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Ligubenzocycloheptanone A, a Novel Tricyclic Butenolide with a 6/7/5 Skeleton from *Ligusticum chuanxiong*

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Ligubenzocycloheptanone A (**1**), a novel tricyclic butenolide with a 6/7/5-membered ring skeleton, was isolated from the rhizome of *Ligusticum chuanxiong*. Its unusual structure was determined using UV, IR, HRESIMS, 1D and 2D NMR data, X-ray diffraction crystallography and by the comparison of experimental and calculated electronic circular dichroism (ECD) spectra. **1** possessed a benzocycloheptanone core featuring butyrolactone, which is rarely observed in nature. A possible biosynthetic pathway was proposed. Ligubenzocycloheptanone A showed strong radical scavenging activity with an IC_{50} value of $2.3 \mu\text{M}$.

Ligusticum chuanxiong Hort (Umbelliferae) is mainly distributed in Sichuan province in China. Its dried rhizome is used in traditional Chinese medicine for the treatment of headaches, rheumatic arthralgia, menstrual disorders, swelling pain due to traumatic injury, and cardiovascular and cerebrovascular diseases^{1–3}. Phytochemical investigations of this plant have reported the presence of phenolic acids⁴, alkaloids⁵, and phthalides^{6–8}. Among these compounds, phthalides are usually considered to be the active constituents of *Ligusticum chuanxiong* with the effects of inhibiting vasoconstriction, antiproliferation, antioxidation, and antiinflammation^{9–12}. In our search for additional active constituents from this plant, a novel tricyclic butenolide with an uncommon 6/7/5-membered ring skeleton, named ligubenzocycloheptanone A (Fig. 1), was isolated from the rhizome of *Ligusticum chuanxiong*. Its structure was determined using UV, IR, HRESIMS, 1D and 2D NMR data, X-ray diffraction crystallography, and by comparing experimental and calculated electronic circular dichroism (ECD) spectra. Ligubenzocycloheptanone A may be derived biosynthetically from a phenyl acrylic acid and a hexose diacid. This may be useful for the exploration of a new synthetic approach to benzocycloheptanone derivatives. Ligubenzocycloheptanone A showed strong radical scavenging activity with an IC_{50} value of $2.3 \mu\text{M}$.

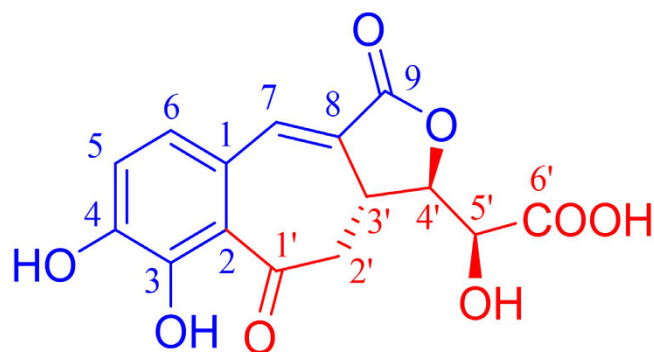
Results

Compound **1** was isolated as a light yellow amorphous powder. Its molecular formula, $C_{15}H_{12}O_8$, was established using HRESIMS with m/z 321.0593 $[M+H]^+$ (calcd 321.0605), which requires 10 degrees of unsaturation. Its IR spectrum exhibited absorptions at 3374.4 cm^{-1} (hydroxy), 1741.4 and 1667.2 cm^{-1} (carbonyl), and 1587.9 cm^{-1} (aromatic rings).

The ^1H NMR spectrum of **1** displayed two *ortho*-coupled aromatic protons at δ_{H} 7.06 (1H, d, $J=8.0$ Hz, H-5) and 7.13 (1H, d, $J=8.0$ Hz, H-6) and one olefinic proton with an allylic coupling at δ_{H} 7.29 (1H, d, $J=3.0$ Hz, H-7). Additionally, a characteristic downfield proton signal at δ_{H} 13.31 (1H, s, 3-OH) suggested the presence of an internal hydrogen bond. Furthermore, two oxymethines at δ_{H} 4.59 (1H, dd, $J=2.0, 6.5$ Hz, H-4') and 4.34 (1H, d, $J=2.0$ Hz, H-5'), one methine at δ_{H} 3.41 (1H, m, H-3'), and one methylene at δ_{H} 2.93 (1H, d, $J=13.0$ Hz, H-2' a) and 3.37 (1H, t, $J=13.0$ Hz, H-2' b) were observed in the ^1H NMR spectrum.

The ^{13}C NMR (Table 1) and HSQC spectra revealed a total of 15 carbon signals: one methylene, six methines, and eight quaternary carbons. Eight sp^2 carbons at δ_{C} 153.7 (C-3), 149.3 (C-4), 135.7 (C-7), 128.9 (C-8), 127.8 (C-6), 125.2 (C-1), 119.5 (C-5), and 118.5 (C-2) could be attributed to six aromatic carbons and two olefinic carbons, suggesting the presence of a tetrasubstituted benzene ring and an olefinic moiety. The substitution pattern

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Ligubenzocycloheptanone A (1)

Figure 1. Structure of ligubenzocycloheptanone A.

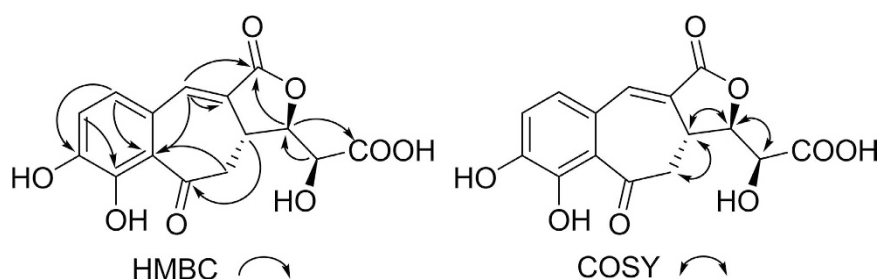


Figure 2. Key HMBC and COSY correlations of 1.

of the benzene ring was determined mainly by HMBC correlations (Fig. 2). In the HMBC spectrum, one aromatic proton at δ_{H} 7.06 (1H, d, $J = 8.0$ Hz, H-5) showed strong HMBC correlations with two carbons at δ_{C} 125.2 (C-1) and 153.7 (C-3), and the other one at δ_{H} 7.13 (1H, d, $J = 8.0$ Hz, H-6) showed correlations with three carbons at δ_{C} 118.5 (C-2), 149.3 (C-4), and 135.7 (C-7). In combination with the key correlations from the olefinic proton at δ_{H} 7.29 (1H, d, $J = 3.0$ Hz, H-7) to C-1, C-2, C-6, C-8, and C-9, a 3,4-dihydroxycinnamoyl moiety in **1** was clearly established.

In the ^1H - ^1H COSY experiment (Fig. 2), the correlations of H-3' with H-2' and H-4', and H-4' with H-3' and H-5', suggested the presence of $-\text{CH}_2\text{-CH-CH-CH}-$ in **1**. Combined with the HMBC correlations from H-2' and H-3' to C-1' at δ_{C} 204.8 and H-4' and H-5' to C-6' at δ_{C} 172.7, these results suggested the existence of a hexaric acid moiety. Furthermore, in the HMBC experiment, the correlation of H-2'a with C-2 confirmed that the hexaric acid moiety was connected to C-2 of the tetrasubstituted benzene ring by a C-C bond, while the HMBC correlations of H-3' with C-7 and C-8 and H-2' with C-8 suggested that C-3' was connected to C-8. A benzocycloheptanone skeleton was formed. The absence of other sp or sp^2 carbon signals and the remaining 1 degree of unsaturation implied that **1** contained a lactone ring, which was further confirmed by the HMBC correlation of H-4' with C-9.

The relative configuration of **1** was deduced through the ROESY experiment. In the ROESY experiment (Fig. 3), the key correlations of H-4' with H-2'a and H-2'b indicated the *trans* configuration of H-3' and H-4'.

The absolute configuration of C-3', C-4', and C-5' in **1** was established by comparing the experimental CD spectrum and the calculated ECD data (Supporting Information). Considering the *trans* configuration of H-3' and H-4', **1** has only two pairs of enantiomers (**1a**: 3'S,4'S,5'R and **1b**: 3'R,4'R,5'S; **1c**: 3'S,4'S,5'S and **1d**: 3'R,4'R,5'R). A systematic conformational analysis was performed for **1a** and **1c** using a MMFF94 molecular mechanics force field calculation. The optimized conformations of **1a** and **1c** were obtained using the time-dependent density functional theory (TD-DFT) method at the B3LYP/6-31G(d) level. The overall calculated ECD spectra of **1a**, **1b**, **1c**, and **1d** were generated by Boltzmann weighting of their lowest energy conformers. The overall pattern of the calculated ECD spectrum of **1b** attributable to the 3'R,4'R,5'S-isomer was consistent with the experimental data for **1** throughout the entire range of wavelengths under investigation (Fig. 4). Accordingly, the dihedral angles of H2'aC2'/C3'H3' (-71°), H2'bC2'/C3'H3' (170°), H3'C3'/C4'H4' (145°), and H4'C4'/C5'H5' (-59°) in the optimized conformation (Fig. 3) favored the coupling constants of H-2'a (d, $J = 13.0$ Hz), H-2'b (t, $J = 13.0$ Hz), H-4' (dd, $J = 2.0, 6.5$ Hz), and H-5' (d, $J = 2.0$ Hz).

Fortunately, the crystal of **1** was obtained in the solvent of MeOH:H₂O (9:1). Analysis of single crystal X-ray diffraction unambiguously proved the 3'R,4'R,5'S configurations for **1** (Fig. 5). Based on the above evidence, the structure of **1** was determined to be as shown in Fig. 1 and was named ligubenzocycloheptanone A.

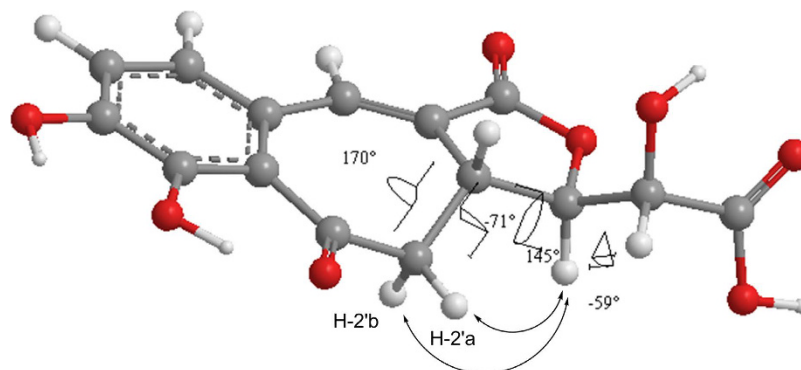


Figure 3. The optimized conformation and the key ROESY correlations of **1**.

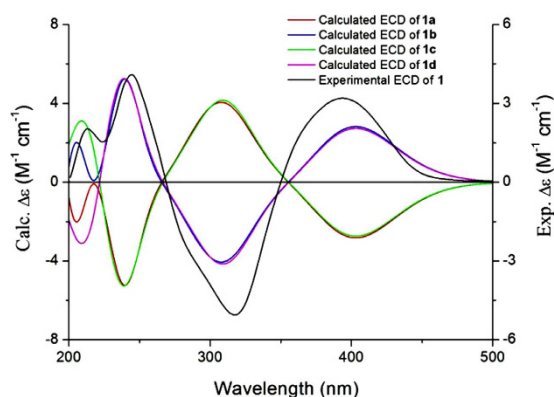


Figure 4. Experimental ECD spectrum of **1** and calculated ECD of **1a**, **1b**, **1c** and **1d** in MeOH.

Position	δ_{H}	δ_{C}	HMBC
1		125.2	
2		118.5	
3		153.7	
4		149.3	
5	7.06, d (8.0)	119.5	C-1, 3, 4, 6
6	7.13, d (8.0)	127.8	C-1, 2, 4, 5, 7
7	7.29, d (3.0)	135.7	C-1, 2, 6, 8, 9, 3'
8		128.9	
9		169.4	
1'		204.8	
2'a	2.93, d (13.0)	44.9	C-2, 8, 1', 3', 4'
2'b	3.37, t (13.0)		
3'	3.41, m	33.7	C-7, 8, 9, 1', 2', 4', 5'
4'	4.59, dd (2.0, 6.5)	81.9	C-9, 2', 3', 5', 6'
5'	4.34, d (2.0)	68.6	C-3', 4', 6'
6'		172.7	

Table 1. NMR data of compound **1** at 500 MHz in DMSO- d_6 .

Discussion

Recently, benzotropone and benzocycloheptanone derivatives have attracted substantial attention^{13–19}. Obviously, **1** represents a novel tricyclic butenolide with a benzocycloheptanone core. Its most intriguing feature from a biosynthetic pathway perspective is that **1** may be derived from a phenyl acrylic acid and a hexose diacid. 2-Deoxy-D-galactose and caffeic acid are considered to be precursors in a plausible biogenetic pathway of **1** (Fig. 6). One molecule of **I** formed from 2-deoxy-D-galactose by oxidation reacted with caffeic acid in a manner similar to a Friedel-Crafts acylation to give **II**, followed by dehydration, intramolecular nucleophilic addition, and hydride shift to give **V**. Finally, a five-membered lactone ring was formed by intramolecular nucleophilic

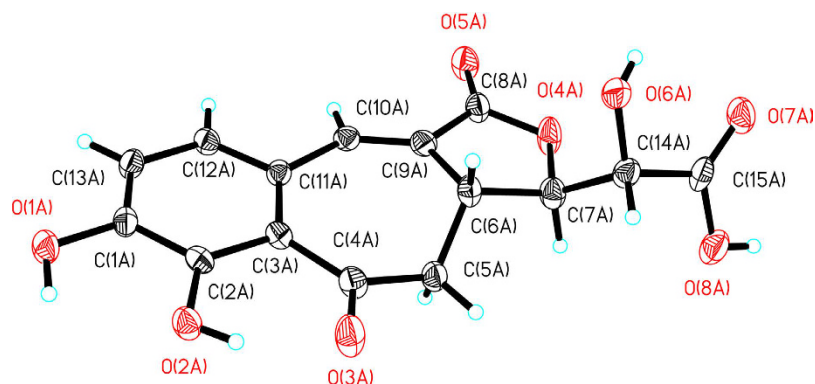


Figure 5. X-ray crystal structure of **1**.

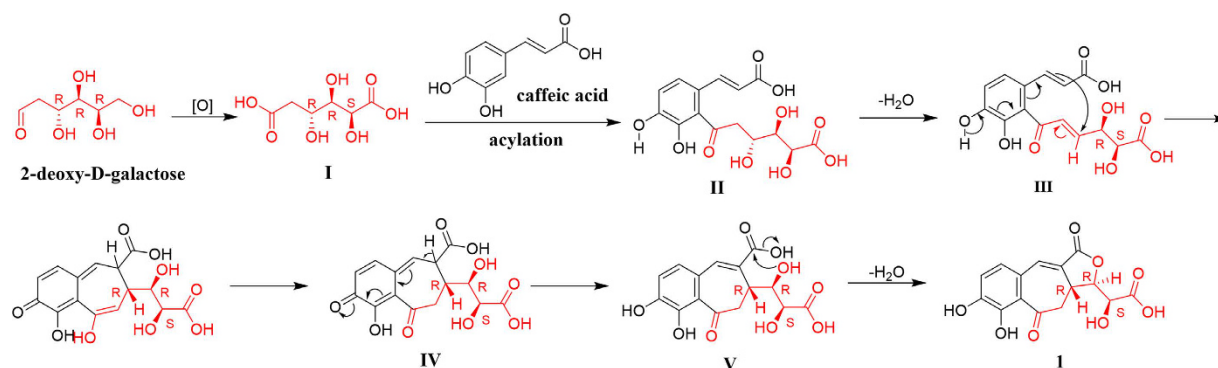


Figure 6. Plausible biogenetic pathway of **1**.

substitution. The key process was the formation of the cycloheptanone moiety by the addition of the double bond in the caffeic acid unit to an α,β -unsaturated ketone. This information may be of considerable interest for the development of a new and efficient synthetic approach to benzocycloheptanone derivatives. In the *in vitro* bioactivity assays²⁴ (Supporting Information), **1** showed strong radical scavenging activity with an IC_{50} value of $2.3 \mu M$, using ascorbic acid as a positive control.

Methods

General experimental procedures. The optical rotations were measured on a Jasco P-2000 polarimeter. IR spectra were recorded on an IMPACT 400 (KBr) spectrometer. 1H NMR (500 MHz), ^{13}C NMR (125 MHz), and 2D-NMR spectra were run on INOVA 500 spectrometers. HRESIMS were performed on Agilent 6520 LC-Q-TOF mass spectrometer (Agilent Technologies, Waldbronn, Germany). Column chromatography was performed with macroporous resin (Diaion HP-20, Mitsubishi Chemical Corp., Tokyo, Japan), Rp-18 ($50 \mu m$, YMC, Kyoto, Japan), Sephadex LH-20 (Pharmacia Fine Chemicals, Uppsala, Sweden). Preparative HPLC was carried out on a Shimadzu LC-6AD instrument with an SPD-20A detector, using a YMC-Pack ODS-A column ($250 \text{ mm} \times 20 \text{ mm}$, $5 \mu m$). HPLC-diode array detection (DAD) analysis was performed on an Agilent 1200 series system with an Apollo C18 column ($250 \text{ mm} \times 4.6 \text{ mm}$, $5 \mu m$, Alltech Corp., Kentucky, USA).

Plant material. The rhizomes of *Ligusticum chuanxiong* Hort. were collected from Pengzhou Town, Sichuan Province in People's Republic of China, in Jun 2013. The plant material was identified by Ma Lin (Institute of Materia Medica, Peking Union Medical College and Chinese Academy of Medical Sciences, Beijing 100050, P R. China). A voucher specimen (ID-S-2594) was deposited at the Institute of Materia Medica, Peking Union Medical College and Chinese Academy of Medical Sciences, Beijing 100050, People's Republic of China.

Extraction and isolation. Air-dried powder rhizome of *Ligusticum chuanxiong* Hort. (100.0 kg) were exhaustively extracted with 80% EtOH under refluxed conditions. After the solvent was evaporated under reduced pressure, the residue (23.1 kg) was suspended in water (50 L) and partitioned successively with EtOAc and *n*-BuOH. The *n*-BuOH-soluble portion (1300 g) was chromatographed on macroporous adsorption resins (HP-20) column, eluting with H_2O , 15% ethanol, 30% ethanol, 50% ethanol, and 95% ethanol to give fractions A–E, respectively. Fr. C (103.0 g) was chromatographed over reversed phase silica gel column eluting with H_2O -MeOH (from 100:0 to 0:100) to give 16 fractions (Fr. C-1–C-16). Fr. C-14 was further purified by Sephadex LH-20 column chromatography and preparative HPLC to yield compound **1** (19 mg).

ligubenzocycloheptanone A (**1**): $[\alpha]_D^{20}$ 44.5° (*c* 0.10, MeOH); IR ν_{\max} 3374, 2955, 2708, 1741, 1667, 1618, 1588, 1435, 1376, 1302, 1198, 1021 cm^{-1} ; UV (MeOH) λ_{\max} (log ϵ): 200 (4.04), 247 (4.18), 307 (3.81), 398 (3.82) nm; For ^1H and ^{13}C NMR spectroscopic data, see Table 1; HRESIMS *m/z*: 321.0593 (calcd for $\text{C}_{15}\text{H}_{12}\text{O}_8$, 321.0605).

References

- Chinese Pharmacopoeia Committee. *Pharmacopoeia of the People's Republic of China* Vol. 1, 38 (China Medical Science Press, 2010).
- Peng, C., Xie, X. F., Wang, L., Guo, L. & Hu, T. L. Pharmacodynamic action and mechanism of volatile oil from *Rhizoma Ligusticum chuanxiong* Hort. *Phytomedicine* **16**, 25–34 (2009).
- Chan, S. S. K., Choi, A. O. K., Jones, R. L. & Lin, G. Mechanisms underlying the vasorelaxing effects of butylidenephthalide, an active constituent of *Ligusticum chuanxiong*, in rat isolated aorta. *Eur J Pharmacol* **537**, 111–117 (2006).
- Li, S. L. *et al.* Simultaneous Analysis of Seventeen Chemical Ingredients of *Ligusticum chuanxiong* by On-Line High Performance Liquid Chromatography-Diode Array Detector-Mass Spectrometry. *Planta Med* **69**, 445–451 (2003).
- Kim, M. *et al.* Tetramethylpyrazine, a natural alkaloid, attenuates pro-inflammatory mediators induced by amyloid β and interferon- γ in rat brain microglia. *Eur J Pharmacol* **740**, 504–511 (2014).
- Naito, T. *et al.* Two phthalides from *Ligusticum chuanxiong*. *Phytochemistry* **31**, 639–642 (1992).
- Naito, T., Niitsu, K., Ikeya, Y., Okada, M. & Mitsuhashi, H. A phthalide and 2-famesyl-6-methyl benzoquinone from *Ligusticum chuanxiong*. *Phytochemistry* **31**, 1787–1789 (1992).
- Naito, T., Ikeya, Y., Okada, M., Mitsuhashi, H. & Maruno, M. Two phthalides from *Ligusticum chuanxiong*. *Phytochemistry* **41**, 233–236 (1996).
- Liang, M. J., He, L. C. & Yang, G. D. Screening, analysis and *in vitro* vasodilatation of effective components from *Ligusticum chuanxiong*. *Life Sci* **78**, 128–133 (2005).
- Lee, T. F., Lin, Y. L. & Huang, Y. T. Studies on antiproliferative effects of phthalides from *Ligusticum chuanxiong* in hepatic stellate cells. *Planta Med* **73**, 527–534 (2007).
- Qi, H. Y. *et al.* Senkyunolides reduce hydrogen peroxide-induced oxidative damage in human liver HepG2 cells via induction of heme oxygenase-1. *Chem Bio Interact* **183**, 380–389 (2010).
- Huang, J. *et al.* Anti-inflammatory ligustilides from *Ligusticum chuanxiong* Hort. *Fitoterapia* **91**, 21–27 (2013).
- Aïssa, C. & Fürstner, A. A Rhodium-Catalyzed C-H Activation/Cycloisomerization Tandem. *J Am Chem Soc* **129**, 14836–14837 (2007).
- Braga, D. *et al.* 1,4-Hydroxybiradical Behavior Revealed through Crystal Structure-Solid-State Reactivity Correlations. *J Am Chem Soc* **126**, 3511–3520 (2004).
- Satoh, T., Kimura, T., Sasaki, Y. & Nagamoto, S. *Synthesis* **44**, 2091–2101 (2012).
- Yadagiri, B. *et al.* Synthesis of novel building blocks of benzosuberone bearing coumarin moieties and their evaluation as potential anticancer agents. *Eur J Med Chem* **79**, 260–265 (2014).
- Zhang, T. X., Huang, X., Xue, J. & Sun, S. Ring expansion reaction of α -sulfonyl cyclic ketones via insertion of arynes into C–C: a facile and mild access to medium- and large-sized benzannulated carbocycles. *Tetrahedron Lett* **50**, 1290–1294 (2009).
- Beletskiy, E. V., Sudheer, C. & Douglas, C. J. Cooperative Catalysis Approach to Intramolecular Hydroacylation. *J Org Chem* **77**, 5884–5893 (2012).
- Blois, M. S. Antioxidant Determinations by the Use of a Stable Free Radical. *Nature* **181**, 1199–1200 (1958).

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Author Contributions

P.-C.Z. and Y.-N.Y. conceived and designed the study. B.H. and X.Z. isolated and identified these compounds. Z.-M.F., L.L. and J.-S.J. participated on the analysis these structures. Y.-N.Y. and P.-C.Z. drafted the paper with contributions from all authors.

Additional Information

Supplementary information accompanies this paper at <http://www.nature.com/srep>

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