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Anti-inflammatory components of *Chrysanthemum indicum* flowers

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ABSTRACT

One new octulosonic acid derivative, chrysannol A (**1**), along with 17 known compounds (**2**–**18**), were isolated from *Chrysanthemum indicum* flowers. Their structures were determined from 1D NMR, 2D NMR, HR-ESI-MS spectral data, and comparisons with previous reports. The effects of these compounds on lipopolysaccharide (LPS)-induced nitric oxide (NO) and tumor necrosis factor alpha (TNF-α) production by RAW 264.7 cells were investigated. Compound **8** showed the highest inhibition of NO production of 46.09% at a concentration of 10.0 μM. Compounds **7**, **10**, **11**, and **16** inhibited TNF-α secretion at all concentration tested (0.4, 2.0, and 10.0 μM), with inhibition values ranging from 22.27% to 33.13%. In addition, compound **8** and **9** decrease COX-2 and iNOS protein on Western blot analysis in dose dependent manner.

Chrysanthemum indicum L. (Compositae), which is widespread in Korea, is a well-known herb and medicinal plant with small yellow flowers. The flowers of *C. indicum* have been used in mixed spices, as a food additives for masking flavors, and used in teas and alcoholic beverages in Korea since ancient times.[1] *C. indicum* has a long history of use in traditional Korea and Chinese medicines for the treatment of infectious diseases, including pneumonia and pertussis, and in the treatment of colitis, stomatitis, cancer, fever, sores, vertigo, inflammation, and hypertension. In terms of the chemical constituents of this plant, several sesquiterpenes, flavonoids, and phenolic compounds have been isolated and found to exhibit inhibitory activity against rat lens aldose reductase.[2–4] Additionally, its extracts have been reported to have central and peripheral analgesic properties, to reduce blood pressure, have anti-inflammatory and immunomodulatory activities, acetylcholinesterase inhibitory activity, and inhibitory activity against various bacteria and viruses.[5–7]

In this study, we report a new compound (**1**) and 17 known compounds (**2**–**18**) isolated from the flowers of *C. indicum* (see Fig. 1). All compounds were assessed for inhibitory activity against LPS-induced NO and TNF-α production in RAW 264.7 cells. In addition, the effects on LPS-induced COX-2 and iNOS in RAW 264.7 cells of compound **8** and **9** were evaluated using Western blot analysis.

Compound **1**[8] was obtained as a colorless oil. The molecular formula of **1** was determined to be $C_{14}H_{22}O_8$ by HR-ESI-MS at *m*/*z* 353.1008 $[M+Cl]^-$ (Calcd $C_{14}H_{22}O_8Cl$ for 353.1003). The ^1H NMR spectrum of **1** showed signals assignable to four oxygenated methine protons H-1 (δ_H 4.43, t, J = 4.8 Hz), H-2 (δ_H 4.00, t, J = 4.8 Hz), H-3 (δ_H 4.12, br t, J = 4.8 Hz), and H-7 (δ_H 4.31, m), one oxygenated methylene group H-9a (δ_H 4.68, dd, J = 3.6, 12.3 Hz) and H-9b (δ_H 4.85, dd, J = 8.4, 12.3 Hz), one methoxy at δ_H 3.78 (s), two methylenes H-4*ax* (δ_H 2.21, dd, J = 4.8, 15.0 Hz), H-4*eq* (δ_H 2.26, dd, J = 1.2, 15.0 Hz), and H-3′a (δ_H 1.47, m) and H-3′b (δ_H 1.67, m), one methine H-2′ (δ_H 2.40, m), and two methyl groups H-4′ (δ_H 0.91, t, J = 7.2 Hz) and H-5′ (δ_H 1.13, d, J = 7.2 Hz) (see Table 1). The ^{13}C NMR, DEPT and HMQC spectra of **1** showed the presence of 14 carbon resonances, consisting of three methyls, three methylenes, four oxygenated methines, and four quaternary carbons. Two carbon resonance signals at δ_C 178.3 (C-1′), 169.6 (C-10) were assigned to two carbonyl groups. Eight carbon resonance signals at δ_C 104.8, 40.3, 65.8, 69.5, 78.4, 81.1, 64.3, and 169.6 and the absence of an anomeric proton indicated the presence of 3-deoxy-2-octulosonic acid.[9] Examination of the COSY spectra of **1** showed two spin systems, H9/H7/H1/H2/H3/H4 and H5′/H2′/H3′/H4′ (see Fig. 2). HMBC correlation were observed between methylene proton H-4*eq*/H-4*ax* (δ_H 2.26/2.21) and C-2 (δ_C 69.5), C-3 (δ_C 65.8), between H-1 (δ_H 4.43) and C-5 (δ_C 104.8), and between H-3 (δ_H 4.12) and C-5 (δ_C 104.8). The quaternary carbon C-10 (δ_C 169.6) and the exclusive methoxy group 10-OCH_3 (δ_C 53.2) were assigned to a methyl carboxylate on the basis of the

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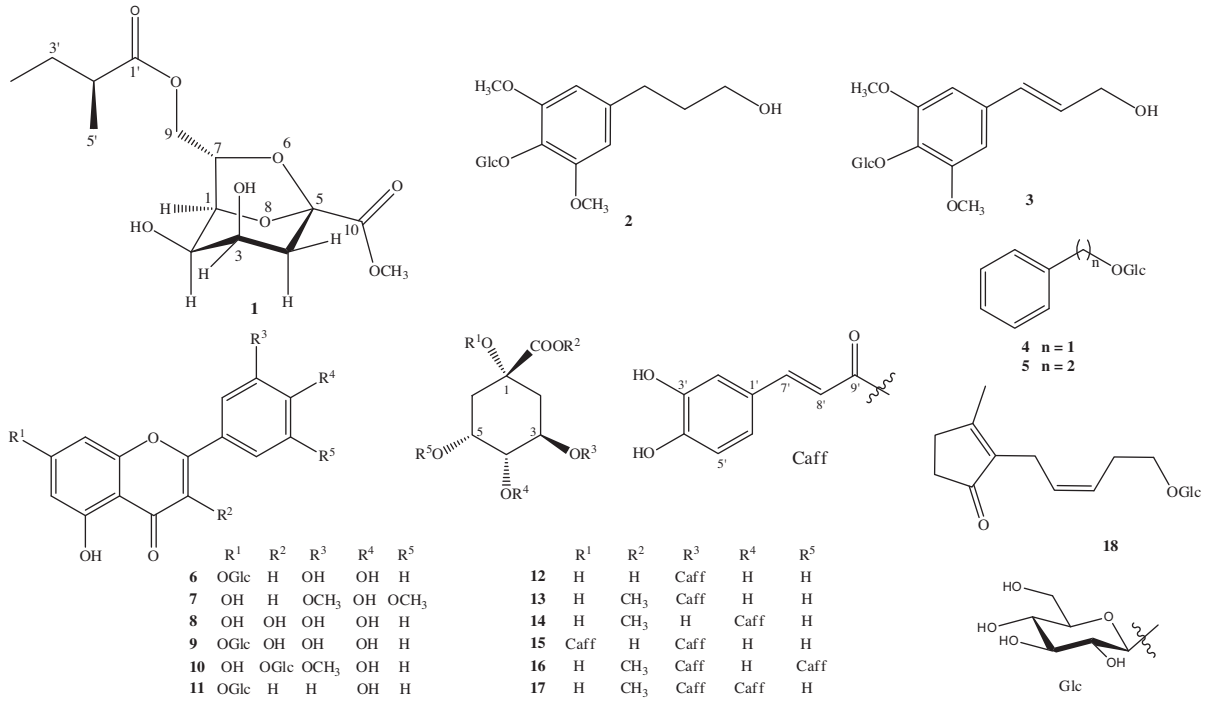

Figure 1. The structure of isolated compounds from the flowers of *Chrysanthemum indicum*.

Table 1
The NMR spectroscopic data for compound **1** (in CD_3OD)

Pos.	1	
	δ_C^a	δ_H^b (mult., *J* in Hz)
1	78.4	4.43 (t, 4.8)
2	69.5	4.00 (t, 4.8)
3	65.8	4.12 (br t, 4.8)
4	40.3	2.21 (dd, 4.8, 15.0)
		2.26 (dd, 1.2, 15.0)
5	104.8	–
7	81.1	4.31 (m)
9	64.3	4.68 (dd, 3.6, 12.3)
		4.85 (dd, 8.4, 12.3)
10	169.6	–
OCH_3	53.2	3.78 (s)
1′	178.3	–
2′	42.3	2.40 (m)
3′	27.8	1.47 (m)
		1.67 (m)
4′	11.9	0.91 (t, 7.2)
5′	17.0	1.13 (d, 7.2)

Assignments were done by DEPT, HMQC, HMBC, COSY, and NOESY experiments. Measured at [a]150 MHz, [b]600 MHz.

HMBC correlation of the methoxy protons at δ_H 3.78 and C-10. The downfield shift of the signals of two oxygenated methine carbons at C-1 (δ_C 78.4) and C-7 (δ_C 81.1) suggested the presence of a bicyclo-ketal group in the methyl octulosonate moiety. These findings suggested a methyl 2,3-dihydroxy-6,8-dioxabicyclo[3.2.1]octane-5-carboxylate moiety.[10] In addition, analysis of COSY spectra together with HMBC correlations of the methyl protons H-5′ (δ_H 1.13, d, J = 7.2 Hz) and C-1′ (δ_C 178.3), C-2′ (δ_C 42.3), and C-3′ (δ_C 27.8) confirmed the presence of a 2′-methyl butanoic acid moiety. The configuration of the 2′-methyl butanoic acid substituent was identified as the *S*-isomer by analyzing the ester formed with (*R*)-(+)-1-phenylethanol by GC (see Supporting information). The HMBC correlation of the methylene protons H_2-9 (δ_H 4.85, and 4.68) and carboxylic group C-1′ (δ_C 178.3) indicated that the 2′*S*-methyl butanoyl moiety was attached to the methyl octulosonate moiety at C-9 via an ester linkage. The absolute configuration of **1** was determined based on the NOESY spectrum, the three-bond ($^3J_{H-H}$) ^1H–^1H spin coupling, and circular dichroism (CD) spectrum. The coupling constant between protons H-3 (δ_H 4.12) and H-4*ax* (δ_H 2.21) was 4.8 Hz, indicating an *equatorial* configuration of H-3. The observation of a NOESY correlation between proton H-2 (δ_H 4.00) and H-4*ax* (δ_H 2.21) suggested an *axial* orientation of H-2; hence the 4.8 Hz coupling constant between H-2 and H-3 also indicated *equatorial* configuration of H-1. The NOESY interaction of H-1 (δ_H 4.43)/H-9 (δ_H 4.68) supported the same α-configuration of H-1 and oxygenated methylene C-9 (see Fig. 2). Moreover, a

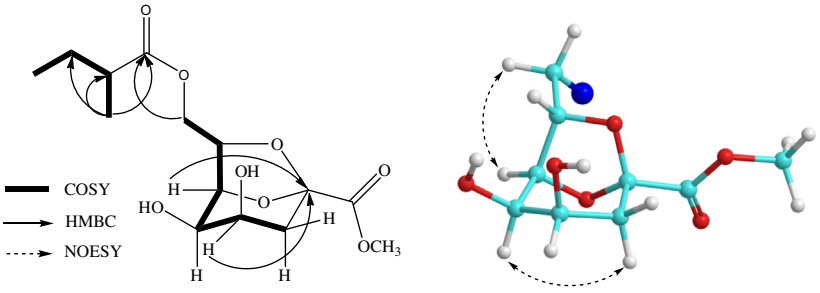

Figure 2. The keys COSY, HMBC, and NOESY correlations for compound **1**.

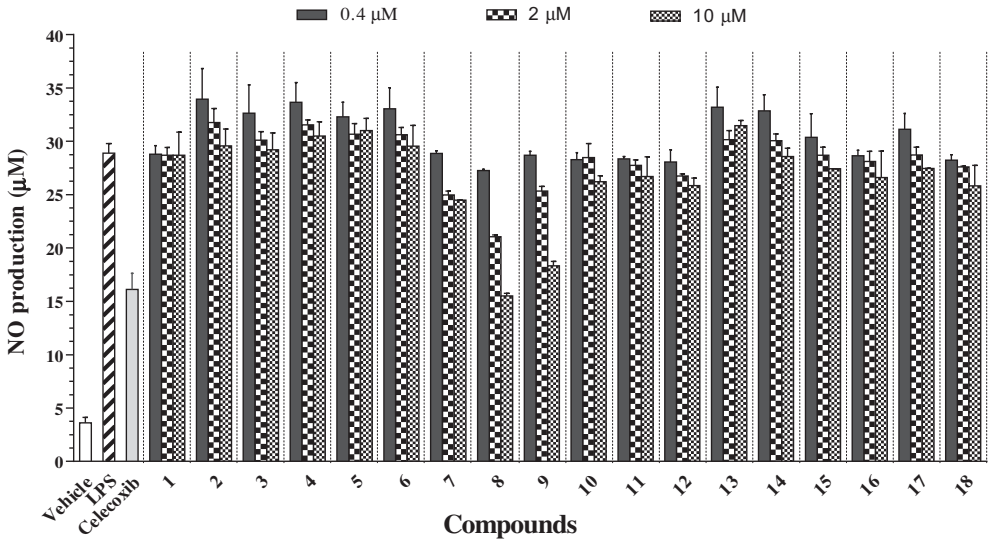

Figure 3. Effect of compounds **1**–**18** on the LPS-induced NO production on RAW 264.7 cells. Data were represented as mean ± SD of at least three independent experiments performed in triplicates. Celecoxib (1.0 μM) was used as a positive control.

positive Cotton effect was observed at 230 nm and indicated the '*S*'-configuration of carbon C-5.[11] Therefore, the structure of compound **1** were determined to be methyl (1*S*,2*R*,3*R*,5*S*,7*R*)-7-(2′*S*-methyl butanoyloxymethyl)-2,3-dihydroxy-6,8-dioxabicyclo [3.2.1] octane-5-carboxylate, a new compound named chrysannol A.

Other known compounds were identified by comparison of their NMR data to previous reports, including dihydrosyringin (**2**),[12] syringin (**3**),[13] benzyl β-D-glucopyranoside (**4**),[14] β-phenylethoxy-β-D-glucopyranoside (**5**),[15] luteoloside (**6**),[16] tricin (**7**),[17] quercetin (**8**),[18] quercimetrin (**9**),[19] isorhamnetin 3-*O*-β-D-glucoside (**10**),[20] apigetrin (**11**),[21] chlorogenic acid (**12**),[22] chlorogenic acid methyl ester (**13**),[23] cryptochlorogenic acid methyl ester (**14**),[23] cynarin (**15**),[24] methyl 3,5-di-*O*-caffeoyl quinate (**16**),[25] methyl 3,4-di-*O*-caffeoyl quinate (**17**),[26] (*Z*)-5′-hydroxyjasmone 5′-*O*-β-D-glucopyranoside (**18**).[27] Eight compounds (**3**, **6**, **7**, **8**, **9**, **11**, **12**, and **15**) were determined previously and other compounds (**2**, **4**, **5**, **10**, **13**, **14**, and **16**–**18**) were isolated for the first time from this plant.

The anti-inflammatory effects of the isolated compounds in RAW 264.7 cells were investigated (see Supporting information). At a concentration of 50.0 μM, the MTT assay results showed that compounds **1**–**18** did not affect cell viability (data not shown). Thus, the effects of compounds **1**–**18** on LPS-induced production of the inflammatory mediators NO and TNF-α in RAW 264.7 cells were evaluated at concentrations lower than 50 μM. NO production was measured using Griess reaction assays. Each compound was screened at concentrations of 0.4, 2.0, and 10.0 μM (see Fig. 3). The results showed that compound **8** exhibited the highest inhibitory activity of 46.09% at 10.0 μM. Compound **9** exhibited potent inhibitory activity of 36.55% at the same concentration. The remaining compounds were weak or inactive. Additionally, aglycone **8** (inhibition value: 46.09%) displayed inhibitory activity that was higher than glycoside, compounds **6** and **9**–**11** (inhibition values decreased from 36.55% to inactive). The lack of a hydroxyl group in compound **7** also caused decreased inhibitory activity. The effect of compounds **8** and **9** on LPS-induced COX-2 and iNOS in RAW 264.7 cells were also investigated using Western blot analysis. The results showed that compounds **8** and **9** decreased COX-2 and iNOS protein expression in dose dependent manner (see Fig. 4).

Similarly, TNF-α secretion levels by cells stimulated with or without compounds were also evaluated (see Fig. 5). Compounds **7**, **10**, **11**, and **16** showed potent inhibitory activities at all of the test concentration (0.4, 2.0, and 10.0 μM) with inhibition values of 22.27% to 33.13%. Compounds **1**, **12**, and **18** displayed significant inhibitory activities of 21.96%, 24.73%, and 23.02% at 10.0 μM, and 14.42%, 16.13%, and 11.78% when diluted to 2.0 μM, respectively. Other compounds showed moderate inhibitory activities with inhibition values of 13.02% to 19.45% at a concentration of 10.0 μM. At 0.4 and 2.0 μM, the remaining compounds were weak or inactive, with the exception of compounds **7**, **10**, **11**, and **16**. Flavonoids (**6**–**11**) significantly inhibited TNF-α production. The glycoside compounds **6** and **9**–**11** showed higher inhibitory activities than the aglycones, **7** and **8**. These implied that the sugar moiety in the flavonoids enhanced the inhibitory activity of these compounds. In addition, the location and number of caffeoyl moieties in structures **12**–**17** are important for the activities of quinic acid derivatives.

In conclusion, our results indicated that the flavonoids and quinic acid derivatives showed marked inhibitory effects on the production of NO and TNF-α secretion. Compounds **8** and **9** also

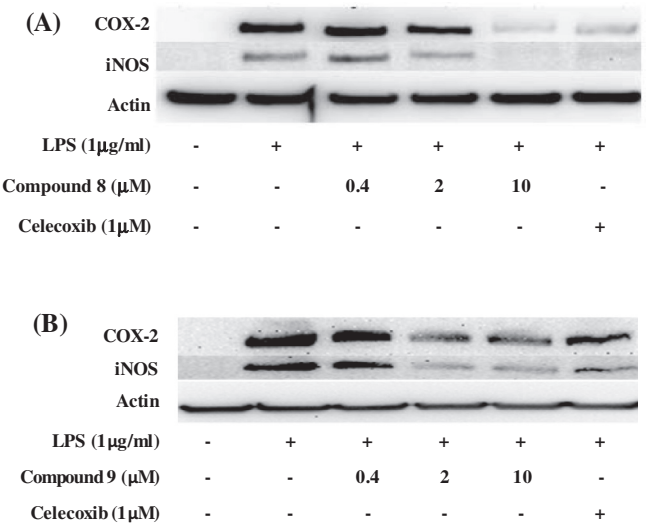

Figure 4. Effects of compounds **8** (A) and **9** (B) on LPS-induced COX-2 and iNOS in RAW 264.7 cells using Western blot analysis.

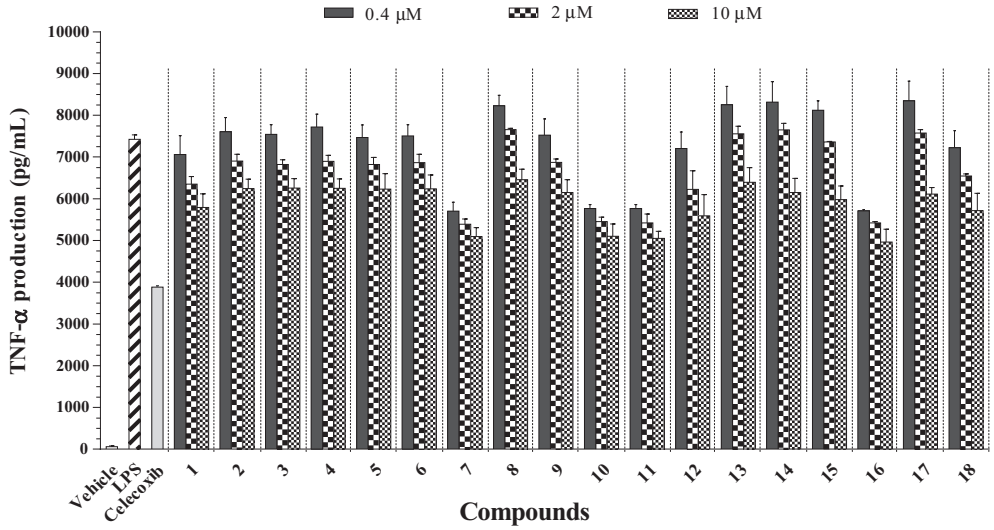

Figure 5. Effects of compounds **1**–**18** on the LPS-induced TNF-α production on RAW 264.7 cells. Data were represented as mean ± SD of at least three independent experiments performed in triplicates. Celecoxib (1.0 μM) was used as a positive control.

decreased COX-2 and iNOS protein expression based on Western blot analysis. Thus, the extract of *C. indicum* was rich in phenolic compounds, which may in part explain the use of this plant in the treatment of inflammatory conditions. These result are important for the development of *C. indicum* as an anti-inflammatory functional food. Further studies should determine the efficacy of combinations of these compounds in the treatment of inflammation.

Acknowledgments

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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.2014.11.054.

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8. *Chrysannol A* (**1**): Colorless oil; $[\alpha]_D^{26}$ −5.76 (*c* 0.08, MeOH); UV (MeOH) λ_{max} (log ε): 208 (2.10) nm; IR (KBr) ν_{max}: 3444, 2968, 1732, 1463, 1178, 1084 cm^{-1}; CD [λ nm, $\Delta\varepsilon$] (MeOH): 230 (+18.58) HR-ESI-MS *m*/*z*: 353.1008 $[M+Cl]^-$ (Calcd $C_{14}H_{24}O_9Cl$ for 353.1003); ^1H NMR (600 MHz, CD_3OD) and ^{13}C NMR (150 MHz, CD_3OD) are given in Table 1.
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