ORGANIC LETTERS

2013
Vol. 15, No. 15
3902–3905

Chukfuransins A–D, Four New Phragmalin Limonoids with β-Furan Ring Involved in Skeleton Reconstruction from *Chukrasia tabularis*

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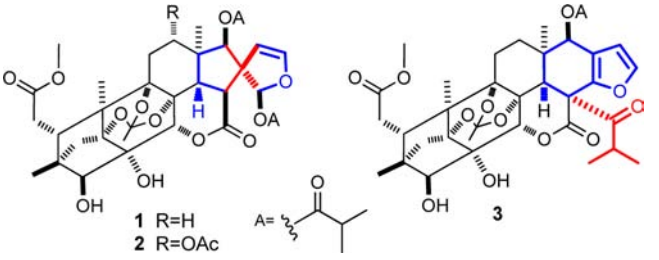
Received June 12, 2013

ABSTRACT



Four new phragmalin limonoids (chukfuransins A–D) were isolated from the twigs and leaves of *Chukrasia tabularis*. Chukfuransins A (1) and B (2) feature a unique C-15/C-20 linkage proposed to be built by a biogenetic pathway involving Michael addition. Chukfuransins C (3) and D (4) feature the C-15/C-21 linkage. Their structures and absolute configurations were established by NMR techniques and X-ray crystallographic analysis.

Phragmalin limonoids are important members of the enormous limonoid family. Since the chemical structure of phragmalin was elucidated in the 1970s,[1] ever-increasing analogues have been derived from many Meliaceae species.[2] Phragmalin limonoids possess characteristic rings of A and B tricyclo[3.3.1^{2,10}.1^{1,4}]decane or tricyclo-[4.2.1^{10,30}.1^{1,4}]decane. The unique bridging framework has motivated researchers to seek synthetic routes by which to attain it.[3] Furthermore, bioassays conducted on these structurally distinctive molecules have demonstrated them to have antifeedent,[4] anti-inflammatory,[5] potassium channel blocking,[6] and antibacterial[7] activities. Genus *Chukrasia* (Meliaceae), comprising only *C. tabularis* and *C. tabularis* var. *velutina*,[8] is a primary source of phragmalin limonoids with nearly 140 possessing miscellaneous skeletons having been reported. Except the common phragmalin limonoids[9] from which they were derived, dominant skeleton types including 16-nor type,[10] 15 enolic acyl 16-nor type,[11] 13/14/18 cyclopropanyl type,[12] and 15-enolic acyl type[13] were all biogenetically formed by alterations confined to D rings compared to the common type. In addition, tabuvelutin A[14] and chukrasone B[6] were the only two cases with C-19 degraded. Chuktabrin B[11]

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10.1021/ol401650m
Published on Web 07/23/2013

represented the only case with the E-ring (furan ring) involved in skeleton reconstruction, featuring C-15/C-21 linkage and an extended C2 unit at C-15. Our research on twigs and leaves of *C. tabularis* afforded chukfuransins A (**1**) and B (**2**) characterized by a C-15/C-20 linkage and the resulting unique 2-oxaspiro[4.4]non-3-ene fragment (blue part). Chukfuransins C (**3**) and D (**4**) featuring C-15/C-21 bonding but bearing C-15 extended C4 unit were also attained (Figure 1). The skeletons of compounds **1** and **3** were both confirmed by X-ray diffraction analysis.

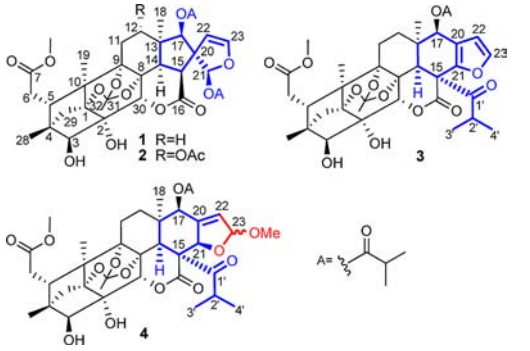

Figure 1. Structures of **1**–**4**.

Chukfuransin A (**1**),[15] colorless crystals, possessed the molecular formula $C_{37}H_{48}O_{14}$ (calcd 716.3044) as determined by HREIMS ([M]$^+$, *m*/*z* 716.3008). ^{13}C DEPT data classified all carbons into 13 quaternary carbons (four ester carbonyl groups, five oxygenated), 11 methines (four oxygenated, two olefinic ones), four methylenes, and nine methyls (Table 1). In addition, one methoxyl [δ_H 3.63 (s); δ_C 51.91 (q)], two isobutyryloxyls [δ_H 1.19 (d), 1.22 (d), 3.11 (m); δ_C 18.14 (q), 20.24 (q), 33.29 (d), 176.05 (s) and δ_H 1.23, 1.23, 2.70 (m); δ_C 18.52 (q), 18.79 (q), 34.03 (d), 175.47 (s)], and an orthoacetate [δ_H 1.62 (s); δ_C 21.19 (q), 118.65 (s)] can be distinguished by analysis of NMR data (Figure 2). The remaining 26 carbons constituting the framework of compound **1** indicated it was a tetranortriterpenoid.

The phragmalin limonoid origin of **1** can be determined since a typical A and B tricyclo[3.3.1^{2,10}.1^{1,4}]decane ring system can be identified through elaborative analysis of the intricate spectroscopic data. Further comparison of these

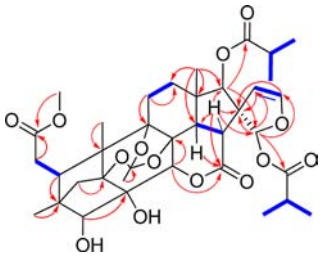

Figure 2. Key HMBC (red arrows) and ^{1}H–^{1}H COSY correlations (blue bonds) of **1**.

data with velutinalide A[16] revealed that they shared the same structure of A, B, C rings and C-6–C-7 side chain, as well as the 1,8,9-substituted pattern of the orthoacetate. Three carbons at δ_C 46.89 (s), 51.32 (d), 84.77 (d) correlating with H_3-18 in the HMBC spectrum were recognized as C-13, C-14, and C-17, respectively. A methine proton at δ_H 3.91 (d) correlating with C-14 in HMBC spectrum as well as with H-14 in COSY spectrum was assigned to be H-15. Through HMBC spectrum, the carbonyl carbon (δ_C 168.98) correlated with both H-14 and H-15 was assigned as C-16 and its correlation with H-30 indicated the existence of a 16, 30-δ-lactone ring in **1**. Apart from the above determined part, only one quaternary carbon at δ_C 65.83 (C-20), one acetal carbon at δ_C 99.40 (C-21) and a pair of olefinic carbons at δ_C 103.07 (C-22) and δ_C 145.19 (C-23) remained undetermined. These four carbon signals could be attributed to a 2*H*-furanyl group attached to C-17 by HMBC correlations from H-21, H-22, H-23 to C-20 and from H-17 to C-21. Furthermore, the quaternary carbon at C-20 gave us the hint of C-15/C-20 linkage, which was further proved by the HMBC correlations of H-15 with C-17, C-20, and C-21. Accordingly, the formation of a unique 2-oxaspiro[4.4]non-3-ene moiety was determined. Two isobutyryloxyls were located at C-17 and C-21 according to HMBC correlations of H-17/δ_C 176.05 and H-21/δ_C 175.47. H-14/H-15, H-15/H-17, and H-17/H-21 correlations in ROESY spectrum showed that these protons were all cofacial.

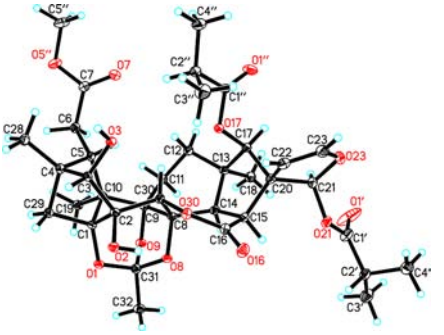

Figure 3. X-ray crystallographic structure of **1**.

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(15) Chukfuransin A (**1**): colorless crystals; mp 262–264 °C; $[\alpha]^{20}_D$ −78 (*c* 0.110, $CHCl_3$); UV ($CHCl_3$) λ_{max} (log ε) 240 (2.95) nm; IR (KBr) ν_{max} 3432, 1739, 1633, 1139 cm^{-1}; for ^{1}H and ^{13}C DEPT data, see Table 1; ESI(+)MS *m*/*z* 739 [M + Na]$^+$; HREIMS *m*/*z* 716.3008 [*M*]$^+$ (calcd for $C_{37}H_{48}O_{14}$, 716.3044).
(16) Yang, W.; Kong, L.; Zhang, Y.; Tang, G.; Zhu, F.; Li, S.; Guo, L.; Cheng, Y.; Hao, X.; He, H. *Planta Med.* **2012**, *78*, 1676.

Table 1. ^{1}H NMR and ^{13}C DEPT data for **1** and **2** in $CDCl_3$ (δ in ppm)[a]

	1		**2**	
no.	δ_H (mult, J in Hz)	δ_C	δ_H (mult, J in Hz)	δ_C
1		84.90 s		85.00 s
2		78.25 s		78.71 s
3	3.68 (s)	83.61 d	3.70 (s)	84.67 d
4		45.62 s		45.72 s
5	2.75 (dd, 9.5, 3.6)	35.12 d	2.76 (d, 11.1)	34.80 d
6a	2.24 (dd, 16.3, 3.6)	33.64 t	2.41 (dd, 18.0, 11.1)	32.98 t
6b	2.43 (dd, 16.3, 9.5)		2.54 (d, 18.0)	
7		172.89 s		173.96 s
8		81.33 s		80.77 s
9		85.08 s		85.74 s
10		45.62 s		45.63 s
11a	1.35 (m)	25.57 t	1.90 (m)	32.37 t
11b	1.62[b]		2.17 (br. d, 11.9)	
12a	1.62[b]	26.36 t	4.96 (br. d, 11.9)	66.89 d
12b	2.12 (m)			
13		46.89 s		50.10 s
14	2.11 (d, 10.6)	51.32 d	2.36 (d, 10.6)	53.29 d
15	3.91 (d, 10.6)	44.10 d	3.93 (d, 10.6)	44.14 d
16		168.98 s		168.36 s
17	5.17 (s)	84.77 d	5.20 (s)	82.53 d
18	1.23[b]	27.17 q	1.29 (s)	22.07 q
19	1.17 (s)	15.95 q	1.13 (s)	15.99 q
20		65.83 s		65.94 s
21	6.61 (s)	99.40 d	6.57 (s)	99.54 d
22	4.84 (d, 2.9)	103.07 d	4.85 (d, 2.9)	103.08 d
23	6.40 (d, 2.9)	145.19 d	6.40 (d, 2.9)	145.60 d
28	0.99 (s)	14.64 q	1.02 (s)	14.42 q
29a	1.66 (d, 10.7)	39.68 t	1.71 (d, 10.7),	40.46 t
29b	1.85 (d, 10.7)		1.85 (d, 10.7)	
30	5.70 (s)	74.26 d	5.73 (s)	74.12 d
31		118.65 s		119.26 s
32	1.62 (s)	21.19 q	1.63 (s)	21.32 q
7-OMe	3.63 (s)	51.91 q	3.68 (s)	52.09 q
17-isobutyryloxy		176.05 s		175.77 s
	3.11 (m)	33.29 d	2.71 (m)	34.26 d
	1.19 (d)	18.14 q	1.25[b]	18.75 q
	1.22[b]	20.24 q	1.25[b]	19.00 q
21- isobutyryloxy		175.47 s		175.82 s
	2.70 (m)	34.03 d	3.01 (m)	33.59 d
	1.23[b]	18.52 q	1.07 (d, 6.6)	18.12 q
	1.23[b]	18.79 q	1.23 (d, 6.6)	20.22 q
12-OAc				169.97 s
			1.96 (s)	21.11 q

[a] Recorded on Bruker AV-600 spectrometer. [b] Overlapped peaks.

Finally, suitable crystals of **1** for an X-ray diffraction experiment were obtained,[17] and the complete structure and stereochemistry were established (Figure. 3). The final refinement on the Cu Kα data resulted in a small Flack parameter of 0.05(10), allowing unambiguous assignment of the absolute configuration of **1** as 14*R*,15*S*,17*R*, 20*R*,21*S*.

Chukfuransin B (**2**),[18] white amorphous powder, has the molecular formula $C_{39}H_{50}O_{16}$ (calcd 774.3099) as revealed by HREIMS ($[M]^+$, m/z 774.3096). The 1D NMR data of **2** exhibited high similarity to those of **1**, except for the presence of an additional acetoxyl group in **2**. A methine at δ_C 66.89 correlating with H_3-18 in HMBC spectrum was recognized as C-12 and the weak crosspeak of H-12/δ_C 169.97 (s) showed that the extra acetoxyl was attached to C-12. The crosspeaks of H-5β/H-30β and H-30β/H-12 were observed in the ROESY spectrum, indicating that H-12 was β-oriented.

Chukfuransin C (**3**)[19] was obtained as colorless crystals. The molecular formula $C_{37}H_{46}O_{13}$ (calcd 698.2938) was determined by HREIMS ($[M]^+$, m/z 698.2948). Extensive analysis of the 1D (Supporting Information Table 1) and 2D NMR spectra uncovered that **3** share a large part (black part) with **1**. The furan ring attached to C-17 in **3** was different with that of common phragmalin limonoids in that C-21 (δ_C 148.69, s) of **3** was a quaternary carbon. HMBC correlations from H-14 to C-15 (δ_C 57.54, s) and C-21 revealed C-15-C-21 linkage as in chuktabrin B.[11] Through the HMBC spectrum, C-16 was determined by

its correlation with H-14, and the crosspeak of H-30 [δ_H 5.73 (s)]/C-16 disclosed the presence of a 16, 30-δ-lactone ring in **3**. HMBC correlations from H_3-3′ (δ_H 1.31, d, J = 6.7 Hz) and H_3-4′ (δ_H 1.13, d, J = 6.7 Hz) to C-1′ (δ_C 208.55, s) and C-2′ (δ_C 38.67, d) and from H-2′ to C-1′ proved the existence of an isobutyryl. And H-14/C-1′ HMBC correlation indicated the isobutyryl was located at C-15, different with the acetyl attached to C-15 in chuktabrin B. The only isobutyryloxyl was placed at C-17 on the basis of HMBC correlation between H-17 and δ_C 177.44 (s). Correlations from H-18α to H-14 and H-17 in ROESY spectrum indicated H-14 and H-17 were both α-oriented.

The absolute configuration of **3** was finally determined as14*S*,15*S*,17*S* by single-crystal X-ray diffraction analysis[20] (Figure. 4).

Chukfuransin D (**4**),[21] white amorphous powder, has the molecular formula $C_{38}H_{50}O_{14}$ as revealed by HREIMS. Compound **4** was not stable in $CDCl_3$ and could transform into **3**. Because of this, **4** was dissolved in CD_3OD (in which it was relatively stable) to be measured on NMR instruments. Comprehensive comparison of 1D data of **4** (Supporting Information Table 1) with those of **3** (Supporting Information Table 1) disclosed that they were extremely similar except that the 2,3-disubstituted furan ring in **3** was replaced by a 2,3-disubstituted 5-methoxy-2,5-dihydrofuran motif in **4**, which was supported by HMBC correlations from H-17 (δ_H 5.53, s) to C-20 (δ_C 143.38, s), C-22 (δ_C 129.32, d), C-21 (δ_C 88.34, d), and from H-21 (δ_H 5.53, s) to C-16 (δ_C 170.07, s), C-20, and from H-23 (δ_H 5.87, d) to 23-OMe (δ_C 54.86, q), C-22. The only isobutyryloxyl was placed at C-17 by HMBC correlation between H-17 and δ_C 178.97 (s). As seen in the planar structure proposed, the instability of acetal (red part) in acid solution may account for the instability of compound **4** in the weak acid $CDCl_3$. ROESY correlations including H-17/H-18α, H-18α/H-21, H-14/H-21, and H-14/H-18α suggested that H-14, H-17, and H-20 were all α-oriented. However, the relative configuration of H-23 remained undetermined due to the lack of ROESY correlations.

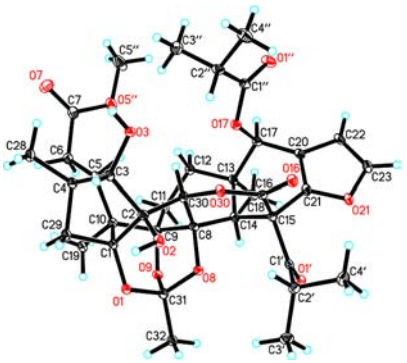

Figure 4. X-ray crystallographic structure of **3**.

It is proposed that compounds **1** and **2** were biogenetically formed via a Michael addition mechanism involving a meliacin butenolide intermediate, a type of limonoid reported in *C. tabularis*,[9] and a few other Meliaceae species such as *Trichilia estipulata*.[22] A plausible biogenetic pathway of **1** and **2** was illustrated in Supporting Information Scheme 1.

Cytotoxicity reports of phragmalin limonoids from genus *Chukrasia* are rare.[23] In our study, **1** was found moderately cytotoxic to five tested human cancer cell lines while **3** was found inactive (Supporting Information).

Acknowledgment. We acknowledge financial support by NSFC (No.81202437), the NKIG-CAS (No. KSCX2-YW-G-038; Y1235211Q1), and the MOST (SB2007FY400).

Supporting Information Available. ^{1}H NMR and ^{13}C DEPT data for **3** and **4**; plausible biogenetic pathway of **1** and **2**; 1D, 2D NMR, HREI-MS, IR, UV, and OR spectra of **1**–**4**; detailed experimental procedures of **1**–**4**; X-ray data for compounds **1** and **3** (CIF). This material is available free of charge via the Internet at http://pubs.acs.org

The authors declare no competing financial interest.

(17) Crystal data for **1**: $C_{37}H_{48}O_{14}$, M = 716.75, monoclinic, a = 13.1611(2) Å, b = 10.2808(2) Å, c = 13.5576(2) Å, α = 90.00°, β = 111.2970(10)°, γ = 90.00°, V = 1709.16(5) Å^3, T = 100(2) K, space group $P21$, Z = 2, μ(Cu Kα) = 0.891 mm^{-1}, 14070 reflections measured, 5320 independent reflections (R_{int} = 0.0251). The final R_1 values were 0.0275 ($I > 2\sigma(I)$). The final $wR(F^2)$ values were 0.0724 ($I > 2\sigma(I)$). The final R_1 values were 0.0278 (all data). The goodness of fit on F^2 was 1.024. Flack parameter = 0.05(10). The Hooft parameter is 0.14(4) for 2158 Bijvoet pairs. The crystal structures of **1** (and the following **3**) was solved by direct method SHELXS-97 (Sheldrick, G. M. University of Gottingen: Gottingen, Germany, 1997) and the full matrix least-squares calculations. Crystallographic data for the structures of **1** and **3** have been deposited in the Cambridge Crystallographic Data Centre (deposition no. CCDC 938758 and CCDC 938757). Copies of these data can be obtained free of charge via the Internet at www.ccdc.cam.ac.uk/conts/retrieving.html (or from the Cambridge Crystallographic Data Centre, 12 Union Road, Cambridge CB21EZ, U.K.; fax (+44) 1223 336 033; or deposit@ccdc.cam.ac.uk).

(18) Chukfuransin B (**2**): white amorphous powder; $[\alpha]^{20}_D$ −78 (c 0.060, $CHCl_3$); UV ($CHCl_3$) λ_{max} (log ε) 240 (3.12) nm; IR (KBr) ν_{max} 3431, 1746, 1134 cm^{-1}; for ^{1}H and ^{13}C DEPT data, see Table 1; ESI(+)MS m/z 797 $[M + Na]^+$; HREIMS m/z 774.3096 $[M]^+$ (calcd for $C_{39}H_{50}O_{16}$, 774.3099).

(19) Chukfuransin C (**3**): colorless crystals; mp 270–273 °C; $[\alpha]^{20}_D$ +129 (c 0.168, $CHCl_3$); UV ($CHCl_3$) λ_{max} (log ε) 240 (3.53) nm; IR (KBr) ν_{max} 3489, 2968, 1739, 1469, 1385, 1312, 1139, 1051, 907 cm^{-1}; for ^{1}H and ^{13}C DEPT data, see the Supporting Information (SI); ESI(+)MS m/z 721 $[M + Na]^+$; HREIMS m/z 698.2948 $[M]^+$ (calcd for $C_{37}H_{46}O_{13}$, 698.2938).

(20) Crystal data for **3**: $C_{37}H_{46}O_{13}$, M = 698.74, monoclinic, a = 9.5986(7) Å, b = 16.7235(12) Å, c = 10.4059(7) Å, α = 90.00°, β = 100.665(2)°, γ = 90.00°, V = 1641.5(2) Å^3, T = 100(2) K, space group $P21$, Z = 2, μ(Cu Kα) = 0.890 mm^{-1}, 11730 reflections measured, 5250 independent reflections (R_{int} = 0.0383). The final R_1 values were 0.0424 ($I > 2\sigma(I)$). The final $wR(F^2)$ values were 0.1186 ($I > 2\sigma(I)$). The final R_1 values were 0.0424 (all data). The goodness of fit on F^2 was 1.069. Flack parameter = 0.01(14). The Hooft parameter is −0.01(5) for 2310 Bijvoet pairs.

(21) Chukfuransin D (**4**): white amorphous powder; $[\alpha]^{14}_D$ +67 (c 0.173, MeOH); UV (MeOH) λ_{max} (log ε) 204 (3.92) nm; IR (KBr) ν_{max} 3445, 2967, 1738, 1140 cm^{-1}; for ^{1}H and ^{13}C DEPT data, see the Supporting Information; ESI(+)MS m/z 753 $[M + Na]^+$; HREIMS m/z 730.3188 $[M]^+$ (calcd for $C_{38}H_{50}O_{14}$, 730.3201).

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