartilagineous and the horny skeleton is conspicuous. The choanosome is fleshy and shows large and tortuous canals, a feature that reflects the specific name. A voucher specimen is maintained in the Arco Felice Institute collection (voucher No. S1CD/94).

EXTRACTION AND ISOLATION.—The frozen sponge (70 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 (3.2 g), which was applied to a column of Si gel. The column was eluted with a solvent gradient system from petroleum ether (40–70°) to Et_2O. Cacospongionolide [**2a**] (315 mg) was recovered from fractions eluted with petroleum ether-Et_2O (7:3), after crystallization from MeOH.

Cacospongionolide B [**2a**].—Mp 116–118° (MeOH); $[\alpha]_D$ +28.2° (c=2.8, $CHCl_3$); uv λ max (MeOH) 223 (ε 4500) nm; ir ν max ($CHCl_3$) 3380 (br), 1785, 1762 cm^{-1}; eims *m/z* [M]$^+$ 400.2633 ($C_{25}H_{36}O_4$ 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); nmr data, see Table 1.

ACETYLATION OF **2**.—A solution of **2a** (50 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 partitioned 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 **2b** and **2c** (47 mg), which were separated by Si gel cc, with petroleum ether-Et_2O (4:1) as eluent, to obtain acetates **2b** (28 mg) and **2c** (17 mg).

Acetate **2b**.—Mp 206–207° (MeOH); uv λ max (MeOH) 206 (ε 7500) nm; ir ν max ($CHCl_3$) 1785, 1750 cm^{-1}; eims *m/z* [M]$^+$ 442 (6), [M−HAc]$^+$ 382 (11), 205 (15), 192 (100), 191 (90), 189 (85), 177 (22); ^{1}H nmr ($CDCl_3$) δ 6.94 (1H, br s, H-25), 6.16 (1H, br s, H-18), 5.52 (1H, br d, J=3.8 Hz, H-14), 4.51 (1H, br s, H-20a), 4.48 (1H, br s, H-20b), 4.32 (1H, ddd, J=10.5, 4.2, and 1.5 Hz, H-16), 4.16 (2H, ABq, J=15.7 Hz, H-24), 2.16 (3H, s, $COCH_3$), 1.09 (3H, s, H_3-21), 0.92 (3H, s, H_3-23), 0.90 (3H, d, J=7.1 Hz, H-22); ^{13}C nmr ($CDCl_3$) δ 169.3 (s, $COCH_3$), 166.4 (s, C-19), 160.6 (s, C-4), 159.5 (s, C-17), 138.9 (s, C-13), 118.1 (d, C-18), 115.7 (d, C-14), 102.1 (t, C-20), 92.5 (d, C-25), 69.6 (d, C-16), 68.5 (t, C-24), 47.8 (d, C-10), 40.4 (s, C-5), 38.1 (s, C-9), 37.9 (t, C-11), 35.5 (d, C-8), 32.9 (t, C-3), 30.2 (t, C-2), 29.8 (t, C-15), 28.6 (t, C-7), 26.1 (t, C-12), 25.6 (t, C-6), 21.3 (t, C-1), 21.3 (q, C-21), 20.7 (q, $COCH_3$), 20.5 (q, C-23), 14.9 (q, C-22).

Acetate **2c**.—Oil; uv λ max (MeOH) 206 (ε 7500) nm; ir ν max ($CHCl_3$) 1785, 1750 cm^{-1}; eims *m/z* [M]$^+$ 442 (4), [M−HAc]$^+$ 382 (19), 205 (18), 192 (100), 191 (78), 189 (78), 177 (31); ^{1}H nmr ($CDCl_3$) δ 7.04 (1H, br s), 6.09 (1H, br s), 5.54 (1H, br d, J=3.8 Hz), 4.52 (1H, br s), 4.48 (1H, br s), 4.30 (1H, br dd, J=10.5 and 4.2 Hz), 4.10 (2H, ABq, J=15.7 Hz), 2.17 (3H, s), 1.10 (3H, s), 0.92 (3H, s), 0.90 (3H, d, J=7.1 Hz); ^{13}C nmr ($CDCl_3$) δ 169.5 (s, $COCH_3$), 166.1 (s), 160.5 (s), 159.4 (s), 138.6 (s), 118.5 (d), 115.5 (d), 102.2 (t), 92.8 (d), 68.6 (d), 68.4 (t), 47.8 (d), 40.4 (s), 38.1 (s), 37.9 (t), 35.5 (d), 32.9 (t), 30.2 (t), 30.5 (t), 28.6 (t), 26.1 (t), 25.6 (t), 21.3 (t), 21.3 (q), 20.7 (q, $COCH_3$), 20.4 (q), 14.8 (q).

BIOLOGICAL EVALUATIONS.—a. *Antimicrobial and antifungal activities*.—Gram-positive bacteria [*Bacillus subtilis* (DSM 347) and *Micrococcus luteus* (DSM 348)], a Gram-negative bacterium [*Escherichia coli* (DSM 1103)], and a fungus [*Candida albicans* (DSM 1665)] were used for the antimicrobial assays, as described already (11). Cacospongionolide B [**2a**] showed activity only against the Gram-positive bacteria, with minimum inhibitory concentration (MIC) values of 0.78 μg/ml (*B. subtilis*) and 0.78 μg/ml (*M. luteus*).

b. *Brine shrimp lethality*.—The brine shrimp (*Artemia salina*) lethality assay performed as already described (3,4), gave an LC_{50} value of 0.25 ppm (0.53/0.12, 95% confidence limits).

c. *Fish lethality.*—A toxicity test with mosquito fish (*Gambusia affinis*) was performed as described previously (11). Compound **2a** gave an LC_{50} value of 1.05 ppm.

ACKNOWLEDGMENTS

This research was supported by the Progetto Strategico Tecnologie Chimiche Innovative, CNR., Rome, and by Self-Management Community of Interest for Scientific Research of Croatia. Mass spectra and nmr spectra were provided by the Servizio di Spettrometria di Massa del CNR-Napoli and the Servizio NMR, ICMIB-CNR, respectively. The assistance of the staff of both services is gratefully acknowledged.

LITERATURE CITED

1. S. De Rosa, S. De Stefano, and N. Zavodnik, *J. Org. Chem.*, **53**, 5020 (1988).
2. R. Puliti, S. De Rosa, C.A. Mattia, and L. Mazzarella, *Acta Cryst.*, **C46**, 1533 (1990).
3. B.N. Meyer, N.R. Ferrigni, J.E. Putnam, L.B. Jacobsen, D.E. Nichols, and J.L. McLaughlin, *Planta Med.*, **45**, 31 (1982).
4. A. De Giulio, S. De Rosa, G. Di Vincenzo, and G. Strazzullo, *Tetrahedron*, **46**, 7971 (1990).
5. G. Cimino, S. De Stefano, and L. Minale, *Experientia*, **30**, 846 (1974).
6. F. Cafieri, L. De Napoli, A. Iengo, and C. Santacroce, *Experientia*, **35**, 157 (1979).
7. S. De Rosa, R. Puliti, A. Crispino, A. De Giulio, C.A. Mattia, and L. Mazzarella, *J. Nat. Prod.*, **57**, 256 (1994).
8. E.D. de Silva and P.J. Scheuer, *Tetrahedron Lett.*, **22**, 3147 (1981).
9. J. Kobayashi, C. Zeng, M. Ishibashi, and T. Sasaki, *J. Nat. Prod.*, **56**, 436 (1993).
10. P.R. Bergquist, *N.Z.J. Zool.*, **7**, 443 (1980).
11. S. De Rosa, A. De Giulio, and C. Iodice, *J. Nat. Prod.*, **57**, 1711 (1994).

Received 25 April 1995