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Flavonoid glycosides from the leaves of *Allium victorialis* var. *platyphyllum* and their anti-neuroinflammatory effects

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ABSTRACT

Eight new flavonoid glycosides, named allivictoside A–H (**1**–**8**), together with twelve known flavonoids (**9**–**20**) were isolated from the leaves of *Allium victorialis* var. *platyphyllum*. The structures of **1**–**8** were determined by chemical and spectroscopic methods, including 1D, 2D NMR analyses and HR-MS. To evaluate the anti-neuroinflammatory activities of all isolates, we measured the secreted nitric oxide levels in murine microglia BV-2 cells stimulated by lipopolysaccharide. In this study, compounds **2**, **6**, **10**, and **18** significantly inhibited nitric oxide production (IC_{50} values of 20.67, 20.42, 21.48 and 19.80 μM, respectively) without cell toxicity. Therefore, we suggest that allivictoside B (**2**) and F (**6**), 3-*O*-β-D-glucosyl-7-*O*-β-D-(2-*O*-feruloyl)glucosylkaempferol (**10**) and quercetin 3-*O*-β-D-glucopyranoside (**18**) may be considered as candidates for the treatment of diseases associated with neuroinflammation.

Neuroinflammation is a general innate immune response of the brain aimed at protecting the central nervous system (CNS) against infectious pathogens and injuries.[1] In most cases, it plays important roles in the defense against injury and restoring homeostasis. However, chronic neuroinflammation may contribute to the progressive neuronal damage observed in many neurodegenerative disorders, most notably parkinson's disease, alzheimer's disease and neuronal injury associated with stroke.[2–4] Therefore, the role of neuroinflammation has become a prominent theme for investigation in various neurological diseases and neuroprotective drug development. Microglia are the immune cells present in the CNS. However, uncontrolled and chronic activated microglia contribute to produce various proinflammatory factors such as nitric oxide (NO), tumor necrosis factor α (TNF-α), and prostaglandin E_2 (PGE_2). These factors become important causes of inflammation in the brain, and finally contribute to neuroinflammation.[5] Thus, many studies have been focusing on the development of materials that can inhibit microglia activation in a safer and more effective fashion.

The genus *Allium* contains about 20 species. Among the species, *Allium victorialis* var. *platyphyllum* (Liliaceae), better known as 'Myung-i' in Korea, is widely distributed in the northern part of Korea. The leaves of *A. victorialis* var. *platyphyllum* are used as vegetable, such as pickles in soy sauce, wrapped pock, and kim-chi and also as a Korean traditional medicine for the treatment of gastritis and heart failures.[6] It has been reported that the EtOH extract of *A. victorialis* var. *platyphyllum* showed several pharmacological activities including anti-hyperlipidemic, anti-obesity,[7] anti-atherogenic,[8] cytotoxic,[9] and anti-hepatotoxic effects.[10] Previous phytochemical studies on this plant reported the isolation of flavonoids, steroidal saponins, and sulfur compounds.[11–13] However, the effects of flavonoids isolated from *A. victorialis* var. *platyphyllum* on the regulation of neuroinflammation have not yet been reported.

Herein, we describe the structural elucidation of new isolated flavonoid glycosides (**1**–**8**), named allivictoside A–H (**1**–**8**) (Figure 1), on the basis of 1D, 2D NMR data and chemical methods, and evaluation of the anti-neuroinflammatory activities of all isolated compounds by measuring the production of proinflammatory factor and nitric oxide (NO) in lipopolysaccharide (LPS)-activated murine microglia BV-2 cells.

The half dried leaves of *A. victorialis* var. *platyphyllum* (2.7 kg) were extracted with 80% MeOH three times at room temperature and evaporated under reduced pressure to give a residue (314.0 g), which was dissolved in water (800 ml) and partitioned with solvent to give *n*-hexane (17.0 g), $CHCl_3$ (2.2 g), EtOAc (3.4 g) and *n*-BuOH (50.0 g) soluble portions. Purification of the EtOAc and *n*-BuOH-soluble fractions by multiple chromatographic steps (Supplementary data) led to the isolation of eight new flavonoid glycosides, together with twelve known flavonoid derivatives. The isolated known compounds were identified as kaempferol 7-*O*-β-D-glucopyranoside (**9**), 3-*O*-β-D-glucosyl-7-*O*-β-D-(2-*O*-feruloyl)glucosylkaempferol (**10**), kaempferol 3,7,4′-tri-*O*-β-D-glucopyranoside

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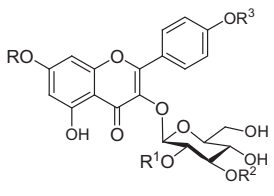

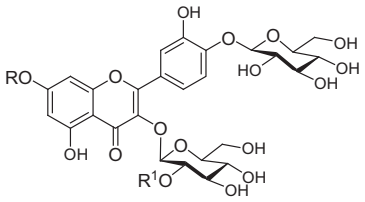

	R	R^1	R^2	R^3
1	H	(*E*)-*p*-coumaroyl	H	Glc
2	H	(*E*)-feruloyl	H	Glc
3	Glc	(*E*)-*p*-coumaroyl	H	Glc
4	Glc	(*Z*)-*p*-coumaroyl	H	Glc
5	Glc	H	(*E*)-*p*-coumaroyl	Glc
6	Glc	(*E*)-feruloyl	H	H

	R	R^1
7	H	(*E*)-*p*-coumaroyl
8	Glc	(*E*)-feruloyl

Figure 1. Chemical structures of compounds **1**–**8** from *A. victorialis* var. *platyphyllum*.

Table 1
^{1}H (500 MHz) data of compounds **1–8** in DMSO-d_6

H	1[a]	2[a]	3[a]	4[a]	5[a]	6[a]	7[b]	8[a]
Flavonol								
6	6.09 (s)	6.07 (s)	6.42 (s)	6.44 (s)	6.46 (s)	6.41 (s)	6.13 (s)	6.42 (s)
8	6.35 (s)	6.29 (s)	6.80 (s)	6.80 (s)	6.82 (s)	6.75 (s)	6.31 (s)	6.78 (s)
2′	8.07 (d, 9.0 Hz)	8.07 (d, 9.0 Hz)	8.17 (d, 7.5 Hz)	8.14 (d, 9.0 Hz)	8.15 (d, 9.0 Hz)	8.04 (d, 9.0 Hz)	7.60 (d, 2.5 Hz)	7.64 (br s)
3′	7.13 (d, 9.5 Hz)	7.13 (d, 9.0 Hz)	7.17 (d, 7.5 Hz)	7.17 (d, 9.0 Hz)	7.18 (d, 9.0 Hz)	6.88 (d, 9.0 Hz)	—	—
5′	7.13 (d, 9.5 Hz)	7.13 (d, 9.0 Hz)	7.17 (d, 7.5 Hz)	7.17 (d, 9.0 Hz)	7.18 (d, 9.0 Hz)	6.88 (d, 9.0 Hz)	7.27 (d, 8.0 Hz)	7.22 (d, 9.0 Hz)
6′	8.07 (d, 9.0 Hz)	8.07 (d, 9.0 Hz)	8.17 (d, 7.5 Hz)	8.14 (d, 9.0 Hz)	8.15 (d, 9.0 Hz)	8.04 (d, 9.0 Hz)	7.62 (dd, 9.0, 2.5 Hz)	7.64 (overlap)
3-*O*-glc								
1″	5.73 (d, 8.0 Hz)	5.75 (d, 8.0 Hz)	5.76 (d, 8.5 Hz)	5.71 (d, 8 Hz)	5.63 (d, 7.5 Hz)	5.74 (d, 8.5 Hz)	5.63 (d, 8.0 Hz)	5.74 (d, 8.0 Hz)
2″	4.84 (dd. 9.0, 8.0 Hz)	4.85 (dd, 9.5, 8.0 Hz)	4.88 (t, 8.5 Hz)	4.84 (t, 9.0 Hz)	3.44 (m)	4.88 (dd, 9.5, 8.5 Hz)	5.02 (dd, 9.0, 9.0 Hz)	4.91 (overlap)
3″	3.49 (m)	3.49 (m)	3.50 (m)	3.50 (m)	4.97 (t, 9.5 Hz)	3.49 (m)	3.60 (m)	3.50 (m)
4″	3.21 (m)	3.19 (m)	3.18 (m)	3.20 (m)	3.44 (m)	3.18 (m)	3.43 (m)	3.21 (m)
5″	3.40 (m)	3.40 (m)	3.43 (m)	3.45 (m)	3.69 (m)	3.43 (m)	3.48 (m)	3.45 (m)
6″	3.69 (brd, 11.5 Hz)	3.69 (brd, 11.0 Hz)	3.69 (m)	3.71 (m)	3.70 (m)	3.67 (brd, 10.5 Hz)	3.95 (dd, 12.5, 2.5 Hz)	3.73 (brd, 11.5 Hz)
	3.49 (m)	3.49 (m)	3.50 (m)	3.50 (m)	3.46 (m)	3.49 (m)	3.74 (m)	3.50 (m)
7-*O*-glc								
1‴	—	—	5.07 (d, 7.5 Hz)	5.08 (d, 8.0 Hz)	5.10 (d, 7.5 Hz)	5.04 (d, 7.0 Hz)	—	5.08 (d, 7.5 Hz)
2‴	—	—	3.24 (m)	3.25 (m)	3.26 (m)	3.26 (m)	—	3.24 (m)
3‴	—	—	3.26 (m)	3.28 (m)	3.28 (m)	3.28 (m)	—	3.29 (m)
4‴	—	—	3.16 (m)	3.16 (m)	3.16 (m)	3.16 (m)	—	3.16 (m)
5‴	—	—	3.43 (m)	3.43 (m)	3.44 (m)	3.44 (m)	—	3.43 (m)
6‴	—	—	3.67 (m)	3.69 (m)	3.69 (m)	3.57 (brd, 11.5 Hz)	—	3.67 (brd, 10 Hz)
	—	—	3.45 (m)	3.45 (m)	3.46 (m)	3.30 (m)	—	3.43 (m)
4′-*O*-glc								
1⁗	5.02 (d, 7.5 Hz)	5.02 (d, 7.5 Hz)	5.06 (d, 7.5 Hz)	5.05 (d, 7.5 Hz)	5.04 (d, 7.5 Hz)	—	4.95 (d, 8.0 Hz)	4.90 (d, 7.0 Hz)
2⁗	3.27 (m)	3.28 (m)	3.29 (m)	3.28 (m)	3.29 (m)	—	3.55 (m)	3.26 (m)
3⁗	3.18 (m)	3.19 (m)	3.22 (m)	3.22 (m)	3.16 (m)	—	3.52 (m)	3.21 (m)
4⁗	3.21 (m)	3.29 (m)	3.24 (m)	3.25 (m)	3.24 (m)	—	3.45 (m)	3.24 (m)
5⁗	3.40 (m)	3.39 (m)	3.36 (m)	3.33 (m)	3.39 (m)	—	3.60 (m)	3.36 (m)
6⁗	3.59 (brd, 11.5 Hz)	3.59 (brd, 11.0 Hz)	3.60 (m)	3.59 (m)	3.57 (m)	—	3.79 (dd, 11.0, 2.0 Hz)	3.62 (brd, 11.5 Hz)
	3.32 (m)	3.36 (m)	3.35 (m)	3.35 (m)	3.39 (m)	—	3.61 (m)	3.36 (m)
Coum/fer								
2⁗′	7.51 (d, 8.5 Hz)	7.28 (d, 1.5 Hz)	7.54 (d, 8.5 Hz)	7.66 (d, 8.5 Hz)	7.56 (d, 8.0 Hz)	7.30 (d, 1.5 Hz)	7.44 (d, 8.0 Hz)	7.30 (br s)
3⁗′	6.77 (d, 9.0 Hz)	—	6.79 (d, 8.5 Hz)	6.68 (d, 8.5 Hz)	6.80 (d, 8.5 Hz)	—	6.80 (d, 8.0 Hz)	—
5⁗′	6.77 (d, 9.0 Hz)	6.77 (d, 8 Hz)	6.79 (d, 8.5 Hz)	6.68 (d, 8.5 Hz)	6.80 (d, 8.5 Hz)	6.77 (d, 8.5 Hz)	6.80 (d, 8.0 Hz)	6.79 (d, 7.0 Hz)
6⁗′	7.51 (d, 8.5 Hz)	7.08 (dd, 8.5, 1.5 Hz)	7.54 (d, 8.5 Hz)	7.66 (d, 8.5 Hz)	7.56 (d, 8.0 Hz)	7.09 (dd, 8.5, 2.0 Hz)	7.44 (d, 8.0 Hz)	7.09 (d, 8.0 Hz)
7⁗′	7.56 (d, 15.5 Hz)	7.56 (d, 16.0 Hz)	7.58 (d, 16.0 Hz)	6.86 (d, 13.0 Hz)	7.57 (d, 18.0 Hz)	7.57 (d, 16.0 Hz)	7.64 (d, 16.0 Hz)	7.57 (d, 15.5 Hz)
8⁗′	6.36 (d, 16.0 Hz)	6.45 (d, 16.0 Hz)	6.40 (d, 17.0 Hz)	5.82 (d, 12.5 Hz)	6.40 (d, 16.0 Hz)	6.48 (d, 16.0 Hz)	6.36 (d, 15.5 Hz)	6.48 (d, 16.0 Hz)
OCH_3	—	3.80 (s)	—	—	—	3.80 (s)	—	3.81 (s)

[a] 125 MHz, DMSO-d_6.
[b] 225 MHz, CD_3OD.

(**11**), 3-*O*-β-D-(2-*O*-feruloyl)glycosyl-7,4′-di-*O*-β-D-glucosylkaempferol (**12**),[14] kaempferol 3-*O*-β-D-glucopyranoside (**13**), kaempferol 3-*O*-α-L-rhamnopyranosyl-(1→6)-β-D-glucopyranoside (**14**),[15] kaempferol 3,4′-di-*O*-β-D-glucopyranoside (**15**),[6] kaempferol 3,7-di-*O*-β-D-glucopyranoside (**16**),[13] quercetin 3,4′-di-*O*-β-D-glucopyranoside (**17**),[16] quercetin 3-*O*-β-D-glucopyranoside (**18**),[17] quercetin 7,4′-di-*O*-β-D-glucopyranoside (**19**),[18] and kaempferol 3-*O*-(2″-(*E*)-*p*-coumaroylglucoside)-7-*O*-β-D-glucoside (**20**)[19] by comparison of their spectroscopic data with previously reported values.

Compound (**1**) was obtained as a yellowish gum. The molecular formula of **1** was determined to be $C_{36}H_{36}O_{18}$ by the positive mode HR-FABMS data at *m*/*z* 757.1982 $[M+H]^+$ (calcd for $C_{36}H_{37}O_{18}$, 757.1980). The IR spectrum of **1** displayed absorption bands at 3357 and 1658 cm^{-1}, ascribable to hydroxyl and α,β-unsaturated ketone groups, respectively. In the ^1H and ^{13}C NMR spectra, the six aromatic protons [δ_H 8.07 (2H, d, *J* = 9.0 Hz, H-2′, 6′); 7.13 (2H, d, *J* = 9.5 Hz, H-3′, 5′); 6.35 (1H, s, H-8); 6.09 (1H, s, H-6)] (Table 1) and fifteen carbons [δ_C 177.6 (C-4), 162.8 (C-7), 161.8 (C-5), 159.9 (C-4′), 157.2 (C-9), 155.8 (C-2), 133.7 (C-3), 131.2 (C-2′, 6′), 124.3 (C-1′), 116.5 (C-3′, 5′), 104.1 (C-10), 99.9 (C-6), 94.6 (C-8)] (Table 2) were obtained, indicating that **1** was a kaempferol skeleton.[20] In addition, two glucose groups [δ_H 5.73 (1H, d, *J* = 8.0 Hz, H-1″), 5.02 (1H, d, *J* = 7.5 Hz, H-1‴), 4.84 (1H, dd, *J* = 9.0, 8.0 Hz, H-2″); δ_C 100.7 (C-1‴), 98.9 (C-1″), 78.4 (C-3‴), 77.7 (C-5″, 5‴), 74.7 (C-2″, 3″), 73.9 (C-2‴), 70.2 (C-4″, 4‴), 61.3 (C-6″, 6‴)][14] and a coumaroyl moiety [δ_H 7.56 (1H, d, *J* = 15.5 Hz, H-7⁗), 7.51 (2H, d, *J* = 8.5 Hz, H-2⁗, 6⁗), 6.77 (1H, d, *J* = 9.0 Hz, H-3⁗, 5⁗), 6.36 (1H, d, *J* = 16.0 Hz, H-8⁗); δ_C 166.4 (C-9⁗), 160.5 (C-4⁗), 145.6 (C-7⁗), 130.9 (C-2⁗, 6⁗), 125.7 (C-1⁗), 116.5 (C-3⁗, 5⁗), 114.9 (C-8⁗)][13] were observed. The *J* values of anomeric protons [δ_H 5.73 (d, *J* = 8.0 Hz), 5.02 (d, *J* = 7.5 Hz)] indicated the β-configuration of D-glucose.[21] The linkage of glucoses with the aglycone was identified by HMBC correlations; δ_H 5.73 (1H, d, *J* = 8.0 Hz) of 3-*O*-glc to δ_C 133.7 (C-3); δ_H 5.02 (1H, d, *J* = 7.5 Hz) of 4′-*O*-glc to δ_C 159.9 (C-4′) (Figure 2). The HMBC spectrum also showed that the downfield shifted methine proton at δ_H 4.84 (1H, dd, *J* = 9.0, 8.0 Hz, H-2″) correlated with the carbonyl carbon at δ_C 166.4 (C-9⁗), indicating that the (*E*)-*p*-coumaroyl moiety was connected at C-2″ (Figure 2). Alkaline hydrolysis of **1** gave compound **15** and (*E*)-*p*-coumaric acid, of which the former was identified by comparison of the ^1H NMR data with the previous reference[6] and the latter by co-TLC ($MeOH/H_2O$ = 4/6, R_f value: 0.45) with a standard (Aldrich Co., USA) and the ^1H NMR spectrum.[22] Thus, the structure of **1** was determined as kaempferol 3-*O*-β-D-[2″-(*E*)-*p*-coumaroylglucopyranosyl]-4′-*O*-β-D-glucopyranoside and named allivictoside A.

Allivictoside B (**2**) was isolated as a yellowish gum, whose molecular formula was deduced to be $C_{37}H_{38}O_{19}$ by HR-FABMS at *m*/*z* 809.1902 $[M+Na]^+$ (Calcd for $C_{37}H_{38}NaO_{19}$, 809.1905). The ^1H and ^{13}C NMR spectra of **2** were very close to those of **1**, expect for the phenylpropanoyl moiety; the coumaroyl moiety in **1** was replaced with the feruloyl group [δ_H 7.56 (1H, d, *J* = 16.0 Hz, H-7⁗), 7.28 (1H, d, *J* = 1.5 Hz, H-2⁗), 7.08 (1H, dd, *J* = 8.5, 1.5 Hz, H-6⁗), 6.77 (1H, d, *J* = 8.0 Hz, H-5⁗), 6.45 (1H, d, *J* = 16.0 Hz, H-8⁗), 3.80 (3H, s, 3⁗–OCH_3); δ_C 166.5 (C-9⁗), 150 (C-4⁗), 148.6 (C-3⁗), 146.0 (C-7⁗), 126.2 (C-1⁗), 123 (C-6⁗), 116.2 (C-5⁗), 115.2 (C-8⁗), 111.8 (C-2⁗), 55.3 (3⁗–OCH_3)] in **2.**[23] The NMR assignments and connectivites of **2** were confirmed by ^1H–^1H COSY, HMQC, and HMBC spectroscopic data (Figure 2). Alkaline methanolysis of **2** with 3% KOH in MeOH (3 mL) afforded compound **15**[6] and methyl (*E*)-ferulate.[24] Therefore, the structure of **2** was established to be kaempferol 3-*O*-β-D-[2″-(*E*)-feruloylglucopyranosyl]-4′-*O*-β-D-glucopyranoside.

Allivictoside C (**3**), a yellowish gum, displayed a molecular ion peak at *m*/*z* 941.2325 $[M+Na]^+$ (calcd for $C_{42}H_{46}NaO_{23}$, 941.2328) in positive HR-FABMS analysis, corresponding to a molecular formula of $C_{42}H_{46}O_{23}$. The ^1H and ^{13}C NMR data of **3** were similar to those of **1**. The major difference was the presence of an additional glucose group [δ_H 5.07 (1H, d, *J* = 7.5 Hz, H-1‴); δ_C 99.6 (C-1‴), 76.5 (C-3‴), 77.1 (C-5‴), 73.2 (C-2‴), 69.5 (C-4‴), 60.6 (C-6‴)] in **3**. The HMBC spectrum showed that the anomeric proton at δ_H 5.07 (H-1‴) is correlated to C-7 (δ_C 162.9) (Figure 2). Alkaline hydrolysis of **5** yielded compound **11**[14] and (*E*)-*p*-coumaric acid.[22] Thus, the structure of **3** was elucidated to be kaempferol 3-*O*-β-D-[2″-(*E*)-*p*-coumaroylglucopyranosyl]-4′,7-*O*-β-D-diglucopyranoside.

Compound **4**, named allivictoside D, was isolated as a yellowish gum. It exhibited a molecular formula of $C_{42}H_{46}O_{23}$ from its positive HR-FABMS *m*/*z*: 919.2507 $[M+H]^+$ (calcd for $C_{42}H_{47}O_{23}$, 919.2505). The ^1H and ^{13}C NMR spectra of **4** were very close to those of **3**, except the coupling constants of H-7⁗′ at δ_H 6.86 (1H, d, *J* = 13.0 Hz) and H-8⁗′ at δ_H 5.82 (1H, d, *J* = 12.5 Hz), indicating that (*E*)-*p*-coumaroyl group in **3** was replaced with the (*Z*)-*p*-coumaroyl moiety in **4**. Alkaline methanolysis of **4** with 3% KOH in MeOH (4 mL) yielded compound **11**[14] and methyl (*Z*)-coumarate.[25] These data deduced the structure of **4** to be kaempferol 3-*O*-β-D-

Table 2
^{13}C NMR Data of Compounds **1**–**8**

C	1[a]	2[a]	3[a]	4[a]	5[a]	6[a]	7[b]	8[a]
Flavonol								
2	155.8	155.7	156.1	156.1	156.1	156.6	157.6	156.3
3	133.7	133.6	133.4	133.4	133.8	133.4	135.7	133.6
4	177.6	177.4	177.4	177.4	177.6	177.9	179.0	177.4
5	161.8	161.7	160.8	161.0	160.8	161.5	161.4	160.7
6	99.9	100.7	99.4	99.4	100.4	100.0	100.4	99.4
7	162.8	162.8	162.9	162.9	162.9	163.5	163.2	162.9
8	94.6	94.7	94.4	94.5	94.4	95.1	95.1	94.4
9	157.2	157.2	156.0	156.0	156.1	157.4	158.6	156.0
10	104.1	104.0	105.7	105.7	105.7	106.3	105.8	105.7
1′	124.3	124.3	123.3	123.3	123.4	123.9	126.8	124.1
2′	131.2	131.1	130.6	130.7	130.6	131.6	117.9	116.6
3′	116.5	116.5	115.8	115.8	115.7	116.2	147.9	146.2
4′	159.9	159.8	159.4	159.4	159.3	161.5	149.1	147.8
5′	116.5	116.5	115.8	115.8	115.7	116.2	117.5	115.4
6′	131.2	131.1	160.6	130.7	130.6	131.6	123.0	121.2
3-*O*-glc								
1″	98.9	98.9	98.2	98.1	99.9	98.7	100.9	98.3
2″	74.7	74.7	74.0	73.9	72.2	74.7	75.9	74.1
3″	74.7	74.8	73.9	73.9	77.3	73.7	76.4	74.0
4″	70.2	70.2	70.1	69.5	67.8	70.2	71.6	70.1
5″	77.7	77.7	77.0	77.0	77.1	77.8	78.5	77.1
6″	61.3	61.2	60.6	60.5	60.5	61.3	62.6	60.6
7-*O*-glc								
1‴	–	–	99.6	99.6	99.4	100.4	–	99.6
2‴	–	–	73.2	73.2	73.1	73.7	–	73.3
3‴	–	–	76.5	76.4	76.5	77.1	–	76.4
4‴	–	–	69.5	69.5	69.5	70.2	–	69.5
5‴	–	–	77.1	77.0	77.1	77.1	–	77.2
6‴	–	–	60.6	60.5	60.5	61.3	–	60.6
4′-*O*-glc								
1⁗	100.7	100.7	99.9	99.4	99.6	–	103.4	101.5
2⁗	73.9	73.9	76.4	76.4	76.4	–	75.0	75.7
3⁗	78.4	78.4	77.8	77.7	77.3	–	78.9	77.9
4⁗	70.2	70.8	69.5	69.5	69.5	–	71.6	69.7
5⁗	77.7	77.2	77.1	77.0	77.0	–	78.5	77.2
6⁗	61.3	61.3	60.5	60.5	60.5	–	62.5	60.6
Coum/fer								
1⁗′	125.7	126.2	124.9	125.3	125.1	126.1	127.4	125.5
2⁗′	130.9	111.8	130.2	132.6	132.6	111.8	131.3	111.0
3⁗′	116.5	148.6	115.8	114.8	115.7	146.0	116.9	147.9
4⁗′	160.5	150.0	160.8	158.8	160.8	148.7	160.0	149.4
5⁗′	116.5	116.2	115.8	114.8	115.7	116.5	116.9	115.8
6⁗′	130.9	123.8	130.2	132.6	132.6	123.9	131.3	123.1
7⁗′	145.6	146.0	145.0	143.2	144.4	146.0	147.1	145.0
8⁗′	114.9	115.2	114.1	115.4	114.6	115.1	115.3	114.4
9⁗′	166.4	166.5	165.8	165.1	166.0	166.5	168.5	165.7
OMe	–	55.3	–	–	–	56.3	–	55.6

[a] 125 MHz, DMSO-d_6.
[b] 225 MHz, CD_3OD.

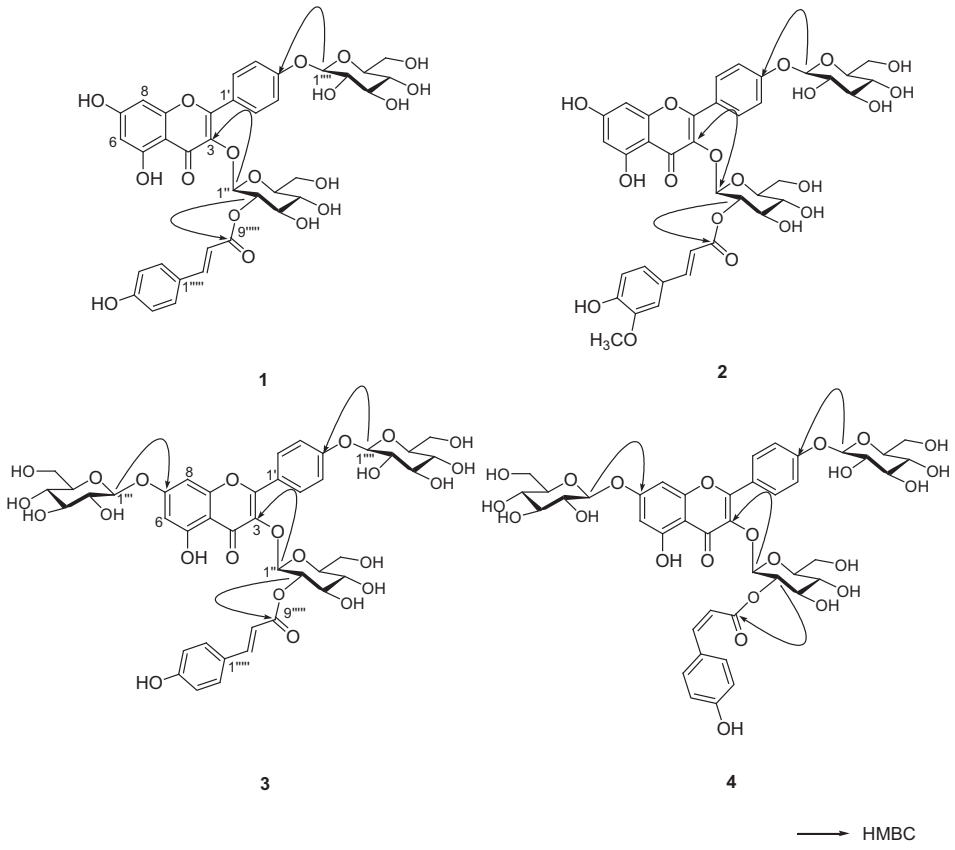

Figure 2. Key HMBC (→) correlations of **1–4**.

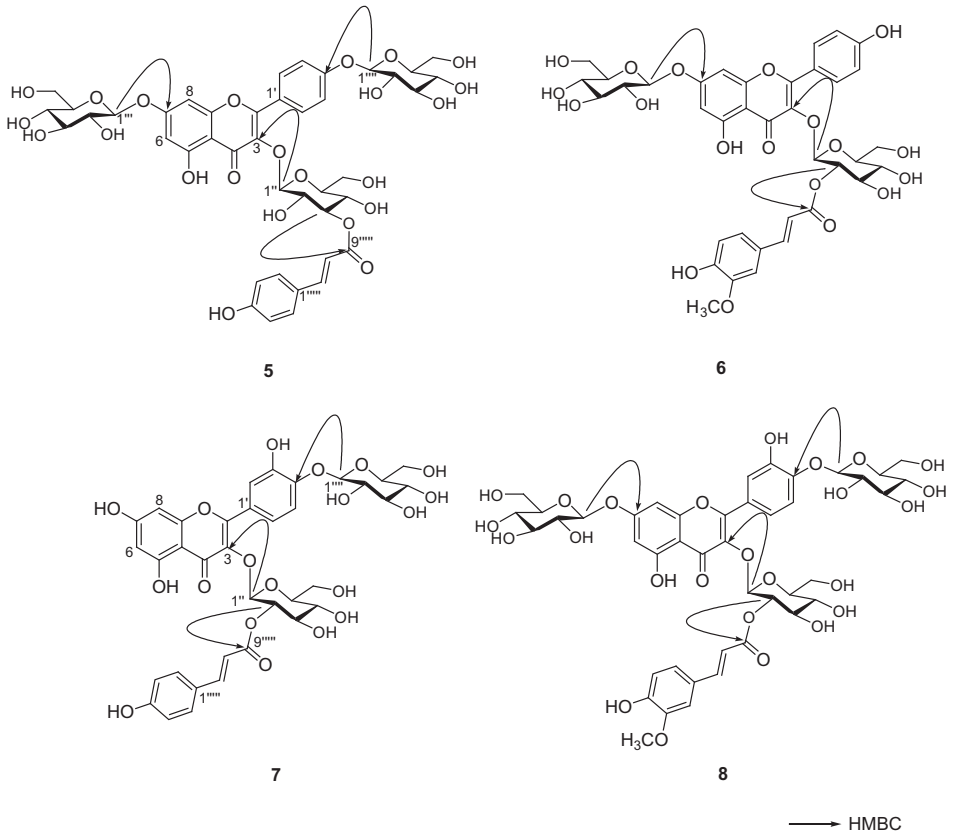

Figure 3. Key HMBC (→) correlations of **5–8**.

[2″-(*Z*)-*p*-coumaroylglucopyranosyl]-4′,7-*O*-β-D-diglucopyranoside.

Allivictoside E (**5**), yellowish gum, was assigned a molecular formula of $C_{42}H_{46}O_{23}$ on the basis of HR-FABMS at *m/z* 919.2504 $[M+H]^+$ (calcd for $C_{42}H_{47}O_{23}$, 919.2500). Its NMR spectra were analogous to those of **3**, except for the attached location of the coumaroyl moiety. The upfield shifted vicinal carbon signals at δ_C 72.2 (C-2″) and 67.8 (C-4″) indicated that the (*E*)-*p*-coumaroyl moiety was connected to C-3″ (δ_C 77.3) of **5**. In addition, the linkage of the coumaroyl group was confirmed to be H-3″ (δ_H 4.97) by the HMBC correlation of C-9‴′ (δ_C 166.0) (Figure 3). Alkaline hydrolysis of **5** yielded compound **11**[14] and (*E*)-*p*-coumaric acid.[22] Thus, **5** was established to be kaempferol 3-*O*-β-D-[3″-(*E*)-*p*-coumaroylglucopyranosyl]-4′,7-*O*-β-D-diglucopyranoside.

Allivictoside F (**6**) was obtained as a yellowish gum, and its molecular formula $C_{37}H_{38}O_{19}$ was inferred from the positive ion HR-FABMS *m/z* 787.2083 $[M+H]^+$ (calcd for $C_{37}H_{39}O_{19}$, 787.2086). The ^1H and ^{13}C NMR spectra of **6** were very similar to those of kaempferol 3-*O*-(2″-(*E*)-*p*-coumaroylglucoside)-7-*O*-β-D-glucoside, which was isolated from *Ononis vaginalis*,[19] except for the phenylpropanoyl signals [δ_H 7.57 (1H, d, *J* = 16.0 Hz, H-7‴′), 7.30 (1H, d, *J* = 1.5 Hz, H-2‴′), 7.09 (1H, dd, *J* = 8.5, 2.0 Hz, H-6‴′), 6.77 (1H, d, *J* = 8.5 Hz, H-5‴′), 6.48 (1H, d, *J* = 16.0 Hz, H-8‴′), 3.80 (3H, s, 3‴′-OCH_3); δ_C 166.5 (C-9‴′), 148.7 (C-4‴′), 146.0 (C-3‴′, 7‴′), 126.1 (C-1‴′), 123.9 (C-6‴′), 116.5 (C-5‴′), 115.1 (C-8‴′), 111.8 (C-2‴′), 56.3 (3‴′-OCH_3)], indicating that the (*E*)-feruloyl moiety in **6** was replaced with coumaroyl group in **20**. The above evidence was confirmed by the HMBC spectrum (Figure 3). In addition, alkaline methanolysis of **6** with 3% KOH in MeOH gave compound **16**[13] and methyl (*E*)-ferulate.[24] Taken together, the structure of **6** was determined as kaempferol 3-*O*-β-D-[2″-(*E*)-feruloylglucopyranosyl]-7-*O*-β-D-glucopyranoside.

Allivictoside G (**7**), a yellowish gum, gave the positive HR-FABMS *m/z* 773.1929 $[M+H]^+$, consistent with the molecular formula $C_{36}H_{36}O_{19}$. The characteristic signals of quercetin skeleton were observed at δ_H 7.62 (1H, dd, *J* = 9.0, 2.5 Hz, H-6′), 7.60 (1H, d, *J* = 2.5 Hz, H-2′), 7.27 (1H, d, *J* = 8.5 Hz, H-5′), 6.31 (1H, s, H-8), 6.14 (1H, s, H-6) in the ^1H NMR spectrum (Table 1) and at δ_C 179.0 (C-4), 163.2 (C-7), 161.4 (C-5), 158.6 (C-9), 157.6 (C-2), 149.1 (C-4′), 147.9 (C-3′), 135.7 (C-3), 126.8 (C-1′), 123.0 (C-6′), 117.9 (C-2′), 117.5 (C-5′), 105.8 (C-10), 100.4 (C-6), 95.1 (C-8) in the ^{13}C NMR spectrum (Table 2).[26] In addition, two glucose moieties [δ_H 5.63 (d, *J* = 8.0 Hz, H-1″), 5.02 (1H, dd, *J* = 9.0, 9.0 Hz, H-2″), 4.95 (d, *J* = 8.0 Hz, H-1‴′); δ_C 103.4 (C-1‴′), 100.9 (C-1″), 78.9 (C-3‴′), 78.5 (C-5″, 5‴′), 76.4 (C-3″), 75.9 (C-2″), 75.0 (C-2‴′), 71.6 (C-4″, 4‴′), 62.6 (C-6″), 62.5 (C-6‴′)][16] and a coumaroyl group [δ_H 7.64 (1H, d, *J* = 16.0 Hz, H-7⁗′), 7.44 (2H, d, *J* = 8.0 Hz, H-2⁗′, 6⁗′), 6.80 (1H, d, *J* = 8.0 Hz, H-3⁗′, 5⁗′), 6.36 (1H, d, *J* = 15.5 Hz, H-8⁗′); δ_C 168.5 (C-9⁗′), 160.0 (C-4⁗′), 147.1 (C-7⁗′), 131.3 (C-2⁗′, 6⁗′), 127.4 (C-1⁗′), 116.9 (C-3⁗′, 5⁗′), 115.3 (C-8⁗′)][13] were shown in the ^1H and ^{13}C NMR spectra. The coupling constant (*J* = 8.0 Hz) of the two anomeric protons of D-glucose was indicated to be β-form.[27] The HMBC spectrum showed correlations between H-1″ (δ_H 5.63) of 3-*O*-Glc and C-3 (δ_C 135.7) and H-1‴′ (δ_H 4.95) of 4′-*O*-Glc and C-4′ (δ_C 149.1). The linkage of the coumaroyl group was confirmed by the HMBC spectrum, in which a correlation was revealed between the H-2″ (δ_H 5.02) of 3-*O*-glc and the C-9⁗′ (δ_C 168.5) as shown in Figure 3. Alkaline hydrolysis of **7** with 0.05 M KOH in H_2O (3 mL) afforded compound **17**[16] and (*E*)-*p*-coumaric acid.[22] Accordingly, the structure of **7** was characterized as quercetin 3-*O*-β-D-[2″-(*E*)-*p*-coumaroylglucopyranosyl]-4′-*O*-β-D-glucopyranoside.

Allivictoside H (**8**) was isolated as a yellowish gum. The molecular formula of **8** was determined to be $C_{43}H_{48}O_{25}$ by the positive mode HR-FABMS data at *m/z* 987.2380 $[M+Na]^+$ (calcd for $C_{43}H_{48}NaO_{25}$, 987.2382). The proton and carbon signals of **8** were very similar to those of quercetin 3,7,4′-*O*-β-D-triglucopyranoside, which was isolated from *Allium cepa*.[27] The major differences were the signals from an additional feruloyl moiety [δ_H 7.57 (1H, d, *J* = 15.5 Hz, H-7⁗′), 7.30 (1H, br s, H-2⁗′), 7.09 (1H, brd, *J* = 8.0 Hz, H-6⁗′), 6.79 (1H, d, *J* = 7.0 Hz, H-5⁗′), 6.48 (1H, d, *J* = 16.0 Hz, H-8⁗′), 3.81 (3H, s, 3⁗′-OCH_3); δ_C 165.7 (C-9⁗′), 149.4 (C-4⁗′), 147.9 (C-3⁗′), 145.0 (C-7⁗′), 123.1 (C-6⁗′), 115.8 (C-5⁗′), 114.4 (C-8⁗′), 111.0 (C-2⁗′), 55.6 (3⁗′-OCH_3)] in the ^1H and ^{13}C NMR spectra. The HMBC spectrum exhibited that the downfield shifted proton at δ_H 4.91 (1H, H-2″) correlated with the carbonyl carbon at δ_C 165.7 (C-9⁗′), suggesting that the (*E*)-feruloyl group was attached at C-2″ (Figure 3). Alkaline methanolysis of **8** gave methyl (*E*)-ferulate,[24] together with quercetin 3,7,4′-*O*-β-D-triglucopyranoside, which was confirmed by comparison with those of the reported data.[27] From the above evidence, the structure of **8** was established to be quercetin 3-*O*-β-D-[2″-(*E*)-feluroylglucopyranosyl]-7,4′-*O*-β-D-diglucopyranoside.

In accordance with this ongoing research, we investigated the inhibitory activities of isolated compounds (**1**-**20**) from *A. victorialis* var. *platyphyllum* on neuroinflammation by measurement of produced NO levels in LPS-activated BV-2 cells. In the present study, compounds **2**, **6, 10**, and **18** exhibited strong inhibitory activities showing NO production with IC_{50} values of 20.67, 20.42, 21.48 and 19.80 μM respectively, without any influence on cell viability (Table 3). Among the new compounds (**1**-**8**), kaempferol 3-*O*-β-D-[2″-(*E*)-feruloylglucopyranosyl]-4′-*O*-β-D-glucopyranoside (**2**) and kaempferol 3-*O*-β-D-[2″-(*E*)-feruloylglucopyranosyl]-7-*O*-β-D-glucopyranoside (**6**) were more active than other isolates. We suggest that the 2″-(*E*)-feruloylglucopyranosyl group may be the necessary functional group responsible for the anti-neuroinflammatory properties of kaempferol.

In the course of our research for active components responsible for the inhibition of NO production, we conducted a phytochemical experiment of the leaves of *A. victorialis* var. *platyphyllum*. Column chromatographic purification of its MeOH extract resulted in the isolation of eight new flavonoid glycosides, named allivictoside A–H (**1**–**8**), together with twelve known ones (**9**–**20**). Among them,

Table 3
Inhibitory effect on NO production of compounds **1**–**20** in LPS-activated BV-2 cells

compounds	IC_{50}[a] (μM)	Cell viability[b] (%)
1	55.56	108.9 ± 3.6
2	20.67	107.1 ± 4.5
3	43.96	104.3 ± 2.0
4	48.48	100.4 ± 1.2
5	49.48	103.8 ± 3.4
6	20.42	100.0 ± 5.6
7	>200	101.3 ± 0.7
8	29.95	105.3 ± 3.2
9	39.06	106.6 ± 1.5
10	21.48	105.9 ± 0.5
11	32.96	102.3 ± 1.6
12	43.35	100.7 ± 2.1
13	77.36	106.4 ± 2.0
14	121.72	103.8 ± 2.0
15	170.06	99.1 ± 1.7
16	28.89	106.0 ± 1.9
17	45.47	100.5 ± 2.0
18	19.80	97.3 ± 3.4
19	51.73	105.3 ± 2.2
20	47.20	104.9 ± 3.0
NMMA[c]	16.27	98.2 ± 2.6

[a] IC_{50} value of each compound was defined as the concentration (μM) that caused 50% inhibition of NO production in LPS-activated BV-2 cells.

[b] Cell viability after treatment with 20 μM of each extract was expressed as a percentage (%) of the LPS only treatment group. The results are averages of three independent experiments, and the data are expressed as mean ± SD.

[c] NMMA as a positive control.

Compounds **2**, **6**, **10**, and **18** from the leaves of *A. victorialis* var. *platyphyllum* significantly inhibited NO production in LPS-activated BV-2 cells. These results indicate that flavonoid derivatives from *A. victorialis* var. *platyphyllum* have anti-neuroinflammatory effects and have potential to be used as candidates for the treatment of various neurodegenerative diseases.

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Supplementary data

Supplementary data associated with this article can be found, in the online version, at http://dx.doi.org/10.1016/j.bmcl.2012.10.043.

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