Erigeroflavanone, a Flavanone Derivative from the Flowers of *Erigeron annuus* with Protein Glycation and Aldose Reductase Inhibitory Activity

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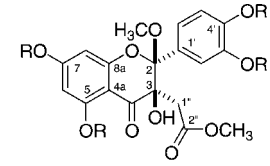
A novel 2,3-dioxygenated flavanone, erigeroflavanone (**1**), as well as eight known flavonoids and two known γ-pyranone derivatives, were isolated from an ethyl acetate-soluble extract of the flowers of *Erigeron annuus*. The structure of compound **1** was elucidated by interpretation of spectroscopic data. All of the isolates were subjected to *in vitro* bioassays to evaluate their inhibitory activity against advanced glycation end products formation and rat lens aldose reductase.

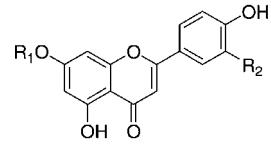
The genus *Erigeron* (Compositae) comprises more than 400 species that occur widely in temperate North America, Europe, and South America.[1] *Erigeron annuus* (L.) Pers., as a naturalized weed, is also found throughout urban and rural areas of Korea and China and has been used in traditional medicine for the treatment of indigestion, enteritis, epidemic hepatitis, and hematuria.[2] Previous phytochemical investigations on this plant resulted in the isolation of γ-pyranone derivatives,[3–5] cyclopentenone derivatives,[6] sesquiterpenoids,[7,8] flavonoids,[3] triterpenoids,[3] and phenolic derivatives.[4,5] In an ongoing project directed toward the discovery of novel treatments for diabetic complications from traditional herbal medicines,[9,10] flowers of *E. annuus* were chosen for more detailed investigation. The ethyl acetate-soluble extract was found to inhibit both advanced glycation end products (AGEs) formation and aldose reductase (AR) *in vitro*. The design and discovery of inhibitors of AGEs formation or AR may offer a promising therapeutic approach for the treatment of diabetic or other pathogenic complications.[11,12]

Repeated chromatography of the ethyl acetate-soluble residue from flowers of *E. annuus* led to the isolation and characterization of a novel flavanone (**1**), eight flavonoids, and two γ-pyranone derivatives. All isolates obtained in the present study were evaluated for their potential to inhibit AGEs formation and AR. The structure elucidation of **1** and the biological evaluation of the isolates are described herein.

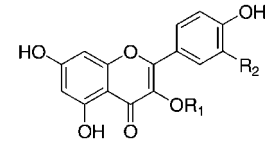
Compound **1** was obtained as a pale yellow powder and gave a sodiated molecular ion $[M + Na]^+$ at *m/z* 429.0781 by HRESIMS, consistent with a molecular formula of $C_{19}H_{18}O_{10}$. Unambiguous NMR assignments were made by application of one- and two-dimensional NMR experiments (^{1}H NMR, ^{13}C NMR, DEPT 135, COSY, NOESY, HMQC, and HMBC). The ^{1}H NMR and COSY spectra of **1** (in CD_3OD) revealed a set of ABX-type signals [δ 7.16 (1H, br s), 6.84 (1H, d, J = 8.1 Hz), and 7.06 (1H, br d, J = 8.1 Hz)], two *meta*-coupled doublets [δ 6.00 (1H, J = 2.1 Hz) and 6.06 (1H, J = 1.8 Hz)], two aliphatic methoxy signals (δ 3.03 and 3.48, 3H each signal), and an AB system [δ 2.23 (1H, J = 13.5 Hz) and 2.72 (1H, J = 13.8 Hz)]. The ^{13}C NMR and DEPT spectra of **1** showed signals for 19 carbon signals, which included two methoxy groups, one methylene, five methines, and 11 quaternary carbons. The chemical shifts of the latter indicated one carbonyl (δ 198.8), one ester carbonyl (δ 171.4), five oxygenated aromatic (δ 168.5, 164.5, 159.0, 147.7, and 146.0), a doubly oxygenated sp^3 (δ 109.6), an oxygenated aliphatic (δ 79.5), and two other aromatic carbons (δ 126.3 and 102.2). Comparison of the above with data in the literature[13,14] suggested that **1** was a 2,3-dioxygenated flavanone bearing five OH groups, two methoxy groups, and an acetic acid group. These assignments were confirmed using 2D NMR techniques. The ^{1}H NMR spectrum of **1** (in DMSO-d_6) included five OH signals at δ 11.22 (OH-5), 10.85 (OH-7), 9.16 (OH-4′), 9.03 (OH-3′), and 5.74 (OH-3). The HMBC correlations (Figure 1) for H_2-1″/C-2, C-3, C-4, C-1″, and OCH_3-1″; 3-OH/C-2, C-3, C-4, and C-1″; OCH_3-2/C-2; and OCH_3-1″/C-2″ confirmed the assignments of all proton and carbon resonances and the location of the acetic acid (C-3), hydroxy (C-3, C-5, C-7, C-3′, and C-4′), and methoxy groups (C-2 and C-1″). Although the absolute configuration at C-2 and C-3 could not be ascertained, the OCH_3-1″ and OH-3 groups were shown to be *cis*-oriented on the basis of NOESY correlations from δ_H 2.72/2.23 (H-1″a/b) to δ_H 7.16 (H-2′) and 7.06 (H-6′). Thus, compound **1** was determined to be *rel*-(2*R*,3*R*)-3,5,7,3′,4′-pentahydroxy-2-methoxy-flavanone-3-acetic acid methyl ester; {methyl 2-[*rel*-(2*R*,3*R*)-3,4-dihydro-3,5,7-trihydroxy-2-(3,4-dihydroxyphenyl)-2-methoxy-4-oxo-2*H*-chromen-3-yl]acetate} and was named erigeroflavanone. Compound **1** was acetylated to afford a tetraacetate derivative (**1a**). Flavonoids oxygenated at both C-2 and C-3 appear to be rare in nature.[14] Some 2,3,5-trihydroxyflavanone isomers and 2,5-dihydroxyflavanone isomers have been reported from *Leptospermum polygalifolium* subsp. *polygalifolium*. The author proposed that the isomers could be interconverted and that the cyclic hemiketal in the flavanones could be opened easily, leading to racemization in solution.[14] Therefore, the small optical rotation ($[\alpha]_D^{25}$ +0.83) of **1** may be due to racemization or to the presence of trace impurities. Although flavone-8-acetic acid (FAA) and its derivatives have been



1 R = H
1a R = Ac



2 $R_1 = R_2 = H$
3 $R_1 = H, R_2 = OH$
4 $R_1 = Glu, R_2 = H$
5 $R_1 = Glm, R_2 = H$



6 $R_1 = R_2 = H$
7 $R_1 = H, R_2 = OH$
8 $R_1 = Glc, R_2 = H$
9 $R_1 = Rha, R_2 = OH$

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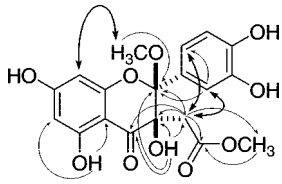

Figure 1. Selected correlations observed in the HMBC (→) and NOESY (↔) NMR spectra of **1** (in DMSO-d_6).

synthesized,[15,16] to the best of our knowledge this is the first report of the isolation of a 2,3-dioxygenated flavanone of this type from nature.

In addition to erigeroflavanone (**1**), 10 known compounds, apigenin (**2**),[13] luteolin (**3**),[13] apigenin-7-*O*-*β*-D-glucuronide (**4**),[13] apigenin-7-*O*-*β*-D-glucuronide methyl ester (**5**),[17] kaempferol (**6**),[18] quercetin (**7**),[13] astragalin (**8**),[18] quercitrin (**9**),[17] 3-hydroxypyran-4-one,[3] and erigeroside,[3] were also isolated in this study. The structures of the known compounds were identified by physical (mp, $[\alpha]_D$) and spectroscopic data (^{1}H NMR, ^{13}C NMR, 2D NMR, and MS) measurement and by comparison with published values.

All isolates obtained in the present study were evaluated for their potential to inhibit advanced glycation end products (AGEs) formation and rat lens aldose reductase (RLAR). Of these, flavonoids with the 3′,4′-dihydroxy group [i.e., the new **1**, luteolin (**3**), quercetin (**7**), and quercitrin (**9**)] exhibited the most inhibitory activity against AGEs formation after incubating at 37 °C for 14 days, with IC_{50} values of 22.7, 23.9, and 28.4 μM, respectively, in agreement with a previous structure–activity study for flavonoids.[19] Compounds **2**, **4**, and **8** also showed activity, with IC_{50} values of 89.9, 107.1, and 178.8 μM, respectively. The positive control, aminoguanidine, had an IC_{50} value of 961 μM. In the RLAR assay, quercitrin (**9**, IC_{50} value of 0.16 μM) exhibited the most potent inhibitory activity, comparable to that of the commercial synthetic aldose reductase inhibitor epalrestate (IC_{50} value of 0.07 μM). Compounds **2**−**8** also showed significant RLAR inhibitory activity, with IC_{50} values of 6.67, 1.54, 1.28, 9.02, 1.33, 7.21, and 5.09 μM, respectively. Compound **1** exhibited weak activity (IC_{50} value of 46.9 μM). Although various flavonoids, including **9**, have been reported as natural AR or protein glycation inhibitors,[19−21] this is the first report of compounds **4** (apigenin-7-*O*-*β*-D-glucuronide) and **5** (apigenin-7-*O*-*β*-D-glucuronide methyl ester) having inhibitory activity for AR or AGEs formation. Compounds **1**, **4**, and **5** may be worth further study of their potential to reduce diabetic complications.

Experimental Section

General Experimental Procedures. Melting points were measured on an IA9100 melting point apparatus (Barnstead International) and are uncorrected. Optical rotations were obtained using a P-2000 digital polarimeter (Jasco, Japan). UV spectra were recorded with a Jasco V-530 spectrometer. IR spectra were recorded using a Jasco FTIR-4100 spectrophotometer. LR- and HR-ESIMS were recorded on a Mariner mass spectrometer (Perspective Biosystem). NMR experiments were conducted on a DRX-300 or AVANCE II 900 FT-NMR (Bruker, Germany), and the chemical shifts were referenced to the residual solvent signals. TLC analyses was performed on Kieselgel 60 F_{254} (Merck) plates (silica gel, 0.25 mm layer thickness); compounds were visualized by dipping plates into 10% (v/v) H_2SO_4 reagent (Aldrich) and then heated at 110 °C for 5–10 min. Silica gel (Merck 60A, 70–230 or 230–400 mesh ASTM), Sephadex LH-20 (Amersham Pharmacia Biotech), and reversed-phase silica gel (YMC Co., ODS-A 12 nm S-150 μm) were used for column chromatography. All solvents used for the chromatographic separations were distilled before use.

Plant Material. Flowers of *Erigeron annuus* were collected in Jeonmin-dong, Yuseong-gu, Daejeon, Korea, in June 2006 and were identified by Professor J.-H. Kim, Division of Life Science, Daejeon University, Daejeon 300-716, Korea. A voucher specimen has been deposited at the Herbarium of the Department of Herbal Pharmaceutical Development (KIOM-ErAn1), Korea Institute of Oriental Medicine, Korea.

Advanced Glycation End Products (AGEs) and Rat Lens Aldose Reductase (RLAR) Assays. Evaluation of plant extracts, solvent partitions, and pure isolates as inhibitors of AGEs formation and RLAR *in vitro* was conducted as described previously.[9,10,22]

Extraction and Isolation. The dried and milled plant material (2.6 kg) was extracted with MeOH (3 × 18 L) by maceration. The extracts were combined and concentrated *in vacuo* at 40 °C to give a MeOH extract (365 g, 14.0%). The extract was suspended in H_2O (2 L) and successively extracted with *n*-hexane (3 × 2 L), EtOAc (3 × 2 L), and BuOH (3 × 2 L) to give *n*-hexane- (62 g), EtOAc- (50 g, 1.92%), BuOH- (79 g), and water-soluble extracts (174 g), respectively. The EtOAc-soluble extract showed the greatest inhibitory activity on AGEs formation (IC_{50} value of 18.6 μg/mL).

The EtOAc-soluble extract was chromatographed over silica gel using a $CHCl_3$−MeOH gradient (from 20:1 to 0:1 v/v) to afford 11 pooled fractions (E1−E11). 3-Hydroxypyran-4-one (537 mg, 0.021%), was purified by recrystallization (MeOH) from fraction E2 [eluted with $CHCl_3$−MeOH (20:1 v/v); 10.38 g]. Fraction E4 [eluted with $CHCl_3$−MeOH (10:1 v/v); 1.34 g] was further fractionated using Sephadex column chromatography (CC) (Φ 3.5 × 74 cm, $CHCl_3$−MeOH = 1:1 v/v) to give apigenin (**2**, 56 mg, 0.0022%) and kaempferol (**6**, 7.4 mg, 0.00028%). Luteolin (**7**, 17 mg, 0.00065%) was obtained from fraction E5 [eluted with $CHCl_3$−MeOH (10:1 v/v); 930 mg] through Sephadex CC (Φ 3.5 × 74 cm, $CHCl_3$−MeOH = 1:1 v/v). Apigenin-7-*O*-*β*-D-glucuronide methyl ester (**5**, 10.5 mg, 0.00040%) was purified by recrystallization (MeOH) from fraction E6 [eluted with $CHCl_3$−MeOH (4:1 v/v); 3.35 g]. The rest of fraction E6 was purified further over a Sephadex column (Φ 5.0 × 65 cm) with MeOH, yielding the new compound (**1**, 80.0 mg, 0.0031%) and quercetin (**7**, 11.0 mg, 0.00042%). Astragalin (**8**, 33 mg, 0.0013%), erigeroside (27 mg, 0.0010%), quercitrin (**9**, 11 mg, 0.00042%), and apigenin-7-*O*-*β*-D-glucuronide (**4**, 67 mg, 0.0026%) were purified from fractions E7, E8, E10, and E11, respectively, by repeated CC.

Erigeroflavanone (1): pale yellow powder (*n*-hexane−EtOAc); mp 205–207 °C; $[\alpha]_D^{25}$ +0.83 (*c* 0.25, MeOH); UV (MeOH) λ_{max} (log ϵ) 290 (4.26) nm; IR ν_{max} (NaCl) 3414, 2922, 2848, 1711, 1664, 1631, 1611, 1582, 1440, 1288, 1201, 1140, 1059, 984, 808, 678 cm^{-1}; ^{1}H NMR (300 MHz, CD_3OD) δ 7.16 (1H, br s, H-2′), 7.06 (1H, br d, J = 8.1 Hz, H-6′), 6.84 (1H, d, J = 8.1 Hz, H-5′), 6.06 (1H, d, J = 1.8 Hz, H-8), 6.00 (1H, d, J = 2.1 Hz, H-6), 3.48 (3H, s, OCH_3-2″), 3.03 (3H, s, OCH_3-2), 2.72 (1H, d, J = 13.8 Hz, H-1″a), 2.23 (1H, d, J = 13.5 Hz, H-1″b); ^{1}H NMR (900 MHz, DMSO-d_6) δ 11.22 (1H, br s, OH-5), 10.85 (1H, br s, OH-7), 9.16 (1H, br s, OH-4′), 9.03 (1H, br s, OH-3′), 7.03 (1H, br s, H-2′), 6.93 (1H, br s, H-6′), 6.80 (1H, d, J = 8.1 Hz, H-5′), 6.04 (1H, d, J = 1.8 Hz, H-8), 6.00 (1H, d, J = 1.8 Hz, H-6), 5.78 (1H, br s, OH-3), 3.39 (3H, s, OCH_3-2″), 2.91 (3H, s, OCH_3-2), 2.63 (1H, d, J = 13.5 Hz, H-1″a), 2.05 (1H, d, J = 13.5 Hz, H-1″b); ^{13}C NMR (75 MHz, CD_3OD) δ 198.8 (C, C-4), 171.4 (C, C-2″), 168.5 (C, C-7), 164.5 (C, C-5), 159.0 (C, C-8a), 147.7 (C, C-4′), 146.0 (C, C-3′), 126.3 (C, C-1′), 121.7 (CH, C-6′), 117.2 (CH, C-2′), 115.8 (CH, C-5′), 109.6 (C, C-2), 102.2 (C, C-4a), 98.0 (CH, C-6), 97.2 (CH, C-8), 79.5 (C, C-3), 52.4 (CH_3, OCH_3-2), 50.0 (CH_3, OCH_3-2″), 42.0 (CH_2, C-1″); ^{13}C NMR (75 MHz, DMSO-d_6) δ 197.2 (C, C-4), 168.7 (C, C-2″), 166.3 (C, C-7), 162.1 (C, C-5), 156.9 (C, C-8a), 146.2 (C, C-4′), 144.4 (C, C-3′), 124.0 (C, C-1′), 119.7 (CH, C-6′), 116.0 (CH, C-2′), 114.7 (CH, C-5′), 108.1 (C, C-2), 100.3 (C, C-4a), 96.6 (CH, C-6), 95.8 (CH, C-8), 77.8 (C, C-3), 51.3 (CH_3, OCH_3-2), 50.4 (CH_3, OCH_3-2″), 41.1 (CH_2, C-1″); ESIMS m/z 429 ([M + Na]$^+$, 38), 375 (100), 157 (24), 135 (33), 115 (48); HRESIMS m/z 429.0781 ([M + Na]$^+$, calcd for $C_{19}H_{18}O_{10}Na$, 429.0792).

Erigeroflavanone 5,7,3′,4′-tetraacetate (1a). A solution of **1** (6.2 mg) in Ac_2O−pyridine (1:1) was stirred overnight. The reaction mixture was evaporated to give a tetracetate (**1a**) as a pale yellow solid: $[\alpha]_D^{25}$ +0.16 (*c* 0.3, MeOH); ^{1}H NMR (300 MHz, CD_3OD) δ 7.62 (1H, br d, J = 8.4 Hz, H-6′), 7.60 (1H, br s, H-2′), 7.35 (1H, d, J = 8.7 Hz, H-5′), 7.00 (1H, d, J = 2.1 Hz, H-8), 6.76 (1H, d, J = 2.1 Hz, H-6), 3.47 (3H, s, OCH_3-2″), 3.07 (3H, s, OCH_3-2), 2.90 (1H, d, J = 14.4 Hz, H-1″a), 2.35 (3H, s, OAc), 2.31 (3H, s, OAc), 2.30 (6H, s, OAc × 2), 2.24 (1H, d, J = 14.4 Hz, H-1″b).

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Supporting Information Available: The ^{1}H and ^{13}C NMR spectra for compound **1**; this material is available free of charge via the Internet at http://pubs.acs.org.

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