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Norsesquiterpene and Steroid Constituents of *Humulus japonicus*

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Abstract – Five steroids and two norsesquiterpene glycosides were isolated from the methanol extract of *H. japonicus*. Their structures were determined by means of physio-chemical and spectral data to be friedelin (**1**), stigmast-5-en-3-β-ol (β-sitosterol) (**2**), 7-keto-β-sitosterol (**3**), 6β-hydroxy-4-stigmasten-3-one (**4**), 7α-hydroxy-β-sitosterol (**5**), 3-hydroxy-4,4-dimethyl-4-butyrolactone (**6**), daucosterol (**7**), (6*S*, 9*S*)-roseoside (**8**), and (9*S*)-drummondol-9-*O*-β-D-glucopyranoside (spinoside B) (**9**). The compounds **1**, **3**, **4**, and **6 - 9** were first isolated from this plant source.
Keywords - *Humulus japonicus*, steroid, norsesquiterpene

Introduction

Humulus japonicus Sieboid & Zucc (Cannabaceae), a perennial herb, has been used for the treatment of pulmonary tuberculosis and hypertension in Korean traditional medicine and found to possess antioxidant, antibacterial (Park *et al*., 1994) and antimutagenic effect (Park *et al*., 1995). Terpenes, lupulones and flavonoids have been reported as constituents of this plant (Naya and Kotake, 1970; Aritomi, 1962). We isolated cytotoxic phenolic compounds from this plant (Yu *et al*., 2007). In continuation of our study on this plant source, we have further isolated five steroids, three terpenoids and a lactone by column chromatographic separation of the MeOH extract of this plant. Their structures were determined by spectroscopic methods. The compounds **1**, **3**, **4**, and **6 - 9** were first isolated from this plant source.

Experimental

General – All melting points were determined on a Gallenkamp melting point apparatus and uncorrected. Optical rotations were measured on a Jasco P-1020 Polarimeter. UV spectra were recorded with Shimadzu UV 1601 and Varian cary 5000 spectrophotometer. IR spectra were recorded with Bruker IFS-66/S. NMR spectra were recorded on either Bruker (Avance-300, Avance-600) or Varian (Unity Inova 300 Nb, Unity Inova 500 Nb). MS were recorded on JEOL JMS-700 mass spectrometer. Preparative HPLC used a Knauer K-1001 instrument with refractive index detector and UV detector, Alltech Econosil silica 5 μm (length: 250 mm, I.D 22 mm) column and Alltech Econosil RP-C18 5 μm (length: 250 mm, I.D 4.6 mm) column. For open column chromatography, silica gel (Merck, 70 - 230), ODS (Cosmosil 140 C_{18}) and Sephadex LH-20 (Pharmacia) were used. Low pressure liquid chromatography was carried out over Merck LiChroprep Lobar®-A Si 60 or Merck LiChroprep Lobar®-A RP-C18 column with FMI QSY pump (Isco).

Plant material – The aerial part of *H. japonicus* (3.4 kg) were collected at Yang Yang, Gang Won province, Korea in Aug 2005. A voucher specimen of the plants (SKKU-2005-07) was deposited at the College of Pharmacy, Sungkyunkwan University, Korea.

Extraction and Isolation – The dried and chopped aerial parts of *H. japonicus* (3.2 kg) were extracted three times with 80% MeOH. The MeOH extract (430 g) was suspended in distilled water (1.6 L) and successively partitioned with *n*-hexane, methylene chloride, ethyl acetate and *n*-butanol followed by evaporation to afford 30 g, 30 g, 10 g and 45 g, respectively. The *n*-hexane fraction (30 g) was chromatographed over a silica gel column using *n*-hexane : ethyl acetate = 10 : 1, 5 : 1, 3 : 1, 1 : 1 to afford nine subfractions (H1 ~ H9). The H1 subfraction (6.5 g) was divided by six subfractions (H1-1 ~ H1-6) with low pressure liquid column chromatography using *n*-hexane : ethyl acetate = 40 : 1. The H1-5 fraction (30 mg) was further purified by Sephadex LH-20 gel filtration (methylene chloride : methanol = 1 : 1) and HPLC (Econosil silica 5 μm, *n*-hexane : chloroform : ethyl acetate = 90 : 10 : 1) to afford **1** (26 mg). The H5 subfraction

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(0.9 g) was filtrated using 100% MeOH and then chromatographed (*n*-hexane : chloroform : ethyl acetate = 12 : 20 : 1) to get five subfractions (H5-1 ~ H5-5). The H5-5 subfraction (200 mg) was purified by HPLC (Econosil silica 5 μm, *n*-hexane : ethyl acetate = 5 : 1) to furnish **2** (180 mg). The H8 subfraction (1.9 g) was chromatographed over a silica gel column using *n*-hexane : methylene chloride : ethyl acetate = 10 : 10 : 3 to give four subfractions (H8-1 ~ H8-4). The H8-4 fraction (800 mg) was purified by Sephadex LH-20 (100% MeOH) and RP-18 Lobar®-A column (acetonitrile : acetone = 4 : 1) to give **3** (5 mg) and **4** (5 mg). The H9 fraction (1 g) was chromatographed over a silica gel column with gradient solvent system (*n*-hexane : methylene chloride : ethyl acetate = 20 : 10 : 3, 10 : 10 : 3) to give four subfractions (H9-1 ~ H9-4). The H9-1 (350 mg) fraction was chromatographed by Sephadex LH-20 (methylene chloride : methanol = 1 : 1) and then further purified by HPLC (Econosil silica 5 μm, methylene chloride : methanol = 40 : 1) to afford **5** (4 mg). The ethyl acetate extract (10 g) was chromatographed over a silica gel column using with gradient solvent system (chloroform : acetone = 20 : 1, 10 : 1, 5 : 1, chloroform : methanol = 5 : 1, 3 : 1, 1 : 1) to give four subfractions (E1 ~ E4). The E1 fraction (1.0 g) was chromatographed over a silica gel column using methylene chloride : methanol = 40 : 1 to afford four subfractions (E1-1 ~ E1-4). The E1-1 subfraction (140 mg) was purified by Sephadex LH-20 (100% MeOH) and semi-prep HPLC (Econosil silica 5 μm, methylene chloride : methanol = 40 : 1) to afford **6** (100 mg). The E2 fraction (1.0 g) afforded three subfractions (E2-1 ~ E2-3) by silica gel column chromatography (methylene chloride : methanol = 30 : 1, 20 : 1, 10 : 1, 5 : 1). The E2-1 subfraction (230 mg) was recrystalized with methanol : acetone = 4 : 1 to afford **7** (6 mg). The E3 fraction (3.6 g) was chromatographed over a RP C-18 column (30% → 60% methanol) to afford ten subfractions (E3-1 ~ E3-10). The E3-4 subfraction (70 mg) was futher purified by Sephadex LH-20 gel filtration and HPLC (Econosil silica 5 μm, chloroform : methanol = 10 : 1) to get **8** (6 mg). The E2-2 subfraction (300 mg) was chromatographed over a silica gel column using chloroform : methanol = 8 : 1, 9 : 2, 2 : 1 and then purified with HPLC (Econosil silica 5 μm, chloroform : methanol = 7 : 1) to afford **9** (26 mg).

Friedelin (**1**) White powder, mp. : 256 °C; $[\alpha]_D^{25}$: –26.1° (*c* = 0.08, $CHCl_3$); FAB-MS (*m*/*z*) : 449 $[M + Na]^+$; ^{1}H-NMR ($CDCl_3$, 500 MHz, δ ppm) : δ 0.74 and 0.89 (each 3H, s, H-24, 25), 0.90 (3H, d, *J* = 6.8 Hz, H-23), 0.97 (3H, s, H-30), 0.97 (1H, m, H-22,), 1.02, 1.02, 1.05 and 1.20 (each 3H, s, H-26, 29, 27, 28), 1.21 and 1.24 (each 1H, d, *J* = 5.8 Hz, H-19b, 11b), 1.28 - 1.58 (16H, m), 1.71 (1H, dd, *J* = 4.8, 12.7 Hz, H-1a), 1.78 (1H, dd, *J* = 2.9, 12.7 Hz, H-6a), 1.98 (1H, m, H-1a), 2.27 and 2.31 (each 1H, dd, *J* = 6.8, 13.6 Hz, H-4, 2b), 2.41 (1H, ddd, *J* = 1.9, 4.9, 13.6 Hz, H-2a), ^{13}C-NMR ($CDCl_3$, 125 MHz, δ ppm) : δ 7.04 (C-23), 14.89 (C-24), 18.18 (C-25), 18.48 (C-7), 18.89 (C-27), 20.49 (C-26), 22.51 (C-1), 28.41 (C-20), 30.24 (C-17), 30.74 (C-12), 32.02 (C-29), 32.33 (C-28), 32.67 (C-21), 33.03 (C-15), 35.24 (C-30), 35.59 (C-19), 35.87 (C-11), 36.26 (C-16), 37.70 (C-9), 38.54 (C-14), 39.49 (C-22), 39.94 (C-13), 41.55 (C-6), 41.76 (C-2), 42.37 (C-5), 43.06 (C-18), 53.35 (C-8), 58.48 (C-4), 59.74 (C-10), 213.35 (C-3)

***β*-Sitosterol** (**2**) White powder, mp. : 136 °C; $[\alpha]_D^{25}$: –30.5° (*c* = 0.025, $CHCl_3$); EI-MS (*m*/*z*) : 414 $[M]^+$; ^{1}H-NMR ($CDCl_3$, 500 MHz, δ ppm) : δ 0.70 (3H, s, H-18), 0.83 (3H, d, *J* = 6.8 Hz, H-27), 0.85 (3H, d, *J* = 6.8 Hz, H-26), 0.87 (3H, t, *J* = 7.5 Hz, H-29), 0.94 (3H, d, *J* = 6.8 Hz, H-21), 1.03 (3H, s, H-19), 2.28 (2H, m), 3.54 (1H, m, H-3), 5.36 (1H, d, *J* = 5.9 Hz, H-6); ^{13}C-NMR ($CDCl_3$, 125MHz, δ ppm) : δ 12.10 (C-29), 12.22 (C-18), 19.01 (C-21), 19.28 (C-27), 19.63 (C-19), 20.05 (C-26), 21.33 (C-11), 23.32 (C-28), 24.54 (C-15), 26.35 (C-23), 28.48 (C-16), 29.42 (C-25), 31.92 (C-2), 32.16 (C-7), 32.16 (C-8), 34.20 (C-22), 36.38 (C-20), 36.75 (C-10), 37.50 (C-1), 40.03 (C-12), 42.56 (C-4), 42.56 (C-13), 46.10 (C-24), 50.39 (C-9), 56.31 (C-17), 57.02 (C-14), 72.05 (C-3), 121.95 (C-6), 141.01 (C-5)

7-Keto-*β*-sitosterol (**3**) White powder, mp. : 124 °C; $[\alpha]_D^{25}$: –91.8° (*c* = 0.17, $CHCl_3$); FAB-MS (*m*/*z*) : 429 $[M + H]^+$; ^{1}H-NMR ($CDCl_3$, 500 MHz, δ ppm) : δ 0.70 (3H, s, H-18), 0.84 (3H, d, *J* = 6.8 Hz, H-27), 0.86 (3H, d, *J* = 6.8 Hz, H-26), 0.87 (3H, t, *J* = 7.28 Hz, H-29), 0.95 (3H, d, *J* = 5.9 Hz, H-21), 1.22 (3H, s, H-19), 3.69 (1H, m, H-3), 5.71 (1H, d, *J* = 2.0 Hz, H-6); ^{13}C-NMR ($CDCl_3$, 125 MHz, δ ppm) : δ 12.19 (C-18), 12.19 (C-29), 17.55 (C-19), 19.17 (C-21), 19.29 (C-27), 20.01 (C-26), 21.43 (C-11), 23.28 (C-28), 26.39 (C-15), 26.54 (C-23), 28.85 (C-16), 29.45 (C-25), 31.48 (C-2), 34.22 (C-22), 36.36 (C-20), 36.60 (C-1), 38.50 (C-10), 38.95 (C-12), 42.05 (C-4), 43.34 (C-13), 45.68 (C-8), 46.04 (C-24), 50.20 (C-9), 50.21 (C-14), 54.96 (C-17), 70.77 (C-3), 126.37 (C-6), 165.22 (C-5), 202.44 (C-7)

6*β*-Hydroxy-4-stigmasten-3-one (**4**) White powder, mp. : 205 °C; $[\alpha]_D^{25}$: +26.7° (*c* = 0.06, $CHCl_3$); FAB-MS (*m*/*z*) : 429 $[M + H]^+$; ^{1}H-NMR ($CDCl_3$, 500 MHz, δ ppm) : δ 0.76 (3H, s, H-18), 0.84 (3H, d, *J* = 6.8 Hz, H-27), 0.86 (3H, d, *J* = 6.8 Hz, H-26), 0.87 (3H, t, *J* = 7.2 Hz, H-29), 0.95 (3H, d, *J* = 6.8 Hz, H-21), 1.40 (3H, s, H-

19), 4.39 (1H, br.s, H-6), 5.81 (1H, s, H-4); ^{13}C-NMR ($CDCl_3$, 125 MHz, δ ppm) : δ 12.20 (C-29), 12.24 (C-18), 18.96 (C-21), 19.27 (C-27), 19.74 (C-19), 20.03 (C-26), 21.22 (C-11), 23.34 (C-28), 24.39 (C-15), 26.39 (C-23), 28.40 (C-16), 29.44 (C-25), 29.97 (C-8), 34.16 (C-22), 34.50 (C-2), 36.35 (C-20), 37.36 (C-1), 38.23 (C-10), 38.83 (C-7), 39.86 (C-12), 42.76 (C-13), 46.11 (C-24), 53.88 (C-9), 56.14 (C-17), 56.33 (C-14), 73.57 (C-6), 126.59 (C-4), 168.55 (C-5), 200.50 (C-3)

7α-Hydroxy-β-sitosterol (**5**) White powder, mp. : 215 °C ; $[\alpha]_D^{25}$: –72.4° (c = 0.04, $CHCl_3$); EI-MS (m/z) : 430 $[M]^+$; ^{1}H-NMR ($CDCl_3$, 500 MHz, δ ppm) : δ 0.70 (3H, s, H-18), 0.83 (3H, d, J = 6.8 Hz, H-27), 0.85 (3H, d, J = 6.8 Hz, H-26), 0.87 (3H, t, J = 7.8 Hz, H-29), 0.95 (3H, d, J = 6.8 Hz, H-21), 1.02 (3H, s, H-19), 1.65-1.77 (2H, m), 1.84-1.96 (3H, m), 1.98-2.05 (1H, m), 2.20-2.40 (2H, m), 3.61(1H, m, H-3), 3.87(1H, br.s, H-7), 5.63(1H, d, J = 5.9 Hz, H-6); ^{13}C-NMR ($CDCl_3$, 125 MHz, δ ppm) : δ 11.87 (C-29), 12.23 (C-18), 18.48 (C-19), 19.04 (C-21), 19.27 (C-27), 20.02 (C-26), 20.95 (C-11), 23.32 (C-28), 24.54 (C-15), 26.22 (C-23), 28.51 (C-16), 29.41 (C-25), 31.68 (C-2), 34.18 (C-22), 36.35 (C-20), 37.26 (C-1), 37.64 (C-10), 37.78 (C-8), 39.43 (C-12), 42.27 (C-4), 42.39 (C-13), 42.53 (C-9), 46.10 (C-24), 46.68 (C-14), 55.98 (C-17), 65.59 (C-7), 71.60 (C-3), 124.13 (C-6), 146.49 (C-5)

3-Hydroxy-4,4-dimethyl-4-butyrolactone (**6**) Colorless oil, $[\alpha]_D^{25}$: –20.4° (c = 0.37, MeOH); ESI-MS (m/z) : 153 $[M + Na]^+$; ^{1}H-NMR (CD_3OD, 300 MHz, δ ppm) : δ 1.35 (3H, s, H-5), 1.38 (3H, s, H-6), 2.42 (1H, dd, J = 3.5, 18.0 Hz, H-2b), 3.02 (1H, dd, J = 6.5, 18.0 Hz, H-2a), 4.13 (1H, dd, J = 3.5, 6.5 Hz, H-3); ^{13}C-NMR (CD_3OD, 125 MHz, δ ppm) : δ 20.34 (C-6), 24.96 (C-5), 37.90 (C-2), 73.13 (C-3), 88.56 (C-4), 176.61 (C-1)

Daucosterol (**7**) White powder, mp. : 286 °C; FAB-MS (m/z) : 599 $[M + Na]^+$; ^{1}H-NMR (pyridine-d_5, 500 MHz, δ ppm) : δ 0.67 (3H, s, H-18), 0.88 (3H, d, J = 6.8 Hz, H-26), 0.90 (3H, d, J = 6.8 Hz, H-27), 0.92 (3H, t, J = 7.8 Hz, H-29), 0.96 (3H, s, H-19), 1.01 (3H, d, J = 5.9 Hz, H-21), 1.99 (1H, m), 2.15 (1H, m), 2.50 (1H, t, J = 11.7 Hz), 2.75 (1H, m), 3.98 (2H, m), 4.07 (1H, t, J = 6.8 Hz), 4.29 (2H, m), 4.43 (1H, m), 4.58 (1H, d, J = 11.7 Hz), 5.07 (1H, d, J = 6.8 Hz, H-1'), 5.37 (1H, d, J = 4.9 Hz, H-6); ^{13}C-NMR (pyridine-d_5, 75 MHz, δ ppm) : δ 12.48 (C-18), 12.67 (C-29), 19.52 (C-21), 19.72 (C-19), 19.93 (C-26), 20.48 (C-27), 21.79 (C-11), 23.90 (C-28), 25.02 (C-15), 26.91 (C-23), 29.04 (C-16), 29.98 (C-25), 30.77 (C-2), 32.57 (C-8), 32.68 (C-7), 34.72 (C-22), 36.89 (C-20), 37.44 (C-10), 37.99 (C-1), 39.85 (C-4), 40.46 (C-12), 42.99 (C-13), 46.56 (C-24), 50.86 (C-9), 56.76 (C-17), 57.34 (C-14), 63.35 (C-6'), 72.22 (C-4'), 75.85 (C-2'), 78.63 (C-5'), 78.98 (C-3'), 79.11 (C-3), 103.09 (C-1'), 122.42 (C-6), 141.43 (C-5)

(6*S*, 9*S*)-Roseoside (**8**) White powder, $[\alpha]_D^{25}$: +72.0° (c = 0.91, MeOH); LC-ESI MS (m/z) : 409 $[M + Na]^+$, 386 M^+; ^{1}H-NMR (CD_3OD, 500 MHz, δ ppm) : δ 1.03[a] (3H, s, H-12), 1.06[a] (3H, s, H-11), 1.31 (3H, d, J = 5.9 Hz, H-10), 1.96 (3H, s, H-13), 2.19 (1H, d, J = 17.6 Hz, H-2a), 2.62 (1H, d, J = 17.6 Hz, H-2b), 3.16 (1H, ddd, J = 2.7, 6.8, 8.8 Hz, H-5), 3.21 (1H, dd, J = 7.8, 8.8 Hz, H-2'), 3.27[b] (1H, t, J = 8.8 Hz, H-4'), 3.28[b] (1H, m, H-3'), 3.65 (1H, dd, J = 5.9, 11.7 Hz, H-6'a), 3.86 (1H, dd, J = 2.8, 11.7 Hz, H-6'b), 4.29 (1H, d, J = 7.8 Hz, H-1'), 4.55 (1H, quint. J = 6.8 Hz, H-9), 5.75 (1H, dd, J = 6.8, 15.6 Hz, H-8), 5.88 (1H, br.s, H-4), 5.99 (1H, d, J = 15.6 Hz, H-7); ^{13}C-NMR (CD_3OD, 75 MHz, δ ppm) : δ 19.71 (C-13), 22.40 (C-10), 23.63 (C-11), 24.85 (C-12), 42.59 (C-1), 50.91 (C-2), 62.99 (C-6'), 71.85 (C-4'), 74.79 (C-9), 75.12 (C-2'), 78.37 (C-5'), 78.53 (C-3'), 80.17 (C-6), 101.41 (C-1'), 127.28 (C-4), 133.86 (C-7), 133.93 (C-8), 167.27 (C-5), 201.42 (C-3); [a, b] : Assignments may be reversed.

(9*S*)-Drummondol-9-*O*-β-D-glucopyranoside (**9**) White powder, $[\alpha]_D^{25}$: –53.2° (c = 0.97, MeOH); FAB-MS (m/z) : 425 $[M + Na]^+$; ^{1}H-NMR (CD_3OD, 300 MHz, δ ppm) : δ 0.96 (3H, s, H-12), 1.19 (3H, s, H-13), 1.33 (3H, d, J = 6.5 Hz, H-10), 2.31 (1H, dd, J = 2.0, 18.0 Hz, H-2a), 2.43 (1H, dd, J = 3.0, 18.0 Hz, H-4a), 2.72 (1H, dd, J = 2.0, 18.0 Hz, H-2b), 2.84 (1H, d, J = 18.0 Hz, H-4b), 3.19[a] (2H, m, H-2', H-5'), 3.24 (1H, m, H-4'), 3.26[a] (1H, m, H-3'), 3.62 (1H, dd, J = 6.0, 12.0 Hz, H-6'a), 3.64 (1H, d, J = 7.5 Hz, H-11a), 3.86 (1H, dd, J = 2.5, 12.0 Hz, H-6'b), 3.92 (1H, dd, J = 3.0, 7.5 Hz, H-11b), 4.29 (1H, d, J = 7.5 Hz, H-1'), 4.58 (1H, d, J = 6.5 Hz, H-9), 6.02 (1H, dd, J = 6.5, 15.0 Hz, H-8), 6.28 (1H, dd, J = 1.0, 15.0 Hz, H-7); ^{13}C-NMR (CD_3OD, 125 MHz, δ ppm) : δ 15.80 (C-12), 19.62 (C-13), 22.62 (C-10), 48.98 (C-1), 53.41 (C-2), 54.12 (C-4), 62.97 (C-6'), 71.81 (C-4'), 74.65 (C-9), 75.17 (C-2'), 78.33 (C-3'), 78.39 (C-5'), 78.49 (C-11), 82.78 (C-6), 87.70 (C-5), 101.39 (C-1'), 129.36 (C-7), 137.34 (C-8), 211.45 (C-3); HMBC (CD_3OD, 500MHz) : see Fig. 1; [a] : Assignments may be reversed.

Results and Discussion

Five steroids, three terpenoids and a lactone were isolated by column chromatography from the MeOH extract. Their structures were identified by spectroscopic methods to be friedelin (**1**) (Mahato and Kundu, 1994; Klass *et al*. 1992), â-sitosterol (**2**) (Kim *et al*., 2006), 7-

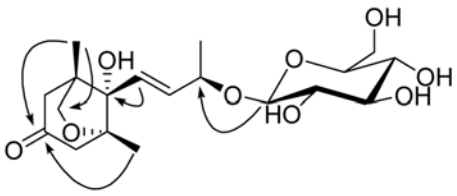

Fig. 1. The HMBC ($^1H \rightarrow {}^{13}C$) correlations of **9**.

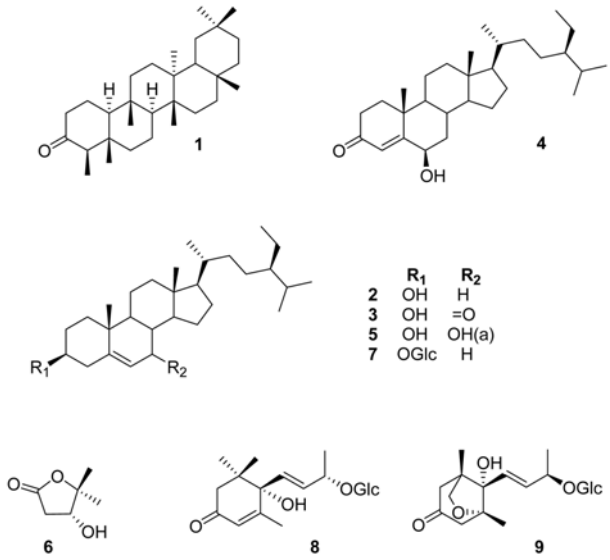

Fig. 2. The structure of isolated compounds (**1 - 9**) from *H. japonicus*.

keto-β-sitosterol (**3**) (Della *et al*., 1990), 6β-hydroxy-4-stigmasten-3-one (**4**) (Arai *et al*., 1998; Achenbach and Lowel, 1995), 7α-hydroxy-β-sitosterol (**5**) (Zhao *et al*., 2005), 3-hydroxy-4,4-dimethyl-4-butyrolactone (**6**) (Ahmed *et al.,* 2004), and daucosterol (**7**) (Yokosuka *et al*., 2005; Lee *et al*., 2006). The compounds **1**, **3**, **4**, and **6 - 9** were isolated for the first time from this plant source. Although the compounds **8** and **9** were previously reported, the compounds were not so common in natural sources. Therefore, we discuss herein the determination of the structures.

Compound **8** was isolated as an amorphous powder. The ^{1}H-NMR spectrum showed four methyl groups at δ 1.03 (3H, s), 1.06 (3H, s), 1.31 (3H, d, J = 5.9 Hz), and 1.96 (3H, s). Three olefinic protons were observed at δ 5.88 (1H, brs), 5.75 (1H, dd, J = 6.8, 15.6 Hz) and 5.99 (1H, d, J = 15.6 Hz). The signals at δ 3.16 (1H, ddd, J = 2.7, 6.8, 8.8 Hz), 3.21 (1H, dd, J = 7.8, 8.8 Hz), 3.27 (1H, t, J = 8.8 Hz), 3.28 (1H, m), 3.65 (1H, dd, J = 5.9, 11.7 Hz), 3.86 (1H, dd, J = 2.8, 11.7 Hz) and 4.29 (1H, d, J = 7.8 Hz) were assignable to D-glucose. The coupling constant (J = 7.8 Hz) of the anomeric proton of D-glucose indicated the β-form. In the ^{13}C-NMR spectrum, nineteen carbon signals including four olefinic and a carbonyl carbon signals at δ 127.28, 133.86, 133.93, 167.27 and 201.42 were observed. The above mentioned data suggested **8** to be the ionol type of norsesquiterpene (Champavier *et al*., 1999; Yamano and Ito, 2005). Based on the above consideration and the comparison of the data with those in the previous papers (Champavier *et al*., 1999; Yamano and Ito, 2005), the structure of **8** was assigned as (6S, 9S)-roseoside.

Compound **9** was obtained as white powder ($[\alpha]_D^{25}$: –53.2° (c = 0.97, MeOH)). The ^{1}H-NMR spectrum showed three methyl signals at δ 0.96 (3H, s), 1.19 (3H, s), and 1.33 (3H, d, J = 6.5 Hz). Two olefinic and an oxymethine proton signals were observed at δ 6.02 (1H, dd, J = 6.5, 15.0 Hz), 6.28 (1H, dd, J = 1.0, 15,0 Hz) and 4.58 (1H, d, J = 6.5 Hz). The signals at δ 3.19 (2H, m), 3.24 (1H, m), 3.26 (1H, m), 3.62 (1H, dd, J = 6.0, 12.0 Hz), 3.86 (1H, dd, J = 2.5, 12.0 Hz) and 4.29 (1H, d, J = 7.5 Hz) were assignable to D-glucose. The ^{13}C-NMR spectrum indicated the presence of nineteen carbon signals. In the HMBC spectrum (Fig. 1), anomeric proton signal of sugar at δ 4.29 (J = 7.5 Hz) were correlated with methine carbon signal at δ 74.65 (C-9). The above mentioned data suggested **9** to be the norsesquiterpene (Champavier *et al*., 1999; Yamano and Ito, 2005). Based on the above consideration and the comparison of the data with those in the previous papers (Calis *et al*., 2002; Cheng *et al*., 2002), the structure was identified as drummondol-9-*O*-β-D-glucopyranoside (spinoside B) (Cheng *et al*., 2002), which was isolated for the first time in this plant.

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