# Structure and stereochemistry of brianolide, a new antiinflammatory diterpenoid from the Okinawan gorgonian *Briareum* sp.

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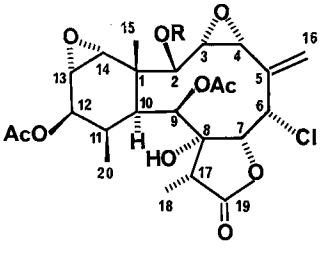
*Summary.* Brianolide (**1**), a new antiinflammatory diterpenoid of the briarein class, possessing a $\beta$ substituent at C-12 (R), has been isolated from the Okinawan gorgonian *Briareum* sp. Its structure has been established from spectral data in conjunction with a single crystal X-ray analysis.

*Key words.* Gorgonian; brianolide; diterpene; *Briareum* sp.; antiinflammatory activity.

Previous studies on gorgonian corals of the genus *Briareum* resulted in the isolation of a series of novel secondary metabolites, represented by briareins[1], astesinins[2] and gorgosterol[3]. Diterpenoids of the briarein class, which are among the more complex marine natural products[4], have also been isolated from other coelenterates like a true soft coral, a sea pansy, and sea pens[5,6]. As part of our survey of bioactive compounds from Okinawan marine organisms[7], pharmacologically active metabolites from some octocorals were investigated. In this paper we report the isolation and structure of a new chlorinated diterpenoid, named brianolide (**1**), with antiinflammatory activity from the Okinawan gorgonian *Briareum* sp.

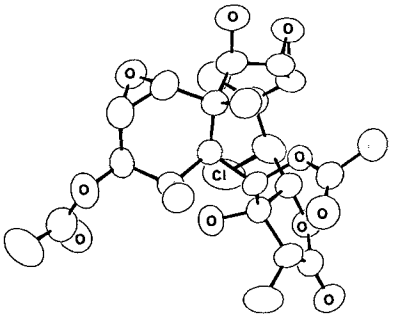
The ethyl acetate soluble materials from methanol extract of *Briareum* sp. collected at Ie Island, Okinawa, in 1986 by SCUBA diving, were chromatographed on a silica gel column ($MeOH/CHCl_3$, 6:94) yielding brianolide (**1**)[8] (0.06% wet wt) as colorless plates (from $MeOH/H_2O$, 2:1): mp 225–226 °C; $[\alpha]_D^{23}$ −15° (*c* 0.1, MeOH). Brianolide (**1**) showed a quasimolecular ion peak at *m/z* 515.1683 in the HRFABMS spectrum, corresponding to the molecular composition of $C_{24}H_{31}O_{10}Cl$ [$C_{24}H_{32}O_{10}Cl$ ($M^+ + H$) requires 515.1684, $\Delta$ −0.1 mmu]. The IR spectrum of **1** showed that it contained a




**1** R = H

**2** R = Ac

Perspective view of brianolide (**1**)




$\gamma$-lactone (1780 $cm^{-1}$), acetyl esters (1740 and 1720 $cm^{-1}$), and hydroxy groups (3490 $cm^{-1}$).

Acetylation of **1** gave the corresponding monoacetate **2**[9]: mp 172–174 °C; $[\alpha]_D^{25}$ −29° (*c* 0.1, MeOH). The $^1H$ and $^{13}C$ NMR data of **2** (see table) implied the presence of two epoxides and three acetate units, and the remaining 20 carbons suggested a diterpene structure. The $^1H$-$^1H$ COSY spectra showed the following cross peaks; H-2/H-3, H-3/H-4, H-4/H-16, H-6/H-7, H-6/H-16, H-6/H'-16, H-9/H-10, H-10/H-11, H-11/H-12, H-11/H-20, H-12/H-13, H-13/H-14, and H-17/H-18. These correlations revealed that **2** possessed a similar partial structure to those of briarein A[2] or briantheins[10]. The most notable difference between brianthein Z[10] and **2** was that H-12 in the former showed a clear coupling to epoxy proton H-13 (J = 5.8 Hz), while these two protons did not couple with each other in **2**. Furthermore, the olefin ($\delta$ 131.1d and 127.9d) at C-3 and C-4 of briathein Z appeared to have been oxidized to an epoxide ($\delta$ 59.9d and 56.9d) in **2**. Therefore the structure of **2** was assigned as the 3,4-epoxy form of brianthein Z with different relative stereochemistry at C-12[10]. A secondary hydroxy group in **1** was assigned to be located at C-2, since acetylation of **1** resulted in a marked lower-field shift of H-2 ($\delta$ 4.95 (**1**) → $\delta$ 5.77 (**2**) in [$^2H_5$]-pyridine).

The structure, including the absolute stereochemistry of **1**, was unambiguously confirmed by a single crystal X-ray diffraction analysis. It is noted that **1** possesses a $\beta$

$^1H$ and $^{13}C$ NMR data for brianolide (**1**) and its acetate (**2**)[a]

| | **1** | | | **2**[b] | | |
|---|---|---|---|---|---|---|
| | $^1H$[c] | $J_{HH}$ | $^{13}C$[d] | $^1H$ | $J_{HH}$ | $^{13}C$ |
| 1 | | | 38.2 s[e] | | | 38.2 s[e] |
| 2 | 4.26 d | 8.9 | 72.0 d | 5.13 d | 9.3 | 74.9 d |
| HO-2 | 4.95 brs | | | | | |
| 3 | 3.94 dd | 8.9, 4.0 | 60.0 d | 3.40 dd | 9.3, 3.8 | 59.9 d |
| 4 | 4.23 d | 4.0 | 56.7 d | 3.64 d | 3.8 | 56.9 d |
| 5 | | | 137.7s | | | 133.7 s |
| 6 | 5.80 m | | 62.2 d | 5.38 ddd | 3.6, 2.5, 2.5 | 61.0 d |
| 7 | 5.67 d | 3.8 | 77.6 d | 5.05 d | 3.6 | 76.5 d |
| 8 | | | 82.6 s | | | 83.8 s |
| HO-8 | 3.59 s | | | 3.56 s | | |
| 9 | 5.76 d | 8.5 | 68.2 d | 5.34 d | 8.5 | 69.1 d |
| 10 | 2.39 dd | 8.5, 2.1 | 35.6 d | 1.74 dd | 8.5, 2.3 | 37.2 d |
| 11 | 3.04 m | | 36.2 d | 2.27 ddd | 7.1, 4.7, 2.3 | 35.7 d |
| 12 | 5.10 d | 4.8 | 71.8 d | 4.60 d | 4.7 | 71.7 d |
| 13 | 3.72 d | 3.5 | 56.4 d | 3.12 d | 3.3 | 56.5 d |
| 14 | 3.40 d | 3.5 | 62.2 d | 2.89 d | 3.3 | 61.3 d |
| 15 | 1.49 s | | 15.5 q | 1.22 s | | 16.3 q |
| $CH_2$ = 16 | 5.52 d | 2.2 | 115.4 t | 6.02 d | 2.5 | 120.3 t |
| | 5.96 d | 2.3 | | 6.11 d | 2.5 | |
| 17 | 3.15 q | 7.1 | 43.8 d | 2.46 q | 7.1 | 45.2 d |
| 18 | 1.37 d | 7.1 | 5.6 q | 1.16 d | 7.1 | 6.0 q |
| 19 | | | 174.5 s | | | 174.2 s |
| 20 | 1.28 d | 7.1 | 9.5 q | 1.04 d | 7.1 | 9.5 q |
| | 2.27 s | | 20.7 q | 2.04 s | | 20.8 q |
| | 2.31 s | | 21.8 q | 2.14 s | | 20.7 q |
| | | | | 2.22 s | | 21.7 q |
| Ac | | | 169.7 s | | | 168.8 s |
| Ac | | | 170.6 s | | | 169.7 s |
| Ac | | | | | | 169.8 s |

[a] recorded on a Bruker AM-500 (500 MHz for $^1H$ NMR) and JEOL FX-90Q (22.5 MHz for $^{13}C$ NMR) spectometers; [b] in $CDCl_3$; [c] in [$^2H_5$]pyridine; [d] in [$^2H_6$]dimethyl sulphoxide; [e] assigned by DEPT and $^1H$-$^{13}C$ COSY data.

acetoxy substituent at C-12 (R), while almost all briarein-class diterpenoids reported so far have an α-substituted pattern if there is any substituent at this carbon[5, 6]. This suggests that the unambiguous assignment of the C-12 stereochemistry of briarein class diterpenoids should be made by careful interpretation of the NMR data[11] or by X-ray analysis.

Brianolide **1** exhibits modest antiinflammatory activity. Potencies of 37% reduction of edema were observed at concentrations in the range of 25 μg (mouse ear assay).

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8 CD (MeOH) $\Delta\varepsilon_{220}$ −1.52; IR (KBr) $\nu_{max}$ 3490, 2980, 2950, 2850, 1780, 1760, 1740, 1720, 1480, 1250, 1230, 1100, 1070, 1020, 930, and 740 $cm^{-1}$.
9 IR (KBr) $\nu_{max}$ 3500, 2980, 2925, 2850, 1780, 1740, 1370, 1220, 1100, 1020, and 750 $cm^{-1}$; FABMS (glycerol as matrix) *m/z* 557 ($M^+$ + H) (Found: $M^+$ + H, 557.1797. Calc. for $C_{26}H_{34}O_{11}Cl$: M + H, 557.1789).
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11 The relative stereochemistry at C-12 might be assigned by the vicinal coupling values between H-12 and H-13 to be α ($J = 4 \sim 6$ Hz) or β ($J = \sim 0$ Hz).

0014-4754/91/050501-02$1.50 + 0.20/0