Two New Diterpenoids, Sarcophytins B and C, from the Indian Ocean Soft Coral Sarcophyton Species

ARTICLE *in* JOURNAL OF NATURAL PRODUCTS · FEBRUARY 2000

Impact Factor: 3.8 · DOI: 10.1021/np990179v · Source: PubMed

9 AUTHORS, INCLUDING:

Andrey S. Dmitrenok
N. D. Zelinsky Institute of Organic Chemistry
49 PUBLICATIONS **503** CITATIONS

SEE PROFILE

Radhika Parvataneni
Andhra University
34 PUBLICATIONS **333** CITATIONS

SEE PROFILE

Volodymyr V Nesterov
New Mexico Highlands University
318 PUBLICATIONS **1,367** CITATIONS

SEE PROFILE

Notes

Two New Diterpenoids, Sarcophytins B and C, from the Indian Ocean Soft Coral *Sarcophyton* Species

Vallurupalli Anjaneyulu,[†] Tatyana N. Makarieva,[‡] Sergey G. Ilyin,[‡] Andrei S. Dmitrenok,[‡] Parvataneni Radhika,[†] Potluru V. Subbarao,[†] Vladimir V. Nesterov,[§] Michael Yu. Antipin,[§] and Valentin A. Stonik*,[‡]

Andra University, Visakhapatnam-53003, India, Pacific Institute of Bioorganic Chemistry of the Far East Branch of the Russian Academy of Science, Vladivostok-22, Russian Federation, and A.N. Nesmeyanov Institute of Organoelement Compounds of the Russian Academy of Science, 117813, Moscow, Russian Federation

Received April 16, 1999

Two new diterpenoids, sarcophytins B and C (**1**, **2**), and the previously known sarcophytin (**4**) have been isolated from the Indian Ocean soft coral *Sarcophyton* sp. Structures of **1** and **2** were established by spectral data and supported by X-ray analysis of **1**.

Soft corals (phylum Cnidaria, class Anthozoa, subclass Octocorallia, order Alcyonaceae) have proven to be an especially valuable source of structurally diverse diterpenoids.[1] Some of them, such as the PAF antagonist chatancin (**3**),[2] and many cembranoids[1,3,4] isolated from soft corals belong to the genus *Sarcophyton*. In the course of our continuing studies on marine natural products,[5] we have studied extracts of a *Sarcophyton* sp. collected near Nicobar Islands. We report herein the isolation and structure elucidation of two new diterpenoids, sarcophytins B (**1**) and C (**2**), and identification of the known sarcophytin (**4**) from this species.

Specimens of the soft coral were hand-picked from the intertidal zone in rocky areas of Kalipur Island coast line, cut into thin slices, and preserved in ethanol. The ethanol-soluble materials were concentrated in vacuo, and the dark residue was re-extracted with ethyl acetate. The ethyl acetate-soluble materials were subjected to column chromatography on Si gel followed by HPLC on an Ultrasphere-Si column to obtain the individual compounds **1**, **2**, and **4**.

Sarcophytin B (**1**) had the molecular formula of $C_{21}H_{28}O_5$, as determined by EIMS (M^+ at *m*/*z* 360), FABMS, positive mode ($M^+ + H$ at *m*/*z* 361), ^1H and ^{13}C NMR data. It was evident from its NMR spectra that the functionality within **1** clearly consisted of a ketone (δ_C 209.3, s), one methylcarboxylate group (*C*OOMe, δ_C 165.5 s; and COO*Me*, δ_C 51.7, q, δ_H 3.33, s), a hemiketal (δ_C 105.7, s), a quaternary oxygenated carbon (δ_C 77.8, s), and two trisubstituted double bonds (δ_C 145.5, d, δ_H 6.98, s and 131.8, s; δ_C 121.5, d, δ_H 5.37, br s and 131.4, s). Additionally it had four methyls, three methylenes, four methines, and a quaternary carbon, which did not belong to the groups mentioned above (Table 1).

The molecular formula and the presence of two carbonyl groups and two double bonds indicated the tetracyclic nature of sarcophytin B. The UV spectrum showed an absorbance maximum at λ_{max} 245 nm (ϵ 3600), confirming conjugation between a carbonyl group and a double bond. The comparison of NMR spectra of **1** and **3** suggested that sarcophytin B could be a 4-keto-6(7)-dehydroderivative of **3**. However, detailed analysis of chemical shifts and spin-decoupling constants, using INDOR and difference spin-decoupling techniques, revealed that an isopropyl group in **1** is equatorial ($\delta_{H\text{-}2}$ 2.43 ddd, $J_{2,3} = 13.0$, $J_{2,3'} = 5.4$, $J_{2,11} = 9.0$ Hz) and was α, rather than β, to a ketone. The signals of H-10a (δ 3.05), indicated by INDOR by irradiation of the olefinic proton at δ 6.95 (H-9), and especially of the signals of H-2 (2.43) and C-2 (55.8), were downfield in comparison to those in the spectra of chatancin (**3**) ($\Delta\delta$ = 0.4, 1.06, and 5.0, respectively).

These and other data, obtained by spin-decoupling experiments, as well as the absence of acetylation of a hemiketal hydroxyl on treatment with acetic anhydride in pyridine suggested that **1** should contain a hemiketal fragment at C-4, shielded by a methyl group, while a ketone group should be at C-1. In accordance with this suggestion, the downfield shift of the hemiketal carbon (105.7 in **1** instead of 99.9 in **3**) could be explained by β-effect of methyl group at 4a.[6]

A single-crystal X-ray analysis confirmed this suggestion and established the structure of **1** for sarcophytin B. Sarcophytin B was crystallized from methanol as colorless prisms with one molecule of $C_{21}H_{28}O_5$ in the asymmetric unit. A computer-generated perspective drawing of the final X-ray model is shown in Figure 1.

The X-ray analysis showed also the following conformational peculiarities of **1**: the three six-membered rings of this diterpenoid have chair and sofa + sofa conformations, respectively; the five-membered cycle has an envelope conformation. Ring-junction configurations for the A/B and B/C ring are *cis*; the oxygen-containing five-membered ring and isopropyl groups are oriented on opposite sides of ring A.

The minor compounds **2** and **4** were isolated along with **1** from the soft coral. When the structure elucidation of sarcophytin B was almost completed, the 6(7)-dihydroderivative of **1** named as sarcophytin (**4**) was described from

* To whom correspondence should be addressed. Tel.: 7 4232 311409. Fax: 7 4232 314050. E-mail: piboc@stl.ru.
[†] Andra University.
[‡] Pacific Institute of Bioorganic Chemistry.
[§] A.N. Nesmeyanov Institute of Organoelemental Compounds.

10.1021/np990179v CCC: $19.00
Published on Web 12/08/1999

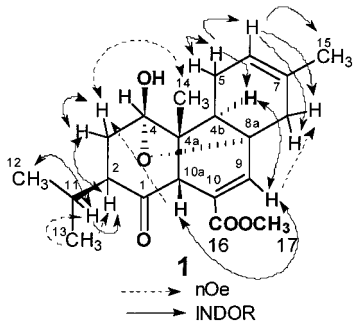
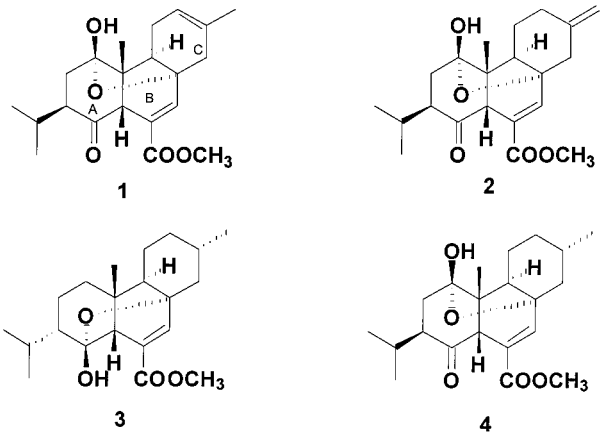


Figure 1. Computer-generated perspective drawing of the final X-ray model of sarcophytin B (**1**).

the Indian Ocean soft coral *Sarcophyton elegans*.[7] We have identified the isolated **4** as sarcophytin by comparison of ^{1}H NMR spectral data. The spectra of **1** and **4** were similar, except for the difference concerning the presence of an additional 6(7)-double bond in the ring C of **1**.



Another minor constituent, **2**, proved to be an isomer of **1**. EIMS of **2** also showed a peak of M^+ ion at *m*/*z* 360. Its ^{1}H NMR spectrum was similar to the spectra of **1** and **4**, but differed from those in having the signals for an *exo* double bond. Therefore, minor diterpenoid **2**, named by us as sarcophytin C, is 7(15)-dehydrosarcophytin.

It is of special interest that an absorption band with λ_{max} 245 nm in the UV spectrum of **1** was shifted approximately 25 nm in comparison with that of other natural products having an α,β-unsaturated ester, for instance chatancin (λ_{max} 218 nm)[2] and sarcophytin (λ_{max} 219 nm).[7] This may be a consequence of nonconjugated interaction of two chromophores: the double bond of the α,β-unsaturated ester and the 6(7)-double bond; the two double bonds are close in space, and the four carbons are nearly coplanar (see Figure 1). It is known that the decrease of intensity and the bathochromic shift of the absorption band by 12−25 nm may be observed in such cases.[8]

Structural features of the sarcophytins, such as 14 carbons in rings A, B, and C, as well as the presence of one isopropyl and three one-carbon substituents, indicate that the sarcophytins, as well as some other tricyclic and tetracyclic products from *Sarcophyton* spp., are derived from cembranoids, which are widely distributed secondary metabolites in soft corals belonging to the genus *Sarcophyton*.[1]

Experimental Section

General Experimental Procedures. ^{1}H and ^{13}C NMR spectra were recorded on a Bruker WM-250 spectrometer at 250 and 62.9 MHz, respectively, with tetramethylsilane as an internal standard. The optical rotations $[\alpha]_D$ were determined on a Perkin−Elmer 141 polarimeter. HPLC separation was conducted on a Du Pont 8800 chromatograph, fitted with RIDK-102 differential refractometer. The sarcophytin fractions were separated on an Ultrasphere-Si column (250 × 10 mm). Melting points were determined on a Boethius apparatus. EIMS were measured on a LKB 9000S spectrometer (ionizing energy 70 eV); FABMS were recorded on LKB 2091 with ion tech saddle gun, 10 mA, 8 kV, using glycerol as matrix. TLC and LPLC were performed on 5/40 and 40/100 μm Si gel L (Chemapol, the former Czechoslovakia), respectively.

Animal Material. The sample has a distinct stalk and a rounded capitulum marginally folded and bearing abundant dimorphic retractile polyps. Interior sclerites are spindle shaped; surface ones include clubs, indicating the sample could be from a colony of an unindentified species belonging to the genus *Sarcophyton*. The sample (dry wt 1.3 kg) was collected off Kalipur Island, Diglipur, 93° 02′ E, 13° 20′ N in March 1992. A voucher specimen MF-VA/50 is on deposit in the collection of the Andra University, Visakhapatnam, India.

Extraction and Isolation. The extraction was carried out using ethanol by percolation every 48 h (8 times). The combined extracts were concentrated in vacuo. The dark oily residue was re-extracted with EtOAc several times, and the combined EtOAc extracts were concentrated in vacuo to give 40 g of EtOAc-soluble materials. This fraction was chromatographed over a Si gel column using petroleum ether−EtOAc (19:1 → 3:7). Petroleum ether−EtOAc (4:1) eluates gave a mixture of compounds **1**, **2**, **4** (50 mg) after the removal of solvents in vacuo. The mixture (26 mg) was subjected to HPLC on an Ultrasphere-Si column using hexane−EtOAc (5:1) as a mobile phase, with a flow rate of 0.65 mL/min, to give the individual diterpenoids **1** (12 mg), **2** (1.0 mg), and **4** (0.7 mg).

Sarcophytin B (1): 12 mg; colorless prisms (MeOH); mp 185−188 °C; $[\alpha]_D$ +206° (*c* 0.43, $CHCl_3$); IR ($CHCl_3$) ν_{max} 3608, 1721 cm^{-1}, in KBr ν_{max} 3402, 1732, 1702 cm^{-1}; UV (EtOH) λ_{max} (ϵ) 245 (3600) nm; ^{1}H NMR (CD_3OD) δ 2.43 (1H, ddd, J = 13.0, 9.0, 5.4 Hz, H-2), 2.40 (1H, dd, J = 13.0, 5.4 Hz, H-3), 1.61 (1H, t, J = 13.0 Hz, H-3), 2.38 (1H, br dd, J = 10.0, 5.0 Hz, H-4b), 2.50 (1H, m, H-5), 1.57 (1H, m, H-5), 5.38 (1H, br s, H-6), 2.34 (1H, m, H-8); 2.48 (1H, m, H-8); 6.95 (1H, t, J = 1.5 Hz, H-9), 3.05 (1H, d, J = 1.5 Hz, H-10a), 1.87 (1H, m, H-11), 0.83 (3H, d, J = 6.5 Hz, H-12), 0.77 (3H, d, J = 6.5 Hz, H-13), 1.05 (3H, s, H-14), 1.77 (3H, br s, H-15); 3.68 (3H, s, H-17); ($CDCl_3$) δ 2.50 (1H, m, H-2), 2.39 (1H, m, H-3), 1.70 (1H, t, J = 13.0 Hz, H-3), 2.40 (1H, m, H-4b), 2.40 (1H, m, H-5), 2.06 (1H, m, H-5), 5.37 (1H, br s, H-6), 6.98 (1H, br s, H-9), 3.10 (1H, d, J = 1.5 Hz, H-10a), 1.95 (1H, m, H-11), 0.86 (3H, d, J = 6.5 Hz, H-12), 0.81 (3H, d, J = 6.5 Hz, H-13), 1.11 (3H, s, H-14), 1.76 (3H, br s, H-15), 3.71 (3H, s, H-17); in C_6D_6, see Table 1; EIMS *m*/*z* 360 [M^+], 342 [$M^+ - H_2O$], 329 [$M^+ - CH_3$-OH], 82, 266, 217 (85%), 202, 185, 158 (100%), 143, 109, 105, 97, 73, 69, 59.

Sarcophytin C (2): 1.0 mg; $[\alpha]_D$ +169° (c 0.10, $CHCl_3$); UV (EtOH) λ_{max} (ϵ) 219 (3800) nm; ^{1}H NMR, see Table 1; EIMS *m*/*z* 360 [M^+], 342 [$M^+ - H_2O$], 328 [$M^+ - CH_3OH$], 309, 299, 282, 266, 257, 217 (100%), 202, 185, 162, 158, 142.

Sarcophytin (4): 0.7 mg; thin colorless crystals (MeOH); mp 160−162 °C; $[\alpha]_D$ +122° (*c* 0.07, $CHCl_3$); ^{1}H NMR data identical with those reported by Anjaneyulu;[7] EIMS *m*/*z* 362 [M^+], 344 [$M^+ - H_2O$], 330 [$M^+ - CH_3OH$], 312, 302, 231, 219 (100%), 204, 187, 158, 142, 119, 105.

X-ray Structure Determination. Crystallization of **1** from MeOH yielded colorless, clear prisms suitable for X-ray analysis. A crystal of 0.30 × 0.30 × 0.20 mm was used for all X-ray measurements. Cell dimensions were determined by a least-squares fit to $\pm 2\theta$ of 24 reflections (10° < θ < 11°) measured at −120 °C using Mo Kα radiation. Crystal data: $C_{21}H_{28}O_5$, fw = 360.43, monoclinic, $P2_1$, a = 11.667(5) Å, b = 6.532(2) Å, c = 12.479(4) Å, β = 102. 92(3)°, V = 926.9(5) Å^3, Z = 2, D_x = 1.291 g/cm^3, μ(Mo Kα) = 0.091 cm^{-1}, λ = 0.71073 Å.

Intensities of 2711 unique reflections within 1.79° < θ < 30.05° were collected on an Simens P3 diffractometer using

Table 1. NMR Data of Sarcophytins B (**1**) and C (**2**)[a]

	1		**2**
position	δ_C ($CDCl_3$)	δ_H (C_6D_6)	δ_H (C_6D_6)
1	209.3 s		
2	55.8 d	2.65 ddd (13.0, 9.0, 5.4)	2.61 m
3	40.5* t	dd (13.0, 5.4); 1.59, t (13.0)	dd (13.0, 5.4); 1.59 t (13.0)
4	105.7 s		
4a	51.0 s		
4b	45.1 d	2.13 m	
5	23.4 t	1.24 m	
6	121.5 d	5.09 br s	2.60 m and 2.23 m
7	131.4 s**		
8	41.5* t	2.22 m	2.22 m
8a	77.8 s		
9	145.5 d	7.09 t (1.5)	under solvent
10	131.8 s**		
10a	48.7 d	3.26 d (1.5)	3.11 d (1.5)
11	25.9 d	2.11 m	2.07 m
12	19.0 q	0.94 d (6.5)	0.95 d (6.5)
13	21.6 q	0.72 d (6.5)	0.71 d (6.5)
14	17.3 q	1.08 s	1.08 s
15	23.8 q	1.46 br s	4.63 m
16	165.5 s		
17	51.7 q	3.33 s	3.31 s

[a] *,**: Assignments with the same superscript may be interchanged.

Mo Kα radiation. Intensities were corrected for Lorentz and polarization factors, but no absorption correction was made. The structure was solved by direct methods using the program SHELX-86[9] and refined by a full-matrix least-squares routine using the program SHELX-93[10,11] to $wR_1 = 0.1146$, $R_{all} = 0.0608$, $R_{obs} = 0.0457$ [$I > 2\sigma(I)$], S = 1.095, $\rho_{max} = 0.357$e/Å^3.

Acknowledgment. We are grateful to Drs. P. S. Dmitrenok and Mrs. O. P. Moiseenko for FABMS and EIMS measurements and to Dr. I. A. Gorshkova for assistance. Authors from Anhdra University thank the Council of Scientific & Industrial Research (CSIR), New Delhi, for the award of a Research Associateship to P.R.

References and Notes

(1) Faulkner, D. J. *Nat. Prod. Rep.* **1995**, *2*, 223−269; **1996**, *13*, 75−125; **1998**, *15*, 113−158, and the earlier references therein.
(2) Sugano, M.; Shindo, T.; Jijima, Y.; Oshima, T.; Kuwano, H.; Hata, T. *J. Org. Chem.* **1990**, *55*, 5803−5805.
(3) W. Fenical. In *Natural Products Chemical and Biological Perspectives*; Scheuer, P. J., Ed. Academic Press: New York, 1978; Vol. II, pp 187−200, and earlier references therein.
(4) Anjaneyulu, A. S. R.; Sagar K. S.; Rao G. V. *J. Nat. Prod.* **1997**, *60*, 9−12.
(5) Makarieva, T. N.; Ilyin, S. G.; Stonik, V. A.; Lyssenko, K. A.; Denisenko, V. A. *Tetrahedron Lett.* **1999**, *40*, 1591−1594.
(6) Levy, G. C., Nelson, G. L. *Carbon-13 Nuclear Magnetic Resonance for Organic Chemists*; Wiley-Interscience: New York, 1972.
(7) Anjaneyulu, A. S. R.; Venugopal, M. J. R. V.; Sarada, P.; Rao, G. V.; Clardy, J.; Lobkovsky, E. *Tetrahedron Lett.* **1998**, *39*, 135−138.
(8) Brend, J.; Scott A. I. in *Technique of Organic Chemistry. Elucidation of Structures by Physical and Chemical Methods*; Weissberger, E., Ed. Interscience Publishers: New York, 1963; Vol. XI, Part 1, pp 81−151.
(9) Sheldrick, G. M. In *Crystallographic Computing 3*. Sheldrick, G. M., Kruger, C., Goddard, R., Eds.; Oxford University Press: Oxford, 1985; p. 175.
(10) Sheldrick, G. M. *SHELX-93*: Program for the Refinement of Crystal Structures; University of Göttingen, 1993.
(11) The final atomic coordinates, thermal parameters, bond distances and angles, and observed and calculated structure factors have been deposited with the Cambridge Crystallographic Data Center and can be obtained upon request from the Director, University Chemical Laboratory, 12 Union Road, Cambridge CB2 1E2, UK.

NP990179V