Notes

A New Phloroglucinol Glycoside from *Aster subulatus* Michx

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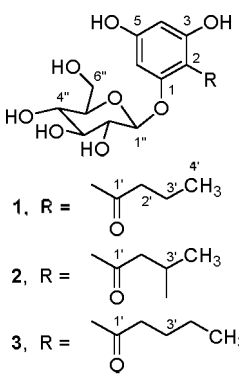
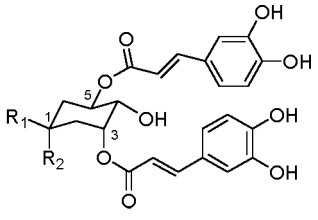
Aster subulatus Michx. (Compositae) is an annual grass native to America. In Korea, this species is widely distributed and now been accepted as a naturalized plant.[1] Previous phytochemical study on this plant has resulted in the isolation of flavonoids and their glycosides as well as chlorogenic acid.[2] As a part of our continuing efforts in search of biologically active compounds from plants in Jeju Island,[3] we have found DPPH radical scavenging activities for the ethanol extract of *A. subulatus*. This prompted us to undertake a phytochemical investigation of this plant for its active constituents. Herein, we report the isolation and characterization of a new compound, 1-[(butanoyl)phloroglucinyl]-β-D-glucopyranoside (**1**) together with two known compounds, 3,5-dicaffeoylquinic acid (**4**) and its epimer, 3,5-dicaffeoyl-*epi*-quinic acid (**5**).

The ethanol extract was prepared from the whole plant of *A. subulatus*. The ethyl acetate soluble fraction of the extract was chromatographed over silica gel and reversed-phase silica gel to yield a new phloroglucinol glycoside (**1**) besides two known quinic acid derivatives (**4**, **5**).





4, R_1 = COOH, R_2 = OH

5, R_1 = OH, R_2 = COOH

Compound **1**, obtained as an amorphous powder, showed a $[M+Na]^-$ peak at *m/z* 381.1160 (calcd *m/z* 381.1162) in the HR-FAB-MS, consistent with the molecular formula $C_{16}H_{22}O_9$ (six unsaturations). This was supported by ^{13}C and DEPT NMR spectra, which showed signals for all the 16 carbons, including six aromatic, one carbonyl, and nine aliphatic carbons (Table 1). The UV absorption maxima of **1** in MeOH at 228 and 286 nm suggested the presence of an aromatic ring. The aromatic ring is inferred to be phloroglucinol (1,3,5-trihydroxybenzene) moiety based on the observation of highly downfield shifts of three ^{13}C signals (δ 162.4, 165.9, 167.8) and upfield shifts of other three ^{13}C signals (δ 95.5, 98.4, 106.9), typical δ_C pattern appeared in phloroglucinol analogue. The aromatic protons at δ 6.17 (d, J = 2.0 Hz) and 5.94 (d, J = 2.0 Hz) were placed at H-6 and H-4 based on their HMQC correlation with carbons at δ 95.5 (C-6) and 98.4 (C-4), respectively. The upfield shift and smaller coupling constant of these protons showed that these *meta* coupled protons are between oxygenated quaternary carbons. Since only two aromatic protons were observed, there should be one substituent connected to aromatic carbon in this 1,3,5-trioxybenzene unit.

The ^1H NMR spectrum showed signals at δ 0.97 (3H, t, J = 7.5 Hz), 3.16 (1H, ddd, J = 16.5, 7.5, 6.5 Hz) and 3.09 (1H, ddd, J = 16.5, 9.0, 7.0 Hz), and 1.69 (2H, m). These signals were respectively assignable to one methyl and two methylene groups, revealing a propyl side chain in **1**, which was further confirmed by COSY experiment. The propyl group is connected to carbonyl to construct a butanoyl unit, which was verified by HMBC data (Table 1). The carbonyl carbon (C-1') is attached to the aromatic carbon (C-2) of 1,3,5-trioxybenzene nucleus, based on long range (^4J) HMBC correlation of H-6 with C-1', probably due to the conjugated π-system of the benzene ring. The presence of a sugar was suggested by the appearance of six oxygen-bearing sp^3 carbons at δ 101-62 in combination with proton signals at δ 5.03 and 3.4-3.9. The large coupling constant (J = 7.5 Hz) for the anomeric proton at δ 5.03 (H-1") having HMQC cross peak with δ 101.9 indicated the sugar was in β- configuration. The sugar protons at δ 3.53 (dd, J = 9.0, 7.5 Hz, H-2") and δ 3.41 (dd, J = 9.0, 9.0 Hz, H-4") all showed axial-axial coupling constants, which suggested that all substituents in this hexose are in equatorial positions. Therefore, the sugar was identified as glucose. In butanoyl substituted phloroglucinols, the glucose unit can be attached to either 1-OH (3-OH) or 5-OH positions. If the substitution is made at 5-OH, it leads to a symmetric benzene nucleus, which show only four aromatic ^{13}C NMR signals. Since it is not observed in **1**, the glucose moiety should be

Table 1. 1D and 2D NMR data for **1** in CD_3OD

No	δ_C (mult)[a]	δ_H (int, mult, *J* in Hz)	HMBC (H→C)
1	162.4 (s)	-	-
2	106.9 (s)	-	-
3	167.8 (s)	-	-
4	98.4 (d)	5.94 (1H, d, 2.0)	C-2
5	165.9 (s)	-	-
6	95.5 (d)	6.17 (1H, d, 2.0)	C-1, C-2, C-5, C-1'
1'	207.6 (s)	-	-
2'	47.3 (t)	3.16 (1H, ddd, 16.5, 7.5, 6.5) 3.09 (1H, ddd, 16.5, 9.0, 7.0)	C-1', C-3', C-4'
3'	19.3 (t)	1.69 (2H, m)	C-1', C-2', C-4'
4'	14.4 (q)	0.97 (3H, t, 7.5)	C-2', C-3'
1''	101.9 (d)	5.03 (1H, d, 7.5)	C-1
2''	74.9 (d)	3.53 (1H, dd, 9.0, 7.5)	
3''	78.7 (d)	3.46 (1H, m)	
4''	71.3 (d)	3.41 (1H, dd, 9.0, 9.0)	C-6''
5''	78.5 (d)	3.46 (1H, m)	
6''	62.6 (t)	3.91 (1H, dd, 12.5, 2.5) 3.72 (1H, dd, 12.5, 5.0)	

[a]Determined by DEPT experiments.

attached to 1-OH. The HMBC correlation of H-1''/C-1 further confirms the placement of the glucose moiety to 1-OH. From the above spectral data, compound **1** was identified as 1-[(butanoyl)phloroglucinyl]-*β*-D-glucopyranoside. A 3-methylbutanoyl phloroglucinol analogue (**2**) has been reported previously from *Fragaria ananassa*[4] having cytochrome P450 enzyme inhibition activities. In addition, a pentanoyl phloroglucinol glycoside (**3**) has been identified from *Indigofera hetrantha*[5] as a strong lipoxygenase enzyme inhibitor. Except the butanoyl side chain, the NMR data of **1** is almost superimposable over **2** and **3**.

The known compounds, 3,5-dicaffeoylquinic acid (**4**) and its epimer, 3,5-dicaffeoyl-*epi*-quinic acid (**5**) were also isolated in the present study. Both the epimers, **4** and **5** differ in their structures only at C-1 configuration. Compound **4** has long been identified from natural sources,[6] while its epimer, **5** has recently been isolated from *Chrysanthemum morifolium*.[7] Identification of these compounds are made by comparison of the NMR data to those reported in the literature.[7]

The antioxidative activities were determined for compounds **1**, **4**, and **5** using DPPH radicial scavenging method.[8] The 50% scavenging concentration (RS_{50}) for **1** was 78.2 μg/mL. The compounds **4** and **5**, whose RR_{50} were respectively 9.0 and 8.5 μg/mL, showed more strong activities than the positive control BHA (butylated hydroxyanisole, RS_{50} = 10.7 μg/mL).

In conclusion, a new phloroglucinol glycoside (**1**), together with two known dicaffeoylquinic acids (**4, 5**) was isolated from the whole plant of *A. subulatus*. Compounds **4** and **5** were isolated for the first time from this plant. The isolated compounds, **1**, **4** and **5** showed moderate to strong radical scavenging activities.

Experimental Section

Reagents and Instruments. All solvents were of analytical grade. Optical rotations were measured on a Jasco P-1030 automatic polarimeter. UV spectra were recorded on a Biochrom Libra S22 spectrophotometer. ^1H (500 MHz) and ^{13}C (125 MHz) NMR spectra were recorded on Bruker Ultrashield Plus 500 spectrometer. The chemical shift values are reported in ppm relative to the solvent used. Column chromatography was performed on silica gel 60 (0.040-0.063 mm) and RP-C_{18} silica gel (230-400 mesh) purchased from Merck. TLC was performed on silica gel GF_{254} plates (0.50 mm, Merck).

Plant Material. The whole plant of *A. subulatus* was collected from Shinpyung, Jeju Island in 2004, August. A voucher specimen (04-347) is deposited at Jeju Bio-Industry Development Center, Hi-Tech Industry Development Institute, Jeju, Korea.

Extraction and Isolation. The shade dried whole plant of *A. subulatus* (364.7 g) was extracted with 70% aqueous ethanol under stirring for 2 days at room temperature. The filtrate was concentrated under reduced pressure and freeze-dried to give a powder. The powdered extract (30.2 g) was then suspended in water (1.0 L) and successively partitioned with *n*-hexane, methylene chloride, ethyl acetate and *n*-butanol. The ethyl acetate fraction (1.9 g) was subjected to reversed-phase silica gel column (6.5 × 22 cm) chromatography using step gradient solvents (water and methanol) to give 10 fractions (Frs. EA-I to EA-X). Fraction EA-V was further purified with silica gel column (2.2 × 64 cm) chromatography eluting with chloroform-methanol (3:1) system to afford three subfractions (Frs. EA-V-1 to EA-V-3). The fractions EA-V-1 and EA-V-3 were identified as the compound **1** (9.9 mg) and the compound **4**

(131 mg), respectively. The fraction EA-VI was purified by short silica gel column with chloroform-methanol (3:1) to give the compound **5** (68.6 mg).

1-[(Butanoyl)phloroglucinyl]-β-D-glucopyranoside (1). Amorphous powder; UV (CH_3OH): λ_{max} 286, 228 nm; $[\alpha]_D^{20}$ - 46.2° (*c* 0.011, MeOH); ^1H and ^{13}C NMR data: Table 1; HR-FAB-MS: *m/z* 381.1160 $[M+Na]^-$ (calcd for $C_{16}H_{22}O_9Na$ 381.1162, △-0.2 mmu).

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References

1. Kim, H. S.; Lim, D. O.; Park, M. S. *Korean J. Plant Res.* **2007**, *20*, 353.
2. El-Sayed, N. H.; Lenherr, A.; Sundberg, S.; Mabry, T. J. *Biochem. Syst. Ecol.* **1987**, *15*, 549.
3. (a) Sultana, N.; Lee, N. H. *Phytother. Res.* **2007**, *21*, 1171. (b) Ham, Y. M.; Baik, J. S.; Hyun, J. W.; Lee, N. H. *Bull. Korean Chem. Soc.* **2007**, *28*, 1595. (c) Kim, Y. H.; Kim, K. S.; Han, C. S.; Yang, H. C.; Park, S. H.; Ko, K. I.; Lee, S. H.; Kim, K. H.; Lee, N. H.; Kim, J. M.; Son, K.-H. *J. Cosmet. Sci.* **2007**, *58*, 19. (d) Ko, R. K.; Lee, N. H. *Bull. Korean Chem. Soc.* **2008**, *29*, 2531.
4. Tsukamoto, S.; Tomise, K.; Aburatani, M.; Onuki, H.; Hirorta, H.; Ishiharajima, E.; Ohta, T. *J. Nat. Prod.* **2004**, *67*, 1839.
5. Rehman, A.-U.; Malik, A.; Riaz, N.; Ahmad, H.; Nawaz, S. A.; Choudhary, M. I. *Chem. Pharm. Bull.* **2005**, *53*, 263.
6. Scarpati, M. L.; Guiso, M. *Tetrahedron Lett.* **1964**, *5*, 2851.
7. Kim, H. J.; Lee, Y. S. *Planta Med.* **2005**, *71*, 871.
8. Blois, M. S. *Nature* **1958**, *29*, 1199.