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# Two new benzofurans and other constituents from *Ligularia przewalskii*

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Two new benzofurans, 2-(1,2-dihydroxyisopropyl)-5,6-dimethoxybenzofuran (**1**) and 2-(1-*O*-feruloyl-2-hydroxyisopropyl)-5,6-dimethoxybenzofuran (**2**), along with eleven known compounds (**3–13**) were isolated from the roots of *Ligularia przewalskii*. Their structures were established on the basis of spectroscopic methods. The antibacterial activity of compounds **1** and **3–5** was tested.

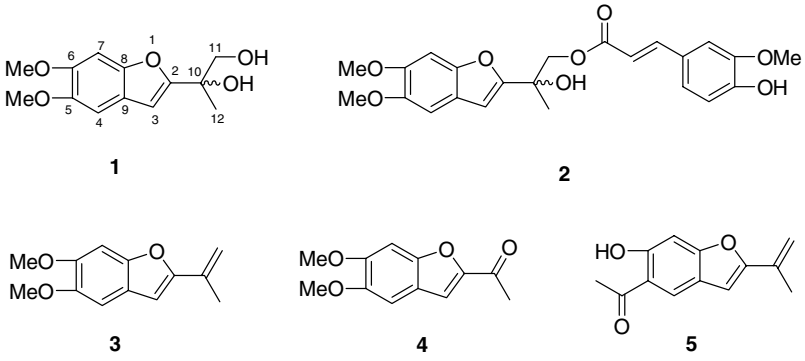
## 1. Introduction

*Ligularia przewalskii* (Maxin.) Diels (Compositae) is mainly distributed in western China. The root of it was used as folk medicine in northwest China for treatment of asthma, hemoptysis, hepatitis and pulmonary tuberculosis (Gou 1987). Earlier studies of this plant showed that its main constituents are eremophilane sesquiterpenoids (Zhao et al. 1994) and benzofurans (Jia and Zhao 1994). With the aim of isolating biologically active substances and discovering the relations between chemical constituents and ecological circumstances, we investigated the constituents of this plant collected in Qingyang County, Gansu Province of China. The isolation and structural elucidation of two new benzofurans (**1** and **2**) as well as eleven known compounds (**3–13**) were reported here. The antibacterial activity of compounds **1** and **3–5** is also described.

## 2. Investigations, results and discussion

The air-dried and pulverized roots of *L. przewalskii* were extracted with petroleum ether-$Et_2O$–$CH_3OH$ (1 : 1 : 1). After chromatographic separation, two new benzofurans, 2-(1,2-dihydroxyisopropyl)-5,6-dimethoxybenzofuran (**1**) and 2-(1-*O*-feruloyl-2-hydroxyisopropyl)-5,6-dimethoxybenzofuran (**2**), were isolated together with eleven known compounds. By comparing the spectral data with those reported in the literature, the known compounds **3–12** were identified, as 2-isopropenyl-5,6-dimethoxybenzofuran (Murae et al. 1968), 2-acetyl-5,6-dimethoxybenzofuran (Jia and Zhao 1994), euparin (Steelink et al. 1979), eremophil-7(11)-en-6α,15;8α,12-diolide (Yoshihiko and Takeyoshi 1976), 8β-hydroxyeremophil-7(11)-en-6α,15;8α, 12-diolide (Yoshihiko and Takeyoshi 1976), 8β-methoxyeremophil-7(11)-en-6α,15;8α,12-diolide (Zhao et al. 1994), eremophil-8(9),7(11)-dien-6α,15;8,12-diolide (Zhao et al. 1994), 8β-hydroxy-6β-angeloyloxyeremophil-7(11)-en-8α, 12-olide-15-oic acid (Zhao et al. 1994), sitoindoside I (Chaurasia and Wichtl 1987) and friedelin (Joy and Winston 1992). Compound **13** was identified as β-sitosterol on the basis of m.p. and TLC comparison with an authentic sample.

Compound **1** was isolated as a gum. Its IR spectrum showed hydroxyl absorption at 3420 $cm^{-1}$ and aromatic ring absorption at 1622, 1584 and 1487 $cm^{-1}$. Its EIMS spectrum showed the molecular ion peak at m/z 252 $[M]^+$, combined with the $^1H$ and $^{13}C$ NMR (DEPT) data, the molecular formula was deduced to be $C_{13}H_{16}O_5$. Its $^1H$ NMR spectrum contains five singlets, three of which were aromatic protons at δ 6.99, 6.95, 6.58. One was the signal of two methoxy at δ 3.88 (6H) and the other one was methyl at δ 1.56. Apart from these signalets, there were two doublets resonating at δ 3.96 (1 H, d, J = 10.5 Hz) and 3.66 (1 H, d, J = 10.5 Hz) which were assigned to an oxygened methylene. The $^{13}C$ NMR spectrum of **1**




**1** **2** **3** **4** **5**

**Table: Antibacterial activity of compounds 1 and 3–5[a, b]**

| Compd. | *Escherichia coli* | *Staphylococcus aureus* | *Bacillus subtilis* |
|---|---|---|---|
| **1** | − | ++ | + |
| **3** | + | ++ | + |
| **4** | ++ | ++ | − |
| **5** | ++ | + | + |
| Chloramphenicol | +++ | +++ | +++ |

[a] zone diameter of growth inhibition: <10 mm (−), 10–12 mm (+), 13–15 mm (++) and 16–20 mm (+++)
[b] the concentrations of each tested compound and chloramphenicol were 100 μg/ml

showed resonances for three aliphatic carbons (δ 72.4, 69.2, 23.6), two methoxy (δ 56.4, 56.6) and eight aromatic carbons. Comparing the $^1$H and $^{13}$C NMR (DEPT) data with those of 2-isopropenyl-5, 6-dimethoxybenzofuran (**3**), it can be seen that there is a substructure of 5,6-dimethoxybenzofuran in compound **1**. In the HMBC spectrum, the correlations of $\delta_H$ 6.99 with $\delta_C$ 120.0 (C-9), 147.9 (C-5), 146.7 (C-6), $\delta_H$ 6.95 with $\delta_C$ 103.1 (C-3), 147.9 (C-5), 146.7 (C-6) and $\delta_H$ 6.58 with $\delta_C$ 120.0 (C-9), 149.6 (C-8), 159.3 (C-2) further confirmed the presence of 5,6-dimethoxybenzofuran. The signals of δ 3.96 (1 H, d, J = 10.5 Hz), 3.66 (1 H, d, J = 10.5 Hz), 1.56 (3 H, s) in $^1$H NMR spectrum and the signals of δ 72.4 (C), 69.2 ($CH_2$), 23.6 ($CH_3$) in $^{13}$C NMR (DEPT) attributed to 1,2-dihydroxyisopropyl. The correlation $\delta_H$ 1.56/$\delta_C$ 159.3 in HMBC spectrum indicated that the 1,2-dihydroxyisopropyl was attached to C-2 of furan ring. Therefore compound **1** was established as 2-(1,2-dihydroxyisopropyl)-5,6-dimethoxybenzofuran. The configuration of C-10 cannot be determined for minor amount.

The EIMS spectrum of compound **2** showed the quasi-molecular ion peak at m/z 410 $[M-H_2O]^+$. The $^1$H NMR spectrum of **2** was similar to that of **1**, except for the presence of a feruloyl group of which protons resonating at $\delta_H$ 7.57 (1 H, d, J = 15.6 Hz, H-7′), 7.07 (1 H, d, J = 8.4 Hz, H-6′), 7.06 (1 H, s, H-2′), 6.95 (1 H, d, J = 8.4 Hz, H-5′), 6.27 (1 H, d, J = 15.6 Hz, H-8′), 3.91 (3 H, s, −OMe). The EIMS and $^1$H NMR spectrum indicated that **2** is a feruloyl derivative of compound **1**. Compared with **1**, the signals of methylene lower field shift from $\delta_H$ 3.96 and 3.66 to $\delta_H$ 4.58 and 4.12 respectively in **2**, which suggested that feruloyl was attached to the 11-hydroxy. The structure of **2** was established as 2-(1-*O*-feruloyl-2-hydroxyisopropyl)-5,6-dimethoxybenzofuran.

The antibacterial activity of **1** and **3–5** was determined against *Escherichia coli*, *Staphylococcus aureus* and *Bacillus subtilis*, and compared with chloramphenicol (Table). The results indicate that **4** and **5** possess medium antibacterial activity against *E. coli*. Compounds **1**, **3** and **4** exhibit medium antibacterial activity against *S. aureus*. All tested compounds have no or minor antibacterial activity against *B. subtilis*.

## 3. Experimental

### *3.1. Apparatus*

Melting points were determined on a Kofler apparatus and are uncorrected. IR spectra were recorded with a Nicolet NEXUS 670 FT-IR spectrometer. Optical rotations were measured on Perkin-Elmer 341 Polarimeter. NMR were recorded on Varian Mercury plus-300 and plus-400 spectrometer. EIMS were recorded on HP-5988A GC/MS instrument. Silica gel (200–300, 300–400 mesh) for CC and silica $GF_{254}$ for TLC were supplied by the Qingdao Marine Chemical factory.

### *3.2. Plant material*

The roots of *Ligularia przewalskii* (Maxim.) Diels. was collected in Qingyang county, Gansu Province of China in August 2002. It was identified by Prof. Guo-Liang Zhang, School of Life Science, Lanzhou University. A voucher specimen (No. 020826) was deposited in the Institute of Organic Chemistry, Lanzhou University.

### *3.3. Extraction and isolation*

The air-dried roots of *Ligularia przewalskii* (1.4 kg) were pulverized and extracted with petroleum ether (60–90 °C)-$Et_2O$−$CH_3OH$ (1 : 1 : 1) three times (7 days each time) at room temperature. The extract was concentrated under reduced pressure to afford a residue (24 g). This residue was subjected to a silica gel column chromatography (200–300 mesh, 240 g) with a gradient of petroleum ether-acetone (20 : 1, 10 : 1, 5 : 1, 2 : 1) as eluent. Four fractions were collected according to TLC analysis. Fraction 1 (petroleum ether-acetone 20 : 1, 2.6 g) was separated on a silica gel column chromatography (300–400 mesh, 26 g) with petroleum ether-$CHCl_3$ (10 : 1, 5 : 1) as eluent. The petroleum ether-$CHCl_3$ (10 : 1) part was purified by PTLC with petroleum ether-EtOAC (20 : 1) to afford **3** (Rf = 0.82, 5 mg). The petroleum ether-$CHCl_3$ (5 : 1) part was separated by PTLC with petroleum ether-EtOAC (20 : 1) to afford **5** (Rf = 0.66, 6 mg) and **12** (Rf = 0.45, 2 mg). From fraction 2 (petroleum ether-acetone 10 : 1), crude crystals of **13** and **8** were obtained successively, then recrystallized from $CHCl_3$ to give **13** (128 mg) and **8** (28 mg), the residue (1.8 g) was subjected to silica gel column chromatography (300–400 mesh, 20 g) with petroleum ether-acetone (10 : 1) as eluent to afford **2** (0.8 mg), **4** (6 mg) and **9** (12 mg). Fraction 3 (petroleum ether-acetone 5 : 1, 1.6 g) was subjected to silica gel column chromatography (300–400 mesh, 32 g) with petroleum ether-acetone (7 : 1) as eluent to afford **6** (16 mg) and **7** (12 mg). Fraction 4 (petroleum ether-acetone 2 : 1, 2.8 g) was separated by silica gel column chromatography (300–400 mesh, 30 g) with $CHCl_3$-acetone (50 : 1) as eluent to afford **1** (6 mg), **10** (18 mg) and **11** (26 mg).

#### *3.3.1. 2-(1,2-Dihydroxyisopropyl)-5, 6-dimethoxybenzofuran* (**1**)

Gum, $[\alpha]_D^{27}$ −21.5° (c, 9.3, $CHCl_3$), IR (KBr): $\nu_{max}$ = 3420, 2936, 1622, 1487, 1466, 1443, 1319, 1210, 1121, 1045, 1002 $cm^{-1}$; EIMS (rel. int.): m/z = 252 (7) $[M]^+$, 234 (1) $[M-H_2O]^+$, 221 (79) $[M-CH_3O]^+$, 222 (11), 205 (6), 177 (6), 164 (12), 84 (18), 69 (15), 43 (100); $^1$H NMR (300 MHz, $CDCl_3$, TMS): $\delta_H$ = 6.99 (1 H, s, H-7), 6.95 (1 H, s, H-4), 6.58 (1 H, s, H-3), 3.96 (1 H, d, J = 10.5 Hz, H-11), 3.88 (6 H, s, 2X−OMe), 3.66 (1 H, d, J = 10.5 Hz, H-11), 1.56 (3 H, s, H-12); $^{13}$C NMR (75 MHz, $CDCl_3$, TMS) and DEPT: $\delta_C$ = 159.3 s (C-2), 103.1 d (C-3), 102.5 s (C-4), 147.9 s (C-5), 146.7 s (C-6), 95.5 d (C-7), 149.6 s (C-8), 120.0 s (C-9), 72.4 s (C-10), 69.2 t (C-11), 23.6 q (C-12), 56.4 q (−OMe), 56.6 q (−OMe).

#### *3.3.2. 2-(1-O-Feruloyl-2-hydroxyisopropyl)-5,6-dimethoxybenzofuran* (**2**)

Gum, $[\alpha]_D^{27}$ −100° (c, 0.5, $CHCl_3$), EIMS (rel. int.): m/z = 410 (7) $[M-H_2O]^+$, 395 (1), 385 (1), 379 (1), 354 (2), 318 (4), 290 (4), 275 (2), 259 (4), 231 (7), 218 (94), 205 (14), 203 (34), 132 (13), 83 (24), 69 (87), 57 (53), 55 (55), 43 (100); $^1$H NMR (400 MHz, $CDCl_3$, TMS): $\delta_H$ = 7.57 (1 H, d, J = 15.6 Hz, H-7′), 7.07 (1 H, d, J = 8.4 Hz, H-6′), 7.06 (1 H, s, H-2′), 7.03 (1 H, s, H-7), 6.95 (1 H, d, J = 8.4 Hz, H-5′), 6.92 (1 H, s, H-4), 6.60 (1 H, s, H-3), 6.27 (1 H, d, J = 15.6 Hz, H-8′), 4.58 (1 H, d, J = 11.6 Hz, H-11), 4.12 (1 H, d, J = 11.6 Hz, H-11), 3.91 (3 H, s, −OMe), 3.87 (3 H, s, −OMe), 3.78 (3 H, s, −OMe), 1.75 (3 H, s, H-12).

#### *3.3.3. 2-Isopropenyl-5,6-dimethoxybenzofuran* (**3**)

Colorless gum, EIMS (rel. int.): m/z = 218 (100) $[M]^+$, 203 (45) $[M-Me]^+$, 175 (13) $[M-Me-CO]^+$, 160 (9); $^1$H NMR (300 MHz, $CDCl_3$, TMS): $\delta_H$ = 7.02 (1 H, s, H-7), 6.96 (1 H, s, H-4), 6.53 (1 H, s, H-3), 5.67 (1 H, d, J = 1.8 Hz, H-11a), 5.08 (1 H, d, J = 1.8 Hz, H-11b), 3.92 (3 H, s, −OMe), 3.91 (3 H, s, −OMe), 2.16 (3 H, s, H-12); $^{13}$C NMR (75 MHz, $CDCl_3$, TMS) and DEPT: $\delta_C$ = 156.3 s (C-2), 103.1 d (C-3), 102.9 s (C-4), 149.7 s (C-5), 148.4 s (C-6), 95.2 d (C-7), 146.5 s (C-8), 120.9 s (C-9), 133.0 s (C-10), 111.7 t (C-11), 19.5 q (C-12), 56.4 q (−OMe), 56.5 q (−OMe).

#### *3.3.4. 2-Acetyl-5, 6-dimethoxybenzofuran* (**4**)

Colorless needles ($CHCl_3$), $^1$H NMR (400 MHz, $CDCl_3$, TMS): $\delta_H$ = 7.43 (1 H, s, H-3), 7.05 (1 H, s, H-7), 7.04 (1 H, s, H-4), 3.96 (3 H, s, −OMe), 3.93 (3 H, s, −OMe), 2.56 (3 H, s, H-11); $^{13}$C NMR (100 MHz, $CDCl_3$, TMS) and DEPT: $\delta_C$ = 147.9 s (C-2), 114.0 d (C-3), 102.9 s (C-4), 151.6 s (C-5), 151.8 s (C-6), 95.2 d (C-7), 152.2 s (C-8), 119.9 s (C-9), 187.8 s (C-10), 26.3 q (C-11), 56.4 q (2 × −OMe).

#### *3.3.5. Euparin* (**5**)

Yellow needles ($CHCl_3$), m.p. 135–136 °C, $^1$H NMR (400 MHz, $CDCl_3$, TMS): $\delta_H$ = 12.52 (1 H, brs, 6-OH), 7.89 (1 H, s, H-4), 6.97 (1 H, s, H-7),

6.53 (1 H, s, H-3), 5.75 (1 H, d, J = 1.6 Hz, H-11a), 5.18 (1 H, d, J = 1.6 Hz, H-11b), 2.67 (3 H, s, H-14), 2.09 (3 H, s, H-12); $^{13}$C NMR (100 MHz, $CDCl_3$, TMS) and DEPT: $\delta_C$ = 158.0 s (C-2), 102.6 d (C-3), 123.7 s (C-4), 117.0 s (C-5), 159.8 s (C-6), 99.6 d (C-7), 161.7 s (C-8), 122.2 s (C-9), 132.3 s (C-10), 113.9 t (C-11), 19.4 q (C-12), 204.1 s (C-13), 27.0 q (C-14).

### *3.4. Antibacterial assay*

The antibacterial assay was carried employing the cup-plate method. Chloramphenicol was used as a positive control. Strains of *Escherichia coli*, *Staphylococcus aureus* and *Bacillus subtilis* were cultured in beef broth and incubated at 37 °C for 24 h. After dilution of beef broth, the three bacteria were cultured in agar medium dishes respectively, six cups (8 × 10 mm) were put onto the dishes, and each tested compound (0.2 ml of 100 μg/ml) was added in to the cups under aseptic conditions. The dishes were cultured at 37 °C for 24 h. The zone of inhibition of the growth of bacteria, produced by diffusion of the compounds from the cup into the surrounding medium, was measured to evaluate the antibacterial activity.

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