Pergamon

Tetrahedron 57 (2001) 8951–8957

TETRAHEDRON

Brianthein A, a novel briarane-type diterpene reversing multidrug resistance in human carcinoma cell line, from the gorgonian *Briareum excavatum*

Shunji Aoki,[a] Masanori Okano,[a] Kouhei Matsui,[a] Takuya Itoh,[a] Rachmaniar Satari,[b] Shin-ichi Akiyama[c] and Motomasa Kobayashi[a,*]

[a]*Graduate School of Pharmaceutical Sciences, Osaka University, Yamada-oka 1-6, Suita, Osaka 565-0871, Japan*
[b]*Research and Development Centre for Oceanology, LIPI, JL. Pasir Putih I, Ancol Timur, Jakarta 11048, Indonesia*
[c]*Department of Cancer Chemotherapy, Faculty of Medicine, Institute for Cancer Research, Kagoshima University, 8-35-1 Sakuragaoka, Kagoshima 890-8520, Japan*

Received 23 July 2001; accepted 4 September 2001

Abstract—A novel briarane-type diterpene named brianthein A (**1**), which has been isolated from the gorgonian *Briareum excavatum*, reversed multidrug resistance (MDR) in human carcinoma cell lines, KB-C2, overexpressing P-glycoprotein (P-gp). The absolute stereostructure of **1** was elucidated by the detailed 2D-NMR analysis of **1** and the application of the modified Mosher's method to the partially deacetylated derivative of **1**. Furthermore, novel analogous compounds, briantheins B (**8**) and C (**9**), were also isolated from the same gorgonian. From the structure-activity relationship study, each of the 2, 3 and 14-acetoxyl groups and 11,12-olefin in **1** were found to be crucial for the MDR reversing activity.

1. Introduction

The development of multidrug resistance (MDR) in tumor cells is a serious problem in the treatment of human cancer. A major mechanism underlying this multidrug resistance is due to overexpression of the membrane glycoprotein named P-glycoprotein (P-gp). P-gp encoded by MDR1 gene functions as an ATP-dependent efflux pump of anti-cancer drugs such as doxorubicin and vincristine.[1] Accordingly, substances which restore the inherent potency of an antitumor agent by inhibiting the action of P-gp are expected to have high potential to realize successful cancer chemotherapy. In the course of our study of bioactive substances from marine organisms,[2] we have been engaged in a search for reversing substances of MDR in tumor cells and isolated brianthein A (**1**) from the Indonesian gorgonian *Briareum excavatum*. Compound **1** was a novel briarane-type diterpene and completely reversed MDR caused by overexpression of P-gp at 10 $\mu g\ ml^{-1}$. We further isolated briantheins B (**8**) and C (**9**) as novel briarane-type diterpenes together with known briarane-type diterpenes, **10**[3] and excavatolide C (**11**),[4] from the same gorgonian. In addition, we synthesized several derivatives of **1** and investigated the structure–activity relationship.

Keywords: briarane diterpene; multidrug resistance; P-glycoprotein; gorgonian.

* Corresponding author. Tel.: +81-6-6879-8215; fax: +81-6-6879-8219; e-mail: kobayasi@phs.osaka-u.ac.jp

2. Results and discussion

The MeOH extract of the titled dried gorgonian showed MDR reversing activity at 10 $\mu g\ ml^{-1}$ concentration against human carcinoma cell lines, KB-C2,[5] overexpressing P-gp. The MeOH extract was partitioned into a water–AcOEt mixture to provide the AcOEt soluble portion. Then, the active AcOEt soluble portion was subjected to bioassay-guided separation. Repeated SiO_2 column chromatography (*n*-hexane–AcOEt) and HPLC (ODS, MeOH–H_2O, and SiO_2, *n*-hexane–AcOEt) furnished a novel briarane-type diterpene, brianthein A (**1**), as a major active component. Furthermore, we investigated the chemical constituents of the AcOEt soluble portion and isolated novel briarane-type diterpenes, briantheins B (**8**) and C (**9**), and known briarane-type diterpenes, **10** and excavatolide C (**11**), which showed much weaker MDR reversing activity than that of **1**.

Brianthein A (**1**) was obtained as a colorless powder. The positive ion FAB MS of **1** gave a quasi-molecular ion $[(M+Na)^+]$ peak at *m/z* 497 and the molecular formula was determined as $C_{26}H_{34}O_8$ by HR-positive ion FAB MS. The IR spectrum of **1** showed strong absorption bands due to ester groups (1738 cm^{-1}), and α,β-unsaturated lactone group (1667 cm^{-1}). The ^1H NMR spectrum of **1** showed three acetyl methyl signals (δ 2.09, 2.08, 1.93), three olefinic methyl signals (δ 2.05, 1.90, 1.59), and a tertiary methyl signal (δ 0.92). The ^{13}C NMR spectrum of **1** also disclosed the presence of four carbonyl carbons (δ_C 174.0,

0040–4020/01/$ - see front matter
PII: S0040-4020(01)00894-8

Table 1. ^{1}H and ^{13}C NMR data for briantheins A (**1**), B (**8**) and C (**9**). (500 MHz and 125 MHz in $CDCl_3$)

No.	**1**		**8**		**9**	
	^{13}C δ_C	^{1}H δ (mult., J (Hz))	^{13}C δ_C	^{1}H δ (mult., J (Hz))	^{13}C δ_C	^{1}H δ (mult., J (Hz))
1	41.5 (s)	–	41.5 (s)	–	46.5 (s)	–
2	72.9 (d)	4.83 (d, 1.8)	73.1 (d)	4.61 (d, 2.4)	72.7 (d)	3.53 (d, 9.9)
3	68.5 (d)	5.38 (ddd, 12.8, 5.5, 1.8)	68.6 (d)	5.62 (ddd, 12.2, 5.4, 2.4)	71.6 (d)	5.49 (dd, 12.1, 5.8)
4	36.1 (t)	2.68 (dd, 12.8, 5.5)	35.7 (t)	2.69 (dd, 12.2, 5.4)	34.6 (t)	2.97 (dd, 13.7, 5.8)
		2.38 (dd, 12.8, 12.8)		2.38 (dd, 12.8, 12.2)		2.07 (m)
5	137.9 (s)	–	144.5 (s)	–	140.0 (s)	–
6	125.4 (d)	5.22 (d, 9.8)	127.6 (d)	5.28 (d, 9.7)	120.9 (d)	5.43 (d, 9.6)
7	80.6 (d)	5.68 (d, 9.8)	80.1 (d)	5.67 (d, 9.7)	74.4 (d)	5.64 (d, 9.6)
8	159.7 (s)	–	158.6 (s)	–	71.0 (s)	–
9	29.1 (t)	3.00 (d, 15.3)	30.7 (t)	3.00 (d, 14.1)	69.5 (d)	5.74 (d, 3.3)
		2.54 (dd, 15.3, 6.3)		2.55 (d, 14.1)		
10	38.0 (d)	2.65(m)	37.4 (d)	2.99 (s-like)	42.8 (d)	2.26 (d, 3.3)
11	136.0 (s)	–	136.5 (s)	–	62.4 (s)	–
12	116.8 (d)	5.21 (brs)	82.5 (d)	4.38 (d, 7.9)	60.5 (d)	3.01 (d, 6.3)
13	26.1 (t)	2.22 (brd, 18.4)	26.7 (t)	2.09 (ddd, 11.0, 7.9, 1.8)	24.9 (t)	2.16 (m)
		2.04 (m)		1.90 (m)		2.06 (m)
14	72.3 (d)	4.94 (s-like)	71.1 (d)	4.82 (dd, 4.2, 1.8)	74.6 (d)	5.17 (d, 1.4)
15	14.3 (q)	0.92 (s)	13.2 (q)	1.00 (s)	13.9 (q)	0.88 (s)
16	26.7 (q)	2.05 (s)	30.4 (q)	1.93 (s)	27.4 (q)	1.90 (s)
17	125.0 (s)	–	126.0 (s)	–	64.0 (s)	–
18	174.0 (s)	–	173.9 (s)	–	170.9 (s)	–
19	9.7 (q)	1.90 (s)	8.4 (q)	1.73 (s)	10.1 (q)	1.69 (s)
20	21.5 (q)	1.59 (s)	116.2 (t)	4.91 (d, 3.0)	24.5 (q)	1.39 (s)
				4.71 (d, 3.0)		
2-Ac	20.9 (q)	1.93 (s)	19.7 (q)	1.97 (s)	–	–
	171.0 (s)	–	170.6 (s)	–	–	–
3-Ac	21.1 (q)	2.08 (s)	20.6 (q)	1.99 (s)	21.2 (q)	2.13 (s)
	170.5 (s)	–	169.8 (s)	–	169.4 (s)	–
9-Ac	–	–	–	–	21.0 (q)	2.06 (s)
	–	–	–	–	167.9 (s)	–
14-Ac	20.4 (q)	2.09 (s)	20.1 (q)	1.78 (s)	21.0 (q)	2.06 (s)
	170.6 (s)	–	169.9 (s)	–	170.1 (s)	–
2-OH	–	–	–	–		2.65 (d, 9.9)
12-OOH	–	–	–	8.00 (brs)	–	–

171.0, 170.6, 170.5) and six olefinic carbons (δ_C 159.7, 137.9, 136.0, 125.4, 125.0, 116.8). All the proton and carbon signals of **1** were assigned by 2D-NMR (COSY, HMQC) analysis of **1** (Table 1) and four partial structures were elucidated (Fig. 1). Connectivities of these partial structures were clarified by the HMBC analysis (Fig. 1) and the plane structure of brianthein A was determined as **1**.

Next, we tried to elucidate the absolute stereostructure of **1**. The relative stereostructure of **1** was elaborated on the basis of the NOESY correlations (Fig. 2). Furthermore, **1** was treated with sodium methoxide in MeOH to give three di-deacetyl derivatives, **2**, **3**, and **4**, and mono-deacetyl derivative **5**, and the modified Mosher's method[6] was applied to the 3-hydroxyl group in **5**. A comparative analysis of all proton signals of *R*-(+)-2-methoxy-2-phenyl-2-trifluoromethylacetic acid (MTPA) ester **6a** and *S*-(−)-MTPA ester **6b** clarified 3-*R* configuration in **1** (Fig. 3). Consequently, the absolute stereostructure of **1** was determined as shown in Chart 1.

The FAB-MS of brianthein B (**8**) showed a quasi-molecular $(M+H)^+$ ion peak at *m/z* 507 and the molecular formula was

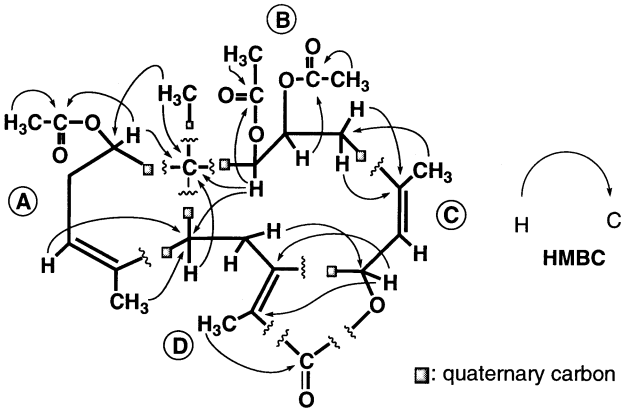


Figure 1. Partial structures of brianthein A (**1**) with key HMBC correlations.

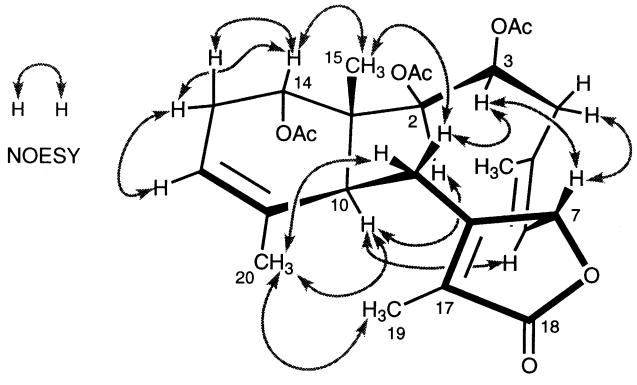


Figure 2. NOESY correlations in brianthein A (**1**).

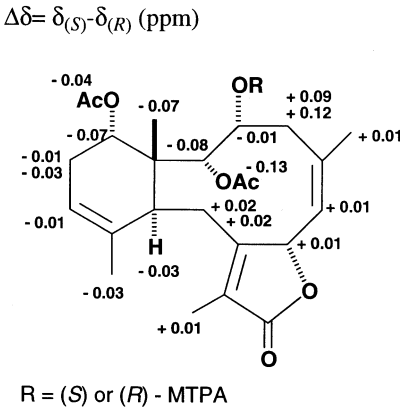
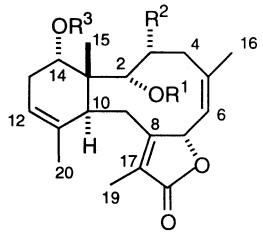


Figure 3. Application of modified Mosher's method to mono-deacetyl derivative **5** of **1**.

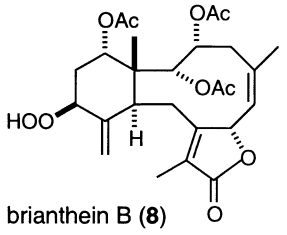
determined as $C_{26}H_{34}O_{10}$ by HR-FAB MS. The IR spectrum of **8** showed strong absorption bands due to a hydroxyl group (3354 cm^{-1}) in addition to ester (1739 cm^{-1}) and α,β-unsaturated lactone group (1670 cm^{-1}). The ^1H and ^{13}C NMR spectra of **8** (Table 1) were closely similar to those of **1**, except for the signals assignable to exomethylene [δ 4.91, 4.71 (both d, J=3.0 Hz), δ_C 116.2 (t)] and peroxygenated methine [δ 4.38 (d, J=7.9 Hz), δ_C 82.5 (d)]. On the basis of 2D-NMR analysis of **8** in conjunction with the molecular formula, the plane structure of brianthein B (**8**) was presumed to be the hydroperoxygenated analogue of **1**. The presence of the hydroperoxyl group in **8** was also supported by positive response to *N*,*N*-dimethyl-*p*-phenylenediammonium dichloride reagent[7] and the ^1H NMR signal observed at δ 8.00 ppm as a broad singlet. The relative stereostructure of **8** was elucidated on the basis of the NOESY correlations (e.g., correlations between 12-H and 13α-H, 20-H; β-H (δ 1.90) and 15-CH_3).

The FAB-MS of brianthein C (**9**) showed a quasi-molecular $(M+H)^+$ ion peak at *m/z* 523 and the molecular formula was determined as $C_{26}H_{34}O_{11}$ by HR-FAB MS. The IR spectrum of **9** showed strong absorption bands due to a hydroxyl (3514 cm^{-1}), ester (1736 cm^{-1}), and lactone (1784 cm^{-1}) groups. In the ^1H and ^{13}C NMR spectra of **9** (Table 1), the H-2 proton signal was observed in higher field compared with those of **1**, while the H-9 proton and the C-9 carbon signals were observed in lower field due to substitution of the acetoxyl group. Furthermore, the ^1H and ^{13}C NMR spectra of **9** defined the presence of two epoxide groups (C-8, C-17 and C-11, C-12). Finally, the plane structure of brianthein C (**9**) was determined as shown in Chart 1 by 2D-NMR analysis and NMR comparison with those of the relative compound, stecolide C.[8] The relative stereostructures of **9** were elaborated on the basis of the NOESY correlations (e.g. correlations between 20-CH_3 and 10-H, 9-H; 12-H and 13a-H; 19-CH_3 and 9-H) and NMR comparison with stecolide C.[8]

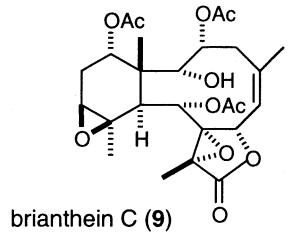
Many briarane-type diterpenes have been isolated from gorgonians,[8–10] and brianthein A (**1**) is characterized as the 3-acetoxyl analogue of brianthein W (**7**) isolated from



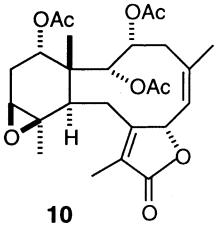
	R^1	R^2	R^3
brianthein A (**1**)	Ac	OAc	Ac
2	Ac	OH	H
3	H	OH	Ac
4	H	OAc	H
5	Ac	OH	Ac
6a	Ac	O-(*R*)-MTPA	Ac
6b	Ac	O-(*S*)-MTPA	Ac
brianthein W (**7**)	Ac	H	Ac



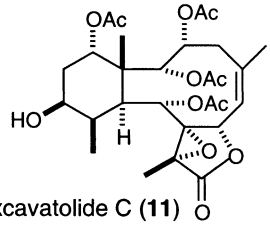
brianthein B (**8**)



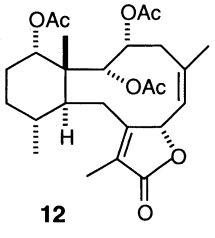
brianthein C (**9**)



10



excavatolide C (**11**)



12

Chart 1.

Table 2. Reversal of MDR in KB-C2 cells by briantheins and its derivatives

No.	Dose ($\mu g\ ml^{-1}$)	Growth inhibition (%)	
		KB 3-1	KB-C2
1	10	27±5	84±3
	3	11±6	60±2
2	10	0±0	36±9
	3	0±0	12±5
3	10	28±1	14±4
	3	14±1	10±5
4	10	25±3	25±6
	3	10±1	0±0
5	10	14±2	39±10
	3	4±1	12±3
8	10	26±4	37±6
	3	5±1	26±4
9	10	17±6	15±2
	3	11±1	0±0
10	10	34±8	28±3
	3	15±6	0±0
11	10	29±5	0±0
	3	19±2	0±0
12	10	36±8	31±7
	3	18±6	16±3

The value to KB 3-1 cells shows the cytotoxicity of each compound. The value to KB-C2 cells shows the growth inhibition in the presence of each compound and 0.1 $\mu g\ ml^{-1}$ of colchicine. Colchicine was not cytotoxic against KB-C2 cells at 0.1 $\mu g\ ml^{-1}$ concentration. Each value presents mean±S.D.

the gorgonian *Briareum polyanthes*.[11] So far, there is no report that those briarane-type diterpenes show modulating activity to MDR in tumor cells. As shown in Table 2, brianthein A (**1**) completely reversed the resistance to colchicine in KB-C2 cells and showed little cytotoxicity at 10 $\mu g\ ml^{-1}$ concentration. We further examined the reversing activity of related compounds of **1** (Table 2). Compounds **2**, **3**, **4**, and **5** were prepared from **1** by partial deacetylation using NaOMe/MeOH and compound **12** was also prepared from **1** by catalytic reduction. The stereochemistry at the C-11 position in **12** was determined by NOESY analysis (e.g., correlations between 19-CH_3 and 20-CH_3; H-11 and 15-CH_3). Compounds **2**, **3**, **4** and **5** lacking a 2- or 3- or 14-acetyl group showed much weaker activity than that of **1**. Furthermore, compound **12** lacking 11,12-olefin was also less active than **1**. Thus, these three acetyl groups and 11,12-olefin are presumed to be crucial for the reversing activity of **1**.

On the other hand, we have isolated a polyhydroxylated sterol acetate named agosterol A (**13**)[12] as a potent reversing substance to both P-gp- and multidrug resistance associated protein (MRP1)-mediated MDR from a marine sponge of *Spongia* sp. Both agosterol A (**13**) and brianthein A (**1**) have three acetoxyl groups, which have been clarified to be important for the excellent MDR reversing activity.[13] So, we focused on the conformational similarity of the two compounds and tried to verify the spatial arrangement of the three acetoxyl groups in both compounds by restrained molecular dynamic calculation. The three-dimensional structures of brianthein A (**1**) and agosterol A (**13**), which satisfy the distance restraints estimated from the NOE cross-peaks, were constructed by restrained molecular dynamics using NMRchitect-Discover software package (Molecular Simulations Inc. (MSI)) as described in our previous report.[14] The calculated structures of **1** and **13** exhibited small deviations from the distance restraints (0.22±0.04 and 0.24±0.03 Å for the ring part of the 10 lowest energy structures, respectively) (Fig. 4), and the three acetyl groups in the calculated lowest energy structures of both compounds were superimposed on each other. As shown in Fig. 5, the three acetoxyl groups in **1** and **13** showed similar spatial arrangement. This finding may suggest the importance of spatial arrangement of the three acetoxyl groups for the binding affinity of these compounds to P-gp.

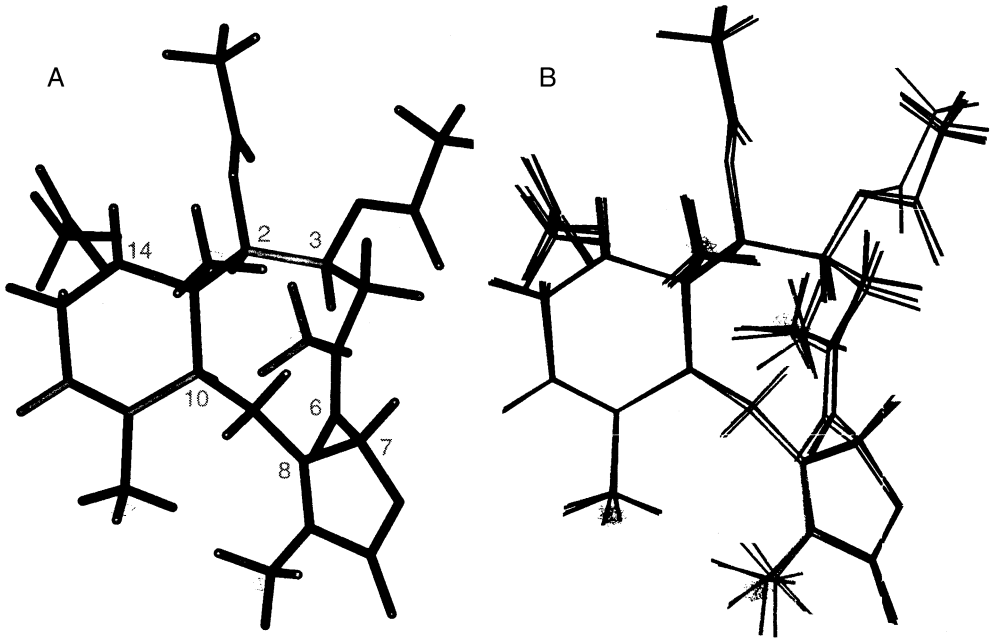


Figure 4. Conformation of brianthein A (**1**) constructed by restrained molecular dynamic calculation. (A) The lowest energy structure of **1** as a tube model. (B) The superimposed 10 lowest energy structures of **1**. Structures are shown as a wire frame model.

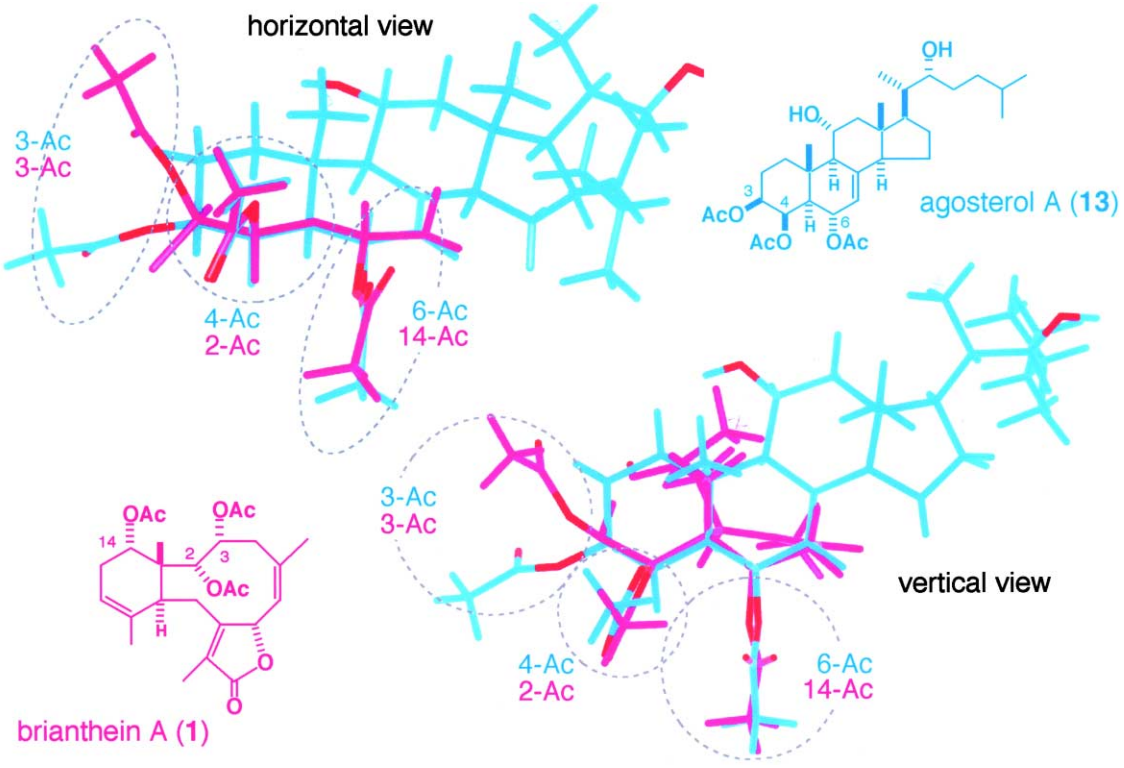


Figure 5. The conformational comparison of three acetoxyl groups in brianthein A (**1**) and agosterol A (**13**).

3. Experimental

3.1. Isolation

The dried gorgonian *Briareum excavatum* (500 g), which was collected in July 1998 at Sulawesi Island, Indonesia, was initially steeped in MeOH. The residue obtained by evaporation of the solvent under reduced pressure was partitioned into an AcOEt–water mixture (1:1), and the AcOEt layer was taken and evaporated to give the AcOEt soluble portion (20 g). The AcOEt soluble portion (5 g) was separated by SiO_2 column chromatography (*n*-hexane–AcOEt) to give five fractions (Fr. A–Fr. E). Fr. B (1076 mg) was subjected to HPLC (Mightysil RP-18 GP, MeOH–H_2O=9:1 and Cosmosil 5SL, *n*-hexane–AcOEt=1:1) to afford brianthein A (**1**, 395 mg, 7.9% yield from the AcOEt soluble portion), brianthein B (**8**, 15 mg, 0.3%), and **10** (65 mg, 1.3%). Fr. C (902 mg) was separated by SiO_2 open column (*n*-hexane–AcOEt) and further purified by HPLC (Cosmosil 5SL, *n*-hexane–AcOEt=1:2 and Mightysil RP-18 GP, MeOH–H_2O=7:3) to obtain excavatolide C (**11**, 25 mg, 0.5%), and brianthein C (**9**, 10 mg, 0.2%). Brianthein A (**1**): $[\alpha]^{20}_D$=+118.7 (*c*=0.4, EtOH). HR-FAB MS: Obsd; *m/z* 497.2122. Calcd for $C_{26}H_{34}O_8Na$; *m/z* 497.2152 $(M+Na)^+$. IR (KBr): 1738, 1667 cm^{-1}. ^1H and ^{13}C NMR spectra: as shown in Table 1. Brianthein B (**8**): $[\alpha]^{20}_D$=+81.7 (*c*=0.4, EtOH). HR-FAB MS: Obsd; *m/z* 507.2222. Calcd for $C_{26}H_{35}O_{10}$; *m/z* 507.2230 $(M+H)^+$. IR (KBr): 3354, 1739, 1670 cm^{-1}. ^1H and ^{13}C NMR spectra: as shown in Table 1. Brianthein C (**9**): $[\alpha]^{20}_D$=+32.6 (*c*=0.4, EtOH). HR-FAB MS: Obsd; *m/z* 523.2202. Calcd for $C_{26}H_{35}O_{11}$; *m/z* 523.2179 $(M+H)^+$. IR (KBr): 3514, 1784, 1736 cm^{-1}. ^1H and ^{13}C NMR spectra: as shown in Table 1.

3.1.1. Di-deacetyl derivative 2–4 and mono-deacetyl derivative 5 of brianthein A (1). Compound **1** (30 mg) was treated with 0.05% NaOMe in MeOH (3.0 ml) and stirred at 0°C for 21 h. The reaction mixture was neutralized with Dowex HCR-W2 and further purified by HPLC (Cosmosil 5SL, *n*-hexane–AcOEt=1:5) to afford 3,14-di-deacetyl derivative **2** (2.8 mg), 2,3-di-deacetyl derivative **3** (2.5 mg), 2,14-di-deacetyl derivative **4** (2.3 mg) and 3-mono-deacetyl derivative **5** (4.1 mg). **2**: HR-FAB MS: Obsd; *m/z* 391.2134. Calcd for $C_{22}H_{31}O_6$; 391.2121 $(M+H)^+$. ^1H NMR ($CDCl_3$, δ): 5.57 (1H, d, *J*=9.4 Hz, H-7), 5.24 (1H, d, *J*=9.4 Hz, H-6), 5.12 (1H, brs, H-12), 4.80 (1H, d, *J*=6.7 Hz, H-2), 3.67 (1H, t-like, *J*=5.5 Hz, H-3), 3.38 (1H, s-like, H-14), 2.02 (3H, s, 2-OAc), 1.81 (3H, s, H-16), 1.74 (3H, s, H-19), 1.44 (3H, s, H-20), 0.89 (3H, s, H-15). **3**: HR-FAB MS: Obsd; *m/z* 391.2101. Calcd for $C_{22}H_{31}O_6$; *m/z* 391.2121 $(M+H)^+$. ^1H NMR ($CDCl_3$, δ): 5.56 (1H, d, *J*=9.8 Hz, H-7), 5.28 (1H, d, *J*=6.1 Hz, H-12), 5.19 (1H, brs, H-14), 5.12 (1H, d, *J*=9.8 Hz, H-6), 4.13 (1H, m, H-3), 3.52 (1H, d, *J*=8.5 Hz, H-2), 2.07 (3H, s, 14-OAc), 1.89 (3H, s, H-16), 1.81 (3H, s, H-19), 1.62 (3H, s, H-20), 1.01 (3H, s, H-15). **4**: HR-FAB MS: Obsd; *m/z* 391.2122. Calcd for $C_{22}H_{31}O_6$; *m/z* 391.2121 $(M+H)^+$. ^1H NMR ($CDCl_3$, δ): 5.68 (1H, d, *J*=9.8 Hz, H-7), 5.22 (1H, dd, *J*=12.2, 4.9 Hz, H-3), 5.18 (1H, d, *J*=6.1 Hz, H-12), 5.07 (1H, d, *J*=9.8 Hz, H-6), 3.45 (1H, d, *J*=8.6 Hz, H-2), 3.42 (1H, s-like, H-14), 2.06 (3H, s, 3-OAc), 1.91 (3H, s, H-16), 1.79 (3H, s, H-19), 1.52 (3H, s, H-20), 0.70 (3H, s, H-15). **5**: HR-FAB MS: Obsd; *m/z* 433.2210. Calcd for $C_{24}H_{33}O_7$; *m/z*

433.2227 $(M+H)^+$. ^1H NMR ($CDCl_3$, δ): 5.53 (1H, d, J=9.8 Hz, H-7), 5.24 (1H, d, J=5.5 Hz, H-12), 5.19 (1H, d, J=9.8 Hz, H-6), 4.89 (1H, s-like, H-2), 4.21 (1H, m, H-3), 3.42 (1H, s-like, H-14), 2.12 (3H, s, 14-OAc), 2.07 (3H, s, H-16), 1.96 (3H, s, 2-OAc), 1.92 (3H, s, H-19), 1.70 (3H, s, H-20), 1.09 (3H, s, H-15).

3.1.2. 3-*R*-(+)-MTPA ester 6a and 3-*S*-(−)-MTPA ester 6b of 5. A solution of **5** (0.7 mg) in CH_2Cl_2 (0.5 ml) was treated with (*R*)-(+)-MTPA (4.6 mg), 1-[3-(dimethylamino)propyl]-3-ethylcarbodiimide (EDCI, 11.4 mg), and *N*,*N*-dimethylaminopyridine (DMAP, 1.2 mg) at room temperature for 48 h under an Ar atmosphere. The reaction was quenched by saturated aq. NH_4Cl and the whole was extracted with AcOEt. The AcOEt phase was washed with 5% HCl, saturated aq. $NaHCO_3$, and saturated aq. NaCl. After evaporation of the solvent, the residue was purified by SiO_2 HPLC (Cosmosil 5SL, *n*-hexane–AcOEt=3:1) to furnish 3-*R*-(+)-MTPA ester **6a** (0.5 mg). A solution of **5** (0.8 mg) in dichloromethane (0.5 ml) was similarly treated with (*S*)-(−)-MTPA (4.6 mg), EDCI (11.4 mg), and DMAP (1.2 mg) to afford 3-*S*-(−)-MTPA ester **6b** (0.5 mg). **6a**: HR-FAB MS: Obsd; *m*/*z* 649.2615. Calcd for $C_{34}H_{40}O_9F_3$; *m*/*z* 649.2625 $(M+H)^+$. ^1H NMR ($CDCl_3$, δ): 5.67 (1H, d, J=9.8 Hz, H-7), 5.62 (1H, m, H-3), 5.28 (1H, d, J=9.8 Hz, H-6), 5.25 (1H, d, J=6.1 Hz, H-12), 4.99 (1H, dd, J=2.4, 2.4 Hz, H-14), 4.93 (1H, d, J=1.8 Hz, H-2), 3.00 (1H, d, J=16.5 Hz, H-9a), 2.75 (1H, dd, J=12.8, 5.5 Hz, H-4a), 2.70 (1H, m, H-10), 2.60 (1H, dd, J=16.5, 6.7 Hz, H-9b), 2.44 (1H, dd, J=12.8, 12.8 Hz, H-4b), 2.25 (1H, brd, J=19.5 Hz, H-13a), 2.07 (3H, s, H-16), 2.04 (1H, m, H-13b), 1.97 (3H, s, 14-OAc), 1.94 (3H, s, 2-OAc), 1.90 (3H, s, H-19), 1.62 (3H, s, H-20), 0.97 (3H, s, H-15). **6b**: HR-FAB MS: Obsd; *m*/*z* 649.2635. Calcd for $C_{34}H_{40}O_9F_3$; *m*/*z* 649.2625 $(M+H)^+$. ^1H NMR ($CDCl_3$, δ): 5.68 (1H, d, J=9.7 Hz, H-7), 5.61 (1H, ddd, J=12.8, 5.5, 1.8 Hz, H-3), 5.29 (1H, d, J=9.7 Hz, H-6), 5.24 (1H, d, J=6.1 Hz, H-12), 4.92 (1H, dd, J=2.1, 2.1 Hz, H-14), 4.85 (1H, d, J=1.8 Hz, H-2), 3.02 (1H, d, J=15.3 Hz, H-9a), 2.84 (1H, dd, J=12.8, 5.5 Hz, H-4a), 2.67 (1H, m, H-10), 2.62 (1H, dd, J=15.3, 6.3 Hz, H-9b), 2.56 (1H, dd, J=12.8, 12.8 Hz, H-4b), 2.23 (1H, brd, J=19.5 Hz, H-13a), 2.08 (3H, s, H-16), 2.01 (1H, m, H-13b), 1.93 (3H, s, 14-OAc), 1.91 (3H, s, H-19), 1.81 (3H, s, 2-OAc), 1.59 (3H, s, H-20), 0.90 (3H, s, H-15).

3.1.3. Catalytic reduction of brianthein A (1). A solution of **1** (10 mg) in MeOH (5 ml) was treated with 10% Pd–C (5 mg) at room temperature for 24 h under a H_2 atmosphere. The reaction mixture was filtered, evaporated and purified by reverse phase HPLC (Mightysil RP-18 GP, MeOH–H_2O=7:3) to furnish **12** (2.5 mg). **12**: HR-FAB MS: Obsd; *m*/*z* 477.2463. Calcd for $C_{26}H_{37}O_8$; *m*/*z* 477.2489 $(M+H)^+$. ^1H NMR ($CDCl_3$, δ): 5.64 (1H, d, J=9.7 Hz, H-7), 5.50 (1H, ddd, J=12.8, 5.5, 1.8 Hz, H-3), 5.19 (1H, d, J=9.7 Hz, H-6), 4.97 (1H, d, J=1.8 Hz, H-2), 4.89 (1H, d, J=2.1 Hz, H-14), 3.05 (1H, d, J=15.3 Hz, H-9a), 2.64 (1H, dd, J=5.5, 12.8 Hz, H-4a), 2.40 (1H, dd, J=12.8, 12.8 Hz, H-4b), 2.39 (1H, dd, J=15.3, 6.3 Hz, H-9b), 2.10 (3H, s, H-16), 2.09 (3H, s, 3-OAc), 2.07 (3H, s, 14-OAc), 2.00 (3H, s, 2-OAc), 1.90 (3H, s, H-19), 1.71 (1H, m, H-13a), 1.62 (1H, m, H-13b), 1.57 (1H, m, H-10), 1.43 (1H, m, H-11), 1.40 (1H, m, H-12a), 1.31 (1H, m, H-12b), 0.96 (3H, s, H-15), 0.91 (3H, d, J=6.1 Hz, H-20).

3.2. Conformational analysis of brianthein A (1) and agosterol A (13)

Brianthein A (**1**) and agosterol A (**13**) were dissolved into $CDCl_3$ at a concentration of 15 mg ml^{-1}. The NOESY spectrum with 1500 ms mixing time was recorded on an INOVA 600 spectrometer (600 MHz, Varian) at 25°C. Data points of $1024(t_1)\times512(t_2)$ in complex points were acquired with a phase-sensitive mode. The spectral width in both axes was 5000 Hz. The relaxation delay was 3.0 s. Such time domain data were zero-filled once and a $\pi/3$ shifted squared sine bell was multiplied in both axes, then Fourier transformed up to $2048(f_1)\times2048(f_2)$ real points. Restrained molecular dynamic calculations were carried out on an O_2 R10000 (Silicon Graphics). NOEs were classified into three classes depending on their intensities, and then were translated into distance restraints (upper bound of distance: 2.8, 3.5 and 5.0 Å). During molecular dynamics, maximum force restraints of bond distance and dihedral angle were set at 25 kcal mol^{-1} A^{-2} and 50 kcal mol^{-1} rad^{-2}, respectively. Non-bonded repulsion function was cut off at 10.0 Å. To maintain correct chirality, chiral restraints were applied to each chiral carbon with 10 kcal mol^{-1} of the force constant apparent for the $F_{kchiral}$ parameter in the NMRchitect-Discover package (MSI). In the first step, an initial structure of **1** and **13** were built with a complete random array of atoms. For all energy minimizations the conjugate-gradient method was used and 1500 steps were needed for convergence. Then, simulated annealing was executed for 30 ps at 1000 K using RA-VCF method.[15,16] While the temperature was cooled down stepwise to 300 K, further 30 ps of dynamics were executed. Then, the obtained structure was again refined by energy-minimization once again. RMSD value and other resulting values were obtained using Insight-II (MSI) and in-house C shell and awk programs.

3.3. Bioassay

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^{-1} of glutamine, 50 μg ml^{-1} of kanamycin sulfate, supplemented with 10% newborn calf serum. MDR cell line, KB-C2, was selected from KB-3-1 cells[13] and maintained in the medium containing 2 μg ml^{-1} of colchicine. Reversing activity and cytotoxicity were measured by means of MTT colorimetric assay performed in 96-well plates. Equal numbers of cells (10000) were inoculated into each well with 100 μl of the culture medium. After 24 h preincubation (37°C, 5% CO_2), a 50 μl solution of an anticancer agent (colchicine to KB-C2) and testing sample were added to each well and the whole were further incubated for 48 h. Thereafter, 25 μl of MTT solution (2 mg ml^{-1} in PBS) was added to each well and incubated for further 3 h. After removing the medium by aspiration, the resulting formazan was dissolved in 200 μl of dimethylsulfoxide. 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.

Acknowledgements

The authors thank Dr Yehuda Benayahu, Department of Zoology, Faculty of Life Sciences, Tel Aviv University, for identification of the gorgonian. The authors are also grateful to the Houansha Foundation, and the Ministry of Education, Science, Sports and Culture of Japan for financial support.

References

1. Gerlach, J. H.; Endicott, J. A.; Juranka, P. F.; Henderson, G.; Sarangi, F.; Deuchars, K. L.; Ling, V. *Nature* **1986**, *324*, 485–489.
2. Aoki, S.; Matsui, K.; Tanaka, K.; Rachmaniar, S.; Kobayashi, M. *Tetrahedron* **2000**, *56*, 9945–9948 and preceding papers.
3. Bowden, B. F.; Coll, J. C.; Vasilescu, I. M. *Aust. J. Chem.* **1989**, *42*, 1705–1726.
4. Sheu, J. H.; Sung, P.-J.; Cheng, M.-C.; Liu, H.-Y.; Fang, L.-S.; Duh, C.-Y.; Chiang, M. Y. *J. Nat. Prod.* **1998**, *61*, 602–608.
5. Akiyama, S.; Fojo, A.; Hanover, J. A.; Pastan, I.; Gottesman, M. M. *Somat. Cell. Mol. Genet.* **1985**, *11*, 117–126.
6. Kusumi, T.; Ohtani, I.; Inoue, M.; Kakisawa, H. *Tetrahedron Lett.* **1988**, *29*, 4731–4734.
7. (a) Knappe, E.; Peteri, D. Z. *Anal. Chem.* **1962**, *190*, 386–389. (b) Kobayashi, M.; Son, B. W.; Kyogoku, Y.; Kitagawa, I. *Chem. Pharm. Bull.* **1984**, *32*, 1667–1670.
8. Bloor, S. J.; Schmitz, F. J.; Hossain, M. B.; van der Helm, D. *J. Org. Chem.* **1992**, *57*, 1205–1216.
9. Guerriero, A.; D'Ambrosio, M.; Pietra, F. *Helv. Chim. Acta* **1995**, *78*, 1465–1478.
10. Neve, J. E.; McCool, B. J.; Bowden, B. F. *Aust. J. Chem.* **1999**, *52*, 359–366.
11. Cardellina, II, J. H.; James, Jr., T. R.; Chen, M. H. M.; Clardy, J. *J. Org. Chem.* **1984**, *49*, 3398–3399.
12. (a) Aoki, S.; Yoshioka, Y.; Miyamoto, Y.; Higuchi, K.; Setiawan, A.; Murakami, N.; Chen, Z. S.; Sumizawa, T.; Akiyama, S.; Kobayashi, M. *Tetrahedron Lett.* **1998**, *39*, 6303–6306. (b) Aoki, S.; Setiawan, A.; Yoshioka, Y.; Higuchi, K.; Fudetani, R.; Chen, Z-S.; Sumizawa, T.; Akiyama, S.; Kobayashi, M. *Tetrahedron* **1999**, *55*, 13965–13972.
13. Aoki, S.; Chen, Z. S.; Higashiyama, K.; Setiawan, A.; Akiyama, S.; Kobayashi, M.; Jpn, J. *Cancer Res.* **2001**, *92*, 886–895.
14. Aoki, S.; Nemoto, N.; Kobayashi, Y.; Kobayashi, M.; Kitagawa, I. *Tetrahedron* **2001**, *57*, 2289–2292.
15. Fujikawa, A.; In, Y.; Ishida, T.; Inoue, M.; Nemoto, N.; Kobayashi, Y.; Kataoka, R.; Ikai, K.; Takesako, K.; Kato, I. *J. Org. Chem.* **1994**, *59*, 570–578.
16. Nilges, M.; Clore, G. M.; Gronenborn, A. M. *FEBS Lett.* **1988**, *239*, 129–136.