Dolabellane-Type Diterpenoids with Antiprotozoan Activity from a Southwestern Caribbean Gorgonian Octocoral of the Genus *Eunicea*

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Ten new diterpenes, **1**−**10**, having a dolabellane skeleton were isolated from a Colombian gorgonian coral of the genus *Eunicea*. Their structures, as well as those of known compounds **11**−**18**, were determined on the basis of spectroscopic analysis and, in some instances, by chemical conversion and X-ray crystallographic analysis. The absolute structure of **7** was established by chemical conversion from **11**, a co-occurring dolabellane congener of known absolute structure. Most of these diterpenoids showed antimalarial activity against the protozoan parasite *Plasmodium falciparum*.

Gorgonian octocorals belonging to the genus *Eunicea*, which can be found in great abundance in waters of the Caribbean Sea, have been recognized as rich sources for marine natural products, most of which possess unique structural features and remarkable biological activities.[1,2] A variety of chemically complex diterpenoids, such as cembrane lactones, dolabellanes, dolastanes, cubitanes, dilophols, and fuscols, have been isolated since the early 1960s from several species of *Eunicea*, including *E. succinea*, *E. mammosa*, *E. laciniata*, *E. palmeri*, *E. calyculata*, *E. asperula*, *E. fusca*, and *E. tourneforti*.[1−10] As part of a study on the chemical constituents of Caribbean gorgonians of this genus, we reported in 2004 the isolation and structure characterization of five new representatives of the cembrane class of diterpenes from an unknown species of *Eunicea* collected near the Colombian Southwestern Caribbean Sea.[11] During our continuing studies on the chemical constituents of this gorgonian species, 10 new dolabellane-type diterpenoids (**1**−**10**) were isolated, as well as eight known dolabellane and dolastane congeners (**11**−**18**). This paper describes the isolation and structure determination of these compounds.[12] The structures of **1**−**18** were established by detailed spectroscopic analysis, including extensive examination of 2D NMR (^{1}H−^{1}H COSY, HMQC, HMBC, and NOESY) correlations, chemical conversion, and, in the case of metabolites **1**, **2**, **8**, and **12**, X-ray crystallographic analysis. Identification of known compounds was based on comparison of their physical properties (mp, $[\alpha]_D$, MS, and NMR) with those reported in the literature, by direct comparison with authentic samples, and, in the case of **12**, by X-ray crystallography. It is to be noted that, except for **7**, only the relative configuration of the new compounds has been determined. However, in light of the established absolute structures of the co-occurring **11** and **16**, the configurations shown are considered more probable than the enantiomeric ones.[13,14] The antiprotozoan activity of **1**−**17** against the *Plasmodium falciparum* W2 strain (chloroquine resistant) was also studied. The results displayed that these compounds are generally toxic toward *P. falciparum*, with **9** being the most potent.

The HREIMS (*m*/*z* 302.2252) of **1** established the molecular formula $C_{20}H_{30}O_2$, appropriate for six degrees of unsaturation, and the IR spectrum revealed the presence of carbonyl (1700 cm^{-1}) and olefin (1621 cm^{-1}) groups. The ^{13}C NMR (Table 3) and DEPT spectroscopic data showed signals of five methyls, six sp^3 methylenes, two sp^3 methines, one sp^2 methine, and one sp^3 and five sp^2 quaternary carbons (including two ketone carbonyls). The NMR signals (Tables 1 and 3) observed at δ_C 206.6 (C, C-13), 147.9 (C, C-18), 137.6 (C, C-12), 24.3 (CH_3, C-19), and 21.5 (CH_3, C-20) and δ_H 2.20 (s, 3H, H_3-20) and 1.88 (s, 3H, H_3-19) revealed the presence of the α-isopropylidenyl ketone functionality.[3] Furthermore, the chemical shift for the C-13 carbonyl carbon in the ^{13}C NMR spectrum of **1** (206.6 ppm), the IR absorption at 1700 cm^{-1}, and the UV absorption data were virtually identical with those reported for known compounds **11**−**14**, thus allowing the further assignment of this chromophore as α-isopropylidenylcyclopentanone. Signals appearing at δ_C 135.7 (C, C-4), δ_C 125.1 (CH, C-3), δ_H 5.27 (dd, 1H, J = 3.2, 11.8 Hz, H-3), and δ_H 1.63 (s, 3H, H_3-16) revealed the presence of one trisubstituted carbon−carbon double bond. One α-methylene-α′-methyl-substituted ketone was also identified from the NMR signals at δ_C 215.2 (C, C-7), 47.2 (CH, C-8), 40.3 (CH_2, C-6), and 18.0 (CH_3, C-17); δ_H 2.89 (dt, 1H, J = 2.7, 14.0 Hz, H-6β), 2.66 (m, 1H, H-8), 2.38 (dq, 1H, J = 2.1, 14.0 Hz, H-6α), and 1.05 (d, 3H, J = 7.1 Hz, H_3-17). In the ^{1}H−^{1}H COSY spectrum, it was possible to identify three different structural units, which were assembled with the assistance of an HMBC experiment (Figure 1). Key HMBC correlations of H_3-15 to C-1, C-2, C-11, and C-14; H_3-16 to C-5; H_2-6 to C-7; H_3-17 to C-7, C-8, and C-9; and H-11 to C-1, C-10, C-12, C-13, C-14, and C-18 permitted connection of the carbon skeleton. Furthermore, the geminal methyl groups positioned at C-18 were confirmed from the simultaneous HMBC correlations of H_3-19 and H_3-20 (δ_H 1.88 and 2.20, respectively) to the sp^2 quaternary carbons C-18 (δ_C 147.9) and C-12 (δ_C 137.6). On the basis of the above analysis, the planar structure of **1** was established unambiguously.

The relative configurations of three asymmetric centers (C-1, C-8, and C-11), as well as the configuration of the trisubstituted double bond in **1**, were determined by X-ray crystallographic analysis on a single crystal of **1**. The results of the X-ray analysis is shown in Figure 2, disclosing the 1*R**, 8*R**, and 11*S** relative configurations for the asymmetric centers as well as the *E* configuration for the double bond. Compound **1** was thus elucidated to be 7,13-diketo-1*R**,8*R**,11*S**-dolabell-3*E*,12(18)-diene, a derivative most likely stemming from the acid-promoted epoxide rearrangement of **12**, the main compound isolated during this investigation.[3]

The molecular formula of $C_{22}H_{32}O_4$ for **2** was demonstrated by HREIMS and the ^{13}C NMR spectrum. All 22 carbons appeared in the ^{13}C NMR spectrum, and DEPT experiments indicated the presence of five methyls (including one acetate methyl), six sp^3 methylenes, one sp^2 methylene, three sp^3 methines (including one oxymethine), and one sp^3 and six sp^2 quaternary carbons (including one ester and two ketone carbonyls). The ^{1}H and ^{13}C NMR spectra (Tables 1 and 3) showed the presence of an α-isopropylidenecyclopentanone system [δ_C 205.9 (C, C-13), 150.4 (C, C18), 136.6 (C, C-12), 54.6 (CH_2, C-14), 25.0 (CH_3, C-19), 21.3 (CH_3, C-20); δ_H 2.37 (d, 1H, J = 18.0 Hz, H-14α), 1.92 (d, 1H, J = 18.0 Hz, H-14β), 2.17 (s, 3H, H_3-20), 1.69 (s, 3H, H_3-19)], which was demonstrated to be the same as that in **1** by the UV [λ_{max} (log ε) 203 (3.4), 255 (3.2) nm] and IR (1698, 1622 cm^{-1}) spectra. ^{1}H

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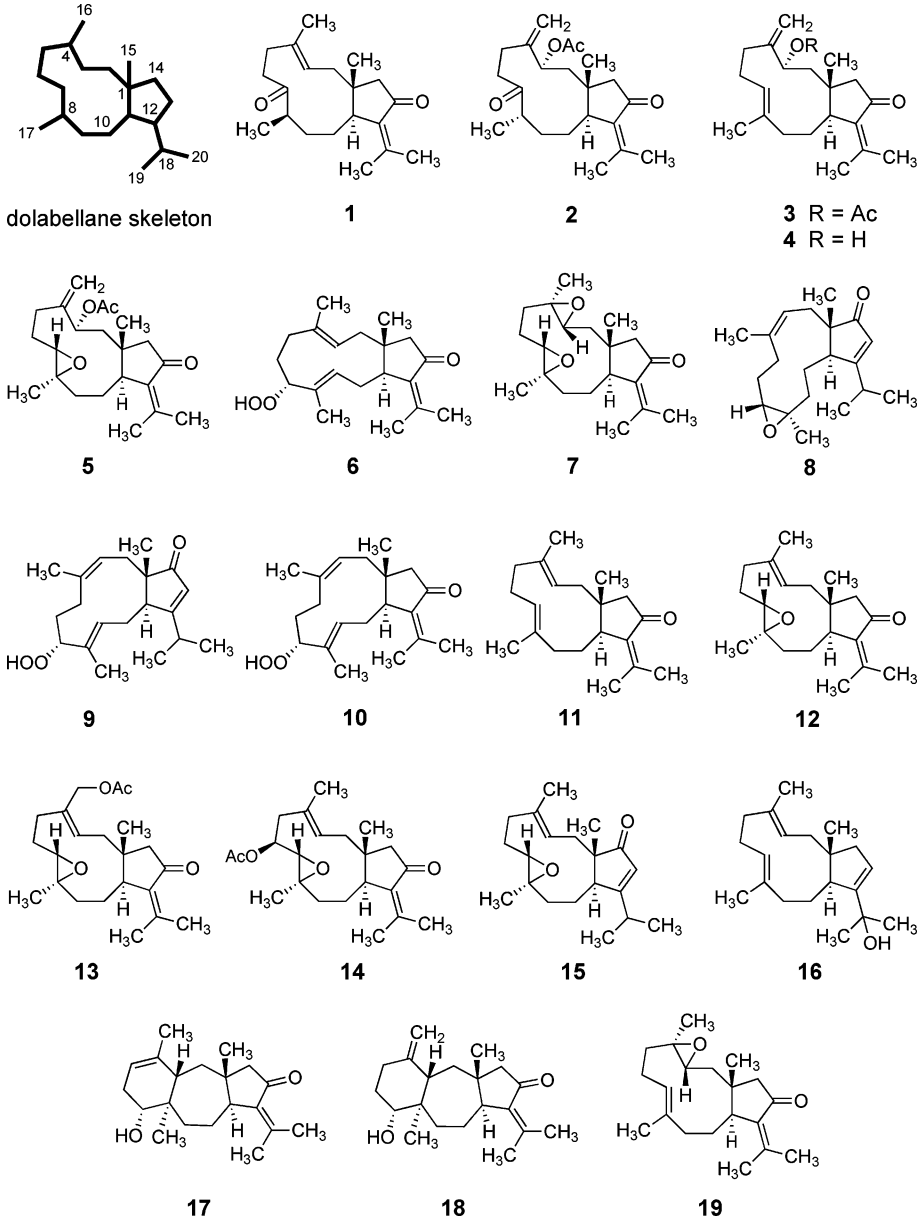
Chart 1



and ^{13}C NMR spectra also showed signals due to a terminal olefin [δ_C 145.8 (C, C-4), 118.7 (CH_2, C-16); δ_H 5.32 (br s, 1H, H-16α), 5.18 (br s, 1H, H-16*β*)] and due to an α-methylene-α′-methyl-substituted ketone [δ_C 211.7 (C, C-7), 45.8 (CH, C-8), 37.2 (CH_2, C-6), 14.5 (CH_3, C-17); δ_H 3.28 and 2.40 (each, m, 1H, H_2-6), 2.67 (m, 1H, H-8), 1.08 (d, 3H, J = 6.8 Hz, H_3-17)]. The presence of a secondary acetate group was demonstrated by the IR absorption (1735 cm^{-1}), the low-field ^1H signal at δ 5.25 (m, 1H) due to the oxymethine proton H-3, and the ^1H and ^{13}C NMR data [δ_H 1.99 (s, 3H, H_3-22); δ_C 170.7 (C, C-21), 70.9 (CH, C-3), 21.4 (CH_3, C-22)]. These spectroscopic findings coupled with $^1H-^1H$ correlations observed in the $^1H-^1H$ COSY spectrum gave several partial structures, as shown in Figure 1. After assignments of the direct $^{13}C-^1H$ correlations were made on the basis of the HMQC analysis, the HMBC spectrum of **2** was measured and analyzed to obtain the gross structure of **2** (Figure 1). The relative configuration of the four asymmetric centers (C-1, C-3, C-8, and C-11) was determined by X-ray crystallographic analysis on a single crystal of **2**. The result of the X-ray analysis is shown in Figure 2, demonstrating 1*R**, 3*R**, 8*S**, and 11*S** relative configurations for the asymmetric centers.

Compound **3** analyzed for $C_{22}H_{32}O_3$, as revealed by the HREIMS (*m*/*z* 344.2346) and ^{13}C NMR data, a molecular formula possessing one oxygen atom less in comparison to that of compound **2**. Of the seven degrees of unsaturation implied by the molecular formula, five were accounted for by multiple bonds, three carbon−carbon double bonds [δ_C 148.7 (C, C-4), 148.1 (C, C-18), 137.9 (C, C-12), 134.6 (C, C-8), 127.7 (CH, C-7), 115.0 (CH_2, C-16)], a keto group [δ_C 206.9 (C, C-13)], and an acetate function [δ_C 170.6 (C, C-21), 21.4 (CH_3, C-22)], indicating **3** to be bicyclic. Comparison of all spectroscopic data of **3** with those of **2**, and the results of a 2D $^1H-^1H$ COSY experiment, revealed that the two molecules were identical, except for the fact that the keto group at C-7 in **2** was replaced by a carbon−carbon double bond in **3**. On the basis of the overall 2D NMR data it was apparent that the new olefin functionality in compound **3** had to reside between carbons C-7 and C-8. From the NOESY spectrum, it was found that H_3-15 (δ 1.30, s, 3H) showed an NOE interaction with H-3 (δ 5.39, dd, 1H, J = 2.2, 10.6 Hz), but not with H-11 (δ 3.28, br d, 1H, J = 9.4 Hz), revealing the *β*-orientation of H-3. Furthermore, we concluded that the acetate function had to be α, based on a 10.6 Hz coupling between H-2α (δ 2.17) and H-3, as well as a 2.2 Hz coupling

Table 1. ^{1}H NMR (300 MHz) Assignments for Compounds **1**−**5** in $CDCl_3$ [δ_H, mult (*J* in Hz)][a]

H	1	2	3	4	5
2α	2.06, m	2.06, m	2.17, m	2.19, m	2.20, m
2*β*	1.72, dd (13.1, 4.2)	1.35, m	1.28, m	1.85, m	1.30, m
3	5.27, dd (11.8, 3.2)	5.25, m	5.39, dd (10.6, 2.2)	4.01, dd (5.1, 3.9)	5.48, dd (11.4, 1.5)
5α	2.07, m	2.35, m	2.49, m	2.13, m	2.63, m
5*β*	2.72, m	3.04, m	2.15, m	2.03, m	2.22, m
6α	2.38, dq (14.0, 2.1)	2.40, m	2.38, m	2.28, m	2.10, m
6*β*	2.89, dt (14.0, 2.7)	3.28, m	2.01, m	1.94, m	1.82, m
7			5.18, m	5.34, br s	3.00, d (8.1)
8	2.66, m	2.67, m			
9α	2.24, m	2.28, m	2.28, m	2.18, m	2.08, m
9*β*	1.38, m	1.76, m	2.12, m	2.18, m	1.40, m
10α	1.54, m	1.58, m	1.68, m	1.72, m	1.75, m
10*β*	1.26, m	1.32, m	1.35, m	1.56, m	1.41, m
11	2.30, m	2.29, m	3.28, br d (9.4)	2.48, m	3.23, br d (12.2)
14α	2.28, d (17.6)	2.37, d (18.0)	2.36, d (18.2)	2.25, br s	2.45, br d (18.2)
14*β*	2.11, d (17.6)	1.92, d (18.0)	2.00, d (18.2)	2.25, br s	2.00, br d (18.2)
15	1.06, s	1.42, s	1.30, s	1.16, s	1.34, s
16α	1.63, s	5.32, br s	5.18, br s	5.27, br s	5.34, br s
16*β*		5.18, br s	5.14, br s	5.02, br s	5.28, br s
17	1.05, d (7.1)	1.08, d (6.8)	1.62, s	1.54, s	1.31, s
19	1.88, s	1.69, s	1.76, s	1.83, s	1.82, s
20	2.20, br s	2.17, s	2.21, s	2.17, s	2.24, s
22		1.99, s	2.00, s		2.01, s

[a] Chemical shift values are in ppm relative to TMS. Spectra were recorded at 25 °C. Proton assignments were aided by ^{1}H−^{1}H COSY, HMQC, HMBC, and NOESY experiments.

Table 2. ^{1}H NMR (300 MHz) Assignments for Compounds **6**−**10** in $CDCl_3$ [δ_H, mult (*J* in Hz)][a]

H	6	7	8	9	10
2α	2.20, m	1.77, m	2.51, m	2.47, br d (14.5)	2.42, dd (11.8, 11.6)
2*β*	1.97, m	1.37, m	2.34, m	2.18, dd (14.6, 12.3)	1.85, m
3	5.09, dd (11.6, 4.5)	3.06, dd (11.2, 2.9)	4.68, br d (11.7)	4.66, br d (12.2)	5.08, br d (11.3)
5α	2.33, m	2.27, m	1.89, m	1.78, m	1.79, m
5*β*	2.22, m	1.42, m	2.53, m	2.30, m	2.27, m
6α	1.92, m	1.76, m	1.48, m	1.83, m	1.83, m
6*β*	1.59, m	1.47, m	2.03, m	1.83, m	1.83, m
7	4.13, dd (10.7, 2.6)	2.96, br d (7.7)	2.93, br d (10.3)	4.02, dd (10.0, 5.2)	4.03, dd (10.0, 5.1)
9α	5.41, t (7.8)	2.12, m	2.15, m	5.68, t (7.4)	5.66, t (7.4)
9*β*		1.43, m	1.42, m		
10α	2.40, m	1.78, m	1.94, m	2.62, m	2.80, m
10*β*	1.78, m	1.57, m	1.53, m	1.95, m	1.82, m
11	2.77, br d (11.8)	2.81, br d (11.4)	2.98, br d (11.0)	2.98, dt (10.2, 2.6)	3.01, br d (11.1)
13			5.97, br s	5.83, br s	
14α	2.37, d (16.3)	2.44, d (18.4)			2.53, d (15.7)
14*β*	2.10, d (16.3)	2.13, d (18.4)			1.81, d (15.7)
15	1.08, s	1.33, s	1.04, s	1.06, s	1.01, s
16	1.62, s	1.21, s	1.64, s	1.64, s	1.75, s
17	1.68, s	1.43, s	1.30, s	1.58, s	1.62, s
18			2.62, m	2.62, m	
19	1.96, s	1.94, s	1.13, d (6.8)	1.18, d (6.3)	1.98, br s
20	2.14, s	2.24, s	1.20, d (6.8)	1.20, d (6.7)	2.20, br s

[a] Chemical shift values are in ppm relative to TMS. Spectra were recorded at 25 °C. Proton assignments were aided by ^{1}H−^{1}H COSY, HMQC, HMBC, and NOESY experiments.

between H-2*β* (δ 1.28) and H-3. The *E* geometry of the trisubstituted double bond was also assigned from the NOE correlation of H_3-17 (δ 1.62, s, 3H) with H-6α (δ 2.38), but not with olefinic proton H-7 (δ 5.18, m, 1H), and also the upper field chemical shift of C-17 (δ 16.8). On the basis of the above findings and detailed examination of other NOE correlations (Figure 3), we concluded that the relative structure of compound **3** is 3*R**-acetoxy-13-keto-1*R**,11*S**-dolabell-4(16),7*E*,12(18)-triene.

Compound **4** was shown by HREIMS to possess the molecular formula $C_{20}H_{30}O_2$ (*m*/*z* 302.2242). The IR spectrum of **4** revealed the presence of hydroxy (3434 cm^{-1}), ketone carbonyl (1701 cm^{-1}), and olefin (1620 cm^{-1}) groups. Comparison of the ^{1}H and ^{13}C NMR data (Tables 1 and 3) of compounds **3** and **4** showed that the structure of **4** should be very close to that of **3**, with the exception of signals assigned to C-3, where an acetate methyl (δ 2.00) and acetoxymethine (δ 5.39, dd, 1H, $J = 2.2, 10.6$ Hz) in **3** are replaced by a hydroxymethine (δ 4.01, dd, 1H, $J = 3.9, 5.1$ Hz) in **4**. The overall planar structure of **4** was fully established by analyzing the ^{1}H−^{1}H COSY and HMBC correlations (Figure 1). The relative configuration of **4** was confirmed to be 1*R**, 3*R**, and 11*S** from the following NOESY correlations: both H-3 (δ 4.01) and H_3-15 (δ 1.16) with H-7 (δ 5.34), and H_3-15 with H-3. The $\Delta^{7,8}$ double bond was assigned with the *E* configuration on the basis of the ^{13}C NMR chemical shift of C-17 (δ_C 15.9), which is in the same range as that of compound **3**. These results, together with other detailed 2D NMR correlations of **4** (Figure 1), unambiguously established the structure of this secondary metabolite as shown in formula **4**. The structural relationship of compounds **3** and **4** was decisively demonstrated when **4**, upon acetylation at room temperature, yielded acetate **3** quantitatively. Thus, **4** is the 3-deacetyl derivative of **3**.

Compound **5** possessed the same molecular formula ($C_{22}H_{32}O_4$) as that of compound **2**, as revealed from HREIMS, suggesting **5** to be an isomer of **2**. Furthermore, it was found that the NMR spectroscopic data of **5** were very similar to those of **2**. The four oxygen atoms in **5** were readily assigned to an acetate, a trisubstituted epoxide, and an α,*β*-unsaturated ketone constellation by

Table 3. ^{13}C NMR (75 MHz) Assignments for Compounds **1**−**10** in $CDCl_3$ [δ_C, mult][a]

C	1	2	3	4	5	6	7	8	9	10
1	40.5, C	38.1, C	38.0, C	40.4, C	38.3, C	39.3, C	37.9, C	50.8, C	53.5, C	39.0, C
2	40.3, CH_2	45.3, CH_2	43.8, CH_2	41.0, CH_2	43.5, CH_2	42.8, CH_2	40.3, CH_2	36.4, CH_2	33.8, CH_2	37.4, CH_2
3	125.1, CH	70.9, CH	74.4, CH	74.8, CH	75.0, CH	125.1, CH	63.0, CH	122.4, CH	122.9, CH	121.6, CH
4	135.7, C	145.8, C	148.7, C	153.9, C	148.3, C	133.8, C	60.3, C	136.7, C	134.8, C	135.0, C
5	35.6, CH_2	30.7, CH_2	35.1, CH_2	34.5, CH_2	33.6, CH_2	38.5, CH_2	37.3, CH_2	28.9, CH_2	27.1, CH_2	27.4, CH_2
6	40.3, CH_2	37.2, CH_2	28.5, CH_2	34.4, CH_2	29.3, CH_2	28.0, CH_2	23.4, CH_2	26.1, CH_2	30.6, CH_2	27.6, CH_2
7	215.2, C	211.7, C	127.7, CH	123.5, CH	64.4, CH	80.6, CH	63.8, CH	67.8, CH	80.6, CH	80.6, CH
8	47.2, CH	45.8, CH	134.6, C	136.7, C	60.7, C	137.5, C	60.6, C	60.6, C	138.8, C	138.2, C
9	30.9, CH_2	31.9, CH_2	37.9, CH_2	37.9, CH_2	36.3, CH_2	129.3, CH	36.7, CH_2	35.9, CH_2	124.0, CH	125.4, CH
10	28.6, CH_2	27.0, CH_2	27.5, CH_2	30.2, CH_2	28.0, CH_2	30.0, CH_2	27.9, CH_2	23.6, CH_2	23.6, CH_2	30.4, CH_2
11	44.4, CH	43.2, CH	42.4, CH	44.0, CH	42.1, CH	47.9, CH	43.0, CH	45.5, CH	46.7, CH	43.2, CH
12	137.6, C	136.6, C	137.9, C	137.6, C	137.4, C	137.0, C	137.1, C	190.4, C	187.2, C	134.5, C
13	206.6, C	205.9, C	206.9, C	206.3, C	206.2, C	205.5, C	205.4, C	125.6, CH	123.6, CH	205.5, C
14	55.3, CH_2	54.6, CH_2	55.7, CH_2	56.0, CH_2	54.8, CH_2	57.5, CH_2	54.8, CH_2	213.4, C	212.4, C	52.8, CH_2
15	21.3, CH_3	23.0, CH_3	23.0, CH_3	22.1, CH_3	23.2, CH_3	23.1, CH_3	23.6, CH_3	21.1, CH_3	24.2, CH_3	25.0, CH_3
16	15.6, CH_3	118.7, CH_2	115.0, CH_2	110.7, CH_2	117.4, CH_2	15.5, CH_3	15.6, CH_3	23.9, CH_3	22.9, CH_3	23.3, CH_3
17	18.0, CH_3	14.5, CH_3	16.8, CH_3	15.9, CH_3	17.3, CH_3	11.2, CH_3	17.5, CH_3	17.5, CH_3	10.1, CH_3	10.6, CH_3
18	147.9, C	150.4, C	148.1, C	147.8, C	148.6, C	145.9, C	149.5, C	29.0, CH	29.4, CH	148.4, C
19	24.3, CH_3	25.0, CH_3	24.7, CH_3	24.1, CH_3	25.1, CH_3	23.8, CH_3	25.2, CH_3	22.5, CH_3	21.7, CH_3	24.7, CH_3
20	21.5, CH_3	21.3, CH_3	21.3, CH_3	21.6, CH_3	21.3, CH_3	21.4, CH_3	21.7, CH_3	21.2, CH_3	20.7, CH_3	22.5, CH_3
21		170.7, C	170.6, C		170.5, C					
22		21.4, CH_3	21.4, CH_3		21.4, CH_3					

[a] Chemical shift values are in ppm relative to TMS. Spectra were recorded at 25 °C. Atom multiplicities were obtained from DEPT NMR experiments. Assignments were aided by $^1H-^1H$ COSY, HMQC, HMBC, and NOESY experiments.

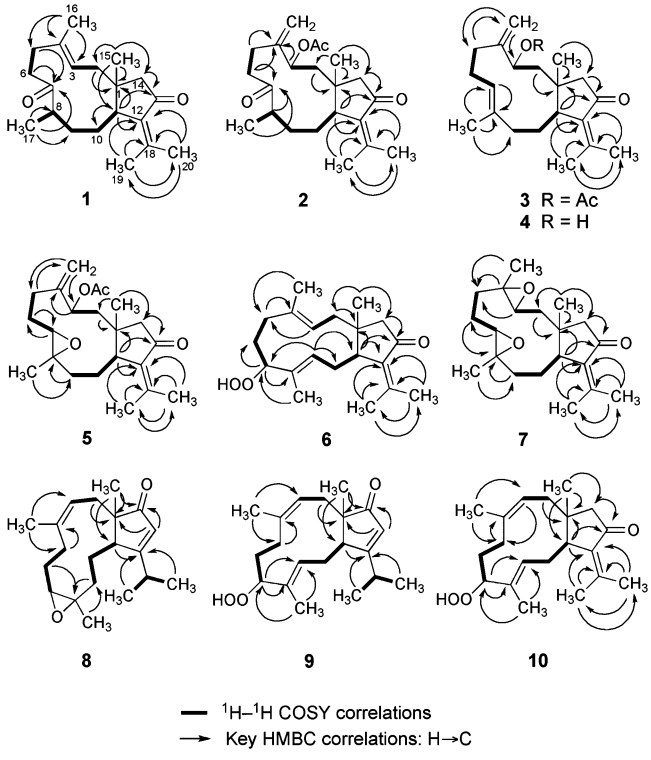


Figure 1. $^1H-^1H$ COSY and HMBC correlations for compounds **1**−**10**.

evaluation of spectral information. ^{13}C NMR bands (Table 3) at δ 64.4 (CH, C-7) and 60.7 (C, C-8) in conjunction with ^1H NMR resonances at δ 3.00 (d, 1H, J = 8.1 Hz, H-7) and 1.31 (s, 3H, H_3-17) illustrated **5** to possess a methyl-substituted epoxide group. By 2D NMR spectroscopic data, including $^1H-^1H$ COSY, HMQC, and HMBC, compound **5** was shown to possess many of the same structural features as those of **2**. Comparison of the ^{13}C NMR data for the two compounds revealed that the major differences between them lay in the vicinity of the C-7,8 junction (see Table 3). On the basis of $^1H-^1H$ couplings observed in the ^1H NMR spectrum and overall HMBC spectral data of **5** (Figure 1) it was apparent that the trisubstituted epoxide group had to reside at the C-7,8 position. The HMBC spectrum of **5** showed heteronuclear couplings between C-8 (δ_C 60.7, C) and H_2-9 (δ_H 2.08 and 1.40), as well as from C-7 (δ_C 64.4, CH) to H_2-6 (δ_H 2.10 and 1.82), which allowed the epoxide group to be unambiguously positioned across C-7 and C-8. The relative configuration of **5** at C-1, C-3, and C-11 was identical to that of **2** on the basis of comparable NOE interactions (Figure 3) and interproton coupling patterns. For C-7 and C-8 it was concluded that the epoxide function had to possess the 7*R**, 8*R** relative configuration, on the basis of a 8.1 Hz coupling between H-7 (δ 3.00) and H-6α (δ 2.10), as well as strong NOE effects observed between H_3-15 (δ 1.34), H-3 (δ 5.48), and H-7 (δ 3.00) and between H-11 (δ 3.23) and H_3-17 (δ 1.31). Thus, compound **5**, a likely precursor to **2** upon acid-promoted epoxide rearrangement, is 3*R**-acetoxy-7*R**,8*R**-epoxy-13-keto-1*R**,11*S**-dolabell-4(16),12(18)-diene.

Mass spectrometry and NMR data revealed compound **6** to have a molecular formula of $C_{20}H_{30}O_3$. The presence of seven sp^2-hybridized carbon atoms in the molecule, as deduced from the ^{13}C and DEPT NMR spectra, being for three carbon−carbon double bonds and one carbon−oxygen bond as the only multiple bonds, indicated compound **6** to be bicyclic. The IR spectrum showed the presence of hydroxy and α,β-unsaturated ketone functionalities (ν_{max} 3397, 1704 cm^{-1}). The ^1H and ^{13}C NMR spectra (Tables 2 and 3) resembled those of known dolabellatrienone (**11**).[3] However, a secondary hydroperoxy [δ_H 4.13 (dd, 1H, J = 2.6, 10.7 Hz, H-7); δ_C 80.6 (CH, C-7)] adjacent to a methyl bearing an *E* trisubstituted double bond [δ_H 5.41 (t, 1H, J = 7.8 Hz, H-9), 1.68 (s, 3H, H_3-17); δ_C 137.5 (C, C-8), 129.3 (CH, C-9), 11.2 (CH_3, C-17)] in **6** replaced the *E* trisubstituted $\Delta^{7,8}$ double bond in **11**. The presence of a secondary hydroperoxy group in **6** was confirmed by the EIMS data, which showed pronounced fragment ions at *m*/*z* 301 and 284 through elimination of HO· and H_2O_2, respectively, from the molecular ion of **6** at *m*/*z* 318. $^1H-^1H$ COSY cross-peaks between H-7 (δ 4.13) and H_2-6 (δ 1.92 and 1.59) and between H-9 (δ 5.41) and H_2-10 (δ 2.40 and 1.78), as well as HMBC correlations between H-7 and C-6, C-8, C-9, C-17; H-9 and C-7, C-8, C-10, C-11, and C-17 (Figure 1), positioned the secondary hydroperoxy group at C-7 and the nearby trisubstituted double bond between C-8 and C-9. In the NOESY spectrum (Figure 4), NOEs between H-7 and H-9, between H-7 and H_3-15, between H-9 and H_3-15, and between H-11 and H_3-17, which indicated that the 11-membered ring in **6** adopts a crown conformation, allowed the configuration of the hydroperoxy and $\Delta^{8,9}$ moieties to be assigned as 7*R** and *E*, respectively. Correspondingly, the triene **6** was fully characterized

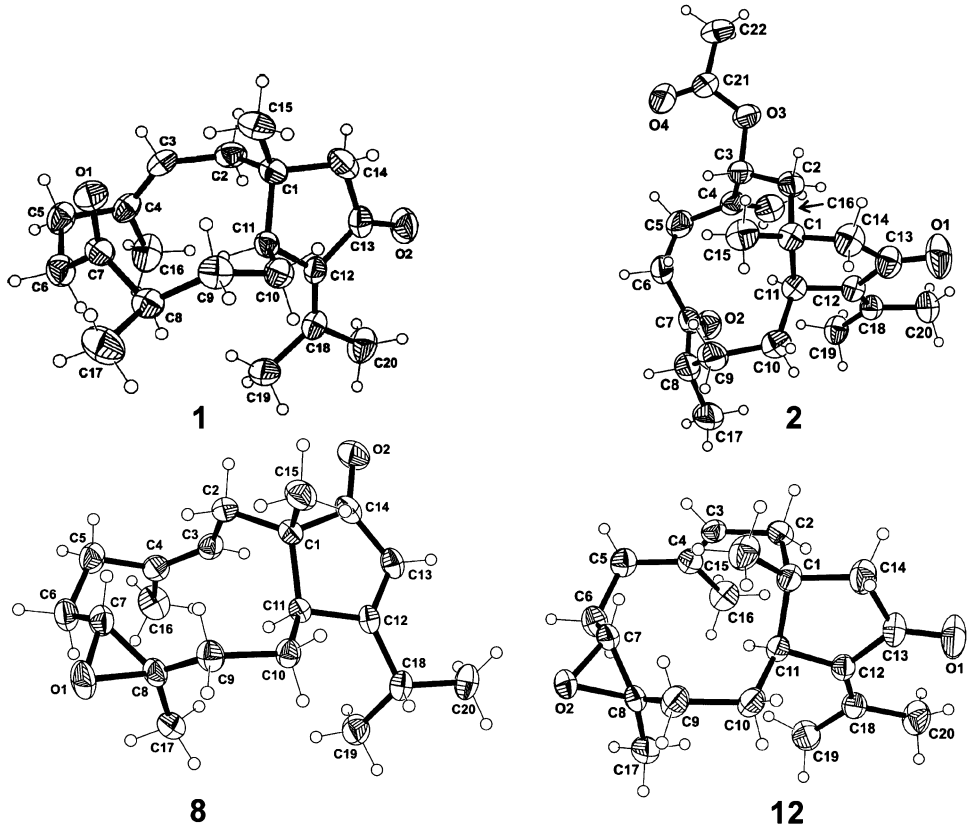


Figure 2. Molecular structures of compounds **1**, **2**, **8**, and **12**, which define only the relative configuration, with atom-labeling scheme. The carbon and oxygen atoms are drawn as 30% thermal ellipsoids.

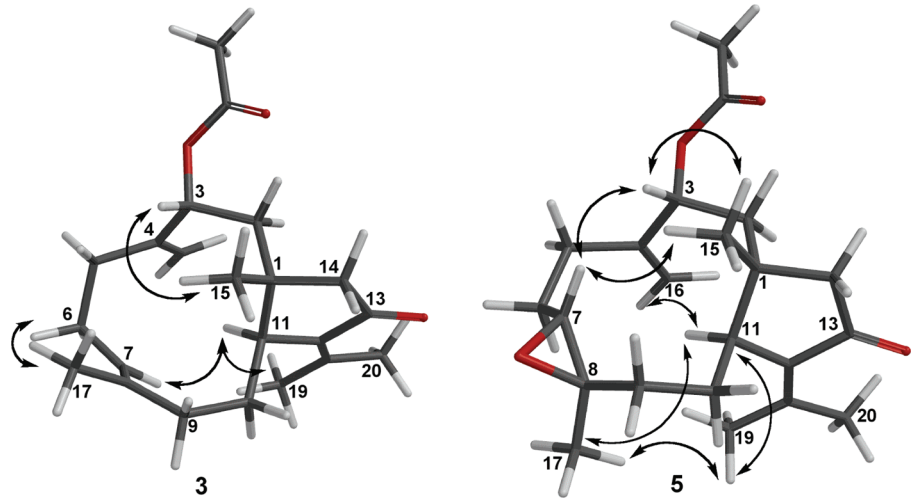


Figure 3. Key NOESY correlations for **3** and **5**.

as 7*R**-hydroperoxy-13-keto-1*R**,11*S**-dolabell-3*E*,8*E*,12(18)-triene. The co-occurrence of **6** and **11** within the same *Eunicea* sp. extract suggests that **6** might be formed from **11** during the isolation process upon air oxidation of the double bond at C-7 of **11**.

Compound **7** was isolated as a colorless oil. HREIMS, ^{13}C NMR, and DEPT spectra established the molecular formula of **7** as $C_{20}H_{30}O_3$. The IR and UV absorptions clearly defined the analogous α-isopropylidenylcyclopentanone group as in **1**−**6**. The NMR data of compound **7** were strongly reminiscent of those of known epoxide **12**, apart from the replacement of the NMR bands of the trisubstituted olefin [δ_H 5.43 (dd, 1H, J = 4.7, 11.6 Hz, H-3), 1.57 (br s, 3H, H_3-16); δ_C 124.7 (CH, C-3), 135.9 (C, C-4), 15.7 (CH_3, C-16)][3] by those of an additional epoxide [δ_H 3.06 (dd, 1H, J = 2.9, 11.2 Hz, H-3), 1.21 (s, 3H, H_3-16); δ_C 63.0 (CH, C-3), 60.3 (C, C-4), 15.6 (CH_3, C-16)]. On the basis of this very favorable comparison, dolabellanone **7** was formulated as the C-3,4 epoxy derivative of **12**. The proposed structural type and substitution pattern of **7** were confirmed by means of 2D NMR correlated spectroscopy including HMQC, HMBC, and ^{1}H−^{1}H COSY (Figure 1). The relative structure of diepoxide **7** was elucidated by the analysis of NOE correlations, as shown in Figure 4. It was found that epoxymethine protons H-3 (δ 3.06) and H-7 (δ 2.96) each showed NOE interactions with H_3-15 (δ 1.33); therefore, assuming a β-orientation of H_3-15, H-3 and H-7 should also be positioned on the β-face. NOE correlations observed between H-11 (δ 2.81) and H_3-16 (δ 1.21), and between H-11 and H_3-17 (δ 1.43), reflected the α-orientations of H_3-16 and H_3-17. Furthermore, H-3 exhibited an interaction with H-7, and H_3-19 (δ 1.94) exhibited a NOE

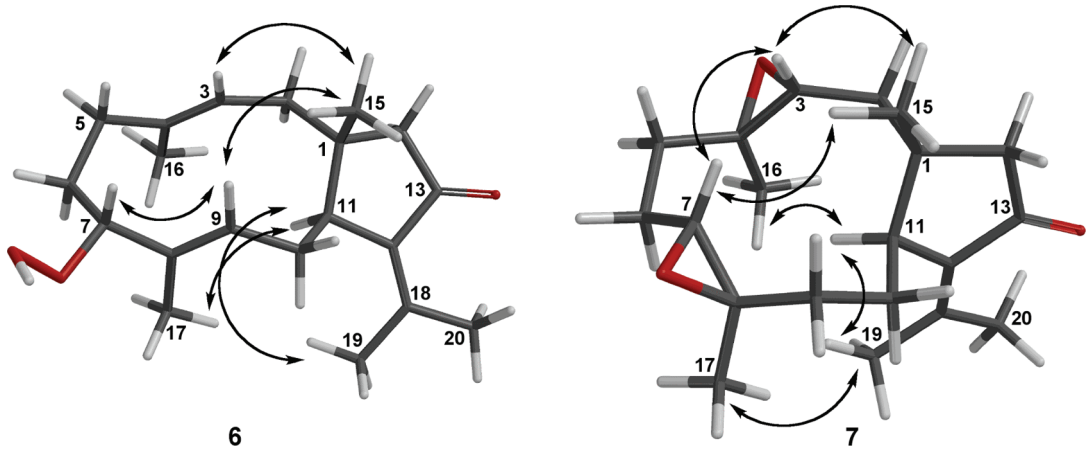


Figure 4. Key NOESY correlations for **6** and **7**.

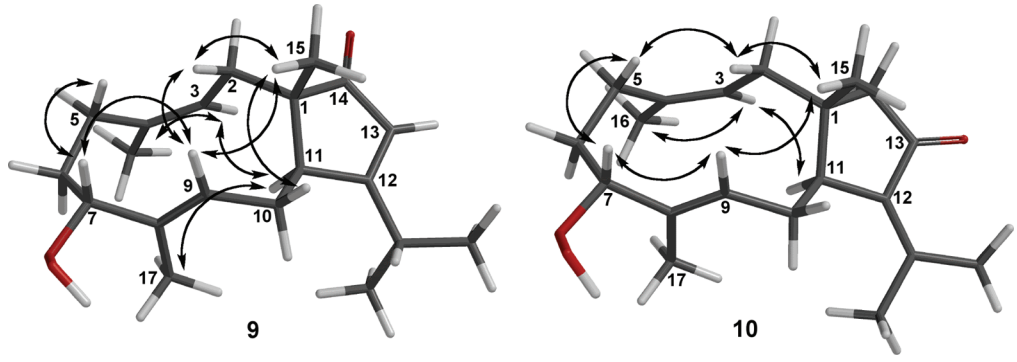


Figure 5. Key NOESY correlations for **9** and **10**.

interaction with H_3-17, all of which pointed toward identical *R** relative configurations at C-3, C-4, C-7, and C-8. To confirm this contention, a solution of known dolabellatrienone (**11**) in CH_2Cl_2 was treated with *m*-CPBA at 25 °C to find a 20% conversion of **11** to **7**, whose NMR data and the $[\alpha]_D$ value were identical to those of **7** obtained by the above-mentioned isolation. Because the absolute configuration of **11** was previously established by synthesis,[13] the absolute configuration of **7** was determined by correlation. Therefore, **7** was found to be 3*R*,4*R*,7*R*,8*R*-diepoxy-13-keto-1*R*,11*S*-dolabell-12(18)-ene.

The molecular formula of compound **8** was established as $C_{20}H_{30}O_2$ from its HREIMS data. The ^{1}H and ^{13}C NMR spectra suggested the same dolabellane diterpenoid skeleton of **1**−**7** with signals for an epoxy functionality, five methyl groups, two trisubstituted double bonds, and one carbonyl group [δ 213.4 (C, C-14)]. While the ^{1}H NMR spectrum of **8** (Table 2) displayed an unusually shielded broad doublet at δ 4.68 (1H, J = 11.7 Hz) for H-3 (cf. δ 5.27 for H-3 of **1**), it contained an additional one-proton vinyl singlet at δ 5.97 and an additional one-proton multiplet at δ 2.62. The usual methyl signals near δ 2.20 and 1.90, ascribable to the vinyl methyl groups of the α-isopropylidenylcyclopentanone moiety of **1**−**7** (H_3-19 and H_3-20), were conspicuously absent in the ^{1}H NMR spectrum of **8** and showed in their place two doublets at δ 1.13 and 1.20 (each, 3H, J = 6.8 Hz). Coupling was observed in the ^{1}H−^{1}H COSY spectrum between these methyl signals and the multiplet at δ 2.62, indicating the presence of an isopropyl group at C-12 of the dolabellane skeleton. The ^{13}C NMR spectrum (Table 3) showed, in addition to the carbonyl absorption at δ 213.4, sp^2 carbon signals at δ 190.4 (C, C-12), 136.7 (C, C-4), 125.6 (CH, C-13), and 122.4 (CH, C-3). All of these features were suggestive of a structure isomeric with that of known compound **15**. Further analysis of the 2D NMR (^{1}H−^{1}H COSY, HMQC, and HMBC) correlations showed compound **8** to possess the same planar structure as that of **15** (Figure 1); thus the differences between them must be stereochemical. The relative configuration, 1*R**, 7*R**, 8*R**, 11*S**, as well as the *Z* configuration of the $\Delta^{3,4}$ trisubstituted double bond in **8** were determined by X-ray crystallographic analysis on a single crystal of **8** (Figure 2). Compound **8** was thus elucidated to have a structure corresponding to the $\Delta^{3,4}$ *Z*-isomer of **15**.

The molecular formula of $C_{20}H_{30}O_3$ for **9** was determined by HRESIMS [found *m*/*z* 319.2259 $(M + 1)^+$; calcd for $C_{20}H_{31}O_3$ 319.2273]. All 20 carbons appeared in the ^{13}C NMR spectrum, and DEPT experiments indicated the presence of five methyls, four sp^3 methylenes, three sp^3 methines, three sp^2 methines, one sp^3 quaternary carbon, and four sp^2 quaternary carbons (Table 3). The presence of a β-isopropyl-α,β-cyclopentenone system, the same as that in compound **8**, was demonstrated by UV, IR, ^{13}C NMR (Table 3), and ^{1}H NMR (Table 2) data. The ^{1}H and ^{13}C NMR spectra showed signals due to two methyl-bearing trisubstituted olefins [δ_C 134.8 (C, C-4), 122.9 (CH, C-3), 22.9 (CH_3, C-16); δ_H 4.66 (br d, 1H, J = 12.2 Hz, H-3), 1.64 (s, 3H, H_3-16)] and [δ_C 138.8 (C, C-8), 124.0 (CH, C-9), 10.1 (CH_3, C-17); δ_H 5.68 (t, 1H, J = 7.4 Hz, H-9), 1.58 (s, 3H, H_3-17)] and due to a secondary hydroperoxide group [δ_C 80.6 (CH, C-7); δ_H 4.02 (dd, 1H, J = 5.2, 10.0 Hz, H-7)]. These spectroscopic findings coupled with proton−proton and long-range ^{13}C−^{1}H correlations observed in the ^{1}H−^{1}H COSY and HMBC spectra gave a series of partial structures, as shown in Figure 1, to give a gross structure for **9**. In the NOESY experiment (Figure 5), the secondary hydroperoxide at C-7 was assigned as α on the basis of NOEs between H-7 and H-9, H-5β; between H-11 and H-3, H_3-17; between H_3-15 and H-9, H-10β; and between H-2β and H-9, H_3-15. The geometries of the trisubstituted $\Delta^{3,4}$ and $\Delta^{8,9}$ double bonds were certified to be *Z* and *E*, respectively, on the basis of NOE experiments and the ^{13}C chemical shift values of the corresponding vinyl methyls. Compound **9** is thus 7*R**-hydroperoxy-14-keto-1*S**,11*S**-dolabell-3*Z*,8*E*,12*Z*-triene.

Compound **10** was shown by HREIMS to possess the same molecular formula ($C_{20}H_{30}O_3$) as those of **6** and **9**. Furthermore, it

was found that the NMR spectroscopic data of **10** were very similar to those of **6**, suggesting that these compounds possess the same planar structure. Comparison of the ^{1}H and ^{13}C NMR data (Tables 2 and 3) of compounds **6** and **10** showed that the structure of **10** should be very close to that of **6**, with the exception of signals assigned to the methyl bearing *E*-trisubstituted olefin $\Delta^{3,4}$ in **6**, which were replaced by resonances for a *Z*-trisubstituted olefin [δ_H 5.08 (br d, 1H, J = 11.3 Hz, H-3), 1.75 (s, 3H, H_3-16); δ_C 135.0 (C, C-4), 121.6 (CH, C-3), 23.3 (CH_3, C-16)] in **10**. The planar structure of **10** was fully established by analyzing the ^{1}H−^{1}H COSY and HMBC correlations (Figure 1), and its relative configuration was confirmed to be 1*R**, 7*R**, and 11*S** from the following NOESY correlations: both H-5β (δ 2.27) and H-9 (δ 5.66) with H-7 (δ 4.03), both H-11 (δ 3.01) and H-14α (δ 2.53) with H-3 (δ 5.08), and H_3-16 (δ 1.75) with H-3. These results, together with other detailed NOE correlations of **10** (Figure 5), unambiguously established that compound **10** is 7*R**-hydroperoxy-13-keto-1*R**,11*S**-dolabell-3*Z*,8*E*,12(18)-triene.

The spread of drug resistance in *Plasmodium falciparum* has made it essential to look into new effective chemotherapeutic agents that possess antimalarial activity with favorable pharmacokinetic properties.[15] Thus, *Eunicea* sp. dolabellanes **1**−**16** as well as dolastane **17** were tested for their inhibitory activity toward the growth of *P. falciparum* W2 (chloroquine-resistant). Interestingly, most of the compounds were active with IC_{50} values ranging from 9.4–59.6 μM. The most active metabolite was **9** (IC_{50} = 9.4 μM), and the least active compound was epoxide **8** ($IC_{50} \geq$ 100 μM).[16] Some of the present isolates (**1**, **3**, **6**, **8**, **9**, **11**, **12**, **16**, **17**, and **18**) were also tested for their inhibitory activity toward the growth of *Mycobacterium tuberculosis*, but were deemed inactive (% of inhibition at 6.25 μg/mL ≤ 50%). Furthermore, dolabellanes **1**, **7**, **11**, and **12** were evaluated in a three-cell-line panel consisting of MCF-7 breast cancer, NCI-H460 non-small-cell lung cancer, and SF-268 (CNS). However, results from the one-dose primary anticancer assay showed a lack of significant cytotoxicity when compared to the untreated control cells. Likewise, at a concentration of 30 mM, compounds **1**, **6**, **9**, **11**, and **16** were not active against the hepatitis B virus (HBV).

Experimental Section

General Experimental Procedures. Optical rotations were recorded with a Rudolph Autopol IV polarimeter and melting point determinations with an electrothermal IA 900 digital apparatus. The UV spectra were recorded with a Shimadzu UV-2401PC spectrometer, and the IR analyses were performed with a Nicolet Magna IR 750FT-IR spectrometer. 1D and 2D NMR data were recorded on a Bruker DRX-300 spectrometer. ^{1}H NMR and ^{13}C NMR chemical shifts were recorded at 25 °C with respect to TMS. Mass spectrometric measurements were generated at the Mass Spectrometry Laboratory of the University of Illinois at Urbana−Champaign. Column chromatography was performed using Si gel (35−75 mesh); TLC analysis was carried out using glass precoated Si gel plates, and the spots were visualized using a UV lamp at λ = 254 nm or by exposure to I_2 vapor. HPLC was performed using either an Ultrasphere polar-bonded Cyano semipreparative column (5 μm, 10 mm × 25 cm), an Ultrasphere normal-phase Si gel semipreparative column (5 μm, 10 mm × 25 cm), or an Ultrasphere ODS reversed-phase Si gel semipreparative column (5 μm, 10 mm × 25 cm). All HPLC separations were monitored simultaneously with a refractive index detector and a UV detector set at λ = 220 nm using a flow rate of 2 mL/min with isocratic elution of the mobile phase. All solvents were distilled from glass prior to use. The percentage yield of each compound is based on the weight of the hexane extract.

Animal Material. A detailed taxonomical description of the biological specimens used in this investigation has been disclosed previously.[11] A photograph of the gorgonian coral voucher specimen is available as Supporting Information.

Collection, Extraction, and Isolation. Medium to large colonies (0.5−1.3 m) of the gorgonian coral *Eunicea* sp. (undescribed species; order: Gorgonacea; family: Gorgoniidae, phylum: Cnidaria) were collected by scuba at depths of 23−26 m from the coral reefs off Old Providence Island (March 2002), Colombia, located off the Nicaraguan shelf in the southwestern Caribbean Sea. A voucher specimen (No. *Eunicea* sp. 2) is on deposit at the Chemistry Department of the University of Puerto Rico. The gorgonian specimens were partially air-dried, freeze-dried, and then kept frozen prior to extraction. The dried animal (1.7 kg) was blended with a mixture of 1:1 MeOH/CH_2Cl_2 (5 × 4 L), and after filtration, the combined extracts were concentrated *in vacuo* to afford a gummy, green residue (74 g). The extract was suspended in H_2O (1 L) and extracted successively with hexane (4 × 2 L), CH_2Cl_2 (4 × 2 L), and EtOAc (3 × 2 L). The hexane extract (46 g) was chromatographed on a large Si gel column by stepwise elution with 100% hexane, hexane/EtOAc mixtures (100−0%), and then 100% MeOH. Fractions were pooled on the basis of their TLC and NMR profiles to yield 15 primary fractions, denoted I−XV. Purification of fraction III (1.5 g) by Si gel CC with 100:1 hexane/EtOAc led to the isolation of known compounds dolabellatrienone (**11**, 7.6 mg, 0.02% yield), epoxide **12** (800 mg, 1.8% yield), and palominol (**16**, 5.3 mg, 0.01% yield).[3−5] A solution of fraction VII (420 mg) in toluene was filtered under vacuum and purified by size-exclusion chromatography on a Bio-Beads SX-3 (90 g) column with toluene as eluant. The combined portions (NMR and TLC guided) were concentrated to yield four subfractions, designated as A (80 mg), B (139 mg), C (155 mg), and D (40 mg). Subfraction C was purified successively by Si gel (18 g) CC with 80:1 hexane/acetone and reversed-phase HPLC (Ultrasphere ODS column with 65:35 MeOH/H_2O) to afford pure compound **1** (33.0 mg, 0.07% yield) as a white crystalline solid. Plate crystals of dolabellanone **1** suitable for X-ray crystallographic analysis were obtained upon recrystallization from a 90:10 MeOH/H_2O mixture. Purification of fraction IX (0.6 g) by size-exclusion chromatography on a Bio-Beads SX-3 column (toluene) led to four subfractions. The penultimate subfraction was eluted through a Si gel column (100:1 hexane/acetone), and then the mixture obtained was separated by successive HPLC analyses (Ultrasphere Si gel column with 95:5 hexane/2-propanol followed by a Cyano column with 97:3 hexane/2-propanol), affording pure compounds **3** (5 mg, 0.01% yield) and **10** (4 mg, 0.009% yield). Fraction X (0.6 g) was chromatographed directly over Si gel (30 g) and separated by stepwise elution with hexane/acetone (2−3%) to yield three fractions (A, B, and C). Fraction B (0.1 g) was purified successively by Si gel CC with 125:1 hexane/2-propanol and then by HPLC (Cyano column with 95:5 hexane/2-propanol) to afford pure compound **6** (15 mg, 0.03% yield) along with the known compounds **12** (10 mg, 0.02% yield), **17** (1 mg, 0.002% yield), and **18** (1 mg, 0.002% yield).[3] As crystals of suitable quality were obtained for previously reported compound **12**, a single-crystal X-ray analysis was undertaken (Figure 2).[17] Fraction XI (1.2 g) was purified by size exclusion chromatography (Bio-Beads SX-3, toluene) to yield five fractions, denoted A−E. Fraction D (0.5 g) was fractionated successively by normal-phase HPLC (Si gel column with 95:5 hexane/2-propanol), polar-bonded HPLC (Cyano column with 95:5 hexane/2-propanol), and reversed-phase HPLC (Ultrasphere ODS column with 75:25 MeOH/H_2O). The main fraction obtained (100 mg) was loaded onto a Si gel column and separated by stepwise elution with hexane/acetone (2−100%) to afford pure dolabellanones **8** (14 mg, 0.03% yield) and **9** (28 mg, 0.06% yield). Needle crystals of dolabellanone **8** suitable for X-ray crystallographic analysis were subsequently obtained upon recrystallization from a 80:20 MeOH/$CHCl_3$ mixture. Size exclusion CC (Bio-Beads SX-3 with toluene) of fraction XII (614 mg) followed by successive reversed-phase HPLC (Ultrasphere ODS column with 75:25 MeOH/H_2O) and Si gel CC (with 50:1 hexane/acetone) yielded known dolabellane **15** (3.4 mg, 0.007% yield).[8] Fraction XIII (900 mg) was purified upon elution through a Bio-Beads SX-3 column with toluene. The penultimate subfraction (564 mg) was subsequently purified by Si gel CC and normal-phase HPLC (Cyano column with 97:3 hexane/2-propanol) to yield pure compound **2** (8 mg, 0.02% yield) and known dolabellane **14** (3.6 mg, 0.008% yield).[8] Recrystallization of dolabellanone **2** from 90:10 MeOH/$CHCl_3$ afforded white cubic crystals. After fraction XIV (1.5 g) was fractionated by size-exclusion chromatography on a Bio-Beads SX-3 column (toluene), the penultimate fraction eluted (0.5 g) was purified by Si gel CC and separated by stepwise elution with hexane/acetone mixtures (5−100%). The second subfraction was purified by HPLC (Cyano column with 97:3 hexane/2-propanol) to yield pure dolabellanone **5** (13 mg, 0.03% yield). The third subfraction was purified by HPLC (Cyano column with 95:5 hexane/2-propanol) to afford dolabellanone **7** (15 mg, 0.03% yield) along with known compound **13** (10 mg, 0.02% yield),[8] and the last

subfraction was purified by Si gel CC with 20:1 hexane/acetone followed by HPLC (Cyano column with 93:7 hexane/2-propanol), yielding dolabellanone **4** (8 mg, 0.02% yield).

Dolabellanone 1: white crystalline solid; mp 128.5−128.9 °C; $[\alpha]^{25}_D$ +41.2 (*c* 1.0, $CHCl_3$); UV (MeOH) λ_{max} (log ε) 203 (3.1), 254 (3.7) nm; IR (film) ν_{max} 2975, 2957, 2929, 2873, 1700, 1621, 1459, 1406, 1369, 1273, 1182 cm^{-1}; ^1H NMR (300 MHz, $CDCl_3$) and ^{13}C NMR (75 MHz, $CDCl_3$), see Tables 1 and 3, respectively; EIMS *m/z* $[M]^+$ 302 (100), 287 (7), 269 (3), 231 (7), 219 (14), 163 (53), 151 (42), 137 (36), 121 (23), 109 (24), 93 (33); HREIMS *m/z* $[M]^+$ 302.2252 (calcd for $C_{20}H_{30}O_2$, 302.2245).

Dolabellanone 2: white crystalline solid; mp 169−170 °C; $[\alpha]^{25}_D$ +26.3 (*c* 1.3, $CHCl_3$); UV (MeOH) λ_{max} (log ε) 203 (3.4), 255 (3.2) nm; IR (film) ν_{max} 2981, 2969, 2930, 1735, 1698, 1622, 1441, 1412, 1371, 1242, 1224, 1186, 1014, 958, 912 cm^{-1}; ^1H NMR (300 MHz, $CDCl_3$) and ^{13}C NMR (75 MHz, $CDCl_3$), see Tables 1 and 3, respectively; EIMS *m/z* $[M]^+$ 360 (21), 318 (29), 300 (95), 285 (14), 189 (23), 163 (58), 151 (100), 136 (50), 121 (34), 107 (49); HREIMS *m/z* $[M]^+$ 360.2306 (calcd for $C_{22}H_{32}O_4$, 360.2301).

Dolabellanone 3: colorless oil, $[\alpha]^{25}_D$ +5.7 (*c* 1.3, $CHCl_3$); UV (MeOH) λ_{max} (log ε) 203 (3.5), 247 (3.2) nm; IR (film) ν_{max} 3077, 2963, 2931, 2869, 1737, 1702, 1624, 1447, 1370, 1280, 1243, 1180, 1016, 966, 906 cm^{-1}; ^1H NMR (300 MHz, $CDCl_3$) and ^{13}C NMR (75 MHz, $CDCl_3$), see Tables 1 and 3, respectively; EIMS *m/z* $[M]^+$ 344 (9), 302 (18), 284 (87), 269 (21), 241 (26), 201 (38), 187 (30), 150 (96), 135 (74), 121 (82), 93 (100), 81 (76), 69 (55); HREIMS *m/z* $[M]^+$ 344.2346 (calcd for $C_{22}H_{32}O_3$, 344.2351).

Dolabellanone 4: colorless oil, $[\alpha]^{25}_D$ +11.8 (*c* 0.83, $CHCl_3$); UV (MeOH) λ_{max} (log ε) 202 (3.9), 235 (3.7) nm; IR (film) ν_{max} 3434, 2933, 2873, 1701, 1620, 1453, 1373, 1242, 1188, 1037, 903 cm^{-1}; ^1H NMR (300 MHz, $CDCl_3$) and ^{13}C NMR (75 MHz, $CDCl_3$), see Tables 1 and 3, respectively; EIMS *m/z* $[M]^+$ 302 (16), 284 (6), 189 (19), 175 (25), 161 (40), 150 (54), 135 (47), 121 (54), 107 (61), 93 (87), 81 (70), 61 (100); HREIMS *m/z* $[M]^+$ 302.2242 (calcd for $C_{20}H_{30}O_2$, 302.2246).

Dolabellanone 5: colorless oil, $[\alpha]^{25}_D$ −7.4 (*c* 1.3, $CHCl_3$); UV (MeOH) λ_{max} (log ε) 204 (3.9), 235 (3.4) nm; IR (film) ν_{max} 3079, 2971, 2933, 2864, 1735, 1706, 1626, 1454, 1372, 1282, 1247, 1190, 1018, 978, 931, 914, 756 cm^{-1}; ^1H NMR (300 MHz, $CDCl_3$) and ^{13}C NMR (75 MHz, $CDCl_3$), see Tables 1 and 3, respectively; EIMS *m/z* $[M]^+$ 360 (2), 318 (1), 300 (3), 257 (1), 215 (2), 189 (4), 163 (5), 149 (11), 135 (7), 107 (11), 91 (11), 61 (100); HREIMS *m/z* $[M]^+$ 360.2298 (calcd for $C_{22}H_{32}O_4$, 360.2301).

Dolabellanone 6: colorless oil, $[\alpha]^{25}_D$ −29.5 (*c* 1.0, $CHCl_3$); UV (MeOH) λ_{max} (log ε) 206 (3.3), 234 (3.7), 235 (3.6) nm; IR (film) ν_{max} 3397, 2969, 2935, 2874, 1704, 1619, 1457, 1376, 1040 cm^{-1}; ^1H NMR (300 MHz, $CDCl_3$) and ^{13}C NMR (75 MHz, $CDCl_3$), see Tables 2 and 3, respectively; EIMS *m/z* $[M]^+$ 318 (21), 302 (36), 301 (22), 284 (64), 189 (24), 149 (43), 136 (100), 121 (26), 109 (30), 83 (36); HRESIMS *m/z* $[M - 1]^-$ 317.2102 (calcd for $C_{20}H_{29}O_3$, 317.2117).

Dolabellanone 7: colorless oil, $[\alpha]^{25}_D$ −26 (*c* 0.9, $CHCl_3$); UV (MeOH) λ_{max} (log ε) 203 (3.9), 254 (3.8) nm; IR (film) ν_{max} 2968, 2932, 2859, 1701, 1621, 1454, 1385, 1272, 1256, 1188, 1086, 1057, 945, 888 cm^{-1}; ^1H NMR (300 MHz, $CDCl_3$) and ^{13}C NMR (75 MHz, $CDCl_3$), see Tables 2 and 3, respectively; EIMS *m/z* $[M]^+$ 318 (54), 163 (37), 149 (79), 135 (73), 121 (59), 109 (79), 93 (72), 81 (70), 69 (80), 55 (100); HREIMS *m/z* $[M]^+$ 318.2197 (calcd for $C_{20}H_{30}O_3$, 318.2195). The physical data of dolabellanone **7** were found to be identical with those of a diepoxide that was chemically derived from dolabellatrienone (**11**) isolated from the Caribbean gorgonian *Eunicea calyculata* and reported with incomplete relative configuration about the epoxides.[3]

Dolabellanone 8: white crystalline solid, mp 102.2−102.7 °C, $[\alpha]^{20}_D$ + 93.0 (*c* 1.0, $CHCl_3$); UV (MeOH) λ_{max} (log ε) 229 (3.7) nm; IR (film) ν_{max} 3077, 2986, 2957, 2919, 2862, 1686, 1601, 1447, 1376, 1266, 1217, 1190, 1162, 1095, 1083, 1024, 868 cm^{-1}; ^1H NMR (300 MHz, $CDCl_3$) and ^{13}C NMR (75 MHz, $CDCl_3$), see Tables 2 and 3, respectively; EIMS *m/z* $[M]^+$ 302 (19), 287 (8), 269 (5), 241 (8), 217 (9), 203 (20), 189 (15), 161 (33), 150 (100), 135 (54), 121 (41), 107 (41), 95 (45), 81 (51), 55 (69); HREIMS *m/z* $[M]^+$ 302.2252 (calcd for $C_{20}H_{30}O_2$, 302.2246).

Dolabellanone 9: colorless oil, $[\alpha]^{25}_D$ +43.1 (*c* 1.0, $CHCl_3$); UV (MeOH) λ_{max} (log ε) 203 (4.2), 229 (4.1) nm; IR (film) ν_{max} 3419, 3075, 2969, 2932, 2875, 1697, 1600, 1458, 1381, 1341, 1264, 1214, 1190, 1110, 1090, 1040, 1014, 864, 825, 810, 758 cm^{-1}; ^1H NMR (300 MHz, $CDCl_3$) and ^{13}C NMR (75 MHz, $CDCl_3$), see Tables 2 and 3, respectively; EIMS *m/z* $[M]^+$ 318 (1), 302 (2), 301(1), 284 (1), 269 (2), 241 (2), 203 (2), 175 (3), 161 (5), 150 (37), 135 (11), 121 (6), 85 (72), 83 (100); HRESIMS *m/z* $[M + 1]^+$ 319.2259 (calcd for $C_{20}H_{31}O_3$, 319.2273).

Dolabellanone 10: colorless oil, $[\alpha]^{25}_D$ −15.0 (*c* 1.0, $CHCl_3$); UV (MeOH) λ_{max} (log ε) 202 (3.3), 238 (3.1) nm; IR (film) ν_{max} 3426, 2965, 2935, 2874, 1712, 1621, 1452, 1377, 1242, 1038, 756 cm^{-1}; ^1H NMR (300 MHz, $CDCl_3$) and ^{13}C NMR (75 MHz, $CDCl_3$), see Tables 2 and 3, respectively; EIMS *m/z* $[M]^+$ 318 (4), 302 (18), 301 (5), 284 (12), 269 (7), 189 (22), 150 (84), 136 (45), 121 (35), 107 (46), 95 (39), 83 (100), 69 (44), 55 (63); HREIMS *m/z* $[M - H_2O_2]^+$ 284.2131 (calcd for $C_{20}H_{28}O$, 284.2140).

Acetylation of Dolabellanone 4. Compound **4** (3.0 mg, 0.01 mmol) was dissolved in a mixture of dry pyridine (500 μL) and acetic anhydride (500 μL) and stirred at rt for 12 h. The reaction mixture was concentrated *in vacuo*, and the oily residue obtained was purified by CC over Si gel (1 g) using 5% EtOAc in hexane to yield a homogeneous oil (3.3 mg, quantitative yield) whose NMR (^1H and ^{13}C) data, TLC retention time, and $[\alpha]_D$ value were identical to those of dolabellanone **3**.

Epoxidation of Dolabellatrienone (11). We followed the general procedure reported by Look and Fenical.[3] A mixture of dolabellatrienone (**11**, 0.33 g, 1.2 mmol), anhydrous Na_2HPO_4 (0.17 g, 1.2 mmol), and *m*-chloroperbenzoic acid (0.20 g, 1.2 mmol) in dry CH_2Cl_2 (54 mL) was stirred at 25 °C for 1 h. The reaction mixture was diluted and washed with 10% Na_2SO_3 (2 × 25 mL), 5% $NaHCO_3$ (2 × 25 mL), and brine (2 × 25 mL). The organic layer was dried over $MgSO_4$, filtered, and concentrated. The product mixture (305 mg) was chromatographed on a Si gel (15 g) column by elution with benzene/EtOAc (95:5) to afford pure diepoxide **7** (73.7 mg, 20%), C-7*R*,8*R* monoepoxide **12** (71.2 mg, 21%), and claenone (**19**, 145 mg, 42%);[18,19] partial data for claenone (**19**) [3*R*,4*R*-epoxy-13-keto-1*R*,11*S*-dolabell-7*E*,12(18)-diene]: $[\alpha]^{25}_D$ −64 (*c* 0.2, $CHCl_3$); ^{13}C NMR (75 MHz, $CDCl_3$) δ 205.9 (C, C-13), 148.5 (C, C-18), 137.0 (C, C-12), 132.8 (C, C-8), 128.2 (CH, C-7), 63.6 (CH, C-3), 61.2 (C, C-4), 55.4 (CH_2, C-14), 42.2 (CH, C-11), 40.7 (C, C-1), 38.4 (CH_2, C-9), 37.4 (CH_2, C-2), 37.1 (CH_2, C-5), 27.2 (CH_2, C-6), 24.4 (CH_3, C-19), 24.3 (CH_2, C-10), 23.2 (CH_3, C-15), 21.2 (CH_3, C-20), 16.5 (CH_3, C-17), 15.4 (CH_3, C-16). The $[\alpha]_D$, IR, UV, HREIMS, and ^1H NMR ($CDCl_3$) data for **19** have been previously described.[3,18]

Single-Crystal X-ray Analysis of Dolabellanones 1, 2, 8, and 12. The X-ray data were collected at 298 K with a Bruker SMART 1 K CCD diffractometer equipped with a graphite monochromator and Mo Kα radiation (λ = 0.71073 Å) using the SMART software. Final values of the cell parameters were obtained from least-squares refinement. The frames were processed using the SAINT software to give the *hkl* file corrected for Lorentz and polarization effects. No absorption correction was applied. The structures were solved by direct methods with the SHELX-90 program and refined by least-squares methods on F^2, SHELXTL-93, incorporated in SHELXTL, Version 5.1.[20] The initial E-maps yielded all non-hydrogen atom positions. All non-hydrogen atoms were refined anisotropically, and the H atoms were positioned geometrically and treated as riding, with C−H distances in the range 0.93−0.98 Å and with $U_{iso}(H) = 1.2$ or $1.5 \times U_{eq}(C)$. The crystallographic data for dolabellanones **1**, **2**, **8**, and **12** reported in this article (Table 4) have been deposited at the Cambridge Crystallographic Data Centre, under the reference numbers CCDC 709008−709011. Copies of the data can be obtained, free of charge, on application to the Director, CCDC, 12 Union Road, Cambridge CB2 1EZ, UK (fax: +44 1223 336033 or e-mail: deposit@ccdc.cam.ac.uk). ORTEP representations of **1**, **2**, **8**, and **12** (Figure 2) show the relative configuration for each of these molecules.

Biological Screening Assays. For a general description of the approach used by the NIAID's Antimicrobial Acquisition and Coordinating Facility (AACF) for determining antiviral activity and toxicity for hepatitis B virus, visit http://niaid-aacf.org/protocols/HBV.htm. Anticancer activity screening by the Developmental Therapeutics Program (DTP) of the National Cancer Institute is conducted following this general protocol: most of the compounds screened have no antiproliferative activity (up to 85%). In order to avoid screening inactive compounds across all the cell lines, a prescreen is done using three highly sensitive cell lines (breast MCF-7, lung NCI-H640, CNS SF-268). Antiproliferative activity must be seen in these cell lines in order to continue to the 60-cell-line panel. The 60 different human tumor lines are incubated with five different doses of compound, and

Table 4. Single-Crystal X-ray Diffraction Data for Compounds **1**, **2**, **8**, and **12**[a]

parameter	**1**	**2**	**8**	**12**
empirical formula	$C_{20}H_{30}O_2$	$C_{22}H_{32}O_4$	$C_{20}H_{30}O_2$	$C_{20}H_{30}O_2$
fw	302.44	360.48	302.44	302.44
temperature (K)	299 (2)	298 (2)	299 (2)	298 (2)
wavelength (Å)	0.71073	0.71073	0.71073	0.71073
cryst syst	monoclinic	orthorhombic	orthorhombic	monoclinic
space group	$P2_1$ (No.4)	$P2_12_12_1$ (No. 19)	$P2_12_12$ (No. 18)	$P2_1$ (No. 4)
unit cell dimens				
a (Å)	9.777(2)	8.994(2)	11.149(2)	8.267(2)
b (Å)	10.719(2)	12.140(2)	21.716(3)	8.670(2)
c (Å)	17.521(3)	18.745(3)	7.590(2)	12.790(3)
α (deg)	90	90	90	90
β (deg)	90.174(3)	90.174(3)	90	90.451(4)
γ (deg)	90	90	90	90
volume (Å^3)	1836.2(5)	2046.8(6)	1837.5(4)	904.3(3)
Z	4	4	4	2
density (calcd) (Mg m^{-3})	1.094	1.170	1.093	1.111
absorp coeff (mm^{-1})	0.068	0.079	0.068	0.069
F(000)	664	784	664	332
cryst size (mm)	0.15 × 0.14 × 0.14	0.46 × 0.09 × 0.05	0.34 × 0.29 × 0.24	0.19 × 0.16 × 0.08
θ range for data collection (deg)	1.16 to 23.25	2.00 to 25.52	1.88 to 27.99	1.61 to 23.33
index ranges	$-10 \le h \le 10$, $-11 \le k \le 9$, $-19 \le l \le 18$	$-10 \le h \le 10$, $-12 \le k \le 14$, $-22 \le l \le 21$	$-14 \le h \le 14$, $-26 \le k \le 28$, $-10 \le l \le 8$	$-7 \le h \le 9$, $-9 \le k \le 9$, $-14 \le l \le 14$
reflns collected	8096	11 514	12 536	4050
indep reflns	4306 [R(int) = 0.0235]	3795 [R(int) = 0.0688]	4358 [R(int) = 0.0217]	2282 [R(int) = 0.0379]
indep reflns [$I > 2\sigma(I)$]	3121 [R(int) = 0.0372]	2439 [R(int) = 0.0630]	3596 [R(int) = 0.0206]	1913 [R(int) = 0.0397]
max. and min. transmn		0.0233, 0.0075		
refinement method		full-matrix least-squares on F^2		
data/restraints/params	4306/1/407	3795/0/240	4358/0/205	2282/1/204
goodness-of-fit on F^2	0.976	1.263	1.036	1.096
final R indices [$I > 2\sigma(I)$]	R1 = 0.0418, wR2 = 0.0942	R1 = 0.0657, wR2 = 0.1278	R1 = 0.0416, wR2 = 0.0973	R1 = 0.0479, wR2 = 0.1168
R indices (all data)	R1 = 0.0675, wR2 = 0.1049	R1 = 0.1405, wR2 = 0.1435	R1 = 0.0541, wR2 = 0.1035	R1 = 0.0618, wR2 = 0.1356
largest diff peak and hole (e Å^{-3})	0.175 and −0.119	0.139 and −0.114	0.175 and −0.119	0.148 and −0.131

[a] Atomic coordinates for all X-ray structures can be obtained free of charge from The Cambridge Crystallographic Data Centre via www.ccdc.cam.ac.uk/data_request/cif.

a sulforhodamine blue (SRB) assay is performed after 48 h to determine cytotoxicity. From the five-point curve, the following concentrations are extrapolated: GI_{50} (inhibits growth by 50%), TGI (totally inhibits growth), LC_{50} (kills 50% of cells). For the specific screening methods from the DTP Web site, visit http://www.dtp.nci.nih.gov/branches/btb/ivclsp.html. Compounds shown to have anticancer activity in cell lines within the NCI 60 panel may then move on to animal trials and, if successful, may eventually move on to be tested in clinical trials. Additional experimental details for our primary *in vitro* antimicrobial assays against *Mycobacterium tuberculosis* and *Plasmodium falciparum* have been previously described.[21,22]

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Supporting Information Available: ^{1}H and ^{13}C NMR spectra in $CDCl_3$, including representative 2D NMR spectra, for compounds **1**−**10**, as well as a photograph of the air-dried voucher specimen *Eunicea* sp. 2. This material is available free of charge via the Internet at http://pubs.acs.org.

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