Sesquiterpenoid Metabolites with Antiplasmodial Activity from a Caribbean Gorgonian Coral, *Eunicea* sp.

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A chemical investigation of the Caribbean gorgonian octocoral *Eunicea* sp. collected along the northwest coast of Puerto Rico has afforded seven new secondary metabolites, **1**−**7**, belonging to several types of sesquiterpenes, including elemane, eudesmane, and germacrane types, along with the known steroidal glycoside **8**. Some of the new metabolites, **4**−**7**, carry an unusual ester side chain at the C-6 position. The structures of all compounds, including their relative stereochemistry, were determined by combined spectroscopic methods. The present compounds exhibited a significant inhibitory effect upon the growth of the malarial parasite *Plasmodium falciparum*.

Gorgonian octocorals of the taxonomically complex genus *Eunicea* are known to produce a wealth of natural products of a number of structural types, including cembrane-, dolabellane-, cubitane-, and elemane-type diterpenoids, as well as sterols, sesquiterpenes, and secondary metabolites of mixed biogenesis.[1] As part of an ongoing search for novel antimalarial agents from marine invertebrates from the Caribbean Sea, we have investigated the secondary metabolite composition of the sea whip *Eunicea* sp., collected along the northwest coast of Puerto Rico off the shores of Aguadilla. The extract of a relatively small specimen collected was found active in inhibiting the growth of *Plasmodium falciparum* and, thus, merited further chemical investigation.

The freshly collected specimen was stored frozen, freeze-dried, and subsequently extracted with a 1:1 mixture of MeOH−$CHCl_3$. Compounds **1**−**8** were purified by flash silica gel chromatography of the crude extract using gradient elution with hexane−EtOAc−MeOH followed by further column chromatography of several of the relatively nonpolar fractions on both normal- and reversed-phase silica gel. The new compounds **1**−**7**, whose structures were established by a study of their physical and spectral [UV, IR, ^{1}H and ^{13}C NMR, DEPT, and 2D NMR (^{1}H−^{1}H COSY, HMQC, HMBC, NOESY) and mass] data, belong to the elemane, eudesmane, and germacrane classes of sesquiterpenes. The known saponin **8** was characterized by a comparative study of its physical and spectral characteristics with those reported in the literature. Similar sesquiterpene hydrocarbons as well as related diterpenoids of the dilophol and fuscol classes have been observed by others from several gorgonians identified as *Eunicea mammosa*, *E. asperula*, and *E. fusca*.[2,3]

Compound **1** was eluted as a white amorphous solid, $[\alpha]_D^{25}$ +18.8°. Its molecular formula was fixed as $C_{15}H_{20}O_2$ by HRFABMS and ^{13}C NMR spectrometry (Table 1). Thus, 6 degrees of unsaturation were determined for **1**. The IR spectrum of **1** exhibited the presence of a carbonyl group of an α,β-unsaturated γ-lactone (ν_{max} 1751 cm^{-1}) and an olefinic unsaturation (ν_{max} 3080, 1688, 1638, 1456, 893 cm^{-1}). Its UV spectrum showed an absorption at 220 nm, indicating the presence of an α,β-unsaturated carbonyl system. In the ^{1}H NMR spectrum of **1** proton resonances at δ 5.08 (1H, br t, J = 1.4 Hz), 4.79 (1H, br s), and 1.79 (3H, dd, J = 0.7, 1.2 Hz), which were mutually coupled, along with carbon resonances at δ 141.6 (s), 114.6 (t), and 24.7 (q), were interpreted to indicate an isopropenyl group. Another cluster of olefin protons at δ 5.71 (1H, dd, J = 10.8, 17.4 Hz), 5.00 (1H, dd, J = 0.8, 10.8 Hz), and 4.96 (1H, dd, J = 0.8, 17.4 Hz), which correlated with carbons at δ 146.2 (d) and 111.9 (t), were readily assigned to a terminal vinyl group. The remaining low-field resonance at δ 4.92 (1H, d, J = 11.5 Hz) was concluded to originate from a proton attached to an oxygen-bearing carbon. A direct carbon−

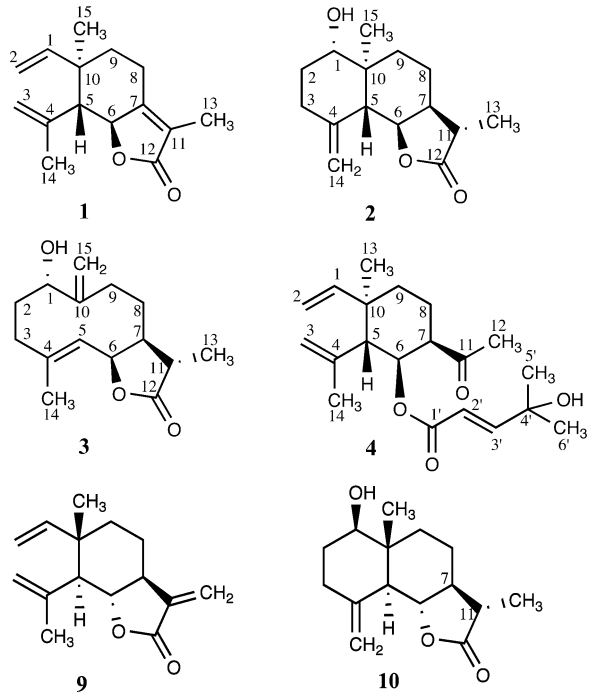

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Table 1. ^{1}H (300 MHz) and ^{13}C (75 MHz) NMR Spectral Data for Compounds **1**−**3** in $CDCl_3$ [δ_H, mult, J (Hz); δ_C (mult)][a]

	compound **1**		compound **2**		compound **3**	
atom	δ_H	δ_C	δ_H	δ_C	δ_H	δ_C
1	5.71, dd, 10.8, 17.4	146.2 (d)	3.41, dd, 4.6, 11.5	78.0 (d)	3.96, dd, 4.0, 8.1	73.5 (d)
2α	5.00, dd, 0.8, 10.8	111.9 (t)	1.92, m	31.3 (t)	2.01, m	33.4 (t)
2β	4.96, dd, 0.8, 17.4		1.86, m		2.01, m	
3α	5.08, br t, 1.4	114.6 (t)	2.42, m	34.0 (t)	2.18, m	37.4 (t)
3β	4.79, br s		2.34, m		2.18, m	
4		141.6 (s)		142.6 (s)		140.7 (s)
5	1.88, d, 11.5	59.0 (d)	1.83, d, 10.5	49.1 (d)	5.27, d, 8.9	121.1 (d)
6	4.92, d, 11.5	80.6 (d)	4.72, dd, 7.5, 10.5	75.9 (d)	5.10, dd, 7.5, 10.0	77.5 (d)
7		161.6 (s)	2.42, m	41.7 (d)	2.24, m	46.9 (d)
8α	2.43, m	22.6 (t)	1.85, m	19.6 (t)	1.67, m	26.5 (t)
8β	2.76, dddd, 2.1, 4.6, 7.6, 14.4		1.76, m		2.05, m	
9α	1.69, dddd, 2.2, 5.6, 7.8, 14.5	39.1 (t)	1.36, m	30.4 (t)	2.38, m	32.2 (t)
9β	1.56, dd, 4.7, 14.2		1.25, m		2.38, m	
10		41.3 (s)		40.9 (s)		152.8 (s)
11		120.0 (s)	2.55, dq, 6.8, 13.5	35.6 (d)	2.40, dq, 7.1, 8.8	40.2 (d)
12		174.6 (s)		179.2 (s)		179.4 (s)
13	1.82, br t, 1.6	8.3 (q)	1.22, d, 6.8	13.4 (q)	1.27, d, 7.1	14.6 (q)
14α	1.79, dd, 0.7, 1.2	24.7 (q)	4.99, s	109.7 (t)	1.62, br d, 1.2	17.2 (q)
14β			4.84, s			
15α	1.18, s	16.9 (q)	0.75, s	10.0 (q)	5.12, br s	110.6 (t)
15β					4.88, br s	

[a] Chemical shift values are in ppm relative to TMS. Spectra were recorded at 25 °C. ^{13}C NMR multiplicities were obtained from DEPT NMR experiments.

proton NMR correlation experiment (HMQC) confirmed this assumption by illustrating that the latter proton corresponded with a carbon at δ 80.6 (d). The proton at δ 4.92 was coupled (J = 11.5 Hz) with an adjacent allylic proton [δ 1.88 (1H, d, J = 11.5 Hz)], which showed no further coupling. The ^{1}H NMR spectrum of **1** further showed a tertiary methyl at δ 1.18 (3H, s) and a second methyl on a double bond at δ 1.82 (3H, br t, J = 1.6 Hz). The ^{13}C NMR spectrum of **1**, which exhibited signals for all 15 carbons and their multiplicities, also showed low-field signals at δ 174.6 (s), 161.6 (s), and 120.0 (s) that might be assigned to a fully substituted α,β-unsaturated γ-lactone. Thus, of the six double-bond equivalents required for the molecular formula, four were accounted for, one in carbonyl and three in double bonds. The molecule should, therefore, be bicyclic. Further consideration of ^{1}H−^{1}H COSY data showed that all of the protons in **1** belong to four isolated spin systems, suggesting four partial structures. With the help of HMQC data (all of the 10 proton-bearing carbons and their protons were precisely matched) three of such four units were confidently assigned as a terminal vinyl, an isopropylene side chain, and a α-methyl-α,β-unsaturated butenolide moiety. The remaining component of **1** was assigned as a six-membered ring on the basis of typical ^{1}H and ^{13}C NMR features. Careful analysis of the HMBC data allowed all four partial structures to be confidently combined. Thus, the above functionalities could be accommodated in structure **1**, which can be best described as a bicyclic sesquiterpene lactone possessing an "elemene" divinylcyclohexane ring.

Determination of the relative stereochemistry at the three asymmetric carbons (C-5, C-6, and C-10) of **1** was accomplished by NOE studies and proton−proton coupling constant analysis. NOESY correlations of H_3-15/H_2-8α, H-6/H_2-8α, and H_3-15/H-6 indicated that these protons are all α-oriented (1,3-diaxial substituents), while the C-5 proton showed no enhancement with either one of these protons. Furthermore, analysis of the coupling constant for the H-5 (δ 1.88) bridgehead proton revealed a J value of 11.5 Hz, assignable to an axial−axial (*trans*) configuration between the H-5 and H-6 protons. Interestingly, a closely related isomer of compound **1**, elemanolide **9**, has been detected in the South American liverworts *Clasmatocolea humilis* and *Plagiochila hondurensis*.[4]

The mass spectrum of compound **2** showed a molecular peak at *m/z* 250.1577 $[M]^+$, which agreed with the molecular formula $C_{15}H_{22}O_3$. Its ^{1}H and ^{13}C NMR spectra (Table 1) showed typical signals that suggested an eudesmanolide framework with features common to some natural products isolated from various plant sources. For instance, the analysis of the NMR spectra with the aid of ^{1}H−^{1}H COSY, DEPT, HMQC, and HMBC showed that **2** has an eudesmanolide nucleus with identical functionalization (but distinct relative stereochemistry) to that of 11β,13-dihydroreynosin (**10**), a known plant metabolite isolated from the dried roots of *Saussurea lappa*.[5] For compound **2** the coupling patterns and the magnitude of the coupling constants of H-1, H-5 to H-7, and H-11 were in full agreement with the β-stereochemistry for H-1, a *cis*-disposition of H-6/H-7, and a *trans*-disposition of H-5/H-6 and H-7/H-11 (Table 1). Further evidence of the relative stereochemistry of the molecule was offered by NOE measurements. Observation of a NOE between H-1/H-5, H-1/H-9β, H-11/H-8β, H_3-15/H-8α, H_3-15/H-6, and H-6/H-7 indicates that these proton pairs are on the same face of the molecule, and most importantly, the fact that no NOE was observed between H-5 and proton H_3-15 or H-6 confirmed the *trans*-disposition of H_3-15/H-5 and H-5/H-6. Judging from the NMR data, compound **10** differs from the compound under study only in the configuration of C-7 and C-11, which is unequivocally H-7α and H-11β in the present case (value of the coupling constant $^3J_{7,11}$ = 13.5 Hz). Structure **2** is, thus, proposed for the new compound.

Compound **3** was isolated as an optically active oil, and its molecular formula, $C_{15}H_{22}O_3$ (M^+ at *m/z* 250.1574), indicated 5 degrees of unsaturation. Compound **3** was revealed by the following spectroscopic evidence to be a bicyclic germacrane-type of sesquiterpene lactone. The presence of a γ-lactone was implied by the IR band at ν_{max} 1765 cm^{-1} and confirmed by the signals in the ^{13}C NMR at δ 179.4 (s) and 77.5 (d) and the ^{1}H NMR resonance at δ 5.10 (1H, dd, J = 7.5, 10.0 Hz) (Table 1). The ^{1}H NMR and HMQC spectra also indicated a terminal methylene group [δ 5.12 (1H, br s), 4.88 (1H, br s)], a methyl-bearing

Table 2. ^{1}H (300 MHz) and ^{13}C (75 MHz) NMR Spectral Data for Compounds **4**−**7** in $CDCl_3$ [δ_H, mult, J (Hz); δ_C (mult)][a]

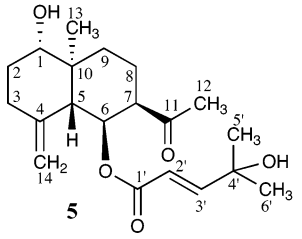
	compound **4**		compound **5**		compound **6**		compound **7**	
atom	δ_H	δ_C	δ_H	δ_C	δ_H	δ_C	δ_H	δ_C
1	5.83, dd, 11.0, 17.2	148.4 (d)	3.57, dd, 4.8, 11.5	78.2 (d)	3.87, br d, 9.1	74.3 (d)		204.7 (s)
2α	4.91, dd, 1.1, 17.2	110.6 (t)	1.87, m	31.8 (t)	1.94, m	34.1 (t)	3.26, ddd, 5.3, 7.6, 11.4	34.3 (t)
2*β*	4.90, dd, 1.1, 11.0		1.87, m		2.10, m		2.27, m	
3α	4.62, br s	113.4 (t)	2.25, m	34.6 (t)	2.11, m	36.8 (t)	2.52, ddd, 4.7, 6.9, 11.4	38.1 (t)
3*β*	4.90, br s		2.15, m		2.11, m		2.25, m	
4		142.7 (s)		143.9 (s)		135.3 (s)		133.6 (s)
5	2.92, d, 11.3	50.9 (d)	2.89, d, 11.6	46.0 (d)	5.22, br d, 6.4	125.9 (d)	5.08, br d, 6.8	128.0 (d)
6	5.28, dd, 5.6, 11.3	72.2 (d)	5.24, dd, 5.8, 11.6	70.0 (d)	5.98, dd, 2.3, 6.4	71.5 (d)	5.91, dd, 2.1, 6.8	70.9 (d)
7	3.35, m	48.4 (d)	3.43, m	48.0 (d)	2.81, ddd, 2.3, 3.5, 7.2	51.9 (d)	2.27, m	54.3 (d)
8α	1.86, m	21.9 (t)	1.81, m	21.7 (t)	2.15, m	20.1 (t)	2.27, m	19.3 (t)
8*β*	1.86, m		1.81, m		1.98, m		1.82, m	
9α	1.23, m	33.5 (t)	1.68, m	30.6 (t)	2.27, br t, 5.4	33.9 (t)	2.92, m	32.1 (t)
9*β*	1.87, m		1.57, m		2.42, dddd, 4.2, 8.5, 11.7, 15.5		1.81, m	
10		40.9 (s)		41.8 (s)		151.8 (s)		149.6 (s)
11		209.3 (s)		209.5 (s)		207.6 (s)		206.4 (s)
12	2.11, s	31.6 (q)	2.09, s	31.9 (q)	2.21, s	28.5 (q)	2.20, s	28.2 (q)
13α	1.05, s	18.5 (q)[b]	0.77, s	10.9 (q)	5.26, br s	111.5 (t)	5.85, br s	126.2 (t)
13*β*					4.86, br s		5.64, br s	
14α	1.68, s	25.2 (q)	4.24, br s	107.6 (t)	1.64, s	17.4 (q)	1.76, s	16.7 (q)
14*β*			4.80, br s					
1′		166.4 (s)		166.7 (s)		166.0 (s)		166.0 (s)
2′	5.93, d, 15.7	117.6 (d)	5.94, d, 15.7	117.5 (d)	5.98, d, 15.7	117.5 (d)	5.92, d, 15.7	117.4 (d)
3′	6.97, d, 15.7	155.3 (d)	6.97, d, 15.7	155.6 (d)	6.97, d, 15.7	155.4 (d)	6.92, d, 15.7	155.5 (d)
4′		70.9 (s)		70.8 (s)		70.9 (s)		70.9 (s)
5′	1.35, s	29.2 (q)	1.34, s	29.2 (q)	1.35, s	29.2 (q)	1.34, s	29.2 (q)
6′	1.35, s	29.2 (q)	1.34, s	29.2 (q)	1.36, s	29.1 (q)	1.34, s	29.2 (q)

[a] Chemical shift values are in ppm relative to TMS. Spectra were recorded at 25°C. ^{13}C NMR multiplicities were obtained from DEPT NMR experiments. [b] The precise chemical shift value of this broad low-intensity resonance could not be measured.

trisubstituted olefin [δ 5.27 (1H, d, J = 8.9 Hz), 1.62 (3H, br d, J = 1.2 Hz)], and a secondary hydroxyl group [δ 3.96 (1H, dd, J = 4.0, 8.1 Hz)]. The presence of a bicyclic ring system in the molecule could be deduced on the basis of the unsaturatation values and the unsaturated groups evident, namely, two olefins and one γ-lactone carbonyl function. The ^{1}H−^{1}H COSY and HMQC spectra readily established the presence of two structural fragments: −CH(−O−)−CH_2−CH_2− (**a**) and −C═CH−CH(−O−)−CH(CHCH_3)−CH_2−CH_2− (**b**). The skeleton of **3** was elucidated from the HMBC experiment. The ^2J and ^3J correlations between H_3-14 at δ 1.62 and the carbons at δ 140.7 (C-4), 121.1 (C-5), and 37.4 (C-3) established the connectivity of fragments **a** and **b** and a methyl group at C-4. Other significant correlations in the HMBC spectrum of **3** observed from δ 5.12/4.88 (H_2-15) to 152.8 (C-10), 32.2 (C-9), and 73.5 (C-1) suggested linkages between fragments **a** and **b** and a terminal methylene group at C-10. The linkage between fragments **a** and **b** was confirmed by the HMBC correlations between δ 140.7 (s) and 5.10 (H-6). Therefore, the planar structure of **3** could be deduced as a germacrane-type sesquiterpene lactone with a hydroxyl function at C-1. On the other hand, the relative stereochemistry of H-6 and H-7 was determined to be *syn* on the basis of the cross-peak between H-7 (δ 2.24) and H-6 (δ 5.10) in the NOESY experiment. Furthermore, the configuration of H-1 was determined as β due to the simultaneous appearance of cross-peaks between H-1 and H-5 and between H-5 and H-11. Finally, the combination of the overall NOE correlations observed in the NOESY spectrum of **3** and the shielded methyl carbon resonance at δ 17.2 established that $\Delta^{4,5}$ was in the *E* form.

Compound **4** was obtained as an oil, $[\alpha]_D^{25}$ +12.3° (*c* 1.0, $CHCl_3$), which analyzed for $C_{20}H_{30}O_4$ by ^{13}C NMR (Table 2), ^{13}C DEPT NMR, and HRFABMS experiments. The ^{13}C NMR and ^{13}C DEPT NMR spectra indicated that **4** contained five CH_3, four CH_2, six CH carbons, and five quaternary carbons. Its IR spectrum showed bands at ν_{max} 1716 (methyl ketone and α,*β*-unsaturated ester), 1654, 1374, 981, and 910 cm^{-1} (vinyl and olefin unsaturation). A strong UV absorption at λ_{max} 206 nm also suggested the presence of an α,*β*-unsaturated carbonyl functionality. The features of the ^{1}H and ^{13}C NMR spectra (Table 2) suggested a nonlactonic 12-*nor*-11-keto-elemane framework with an oxygenated function at C-6. The presence of an acyl functionality at C-7 was inferred from the methyl singlet at δ 2.11 (3H, H_3-12) and multiplet at δ 3.35 (1H, H-7). A double doublet at δ 5.28 (1H, J = 5.6, 11.3 Hz) indicated the presence of an ester moiety at C-6, whereas the presence of an unusual 4-hydroxyl-4-methyl-2(*E*)-pentenoyl ester side chain attached to C-6 was deduced from the signals at δ 6.97 (1H, d, J = 15.7 Hz) and 5.93 (1H, d, J = 15.7 Hz) for H-3′ and H-2′, respectively, and δ 1.35 (6H, s) for H_3-5′ and H_3-6′. An intense peak at *m/z* 204 in the EI mass spectrum of **4** was consistent with the rapid loss of 4-hydroxyl-4-methyl-2(*E*)-pentenoic acid from the molecular ion species at *m/z* 334. The location of the ester side chain, the tertiary hydroxyl, and the acyl group was confirmed by conducting a HMBC experiment, which indicated connections between δ 5.28 (H-6) and 166.4 (C-1′), 50.9 (C-5), and 48.4 (C-7) ppm; δ 6.97 (H-3′) and 166.4 (C-1′), 70.9 (C-4′), and 29.2 (C-5′ and C-6′) ppm; and δ 2.11 (H_3-12) and 209.3 (C-11) and 48.4 (C-7) ppm. The chemical shifts, the multiplicities of the signals, and the absolute values of the coupling constants in the ^{1}H NMR spectrum (Table 2) were in good agreement with a *cis*-disposition of H-6/H-7 as well as with the *trans*-disposition of H-5/H-6 and H-2′/H-3′. A NOESY experiment supported the α-orientations of the methyl group at C-10 and the methine protons at C-6 and C-7, while the ester moiety at C-6 and the bridgehead proton at C-5 had a *β*-orientation.

Compound **5** was obtained as a UV-active (λ_{max} 204 nm) colorless oil, whose molecular formula, $C_{20}H_{30}O_5$, was revealed by HRFABMS. The IR spectrum exhibited ab-



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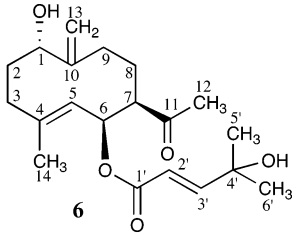

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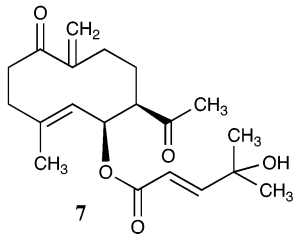

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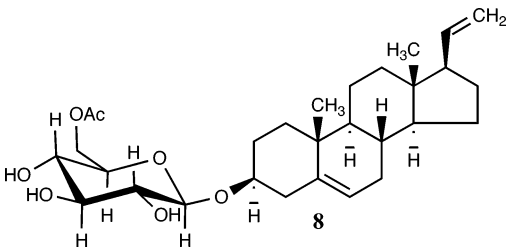
sorption bands at 3421 cm^{-1} for hydroxy functions, 1648 and 894 cm^{-1} for an exomethylene double bond, and 1712 cm^{-1} for methyl ketone and α,β-unsaturated ester carbonyl groups. The ^{13}C and DEPT NMR spectra of **5** showed the presence of 20 carbons (Table 2), which were assigned to four methyls, five methylene (one olefinic at δ 107.6), six methine (two olefinic at δ 155.6 and 117.5 and two oxygenated at δ 78.2 and 70.0), and five quaternary carbons (one ketone at δ 209.5, one α,β-unsaturated ester at δ 166.7, and one oxygenated at δ 70.8). The ^1H NMR spectrum exhibited four olefinic proton groups at δ 6.97 (1H, d, J = 15.7 Hz), 5.94 (1H, d, J = 15.7 Hz), 4.80 (1H, br s), and 4.24 (1H, br s), two oxymethine protons at δ 5.24 (1H, dd, J = 5.8, 11.6 Hz) and 3.57 (1H, dd, J = 4.8, 11.5 Hz), and four tertiary methyl signals at δ 2.09, 1.34 (two overlapped singlets), and 0.77. The connectivity of ^1H and ^{13}C NMR signals was determined by a HMQC spectrum, and the further correlation of ^1H and ^{13}C chemical shifts was assigned by inspection of $^1H-^1H$ COSY and HMBC spectra.[6] The above data suggested that **5** was a nonlactonic 12-*nor*-11-keto eudesmane-type sesquiterpene linking through C-6 with a 4-hydroxyl-4-methyl-2(*E*)-pentenoyl group. The relative stereochemistry of compound **5** was assigned readily by analyses of the NOESY spectrum, most notably, by the correlations of H-6 with H-7 and H_3-13, and H-1 with H-5, suggesting that H-6, H-7, and H_3-13 had α-orientations and H-1 and H-5 had axial orientations.

The HRFAB mass spectral data for metabolite **6**, isolated as a pale yellow oil, established its molecular formula as $C_{20}H_{30}O_5$. The UV absorption at λ_{max} 206 nm (ε 11 800) and IR absorption band at ν_{max} 1713 cm^{-1} suggested the presence of ketone and α,β-unsaturated ester groups. This was further supported by a pair of characteristic ^{13}C NMR resonances at δ 207.6 (s) and 166.0 (s). The ^{13}C NMR spectrum of **6** showed a total of 20 carbon atoms, two in the carbonyl region, six in the olefin region, and four in the methyl region. The ^1H and ^{13}C NMR spectra (Table 2) indicated the presence of three sets of double bonds: one was terminal [δ_H 5.26 (1H, br s) and 4.86 (1H, br s); δ_C 151.8 (s), 111.5 (t)]; one was *trans* disubstituted [δ_H 6.97 (1H, d, J = 15.7 Hz) and 5.98 (1H, d, J = 15.7 Hz); δ_C 155.4 (d), 117.5 (d)]; and one was a trisubstituted olefin [δ_H 5.22 (1H, br d, J = 6.4 Hz); δ_C 135.3 (s), 125.9 (d)]. The presence of these three double bonds and the two carbonyls suggested a monocyclic structure for **6**. Further comparison of the ^1H and ^{13}C NMR spectra of **6** with those of compounds **3**–**5** provided strong evidence that **6** was a nonlactonic 12-*nor*-11-keto germacrane-type sesquiterpene bearing a hydroxyl and 4-hydroxyl-4-methyl-2(*E*)-pentenoyl groups at C-1 and C-6, respectively. The hydroxy groups were displayed by a broad IR absorption band at ν_{max} 3376 cm^{-1}, and two oxymethine signals at δ 3.87 (1H, br d, J = 9.1 Hz) and 5.98 (1H, dd, J = 2.3, 6.4 Hz) were attributable to H-1 and H-6, respectively. The $^1H-^1H$ COSY spectrum established the presence of partial structures $-CH(-O-)-CH_2-CH_2-$ and $-C{=}CH-CH(-O-)-CH(COCH_3)-CH_2-CH_2-$, the linkage of which was achieved primarily by HMBC experiments.[7] On the basis of the above information, the planar structure of metabolite **6** was assigned. The presence of NOEs of H-6 with H-7 and H_3-14 indicated that H-6, H-7, and H_3-14 have *syn* configurations. Furthermore, NOE correlations of H-1 and H-5, and most importantly, the absence of NOEs between H-5 and H_3-14, showed H-1 and H-5 to be β-oriented and that $\Delta^{4,5}$ was in the *E* form. That the trisubstituted olefin had indeed the *E* configuration was confirmed by the shielded vinyl methyl carbon resonance at δ 17.4.

Compound **7** was isolated as an optically active colorless oil, $[\alpha]^{25}_D$ +12.3° (*c* 1.0, $CHCl_3$). Its molecular formula, $C_{20}H_{28}O_5$, established by HRFABMS and ^{13}C NMR spectra, contained two hydrogen atoms less than that of compound **6**. Interestingly, although the ^{13}C NMR spectrum of **7** showed the presence of three carbonyl carbons by signals at δ 206.4 (s), 204.7 (s), and 166.0 (s) (Table 2), a single, very intense carbonyl absorption band at ν_{max} 1717 cm^{-1} was visible in the IR spectrum. Likewise, the UV spectrum consisted of a single maximum at λ_{max} 206 nm (ε 13 400), consistent with the presence in **7** of α,β-unsaturated carbonyl systems. The ^1H NMR spectrum of **7** was very similar to that of **6**, except for the noticeable downfield shift experienced by the signals attributed to the exomethylene protons (δ 5.85 and 5.64 in **7** vs δ 5.26 and 4.86 in **6**) and the absence in the ^1H NMR spectrum of **7** of a signal ascribable to a hydroxyl-bearing methine proton. Furthermore, the ^{13}C NMR spectra of compounds **6** and **7** were generally quite similar, except for the observation that the oxymethine carbon at δ 74.3 in **6** had been replaced by a new carbonyl resonance at δ 204.7 in **7**. On the basis of the above spectral differences and similarities, we suggest that compound **7** is the oxidized version of **6** at C-1. On the other hand, the NOESY spectrum of **7** showed a strong correlation between the two protons at C-6 and C-7, thus revealing that the relative stereochemistry of **7** was the same as that of **6**. The *cis*-disposition of H-6 and H-7 and the *trans*-disposition of H-2′ and H-3′ in **7** were supported by their respective coupling constants ($^3J_{6,7}$ = 2.1 Hz and $^3J_{2',3'}$ = 15.7 Hz).



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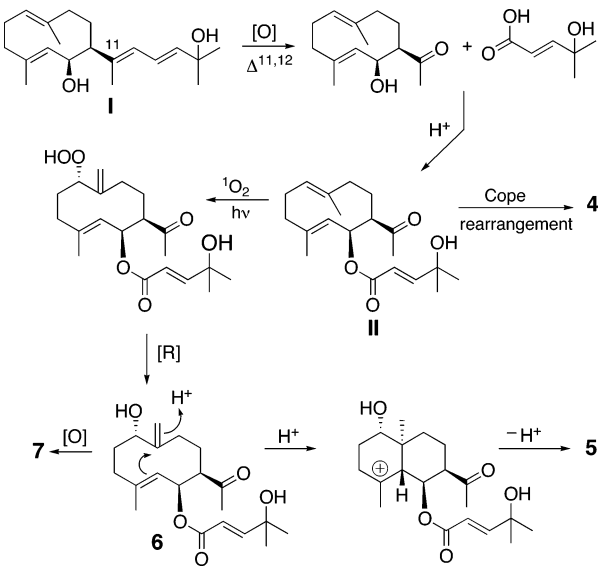
The known compound 6′-*O*-acetyl-3β-pregna-5,20-dienyl-β-galactopyranoside (**8**) was also isolated and characterized during this investigation. A comparison of its spectroscopic data (IR, ^1H and ^{13}C NMR, HRFABMS) with literature values quickly established its identity.[8] Structurally similar steroidal glycosides have been previously isolated from various species of Caribbean gorgonian octocorals.[9]

Scheme 1



A diterpenoid as yet to be discovered of the dilophol class, such as (I), should not be excluded as a possible precursor to *nor*-sesquiterpenes **4**−**7**. Structurally similar compounds, i.e., asperketals A−E, that are ketals and hemiketals (at the C-12 position) related to the dilophol class of 10-membered ring diterpenoids have been isolated from the Caribbean gorgonian *Eunicea asperula*.[3a] Loss of a C_6 fragment from (I) by enzymatic oxidative modifications could provide a key 12-*nor*-germacradiene intermediate such as (II), and from the latter intermediate the other skeletal types of nor-sesquiterpernes shown in Scheme 1 could be derived.

The isolated compounds were tested for antiplasmodial activity against the malarial parasite *Plasmodium falciparum* W2 (chloroquine-resistant) strain. Compounds **2**, **3**, **5**, **6**, and **7** were very active, with IC_{50} values of 14, 18, 10, 14, and 16 μg/mL, respectively. Quite disappointingly, compound **1** was inactive in this assay, with an IC_{50} value > 50 μg/mL. Furthermore, in vitro screening of compounds **2**, **4**, **5**, and **7** against *Mycobacterium tuberculosis* $H_{37}Rv$ showed no significant inhibitory activity at a concentration of 6.25 μg/mL. Compounds **6** and **7** also proved to be inactive as potential inhibitors of the cell cycle regulators cdc2/cyclin B kinase and cdc25 phosphatase.[10] On the other hand, compound **3** weakly inhibited the growth of human cancer cells CCRF-CEM, HL-60 (TB), MOLT-4, RPMI-8226, and MCF7 (IC_{50}'s 31.3, 51.5, 19.6, 90.4, and 82.2 μg/mL, respectively).

Experimental Section

General Experimental Procedures. Optical rotations were determined with a Perkin-Elmer polarimeter Model 243B. IR and UV spectra were recorded with a Nicolet Magna 750 FT-IR and a Hewlett-Packard diode array spectrophotometer Model 8452A, respectively. NMR spectra were recorded with a Bruker DPX-300 spectrometer operating at 300 MHz for ^{1}H NMR and 75 MHz for ^{13}C NMR. NMR chemical shifts are referenced to the residual $CHCl_3$ in the deuterated solvent (7.26 ppm for ^{1}H and 77.0 ppm for ^{13}C NMR). HREIMS and HRFABMS analyses were obtained from the Mass Spectrometry Laboratory of the University of Illinois at Urbana−Champaign. Column chromatography was performed on Si gel (35−75 mesh), and TLC analyses were carried out using Analtech glass precoated Si gel plates. All solvents used were spectral grade or were distilled from glass prior to use.

Animal Material. *Eunicea* sp. (Plexauridae: Octocoralia: Cnidaria) collected near Aguadilla, Puerto Rico, has colonies with terminal branches of 10−12 mm without the calyx. The calyx are conical and slightly paler than the surrounding tissue in dry samples. The surface layer presents mostly small foliate and irregular sclerites (clubs) between 0.07 and 0.11 mm in length. The specimen resembles *E. tourneforti* though their branches are not elliptical in section and the club sclerites, a diagnostic character, are rather small. Consequently, this species should be considered as part of the "tourneforti" complex (*E. tourneforti*, *E. asperula*, and *E. laciniata*), whose species are sympatric but most of their morphological traits and diagnostic characters greatly overlap. A voucher specimen (No. ET-PR-03) has been deposited at the Chemistry Department of the University of Puerto Rico, Río Piedras campus.

Collection, Extraction, and Isolation. *Eunicea* sp. was collected near Aguadilla, Puerto Rico, at a depth of 20−40 ft on July 7, 2001, and kept frozen prior to its extraction. The freeze-dried gorgonian (493 g) was extracted with $CHCl_3$−MeOH (1:1) (5 × 0.2 L) to give 8.5 g of a dark green oil. This material was fractionated by column chromatography over Si gel (290 g) using gradient elution with hexane−EtOAc−MeOH to yield 40 fractions. Fraction 21 (431 mg) was purified further by column chromatography over Si gel (15 g) with 30% EtOAc in hexane to afford compound **1** (6 mg). Fractions 23 (509 mg) and 24 (348 mg) were combined and purified over Si gel (30 g) using a $CHCl_3$−EtOAc mixture (95.5:0.5) to yield 25 subfractions. Subfraction 9 (117 mg) was purified further by successive reversed-phase column chromatography over C_{18} Si gel (4 g) using 20% H_2O in MeOH and normal-phase column chromatography with 2% EtOAc in $CHCl_3$ to yield compound **4** (5 mg). Fractions 25 (345 mg) and 26 (147 mg) were combined and purified over Si gel (17 g) using 10% EtOAc in $CHCl_3$ to give 24 subfractions. Subfractions 9 and 21 consisted of pure compounds **3** (8 mg) and **5** (15 mg), respectively. Compound **2** (6 mg) was isolated from subfraction 7 (9 mg) after purification by reversed-phase column chromatography over C_{18} Si gel (2 g) using 10% H_2O in MeOH. Fraction 27 (87 mg) was chromatographed over Si gel (5 g) using a mixture of 20% EtOAc in $CHCl_3$ to afford compounds **6** (9 mg) and **7** (2 mg), respectively. Compound **8** (6 mg) was obtained from fraction 31 (283 mg) after purification by column chromatography over Si gel (10 g) using 15% EtOAc in $CHCl_3$.

Compound 1: white amorphous solid; $[\alpha]^{25}_D$ +18.8° (*c* 1.2, $CHCl_3$); UV (MeOH) λ_{max} 220 nm (ϵ 9400); IR (film, NaCl) ν_{max} 3080, 2927, 2855, 1751, 1688, 1638, 1456, 1375, 1221, 1095, 1008, 981, 893 cm^{-1}; ^{1}H NMR ($CDCl_3$, 300 MHz) and ^{13}C NMR ($CDCl_3$, 75 MHz) (see Table 1); HRFABMS (3-NBA) *m/z* [M + Na]$^+$ 255.1363 (calcd for $C_{15}H_{20}O_2Na$, 255.1361).

Compound 2: colorless oil; $[\alpha]^{25}_D$ +25.8° (*c* 1.1, $CHCl_3$); IR (film, NaCl) ν_{max} 3452, 2971, 2938, 2875, 1765, 1677, 1651, 1455, 1383, 1173, 1073, 994, 752 cm^{-1}; ^{1}H NMR ($CDCl_3$, 300 MHz) and ^{13}C NMR ($CDCl_3$, 75 MHz) (see Table 1); EIMS *m/z* 250 [M]$^+$ (4), 232 (100), 217 (16), 177 (21), 159 (71), 147 (23), 133 (44), 119 (40), 107 (46), 91 (48); HREIMS *m/z* 250.1577 (calcd for $C_{15}H_{22}O_3$, 250.1569).

Compound 3: colorless oil; $[\alpha]^{25}_D$ +24.6° (*c* 1.1, $CHCl_3$); IR (film, NaCl) ν_{max} 3438, 2933, 2875, 1765, 1671, 1453, 1380, 1176, 978 cm^{-1}; ^{1}H NMR ($CDCl_3$, 300 MHz) and ^{13}C NMR ($CDCl_3$, 75 MHz) (see Table 1); EIMS *m/z* 250 [M]$^+$ (11), 232 (46), 207 (93), 177 (54), 159 (95), 147 (46), 133 (59), 119 (70), 107 (68), 93 (78), 55 (100); HREIMS *m/z* 250.1574 (calcd for $C_{15}H_{22}O_3$, 250.1569).

Compound 4: colorless oil; $[\alpha]^{25}_D$ +12.3° (*c* 1.0, $CHCl_3$); UV (MeOH) λ_{max} 206 nm (ϵ 13 400); IR (film, NaCl) ν_{max} 3412, 2919, 2850, 1716, 1654, 1557, 1456, 1374, 1283, 1184, 1152, 1011, 981, 910 cm^{-1}; ^{1}H NMR ($CDCl_3$, 300 MHz) and ^{13}C NMR ($CDCl_3$, 75 MHz) (see Table 2); EIMS *m/z* 334 [M]$^+$ (1), 204 (42), 189 (10), 171 (4), 161 (100), 146 (32), 135 (24), 113 (55), 95 (44), 81 (58); HRFABMS (3-NBA) *m/z* [M + Na]$^+$ 357.2032 (calcd for $C_{20}H_{30}O_4Na$, 357.2042).

Compound 5: colorless oil; $[\alpha]^{25}_D$ +32.0° (*c* 1.1, $CHCl_3$); UV (MeOH) λ_{max} 204 nm (ϵ 14 000); IR (film, NaCl) ν_{max} 3421, 2916, 2850, 1712, 1648, 1461, 1377, 1283, 1183, 1156, 1062, 994, 894, 803 cm^{-1}; ^{1}H NMR ($CDCl_3$, 300 MHz) and ^{13}C NMR

($CDCl_3$, 75 MHz) (see Table 2); HRFABMS (M.B.) *m/z* [M + H]$^+$ 351.2172 (calcd for $C_{20}H_{31}O_5$, 351.2171).

Compound 6: pale yellow oil; $[\alpha]^{25}_D$ +77.6° (*c* 1.2, $CHCl_3$); UV (MeOH) λ_{max} 206 nm (ϵ 11 800); IR (film, NaCl) ν_{max} 3376, 3074, 2925, 2852, 1713, 1653, 1451, 1353, 1282, 1184, 1149, 1005, 941, 912 cm^{-1}; ^{1}H NMR ($CDCl_3$, 300 MHz) and ^{13}C NMR ($CDCl_3$, 75 MHz) (see Table 2); HRFABMS (3-NBA) *m/z* [M + Na]$^+$ 373.1996 (calcd for $C_{20}H_{30}O_5Na$, 373.1991).

Compound 7: colorless oil; $[\alpha]^{25}_D$ +12.3° (*c* 1.0, $CHCl_3$); UV (MeOH) λ_{max} 206 nm (ϵ 13 400); IR (film, NaCl) ν_{max} 3454, 2976, 2922, 2854, 1717, 1671, 1446, 1369, 1281, 1181, 1153, 1018, 982 cm^{-1}; ^{1}H NMR ($CDCl_3$, 300 MHz) and ^{13}C NMR ($CDCl_3$, 75 MHz) (see Table 2); HRFABMS (3-NBA) *m/z* [M + Na]$^+$ 371.1825 (calcd for $C_{20}H_{28}O_5Na$, 371.1834).

Antimalaria Inhibition Bioassay. Compounds **2**, **3**, **5**, **6**, and **7** were tested for antimalarial activity against a chloroquine-resistant *Plasmodium falciparum* strain using a novel fluorometric method, based on the intercalation of the fluorochrome PicoGreen in the parasite DNA, as described by Corbett et al.[11]

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(6) Selected correlations observed in the HMBC spectrum of compound **5**: C-1 [H_3-13], C-3 [H_2-14$\alpha\beta$], C-5 [H_3-13, H_2-14$\alpha\beta$], C-9 [H_3-13], C-10 [H_3-13], C-11 [H_3-12], C-1′ [H-2′, H-3′], C-3′ [H_3-5′, H_3-6′], C-4′ [H-2′, H-3′, H_3-5′, H_3-6′], C-5′ [H_3-6′], C-6′ [H_3-5′].
(7) Selected correlations observed in the HMBC spectrum of compound **6**: C-1 [H-3$\alpha\beta$, H_2-13$\alpha\beta$], C-3 [H-5, H_3-14], C-4 [H_3-14], C-5 [H_3-14], C-8 [H-6], C-9 [H_2-13$\alpha\beta$], C-11 [H_3-12], C-1′ [H-2′, H-3′], C-3′ [H_3-5′, H_3-6′], C-4′ [H-2′, H-3′, H_3-5′, H_3-6′], C-5′ [H_3-6′], C-6′ [H_3-5′].
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