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LEONTICINS D–H, FIVE TRITERPENE SAPONINS FROM *LEONTICE KIANGNANENSIS*

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Key Word Index—*Leontice kiangnanensis*; Berberidaceae; triterpene saponins; leonticins D–H; caulophyllogenin; oleanolic acid; hederagenin; echinocystic acid.

Abstract—Five new triterpene saponins, leonticins D–H, were isolated from the tubers of *Leontice kiangnanensis*. Based on a combination of chemical degradation and spectroscopic analysis (negative ion FAB mass spectrometry and 2D NMR experiments), their structures were characterized as 3-*O*-α-L-arabinopyranosyl-caulophyllogenin 28-*O*-α-L-rhamnopyranosyl-(1→4)-β-D-glucopyranosyl-(1→6-β-D-glucopyranoside, 3-*O*-[β-D-glucopyranosyl-(1→3)]-[β-D-glucopyranosyl-(1→2)]-α-L-arabinopyranosyl-oleanolic acid 28-*O*-α-L-rhamnopyranosyl-(1→4)-β-D-glucopyranosyl-(1→6)-β-D-glucopyranoside, 3-*O*-[β-D-glucopyranosyl-(1→3)]-[β-D-glucopyranosyl-(1→2)]-α-L-arabinopyranosyl-hederagenin 28-*O*-α-L-rhamnopyranosyl-(1→4)-β-D-glucopyranosyl-(1→6)-β-D-glucopyranoside, 3-*O*-β-D-xylopyranosyl-(1→3)-β-D-galactopyranosyl-(1→4)-β-D-glucopyranosyl-(1→3)-α-L-arabinopyranosyl-oleanolic acid 28-*O*-α-L-rhamnopyranosyl-(1→4)-β-D-glucopyranosyl-(1→6)-β-D-glucopyranoside and 3-*O*-β-D-xylopyranosyl-(1→3)-β-D-galactopyranosyl-(1→4)-β-D-glucopyranosyl-(1→3)-α-L-arabinopyranosyl-echinocystic acid 28-*O*-α-L-rhamnopyranosyl-(1→4)-β-D-glucopyranosyl-(1→6)-β-D-glucopyranoside, respectively. Copyright © 1997 Elsevier Science Ltd

INTRODUCTION

In our search for biologically active natural products, we investigated the saponin constituents of the tubers of *Leontice kiangnanensis* P. L. Chiu, from which three octasaccharide saponins were isolated and recently reported by us [1]. This paper describes the characterization of five additional new saponins, leonticins D–H (**1**–**5**), all of which are bisdesmosidic saponins with an identical trisaccharide unit linked at C-28 of the aglycones.

RESULTS AND DISCUSSION

Leonticin D (**1**) was obtained as needles, mp 219–220°. The negative ion mode FAB mass spectrum exhibited an $[M-H]^-$ ion at *m/z* 1089 ($C_{53}H_{86}O_{23}$), which is consistent with a tetrasaccharide glycoside containing one pentose, one deoxyhexose, two hexoses and one aglycone with molecular mass of 488 [*m/z* 1089 $(M-H)^-$, 943 $(M-H-146)^-$, 781 $(M-H-146-162)^-$, 619 $(M-H-146-162-162)^-$ and 487 $(M-H-146-162-162-132)^-$]. The NMR spectral data revealed the feature of an oleanane type triterpene saponin whose hydroxyl group at C-3 and carboxyl group at C-28 are glycosylated [2, 3]. Acid hydrolysis of **1** afforded arabinose, rhamnose and glucose as the sugar components identified on TLC by comparison with authentic samples. Alkaline hydrolysis of **1** yielded prosapogenin **1a**, which showed an $[M-H]^-$ ion at *m/z* 619 and a further fragment at *m/z* 487 generated by the loss of $[M-H-132]^-$, suggesting a pentose was linked at the C-3 of the aglycone.

The ^{1}H NMR spectrum of **1** displayed four anomeric proton resonances at δ 6.26 (*d*, *J* = 8.1 Hz), 5.87 (*br s*), 4.99 (*d*, *J* = 7.8 Hz) and 4.90 (*d*, *J* = 7.3 Hz). The DQF-COSY spectrum, which maps the vicinal connectivities starting from the relatively well resolved anomeric protons, in conjunction with the ROESY experiment of **1**, allowed the identification of the spin systems of the four monosaccharides and a complete assignment of the proton resonances to individual monosaccharides. The shifts of the sugar resonances, summarized in Table 1, are attributable to two inner glucose residues, one terminal rhamnose and one terminal arabinose residue, respectively [4]. Subsequent examination of the inter-residue ROEs in the ROESY spectrum defined the

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Table 1. ^{1}H NMR chemical shifts for sugar moieties of compounds **1–5**, **2a** and **3a**

	1	**2**	**3**	**4**	**5**	**2a**	**3a**		**1**	**2**	**3**	**4**	**5**
C-3								Xyl (1-3) Gal					
Ara 1	4.90 *d* (7.3)	4.81 *d* (6.2)	4.94 *d* (6.7)	4.74 *d* (7.4)	4.76 *d* (7.4)	4.80 *d* (6.9)	5.00 *d* (6.8)	1				5.22 *d* (7.6)	5.24 *d* (7.7)
2	4.38 *t* (8)	4.76 *t* (9)	4.64 *t* (9)	4.49 *t* (8)	4.48 *t* (9)	4.75 *t* (9)	4.68 *t* (9)	2				3.98 *t* (9)	4.02*
3	4.11*	4.36*	4.32*	4.26*	4.24*	4.34*	4.32*	3				4.16*	4.15*
4	4.31 *br s*	4.54 *br s*	4.49 *br s*	4.47*	4.49 *br s*	4.51 *br s*	4.52 *br s*	4				4.17*	4.18*
5A	3.73 *d* (11)	3.72 *d* (11)	3.69 *d* (11)	3.76 *d* (12)	3.78 *d* (11)	3.69 *d* (11)	3.66 *d* (11)	5A				3.72 *d* (12)	3.71 *d* (11)
5B	4.21*	4.18*	4.17*	4.18*	4.20*	4.20*	4.18*	5B				4.26*	4.33*
Glc (1-2) Ara								C-28 Glc″					
1		5.52 *d* (7.7)	5.49 *d* (7.8)			5.53 *d* (7.8)	5.50 *d* (7.8)	1	6.26 *d* (8.1)	6.25 *d* (8.2)	6.23 *d* (8.1)	6.23 *d* (8.1)	6.25 *d* (8.1)
2		4.06*	3.99*			4.08 *t* (8)	4.04 *t* (8)	2	4.05 *t* (9)	4.13*	4.11 *t* (9)	4.10*	4.09 *t* (9)
3		4.20*	4.19*			4.22*	4.17*	3	4.19 *t* (9)	4.21*	4.19*	4.20*	4.21 *t* (9)
4		4.16 *t* (19)	4.15*			4.20*	4.14*	4	4.21*	4.22*	4.20*	4.23*	4.23*
5		3.72 *m*	3.71 *m*			3.75 *m*	3.66 *m*	5	4.11*	4.10*	4.08*	4.10 *m*	4.10 *m*
6A		4.29*	4.27*			4.30 *dd* (11, 4)	4.25 *dd* (12, 5)	6A	4.32*	4.32*	4.31*	4.32 *dd* (12, 4)	4.34 *dd* (12, 5)
6B		4.38*	4.36*			4.37 *dd* (12, 3)	4.34 *dd* (12, 2)	6B	4.69 *d* (12)	4.68*	4.66 *d* (12)	4.68 *d* (12)	4.66*
Glc′ (1-3) Ara								Glc‴ (1-6) Glc″					
1		5.32 *d* (7.8)	5.33 *d* (7.9)	5.31 *d* (7.8)	5.31 *d* (7.9)	5.33 *d* (7.8)	5.23 *d* (7.8)	1	4.99 *d* (7.8)	5.01 *d* (7.8)	4.99 *d* (7.9)	4.98 *d* (7.8)	4.99 *d* (7.8)
2		4.01 *t* (8)	3.95 *t* (9)	4.02 *t* (9)	4.00*	4.02 *t* (8)	3.97 *t* (9)	2	3.92 *t* (8)	3.95*	3.93 *t* (9)	3.93 *t* (9)	3.92 *t* (9)
3		4.21*	4.19*	4.17*	4.19*	4.24*	4.20*	3	4.14*	4.15*	4.14*	4.13*	4.15*
4		4.20 *t* (9)	4.18*	4.28*	4.30*	4.21*	4.18*	4	4.36*	4.40 *t* (9)	4.38*	4.39*	4.39*
5		3.95 *m*	3.92 *m*	3.91 *m*	3.91 *m*	3.96 *m*	3.92 *m*	5	3.72 *d* (10)	3.68 *m*	3.71 *m*	3.68 *m*	3.69 *m*
6A		4.35*	4.33*	4.46*	4.47*	4.35 *dd* (11, 3)	4.30 *dd* (12, 5)	6A	4.11*	4.07*	4.10*	4.09*	4.10*
6B		4.49 *br d* (11)	4.47 *d* (12)	4.46*	4.47*	4.49 *dd* (12, 3)	4.44 *dd* (12, 2)	6B	4.27*	4.25*	4.21*	4.25*	4.23*
Gal (1-4) Glc′								Rha (1-4) Glc‴					
1				5.13 *d* (7.7)	5.12 *d* (7.8)			1	5.87 *br s*	5.85 *br s*	5.85 *br s*	5.83 *br s*	5.86 *br s*
2				4.57 *t* (9)	4.59 *t* (9)			2	4.64 *br s*	4.62 *br s*	4.61 *br s*	4.61 *br s*	4.62 *br s*
3				4.21*	4.20*			3	4.50 *dd* (9, 2)	4.49*	4.47*	4.51 *dd* (9, 3)	4.49 *dd* (9, 3)
4				4.66 *br s*	4.69 *br s*			4	4.29*	4.25*	4.26*	4.25*	4.25*
5				4.17*	4.17 *m*			5	4.87 *m*	4.84 *m*	4.85 *m*	4.85 *m*	4.84 *m*
6A				4.31*	4.29*			6	1.72 *d* (6)	1.70 *d* (6)	1.71 *d* (6)	1.70 *d* (6)	1.72 *d* (6)
6B				4.40 *dd* (12, 4)	4.42*								

Recorded on Bruker AMX-600 in pyridine-d_5–methanol-d_4. *J* values (in Hz) in parentheses.
*Overlapped signals.

sequence and the linkage sites of the sugar moieties. The resonance at δ 4.90 (Ara H-1) shows a cross-peak with a signal at δ 4.15 (aglycone H-3), indicating that arabinose is the pentose residue linked at the C-3 of the aglycone as initially inferred from the FAB mass spectral data. Similarly, the characterization of the remaining trisaccharide unit at C-28 was achieved by the observation of ROEs between Glc‴ H-1 and the preceding sugar Glc″ H-6, and Rha H-1 and Glc‴ H-4 as illustrated in Fig. 1. The carbon resonances, which were readily assigned from an HSQC spectrum, are in good agreement with the suggested structure (Table 2). The glycosidic connectivity was further confirmed by an HMBC spectrum, which displayed correlations between Ara H-1 and C-3 of the aglycone (δ 82.5), between a terminal Rha H-1 (δ 5.87) and Glc‴ C-4 (δ 78.8), Glc‴ H-1 (δ 4.99) and inner Glc″ C-6 (δ 69.4), and between Glc″ H-1 (δ 6.26) and a carbonyl ^{13}C resonance at δ 176.4, thereby providing evidence for an ester linkage of the trisaccharide unit and the aglycone (Fig. 1). The relative stereochemistry of each of the monosaccharides was determined as β-D-glucopyranose, α-L-rhamnopyranose and α-L-arabinopyranose based on the characteristic $J_{1,2}$ coupling constants and the evidence from intraresidue ROEs. Thus, the sugar moieties of **1** are characterized as 3-*O*-α-L-arabinopyranosyl and 28-*O*-α-L-rhamnopyranosyl-(1→4)-β-D-glucopyranosyl-(1→6)-β-D-glucopyranoside.

The aglycone moiety region in the ^{1}H and ^{13}C NMR spectra of **1** showed a great similarity to those of hederagenin except for the resonances of position C-16 and a high-frequency shift of the axial methyl group at C-14 (H-27), implying that there is an additional hydroxyl group at C-16 of **1** [5]. This interpretation was unambiguously confirmed by the HMBC spectrum. Figure 2 presents key correlations to establish the structure of the aglycone. In particular, the observation of cross-peaks between the proton resonance at δ 5.25 (*br s* H-16) and the four carbon signals at δ 42.4, 49.5, 41.6 and 32.4 (C-14, C-17, C-18 and C-22), respectively, strongly indicates the presence of a hydroxyl group

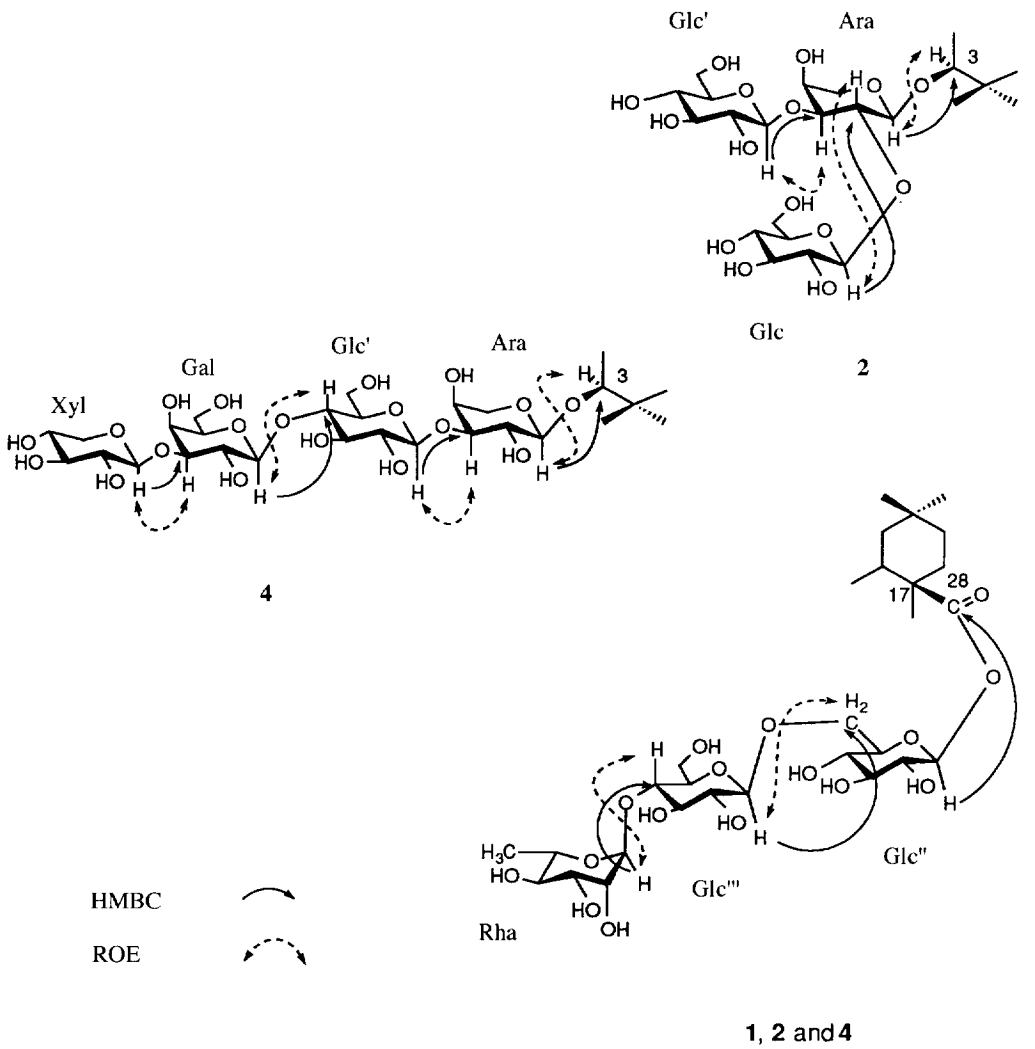


Fig. 1. Key ROE and HMBC correlations for establishing the sugar sequence and linkage sites of saponins **1**, **2** and **4**.

Table 2. ^{13}C NMR chemical shifts for sugar moieties of compounds **1–5**, **2a** and **3a**

	1	**2**	**3**	**4**	**5**	**2a**	**3a**		**1**	**2**	**3**	**4**	**5**
C-3								Xyl (1-3) Gal					
Ara-1	106.8	105.7	105.8	107.5	107.3	106.0	104.2	1				107.0	107.3
2	73.2	77.6	77.3	72.1	72.1	77.9	77.3	2				75.4	75.7
3	74.8	83.6	82.8	84.1	84.3	83.7	83.4	3				78.1	78.6
4	69.8	69.0	68.6	69.5	69.6	69.2	68.5	4				71.2	71.4
5	67.1	66.2	66.0	67.1	67.3	66.5	65.7	5				67.4	67.7
Glc (1-2) Ara								C-28 Glc″					
1		104.6	104.5			105.4	104.2	1	96.0	95.9	95.8	95.9	96.0
2		76.4	76.3			76.6	75.9	2	74.0	74.1	73.6	74.0	74.2
3		78.8	78.6			79.0	78.5	3	78.7	78.8	78.6	78.7	78.7
4		72.6	72.5			72.8	71.9	4	70.9	71.1	70.8	71.0	71.2
5		77.8	77.6			78.0	77.6	5	78.3	78.3	77.8	78.3	78.3
6		63.5	63.0			63.6	62.9	6	69.4	69.5	69.3	69.4	69.7
Glc′ (1-3) Ara								Glc‴ (1-6) Glc″					
1		105.2	105.3	105.8	105.8	105.5	104.8	1	104.9	105.1	105.0	105.1	105.2
2		75.5	75.2	75.3	75.3	75.7	75.1	2	75.4	75.5	75.3	75.5	75.7
3		78.6	78.3	76.4	76.5	78.7	78.1	3	76.7	76.8	76.5	76.7	77.0
4		71.1	71.4	81.5	81.6	71.9	71.4	4	78.8	78.6	78.4	78.9	78.8
5		78.7	78.4	76.8	76.9	79.0	78.4	5	77.3	77.4	77.1	77.3	77.6
6		62.8	62.5	61.9	62.2	63.0	62.4	6	61.5	61.6	61.4	61.6	61.7
Gal (1-4) Glc′								Rha (1-4) Glc‴					
1				105.3	105.4			1	103.0	103.0	102.9	103.0	103.2
2				71.6	71.8			2	72.7	72.8	72.6	72.7	72.7
3				84.5	84.7			3	72.7	73.0	72.8	72.7	77.7
4				69.7	69.8			4	74.0	74.2	73.9	74.0	74.2
5				77.2	77.6			5	70.6	70.6	70.4	70.7	70.8
6				62.1	62.2			6	18.6	18.8	18.6	18.6	18.9

Recorded on Bruker AMX-600 in pyridine-d_5–methanol-d_4.

at the C-16 position of the aglycone in **1**. The α-configuration of the hydroxyl group was supported by the detection of a ROE between H-16 and H-15β as well as the small J coupling of H-16 (t-like $br\,s$), characteristic of an equatorial proton. Thus, the aglycone of **1** was concluded to be caulophyllogenin [6], which is rarely found in saponins [7]. The chemical structure of **1** was then elucidated as 3-*O*-α-L-arabinopyranosyl-caulophyllogenin 28-*O*-α-L-rhamnopyranosyl-(1→4)-β-D-glucopyranosyl-(1→6)-β-D-glucopyranoside.

Leonticin E (**2**) was obtained an amorphous powder, mp 223–225°. The deprotonated molecule of **2** was observed at m/z 1381 ($C_{65}H_{106}O_{31}$) in the negative ion FAB mass spectrum. In addition, the ion at m/z 911 $[M-H-470]^-$, taken as evidence for the direct elimination of the combined masses of two hexoses and one deoxyhexose, was particularly abundant, reflecting the presence of a similar trisaccharide unit at C-28 of the aglycone in **2** as in **1**. Analysis of the ^1H and ^{13}C NMR data indicated that **2** was a hexasaccharide saponin with oleanolic acid as aglycone. Alkaline hydrolysis gave a trisaccharide prosapogenin **2a**, which showed an $[M-H]^-$ ion at m/z 911 corresponding to the composition of one oleanolic acid, one pentose and two hexoses, which were identified as arabinose and glucose on TLC acid hydrolysis.

The characterization of the sugar moieties in **2** was accomplished by the analysis of the NMR data obtained from DQF-COSY, TOCSY, T-ROESY [8, 9], HSQC

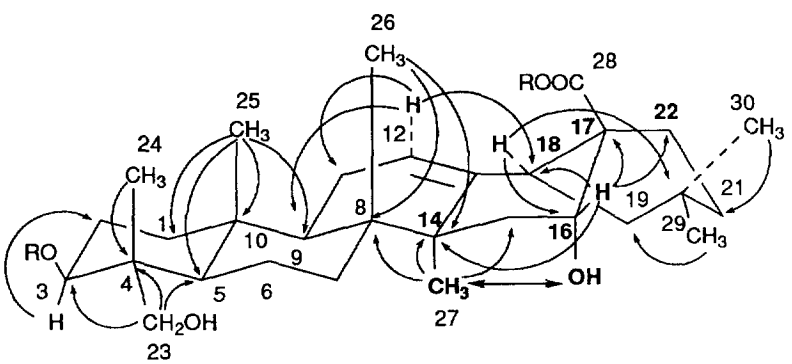


Fig. 2. Key HMBC correlations and the 1,3-diaxial interaction between 16α-OH and 14α-CH_3 from the aglycone of **1**.

and HMBC spectra. The spin systems corresponding to the six monosaccharides were clearly discernible in the TOCSY spectrum of **2**, walking along pivotal anomeric proton resonances. The complementary data from the DQF-COSY spectrum was used to obtain a full assignment of the proton resonances (Table 1) and to characterize the relative stereochemistry of each of the sugar residues in **2** as β-D-glucopyranose, α-L-rhamnopyranose and α-L-arabinopyranose, respectively. The sequential assignments of the sugar moieties were derived from a cross-relaxation measurement (T-ROESY) and further verified by the HMBC experiment in the same way as discussed above. The arabinose showed ROEs with H-3 of the aglycone as well as with two terminal glucose residues, indicating the branched nature of the trisaccharide connected at C-3 of the aglycone (Fig. 1). Furthermore, the intra-residue ROE correlations arising from the 1,3-diaxial interaction between the anomeric proton and H-3 in glucose residues discriminated the resonances of the glucose H-3 and H-4 protons, which overlapped in the DQF-COSY and TOCSY spectra. It is noteworthy that the disturbing positive TOCSY artefacts in conventional ROESY spectra obtained with a CW spin lock, were completely eliminated in the T-ROESY counterparts, which displayed almost exclusively genuine ROE signals with opposite phase to the diagonal. More importantly, in conventional spectra, overlapping positive TOCSY and negative ROE signals cancelled and thus masked cross relaxation effects that were clearly visible in the T-ROESY spectrum. The ^{13}C resonances of **2**, assigned from the HSQC spectrum, are given in Table 2. The chemical structure of **2** was therefore established as 3-*O*-[β-D-glucopyranosyl-(1 $\rightarrow$ 3)]-[β-D-glucopyranosyl-(1 $\rightarrow$ 2)]-α-L-arabinopyranosyl-oleanolic acid 28-*O*-α-L-rhamnopyranosyl-(1 $\rightarrow$ 4)-β-D-glucopyranosyl -(1 $\rightarrow$ 6)-β-D-glucopyranoside.

Leonticin F (**3**) was found to have the molecular formula $C_{65}H_{106}O_{32}$, as deduced by its negative FAB mass spectrum (*m/z* 1397 $[M-H]^-$), which differs from the formula of **2** by 16 mass units. Alkaline hydrolysis liberated a trisaccharide prosapogenin **3a** with an $[M-H]^-$ ion at *m/z* 927. The fragment ions at *m/z* 927, 765, 603 and 471 in **3a** are analogous to those at *m/z* 911, 749, 587 and 455 in **2a**, inferring a similar composition and sequence of the monosaccharides in both compounds, but with a different aglycone. The analysis of the ^1H and ^{13}C NMR spectra disclosed the identity of sugar moieties in **3** and **2** and the nature of the hederagenin of **3**. The assignment of the carbon resonances was made by comparing the 1D ^{13}C NMR spectrum of **3** with that of **2**, whereas the proton resonances were identified from the DQF-COSY correlations of **3a** as well as from the ^1H–^{13}C correlation experiments (HSQC) of **3** and **3a**. The anomeric configuration of the sugar residues in **3** were identical to those of **2** since the chemical shifts and the coupling constants in both compounds were similar. Thus, the structure of **3** was identified as 3-*O*-[β-D-glucopyranosyl-(1 $\rightarrow$ 3)]-[β-D-glucopyranosyl-(1 $\rightarrow$ 2)]-α-L-arabinopyranosyl-hederagenin 28-*O*-α-L-rhamnopyranosyl-(1 $\rightarrow$ 4)-β-D-glucopyranosyl-(1 $\rightarrow$ 6)-β-D-glucopyranoside. Compound **3** has a similar structure to leontoside E, from the tubers of *L. eversmannii* as reported earlier [10, 11], but a different sugar sequence.

Leonticin G (**4**) was obtained as a white amorphous powder. Inspection of the NMR spectral data suggested the presence of oleanolic acid and seven monosaccharides, which were identified as arabinose, xylose, rhamnose, galactose and glucose from TLC analysis with authentic samples after acid hydrolysis. As expected, the $[M-H]^-$ ion was observed at *m/z* 1513 in the negative ion FAB mass spectrum, which was consistent with the molecular formula $C_{70}H_{114}O_{35}$.

The assignment of the principle fragments from the negative ion FAB mass spectrum of **4** revealed that the predominant ion at *m/z* 1043 is formed by the elimination of a fragment of mass corresponding to 470, which established the nature of the trisaccharide unit linked at C-28 of the aglycone to be the same as outlined in the preceding compounds. In contrast to the compounds **2** and **3** described above, a linear sequence of the

Table 3. ^1H NMR chemical shifts for aglycone moieties of compounds **1–5**, **2a** and **3a**

H	1	2	3	4	5	2a	3a
3	4.15*	3.27 *dd* (12, 3)	4.13*	3.33 *dd* (12, 4)	3.38 *dd* (12, 4)	3.32 *dd* (12, 3)	4.16*
12	5.61 *br s*	5.43 *br s*	5.42 *br s*	5.41 *br s*	5.57 *br s*	5.49 *br s*	5.45 *br s*
16	5.25 *br s*				5.31 *br s*		
18	3.52 *dd* (14, 4)	3.20 *dd* (13, 4)	3.23 *br d* (13)	3.18 *dd* (14, 4)	3.53 *dd* (13, 4)	3.28 *dd* (13, 4)	3.26 *br d* (13)
23	3.64 *d* (11) 4.17*	1.26 *s*	3.66 (11) 4.19*	1.30 *s*	1.27 *s*	1.29 *s*	3.68 *d* (11) 4.23*
24	1.00 *s*	1.02 *s*	1.02 *s*	1.00 *s*	1.01 *s*	1.01 *s*	1.05 *s*
25	0.94 *s*	0.88 *s*	0.93 *s*	0.89 *s*	0.91 *s*	0.84 *s*	0.91 *s*
26	1.03 *s*	1.10 *s*	1.01 *s*	1.09 *s*	1.02 *s*	1.04 *s*	1.00 *s*
27	1.79 *s*	1.27 *s*	1.25 *s*	1.26 *s*	1.76 *s*	1.31 *s*	1.22 *s*
29	1.02 *s*	0.92 *s*	0.97 *s*	0.91 *s*	1.02 *s*	1.01 *s*	0.94 *s*
30	1.10 *s*	0.92 *s*	0.98 *s*	0.91 *s*	1.12 *s*	0.98 *s*	1.00 *s*

Recorded on Bruker AMX-600 in pyridine-d_5–methanol-d_4. *J* values (in Hz) in parentheses.
*Overlapped signals.

Table 4. ^{13}C NMR chemical shifts for aglycone moieties of compounds **1**–**5** and **1a**–**3a**

C	1	2	3	4	5	1a	2a	3a
1	39.3	39.1	39.0	39.3	39.5	39.3	39.3	38.8
2	26.4	26.9	26.2	27.1	27.2	26.6	27.1	26.0
3	82.5	89.3	82.3	89.4	89.3	82.4	89.5	82.5
4	43.8	40.0	43.7	40.0	40.2	43.9	40.2	43.6
5	48.1	56.2	47.2	56.4	56.7	47.8	56.4	47.9
6	18.6	18.9	18.6	18.6	19.0	18.6	19.0	18.2
7	33.6	32.8	32.7	33.0	33.8	33.6	33.7	32.9
8	40.5	40.2	40.1	40.4	40.6	40.4	40.2	39.7
9	47.7	48.3	47.9	47.5	47.7	47.7	48.5	48.1
10	37.4	37.3	37.1	37.5	37.6	37.4	37.5	36.9
11	24.3	24.1	24.0	24.2	24.3	24.3	24.3	23.8
12	123.1	123.1	123.2	123.3	123.0	123.2	123.6	123.4
13	144.8	144.4	144.4	145.5	145.3	145.0	145.3	144.9
14	42.4	42.4	42.3	42.6	42.6	42.6	42.6	42.2
15	36.4	28.6	28.5	28.7	36.5	36.6	28.8	28.3
16	74.6	23.7	23.5	23.8	74.8	75.1	24.2	23.7
17	49.5	47.3	47.2	47.5	49.2	48.2	47.2	47.1
18	41.6	42.0	41.9	42.1	41.8	41.7	42.5	42.0
19	47.6	46.5	46.4	46.7	47.7	47.6	47.0	46.8
20	31.1	31.1	30.9	31.2	31.3	31.4	31.5	30.9
21	36.3	34.3	34.2	34.5	36.4	36.6	34.7	34.2
22	32.4	33.4	33.2	33.6	32.6	33.6	33.7	33.2
23	64.7	28.3	65.9	28.5	28.6	65.0	28.5	64.6
24	13.8	17.0	13.6	17.3	17.5	14.0	17.3	13.3
25	14.5	15.9	16.3	16.0	16.3	14.6	16.0	16.0
26	16.7	17.8	17.5	17.9	18.1	16.8	17.9	17.5
27	27.5	26.3	26.2	26.4	27.7	27.6	26.7	26.1
28	176.4	176.8	176.8	177.0	176.4	180.5	180.6	180.2
29	33.6	33.4	33.2	33.5	33.7	33.7	33.8	33.2
30	25.0	24.0	23.8	24.1	25.2	24.8	24.3	23.8

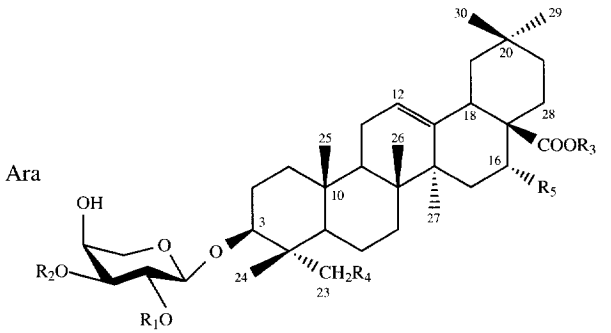
Recorded on Bruker AMX-600 in pyridine-d_5–methanol-d_4.

tetrasaccharide unit linked at C-3 of the aglycone was implied by a set of glycosidic cleavage fragments at *m/z* 1043 $[M-H-470]^-$, 911 $[M-H-470-132]^-$, 749 $[M-H-470-132-162]^-$, 587 $[M-H-470-132-162-162]^-$ and 455 $[M-H-470-132-162-162-132]^-$ in **4**. The unambiguous identification of the individual spin systems associated with the seven monosaccharides and the assignments of the NMR resonances were accomplished by a combination of TOCSY, DQF-COSY, T-ROESY, HSQC and HMBC experiments. The connectivity of the sugar moieties was established from the inter-residual ROEs and HMBC cross-peaks arising from the anomeric protons to the signals involved in the glycosidic linkage. The determination of the anomeric configuration for individual monosaccharides is based on the magnitude of the coupling constants and the appearance of intra-residual ROEs. Consequently, the structure of **4** was characterized as 3-*O*-*β*-D-xylopyranosyl-(1→3)-*β*-D-galactopyranosyl-(1→4)-*β*-D-glucopyranosyl-(1→3)-*α*-L-arabinopyranosyl-oleanolic acid 28-*O*-*α*-L-rhamnopyranosyl-(1→4)-*β*-D-glucopyranosyl-(1→6)-*β*-D-glucopyranoside.

Leonticin H (**5**) was obtained as a minor component. The NMR data clearly showed the presence of echinocystic acid and seven anomeric resonances which were very similar to those of **4**. In addition, both compounds displayed similar fragmentation patterns in the negative ion FAB mass spectra. The $[M-H]^-$ ion of **5** was observed at *m/z* 1529 ($C_{70}H_{114}O_{36}$), 16 units higher than that of **4**. Analogous fragments to those of **4** were observed at *m/z* 1059 $[M-H-470]^-$, 927 $[M-H-470-132]^-$, 765 $[M-H-470-132-162]^-$, 603 $[M-H-470-132-162-162]^-$ and 471 $[M-H-470-132-162-162-132]^-$, suggesting a very close structural similarity in both compounds. The structural elucidation was straightforward from the detailed comparison of the proton and carbon spectra with those of **4**. The assignment for carbon signals derived from comparison were in turn used to interpret proton data through the $^1H-^{13}C$ correlation spectrum (HSQC) of **5** (Tables 1–4). The relative stereochemistry of **5** appeared to be the same as in **4**, based on the diagnostic coupling constants. Finally, the structure of **5** was deduced to be 3-*O*-*β*-D-xylopyranosyl-(1→3)-*β*-D-galactopyranosyl-(1→4)-*β*-D-glucopyranosyl-(1→3)-*α*-L-arabinopyranosyl-echinocystic acid 28-*O*-*α*-L-rhamnopyranosyl-(1→4)-*β*-D-glucopyranosyl-(1→6)-*β*-D-glucopyranoside.

EXPERIMENTAL

General. Mps (uncorr.) were measured on a Mettler FP 52. Optical rotations were determined on a Perkin–



	R_1	R_2	R_3	R_4	R_5
1	H	H	Rha (1-4) Glc (1-6) Glc	OH	OH
1a	H	H	H	OH	OH
2	Glc	Glc	Rha (1-4) Glc (1-6) Glc	H	H
2a	Glc	Glc	H	H	H
3	Glc	Glc	Rha (1-4) Glc (1-6) Glc	OH	H
3a	Glc	Glc	H	OH	H
4	H	Xyl (1-3) Gal (1-4) Glc	Rha (1-4) Glc (1-6) Glc	H	H
5	H	Xyl (1-3) Gal (1-4) Glc	Rha (1-4) Glc (1-6) Glc	H	OH

Elmer 241 polarimeter. FABMS were obtained in a glycerol matrix in the negative ion mode using a VG ZAB_2-Seq spectrometer and EI-MS on a VG Tribrid spectrometer. NMR spectra were measured at 300 K in pyridine-d_5 with 2 drops of MeOH-d_4 on a Bruker AMX-600 instrument, and chemical shifts were referenced to TMS. HPLC was performed on a Waters 590 programmable pump with a Knauer differential refractometer. VLC was carried out with silica gel 60 (0.040–0.063 mm, Merck). TLC was performed on silica gel 60 F_{254} (Merck) and RP-18 F_{254} S (Merck).

Isolation. The tubers of *L. kiangnanensis* were collected in the Anhui province of China in May 1992 by D. Q. Wang. A voucher specimen was deposited in the herbarium of the Shanghai Institute of Pharmaceutical Industry, Shanghai. The extraction and fractionation was performed as described previously [1]. The 50% EtOH eluate (32 g) from a styrene polymer resin SIP 1100 column was repeatedly fractionated by VLC on silica gel 60 using $CHCl_3$–MeOH–H_2O (12:7:1) as eluent. Final purification was carried out by HPLC on a Spherisorb C_{18} column [5 μm, 16 × 250 mm, MeOH–H_2O (7:3), flow rate 6 ml min^{-1}] to afford **1** (88 mg), **2** (114 mg), **3** (38 mg), **4** (21 mg) and **5** (6 mg).

Acid hydrolysis of **1–5**. Compound **1** was refluxed with 2 M HCl in MeOH (2 ml) for 4 hr. The reaction soln was evapd under red. pres. and the hydrolysate was extracted with Et_2O. The Et_2O extract was evapd to afford the aglycone caulophyllogenin. The H_2O layer was neutralized with alkali soln and concd under red. pres. The residues were compared with standard sugars by silica gel TLC [$CHCl_3$–MeOH–H_2O (15:6:2), 9 ml of lower layer + 1 ml HOAc], which showed the sugars to be arabinose, rhamnose and glucose for **1**. Compounds **2–5** were hydrolysed on TLC plates in conc. HCl vapour at 80° for 20 min.

Alkaline hydrolysis of **1–3**. Saponins **1–3** were hydrolysed with 0.5 M KOH (4 ml) at 80° for 2 hr, and on usual work-up the prosapogenins **1a**, **2a** and **3a** were obtained.

Leonticin D (**1**). Needles, mp 219–220°, $[\alpha]_D^{20}$ − 18.9° (MeOH; *c* 0.96); ^{1}H NMR (pyridine-d_5–MeOH-d_4, 600.13 MHz): Tables 1 and 3; ^{13}C NMR (pyridine-d_5–MeOH d_4, 150.91 MHz): Tables 2 and 4; negative ion FABMS *m/z*: $[M-H]^-$ 1089, $[M-Rha-H]^-$ 943, $[M-Rha-Glc-H]^-$ 781, $[M-Rha-2Glc-H]^-$ 619, $[M-Rha-2Glc-Ara-H]^-$ 487.

Prosapogenin **1a**. Needles, mp 278–280°, $[\alpha]_D^{20}$ + 2° (MeOH; *c* 0.54); ^{13}C NMR (pyridine-d_5–MeOH-d_4, 150.91 MHz): Table 4; negative ion FABMS *m/z*: $[M-H]^-$ 619, [M–Ara–H]$^-$ 487.

Caulophyllogenin. Needles, mp 275–276°; EI-MS *m/z*: $[M]^+$ 488, $C_{30}H_{48}O_5$, $[M-CH_2O_2]^+$ 442 (52), $[M-CO_2-H_2O]^+$ 426 (81), 411 (100).

Leonticin E (**2**). Amorphous powder, mp 223–225°, $[\alpha]_D^{20}$ − 4.42° (MeOH; *c* 1.29); ^{1}H NMR (pyridine-d_5–MeOH-d_4, 600.13 MHz): Tables 1 and 3; ^{13}C NMR (pyridine-d_5–MeOH-d_4, 150.91 MHz): Tables 2 and 4; negative ion FABMS *m/z*: $[M-H]^-$ 1381, [M-Rha-H]$^-$ 1235, $[M-Glc-H]^-$ 1219, [M − Rha − Glc −

H]⁻ 1073, [M − Rha − 2Glc − H]⁻ 911, [M − Rha − 3Glc − H]⁻ 749, [M − Rha − 4Glc − H]⁻ 587, [M − Rha − 4Glc − Ara − H]⁻ 455.

Prosapogenin **2a**. Amorphous powder, mp 247–249°, $[\alpha]_D^{20}$ + 31.78° (MeOH; *c* 0.45); ^{1}H NMR (pyridine-d_5–MeOH-d_4, 600.13 MHz): Tables 1 and 3; ^{13}C NMR (pyridine-d_5–MeOH-d_4, 150.91 MHz): Tables 2 and 4; negative ion FABMS *m/z*: [M − H]⁻ 911, [M − Glc − H]⁻ 749, [M − 2Glc − H]⁻ 587, [M − 2Glc − Ara − H]⁻ 455.

Leonticin F (**3**). Amorphous powder, mp 231–233°, $[\alpha]_D^{20}$ − 9.57° (MeOH; *c* 0.47); ^{1}H NMR (pyridine-d_5–MeOH-d_4, 600.13 MHz): Tables 1 and 3; ^{13}C NMR (pyridine-d_5–MeOH-d_4, 150.91 MHz): Tables 2 and 4; negative ion FABMS *m/z*: [M − H]⁻ 1397, [M − Rha − H]⁻ 1251, [M − Glc − H]⁻ 1235, [M − Rha − Glc]⁻ 1089, [M − Rha − 2Glc − H]⁻ 927, [M − Rha − 3Glc − H]⁻ 765, [M − Rha − 4Glc − H]⁻ 603, [M − Rha − 4Glc − Ara − H]⁻ 471.

Prosapogenin **3a**. Amorphous powder, mp 263–265°, $[\alpha]_D^{20}$ + 30.68° (MeOH; *c* 0.44); ^{1}H NMR (pyridine-d_5–MeOH-d_4, 600.13 MHz): Tables 1 and 3; ^{13}C NMR (pyridine-d_5–MeOH-d_4, 150.91 MHz): Tables 2 and 4; negative ion FABMS *m/z*: [M − H]⁻ 927, [M − Glc − H]⁻ 765, [M − 2Glc − H]⁻ 603, [M − 2Glc − Ara − H]⁻ 471.

Leonticin G (**4**). Amorphous powder, mp 219–221°, $[\alpha]_D^{20}$ − 4.38° (MeOH; *c* 1.05); ^{1}H NMR (pyridine-d_5–MeOH-d_4, 600.13 MHz): Tables 1 and 3; ^{13}C NMR (pyridine-d_5–MeOH-d_4, 150.91 MHz); Tables 2 and 4; negative ion FABMS *m/z*: [M − H]⁻ 1513, [M − Xyl − H]⁻ 1381, [M − Rha − H]⁻ 1367, [M − Xyl − Gal − H]⁻ 1219, [M − Xyl − Gal − Glc − H]⁻ 1057, [M − Rha − 2Glc]⁻ 1043, [M − Rha − 2Glc − Xyl − H]⁻ 911, [M − Rha − 2Glc − Xyl − Gal − H]⁻ 749, [M − Rha − 3Glc − Xyl − Gal − H]⁻ 587, [M − Rha − 3Glc − Xyl − Gal − Ara − H]⁻ 455.

Leonticin H (**5**). Amorphous powder, mp 207–209°, $[\alpha]_D^{20}$ − 8.79° (MeOH; *c* 0.58); ^{1}H NMR (pyridine-d_5–MeOH-d_4, 600.13 MHz): Tables 1 and 3; ^{13}C NMR (pyridine-d_5–MeOH-d_4, 150.91 MHz): Tables 2 and 4; negative ion FABMS *m/z*: [M − H]⁻ 1529, [M − Xyl − H]⁻ 1397, [M − Rha − H]⁻ 1383, [M − Xyl − Gal − H]⁻ 1235, [M − Xyl − Gal − Glc − H]⁻ 1073, [M − Rha − 2Glc]⁻ 1059, [M − Rha − 2Glc − Xyl − H]⁻ 927, [M − Rha − 2Glc − Xyl − Gal − H]⁻ 765, [M − Rha − 3Glc − Xyl − Gal − H]⁻ 603, [M − Rha − 3Glc − Xyl − Gal − Ara − H]⁻ 471.

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