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Short Communication

Isolation of Steroids from the Kalopanacis Cortex

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Kalopanacis Cortex is the stem bark of *Kalopanax pictus* Nakai belonging to the family of Araliaceae, which is distributed mainly in Korea [Lee, 1993]. It has been used as a traditional Korean medicine for the remedy of paralysis, arthritis, rheumatism, neuralgia, lumbago, diabetes, and tonic [Lee, 1966]. It was also reported that *K. pictus* Nakai extract has anti-nociceptive and anti-rheumatoidal [Choi *et al.*, 2002], anti-inflammatory [Lee *et al.*, 2001], and anti-lipid peroxidative [Choi *et al.*, 2001] effects. A number of researchers reported the chemical constituents of Kalopanacis Cortex such as saponins [Sun *et al.*, 1990; Cho *et al.*, 1991; Sano *et al.*, 1991], fatty acids [Sano *et al.*, 1991; Lee *et al.*, 1995], lignans [Sano *et al.*, 1991; Porzel *et al.*, 1992; Hong *et al.*, 2001], and phenolic compounds [Sano *et al.*, 1991] among others, with saponins being especially important as the principal component manifesting pharmacological activities [Lee *et al.*, 2000; Choi *et al.*, 2002].

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Abbreviations: cc, column chromatography; $CHCl_3$, chloroform; EI-MS, electron ionization mass spectrometry; EtOAc, ethyl acetate; EtOH, ethanol; FAB-MS, fast atom bombardment mass spectrometry; IR, infra-red; MeOH, methanol; TLC, thin layer chromatography

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Except for saponins, however, there are only few reports on other pharmacologically active compounds of Kalopanacis Cortex. We, therefore, initiated the present study to identify other principal, low molecular-weight compounds of Kalopanacis Cortex. Here the isolation and identification of four steroids from Kalopanacis Cortex are reported.

The Kalopanacis Cortex was purchased from Kyungdong Market, Seoul, Korea, in June 2006, and identified by Prof. Dae-Keun Kim, College of Pharmacy, Woosuk University, Jeonju, Korea. A voucher specimen (KHU061230) is reserved at the Laboratory of Natural Products Chemistry, Kyung Hee University, Yongin, Korea.

Kalopanacis Cortex (10 kg) was extracted three times at room temperature with 80% aqueous EtOH (18 L×3). The filtrate was concentrated *in vacuo* at 40°C to render the MeOH extracts. The concentrates were poured into 3 L water and extracted with 3 L EtOAc, obtaining total 220 g. EtOAc extract (100 g) was applied to a silica gel cc (9×17 cm) and eluted with a gradient of *n*-hexane-EtOAc (10:1→7:1→5:1→1:1, 1 L of each) and $CHCl_3$-MeOH (12:1→10:1, 2 L of each). The eluting solutions were monitored by TLC to produce twenty five fractions (KCE1 to KCE25). Fraction KCE5 [3.1 g, V_e/V_t (elution volume/total volume) 0.15-0.17] was subjected to a silica gel cc (3.5×17 cm) eluted with *n*-hexane:EtOAc (6:1→3:1, 2 L of each), yielding twenty-two fractions (KCE5-1 to KCE5-22) to ultimately produce compound **1** [KCE5-8, 185.2 mg, V_e/V_t 0.21-0.25, TLC (SiO_2 F_{254}) R_f 0.38, *n*-hexane-EtOAc=4:1] and compound **2** [KCE5-9, 114.4 mg, V_e/V_t 0.25-0.28, TLC (SiO_2 F_{254}) R_f 0.25, *n*-hexane-EtOAc=3:1]. Fraction KCE13 [4.6 g, V_e/V_t 0.35-0.4] was subjected to a silica gel cc (4.5×15 cm) eluted with $CHCl_3$-MeOH (50:1→20:1, 2 L of each), yielding fifteen fractions (KCE13-1 to KCE13-15). Fraction KCE13-15 [893 mg, V_e/V_t 0.95-1] was subjected to a silica gel cc (4.5×16 cm) eluted with $CHCl_3$-MeOH-H_2O (20:3:1→10:3:1, 1 L of each), yielding nineteen fractions (KCE13-15-1 to KCE13-15-19). Fraction KCE13-15-6 [51 mg, V_e/V_t 0.09-0.14] was precipitated in MeOH/$CHCl_3$ ultimately to produce compound **3** [KCE13-15-6-2, 11 mg, TLC (ODS F_{254}) R_f 0.2, MeOH-H_2O=20:1]. Fraction KCE16 [6.2 g, V_e/V_t 0.48-0.52] was subjected to a silica gel cc (6×15 cm) eluted with $CHCl_3$-MeOH (15:1, 7.8 L), yielding fifteen fractions (KCE16-1 to KCE16-13). Fraction KCE16-8 was precipitated in MeOH/$CHCl_3$ ultimately to produce compound **4** [KCE13-15-6-2, 11 mg, TLC (ODS F_{254}) R_f 0.2, MeOH-H_2O=20:1]. Structural identification of these compounds was carried out based on the interpretation of the extensive NMR, MS, and IR spectroscopic data.

β-Sitosterol (**1**): white powder ($CHCl_3$); m.p. 140-142°C; $[\alpha]_D$ =−29.2° (*c*=0.2, $CHCl_3$); EI-MS *m/z*: 414 $[M]^+$; IR (KBr, ν)

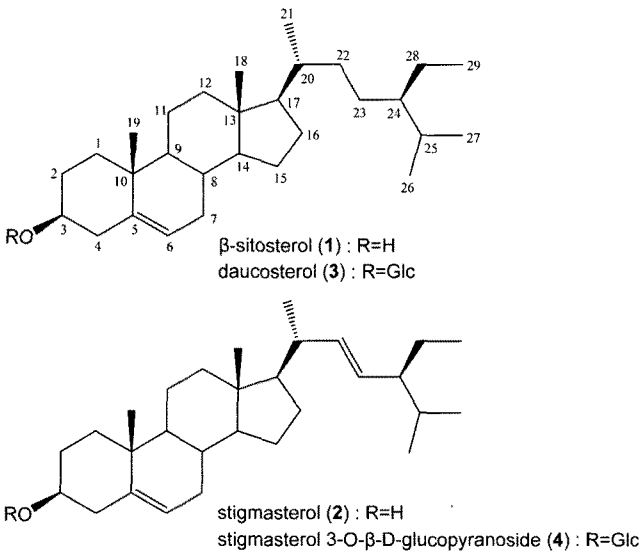

Fig. 1. Chemical structures of compounds 1~4 isolated from Kalopanacis Cortex.

3400, 1640, 1050, 802, 845, 830 cm^{-1}; ^{1}H-NMR (400 MHz, $CDCl_3$, δ) 5.26 (1H, br. d, *J*=4.4 Hz, H-6), 3.40 (1H, m, H-3), 0.93 (3H, s, H-19), 0.85 (3H, d, *J*=6.4 Hz, H-21), 0.77 (3H, t, *J*=7.2 Hz, H-29), 0.74 (3H, d, *J*=7.2 Hz, H-26), 0.72 (3H, d, *J*=6.8 Hz, H-27), 0.61 (3H, s, H-18); ^{13}C-NMR (100 MHz, $CDCl_3$, δ); see Table 1.

Stigmasterol (**2**): white powder ($CHCl_3$); m.p. 163-165°C; $[\alpha]_D$=–42.9° (*c*=0.2, $CHCl_3$); EI-MS *m/z*: 412 $[M]^+$; IR (KBr, ν) 3460, 2930, 1650, 1550, 1330, 1060 cm^{-1}; ^{1}H-NMR (400 MHz, $CDCl_3$, δ) 5.30 (1H, br. d *J*=4.8 Hz, H-6), 5.11 (1H, dd, *J*=15.2, 8.8 Hz, H-22), 4.98 (1H, dd, *J*=15.2, 8.8 Hz, H-23), 3.48 (1H, m, H-3), 0.99 (3H, d, *J*=7.2 Hz, H-21), 0.97 (3H, s, H-19), 0.82 (3H, d, *J*=7.2 Hz, H-26), 0.79 (3H, t, *J*=7.6 Hz, H-29), 0.76 (3H, d, *J*=6.8 Hz, H-27), 0.66 (3H, s, H-18); ^{13}C-NMR (100 MHz, $CDCl_3$, δ); see Table 1.

Daucosterol (**3**): colorless crystals ($CHCl_3$-MeOH); m.p. 282-286°C; $[\alpha]_D$=–29.0° (*c*=0.06, pyridine); FAB-MS *m/z*: 577 $[M+1]^+$; IR (KBr, ν) 3320, 3030, 2935, 1645 cm^{-1}; ^{1}H-NMR (400 MHz, pyridine-d_5, δ) 5.34 (1H, br. d, *J*=4.4 Hz, H-6), 5.06 (1H, d, *J*=7.6 Hz, H-1'), 3.95 (1H, m, H-3), 0.97 (3H, d, *J*=6.8 Hz, H-21), 0.92 (3H, s, H-19), 0.90 (3H, d, *J*=6.4 Hz, H-26), 0.87 (3H, d, *J*=8.4 Hz, H-27), 0.85 (3H, t, *J*=7.2 Hz, H-29), 0.64 (3H, s, H-18); ^{13}C-NMR (100 MHz, pyridine-d_5, δ); see Table 1.

Stigmasterol 3-*O*-β-D-glucopyranoside (**4**): colorless crystals ($CHCl_3$-MeOH) m.p. 298-299°C; $[\alpha]_D$=–48.2° (*c*=1.0, pyridine); FAB-MS *m/z* 597 $[M+Na]^+$; IR (KBr, ν) 3476, 2944, 1646, 1556, 1370, 1340, 1214, 1168, 1114, 1062, 1026 cm^{-1}; ^{1}H-NMR (400 MHz , pyridine-d_5, δ) 5.35 (1H, br. d, *J*=4.8 Hz, H-6), 5.21 (1H, dd, *J*=15.2, 8.4 Hz, H-22), 5.05 (1H, dd, *J*=15.2, 8.4 Hz, H-23), 5.05 (1H, d, *J*=7.6 Hz, H-1'), 3.96 (1H, m, H-3), 1.07 (3H, d, *J*=6.8 Hz, H-21), 0.93 (3H, s, H-19), 0.91 (3H, d, *J*=6.8 Hz, H-26), 0.89 (3H, t, *J*=7.6 Hz, H-29), 0.87 (3H, d, *J*=7.2 Hz, H-27), 0.67 (3H, s, H-18); ^{13}C-NMR (100 MHz, pyridine-d_5, δ); see Table 1.

Table 1. ^{13}C-NMR chemical shifts (100 MHz) of steroids from the Kalopanacis Cortex (1, 2 in $CDCl_3$ and 3, 4 in pyridine-d_5)

No. of Carbon	Compound **1**	Compound **2**	Compound **3**	Compound **4**
1	37.20	37.27	37.61	37.63
2	31.82	29.73	29.61	30.42
3	71.43	71.69	78.66	78.69
4	42.23	42.31	40.08	39.49
5	140.58	140.60	140.88	140.90
6	121.38	121.52	121.92	121.92
7	31.82	31.55	30.39	30.42
8	31.31	31.91	30.10	32.22
9	50.07	50.12	50.46	50.48
10	36.42	36.51	37.06	37.09
11	21.03	21.12	21.44	21.47
12	39.70	39.69	39.46	40.11
13	41.97	42.19	42.60	42.62
14	56.66	56.84	56.93	56.95
15	24.24	24.39	24.65	24.71
16	28.19	28.94	28.68	29.47
17	55.97	55.93	56.35	56.37
18	11.80	12.08	12.14	12.16
19	19.75	19.42	19.57	19.59
20	36.08	40.50	36.52	40.92
21	18.73	21.26	19.17	21.65
22	33.85	138.16	34.34	138.82
23	26.01	129.12	26.54	129.48
24	45.72	51.23	46.16	51.56
25	29.08	31.91	29.61	32.22
26	19.32	21.12	19.37	21.47
27	18.98	19.02	20.13	19.40
28	23.00	25.43	23.55	25.87
29	11.92	12.30	12.32	12.35
1'			102.59	102.63
2'			75.40	75.43
3'			78.19	78.18
4'			71.77	71.79
5'			78.53	78.69
6'			62.92	62.95

Compounds **1** and **3** were identified as stigmast-5-en-3β-ol (β-sitosterol) and β-sitosterol 3-*O*-β-D-glucopyranoside (daucosterol), respectively, through the comparison of several physical and spectroscopic data with those of the literatures [Lim *et al.*, 2005; Yoo *et al.*, 2006].

Compound **2**, white powder, showed absorbance bands due to hydroxyl (3460 cm^{-1}) and olefine (1650 cm^{-1}) groups in the IR spectrum and a molecular ion peak $[M]^+$ at *m/z* 412 in the EI-MS. In the ^{1}H-NMR spectrum, three olefinic methine proton (δ_H 5.30, 5.11, and 4.98) and one oxygenated methine proton (δ_H 3.48) signals were observed. Moreover, in the high magnet field region, a number of methylene and methine proton (δ_H 2.25-1.00), two singlet methyl proton (δ_H 0.97 and 0.66), three

doublet methyl proton (δ_H 0.99, 0.82, and 0.76), and one triplet methyl proton (δ_H 0.79) signals were observed. In the ^{13}C-NMR spectrum, twenty-nine carbon signals consisting of one olefinic quaternary carbon (δ_C 141.60), three olefinic methine carbon (δ_C 138.16, 129.12, and 121.52), one oxygenated methine carbon (δ_C 71.69), and six methyl carbon (δ_C 21.26, 21.12, 19.42, 19.02, 12.30, and 12.08) signals were observed, leading to the conclusion that compound **2** was also a stigmastane sterol with two double bonds, one oxygenated carbon, as well as two singlet methyl, three doublet methyl, and one triplet methyl signals. Compound **2** was finally identified as stigmasta-5,22-dien-3β-ol (stigmasterol), through the comparison of several physical and spectroscopic data with those of the literatures [Forgo and Kover, 2004; Lim *et al.*, 2005]. These two steroids were isolated from Kalopanacis Cortex for the first time in the present study.

Compound **4**, colorless crystals, showed absorbance bands resulting from the hydroxyl (3476 cm^{-1}) and olefine (1646 cm^{-1}) groups in the IR spectrum and a pseudomolecular ion peak $[M+Na]^+$ at *m/z* 597 in the FAB-MS spectrum. The NMR spectra of compound **4** were very similar to those of compound **2**, except for the presence of sugar signals, such as anomeric proton (δ_H 5.05) and carbon (δ_C 102.63) signals. In the ^{13}C-NMR spectrum, 35 carbon signals were observed, and the sugar signals were identified as D-glucopyranose from the chemical shifts of sugar moiety carbon signals. In the ^{1}H-NMR spectrum, the coupling constant of the anomeric proton signal was 7.6 Hz, indicating the glucopyranose to have β-glucosidic linkage. Compound **4** was eventually identified as stigmasterol 3-*O*-β-D-glucopyranoside [Alam *et al.*, 1996].

From the result of spectroscopic data including NMR, MS, and IR, the chemical structures of the isolated compounds **1**~**4** were determined. These steroids were isolated from Kalopanacis Cortex for the first time in the present study. All compounds were stigmastane sterols with hydroxyl group. Such steroids have been reported to exhibit several biological activities. β-Sitosterol (**1**) was reported to have uterotrophic effect through acceleration of the alkaline and acid phosphate activities [Malini *et al.*, 1991], antivirus activity against tobamovirus [Kahn *et al.*, 1991], antiinflammatory and antipyretic activities [Gupta *et al.*, 1980]. Stigmasterol (**2**), reported to exhibit antivirus activity against tobamovirus [Kahn *et al.*, 1991], was examined for its blood cholesterol level-reducing ability [Pollak *et al.*, 1981]. Daucosterol (**3**) was examined for its cytotoxity on cancer cells [Hyun *et al.*, 1996], FPTase inhibitory activity [Kim *et al.*, 2004], and insecticidal and antifeedant effects [Carlos *et al.*, 2005]. Stigmasterol 3-*O*-β-D-glucopyranoside (**4**) was examined for its cytotoxity on cancer cells [Hyun *et al.*, 1996] and antivirus activity against tobamovirus [Kahn *et al.*, 1991].

Kalopanacis Cortex has been used as an eastern traditional medicine. Through continued research on these components, Kalopanacis Cortex could be useful as functional food and medicinal material.

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