HETEROCYCLES, Vol. 85, No. 1, 2012, pp. 135 - 145. © 2012 The Japan Institute of Heterocyclic Chemistry
Received, 31st October, 2011, Accepted, 30th November, 2011, Published online, 6th December, 2011
DOI: 10.3987/COM-11-12383

BRIAROXALIDES : NOVEL DIEPOXYBRIARANE DITERPENES FROM AN OKINAWAN GORGONIAN *BRIAREUM* SP.

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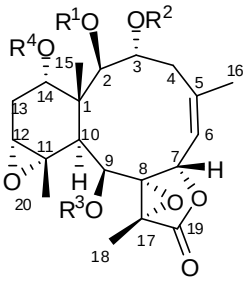
Abstract – Seven new 8,17- and 11,12-diepoxybriarane diterpenoids, briaroxalides A-G (**1**-**7**), were isolated from an Okinawan gorgonian *Briareum* sp. The structures of the diterpenoids were determined on the basis of spectroscopic analysis, chemical conversions and X-ray diffraction analysis.

INTRODUCTION

Briarane-type diterpenes have received a great deal of interest due to their structural features and biological activities. These diterpenoids are characterized by a highly oxygenated bicyclo[8.4.0]tetradecane skeleton, and most also contain a γ-lactone moiety. The range of activities reported for some briarane-type diterpenes include cytotoxic,[1-3] antiviral,[4,5] anti-inflamatory,[6,7] insecticidal,[8,9] immunomodulation,[10] and reversal of multidrug resistance.[11] We examined the chemical constituents of *Briareum* sp., collected at Ishigaki Island in the region of Okinawa Prefecture in Japan.[12] During the course of our investigations, seven new 8,17-, and 11,12-diepoxybriarane diterpenoids **1**-**7**, designated as briaroxalides A-G,[13] have been isolated. Herein we report on the isolation and structural elucidation of these new briarane diterpenoids, including the determination of absolute configuration based on chemical conversions and X-ray diffraction analysis.

RESULTS AND DISCUSSION

Gorgonian specimens of *Briareum* sp. (177 g), collected from the coral reef of Ishigaki Island, Okinawa, Japan in 2004, were immersed in MeOH. Repeated chromatographic separation of the combined extracts led to the purification and subsequent characterization of seven new 8,17- and 11,12-diepoxybriaranes, briaroxalides A-G (**1**-**7**) (Figure 1), along with known diterpenoids brianthein A,[11] violide G[14] and briarlide R.[15]



briaroxalide A (**1**) : R^1 = H , R^2 = Ac, R^3 = H , R^4 = Ac
briaroxalide B (**2**) : R^1 = H , R^2 = H , R^3 = Ac, R^4 = Ac
briaroxalide C (**3**) : R^1 = H , R^2 = Ac, R^3 = Ac, R^4 = Ac
briaroxalide D (**4**) : R^1 = H , R^2 = H , R^3 = Ac, R^4 = H
briaroxalide E (**5**) : R^1 = H , R^2 = Ac, R^3 = Ac, R^4 = H
briaroxalide F (**6**) : R^1 = Ac, R^2 = Ac, R^3 = Ac, R^4 = H
briaroxalide G (**7**) : R^1 = Ac, R^2 = H , R^3 = Ac, R^4 = Ac

Figure 1. Structures of briaroxalides A-G (**1**-**7**)

Briaroxalide A (**1**) was obtained as colorless needles and the molecular formula was found to be $C_{24}H_{32}O_{10}$ as determined from high-resolution ESIMS. The IR spectrum of **1** suggested the presence of a γ-lactone (1771 cm^{-1}), ester (1740 cm^{-1}) and hydroxy group (3537, 3449 cm^{-1}). The ^{13}C, ^{1}H NMR (Table 1 and 2) and DEPT spectra showed signals indicating six methyl, two sp^3 methylene, one sp^3 methine, six sp^3 oxymethine, one sp^3 quaternary carbon, three sp^3 quaternary carbons bearing an oxygen functional group, two sp^2 carbons, and three carbonyl carbons. These spectral data, coupled with the degree of unsaturation (9), suggested that compound **1** is a tricyclic diterpenoid possessing a γ-lactone [δ_C 171.8 (C)], two epoxide [δ_C 71.1 (C), δ_C 64.6 (C), δ_C 60.4 (C), δ_C 59.9 (CH)], two acetoxyl [δ_H 2.10 (3H, s), δ_H 2.03 (3H, s), δ_C 171.3 (C), δ_C 170.8 (C)], and a trisubstituted olefin [δ_H 5.27 (1H, d, J = 9.1 Hz), δ_C 140.4 (C), δ_C 120.3 (CH)] moiety. COSY cross-peaks indicated sequences of C-3 to C-4, C-6 to C-7, C-9 to C-10, and C-12 to C-14. The planar structure of **1** was determined on the basis of the following correlations (Figure 2) in the HMBC spectrum: H-2/C-1, C-4, C-10, C-15; H-3/C-3-Ac (C=O); H-4 (δ_H 2.99)/C-2, C-5, C-6; H-6/C-4, C-16; H-7/C-5, C-19; H-9/C-7, C-8, C-11, C-17; H-10/C-1, C-8, C-11, C-15; H-13 (δ_H 2.24)/C-11; H-14/C-1, C-2, C-10, C-14-Ac (C=O); H-15/C-1, C-2, C-10, C-14; H-16/C-4, C-5, C-6; H-18/C-8, C-17, C-19; H-20/ C-10, C-11, C-12; H-3-Ac (Me)/C-3-Ac (C=O); H-14-Ac (Me)/C-14-Ac (C=O).

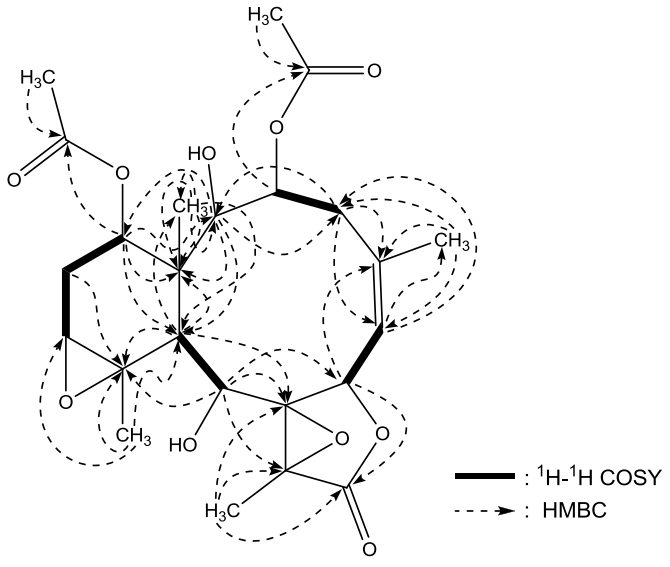


Figure 2. Selective ^{1}H-^{1}H COSY and HMBC correlations of **1**

Table 1. ^{13}C-NMR[a] spectral data (150 MHz, $CDCl_3$) for compounds **1**-**7**

no	**1**	**2**	**3**	**4**	**5**	**6**	**7**
1	45.9 (C)	46.1 (C)	45.7 (C)	45.5 (C)	46.0 (C)	45.7 (C)	44.5 (C)
2	69.0 (CH)	68.6 (CH)	69.5 (CH)	72.5 (CH)	72.6 (CH)	73.8 (CH)	73.3 (CH)
3	73.0 (CH)	68.4 (CH)	72.0 (CH)	68.3 (CH)	72.2 (CH)	71.3 (CH)	68.9 (CH)
4	34.4 (CH_2)	39.4 (CH_2)	34.4 (CH_2)	39.6 (CH_2)	34.4 (CH_2)	34.9 (CH_2)	39.4 (CH_2)
5	140.4 (C)	141.3 (C)	139.7 (C)	142.3 (C)	141.2 (C)	141.5 (C)	141.9 (C)
6	120.3 (CH)	119.3 (CH)	120.9 (CH)	118.5 (CH)	119.8 (CH)	120.2 (CH)	119.0 (CH)
7	75.5 (CH)	74.7 (CH)	74.8 (CH)	74.6 (CH)	74.8 (CH)	74.5 (CH)	74.6 (CH)
8	71.1 (C)	70.3 (C)	70.3 (C)	71.1 (C)	71.1 (C)	70.8 (C)	70.3 (C)
9	69.2 (CH)	68.8 (CH)	69.2 (CH)	67.6 (CH)	68.0 (CH)	67.5 (CH)	68.0 (CH)
10	44.5 (CH)	43.4 (CH)	44.0 (CH)	43.7 (CH)	43.8 (CH)	42.1 (CH)	43.0 (CH)
11	60.4 (C)	59.7 (C)	59.5 (C)	61.6 (C)	61.5 (C)	61.2 (C)	58.9 (C)
12	59.9 (CH)	59.3 (CH)	59.3 (CH)	62.4 (CH)	62.3 (CH)	62.0 (CH)	58.8 (CH)
13	26.7 (CH_2)	26.4 (CH_2)	26.1 (CH_2)	26.0 (CH_2)	26.1 (CH_2)	25.8 (CH_2)	24.9 (CH_2)
14	73.3 (CH)	73.7 (CH)	73.0 (CH)	72.9 (CH)	72.2 (CH)	72.3 (CH)	72.1 (CH)
15	13.3 (CH_3)	13.8 (CH_3)	13.4 (CH_3)	15.0 (CH_3)	14.4 (CH_3)	15.8 (CH_3)	15.1 (CH_3)
16	26.5 (CH_3)	27.2 (CH_3)	26.9 (CH_3)	27.9 (CH_3)	27.5 (CH_3)	26.8 (CH_3)	26.6 (CH_3)
17	64.6 (C)	65.1 (C)	65.8 (C)	63.8 (C)	64.3 (C)	63.7 (C)	64.1 (C)
18	10.5 (CH_3)	10.6 (CH_3)	11.2 (CH_3)	9.6 (CH_3)	10.0 (CH_3)	9.6 (CH_3)	9.9 (CH_3)
19	171.8 (C)	170.4 (C)	170.1 (C)	170.6 (C)	170.5 (C)	170.7 (C)	170.5 (C)
20	22.9 (CH_3)	24.7 (CH_3)	23.7 (CH_3)	26.0 (CH_3)	25.9 (CH_3)	26.3 (CH_3)	25.9 (CH_3)
2-Ac						169.9 (C)	169.6 (C)
						20.7 (CH_3)	20.8 (CH_3)
3-Ac	170.8 (C)		169.4 (C)		170.1 (C)	170.4 (C)	
	21.4 (CH_3)		21.2 (CH_3)		21.3 (CH_3)	21.2 (CH_3)	
9-Ac		169.1 (C)	169.5 (C)	169.7 (C)	169.7 (C)	169.8 (C)	169.4 (C)
		21.1 (CH_3)	20.9 (CH_3)	21.4 (CH_3)	21.0 (CH_3)	21.1 (CH_3)	21.1 (CH_3)
14-Ac	171.3 (C)	172.7 (C)	170.6 (C)				170.9 (C)
	21.1 (CH_3)	21.2 (CH_3)	21.1 (CH_3)				21.4 (CH_3)

[a] Carbon multiplicities were determined by DEPT experiments.

Table 2. ^{1}H-NMR[a] spectral data (600 MHz, $CDCl_3$) for compounds **1**-**7** (J = Hz)

no	**1**	**2**	**3**	**4**	**5**	**6**	**7**
2	3.87 (d, 10.2)	3.40 (d, 6.5)	3.92 (s)	3.84 (d, 5.9)	4.05 (d, 7.4)	5.28 (s)	5.09 (s)
3	6.05 (dd, 7.6, 11.0)	4.63 (m)	5.69 (dd, 7.4, 11.0)	4.53 (m)	5.53 (dd, 6.2, 12.0)	5.55 (dd, 5.6, 12.3)	4.68 (m)
4	2.99 (m)	2.74 (m)	3.03 (dd, 7.4, 14.0)	2.83 (dd, 4.6, 14.1)	2.99 (dd, 6.2, 13.7)	2.90 (m)	2.64 (m)
	2.03 (m)	2.18 (m)	2.03 (m)	2.04 (m)	2.13 (m)	2.24 (m)	1.97 (m)
6	5.27 (d, 9.1)	5.22 (d, 9.0)	5.27 (m)	5.32 (d, 9.6)	5.35 (d, 9.5)	5.44 (d, 9.6)	5.35 (d, 9.1)
7	5.54 (d, 9.1)	5.16 (m)	5.27 (m)	5.36 (d, 9.6)	5.61 (d, 9.5)	5.75 (d, 9.6)	5.27 (d, 9.1)
9	4.59 (d, 3.7)	5.72 (s)	5.64 (s)	5.78 (d, 3.1)	5.82 (d, 2.4)	5.87 (d, 3.7)	5.89 (d, 2.5)
10	2.37 (s)	2.54 (d, 1.6)	2.60 (s)	2.57 (d, 3.1)	2.59 (d, 2.4)	2.83 (d, 3.7)	2.78 (d, 2.5)
12	2.96 (d, 3.5)	2.96 (s)	2.93 (d, 3.1)	3.21 (s)	3.18 (s)	3.17 (s)	2.93 (s)
13	2.24 (m)	2.18 (m)	2.23 (m)	2.23 (m)	2.24 (d, 16.3)	2.24 (m)	2.22 (d, 16.8)
	2.17 (m)	2.13 (d, 16.0)	2.16 (m)	2.16 (d, 16.4)	2.13 (m)	2.11 (m)	2.09 (m)
14	5.11 (d, 5.8)	5.16 (m)	5.12 (d, 5.3)	3.73 (s)	3.89 (d, 10.8)	3.46 (m)	4.57 (d, 2.6)
15	1.03 (s)	1.11 (s)	0.95 (s)	0.93 (s)	0.78 (s)	0.85 (s)	1.14 (s)
16	1.85 (s)	1.78 (s)	1.85 (s)	1.87 (s)	1.91 (s)	2.09 (s)	1.97 (s)
18	1.62 (s)	1.71 (s)	1.71 (s)	1.61 (s)	1.63 (s)	1.60 (s)	1.67 (s)
20	1.53 (s)	1.47 (s)	1.39 (s)	1.59 (s)	1.56 (s)	1.60 (s)	1.53 (s)
2-Ac						2.13 (s)	2.11 (s)
3-Ac	2.10 (s)		2.11 (s)		2.13 (s)	2.09 (s)	
9-Ac		2.24 (s)	2.16 (s)	2.23 (s)	2.10 (s)	2.09 (s)	2.25 (s)
14-Ac	2.03 (s)	2.10 (s)	2.03 (s)				1.97 (s)

[a] Proton and carbon assignments were made based on the results of HSQC and HMBC experiments.

Furthermore, the *p*-bromobenzoate derivative (**8**), which would be amenable to X-ray diffraction analysis for determination of the relative and absolute configuration, was prepared by treatment of **1** with *p*-bromobenzoic acid in the presence of 1-ethyl-3-(3-dimethylaminopropyl)carbodiimide hydrochloride (EDC·HCl) and DMAP (Figure 3). Results showed that the absolute configuration of *p*-bromobenzoate **8**was 1*S*, 2*R*, 3*R*, 7*S*, 8*R*, 9*S*, 10*S*, 11*S*, 12*R*, 14*S*, 17*R* on the basis of the Flack parameter [0.003 (3)] in the X-ray analysis (Figure 4). This indicated that briaroxalide A (**1**) possesses the same 1*S*, 2*R*, 3*R*, 7*S*, 8*R*, 9*S*, 10*S*, 11*S*, 12*R*, 14*S*, 17*R* configuration.

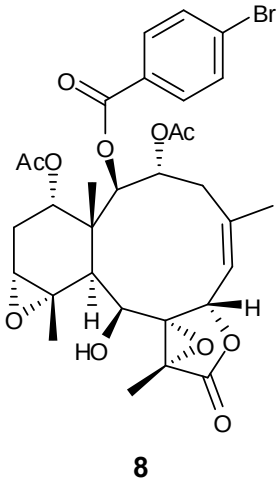


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Figure 3. Structure of *p*-bromobenzoate **8**

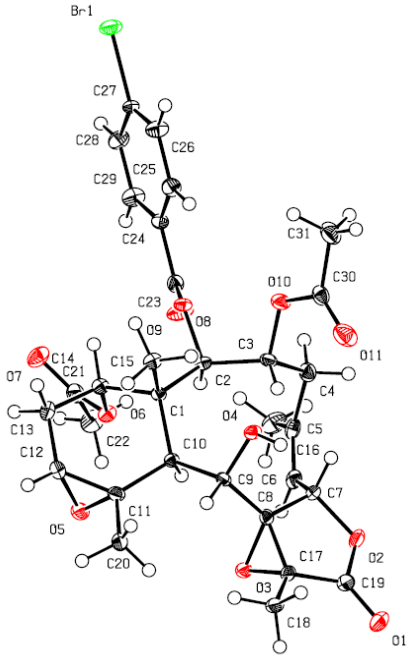

Figure 4. ORTEP drawing of *p*-bromobenzoate **8**

Briaroxalide B (**2**), obtained as a white amorphous compound with absorption bands at 1777 (γ-lactone), 1730 (ester) and 3388 cm^{-1} (OH) in its IR spectrum. The molecular formula of **2** was determined as $C_{24}H_{32}O_{10}$ from high-resolution ESIMS. Comparison of the ^{13}C and ^{1}H NMR data of **2** with those of **1** (Tables 1 and 2) showed the presence of a 20-carbon skeleton of a briarane diterpenoid having two hydroxy and two acetoxy groups. Furthermore, the HMBC correlation between H-9 (δ_H 5.72) and the carbonyl carbon (δ_C 169.1) of the acetyl group, and between H-14 (δ_H 5.16) and the carbonyl carbon (δ_C 172.7) of the acetyl group revealed the position of two acetates attached to C-9 and C-14.

Briaroxalide C (**3**), obtained as colorless needles, was found to have the molecular formula $C_{26}H_{34}O_{11}$ as determined from high-resolution ESIMS. The IR spectrum of **3** suggested the presence of a γ-lactone (1784 cm^{-1}), ester (1723 cm^{-1}) and hydroxy group (3513 cm^{-1}). Comparison of the ^{13}C and ^{1}H NMR data of **3** with those of **1** (Tables 1 and 2) showed the presence of a briarane diterpenoid having a hydroxy and

three acetoxy groups. Furthermore, the HMBC correlation between H-3 (δ_H 5.69) and the carbonyl carbon (δ_C 169.4) of the acetyl group, H-9 (δ_H 5.64) and the carbonyl carbon (δ_C 169.5) of the acetyl group, and between H-14 (δ_H 5.12) and the carbonyl carbon (δ_C 170.6) of the acetyl group revealed the position of three acetates, one each attached to C-3, C-9 and C-14. Thus, the structure of **3** represents the diastereomer at C-11, 12 of the known briarane-type diterpene, brianthein C.[11]

Briaroxalide D (**4**), obtained as a colorless oil with absorption bands at 1782 (γ-lactone), 1758 (ester) and 3457 cm^{-1} (OH) in its IR spectrum. The molecular formula of **4** was determined as $C_{22}H_{30}O_9$ from high-resolution ESIMS. Comparison of the ^{13}C and ^1H NMR data of **4** with those of **1** (Tables 1 and 2) showed the presence of a briarane diterpenoid having three hydroxy and an acetoxy groups. Furthermore, the HMBC correlation between H-9 (δ_H 5.78) and the carbonyl carbon (δ_C 169.7) of the acetyl group revealed the position of one acetate attached to C-9.

Briaroxalide E (**5**) was obtained as a colorless oil with molecular formula $C_{24}H_{32}O_{10}$ as determined from high-resolution ESIMS. The IR spectrum of **5** suggested the presence of a γ-lactone (1783 cm^{-1}), ester (1732 cm^{-1}) and hydroxy group (3478 cm^{-1}). Comparison of the ^{13}C and ^1H NMR data of **5** with those of **1** (Tables 1 and 2) showed the presence of a briarane diterpenoid having two hydroxy and two acetoxy groups. Furthermore, the HMBC correlation between H-3 (δ_H 5.53) and the carbonyl carbon (δ_C 170.1) of the acetyl group, and between H-9 (δ_H 5.82) and the carbonyl carbon (δ_C 169.7) of the acetyl group revealed the position of two acetates, one each attached to C-3 and C-9.

Briaroxalide F (**6**), a white amorphous compound, and briaroxalide G (**7**), a colorless oil were assigned as $C_{26}H_{34}O_{11}$ on the basis of high-resolution ESIMS. Comparison of the ^{13}C and ^1H NMR data of **6** and **7** with those of **1** (Tables 1 and 2) showed the presence of a briarane diterpenoid having three hydroxy and an acetoxy groups. Moreover, the decision of the position of three acetates was similar to that of other briaroxalides.

The relative and absolute configurations of briaroxalides B-G (**2**-**7**) were determined by chemical conversion and comparison with the spectral data for tetraacetate **9** derived from briaroxalide A (**1**), the absolute configuration of which had been determined. Briaroxalides B-G (**2**-**7**) and briaroxalide A (**1**) were treated with acetic anhydride, pyridine and a catalytic amount of DMAP (except for **2** and **3**) to afford tetraacetate **9** (Figure 5). The ^1H and ^{13}C NMR spectra, $[\alpha]_D$ value, and melting point of tetraacetate **9** were identical with those of **9**. These results clearly indicated that each of the briaroxalides A-G (**1**-**7**) possess the same absolute configuration. The biological activity of the seven new briarane diterpenoids briaroxalides A-G (**1**-**7**) reported here is currently under investigation.

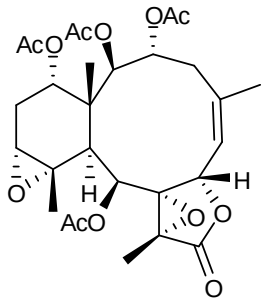

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Figure 5. Structure of tetraacetate **9**

EXPERIMENTAL

General Experimental Procedures. Optical rotations were measured with a JASCO P-1030 polarimeter. IR spectra were recorded with a JASCO FT-IR/620 spectrometer. ^{1}H and ^{13}C NMR spectra were taken with Bruker Biospin AV-600 spectrometer. Chemical shifts were expressed on a δ (ppm) scale with tetramethylsilane (TMS) as the internal standard (s, singlet; d, doublet; t, triplet; m, multiplet; br, broad). High resolution ESIMS (HRESIMS) spectra were obtained using a Micromass LCT spectrometer. Single crystal X-ray diffraction was recorded using a Mac Science Co., Ltd DIP 2020 Image Plate. Flash column chromatography was carried out on Kanto Chemical silica gel 60N (spherical, neutral) 40-50 μm.

Animal Material. The gorgonian specimens of *Briareum* sp. were obtained from the coral reef of Ishigaki Island, Okinawa, Japan, in 2004. A voucher specimen has been deposited at Tokyo University of Pharmacy and Life Sciences (SC-04-5).

Extraction and Isolation. Wet specimens (177 g) were cut into small pieces and extracted with MeOH (900 mL×3). The combined extracts were concentrated to give a green residue (13.4 g). The part of MeOH-extracted portion (1.01 g) was chromatographed on silica gel using an AcOEt-MeOH (2 : 1, 1 : 1, 1 : 2) gradient and MeOH as eluent to produce fraction 1 (373 mg), and 2 (136 mg). Fraction 1 was subjected to flash silica gel column chromatography using a hexane-AcOEt (2 : 1, 1 : 1, 1 : 5, 0 : 1) gradient and MeOH to give fractions 1-1 (23.0 mg), 1-2 (18.4 mg), 1-3 (91.9 mg), 1-4 (152 mg), 1-5 (33.1 mg), and 1-6 (29.2 mg). Fraction 1-3 was subjected to flash silica gel column chromatography (elution with hexane-AcOEt (1 : 1, 1 : 5, 0 : 1)) to give briaroxalide A (**1**) (5.4 mg) and violide G (5.9 mg). Fraction 1-4 was subjected to flash silica gel column chromatography (elution with $CHCl_3$-AcOEt (2 : 1)) to give briaroxalide A (**1**) (21.6 mg), briaroxalide B (**2**) (6.8 mg), and briaroxalide C (**3**) (36.4 mg). Fraction 1-5 was subjected to flash silica gel column chromatography (elution with hexane-AcOEt (1 : 5)) to give briaroxalide D (**4**) (14.1 mg). Fraction 1-6 was subjected to flash silica gel column chromatography (elution with $CHCl_3$-MeOH (30 : 1)) to give briaroxalide E (**5**) (39.1 mg). And then the rest of MeOH-extracted portion (12.4 g) was chromatographed on silica gel using an AcOEt-MeOH (2 : 1,

1 : 1, 1 : 2) gradient and MeOH as eluent to produce fraction 3 (4.50 g), and 4 (804 mg). Fraction 3 was subjected to flash silica gel column chromatography using a hexane-AcOEt (2 : 1, 1 : 2, 0 : 1) gradient and MeOH to give fractions 3-1 (359 mg), 3-2 (1.83 g), 3-3 (2.02 g), and 3-4 (282 mg). Fraction 3-1 was subjected to flash silica gel column chromatography (elution with $CHCl_3$-AcOEt (7 : 1)) to give brianthein A (26.5 mg). Fraction 3-2 was subjected to flash silica gel column chromatography using a hexane-AcOEt (2 : 1, 1 : 2, 0 : 1) gradient and MeOH to give fractions 3-2-1 (86.9 mg), 3-2-2 (215 mg), and 3-2-3 (1.47 g). Fraction 3-2-2 was subjected to flash silica gel column chromatography (elution with $CHCl_3$-AcOEt (5 : 1)) to give violide G (88.2 mg), and briarlide R (10.3 mg). Fraction 3-2-3 was subjected to flash silica gel column chromatography using a $CHCl_3$-AcOEt (5 : 1) gradient and MeOH to give fractions 3-2-3-1 (462 mg), 3-2-3-2 (338 mg), 3-2-3-3 (89.5 mg), 3-2-3-4 (447 mg), and 3-2-3-5 (234 mg). Fraction 3-2-3-1 was subjected to flash silica gel column chromatography (elution with $CHCl_3$-AcOEt (10 : 1)) to give briaroxalide C (**3**) (238 mg), briaroxalide F (**6**) (34.0 mg), and briarlide R (38.8 mg). Fraction 3-2-3-2 was subjected to flash silica gel column chromatography (elution with hexane-AcOEt (1 : 1)) to give briaroxalide C (**3**) (322 mg). Fraction 3-2-3-4 was subjected to flash silica gel column chromatography (elution with $CHCl_3$-MeOH (40 : 1)) to give briaroxalide A (**1**) (222 mg), and briaroxalide G (**7**) (18.9 mg).

Briaroxalide A (1): colorless needles (Et_2O); mp 220-222 ˚C; $[\alpha]^{25}_D$ +132.3 (*c* 0.27, MeOH); IR (KBr) νmax: 3537, 3449, 1771, 1740, 1248 cm^{-1}; ^{13}C-NMR and ^{1}H-NMR, see Table 1 and 2; COSY correlarions (H/H) H-3/H-4 (δ_H 2.03); H-3/H-4 (δ_H 2.99); H-6/H-7; H-9/H-10; H-12/H-13 (δ_H 2.24); H-14/H-13 (δ_H 2.24); HMBC correlations (H/C) H-2/C-1, C-4, C-10, C-15; H-3/C-3-Ac (C=O); H-4 (δ_H 2.99)/C-2, C-3, C-5, C-6; H-6/C-4, C-16; H-7/C-5, C-6, C-19; H-9/C-7, C-8, C-11, C-17; H-10/C-1, C-3, C-8, C-9, C-11, C-15; H-12/C-13, C-14; H-13 (δ_H 2.24)/C-11, C-12, C-14; H-14/C-1, C-2, C-10, C-12, C-13, C-14-Ac (C=O); H-15/C-1, C-2, C-10, C-14; H-16/C-4, C-5, C-6; H-18/C-8, C-17, C-19; H-20/C-9, C-10, C-11, C-12; H-3-Ac (Me)/C-3-Ac (C=O); H-14-Ac (Me)/C-14-Ac (C=O); ESIMS *m*/*z* 503 [M^++Na] (100); HRESIMS *m*/*z* 503.1892 (calcd for $C_{24}H_{32}O_{10}Na$: M^++Na, 503.1893).

Briaroxalide B (2): white amorphous; mp 208-211 ˚C; $[\alpha]^{25}_D$ +32.7 (*c* 0.34, $CHCl_3$); IR (KBr) νmax: 3388, 2993, 1777, 1730 cm^{-1}; ^{13}C-NMR and ^{1}H-NMR, see Table 1 and 2; COSY correlarions (H/H) H-3/H-4 (δ_H 2.18); H-3/H-4 (δ_H 2.74); H-6/H-7; H-9/H-10; H-12/H-13 (δ_H 2.13), H-13 (δ_H 2.18); H-14/H-13 (δ_H 2.13), H-13 (δ_H 2.18); HMBC correlations (H/C) H-2/C-1, C-2, C-4, C-10, C-14, C-15; H-3/C-1, C-3, C-4; H-4 (δ_H 2.18)/C-3, C-5, C-6, C-16; H-4 (δ_H 2.74)/C-2, C-3, C-5, C-16; H-6/C-4, C-16; H-7/C-5, C-6, C-19; H-9/C-1, C-7, C-8, C-10, C-11, C-17, C-9-Ac (C=O); H-10/C-1, C-5, C-11; H-12/C-13, C-20; H-13 (δ_H 2.13)/C-1, C-11, C-12, C-14; H-14/C-10, C-12, C-13, C-14-Ac (C=O); H-15/C-1, C-2, C-10, C-14; H-16/C-4, C-5, C-6; H-18/C-8, C-17, C-19; H-20/C-9, C-10, C-11, C-12;

H-9-Ac (Me)/C-9-Ac (C=O); H-14-Ac (Me)/C-14-Ac (C=O); ESIMS *m*/*z* 481 [M^++H] (100); HRESIMS *m*/*z* 481.2103 (calcd for $C_{24}H_{33}O_{10}$: M^++H, 481.2074).

Briaroxalide C (3): colorless needles (Et_2O); mp 198-201 ˚C; $[\alpha]^{25}_D$ +130.0 (*c* 0.20, EtOH); IR (KBr) νmax: 3513, 1784, 1723, 1249 cm^{-1}; ^{13}C-NMR and ^{1}H-NMR, see Table 1 and 2; COSY correlarions (H/H) H-3/H-4 (δ_H 2.03); H-3/H-4 (δ_H 3.03); H-6/H-7; H-9/H-10; H-12/H-13 (δ_H 2.23), H-13 (δ_H 2.16); H-14/H-13 (δ_H 2.23), H-13 (δ_H 2.16); HMBC correlations (H/C) H-2/C-1, C-15; H-3/C-1, C-4, C-3-Ac (C=O); H-4 (δ_H 2.03)/C-3, C-5, C-6, C-16; H-4 (δ_H 3.03)/C-2, C-3, C-5, C-6, C-16; H-6/C-4, C-16; H-7/C-5, C-6, C-19; H-9/C-1, C-7, C-8, C-10, C-11, C-9-Ac (C=O); H-10/C-1, C-2, C-11, C-14, C-15; H-12/C-13, C-14; H-13 (δ_H 2.16)/C-1, C-12, C-14; H-14/C-1, C-2, C-10, C-12, C-13, C-14-Ac (C=O); H-15/C-1, C-2, C-10, C-14; H-16/C-4, C-5, C-6; H-18/C-7, C-17, C-19; H-20/C-9, C-10, C-11, C-12; H-3-Ac (Me)/C-3-Ac (C=O); H-9-Ac (Me)/C-9-Ac (C=O); C-14-Ac (Me)/C-14-Ac (C=O); ESIMS *m*/*z* 545 [M^++Na] (100); HRESIMS *m*/*z* 545.2015 (calcd for $C_{26}H_{34}O_{11}Na$: M^++Na, 545.1999).

Briaroxalide D (4): colorless oil; $[\alpha]^{25}_D$ –39.7 (*c* 0.19, $CHCl_3$); IR (neat) νmax: 3457, 2917, 1782, 1758 cm^{-1}; ^{13}C-NMR and ^{1}H-NMR, see Table 1 and 2; COSY correlarions (H/H) H-3/H-4 (δ_H 2.04); H-3/H-4 (δ_H 2.83); H-6/H-7; H-9/H-10; H-12/H-13 (δ_H 2.16), H-13 (δ_H 2.23); H-14/H-13 (δ_H 2.16), H-13 (δ_H 2.23); HMBC correlations (H/C) H-2/C-1, C-4, C-10, C-15; H-3/C-1, C-4; H-4 (δ_H 2.04)/C-3, C-5, C-6, C-16; H-4 (δ_H 2.83)/C-2, C-3, C-5, C-6; H-6/C-4, C-16; H-7/C-5, C-6, C-19; H-9/C-7, C-8, C-10, C-11, C-9-Ac (C=O); H-10/C-1, C-2, C-8, C-9, C-11, C-15; H-12/C-14, C-20; H-14/C-10, C-12; H-15/C-10, C-14; H-16/C-4, C-5, C-6; H-18/C-8, C-17, C-19; H-20/C-9, C-11, C-12; H-9-Ac (Me)/C-9-Ac (C=O); ESIMS *m*/*z* 439 [M^++H] (100); HRESIMS *m*/*z* 439.2042 (calcd for $C_{22}H_{31}O_9$: M^++H, 439.1968).

Briaroxalide E (5): colorless oil; $[\alpha]^{25}_D$ +44.1 (*c* 0.37, $CHCl_3$); IR (neat) νmax: 3478, 2977, 1783, 1732 cm^{-1}; ^{13}C-NMR and ^{1}H-NMR, see Table 1 and 2; COSY correlarions (H/H) H-3/H-4 (δ_H 2.13); H-3/H-4 (δ_H 2.99); H-6/H-7; H-9/H-10; H-12/H-13 (δ_H 2.13), H-13 (δ_H 2.24); H-14/H-13 (δ_H 2.13), H-13 (δ_H 2.24); HMBC correlations (H/C) H-2/C-1, C-2, C-4, C-10, C-15; H-3/C-1, C-4, C-3-Ac (C=O); H-4 (δ_H 2.13)/C-3, C-5, C-6, C-14, C-16; H-4 (δ_H 2.99)/C-5, C-16; H-6/C-4, C-16; H-7/C-5, C-6, C-19; H-9/C-7, C-8, C-10, C-11, C-9-Ac (C=O); H-10/C-1, C-9, C-11, C-15; H-12/C-13, C-14; H-13 (δ_H 2.24)/C-1, C-3, C-11, C-14; H-14/C-10, C-12, C-13; H-15/C-1, C-2, C-3, C-10, C-14; H-16/C-4, C-5, C-6; H-18/C-8, C-17, C-19; H-20/C-9, C-10, C-11; H-3-Ac (Me)/C-3-Ac (C=O); H-9-Ac (Me)/C-9-Ac (C=O); ESIMS *m*/*z* 503 [M^++Na] (100); HRESIMS *m*/*z* 503.1916 (calcd for $C_{24}H_{32}O_{10}Na$: M^++Na, 503.1916).

Briaroxalide F (6): white amorphous; mp 187-190 ˚C; $[\alpha]^{25}_D$ +70.6 (*c* 0.32, $CHCl_3$); IR (KBr) νmax: 3490, 3001, 1792, 1747 cm^{-1}; ^{13}C-NMR and ^{1}H-NMR, see Table 1 and 2; COSY correlarions (H/H) H-2/H-3, H-3/H-4 (δ_H 2.24); H-3/H-4 (δ_H 2.90); H-6/H-7; H-9/H-10; H-12/H-13 (δ_H 2.11), H-13 (δ_H

2.24); H-14/H-13 (δ_H 2.11), H-13 (δ_H 2.24); HMBC correlations (H/C) H-2/C-1, C-4, C-10, C-14, C-15, C-2-Ac (C=O); H-3/C-1, C-4, C-3-Ac (C=O); H-4 (δ_H 2.24)/C-3, C-5, C-16; H-4 (δ_H 2.90)/C-2, C-3, C-5; H-6/C-4; H-7/C-6, C-19; H-9/C-7, C-8, C-10, C-11, C-9-Ac (C=O); H-10/C-1, C-2, C-5, C-8, C-9; H-12/C-13, C-14; H-14/C-10, C-12, C-13; H-15/C-1, C-2, C-10, C-14; H-16/C-4; H-18/C-7, C-8, C-17; H-20/C-9, C-10, C-11; ESIMS *m*/*z* 523 [M^++H] (100); HRESIMS *m*/*z* 523.2171 (calcd for $C_{26}H_{35}O_{11}$: M^++H, 523.2179).

Briaroxalide G (7): colorless oil; $[\alpha]^{25}_D$ +47.8 (*c* 0.095, $CHCl_3$); IR (neat) νmax: 3524, 2979, 1782, 1748 cm^{-1}; ^{13}C-NMR and ^{1}H-NMR, see Table 1 and 2; COSY correlarions (H/H) H-3/H-4 (δ_H 1.97); H-3/H-4 (δ_H 2.64); H-6/H-7; H-9/H-10; H-12/H-13 (δ_H 2.09), H-13 (δ_H 2.22); H-14/H-13 (δ_H 2.09), H-13 (δ_H 2.22); HMBC correlations (H/C) H-2/C-1, C-4, C-14, C-15, C-2-Ac (C=O); H-3/C-1, C-4; H-4 (δ_H 1.97)/C-3, C-5, C-6, C-16; H-4 (δ_H 2.64)/C-2, C-3, C-5, C-6; H-6/C-4, C-16; H-7/C-5, C-6, C-19; H-9/C-7, C-8, C-11, C-17, C-9-Ac (C=O); H-10/C-1, C-2, C-8, C-9, C-11, C-12, C-15; H-12/C-13, C-14; H-13 (δ_H 2.22)/C-1, C-12, C-14; H-14/C-2, C-10, C-12, C-13, C-14-Ac (C=O); H-15/C-1, C-2, C-10, C-14; H-16/C-3, C-4, C-5, C-6; H-18/C-8, C-17, C-19; H-20/C-9, C-10, C-11, C-12; H-9-Ac (Me)/C-9-Ac (C=O), H-14-Ac (Me)/C-14-Ac (C=O); ESIMS *m*/*z* 523 [M^++H] (100), 463 (M^+–Ac); HRESIMS *m*/*z* 523.2180 (calcd for $C_{26}H_{35}O_{11}$: M^++H, 523.2179).

Synthesis of *p*-bromobenzoate 8 from briaroxalide A (1). To a solution of briaroxalide A (**1**) (11.5 mg, 23.9 μmol) in CH_2Cl_2 (400 μL) were added *p*-bromobenzoic acid (96.2 mg, 479 μmol), EDC·HCl (91.8 mg, 479 μmol), and DMAP (0.1 mg, 0.820 μmol). The mixture was stirred at room temperature for 24 h. The reaction mixture was diluted with Et_2O, washed with H_2O and saturated aqueous NaCl, dried over Na_2SO_4, and concentrated under reduced pressure. The residue was purified by silica gel column chromatography (elusion with hexane-AcOEt (1 : 1)) to give *p*-bromobenzoate **8** (10.3 mg, 65% yield): colorless scales (toluene); mp 168-170 ˚C; $[\alpha]^{25}_D$ +145.9 (*c* 0.39, $CHCl_3$); IR (KBr) νmax: 3450, 2934, 1782, 1728 cm^{-1}; ^{1}H-NMR (600 MHz, $CDCl_3$) δ ppm: 7.87 (2H, m), 7.60 (2H, m), 6.11 (1H, dd, *J* = 5.9, 12.3 Hz), 5.74 (1H, d, *J* = 8.8 Hz), 5.52 (1H, s), 5.36 (1H, d, *J* = 8.8 Hz), 4.68 (1H, s), 4.59 (1H, d, *J* = 3.8 Hz), 3.93 (1H, s), 2.93 (1H, d, *J* = 2.8 Hz), 2.84 (1H, s), 2.68 (1H, s), 2.17 (2H, s), 2.12 (3H, s), 2.01 (3H, s), 1.93 (3H, s), 1.90 (1H, m), 1.69 (3H, s), 1.53 (3H, s), 1.26 (3H, s); ^{13}C-NMR (150 MHz, $CDCl_3$) δ ppm: 171.6, 171.5, 170.9, 164.6, 139.9, 131.9×2, 131.2×2, 128.8, 128.4, 120.4, 75.6, 72.8, 72.0, 71.9, 71.1, 69.1, 64.3, 59.7, 59.6, 45.7, 43.6, 34.6, 30.9, 26.4, 26.0, 24.2, 21.5, 15.1, 10.3; ESIMS *m*/*z* 685 [M^++Na] (100); HRESIMS *m*/*z* 685.1260 (calcd for $C_{31}H_{35}O_{11}BrNa$: M^++Na, 685.1260).

Synthesis of tetraacetate 9 from briaroxalides A-G (1-7). To a solution of briaroxalides A-G (**1**-**7**) (3.30-11.0 μmol) in pyridine (200 μL) were added acetic anhydride (100 μL) and catalytic amount of DMAP (except for **2** and **3**). The mixture was stirred at room temperature for 3-96 h. The reaction mixture

was concentrated under reduced pressure. The residue was purified by silica gel column chromatography (elusion with hexane-AcOEt (1 : 1)) to give tetraacetate **9** (44-89% yield) as a white amorphous.

Tetraacetate 9 from briaroxalide A (1): mp 183-184 ˚C; $[\alpha]^{25}_{D}$ +101.1 (*c* 0.15, $CHCl_3$); IR (KBr) νmax: 2924, 1779, 1730 cm^{-1}; ^{1}H-NMR (600 MHz, $CDCl_3$) δ ppm: 5.86 (1H, d, *J* = 2.2 Hz), 5.62 (1H, dd, *J* = 6.0, 11.6 Hz), 5.55 (1H, d, *J* = 8.9 Hz), 5.37 (1H, d, *J* = 9.0 Hz), 5.19 (1H, s), 4.71 (1H, d, *J* = 3.5 Hz), 2.93 (1H, s), 2.85 (1H, m), 2.80 (1H, d, *J* = 2.3 Hz), 2.21 (1H, m), 2.14 (3H, s), 2.11 (3H, s), 2.08 (3H, s), 2.04 (1H, m), 1.98 (3H, s), 1.95 (3H, s), 1.68 (3H, s), 1.49 (3H, s), 1.03 (3H, s); ^{13}C-NMR (150 MHz, $CDCl_3$) δ ppm: 170.8, 170.5, 170.3, 170.1, 169.7, 140.4, 120.5, 74.8, 72.1, 71.8, 71.4, 70.2, 68.4, 64.6, 59.0, 58.8, 44.7, 42.8, 35.0, 26.6, 25.3, 25.2, 21.3, 21.3, 21.0, 20.7, 15.0, 10.3; ESIMS *m*/*z* 587 [M^++Na] (100); HRESIMS *m*/*z* 587.2108 (calcd for $C_{28}H_{36}O_{12}Na$: M^++Na, 587.2104).

Tetraacetate 9 from briaroxalide B (2): mp 177-179 ˚C; $[\alpha]^{25}_{D}$ +141.0 (*c* 0.085, $CHCl_3$).

Tetraacetate 9 from briaroxalide C (3): mp 184-185 ˚C; $[\alpha]^{25}_{D}$ +130.6 (*c* 0.16, $CHCl_3$).

Tetraacetate 9 from briaroxalide D (4): mp 184-185 ˚C; $[\alpha]^{25}_{D}$ +106.6 (*c* 0.060, $CHCl_3$).

Tetraacetate 9 from briaroxalide E (5): mp 182-184 ˚C; $[\alpha]^{25}_{D}$ +109.9 (*c* 0.16, $CHCl_3$).

Tetraacetate 9 from briaroxalide F (6): mp 180-181 ˚C; $[\alpha]^{25}_{D}$ +111.5 (*c* 0.085, $CHCl_3$).

Tetraacetate 9 from briaroxalide G (7): mp 185-186 ˚C; $[\alpha]^{25}_{D}$ +149.2 (*c* 0.085, $CHCl_3$).

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