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THREE NEW D-LACTONE RING ISOMERIC PHRAGMALIN-TYPE LIMONOIDS FROM *CHUKRASIA TABULARIS* VAR. *VELUTINA*

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Abstract – Three new D-lactone ring isomeric phragmalin-type limonoids, tabulalins G-I (**1**-**3**), were isolated from the stem barks of *Chukrasia tabularis* var. *velutina*. Compound **1** possesses C-16/C-30 δ-lactone ring (D), which was unusual in phragmalins, and that of **2** and **3** were C-16/C-17. The structures of these three new compounds were elucidated on HR-ESI-MS, 1D and 2D NMR including HSQC, HMBC, and ROESY experiments.

Chukrasia tabularis var. *velutina* (Wall.) King (Maliaceae) is a timber tree growing mainly in tropical areas of Asia, e.g., India and southern mainland China.[1] Its stem barks have been traditionally used to treating cold and febrile in China.[2] Chemical constituent studies on plants of genus *chukrasia* indicated that phragmalin-type limonoids were their main constituents,[3,4] and many kinds of phragmalins with different skeletons were isolated in recent 5 years.[5-10] In most reported phragmalins, D ring is a δ-lactone ring forming between COOH-16 and C-17. But in our research, many kinds of phragmalins with C-16/C-30 δ-lactone ring were isolated, such as C-15-acyl phragmalin-type limonoids orthoesters,[8] 13/14/18-cyclopropanyl phragmalins,[10] and normal phragmalin-type orthoesters.[11] Further investigation on the limonoids of title plant led to the isolation of three new D-lactone ring isomeric phragmalin-type limonoids, tabulalins G-I (**1**-**3**). The main difference between compounds **1**-**3** was the location of D-lactone ring, which of compound **1** was C-16/C-30 δ-lactone ring and compounds **2**-**3** was C-16/C-17 ones. The structures of these new compounds were elucidated on their extensive 1D and 2D spectroscopic analysis (HSQC, HMBC, and ROESY) and HR-ESI-MS. Herein, the isolation and structural elucidation of them are reported.

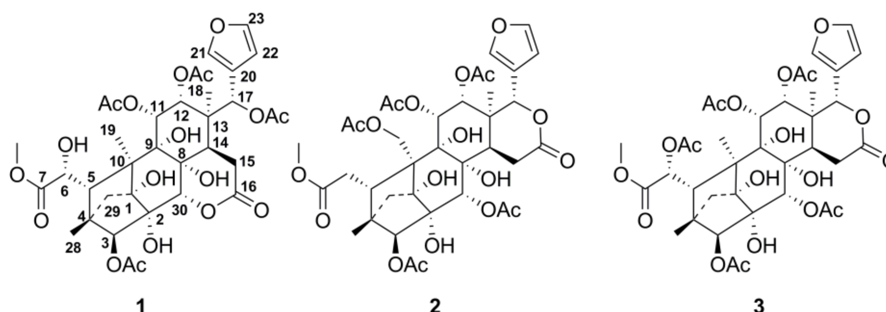

Figure 1. Structures of compounds **1-3**

Tabulalin G (**1**), white amorphous powder, has a molecular formula $C_{35}H_{44}O_{18}$ as established by the HRESIMS ion at *m/z* 751.2457 ($[M-H]^-$, $C_{35}H_{43}O_{18}$; calc. 751.2455). The IR absorption bands at 3432, 1732 cm^{-1} suggested the presence of OH and ester groups. In ^1H and ^{13}C-NMR spectra of **1**, the presence of three characteristic olefinic proton signals at δ_H 6.59, 7.63, and 7.76, four olefinic carbons at δ_C 121.7, 109.7, 143.2, and 141.3 indicated that compound **1** possesses a β-substituted furan ring moiety. A pair of characteristic double proton signals at δ_H 2.11 and 1.44 with coupling constant at 11.0 Hz suggested the presence of a 4,29,1-bridge moiety.[12] Aforementioned and other 2D-NMR information indicated that the basic carbon skeleton of **1** was phragmalin-type limonoid.

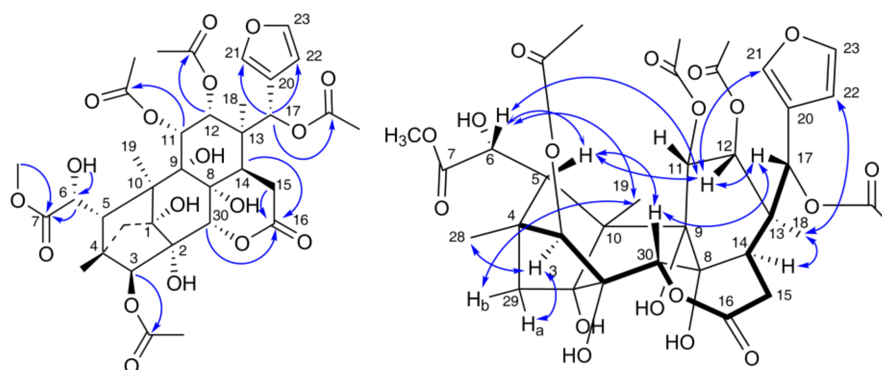

Figure 2. Key HMBC and ROESY correlations of **1**

In HMBC spectra of compound **1**, obvious correlations from H-17 (δ_H 5.94) to an acetyl CO group (δ_C 168.8) and H-30 (δ_H 4.99) to COOH-16 (δ_C 169.0) suggested the opening of normal C-16/C-17 δ-lactone ring in phragmalins and formation of a new C-16/C-30 δ-lactone ring like tabulalin A.[11] HMBC correlations from a double proton signal at δ_H 3.97 (H-6) and methoxyl signal at δ_H 3.66 to a carbonyl carbon at δ_C 174.5 (C-7) indicated that the C-6 in compound **1** was oxygenated, which was the difference between methylene of C-6 in tabulalin A.[11] HMBC correlations from H-3 (δ_H 4.60), H-11 (δ_H 5.06), H-12 (δ_H 5.09) to carbonyl carbon signals at δ_C 169.9, 170.1, 169.0 suggested that the other three acetyl groups were located at OH-3, OH-11, and OH-12, respectively. Hitherto, the planar structure of compound **1** was determined as shown in Figure 1.

The ROESY correlations (Figure 2), from H-5 to H-12 and H-30, from H-17 to H-12 and H-30, from H-12 to H-21, from Me-18 to H-14 and H-22, from H-3 to Me-28 and H-29a, from Me-19 to H-29b indicated the basic skeleton of **1** to be the same as that of tabulalin A,[11] that is β-orientation of H-5, H-12, H-17, H-30, and Me-28, an α-orientation of H-3, H-14, Me-18, Me-19, and H-29. The H-6 proton showed NOE correlations with H-5, H-12, and Me-19, which suggested the dihedral angle with H-5 was near 90° and adopted an α-orientation.[13] Thus, the structure of **1** was established as shown in Figure 1.

Tabulalin H (**2**), white amorphous powder, has a molecular formula $C_{37}H_{46}O_{19}$ as established by the HRESIMS ion at *m/z* 793.2562 ($[M-H]^-$, $C_{37}H_{45}O_{19}$; calc. 793.2561). The similarity of the ^1H and ^{13}C NMR spectroscopic data of **2** to those of **1** (Table 1) indicated that compound **2** possessed the same phragmalin-type limonoid skeleton. Strong down-field shift of C-17 (Δ5.6) and up-field shift of C-30 (Δ-5.2) suggested that the C-16/C-30 δ-lactone ring in **1** changed to C-16/C-17 one as tabulalide E,[14] which was confirmed by the HMBC correlation from H-30 (δ_H 5.49) to an acetyl CO carbon singal (δ_C 170.1). Compared the NMR data between **2** and tabulalide E indicated that the former was an acetyl derivative of the latter, which was confirmed by one more C_2H_2O unit from molecular formula. HMBC correlations from H-3 (δ_H 4.53), H-11 (δ_H 5.31), H-12 (δ_H 5.44), and H-30 (δ_H 5.49) to carbonyl carbon signals at δ_C 168.3, 170.3, 169.3, and 170.1 suggested that the other four acetyl groups were located at OH-3, OH-11, OH-12, and OH-30, respectively, which indicated one more acetyl group at OH-12. The relative configuration of **2**, as same as those of tabulalide E,[14] was determined by its key ROESY correlations, such as from H-11 to H-5, H-12, from H-17 to H-22 and H-30, from H-21 to H-12, from H-3 to Me-28 and H-29a. Thus, the structure of **2** was established as 12-*O*-acetyl derivative of tabulalide E.

Tabulalin I (**3**) was isolated as white amorphous powder, and its molecular formula was established as $C_{37}H_{46}O_{19}$ by the HRESIMS ion at *m/z* 793.2569 ($[M-H]^-$, $C_{37}H_{45}O_{19}$; calc. 793.2561), an isomer of compound **2**. The ^1H and ^{13}C NMR data (Table 1) indicated that compound **3** possesses the same phragmalin-type skeleton as **2** with C-16-O-C-17 δ-lactone ring and five acetyl moieties, and the difference was the location of acetyl groups. A singlet proton signal at δ_H 5.52 (H-6) showed HMBC correlations with carbon signals at δ_C 45.0 (C-5), δ_C 169.8 (C-7), and δ_C 169.6 (OAc-6), which suggested that C-6 was acetylated. The HMBC correlation from H-3 (δ_H 4.50), H-11 (δ_H 5.20), H-12 (δ_H 5.50), and H-30 (δ_H 5.46) to carbonyl carbon signals at δ_C 168.0, 170.5, 169.3,and 170.1 suggested that the other four acetyl groups was located at OH-3, OH-11, OH-12, and OH-30, respectively. Aforementioned analysis indicated that the acetoxy moiety at C-19 in **2** have changed to C-6 in **3**. The relative configuration of **3** was determined to be the same as those of **2** by its key ROESY correlations. Thus, the structure of **3** was demonstrated as 19-*O*-deacetyl, 6-*O*-acetyl derivative of **2**.

Table 1. ^{1}H NMR (500 MHz) and ^{13}C NMR (125 MHz) data of **1-3** (in DMSO-d_6)

No.	**1**		**2**		**3**	
	δ_H (multi, *J* in Hz)	δ_C	δ_H (multi, *J* in Hz)	δ_C	δ_H (multi, *J* in Hz)	δ_C
1		83.0		83.7		83.9
2		74.6		75.2		75.0
3	4.60 (s)	86.6	4.53 (s)	85.3	4.50 (s)	85.7
4		43.6		43.6		43.4
5	2.66 (s)	44.7	2.52 (br s)	41.2	2.72 (br s)	45.0
6a	3.97 (d, 5.5)	69.3	2.71*	32.8	5.52 (br s)	71.4
6b			2.52*		2.52*	
7		174.5		173.1		169.8
8		71.9		78.2		78.3
9		76.6		80.2		79.1
10		52.9		53.4		53.6
11	5.06 (d, 3)	70.6	5.31 (d, 4.0)	70.8	5.20 (d, 4.0)	71.8
12	5.09 (d, 3)	71.2	5.44 (d, 4.0)	69.7	5.50 (d, 4.0)	69.5
13		42.2		37.9		38.1
14	2.62 (d, 9.5)	40.2	2.77 (d, 9.0)	41.6	2.83 (d, 8.5)	41.6
15a	2.83 (dd, 18, 9.5)	27.8	3.12 (d, 19.0)	27.4	3.13 (d, 19.5)	27.5
15b	2.73 (d, 18)		2.63 (dd, 19.0, 9.0)		2.58 (dd, 19.5, 8.5)	
16		168.9		169.0		169.0
17	5.94 (s)	70.4	5.77 (s)	76.0	5.77 (s)	76.0
18	0.98 (s, 3H)	18.6	0.75 (s, 3H)	18.8	0.74 (s, 3H)	18.6
19	1.42 (s, 3H)	15.1	4.39 (s, 2H)	66.2	1.13 (s, 3H)	17.2
20		121.7		121.6		121.6
21	7.76 (s)	141.3	7.64 (br s)	140.4	7.66 (br s)	140.3
22	6.59 (s)	109.7	6.61 (d, 1.0)	109.5	6.65 (d, 1.0)	109.4
23	7.63 (s)	143.2	7.74 (t-like 1.5)	144.0	7.75 (t-like 1.0)	144.0
28	0.84 (s, 3H)	15.6	0.62 (s, 3H)	15.9	0.77 (s, 3H)	16.1
29a	1.44 (d, 11)	41.8	2.26 (d, 10.5)	40.0	2.00 (d, 9.5)	41.4
29b	2.11 (d, 11)		1.60 (d, 10.5)		1.64 (d, 9.5)	
30	4.80 (s)	74.4	5.49 (s)	69.2	5.46 (s)	69.4
7-OMe	3.66 (s, 3H)	51.7	3.54 (s, 3H)	51.4	3.62 (s, 3H)	52.3
1-OH	6.55 (br s)		6.50 (s)		6.25 (s)	
2-OH	4.79 (s)		4.96 (s)		5.46 (s)	
6-OH	5.73 (d, 5.5)					
8-OH	4.53 (s)				5.28 (s)	

9-OH	6.22 (br s)				5.52 (s)	
3-OAc		169.9		168.3		168.0
	2.14 (s, 3H)	20.4	2.10 (s, 3H)	20.5	2.07 (s, 3H)	20.6
6-OAc						169.6
					2.17 (s, 3H)	20.6
11-OAc		170.1		170.3		170.5
	2.01 (s, 3H)	20.4	2.02 (s, 3H)	20.7	2.03 (s, 3H)	20.8
12-OAc		169.0		169.3		169.3
	1.91 (s, 3H)	20.8	1.80 (s, 3H)	20.8	1.85 (s, 3H)	20.9
17-OAc		168.8				
	1.96 (s, 3H)	20.9				
19-OAc				170.0		
			2.05 (s, 3H)	20.8		
30-OAc				170.1		170.1
			1.92 (s, 3H)	21.2	1.92 (s, 3H)	21.1

* Resonance pattern unclear due to overlapping.

EXPERIMENTAL SECTIONS

Optical rotations were measured with a JASCO P-1020 polarimeter. IR (KBr disks) spectra were recorded on a Bruker Tensor 27 spectrometer. NMR spectra were recorded on Bruker ACF-500 NMR instrument, (^{1}H: 500 MHz, ^{13}C: 125 MHz), with TMS as internal standard. Mass spectra were obtained on a MS Agilent 1100 Series LC/MSD ion-trap mass spectrometer (ESIMS) and a Mariner ESITOF spectrometer (HRESIMS), respectively. All solvents used in column chromatography were analytical grade (Jiangsu Hanbang Science and Technology. Co., Ltd.). Silica gel (Qingdao Haiyang Chemical Co., Ltd.), Sephadex LH-20 (Pharmacia), and RP-C_{18} (40–63 μm, FuJi) were used for column chromatography. Preparative HPLC was carried out using an Agilent 1100 Series instrument with a Shim-park RP-C_{18} column (20 × 200 mm) and a 1100 Series multiple wavelength detector.

Plant Material. The air-dried stem bark of *Chukrasia tabularis* var. *velutina* (Wall.) King was collected from Xishuangbanna, Yunnan Province, People`s Republic of China, in March 2007, and was authenticated by Professor Mian Zhang of the Research Department of Pharmacognosy, China Pharmaceutical University. A voucher specimen (no. 2006-MML) has been deposited in the Department of Natural Medicinal Chemistry, China Pharmaceutical University.

Extraction and Isolation. The air-dried stem bark (10 kg) was extracted by refluxing with 95% EtOH

three times. The EtOH extract was concentrated under reduced pressure (2000 g) and then partitioned with $CHCl_3$ to give a chloroform part (300 g). The oily chloroform part was dissolved in 2 L MeOH-H_2O (50:50, v/v) and then extracted with petroleum ether. After removal of the fatty components, 210 g of residue were obtained, which was subjected to a silica gel column eluted with $CHCl_3$-MeOH in a gradient from 1:0 to 1:2 to afford eight fractions (Fr. A–H). Fr. F (13 g) was chromatographed on a column of silica gel eluted successively with a gradient of petroleum ether-EtOAc (1:1 to 1:4) to give four sub-fractions (Fr. F1–4). Fr. F3 was chromatographed on a column of reversed-phase C_{18} silica gel eluted with MeOH-H_2O (2:3 to 7:3) to give four sub-fractions (Fr. F3a–d). Fr. F3d was separated by preparative HPLC using CH_3OH-H_2O (52:48, 10 mL/min) as the mobile phase to give **1** (6 mg). Fr. F4 was chromatographed on a column of reversed-phase C_{18} silica gel eluted with MeOH-H_2O (2:3 to 7:3) to give four sub-fractions (Fr. F4a–d). Fr. F4d was separated by preparative HPLC using CH_3OH-H_2O (55:45, 10 mL/min) as the mobile phase to give **2** (3 mg) and **3** (10 mg).

Tabulalin G (1) White, amorphous powder; $[\alpha]^{25}_{D}$ +20 (*c* 0.10, MeOH); IR (KBr) cm^{-1}: 3432, 2974, 1804, 1732, 1639, 1375, 1245, 1060; ^{1}H and ^{13}C NMR, see Table 1; negative ESIMS *m/z*: 787.5 $[M+Cl]^-$ (100); positive ESIMS *m/z*: 770.4 $[M+NH_4]^+$ (100); HRESIMS *m/z*: 751.2457 $[M-H]^-$ (calcd: $C_{35}H_{43}O_{18}$, 751.2455).

Tabulalin H (2) White, amorphous powder; $[\alpha]^{25}_{D}$ -21 (*c* 0.10, MeOH); IR (KBr) ν_{max} cm^{-1}: 3450, 2978, 1733, 1640, 1377, 1245, 1041; ^{1}H and ^{13}C NMR, see Table 1; negative ESIMS *m/z*: 829.5 $[M+Cl]^-$ (100); positive ESIMS *m/z*: 812.3 $[M+NH_4]^+$ (100); HRESIMS *m/z*: 793.2562 $[M-H]^-$ (calcd: $C_{37}H_{45}O_{19}$, 793.2561).

Tabulalin I (3) White, amorphous powder; $[\alpha]^{25}_{D}$ -27 (*c* 0.13, MeOH); IR (KBr) cm^{-1}: 3448, 2978, 1738, 1641, 1465, 1378, 1239, 1167; ^{1}H and ^{13}C NMR, see Table 1; negative ESIMS *m/z*: 829.5 $[M+Cl]^-$ (100); positive ESIMS *m/z*: 812.3 $[M+NH_4]^+$ (100); HRESIMS *m/z*: 793.2569 $[M-H]^-$ (calcd: $C_{37}H_{45}O_{19}$, 793.2561).

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