ORIGINAL ARTICLE

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Rec. Nat. Prod. 9:2 (2015) 190-195

records of natural products

Three New C-16/C-30 δ-Lactone Ring Phragmalin-Type Limonoids from *Chukrasia tabularis* Var. *velutina*

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(Received January 14, 2014; Revised July 28, 2014; Accepted August 20, 2014)

Abstract: Three new phragmalin-type limonoids, with isomeric D-lactone ring from C-16/C-17 to C-16/C-30, tabulalin K(**1**),L(**2**),M(**3**) were isolated from the stem barks of *Chukrasia tabularis* var. *velutina* during our ongoing research work in this area. Their structures were elucidated on HR-ESI-MS, ^{1}H and ^{13}C-NMR, HSQC, HMBC, and ROESY experiments.

Keywords: Phragmalin-type limonoids; C-16/C-30 δ-lactone ring; *Chukrasia tabularis* var. *velutina.*

1. Introduction

Chukrasia tabularis var. *velutina* (Meliaceae) as a timber tree, is mainly distributed in the tropical areas of Asia, such as India, Malaysia, and southern China [1]. Its barks have been traditionally used for their astringent, antidiarrheal, and anti-influenza properties in China and India [2]. Phragmalin-type limonoids are the main constituents in genus *chukrasia* [3,4], and many kinds of phragmalins with different skeletons were reported in recent 6 years, such as normal phragmalins and their orthoesters, C(15)-acyl phragmalins, 16-norphragmalins, 13/14/18-cyclopropanyl phragmalin-type orthoesters, and 16,19-dinor phragmalins [5-16]. Ongoing investigation on the limonoids of title plant led to the discovery of three new C-16/C-30 δ-lactone ring phragmalin-type limonoids, tabulalin K-M. 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.

2. Materials and Methods

2.1. General

All solvents used were analytical grade (Jiangsu Hanbang Science and Technology. Co., Ltd.). Silica gel (Qingdao Haiyang Chemical Co., Ltd.), Sephadex LH-20 (Pharmacia), and RP-C18 (40–63 μm, FuJi) were used for column chromatography. Preparative HPLC was carried out using an SHMADZU LC-6AD series instrument with a Shim-park RP-C_{18} column (20×200 mm) and a SHMADZU SPD-20A detector. 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

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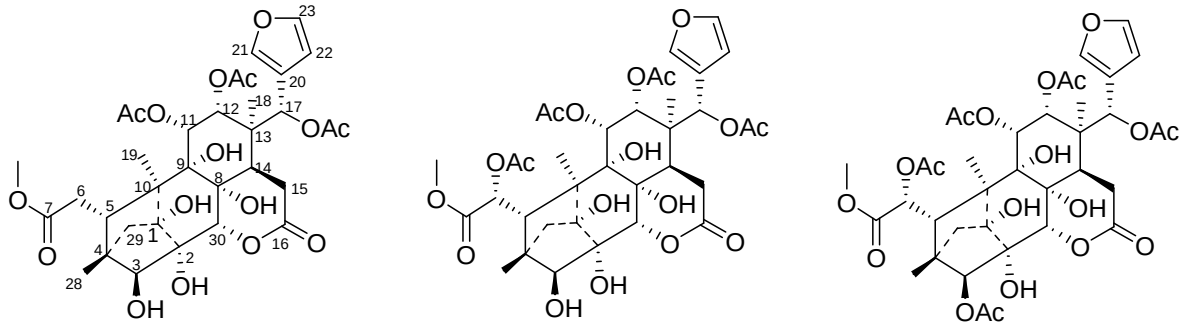
standard. Mass spectra were determined with a MS Agilent 1100 Series LC/MSD ion-trap mass spectrometer (ESI-MS) and an Agilent 6520B Q-TOF MS (HR-ESI-MS), respectively.



1 **2** **3**

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

2.2. 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.

2.3. Extraction and Isolation

The air-dried stem bark (10 kg) was extracted by refluxing with 95% ethanol three times. The EtOH extract was concentrated under reduced pressure (2000 g) and then extracted with $CHCl_3$ to give a chloroform part (300 g). The oily chloroform extract was dissolved in 2 L of 50% MeOH and H_2O and then extracted with petroleum ether. After removal of the fatty components, 210 g of extract was obtained, which was subjected to passage over a silica gel column eluted with $CHCl_3$-MeOH in a gradient (1:0 to 1:2), to afford eight fractions (Fr. A-H), monitored by TLC**.** 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. F4 (5g) was chromatographed on a column of reversed-phase C18 silica gel eluted with MeOH-H_2O (3:7 to 1:0) to give eight sub-fractions (Fr. F4a–h). Fr. F4c was separated by Sephadex LH-20 using CH_3OH as the mobile phase to give **1** (26 mg) (as the Figure 1 and 2). Fr. F4d was chromatographed on a silica gel column eluted with $CHCl_3$-MeOH in a gradient from 40:1 to 10:1 to give three sub-fractions (Fr. F4d1–d3). Fr. F4d3 was separated by preparative HPLC using ACN-H_2O (40:60, 10 mL/min) as the mobile phase and 210nm as the detection UV λ to give **2** (3 mg) and **3** (10 mg) (as the Figure 1).

Tabulalin K (**1**)White, amorphous powder; $[\alpha]^{25}{}_{D}$ -14.9 (c0.13,MeOH); IR (KBr) cm^{-1}: 3444, 1742, 1679, 1639, 1398, 1384, 1079, 1028; ^{1}H and ^{13}C NMR, see Table 1; negative ESIMS *m/z*: 693.5 $[M-H]^-$ (100); positive ESIMS *m/z*: 712.5 $[M+NH_4]^+$(100); HRESIMS *m/z*: 712.2813 ($[M+NH_4]^+$, $C_{33}H_{46}NO_{16}$; calc. 712.2811)

Tabulalin L (**2**)White, amorphous powder; $[\alpha]^{25}{}_{D}$ +8.2 (c0.09,MeOH); IR (KBr) cm^{-1}: 3450, 1747, 1638, 1384, 1080, 1027; ^{1}H and ^{13}C NMR, see Table 1; negative ESIMS *m/z*: 787.4$[M+Cl]^-$ (100); positive ESIMS *m/z*: 770.5 $[M+NH_4]^+$(100); HRESIMS *m/z*: 770.2869 ($[M+NH_4]^+$, $C_{35}H_{48}NO_{18}$; calc.770.2866)

Tabulalin M (**3**)White, amorphous powder; $[\alpha]^{25}{}_{D}$ +1.8 (c0.10,MeOH); IR (KBr) cm^{-1}: 3423, 1748, 1635, 1399, 1384, 1031;^{1}H and ^{13}C NMR, see Table 1; negative ESIMS *m/z*: 793.5 $[M-H]^-$ (100);

positive ESIMS *m/z*: 812.6 $[M+NH_4]^+$(100); HRESIMS *m/z*: 812.2973 ($[M+NH_4]^+$, $C_{37}H_{50}NO_{19}$; calc. 812.2972)

3. Results and Discussion

Tabulalin K (**1**)was obtained as white amorphous powder, which had a molecular formula $C_{33}H_{42}O_{16}$ as established by the HRESIMS ion at *m/z* 712.2813 ($[M+NH_4]^+$, $C_{33}H_{46}NO_{16}$; calc. 712.2811), indicating thirteen degrees of unsaturation. In 1D-NMR spectra of **1**, the presence of three characteristic olefinic proton signals at δ_H 6.54, 7.59, and 7.72, four olefinic carbons at δ_C 122.1, 110.0, 142.9, and 141.5 indicated that compound **1** possesses a β-substituted furan ring moiety. A pair of H-atoms with characteristic signals at δ_H 1.81 and 1.33 (d, J=10.5) assigned to a C-atom with a signal at δC 40.2 showed that the basic carbon skeleton of **1** was phragmalin-type limonoid, which was proved by their HMBC correlations to C-1 (δ_C 83.1), C-2(δ_C 75.3),C-3 (δ_C 85.2), C-4 (δ_C 44.0),C-5 (δ_C 38.2), and C-10 (δ_C 51.2).

In HMBC spectra of compound **1**, obvious correlations from H-17 (δ_H 6.03) to an acetyl CO group (δ_C 168.1) and H-30 (δ_H 5.17) to C-16 (δ_C 169.5) 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 [17]. Comparison of the NMR data and molecular formula suggested that **1** was a deacetyl derivative of tabulalin J [18].A significant upfield shift for H-3 (δ_H 3.27), when compared with tabulalin J, and the HMBC correlations from OH-3(δ_H 5.41) to C-2(δ_C 75.3) determined the position of the hydroxyl groups at C-3. The ROESY correlations from H-17 to H-30, H-11, and H-12, from H-30 to H-5 indicated that these H-atoms were β-oriented [7]. Correlations from H-14 to Me(18), from Me(19) to H_a-29, and from H-3 to H_b-29 revealed that these H-atoms were α-oriented. Hitherto, the planar structure of compound **1** was determined as shown in Figure 1.

Tabulalin L (**2**) was obtained as white amorphous powder, which had the molecular formula of $C_{35}H_{44}O_{18}$ deduced from its HR-ESI-MS (m/z 770.2869 ($[M+NH_4]^+$, $C_{35}H_{48}NO_{18}$; calc.770.2866)), indicating fourteen degrees of unsaturation. The 1D-NMR spectral features (Tables 1 and 2) and the key HMBC correlations from H-30 (δ_H 5.07) to C-16 (δ_C 169.3) indicated that **2** was also a phragmalin-type limonoid with a C(16)/C(30) δ-lactone ring as **1**, and that the difference was an additional AcO group signals. In the HMBC spectrum of **2**, a single H-atom signal at δ_H 5.14 was assigned to H-C(6) from its correlations with the quaternary C-atom signals at δ_C 42.8 (C-5), δ_C 169.6 (C-7), and δ_C 169.5 (AcO-C6), which also suggested that the location of the additional AcO group was C-6. The ROESY correlations from H-17 to H-30, H-11, and H-12, from H-30 to H-5 indicated that these H-atoms were β-oriented [7]. 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 β-orientation [19]. Correlations from H-14 to Me(18), from Me(18) to H-29, from Me(19) to H_a-29, and from H-3 to H_b-29 revealed that these H-atoms were α-oriented . Thus, the structure of **2** was established to be the 6-O-Ac derivative of **1**.

Tabulalin M (**3**) was obtained as white amorphous powder, which had the molecular formula of $C_{37}H_{46}O_{19}$ deduced from its HR-ESI-MS (m/z 812.2973 ($[M+NH_4]^+$, $C_{37}H_{50}NO_{19}$; calc. 812.2972)), indicating fifteen degrees of unsaturation. The 1D-NMR and 2D-NMR spectral features (Tables 1 and 2) showed that **3** was also a phragmalin-type limonoid with a C(16)/C(30) δ-lactone ring as **1** and **2**. Comparing the NMR data between **3** and **2** indicated that the former was an acetyl derivative of the later, which was confirmed by one more C_2H_2O unit from molecular formula. A singlet proton signal at δ_H 4.68 (H-3) showed HMBC correlations with carbon signals at δ_C 74.7 (C-2), δ_C 43.8 (C-4), δ_C 15.5 (C-28), and δ_C 169.8 (OAc-3), which suggested that C-3 was acetylated. The HMBC correlation from H-6 (δ_H 5.16), H-11 (δ_H 5.08), H-12 (δ_H 5.13), and H-17 (δ_H 5.91) to carboxyl carbon signals at δC169.5, 170.1, 169.3,and 168.8 suggested that the other four acetyl groups was located at OH-6, OH-11, OH-12, and OH-17, respectively.

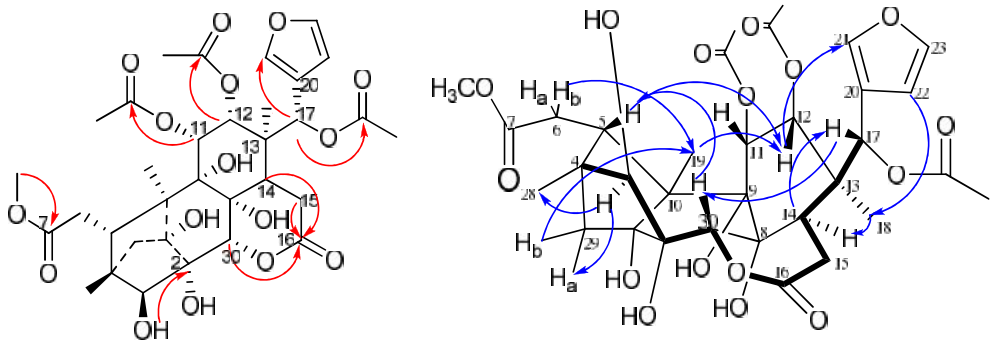


Figure 2. Key HMBC and ROESY correlations of **1**

Table 1. ^{1}H (500 MHz) and ^{13}C (125 MHz) NMR data of **1-3** in DMSO-*d*6.

No.	**1**		**2**		**3**	
	δ_H (*J* in Hz)	δ_C	δ_H (*J* in Hz)	δ_C	δ_H (*J* in Hz)	δ_C
1		83.1		82.8		82.9
2		75.3		75.1		74.7
3	3.27, d (5.0)	85.2	3.20, d (4.5)	85.3	4.68, s	86.2
4		44.0		43.8		43.8
5	2.54, br d (11.5)	38.2	2.80, br s	42.8	2.86, br s	43.6
6a	2.33,br d (18.0)					
6b	2.32,dd(18.0,11.5)	32.7	5.14, br s	70.9	5.16, br s	70.4
7		172.4		169.6		169.5
8		72.0		72.1		71.9
9		76.5		76.6		76.5
10		51.2		52.8		53.0
11	5.18, d (3.0)	70.9	5.16, d (3.5)	70.9	5.08, d (3.5)	70.5
12	5.02, d (3.0)	71.7	5.20, d (3.5)	71.6	5.13, d (3.5)	71.1
13		42.3		42.2		42.2
14	2.56,d (9.0)	40.1	2.64, d (9.0)	40.1	2.65, d (9.0)	40.2
15a	2.79,dd(18.0,9.0)	28.1	2.78,dd(18.0,9.0)	27.8	2.85,dd(18.0,9.0)	75.9
15b	2.73,d (18.0)		2.73,d (18.0)		2.76, d (18.0)	
16		169.5		169.3		168.7
17	6.03, s	70.6	6.01, s	70.5	5.91, s	70.1
18	0.94, s, 3H	19.1	0.94, s, 3H	19.2	1.01, s, 3H	18.6
19	1.09, s, 3H	15.8	1.15, s, 3H	14.5	1.18, s, 3H	14.3
20		122.1		122.3		121.7
21	7.72, br s	141.5	7.77, br s	141.4	7.78, br s	141.3

22	6.54, d (1.0)	110.0	6.60, d (1.0)	109.9	6.60, d (1.0)	109.7
23	7.59, t-like (1.5)	142.9	7.62, t-like (1.5)	142.9	7.65, t-like (1.5)	143.3
28	0.71, s, 3H	15.1	0.90, s, 3H	16.0	0.86, s, 3H	15.5
29a	1.81, d (10.5)		1.97, d (10.0)		2.11, d (11.0)	
29b	1.33, d (10.5)	40.2	1.42, d (10.0)	42.0	1.59, d (11.0)	41.7
30	5.17, s	73.3	5.07, s	73.2	4.92, s	74.3
OH-1	6.23, s		6.13, s		6.26, s	
OH-2	4.43, s		4.44, s		4.81, s	
OH-3	5.41, d (5.0)		5.56, d (4.5)			
OH-8	6.47, s		6.38, s		6.57, s	
OH-9	4.20, s		4.46, s		4.70, s	
OCH_3-7	3.56, s, 3H	51.9	3.66, s, 3H	52.3	3.72, s, 3H	52.7
OAc-3						169.8
					2.17,,s, 3H	20.4
OAc-6				169.5		169.5
			2.15, s, 3H	20.7	2.17, s, 3H	20.4
OAc-11		168.9		170.1		170.1
	1.86, s, 3H	20.5	2.01, s, 3H	20.4	1.98, s, 3H	20.5
OAc-12		170.1		169.1		169.3
	1.98, s, 3H	21.0	1.94, s, 3H	20.8	1.95, s, 3H	20.7
OAc-17		168.1		168.0		168.8
	1.95, s, 3H	20.7	1.96, s, 3H	20.8	1.96, s, 3H	20.9

Acknowledgments

This research work was supported by Program for Changjiang Scholars and Innovative Research Team in University (IRT1193), A Project Funded by the Priority Academic Program Development of Jiangsu Higher Education Institutions(PAPD), the National Natural Sciences Foundation of China (81202899), the youth fund project of basic research program of Jiangsu Province (Natural Science Foundation, BK2012351) and the Natural High Technology Research and Development Program of China (863 Program) (2013AA093001).

Supporting Information

Supporting Information accompanies this paper on http://www.acgpubs.org/RNP

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