J. Appl. Biol. Chem. **54**(4), 309-312 (2011)

Short Communication

Sterols from *Lindera glauca* Blume Stem Wood

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Received August 2, 2011; Accepted October 25, 2011

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 H_2O. 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

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 SiO_2 and ODS column chromatography. Their phytochemical structures were elucidated using spectroscopic methods such as NMR, electron ionization mass spectrometry (EI/MS), and IR.

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http://dx.doi.org/10.3839/jabc.2011.050

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 H_2O (3 L). The obtained EtOAc (LGBE, 35 g), the *n*-BuOH (LGBB, 42 g), and H_2O (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 SiO_2 c.c. (Ø 8 cm × 19 cm) and eluted with *n*-hexane-EtOAc (1:1, 12 L) and chloroform ($CHCl_3$)-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 (V_e/V_t) 0.02-0.06] was purified using an ODS c.c. (Ø 3.5 cm × 5.5 cm) and was eluted with acetone-H_2O (9:1, 1.4 L) to produce compound **1** [LGBE-2-13, 187 mg, V_e/V_t 0.61–0.86, TLC (ODS F_{254S}) R_f 0.60, acetone-H_2O=3:2]. Fraction LGBE-3 (525 mg, V_e/V_t 0.06–0.11) was subjected to a SiO_2 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_e/V_t 0.36–0.44) was purified using an ODS c.c. (Ø 3 cm × 4.5 cm) and was eluted with MeOH-H_2O (20:1, 245 mL) to produce compound **2** [LGBE-3-13-7, 26 mg, V_e/V_t 0.35–0.70, TLC (ODS F_{254S}) R_f 0.13, MeOH-H_2O=20:1]. Fraction LGBE-3-17 (64 mg, V_e/V_t 0.68-0.77) was purified using the ODS c.c. (Ø 3.5 cm × 3.5 cm) and was eluted with MeOH-H_2O (10:1, 696 mL) to obtain compound **3** [LGBE-3-17-9, 16 mg, V_e/V_t 0.44–0.77, TLC (SiO_2 F_{254}) R_f 0.45, *n*-hexane-EtOAc=1:3]. Fraction LGBE-6 (1.2 g, V_e/V_t 0.24–0.28) was recrystallized using $CHCl_3$-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 (SiO_2 F_{254}) R_f 0.50, $CHCl_3$-MeOH=5:1].

Compound **1** (β-sitosterol): white powder ($CHCl_3$); m.p. 140°C; $[\alpha]_D$ 37.0° (*c*=0.6, $CHCl_3$); IR (KBr, ν) 3362, 1653 cm^{-1}; EI/MS *m/z* 414$[M]^+$; ^{1}H-NMR (400 MHz, $CDCl_3$, δ_H) 5.33 (1H, br d, *J*=4.8 Hz, H-6), 3.49 (1H, m, H-3), 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); ^{13}C-NMR (100 MHz, $CDCl_3$, δ_C); see Table 1.

Compound **2** (7-ketositosterol): white powder ($CHCl_3$); m.p. 140°C; $[\alpha]_D$ 29.2° (*c*=0.2, $CHCl_3$); IR (KBr, ν) 3440, 1680, 1480 cm^{-1}; EI/MS *m/z* 428$[M]^+$; ^{1}H-NMR (400 MHz, $CDCl_3$, δ_H) 5.66 (1H, br s, H-6), 3.64 (1H, m, H-3), 1.17 (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); ^{13}C-NMR (100 MHz, $CDCl_3$, δ_C) see Table 1.

Compound **3** (7β-hydroxysitosterol): white powder ($CHCl_3$); m.p. 157–158°C; $[\alpha]_D$ 15.9° (*c*=0.30, $CHCl_3$); IR (KBr, ν) 3403, 1663 cm^{-1}; EI/MS *m/z* 430 $[M]^+$; ^{1}H-NMR (400 MHz, $CDCl_3$, δ_H) 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); ^{13}C-NMR (100 MHz, $CDCl_3$, δ_C) see Table 1.

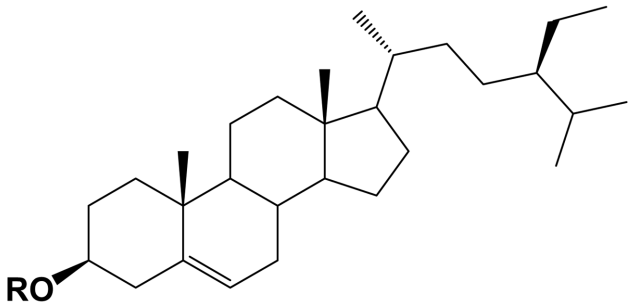
Compound **4** (daucosterol): colorless crystal ($CHCl_3$-MeOH); m.p. 285–288°C; $[\alpha]_D$ 43.7° (*c*=0.90, pyridine); IR (KBr, ν) 3320, 3030, 2937, 1640 cm^{-1}; positive FAB/MS *m/z* 577 $[M+H]^+$; ^{1}H-NMR (400 MHz, C_5D_5N, δ_H) 5.33 (1H, br d, *J*=3.6 Hz, H-6), 5.05 (1H, d, *J*=7.6 Hz, H-1'), 4.56 (1H, br d, *J*=11.3 Hz, H-6'b), 4.41 (1H, dd, *J*=11.3, 5.0 Hz, H-6'a), 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); ^{13}C-NMR (100 MHz, C_5D_5N, δ_C) 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 SiO_2 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].

Table 1. ^{13}C-NMR chemical shifts (100 MHz) of sterols 1–4 from the stem wood of *L. glauca* (compounds 1–3 in CD_3OD-d_4 and compound 4 in pyridine-d_5)

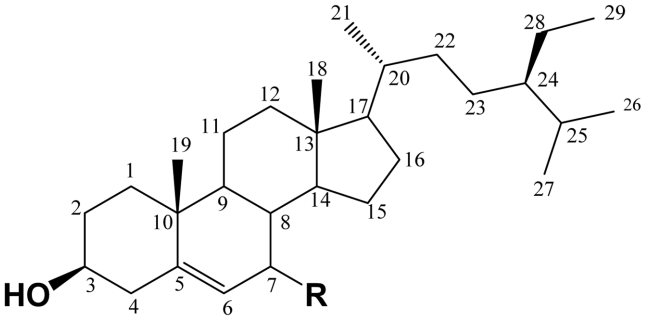
No. of Carbon	β-sitosterol (**1**)	7-ketositosterol (**2**)	7β-hydroxysitosterol (**3**)	daucosterol (**4**)
1	37.3	36.3	37.0	37.6
2	31.7	31.1	31.6	30.4
3	71.8	70.4	71.4	78.1
4	42.3	41.8	41.7	40.0
5	140.6	165.2	143.3	140.8
6	121.6	125.9	125.3	121.9
7	31.9	202.4	73.3	32.3
8	31.9	45.4	39.6	32.2
9	50.1	49.8	48.3	50.4
10	36.5	38.6	36.5	37.0
11	21.1	21.2	21.1	21.4
12	39.8	38.8	42.9	39.4
13	42.3	43.1	40.9	42.6
14	56.8	49.9	55.9	56.9
15	24.4	25.9	26.4	24.6
16	28.3	28.6	28.6	28.7
17	56.0	54.6	55.3	56.3
18	11.9	12.0	11.9	12.1
19	19.9	17.3	19.2	20.1
20	36.2	36.1	36.1	36.5
21	18.9	18.9	19.1	19.2
22	34.0	33.9	34.0	34.3
23	26.1	26.3	29.1	26.5
24	45.8	45.7	45.8	46.1
25	29.2	29.0	26.1	29.6
26	19.1	20.2	18.9	19.3
27	19.5	19.0	19.9	19.6
28	23.1	23.0	23.1	23.5
29	12.1	12.0	12.1	12.3
1'				102.6
2'				75.4
3'				71.7
4'				78.7
5'				78.6
6'				62.9

Compound **2** was isolated as a white powder and displayed a yellow color on the ODS TLC when sprayed with 10% aqueous sulfuric acid (H_2SO_4) 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 ^{1}H-NMR (400 MHz, $CDCl_3$) spectrum exhibited an olefin



β-sitosterol (**1**) : R = H

daucosterol (**4**) : R = glucopyranosyl



7-ketositosterol (**2**) : R = O

7*β*-hydroxysitosterol (**3**) : R = 7*β*-OH

Figure 1. Chemical structures of sterols 1–4 isolated from the stem wood of *L. glauca*.

proton signal at δ_H 5.66 (1H, br s, H-6), an oxygenated methine proton signal at δ_H 3.64 (1H, m, H-3), two singlet methyl proton signals at δ_H 1.17 (3H, s, H-19) and 0.67 (3H, s, H-18), three doublet methyl proton signals at δ_H 0.89 (3H, d, *J*=6.0 Hz, H-21), 0.79 (3H, d, *J*=7.2 Hz, H-26), and 0.78 (3H, d, *J*=7.2 Hz, H-27), and a triplet methyl proton signal at δ_H 0.80 (3H, t, *J*=8.4 Hz, H-29). The ^{13}C-NMR (100 MHz, $CDCl_3$) spectrum revealed 29 carbon signals including a conjugated ketone carbon signal at δ_C 202.4, an olefin quaternary carbon signal at δ_C 165.2, an olefin methine carbon signal at δ_C 125.9, an oxygenated methine carbon signal at δ_C 70.4, and six methyl carbon signals at δ_C 20.2 (C-26), 19.00 (C-27), 18.9 (C-21), 17.3 (C-19), and 12.0 (C-18, 29). 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 literature [Kolak *et al*., 2005; Zhao *et al*., 2005; Cui *et al*., 2011]. Finally, compound **2** was identified as 7-ketositosterol (3β-hydroxystigmast-5-en-7-one), which was isolated for the first time from *L. glauca* in this experiment (Fig. 1).

Compound **3** was isolated as a white powder that turned blue on the ODS TLC when sprayed with 10% aqueous H_2SO_4 and heated. EI/MS spectrum produced a molecular ion peak at *m/z* 430$[M]^+$. IR absorption bands at 3403 and 1663 cm^{-1} corresponded to hydroxyl and olefin groups, respectively. ^{1}H-NMR (400 MHz, $CDCl_3$) and ^{13}C-NMR (100 MHz, $CDCl_3$) spectra of compound **3** were similar to those of compound **2** with the exception of an additional oxygenated methine carbon signal instead of a conjugated ketone signal. The additional signals of compound **3** were observed at δ_H 3.82 (br d, *J*=7.6 Hz) and δ_C 73.3. Consequently, compound **3** was deduced to have a hydroxyl group in the β configuration instead of a conjugated ketone group. The chemical structure was identified by interpretation and comparison of spectroscopic data with those described in the literatures [Chaurasia and Wicht, 1987; Cui *et al*., 2011]. Therefore, compound **3** was identified as 7β-hydroxysitosterol (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].

Acknowledgment

This research was financially supported by the Next Generation Bio-Green 21(PJ008020) Project from Rural Development Administration, Republic of Korea.

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