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Bi-linderone, a Highly Modified Methyl-linderone Dimer from *Lindera aggregata* with Activity toward Improvement of Insulin Sensitivity in Vitro

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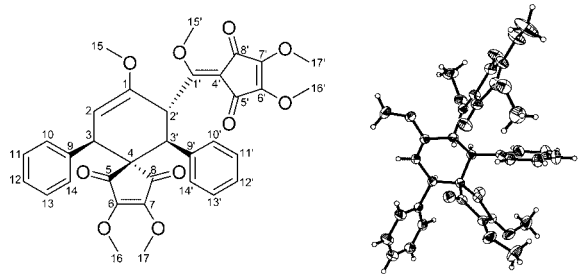
ABSTRACT



Bi-linderone (1) was isolated as racemate from the traditional Chinese medicinal plant *Lindera aggregata*. The structure elucidation of bi-linderone was reported on the basis of extensive analysis of NMR spectra and crystal X-ray diffraction. Bi-linderone has an unprecedented spirocyclopentenedione-containing carbon skeleton and showed significant activity against glucosamine-induced insulin resistance in HepG2 cells at a concentration of 1 μg/mL.

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 acesodyne and antispasmodic.[1] Sesquiterpenoids,[2] alkaloids,[3] flavonoids,[4] lignans,[5] butanolides,[6] and unique cyclopentenedione derivatives[7] were reported in previous chemical investigations on *Lindera* species. Pharmacological studies on this plant have shown various bioactivities such as antioxidation,[8] protection against post-ischemic myocardial dysfunction,[9] antiviral (SARS-associated coronavirus) activity,[10] cytotoxicity,[1] and slowing the progression of diabetic nephropathy in db/db mice.[11]

As part of our effort to assemble a large-scale natural compound library of thousands of structures derived from plants and micro-organisms,[12,13] we report herein the struc-

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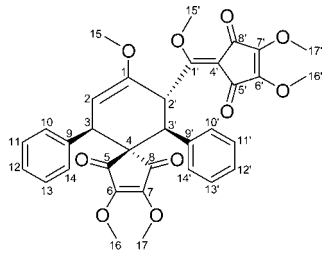
Table 1. NMR Spectroscopic Data for Bi-linderone (**1**) in $CDCl_3$

no.	^{1}H NMR	^{13}C NMR	HMBC (from H to C)
1		154.6 (C)	
2	4.84 (1H, br s)	96.3 (CH)	1, 3, 4, 9, 2′
3	4.32 (1H, br s)	45.8 (CH)	1, 2, 4, 5/8, 9, 10, 14
4		58.8 (C)	
5, 8		196.1, 194.7 (each C)	
6, 7		153.4, 152.8 (each C)	
9		140.1 (C)	
10−14	7.00−7.24 (m)	127.4−129.0	3
15	3.58 (3H, s)	54.9 (CH_3)	1, 2*
16, 17	3.75, 3.61 (each 3H, s)	59.3, 59.5 (each CH_3)	6, 7 (res.)
1′		172.6 (C)	
2′	6.14 (1H, br d, 11.5)	43.1 (CH)	
3′	3.88 (1H, d, 11.5)	48.2 (CH)	3, 4, 5/8, 1′, 2′, 9′, 10′, 14′
4′		111.0 (C)	
5′, 8′		186.7, 183.9 (each C)	
6′, 7′		147.7, 146.7 (each C)	
9′		136.1 (C)	
10′−14′	7.00−7.24 (m)	127.4−129.0	
15′	3.99 (3H, s)	65.0 (CH_3)	1′
16′, 17′	4.11, 4.04 (each 3H, s)	59.5, 59.9 (each CH_3)	6′, 7′ (res.)

ture elucidation of bi-linderone (**1**) isolated from the root of *L. aggregata*. It has an unprecedented spirocyclopentenedione-containing carbon skeleton. To the best of our knowledge, no other structure with this skeleton has been reported to date. Bi-linderone was tested using an antidiabetes assay. It showed significant activity against glucosamine-induced insulin resistance in HepG2 cells at a concentration of 1 μg/mL.



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Compound **1** was obtained as colorless crystals. The ion at *m*/*z* 623.1908 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 ^{1}H and ^{13}C NMR spectroscopic data (Table 1). The IR spectrum exhibited the absorption bands of unsaturated ketone and phenyl groups at 3005, 1684, 1672, 1639, 1619, 1494 cm^{-1}. The ^{1}H NMR spectrum of **1** suggested the presence of two monosubstituted benzene rings at δ_H 7.00−7.24 (10H, m) and six methoxyl groups at δ_H 3.58, 3.61, 3.75, 3.94, 4.04, and 4.11 (each 3H, s); protons at δ_H 4.84 (1H, br s) and 4.32 (1H, br s), as well as two intercoupling protons at δ_H 6.14 (1H, br d, J = 11.5) and 3.88 (1H, d, J = 11.5 Hz). The ^{13}C NMR spectrum showed a total of 34 carbon signals, including four unsaturated ketone carbonyl resonances at δ_C 196.1, 194.7, 186.7, and 183.9; a set of signals due to two monosubstituted phenyls at δ_C 127.4−129.0 (d), 136.1 (s), and 140.1 (s); eight olefinic carbons at δ_C 172.6 (s), 154.6 (s), 153.4 (s),152.8 (s), 147.6 (s), 146.7 (s), 111.0 (s), and 96.3 (d); and six methoxyls at δ_C 65.0 (q), 59.9 (q), 59.5 (q), 59.5 (q), 59.3 (q), and 54.9 (q), as well as four remaining carbons at δ_C 43.1 (d), 45.8 (d), 48.2 (d), and 58.8 (s). Detailed interpretation of the HMBC correlations (Table 1) allowed the construction of the fragments. 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 configurations of **1**. The perspective presentation of the final structure is shown in Figure 1. It occurs as a racemate, also evidenced by the lack of optical activity. Subsequent HPLC separation on a Chiralcel OD column (250 mm × 10 mm) for two enantiomers, (+)-**1** and (−)-**1**, was not successful.

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(14) **(±)-Bi-linderone (1).** Colorless crystals ($CHCl_3$/MeOH), mp 201−202°C. $[\alpha]^{23}_D$ 0 (*c* 0.10, $CHCl_3$). UV λ_{max} ($CHCl_3$) (log ε) nm: 251 (4.40), 298 (4.36). IR (KBr) ν_{max} cm^{-1}: 3005, 2949, 1684, 1672, 1639, 1619, 1601, 1494, 1462, 1430, 1326, 1209, 1199, 1122. EI-MS *m*/*z*: 600 $[M]^+$ (52), 585 (7), 568 (59), 553 (38), 536 (32), 521 (16), 356 (26), 341 (84), 325 (59), 313 (26), 297 (46), 279 (58), 131 (100). ESI-MS (pos.) *m*/*z*: 623 $[M + Na]^+$. HR-ESI-MS (pos.) *m*/*z*: 623.1908 (calcd for $C_{34}H_{32}O_{10}Na$, 623.1893).

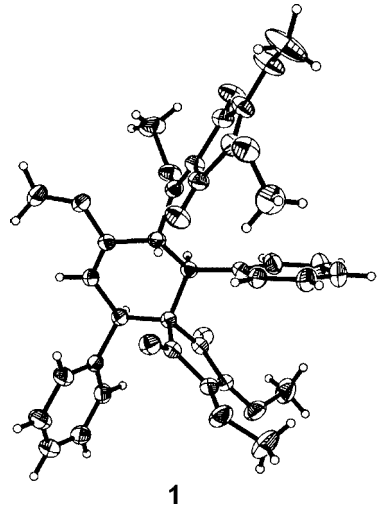

Figure 1. X-ray crystal structure of bi-linderone (**1**).

Bi-linderone (**1**) was isolated from a famous traditional Chinese medicinal 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 bi-linderone represents the first member of an unprecedented class of spirocyclopentene diones. Although it shares its structural features with the cyclopentenedione derivative methyl-linderone (**2**),[15] it has a backbone with 34 carbon atoms that includes an unique spiro ring, 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 (Figure 2).

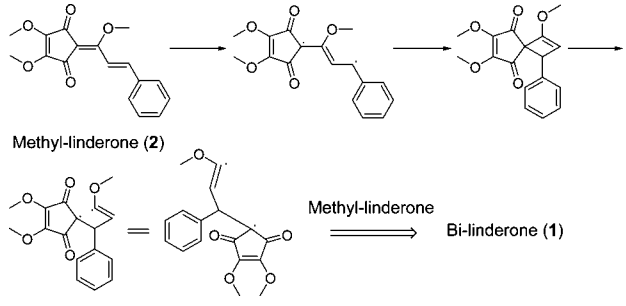

Figure 2. Proposed biosynthetic pathway of bi-linderone (**1**).

To investigate the effect of bi-linderone (**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. HepG2 cells were treated with **1** at 1, 10, 25 μg/mL for 24 h, and then insulin resistance was induced 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. Bi-linderone markedly elevated phosphorylation of InsR, Akt, and GSK-3β in HepG2 cells and improved insulin signaling under the insulin-resistant conditions. The roots of *Lindera aggregata* were collected in Ning'er County of Yunnan Province, China, in February 2008 and were identified by Mr. Yu Chen of Kunming Institute of Botany, Chinese Academy of Sciences. A voucher specimen (BBP2010008LA) has been deposited at BioBioPha Co., Ltd. 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), which 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 bi-linderone (**1**, 4 mg, t_R = 7.1 min). The retention time for analysis-type HPLC was 6.0 min (50%−100% MeCN in H_2O over 5 min followed by 100% MeCN to 10 min, 1 mL/min, 25 °C).

Optical rotation was measured on a Jasco P-1020 (Jasco International Co., Ltd., Tokyo, Japan) automatic digital polarimeter. The IR spectrum was recorded using a Bruker Tensor 27 FT-IR (Bruker Optics GmbH, Ettlingen, Germany) spectrometer with KBr pellets. UV spectroscopic data were obtained from online HPLC analysis. NMR spectra were carried out on a Bruker DRX-500 (Bruker BioSpin GmbH, Rheinstetten, Germany) spectrometer with deuterated chloroform signals (δ_H 7.26 ppm, δ_C 77.0 ppm) as the internal standard. EIMS and ESIMS (including HRESIMS) were measured on Finnigan-MAT 90 (Thermo Fisher Scientific Inc., Waltham, MA, U.S.) and API QSTAR Pulsar i (MDS Sciex, Concord, Ontario, Canada) mass spectrometers, respectively. Silica gel (200−300 mesh, Qingdao Marine Chemical Inc., Qingdao, China) and Sephadex LH-20 (Amersham Biosciences, Uppsala, Sweden) were used for normal pressure column chromatography. Fractions were monitored and analyzed using an Agilent 1100 series HPLC system (Zorbax SB-C18 column, 5 μm, 4.6 mm × 150 mm) in combination

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(16) **X-ray Crystal Data of 1.** $C_{34}H_{32}O_{10}$, MW = 600.61, orthorhombic, space group *Pbca*, with a = 27.4563(4) Å, b = 13.6040(17) Å, c = 16.2662(2) Å, β = 90.000°, V = 6075.7(13) Å^3, Z = 9, D_{calcd} = 1.313 g/cm3, λ = 0.71073 Å, μ(Mo Kα) = 0.097 mm^{-1}, F(000) = 2528, and T = 298(2) K. A colorless block crystal of dimensions 0.16 × 0.19 × 0.23 mm^3 was selected for X-ray analysis. A total of 37866 reflections, collected in the range 1.48° ≤ θ ≤ 28.28°, yielded 7274 unique reflections. The structure was solved using direct methods and was refined by full-matrix least-squares on $F2$ values for 2698 $I > 2\sigma(I)$. Hydrogen atoms were fixed at calculated positions. The final indices were R_1 = 0.0595, wR_2 = 0.1409 with goodness-of-fit = 0.866.

with TLC (silica gel, petroleum ether/acetone 2:1, R_f 0.6). Preparative HPLC was performed using an Agilent 1100 series (Zorbax SB-C18 column, 5 μm, 9.4 mm $\times$ 150 mm).

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

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