Leonticins A−C, Three Octasaccharide Saponins from *Leontice kiangnanensis*†

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Three octasaccharide saponins, leonticins A, B, and C (**1**−**3**), were isolated from the tubers of *Leontice kiangnanensis*. Their structures were elucidated by a combination of chemical degradation and spectral methods including negative FABMS and NMR measurements as 3-*O*-*β*-D-glucopyranosyl(1→2)-α-L-arabinopyranosylhederagenin 28-*O*-α-L-rhamnopyranosyl(1→4)-*β*-D-glucopyranosyl(1→6)-*β*-D-glucopyranosyl(1→4)-α-L-rhamnopyranosyl(1→4)-*β*-D-glucopyranosyl-(1→6)-*β*-D-glucopyranoside (**1**), 3-*O*-[*β*-D-xylopyranosyl(1→3)-*β*-D-galactopyranosyl(1→4)-*β*-D-glucopyranosyl(1→3)][*β*-D-glucopyranosyl(1→2)]-α-L-arabinopyranosyloleanolic acid 28-*O*-α-L-rhamnopyranosyl(1→4)-*β*-D-glucopyranosyl(1→6)-*β*-D-glucopyranoside (**2**), and 3-*O*-[*β*-D-xylopyranosyl(1→3)-*β*-D-galactopyranosyl(1→4)-*β*-D-glucopyranosyl(1→3)][*β*-D-glucopyranosyl(1→2)]-α-L-arabinopyranosylechinocystic acid 28-*O*-α-L-rhamnopyranosyl(1→4)-*β*-D-glucopyranosyl-(1→6)-*β*-D-glucopyranoside (**3**), respectively. The complete assignments of the proton and carbon resonances for **1**−**3** were achieved based on extensive 2D NMR analysis (DQF-COSY, TOCSY, ROESY, HSQC, and HMBC).

Leontice kiangnanensis P.L. Chiu, identified as a new species of the genus *Leontice* (Berberidaceae) in 1987,[1] is one of the four species of this genus distributed in China. Its tubers have been used as a folk medicine in southeastern China for the treatment of rheumatism and hemorrhages associated with gastric ulcers. As part of our screening program aimed at the discovery of new bioactive agents from plant sources, the 70% EtOH extract of this crude drug exhibited cytotoxic activity against P-388 lymphocytic leukemia in vitro and anti-inflammatory effects on carrageenin-induced swelling of the ankle in rats and xylene-induced inflammation of the ear in mice. Bioassay-guided fractionation showed that the *n*-BuOH extract was responsible for the anti-inflammatory activity, while the most prominent cytotoxic fraction (ED_{50} = 1.21 μg/mL) was located in an aqueous effluent from a styrene polymer gel purification of the aqueous phase after solvent partition. Chemical investigation of this cytotoxic aqueous fraction resulted in the isolation of several saponins.[2] This paper reports the isolation and structure elucidation of three octasaccharide saponins obtained in our continuing efforts to reveal the chemical constituents from this saponin-rich fraction.

Results and Discussion

Leonticin C (**3**) was obtained as a colorless amorphous powder, mp 226−228 °C; $[\alpha]^{20}_D$ −13.49° (*c* 0.43, MeOH). Compound **3** exhibited a $[M - H]^-$ ion at *m/z* 1691 in the negative-ion FABMS corresponding to a molecular formula of $C_{76}H_{124}O_{41}$. Analysis of the ^{1}H- and ^{13}C-NMR spectra indicated that leonticin C is a C-3 and C-28 bisdesmosidic octasaccharide saponin with echinocystic acid as the aglycon. Acid hydrolysis of **3** afforded arabinose, xylose, rhamnose, galactose, and glucose as the sugar components on TLC by comparison with authentic samples. Alkaline hydrolysis gave the pentasaccharide prosapogenin (**3a**), along with rhamnose and glucose identified in the same manner as above.

The negative-ion FABMS spectra of **3** and **3a** provided the diagnostic fragment ions generated from glycosidic cleavage along the sugar chain so that the composition and the sequence of monosaccharides in the molecule of **3** were deduced from the characteristic fragmentation pattern. In addition, the weak ions at *m/z* 1089 $[M - 132 - H]^-$ and 1059 $[M - 162 - H]^-$ in **3a** (Figure 1), corresponding to the separate loss of either a pentose or a hexose residue, suggested that the pentasaccharide unit at C-3 of **3** was branched.

The ^{1}H-NMR spectrum of **3** was typical of a saponin. The sugar proton resonances, except for the relatively well-resolved eight anomeric proton resonances, partially or completely overlapped between 3.4 and 4.4 ppm. The signals arising from the aglycon moiety were in good agreement with that of echinocystic acid from the observation of seven methyl singlets, one of which was shifted downfield to *δ* 1.81 (CH_3-27) due to 1,3-diaxial interaction in the presence of the axially oriented 16α-OH and the characteristic broad singlet (H-16_{eq}) at *δ* 5.25.[3] Identification of the spin-systems of individual monosaccharides and the complete assignment of proton resonances were achieved through the TOCSY experiment (t_{mix} = 106 ms) complemented by a DQF-COSY spectrum. The expanded sugar proton region of the TOCSY spectrum of **3** identified most of the spin-systems associated with the eight individual monosaccharides. Spin-systems with consistently large vicinal

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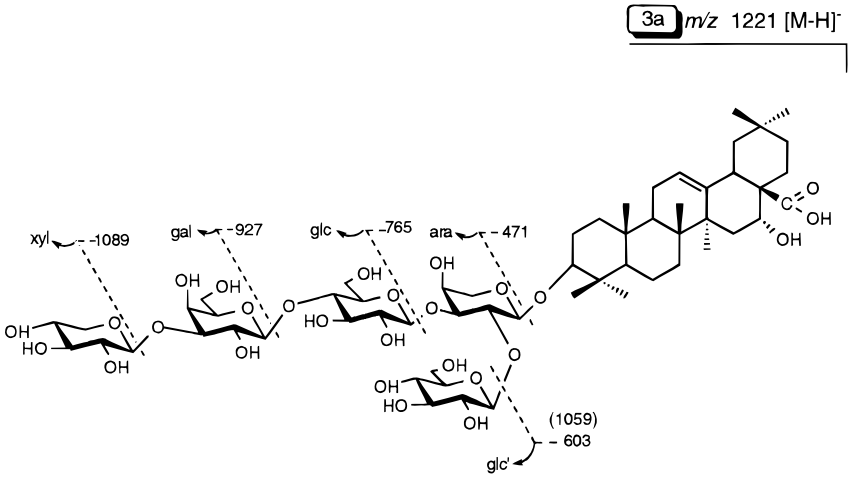


Figure 1. FABMS (negative) fragments of **3a**.

Table 1. ^{1}H-NMR Chemical Shifts and HMBC Correlations for the Aglycon Moieties of **1**−**3** and **1a**−**3a**[a,b]

proton	1	2	3	1a	2a	3a	HMBC
3	4.16[c]	3.25 dd (12, 4)	3.26 dd (12, 4)	4.18[c]	3.24 dd (12, 4)	3.26 dd (12, 4)	C: 1, 2, 4, 5, Ara C1
12	5.44 br s	5.45 br s	5.60 br s	5.47 br s	5.47 br s	5.61 br s	C: 9, 11, 14, 18
16			5.25 brs			5.23 br s	C: 14, 15, 17, 18, 22
18	3.21 dd (13, 4)	3.22 dd (13, 3)	3.50 dd (14, 3)	3.28 dd (14, 3)	3.32 dd (14, 4)	3.68[c]	C: 14, 16, 17, 20
23	3.69 d (11)	1.26 s	1.24 s	3.71 d (11)	1.27 s	1.25 s	C: 3, 4, 5, 24
	4.13[c]			4.28 d (12)			
24	0.95 s	1.09 s	1.09 s	1.05 s	1.10 s	1.09 s	C: 3, 4, 5, 23
25	0.98 s	0.87 s	0.90 s	0.95 s	0.85 s	0.87 s	C: 1, 5, 9, 10
26	1.00 s	0.97 s	0.99 s	1.04 s	1.00 s	1.02 s	C: 7, 8, 9, 14
27	1.26 s	1.32 s	1.81 s	1.24 s	1.31 s	1.83 s	C: 8, 13, 14, 15
29	0.96 s	0.99 s	1.05 s	0.95 s	0.98 s	1.07 s	C: 19, 20, 21, 30
30	0.96 s	0.99 s	1.12 s	1.02 s	1.03 s	1.20 s	C: 19, 20, 21, 29

[a] Recorded at 600.13 MHz in pyridine-d_5/MeOH-d_4. [b] J values in Hz in parentheses. [c] Overlapped signals.

coupling constants ($^3J = 7-9$ Hz) in xylose and glucose displayed a magnetization transfer from the anomeric protons (δ 6.24, 5.51, 5.27, 5.23, and 4.97) up to H-5 or H-6A and H-6B. In contrast, magnetization transfer is inefficient in arabinose, galactose, and rhamnose (δ 4.78, 5.13, and 5.83 ppm) ring systems because of the small coupling $^3J_{4,5} < 1.5$ Hz[4] for the former two and the weak coupling $^3J_{2,3}$ in the latter, which block the transfer from H-4 to H-5 and from H-2 to H-3, respectively. This J-network information allowed us to discern the individual sugar residue types. However, an unambiguous assignment of the sugar proton resonances to specific protons in a given residue could not be determined at this stage. Therefore, the DQF-COSY experiment was utilized to trace each magnetization transfer between the vicinal coupled protons starting from each well resolved anomeric proton (H-1). Using this approach, the assignment of the sugar proton signals of **3** were established as shown in Table 3.

With the proton resonances assigned, the sugar sequence and the linkage sites of **3** and **3a** were examined by cross-relaxation experiments. Interresidual cross peaks in the ROESY spectrum of **3a** (t_{mix} = 300 ms) showed the following proton pairs to be close in space (Figure 2): ara H-1 (δ 4.76) and aglycon H-3 (δ 3.26), glc′ H-1 (δ 5.50) and ara H-2 (δ 4.72), glc H-1 (δ 5.26) and ara H-3 (δ 4.27), gal H-1 (δ 5.13) and glc H-4 (δ 4.27), and xyl H-1 (δ 5.23) and gal H-3 (δ 4.19). The three correlations arising from the arabinose residue clearly indicated it to be (a) the inner pentose residue bonded to the aglycon and (b) the branched center for the unit of five monosaccharides at C-3. The combination of ROE data and the sequence information obtained from FABMS (negative) spectrum of **3a** enabled us to establish the partial structure of **3**. Analogously, the sequence and linkage sites of the remaining trisaccharide unit linked at C-28 were solved by using a similar approach. In addition to the information of the connectivities, the intraresidual ROE correlation, particularly the 1,3-diaxial dipolar interaction in the xylose and glucose residues, allowed the verification of the proton assignment based on J-coupling information. Furthermore, ROE data and the diagnostic coupling constants of the anomeric protons of **3** permitted us to establish their relative stereochemistry as α-arabinopyranose, α-rhamnopyranose, β-xylopyranose, β-galactopyranose, and β-glucopyranose, as would be expected for most naturally occurring carbohydrates.

The ^{13}C-NMR chemical shifts of **3**, which were readily assigned by a heteronuclear single-quantum correlation (HSQC) spectrum, were found to agree well with these results. It is worthy of note that, compared with the routinely used HMQC experiment, the HSQC spectrum exhibited better resolution in the F_1 dimension,[5,6] which was particularly useful to distinguish signals in extensively overlapped regions in both the ^{13}C- and ^{1}H-NMR spectra. The glycosidation shifts obtained from the data analysis fully supported the linkage site assignment based on the ROESY spectrum (Table 4).[7,8] Final confirmation of the connectivities and assignments for both aglycon and sugar moieties was obtained by exhaustive analysis of an HMBC spectrum of **3**. The observed $^2J_{H,C}$ and $^3J_{H,C}$ long-range correlations arising from the separately resolved H-12, H-16, H-18, and methyl protons in the aglycon are summarized in Table 1. Similarly, the detection of all possible two- and three-

Table 2. ^{13}C-NMR Chemical Shifts for the Aglycon Moieties of **1**−**3** and **1a**−**3a**[a]

carbon	**1**	**2**	**3**	**1a**	**2a**	**3a**	DEPT[b]
1	39.23	39.37	39.46	39.26	39.29	39.47	t
2	26.34	27.01	27.04	26.49	27.18	27.27	t
3	82.74	90.11	90.18	82.71	89.53	89.60	d
4	43.88	40.18	40.19	44.05	40.25	40.32	s
5	48.19	56.47	56.55	48.45	56.42	56.58	d
6	18.67	19.02	19.04	18.75	19.04	19.13	t
7	32.99	32.99	33.91	33.41	33.84	34.11	t
8	40.35	40.37	40.54	40.28	40.28	40.49	s
9	48.58	48.58	47.69	48.65	48.57	47.81	d
10	37.36	37.50	37.54	37.46	37.55	37.62	s
11	24.31	24.28	24.30	24.39	24.34	24.42	t
12	123.28	123.32	123.13	123.05	123.03	122.78	s
13	144.59	144.56	144.80	145.31	145.33	145.83	s
14	42.61	42.61	42.50	42.68	42.69	42.72	s
15	28.73	28.74	36.35	28.87	28.87	36.71	t
16	23.80	23.84	74.67	24.22	24.26	75.44	t, d
17	47.52	47.55	49.63	47.18	47.22	49.02	s
18	42.15	42.18	41.71	42.51	42.54	42.18	d
19	46.73	46.79	47.69	46.95	47.03	47.94	t
20	31.19	31.23	31.22	31.48	31.52	31.61	s
21	34.46	34.50	36.30	34.74	34.79	36.83	t
22	33.16	33.58	32.47	33.78	33.74	33.18	t
23	65.03	28.38	28.36	65.41	28.58	28.57	t, q
24	13.72	17.04	17.05	13.99	17.29	17.33	q
25	16.56	16.00	16.13	16.60	16.02	16.19	q
26	17.94	17.89	17.96	17.99	17.95	18.13	q
27	26.43	26.43	27.53	26.67	26.71	27.85	q
28	177.02	177.03	176.44	180.66	180.74	181.18	s
29	33.47	33.50	33.52	33.78	33.84	33.99	q
30	24.07	24.09	25.05	24.31	24.34	25.58	q

[a] Recorded at 150.91 MHz in pyridine-d_5/MeOH-d_4. [b] Multiplicity by DEPT.

Table 3. ^{1}H-NMR Chemical Shifts for the Sugar Moieties of Compounds **2**, **3**, **2a**, and **3a**[a,b]

proton	**2**	**3**	**2a**	**3a**	proton	**2**	**3**
C-3					C-28		
Ara 1	4.78 d (6.6)	4.78 d (6.8)	4.75 d (7.0)	4.76 d (6.9)	Glc″ 1	6.25 d (8.2)	6.24 d (8.2)
2	4.72 t (7, 8)	4.71 t (7, 8)	4.72 t (7, 8)	4.72 t (7, 8)	2	4.10[c]	4.04[c]
3	4.34[c]	4.32[c]	4.28[c]	4.27[c]	3	4.21[c]	4.17[c]
4	4.54 br s	4.53 br s	4.45 br s	4.46 br s	4	4.24 t (8)	4.16[c]
5A	3.72 d (11)	3.72 d (11)	3.67 d (10)	3.68[c]	5	4.10[c]	4.09[c]
5B	4.26[c]	4.26[c]	4.16[c]	4.18[c]	6A	4.34[c]	4.32[c]
					6B	4.70 d (10)	4.68 d (12)
Glc′ (1→2) Ara					Glc‴ (1→6) Glc″		
1	5.53 d (8.2)	5.51 d (7.8)	5.52 d (7.9)	5.50 d (7.7)	1	4.99 d (7.9)	4.97 d (7.9)
2	3.97[c]	3.95 t (8, 9)	4.03 t (8)	4.02 t (8)	2	3.91 t (8, 9)	3.91 t (8, 9)
3	4.15[c]	4.13[c]	4.21[c]	4.18[c]	3	4.13[c]	4.11[c]
4	4.05 t (9)	4.02[c]	4.13[c]	4.14[c]	4	4.33[c]	4.32[c]
5	3.77 m	3.76 m	3.72 m	3.72 m	5	3.73[c]	3.71[c]
6A	4.24[c]	4.22[c]	4.29 dd (11, 3)	4.28[c]	6A	4.12[c]	4.12[c]
6B	4.40 br d (12)	4.40 br d (10)	4.37 dd (11, 3)	4.38 dd (12, 3)	6B	4.27[c]	4.27[c]
Glc (1→3) Ara					Rha (1→4) Glc‴		
1	5.27 d (7.8)	5.27 d (7.9)	5.26 d (7.8)	5.26 d (8.0)	1	5.85 br s	5.83 br s
2	4.00 t (8)	4.00[c]	3.98 t (8)	3.98 t (8, 9)	2	4.62 br s	4.61 br s
3	4.20[c]	4.19[c]	4.22 t (9)	4.22 t (9)	3	4.48[c]	4.47[c]
4	4.27[c]	4.26[c]	4.27 t (9)	4.27 t (9)	4	4.26 t (8)	4.24[c]
5	3.92[c]	3.91[c]	3.87 m	3.89 m	5	4.85 m	4.85 m
6A	4.46[c]	4.47[c]	4.41 dd (11, 3)	4.42 dd (11, 3)	6	1.71 d (6)	1.71 d (6)
6B	4.52[c]	4.47[c]	4.48 dd (12, 4)	4.48 dd (11, 3)			
Gal (1→4) Glc							
1	5.13 d (7.7)	5.13 d (7.9)	5.11 d (7.6)	5.13 d (7.7)			
2	4.59 t (8, 9)	4.58 t (8, 9)	4.66 t (8)	4.66 t (8)			
3	4.20[c]	4.20[c]	4.18[c]	4.19[c]			
4	4.68 br s	4.68 br s	4.67 br s	4.67 br s			
5	4.19[c]	4.19[c]	4.17[c]	4.16[c]			
6A	4.33[c]	4.32[c]	4.33[c]	4.33 dd (12, 4)			
6B	4.44[c]	4.43 dd (12, 3)	4.46 d (12)	4.44[c]			
Xyl (1→3) Gal							
1	5.24 d (7.5)	5.23 d (7.5)	5.23 d (7.6)	5.23 d (7.7)			
2	3.97 t (8)	3.97 t (8, 9)	3.99 t (8, 9)	3.99 t (8, 9)			
3	4.10[c]	4.10[c]	4.15[c]	4.15[c]			
4	4.13[c]	4.12[c]	4.18[c]	4.17[c]			
5A	3.70 d (11)	3.70 d (10)	3.67 d (11)	3.69[c]			
5B	4.32[c]	4.31[c]	4.30[c]	4.30[c]			

[a] Recorded at 600.13 MHz in pyridine-d_5/MeOH-d_4. [b] J values (in Hz) in parentheses. [c] Indicates overlapped signals.

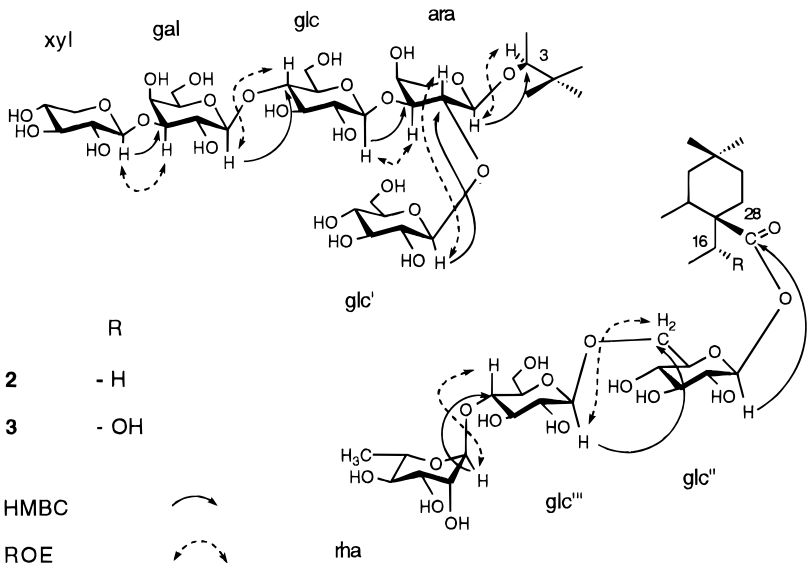


Figure 2. Key HMBC and ROE correlations for establishing the sugar linkage sites of compounds of **2** and **3**.

Table 4. ^{13}C-NMR Chemical Shifts for the Sugar Moieties of Compounds **2**, **3**, **2a**, and **3a**[a]

carbon	**2**	**3**	**2a**	**3a**	carbon	**2**	**3**
C-3					C-28		
Ara 1	106.19	106.18	106.10	106.12	Glc″ 1	96.32	96.42
2	77.64	77.61	77.97	77.92	2	74.40	74.43
3	84.21	84.24	83.87	83.93	3	79.06	79.03
4	69.67	69.66	69.49	69.50	4	71.33	71.29
5	66.81	66.81	66.67	66.71	5	78.62	78.62
					6	69.78	69.81
Glc′ (1→2) Ara					Glc‴ (1→6) Glc″		
1	104.69	104.65	104.85	104.82	1	105.23	105.27
2	76.69	76.66	76.79	76.77	2	75.84	75.76
3	79.06	79.03	79.15	79.14	3	77.09	77.06
4	72.87	72.86	72.88	72.88	4	79.22	79.27
5	78.46	78.44	78.09	78.14	5	77.64	77.61
6	63.85	63.85	63.76	63.78	6	61.95	61.95
Glc (1→3) Ara					Rha (1→4) Glc‴		
1	105.35	105.35	105.26	105.28	1	103.48	103.48
2	75.31	75.30	75.21	75.28	2	73.04	72.97
3	76.88	76.86	76.91	76.89	3	73.09	73.01
4	81.96	81.89	82.28	82.20	4	74.40	74.37
5	77.08	77.05	77.07	77.07	5	71.03	71.03
6	62.29	62.25	62.31	62.27	6	18.95	18.92
Gal (1→4) Glc							
1	105.73	105.70	105.89	105.85			
2	71.98	71.96	71.89	71.89			
3	84.83	84.81	84.89	84.88			
4	70.12	70.11	70.08	70.08			
5	77.57	77.54	77.66	77.65			
6	62.56	62.56	62.48	62.49			
Xyl (1→3) Gal							
1	107.47	107.43	107.66	107.60			
2	75.88	75.83	75.95	75.94			
3	78.46	78.44	78.81	78.78			
4	71.52	71.50	71.59	71.59			
5	67.71	67.69	67.79	67.78			

[a] Recorded at 150.91 MHz in pyridine-d_5/MeOH-d_4.

bond inter- and intra-residual correlations of the anomeric protons confirmed unambiguously the glycosidic linkages (Figure 2) and the assignments for proton and carbon resonances. Thus, the chemical structure of leonticin C was established as 3-*O*-[*β*-D-xylopyranosyl-(1→3)-*β*-D-galactopyranosyl(1→4)-*β*-D-glucopyranosyl-(1→3)][*β*-D-glucopyranosyl(1→2)]-α-L-arabinopyranosyl-echinocystic acid 28-*O*-α-L-rhamnopyranosyl(1→4)-*β*-D-glucopyranosyl(1→6)-*β*-D-glucopyranoside (**3**).

Leonticin B (**2**) was found to have a molecular formula of $C_{76}H_{124}O_{40}$ [*m*/*z* 1675 $(M - H)^-$]. Alkaline hydrolysis gave the pentasaccharide prosapogenin (**2a**), with a molecular weight of 1206 [*m*/*z* 1205, $(M - H)^-$]. Full spectroscopic characterization indicated that **2** was similar to **3**, except that **2** has oleanolic acid as the aglycon. The complete assignments of the proton and carbon resonances of **2** were based on the heteronuclear correlation experiments (HSQC and HMBC) of **2** and homonuclear correlations (DQF-COSY and ROESY spectra) of **2a**. Consequently, the determination of the connectivities and the relative stereochemistry of the sugar moieties in **2** were achieved by the methods described above. The chemical structure of leonticin B was thus deduced as 3-*O*-[*β*-D-xylopyranosyl(1→3)-*β*-D-

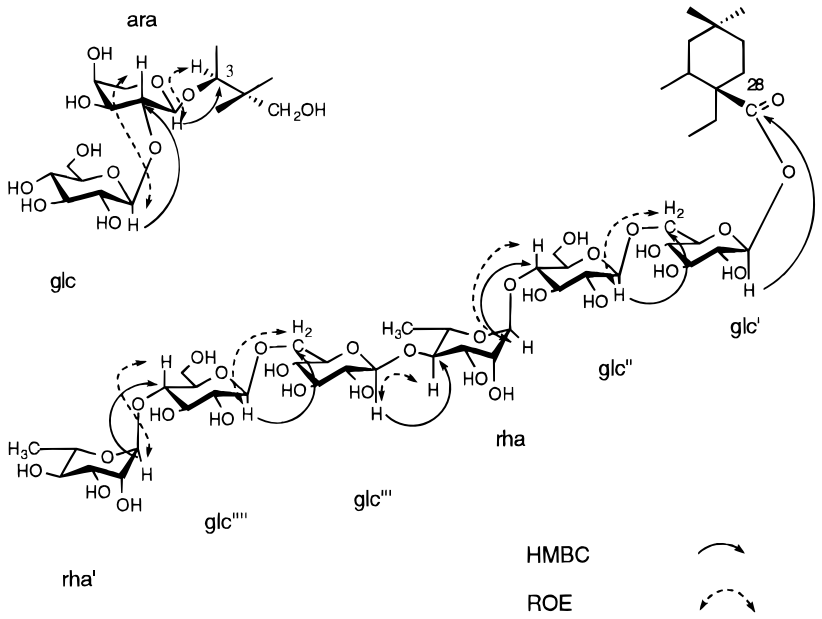
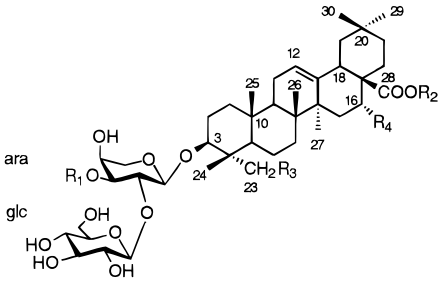


Figure 3. Key HMBC and ROE correlations for establishing the sugar linkage sites of compound **1**.

galactopyranosyl(1→4)-*β*-D-glucopyranosyl(1→3)][*β*-D-glucopyranosyl(1→2)]-α-L-arabinopyranosyloleanolic acid 28-*O*-α-L-rhamnopyranosyl(1→4)-*β*-D-glucopyranosyl-(1→6)-*β*-D-glucopyranoside (**2**).

Leonticin A (**1**) showed a molecular ion at *m/z* 1705 [(M − H)$^-$, $C_{77}H_{126}O_{41}$] along with the characteristic fragments for a successive loss of monosaccharide residues in the negative-ion FABMS spectrum. Acid hydrolysis of **1** afforded hederagenin, arabinose, rhamnose, and glucose. Alkaline hydrolysis yielded the disaccharide glycoside cauloside C[9] as prosapogenin (**1a**), indicating that a hexasaccharide unit is attached at C-28 of **1**.



	R_1	R_2	R_3	R_4
1	H	rha (1→4) glc (1→6) glc (1→4) rha (1→4) glc (1→6) glc	OH	H
1a	H	H	OH	H
2	xyl (1→3) gal (1→4) glc	rha (1→4) glc (1→6) glc	H	H
2a	xyl (1→3) gal (1→4) glc	H	H	H
3	xyl (1→3) gal (1→4) glc	rha (1→4) glc (1→6) glc	H	OH
3a	xyl (1→3) gal (1→4) glc	H	H	OH

Analysis of the sugar region of the ^{1}H-NMR and DEPT spectra of **1** indicated the presence of one arabinose, two rhamnose, and five glucose residues in the molecule, which were readily recognized by the characteristic two methyl doublets at 1.71 and 1.83 ppm (rha CH_3-6) in the proton spectrum and the multiplicities of the carbon signals (60−70 ppm) in the DEPT spectrum, although only six anomeric protons could be seen in the 1D proton spectrum. The HSQC spectrum disclosed that the anomeric protons of two glucose and two rhamnose residues resonate both at *δ* 4.99 (2H, d, *J* = 7.7 Hz) and *δ* 5.85 (2H, br s), respectively. The overlap of both ^{1}H- and ^{13}C-NMR signals due to the repetition of similar structural moieties prevented the precise characterization of the monosaccharide ring systems from the homonuclear *J*-coupling correlations as described above. The unambiguous assignments of the proton and carbon resonances and the connectivities of **1** were established by a careful study of the HMBC spectrum. The anomeric configuration of each sugar component was determined from the magnitude of the coupling constant of the anomeric proton with the aid of ROEs detected in the ROESY spectrum (Figure 3). Thus, the structure of leonticin A was elucidated as 3-*O*-*β*-D-glucopyranosyl(1→2)-α-L-arabinopyranosylhederagenin 28-*O*-α-L-rhamnopyranosyl(1→4)-*β*-D-glucopyranosyl(1→6)-*β*-D-glucopyranosyl(1→4)-α-L-rhamnopyranosyl(1→4)-*β*-D-glucopyranosyl(1→6)-*β*-D-glucopyranoside (**1**).

Leonticins A−C (**1**−**3**) and the prosapogenins (**1a**−**3a**) were tested for cytotoxic activity against KB cells.[10] None of them was found to be active at the concentration of 4 *μ*g/mL.

Experimental Section

General Experimental Procedures. Optical rotations were determined on a Perkin-Elmer 241 polarimeter. FABMS were recorded in a glycerol matrix in the negative-ion mode on a VG ZAB_2-Seq spectrometer and EIMS on a VG Tribrid spectrometer. All NMR experiments were performed on a Bruker AMX-600 spectrometer in pyridine-d_5 with two drops of MeOH-d_4 at 300 K, and chemical shifts were referenced to TMS. HPLC was performed on a Waters 590 programmable pump with a Knauer differential refractometer. Vacuum liquid chromatography was carried out with Si gel 60 (0.040−0.063 mm, Merck). TLC was conducted on Si gel 60 F_{254} (Merck) and RP-18 F_{254} S (Merck).

Table 5. NMR Chemical Shifts for the Sugar Moieties of Compounds **1** and **1a**[a,b]

	1 (δ_H)	**1a** (δ_H)	**1** (δ_C)	**1a** (δ_C)		**1** (δ_H)	**1** (δ_C)
C-3					Rha (1→4) Glc″		
Ara 1	5.14 d (6.0)	5.17 d (6.0)	104.49	104.51	1	5.85 br s	103.38
2	4.56 t (6, 7)	4.58 t (6, 7)	81.33	81.92	2	4.61 br s	72.63
3	4.31[c]	4.28[c]	74.01	74.18	3	4.62 m	73.03
4	4.37[c]	4.33 br s	68.82	68.81	4	4.43 t (9)	84.84
5A	3.71 d (10)	3.75 d (11)	65.44	65.41	5	4.95 m	69.22
5B	4.23[c]	4.24[c]			6	1.83 d (6)	19.09
Glc (1→2) Ara					Glc‴ (1→4) Rha		
1	5.21 d (7.5)	5.18 d (7.4)	106.22	106.54	1	5.29 d (7.9)	106.67
2	4.05 t (8)	4.08 t (8)	76.62	76.79	2	3.99 t (8)	76.61
3	4.14[c]	4.19 t (8)	78.60	78.75	3	4.11[c]	78.89
4	4.15[c]	4.22 t (8)	71.91	71.90	4	4.13[c]	71.77
5	3.83[c]	3.82 m	78.88	78.82	5	3.91[c]	77.86
6A	4.34[c]	4.36 dd (12, 4)	63.07	63.04	6A	4.29[c]	70.61
6B	4.47[c]	4.46 dd (11, 3)			6B	4.67 d (12)	
C-28					Glc⁗ (1→6) Glc‴		
Glc′ 1	6.26 d (8.0)		96.31		1	4.99 d (7.7)	105.71
2	4.10[c]		74.40		2	3.93 t (9)	75.85
3	4.21[c]		79.06		3	4.13[c]	77.02
4	4.26[c]		71.30		4	4.36[c]	78.49
5	4.11[c]		78.57		5	3.70 m	77.65
6A	4.34[c]		69.81		6A	4.08[c]	61.90
6B	4.69 d (12)				6B	4.27[c]	
Glc″ (1→6) Glc′					Rha′ (1→4) Glc⁗		
1	4.99 d (7.7)		105.32		1	5.85 br s	102.83
2	3.92 t (8, 9)		76.05		2	4.61 br s	73.03
3	4.13[c]		77.05		3	4.47[c]	73.03
4	4.33[c]		79.16		4	4.25[c]	74.40
5	3.70[c]		77.71		5	4.88 m	71.02
6A	4.08[c]		62.03		6	1.71 d (6)	18.96
6B	4.27[c]						

[a] Recorded at 600.13 MHz and 150.91 MHz in pyridine-d_5/MeOH-d_4. [b] *J* values (in Hz) in parentheses. [c] Indicates overlapped signals.

Plant Material. The tubers of *L. kiangnanensis* were collected in Anhui Province of the People's Republic of China in May 1992. A voucher specimen (No. N 88006) has been deposited in the herbarium of Shanghai Institute of Pharmaceutical Industry, Shanghai, People's Republic of China.

Extraction and Isolation. The dried and pulverized tubers (1 kg) were extracted with 70% EtOH. The concentrated aqueous extract was subjected to solvent partition and gel chromatography as previously described.[2] The H_2O effluent obtained from the porous polymer gel column was rechromatographed on the same column. Fractions eluting with 30% aqueous EtOH were combined for further fractionation by vacuum liquid chromatography on Si gel 60 using $CHCl_3$–MeOH–H_2O (5:5:1) as eluent. Final purification was performed with HPLC on Spherisorb C_{18} column [5 μm, 16 × 250 mm, MeOH–H_2O (6:4), flow rate 5 mL/min] to afford **1** (56 mg), **2** (63 mg), and **3** (31 mg).

Acid Hydrolysis of 1–3. A 5-mg quantity of each sample was refluxed with 2 M HCl in MeOH (2 ml) for 4 h. The reaction solution was evaporated under reduced pressure, and the hydrolysate was extracted with ether. The ether extract was evaporated to afford the aglycon, which was identified by a combination of EIMS measurement[11] and comparison of its ^{13}C-NMR data with published values.[12,13] The H_2O layer was neutralized with alkali solution and concentrated at reduced pressure. The residues were compared with standard sugars on Si gel TLC [$CHCl_3$–MeOH–H_2O (30:12:4), 9 mL of lower layer + 1 mL of HOAc], which showed the sugars to be arabinose, rhamnose, and glucose for compound **1** and arabinose, xylose, rhamnose, galactose, and glucose for **2** and **3**.

Alkaline Hydrolysis of 1–3. Compounds **1–3** were each hydrolyzed with 0.5 M KOH (4 mL) at 80 °C for 2 h, and after the usual workup the prosapogenins **1a**, **2a**, and **3a** were obtained.

Leonticin A (1): white amorphous powder; mp 219–221 °C; $[\alpha]^{20}_D$ −16.31° (*c* 1.03, MeOH); ^{1}H NMR (pyridine-d_5/MeOH-d_4, 600.13 MHz), see Tables 1 and 5; ^{13}C NMR (pyridine-d_5/MeOH-d_4, 150.91 MHz), see Tables 2 and 5; negative-ion FABMS *m/z* [M − H]$^-$ 1705, [M − rha − H]$^-$ 1559, [M − glc − H]$^-$ 1543, [M − rha − glc − H]$^-$ 1397, [M − rha − 2glc − H]$^-$ 1235, [M − 2rha − 2glc − H]$^-$ 1089, [M − 2rha − 3glc − H]$^-$ 927, [M − 2rha − 4glc − H]$^-$ 765, [M − 2rha − 5glc − H]$^-$ 603, [M − 2rha − 5glc − ara − H]$^-$ 471.

Prosapogenin 1a: white amorphous powder; mp 253–255 °C; ^{1}H NMR (pyridine-d_5/MeOH-d_4, 600.13 MHz), see Tables 1 and 5; ^{13}C NMR (pyridine-d_5/MeOH-d_4, 150.91 MHz), see Tables 2 and 5; negative-ion FABMS *m/z* [M − H]$^-$ 765, [M − glc − H]$^-$ 603, [M − glc − ara − H]$^-$ 471.

Hederagenin: white needles; mp 304–305 °C; EIMS *m/z* [M]$^+$ 472 (2), 426 (3), 248 (100), 203 (62).

Leonticin B (2): white amorphous powder; mp 244–246 °C; $[\alpha]^{20}_D$ −4.06° (*c* 0.96, MeOH); ^{1}H NMR (pyridine-d_5/MeOH-d_4, 600.13 MHz), see Tables 1 and 3; ^{13}C NMR (pyridine-d_5/MeOH-d_4, 150.91 MHz), see Tables 2 and 4; negative-ion FABMS *m/z* [M − H]$^-$ 1675, [M − xyl − H]$^-$ 1543, [M − xyl − glc − H]$^-$ 1381, [M − xyl − gal − glc − H]$^-$ 1219, [M − rha − 2glc]$^-$ 1205, [M − rha − 2glc − xyl − H]$^-$ 1073, [M − rha − 3glc − H]$^-$ 1043, [M − rha − 3glc − xyl − H]$^-$ 911, [M − rha − 3glc − gal − xyl − H]$^-$ 749, [M − rha − 4glc − xyl − gal − H]$^-$ 587, [M − rha − 4glc − xyl − gal − ara − H]$^-$ 455.

Prosapogenin 2a: white amorphous powder; mp 264–266 °C; $[\alpha]^{20}_D$ +22.30° (*c* 1.00, MeOH); ^{1}H NMR (pyridine-d_5/MeOH-d_4, 600.13 MHz), see Tables 1 and 3; ^{13}C NMR (pyridine-d_5/MeOH-d_4, 150.91 MHz), see Tables 2 and 4; negative-ion FABMS *m/z* [M − H]$^-$

1205, [M − xyl − H]$^-$ 1073, [M − glc − H]$^-$ 1043, [M − xyl − glc − H]$^-$ 911, [M − xyl − gal − glc − H]$^-$ 749, [M − xyl − gal − 2glc − H]$^-$ 587, [M − xyl − gal − 2glc − ara − H]$^-$ 455.

Oleanolic acid: white amorphous powder; mp 290−292 °C; EIMS *m/z* [M]$^+$ 456 (1), 248 (100), 203 (46), 189 (24).

Leonticin C (3): white amorphous powder; mp 226−228 °C; $[\alpha]^{20}_D$ −13.49° (*c* 0.43, MeOH); ^{1}H NMR (pyridine-d_5/MeOH-d_4, 600.13 MHz), see Tables 1 and 3; ^{13}C NMR (pyridine-d_5/MeOH-d_4, 150.91 MHz), see Tables 2 and 4; negative-ion FABMS *m/z* [M − H]$^-$ 1691, [M − xyl − H]$^-$ 1559, [M − xyl − glc − H]$^-$ 1397, [M − xyl − gal − glc − H]$^-$ 1235, [M − rha − 2glc]$^-$ 1221, [M − rha − 2glc − xyl − H]$^-$ 1089, [M − rha − 3glc − H]$^-$ 1059, [M − rha − 3glc − xyl − H]$^-$ 927, [M − rha − 3glc − gal − xyl − H]$^-$ 765, [M − rha − 4glc − xyl − gal − ara − H]$^-$ 471.

Prosapogenin 3a: white amorphous powder; mp 249−250 °C; $[\alpha]^{20}_D$ +10.81° (*c* 0.74, MeOH); ^{1}H NMR (pyridine-d_5/MeOH-d_4, 600.13 MHz), see Tables 1 and 3; ^{13}C NMR (pyridine-d_5/MeOH-d_4, 150.91 MHz), see Tables 2 and 4; negative-ion FABMS *m/z* [M − H]$^-$ 1221, [M − xyl − H]$^-$ 1089, [M − glc − H]$^-$ 1059, [M − xyl − glc − H]$^-$ 927, [M − xyl − gal − glc − H]$^-$ 765, [M − xyl − gal − 2glc − H]$^-$ 603, [M − xyl − gal − 2glc − ara − H]$^-$ 471.

Echinocystic acid: white amorphous powder; mp 297−299 °C; EIMS *m/z* [M]$^+$ 471 (1), 428 (2), 410 (42), 395 (100), 187 (21).

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