Two New Phenolic Glycosides from *Sorbus commixta*

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From the stem bark of *Sorbus commixta*, two new phenolic glycosides, sorcomisides A and B (1 and 2), were isolated along with 10 known compounds. The structures of the isolates were determined by analysis of one-dimensional and two-dimensional NMR (1D- and 2D-NMR) data and high resolution (HR)-MS, chemical reaction, and computational methods. All the isolated compounds (1–12) were tested for their neuroprotective, anti-inflammatory, and cytotoxic activities.

Key words  *Sorbus commixta*; phenolic glycoside; DP4+

*Sorbus commixta* Hedl. is a deciduous tree belonging to the family Rosaceae, and widely distributed throughout Korea and Japan. This plant has been used as a traditional medicine in Korea to treat cough, asthma, bronchial disorder, gastritis, and dropsy.1,2 Although there has been research on the pharmacological activities of *S. commixta* such as antioxidative, anti-atherogenic, anti-inflammatory, anti-sclerotic, and vascular relaxant activities, only a few investigations of the bioactive constituents of this plant have been carried out.3) We previously reported 10 fatty acids and their neuroprotective and anti-inflammatory activities from *S. commixta*,4) and herein, further identified 12 compounds including two new phenolic glycosides (Fig. 1). Their chemical structures were determined through conventional NMR and high resolution (HR)-MS data analysis, computational analysis coupled with advanced statistics, and chemical reaction. All these phytochemicals (1–12) were tested for their neuroprotective, anti-inflammatory, and cytotoxic activities.

Results and Discussion

Sorcomiside A (1) was purified as a colorless gum and its molecular formula was determined as C_{21}H_{22}NO_{7} based on the [M+H]^+ ion peak m/z 400.1389 (Calcd for C_{21}H_{22}NO_{7} 400.1391) acquired by HR electrospray ionization (ESI)-MS. The 1H-NMR spectrum of 1 showed 10 aromatic signals (δ_H 8.12 (2H, m), 7.63 (1H, m), 7.51 (4H, overlap), and 7.40 (3H, overlap), a singlet (δ_H 5.73 (1H, s)), and a set of protons peaks for a β-glucopyranosyl moiety (δ_H 4.38 (1H, m), 3.32 (1H, overlap), 3.33 (1H, overlap), 3.43 (1H, m), 3.57 (1H, ddd, J=9.8, 6.3, 2.2 Hz), 4.73 (1H, dd, J=11.9, 2.2 Hz), and 4.47 (1H, dd, J=11.9, 6.3 Hz). The 13C-NMR spectrum of 1 exhibited 21 peaks characteristic for two monosubstituted aromatic rings (δ_C 135.0, 129.0 (×2), 130.2 (×2), and 131.1, 131.5, 130.9 (×2), 129.8 (×2), and 134.5), a β-glucopyranosyl moiety (δ_C 102.9, 74.9, 77.9, 71.8, 76.0, and 65.3), a cyanide (δ_C 119.3), and an oxygenated carbon (δ_C 69.4). These spectroscopic data of 1 (Table 1) were similar to those of 6′-O-galloylsambunigrin and 6′-O-galloylprunasin5) except for the presence of the signals for a monosubstituted aromatic ring in 1 instead of those for a 3,4,5-trihydroxy aromatic ring in 6′-O-galloylsambunigrin and 6′-O-galloylprunasin. The analysis of two-dimensional NMR (2D-NMR) data including correlation spectroscopy (COSY), heteronuclear single quantum coherence (HSQC), and heteronuclear multiple bond connectivity (HMBC) spectra confirmed the 2D structure of 1 (Fig. 2). The HMBC correlations of H-7/C-1′ and H-6′/C-7′ determined the connections of the β-glucopyranosyl moiety with the phenylacetonitrile group and the benzoic acid, respectively. The absolute configuration of the glucopyranosyl moiety was established through chiral derivatization and LC/MS analysis.6,7) The retention time of the derivative of 1 (13.6 min) matched that of standard d-glucopyranose (13.6 min), not L-glucopyranose (12.6 min), which confirmed the absolute configuration of the glucopyranose moiety of 1 as β-configuration. To assign the absolute configuration of C-7, we compared the 1H-NMR data of 1 with those of prunasin and sambunigrin.8) The only structural difference is that these two compounds possess a free hydroxy group at C-6′ instead of a benzoyl group in 1, and prunasin has 7R configuration while sambunigrin has 7S configuration. The anomeric proton peak in 1H-NMR of prunasin showed a higher-order splitting pattern (multiplet) which was consistent with that of 1 (multiplet) whereas clear doublet (7.81 Hz) was observed in sambunigrin.8) In addition, the chemical shift of anomeric proton of 1 (δ_H 4.38) was closer to that of prunasin (δ_H 4.25) than that of sambunigrin (δ_H 4.67).8) To corroborate the R configuration of C-7, we used DP4+ method featuring quantum mechanics (QM)-based calculations of NMR shielding tensors with support of advanced statistics.9) The calculated 1H and 13C chemical shifts of two possible epimers 1R and 1S (Table S1) were subjected to DP4+ analysis with their respective experimental values. The statistical results demonstrated the more likely structure for 1, of the two suggested epimers, was the epimer 1R with 69.4% probability (Figs. 3 and S13). Collectively, we confirmed the absolute configuration of C-7 as R. Therefore, the structure of 1 was established as 6′-O-benzoylprunasin.
Sorcomiside B (2) had the molecular formula C_{22}H_{26}O_{10}, determined via HR-FAB-MS analysis. The 1H-NMR data of 2 were similar to those of 1, except for the presence of an aromatic singlet [δ_H 6.44 (2H, s)] and two methoxy singlets [δ_H 3.70 (3H, s) and 3.60 (6H, s)] in 2 and the absence of a set of monosubstituted aromatic protons [δ_H 7.51 (2H, overlap, H-2,6) and 7.40 (3H, m, H-3,4,5)] and a singlet [δ_H 5.73 (1H, s, H-7)] in 1. The presence and the location of a 3,4,5-trimethoxyphenyl group was confirmed by the HMBC correlations of OCH_3-4/C-4, OCH_3-3,5/C-3,5, H-2,6/C-1 and C-4, and H-1/C-1 (Fig. 2). The d-configuration of glucopyranose moiety was confirmed by the same method as 1. Therefore, the structure of 2 was elucidated as 3,4,5-trimethoxyphenyl-(6'-O-benzoyl)-O-β-D-glucopyranoside.
The 10 known compounds were identified as (+)-lyoniresinol (3),\textsuperscript{10} tyloside (4),\textsuperscript{10} tiliamuroides A (5),\textsuperscript{11} nudiposide (6),\textsuperscript{12} ssioriside (7),\textsuperscript{13} prupaside (8),\textsuperscript{13} (7S,8R)-dihydrodiconiferyl alcohol 9-\Oα-L-rhamnoside (9),\textsuperscript{14} methyl syringate \Oα-L-rhamnoside (10),\textsuperscript{15} 2,3-dihydroxy-1-(4-hydroxy-3,5-dimethoxyphenyl)-1-propanone (11),\textsuperscript{15} and cecropiacic acid (12)\textsuperscript{16} based on their observed and reported spectroscopic data.

All the isolated compounds (1–12) were tested for their neuroprotective, anti-inflammatory, and cytotoxic activities, but none of them showed significant activities.

Experimental

General Experimental Procedures See our previous paper.\textsuperscript{18}

Plant Material The stem bark of \textit{S. commixta} was collected in Yangyang, Gangwon-do, Republic of Korea in June 2012, and the plant was identified by one of the authors (K.R.L.). A voucher specimen (SKKU-NPL 1210) has been deposited in the herbarium of the School of Pharmacy, Sungkyunkwan University, Suwon, Republic of Korea.

Extraction and Isolation The stem bark of \textit{S. commixta} (8.0 kg) was extracted with 80% aqueous MeOH under reflux and filtered. The filtrate was evaporated under reduced pressure to obtain the MeOH extract (1.3 kg), which was suspended in distilled H\textsubscript{2}O and successively partitioned with \n-hexane, CHCl\textsubscript{3}, EtOAc, and \n-BuOH, yielding 5, 13, 43, and 160 g, respectively. The CHCl\textsubscript{3}-soluble fraction (13 g) was separated over a silica gel column (CHCl\textsubscript{3}–MeOH, 50 : 1 \textsuperscript{9}n) to yield 12 fractions (C1–C12). Fraction C8 (500 mg) was chromatographed on an RP-C18 silica gel column with 50% aqueous MeOH to give 14 subfractions (C8-1–C8-14). Fractions C8-1 (15 mg) and C8-2 (20 mg) were purified by semi-preparative HPLC (2 mL/min, 20% aqueous MeOH) to give 14 subfractions (C8-1–C8-14). Fractions C8-1 (15 mg) and C8-2 (20 mg) were purified by semi-preparative HPLC (2 mL/min, 20% aqueous MeOH) to give 11 (2 mg) and 2 (2 mg), respectively. Fractions C8-3 (100 mg), C8-5 (40 mg), C8-6 (30 mg), and C8-10 (35 mg) were purified by semi-preparative HPLC with 50%–40% aqueous MeCN to yield 1 (2 mg), 3 (27 mg), and 10 (2 mg). Fraction C11 (900 mg) was separated on an RP-C18 silica gel column with 50% aqueous MeOH to give 12 subfractions (C11-1–C11-12). Compounds 4 (8 mg), 5 (35 mg), 6 (3 mg), 7 (5 mg), 8 (3 mg), 9 (4 mg), and 12 (4 mg) were obtained from fractions C11-1 (200 mg), C11-2 (80 mg), C11-4 (20 mg), and C11-11 (50 mg) by semi-preparative HPLC with 40–70% aqueous MeOH.

Structures A (1): Colorless gum; \[\delta\textsubscript{d}^\text{H} = 19\text{ (c 0.10, MeOH); H- (500 MHz) and }^{13}\text{C-NMR (125 MHz) data, see Table 1; HR-ESI-MS (positive-ion mode}} m/z 451.1573 [M+H]\textsuperscript{+} (Calcd for C\textsubscript{25}H\textsubscript{18}O\textsubscript{10}, 451.1599).

Structures B (2): Colorless gum; \[\delta\textsubscript{d}^\text{H} = 19\text{ (c 0.15, MeOH); H- (700 MHz) and }^{13}\text{C-NMR (175 MHz) data, see Table 1; HR-ESI-MS (positive-ion mode}} m/z 451.1573 [M+H]\textsuperscript{+} (Calcd for C\textsubscript{25}H\textsubscript{18}O\textsubscript{10}, 451.1599).

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Conflict of Interest The authors declare no conflict of interest.

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References
