Nine compounds from the root bark of *Lycium chinense* and their anti-inflammatory activities

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**KEY WORDS**

*Lycium chinense*; Lyciumlignan D; Lyciumphenylpropanoid A; Structure elucidation; Anti-inflammatory effect

**Abstract**

Two new compounds, named lyciumlignan D (1) and lyciumphenyl propanoid A (2), along with seven known compounds, were isolated from the root bark of *Lycium chinense*. Their structures were elucidated using spectroscopic data (UV, IR, HR-ESI-MS, 1D and 2D NMR, CD), as well as by comparison with those of the literature. Compounds 3–9 were isolated from this genus for the first time. In the in vitro assay, compounds 3, 6, and 7 exhibited stronger anti-inflammatory effects than the positive control curcumin at a concentration of 10 μmol/L.

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1. Introduction

The root bark of *Lycium chinense* Mill. or *Lycium barbarum* L., named Digupi in China, is a famous traditional Chinese medicine. During the recent decades, it has been used for treating diabetes, cough, hypertension, and fever. Previous phytochemical investigations have been reported for different types of chemical constituents, including alkaloids, cyclopeptides, lignans, anthraquinones, coumarines, flavonoids, terpenoids, sterols, and other compounds. As part of an ongoing effort for bioactive constituents from the root bark of *L. chinense*, two new compounds (lyciumlignan D and lyciumphenyl propanoid A, Fig. 1) and seven known compounds (3–9) were isolated. In addition, all of the isolates were tested for anti-inflammatory activity by suppressing the production of NO in lipopolysaccharide-induced BV2 cells. In this paper, we reported the isolation, structure elucidation, and bioactivity evaluation of the compounds isolated from the root bark of *L. chinense*.

2. Results and discussion

Compound 1 was obtained as a white amorphous powder, whose molecular formula was confirmed to be C_{37}H_{36}O_{13} by the sodiated molecular ion peak observed at m/z 607.2000 [M + Na]^+ in the HR-ESI-MS. In the IR spectrum, absorption bands for hydroxyl, aromatic ring (1616, 1518, and 1463 cm$^{-1}$) were observed. In the upfield region, four oxygenmethine protons at $\delta_{\text{H}}$ 6.63 (2 H, s), 6.87 (1 H, d, $J = 1.5$ Hz), and 6.86 (1 H, d, $J = 1.5$ Hz), revealing the presence of two tetra-substituted aromatic rings. In the 1H NMR spectrum (Table 1), a set of ABX spin-system aromatic protons at $\delta_{\text{H}}$ 6.37 (1 H, m), 3.60 (1 H, m), 3.35 (1 H, m), and 3.16 (1 H, m), three oxygenmethine protons at $\delta_{\text{H}}$ 5.39 (1 H, d, $J = 7.0$ Hz), 4.55 (1 H, d, $J = 6.0$ Hz), and 3.63 (1 H, m), and one methine proton at $\delta_{\text{H}}$ 3.46 (1 H, m) were detected. In addition, three methoxyl groups at $\delta_{\text{H}}$ 3.72 (6 H, s) and 3.76 (3 H, s) and a glucopyranosyl anemic proton at $\delta_{\text{H}}$ 4.39 (1 H, d, $J = 7.5$ Hz) were observed. The $^{13}$C NMR spectrum (Table 1) of 1 exhibited 27 carbon signals, apart from three methoxyl groups and six carbon signals of one O-glucose unit, and the remaining 18 carbon signals could be attributed to a lignan skeleton. The HMBC correlations (Fig. 2) from H-7 (C-1, C-2, C-6, C-8, and C-9) and from H-7 (C-1′, C-2′, C-6′, C-8′, and C-9′) confirmed the presence of two C6-C3 units. Furthermore, the key correlations from H-8 (C-1, C-2, C-3, C-4, C-5, and C-6) and from H-7 (C-1, C-2, C-3, C-4, C-5, C-6, C-7, C-8, and C-9) verified that 1 was a benzofuran-type lignan. The locations of three methoxyl groups were determined to be at C-3, C-5, and C-3′ by the HMBC correlations of C-7, C-3, C-5, and C-3′. Based on the HMBC correlation of the anemic proton H-1′ correlated with C-7′, the glucose unit was determined to be located at C-7′. The trans configuration between H-7 and H-8 was identified by a big coupling constant ($J_{7,8} = 7.0$ Hz). In combination with positive Cotton effect at 243 and 286 nm, the 7.8′-three configuration of 1 was established. Furthermore, the 7.8′-three configuration was confirmed by the coupling constant of H-7′ ($J_{7,8} = 6.0$ Hz). Unfortunately, the absolute configurations of C-7′ and C-8′ were not identified. From the above analysis, the structure of 1 was elucidated as shown, and was accorded the trivial name lyciumlignan D.

Compound 2 was isolated as a white amorphous powder. Its molecular formula (C_{24}H_{36}O_{14}) was deduced from positive HR-ESI-MS (m/z 571.0077 [M + Na]^+). The IR spectrum similarly revealed the existence of hydroxyl, –CH$_2$–, and aromatic ring. In the 1H NMR spectrum (Table 1), a set of ABX spin-system aromatic protons at $\delta_{\text{H}}$ 7.03 (1 H, d, $J = 1.5$ Hz), 7.00 (1 H, d, $J = 1.5$ Hz), and 6.88 (1 H, d, $J = 8.0$ Hz), a set of typical double bond resonances at $\delta_{\text{H}}$ 6.45 (1 H, d, $J = 16.0$ Hz) and

![Figure 1](https://example.com/figure1.png)

**Figure 1** Structures of compounds 1–9.
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Table 1. 

| Position | 1 | 2 |
|----------|---|---|
| δ_H | δ_C | δ_H | δ_C |
| 1 | 6.63, s | 131.3 | 130.6 |
| 2 | 6.3, s | 103.7 | 7.03, d (1.5) | 109.9 |
| 3 | 147.9 | 3.76, s | 55.7 |
| 4 | 135.3 | 128.5 |
| 5 | 147.9 | 7.00, d (8.0) | 115.8 |
| 6 | 6.63, s | 103.7 | 6.88, dd (1.5, 8.0) | 119 |
| 7 | 5.39, d (7.0) | 87.3 | 6.45, d (16.0) | 128.5 |
| 8 | 3.46, m | 53 | 6.25, dt (16.0, 5.0) | 128.8 |
| 9 | 3.67, m | 62.7 | 4.08, brd (5.0) | 61.6 |
| 10 | 3.60, m | 68 |
| 11 | 4.34, m | 78.8 |
| 12 | 3.58, m | 60 |
| 1' | 133.5 | 4.19, d (8.0) | 103.3 |
| 2' | 6.87, d (1.5) | 115.7 | 2.95, t (8.5) | 73.3 |
| 3' | 142.9 | 3.13, m | 76.6 |
| 4' | 146.6 | 3.00, t (9.0) | 70 |
| 5' | 128.7 | 3.24, m | 75.4 |
| 6' | 6.86, d (1.5) | 112.2 | 3.41, m | 67.5 |
| 7' | 4.55, d (6.0) | 82 |
| 8' | 3.63, m | 75.2 |
| 9' | 3.35, m | 61.9 |
| 1'' | 3.16, m | 61 |
| 2'' | 3.60, m | 74.2 | 3.75, m | 75.8 |
| 3'' | 3.02, m | 76.9 |
| 4'' | 3.04, m | 69.9 | 3.56, m | 73.3 |
| 5'' | 3.14, m | 76.4 | 3.32, m | 63.2 |
| 6'' | 3.57, m | 61 |
| 3-CH_3O | 3.72 (s) | 56 | 3.76, s | 55.5 |
| 5-CH_3O | 3.72 (s) | 56 |
| 3'-CH_3O | 3.76 (s) | 55.7 |

6.25 (1 H, dt, J = 16.0, 5.0 Hz), two oxymethylene protons at δ_H 4.08 (2 H, brd, J = 5.0 Hz), and a methoxy group at δ_H 3.76 (3 H, s) were displayed, which indicated the presence of a coniferyl alcohol unit. Additionally, four oxymethylene protons at δ_H 3.84 (1 H, m), 3.68 (1 H, m), and 3.58 (2 H, m), together with an oxymethine proton at δ_H 4.34 (1 H, m) confirmed the existence of a glycerol moiety. Two anomeric protons at δ_H 4.19 (1 H, d, J = 8.0 Hz) and 4.83 (1 H, d, J = 2.5 Hz) were also observed in the upfield region, which could be assigned to a glucose moiety and an apiose moiety. The 13C NMR spectrum (Table 1) of 2 exhibited 24 carbon signals, including a coniferyl alcohol moiety, a glycerol moiety, a glucose moiety, and an apiose moiety. The connections between these moieties were established by the HMBC correlation peaks (Fig. 2). An obvious HMBC correlation from H-11 (δ_H 4.34) to C-4 (δ_C 146.6) indicating the C11-O-C4 connection between coniferyl alcohol and glycerol moiety. Moreover, the HMBC correlation of H-1' (δ_H 4.19) with C-10 (δ_C 68.0) revealed that the glucopyranose unit was attached to C-10, while the correlation of H-1'' (δ_H 4.83) with C-6' (δ_C 67.5) suggested that the apiofuranose unit was attached to C-6'. Consequently, the structure of 2 was identified as shown, and was accorded the trivial name lyciumphenylpropanoid A.

The structures of seven known compounds (3–9) isolated from the root bark of *L. chinense*, were also identified by comparison of their spectroscopic data to those found in the literature. They were elucidated as: periplocoside M (3), intrapilosin I (4), (-)-lyoniresinosinol-4,9-di-O-β-g-lucopyranoside (5), cedrusin (6), smiliglabrol (7), 2',5-di-hydroxy-6,7,8,6'-tetramethoxy flavone (8) and lycifuranone A (9).

All of the compounds were tested for their anti-inflammatory activities by suppressing the production of NO in lipopolysaccharide-induced BV2 cells. As a result, compounds 3, 6, and 7 exhibited 87.5%, 83.6%, and 75.0% inhibition at 10 μmol/L, respectively, whereas the positive control curcumin showed 65.75% inhibition at 10 μmol/L. Then, a further test of compounds 3, 6, and 7 was performed at 1 μmol/L, which demonstrated 60.0%, 56.9%, and 60.0% inhibition, respectively.

3. Experimental

3.1. General experimental procedure

Optical rotations were measured on a Jasco P-2000 polarimeter (Jasco Corp; Tokyo, Japan). The UV spectra were measured on a Jasco V650 spectrophotometer (Jasco). The IR spectra were recorded on a Nicolet 5700 spectrometer (Thermo Scientific, FL). The CD spectra were measured on a Jasco J-815 CD spectrometer. The high-resolution electrospray ionization mass spectrometry (HR-ESI-MS) was performed on an Agilent 6520 HPLC-Q-TOF (Agilent Technologies, Waldbronn, Germany). 1H NMR (500 MHz), 13C NMR (125 MHz), and 2D NMR spectra were recorded with a Bruker 500 MHz spectrometer (Bruker-Biospin, Billerica, MA, USA) and values were given in ppm (δ).

Figure 2. Key HMBC (H→C) correlations of compounds 1 and 2.
Japan) and Sephadex LH-20 (Pharmacia Fine Chemicals, Uppsala, Sweden). Flash chromatography was conducted using Combiflash RF200 (Teledyne Isco Corp., Nebraska, USA). Preparative HPLC was carried out on a Shimadzu LC-10A instrument with a SPD-20A detector (Shimadzu Corp., Tokyo, Japan), using YMC-Pack ODS-A column (250 mm × 20 mm, 5 μm, YMC Corp., Kyoto, Japan). HPLC-DAD analysis was set up on Agilent 1200 series system (Agilent Technologies) with an Apollo C18 column (250 mm × 4.6 mm, 5 μm, Alltech Corp., Kentucky, USA).

3.2. Plant material

The root bark of the *L. chinense* was collected from Ninging Town, Zhongning County, Ningxia Hui autonomous region, People’s Republic of China, in March 2012. A voucher specimen (ID-S-2592) was deposited in the Herbarium of the Institute of Materia Medica, Chinese Academy of Medical Science, Beijing.

3.3. Extraction and isolation

The powdered plant material (100 kg) was extracted three times with 80% EtOH (600 L) under reflux. The solvent was evaporated and the crude residue (8.0 kg) was partitioned with EtOAc (3 × 45 L). The EtOAc and H₂O solvent were removed under reduced pressure, which yielded Fr. 1 and Fr. 2, respectively. Fr. 1 (1.5 kg) was subjected to column chromatography on silica gel and continuously eluted with petroleum ether, EtOAc, acetone, and methanol, which gave fractions I–IV. Fraction II was further separated on silica gel chromatography column, and eluted with petroleum ether–EtOAc (from 100 : 0 to 0 : 100) in a gradient to afford 10 fractions (fractions II-1–II-10). Fr. II-2 was chromatographed over Sephadex LH-20 and eluted with gradient mixtures of MeOH–H₂O (from 60:40 to 100:0) to yield Fr. II-2-1–II-2-16. Fr. II-2-5 was further purified with pHPLC (MeOH–H₂O, 65:35) to yield 8 (9 mg). Fr. II-6 was subjected to Combiflash RF200 apparatus with a C18 column (55 cm × 8 cm, 50 μm) and eluted with MeOH–H₂O (from 5:95 to 100:0) to provide Fr. II-6-1–Fr. II-6-20. Compound 3 (20 mg) was obtained from Fr. II-6-12 by recrystallization. Fr. II-8 was purified using reverse-phase preparative HPLC with MeOH–H₂O (60:40) as the mobile phase to give 4 (19 mg).

Fr. 2 (1.375 kg) was chromatographed with a macroporous resin column (HP-20, 200 cm × 15 cm) and eluted successively with H₂O, 15%, 30%, 50%, 70%, and 95% EtOH. The 30% EtOH fraction was concentrated and was further chromatographed over a macroporous resin (SP-700, 200 cm × 15 cm) and eluted successively with 15%, 20%, 25%, 30%, 45%, 50%, and 95% EtOH, which yielded fractions A–G. Fr. C (72 g) was subjected to Combiflash RF200 apparatus with a C18 column (55 cm × 8 cm, 50 μm) and eluted with MeOH–H₂O (from 5:95 to 100:0) to provide Fr. C-1–Fr. C-25. Fr. C2 (4 g) was chromatographed over Sephadex LH-20 and eluted with gradient mixtures of MeOH–H₂O (from 10:90 to 95:5) to yield Fr. C2-1–C2-19. Fr. C2-6 was purified with reverse-phase preparative HPLC (MeOH–H₂O, 30:70) to yield 1 (20 mg) and 2 (35 mg). Fr. C6 was separated on Sephadex LH-20 column (H₂O) and then further purified by reverse-phase preparative HPLC with MeOH–H₂O (40:60) to afford 5 (13 mg), 6 (8 mg), and 7 (6 mg). Similar to Fr. C6, compound 9 (20 mg) was isolated from Fr. C8.

3.3.1. *Lyciumlignan D (1)*

White amorphous powder, [α]D20 + 22.9 (c 0.10 MeOH); UV (MeOH) λmax (logε): 238 (4.02), 282 (3.53) nm; CD (MeOH) λmax (Δε): 217 (+5.03), 242 (+4.14), 286 (+0.89) nm; IR (KBr) νmax: 3382, 2929, 2881, 1616, 1518, 1463, 1332, 1217, 1114, 1068 cm⁻¹; HR-ESI-MS: m/z 607.2000 [M+Na]⁺ (Calcd. for C24H33O13Na, 607.2007). For 1H NMR and 13C NMR spectroscopic data, see Table 1.

3.3.2. *Lyciumphenylpropanoid A (2)*

White amorphous powder, IR (KBr) νmax: 3354, 2934, 1576, 1511, 1420, 1266, 1229, 1048, 859, 778 cm⁻¹; HR-ESI-MS: m/z 571.007 [M+Na]⁺ (Calcd. for C24H32O14Na, 571.1997). For 1H NMR and 13C NMR spectroscopic data, see Table 1.

3.4. Determination of the absolute configuration of sugar

Compounds 1 and 2 (2 mg) were separately dissolved in 1 mol/L CF₃COOH–H₂O (5 mL) and refluxed for 6 h. The mixture was concentrated under vacuum, and the residue was suspended in H₂O and extracted three times with EtOAc. The aqueous layer was evaporated under vacuum, diluted repeatedly with H₂O and evaporated under vacuum to produce a neutral residue. The residue was dissolved in fresh anhydrous pyridine (1.0 mL). t-Cysteine methyl ester hydrochloride (2 mg) was added, and the reaction mixture was incubated at 60 °C for 2 h. After that, N-trimethylsilylimidazole (0.2 mL) was added into the mixture which had been dried by nitrogen current. The reaction mixture was incubated at 60 °C for 2 h and partitioned between n-hexane and H₂O (2 mL each). The n-hexane extract was subjected to GC analysis under the following conditions: capillary column, HP-5 (60 m × 0.25 mm, with a 0.25 μm film, Dikma); Detection, FID; detector temperature, 300 °C; injection temperature, 300 °C; initial temperature 200 °C, then raised to 260 °C at 10 °C/min, final temperature maintained for 30 min; carrier, N₂ gas. The d-apiose and d-glucose were confirmed by comparing the retention time of their derivatives to the standard sugar derivatized, which exhibited retention times of 18 and 29 min, respectively.

3.5. Anti-inflammatory effects of compounds

Compounds 1–9 were tested for anti-inflammatory activity on BV2 microglial cells, using the same method as described in the Refs. 22 and 23.

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Appendix A. Supporting information

Supplementary data associated with this article can be found in the online version at http://dx.doi.org/10.1016/j.apsb.2017.04.004.

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