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Communication

(±)-6-3'a,7-6'-Isowallichilide: A pair of enantiomeric phthalide dimers from *Ligusticum chuanxiong* with new 6-3'a,7-6' dimerization sites

Xin Fang, Qiang Ma, Yi Feng, Shuang Liang*

Innovation Research Institute of Traditional Chinese Medicine, Shanghai University of Traditional Chinese Medicine, Shanghai 201203, China

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ABSTRACT

(+)-6-3'a,7-6'-Isowallichilide and (−)-6-3'a,7-6'-isowallichilide, a pair of enantiomeric phthalide dimers featuring new 6-3'a,7-6' dimerization sites, were isolated from *Ligusticum chuanxiong*. The structures were elucidated by NMR spectroscopy and X-ray diffraction analysis. Furthermore, the absolute configurations were assigned using experimental and theoretical electronic circular dichroism methods. Their nitric oxide inhibition, antiplatelet aggregation and antioxidant activities were investigated.

Ligusticum chuanxiong Hort. (Umbelliferae), a well-known traditional Chinese medicine, has been used to treat cardiac arrhythmia, angina pectoris, stroke, and hypertension in clinical applications for millennia [1,2]. To date, more than 100 phthalides have been isolated and elucidated from this herb, and confirmed to possess significant pharmacological activities, such as anticoagulation, antiplatelet aggregation, antioxidant, anti-inflammation, antimicrobial, and antitumor effects [3–8]. Meanwhile, phthalide dimers with complex polycyclic skeletons and multiple chiral centers have become a hot research topic owing to their interesting chemical structures and significant pharmacological activities [9–15]. As part of a continuing effort to search for interesting phthalide dimers from *L. chuanxiong*, a pair of enantiomeric phthalide dimers with new dimerization sites, named (+)-6-3'a,7-6'-isowallichilide and (−)-6-3'a,7-6'-isowallichilide, were obtained (Fig. 1), and their nitric oxide inhibition, antiplatelet aggregation and antioxidant activities were screened.

6-3'a,7-6'-Isowallichilide (**1**) was obtained as colorless needle-like crystals. The molecular formula was determined to be $C_{25}H_{32}O_5$ (*m/z* 435.2144 $[M+Na]^+$, calcd. for 435.2147) by HR-ESI-MS, with ten degrees of unsaturation. In the IR spectrum, carbonyl groups were indicated by the strong absorption peaks at 1750 and 1702 cm^{-1}, while the presence of alkene bonds was shown by the absorption peaks at 1639 and 1607 cm^{-1}. The ^{13}C and DEPT NMR data showed 25 carbon signals, including eight C, six CH, eight CH_2, and three CH_3 groups. The ^1H and ^{13}C NMR spectroscopic data showed one carbonyl group [δ_C 210.5 (C-3')], two lactone groups [δ_C 169.6 (C-1) and 166.4 (C-1')], three alkene bonds [δ_H 7.15, 1H, d, J = 7.2 Hz (H-7'); δ_H 5.15, 1H, t, J = 8.0 Hz (H-8) and δ_C 111.9 (C-8), 126.8 (C-7a), 136.8 (C-7'a), 145.2 (C-7'), 148.3 (C-3), 153.7 (C-3a)], one methoxyl group [δ_H 3.72, 3H, s (H-1") and δ_C 52.1 (C-1")], and two methyl groups [δ_H 0.92, 3H, t, J = 7.2 Hz (CH_3-11), δ_C 14.0 (CH_3-11) and δ_H 0.91, 3H, t, J = 7.2 Hz (CH_3-11'), δ_C 14.0 (CH_3-11')] (Table 1). The above data characterized this compound as a phthalide dimer similar to wallichilide [16], except for the dimerization sites, which were further demonstrated by 2D-NMR. One of the dimerization sites was established by the key ^1H–^1H COSY cross peaks of H-4'/H-5'/H-6'/H-7, which was also supported by HMBC cross peaks from H-6' (δ_H 3.68) to C-4'/C-6/C-7/C-7a/C-7'/C-7'a. The other dimerization site was confirmed by the HMBC cross peaks from H-6 (δ_H 2.71) to C-4'/C-6'/C-7'a. Therefore, the planar structure of **1** was deduced to be a phthalide dimer linked at 6-3'a,7-6' through a Diels–Alder reaction. The relative configuration of **1** was inferred from the NOE correlations of H-7/H-5'a,6' and H-6'/H-5'a,7' (Fig. S1 in Supporting information). Single-crystal X-ray diffraction analysis unambiguously defined the relative configuration of **1** as 6β,7α,6'α (Fig. 1 and Table S1 in Supporting information). However, the crystal structure of **1** was found to exhibit space group $P2_{1/c}$, combined with a specific optical rotation of $[\alpha]_D^{20}$: 0 (MeOH), suggesting its crystallization as a racemate. Two monomer compounds, (+)-**1** and (−)-**1**, were separated by chromatography using a chiral column (Fig. S2 in Supporting information). The rotatory values of the two compounds were the inverse of each other, and the CD spectra showed the positive and negative cotton effects of the mirror-image relationship, further confirming that the compounds were

* Corresponding author.
E-mail address: ls7312@163.com (S. Liang).

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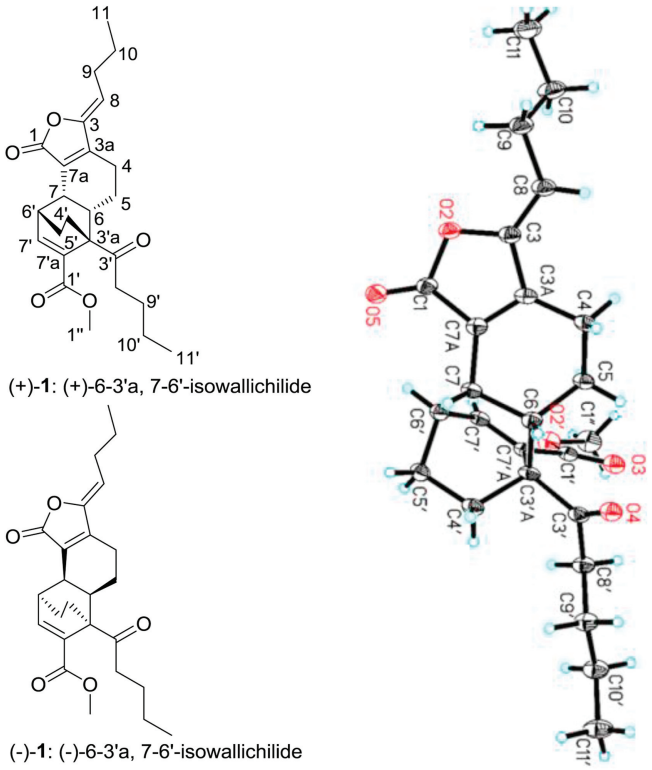


Fig. 1. Absolute configurations of (+)-**1** and (−)-**1** and X-ray crystallographic structure of **1**.

Table 1
NMR data for 6-3'a,7-6'-isowallichilide (400 MHz, $CDCl_3$; *J* in Hz).

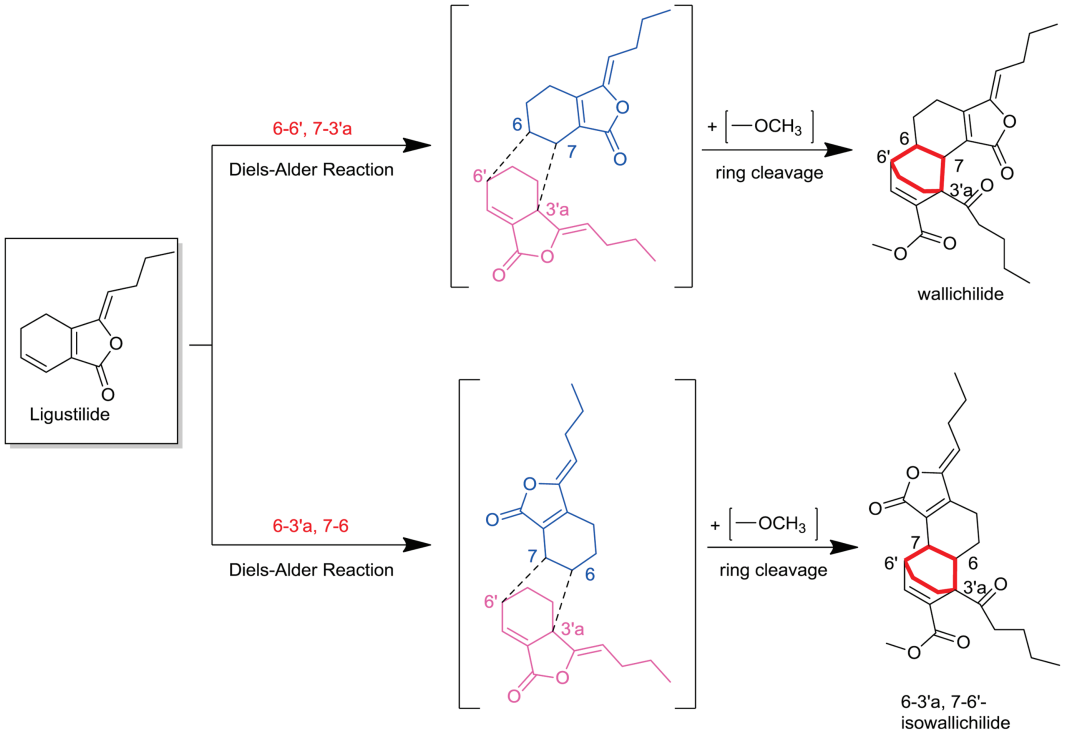
No.	δ_C	δ_H	No.	δ_C	δ_H
1	169.6		1'	166.4	
3	148.3		3'	210.5	
3a	153.6		3'a	56.9	
4	19.9	2.28, m, Ha	4'	29.0	1.78, td (12.4, 3.2), Ha
		2.09, m, Hb			1.62, m, Hb
5	22.7	2.25, m, Ha	5'	26.9	1.92, m, Ha
		0.92, m, Hb			1.46, m, Hb
6	41.0	2.71, m, 1H	6'	33.1	3.68, m, 1H
7	35.3	2.69, m, 1H	7'	145.2	7.14, d (7.2),1H
7a	126.8		7'a	136.8	
8	111.9	5.15, t (8.0), 1H	8'	40.4	2.37, m, 2H
9	28.1	2.32, d (7.2), 2H	9'	27.4	1.62, m, 2H
10	22.5	1.46, m, 2H	10'	22.8	1.32, m, 2H
11	14.0	0.92, t (7.2), 3H	11'	14.0	0.91, t (7.2), 3H
			1"	52.1	3.72, s, 3H

enantiomers (Fig. S15 in Supporting information). Quantum chemical calculations of the electronic circular dichroism (ECD) spectra were used to determine the absolute configurations of (+)-**1** and (−)-**1** as 6*S*,3'a*S*,7*S*,6'*R* and 6*R*,3'a*R*,7*R*,6'*S* by comparing experimental and predicted ECD curves (Fig. S4 in Supporting information). Consequently, the structures of compounds (+)-**1** and (−)-**1** were finally identified as (+)-6-3'a,7-6'-isowallichilide and (−)-6-3'a,7-6'-isowallichilide, respectively. To our knowledge, this is the first example of a phthalide dimer skeleton with 6-3'a,7-6' dimerization sites obtained from herbs. This discovery further enriches the known dimerization sites of phthalide dimers, which adds understanding of phthalide dimerization modes.

A plausible biosynthetic pathway of 6-3'a,7-6'-isowallichilide was proposed, as shown in Scheme 1. Similar to wallichilide formation, a ligustilide was flipped in the Diels-Alder reaction process, which changed the dimerization site to form 6-3'a,7-6'-isowallichilide.

According to the active characteristics of phthalide dimers, *in vitro* inhibitory effect against nitric oxide release in RAW 264.7 macrophages stimulated by lipopolysaccharides (LPS), antiplatelet aggregation induced by adenosine diphosphate (ADP) or arachidonic acid (AA), and oxidative damage models using SH-SY5Y and PC12 cells induced by H_2O_2 were selected to investigate the activity of **1**, (+)-**1**, and (−)-**1**. Among them, **1**, (+)-**1** and (−)-**1** displayed significant inhibitory effects against LPS-stimulated NO release with EC_{50} values of 4.76, 9.44, and 2.80 μmol/L, respectively (Table 2). Unfortunately, none showed significant antiplatelet aggregation and antioxidant activities.

Phthalide is an important active compound with an unstable structure from *L. chuanxiong* [17]. Previous studies have suggested that phthalide could translate into various dimers or trimers in plants and during harvesting, drying, storage, and decoction processes [18]. Compound **1** was also detected in the transformation of phthalide by HPLC-MS, which further confirmed that **1** was a biotransformation or artificial compound from phthalide. Furthermore, previous research has shown that phthalide dimers not only have similar biological activities to phthalide, but also have higher stability than phthalide [19,20]. This suggests that isolating new phthalide dimers, elucidating their structures, and evaluating their biology activity is important. Phthalide dimers are mainly polymerized through [4 + 2] or [2 + 2] cycloadditions, and usually have complex configurations with more than four chiral carbons, which makes elucidating their stereoscopic structures difficult. Our present investigation obtained optically pure phthalide dimers from the racemate by chiral column chromatography. The absolute configurations of (+)-**1** and (−)-**1** were established using ECD calculations, which provide reliable references for the structural elucidation of new dimers in future.



Scheme 1. Plausible biosynthetic pathway of 6-3'a,7-6'-isowallichilide.

Table 2
Inhibitory effects and cycotoxicity of **1**, (+)-**1**, and (−)-**1** against LPS-induced NO production in RAW 264.7 macrophages ($\bar{x} \pm S$, n = 3).

Compound	NO	Cycotoxicity
	EC_{50} value (μmol/L)	IC_{50} value (μmol/L)
Captopril	1.44	>100
Wallichilide	78.89	>100
1	4.76	29.992
(+)-**1**	9.44	34.914
(−)-**1**	2.80	27.542

Declaration of competing interest

The authors declare that they have no known competing financial interests or personal relationships that could have appeared to influence the work reported in this paper.

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Appendix A. Supplementary data

Supplementary material related to this article can be found, in the online version, at doi:https://doi.org/10.1016/j.cclet.2019.12.012.

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