ORGANIC LETTERS

2012
Vol. 14, No. 2
486–489

Walsucochinoids A and B: New Rearranged Limonoids from *Walsura cochinchinensis*

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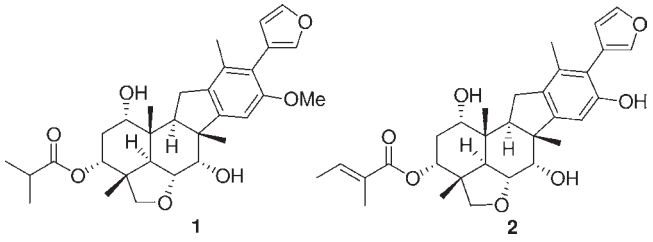
Received November 16, 2011

ABSTRACT



Walsucochinoids A (1) and B (2), two rearranged limonoids possessing an unprecedented carbon framework, were isolated from *Walsura cochinchinensis*. Their configurations were assigned as 1*S*, 3*R*, 4*R*, 5*R*, 6*R*, 7*S*, 8*R*, 9*R*, and 10*R* on the basis of a detailed examination of spectroscopic data, single crystal X-ray diffraction analysis, and CD experiments.

The genus *Walsura* Roxb (Family Meliaceae), an important component of the tropical vegetation in Asia, comprises 30 to 40 species mainly distributed in China, India, Malaysia, and Indonesia.[1] Chemical and pharmacological studies of the plants from this genus have been very active in the past decade, resulting in the separation and identification of over 50 compounds with a variety of biological properties such as antifeeding, antibacterial, antioxidative, and antimalarial activities.[2]

Wasura cochinchinensis (Baill.), a widespread species in Vietnam, is also found in the southern area of China such as Guangxi, Guangdong, Yunnan, and Hainan provinces.[1] A previous investigation into this plant in our research group led to the isolation of two unusual C_{24} nortriterpenoids, walsucochins A and B, with remarkable neuroprotective activity.[3] As a continuation of our work on structurally interesting and biologically significant secondary metabolites of plant origin, we collected the twigs and leaves of *W. cochinchinensis* and conducted a more intensive study into its chemical constituents. This work yielded two additional new compounds, walsucochinoids A (**1**) and B (**2**). The absolute structures of **1** and **2** were characterized on the basis of spectroscopic data, X-ray crystallographic analysis, and CD experiments. Unlike most other limonoids incorporating a six-membered C-ring and a five-membered or a δ-lactonyl D-ring, walsucochinoids A (**1**) and B (**2**) are noteworthy in that they feature a rearranged motif of C/D rings, with a five-membered C ring fused with a

(1) Chen, S. K.; Chen, B. Y.; Li, H. In *Flora of China (Zhongguo Zhiwu Zhi)*; Science Press: Beijing, 1997; Vol. 43 (3), p 62.

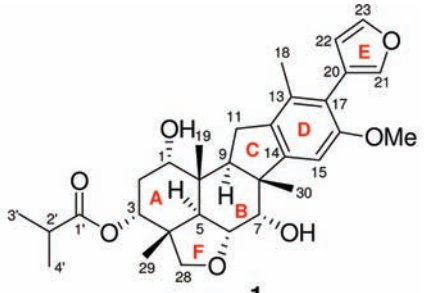
(2) (a) For a review, see: Jiang, J. H.; Pan, Y. Q.; Ma, Y. J.; Chen, Y. G. *Anhui Nongye Kexue* **2008**, *36*, 11142–11143 and references cited therein. (b) Voravuthikunchai, S. P.; Kanchanapoom, T.; Sawangjaroen, N. Y.; Hutadilok-Towatana, N. *Nat. Prod. Sci.* **2010**, *24*, 813–824. (c) Mohamad, K.; Hirasawa, Y.; Lim, C. S.; Awang, K.; Hadi, A. H. A.; Takeya, K.; Morita, H. *Tetrahedron Lett.* **2008**, *49*, 4276–4278. (d) Awang, K.; Yusoff, M.; Mohamad, K.; Chong, S. L.; Ng, S. W. *Acta Crystallogr., Sect. E: Struct. Rep. Online* **2009**, *65*, o1166. (e) Mohamad, K.; Yusoff, M.; Awang, K.; Ahmad, K.; Ng, S. W. *Acta Crystallogr., Sect. E: Struct. Rep. Online* **2009**, *65*, o1317.

(3) Zhou, Z. W.; Yin, S.; Zhang, H. Y.; Fu, Y.; Yang, S. P.; Wang, X. N.; Wu, Y.; Tang, X. C.; Yue, J. M. *Org. Lett.* **2008**, *10*, 465–468.

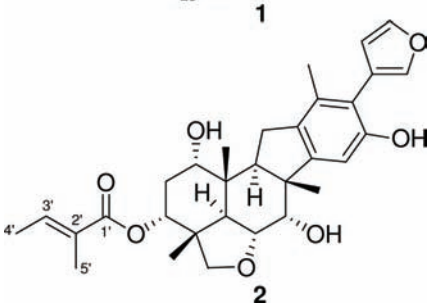
(4) **Walsucochinoid A (1):** colorless crystals; mp 168−170 °C; $[\alpha]^{21}_{D}$ −3.8 (*c* 0.105, MeOH); UV (MeOH) λ_{max} (log ε) 286 (3.10) nm; CD (MeOH) λ ($\Delta\varepsilon$) 213 (−0.59), 249 (0.20) nm; IR (KBr) ν_{max} 3433, 2966, 2935, 1728, 1631, 1610, 1506, 1464, 1425, 1389, 1313, 1157, 1080, 1038, 1001, 874, 598 cm^{-1}; ^1H and ^{13}C NMR data, see Table 1; ESI(+)MS *m*/*z* 525.4 $[M + H]^+$, 547.4 $[M + Na]^+$, 1071.7 $[2 M + Na]^+$; HR-ESI(+)MS *m*/*z* 547.2661 $[M + Na]^+$ (calcd for $C_{31}H_{40}O_7Na$, 547.2672).

10.1021/ol203082c
Published on Web 12/22/2011

six-membered aromatic D ring. Adding to their structural complexity, an extra F ring of tetrahydrofuran is also formed connecting C-6 and C-28. This paper describes the extraction, isolation, and structure characterization of the two new limonoids.



1



2

The air-dried plant material of *W. cochinchinensis*, collected from Hainan province, P. R. China, was extracted with 95% ethanol at rt to return, after condensation *in vacuo*, 280 g of crude extract which was further partitioned between H_2O and EtOAc. The EtOAc partition (130 g) was fractionated by an MCI gel column eluted with MeOH/H_2O (40% to 90%) to return seven fractions, and fraction 4 (20 g) was subjected to repeated column chromatography (silica gel, Sephadex LH-20 and reversed-phase HPLC) to yield walsucochinoids A (**1**, 7 mg) and B (**2**, 23 mg).

High resolution ESI(+)MS analysis of walsucochinoid A (**1**)[4] revealed a sodiated molecular ion $[M + Na]^+$ at *m*/*z* 547.2661 (cacld 547.2672) consistent with a molecular formula of $C_{31}H_{40}O_7$ incorporating 12 degrees of unsaturation. Its IR spectrum displayed absorption bands of hydroxyl (3433 cm^{-1}), ester carbonyl (1728 cm^{-1}), and phenyl (1631, 1610, and 1506 cm^{-1}) groups. The NMR data ($CDCl_3$) of **1** (Table 1) revealed resonances suggestive of an isobutyryloxyl, a pentasubstituted benzene (δ_H 6.66, s, H-15; δ_C 101.6, C-15; 119.3, C-17; 134.0, C-12; 135.0, C-13; 149.1, C-14; 156.6, C-16) bearing a methyl (δ_H 2.12, s; δ_C 17.5; Me-18) and a methoxyl (δ_H 3.76, s; δ_C 56.0; 16-OMe), a typical β-substituted furan, and three tertiary methyls (δ_H 1.13, s, H_3-19; 1.24, s, H_3-29; 1.14, s, H_3-30) as well as two exchangeable protons (δ_H 2.53, 1-OH; 2.16, s, 7-OH) that were distinguished from the others by an HSQC spectrum (Supporting Information (SI), Figure S3). The above observations accounted for 8 degrees of unsaturation and required that **1** possessed four additional rings.

Analysis of $^1H-^1H$ COSY (Figure 1a) data for **1** revealed the presence of key structural fragments as shown in bold lines (Figure 1a), based on the correlations of H-1 (δ_H 3.62) via H_2-2 (δ_H 2.02 and 2.38) to H-3 (δ_H 5.12), H-5 (δ_H 2.53)

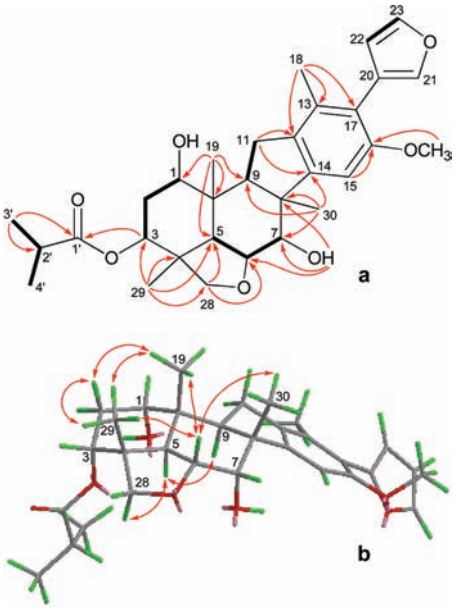


Figure 1. Key $^1H-^1H$ COSY (a: bold —), HMBC (a: →), and ROESY (b: ↔) correlations of **1**.

via H-6 (δ_H 4.36) to H-7 (δ_H 4.54), and H-9 (δ_H 2.94) to H_2-11 (δ_H 2.51 and 2.68). The HMBC correlation networks (Figure 1a) of H_3-19 to C-1 (δ_C 72.8), C-5 (δ_C 40.3), C-9 (δ_C 48.0), and C-10 (δ_C 39.2); H_3-29 to C-3 (δ_C 73.4), C-4 (δ_C 42.2), C-5, and C-28 (δ_C 78.1); H_3-30 to C-7 (δ_C 69.9), C-8 (δ_C 53.6), C-10, and C-14 (δ_C 149.1); and H-11α (δ_H 2.68) to C-12 and C-14 defined the conjunction of A/B/C/D rings in **1** as drawn. Other critical HMBC correlations from H-3 to C-1′ (δ_C 175.4); H-28α (δ_H 3.54) to C-6 (δ_C 75.7); 7-OH to C-6, C-7, and C-8; H_3-18 to C-12, C-13, and C-17; and OC$\underline{H_3}$-16 to C-16 indicated the locations of the 3-isobutyryloxyl, the ether bridge bond between C-6 and C-28 (δ_C 78.1), the7-OH, the Me-18, and the 16-OMe. In addition, the presence of a 1-OH was indicated, although it could not be located by HMBC data due to the overlapping nature of the nearby resonances, was also evident from the chemical shifts for H-1 (δ_H 3.62) and C-1 (δ_C 72.8). Finally, with the aforementioned establishment the remaining furan ring could only be assigned to C-17, thus defining the planar structure of **1** as shown.

The relative stereochemistry of **1** was established by a detailed explanation of both $^1H-^1H$ couplings and ROESY data (Figure 1b). More specifically, strong ROESY correlations of H-2β/H_3-19, H-2β/H_3-29, and H_3-29/H-6 were indicative of an axial position for H-2β, Me-19, Me-29, and H-6, and they were arbitrarily assigned as β-oriented. Furthermore, the coupling constants of H-1/H-2β (3.4 Hz) and H-2β/H-3 (2.9 Hz) suggested that H-1 and H-3 were equatorially bonded, thus leaving 1-OH and 3-isobutyryloxyl axially positioned and α-oriented, while the magnitudes of $J_{5,6}$ (12.2 Hz) and $J_{6,7}$ (2.8 Hz) were supportive of an axial H-5 and an equatorial H-7 consistent

Table 1. NMR Data ($CDCl_3$, 400 MHz) for **1** and **2**

	1		**2**	
no.	δ_C	δ_H	δ_C	δ_H
1	72.8	3.62[b]	72.9	3.61[b]
2	29.9	α 2.02 (ddd, 16.1, 2.9, 2.5)	30.2	α 2.04 (ddd, 16.1, 2.8, 2.4)
		β 2.38 (ddd, 16.1, 3.4, 2.9)		β 2.39 (ddd, 16.1, 3.5, 2.8)
3	73.4	5.12 (dd, 2.9, 2.9)	73.5	5.21 (dd, 2.8, 2.8)
4	42.2		42.5	
5	40.3	2.53[b]	40.5	2.58 (d, 12.4)
6	75.7	4.36 (dd, 12.2, 2.8)	75.9	4.35 (dd, 12.4, 2.8)
7	69.9	4.54 (d, 2.8)	70.1	4.51 (d, 2.8)
8	53.6		53.6	
9	48.0	2.94 (dd, 12.2, 6.4)	47.9	2.98 (dd, 12.4, 6.5)
10	39.2		39.4	
11	25.1	α 2.68 (dd, 13.9, 6.4)	25.1	α 2.66 (dd, 13.7, 6.5)
		β 2.51[b]		β 2.51 (dd, 13.7, 12.4)
12	134.0		133.6	
13	135.0		134.8	
14	149.1		150.4	
15	101.6	6.66 (s)	105.4	6.70 (s)
16	156.6		152.6	
17	119.3		116.3	
18	17.5	2.12 (s)	17.4	2.07 (s)
19	16.2	1.13 (s)	16.4	1.13[b] (s)
20	120.3		119.2	
21	140.9	7.36 (dd, 1.6, 0.7)	141.3	7.44 (br s)
22	112.9	6.40 (dd, 1.6, 0.7)	112.6	6.39 (br s)
23	141.9	7.48 (dd, 1.6, 1.6)	144.2	7.60 (dd, 1.6, 1.6)
28	78.1	α 3.54 (d, 7.9)	78.3	α 3.57 (d, 8.0)
		β 3.63[b] (d, 7.9)		β 3.63[b] (d, 8.0)
29	18.8	1.24 (s)	19.1	1.25 (s)
30	22.8	1.14[b] (s)	22.9	1.13[b] (s)
1-OH		2.53[b]		2.44 (d, 9.1)
7-OH		2.16 (s)		2.13 (s)
16-OH/OMe	56.0	3.76 (s)		5.03 (s)
1′	175.4		166.7	
2′	34.2	2.52[b]	128.1	
3′	19.1[a]	1.152[a] (d, 7.1)	138.8	6.77 (br q, 7.1)
4′	19.0[a]	1.146[a,b]	12.4	1.75 (br d, 7.1)
5′			14.8	1.80 (br s)

[a] Interchangeable signals within the same column. [b] Overlapping signals within the same column.

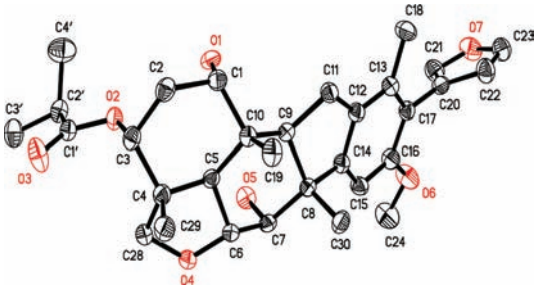


Figure 2. Single-crystal X-ray structure of **1**.

with an α-configuration for both H-5 and 7-OH. The tentative assignments of an α-configuration for H-9 and a β-orientation for Me-30 based on biosynthetic considerations, though complicated by the severe overlapping proton signals of H_3-19/H_3-30/H_3-3′/H_3-4′ and 1-OH/H-5/H-11β/H-2′, were eventually secured by single-crystal diffraction analysis (Figure 2). Moreover, the successful performance of an X-ray diffraction experiment with Cu Kα (λ = 1.54178 Å) radiation also allowed the assignment of the absolute configuration of **1** as 1*S*, 3*R*, 4*R*, 5*R*, 6*R*, 7*S*, 8*R*, 9*R*, and 10*R* as drawn [absolute structure parameter: 0.03(17)].[5]

(5) Flack, H. D. *Acta Crystallogr., Sect. A* **1983**, *A39*, 876–881.

(6) **Walsucochinoid B (2):** colorless crystals; mp 166−168 °C; $[\alpha]^{20}_D$ 8.1 (*c* 0.135, MeOH); UV (MeOH) λ_{max} (log ε) 288 (3.26) nm; CD (MeOH) λ ($\Delta\varepsilon$) 211 (sh) (−1.25), 224 (−2.12), 248 (−0.49) nm; IR (KBr) ν_{max} 3438, 2929, 1703, 1649, 1618, 1506, 1435, 1389, 1313, 1157, 1038, 874, 735, 598 cm^{-1}; ^1H and ^{13}C NMR data, see Table 1; ESI(+)MS *m*/*z* 523.3 $[M + H]^+$, 545.3 $[M + Na]^+$, 1067.7 $[2 M + Na]^+$; HR-ESI(+)MS *m*/*z* 545.2525 $[M + Na]^+$ (calcd for $C_{31}H_{38}O_7Na$, 545.2515).

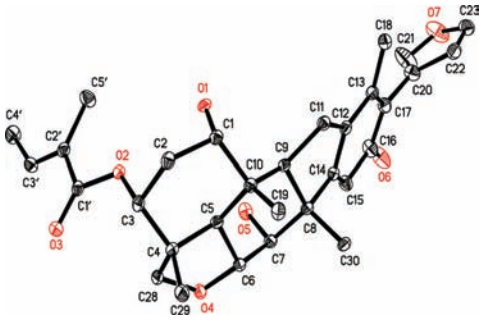


Figure 3. Single-crystal X-ray structure of **2**.

High resolution ESI(+)MS analysis of walsucochinoid B (**2**)[6] revealed a sodiated molecular ion at m/z 545.2525 $[M + Na]^+$ (cacld 545.2515) consistent with a molecular formula of $C_{31}H_{38}O_7$. Analyses of the 1D (Table 1) and 2D NMR data ($CDCl_3$) for **2** suggested that it was a structural congener of **1**, and the differences were likely the presence of a tiglyloxyl group (δ_H 1.75, H-4′; 1.80, H-5′; 6.77, H-3′; δ_C 12.4, C-4′; 14.8, C-5′; 128.1, C-2′; 138.8, C-3′; 166.7, C-1′) and a phenol hydroxyl (δ_H 5.03, 16-OH) in **2** replacing the corresponding 3-isobutyryloxyl and 16-OMe in **1**. The presence of a tiglyloxyl group was confirmed by HMBC correlations within this motif, and it was assigned to C-3 by the HMBC correlation (SI, Figure S13) from H-3 to C-1′. This assignment was further supported by the remarkably deshielded proton resonance of H-3 at δ_H 5.21. Furthermore, the existence of a 16-OH was also confirmed by the HMBC correlations from the proton of 16-OH to C-15 (δ_C 105.4), C-16 (δ_C 152.6), and C-17 (δ_C 116.3). Excellent resemblances between the remaining NMR data for **2** and **1** supported the assignment of a common limonoid core for the two coexisting metabolites. The same relative stereochemistry for **2** as **1** was also assigned by a ROESY experiment (SI, Figure S15).

A single-crystal X-ray diffraction study (Figure 3) for **2**, although it failed to establish the absolute configuration of **2** owing to the limited data points [absolute structure parameter: 0.14(18)],[5] confirmed the above demonstration. Finally **2** was assigned the same absolute stereochemistry as **1** by comparing their CD spectra (Figure 4). In addition to the newly introduced Cotton effect at 224 nm relating to the tiglyloxyl group, the CD data of **2** revealed comparable Cotton effects at 211 (shoulder) and 248 nm corresponding to those of **1** (213 and 249 nm) caused by the chromophore of the C- and D-ring system. This assignment was also consistent with biogenetic considerations.

An *in vitro* SH-SY5Y cell protecting assay (H_2O_2 induced injury) showed that compounds **1** and **2** were inactive. Compounds **1** and **2** were also evaluated *in vitro* in an 11β-HSD1 inhibitory assay, but neither of them were active (SI, Tables S1 and S2).

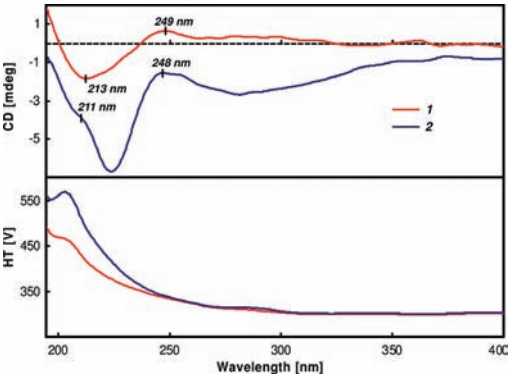


Figure 4. CD spectra of **1** and **2**.

Acknowledgment. Financial support of the National Natural Science Foundation (Grant Nos. 81021062; 20932007), and National Science & Technology Major Project "Key New Drug Creation and Manufacturing program (Number: 2011ZX09307-002-03) of the People's Republic of China are gratefully acknowledged. We thank Prof. S.-M. Huang of Hainan University for the identification of the plant material and Prof. H.-Y. Zhang and Prof. Y. Leng of Shanghai Institute of Materia Medica for biological tests.

Supporting Information Available. Experimental section (general experimental procedures, collection and taxonomy of plant material and detailed extraction and isolation process, biological experiments and the tabulated results), and spectroscopic data (UV, IR, NMR, X-ray and MS) for compounds **1** and **2** were provided. This material is available free of charge via the Internet at http://pubs.acs.org.