# Junceols D—H, New Polyoxygenated Briaranes from Sea Whip Gorgonian Coral *Junceella juncea* (Ellisellidae)

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**Chemical investigations on the sea whip gorgonian coral *Junceella juncea* have led to the isolation of five new 8-hydroxybriarane diterpenoids, junceols D—H (1—5). The structures of briaranes 1—5 were determined on the basis of spectroscopic methods and the methylenecyclohexane rings were found to exist in boat form in these new diterpenoids. Junceols D (1) and F—H (3—5) exhibited cytotoxicity toward CCRF-CEM and DLD-1 tumor cells and junceols E—H (2—5) displayed weak inhibitory effects on superoxide anion generation by human neutrophils.**

**Key words** *Junceella juncea*; junceol; briarane; cytotoxicity

Previous chemical investigations on the gorgonian coral *Junceella juncea* (Ellisellidae) have yielded a series of interesting new natural products including briarane-type diterpenoids,[1—12] steroidal glycosides,[13,14] glycerol,[14] and sphingolipid derivatives.[15] In continuation of our search for anti-inflammatory and cytotoxic natural products from the invertebrates collected off Taiwanese waters, we have further isolated five new 8-hydroxybriaranes, junceols D—H (**1**—**5**), from the gorgonian coral *J. juncea*. Briarane-type natural products are found only in marine organisms and mainly from octocorals.[16,17] The compounds of this type are suggested to be originally synthesized by host corals,[18,19] and proven to possess various activities.[16,17] In this paper, we describe the isolation, structure determination, and biological activity of the above new briaranes. The structures, including the relative configurations of briaranes **1**—**5** were elucidated mainly by spectroscopic methods.

## Results and Discussion

Junceol D (**1**) was obtained as a white powder. From HR-ESI-MS, the molecular formula of **1** was determined to be $C_{35}H_{50}O_{13}$ with *m*/*z* 701.3154 (Calcd for $C_{35}H_{50}O_{13}Na$, 701.3149), indicating 11 degrees of unsaturation. The IR absorptions of **1** showed the presence of 3453, 1775, and 1744 $cm^{-1}$, consistent with the presence of hydroxy, $\gamma$-lactone, and ester groups. From the $^{13}$C-NMR data of **1** (Table 1), the presence of a trisubstituted olefin and an exocyclic carbon–carbon double bond were deduced from the signals of four carbons resonating at $\delta_C$ 146.9 (s, C-11), 144.6 (s, C-5), 123.3 (d, CH-6), 115.6 (t, $CH_2$-20), and further supported by three olefin proton signals at $\delta_H$ 5.68 (1H, ddd, $J$=10.0, 1.2, 1.2 Hz, H-6), 5.26 (1H, s, H-20a), 5.11 (1H, s, H-20b) in the $^1$H-NMR spectrum of **1** (Table 2). Furthermore, in the $^{13}$C-NMR spectrum, six carbonyl resonances appeared at $\delta_C$ 176.3 (s), 175.6 (s), 172.2 (s), 170.1 (s), 170.1 (s), and 169.3 (s), confirming the presence of a $\gamma$-lactone and five esters in **1**. In the $^1$H-NMR spectrum, three acetyl methyls ($\delta_H$ 2.21, 1.98, 1.94, each 3H×s), an isobutyryl group ($\delta_H$ 1.17, 3H, d, $J$=7.2 Hz; 1.16, 3H, d, $J$=7.2 Hz; 2.50, 1H, septet, $J$=7.2 Hz), and an isovaleryl group ($\delta_H$ 0.95, 3H, d, $J$=6.8 Hz; 0.95, 3H, d, $J$=6.8 Hz; 2.07, 1H, m; 2.19, 2H, d, $J$=7.6 Hz) were observed. The $^1$H-NMR spectrum of **1** also showed the presence of a vinyl methyl ($\delta_H$ 2.21, 3H, d, $J$=1.2 Hz, $H_3$-16), a secondary methyl ($\delta_H$ 1.13, 3H, d, $J$=7.2 Hz, $H_3$-18), a tertiary methyl ($\delta_H$ 1.12, 3H, s, $H_3$-15), two aliphatic methines ($\delta_H$ 3.32, 1H, d, $J$=3.6 Hz, H-10; 2.55, 1H, q, $J$=7.2 Hz, H-17), six oxymethines ($\delta_H$ 5.57, 1H, d, $J$=10.0 Hz, H-7; 5.54, 1H, d, $J$=3.6 Hz, H-9; 5.12, 1H, ddd, $J$=14.0, 5.6, 1.2 Hz, H-4; 5.02, 1H, dd, $J$=6.4, 6.0 Hz, H-13; 5.02, 1H, s, H-14; 4.88, 1H, d, $J$=6.0 Hz, H-2), and two aliphatic methylenes ($\delta_H$ 2.83, 1H, dd, $J$=14.0, 14.0 Hz; 1.93, 1H, m, $H_2$-3; 2.58, 1H, dd, $J$=13.6, 6.4 Hz; 2.31, 1H, dd, $J$=13.6, 6.0 Hz, $H_2$-12) in the $^1$H-NMR spectrum of **1**.

From the $^1$H–$^1$H COSY spectrum and coupling constants analysis of **1**, it was possible to establish the separate spin systems between H-2/$H_2$-3; $H_2$-3/H-4; H-4/H-6 (by allylic coupling); H-6/H-7; and H-9/H-10 (Fig. 1). These data, together with the HMBC correlations between H-2/C-1, -4; $H_2$-3/C-1, -4, -5; H-4/C-5, -6; H-6/C-4; H-7/C-5, -6, -8; H-9/C-7, -8; and H-10/C-1, -8 (Fig. 1, Table 3), established the connectivity from C-1 to C-10 within the 10-membered ring. A vinyl methyl attached at C-5 was confirmed by the allylic coupling between $H_3$-16 and H-6 ($J$=1.2 Hz) and by the HMBC correlations between H-4/C-16; H-6/C-16; and $H_3$-16/C-4, -5, -6. The methylenecyclohexane ring, which is fused to the 10-membered ring at C-1 and C-10, was established by the HMBC correlations between H-9/C-11; H-10/C-11; $H_2$-12/C-10, -11, -13, -20; H-13/C-12; and H-14/C-10, -12, -13. The exocyclic carbon–carbon double bond, which is attached to the six-membered ring at C-11, was elucidated by the HMBC correlations between H-10/C-11; $H_2$-12/C-11, -20; and $H_2$-20/C-10, -11, -12. The ring junction C-

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Table 1. $^{13}$C-NMR Data for Diterpenoids **1**—**5**[a)]

| Position | 1 | 2 | 3 | 4 | 5 |
|---|---|---|---|---|---|
| 1 | 47.7 (s)[b)] | 47.5 (s) | 47.4 (s) | 46.1 (s) | 46.9 (s) |
| 2 | 71.3 (d) | 71.4 (d) | 71.3 (d) | 73.0 (d) | 72.9 (d) |
| 3 | 37.6 (t) | 38.1 (t) | 38.3 (t) | 30.6 (t) | 30.4 (t) |
| 4 | 72.2 (d) | 72.8 (d) | 72.9 (d) | 29.1 (t) | 28.9 (t) |
| 5 | 144.6 (s) | 144.6 (s) | 144.6 (s) | 145.7 (s) | 145.7 (s) |
| 6 | 123.3 (d) | 123.8 (d) | 123.8 (d) | 119.7 (d) | 119.7 (d) |
| 7 | 77.2 (d) | 76.9 (d) | 77.2 (d) | 78.1 (d) | 78.1 (d) |
| 8 | 82.2 (s) | 83.0 (s) | 83.0 (s) | 81.8 (s) | 81.8 (s) |
| 9 | 71.8 (d) | 71.1 (d) | 71.0 (d) | 71.5 (d) | 71.7 (d) |
| 10 | 41.8 (d) | 42.3 (d) | 42.3 (d) | 41.4 (d) | 41.4 (d) |
| 11 | 146.9 (s) | 151.2 (s) | 151.3 (s) | 147.0 (s) | 146.9 (s) |
| 12 | 35.7 (t) | 25.7 (t) | 25.6 (t) | 34.9 (t) | 35.0 (t) |
| 13 | 68.3 (d) | 27.4 (t) | 27.3 (t) | 68.6 (d) | 68.2 (d) |
| 14 | 73.1 (d) | 73.9 (d) | 74.0 (d) | 73.8 (d) | 73.9 (d) |
| 15 | 14.6 (q) | 15.2 (q) | 15.2 (q) | 15.4 (q) | 15.7 (q) |
| 16 | 26.0 (q) | 26.1 (q) | 26.1 (q) | 26.3 (q) | 26.4 (q) |
| 17 | 43.1 (d) | 42.4 (d) | 42.4 (d) | 43.1 (d) | 43.2 (d) |
| 18 | 6.4 (q) | 6.4 (q) | 6.4 (q) | 6.6 (q) | 6.6 (q) |
| 19 | 175.6 (s) | 175.9 (s) | 176.4 (s) | 175.7 (s) | 175.8 (s) |
| 20 | 115.6 (t) | 112.9 (t) | 112.9 (t) | 115.6 (t) | 115.5 (t) |
| Acetyl | 21.6 (q) | 21.8 (q) | 21.9 (q) | 21.7 (q) | 21.7 (q) |
| groups | 169.3 (s) | 169.4 (s) | 169.4 (s) | 169.4 (s) | 169.4 (s) |
| | 20.9 (q) | 21.2 (q) | 21.2 (q) | 21.0 (q) | 21.0 (q) |
| | 170.1 (s) | 170.2 (s) | 170.2 (s) | 170.2 (s) | 170.2 (s) |
| | 20.9 (q) | 21.2 (q) | 21.2 (q) | 20.9 (q) | 20.9 (q) |
| | 170.1 (s) | 170.1 (s) | 170.1 (s) | 170.3 (s) | 170.3 (s) |
| Isobutyryl | 18.9 (q) | 18.9 (q) | | | 19.1 (q) |
| groups | 18.2 (q) | 18.5 (q) | | | 18.2 (q) |
| | 34.0 (d) | 34.0 (d) | | | 34.0 (d) |
| | 176.3 (s) | 176.2 (s) | | | 176.3 (s) |
| Isovaleryl | 22.4 (q) | | | | |
| group | 22.3 (q) | | | | |
| | 25.7 (d) | | | | |
| | 43.4 (t) | | | | |
| | 172.2 (s) | | | | |
| 2-Methylbutanoates | | | 11.6 (q) | 11.5 (q) | |
| | | | 26.1 (t) | 25.8 (t) | |
| | | | 16.4 (q) | 16.1 (q) | |
| | | | 41.2 (d) | 41.0 (d) | |
| | | | 175.9 (s) | 175.4 (s) | |

*a*) Spectra measured at 100 MHz in $CDCl_3$ at 25 °C. *b*) Multiplicity deduced by DEPT and HMQC spectra and indicated by usual symbols.

15 methyl group was positioned at C-1 from the HMBC correlations between H-2/C-15; H-10/C-15; and $H_3$-15/C-1, -2, -10, -14. In addition, the carbon signal appearing at $\delta_C$ 172.2 (s) was correlated with the signals of the methylene and methine protons at $\delta_H$ 2.19 and 2.07, respectively, in the HMBC spectrum and was assigned as the carbon atom of isovalerate carbonyl. The isovaleroxy group attached at C-4 was confirmed by the connectivity between H-4 ($\delta_H$ 5.12) and the carbonyl carbon ($\delta_C$ 172.2) of isovaleroxy group. The acetoxy groups positioned at C-9, C-13, and C-14 were confirmed from the HMBC correlations between $\delta_H$ 5.54 (H-9), 5.02 (H-13), 5.02 (H-14) and the acetate carbonyl carbons resonating at $\delta_C$ 169.3 (s), 170.1 (s), 170.1 (s), respectively. Furthermore, the carbon signal appearing at $\delta_C$ 176.3 (s) was correlated with the signals of an aliphatic methine ($\delta_H$ 2.50), two methyls ($\delta_H$ 1.17, 1.16), and an oxymethine ($\delta_H$ 4.88, H-2), in the HMBC experiment of **1**, indicating that the isobutyroxy group should be attached at C-2. Thus, the remaining hydroxy group had to be positioned at C-8, an oxygenated quaternary carbon resonating at $\delta_C$ 82.2 (s). These data, together with the HMBC correlations between H-9/C-17; H-17/C-8, -18, -19; and $H_3$-18/C-8, -17, -19 unambiguously established the molecular framework of **1**.

In previous studies, all the naturally occurring briarane-type natural products have the C-15 methyl group which is *trans* to H-10.[16,17)] The relative stereochemistry of **1** was deduced mainly with a NOESY experiment and by vicinal $^1$H–$^1$H coupling constant analysis. In the NOESY experiment of **1** (Fig. 2), H-10 gives NOE correlations to H-2 and H-9, but not to $H_3$-15, indicating that H-2, H-9, and H-10 are located on the same face of the molecule and assigned as $\alpha$-protons, since C-15 methyl group is the $\beta$-substituent at C-1. $H_3$-15 was found to exhibit NOE correlations with H-13 and H-14, showing that the acetoxy groups at C-13 and C-14 were $\alpha$-oriented, respectively. A proton of C-3 methylene ($\delta_H$ 2.83, H-3$\beta$) showed NOE responses with $H_3$-15 and H-7 which reflected the $\beta$-orientation of H-7. The *Z*-configuration of C-5/6 double bond was elucidated by an NOE response between C-6 olefin proton and C-16 vinyl methyl. H-4 exhibited an NOE interaction with $H_3$-16, and the coupling constants 14.0 and 5.6 Hz were found between H-4 and C-3 methylene protons, indicating that the isovaleroxy group attaching at C-4 was $\beta$-oriented. Furthermore, H-9 was found to exhibit NOE responses with H-17 and $H_3$-18, and H-17 correlated with H-7. From consideration of molecular models, H-17 was found to be reasonably close to H-7 and H-9, thus it was concluded that H-17 should be placed on the $\beta$ face in $\gamma$-lactone moiety. Due to the signal for hydroxy proton not being observed in the $^1$H-NMR spectrum of **1**, the stereochemistry of C-8 hydroxy group cannot be determined by NOESY experiment.

In the configuration of methylenecyclohexane ring of **1**, a proton of C-20 methylene ($\delta_H$ 5.26, H-20a) was found to exhibit NOE correlations with H-9 and H-10, but not with $H_3$-15; $H_3$-15 showed an NOE correlation with a proton of C-12 methylene ($\delta_H$ 2.58, H-12$\beta$); and H-12$\alpha$ ($\delta_H$ 2.31) correlated with H-20b ($\delta_H$ 5.11), but not with H-10, indicating that the methylenecyclohexane ring of **1** should be presented as a boat rather than a chair conformation for **1**. Based on the above observations, the structure of **1** could be similar to that of a known 8-hydroxybriarane derivative, junceol A (**6**), which was proven to possess a methylenecyclohexane moiety with boat conformation.[12)] By comparing the $^{13}$C-NMR data of C-8 in **1** ($\delta_C$ 82.2, s) with those of **6** ($\delta_C$ 82.9, s), it was revealed that the C-8 hydroxy group in **1** must be $\alpha$-oriented and all the chiral centers of **1** are assigned as 1*S**, 2*S**, 4*R**, 5*Z*, 7*S**, 8*R**, 9*S**, 10*S**, 13*S**, 14*R**, and 17*R**.

Junceol E (**2**) had the molecular formula $C_{30}H_{42}O_{11}$ (HR-ESI-MS, *m/z* 601.2628, Calcd for $C_{30}H_{42}O_{11}Na$, 601.2625), and its IR absorptions at 3448, 1777, and 1734 cm$^{-1}$, typical for hydroxy, $\gamma$-lactone, and ester functionalities. Both the $^{13}$C- and $^1$H-NMR data of **2** (Tables 1, 2) indicated three acetates ($\delta_C$ 21.8, 21.2, 21.2, 3×q; $\delta_H$ 2.25, 2.05, 1.89, each 3H×s; $\delta_C$ 169.4, 170.2, 170.1, 3×s) and an isobutyrate ester ($\delta_H$ 2.49, 1H, septet, $J$=6.8 Hz; 1.15, 3H, d, $J$=6.8 Hz; 1.12, 3H, d, $J$=6.8 Hz; $\delta_C$ 34.0, d; 18.9, 18.5, 2×q; 176.2, s). Besides the above ester carbonyls, the carbon signal at $\delta_C$ 175.9 (s, C-19) was assigned to a $\gamma$-lactone ring along with an oxymethine ($\delta_H$ 5.59, 1H, d, $J$=10.0 Hz; $\delta$ 76.9, d, CH-7). The two proton singlets at $\delta_H$ 5.02 and 4.87 correlating to the methylene signal at $\delta_C$ 112.9 (t) were ascribed to an exocyclic carbon–carbon double bond. The tertiary methyl sin-

Table 2. $^1$H-NMR Data for Diterpenoids **1**—**5**[a)]

| Position | **1** | **2** | **3** | **4** | **5** |
|---|---|---|---|---|---|
| 2 | 4.88 d (6.0)[b)] | 4.80 dd (5.6, 1.6) | 4.81 dd (5.6, 1.6) | 4.92 br s | 4.92 br s |
| 3$\alpha$ | 1.93 m | 1.94 m | 1.81 m | 1.78 m | 1.79 m |
| $\beta$ | 2.83 dd (14.0, 14.0) | 2.78 ddd (15.2, 12.8, 1.6) | 2.78 ddd (15.2, 12.8, 1.6) | 2.37 m | 2.36 m |
| 4$\alpha$ | 5.12 ddd (14.0, 5.6, 1.2) | 5.16 ddd (12.8, 6.0, 1.2) | 5.19 ddd (12.8, 6.0, 1.2) | 2.07 m | 2.05 m |
| $\beta$ | | | | 2.61 m | 2.60 m |
| 6 | 5.68 ddd (10.0, 1.2, 1.2) | 5.80 ddd (10.0, 1.2, 1.2) | 5.81 ddd (10.4, 1.2, 1.2) | 5.53 dd (5.2, 1.2) | 5.52 ddd (9.2, 1.2, 1.2) |
| 7 | 5.57 d (10.0) | 5.59 d (10.0) | 5.59 d (10.4) | 5.29 d (5.2) | 5.30 d (9.2) |
| 9 | 5.54 d (3.6) | 5.25 d (5.2) | 5.24 d (5.6) | 5.54 d (4.4) | 5.56 d (6.0) |
| 10 | 3.32 d (3.6) | 3.35 d (5.2) | 3.36 d (5.6) | 3.44 d (4.4) | 3.45 d (6.0) |
| 12$\alpha$ | 2.31 dd (13.6, 6.0) | 2.15 m | 2.15 m | 2.68 dd (13.2, 7.2) | 2.68 dd (13.2, 7.2) |
| $\beta$ | 2.58 dd (13.6, 6.4) | 2.27 m | 2.25 m | 2.34 m | 2.36 m |
| 13$\alpha$ | | 1.79 m | 1.82 m | | |
| $\beta$ | 5.02 dd (6.4, 6.0)[c),d)] | 2.02 m | 2.04 m | 5.03 ddd (7.2, 7.2, 3.6) | 5.03 ddd (7.2, 7.2, 2.8) |
| 14 | 5.02 s[c)] | 4.62 dd (5.2, 1.2) | 4.60 d (4.4) | 4.99 d (3.6) | 5.00 d (2.8) |
| 15 | 1.12 s | 1.11 s | 1.11 s | 1.16 s | 1.16 s |
| 16 | 2.21 d (1.2) | 2.24 d (1.2) | 2.24 d (1.2) | 2.03 d (1.2) | 2.02 d (1.2) |
| 17 | 2.55 q (7.2) | 2.48 q (6.8) | 2.47 q (7.2) | 2.50 q (7.2) | 2.52 q (6.8) |
| 18 | 1.13 d (7.2) | 1.12 d (6.8) | 1.13 d (7.2) | 1.15 d (7.2) | 1.15 d (6.8) |
| 20a | 5.26 s | 5.02 s | 5.02 s | 5.20 s | 5.20 s |
| b | 5.11 s | 4.87 s | 4.87 s | 5.11 s | 5.11 s |
| OH-8 | n.o.[e)] | n.o. | n.o. | 2.13 s | 2.18 s |
| Acetyl groups | 2.21 s | 2.25 s | 2.25 s | 2.20 s | 2.20 s |
| | 1.98 s | 2.05 s | 2.05 s | 1.97 s | 1.97 s |
| | 1.94 s | 1.89 s | 1.89 s | 1.95 s | 1.95 s |
| Isobutyryl groups | 1.17 d (7.2) | 1.15 d (6.8) | | | 1.13 d (6.8) |
| | 1.16 d (7.2) | 1.12 d (6.8) | | | 1.10 d (6.8) |
| | 2.50 septet (7.2) | 2.49 septet (6.8) | | | 2.46 septet (6.8) |
| Isovaleryl group | 0.95 d (6.8) | | | | |
| | 0.95 d (6.8) | | | | |
| | 2.07 m | | | | |
| | 2.19 d (7.6) | | | | |
| 2-Methylbutanoates | | | 0.94 t (7.2) | 0.91 t (7.2) | |
| | | | 1.42 m (1H) | 1.40 m (1H) | |
| | | | 1.67 m (1H) | 1.66 m (1H) | |
| | | | 1.14 d (6.4) | 1.12 d (7.2) | |
| | | | 2.28 m | 2.25 m | |

*a*) Spectra measured at 400 MHz in $CDCl_3$ at 25 °C. *b*) *J* values (in Hz) in parentheses. *c*) Signals overlapping. *d*) The coupling constants for H-13 in **1** were deduced from the coupling patterns and correlations observed between H-13 and C-12 methylene protons. *e*) n.o.=not observed.

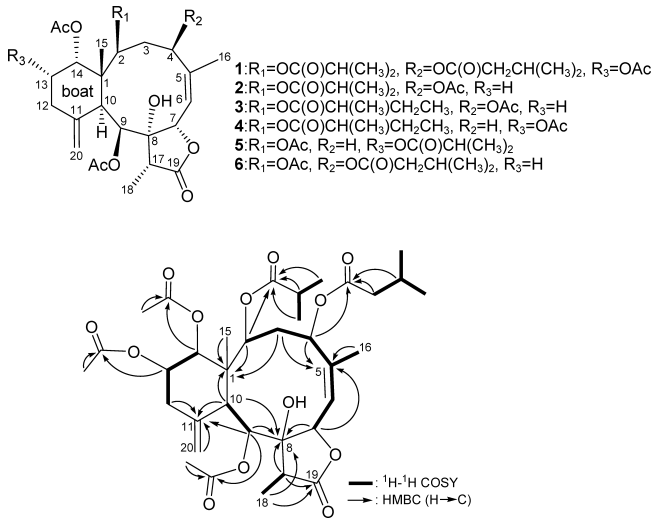


Fig. 1. The $^1$H–$^1$H COSY and Selective HMBC Correlations (Protons and Quaternary Carbons) of **1**

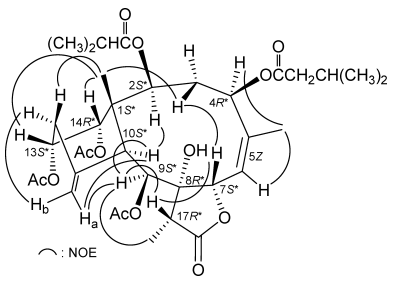


Fig. 2. Selective NOESY Correlations of **1**

glet at $\delta_H$ 1.11 (3H, s) was assigned to $H_3$-15 while the secondary methyl doublet at $\delta_H$ 1.12 (3H, d, $J$=6.8 Hz) was assigned to $H_3$-18.

The carbon skeleton from C-1 to C-14 of **2** was established by 2D-NMR studies and mainly by HMBC experiment (Table 3). The exocyclic double bond attached at C-11 was elucidated by the HMBC correlations between $H_2$-20/C-10, -11, -12; H-10/C-11, -20; and $H_2$-12/C-11, -20. Me-15 and Me-16 groups attached at C-1 and C-5 were deduced from the HMBC correlations between $H_3$-15/C-1, -2, -10, -14; H-2/C-15; H-10/C-15; H-14/C-15; and $H_3$-16/C-4, -5, -6; H-4/C-16; H-6/C-16, respectively. Furthermore, five oxymethines observed at $\delta_H$ 5.59, 5.25, 5.16, 4.80, 4.62 were $^1J$-correlated to the carbons $\delta_C$ 76.9, 71.1, 72.8, 71.4, 73.9, and assigned to C-7, -9, -4, -2, -14, respectively. The isobutyrate ester positioned at C-2 was confirmed from the connectivity

Table 3. The HMBC Correlations (H→C) for Diterpenoids **1**—**5**

| Position | 1 | 2 | 3 | 4 | 5 |
|---|---|---|---|---|---|
| H-2 | C-1, -4, -15, isobutryate carbonyl | C-1, -3, -4, -10, -15, isobutyrate carbonyl | C-1, -4, -10, -15, 2-methylbutanoate carbonyl | 2-Methylbutanoate carbonyl | Acetate carbonyl |
| H-3 | C-1, -4, -5 | C-1, -2, -4, -5 | C-1, -2, -4, -5 | n.o. | n.o. |
| H-4 | C-5, -6, -16, isovalerate carbonyl | C-3, -5, -6, -16, acetate carbonyl | C-5, -6, -16, acetate carbonyl | C-5 | C-5 |
| H-6 | C-4, -16 | C-4, -16 | C-4, -16 | C-8, -16 | C-4, -16 |
| H-7 | C-5, -6, -8 | C-5, -6, -8 | C-5, -6, -8 | C-5 | C-5, -6 |
| H-9 | C-7, -8, -11, -17, acetate carbonyl | C-7, -8, -10, -11, acetate carbonyl | C-7, -8, -10, -11, acetate carbonyl | C-7, -8, -10, -11, acetate carbonyl | C-1, -7, -8, -10, -11, -17, acetate carbonyl |
| H-10 | C-1, -8, -11, -15 | C-1, -2, -8, -9, -11, -12, -15, -20 | C-1, -2, -8, -9, -11, -12, -14, -15, -20 | C-1, -8, -9, -11, -20 | C-1, -2, -8, -9, -11, -15, -20 |
| H-12 | C-10, -11, -13, -20 | C-10, -11, -13, -20 | C-10, -11, -20 | C-10, -11, -13, -14, -20 | C-10, -11, -13, -14, -20 |
| H-13 | C-12, acetate carbonyl | C-1, -12, -14 | n.o. | Acetate carbonyl | C-12 |
| H-14 | C-10, -12, -13, acetate carbonyl | C-1, -2, -10, -12, -13, -15, acetate carbonyl | C-1, -10, -12, -15, acetate carbonyl | C-12, acetate carbonyl | C-10, acetate carbonyl |
| H-15 | C-1, -2, -10, -14 | C-1, -2, -10, -14 | C-1, -2, -10, -14 | C-1, -2, -10, -14 | C-1, -2, -10, -14 |
| H-16 | C-4, -5, -6 | C-4, -5, -6 | C-4, -5, -6 | C-4, -5, -6 | C-4, -5, -6 |
| H-17 | C-8, -18, -19 | C-8, -18, -19 | C-8, -18, -19 | C-8, -18, -19 | C-18, -19 |
| H-18 | C-8, -17, -19 | C-8, -17, -19 | C-8, -17, -19 | C-8, -17, -19 | C-8, -17, -19 |
| H-20 | C-10, -11, -12 | C-10, -11, -12 | C-10, -11, -12 | C-10, -11, -12 | C-10, -12 |
| OH-8 | n.o.[a)] | n.o. | n.o. | C-7, -8, -9, -17 | C-7, -8, -9, -17 |

*a*) n.o.=not observed.

between H-2 ($\delta_H$ 4.80) and isobutyrate carbonyl ($\delta_C$ 176.2). At the same time, H-4, H-9, and H-14, showed HMBC correlations with acetate carbonyls at $\delta_C$ 170.2, 169.4, and 170.1, confirming the acetoxy groups were attached at these three positions and the remaining hydroxy group had to be positioned at C-8 ($\delta_C$ 83.0, s).

The relative stereochemistry of **2** was elucidated from the NOE interactions observed in a NOESY experiment. Due to the $\alpha$-orientation of H-10, the ring junction C-15 methyl group should be $\beta$-oriented as no NOE correlation was observed between H-10 and $H_3$-15. The correlation between $H_3$-15 and H-14 indicated the $\beta$-orientation of H-14. In addition, the NOE correlations between H-10 and H-2, H-9 suggested the $\alpha$-orientation of these protons. A proton of C-3 methylene ($\delta_H$ 2.78, H-3$\beta$) showed NOE correlations with $H_3$-15 and H-7, suggesting H-7 is on the $\beta$ face. Moreover, H-9 showed NOE responses with H-17 and $H_3$-18, and H-17 exhibited NOE response with H-7, indicating that H-17 was $\beta$-oriented in the $\gamma$-lactone ring by molecular modeling. The boat conformation for the methylenecyclohexane unit and the 8$\beta$-hydroxy group of **2** were elucidated by the NOE correlations and by comparing the $^1$H- and $^{13}$C-NMR data of **2** with those of **1** and junceol A (**6**).[12)] Based on the above findings, the configurations of all chiral centers of **2** were assigned as 1*R**, 2*S**, 4*R**, 5*Z*, 7*S**, 8*R**, 9*S**, 10*S**, 14*S**, and 17*R**.

Junceol F (**3**) had the molecular formula $C_{31}H_{44}O_{11}$ as determined by HR-ESI-MS (*m*/*z* 615.2785, Calcd for $C_{31}H_{44}O_{11}Na$, 615.2781). The IR absorptions of **3** showed the presence of hydroxy (3458 cm$^{-1}$), $\gamma$-lactone (1777 cm$^{-1}$), and ester carbonyl (1731 cm$^{-1}$) groups. Carbonyl resonances in the $^{13}$C-NMR spectrum of **3** at $\delta_C$ 176.4 (s), 175.9 (s), 170.2 (s), 170.1 (s), and 169.4 (s), confirmed the presence of a $\gamma$-lactone and four esters (Table 1). In the $^1$H-NMR spectrum of **3** (Table 2), three acetyl methyls ($\delta_H$ 2.25, 2.05, 1.89, each 3H×s) and a 2-methylbutanoxy group [–OC(O)CH($CH_3$)$CH_2CH_3$, $\delta_H$ 2.28, 1H, m; 1.14, 3H, d, *J*=6.4 Hz; 1.42, 1H, m; 1.67, 1H, m; 0.94, 3H, t, *J*=7.2 Hz] were observed. It was found that the NMR data of **3** were very similar to those of **2**, except that the signals corresponding to an isobutyroxy group in **2** were replaced by a 2-methylbutanoxy group in **3**. In the HMBC spectrum of **3** (Table 3), the 2-methylbutanoxy group positioned at C-2 was confirmed by the connectivity between H-2 ($\delta_H$ 4.81) with the carbonyl carbon ($\delta_C$ 175.9) of 2-methylbutanoxy group. Furthermore, the HMBC correlations also revealed that the acetoxy groups were attached to C-4, C-9, and C-14. The other various HMBC correlations observed fully supported the location of functional groups, and hence junceol F (**3**) was assigned as structure **3** with the same relative stereochemistry as in **2**, and the chiral centers of **3** were assigned as 1*R**, 2*S**, 4*R**, 5*Z*, 7*S**, 8*R**, 9*S**, 10*S**, 14*S**, and 17*R**.

Junceol G (**4**) had the same molecular formula as that of **3**, $C_{31}H_{44}O_{11}$, as determined by HR-ESI-MS (*m*/*z* 615.2783, Calcd for $C_{31}H_{44}O_{11}Na$, 615.2781), indicating that compounds **3** and **4** are isomers, and these two briaranes were found to possess the same substituents (a 2-methylbutanoxy, a hydroxy, and three acetoxy groups) by NMR data analysis (Tables 1, 2). In the HMBC experiment of **4** (Table 3), the correlations revealed connectivity between H-2 ($\delta_H$ 4.92) and the carbonyl carbon ($\delta_C$ 175.4) of 2-methylbutanoxy unit and demonstrated that the location of 2-methylbutanoxy group was at C-2. The other three acetoxy groups were positioned at C-9, C-13, and C-14, as indicated by analysis of key HMBC correlations. The signal for hydroxy proton was observed in the $^1$H-NMR spectrum of **4** ($\delta_H$ 2.13, 1H, s) and the hydroxy group had to be positioned at C-8, as was supported by the HMBC correlations observed between OH-8/C-7, -8, -9, -17.

The relative stereochemistry of **4** was deduced by analysis of NOE correlations. In the NOESY spectrum of **4**, the correlations between $H_3$-15 with H-13, H-14, and a proton of C-3 methylene ($\delta_H$ 2.37, H-3$\beta$), suggested that these protons were all in $\beta$-orientation. Meanwhile, correlations of H-10 with H-2, H-9, and OH-8 indicated the $\alpha$ orientation of these

protons. H-7 showed NOE responses with H-3$\beta$ and H-17. From consideration of molecular models, H-7 was found to be reasonably close to H-3$\beta$ and H-17, while these three protons were $\beta$-oriented. Like those of briaranes **1**—**3**, the methylenecyclohexane ring of **4** existed in boat form by the following NOE correlations: a proton of C-20 methylene ($\delta_H$ 5.20, H-20a) with H-9, H-10, and OH-8; $H_3$-15 with a proton of C-12 methylene ($\delta_H$ 2.34, H-12$\beta$); and H-12$\alpha$ ($\delta_H$ 2.68) correlated with H-20b ($\delta_H$ 5.11), but not with H-10. Based on the above findings, the chiral centers of **4** are assigned as 1*S**, 2*S**, 5*Z*, 7*S**, 8*S**, 9*S**, 10*S**, 13*S**, 14*R**, and 17*R**. To the best of our knowledge, junceols F (**3**) and G (**4**) are the first briarane derivatives possessing 2-methylbutanoxy groups in structures.

Junceol H (**5**) was isolated as a white powder and had the molecular formula $C_{30}H_{42}O_{11}$ on the basis of HR-ESI-MS (*m/z* 601.2622, Calcd for $C_{30}H_{42}O_{11}Na$, 601.2625). By detailed analysis, the spectral data of **5** were very close to those of junceol G (**4**), except that the acyloxy groups at C-2 and C-13 of **4** were replaced by acetoxy and isobutyroxy groups, respectively. The relative configurations of chiral centers of **5** were assigned as those of **4** by NOE correlations.

The cytotoxicity of new briaranes **1**—**5** toward CCRF-CEM (human T-cell acute lymphoblastic leukemia) and DLD-1 (human colon adenocarcinoma) tumor cells were studied and the results are shown in Table 4. These data showed that junceols D (**1**) and F—H (**3**—**5**) exhibited significant and modest cytotoxicity against CCRF-CEM cells, respectively, and junceols D (**1**) and H (**5**) showed modest cytotoxicity toward DLD-1 cells. In addition, junceols E—H (**2**—**5**) were found to possess weak inhibitory effects on superoxide anion generation by human neutrophils at a concentration of 10 $\mu$g/ml (Table 5). Although junceol D (**1**) was not active in inhibition of superoxide anion generation, it was the only compound to show significant cytotoxicity in this study. The structure–activity relationships among these briarane derivatives will be studied if enough material is obtained in the future.

Table 4. Cytotoxic Data of Briaranes **1**—**5**

| Compound | Cell lines $LD_{50}$ ($\mu$g/ml)[a] | |
|---|---|---|
| | CCRF-CEM | DLD-1 |
| **1** | 1.3 | 10.0 |
| **2** | >40.0 | >40.0 |
| **3** | 4.9 | >40.0 |
| **4** | 4.4 | >40.0 |
| **5** | 7.2 | 17.0 |

*a*) For significant activity of pure compounds, the values of $LD_{50} \leq 4.0$ $\mu$g/ml is required. See Geran *et al.*[26]

Table 5. Inhibitory Effects of Briaranes **1**—**5** on Superoxide Anion Generation by Human Neutrophils in Response to fMet-Leu-Phe/cytochalastin B

| Compound | Superoxide generation inhibition[a] (%) |
|---|---|
| **1** | 6.0±0.3 |
| **2** | 25.6±5.5 |
| **3** | 23.5±6.0 |
| **4** | 17.3±1.2 |
| **5** | 19.4±7.2 |

*a*) Percentage of inhibition (Inh %) at 10 $\mu$g/ml concentration. Results are presented as means±S.E.M. ($n$=3).

## Experimental

**General** Melting points were measured on a Fargo apparatus and were uncorrected. Optical rotation values were measured with a Jasco P-1010 digital polarimeter. IR spectra were obtained on a Varian DIGLAB FTS 1000 FT-IR spectrophotometer. NMR spectra were recorded on a Varian MERCURY PLUS 400 FT-NMR at 400 MHz for $^1$H-NMR and 100 MHz for $^{13}$C-NMR, respectively. Proton chemical shifts were referenced to the residual $CHCl_3$ signal ($\delta_C$ 7.26 ppm). $^{13}$C-NMR spectra were referenced to the center peaks of $CDCl_3$ at $\delta$ 77.0 ppm. ESI-MS and HR-ESI-MS data were recorded on a Bruker APEX II mass spectrometer. Gravity column chromatography was performed on silica gel (230—400 mesh, Merck, Darmstadt, Germany). TLC was carried out on precoated Kieselgel 60 $F_{254}$ (0.2 mm, Merck) and spots were visualized by spraying with 10% $H_2SO_4$ solution followed by heating. HPLC was performed using a system comprised of a Hitachi L-7100 pump, a Hitachi photodiode array detector L-7455, and a Rheodyne 7725 injection port. A normal phase semi-preparative column (Hibar 250—25 mm, LiChrospher Si 60, 5 $\mu$m) and a semi-preparative reverse phase column (Hibar 250—10 mm, Purospher Star RP-18e, 5 $\mu$m) were used for HPLC, respectively.

**Animal Material** Specimens of the gorgonian coral *J. juncea* were collected by hand using scuba gear off the southern Taiwan coast in September 2006. This organism was identified by comparison with previous descriptions.[20—22] The living reference specimens are being maintained in the authors' marine organisms cultivating tank and a voucher specimen has been deposited in the National Museum of Marine Biology & Aquarium (NMMBA), Taiwan.

**Extraction and Isolation** The freeze-dried and minced material of *J. juncea* (wet weight 369 g, dry weight 108 g) was extracted with a mixture of MeOH and $CH_2Cl_2$ (1 : 1) at room temperature. The residue was partitioned between EtOAc and $H_2O$. The EtOAc layer (2.57 g) was separated on silica gel and eluted using hexane/EtOAc (stepwise, 10 : 1–pure EtOAc) to yield 22 fractions (Fr. 1—22). Fr. 16 was separated on silica gel and eluted using hexane/EtOAc (stepwise, 5 : 1–pure EtOAc) to yield Fr. 16A—I. Fr. 16C was repurified by normal phase HPLC, using the mixtures of $CH_2Cl_2$ and EtOAc to afford 8 fractions, one of which (Fr. 16C-8) was further separated by reverse phase HPLC using the mixtures of MeOH/$H_2O$ (7 : 3) to afford **1** (0.7 mg). Fr. 17 and 18 were combined and separated by normal phase HPLC, using the mixtures $CH_2Cl_2$ and acetone to yield 13 fractions Fr. 17A—M and Fr. 17G—I were further repurified by reverse phase HPLC, respectively, using the mixtures of MeOH and $H_2O$ (7 : 3) to afford briaranes **2** (1.7 mg), **3** (0.6 mg) (from Fr. 17G), **4** (0.6 mg, from Fr. 17H), and **5** (0.7 mg, from Fr. 17I).

Junceol D (**1**): White powder; mp 105—108 °C; $[\alpha]_D^{23}$ +29° ($c$=0.04, $CHCl_3$); IR (neat) $\nu_{max}$ 3453, 1775, 1744 cm$^{-1}$; $^1$H-NMR ($CDCl_3$, 400 MHz) and $^{13}$C-NMR ($CDCl_3$, 100 MHz) data, see Tables 1 and 2; ESI-MS *m/z* 701 $(M+Na)^+$; HR-ESI-MS *m/z* 701.3154 (Calcd for $C_{35}H_{50}O_{13}Na$, 701.3149).

Junceol E (**2**): White powder; mp 111—113 °C; $[\alpha]_D^{23}$ −6° ($c$=0.09, $CHCl_3$); IR (neat) $\nu_{max}$ 3448, 1777, 1734 cm$^{-1}$; $^1$H-NMR ($CDCl_3$, 400 MHz) and $^{13}$C-NMR ($CDCl_3$, 100 MHz) data, see Tables 1 and 2; ESI-MS *m/z* 601 $(M+Na)^+$; HR-ESI-MS *m/z* 601.2628 (Calcd for $C_{30}H_{42}O_{11}Na$, 601.2625).

Junceol F (**3**): White powder; mp 116—118 °C; $[\alpha]_D^{23}$ −9° ($c$=0.03, $CHCl_3$); IR (neat) $\nu_{max}$ 3458, 1777, 1731 cm$^{-1}$; $^1$H-NMR ($CDCl_3$, 400 MHz) and $^{13}$C-NMR ($CDCl_3$, 100 MHz) data, see Tables 1 and 2; ESI-MS *m/z* 615 $(M+Na)^+$; HR-ESI-MS *m/z* 615.2785 (Calcd for $C_{31}H_{44}O_{11}Na$, 615.2781).

Junceol G (**4**): White powder; mp 240—243 °C (decomposed); $[\alpha]_D^{23}$ −21° ($c$=0.02, $CHCl_3$); IR (neat) $\nu_{max}$ 3451, 1772, 1731 cm$^{-1}$; $^1$H-NMR ($CDCl_3$, 400 MHz) and $^{13}$C-NMR ($CDCl_3$, 100 MHz) data, see Tables 1 and 2; ESI-MS *m/z* 615 $(M+Na)^+$; HR-ESI-MS *m/z* 615.2783 (Calcd for $C_{31}H_{44}O_{11}Na$, 615.2781).

Junceol H (**5**): White powder; mp 272—275 °C (decomposed); $[\alpha]_D^{23}$ −33° ($c$=0.03, $CHCl_3$); IR (neat) $\nu_{max}$ 3443, 1771, 1732 cm$^{-1}$; $^1$H-NMR ($CDCl_3$, 400 MHz) and $^{13}$C-NMR ($CDCl_3$, 100 MHz) data, see Tables 1 and 2; ESI-MS *m/z* 601 $(M+Na)^+$; HR-ESI-MS *m/z* 601.2622 (Calcd for $C_{30}H_{42}O_{11}Na$, 601.2625).

**Cytotoxicity Testing** The cytotoxicity of tested compounds **1**—**5** was assayed with a modification of MTT [3-(4,5-dimethylthiazol-2-yl)-2,5-diphenyltetrazolium bromide] colorimetric method. Cytotoxicity assays were carried out according to the procedures described previously.[23]

**Human Neutrophil Superoxide Anion Generation** Human neutrophils were obtained by means of dextran sedimentation and Ficoll centrifugation. Superoxide generation was carried out according to the procedures de-

scribed previously.[24,25] Briefly, superoxide anion production was assayed by monitoring the superoxide dismutase-inhibitable reduction of ferricytochrome *c*.

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