New Cembranoid Diterpenes from the Soft Coral *Sarcophyton ehrenbergi*[1]

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From the dichloromethane extract of the soft coral *Sarcophyton ehrenbergi*, three new [(1*R**,4*E*,8*E*,12*S**,13*E*)-cembra-4,8,13-triene-1,12-diol (sarcophytol T, **1**), (1*E*,3*E*,7*E*,11*R**12*R**)-15-(acetoxymethyl)cembra-11,12-epoxy-1,3,7-triene (**2**), [1*E*,3*R**,4*R**,7*E*,11*R**,12*R** or (11*S**,-12*S**)]-15-(acetoxymethyl)cembra-3,4:11,12-diepoxy-1,7-diene (**3**)] and two known [isoneocembrene A (**4**) and (2*S**,11*R**,12*R**)-isosarcophytoxide (**5**)] cembranoid-based diterpenes were isolated.

Cembranoid diterpenes are secondary metabolites characteristic of several genera of soft corals, in particular *Lobophytum*, *Sinularia*, and *Sarcophyton*.[2] Ecologically, these compounds have been found to assist the producing animal when it is in direct competition with other reef organisms.[3,4] Screening of cembranoids in therapeutically relevant assay systems has shown some to have cytotoxic,[5] cancer chemopreventative, and antiinflammatory[6] potential. Recently, the discovery of the potent inhibition of Ras farnesyl transferase by a cembranolide from *Lobophytum christagalli* (IC_{50} 0.15 μM)[7] has further enhanced the interest in this group of secondary metabolites. The current report focuses on the isolation and characterization of three new and two known cembranoid diterpenes from the CH_2Cl_2 solubles of an Australian sample of *Sarcophyton ehrenbergi* von Marenzeller (1886) (Alcyoniidae, Octocorallia).

MS and ^{13}C NMR analysis of **1** indicated it to have the molecular formula $C_{20}H_{34}O_2$. From its ^1H, ^{13}C NMR, MS, and IR data it was evident that the molecule contained two tertiary alcohol functions (78.7 s, 74.2 s ppm, 3500 cm^{-1}), and three nonconjugated (the molecule lacks UV activity at 254 nm) carbon−carbon double bonds (126.4 d, 127.6 d, 129.0 d, 133.3 s, 136.4 s, 137.6 d ppm). As the molecular formula of **1** required it to have four elements of unsaturation, **1** had to be monocyclic. More detailed analysis of its spectroscopic data, particularly the NMR data, suggested **1** to be a cembranoid-based diterpene. To resolve the positioning of the deduced functionality extensive 2D NMR measurements were necessary, as many of the proton resonances were degenerate. As the resonance for H-15 [δ 1.58 (sep, J = 7.0 Hz)] showed coupling only to the resonances for H_3-16 [δ 0.85 (d, J = 7.0 Hz)] and H_3-17 [δ 0.85 (d, J = 7.0 Hz)], C-1 must be fully substituted. Because H_3-16, H_3-17, H-13 [δ 5.94 (d, J = 15.5 Hz)], and H-14 [δ 5.64 (d, J = 15.5 Hz)] all showed long-range $^1H-^{13}C$ coupling to the carbon resonance at 78.7 (s) ppm, it was evident that this signal originated from C-1 and was thus the site of one of the OH groups. Further, long-range $^1H-^{13}C$ couplings from H_3-20 (δ 1.32 s), H-13, and H-14 to a carbon with resonance at 74.2 s ppm indicated C-12 to be the location of the second hydroxyl function. The resonance for H_3-20 had a cross peak with the resonances for C-11 (44.8 t ppm), placing the C-11 methylene group adjacent to C-12. In the $^1H-^1H$ COSY spectrum of **1** couplings between H_2-11 (δ 1.58 m, 1.94 m, and H_2-10 (δ 2.02 m, 2.34 m), H_2-10 and H-9 [δ 5.32 (br t, J = 7.4 Hz)], and H-9 and H_3-19 (δ 1.59 s), confirmed a further part of the molecule, C-12 through C-8 to C-19. C-19 (14.8 q ppm) long-range coupled to H_2-7 (δ 1.93 m, 2.18 m), from where a continuous chain of $^1H-^1H$ couplings could be traced to H_2-6 (δ 2.01 m, 2.23 m), H-5 [δ 5.02 (d, J = 6.6 Hz)], and H_3-18. The resonance for H_3-18 showed long-range coupling, in the HMBC spectrum, with C-4 (136.4 s ppm) and C-3 (36.2 t ppm), with H_2-3 $^1H-^1H$ coupling to H_2-2. The remaining connection between C-1 and C-2 followed from cross peaks in the HMBC spectrum of **1** between the resonance for C-1 and H_2-2 and H_2-3. With the 2D structure of **1** established, the geometry of three carbon−carbon double bonds and the configurations at two chiral centers remained to be assigned. $\Delta^{13,14}$ was assigned the E geometry as $J_{H13-H14}$ = 15.5 Hz. On the basis of the ^{13}C NMR chemical shifts for CH_3-18 and CH_3-19 (<20 ppm),[8] both $\Delta^{4,5}$ and $\Delta^{8,9}$ were also defined as E. The relative configurations at C-1 and C-12 were deduced from the NOESY spectral data of **1**. Thus, cross peaks observed between 1-O*H* (δ 2.36 s), H-13 [δ 5.94 (d, J = 15.5 Hz)], and H-5 [δ 5.20 (d, J = 6.6 Hz)], and between H-5 and H-13 and H-14 [δ 5.64 (d, J = 15.5 Hz)], as well as between H_3-20 (δ 1.32 s) and H-13, indicated the hydroxyl at C-1 and CH_3-20 both to be on the same side of **1**. Thus, in a relative sense, C-1 and C-12 were assigned the R* and S* configurations, respectively. Hence, **1** is (1*R**,4*E*,8*E*,12*S**,13*E*)-cembra-4,8,13-triene-1,12-diol, for which the trivial name of sarcophytol T is proposed.

Mass spectral analysis of **2** indicated it to have the molecular formula $C_{22}H_{34}O_3$. The six elements of unsaturation implied by the molecular formula of **2** were accounted for by the presence of a carbonyl function (part of an acetate), three carbon−carbon double bonds, a three-membered epoxide, and one other ring, as deduced from its ^1H, ^{13}C NMR, IR, and MS data. These spectroscopic data also indicated **2** to be a cembranoid diterpene, hence accounting for the second ring. Com-

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Table 1. ^{13}C NMR (75.5 MHz, $CDCl_3$) Data for Compounds **1**−**3** and **5**

carbon	1	2	3	5
1	78.7 s[a]	141.4 s	145.9 s	132.6 s
2	33.1 t	121.5 d	123.2 d	83.4 d
3	36.2 t	120.7 d	59.1 d	126.6 d
4	136.4 s	137.1 s	61.5 s	139.9 s
5	126.4 d	38.6 t	37.4 t	38.9 t
6	23.9 t	25.3 t	22.2 t	24.3 t
7	39.0 t	126.9 d	126.1 d	125.7 d
8	133.3 s	133.6 s	134.8 s	133.2 s
9	127.6 d	36.7 t	36.7 t	36.7 t
10	23.3 t	24.5 t	24.5 t	23.8 t
11	44.8 t	60.1 d	62.1 d	62.3 d
12	74.2 s	60.8 s	61.1 s	61.4 s
13	137.6 d	36.3 t	39.0 t	37.4 t
14	129.0 d	24.7 t	27.7 t	22.5 t
15	38.7 d	38.8 d	39.5 d	128.3 s
16	16.6 q	68.4 t	67.5 t	78.3 t
17	17.6 q	17.3 q	17.1 q	9.9 q
18	14.7 q	17.3 q	18.2 q	14.6 q
19	14.8 q	15.0 q	14.8 q	14.7 q
20	29.3 q	18.2 q	16.2 q	15.7 q
OAc		171.1 s	170.9 s	
		21.0 q	20.9 q	

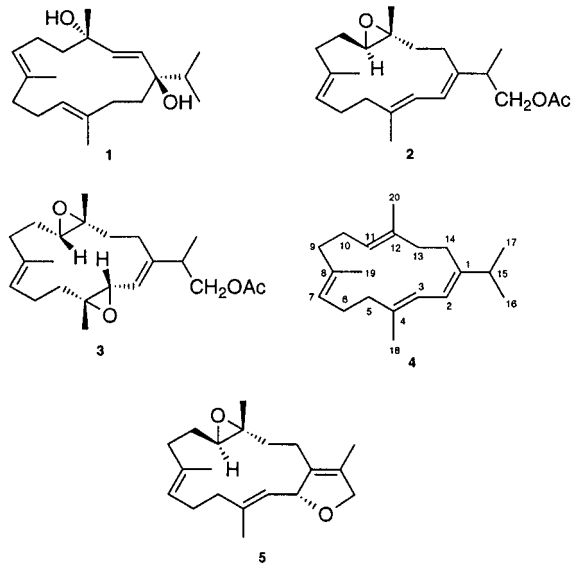
[a] Multiplicity by DEPT, s = C, d = CH, t = CH_2, q = CH_3.

pound **2** had many structural features in common with the known metabolites **4** and **5**, in particular, the conjugated diene system (C-1 to C-4), the 11,12 epoxy function, and the $\Delta^{7,8}$ double bond. Analysis of the 1D and 2D NMR data (see Tables 1 and 2), supported these deductions and allowed the position of the acetate function to be determined. Thus, from the HMBC spectrum it was evident that H_2-16 [δ 3.93 (dd, J = 7.6, 10.7 Hz), 4.04 (dd, J = 6.6, 10.7 Hz)] long-range coupled to C-21 (171.1 ppm); and from the $^1H-^1H$ COSY spectrum, that H_2-16 coupled with H-15 [δ 2.49 (ddq, J = 6.6, 7.1, 7.6 Hz)], which further coupled to H_3-17 [δ 1.07 (d, J = 7.1 Hz]. Further, in the HMBC spectrum cross peaks also indicated that H_3-17 long-range coupled to C-1 (141.4 s ppm), C-15 (38.8 d ppm), and C-16 (68.4 t ppm), thus confirming the position of the acetylated isopropyl moiety. The geometry of the three carbon−carbon double bonds was 1*E*, 3*E*, and 7*E*, on the basis of ^{13}C NMR data comparison made with equivalent data for **4**, as well as with those reported by Kobayashi et al., for sarcophytols K and P.[9] The configurations at C-11 and C-12, and hence of the epoxide, were both concluded to be *R** on the basis of the NOE interaction observed between H-11 and H_2-13. Thus, the new natural product **2** is best described as (1*E*,3*E*,7*E*,11*R**,-12*R**)-15-(acetoxymethyl)cembra-11,12-epoxy-1,3,7-triene.

^1H, ^{13}C NMR, IR, and MS analysis ($C_{22}H_{34}O_4$) of **3** showed it to be a monoacetylated cembranoid diterpene, which contained two epoxide functions and two carbon−carbon double bonds. Comparison of the ^1H and ^{13}C NMR data of **3** with those of **2** indicated the two molecules to be closely related. The obvious spectroscopic differences between the two resulted from the presence of a second epoxide function in **3**, instead of one of the double bonds found in **2**. As **3** was not UV-active, and the fact that the resonance assigned to H-2 [δ 5.04 (d, J = 6.6 Hz)] showed a cross peak in the $^1H-^1H$ COSY spectrum to the signal at δ 3.32 (d, J = 6.6 Hz), an epoxy proton (H-3) other than H-11, the second epoxy-bridge was placed between C-3 and C-4. The geometries of the two carbon−carbon bonds were deduced both to be *E* on the basis of NOE interactions between H-2 and H_2-16 for $\Delta^{1,2}$, and from the ^{13}C NMR chemical shift of CH_3-19 (14.8 q ppm) for $\Delta^{7,8}$.[8] From the NOESY spectrum of **3**, cross peaks between H-3 and H_2-5, H-11, and H_2-13, and between H_3-18 and H-2, as well as between H-11 and H_2-13 clearly gave the *R** configuration to C-3 and C-4, and either the *R** to both C-11 and C-12 or *S** to both. Thus, in a relative sense **3** is [1*E*,3*R**,4*R**,7*E*,11*R**,12*R** or (11*S**,12*S**)]-15-(acetoxymethyl)cembra-3,4:11,12-diepoxy-1,7-diene. The structural representation of **3** shows only the 3*R**,4*R**,-11*R**,12*R** configuration.

Compound **4** had physical and spectroscopic characteristics consistent with it being isoneocembrene A,[10,11] an isomer of the termite trail pheromone neocembrene A. Compound **5** was readily identified from its ^1H and ^{13}C NMR (see Table 1) spectroscopic data as (2*S**,11*R**,-12*R**)-isosarcophytoxide (**5**).[12,13]

The most commonly encountered functionalizations of the isopropyl (C-15, C-16, and C-17) part of soft-coral-derived cembranes occur through lactonization or epoxidation to generate either γ-lactones[2] or furans.[12,13] The acetoxyl group, found at C-16 of the isopropyl moiety in compounds **2** and **3**, is, to date, without precedent.



Experimental Section

General Experimental Procedures. The procedures were as previously described.[14]

Animal Material. All animals were collected in May 1983, from Old Reef, Great Barrier Reef, Queensland, Australia, from a depth of 9 m. After collection, animals were deep frozen and freeze-dried prior to extraction. The voucher specimen is stored at the Museum and Art Galleries of the Northern Territory, Darwin, Australia, voucher no. NTM C12522.

Extraction and Isolation. Freeze-dried material (35.3 g) was extracted with CH_2Cl_2 (2 L) followed by MeOH (2 L). From both extracts 5.67 g (16.1%) of CH_2Cl_2 solubles were obtained. Initial separation of this extract by VLC over Si gel, employing hexane containing increasing proportions of EtOAc as eluent, gave 10 fractions, each of 90 mL. ^1H NMR and TLC analysis of all VLC fractions indicated fractions 1 and 5 to be of

Table 2. ^{1}H NMR (300 MHz, $CDCl_3$) Data for Compounds **1**−**3**[a]

proton	1	2	3
2	1.63 (br d, J = 13.1 Hz) 1.85 (ddd, J = 3.3, 12.9, 13.1 Hz)	5.96 (d, J = 10.7 Hz)	5.04 (d, J = 6.6 Hz)
3	2.07 m, 2.25 m	5.84 (d, J = 10.7 Hz)	3.32 (d, J = 6.6 Hz)
5	5.20 (d, J = 6.6 Hz)	2.17 m	1.50 m, 2.04 m
6	2.01 m, 2.23 m	2.23 m	1.38 m, 2.14 m
7	2.18 m, 1.93 m	5.22 (br dd, J = 5.8, 6.6 Hz)	5.28 (br t, J = 6.1 Hz)
9	5.32 (br t, J = 7.4 Hz)	2.13 m, 2.27 m	2.08 m, 2.28 m
10	2.02 m, 2.34 m	1.57 m, 1.72 m	1.31 m, 2.11 m
11	1.58 m, 1.94 m	2.87 (dd, J = 6.2, 6.2 Hz)	2.66 (dd, J = 3.3, 10.2 Hz)
13	5.94 (d, J = 15.5 Hz)	1.49 m, 2.00 m	1.13 m, 2.18 m
14	5.64 (d, J = 15.5 Hz)	1.99 m, 2.21 m	2.05 m, 2.25 m
15	1.58 (sep, J = 7.0 Hz)	2.49 (ddq, J = 6.6, 7.1, 7.6 Hz)	2.48 (ddq, J = 6.6, 6.7, 6.9 Hz)
16	0.85 (d, J = 7.0 Hz)	3.93 (dd, J = 7.6, 10.7 Hz) 4.04 (dd, J = 6.6, 10.7 Hz)	3.95 (dd, J = 6.7, 10.8 Hz) 4.04 (dd, J = 6.6, 10.8 Hz)
17	0.85 (d, J = 7.0 Hz)	1.07 (d, J = 7.1 Hz)	1.06 (d, J = 6.9 Hz)
18	1.67 br s	1.72 (d, J = 1.2 Hz)	1.24 s
19	1.59 s	1.58 br s	1.64 s
20	1.32 s	1.26 s	1.28 s
OH	2.07 br s, 12-O*H* 2.37 s, 1-O*H*		
acetate CH_3		2.03 s	2.03 s

[a] All assignments are based on extensive 1D and 2D NMR experiments, including COSY90, HMQC, HMBC, HETCOR, NOE difference, and NOESY.

further interest. Normal-phase HPLC separation (Me_2CO−hexane, 7:93) of VLC fraction 5 yielded three cembranoid diterpenes, **1**−**3**. HPLC separation (hexane) of VLC fraction 1 employing normal-phase silica yielded **4**. The residue from the first HPLC separation (Me_2CO−hexane, 7:93) of VLC fraction 5 was collected and rechromatographed, again using normal-phase silica (EtOAc−hexane, 7:93) to yield **5**.

Sarcophytol T (1): 10.3 mg, 0.029%, an oil; $[\alpha]^{25}_D$ −6.5° (*c* 1.0, $CHCl_3$); IR υ_{max} 3500, 2930, 1470 cm^{-1}; ^{1}H NMR, see Table 1; ^{13}C NMR, see Table 2; EIMS *m*/*z* (rel int); 288 ([M − H_2O]$^+$, 1), 270 (12), 245 (14),227 (62), 159 (25); HREIMS *m*/*z* 288.2378 (calcd for $C_{20}H_{32}O$ 288.2455).

(1*E*,3*E*,7*E*,11*R,12*R**)-15-(Acetoxymethyl)cembra-11,12-epoxy-1,3,7-triene (2):** 5.1 mg, 0.015%, an oil; $[\alpha]25_D$ +54.6° (*c* 0.5, $CHCl_3$); IR υ_{max} 2930, 1740, 1445 cm^{-1}; UV λ_{max} EtOH 245, 249, 259 (sh) (e 8550, 8810, 5710) nm; ^{1}H NMR, see Table 2; ^{13}C NMR, see Table 1; EIMS *m*/*z* (rel int); 346 ([M]$^+$, 1), 328 (2), 302 (15), 286 (38), 243 (40); HREIMS 346.2464 (calcd for $C_{22}H_{34}O_3$ 346.2509).

[1*E*,3*R,4*R**,7*E*,11*R**,12*R** or (11*S**,12*S**)]-15-(Acetoxymethyl)cembra-3,4:11,12-diepoxy-1,7-diene (3):** 5.0 mg, 0.015%, an unstable oil; $[\alpha]_{25}^D$ +1.8° (*c* 0.5, $CHCl_3$); IR υ_{max} 2930, 1740, 1455, 1374 cm^{-1}; ^{1}H NMR, see Table 1; ^{13}C NMR, see Table 2; EIMS *m*/*z* (rel int); 362 ([M]$^+$, >1), 302 (>1), 284 (>1), 243 (1), 149 (60); HREIMS compound decomposed prior to this measurement.

Isoneocembrene A (4): 6.7 mg, 0.019%, an oil with identical physical and spectroscopic properties to those previously published.[10,11]

(2*S,11*R**,12*R**)-Isosarcophytoxide (5):** 166.3 mg, 0.47%, an oil with identical physical and spectroscopic properties to those previously published.[12,13]

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