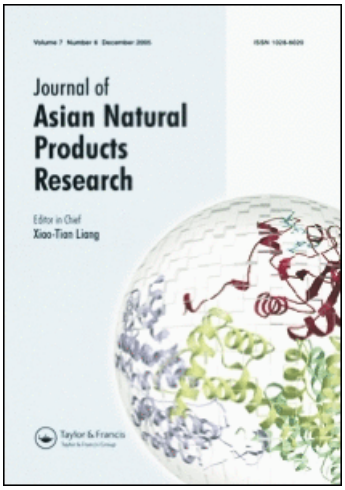

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Two new compounds from the roots of *Ligusticum chuanxiong*

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Two new compounds, named ligusticoside A (**1**), a novel phthalide derivative with a lactone ring, and 4-pentylcyclohex-3-ene-1α,2β-diol (**2**), were isolated from the rhizomes of *Ligusticum chuanxiong*. Their structures were determined by spectral methods (MS, IR, UV, 1D and 2D NMR). An X-ray diffraction experiment was performed to confirm the structure of compound **1**.

Keywords: *Ligusticum chuanxiong*; phthalide derivatives; ligusticoside A; 4-pentylcyclohex-3-ene-1α,2β-diol

1. Introduction

Ligusticum chuanxiong Hort., a member of the Umbelliferae family, is mainly cultivated in Sichuan Province of China, and its root is an important traditional Chinese medicine being used to treat headaches, anemia, and irregular menstrual cycles [1]. Previous chemical analyses on the roots of *L. chuanxiong* have reported the occurrence of phthalides, dimeric phthalides, terpenes, alkaloids, and organic acids [2–10]. In order to further understand its chemical diversities, the water extract of *L. chuanxiong* was phytochemically investigated to afford two new compounds, ligusticoside A (**1**) and 4-pentylcyclohex-3-ene-1α,2β-diol (**2**) (Figure 1). This paper describes the isolation and structural elucidation of the two new compounds. The structure of ligusticoside A (**1**) was also confirmed by an X-ray diffraction experiment.

2. Results and discussion

Compound **1**, $[\alpha]_D^4$ +72.33 ($c = 0.26$, MeOH), was obtained as a colorless prism (MeOH). The molecular formula was deduced to be $C_{18}H_{28}O_8$ by negative HR-ESI-MS at *m/z* 371.1704 $[M - 1]^-$, indicating the presence of five degrees of unsaturation in the molecule. The IR spectrum displayed absorptions ascribable to hydroxyl (3486 cm^{-1}) and ester carbonyl (1752 cm^{-1}) functionalities. The ^1H NMR spectrum (Table 1) exhibited two olefinic proton signals at δ_H 5.88 (dd, $J = 8.6$, 7.8 Hz), 5.69 (br d, $J = 8.6$ Hz), a triplet methyl group at δ_H 0.82 (t, $J = 6.5$ Hz), as well as an anomeric proton at δ_H 5.06 (1H, d, $J = 7.8$ Hz), indicating that there was a β-configuration sugar moiety in the molecule. The ^{13}C NMR (DEPT) spectrum (Table 1) showed 18 carbon signals: one ester carbonyl group (δ_C 177.5), one

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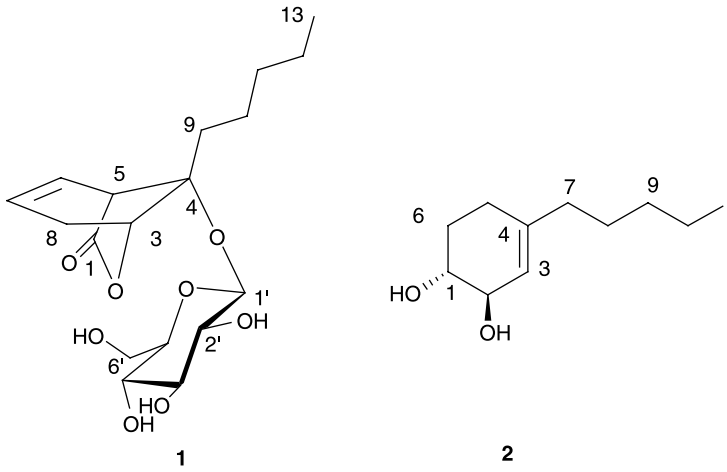

Figure 1. The structures of compounds **1** and **2**.

quaternary carbon (δ_C 85.7), four methines including two olefinic carbons at δ_C 128.8 and 122.4, and one oxygen-bearing methine at δ_C 81.2, and five aliphatic methylenes at δ_C 32.5, 31.6, 28.0, 22.8, and 22.6, along with one glucopyranosyl unit at δ_C 99.1, 74.8, 78.8, 72.2, 78.3, and 63.8 which was identified by the comparison of its NMR spectral data with those of a β-D-glucopyranosyl moiety of (4*S*)-*p*-menth-1-ene-4,7-diol 4-*O*-β-D-glucopyranoside [11].

Table 1. ^{1}H and ^{13}C NMR spectral data of compounds **1** and **2** in C_5D_5N[a].

	1		**2**	
No.	^{1}H (δ_H, mult., *J* in Hz)	^{13}C (δ_C)	^{1}H (δ_H, mult., *J* in Hz)	^{13}C (δ_C)
1	–	177.5 (s)	3.54–3.49 (m)	73.8 (d)
2	–	–	3.92–3.90 (m)	73.6 (d)
3	4.83 (br s)	81.2 (d)	5.27 (br s)	123.7 (d)
4		85.7 (s)	–	141.8 (s)
5	3.65 (d, 7.3)	48.8 (d)	2.07–2.04 (m)	28.1 (t)
6	5.88 (dd, 8.6, 7.3)	124.4 (d)	1.90–1.84 (m), 1.64–1.57 (m)	29.8 (t)
7	5.69 (br d, 8.6)	128.8 (d)	1.98–1.95 (t, 7.5)	37.9 (t)
8	2.53–2.40 (m)	31.6 (t)	1.46–1.35 (m)	28.4 (t)
9	1.98–1.81 (m)	28.0 (t)	1.35–1.24 (overlapped)	32.7 (t)
10	2.07–2.04 (m), 1.44–1.40 (m)	22.6 (t)	1.35–1.24 (overlapped)	23.6 (t)
11	1.24 (m)	32.5 (t)	0.89 (t, 6.9)	14.4 (q)
12	1.24 (m)	22.8 (t)		
13	0.82 (t, 6.5)	14.3 (q)		
1′	5.06 (d, 7.8)	99.1 (d)		
2′	4.04–3.92 (overlapped)	74.8 (d)		
3′	4.21 (t, 8.7)	78.8 (d)		
4′	4.04–3.92 (overlapped)	72.2 (d)		
5′	4.04–3.92 (overlapped)	78.3 (d)		
6′	4.53 (br d, 10.4), 4.15–4.10 (m)	63.8 (t)		

[a]Assignments were performed on the HSQC and HMBC spectra.

Except for three degrees of unsaturation due to one carbonyl group, one olefin, and one sugar, the remaining two degrees of unsaturation could be assigned to a bicyclic system in compound **1**, which was substantiated by 2D NMR (^{1}H–^{1}H COSY, HSQC, HMBC) analyses. The correlated proton signals H-5/H-6, H-6/H-7, H-7/H-8, and H-8/H-3 in the ^{1}H–^{1}H COSY spectrum of compound **1** (Figure 2), together with the correlations H-3/C-4, H-5/C-4, H-8/C-3, H-8/C-7, and H-5/C-6 in the HMBC spectrum (Figure 2), suggested the existence of a ring between C5—C6—C7—C8—C3—C4. The presence of *n*-pentyl (C-9 to C-13) could also be deduced by the ^{1}H–^{1}H COSY and HMBC analyses (Figure 2). The correlations between H-3 (δ_H 4.83), H-5 (δ_H 3.65) and C-1 (δ_C 177.5), H-9 (δ_H 1.98–1.81), H-1′ (δ_H 5.06) and C-4 (δ_C 85.7) in the HMBC spectrum (Figure 2) established that C-3 and C-5 were connected through an ester group and the β-D-glucopyranosyl and *n*-pentyl units were attached at C-4. Thus, compound **1** was characterized as shown in Figure 1.

The compound **1** is a new rearranged phthalide derivative featuring quaternary carbon glycoside. To further confirm the structure of compound **1**, an X-ray diffraction experiment was performed (Figure 3) through which the structure of compound **1** was corroborated as depicted in Figure 1.

Compound **2** was obtained as a colorless oil, $[\alpha]_D^{23}$ −2.01 ($c = 0.74$, MeOH). The molecular formula was determined to be $C_{11}H_{20}O_2$ by positive HR-ESI-MS at *m/z* 207.1353 $[M+Na]^+$, with two degrees of unsaturation. The IR spectrum showed the presence of the hydroxyl group at 3420 cm^{-1}. In the ^{1}H NMR spectrum, one triplet methyl signal at δ_H 0.89 (3H, t, $J = 6.9$ Hz, H-11) was observed, as well as an olefinic proton signal at δ_H 5.27 (1H, br s). The ^{13}C NMR (DEPT) spectrum (Table 1) suggested that there were two olefinic carbons, two oxygenated methines, six aliphatic methylenes, and one methyl in compound **2**. In consideration of two degrees of unsaturation in compound **2**, it could be deduced that compound **2** contained one ring, which was supported by the HMBC correlations (Figure 2) of H-2/C-1, H-2/C-3, H-5/C-4, H-6/C-1, and H-6/C-5, suggesting the existence of a C1—C2—C3—C4—C5—C6 (cyclohex-3-ene-1,2-diol) ring. The HMBC spectrum (Figure 2), combined with the ^{1}H–^{1}H

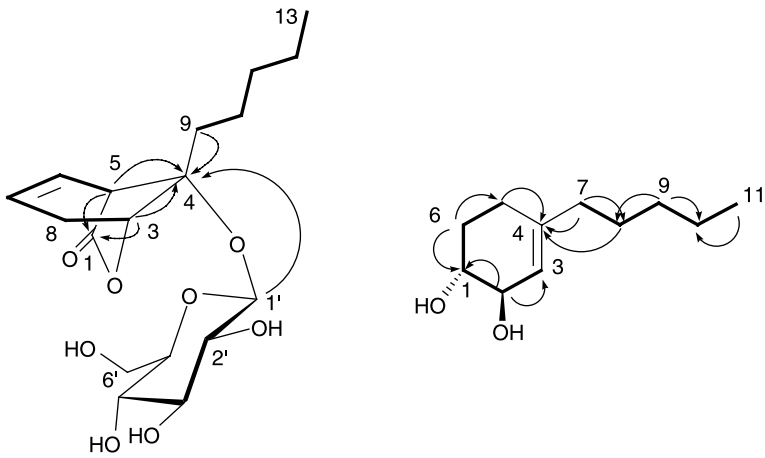

Figure 2. ^{1}H–^{1}H COSY (—) and selected HMBC (H → C) correlations of compounds **1** and **2**.

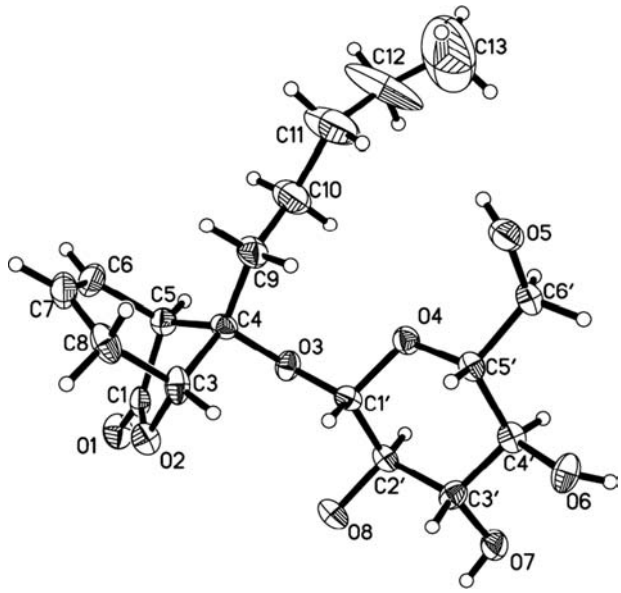

Figure 3. The X-ray crystal structure of compound **1**.

COSY experiment (Figure 2), also established the presence of an *n*-pentyl group in the molecule of compound **2**. The assignment of the *n*-pentyl group attached at C-4 was performed through the HMBC correlations between H-7, 8 and C-4. Thus, compound **2** was determined as 4-pentylcyclohex-3-ene-1,2-diol. In order to interpret the relative stereochemistry of compound **2**, a ROESY experiment was conducted. The absent correlation between H-1, assumed to be β-oriented, and H-2 in the ROESY spectrum demonstrated that H-1 and H-2 were in antarafacial. Thus, the structure of compound **2** was deduced as 4-pentylcyclohex-3-ene-1α,2β-diol (**2**).

3. Experimental

3.1 General experimental procedures

Optical rotations were carried out on a HORIBA SEPA-300 high-sensitive polarimeter. IR spectra were recorded on a Bio-Rad FTS-135 spectrometer with KBr pellets, ν in cm^{-1}. 1D and 2D NMR experiments were performed on a Bruker-AM-400 (^1H and ^{13}C at 400 and 100 MHz, respectively) spectrometer, with TMS as the internal reference, *J* in Hz. Mass spectra were recorded on a VG-Auto-Spec-3000 instrument. Silica gel (200–300 mesh) for column chromatography was obtained from Qingdao Marine Chemical Factory (Qingdao, China); D_{101} macroporous resins were obtained from Tianjin Pesticide Chemical Company (Tianjin, China); MCI gel CHP-20P (70–150 μm) was purchased from Mitsubishi Chemical Corporation (Tokyo, Japan); Lichrospher Rp-18 gel was purchased from Merck Chemical Ltd (40–63 μm; Darmstadt, Germany). Detection was performed by silica gel TLC on which 10% H_2SO_4 in EtOH was sprayed, followed by heating.

3.2 Plant material

The roots of *L. chuanxiong* used in this experiment were collected in 9 October 2004 in Sichuan Province, China, and identified as *L. chuanxiong* Hort. by Prof. Dr Li-Gong Lei from the Kunming Institute of Botany, Chinese Academy of

Sciences. A voucher specimen (No. 2004-10-09) has been deposited in the Kunming Institute of Botany, Chinese Academy of Sciences.

3.3 Extraction and isolation

The air-dried and powdered roots of *L. chuanxiong* (53 kg) were extracted with boiling water. The extract was obtained after evaporation of the solvent under vacuum, which was suspended in water and partitioned between EtOAc and *n*-BuOH, respectively, to afford the EtOAc and *n*-BuOH fractions. The *n*-BuOH fraction (1.6 kg) was applied onto a D_{101} macroporous resin column and eluted with H_2O, 20% EtOH, 50% EtOH, and 95% EtOH to give the corresponding fractions. The fraction eluted by 50% EtOH (400 g) was separated on a silica gel column ($CHCl_3$–MeOH–H_2O, 70:30:5 v/v) to give six fractions (1–6). Fraction 5 (16 g) was performed on an MCI CHP-20P gel column (MeOH–H_2O, 80:20) and subsequently purified on an Rp-18 column with an eluent of MeOH–H_2O (80:20) to yield compound **1** (100 mg). Fraction 3 (30 g) was subjected to a silica gel column (400 g, 200–300 mesh) eluted with $CHCl_3$–MeOH–H_2O (85:15:2 v/v) to furnish three subfractions (3.1–3.3). Subfraction 3.1 (1.5 g) was purified on an Rp-18 column (MeOH–H_2O, 85:15) followed by Sephadex LH-20 (MeOH) to obtain compound **2** (50 mg).

3.3.1 Ligusticoside A (*1*)

A colorless prism (MeOH); $[\alpha]_D^{14}$ + 72.33 ($c = 0.26$, MeOH); UV λ_{max} (MeOH) nm (log ε): 218 (3.26), 197 (3.06), 193 (3.06); IR (KBr) ν_{max} (cm^{-1}): 3486, 3363, 1752, 1428, 1350, 1098, 1035; ^{1}H NMR (C_5D_5N, 400 MHz) and ^{13}C NMR (C_5D_5N, 100 MHz) spectral data, see Table 1. FAB-MS (−) *m/z*: 371 $[M - 1]^-$ (90). HR-ESI-MS (−): 371.1704 $[M - 1]^-$ (calcd for $C_{18}H_{27}O_8$, 371.1705).

3.3.2 Crystallographic data for compound *1*

$C_{18}H_{28}O_8$, $M = 372.40$, orthorhombic, space group $P2$ (1), $a = 6.5066$ (14) Å, $b = 34.978$ (7) Å, $c = 8.2676$ (17) Å, $\beta = 90.00°$, $V = 1881.6$ (7) Å^3, $Z = 4$. Crystal dimensions ($0.22 \times 0.20 \times 0.14$ mm^3) were used for the measurement on a MAC DIP-2030K diffractometer with a graphite monochromator (ϕ and ω scans, 2θmax = 57.5°), Mo Kα radiation. The total number of independent reflections was 8407, of which 2882 were observed ($|F|^2 \geq 2\sigma|F|^2$). Final indices: $R_f = 0.0793$, $wR_2 = 0.1524$ ($w = 1/\sigma|F|^2$), $S = 0.916$. The crystal structure of compound **1** was solved by the direct method SHELXS-97 and expanded using geometrical calculations and difference Fourier techniques, refined by the program SHELXS-97 (Sheldrick, G. M. University of Gottingen: Gottingen, Germany, 1997) and the full-matrix least-squares calculations. Crystallographic data (excluding structure factors) for the structures in this paper have been deposited with the Cambridge Crystallographic Data Centre as supplementary publication No. CCDC 656110. Copies of the data can be obtained, free of charge, on application to CCDC, 12 Union Road, Cambridge CB2 1EZ, UK (fax: +44 (0)1223 336033 or e-mail: deposit@ccdc.cam.ac.uk; http://www.ccdc.cam.ac.uk).

3.3.3 4-Pentylcyclohex-3-ene-1α,2β-diol (*2*)

A colorless oil; $[\alpha]_D^{23}$ −2.01 ($c = 0.74$, MeOH); IR (KBr) ν_{max} (cm^{-1}): 3420, 1621, 1457, 1434, 1065, 1034, 909; ^{1}H NMR (C_5D_5N, 400 MHz) and ^{13}C NMR (C_5D_5N, 100 MHz) spectral data, see Table 1. EI-MS *m/z*: 184 $[M]^+$(5), 166 (2), 125 (13), 113 (13), 95 (18), 84 (100). HR-ESI-MS (+): 207.1353

$[M+Na]^+$ (calcd for $C_{11}H_{20}O_2Na$, 207.1360).

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