# Briarane Diterpenes from Two Species of Octocorals, Ellisella sp. and Pteroeides sp. ‖

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# Briarane Diterpenes from Two Species of Octocorals, *Ellisella* sp. and *Pteroeides* sp.[||]

Chiaki Tanaka,[†] Yumiko Yamamoto,[†] Masahito Otsuka,[†] Junichi Tanaka,[†] Toshio Ichiba,[‡] Gerard Marriott,[§] Rachmaniar Rachmat,[⊥] and Tatsuo Higa*,[†]

*Department of Chemistry, Biology, and Marine Science, University of the Ryukyus, Nishihara, Okinawa 903-0213, Japan, Okinawa Industrial Technology Center, 12-2 Suzaki, Gushikawa, Okinawa 904-2234, Japan, Department of Physiology, University of Wisconsin–Madison, 1300 University Avenue, Madison, Wisconsin 53706, and Research and Development Centre for Oceanology, LIPI, Jl. Pasir Putih 1, Ancol Timur, Jakarta 11048, Indonesia*

Eight new briarane diterpenes (**1**−**4**, **7**−**10**) have been isolated from two species of octocorals and the structures elucidated by spectroscopic analysis. Two diterpenes (**2**, **3**) from the gorgonian *Ellisella* sp. inhibited cytokinesis, causing multinuclei formation on NBT-II cells, while a known briarane (**12**) from the sea pen *Pteroeides* sp. showed reversal of multidrug resistance.

Since the first successful structure elucidation of briarein A in 1977, a unique bicyclic diterpene isolated earlier from the gorgonian *Briareum asbestinum*, more than 300 briarane diterpenes have been reported from octocorals.[1] Over half of these diterpenes have been described from species of the single genus *Briareum*. Other octocorals containing briaranes include gorgonians of the genera *Ellisella*, *Junceella*, *Solenopodium*, and *Erythropodium* and sea pens of the genera *Stylatula*, *Pteroides,* and *Ptilosarcus*. The briarane diterpenes are characterized by a highly oxygenated bicyclo[8.4.0]tetradecane skeleton, with most also containing a γ-lactone, a chlorine atom, and two or more acetoxy functions. Some briaranes have been reported to have such activities as antiinflammatory,[2] immunomodulatory,[3] and reversal of multidrug resistance.[4] In our research on bioactive compounds from coral reef invertebrates, we recently examined the constituents of a sea whip, *Ellisella* sp., and a sea pen, *Pteroeides* sp., and isolated a total of eight new briaranes (**1**−**4**, **7**−**10**) together with some known ones (**5**, **6**, **11**, **12**). Two (**2**, **3**) of the new compounds showed inhibition of cytokinesis and one (**12**) reversal of multidrug resistance. We herein report the isolation, structure elucidation, and biological activity of these compounds.

## Results and Discussion

A sample of *Ellisella* sp. collected in Okinawa was extracted with acetone, and the extract was purified by silica gel column chromatography and HPLC to yield six compounds (**1**−**6**). Compounds **5** and **6** were identified as the known briaranes umbraculolide A[5] and gemmacolide C,[6] respectively, by comparing NMR data. Compounds **1**−**4** were found to be new entities.

Compound **1** had a molecular formula $C_{26}H_{31}ClO_{10}$ as determined by HRESIMS. The formula and the presence of three acetyl groups as shown by NMR data (Table 1, 2) indicated that **1** is a briarane derivative. The NMR and
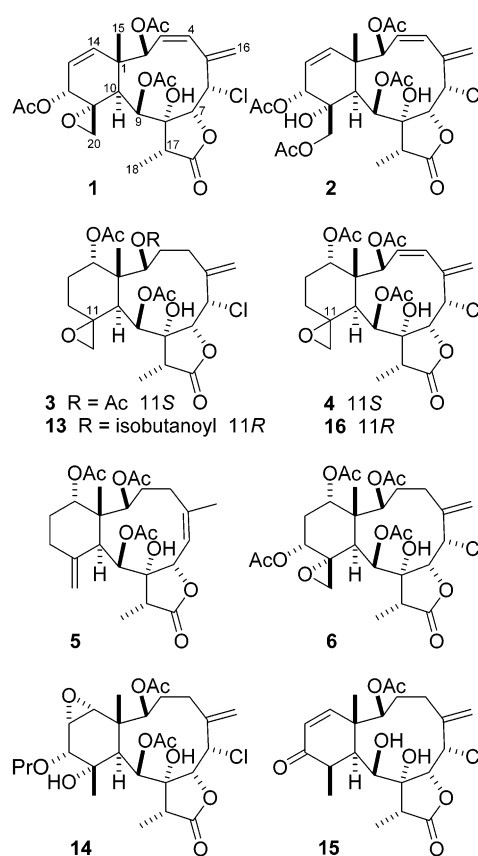
IR data revealed the presence of other typical functionalities: a γ-lactone, an exomethylene, two *cis* double bonds, an oxirane, and a hydroxyl group. These functional groups and a chlorine atom were placed on the briarane skeleton by 2D NMR analysis and by comparison of the data with those of known briaranes to determine the planar structure of **1**. The relative stereochemistry was deduced from NOE measurements. When the signal (δ 0.98) for the angular methyl protons (H-15) was irradiated, the resonances for H-3, -14, -20b, and a methyl group of an acetoxyl at C-9 were enhanced, indicating their spatial proximity and the relative configurations at C-1, -7, -9, and -11, as shown in **1**. Irradiation of the signal at δ 3.65 (H-10) showed enhancement of the signal at δ 6.11 (H-2), suggesting that these two protons should be located at the opposite side

OAc OAc
14 15
1 4 16
11 9 OH
O Cl
20 H 6''
AcO 17
18 O
**7**

OAc OAc
3 OAc
OH 6
O
AcO O
O
**8**

OAc OAc
12 Cl
OH
HO H
O
O
**9**

OAc OAc OAc
CO₂Me
OAc
OH
H
O
O
**10**

OAc OAc OH
RO
H
AcO O
O
**11** R = Ac
**12** R = Bz

OAc OAc OAc
CO₂Me
OAc
OH
HO H
O
O
**17**
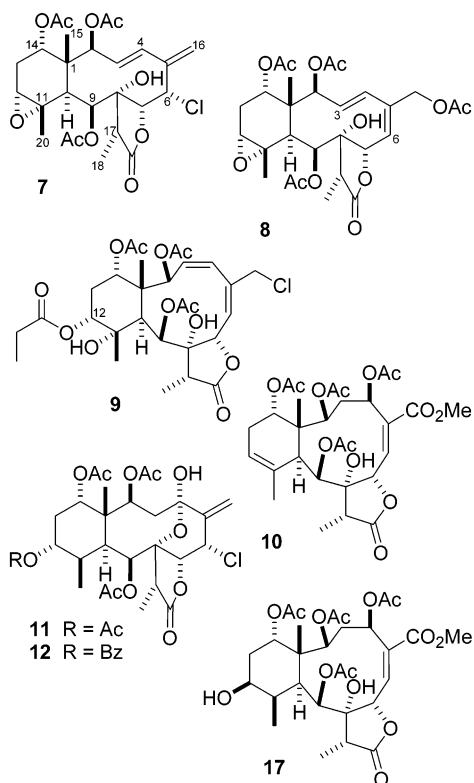
(α) of the angular methyl group at C-1. More NOE observations between OH-8 and H-18, H-7 and H-17, H-12 and H-20a, and H-9 and H-18 supported the relative stereochemistry of **1** as shown.

Compound **2** analyzed for $C_{28}H_{35}ClO_{12}$ by HRESIMS. The spectral data indicated the presence of the same functional groups, except for the oxirane. In place of the latter it contained a hydroxyl at C-11 ($\delta$ 76.9 s) and an acetoxyl at C-20 ($\delta$ 65.4 t; $\delta$ 4.32 and 4.41 d), the placement of which

was shown by 2D NMR analysis (see Experimental Section and Tables 1 and 2 for the NMR signal assignment). The rest of the molecule was the same as that of **1** including the relative stereochemistry as demonstrated by NOE observation (see Experimental Section).

Compound **3**, which revealed the formula $C_{26}H_{35}ClO_{10}$, having two less sites of unsaturation than **1**, also contained these common functionalities: a chlorine, three acetates, a γ-lactone ($\delta$ 175.4 s, 1780 cm⁻¹), an exomethylene ($\delta$ 5.66 s, 5.94 s), an epoxide ($\delta$ 2.84 d, 2.88 dd), and a hydroxyl group ($\delta$ 4.75 s). The presence of two sets of $-CH(OAc)-$ $CH_2-CH_2-$ moieties was shown by COSY, indicating saturation of the two double bonds in **1**. The HMBC correlation of H-15/C-14 clearly demonstrated the presence of an acetoxyl group at C-14 ($\delta$ 73.6 d). The spectrum (see Experimental Section) also showed the same arrangement of the functional groups on the 10-membered ring portion as those of umbraculolide A (**5**), juncenolide A (**13**),[7] 3,4-dihydro-11-hydroxybrianthein U (**14**),[8] and solenolide E (**15**).[9] NOE observation between either of H₂-20 and H-10 but not H-15 suggested that the configuration at C-11 was opposite that of **1**, and that observed for H-15/H-14 indicated the C-14 configuration as shown.

The molecular formula of compound **4**, $C_{26}H_{33}ClO_{10}$, indicated an additional unsaturation compared to that of **3**. In fact, the presence of a *cis* double bond ($\delta$ 5.53 dd, 5.93 brd, $J_{3,4} = 11.9$ Hz) was observed as in **1**. Again, 2D NMR analysis (Tables 1 and 2) and comparison of the spectral data with those of **1** and **3** suggested the gross structure. NOE measurement revealed the same relative stereochemistry as that of **3**. Thus, the structure of **4** is the 11-epimer of the known juncin A (**16**).[10]

An EtOAc extract from a sea pen, *Pteroeides* sp., was successively chromatographed to give compounds **7−12**. Two were identified to be pteroidine (**11**) and 12-*O*-desacetyl-12-*O*-benzoylpteroidine (**12**) reported from *Pteroides laboutei*,[11] while compounds **7−10** were elucidated as new briaranes.

**Table 1.** ¹H NMR Data for Briaranes **1−4** in CDCl₃ (*J* in Hz)

| H# | 1 | 2 | 3 | 4 |
|---|---|---|---|---|
| 2 | 6.11 (d, 9.2) | 6.01 (d, 9.2) | 5.12 (brt, 3.0) | 6.23 (d, 8.8) |
| 3a | 5.54 (dd, 11.6, 9.2) | 5.56 (dd, 11.9, 9.2) | 1.64 (m) | 5.53 (dd, 11.9, 8.8) |
| 3b | | | 2.18 (m) | |
| 4a | 5.96 (brd, 11.6) | 5.94 (d, 11.9) | 2.19 (m) | 5.93 (brd, 11.9) |
| 4b | | | 2.84 (m) | |
| 6 | 5.14 (m) | 5.08 (m) | 4.87 (d, 3.7) | 5.03 (m) |
| 7 | 4.89 (brd, 3.7) | 5.02 (dd, 4.3, 1.2) | 5.10 (d, 3.7) | 4.98 (dd, 4.3, 1.2) |
| 8-OH | 3.32 (d, 1.2) | 5.15 (s) | 4.75 (s) | 5.12 (d, 1.2) |
| 9 | 4.76 (d, 7.9) | 5.74 (d, 6.4) | 5.50 (d, 6.1) | 5.55 (d, 7.3) |
| 10 | 3.65 (d, 7.9) | 3.38 (d, 6.4) | 2.42 (brd, 6.1) | 2.65 (d, 7.3) |
| 11-OH | | 3.96 (s) | | |
| 12a | 4.58 (brd, 6.1) | 5.22 (d, 6.4) | 1.17 (m) | 1.08 (m) |
| 12b | | | 2.33 (m) | 2.35 (m) |
| 13a | 5.87 (dd, 10.1, 6.1) | 5.74 (dd, 10.1, 6.4) | 1.79 (m) | 1.81 (m) |
| 13b | | | 2.14 (m) | 2.16 (m) |
| 14 | 5.76 (d, 10.1) | 5.82 (d, 10.1) | 4.86 (s) | 4.95 (d, 4.9) |
| 15 | 0.98 (s) | 1.10 (s) | 1.11 (s) | 1.10 (s) |
| 16a | 6.06 (d, 2.0) | 5.95 (d, 2.8) | 5.66 (s) | 5.97 (d, 2.4) |
| 16b | 6.13 (brs) | 6.18 (brs) | 5.94 (s) | 6.09 (s) |
| 17 | 2.28 (q, 7.0) | 2.33 (q, 7.0) | 2.28 (q, 7.0) | 2.29 (q, 7.0) |
| 18 | 1.16 (d, 7.0) | 1.21 (d, 7.0) | 1.22 (d, 7.0) | 1.22 (d, 7.0) |
| 20a | 2.83 (d, 2.4) | 4.32 (d, 11.9) | 2.84 (d, 4.0) | 2.83 (d, 4.0) |
| 20b | 3.38 (d, 2.4) | 4.41 (d, 11.9) | 2.88(dd, 4.0, 1.5) | 2.95 (dd, 4.0, 1.8) |
| 2-Ac | 2.13 (s) | 2.11 (s) | 2.01 (s) | 2.06 (s)[a] |
| 9-Ac | 2.16 (s) | 2.17 (s) | 2.21 (s) | 2.18 (s)[a] |
| 12-Ac | 2.13 (s) | 2.12 (s) | | |
| 14-Ac | | | 2.02 (s) | 2.04 (s)[a] |
| 20-Ac | | 2.16 (s) | | |

[a] Signals are exchangeable.

**Table 2.** $^{13}C$ NMR Data for **1**–**4** and **7**–**10** in $CDCl_3$

| C# | 1 | 2 | 3 | 4 | 7 | 8 | 9 | 10 |
|---|---|---|---|---|---|---|---|---|
| 1 | 47.4 qC | 45.4 qC | 45.9 qC | 46.8 qC | 45.3 qC | 45.4 qC | 45.5 qC | 44.3 qC |
| 2 | 76.4 CH | 77.3 CH | 73.6 CH* | 71.6 CH | 75.5 CH | 76.6 CH | 75.3 CH | 72.6 CH |
| 3 | 129.5 CH | 128.7 CH | 29.1 CH$_2$ | 129.1 CH | 133.0 CH | 137.7 CH | 131.9 CH | 37.6 CH$_2$ |
| 4 | 129.2 CH | 129.5 CH | 34.3 CH$_2$ | 129.8 CH | 129.4 CH | 127.5 CH | 127.9 CH | 67.3 CH |
| 5 | 137.9 qC | 137.1 qC | 141.4 qC | 138.4 qC | 142.3 qC | 137.6 qC | 139.2 qC | 138.8 qC |
| 6 | 60.4 CH | 61.5 CH | 64.2 CH | 61.0 CH | 66.0 CH | 125.8 CH | 127.3 CH | 136.7 CH |
| 7 | 78.6 CH | 78.9 CH | 78.8 CH | 79.0 CH | 80.9 CH | 80.6 CH | 79.7 CH | 77.3 CH |
| 8 | 83.4 qC | 82.8 qC | 81.9 qC | 82.1 qC | 84.5 qC | 81.1 qC | 81.1 qC | 82.4 qC |
| 9 | 64.6 CH | 68.6 CH | 69.0 CH | 68.1 CH | 72.3 CH | 70.0 CH | 69.1 CH | 69.7 CH |
| 10 | 33.9 CH | 38.3 CH | 39.8 CH | 40.6 CH | 41.2 CH | 41.2 CH | 38.0 CH | 40.3 CH |
| 11 | 60.3 qC | 76.9 qC | 62.7 qC | 62.2 qC | 59.6 qC | 59.8 qC | 77.3 qC | 134.2 qC |
| 12 | 70.7 CH | 68.1 CH | 23.5 CH$_2$ | 23.7 CH$_2$ | 59.3 CH | 59.3 CH | 73.5 CH | 121.1 CH |
| 13 | 121.4 CH | 120.5 CH | 24.0 CH$_2$ | 23.9 CH$_2$ | 26.5 CH$_2$ | 25.9 CH$_2$ | 26.4 CH$_2$ | 26.6 CH$_2$ |
| 14 | 142.8 CH | 142.6 CH | 73.7 CH* | 72.9 CH | 75.4 CH | 78.0 CH | 73.0 CH | 72.9 CH |
| 15 | 15.0 CH$_3$ | 15.5 CH$_3$ | 15.4 CH$_3$ | 14.1 CH$_3$ | 14.9 CH$_3$ | 15.4 CH$_3$ | 14.1 CH$_3$ | 14.1 CH$_3$ |
| 16 | 117.8 CH$_2$ | 116.8 CH$_2$ | 122.1 CH$_2$ | 117.2 CH$_2$ | 116.8 CH$_2$ | 66.4 CH$_2$ | 46.1 CH$_2$ | 168.0 qC |
| 17 | 44.6 CH | 45.5 CH | 43.6 CH | 44.4 CH | 48.7 CH | 45.1 CH | 44.8 CH | 44.0 CH |
| 18 | 6.4 CH$_3$ | 7.0 CH$_3$ | 7.2 CH$_3$ | 6.6 CH$_3$ | 7.5 CH$_3$ | 8.1 CH$_3$ | 6.6 CH$_3$ | 6.9 CH$_3$ |
| 19 | 174.4 qC | 174.7 qC | 175.4 qC | 175.0 qC | 174.3 qC | 175.2 qC | 175.8 qC | 175.3 qC |
| 20 | 47.5 CH$_2$ | 65.4 CH$_2$ | 58.3 CH$_2$ | 58.2 CH$_2$ | 25.3 CH$_3$ | 25.4 CH$_3$ | 25.8 CH$_3$ | 24.6 CH$_3$ |
| 2-Ac | 21.8 CH$_3$ | 21.1 CH$_3$ | 21.0 CH$_3$ | 22.0 CH$_3^a$ | 21.0 CH$_3^a$ | 20.9 CH$_3$ | 21.3 CH$_3^a$ | 20.9 CH$_3$ |
|  | 169.9 qC | 169.6 qC | 170.8 qC | 170.2 qC$^b$ | 169.9 qC | 170.0 qC | 169.2 qC | 170.3 qC |
| 4-Ac |  |  |  |  |  |  |  | 21.3 CH$_3^a$ |
|  |  |  |  |  |  |  |  | 169.7 qC$^b$ |
| 9-Ac | 20.8 CH$_3$ | 21.9 CH$_3$ | 21.8 CH$_3$ | 21.0 CH$_3^a$ | 21.5 CH$_3$ | 21.6 CH$_3$ | 21.6 CH$_3$ | 21.5 CH$_3$ |
|  | 170.3 qC | 170.1 qC | 170.0 qC | 170.2 qC$^b$ | 170.3 qC | 170.6 qC | 169.6 qC | 169.4 qC |
| 12-Ac | 21.1 CH$_3$ | 20.8 CH$_3$ |  |  |  |  | 9.1 CH$_3$ |  |
| (12-Pr) | 170.2 qC | 171.8 qC |  |  |  |  | 28.0 CH$_2$ |  |
|  |  |  |  |  |  |  | 176.7 qC |  |
| 14-Ac |  |  | 21.0 CH$_3$ | 20.9 CH$_3^a$ | 21.4 CH$_3^a$ | 21.7 CH$_3$ | 21.2 CH$_3^a$ | 21.2 CH$_3^a$ |
|  |  |  | 169.6 qC | 169.8 qC$^b$ | 170.2 qC | 169.8 qC | 170.4 qC | 171.0 qC$^b$ |
| 16-Ac |  |  |  |  |  | 20.9 CH$_3$ |  |  |
|  |  |  |  |  |  | 170.6 qC |  |  |
| 16-OMe |  |  |  |  |  |  |  | 53.1 CH$_3$ |
| 20-Ac |  | 21.0 CH$_3$ |  |  |  |  |  |  |
|  |  | 170.6 qC |  |  |  |  |  |  |

$^{a,b}$ Signals with the same footnote are exchangeable in the same column.

Compound **7**, $C_{26}H_{33}ClO_{10}$, contained common features of a briarane: three acetoxyls, a $\gamma$-lactone, an exomethylene, a *trans* double bond, a hydroxyl, a trisubstituted epoxide, and a chlorine atom. The gross structure was elucidated by 2D NMR analysis and relative stereochemistry by NOE measurements and coupling constants (see Experimental Section). The coupling constant ($J = 16.0$ Hz) between H-3 and H-4 clearly demonstrated $E$ geometry of the double bond.

Compound **8**, $C_{28}H_{36}O_{12}$, contained structural features similar to those of **7** except for the absence of a chlorine atom. The NMR data (Tables 2 and 3) showed the presence of an additional acetoxyl group attached to a methylene ($\delta$ 66.4 t) and trisubstitued double bond ($\delta$ 137.6 s, 125.8 d) instead of an exomethylene, suggesting structural modification around the C-4/C-7 region. The structure for this portion was deduced from an allylic coupling ($J = 1.2$ Hz) between H-6 and H-16ab and HMBC correlation of H-4/C-6, H-7/C-5, H-16ab/C-6, and H-16ab/16-acetoxy carbonyl. The spectral data indicated that the remaining portion of the molecule was the same as that of **7**.

Compound **9**, $C_{29}H_{39}ClO_{12}$, was shown to contain two double bonds ($\delta$ 127.3 d, 127.9 d, 131.9 d, 139.2 s), a $\gamma$-lactone ($\delta$ 175.8 s), two hydroxyls ($\delta$ 2.94 s, 4.28 s; 3400 cm$^{-1}$), three acetates ($\delta$ 1.96 s, 2.03 s, 2.21 s), and a propionate ($\delta$ 1.16 t (3H), 2.35 dq, 2.44 dq). The position of the propanoyloxy group was assigned at C-12 by observing HMBC correlations between H-12 ($\delta$ 4.96) and the ester carbonyl ($\delta$ 176.7), which showed cross-peaks with $\alpha$ methylene protons. The stereochemistry of **9** was determined as shown by NOE analysis. The structure of **9** was reminiscent of briarein J,[12] in which the propanoyloxyl group at C-12 was replaced by an acetate.

Compound **10**, $C_{29}H_{38}O_{13}$, contained four acetates ($\delta$ 1.90, 2.00, 2.09, 2.20), a $\gamma$-lactone ($\delta$ 175.3 s), an $\alpha,\beta$-unsaturated methyl ester ($\delta$ 3.83 s, 6.90 dd; $\delta$ 168.0 s, 138.8 s, 136.7 d), a trisubstituted double bond ($\delta$ 121.1 d, 134.2 s), and a hydroxyl group ($\delta$ 82.4 s, C-8). A connectivity study (see Experimental Section) allowed us to assign all the $^1H$ and $^{13}C$ NMR signals (Tables 2 and 3). Comparison of the NMR data with those of the known milolide J (**17**)[13] showed their close structural relationship. The chemical shifts for C-1 to C-8, and C-15 to C-19, matched within $\pm 1.3$ ppm, suggesting the same stereochemistry for these portions, which is in good agreement with the result of NOE measurements.

Compounds **2** and **3** induced formation of multinuclear cells at concentrations between 0.5 and 2 $\mu$g/mL against NBT-II cells, indicating inhibition of cytokinesis. When compounds **7**–**12** were screened for multidrug resistance, *O*-desacetyl-12-*O*-benzoylpteroidine (**12**) showed reversal of multidrug resistance against KB-C2 (P-Gp type) and KB-CV60 cells (MRP-1 type), while new compounds **7**–**9** were inactive.

## Experimental Section

**General Experimental Procedures.** The optical rotations were recorded on a Jasco DIP-1000 digital polarimeter. IR spectra were measured on a Jasco FT IR-300 spectrometer. NMR spectra were taken on a JEOL $\alpha$ 500 FT NMR spectrometer in $CDCl_3$ and referenced to the TMS signal at $\delta$ 0.00

**Table 3.** $^1$H NMR Data for Briaranes **7**–**10** in CDCl$_3$ ($J$ in Hz)

| H# | 7 | 8 | 9 | 10 |
|---|---|---|---|---|
| 2 | 5.51 (dd, 6.4, 1.5) | 5.51 (dd, 6.1, 1.2) | 5.54 (d, 10.1) | 4.81 (d, 8.9) |
| 3a | 6.17 (dd, 16.0, 6.4) | 6.06 (dd, 17.6, 6.1) | 5.67 (dd, 10.1, 10.7) | 2.20 (m) |
| 3b | | | | 2.74 (dd, 12.2, 15.0) |
| 4 | 6.62 (d, 16.0) | 6.15 (d, 17.6) | 6.36 (d, 10.7) | 5.83 (ddd, 12.2, 6.1, 0.9) |
| 6 | 5.19 (d, 2.1) | 5.54 (dt, 3.4, 1.2) | 5.94 (d, 8.2) | 6.90 (dd, 10.1, 0.9) |
| 7 | 4.41 (d, 2.1) | 5.83 (m) | 4.98 (m) | 5.65 (d, 10.1) |
| 8-OH | 3.80 (s) | 2.87 (s) | 4.28 (s) | 3.35 (s) |
| 9 | 6.00 (d, 5.5) | 5.95 (d, 7.0) | 5.77 (d, 4.0) | 6.12 (d, 2.1) |
| 10 | 3.24 (d, 5.5) | 2.96 (d, 7.0) | 3.09 (d, 4.0) | 2.95 (brs) |
| 11-OH | | | 2.94 (s) | |
| 12 | 2.95 (d, 3.0) | 2.92 (brt, 0.9) | 4.96 (m) | 5.44 (m) |
| 13a | 2.21 (m) | 2.13 (m) | 2.06 (m) | 2.00 (m) |
| 13b | 2.28 (brd, 17.0) | 2.36 (d, 17.1) | 2.12 (m) | 2.20 (m) |
| 14 | 4.84 (dd, 5.2, 1.5) | 4.83 (d, 4.6) | 4.77 (t, 3.0) | 4.79 (brs) |
| 15 | 0.97 (s) | 0.83 (s) | 1.10 (s) | 0.95 (s) |
| 16a | 5.22 (s) | 4.53 (brd, 13.4) | 4.45 (d, 12.8) | |
| 16b | 5.34 (s) | 4.75 (brd, 13.4) | 4.63 (d, 12.8) | |
| 17 | 2.64 (q, 7.3) | 2.50 (q, 7.0) | 2.37 (q, 7.0) | 2.55 (q, 7.3) |
| 18 | 1.32 (d, 7.3) | 1.38 (d, 7.0) | 1.17 (d, 7.0) | 1.30 (d, 7.3) |
| 20 | 1.51 (s) | 1.58 (s) | 1.55 (s) | 2.00 (brs) |
| 2-Ac | 2.09 (s) | 2.11 (s) | 1.96 (s) | 2.00 (s) |
| 4-Ac | | | | 2.09 (s)$^a$ |
| 9-Ac | 2.23 (s) | 2.29 (s) | 2.21 (s) | 2.20 (s) |
| 12-Pr | | | 2.35 (dq, 16.5, 7.6) | |
| | | | 2.44 (dq, 16.5, 7.6) | |
| | | | 1.16 (t, 7.6) | |
| 14-Ac | 2.09 (s) | 2.16 (s) | 2.03 (s) | 1.90 (s)$^a$ |
| 16-Ac | | 2.11 (s) | | 3.83 (s) (OMe) |

$^a$ Signals are exchangeable in the same column.

for $^1$H NMR and the CDCl$_3$ signal at $\delta$ 77.0 for $^{13}$C NMR spectra. Multiplicities of $^{13}$C NMR data were determined by DEPT experiments. ESI, APCI, and FAB mass spectra were measured on a JEOL JMS-300 instrument. HPLC separations were carried out on a Hitachi L-6000 pump equipped with a Hitachi L-4000 UV detector and Waters R401 differential refractometer. Columns used for HPLC were normal-phase silica (250 × 10 mm, LiChrosorb) or reversed-phase silica (250 × 10 mm, 5C$_{18}$-ARII). Kieselgel 60 (230–400 mesh) was used for column chromatography. TLC was carried out on precoated silica 60 F254 plates and visualized with vanillin–EtOH–5% H$_2$SO$_4$.

**Animal Material.** The gorgonian *Ellisella* sp. (suborder Calcaxonia, family Ellisellidae) was collected by hand using scuba at a depth of 30 m at Hedo, Okinawa, in March 2002. The specimen was examined by J.T. in consultation with Prof. Y. Benayahu, Tel Aviv University, Ramat Aviv, Tel Aviv, Israel. The gorgonian was pale red in color and had branching. A voucher specimen (CT-1) is kept at the Department of Chemistry, Biology, and Marine Science, University of the Ryukyus.

A few specimens of the sea pen *Pteroeides* sp. (order Pennatulacea, family Pteroeididae) were collected off Flores Island, Indonesia, in August 2001. The specimen was examined by J.T. in consultation with Dr. G. C. Williams, California Academy of Science, San Francisco. The sea pen was robust and fleshy, and the height was around 40 cm. A voucher specimen (01Z28) is kept at the Department of Chemistry, Biology, and Marine Science, University of the Ryukyus.

**Extraction of *Ellisella* sp. and Isolation of Briaranes 1–6.** A fresh sample of the gorgonian (0.5 kg) was cut into small pieces (5 cm) and soaked in acetone. After decantation, fresh solvent was added, and the procedure was repeated three times. The combined extracts were concentrated and partitioned between EtOAc and H$_2$O. The organic layer was concentrated to give an oil (4.8 g), which was chromatographed on silica gel by eluting with a step gradient of hexane–EtOAc–MeOH. On the basis of the characteristic signals observed in $^1$H NMR spectra, fractions 5–7 were selected for further purification. Compound **5** (5.4 mg) was isolated from fraction 5 (85 mg) by silica HPLC (hexane–EtOAc). Fraction 6 (129

mg) was separated by silica HPLC (hexane–EtOAc) to give compounds **3** (16.9 mg) and **1** (14.9 mg). Fraction 7 (80 mg) was similarly separated to give compounds **2** (7.7 mg), **6** (1.1 mg), and **4** (2.9 mg).

**Extraction of *Pteroeides* sp. and Isolation of Briaranes 7–12.** The specimen (90 g, wet weight) was extracted with acetone (2 L, 3 times). The extract was concentrated, and its lipophilic portion (EtOAc) was taken to give 2.2 g of an extract. This extract was separated on a silica gel column to give seven fractions using a stepwise gradient of hexanes/CH$_2$Cl$_2$/CH$_2$-Cl$_2$–MeOH/MeOH. The fourth fraction (502 mg) gave crystals by adding MeOH. The crystals were collected to obtain **11** (232 mg). The aliquot was separated by reversed-phase HPLC (5C$_{18}$-ARII, MeOH–H$_2$O, 2:1) to yield **11** (30.5 mg), **12** (15.0 mg), and **7** (66.6 mg). The fifth fraction (10.8 mg) from HPLC was separated again on reversed-phase HPLC to give **8** (5.2 mg). Fraction 9 (9.9 mg) from HPLC was similary separated to give **9** (4.8 mg) and **10** (1.5 mg).

**Compound 1:** white solid; [α]$^{26}$$_D$ −59° (*c* 0.106, CHCl$_3$); IR (neat) $\nu_{max}$ 3502, 1772, 1734 cm$^{-1}$; $^1$H and $^{13}$C NMR, see Tables 1 and 2, COSY H-2/H-3, -4, H-3/H-4, H-4/H-16b, H-6/H-7, -16ab, H-7/OH-8, H-9/H-10, H-12/H-13, H-13/H-14, H-16a/H-16b, H-17/H-18, H-20a/H-20b; HMBC H-2/C-1, -3, -14, -15, -2-Ac, H-3/C-5, H-4/C-2, -3, -6, -16, H-6/C-5, -7, -8; H-7/C-6, OH-8/C-7, -8, H-9/C-7, -8, -10, -17, -9-Ac, H-10/C-1, -2, -8, -9, -11, -12, -15, -20, H-12/C-11, -13, -14, -20, -12-Ac, H-13/C-1, -11, -12, H-14/C-1, -2, -10, -12, -15, H-15/C-1, -2, -10, -14, H-16a/C-4, -5, -6, H-16b/C-4, -6, H-17/C-8, -9, -18, H-18/C-8, -17, -19, H-20a/C-11, H-20b/C-11, -12; NOE: H-2/H-10, H-3/H-15, H-7/H-4, -6, -17, OH-8/H-10, -18, H-9/H-15, -18, -20b, H-12/H-20a, H-14/H-15, H-15/9-Ac, H-20b; ESIMS *m/z* 561 [M + Na]$^+$, 360; HRESIMS *m/z* 561.1525 (calcd for C$_{26}$H$_{31}$ClNaO$_{10}$, 561.1503).

**Compound 2:** white solid; [α]$^{26}$$_D$ −50° (*c* 0.462, CHCl$_3$); IR (neat) $\nu_{max}$ 3502, 1770, 1732, 1232 cm$^{-1}$; $^1$H and $^{13}$C NMR, see Tables 1 and 2; COSY H-2/H-3, H-3/H-4, H-4/H-6, H-6/H-7, -16b, H-9/H-10, H-12/H-13, H-13/H-14, H-17/H-18, H-20a/H-20b; HMBC H-2/C-1, -3, -4, -15, -2-Ac, H-3/C-4, H-4/C-3, -5, -16, H-7/C-6, OH-8/C-7, -8, -9, H-9/C-7, -8, -10, -11, -8-Ac, H-10/C-1, -8, -9, -11, -15, -20, OH-11/C-10, -11, H-12/C-10, -11, -13, -14, -12-Ac, H-13/C-1, -12, H-14/C-1, -10, -12, -15, H-15/C-1, -2, -10, -14, H-16a/C-4, -5, -6, H-16b/C-4, -6, H-17/C-8, -9, -18,

H-20a/C-12, -20-Ac, H-20b/C-11, -12, -20-Ac; NOE H-2/H-10, H-3/H-4, -15, H-4/H-6, -7, OH-8/H-10, H-9/OH-11, H-12, -15, -17, -20b, H-12/H-13, OH-11, H-14/H-15, H-15/H-20a; ESIMS $m/z$ 621 [M + Na]$^+$; HRESIMS $m/z$ 621.1703 (calcd for $C_{28}H_{35}$-ClNaO$_{12}$ 621.1715).

**Compound 3:** white solid; $[\alpha]^{26}{}_D$ −26.8° ($c$ 1.038, CHCl$_3$); IR (neat) $\nu_{max}$ 3290, 1780, 1732 cm$^{-1}$; $^1$H and $^{13}$C NMR, see Tables 1 and 2; COSY H-2/H-3, H-3/H-4, H-6/H-7, -16ab, H-9/H-10, H-12a/H-12b, -13ab, H-12b/H-13b, -20a, H-13a/H-12b, -13b, -14, H-17/H-18, H-20a/H-20b; HMBC: OH-8/C-7, -8, H-9/C-7, -8, -10, -11, -17, -9-Ac, H-10/C-2, -8, -9, -11, H-14/14-Ac, H-15/C-1, -2, -10, -14, H-16ab/C-6, H-17/C-18, H-18/C-8, -17, -19; NOE: H-2/H-10, -16a, H-3b/H-6, H-6/H-7, -16b, H-7/H-9, -17, 8-OH/H-10, H-9/H-15, -17, H-10/H-16b, -20b, H-14/H-15; ESIMS $m/z$ 565 [M + Na]$^+$, 360; HRESIMS $m/z$ 565.1826 (calcd for $C_{26}H_{35}$ClNaO$_{10}$ 565.1816).

**Compound 4:** white solid; $[\alpha]^{26}{}_D$ −127° ($c$ 0.175, CHCl$_3$); IR (neat) $\nu_{max}$ 3275, 1780, 1734 cm$^{-1}$; $^1$H and $^{13}$C NMR, see Tables 1 and 2; COSY H-2/H-3, -4, H-3/H-4, H-4/H-16b, H-6/H-7, -16ab, H-9/H-10, H-10/H-12a, -14, -15, -20b, H-12a/H-12b, -13a, -14, H-12b/H-13a, -20b, H-13a/H-13b, -14, H-13b/H-14, H-14/H-15, H-16a/H-16b, H-17/H-18, H-20a/H-20b; HMBC H-2/C-1, -3, -10, -15, H-3/C-5, H-4/C-2, -5, H-6/C-5, -7, -8, H-7/C-6, OH-8/C-7, -8, H-9/C-7, -8, -10, -11, -17, H-10/C-1, -2, -11, -12, -15, -20, H-12a/C-11, -20, H-12b/H-11, -13, -20, H-13a/C-1, -12, -14, H-13b/C-11, H-14/C-10, -13, H-15/C-1, -2, -10, -14, H-16a/C-4, -5, -6, H-16b/C-6, H-17/C-18, -19, H-18/C-8, -17, -19, H-20a/C-10, -11, -12, H-20b/C-10, -11; NOE: H-2/H-10, -16a, H-3/H-15, H-4/H-7, -9, H-6/H-7, -16a, OH-8/H-9, -10, -20a, H-9/H-15, -17, H-10/H-20a, H-16a/H-16b; -18, H-20a/H-20b; FABMS $m/z$ 541 [M + H]$^+$, 481; HRFABMS $m/z$ 541.1872 (calcd for $C_{26}H_{34}$ClO$_{10}$ 541.1841).

**Compound 5:** white solid; $[\alpha]^{26}{}_D$ −29° ($c$ 0.356, CHCl$_3$); lit. $[\alpha]^{30}{}_D$ −37° (CHCl$_3$);[5] IR (neat) $\nu_{max}$ 3444, 1780, 1731 cm$^{-1}$. NMR data were identical with those reported for umbraculolide A.[5]

**Compound 6:** white solid; $[\alpha]^{26}{}_D$ +18° ($c$ 0.075, CHCl$_3$); lit. $[\alpha]^{30}{}_D$ +14° (CHCl$_3$);[6] IR (neat) $\nu_{max}$ 3477, 1790, 1738 cm$^{-1}$. NMR data were identical with those reported for gemmacolide C.[6]

**Compound 7:** white solid; $[\alpha]^{26}{}_D$ +38° ($c$ 2.33, CHCl$_3$); IR (neat) $\nu_{max}$ 3300, 1780, 1740, 1371, 1230 cm$^{-1}$; $^1$H and $^{13}$C NMR, see Tables 2 and 3; COSY: H-2/H-3, -4, H-3/H-4, H-4/H-16ab, H-6/H-7, H-7/OH-8, H-9/H-10, H-10/H-15, H-12/H-13ab, -14, H-13b/H-13a, -14, H-16a/H-16b, H-17/OH-8, H-18; HMBC H-2/C-1, -3, -4, -10, -14, -15, -2-Ac, H-3/C-1, -2, -5, H-4/C-2, -3, -5, -16, H-6/C-4, -5, -7, -8, -16, H-7/C-5, -6, OH-8/C-7, -8, -9, -17, H-9/C-1, -7, -8, -10, -11, -17, -9-Ac, H-10/C-1, -2, -8, -9, -11, -15, H-12/C-11, -13, -14, -20, H-13a/C-12, H-13b/H-1, -12, -14, H-14/C-1, -2, -10, -12, -13, -14-Ac, H-15/C-1, -2, -10, -14, H-16ab/C-4, -5, -6, H-17/C-8, -9, -18, -19, H-18/C-8, -17, -19, H-20/C-10, -11, -12; NOE H-2/H-3, -4, -10, -14, H-4/OH-8, -10, -16a, H-6/H-7, -16b, H-7/H-17, OH-8/H-4, -10, -18, H-9/H-10, -17, -18, H-10/H-18, H-12/H-20, H-14/H-15, H-17/H-18, H-18/H-20; ESIMS $m/z$ 579 ([M + K]$^+$); HRESIMS $m/z$ 579.1390 (calcd for $C_{26}H_{33}$ClKO$_{10}$ 579.1399).

**Compound 8:** white solid; $[\alpha]^{26}{}_D$ −62° ($c$ 0.383, CHCl$_3$); IR (neat) $\nu_{max}$ 3400, 1780, 1740, 1375, 1230 cm$^{-1}$; $^1$H and $^{13}$C NMR, see Tables 2 and 3; COSY H-2/H-3, -4, H-3/H-4, H-6/H-7, -16b, H-7/OH-8, H-9/H-10, H-12/H-13ab, H-13ab/H-14, H-16a/H-16b, H-17/H-18; HMBC H-2/C-1, -3, -4, -14, -2-Ac, H-3/C-4, H-4/C-3, -5, -6, H-6/C-4, H-7/C-5, OH-8/C-7, -17, H-9/C-1, -7, -10, -11, -17, -9-Ac, H-10/C-1, -2, -8, -9, -11, H-12/C-14, H-13b/C-12, -14, H-14/C-10, -12, -14-Ac, H-15/C-1, -2, -10, -14, H-16ab/C-5, -6, -16-Ac, H-17/C-18, H-18/C-8, -17, -19, H-20/C-10, -11, -12; NOE H-2/H-14, -15, H-3/H-7, H-4/H-7, -10, H-6/H-7, -16ab H-7/H-17, H-9/H-10, -18, -20, H-12/H-20, H-14/H-15, H-16a/H-16b, H-18/H-17, -20; APCIMS $m/z$ 563 ([M − H]$^-$); HRAPCIMS $m/z$ 563.2121 (calcd for $C_{28}H_{35}O_{12}$ 563.2129).

**Compound 9:** white solid; $[\alpha]^{26}{}_D$ +12° ($c$ 0.35, CHCl$_3$); IR (neat) $\nu_{max}$ 3400, 1780, 1740, 1370 cm$^{-1}$; $^1$H and $^{13}$C NMR, see Tables 2 and 3; COSY H-2/H-3, H-3/H-4, H-4/H-6, -16b, H-6/H-7, -16a, H-7/OH-8, H-9/H-10, H-12/H-13ab, H-13a/H-13b, -14, H-13b/H-14, H-16a/H-16b, H-17/H-18; HMBC H-2/C-1, -3, -4, -15, -2-Ac, H-3/C-2, -5, H-7/C-5, OH-8/C-7, H-9/C-7, -8, -10,
-11, -17, -9-Ac, H-10/C-1, -2, -8, -9, -11, -15, -20, H-12/C-11, -14, H-13b/C-1, -11, H-14/C-1, -10, -12, -15, -14-Ac, H-15/C-1, -2, -10, -14, H-16a/C-4, -5, -6, H-16b/C-5, -6, H-17/C-18, H-18/C-8, -17, -19, H-20/C-10, -11, -12; NOE H-2/H-10, H-3/H-15, H-6/H-16a, OH-8/H-9, -10, H-9/H-10, -17, -20, OH-11/H-9, -10, H-12/H-20, H-14/H-15, H-15/H-20, -9-Ac; APCIMS $m/z$ 613 ([M − H]$^-$); HRAPCIMS $m/z$ 613.2064 (calcd for $C_{29}H_{38}$ClO$_{12}$ 613.2052).

**Compound 10:** white solid; $[\alpha]^{26}{}_D$ +23° ($c$ 0.083, CHCl$_3$); IR (neat) $\nu_{max}$ 3400, 1780, 1740, 1370 cm$^{-1}$; $^1$H and $^{13}$C NMR, see Tables 2 and 3; COSY H-3a/H-2, -3b, -4, H-3b/H-4, H-6/H-7, H-10/H-9, -12, H-13a/H-12, -13b, -14, H-14/H-12, -13b, H-17/H-18; HMBC H-2/C-4, -10, -15, -2-Ac, H-4/C-2, H-6/C-16, OH-8/C-17, H-9/C-7, -8, -11, -9-Ac, H-15/C-1, -2, -10, -14, H-17/C-19, H-18/C-8, 17, -19, H-20/C-10, -11, -12, 16-OMe/C-16; NOE H-2/H-4, -10, H-4/16-OMe, H-6/H-7, H-7/H-17, H-9/H-17, -18, -20, H-14/H-15, H-15/9-Ac, H-17/H-18; APCIMS $m/z$ 633 ([M + K]$^+$); HRAPCIMS $m/z$ 633.1944 (calcd for $C_{29}H_{38}$-KO$_{13}$ 633.1950).

**Compound 11:** white solid; $[\alpha]^{26}{}_D$ −10° ($c$ 1.26, CHCl$_3$); lit. $[\alpha]^{26}{}_D$ −10° (CHCl$_3$);[11] EIMS $m/z$ 602 (M$^+$); IR (neat) $\nu_{max}$ 3300, 1780, 1740, 1375 cm$^{-1}$. NMR data were identical with those reported for pteroidine.[11]

**Compound 12:** white solid; $[\alpha]^{26}{}_D$ −2.2° ($c$ 0.925, CHCl$_3$); lit. $[\alpha]_D$ −4° (CHCl$_3$);[11] EIMS $m/z$ 664 (M$^+$); IR (neat) $\nu_{max}$ 3300, 1780, 1740, 1600, 1375 cm$^{-1}$. NMR data were identical with those reported for $O$-desacetyl-12-$O$-benzoylpteroidine.[11]

**Cell Assay.** Rat bladder epithelial NBT-II cells were seeded in 1 mL of modified Eagle's media supplemented with 10% heat-inactivated fetal bovine serum, streptomycin, amphotericin B, and glutamic acid. Cells were exposed to graded concentrations of the briaranes at 37 °C for 72 h and observed under microscope to observe the effects at 48 and 72 h.

**Multidrug Resistance Assay.** Human epidermoid carcinoma KB cells (KB-3-1) were used as the parental cell line for the present study. KB-3-1 cells were cultured in RPMI 1640 medium with 0.44 mg/mL of glutamine and 50 $\mu$g/mL of kanamycin sulfate, supplemented with 10% newborn calf serum. MDR cell line KB-C2 was selected from KB-3-1 cells and maintained in the medium containing 2 $\mu$g/mL of colchicines. KB-CV60 cells were similarly selected and maintained in a medium containing 1 $\mu$g/mL of cepharanthine and 60 ng/mL of vincristine. Reversing activity and cytotoxicity were measured by means of MTT colorimetric assay performed in 96-well plates. Equal numbers of cells (10 000) were inoculated into each well with 100 $\mu$L of the culture medium. After 24 h preincubation (37 °C, 5% CO$_2$), a 50 mL solution of an anticancer agent (colchicines to KB-C2) and testing samples were added to each well, and the whole were further incubated for 48 h. Thereafter, 25 $\mu$L of MTT solution (2 mg/mL in PBS) was added to each well and incubated for additional 3 h. After removing the medium by aspiration, the resulting formozan was dissolved in 200 $\mu$L of dimethyl sulfoxide. The percentage of cell growth inhibition was calculated from the absorbance at 540 nm. The cytotoxic activity of the testing sample was also examined by MTT assay using parental KB 3-1 cells.

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