Tetrahedron 67 (2011) 782–792

Contents lists available at ScienceDirect

Tetrahedron

journal homepage: www.elsevier.com/locate/tet

Absolute stereostructure of Andirolides A–G from the flower of *Carapa guianensis* (Meliaceae)

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ARTICLE INFO

Article history:
Received 30 August 2010
Received in revised form 5 November 2010
Accepted 5 November 2010
Available online 13 November 2010

Keywords:
Carapa guianensis
Limonoid
Andirolides A–G
CD spectrum
X-ray analysis

ABSTRACT

A new gedunin, three new mexicanolides and three new phragmalin-type limonoids named Andirolides A (**1**), B (**2**), C (**3**), D (**4**), E (**5**), F (**6**), and G (**7**) were isolated from oil of the flower of *Carapa guianensis* Aublet (Meliaceae). Their absolute stereostructures were determined by 2D NMR and CD spectra, and single-crystal X-ray analysis, and all compounds were confirmed to have the C-17β*H* configuration. Considering the similarity in CD spectra between Andirolide G (**7**) and the xyloccensins reported by Wu, we concluded that the structures of xyloccensins should be revised so as to have the absolute configuration of 17*R*.

1. Introduction

Carapa guianensis Aublet (Meliaceae), referred to andiroba or Brazilian mahogany in Brazil and Colombia, is a towering tree, which grows up to 40 m high in the tropical rainforests of South America. The tree has been used not only as timber but also as material for herbal medicines by indigenous people in the Amazon. In particular, the oil collected from the seeds is used as an insect repellant and as a standing medicine for the treatment of wounds and bruises. Interestingly, gedunin-type limonoids bearing the 4,4,8-trimethyl-17-furanylsteroid (tetra-*nor*-triterpene) skeleton, such as andirobin,[1] 7-deacetoxy-7-oxogedunin,[2] 11β-acetoxygedunin and 6α,11β-diacetoxygedunin,[3] 6α-acetoxy-epoxyazadiradione, 6α-acetoxygedunin and 6α-hydroxygedunin,[4] 6β,11β-diacetoxygedunin and 6α-acetoxygedunin,[5] and 1,2-dihydro-3β-hydroxy-7-deacetoxy-7-oxogedunin,[6] were isolated from the seeds or wood; however, the presence of mexicanolide- or phragmalin-type limonoids, which are biosynthesized via gedunin-type limonoids, have not been reported to date. As our initial hypothesis, the flower moiety, the most highly differentiated organ in the plant body, could contain novel metabolites produced downstream of the biosynthetic pathways. Thus, we investigated the components of the flower oil of *C. guianensis* as a part of our study on bioactive limonoids from Maliaceae plants. As expected, three new mexicanolide and three new phragmalin-type of limonoids named Andiloride B (**2**), C (**3**), D (**4**), E (**5**), F (**6**), and G (**7**), respectively, were obtained as well as a novel gedunin-type Andiloride A (**1**). In the present paper, we report the details of their isolation, structure determination, and cytotoxic activities against P388, KB, L1210, and HL-60 cell lines. Moreover, we discuss the absolute structures of these compounds and propose the necessity of reexaming those of xyloccensins Q–V in the literature.[7,8]

2. Results and discussion

Flower oil of *C. guianensis* was dissolved in $CHCl_3$, the extract of which was separated by silica gel column chromatography, medium-pressure liquid chromatography (MPLC), and reverse phased HPLC to obtain seven new limonoids (**1**–**7**) along with the known 7-deacetoxy-7-oxogedunin (**8**)[9] and 6α-acetoxygedunin (**9**).[4,10]

Andirolide A (**1**) was isolated as colorless needles and demonstrated to have the molecular formula $C_{30}H_{36}O_9$ ($[M]^+$+H; *m*/*z* 541.2442, calcd for 541.2437) by HRFABMS. The ^1H and ^{13}C NMR spectra, which were characteristic of a gedunin limonoid such as 6α-acetoxygedunin (**9**),[4,10] indicated the presence of five tertiary methyls [δ_H 1.16, 1.20, 1.24, 1.27, and 1.47], two acetyl methyls [δ_H 2.02, 2.07], two methylenes, four sp^3 methines including two oxymethines [δ_H 5.47 (dd), 5.50 (d)], an α,β-unsaturated ketone [δ_H 5.94 (d), 7.06 (d), δ_C 204.0 (s)], a β-substituted furan ring [δ_H 6.47 (dd), 7.43 (t), 7.57 (dd)], five sp^3 quaternary carbons including an

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doi:10.1016/j.tet.2010.11.028

acetal or hemiacetal carbon [δ_C 104.0 (s)], and an α,β-unsaturated lactone [δ_C 111.3 (d), 163.2 (s), 169.3 (s)] (Table 1). The IR spectrum showed the presence of a hydroxyl group at ν_{max} 3448 cm^{-1}. In addition, a combination of the IR absorption at ν_{max} 1672 cm^{-1} and the UV absorption λ_{max} at 236 nm (log ε 3.49) supported the

Table 1
^1H NMR and ^{13}C NMR spectroscopic data of compound **1**

Position		**1**		
		$^1H^a$ (*J*, Hz)		$^{13}C^b$
1		7.06	d 10.2 (2)	155.8
2		5.94	d 10.2 (1)	126.7
3				204.0
4				44.8
5		2.51	d 12.2 (6)	47.5
6		5.47	dd 12.2 (5) 2.4 (7)	69.6
7		5.50	d 2.4 (6)	73.1
8				45.0
9		2.24	dd 11.7 (11β), 8.6 (11α)	36.2
10				41.2
11	α	1.99	m	15.3
	β	1.83	m	
12	α	1.60	m	23.4
	β	2.28	m	
13				42.1
14				169.3
15		5.67	s	111.3
16				163.2
17				104.0
18		1.16	s	23.7
19		1.24	s	20.7
20				125.2
21		7.57	dd 2.2 (23), 1.2 (22)	141.8
22		6.47	dd 2.2 (23), 1.2 (21)	109.9
23		7.43	t 2.2 (22, 21)	143.2
28		1.27	s	31.6
29		1.20	s	20.5
30		1.47	s	23.7
6′				170.3
6"		2.07	s	21.3
7′				170.0
7"		2.02	s	20.7

[a] Measured at 500 MHz in $CDCl_3$.
[b] Measured at 125 MHz in $CDCl_3$.

presence of α,β-unsaturated carbonyl groups. Next, the results of the $^1H-^1H$ COSY analysis of **1** revealed the partial structures shown by bold-faced lines in Fig. 2. Herein, it should be particularly noted that H-6 (δ_H 5.47) was correlated with H-5 (δ_H 2.51) and H-7 (δ_H 5.50). In the HMBC spectrum, cross-peaks were observed from Me-18/C-12, C-13, C-14, and C-17 [δ_C 104.0 (s)]; Me-19/C-1, C-5, C-9, and C-10; H-5/C-4, C-6 [δ_C 69.6 (d)], and C-7 [δ_C 73.1 (d)]; H-6/C-5, C-6′ [δ_C 170.3 (s)], C-7 [δ_C 73.1 (d)], and C-8; H-15/C-13, and C-16 [δ_C 163.2 (s)] (Fig. 2). Furthermore, two methyl groups at δ_H 2.02 (3H, s) and 2.07 (3H, s) showed HMBC correlations with carbonyl groups δ_C 170.0 (s) and 170.3 (s), respectively, indicating the presence of the two-acetyl groups. Finally, the hydroxyl group was attached at C-17 to satisfy its molecular formula ($C_{30}H_{36}O_9$) and the presence of a hemiacetal carbon at δ_C 104.0 (s). Regarding the relative configuration of chiral centers, the hydroxyl group at C-17 was assigned as β, because NOEs were observed between Me-18 and H-9α, H-12α and H-21 (Fig. 3). The relative configuration at C-6 was determined to be α because of the significant NOEs between H-6 and Me-19, Me-29 and Me-30, and the coupling constants of H-6 [δ_H 5.47 (dd, $J_{6\beta,5\alpha}$=12.2 Hz, $J_{6\beta,7\beta}$=2.4 Hz)]. The configuration of C-7 was α due to the significant NOEs between H-7 and H-15 and Me-30, and the coupling constants of H-7 [δ_H 5.50 (d, $J_{7\beta,6\beta}$=2.4 Hz)], the same as **9**.[4,10] HMBC and NOESY spectra showing the relative structure of **1** are shown in Figs. 2 and 3.

Andirolide B (**2**), isolated as colorless needles, had the molecular formula $C_{31}H_{38}O_{11}$ ($[M]^+$+H; *m/z* 587.2493, calcd for 587.2492) as determined by HRFABMS. The ^1H and ^{13}C NMR spectra, which were characteristic of mexicanolide,[11–13] indicated the presence of four tertiary methyls [δ_H 0.74, 0.90, 1.18, 1.32], two acetyl methyls (δ_H 2.18, 2.22), a methyl ester group (δ_H 3.67), four methylenes, four sp^3 methines including two oxymethines [δ_H 5.24 (s), 5.32 (s)], five sp^3 quaternary carbons including two oxycarbons [δ_C 72.1 (s), 83.5 (s)], four sp^2 methines including a furan ring [δ_H 6.50 (dd), 7.44 (t), 7.49 (br s)], and seven sp^2 quaternary carbons including two acetyl C=O [δ_C 169.8 (s), 171.2 (s)], a $COOCH_3$ [δ_C 173.3 (s)], and an α,β-unsaturated δ-lactone [δ_C 165.3 (s)] (Table 2). The IR absorption at ν_{max} 3448 cm^{-1} showed the presence of a hydroxyl group. The combination of IR absorption at ν_{max} 1672 cm^{-1} and UV absorption λ_{max} at 236 nm (log ε 3.49) supported the presence of α,β-unsaturated carbonyl groups. After assignments of HMQC, $^1H-^1H$ COSY and HMBC spectra (Fig. 4), it became clear that **2** was a common mexicanolide.[11–13] In the NOESY spectrum, significant NOEs were observed between H-5β and H-11β and H-30β; between H-9α and H-12α; between H-11α and Me-19; between H-12β and H-17β; between H-15 and H-30α. The relative structure was confirmed from the NOESY spectrum (Fig. 5).

The molecular formula of Andirolide C (**3**) and Andirolide D (**4**) was assigned as $C_{33}H_{42}O_{11}$ ($[M]^+$+H; *m/z* 615.2800, calcd for 615.2805) and $C_{34}H_{42}O_{11}$ ($[M]^+$+H; *m/z* 627.2798, calcd for 627.2805), respectively, based on HRFABMS. The UV and IR spectra of **3** and **4** showed the presence of a hydroxyl (ν_{max} 3445 cm^{-1} in **3**; ν_{max} 3439 cm^{-1} in **4**), an α,β-unsaturated δ-lactone (ν_{max} 1680 cm^{-1}; λ_{max} 224.6 nm in **3**; λ_{max} 227.5 nm in **4**) and ester groups (ν_{max} 1259 cm^{-1}). According to the structures of **3** and **4**, their UV, IR, and ^1H and ^{13}C NMR spectra were very similar to those of **2** except for the absence of an acetyl group at C-3 and the presence of a 2-methylpropanoyl group [δ_H 1.25 and 1.26 (each 3H, d, *J*=6.9 Hz); 2.73 (1H, sept. *J*=6.9 Hz); 5.21 (1H, s)] in **3**, and the presence of a tigloyl group [δ_H 1.89 (3H, d, *J*=7.0 Hz), 1.94 (3H, s), 6.97 (1H, qd, *J*=7.0, 1.4 Hz)] in **4** (Table 2).[14] In the HMBC spectrum of **3**, cross-peaks were observed from H-3/C-3′ and isopropyl methyl/C-3′, and cross-peaks were observed from H-3/C-3′, tigloyl group/C-3′ in compound **4**; therefore, the relative structures of **3** and **4** were established as shown in Fig. 1.

Andirolide E (**5**), isolated as colorless prisms, had the molecular formula $C_{35}H_{40}O_{14}$ ($[M]^+$+H; *m/z* 685.2493, calcd for 685.2496) as determined by HRFABMS. The ^1H and ^{13}C NMR spectra indicated the presence of two angular methyls [δ_H 0.98, 1.13 (each 3H, s)], two acetyl methyls [δ_H 2.15 and 2.24 (each 3H, s)], a primary methyl group [δ_H 1.05 (3H, t)], another methyl group [δ_H 1.69 (3H, s), δ_C 21.6 (q)], seven methylenes including an oxymethylene [δ_H 4.38 and 4.77 (each d, *J*=13.8 Hz)], five sp^3 methines including three oxymethines [δ_H 5.16 (s), 5.35 (s), 6.10 (s)], eight sp^3 quaternary carbons, a furan ring [δ_H 6.41 (d), 7.44 (t), 7.49 (s)], and two lactones [δ_C 169.8 (s), 171.0 (s)] (Table 3), which were characteristic of phragmalin limonoids.[7,8,15] Alkaline hydrolysis of **5** with KOH/MeOH yielded a triol (**5a**), in which the two carbinolic methine proton signals were considerably up-field shifted at [δ_H 3.61 and 4.49 (each s)]. In the HMBC spectrum, cross-peaks were observed from Me-18/C-12, C-13, C-14, and C-17; H-3/C-1, C-4, and C- 3′; H-14/C-8, and C-16; H-17/C-13, C-20, C-21, and C-22; H-30/C-9, C-30, and C-30′ (Fig. 6). Two methyl groups at δ_H 2.15 and 2.24 (each 3H, s) showed HMBC correlations with carbonyl groups δ_C 170.2 (s) and 169.5 (s), respectively, indicating the presence of the two-acetyl groups. In addition, the COSY correlation between a primary methyl [δ_H 1.05 (3H, t)] and methylene signals [δ_H 2.44 (1H, dq), 2.46 (1H, dq)], correlated with the carbonyl carbon [δ_C 172.1 (s)], indicated the presence of a propanoyl group.

A quaternary carbon at δ_C 119.4 (C-31) showing an HMBC correlation with H_3-32 [δ_H 1.69 (s)], suggested the presence of an orthoacetyl group. A pair of geminal doublets at δ_H 1.73 (d, *J*=11.8 Hz) and 2.33 (d, *J*=11.8 Hz) was assigned to H_2-29 in A-ring.

Table 2
^{1}H NMR and ^{13}C NMR spectroscopic data of compounds **2**–**4**

Position		**2**			**3**			**4**		
		^{1}H[a] (*J*, Hz)		^{13}C[b]	^{1}H[a] (*J*, Hz)		^{13}C[b]	^{1}H[a] (*J*, Hz)		^{13}C[b]
1				208.3			208.2			208.4
2				83.5			83.6			83.7
3		5.24	s	81.8	5.21	s	81.6	5.31	s	81.8
4				40.3			40.3			40.6
5		3.08	t 5.7 (6)	41.4	3.10	t 5.5 (6)	41.5	3.14	t 5.9 (6)	41.4
6		2.34	d 5.7 (5)	33.3	2.34	d 5.5 (5)	33.3	2.34	d 5.9 (5)	33.3
7				173.3			173.3			173.3
8				72.1			72.0			72.0
9		2.15	dd 13.0 (11β), 4.6 (11α)	64.6	2.16	dd 13.0 (11β), 4.6 (11α)	64.6	2.16	dd 13.1(11β), 4.1(11α)	64.6
10				50.2			50.3			50.2
11	α	1.70	dtd 13.0 (11β), 4.6 (9, 12β), 2.0 (12α)	21.0	1.70	dtd 13.0 (11β), 4.6 (9, 12β), 2.0 (12α)	30.3	1.70	dtd 13.1 (11β), 4.1 (9, 12β), 2.2 (12α)	21.0
	β	1.42	qd 13.0 (9, 11α, 12α), 2.0 (12β)		1.42	qd 13.0 (9, 11α, 12α), 2.0 (12β)		1.43	dq 13.1(9, 11α, 12α), 2.2 (11α)	
12	α	1.53	td 13.0 (11β, 12β), 2.0 (12β)	30.4	1.54	td 13.0 (11β, 12β), 2.0 (11α)	21.0	1.53	td 13.1 (11β, 12β), 2.2 (11α)	30.5
	β	1.83	ddd 13.0 (12α), 4.6 (11α), 2.0 (11β)		1.83	ddd 13.0 (12α), 4.6 (11α), 2.0 (11β)		1.83	ddd 13.1 (12α), 4.1 (11α), 2.2 (11β)	
13				39.5			39.6			39.6
14				166.8			166.8			167.0
15		6.26	s	115.7	6.22	s	115.6	6.19	s	115.6
16				165.3			165.2			165.1
17		5.32	s	79.2	5.33	s	79.1	5.33	s	79.1
18		1.32	s	21.6	1.30	s	21.5	1.31	s	21.6
19		1.18	s	17.2	1.17	s	17.1	1.18	s	17.2
20				120.1			120.1			120.1
21		7.49	br s	141.8	7.49	br s	141.8	7.49	br s	141.8
22		6.50	dd 1.8 (23), 0.9 (21)	110.6	6.50	dd 1.8 (23), 0.7 (21)	110.6	6.50	dd 1.6 (23), 0.7 (21)	110.6
23		7.44	t 1.8 (21, 22)	143.0	7.44	t 1.8 (21, 22)	143.0	7.44	t 1.6 (21, 22)	143.1
28		0.74	s	22.3	0.72	s	22.4	0.73	s	23.1
29		0.90	s	23.3	0.91	s	23.3	0.93	s	22.5
30	α	2.39	d 17.1 (30β)	44.6	2.38	d 17.2 (30β)	44.7	2.42	d 17.2 (30β)	44.6
	β	4.14	d 17.1 (30α)		4.19	d 17.2 (30α)		4.22	d 17.2 (30α)	
2′				171.2			171.2			171.3
2″		2.18	s	21.7	2.16	s	21.6	2.17	s	21.7
3′				169.8			175.8			166.6
3″		2.22	s	20.9	2.73	sept. 6.9 (3‴, 3⁗)	34.3			127.8
3‴					1.26	d 6.9 (3")	18.9	1.94	s	12.4
3⁗					1.25	d 6.9 (3")	19.4	6.97	qd 7.0 (3‴) 1.4	139.3
3‴								1.89	d 7.0 (3⁗)	14.7
7′		3.67	s	52.2	3.68	s	52.2	3.67	s	52.2
8-OH		5.03	s		5.08	s		5.03	s	

[a] Measured at 500 MHz in $CDCl_3$.
[b] Measured at 125 MHz in $CDCl_3$.

The IR spectrum showed broad ester bands at ν_{max} 1751, 1723, and 1243 cm^{-1}, which could be assigned to two acetyl and a propanoyl esters. Thus, the planar structure of **5** was established as phragmalin-1,8,9-orthoacetate,[16] and the positions of a propanoyl group, two acetyl groups, and two δ-lactones were determined from HMBC and ^{1}H–^{1}H COSY correlations (Fig. 6). The location of an orthoacetate and the relative structure of **5** were determined from the NOESY spectrum (Fig. 7) and by single-crystal X-ray diffraction (Fig. 8). In the NOESY spectrum, significant NOEs were observed between H-5β and H-12β, H-30β and Me-28; between H-15β and H-30β; between H-17β and H-12β, H-22 and H-30β; and between H-30β and H-12β, H-15β, and H-17β; therefore, the C-ring adopted a boat-like conformation similar to switenialide D.[17]

Andirolide F (**6**), obtained from colorless needles, had the molecular formula $C_{35}H_{38}O_{14}$ ($[M]^+$+H; *m*/*z* 683.2335, calcd for 683.2340) as determined by HRFABMS. The UV and IR spectra showed α,β-unsaturated δ-lactone and ester groups. The IR, ^{1}H, and ^{13}C NMR spectra were very similar to those of **5** except for a double bond at C-14:15 [δ_H 6.05 (s), δ_C 121.0 (d), 159.6 (s)] (Table 3). NOESY experiments revealed the relative stereochemistry of **6** to have the same conformation as **5**.

Andirolide G (**7**), isolated as colorless needles, had the molecular formula $C_{34}H_{40}O_{14}$ ($[M]^+$+H; *m*/*z* 673.2505, calcd for 673.2495) as determined by HRFABMS. The IR spectrum showed the presence of a hydroxyl at ν_{max} 3568 cm^{-1}, and ester groups at ν_{max} 1729 cm^{-1}. The ^{1}H and ^{13}C NMR spectra indicated the presence of two angular methyls [δ_H 1.32, 1.48 (each 3H, s)], an acetyl methyl [δ_H 2.09 (3H, s)], a propanoyl group [δ_H 1.16 (3H, t), 2.44 (1H, dq), 2.46 (1H, dq)], a methyl ester group [δ_H 3.71 (3H, s), δ_C 173.8 (s)], an α,β-unsaturated lactone group [δ_H 6.62 (1H, s), δ_C163.5 (s)], an orthoacetate [δ_H 1.70 (3H, s), δ_C 16.5 (q), 119.7 (s)], a secondary hydroxyl group [δ_H 3.87 (ddd)], a tertiary hydroxyl group [δ_C 84.1 (s)] and a furan ring [δ_H 6.61 (dd), 7.53 (t), 7.64 (br s)] (Table 3). In the HMBC spectrum, cross-peaks were observed from H-3 [δ_H 5.22 (s)]/C-4, C-28, C-30 [δ_C 74.3 (d)], and C-3′ [δ_C 169.1 (s)]; H-5/C-3 [δ_C 85.0 (s)], C-4, C-6, C-7 [δ_C 173.8 (s)], C-9 [δ_C 86.1 (s)] and C-29; 1-OH [δ_H 3.40 (s)]/C-1 and C-29; H-30 [δ_H 5.35 (s)]/C-1, C-2, C-3 [δ_C 85.0 (s)], C-8 [δ_C 83.6 (s)], C-9, C-14 [δ_C 153.8 (s)], and C-31 [δ_C 119.7 (s)]. The positions of an acetoxyl, a propanoyl, a hydroxyl, a carbomethoxyl, and an orthoacetyl were located by detailed ^{1}H–^{1}H COSY and HMBC correlations (Fig. 9). In particular, the location of the orthoacetate was confirmed by the HMBC correlation between H-30 and C-31, in addition to the correlation between the hydroxyl proton at C-1 and C-29. In the NOESY spectrum, significant NOEs (Fig. 10) were observed between H-11α and Me-18; between H-11β and Me-19; between H-12β and H-5β, H-17β and H-21, therefore, the C-12 hydroxyl group [δ_H 3.87 (1H, dd, *J*=13.0 (11α), 4.5 (11β))] was attached at the α equatorial. The relative structure was established as a phragmalin 8,9,30-orthoacetate analog, which was once isolated from *Xylocarpus granatum*.[7,8,15]

Next, Andirolide G (**7**) showed two positive Cotton effects at 213 nm (Δε=+8.5) and 264 nm (Δε=+2.8), as did xyloccensin Q.

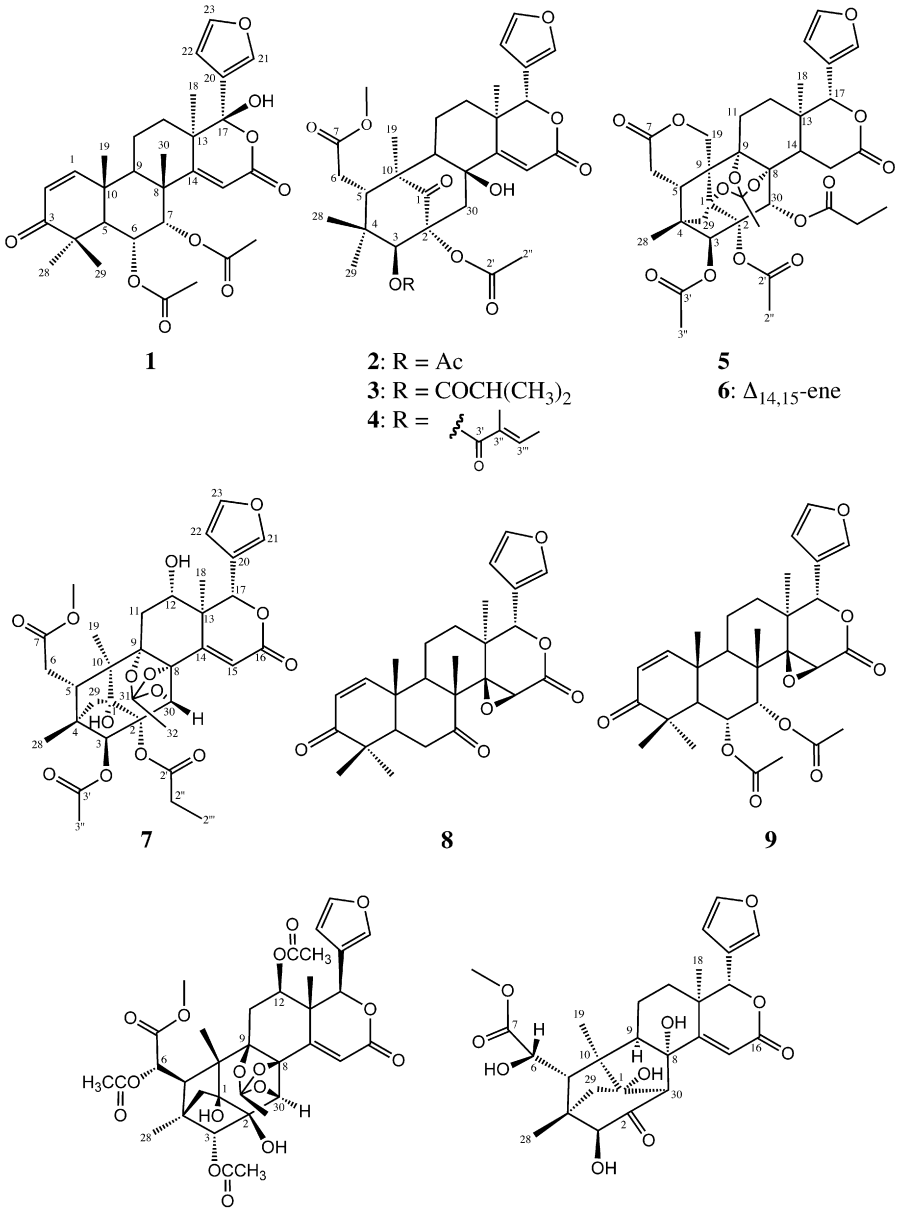


Fig. 1. Structures of compounds **1**–**9** isolated from *C. guianensis*, xyloccensins Q, and Khayanolide C.

Although both compounds should have the same absolute structure having the configuration of C-17*R*, as claimed by Wu, limonoids are known to be biosynthesized via euphane-type triterpenoid intermediates with 17β*H*; thus, it was necessary to reconfirm the absolute configuration of xyloccensins. Herein, CD spectra of compounds **1**–**4** and **6**–**7** were measured, which afforded the following results; compound **1** [235 ($\Delta\varepsilon$=+9.4), 264 ($\Delta\varepsilon$=+1.4), 299 ($\Delta\varepsilon$=−1.4), 340 ($\Delta\varepsilon$=−2.0)]; **2** [220 ($\Delta\varepsilon$=+13.5), 261 ($\Delta\varepsilon$=+1.8), 297 ($\Delta\varepsilon$=−4.4)]; **3** [219 ($\Delta\varepsilon$=+11.3), 260 ($\Delta\varepsilon$=+1.6), 297 ($\Delta\varepsilon$=−3.5)]; **4** [223 ($\Delta\varepsilon$=+13.7), 260 ($\Delta\varepsilon$=+1.2), 297 ($\Delta\epsilon$=−3.8)]; **6** [224 ($\Delta\varepsilon$=+7.6), 270 ($\Delta\varepsilon$=+6.1)]; **7** [213 ($\Delta\varepsilon$=+8.5), 268 ($\Delta\varepsilon$=+2.8)] (Fig. 11).

Since compound **1** had an α,β-unsaturated ketone on a six-membered ring and compounds **2**–**4** had a ketone on a six-membered ring in each molecule, some interactions between the ketones and the α,β-unsaturated lactone on C-17 would cause negative Cotton effects at 299 nm ($\Delta\varepsilon$=−1.4) and 340 nm ($\Delta\varepsilon$=−2.0) in **1** and at 297 nm in **2**–**4** ($\Delta\varepsilon$=−4.4,−3.5, and −3.8, respectively). This assumption was supported by the finding that compounds **6**–**7**, which have no functional groups to affect the CD spectra, showed no Cotton effects in the region of wavelengths longer than 300 nm (Fig. 12). Moreover, X-ray diffraction analysis of the 16-*p*-bromobenzoate **8d** derived from the most abundant limonoid, 7-deacetoxyl-7-oxogedunin (**8**) (Scheme 1), revealed the structure to have the absolute configuration as shown in Fig. 13. As a result, the absolute stereochemistry of Andirolides A–G (**1**–**7**) and **8** was determined as shown in Fig. 1.

Wu may have mistaken the stereochemistry of xyloccensins because the presence of the C-2 ketone was not considered, which could affect the CD spectrum of Khayanolide C. In conclusion, we estimated that the Cotton effects could be potentially affected by the interactions of a chromophore and a carbonyl group. Namely, the carbonyl groups of the α,β-unsaturated δ-lactone and the C-2 ketone were orientated in the same direction in Khayanolide C, while the carbonyl groups of the α,β-unsaturated lactone and ketone at the C-1 or C-3 position in compounds **1**–**4** faced in different directions (Fig. 12). Thus, the

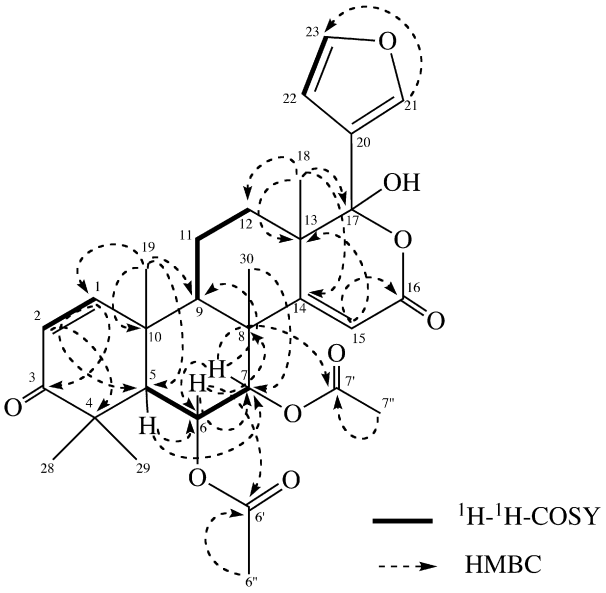


Fig. 2. Selected ^{1}H–^{1}H COSY and HMBC correlations in Andirolide A (**1**).

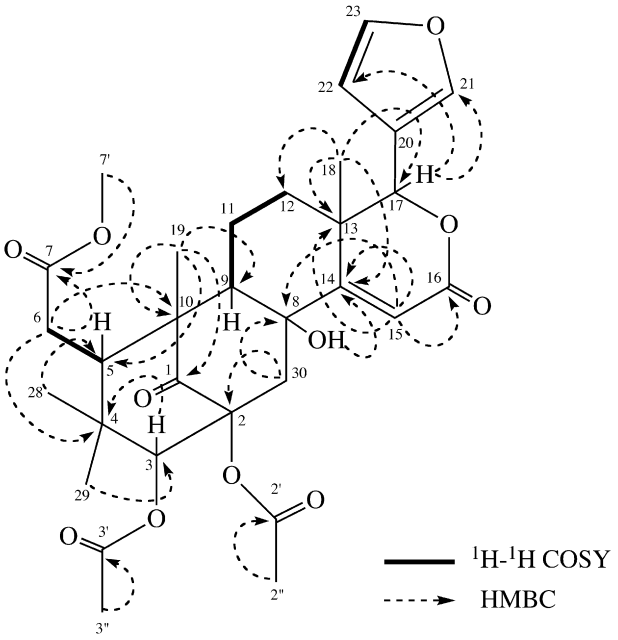


Fig. 4. Selected ^{1}H–^{1}H COSY and HMBC correlations in Andirolide B (**2**).

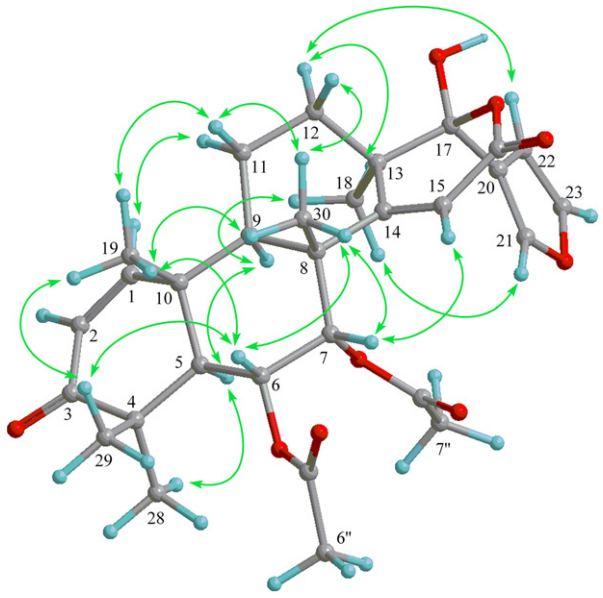


Fig. 3. Key NOESY correlations for Andirolide A (**1**).

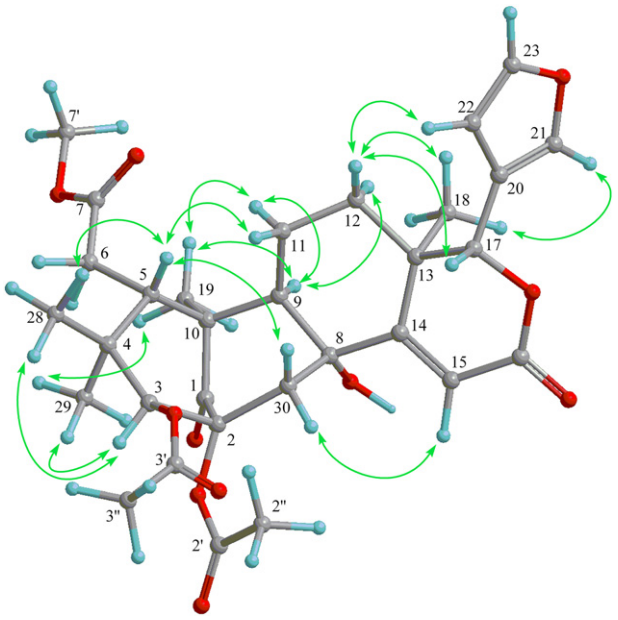


Fig. 5. Key NOESY correlations for Andirolide B (**2**).

negative Cotton effect at 245 nm ($\Delta\varepsilon$=− 4.3) in the CD spectrum of Khayanolide C would be caused by the interaction between parallel orientated carbonyl groups. Moreover, the first Cotton effect for the furan ring chromophore at 225 nm should have had a negative Cotton effect, if xyloccensins were enantiomers.

Wu took the reversed sign of $\Delta\delta$ values in applying the Mosher method using (*R*)-(−)- and (*S*)-(+)-MTPACl to determine the absolute structure of xyloccensin Q, i.e., (*R*)-MTPACl should afford (*S*)-esters; however, it was stated that the (*R*)-ester was generated.[18,19] The isolated compounds **1**–**7** were subjected to assays of growth inhibition using various cancer cell lines. As a primary screening for antitumor activities, the cell growth inhibitory properties of compounds **1**–**7** were examined using the murine P388 leukemia cell line, the human HL-60 leukemia cell line, the murine L1210 leukemia cell line and the human KB epidermoid carcinoma cell line. Compound **1** exhibited significant cytotoxic activity against all cell lines (Table 4).[20] Compound **6** also showed moderate cytotoxic activity.

3. Experimental

3.1. General procedures

Melting points were determined on a Yanagimoto micro-melting point apparatus and are uncorrected. Optical rotations were measured with a JASCO DIP-1000 digital polarimeter. IR spectra were recorded on a Perkin–Elmer 1720X FTIR spectrophotometer. ^{1}H and ^{13}C NMR spectra were recorded on a Varian INOVA 500 spectrometer with standard pulse sequences, operating at 500 and 125 MHz, respectively. $CDCl_3$ was used as the solvent and TMS as the internal standard. FABMS was recorded on a JEOL JMS-700 mass spectrometer. Column chromatography (silica gel, 70–230 mesh; Merck) and medium-pressure liquid chromatography (MPLC; silica gel,

Table 3
^{1}H NMR and ^{13}C NMR data for compounds **5**–**7**

Position		**5**			**6**			**7**		
		^{1}H[a] (*J*, Hz)		^{13}C[b]	^{1}H[a] (*J*, Hz)		^{13}C[b]	^{1}H[a] (*J*, Hz)		^{13}C[b]
1				85.9			83.9			84.1
2				85.2			84.6			83.4
3		5.16	s	81.3	5.27	s	81.3	5.22	s	85.0
4				46.2			46.4			44.6
5		2.62	dd 6.2 (6β), 4.0 (6α)	33.0	2.72	m	33.7	2.12	brd 10.5 (6)	39.9
6	α	2.63	dd 18.5 (6β), 4.0 (5)	30.8	2.58	m	31.4	2.35	m	33.7
	β	2.45	dd 18.5 (6α), 6.2 (5)		2.62	m				
7				171.0			170.9			173.8
8				85.7			84.2			83.6
9				85.1			82.6			86.1
10				45.1			46.9			48.1
11	α	1.88	m	25.7	2.16	m	25.6	1.98	dd 17.0 (11β), 13.0 (12)	34.6
	β	2.26	m		2.34	m		2.22	dd 17.0 (11α), 4.5 (12)	
12	α	1.49	m	29.1	1.49	m	26.5	3.87	ddd 13.0 (11α), 4.5 (11β), 2.0 (12-OH)	66.6
	β	1.42	m		1.64	m				
13				34.4			37.6			44.8
14		2.04	dd 10.5 (15α), 1.6 (15β)	42.8			159.6			153.8
15	α	2.72	dd 18.9 (15β), 10.5 (14)	26.5	6.05	s	121.0	6.62	s	123.7
	β	3.22	br s 18.9 (15α)							
16				169.8			163.0			163.5
17		5.35	s	78.6	5.10	s	80.4	5.90	s	78.8
18		1.13	s	20.7	1.14	s	18.6	1.48	s	13.0
19	α	4.77	d 13.8 (19β)	68.8	4.87	d 13.7 (19α)	68.6	1.32	s	15.4
	β	4.38	d 13.8 (19α)		4.36	d 13.7 (19β)				
20				120.9			119.4			121.4
21		7.49	s	140.9	7.52	br s	141.4	7.64	br s	142.4
22		6.41	d 1.6 (23)	109.6	6.44	dd 1.9 (23) 0.7 (21)	109.7	6.61	dd 1.6 (23) 0.7 (21)	109.6
23		7.44	t 1.6 (21, 22)	143.4	7.44	t 1.9 (21, 22)	143.3	7.53	t 1.6 (21, 22)	144.8
28		0.98	s	13.7	1.02	s	14.1	0.74		14.4
29	*pro-R*	1.73	d 11.8 (29β)	39.0	1.78	d 11.4 (29β)	39.2	1.72	d 10.7 (29β)	39.8
	pro-S	2.33	d 11.8 (29α)		2.38	d 11.4 (29α)		1.96	d 10.7 (29α)	
30		6.10	s	68.5	5.78	s	68.1	5.35	s	74.3
31				119.4			121.0			119.7
32		1.69	s	21.6	1.68	s	20.9	1.70	s	16.5
2′				170.2			170.0			173.9
2″	A	2.15	s	20.9	2.17	s	21.8	2.44	dq 13.8 (2"B), 7.6 (2‴)	28.1
	B							2.46	dq 13.8 (2"A), 7.6 (2‴)	
2‴								1.16	t 7.6 (2")	8.9
3′				169.5			169.1			169.1
3″		2.24	s	21.2	2.03	s	20.8	2.09	s	21.7
7′								3.71	s	52.3
30′				172.1			173.3			
30″	A	2.19	dq 11.2 (30″B), 7.3 (30‴)	27.7	2.24	m	27.4			
	B	2.21	dq 11.2 (30″A), 7.3 (30‴)		2.34	m				
30‴		1.05	t 7.3 (30″)	8.3	1.07	t 7.4 (30″)	8.5			
1-OH								3.40	s	
12-OH								1.23	d 2.0 (12)	

[a] Measured at 500 MHz in $CDCl_3$.
[b] Measured at 125 MHz in $CDCl_3$.

230–400 mesh; Merck) were conducted. HPLC was run on a JASCO PU-1586 instrument equipped with a differential refractometer (RI 1531). Fractions obtained from column chromatography were monitored by TLC (silica gel 60 F_{254}; Merck). Preparative TLC was carried out on Merck silica gel F_{254} plates (20×20 cm, 0.5 mm thick).

3.2. Materials

The oil of the flower of *C. guianensis* Aublet (Meliaceae), was collected in Amazon, Brazil, in March, 2006. A voucher specimen (CG-01-1) was deposited in the Herbarium of the Laboratory of Medicinal Chemistry, Osaka University of Pharmaceutical Sciences.

3.3. Isolation of compounds 1–8

The flower oil of *C. guianensis* Aublet (Meliaceae) (500 g) was dissolved in $CHCl_3$, and the $CHCl_3$ solution was subjected to CC (silica gel (7 kg); $CHCl_3$) affording a yellow oil (Fr. No. 17–21, 164.9 g), and a crystalline solid (Fr. No. 29–30, 31.3 g). The crystalline solid was repeatedly recrystallized from MeOH/$CHCl_3$ to give 7-deacetoxy-7-oxogedunin (**8**) (10.1 g). The yellow oil was rechromatographed over silica gel (2 kg) giving an amorphous solid (Fr. No. 19–31, 6.0 g), which was subjected to CC using *n*-hexane/AcOEt 5:1 to afford the residue F1 (Fr. No. 43–51, 900 mg), subjected to CC with *n*-hexane/AcOEt 3:1 giving the residue F2 (Fr. No. 65–76, 433 mg), subjected to CC with *n*-hexane/AcOEt 2:1 affording the residues F3 (Fr. No. 80–86, 1.0 g) and F4 (Fr. No. 87–100, 900 mg), and subjected to CC with *n*-hexane/AcOEt 1:1 to give F5 (Fr. No. 101–112, 380 mg). Residue F1 was subjected to CC (silica gel (230–400 mesh, 100 g); *n*-hexane/AcOEt 5:1) giving a crystalline solid (40 mg, Fr. No. 51–60), which was separated by HPLC (ODS, 60% CH_3CN) to afford compounds **3** (12 mg) and **4** (13 mg). Residue F2 was subjected to CC (silica gel (230–400 mesh, 100 g); *n*-hexane/AcOEt, 5:1–3:1) giving an amorphous solid (93 mg, Fr. No. 19–51), which was purified by HPLC (ODS, 60% MeOH) to afford compound **1** (15 mg). Residue F3 was subjected to CC (silica gel (230–400 mesh, 100 g); *n*-hexane/AcOEt, 5:1) to give a crystalline solid (1.0 g, Fr. No. 80–86), which was twice subjected to CC (silica gel (230–400 mesh, 100 g); *n*-hexane/

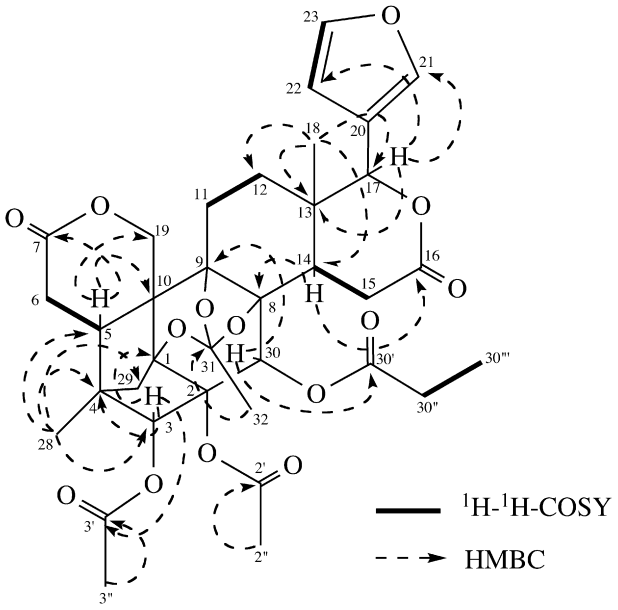


Fig. 6. Selected ^{1}H–^{1}H COSY and HMBC correlations in Andirolide E (**5**).

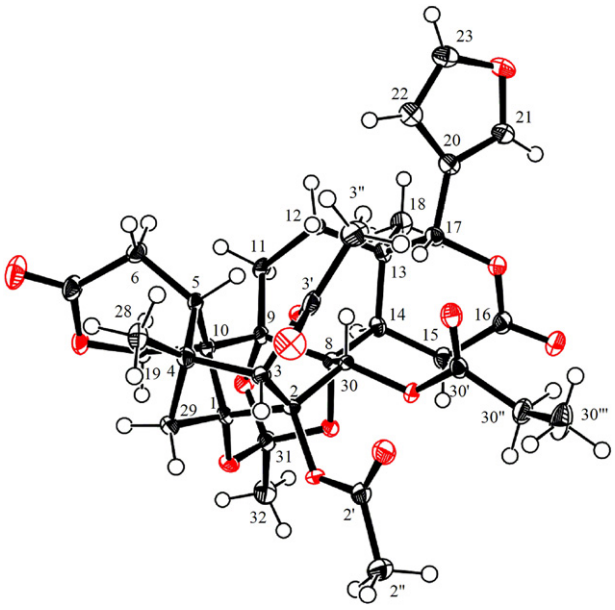


Fig. 8. ORTEP drawing of Andirolide E (**5**).

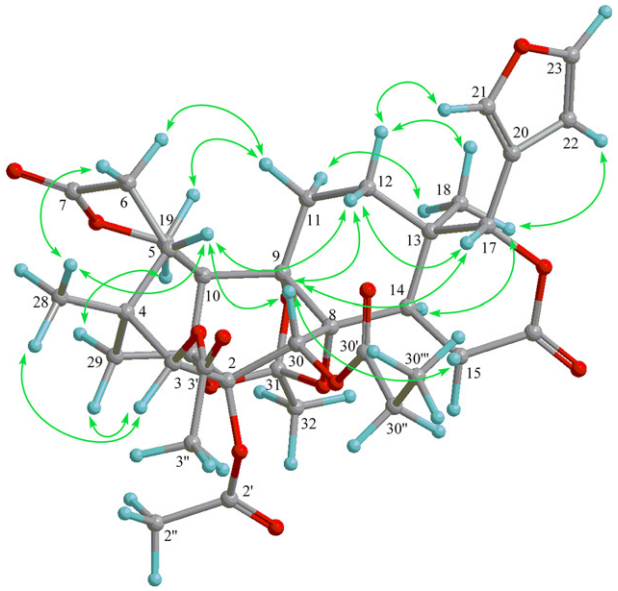


Fig. 7. Key NOESY correlations for Andirolide E (**5**).

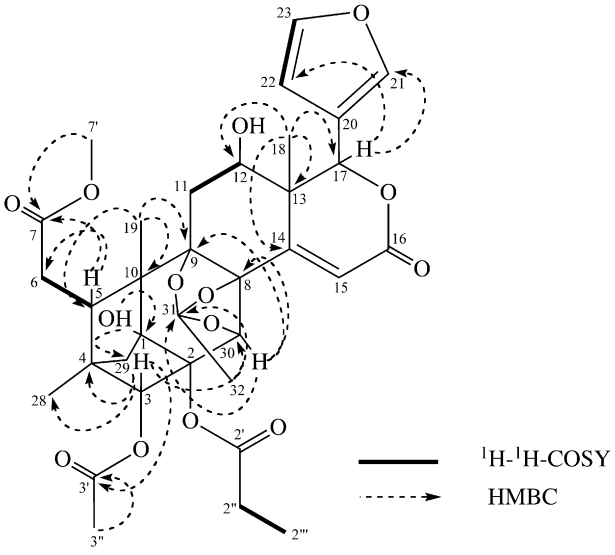


Fig. 9. Selected ^{1}H–^{1}H COSY and HMBC correlations in Andirolide G (**7**).

AcOEt, 5:1) to give a colorless solid (240 mg, Fr. No. 97–109), which in turn was separated by HPLC (ODS, 50% CH_3CN) to afford compounds **2** (10 mg) and **6** (12 mg). Residue F4 was subjected to CC (silica gel (230–400 mesh, 100 g); *n*-hexane/AcOEt 2:1) to give an amorphous solid (250 mg, Fr. No. 65–72), which was purified by HPLC (ODS, 60% MeOH) to afford compound **5** (100 mg). Residue F5 was subjected to CC (silica gel (230–400 mesh, 100 g); *n*-hexane/AcOEt, 2:1) to give an amorphous solid (78 mg, Fr. No. 86–120), which was purified by HPLC (silica gel, $CHCl_3$/MeOH, 50:1) to afford compound **7** (15 mg).

*3.3.1. Andirolide A (**1**).* Colorless needles; mp 151–153°C (MeOH/$CHCl_3$); $[\alpha]_D^{23}$ +3.1 (*c* 0.20, $CHCl_3$); HRFABMS *m*/*z*: 541.2442 $[M+H]^+$ ($C_{30}H_{37}O_9$, calcd for 541.2437); FABMS *m*/*z* (rel.int.):541 ($[M+H]^+$, 64), 523 (36), 481 (7), 463 (6), 421 (25), 403 (7); UV λ_{max} nm (log ε): 236 (3.49); IR ν_{max} cm^{-1}: 3448 (OH), 2979, 1742, 1723, 1672, 1459, 1370, 1246, 1031, 973, 875; CD λ nm ($\Delta\varepsilon$)[*c* 7.39×10^{-4} M, CH_3CN]: 235 (9.4), 251 (0.7), 264 (1.4), 281 (0), 299 (−1.4), 340 (−2.0), 393 (0). ^{1}H and ^{13}C NMR data, see Table 1.

*3.3.2. Andirolide B (**2**).* Colorless needles; mp 138–140°C (MeOH/$CHCl_3$); $[\alpha]_D^{23}$ −16.5 (*c* 0.13, $CHCl_3$); HRFABMS *m*/*z*: 587.2493 $[M+H]^+$ ($C_{31}H_{39}O_{11}$, calcd for 587.2492); FABMS *m*/*z* (rel int.): 587 ($[M+H]^+$, 100), 569 (12), 527 (9), 509 (8), 467 (56), 449 (11); UV λ_{max} nm (log ε): 223 (3.73); IR ν_{max} cm^{-1}: 3448 (OH), 2926, 1734, 1680, 1458, 1375, 1240, 1030, 875; CD λ nm ($\Delta\epsilon$)[*c* 4.37×10^{-4} M, CH_3CN]: 220 (13.5), 238 (0), 243 (−0.7), 249 (0), 261 (1.8), 273 (0), 297 (−4.4), 319 (0). ^{1}H and ^{13}C NMR data, see Table 2.

*3.3.3. Andirolide C (**3**).* Colorless needles; mp 138–140°C (MeOH/$CHCl_3$); $[\alpha]_D^{23}$ −37.3 (*c* 0.08, $CHCl_3$); HRFABMS *m*/*z*: 615.2800 $[M+H]^+$ ($C_{33}H_{43}O_{11}$, calcd for 615.2805); FABMS *m*/*z* (rel int.): 615 ($[M+H]^+$, 87%), 597 (19), 555 (5), 537 (5), 527 (6), 467 (68), 449 (17); UV ν_{max} nm (log ε): 224.6 (3.94); IR ν_{max} cm^{-1}: 3445 (OH),

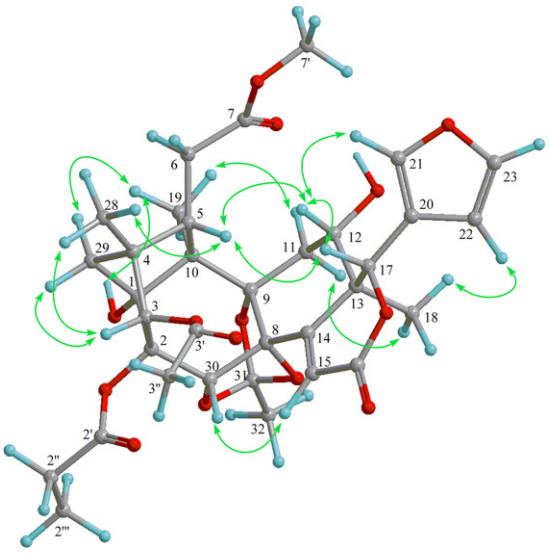


Fig. 10. Key NOESY correlations for Andirolide G (**7**).

2973, 1727, 1680, 1459, 1374, 1259, 1026, 875; CD λ nm ($\Delta\epsilon$)[c 4.15×10^{-4} M, CH_3CN]: 219 (11.3), 243 (−0.2), 260 (1.6), 274 (0), 297 (−3.5), 320 (0); ^{1}H and ^{13}C NMR data, see Table 2.

*3.3.4. Andirolide D (**4**).* Colorless needles; mp 131–133°C (MeOH/$CHCl_3$); $[\alpha]_D^{23}$ −48.6 (c 0.08, $CHCl_3$); HRFABMS m/z: 627.2798 $[M+H]^+$ ($C_{34}H_{43}O_{11}$, calcd for 627.2805); FABMS m/z (rel int.): 627 $[M+H]^+$, (25), 609 (5), 567 (2), 549 (3), 527 (3), 509 (1), 467 (13), 449 (4); UV ν_{max} nm (log ε): 227.5 (4.12); IR ν_{max} cm^{-1}: 3439 (OH), 2952, 1727, 1680, 1459, 1374, 1259, 1027, 876; CD λ nm ($\Delta\epsilon$)[c 5.44×10^{-4} M, CH_3CN]: 223 (13.7), 245 (−0.1), 260 (1.2), 272 (0), 297 (−3.8), 323 (0); ^{1}H and ^{13}C NMR data, see Table 2.

*3.3.5. Andirolide E (**5**).* Colorless prisms; mp 180–182°C (MeOH/$CHCl_3$); $[\alpha]_D^{23}$ −24.3 (c 0.10, $CHCl_3$); HRFABMS m/z: 685.2493 $[M+H]^+$ ($C_{35}H_{41}O_{14}$, calcd for 685.2496); FABMS m/z (rel int.): 685 $[M+H]^+$, (69), 625 (6), 551 (3), 491 (12), 449 (34); IR ν_{max} cm^{-1}: 2979, 1751(δ-lactone), 1723, 1638, 1374, 1243, 1146, 1083, 1048, 1022, 874; ^{1}H and ^{13}C NMR data, see Table 2.

*3.3.6. Crystal data of **5**.* $C_{35}H_{42}O_{14}$, M_r 686.69, orthorhombic, space group: $P2_12_12_1$, a=9.844 (6) Å, b=15.145 (10) Å, c=21.469 (13) Å, α=90.00°, β=90.00°γ=90.00°, V=3200.7 (3) Å^3, D_x=1.425 g/cm^{-3},

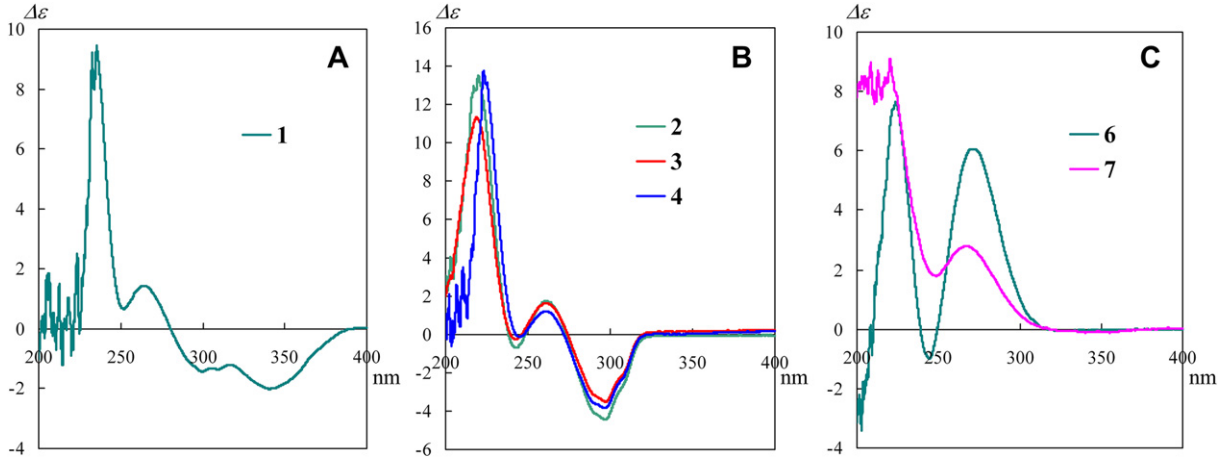
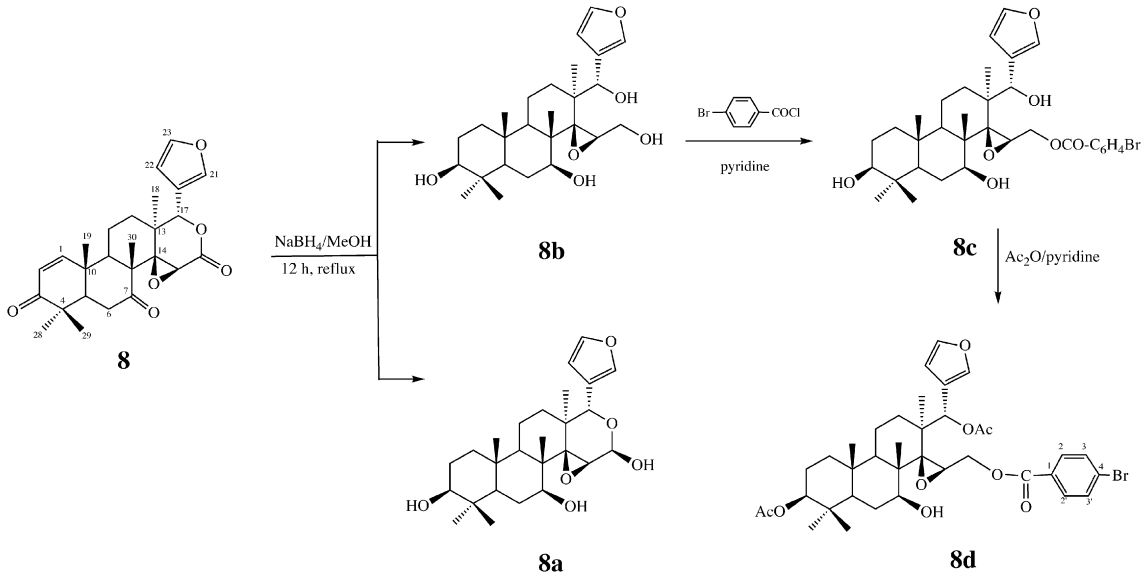


Fig. 11. (A) CD spectrum of Andirolide A (**1**). (B) CD spectra of Andirolide B (**2**), C (**3**), D (**4**). (C) CD spectra of Andirolide F (**6**), G (**7**).



Scheme 1. Synthesis of **8d** from 7-deacetoxy-7-oxogedunin (**8**).

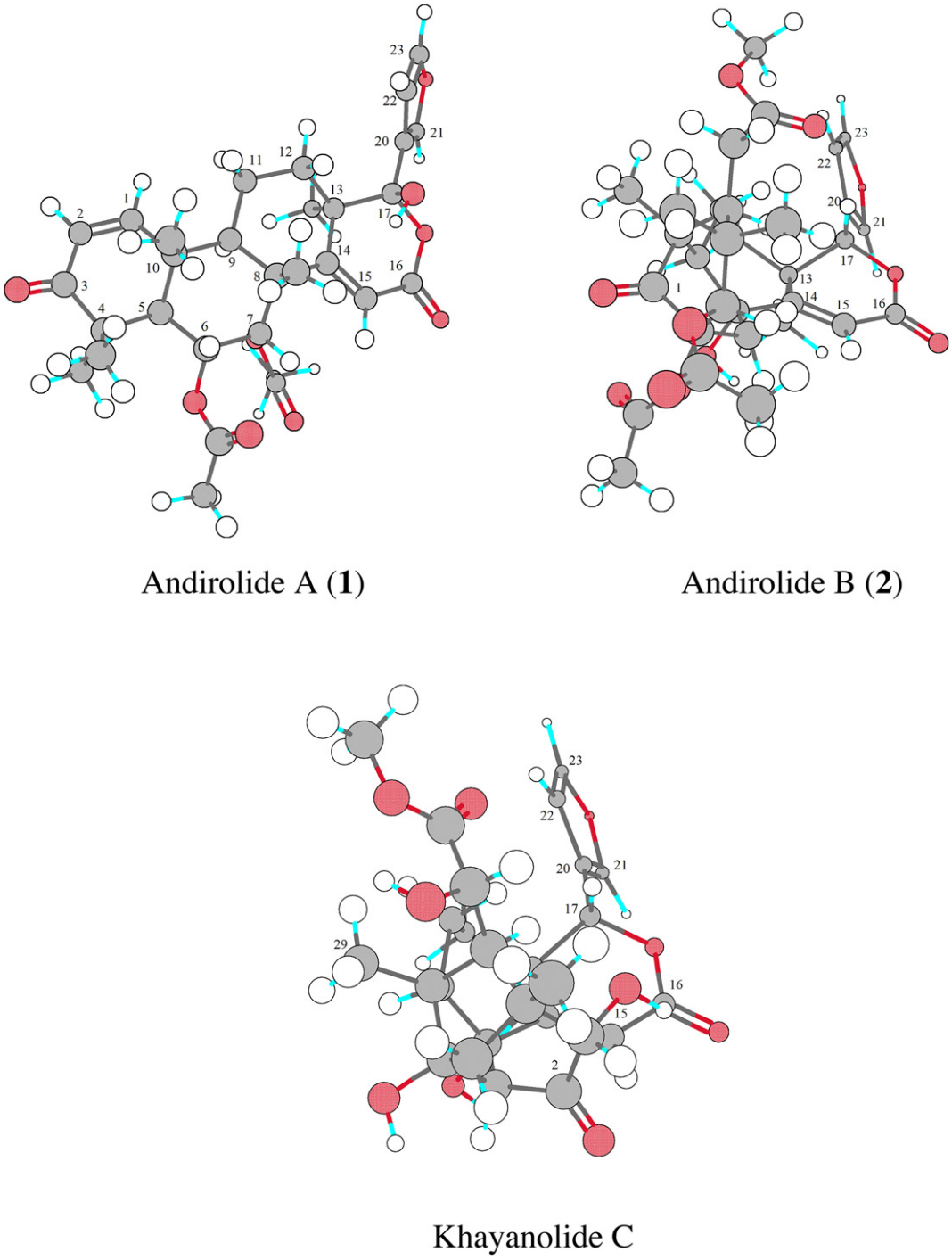


Fig. 12. The stable conformations of Andirolides A (**1**), B (**2**), and Khayanolide C calculated on Chem 3D.

Z=4. F(000)=1456, μ(Mo Kα)=0.110 mm^{-1}, measured independent reflections 7679, reflections 7329 ($I>2\sigma$ (I)), parameters used for refinement 442, R_1=0.0598 (for $I>2\sigma$ (I)), wR_2=0.1398 (for all data). X-ray diffraction data were collected with a Bruker AXS SMART APEX CCD camera using graphite-monochromated Mo Kα radiation (λ=0.71069) at 120 K for **5**. The crystal structures were solved by a direct method using the SHELXS-97 program.[21] Atomic scattering factors were taken from the International Tables for X-ray Crystallography.[22] Positional parameters of non-H-atoms were refined by a full-matrix least-squares method with anisotropic thermal parameters using the SHELXL-97 program.[21] The structural data were deposited with the following designation: **5**: CCDC-773260. These can be obtained free of charge at www.ccdc.cam.ac.uk/conts/retrieving.html (or from the Cambridge Crystallograpic Date Centre, 12 Union Road, Cambridge CB21EZ, U.K.; fax: +44 1223 336 033; e-mail: deposit@ccdc.cam.ac.uk). The H-atoms were calculated assuming idealized geometries but were not refined.

*3.3.7. Alkaline hydrolysis of Andirolide E (**5**).* Compound **5** (7.6 mg) was refluxed with a solution of 0.03 mol dm^{-3} KOH in MeOH over a steam bath for 6 h. Evaporation of the solvent under reduced pressure afforded a residue, which was subjected to HPLC [ODS, MeOH/H_2O (60:40)] to afford compound **5a** (4.6 mg): HRFABMS m/z: 545.2030 [M+H]$^+$ ($C_{28}H_{33}O_{11}$, calcd for 545.2023); FABMS m/z

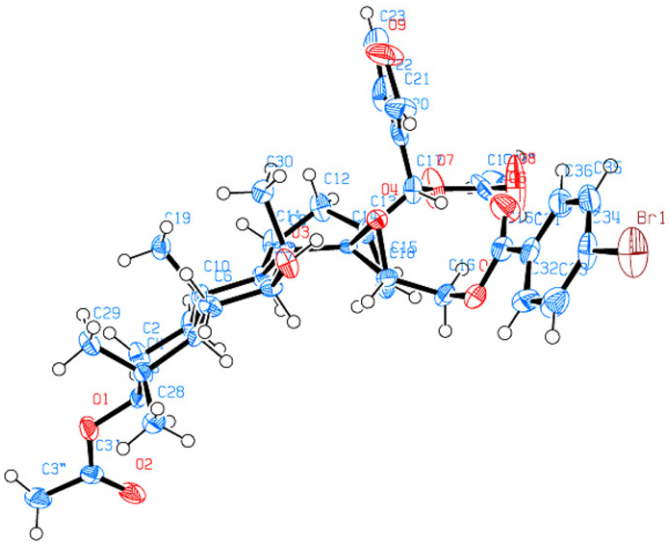


Fig. 13. ORTEP drawing of **8d**.

Table 4
Cytotoxicity of Andirolides A–G (1–7) against the P388, HL-60, L1210 and KB cell lines

Compounds	Cell line P388 IC50 (mM)[a]	Cell line HL-60 IC50 (mM)[a]	Cell line L1210 IC50 (mM)[a]	Cell line KB IC50 (mM)[a]
1	3.3	19.4	16.7	11.4
2	>100	>100	>100	>100
3	>100	>100	>100	>100
4	>100	79.9	>100	>100
5	>100	>100	>100	>100
6	14.4	16.1	27.0	29.3
7	50.6	>100	>100	68.5
5-Fluoro uracil[b]	0.8	0.9	0.4	7.7

[a] DMSO was used as vehicle.
[b] Positive control.

(rel int.): 545 $[M+H]^+$ (100), 527 (2), 509 (1), 485 (4), 425 (11); ^1H NMR δ ppm ($CDCl_3$): 1.08 (3H, s, H_3-28), 1.10 (3H, s, H_3-18), 1.42 (1H, m, H-12β), 1.49 (1H, m, H-12α), 1.62 (3H, s, H_3-32), 1.63 [1H, d, *J*=11.0 Hz, H-29 *pro-R*],1.77(1H, m, H-11α),1.93[1H, dd, *J*=9.8Hz (15α), 1.4 Hz (15β), H-14], 2.18 [1H, d, *J*=11.0 Hz, H-29 *pro-S*], 2.24 (1H, m, H-11β), 2.42 [1H, dd, *J*=16.7 Hz (6α), 5.1 Hz (5), H-6β], 2.61 [1H, dd, *J*=16.7 Hz (6β), 4.0 Hz (5), H-6α],2.62[1H, dd, *J*=19.0 Hz (15β), 9.8 Hz (14), H-15α], 2.76 [1H, dd, *J*=5.1 Hz (6β), 4.0 Hz (6α), H-5], 2.98 (1H, s, 2-OH), 3.22 [1H, dd, *J*=19.0 Hz (15α), 1.4 Hz (14), H-15β], 3.61 (1H, s), 3.64 [1H, d, *J*=7.6 Hz (30), 30-OH], 4.36 [1H, d, *J*=13.7 Hz (19α), H-19β], 4.49 [1H, d, *J*=7.6 Hz (30-OH), H-30], 4.76 [1H, d, *J*=13.7 Hz (19β), H-19α], 5.45 (1H, s, H-17), 6.38 [1H, d, *J*=1.7 Hz (23), H-22], 7.36 [1H, t, *J*=1.7 Hz (21, 22), H-23], 7.44 [1H, d, *J*=1.7 Hz (23), H-21].

*3.3.8. Andirolide F (**6**).* Colorless needles; mp 188–190°C (MeOH/$CHCl_3$); $[\alpha]_D^{23}$ +15.4 (*c* 0.06, $CHCl_3$); HRFABMS *m/z*: 683.2335 $[M+H]^+$ ($C_{35}H_{39}O_{14}$, calcd for 683.2340); FABMS *m/z* (rel int.): 683 $[M+H]^+$ (100), 623 (4), 507 (10), 447 (7); UV λ_{max} nm (log ε): 225 (3.94); IR ν_{max} cm^{-1}: 3445 (OH), 2973, 1727, 1680, 1459, 1374, 1259, 1026, 875; CD λ nm ($\Delta\varepsilon$)[*c* 3.59×10^{-4} M, CH_3CN]: 224 (7.6), 239 (0), 244 (−1.0), 249 (0), 270 (6.1), 317 (0); ^1H and ^{13}C NMR data, see Table 3.

*3.3.9. Andirolide G (**7**).* Colorless needles; mp 145–147°C (MeOH/$CHCl_3$); $[\alpha]_D^{23}$ +3.2 (*c* 0.17, $CHCl_3$); HRFABMS *m/z*: 673.2505 $[M+H]^+$ ($C_{34}H_{41}O_{14}$, calcd for 673.2495); FABMS *m/z* (rel.int.): 673 $[M+H]^+$ (17), 655 (5), 613 (6), 599 (69), 479 (7); UV λ_{max} nm (log ε): 229 (3.68); IR ν_{max} cm^{-1}: 3568 (OH), 2929, 1729, 1459, 1236, 1160, 1022, 875; CD λ nm ($\Delta\varepsilon$)[*c* 4.46×10^{-4} M, CH_3CN]: 213 (8.5), 249 (1.8), 268 (2.8), 323 (0); ^1H and ^{13}C NMR data, see Table 3.

*3.3.10. 7-Deacetoxy-7-oxogedunin (**8**).* Colorless prisms; mp 264–266°C (MeOH/$CHCl_3$); $[\alpha]_D^{23}$ −38.8 (*c* 0.178, $CHCl_3$). Compound **8** was identified from published data.[2]

*3.3.11. $NABH_4$ reduction of **8**.* To a solution of **8** (104.0 mg) in MeOH (20 ml), $NaBH_4$ (95 mg) was added and the mixture was refluxed for 12 h. The addition of AcOEt and H_2O was followed by the usual work-up. Evaporation of the solvent under reduced pressure afforded a residue (83.0 mg), which was subjected to HPLC (ODS, MeOH/H_2O, 60:40) to give **8a** (55.2 mg) and **8b** (19.0 mg). Compound **8a**: HRFABMS *m/z*: 429.2637 $[M-OH]^+$ ($C_{26}H_{37}O_5$, calcd for 429.2641); FABMS *m/z* (rel int.): 429 ([M−OH], 12%), 411 (3). Compound **8b**: HRFABMS *m/z*: 431.2796 $[M-OH]^+$ ($C_{26}H_{39}O_5$, calcd for 431.2798); FABMS *m/z* (rel int.): 431 ([M−OH], 87%), 413 (41), 395 (2); ^1H NMR δ ppm ($CDCl_3$): 0.79 (3H, s, H_3-29), 0.86 [1H, dd, *J*=12.3Hz (6β), 2.1 Hz (6α), H-5], 0.90 (3H, s, H_3-19), 0.94 (1H, m, H-1α), 1.00 (3H, s, H_3-28), 1.05 (3H, s, H_3-18), 1.15 (3H, s, H_3-30), 1.18 (1H, m, H-9), 1.20 (1H, m, H-12α), 1.50 (1H, m, H-6α), 1.52 (1H, m, H-11β), 1.56 (1H, m, H-2α), 1.60 (1H, m, H-11α), 1.64 (1H, m, H-1β), 1.65 (1H, m, H-6β), 1.66 (1H, m, H-2β), 1.91 (1H, m, H-12β), 3.20 [1H, dd, *J*=11.4 Hz (2β), 4.3 Hz (2α), H-3], 3.67 [1H, dd, *J*=10.7Hz (6β), 4.3 Hz (6α), H-7], 3.91 [1H, dd, *J*=7.8Hz (16β), 2.8 Hz (16α), H-15], 4.11 [1H, dd, *J*=12.4Hz (16β), 2.8 Hz (15), H-16α], 4.25 [1H, dd, *J*=12.4Hz (16α), 7.8 Hz (15), H-16β], 4.99 (1H, s, H-17), 6.42 [1H, dd, *J*=1.6Hz (23), 0.7 Hz (21), H-22], 7.36 [1H, t, *J*=1.6Hz (22, 21)], 7.42 (1H, br s, H-21).

*3.3.12. Formation of mono-p-bromobenzoate of **8b**.* *p*-Bromobenzoylchloride (39 mg) and DMAP (3 mg) were added to a pyridine solution (2 ml) of **8b** (19.0 mg), and the reaction mixture was refluxed for 2 h. AcOEt and H_2O were added and a standard work-up followed. The solvent was evaporated under reduced pressure, and the residue (83.0 mg) was purified by HPLC (ODS, MeOH/H_2O, 80:20) to give **8c** (5.6 mg).Compound **8c**: HRFABMS *m/z*: 630.4590 $[M+H]^+$($C_{33}H_{44}{}^{79}BrO_7$, calcd for 630.4591).

*3.3.13. Acetylation of **8c**.* To a solution of **8c** (5.6 mg) in pyridine (0.5 ml) was added Ac_2O (1.0 ml), and the reaction mixture was left at room temperature overnight. The mixture was concentrated dry under reduced pressure, and the residue was purified by HPLC (ODS, MeOH/H_2O, 85:15) to give **8d** (3.8 mg).

Compound **8d**: Colorless prisms; mp 241–243°C (MeOH/$CHCl_3$); $[\alpha]_D^{23}$ +4.1 (*c* 0.03, $CHCl_3$); HRFABMS *m/z*: 715.2484 $[M+H]^+$ ($C_{37}H_{48}{}^{79}BrO_9$, calcd for 715.2481); FABMS *m/z* (rel int.): 715, $[M+H]^+$ (10), 657 (19) 655 (19), 639 (5), 637 (4), 455 (12) 395 (6), 377 (7). ^1H NMR δ ppm ($CDCl_3$): 0.81 (3H, s, H_3-30), 0.84 (3H, s, H_3-29), 0.86 (3H, s, H_3-28), 0.90 (3H, s, H_3-19), 0.94 [1H, dd, *J*=13.0Hz (6β), 2.0 Hz (6α), H-5], 1.03 (1H, m, H-1α), 1.17 (3H, s, H_3-18), 1.28 (1H, m, H-12α), 1.42 (1H, m, 6α), 1.52 (1H, m, H-12β), 1.54 (1H, m, H-2α), 1.55 (1H, m, H-11α), 1.61 (1H, m, 1β), 1.65 (1H, m, 6β), 1.68 (1H, m, 2β), 1.70 (1H, m, 11β), 2.04 (3H, s, Ac), 2.07 (3H, s, Ac), 3.59 [1H, dd, *J*=10.8Hz (6β), 4.6 Hz (6α), H-7], 3.92 [1H, dd, *J*=8.7Hz (16β), 2.6 Hz (16α), H-15], 4.45 [1H, dd, *J*=11.6 Hz (2β), 4.6 Hz (2α), H-3], 4.84 [1H, dd, *J*=12.3 Hz (16α), *J*=8.7 Hz (15), H-16β], 4.96 [1H, dd, *J*=12.3Hz (16β), 2.6 Hz (15), H-16α], 6.13 (1H, s, H-17), 6.50 [1H, dd, *J*=1.8Hz (23), 0.7 Hz (21), H-22], 7.35 [1H, t, *J*=1.8Hz (22, 21), H-23], 7.55 (1H, br s, H-21) 7.62 [each 1H, d, *J*=8.7 Hz, H-2, 2′], 7.98 [each 1H, d, *J*=8.7 Hz, H-3, 3′].

*3.3.14. Crystal data of **8d**.* $C_{37}H_{47}BrO_9$, M_r 715.66, monoclinic, space group: $P2_1$, *a*=7.1529 (12) Å, *b*=27.995 (5) Å, *c*=9.411 (17) Å, α=90.00°, β=112.017 (3)°γ=90.00°, *V*=1747.0 (5) $Å^3$, D_x=1.360 g/cm^{-3}, *Z*=2. *F* (000)=752, μ (Mo Kα)=1.230 mm^{-1}, measured independent reflections 6241, reflections 3635 ($I>2\sigma$ (*I*)), parameters

used for refinement 423, R_1=0.0645 (for $I>2\sigma(I)$), wR_2=0.1507 (for all date), Flack χ parameter=0.025(12). X-ray diffraction data were collected with a Bruker AXS SMART APEX CCD camera using graphite-monochromated Mo Kα radiation (λ=0.71069) at 293 K for **8d**. The crystal structures were solved by a direct method using the SHELXS-97 program.[16] Atomic scattering factors were taken from the International Tables for X-ray Crystallography.[17] Positional parameters of non-H-atoms were refined by a full-matrix least-squares method with anisotropic thermal parameters using the SHELXL-97 program.[18] The structural data were deposited with the following designation: **8d**: CCDC-773261. These can be obtained free of charge at www.ccdc.cam.ac.uk/conts/retrieving.html (or from the Cambridge Crystallograpic Date Centre, 12 Union Road, Cambridge CB21EZ, U.K.; fax: +44 1223 336 033; e-mail: deposit@ccdc.cam.ac.uk). The H-atoms were calculated assuming idealized geometries but were not refined.

3.3.15. Cytotoxic assay against P388, HL-60, L1210, and KB cell lines. Cytotoxic activities of compounds **1**–**6** were examined by the 3-(4,5-dimethyl-2-thiazolyl)-2, 5-diphenyl-2H-tetrazolium bromide (MTT) method. P388, HL-60, L1210, and KB cells were cultured in Eagle's Minimum Essential Medium (10% fetal calf serum) at 37°C in 5% CO_2. The test material was dissolved in dimethyl sulfoxide (DMSO) to give a concentration of 10 mM, and the solution was diluted with Essential Medium to give concentrations of 200, 20, and 2 μmol, respectively. Each solution was combined with each cell suspension (1×10^5 cells/ml) in the medium, respectively. After incubation at 37°C for 72 h in 5% CO_2, the grown cells were labeled with 5 mg/ml MTT in phosphate-buffered saline (PBS), and then the absorbance of formazan dissolved by 20% sodium dodecyl sulfate (SDS) in 0.1 N HCl was measured at 540 nm using a microplate reader (Model 450; BIO-RAD). Each absorbance value was expressed as a percentage relative to the control cell suspension, which was prepared without the test substance by the same procedure as described above. All assays were performed three times, semi-logarithmic plots were constructed from the averaged data, and the effective dose of the substance required to inhibit cell growth by 50% (IC_{50}) was determined.

Acknowledgements

We thank Dr. Katsuhiko Minoura and Mrs. Mihoyo Fujitake (this university) for NMR and MS measurements. This work was supported by a Grant-in-Aid for High Technology from the Ministry of Education, Sciences, Sports and Culture, Japan.

Supplementary data

Supplementary data related to this article can be found online version, at doi:10.1016/j.tet.2010.11.028.

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