Three New Flavonol Glycosides from the Aerial Parts of *Rodgersia podophylla*

Young-Won CHIN and Jinwoong KIM*

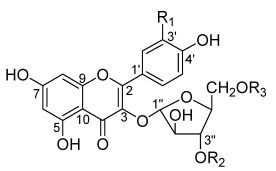
College of Pharmacy and Research Institute of Pharmaceutical Science, Seoul National University; Seoul 151–742, Korea.
Received August 4, 2005; accepted October 25, 2005

Three new flavonol glycosides were isolated together with five known flavonoids and six bergenin derivatives from the aerial parts of *Rodgersia podophylla*. The structures of these new compounds were elucidated by spectral methods as kaempferol-3-*O*-α-L-5″-acetyl-arabinofuranoside (1), kaempferol 3-*O*-α-L-3″-acetyl-arabinofuranoside (2), and quercetin-3-*O*-α-L-3″,5″-diacetyl-arabinofuranoside (3).

Key words *Rodgersia podophylla*; flavonoid; bergenin derivative; kaempferol-3-*O*-α-L-5″-acetyl-arabinofuranoside; kaempferol 3-*O*-α-L-3″-acetyl-arabinofuranoside; quercetin-3-*O*-α-L-3″,5″-diacetyl-arabinofuranoside

Rodgersia podophylla (Saxifragaceae) is a medicinal plant, the rhizomes of which have been used for the treatment of enteritis and bacillary dysentery in China and Korea.[1)] Several flavonol glycosides, lignans, monoterpenes, tannins, and triterpenenes have been isolated as chemical constituents of this plant.[1—4)] A previous bioactivity-guided investigation for the aerial parts of this plant demonstrated that flavonol glycosides possess hepatoprotective activity in primary cultures of rat hepatocytes injured by H_2O_2.[1)] Thus, the aerial parts of *R. podophylla* were recollected and reinvestigated to search for other active compounds. From EtOAc- and *n*-BuOH-soluble extracts, three new acetylated flavonol glycosides (**1**—**3**), along with eleven known compounds, were isolated and characterized.

Compound **1** was obtained as a dark yellow powder and gave a quasimolecular ion $[M+H]^+$ at *m*/*z* 461.1091 in high resolution fast atom bombardment mass spectroscopy (HR-FAB-MS), consistent with an elemental formula of $C_{22}H_{20}O_{11}$. The ^{1}H-NMR spectrum of **1** showed two broad singlet proton signals at δ 6.15 (1H, br s, H-6) and 6.38 (1H, br s, H-8), and two doublet proton signals at δ 6.88 (2H, d, J=8.8 Hz, H-3′, 5′) and 8.05 (2H, d, J=8.8 Hz, H-2′, 6′), assignable to a kaempferol moiety. An anomeric proton appeared at δ 5.51 (1H, br s, H-1″) along with other glycosidic protons in the range of δ 3.61 to 4.15. In addition to these proton signals, a methyl proton signal was observed at δ 1.90 due to the presence of an acetyl group. These data suggested that compound **1** contained an acetylated sugar. By comparison of ^{1}H- and ^{13}C-NMR data of a sugar in **1** with those in the literature,[1)] the sugar of **1** proved to be 5″-acetyl-arabinofuranoside and the position of an acetyl group was confirmed by the HMBC correlations from H_2-5″ (δ 3.78, 4.02) to a carbonyl group (δ 169.4). The position of sugar attachment to the aglycone was found to be the C-3 position through an ether linkage based on the observed ^3J correlations between H-1″ (δ 5.51) and C-3 (δ 133.2) in the heteronuclear multiple-bond correlation spectroscopy (HMBC) data. The stereochemistry of the sugar in **1** was determined to be L configuration by GC analysis of acid hydrolysate. Therefore, the structure of compound **1** was elucidated as kaempferol-3-*O*-α-L-5″-acetyl-arabinofuranoside.



Compound	R_1	R_2	R_3
1	H	H	Ac
2	H	Ac	H
3	OH	Ac	Ac

Fig. 1. The Structures of Compounds **1**—**3**

A molecular formula of $C_{22}H_{20}O_{11}$ of compound **2** was deduced from its HR-FAB-MS (Found at *m*/*z* 461.1081 $[M+H]^+$, Calcd for $C_{22}H_{21}O_{11}$ 461.1084). The ^{1}H- and ^{13}C-NMR spectral data of **2** were similar to those of **1**, and permitted the assignment of the aglycone and sugar moiety in **2** except for the position of an acetyl group in the sugar. The signal of C-2″ appeared to move upfield and that of C-3″ was observed in the downfield region when compared to those of **1** in the ^{13}C-NMR spectrum. This observation suggested the location of an acetyl group on the C-3″ of sugar, which was ascertained by the cross peak between δ 4.71 (H-3″) and 170.0 (C=O) in the HMBC data. Based on the above data, the structure of compound **2** was determined as kaempferol 3-*O*-α-L-3″-acetyl-arabinofuranoside.

Compound **3** displayed a sodiated ion at *m*/*z* 541.0957 $[M+Na]^+$ corresponding to a molecular formula of $C_{24}H_{22}O_{13}Na$ in the HR-FAB-MS. The signals belonging to a quercetin skeleton were observed at δ 6.18 (1H, br s, H-6), 6.37 (1H, br s, H-8), δ 6.84 (1H, d, J=8.7 Hz, H-5′), 7.38 (1H, d, J=8.7 Hz, H-6′), and 7.39 (1H, br s, H-2′) in the ^{1}H-NMR spectrum. Also, two methyl proton peaks appeared at δ 1.92 and 2.09 suggesting that two acetyl groups were present in **3**. When the chemical shifts of this compound were compared to those of an arabinofuranoside in the literature,[1)] two signals at δ 63.3 (C-5″) and 79.6 (C-3″) appeared to be shifted downfield. These findings suggested that two acetyl groups were attached to C-3″ and 5″. Furthermore, the HMBC data confirmed these substitutions by observation of long range correlations between δ 4.66 (H-3″) and 170.2 (C=O) as well as δ 3.89 and 4.11 (H_2-5″) and δ 170.0 (C=O). These data enabled us to assign the structure of **3** as quercetin 3-*O*-α-L-3″,5″-diacetyl-arabinofuranoside.

Other isolates were elucidated as kaempferol,[5)] quercetin,[5)] myricetin-3-*O*-α-L-arabinofuranoside,[6)] quercetin-3-*O*-β-D-

* To whom correspondence should be addressed. e-mail: jwkim@snu.ac.kr

Table 1. The ^{1}H- and ^{13}C-NMR Chemical Shifts of Compounds **1**—**3** in DMSO-d_6

	1		**2**		**3**	
	^{1}H	^{13}C	^{1}H	^{13}C	^{1}H	^{13}C
2		156.5		156.5		156.5
3		133.2		133.0		133.1
4		177.3		176.9		177.2
5		161.1		161.1		161.2
6	6.15 br s	99.1	6.09 br s	99.4	6.18 br s	99.0
7		165.7		165.3		165.1
8	6.38 br s	93.9	6.31 br s	94.1	6.37 br s	93.8
9		157.0		156.6		157.3
10		103.5		103.1		103.8
1′		120.7		120.1		120.7
2′	8.05 (d 8.8)	130.7	7.92 (d 8.7)	130.7	7.39 br s	115.3
3′	6.88 (d 8.8)	115.4	6.89 (d 8.7)	115.3		145.2
4′		160.0		160.0		148.7
5′	6.88 (d 8.8)	115.4	6.89 (d 8.7)	115.3	6.84 (d 8.7)	115.8
6′	8.05 (d 8.8)	130.7	7.92 (d 8.7)	130.8	7.38 (d 8.7)	121.4
1″	5.51 br s	108.1	5.67 br s	108.0	5.58 br s	108.1
2″	4.15 br s	81.9	4.25 br s	79.1	4.32 br s	78.9
3″	3.61 m	77.7	4.71 (d 3.3)	79.6	4.66 (d 4.0)	79.6
4″	3.61 m	82.3	3.57 m	85.5	3.74 m	82.2
5″	3.78 (dd 9.6, 6.6)	63.6	3.32 m	61.3	3.89 (dd 11.7, 6.7)	63.3
	4.02 (dd 9.6, 2.6)				4.11 (dd 11.7, 3.3)	
CO		169.4		170.0		170.0
						170.2
CH_3	1.90 s	20.5	2.10 s	20.8	1.92 s	20.5
					2.09 s	20.7

glucopyranoside,[6] quercetin-3-*O*-*β*-D-xylopyranoside,[6] (−)-bergenin,[7] (−)-norbergenin,[8] (+)-11-*O*-galloylbergenin,[8] (+)-11-*O*-galloylnorbergenin,[7] (−)-4-*O*-galloylbergenin,[8] and (+)-4,11-di-*O*-galloylbergenin[8] by comparison of their spectroscopic data with the reported data. Among these known compounds, 4,11-di-*O*-galloylbergenin showed the positive value of specific rotation, $[\alpha]_D^{20}$ +12.3° (c=0.20, MeOH), in contrast to the negative value of 4,11-di-*O*-galloylbergenin that was reported previously in the literature.[8] Thus, this compound was designated as (+)-4,11-di-*O*-galloylbergenin.

Experimental

General Procedure The optical rotation was measured with a JASCO DIP-1000 digital polarimeter (JASCO, Japan). HR-FAB-MS spectra were obtained on a JEOL JMS-AX505WA spectrometer. UV and IR spectra were recorded on a Shimadzu UV-2101 and Perkin Elmer 1710 spectrometer, respectively. ^{1}H- and ^{13}C-NMR spectra were recorded on a Bruker spectrometer at 400 MHz and 100 MHz, respectively. GC analysis was conducted on GC 353B (GL Sciences, Japan) with a BPX 50 column (30×0.25 mm i.d., SGE Australia, Australia). Column chromatography was performed using Sephadex LH-20 (Pharmacia, NJ, U.S.A.) and Kiesegel 60 (Art. 7734; Merck, Darmstadt, Germany). TLC was conducted on pre-coated Kiesegel 60 F_{254} plates (Art. 5715; Merck, Darmstadt, Germany). Spots on TLC were detected under UV radiation.

Plant Material The aerial parts of *R. podophylla* were recollected from Jinbu, Gangwon province, South Korea in 2003. A voucher specimen (SNUPC-011-1) was deposited at Seoul National University.

Extraction and Isolation Dried materials (3.2 kg) were extracted with MeOH by sonication for 90 min three times at room temperature. After filtration and evaporation of the solvent under reduced pressure, the combined crude methanolic extract (300 g) was suspended in H_2O, then partitioned sequentially with *n*-hexane (3×1000 ml), CH_2Cl_2 (3×1000 ml), EtOAc (3×1000 ml) and *n*-BuOH (3×1000 ml), to afford *n*-hexane- (70 g), CH_2Cl_2- (43 g), EtOAc- (21 g) and *n*-BuOH- (50 g), and H_2O-residues.

The EtOAc-soluble extract (21 g) was subjected to silica gel column chromatography and eluted with CH_2Cl_2–MeOH (from 10 : 1 to 1 : 1) to give nine fractions (RE1—9). Fraction RE2 (1.6 g) was chromatographed on Sephadex LH-20 (MeOH) to give six sub-fractions (RE21—26). Sub-fraction RE24 was further purified by HPLC (MeCN–H_2O=25 : 75 (v/v), 2 ml, YMC J'sphere ODSH80), which resulted in the isolation of compounds **1** (6.0 mg, t_R 30.2 min), **2** (5.4 mg, t_R 36.1 min) and **3** (15.0 mg, t_R 40.3 min). RE26 was applied on Sephadex LH-20 (MeOH) and then gave kaempferol and quercetin. Fraction RE5 (2.4 g) was fractionated by using Sephadex LH-20 (MeOH) to give five sub-fractions (RE51—55). Sub-fraction RE54 was applied to HPLC (MeCN–H_2O=20 : 80 (v/v), 2 ml, YMC J'sphere ODSH80), which resulted in the isolation of myricetin-3-*O*-*α*-L-arabinofuranoside (5.2 mg, t_R 28.2 min), quercetin-3-*O*-*β*-D-glucopyranoside (3.8 mg, t_R 32.1 min), and quercetin-3-*O*-*β*-D-xylopyranoside (4.6 mg, t_R 35.7 min). The *n*-BuOH-soluble extract (50 g) was subjected to HP-20 gel (Diaion, 500 g) chromatography (10%, 20%, 30%, 40%, 50%, 60%, 70%, 80%, 100% acetone, 2L each) which gave nine fractions (RB1—9). A small portion (200 mg) of RB1 (10.2 g) was subjected to an HPLC (J'sphere ODS-H80) with an MeCN–H_2O (10 : 90 (v/v), 2 ml/min) solvent system to provide (−)-norbergenin (7.0 mg, t_R 10.2 min). The RB3 (3.4 g) was applied to silica gel (230—400 mesh, Merck, 90 g) column chromatography using a CH_2Cl_2–MeOH (10 : 1, 7 : 1, 5 : 1, 4 : 1, 3 : 1, 2 : 1, 1 : 1, 0 : 1, 900 ml each) gradient that afforded eight fractions (RB31—38). The RB32 was subjected to HPLC (J'sphere ODS-H80) with an MeCN–H_2O (20 : 80 (v/v), 2 ml/min) solvent system to provide (−)-bergenin (20 mg, t_R 17.4 min). From the RB35, (+)-11-*O*-galloylbergenin (5.0 mg, t_R 15.3 min) was purified using HPLC (YMC-Pack Ph, MeCN–H_2O=13 : 87 (v/v), 2 ml/min) followed by Sephadex LH-20 (MeOH). (−)-4-*O*-Galloylbergenin (2.3 mg, t_R 19.5 min) from RB36 was separated by HPLC (J'sphere ODS-H80, MeCN–H_2O=17 : 83 (v/v), 2 ml/min). (+)-11-*O*-Galloylnorbergenin (3.5 mg, t_R 12.0 min) was obtained from RB38 using Sephadex LH-20 (MeOH) and HPLC (YMC-Pack Ph, MeCN–H_2O=13 : 87 (v/v), 2 ml/min). The RB4 (4.2 g) was applied to Sephadex LH-20 (MeOH) and then HPLC (J'sphere ODS-H80, MeCN–H_2O=15 : 85 (v/v), 2 ml/min), affording (+)-4,11-di-*O*-galloylbergenin (6.3 mg, t_R 22.8 min).

Compound **1**: ^{1}H- and ^{13}C-NMR data see Table 1. IR ν_{max} (KBr) cm^{-1}: 3267, 1738, 1654, 1606, 1504, 1177. UV λ_{max} (MeOH) nm (log ε): 265 (4.28), 342 (4.15). A dark yellow powder, $[\alpha]_D^{20}$ −91.3° (c=0.03, MeOH). HR-FAB-MS m/z: 461.1091 $[M+H]^+$ (Calcd for $C_{22}H_{21}O_{11}$ 461.1084).

Compound **2**: ^{1}H- and ^{13}C-NMR data see Table 1. IR ν_{max} (KBr) cm^{-1}: 3363, 1735, 1650, 1607, 1505, 1177. UV λ_{max} (MeOH) nm (log ε): 265 (4.48), 342 (4.35). A dark yellow powder, $[\alpha]_D^{20}$ −231.6° (c=0.01, MeOH). HR-FAB-MS m/z: 461.1081 $[M+H]^+$ (Calcd for $C_{22}H_{21}O_{11}$ 461.1084).

Compound **3**: ^{1}H- and ^{13}C-NMR data see Table 1. IR ν_{max} (KBr) cm^{-1}: 3267, 1736, 1653, 1605, 1505, 1199. UV λ_{max} (MeOH) nm (log ε): 256 (4.38), 354 (4.25). A dark yellow powder, $[\alpha]_D^{20}$ −102.3° (c=0.17, MeOH). HR-FAB-MS m/z: 541.0957 $[M+Na]^+$ (Calcd for $C_{24}H_{22}O_{13}Na$ 541.0958).

Sugar Analysis A solution of **1** (1.0 mg) in 1 N HCl (dioxane–H_2O= 1 : 1, 1.0 ml) was heating at 80 °C for 2 h. The reaction mixture was neutralized with Ag_2CO_3 and was concentrated to dryness under N_2 flow. The residue was dissolved in pyridine (0.1 ml) and 0.1 M L-cysteine methyl ester hydrochloride in pyridine (0.1 ml) was added. After heated for 2 h, trimethylsilylimidazole (0.1 ml) was added and the mixture was heated at 60 °C for 1 h. After drying this mixture, it was partitioned with hexane and water (each 1 ml). The organic layer was analyzed by GC; column BPX 50 (30 m×0.25 mm i.d.); column temperature 210 °C; carrier gas, He (1.0 ml/min); injection temperature, 270 °C; detection temperature, 300 °C. Derivatives of authentic sugars were detected at t_R 7.57 min (L-arabinose), 7.65 (D-xylose), 8.40 (L-xylose), 8.47 (D-arabinose), 10.24 (L-glucose), and 11.36 (D-glucose). The L-arabinose in hydrolysate of **1** gave a peak at t_R 7.56 min. Analyses of compounds **2** and **3** were treated in the same manner and gave peaks at t_R 7.58 and 7.55, respectively. The known compounds **6**—**8** were also hydrolyzed and derivatized for GC analysis, which exhibited peaks at t_R 7.58, 11.36, and 7.68 min, respectively.

Acknowledgements This work was supported by a grant from the Korea Health 21 R&D Project, Ministry of Health & Welfare, Republic of Korea. (00-PJ1-PG1-CH14-0006).

References

1) Chin Y.-W., Lim S. W., Kim Y. C., Choi S. Z., Lee K. R., Kim J., *Planta Med.*, **70**, 576—577 (2004).
2) Chin Y.-W., Kim J., *Tetrahedron Lett.*, **45**, 339—341 (2004).
3) Kim Y. L., Chin Y.-W., Kim J., *Chem. Pharm. Bull.*, **53**, 103—104 (2005).
4) Takada Y., Machida K., Kikuchi M., *J. Tohoku Pharmaceutical University*, **50**, 89—93 (2003).
5) Agrawal P. K., Bansal M. C., "Carbon-13 NMR of Flavonoids," ed. by Agrawal P. K., Elsevier, Amsterdam, 1989, pp. 335—338.
6) Agrawal P. K., Thakur R. S., Bansal M. C., "Carbon-13 NMR of flavonoids," ed. by Agrawal P. K., Elsevier, Amsterdam, 1989, pp. 152—154.
7) Yoshida T., Seno K., Takama Y., Okuda T., *Phytochemistry*, **21**, 1180—1182 (1982).
8) Saijo R., Nonaka G.-I., Nishioka I., *Phytochemistry*, **29**, 267—270 (1990).