New Cembrane Diterpenes of the Marine Octocoral *Eunicea tourniforti* from the Eastern Caribbean

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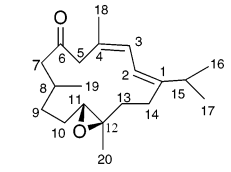
An investigation of the Caribbean octocoral *Eunicea tourniforti* collected along the southwest coast of Barbados afforded four new cembrane diterpenes, **1**, **2**, **4**, and **5**, along with the known cembranolide **3**. The structures of all metabolites were determined by spectroscopic methods. Seasonal effects on the secondary metabolite content of the specimens collected were also observed.

A major component of the Caribbean water invertebrate fauna are marine octocorals of the genus *Eunicea* (order Gorgonacea, phylum Cnidaria), which are commonly referred to as sea whips.[1] These animals are a rich source of natural products that are biologically active as well as structurally unique.[2a,b] Diterpenoids as a single class represent the largest percentage of natural products isolated from the genus *Eunicea*, with cembrane derivatives representing the most commonly reported class.[2a] It has been reported that the secondary metabolite content of this genus varies due to change in geographical location or environmental conditions.[4] These factors led to the undertaking of a study of two specimens of *Eunicea tourniforti*, Milne Edwards and Haime (Plexauridae), from the Eastern Caribbean, since no previous studies were reported from this location. This led to the isolation of the cembrene diterpenes **1**−**5**. Compound **3**, (3*E*,7*S*,11*Z*)-7-hydroxy-3,11,15-cembratrien-20,28-olide, was previously isolated from *E. tourniforti* collected off the coast of St. Thomas, U.S. Virgin Islands.[5]



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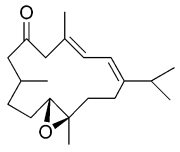

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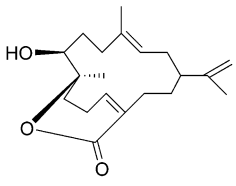

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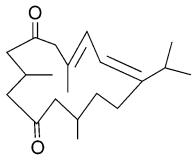

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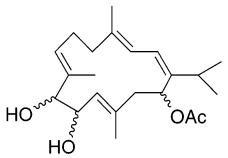

5

Compound **1**, $C_{20}H_{32}O_2$, was isolated as a yellow oil and had IR absorptions due to ketone (1707 cm^{-1}) and epoxide (1228 cm^{-1}) functionalities. A UV absorption at 240 nm (log ϵ 4.33) was attributed to conjugated double bonds. Four low-field ^{13}C NMR resonances at δ 147.7 (s), 129.6(s), 125.0 (d), and 118.8 (d) supported the presence of two double bonds, while a resonance at δ 209.9 confirmed the presence of a saturated ketone. A trisubstituted epoxide had ^{13}C NMR resonances at δ 63.6 (d) and 60.2 (s).

The ^1H NMR spectrum of **1** was characteristic of cembrane diterpenes and had methyl doublets at δ 0.90 (J = 6.7 Hz, H_3-19), 1.01 (J = 6.9 Hz, H_3-17), and 1.11 (J = 6.9 Hz, H_3-16), a methyl singlet at δ 1.32 (H_3-20), and an olefinic methyl at δ 1.90 (H_3-18). Both methyl doublets at δ 1.01 and 1.11 showed HMBC correlations to the olefinic carbon at δ 147.7 (C-1) and a methine carbon at δ 32.0 (C-15), in addition to their respective carbons at δ 20.9 (C-16) and 23.5 (C-17), and confirmed that an isopropyl group was attached to C-1. The carbonyl carbon at δ 209.9 showed long-range correlations to the C-5 methylene protons at δ 2.75 and 3.68 and the C-7 methylene protons at δ 1.89 and 2.50. The C-10 (δ 24.9) and C-13 (δ 37.3) protons showed correlations to the epoxide carbons at δ 60.2 and 63.6, whereas the slightly nonequivalent protons attached to C-9 (δ 33.8) showed HMBC correlations only to the protonated carbon at δ 63.6.

The H-2 proton at δ 6.08 had ROESY cross-peaks to H-5a (δ 3.68), H_3-17 (1.01), and H_3-16 (δ 1.11). Further, ROESY cross-peaks between H-3 (δ 6.30) and H-5b (δ 2.75) and H_3-18 (δ 1.90) allowed the assignment of the configuration of the diene as 1*E*, 3*Z*. The stereochemistry at C-8, C-11, and C-12 remains undefined. Compound **1** was thus identified as (1*E*,3*Z*)-11,12-epoxycembra-1,3-dien-6-one. The 3*Z* stereochemistry is the same as in asperdiol, a cembrane diterpenoid previously isolated from *E. asperula* and *E. tourneforti*.[6,7]

Compound **2** was isolated as a yellow oil, and a molecular formula $C_{20}H_{32}O_2$ isomeric with that of **1** was established by HREIMS. Comparison of the NMR spectral data of **1** and **2** (Tables 1 and 2) showed similarities, but there were ^{13}C NMR differences for C-18. In compound **2** C-18 resonated at δ 17.3, and thus an *E*-trisubstituted double bond was present at C-3/C-4. Compound **2** was thus assigned as the geometric isomer of compound **1** and was identified as (1*E*,3*E*)-11,12-epoxycembra-1,3-dien-6-one. The difference in configuration of the C-3/C-4 double bond is believed to be responsible for the changes in the chemical shifts observed for C-11, C-12, and C-20.

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Table 1. ^{1}H NMR Assignments for Compounds **1**−**5**[a]

H	1	2	3	4	5
1			1.82 (m)		
2	6.08 (d, 11.4)	6.07 (d, 10.0)	⟨1.93⟩[a] (m)	5.98 (d, 11.2)	6.14 (d, 11.5)
3	6.30 (d, 11.4)	6.23 (d, 10.0)	5.17 (t, 8.0)	5.94 (d, 11.2)	5.78 (d, 11.5)
4					
5	3.68 (d, 14.4)	3.33 (d, 14.0)	⟨2.15⟩ [a](m)	3.10 (d, 14.4)	2.24 (m)
	2.75 (d, 14.4)	2.95 (d, 14.0)		2.95 (d, 14.4)	2.19 (m)
6			1.90 (m)		2.38 (m)
			1.48 (m)		2.20 (m)
7	2.50 (dd, 13.7, 6.6)	2.62 (dd, 12.8, 6.4)	4.15 (d, 8.0)	2.69 (dd, 16.8, 4.8)	5.23 (m)
	1.89 (dd, 13.7, 8.3)	2.00 (dd, 12.8, 8.0)		2.18 (m)	
8	2.08 (m)	2.30 (m)		2.45 (m)	
9	⟨1.34⟩[a] (m)	⟨1.45⟩[a] (m)	2.25 (m)	⟨2.43⟩ [a](m)	3.81 (d, 8.9)
			2.04 (m)		
10	1.58 (m)	1.57 (m)	2.73 (m)		4.36 (t, 9.3)
	1.35 (m)	1.50 (m)	2.48 (m)		
11	2.48 (m)	2.91(dd, 9.9, 4.8)	6.17 (t, 6.0)	2.55 (dd, 16.8, 8.6)	5.01 (d, 9.8)
				1.16 (m)	
12				1.94 (m)	
13	2.16 (m)	2.19 (m)	2.91(dd, 13.2, 8.8)	1.33 (m)	2.50 (dd, 15.2, 4.8)
	1.30 (m)	1.54 (m)	1.68 (m)	1.28 (m)	2.26 (dd, 15.2, 4.8)
14	2.70 (br d, 3.8)	2.90 (m)	1.58 (m)	2.28 (m)	5.76 (t, 4.8)
	2.07 (m)	2.07 (m)	1.08 (m)	1.97 (m)	
15	2.29 (sept, 6.9)	2.38 (sept, 6.9)		2.30 (m)	2.46 (sept, 6.9)
16	1.11 (d, 6.9)	1.13 (d, 6.9)	4.74 (brs)	1.04 (d, 6.9)	1.04 (d, 6.9)
			4.69 (brs)		
17	1.01 (d, 6.9)	1.06 (d, 6.9)	1.70 (s)	1.01 (d, 6.9)	1.11 (d, 6.9)
18	1.90 (s)	1.82 (m)	1.63 (s)	1.76 (s)	1.76 (s)
19	0.90 (d, 6.7)	0.99 (d, 6.8)	1.34 (s)	1.06 (d, 6.9)	1.64 (s)
20	1.32 (s)	1.21 (s)		0.95 (d, 6.7)	1.80 (s)
21					
22					2.06 (s)

[a] Average values for slightly nonequivalent protons. All assignments were based on 1D and 2D experiments including G-COSY, G-HSQC, G-HMBC, and NOESY.

Table 2. ^{13}C NMR Assignments for Compounds **1**−**5**

C	1	2	3	4	5
1	147.7	148.5	46.6	148.8	144.2
2	118.8	117.9	32.8	118.2	119.9
3	125.0	125.3	126.2	123.9	118.8
4	129.6	130.4	134.3	129.9	137.4
5	47.2	53.7	33.9	52.9	37.9
6	209.9	210.6	29.8	209.2	24.0
7	48.5	49.6	67.6	46.9	131.2
8	28.4	28.7	83.0	26.6	134.0
9	33.8	33.5	35.0	48.9	82.4
10	24.9	24.6	27.2	210.2	69.6
11	63.6	61.0	140.1	48.9	125.8
12	60.2	60.5	133.5	28.2	139.6
13	37.3	35.9	34.8	35.3	46.7
14	26.0	25.2	28.4	26.2	74.0
15	32.0	32.2	148.7	34.6	28.9
16	20.9	21.3	110.7	21.7	24.4
17	23.5	23.0	18.6	22.5	23.7
18	25.3	17.3	16.9	17.9	17.6
19	20.0	20.3	22.4	20.5	11.9
20	16.9	18.7	167.1	20.7	16.4
21					170.1
22					21.2

In an attempt to isolate further quantities of compounds **1** and **2**, another collection of the gorgonian specimen from the same area was carried out at a later date. The specimen was treated in the same manner as the first collected specimen, but compounds **1** and **2** were not reisolated; instead compound **4** was isolated as the major metabolite along with smaller quantities of compound **5**.

Compound **4** was isolated as white needles, mp 95−96 °C and had a molecular formula $C_{20}H_{32}O_2$, as determined by HREIMS. A UV absorption at 239 nm revealed the presence of conjugation, while a sharp IR absorption due to the presence of carbonyl groups was observed at 1715 cm^{-1}. The ^{1}H NMR spectrum of compound **4** showed signals at δ 5.98 (H-2, d, J = 11.2 Hz) and 5.94 (H-3, d, J = 11.2 Hz) due to olefinic protons. Five methyl signals at δ 1.76 (H_3-18), 1.06 (H_3-19, d, J = 6.9 Hz), 1.04 (H_3-16, d, J = 6.9 Hz), 1.01 (H_3-17, d, J = 6.9 Hz), and 0.95 (H_3-20, d, J = 6.7 Hz) were also observed. The ^{13}C NMR spectrum had four signals at δ 129.9 (C-4), 123.9 (C-3), 118.2 (C-2), and 148.8 (C-1), due to olefinic carbons, while carbonyl carbons had resonances at δ 209.2 and 210.3.

HMBC correlations observed between the methyl protons at δ 1.04 and 1.01, in addition to correlations involving a methine proton at δ 2.30, established the presence of an isopropyl group at C-1 as in compounds **1** and **2**. HMBC correlations confirmed the presence of two ketone moieties at C-6 and C-10, both positioned between two methylene carbons. The position of these carbonyl groups explains the high downfield chemical shift of C-5, C-7, C-9, and C-11. The configuration of the olefinic bond at C-1 was determined as 1*Z* since no NOESY cross-peaks between the olefinic proton at δ 5.98 (H-2) and the methyl protons at δ 1.04 (H_3-17) and 1.01 (H_3-16) were observed. The chemical shift of C-18 was δ 17.9, and this inferred an *E*-trisubstituted olefin at C-3. Compound **4** was thus identified as (1*Z*,3*E*)-cembra-1,3-diene-6,10-dione.

Compound **5** was isolated as a moderately stable oil and did not give a molecular ion in the EIMS, but there were fragment ions due to M − $2H_2O$ and M − OAc. The molecular formula, $C_{22}H_{34}O_4$, was established by ESI-MS and ^{13}C NMR spectroscopy. The IR spectrum showed peaks characteristic of hydroxy (3446 cm^{-1}) and olefinic functionalities (1647 cm^{-1}). The presence of conjugation in compound **5** was indicated by a UV absorption at 248 nm. Signals at δ 6.14 (H-2, J = 11.5 Hz) and 5.01 (H-11, J = 9.8 Hz), 5.78 (H-3), and 5.23 (H-7) were determined to be olefinic protons on the basis of HSQC data. The HSQC data also showed that oxymethine protons had resonances at δ

3.81 (H-9, J = 8.9 Hz) and 4.36 (H-10, J = 9.3 Hz). Five methyl signals at δ 1.80 (H_3-20), 1.76 (H_3-18), 1.64 (H_3-19), 1.11 (H_3-17, d, J = 6.9 Hz), and 1.04 (H_3-16, d, J = 6.9 Hz) were also observed. A methyl singlet at δ 2.06, indicative of an acetate group, and an associated oxymethine proton at δ 5.76 (H-14, d, J = 4.8 Hz) were also present.

HMBC correlations observed for the methyl protons at δ 1.11 and 1.04 confirmed the presence of an isopropyl group in compound **5**. The H−H COSY spectrum showed cross-peaks between the oxymethine protons at δ 3.81 and 4.36, while the HSQC spectrum indicated that these protons were directly attached to the carbons at δ 82.4 (C-9) and 69.6 (C-10), respectively. HMBC correlations were also observed between the oxymethine proton at δ 3.81 and the olefinic carbons at δ 134.0 (C-8), 131.2 (C-7), and 125.8 (C-11), in addition to the carbon at δ 69.6 (C-10) and the methyl carbon at δ 11.9 (C-19). The oxymethine proton at δ 4.36 showed HMBC connectivities to the olefinic carbon at 139.6 (C-12) and the other carbon at δ 82.4 (C-9). These spectral data confirmed that the secondary hydroxy groups at C-9 and C-10 were vicinal and that each hydroxy group was in an allylic position.

There were ROESY cross-peaks observed between H-2 (δ 6.14) and H_3-16 (δ 1.04), H_3-17 (δ 1.11), and H_3-18 (δ 1.76). Other ROESY cross-peaks were observed between H-3 (δ 5.78) and H-6a (δ 2.38), H-5a (δ 2.24), and Me-20 (δ 1.80). Thus the configuration of the diene was assigned as 1*Z*, 3*E*. The methyl protons at δ 1.64 (H_3-19) showed ROESY cross-peaks to the oxymethine proton at δ 4.36 (H-10), the methylene proton at δ 2.38, and the methyl protons at δ 1.80 (H_3-20). No cross-peaks were observed between H-7 and H_3-19. The olefinic group between C-7 and C-8 was therefore assigned an *E* configuration. ROESY cross-peaks were also observed between the protons at δ 1.80 (H_3-20) and the oxymethine proton at δ 4.36, the olefinic proton at δ 5.76 (H-14), the methylene proton at δ 2.50 (H-13a), and the methyl protons at δ 1.64. The fourth trisubstituted double bond at C-11/C-12 was therefore assigned an *E* configuration as well. Compound **5** was thus identified as (1*Z*,3*E*,7*E*,11*E*)-14-acetoxycembra-1,3,7,11-tetraene-9,10-diol. Seasonal variation may be responsible for the change in cembrane diterpenoid content in *E. tourniforti* collected from the eastern Caribbean. Previous investigations of the organism from the northern Caribbean resulted in the isolation of both cembrane and dolabellane diterpenoids.[5−7]

Experimental Section

General Experimental Procedures. Melting points were determined using a Fisher/Johns melting point apparatus. Optical rotations were recorded on a Perkin-Elmer 341 polarimeter in $CHCl_3$ solutions. UV spectra were obtained on a Hewlett-Packard 8452A spectrophotometer in MeOH. Infrared spectra were recorded on a Nicolet Nexus 870 FT-IR spectrometer. NMR spectra were recorded on a Varian Unity 500 MHz or a Bruker Avance DRX 400 MHz spectrometer in $CDCl_3$ solutions using TMS as an internal standard. Mass spectra were recorded on a VG 70-25S mass spectrometer operating at 70 eV. Electrospray ionization (ESI-MS) spectral results were obtained using a micromass platform mass spectrometer, and infusion was carried out via a Cole-Palmer 74900 syringe pump. HPLC was performed using a Beckman instrument with a Supelcosil LC-18 column (25 cm × 21.2 mm, 5 μm).

Animal Material. The specimens of *Eunicea tourneforti* were collected in October 1999 and again in September 2001 off Folkstone Beach on the west coast of Barbados at −38 ft. Voucher specimens are kept in the Department of Biological and Chemical Sciences, University of the West Indies, Cave Hill Campus, Barbados.

Extraction and Isolation. The first *E. tourneforti* specimen (dry weight 403 g) was blended with acetone (3 L), and the extract was evaporated under vacuum to produce an aqueous suspension, which was extracted with CH_2Cl_2 (3 × 200 mL) to give a brown solid (20 g). A portion (8 g) of the extract was chromatographed over silica gel using hexane/acetone (4:1) to give a major nonpolar fraction (1.52 g), which was further separated by preparative TLC using hexane/acetone (9:1) as the mobile phase. The major band collected was then subjected to reversed-phase HPLC (75:25, MeOH/H_2O), leading to the isolation of compounds **1** (2.1 mg), **2** (2.4 mg), and **3** (12.4 mg).

The second specimen of *E. tourneforti* (dry weight 120 g) was extracted with acetone (9 L) and the solvent evaporated under vacuum to produce an aqueous suspension, which was extracted with CH_2Cl_2 (3 × 600 mL) to yield a dark green gum (2.6 g). The extract was chromatographed on silica gel to yield three major fractions. Preparative TLC on the second fraction using hexane/acetone (5:1) as the eluent yielded compound **4** as white crystals (6 mg) after recrystallization from EtOAc, while preparative TLC on the third fraction gave compound **5** as an oil (1.2 mg).

Compound 1: yellow oil; $[\alpha]_D$ + 150.9° (*c* 0.21, $CHCl_3$); IR ν_{max} (film) 2962, 2872, 1704, 1216 cm^{-1}; UV λ_{max} (MeOH) 240 nm (log ϵ 4.33); ^1H NMR ($CDCl_3$, 500 MHz), see Table 1; ^{13}C NMR ($CDCl_3$, 100 MHz), see Table 2; EIMS *m*/*z* 304 $[M]^+$ (5), 289 (4), 261 (9), 203 (14), 177 (25), 151 (40), 135 (49), 121 (84), 109 (88), 69 (100); HREIMS *m*/*z* 304.2396 $[M]^+$ (calcd for $C_{20}H_{32}O_2$ 304.2402).

Compound 2: yellow oil; $[\alpha]_D$ −14.4° (*c* 0.24, $CHCl_3$); IR ν_{max} (film) 2961, 2872, 1707, 1229 cm^{-1}; UV λ_{max} (MeOH) 240 nm (log ϵ 4.27); ^1H NMR ($CDCl_3$, 500 MHz), see Table 1; ^{13}C NMR ($CDCl_3$, 100 MHz), see Table 2; EIMS *m*/*z* 304 $[M]^+$ (4), 289 (3), 261 (7), 203 (5), 177 (12), 151 (18), 135 (36), 121 (100), 109 (64), 69 (64); HREIMS *m*/*z* 304.2406 $[M]^+$ (calcd for $C_{20}H_{32}O_2$ 304.2402).

Compound 3: yellow oil; $[\alpha]_D$ +196.5° (*c* 2.1, $CHCl_3$); IR ν_{max} (film) 3390, 1731 cm^{-1}; UV λ_{max} (MeOH) 214 nm (log ϵ 2.84); ^1H NMR ($CDCl_3$, 400 MHz), see Table 1; ^{13}C NMR ($CDCl_3$, 125 MHz), see Table 2; EIMS *m*/*z* 318 (38), 305 (35), 302 (100), 287 (36), 275 (56), 261 (62), 247 (52); HREIMS *m*/*z* 318.2193 $[M]^+$ (calcd for $C_{20}H_{30}O_3$ 318.2195).

Compound 4: white needles, mp 95−96 °C; $[\alpha]_D$ −2.5° (*c* 0.22, $CHCl_3$); IR ν_{max} (film) 2953, 2920, 1715, 1647, 1454, 1378 cm^{-1}; UV λ_{max} (MeOH) 239 nm (log ϵ 3.20); ^1H NMR ($CDCl_3$, 400 MHz), see Table 1; ^{13}C NMR ($CDCl_3$, 100 MHz), see Table 2; EIMS *m*/*z* 304 $[M]^+$ (3), 289 (8), 261 (5), 233 (8), 191 (18), 151 (29), 121 (51), 109 (72), 93 (100), 69 (71); HREIMS *m*/*z* 304.2416 $[M]^+$ (calcd for $C_{20}H_{32}O_2$, 304.2402).

Compound 5: yellow oil; $[\alpha]_D$ +42.9° (*c* 0.06, $CHCl_3$); IR ν_{max} (film) 3446, 2919, 2850, 1710, 1647 cm^{-1}; UV λ_{max} (MeOH) (log ϵ) 248 (3.42), 212 nm (3.38); ^1H and ^{13}C NMR, see Tables 1 and 2; EIMS *m*/*z* 326 $[M - 2H_2O, 4\%]^+$, 303 (6), 285 (7), 219 (25), 201 (12), 175 (27), 137 (61), 123 (42), 109 (100), 95 (59); ESI *m*/*z* 385.4 $[M + Na]^+$.

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