

Triterpenoid Saponins from *Albizia mollis*Zhong-Quan Cheng,^{‡,§} Dan Yang,[§] Qing-Yun Ma,[†] Xin-Hui Yi,[§] Nen-Ling Zhang,[‡] Jun Zhou,^{‡,*} and You-Xing Zhao^{†,‡,*}[†]Institute of Tropical Bioscience and Biotechnology, Chinese Academy of Tropical Agricultural Sciences, Haikou 571101, P. R. China. *E-mail: zhaoyx1011@163.com[‡]State Key Laboratory of Phytochemistry and Plant Resources in West China, Kunming Institute of Botany, the Chinese Academy of Sciences, Kunming 650204, P. R. China[§]Guilin Normal College, Guilin 541001, P. R. China

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Plant species of *Albiziaceae* are known for their traditional medicinal uses as medicaments of mind unrest, insomnia, physique damage, carbuncle gall in many parts of tropic zone. *Albizia mollis*, popularly named “maoyehedian” in China, is one of representative plant species of this family. This plant is well known for its sedative and sleeping pill properties.¹ The previous chemical investigation of this family has led to the isolation of lignans,²⁻⁵ flavonoids,⁶⁻⁹ saponins,¹⁰ alkaloids,¹¹ together with other secondary metabolites.¹² In search for new and bioactive compounds from medicinal plant, the chemical study on *A. mollis*, collected from Kunming Botanical Garden, Yunnan Province, Peoples Republic of China, led to the isolation of four saponins including two new ones. This paper describes the isolation and structural elucidation of one new bisdesmosidic triterpene glycoside (molliside A, **1**) and one new trisdesmosidic triterpene glycoside (molliside B, **2**), together with two known compounds concinnoside A (**3**), albiziasaponin A (**4**).

Molliside A (**1**) was isolated as white powder. Its molecular formula was determined to be C₄₂H₆₆O₁₃ with ten unsaturation degrees by HRESIMS (*m/z* 777.4428 [M-1]⁻) and ¹³C NMR spectra. The ¹H NMR spectrum of compound **1** clearly showed eight methyls at δ 0.86 (3H, s), 0.92 (3H, s), 0.95 (3H, s), 1.00 (3H, s), 1.00 (3H, s), 1.06 (3H, s), 1.24 (3H, s), 1.26 (d, *J* = 6.5 Hz), two anomeric protons of β-type sugar at δ 4.30 (1H, d, *J* = 7.0 Hz), 4.31 (d, *J* = 6.0 Hz) and one olefinic proton at δ 5.42 (1H, s). The ¹³C NMR (DEPT) spectroscopic data (Table 1) revealed the presence of 8 methyls, 9 methylenes, 17 methines and 8 quaternary carbons. The IR absorption band at 1766 cm⁻¹ and the peak at δ 181.6 in the ¹³C NMR spectrum showed the presence of γ-lactone ring. The gross structure of the aglycone of **1** was determined by analysis of NMR data and by referring to the NMR data of acacic acid lactone.¹³ The formation of lactone ring with the carboxyl group at C-17 was confirmed by the key HMBC correlation of H-21 (δ 4.26) with carboxyl carbon (C-28, δ 181.6). In the ROESY experiment, the key correlations of H-16/H-26, H-16/H-18 indicated β-orientations of H-16, H-18 and H-3/H-23, H-3/H-5, H-5/H-9, H-21/H-29 indicated α-orientations of H-3, H-5, H-9 and H-21, from which the relative configuration of the aglycone in **1** was elucidated (see Figure 3).

Comparing the NMR data of **1** with those of compound **3**¹⁴ indicated that **1** was similar to **3** except for one rhamnose unit in **1** replacing the arabinose in the literature. The linkages of interglycosides and the saccharide part to the aglycone were respectively established by the key HMBC correlations of H-1'' (δ 4.30) with C-6' (δ 68.0) and H-1' (δ 4.31) with C-3 (δ 89.0). Moreover, the presence of rhamnose and glucose were further evidenced by the acid hydrolysis and the fragment ion peaks at *m/z* 631 [M-1-Rha]⁻, 469 [M-1-Rha-Glc]⁻ in the negative FABMS spectrum of **1**. Based on the above evidences, compound **1** was identified as acacic acid lactone 3-*O*-β-L-rhamnopyranosyl-(1→6)-β-D-glucopyranoside, named molliside A.

Molliside B (**2**) was established a molecular formula of C₄₇H₇₄O₁₇ determined from a pseudomolecular ion peak at *m/z* 909.4863 [M-H]⁻ by the HRESIMS. All the ¹H and ¹³C NMR signals of its aglycone moiety were consistent with the corresponding resonances of **1**. The relative configuration of the aglycone in **2** was same as **1** with β-orientations of H-16 and H-18 and α-orientations of H-3, H-5, H-9 and H-21 and were elucidated by ROESY experiment. Comparing the NMR data of **2** with those of **1** indicated that the distinct difference between compounds **1** and **2** was the additional chemical shift at δ 107.0, 77.4, 72.6, 71.7, and 69.8 which are combined to be one xylose (see Table 1). The linkages of interglycosides were determined by key HMBC correlations from H-1''' (δ 4.46) of xlyopyranosyl to C-4'' (δ 78.1) of rhamnopyranosyl and from H-1'' (δ 4.30) of rhamnopyranosyl to C-6' (δ 67.3) of glucose. The linkage of the saccharide part to the aglycone were established by the key HMBC correlation of H-1' (δ 4.31) with C-3 (δ 89.4). Furthermore, the presence of xylose, rhamnose and glucose were further evidenced by the acid hydrolysis and the fragment ion peaks at *m/z* 777 [M-H-xy]⁻ and 631 [M-xy-rha]⁻ in the negative FABMS spectrum of **2**.

Based on the above evidences, the structure of **2** was determined to be as acacic acid lactone 3-*O*-β-D-xylopyranosyl-(1→4)-β-L-rhamnopyranosyl-(1→6)-β-D-glucopyranoside, named molliside B.

Two known compounds **3** and **4** had the similar spectral characteristic as triterpene glycosides **1** and **2** with the same aglycone. The spectral data of compound **3** were consistent

Table 1. ^1H and ^{13}C NMR spectroscopic data for molliside A (**1**) and molliside B (**2**) (400 and 100 MHz, respectively, CD_3OD)

1			2		
	$\delta\text{ C}$	$\delta\text{ H}$	$\delta\text{ C}$	$\delta\text{ H}$	
1	38.2	1H, 1.00, m; 1H, 1.61, m	39.7	1H, 1.11, m; 1H, 1.63, m	
2	25.6	2.18, d, 12 Hz	27.2	1H, 1.54, d, 9.2 Hz; 1H, 1.95, d, 6.7 Hz	
3	89.0	2.32, m	89.4	3.34, d, 4.4 Hz	
4	36.3	-	37.9	-	
5	55.6	0.80, t, 11 Hz	57.1	0.82, m	
6	17.7	1H, 1.51, m; 1H, 1.61, m	19.2	1.50, m	
7	31.8	1.53, m	33.4	1.52, m	
8	39.8	-	41.4	-	
9	46.8	1.54, m	48.4	1.57, m	
10	38.6	-	40.3	-	
11	23.0	1H, 1.95, m; 1H, 0.97, m	24.6	1.96, m	
12	124.5	5.42, s	126.0	5.41, s	
13	139.1	-	140.7	-	
14	42.8	-	44.3	-	
15	36.6	1H, 2.04, dd, 5.0 Hz, 12.1 Hz; 1H, 1.11, m	38.2	1H, 1.14, m; 1H, 2.03, m	
16	66.4	1H, 3.90, dd, 5.0 Hz, 12.1 Hz	68.0	3.90, dd, 5.2 Hz, 12.4 Hz	
17	49.2	-	50.7	-	
18	41.2	2.51, m	42.7	2.50, dd, 6.4 Hz, 12.0 Hz	
19	42.8	1H, 1.37, m; 1H, 1.81, t, 13.5 Hz	44.3	1H, 1.38, m; 1H, 1.82, m	
20	33.3	-	34.9	-	
21	83.6	4.26, m	85.2	3.34, m	
22	27.2	2.18, m; 2.33, m	27.4	1H, 2.18, d, 12.0 Hz; 1H, 2.32, m	
23	26.9	1.06, s	28.3	1.06, s	
24	15.4	0.86, s	17.0	0.86, s	
25	14.6	0.95, s	16.2	0.95, s	
26	15.1	0.92, s	16.7	0.92, s	
27	27.7	1.24, s	29.4	1.25, s	
28	181.6	-	183.1	-	
29	27.4	1.00, s	29.0	1.00, s	
30	22.6	1.00, s	24.2	0.99, s	
Glc			Glc		
1'	105.2	4.31, d, 6.0	106.5	4.31, d, 8.0	
2'	74.1	3.20, m	77.1	3.48, m	
3'	76.5	3.30, m	71.1	3.49, m	
4'	73.5	3.45, m	71.7	3.61, m	
5'	75.3	3.40, m	75.0	3.62, m	
6'	68.0	1H, 3.73, m; 1H, 4.70, d, 10 Hz	67.3	1H, 3.25, d, 10.8 Hz; 1H, 3.95, dd, 5.2 Hz, 10.8 Hz	
Rha			Rha		
1''	103.6	4.30, d, 7.0 Hz	103.8	4.30, d, 7.0 Hz	
2''	70.8	3.48, m	75.7	3.48, m	
3''	69.9	3.32, m	75.0	3.58, m	
4''	71.5	3.58, m	78.1	3.32, m	
5''	70.4	3.60, m	72.6	3.60, m	
6''	15.2	3H, 1.26, d, 6.5 Hz	16.8	3H, 1.26, d, 6.5 Hz	
			Xyl		
1'''			107.0	4.46, d, 7.2 Hz	
2'''			77.4	3.94, m	
3'''			72.6	4.38, m	
4'''			71.7	3.61, m	
5'''			69.8	1H, 3.73, dd, 6.4 Hz, 12.0 Hz; 1H, 4.05, d, 12.0 Hz	

with those of concinnoside A reported in the literature.¹⁴ The linkage of arabinose to glucose at C-6-Glc were evidenced by the fragment ion peak at m/z 631 $[\text{M}-1-\text{Ara}]^-$ in the negative FABMS spectrum of **3** and the downfield shift of δ_{C} 69.4 (C-6-Glc). Base on above analysis, compound **3** was

identified to be acacic acid lactone 3-*O*- β -D-arabinopyranosyl-(1 $\rightarrow$ 6)- β -D-glucopyranoside (named concinnoside A). Compound **4** had the similar spectral data with those of albiziasaponin A reported in the literature.¹⁵ The linkages of xylose to arabinose at C-2-Ara and arabinose to glucose at

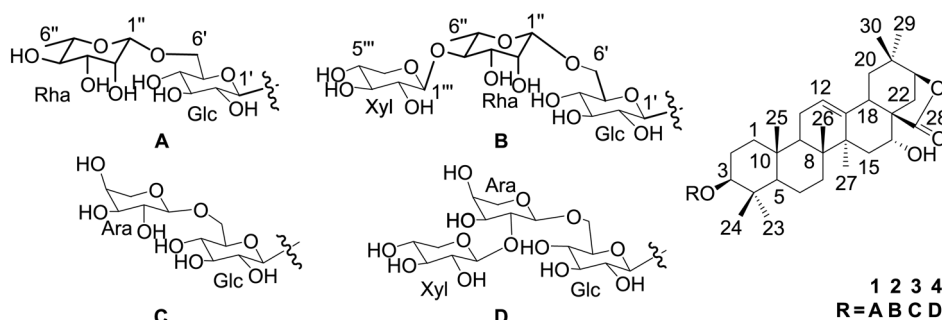


Figure 1. Structures of compounds 1-4.

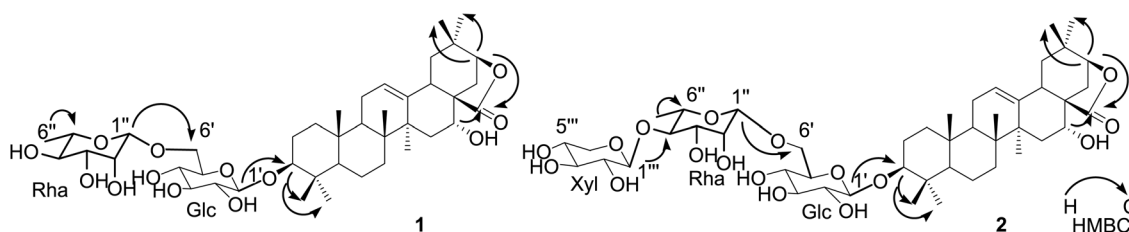


Figure 2. Key HMBC correlations of compounds 1 and 2.

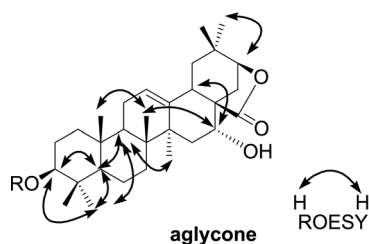


Figure 3. Key ROESY correlations of aglycone.

C-6-Glc were further proposed by the fragment ion peak at m/z 763 $[M-1-Xyl]^-$ and 631 $[M-1-Xyl-Ara]^-$ in the negative FABMS spectrum of 4 and the downfield shifts of δ_c 81.4 (C-2-Ara) and 69.5 (C-6-Glc). Thus, compound 4 was identified to be acacic acid lactone 3-*O*- β -D-xylopyranosyl-(1 $\rightarrow$ 2)- β -L-arabinopyranosyl-(1 $\rightarrow$ 6)]- β -D-glucopyranoside (named albiziasaponin A).

Experimental Section

General Experimental Procedures. Melting points were measured on a XRC-1 micro-melting point apparatus and were uncorrected. MS spectra were obtained on a VG Auto Spec-3000 mass spectrometer. 1D and 2D NMR spectra were recorded on Bruker AM-400 MHz spectrometers, with chemical shifts (δ) in ppm relative to TMS as internal standard and coupling constants in hertz (Hz). IR spectra were measured with a Bio-Rad FTS-135 spectrometer with KBr pellets. UV spectra were measured on a Hitachi UV-3210 spectrophotometer. Silica gel (200-300 mesh) for column chromatography was product of the Qingdao Marine Chemical Ltd., Qingdao, P. R. China. Sephadex LH-20 for chromatography was purchased from Amersham Biosciences. Reversed-phase chromatography was with RP-

18 (LiChroprep, 40-63 μ m, Merck, Darmstadt, Germany).

Plant Materials. The bark of *A. mollis* was collected in Kunming, Yunnan Province, Peoples Republic of China, in September 2008, and authenticated by Professor Xun Gong. A voucher specimen (ZM080920) has been deposited in the Herbarium of Kunming Institute of Botany, Chinese Academy of Sciences.

Extraction and Isolation. The air-dried and powdered bark of *A. mollis* (18.0 kg) were extracted three times each with 20 L of 95% EtOH under reflux for 3 h. The extracts were evaporated and the residue was resuspended in 15 L of H₂O and partitioned successively with EtOAc (5 L $\times$ 3) and *n*-BuOH (5 L $\times$ 3) to yield EtOAc extract (300 g), *n*-BuOH extract (640 g), respectively. Part of *n*-BuOH extract (500 g) was applied to a silica gel column chromatography (200-300 mesh) eluted with CHCl₃/CH₃OH/H₂O (10:3:0.5, v/v) to give five fractions. Fraction 1 (45 g) was purified by RP-18 with 10% $\rightarrow$ 100% aqueous CH₃OH and give five fractions. Sub-fraction 2 (96 mg) was purified on Sephadex LH-20 with CH₃OH/CH₃Cl 1:1 and then subjected to semipreparative HPLC (CH₃OH/H₂O, 62/38, 2 mL/min, 20 $^{\circ}$ C, detector wavelength 210 nm), which gave compounds 2 (11 mg) and compounds 4 (34 mg); Sub-fraction 3 (28 mg) was subjected to semipreparative HPLC (CH₃CN/H₂O, 32/68, 2 mL/min, 20 $^{\circ}$ C, detector wavelength 210 nm) to give compounds 1 (6 mg) and 3 (10 mg).

Molliside A (1): White powder, mp 209-211 $^{\circ}$ C; $[\alpha]_D^{21} +6.3116^{\circ}$ (c 0.500, CH₃OH); UV (CH₃OH) λ_{max} (log ϵ): 191 (3.519), 204 (3.712), 252 (3.260), 373 (2.508); IR (KBr) ν 3425, 1766, 1602, 1063, 1045 cm^{-1} ; 1H NMR (400 MHz, CD₃OD) and ^{13}C NMR (100 MHz, CD₃OD) see Table 1; HRESIMS (negative ion) m/z 777.4428 $[M-1]^-$ (Calcd. for C₄₂H₆₅O₁₃, 777.4789); FABMS (negative ion) m/z (%) 777 $[M-1]^-$ (100), 631 (3), 469 (3), 437 (5).

Molliside B (2): White powder, mp 205–207 °C, $[\alpha]_D^{21}$ –24.8750° (c 0.320, CH₃OH); UV (CH₃OH) $\lambda_{\max}$ (log ϵ): 203 (3.892), 252 (3.005), 383 (2.194); IR (KBr) ν 3423, 1762, 1639, 1165, 1046 cm^{–1}; ¹H NMR (400 MHz, CD₃OD) and ¹³C NMR (100 MHz, CD₃OD) see Table 1; HRESIMS (negative ion) m/z 909.4863 [M–1][–] (Calcd. for C₄₇H₇₃O₁₇, 909.4847); FABMS (negative ion) m/z (%) 909 [M–1][–] (100), 777 (12), 631 (5), 437 (4).

Concinnoside A (3): white powder, ¹H NMR (400 MHz, CD₃OD) δ 0.85 (3H, s, H-24), 0.86 (3H, s, H-26), 0.92 (3H, s, H-25), 0.99 (3H, s, H-30), 1.00 (3H, s, H-29), 1.06 (3H, s, H-23), 1.24 (3H, s, H-27), 4.26 (1H, d, J = 7.8 Hz, H-1-Ara), 4.31 (1H, d, J = 6.9 Hz, H-1-Glc), 5.41 (1H, s, H-12); ¹³C NMR (100 MHz, CD₃OD) δ 37.9 (t, C-1), 26.9 (t, C-2), 85.2 (d, C-3), 40.2 (s, C-4), 57.1 (d, C-5), 19.0 (t, C-6), 32.6 (t, C-7), 40.2 (s, C-8), 48.4 (d, C-9), 37.8 (s, C-10), 24.5 (t, C-11), 126.1 (d, C-12), 140.1 (s, C-13), 44.3 (s, C-14), 37.9 (t, C-15), 67.9 (d, C-16), 50.7 (s, C-17), 40.2 (d, C-18), 42.7 (t, C-19), 37.8 (s, C-20), 85.2 (d, C-21), 27.5 (t, C-22), 29.0 (q, C-23), 16.2 (q, C-24), 15.2 (q, C-25), 16.8 (q, C-26), 27.0 (q, C-27), 183.1 (s, C-28), 29.2 (q, C-29), 24.1 (q, C-30), 106.8 (d, Glc. C-1), 75.5 (d, Glc. C-2), 78.0 (d, Glc. C-3), 71.6 (d, Glc. C-4), 76.7 (d, Glc. C-5), 69.4 (t, Glc. C-6), 105.0 (d, Ara. C-1), 72.4 (d, Ara. C-2), 74.1 (d, Ara. C-3), 67.9 (d, Ara. C-4), 66.4 (t, Ara. C-5); FAB-MS (negative ion) m/z 763 [M–1][–] (72), 631 (5), 347 (6).

Albiziasaponin A (4): white powder, ¹H NMR (400 MHz, CD₃OD) δ 0.86 (3H, s, H-24), 0.91 (3H, s, H-26), 0.92 (3H, s, H-25), 0.99 (3H, s, H-30), 1.00 (3H, s, H-29), 1.06 (3H, s, H-23), 1.26 (3H, s, H-27), 4.32 (1H, d, J = 7.8 Hz, H-1-Glc), 4.54 (1H, d, J = 6.7 Hz, H-1-Xyl), 4.26 (1H, d, J = 5.4 Hz, H-1-Ara), 5.40 (1H, s, H-12); ¹³C NMR (100 MHz, CD₃OD) δ 38.0 (t, C-1), 26.0 (t, C-2), 89.8 (d, C-3), 38.2 (s, C-4), 57.1 (d, C-5), 19.1 (t, C-6), 32.8 (t, C-7), 41.3 (s, C-8), 48.3 (d, C-9), 37.9 (s, C-10), 24.3 (t, C-11), 125.9 (d, C-12), 140.6 (s, C-13), 44.3 (s, C-14), 40.2 (t, C-15), 66.9 (d, C-16), 50.7 (s, C-17), 42.5 (d, C-18), 44.3 (t, C-19), 34.8 (s, C-20), 85.1 (d, C-21), 27.3 (t, C-22), 28.4 (q, C-23), 17.0 (q, C-24), 16.2 (q, C-25), 16.7 (q, C-26), 29.4 (q, C-27), 183.1 (s, C-28), 29.0 (q, C-29), 24.2 (q, C-30), 106.6 (d, Glc. C-1), 75.6 (d, Glc. C-2), 78.0 (d, Glc. C-3), 71.8 (d, Glc. C-4), 75.8 (d, Glc. C-5), 69.5 (t, Glc. C-6), 103.3 (d, Ara. C-1), 81.4 (d, Ara. C-2), 72.8 (d, Ara. C-3), 67.8 (d, Ara. C-4), 66.5 (t, Ara. C-5), 106.6 (d, Xyl. C-1), 75.6 (d, Xyl. C-2), 77.5 (d, Xyl. C-3), 71.1 (d, Xyl. C-4), 67.5 (t, Xyl. C-5); FABMS (negative ion) m/z 895 [M–1][–] (100), 763 (120), 631 (40).

Acid Hydrolysis of Compounds 1 and 2. Compounds **1** (3 mg) and **2** (3 mg) in 5% EtOH (2 mL) containing 5% H₂SO₄ was each heated in a boiling water-bath for 5 h, then cooled for few minutes. The mixture was washed with EtOAc (2 mL $\times$ 2). The H₂O layer was concentrated in vacuo to give a residue. The residue and authentic sugar were dotted to the plate developed with EtOAc/pyridine/ethanol/H₂O (8/1/1/2, v/v), and phenylene diamine-aniline-phosphoric acid used as spray reagent, followed by heating at 80. From compounds **1** and **2** glucose was detected, R_f : glucose 0.38, xylose 0.55, rhamnose 0.76, respectively.

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