

A NEW LIGNAN FROM THE ROOTS OF *Ginkgo biloba*

Xue-li Wei,^{1,2} Yin Chen,¹ Xing-yi Chen,¹
Jing-yu Liang,^{1*} and Wei Qu^{1*}

A new lignan, named ginkgool (**1**), was isolated from the roots of *Ginkgo biloba* together with four known lignans, isolariciresinol (**2**), cycloolivil (**3**), pinoresinol (**4**), and sesamin (**5**). The new lignan was characterized as 4 β -(5-hydroxy-3-methoxybenzyl)-2 α -(4-hydroxy-3-methoxyphenyl)-3 β -(hydroxymethyl)-tetrahydrofuran-4 α -ol (**1**). The structures of the five compounds were determined on the basis of physicochemical and spectrometric data.

Keywords: *Ginkgo biloba*, ginkgool, lignans, structure elucidation.

Ginkgo biloba L., known as “living fossil,” has been used as a traditional Chinese medicines for a long time [1]. Lots of studies have been carried out on the leaves of *G. biloba*, and many chemical constituents with significant bioactivities, such as terpene lactones and flavonoids, have been isolated. In the process of our study, a new lignan from the roots of *G. biloba* and four known compounds were isolated, whose structures were established primarily on the basis of spectroscopic techniques, including 1D and 2D NMR methods.

Compound **1** was obtained as white needle crystals. (–)-HR-ESI-MS confirmed the molecular formula to be C₂₀H₂₄O₇ based on its ion peak at 375.1447 [M – H][–] (calcd 375.1444), which indicated nine degrees of unsaturation. In its ¹H NMR spectrum (Table 1), two typical peaks δ 3.43 (1H, d, J = 8.8 Hz) and 3.61 (1H, d, J = 8.9 Hz) and the typical signal at 4.59 (1H, d, J = 9.7 Hz) indicated that **1** was a tetrahydrofuran lignan. Signals at δ 7.03 (1H, d, J = 1.8 Hz), 6.68 (1H, d, J = 8.1 Hz), and 6.79 (1H, dd, J = 8.1, 1.8 Hz) indicated an AMX benzene system, and signals at δ 6.85 (1H, br.s), 6.65 (1H, s), and 6.65 (1H, s) suggested the presence of a meta-substituted phenyl group. Two phenolic hydroxyl signals at δ 8.73 (1H, s) and 8.59 (1H, s) and two methoxy groups at δ 3.74 and 3.73 (each 3H, s) were on each of the two aromatic rings. The ¹³C NMR spectrum of compound **1** (Table 1) showed 20 carbon signals, including two aromatic rings (δ 111.0–147.2), one oxymethine (δ 83.2), one oxygenated quaternary carbon (δ 80.5), two oxygenated methylenes (δ 76.1, 60.5), one methane carbon (δ 58.9), one methylene carbon (δ 39.1), and two methoxy groups (δ 55.5). This further suggested that compound **1** is a 7-*O*-9-type tetrahydrofuran lignan. The ¹H NMR and ¹³C NMR data of compound **1** were completely assigned with the HMBC data.

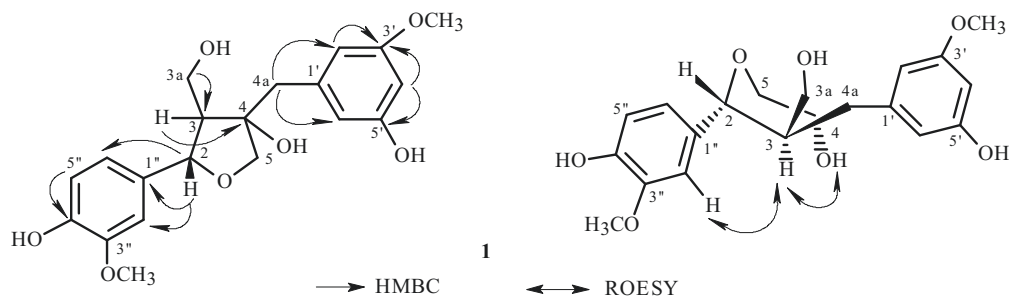
In the HMBC spectra, the correlations (Fig. 1) of H-2 (δ 4.59) with C-3 (δ 58.9) and C-3a (δ 60.5) led to the connection of C-3a and C-3 of compound **1**. The correlations of 4-OH (δ 4.57) with C-4 (δ 80.5) and C-5 (δ 76.1) indicated that the hydroxy was placed at C-4. The correlation of H-6'' (δ 6.79) and H-2'' (δ 7.03) with C-2 (δ 83.2) confirmed that C-2 was attached to the 1,3,4-substituted benzene ring. The HMBC correlations of the proton signals at H-2' (δ 6.85) and H-4' (δ 6.65) with C-4a (δ 39.1) showed that C-4a was attached to the 1,3,5-substituted benzene ring. The correlations of 3''-OCH₃/C-3'' and 3'-OCH₃/C-3' and all data mentioned above established the planar structure of compound **1**.

The relative configuration was established by the ROESY correlations. The correlations (Fig. 1) of H-2 with H-3a and 4-OH with H-3 showed that H-3 and 4-OH were α -oriented and H₂-3a, H₂-4a, and H-2 were β -oriented (Fig. 1).

1) Department of Natural Medicinal Chemistry, China Pharmaceutical University, 210009, Nanjing, P. R. China, e-mail: jyliang08@126.com; popoqzh@126.com; weiqcpu@126.com; 2) Center of Drug Analysis and Testing, Zibo Biomedical R&D Institute of Shandong University, 255000, Zibo, P. R. China. Published in *Khimiya Prirodnykh Soedinenii*, No. 5, September–October, 2015, pp. 704–706. Original article submitted January 2, 2014.

TABLE 1. ^1H (500 MHz), ^{13}C (125 MHz) NMR and HMBC ($\text{H}\rightarrow\text{C}$) Data for Compound **1** (DMSO-d_6 , δ , ppm, J/Hz)

C atom	δ_{H}	δ_{C}	HMBC
2	4.59 (1H, d, $J = 9.7$)	83.2	1'', 2'', 6'', 3, 3a
3	2.14 (1H, q, $J = 6.1$)	58.9	2, 4, 1''
4		80.5	
5	3.61 (1H, d, $J = 8.9$)	76.1	2, 3, 4, 4a
3a	3.43 (1H, d, $J = 8.8$)		
	3.65 (1H, m)	60.5	2, 3, 4
	3.55 (1H, m)		
4a	2.84 (1H, d, $J = 13.9$)	39.1	4, 5, 1', 2', 6'
	2.75 (1H, d, $J = 13.9$)		
1'		129.1	
2'	6.85 (1H, br.s)	114.7	4a, 1', 4', 3', 6'
3'		146.8	
4'	6.65 (1H, s)	114.7	2', 3', 5'
5'		144.6	
6'	6.65 (1H, s)	122.4	1', 2', 4', 5', 4a
3''-OCH ₃	3.74 (3H, s)	55.5	3'
1''		134.4	
2''	7.03 (1H, d, $J = 1.8$)	111.0	2, 1'', 3'', 4'', 6''
3''		147.2	
4''		145.5	
5''	6.68 (1H, d, $J = 8.1$)	114.8	1'', 2'', 3'', 4''
6''	6.79 (1H, dd, $J = 8.1, 1.8$)	119.1	2, 2'', 4'', 5''
3''-OCH ₃	3.74 (3H, s)	55.5	3''


 Fig. 1. Key HMBC and ROESY correlations of compound **1**.

To support this, we found that H-3 showed a NOE with H-2'', proving the α -orientation of 4-OH and H-3. Thus, the structure of compound **1** was established as 4 β -(5-hydroxy-3-methoxybenzyl)-2 α -(4-hydroxy-3-methoxyphenyl)-3 β -(hydroxymethyl)-tetrahydrofuran-4 α -ol, named ginkgool, which was similar to (2*R*,3*S*,4*S*)-4-(4-hydroxy-3-methoxybenzyl)-2-(5-hydroxy-3-methoxyphenyl)-3-(hydroxymethyl)-tetrahydrofuran-3-ol from *Saussurea cordifolia* [2].

Compounds **2–5** were known ligands. Their structures were identified as isolariciresinol (**2**) [3], cycloolivil (**3**) [4], pinioresinol (**4**) [5], and sesamin (**5**) [6] by comparison of their spectroscopic data with those in the related literature.

EXPERIMENTAL

General. NMR spectra were measured on a Bruker AV 500M NMR spectrometer. ESI-MS and HR-ESI-MS were measured on an Agilent 6224 TOF LC/MS spectrometer. Silica gel (100–200 mesh, 200–300 mesh, Qingdao Haiyang Chemical Co., Ltd.) was used. All solvents were of analytical grade (Tianjin BOYOU Chemical Plant).

Plant Material. The roots of *Ginkgo biloba* were collected in 2007 from Jiangsu Province, China and identified by Prof. Xue-hua Song (China Pharmaceutical University). A voucher specimen was deposited at the Department of Natural Medicinal Chemistry, China Pharmaceutical University, Nanjing 210009, China.

Extraction and Isolation. The roots of *Ginkgo biloba* (5 kg) were extracted three times by 95% ethanol. The extracted solvent was concentrated by a rotary vacuum evaporator, then extracted with ethyl acetate after suspension in water. The ethyl acetate extract (109 g) was separated on a silica gel column eluted with a gradient of petroleum ether–acetone (1:0–0:1, v/v) to afford four fractions (A–D). Fractions B (60 g) was separated on ODS using 30, 50, 70, and 90% MeOH–water and MeOH in sequence to afford five fractions (B1–B5). Fraction B3 (6 g) was separated on a Sephadex LH-20 column eluted with MeOH to afford **1** (100.6 mg). Fraction B2 was further chromatographed on a silica gel column eluted with a gradient solvent system of CH₂Cl₂–MeOH to afford several subfractions. After repeated silica gel column chromatography (CH₂Cl₂–MeOH, petroleum ether–EtOAc, petroleum ether–acetone), Sephadex LH-20 gel column chromatography (MeOH), and ODS reverse-phase column chromatography (MeOH–H₂O), we obtain the four known lignans.

Ginkgool (1). White needle crystals. For ¹H and ¹³C NMR, see Table 1. HR-ESI-MS (negative) *m/z* [M – H][–] 375.1447 (calcd for C₂₀H₂₄O₇, 375.1444).

Isolariciresinol (2). White granular crystals. ¹H NMR (500 MHz, DMSO-d₆, δ, ppm, J/Hz): 8.67 (1H, s, OH), 8.36 (1H, s, OH), 6.68 (1H, d, J = 8.0, H-6), 6.63 (1H, s, H-2), 6.60 (1H, s, H-2'), 6.49 (1H, d, J = 8.1, H-5), 6.10 (1H, s, H-5'), 3.74 (1H, d, J = 10.2, H-7), 3.70 (3H, s, OCH₃), 3.69 (3H, s, OCH₃), 3.56 (1H, m, H-9'), 3.45 (1H, m, H-9), 3.43 (1H, m, H-9'), 3.17 (1H, m, H-9), 2.65 (2H, m, H-7'), 1.84 (1H, m, H-8'), 1.62 (1H, m, H-8). ¹³C NMR (125 MHz, DMSO-d₆, δ, ppm): 147.3 (C-3), 145.5 (C-3'), 144.6 (C-4), 144.1 (C-4'), 137.1 (C-1), 132.7 (C-6'), 127.1 (C-1'), 121.5 (C-6), 116.2 (C-5'), 115.2 (C-5), 113.3 (C-2), 111.8 (C-2'), 63.6 (C-9'), 59.8 (C-9), 55.6 (OCH₃), 55.5 (OCH₃), 45.9 (C-7, 8), 38.1 (C-8'), 32.2 (C-7'). ESI-MS *m/z* 378 [M + NH₄]⁺.

Cyclooolivil (3). White needle crystals. ¹H NMR (500 MHz, DMSO-d₆, δ, ppm, J/Hz): 8.69 (1H, s, OH), 8.32 (1H, s, OH), 6.70 (1H, d, J = 8.0, H-5), 6.65 (1H, d, J = 1.8, H-2), 6.55 (1H, s, H-2'), 6.53 (1H, dd, J = 8.0, 1.8, H-6), 6.05 (1H, s, H-5'), 4.88 (1H, t, J = 4.3, OH), 4.65 (1H, t, J = 5.8, OH), 4.28 (1H, s, OH), 3.85 (1H, d, J = 11.7, H-7), 3.70 (3H, s, OCH₃), 3.69 (3H, s, OCH₃), 3.61 (2H, m, H-9'), 3.35 (2H, m, H-9), 3.09 (1H, d, J = 16.6, H-7'), 2.44 (1H, d, J = 16.6, H-7'), 1.88 (1H, d, J = 11.7, H-8). ¹³C NMR (125 MHz, DMSO-d₆, δ, ppm): 147.3 (C-3), 145.7 (C-3'), 144.7 (C-4), 143.9 (C-4'), 136.9 (C-1), 132.1 (C-1'), 125.1 (C-6'), 121.8 (C-6), 116.1 (C-5'), 115.3 (C-5), 113.4 (C-2), 112.3 (C-2'), 72.9 (C-8'), 68.0 (C-9'), 59.0 (C-9), 55.7 (OCH₃), 55.5 (OCH₃), 45.6 (C-8), 43.0 (C-7), 40.0 (C-7'). ESI-MS *m/z* 399 [M + Na].

Pinoresinol (4). Yellow gum. ¹H NMR (500 MHz, CDCl₃, δ, ppm): 6.88 (4H, d, J = 8.0, H-2, 5, 2', 5'), 6.82 (2H, dd, J = 1.3, 8.0, H-6, 6'), 4.74 (2H, d, J = 4.5, H-7, 7'), 4.24 (2H, dd, J = 9.3, 6.9, H-9, 9'), 3.89 (6H, s, OCH₃), 3.10 (2H, m, H-8, 8'). ¹³C NMR (75 Hz, CDCl₃, δ, ppm): 146.7 (C-3, 3'), 145.2 (C-4, 4'), 132.9 (C-1, 1'), 118.9 (C-5, 5'), 114.3 (C-6, 6'), 108.6 (C-2, 2'), 85.7 (C-7, 7'), 71.6 (C-6, 6'), 55.9 (C-9, 9'), 54.3 (C-8, 8'). ESI-MS *m/z* 357 [M – H][–].

Sesamin (5). White granular crystals. ¹H NMR (300 MHz, CDCl₃, δ, ppm, J/Hz): 6.86 (2H, d, J = 10.4, H-5, 5'), 6.84 (2H, s, H-2, 2'), 6.76 (2H, d, J = 8.2, H-6, 6'), 5.90 (4H, s, 2 × OCH₂), 4.71 (2H, d, J = 4.1, H-7, 7'), 4.22 (2H, dd, J = 6.8, 9.0, H-9b, 9b'), 3.85 (2H, dd, J = 3.4, 9.2, H-9a, 9a'), 3.04 (2H, m, H-8, 8'). ¹³C NMR (75 MHz, CDCl₃, δ, ppm): 148.0 (C-4, 4'), 147.1 (C-3, 3'), 135.1 (C-1, 1'), 119.3 (C-6, 6'), 108.2 (C-5, 5'), 106.5 (C-2, 2'), 101.0 (2 × OCH₂), 85.8 (C-7, 7'), 54.3 (C-8, 8').

ACKNOWLEDGMENT

This work was supported by the Natural Science Foundation of Jiangsu Province (No. BK2011628) in China and the College Graduate Research and Innovation Programs Funded Project of Jiangsu Province (CXLX11_0809).

REFERENCES

1. P. Yu and J. Liang, *Chin. Chem. Lett.*, **23**, 1165 (2012).
2. X. W. Li, Z. T. Guo, Y. Zhao, Z. Zhao, and J. F. Hu, *Phytochemistry*, **71**, 682 (2010).
3. Y. Liu, S. J. Song, S. X. Xu, and X. H. Fu, *J. Shenyang Pharm. Univ.*, **02**, 101 (2003).
4. X. H. Zheng and L. Y. Kong, *Chin. Trad. Herb. Drugs*, **11**, 964 (2002).
5. D. M. Zhang, L. H. Hu, W. C. Ye, and S. X. Zhao, *Chin. J. Nat. Med.*, **2**, 80 (2003).
6. Seon-Mi Seo, Hak-Ju Lee, Oh-Kyu Lee, Hyun-Jin Jo, Ha-Young Kang, Don-Ha Choi, Ki-Hyon Paik, and M. Khan, *Chem. Nat. Compd.*, **44**, 419 (2008).