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Chukrasones A and B: Potential Kv1.2 Potassium Channel Blockers with New Skeletons from *Chukrasia tabularis*

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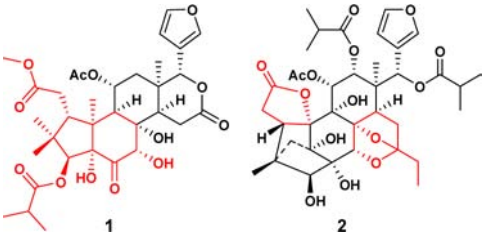
ABSTRACT



Two new limonoids, chukrasone A (1) incorporating a highly rearranged A/B ring system and chukrasone B (2) possessing the first 16,19-dinor limonoid backbone with an extended C3 unit at C-15, were isolated from *Chukrasia tabularis*. Their structures were characterized on the basis of detailed spectroscopic analysis. Compounds 1 and 2 exhibited potential inhibition of the delayed rectifier (I_K) K^+ current.

Plants of the Meliaceae family, which are rich sources of limonoids with both fascinating structures and potential bioactivities, have been studied comprehensively in the past half century. *Chukrasia tabularis* A. Juss, one of the only two Chinese species of the genus *Chukrasia*,[1] has proved its ability to produce a variety of phragmalin-type limonoids.[2] Our previous chemical investigation of the stem bark of *C. tabularis* led to the isolation and elucidation of a series of phragmalin-type limonoids that showed significant inhibition toward the delayed rectifier (I_K) K^+ current.[3] As a result of our continuing search for structurally interesting metabolites from this plant, two additional limonoids, chukrasones A (**1**) and B (**2**) with unprecedented carbon skeletons, were further isolated and elucidated. Chukrasone A (**1**) represents a unique rearranged scaffold which could be originated from the mexicanolide-type limonoid,[4] while chukrasone B (**2**) incorporates the first 16,19-dinor limonoid backbone featuring a biosynthetically extended unusual 2,7-dioxabicyclo[2.2.1]heptane motif. We present herein the isolation and structural characterization of the two new limonoids as well as their blocking activities against the Kv1.2 potassium channel.

A further chemical investigation into the EtOH extract generated from the stem bark of *C. tabularis*, using solvent partitioning, normal- and reversed-phase column chromatography, and semipreparative HPLC, yielded chukrasones A (**1**) and B (**2**), respectively.

Chukrasone A (**1**),[5] a white amorphous powder, was assigned a molecular formula of $C_{33}H_{44}O_{13}$ as determined by HR-ESI(+)MS displaying a *quasi* molecular ion at *m/z* 671.2699 $[M + Na]^+$ (calcd for $C_{33}H_{44}O_{13}Na$, 671.2680) with 12 degrees of unsaturation. The IR spectrum showed the presence of hydroxyl (3446 cm^{-1}) and carbonyl (1741 cm^{-1}) functionalities. Analysis of the NMR data (Table 1) for **1** revealed resonances for 33 carbons including eight methyls (one oxygenated), three methylenes, eleven methines (four oxygenated and three olefinic), and eleven

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(2) Kaur, R.; Arora, S. *J. Med. Plants Res.* **2009**, *3*, 196–216.
(3) Liu, H.-B.; Zhang, H.; Li, P.; Wu, Y.; Gao, Z.-B.; Yue, J.-M. *Org. Biomol. Chem.* **2012**, *10*, 1448–1458.
(4) Tan, Q. G.; Luo, X. D. *Chem. Rev.* **2011**, *111*, 7437–7255.

(5) White amorphous powder; $[\alpha]^{20}_D$ −112 (*c* 0.11, $CHCl_3$); UV (MeOH) λ_{max} (log ε) 207 (3.52) nm; IR (KBr) ν_{max} 3446, 2974, 1741, 1230, 1170, 1030, 878, 615 cm^{-1}; for ^1H and ^{13}C NMR data, see Table 1; ESI(+)MS *m/z* 671.3 $[M + Na]^+$, 1297.4 $[2\ M + H]^+$; HR-ESI(+)MS *m/z* 671.2699 $[M + Na]^+$ (calcd for $C_{33}H_{44}O_{13}Na$, 671.2680).

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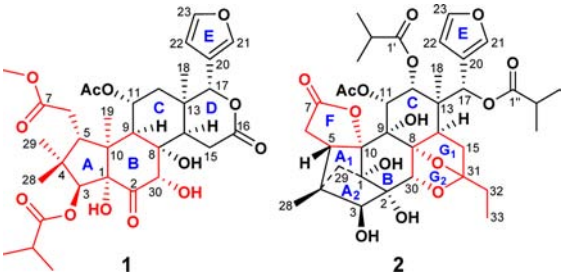
quaternary carbons (five carbonyls, one olefinic, and five sp^3). In particular, one methoxyl (δ_H 3.71 and δ_C 52.8), one acetyl (δ_H 2.07; δ_C 21.3 and 170.2), one isobutyryl (δ_H 1.25, 1.29, and 2.71; δ_C 18.5, 19.3, 34.2, and 175.4), and a β-substituted furan (δ_H 6.56, 7.39, and 7.63; δ_C 110.8, 119.9, 142.3, and 142.6) were also distinguished (Table 1). Three exchangeable signals (δ_H 2.78, 2.88, and 4.27) belonging to the hydroxyls were supported by the HSQC experiment. The above observations accounted for 8 out of 12 degrees of unsaturation suggestive of a limonoid analogue with four additional rings for **1**.



The $^1H-^1H$ COSY and HSQC experiments further established six structural fragments **a** (C-5 to C-6), **b** (C-9 to C-12 via C-11), **c** (C-14 to C-15), **d** (CH-30−OH), **e** (C-22 to C-23 of the furan), and **f** (C-3′/4′ to C-2′ of the isobutyryl) as drawn in bold bonds (Figure 1A). On the basis of the existence of fragments **a**, **b**, and **d**, and the HMBC cross-peaks (Figure 1A) of H_3-19/C-1, C-5, C-9, and C-10; H_3-28 (29)/C-3, C-4, and C-5; H-3/C-1 and C-2; OH-1/C-1, C-2, and C-10; H-30/C-2; OH-30/C-8 and C-30; and OH-8/C-8, C-9, and C-14, the rings A and B were established in **1**. In addition, the multiple HMBC correlations of H_3-18/C-12, C-13, C-14, and C-17; H-17/C-16; and H-15/C-13 and C-16 established rings C and D as shown. Furthermore, the HMBC correlations from H-17 (δ_C 5.41, s) to C-20 (δ_C 119.9) and C-22 (δ_C 110.8) revealed that the β-furyl group was attached to C-17 (δ_C 77.0). The presence of a 3-isobutyryloxyl, 6-methoxycarbonyl, and 11-acetoxyl were also confirmed by HMBC correlations of H-3/C-1′, H_2-6 and CH_3O/C-7, and H-11/CH_3CO, respectively. Three proton resonances assignable to their corresponding OH groups displayed HMBC correlations from 1-OH (δ_H 4.27, s) to C-1, C-2, and C-10, from 8-OH (δ_H 2.78, s) to C-8, C-9, and C-14, and from 30-OH (δ_H 2.88, d, J = 10.9 Hz) to C-8 and C-30, respectively. The planar structure of **1** was thereby defined to have a new carbon scaffold that likely derived from a mexicanolide-type limonoid.[4]

The relative configuration of **1** was elucidated by the examination of its ROESY data (Figure 1B). H-15β (δ_H 3.51) showed strong ROESY correlations with both H-11 (δ_H 5.06) and H-17 (δ_H 5.41) suggesting that both C and D rings adopted a boat-like conformation, and H-11, H-15β, and H-17 were assigned a β-configuration consistent with that of H-17 in all limonids with the δ-lactone D ring.[4] A 1,3 diaxial relationship for H-11 and CH-17 within the C ring was revealed by the strong ROESY correlation between them, thus leaving Me-18 equatorially located, while a 1,2 diaxial relationship for both H-9/H-11 and H-14/H-15β was indicated by the magnitude of $J_{9,11}$ (11.8 Hz) and $J_{14,15\beta}$ (15.4 Hz), respectively, suggestive of an α-orientaion for both H-9 (δ_H 2.35) and H-14 (δ_H 2.11). ROESY correlation of H-30/H-15α suggested that H-30 (δ_H 5.24) and CH_2-15 were on the same side (a *pseudo* 1,3 diaxial relationship) of the C/D ring hence resulting in 8-OH being oppositely directed and α-configured, as further confirmed by the strong correlation of H-9/8-OH. Similarly, the correlation of H-11/H-5 was supportive of H-11 and CH-5 being on the same side of the B/C ring thus requiring CH_3-19 to be on the other side and α-oriented. Further observation of ROESY cross-peaks of H_3-19/H-6b and H_3-19/1-OH indicated the α-configuration for the C6−C7 fragment and 1-OH. Finally, H-3 was assigned to be α-configured on the basis of the following observations: H-5 displayed a strong correlaion with H_3-28 but no correlation with H_3-29 suggesting that both H-5 and CH_3-29 were *quasi*-axially bonded and were on the other side of ring A, while H-3 showed ROESY correlations to both H_3-29 and H_3-28 with the former being much stronger than the latter, indicating that H-3 was cofacial with H_3-29. The relative stereochemistry of **1** was thus fully characterized.

Chukrasone B (**2**)[6] was obtained as a white amorphous powder. The HR-ESI(+)MS analysis revealed a *quasi* molecular ion at m/z 755.2906 $[M + Na]^+$ (calcd for $C_{37}H_{48}O_{15}Na$, 755.2891) consistent with a molecular formula of $C_{37}H_{48}O_{15}$ requiring 14 degrees of unsaturation.

Analysis of the NMR data (Table 1) for **2** revealed resonances for 37 carbons including eight methyls, four methylenes, twelve methines (five oxygenated and three olefinic), and thirteen quaternary carbons (four carbonyls, one olefinic, and eight sp^3). In particular, one ketal (δ_C 112.7), one acetyl (δ_C 20.5 and 169.3), one ethyl (δ_C 8.0 and 26.1), two isobutyryls, and a β-substituted furan were also clearly resolved (Table 1). Four hydroxyl protons (δ_H 3.47, 3.53, 3.55, and 4.27) were distinguished from the other hydrogens by the HSQC experiment. The aforementioned discussion accounted for 7 out of 14 degrees of unsaturation suggestive of the presence of seven additional rings in **2** and indicative of a limonoid analogue.

Comprehensive analysis of the 2D NMR data of **2**, especially HMBC correlations (Figure 2A), established that **2** was the first 16,19-dinor-limonoid, i.e. a very rare hexanortriterpenoid. Detailed analysis of the NMR data of **2** further revealed that the A, B, C, and E rings of a phragmalin-type limonoid were kept intact; the typical D ring of a δ-lactone for phragmalin limonoids was missing, and in particular, a biosynthetically extended C3 unit at C-15 forming an unusual 2,7-dioxabicyclo[2.2.1]heptane moiety[7] was present, which was confirmed by the HMBC correlations of H-30, H_2-15, and H_3-33 to C-31 as well as the deshielded chemical shift of C-8 (δ_C 88.4). The HMBC correlations from H-6a and H_2-29 to the oxygenated and remarkably deshielded C-10 (δ_C 91.2) confirmed the loss of C-19 and the formation

(6) White amorphous powder; $[\alpha]^{20}_D$ +200 (c 0.015, $CHCl_3$); UV (MeOH) λ_{max} (log ε) 206 (3.68) nm; IR (KBr) ν_{max} 3444, 2976, 1786, 1740, 1232, 1198, 1148, 1049, 1028, 930, 602 cm^{-1}; for ^1H and ^{13}C NMR data, see Table 1; ESI(+)MS m/z 755.3 $[M + Na]^+$, 1487.6 $[2\,M + Na]^+$; HR-ESI(+)MS m/z 755.2906 $[M + Na]^+$ (calcd for $C_{37}H_{48}O_{15}Na$, 755.2891).

(7) Zhang, C.-R.; Yang, S.-P.; Liao, S.-G.; Fan, C.-Q.; Wu, Y.; Yue, J.-M. *Org. Lett.* **2007**, *9*, 3383–3386.

Table 1. ^{1}H and ^{13}C NMR spectrosopic data of **1** and **2** (in $CDCl_3$)[a]

	1		**2**	
no.	δ_H (multi, J in Hz)	δ_C	δ_H (multi, J in Hz)	δ_C
1		88.7		85.0
2		204.3		73.2
3	4.99 (*s*)	80.1	3.84 (*d*, 5.2)	85.7
4		43.0		42.9
5	2.69 (*m*)[b]	39.1	2.95 (*br d*, 9.3)	43.1
6	a 2.37 (*dd*, 18.6, 6.6)		a 2.50 (*dd*, 19.4, 3.6)	
	b 2.22 (*br d*, 18.6)	31.2	b 2.36 (*dd*, 19.4, 9.3)[c]	30.9
7		174.5		175.3
8		82.5		88.4
9	2.35 (*d*, 11.8)	67.8		73.4
10		54.9		91.2
11	5.06 (*ddd*, 11.8, 11.8, 2.4)	68.2	5.46 (*d*, 3.6)	72.1
12	α 1.58 (*dd*, 13.3, 11.8)	38.6	5.19 (*d*, 3.6)	71.0
	β 1.84 (*dd*, 13.3, 2.4)			
13		38.6		41.9
14	2.11 (*dd*, 15.4, 5.1)	43.5	3.19 (*dd*, 11.4, 8.9)	44.4
15	α 2.71 (*m*)[b]		α 1.99 (*m*)[d]	
	β 3.51 (*dd*, 15.4, 14.2)	32.0	*β* 2.36 (*d*, 8.9)[c]	33.9
16		172.6		
17	5.41 (*s*)	77.0	6.38 (*s*)	70.7
18	1.07 (*s*, 3H)	25.6	1.08 (*s*, 3H)	17.3
19	1.09 (*s*, 3H)	19.3		
20		119.9		120.9
21	7.63 (*br s*)	142.3	7.64 (*br s*)	142.0
22	6.56 (*dd*, 1.7, 0.7)	110.8	6.45 (*br s*)	109.5
23	7.39 (*br t*, 1.7)	142.6	7.38 (*br s*)	143.3
28	0.88 (*s*, 3H)	24.9	1.08 (*s*, 3H)	15.7
29	0.92 (*s*, 3H)	21.5	a 1.93 (*dd*, 11.6, 1.6)	37.2
			b 1.56 (*d*, 11.6)	
30	5.24 (*d*, 10.9)	74.1	4.30 (*s*)	70.5
31				112.7
32			2.04 (*m*)[d]	26.1
33			1.18 (*t*, 7.5, 3H)	8.0
7-OMe	3.71 (s, 3H)	52.8		
11-OAc		170.2		169.3
	2.07 (*s*, 3H)	21.3	2.02 (*s*, 3H)	20.5
hydroxyl	1-OH, 4.27 (*s*); 8-OH, 2.78 (*s*)		1-OH, 4.27 (*s*); 2-OH, 3.55 (*s*)	
	30-OH, 2.88 (*d*, 10.9)		3-OH, 3.47 (*d*, 5.2); 9-OH, 3.53 (*s*)	
1′		175.4		176.4
2′	2.71 (*m*)[b]	34.2	2.57 (*m*)	34.0
3′	1.29 (*d*, 7.0, 3H)	19.3	1.15 (*d*, 7.1, 3H)	19.0
4′	1.25 (*d*, 7.0, 3H)	18.5	1.12 (*d*, 6.8, 3H)	19.0
1″				176.8
2″			2.59 (*m*)	34.5
3″			1.18 (*d*, 7.1, 3H)	18.9
4″			1.18 (*d*, 7.1, 3H)	18.5

[a] Data were measured in $CDCl_3$ at 400 MHz (^{1}H) and 100 MHz (^{13}C); Chemical shifts (δ) are in ppm relative to TMS. [b−d] Overlapping signals within the same column.

of the five-membered lactonyl F ring. The degrees of unsaturation of **2** and the intense $\nu_{C=O}$ absorption bond at 1786 cm^{-1} in the IR spectrum further supported the presence of a 7,10-γ-lactone. The β-substituted furyl ring E was attached to C-17 by the HMBC correlations from H-17 to C-20, C-21, and C-22. Two isobutyryloxyls were assigned to C-12 (δ_C 71.0) and C-17 (δ_C 70.7) by the HMBC correlations from H-12 (δ_H 5.19) and H-17 (δ_H 6.38) to C-1′ and C-1″, respectively, while the acetoxyl was attached to C-11 according to the HMBC correlation from H-11 (δ_H 5.46) to the acetyl carbonyl. Three hydroxyls were assigned to C-1 (δ_C 85.0), C-2 (δ_C 73.2), and C-9 (δ_C 73.4) on the basis of the HMBC correlations from 1-OH to C-1 and C-29, from 2-OH to C-1, C-2, and C-3, and from 9-OH to C-8 and C-9, respectively. Notwithstanding the absence of HMBC correlations from the remaining OH to any carbons, it was assigned to C-3 as determined by its coupling to H-3 (δ_H 3.84, d, J = 5.2 Hz). The planar structure of **2** was thus elucidated.

The relative configuration of **2** was established on the basis of a detailed examination of the ROESY data (Figure 2B) in

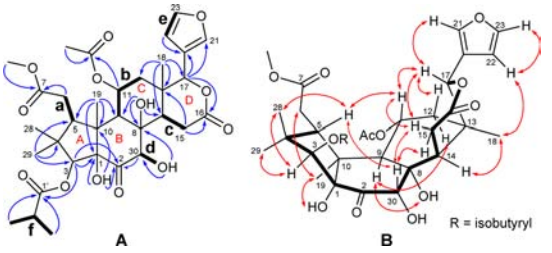


Figure 1. $^{1}H-^{1}H$ COSY (**A**: bold —), selected HMBC (**A**: →), and ROESY (**B**: ↔) correlations of **1**.

which significant correlations of H-5/H-11, H-11/H-17, H-17/H-30, and H-30/3-OH, H-11 and H-15β across the whole molecule suggested a highly bending and pocket-shaped conformation for **2** as shown, and these protons were assigned to be β-oriented following the configuration of H-17 in **1**. Consequently, it was very much self-evident that the C-1−C-29 carbon bridge, F ring (C-6−C-7−O frament), 9-OH, 11-acetoxyl, CH_3-18, H-14, G_2 ring, and 2-OH were α-positioned, which was further supported by ROESY correlations of H-29a/H-3, 2-OH/H-3 and 1-OH, H-14/H-15α and H_3-18, and H-6a/H-29b. H-12 was finally determined to be equatorially located and thus β-configured based on the magnitude of $J_{11,12}$ (3.6 Hz), since H-11 showing marked ROESY correlation with H-17 (1,3 diaxial relationship for H-11 and CH-17) was axially positioned. The relative stereochemistry of compound **2** was then unambiguously characterized.

The structures of compounds **1** and **2** were computer-modeled using ChemDraw Ultra 9.0, and MM2 calculation was used for energy minimization. The modeling results match quite well with the key correlations in their ROESY spectra (Supporting Information Figure S17 and Table S1).

Plausible biogenetic pathways to compounds **1** and **2** are proposed as shown in Schemes 1 and 2. The biosynthetic precursor of **1** could be traced back to a proposed mexicanolide-type limonoid **1a**. After protonation, **1a** would undergo Pinacol rearrangement to give the key intermediate **1b** which would readily produce **1** by loss of a proton (Scheme 1).

Compound **2** might derive from precursor **2a** of the abundant phragmalin-type limonoids of *Chukrasia* genus.[2,3]

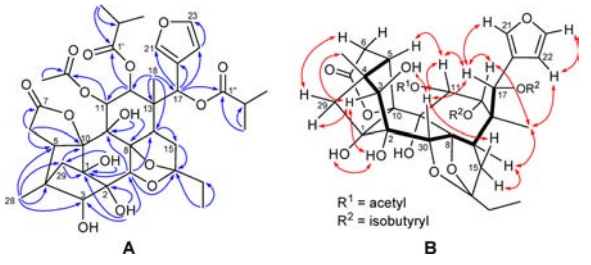
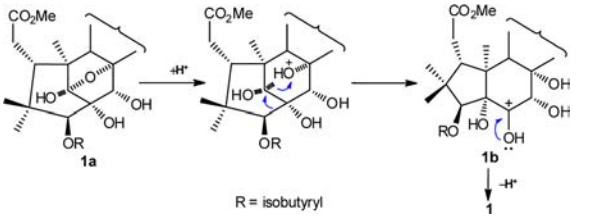
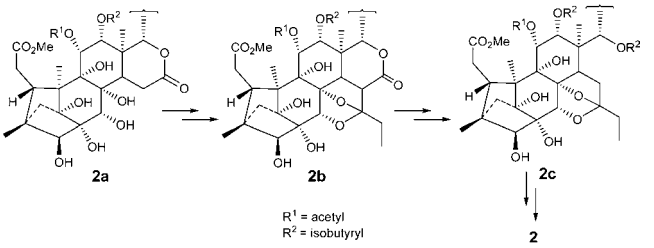


Figure 2. Selected HMBC (**A**: →) and ROESY (**B**: ↔) correlations of **2**.

Scheme 1. Plausible Biogenetic Origin of **1**



Scheme 2. Plausible Biogenetic Origin of **2**



The introduction of a C3 unit to C-15 of **2a** by incorporating a propionyl-CoA was followed by ketal formation yielding intermediate **2b**[7] which after ester hydrolysis and decarboxylation would yield the crucial intermediate **2c**. Then the Me-19 of **2c** could undergo a cascade of oxidation, degradation, and the final formation of the lactonyl ring F to produce **2** (Scheme 2).

Compounds **1** and **2** were tested on the voltage-gated potassium (Kv1.2) channel using whole-cell voltage-clamp recording in Chinese hamster ovary cells, and 4-aminopyridine was used as the positive control. The assay established that **1** and **2** displayed inhibitory rates of 0.49 ± 0.07 and 0.38 ± 0.03, respectively, at the concentration 30 μM (Supporting Information Figure S16).

In conclusion, our intensive chemical study on *C. tabularis* identified chukrasones A (**1**) and B (**2**) with new carbon skeletons and revealed the robust power of this plant in producing structurally unique and diverse limonoids. The proposed biosynthetic routes for **1** and **2** suggest new avenues of chemical transformation for this versatile class.

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Supporting Information Available. Experimental Section; IR, LR and HR-ESIMS, and 1D and 2D NMR spectra as well as computer modeling data for chukrasones A (**1**) and B (**2**) were included. This material is available free of charge via the Internet at http://pubs.acs.org.

The authors declare no competing financial interest.