

Antioxidant Polyphenol Glycosides from the Plant *Draba nemorosa*

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Bioassay-directed fractionation of *Draba nemorosa* led to the isolation of two new phenolic glycosides, 1-O-(sinapoyl)-glucitol (**2**) and 1,3-disinapoylgentiobiose (**5**) along with five known phenolic glycosides (**1**, **3**, **4**, **6**, and **7**). Their structures were characterized based on spectroscopic methods (2D NMR, HRTOFMS, IR, and UV). The isolated compounds showed antioxidant activities (IC₅₀) in the range of 14-98 mM which were estimated by DPPH radical-scavenging assay.

Key Words : *Draba nemorosa*, Sinapoylglucitol, Disinapoylgentiobiose, Antioxidants

Introduction

In recent years, oxygen-derived free radicals have been reported to be closely involved in many biological symptoms such as inflammation, cancer, atherosclerosis, and coronary heart disease.^{1,2} Almost all organisms are protected from free radical attack by defense mechanisms. One such mechanism is a preventive antioxidant system that reduces the rate of radical formation, and another is a system to produce chain-breaking antioxidants that scavenge and stabilize free radicals. The free radical production rate may exceed the capacity of the antioxidant defense mechanisms which result in substantial tissues injury.³ Recent studies with regard to these aspects have suggested that the antioxidant activities of various agents such as antiallergic, nonsteroidal, and anti-inflammatory drugs, and traditional medicinal plants may provide clinically beneficial actions.^{4,5} Thus, studies have been focused on the potential of plant products to find antioxidants against various diseases induced by free radicals.⁶ Additionally, it has been determined that the antioxidant effect of plant products is mainly due to radical-scavenging activity of phenolic compounds such as flavonoids, polyphenols, tannins, and phenolic terpenes.^{7,8}

In order to search compounds with antioxidant activity from the plants, the crude methanolic extracts from over two hundred Korean medicinal plants were prepared and tested using 2,2-diphenyl-1-picrylhydrazyl (DPPH) assay method. Of those plant extracts tested, an annual herb, *Draba nemorosa* (gotdazi, Korean common name), showed significant antioxidant activity (IC₅₀ 250 µg/mL). This herb has been known to exhibit expectorant, stomachic, diuretic, antiscorbutic, anticarcinogenic, antiviral, and antihypertensive properties.⁹⁻¹¹ However, the potential health benefits of the extracts of *Draba nemorosa* have not been studied in detail to date and phytochemical investigations have little been reported.^{12,13} Therefore, this plant might be a good candidate for further development as a nutraceutical or for its antioxidant remedies. These observations influenced us to characterize antioxidant ingredients of the plant as a part of our ongoing phytochemical and biological investigations on Korean medicinal plants.

This paper describes the isolation and structure elucidation of two new compounds, 1-O-(sinapoyl)-glucitol (**2**) and 1,3-disinapoylgentiobiose (**5**) together with five known compounds, sinapic acid (**1**), β-D-fructofuranosyl-α-D-(6-O-sinapoyl)-glucopyranoside (**3**), 1,2-disinapoyl-β-D-glucopyranoside (**4**), 1,2-disinapoylgentiobiose (**6**), and 1,2,2'-trisinapoylgentiobiose (**7**). Antioxidant activities of the isolates evaluated by DPPH activity assay are also reported.

Experimental Section

The melting point was measured on a Fisher melting point apparatus and is uncorrected. High resolution TOF mass spectra were measured on a Waters LCT Premier mass spectrometer coupled with a Waters AQUITY HPLC system and data acquisition was achieved using MassLynx version 4.0 software. Optical rotations were measured on a Perkin Elmer's 341-LC polarimeter. UV and IR spectra were measured on a Shimadzu UV-2401 PCR spectrometer and a Perkin-Elmer BXFT-IR spectrometer, respectively. NMR spectra were recorded on a Varian Mercury 400 spectrometer with standard pulse sequences operating at 400 and 100 MHz in ¹H and ¹³C NMR, respectively. ¹H and ¹³C spectra were referenced relative to either methanol-*d*₄ (δ = 3.30 and 49.15 ppm for ¹H and ¹³C NMR, respectively) or DMSO-*d*₆ (δ = 2.50 and 39.51 for ¹H and ¹³C NMR, respectively). 2D NMR spectra (COSY, TOCSY, HSQC, HMBC) were recorded using the manufacturer's software VNMR 6.1C. Flash column chromatography was carried out on C₁₈ (40-63 µm, 90 id × 70 mm, Merck). Medium-pressure liquid chromatography (MPLC) was carried out on a FMI lab pump system using silica gel 60 (25-40 µm, Daisogel, 30 id × 300 mm, eluent: 85:14.5:0.5 to 80:18:2 CH₂Cl₂-MeOH-H₂O, flow rate: 8 mL/min). Thin-layer chromatography (TLC) was performed on precoated silica gel plates (Kieselgel 60 F₂₅₄, 20 × 20 cm, 0.25 mm thick, Merck). Spots were detected under UV light at 254 and 365 nm or by charring (dipping in methanol solution of *p*-anisaldehyde-sulfuric acid followed by heating). Reversed phase HPLC was performed on a Waters 600 model system with a photodiode array UV detector 996 using a C₁₈ reverse

phased silica gel column (Senshu pak, Pegasil ODS, 20 id × 250 mm) with a gradient elution of 30 to 51% aqueous MeOH over 10 min and 51 to 65% aqueous MeOH over 80 min with a flow rate of 7 mL/min.

Plant Material. The seeds of *Draba nemorosa* were purchased from the local market at Geumsan, Daejeon, Korea in August 2005 and identified by Dr. Eunkyun Lim at the Busong Clinic of Medicinal Herbs (Iksan, Korea). A voucher specimen is deposited at the Natural Product Chemistry Lab, Department of Chemistry, Kongju National University, Korea (identification number: SM1372).

Extraction and Isolation. The seeds (6 kg) were pulverized and soaked with a series of extraction solvents: 80% aqueous MeOH (8 L) at room temperature for one week, MeOH (8 L) for one week, and CH₂Cl₂ (7 L) for 5 days. The extracts were pooled and evaporated under reduced pressure to yield brownish oily syrup (402 g). The residue was suspended in 30% aqueous MeOH (1.4 L) and was extracted with hexane (900 mL × 9). Aqueous methanolic layer was concentrated to give brownish residue (190 g) that was partitioned between H₂O (1.2 L) and butanol (800 mL × 4). Butanol fraction (50 g) was chromatographed on a C₁₈ flash column eluting with 100% H₂O to 100% MeOH to give twelve fractions.

Fraction 4 (3.2 g) was triturated with EtOAc to give compound **1** (150 mg) as a white solid and the filtrate (3 g) was further fractionated by silica gel MPLC to give ten subfractions. Subfractions, 6 and 7, were subjected to silica MPLC and C₁₈ HPLC to afford **2** (9 mg) as pale yellowish oil and **3** (55 mg) as yellowish powder.

Fraction 6 (4.5 g) was further chromatographed using silica MPLC to give thirteen subfractions. Subfractions, 5, 6, 7, and 9, were further purified using C₁₈ HPLC to afford **4** (462 mg) as pale yellow amorphous solid, **7** (48 mg) as brownish oil, **5** (46 mg) as pale yellowish oil, and **6** (900 mg) as yellowish oil, respectively.

1-O-(Sinapoyl)-glucitol (2): pale yellowish oil; $[\alpha]_D^{20}$: +5.13 (c 0.13, CH₃OH); UV (CH₃OH): λ_{max} (log ϵ) 326 (4.95), 238 (4.90), 203 (4.97) nm; IR (NaCl plate): ν_{max} 3417, 2948, 2849, 1704, 1633, 1603, 1516, 1462, 1427, 1286, 1224, 1115, 1018 cm⁻¹; ¹H NMR (CD₃OD, 400 MHz): δ 7.66 (1H, d, J = 16.0 Hz, H-3'), 6.91 (2H, s, H-5'', 9''), 6.43 (1H, d, J = 16.0 Hz, H-2'), 4.46 (1H, dd, J = 11.6, 6.4 Hz, H_a-1), 4.26 (1H, dd, J = 11.6, 2.8 Hz, H_b-1), 3.97 (1H, ddd, J = 8.4, 6.4, 2.8 Hz, H-2), 3.91 (1H, dd, J = 4.8, 2.4 Hz, H-4), 3.89 (6H, s, OCH₃), 3.80 (1H, ddd, J = 6.0, 4.8, 4.0 Hz, H-5), 3.70 (1H, dd, J = 11.2, 4.0 Hz, H_a-6), 3.68 (1H, dd, J = 8.4, 2.4 Hz, H-3), 3.61 (1H, dd, J = 11.2, 6.0 Hz, H_b-6) ppm; ¹³C NMR (CD₃OD, 100 MHz): δ 169.2 (C, C-1'), 149.3 (C, C-6', 8'), 147.0 (CH, C-3'), 139.5 (C, C-7'), 126.6 (C, C-4'), 115.9 (CH, C-2'), 106.9 (CH, C-5', 9'), 75.2 (CH, C-5), 73.4 (CH, C-3), 70.8 (CH, C-2), 70.7 (CH, C-4), 67.6 (CH₂, C-1), 64.3 (CH₂, C-6), 56.9 (CH₃) ppm; HRTOFMS: m/z 389.1448 [M+H]⁺ (calcd. for C₁₇H₂₃O₁₀+H, 389.1447); TLC (80:18:2 CH₂Cl₂-MeOH-H₂O): R_f 0.24.

6-O- β -D-Glucopyranosyl- β -D-(1-O-sinapoyl,3-O-sinapoyl)-glucopyranose (5): pale yellowish oil; $[\alpha]_D^{20}$: +1.41 (c 0.92,

CH₃OH); UV (CH₃OH): λ_{max} (log ϵ) 328 (4.51), 240 (4.44), 202 (4.52) nm; IR (NaCl plate): ν_{max} 3363, 2940, 1704, 1633, 1604, 1515, 1456, 1427, 1339, 1285, 1226, 1156, 1115, 910, 828, 766 cm⁻¹; ¹H NMR (CD₃OD and DMSO-*d*₆, 2:1 ratio, 400 MHz): δ 7.71 (1H, d, J = 16.0 Hz, H-3'''), 7.66 (1H, d, J = 16.0 Hz, H-3''), 6.92 (2H, s, H-5''', 9'''), 6.91 (2H, s, H-5'', 9''), 6.54 (1H, d, J = 16.0 Hz, H-2'''), 6.44 (1H, d, J = 16.0 Hz, H-2''), 5.75 (1H, d, J = 8.8 Hz, H-1), 5.22 (1H, dd, J = 9.2, 8.8 Hz, H-3), 4.36 (1H, d, J = 8.0 Hz, H-1'), 4.21 (1H, brd, J = 10.4 Hz, H_a-6), 3.88 (12H, s, OCH₃ at C-6'', C-8'', C-6''', C-8'''), 3.86 (1H, dd, J = 10.4, 4.0 Hz, H_b-6), 3.85 (1H, d, J = 12.0 Hz, H_a-6'), 3.80 (1H, dd, J = 9.2, 8.8 Hz, H-4), 3.78 (1H, m, H-5), 3.74 (1H, dd, J = 9.2, 8.8 Hz, H-2), 3.64 (1H, dd, J = 12.0, 4.0 Hz, H_b-6'), 3.35 (1H, t, J = 9.2 Hz, H-3'), 3.26 (2H, m, H-4', H-5'), 3.23 (1H, dd, J = 9.2, 8.0 Hz, H-2') ppm; ¹³C NMR (2:1 CD₃OD-DMSO-*d*₆, 100 MHz): δ 168.3 (C, C-1'''), 167.0 (C, C-1''), 149.2 (C, C-6''', C-6''', C-8'', C-8'''), 148.5 (CH, C-3'''), 147.0 (CH, C-3''), 139.8 (C, C-7'''), 139.4 (C, C-7''), 126.5 (C, C-4'''), 126.1 (C, C-4''), 116.3 (CH, C-2'''), 115.0 (CH, C-2''), 107.2 (CH, C-5''', 9'''), 106.9 (CH, C-5'', 9''), 104.6 (CH, C-1'), 95.7 (CH, C-1), 78.6 (CH, C-3), 78.0 (2CH, C-3', C-5'), 77.6 (CH, C-5), 75.1 (CH, C-2'), 72.4 (CH, C-2), 71.6 (CH, C-4'), 69.3 (CH₂, C-6), 69.2 (CH, C-4), 62.7 (CH₂, C-6'), 57.0 (CH₃ at C-6'', C-6''', C-8'', C-8''') ppm; HRTOFMS: m/z 777.2197 [M+Na]⁺ (calcd. for C₃₄H₄₂O₁₉+Na, 777.2218); TLC (80:19:1 CH₂Cl₂-MeOH-H₂O): R_f 0.51.

Determination of Radical Scavenging Activity. Test compounds (20 μ L) at concentration of 1.5 μ g/mL to 100 μ g/mL were mixed with ethanolic solution (80 μ L) of 2,2-diphenyl-1-picrylhydrazyl (DPPH, 59 μ g/mL) in 96 well plate. Changes in absorbance were measured at 517 nm (at 24 °C after 5 min shaking) in a 96-well plate reader (Tecan, A-5082, Salzburg, Austria). Radical scavenging activity was expressed in terms of IC₅₀ (concentration required for a 50% decrease in absorbance of a control solution of DPPH) in mM. Vitamin C (L-ascorbic acid) was served as a reference compound.

Hydrolysis of Compound 5 and Identification of Monosaccharides.¹⁴ Compound **5** (2 mg) was dissolved in MeOH (0.6 mL) and treated with a solution of aqueous KOH (2 M, 0.2 mL) at room temperature for 1 h. The reaction mixture was neutralized with 2 M HCl and dried under vacuum. The residue was dissolved in 2 M HCl (1 mL) and heated at 100 °C for 4 h. After cooling and concentration the hydrolysate was passed through a short column of C₁₈ (40-63 μ m) by stepwise gradient elution of a mixture of H₂O to MeOH. The H₂O eluate was analyzed on silica gel TLC (3:1:1 isopropanol-AcOH-H₂O) and compared with authentic D-glucose (R_f 0.72) and other monosaccharides. Compound **5** and **6** provided only glucose after hydrolysis.

Results and Discussion

The powdered seeds were successively extracted with 80% aqueous MeOH, MeOH, and CH₂Cl₂. The combined extracts were suspended in a mixture of hexane-aqueous

MeOH. The aqueous MeOH layer separated was partitioned between butanol and H₂O. The butanol layer was fractionated on a C₁₈ silica gel flash column. The fractions were further subjected to a series of chromatography (silica gel MPLC and C₁₈ HPLC) to afford compounds, **1**, **2**, **3**, **4**, **5**, **6** and **7**, in the yield of 150, 9, 55, 462, 46, 900, and 48 mg, respectively.

Compound **2** was obtained as pale yellowish oil. The mass spectrum (HRTOFMS) displayed a protonated molecular ion [M+H]⁺ at *m/z* 389.1448 (calcd. for M+H, 389.1447), indicating a molecular formula of C₁₇H₂₄O₁₀. UV maxima in MeOH were observed at 326, 238 and 203 nm. The infrared (IR) spectrum showed the presence of hydroxyl group (3417 cm⁻¹), ester (1704 cm⁻¹), double bond (1633 cm⁻¹), and aromatic ring (1603, 1516 cm⁻¹). The ¹H NMR spectrum (methanol-*d*₄) showed a sharp signal at δ 3.89 for two methoxy protons, two sets of doublets at δ 7.66 and 6.43 (*J* = 16 Hz) for *trans*-configured olefinic protons, and a sharp singlet at δ 6.91 for two aromatic protons. These data indicate the presence of a sinapoyl moiety.^{15,16} The remaining part of the ¹H NMR spectrum showed the presence of eight protons coupled with six carbon signals from HSQC experiment: two methylene protons at δ 4.26 and 4.46 (δ_c 67.6), four methine protons at δ 3.97 (δ_c 70.8), 3.68 (δ_c 73.4), 3.91 (δ_c 70.7), and 3.80 (δ_c 75.2), two methylene protons at δ 3.61 and 3.70 (δ_c 64.3), which is characteristic of a saccharide or polyhydroxy chain. The ¹H-¹H COSY spectrum showed that geminally coupled methylene protons at δ 4.26 and 4.46 were coupled with the proton at δ 3.97, which was in turn coupled with the proton at δ 3.68. Similarly, the remaining methylene protons at δ 3.61 and 3.70 were coupled with the proton at δ 3.80 (ddd, *J* = 6.0, 4.8, 4.0 Hz) which showed correlation with the methine proton at δ 3.91 (1H, dd, *J* = 4.8, 2.4 Hz), which was in turn coupled with the proton at δ 3.68. From this spin coupling network and the coupling constant values, the polyoxygenated chain was suggested to be glucitol (sorbitol). The ¹H-¹H COSY correlations of the polyhydroxy chain with their coupling constants are shown in the Figure 1.

The glucitol moiety was further confirmed by the HMBC spectrum. The terminal methylene carbon (δ 67.6) was correlated with two methine protons at δ 3.97 and 3.68. Correlations of the remaining methylene carbon (δ 64.3) with δ 3.80 (H-5) and 3.91 (H-4) were also confirmed by HMBC analysis (Figure 2). Finally, the connectivity between two partial structures, sinapoyl and glucitol moiety, were determined by the HMBC spectrum. The proton signal at δ 4.26 of glucitol moiety showed a ³*J* correlation with the carbonyl carbon (δ 169.2) of sinapoyl moiety, indicating the

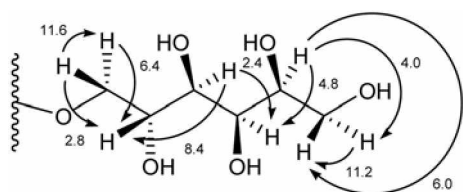


Figure 1. gCOSY with coupling constants of glucitol part of **2**.

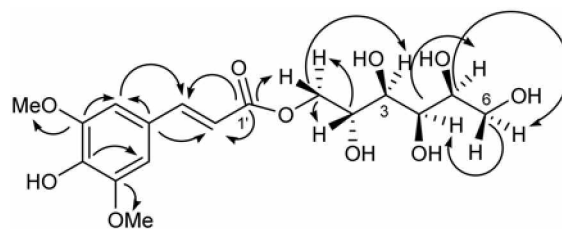


Figure 2. ¹³C-¹H correlations in gHMBC of **2**.

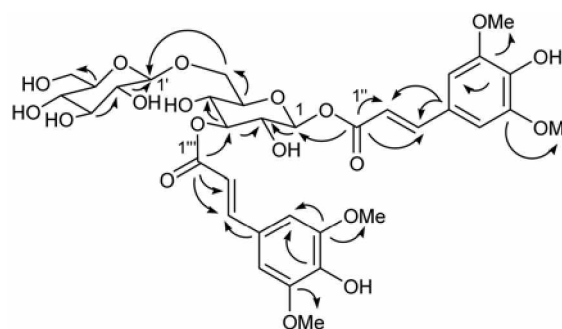


Figure 3. ¹³C-¹H correlations in gHMBC of **5**.

glucitol unit was attached to the carbonyl carbon forming ester linkage. Although the optical rotation of compound **2** was measured to be +5.13 (*c* 0.13, CH₃OH), we were not able to determine the absolute stereochemistry of the glucitol moiety due to limited material availability. Thus, the structure of **2** was determined to be 1-O-(sinapoyl)-glucitol.

Compound **5** was obtained as yellowish oil. [α]_D²⁰ +1.41 (*c* 0.92, CH₃OH) and its molecular formula of C₃₄H₄₂O₁₉ was derived from HRTOFMS (found *m/z* 777.2197, calcd 777.2218 for [M+Na]⁺). UV maxima in MeOH were observed at 328 (4.51), 240 (4.44) and 202 (4.52) nm. The infrared (IR) spectrum of **5** showed the presence of hydroxyl group, ester, double bond, and aromatic ring. The ¹H NMR spectrum of **5** showed the signals for four aromatic protons at δ 6.92 and 6.91 (two each, s), two sets of *trans*-olefinic protons at δ 7.71 and 6.54 (both *J* = 16.0 Hz); 7.66 and 6.44 (both *J* = 16.0 Hz), and one singlet for four methoxy groups at δ 3.88. These data indicate compound **5** contains two sinapoyl moieties. The remaining parts of ¹H NMR showed two anomeric protons (δ 5.75 and 4.36, each 1H, d, *J* = 8.8, 8.0 Hz, respectively), one proton at δ 5.22, and overlapped eleven protons in the range of δ 3.23-4.21. The ¹³C NMR showed twelve oxygenated carbon signals. ¹H and ¹³C NMR assignments from HSQC experiment indicated that these twelve oxygenated carbon signals are from two monosaccharide units. To identify the nature of the monosaccharides, compound **5** was hydrolyzed under acidic condition to yield only glucose by comparison of the *R_f* value of the hydrolysate with authentic glucose. Thus, it was suggested that the compound **5** was an ester of *trans*-sinapic acid with two glucose units.

The ¹H-¹H COSY spectrum showed that the protons at δ 5.75 and δ 4.36 were correlated with the protons at δ 3.74 and δ 3.23, respectively. For the rest of the ¹H NMR assignments, 1D TOCSY experiments were conducted to assign

signals of glucose units from overlapped proton signals. In 1D TOCSY, one of the anomeric protons at δ 5.75 (d, $J = 8.8$ Hz) was correlated with protons at δ 5.22 (dd, $J = 9.2, 8.8$ Hz), 4.21 (brd, $J = 10.4$ Hz), 3.86 (dd, $J = 10.4, 4.0$ Hz), 3.74 (dd, $J = 9.2, 8.8$ Hz), 3.80 (dd, $J = 9.2, 8.8$ Hz), and 3.78 (m). Similarly, the other anomeric proton at δ 4.36 (d, $J = 8.0$ Hz) was correlated with the protons at δ 3.23-3.35 (overlapped), 3.85 (d, $J = 12.0$ Hz), and 3.64 (dd, $J = 12.0, 4.0$ Hz).

In HMBC spectrum, the anomeric proton of one of the glucose moieties at δ_{H} 5.75 was correlated with the carbonyl carbon at δ_{C} 167.0 of one of the *trans*-sinapoyl moieties, indicating the attachment of sinapoyl group to anomeric position. The protons and carbons of the glucose were assigned from interpretation of HSQC and HMBC spectrum (HMBC correlation: C-1 to H-2; C-2 to H-3; C-3 to H-2 and H-4; C-4 to H-3 and H-5; C-6 to H-5). Downfield shifts of the proton H-3 (δ 5.22) suggested the attachment of the remaining sinapoyl moiety at this position, which was further confirmed from the HMBC correlation of the carbon of the sinapoyl carbonyl group at δ 168.3 with the H-3 proton. Thus, both of the sinapoyl moieties were connected to the same glucose. The H-6 protons (δ 4.21 and 3.86) of the glucose were correlated with the remaining anomeric carbon at δ 104.6 in the HMBC spectrum. Thus, one glucose was connected at C-6 position of the other. Figure 3 showed the HMBC correlation of compound **5**. The structure of compound **5** was determined to be 6-O- β -D-glucopyranosyl- β -D-(1-O-sinapoyl, 3-O-sinapoyl)glucopyranose or 1,3-disinapoylgentiobiose.

Compound **6** was obtained as yellowish oil and showed similar UV and IR with compound **5**. Acidic hydrolysis of **6** provided only glucose as it was for **5**. Mass spectra indicated compound **6** was an isomer of compound **5**. However, the R_f value for **5** was 0.51 whereas for **6**, 0.38 (TLC, silica gel, 80:19:1, CH_2Cl_2 -MeOH- H_2O). Spectroscopic analysis and comparison with reported data showed the compound **6** was

6-O- β -D-glucopyranosyl- β -D-(1-O-sinapoyl, 2-O-sinapoyl)glucopyranose²⁰ where sinapoyl groups were attached at C-1 and C-2 position of the same glucose unit, contrary to compound **5**.

Sinapoyl esters, especially sinapoylated carbohydrates are widely distributed in plants, as antioxidant principles, most of them are ester of glucose, fructose, anhydroglucitol or gentiobiose,¹⁵⁻¹⁷ but there is no any report on glucitol ester. Thus, to the best of our knowledge compound **2**, 1-O-(sinapoyl)-D-glucitol, is a new natural product. Although many gentiobiose derivatives have been isolated from plants,^{18,19} almost all have acyl-substitutions at C-1, C-2, C-2' or C-6' of gentiobiose such as sinapoyl, feruloyl or cinnamoyl moiety. 1,4-disinapoylgentiobiose has been reported²⁰ but there is no report on C-3 substitution. Thus, compound **5**, 1,3-disinapoylgentiobiose, is a novel natural product.

In addition to the compound **2**, **5**, and **6**, four more sinapic acid derivatives were also isolated and were identified as sinapic acid (**1**),²¹ β -D-fructofuranosyl- β -D-(6-O-sinapoyl)glucopyranoside (**3**),¹⁵ 1,2-disinapoyl- β -D-glucopyranose (**4**),¹⁸ and 6-O- β -D-(2'-O-sinapoyl)glucopyranosyl- β -D-(1-O-sinapoyl, 2-O-sinapoyl)glucopyranose (**7**).¹⁹ (Figure 4) Their structures were characterized by comparison with

Table 1. Radical scavenging activity

Compound	IC ₅₀ in mM
1	21.87
2	98.88
3	35.60
4	14.84
5	65.50
6	17.99
7	83.14
L-Ascorbic acid	11.53

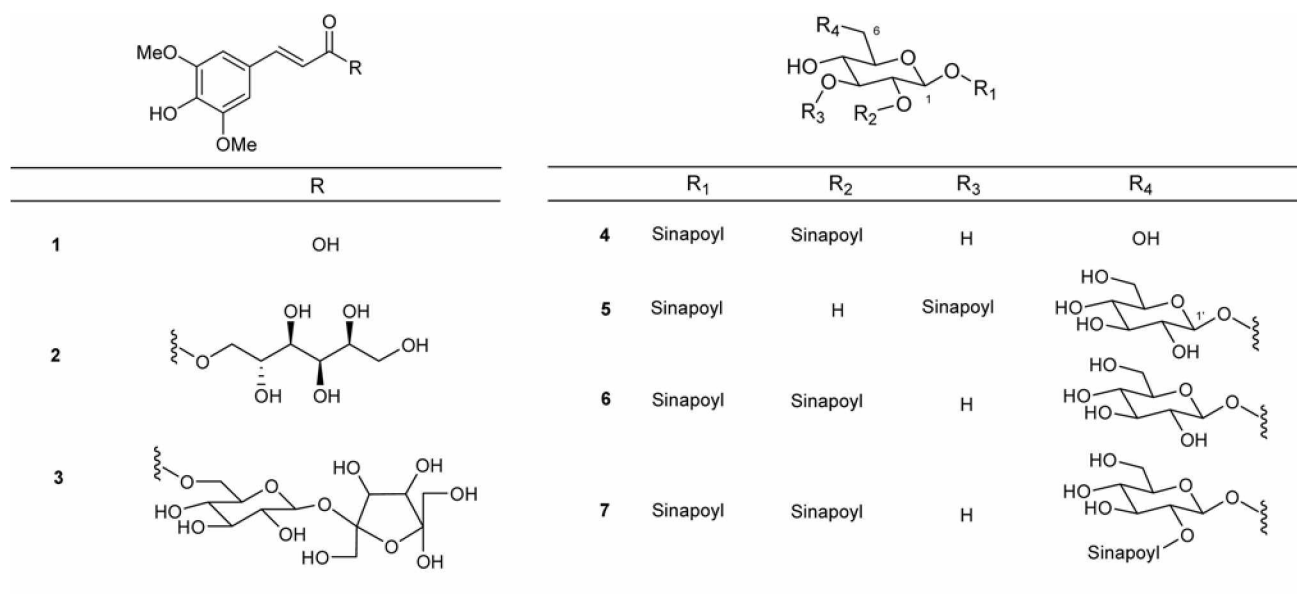


Figure 4. Structures of the isolates from *Draba nemorosa*.

known compounds from literatures. However, these are the first isolates from the plant *Draba nemorosa*.

The antioxidant potential of the isolated sinapoyl derivatives have been evaluated by their activity to scavenge a free radical, DPPH, according to Blois.²² The free radical scavenging activities of the isolates were found in the range of 14.84 to 98.88 mM. Compound 4 and 6 showed comparable activities to a reference compound, Vitamin C, with IC₅₀ 14.84 and 17.99 mM, respectively (Table 1). When sinapoyl moiety is substituted at C-3 position instead of at C-2 position, the activity decreases approximately five times (5 vs 6). The data obtained suggested that the major determinants for radical-scavenging capability are the presence of sinapoyl groups, nature of sugar units as well as the positions of substitutes on sugar moiety.

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