DOI 10.1007/s10600-019-02898-y
Chemistry of Natural Compounds, Vol. 55, No. 6, November, 2019

A NEW SESQUITERPENOID FROM *Chrysanthemum indicum*

Jia Chen,[1,2] Xu Yang,[3] Bo Li,[4] Kun Yang,[3] Yiqin Wang,[2*] Kexing Sun,[1] Yong Zhang,[4*] and Weiliang Zhu[4]

*A novel glucoside sesquiterpenoid (**1**) was isolated from* Chrysanthemum indicum *L. flowers, together with 14 known compounds (**2–15**), among which eight compounds (**3**, **4**, **8**, and **10–14**) were isolated from the plant for the first time. Their structures were determined on the basis of the interpretation of spectroscopic data,* viz.*, ESI-MS, HR-TOF-MS, IR, and NMR. The effects of these compounds on lipopolysaccharide-induced nitric oxide production by RAW 264.7 cells were investigated. Compounds **2**, **3**, and **7** showed strong or moderate inhibitory effects at 100 μM, and the IC_{50} values of **3** and **7** were 36.09 and 9.59 μM, respectively.*

Keywords: *Chrysanthemum indicum*, sesquiterpenoid, glucoside, nitric oxide production.

Dendranthema indicum is the dried flowers and inflorescences of *Chrysanthemum indicum* L. As a traditional Chinese medicine, it has been used for heat-clearing and detoxifying, detumescence, and removing intensive heat from the liver and improving acuity of sight [1]. *C. indicum* has many pharmacological effects such as anti-inflammatory [2–4], antibacterial [5], and immunomodulatory properties [6]. The flowers of this herb are also commonly used historically as tea to treat some eye diseases [7]. Phytochemical investigation on the plant has shown the presence of sesquiterpenoids, flavonoids, and phenolic compounds [1–3].

So far, there are only few studies focused on water-soluble chemical constituents of *C. indicum.* In our previous study, the water extract of *C. indicum* showed stronger anti-inflammatory activity than the other extractions [3]. Herein, we present the isolation and structure elucidation of one novel glucoside sesquiterpenoid (**1**) and eight compounds (**3**, **4**, **8**, and **10–14**) from *C. indicum* for the first time. In addition, all compounds were tested for their abilities to inhibit nitric oxide production, which is one of the indicators of inflammatory activity.

Compound **1** was obtained as a white amorphous powder. The molecular formula was determined to be $C_{21}H_{32}O_9$ from the quasimolecular ion peak at *m/z* 427.1979 $[M - H]^-$ (calcd 427.1974) in the HR-ESI-MS. Two fragment ion peaks of the negative ion ESI-MS at *m/z* 265.1 and 247.0 were attributed to $[M - C_6H_{10}O_5 - H]^-$ and $[M - C_6H_{10}O_5 - H_2O - H]^-$, respectively, which suggested the presence of one glucose moiety ($C_6H_{12}O_6$) in the molecule. The IR spectrum (KBr) exhibited absorptions for hydroxyl (3425 cm^{-1}), ester carbonyl (1767 and 1735 cm^{-1}), and olefinic functionalities (1654 cm^{-1}). The ^1H NMR spectrum of **1** (Table 1) showed the proton signals of three methyl protons at δ 1.83 (3H, s), 1.40 (3H, d, J = 6.7 Hz), and 1.17 (3H, s), an anomeric proton signal at δ 4.43 (1H, d, J = 7.8 Hz) and signals of one methylene group at δ 3.86 (1H, dd, J = 11.9, 2.1 Hz) and 3.72 (1H, dd, J = 11.9, 4.8 Hz) corresponding to a β-configuration of the glucose. The ^{13}C NMR spectrum (broadband and DEPT 135°) showed 21 signals comprising three methyl groups, three methylenes, 12 methines, and three quaternary carbons in an HSQC experiment. Five tertiary C-atoms (δ 105.2, 78.4, 77.9, 75.4, 71.3) and one methylene signal (δ 62.5) showed the presence of a glucose unit.

1) Shanghai Children's Medical Center, Shanghai Jiao Tong University, 200127, Shanghai, P. R. China; 2) Shanghai University of Traditional Chinese Medicine, 201203, Shanghai, P. R. China; 3) Chengdu University of Traditional Chinese Medicine, 611137, Chengdu, P. R. China; 4) Drug Discovery and Design Centre & CAS Key Laboratory of Receptor Research, Shanghai Institute of Materia Medica, Chinese Academy of Sciences, 201203, Shanghai, P. R. China, e-mail: zhangyong109@simm.ac.cn. Published in *Khimiya Prirodnykh Soedinenii*, No. 6, November–December, 2019, pp. 926–929. Original article submitted November 22, 2018.

TABLE 1. ^{1}H and ^{13}C NMR Data for **1** (CD_3OD, δ, ppm, J/Hz)

C atom	δ_H	δ_C	C atom	δ_H	δ_C
1	2.57 (1H, dd, J = 16.1, 8.0)	54.6	11	2.66 (1H, dd, J = 13.6, 6.8)	43.0
2	2.32 (1H, overlapped)	34.4	12	–	181.9
	2.24 (1H, m)		13	1.40 (3H, d, J = 6.7)	16.5
3	5.48 (1H, br.s)	127.0	14	1.17 (3H, s)	30.2
4	–	144.0	15	1.83 (3H, s)	17.6
5	2.70 (1H, t, J = 8.9)	56.0	Glc-1′	4.43 (1H, d, J = 7.8)	105.2
6	4.18 (1H, t, J = 10.1)	82.5	2′	3.22 (1H, m)	75.4
7	2.61 (1H, m)	54.5	3′	3.29–3.39 (1H, m, overlapped)	78.4
8	3.94 (1H, m)	83.2	4′	3.29–3.39 (1H, m, overlapped)	71.3
9	2.32 (1H, dd, J = 15.2, 5.0)	44.3	5′	3.29–3.39 (1H, m, overlapped)	77.9
	2.12 (1H, dd, J = 15.2, 4.3)		6′	3.86 (1H, dd, J = 11.9, 2.1)	62.5
10	–	74.2		3.72 (1H, dd, J = 11.9, 4.8)	

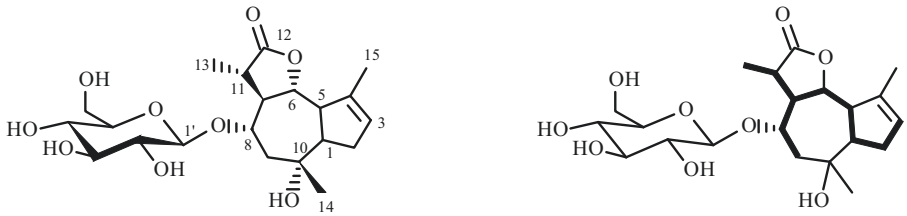

Fig. 1. Structure and $^{1}H–^{1}H$ COSY correlations of **1**.

These characteristic signals, in combination with the ESI-MS data (*m/z* 265.1 $[M - C_6H_{10}O_5 - H]^-$), implied that **1** is a glucoside guaianolide-type sesquiterpenoid. The NMR data of compound **1** were comparable to the known compound chrysanolide B [8]. The differences in NMR data for **1** and chrysanolide B were at C-8 and the signals of glucose (Table 1). The signals of the glucose unit and low-frequency shift of the 8-position by 83.2 ppm further revealed the presence of the sugar moiety at C-8, which was also substantiated by an HMBC experiment. The entire sequence of protons attached to the guaianolide skeleton was established by an $^{1}H–^{1}H$ COSY experiment (Fig. 1). The absolute configuration of the sugar was determined by acid hydrolysis followed by GC analysis. In the HMBC spectrum, the long-range correlations of H-1/C-2, C-5, and C-10; H-2/C-1 and C-3; H-3/C-2, C-4, and C-15; H-5/C-1, C-4, C-6, and C-10; H-6/C-8; H-7/C-6, C-8, and C-11; H-8/C-7 and C-1′; H-9/C-8 and C-10; H-11/C-7, C-12, and C-13; H-13/C-7, C-11, and C-12; H-14/C-1 and C-10; H-15/C-3, C-4, and C-5 were used to establish the molecular skeleton of **1** as being the same as the typical guaianolide sesquiterpene [8, 9]. The relative configuration of **1** was determined from its ROESY spectrum and *J*-based configurational analysis. The ROESY correlations of H-6/H-11, H-8/H-6, H-14, and H-1′ suggest that CH-6, CH-8, CH-11, CH_3-14, and CH-1′ are in the *β*-orientation. The correlations of H-1/H-5 and H-7/H-13 suggest that CH-1, CH-5, CH-7, and CH_3-13 are in the *α*-orientation. Thus, compound **1** was elucidated as 10*α*-hydroxy-8*α*-*O*-(*β*-D-glucopyranosyl)-1*αH*,5*αH*,6*βH*,8*βH*,7*αH*,11*βH*,11*α*-methylguaia-3-enolide.

The 14 known compounds (**2–15**) were identified as eriodictyol-7-*O*-*β*-D-glucopyranosiduronic acid (**2**) [7], luteolin-7-*O*-*β*-D-glucopyranosiduronic acid (**3**) [10], diosmin (**4**) [11], apigenin-7-*O*-*β*-D-rutinoside (**5**) [12], buddleoside (**6**) [10], luteolin-7-*O*-*β*-D-glucoside (**7**) [10], 5-hydroxy-4′-methoxyflavone-7-*O*-*α*-L-rhamnopyranosyl(1→6) [2-*O*-acetyl-*β*-D-glucopyranosyl(1→2)]-*β*-D-glucopyranoside (**8**) [13], 4-*O*-caffeoylquinic acid (**9**) [14], guanosine (**10**) [15], adenosine (**11**) [15], thymidine (**12**) [16], uridine (**13**) [15], bis(2-ethylhexyl)phthalate (**14**) [17], and uracil (**15**) [18] by comparison of their spectroscopic data with those reported in the literature.

To investigate whether compounds **1–15** have anti-inflammatory effects, inhibitory activities of the isolates on lipopolysaccharide induced nitric oxide production in mouse macrophage RAW264.7 cells were evaluated. Before the assay, the cytotoxicity of each isolate was detected, and no isolate was found that showed cytotoxicity to RAW264.7 cells (data not shown). As shown in Fig. 2, compounds **2**, **3**, and **7** showed strong or moderate inhibitory effects at 100 μM. In particular, the IC_{50} value of **7** was 9.59 μM, which was better than the positive control aminoguanidine hydrochloride (IC_{50} = 15.03 μM), and that of **3** was 36.09 μM.

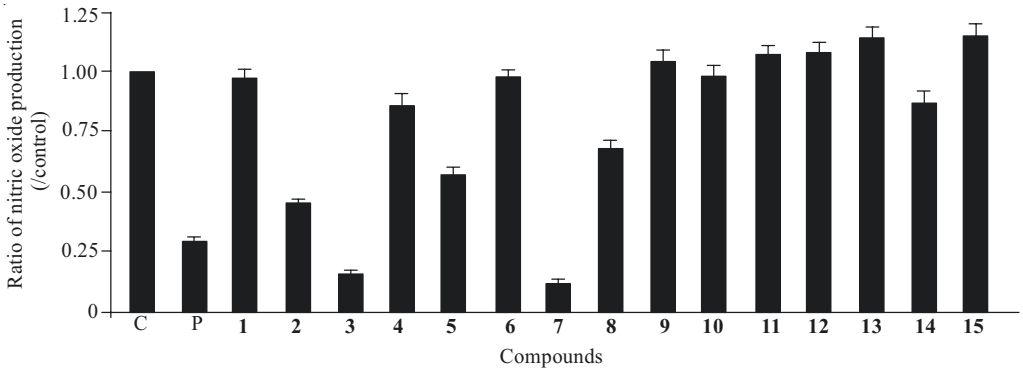

Fig. 2. Effect of compounds **1–15** on the LPS-induced nitric oxide production on RAW 264.7 cells. Data are the means ±s.d. of three wells. C – control; P – positive control (50 mM aminoguanidine hydrochloride).

EXPERIMENTAL

General Experimental Procedures. Macroporous resin Diaion HP20 (Mitsubishi Chemical Corporation, Japan), MCI (GEL CHP 20P, Mitsubishi Chemical Corporation, Japan), Sephadex LH-20 (Pharmazia, Uppsala, Sweden), and ODS (50 μm, YMC Co., Ltd, Kyoto, Japan) were used for column chromatography. Optical rotations were determined on a Rudolph Autopol VI 90079 polarimeter (Rudolph Research Analytical, Hackettstown, NJ, USA). IR spectra were recorded on a Thermo Nicolet 6700 spectrophotometer (Thermo Fisher Scientific, MA, USA). NMR spectra were obtained on a Bruker AV-400 spectrometer (Bruker Corporation, Rheinstetten, Switzerland). Chemical shifts are given in δ values (ppm) relative to tetramethylsilane as an internal standard. 2D NMR spectra were recorded on a Bruker AV-500 spectrometer (Bruker Corporation, Rheinstetten, Switzerland). ESI-MS were measured on a Finnigan LCQ-DECA spectrometer (Thermo Fisher, Pittsburgh, PA, USA). HR-ESI-MS were measured on a Waters Q-TOF Ultima Premier instrument (Waters, Milford, MA, USA).

Plant Material. The dried flowers and inflorescences of *Chrysanthemum indicum* L. were purchased from the market of Lotus Pond Medicinal Materials from Chengdu, China in 2016. The plants were identified by Dr. Yan-Hong Shi, Institute of Chinese Materia Medica, Shanghai University of Traditional Chinese Medicine. A voucher specimen (No. YJ-20160923) was deposited in the Drug Discovery and Design Centre, Shanghai Institute of Materia Medica, Chinese Academy of Sciences.

Extraction and Isolation. Dried flowers and inflorescences of *Chrysanthemum indicum* L. (5 kg) were crushed and reflux-extracted with water (40 L × 2 times, 1 h for each). The solvent was evaporated under reduced pressure and concentrated to yield 8 L of concentration liquid, which was extracted with EtOAc and *n*-BuOH. The *n*-BuOH extract (240 g) was subjected to HP20 (Diaion) column chromatography, with EtOH–H_2O (0% to 100%) to yield five fractions (Frs. 1–5), all fractions being monitored by TLC (*n*-BuOH–acetic acid–water, 4:1:5).

Fraction 2 (3.3 g, 20% EtOH) was subjected to repeated CC on ODS and eluted with MeOH–H_2O (0% to 60%) to yield six subfractions. Subfraction 3 was further separated by Sephadex LH-20 and eluted with 30% MeOH to afford compound **1** (7.6 mg). Subfraction 4 was also purified by Sephadex LH-20 (30% MeOH) to provide two subfractions (Subfrs. A and B). Subfraction B was separated by ODS with MeOH–H_2O (10%, 20%, and 30%) to give compounds **2** (14.3 mg) and **3** (48.2 mg). Then Subfr. A was separated by ODS with MeOH–H_2O (0% and 10%) to obtain compounds **12** (15.3 mg) and **15** (4.6 mg). In addition, Fr. 1 was separated by ODS with MeOH–H_2O (0% and 10%) to afford compounds **10** (21.4 mg), **11** (7.1 mg), and **13** (9.6 mg).

Fraction 3 (4.0 g, 40% EtOH) was subjected to MCI and eluted with MeOH–H_2O (20% to 100%) to yield five subfractions. Subfraction 2 (0.9 g, 40% MeOH) was further purified by ODS and eluted with MeOH–H_2O (20% to 60%) to obtain compounds **4** (8.0 mg), **5** (26.6 mg), and **6** (74.2 mg).

Fraction 4 (8.2 g, 60% EtOH) was also subjected to MCI and eluted with MeOH–H_2O (20% to 100%) to yield four subfractions. Subfraction 3 (1.2 g) was purified by ODS with MeOH–H_2O (20% to 80%) to give compounds **7** (122.1 mg) and **9** (14.8 mg). Subfraction 4 was further separated by Sephadex LH-20 with 60% MeOH to afford compounds **8** (9.7 mg) and **14** (15.4 mg).

10α-Hydroxy-8α-*O*-(β-D-glucopyranosyl)-1α*H*,5α*H*,6β*H*,8β*H*,7α*H*,11β*H*,11α-methylguaia-3-enolide (1). White amorphous powder; $[\alpha]_D^{25}$ –3.8° (*c* 0.5, MeOH). IR (KBr, ν_{max}, cm^{-1}): 3425, 1767, 1735, 1654, 1384, 1077, 1048, 1500, 1462, 1365, 1201, 1072. For ^1H and ^{13}C NMR (CD_3OD) spectral data, see Table 1. HR-ESI-MS *m/z* 427.1979 $[M - H]^-$ (calcd for $C_{21}H_{31}O_9$, 427.1974).

Determination of Sugar Components. The acid hydrolysis and detection of sugars were conducted according to the method described in [19]. Briefly, compound **1** (1 mg) was refluxed in 2 mL 10% HCl–dioxane (1:1) for 2 h, and the solution

was evaporated under N_2. The residue was dissolved in anhydrous pyridine (100 μL), 0.1 mL cysteine methyl ester hydrochloride (200 μL) was added, and the mixture was warmed at 60°C for 1 h. Next, 2.6 mL of the trimethylsilylation reagent HMDS–TMCS (hexamethyldisilazane–Me_3SiCl–pyridine, 2:1:10) was added, with warming at 60°C for another 30 min. The mixture was partitioned between cyclohexane and H_2O (each 2 mL). The cyclohexane extract was subjected to GC analysis to identify the sugars. GC conditions: cap. column, DB-5 (30 m × 0.25 mm × 0.25 μm); detection, FID; detector temp., 280°C; injection temp., 280°C; initial temp. maintained at 100°C for 5 min and then raised to 280°C at the rate of 35°C/min, final temp. maintained for 9 min; carrier, N_2 gas. The absolute configurations of the sugars were determined by comparing the retention times (t_R) of derivatives of the sugars with those of authentic sugars prepared in a similar way. The t_R values of the derivatives were 14.16 min (D-glucose).

Anti-inflammatory Effects Assay. Cell Culture. RAW 246.7 cells were maintained in Dulbecco′s modified Eagle′s medium (high-glucose condition) supplemented with 10% fetal calf serum, 100 U/mL penicillin, and 100 μg/mL streptomycin at 37°C in 5% CO_2.

Measurement of Nitric Oxide Production. RAW 246.7 cells were seeded in 96-well plates at a density of 50.000 cells/well. After incubation for 24 h, cells were treated with the isolates (100 μM) for 30 min and then stimulated with LPS (100 ng/mL) for 24 h. The culture supernatant (50 μL) was transferred to another 96-well plate and reacted with Griess reagent (50 μL) for 10 min. Then, the absorbance at 540 nm was measured using a microplate spectrophotometer. The compounds were dissolved in Me_2SO, and the final concentration of Me_2SO was < 0.1%. Aminoguanidine hydrochloride, which inhibits nitric oxide synthase activity, was used as the positive control.

ACKNOWLEDGMENT

This work was supported by the Natural Science Foundation of Young Scholars of China (81603270), the China Postdoctoral Science Foundation (2017M611608), and the Shanghai Sailing Program (16YF1414100), which are gratefully acknowledged. In addition, Jia Chen and Xu Yang contributed equally to this work.

REFERENCES

1. J. S. Wang, J. Zhou, and L. Y. Kong, *Fitoterapia*, **83**, 1675 (2012).
2. G. M. Xue, X. Q. Li, C. Chen, K. Chen, X. B. Wang, Y. C. Gu, J. G. Luo, and L. Y. Kong, *J. Nat. Prod.*, **81**, 378 (2018).
3. L. B. Luyen, B. H. Tai, N. P. Thao, J. Y. Cha, H. Y. Lee, Y. M. Lee, and Y. H. Kim, *Bioorg. Med. Chem. Lett.*, **25**, 266 (2015).
4. J. Chen, M. Guo, L. A. Zhou, Y. H. Shi, J. Guo, and Y. Q. Wang, *Chin. Trad. Patent Med.,* **40**, 440 (2018).
5. A. N. Ren, Z. G. Wang, Z. C. Lu, L. W. Wang, and Y. L. Wu, *Pharm. Biotechnol.*, **6**, 241 (1999).
6. Z. G. Wang, A. N. Ren, L. Xu, X. J. Sun, and X. B. Hua, *Chin. J. Trad. Med. Sci. Technol*., **2**, 92 (2000).
7. H. Matsuda, T. Morikawa, I. Toguchida, S. Harima, and M. Yoshikawa, *Chem. Pharm. Bull.*, **50**, 972 (2002).
8. Q. Gu, Y. Y. Chen, H. Cui, D. Huang, J. W. Zhou, T. Z. Wu, Y. P. Chen, L. N. Shi, and J. Xu, *RSC Adv.*, **3**, 10168 (2013).
9. M. Ibrahim, M. Imran, B. Ali, R. Hussain, F. S. Rehmani, and A. Malik, *J. Asian Nat. Prod. Res.*, **14**, 281 (2012).
10. Z. O. Toshmatov, K. A. Eshbakova, and H. A. Aisa, *Chem. Nat. Compd.*, **54**, 358 (2018).
11. G. Lewin, A. Maciuk, A. Moncomble, and J. P. Cornard, *J. Nat. Prod.*, **76**, 8 (2013).
12. F. A. Moharram, M. S. Marzouk, S. M. El-shenawy, A. H. Gaara, and W. M. Kady, *J. Pharm. Pharmacol.*, **64**, 1678 (2012).
13. Y. X. Shen, L. H. Quan, L. Guan, and J. M. Chen, *Acta Pharm. Sin.*, **32**, 451 (1997).
14. I. Dini, G. C. Tenore, and A. Dini, *J. Agric. Food Chem.*, **54**, 8733 (2006).
15. V. G. Nebieridze, A. V. Skhirtladze, E. P. Kemertelidze, and M. Ganzera, *Chem. Nat. Compd.*, **53**, 1010 (2017).
16. J. F. Sun, Y. Wu, B. Yang, and Y. H. Liu, *Chem. Nat. Compd.*, **51**, 975 (2015).
17. S. S. Hong, H. J. Suh, and J. S. Oh, *Chem. Nat. Compd.*, **53**, 359 (2017).
18. S. Kan, G. Y. Chen, C. R. Han, Z. Chen, X. M. Song, M. Ren, and H. Jiang, *Nat. Prod. Res.*, **25**, 1243 (2011).
19. Y. Zhang, C. H. Tan, J. J. Tan, P. M. Yang, S. H. Jiang, X. Ni, and D. Y. Zhu, *Helv. Chim. Acta*, **93**, 1407 (2010).