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MECHANISM-BASED ANTITUMOR SCREENING OF CARIBBEAN MARINE ORGANISMS: ISOLATION AND STRUCTURE DETERMINATION OF NOVEL DITERPENOIDS FROM THE GORGONIAN *EUNICEA TOURNEFORTI*

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ABSTRACT.—As part of a collaborative research effort between the University of the Virgin Islands and Virginia Polytechnic Institute and State University, we carried out the extraction and bioassay of 87 marine organisms in a mechanism-based assay involving genetically altered yeast strains. Of these, nineteen showed differential activity between the mutant and wild-type yeast strains indicating the presence of potential DNA interacting agents. We now report the isolation and characterization of five new diterpenoids, **2–6**, together with the previously known diterpenoid **1**, from the bioactive extracts of the gorgonian *Eunicea tourneforti* forma *atra*. The structures of the isolated compounds were determined by employing a variety of one- and two-dimensional nmr methods.

A mechanism-based bioassay involving DNA repair or recombination-deficient yeast mutants developed by SmithKline Beecham (SKB) Pharmaceuticals has been employed as a primary screen for potential antitumor activity and to monitor the isolation of active compounds (1–7). More than 5000 plant, marine, and microbial extracts have been screened for antitumor activity using this assay, primarily by the SKB group and the Virginia Polytechnic Institute and State University group. In a collaborative effort to isolate and characterize potential antitumor agents from marine organisms from the Caribbean we screened 87 species using this assay. The crude extracts were screened using the mechanism-based bioassay involving three strains of the yeast *Saccharomyces cerevisiae: rad52* and RS 321, which lack specific DNA-damage repair genes, and the wild-type yeast, *rad*+, which possesses these genes (1). Potential antitumor activity was presumed when an extract showed inhibition of the growth of one or more of the mutant strains and no inhibition of the growth of the wild-type strain, or when it showed a lower IC_{12} (defined as the dose that gives an inhibition zone of 12 mm using a 6-mm diameter, 100-μl well in an agar plate) towards one or more of the mutant yeast strains than the wild-type yeast.

RESULTS AND DISCUSSION

The organisms (65 sponges, 16 gorgonians, and 6 others) were collected from the Virgin Islands and extracted by sequentially soaking in MeOH and CH_2Cl_2. The crude extracts of nineteen organisms (15 sponges and 4 gorgonians) showed differential activity between a mutant strain and the wild-type strain. From one of the gorgonians showing activity in the initial screen, *Eunicea tourneforti* forma *atra* (Gorgoniidae), eight compounds have been isolated employing a variety of chromatographic techniques, including hplc and prep. tlc. This organism was relatively abundant at the original collection site in the Virgin Islands and was therefore chosen for further studies.

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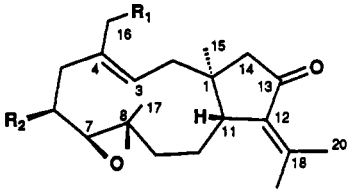
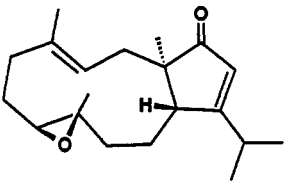
Eunicea tourneforti was recollected by scuba at a depth of 20 meters off the southwest coast of St. Thomas, U.S. Virgin Islands, and immediately frozen. The frozen specimen was thawed, cut into small pieces, and extracted extensively with MeOH and CH_2Cl_2. The combined concentrates selectively inhibited the growth of the mutant RS 321 strain of *Saccharomyces cerevisiae* (IC_{12}=2570 μg/ml). The crude extract was partitioned between hexane and MeOH-H_2O (8:2) and the active aqueous methanol layer was diluted to MeOH-H_2O (6:4) and extracted with $CHCl_3$. The $CHCl_3$ fraction (3.2 g), which continued to show selective inhibition of the RS 321 strain in the yeast bioassay, was then subjected to gel filtration chromatography on Sephadex LH-20 employing the procedure developed by Cardellina (8). The fractions eluting with hexane-CH_2Cl_2 (1:4) and CH_2Cl_2-Me_2CO (3:2) gave inhibition zones of 10–14 mm against the RS 321 strain at 500 μg/ml. The combined active fractions (2.5 g) were subjected to a series of Si gel vacuum flash and medium pressure flash chromatographic separations using hexane/EtOAc/MeOH gradient elution. Regrettably, the activity was distributed among all the fractions from these separations indicating that this organism produced several weakly active constituents rather than one or two strongly active compounds. Because of the interesting structures of compounds previously isolated from *Eunicea* (9–30) we elected to carry out a chemical study so as to provide further insight into the diversity of structures produced by this organism.

The known dolabellane diterpene **1**, which was previously isolated from *Eunicea laciniata* (9,10), was obtained as a white solid (600 mg, 8.2% of the extract) from one of the final chromatographic fractions described above. Its ^{1}H- and ^{13}C-nmr and mass spectra were identical with those reported (9). Compounds **2–6** were isolated by further chromatography (Si gel flash, prep. tlc, and reversed-phase hplc) of a second chromatographic fraction (see Experimental). Their yields were: **2**, 0.30%; **3**, 0.082%; **4**, 0.095%; **5**, 0.041%; and **6**, 0.068% of the crude extract.

The molecular formula of compound **2** was established by hr-cims as $C_{22}H_{32}O_4$. The ^{1}H-nmr data (Table 1) looked similar to those of **1** except for an additional AB quartet at δ 4.45, an additional methyl singlet at δ 2.04, and the absence of a methyl singlet at δ 1.57 (assigned to H_3-16 in **1**). The ^{13}C-nmr spectrum (Table 1), also similar to that



1 R_1=R_2=H
2 R_1=$OCOCH_3$, R_2=H
3 R_1=H, R_2=$OCOCH_3$



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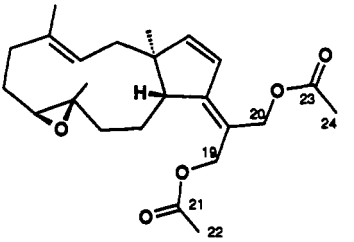

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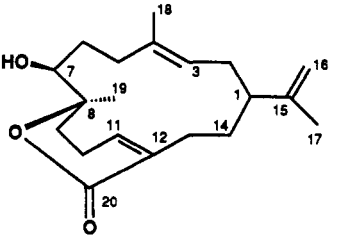

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TABLE 1. Nmr Data for Compounds **2** and **3**.

Compound **2**				Compound **3**			
Position	δ ^{1}H[a]	δ ^{13}C[b]	HMBC Correlations	Position	δ ^{1}H[a]	δ ^{13}C[b]	HMBC Correlations
1	—	40.58 (s)		1	—	40.95 (s)	
2	2.24 (dd, 12, 12)	39.74 (t)		2	2.17 (dd, 12, 12)	39.91 (t)	
	1.71 (dd, 12, 5)				1.66 (dd, 12, 5)		
3	5.68 (dd, 12, 5)	130.9 (d)	C-4, C-16	3	5.55 (dd, 12, 5)	128.3 (d)	C-1, C-2, C-16
4	—	136.9 (s)		4	—	131.0 (s)	
5	[c]	36.78 (t)		5	2.63 (dd, 11, 5), 2.42 (d, 11)	44.42 (t)	
6	1.68 (m), 1.49 (m)	27.43 (t)		6	5.05 (ddd, 11, 9, 5.5)	66.64 (d)	C-7, C-8, C-21
7	2.84 (d, 9)	65.49 (d)		7	3.05 (d, 9)	65.82 (d)	C-8
8	—	60.43 (s)		8	—	62.03 (s)	
9	[c]	33.30 (t)		9	2.02 (m), 1.41 (m)	36.66 (t)	
10	2.28 (m), 1.74 (m)	23.00 (t)		10	[c]	27.28 (t)	
11	2.51 (t, 13)	42.07 (d)		11	2.61 (br d, 12)	42.40 (d)	
12	—	134.8 (s)		12	—	137.2 (s)	
13	—	205.8 (s)		13	—	206.2 (s)	
14	2.40 (d, 18.5), 2.14 (d, 18.5)	54.31 (t)		14	2.44 (d, 19), 2.14 (d, 19)	54.38 (t)	C-1, C-2, C-13
15	1.19 (s)	23.42 (q)		15	1.18 (s)	23.45 (q)	C-1, C-2, C-11, C-14
16	4.45 (dd, 18.5, 12)	60.22 (t)	C-3, C-4	16	1.62 (s)	17.12 (q)	C-3, C-4, C-5
17	1.35 (s)	17.61 (q)		17	1.50 (s)	18.06 (q)	C-7, C-8, C-9
18	—	150.1 (s)		18	—	149.7 (s)	
19	1.95 (s)	24.82 (q)		19	1.92 (s)	24.93 (q)	C-12, C-18, C-20
20	2.24 (s)	21.78 (q)		20	2.25 (s)	22.01 (q)	C-12, C-18, C-19
21	—	171.0 (s)		21 (Ac)	—	170.4 (s)	
22	2.04 (s)	20.90 (q)		22 (Ac)	2.08 (s)	21.09 (q)	C-21

[a]Assignments made from HETCOR and TOCSY and by comparison of chemical shifts with those of **1**.
[b]Multiplicities determined from a DEPT spectrum.
[c]Not assigned, part of a complex signal.

of **1**, showed 22 signals including carbonyl absorptions at δ 205.8 for a ketone group (cf. δ 206.4 ppm for **1**) and δ 171.0 for an ester carbonyl. Also present in the ^{13}C-nmr spectrum were four other sp^2 carbon signals at δ 150.1, 136.9, 134.8, and 130.9, as well as two epoxide carbon signals at δ 65.49 and 60.43. The notable addition in the aliphatic region was the additional oxygenated methylene carbon signal at δ 60.22 (multiplicity was established by DEPT), which correlated with the AB quartet at δ 4.45 in a HETCOR spectrum. Conspicuously absent from the ^{13}C-nmr spectrum was the methyl signal at δ 15.6 assigned to C-16 of **1**. A TOCSY spectrum revealed that the protons of the AB quartet at δ 4.45 were in an isolated spin-system. The uv spectrum showed an absorption maximum at 252 nm indicating a conjugated enone moiety. All of these data suggested that **2** had the same dolabellane skeleton as **1** except for the oxygenation and esterification of C-16. The AB quartet at 4.45 was assigned to H_2-16. There is precedence for such a methylene group to have diastereotopic hydrogens in a dolabellane system (31). The HMBC correlations from H-3 (δ 5.68) to C-16 (δ 60.22) and from H_2-16 (δ 4.45) to C-3 (δ 130.9) and C-4 (δ 136.9) conclusively established that this diterpene possessed structure **2**. The Δ^3 olefin was assigned the *E* configuration based on the coupling constants for H-3, H-2_α, and H-2_β. These are similar to those reported for **1**, which were established using difference decoupling nmr experiments. Similarly, the configurations of the angular positions C-1 and C-11 were assigned as α and β, respectively, based on the ^{1}H- and ^{13}C-nmr chemical shifts and the coupling constant for H-11, which are similar to those reported for **1** (9).

Compound **3** also had the molecular formula $C_{22}H_{32}O_4$ as determined by hreims. Its lreims also showed a similar fragmentation pattern to that of **2**, implying a similarity in structure. The ^{1}H- and ^{13}C-nmr spectra of **3** also closely resembled those of **2** (Table 1), with the following notable exceptions: the AB quartet at δ 4.45 observed for H_2-16 in the ^{1}H-nmr spectrum of **2** was replaced by a methyl singlet at δ 1.62, a one-proton multiplet at δ 5.05 (ddd; J=11 Hz, 9 Hz, and 5.5 Hz), and the doublet (J=9 Hz) due to H-7 had shifted downfield to δ 3.00 ppm (cf. 2.83 ppm for H-7 of **2**). The nature of the coupling constants suggested the proximity of these protons and this was confirmed by the coupling observed in the DQCOSY spectrum. The DQCOSY spectrum also showed couplings between the proton signal at δ 5.05 ppm and proton signals at δ 2.63 and 2.43 ppm (H_2-5). The ^{13}C-nmr spectrum of **3** showed a ketone carbonyl absorption at δ 206.2, an ester carbonyl at δ 170.4, and four double-bond carbon signals at δ 149.7, 137.2, 131.0, and 128.3 ppm (Table 1). A uv spectrum showed an absorption maximum at 250 nm. All of these suggested structure **3** for this compound. This structure was confirmed by the HMBC correlations (Table 1) observed from H-6 (δ 5.05) to C-7 (δ 65.82), C-8 (δ 62.03), and C-21 (δ 170.4), and from H_3-22 (δ 2.08) to C-21 (δ 170.4). The configuration of the acetoxy group at C-6 is assumed to be β based on the coupling constant of H-6 with H-7. H-7 is a doublet with J=9 Hz, a value closer to the axial-equatorial couplings in cyclohexanes. It has been well-established that the epoxide is β using selective nOe experiments (9) and, in order for H-6 to have an axial-equatorial relationship with H-7, it would have to occupy the α-configuration.

This was further confirmed by a difference nOe experiment. Irradiation of the Me singlet at δ 1.50 ppm (Me-17) resulted in the enhancement of the signal at δ 5.05 for H-6. The Δ^3 olefin was assigned the *E* configuration based on the coupling constants for H-3, H-2_α, and H-2_β. These are similar to those reported for **1**, which were established using difference decoupling experiments. Similarly, the configurations of the angluar positions C-1 and C-11 were assigned as α and β, respectively, based on the ^{1}H- and ^{13}C-nmr chemical shifts and the coupling constant for H-11, which are similar to those reported for **1** (9).

The molecular formula of compound **4** was established as $C_{20}H_{30}O_2$ from its hreims. The ^{1}H- and ^{13}C-nmr spectra suggested the same dolabellane diterpenoid skeleton of **1–3** with signals for the epoxide functionality, five methyl groups, two double bonds, and one carbonyl group (δ 213.0 in the ^{13}C-nmr spectrum). All of these were suggestive of a structure isomeric with that of **1**. The ^{1}H-nmr spectrum of **4** (Table 2), while having the same doublet of doublets at δ 5.47 for H-3 (cf. δ 5.41 for H-3 of **1**), contained an additional one-proton vinyl singlet at δ 5.85, and an additional multiplet at δ 2.55. The methyl signals at δ 1.93 and 2.26, characteristic of the vinyl methyl groups (H_3-19 and H_3-20) of **1** were conspicuously absent in the ^{1}H-nmr spectrum of **4** and present in their place were two doublets at δ 1.17 and 1.20. Coupling was observed in a DQCOSY spectrum between these methyl signals and the multiplet at δ 2.55 indicating the presence of an isopropyl group at C-12 of the dolabellane skeleton. The ^{13}C-nmr spectrum (Table 2) showed, in addition to the carbonyl absorption at δ 213.0, sp^2 carbon signals at δ 189.9, 136.2, 123.9, and 123.0. The DEPT spectrum gave the multiplicities of the signals at δ 123.9 and 123.0 as doublets and the signals at δ 189.9 and 136.2 as singlets. A HETCOR spectrum revealed the connectivity of the carbon signal at δ 123.9 to the proton singlet at δ 5.85 and the carbon signal at δ 123.0 to the proton signal at δ 5.47. This supported the proposed structure **4** and the assignment of the δ 5.85 ppm signal to H-13. An HMBC spectrum showed the expected two-bond and three-bond correlations (Table 2), notably from H-13 (δ 5.85) to C-1 (δ 53.30), C-11 (δ 48.03), C-12 (δ 189.9), and C-14 (δ 213.0), thus confirming the proposed structure. It is proposed that the relative configuration of the methyl group at C-1 be assigned as α, by comparison of its ^{13}C-nmr chemical shift (δ 15.56 ppm) to that of the α-methyl group (δ 15.9 ppm) in 3,4-epoxy-14-oxo-7,18-dolabelladiene isolated from the marine alga *Dictyota dichotoma* by Amico *et al.* (32).

Compound **5** was obtained by reversed-phase hplc from the same mixture that gave compound **3**. The molecular formula was found to be $C_{24}H_{34}O_5$ from its hreims spectrum. The ^{1}H-nmr spectrum (Table 2) showed the now familiar signals for a dolabellane skeleton: a one-proton doublet of doublets at δ 5.38 ppm (for H-3), a broad doublet at δ 2.79 for H-7, and methyl singlets at δ 1.17, 1.33, and 1.60. Notable differences were the mutually coupled doublets at δ 6.34 and 5.90, the mutually coupled doublets at δ 4.81 and 4.59, the broad singlet at δ 4.71, and two methyl singlets at δ 2.04. The chemical shifts and coupling constant (5.5 Hz) of the two doublets at δ 6.34 and 5.90 were suggestive of a cis double bond that is part of a conjugated diene. Further evidence for the conjugated diene came from the λ max of 243 nm observed in its uv spectrum. The mutually coupled doublets at δ 4.81 and 4.59 were suspected to be due to an oxomethylene group. The singlet at δ 4.71 integrated for two protons and was suggestive of another oxomethylene group with magnetically equivalent protons. The ^{13}C-nmr spectrum showed 23 signals including an ester carbonyl at δ 171.1, other sp^2 carbons at δ 158.4, 150.8, 135.9, 126.4, 123.7, and 118.8, and oxygenated sp^3 carbons at δ 66.52, 62.82, 62.77, and 60.41. A DEPT spectrum gave the multiplicities of the carbon signals and together with a HETCOR spectrum most of the C-H correlations were established with the exception of the carbon signals (doublets from DEPT) at δ 150.8 and 126.4 ppm. Neither the HETCOR nor the HMQC spectrum provided the C-H correlations for these signals (it must be noted that we only had about 3 mg of this compound). Inspection of lreims data showed the loss of two successive HOAc fragments from the molecular ion at *m/z* 402 (fragments with *m/z* 342 and 282, respectively). This, together with the nmr information, that is, the presence of an ester carbonyl at δ 171.0 and two acetate methyls at δ 21.02 and 20.90 (correlated with the δ 2.04 ppm proton signals in both HETCOR and HMQC), suggested the presence of two acetoxy groups.

TABLE 2. Nmr Data for Compounds **4** and **5**.

Compound **4**				Compound **5**			
Position	δ $^1H^a$	δ $^{13}C^b$	HMBC Correlations	Position	δ $^1H^a$	δ $^{13}C^b$	HMBC Correlations
1	—	53.30 (s)		1	—	54.02 (s)	
2	1.90 (m)	38.02 (t)	C-3, C-4	2	1.85 (m), 1.63 (m)	39.56 (t)	C-3, C-14
3	5.47 (dd, 11, 5)	123.0 (d)		3	5.38 (dd, 11, 7)	123.7 (d)	
4	—	136.2 (s)		4	—	135.9 (s)	
5	2.30 (m)	37.77 (t)		5	[c]	37.98 (t)	
6	1.90 (m), 1.52 (m)	23.38 (t)		6	[c]	22.91 (t)	
7	2.74 (d, 10)	67.34 (d)	C-6, C-8	7	2.79 (br d, 9)	66.52 (d)	C-6, C-17
8	—	60.42 (s)		8	—	60.41 (s)	
9	1.63 (m)	36.17 (t)	C-17	9	[c]	36.64 (t)	
10	1.78 (m), 1.29 (m)	27.20 (t)		10	[c]	29.77 (t)	
11	2.54 (br d, 11)	48.03 (d)		11	2.56 (br d, 12.5)	44.25 (d)	C-14
12	—	189.9 (s)		12	—	158.4 (s)	
13	5.85 (s)	123.9 (d)	C-1, C-11, C-12, C-14	13	5.90 (d, 5.5)	126.4 (d)	C-12
14	—	213.0 (s)		14	6.34 (d, 5.5)	150.8 (d)	C-1, C-11, C-12, C-15
15	1.15 (s)	15.52 (q)	C-1, C-2, C-11, C-14	15	1.17 (s)	20.19 (q)	C-1, C-2, C-11, C-14
16	1.60 (s)	16.15 (q)	C-3, C-4, C-5	16	1.60 (s)	15.49 (q)	C-3, C-4, C-5
17	1.30 (s)	17.38 (q)	C-7, C-8	17	1.33 (s)	17.57 (q)	C-7, C-8, C-9
18	2.55 (m)	29.53 (d)		18	—	118.8 (s)	
19	1.17 (d, 7)	22.60 (q)	C-12, C-18, C-20	19	4.81 (d, 12), 4.59 (d, 12)	62.82 (t)	C-12, C-18, C-20, C-21 (OAc)
20	1.20 (d, 7)	21.57 (q)	C-12, C-18, C-19	20	4.71 (br s)	62.77 (t)	C-12, C-18, C-19, C-22 (OAc)
				21 (Ac)	—	171.1 (s)	
				22 (Me/Ac) .	2.04 (s)	20.90 (q)	C-21
				23 (Ac)	—	171.1 (s)	
				24 (Me/Ac) .	2.04 (s)	21.02 (q)	C-23

[a]Assignments made from HETCOR, HMQC, and DQCOSY.
[b]Multiplicities determined from DEPT nmr spectrum.
[c]Not assigned, part of a complex signal.

The possibility that these acetoxy groups could be at C-15, C-16, or C-17 was ruled out because the ^{1}H- and ^{13}C-nmr chemical shifts of the three methyl groups corresponded well with these three methyls in dolabellanes like **1** and **4**. This led to proposed structure **5** with the two acetoxy groups at C-19 and C-20 and double bonds between C-13 and C-14 and C-12 and C-18. This diene moiety and the diacetoxy structure are unprecedented in a dolabellane skeleton. Support for the proposed structure came from HMBC correlations (Table 2), most notably: H-14 (δ 6.34) to C-1 (δ 54.02), C-11 (δ 44.25), C-12 (δ 158.4), C-15 (δ 20.19); H-13 (δ 5.90) to C-12 (δ 158.4); H-19 (δ 4.81, 4.59) to C-12 (δ 158.4), C-18 (δ 118.8), C-20 (δ 62.77), C-21 (δ 171.1); H-20 (δ 4.71) to C-12 (δ 158.4), C-18 (δ 118.8), C-19 (δ 62.82), and C-23 (δ 171.1). The basis for assigning the α-configuration to C-15 is that the other dolabellanes isolated from this organism [**1–4**] all have an α orientation for this methyl group and that the observed ^{13}C-nmr chemical shift of δ 20.19 is similar to the chemical shift of C-15 in **1–3**.

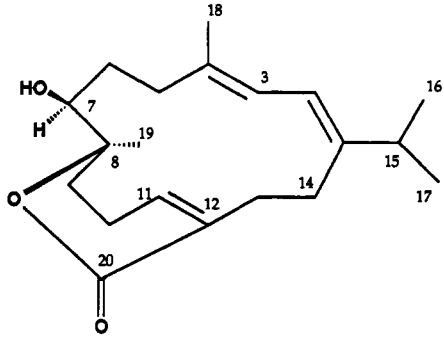
Compound **6** was obtained by reversed-phase hplc from the same fraction that yielded **2** and was found to have the molecular formula $C_{20}H_{30}O_3$ from its hreims spectrum. An indication that **6** was not a dolabellane came from its ^{1}H-nmr spectrum (Table 3), which lacked many of the characteristic signals seen in the ^{1}H-nmr spectra of **1–5**. It contained five downfield signals: triplets at δ 6.15 (J=4 Hz) and 5.13 (J=8 Hz), the doublet of doublets at δ 4.71 (J=2 and 1 Hz) and 4.12 (J=10 and 7 Hz), and a doublet at δ 4.66 (J=1 Hz). Three methyl singlets were present at δ 1.66, 1.59, and 1.32 ppm. The ^{13}C-nmr spectrum (Table 3) showed an ester carbonyl signal at δ 167.1, six other sp^2 carbon signals at δ 148.7, 139.9, 134.3, 133.6, 126.2, and 110.7, and two oxygenated sp^3 carbon signals at δ 82.92 and 67.66. A DEPT spectrum gave the multiplicities of the protonated carbons and a HETCOR spectrum showed C-H correlations of all the important carbon signals (see Table 3). Proton-proton couplings were observed in a DQ/COSY spectrum between the triplet at δ 6.15 and multiplets at δ 2.65 and 2.49, between the triplet at δ 5.13 and the multiplet at δ 1.89, between the

TABLE 3. Nmr Data for Compound **6**.

Position	δ ^{1}H[a]	δ ^{13}C[b]	HMBC Correlations
1	1.75 (m)	46.60 (d)	
2	1.89 (m)	32.85 (t)	C-3, C-4, C-14
3	5.13 (br t, 8)	126.2 (d)	C-4, C-5, C-18
4	—	134.3 (s)	
5	2.07 (m)	33.92 (t)	C-3, C-18
6	1.82 (m), 1.41 (m)	29.91 (t)	
7	4.12 (dd, 10, 7)	67.66 (d)	C-6, C-8, C-19
8	—	82.92 (s)	
9	[c]	35.02 (t)	
10	2.65 (m), 2.49 (m)	27.19 (t)	
11	6.15 (t, 4)	139.9 (d)	C-10, C-12, C-13, C-20
12	—	133.6 (s)	
13	2.88 (dd, 14, 8), 1.62 (m)	34.82 (t)	C-1, C-11, C-12, C-14, C-20
14	1.45 (m), 1.05 (m)	28.38 (t)	
15	—	148.7 (s)	
16	4.71 (dd, 2, 1), 4.66 (br d, 1)	110.7 (t)	C-1, C-15, C-17
17	1.66 (br s)	18.57 (q)	C-1, C-15, C-16
18	1.59 (s)	16.94 (q)	C-3, C-4, C-5
19	1.32 (s)	22.44 (q)	C-7, C-8
20	—	167.1 (s)	

[a]Assignments made from HETCOR and DQCOSY.
[b]Multiplicities determined from DEPT nmr spectrum.
[c]Not assigned, part of a complex signal.

doublet of doublets at δ 4.71 and the doublet at δ 4.66, and between the doublet of doublets at δ 2.88 and the multiplet at δ 1.62. The proton resonances at δ 4.71 and 4.66 were correlated with the carbon absorption at δ 110.7 in the HETCOR spectrum; this, together with the chemical shift of δ 148.7 for one of the non-protonated sp^2 carbons and the methyl absorption at δ 1.66, suggested an isopropenyl group. Comparison of these nmr data with those of other diterpenoids from marine organisms led to the proposed cembranolide structure **6** with an unusual ϵ-lactone formed by the oxidized methyl group at C-12 and a hydroxyl at C-8. This structure explained several of the unusual characteristics in both the ^{1}H- and ^{13}C-nmr spectra: the quaternary carbon at δ 82.92, the carbonyl absorption at δ 167.1, and the downfield vinyl proton at δ 6.15. Extensive two- and three-bond H-C correlations were observed in an HMBC spectrum (Table 3) all of which supported the proposed structure. The key correlations were: from H-11 (δ 6.15) to C-10 (δ 27.19), C-12 (δ 133.6), C-13 (δ 34.82), C-20 (δ 167.1); from H-7 (δ 4.12) to C-6 (δ 29.91), C-8 (δ 82.92), C-19 (δ 22.44); from H-13 (δ 2.88 and 1.62) to C-1 (δ 46.6), C-11 (δ 139.9), C-12 (δ 133.6), C-14 (δ 28.38), C-20 (δ 167.1); from H-16 (δ 4.71 and 4.66) to C-1 (δ 46.6), C-15 (δ 148.7), C-17 (δ 18.57). There are two previous reports of marine-derived cembranolides with the same ϵ-lactone ring as in **6** (33,34), but both describe the isolation of cembranolides from Pacific soft corals, and both have an upper right-hand quadrant different from that of **6**. To our knowledge, this is the first instance where this functional group has been encountered in a Caribbean gorgonian. A β-configuration is assigned for the hydroxyl group at C-7 based on the chemical shift and shape of the ^{1}H-nmr signal of H-7 as compared to those reported for the corresponding proton in the natural product derivative **7** (34). The signal for H-7 of **7** is a doublet of doublets with δ 4.15, while that for its epimer appears as a broad doublet at δ 4.30 (34).



7

In the Caribbean, sea whips of the genus *Eunicea* are particularly abundant and a number of chemical investigations of *Eunicea* spp. have been reported (9–30). More than 50 diterpenoids have been isolated from this genus and most of them possess the cembrane skeleton. Only nine dolabellanes have been reported so far from *Eunicea* and, therefore, they should still be considered somewhat uncommon. In addition, to our knowledge, there have not been any reports of the isolation of dolabellanes from *E. tourneforti*; the only previous report on the secondary chemistry of *E. tourneforti* was the isolation of the cembranoid diterpene, asperdiol, by Weinheimer *et al.* (28). This is also the first instance of the occurrence of a cembranolide with an ϵ-lactone ring in a Caribbean gorgonian. More interestingly, this is the first account of the occurrence of dolabellanes and a cembrane coexisting within the same *Eunicea* species. This could

support a hypothesis that the cembrane skeleton is a logical biosynthetic precursor to the dolabellane ring system.

The diterpenoids **1–6** were either inactive or only weakly active in the yeast-based bioassay. The IC_{12} values against RS 321 (the only strain that was inhibited) were: **1**, 1500 μg/ml; **2**, 1800 μg/ml; **3**, >3000 μg/ml; **4**, 2600 μg/ml; **5**, >3000 μg/ml; and **6**, >3000 μg/ml.

EXPERIMENTAL

GENERAL EXPERIMENTAL PROCEDURES.—Nmr spectra were recorded on a Varian Unity 400 spectrometer at 399.95 MHz for ^1H and 100.58 MHz for ^{13}C and built-in Varian pulse sequences were employed. All samples were run in $CDCl_3$. The chemical shifts were referenced to the residual $CHCl_3$ signal at 7.24 ppm for ^1H-nmr spectra and the $CDCl_3$ signal at 77.00 ppm for ^{13}C-nmr spectra. All extractions and chromatography were conducted using commercially available hplc-grade solvents. Mps were determined using a Mel-Temp apparatus and optical rotations were measured using a Perkin-Elmer 241 polarimeter in $CHCl_3$. Mass spectra were obtained from the Virginia Tech Mass Spectrometry Center using a VG-7070 E-HF instrument with direct probe insertion (compounds **2–6**) or a VG-Fisons Quattro (compound **1**). Uv spectra were measured in MeOH using a Perkin-Elmer Lambda B UV spectrometer.

BIOLOGICAL SCREENING PROCEDURE.—The experimental procedures used for the mechanism-based bioassay have been described previously (7). The IC_{12} values refer to the concentration in μg/ml required to produce a zone of inhibition of 12 mm around a 100-μl well of 6-mm diameter during a 48 h incubation period at 37° determined from dose-response experiments.

ANIMAL MATERIAL.—The marine organisms were collected in September 1993, by scuba at depths of between 12 and 20 meters from two locations off the southwest and northwest coasts of St. Thomas, U.S. Virgin Islands. A second collection of *Eunicea tourneforti* was made in March 1994, at the original site. Voucher specimens are stored at the University of the Virgin Islands (voucher no. 87-SA-93 for *E. tourneforti*). The specimens were frozen immediately and stored. Dr. William Gladfelter, Visiting Professor of Marine Biology, University of the Virgin Islands, carried out the preliminary identification of the collected specimens, including *E. tourneforti*. The final taxonomic verification of *E. tourneforti* was carried out by Dr. Paul Yoshioka of the University of Puerto Rico, Mayaguez.

EXTRACTION AND ISOLATION.—Freshly thawed samples of the specimens were extracted by sequential soaking in MeOH and CH_2Cl_2. Each sample was cut into small pieces and was extracted overnight twice with MeOH and twice with CH_2Cl_2. The first MeOH extract containing a significant proportion of H_2O was concentrated to remove the MeOH, extracted twice with $CHCl_3$, and once with EtOAc. These $CHCl_3$ and EtOAc extracts were combined with the second MeOH extract and subsequent CH_2Cl_2 extracts and concentrated to obtain the crude extract, which was screened in the mechanism-based bioassay at a concentration of 2000 μg/ml. The aqueous layer from the first MeOH extraction was also tested for bioactivity.

The freshly thawed specimen (700 g wet wt; 274 g dry wt) of *E. tourneforti* was sequentially extracted with MeOH (2×1 liter) and CH_2Cl_2 (3×1 liter) by the general procedure described above to obtain 7.36 g of crude extract. This extract showed no inhibition of *rad*+ and *rad 52*Y but inhibited the growth of the RS 321 strain at 2000 μg/ml. A dose response analysis gave an IC_{12} value of 2570 μg/ml.

ISOLATION OF DITERPENOIDS **1–6**.—The crude extract (7.1 g) was suspended in 80% aqueous MeOH (500 ml), sonicated for 5 min, and extracted twice with hexane (250 ml each). The hexane layer was backwashed twice with 80% aqueous MeOH (150 ml each), dried with anhydrous $MgSO_4$, concentrated, and tested for biological activity. The aqueous MeOH layer was diluted to 60% aqueous MeOH and extracted three times with $CHCl_3$ (400 ml each). The combined $CHCl_3$ extracts were washed twice with 60% aqueous MeOH (200 ml each), dried with $MgSO_4$, concentrated, and tested for biological activity. The aqueous MeOH layer was concentrated and extracted with *n*-BuOH (3×200 ml) and the *n*-BuOH extract was tested for biological activity. The $CHCl_3$ extract (3.18 g) continued to show moderate inhibition of the RS 321 yeast strain, while the hexane and *n*-BuOH extracts only had slight activities.

The $CHCl_3$ extract (3.18 g) was fractionated by gel-filtration chromatography on Sephadex LH-20 (30 g) using the procedure of Cardellina (8). Ten fractions were collected, and the first four fractions were combined based on their activity against RS 321, and tlc (Si gel, hexane/EtOAc, 1:1). This combined material (2.5 g) was further separated by vacuum-flash chromatography on Si gel (50 g, hexane/EtOAc/MeOH gradient) to obtain nine fractions. Bioassay indicated that the activity was distributed among all the fractions and it was decided to discontinue the use of bioassay to guide further fractionations. Inasmuch as the fourth fraction contained more than 50% of the material (1.32 g), its ^1H-nmr spectrum was obtained,

which showed characteristic terpenoid signals. This fraction was again chromatographed on Si gel (20 g, vacuum flash, hexane/EtOAc/MeOH gradient) to nine fractions. The second and third fractions contained most of the material (1.25 g) and their ^{1}H-nmr spectra continued to show the terpenoid signals. The combined fractions were again chromatographed on Si gel (medium-pressure flash, 20 g, hexane/EtOAc/MeOH gradient). The fourth fraction (786 mg) upon crystallization from Me_2CO/hexane produced a white solid, which was identified as **1** from its ^{1}H- and ^{13}C-nmr and lreims data.

Fractions 5 and 6 from the above chromatographic separation, similar by ^{1}H-nmr and tlc data, were combined (344 mg) and chromatographed on Si gel (medium pressure, 10 g, CH_2Cl_2/EtOAc gradient). Fractions 2 and 3, showing similar bright pink spots on tlc (Si gel, 20% Me_2CO/hexane, vanillin/H_2SO_4 spray and charring) and characteristic terpenoid signals in their ^{1}H-nmr spectra, were combined (146 mg) and separated by prep. tlc (Si gel, 1000 μm, double elution with 20% Me_2CO/hexane). Four fractions were collected and the first fraction (20 mg) was found to contain mainly **1**. The remaining fractions were separated by reversed-phase hplc to obtain **2–6**.

7,8-Epoxy-3,12-dolabelladien-13-one [**1**].—White solid, 600 mg, mp 88–89°; $[\alpha]^{24}D$ +100° (c=2 mg/ml); ^{1}H nmr (400 MHz, $CDCl_3$) δ 5.41 (1H, dd, J=12 and 5 Hz), 2.89 (2H, d, J=9 Hz), 2.67 (1H, d, J=13 Hz), 2.38 (1H, d, J=13 Hz), 2.24 (3H, s), 2.15 (1H, m), 2.12 (1H, d, J=13 Hz), 2.00 (1H, m), 1.91 (3H, s), 1.66 (1H, m), 1.62 (1H, m), 1.43 (1H, m), 1.55 (3H, s), 1.34 (3H, s), 1.31 (1H, m), 1.17 (3H, s); ^{13}C nmr (100 MHz, $CDCl_3$) δ 206.5, 149.1, 137.6, 136.0, 124.7, 65.71, 60.45, 54.35, 42.07, 41.04, 39.78, 37.97, 36.88, 27.47, 24.98, 23.32, 22.84, 21.79, 17.67, 15.65; lreims, m/z $[M]^+$ 302 (for $C_{20}H_{30}O_2$), 287, 259, 245; uv λ max (MeOH), 250 nm (ε 10,100).

7,8-Epoxy-3,12-dolabelladien-14-one [**4**].—The second fraction (14 mg) from the prep. tlc above was further purified by reversed-phase hplc (Dynamax C_{18} column; 10×250 mm; 90% MeOH/H_2O; 2 ml/min; Waters photodiode array detector set at 210 nm) to obtain **4** (7 mg), mp 134–136°; $[\alpha]^{24}D$ +10° (c=6 mg/ml); ^{1}H- and ^{13}C-nmr data, see Table 2; hreims, m/z $[M]^+$ 302.22580, $C_{20}H_{30}O_2$ requires 302.22458; lreims, m/z 302, 287, 259, 241; uv λ max (MeOH), 230 nm (ε 7,000).

6-Acetoxy-7,8-epoxy-3,12-dolabelladien-13-one [**3**] *and 19,20-diacetoxy-7,8-epoxy-3,12,13-dolabellatriene* [**5**].—The third fraction (18 mg) was further purified by reversed-phase hplc (Dynamax C_{18} column; 10×250 mm; 80% MeOH/H_2O; 3 ml/min; Waters photodiode array detector set at 210 nm) to obtain **3** (6 mg) and **5** (3 mg). Compound **3**: white solid, mp not determined; $[\alpha]^{24}D$ +90° (c=2 mg/ml); ^{1}H- and ^{13}C-nmr data, see Table 1; hreims m/z $[M]^+$ 360.22899, $C_{22}H_{32}O_4$ requires 360.23006; lreims, m/z 360, 318, 300, 285; uv λ max (MeOH), 250 nm (ε 10,600). Compound **5**: glassy solid; $[\alpha]^{24}D$ 0° (c=1 mg/ml); ^{1}H- and ^{13}C-nmr data, see Table 2; hreims, m/z $[M]^+$ 402.24171, $C_{24}H_{34}O_5$ requires 402.24063; lreims, m/z 402, 387, 342, 316, 300, 282; uv λ max (MeOH), 243 nm (ε 3,200).

16-Acetoxy-7,8-epoxy-3,12-dolabelladien-13-one [**2**] *and (3E,7S,11Z)-7-hydroxy-3,11,15-cembratrien-20,8-olide* [**6**].—The fourth fraction from prep. tlc (64 mg) gave four major peaks in its reversed-phase hplc (Dynamax C_{18} column; 10×250 mm; 80% MeOH/H_2O; 2 ml/min; Waters photodiode array detector set at 210 nm). The first and third peaks from repeated injections were collected to obtain **2** (22 mg) and **6** (5 mg), respectively. The other two peaks were also due to diterpenes (<2 mg each) as suggested by ^{1}H-nmr spectra, but their identities have not been determined due to difficulties in obtaining additional nmr data with such small amounts. Compound **2**: glassy solid; $[\alpha]^{24}D$ +40° (c=4.5 mg/ml); ^{1}H- and ^{13}C-nmr data, see Table 1; hrcims, m/z $[M+H]^+$ 361.23788, $C_{22}H_{33}O_4$ requires 361.23877; lreims, m/z 360, 345, 342, 318, 300; uv λ max (MeOH), 252 nm (ε 16,000). Compound **6**: glassy solid; $[\alpha]^{24}D$ +3° (c=1 mg/ml); ^{1}H- and ^{13}C-nmr data, see Table 3; hreims, m/z $[M]^+$ 318.21851, $C_{20}H_{30}O_3$ requires 318.21950; lreims m/z 318, 300, 285, 272; uv λ max (MeOH), 227 nm (ε 10,700).

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