Characterization of Caffeoylglucoside Derivatives and Hypouricemic Activity of the Ethyl Acetate Fraction from *Aster glehni*

Min Jeoung Son,[†,‡] Changbae Jin,[†] Yong Sup Lee,[§] Jae Yeol Lee,[‡] and Hyoung Ja Kim[†,*]

[†]*Molecular Recognition Research Center, Future Convergence Research Division, Korea Institute of Science and Technology, Seoul 136-791, Korea. *E-mail: khj@kist.re.kr*
[‡]*Research Institute for Basic Sciences and Department of Chemistry, College of Sciences, Kyung Hee University, Seoul 130-701, Korea*
[§]*Department of Pharmacy, College of Pharmacy, Kyung Hee University, Seoul 130-701, Korea*
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In the search for xanthine oxidase (XOD) inhibitors as anti-gout agents from natural products, various chromatographic separations of the ethyl acetate-soluble fraction of *Aster glehni* (AGEF) led to the isolation of five new caffeoylglucoside derivatives, namely 6′-*O*-caffeoyl-(6*S*,9*R*)-roseoside (**1**), 6′-*O*-caffeoylampelopsisionoside (**2**), 6′-*O*-caffeoylsonchuinoside C (**3**), 6′-*O*-caffeoyldihydrosyringin (**4**), and (2*E*)-2-methyl-but-2-ene-1,4-diol-6′-*O*-caffeoyl-1-*O*-β-glucopyranoside (glehnoside, **5**), together with 13 known compounds. The absolute stereochemistry of the 6′-*O*-caffeoylsonchuinoside C (**3**) was established with the help of spectroscopic analyses, enzymatic hydrolysis, and Mosher's method, as well as in comparison with literature data. All isolated substances were determined for their inhibitory activities on uric acid production by the xanthine/XOD system. Among them, 4,5-*O*-dicaffeoylquinic acid methyl ester (**12**) showed the most potent inhibitory activity with an IC_{50} value of 2.6 ± 0.1 μM, which was comparable to that of allopurinol used as a positive control. Furthermore, hypouricemic effects of AGEF were assessed by measuring serum uric acid levels 3 h after potassium oxonate treatment (250 mg/kg, i.p.) to induce hyperuricemia in rats. When preadministered orally once a day at doses of 50, 100, and 300 mg/kg for 7 days, AGEF reduced the potassium oxonate-induced elevated serum uric acid level by 15.4, 39.8, and 32.3%, respectively. The results suggest that AGEF has the potential to be a new source of agents for the prevention and/or treatment of hyperuricemia and gout.

Keywords: *Aster glehni*, Compositae, Caffeoylglucoside derivatives, Quinic acid derivatives, Hyperuricemia

Introduction

Gout is one of the most common metabolic disorders affecting humans. It is characterized by hyperuricemia, leading to the deposition of urate monohydrate crystals in joints and kidney, which results in gouty arthritis and uric acid nephrolithiasis, respectively.[1] Gout and hyperuricemia increase the risk for inflammation, gouty arthritis, and renal failure, as well as cardiovascular diseases.[2–5] Clinically, therapeutic agents for the treatment of gout and hyperuricemia could help reduce the elevated uric acid levels in the blood.[6] These include xanthine oxidase (XOD) inhibitors such as oxypurinol, tisopurine, and allopurinol, the last being the most often prescribed one. However, these agents available for the treatment of gout patients with hyperuricemia are still limited because of undesirable effects such as hepatitis, nephropathy, and allergic reactions in some patients.[7–9] Natural products have long been recognized as an important source of therapeutically effective agents. Thus, safer and effective XOD inhibitors are still needed for gout treatment. As natural products have been versatile sources in providing lead drugs for further development, exploration of potential XOD inhibitors from medicinal plants has been attempted in this study.

Aster glehni Fr. Schm (Compositae) is the principal endemic vegetable for food distributed in Ulleung Island, Korea. The leaves of *A. glehni* have been reported to exhibit anticonvulsant, sedative, antioxidant, anti-inflammatory, and antiadipogenic effects, and has been shown to prevent increase in the atherogenic index and body weight in high-fat-diet-induced rats.[10–13] Previous investigations have shown that numerous terpenoids, quinic acid derivatives, and flavonoids could be isolated from this plant.[10,14]

In our preliminary study, the ethyl acetate fraction from aerial parts of *A. glehni* (AGEF) was found to show XOD inhibitory effects. Accordingly, in this work, AGEF was subjected to repeated chromatographic separations to examine which constituents possessed XOD inhibitory activities. The chromatographic separations yielded five new caffeoylglucoside derivatives: 6′-*O*-caffeoyl-(6*S*,9*R*)-roseoside (**1**), 6′-*O*-caffeoylampelopsisionoside (**2**), 6′-*O*-caffeoylsonchuinoside C (**3**), 6′-*O*-caffeoyldihydrosyringin (**4**), and (2*E*)-2-methyl-but-2-ene-1,4-diol-6′-*O*-caffeoyl-1-*O*-β-glucopyranoside (glehnoside, **5**), together with 13 known compounds: 5-*O*-caffeoylqunic acid (**6**), 3,4-*O*-dicaffeoylquinc acid (**7**), 3,5-*O*-dicaffeoylquinc acid (**8**), 3,5-*O*-*epi*-dicaffeoylquinc acid (**9**), 3,5-*O*-dicaffeoylquinc acid methyl ester (**10**), 4,5-*O*-

dicaffeoylquinc acid (**11**), 4,5-*O*-dicaffeoylquinc acid methyl ester (**12**),[15–17] 6-*O*-caffeoylglucose (**13**),[18] quercetin 3-*O*-glucopyranoside (**14**), kaempferol 3-*O*-glucopyranoside (**15**),[19] (*Z*)-3-hexenyl-β-D-glucopyranoside (**16**),[20] benzyl-β-D-glucopyranoside (**17**), and α-spinasterol 3-*O*-glucopyranoside (**18**) (Figure 1).[21,22] Here we report the isolation and structure elucidation of five new caffeoylglucoside derivatives along with the inhibitory activities of all isolated substances on XOD. Furthermore, hypouricemic effects of AGEF were evaluated using a rat model of potassium oxonate-induced hyperuricemia.

Experimental

Plant Material. Aerial parts of *A. glehni* were collected from Ulleung Island, Korea, during August, 2007 and identified by Prof. (Emer.) Chang-Soo Yook (Department of Pharmacognosy, Kyung Hee University). The voucher specimens (971-12A) were deposited in the herbarium of the Korea Institute of Science and Technology.

Extraction and Isolation. Chopped and air-dried aerial parts of *A. glehni* (2.7 kg) were extracted three times with methanol (26 L) at room temperature to give a methanol-soluble extract. The dried extract residue (394.8 g) was suspended in water and then partitioned in turn with dichloromethane, ethyl acetate, and *n*-butanol. The ethyl acetate fraction was evaporated under reduced pressure to yield 22.0 g of a residue. A part of this residue (14.6 g) was divided into nine fractions (E1–E9) by Sephadex LH-20 CC using MeOH as eluent. Fraction E3 (1.48 g) was purified by Sephadex LH-20 CC using MeOH/H_2O (70:30, v/v) as eluent to give four subfractions (E3a–E3j). The subfraction E3d (614.4 mg) was subjected to LiChroprep RP-18 eluted with MeOH/H_2O (45:55→75:25, v/v) and purified by silica gel CC using CH_2Cl_2/MeOH/H_2O (6.5:1:0.1→5:1:0.1, v/v) as eluent to give compounds **4** (8.7 mg) and **5** (15.6 mg). Fraction E3d5 (112.1 mg) was purified by silica gel CC using CH_2Cl_2/MeOH (6.5:1, v/v) as eluent to give compound **1** (11.8 mg). Fraction E3d6 (54.4 mg) was purified using preparative HPLC (MeOH/H_2O, 50:50, v/v) to give compounds **2** (5.7 mg) and **3** (9.2 mg). Subfraction

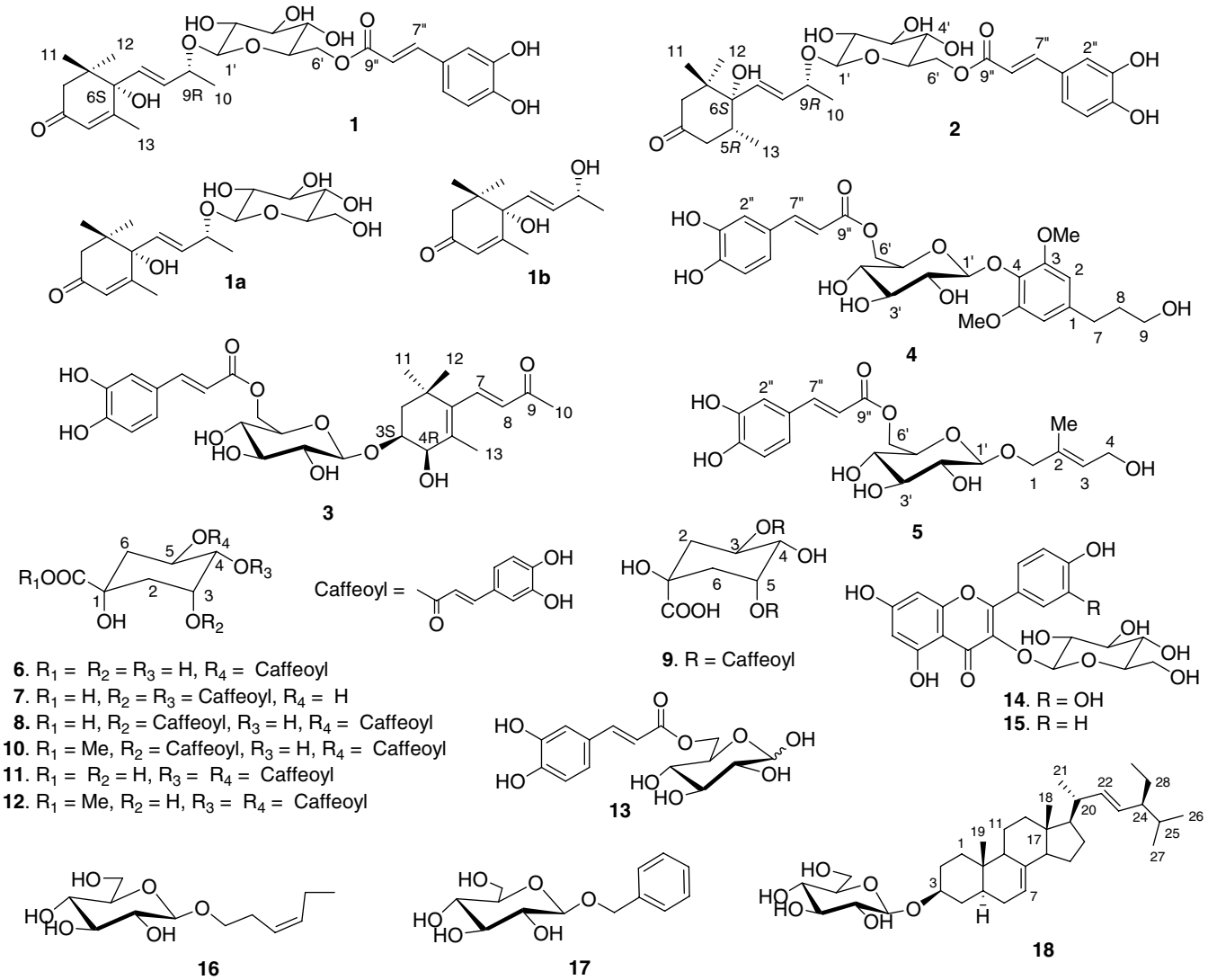

Figure 1. Structures of compounds **1–18** isolated from *Aster glehni.*

E3c (385.7 mg) was further purified by LiChroprep RP-18 using MeOH/H_2O (45:55→75:25, v/v) as eluting solvent to give six fractions (E3c1–E3c6). Fraction E3c3 (37.0 mg) was purified by preparative HPLC (MeOH/H_2O, 50:50, v/v) to give compounds **16** (4.6 mg) and **17** (5.7 mg). Fraction E4 (10.7 g) was divided into 11 fractions (E4a–E4k) by Sephadex LH-20 CC using MeOH/H_2O (70:30, v/v) as eluent. Compounds **6** (14.2 mg) and **13** (7.0 mg) were obtained from Fr. E4d (68.2 mg) using preparative RP-18 TLC (MeOH/H_2O, 30:70). Fraction E4e (3.23 g) was subjected to Sephadex LH-20 CC using MeOH/H_2O (70:30, v/v) as eluent to give four subfractions (E4e1–E4e4). Fraction E4e2 (1.60 g) was further purified by LiChroprep RP-18 CC using MeOH/H_2O (30:70→45:55, v/v) as eluent to give compounds **10** (611.7 mg), **14** (560.5 mg), and **15** (147.2 mg). Fraction E4f (4.68 g) was subjected to Sephadex LH-20 CC using MeOH/H_2O (70:30, v/v) to give six subfractions (E4f1–E4f6). Fraction E4f1 (680 mg) was purified by LiChroprep RP-18 CC using MeOH/H_2O (30:70→45:55, v/v) as eluent to give compounds **7** (7.7 mg) and **8** (451.5 mg). Fraction E4f3 (3.98 mg) was subjected to LiChroprep RP-18 CC eluted with MeOH/H_2O (35:65→45:55, v/v) to give compounds **8** (2.44 g), **9** (15.0 mg), **10** (486.3 mg), and **12** (315.5 mg). Subfraction E4g (1.59 g) was purified by LiChroprep RP-18 CC using MeOH/H_2O (30:70→45:55, v/v) as eluting solvent to afford compound **11** (741.5 mg). Compound **18** (26.1 mg) was obtained from the ethyl acetate fraction by crystallization in MeOH. Thirteen known compounds (**6–18**) were identified by comparison of their physical and spectroscopic data ($[\alpha]_D$, IR, ^{1}H and ^{13}C NMR, and MS) with those found in the literature.

6′-*O*-Caffeoyl-(6*S*, 9*R*)-roseoside (1): Yellowish amorphous powder; $[\alpha]_D^{20}$ +42.8° (*c* 0.5, MeOH); UV (MeOH) λ_{max}: 222, 323 nm; IR (KBr) ν_{max}: 2955, 2916, 2849, 1733, 1463, 1377, 1217, 1022, 761 cm^{-1}; CD (MeOH) Δε (nm): +24.0 (240), −2.57 (314); ^{1}H NMR (CD_3OD, 400 MHz) and ^{13}C NMR (CD_3OD, 100 MHz), see Table 1; ^{1}H NMR (pyridine-d_5, 400 MHz) δ 7.89 (1H, d, *J* = 16.0 Hz, H-7″),

Table 1. ^{1}H (400 MHz) and ^{13}C NMR (100 MHz) spectroscopic data of compounds **1–3** (CD_3OD, δ in ppm, *J* in Hz).

	1		**2**		**3**	
No.	δ_H	δ_C	δ_H	δ_C	δ_H	δ_C
1	—	42.1	—	43.9	—	37.9
2α	2.06 (d, 16.8)	50.4	1.69 (dd, 2.4, 13.6)	52.4	1.54 (dd, 2.4, 12.4)	40.2
2β	2.40 (d, 16.8)		2.75 (d, 13.6)		1.82 (d, 12.8)	
3	—	200.9	—	214.9	3.86 (td, 3.6, 12.8)	75.9
4	5.78 (br s)	126.9	1.98 (ddd, 2.0, 4.4, 14.0) 2.32 (t, 13.6)	46.1	4.01 (d, 3.2)	70.2
5	—	166.8	2.14 (dq, 14.0, 6.4)	37.9	—	132.5
6	—	79.7	—	78.0	—	141.2
7	5.77 (d, 2.4)	131.4	5.61 (d, 16.0)	134.5	7.11 (d, 16.8)	144.2
8	5.77 (d, 2.4)	134.5	5.79 (dd, 6.4, 15.6)	134.5	6.01 (d, 16.4)	134.6
9	4.33 (overlapped)	76.8	4.34 (overlapped)	78.0	—	200.9
10	1.23 (d, 6.4)	20.9	1.24 (d, 6.4)	21.7	2.21 s	27.3
11	0.95 s	23.1	0.796 s	25.0	1.01 s	27.9
12	0.93 s	24.3	0.883 s	25.3	0.97 s	30.2
13	1.81 (d, 1.2)	19.4	0.77 (d, 6.4)	16.4	1.78 s	20.0
1′	4.33 (d, 8.0)	102.7	4.31 (d, 7.6)	103.0	4.45 (d, 7.6)	103.2
2′	3.14 (dd, 8.0, 9.2)	74.9	3.15 (dd, 7.8, 9.1)	75.3	3.20 (dd, 8.0, 9.2)	75.3
3′	3.28 m	77.6	3.31 m	77.9	3.49 (t, 9.2)	77.8
4′	3.25 m	71.2	3.29 m	71.6	3.26 (overlapped)	72.0
5′	3.41 m	75.1	3.41 m	75.4	3.51 m	75.6
6′a	4.23 (dd, 5.6, 12.0)	64.2	4.19 (dd, 5.6, 11.6)	64.3	4.30 (dd, 6.8, 12.0)	64.8
6′b	4.38 (dd, 2.0, 12.0)		4.37 (dd, 2.0, 12.0)		4.41 (dd, 2.4, 12.0)	
1″	—	127.3	—	127.7	—	127.6
2″	6.98 (d, 2.0)	114.9	6.97 (d, 2.0)	115.2	6.94 (d, 2.0)	115.1
3″	—	146.5	—	146.9	—	146.9
4″	—	149.3	—	149.7	—	149.7
5″	6.71 (d, 8.0)	116.2	6.69 (d, 8.0)	116.5	6.70 (d, 8.0)	116.5
6″	6.89 (dd, 2.0, 8.4)	122.7	6.87 (dd, 2.0, 8.0)	122.9	6.84 (dd, 2.0, 8.4)	123.0
7″	7.50 (d, 16.0)	146.9	7.48 (d, 15.6)	147.2	7.48 (d, 16.0)	147.2
8″	6.23 (d, 15.6)	114.6	6.24 (d, 16.0)	114.9	6.18 (d, 15.6)	114.9
9″	—	168.9	—	169.2	—	168.9

7.52 (1H, d, J = 2.0 Hz, H-2″), 7.18 (1H, d, J = 8.0 Hz, H-5″), 7.07 (1H, dd, J = 2.0, 8.0 Hz, H-6″), 6.56 (1H, d, J = 16.0 Hz, H-8″), 6.27 (1H, dd, J = 6.0, 16.0 Hz, H-8), 6.17 (1H, d, J = 16.0 Hz, H-7), 6.05 (1H, br s, H-4), 4.97 (1H, dd, J = 2.0, 12.0 Hz, H-6′a), 4.93 (1H, d, J = 7.6 Hz, H-1′), 4.89 (1H, dd, J = 5.2, 12.0 Hz, H-6′b), 4.70 (1H, quintet, J = 6.4 Hz, H-9), 4.22 (1H, t, J = 8.8 Hz, H-3′), 4.16 (1H, t, J = 9.6 Hz, H-4′), 4.03 (1H, t, J = 8.0 Hz, H-2′), 3.98 (1H, t, m, H-5′), 2.66 (1H, d, J = 16.8 Hz, H-2a), 2.38 (1H, d, J = 16.8Hz, H-2a), 1.96 (3H, d, J = 1.2 Hz, H-13), 1.36 (3H, d, J = 6.4 Hz, H-10), 1.22, 1.13 (each 3H, s, H-11, 12); HR-ESI-MS *m/z* 571.2159 $[M + Na]^+$ (calcd for $C_{28}H_{36}O_{11}Na$, 571.2155).

6′-*O*-Caffeoyl-(5*R*, 6*S*, 9*R*)-ampelopsisionoside (2): Yellowish amorphous powder; $[\alpha]_D^{20}$ −9.4° (*c* 0.4, MeOH); UV (MeOH) λ_{max}: 218, 324 nm; IR (KBr) ν_{max}: 2955, 2917, 2850, 1733, 1463, 1377, 1215, 1022, 761 cm^{-1}; CD (MeOH) Δε (nm): +1.13 (248), −1.93 (328); ^{1}H NMR (CD_3OD, 400 MHz) and ^{13}C NMR (CD_3OD, 100 MHz), see Table 1; HR-ESI-MS *m/z* 573.2311 $[M + Na]^+$ (calcd for $C_{28}H_{38}O_{11}Na$, 573.2312).

6′-*O*-Caffeoyl-(3*S*, 4*R*)-sonchuionoside C (3): Yellowish amorphous powder; $[\alpha]_D^{20}$ −35.6° (*c* 0.4, MeOH); UV (MeOH) λ_{max}: 217, 293, 326 nm; IR (KBr) ν_{max}: 2955, 2917, 2850, 1735, 1462, 1377, 1217, 1018, 761 cm^{-1}; CD (MeOH) Δε (nm): +1.30 (268.6), −2.44 (300.0); ^{1}H NMR (CD_3OD, 400 MHz) and ^{13}C NMR (CD_3OD, 100 MHz), see Table 1; HR-ESI-MS *m/z* 571.2160 $[M + Na]^+$ (calcd for $C_{28}H_{36}O_{11}Na$, 571.2155).

6′-*O*-Caffeoyldihydrosyringin (4): Yellowish amorphous powder; $[\alpha]_D^{20}$ −51.2° (*c* 0.5, MeOH); UV (MeOH) λ_{max}: 217, 237, 330 nm; IR (KBr) ν_{max}: 2955, 2916, 2849, 1734, 1463, 1377, 1215, 1024, 760 cm^{-1}; ^{1}H NMR (CD_3OD, 400 MHz) and ^{13}C NMR (CD_3OD, 100 MHz), see Table 2; HR-ESI-MS *m/z* 559.1793 $[M + Na]^+$ (calcd for $C_{26}H_{32}O_{12}Na$, 559.1791).

Glehnoside (5): Yellowish amorphous powder; $[\alpha]_D^{20}$ −26.2° (*c* 0.7, MeOH); UV (MeOH) λ_{max}: 216, 243, 326 nm; IR (KBr) ν_{max}: 2955, 2917, 2849, 1737, 1466, 1378, 1217, 1020, 763 cm^{-1}; ^{1}H NMR (CD_3OD, 400 MHz) and ^{13}C NMR (CD_3OD, 100 MHz), see Table 2; HR-ESI-MS *m/z* 449.1422 $[M + Na]^+$ (calcd for $C_{20}H_{26}O_{10}Na$, 449.1424).

Enzymatic Hydrolysis of 1 and 3. A solution of compound **1** (6.0 mg) and cellulase (15.0 mg) in water (1 mL) was shaken for 12 h at 37 °C. The reaction mixture was extracted in turn with CH_2Cl_2 and EtOAc three times each, and evaporating the organic solvents *in vacuo*. The organic residues were

Table 2. ^{1}H (400 MHz) and ^{13}C NMR (100 MHz) spectroscopic data of compounds **4** and **5** (CD_3OD, δ in ppm, J in Hz).

	4		**5**	
No.	δ_H	δ_C	δ_H	δ_C
1	—	140.4	4.12, 4.00 (each d, 12.0)	75.1
2	6.43 s	107.0	—	135.4
3	—	154.1	5.59 (t-like, 6.4)	128.5
4	—	133.9	4.06 (dd, 2.4, 6.4)	59.1
5	—	154.1	1.63 s	14.1
6	6.43 s	107.0	—	—
7	2.49 (dd, 7.6, 8.0)	33.2	—	—
8	1.70 m	35.2	—	—
9	3.45 (overlapped)	62.1	—	—
OMe	3.74	56.8	—	—
1′	4.71 (d, 7.6)	105.3	4.24 (d, 7.6)	102.8
2′	3.45 (overlapped)	75.5	3.17 (t, 8.4)	75.4
3′	3.67 m	77.7	3.30 m	78.0
4′	3.67 m	71.8	3.28 m	71.8
5′	3.67 m	75.5	3.43 m	75.3
6′a6′b	4.27 (dd, 5.2, 11.2) 4.38 (dd, 2.0, 12.4)	64.4	4.27 (dd, 6.0, 11.6) 4.42 (dd, 2.0, 11.6)	64.6
1″	—	127.6	—	127.8
2″	6.96 (d, 2.0)	114.9	6.98 (d, 2.0)	115.1
3″	—	146.7	—	146.9
4″	—	149.5	—	149.7
5″	6.73 (d, 8.0)	116.4	6.71 (d, 8.4)	116.5
6″	6.85 (dd, 2.0, 8.4)	122.9	6.88 (dd, 2.0, 8.0)	123.0
7″	7.42 (d, 16.0)	146.8	7.51 (d, 16.0)	147.2
8″	6.13 (d, 16.0)	114.8	6.23 (d, 16.0)	114.9
9″	—	168.8	—	169.3

purified by preparative TLC on RP-18 developed with MeOH/H_2O (40:60, v/v) to yield the roseoside **1a** (1.0 mg) from the CH_2Cl_2 residue, and aglycone **1b** (0.5 mg) and (*E*)-caffeic acid from the EtOAc residue. Compound **3** (3.0 mg) was hydrolyzed in the same way to yield the aglycone **3a** (1.6 mg) from CH_2Cl_2 residue and (*E*)-caffeic acid from EtOAc residue. The aqueous layer was passed through Sephadex LH-20 (MeOH) to give D-glucose as a syrup, which was identified by comparison of the ^{1}H NMR spectral data and the optical rotation value $\{[\alpha]_D^{25} +51.3^\circ$ (*c* 0.3, $H_2O)\}$ with the authentic sample.

Compound 1a: Colorless amorphous powder, ^{1}H NMR (pyridine-d_5, 400 MHz) δ 6.29 (1H, dd, *J* = 6.8, 16.0 Hz, H-8), 6.10 (1H, d, *J* = 16.0 Hz, H-7), 6.04 (1H, br s, H-4), 4.98 (1H, overlapped, H-1′), 4.70 (1H, quintet, *J* = 6.8 Hz, H-9), 4.52 (1H, dd, *J* = 1.6, 12.0 Hz, H-6′a), 4.27 (1H, dd, *J* = 6.0, 12.0 Hz, H-6′b), 4.22 (1H, t, *J* = 9.0 Hz, H-3′), 4.13 (1H, t, *J* = 8.8 Hz, H-4′), 4.01 (1H, t, *J* = 8.4 Hz, H-2′), 3.88 (1H, t, m, H-5′), 2.64 (1H, d, *J* = 16.4 Hz, H-2b), 2.36 (1H, d, *J* = 16.4Hz, H-2a), 1.97 (3H, d, *J* = 1.2 Hz, H-13), 1.34 (3H, d, *J* = 6.4 Hz, H-10), 1.22, 1.12 (each 3H, s, H-11, 12).

Compound 1b: Colorless, ^{1}H NMR (CD_3OD, 600 MHz) δ 5.82 (1H, br s, H-4), 5.75 (1H, dd, *J* = 4.8, 15.6 Hz, H-8), 5.71 (1H, d, *J* = 15.6 Hz, H-7), 4.26 (1H, dd-like, *J* = 4.4, 6.8 Hz, H-9), 2.42 (1H, d, *J* = 17.2 Hz, H-2), 2.10 (1H, d, *J* = 17.2 Hz, H-2), 2.85 (3H, d, *J* = 1.2 Hz, H-13), 1.18 (3H, d, *J* = 6.4 Hz, H-10), 0.98, 0.96 (each 3H, s, H-11, 12).

Compound 3a: Colorless amorphous powder, $[\alpha]^{25}$ –42.5° (*c* 0.08, MeOH); CD (MeOH) Δε (nm): +3.65 (223.8), +5.29 (275.0), –3.30 (314.4); ^{1}H NMR (CD_3OD, 400 MHz) δ: 7.21 (1H, d, *J* = 16.8 Hz, H-7), 6.08 (1H, d, *J* = 16.4 Hz, H-8), 3.81 (1H, d, *J* = 4.0 Hz, H-4), 3.72 (1H, dt, *J* = 12.8, 4.0 Hz, H-3), 2.25 (3H, s, H-10), 1.76 (1H, br t, *J* = 12.4 Hz, H-2β), 1.81 (3H, s, H-13), 1.41 (1H, dd, *J* = 12.4, 2.0 Hz, H-2α), 1.06 (3H, s, H-11), 1.02 (3H, s, H-12); HR-ESI-MS *m/z* 247.1311 $[M + Na]^+$ (calcd for $C_{13}H_{20}O_3Na$, 247.1310).

Preparation of (*R*)-MTPA Ester (3b) and (*S*)-MTPA Ester (3c) of 3a. A stirred solution of **3a** (0.8 mg) in pyridine (500 μL) was treated with (*S*)-(+)-α-methoxy-α-trifluoromethylphenyl acetic acid chloride (MTPA-Cl, 5 μL) in the presence of 1-ethyl-3-(3-dimethoxylaminopropyl)-carbodiimide hydrochloride (EDC·HCl, 1.5 mg) and 4-methylaminopyridine (4-DMAP, 0.7 mg), and the mixture was stirred at room temperature for 14 h. After cooling, the reaction mixture was poured into water and extracted with CH_2Cl_2. The CH_2Cl_2 extract was successively washed with 5% aqueous HCl, saturated aqueous $NaHCO_3$, and brine, then dried over anhydrous Na_2SO_4 and filtered. Removal of the solvent from the filtrate under reduced pressure furnished a residue, which was separated by preparative TLC on silica gel developed with CH_2Cl_2–MeOH (30:1, v/v) to give the (*R*)-MTPA ester **3b** (0.4 mg). The (*S*)-MTPA ester **3c** (0.4 mg) was obtained through a similar procedure from **3a** using (*R*)-(−)-MTPA-Cl, EDC · HCl and 4-DMAP.

Compound 3b: ^{1}H NMR (CD_3OD, 600 MHz) δ 7.203 (1H, d, *J* = 16.2 Hz, H-7), 6.111 (1H, d, *J* = 16.2 Hz, H-8), 5.121 (1H, dt, *J* = 12.6, 4.2 Hz, H-3), 4.165 (1H, d, *J* = 4.2 Hz, H-4), 2.273 (3H, s, H-10), 1.940 (1H, br t, *J* = 13.2 Hz, H-2β), 1.753 (3H, s, H-13), 1.495 (1H, ddd, *J* = 13.2, 3.6, 1.2 Hz, H-2α), 1.136 (3H, s, H-11), 1.101 (3H, s, H-12).

Compound 3c: ^{1}H NMR (CD_3OD, 600 MHz) δ 7.252 (1H, d, *J* = 16.8 Hz, H-7), 6.152 (1H, d, *J* = 16.2 Hz, H-8), 5.176 (1H, dt, *J* = 12.6, 4.2 Hz, H-3), 4.103 (1H, d, *J* = 4.2 Hz, H-4), 2.314 (3H, s, H-10), 2.075 (1H, br t, *J* = 12.6 Hz, H-2β), 1.857 (3H, s, H-13), 1.702 (1H, ddd, *J* = 12.6, 3.6, 1.2 Hz, H-2α), 1.136 (3H, s, H-11), 1.121 (3H, s, H-12).

Superoxide Anion Radical Scavenging Assay. The superoxide anion radical scavenging activity was determined by the method previously described.[23] The reaction mixture contained 0.1 M potassium phosphate buffer (pH 7.5), 1 mM xanthine, 1 mM EDTA, 250 μM NBT, and various concentrations of extracts or compounds in the 96-well plates. Reaction was started by adding 0.05 U/mL XOD (EC 1.2.3.2), and the reaction mixture was incubated at 37 °C for 20 min. The absorbance of each sample was measured spectrophotometrically at 540 nm, and IC_{50} values denote the concentration of samples required to scavenge 50% superoxide anion radicals.

XOD Inhibitory Assay. The inhibitory activity on XOD was measured spectrophotometrically at 295 nm under aerobic condition according to the method described with slight modifications.[24] The assay mixture consisting of 10 μL of sample solution, 50 μL of 0.1 M phosphate buffer (pH 7.5), and 10 μL of enzyme solution was prepared immediately before use. After preincubation at 25 °C for 10 min, the reaction was initiated by adding 30 μL of 0.1 mM xanthine solution. This assay mixture was incubated at 25 °C for 30 min. The reaction was stopped by adding 10 μL of 1 N HCl, and the absorbance of the mixture at 295 nm was measured spectrophotometrically. The inhibitory activity on XOD was assessed as the percentage inhibition, and the IC_{50} values denote the concentration of samples required to inhibit 50% XOD activity.

Hypouricemic Effects of AGEF on Serum Uric Acid Levels in Potassium Oxonate-induced Hyperuricemic Rats. Male Sprague–Dawley rats of age 12–16 weeks (200 g ± 20% in weight), purchased from Oriental-Bio Co. (Gyeonggi, Korea), were kept in the animal polycarbonate house with three animals per cage. The animals were maintained on a 12-h light/dark cycle, at a temperature of 23 ± 3 °C and a relative humidity of 55 ± 15%, and were allowed free access to standard food pellets (DreamBio Co., Seoul, Korea) and tap water for 1 week prior to the experiment. All animals were housed and all experiments performed according to the policies and guidelines of the Institutional Animal Care and Use Committee of the Korea Animal Medicinal Science Institute (KAMSI-IACUC), Gyeonggi, Korea (publication no: 14-KE-161).

Following adaption for a week, the animals were treated orally once a day with vehicle (sterile water for injection), AGEF, or allopurinol for 6 days. On the seventh day, potassium oxonate (250 mg/kg, i.p.) was injected to induce hyperuricemia according to the method described with slight modifications[7,8] 1 h before final administration of the samples. Rats were randomly divided into six groups of 10 animals

each. Group I served as normal control without any drug treatment and injected only with the vehicle. Group II was injected with potassium oxonate following oral administration of the vehicle. Groups III, IV, and V were injected with potassium oxonate following oral administration of AGEF at doses of 50, 100, and 300 mg/kg. Group VI was injected with potassium oxonate following oral administration of allopurinol used as a positive control at a dose of 50 mg/kg. All sample solutions were administered at a final volume of 2 mL/kg. Three hours after potassium oxonate injection, blood samples were taken from the jugular vein and allowed to clot for 15 min at ambient temperature, and centrifuged at 3000 rpm for 10 min to obtain serum, which was stored at −20 °C until assayed. Serum uric acid concentrations were measured by the auto blood analyzer (7020 Hitachi, Japan).

Statistical Analysis. Data were expressed as mean ± SEM. Statistical significance was assessed by the one-way analysis of variance test followed by the Newman–Keuls test for multiple comparisons. A value of $p < 0.01$ was considered statistically significant.

Results and Discussion

Identification of New Compounds 1–5. Compound **1** was isolated as an amorphous powder. The molecular formula of **1** was deduced to be $C_{28}H_{36}O_{11}$ by high-resolution electrospray ionization mass spectrometry (HR-ESI-MS) at *m/z* 571.2159 $[M + Na]^+$ (calcd for $C_{28}H_{36}O_{11}Na$: 571.2155) and ^{13}C NMR data. Its ^1H NMR spectrum showed four methyl groups: the two singlets at δ_H 0.93 and 0.95, and the two doublets at δ_H 1.23 (J = 6.4 Hz) and 1.81 (J = 1.2 Hz). The ^1H and ^{13}C NMR spectra (Table 1) of **1** showed three aromatic protons at δ_H 6.98 (d, J = 2.0 Hz, H-2″), 6.89 (dd, J = 8.4, 2.0 Hz, H-6″), 6.71 (d, J = 8.0 Hz, H-5″) and two doublets at δ_H 7.50 (d, J = 16.0 Hz, H-7″), 6.23 (d, J = 15.6 Hz, H-8″) of trans double bond, and a carboxyl group (δ_C 168.9), and two hydroxyl bearing aromatic carbons (δ_C 149.3 and 146.5). These findings suggested the presence of signals corresponding to a caffeoyl moiety, which was confirmed by heteronuclear multiple bond correlation (HMBC) experiments. In the HMBC spectrum, the signal at δ_C 168.7 (C-9″) of the caffeoyl moiety was correlated with the signals at δ_H 4.38 and 4.23 (H-6′) of a sugar moiety, indicating that the caffeoyl group was located at the C-6′ position. These observations supported that compound **1** revealed a structural feature similar to that of roseoside isolated from *Alangium premnifolium* except for the caffeoyl moiety.[25] Furthermore, the signal at δ_H 4.33 (H-1′) assigned to the anomeric proton was found to be correlated with the signal at δ_C 76.7 (C-9), suggesting presence of a sugar moiety at the C-9 of the aglycone. The signal of the anomeric proton appeared at δ_H 4.33 (d, J = 8.0 Hz) with coupling constants characteristic of a β-configuration. In particular, by the chemical shift of the carbons at δ_C 102.7 (C-1′), 74.9 (C-2′), 77.6 (C-3′), 71.2 (C-4′), 75.1 (C-5′), and 64.2 (C-6′) in the ^{13}C NMR spectrum of **1** (Table 1), the sugar was identified as β-glucopyranoside.[26] The broad singlet at δ_H 5.78 (H-4) and signal at δ_H 5.77 (2H, d, J = 2.4 Hz, H-7, 8) corresponded to substituted vinyl and the olefin groups. To examine the conformation of H-7/8, more comprehensive ^1H NMR studies on **1** were performed. Its ^1H NMR spectrum in pyridine-d_5 exhibited the resonances of H-8 at δ_H 6.27 (J = 6.0 and 16.0 Hz) and H-7 at δ_H 6.17 (J = 16.0 Hz) (see Figure S2 in Supporting Information and *Experimental* section), indicating that the olefin group of the aglycone was in *trans*-conformation. For further evaluation, enzymatic hydrolysis of **1** with cellulase was carried out, and then yielded roseoside (**1a**), aglycone (vomifoliol, **1b**), and D-glucose along with caffeic acid. Hydrolysates **1a** and **1b** were identified as roseoside and vomifoliol by comparison of their ^1H NMR spectra with those in the literature (see *Experimental* section).[27–30]

The NMR spectra of four stereoisomers of (6*S*,9*S*)-, (6*R*,9*R*)-, (6*S*,9*R*),- and (6*R*,9*S*)-roseoside were similar to each other, but showed slight differences around the C-9 chemical shifts. In the literature, the ^{13}C NMR chemical shifts of the (9*R*)- and (9*S*)-configuration are 77.0 and 74.7, respectively.[30] The absolute configuration at the C-9 of **1** was assigned to the *R*-configuration because the C-9 chemical shift was observed at δ_C 76.7. Stereochemistry at C-6 was established on the basis of circular dichroism (CD) with comparison to literature data.[27,30] It was reported that the (6*S*)-isomers exhibited a positive maximum at 243 nm. The CD extreme values of **1** [240 (Δε +24.0), 314 (−2.27) nm] were similar to those of the (6*S*)-roseoside isomers. Based on the results, the stereochemistry at C-6 in **1** was assigned the *S*-configuration. Therefore, on the basis of the evidence above, structure **1** was finally determined to be 6′-*O*-caffeoyl-(6*S*,9*R*)-roseoside.

Compound **2** was obtained as an amorphous powder with the molecular formula of $C_{28}H_{38}O_{11}$ as deduced from HR–ESI–MS ($[M + Na]^+$ at *m/z* 573.2311). Confirmation of the above molecular formula was obtained by the analysis of the ^{13}C NMR spectroscopic data. The ^1H and ^{13}C NMR spectra (Table 1) of **2** indicated a structural feature similar to that of ampelopsisionoside,[31] except for the presence of the signals of an extra 1,3,4-trisubstituted aromatic ring [δ_H 6.97 (d, J = 2.0 Hz, H-2″), 6.87 (dd, J = 8.0, 2.0 Hz, H-6″), 6.69 (d, J = 8.0 Hz, H-5″)] and two extra doublets at δ_H 7.48 (d, J = 15.6 Hz, H-7″) and 6.24 (d, J = 16.0 Hz, H-8″) in **2**. Its ^{13}C NMR spectrum (Table 1) exhibited one carboxyl group (δ_C 169.2) and two hydroxyl bearing aromatic carbons (δ_C 149.7 and 146.9). These findings suggested the presence of a caffeoyl moiety. Its HMBC correlations (Figure 2) between δ_H 4.19 (H-6′) and δ_C 169.2 (C-9″) suggested that the caffeoyl moiety was connected to C-6′ of glucose through an ester bond. In its ^1H NMR spectrum, the large couplings of H-4_{ax} with H-5 (J = 14.0 Hz) implied that H-5 should be in the axial position and that the CH_3-13 had an equatorial orientation. In addition, the resonances of the axial protons at positions the 2 and 4 were shifted downfield as a result of the 1,3-diaxial interaction of the hydroxyl group at the C-6 position.[31] Furthermore, the 5β-H orientation was assigned by analysis of the nuclear Overhauser enhancement spectroscopy (NOESY) experiments (Figure 2). Correlations between the H-5 with H-7 and

CH_3-11 suggested that CH_3-13 had an equatorial orientation. Therefore, the absolute configurations of the 6,9-dihydroxy megastigman-3-one-7-ene moiety were determined to be 5*R* and 6*S*. The absolute configuration of C-9 was reflected by reported chemical shifts.[30,32] The C-9 chemical shift in **2** was observed at δ_C 78.0 (Table 1), indicating that the absolute configuration at position 9 was determined to be the *R*-configuration. Thus, structure **2** was determined to be 6′-*O*-caffeoyl-(5*R*,6*S*,9*R*)-ampelopsisionoside.

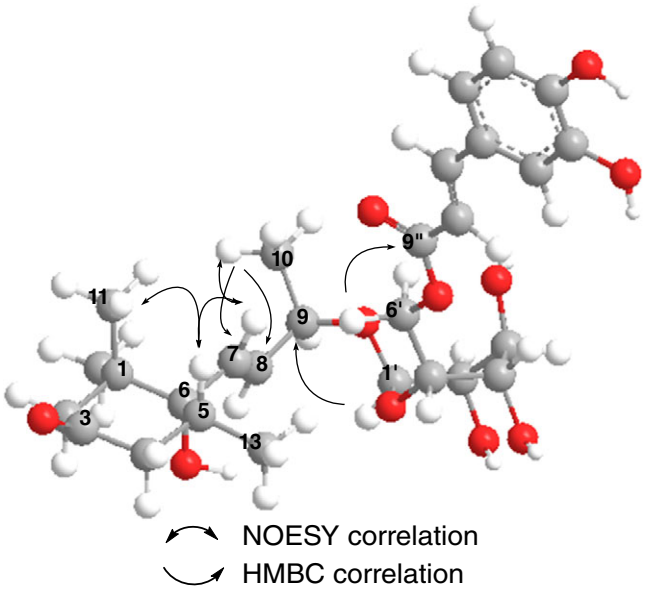

Figure 2. Key HMBC and NOE correlations of compound **2**.

Compound **3** was isolated as an amorphous powder with $[\alpha]_D^{20}$ −35.6° (*c* 0.4, MeOH). The molecular formula of **3** was deduced from HR–ESI–MS ($[M+Na]^+$ at *m/z* 571.2160) and ^{13}C NMR data. The ^{1}H and ^{13}C NMR spectra (Table 1) of **3** showed a structural feature similar to that of sonchuionoside C except for the signals of a caffeoyl moiety.[33] Its ^{1}H NMR spectrum showed a monoterpene moiety: four methyl groups [δ_H 2.21 (s, H-10), 1.01 (s, H-11), 0.97 (s, H-12), 1.78 (s, H-13)], two oxymethines [δ_H 4.01 (d, *J* = 3.2 Hz, H-4), 3.86 (td, *J* = 3.6, 12.8 Hz, H-3)], one methylene [δ_H 1.82 (d, *J* = 12.8 Hz, H-2β), 1.54 (dd, *J* = 12.4, 2.4 Hz, H-2α)], and two doublets at δ_H 7.11 (d, *J* = 16.8 Hz, H-7), 6.01 (d, *J* = 16.4 Hz, H-8) of trans double bond. The composition of the monoterpene unit was determined by its HMBC spectrum, which showed long-range correlations between the following protons and carbons: H-6′ (δ_H 4.41 and 4.30)/C-9″ (δ_C 168.9) and H-1′ (δ_H 4.45)/C-3 (δ_C 75.9). Enzymatic hydrolysis of **3** with cellulase yielded 3,4-dihydroxy-5,7-megastigmadien-9-one (**3a**) and D-glucose along with caffeic acid, which were in good agreement with those reported spectral values.[33] The absolute configurations at C-3 of **3** and **3a** were determined by a modified Mosher's method.[34] Treatment of **3a** with (*S*)- or (*R*)-MTPA-Cl in the presence of 1-ethyl-3-(3-dimethylaminopropyl)carbodiimide (EDC·HCl) and 4-dimethylaminopyridine (4-DMAP) selectively yielded the 3-mono-(*R*)- or (*S*)-MTPA esters (**3b** or **3c**), respectively (Figure 3). Based on the Mosher's result, the absolute configuration at C-3 in **3a** was determined as the *S*-form. From the small coupling constants at δ_H 4.01 (d, *J* = 3.2 Hz, H-4) and δ_H 3.86 (dt, *J* = 12.8, 3.6 Hz, H-3), it is suggested that two hydroxyl groups at C-3 and C-4 are in the *cis*-configuration.[35] Thus, structure **3** was determined to be 6′-*O*-caffeoyl-(3*S*,4*R*)-sonchuionoside C.

Compound **4** was isolated as an amorphous powder, and its molecular formula was established as $C_{26}H_{32}O_{12}$ based on the molecular ion peak at *m/z* 559.1793 $[M+Na]^+$ in the HR-ESI-MS and ^{13}C NMR data. Its ^{1}H NMR spectrum (Table 2) showed five aromatic protons at δ_H 6.96 (d, *J* = 2.0 Hz, H-2″), 6.85 (dd, *J* = 8.4, 2.0 Hz, H-6″), 6.73 (d, *J* = 8.0 Hz, H-5″) of ABX spin system, and one singlet integrated for two protons at δ_H 6.43 (H-2,6) of a 1,2,3,6-tetrasubstituted benzene ring, as well as two doublets at δ_H 7.42 (d, *J* = 16.0 Hz, H-7″), 6.13 (d, *J* = 16.0 Hz, H-8″) of the trans double bond. The ^{1}H and ^{13}C NMR spectra of **4** showed a glucopyranosyl moiety: an anomeric proton [δ_H 4.71 (d, *J* = 7.6 Hz, H-1′)], one methylene [δ_H 4.27 (dd, *J* = 11.2, 5.2 Hz, H-6′a), 4.38 (dd, *J* = 12.4, 2.0 Hz, H-6′b)] and another four methines [δ_H 3.45 (m, H-2′), 3.67 (m, H-3′, 4′, 5′)], and at δ_C 105.3 (C-1′), 75.5 (C-2′), 77.7 (C-3′), 71.8 (C-4′), 75.5 (C-5′), 64.4 (C-6′). The coupling constant (*J* = 7.6 Hz) of the anomeric proton of glucose indicated it to be the β-form. Its ^{13}C NMR spectrum exhibited one carboxyl group (δ_C 168.8) and two hydroxyl bearing aromatic carbons (δ_C 149.6 and 146.7), indicating presence of a caffeoyl moiety. Comparison of its ^{1}H and ^{13}C NMR spectroscopic data (Table 2) with those of dihydroxysyringin isolated from *Ligustrum lucidum* implied that their structures were closely related.[36] The assignment of **4** was further confirmed by the HMBC correlations between H-6′ (δ_H 4.27 and 4.38)/C-9″ (δ_C 168.8), H-1′ (δ_H 4.71)/C-4 (δ_C 133.9), H-7 (δ_H 2.49) /C-1 (δ_C 140.4) and C-2,6 (δ_C 107.0), and H-OCH_3 (δ_H 3.74)/C-3,5 (δ_C 154.1). Thus, structure **4** was determined to be 6′-*O*-caffeoyldihydroxysyringin.

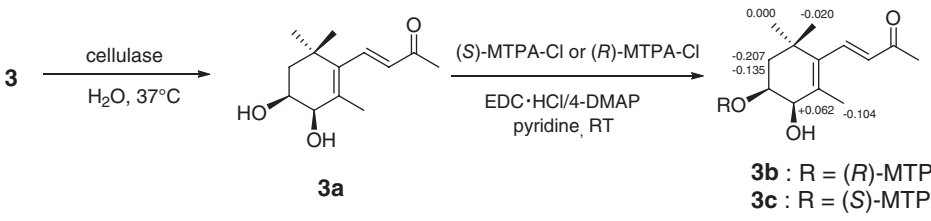

Figure 3. Results of enzymatic hydrolysis and the following Mosher's method of **3a**. Δδ values are in ppm ($\delta_S - \delta_R$).

Compound **5** was isolated as an amorphous powder, and its molecular formula was established as $C_{20}H_{26}O_{10}$ based on the molecular ion peak at *m/z* 449.1422 $[M + Na]^+$ in the HR–ESI–MS and ^{13}C NMR data. The ^1H and ^{13}C NMR spectra (Table 2) of **5** showed a glucopyranosyl moiety, linked in the β-configuration at anomeric position [δ_H 4.24 (d, J = 7.6 Hz, H-1′) and at δ_C 102.8 (C-1′)], and showed hemiterpene moiety: two oxymethylenes [δ_H 4.12, 4.00 (each d, J = 12.0 Hz, H-1) and 4.06 (dd, J = 6.4, 2.4 Hz, H-4)], a vinyl methyl group [δ_H 1.63 (s, H-5)], and an olefinic proton at δ_H 5.59 (t-like, J = 6.4 Hz, H-3). Its ^{13}C NMR spectrum exhibited one methyl carbon (δ_C 14.1), two oxymethylene carbons (δ_C 75.1, 59.1), and two olefinic carbons (δ_C 135.4, 128.5). Its ^1H NMR spectrum further provided evidence for the presence of a caffeoyl group. Comparison of its NMR spectroscopic data with those of (2*E*)-2-methyl-but-2-ene-1,4-diol-1-*O*-β-glucopyranoside isolated from *Ornithogalum montanum* implied that the only difference is in the presence of a caffeoyl group at C-6′ of glucose,[37] which was further confirmed by the HMBC correlations between H-6′ (δ_H 4.27)/C-9″ (δ_C 169.3) and H-1′ (δ_H 4.24)/C-1 (δ_C 75.1). Thus, structure **5** was determined to be (2*E*)-2-methyl-but-2-ene-1,4-diol-6′-*O*-caffeoyl-1-*O*-β-glucopyranoside and trivially named as glehnoside.

Bioactivities. The organic fractions and isolated compounds from *A. glehni* were tested for their XOD inhibitory activities along with superoxide anion radical scavenging effects (Table 3). Among the fractions, AGEF exhibited a marked quenching effect on superoxide anion radicals with an IC_{50} value of 3.3 ± 0.1 μg/mL, and showed the most potent inhibitory effect on XOD (IC_{50} = 4.7 ± 0.1 μg/mL). Among the isolated compounds, 4,5-dicaffeoylquinic acid methyl ester (**12**) displayed the most potent superoxide anion radical scavenging (IC_{50} = 2.5 ± 0.2 μM) and XOD inhibitory (IC_{50} = 2.6 ± 0.1 μM) activities. Surprisingly, the inhibitory activity on XOD was comparable to that of allopurinol (IC_{50} = 3.0 ± 0.3 μM), a well-known XOD inhibitor clinically used for the treatment of gout. 3,5-Dicaffeoylquinic acid methyl ester (**10**), a regioisomer of **12**, also showed potent superoxide anion radical scavenging (IC_{50} = 4.6 ± 0.3 μM) and XOD inhibitory (IC_{50} = 7.2 ± 0.2 μM) activities, although the activities were

Table 3. Superoxide anion radical scavenging and xanthine oxidase (XOD) inhibitory effects of organic fractions and compounds **1–18** from *A. glehni.*

Sample	O_2^- Scavenging activity (IC_{50}, μg/mL)[a]	XOD Inhibitory activity (IC_{50}, μg/mL)[b]
MeOH extract	20.7 ± 0.6	93.2 ± 3.6
CH_2Cl_2 fraction	>500	69.3 ± 3.3
EtOAc fraction	3.3 ± 0.1	4.7 ± 0.1
BuOH fraction	6.4 ± 0.1	33.2 ± 2.4
Compound	O_2^- Scavenging activity (IC_{50}, μM)[a]	XOD Inhibitory activity (IC_{50}, μM)[b]
1	10.9 ± 0.3	42.0 ± 1.4
2	11.4 ± 0.3	93.0 ± 1.7
3	9.1 ± 0.1	73.2 ± 5.1
4	9.7 ± 0.2	71.1 ± 3.9
5	11.0 ± 0.1	80.8 ± 4.9
6	13.6 ± 0.9	82.8 ± 1.0
7	4.3 ± 0.5	35.7 ± 0.5
8	3.5 ± 0.4	36.3 ± 3.5
9	5.1 ± 0.5	40.2 ± 1.5
10	4.6 ± 0.3	7.2 ± 0.2
11	3.6 ± 0.1	11.0 ± 0.6
12	2.5 ± 0.2	2.6 ± 0.1
13	10.7 ± 0.7	75.3 ± 1.3
14	10.7 ± 0.2	76.3 ± 3.9
15	>200	58.7 ± 3.9
16	89.4 ± 0.6	>100
17	>200	>100
18	>200	>100
Ascorbic acid[c]	>200	Not tested
Quercetin[c]	4.9 ± 0.1	Not tested
Allopurinol[c]	Not tested	3.0 ± 0.3

All data are expressed as mean ± SEM.
[a] IC_{50} is the concentration of a sample needed to scavenge 50% of superoxide anion radicals.
[b] IC_{50} is the concentration of a sample needed to inhibit 50% of XOD.
[c] Positive controls.

Table 4. Hypouricemic effects of the ethyl acetate fraction from *A. glehni* (AGEF) and allopurinol on increases in serum uric acid levels induced by potassium oxonate (PO) in rats.

Group	Treatment	Dose (mg/kg/day)	Serum uric acid level (mg/dL)	Reduction (%) [a]
I	Normal control	—	1.01 ± 0.03	—
II	Vehicle + PO	—	2.54 ± 0.12**	—
III	AGEF + PO	50	2.30 ± 0.12	15.4
IV	—	100	1.93 ± 0.14##	39.8
V	—	300	2.04 ± 0.14##	32.3
VI	Allopurinol + PO	50	0.61 ± 0.05##	125.9

[a] % reduction of the serum uric acid level elevated by PO from the normal control level.
Rats were treated orally once a day with vehicle (sterile water for injection), AGEF, or allopurinol for 6 days. On the seventh day, potassium oxonate (250 mg/kg, i.p.) was injected to induce hyperuricemia 1 h before final administration of the samples. Three hours after potassium oxonate injection, blood samples were analyzed for uric acid levels. Data were expressed as mean ± SEM (n = 10). Data were statistically analyzed using the Newman–Keuls test for multiple comparisons.
** $p < 0.01$ compared with the normal control group.
$p < 0.01$ compared with the vehicle-treated hyperuricemic group.

approximately two- to threefold weaker than those of **12**. However, other dicaffeoylquinic acid derivatives (**7–9**) exhibited moderate inhibitory activities on XOD (IC_{50} = 35.7 ± 0.5, 36.3 ± 3.5 and 40.2 ± 1.5 μM, respectively) with still potent superoxide anion radical scavenging activities (IC_{50} = 3.5–5.1 μM). The new compounds **1–5** exhibited moderate superoxide anion radical scavenging activities with IC_{50} values of ~9–11 μM, whereas they showed relatively weak XOD inhibitory activities (IC_{50} = 71.7–93.0 μM), except for **1** (IC_{50} = 42.0 ± 1.4 μM). These results suggest that the structural feature responsible for XOD inhibitory activities of these compounds may be the methyl esters of quinic acid containing two caffeoyl functionalities.

In addition, hypouricemic effects of AGEF were assessed by measuring serum uric acid levels 3 h after potassium oxonate treatment (250 mg/kg, i.p.) to induce hyperuricemia in rats, following subacute oral administration of AGEF (Table 4). Potassium oxonate, a competitive uricase inhibitor, blocks the effect of hepatic uricase and is most frequently employed to create an animal model of hyperuricemia in medical and nutritional investigations.[38] The serum uric acid level of normal control rats was only 1.01 ± 0.03 mg/dL, whereas the level was significantly elevated to 2.54 ± 0.12 mg/dL 3 h after potassium oxonate injection. When administered orally once a day at doses of 50, 100, and 300 mg/kg for 7 days (6-day pretreatment + 1-h post-treatment), AGEF reduced the serum uric acid level elevated by potassium oxonate from the normal control level by 15.4, 39.8, and 32.3%, respectively, with significance only at the higher two doses, compared to the vehicle-treated hyperuricemic group. Moreover, the positive reference drug, allopurinol, treated in the same manner at a dose of 50 mg/kg, remarkably reduced the serum uric acid level to 0.61 ± 0.05 mg/dL which was lower than the normal control level, suggesting substantial inhibition of XOD by allopurinol. These *in-vitro* and *in-vivo* studies suggest that AGEF possesses significant XOD inhibitory activity and can reduce serum uric acid levels to some extent against potassium oxonate-induced hyperuricemia in rats.

Conclusion

In this study, to identify the compounds responsible for XOD inhibition, various chromatographic separations of AGEF led to isolation of five new caffeoylglucoside derivatives **1–5** together with 13 known compounds. All isolated substances were determined for their inhibitory activities on XOD along with superoxide anion radical scavenging activities. Among the isolates, 4,5-*O*-DCQA methyl ester (**12**) showed the most potent XOD inhibitory activity, which was comparable to that of allopurinol used as a positive control. The active principle in AGEF was the 4,5-dicaffeoylquincic acid methyl ester, which was in agreement with a previous report showing that this ester was the principal hypouricemic component in the flowers of *Chrysanthemum sinense*.[39] Furthermore, caffeoylquinic acid derivatives (CQAs) have also been reported to possess hepatoprotective, radical scavenging, and XOD inhibitory activities.[16,40] CQAs are naturally occurring phenolic acids that are distributed widely in plants. However, CQAs and CQA-rich extracts from natural products appear to be valuable therapeutic candidates because of their various biological functions.[41] As AGEF contains high amounts of dicaffeoylquinic acid derivatives, AGEF might be a potential drug candidate for the prevention and/or treatment of hyperuricemia and gout. Although the mechanism of the hypouricemic action of AGEF is not fully elucidated, these natural compounds appear to be a possible alternative to allopurinol.

In conclusion, the results of the present study indicate that orally administered AGEF reduces serum uric acid levels in rats with hyperuricemia induced by potassium oxonate. However, further studies are needed to develop AGEF as the natural XOD inhibitors for the possible health benefit in the prevention and/or treatment of gout.

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Supporting Information. Additional supporting information is available in the online version of this article.

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