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A New Lignan Glucoside from *Lagochilus ilicifolius*

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ABSTRACT

Background: The whole herb of *Lagochilus ilicifolius* has been used as a folk medicine for treating hemostatic, inflammation and ulcer in China. There were only limited reports on its chemical constituents, and no reports on its pharmacology study. **Objective:** To isolate compounds from the whole herb of *L. ilicifolius* and evaluate their cytotoxic activity. **Materials and Methods:** The column chromatographic techniques were used for separating the constituents of the *n*-butanol-soluble fraction of the 95% ethanol extract from the whole plant of *L. ilicifolius*. The structures of one new lignan and two known lignans were elucidated on the basis of spectroscopic analyses and comparison with literature data. The cytotoxic activities of these three lignans were evaluated using the MTT-assay against PC12 cell line derived from rat adrenal pheochromocytoma. **Results:** The new lignan was identified as *erythro*-1-[(4-*O*-*β*-D-glucopyranosyl-3-methoxyl)- phenyl]-2-[(5'-methoxyl)-pinoresinol]-propane-1,3-diol (1), and two known lignans were identified as tortoside C (2) and sisymbrifolin (3). The new lignan exhibited significant cytotoxic activity against PC12 cell line with IC_{50} value of 1.22 ± 0.03 μmol/L. **Conclusions:** A new lignan, *erythro*-1-[(4-*O*-*β*-D-glucopyranosyl -3-methoxyl)-phenyl]-2-[(5'-methoxyl)-pinoresinol]-propane-1,3-diol and two known lignans were isolated from the whole herbs of *L. ilicifolius*. The two known lignans were reported for the first time in the genus *Lagochilus.* Three lignans were evaluated for *in vitro* cytotoxic activity. The new lignan showed relatively strong cytotoxicity against PC12 cell line, while sisymbrifolin and tortoside C exibited no cytotoxicity.

Keywords: Cytotoxic activity, Labiatae, *Lagochilus ilicifolius*, lignans

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INTRODUCTION

The genus *Lagochilus*, belonging to Labiatae family, comprises 35 species distributed mainly in central Asia such as Turkistan, Iran, Afghanistan, Russian, Mongolia and China**,** of which 14 species have been found to distribute wild in China.[1,2] Some plants in this genus, including *L. inebrians* and *L. lanatonodus*, are employed as infusion or tincture as antihemorrhagic for their hemostatic effects.[3-5] Some plants were also applied for the treatment of allergic dermatosis.[6-8] Previous phytochemical studies revealed the presence of diterpenoids, flavonoids, coumarins, iridoid glycosides and polysaccharides in this genus.[6,7,9-24] Pharmacological results indicated that diterpenoids from this genus, including lagochiline and its derivatives, could have the ability of hemostatic.[20,25]

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L. ilicifolius, a folk medicine used for the treatment of hemostatic, inflammation and ulcer, distributes in northwest regions in China.[26] In our previous studies, we have described a lignan from this plant.[27] In a continued search for bioactive constituents from this plant, one new lignan, *erythro*-1-[(4-O-*β*- D- glucopyranosyl- 3- methoxyl)-phenyl]- 2- [(5'-methoxyl)-pinoresinol]-propane-1,3-diol (1, Figure 1), and two known lignans tortoside C (2, Figure 1) and sisymbrifolin (3, Figure 1), were isolated from the whole herbs of *L. ilicifolius*. In the present paper, the structure elucidation of the new lignan is reported.

MATERIALS AND METHODS

General procedure and reagents Optical rotations were measured on a KRÜSS P8000-T digital polarimeter. UV spectra were measured with a UV-1901 recording spectrophotometer (Beijing Puxi General Instrument Co., Ltd., Beijing, China). IR spectra were recorded on Nicolet™-380 spectrophotometer from Thermo Electron. NMR spectra were recorded on Brucker AV-500

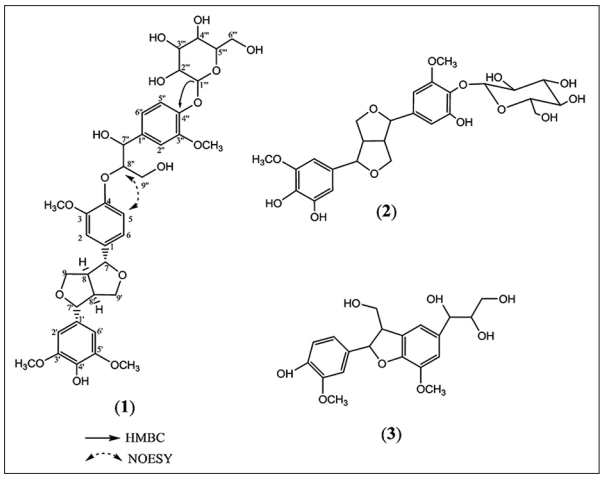

Figure 1: Structure of isolated compounds 1–3 and selected HMBCs of the new lignan (compound 1)

(Switerland, Bruker) with TMS as internal reference. HR-ESI-MS were obtained on Brucker APEXIII 7.0 TESLA FTMS (Switerland, Bruker).

Column chromatography (CC): silica gel (200-300 mesh, Qingdao Haiyang Chemical Co., Ltd., Qingdao, China), Sephadex LH-20 (GE-Healthcare Bio-Sciences AB, Uppsala, Sweden), D101 macroporous resin (Sinopharm Chemical Reagent Co., Ltd., Shanghai, China), microporous resin (MCI) (75-150 μm, Mitsubishi Chemical Corporation, Tokyo, Japan) and octadecylsilyl (ODS) (40-60 μm, Sepax Technologies Inc., USA). All reagents were of analytical grade.

Plant materical

The whole herbs of *L. ilicifolius* were collected in Yinchuan, Ningxia, China, in July 2009. A voucher specimen (No. 2009001) was identified by Professor Xu Hong, and has been deposited in the herbarium of Institute of Chinese Materia Medica, Shanghai University of Traditional Chinese Medicine.

Extraction and isolation

The whole herbs of *L. ilicifolius* (13 kg) were ground and exhaustively extracted with 95% ethanol at 80°C (for 2 h, 3 times). The solvent was evaporated *in vacuo* to yield a dry residue (489.8 g). The residue was then suspended in water and extracted successively with petroleum ether, dichloromethane and *n*-butanol to give four extracts including petroleum ether extract (163.0 g), dichloromethane extract (22.8 g), *n*-butanol extract (73.0 g) and H_2O extract (231.0 g).

The *n*-butanol extract (73.0 g) was subjected to D101 column chromatography and eluted with H_2O, 30% EtOH, 60% EtOH and 90% EtOH to give four fractions including Fr. H_2O (54.0 g), Fr. 30% EtOH (12.0 g), Fr. 60% EtOH (5.0 g) and Fr. 90% EtOH (2.0 g).

Fr. 30% EtOH (12.0 g) was subjected to silica gel column chromatography and eluted by CH_2Cl_2-MeOH = 20:1 to yield Subfr. 1-2. Subfr. 1 was subsequently subjected to MCI and silica gel column chromatography (EtOAc-MeOH-H_2O = 20:2:1) to afford compound tortoside C (2).

Fr. 60% (5.0 g) was subjected to MCI column chromatography to yield Subfr. 1-6. Subfr. 3 was subjected to Sephadex LH-20 column chromatography (eluted with MeOH) to afford compound sisymbrifolin (3). Subfr. 5 was subjected to silica gel (EtOAc- MeOH-H_2O = 12:1:0.5) and ODS column chromatography (eluted with 40% MeOH), and further purified by Sephadex LH- 20 column chromatography (eluted with MeOH) to give compound erythro - 1- [(4 -O-β-D- glucopyranosyl-3-methoxyl)-phenyl]-2-[(5'-methoxyl)-pinoresinol]- propane -1,3-diol (1).

Cytotoxicity assay

PC12 cell line, derived from a rat pheochromocytoma, was obtained from the American Type Culture Collection (ATCC, Manassas, VA). The cells were maintained in Dulbecco's modified Eagles medium (DMEM) supplemented with 6% fetal bovine serum, 6% horse serum, 100 U/mL penicillin, and 100 mg/mL streptomycin at 37°C in a water-saturated 7.5% CO_2 incubator. Cultured PC12 cells in 96-well-plate (15,000 cells/well) were pre-treated with various concentrations (0.1, 0.3, 1, 3, 10, 30, 100 μM) for 48 h. Cell viability test was performed with the addition of thiazolyl blue tetrazolium bromide (MTT) (Sigma, USA) in PBS at a final concentration of 0.5 mg/mL for 1 h. After the solution was removed, the purple precipitate inside the cells was re-suspended in DMSO and then measured at 570 nm absorbance.

RESULTS

Compound 1 white amorphous solid (CH_3OH), [α]–16.7(c, 0.191, CH_3OH). UV (CH_3OH) λ_{max}: 291 nm. IR (KBr) ν cm^{-1}: 3 417, 2 930, 1 594, 1 514, 1 463, 1 422, 1 267, 1 224, 1 125, 1 075, 635. HR-ESI-MS: *m/z* 745.2711 $[M–H]^-$ (calcd. 746.2786). ^1H NMR and ^{13}C NMR data were shown in Table 1.

Compound 2 white amorphous solid (CH_3OH). EI-MS: *m/z* 553 $[M–H]^-$. ^1H NMR (Pyr, 500 MHz) δ_H:7.05 (2H, d, *J* = 13.5Hz, H-2', 6'), 5.06 (2H, *J* = 4.5Hz, H-2, 6), 4.13 (2H, dd, *J* = 9.0, 4.5Hz, H-4a, 8a), 4.02 (2H, dd, *J* = 4.5, 4.5Hz, H-4e, 8e), 3.88 (6H, s, 2 × OCH_3), 3.23-3.34 (H of sugar).

Table 1: NMR chemical shifts of compound 1 (125 MHz for ^{13}C and 500 MHz for ^{1}H)

Position	δ (H), (*J*) [Hz] (CD_3OD)	δ (C)	HMBC ($^{1}H \rightarrow ^{13}C$)
1		133.7	
2	6.94 *d* (1.5)	111.3	C-1, C-3, C-4
3		149.4	
4		147.3	
5	6.81*d* (8.5)	116.1	C-1, C-3, C-4
6	6.77 *dd* (8.5, 1.5)	120.0	C-4, C-7
7	4.72 *d* (4.5)	87.3	C-2, C-6
8	3.12 *brs*	56.0	
9	4.25 *m* 3.90 *m*	73.2	
1'		136.4	
2'	6.65 *s*	104.5	
3'		154.7	
4'		139.2	
5'		154.7	
6'	6.65 *s*	104.5	C-1', C-3', C-4', C-7'
7'	4.70 *d* (4.5)	87.4	C-6', C-8', C-9'
8'	3.12 *brs*	56.0	
9'	4.25 *m* 3.90 *m*	73.0	
1"		137.6	
2"	7.04 *d* (1.5)	112.5	C-1", C-3", C-4"
3"		150.7	
4"		147.5	
5"	7.08 *d* (8.5)	117.5	C-1", C-3", C-4"
6"	6.88 *d* (8.5,1.5)	121.2	C-3", C-4", C-7"
7"	4.90 *d* (5.5)	74.1	C-1", C-2", C-6", C-8", C-9"
8"	4.25 *brs*	87.3	
9"	3.67 *dd* (12, 4.5) 3.88 *dd* (12,4.5)	62.0	
3-OMe	3.85 *s*	56.8	C-3
3"-OMe	3.85 *s*	56.9	C-3'
5'-OMe	3.85 *s*	56.9	C-5'
3"-OMe	3.84 *s*	56.9	C-3"
Glu			
1	4.86 *d* (7.5)	103.1	C-4'
2	3.46 *t* (7.5,7.5)	75.2	
3	3.65 *m*	78.5	
4	3.38 *m*	71.7	
5	3.60 *m*	78.1	
6	3.88 *dd* (12.0, 4.5) 3.68 *dd* (12.0,4.5)	62.9	

NMR: Nuclear magnetic resonance; HMBC: Heteronuclear multiple bond correlation

^{13}C NMR (Pyr, 125MHz) δ_C: 138.50 (C-5', 5"), 135.4 (C-4', 4"), 105.1 (C-glc-1), 105.0 (C-6', 6"), 86.4 (C-2, 6), 78.8 (C-glc-5), 78.5 (C-glc-3), 76.2 (C-4, 8), 72.4 (C-glc-2), 71.7 (C-glc-4), 62.7 (C-glc-6), 56.8 (OCH_3), 54.9 (C-1, 5). The ^{1}H and ^{13}C NMR data are in accordance with those in literature,[28] so compound 2 was identified as tortoside C.

Compound 3 white powder (CH_3OH). EI-MS: *m/z* 393 $[M–H]^-$. ^{1}H NMR (CD_3OD, 500 MHz) δ_H: 6.85 (1H, d = 2.5 Hz, H-2), 6.85 (1H, s, H-2'), 6.84 (1H, s, H-6'), 6.81 (1H, dd, *J* = 8.5, 2.0 Hz, H-6), 6.67 (1H, d, H = 8.5 Hz, H-5), 5.44 (1H, d, *J* = 6.0 Hz, H-7), 4.48 (1H, d, *J* = 4.0 Hz, H-7'), 3.77 (3H, s, 3-OCH_3), 3.71 (3H, s, 3'-OCH_3), 3.75 (2H, m, H-9), 3.59 (1H, m, H-8'), 3.45 (1H, d, *J* = 11.0 Hz, H-9a'), 3.40 (1H, d, *J* = 6.0Hz, H-8), 3.32 (1H, d, *J* = 11.5Hz, H-9b'). ^{13}C NMR (CD_3OD, 125MHz) δ_C: 136.9 (C-1), 110.5 (C-2), 147.5 (C-4), 116.1 (C-5), 119.6 (C-6), 89.1 (C-7), 55.3 (C-8), 64.8 (C-9), 129.7 (C-1'), 112.5 (C-2'), 145.2 (C-3'), 149.1 (C-4'), 134.6 (C-5'), 116.6 (C-6'), 75.3 (C-7'), 77.6 (C-8'), 64.2 (C-9'). The ^{1}H and ^{13}C NMR data are in accordance with those in literature,[29] so compound 3 was identified as sisymbrifolin.

DISCUSSION

Compound 1 was a white amorphous solid, [α]–16.7(c 0.191, CH_3OH). The molecular formula was deduced as $C_{37}H_{46}O_{16}$ from negative HRESI-MS $[M–H]^-$ at *m/z* 745.2711. The UV spectrum displayed a maximum absorption at 291 nm (CH_3OH). The IR spectrum showed the presence of hydroxyl at 3417 cm^{-1}, aromatic rings at 1594 and 1514 cm^{-1} and C–O–C bond at 1224 and 1075 cm^{-1}.

The NMR data [Table 1] showed that 1 contained two ABX spin systems assignable to two 1,3,4-trisubstituted benzene rings at δ_H 6.81 (1H, *d*, *J* = 8.5 Hz, H-5), 6.77 (1H, *dd*, *J* = 8.5, 1.5 Hz, H-6), and 6.94 (1H, *d*, *J* = 1.5 Hz, H-2); and 7.08 (1H, *d*, *J* = 8.5 Hz, H-5"), 6.88 (1H, *d*, *J* = 8.5, 1.5 Hz, H-6") and 7.04 (1H, *d*, *J* = 1.5 Hz, H-2"); two magnetic equivalent aromatic protons at δ_H 6.65 (2H, s) were indicative of one symmetrical 1,3,4, 5-tetrasubstituted benzene ring. The ^{1}H-NMR chemical shifts observed for the three benzene systems together with the presence of four aromatic methoxyl group signals at δ_H 3.85 (9H, s, 3'-OMe, 5'-OMe and 3-OMe) and 3.84 (3H, s, 3"-OMe) suggested the presence of two guaiacyl (3-methoxy-4-hydroxyphenyl) groups and one 3,5-dimethoxy-4-hydroxyphenyl in this compound.[30] In addition, the NMR spectral data of 1 also established one unit of β-glucose, a *bis*-tetrahydrofuran ring[31] and a propan-1,2,3-triol moiety.[32] Detailed analysis of the above data of 1 suggested that compound 1 was a sesquilignan monoglucoside consisting of (+)-medioresinol,[33] 3-(4-hydroxy-3-methoxyphenyl)-propan-1,2,3-triol and β-D-glucopyranose.

The NOESY correlation of the H-8" signal δ_H 4.25 (1H, brs) with the H-5 signal δ_H 6.81 (1H, d, *J* = 8.5 Hz) of the guaiacyl group in (+)-medioresinol indicated that 3-(4-hydroxy-3-methoxyphenyl)-propan-1,2,3-triol was

connected to the C-4 position of (+)-medioresinol. The relatively small coupling constant of H-7" signal δ_H 4.90 (1H, *d*, J = 5.5 Hz) indicated that the glycerol moiety was in the *erythro*-configuration.[34] The attached position of β-D-glucopyranose was determined by the HMBC correlation of the anomeric proton at δ_H 4.86 (1H, d, J = 7.5 Hz) with the signal at δ_C 147.5 (C-4"). The NMR spectral data of 1 was similar to that of a known sesquilignan monoglucoside *erythro*-1- (4-*O*-β-D-glucopyranosyl-3,5-dimethoxy-phenyl)-2-syring aresinoxyl- propane-l, 3-diol.[31] The only difference was that two methoxyl groups at δ_H 3.88 (5-OMe) and δ_H 3.84 (5"-OMe) present in the known sesquilignan monoglucoside had disappeared in 1; instead, two aromatic proton signals were observed at δ_H 6.81 (1H, d, J = 8.5, H-5) and δ_H 7.08 (1H, d, J = 8.5, H-5"). Based on similar chemical shifts and coupling constants of H-7 (δ 4.72, 1H, d, J = 4.5 Hz) and H-8 (δ 3.12, 1H, brs), H-7' (δ 4.70, 1H, d, J = 4.5 Hz) and H-8' (δ 3.12, 1H, brs) to those of *erythro*-1-(4-*O*-β-D-glucopyranosyl-3,5-dimet hoxy- phenyl)-2-syringaresinoxyl-propane-l, 3-diol, the relative configuration of C-7, C-8, C-7' and C-8' of 1 was proposed to be the same as those of *erythro*-1-(4-*O*-β-D-glucopyran osyl-3,5-dimethoxy-phenyl)-2- syringaresinoxyl-propane-l, 3-diol with H-7, H-8, H-7', H-8' as *b*, *a*, *b* and *a*, respectively.[31] Therefore, compound **1** was identified as *ery thro*-1-[(4-*O*-β-D-glucopyranosyl-3-methoxyl) phenyl]-2-[(5'-methoxyl)-pinoresinol]-propane-1,3-diol. [Figure 1].

Compound 1-3 were evaluated *in vitro* for cytotoxicity against PC12 cell line derived from a transplantable rat pheochromocytoma employing a MTT-assay. The new lignan (1) exhibited significant cytotoxicity with the IC_{50} value of 1.22 ± 0.03 mol/L.

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