**Sterols from *Lindera glauca* Blume Stem Wood**

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Chipped stem wood from *Lindera glauca* was extracted repeatedly with 80% aqueous methanol at room temperature, and the concentrated methanolic extract was successively partitioned with ethyl acetate (EtOAc), *n*-butyl alcohol, and H₂O. From the EtOAc fraction, four sterols were isolated through a repeated silica gel and octadecyl silica gel column chromatography. The chemical structures of the sterols were elucidated as β-sitosterol (1), 7-ketositosterol (2), 7β-hydroxysitosterol (3), and daucosterol (4). Among them, compounds 2 and 3 were isolated for the first time from the stem woods of this plant.

**Key words:** daucosterol, 7β-hydroxysitosterol, 7-ketositosterol, *Lindera glauca*, NMR, β-sitosterol

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**Lindera glauca** Blume is a deciduous shrub that belongs to the Lauraceae family and is widely distributed in the mountainous districts of China, Japan, Korea, and Taiwan [Lee, 1998]. The fruits of *L. glauca* have been used as traditional medicines to treat symptoms of paralysis including abdominal pain and speech disorder. The roots of the species have been traditionally employed as pharmaceuticals as a remedy for extravasation, contusion, and pain due to rheumatoid arthritis. Also, the leaves of the plant have been used as a folk cure to counteract the effect of a poison and to arrest bleeding [Chung and Shin, 1990]. Thirteen alkaloids, eleven butanolides, nine sesquiterpenoids, four flavonoids, seven phenolic compounds, and four steroids, which are phytochemicals, have been isolated from the aerial parts of *L. glauca* in previous studies [Nii et al. 1983a; 1983b; Seki et al. 1994; 1995; Chang et al., 2000; 2001]. And the nitrogen-containing compounds and monoterpenes of *L. glauca* manifested anti-tumor metastatic activities to approach tumor-inhibiting drugs in recent research [Wang et al., 2011]. Nevertheless, the investigation of phytochemical constituent of the stem wood of this plant has been scarcely carried out.

Therefore, our study focused on the isolation and identification of secondary metabolites from the stem wood of this species. Four sterols were isolated using a SiO₂ and ODS column chromatography. Their phytochemical structures were elucidated using spectroscopic methods such as NMR, electron ionization mass spectrometry (EI/MS), and IR.

Dried stem wood from *L. glauca* Blume was provided by GFC Co., Ltd. (Suwon, Korea) in 2009 and identified by Prof. Dae-Geun Kim, College of Pharmacy, Woosuk University, Jeonju, Korea. A voucher specimen (KHU-09-1125) was stored at the Natural Products Chemistry Laboratory, Kyung Hee University, Yongin, Korea.

The dried and chipped stem wood of *L. glauca* (4.9 kg) was extracted repeatedly with 80% aqueous MeOH (45 L × 3) at room temperature and then the concentrated methanolic extract (179 g) was successively partitioned with ethyl acetate (EtOAc) (3 L × 3), *n*-butyl alcohol (*n*-BuOH) (2.7 L × 3), and H₂O (3 L). The obtained EtOAc (LGBE, 35 g), the *n*-BuOH (LGBB, 42 g), and H₂O (LGBW, 102 g) fractions were evaporated at 40°C and the dried EtOAc fraction was redissolved in *n*-hexane-EtOAc (1:1).

Next, the EtOAc fraction was applied to a SiO₂ c.c. (Ø 8 cm × 19 cm) and eluted with *n*-hexane-EtOAc (1:1, 12 L) and chloroform (CHCl₃)-MeOH (10:15:1, 20.7 L of each). The eluted solutions were monitored by thin layer chromatography (TLC) and produced 13 fractions (LGBE-1 to LGBE-13). Fraction LGBE-2 [1.6 g, elution volume/total volume (*Vᵣ*/*Vₜ*) 0.02-0.06] was purified using an ODS c.c. (Ø 3.5 cm × 5.5 cm) and was eluted with acetone-H₂O (9:1, 1.4 L) to produce compound I [LGBE-2-13, 187 mg, *Vᵣ/Vₜ* 0.61-0.86, TLC (ODS F₂₅₄) *Rₑ* 0.60, acetone-H₂O=3:2]. Fraction LGBE-3 (352 mg, *Vᵣ/Vₜ* 0.06-0.11) was subjected to a SiO₂ c.c. (Ø 4 cm × 12.5 cm) and was eluted with *n*-hexane-EtOAc (4:1→2:1, 3.5 L of...
each), yielding 24 fractions (LGBE-3-1 to LGBE-3-24).
Fraction LGBE-3-13 (44 mg, V/V, 0.36–0.44) was purified using an ODS c.c. (Ø 3 cm × 4.5 cm) and was eluted with MeOH-H₂O (20:1, 245 mL) to produce compound 2 [LGBE-3-13-7, 26 mg, V/V, 0.35–0.70, TLC (ODS F₂₅₄); R₂₀ 0.13, MeOH-H₂O (0:20)].
Fraction LGBE-3-17 (64 mg, V/V, 0.68-0.77) was purified using the ODS c.c. (Ø 3.5 cm × 3.5 cm) and was eluted with MeOH-H₂O (10:1, 696 mL) to obtain compound 3 [LGBE-3-17-9, 16 mg, V/V, 0.44–0.77, TLC (SiO₂ F₂₅₄); R₂₀ 0.45, n-hexane-乙OAc=1:3].
Fraction LGBE-6 (1.2 g, V/V, 0.24–0.28) was recrystallized using CHCl₃-MeOH (1:1) and was filtered through Whatman Grade 2 filter paper with an 11 cm diameter (Whatman # 1002-11, Maidstone, England) to acquire compound 4 [LGBE-6-P, 13 mg, TLC (SiO₂ F₂₅₄); R₂₀ 0.50, CHCl₃-MeOH=5:1].

Compound 1 (β-sitosterol): white powder (CHCl₃); m.p. 140°C; [α]D 370.0° (c=0.6, CHCl₃); IR (KBr, v) 3362, 1653 cm⁻¹; EI/MS m/z 414[M⁺]; 1H-NMR (400 MHz, CDCl₃, δ₀) 5.33 (1H, br d, J=4.8 Hz, H-6), 3.49 (1H, m, H-5), 0.98 (3H, s, H-19), 0.89 (3H, d, J=6.4 Hz, H-21), 0.82 (3H, t, J=7.4 Hz, H-29), 0.81 (3H, d, J=7.6 Hz, H-26), 0.79 (3H, d, J=7.6 Hz, H-27), 0.66 (3H, s, H-18); 1³C-NMR (100 MHz, CDCl₃, δ₀); see Table 1.

Compound 2 (7-ketositoerol): white powder (CHCl₃); m.p. 140°C; [α]D 29.2° (c=0.2, CHCl₃); IR (KBr, v) 3440, 1680, 1480 cm⁻¹; EI/MS m/z 428[M⁺]; 1H-NMR (400 MHz, CDCl₃, δ₀) 5.66 (1H, br s, H-6), 3.64 (1H, m, H-3), 3.17 (3H, s, H-19), 0.89 (3H, d, J=6.0 Hz, H-21), 0.80 (3H, t, J=8.4 Hz, H-29), 0.79 (3H, d, J=7.2 Hz, H-26), 0.78 (3H, d, J=7.2 Hz, H-27), 0.67 (3H, s, H-18); 1³C-NMR (100 MHz, CDCl₃, δ₀); see Table 1.

Compound 3 (7β-hydroxysitoerol): white powder (CHCl₃); m.p. 157–158°C; [α]D 15.9° (c=0.30, CHCl₃); IR (KBr, v) 3403, 1663 cm⁻¹; EI/MS m/z 430 [M⁺]; 1H-NMR (400 MHz, CDCl₃, δ₀) 5.26 (1H, br s, H-6), 3.82 (1H, br d, J=7.6 Hz, H-7), 3.52 (1H, m, H-3), 1.02 (3H, s, H-19), 0.90 (3H, d, J=6.4 Hz, H-21), 0.82 (3H, t, J=7.4 Hz, H-29), 0.81 (3H, d, J=7.6 Hz, H-27), 0.78 (3H, d, J=7.6 Hz, H-26), 0.67 (3H, s, H-18); 1³C-NMR (100 MHz, CDCl₃, δ₀) see Table 1.

Compound 4 (daucosterol): colorless crystal (CHCl₃-MeOH); m.p. 285–288°C; [α]D 43.7° (c=0.90, pyridine); IR (KBr, v) 3320, 3030, 2937, 1640 cm⁻¹; positive FAB/MS m/z 577 [M+H⁺]; 1H-NMR (400 MHz, C₂D₅N, δ₀) 5.33 (1H, br d, J=3.6 Hz, H-6), 5.05 (1H, d, J=7.6 Hz, H-1), 4.56 (1H, br d, J=11.3 Hz, H-6b), 4.41 (1H, dd, J=11.3, 5.0 Hz, H-6a), 3.93 (1H, m, H-3), 0.96 (3H, d, J=6.4 Hz, H-21), 0.91 (3H, s, H-19), 0.87 (3H, t, J=8.0 Hz, H-29), 0.86 (3H, d, J=7.6 Hz, H-27), 0.84 (3H, d, J=7.6 Hz, H-26), 0.63 (3H, s, H-18); 1³C-NMR (100 MHz, C₂D₅N, δ₀) see Table 1.

In this investigation, biologically active compounds from the stem wood of L. glauca were identified by extracting stem wood with MeOH and partitioning the extract into EtOAc, n-BuOH, and distilled water fractions through solvent partitioning. Successive repeated SiO₂ and ODS c.c. of the acquired EtOAc fraction led to the isolation of four sterols. Compounds 1 and 4 are very common sterols in the plant and had been previously isolated from the aerial parts of L. glauca and reported as antioxidant [Chang et al., 2000; El-Desouki and Al-Omair, 2007; Luo et al., 2009].

Compound 2 was isolated as a white powder and displayed a yellow color on the ODS TLC when sprayed with 10% aqueous sulfuric acid (H₂SO₄) and heated. EI/MS spectrum produced a molecular ion peak at m/z 428[M⁺]. IR absorption bands at 3440, 1680, and 1480 cm⁻¹ characterized the hydroxyl, conjugated ketone, and double bond functional groups, respectively. The 1H-NMR (400 MHz, CDCl₃) spectrum exhibited an olefin

<table>
<thead>
<tr>
<th>No. of Carbon</th>
<th>β-sitosterol (1)</th>
<th>7-β-ketosterol (2)</th>
<th>7β-hydroxysitosterol (3)</th>
<th>daucosterol (4)</th>
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<tr>
<td>1'</td>
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<tr>
<td>6'</td>
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</table>

Table 1. 1³C-NMR chemical shifts (100 MHz) of sterols 1–4 from the stem wood of L. glauca (compounds 1–3 in CD₃OD-d₅, and compound 4 in pyridine-d₅)
carbon signal at δ29 carbon signals including a conjugated ketone carbon signal at proton signal at δ29. The doublet methyl proton signals at δ29. Consequently, it was assumed that compound 2 was a stigmastane sterol with a ketone, an oxygenated methine, and a double bond. The chemical structure was elucidated through interpretation of IR and NMR spectroscopic data as well as MS data and was confirmed by comparison of chemical shifts as well as coupling constants of NMR data to those described in the literatures [Chaurasia and Wicht, 1987; Cui et al., 2011]. Therefore, compound 3 was identified as 7β-hydroxystigmasterol (stigmast-5-en-3β,7β-diol), which was isolated for the first time from this plant (Fig. 1). Also, compounds 2 and 3 are uncommon natural products in plants and were previously reported as antioxidant [Cui et al., 2011].

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**References**


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