A New Monoterpene Glucoside from *Portulaca oleracea*

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Three monoterpene glucosides (**1-3**), including one new compound (**3**), have been isolated from the methanol extract of *Portulaca oleracea*. Structures of these compounds were determined to be (3*S*)-3-O-(β-D-glucopyranosyl)-3,7-dimethylocta-1,6-dien-3-ol (**1**), (3*S*)-3-O-(β-D-glucopyranosyl)-3,7-dimethylocta-1,5-dien-3,7-diol (**2**) and (3*S*)-3-O-(β-D-glucopyranosyl)-3,7-dimethyl-7-hydroperoxyocta-1,5-dien-3-ol (**3**), respectively, by a combination of spectral analyses. Their stereochemistries were established by measurement of NOE and vicinal proton-proton coupling constants as well as comparisons of spectral data with those of previously related compounds.

Key Words : *Portulaca oleracea*, Portuloside B, Monoterpene glucosides, Linalool

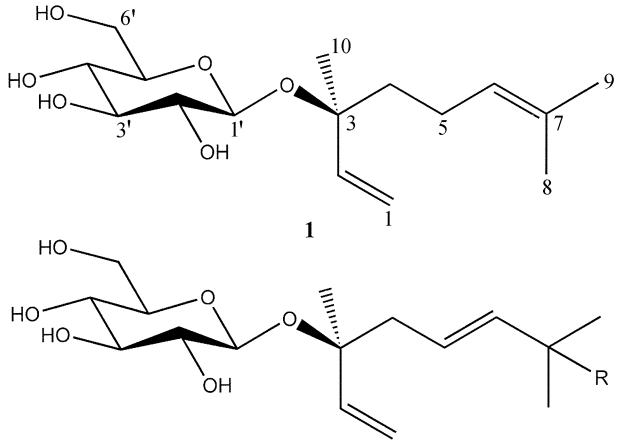
Introduction

Portulaca oleracea Linne, known as purslane, is a succulent herb which inhabits various parts of temperate regions. It has been not only used as vegetable[1] but also widely used in traditional folk medicine to treat sore nipples, gastric ulcers, tonsillitis, and so on.[2,3] A number of investigators have examined extracts of *P. oleracea* on diverse bioactivities such as hepatoprotective,[4] diuretic,[4] anti-inflammatory,[4,5] and antimicrobial[6] activities as well as anti-cancer[7] and anti-gastric ulcer.[8] Therefore, it is generally conceived to contain various bioactive compounds. During the course of our search for novel metabolites from herbal medicines, we recently reported the structures of two biophenolic glycosides from the purslane.[9] In addition to these compounds, ^{1}H NMR spectral analysis of moderately polar chromatographic fractions revealed the presence of structurally different group of metabolites. Herein we report the isolation and structural determination of compounds **1-3**, monoterpene glucosides.

Results and Discussion

The methanol extract of *P. oleracea* was partitioned between methylene chloride and water. The organic layer was re-partitioned between 15% aqueous MeOH and *n*-hexane. Purification of the aqueous MeOH fraction was done using C18 reversed-phase gravity column, followed by reversed-phase HPLC, resulting in the isolation of monoterpene glucosides **1-3**.

Compound **1** was isolated as a colorless gum which was analyzed for $C_{16}H_{28}O_6$ by a combination of HRFABMS and ^{13}C NMR spectrometry. In the ^{1}H NMR spectrum of **1**, a signal for an anomeric proton at δ 4.34 (1H, d, 7.7 Hz) was correlated with a carbon signal at δ 99.45 by an HMQC



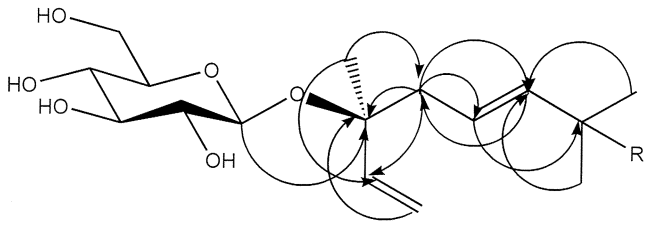
1

2: R=OH
3: R=OOH

Figure 1. The Structure of compounds **1-3**.

experiment, and were diagnostic for the presence of a sugar moiety, which was also supported by the presence of carbon signals in the region of δ 80-60 in the ^{13}C NMR spectrum.

The overall structure of **1** was determined by a combination of ^{1}H COSY, TOCSY, NOESY, HSQC, and HMBC experiments. The HMBC data were particularly helpful in determining both the partial structures and connectivities among them. The presence of a terminal trisubstituted double bond was revealed by the long-ranged coupling of the olefinic proton signal (δ 5.08, br t, 7.2) with those of vinyl methyl protons (δ 1.59, br s; 1.66, br s) in the ^{1}H COSY experiment. Also, the ^{1}H COSY and HSQC experiments showed the presence of a monosubstituted double bond and a CH_2CH_2 unit. Determination of connectivities between these partial structures as well as an spin-isolated methyl group (δ 1.34, 3H; 23.20 q) and a quaternary carbon



2: R=OH
3: R=OOH

Figure 2. Key HMBC correlation of compounds **2** and **3**.

(δ 81.37) signal by HMBC experiment defined the aglycon of **1** as linalool moiety.[10]

The planar structure of the sugar moiety was elucidated on the basis of ^{1}H COSY and HMBC experiments. Its stereochemistry was determined to be β-glucopyranose by measurement of vicinal proton-proton coupling constants ($J_{1',2'}$ = 7.7, $J_{2',3'}$ = 8.8, $J_{3',4'}$ = 8.3, $J_{4,5}$ = 8.3 Hz)[11] and NOE. The orientations of H-1' (δ 4.34), H-3' (δ 3.33), and H-5' (δ 3.17) were assigned axial, axial, and axial, respectively, by the mutual NOESY correlations among them. A linkage between the aglycon and sugar moiety was established by a HMBC correlation of the H-1' anomeric proton of glucopyranose at δ 4.34 with C-3 of linalool part at δ 81.37. With the aid of all this information, a literature survey revealed that **1** was identical to (3*S*)-3-O-(β-D-glucopyranosyl)-3,7-dimethylocta-1,6-dien-3-ol (linaloyl glucoside), previously isolated from *Pluchea indica* and *Cunila spicata*.[10,11] Comparison of spectral data showed very good correlation with published data for this compound.

A closely related metabolite, compound **2**, was isolated as a colorless gum which analyzed for $C_{16}H_{28}O_7$ by HRFABMS and ^{13}C NMR analyses. Spectral data for this compound were similar to those obtained for **1**. However, there are significant differences in the ^{13}C NMR spectrum. The most noticeable change is the replacement of the methylene carbon signal at δ 23.69 by an oxygen-bearing quaternary carbon signal at δ 71.27. Also, trisubstituted olefinic carbon signals (131.99 s and 125.55 d) are changed to disubstituted olefinic ones (142.20 d and 123.04 d). Corresponding differences were found in ^{1}H NMR spectrum in which two methylene protons at δ 1.58 (2H, t, 8.4) and 2.02 (2H, dt, 7.2, 8.4) merged into one methylene protons signal at δ 2.32 (2H, d, 6.0), and new downfield signals appeared at δ 5.66 (1H, dt, 15.4, 6.0) and 5.58 (1H, d, 15.4) instead of the olefinic proton at δ 5.08 (1H, br t, 7.2). In addition, two methyl signals at δ 1.59 (3H, s) and 1.66 (3H, s) were shifted downfield to δ 1.25 (6H, s). On the basis of the ^{1}H COSY and HSQC experiments, the locations of the double bond and hydroxyl group were assigned to C-5 and C-7, respectively, forming an en-ol functionality. This interpretation was further supported by HMBC data in which several two- and three-bond correlations were observed between the C-5 and C-6 olefinic carbons and neighboring protons. The geometry of the double bond was assigned as E by measuring a vicinal coupling constant (15.4 Hz) between the olefinic protons. Thus, the structure of **2** was determined as (3*S*)-3-O-(β-D-glucopyranosyl)-3,7-dimethylocta-1,5-dien-3,7-diol. Literature survey revealed that compound **2** has not appeared as a natural product. Indeed this compound has been reported as a synthetic product of reductive cleavage of (3*S*)-3-O-(3',4'-diangeloyl-β-D-glucopyranosyloxy)-7-hydroperoxy-3,7-dimethylocta -1,5-diene.[12]

Another related compound **3**, designated as portuloside B, was obtained as a colorless gum. HRFABMS and ^{13}C NMR

Table 1. Carbon NMR Assignments for Compounds **1**-**3**[a]

no	**1**		**2**		**3**	
	δ_H	δ_C	δ_H	δ_C	δ_H	δ_C
1	5.21 (1H, dd, 17.6, 1.1)	115.65 t	5.20 (1H, dd, 17.9, 1.1)	116.05 t	5.20 (1H, dd, 17.9, 1.4)	115.99 t
	5.19 (1H, dd, 10.8, 1.1)		5.19 (1H, dd, 10.7, 1.1)		5.19 (1H, dd, 10.7, 1.4)	
2	5.91 (1H, dd, 17.6, 10.8)	144.31 d	5.91 (1H, dd, 17.9, 10.7)	143.84 d	5.92 (1H, dd, 17.9, 10.7)	143.91 d
3		81.37 s		81.32 s		81.37 s
4	1.58 (2H, t, 8.4)[b]	42.66 t	2.32 (2H, d, 6.0)	45.34 t	2.35 (2H, d, 6.2)	45.50 t
5	2.02 (2H, dt, 7.2, 8.4)	23.69 t	5.66 (1H, dt, 15.4, 6.0)	123.04 d	5.67 (1H, dt, 16.0, 6.2)	126.68 d
6	5.08 (1H, br t, 7.2)	125.55 d	5.58 (1H, d, 15.4)	142.20 d	5.59 (1H, d, 16.0)	138.63 d
7		131.99 s		71.27 s		82.50 s
8	1.59 (3H, s)	16.11 q	1.25 (3H, s)	29.97 q	1.27 (3H, s)	23.41 q
9	1.66 (3H, s)	25.92 q	1.25 (3H, s)	29.97 q	1.27 (3H, s)	24.98[c] q
10	1.34 (3H, s)	23.20 q	1.34 (3H, s)	23.32 q	1.34 (3H, s)	24.93[c] q
1'	4.34 (1H, d, 7.7)	99.45 d	4.36 (1H, d, 7.7)	99.41 d	4.36 (1H, d, 7.7)	99.47 d
2'	3.15 (1H, dd, 8.3, 7.7)[b]	75.15 d	3.15 (1H, dd, 8.8, 7.7)[b]	75.10 d	3.15 (1H, dd, 8.7, 7.7)[b]	75.13 d
3'	3.33 (1H, dd, 8.8, 8.3)[b]	78.28 d	3.33 (1H, dd, 8.8, 8.3)[b]	78.08 d	3.33 (1H, dd, 8.7, 8.0)[b]	78.15 d
4'	3.27 (1H, dd, 8.3, 8.3)	71.69 d	3.27 (1H, dd, 8.3, 8.3)	71.64 d	3.26 (1H, dd, 8.0, 8.0)	71.68 d
5'	3.17 (1H, 1H, m)	77.55 d	3.16 (1H, 1H, m)	77.54 d	3.17 (1H, 1H, m)	77.60 d
6'	3.79 (1H, dd, 11.8, 2.5)	62.79 t	3.80 (1H, dd, 12.1, 2.5)	62.71 t	3.80 (1H, dd, 11.8, 2.2)	62.76 t
	3.62 (1H, dd, 11.8, 5.5)		3.62 (1H, dd, 12.1, 5.7)		3.62 (1H, dd, 11.8, 5.5)	

[a]H and ^{13}C NMR spectra were recorded in CD_3OD solution at 300 and 75 MHz, respectively. Assignments were based upon ^{1}H COSY, HSQC, and HMBC experiments. [b]Coupling constants were measured by 1D TOCSY experiment. [c]Exchangeable.

spectral data of this compound showed the molecular formula $C_{16}H_{28}O_8$. ^1H NMR spectral data for this compound were almost the same as those of **2**. The only significant difference in the ^{13}C NMR spectrum was the downfield shift of a carbon signal from δ 71.27 for **2** to δ 82.50. Its neighboring carbon signals also exhibited considerable changes compared with **2** ($\Delta\delta$ 3-5 ppm for C-5, -6, -8, and -9). These changes were accommodated by replacement of the hydroxy group with hydroperoxy one which naturally influences stronger electron-withdrawing effect than the former group. This interpretation was also supported by HMBC correlations between the carbon signal at δ 82.50 and its neighboring protons (H-5, -8, and -9). In addition, NMR spetral data for **3** were well-matched with those for 3',4'-diangeloate of **3**, previously isolated from *Aster scaber*.[12] Thus, the structure of portuloside B was confirmed as (3*S*)-3-O-(β-D-glucopyranosyl)-3,7-dimethyl-7-hydroperoxyocta-1,5-dien-3-ol.

Experimental Section

General Experimental Procedures. Optical rotations were obtained on a Jasco digital polarimeter. NMR spectra were recorded in CD_3OD on Varian Mercury 300 spectrometers using standard pulse sequence programs. Proton and carbon NMR spectra were measured at 300 and 75 MHz, respectively. Chemical shifts were recorded with respect to CD_3OD as an internal standard. Mass spectral data were obtained at the Korean Basic Science Institute, Taejeon, Korea. High performance liquid chromatography (HPLC) was performed with a Dionex P580 with a Varian 350 RI detector. All solvents used were spectral grade or were distilled from glass prior to use.

Extraction and Isolation. Dried samples (0.5 kg) of *P. oleracea* were ground to a powder and repeatedly extracted for 2 days with MeOH (3 L). The combined crude extracts (29.8 g) were evaporated to dryness and partitioned between CH_2Cl_2 and water. The organic layer was further partitioned between 15% aqueous MeOH and *n*-hexane to give a 15% aqueous MeOH fraction (12.1 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 (4.7 g) eluted with 30% aqueous MeOH was dried and the residue was separated with Si gel chromatography using a gradient solvent system of $CHCl_3$-MeOH. Further purification of 20% MeOH in chloroform fraction (0.9 g) by PTLC on a Si gel with MeOH/$CHCl_3$ (1 : 20) as a solvent system followed by semi-preparative C_{18} HPLC (YMC ODS-A column, 1 cm × 25 cm, 90% aqueous MeOH) gave compounds **1** (10.8 mg), **2** (3.2 mg), and **3** (2.1 mg).

(3*S*)-3-O-(β-D-Glucopyranosyl)-3,7-dimethylocta-1,5-dien-3-ol (1): $[\alpha]_D^{25}$ -12.5° (c 1.1, $CHCl_3$) (lit.[10] $[\alpha]_D^{24}$ -23.4); FABMS *m/z* 339.1782 $(M+Na)^-$ (calcd for $C_{16}H_{28}O_8Na$, 339.1784); HMBC correlations H-1/C-2, C-3; H-4/C-2, C-3, C-5; H-5/C-6, C-7; H-8/C-6, C-7, C-9; H-9/C-6, C-7, C-8; H-10/C-2, C-3, C-5; H-1'/C-3; ^1H and ^{13}C NMR spectral data, see Table 1.

(3*S*)-3-O-(β-D-Glucopyranosyl)-3,7-dimethylocta-1,5-dien-3,7-diol (2): a colorless gum; $[\alpha]_D^{25}$ -12.4° (c 0.3, MeOH); HRFABMS *m/z* 355.1731 $(M+Na)^-$ (calcd for $C_{16}H_{28}O_7Na$, 355.1733); HMBC correlations H-1/C-2, C-3; H-4/C-2, C-3, C-5; H-5/C-7; H-6/C-4; H-8/C-6, C-7; H-9/C-6, C-7; H-10/C-2, C-3, C-4; H-1'/C-3; ^1H and ^{13}C NMR spectral data, see Table 1.

Portuloside B, (3*S*)-3-O-(β-D-Glucopyranosyl)-3,7-dimethyl-7-hydroperoxyocta-1,5-dien-3-ol (3): a colorless gum; $[\alpha]_D^{25}$ -3.1° (c 0.2, MeOH); HRFABMS *m/z* 371.1681 $(M+Na)^-$ (calcd for $C_{16}H_{28}O_8Na$, 371.1682); HMBC correlations H-1/C-2, C-3; H-4/C-2, C-3, C-5; H-5/C-7; H-6/C-4; H-8/C-6, C-7; H-9/C-6, C-7; H-10/C-2, C-3, C-4; H-1'/C-3; ^1H and ^{13}C NMR spectral data, see Table 1.

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