Limonoids from the Root of *Dictamnus radicis* Cortex

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Chemical investigation of the roots of *Dictamnus radicis* Cortex led to the isolation of a new limonoid isodictamdiol (1) and a known dictamdiol (2), the first 5*S*/9*S*-type degraded limonoids, together with other six known limonoids (3—8). The chemical structures were identified on the basis of modern spectroscopic methods, including IR, MS, NMR (^{1}H-NMR, ^{13}C-NMR, ^{1}H–^{1}H COSY, HMQC, HMBC, NOESY). Additionally, the absolute configurations of limonoid isodictamdiol (1) and dictamdiol (2) were separately elucidated by single crystal X-ray diffraction, as well as their circular dichroism spectra. Furthermore, all compounds were evaluated for antibacterial activity against three bacterial cultures.

Key words *Dictamnus radicis* Cortex; isodictamdiol; absolute configuration; antibacterial activity

Dictamnus radicis Cortex is a traditional folk herb belonging to Rutaceae. Its dried roots have been called the Chinese drug, Bai–Xian–Pi, which has been used for the treatment of jaundice, rheumatism and various other diseases.[1)] Previously phytochemical studies on the species revealed the presence of limonoids,[2—8)] furoquinoline alkaloids,[9,10)] trinorguaianetype sesquiterpenes,[11)] and flavonoids.[12)]

During our study on limonoid constituents from *D. radicis* Cortex, a pair of diastereomers named isodictamdiol (**1**) and dictamdiol (**2**), the first 5*S*/9*S*-type degraded limonoids, were isolated.[2)] Six known limonoids, fraxinellone (**3**),[3,4)] fraxinellonone (**4**),[5)] evodol (**5**),[7)] rutaevine (**6**),[7)] limonin (**7**),[7)] and obacunone (**8**)[7,8)] were also isolated during this study. Among the eight isolated compounds, isodictamdiol (**1**) had a new structure. In addition, the spectroscopic data of dictamdiol (**2**) were re-assigned and the absolute configurations were elucidated for the first time.

Results and Discussion

Isodictamdiol (**1**), $[\alpha]_D^{20}$ −22° (c=0.57 MeOH), was obtained as colorless crystals. Its IR absorptions exhibited the existence of two hydroxyl groups (3362, 3259 cm^{-1}), a δ-lactone (1724 cm^{-1}), a double bond (1560 cm^{-1}), and a furanyl ring (1506, 873 cm^{-1}). The molecular formula was deduced to be $C_{15}H_{18}O_5$ from HR-ESI-MS ($[M+NH_4]^+$ m/z 296.1488, Calcd 296.1492) with seven degrees of unsaturation. Analysis of the proton and carbon NMR spectra of **1** indicated the presence of a β-substituted furanyl ring [δ_H 7.65, 7.59, 6.53 (each br s, 1H), δ_C 110.9 (CH), 121.4 (C), 142.3 (CH), and 144.0 (CH)], a tertiary methyl [δ_H 1.02 (s, 3H)], a vinyl methyl [δ_H 1.86 (s, 3H)], two oxygen-bearing methines [δ_H 4.02 (br s, 1H), δ_H 4.82 (d, 1H, J=4.8 Hz)], two secondary hydroxyl groups [δ_H 4.02 (br s, 1H) and δ_H 5.33 (d, 1H, J=4.8 Hz)], one ester carbonyl group, and one tetrasubstituted double bond (Table 1). The assignments of all the direct ^{1}H–^{13}C bondings were made on the basis of the heteronuclear multiple quantum coherence (HMQC) spectrum and the planar structure was determined by ^{1}H–^{1}H correlation spectroscopy (^{1}H–^{1}H COSY). The heteronuclear multiple bond correlations (HMBC) were determined as follows: H-14/C-1, 2, 6; H-15/C-4, 5, 6, 9; H-9/C-10, 11, 13; H-7/C-1, 5, 6, 8; 2-OH/C-2, and 7-OH/C-7. The cross-peaks observed in the NOESY experiments between the protons H-9/H-4*b*, H-4*b*/H-3*b*, H-3*b*/H-2, and H-2/H-4*b* indicated that H-2 and H-9 were on the same side and were arbitrarily assigned β-orientations while the 2-OH and furanyl ring were in α-configurations. Meanwhile, the large coupling constant between H-2 and H-3a [$J_{2,3a}$=9.2 Hz, (the peak of H-2 was split after adding D_2O in the HMBC experiments)] also implied that H-2 should be axial (in β-orientation). The NOESY correlation pairs of H-15/H-3*a*, and H-15/H-4*a* suggested that H-15 had an α-orientation (Fig. 1). Although the stereochemistry at C-7 could not be determined by analysis of the available spectral data since the splitting pattern and relevant NOESY correlations did not provide sufficient information, fortunately, we obtained a single crystal (petroleum ether–acetone 1 : 1) and X-ray crystallography (Fig. 2) showed that the 7-OH was in the β-configuration. Furthermore, the single crystal X-ray diffraction for **1** showed that the δ-lactone ring was in a boat conformation (H-9 and 7-OH were *cis*, and H-9 was axial), which corresponded to the peak centered at λ_{max} 222 nm in the CD spectrum.[14)] According to the Klyne sector rule for the saturated lactone,[15)] the positive Cotton effect, $[\theta]_{222}$=+110000, indicated that the absolute configuration at C-5 was assigned to *S*. Then C-2, C-7, and C-9 were fixed to be *S*, *R*, and *S*, respectively. Therefore, the structure of **1** was

Table 1. ^{1}H- and ^{13}C-NMR Spectral Data for Compound **1** (CD_3COCD_3, δ in ppm, J in Hz)

Position	δ_H	δ_C	Position	δ_H	δ_C
1		139.5 s	9	5.51 s	79.7 d
2	4.02 br s	70.2 d	10		121.4 s
3a	1.76 m	28.9 t	11	7.65 br s	142.3 d
3b	2.05 m		12	7.59 br s	144.0 d
4a	1.14 dt (13.2, 3.2)	32.4 t	13	6.53 br s	110.9 d
4b	1.54 ddd (14.0, 13.2, 3.2)		14	1.86 s	14.6 q
5		40.0 s	15	1.02 s	18.9 q
6		135.1 s	2-OH	4.02 br s	
7	4.82 d (4.8)	67.9 d	7-OH	5.33 d (4.8)	
8		171.1 s			

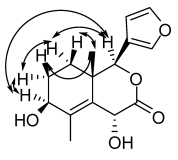

Fig. 1. Key NOESY Correlations of Compound **1**

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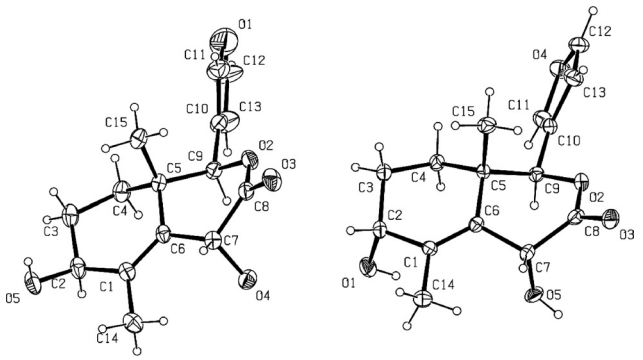

1 **2**

Fig. 2. X-Ray Structures of **1** and **2**

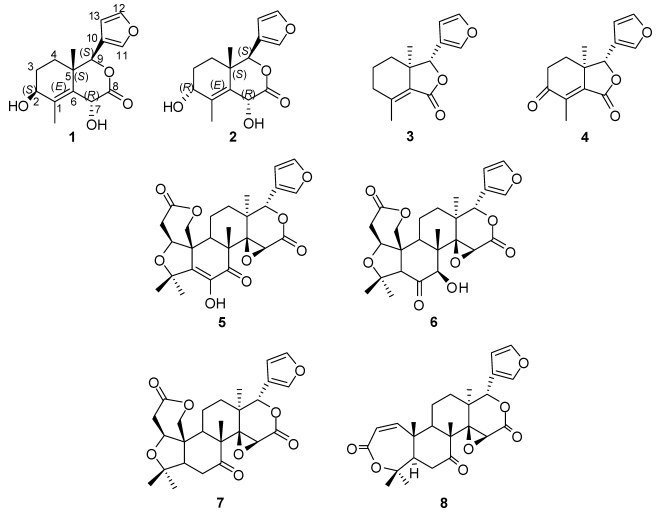

Fig. 3. The Structures of Compounds **1**—**8**

assigned as shown in Fig. 3.

Dictamdiol (**2**), $[\alpha]_D^{20}$ −114° (c=0.42 MeOH) and colorless crystals, showed a molecular formula of $C_{15}H_{18}O_5$ as determined by HR-ESI-MS ($[M+Na]^+$ at m/z 301.1048, Calcd 301.1046) with seven degrees of unsaturation. The IR spectrum exhibited absorptions for two hydroxyl groups (3422, 3342 cm^{-1}), a δ-lactone (1732 cm^{-1}), a double bond (1592 cm^{-1}), and a furanyl ring (1503, 876 cm^{-1}). This compound gave the same $[M]^+$ and fragment peaks in the EI-MS, and had similar features in the ^{1}H- and ^{13}C-NMR spectra to those of **1** (Table 1). In comparison with the NMR data of **1**, the H-2 and 2-OH proton signals of **2** exhibited an upfield shift from δ 4.02 to δ 3.90 and a downfield shift from δ 4.02 to δ 4.13, respectively. The signal of C-2 in the ^{13}C spectrum was shifted upfield from δ 70.2 to δ 67.7, and the signal at C-14 was shifted downfield from δ 14.6 to δ 17.1, indicating that the stereochemistry at C-2 in **2** and **1** was different. From the ^{1}H-NMR coupling constants, H-2 should be equatorial because of the small coupling constant between H-2 and H-3b ($J_{2,3b}$=4.8 Hz). To sum up above deduction, **2** was elucidated to be dictamdiol which was previously isolated from *D. angustifolius*[2] and *D. dasycarpus*[13] separately. The spectra of compound **2** were assigned in the lit. 2, and the spectroscopic data were re-assigned in the ref. 13 on the basis of 2D-NMR experiments, but the assignment for chemical shifts of C-10 and C-13 (δ 110.1, 120.3) were still found to be incorrect in ref. 13, and ^{13}C-NMR were definitely re-assigned again to be (δ 121.7, 110.9) in this manuscript (Table 2). Additionally, the reported specific rotation[2] was of the same sign {$[\alpha]_D^{20}$ −151° (c=0.1 MeOH)} but somewhat higher than that measured for our sample {$[\alpha]_D^{20}$ −114° (c=0.42 MeOH)}. The single crystal X-ray diffraction (Fig. 2) showed that the δ-lactone ring for **2** was also in boat conformation, which corresponded to the λ_{max} 223 nm in the CD spectra, just as that in the spectrum of compound **1**. Thus, the stereochemistry of C-2, C-5, C-7, and C-9 were fixed to be *R*, *S*, *R*, and *S*, respectively. The structure of **2** was determined and named (2*R*,5*S*,7*R*,9*S*)-dictamdiol (Fig. 3).

To the best of our knowledge, the absolute configurations at C-5 and C-9 as *S* and *S* of the limonoids were reported for the first time.

The known compounds, **3**—**8**, were determined as fraxinellone (**3**),[3,4] fraxinellonone (**4**),[5] evodol (**5**),[7] rutaevine (**6**),[7] limonin (**7**),[7] and obacunone (**8**)[7,8] by comparison of their spectral data with those reported.

Table 2. ^{1}H- and ^{13}C-NMR Assignment for Compound **2** (δ in ppm, J in Hz)

Position	δ_H[a]	δ_C[a]	δ_C[b]	δ_C[c]
1		137.4 s	136.0	135.6
2	3.90 t (4.8)	67.7 d	65.8	68.0
3a	1.78—1.71 m	27.8 t	27.1	26.9
3b	1.96 tt (12.8, 3.2)			
4a	0.96 dt (12.8, 3.2)	28.0 t	26.9	26.9
4b	1.71—1.64 m			
5		40.0 s	38.8	39.2
6		136.0 s	134.3	136.7
7	4.81 d (5.2)	67.6 d	65.9	66.7
8		171.1 s	170.8	170.9
9	5.48 s	79.6 d	78.4	79.8
10		121.7 s	110.1	120.0
11	7.64 br s	142.2 d	141.5	142.9
12	7.58 br s	144.0 d	143.4	141.3
13	6.52 br s	110.9 d	120.3	109.9
14	1.89 s	17.1 q	17.0	17.0
15	0.92 s	17.5 q	16.6	17.2
2-OH	4.13 br s			
7-OH	5.36 d (5.2)			

a) The data were measured in CD_3COCD_3. *b*) The data in ref. 13. *c*) The data in ref. 2.

Table 3. Antibacterial Activities of Compounds **1**—**8**[a,b]

Compounds	*Staphylococcus aureus*	*Escherichia coli*	*Bacillus subtilis*
1	−	−	+
2	−	−	−
3	−	−	−
4	−	+	−
5	−	−	−
6	−	−	+
7	+	−	−
8	−	−	−
Chloramphenicol	+++	+++	+++
H_2O	−	−	−

a) Zone diameter of growth inhibition: <10 mm (−), 10—12 mm (+), 13—15 mm (++), and 16—20 mm (+++). *b*) Each tested compound as well as chloramphenicol was 0.2 ml of 100 mg·ml^{-1}.

The antimicrobial activities of compounds **1**—**8** were tested. Among them, compounds **1**, **4** and **7** exhibited weak antibacterial activities against *Bacillus subtilis*, *Escherichia coli* and *Staphylococcus aureus*, respectively (Table 3).

Experimental

General Experimental Procedures Melting points were determined on a SWG X-4 melting point apparatus and were uncorrected. Optical rotations were measured on a Perkin-Elmer 341 polarimeter. IR spectra were measured on a Nicolet 170SX FT-IR instrument (neat). One-dimensional (1D) and two-dimensional (2D) NMR spectra were measured on a Varian Mercury-400BB NMR spectrometer with TMS as the internal standard. Electron impact ionization mass spectrometry (EI-MS) data were taken on VG ZABHS mass spectrometer at 70 eV. HR-ESI-MS were recorded on a Bruker APEX II. UV spectra were obtained using a Shimadzu UV-260 spectrophotometer. Single crystal X-ray diffraction was measured on a D8 SMART APEX II X-ray single crystal instrument. The CD spectra were recorded on an OLIS DSM 1000 bisbeam CD spectrometer. Silica gel (200—300 mesh) used for column chromatography, and silica GF_{254} (10—40 μ) for TLC were supplied by the Qing-dao, Marine Chemical Factory, Qingdao, P. R. China. TLC spots were detected under a UV lamp or by heating after being sprayed with 5% H_2SO_4 in EtOH (v/v).

Plant Material The roots of *Dictamnus radicis* Cortex were purchased from Focitang Medicine Material Corporation in Lanzhou, and identified by Prof. Peijun Yu, of the Second Hospital of Lanzhou University. A voucher specimen (No. 20050301) was deposited in the Institute of Organic Chemistry, Lanzhou University.

Extraction and Separation The dried and powdered underground parts of *D. radicis* Cortex (10.0 kg) was percolated three times with petroleum ether–EtOAc–MeOH (1 : 1 : 1, 3×20 l) at room temperature. After evaporated to dryness under reduced pressure, a residue (366.4 g) was obtained and redissolved in water (600 ml). The aqueous solution was extracted with petroleum ether (30—60 °C, 600 ml×3), EtOAc (600 ml×3) and *n*-BuOH (600 ml×3) to give extracts 50.5 g, 101.0 g and 56.3 g, respectively. The EtOAc extracts were subjected to column chromatography on silica gel and eluted with $CHCl_3$–MeOH (1 : 99—50 : 50 v/v) to give fractions A—F. Fractions A, B, and C were separated by sequential column chromatography on silica gel and then purified by Sephadex LH-20 column ($CHCl_3$–MeOH 2 : 1 v/v) and preparative TLC to afford **3** (696 mg), **4** (7 mg), **5** (8 mg), **6** (10 mg), **7** (456 mg), and **8** (816 mg). Fraction D was chromatographed over repeated silica gel column chromatography and Sephadex LH-20 column ($CHCl_3$–MeOH 2 : 1 v/v) and was recrystallized with petroleum ether–acetone (1 : 1) to give **1** (47 mg) and **2** (51 mg).

Isodictamdiol (**1**): $C_{15}H_{18}O_5$, colorless rhomboid crystals, mp 174—176 °C. $[\alpha]_D^{20}$ −22° (c=0.57 MeOH). CD (MeOH) $[\theta]_{222}$=+110000. IR (KBr): 3362, 3259, 1724, 1560, 1506, 1021, 873, and 800 cm^{-1}. EI-MS *m*/*z*: 278 $[M]^+$ (0.49), 260 (0.51), 152 (24.7), 136 (100), 121 (24.7), 93 (33.8). HR-ESI-MS *m*/*z*: 296.1488 $[M+NH_4]^+$ (Calcd for 296.1492). ^{1}H- and ^{13}C-NMR: see Table 1.

Dictamdiol (**2**): $C_{15}H_{18}O_5$, colorless rhomboid crystals, mp 148—150 °C. $[\alpha]_D^{20}$ −114° (c=0.42 MeOH). CD (MeOH) $[\theta]_{223}$=+12500. IR (KBr): 3422, 3342, 1732, 1592, 1503, and 876 cm^{-1}. EI-MS *m*/*z*: 278 $[M]^+$ (0.9), 260 (0.02), 136 (100), 121 (26.0), 93 (35.0). HR-ESI-MS *m*/*z*: 301.1048 $[M+Na]^+$ (Calcd for 301.1046). ^{1}H- and ^{13}C-NMR: see Table 2.

X-Ray Crystallography of Isodictamdiol (**1**): Colorless crystal (0.06×0.31×0.44 mm) grown from petroleum ether–acetone, $C_{15}H_{18}O_5$, Mw=278.29, monoclinic, space group $P2(1)$, Z=4, a=7.5269(5), b=10.5031(7), c=18.8455(12) Å, α=90.00°, β=97.971(3)°, γ=90.00°, V=1475.45(17) Å^3, dc=1.253 g·cm^{-3}, F(000)=592, λ (Mo$K\alpha$)=0.7107 Å, μ=0.094 mm^{-1}; 8974 measured intensities (−9≤h≤9, −13≤k≤9, −24≤I≤24), 4697 unique (R_{int}=0.0351) of which 2995 observed with $I\geq 2.0\ \sigma(I)$.

X-Ray Crystallography of Dictamdiol (**2**): Colorless crystal (0.20×0.21×0.23 mm) grown from petroleum ether–acetone, $C_{15}H_{18}O_5\cdot H_2O$, Mw=296.31, monoclinic, space group $P2(1)$, Z=4, a=11.0906(6), b=9.1801(5), c=15.5766(8) Å, α=90.00°, β=109.752(3)°, γ=90.00°, V=1492.59(14) Å^3, dc=1.319 g·cm^{-3}, F(000)=632, λ (Mo$K\alpha$)=0.7107 Å, μ=0.102 mm^{-1}; 8062 measured intensities (−13≤h≤13, −8≤k≤11, −18≤I≤15), 5037 unique (R_{int}=0.0298) of which 3816 observed with $I\geq 2.0\ \sigma(I)$.

Antibacterial Assays The antibacterial screening was carried out by employing the cup-plate method. Three strains of bacteria (*Staphylococcus aureus*, *Escherichia coli*, and *Bacillus subtilis*) were incubated in beef broth and incubated at 37 °C for 24 h. After dilution of beef broth, with three microorganisms were cultured in agar medium dishes, respectively; four cups (8×10 mm) were put onto the dishes, and each tested compound (0.2 ml of 100 mg/ml) was added into the cups under aseptic conditions. Then the dishes were incubated at 37 °C for 24 h. The zone of inhibition of the growth of bacteria, which were produced by diffusion of the compounds from the cup into the surrounding medium, was measured to evaluate the antibacterial activity. Each experiment was repeated twice. Chloramphenicol was used as a positive control for all experiments.

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