Tetrahedron Vol. 49, No. 3, pp. 515-524, 1993
Printed in Great Britain

0040-4020/93 $5.00+.00

New Cembradiene Diterpenoids from an Undescribed Caribbean Gorgonian of the Genus *Eunicea*

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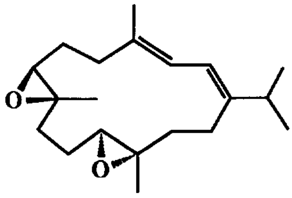
(*Received in USA* 29 *September* 1992; *accepted* 30 *October* 1992)

Abstract: Five new cembradiene diterpenoids (**1-5**) and two previously known compounds (**7** and **8**) have been isolated from an undescribed Caribbean gorgonian of the genus *Eunicea*. The structures of these compounds were assigned on the basis of chemical and spectral studies, and the relative stereochemistry of cembradiene **3** was defined by X-ray crystallographic methods.

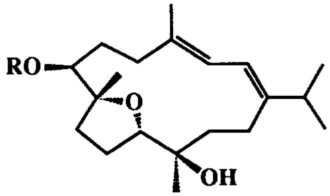
Marine octocorals of the order Gorgonacea, the sea whips and sea fans (Phylum Cnidaria) produce a wide variety of both biologically-active and structurally-unique secondary metabolites.[2,3] In the Caribbean Sea, sea whips of the genus *Eunicea* (Family Plexauridae) are particularly diverse and abundant,[4] and they have been recognized for many years as attractive targets for chemical study. Early reports revealed that these organisms produce diterpenoids of the cembrene class,[5-14] some of which appear to possess defensive properties.[15] Despite their abundance, *Eunicea* species have not been comprehensively investigated due to difficulties in collecting taxonomically-homogeneous samples. Through exhaustive chemical analyses of single *Eunicea* colonies, we have been able to combine "chemotypes" thus providing access to new species or chemical strains which produce unique metabolites.[16-21]

In this paper we report the structures of five new cembrenoid diterpenoids (**1-5**) from an undescribed *Eunicea* sp.[22] In addition, we report the isolation of two diterpenoids (**7** and **8**), isolated previously from Australian soft corals.[23-26]

Eunicea sp.(Voucher # CI86-222)[22] was collected in 1986, as part of an expedition to the Tobago Cays in the eastern Caribbean Sea. Freshly collected animals were stored frozen and subsequently exhaustively extracted with dichloromethane. Compounds **1-5**, **7** and **8** were purified by vacuum flash chromatography of the crude extract followed by silica high performance liquid chromatography (HPLC) of several of the nonpolar and moderately polar fractions.

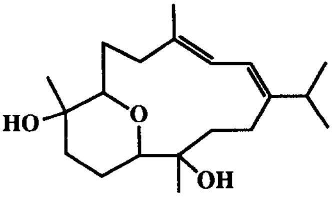


1



2, R=H

3, R=Ac



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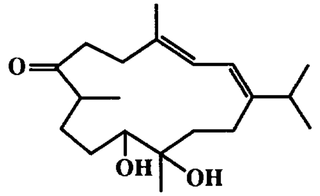

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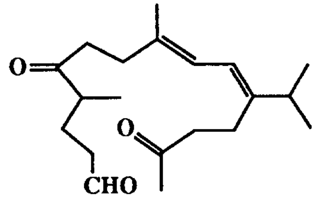

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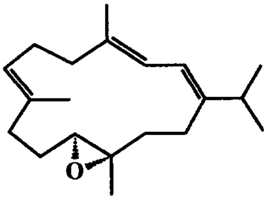

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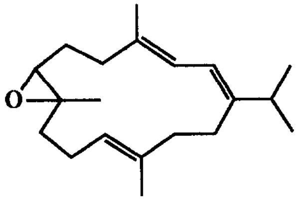

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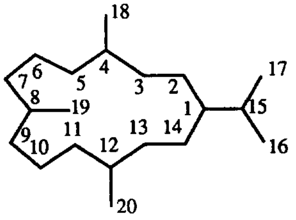

Cembradiene **1** was isolated as a white solid (2% of the extract) which was analyzed for $C_{20}H_{32}O_2$ by high resolution mass and ^{13}C NMR spectrometry. Four low field ^{13}C NMR resonances (Table I) at δ 147.8 (C), 135.2 (C), 122.1 (CH), and 118.0 (CH) revealed that **1** possessed two double bonds. Absorption maxima at 249 and 242 nm in the UV spectrum of **1** indicated that the double bonds were conjugated. Another four ^{13}C NMR bands at δ 63.8 (CH), 61.2 (C), 60.7 (C) and 59.9 (CH), and corresponding ^1H NMR (Table II) resonances at δ 2.96 (dd, J = 8.0, 4.2) and 2.86 (dd, J = 8.8, 3.4) showed that two trisubstituted epoxide rings were present. On the basis of these data, cembrenoid **1** was concluded to be monocarbocyclic. Proton NMR COSY experiments revealed several isolated spin systems. Two methyl groups at δ 1.01(J = 6.8) and 1.00 (J = 6.8) were coupled to an isolated methine proton at δ 2 19 (heptet, J = 6.8) indicating the presence of an isopropyl group. An olefinic methyl resonance at δ 1.56 (brs) showed long range coupling to one of the diene protons at δ 6.15 (brd, J = 10.9). Both of the epoxide methine protons were coupled to high field resonances indicating adjacent methylene groups. Finally, an isolated spin system of four protons (δ 2.42, 1H; 2.0-1.9,

Table I. ^{13}C NMR Assignments for Cembradienes **1-6**.

C#	**1**	**2**	**3**	**4**	**5**	**6**
1	147.8 (C)	147.4 (C)	147.2 (C)	150.9 (C)	146.2 (C)	145.8 (C)
2	118.0 (CH)	117.7 (CH)	118.7 (CH)	119.0 (CH)	117.5 (CH)	118.8 (CH)
3	122.1 (CH)	120.2 (CH)	121.4 (CH)	122.4 (CH)	118.3 (CH)	121.0 (CH)
4	135.2 (C)	133.1 (C)	132.6 (C)	134.6 (C)	135.8 (C)	135.7 (C)
5	37.0 (CH2)	32.2 (CH2)	32.4 (CH2)	40.5 (CH2)	37.1 (CH2)^a	34.0 (CH2)
6	26.5 (CH2)	27.7 (CH2)	26.0 (CH2)	22.8 (CH2)	39.6 (CH2)^a	43.7 (CH2)^a
7	63.8 (CH)	73.9 (CH)	74.8 (CH)	87.9 (CH)	212.9 (C)	213.1 (C)
8	60.7 (C)	84.9 (C)	83.6 (C)	71.0 (C)	45.6 (CH)	45.2 (CH)
9	35.2 (CH2)	36.7 (CH2)	37.0 (CH2)	33.0 (CH2)	27.8 (CH2)^b	24.6 (CH2)^b
10	23.7 (CH2)	27.0 (CH2)	26.9 (CH2)	21.1 (CH2)	29.1 (CH2)^b	39.7 (CH2)^a
11	59.9 (CH)	88.1 (CH)	89.1 (CH)	70.9 (CH)	75.2 (CH)	201.6 (CH)
12	61.2 (C)	72.4 (C)	72.1 (C)	75.6 (C)	74.6 (C)	208.5 (C)
13	34.9 (CH2)	35.5 (CH2)	35.7 (CH2)	41.4 (CH2)	30.9 (CH2)^b	41.4 (CH2)^a
14	23.7 (CH2)	24.7 (CH2)	25.1 (CH2)	24.1 (CH2)	24.9 (CH2)	23.5 (CH2)^b
15	33.0 (CH)	36.8 (CH)	37.2 (CH)	37.8 (CH)	34.4 (CH)	35.3 (CH)
16	22.6 (CH3)	21.7 (CH3)	21.9 (CH3)	22.2 (CH3)	22.8 (CH3)	22.0 (CH3)
17	22.1 (CH3)	21.6 (CH3)	21.7 (CH3)	22.1 (CH3)	22.0 (CH3)	22.0 (CH3)
18	16.1 (CH3)	18.9 (CH3)	19.2 (CH3)	18.1 (CH3)	18.5 (CH3)	16.5 (CH3)
19	16.8 (CH3)	20.2 (CH3)	21.5 (CH3)	27.3 (CH3)	17.2 (CH3)	16.5 (CH3)
20	18.9 (CH3)	25.8 (CH3)	26.1 (CH3)	23.8 (CH3)	25.0 (CH3)	29.9 (CH3)
OAc			170.2 (C)			
			20.6 (CH3)			

Carbon NMR data were obtained at 50MHz. Spectra for **1**, **2**, **5** and **6** were obtained in $CDCl_3$ solution. Spectra for **3** and **4** were obtained in C_6D_6 and CD_3OD solutions, respectively. Numbers of attached protons were determined by DEPT experiments. Assignments for **1**, **3** and **4** were aided by XHCORR and COLOC experiments Assignments for others were aided by comparison with **1**, **3** and **4**. ^{a,b} Signals within a column may be reversed.

2H; 1.60, 1H), was assigned to a blocked ethylene unit

Direct (XHCORR) and long range COLOC (see Table III) carbon-proton NMR correlation experiments allowed these latter spin systems to be combined. Several long range couplings between protons on the diene and the isopropyl group carbons revealed that the isopropyl group was positioned at C-1 of the diene. In the same way, a vinyl methyl carbon (δ 16.1, C-18) and a methylene carbon (δ 37.0, C-5) were coupled to an olefinic proton at δ 6.15 (C-3), positioned at the terminal end of the diene. These experiments also served to position the trisubstituted epoxides at C-7, -8 and C-11, -12. On the basis of these data, compound **1** was thus assigned as 7, 8; 11, 12-diepoxycembra-1,3-diene, without assignment of stereochemistry at the olefinic bonds or epoxide centers. Data derived from compound **3** (discussed later) allowed these assignments to be made, completing the structure assignment of **1**.

Table II. ^{1}H NMR Assignments for Cembradienes **1-5**.

#	**1**^{a}	**2**^{b}	**3**^{a}	**4**^{c}	**5**^{b}
2	6.07 (1H,brd,10.9)	6.02 (1H,brd,11.2)	6.27 (1H,brs)	5.82 (1H,brd,8.6)	6.02 (1H,brd,10.8)
3	6.15 (1H,brd,10.9)	6.13 (1H,brd,11.2)	6.27 (1H,brs)	6.05 (1H,brd,8.6)	5.55 (1H,brd,10.8)
5a	2.2 - 2.0 (2H, m)	2.1 - 1.9 (2H, m)	2.28 (1H, m)	2.49 (1H,brdd,17.0,7.9)	2.66 - 2.56 (1H, m)
5b			1.86 (1H, m)	2.05 (1H,brdd,17.0,10.9)	2.20 (1H, m)
6a	1.5 - 1.4 (1H, m)	2.4 - 2.3 (1H, m)	2.30 (1H,m)	2.21 (1H,ddd,15.4,10.9,9.3)	2.93 (1H,ddd,19.0,11.2,1.5)
6b		1.4 - 1.3 (1H, m)	1.49 (1H, m)	1.50 - 1.46 (1H, m)	2.66 - 2.56(1H, m)
7	2.86 (1H,dd,8.8,3.4)	3.49 (1H,d,9.8)	5.15 (1H,d,10.2)	3.45 (1H,brd,9.3)	
8					2.81 (1H, m)
9a		2.28 (1H,m)	1.81 (1H, m)	1.95 - 1.82 (1H, m)	2.06 (1H, m)
9b		1.74 (1H, m)	1.30 (1H, m)	1.64 (1H, m)	1.75 - 1.67 (1H, m)
10a	1.4 - 1.3 (1H, m)	1.69 (1H, m)	1.44 (1H, m)	1.95 - 1.82 (1H, m)	1.75 - 1.67 (1H, m)
10b		1.56 (1H, m)	1.26 (1H, m)	1.56 (1H, m)	1.44 (1H, m)
11	2.96 (1H,dd,8.0,4.2)	3.94 (1H,dd,11.3,4.3)	3.81 (1H,dd,11.3,4.5)	3.90 (1H,dd,11.6,2.8)	3.48 (1H, m)
13a	2.0 - 1.9 (1H, m)	2.4 - 2.3 (1H, m)	2.23 (1H, m)	1.82 - 1.70 (1H, m)	1.96 (1H, m)
13b	1.60 (1H, m)	2.10 (1H, m)	1.34 (1H, m)	1.52 (1H, m)	1.64 (1H, m)
14a	2.42 (1H, m)	2.4 - 2.3 (1H, m)	2.60 (1H,ddd,13.2,9.2,2.8)	2.35 (1H,brdd,12.6,12.6)	2.11 (1H, m)
14b	2.0 - 1.9 (1H, m)	1.4 - 1.3 (1H, m)	2.13 (1H, m)	1.95 - 1.82 (1H, m)	1.48 (1H, m)
15	2.19 (1H,h,6.8)	2.4 - 2.3 (1H, m)	2.36 (1H,h,6.8)	2.30 (1H,h,6.8)	2.29 (1H, m)
16	1.01 (3H,d,6.8)	1.04 (3H,d,6.7)	1.09 (3H,d,6.8)	1.03 (3H,d,6.8)	1.04 (3H,d,7.0)
17	1.00 (3H,d,6.8)	1.03 (3H,d,6.7)	1.09 (3H,d,6.8)	1.03 (3H,d,6.8)	1.02 (3H,d,7.8)
18	1.56 (3H, brs)	1.77 (3H, brs)	1.82 (3H, brs)	1.68 (3H, brs)	1.81 (3H, brs)
19	1.07 (3H, s)	1.22 (3H, s)	1.23 (3H, s)	1.13 (3H, s)	1.14 (3H,d,7.1)
20	1.09 (3H, s)	1.15 (3H, s)	1.07 (3H, s)	1.15 (3H, s)	1.27 (3H, s)
OAc			1.66 (3H, s)		

[a,b,c] Spectra were recorded at 360 MHz in C_6D_6, $CDCl_3$, and CD_3OD solutions, respectively. Assignments were aided by one-dimensional decoupling and COSY experiments. Blank spaces indicate protons were either not present or not resolved.

Table III. Results of Two-Dimensional Carbon-Proton Long-Range Correlation Experiments (COLOC) with Cembradienes **1**, **3** and **4**.*

C#	1(H at C#)	3	4
1	14a, 16, 17	14a, 16, 17	3, 14a, 16, 17
2	----	14a, 14b	14a
3	18	18	5b, 18
4	18	18	18
5	3, 18	3, 7, 18	3, 7, 18
6	----	----	5a, 7
7	19	19	5b, 6a, 19
8	19	7, 19	7, 19
9	19	----	19
10	----	----	----
11	20	20	7, 20
12	20	11, 14b, 20	11, 14a, 20
13	14a, 20	14a, 14b, 20	20
14	2	2	----
15	2, 14a, 17	2, 14a, 14b, 16, 17	2, 14a, 16, 17
16	17	17	17
17	16	16	16
18	3	3	3
19	----	7	----
20	----	----	11
carbonyl		7, CH_3	

* COLOC experiments with **1** and **3** were performed at 50 MHz in C_6D_6 solution. The experiment with **4** was performed at 50 MHz in CD_3OD solution. Parameters were optimized for couplings of 8 Hz for **1**, and 6 Hz for **3** and **4**.

Cembradiene **2** was isolated as a white solid (0.4% of the extract) which was analyzed for $C_{20}H_{34}O_3$ by high resolution mass and ^{13}C NMR spectrometry. Comparison of ^1H, ^{13}C NMR and UV spectra showed that **2** possessed the same diene chromophore as cembradiene **1**. Instead of the epoxide carbon resonances however, the ^{13}C NMR spectrum of **2** showed four carbon bands at far lower field; δ 88.1 (CH), 84.9 (C), 73.9 (CH), and 72.4 (C) (Table I). In addition, the IR spectrum of **2** showed a strong hydroxyl absorption band at 3400 cm^{-1}. Consideration of the molecular formula led to the conclusion that two hydroxyl groups and one ether ring were present. Although the chemical shifts of many proton resonances were different, proton NMR COSY experiments showed very similar coupling patterns between **2** and **1**, suggesting that the same carbons were oxygenated. Acetylation of **2**, under normal conditions, provided the monoacetate **3** in good yield. Compound **3**, also isolated as a natural product, is discussed below.

Cembradiene **3** was isolated as a crystalline solid, mp 96-98°, which was analysed for $C_{22}H_{36}O_4$ by high resolution mass and ^{13}C NMR methods. Cembradiene **3** yielded readily interpretable NMR spectra (see Tables) which allowed the assignment of a tetrahydrofuranyl substituent between C-8 and C-11. Confirmation of this assignment, and the relative stereochemistry of the asymmetric carbon centers, was achieved by X-ray crystallographic methods. The X-ray drawing of compound **3**, shown in the Figure, illustrates an overall stereochemistry of 1E, 3E, 7S*, 8R*, 11S*, and 12 R*. The absolute stereochemistry of **3** was not determined in the X-ray experiment. The final refinement of the X-ray structure proved problematic and will be the subject of a forthcoming publication.

Cembradiene **4** was analyzed for $C_{20}H_{34}O_3$, a formula isomeric with that of **2**, by high resolution mass and ^{13}C NMR spectrometry. Comparison of spectral data showed similarities, but distinct ^{13}C NMR differences between 90 and 70 ppm indicated an alternative array of ether and hydroxyl functionalities. Several attempts to prepare an acetyl derivative of **4** failed indicating that both hydroxyl groups were tertiary. These data suggested that cembradiene **4** possessed an ether linkage between C-7 and -11, and two tertiary hydroxyl groups at C-8 and C-12. The high field shift of the C-11 carbon was rationalized by a change of ring size from 5- to 6-membered. Another experiment supporting this assignment was the reaction of compounds **2**, **3**, and **4** with trichloroacetyl isocyanate (see Experimental), followed by comparison of the chemical shifts of relevant proton signals. Proton resonances at C-7 and C-11 of **4** were shifted downfield much less than in **2** and **3**, which suggested that they were not hydroxyl methine but ether methine protons. Thus, the structure of compound **4**, void of stereochemical features at C-7, C-8, C-11 and C-12, was assigned as a cembradiene diterpenoid possessing a 6-membered ether ring.

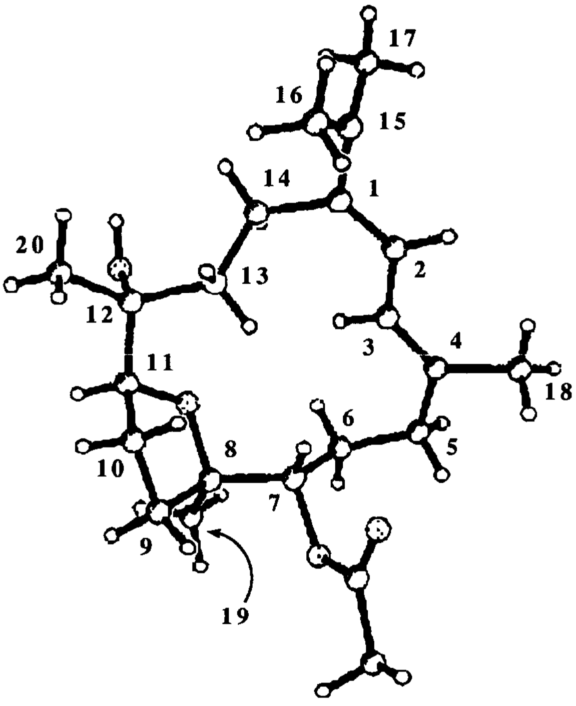

Figure A computer-generated drawing of the X-ray structure of cembradiene 3. The absolute stereochemistry was not determined in the X-ray experiment and the enantiomer shown was randomly chosen.

Confirmation of the spectral assignment of **4**, as well as that of the diepoxide **1**, was achieved by chemical interconversion. Acid catalyzed hydrolysis of **1** gave **2** and **4** as the sole products, confirming their oxidation site relationships. Based upon the X-ray structure of cembradiene **3**, and assuming that the stereochemistry at C-7 and C-11 remain unchanged in the acid catalysed ring opening, cembradiene **1** should have the relative stereochemistry shown. Since compound **4** could be produced from **1** through several pathways, the stereochemistry of this compound could not be assigned. Comprehensive NOEDS experiments with **4** failed to lead to significant stereochemical information.

Because acid treatment converts the diepoxide **1** into cembradienes **2** and **4**, it is difficult to guarantee that the latter compounds are true natural products and not artefacts of an unknown acid catalysed hydrolysis occurring during workup The isolation of the acetate derivative **3**, the fact that cembradiene **5** is not produced in the hydrolysis,and the rather vigorous conditions (conc. H_2SO_4) required to effect the hydrolysis, suggest, however, that cembradienes **2** and **4** are produced by enzymatic processes.

Cembradiene **5** was analyzed for $C_{20}H_{34}O_3$ by high resolution mass and ^{13}C NMR spectrometry. The infrared spectrum showed strong absorption bands at 3630 and 1715 cm^{-1}, which in conjunction with ^{13}C NMR bands at δ 212.9 (C), 75.2 (CH) and 74.6 (C), showed that **5** possessed a ketone and two hydroxyl groups. Proton NMR COSY experiments showed **5** possessed the identical diene and isopropyl groups as found in the other metabolites. The presence of a low field methylene proton (δ 2.93) with a very large coupling constant (J = 19.0 Hz), assigned at C-6, indicated the ketone was at C-7. Proton NMR data also showed that the ethylene bridge between C-1 and C-12 was intact. This ethylene group must be directly connected to the only remaining quaternary carbon center (C-12) which bears methyl and hydroxyl substituents. To assign the location of the secondary hydroxyl group, compound **5** was oxidized with pyridinium chlorochromate in dichloromethane to yield the unexpected diketo-aldehyde **6**. The structure of this acyclic derivative showed that the hydroxyl group had to be placed at the C-11 position in **5**. Although the planar structure of cembradiene **5** could be assigned with confidence, the stereochemistry at C-11 and C-12 remained undetermined. Several attempts to crystallize **5** and several derivatives for X-ray crystallographic studies failed.[27]

Two previously reported diterpenoids, **7** and **8**, were also isolated in small amounts. These compounds showed ^1H and ^{13}C NMR spectra in full accord with published data. Compound **7** has been isolated from the Australian soft corals, *Sinularia grayi*,[23] *Nephthea* sp ,[24] and *Lobophytum* sp.[25] Cembrenoid **8** was isolated from the soft coral *Sarcophyton* sp.[26] collected from the same area.

EXPERIMENTAL

General. Proton NMR and COSY spectra were recorded at 360 MHz, using a spectrometer constructed from an Oxford magnet and Nicolet-1180E Fourier transform data system. Carbon-13 NMR, XHCORR and COLOC spectra were recorded at 50 MHz. High resolution mass measurements were supplied by the University of California, Riverside Mass Spectrometry Center. Optical rotations were measured with a 10-cm microcell. Melting points are reported uncorrected, and all solvents used were either spectral grade or distilled from glass prior to use.

Collection and Extraction. *Eunicea* sp.(specimen number CI 86-222[22]) was collected by hand using SCUBA at 20 to 25 m depth in July, 1986, along the offshore islands of the Tobago Cays, eastern Caribbean Sea. The collection was surface air-dried in the shade and immediately frozen. The gorgonian was next repeatedly extracted with CH_2Cl_2 and the combined extracts were evaporated to yield 15g of crude organic materials (from 1 kg, dry weight of the gorgonian). Compounds **1-5**, **7** and **8** were eluted from a vacuum flash silica column with sequential mixtures of EtOAc and isooctane and further purified by preparative silica HPLC.

Cembradiene **1**. The diterpenoid **1** was isolated as a white solid after final purification by silica HPLC (30% EtOAc in isooctane). Recrystallization from CH_2Cl_2 gave 300 mg (2.0% of the crude extract) of **1**, mp. 99-101° Compound **1** displayed the following spectral features: $[\alpha]_D$ -76.4° (c 0.4, methanol); UV (methanol) λmax 249 nm (ε 27000), 242 nm (ε 25000); IR (film, NaCl) 2960, 2940, 1670, 1460, 1380, 1070 cm^{-1}; HRMS: M^+ m/z obsd 304.2426, $C_{20}H_{32}O_2$ requires 304.2402.

Cembradiene **2**. The diterpenoid **2** was isolated as a white solid after final purification by silica HPLC (50% EtOAc in isooctane). Recrystallization from acetone gave 60 mg (0.4% of the crude extract) of **2**, mp 131-132°. Compound **2** showed the following spectral features: $[\alpha]_D$ -85.8° (c 0.8, methanol); UV (methanol) λmax 249 nm (ε 27000), 243 nm (ε 25000); IR (film, NaCl) 3400, 2980, 1620, 1460, 1380, 1070 cm^{-1}; HRMS: M^+ m/z obsd 322.2500, $C_{20}H_{34}O_3$ requires 322.2508.

Cembradiene **3**. The diterpenoid **3** was isolated as a white solid after final purification by silica HPLC

(40% EtOAc in isooctane). Recrystallization from a mixture of hexane and acetone gave 80 mg (0.7% of the crude extract) of **3**, mp 96-98°. Compound **3** displayed following spectral features: $[\alpha]_D$ -35.3° (c 0.5, methanol); UV (methanol) λmax 249 nm (ε 33000), 241 nm (ε 25000); IR (film, KBr) 3450, 2960, 1740, 1660, 1610, 1440, 1370, 1030 cm^{-1}; HRMS: M^+ m/z obsd 364.2619, $C_{22}H_{36}O_4$ requires 364.2614.

Cembradiene **4**. The diterpenoid **4** was isolated as a oil after final purication by HPLC (70% EtOAc in isooctane); yield 50 mg (0.3% of the crude extract). Compound **4** displayed the following spectral features: $[\alpha]_D$ -85.8° (c 0.9, methanol); UV (methanol) λmax 250 nm (ε 23000), 243 nm (ε 22000); IR (film, NaCl) 3440, 2960, 1660, 1460, 1370, 1070 cm^{-1}; HRMS: M^+ m/z obsd 322.2534, $C_{20}H_{34}O_3$ requires 322.2508.

Cembradiene **5**. The diterpenoid **5** was isolated as a white solid after final purification by HPLC (70% EtOAc in isooctane). Recrystallization from methanol gave 100 mg (0.7% of the crude extract); mp 156-157°. Compound **5** showed the following spectral features: $[\alpha]_D$ +11.3° (c 0.7, $CHCl_3$); UV (methanol) λmax 246 nm (ε 38000), 241 nm (ε 37000); IR ($CHCl_3$) 3630, 3030, 2980, 1715, 1620, 1420, 1050 cm^{-1}; HRMS: M^+ m/z obsd 322.2507, $C_{20}H_{34}O_3$ requires 322.2508.

Acid Hydrolysis of **1**. To a stirred emulsion of 35 mg of **1** (0.12 mmol) in 1.5 ml of acetone and distilled water (2:1, v/v), 0.1 ml of conc. H_2SO_4 was added. After 15 min, the solution was extracted with 2 x 20 ml of Et_2O. The ether layer was washed with satd $NaHCO_3$ (2 x 15 ml), water (2 x 15 ml) and dried under vacuum. Purification by HPLC (50% EtOAc in isooctane) gave 15.4 mg (0.05 mmol, 42% yield) of **2** and 13.0 mg (0.04 mmol, 35% yield) of **4**. The ^1H and ^{13}C NMR spectra of the products were identical with those from the natural compounds.

Acetylation of **2**. To a stirred solution of 10 mg (0.03 mmol) of **2** in 1.0 ml of dry pyridine, 0.5 ml of acetic anhydride was added. After 2 hrs, pyridine and excess acetic anhydride were removed under vacuum. Compound **3** was obtained as the sole product in quantitative yield. The ^1H and ^{13}C NMR spectra of **3** were identical with the natural compound.

Reaction of cembradienes **2**, **3** and **4** with trichloroacetyl isocyanate. All reactions were performed in NMR tubes. After measuring relevant chemical shifts in C_6D_6 solution, one drop of neat isocyanate was added. Products formed instantly, and the number of derivatives formed were determined by counting the number of amide NH protons observed. Tabulated below are the reactions of compounds **2-4** and the differences of chemical shifts of the key protons. **R** is $Cl_3CCONHC(=O)$-. Δ refers to the shifts noted for the relevant protons.

Cmpd	H-7	H-11	H-19	H-20
2	3.30	3.78	1.14	1.09
2+2R	5.28	4.52	1.29	1.43
3	5.15	3.81	1.25	1.04
3+R	5.25	4.55	1.21	1.42
4	3.42	3.76	1.05	1.04
4+2R	4.48	4.14	1.29	1.65
Δ2	-1.98	-0.74	-0.15	-0.34
Δ3	-0.10	-0.74	+0.04	-0.35
Δ4	-1.06	-0.38	-0.24	-0.61

Oxidation of **5**. To a stirred solution of 21 mg (0.06 mmol) of **5** in 2 ml of dry CH_2Cl_2, 30 mg (0.13 mmol) of pyridinium chlorochromate and 6 mg (0.1 mmol) of sodium acetate were added. After stirring the mixture for 3 hr, crude product **6** was obtained by silica column chromatography (ether eluent). Final purification by silica HPLC (40% EtOAc in isooctane) gave 9 mg (0.03 mmol, 43% yield) of **6** as an oil; HRMS: M^+ m/z obsd 320.2357, $C_{20}H_{32}O_3$ requires 320.2353. ^1H NMR ($CDCl_3$) data for **6**: δ 9.75 (1H, brs), 6.00 (1H, d, 11.3), 5.94 (1H, brd, 11.5), 2.6-2.2 (11H, m), 2.16 (3H, brs), 1.99 (1H, m), 1.75 (3H, brs), 1.65 (1H, m), 1.11 (3H, d, 7.0), 1.03 (6H, d, 6.8). ^{13}C NMR data for **6** are shown in Table I.

Isolation of 7 and 8. Compounds **7** and **8** were isolated, as oils, by silica HPLC (10% EtOAc in isooctane); yield 25 mg each (0.08 mmol). The ^1H and ^{13}C NMR spectra for these metabolites were in full accord with published data.[23-26]

ACKNOWLEDGEMENTS

This research is a result of combined research and ship funding from the National Science Foundation, Chemistry and Oceanography Divisions, under Grant CHE86-20217, and from the California Sea Grant Program, under Grant NA89AA-D-SG140 (project Nos R/MP-39 and -48), and in part by the California State Resources Agency. We thank the captain and crew of the research vessel Columbus Iselin (University of Miami) for their aid and assistance. We gratefully acknowledge the government of the Grenadines for permission to perform research in their territorial waters, and we thank Dr. Frederick M. Bayer, Smithonian Institution, Washington, D.C., for his guidance with *Eunicea* systematics.

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