Two New Phragmalin-Type Limonoids from *Chukrasia tabularis* var. *velutina*

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Abstract: Two new phragmalin-type limonoids with different structural skeletons, chuktabrin K (1) and tabulalin J (2), were isolated from the stem barks of *Chukrasia tabularis* var. *velutina* in the course of our ongoing research work in this area. Compound 1 was a 16-norphragmalin with an enolic alkyl appendage at C-15, and the carbonate moiety in 1 was also rare in natural organic molecules. The basic skeleton of compound 2 was a D-ring-opened phragmalin. Their structures were elucidated on HR-ESI-MS, 1H and 13C-NMR, HSQC, HMBC, and ROESY experiments.

Keywords: phragmalin-type limonoids; 16-norphragmalin; carbonate moiety; *Chukrasia tabularis* var. *velutina*

1. Introduction

The stem barks of plants of the genus *Chukrasia*, traditionally used in Southern China to treat cold and fever [1], have been a research focus for natural products chemistry in recent years, and a series of phragmalin-type limonoids with novel and diverse structures have been isolated [2–6]. In our previous research on limonoids from the stem barks of the title plant, many kinds of phragmalins with different skeletons were isolated, such as 16-norphragmalin with ketonic, enolic, or ketal alkyl appendages at C-15 [5,7–9], phragmalin orthoesters with enolic alkyl appendages at C-15 [10], phragmalin with an
unprecedented 8-oxatricyclo[4,3,11,6]decane moiety [11], and normal phragmalin and its orthoester derivative [12–16]. Further investigation on the phragmalin-type limonoids of this plant led to the isolation of two new phragmalin-type limonoids (Figure 1) with different structural skeletons. Chuktabrin K (1) was a rare 16-norphragmalin with enolic alkyl appendage at C-15, and the carbonate moiety in 1 is also rare in natural organic molecules. Tabulalin J (2) was a normal phragmalin with a C-16/C-30 δ-lactone ring. Their structures were elucidated on their extensive 1D and 2D spectroscopic analysis (HSQC, HMBC, and ROESY) and HR-ESI-MS. Herein, their isolation and structural elucidation are reported.

2. Results and Discussion

Chuktabrin K (1) was isolated as a white amorphous powder, and its molecular formula was determined as C₃₁H₃₆O₁₄ by its HRESIMS ion at m/z 667.1809 ([M+Cl]⁻, C₃₁H₃₆O₁₄Cl; calc. 667.1799). Characteristic 1D-NMR spectra, i.e., three upfield proton signals at δH 6.42, 7.59, and 7.68 and a set of double proton signals at δH 1.92 and 1.46 with an 11.0 Hz coupling constant in the ¹H-NMR (Table 1), and four olefinic carbons at δC 109.9, 121.5, 141.1, and 143.7 in the ¹³C-NMR (Table 1), indicated that compound 1 was a phragmalin-type limonoid possessing an α,β-substituted furan ring and a 4,29,1-bridge moiety [8]. The presence of a carbon signal at δC 92.4 showing a HSQC correlation with the proton signal at δH 4.67 and two down-field carbon signals at δC 152.0 and 152.8 suggested that compound 1 was a 16-norphragmalin with an enolic alkyl appendage at C-15 and a characteristic carbonate moiety like chuktabrins C-H [8]. The obvious HMBC correlations (Figure 2a) from a set of ethyl proton signals [δH 2.10, q, (7.5), 2H; δH 1.04, t (7.5), 3H] and proton signal of H-15 (δH 4.67) to a carbon signal at δC 152.8 (C-1’) indicated that a propionyl group was attached at C-15 biosynthetically as in the chuktabrins C-H [8].

Comparison of the NMR data between 1 and chuktabrin H indicated that the former was a deacetylated derivative of the latter, which was also confirmed from the molecular formula by the absence of one C₂H₂O unit. An obvious HMBC correlation (Figure 2a) from H-17 (δH 5.79) to the ¹³C signal for an acetoxy group (MeCOOR) at δC 167.9 suggested that the only acetyl group was located at OH-17. Hitherto, the planar structure of compound 1 was determined, except for the location of the carbonate moiety and the ether linkage of C-1’ due to a lack of direct HMBC evidence. Hydroxyl groups must be connected at C-1, C-2, C-3, C-11 and C-12 due to the observed correlations between the 1-OH signal (δH 5.35) to the ¹³C signal for C-1 at δC 82.3 and C-2 at δC 74.0; 2-OH (δH 4.23) to C-1 at δC 82.3, C-3...
at δC 85.7 and C-30 at δC 65.7; 3-OH (δH 5.92) to C-2 at δC 74.0, C-3 at δC 85.7 and C-4 at δC 43.1; 11-OH (δH 5.67) to C-11 at δC 67.3 and C-9 at δC 86.6; 12-OH (δH 5.14) to C-11 at δC 67.3, C-12 at δC 73.4 and C-13 at δC 43.9. The 1H signal for H-30 at δH 4.57 (δC 65.7) showed correlation to the 13C signal at δC 83.2 (C-8). Thus, these correlation required the presence of -OR substituent at C-8, C-9 and C-30. The 13C-NMR data for C-30, C-9 and C-8 were similar to those for chuktabrin C, which was determined by single-crystal X-ray diffraction [8], determining the position of enol ether at C-30 and the carbonate moiety at C-8 and C-9.

Figure 2. HMBC correlations of compound 1. (a) carbon skeleton (b) hydroxyl groups.

The key ROESY correlations (Figure 3), from H-5 to H-11, H-17, and H-21, H-17 to H-5, H-11, H-21, and H-30, Me-18 with H-14 and H-22, H-29a with H-3, and H-29b with H-19b, indicated that the relative stereochemistry of the key asymmetric carbons of 1 was well matched with those of chuktabrin C obtained by X-ray crystallography [8]. Thus, the structure of 1 was established as shown in Figure 1, namely as a 12-deacetyl derivate of chuktabrin H [8].

Figure 3. Key ROESY correlations of compound 1.
Table 1. $^1$H (500 MHz) and $^{13}$C (125 MHz) NMR data of 1 and 2 in DMSO-$d_6$.

| No. | $^1$H (multi, $J$ in Hz) | $^1$H (multi, $J$ in Hz) | $^1$H (multi, $J$ in Hz) | $^1$H (multi, $J$ in Hz) |
|-----|-------------------------|-------------------------|-------------------------|-------------------------|
| 1   | 82.3                    | 82.9                    |                        |                        |
| 2   | 74.0                    | 74.8                    |                        |                        |
| 3   | 3.35 (d 5.5)            | 85.7                    | 4.73 (s)                | 86.1                    |
| 4   | 43.1                    | 43.9                    |                        |                        |
| 5   | 2.44 (m)                | 36.7                    | 2.60 *                  | 39.2                    |
| 6a  | 2.38 (dd 16.0, 3.3)     | 29.6                    | 1.94 *                  | 31.9                    |
| 6b  | 2.94 (dd 16.0, 6.0)     |                        | 2.47 (dd 18.0, 11.0)    |                        |
| 7   |                        | 172.8                   |                        | 172.4                   |
| 8   | 83.2                    | 71.7                    |                        |                        |
| 9   | 86.6                    | 76.4                    |                        |                        |
| 10  | 48.1                    | 51.8                    |                        |                        |
| 11  | 3.88 (dd 3.0, 8.6)      | 67.3                    | 5.11 (d 3.0)            | 71.2                    |
| 12  | 3.17 (dd 3.0, 5.0)      | 73.4                    | 4.96 (d 3.0)            | 70.8                    |
| 13  |                        | 43.9                    |                        | 42.3                    |
| 14  | 2.52 (d 4.5)            | 43.4                    | 2.60 *                  | 40.3                    |
| 15a | 4.67 (d 4.5)            | 92.4                    | 2.76 (d 18.5)           | 27.8                    |
| 15b |                        | 2.88 (dd 18.5, 9.0)     |                        |                        |
| 16  |                        | 168.9                   |                        |                        |
| 17  | 5.79 (s)                | 67.5                    | 6.00 (s)                | 70.2                    |
| 18  | 1.29 (s, 3H)            | 17.1                    | 1.04 (s 3H)             | 18.4                    |
| 19a | 4.57 (d 12.5)           | 67.9                    | 1.19 (s 2H)             | 15.3                    |
| 19b | 5.27 (d 12.5)           |                        |                        |                        |
| 20  |                        | 121.5                   |                        | 121.4                   |
| 21  | 7.59 (s)                | 141.1                   | 7.75 (s)                | 141.7                   |
| 22  | 6.42 (s)                | 109.9                   | 6.57 (t 1.0)            | 109.7                   |
| 23  | 7.68 (s)                | 143.7                   | 7.65 (t 1.5)            | 143.2                   |
| 28  | 0.88 (s 3H)             | 14.9                    | 0.70 (s 3H)             | 14.6                    |
| 29a | 1.46 (d 11.0)           | 41.4                    | 1.51 (d 11.0)           | 40.8                    |
| 29b | 1.92 (d 11.0)           |                        | 1.98 (d 11.0)           |                        |
| 30  | 4.57 (s)                | 65.7                    | 4.98 (s)                | 74.4                    |
| 31  |                        | 152.0                   |                        |                        |
| 1'  |                        | 152.8                   |                        |                        |
| 2'  | 2.10 (q 7.5)            | 26.0                    |                        |                        |
| 3'  | 1.04 (t, 7.5)           | 10.9                    |                        |                        |
| 7-Ome|                        | 3.62 (s 3H)             |                        | 51.4                    |
| 1-OH | 5.35 (s)                |                        | 6.46 (s)                |                        |
| 2-OH | 4.23 (s)                |                        | 5.06 (s)                |                        |
| 3-OH | 5.92 (d5.5)             |                        |                        |                        |
| 8-OH |                        | 6.64 (s)                |                        |                        |
| 9-OH |                        | 4.42 (s)                |                        |                        |
| 11-OH | 5.67 (d 8.6)           |                        |                        |                        |
| 12-OH | 5.14 (d 5.0)           |                        |                        |                        |
| 3-OAc|                        | 170.0                   |                        |                        |
| 11-OAc|                        | 2.22 (s 3H)             | 20.4                    |
| 12-OAc|                        | 2.03 (s 3H)             | 20.5                    |
| 17-OAc|                        | 1.91 (s 3H)             | 20.4                    |
| 17-OAc|                        | 167.9                   | 168.6                   |

* Resonance pattern unclear due to overlapping.
Tabulalin J (2), was obtained as a white amorphous powder, and its molecular formula was established as C_{35}H_{44}O_{17} by the HRESIMS ion at \textit{m/z} 735.2516 ([M–H]−, C_{35}H_{43}O_{17}; calc. 735.2506). The similarity of the \textsuperscript{1}H and \textsuperscript{13}C-NMR spectroscopic data of 2 (Table 1) to those of tabulalin A, a phragmalin-type limonoid isolated in our previous research [13], indicated that these two molecules possessed the same carbon framework. Obvious HMBC correlations (Figure 4a) from H-17 (δ\textsubscript{H} 6.00) to the \textsuperscript{13}C signal for the acetoxy group (MeCOOR) at δ\textsubscript{C} 168.6 and H-30 (δ\textsubscript{H} 4.98) to C-16 (δ\textsubscript{C} 168.9) indicated that compound 2 possesses the same phragmalin skeleton with a C-16/C-30 δ-lactone ring as tabulalin A [13]. Comparison of the NMR data and molecular formula suggested that 2 was a bisacetyl derivative of tabulalin A [13]. A significant downfield shift for H-11 (δ\textsubscript{H} 5.11) and H-12 (δ\textsubscript{H} 4.96), when compared with tabulalin A, determined the position of the acetoxy groups at C-11 and C-12. In comparison with the \textsuperscript{13}C-NMR spectrum of the parent compound tabulalin A (C-9 δ\textsubscript{C} 79.3, C-11 δ\textsubscript{C} 67.7, C-12 δ\textsubscript{C} 76.0), the acetylation of hydroxyl groups at C-11 and C-12 resulted in 2.9 ppm upfield shifts for the C-9 and 5.2 ppm for C-12 resonances, and 3.5 ppm downfield shifts for C-11 position, confirming the acetoxy groups at C-11 and C-12. Thus, the planar structure of 2 was determined. The relative configuration of 2 was determined to be the same as tabulalide A [13] by its key ROESY correlations, such as from H-11 to H-5, H-12, H-17, and H-30, from H-17 to H-21 and H-30, from H-21 to H-12 and H-30, from H-30 to H-5, from H-3 to Me-28 and H-29a, H-29b to Me-19. Thus, the structure of 2 was demonstrated as 11,12-bisacetyl derivative of tabulalin A [13].

**Figure 4.** Key HMBC correlations of compound 2.

3. Experimental

3.1. General

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, (\textsuperscript{1}H: 500 MHz, \textsuperscript{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 Agilent UPLC (1290)-TOFMS (6520B) (HR-ESI-MS), respectively. All solvents used in column chromatography were analytical grade (Jiangsu Hanbang Science and Technology Co., Ltd., Huanan, China). Silica gel (Qingdao Haiyang Chemical Co., Ltd., Qingdao, China), Sephadex LH-20 (Pharmacia, Uppsala, Sweden), and RP-C\textsubscript{18} (40–63 μm, Fuji, Aichi, Japan) were used for column chromatography. Preparative HPLC was carried out using an Agilent 1100 Series instrument (Agilent Technologies,
Santa Clara, CA, USA) equipped with a Shim-park RP-C\textsubscript{18} column (20 × 200 mm) and a 1100 Series multiple wavelength detector.

3.2. 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.

3.3. Extraction and Isolation

The air-dried stem bark (10 kg) was extracted by refluxing with 95% ethanol (40 L) three times. The EtOH extract was concentrated under reduced pressure (2,000 g) and then extracted with CHCl\textsubscript{3} to give a chloroform extract (300 g). The oily chloroform extract was dissolved in 2 L MeOH/H\textsubscript{2}O (50:50, v/v) and then extracted with petroleum ether (6 L, 60–90 °C, ×3). After removal of the fatty components, 210 g of extract were obtained, which was subjected to silica gel column chromatography eluting with CHCl\textsubscript{3}/MeOH in a gradient from 1:0 to 1:2 to afford eight fractions (Fractions A–H). Fraction E (20 g) was chromatographed on a column of reversed-phase C\textsubscript{18} silica gel eluted with MeOH/H\textsubscript{2}O (4:6 to 7:3) to give six sub-fractions (Fractions E1–6). Fraction E6 (7 g) was chromatographed on a column of reversed-phase C\textsubscript{18} silica gel eluted with MeOH/H\textsubscript{2}O (2:3 to 7:3) to give four sub-fractions (Fractions E6a–d), Fraction E6c was separated by prep-HPLC using MeOH/H\textsubscript{2}O (55:45) as the mobile phase to give compound 2 (3 mg). Fraction 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 (Fractions F1–4). Fraction F3 was chromatographed on a column of reversed-phase C\textsubscript{18} silica gel eluted with MeOH/H\textsubscript{2}O (2:3 to 7:3) to give four sub-fractions (Fractions F3a–d). Fraction F2a was separated by preparative HPLC using CH\textsubscript{3}OH/H\textsubscript{2}O (52:48, 10 mL/min) as the mobile phase to give 1 (4 mg).

**Chuktabrin K** (1), White, amorphous powder; [\(\alpha\)]\textsubscript{D}\textsuperscript{25} +46 (c 0.10, CH\textsubscript{3}OH); IR (KBr) cm\textsuperscript{−1}: 3450, 2976, 1800, 1736, 1640, 1378, 1238, 1033; \textsuperscript{1}H and \textsuperscript{13}C-NMR, see Table 1; negative ESIMS \textit{m/z}: 667.3 [M+Cl]\textsuperscript{−} (100); positive ESIMS \textit{m/z}: 650.2 [M+NH\textsubscript{4}]\textsuperscript{+} (100); HRESIMS \textit{m/z}: 667.1809 ([M+Cl]\textsuperscript{−}, C\textsubscript{31}H\textsubscript{36}O\textsubscript{14}Cl; calc. 667.1799).

**Tabulalin J** (2), White, amorphous powder; [\(\alpha\)]\textsubscript{D}\textsuperscript{25} −23 (c 0.15, CH\textsubscript{3}OH); IR (KBr) cm\textsuperscript{−1}: 3450, 2973, 1740, 1640, 1463, 1376, 1242, 1169; \textsuperscript{1}H and \textsuperscript{13}C-NMR, see Table 1; negative ESIMS \textit{m/z}: 771.1 [M+Cl]\textsuperscript{−} (100); positive ESIMS \textit{m/z}: 754.3 [M+NH\textsubscript{4}]\textsuperscript{+} (100); HRESIMS \textit{m/z}: 735.2516 [M−H]\textsuperscript{−} (calcld: C\textsubscript{37}H\textsubscript{45}O\textsubscript{17}, 735.2506).

4. Conclusions

Two new phragmalin-type limonoids with different structure skeletons, chuktabrin K (1) with a 16-norphragmalin skeleton and tabulalin J (2) with a normal phragmalin skeleton, were isolated from
the stem barks of *Chukrasia tabularis* var. *velutina*. The complex and new structures of these two phragmalins represent new additions to the molecular diversity of natural organic limonoid molecules.

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*Sample Availability*: Samples of the compounds 1 and 2 are available from the authors.

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