Two New Hepatoprotective Stilbene Glycosides from *Acer mono* Leaves

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Received June 24, 2004

Two new stilbene glycosides, 5-*O*-methyl-(*E*)-resveratrol 3-*O*-*β*-D-glucopyranoside (**1**) and 5-*O*-methyl-(*E*)-resveratrol 3-*O*-*β*-D-apiofuranosyl-(1→6)-*β*-D-glucopyranoside (**2**), were isolated from the leaves of *Acer mono*, along with seven known compounds. Among these compounds, **1**, **2**, and quercetin (**3**) showed significant hepatoprotective activities against H_2O_2-induced toxicity in primary cultures of rat hepatocytes.

Acer mono Maximowicz (Aceraceae; "Gorosoe" in Korean) is widely distributed in Korea, the People's Republic of China, and Japan.[1] The leaves of *A. mono* have been used in Korean folk medicine for hemostasis, and the roots have been used for the treatment of arthralgia and cataclasis. The sap of *A. mono* has been employed for the treatment of difficulty in urination, constipation, other gastroenteric disorders, gout, and neuralgia.[1] Coumarinolignans, diarylheptanoids, flavonoids, sterols, and triterpenoids were reported as secondary metabolic constituents of *Acer* species.[2−4] To date, however, there have been no previous studies on the bioactive principles of *A. mono*.

In the course of our search for hepatoprotective natural products using, as a screening system, primary cultures of rat hepatocytes injured with hydrogen peroxide (H_2O_2), we found that the methanolic extract of *A. mono* leaves showed significant hepatoprotective activity. Bioactivity-guided fractionation of the methanolic extract of *A. mono* leaves revealed that the EtOAc-soluble fraction showed the most significant hepatoprotective activity (97.3 ± 1.2% protection against toxicity induced by H_2O_2 at 50 μg/mL, $p < 0.001$). Further fractionation of this EtOAc fraction by several chromatographic methods yielded two new stilbene glycosides, 5-*O*-methyl-(*E*)-resveratrol 3-*O*-*β*-D-glucopyranoside (**1**) and 5-*O*-methyl-(*E*)-resveratrol 3-*O*-*β*-D-apiofuranosyl-(1→6)-*β*-D-glucopyranoside (**2**), along with seven known compounds, quercetin (**3**), quercitrin, eriodictyol, naringenin, eriodictyol-7-*O*-*β*-D-glucopyranoside, 5,7-dihydroxychromone 7-*O*-*β*-D-glucopyranoside, and naringenin 7-*O*-*β*-D-glucopyranoside. The known compounds were identified by spectroscopic data comparison with published values.[5−8]

Compound **1** was isolated as a pale yellowish powder. The molecular formula of **1** was determined to be $C_{21}H_{24}O_8$ from the HRFABMS at *m/z* 404.1470 $[M]^+$ (calcd for $C_{21}H_{24}O_8$, *m/z* 404.1471). The fragment ion peak at *m/z* 242 ($[M - 162]^+$) indicated the loss of one 2-deoxy sugar unit. The IR absorption bands at 3429, 1600, and 1455 cm^{-1} suggested the presence of OH groups and aromatic rings in **1**. The ^1H and ^{13}C NMR (Table 1) spectra indicated that **1** has an (*E*)-resveratrol aglycon moiety from the observed signals consistent with those published for (*E*)-resveratrol 3-*O*-*β*-D-glucopyranoside.[9] The ^1H NMR spectrum showed the presence of three 1,3,5-trisubstituted aromatic protons at *δ* 6.60 (H-4), 6.75 (H-6), and 6.93 (H-2), signals for 1,4-disubstituted aromatic protons at *δ* 7.40 (2H, d, $J = 8.5$ Hz, H-2′, 6′) and 6.79 (2H, d, $J = 8.5$ Hz, H-3′, 5′), and two *trans*-olefinic protons at *δ* 6.91 (1H, d, $J = 16.2$ Hz, H-α) and 7.09 (1H, d, $J = 16.2$ Hz, H-*β*). Moreover, HMBC correlations were observed between *δ* 6.91 (H-α) and C-2, 6 (*δ* 107.9, 107.2, respectively) as well as *δ* 7.09 (H-*β*) and C-2′, 6′ (*δ* 129.0), indicating that **1** has an (*E*)-resveratrol aglycon moiety. In addition, the HMBC correlation between the anomeric proton at *δ* 4.94 (1H, d, $J = 7.2$ Hz) and C-3 (*δ* 160.5) indicated the presence of a *β*-glucopyranosyl moiety, attached to C-3 of the (*E*)-resveratrol moiety through the anomeric carbon. Furthermore, the D configuration of the glucose unit was determined by acid hydrolysis of **1** followed by HPLC analysis. Additionally, a HMBC correlation between the methoxy group at *δ* 3.81 and C-5 (*δ* 162.3) indicated the position of this methoxy group to be C-5. Therefore, **1** was assigned as 5-*O*-methyl-(*E*)-resveratrol 3-*O*-*β*-D-glucopyranoside, and it is a new stilbene glycoside reported for the first time.

Table 1. ^1H and ^{13}C NMR Data of **1** and **2**[a]

	1		**2**	
position	δ_H	δ_C	δ_H	δ_C
1		141.4		141.4
2	6.93 brs	107.9	6.86 brs	108.4
3		160.5		160.4
4	6.60 brs	102.9	6.61 brs	103.1
5		162.3		162.3
6	6.75 brs	107.2	6.78 brs	106.7
α	6.91 d ($J = 16.2$)	126.5	6.93 d ($J = 16.3$)	126.6
β	7.09 d ($J = 16.2$)	130.3	7.08 d ($J = 16.3$)	130.3
1′		130.2		130.2
2′, 6′	7.40 d ($J = 8.5$)	129.0	7.41 d ($J = 8.6$)	129.0
3′, 5′	6.79 d ($J = 8.5$)	116.5	6.80 d ($J = 8.6$)	116.5
4′		158.5		158.6
−OCH_3	3.81 s	55.8	3.84 s	55.9
1″	4.94 d ($J = 7.2$)	102.5	4.91 d ($J = 7.5$)	102.5
2″	3.40 m	75.0	3.48 m	74.9
3″	3.50 m	78.0	3.48 m	77.9
4″	3.50 m	71.5	3.40 m	71.6
5″	3.50 m	78.3	3.64 m	76.9
6″	3.96 dd ($J = 1.8, 12.1$), 3.73 dd ($J = 6.1, 12.1$)	62.6	4.06 m, 3.64 m	68.7
1‴			4.99 d ($J = 2.4$)	110.9
2‴			3.91 d ($J = 2.4$)	78.1
3‴				80.5
4‴			3.99 d ($J = 9.7$), 3.77 d ($J = 9.7$)	75.1
5‴			3.56 s	65.8

[a] Measured in CD_3OD at 500 and 125 MHz, respectively.

Compound **2** was also obtained as a pale yellowish powder. The molecular formula of **2** was determined to be $C_{26}H_{32}O_{12}$ from the HRFABMS at *m/z* 536.1896 $[M]^+$ (calcd for $C_{26}H_{32}O_{12}$, *m/z* 536.1894). The fragment ions at *m/z* 405 ($[M + H - 132]^+$) and 242 ($[M - 132 - 162]^+$) indicated that **2** contains a hexose−pentose moiety. The ^1H and ^{13}C NMR (Table 1) spectra of **2** were very similar to those of **1**.

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10.1021/np0497907 CCC: $30.25
Published on Web 12/21/2004

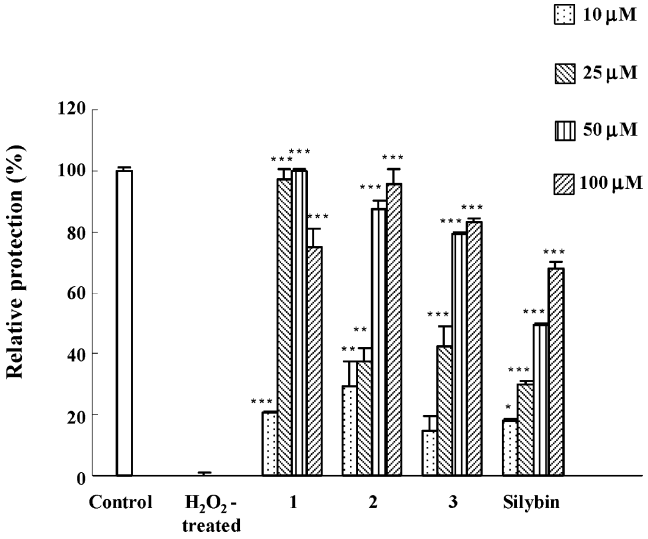
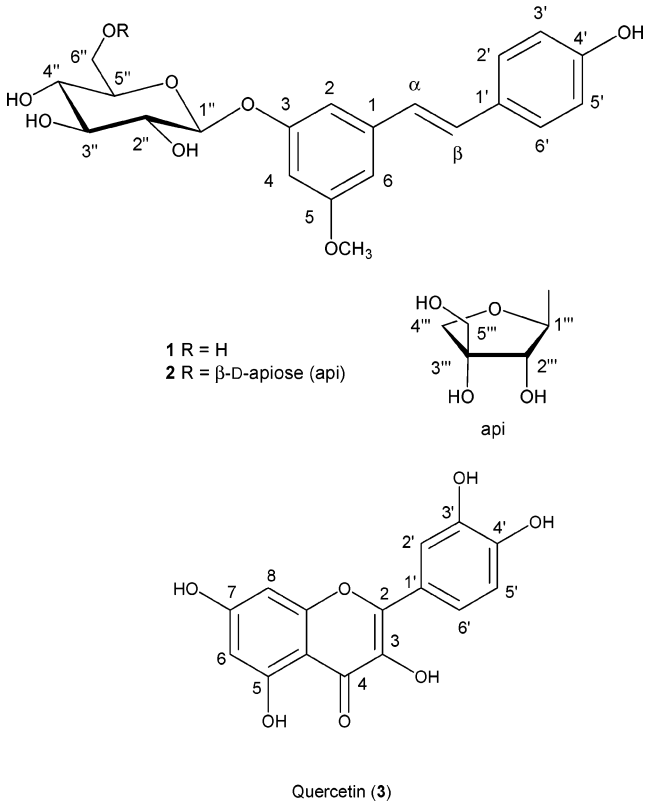


Figure 1. Hepatoprotective activities of **1**−**3** on primary cultures of rat hepatocytes injured by H_2O_2. The control is the value of hepatocytes not challenged with H_2O_2. The control and H_2O_2-treated values for GPT were 26.3 ± 3.0 IU/L and 65.0 ± 2.2 IU/L, respectively. Each value represents the mean ± SD ($n = 3$). Silybin, the positive control, showed optimal hepatoprotective activity at a concentration of 100 μM (38.7 ± 2.1 IU/L). Significant difference from control values for H_2O_2 − treated samples: $^*p < 0.05$, $^{**}p < 0.01$, $^{***}p < 0.001$.



However, another anomeric proton at δ 4.99 (1H, d, J = 2.4 Hz, H-1‴) and ^{13}C NMR signals at δ 110.9 (C-1‴), 78.1 (C-2‴), 80.5 (C-3‴), 75.1 (C-4‴), and 65.8 (C-5‴) indicated the presence of a β-apiofuranosyl moiety.[10] The D configurations of the glucose and apiose units were determined by HPLC and optical rotation analysis after acid hydrolysis of **2**. HMBC correlations between δ 4.91 (H-1″) and C-3 (δ 160.4) and δ 4.99 (H-1‴) and C-6″ (δ 68.7) showed that **2** contains a β-D-apiofuranosyl-(1→6)-β-D-glucopyranosyl moiety. Thus, **2** was assigned as 5-*O*-methyl-(*E*)-resveratrol 3-*O*-β-D-apiofuranosyl-(1→6)-β-D-glucopyranoside, which is also a new stilbene glycoside.

Hepatoprotective activities of these two new compounds and seven known compounds were assessed by measuring their effects on the release of glutamic pyruvic transaminase (GPT) into the culture media from primary cultures of rat hepatocytes injured with H_2O_2. Among these, compounds **1**−**3** reduced the GPT release from H_2O_2-damaged hepatocytes and were more potent in this regard than silybin used as a positive control (Figure 1).[11] The other six known compounds from *A. mono* leaves were inactive in this assay (data not shown). Since compounds **1** and **2** showed excellent hepatoprotective activities, we are currently investigating in more detail the protective mechanism of these compounds against H_2O_2-induced oxidative damage.

Experimental Section

General Experimental Procedures. Melting points were measured using a Fisher-Johns 12-144 melting point apparatus with a 12-142T thermometer. Optical rotations were measured on a JASCO DIP-1000 polarimeter. UV spectra were obtained on a Shimadzu UV-201 spectrophotometer and IR spectra on a Perkin-Elmer 1710 spectrophotometer. The ^{1}H and ^{13}C NMR measurements were carried out on a JEOL LA-300 NMR spectrometer operating at 300 and 75 MHz, a GSX-400 NMR spectrometer operating at 400 and 100 MHz, or a Bruker AMX 500 NMR spectrometer operating at 500 and 125 MHz, respectively. Data processing was carried out an Aspect ×32 computer with UXNMR software with Bruker microprograms. Standard pulse sequences were used for HMBC [1/2J = 70 ms for $J_{C,H}$ = 7 Hz]. The experiments were carried out at 300 K unless stated otherwise. An internal lock was applied, and the reference was set to the solvent peak (CD_3OD, 3.34 for ^{1}H and 49.0 for ^{13}C). HMBC and HMQC spectra were recorded on a JEOL GSX 400 NMR spectrometer. EIMS and FABMS were obtained on a JEOL JMS 700 spectrometer and HRFABMS on a JEOL JMS AX 505 WA spectrometer with direct inlet system using *m*-nitrobenzyl alcohol as matrix. ODS RP (Lichroprep ODS RP-18, 40−63 μm, EM Science) was used for column chromatography. TLC was carried out on precoated Kieselgel 60 F_{254} (0.25 mm, Merck) and RP-18 (0.25 mm, Merck) plates, and spots were visualized by heating after spraying with anisaldehyde-H_2SO_4 or aniline phthalate. HPLC was performed with an L-7100 pump (Hitachi, Japan), an L-7400 UV detector (Hitachi, Japan), a Shodex RI-71 refractive index detector (Showa-Denko, Japan), and a Watchers 120 ODS-BP (DAISO, Japan), LUNA 10 μm phenylhexyl (Phenomenex, Torrance, CA), and carbohydrate analysis columns (Waters, Milford, MA).

Plant Material. The leaves of *A. mono* were collected at Mt. Baekwoon (Kwangyang, Korea) in June 2001 and identified by the late Dr. Dae S. Han, an emeritus professor of the College of Pharmacy, Seoul National University. A voucher specimen (SNUPH-CS11-1) has been deposited in the Herbarium of the Medicinal Herb Garden, College of Pharmacy, Seoul National University.

Extraction and Isolation. The air-dried plant material (5.2 kg) was extracted four times with 80% MeOH in an ultrasonic apparatus. Removal of the solvent in vacuo yielded a methanolic extract (783 g). After evaporation of the solvent, the concentrated methanolic extract was suspended in distilled water and partitioned successively with *n*-hexane, $CHCl_3$, EtOAc, and *n*-BuOH (saturated with water). The EtOAc fraction (98 g) was fractionated by chromatography on an ODS reversed-phase column with a H_2O−MeOH step-gradient (100% H_2O → 100% MeOH) to yield six subfractions (1−6). Fraction 4 was subjected to repeated column chromatography and reversed-phase HPLC to yield 5-*O*-methyl-(*E*)-resveratrol 3-*O*-β-D-glucopyranoside (**1**) (106 mg, ODS, H_2O−MeOH, 50:50, 2 mL/min, t_R = 28.27 min) and 5-*O*-methyl-(*E*)-resveratrol 3-*O*-β-D-apiofuranosyl-(1→6)-β-D-glucopyranoside (**2**) (123 mg, phenylhexyl, H_2O−AcCN−MeOH, 50:5:45, 2 mL/min, t_R =16.04

min), with UV detection at 220 nm. Quercetin (**3**), quercitrin, eriodictyol, naringenin, eriodictyol-7-*O*-*β*-D-glucopyranoside, 5,7-dihydroxychromone 7-*O*-*β*-D-glucopyranoside, and naringenin 7-*O*-*β*-D-glucopyranoside were also obtained from fraction 4 by column chromatography and recrystallization.

5-*O*-Methyl-(*E*)-resveratrol 3-*O*-*β*-D-glucopyranoside (1): pale yellowish powder; mp 128−129 °C; $[\alpha]^{25}_{D}$ −46.4° (*c* 0.2, MeOH); UV (MeOH) λ_{max} (log ϵ) 215 (4.32), 305 (4.27), 320 (4.27) nm; IR (KBr) ν_{max} 3429, 1600, 1455, 1170, 1073 cm^{-1}; ^1H and ^{13}C NMR data, see Table 1; positive FABMS *m/z* 427 $[M + Na]^+$, 404 $[M]^+$, 242 $[M - 162]^+$; HRFABMS *m/z* 404.1470 (calcd for $C_{21}H_{24}O_8$, 404.1471).

5-*O*-Methyl-(*E*)-resveratrol 3-*O*-*β*-D-apiofuranosyl-(1→6)-*β*-D-glucopyranoside (2): pale yellowish powder; mp 124−126 °C; $[\alpha]^{25}_{D}$ −76.4° (*c* 0.2, MeOH); UV (MeOH) λ_{max} (log ϵ) 216 (4.48), 308 (4.55), 321 (4.55) nm; IR (KBr) ν_{max} 3429, 1600, 1456, 1168, 1060 cm^{-1}; ^1H and ^{13}C NMR data, see Table 1; positive FABMS *m/z* 559 $[M + Na]^+$, 536 $[M]^+$, 405 $[M + H - 132]^+$, 242 $[M - 132 - 162]^+$; HRFABMS *m/z* 536.1896 (calcd for $C_{26}H_{32}O_{12}$, *m/z* 536.1894).

Acid Hydrolysis of 1 and 2. The absolute configurations of sugar units were assigned by HPLC and optical rotation analysis after total acid hydrolysis of each compound. The sugars were compared with those of the authentic samples prepared in a modified manner.[12,13] In brief, **1** and **2** (10 mg each) were dissolved in 1.1 mL of 2 N HCl and then refluxed in a water bath at 90 °C for 3 h, respectively. After cooling, the reaction mixtures were evaporated in vacuo and the residues were dissolved in H_2O and extracted with EtOAc. The aqueous layers were neutralized with Ag_2CO_3 powder and then filtered to remove the inorganic materials. Each product, obtained by evaporation of the solvent from the filtrate in vacuo, was analyzed by HPLC under the following conditions: column, carbohydrate analysis column (3.9 mm × 300 mm, 5 μm); solvent, AcCN−H_2O (17:3); flow rate, 1.0 mL/min; detection, RI. The absolute configuration of each sugar was identified by the comparison of retention time and optical rotation: t_R 6.58 min (D-apiose, positive polarity), 7.33 min (D-glucose, positive polarity). Aniline phthalate was employed as a spray for color detection of sugar.

Biological Evaluation. Isolated rat hepatocytes were prepared from male Wistar rats by a collagenase perfusion technique as described previously.[14] One day after the isolated rat hepatocytes were plated, the cultured cells were treated either with or without each compound for 1 h. Then, the hepatocytes were exposed to 12 mM H_2O_2 for 1.5 h to induce hepatotoxicity.[15] The activity of GPT released into the culture medium was determined by the method of Reitman−Frankel.[16] Data were expressed as the value of relative protection (%) calculated as 100 × (value for the H_2O_2-treated − value of sample)/(value for the H_2O_2-treated − value of control).

Statistical Analysis. All data are expressed as the mean ± SD. The evaluation of statistical significance was determined by "the one-way ANOVA" using a computerized statistical package. The data were considered to be statistically significant if the probability had a value of 0.05 or less.

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

Supporting Information Available: Extraction details for known compounds and NMR spectra for compounds **1** and **2**. This information is available free of charge via the Internet at http://pubs.acs.org.

References and Notes

(1) Bae, K. H. *Medicinal Plants of Korea*; Kyo-Hak Publishing Co., Ltd.: Seoul, 2002; p 302.
(2) Morikawa, T.; Tao, J.; Toguchida, I.; Matsuda, H.; Yoshikawa, M. *J. Nat. Prod.* **2003**, *66*, 86−91.
(3) Abou-Zaid, M. M.; Helson, B. V.; Nozolillo, C.; Arnason, J. T. *J. Chem. Ecol*. **2001**, *27*, 2517−2526.
(4) Shiratori, S.; Nagumo, S.; Inoue, T.; Nagai, M.; Chi, H. J. *Chem. Pharm. Bull.* **1994**, *42*, 960−962.
(5) Markham, K. R.; Geiger, H. In *The Flavonoids: Advances in Research*; Harborne, J. B., Ed.; Chapman and Hall: London, 1994; pp 452−458.
(6) Agrawal, P. K.; Thakur, R. S.; Bansal, M. C. In *Carbon-13 NMR of Flavonoids*; Agrawal, P. K., Ed.; Elsevier: Amsterdam, 1989; pp 102−154.
(7) Agrawal, P. K.; Bansal, M. C. In *Carbon-13 NMR of Flavonoids*; Agrawal, P. K., Ed.; Elsevier: Amsterdam, 1989; pp 314−336.
(8) Simon, A.; Chulia, A. J.; Kaouadji, M.; Delage, C. *Phytochemistry* **1994**, *36*, 1043−1045.
(9) Wanjala, C. C. W.; Majinda, R. R. T. *Fitoterapia* **2001**, *72*, 649−655.
(10) Řezanka, T.; Guschina, I. A. *Phytochemistry* **2000**, *54*, 635−645.
(11) Hikino, H.; Kiso, Y.; Wagner, H.; Fiebig, M. *Planta Med.* **1984**, *50*, 248−250.
(12) Xiao, K.; Xuan, L.; Bai, K. *J. Nat. Prod*. **2000**, *63*, 1373−1376.
(13) Mimaki, Y.; Watanabe, K.; Sakuma, C.; Sakagami, H.; Sashida, Y. *Helv. Chim. Acta* **2003**, *86*, 398−407.
(14) Lee, E. J.; Kim, S. R.; Kim, J.; Kim, Y. C. *Planta Med*. **2002**, *68*, 407−411.
(15) Kanno, S.; Ishikawa, M.; Takayanagi, M.; Takayanagi, Y.; Sasaki, K. *Biol. Pharm. Bull.* **2000**, *23*, 37−42.
(16) Reitman, S.; Frankel, S. A. *Am. J. Clin. Pathol.* **1957**, *28*, 56−63.

NP0497907