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STUDIES OF AUSTRALIAN SOFT CORALS, XLIX.[1] A NEW BISCEMBRANOID AND ITS PROBABLE BIOSYNTHETIC PRECURSORS FROM THE SOFT CORAL *SARCOPHYTON TORTUOSUM*

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ABSTRACT.—The isolation of the new biscembranoid methyl neosartortuate acetate [**3**] and its proposed biosynthetic precursors, methyl sarcoate [**2**] and the novel bisepoxide **4**, from the soft coral *Sarcophyton tortuosum* is reported. Structures were elucidated by 1D and 2D nmr spectroscopic techniques.

Soft corals of the genus *Sarcophyton* have been reported to contain a variety of diterpenes, of which cembranoids are the most commonly encountered structural type (2). Unusual dimeric terpenoid skeletons have also been isolated from this genus, and these structures have been proposed to originate by Diels-Alder addition of two different cembranoid units (3). The first such biscembranoid, called methyl isosartortuate [**1**], was isolated by Jingyu *et al.* (3), from *Sarcophyton tortuosum* Tix.-Dur. (Alcyoniidae). This was followed by the isolation of a related compound, methyl sartortuate, by the same authors (4). Two other biscembranoids, methyl sarcophytoate and methyl chlorosarcophytoate, were subsequently reported by Kusumi *et al.* (5) from *Sarcophyton glaucum*. This research group also isolated methyl sarcoate [**2**] (6), a proposed biosynthetic precursor of the two biscembranoids previously isolated from the same coral.

RESULTS AND DISCUSSION

Here we report the isolation and structural elucidation of a new pentacyclic biscembranoid methyl neosartortuate acetate [**3**] together with its proposed biosynthetic precursors methyl sarcoate [**2**] and the novel bisepoxide **4**, from *S. tortuosum*.

The yellow soft coral was first collected at Rib Reef, 80 km north of Townsville, Australia. The sample was freeze-dried and then extracted exhaustively with CH_2Cl_2. Three major terpenoid components were obtained after Si gel chromatography of the crude extract.

Compound **2** was detected in the less polar chromatographic fractions and was identified as methyl sarcoate by comparison of its ^{1}H- and ^{13}C-nmr data with those previously reported (6).

The more polar component **3** was purified by hplc as a highly viscous oil, representing 0.07% of the dried coral. The ^{1}H-nmr spectrum of **3** contained signals for an isopropyl group with doublets at δ 0.85 and 0.96, and a multiplet at 2.00, two trisubstituted epoxide groups with methyl singlets at δ 1.24 and 1.26, and epoxymethine multiplets at δ 2.42 and 3.07, and ^{13}C nmr signals at 59.4, 59.6, 60.1, 60.3 ppm. The presence of one saturated and two conjugated ketonic functions and two ester groups was indicated by signals in the ^{13}C-nmr spectrum of **3** at 210.0, 199.1, 198.7, 170.9, 169.9 ppm. One ester group was a secondary acetate (δ 2.07, three-proton singlet, 5.99, one-

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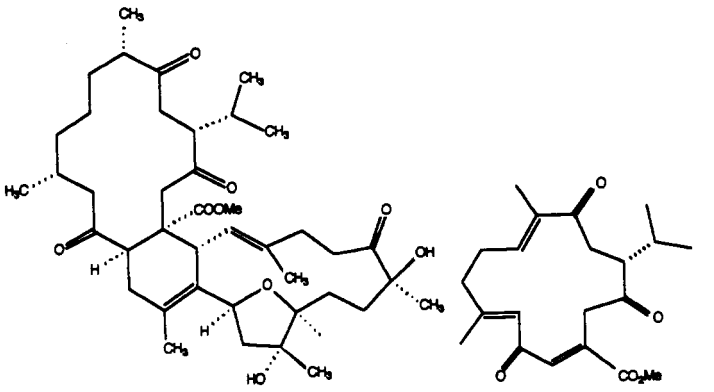

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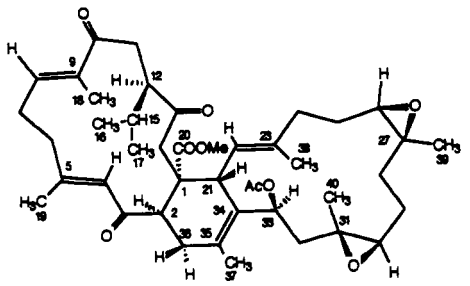

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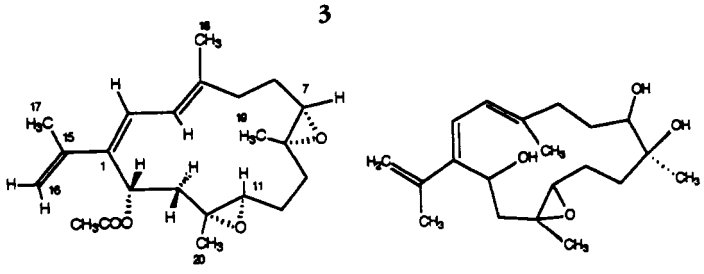

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proton multiplet; 21.3, 68.1, 169.9 ppm), while the other was a methyl ester (δ 3.35, three-proton singlet; 50.8, 170.9 ppm). The ^{13}C-nmr spectrum also indicated the presence of three trisubstituted and one tetra-substituted double bond (CH signals at 127.4, 128.3, 141.0; C signals at 127.3, 131.0, 134.8, 139.2, and 157.6 ppm). Three normal allylic methyl groups resonated as singlets at δ 1.60, 1.68, and 1.70, while a more deshielded methyl singlet at δ 2.13 was assigned to an allylic methyl group, β to a carbonyl group. Three olefinic proton signals appeared as a doublet at δ 5.19 (J=11.1 Hz), a singlet at 5.91, and a multiplet at 6.59. The olefinic proton signal at δ 6.59 was apparently β to a carbonyl group, while the one at δ 5.91 was α to a carbonyl group. The proton signal at δ 4.13 (d, J=11.1 Hz) attached to the carbon atom resonating at 37.1 ppm (XHCORRD), was assigned to a doubly allylic bridgehead position.

The carbon connectivity was determined by analyses of 1D and 2D nmr spectra, including COSY, COSY+RCT, XHCORRD, and COLOC. Compound **3** was shown to have the same carbon framework as that of the previously reported biscembranoids, methyl isosartortuate, methyl sartortuate, methyl sarcophytoate, and methyl chlorosarcophytoate. Full ^{1}H and ^{13}C assignments for **3** are presented in Table 1.

Although present as the major terpenoid component (estimated 0.6% of dried coral), the highly reactive nature of compound **4** and its low tolerance to Si gel

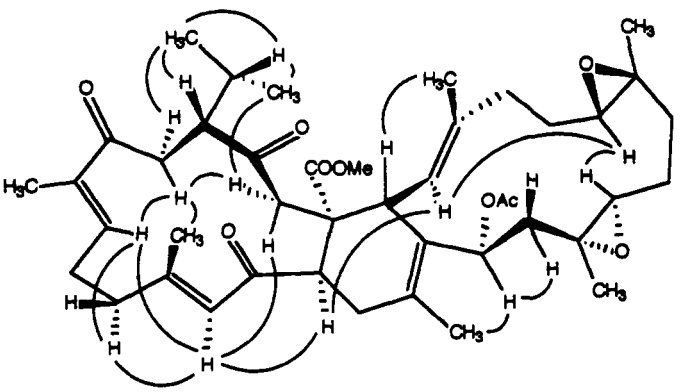
TABLE 1. ^{13}C- and ^{1}H-nmr ($CDCl_3$) Assignments for Methyl Neosartortuate Acetate [**3**] and Compound **4**.

Position	Compound					
	3			**4**		
	δ_C	δ_H mult.	*J* (Hz)	δ_C	δ_H mult.	*J* (Hz)
1	49.7			140.2		
2	48.3	3.05 m		126.3	6.40 d	11.5
3	198.7			122.0	6.53 d	11.5
4	127.4	5.91 bs		141.9		
5	157.6			37.5	2.30 m	
					2.50 dd	2.7, 13.2
6	40.1	2.31 m		26.1	1.55 m	
		2.51 m			2.05 m	
7	26.1	2.53 m		64.4	2.85 dd	2.3, 6.2
		2.67 m				
8	141.0	6.59 m		60.9		
9	139.2			23.3	1.40 m	
10	199.1			34.6	1.40 m	
					1.98 m	
11	31.6	2.11 m		58.4	2.76 dd	3.0, 9.3
		3.48 m				
12	54.6	2.88 m		58.9		
13	210.0			39.5	2.12 dd	2.3, 15.0
					2.60 dd	10.0, 15.0
14	46.1	2.59 m		66.9	5.76 dd	2.3, 10.0
		2.85 m				
15	30.4	2.00 m		136.4		
16	18.3	0.85 d	6.8	115.7	5.08 bs	
					5.27 bs	
17	21.5	0.96 d	6.8	22.7	1.91 s	
18	11.4	1.68 s		15.7	1.78 s	
19	19.2	2.13 s		16.6	1.17 s	
20	170.9			19.4	1.20 s	
21	37.1	4.13 d	11.1	169.5		
22	128.3	5.19 d	11.1	20.9	1.97 s	
23	134.8					
24	35.6	2.13 m				
		2.17 m				
25	26.1	1.60 m				
		1.73 m				
26	61.0	3.07 m				
27	59.4					
28	36.2	1.13 m				
		2.10 m				
29	23.7	1.60 m				
30	60.3	2.38 m				
31	59.6					
32	37.5	1.80 m				
		2.20 m				
33	68.1	5.99 dd	2.7, 11.0			
34	127.3					
35	131.0					
36	32.4	1.88 m				
		2.45 m				
37	19.1	1.78 s				
38	18.7	1.60 s				
39	15.7	1.26 s				
40	18.5	1.24 s				
41	50.8	3.35 s				
42	169.9					
43	21.3	2.07 s				

chromatography made its purification difficult. Reversed-phase hplc separations proved more suitable for purification of this unstable molecule. Compound **4** was identified as being one of the precursors of **3**. In its ^{1}H-nmr spectrum, an AB quartet (doublets at δ 6.40 and 6.53, J=11.5 Hz) indicated the presence of a conjugated diene system, while a pair of broad singlets at 5.08 and 5.27 suggested an exocyclic methylene group. Two allylic methyl groups gave rise to signals at δ 1.78 and 1.91. The remaining functionality was the same as that observed in one half of **3**. Thus, a secondary acetate function was present (δ 5.76, dd, J=2.3, 10.0 Hz; 1.97, s, methyl). The two epoxymethine proton signals resonated at 2.76 (dd, J=3.0, 9.3 Hz) and 2.85 (dd, J=2.3, 6.2) with their respective methyls at δ 1.17 and 1.20. Again, the connectivity was determined by 2D nmr$J$ and shift-correlated spectroscopy (COSY, XHCORRD, and COLOC). Full ^{1}H and ^{13}C assignments for **4** are given in Table 1.

Despite many attempts, neither **3** nor **4** was obtained in crystalline form, and all the relative stereochemistry of each was determined by 2D phase-sensitive NOESY and 1D nOe difference experiments (Figure 1).

The proposed stereochemistry for the isopropyl group in **3** is worthy of comment. It bears the same relative stereochemical relationship to the ring junction as was reported for methyl sartortuate and methyl isosartortuate, whose structures were supported by X-ray analyses (3,4). These X-ray structures indicate that the stereochemistry at C-1 and C-12 is 1R*,12R*. However, for methyl sarcophytoate and methyl chlorosarcophytoate,



3

4

FIGURE 1. NOe's observed in the phase-sensitive NOESY and 1D nOe difference spectra of methyl neosartortuate acetate [**3**] and its proposed precursor **4**. The nOe's not essential for elucidation of the relative stereochemistry have been omitted.

whose structures are based on nmr data (5,6), the structures proposed indicate the relative stereochemistry at C-1 and C-12 as 1*R**,12*S**. (The authors made no comment that this regiochemistry differs from that of the other published dimeric cembranoids.) Assignment was based on observed phase-sensitive NOESY results, which placed the isopropyl group on the α- face of the molecule.

The 1D nOe difference spectra and 2D phase-sensitive NOESY results for methyl neosartortuate acetate [**3**] also placed the isopropyl group on the α face of the molecule. However, a conformation significantly different to that proposed for methyl sarcophytoate (and methyl chlorosarcophytoate) was implied. We have attempted to indicate the suggested conformation in Figure 1. It is clear that the conformation of the A ring differs from that proposed for methyl sarcophytoate, since significant ^{13}C chemical shift differences (on average ca. 2 ppm for the majority of the atoms around the ring) were observed when the spectra of the two compounds were compared.

The Diels-Alder reaction between **2** and **4** arises from the Supra-Supra transition state shown in Figure 2 (i.e., bonding on the α face of **2** and the β face of **4**). This addition mode not only gives the correct regiochemistry for the product but also explains the cis stereochemistry of the bridgehead derived from the dienophile, in addition to the trans geometry of the carbomethoxyl group relative to the doubly allylic bridgehead proton. The proposed transition state corresponds to endo cycloaddition with respect to the α,β-unsaturated ester. Exo cycloaddition (i.e., endo with respect to the α,β-unsaturated ketone), would involve an impossibly encumbered transition state.

It is interesting to note that methyl sarcoate [**2**] appears to be a precursor of all previously described biscembranoids. It is tempting to suggest that the bisepoxide system present in methyl neosartortuate acetate could readily lead to either the tetrahydrofuran system observed in methyl isosartortuate, the tetrahydropyran rings of methyl sartortuate and methyl chlorosarcophytoate, or the dihydropyran ring of methyl sarcophytoate. Indeed, in their paper on methyl sartortuate, Jingyu *et al.* (4) proposed a cembranoid precursor **5** remarkably similar to **4**, although they had not in fact isolated any such precursor. A hydroxyl group at C-33 in the biscembranoid could, by nucleophilic attack at C-30, open the epoxide ring with inversion at that center to yield the tetrahydrofuran system with the stereochemistry observed in methyl isosartortuoate [**1**]. Moreover, the stereochemistry at C-27 for methyl neosartortuate acetate is also the same as for methyl isosartortuate.

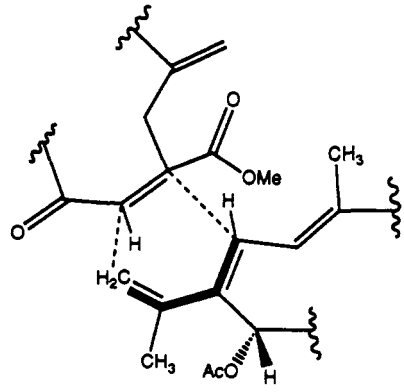


FIGURE 2. Proposed transition state for Diels-Alder reaction for **2** and **4**.

EXPERIMENTAL

GENERAL EXPERIMENTAL PROCEDURES.—Hreims was carried out by the Mass Spectrometry Unit, University of Queensland, and low resolution spectra were determined on a Shimadzu GCMS-QP 2000.

Nmr spectra were recorded on a Bruker AM 300 spectrometer in $CDCl_3$ solutions, using the 1D programs DEPT, INAPT, and NOEDIFF, and 2D programs COSYDQF, COSYRCT, NOESYPH, XHCORRD, and COLOC. Ir spectra (in CCl_4) were recorded on a Perkin-Elmer 1600 series Ft-ir spectrometer and uv spectra (in EtOH) on a Varian uv-visible series 634 spectrophotometer. Optical rotation measurements were made on a Perkin-Elmer 141 polarimeter in CCl_4 solutions. Si gel type 60 (Merck) was used for vlc, and plastic-backed plates coated with Si gel F_{254} (Merck) were used for tlc. Glass plates coated with RP-18 F_{254s} (Merck) were used for reversed-phase tlc. Reversed-phase hplc separations were performed with either an Activon Techsphere 5 μm C-18 or a Whatman Partsil ODS 10 μm column.

CORAL MATERIAL.—The sample of *S. tortuosum* was collected at Rib Reef, 80 km north of Townsville, Australia by scuba diving. A voucher sample (NTM C10794) is lodged in the Northern Territory Museum of Arts and Science, Darwin, NT, Australia.

EXTRACTION AND ISOLATION.—The coral, freed from its substrate, was initially frozen and then freeze-dried. The freeze-dried soft coral (174.0 g) was exhaustively extracted with CH_2Cl_2 to afford the crude extract (8.5 g, 4.9%). This extract was chromatographed on Si gel (vlc) (7) using stepped gradient elution from petroleum ether to CH_2Cl_2, then from CH_2Cl_2 to EtOAc. Fractions which appeared similar by tlc were combined to yield three main fractions which contained terpenoid compounds. These fractions (approximately 1.0 g each) were purified by reversed-phase hplc using MeCN-H_2O (60:40).

Methyl sarcoate [**2**] was isolated from the less polar fractions, and ^1H- and ^{13}C-nmr data were consistent with those reported previously (6). The more polar fractions yielded methyl neosartortuate acetate [**3**] (120 mg), while **4** was isolated from the fractions with intermediate polarity.

Methyl neosartortuate acetate [**3**].—Colorless oil: $[\alpha]_D$ +142° (10 mg/ml, CCl_4); uv λ max (EtOH) 220 nm (10224); ir ν max (CCl_4) 2970, 2934, 1750, 1706, 1684, 1618, 1456, 1404, 1382, 1257, 904 cm^{-1}; ^{13}C and ^1H nmr see Table 1; eims $[M]^+$ *m/z* 720.422 ($C_{43}H_{60}O_9$ requires 720.424) and fragments 702.423 ($C_{43}H_{58}O_8$), 692.434 ($C_{42}H_{60}O_8$), 674.413 ($C_{42}H_{58}O_7$), 661.404 ($C_{41}H_{57}O_7$), 660.404 ($C_{41}H_{56}O_7$), 643.337 ($C_{39}H_{47}O_8$), 642.375 ($C_{37}H_{54}O_9$).

(7R,8R*,11S*,12S*,14R*,1E,3E)-14-Acetoxy-7,8:11,12-Diepoxy-1-isopropylene-4,8,12-trimethylcyclotetradeca-1,3-diene* [**4**].—Compound **4** (20 mg), was obtained as a colorless oil: $[\alpha]_D$ +110° (10 mg/ml, CCl_4); uv λ max (EtOH) 250.2 nm (8765), ir ν max (CCl_4) 2964, 2920, 2393, 2366, 1750, 1446, 1366, 1232, 1015 cm^{-1}; ^{13}C and ^1H nmr see Table 1; low resolution fabms (glycerol matrix) *m/z* $[C_{22}H_{32}O_4+H]^+$ 361, $[C_{22}H_{32}O_4-HOAc+H]^+$ 301.

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