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A Rare Secoiridoid Dimer Derivative from *Ligustri lucidi fructus*

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Abstract: A secoiridoid glucoside dimer derivative, named as 4', 5'-(2'-hydroxy ligustrosidic acid) dimer (**1**), together with two known secoiridoids were isolated from the aqueous extract of *Ligustri lucidi fructus*. Structures of these compounds were elucidated by analysis of spectroscopic data including 1D-, 2D-NMR and HR-ESI-MS, and the reported literature data comparison. This is the first report on iridoid dimer derivative isolation from the genus *Ligustrum*. Their antioxidant activities were evaluated by using 1, 1-diphenyl-2-picrylhydrazyl (DPPH) radical scavenging assay. Compound **1** exhibited moderate antioxidant activity.

Keywords: *Ligustri lucidi fructus*; Secoiridoid dimer derivative; Antioxidant activity. © 2015 ACG Publications. All rights reserved.

1. Introduction

Ligustri lucidi fructus (LLF) which belongs to the Oleaceae family [1] naturally occurs in warm and humid climates with rich resources, *e.g.* the east, south and southwest of China, south Korea and India [2]. As a traditional Chinese medicine, LLF has been used for two thousands of years in China. It was firstly described in the Chinese Materia Medica, *Shennong-Bencao-Jing* (Anonymous, ca. 200 B.C.). In Chinese Pharmacopoeia (Edition 2010), its main function is to nourish liver and kidneys, improve eyesight and make the hair black [3]. Previous phytochemical investigation indicated the presence of triterpenoids, iridoids [4-5], flavonoids, phenylethanoid glycosides and others, responsible for a variety of pharmacological effects *e.g.* anti-tumor, hepatoprotective, immune regulating, antioxidative and anti-aging effect, anti-inflammation and reducing hypercholesterolemia, *etc*.

In order to further study the genus *Ligustrum* and search for novel bioactive metabolites from it, in this paper, the chemical constituents from LLF were systematically studied. One new antioxidant secoiridoid glucoside dimer derivative, 4', 5'-(2'-hydroxy ligustrosidic acid) dimer (**1**), together with two known compounds, (8*E*)-nuezhenide (**2**) and specnuezhenide (**3**) (Figure 1) were obtained and identified. Details of the isolation and structure elucidation of the chemical constituents **1**-**3** are described, and their antioxidant activities were also tested by DPPH radical scavenging assay using ascorbic acid as a standard antioxidant.

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2. Materials and Methods

2.1. General experimental procedures

Melting point (MP) was determined on an X-4 micromelting point apparatus (Cany Precision Instruments Co., Ltd., Shanghai, China). IR spectra were recorded on a Nicolet 5700 FT-IR spectrometer using methanol as solvent (Thermo Nicolet Corporation, Madison, USA). UV spectra were measured on T6 New Century UV-vis spectrophotometer (Pgeneral, Beijing, China). NMR spectra were acquired with Bruker Advance 600 spectrometer (Bruker, Fallanden, Switzerland). HRESIMS data were recorded on an Agilent Technologies 6250 Accurate-Mass Q-TOF LC/MS spectrometer (Santa Clara, CA, USA). Preparative HPLC was performed on a LC 3000 instrument (Beijing Chuangxintongheng Science and Technology Co., Ltd., Beijing, China) connected to a UV 3000 detector, using an Intersil-ODS column (250 × 20 mm, 10 μm; GL Sciences Inc. Japan). TLC plate precoated with silica gel GF_{254} (20 × 20 cm) was purchased from Merck (Darmstadt, Germany). ODS (Octadecylsilyl, 50 μm, YMC Co., Ltd., Kyoto, Shimogyo-ku, Japan), silica gel (200-300 mesh, Qingdao Marine Chemical Industry, Qingdao, China) and AB-8 macroporous adsorption resin (The Chemical Plant of Nankai University, Tianjin, China) were used for column chromatography (CC). Spots were visualized under UV light or by spraying with vanillic aldehyde-10% concentrated sulfuric acid ethanol solution (5:95, v/v) followed by heating. Solvents [petroleum ether (60-90℃), $CHCl_3$, EtOAc, MeOH, CH_2Cl_2 and EtOH] were of analytical grade and purchased from Beijing Chemical Company, Beijing, China.

2.2. Plant material

Plant material as fruits of *Ligustrum lucidum* Ait. were collected from Jiangsu province, China, in January 2013 and identified by Researcher Zhimin Wang of Institute of Chinese Materia Medica, China Academy of Chinese Medical Sciences. A voucher specimen (No. 20130120) has been deposited there.

2.3. Extraction and isolation

The dry fruits (LLF; 5 Kg) were decocted three times with hot water (60℃, 50 L, 2 h each time), and the combined solution was concentrated under reduced pressure to yield an extract (400 g). Then the extract was subjected to AB-8 macroporous resin column chromatography eluted with EtOH-H_2O (0:100 and 50:50, v/v) and the fractions eluted with 50% EtOH afforded the total iridoids of LLF (LFI). The LFI portion was subjected to column chromatography on silica gel with a stepped gradient of $CHCl_3$-MeOH (100:10 to 100:50, v/v) to yield 5 fractions (Fractions 1-5). Fraction 4 was further separated by ODS column chromatography using MeOH-H_2O (10:90 to 100:0, v/v) to afford subfractions 1-4. Subfraction 4 was subjected to silica gel column chromatography gradiently eluted with $CHCl_3$-MeOH (100:1 to 100:50, v/v) to give 20 fractions (subfractions 4-1 - 4-20). Subfraction 4-4 was purified by preparative HPLC and compound **1** (5.4 mg) was obtained after Sephadex LH-20 column chromatography eluted with MeOH. Subfraction 4-10 was fractionated into two parts by preparative HPLC, and the first part yielded compound **2** (45.5 mg); The second part was subjected to sephadex LH-20 column chromatography eluted with MeOH to give compound **3** (200mg).

2.4. 4', 5'-(2'-hydroxy ligustrosidic acid) dimer (1)

Amorphous powder, mp 172-175°C. UV (MeOH) λmax: 193nm. IR *ν*max (KBr): 3429, 2952, 1713, 1633 cm^{-1}. HR-ESI-MS *m/z*: 1159.2758 $[M+Na]^+$ (calcd. for $C_{50}H_{56}O_{30}$, 1159.2754). ^{1}H-NMR, ^{13}C-NMR data in Table 1.

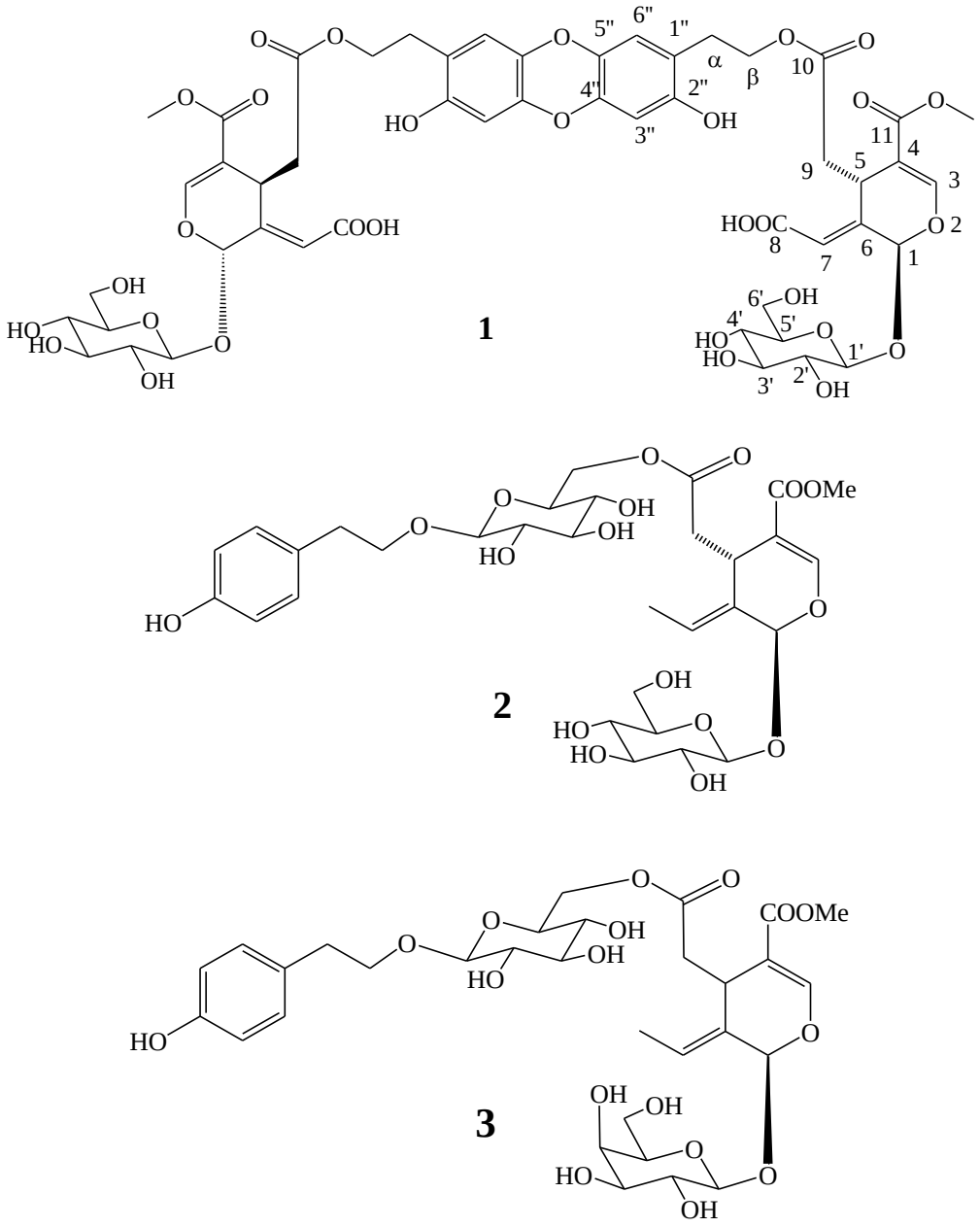

Figure 1. Chemical structures of compound **1-3**.

3. Results and Discussion

3.1. Structure elucidation

Compound **1** (Figure 1) was obtained as pale amorphous power. Its molecular formula was determined as $C_{50}H_{56}O_{30}$ with twenty-three degrees of unsaturation based on the observed pseudomolecular ion $[M + Na]^+$ at *m/z* 1159.2758 (calcd. 1159.2754) by HR-ESI-MS, which was supported by its NMR data. The IR spectrum showed the presence of hydroxyl (3429 cm^{-1}), carbonyl (1713 cm^{-1}), double bond (1633 cm^{-1}) groups. The ^1H and ^{13}C-NMR spectrum of **1** together with an HSQC experiment showed the presence of a 1, 2, 4, 5-tetrasubstituted benzene ring [δ_H 6.60 (1H, s), 6.42 (1H, s)], two double bonds [δ_H 7.52 (1H, s), 6.41 (1H, brs) corresponding to δ_C 154.7, 119.9], a

methoxy signal [δ_H 3.62 (3H, s), δ_C 52.0], three carbonyl groups (δ_C 165.7, 167.8, 172.5) and one six-carbon sugar (δ_C 101.2, 74.7, 77.9, 71.5, 78.6, 62.8).

Table1. ^{1}H NMR (600 MHz) and ^{13}C NMR (150 MHz) spectral data of compound **1** and ^{13}C NMR (150 MHz) spectral data of compounds **2**, **3** (CD_3OD, δ/ppm, *J*/Hz).

Position	**1**		**2**	**3**
	δ_H	δ_C	δ_C	δ_C
1	6.09 (1H, brd)	93.7	95.2	92.9
2				
3	7.52 (1H, s)	154.7	155.2	153.
4		109.2	109.5	129.
5	4.84 (1H, dd, *J*=3.6, 12.0 Hz)	34.3	31.8	30.0
6		147.2	130.6	107.
7	6.41 (1H, brs)	119.9	125.0	123.
8		165.7	13.8	13.0
9	2.96 (1H, dd, *J*=3.6, 13.2 Hz), 2.26 (1H, t, *J*=12.6 Hz)	42.2	41.4	39.5
10		172.5	173.1	170.
11		167.8	168.7	166.
α	2.89 (1H, td, *J*=2.4, 13.8 Hz), 2.30 (1H, dd, *J*=1.8, 15.0	30.8	36.5	34.8
β	4.67(1H, dt, *J*=3.6, 11.2 Hz)	68.3	72.3	70.1
1′	4.78 (1H, d, *J*=7.8 Hz)	101.2	100.9	99.0
2′	3.27 (1H, m)	74.7	74.8	73.2
3′	3.33 (1H, t, *J*=9.6Hz)	77.9	78.0	73.5
4′	3.23 (1H, m)	71.5	71.6	69.9
5′	3.27 (1H, m)	78.6	78.5	76.4
6′	3.82 (1H, dd, *J*=2.4, 12.0 Hz), 3.64 (1H, dd, *J*=1.8, 12.0	62.8	62.8	61.1
1″		123.3	130.8	128.
2″		142.3	131.0	129.
3″	6.42 (1H, s)	110.6	116.2	115.
4″		145.7	156.9	155.
5″		145.0	116.2	115.
6″	6.60 (1H, s)	117.8	131.0	129.
CH_3	3.62 (3H, s)	52.0	52.0	51.2
1‴			102.2	102.
2‴			72.6	73.2
3‴			75.6	77.3
4‴			69.7	70.1
5‴			73.1	76.5
6‴			63.8	64.0

The ^{1}H- and ^{13}C-NMR data of **1** were similar to those of a secoiridoid, oleuropeinic acid [6] except the resonance signals belonging to the benzene ring. In combination with the HMBC experiment (Figure 2), the part A of **1** was determined. According to molecular formula $C_{50}H_{56}O_{30}$, compound **1** was assigned as 4', 5'-(2'-hydroxy ligustrosidic acid) dimer.

Additionally, two known secoiridoids, (8*E*)-nuezhenide (**2**) [6-8] and specnuezhenide (**3**) [9] were obtained and identified by comparing their spectroscopic data with those reported in literatures.

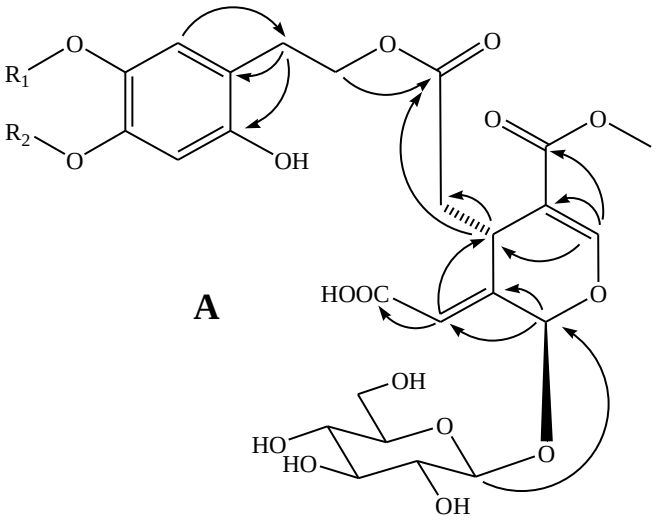

Figure 2. Key HMBC correlations of compound **1**.

3.2 Antioxidant activity

All the three compounds were tested for their antioxidant activity in the diphenylpicrylhydrazyl (DPPH) radical scavenging assay using a modified established protocol [10-11]. The IC_{50} values for compounds (**1**-**3**) were found to be 7.83, 391.13, 1100 µg/mL, respectively. The assay was done in comparison to ascorbic acid (IC_{50} = 2.45 µg/mL) which was taken as positive control.

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Supporting Information

Supporting Information accompanies this paper on http://www.acgpubs.org/RNP

References

[1] Pharmacopoeia Commission of the Ministry of Public Health, "Chinese Pharmacopoeia, Part I," 2010 edition, Chemical Industrial Press, Beijing, China, 2010.

[2] X. Yang and G. h. Jiang (2008). Current Situation and Prospect of Research and Development on the Fruit of Glossy Privet, *Lishizhen Med. Materia. Medica. Res.* **19**, 2987-2990.

[3] W. Huang and Y. F. Yang (2003). Pharmacological and Clinical Research Progress of Fructus Ligustri Lucidi and its effective components, *Modern J. Integ. Trad. Chin. Wester. Med.* **12**, 772-774.

[4] K. Inoue, T. Nishioka, T. Tanahashi and H. Inouye (1982). Three secoiridoid glucosides from *Ligustrum japonicum*, Phytochemistry. **21**, 2305-2311.

[5] M. Kikuchi and R. Kakuda (1999). Studies on the Constituents of *Ligustrum* Species. XIX. : Structures of Iridoid Glucosides from the Leaves of *Ligustrum lucidum* AIT. Yakugaku Zasshi. **119**, 444-450.

[6] X. J. Huang, Y. Wang, Z. Q. Yin and W. C. Ye (2010). Two new dimeric secoiridoid glycosides from the fruits of *Ligustrum lucidum*, *J. Asian Nat. Prod. Res.* **12**, 685-690.

[7] S. C. Ma, Z. D. He, X. L. Deng, P. P. But, V. E. Ooi, H. X. Xu, S. H. Lee and S. F. Lee (2001). In vitro evaluation of secoiridoid glucosides from the fruits of *Ligustrum lucidum* as antiviral agents, *Chem. Pharm. Bull.* **49**, 1471-1473.

[8] H. Inouye and T. Nishioka (1972). U¨ber die monoterpenglucoside und verwandte naturstoffe—XIX: U¨ber die struktur des nu¨zhenids, eines bitter schmeckenden glucosids aus Ligustrum lucidum sowie Ligustrum japonicum, *Tetrahedron* **28**, 4231-4237.

[9] L. F. Shi, Y. Y. Cao, H. S. Chen and J. P. Dong (1997). Isolation and identification of two new secoiridoids of water-soluble chemical constituents from the fruits of *Ligustrum lucidum* Ait., *Acta Pharm. Sin.* **32**, 442-446.

[10] M. S. Blois (1958). Antioxidant determinations by the use of a stable free radical, *Nature* **26**, 1199-1200.

[11] A. Yildirim, A. Mavi and A. A. Kara (2003). Antioxidant and antimicrobial activities of *Polygonum cognatum* Meissn extracts, *J. Sci. Food Agric.* **83**, 64-69.

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