Three New Lignan Glycosides with IL-6 Inhibitory Activity from *Akebia quinata*

Hong-Guang Jin,[a,c] A Ryun Kim,[a] Hae Ju Ko,[a] Sang Kook Lee,[b] and Eun-Rhan Woo*[,a]

[a] *College of Pharmacy, Chosun University; Gwangju 501–759, Republic of Korea:* [b] *College of Pharmacy, Natural Products Research Institute, Seoul National University; Seoul 151–742, Republic of Korea: and* [c] *College of Pharmacy, Jilin Medical College; Jilin 132013, China.*

Received August 22, 2013; accepted December 24, 2013

Three new lignan glycosides, akeqintoside A [(7*S*,8*S*)-7,8-dihydro-8-hydroxymethyl-7-(4-hydroxy-3-methoxyphenyl)-1′-benzofuranpropanol 2′-*O*-*β*-D-glucopyranoside] (1), akeqintoside B [(7*R*,8*R*)-7,8-dihydro-8-hydroxymethyl-7-(4-hydroxy-3-methoxyphenyl)-1′-(9′-methoxy-7′-propenyl) benzofuran 2′-*O*-*β*-D-glucopyranoside] (2), and akequintoside C [7*R,8*R**-dihydroxy-7-(4-hydroxy-3-methoxyphenyl)-glycerol 9-*O*-*β*-D-(6′-*O*-caffeoyl)-glucopyranoside] (3) were isolated from *Akebia quinata* along with five known compounds, syringin (4), vanilloloside (5), salidroside (6), 3,4-dihydroxyphenylethyl alcohol 8-*O*-*β*-D-glucopyranoside (7), and calceolarioside B (8). The structures of the compounds were identified based on one dimensional (1D)- and 2D-NMR, including ^{1}H–^{1}H correlation spectroscopy (COSY), heteronuclear single quantum coherence (HSQC), heteronuclear multiple bond connectivity (HMBC) and nuclear Overhauser effect spectroscopy (NOESY) spectroscopic analyses. The inhibitory activity of these isolated compounds against interleukin-6 (IL-6) production in tumor necrosis factor-alpha (TNF-*α*) stimulated MG-63 cells was also examined.**

Key words lignan glycoside; interleukin-6 (IL-6) inhibitory effect; Lardizabalaceae; *Akebia quinata*

Akebia quinata Decaisene (Lardizabalaceae) is a creeping woody vine that is widely distributed in East Asia, including Korea, China, and Japan.[1] Traditionally, its dried stem is used mainly as a diuretic agent for the treatment of edema and rheumatic pain.[2,3] Previous phytochemical investigations resulted in the isolation of triterpenes, triterpene glycosides, and phenylethanoid glycosides.[4–6] Regarding the biological activity of *A. quinata*, only the cytotoxic effect of oleanane disaccharides has been reported so far.[7] The anti-inflammatory activity of this plant has not been explored yet. In an ongoing investigation into anti-inflammatory compounds from this plant, the methanol extract of *A. quinata* was investigated. By means of repeated column chromatography using silica gel, Sephadex LH-20, and LiChroprep RP-18, three new lignan glycosides, akequintoside A (**1**), akequintoside B (**2**), and akequintoside C (**3**), along with five known compounds, were isolated. The structures of the known compounds were identified as syringin (**4**),[8,9] vanilloloside (**5**),[10] salidroside (**6**),[11] 3,4-dihydroxyphenylethyl alcohol 8-*O*-*β*-D-glucopyranoside (**7**),[12] and calceolarioside B (**8**),[13] by comparing their spectroscopic data with those reported in the literature (Fig. 1). Furthermore, these five known compounds were isolated from this plant for the first time. The inhibitory activity of the isolated compounds against interleukin-6 (IL-6) production in tumor necrosis factor-alpha (TNF-*α*) stimulated MG-63 cells was examined. This paper reports the isolation and structural characterization of these compounds and their inhibitory activities against IL-6 production.

Results and Discussion

Akequintoside A (**1**) was obtained as a white amorphous powder, $[\alpha]_D^{25}$ −35.1 (MeOH). Its molecular formula was identified as $C_{25}H_{32}O_{11}$ by positive mode high resolution electrospray ionization mass spectrometry (HR-ESI-MS) data at *m/z* 531.1847 $[M+Na]^+$ (Calcd for $C_{25}H_{32}O_{11}Na$: 531.1842). In the IR spectrum, absorption bands for hydroxyl (3380 cm^{-1}) and aromatic ring (1605, 1518 cm^{-1}) groups were observed. The ^{1}H-NMR spectrum (Table 1) of **1** showed 1,3,4-trisubstituted aromatic protons at δ_H 6.97 (1H, d, *J*=2.0 Hz, H-2), 6.85 (1H, dd, *J*=2.0, 8.0 Hz, H-6) and 6.78 (1H, d, *J*=8.0 Hz, H-5), 1,2,4,5-tetrasubstituted aromatic protons at δ_H 6.92 (1H, s, H-3′) and 6.82 (1H, s, H-6′), hydroxyl propyl protons at δ_H 3.57 (2H, t, *J*=6.5 Hz, H-9′), 2.62 (2H, t, *J*=6.5 Hz, H-7′) and 1.82 (2H, m, H-8′), hydroxyl methyl protons at δ_H 3.84 (1H, m, H-9a) and 3.76 (1H, m, H-9b), an oxygenated methine proton at δ_H 5.52 (1H, d, *J*=6.0 Hz, H-7), a methine proton at δ_H 3.52 (1H, m, H-8), methoxyl protons at δ_H 3.82 (3H, s, OCH_3), in addition to a glucosyl anomeric proton at δ_H 5.01 (1H, d, *J*=8.0 Hz, H-1″). Acid hydrolysis of **1** in refluxing 1 N-HCl/MeOH afforded D-(+)-glucose which was detected by direct comparison with an authentic sample using co-TLC.[14] Furthermore, the configuration of the glucosidic linkage was determined to be *β* based on the coupling constant (*J*=8.0 Hz).[15] In the heteronuclear multiple bond correlation (HMBC) experiment, long-range correlations (Fig. 2) were observed between each of H-2/H-6 and C-7, H-6′ and C-7′. These results indicated that **1** had two phenylpropanoid units.[16,17] Significant HMBC correlations were also observed between H-7/C-5′ and H-8/C-4′. Therefore, it could be concluded that two phenylpropanoids formed a 7,8-dihydro-8-hydroxymethyl-7-phenyl-1′-benzofuranpropanol skeleton.[16,17] In the ^{1}H–^{1}H-correlation spectroscopy (COSY) spectrum, the oxymethine proton at δ_H 5.52 showed coupling with H-8, in addition methylene protons at δ_H 2.62 showed coupling with H-8′ and H-9′. The glycosidic linkage was identified at C-2′ by nuclear Overhauser enhancement spectroscopy (NOESY) experiment and HMBC correlations as shown in Fig. 2. The HMBC correlation between H-1″ and C-2′ indicated that the glucopyranosyl moiety was connected at C-2′. The relative configuration of H-7 and H-8 was identified as *cis* form from the distinct NOE correlation between H-7 and H-8, H-8 and H-2/H-6, also based on the reported NMR data.[17,18] The absolute stereochemistry at C-7

The authors declare no conflict of interest.

* To whom correspondence should be addressed. e-mail: wooer@chosun.ac.kr

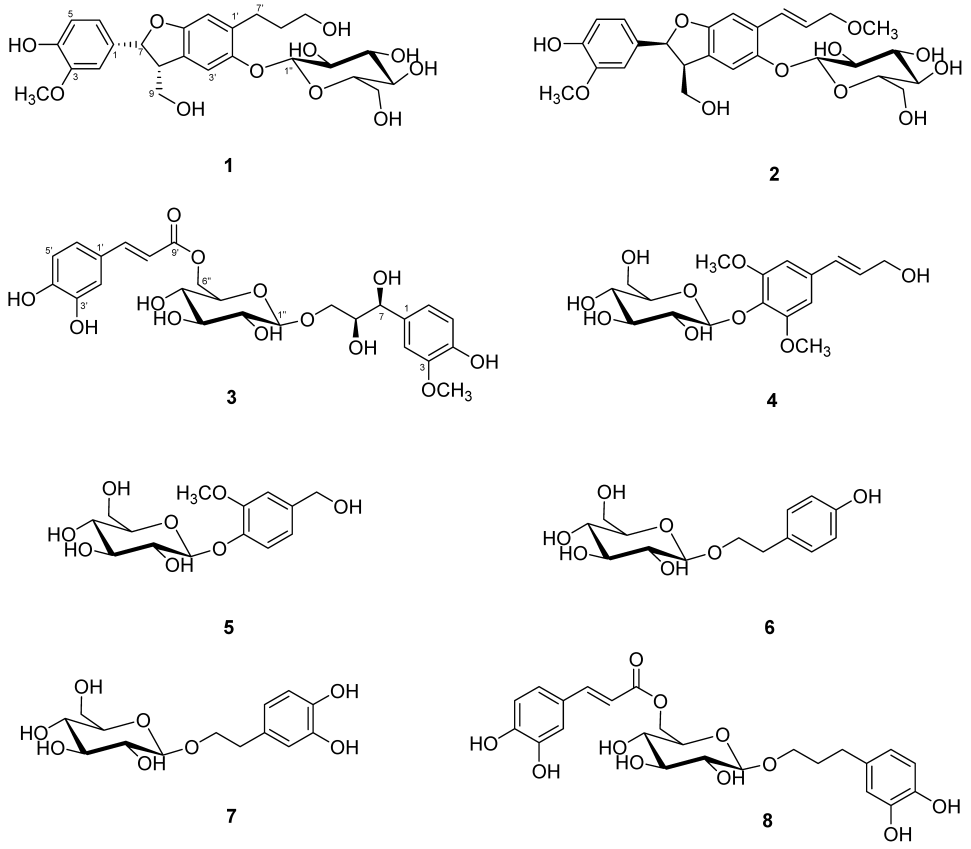

Fig. 1. The Structures of Compounds **1**–**8** from *A. quinata*

and C-8 were assigned to be both *S*, on the basis of negative Cotton effect at 246 and 291 nm in its circular dichroism (CD) spectrum.[17,18] Accordingly, the structure of **1** was identified as (7*S*,8*S*)-7,8-dihydro-8-hydroxymethyl-7-(4-hydroxy-3-methoxyphenyl)-1′-benzofuranpropanol 2′-*O*-*β*-D-glucopyranoside, named akeqintoside A, as drawn in Fig. 1.

Akequintoside B (**2**) was obtained as a white amorphous powder, $[\alpha]_D^{25}$ −50.4 (MeOH). Its molecular formula was identified as $C_{26}H_{32}O_{11}$ based on positive mode HR-ESI-MS data at *m/z* 543.1841 $[M+Na]^+$ (Calcd for $C_{26}H_{32}O_{11}Na$: 543.1843). In the IR spectrum, absorption bands for hydroxyl (3370 cm^{-1}) and aromatic ring (1610, 1520 cm^{-1}) groups were observed. The ^{1}H-NMR spectrum (Table 1) of **2** showed five aromatic protons at δ_H 7.18 (1H, s, H-3′), 7.07 (1H, s, H-6′), 6.98 (1H, d, *J*=2.0 Hz, H-2), 6.86 (1H, dd, *J*=2.0, 8.5 Hz, H-6) and 6.78 (1H, d, *J*=8.5 Hz, H-5), an oxygenated methine proton at δ_H 5.56 (1H, d, *J*=6.0 Hz, H-7), a methine proton at δ_H 3.54 (1H, m, H-8), a glucosyl anomeric proton at δ_H 5.04 (1H, d, *J*=7.5 Hz, H-1″), hydroxymethyl protons at δ_H 3.79–3.83 (2H, m), disubstituted *trans* double bond protons at δ_H 6.56 (1H, d, *J*=15.5 Hz, H-7′) and 6.18 (1H, dt, *J*=6.5, 15.5 Hz, H-8′), two methoxyl protons at δ_H 3.82 and 3.35, in addition to a glucosyl anomeric proton at δ_H 5.04 (1H, d, *J*=7.5 Hz, H-1″). Furthermore, acid hydrolysis of **2** in refluxing 1 N-HCl/MeOH afforded D-(+)-glucose which was detected by direct comparison with an authentic sample using co-TLC.[14] The ^{13}C-NMR and HSQC spectral data (Table 1) displayed 26 carbon signals, including one oxygenated methine carbon at δ_C 90.0 (C-7), olefinic carbons at δ_C 134.1 (C-7′) and 124.8 (C-8′), one oxygenated methylene carbon at δ_C 64.9 (C-9), and D-glucosyl carbons at δ_C 103.0 (C-1″), 78.4 (C-5″), 77.9 (C-3″), 75.1 (C-2″), 71.5 (C-4″), 62.6 (C-6″). The ^{1}H-, ^{13}C-NMR and ^{1}H–^{1}H COSY spectral data indicated that compound **2** was a dihydrobenzofuran-type lignan glucoside formed by two phenylpropanoid units.[19,20] Closer examination of the ^{1}H- and ^{13}C-NMR data revealed the structure of **2** to be quite similar to that of **1** except for the presence of an additional methoxyl group at δ_C 58.1(δ_H 3.35) and an olefinic bond [δ_C 134.1 (δ_H 6.56, H-7′); δ_C 124.8 (δ_H 6.18, H-8′)] in **2**. Long-range correlations between each of the two methoxyl protons at δ_H 3.35, 3.82 and δ_C 74.4 (C-9′), 149.3 (C-3), respectively, indicated the position of the methoxyl groups at C-10′ and C-3. Meanwhile, the coupling constant of the anomeric proton (*J*=7.5 Hz) suggested that the sugar was *β* form.[15] These observations suggested that the structure of **2** was dehydrodiconiferyl alcohol 9′-methoxy-2′-*O*-*β*-D-glucopyranoside.[21] The relative configuration of C-7 and C-8 was characterized by a NOESY experiment, which showed distinct NOE correlations between H-7 and H-8, H-8 and H-2/H-6. These observations suggested that the relative configurations at C-7 and C-8 are in a *cis* arrangement. In addition, the CD cotton effect of **2** was entirely opposite to that of **1**, suggesting that the aglycone of **2** is the enantiomer of **1**. The CD spectrum of **2** showed a positive Cotton effect at 246 and 291 nm, which indicated the absolute configurations of **2** to be 7*R*,8*R*-configurations.[17,18] Accordingly, the structure of **2** was identified as (7*R*,8*R*)-7,8-dihydro-8-hydroxymethyl-7-(4-hydroxy-3-methoxyphenyl)-1′-(9′-methoxy-7′-propenyl) benzofuran 2′-*O*-*β*-D-glucopyranoside, named akeqintoside B, as drawn in Fig. 1.

Akequintoside C (**3**) was obtained as a white amorphous powder, $[\alpha]_D^{25}$ −26.7 (MeOH). Its molecular formula was identified as $C_{25}H_{30}O_{13}$ by positive mode HR-ESI-MS data at *m/z*

Table 1. ^{1}H- and ^{13}C-NMR Data of **1**, **2** and **3**

No.	**1**		**2**		**3**	
	δ_H	δ_C	δ_H	δ_C	δ_H	δ_C
1		134.4		134.2		134.6
2	6.97 (d, 2.0)	110.9	6.98 (d, 2.0)	110.8	6.98 (d, 2.0)	111.9
3		149.3		149.3		148.9
4		147.9		148.0		147.1
5	6.78 (d, 8.0)	116.4	6.78 (d, 8.5)	116.4	6.72 (d, 8.0)	115.9
6	6.85 (dd, 2.0, 8.0)	120.2	6.86 (dd, 2.0, 8.5)	120.2	6.79 (dd, 2.0, 8.0)	121.1
7	5.52 (d, 6.0)	89.6	5.56 (d, 6.0)	90.0	4.56 (d, 6.0)	75.6
8	3.52 (m)	55.2	3.54 (m)	55.0	3.91 (dt, 3.0, 6.0)	75.6
9	3.76 (m)	65.0	3.79 (m)	64.9	3.62 (dd, 6.0, 10.0)	72.8
	3.84 (m)		3.83 (m)		4.01 (dd, 3.0, 10.0)	
1′		137.2		132.6		127.8
2′		142.6		142.9	7.04 (d, 2.0)	115.2
3′	6.92 (s)	118.1	7.18 (s)	116.7		147.0
4′		130.7		131.3		150.0
5′		147.6		149.5	6.77 (d, 8.0)	116.6
6′	6.82 (s)	119.7	7.07 (s)	118.2	6.94 (d, 2.0, 8.0)	123.3
7′	2.62 (t, 6.5)	32.9	6.56 (d, 15.5)	134.1	7.56 (d, 16.0)	147.4
8′	1.82 (m)	35.8	6.18 (dt, 6.5, 15.5)	124.8	6.27 (d, 16.0)	114.9
9′	3.57 (t, 6.5)	62.3	4.05 (d, 6.5)	74.4		169.2
10′			3.35 (s)	58.1		
1″	5.01 (d, 8.0)	102.9	5.04 (d, 7.5)	103.0	4.33 (d, 7.5)	105.2
2″	3.45 (dd, 8.0, 8.5)	75.0	3.45 (dd, 8.0, 8.5)	75.1	3.26 (dd, 7.5, 9.0)	75.3
3″	3.44 (dd, 8.5, 8.5)	77.9	3.44 (dd, 8.5, 8.5)	77.9	3.39 (dd, 9.0, 9.0)	77.8
4″	3.40 (dd, 8.5, 8.5)	71.5	3.40 (dd, 8.5, 8.5)	71.5	3.36 (dd, 9.0, 9.0)	71.8
5″	3.39 (ddd, 2.5, 5.5, 8.5)	78.3	3.40 (ddd, 2.5, 5.5, 8.5)	78.4	3.52 (ddd, 2.0, 6.0, 9.0)	75.6
6″	3.68 (dd, 5.5, 12.0)	62.6	3.68 (dd, 5.5, 12.0)	62.6	4.30 (dd, 6.0, 12.0)	64.7
	3.87 (dd, 2.5, 12.0)		3.84 (dd, 2.5, 12.0)		4.50 (dd, 2.0, 12.0)	
O<u>CH</u>$_3$	3.82 (s)	56.6	3.82 (s)	56.6	3.82 (s)	56.5

600 MHz, CD_3OD; chemical shifts in ppm relative to TMS; coupling constants (J) in Hz.

561.1581 $[M+Na]^+$ (Calcd for $C_{25}H_{30}O_{13}Na$: 561.1584). In the IR spectrum, absorption bands for hydroxyl (3400 cm^{-1}), carbonyl (1700 cm^{-1}), and aromatic ring (1513, 1435 cm^{-1}) groups were observed. The ^{1}H-NMR spectrum (Table 1) of **3** showed two sets of ABX aromatic protons at δ_H 7.04 (1H, d, J=2.0 Hz, H-2′)/6.98 (1H, d, J=2.0 Hz, H-2), 6.94 (1H, dd, J=2.0, 8.0 Hz, H-6′)/6.79 (1H, dd, J=2.0, 8.0 Hz, H-6), 6.77 (1H, d, J=8.0 Hz, H-5′)/6.72 (1H, d, J=8.0 Hz, H-5), two oxymethine protons at δ_H 4.56 (1H, d, J=6.0 Hz, H-7) and 3.91 (1H, dt, J=3.0, 6.0 Hz, H-8), one oxymethylene protons at δ_H 4.01 (1H, dd, J=3.0, 10.0 Hz, H-9a) and 3.62 (1H, dd, J=6.0, 10.0 Hz, H-9b), disubstituted *trans* double bond protons at δ_H 7.56 (1H, d, J=16.0 Hz, H-7′) and 6.27 (1H, d, J=16.0 Hz, H-8′), and one methoxyl group at δ_H 3.82 (3H, s). In the ^{13}C-NMR spectrum (Table 1), 25 carbon signals appeared besides those of the sugar unit, including one carbonyl carbon at δ_C 169.2 (C-9′), two oxygenated methine carbons at δ_C 75.6 (C-7) and 75.6 (C-8), olefinic carbons at δ_C 147.4 (C-7′) and 114.9 (C-8′), and one oxygenated methylene carbon at δ_C 72.8 (C-9). Based on the ^{1}H- and ^{13}C-NMR data, the structure of **3** was closely related to guaiacylglycerol 4-*O*-*β*-D-glucopyranoside, which was isolated from *Osmanthus asiaticus*[8] except for the presence of a caffeoyl group in the sugar unit and the different location of the sugar in **3**. The signals from the sugar unit appeared at δ_H 4.33 (1H, d, J=7.5 Hz, H-1″), 3.26 (1H, dd, J=7.5, 9.0 Hz, H-2″), 3.39 (1H, dd, J=9.0, 9.0 Hz, H-3″), 3.36 (1H, dd, J=9.0, 9.0 Hz, H-4″), 3.52 (1H, ddd, J=2.0, 6.0, 9.0 Hz, H-5″), 4.50 (1H, dd, J=2.0, 12.0 Hz, H-6″a), 4.30 (1H, dd, J=6.0, 12.0 Hz, H-6″b) [δ_C 105.2 (C-1″), 77.8 (C-3″), 75.6 (C-5″), 75.3 (C-2″), 71.8 (C-4″), 64.7 (C-6″)], and acid hydrolysis experiment supported the presence of D-glucopyranose.[8,15] The coupling constant (J=7.5 Hz) of the anomeric proton of D-glucose indicated it to be the *β*-form.[15] The glycosidic linkage was established by a HMBC experiment and comparison of the reported NMR data.[8,15] The downfield shift of C-6′ and the HMBC correlation between H-6″ and C-9′ confirmed that the caffeoyl group was attached to C-6″ of glucose in **3**. In addition, the HMBC correlation between H-1″ and C-9 suggested that glucose was attached to C-9 of the guaiacylglycerol moiety. Furthermore, long-range correlations were observed between the following protons and carbons (H-2/H-6 and C-7; H-7 and C-9; H-9 and C-1″; H-6″ and C-3″/C-9′; H-7′ and C-2′/C-6′/C-9′). In the ^{1}H–^{1}H COSY spectrum, the glycosylated proton of H-9 showed couplings with H-8 and H-7 (Fig. 2). The relative configuration of **3** was proposed from a NOESY experiment (Fig. 2), and a comparison of the observed and reported NMR data.[8,16] In the NOESY spectrum, correlations between H-1″, assigned an *α*-orientation, and H-8, H-7 indicated that these are on the same side (*α*). These NOE correlations suggested the relative configuration at C-7 and C-8 as shown in **3**. Accordingly, the structure of **3** is proposed to be 7*R**,8*R**-dihydroxy-7-(4-hydroxy-3-methoxyphenyl)-glycerol 9-*O*-*β*-D-(6′-*O*-caffeoyl)-glucopyranoside, named akequintoside C.

IL-6 is a cytokine, originally identified as a T-cell derived

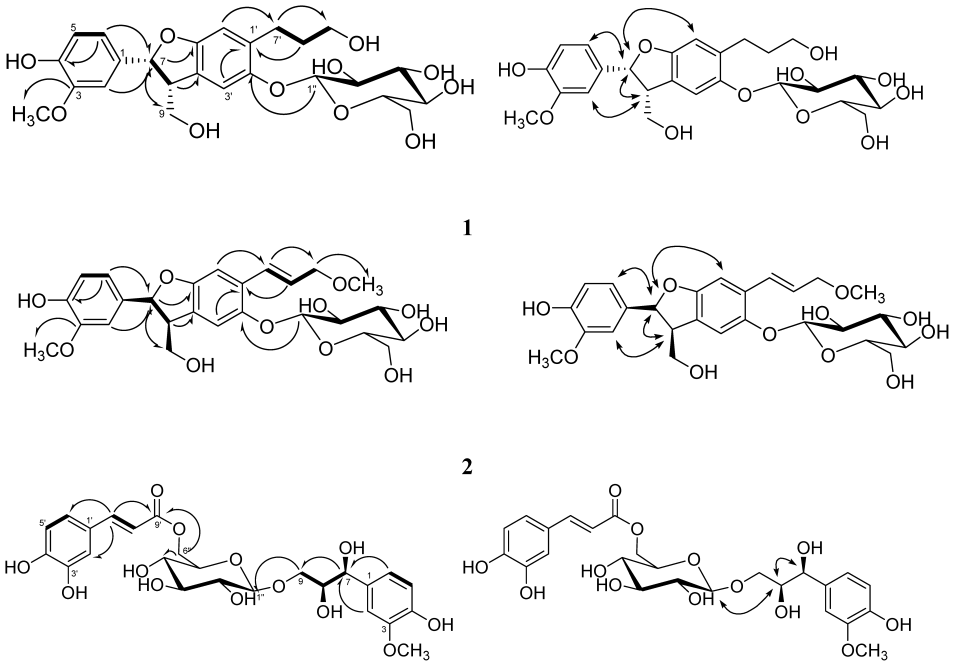

Fig. 2. Key ^{1}H–^{1}H COSY (—) HMBC (H→C), and NOESY Correlations of **1**, **2** and **3**

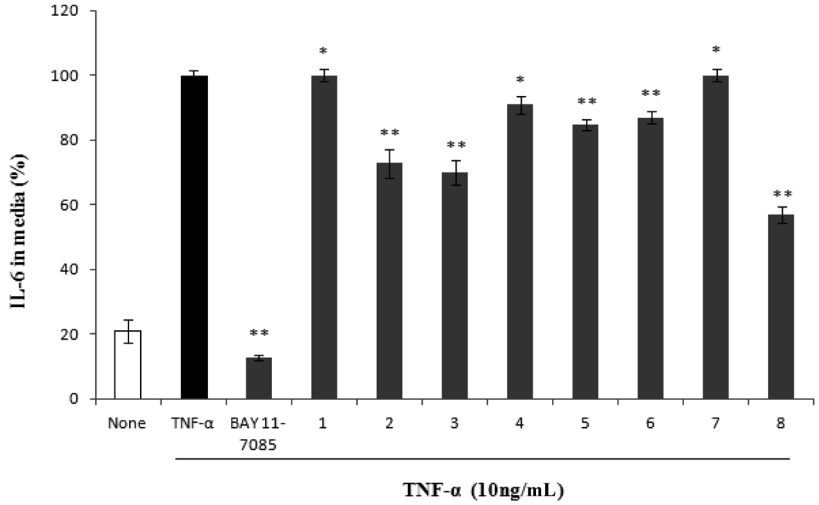

Fig. 3. Inhibitory Effect of Compounds **1**–**8** against IL-6 Production in TNF-α Sitimulated MG-63 Cells

MG-63 cells (3×10^4) were incubated for 24 h. Cultures were incubated with or without compounds (100 μg/mL) for 30 min and then stimulated with TNF-α (10 ng/mL) for 24 h. IL-6 in the supernatant was measured by ELAISA as described in Experimental. Results are expressed as the mean±S.E. from three different experiments. BAY 11–7085 was used as a positive control. *$p<0.05$ or **$p<0.01$ compared with TNF-α treated value.

factor that regulates B-cell growth and differentiation.[22] Human IL-6 is an important component of the inflammatory cascade. Dysregulation of IL-6 production has been implicated in a variety of inflammatory/autoimmune disease states, including rheumatoid arthritis, cardiac myxoma, Castleman's disease, and mesangial proliferative glomerulonephritis.[22] The proinflammatory cytokines IL-1 and TNF-α markedly stimulate the production IL-6.[23]

The inhibitory activity of the isolated compounds (**1**–**8**) against IL-6 production in TNF-α stimulated MG-63 cells was examined. Among them, compounds **2**, **3** and **8** showed moderate inhibitory activity against IL-6 production in TNF-α stimulated MG-63 cells, while compounds **1** and **4**–**7** showed no activity (Fig. 3, Table 2).

In conclusion, this paper reports the isolation, characterization, and inhibitory activity of 8 isolates, including three new compounds and five known compounds, from *A. quinata*.

Experimental

General Experimental Procedures Optical rotations were measured using an Autopol-IV polarimeter. UV spectra were obtained on a Shimadzu UV/Visible Spectrophotometer. IR spectra were recorded on an IMS 85 (Bruker). CD spectra were recorded on a JASCO J-810 spectropolarimeter. HR-ESI-MS was obtained on a Q-TOF (Synapt HDMS system, Waters, U.S.A.) mass spectrometer. NMR spectra, including NOESY, COSY, heteronuclear multiple quantum coherence (HMQC) and HMBC experiments, were recorded on a Varian VNMRS 600 MHz spectrometer (KBSI-Gwangju center) operating at 600 MHz (^{1}H) and 150 MHz (^{13}C), respectively,

Table 2. Inhibitory Effect of Compounds **1**–**8** against IL-6 Production in TNF-α Stimulated MG 63 Cells

Treatment	IL-6 (pg/mL)	Inhibition (%)
None	52.6±9.0	—
TNF-α	250.6±4.3	—
BAY 11–7085	30.2±2.1**	87.9**
1	390.9±7.8*	0.0*
2	182.9±5.4**	27.3**
3	175.4±8.6**	30.1**
4	228.0±4.9*	9.09**
5	213.0±2.3**	15.3**
6	218.0±6.2**	13.07**
7	285.7±11.1*	0.0*
8	142.8±6.3**	43.18**

MG-63 cells (3×10^4) were incubated for 24 h. Cultures were incubated with or without compounds (100 μg/mL) for 30 min and then stimulated with TNF-α (10 ng/mL) for 24 h. IL-6 in the supernatant was measured by ELAISA as described in Experimental. Results are expressed as the mean±S.E. from three different experiments. BAY 11–7085 was used as a positive control. *$p<0.05$ or **$p<0.01$ compared with TNF-α treated value.

with chemical shifts given in ppm (δ). Semi-preparative HPLC was performed using a Waters HPLC system equipped with Waters 600 Q-pumps, a 996 photodiode array detector, and a YMC-Pack ODS-A column (250×10 mm i.d., 5 μm), flow rate 4.0 mL/min. TLC was carried out on precoated Kieselgel 60 F_{254} (art. 5715, Merck) and RP-18 F_{254}s (art. 15389, Merck) plates. Column chromatography was performed on silica gel 60 (40–63 and 63–200 μm, Merck), MCI gel CHP20P (75–150 μm, Mitsubishi Chemical Co.), and Sephadex LH-20 (25–100 μm, Sigma). Silver carbonate (Ag_2CO_3, Aldrich Co.) and D-(+)-glucose ($C_6H_{12}O_6$, Sigma) were used as neutralization reagent and standard sugar on acid hydrolysis experiment, respectively. BAY 11–7085 (Sigma) was used as a positive control on IL-6 bioassay. Low pressure liquid chromatography was carried out over a Merck Lichroprep Lobar®-A RP-18 (240×10 mm) column with a FMI QSY-0 pump (ISCO).

Plant Materials The stems of *A. quinata* were collected in Gyeongju, Gyeongbuk province, Korea, in August 2011 and identified by Dr. J. H. Lee, Professor of the department of Korean Medicine, Dongguk University. A voucher specimen (CSU-877-17) was deposited in the Herbarium of the College of Pharmacy, Chosun University.

Extraction and Isolation The air-dried stem of *A. quinata* (11 kg) were cut and extracted with MeOH three times for 4 h at 80°C. The resultant MeOH extract (480 g) was suspended in water (1.5 L×3) and then partitioned sequentially with equal volumes of dichloromethane, ethyl acetate, and *n*-butanol. Each fraction was evaporated *in vaccuo* to yield the residues of CH_2Cl_2 (45.2 g), EtOAc (11.0 g), *n*-BuOH (57.0 g), and water (150.3 g) extracts. The *n*-BuOH soluble fraction (57.0 g) was subjected to column chromatography (CC) over a diaion HP 20 column and eluted with H_2O–MeOH (100 : 0→0 : 100) gradient system. The fractions were combined based on their TLC pattern to yield subfractions designated B1–B6. Fraction B2 (3.47 g), containing **5**, **6**, and **7** was purified by MCI gel CC (MeOH–H_2O, 1 : 9→2 : 8), followed by Sephadex LH 20 CC (MeOH–H_2O, 1 : 20), and finally by silica gel CC ($CHCl_3$–MeOH–H_2O, 4 : 1 : 0.2→2 : 1 : 0.2) to give **5** (3.2 mg), **6** (3.0 mg), and **7** (1.2 g). Fraction B3 (5.6 g) was subjected to silica gel CC eluting with a $CHCl_3$–MeOH–H_2O (6 : 1 : 0.1→2 : 1 : 0.1) in gradient system to give six subfractions (B31–B36). Subfraction B31 (0.79 g) was then purified by Lichroprep RP 18 CC (MeOH–H_2O, 1 : 3), followed by preparative HPLC eluted with MeOH–H_2O (27 : 75) to give **4** (1.6 mg), and **2** (1.6 mg). Subfracton B33 (0.44g) was purified by Lichroprep RP 18 CC (MeOH–H_2O, 1 : 3.3) to give **3** (4.2 mg), **1** (2.5 mg), and **8** (20.5 mg).

Akeqintoside A (**1**): Amorphous powder (MeOH); $[\alpha]_D^{20}$ −35.1° (c=0.12, MeOH); UV λ_{max} (MeOH) nm (log ε) 217 (4.23), 289 (3.61), 328 (4.07); IR ν_{max} (film) cm^{-1}: 3400, 1702, 1630, 1590, 1516, 1438, 1150; CD (MeOH, c 4.92×10^{-6} M): 246 (−5685.0), 291 (−7818.9) nm; HR-ESI-MS (positive mode) m/z: 531.1847 $[M+Na]^+$ (Calcd for $C_{25}H_{32}O_{11}Na$, 531.1842); ^{1}H-NMR (CD_3OD for 600 MHz): see Table 1; ^{13}C-NMR (CD_3OD for 150 MHz): see Table 1.

Akeqintoside B (**2**): Amorphous powder (MeOH); $[\alpha]_D^{27}$ −50.4° (c=0.77, MeOH); UV λ_{max} (MeOH) nm (log ε) 218 (4.22), 289 (3.63), 328 (4.00); IR ν_{max} (film) cm^{-1}: 3405, 1700, 1630, 1593, 1517, 1440, 1152; CD (MeOH, c 3.07×10^{-6} M): 246 (+0.48), 291 (+0.35) nm; HR-ESI-MS (positive mode) m/z: 543.1841 $[M+Na]^+$ (Calcd for $C_{26}H_{32}O_{11}Na$, 543.1843); ^{1}H-NMR (CD_3OD for 600 MHz): see Table 1; ^{13}C-NMR (CD_3OD for 150 MHz): see Table 1.

Akeqintoside C (**3**): White amorphous solid; $[\alpha]_D^{28}$ −26.7° (c=0.21, MeOH); UV λ_{max} (MeOH) nm (log ε) 218 (4.25), 289 (3.60), 328 (4.05); IR ν_{max} (film) cm^{-1}: 3405, 1702, 1633, 1590, 1513, 1435, 1150; HR-ESI-MS (positive mode) m/z: 561.1581 $[M+Na]^+$ (Calcd for $C_{25}H_{30}O_{13}Na$: 561.1584); ^{1}H-NMR (CD_3OD for 600 MHz): see Table 1; ^{13}C-NMR (CD_3OD for 150 MHz): see Table 1.

Acidic Hydrolysis of 1–3 Compounds **1**–**3** (1 mg each) were dissolved in 1 N HCl (1 mL) and MeOH (1 mL) and refluxed at 90°C for 90 min.[14] The reaction solution was evaporated under reduced pressure, and the hydrolysate was extracted with EtOAc (3 mL×3). The aqueous fraction was neutralized with Ag_2CO_3, filtered, and the filtrate was concentrated under reduced pressure. The residue was compared with standard sugar using TLC ($CHCl_3$–MeOH–H_2O, 6 : 4 : 1), which showed the sugar to be D-(+)-glucose (Rf=0.13) in **1**–**3**.

Bioassay of Human IL-6 IL-6 bioassay was carried out using a slight modification of an established method.[24] Briefly, 500 μL of the MG-63 cells (3×10^4 cells/mL) in Dulbecco's modified Eagle's medium (DMEM) containing 10% fetal bovine serum (FBS) were dispensed into a 24-well plate; the culture was incubated for 24 h at 37°C. Then, 5 μL of TNF-α (10 ng/mL), 5 μL of BAY 11–7085 (10 ng/mL), and 5 μL of the dimethyl sulfoxide (DMSO) with or without the compounds (100 μg/mL) were added. After incubation at 37°C with 5% CO_2 for 24 h, the medium was stored at −20°C until measurement. The IL-6 content of the medium was measured in an enzyme-linked immunosorbent assay (ELISA) procedure. 96-well plates were coated with 100 μL of purified rat anti-human IL-6 monoclonal antibody in 0.1 M $NaHCO_3$ (pH 9.6) by overnight incubation at 4°C. The wells were blocked with 200 μL of 3% bovine serum albumin (BSA) in phosphate buffered saline (PBS) for 2 h at room temperature (RT) and then incubated with 100 μL of specific antibody for 2 h at RT. One hundred microliters of horseradish peroxidase (HRP) conjugated rabbit anti-goat immunoglobulin G (IgG) (1 : 1000 dilution) was added to each well and incubated for 2 h at RT. One hundred microliters of 3,3′,5,5′-tetramethyl-benzidine (TMB) substrate solution was added and incubated for 10 min

at RT. The color reaction was stopped with 50 μL of 0.4 N HCl and the optical density was read at 450 nm using a Microplate Reader (Molecular Devices Co., Ltd., U.S.A.).

Acknowledgments This research was supported by a Grant (12172MFDS989) from Ministry of Food and Drug Safety in 2013. We thank Dr. K. D. Park, and Mr. S.-T. Hong at the Korea Basic Science Institute for Gwangju Center for their aid in obtaining the NMR and mass spectra.

References

1) Lee T. B., "Illustrated Flora of Korea," Hyang-Moon Publishing Co., Korea, 2003, p. 369.
2) Ahan D. K., "Illustrated Book of Korean Medicinal Herbs," Kyo-Hak Publishing Co., Korea, 1998 p. 384.
3) Bensky D., Clavey S., Stöger E., "Chinese Herbal Medicine: Materia Medica," 3rd ed., Eastland Press, Seattle, 2004, p. 283.
4) Gao H., Wang Z., *Phytochemstry*, **67**, 2697–2705 (2006).
5) Mimaki Y., Doi S., Kuroda M., Yokosuka A., *Chem. Pharm. Bull.*, **55**, 1319–1324 (2007).
6) Mimaki Y., Kuroda M., Yokosuka A., Harada H., Fukushima M., Sashida Y., *Chem. Pharm. Bull.*, **51**, 960–965 (2003).
7) Jung H.-J., Lee C. O., Lee K.-T., Choi J., Park H.-J., *Biol. Pharm. Bull.*, **27**, 744–747 (2004).
8) Sugiyama M., Nagayama E., Kikuchi M., *Phytochemistry*, **33**, 1215–1219 (1993).
9) Niwa M., Iwadare Y., Wu Y.-C., Hirata Y., *Chem. Pharm. Bull.*, **36**, 1158–1161 (1988).
10) Ida Y., Satoh Y., Ohtsuka M., Nagasao M., Shoji J., *Phytochemistry*, **35**, 209–215 (1993).
11) Nishimura H., Sasaki H., Morota T., Chin M., Mitsuhashi H., *Phytochemistry*, **29**, 3303–3306 (1990).
12) Park H.-J., Lee M.-S., Lee K.-T., Sohn H.-C., Han Y.-N., Miyamoto K., *Chem. Pharm. Bull.*, **47**, 1029–1031 (1999).
13) Damtoft S., Jensen S. R., *Phytochemistry*, **37**, 441–443 (1994).
14) Kim M. R., Moon H.-I., Chung J. H., Moon Y. H., Hahm K.-S., Woo E.-R., *Chem. Pharm. Bull.*, **52**, 1466–1469 (2004).
15) Ishimaru K., Nonaka G.-I., Nishioka I., *Phytochemistry*, **26**, 1147–1152 (1987).
16) Nakanishi T., Iida N., Inatomi Y., Murata H., Inada A., Murata J., Lang F. A., Iinuma M., Tanaka T., *Phytochemistry*, **65**, 207–213 (2004).
17) Dong L.-P., Ni W., Dong J.-Y., Li J.-Z., Chen C.-X., Liu H.-Y., *Molecules*, **11**, 1009–1014 (2006).
18) Baderschneider B., Winterhalter P., *J. Agric. Food Chem.*, **49**, 2788–2798 (2001).
19) Salama O., Chaudhuri R. K., Sticher O., *Phytochemistry*, **20**, 2603–2604 (1981).
20) Wang C. Z., Jia Z. J., *Phytochemistry*, **45**, 159–166 (1997).
21) Wang C. Z., Jia Z. J., *Planta Med.*, **63**, 241–244 (1997).
22) Hirano T., Akira S., Taga T., Kishimoto T., *Immunol. Today*, **11**, 443–449 (1990).
23) Van Damme J., Opdenakker G., Simpson R. J., Rubira M. R., Cayphas S., Vink A. A., Billiau A. van Snick J., *J. Exp. Med.*, **165**, 914–919 (1987).
24) Liu Q. H., Jeong J.-E., Choi E. J., Moon Y. H., Woo E.-R., *Arch. Pharm. Res.*, **29**, 1109–1113 (2006).