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Original Article

A new feruloyl glyceride from the roots of Asian rice (*Oryza sativa*)

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ABSTRACT

Oryza sativa L., Poaceae, is the most important staple food in the world and provides food for more than half of the world's population. The roots of *O. sativa* have been used as a traditional medicine in Korea. As part of our continuing efforts to explore structurally new compounds from Korean natural resources, two feruloyl glycerides, 2-*O*-(*E*)-feruloyl glyceride (**1**) and 2-*O*-(*Z*)-feruloyl glyceride (**2**), which is a new compound, together with one known flavonoid, 8-hydroxyacacetin (**3**), were isolated from the ethanolic (EtOH) extract of the roots of *O. sativa* using an LC/MS-guided isolation method. The chemical structure of compound **2** was elucidated based on comprehensive 1D and 2D NMR spectroscopic experiments and HR-ESIMS. This study represents the first report of feruloyl glycerides (**1–2**) identified in *O. sativa*. In addition, the identification of compound **3** is reported from Asian rice (*O. sativa*) for the first time. The cytotoxic activities of the isolates **1–3** were evaluated by determining their inhibitory effects on A2780 human ovarian carcinoma cells.

Introduction

Oryza sativa L., Poaceae, is the most important staple food in the world and provides food for more than half of the world's population (Khush, 2005). Its roots, which are called "Na-do-guen" in Korea, have been used as a traditional medicine in Korea and China to improve digestion, promote the production of body fluid, reduce fever, stop cold sweats, and treat diabetes mellitus (Hikino et al., 1986). A variety of therapeutic activities including anti-tumor (Kim et al., 2007), anti-fungal (Koga et al., 1997; Peters, 2006), anti-viral (Peters, 2006), anti-microbial (Prisic et al., 2004), and anti-melanogenic effects (Cho et al., 2015) have been reported for the extracts of *O. sativa*. Additionally, recent studies have examined the pharmacological activities of the roots of *O. sativa* such as skin-whitening (Cho et al., 2015), hypoglycemic (Hikino et al., 1986), and laxative activities (Sangle et al., 2016). Previous phytochemical investigations of this plant have shown the presence of a variety of chemical constituents including glycans (Hikino et al., 1986), phytosterols (Park et al., 2017), polysaccharides (Park et al., 2017), diterpenoids (Koga et al., 1997; Kato et al., 2002; Peters, 2006), gibberellins (Prisic et al., 2004; Peters, 2006), and phenolic compounds (Cho et al., 2015).

Despite several trials investigating the chemical components of *O. sativa*, there have been few reports on the chemical constituents present in the roots. As part of our continuing efforts to explore structurally new compounds from Korean natural resources (Eom et al., 2016; Lee et al., 2016, 2017a,b; Yu et al., 2016a,b, 2017; Kang et al., 2016a,b; Eom et al., 2017; Beemelmanns et al., 2017), we focused on the roots of Asian rice (*O. sativa*), which has been relatively neglected in the phytochemical research field, and investigated the chemical constituents from the EtOH extract of the roots. In the present study, our LC/MS analysis of the EtOH extract of *O. sativa* roots revealed that the EtOH extract contains 2-*O*-feruloyl glyceride, which had not been reported from *O. sativa*. A liquid chromatography (LC)/mass spectrometry (MS) guided isolation technique was applied for the separation of the target constituents to effectively identify potential new compounds. As a result, two feruloyl glycerides (**1–2**) including a new compound, 2-*O*-(*Z*)-feruloyl glyceride (**2**), together with one known flavonoid (**3**) were isolated from the EtOH extract. In the present study, we report the LC/MS-guided isolation of compounds **1–3** and their structural elucidation, along with their cytotoxic effects on a human ovarian carcinoma line.

Materials and methods

General experimental procedures

IR spectra were recorded with a Bruker IFS-66/S FT-IR spectrometer (Bruker, Karlsruhe, Germany). UV spectra were acquired on an Agilent 8453 UV-visible spectrophotometer (Agilent Technologies, Santa Clara, CA, USA). ESI and HR-ESI mass spectra were

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recorded using a Waters Micromass Q-Tof Ultima ESI-TOF mass spectrometer (Waters, New York, NY, USA). NMR spectra, including those from ^{1}H–^{1}H COSY, HSQC, and HMBC, were recorded with a Bruker AVANCE III 700 NMR spectrometer operating at 700 MHz (^{1}H) and 175 MHz (^{13}C) (Bruker, Karlsruhe, Germany), with chemical shifts given in ppm (δ) for ^{1}H and ^{13}C NMR analyses. Semi-preparative HPLC was performed using a Shimadzu Prominence HPLC System with SPD-20A/20AV Series Prominence HPLC UV-Vis detectors (Shimadzu, Tokyo, Japan). LC/MS analysis was performed on an Agilent 1200 Series HPLC system equipped with a diode array detector and 6130 Series ESI mass spectrometer using an analytical Kinetex C18 100 Å column (100 × 2.1 mm i.d., 5 μm; Phenomenex, Torrance, CA, USA). Silica gel 60 (70–230 mesh and 230–400 mesh; Merck, Darmstadt, Germany) and RP-C_{18} silica gel (Merck, 40–63 μm) were used for column chromatography. The packing material for molecular sieve column chromatography was Sephadex LH-20 (Pharmacia, Uppsala, Sweden). Merck precoated silica gel F_{254} plates and RP-18 F_{254s} plates were used for thin-layer chromatography (TLC). Spots were detected after TLC under UV light or by heating after spraying with anisaldehyde-sulfuric acid.

Plant material

The roots of *O. sativa* L., Poaceae, were purchased at Kyungdong Market in Seoul, Korea, in October 2013, and the identity of the material was verified by one of the authors (K.H.K.). A voucher specimen (SKK-BBR-2014) was deposited in the herbarium of the School of Pharmacy, Sungkyunkwan University, Suwon, Korea.

Extraction and isolation

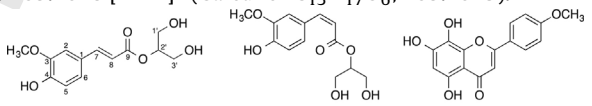
O. sativa roots (500 g) were air-dried and extracted three times with 95% aqueous EtOH at 60 °C for 24 h and then filtered using Whatman filter paper No. 2 (pore size: 8 μm). After evaporation of the filtrate in a laboratory freeze-dryer, 53 g of the resultant dried extract was obtained. The dried EtOH extract powder was dissolved in sterile distilled water, and a small aliquot of the EtOH extract was sequentially injected into LC/MS eluted with a gradient solvent system of MeOH/H_2O (1:9–1:0, flow rate of 0.3 ml/min, UV 254 nm), which revealed the presence of feruloyl glyceride with a molecular ion peak at *m/z* 269 $[M+H]^+$ in positive ESI mode by comparison with our house-built UV library in LC/MS. The EtOH extract in distilled water was solvent-partitioned with hexanes, dichloromethane (DCM), ethyl acetate (EtOAc), *n*-butanol (BuOH), and water (residue). Five fractions with increasing polarity, the hexane-soluble fraction (1.32 g), DCM-soluble faction (3.20 g), EtOAc-soluble fraction (0.41 g), *n*-BuOH-soluble fraction (4.15 g), and water residue, were obtained. All five fractions were subjected to LC/MS and eluted with a gradient solvent system of MeOH/H_2O (1:9–1:0, flow rate of 0.3 ml/min, UV 254 nm) to identify the target constituent, feruloyl glyceride. Based on the LC/MS data, the DCM-soluble fraction containing the target constituent was then separated by silica gel column chromatography (200 g, 3 × 100 cm) into nine fractions (D1-D9) according to the solvent mixture ratio of chloroform/methanol [200:1 (D1), 100:1 (D2), 50:1 (D3), 20:1 (D4), 10:1 (D5), 5:1 (D6), 2:1 (D7), 1:1 (D8), and 0:1 (D9)]. All nine fractions were subjected to LC/MS prior to purification for the target isolation of feruloyl glyceride, which revealed that fraction D3 contained the target constituent. Fraction D3 (350 mg) was further separated into nine fractions (D31–D39) using a Sephadex LH-20 column with 100% MeOH. LC/MS analysis of the nine subfractions indicated the presence of feruloyl glyceride in subfraction D37 (37 mg), which was separated utilizing semi-preparative reversed-phase HPLC with an isocratic solvent system of aqueous 60% MeOH (Phenomenex Luna Phenyl-hexyl column, 250 mm × 10 mm i.d., 10 μm) with a flow rate of 2 ml/min and UV 254 nm to isolate the two feruloyl glycerides, compound (**1**) (1.6 mg, t_R = 32.5 min) and compound (**2**) (0.8 mg, t_R = 34.1 min), together with compound (**3**) (0.8 mg, t_R = 39.5 min).

Table 1
^{1}H (700 MHz) and ^{13}C NMR (175 MHz) spectral data of **1–2** in CD_3OD (δ in ppm).

Position	1		2	
	δ_H	δ_C	δ_H	δ_C
1		127.1		126.8
2	7.16 d (2.0)	110.3	7.83 d (2.0)	114.5
3		148.5		142.2
4		147.3		148.9
5	6.78 d (8.0)	115.7	6.76 d (8.0)	115.2
6	7.06 dd (8.0, 2.0)	123.2	7.08 dd (8.0, 2.0)	124.8
7	7.68 d (16.0)	145.6	6.89 d (12.5)	146.1
8	6.36 d (16.0)	115.0	5.82 d (12.5)	118.6
9		167.8		168.0
1′	3.90 m; 3.86 m	62.3	3.90 m; 3.86 m	62.4
2′	4.29 dd (5.5, 1.5)	75.2	4.29 dd (5.5, 1.5)	75.0
3′	3.90 m; 3.86 m	62.3	3.90 m; 3.86 m	62.4
OCH_3	3.86 s	56.0	3.85 s	56.0

Assignments were based on 2D NMR including COSY, HSQC, and HMBC. Well-resolved couplings are expressed with coupling patterns and coupling constants in Hz in parentheses.

2-*O*-(*Z*)-Feruloyl glyceride (**2**): Colorless gum; UV (MeOH) λ_{max} (log ε) 248 (2.8) 300 (2.7) 328 (3.2) nm; IR (KBr) ν_{max}: 3350, 2942, 2834, 1718, 1452, 1029, 670 cm^{-1}. ^{1}H (700 MHz) and ^{13}C (175 MHz) NMR data, see Table 1; HR-ESIMS (positive-ion mode) *m/z*: 269.1028 $[M+H]^+$ (Calcd for $C_{13}H_{17}O_6$, 269.1025).



Cytotoxicity assay

The A2780 human ovarian carcinoma cell line was purchased from the American Type Culture Collection (ATCC, Manassas, VA, USA) and maintained in Roswell Park Memorial Institute 1640 medium (RPMI 1640) (Cellgro, Manassas, VA, USA) supplemented with 10% fetal bovine serum (Gibco BRL, Carlsbad, CA, USA), 100 units/ml penicillin, and 100 mg/ml streptomycin with incubation at 37 °C in a humidified atmosphere with 5% CO_2. The A2780 cells were seeded at 1×10^4 cells/100 ml in 96-well plates. After incubation for 24 h, the cells were incubated in cell culture medium with or without test samples for an additional 24 h. Cell viability was determined using the MTT cell proliferation assay.

Results and discussion

The dried roots of *O. sativa* were extracted with 95% EtOH and then filtered. After evaporation of the filtrate in a laboratory freeze-dryer, we obtained the resultant dried EtOH extract powder. LC/MS analysis of the extract deduced the presence of feruloyl glyceride with a molecular ion peak at *m/z* 269 $[M+H]^+$ in positive electrospray ionization (ESI) mode, by comparison with our house-built UV library in LC/MS. The major fragment at *m/z* 177 in $ESIMS^2$ indicated $[C_{10}H_9O_3]^+$, and another stable fragment at *m/z* 75 indicated $[C_3H_7O_2]^+$. Based on the molecular weight, fragmentation pattern, and UV absorption, the compound was tentatively identified as feruloyl glyceride. Since feruloyl glyceride has not been previously reported from *O. sativa*, LC/MS-guided isolation was carried out for the target separation of feruloyl glyceride. The high sensitivity and selectivity of the LC/MS-guided isolation method selectively reduced the analysis time and consequently enabled fast isolation of target compounds (Wang et al., 2016; Nørskov

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