A NOVEL DITERPENOID FROM THE
SOFT CORAL *SARCOPHYTON SOLIDUM*

MIN ZHANG,

Guangdong Provincial Institute for Materia Medica, Jinbu Li No. 2, West Huifu Road,
Guangzhou 510180, People's Republic of China

KANGHOU LONG,

Chemistry Department, Zhongshan University, Guangzhou 510275, People's Republic of China

KAN MA, XIAOLING HUANG, and HOUMIN WU*

State Key Laboratory of Bioorganic and Natural Products Chemistry, Shanghai Institute of Organic Chemistry,
Chinese Academy of Sciences, Shanghai 200032, People's Republic of China

ABSTRACT.—A novel diterpenoid, sarsolenone [1], has been isolated from the soft coral *Sarcophyton solidum* collected from the South China Sea. The structure of sarsolenone was assigned on the basis of spectral analysis inclusive of DQF-COSY, TOCSY, HMBC, HMQC, and NOESY nmr experiments.

In the course of studies on *Sarcophyton solidum* Tixier-Durivault (Alcyoniidae), we have previously reported a new diterpenolide, sarsolilide A, isolated from an EtOH extract of *S. solidum* (1). Compounds with the same bicarbocyclic skeleton as sarsolilide A were previously reported from a soft coral, *Cespitularia* sp. (2). Further investigation has now led to the isolation of a new ketonic diterpenoid, sarsolenone [1], with a different carbon skeleton from that of sarsolilide A.

Sarsolenone [1] was isolated as colorless crystals, $[\alpha]^{25}D$ +145.8° (c=0.02, EtOH), mp 219.5–221.0°. It was assigned the molecular formula $C_{22}H_{30}O_5$ on the basis of eims, ^{13}C-nmr, and DEPT experiments. All of the ^1H- and ^{13}C-nmr signals of sarsolenone [1] were unambiguously assigned by DQF-COSY, TOCSY, HMBC, HMQC, and NOESY nmr experiments and are shown in Table 1. The ^1H and ^{13}C-nmr spectra and a DEPT experiment indicated the presence

of a ketone [δ_C 200.1 (s)], an ester [δ_C 169.8 (s)], a tetrasubstituted double bond [δ_C 163.6 (s) and 117.1 (s)], a trisubstituted double bond [δ_H 4.81 (1H, d, J=7.2 Hz), δ_C 147.7 (s), 123.3 (d)], a terminal double bond [δ_H 4.97 (1H, br s) 4.93 (1H, d, J=1.2 Hz), δ_C 137.7 (s), and 112.2 (t)], and three oxygenated carbons [δ 84.7 (s), 75.7 (d), and 75.0 (s)] in the molecule of 1. In addition, the ^{13}C-nmr spectrum and the DEPT experiment showed signals of four methyl groups, six methylene groups, and one methine group. The ir absorption bands at 3415 and 3475 (s) cm^{-1}, together with the eims fragment peak at m/z 357 $(M-OH)^+$ indicated the presence of a hydroxyl group. Of the eight degrees of unsaturation in the molecule, two were assigned to carbonyl groups and three to carbon-carbon double bonds, and thus compound 1 was determined as being tricyclic.

The DQF-COSY nmr spectrum of sarsolenone [1] showed H/H correlations for five partial structures that were isolated by quaternary carbons (Table 1). Connectivities of these partial structures and the quaternary carbons were determined by detailed analysis of the HMBC nmr spectrum of sarsolenone [1] and established the carbobicyclic system present (Figure 1). HMBC correlations of
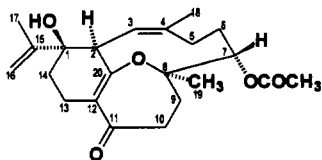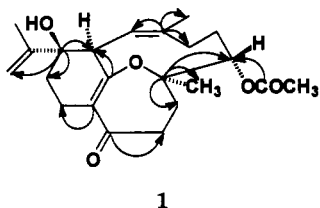

1

TABLE 1. ^{1}H- (600 MHz) and ^{13}C-Nmr Data in CDCl$_3$ for Sarsolenone [1].[a]

Position	δ$_C$ (mult.)	δ$_H$ J (Hz)	DQF-COSY	HMBC	NOESY
1	75.0 s			H-2,H-3,H-16a,b H-17	
2	47.6 d	3.15 d 7.2	3	H-3	H-5a,H-17
3	123.3 d	4.81 d 7.2	2	H-2,H-18	H-18,H-14b
4	147.7 s			H-2,H-18,H-5	
5	29.0 t	1.92 m (Hb)		H-3,H-18	H-6b,H-5a
		3.06 dt 4.6, −14.6 (Ha)	5b,6a		H-2,H-6a,H-5b
6	26.5 t	1.81 dq 2.9,5.8, −15.2 (Hb)	5b	H-5a,b	H-5b,H-7,H-6a
		1.96 m (Ha)	5b		H-5a,H-6b
7	75.7 d	4.69 dd 2.8, 11.5	6b	H-9a,b,H-19	H-6b,H-9b
8	84.7 s			H-7,H-9b,H-19	
9	33.2 t	1.64 t 12.8, −12.8 (Hb)	10a,9a	H-10a,H-19	H-7,H-9a
		1.94 m (Ha)	9b,10b		H-9b,H-19
10	38.0 t	2.59 dd 7.0, −12.8 (Hb)	9a,10a	H-9a	H-10a
		2.67 dd 12.8, −12.8 (Ha)	10b	H-9a	H-10b,H-19
11	200.1 s			H-9a,b,H-10a,b	
12	117.1 s			H-2,H-13a,b,H-10b	
13	20.6 t	2.36 ddd 3.1,5.1, −17.3 (Hb)	13a,14		H-13a
		2.45 dt 8.9, −17.3 (Ha)	13b,14		H-13b
14	26.0 t	1.90 m	13a,13b	H-13a,b,H-2	H-3,H-16
15	137.7 s			H-2,H-17	
16	112.2 t	4.93 d 1.2 (Hb)	16a,17		H-17
		4.97 br s (Ha)	16b,17	H-17	H-14a
17	17.8 q	1.74 br s	16b,16a	H-3,H-5a	H-2,H-16b
18	22.7 q	1.79 s			H-3
19	20.2 q	1.43 s			H-9a,H-10a
20	163.6 s			H-2,H-13a,b	
1'	169.8 s			H-7,H-2'	
2'	21.0 q	2.00 s			

[a]In ppm from internal TMS in CDCl$_3$ solution.

1

FIGURE 1.   HMBC nmr observations for **1** (correlation C→H).

δ_C 75.0 (C-1) to δ_H 1.90 (H-14) and 3.15 (H-2) suggested the connectivity of the oxygenated carbon to C-14 and C-2. Similarly, HMBC correlations of δ_C 117.1 (C-12) to δ 2.45 (H-13a) and 2.36 (H-13b), and δ_C 163.6 (C-20) to δ_H 3.15 (H-2) suggested the connectivities of C-12 to C-13 and of C-20 to C-2, leading to a six-membered ring. The HMBC experiment also showed the correlation of the sp^2 quaternary carbon δ_C 147.7 (C-4) to δ_H 3.06 (H-5a), and an oxygenated quaternary carbon δ_C 84.7 (C-8) to δ_H 4.69 (H-

7), suggesting the connectivities of C-4 to C-5 and C-8 to C-7. The connectivity of C-8 to C-9 was assigned by HMBC correlation of δ 84.7 (C-8) to δ 1.64 (H-9b). Absorptions in the uv spectrum at λ max 265 (log ε 3.84) and 209 (log ε 3.57) nm were consistent with the presence of an α,β-unsaturated-β-ether ketone functionality in **1** (3). The ir spectrum also showed an α,β-unsaturated-β-ether ketone absorption at ν max 1642 cm^{-1} (4). The ^{13}C-nmr signal at δ_C 163.6 (s, C-20) also indicated the presence of an oxygen substituent. Thus, the ketone was bonded to the tetrasubstituted double bond. The downfield signals of H-10a (2.67 ppm) and H-10b (2.59 ppm), together with the long-range correlation of δ_C 200.1 (C-11) to δ_H 2.67 (H-10a), suggested the connectivity of C-10 to the ketone group and established the second carbon ring of sarsolenone [1].

Four singlets for four methyl groups

in the ^{1}H-nmr spectrum of **1** suggested connectivities of these methyl groups to quaternary carbons. In the DQF-COSY spectrum, cross-peaks of the singlet at δ 1.74 (H-17) with the doublet at δ 4.93 (H-16b) and the singlet at δ 4.97 (H-16a) suggested the connectivity of Me-17 to the terminal double bond. HMBC correlations (Figure 1) of $δ_C$ 75.0 (C-1) to $δ_H$ 4.93 and 4.97 permitted the assignment of the location of this isopropenyl on the oxygenated carbon. An HMBC correlation between $δ_C$ 147.7 (C-4) and $δ_H$ 1.79 (H-18) revealed the connectivity of Me-18 to the trisubstituted double bond. The ^{13}C-nmr chemical shift of C-18 at 22.7 ppm and an nOe observed between H-3 and H-18 showed this trisubstituted double bond to have Z-geometry. The methyl group (Me-19) was assigned to C-8 by an HMBC correlation of $δ_C$ 84.7 (C-8) to $δ_H$ 1.43 (H-19). An ir absorption at 1735 (s) cm^{-1} showed the presence of an ester carbonyl group. The signals at $δ_H$ 2.00 (3H, $δ_C$ 169.8 (s), and 21.0 (q), together with eims fragment peaks at m/z 331 [M−CH$_3$CO]$^+$

and 315 [M−CH$_3$−CO$_3$]$^+$, indicated the carbonyl group to be part of an acetate group. The downfield signals of the methine at $δ_H$ 4.69 (1H, dd, J=2.8 and 11.5 Hz, H-7) and $δ_C$ 75.7 (d, C-7) indicated that this methine was attached to the acetate. This connectivity was supported by the HMBC correlation of C-1' to H-7. The chemical shifts of two oxygenated carbons [δ 75.0 (C-1), 84.7 (C-8)] suggested connectivities of C-1 to a hydroxyl group, and C-8 to an ether group which established composition of the heterocyclic ring (5).

In order to obtain a more detailed structure and conformation consistent with its nmr data, the structure of compound **1** was calculated using a minimization-molecular dynamics-minimization procedure *in vacuo*. The MM+ HyperChem software program (6) was used for energy minimization and molecular dynamics optimization of the structures with distance constraints based on the observed nOe data (Table 2). The starting coordinates of the atoms in the molecule were taken from the model
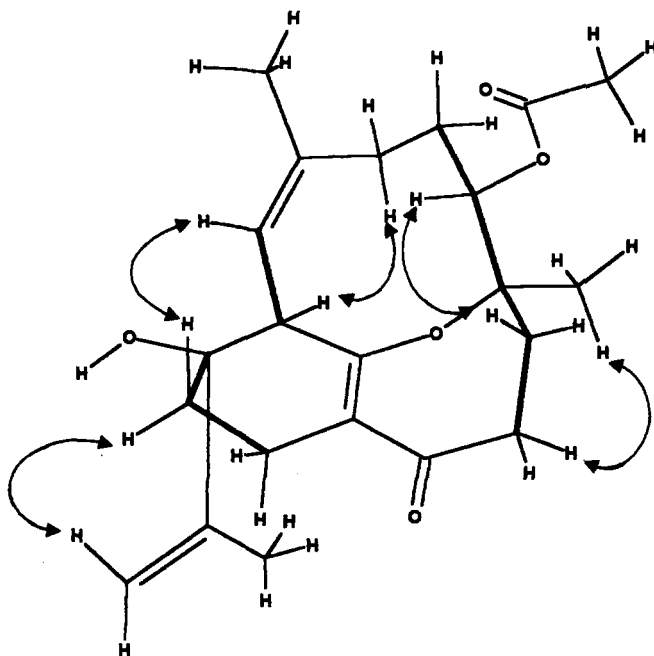

FIGURE 2.   Lowest-energy conformation of **1** as determined by molecular dynamics.

TABLE 2.   Comparison of Proton-Proton
Distances Obtained from NOESY Nmr
Observations Compared with Molecular
Dynamics Calculations.

Number	Protons Involved	(Å)[a]	(Å)[b]
1	H-16a–H-14α	2.34	2.32
2	H-16a–Me-15	2.24	2.30
3	H-3–Me-4	2.58	2.50
4	H-3–H-14β	2.41	2.24
5	H-7–H-9β	2.40	2.43
6	H-7–H-6β	2.47	2.53
7	H-2–Me-4	2.59	2.62
8	H-2–H-5α	2.20	2.18

[a]Proton-proton distances determined experimentally.

[b]Proton-proton distance determined by molecular dynamics calculations.

built structure. After the energy minimization, we performed a 20 psec restrained molecular dynamics run. This run consisted of a 0.5 fsec time-step at 400° K with a strong coupling to the temperature bath. Minimization of several dynamics data-sets resulted in convergence to the lowest energy conformation (Figure 2).

This conformer shows that the bridgehead oxygen and C-20, C-12, C-11, C-13, and C-2 of 1 are nearly coplanar due to a double bond between the A and B fused rings. The A ring is in a "twist boat" conformation, and the B ring adopts an envelope-like conformation with C-9 residing on the upward flap of the envelope. The C-2–C-3 bond is oriented axially to the A ring, making H-3 close to H-14β (2.24 Å). On the other hand, because of the presence of a Z-double bond at C-3 and C-4, C-5 is folded inward. Thus endo-H-5a is adjacent to H-2 (2.18 Å) and the ether group, which results in a low-field chemical shift of this proton (3.06 ppm). These parts of the conformation of the molecule are in good agreement with nOe observations between H-3/H-14β and H-2/H-5α, respectively. In addition, since the angular methyl at C-8 has the α-orientation, which was confirmed by its nOe correla-

tion with H-10α, both β-protons at C-7 and C-9 must be axially oriented. The nOe connectivity between them revealed the α-orientation of the acetyl group at C-7. In addition, the dihedral angle (H3-C3-H2-C2) determined via the Karplus equation (7) from coupling constants $(\theta = 133.8°)$, is close to the value determined by the molecular dynamics calculation $(\theta = 137.7°)$.

All the above data allowed the assignment of the relative stereochemistry of sarsolenone [1].

EXPERIMENTAL

GENERAL EXPERIMENTAL PROCEDURES.—[1]H- and ^{13}C-nmr spectra were recorded on a Bruker AMX-600 MHz and a JEOL FX-90Q instrument. A VG analytical ZAB mass spectrometer, a Nicolet 5DX Ft-ir spectrometer and a Perkin-Elmer 241 polarimeter were also used.

ANIMAL MATERIAL.—The fresh soft coral, *S. solidum*, was collected by hand from the South China Sea near the Xisha islands in July 1987. The animals were dried under the sun and brought back to the laboratory. A voucher specimen has been deposited in the Research Center of Organic Natural Products, Zhongshan University, Guangzhou, People's Republic of China.

EXTRACTION AND ISOLATION.—The specimens (1 kg) were extracted three times with EtOH. The extracts were combined and chromatographed over a column of Si gel eluted stepwise with petroleum ether containing increasing concentrations of EtOAc. The fractions eluted with 30% EtOAc gave sarsolenone [1] (60 mg).

Sarsolenone [1].—Colorless needles, mp 219.5–221.0°, $[\alpha]^{25}D$ 145.8° ($c=0.02$, EtOH); ir (KBr) ν max 3475, 3415, 2970, 2937, 1735, 1642, 1616, 1523, 1443, 1376, 1244, 1197, 1137, 1038, 912 cm^{-1}; ms (m/z) 374 (M$^+$, 74); 357 (26), 346 (24), 331 (6), 315 (19), 297 (10), 286 (10), 245 (16), 233 (27), 215 (18), 120 (77), 95 (74), 69 (100); uv (EtOH) λ max 265 (log ε 3.84) and 209 nm (log ε 3.57).

MOLECULAR DYNAMICS AND MOLECULAR MECHANICS CALCULATIONS ON 1.—Molecular modeling was carried out with the HyperChem program system on an IBM 486 personal computer. Modeling involved a minimization-molecular dynamics-minimization protocol. Molecular mechanics and dynamics calculation were performed with the MM+ package. Initial calculations started with coordinates from the model-built structure using computer/geometry optimi-

zation. The structure was then subjected to HyperChem MM+energy minimization *in vacuo*, which was terminated when the energy value gradient between cycles was less than 0.001 kcal/mol Å^2. Typical molecular dynamics simulations involved a 1 psec heating period during which time the system was heated to 400° K followed by a 1 psec equilibration period and then 20 sec of dynamics simulation at the appropriate elevated temperature. Considering the nOe shown in Table 2, constrained minimizations and dynamics were calculated ($K_{dc}=7$ kcal/mol Å^2). Structures were sampled every 0.1 psec. The snapshots from the dynamics trajectories were then subjected to energy minimization. A snapshot with the lowest energy was selected as a relevant conformation.

ACKNOWLEDGMENTS

LITERATURE CITED

1. M. Zhang, K. Long, S. Huang, K. Shi, and T.C.W. Mak, *J. Nat. Prod.*, **55**, 1672 (1992).
2. B.F. Bowden, J.C. Coll, J.M. Gulbis, M.F. Mackay, and R.H. Willis, *Aust J. Chem.*, **39**, 803 (1986).
3. S. Hong, "Applications of Spectroscopic Methods in Organic Chemistry," Science Press, Beijing, 1981, pp. 377–382.
4. L.J. Bellamy, "The Infra-Red Spectra of Complex Molecules," Chapman and Hall, London, 1975, pp. 149–155.
5. D. Chen, "Applications of ^{13}C-NMR Spectra in Chinese Herbal Medical Chemistry," Renmin Health Publishing House Press, Beijing, 1991, p. 82.
6. HyperChem Release 3 for Windows, Autodesk, Inc., Sausalito, CA, Publication 102097-01, March 1993.
7. M. Karplus, *J. Chem. Phys.*, **308**, 11 (1959).