Two Flavone *C*-Glycosides from the Style of *Zea mays* with Glycation Inhibitory Activity

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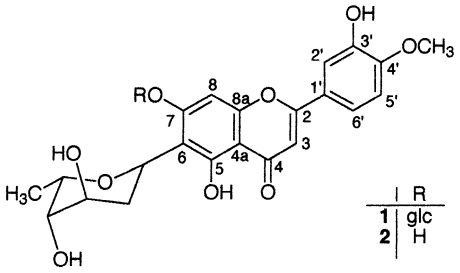
Received June 7, 2002

A new flavone *C*-glycoside, chrysoeriol 6-*C*-*β*-boivinopyranosyl-7-*O*-*β*-glucopyranoside (**1**), and a known flavone *C*-glycoside (**2**) were isolated from the style of *Zea mays*. Each compound contains the rare sugar boivinose. These flavones exhibited glycation inhibitory activity similar to that of aminoguanidine, a known glycation inhibitor.

The style of *Zea mays* L. (Gramineae) is commonly known as "corn silk" and has been used in folk medicine as a decoction for diuretic treatment in Japan and China. Sosa et al. isolated flavonoids and saponins from this material.[1] In previous papers, we reported the effectiveness of Chinese crude drugs and marine algae for the prevention of diabetic complications.[2,3] Some of these complications arise when reducing sugars, such as glucose, react nonenzymatically with the amino groups in proteins, lipids, and nucleic acids through a series of reactions forming Schiff bases and Amadori products to produce AGE (advanced glycation end products) in a process known as glycation. This paper describes the isolation of a new and a known flavone *C*-glycoside and the glycation inhibitory activity of the isolated compounds.

The MeOH extract of the style of *Z. mays* L. was chromatographed on Diaion HP-20, TOYOPAL HW-40F, CPC (centrifuged partition chromatography), and RP-HPLC to give compounds **1** and **2**.



Compound **1** had the molecular formula $C_{28}H_{32}O_{14}$ as determined from its HRFABMS, ^{13}C NMR, and ^{13}C DEPT data. The UV spectrum of **1** showed absorption maxima at 273 and 347 nm. The ^1H NMR spectrum of **1** in CD_3OD showed an ABX system due to a 3′,4′-disubstitution and two singlets at *δ* 7.08 (1H) and 6.72 (1H) attributed respectively to H-8 and H-3. A singlet at *δ* 3.96 (3H) was assigned to an aromatic methoxyl group at the 3′-position in the B-ring through HMBC and NOE difference spectroscopy. The two anomeric protons appeared at *δ* 5.52 (1H, dd, *J* = 2.5, 10.1 Hz) and 4.93 (1H, d, *J* = 7.6 Hz), which correlated respectively with signals at *δ* 67.3 and 104.0 in the HMQC spectrum. The coupling constants of the two anomeric protons indicated that each sugar moiety was connected to the flavone via a *β*-linkage. In the DQF-COSY spectrum a double doublet (*J* = 2.5, 10.1 Hz) due to H-1″ (anomeric proton) at *δ* 5.52 was coupled to two nonequivalent geminal protons at *δ* 3.06 and 1.45, assigned respectively to H-2″ax and H-2″eq. The observation of cross-peaks between the H-2″ax and H-2″eq and a resonance at *δ* 4.03 and between resonances at *δ* 4.03 and 3.37 permitted the assignments of H-3″ and H-4″, respectively. The HMBC and HMQC experiments of **1** indicated connections between *δ* 1.21 and *δ* 4.09, 3.37. In a spin-decoupling experiment of **1**, irradiation of a double doublet at *δ* 5.52 (H-1″) resulted in both an apparent triplet at *δ* 3.06 (H-2″ax) and an apparent doublet at *δ* 1.45 (H-2″eq) becoming a double doublet with the same coupling constant. Furthermore, on irradiation of an apparent doublet at *δ* 4.03 (H-3″), an apparent doublet at *δ* 3.37 (H-4″) was changed to an apparent singlet. Thus, the configurations of H-3″ and H-4″ were suggested to be equatorial. The configuration of H-5″ was elucidated to be axial because of the NOE observed at *δ* 4.09 (H-5″) on irradiation at *δ* 5.52 (H-1″). According to the above data and literature values,[4] the structure of this sugar is *β*-boivinose. In a similar way, HMQC, spin-decoupling, and NOE experiments indicated that another anomeric proton *δ* 4.93 (1H, d, *J* = 7.6 Hz) was that of *β*-glucose. The chemical shift values of these anomeric carbons and HMBC (DMSO-d_6) from these anomeric protons and the hydroxyl group at C-5 indicated that the anomeric carbon (*δ* 67.3) of *β*-boivinose was connected to C-6 through a *C*-linkage and that the anomeric carbon (*δ* 104.0) of *β*-glucose was connected to C-7 through an *O*-linkage (Figure 1). Consequently, compound **1** was determined to be chrysoeriol 6-*C*-*β*-boivinopyranosyl-7-*O*-*β*-glucopyranoside (**1**).

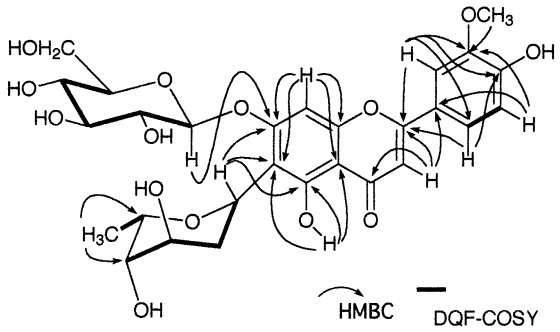


Figure 1. HMBC and DQF-COSY of compound **1**.

Compound **2** had the molecular formula $C_{22}H_{22}O_9$ as determined from its EIMS, ^{13}C NMR, and ^{13}C DEPT data. The UV spectrum of **2** showed absorption maxima at 270 and 346 nm. The ^1H NMR spectrum of **2** was very similar to **1** except for only one anomeric proton *δ* 5.47 (dd, *J* = 2.6, 12.1 Hz). The $^1H-^1H$ COSY spectrum, spin-decoupling, and NOE experiments showed that this glycosyl residue

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10.1021/np020256d CCC: $25.00
Published on Web 04/05/2003

was β-boivinose. The location of the sugar moiety at C-6 was established through the CD spectrum. The HMBC from the hydroxyl group at C-5 was not observed in this case. The CD spectrum showed a positive Cotton effect at 267.6 nm for **2**, indicating that the glycosyl residue is linked to C-6, as opposed to a negative Cotton effect had it been linked to C-8.[5] The absolute configuration of the sugar was determined from Klyne's rule.[6] Comparison of $[\alpha]_D$ of β-methyl-D-boivinopyranoside (−125°)[7] with **2** supported the belief it was β-L-boivinose. Consequently, it was concluded that compound **2** was chrysoeriol 6-*C*-β-L-boivinopyranoside (**2**), althernanthin isolated from *Alternanthera philoxeroides* Griseb. (Amaranthaceae).[4] The spectral data were consistent with those in the literature.[4]

Carrier proteins in blood vessels, structural proteins, and enzymes in the body are modified by glucose in a process called glycation. It is known that accumulation of these glycated proteins (AGE) causes diabetic complications.[8] The inhibitory effects of compounds **1** and **2** on glycation were tested by a fluorescent method.[9−11] Fluorescent AGEs such as pentosidine, crossline, can be detected by this method. Accumulation of fluorescent AGEs in kidneys of diabetic subjects is supported.[12] Percent inhibition was calculated to be 53.0% for **1**, 64.2% for **2**, and 60.5% for aminoguanidine at 1 mM. Thus, **1** and **2** were comparable to aminoguanidine, a known glycation inhibitor which traps reactive dicarbonyls impeding conversion to AGE.[12]

Experimental Section

General Experimental Procedures. Melting points were determined on a Yanako micro-melting point apparatus and were uncorrected. UV spectra and optical rotation were obtained with a Shimadzu UV 1600 spectrophotometer and a JASCO DIP-140 digital polarimeter, respectively. The CD spectrum was obtained in MeOH with a JASCO J-720 spectrophotometer. EIMS and FABMS (positive ion mode, magic bullet matrix) were obtained with a JEOL JMS DX-302 mass spectrometer. ^{1}H and ^{13}C NMR spectra were measured on JEOL BM-400, LA-500, and ECA-600 spectrometers using tetramethylsilane as an internal standard. All chemical shifts (δ) are given in ppm, and the samples were solubilized in CD_3-OD or in DMSO-d_6.

Plant Material. The style of *Zea mays* L. (Gramineae) (Lot: COFQO15) was purchased from Kinokuniya Pharmacy (Osaka, Japan). Voucher specimens were deposited with the laboratory of the Department of Natural Medicine and Phytochemistry at Meiji Pharmaceutical University.

Extraction and Isolation. The dried style of *Zea mays* L. (1 kg) was extracted twice in MeOH (12 L) for 2 h at 50 °C, and the solvent was evaporated in vacuo (13.4 g). This extract was separated by chromatography on Diaion HP-20 (50% MeOH → MeOH → acetone) to yield the corresponding fractions. The aqueous MeOH fraction (3.58 g) was subjected to CPC using EtOAc and H_2O as solvent system. The effluents were combined into eight fractions based on TLC patterns. Fraction 3 was purified by ODS chromatography eluted with a MeOH−H_2O mixture to afford **1** (8.1 mg). The MeOH fraction was subjected to gel filtration chromatography [TOYOPAL HW-40F, MeOH] followed by RP-HPLC (C-8 and 18) eluting with a MeOH−H_2O mixture to afford **2** (12.4 mg).

Compound 1: yellow amorphous solid; mp 196−198; $[\alpha]^{20}_D$ −24.9° (*c* 0.61, MeOH); positive FABMS *m*/*z* 593 $[M + H]^+$, 431 $[M - (glc) + H]^+$; UV λ_{max} (MeOH) 272.5 (log ε 4.23), 347 (log ε 4.55) nm; ^{1}H NMR (CD_3OD, 500 MHz) δ 7.56 (1H, dd, *J* = 8.4, 2.0 Hz, H-6′), 7.52 (1H, d, *J* = 2.0 Hz, H-2′), 7.08 (1H, s, H-8), 6.94 (1H, d, *J* = 8.4 Hz, H-5′), 6.72 (1H, s, H-3), 5.52 (1H, dd, *J* = 10.1, 2.5 Hz, H-1″), 4.93 (1H, d, *J* = 7.6 Hz, H-1‴), 4.09 (1H, q, *J* = 6.4 Hz, H-5"), 4.03 (1H, d-like, *J* = 2.8 Hz, H-3″), 4.02 (1H, dd, *J* = 1.9 Hz, H-6‴a), 3.96 (3H, s, OMe), 3.73 (1H, dd, *J* = 12.2, 6.8 Hz, H-6‴b), 3.61 (1H, t, *J* = 8.7 Hz, H-2‴), 3.61 (1H, m, H-5‴), 3.52 (1H, t, *J* = 9.2 Hz, H-3‴), 3.38 (1H, t, *J* = 9.3 Hz, H-4‴), 3.37 (1H, d-like, *J* = 3.8 Hz, H-4″), 3.06 (1H, t-like, *J* = 3.1 Hz, H-2″$_{ax}$), 1.45 (1H, d-like, *J* = 13.7 Hz, 2″$_{eq}$) and 1.21 (3H, d, *J* = 6.4 Hz, H-6″); ^{13}C NMR (CD_3OD, 125 MHz) δ 184.4 (s, C-4), 166.8 (s, C-2), 164.8 (s, C-7), 160.2 (s, C-5), 158.7 (s, C-8a), 152.5 (s, C-4′), 149.7 (s, C-3′), 123.6 (s, C-1′), 122.2 (d, C-6′), 117.2 (d, C-5′), 114.5 (s, C-6), 110.0 (d, C-2′), 107.2 (s, C-4a), 104.8 (d, C-3), 104.0 (d, C-1‴), 96.6 (d, C-8), 79.0 (d, C-5‴), 77.3 (d, C-3‴), 75.2 (d, C-2‴), 72.5 (d, C-5″), 71.9 (d, C-4‴), 71.3 (d, C-4″), 69.6 (d, C-3″), 67.3 (d, C-1″), 63.0 (t, C-6‴), 56.9 (q, OMe), 31.5 (t, C-2″) and 17.7 (q, C-6″); HRFABMS *m*/*z* 593.1866 $[M + H]^+$ (calcd for $C_{28}H_{32}O_{14}$, 593.1870)

Compound 2: yellow amorphous solid; mp 212−216; $[\alpha]^{20}_D$ +27.1° (*c* 1.18, MeOH); EIMS *m*/*z* 430 (M^+, 6.4), 412 (7.5), 394 (1.42), 367 (7.78), 337 (100), 327 (21.4), 300 (19.6); UV λ_{max} (MeOH) 272.5 (log ε 4.11), 346 (log ε 4.65) nm; ^{1}H NMR (CD_3-OD, 500 MHz) δ 7.48 (1H, dd, *J* = 8.4, 1.9, H-6′), 7.45 (1H, d, *J* = 1.9 Hz, H-2′), 6.91 (1H, d, *J* = 8.4 Hz, H-5′), 6.60 (1H, s, H-3), 6.48 (1H, s, H-8), 5.47 (1H, dd, *J* = 12.1, 2.6 Hz, H-1″), 4.13 (1H, q, *J* = 6.4 Hz, H-5″), 3.99 (1H, d-like, *J* = 3.1 Hz, H-3″), 3.94 (3H, s, OMe), 3.38 (1H, d-like, *J* = 3.1 Hz, H-4″), 2.26 (1H, t-like, *J* = 2.8 Hz, H-2″$_{ax}$), 1.73 (1H, d-like, *J* = 14.1 Hz, H-2″$_{eq}$) and 1.27 (3H, d, *J* = 6.7 Hz, H-6″); ^{13}C NMR (CD_3-OD, 125 MHz) δ 184.0 (s, C-4), 166.3 (s, C-2), 164.8 (s, C-7), 158.8 (s, C-5), 158.3 (s, C-8a), 152.4, (s, C-4′), 149.6 (s, C-3′), 123.6 (s, C-1′), 121.9 (d, C-6′), 116.9 (d, C-5′), 111.6 (s, C-6), 110.7 (d, C-2′), 104.9 (s, C-4a), 104.1 (d, C-3), 96.1 (d, C-8), 72.8 (d, C-5″), 70.7 (d, C-4″), 70.2 (d, C-1″), 68.8 (d, C-3″), 56.7 (q, OMe), 32.9 (t, C-2″), 17.4 (q, C-6″); CD (*c* 1.16 × 10^{-5}; MeOH) $\Delta\epsilon^{24}$:342 (+0.2), 336 (0), 328 (−0.2), 316 (0), 305 (+0.2), 300 (+0.1), 268 (+1.7), 245 (0), 223 (−3), 208 (0)

Inhibition Test on AGE Formation in Vitro. Evaluation for glycation inhibitory activity of isolated compounds was performed as previously described.[9−11] Lysozyme (10 mg/mL) was incubated with 100 mM fructose in the presence or absence of test compound for 7 days in 0.1 M phosphate buffer (pH 7.4) at 37 °C. After incubation, the fluorescence of AGE was measured with a fluorescence spectrophotometer (Ex 360 nm, Em 440 nm). Percent inhibition was calculated as follows: inhibition % = $[1 - (A_s - A_b)/(A_c - A_b)] \times 100$, where A_s is the fluorescence of the incubated mixture with sample, and A_c and A_b are the fluorescence of the incubated mixture without sample as a positive control and the fluorescence of unincubated mixture without sample as a blank control.

Acknowledgment. The authors are grateful to Dr. T. Shimizu of the Institute of Physical and Chemical Research (RIKEN), for measuring of the CD spectra. We also thank JEOL for recording the HMBC. This work was supported in part by a grant for the promotion of the advancement of education and research in graduate schools from the Ministry of Education, Culture, Sports and Technology of Japan.

References and Notes

(1) Sosa, A.; de Ruiz, R. E. L.; Fusco, M. del R.; Ruiz, S. O. *Acta Farm. Bonaerense* **1997**, *16*, 215−218.
(2) Iwahori, Y.; Enomoto, S.; Okada, Y.; Tanaka, J.; Okuyama, T. *Nat. Med.* (Tokyo) **1999**, *53*, 138−140.
(3) Okada, Y.; Miyauchi, N.; Suzuki, K.; Kobayashi, T.; Tsutsui, C.; Mayuzumi, K.; Okuyama, T. *Nat. Med.* (Tokyo) **1994**, *48*, 324−329.
(4) Bing-nan, Z.; Gabor, B.; Geoffrey, A. C. *Phytochemistry* **1988**, *27*, 3633−3636.
(5) William, G.; Robert, M. H.; Bruno G.; Jean C.; Marie-L. B. *Tetrahedron* **1978**, *34*, 3089−3096.
(6) Klyne, W. *Proc. Biochem. Soc.***1950**, *47*, xli−xlii.
(7) Kreis, W.; Tamm, Ch.; Reichstein, T. *Helv. Chim. Acta* **1957**, *40*, 593−604.
(8) Peter, U.; Anthony, C. *Recent Prog. Horm. Res.* **2001**, *56*, 1−21.
(9) Morimitsu, Y.; Yoshida, K.; Esaki, S.; Hirota, A. *Biosci. Biotech. Biochem.* **1995**, *59*, 2018−2021.
(10) Lou, H.; Yuan, H.; Yamazaki, Y.; Sasaki, T.; Oka, S. *Planta Med.* **2001**, *67*, 345−349.
(11) Nakamura, S.; Makita, Z.; Ishikawa, S.; Yasumura, K.; Fujii, W.; Yanagisawa, K.; Kawata, T.; Koike, T. *Diabetes* **1997**, *46*, 895−899.
(12) Singh, R.; Barden, A.; Mori, T.; Beilin, L. *Diabetologia* **2001**, *44*, 129−146.

NP020256D