Apotirucallane and Tirucallane Triterpenoids from *Cedrela sinensis*

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Nine new triterpenoids, 1—9, were isolated from the cortex of *Cedrela sinensis* (Meliaceae), together with six known compounds, sapelin E acetate, grandifoliolenone, azadirone, bourjotinolone A, piscidinol A, and hispidol B. The structures of 1—9 were determined by the 2D NMR experiments, chemical methods, and X-ray crystallography.

Key words *Cedrela sinensis*; Meliaceae; apotirucallane; tirucallane; X-ray crystallography

Cedrela sinensis JUSS. (Meliaceae) has been used in mainland China and Korea as a traditional medicine for the treatment of enteritis, dysentery, and itching. Previously, from the seeds, leaves, stems, and cortex of this plant, we reported on five obacunone-type limonoids,[1] twenty-three apotirucallane-type triterpenoids,[2] and seven gedunin-type limonoids.[3] In the present study, from the cortex of this plant, we isolated nine new apotirucallane and tirucallane triterpenoids along with six known compounds and determined their structures (Fig. 1).

By a series of column chromatography including Diaion HP-20, activated charcoal, and ODS column chromatogra-

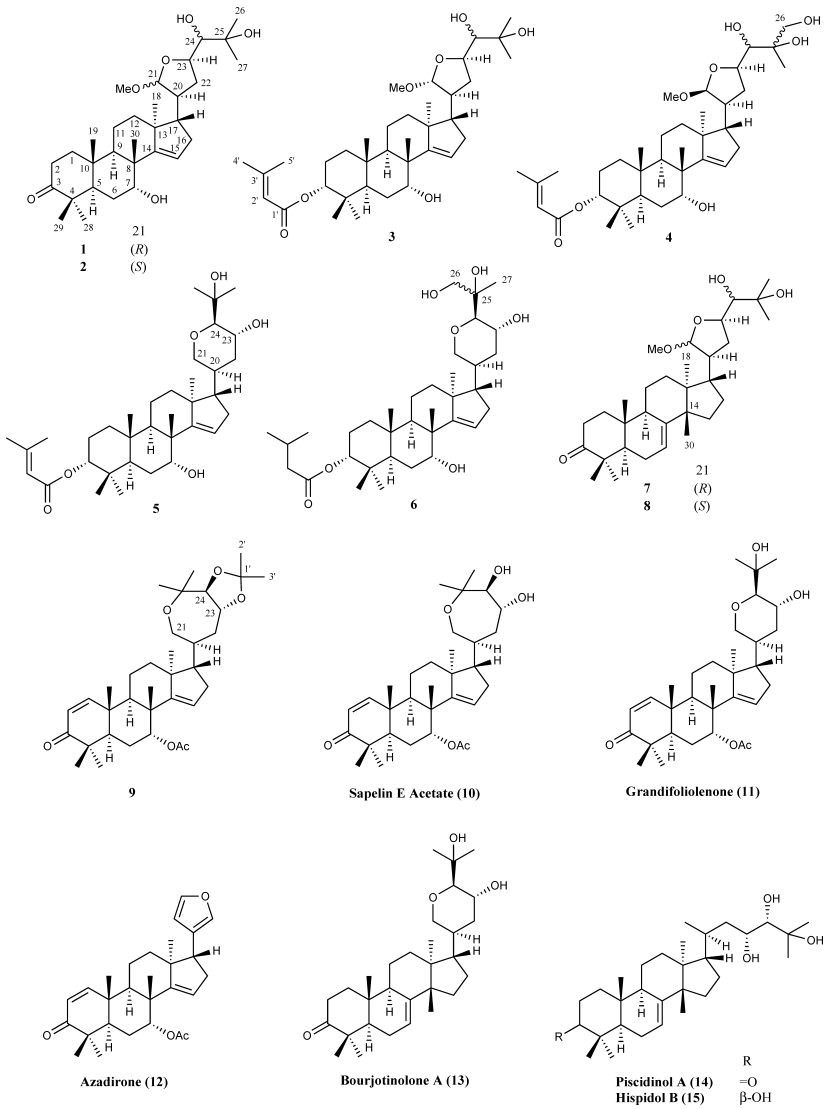

Fig. 1

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Table 1. ^{1}H-NMR (500 MHz) Spectral Data in $CDCl_3$ at 300 K

Position	1	2	3	4	5	6	7	8	9
1 (α)	1.52 (m)	1.52 (m)	1.28 (m)	1.27 (m)	1.26 (m)	1.22 (m)	1.46 (m)	1.46 (td, 14.1, 4.0)	7.16 (d, 10.2)
1 (β)	1.86 (m)	1.87 (m)	1.36 (m)	1.37 (m)	1.37 (dt, 12.8, 3.2)	1.37 (dt, 12.7, 3.2)	1.99 (ddd, 13.4, 5.5, 3.1)	1.98 (m)	
2 (α)	2.43 (ddd, 16.0, 7.3, 3.9)	2.43 (ddd, 15.9, 7.3, 3.8)	1.62 (m)	1.62 (m)	1.62 (m)	1.60 (m)	2.25 (dt, 14.1, 3.5)	2.25 (dt, 14.1, 3.5)	5.85 (d, 10.2)
2 (β)	2.52 (ddd, 16.0, 10.1, 7.3)	2.53 (ddd, 15.9, 10.2, 7.5)	1.91 (m)	1.92 (m)	1.91 (m)	1.90 (m)	2.76 (td, 14.5, 5.5)	2.76 (td, 14.5, 5.5)	
3			4.70 (t, 2.6)	4.70 (t, 2.6)	4.69 (t, 2.6)	4.65 (t, 2.5)			
5	2.09 (dd, 12.4, 3.2)	2.08 (m)	2.02 (m)	2.01 (m)	1.99 (m)	1.98 (dd, 11.9, 3.3)	1.72 (m)	1.72 (dd, 9.9, 7.5)	2.19 (dd, 13.1, 2.1)
6α	1.74—1.88 (m)	1.74—1.89 (m)	1.68—1.79 (m)	1.66—1.79 (m)	1.69—1.78 (m)	1.67—1.78 (m)	2.06—2.13 (m)	2.05—2.15 (m)	1.78 (dt, 14.8, 2.8)
6β	1.74—1.88 (m)	1.74—1.89 (m)	1.68—1.79 (m)	1.66—1.79 (m)	1.69—1.78 (m)	1.67—1.78 (m)	2.06—2.13 (m)	2.05—2.15 (m)	1.95 (m)
7	3.95 (s-like)	3.95 (s-like)	3.91 (s-like)	3.92 (s-like)	3.91 (s-like)	3.91 (s-like)	5.31 (q, 3.1)	5.31 (q, 3.2)	5.23 (s-like)
9	2.04 (dd, 11.9, 7.3)	2.03 (m)	2.03 (m)	1.99 (m)	2.02 (m)	2.01 (m)	2.32 (m)	2.31 (m)	2.21 (m)
11α	1.75 (m)	1.72 (m)	1.73 (m)	1.71 (m)	1.68 (m)	1.69 (m)	1.54—1.66 (m)	1.51—1.65 (m)	1.90 (m)
11β	1.59 (m)	1.61 (m)	1.51 (m)	1.51 (m)	1.49 (m)	1.49 (m)	1.54—1.66 (m)	1.51—1.65 (m)	1.69 (m)
12α	1.82 (m)	1.54 (m)	1.80 (m)	1.50 (m)	1.90 (m)	1.89 (m)	1.57 (m)	1.33 (m)	1.90 (m)
12β	1.48 (m)	1.61 (m)	1.46 (m)	1.59 (m)	1.49 (m)	1.48 (m)	1.76 (m)	1.90 (m)	1.69 (m)
15 (α)	5.47 (d, 2.3)	5.49 (d, 1.6)	5.45 (d, 2.2)	5.48 (br s)	5.49 (d, 2.6)	5.50 (d, 2.5)	1.47—1.60 (m)	1.48—1.62 (m)	5.31 (d, 2.2)
15 (β)							1.47—1.60 (m)	1.48—1.62 (m)	
16α	2.12 (m)	2.14 (m)	2.13 (m)	2.10—2.22 (m)	2.11 (m)	2.12 (m)	1.30 (m)	1.32 (m)	1.98 (m)
16β	2.22 (ddd, 15.6, 7.3, 3.7)	2.17 (m)	2.22 (ddd, 15.5, 7.3, 3.6)	2.10—2.22 (m)	2.35 (ddd, 15.1, 7.1, 3.7)	2.35 (ddd, 15.3, 7.1, 3.7)	1.94 (m)	1.90 (m)	2.27 (ddd, 14.8, 6.9, 3.6)
17	1.74 (m)	2.01 (m)	1.73 (m)	1.98 (m)	2.02 (m)	2.01 (m)	1.79 (m)	2.04 (m)	2.08 (m)
18	1.06 (s, 3H)	1.02 (s, 3H)	1.08 (s, 3H)	1.04 (s, 3H)	1.00 (s, 3H)	1.01 (s, 3H)	0.85 (s, 3H)	0.83 (s, 3H)	0.97 (s, 3H)
19	1.00 (s, 3H)	1.01 (s, 3H)	0.92 (s, 3H)	0.92 (s, 3H)	0.91 (s, 3H)	0.90 (s, 3H)	1.01 (s, 3H)	1.01 (s, 3H)	1.17 (s, 3H)
20	2.34 (m)	2.19 (m)	2.35 (m)	2.20 (m)	1.92 (m)	1.94 (m)	2.17 (m)	1.98 (m)	1.81 (m)
21 (α)		4.76 (d, 4.3)		4.80 (d, 4.2)	3.44 (dd, 11.6, 2.5)	3.46 (dd, 11.5, 2.2)		4.69 (d, 3.7)	3.62 (s, 2H)
21 (β)	4.80 (d, 3.6)		4.80 (d, 3.7)		3.99 (d, 11.6)	4.00 (d, 11.5)	4.78 (d, 3.6)		
22 (α)	1.92 (m)	1.86—2.01 (m)	1.92 (m)	1.87—2.04 (m)	1.55 (m)	1.57 (m)	1.94 (m)	1.85—1.98 (m)	1.56 (m)
22 (β)	1.81 (m)	1.86—2.01 (m)	1.82 (m)	1.87—2.04 (m)	2.05 (m)	2.08 (m)	1.75 (m)	1.85—1.98 (m)	2.10 (m)
23	4.26 (ddd, 10.5, 4.6, 1.4)	4.44 (m)	4.26 (ddd, 10.6, 4.8, 1.6)	4.51 (br t, 8.7)	3.88 (ddd, 10.9, 9.0, 4.7)	3.94 (ddd, 11.1, 9.1, 4.8)	4.22 (ddd, 10.6, 5.0, 1.7)	4.42 (m)	4.03 (ddd, 11.2, 9.0, 2.9)
24	3.25 (br s)	3.17 (d, 6.2)	3.25 (dd, 9.9, 1.7)	3.35 (dd, 7.2, 1.1)	2.90 (d, 9.0)	3.09 (d, 9.1)	3.24 (br s)	3.17 (dd, 8.1, 2.0)	3.66 (d, 9.0)
26 (a)	1.26 (s, 3H)	1.27 (s, 3H)	1.27 (s, 3H)	3.57 (d-like, 6.6, 2H)	1.27 (s, 3H)	3.70 (d, 11.2)	1.26 (s, 3H)	1.26 (s, 3H)	1.17 (s, 3H)
26 (b)						3.49 (d, 11.2)			
27	1.30 (s, 3H)	1.28 (s, 3H)	1.30 (s, 3H)	1.23 (s, 3H)	1.31 (s, 3H)	1.23 (s, 3H)	1.29 (s, 3H)	1.27 (s, 3H)	1.30 (s, 3H)
28	1.10 (s, 3H)	1.10 (s, 3H)	0.86 (s, 3H)	0.86 (s, 3H)	0.86 (s, 3H)	0.85 (s, 3H)	1.11 (s, 3H)	1.12 (s, 3H)	1.07 (s, 3H)
29	1.05 (s, 3H)	1.05 (s, 3H)	0.91 (s, 3H)	0.91 (s, 3H)	0.91 (s, 3H)	0.90 (s, 3H)	1.01 (s, 3H)	1.02 (s, 3H)	1.07 (s, 3H)
30	1.09 (s, 3H)	1.09 (s, 3H)	1.06 (s, 3H)	1.06 (s, 3H)	1.08 (s, 3H)	1.08 (s, 3H)	1.04 (s, 3H)	1.04 (s, 3H)	1.20 (s, 3H)
2′ (a)			5.77 (m)	5.77 (m)	5.77 (m)	2.23 (dd, 14.6, 7.5)			1.38 (s, 3H)
2′ (b)						2.21 (dd, 14.6, 6.9)			
3′						2.10 (m)			1.39 (s, 3H)
4′			1.89 (d, 1.1, 3H)	1.89 (d, 1.2, 3H)	1.89 (d, 1.1, 3H)	0.95 (d, 6.6, 3H)			
5′			2.17 (d, 1.1, 3H)	2.17 (d, 1.2, 3H)	2.17 (d, 1.1, 3H)	0.95 (d, 6.5, 3H)			
OAc									1.94 (s, 3H)
OMe	3.35 (s, 3H)	3.37 (s, 3H)	3.36 (s, 3H)	3.40 (s, 3H)			3.34 (s, 3H)	3.35 (s, 3H)	

Chemical shifts are reported in ppm relative to the residual $CHCl_3$ resonance at 7.26 ppm. Multiplicity and J values in Hz are given in parentheses.

Table 2. ^{13}C-NMR (125 MHz) Spectral Data in $CDCl_3$ at 300 K

Position	1	2	3	4	5	6	7	8	9
1	38.6 (t)	38.6 (t)	33.4 (t)	33.4 (t)	33.4 (t)	33.3 (t)	38.5 (t)	38.6 (t)	158.4 (d)
2	34.0 (t)	34.0 (t)	22.8 (t)	22.8 (t)	22.8 (t)	22.8 (t)	34.9 (t)	34.9 (t)	125.5 (d)
3	217.4 (s)	217.3 (s)	77.1 (d)	77.0 (d)	77.1 (d)	77.8 (d)	216.9 (s)	216.9 (s)	204.7 (s)
4	46.9 (s)	47.0 (s)	36.3 (s)	36.3 (s)	36.3 (s)	36.2 (s)	47.9 (s)	47.9 (s)	44.2 (s)
5	46.5 (d)	46.6 (d)	41.9 (d)	41.9 (d)	41.9 (d)	41.9 (d)	52.4 (d)	52.5 (d)	46.3 (d)
6	24.8 (t)	24.8 (t)	23.6 (t)	23.6 (t)	23.7 (t)	23.7 (t)	24.4 (t)	24.4 (t)	23.9 (t)
7	72.0 (d)	72.0 (d)	72.2 (d)	72.2 (d)	72.5 (d)	72.4 (d)	118.2 (d)	118.2 (d)	74.8 (d)
8	44.1 (s)	44.1 (s)	44.5 (s)	44.5 (s)	44.5 (s)	44.5 (s)	145.6 (s)	145.6 (s)	42.8 (s)
9	41.1 (d)	41.0 (d)	41.7 (d)	41.6 (d)	41.8 (d)	41.7 (d)	48.4 (d)	48.4 (d)	38.6 (d)
10	37.2 (s)	37.2 (s)	37.6 (s)	37.6 (s)	37.6 (s)	37.5 (s)	35.1 (s)	35.1 (s)	39.9 (s)
11	16.3 (t)	16.4 (t)	16.3 (t)	16.4 (t)	16.4 (t)	16.4 (t)	17.8 (t)	17.9 (t)	16.9 (t)
12	32.7 (t)	32.7 (t)	32.8 (t)	32.9 (t)	34.2 (t)	34.2 (t)	31.6 (t)	31.1 (t)	34.0 (t)
13	47.0 (s)	46.6 (s)	47.0 (s)	46.6 (s)	46.7 (s)	46.7 (s)	43.7 (s)	43.6 (s)	46.2 (s)
14	161.8 (s)	161.5 (s)	162.5 (s)	162.1 (s)	162.4 (s)	162.4 (s)	51.0 (s)	50.8 (s)	159.4 (s)
15	119.6 (d)	120.0 (d)	119.3 (d)	119.6 (d)	119.8 (d)	119.8 (d)	33.8 (t)	34.2 (t)	119.2 (d)
16	34.8 (t)	35.0 (t)	34.7 (t)	34.9 (t)	34.9 (t)	34.9 (t)	27.4 (t)	27.4 (t)	34.9 (t)
17	57.6 (d)	52.5 (d)	57.5 (d)	52.3 (d)	52.3 (d)	52.1 (d)	50.3 (d)	45.0 (d)	52.3 (d)
18	19.6 (q)	20.1 (q)	19.4 (q)	19.9 (q)	19.2 (q)	19.2 (q)	22.6 (q)	23.3 (q)	19.9 (q)
19	15.0 (q)	15.0 (q)	15.3 (q)	15.2 (q)	15.3 (q)	15.2 (q)	12.8 (q)	12.8 (q)	19.1 (q)
20	45.9 (d)	44.6 (d)	45.9 (d)	44.6 (d)	35.8 (d)	35.7 (d)	47.8 (d)	46.3 (d)	36.7 (d)
21	109.5 (d)	104.6 (d)	109.6 (d)	104.9 (d)	70.1 (t)	70.0 (t)	109.0 (d)	104.9 (d)	63.6 (t)
22	33.8 (t)	31.4 (t)	33.8 (t)	31.6 (t)	36.4 (t)	36.1 (t)	34.4 (t)	31.6 (t)	35.3 (t)
23	77.0 (d)	78.9 (d)	77.0 (d)	78.0 (d)	64.5 (d)	63.2 (d)	76.8 (d)	78.9 (d)	74.2 (d)
24	75.5 (d)	76.6 (d)	75.5 (d)	75.8 (d)	86.6 (d)	83.2 (d)	75.5 (d)	76.6 (d)	87.3 (d)
25	73.1 (s)	72.9 (s)	73.1 (s)	74.5 (s)	74.2 (s)	74.8 (s)	73.1 (s)	73.0 (s)	73.6 (s)
26	26.4 (q)	26.4 (q)	26.4 (q)	67.3 (t)	24.1 (q)	67.9 (t)	26.4 (q)	26.3 (q)	22.9 (q)
27	26.5 (q)	26.4 (q)	26.5 (q)	20.6 (q)	28.6 (q)	20.4 (q)	26.5 (q)	26.4 (q)	26.6 (q)
28	26.3 (q)	26.3 (q)	27.7 (q)	27.7 (q)	27.7 (q)	27.8 (q)	24.5 (q)	24.5 (q)	27.1 (q)
29	21.2 (q)	21.2 (q)	21.8 (q)	21.8 (q)	21.8 (q)	21.8 (q)	21.6 (q)	21.6 (q)	21.3 (q)
30	27.1 (q)	27.1 (q)	27.8 (q)	27.8 (q)	27.9 (q)	27.9 (q)	27.3 (q)	27.5 (q)	27.4 (q)
1′			166.5 (s)	166.5 (s)	166.5 (s)	173.0 (s)			109.0 (s)
2′			117.1 (d)	117.0 (d)	117.0 (d)	43.8 (t)			26.9 (q)
3′			155.7 (s)	155.8 (s)	155.7 (s)	25.8 (d)			27.5 (q)
4′			27.4 (q)	27.4 (q)	27.4 (q)	22.5 (q)			
5′			20.3 (q)	20.3 (q)	20.3 (q)	22.5 (q)			
OAc (C=O)									170.1 (s)
OAc (Me)									21.2 (q)
OMe	55.6 (q)	55.2 (q)	55.6 (q)	55.4 (q)			55.6 (q)	55.2 (q)	

Chemical shifts are reported in ppm relative to the solvent resonance at 77.03 ppm.

phies, and subsequent purification by preparative HPLC, a MeOH extract of the cortex of *C. sinensis* gave nine new triterpenoids, **1**—**9**, along with six known compounds, **10**—**15**.

Compound **1** was isolated as an amorphous solid. Its molecular formula was determined to be $C_{31}H_{50}O_6$ from the $[M-OMe]^+$ peak at *m/z* 487.3410 (Calcd for $C_{30}H_{47}O_5$, 487.3424) in the HR-ESI-MS. Its NMR spectra generally resembled those of apotirucallane-type triterpenoids.[2] The ^{1}H-NMR spectrum of **1** showed the presence of seven tertiary methyl groups (δ 1.00, 1.05, 1.06, 1.09, 1.10, 1.26, 1.30), one olefinic proton (δ 5.47, d, J=2.3 Hz), one methoxyl group (δ 3.35, s), and one acetal methine proton (δ 4.80, d, J=3.6 Hz) (Table 1). The ^{13}C-NMR spectrum indicated the presence of eight methyls, seven methylenes, nine methines, and seven quaternary carbons (Table 2). Analysis of the HMBC spectrum revealed the presence of a tertiary methyl group at C-13 and a double bond between C-14 and C-15 (Fig. 2). The location of the methoxyl group was shown to be at C-21 by the HMBC cross-peak between C-21 (δ_C 109.5) and the *O*-methyl protons (δ_H 3.35). Further analysis of the ^{13}C-NMR and the HMBC spectra demonstrated the presence of a carbonyl group at C-3, and three hydroxyl groups at C-7, C-24, and C-25 (Fig. 2). The relative stereochemistry of **1** was determined on the basis of NOESY experiments (Fig. 3). NOE correlations between H-5/H-9, H-5/H_3-28, H-7/H_3-30, H-9/H_3-18, H-17/H_3-30, H_3-19/H_3-29, and H_3-19/H_3-30 showed that H-5, OH-7, H-9, and Me-18 were α-oriented, whereas H-17, Me-19, Me-29, and Me-30 were β-oriented. NOE correlations were also observed between H-17/H-21, H_3-18/H-20, H_3-18/H-21, H_3-18/OCH_3-21, H-20/OCH_3-21, H-20/H-23, and OCH_3-21/H-23. These correlations were

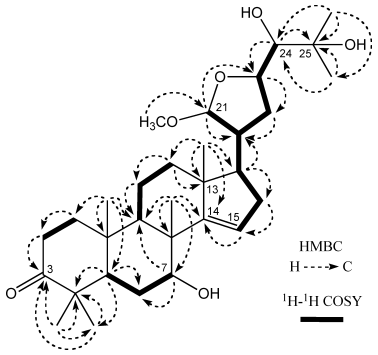

Fig. 2. Selected ^{1}H–^{1}H COSY and HMBC Correlations for **1**

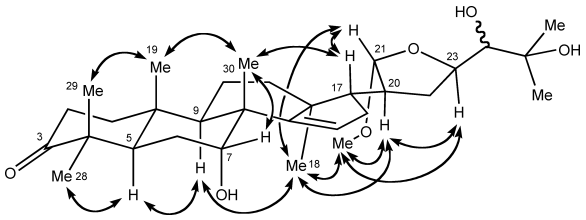

Fig. 3. Selected NOE Correlations for **1**

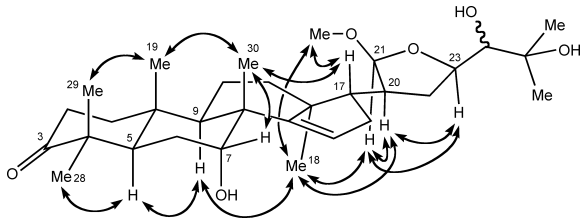

Fig. 4. Selected NOE Correlations for **2**

possible only when **1** possessed 20*S*, 21*R*, and 23*R* configuration. From these observations, the structure of **1** was determined to be as shown in Fig. 1.

Compound **2** was obtained as an amorphous solid. Its molecular formula, $C_{31}H_{50}O_6$, determined from the $[M-OMe]^+$ peak at *m/z* 487.3419 (Calcd for $C_{30}H_{47}O_5$, 487.3424) in the HR-ESI-MS, was the same as that of **1**. Its ^{1}H- and ^{13}C-NMR spectra were very similar to those of **1**, and analysis of the HMQC, HMBC, and ^{1}H–^{1}H COSY spectra demonstrated that **2** and **1** had the same gross structure (Tables 1, 2). The differences observed between them were that **2** gave an NOE correlation between H-21 and H-23, which was not seen in **1** (Figs. 3, 4), and that the ^{13}C-NMR signals of C-17 and C-21 in **2** (δ 52.5, 104.6, respectively) were considerably upfield when compared with the corresponding signals in **1** (δ 57.6, 109.5, respectively), whereas that of C-23 in **2** (δ 78.9) was slightly downfield shifted compared with that in **1** (δ 77.0) (Table 2). These facts indicated that **2** was the epimer of **1** at C-21.[2] NOE correlations between H-17/OCH_3-21, H_3-18/H-20, H_3-18/H-21, and H_3-18/OCH_3-21 indicated that **2** possessed 20*S*, 21*S*, and 23*R* configuration. From these observations, the structure of **2** was determined to be as shown in Fig. 1.

Compound **3** was obtained as an amorphous solid. From the $[M+Na]^+$ peak at *m/z* 625.4049 (Calcd for $C_{36}H_{58}O_7Na$, 625.4080) in the HR-ESI-MS, its molecular formula was determined to be $C_{36}H_{58}O_7$. The ^{1}H- and ^{13}C-NMR spectra of **3** showed close resemblance to those of **1** except for the resonances of ring-A protons and carbons. The difference noted between **3** and **1** was due to a senecioyl ester side chain, whose location was determined to be at C-3 by the cross-peak between C-1′ (δ_C 166.5) and H-3 (δ_H 4.70) in the HMBC spectrum. The NOE correlations between H-3/H_3-29, H-17/H-21, H_3-18/H-20, H_3-18/H-21, H_3-18/OCH_3-21, H-20/OCH_3-21, H-20/H-23, and OCH_3-21/H-23, and the chemical shifts of the C-17 (δ 57.5), C-21 (δ 109.6), C-23 (δ 77.0), and H-3 (δ 4.70, t, J=2.6 Hz) signals revealed that H-3 was β-oriented and C-21 had the *R*-configuration in **3**. Thus, the structure of **3** was determined to be as shown in Fig. 1.

Compound **4** was obtained as an amorphous solid. Its molecular formula was determined to be $C_{36}H_{58}O_8$ from the $[M+Na]^+$ peak at *m/z* 641.4006 (Calcd for $C_{36}H_{58}O_8Na$, 641.4029) in the HR-ESI-MS. The NMR spectra of **4** were generally similar to those of **3**, suggesting that they had the same basic structure. The major difference between **4** and **3** was that C-26 of **4** was a hydroxymethyl group (δ_C 67.3, t) whereas that of **3** was a methyl group. The NOE correlations detected between H-3/H_3-29, H-9/H_3-18, H-17/OCH_3-21, H_3-18/H-20, H_3-18/H-21, H_3-18/OCH_3-21, H-20/H-21, H-20/H-23, and H-21/H-23, and the chemical shift values of the C-17 (δ 52.3), C-21 (δ 104.9), C-23 (δ 78.0), and H-3 (δ 4.70, t, J=2.6 Hz) signals suggested that in **4**, H-3 was β-oriented and C-21 had the *S* configuration. Thus, the structure of **4** was determined to be as shown in Fig. 1.

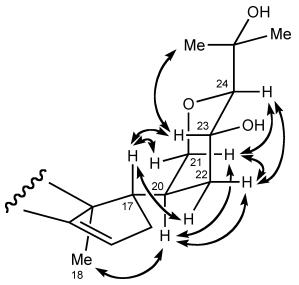

Fig. 5. Selected NOE Correlations for **5**

Compound **5** was isolated as an amorphous solid. Its molecular formula was determined to be $C_{35}H_{56}O_6$ from the $[M+H]^+$ peak at *m/z* 573.4123 (Calcd for $C_{35}H_{57}O_6$, 573.4155) in the HR-ESI-MS. The NMR spectra of **5** were very similar to those of **3**, except for the resonances ascribable to the side chain at C-17. Compound **3** had an acetal methine group at C-21, whereas **5** had a methylene group (δ_C 70.1, t) at C-21. In the HMBC spectrum of **5**, the correlations between the methine carbon at δ 86.6 (C-24) and the methylene protons at δ 3.44 (dd, J=11.6, 2.5 Hz, H-21α) and δ 3.99 (d, J=11.6 Hz, H-21β) suggested that C-21 was linked to C-24 *via* an oxygen bridge to form a cyclic ether. The coupling constant (J=9.0 Hz) between H-23 and H-24 suggested their anti-periplanar relation. The NOE correlations detected between H-17/H-21β, H-17/H-22β, H-17/H-23, H_3-18/H-20, H-20/H-21α, H-20/H-22α, H-21α/H-22α, H-21α/H-24, and H-22α/H-24 revealed that the configuration at C-23 and that at C-24 were both *R* (Fig. 5). Thus, the structure of **5** was determined to be as shown in Fig. 1.

Compound **6** was obtained as an amorphous solid. From the $[M+H]^+$ peak at *m/z* 591.4304 (Calcd for $C_{35}H_{59}O_7$, 591.4261) in the HR-ESI-MS, its molecular formula was determined to be $C_{35}H_{58}O_7$. The NMR spectra of **6** were generally similar to those of **5**, suggesting that **6** was also a triterpenoid of the same series. However, the signals of C-26 (δ 67.9), C-2′ (δ 43.8), and C-3′ (δ 25.8) in the ^{13}C-NMR spectrum of **6** were observed as hydroxymethyl, methylene, and methine carbons, respectively, whereas the signal of C-26 in **5** was of a methyl carbon (Table 2). Thus, the structure of **6** was determined to be as shown in Fig. 1.

Compound **7** was isolated as an amorphous solid. Its molecular formula was determined to be $C_{31}H_{50}O_5$ from the $[M+Na]^+$ peak at *m/z* 525.3531 (Calcd for $C_{31}H_{50}O_5Na$, 525.3556) in the HR-ESI-MS. Its NMR spectra generally resembled those of melianodiol[4] suggesting that **7** had a tiru-

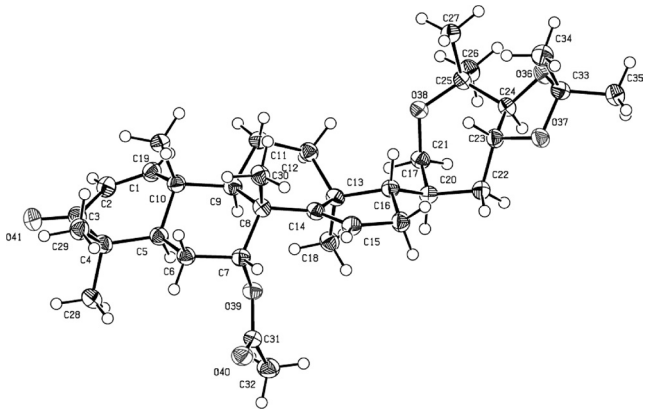

Fig. 6. Crystal Structure of **9**

callane-type skeleton. Analysis of the HMBC spectrum revealed the presence of a carbonyl group at C-3, a double bond between C-7 and C-8, a tertiary methyl group at C-14, a methoxyl group at C-21, and two hydroxyl groups at C-24 and C-25. As regards the relative stereochemistry of **7**, the NOE correlations between H-5/H-9, H-5/H_3-28, H-9/H_3-18, H-17/H-21, H-17/H_3-30, H_3-18/H-20, H_3-18/H-21, H_3-18/OCH_3-21, H-20/OCH_3-21, H-20/H-23, and OCH_3-21/H-23 showed that the configurations at C-21 and C-23 were both *R*, and that at C-20 was *S*. From these observations, the structure of **7** was determined to be as shown in Fig. 1.

Compound **8** was obtained as an amorphous solid. Its molecular formula, $C_{31}H_{50}O_5$, determined from the $[M+Na]^+$ peak at *m*/*z* 525.3569 (Calcd for $C_{31}H_{50}O_5Na$, 525.3556) in the HR-ESI-MS, was the same as that of **7**, and the NMR spectra of **8** were quite similar to those of **7** with the minor difference analogous to that observed between **1** and **2**, suggesting that **8** was the epimer of **7** at C-21. The NOE correlations detected between H-17/OCH_3-21, H-17/H_3-30, H_3-18/H-20, H_3-18/H-21, H_3-18/OCH_3-21, H-20/H-21, H-20/H-23, and H-21/H-23, and the chemical shift values of the C-17 (δ 45.0), C-21 (δ 104.9), and C-23 (δ 78.9) implied that C-21 of **8** had the *S* configuration. Thus, the structure of **8** was determined to be as shown in Fig. 1.

Compound **9** was isolated as colorless prisms. Its molecular formula was determined to be $C_{35}H_{52}O_6$ from the $[M+H]^+$ peak at *m*/*z* 569.3811 (Calcd for $C_{35}H_{53}O_6$, 569.3842) in the HR-ESI-MS. The NMR spectra of **9** were generally similar to those of sapelin E acetate.[5] The only difference between them was that **9** had additional signals due to C-1′ (δ 109.0), C-2′ (δ 26.9), and C-3′ (δ 27.5) carbons that formed an acetonide between the C-23 and C-24 hydroxyls. Treatment of **9** with boron trifluoride diethyl etherate in MeOH–H_2O afforded a product that was shown to be identical to sapelin E acetate from its spectroscopic data. The structure of **9** was confirmed by an X-ray crystallographic analysis (Fig. 6).

Compounds **10**—**15** were identified as sapelin E acetate,[5] grandifoliolenone,[6] azadirone,[7] bourjotinolone A,[8] piscidinol A,[9] and hispidol B,[10] respectively, by the analysis of their spectroscopic data. Full ^{1}H- and ^{13}C-NMR data for **10** and **11** are provided in Experimental.

Compounds **1**—**15** all showed moderate cytotoxicity against P-388 murine leukemia cells, with IC_{50} values of 5.2, 5.2, 8.4, 9.3, 5.9, 4.9, 4.8, 9.9, 9.3, 3.8, 4.2, 4.2, 6.8, 3.3, and 5.2 μg/ml, respectively.

Experimental

General Experimental Procedures Melting points were determined on a Yanagimoto micro-melting point apparatus and are uncorrected. Optical rotations were measured on a JASCO P-1030 digital polarimeter. UV spectra were taken on a JASCO V-530 spectrophotometer, IR spectra on a JASCO FT/IR 620 spectrophotometer, NMR spectra on a Bruker DRX-500 spectrometer at 300 K, and mass spectra on a Micromass LCT spectrometer. Preparative HPLC was performed on a Shimadzu LC-6AD system equipped with a SPD-10A UV detector (at 205 nm) and a reversed-phase column, Wakosil-II 5C18HG prep (5 μm, 20×250 mm), by using a mixed solvent system of MeOH/H_2O or MeCN/H_2O, at a flow rate of 10 ml/min. X-Ray single crystal analysis was carried out on a Mac Science DIP diffractometer with Mo$K\alpha$ radiation (λ=0.71073 Å).

Plant Material The cortex of *C. sinensis* was collected in Jilin Province, People's Republic of China, in September 2000, and the botanical origin was identified by Professor Soo-Cheol Kim of the Agricultural College of Yanbian University. A voucher specimen (00CHI005) has been deposited at the herbarium of Tokyo University of Pharmacy and Life Science.

Extraction and Isolation The cut and air-dried cortex of *C. sinensis* (24 kg) was extracted with hot MeOH (3×90 l). After the removal of the solvent, the MeOH extract (3.2 kg) was placed on a column of HP-20 (8.0 kg) and fractionated into five fractions, by eluting with H_2O, 50% MeOH, 80% MeOH, MeOH, and acetone (each 90 l). The MeOH fraction (209 g) was subjected to activated charcoal (500 g) column chromatography eluting with MeOH, $CHCl_3$–MeOH (1 : 9), and $CHCl_3$–MeOH (1 : 1), each 30 l. The MeOH fraction (51 g) was crystallized from MeOH to give crystalline gedunin[3,11,12] (9.0 g). The mother liquid was further subjected to RP-18 (40 g) column chromatography, eluting with MeOH–H_2O (60 : 40, 70 : 30, 80 : 20, 90 : 10, 100 : 0, each 2 l), to afford five fractions (frs. 1—5). After removal of the solvent to dryness, fr. 1 (511 mg) was subjected to HPLC using MeOH–H_2O (60 : 40) to afford **2** (4.0 mg). Analogously, fr. 2 (2.1 g) was subjected to HPLC using MeOH–H_2O (60 : 40) to afford a triterpenoid-containing fraction, which after subsequent purification by HPLC using MeCN–H_2O (40 : 60), and MeOH–H_2O (53 : 47) afforded **1** (4.3 mg). Fr. 3 (7.0 g) was subjected to HPLC using MeOH–H_2O (65 : 35) to afford three triterpenoid-containing fractions, each of which was subsequently purified by HPLC using MeCN–H_2O (50 : 50) to give **10** (8.8 mg), **11** (15.0 mg), and **9** (141.1 mg), respectively. Fr. 4 (14 g) was subjected to HPLC using MeOH–H_2O (80 : 20) to afford five triterpenoid-containing fractions, each of which was subsequently purified by HPLC using MeCN–H_2O (60 : 40) and MeOH–H_2O (75 : 25), to give **12** (18.4 mg), **4** (1.4 mg), **7** (10.2 mg), **3** (0.7 mg), and **5** (9.3 mg), respectively.

The $CHCl_3$–MeOH (1 : 9) fraction (28 g) was crystallized from MeOH to give crystalline gedunin (7.2 g). The mother liquid was subjected to HPLC using MeOH–H_2O (80 : 20) to afford five triterpenoid-containing fractions, each of which was subsequently purified by HPLC using MeCN–H_2O (60 : 40) and MeOH–H_2O (75 : 25) to give **15** (19.1 mg), **6** (3.4 mg), **14** (348.6 mg), **13** (170.9 mg), and **8** (28.1 mg), respectively.

Compound 1 Amorphous solid. $[\alpha]_D^{27}$ −72° (*c*=0.19, $CHCl_3$). IR (film) cm^{-1}: 3435, 2968, 1643, 1452, 1387, 1209, 1097, 1032, 1003, 949. ^{1}H- and ^{13}C-NMR spectra, see Tables 1 and 2. HR-ESI-MS *m*/*z*: 487.3410 ($[M-OMe]^+$, Calcd for $C_{30}H_{47}O_5$: 487.3424).

Compound 2 Amorphous solid. $[\alpha]_D^{27}$ −6.3° (*c*=0.14, $CHCl_3$). IR (film) cm^{-1}: 3440, 2937, 1701, 1649, 1510, 1458, 1385, 1242, 1155, 1097. ^{1}H- and ^{13}C-NMR spectra, see Tables 1 and 2. HR-ESI-MS *m*/*z*: 487.3419 ($[M-OMe]^+$, Calcd for $C_{30}H_{47}O_5$: 487.3424).

Compound 3 Amorphous solid. $[\alpha]_D^{26}$ −142° (*c*=0.04, $CHCl_3$). UV λ_{max} (MeOH) nm (log ε): 213 (4.26). IR (film) cm^{-1}: 3437, 2933, 1645, 1460, 1385, 1230, 1147, 1034. ^{1}H- and ^{13}C-NMR spectra, see Tables 1 and 2. HR-ESI-MS *m*/*z*: 625.4049 ($[M+Na]^+$, Calcd for $C_{36}H_{58}O_7Na$: 625.4080).

Compound 4 Amorphous solid. $[\alpha]_D^{26}$ −34° (*c*=0.06, $CHCl_3$). UV λ_{max} (MeOH) nm (log ε): 211 (4.21). IR (film) cm^{-1}: 3458, 2933, 1645, 1558, 1458, 1381, 1230, 1147, 1065, 1030, 995. ^{1}H- and ^{13}C-NMR spectra, see Tables 1 and 2. HR-ESI-MS *m*/*z*: 641.4006 ($[M+Na]^+$, Calcd for $C_{36}H_{58}O_8Na$: 641.4029).

Compound 5 Amorphous solid. $[\alpha]_D^{28}$ −78° (*c*=0.18, $CHCl_3$). UV λ_{max} (MeOH) nm (log ε): 213 (4.26). IR (film) cm^{-1}: 3437, 2943, 2868, 1645, 1444, 1385, 1346, 1292, 1232, 1149, 1107, 1074, 1024, 993. ^{1}H- and ^{13}C-NMR spectra, see Tables 1 and 2. HR-ESI-MS *m*/*z*: 573.4123 ($[M+H]^+$,

Calcd for $C_{35}H_{57}O_6$: 573.4155).

Compound 6 Amorphous solid. $[\alpha]_D^{27}$ −107° (c=0.17, $CHCl_3$). IR (film) cm^{-1}: 3431, 2958, 2870, 1643, 1463, 1385, 1296, 1255, 1200, 1184, 1165, 1103, 1068, 1028, 991. ^1H- and ^{13}C-NMR spectra, see Tables 1 and 2. HR-ESI-MS m/z: 591.4304 ($[M+H]^+$, Calcd for $C_{35}H_{59}O_7$: 591.4261).

Compound 7 Amorphous solid. $[\alpha]_D^{27}$ −81° (c=0.45, $CHCl_3$). IR (film) cm^{-1}: 3444, 2970, 1645, 1468, 1387, 1367, 1236, 1213, 1155, 1099, 1065. ^1H- and ^{13}C-NMR spectra, see Tables 1 and 2; HR-ESI-MS m/z: 525.3531 ($[M+Na]^+$, Calcd for $C_{31}H_{50}O_5Na$: 525.3556).

Compound 8 Amorphous solid. $[\alpha]_D^{28}$ −1.6° (c=0.28, $CHCl_3$). IR (film) cm^{-1}: 3734, 3421, 2935, 1703, 1473, 1387, 985, 822. ^1H- and ^{13}C-NMR spectra, see Tables 1 and 2; HR-ESI-MS m/z: 525.3569 ($[M+Na]^+$, Calcd for $C_{31}H_{50}O_5Na$: 525.3556).

Compound 9 Colorless prisms (MeOH). mp 265—268 °C. $[\alpha]_D^{27}$ −17° (c=0.37, $CHCl_3$). UV (MeOH) λ_{max} nm (log ε): 228 (4.00). IR (film) cm^{-1}: 3442, 2979, 2935, 1736, 1670, 1458, 1379, 1300, 1246. ^1H- and ^{13}C-NMR spectra, see Tables 1 and 2. HR-ESI-MS m/z: 569.3811 ($[M+H]^+$, Calcd for $C_{35}H_{53}O_6$: 569.3842).

Sapelin E Acetate (10) Amorphous solid. $[\alpha]_D^{27}$ −7.1° (c=0.89, $CHCl_3$). ^1H-NMR (500 MHz, $CDCl_3$) δ: 7.15 (1H, d, J=10.2 Hz, H-1), 5.85 (1H, d, J=10.2 Hz, H-2), 5.30 (1H, d, J=2.2 Hz, H-15), 5.22 (1H, s-like, H-7), 3.81 (1H, td, J=9.0, 2.9 Hz, H-23), 3.61 (1H, dd, J=12.9, 1.7 Hz, H-21α), 3.50 (1H, dd, J=12.9, 3.0 Hz, H-21β), 3.42 (1H, d, J=9.0 Hz, H-24), 2.26 (1H, ddd, J=14.9, 6.1, 3.5 Hz, H-16β), 2.20 (1H, dd, J=12.3, 5.4 Hz, H-9), 2.18 (1H, dd, J=13.1, 2.1 Hz, H-5), 1.99 (1H, m, H-16α), 1.96 (1H, m, H-22a), 1.94 (3H, s, OAc), 1.94 (1H, m, H-6β), 1.90 (3H, m, H-11α, H-17, H-20), 1.87 (1H, m, H-12α), 1.78 (1H, dt, J=14.5, 2.6 Hz, H-6α), 1.70 (1H, m, H-11β), 1.65 (1H, m, H-12β), 1.62 (1H, m, H-22b), 1.31 (3H, s, H_3-27), 1.19 (3H, s, H_3-30), 1.16 (6H, s, H_3-19, H_3-26), 1.07 (6H, s, H_3-28, H_3-29), 0.98 (3H, s, H_3-18). ^{13}C-NMR (125 MHz, $CDCl_3$) δ: 204.7 (C, C-3), 170.1 (C, O$\underline{C}$OCH$_3$), 159.2 (C, C-14), 158.4 (CH, C-1), 125.5 (CH, C-2), 119.1 (CH, C-15), 80.8 (CH, C-24), 76.2 (C, C-25), 74.7 (CH, C-7), 68.2 (CH, C-23), 64.3 (CH_2, C-21), 54.3 (CH, C-17), 46.4 (C, C-13), 46.2 (CH, C-5), 44.2 (C, C-4), 42.8 (C, C-8), 39.9 (C, C-10), 38.5 (CH, C-9), 37.9 (CH_2, C-22), 36.4 (CH, C-20), 35.0 (CH_2, C-16), 34.1 (CH_2, C-12), 27.4 (CH_3, C-30), 27.1 (CH_3, C-28), 26.3 (CH_3, C-27), 23.9 (CH_2, C-6), 22.4 (CH_3, C-26), 21.3 (CH_3, C-29), 21.2 (CH_3, OCO$\underline{C}$H$_3$), 20.0 (CH_3, C-18), 19.0 (CH_3, C-19), 16.8 (CH_2, C-11); HR-ESI-MS m/z: 529.3505 ($[M+H]^+$, Calcd for $C_{32}H_{49}O_6$: 529.3529).

Grandifoliolenone (11) Amorphous solid. $[\alpha]_D^{27}$ −9.0° (c=0.53, $CHCl_3$). ^1H-NMR (500 MHz, $CDCl_3$) δ: 7.16 (1H, d, J=10.2 Hz, H-1), 5.85 (1H, d, J=10.2 Hz, H-2), 5.32 (1H, d, J=2.3 Hz, H-15), 5.22 (1H, s-like, H-7), 3.96 (1H, d, J=11.6 Hz, H-21β), 3.86 (1H, ddd, J=10.8, 9.2, 4.8 Hz, H-23), 3.44 (1H, dd, J=11.6, 2.2 Hz, H-21α), 2.89 (1H, d, J=9.2 Hz, H-24), 2.28 (1H, ddd, J=14.5, 6.5, 3.6 Hz, H-16β), 2.20 (1H, dd, J=11.7, 5.2 Hz, H-9), 2.18 (1H, dd, J=12.7, 2.1 Hz, H-5), 2.02 (1H, m, H-22β), 2.00 (1H, m, H-17), 1.95 (1H, m, H-16α), 1.94 (3H, s, OAc), 1.94 (1H, m, H-6β), 1.93 (1H, m, H-12α), 1.91 (1H, m, H-11α), 1.88 (1H, m, H-20), 1.78 (1H, dt, J=14.5, 2.7 Hz, H-6α), 1.68 (1H, m, H-11β), 1.64 (1H, m, H-12β), 1.53 (1H, ddd, J=13.1, 10.8, 4.6 Hz, H-22α), 1.30 (3H, s, H_3-27), 1.27 (3H, s, H_3-26), 1.20 (3H, s, H_3-30), 1.15 (3H, s, H_3-19), 1.07 (6H, s, H_3-28, H_3-29), 0.96 (3H, s, H_3-18). ^{13}C-NMR (125 MHz, $CDCl_3$) δ: 204.7 (C, C-3), 170.1 (C, O$\underline{C}$OCH$_3$), 159.0 (C, C-14), 158.4 (CH, C-1), 125.5 (CH, C-2), 119.6 (CH, C-15), 86.6 (CH, C-24), 74.9 (CH, C-7), 74.2 (C, C-25), 70.1 (CH_2, C-21), 64.4 (CH, C-23), 52.4 (CH, C-17), 46.4 (C, C-13), 46.2 (CH, C-5), 44.2 (C, C-4), 42.8 (C, C-8), 39.8 (C, C-10), 38.7 (CH, C-9), 36.3 (CH_2, C-22), 35.9 (CH, C-20), 35.0 (CH_2, C-12), 34.9 (CH_2, C-16), 28.6 (CH_3, C-27), 27.3 (CH_3, C-30), 27.1 (CH_3, C-28), 24.1 (CH_3, C-26), 23.9 (CH_2, C-6), 21.2 (2C, CH_3, C-29, OCO$\underline{C}$H$_3$), 20.3 (CH_3, C-18), 19.1 (CH_3, C-19), 16.9 (CH_2, C-11). HR-ESI-MS m/z: 529.3530 ($[M+H]^+$, Calcd for $C_{32}H_{49}O_6$: 529.3529).

Azadirone (12) Amorphous solid. $[\alpha]_D^{27}$ +22° (c=0.82, $CHCl_3$). HR-ESI-MS m/z: 437.2693 ($[M+H]^+$, Calcd for $C_{28}H_{37}O_4$: 437.2692).

Bourjotinolone A (13) Amorphous solid. $[\alpha]_D^{27}$ −35° (c=0.32, $CHCl_3$). HR-ESI-MS m/z: 473.3639 ($[M+H]^+$, Calcd for $C_{30}H_{49}O_4$: 473.3631).

Piscidinol A (14) Colorless needles (MeOH). mp 197—200 °C. $[\alpha]_D^{27}$ −85° (c=0.14, $CHCl_3$). HR-ESI-MS m/z: 475.3751 ($[M+H]^+$, Calcd for $C_{30}H_{51}O_4$: 475.3787).

Hispidol B (15) Colorless prisms (MeOH). mp 255—257 °C. $[\alpha]_D^{28}$ −58° (c=0.24, $CHCl_3$–EtOH, 1 : 1). HR-ESI-MS m/z: 477.3944 ($[M+H]^+$, Calcd for $C_{30}H_{53}O_4$: 477.3944).

Treatment of 9 with Boron Trifluoride Diethyl Etherate To a solution of **9** (10.8 mg, 0.019 mmol) in MeOH–H_2O (2 : 1, 1.5 ml) was added a catalytic amount of boron trifluoride diethyl etherate, and the mixture was stirred at room temperature for 24 h. The mixture was diluted with $CHCl_3$, washed successively with H_2O and brine, dried over Na_2SO_4, filtrated and concentrated under reduced pressure. The residue was purified by ODS HPLC using MeCN–H_2O (50 : 50) to give a product (9.5 mg, 95%), which was identified as sapelin E acetate by comparison of their ^1H- and ^{13}C-NMR spectra, and HR-ESI-MS.

X-Ray Crystallographic Study of 9 $C_{35}H_{52}O_6$, M=568.77, 0.43×0.43×0.30 mm, orthorhombic, $P2_12_12_1$, a=8.77400(10) Å, b=13.3180(5) Å, c=26.1910(9) Å, V=3060.47(16) Å^3, Z=4, D_x=1.234 Mg m^{-3}, μ(Mo$K\alpha$)=0.082 mm^{-1}, 3596 measured reflections, 3596 unique reflections, 3047 observed reflections [$I>2\sigma(I)$], R1=0.0455, wR2=0.1110 (observed data), GOF=0.999; R1=0.0508, wR2=0.1127 (all data). The structure was solved by direct methods using the maXus crystallographic software package,[13] and refined by full-matrix least-squares on F^2 using the program SHELXL-97.[14]

CCDC 624284 contains the supplementary crystallographic data for compound **9** in this paper. These data can be obtained free of charge *via* http://www.ccdc.cam.ac.uk/data_request/cif, or by e-mailing data_request@ccdc.cam.ac.uk, or by contacting The Cambridge Crystallographic Data Centre, 12 Union Road, Cambridge CB2 1EZ, U.K.; fax: +44 1223 336033.

Assay for Cytotoxic Activity Evaluation of cytotoxicity against P-388 murine leukemia cells was performed as described previously.[15]

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