

GABAergic Phthalide Dimers from *Angelica sinensis* (Oliv.) Diels

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Abstract: The methanol extract of *Angelica sinensis* (Oliv.) Diels roots (Dang Gui) has been shown to exhibit competitive binding to the GABA_A receptor, suggesting the presence of GABAergic ligands. Chromatographic fractionation of the methanol extract led to the isolation of two GABAergic dimeric phthalides **1** and **2**. Gelispirolide (**1**) was elucidated as a new phthalide dimer composed of a *Z*-ligustilide and a *Z*-butylidenephthalide unit on the basis of spectroscopic approaches including one- and two-dimensional NMR, HRESIMS and HRESIMS-MS. Compound **2** was identified as the known dimeric phthalide, riligustilide, by comparison of its spectroscopic data with literature values. Its dimeric linkage and stereochemistry were ascertained by a single crystal X-ray diffraction experiment. Both dimers **1** and **2** were found to be active in an *in vitro* GABA_A receptor-binding assay with IC₅₀ values of 29 and 24 μM, respectively. Copyright © 2006 John Wiley & Sons, Ltd.

Keywords: *Angelica sinensis*; dimeric phthalides; NMR; GABAergic activity; gelispirolide; riligustilide.

INTRODUCTION

The root of *Angelica sinensis* (Oliv.) Diels (Apiaceae), also known as Dang Gui, is one of the most popular traditional herbs used in Asia. The dried root of this plant has been regarded as a gynecological panacea in folklore due to its wide range of applications for women's disorders (China Pharmacopoeia Committee, 2000). As part of the collaborative research program in the UIC/NIH Center for Botanical Dietary Supplements Research for Women's Health, a phytochemical investigation of bioactive principles in *A. sinensis* has been conducted. Previously, we reported on the serotonergic effects of some isolates from *A. sinensis* (Deng *et al.*, 2006). In our current study, a preliminary *in vitro* screening experiment showed that a methanol extract of *A. sinensis* roots exhibited 97% inhibition on the binding of [³H] diazepam to the γ -aminobutyric acid A (GABA_A) neurotransmitter receptor at a concentration

of 50 μg/mL, thus implicating the presence of ligand(s) binding to GABA_A receptors in *A. sinensis* roots. The GABA_A receptor is regarded as an intriguing drug target, mediating anxiolytic, sedative, anticonvulsant, muscle relaxant and amnesic activities (Mohler *et al.*, 2001), and modulators of GABA_A receptors relate to the improvement of depression and anxiety-related symptoms that have been observed in premenstrual syndrome (PMS) and menopausal women (Barbaccia *et al.*, 2000).

In our search for GABAergic compounds, chromatographic fractionation was carried out on the non-polar petroleum ether and chloroform soluble extracts on the basis of an *in vitro* bioassay. Preliminary experiments revealed that the petroleum ether and chloroform soluble extracts exhibited a marked inhibitory effect on the binding of [³H] diazepam to the GABA_A receptor with an inhibition of 92 and 94%, respectively. In addition, the studies on non-polar extracts excluded the possibility of γ -aminobutyric acid itself being the underlying active principle, since this compound has been demonstrated to be present in polar aqueous extracts of this herb only (Chen and Zhang, 1984; Liao *et al.*, 1995). In our current investigation, two dimeric phthalides (**1**, **2**) were isolated from the combined GABAergic active petroleum ether and chloroform extracts. Compound **1**

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was determined to be a new phthalide dimer composed of a *Z*-ligustilide and a *Z*-butyridenephtalide unit on the basis of its spectroscopic data including one- and two-dimensional NMR, HRESIMS and HRESIMS-MS. Compound **2** was identified as the known dimeric phthalide, riligustilide, by comparison of its NMR and MS spectroscopic spectra with those reported in the literature (Kaouadji *et al.*, 1986), and further confirmed by a single crystal X-ray diffraction experiment. Compounds **1** and **2** were evaluated for their activity to bind to the GABA_A receptor, and shown to be active with IC₅₀ values of 29 and 24 μM, respectively. In addition to the two phthalide dimers, **1** and **2**, reported in this paper, the sources, monomer units and linkage of structural skeletons of 16 phthalide dimers from natural sources are summarised in Table 1. All of these dimers were isolated or identified from seven different

species in the family of Apiaceae, including *Ligusticum wallichii*, *L. officinale*, *L. chuanxiong*, *Meum athamanticum*, *A. sinensis*, *A. acutiloba* and *A. glauca*.

EXPERIMENTAL

General experimental procedures

Optical rotations were measured using a Perkin-Elmer (Wellesley, MA, USA) model 241 polarimeter at 20°C. IR spectra were taken on a Jasco (Portland, OR, USA) FT/IR-410 spectrometer, UV λ_{max} values were obtained from the PDA chromatograms of the isolates. One-dimensional (¹H NMR, ¹³C NMR and DEPT) and two-dimensional (COSY, HMQC, HSQC and HMBC) NMR data were recorded at room temperature on

Table 1 Overview of known phthalide dimers, which have been isolated from only three geni of the Apiaceae family (*Angelica*, *Ligusticum*, and *Meum*)

Number	Name	Source plants	Monomer unit(s)	Linkage
1	Gelispirolide	<i>A. sinensis</i>	<i>Z</i> -ligustilide and butyridenephtalide	6-8', 7-3'
2	Riligustilide	<i>L. wallichii</i> (Kaouadji <i>et al.</i> , 1986) <i>A. sinensis</i> (Lin <i>et al.</i> , 1998) <i>L. chuanxiong</i> (Naito <i>et al.</i> , 1991)	<i>Z</i> -ligustilide	6-8', 7-3'
3	Angelicide	<i>A. sinensis</i> (Chen and Zhang, 1984; Lin <i>et al.</i> , 1998)	<i>Z</i> -ligustilide	6-8', 7-3'
4	Levistolide A	<i>L. wallichii</i> (Kaouadji <i>et al.</i> , 1983) <i>L. officinale</i> (Cichy <i>et al.</i> , 1984) <i>M. athamanticum</i> (Kobayashi <i>et al.</i> , 1984) <i>A. sinensis</i> (Lin <i>et al.</i> , 1998) <i>A. acutiloba</i> (Tsuchida <i>et al.</i> , 1987) <i>L. chuanxiong</i> (Naito <i>et al.</i> , 1991)	<i>Z</i> -ligustilide	6-6', 7-3'a
5	Levistolide B	<i>L. officinale</i> (Cichy <i>et al.</i> , 1984) <i>A. sinensis</i> (Su <i>et al.</i> , 2005)	<i>Z</i> -ligustilide and <i>E</i> -ligustilide	6-6', 7-3'a
6	Senkyunolide O	<i>L. chuanxiong</i> (Naito <i>et al.</i> , 1991)	<i>Z</i> -ligustilide and <i>E</i> -ligustilide	6-6', 7-3'a
7	Senkyunolide P	<i>L. chuanxiong</i> (Naito <i>et al.</i> , 1991)	<i>Z</i> -ligustilide and senkyunolide	6-6', 7-3'a
8	<i>Z</i> -3,8-dihydro-6.6', 7.3'a-diligustilide	<i>L. wallichii</i> (Kaouadji <i>et al.</i> , 1986)	<i>Z</i> -ligustilide and senkyunolide	6-6', 7-3a'
9	Wallichilide	<i>L. wallichii</i> (Wang <i>et al.</i> , 1984)	<i>Z</i> -ligustilide	6-6', 7-3'a
10	Tokinolide A	<i>A. acutiloba</i> (Tsuchida <i>et al.</i> , 1987)	<i>Z</i> -ligustilide	6-3'a, 7-7'a
11	<i>Z</i> -3',8',3'a,7'a-tetrahydro-6,3',7,7'a-diligustilide-8'-one	<i>A. sinensis</i> (Su <i>et al.</i> , 2005)	<i>Z</i> -ligustilide	6-3', 7-7'a
12	Angeolide	<i>A. glauca</i> (Banerjee <i>et al.</i> , 1982)	<i>E</i> -ligustilide	3-3'a, 8-6'
13	Tokinolide B	<i>A. acutiloba</i> (Tsuchida <i>et al.</i> , 1987) <i>L. chuanxiong</i> (Naito <i>et al.</i> , 1991)	<i>Z</i> -ligustilide	3-3a', 8-6'
14	<i>Z,Z</i> -3.3',8.8'-Diligustilide	<i>A. sinensis</i> (Lin <i>et al.</i> , 1998)	<i>Z</i> -ligustilide	3-3', 8-8'
15	Angelicolide	<i>A. glauca</i> (Banerjee <i>et al.</i> , 1984)	<i>E</i> -ligustilide	3-3', 8-8'
16	E-232	<i>A. sinensis</i> (Hon <i>et al.</i> , 1990; Lin <i>et al.</i> , 1998)	<i>Z</i> -ligustilide and <i>E</i> -ligustilide	3a-8', 6-3'
17	Sinospirolide	<i>A. sinensis</i> (Deng <i>et al.</i> , 2006)	<i>Z</i> -ligustilide and butyridenephtalide	3-3'a, 8-7'a
18	Ansaspirolide	<i>A. sinensis</i> (Deng <i>et al.</i> , 2006)	<i>Z</i> -ligustilide and butyridenephtalide	3-3'a, 8-6'

Bruker (Billarica, MA, USA) DPX-400 and/or Avance-360 spectrometers using CDCl_3 as a solvent (operating at 400/360 MHz for ^1H NMR and 100/90 MHz for ^{13}C NMR, respectively). The digital resolution was always better than 0.1 Hz, equivalent to 0.0002 ppm (e.g. 32K real datapoints, 8 ppm spectral width for ^1H NMR), in the ^1H and 1.2 Hz, equivalent to 0.008 ppm (32K real data points, 250 ppm spectral width), in the ^{13}C domain. Chemical shifts (δ) were expressed in ppm with reference to tetramethylsilane (TMS) signals. All two-dimensional NMR experiments were performed using standard pulse sequences supplied by the vendor. A Micromass (Manchester, UK) Q-TOF-2 Quadrupole/Time-of-Flight mass spectrometer equipped with electrospray ion source (ESI) was employed for the measurement of exact mass, and for acquisition of tandem mass spectra at a collision energy of 20 eV using argon as the collision gas at a pressure of 2.0×10^{-5} mbar. The spectrometer was operated in positive ion mode. Vacuum liquid chromatography (VLC) was carried out on Merck (Darmstadt, Germany) silica gel 60 (60–400 mesh). Reverse-phase preparative HPLC was conducted on a Waters (Milford, MA, USA) 600 system aided by a photodiode array (PDA) detector and a Waters 717 plus autosampler, using a Watrex (Pittsford, NY, USA) GROM-Sil 120 ODS-4 HE semipreparative column (5 μm , 20×300 mm) with a flow rate of 6 mL/min. Analytical thin-layer chromatography (TLC) was performed on Merck TLC plates (250 μm thickness, KGF Si gel 60 and KGF RP₁₈ Si gel 60) with compounds visualised by spraying the dried plates with sulfuric acid–ethanol (5:95) or *p*-anisaldehyde–sulfuric acid–ethanol (2:2:96) followed by heating at 110°C.

Plant materials

The dried roots of *A. sinensis* (Oliv.) Diels were purchased from Kiu Shun Trading Ltd, Vancouver, Canada in 2000, and identified at UIC (lot no. 927-200110; Deng *et al.*, 2003). A voucher sample (BC 165) was deposited at the UIC/NIH Center for Botanical Dietary Supplements Research, Chicago, IL, USA.

Extraction and isolation

The milled roots (8 kg) of *A. sinensis* were macerated with 20 L of methanol for 24 h at room temperature, and percolated exhaustively with 40 L of methanol. The methanol percolate was concentrated to ca. 2700 mL. A 200 mL portion of the concentrated percolate was dried under vacuum to afford 39 g of residue, which was submitted for the GABA_A binding assay. The remaining 2500 mL of methanol extract was diluted with water to afford an approximately 85% aqueous methanol

extract, which was then partitioned with petroleum ether (2000 mL \times 3). The petroleum ether partition was dried by rotary evaporation, leading to an acquisition of 211 g of the dried petroleum ether residue. The aqueous methanol fraction was concentrated *in vacuo* to ca 2100 mL to remove methanol, and then subjected to further partitioning with chloroform (2000 mL \times 3) to give 57 g of chloroform residue after *in vacuo* evaporation. Both petroleum ether and chloroform partitioning residues showed marked GABAergic activities with 92 and 94% inhibitory effects at a concentration of 50 $\mu\text{g}/\text{mL}$ in the competitive GABA_A binding assay. Thus, the petroleum ether and chloroform partitions were combined for further fractionation and isolation of GABAergic ligands. The combined fraction was chromatographed by vacuum liquid chromatography (VLC) over a silica gel column (5 kg, 65–200 μm) and developed by a step gradient of petroleum ether–ethyl acetate–methanol (100:0:0 \rightarrow 0:0:100) to afford 13 fractions pooled on the basis of their TLC profiles (F1–F13). Fraction F5 (7.0 g) was further chromatographed over a silica gel column (600 g, 60–200 mesh) using VLC and eluted with gradients of petroleum ether–ethyl acetate–methanol (100:0:0 \rightarrow 0:0:100) to give 14 combined subfractions (F5-1 to F5-14). Subfraction F5-5 (4.5 g) was fractionated on a VLC column packed with silica gel (70 g, 230–400 mesh) eluting with a gradient solvent of 100% petroleum ether in ethyl acetate to 100% ethyl acetate, and afforded seven subfractions, F5-5-1 to F5-5-7. Reverse-phase preparative HPLC was employed for further separation of F5-5-4 (450 mg) with an isocratic solvent system of 78% methanol in water at a flow rate of 6 mL/min, resulting in the isolation of compounds **1** (3.8 mg, $t_{\text{R}} = 54$ min) and **2** (3.8 mg, $t_{\text{R}} = 59$ min).

Compound identification

Gelispirolide (1). Colourless oil. $[\alpha]_{\text{D}}^{20} 0^\circ$ (c 0.1, CH_2Cl_2); UV (LC-PDA) λ_{max} 278 nm; IR ν_{max} (CH_2Cl_2) cm^{-1} : 2929, 1761, 1680, 1620, 1079, 946, 752; ^1H NMR (CDCl_3 , 400 MHz) and ^{13}C NMR (CDCl_3 , 100 MHz), see Table 2; HRESIMS $[\text{M} + \text{H}]^+$ 379.1925 m/z calcd for $\text{C}_{24}\text{H}_{26}\text{O}_4$ (with 4.1 ppm error); ESIMSMS product ions m/z (% base peak) 361 (100), 191 (85), 189 (17), 171 (13).

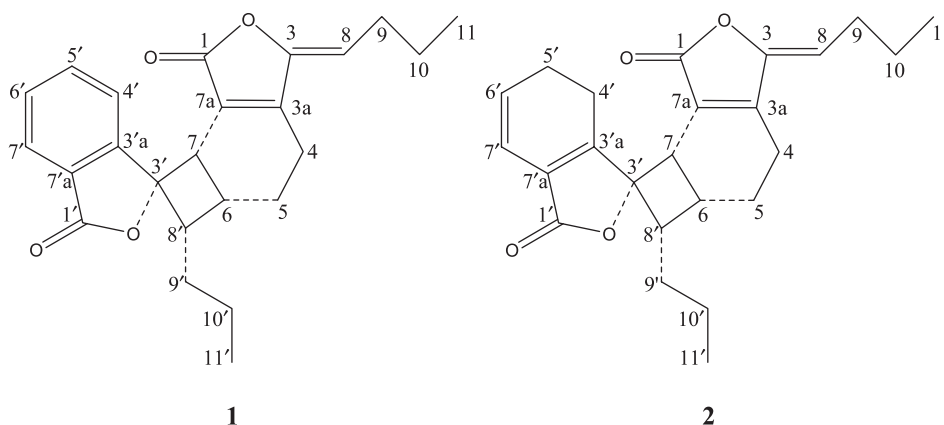
Riligustilide (2). Colourless crystals. m.p. 138°C; $[\alpha]_{\text{D}}^{20} 0^\circ$ (c 0.1, CH_2Cl_2); UV (LC-PDA) λ_{max} 283 nm; IR $\nu_{\text{max}}^{\text{KBr}}$ cm^{-1} : 1754, 1676, 1650, 1632, 1056, 720; ^1H NMR (CDCl_3 , 360 MHz) and ^{13}C NMR (CDCl_3 , 90 MHz), see Table 2; HRESIMS $[\text{M} + \text{H}]^+$ m/z 381.2076 calcd for $\text{C}_{24}\text{H}_{28}\text{O}_4$ (with 3.8 ppm error); ESIMSMS product ions m/z (% base peak) 191 (100); physical and spectroscopical data consistent with the literature (Naito *et al.*, 1991).

Table 2 ^1H and ^{13}C NMR spectral data for **1** and **2** in CDCl_3 (360/400 MHz and 90/100 MHz, respectively)

Position	1		2	
	$\delta_{\text{H}}^{\text{a}}$	$\delta_{\text{C}}^{\text{b}}$	$\delta_{\text{H}}^{\text{a}}$	$\delta_{\text{C}}^{\text{b}}$
1		168.6		168.6
3		149.4		149.1
3a		154.9		154.7
4	1H, Ha: 2.636, m; 1H, Hb: 2.232, m	20.0	1H, Ha: 2.468, m; 1H, Hb: 2.160, m	20.1
5	1H, Ha: 2.180, m; 1H, Hb: 2.114, m	21.3	1H, Ha: 2.205, m; 1H, Hb: 2.020, m	20.9
6	1H, 2.749, m	32.4	1H, 2.515, m	32.3
7	1H, 3.659, br d (8.2)	38.8	1H, 3.475, br d (7.7)	34.9
7a		122.7		122.2
8	1H, 5.231, t (7.8)	112.2	1H, 5.215, t (7.7)	112.2
9	2H, 2.336, m	28.2	2H, 2.336, ddd (15.3, 7.7, 4.2)	27.9
10	2H, 1.489, m	22.6	2H, 1.489, m	22.4
11	3H, 0.950, t (7.4)	14.3	3H, 0.954, t (7.4)	14.2
1'		170.2		170.3
3'		91.1		92.0
3'a		151.0		160.2
4'	1H, 7.639, ddd (7.6, 1.0, 1.0)	121.0	1H, Ha: 2.750, m; 1H, Hb: 2.572, m	19.6
5'	1H, 7.737, ddd (7.6, 7.5, 1.0)	134.8	1H, Ha: 2.612, m; 1H, Hb: 2.478, m	22.5
6'	1H, 7.529, ddd (7.6, 7.5, 1.0)	129.7	1H, 5.932, dt (9.6, 4.0)	128.8
7'	1H, 7.825, ddd (7.6, 1.0, 1.0)	125.0	1H, 6.173, dt (9.6, 1.9)	117.0
7'a		125.8		123.4
8'	1H, 3.136, dt (7.8, 7.8)	47.7	1H, 2.940, dt (7.8, 7.8)	43.9
9'	2H, 1.437, m	26.5	2H, 1.446, ddd (15.3, 7.8, 4.2)	26.3
10'	2H, 0.993, m	20.9	2H, 1.137, m	20.7
11'	3H, 0.762, t (7.4)	14.1	3H, 0.860, t (7.4)	13.9

^a Values in parentheses are the coupling constants J in Hz.

^b Signal assignments are aided by DEPT, HMQC/HSQC and HMBC.

**Figure 1** Chemical structures of the GABAergic dimeric phthalides gelispirolide (**1**) and riligustilide (**2**) from *Angelica sinensis*.

X-ray crystal structure analysis of riligustilide (2) (see **Fig. 2**). Formula $\text{C}_{24}\text{H}_{28}\text{O}_4$, $M = 378.45$, colourless plate $0.01 \times 0.10 \times 0.10$ mm, $a = 6.0766$ (5), $b = 12.6776$ (9), $c = 27.1207$ (16) Å, $\beta = 92.667$ (3)°, $V = 2087.0$ (3) Å³, $\rho_{\text{calc}} = 1.204$ g cm⁻³, $\mu = 0.650$ cm⁻¹, $Z = 4$, monoclinic,

space group $P2_1/c$ (no. 14), $\lambda = 1.54178$ Å, $T = 223$ K, 10,729 reflections collected 2510 unique, averaged ($R_{\text{int}} = 0.091$) 256 refined parameters, $R = 0.110$, GOF = 1.083, max. residual electron density 0.14 (−0.16) e Å⁻³.

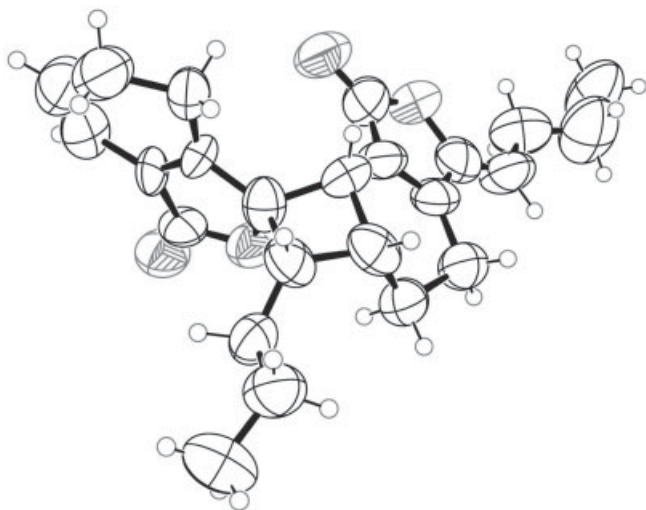


Figure 2 X-ray crystal structure (ORTEP drawing) of riligustilide (**2**).

GABA_A receptor binding assay

Bioassay materials. [³H] flunitrazepam (FNZ) was obtained from Amersham Biosciences (Piscataway, NJ, USA). Diazepam, polyethylenimine (PEI) and bovine albumin were purchased from Sigma (St. Louis, MO, USA).

Membrane preparation. Cerebral cortex was dissected from Sprague–Dawley rats. Brain tissue was washed with ice-cold phosphate-buffered saline (PBS) three times, homogenised by Polytron at setting 6 for 2 min on ice and centrifuged at 20,000g for 30 min at 4°C. The pellet was collected and stored at –80°C. Before use, the membrane pellet was thawed and resuspended in 50 mM Tris hydrochloric acid binding buffer (pH 7.4). Protein concentration was determined using Coomassie protein assay reagents (Pierce, Rockford, IL, USA) and bovine serum albumin as the standard.

GABA_A receptor binding assay. The assay was performed according to the method described previously (Tehrani and Barnes, 1997; Kumar *et al.*, 2003) with minor modifications. Briefly, membranes were incubated with 0.2 nM [³H] FNZ in 50 mM Tris hydrochloric acid buffer (pH 7.4) at 4°C for 90 min (100 µg protein/reaction). Nonspecific binding was determined in the presence of 10 µM diazepam. Reactions were terminated by rapid vacuum filtration with ice-cold PBS through GF/B filters presoaked with 0.2% polyethylenimine (PEI). Filter-bound radioactivity was determined by a liquid scintillation counter (Beckman Coulter Inc., Fullerton, CA, USA). Binding data, expressed in mean ± SD, were analysed using Prism 3.0 (GraphPad Software, San Diego, CA, USA). Diazepam was used as a positive control with its IC₅₀ value of 14.9 nM.

RESULTS AND DISCUSSION

Identification of the GABAergic dimeric phthalides

Liquid–liquid partitions of the initial GABAergic active extract with petroleum ether–aqueous methanol and chloroform–aqueous methanol successively led to active petroleum ether and chloroform fractions in an *in vitro* GABA_A binding assay. Repeated VLC fractionation of the pooled fractions over silica gel followed by reversed-phase HPLC afforded compounds **1** and **2**. Their structures were determined using spectroscopic data and a single crystal X-ray analysis.

The molecular formula of compound **2** was deduced as C₂₄H₂₈O₄ from a protonated molecule of *m/z* 381.2076 (calcd for 381.2066) in the product ion spectrum. A major fragment ion peak at *m/z* 191 in the ESIMSMS fragmentation spectrum suggested that **2** is a dimeric derivative of two ligustilide moieties. By comparison of its ¹H and ¹³C NMR spectral data (Table 2) with those values reported in the literature (Kaouadji *et al.*, 1986), as well as the analysis of its X-ray data (Fig. 2), compound **2** was unambiguously identified as riligustilide. In addition, the previously reported C-7 and C-8' assignments in the ¹³C NMR spectra (Kaouadji *et al.*, 1986) were corrected based on the analysis of the two-dimensional spectra of **2**. In the HMQC spectrum of **2**, the carbon signal at δ 34.9 was correlated to the proton signal at δ 3.475 [H-7, *brd* (7.7)], which in turn displayed a cross-peak with C-1 (168.6). At the same time, the H-10' at δ 1.137 exhibited long-range correlation with the carbon signal at δ 43.9 in the HMBC spectrum. These findings helped us to unambiguously assign the carbon signals at δ 34.9 and 43.9 to C-7 and C-8', respectively, contrary to the assignments proposed in the literature (Kaouadji *et al.*, 1986). The corrected NMR data is presented in Table 2. The stereochemistry of riligustilide (**2**) was established by X-ray crystal structure analysis (Fig. 2).

Compound **1** was obtained as a colourless oil. Its UV spectrum exhibited a maximum absorption at 278 nm, and the IR spectrum showed strong bands at 1761, 1680 and 1620 cm⁻¹. These data suggested the presence of an α,β-unsaturated lactone and an aromatic ring. The molecular formula of **1** was established as C₂₄H₂₆O₄ from a protonated molecule of *m/z* 379.1925 (calcd for 379.1909) in the HRESIMS spectrum, and 12 degrees of unsaturation in the structure were deduced. Two fragment ions of *m/z* 191 and 189 corresponding to two monomers of a ligustilide and a butyridenepthalide were observed in the product ion spectrum, indicating **1** as a dimeric phthalide. The ¹H NMR spectrum of **1** exhibited signals due to two methyl groups [δ 0.950 (3H, *t*, *J* = 7.4, Me-11) and 0.762 (3H, *t*, *J* = 7.4, Me-11')], an olefinic proton [δ 5.231 (1H, *t*, *J* = 7.8, H-8)], four aromatic protons [δ 7.737 (1H, *ddd*,

$J = 7.6, 7.5, 1.0$ Hz, H-5'), 7.825 (1H, *ddd*, $J = 7.6, 1.0, 1.0$ Hz, H-7'), 7.639 (1H, *ddd*, $J = 7.6, 1.0, 1.0$ Hz, H-4'), and 7.529 (1H, *ddd*, $J = 7.6, 7.5, 1.0$ Hz, H-6')], three methine protons [δ 3.659 (1H, *brd*, $J = 8.2$ Hz, H-7), 3.136 (1H, *dt*, $J = 7.8, 7.8$ Hz, H-8') and 2.749 (1H, *m*, H-6)], and six methylenes between δ 0.993 and 2.636. Analysis of the ^{13}C , DEPT and HSQC NMR spectra of **1** established the presence of 24 carbon atoms, comprising of two carbonyl groups (δ 170.2/C-1' and 168.6/C-1), five sp^2 quaternary carbons (δ 154.9/C-3a, 151.0/C-3'a, 149.4/C-3, 125.8/C-7'a and 122.7/C-

7a), five sp^2 methines (δ 134.8/C-5', 129.7/C-6', 125.0/C-7', 121.0/C-4' and 112.2/C-8), four sp^3 quaternary carbons (δ 91.1/C-3', 47.7/C-8', 38.8/C-7 and 32.4/C-6), six sp^3 methylenes (δ 28.2/C-9, 26.5/C-9', 22.6/C-10, 21.3/C-5, 20.9/C-10' and 20.0/C-4) and two sp^3 methyls (δ 14.3/C-11 and 14.1/C-11'). The ^1H and ^{13}C NMR spectra of **1** were almost superimposable on the spectra recorded for **2**, with the major difference being the presence of aromatic signals in **1**. By a comparison of their NMR data (Table 2), it was readily observed that two methylene groups (δ 2.750, 2.572/19.6

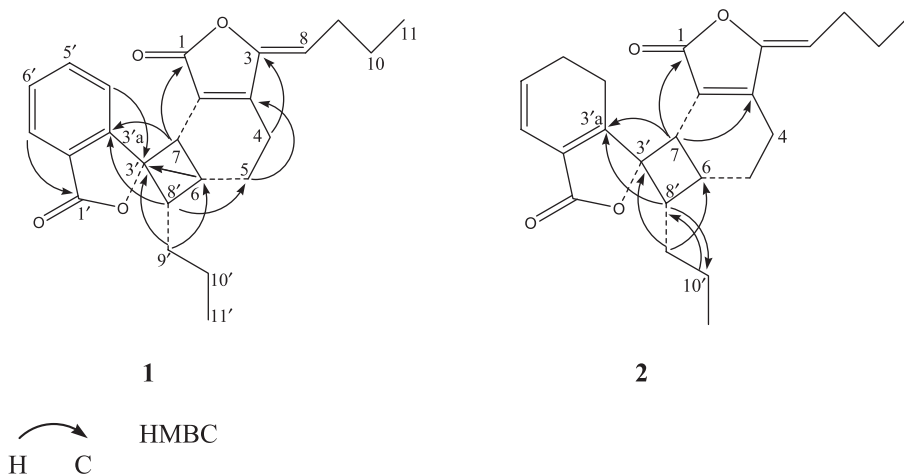


Figure 3 Key HMBC correlations for compounds **1** and **2**.

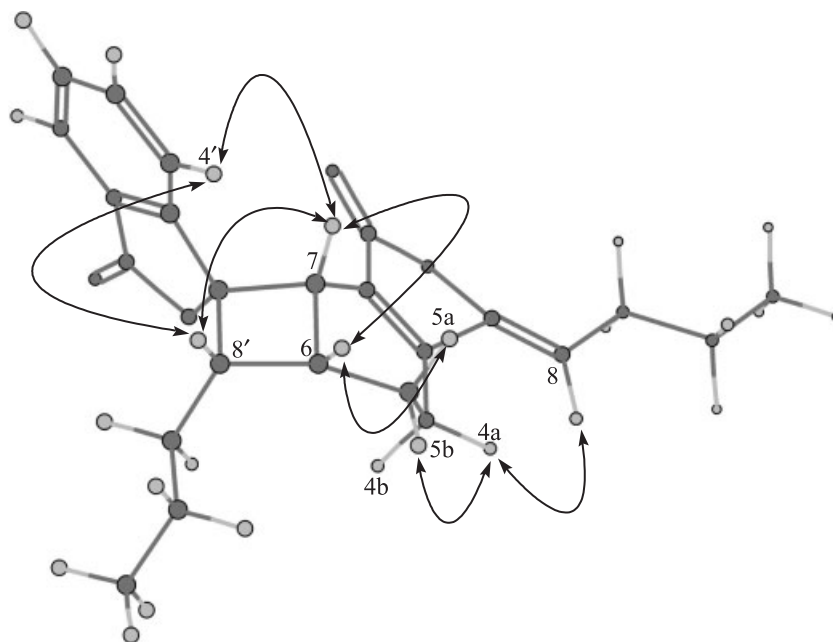


Figure 4 Key NOE correlations (double arrows) for compound **1** from a two-dimensional NOESY map led to the establishment of its relative configuration. The presence of cross-peak correlations between H-6 and H-7, and among H-7, H-4' and H-8' suggested that these protons are in the same plane above the cyclobutane ring. The *Z*-form of the butylidene side chain in the ligustilide moiety was determined by the NOESY correlation between H-8 and H-4a. The configuration of the butyl group was also assigned as a *Z*-form according to an observed correlation between H-4' and H-8'. The structure of **1** was elucidated as the previously unknown phthalide dimer of a *Z*-ligustilide and a *Z*-butylidenephthalide moiety.

and 2.612, 2.478/22.5) present in **2** were missing, with the emergence of an additional double bond (δ 7.639/121.0 and 7.737/134.8) to form an aromatic ring in **1**. This was consistent with the MS data (one more degree of unsaturation and two less protons in the formula of **1**, and fragment ion peaks at m/z 191 and 189). Therefore, it was concluded that **1** was composed of a ligustilide and a butyridenepthalide unit, rather than two units of ligustilide. The linkage between the two units was unambiguously assigned by the following HMBC correlations: H-6 \rightarrow C-3', H-7 \rightarrow C-3'a, H-8' \rightarrow C-5, and H-9' \rightarrow C-6 (Fig. 3). The *Z*-form of the butyridene side chain in the ligustilide moiety was determined by the NOESY correlation between H-8 and H-4a. The relative configuration of **1** was established by the NOESY data by the presence of cross-peak correlations between H-6 and H-7, and among H-7, H-4' and H-8' (Fig. 4). On the basis of the foregoing evidences, the structure of **1** was elucidated as a novel phthalide dimer formed by one *Z*-ligustilide and one *Z*-butyridenepthalide moiety, and was trivially named gelispirolide. Based on the lack of optical activity, both isolates appear to be racemic mixtures.

GABAergic and other biological activity of dimeric phthalides

Compounds **1** and **2**, upon isolation from GABA_A-ergic primary and secondary fractions, were tested in a GABA_A competitive binding assay *in vitro*. The results showed that **1** and **2** exhibited inhibitory effects on the binding of [³H] diazepam to the GABA_A receptors with IC₅₀ values of 29 and 24 μ M, respectively. These results at least partially suggest the rationale of sedative effect of *A. sinensis* for menopausal women. Taking into account the very recently reported serotonergic effects of some isolates from *A. sinensis* (Deng *et al.*, 2006), which were isolated from the same plant material used in this study, the present report of two GABA_A active phthalides establishes both a new pharmacological target for pharmaceutically relevant *Angelica* species and a new structure-activity link to a well-known group of *Angelica* phytoconstituents. Since the observed GABA_A binding potencies of **1** and **2** do not quantitatively match the activity of the crude extract, more studies are required to evaluate the presence of further active principles and/or potential synergy in *A. sinensis* preparations.

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