Flexusines A and B and Epimukulol from the Soft Coral *Sarcophyton flexuosum*

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Three new cembranes, flexusines A (**1**) and B (**2**) and an epimukulol (**3**), were isolated from the soft coral *Sarcophyton flexuosum* collected near Reunion Island, Indian Ocean. Their structures were elucidated using 1D and 2D NMR spectral analyses.

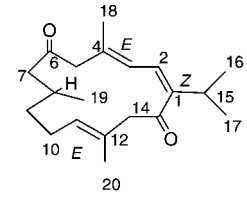
Soft corals are known to contain secondary metabolites with unique structures, and most of them exhibit various biological activities.[1,2] Cembranoid diterpenes are quite common in soft corals, particularly in the genus *Sarcophyton*.[3–7] Many of these compounds are ichthyotoxic,[8] protecting the soft coral from predation,[9–11] and also possess interesting pharmacological properties such as antitumor[12,13] or antimicrobial[14] activities. In our continuing studies to discover bioactive compounds from marine organisms,[15,16] three new cembranes (**1**−**3**) (Chart 1) were isolated and characterized from the soft coral *Sarcophyton flexuosum* collected at the Pointe des Aigrettes at Saint-Gilles in Reunion Island. We herewith describe their isolation and structure elucidation.

Compound **1** was isolated as a colorless oil, $[\alpha]^{25}_{D}$ +290 (*c* 0.26, $CHCl_3$). The mass and ^{13}C NMR spectra established the molecular formula $C_{20}H_{30}O_2$, requiring six degrees of unsaturation. The IR spectrum of **1** revealed the presence of carbonyl groups at 1700 and 1667 cm^{-1} and olefinic groups at 1659 cm^{-1} corresponding to a conjugated double bond. Resonances due to two carbonyl groups (δ 208.2 and 204.9) and three trisubstituted double bonds (δ 150.2, 134.5, 130.2, 126.9, 124.5, and 121.2) in the ^{13}C NMR spectrum accounted for five degrees of unsaturation, indicating that **1** was monocyclic. The molecular formula and the presence of an isopropyl and three methyl groups suggested a cembranoid.[17] Moreover, the ^{13}C NMR and ^{1}H NMR spectra showed the presence of five methyl groups at δ 22.4, 21.7, 21.2, 20.3, and 19.5 (Table 1).

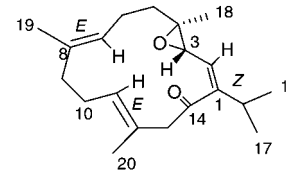
The COSY and HMBC correlations established the planar structure of **1**. The downfield chemical shifts of H-15 (δ 3.05) and the HMBC correlations (Table 1) of H-2, H-15, H-16, and H-17 with C-1 suggested the presence of a double bond at C-1. In the same way, the positions of the two remaining double bonds were located at C-3 and C-11.

Finally, the relative stereochemistry was determined by NOESY experiments (Figure 1). A cross-peak between methyls 16 and 17 and H-2 determined the *Z* configuration of the 1(2) double bond. A NOE between methyl 18 and H-2 as well as a weak correlation between H-3 and H-5 determined an *E* configuration for the 3(4) bond (this is in agreement with the absence of an NOE between methyl 18 and H-3, always seen in the case of the *Z* isomer). Finally, an NOE between methyl 20 and H-10 established the *E* configuration for the third double bond. The chemical shift of C-20 (δ 21.2) is in agreement with a *cis* assignment described in some model olefins.[18] Of interest is a transannular NOE between methyl 19 and H-3, establishing the conformation of the 14-membered ring. This compound is a new structure.

Chart 1. Structure Formulas of Compounds **1** to **3** Isolated from *Sarcophyton flexuosum*



1



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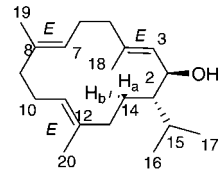

3

Table 1. NMR Spectroscopic Data for Compound **1**[a]

position	δ_C, mult.[b]	δ_H (mult., *J* in Hz)	HMBC
1	150.2 (qC)		
2	121.2 (CH)	6.45 (d, 11.8)	3, 14, 16, 17
3	126.9 (CH)	6.30 (d, 11.8)	1, 2, 4, 18
4	134.5 (qC)		
5a	54.3 (CH_2)	3.55 (d, 12.8)	4, 6, 18
5b		3.25 (d, 12.8)	
6	208.2 (qC)		
7a	49.4 (CH_2)	2.80 (dd, 12.7, 4.4)	19
7b		2.30 (m)	
8	28.8 (CH)	2.35 (m)	19
9a	37.2 (CH_2)	1.85 (m)	19
9b		1.65 (m)	
10	25.4 (CH_2)	2.48 (m)	9, 11
11	130.2 (CH)	5.65 (t, 6.9)	10, 12, 20
12	124.5 (qC)		
13a	53.3 (CH_2)	3.57 (d, 12.2)	12, 14, 20
13b		3.40 (d, 12.2)	
14	204.9 (qC)		
15	31.4 (CH)	3.05 (hept, 6.4)	1, 2, 16, 17
16	21.7 (CH_3)	1.60 (d, 6.4)	1, 15, 17
17	22.4 (CH_3)	1.50 (d, 6.4)	1, 15, 16
18	19.5 (CH_3)	2.15 (s)	3, 4, 5
19	20.3 (CH_3)	1.30 (d, 6.4)	7, 8, 9
20	21.2 (CH_3)	1.88 (s)	11, 12, 13

[a] In benzene-d_6, 500 MHz for ^{1}H and 100 MHz for ^{13}C. [b] Attached protons were determined by DEPT experiments.

Flexusine B (**2**) was obtained as a colorless oil, $[\alpha]^{25}_{D}$ −40 (*c* 0.10, $CHCl_3$). The molecular formula $C_{20}H_{30}O_2$, established by ^{1}H

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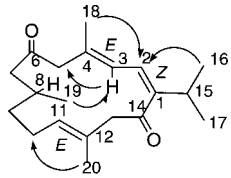

Figure 1. Key NOEs of compound **1**.

Table 2. NMR Spectroscopic Data for Compound **2**[a]

position	δ_C, mult.[b]	δ_H (mult., *J* in Hz)	HMBC
1	150.7 (qC)		
2	142.5 (CH)	5.90 (d, 9.3)	3, 16, 17
3	52.3 (CH)	3.45 (d, 9.3)	1, 2, 4, 18
4	59.4 (qC)		
5a	36.4 (CH_2)	1.91 (m)	4, 6, 18
5b		1.70 (m)	
6	24.0 (CH_2)	2.25 (m)	5, 7
7	123.8 (CH_2)	4.91 (d, 10.8)	6, 8, 19
8	132.6 (qC)		
9a	39.2 (CH_2)	1.92 (m)	8, 10, 19
9b		1.72 (m)	
10	23.9 (CH_2)	2.08 (m)	9, 11, 12
11	129.7 (CH)	5.05 (t, 7.1)	10, 12, 20
12	126.2 (qC)		
13a	52.3 (CH_2)	3.45 (d, 12.4)	12, 14, 20
13b		3.25 (d, 12.4)	
14	205.0 (qC)		
15	29.2 (CH)	3.00 (m)	1, 2, 16, 17
16	19.7 (CH_3)	1.05 (d, 6.8)	1, 15, 17
17	22.1 (CH_3)	1.08 (d, 6.8)	1, 15, 16
18	16.2 (CH_3)	1.15 (s)	3, 4, 5
19	14.0 (CH_3)	1.60 (s)	7, 8, 9
20	15.5 (CH_3)	1.63 (s)	11, 12, 13

[a] In $CDCl_3$, 500 MHz for ^1H and 100 MHz for ^{13}C. [b] Attached protons were determined by DEPT experiments.

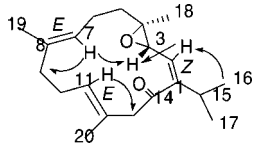

Figure 2. Key NOEs of compound **2**.

and ^{13}C NMR spectra, was identical to that of **1**. The IR absorption of **2** at ν_{max} 1659 cm^{-1} and at ν_{max} 1667 cm^{-1} revealed the presence of olefinic groups and a conjugated carbonyl group. This was further supported by the ^{13}C NMR signals at δ 150.7 (qC), 142.5 (CH), 132.6 (qC), 129.7 (CH), 126.2 (qC), and 123.8 (CH_2) (Table 2), corresponding to three trisubstituted double bonds positioned at 1(2), 7(8), and 11(12) according to the COSY and HMBC correlations.

A ^{13}C NMR resonance at δ 205 ppm was assigned to a carbonyl carbon and located at C-14 by HMBC correlations between H-13 and C-14 (δ 3.45 and 3.25). The main differences between compounds **1** and **2** were signals at δ 59.4 and 52.3 ppm, suggesting the presence of a trisubstituted epoxide in molecule **2**. The chemical shift of H-3 (δ 3.25 ppm) and the HMBC correlations between H-3 and C-2, H-3 and C-4, and H-3 and C-18 indicated that the epoxide was located at C-3. The planar structure of compound **2** was established from the COSY and HMBC correlations (Table 2).

The relative stereochemistry of **2** was established on the basis of NOESY data. NOESY correlations (Figure 2) between H-2 and methyls 16 and 17 established the *Z* configuration of the 1(2) double bond. NOEs between methyl 18 and H-2 and between H-3 and H-7 indicated a *trans* epoxide. The absence of an NOE between methyl 19 and H-7 as well as the chemical shifts of the allylic carbon atoms[19] established the *E* configuration of the 7(8) bond. Finally, an NOE between H-11 and H-13 determined the *E* configuration of 11(12). The NOE between H-3 and H-7 indicates reduced flexibility of the macrocycle.

Table 3. NMR Spectroscopic Data for Compound **3**[a]

position	δ_C, mult.	δ_H (mult., *J* in Hz)	HMBC
1	49.1 (CH)	1.45 (m)	2, 3, 14, 15, 16, 17
2	69.2 (CH)	4.18 (t, 9.3)	1, 3, 4, 15
3	129.2 (CH)	5.21 (d, 9.3)	1, 2, 4, 18
4	136.0 (qC)		
5a	38.7 (CH_2)	2.30 (m)	4, 6, 18
5b		1.98 (m)	
6	24.2 (CH_2)	2.25 (m)	5, 7
7	125.2 (CH)	4.80 (t, 6.3)	6, 9, 19
8	132.5 (qC)		
9a	39.4 (CH_2)	1.98 (m)	8, 10, 19
9b		1.75 (m)	
10	23.4 (CH_2)	2.10 (m)	9, 11
11	122.8 (CH)	5.10 (t, 6.9)	10, 12, 20
12	134.7 (qC)		
13a	38.6 (CH_2)	2.08 (m)	11, 12, 14, 20
13b		1.90 (m)	
14a	26.1 (CH_2)	1.40 (m)	1, 12, 13
14b		1.19 (m)	
15	26.9 (CH)	2.30 (m)	1, 16, 17
16	18.4 (CH_3)	1.01 (d, 6.8)	1, 15, 17
17	20.5 (CH_3)	1.03 (d, 6.8)	1, 15, 16
18	15.1 (CH_3)	1.76 (s)	3, 4, 5
19	14.5 (CH_3)	1.61 (s)	7, 8, 9
20	15.8 (CH_3)	1.63 (s)	11, 12, 13

[a] In benzene-d_6, 500 MHz for ^1H and 100 MHz for ^{13}C. Attached protons were determined by DEPT experiments.

Compound **3** (25 mg) was isolated as a colorless oil, $[\alpha]^{25}_D$ +24 (*c* 0.13, $CHCl_3$). On the basis of its mass and ^{13}C NMR data, the molecular formula was established as $C_{20}H_{34}O$. The IR spectrum of this compound suggested the presence of a hydroxyl group (ν_{max} 3400 cm^{-1}) and olefinic groups (ν_{max} 1659 cm^{-1}). In the ^{13}C NMR spectrum (Table 3), the main difference between compound **3** and **1** and **2** was the absence of carbonyl groups and the presence of a carbon at δ 69.2 (C-2) corresponding to a methinoxy group [δ 4.18 (1H, t, $J = 9.3$ Hz)] (Table 3). The COSY and HMBC experiments established that the planar structure was identical with that of mukulol, reported earlier from guggulu (the resin from *Commiphora mukul*).[20–22]

The *cis* relationship between H-2 and H-16 and H-17 was determined by NOEs. Moreover, Scheuer demonstrated that cembranes from the order Alcyonacea possess an α-oriented isopropyl group at C-1.[23] A NOESY correlation between H-15 (δ 2.30) and the signal at δ 1.19 identified H-14b, which also gave a NOESY correlation to H-2 (δ 4.18). Consequently, protons H-2 and H-14b have the same orientation, and consequently, H-2 and the isopropyl group also have the same orientation. The all-*E* configuration for the three double bonds was confirmed on the basis of the allylic carbon resonances[19] as well as the absence of NOEs between the vinyl-methyls and the neighbor vinyl-protons, *vide supra*. Since the positive optical rotation of **3**, $[\alpha]^{25}_D$ +24, is different from that obtained in the case of mukulol ($[\alpha]^{25}_D$ +53),[21] we can conclude that compound **3** probably corresponds to epimukulol, an epimer of mukulol at the C-2 position. Various syntheses of this cembrane natural product have been achieved.[24,25]

Experimental Section

General Experimental Procedures. Optical rotations were measured on a JASCO DIP-1000 digital polarimeter. IR spectra were recorded on a Hitachi I-2001 infrared spectrophotometer. ^1H and ^{13}C NMR spectra were recorded on Bruker ARX-500 and Avance-400 spectrometers. ^1H, ^{13}C, COSY, HSQC, and HMBC were recorded using standard Bruker pulse sequences. HRMS measurements were recorded on an Autospec Q instrument. Solvents used were HPLC EtOAc and MeOH, SDS CH_2Cl_2, *n*-hexane, and $CHCl_3$. An LH-20 (20 cm, 1.5 × 30 cm) column was used for separation of compounds, and VLC (3.5 cm, 1.5 × 14 cm) columns were used for separation and purification.

Biological Material. The specimen of *Sarcophyton flexuosum* (phylum Cnidaria, class Anthozoa, subclass Octocorallia, order Alcyonacea, family Alcyoniidae) was collected near Reunion Island, Indian Ocean, and immediately frozen. It was collected from 2−6 m water depth at Pointe des Aigrettes.

Extraction and Isolation. The freeze-dried soft coral (190 g dry weight) was extracted with $CHCl_3$/MeOH (1:1) at room temperature. The crude extract (850 mg) was separated over Sephadex LH-20 with *n*-hexane/CH_2Cl_2/MeOH (2:1:1). Further separations were done on silica gel columns (VLC) eluted with *n*-hexane and EtOAc to afford with 15% EtOAc, in the following order, **1** (28 mg), **3** (25 mg), and **2** (5 mg).

Flexusine A (1): colorless oil, $[\alpha]^{25}_D$ +290 (*c* 0.26, $CHCl_3$); IR 1700, 1667, 1659 cm^{-1}; NMR data, see Table 1; HRMS *m*/*z* 302.2250 (calcd for $C_{20}H_{30}O_2$ *m*/*z* 302.2246).

Flexusine B (2): colorless oil, $[\alpha]^{25}_D$ −40 (*c* 0.10, $CHCl_3$); IR 1667, 1659 cm^{-1}; NMR data, see Table 2; HRMS *m*/*z* 302.2252 (calcd for $C_{20}H_{30}O_2$ *m*/*z* 302.2246).

Epimukulol (3): colorless oil, $[\alpha]^{25}_D$ +24 (*c* 0.13, $CHCl_3$); IR 3400, 1659 cm^{-1}; NMR data, see Table 3; HRMS *m*/*z* 290.2618 (calcd for $C_{20}H_{34}O$ *m*/*z* 290.2610).

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