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RESEARCH ARTICLE

A new flavonol glycoside from the aerial part of *Rudbeckia laciniata*

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Abstract The phytochemical investigation of *Rudbeckia laciniata* L. obtained a new flavonol glycoside (**1**), together with four flavonol glycosides (**2**–**5**) and eight quinic acid derivatives (**6**–**13**). The structure was elucidation by means of spectroscopic methods and chemical evidence. The isolated compounds were tested for cytotoxicity against four human tumor cell lines in vitro using the sulforhodamine B bioassay.

Keywords *Rudbeckia laciniata* · Asteraceae · Flavonol glycoside · Cytotoxicity

Introduction

Rudbeckia laciniata L. is a large perennial herb that is widely distributed in the mountainous regions of Korea, Japan, and China (Lee 2003). Previous phytochemical investigation on this herb reported the isolation of sesquiterpenes (Fukushi et al. 1994; Fukushi et al. 1998; Bohlmann et al. 1978; Jakupovic et al. 1986) and triterpenes (Bohlmann et al. 1978). The extract of this plant was reported to exhibit antioxidant, antibacterial, antiviral and antifungal activities and is used to treat the common cold, as well as respiratory and urinary diseases (Barrett 2003).

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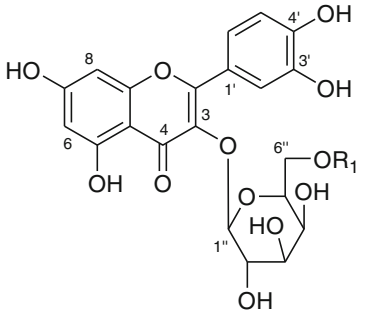
As part of our continuing search for biologically active compounds from medicinal plants, we investigated the constituents of the aerial parts of *R. laciniata* and have reported cytotoxic constituents including lignans (Lee et al. 2013). In continuing research on this source, we performed a phytochemical investigation on the aerial parts of *R. laciniata*. Column chromatography separation of constituents in the MeOH extract of *R. laciniata* resulted in the isolation of a new flavonol glycoside (**1**), together with twelve known compounds (**2**–**13**) (Fig. 1). Their structures were determined by spectroscopic methods. The isolated compounds were tested for cytotoxicity against four human tumor cells in vitro using the sulforhodamine B (SRB) bioassay.

Materials and methods

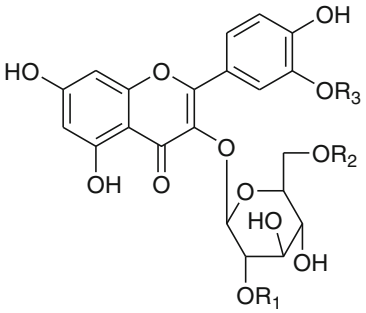
General experimental procedure

IR spectra were recorded on a Bruker IFS-66/S FT-IR spectrometer. UV spectra were recorded with a Shimadzu UV-1601 UV–Visible spectrophotometer. NMR spectra were recorded on a Varian UNITY INOVA 500 NMR spectrometer. FAB and HR-FAB mass spectra were obtained on a JEOL JMS700 mass spectrometer. Preparative HPLC was performed using a Gilson 306 pump with a Shodex refractive index detector and an Apollo Silica 5 μ column (250 × 22 mm) or an Econosil® RP-18 10 μ column (250 × 22 mm). Silica gel 60 (Merck, 70–230 and 230–400 mesh) was used for column chromatography. TLC was performed using Merck precoated Silica gel F_{254} plates and RP-18 F_{254s} plates. The packing material for molecular sieve column chromatography was Sephadex LH-20 (Pharmacia Co.).

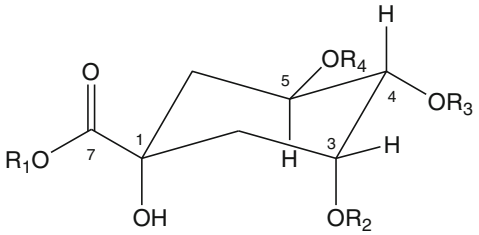
Fig. 1 Structures of the compounds **1–13**



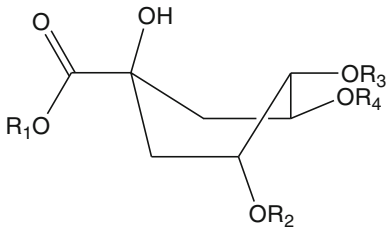
1 R = α-L-Ala (f)
2 R = α-L-Ala (p)



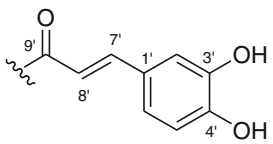
3 R_1 = β-D-Xyl, R_2 = R_3 = H
4 R_1 = R_2 = R_3 = H
5 R_1 = R_2 = H, R_3 = CH_3



	R_1	R_2	R_3	R_4
6	CH_3	Caffeoyl	H	Caffeoyl
7	H	Caffeoyl	H	Caffeoyl
8	CH_3	H	Caffeoyl	Caffeoyl
9	CH_3	Caffeoyl	Caffeoyl	H
10	H	Caffeoyl	Caffeoyl	H
11	CH_3	H	H	Caffeoyl
12	H	Caffeoyl	H	H



	R_1	R_2	R_3	R_4
13	H	Caffeoyl	H	Caffeoyl



Caffeoyl

Plant materials

The aerial parts of *R. laciniata* L. (7.0 kg) were collected on Taebaek mountain in Gangwon-Do province, Korea in May 2009, and the plant was identified by one of the authors (K. R. Lee). A voucher specimen of the plant (SKK-09-06) was deposited at the School of Pharmacy in Sungkyunkwan University.

Extraction and isolation

The half dried aerial parts of *R. laciniata* (7.0 kg) were extracted at room temperature with 80 % MeOH and evaporated under reduced pressure to give a residue (240 g), that was dissolved in water (800 ml three times) and then partitioned into a series of other solvents. These solvents, and the residues found in each were as follows; *n*-hexane (37 g), $CHCl_3$ (1 g), EtOAc (5 g), and *n*-BuOH (30 g). The EtOAc fraction (5 g) was subjected to Sephadex LH-20 column chromatography (CH_2Cl_2:MeOH = 1:1) to give four fractions (E1–E4). Fraction E2 was separated over a silica gel column with a solvent system of ($CHCl_3$:MeOH = 20:1–$CHCl_3$:MeOH = 1:1) to give four subfractions (E21–E24). Subfraction E22 (100 mg) was purified with by RP-C_{18} prep HPLC (50 % MeOH) to yield compounds **9** (20 mg, R_t = 15 min) and **11** (20 mg, R_t = 17 min). Subfraction E24 (150 mg) was purified with by RP-C_{18} prep HPLC (50 % MeOH) to yield compound **5** (35 mg, R_t = 17 min). Fraction E3 was separated over a silica gel column with a solvent system of ($CHCl_3$:MeOH = 25:1–$CHCl_3$:MeOH = 1:1) to give seven subfractions (E31–E37). Subfraction E31 (65 mg) was purified with by RP-C_{18} prep HPLC (55 % MeOH) to yield compounds **1** (5 mg, R_t = 13 min), **2** (4 mg, R_t = 17 min) and **3** (7 mg, R_t = 21 min). Subfraction E36

(150 mg) was purified with by RP-C_{18} prep HPLC (55 % MeOH) to yield compounds **6** (30 mg, R_t = 18 min), **8** (20 mg, R_t = 21 min) and **12** (30 mg, R_t = 25 min). Sub-fraction E37 (100 mg) was purified with by RP-C_{18} prep HPLC (50 % MeOH) to yield compounds **10** (20 mg, R_t = 15 min) and **13** (45 mg, R_t = 18 min). Fraction E4 was purified with by RP-C_{18} prep HPLC (50 % MeOH) to yield compounds **4** (15 mg, R_t = 18 min) and **7** (45 mg, R_t = 22 min).

Quercetin 3-*O*-α-L-arabinofuranosyl-(1‴ → 6″)-β-D-galactopyranoside (**1**)

Yellow gum, IR (KBr) ν_{max} cm^{-1}: 3,401 (OH), 1,650 (C=C); UV (MeOH) nm (log ε): 260 (4.50), 361 (4.45); HR-FAB-MS *m/z* : 619.1274 $[M+Na]^+$ (calcd. 619.1275); ^{1}H-NMR (500 MHz, DMSO-d_6) and ^{3}C-NMR (125 MHz, DMSO-d_6) : see Table 1.

Acid hydrolysis of compound **1**

Compound **1** (1.2 mg) was heated in an ampoule with 2 ml of 1 N HCl(aq) at 90 °C for 1 h. After cooling, the reaction mixture was extracted with EtOAc. The EtOAc solvent was removed under reduced pressure and the residue was purified by HPLC [MeOH-H_2O (50:50, v/v)] to give **1a**. The structure was identified by ^{1}H-NMR and MS spectral analysis. The H_2O layer was subjected to co-TLC analysis ($CHCl_3$:MeOH:H_2O = 6:4:1), and two sugars were identified after comparison with an authentic sample.

Compound **1a**: yellow gum; FAB-MS *m/z* : 303 $[M+H]^+$; ^{1}H-NMR (500 MHz, CD_3OD): δ 7.68 (1H, dd, *J* = 8.5, 2.0, H-2′), 7.55 (1H, d, *J* = 2.0, H-6′), 6.90 (1H, d, *J* = 8.5, H-5′), 6.42 (1H, s, H-8), 6.20 (1H, s, H-6).

Test for cytotoxicity in vitro

SRB bioassays were used as cytotoxicity screening methods (Skehan et al. 1990). Cytotoxicity assays for each compound were performed in vitro against four cultured human tumor cell lines at the Korea Research Institute of Chemical Technology. The cell lines used were A549 (non-small cell lung adenocarcinoma), SK-OV-3 (ovarian cancer cells), SK-MEL-2 (skin melanoma), and HCT15 (colon cancer cells). Doxorubicin was used as the positive control.

Results and discussion

The structures of the known compounds were identified to be quercetin 3-*O*-α-L-arabinopyranosyl-(1‴ → 6″)-β-D-galactopyranoside (**2**) (Takemura et al. 2002), quercetin 3-*O*-β-D-xylopyranosyl-(1‴ → 2″)-β-D-glucopyranoside (**3**) (Yeom et al. 2003), quercetin 3-*O*-β-D-glucopyranoside (**4**) (Kim 2001), isorhamnetin 3-*O*-β-D-glucopyranoside (**5**) (Ahn et al. 2007), 3,5-*O*-*trans*-dicaffeoylquinic acid methyl ester (**6**) (Kim et al. 2007), 3,5-*O*-*trans*-dicaffeoylquinic acid (**7**) (Kim et al. 2007), 4,5-*O*-*trans*-dicaffeoylquinic acid methyl ester (**8**) (Basnet et al. 1996), 3,4-*O*-*trans*-caffeoylquinic acid methyl ester (**9**) (Basnet et al. 1996), 3,4-*O*-*trans*-caffeoylquinic acid (**10**) (Kim et al. 2007), 5-*O*-*trans*-caffeoylquinic acid methyl ester (**11**) (Kim et al. 2007), 3-*O*-*trans*-caffeoylquinic acid (**12**) (Kim et al. 2007), and 3,5-*O*-*trans*-dicaffeoylepiquinic acid (**13**) (Kim et al. 2007) by comparing the spectroscopic data with those in the literature.

Compound **1** was isolated as a yellow gum. The molecular formula $C_{26}H_{28}O_{16}$ was determined by the HR-FAB-MS *m/z* 619.1274 $[M+Na]^+$ (calcd. 619.1275). The

Table 1 ^{1}H- (500 MHz) and ^{13}C-NMR (125 MHz) spectral data of **1** and **2** in DMSO-d_6 (δ in ppm)

Position	**1**		**2**	
	δ_H	δ_C	δ_H	δ_C
2		157.1		157.0
3		134.2		134.2
4		178.1		178.1
5	6.17 (s)	161.9	6.17 (s)	161.9
6		99.4		99.4
7		164.9		164.9
8	6.37 (s)	94.2	6.37 (s)	94.2
9		157.0		157.0
10		104.6		104.6
5-OH	12.57		12.60	
1′		121.8		121.7
2′	7.50 (d, 2.0)	115.9	7.57 (d, 2.0)	115.9
3′		145.5		145.5
4′		149.2		149.2
5′	6.80 (d, 8.5)	116.7	6.86 (d, 8.5)	116.6
6′	7.64 (dd, 8.5, 2.0)	122.7	7.67 (dd, 8.5, 2.0)	122.7
1″	5.31 (d, 7.5)	102.6	5.30 (d, 7.5)	102.5
2″		71.8		71.7
3″		73.7		73.7
4″		68.7		68.9
5″		74.4		75.0
6″		66.3		67.2
1‴	4.65 (s)	108.5	3.99 (d, 6.5)	103.3
2‴		82.5		71.1
3‴		77.7		73.1
4‴		84.5		68.0
5‴		61.9		65.6

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

IR spectrum showed the bands of a hydroxyl group at 3,384 cm^{-1} and an α,β-unsaturated carbonyl group at 1,650 cm^{-1}. UV spectrum absorption maxima at 260 and 361 nm are characteristic of a flavonol group (Mabry et al. 1970). The ^{1}H-NMR spectra of **1** showed three aromatic protons at δ_H 7.64 (dd, $J = 8.5$, 2.0 Hz), 7.50 (d, $J = 2.0$ Hz), and 6.80 (d, $J = 8.5$ Hz) of the B ring and two *meta*-coupled signals at δ_H 6.37 (s) and 6.17 (s) for the A ring. In the ^{13}C-NMR spectrum, 15 carbon signals of an aglycone appeared at δ_C 149.2 (C-4′), 145.5 (C-3′), 122.7 (C-6′), 121.8 (C-1′), 116.7 (C-5′) and 115.9 (C-2′) of the B ring, as well as other signals at δ 178.1 (C-4), 164.9 (C-7), 161.9 (C-5), 157.1 (C-2), 157.0 (C-9), 134.2 (C-3), 104.6 (C-10), 99.4 (C-6), and 94.2 (C-8) of the A and C rings (Table 1). These spectral data suggested that **1** was a quercetin derivative (Young et al. 1991). The NMR spectra data of **1** were very similar to those of **2**. The major difference was the terminal sugar unit; signals from the sugar unit appeared at δ_H 4.65 (1H, s), and 3.80–3.20 (5H, m) in the ^{1}H-NMR spectrum and δ_C = 108.5, 84.5, 82.5, 77.7, and 61.9 in the ^{13}C-NMR spectrum, indicating that **1** had an L-arabinofuranose moiety instead of the L-arabinopyranose moiety of **2** (Takemura et al. 2002; Latza et al. 1996; Zahid et al. 2002). The small coupling constant of the anomeric proton of L-arabinofuranose indicated that it was in the α-form (Latza et al. 1996; Zahid et al. 2002). The α-L-arabinofuranose moiety was identified by comparisons of the spectroscopic data with literature values (Latza et al. 1996; Zahid et al. 2002). The L-arabinofuranose position was established by a HMBC experiment, in which long-range correlations were observed between the H-1‴ (δ 4.65) of L-arabinofuranose and C-6″ (66.3) of D-galatopyranose as shown in Fig. 2 (Nunes et al. 2008). Acid hydrolysis of **1** yielded the aglycone, quercetin (Takemura et al. 2002) and two sugars, L-arabinofuranose and D-galatopyranose (Nunes et al. 2008; Latza et al. 1996; Zahid et al. 2002). The aglycone was confirmed by comparison of its ^{1}H-NMR, and FAB-MS data with literature values (Young et al. 1991), and the sugar was identified by co-TLC with authentic sugars ($CHCl_3$:MeOH:H_2O = 6:4:1, R_f value D-galatose 0.20, L-arabinose 0.50) (Latza et al. 1996; Zahid et al. 2002). Thus, the structure of **1** was determined to be quercetin 3-*O*-α-L-arabinofuranosyl-(1‴ → 6″)-β-D-galactopyranoside.

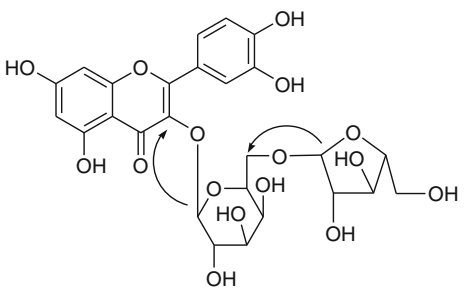


Fig. 2 Key HMBC correlations (H → C) of **1**

The isolated 13 compounds were tested for cytotoxicity against four human tumor cells in vitro using the SRB assay. The results of the bioassay showed that compounds **7**, **8**, **10**, and **13** exhibited weak cytotoxicity against the SK-MEL-2 cell line with IC_{50} values in the range of 16.08–23.71 μM. However, the other compounds showed little cytotoxicity ($IC_{50} > 30$ μM). IC_{50} values for the cytotoxicity of the control compound, doxorubicin, against A549, SK-OV-3, SK-MEL-2, and HCT15 cell lines were 0.074, 0.029, 0.016, and 0.6414 μM, respectively.

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