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ORIGINAL ARTICLE

A New Biphenyl Neolignan from Leaves of *Patrinia villosa* (Thunb.) Juss.

Yan Xin-Jia, Liu Wei, Zhao Ying, Chen Ning, Xu Ying, Wu Jian, Wang Tan[1], Li Yue[1], Xiang Zheng

Harbin University of Commerce, Harbin 150076, [1]Beijing Center for Physical and Chemical Analysis, Beijing 100094, PR China

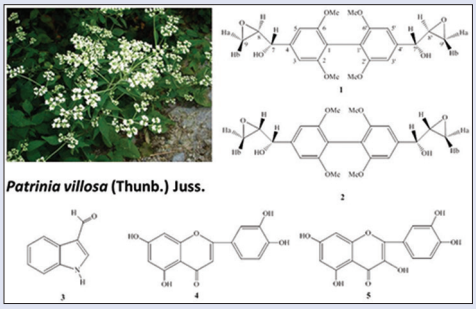
ABSTRACT

Results: One new stereoisomer of biphenylneolignan with four known compounds was isolated from the leaves of Patrinia villosa Juss. **Methods:** The structure of the new compound was elucidated as 2,6,2′,6′-tetramethoxy-4,4′-bis (1,2-trans-2,3-epoxy-1-hydroxypropyl) biphenyl (1) on the basis of spectroscopic analysis and comparison with literature data. The four known compounds were identified as 2,6,2′,6′-tetramethoxy-4,4′-bis(1,2-cis-2,3-epoxy-1-hydroxypropyl)biphenyl (2), 1H-indole-3-carbaldehyde (3), luteolin (4) and quercetin(5) by comparison of their spectral data with the reported data, respectively. **Conclusions:** Compound 1 is a new biphenylneolignan, compound 2 and 3 were isolated for the first time from the plant.

Key words: Biphenyl neolignan, coupling constant, nuclear magnetic resonance, *Patrinia villosa* (Thunb.) Juss., structure

SUMMARY

- One new stereoisomer of biphenylneolignan named 2,6,2′,6′-tetramethoxy-4,4′-bis (1,2-trans-2,3-epoxy-1-hydroxypropyl) biphenyl with four known compounds was isolated from the leaves of Patrinia villosa Juss.



Correspondence:

Dr. Li Yue, Beijing Center for Physical and Chemical Analysis, Beijing 100094, PR China.
E-mail: liyuedl04@126.com,
Dr. Xiang Zheng, Center of Research on Life Science and Environmental Science, Harbin University of Commerce, Harbin 150076, PR China.
E-mail: rainbowaftersnow@hotmail.com

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INTRODUCTION

Patrinia, which belongs to the family of Valerianaceae, is a widely distributed plant grown in East Asia and North America. The genus included more than 20 species, 10 of which growing in China.[1] Usually, *Patrinia* species are used as leaves and vegetables in some areas of China, and research also revealed its leaves with pharmacological properties, especially the species of *Patrinia villosa* (Thunb.) Juss. The *P. villosa* (Thunb.) Juss., is an important ancient herbal medicine widely used for more than 2000 years from Shen Nong Ben Cao Jing, a famous ancient Chinese medicinal literary. It has been used in traditional Chinese medicine for an inflammation, wound healing, and abdominal pain. The previous research on the chemical constituents of *P. villosa* (Thunb.) Juss., have revealed that it contains several compound classes. Pentacyclic triterpenoids, iridoids, and flavonoids are the dominant bioactive constituents in the leaves of *P. villosa* Juss., which displayed potential ability of anti-tumor and anti-inflammatory.[2-5] Other components, such as sterols, fatty acids, and essential oil[2,6-8] were also confirmed in *P. villosa* (Thunb.) Juss.

As part of our search for new natural compounds with anti-tumor activity, we carried out phytochemical investigations on the leaves of *P. villosa* (Thunb.) Juss., collected in China. A new biphenyl neolignan and four known compounds [Figure 1] were isolated from the plant. The present study reports the isolation and structural elucidation of these components.

MATERIALS AND METHODS

General experimental procedures

Optical rotations were measured on a Perkin-Elmer digital polarimeter (USA). nuclear magnetic resonance (NMR) spectra were recorded with tetramethylsilane as internal standard on a Bruck AVANCE 400 FT-NMR spectrometer. Electrospray ionization/mass spectrometry was measured on an Agilent 1100 LC/MSD Trap-SL spectrometer (USA). Column chromatography was performed on silica gel (200–300 mesh) (Marine Chemical Factory, Qingdao, China) and octa decylsilyl silicion (ODS) (ultimate XB-C18, 40–70 μm, Welch Materials, Inc., USA). High-performance liquid chromatography (HPLC) separation was performed with an Agilent 1100 chromatograph apparatus using an ODS column (ultimate XB-C18, 10 × 250, 5 μm, Welch Materials, Inc., USA) and detected with an ultraviolet detector.

Materials and chemicals

The *P. villosa* Juss., leaves were purchased from Hebei Qixin Traditional Chinese Medicine Pellets Co., Ltd., P.R. China, and identified by Prof. Kang Tingguo, College of Pharmacy, Liaoning University of Traditional Chinese Medicine. A voucher specimen (NO. PVJ20130821) has been deposited at the Pharmacognosy Laboratory, Harbin University of

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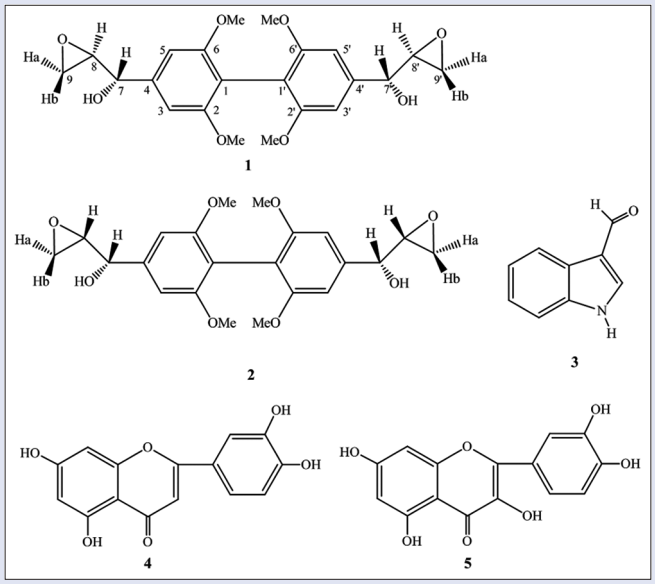

Figure 1: The structures of compounds 1-5

Commerce. Organic solvents (analytical grade or HPLC grade) for the experiment were purchased from Kermel Chemical Co., (Tianjin, China).

Extraction and isolation

The air-dried leaves of *P. villosa* Juss., (15 kg) were extracted three times with 70% EtOH under reflux. Evaporation of the solvent under reduced pressure gave a condensed extract (2.8 kg), which was suspended in H_2O and then partitioned successively with light petroleum, dichloromethane, and *n*-BuOH. The dichloromethane portion (110 g) was then subjected to normal phase silica gel column chromatography and eluted with a gradient of CH_2Cl_2-MeOH (100:0 → 0:100, v/v) to give ten fractions. Fraction 2 (CH_2Cl_2-MeOH, 80:1, v/v) was subjected to further separation using ODS silica gel column chromatography eluted with 20%, 36%, 44%, 60%, and 80% MeOH in water, respectively. Subfraction 2.2 (36% methanol elution) was purified by semi-preparative HPLC (MeCN-H_2O, wavelength 280 nm) to yield compounds 1 (16.5 mg) and 2 (21.3 mg). Subfraction 2.3 (44% methanol elution) was purified by semi-preparative HPLC (MeCN-H_2O, wavelength 280 nm) to give compound 3 (10.4 mg).

The *n*-BuOH portion (280 g) was subjected to normal phase silica gel column chromatography and eluted with a gradient of CH_2Cl_2-MeOH (100:0 → 0:100, v/v) to give eight fractions. Fraction 4 was subjected to further separation using repeated ODS silica gel column and HPLC to give compounds 4 (25.0 mg) and 5 (18.6 mg).

RESULTS AND DISCUSSION

Compound 1 was obtained as a colorless needle crystal ($CHCl_3$), with the molecular formula $C_{22}H_{26}O_8$ as determined by the high-resolution electrospray ionization mass spectrometry (HRESIMS) at m/z 441.1518 $(M + Na)^+$, indicating ten degrees of unsaturation. The ^{1}H-NMR spectrum of compound 1 [Table 1] displayed four aromatic proton signals at δ_H 6.59 (4H, s, H-3, H-3′, H-5, H-5′), which were assigned to two 1,2,4,6-tetrasubstituted benzene rings. In addition, the ^{1}H-NMR spectrum also revealed four oxymethine protons at δ_H 4.74 (2H, d, J = 4.0 Hz, H-7, H-7′) and 3.10 (2H, m, H-8, H-8′), two methylene protons at δ_H 4.28 (2H, m, H-9a, H-9′a) and 3.93 (2H, m, H-9b, H-9′b), four methoxyl groups at δ_H 3.90 (12H, s). The ^{13}C NMR spectrum displayed 22 carbon signals, including 12 aromatic carbons, four oxygen-substituted methine (δ_C 54.4, 54.4, 86.1, 86.1), two oxygen-substituted methylene (δ_C 71.8, 71.8), and four methoxyl carbons (δ_C 56.4), All of the above spectroscopic data were similar to those of 2,6,2′,6′-tetramethoxy-4,4′-bis (1,2-*cis*-2,3-epoxy-1-hydroxypropyl) biphenyl (2) except for difference of coupling constants at H-7, 7′ (J = 4.0 Hz). In the NOESY experiment [Figure 2] on 1, correlations between H-3/H-7 or H-5/H-7, and a strong cross-peak between H-8/H_a-9 suggested the β-configuration for H-7 and H_b-9 and an α-configuration for OH-7. The relative configuration could be proposed as 7,8-*trans*-8,9a-*cis*. Thus, the structure of one was determined as 2,6,2′,6′-tetramethoxy-4,4′-bis (1,2-*trans*-2,3-epoxy-1-hydroxypropyl) biphenyl.

Table 1: ^{1}H-NMR (300 MHz) and ^{13}C-NMR (100 MHz) spectral data of compounds 1 and 2 in $CDCl_3$

Number	1		2	
	δ_H	δ_C	δ_H	δ_C
1,1’		132.1		132.0
2,2’		147.2		147.1
3,3’	6.59 (s)	102.8	6.57 (s)	102.8
4,4’		134.34		134.3
5,5’	6.59 (s)	102.8	6.57 (s)	102.7
6,6’		147.2		147.1
7,7’	4.74 (d, 4.0)	86.1	4.78 (d, 7.0)	86.0
8,8’	3.10 (m)	54.4	3.09 (m)	54.3
9a, 9’a	4.28 (m)	71.8	3.89 (m)	71.8
9b, 9’b	3.93 (m)		4.26 (m)	
-OH	5.52 (s)		5.54 (s)	
-OCH_3	3.90 (s)	56.4	3.90 (s)	56.3

Coupling constants (J) in Hz are given in parentheses; chemical shift values are expressed in ppm. NMR: Nuclear magnetic resonance

Compounds 2–5 were identified as 2,6,2′,6′-tetramethoxy-4,4′-bis (2,3-epoxy-1-hydroxypropyl) biphenyl (2), 1H-indole-3-carbaldehyde (3), luteolin (4), and quercetin (5) on the basis of its spectroscopic data.[9-11]

CONCLUSION

One new stereoisomer of biphenyl neolignan (1) with four known compounds 2–5 was isolated from the leaves of *P. villosa* Juss. The structures of all the compounds were elucidated on the basis of spectroscopic analysis and comparison with literature data.

Compounds

2,6,2′,6′-Tetramethoxy-4,4′-bis (1,2-*trans*-2,3-epoxy-1- hydroxypropyl) biphenyl (1). Colorless needle crystal. HRESIMS m/z 441.1518 $(M + Na)^+$ (calculated for $C_{22}H_{26}O_8Na$, 441.1520). ^{1}H-NMR ($CDCl_3$, 300 MHz): δ_H: 3.10 (2H, m, H-8, H-8′), 4.28 (2H, m, H-9a, H-9′a), 3.90 (12H, s, OMe-2, OMe-2′, OMe-6, OMe-6′), 3.93 (2H, m, H-9b, H-9′b), 4.74 (2H, d, J = 4.0 Hz, H-7, H-7′), 5.52 (2H, brs, OH-7, OH-7′), 6.59 (4H, s, H-3, H-3′, H-5, H-5′). ^{13}C-NMR δ_C: 54.4 (2C, C-8, C-8′), 56.4 (4C, OMe-2, OMe-2′, OMe-6, OMe-6′), 71.8 (2C, C-9, C-9′), 86.1 (2C, C-7, C-7′), 102.8 (4C, C-3, C-3′, C-5, C-5′), 132.1 (2C, C-1, C-1′), 134.3 (2C, C-4, C-4′), 147.2 (4C, C-2, C-2′, C-6, C-6′).

2,6,2′,6′-Tetramethoxy-4,4′-bis (1,2-*cis*-2,3-epoxy-1-hydroxypropyl) biphenyl (2). Colorless needle crystal. ^{1}H-NMR ($CDCl_3$, 300 MHz): δ_H: 3.09 (2H, m, H-8, H-8′), 3.89 (2H, m, H-9a, H-9′a), 3.90 (12H, s, OMe-2, OMe-2′, OMe-6, OMe-6′), 4.26 (2H, m, H-9b, H-9′b), 4.78 (2H, d, J = 7.0 Hz, H-7, H-7′), 5.54 (2H, brs, OH-7, OH-7′) 6.57 (4H, s, H-3, H-3′, H-5, H-5′). ^{13}C-MNR δ_C: 54.3 (2C, C-8, C-8′), 56.3 (4C, OMe-2, OMe-2′, OMe-6, OMe-6′), 71.8 (2C, C-9, C-9′), 86.0 (2C, C-7, C-7′),

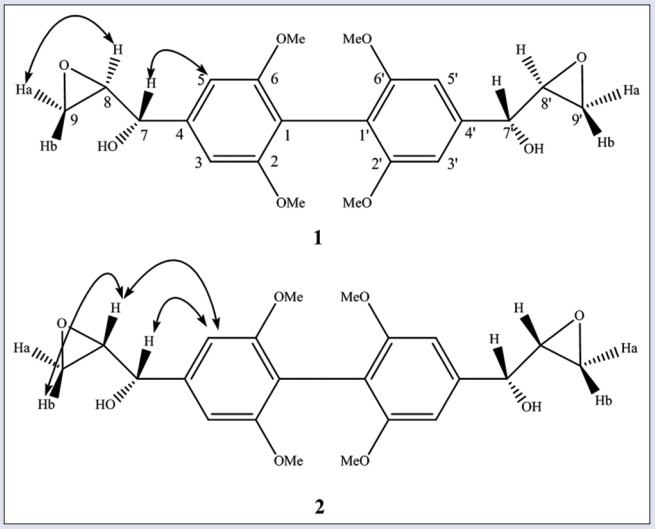

Figure 2: Key NOESY correlations for 1 and 2

102.7 (4C, C-3, C-3′, C-5, C-5′), 132.0 (2C, C-1, C-1′), 134.3 (2C, C-4, C-4′), 147.1 (4C, C-2, C-2′, C-6, C-6′).

1H-indole-3-carbaldehyde (3). Pale yellow needle crystal. ^{1}H-NMR (dimethyl sulfoxide [DMSO], 400 MHz) δ_H: 7.24 (2H, m, 5,6-H), 7.52 (1H, d, J = 8.0 Hz, 7-H), 8.10 (1H, s, 2-H), 8.29 (1H, d, J = 7.0 Hz, 4-H). ^{13}C-NMR δ_C: 185.4 (C-10), 138.9 (C-8), 137.5 (C-2), 124.6 (C-9), 123.9 (C-5), 122.6 (C-6), 121.3 (C-7), 118.64 (C-3), 112.6 (C-4).

Luteolin (4). Pale yellow powder. ^{1}H-NMR (DMSO, 400 MHz) δ_H: 13.02 (1H, brs, 5-OH), 7.45 (1H, dd, J = 8.5, 2.5 Hz, 6′-H), 7.40 (1H, d, J = 2.5 Hz, 2′-H), 6.91 (1H, d, J = 8.5 Hz, 5′-H), 6.71 (1H, s, 3-H), 6.47 (1H, d, J = 2.0 Hz, 6-H), 6.59 (1H, d, J = 1.6 Hz, H-8). ^{13}C-NMR δ_C: 163.8 (C-2), 103.3 (C-3), 181.4 (C-4), 161.4 (C-5), 99.0 (C-6), 164.9 (C-7), 93.9 (C-8), 157.3 (C-9), 103.9 (C-10), 118.8 (C-1′), 113.1 (C-2′), 145.9 (C-3′), 149.9 (C-4′), 116.0 (C-5′), 121.7 (C-6′).

Quercetin (5). Pale yellow powder. ^{1}H-NMR (DMSO, 400 MHz) δ_H: 6.20 (1H, d, J = 2.0 Hz, 6-H), 6.38 (1H, d, J = 2.0 Hz, 8-H), 6.86 (1H, d, J = 8.4 Hz, 5′-H), 7.56 (1H, dd, J = 8.4, 2.0 Hz, 6′-H), 7.65 (1H, d, J = 2.0 Hz, 2′-H), 9.32, 9.38, 9.62, 10.79, 12.49 (1H, s,-OH × 5). ^{13}C-NMR δ_C: 146.9 (C-2), 122.1 (C-3), 175.9 (C-4), 156.3 (C-5), 98.3 (C-6), 164.0 (C-7), 93.4 (C-8), 160.9 (C-9), 103.3 (C-10), 123.3 (C-1′), 115.8 (C-2′), 148.0 (C-3′), 145.2 (C-4′), 115.2 (C-5′), 120.2 (C-6′).

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Conflicts of interest

There are no conflicts of interest.

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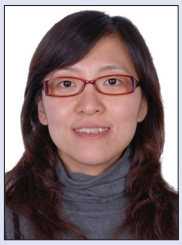

Li Yue

Xiang Zheng

ABOUT AUTHORS

Li Yue, PhD, Research Associate, Beijing Center for Physical and Chemical Analysis, Beijing 100094, PR China, Her major is cancer pharmacology.

Xiang Zheng, PhD, is a dozent at the Center of Research on Life Science and Environmental Science, Harbin University of Commerce, Harbin, P.R. China. His research interest is phytochemistry and cancer pharmacology.