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Polyhydroxylated Steroidal Glycosides from *Paris polyphylla*

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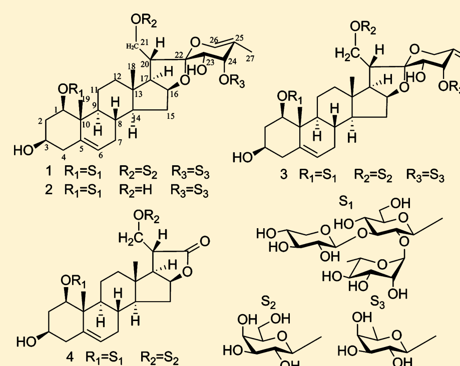
Supporting Information

ABSTRACT: Three new steroidal saponins, parisyunnanosides G–I (**1**–**3**), one new C_{21} steroidal glycoside, parisyunnanoside J (**4**), and three known compounds, padelaoside B (**5**), pinnatasterone (**6**), and 20-hydroxyecdyson (**7**), were isolated from the rhizomes of *Paris polyphylla* Smith var. *yunnanensis*. Compounds **1** and **3** have unique trisdesmoside structures that include a C-21 β-D-galactopyranose moiety. All compounds were evaluated for their cytotoxicity against human CCRF leukemia cells.



The genus *Paris* belongs to the Liliaceae family and includes more than 24 species throughout the world.[1] Steroidal saponins are believed to be the main active ingredients in this family. In recent years, steroidal saponins have attracted scientific attention because of their structural diversity and significant bioactivities, including antitumor, hemostatic, immunotropic, and analgesic properties.[2−8] In this report, we discuss the isolation and identification of four new (**1**–**4**) and three known compounds (**5**–**7**) from the ethanolic extract of *Paris polyphylla* Smith var. *yunnanensis*. We also evaluated the cytotoxicity against human CCRF leukemia cells of all compounds.

The rhizomes of *P. polyphylla* were extracted using 90% aqueous EtOH. The extract was subjected to macroporous resin SP825 followed by silica gel, RP-LC, and preparative HPLC to yield parisyunnanosides G–J (**1**–**4**), padelaoside B (**5**),[3] pinnatasterone (**6**), and 20-hydroxyecdyson (**7**).[9]

Parisyunnanoside G (**1**) was isolated as a white, amorphous powder. The negative HRESIMS showed an $[M - H]^-$ ion at m/z 1223.5344, corresponding to the molecular formula $C_{56}H_{88}O_{29}$ (calcd. for $C_{56}H_{87}O_{29}$, 1223.5339). The ^1H NMR spectrum of **1** showed three methyl singlets at δ 1.04 (s, CH_3-18), 1.33 (s, CH_3-19), and 1.84 (s, CH_3-27), signals from a primary alcohol group at δ 3.97 (1H, dd, J = 6.6, 10.2 Hz, H-21) and 4.48 (1H, m, H-21), five methine protons indicative of secondary alcoholic functions at δ 3.73 (m, H-3), 3.79 (dd, J = 3.6, 12.0 Hz, H-1), 4.12 (m, H-24), 4.58 (m, H-16), and 4.60

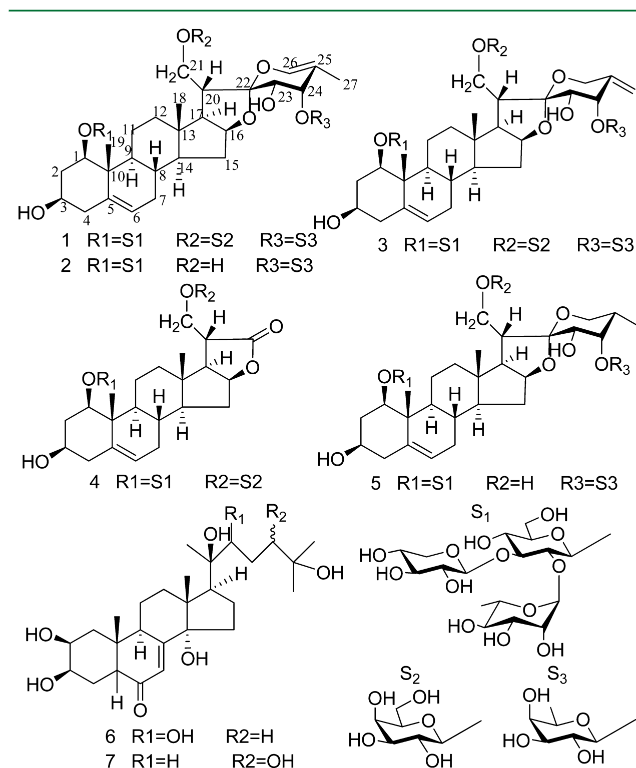


Figure 1. Structures of compounds **1**–**7**.

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Table 1. ^{1}H (600 MHz) and ^{13}C NMR (150 MHz) Data for the Aglycone Moieties of Compounds 1−4 in Pyridine-d_5

	1		2		3		4	
position	δ_C	δ_H (J in Hz)	δ_C	δ_H (J in Hz)	δ_C	δ_H (J in Hz)	δ_C	δ_H (J in Hz)
1	84.4	3.79, dd (3.6, 12.0)	84.0	3.84, dd (3.6, 12.0)	84.7	3.78, dd (3.6, 12.0)	84.0	3.85, m
2	38.0	2.37, q-like (12.0)	37.7	2.35, q-like (12.0)	38.1	2.39, q-like (12.0)	37.8	2.38, q-like (12.0)
		2.64, m		2.63, m		2.62, m		2.62, m
3	68.1	3.73, m	68.0	3.72, m	68.1	3.72, m	68.0	3.76, m
4	43.8	2.52, dd (3.6, 12.0)	43.8	2.52, dd (3.6, 12.0)	43.8	2.52, dd (3.6, 12.0)	43.7	2.54, dd (4.2, 12.0)
		2.63, m		2.64, m		2.64, m		2.63, m
5	139.5		139.5		139.4		139.4	
6	124.7	5.52, d (5.4)	124.7	5.52, d (5.4)	124.8	5.53, br d (5.4)	124.6	5.54, br d (5.4)
7	31.8	1.37, m	31.8	1.38, m	31.8	1.43, m	31.7	1.48, m
		1.65, m		1.64, m		1.75, m		1.77, m
8	33.0	1.34, o	33.1	1.34, m	33.0	1.44, m	32.8	1.43, m
9	50.2	1.53, m	50.1	1.58, m	50.3	1.58, m	50.2	1.59, m
10	42.8		42.8		42.8		42.7	
11	24.0	1.51, m	24.1	1.61, m	24.1	1.48, m	23.6	1.55, m
		2.78, br d (10.2)		2.82, m		2.77, br d (13.2)		2.84, br d (12.6)
12	40.0	1.43, m	40.2	1.46, m	40.1	1.45, o	38.5	1.38, m
		1.81, m		1.93, m		1.89, br d (11.4)		1.59, m
13	41.1		41.2		41.0		41.8	
14	56.9	1.07, m	56.9	1.13, m	57.1	1.15, m	54.6	0.87, m
15	32.5	1.30, m	32.7	1.32, m	32.4	1.42, o	33.3	1.36, m
		1.71, m		1.72, m		1.79, m		1.87, m
16	83.8	4.58, m	84.0	4.64, m	83.4	4.53, o	84.3	4.82, m
17	57.8	1.88, dd (7.2, 7.8)	58.4	1.99, dd (7.2, 7.8)	58.4	1.94, dd (7.2, 8.4)	55.1	2.32, d (7.8)
18	16.8	1.04, s	17.0	1.10, s	16.9	1.03, s	14.1	0.78, s
19	15.1	1.33, s	15.1	1.36, s	15.1	1.33, s	15.0	1.36, s
20	42.5	3.59, m	45.4	3.54, m	43.6	3.42, q-like (6.6)	43.4	2.88, m
21	70.2	3.97, dd (6.6, 10.2)	62.6	4.05, m	70.0	3.95, m	70.6	4.20, m
		4.48, dd (7.7, 10.2)		4.22, m		4.43, m		4.30, m
22	110.8		111.2		111.5		179.5	
23	69.5	4.60, dd (3.4, 9.3)	70.0	4.66, o	71.3	4.34, dd (3.6, 9.6)		
24	76.1	4.12, dd (3.4, 9.3)	76.3	4.29, d (3.6)	81.9	4.67, d (3.6)		
25	109.5		109.5		143.7			
26	138.0	6.21, s	138.2	6.28, s	61.6	3.99, d (11.4)		
						4.84, d (11.4)		
27	15.7	1.84, s	15.8	1.91, s	113.7	5.01, s		
						5.10, s		

(m, H-23), and two olefinic protons at δ 5.52 (1H, br d, H-6) and 6.21 (1H, s, H-26). In the ^{13}C NMR spectrum, four olefinic carbons at δ 139.5 (C-5), 124.7 (C-6), 109.5 (C-25), and 138.0 (C-26) were observed. Comparing the NMR data with those of padelaoside A[3] and bethoside A,[10] five hydroxy groups were determined to be located at C-1, C-3, C-21, C-23, and C-24 on the aglycone of **1**.[11] The presence of a C-25−C-26 double bond was supported by H-26 (δ 6.21) showing long-range correlations with C-27 (δ 15.7), C-24 (δ 76.1), C-25 (δ 109.5), and C-22 (δ 110.8) in the HMBC spectrum. The various ring junctions and relative configurations at C-1, C-3, C-20, C-23, and C-24 could be deduced from the NOESY experiment.[4,10,11] NOE correlations were observed between H-8/H-18, H-18/H-20, H-9/H-14, H-14/H-16/H-17, and H-17/H-20, suggesting the usual *trans* junction for the B/C and C/D rings, a *cis* ring fusion for the D/E rings, and a 20*R* configuration. Additional NOE correlations between H-1/H-9 and H-1/H-2b/H-3 were indicative of β orientations for OH-1 and OH-3. Finally, correlations between H-20/H-23, H-23/H-24, and H-24/H-27, along with the small coupling constant (3.4 Hz) between H-23 and H-24, indicated *S* configurations at C-23 and C-24.[3,10−12] Thus, the aglycone moiety of **1** was deduced as (23*S*,24*S*)-spirost-5,25(26)-diene-1β,3β,21,23α,24α-pentol, a new sapogenin. The anomeric regions in the ^{1}H and ^{13}C NMR spectra of **1** showed five anomeric protons at δ 4.75 (d, J = 7.8 Hz), 6.37 (s), 4.91 (d, J = 7.8 Hz), 4.92 (d, J = 7.8 Hz), and 5.59 (d, J = 8.4 Hz), corresponding to anomeric carbon signals at δ 100.2, 101.8, 105.2, 105.4, and 103.6, respectively. Acidic hydrolysis of **1** produced D-galactose, D-glucose, D-xylose, D-fucose, and L-rhamnose. The combined use of COSY, TOCSY, HSQC, and HMBC experiments allowed for the assignment of all resonances for each monosaccharide moiety. Starting from the anomeric proton of each sugar unit, all of the protons within each spin system were delineated using COSY, with the aid of the TOCSY spectrum. The ^{13}C NMR resonances of each sugar unit were identified by HSQC and further confirmed by HMBC experiments. The HMBC spectrum showed long-range correlations between H-1-Glc (δ 4.75) and C-1 (δ 84.4); H-1-Rha (δ 6.37) and C-2-Glc (δ 76.4); H-1-Xyl (δ 4.91) and C-3-Glc (δ 88.4); H-1-Gal (δ 4.92) and C-21 (δ 70.2); and H-1-Fuc (δ 5.59) and C-24 (δ 76.1). These correlations reveal the sequence of the sugars and their linkage sites to the aglycone moiety. Thus, the structure of **1** was elucidated as (23*S*,24*S*)-

Table 2. ^{1}H (600 MHz) and ^{13}C NMR (150 MHz) Data for the Sugar Portions of Compounds 1−4 in Pyridine-d_5

	1		2		3		4	
position	δ_C	δ_H	δ_C	δ_H	δ_C	δ_H	δ_C	δ_H
Glc								
1	100.2	4.75, d (7.8)	99.9	4.78, d (7.8)	100.3	4.74, d (7.8)	99.7	4.79, d (7.8)
2	76.4	4.09, m	76.4	4.12, m	76.4	4.05, m	76.3	4.14, dd (7.8, 9.0)
3	88.4	4.03, dd (9.0, 9.0)	88.5	4.03, m	88.4	4.02, m	88.6	4.04, m
4	70.0	3.84, dd (9.0, 9.6)	70.2	3.77, m	70.0	3.83, dd (8.4, 9.6)	70.0	3.84, dd (9.6, 9.0)
5	77.7	3.76, m	77.8	3.76, m	77.7	3.74, m	77.8	3.76, m
6a	63.2	4.22, m	63.3	4.14, m	63.2	4.23, m	63.0	4.20, m
6b		4.49, o		4.44, dd (4.8, 10.8)		4.45, m		4.47, o
Rha								
1	101.8	6.37, s	101.7	6.38, s	101.8	6.34, s	101.7	6.43, s
2	72.5	4.76, br d (3.6)	72.5	4.75, o	72.5	4.75, o	72.5	4.77, o
3	72.5	4.56, o	72.5	4.55, m	72.5	4.55, o	72.5	4.56, o
4	74.2	4.29, dd (9.6, 9.6)	74.2	4.29, dd (9.6, 9.6)	74.2	4.28, dd (9.6, 9.6)	74.2	4.30, dd (9.6, 9.6)
5	69.6	4.79, dq (9.6, 6.6)	69.6	4.78, m	69.6	4.77, dd (6.6, 9.6)	69.6	4.80, m
6	19.3	1.72, d (6.6)	19.3	1.72, d (6.6)	19.3	1.72, d (6.6)	19.3	1.73, d (6.0)
Xyl								
1	105.2	4.91, d (7.8)	105.3	4.91, d (7.8)	105.2	4.91, d (7.8)	105.3	4.92, d (7.8)
2	74.8	3.94, dd (7.8, 8.4)	74.8	3.95, dd (7.8, 8.4)	74.8	3.92, m	74.8	3.96, dd (7.8, 8.4)
3	78.4	4.06, m	78.4	4.05, m	78.4	4.03, m	78.4	4.05, m
4	70.6	4.11, o	70.6	4.09, o	70.6	4.09, m	70.6	4.11, o
5a	67.3	3.66, dd (10.8, 10.8)	67.3	3.66, dd (10.8, 10.8)	67.3	3.66, dd (10.8, 10.8)	67.3	3.67, dd (10.2, 11.4)
5b		4.24, dd (4.8, 10.8)		4.23, dd (4.8, 10.8)		4.23, dd (4.8, 10.8)		4.24, dd (5.4, 11.4)
Gal								
1	105.4	4.92, d (7.8)			105.3	4.85, d (7.8)	105.4	4.77, d (7.2)
2	72.7	4.46, o			72.5	4.43, o	72.4	4.44, o
3	75.5	4.17, dd (3.0, 9.6)			75.5	4.12, dd (3.0, 9.6)	75.3	4.12, dd (3.0, 9.6)
4	70.3	4.56, o			70.3	4.56, o	70.2	4.55, o
5	77.1	4.10, o			77.1	4.06, o	77.1	4.02, o
6	62.4	4.44, o			62.4	4.39, o	62.4	4.46, o
Fuc								
1	103.6	5.59, d (8.4)	103.7	5.63, d (8.4)	103.7	5.72, d (8.4)		
2	70.6	4.53, o	70.5	4.58, m	70.6	4.53, o		
3	73.9	4.68, br d (3.2)	74.0	4.68, m	73.5	4.73, m		
4	73.5	4.09, o	73.5	4.09, o	73.5	4.07, m		
5	70.0	4.54, o	70.0	4.54, o	70.0	4.53, o		
6	17.0	1.50, d (6.0)	17.0	1.51, d (6.0)	16.9	1.45, d (6.6)		

spirost-5,25-diene-1β,3β,21,23α,24α-pentol-1-*O*-{α-L-rhamnopyranosyl-(1→2)-[β-D-xylopyranosyl-(1→3)]-β-D-glucopyranosyl}-21-*O*-β-D-galactopyranosyl-24-*O*-β-D-fucopyranoside.

Parisyunnanoside H (**2**) was isolated as a white, amorphous powder. The negative HRESIMS showed an $[M - H]^-$ ion peak at *m/z* 1061.4813 (calcd for $C_{50}H_{77}O_{24}$, 1061.4810), corresponding to the molecular formula $C_{50}H_{78}O_{24}$. A comparison of the NMR and MS data of **2** with those of **1** revealed that they have similar structures but significant differences in lacking the galactose residue. The HMBC spectrum showed long-range correlations between H-1-Glc (δ 4.78) and C-3 (δ 84.0); H-1-Rha (δ 6.38) and C-2-Glc (δ 76.4); H-1-Xyl (δ 4.91) and C-3-Glc (δ 88.5); and H-1-Fuc (δ 5.63) and C-24 (δ 76.3). Thus, **2** was determined to be (23*S*,24*S*)-spirost-5,25-diene-1β,3β,21,23α,24α-pentol-1-*O*-{α-L-rhamnopyranosyl-(1→2)-[β-D-xylopyranosyl-(1→3)]-β-D-glucopyranosyl}-24-*O*-β-D-fucopyranoside.

Parisyunnanoside I (**3**) was isolated as a white, amorphous powder. The negative HRESIMS (*m/z* 1223.5341 $[M - H]^-$, calcd for $C_{56}H_{87}O_{29}$, 1223.5339) indicated that the molecular formula of **3** is $C_{56}H_{88}O_{29}$. The ^{1}H NMR spectrum showed three olefinic protons at δ 5.53 (1H, s, H-6), 5.01 (1H, s, H-26), and 5.10 (1H, s, H-26). Comparing the ^{1}H and ^{13}C NMR data with those of **1** and recurvoside E,[11] it could be deduced that **3** contained the same aglycone as recurvoside E and the same sugar chain linkages at C-1 and C-21 as **1**. The HMBC spectrum showed long-range correlations between H-1-Fuc (δ 5.72) and C-24 (δ 81.9), indicating the sugar linkage site to C-24 of the aglycone. Therefore, the structure of **3** was assigned as (23*S*,24*S*)-spirost-5,25(27)-diene-1β,3β,21,23α,24α-pentol-1-*O*-{α-L-rhamnopyranosyl-(1→2)-[β-D-xylopyranosyl-(1→3)]-β-D-glucopyranosyl}-21-*O*-β-D-galactopyranosyl-24-*O*-β-D-fucopyranoside.

Parisyunnanoside J (**4**) was isolated as a white, amorphous powder with a molecular formula of $C_{45}H_{70}O_{23}$, which was determined by the negative ion HRESIMS (*m/z* 977.4230 $[M - H]^-$). The ^{1}H NMR spectrum showed two methyl singlets at δ 0.78 (s, CH_3-18) and 1.36 (s, CH_3-19). Comparison of the NMR data of **4** with those of **1** and a newly reported saponin[13] indicated **4** contained the same aglycone and sugar chain linkage at C-1 of the aglycone as the reported saponin,[13] and their structural difference could be deduced to be the sugar chain linkage at C-21 of the aglycone. The HMBC correlations between H-1-Gal (δ 4.77) and C-21 (δ 70.6) indicated the

sugar linkage site to the aglycone moiety. Thus, **4** was elucidated as 21-*O*-β-D-galactopyranosyl-1-*O*-{α-L-rhamnopyranosyl-(1→2)-[β-D-xylopyranosyl-(1→3)]-β-D-glucopyranoside}-(20*R*)-1β,3β,21-trihydroxypregn-5-ene-20,16β-carbolactone.

Compounds **1**–**7**, Pa, Pb, gracillin, prosapogenin A of dioscin, Tg, Tb, chonglououside H, dichotomin, Th, pseudoproto-Pb, parisaponin I, parisyunnanoside A, and protogracillin[6] were evaluated for cytotoxicity against human CCRF-CEF leukemia cells. Only dichotomin, parisaponin I, protogracillin, Th, parisyunnanoside A, and pseudoproto-Pb showed moderate to weak activities. IC_{50} values were observed ranging from 0.97 (parisaponin I) to 85.34 μM (pseudoproto-Pb). The spirostanol saponins and sterol showed no cytotoxicity, whereas the furostanol saponins were highly toxic. Comparing the activities of dichotomin, Th, and pseudoproto-Pb, it can be concluded that compounds with a 17α-hydroxy group and $\Delta^{20(22)}$ double bond on the aglycone moiety will have reduced cytotoxicity.

EXPERIMENTAL SECTION

General Experimental Procedures. Optical rotations were measured with a Perkin-Elmer 343 polarimeter. NMR experiments were performed on a Varian ^{UNITY}INOVA 600 spectrometer in pyridine-d_5 (99.95%, Sigma-Aldrich). FAB-MS was performed on a Micromass Zabspec spectrometer. HRESIMS was recorded on a 9.4 T Q-FT-MS Apex Qe (Bruker Co., Germany). HPLC was performed on an Agilent 1100 series; ODS columns (5 μm, 4.6 × 250 mm, YMC); Lichrospher C18 (5 μm, 10 × 250 mm, Hanbon Sci. & Tech.). Column chromatography (CC) was performed with macroporous resin SP825 (Mitsubishi Chemicals, Japan), silica gel (Qingdao Haiyang Chemical Co., Ltd., China), and ODS-A silica gel (120 Å, 50 μm, YMC, Japan).

Table 3. IC_{50} Values against Human CCRF-CEM Leukemia Cells ($\bar{x} \pm$ SD)

compound	IC_{50} (μM)	compound	IC_{50} (μM)
doxorubicin	2.14 ± 0.005	7	
1		dichotomin	0.59 ± 0.11
2		pseudoproto-Pb	6.52 ± 0.29
3		parisyunnanoside A	6.68 ± 0.22
4		Th	5.15 ± 0.16
5		parisaponin I	1.23 ± 0.08
6		proto-gracillin	1.77 ± 0.14

Plant Material. Dried rhizomes of *P. polyphylla* var. *yunnanensis* were collected from the Lijiang region of Yunnan Province, People's Republic of China, in November 2004. The plant was identified by Prof. J. M. Huang (Beijing University of Traditional Chinese Medicine), and a voucher specimen (No. 041120) was deposited in the Herbarium of the Beijing Institute of Radiation Medicine, Beijing.

Extraction and Isolation. Dried rhizomes slices (7.8 kg) were refluxed in 90% EtOH. The extract was chromatographed on macroporous resin SP825, eluting with a gradient mixture of actone–H_2O to produce five fractions. Fr. A-1 (1.5 g) was further separated by ODS silica gel (eluted with actone–H_2O) and preparative HPLC (eluted with actone–H_2O) to yield **6** (15.7 mg) and **7** (11.5 mg). Fr. A-2 (1.1 g) was subjected to CC on ODS silica gel (eluted with actone–H_2O) and preparative HPLC (eluted with actone–H_2O) to yield **1** (19.9 mg) and **3** (18.4 mg). Fr. A-3 (4.9 g) was further separated by silica gel (eluted with $CHCl_3$–MeOH–H_2O), ODS silica gel (eluted with actone–H_2O), and preparative HPLC (eluted with actone–H_2O) to yield **2** (28.6 mg), **4** (18.4 mg), and **5** (13.3 mg).

*Parisyunnanoside G (**1**):* white, amorphous powder; $[\alpha]_D^{20}$ −85.6 (*c* 0.05, pyridine); ^{1}H and ^{13}C NMR data, see Tables 1 and 2; HRESIMS *m/z* 1223.5344 $[M - H]^-$ (calcd for 1223.5339 $[C_{56}H_{87}O_{29}]^-$).

*Parisyunnanoside H (**2**):* white, amorphous powder; $[\alpha]_D^{20}$ −91.8 (*c* 0.05, pyridine); ^{1}H and ^{13}C NMR data, see Tables 1 and 2; HRESIMS *m/z* 1061.4813 $[M - H]^-$ (calcd for 1061.4810 $[C_{50}H_{77}O_{24}]$).

*Parisyunnanoside I (**3**):* white, amorphous powder; $[\alpha]_D^{20}$ −110.2 (*c* 0.04, pyridine); ^{1}H and ^{13}C NMR data, see Tables 1 and 2; HRESIMS *m/z* 1223.5341 $[M - H]^-$ (calcd for 1223.5339 $[C_{56}H_{87}O_{29}]$).

*Parisyunnanoside J (**4**):* white, amorphous powder; $[\alpha]_D^{20}$ −44.6 (*c* 0.04, pyridine); ^{1}H and ^{13}C NMR data, see Tables 1 and 2; HRESIMS *m/z* 977.4230 $[M - H]^-$ (calcd for 977.4235 $[C_{45}H_{69}O_{23}]^-$).

Acidic Hydrolysis. The hydrolysis and GC-MS analysis of the chiral derivatives of the sugars of compounds **1**–**4** were done as previously described.[14] D-Galactose, D-glucose, D-xylose, D-fucose, and L-rhamnose were detected for **1** and **3**; D-glucose, D-xylose, D-fucose, and L-rhamnose were detected for **2**; and D-glucose, D-xylose, L-rhamnose, and D-galactose were detected for **4**.

XTT Proliferation Assay. Compounds were first tested at a fixed concentration of 100 μM. Human CCRF-CEM leukemia cells were maintained as previously described.[15] Inhibition of proliferation was assessed using the XTT assay.[16] The compounds showing inhibition rates of 80% or more were further analyzed by applying them over a range of concentrations from 0.001 to 100 μM, thus generating dose–response curves to determine their 50% inhibition concentrations (IC_{50}). An equivalent concentration of DMSO served as a negative control and was used as a reference for the viability of the compound-treated cells. The established anticancer drug doxorubicin was used as a positive control.[17]

ASSOCIATED CONTENT

Supporting Information

1D and 2D NMR (COSY, HOM2DJ, HSQC, HMBC, TOCSY, and NOESY) spectra, HRESIMS, and FAB-MS for compounds **1**–**4** are available free of charge via the Internet at http://pubs.acs.org.

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Notes

The authors declare no competing financial interest.

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REFERENCES

(1) Huang, X. X.; Gao, W. Y.; Man, S. L.; Zhao, Z. Y. *Chin. Tradit. Herb. Drugs* **2009**, *40*, 483–489.

(2) Zhang, X. F.; Cui, Y.; Huang, J. J.; Zhang, Y. Z.; Zhou, N.; Wang, L. F.; Yan, B. Z.; Tang, Y. L.; Liu, Y. *Bioorg. Med. Chem. Lett.* **2007**, *17*, 2408–2413.

(3) Zhang, T.; Liu, H.; Liu, X. T.; Chen, X. Q.; Wang, Q. *Steroids* **2009**, *74*, 809–813.

(4) Deng, D. W.; Lauren, D. R.; Cooney, J. M.; Jensen, D. J.; Wurms, K. V.; Upritchard, J. E.; Cannon, R. D.; Wang, M. Z.; Li, M. Z. *Planta Med.* **2008**, *74*, 1397–1402.

(5) Zhao, Y.; Kang, L. P.; Liu, Y. X.; Zhao, Y.; Xiong, C. Q.; Ma, B. P.; Dong, F. T. *Magn. Reson. Chem.* **2007**, *45*, 739–744.

(6) Zhao, Y.; Kang, L. P.; Liu, Y. X.; Liang, Y. G; Kang, L. P.; Liu, Y. X.; Tan, D. W.; Yu, Z. Y.; Cong, Y. W.; Ma, B. P. *Planta Med.* **2009**, *75*, 356−363.
(7) Fu, Y. L.; Yu, Z. Y.; Tang, X. M.; Zhao, Y.; Yuan, X. L.; Wang, S. Q.; Ma, B. P.; Cong, Y. W. *J. Thromb. Haemostasis* **2008**, *6*, 524−533.
(8) Guo, L.; Su, J.; Deng, B. W.; Yu, Z. Y.; Kang, L. P.; Zhao, Z. H.; Shan, Y. J.; Chen, J. P.; Ma, B. P.; Cong, Y. W. *Hum. Reprod.* **2008**, *23*, 964−971.
(9) Suksamrarn, A.; Sommechai, C.; Charulpong, P.; Chitkul, B. *Phytochemistry* **1995**, *38*, 473−476.
(10) Hayes, P. Y.; Lehmann, R.; Penman, K.; Kitching, W.; Voss, J. J. D. *Phytochemistry* **2009**, *70*, 105−113.
(11) Takaashi, Y.; Mimaki, Y.; Kuroda, M.; Sashida, Y.; Nikaido, T.; Ohmoto, T. *Tetrahedron* **1995**, *51*, 2281−2292.
(12) Kougan, G. B.; Miyamoto, T.; Tanaka, C.; Paululat, T.; Mirjolet, J. F.; Duchamp, O.; Sondengam, B. L.; Lacaille-Dubois., M. A. *J. Nat. Prod.* **2010**, *73*, 1266−1270.
(13) Huang, Y.; Cui, L. J.; Zhan, W. H.; Dou, Y. H.; Wang, Y. L.; Wang, Q.; Ding, Z. *Chem. Nat. Compd.* **2007**, *43*, 672−677.
(14) Pang, X.; Cong, Y.; Yu, H. S.; Kang, L. P.; Feng, B.; Han, B. X.; Zhao, Y.; Xiong, C. Q.; Tan, D. W.; Song, W.; Liu, B.; Cong, Y. W.; Ma, B. P. *Planta Med.* **2012**, *78*, 276−285.
(15) Efferth, T.; Davey, M.; Olbrich, A.; Rücker, G.; Gebhart, E.; Davey, R. *Blood Cells Mol. Dis.* **2002**, *28*, 160−168.
(16) Scudiero, D. A.; Shoemaker, R. H.; Paull, K. D.; Monks, A.; Tierney, S.; Nofziger, T. H.; Currens, M. J.; Seniff, D.; Boyd, M. R. *Cancer Res.* **1988**, *48*, 4827−4833.
(17) Kuete, V.; Wiench, B.; Hegazy, M. E.; Mohamed, T. A.; Fankam, A. G.; Shahat, A. A.; Efferth, T. *Planta Med.* **2012**, *78*, 193−199.

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