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A Pair of Windmill-Shaped Enantiomers from *Lindera aggregata* with Activity toward Improvement of Insulin Sensitivity

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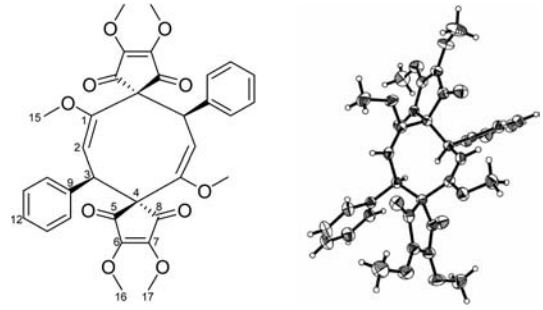
ABSTRACT



(+)-Linderaspirone A and (−)-linderaspirone A, a pair of natural windmill-shaped enantiomers, were isolated from the traditional Chinese medicine plant *Lindera aggregate* by HPLC using a chiral column, achieving over 98% ee. Their structures and absolute configurations were determined on the basis of extensive analysis of NMR spectra, crystal X-ray diffraction, and calculation of the optical rotations (OR). They have an unprecedented carbon skeleton and showed significant activity against glucosamine-induced insulin resistance.

The root of *Lindera aggregata* (Sims) Kosterm. [synonym: *Lindera strychnifolia* (Sieb. et Zucc.) Fern.-Vill.] (Lauraceae), Radix Linderae, has been traditionally used in China (Wu Yao) and Japan (Uyaku) as an analgesic and antispasmodic.[1] Sesquiterpenoids,[2] alkaloids,[3] flavonoids,[4] lignans,[5] butanolides,[6] unique cyclopentenedione derivatives,[7] and bilinderone[8] were isolated in previous chemical investigations on the *Lindera* species. Pharmacological

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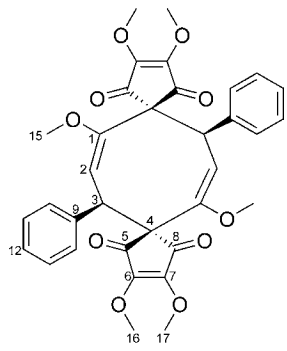
studies on this plant have shown various bioactivities such as improvement of insulin sensitivity,[8] antioxidation,[9] protection against postischemic myocardial dysfunction,[10] antiviral (SARS-associated coronavirus) activity,[11] cytotoxicity,[1] and slowing down the progression of diabetic nephropathy in db/db mice.[12]

As part of our effort to assemble a large-scale natural compound library of thousands of structures derived from plants and micro-organisms,[13] we report herein the structure elucidation and assignment of the absolute configurations to a pair of enantiomers (**1**) isolated from the root of *Lindera aggregate* during our further investigation. These enantiomers have an unprecedented spirocyclopentenedione-containing and windmill-shaped carbon skeleton, for which we propose the name "linderaspirone A". It showed significant activity against glucosamine-induced insulin resistance in HepG2 cells at a concentration of 1 μg/mL. The two enantiomers were separated by HPLC using a chiral column, achieving over 98% ee. The absolute configurations were determined by computational methods via calculation of the optical rotations. To the best of our knowledge, no other structure with this skeleton has been reported to date.



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Air-dried, powdered roots (800 g) of *L. aggregata* were soaked in 95% ethanol (3 × 1.5 L, 3 days each) at room temperature and filtered. The filtrate was concentrated in vacuo to give a residue (~50 g), and linderaspirone was isolated by silica gel column chromatography with gradient elution systems of petroleum ether/acetone (from 95:5 to 0:100). The fraction eluted with 30% acetone was repeatedly separated and purified by silica gel (petroleum ether/acetone from 10:1 to 7:1) and Sephadex LH-20 ($CHCl_3$/MeOH, 1:1) to give a subfraction that mainly contained the target substance, which was further isolated and purified by preparative HPLC (62%−75% MeCN in H_2O over 20 min, 5 mL/min, 300 nm) to yield linderaspirone A (**1**, 15 mg, t_R = 7.1 min). The retention time for analysis-type HPLC was 5.7 min (50%−100% MeCN in H_2O over 5 min followed by 100% MeCN to 10 min, 1 mL/min, 25 °C).

Compound **1** was obtained as yellow crystals. The ion at *m*/*z* 623.1883 in its positive high-resolution ESI mass spectrum (*m*/*z* calcd for $[M + Na]^+$ 623.1893) gave a molecular formula of $C_{34}H_{32}O_{10}$, which was in accordance with the ^1H and ^{13}C NMR spectroscopic data (Table 1). The

Table 1. NMR Spectroscopic Data for Linderaspirone (**1**) in $CDCl_3$

no.	^1H NMR	^{13}C NMR	HMBC (from H to C)
1		153.2 (C)	
2	5.42 (1H, d, 10.3)	102.1 (CH)	1, 3, 4, 9
3	5.54 (1H, d, 10.3)	43.2 (CH)	1, 2, 4, 5, 8, 9, 10, 14
4		66.5 (C)	
5		194.7 (C)[b]	
6		153.9 (C)[c]	
7		151.8 (C)[c]	
8		192.2 (C)[b]	
9		138.0 (C)	
10, 14	7.27−7.30 (2H, m)	129.6 (CH)	3
11, 13	7.27−7.30 (2H, m)	128.4 (CH)	
12	7.22−7.26 (1H, m)	127.7 (CH)	
15	3.50 (3H, s)	55.4 (CH_3)	1, 2*
16	3.94 (3H, s)[a]	59.5 (CH_3)	6
17	3.89 (3H, s)[a]	59.5 (CH_3)	7

[a] Interchangeable. [b] Interchangeable. [c] Interchangeable. * Weak but significant four-bond correlation.

IR spectrum exhibited the absorption bands of unsaturated ketone and phenyl groups at 3008, 1673, 1655, 1620, 1497 cm^{-1}. The ^1H NMR spectrum of **1** suggested the presence of a monosubstituted benzene ring at δ_H 7.27−7.30 (4H, m) and 7.22−7.26 (1H, m) and three methoxyls at δ_H 3.50, 3.89, and 3.94 (each 3H, s), as well as two intercoupling protons at δ_H 5.42 and 5.54 (each 1H, d, J = 10.3 Hz). The ^{13}C NMR spectrum showed a total of 17 carbon signals, including two unsaturated ketone carbonyl resonances at δ_C 192.2 and 194.7, a set of signals due to a monosubstituted phenyl at δ_C 127.7 (d), 128.4 (2 × d), 129.6 (2 × d), and 138.0 (s), four olefinic carbons at δ_C 102.1 (d), 151.8 (s), 153.2 (s), and 153.9 (s), and three methoxyls at δ_C 55.4 (q) and 59.5 (2 × q), as well as two remaining carbons at δ_C 43.2 (d) and 66.5 (s). The above information gave us a clear hint that this molecule possessed a highly symmetrical nature. Detailed interpretation of the HMBC correlations (Figure 1) allowed the construction of the half fragment (**I**) of **1**. However, two potential structures, **IIa** and **IIb** (Figure 1), generated by "head−tail" and "head−head" linkages of **I**, respectively, could not be excluded by the NMR study alone.

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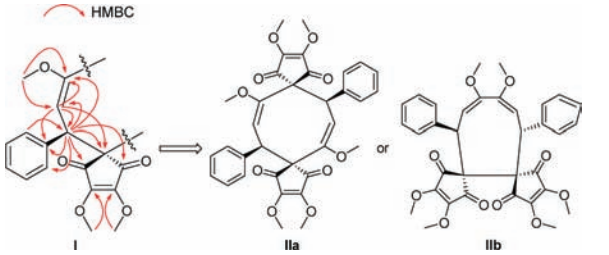

Figure 1. Structural fragment (I) and two possible structures (IIa and IIb) of linderaspirone A.

Slow and careful recrystallization of **1** from $CHCl_3$/MeOH furnished single crystals suitable for X-ray analysis. Consequently, we applied single-crystal X-ray diffraction to determine the final structure and relative configuration, which revealed the molecule as **IIa**, an unprecedented windmill-shaped structure that we named linderaspirone A (**1**). The perspective presentation of the final structure is shown in Figure 2. It is worth noting that the crystals of **1** had the

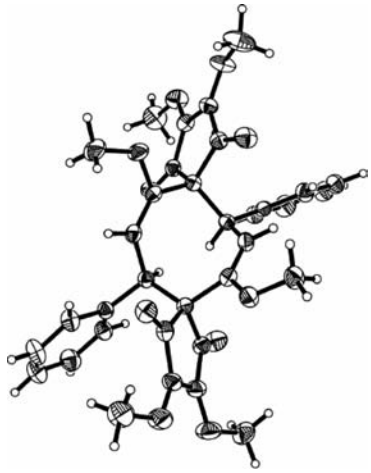

Figure 2. X-ray crystal structure of linderaspirone (**1**).

space group *P*2(1)/*n*, indicative of a racemic nature as also evidenced by the lack of optical activity. Subsequent HPLC of **1** on a chiral phase led to the separation of two enantiomers, (+)-**1** and (−)-**1**, which were opposite in terms of optical rotation (OR) values but with identical NMR spectra.

The measured ORs are +224 and −233.2 for the two respective enantiomers. OR computations were performed for linderaspirone A (3*S*,3′*S*) at B3LYP/6-311++G(2d,p)//B3LYP/6-311+G(d), with a calculated OR value of +301.[14−18] Accordingly, the absolute configurations for (+)-linderaspirone A and (−)-linderaspirone A are 3*S*,3′*S* and 3*R*,3′*R*, respectively.

The biogenetic route to linderaspirone A was proposed to be a formation by a [4 + 4] cycloaddition from the monomer methyl linderone isolated from the same plant. Transition-state (TS) computations were performed using a model molecule (Figure 3). Formation was suggested to occur via

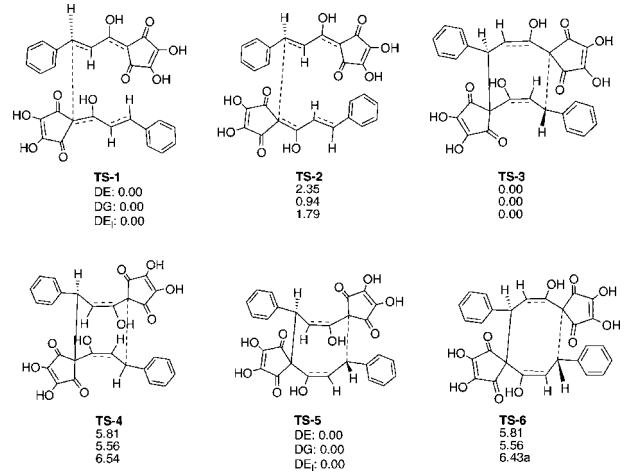

Figure 3. Transition-state (TS) computations using the model molecule.

a stepwise [4 + 4] cycloaddition. The first reaction step can occur by two pathways (**TS-1** and **TS-2**, Figure 3). The lower TS energy was found for **TS-1**. The relative total electronic energy (ΔE), relative free energy (ΔG) at the B3YP/6-31G(d) level, and the relative energy catalyzed by an enzyme (ΔE_l) obtained at the B3LYP/6-311++G(2d,p)//B3YP/6-31G(d) level are summarized in Figure 3. After the first step, there are four possible cycloadditions; however, two will be of the same energy as the other two. The TS structures and energies are illustrated in Figure 3. Only the relative energy in **TS-3** (*S*,*S*) or **TS-5** (*R*,*R*) is lower. It is not possible to form enantiomers (*S*,*R*) (via **TS-4)** or (*R*,*S*) (via **TS-6)**.

Linderaspirone A[19,20] was isolated from a famous traditional Chinese medicine plant belonging to the genus *Lindera* that has been used for thousands of years and investigated by different research groups around the world. It is surprising that linderaspirone A represents the first member of an

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(19) Linderaspirone (**1**): yellow crystals ($CHCl_3$/MeOH); mp 181−182 °C; $[\alpha]^{22}_D$ 0 (*c* 0.11, $CHCl_3$); UV λ_{max} (MeOH) (log ε) nm 300 (4.20); IR (KBr) ν_{max} cm^{-1} 3008, 2955, 1673, 1655, 1620, 1497, 1460, 1435, 1331, 1195, 1143; EI-MS *m/z* 600 $[M]^+$ (25), 585 (9), 568 (25), 553 (15), 536 (12), 356 (76), 341 (55), 325 (42), 313 (86), 297 (91), 279 (80), 269 (69), 244 (100), 215 (73); ESI-MS (positive) *m/z* 623 $[M + Na]^+$, 1224 $[2M + Na + H]^+$; HR-ESI-MS (positive) *m/z* 623.1883 (calcd for $C_{34}H_{32}O_{10}Na$ 623.1893).

unprecedented class of highly symmetrical spirocyclopentene diones. Although it shares its structural features with the cyclopentenedione derivative methyl linderone, it has a backbone with 34 carbon atoms that includes two unique spiro rings, which is unprecedented in the field of natural products. All of the uncommon structural features present in this molecule exhibit an unusual metabolite profile that suggests a unique biogenetic pathway. It could be postulated that the enzyme catalyzing [4 + 4] cycloaddition to form spiro rings may be involved. To confirm this, direct evidence is necessary from biosynthetic studies using purified or partially purified enzymes for naturally occurring [4 + 4] cycloaddition. However, speculation will still help us to understand how nature promotes this unusual transformation and will encourage us to explore its biomimetic syntheses in the future. It is also highly significant that linderaspirone A, which we investigated here, is a novel windmill-shaped molecule isolated from nature.

(20) X-ray crystal data of **1**: $C_{34}H_{32}O_{10}$; MW = 600.61, monoclinic, space group $P2(1)/n$, with a = 12.3804(2) Å, b = 8.6909(14) Å, c = 27.7296(5) Å, β = 92.667(2)°, V = 2980.4(8) Å^3, Z = 7, D_{calcd} = 1.339 g/cm^3, λ = 0.71073 Å, μ(Mo Kα) = 0.099 mm^{-1}, F(000) = 1264, and T = 298(2) K. A yellow block crystal of dimensions 0.10 × 0.11 × 0.12 mm was selected for X-ray analysis. A total of 18891 reflections, collected in the range 1.47° ≤ θ ≤ 28.33°, yielded 7048 unique reflections. The structure was solved using direct methods and was refined by full-matrix least-squares on F_2 values for 2892 $I > 2\sigma(I)$. Hydrogen atoms were fixed at calculated positions. The final indices were R_1 = 0.0568, wR_2 = 0.1380 with goodness-of-fit = 0.858.

To investigate the effect of linderaspirone **(1)** on insulin sensitivity, we used glucosamine to induce insulin resistance in HepG2 cells. Glucosamine significantly inhibited the phosphorylation of insulin receptor and Akt induced by insulin and thus induced insulin resistance in HepG2 cells.[21] HepG2 cells were treated with **1** at 1, 10, and 25 μg/mL for 24 h and then induced insulin resistance with 18 mM glucosamine for 18 h in serum-free DMEM with 5 mM glucose. Subsequently, cells were stimulated with 100 nM insulin for 20 min and harvested for Western blot analysis. Compound **1** markedly elevated phosphorylation of InsR, Akt, and GSK-3β under insulin-resistant condition. Compound **1** improved insulin signaling, including the phosphorylation of InsR, Akt, and GSK-3β in HepG2 cells under insulin-resistant conditions.

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Supporting Information Available: Experimental procedures and NMR spectra of linderaspirone. This material is available free of charge via the Internet at http://pubs.acs.org.

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