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25-Deoxycacospongionolide B and Cacospongionolide C, Two New Terpenoids from the Sponge *Fasciospongia Cavernosa*

Salvatore De Rosa[a]*, Raffaella Puliti[a], Antonio Crispino[a], Alfonso De Giulio[a], Cira De Sena[a], Carmine Iodice[a] and Carlo A. Mattia[b]

[a] Istituto per la Chimica di Molecole di Interesse Biologico CNR, Via Toiano, 6 - 80072 Arco Felice (Napoli) Italy
[b] Dipartimento di Chimica, Università "Federico II", Via Mezzocannone, 4 - 80134 Napoli Italy

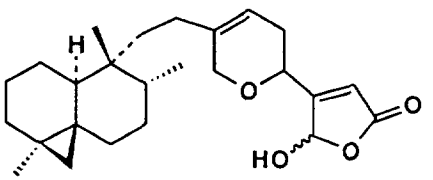
Abstract: 25-Deoxycacospongionolide B (**3**), a new bioactive sesterterpene, and a diterpene, cacospongionolide C (**4**), have been isolated as minor components from the Adriatic sponge *Fasciospongia cavernosa*. The structures were proposed on the basis of spectroscopic data and defined for the former on the basis of X-ray analysis. Brine shrimp and fish lethalities of 25-deoxycacospongionolide B are reported.

In the course of our search for marine natural compounds that have biological activities, we isolated and characterized a sesterterpenoid, cacospongionolide (**1**)[1,2], with high cytotoxic activity, from the sponge *Fasciospongia cavernosa* (erroneously classified as *Cacospongia mollior*)[3] a horny sponge belonging to the family Thorectidae. Subsequently, we made an extensive harvesting of other Mediterranean horny sponges belonging to the same family in order to find compounds related to cacospongionolide.

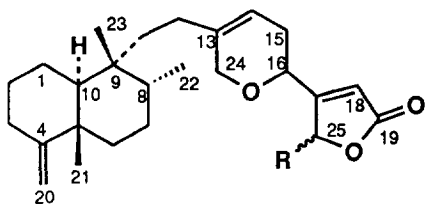
We recently isolated cacospongionolide B (**2**)[3] from some specimens of *F. cavernosa* Schmidt (order Dictyoceratida; family Thorectidae) collected in the North Adriatic. This sesterterpene exhibited cytotoxic and antimicrobial activities and the structure was established by spectral and chemical means[3]. We continued investigation on extracts of this sponge and purification of fraction containing sterols by HPLC gave a new sesterterpene, 25-deoxycacospongionolide B (**3**, 0.02% dry weight), and a diterpenoid, cacospongionolide C (**4**, 0.006%). The isolation, the structure elucidation, including X-ray analysis and some biological activities of **3**, and the structure of **4** are reported in this paper.

Cacospongionolide C (**4**) had $[\alpha]_D$ -18° and a molecular formula $C_{20}H_{36}O_3$ from HRMS of the parent ion. The UV absorption at 220 nm, IR bands at 3380, 1785, 1760 cm^{-1}, ^{1}H-NMR signals at δ 5.99 (brs, 1H) and 5.86 (brs, 1H) and ^{13}C-NMR signals at δ 170.7 (s), 169.3 (s), 117.6 (d) and 99.6 (d) define the γ-hydroxybutenolide moiety, which has previously been encountered in other metabolites isolated from this sponge[1,3]. The analysis of the spectral data established the presence of a saturated acyclic chain from C-4 to C-19, with three additional secondary methyl groups. The presence of fragment ions at *m/z* 263, 221, 193, 151 and 123 in the mass spectrum of **4** located the methyl groups on C-7, C-11 and C-15. No attempt was made to determine the stereochemistry of the methyl-bearing carbons at C-7 and C-11.

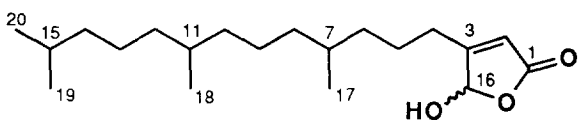
25-Deoxycacospongionolide B (**3**) crystallized from MeOH, mp 159-161°C, had $[\alpha]_D$ 10.5° and a molecular formula $C_{25}H_{36}O_3$ from HRMS of the parent ion. Examination of the spectral data of **3** established that it was closely related to the sesterterpenoid cacospongionolide B (**2**) that was recentely reported[3]. The chemical shifts of the C-1 to C16 region in the ^{1}H- and ^{13}C-NMR spectra of **3** were in excellent agreement with those of the corresponding resonances in the spectra of cacospongionolide B (**2**)[3]. The absence of the characteristic infrared band of hydroxyl group (ν_{max} 3380 cm^{-1}) and the presence of bands at 1745, 1650 cm^{-1} in the IR spectrum of **3** suggested the presence of a butenolide moiety in 25-deoxycacospongionolide B, instead of a γ-hydroxybutenolide moiety showed in **2**. The presence of a methylene [δ 4.87 (ABq, J= 17.9 Hz, H-25); 71.0 (t, C-25)], a methine [δ 5.95 (brs, H-18); 114.7 (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.



1



2 R = OH
3 R = H



4

Careful recrystallization of **3** from MeOH provided crystalline samples suitable for a conclusive X-ray study which determines without ambiguities the structure of 25-deoxycacospongionolide B.

The crystal structure of **3** was solved using SIR92 package[4] and refined to a discrepancy index R of 0.038. The positional parameters and equivalent isotropic temperature factors for non-hydrogen atoms are given in Table 1. A perspective view of the final X-ray model, together with the atomic labelling, is shown in Fig. 1. In the absence of atoms with strong anomalous scattering, the absolute stereochemisty was not determined and the configuration shown was chosen in accordance with that of the cacospongionolide (**1**)[2], on this basis the relative configurations at the chiral centres are C5*S**, C8*R**, C9*R**, C10*S**.

The molecule is characterized by a *trans* fused bicyclic carbon system which is linked to a dihydro pyranose ring through an equatorial ethylene bridge. The pyranose ring is further connected to an α, β-unsaturated-γ-lactone. Compound **3** is structurally similar to cacospongionolide (**1**), but in **3** there is an *extra*-ring CH_2 group at the unsaturated C4 carbon as well as a methyl substituent in the axial β orientation at C5, instead of the cyclopropyl-methylene group joining 4,5 positions of cacospongionolide; in **3** the hydroxyl group at C25 position of the lactone ring is also absent. In both **1** and **3** the *trans* fused carbon-system presents two axial methyl substitutions at C8 (α-oriented) and C9 (β-oriented).

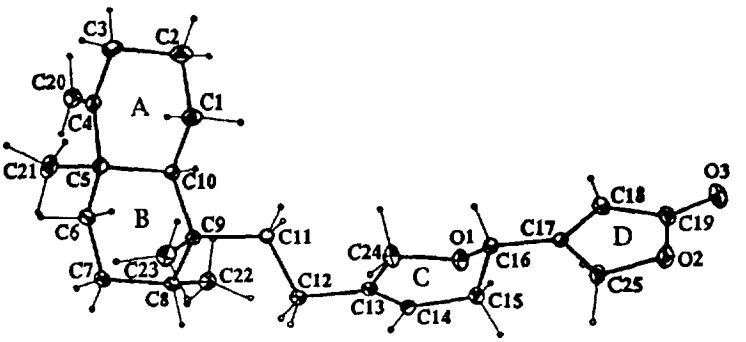


Figure 1. A perspective view of 25-deoxycacospongionolide B (3): labels for H-atoms were omitted for clarity.

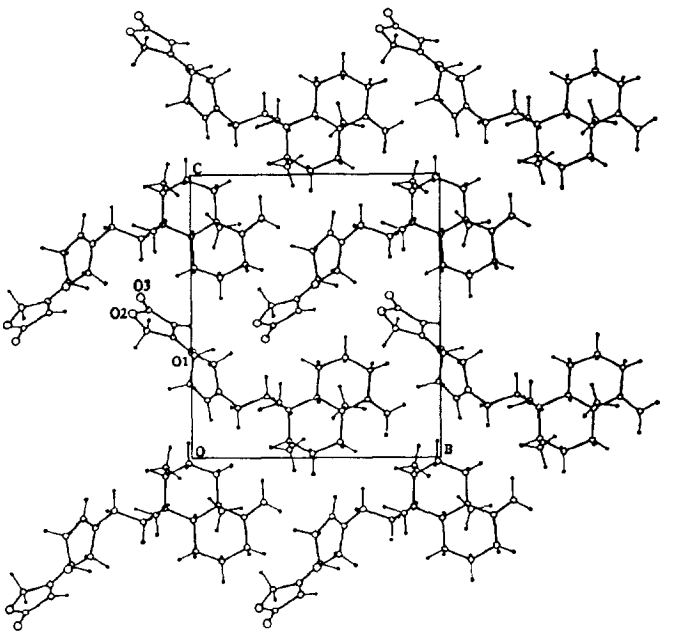


Figure 2. The crystal packing of compound 3 projected onto the *bc* plane: only oxygen atoms are labelled.

A selection of intramolecular geometry is given in Table 2. The trend of bond lengths and bond angles agrees well with the values observed in similar molecules[2,5,6]. In the bicyclic carbon-system, few significant deviations of the geometrical parameters from normal values are probably caused by the atomic crowding. In particular the lengthening of the bond distances along the atomic sequence C21,C5,C10,C9,C23, and the widening of the angles C21-C5-C10, C5-C10-C9, and C10-C9-C23 help to release the short 1,5 interaction

Table 1 - Compound **3**: Fractional coordinates and equivalent isotropic thermal parameters (Å^2) with e.s.d.'s in parentheses for non-H atoms: $Beq = \frac{1}{3} \Sigma_i \Sigma_j b_{ij} a_i^* a_i \, a_j^* a_j$

atom	*x*	*y*	*z*	Beq	atom	*x*	*y*	*z*	Beq
O1	0.5274(3)	0.0000	0.3823(1)	4.68(4)	C12	0.2955(5)	0.1855(2)	0.1783(2)	4.59(5)
O2	0.9394(3)	-0.2354(2)	0.5185(2)	5.80(5)	C13	0.4489(4)	0.1085(2)	0.2365(2)	3.89(4)
O3	1.2789(4)	-0.2023(3)	0.5748(2)	7.47(6)	C14	0.6126(5)	0.0625(2)	0.2048(2)	4.63(6)
C1	0.2365(5)	0.5114(3)	0.3214(2)	5.39(6)	C15	0.7705(5)	-0.0092(3)	0.2660(2)	4.80(6)
C2	0.3472(6)	0.6167(3)	0.3588(2)	6.42(8)	C16	0.7451(4)	0.0095(2)	0.3704(2)	3.60(4)
C3	0.2366(6)	0.7173(3)	0.3108(2)	6.09(7)	C17	0.8645(4)	-0.0734(2)	0.4349(2)	3.65(4)
C4	0.2101(4)	0.7076(2)	0.2041(2)	4.36(5)	C18	1.0644(4)	-0.0693(3)	0.4774(2)	4.52(5)
C5	0.0960(4)	0.6042(2)	0.1634(2)	3.98(5)	C19	1.1159(4)	-0.1709(3)	0.5291(2)	4.98(6)
C6	0.0980(5)	0.5950(2)	0.0555(2)	4.79(5)	C20	0.2845(5)	0.7857(3)	0.1524(2)	5.97(7)
C7	0.0168(5)	0.4844(2)	0.0152(2)	4.92(6)	C21	-0.1392(4)	0.6170(3)	0.1822(3)	6.88(9)
C8	0.1508(4)	0.3900(2)	0.0613(2)	3.77(5)	C22	0.3713(5)	0.3916(3)	0.0289(2)	5.05(6)
C9	0.1522(3)	0.3881(2)	0.1708(2)	3.23(4)	C23	-0.0692(4)	0.3524(3)	0.1937(2)	5.20(6)
C10	0.2201(3)	0.5033(2)	0.2128(2)	3.31(4)	C24	0.4058(4)	0.0882(3)	0.3364(2)	5.25(6)
C11	0.3162(4)	0.3029(2)	0.2175(2)	3.77(4)	C25	0.7714(5)	-0.1796(2)	0.4587(2)	5.08(6)

Table 2 - Compound **3**: Molecular geometry with e.s.d.'s in parentheses

bond lengths (Å)							
O1 - C16	1.416(3)	C3 - C4	1.502(4)	C8 - C9	1.551(3)	C13 - C24	1.498(4)
O1 - C24	1.427(3)	C4 - C5	1.526(4)	C8 - C22	1.528(4)	C14 - C15	1.508(4)
O2 - C19	1.358(4)	C4 - C20	1.327(4)	C9 - C10	1.562(3)	C15 - C16	1.526(3)
O2 - C25	1.436(3)	C5 - C6	1.533(4)	C9 - C11	1.551(3)	C16 - C17	1.496(3)
O3 - C19	1.202(3)	C5 - C10	1.571(3)	C9 - C23	1.544(4)	C17 - C18	1.325(3)
C1 - C2	1.523(5)	C5 - C21	1.556(4)	C11- C12	1.534(4)	C17 - C25	1.481(4)
C1 - C10	1.530(3)	C6 - C7	1.526(4)	C12- C13	1.511(4)	C18 - C19	1.453(4)
C2 - C3	1.525(5)	C7 - C8	1.522(4)	C13- C14	1.311(4)		
bond angles (°)							
C16-O1-C24	110.9(2)	C6 -C5 -C21	108.6(2)	C11-C9 -C23	107.1(2)	O1 -C16-C17	106.8(2)
C19-O2-C25	108.7(2)	C10-C5 -C21	115.7(2)	C1 -C10-C5	110.9(2)	C15-C16-C17	112.3(2)
C2 -C1 -C10	111.6(2)	C5 -C6 -C7	112.9(2)	C1 -C10-C9	114.7(2)	C16-C17-C18	128.4(2)
C1 -C2 -C3	111.3(3)	C6 -C7 -C8	111.6(2)	C5 -C10-C9	116.1(2)	C16-C17-C25	123.2(2)
C2 -C3 -C4	111.4(3)	C7 -C8 -C9	111.5(2)	C9 -C11-C12	116.9(2)	C18-C17-C25	108.3(2)
C3 -C4 -C5	115.3(2)	C7 -C8 -C22	109.9(2)	C11-C12-C13	111.5(2)	C17-C18-C19	109.3(3)
C3 -C4 -C20	120.0(3)	C9 -C8 -C22	114.9(2)	C12-C13-C14	123.9(2)	O2 -C19-O3	120.4(3)
C5 -C4 -C20	124.8(3)	C8 -C9 -C10	109.3(2)	C12-C13-C24	115.5(2)	O2 -C19-C18	108.4(2)
C4 -C5 -C6	111.6(2)	C8 -C9 -C11	110.3(2)	C14-C13-C24	120.6(2)	O3 -C19-C18	131.2(3)
C4 -C5 -C10	107.3(2)	C8 -C9 -C23	109.5(2)	C13-C14-C15	122.9(2)	O1 -C24-C13	113.5(2)
C4 -C5 -C21	105.4(2)	C10-C9 -C11	107.9(2)	C14-C15-C16	108.7(2)	O2 -C25-C17	105.3(2)
C6 -C5 -C10	108.3(2)	C10-C9 -C23	112.6(2)	O1 -C16-C15	109.8(2)		
selected torsion angles (°)							
C4 -C5 -C10-C9	-171.0(2)	C23-C9 -C10-C5	-71.2(2)	O1 -C16-C17-C18	-150.6(2)		
C21-C5 -C10-C9	71.7(3)	C10-C9 -C11-C12	-173.2(2)	C16-C17-C18-C19	-176.9(2)		
C22-C8 -C9 -C10	72.9(3)	C9 -C11-C12-C13	-173.9(2)	C17-C18-C19-O3	177.9(3)		
C11-C9 -C10-C5	170.7(2)	C11-C12-C13-C14	-111.8(3)				

C21···C23 = 3.259(5) Å. The geometry at C4 (*see* Table 2) is significantly distorted with respect to normal C(sp^2) values. Such variations are rather common in similar cyclic systems[7,8] and seem to contribute in preserving the chair conformation of the ring *A*, in spite of the presence of the C4-C20 double bond.

Rings *A* and *B* are in almost ideal chair conformations which deviate towards half-boat forms. Atomic displacements from the respective best planes through the remaining endocyclic atoms are: C3 = 0.629(4), C10 = 0.694(2) Å (ring *A*) and C7 = 0.681(3), C10 = 0.607(2) Å (ring *B*). The pyranose ring (*C*), which blats a double bond in the 13-14 positions, adopts a distorted half-chair conformation with O1 and C16 atoms displaced 0.320(1) and 0.443(2) Å, respectively and on the apposite side of the average plane through the remaining four atoms. For this ring the distortion is essentially toward a twist form, as shown by the puckering parameters[9]: q_2 = 0.397(2) Å, ϕ_2 = 24.1(4)° and θ = 51.7(3)°. γ-Lactone system is planar to within 0.013(3) Å. Conformational details for each ring are given in Table 3 (*deposited*).

In the absence of hydrogen donors the crystal packing (Fig. 2) is governed only by van der Waals interactions, shortest intermolecular contacts being O1···$C18_{(x-1, y, z)}$ = 3.494(3) and C3···$C19_{(x-1, y+1, z)}$ = 3.557(4) Å.

25-Deoxycacospongionolide B showed a high cytotoxicity (LC_{50} = 0.74 μg/ml) comparable to that of **2**[3], in the *Artemia salina* bioassay[10,11] while it was less toxic (no toxicity at 100 μg/ml) than **2**[3], in the fish lethality[12].

EXPERIMENTAL[13]

Isolation: from fraction containing sterols after HPLC purification (Spherisorb S5W; n-exane/ethylacetate, 4:1; flow 3 ml/min) were recovered 25-deoxycacospongionolide B that crystallized from MeOH (14 mg, 0.02% dry weight) and cacospongionolide C (4 mg, 0.006%).

25-Deoxycacospongionolide B (**3**): white crystals, mp 159-161°C; $[\alpha]_D$=10.5° (c= 1.40, $CHCl_3$); IR ν_{max}($CHCl_3$) 1745, 1650, 1110 cm^{-1}; EIMS (70 eV) *m/z* (%) $[M]^+$ 384.2661 ($C_{25}H_{36}O_3$ requires 384.2664) (41), 369 (18), 205 (31), 193 (52), 191 (100), 189 (90), 177 (85); ^{1}H-NMR[14] (500 MHz, in $CDCl_3$) δ 5.95 (1H, brs, H-18), 5.54 (1H, brs, H-14), 4.87 (2H, ABq, J= 17.9 Hz, H-25), 4.51 (1H, brs, H-20a), 4.48 (1H, brs, H-20b), 4.33 (1H, ddd, J= 10.5, 4.2, 1.5 Hz, H-16), 4.16 (2H, ABq, J= 15.7, H-24), 2.30 (1H, ddd, J= 13.6, 13.6, 5.1 Hz, H-3β), 1.15 (1H, dd, J= 12.0, 2.3 Hz, H-10), 1.09 (3H, s, H-21) 0.92 (3H, s, H-23), 0.89 (3H, d, J= 6.9 Hz, H-22); ^{13}C-NMR[14] (125 MHz, in $CDCl_3$) δ 173.5 (s, C-19), 169.8 (s, C-17), 160.4 (s, C-4), 138.4 (s, C-13), 115.7 (d, C-14), 114.7 (d, C-18), 102.1 (t, C-20), 71.0 (t, C-25), 70.3 (d, C-16), 68.3 (t, C-24), 47.8 (d, C-10), 40.4 (s, C-5), 38.0 (s, C-9), 37.8 (t, C-11), 35.4 (d, C-8), 32.9 (t, C-3), 30.1 (t, C-2), 29.9 (t, C-15), 28.7 (t, C-7), 26.0 (t, C-12), 25.5 (t, C-6), 21.3 (t, C-1), 21.3 (q, C-21), 20.5 (q, C-23), 14.8 (q, C-22).

Cacospongionolide C (**4**): colorless oil; $[\alpha]_D$=-18.0° (c= 0.32, $CHCl_3$); IR ν_{max}($CHCl_3$) 3380 (br), 1785, 1760 cm^{-1}; EIMS (70 eV) *m/z* (%) $[M]^+$ 324.2667 ($C_{20}H_{36}O_3$ requires 324.2664) (32), 306 (30), 291 (9), 263 (6), 221 (23), 193 (40), 179 (54), 151 (95), 123 (100); ^{1}H-NMR (500 MHz, in $CDCl_3$) δ 5.99 (1H, brs), 5.86 (1H, brs), 2.40 (2H, brt, J= 6.5 Hz), 0.87 (3H, d, J= 6.5 Hz), 0.86 (6H, d, J= 6.6 Hz), 0.84 (3H, d, J= 6.5 Hz); ^{13}C-NMR (125 MHz, in $CDCl_3$) δ 170.8 (s), 169.3 (s), 117.6 (d), 98.6 (d), 39.4 (t), 37.4 (t), 37.3 (t), 36.6 (t), 32.8 (d), 32.6 (d), 28.0 (d), 27.9 (t), 24.8 (t), 24.4 (t), 24.2 (t), 22.7 (q), 22.6 (q), 19.7 (q), 19.6 (q).

Crystal data and structure determination of **3**: Single crystals of 25-deoxycacospongionolide B were obtained by slow concentration of a methanol solution and a sample of size 0.4x0.4x0.1 mm, was selected for the crystallographic study. Accurate cell parameters were obtained by least-squares refinement of the setting angles of 25 reflections at medium θ (22°< θ < 26°), using Ni-filtered CuKα radiation and Enraf-Nonius CAD-4F diffractometer on line with a MicroVAX Digital computer.

Crystal data: $C_{25}H_{36}O_3$, Mw = 384.56: monoclinic system, space group P21, with Z = 2: a = 6.3294(8), b = 12.197(1), c = 14.162(3) Å, β = 97.95(2)°, V = 1082.8(6) Å^3, D_C = 1.179 g/cm^3. 2303 Independent reflections (θmax = 74°) were collected at room temperature, using ω-2θ scan mode, as suggested by peak-shape analysis. During the data collection, the intensities of three standard reflections were monitored every 4h (4% variation) in order to check the crystal and equipment stability. No significant intensity decay was observed. The intensities were corrected for Lorentz and polarization factors, but not for the absorption effect (μ = 5.55 cm^{-1}). The structure was solved by direct metods using SIR92 package[4]. The positional and anisotropic temperature parameters for the 28 non-hydrogen atoms were refined by full-matrix (on F) least-squares method, *y* coordinate of O1 atom was fixed to define the origin. At convergence, all the hydrogen atoms were unambiguously located on a difference Fourier map and they were included in the last refinement at the calculated positions (C-H distance = 1.02 Å), as fixed atoms with isotropic thermal parameters set equal to Beq of the parent atom. At convergence, the final discrepancy index $R = \Sigma ||Fo| - |Fc|| / \Sigma |Fo|$ was 0.038 for the 2039 observed reflection with $I \geq 2.5\ \sigma(I)$, Rw = 0.045 with $w^{-1} = [\sigma^2(Fo)+(0.02Fo)^2+0.2]$; $S = 0.962$, $(\Delta/\sigma)_{max} < 0.01$; number of refined parameters = 253 including the extinction coefficient[15] = 5.2(4)·10^{-6}. No residual electron density is outside the range -0.11÷0.13 e·Å^{-3}. Atomic scattering factors were taken from Cromer and Waber[16]. For the crystallographic data the equipment of the C.I.M.C.F. of the University "Federico II" of Naples was used. All calculations were performed using Enraf-Nonius *SDP* software[17] on a MicroVAX 3100 computer.

Structure factors, anisotropic thermal parameters, hydrogen atom parameters, and complete molecular geometry, together with conformational details for each ring (Table 3), have been deposited.

Biological evaluations.- As per reference[12].

a.-*Brine shrimp lethality*.- The brine shrimp (*Artemia salina)* lethality assay performed as already described[10,11], gave LC_{50} 0.74 μg/ml (1.26/0.44 μg/ml, 95% confidence limits)

b.-*Fish lethality*.- The fish (*Gambusia affinis*) lethality assay performed as already described[12], gave no toxicity at 100 μg/ml.

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REFERENCES AND NOTES

1. De Rosa, S.; De Stefano, S.; Zavodnik, N. *J. Org. Chem.* **1988**, 53, 5020-5023.
2. Puliti, R.; De Rosa, S.; Mattia, C.A.; Mazzarella, L. *Acta Cryst.* **1990**, C46, 1533-1536.
3. De Rosa, S.; Crispino, A.; De Giulio, A.; Iodice, C.; Pronzato, R.; Zavodnik, N. *J. Nat. Prod.* in press.
4. Altomare, A.; Cascarano, G., Giacovazzo, C.; Guagliardi, A. *J. Appl. Cryst.* **1993**, 26, 343-350.
5. De Rosa, S.; Puliti, R.; Crispino, A.; De Giulio, A.; Mattia, C.A.; Mazzarella, L. *J. Nat. Prod.* **1994**, 57, 256-262.
6. Puliti, R.; Mattia, C.A.; Mazzarella, L. *Acta Cryst.* in press.
7. Kushlan, D.M.; Faulkner, D.J.; Parkanyi, L.; Clardy, J. *Tetrahedron*, **1989**, 45, 3307-3312.
8. Soriano-Garcia, M.; Guerrero, C.; Toscano, R.A.; Villena Iribe, R. *Acta Cryst.* **1990**, C46, 2478-2480.
9. Cremer, D.; Pople, J.A. *J. Amer. Chem. Soc.* **1975**, 97, 1354-1358.
10. Meyer, B.N.; Ferrigni, N.R.; Putnam, J.E.; Jacobsen, L.B.; Nichols, D.E; McLaughlin, J.L. *Planta Med.*, **1982**, 45, 31-34.
11. De Giulio, A.; De Rosa, S.; Di Vincenzo, G.; Strazzullo, G. *Tetrahedron*, **1990**, 46, 7971-7976.
12. De Rosa, S.; De Giulio, A.; Iodice, C. *J. Nat. Prod.*, **1994**, 57, 1711-1716.
13. General experimental details, biological material and extraction of the sponge are the same as those described in the previous report[3].
14. The data are based on COSY, HMQC and HMBC experiments.
15. Stout, G.H.; Jensen, L.H. *X-ray Structure Determination;* Macmillan: New York, 1968.
16. Cromer, D.T.; Waber, J.T. *International Tables for X-ray Crystallography*; Vol. IV. Kynoch Press: Birmingham, (Present distributor Kluwer Academic Publishers: Dordrecht), 1974.
17. B.A. Frenz and Associates, Inc. *SDP Structure Determination Package*; College Station, Texas, USA, and Enraf-Nonius, Delft, The Netherlands, 1985.

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