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쇠비름에서 분리된 2개의 Biophenolic Glycosides

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Two Biophenolic Glycosides from *Portulaca oleracea*

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요 약. 우리나라에 널리 분포하며 오래 동안 약용으로 사용되어 온 쇠비름으로부터 페놀 글리코시드인 3-hydroxy-1-(2-hydroxyethyl)phenyl-4-O-β-D-glucopyranoside (**1**)과 2-(3,4-dihydroxyphenyl)ethyl-O-β-D-glucopyranoside (**2**)를 칼럼 크로마토그래피 및 역상 HPLC로 분리하였으며, NOESY, HMQC, HMBC와 같은 이차원적인 NMR 분광실험에 의해서 이 물질들의 ^{13}C NMR 분광 데이터 값의 지정이 수정되었다. DPPH를 이용하여 이 물질들의 항산화 활성을 측정한 결과 주목할 만한 활성을 나타내었다.

주제어: 쇠비름, 페놀 글리코시드, 항산화 활성

ABSTRACT. From *Portulaca oleracea* which is widely distributed in Korea and has long been used as a folk medicine, two biophenolic glycosides, 3-hydroxy-1-(2-hydroxyethyl)phenyl-4-O-β-D-glucopyranoside (**1**) and 2-(3,4-dihydroxyphenyl)ethyl-O-β-D-glucopyranoside (**2**) were isolated using column chromatography and reversed-phase HPLC. ^{13}C NMR spectral assignment for these compounds was revised by the extensive 2-D NMR experiments such as NOESY, HMQC, and HMBC. These compounds showed a considerable antioxidant effect in DPPH assay system.

Key words: *Portulaca oleracea*, Biophenolic Glycosides, Antioxidant Effect

INTRODUCTION

Portulaca oleracea Linne (also known as purslane) is a species which occurs worldwide and spreads prolifically in gardens and lawns. It has been not only eaten throughout much of Europe and Asia[1] but also used in folk medicine to treat dysentery, beriberi, tonsillitis, and so on[2]. A recent study showed its crude extract is effective in cancer[3]. But a secondary metabolite from the purslane has little been reported though it was recognized as a quite nutritious vegetable to include carbohydrates, amino acids, etc.[1] Only reported compound from this species is portuloside A, a monoterpene glucoside[4]. As a part of our search for new bioactive compounds from natural resources, we have investigated radical scavenging constituents from purslane. The crude extract was subjected to solvent partitioning followed by

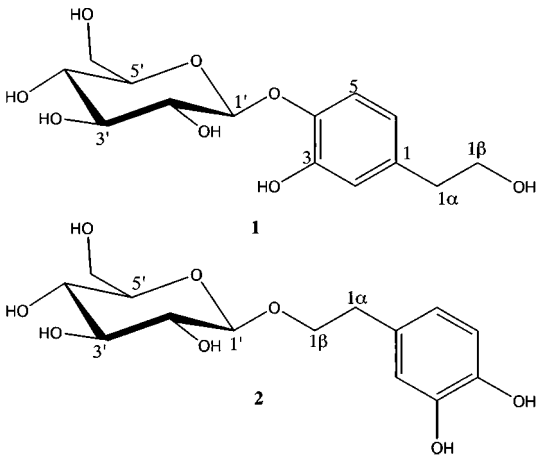

Fig. 1. The structure of compounds **1** and **2**.

C_{18} vacuum flash chromatography of the n-butanol fraction. The chromatographic fraction eluted from the C_{18} column with 50% aq. MeOH showing radical scavenging effect was purified by repeated reversed-phase HPLC to give the two biophenolic glucosides (*Fig.* 1).

RESULT AND DISCUSSION

Compound **1** was obtained as a colorless gum that was determined to have the composition $C_{14}H_{20}O_8$ by HRFABMS and ^{13}C NMR analyses. Six downfield signals in the region of δ150-115 in ^{13}C NMR spectrum and the corresponding signals at δ7.18 (1H, d, 8.3), 6.83 (1H, d, 2.0), and 6.66 (1H, dd, 8.3, 2.0) in ^1H NMR spectrum indicated the existence of an aromatic ring, which was shown by a ^1H-^1H COSY and HMBC experiments to be part of a 3,4-dihydroxyphenethyl alcohol known as a hydroxytyrosol. This interpretation was supported by an absorption maximum at 285 (log ε 3.49) nm in UV spectrum. Similarly, the presence of a sugar moiety was readily recognized by characteristic carbon signals in the region of δ105-60 in the ^{13}C NMR spectrum. Particularly, an anomeric proton signal at 4.73 (1H, d, 7.8), correlated with a carbon signal at δ104.3 by an HMQC experiment, was diagnostic for the presence of a sugar moiety.

The structure of sugar moiety was determined by combined 2-D NMR experiments. A long-range coupling of the C-1′ anomeric carbon at δ104.7 with the H-5′ at δ3.38 revealed that the sugar was a pyranose. This interpretation was also supported by NOESY correlation between H-1′ and H-5′. The orientations of the H-1′ - H-5′ protons were all assigned as axial by measurement of the vicinal proton-proton coupling constants using a mixture of 2:1 of MeOH-d_4 and benzene-d_6 as a NMR experimental solvent. Thus, the sugar moiety was defined as a β-D-glucopyranose.

A linkage between hydroxytyrosol and sugar moieties was established by HMBC and NOESY experiments. A long-range correlation of the H-1′ anomeric proton at δ4.28 with C-4 at δ145.2 placed the attachment of the sugar moiety at C-4 of the aromatic ring. This interpretation was also confirmed by a strong NOESY cross peak between H-1′ and H-5. Thus, the structure of compound **1** was determined as 3-hydroxy-1-(2-hydroxyethyl)phenyl-4-O-β-D-glucopyranoside.

Compound **2** was isolated as a colorless gum, which analyzed for $C_{14}H_{20}O_8$ by a combination of HRFABMS and ^{13}C NMR spectrometry. The spectral data of **2** were very similar to those obtained for **1**. However, careful examination of the ^{13}C NMR data revealed that signals for C-1, -1α, and -1β of **1** were shifted from δ136.1, 39.7, and 64.3, to δ131.5, 36.5, and 72.1, respectively. The corresponding changes were also observed in the ^1H NMR spectrum in which signals for H-5 and -1β of **1** were shifted from 7.09 (1H, d, 8.1) and 3.69 (2H, t, 7.1) to 6.66 (1H, d, 8.3), and 4.01 (1H, m) and 3.69 (1H, m). These changes could be accommodated by introducing a new linkage between sugar and hydroxytyrosol moieties. A combination of HMBC and NOESY experiments determined the location of connectivity to each other at C-1β and -1′. Thus, the structure of **2** was determined as 2-(3,4-dihydroxyphenyl)ethyl-O-β-D-glucopyranoside.

The literature survey revealed that compound **1** was once isolated from *Olea europaea*[5], while compound **2** was already identified in *Olea europaea*, *Prunus grayana*, and *Ricciocarpus natans*.[6] But neither was detected in *P. olerucea*. Their ^1H NMR spec-

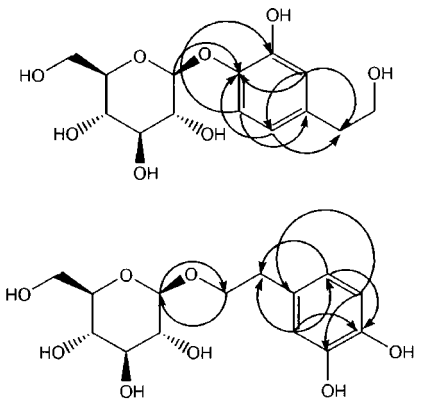

Fig. 2. Key HMBC correlations of **1** and **2**.

tral data were in good agreement with those reported, while ^{13}C NMR spectral assignments for C-2, -3, -4 and -6 of **1** and C-2 and -5 of **2** were corrected by HMBC long-ranged correlations, respectively (*Fig.* 2).[8a]

Phenolic compounds are one of the main classes of secondary metabolites with a large range of structures and functions. Especially, as one of the most important group of natural antioxidants, they possess several common biological and chemical properties, namely, antioxidant activity, the ability to scavenge both active oxygen species and electrophiles, the ability to inhibit nitrosation and to chelate metal ions, and the capability to modulate certain cellular enzyme activities.[7] In our measurement for evaluating antioxidant effect of both compounds **1** and **2** using DPPH radical, they exhibited significantly high activities with EC_{50} value of 290 μg/ml and 26 μg/ml, respectively, compared with BHT (EC_{50}, 62 μg/ml) and α-tocopherol (EC_{50}, 8 μg/ml).

EXPERIMENTAL SECTION

General Experimental Procedures. Optical rotations were taken on a Jasco digital polarimeter. NMR spectra were recorded in CD_3OD on a Varian Unity 500 and Varian Mercury 300 spectrometers using stsandard pulse sequence programs. Mass spectral data were obtained by using a Jeol JMS-HX110 high resolution mass spectrometer at the Korean Basic Science Institute, Taejeon, Korea. UV spectra were measured on a Shimadzu UV1201 spectrophotometric instrument. High performance liquid chromatography (HPLC) was performed with a Dionex P580 with Varian 350 RI detector.

Extraction and Isolation. Dried aerial parts of *P. oleracea* were ground to a powder and repeatedly extracted for 2 days with MeOH and CH_2Cl_2, respectively. The combined crude extracts (29.8 g) were evaporated to dryness and partitioned between CH_2Cl_2 and water. The aqueous layer was further partitioned between n-BuOH and water to afford a n-BuOH fraction (12.121 g), which was subjected to C_{18} reversed-phase vacuum flash chromatography eluting with stepwise gradient mixtures of MeOH and water (50%, 40%, 30%, 20%, 10% aqueous MeOH, and 100% MeOH). The fraction (4.660 g) eluted with 50% aqueous MeOH was dried and the residue was separated on a HP 20 gel chromatography with the solvent system of 50% aqueous MeOH, 50% aqueous acetone, 100% MeOH, and 100% acetone as eluents. Further purification of 50% aqueous MeOH fraction (0.932 g) by semi-preparative C_{18} HPLC (YMC ODS-A column, 1 cm×25 cm, 90% aqueous MeOH) gave compounds **1** (17.8 mg) and **2** (20.8 mg).

3-Hydroxy-1-(2-hydroxyethyl)phenyl-4-O-β-D-glucopyranoside (1). a colorless gum; $[\alpha]_D^{25}$-24.6° (c 0.5, MeOH); UV (MeOH) λ_{max} (logε) 285 (3.49) nm; HRFABMS *m z* 339.1060 $(M+Na)^+$ (calcd for $C_{14}H_{20}O_8Na$, 339.1056); ^1H NMR (CD_3OD+C_6D_6= 2:1); 7.18 (1H, d, J=8.3, H-5), 6.83 (1H, d, J=2.0, H-2), 6.66 (1H, dd, J=8.3, J=2.0, H-6), 4.73 (1H, d, $J_{1',2'}$=7.8, H-1'), 3.93 (1H, dd, $J_{6',6}$=12.0, $J_{6',5'}$=2.4, H-6'), 3.78 (1H, dd, $J_{6,6'}$=12.0, $J_{6,5'}$=5.4, H-6'), 3.71 (2H, t, J=7.2, H-1), 3.60 (1H, dd, $J_{2',3'}$=9.3, $J_{1',2'}$=7.8, H-2'), 3.54 (1H, dd, $J_{2',3'}$=9.3, $J_{3',4'}$=8.3, H-3'), 3.52 (1H, d, $J_{4',5'}$=9.3, $J_{3',4'}$=8.3, H-4'), 3.38 (1H, m, H-5'), 3.71 (2H, t, J=7.2, H-1), 2.73 (2H, t, J=7.2, H-1); ^1H and ^{13}C NMR data for CD_3OD as a NMR solvent, see *Table* 1.

2-(3,4-Dihydroxyphenyl)ethyl-O-β-D-glucopyranoside (2). a colorless gum; $[\alpha]_D^{25}$-20.4° (c 0.6, MeOH); *m z* 339.1057 $(M+Na)^+$ (calcd for $C_{14}H_{20}O_8Na$, 339.1056); ^1H and ^{13}C NMR data for CD_3OD as a NMR solvent, see *Table* 1.

Table 1. NMR Spectral Data for Compounds 1 and 2[a]

No.	1			2		
	δ_H(H, m., Hz)	δ_C(lit.[b])	HMBC	δ_H(H, m., Hz)	δ_C(lit.)	HMBC
1		136.1 (137.9)			131.5 (131.6)	
2	6.72 (1H, d, 2.0)	117.6 (124.1)	C-3, C-4, C-6, C-1α	6.68 (1H, d, 1.0)	117.1 (116.4)	C-4, C-6, C-1α
3		148.3 (145.9)			146.1 (146.1)	
4		145.2 (148.2)			144.5 (144.7)	
5	7.09 (1H, d, 8.1)	119.1 (119.7)	C-3, C-4, C-1	6.66 (1H, d, 8.3)	116.3 (117.2)	C-3, C-1
6	6.64 (1H, dd, 8.1, 2.0)	121.3 (119.8)	C-2, C-4, C-1α	6.54 (1H, d, 1.0)	121.2 (121.3)	C-2, C-4, C-1α
1α	2.70 (2H, t, 7.1)	39.7 (40.0)	C-1, C-2, C-6, C-1β	2.77 (2H, m)	36.5 (36.6)	C-1, C-2, C-6, C-1β
1β	3.69 (2H, t, 7.1)	64.3 (65.3)	C-1, C-1α	4.01 (1H, m), 3.69 (1H, m)	72.1 (72.1)	C-1, C-1α
1′	4.69 (1H, d, 7.3)	104.7 (104.2)	C-4	4.28 (1H, d, 7.8)	104.3 (104.4)	C-1β
2′	3.47 (1H, dd, 8.8, 7.3)	74.9 (75.7)		3.18 (1H, dd, 8.8, 7.8)	75.1 (75.2)	C-1′, C-3′
3′	3.45 (1H, dd, 8.8, 8.3)	77.6 (78.9)		3.35 (1H, dd, 8.8, 8.3)	77.9 (78.0)	
4′	3.39 (1H, m)	71.3 (72.2)		3.28 (1H, dd, 8.8, 9.2)	71.6 (71.7)	
5′	3.38 (1H, m)	78.3 (78.8)		3.25 (1H, m)	78.0 (78.1)	
6′	3.88 (1H, dd, 12.0, 2.0),	62.4 (63.3)		3.85 (1H, dd, 11.5, 1.5),	62.7 (62.8)	
	3.71 (1H, dd, 12.0, 4.9)			3.66 (1H, dd, 11.5, 5.4)		

[a]^{1}H and ^{13}C NMR spectra were measured in CD_3OD at 500 MHz and 125 MHz, respectively. [b] ^{13}C NMR spectra were recorded in D_2O. Assignments were aided by ^{1}H COSY, HMQC, HMBC, and NOESY experiments.

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