Hepatoprotective Flavonol Glycosides from the Aerial Parts of *Rodgersia podophylla*

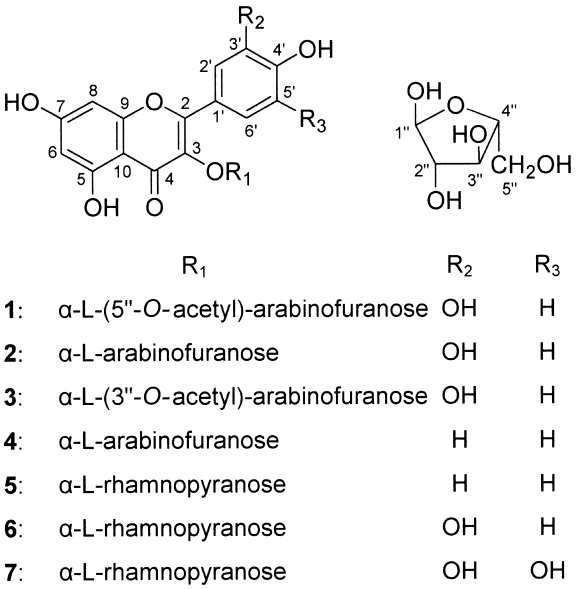
Young-Won Chin[1], Song Won Lim[1], Young Choong Kim[1], Sang Zin Choi[2], Kang Ro Lee[2], Jinwoong Kim[1]

Abstract

A new acylated flavonoid, quercetin 3-*O*-α-L-(5″-*O*-acetyl)-arabinofuranoside (**1**), along with six known flavonoids (**2 – 7**) were isolated from the aerial parts of *Rodgersia podophylla*. The new flavonoid **1** exhibited 50.1 % hepatoprotective activity at a concentration of 100 μM, and the three known compounds **3**, **5** and **6** showed hepatoprotective activities at a concentration of 50 μM (45.7, 50.8 and 57.3 %, respectively) by using the primary cultures of rat hepatocytes injured by H_2O_2.

The rhizomes of *Rodgersia podophylla* A. Gray (Saxifragaceae) have been used to treat enteritis and bacillary dysentery in China and are also known to have antipyretic and analgesic effects [1]. It has been reported that the rhizomes of this plant contained bergenin, β-peltoboykinolic acid, sterols, monoterpenes, fatty acids and neolignans [1]. During our investigation of hepatoprotective materials from Korean plants, the aerial parts of *R. podophylla* exhibited hepatoprotective activity in the primary cultures of rat hepatocytes injured by H_2O_2. Therefore, we conducted an activity-guided separation of this plant and isolated the flavonol glycosides **1 – 7** (Fig. **1**) as the active constituents.

Compound **1**, yellow powder, gave a pseudomolecular ion peak at *m/z* = 477.1016 $[M + H]^+$ corresponding to a molecular formula of $C_{22}H_{21}O_{12}$ in the HR-FAB-MS. ^{1}H-NMR and ^{13}C-NMR spectra of **1** showed characteristics of a quercetin moiety with a glycoside. The sugar moiety was assumed to be an α-L-arabinofuranoside, which was supported by comparison of the ^{13}C-NMR data of **1** with those of quercetin 3-*O*-α-L-arabinofuranoside (**2**) (Table **1**) [2]. Also, the methyl protons of an acetyl group were observed as a singlet peak at δ = 1.89 in the ^{1}H-NMR and the two carbon peaks belonging to an acetyl group at δ = 20.9 and 170.4 in the ^{13}C-NMR spectra. The acetyl group was determined to be attached at the C(5″)-*O* position of arabinofuranoside based on the downfield-shifted C-5″ signal at δ = 63.8 and the upfield-shifted C-4″ signal at δ = 82.3 in the ^{13}C-NMR spectrum [3]. The position of the acetyl group was further confirmed by the cross peaks between δ = 3.72 (H-5″a), 4.05 (H-5″b) and δ = 170.4 ($O\underline{CO}CH_3$) in the HMBC spectrum. Therefore, **1** was elucidated as quercetin 3-*O*-α-L-(5″-*O*-acetyl)-arabinofuranoside which is isolated for the first time from a natural resource.



	R_1	R_2	R_3
1:	α-L-(5"-*O*-acetyl)-arabinofuranose	OH	H
2:	α-L-arabinofuranose	OH	H
3:	α-L-(3"-*O*-acetyl)-arabinofuranose	OH	H
4:	α-L-arabinofuranose	H	H
5:	α-L-rhamnopyranose	H	H
6:	α-L-rhamnopyranose	OH	H
7:	α-L-rhamnopyranose	OH	OH

Fig. **1** Structures of the compounds **1 – 7** isolated from *R. podophylla*.

Table **1** The ^{13}C-NMR chemical shifts of compounds **1** and **2**

No.	*1*	*2*
2	156.8	156.4
3	133.7	133.4
4	177.9	177.7
5	161.7	161.3
6	99.1	98.7
7	164.6	164.2
8	94.0	93.6
9	157.9	157.0
10	104.4	104.0
1′	121.3	121.0
2′	115.8	115.5
3′	145.5	145.1
4′	148.8	148.5
5′	115.9	115.6
6′	122.0	121.8
1″	108.3	107.9
2″	82.3	82.2
3″	78.0	77.0
4″	82.3	85.9
5″	63.8	60.7
C = O	170.4	–
CH_3	20.9	–

The hepatoprotective activities of compounds **1 – 7** were assessed by measuring their effects on the release of glutamic pyruvic transaminase (GPT) from the primary cultures of rat hepatocytes

Affiliation: [1] College of Pharmacy and Research Institute of Pharmaceutical Science, Seoul National University, Seoul, Korea · [2] College of Pharmacy, SungKyunKwan University, Suwon, Korea

Correspondence: Prof. Jinwoong Kim · College of Pharmacy · Seoul National University · San 56–1 · Shinlim-dong · Kwanak-gu · Seoul 151–742 · Korea · Phone: +82-2-880-7853 · Fax: +82-2-887-8509 · E-mail: jwkim@snu.ac.kr

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

Received: October 30, 2003 · **Accepted:** March 7, 2004

Bibliography: Planta Med 2004; 70: 576–577 · © Georg Thieme Verlag KG Stuttgart · New York · DOI 10.1055/s-2004-827163 · ISSN 0032-0943

Table **2** Effects of compounds **1 – 7** on the H_2O_2-induced toxicity in the primary cultures of rat hepatocytes

Compound	*GPT (IU/L) Relative protection[a] (%)*	
	50 μM	*100 μM*
Control	22.19 ± 1.95 (100)*[b]	
H_2O_2-treated	56.15 ± 1.53 (0.00)*	
1	41.05 ± 1.61 (44.5)*	39.15 ± 5.79 (50.1)*
2	49.28 ± 9.50 (20.2)*	45.96 ± 1.81 (30.0)*
3	40.64 ± 1.61 (45.7)*	42.52 ± 4.20 (40.1)*
4	42.66 ± 3.34 (39.7)*	50.67 ± 6.96 (16.1)*
5	38.91 ± 3.61 (50.8)*	49.03 ± 1.13 (21.0)*
6	36.70 ± 1.93 (57.3)*	44.63 ± 3.59 (33.9)*
7	47.80 ± 2.30 (24.6)*	46.60 ± 5.00 (28.2)*
Silibinin[c]	30.70 ± 2.30 (74.9)*	33.00 ± 2.10 (68.2)*

[a] Primary cultures of rat hepatocytes were exposed to 15 mM H_2O_2 with or without each compound.

[b] The value of parenthesis is relative percent. The % of protection is calculated as 100 × (value of H_2O_2 - value of sample)/(value of H_2O_2 - value of control).

[c] Positive control.

* Significant different from positive control at $P < 0.01$. The Each value represents the mean ± SD ($n = 3$).

injured by H_2O_2. Compounds **1, 3, 5** and **6** exhibited significant hepatoprotective activities (Table **2**). Compounds **5** and **6** having an α-L-rhamnopyranosyl moiety at the 3-position were more effective than compounds **2** and **4** having an α-L-arabinofuranosyl moiety at the 3-position. Among the quercetin glycosides **1**, **2** and **3** having an α-L-arabinofuranosyl moiety at the 3-position, the acylated compounds **1** and **3** were more effective than **2**.

Material and Methods

The aerial parts of *Rodgersia podophylla* were collected from Jinbu, Kangwon province in 2001. A voucher specimen (SNUPC-011) was deposited at the Seoul National University. Silibinin was purchased from Sigma Chemical Co. (St. Louis, USA). Dried materials (2 kg) were extracted with MeOH giving a crude extract (173 g). The MeOH extract was partitioned with *n*-hexane, CH_2Cl_2 and *n*-BuOH, successively. The *n*-BuOH extract (44 g) was subjected to HP-20 gel (Diaion, 400 g) chromatography (10%, 20%, 30%, 40%, 50%, 60%, 70%, 80%, 100% acetone, 2 L each) which gave nine fractions and then the fifth fraction (1.8 g) was applied to silica gel (230 – 400 mesh, Merck, 40 g) column chromatography using a CH_2Cl_2-MeOH (7 : 1, 6 : 1, 5 : 1, 4 : 1, 3 : 1, 2 : 1, 1 : 1, 0 : 1, 750 mL each) gradient that afforded eight fractions. The first fraction (320 mg) was subjected to HPLC (J'sphere ODS-H80) with an AcCN-H_2O [17 : 83 (v/v), 2 mL/min] solvent system to provide compounds **1** (5.4 mg, t_R: 35.7 min), **2** (22 mg, t_R: 23.2 min) [2] and **6** (7 mg, t_R: 52.1 min) [4]. From the second fraction (210 mg), compounds **3** (7.8 mg, t_R: 43.1 min) [5], **4** (12.3 mg, t_R: 30.6 min) [6], **5** (13.3 mg, t_R: 35.4 min) and **7** (7.4 mg, t_R: 26.6 min) [4] were isolated by HPLC [YMC-Pack Ph, AcCN-H_2O 17 : 83 (v/v), 2 mL/min].

These compounds were demonstrated to be pure as evidenced by NMR and HPLC analysis (purity > 95%).

Quercetin 3-O-α-L-(5″-O-acetyl)arabinofuranoside (**1**): yellow powder; m. p. 186 °C; $C_{22}H_{20}O_{12}$; HR-FAB-MS: *m/z* = 477.1016 [M + H]$^+$; calcd. for $C_{22}H_{21}O_{12}$: 477.1033; $[\alpha]_D^{20}$: –96.3° (*c* 0.1, MeOH); UV (MeOH): μ_{max} (log ε) = 257 (3.93), 355 nm (3.82); IR (KBr): λ_{max} = 3239, 1738, 1653, 1606, 1199, 1008 cm^{-1}; ^{1}H-NMR (400 MHz, DMSO-*d6*): δ = 1.89 (3H, s, OCOC<u>H</u>$_3$), 3.62 (2H, brs, H-2″, 3″), 3.72 (1H, dd, *J* = 10.7, 6.3 Hz, H-5″a), 4.05 (1H, d, *J* = 10.7 Hz, H-5″b), 4.15 (1H, brs, H-4″), 5.46 (1H, s, H-1″), 6.17 (1H, s, H-6), 6.38 (1H, s, H-8), 6.85 (1H, d, *J* = 8.4 Hz, H-5′), 7.44 (1H, s, H-2′), 7.46 (1H, d, *J* = 8.4 Hz, H-6′); ^{13}C-NMR: Table **1**. Copies of the original spectra are obtainable from the author of correspondence.

Compounds **1**, **2** and **6** were hydrolyzed according to the procedure in the literature [7]. The specific optical rotations of arabinose from **1** and **2** are + 226.2° and + 227.4° (*c* 0.1, H_2O), respectively, whereas rhamnose from **6** has + 13.0° (*c* 0.1, H_2O).

Details of bioassay on hepatoprotective activity have been described elsewhere [8],[9].

References

[1] Chin Y-W, Kim J. Four novel neolignans from *Rodgersia podophylla*. Tetrahedron Lett 2004; 45: 339 – 41

[2] Lu Y, Foo LY. Identification and quantification of major polyphenols in apple pomace. Food Chemistry 1997; 59: 187 – 94

[3] Merfort I. Acylated and other flavonoid glycosides from *Arnica chamissonis*. Phytochemistry 1988; 27: 3281 – 4

[4] Agrawal PK, Bansak MC. Flavonoid Glycosides. In: Agrawal PK, editor. Carbon-13 NMR of Flavonoids Amsterdam: Elsevier, 1989: pp 335 – 8

[5] Takemoto T, Yoshida S, Kobayashi M, Hasegawa M. Studies on the constituents of *Boehmeria tricuspis* Makino. Yakugaku Zasshi 1974; 94: 1597 – 602

[6] Murakami T, Satake T, Hirasawa C, Ikeno Y, Saiki Y, Chen C-M. Chemical and chemotoxonomical studies on Filices. Yakugaku Zasshi 1984; 104: 142 – 6

[7] Plaza A, Cinco M, Tubaro A, Pizza C, Piacente S. New triterpene glycosides from the stem of *Anomospermum grandifolium*. J Nat Prod 2003; 66: 1606 – 10

[8] Kanno S, Ishikawa M, Takayanagi M, Takayanagi Y, Sasaki K. Exposure to hydrogen peroxide induces cell death via apoptosis in primary cultured mouse hepatocytes. Biol. Pharm. Bull. 1999; 22: 1296 – 1300

[9] Reitman S, Frankel SA. A colorimetric method for the determination of serum glutamic pyruvic transaminase. Am. J. Clin. Pathol. 1957; 28: 56 – 63