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Chemical Constituents from *Sorbus commixta*

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Abstract – Two lupane-type triterpenes, lupenone (**1**) and lupeol (**2**), a phytosterol, β-sitosterol (**3**), two ursane-type triterpenes, 3β-acetoxy ursolic acid (**4**) and ursolic acid (**5**), a lignan, (-)-lyoniresinol 3a-*O*-β-D-xylopyranoside (**6**), and two flavanol glycosides, catechin-7-*O*-β-D-xylopyranoside (**7**) and catechin-7-*O*-β-D-apiofuranoside (**8**) were isolated from the stem bark of *Sorbus commixta* (Rosaceae). Their chemical structures were identified by physicochemical and spectroscopic methods.
Keywords – *Sorbus commixta*, Rosaceae, triterpens, phytosterol, lignan, flavanol glycoside

Introduction

Sorbus commixta Hedlund (Rosaceae) is a shrub growing in the base of mountainous regions and usually grows 6-8 m in height. The stem bark of *S. commixta* is used in traditional medicine as a tonic and for the treatment of cough, asthma and other bronchial disorders (Bae, 2000; Chiang, 1977).

Up to now, some compounds such as aucuparin, 2'- and 4'-methoxyaucuparin, and 2'-hydroxyaucuparin, 3-(4-hydroxy-3,5-dimethoxyphenyl)-propanal, parasorboside have been reported from Sorbus genus (Kokubun *et al*, 1995; Malterud *et al*, 1989; Tschesche *et al*, 1971). In our previous report, we already isolated the two compounds, catechin-7-*O*-β-D-xylopyranoside and catechin-7-*O*-β-D-apiofuranoside, from *S. commixta* as their antioxidant active principles (Na *et al*, 2002). As a part of our continuous research to find a novel compound from plants, we studied the EtOAc and BuOH-soluble fractions of the MeOH extract of the stem bark of *S. commixta*. This paper reports further isolation and structure determination of the compounds from *S. commixta*.

Experimental

General – Melting points were measured with an Electrothermal Series IA9100 apparatus and are uncorrected. Optical rotations were measured with a DIP-370 automatic polarimeter (JASCO Co.). IR spectra were recorded on a JASCO infrared spectrophotometer IR Report-100 (JASCO Co.). ^{1}H- and ^{13}C- NMR experiments were run in $CDCl_3$, CD_3OD and DMSO-d_6 containing TMS as an internal standard, using Bruker AC 300F (^{1}H, 300 MHz; ^{13}C, 75 MHz). Silica gel (70-230 mesh, 230-400 mesh; Merck) and Florisil® (60-100 mesh, J. T. Baker) were used for chromatography. TLC was performed on pre-coated Si gel 60 (F_{254}, Merck).

Plant material – The stem bark of *S. commixta* was collected in Mt. Sulak, Korea in June 1998 and identified by Prof. KiHwan Bae, College of Pharmacy, Chungnam National University. A voucher specimen (CNU 1081) has been deposited in the herbarium of the College of Pharmacy, Chungnam National University.

Extraction and isolation – The dried stem bark of *S. commixta* (2.2 kg) was extracted with MeOH by reflux. The MeOH extract (270 g) was suspended in water and then partitioned with EtOAc and BuOH, sequentially. The EtOAc fraction (88 g) was subjected to column chromatography on a silica gel (9×40 cm, 70-230 mesh) eluted with gradient hexane-acetone (10:1→0:1). Seven fractions, Fr. 1 (5.7 g), Fr. 2 (9.8 g), Fr. 3 (15.0 g), Fr. 4 (9.2 g), Fr. 5 (6.5 g), Fr 6 (15.6 g), Fr. 7 (19.2 g), were obtained based on their TLC (silica gel) pattern. Silica gel column chromatography (5×30 cm, 230-400 mesh) of the Fr. 2 was then carried out using a mobile phase of hexane-acetone (20:1) to yield three fractions (Fr. 2-1~Fr. 2-3). The Fr. 2-1 (2.1 g) was recrystallized from MeOH to give compound **1** (480 mg). Fraction 3 (Fr. 3, 15.0 g) was separated by Florisil® column chromatography (6×30 cm, 60-100 mesh) with hexane-acetone (5:1) to yield the compound **2** (5.2 g).

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Fraction 4 (Fr. 4, 9.2 g) was separated by silica gel column chromatography (5×30 cm, 230-400 mesh) with hexane-EtOAc (4:1) to yield five fractions (Fr. 4-1~Fr. 4-5). Compound **3** (115.0 mg) was crystallized in $CHCl_3$ as colorless plate from fr. 4-2. Fraction 4-4 (350 mg) was further fractionated by silica gel column chromatography (3×20 cm, 230-400 mesh) with hexane-EtOAc (4:1) to yield the compound **4** (9.0 mg). Fraction 5 (Fr. 5, 6.5 g) was separated by silica gel column chromatography (5×30 cm, 230-400 mesh) with hexane-EtOAc (7:3) to yield the compound **5** (75.0 mg). The BuOH fraction (70 g) was subjected to column chromatography on a silica gel (9×40 cm, 70-230 mesh) eluted with gradient $CHCl_3$-MeOH (30:1→ 0:1) and as a result, eight fractions were obtained. Silica gel column chromatography (5×30 cm, 230-400 mesh) of the Fr. 2 (12.5 g) was carried out using a gradient mobile phase of $CHCl_3$-MeOH (30:1 → 10:1) to yield three fractions (Fr. 2-1~Fr. 2-3). The Fr. 2-2 (1.6 g) was purified by preparative MPLC on an ULTRA PACK ODS-18 column (YAMAZEN, 26×300 mm; mobile phase: MeOH-H_2O (4:6); flow rate: 12 ml/min; detection: 254 nm) to give compound **6** (350 mg, t_R 15 min). Compound **7** and **8** were isolated from Fr. 5 (7.2 g) as described in the previous report (Na *et al.*, 2002) by silica gel column chromatography [$CHCl_3$-MeOH (7:3)] and preparative MPLC on an ULTRA PACK ODS-18 column (YAMAZEN, 26×300 mm; mobile phase: MeOH-H_2O (3:7); flow rate: 12 ml/min; detection: 254 nm).

Compound 1 (lupenone) – colorless needle ($CHCl_3$-MeOH); mp 169-170°C; $[\alpha]_D$ +62.8° (*c* 1.0, $CHCl_3$); IR ν_{max} cm^{-1}: 3080, 1700, 1648, 890; EIMS *m/z*: 424 $[M]^+$, 409, 313, 218, 205, 189, 161; ^{1}H-NMR (300 MHz, $CDCl_3$) δ: 4.69 (1H, m, H-29β), 4.57 (1H, m, H-29α), 2.41 (1H, m, H-19), 1.68, 1.07, 1.07, 1.02, 0.96, 0.93, 0.80 (each 3H, s, 7×CH_3); ^{13}C-NMR (75 MHz, $CDCl_3$) δ: 39.6 (C-1), 34.1 (C-2), 217.9 (C-3), 47.3 (C-4), 55.0 (C-5), 19.6 (C-6), 33.6 (C-7), 40.9 (C-8), 49.8 (C-9), 36.9 (C-10), 21.5 (C-11), 25.2 (C-12), 38.2 (C-13), 42.9 (C-14), 27.4 (C-15), 35.6 (C-16), 42.9 (C-17), 48.3 (C-18), 47.9 (C-19), 150.7 (C-20), 29.9 (C-21), 40.0 (C-22), 26.6 (C-23), 21.0 (C-24), 15.8 (C-25), 15.9 (C-26), 14.4 (C-27), 18.0 (C-28), 109.2 (C-29), 19.3 (C-30).

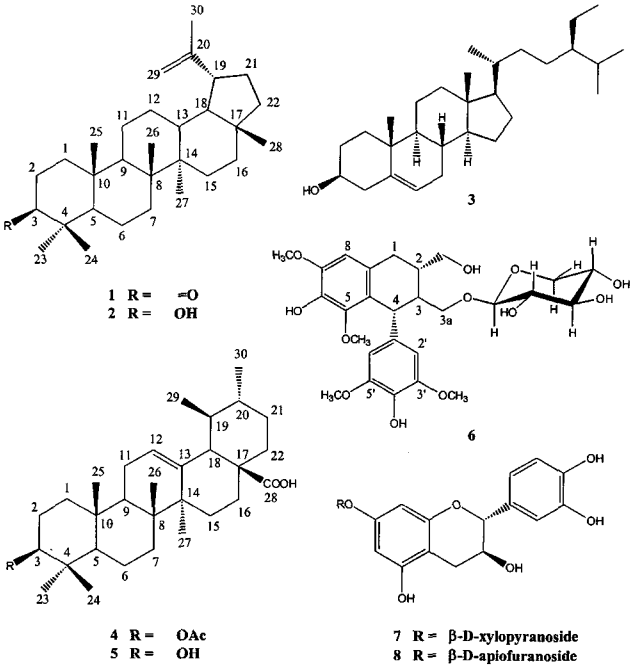
Compound 2 (lupeol) – white amorphous powder; mp 210°C; $[\alpha]_D$ +26.0° (*c* 0.8, $CHCl_3$); IR ν_{max} cm^{-1}: 3235, 1640, 1490, 1382, 1185, 1105, 1040, 984, 943; EIMS *m/z*: 426 $[M]^+$, 218, 207, 189; ^{1}H-NMR (300 MHz, $CDCl_3$) δ: 4.69 (1H, m, H-29β), 4.57 (1H, m, H-29α), 3.18 (1H, dd, H-3), 2.29 (1H, m, H-19), 1.91 (1H, m, H-21), 1.68, 1.03, 0.97, 0.94, 0.83, 0.79, 0.76 (each 3H, s, 7×CH_3); ^{13}C-NMR (75 MHz, $CDCl_3$) δ: 38.6 (C-1), 27.3 (C-2), 78.9 (C-3), 38.8 (C-4), 55.2 (C-5), 18.2 (C-6), 34.2 (C-7), 40.7 (C-8), 50.3 (C-9), 37.1 (C-10), 20.9 (C-11), 25.0 (C-12), 38.0 (C-13), 42.7 (C-14), 27.4 (C-15), 35.5 (C-16), 42.9 (C-17), 48.2 (C-18), 47.9 (C-19), 150.8 (C-20), 29.8 (C-21), 39.9 (C-22), 27.9 (C-23), 15.3 (C-24), 16.1 (C-25), 15.9 (C-26), 14.5 (C-27), 17.9 (C-28), 109.3 (C-29), 19.2 (C-30).

Compound 3 (β-sitosterol) – colorless plate ($CHCl_3$); mp 140-142°C (uncorrected); $[\alpha]_D$ –37° (*c* 2.0, $CHCl_3$); and possessed comparable spectral data to the previously reported one (Chang *et al.*, 1981).

Compound 4 (3β-acetoxy ursolic acid) – white amorphous powder; mp 214-216°C; $[\alpha]_D$ +58° (*c* 1.5, $CHCl_3$); IR ν_{max} cm^{-1}: 3050, 2910, 1450, 1360; EIMS *m/z*: 498 $[M]^+$, 456, 424, 218, 203, 175, 109, 91; ^{1}H-NMR (300 MHz, $CDCl_3$) δ: 5.23 (1H, t, *J* = 3.5 Hz, H-12), 4.50 (1H, dd, *J* = 10.8, 4.8 Hz, H-3α), 2.05 (3H, s, H-OAc), 1.07, 0.96, 0.96, 0.87, 0.85, 0.77 (each 3H, s, 7×CH_3); ^{13}C-NMR (75 MHz, $CDCl_3$+DMSO-d_6) δ: 38.1 (C-1), 23.8 (C-2), 80.8 (C-3), 37.5 (C-4), 55.1 (C-5), 18.0 (C-6), 32.7 (C-7), 39.3 (C-8), 47.3 (C-9), 36.9 (C-10), 23.4 (C-11), 125.5 (C-12), 137.8 (C-13), 41.6 (C-14), 27.9 (C-15), 23.9 (C-16), 47.8 (C-17), 52.3 (C-18), 38.8 (C-19), 38.7 (C-20), 30.4 (C-21), 36.5 (C-22), 27.9 (C-23), 16.9 (C-24), 15.5 (C-25), 16.5 (C-26), 23.9 (C-27), 184.0 (C-28), 16.8 (C-29), 21.0 (C-30), 170.7 (OAc), 21.1 (OAc).

Compound 5 (ursolic acid) – white amorphous powder; mp 261-263°C; $[\alpha]_D$ +63° (*c* 1.0, EtOH); IR ν_{max} cm^{-1}: 3400, 2920, 1680, 1450, 1380; ^{1}H-NMR (300 MHz, $CDCl_3$+DMSO-d_6) δ: 5.17 (1H, t, *J* = 3.5 Hz, H-12), 3.04 (1H, dd, *J* = 10.9, 4.8 Hz, H-3α), 1.07 (3H, s, H-23), 0.94 (6H, s, H-26, 27), 0.90 (3H, s, H-24), 0.84 (3H, d, *J* = 6.4 Hz, H-30), 0.77 (3H, d, *J* = 9.6 Hz, H-29) and 0.72 (3H, s, H-25); ^{13}C-NMR (75 MHz, $CDCl_3$+DMSO-d_6) δ: 38.1 (C-1), 26.9 (C-2), 76.8 (C-3), 36.5 (C-4), 54.7 (C-5), 17.8 (C-6), 32.6 (C-7), 38.7 (C-8), 46.7 (C-9), 36.4 (C-10), 22.7 (C-11), 124.5 (C-12), 137.9 (C-13), 41.5 (C-14), 27.4 (C-15), 23.7 (C-16), 46.9 (C-17), 52.2 (C-18), 38.4 (C-19), 38.2 (C-20), 30.1 (C-21), 36.2 (C-22), 28.1 (C-23), 15.1 (C-24), 15.8 (C-25), 16.8 (C-26), 23.1 (C-27), 178.3 (C-28), 16.7 (C-29), 20.9 (C-30).

Compound 6 [(-)-lyoniresinol 3a-O-β-D-xylopyranoside] – white needle (MeOH-water); mp 169-172°C (uncorrected); $[\alpha]_D$ –66.3° (*c* 0.5, MeOH); IR ν_{max} cm^{-1}: 3400, 2900, 1610, 1515, 1490, 1315, 1212, 1110; UV (MeOH) λ_{max} (log ε): 230 (4.27), 280 (3.60) nm; FAB-MS *m/z* 553 $[M+H]^+$; ^{1}H-NMR (300 MHz, DMSO-d_6) δ: 3.64 (9H, s, OMe-7, 3', 5'), 3.76 (3H, s, OMe-5), 4.12 (1H, d, *J* = 7.4 Hz, xyl-1), 4.26 (1H, d, *J* = 6.4 Hz, H-4), 6.33 (each 1H, s, H-2', H-6'), 6.56 (1H, s, H-8). ^{13}C-NMR (75 MHz, DMSO-d_6)



δ: 32.6 (C-1), 40.6 (C-2), 63.7 (C-2a), 44.6 (C-3), 69.0 (C-3a), 40.9 (C-4), 146.9 (C-5), 137.3 (C-6), 146.5 (C-7), 106.7 (C-8), 128.3 (C-9), 124.9 (C-10), 137.6 (C-1'), 106.0 (C-2'), 147.5 (C-3'), 133.3 (C-4'), 147.5 (C-5'), 106.0 (C-6'), 104.0 (xyl-1), 73.3 (xyl-2), 76.8 (xyl-3), 69.6 (xyl-4), 65.7 (xyl-5), 58.6 (OMe-5), 55.7 (OMe-7), 56.0 (OMe-3', OMe-5').

Compound 7 (catechin-7-*O*-β-D-xylopyranoside) – pale yellow powder; mp 165-167°C; $[\alpha]_D$ –37.9° (*c* 1.6, MeOH); IR ν_{max} (KBr) cm^{-1}: 3400, 2900, 1610, 1590, 1510, 1450, 1280, 1160, 1100, 1040; UV (MeOH) λ_{max} (log ε): 280 nm (3.60); FAB-MS *m/z* 423 $[M+H]^+$; ^{1}H- and ^{13}C-NMR data were reported previously (Na *et al.*, 2002).

Compound 8 (catechin-7-*O*-β-D-apiofuranoside) – pale yellow powder; mp 171-174°C; $[\alpha]_D$ –33.3° (*c* 0.48, MeOH); IR ν_{max} (KBr) cm^{-1}: 3400, 2900, 1610, 1590, 1510, 1450, 1280, 1160, 1100, 1040; UV (MeOH) λ_{max} (log ε): 280 nm (3.60); FAB-MS *m/z* 423 $[M+H]^+$; ^{1}H- and ^{13}C-NMR data were reported previously (Na *et al*, 2002).

Results and Discussion

Compound **1**, obtained as colorless needle from $CHCl_3$-MeOH mixture, showed a molecular ion peak at *m/z* 424, and fragmentation ions at *m/z* 218, 205, 189, which were characteristic to lupane-type triterpenens. Its IR spectrum exhibited carbonyl stretching at 1700 cm^{-1}. The ^{1}H-NMR spectrum showed olefinic protons at δ 4.69 (1H, m) and 4.57 (1H, m), correlated with δ_C 109.2 (C-29) in COLOC spectrum. Seven methyl signals at δ 1.68, 1.07, 1.07, 1.02, 0.96, 0.93, 0.80. The ^{13}C-NMR spectrum showed characteristic peak at δ 150.7 (C-20) and 109.2 (C-29) and thirty carbon peak of lupane-type triterpenes. As a result, compound **1** was expected to lupenone and identified by comparison of their physicochemical and spectral data with those reported (Prashant *et al.*, 1993).

Compound **2**, white amorphous powder, showed the molecular ion peak at *m/z* 426, and characteristic mass fragments at *m/z* 218, 207, 189 of lupane-type triterpenens. Its IR spectrum exhibited hydroxyl group at 3235 cm^{-1}. The ^{1}H- and ^{13}C-NMR spectrum were similar to those of **1**, except that, the peak at δ_C 78.9 was remarkably shifted in comparison with 217.9 (C-3) of **1**. It means that compound **2** could be substituted a hydroxyl group at C-3 position. Thus, compound **2** was expected to lupeol and verified by comparison of their spectral data with those reported (Sholichin *et al.*, 1980).

Compound **3** was identified as β-sitosterol in comparison with their physicochemical and spectral data with those reported data (Chang *et al.*, 1981).

Compound **4**, white amorphous powder, showed the molecular ion peak at *m/z* 498 in the EIMS, and exhibited characteristic carbon peaks at δ_C 125.5 (C-12) and 137.8 (C-13) of ursane-type triterpene. The ^{1}H- and ^{13}C-NMR data were comparable with the reported data of ursolic acid with an additional acetoxy group that could be placed at C-3. Thus, compound **4** was expected to be 3β-acethoxy ursolic acid and confirmed by comparison of their spectral data with those reported one (Kang, 1987).

Compound **5,** white amorphous powder, exhibited characteristic carbon peaks at δ_C 124.5 (C-12) and 137.9 (C-13) of ursane-type triterpenens. By comparison with the authentic sample and those of reported spectral data (Kang, 1983; Nakanishi, 1983), **5** was identified as ursolic acid.

Compound **6**, obtained as white needle from MeOH-water mixture, had a molecular weight of 552, as identified by positive ion FAB-MS ($[M+H]^+$ at *m/z* 553). Its IR spectrum exhibited hydroxyl group at 3400 cm^{-1} and aromatic ring at 1610 and 1515 cm^{-1}. The ^{1}H-NMR signals at δ 6.33 (2H, s) were indicative of H-2' and H-6', and δ 6.56 (1H, s) of H-8 in **6**. In addition, the ^{1}H-NMR spectrum indicated four methoxyl groups at δ 3.76 (3H, s) and 3.64 (9H, s). A doublet at δ 4.12 (1H, d, J = 7.4 Hz) and complicated signals at δ 3.02-3.69 were indicative of a sugar moiety. The ^{13}C-NMR and DEPT specra indicated a tetra- and a penta-substituted aromatic rings, four methoxyl carbons and pentose carbons, which suggested that **6** is a lignan glycoside similar to lyoniresinol. The five signals at δ 104.0 (C-1″), 73.3 (C-2″), 76.8 (C-3″), 69.6 (C-4″) and 65.7 (C-5″) in the ^{13}C-NMR spectrum corresponded to a D-xylopyranose (Fuchino *et al.*, 1995). The coupling

constant (J = 7.4 Hz) supported a β-configuration for the anomeric position. The signal of C-3a was shifted to the lower-field by 7.3 ppm, compared with the corresponding signals in lyoniresinol. This suggested that the xylosyl group was combined at C-3a. in **6**. Thus, the structure of **6** was determined as (−)-lyoniresinol 3a-O-β-D-xylopyranoside. This was confirmed by a physicochemical and spectral data comparison with the published one (Fuchino *et al.*, 1995; Inoshiri *et al.*, 1987).

Compound **7** and **8** were identified as catechin-7-*O*-β-D-xylopyranoside and catechin-7-*O*-β-D-apiofuranoside, respectively, in our previous report (Na *et al.*, 2002).

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