Pergamon

Tetrahedron Letters 43 (2002) 5937–5939

TETRAHEDRON
LETTERS

Two new cembrane diterpenes from a Madagascan soft coral of the genus *Sarcophyton*[†]

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Received 26 June 2002; accepted 27 June 2002

Abstract—Two new cembrane diterpenes have been isolated from an unidentified species of soft coral of the genus *Sarcophyton*, collected along the South coast of Madagascar. Their structures were elucidated on the basis of detailed NMR spectroscopic data.
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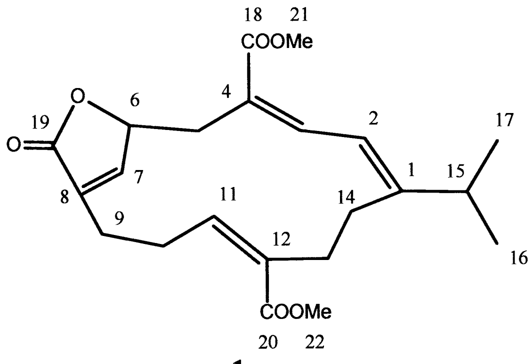
Soft corals (phylum Cnidaria, class Anthozoa, subclass Octocorallia, order Alcyonacea) have proven to be a rich source of terpenoid and polyhydroxy steroid metabolites.[1] The most prolific and the most scattered in the world, which belong to the genus *Sarcophyton* have been widely studied.[2] Among the nearly 30 species from different areas chemically examined, cembranoid-type diterpenes emerge as characteristic constituents of this genus. Most of them have been regarded as defensive, competitive, reproductive or pheromonal substances, playing a functional role in their survival.[3] Some of them exhibited a pharmacological activity.[2] In the course of our ongoing studies on marine natural products, we have recently examined the dichloromethane extract of the soft coral collected off the Tulear coast in the South of Madagascar, identified as *Sarcophyton* sp.[4] and have isolated two new cembrane diterpenes **1** and **2**. The details of characterization and structure elucidation of these two new compounds are presented in this paper.

The CH_2Cl_2 extract (28 g) of air-dried sample of the soft coral *Sarcophyton* sp. (500 g) was fractionated by silica gel chromatography using a linear gradient of acetone in dichloromethane as eluent. The fraction eluted with 5% acetone afforded the two new cembranes **1** (0.0009% dry weight) and **2** (0.0006% dry weight), which were purified on preparative TLC (hexane/AcOEt 65:35).

Compound **1** was isolated as a colourless oil. The molecular formula $C_{22}H_{28}O_6$, determined by FAB HREI MS (M+NH_4^+ at m/z 406.2240, $C_{22}H_{32}O_6N$) and ^{13}C NMR, indicated nine degrees of unsaturation. The presence of several bands in the region of 1735 cm^{-1} in the IR spectrum and the ^{13}C NMR resonances at δ



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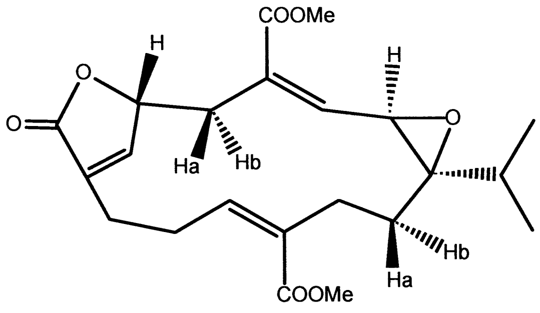

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Keywords: diterpene; cembrane; soft coral; *Sarcophyton* sp..

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[†] Dedicated to the memory of Marie-Thérèse Raharimalala-Rakotoarisoa who collected the specimens of *Sarcophyton* sp.

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PII: S0040-4039(02)01266-2

80.2, 174.0, 167.3, 51.6 and 168.4, 51.5 ppm suggested the presence of a lactone function and esters groups. The ^{13}C NMR spectrum showed low-field signals at δ 157.5, 150.2, 141.9, 141.6, 133.3, 132.8, 120.6 and 119.9 ppm, indicating the presence of four trisubstituted double bonds in the molecule. In addition, ^{1}H–^{1}H COSY spectrum revealed the presence of an isopropyl group due to resonances at δ 1.06 (3H, d, $J=6.8$ Hz), 1.03 ppm (3H, d, $J=6.8$ Hz) and 2.41 (1H, m). The ^{1}H NMR spectrum showed one deshielded aliphatic proton at δ 5.15 ppm and four olefinic protons at δ 5.70 (dd, 11.1, 4.4), 6.50 (d, 11.9), 7.00 (d, 11.9) and 7.54 (d, 1.7) ppm and confirmed the presence of two methoxyl groups at δ 3.76 (s) and 3.67 (s) ppm. According to the molecular formula, the presence of three carbonyl groups and four double bonds suggested that **1** was a bicyclic cembrane. Full NMR data assignments for **1** were obtained by careful analysis of 2D NMR data. The protonated carbons were all assigned by HMQC experiments.

The downfield chemical shift of H-15 (δ 2.41 ppm) suggested the presence of a double bond at C-1.[5] The connectivities H-2/H-3, H-5/H-6, H-9/H-10, H-10/H-11, H-13/H-14 were also afforded by ^{1}H–^{1}H COSY. Connections of these protons were provided by HMBC (see Table 1). Hence, the position of the isopropyl group at C-1 was determined by HMBC correlations of the ethylenic proton at δ 7.00 ppm with C-15 and secured by HMBC correlations of the methine at δ 2.41 ppm with C-1/C-2/C-16/C-17. Further relevant connectivities were furnished by HMBC correlations observed between the methylene protons at δ 3.12–2.27 ppm and the proton at δ 6.50 ppm with C-4 and C-18, which fixed the position of one ester group on C-4. The lactone moiety was supported by significant HMBC correlations of the proton at δ 7.54 ppm with C-6 and C-19. The position of the second ester group was deduced as being on C-12 due to HMBC correlations of the proton at δ 5.70 ppm with C-9/C-20 and HMBC correlations of the methylene protons at δ 2.78–2.29 ppm with C-1/C-11/C-12/C-20.

We have attempted to determine the stereochemistry by NOESY experiments. A NOESY correlation between the proton H-2 at δ 7.00 ppm and one of the methyl groups, indicated a *E* configuration for the first trisubstituted olefin. Furthermore, a NOESY correlation observed between the proton H-2 at δ 7.00 ppm and the methoxyl group in position 21 at δ 3.76 ppm, coupled with the *trans* orientation of protons H-2 and H-3 ($J=11.9$ Hz) implied a *Z* configuration for the second trisubstituted olefin. This was confirmed by the chemical shift of proton H-3 at δ 6.50 ppm.

Compound **2** was isolated as a colourless oil. The high-resolution analysis gave a value of 422.2206 ($C_{22}H_{32}O_7N$) for the $(M+NH_4)^+$ ion at m/z 422, indicating the molecular formula as being $C_{22}H_{28}O_7$. This and spectral data, revealed a close structural relationship with compound **1**. The main difference appeared in ^{13}C NMR spectrum with the absence of one olefin and the appearance of resonances at δ 68.3 and 56.7 ppm (δ 3.33 ppm in ^{1}H NMR spectrum), suggesting the pres-

Table 1. ^{1}H and ^{13}C NMR assignments for **1** and **2** ($CDCl_3$)

N°	1			2		
	^{13}C	^{1}H (mult, J, Hz)	HMBC (H→C)	^{13}C	^{1}H (mult, J, Hz)	HMBC (H→C)
1	157.5			68.3		
2	119.9	7.00 (d, 11.9)	3, 4, 14, 15	56.7	3.33 (d, 9.7)	1, 3, 4
3	141.6	6.50 (d, 11.9)	1, 4, 6, 18	140.8	6.79 (d, 9.7)	4, 5, 6, 18
4	120.6			131.5		
5	40.0	3.12–2.27	3, 4, 6, 7, 18	32.2	3.28–2.38	3, 4, 6, 18
6	80.2	5.15 (m)		78.4	5.14 (m)	
7	150.2	7.54 (d, 1.7)	6, 19	148.1	6.71 (s)	6
8	132.8			133.0		
9	24.4	2.42 (m)	11	24.9	2.67–2.20 (m)	7, 11
10	25.9	3.18–2.33 (m)		27.3	2.72–2.30 (m)	
11	141.9	5.70 (dd, 11.1, 4.4)	9, 13, 20	132.8	5.52 (dd, 10.5, 2.9)	20
12	133.3			134.6		
13	32.7	2.78–2.29 (m)	1, 11, 12, 20	24.0	2.29–2.10 (m)	1, 11, 12, 14
14	29.5	2.48–2.29 (m)	1, 2, 11, 12, 13	23.5	2.10–1.92 (m)	1, 12, 13
15	33.5	2.41 (m)	1, 2, 16, 17	31.7	1.83 (m)	1, 3, 14
16	22.4	1.03 (d, 6.8)	1	19.5*	1.01 (d, 7.1)	1, 15, 17
17	21.3	1.06 (d, 6.8)	1	17.6*	1.11 (d, 6.9)	1, 15, 16
18	167.3			166.8		
19	174.0			174.5		
20	168.4			169.5		
21	51.6	3.76 (s)	18	52.4	3.78 (s)	18
22	51.5	3.67 (s)	20	51.7	3.71 (s)	20

* May be interchanged.

ence of an epoxide. HMQC and HMBC experiments allowed the unambiguous definition of the structure of **2**. Especially, HMBC correlations of the methine proton at δ 1.83 ppm with C-1/C-3, HMBC correlations of the methine proton at δ 3.33 ppm with C-1 and of the methylene protons at δ 2.10–1.92 ppm with C-1 fixed the position of the epoxide on C-1/C-2. An accurate study of HMBC spectrum secured the three trisubstituted olefins (see Table 1).

The relative configuration was established through NOESY spectrum. Starting from a conventional α orientation of C-15 in cembranes from the order of Alcyonacea,[6] a NOESY correlation of the methine proton at δ 1.83 ppm with signal at δ 1.92 ppm allowed identification of H-14b, which also gave a NOESY correlation with the proton H-2 at δ 3.33 ppm. Moreover, H-2 gave a NOESY correlation with H-5b at δ 2.38 ppm, while the proton H-5a at δ 3.28 ppm gave a NOESY correlation with the proton H-6 at δ 5.14 ppm, placing H-6 in β position. Furthermore, chemical shift of H-3 at δ 6.79 ppm was in favour of a *Z* configuration for the 3–4 double bond; chemical shift of H-11 at δ 5.52 ppm suggested a *Z* configuration for the second double bond.[7] The structure of compound **2** was illustrated above.

Compounds **1** and **2** proved inactive until 250 μg/disc in antibiotic assays.

Acknowledgements

We thank Ms. M. J. d'Hondt for identification of the soft coral, Mr. G. Gastine for his assistance in the antibacterial tests, Mr. A. Blond and Miss C. Caux for NMR spectra and Mr. J. P. Brouard and Ms. N. Morin for low- and high- resolution mass spectra, respectively.

References

1. Faulkner, D. J. *Nat. Prod. Rep.* **2001**, *18*, 1–49 and earlier reports in the series.
2. Anjaneyulu, A. S. R.; Venkateswara Rao, G. *J. Indian Chem. Soc.* **1997**, *74*, 272–278.
3. Coll, J. C. C. *Chem. Rev.* **1992**, *92*, 613–631.
4. A voucher specimen is available from the Museum National d'Histoire Naturelle in Paris as collection number oct.s.2002.02.
5. Iwagawa, T.; Nakashima, R.; Takayama, K.; Okamura, H.; Nakatani, M.; Doe, M.; Shibata, K. *J. Nat. Prod.* **1999**, *62*, 1046–1049.
6. Scheuer, P. J. *Marine Natural Products*, *Chemical and Biological Perspectives*, *Terpenoids from Coelenterates*; Academic Press: New York, 1978; pp. 247–296.
7. Jackman, L. M.; Sternhell, S. *Applications of Nuclear Magnetic Resonance Spectroscopy in Organic Chemistry*; Pergamon Press: Braunschweig, 1969; pp. 184–185.