Two New Terpenoids From the Fruits of *Chaenomeles sinensis* (Thouin) Koehne

Meng Li1,2*, Zhi-guang Zhang1,2,3*, Jing-ya Shi1,2, Ya-ge Li1,2, Jing-ke Zhang1,2, Yang Ying1,2, Xiao-yan Deng1,2, Xiao-ke Zheng1,2, and Wei-sheng Feng1,2

Abstract

A new sesquiterpenoid, chaenomelisterpenoid A (1), and a new norisoprenoid, chaenomelisterpenoid B (2), were isolated from the fruits of *Chaenomeles sinensis* (Thouin) Koehne. Their structures were determined by NMR spectroscopy and MS. In addition, the protective effects of the compounds were tested against corticosterone-induced damage in PC-12 cells using real-time cellular analysis (RTCA). Compounds 1 and 2 significantly improved cell viability and corticosterone-induced damage in PC-12 cells with EC50 values of 15.7 and 12.6 µM, respectively.

Keywords

*Chaenomeles sinensis* (Thouin) Koehne, fruits, chemical constituents, terpenoids, protective effect on PC12 cells

Received: October 12th, 2020; Accepted: January 22nd, 2021.

*Chaenomeles sinensis* (Thouin) Koehne, commonly known as Chinese quince or “Guang Pi Mu Gua,” is a deciduous or semi-evergreen tree of the family Rosaceae and native to China.1 Its fruits are of high economic value and reported to be rich in dietary fiber, organic acids, and vitamins,2 while its sugar content is relatively low. After appropriate dilution and supplementation with a sweetener, the fruits can be made into a delicious dietary fiber, organic acids, and vitamins,2 while its sugar content is relatively low. After appropriate dilution and supplementation with a sweetener, the fruits can be made into a delicious food with a unique flavor. *Chaenomeles* fruits are used as medicinal herbs in Korea and China for the treatment of throat diseases, anaphylaxis, viral infection, and neurodegenerative diseases.3 Modern pharmacological investigations have demonstrated significant biological properties such as anti-hyperuricemic, antiacetylcholinesterase, and antidiabetic effects.4 The fruits of *C. sinensis* contain pentacyclic triterpene acids, flavonoids, lignans, and simple phenolic compounds.5 As a continuation of our recent research on the neuroprotective constituents of *C. sinensis* fruits,6 we further isolated a new sesquiterpenoid, chaenomelisterpenoid A (1), and a new norisoprenoid, chaenomelisterpenoid B (2) (Figure 1). Here, we report the isolation, structure elucidation, and evaluation of the protective effects of compounds 1 and 2 on corticosterone-induced pheochromocytoma cell damage in rats (PC-12 cells).

Results and Discussion

Compound 1 was isolated as a white powder. The molecular formula was determined to be C21H30O9 based on the HR-ESI-MS ion at m/z 449.1786 [M + Na]+ (calculated for 449.1782, C21H30O9Na) (Supplemental Figure S8), combined with 1H and 13C NMR spectroscopic data. The UV spectrum of 1 displayed absorption of a conjugated double bond at 266 nm (Supplemental Figure S10). The IR spectrum displayed absorption bands at 3385 (OH), 1654 (conjugated C = O), and 1030 (ether bond) cm⁻¹ (Supplemental Figure S9). The 1H NMR spectrum of 1 showed a pair of trans-olefinic proton signals [δH 6.48 (1H, d, J = 15.7 Hz, H-8) and 6.29 (1H, d, J = 15.7 Hz, H-7)], two olefinic proton signals [δH 5.90 (1H, s, H-4) and 5.86 (1H, s, H-10)], and four methyl groups [δH 2.28 (3H, s, H-15), 1.90 (3H, s, H-14), 1.05 (3H, s, H-13), and 1.00 (3H, s, H-12)]. In addition, a pair of free anomic protons of glucose were observed at δH 5.08 (0.5H, d, J = 3.7 Hz, Hα−1′) and 4.47 (0.5H, d, J = 7.7 Hz, Hβ−1′) (Supplemental Figure S1). The 13C NMR and DEPT spectra indicated the presence of 27 carbons, of which 12 were assigned to the pair of free anomeric protons of glucose. 72.0 (Cα−1′)/73.8 (Cβ−1′), 70.7 (Cα−2′)/76.2 (Cβ−2′), 72.0 (Cα−3′)/73.8 (Cβ−3′), 70.7 (Cα−4′)/71.8 (Cβ−4′), 76.2 (Cα−5′)/77.9 (Cβ−5′), 64.4 (Cα−6′)/64.5 (Cβ−6′) (Supplemental Figure S2 and S3). According to the

1 Henan University of Chinese Medicine, Zhengzhou, China

2 The Engineering and Technology Center for Chinese Medicine Development of Henan Province, China

3 School of Pharmacy, Minzu University of China, Beijing, China

*Dr. Meng Li and Zhi-guang Zhang contributed equally to the work.

Corresponding Authors:

Wei-sheng Feng and Xiao-ke Zheng, Henan University of Chinese Medicine, Zhengzhou 450046, China.

Emails: fwshe@huactcm.edu.cn; zhengxk.2006@163.com

Creative Commons Non Commercial CC BY-NC: This article is distributed under the terms of the Creative Commons Attribution-NonCommercial 4.0 License (https://creativecommons.org/licenses/by-nc/4.0/) which permits non-commercial use, reproduction and distribution of the work without further permission provided the original work is attributed as specified on the SAGE and Open Access pages (https://us.sagepub.com/en-us/nam/open-access-at-sage).
glycosylation shift, the aglycone unit was assumed to be connected to C-6′ of glucose. The other carbons were observed as two carbonyl groups [δC 200.9 (C-3) and 168.3 (C-11)], four quaternary carbons [δC 166.2 (C-5), 153.2 (C-9), 80.3 (C-6), and 42.7 (C-1)], four methine groups [δC 127.5 (C-4), 137.4(C-7), 135.0(C-8), and 120.5(C-10)], a methylene group [δC 50.7 (C-2)] and four methyl groups [δC 24.7(C-12), 23.6(C-13), 19.4(C-14), and 14.3(C-15)], which suggested that the aglycon of 1 might be (2-trans, 4-trans)-abscisic acid except for the additional signals associated with the free glucopyranosyl unit. Acid hydrolysis of 1 with 2 M HCl afforded glucose, revealing the occurrence of a D-glucopyranosyl moiety in 1\textsuperscript{10}. The cross peaks between 4.21 (H\textsubscript{2}−6′) and 4.25 (H\textsubscript{2}−6′) of glucose and C-11 in the HMBC spectrum indicated that C-6′ of the glucose unit is connected to C-11 (Figure 2 and Supplemental Figure S6), which was confirmed by the glycosidation shift.\textsuperscript{11} Thus, compound 1 was identified as (7E, 9\text{Z})-abscisic acid-(α and β)-glucopyranoside and named chaenomelesterpenoid A (Figure 1).

Compound 2, extracted as white powder, was determined to possess a molecular formula of C\textsubscript{11}H\textsubscript{16}O\textsubscript{3} from the HR-ESI-MS [M + Na\textsuperscript{+}] ion at \textit{m/z} 219.0992 (Calcd 219.0991) (Supplemental Figure S18). The UV spectrum of 2 exhibited absorptions at 213 and 265 nm (Supplemental Figure S20). In the \textsuperscript{1}H NMR spectrum of 2, the signals of one olefinic proton [δ\textsubscript{H} 5.80 (1H, s, H-9)], one oxygenated methylene [δ\textsubscript{H} 3.57 (1H, d, J = 11.0 Hz, H-12)], and three methines [δ\textsubscript{H} 2.24 (1H, dd, J = 1.6, 12.5 Hz, H-5\text{α}), 1.43 (1H, dd, J = 5.3, 12.5 Hz, H-5\text{β}), 1.80 (2H, m, H-4), 1.65 (1H, m, H-3\text{α}), and 1.35 (1H, m, H-3\text{β})], and two methyl groups [δ\textsubscript{H} 1.56 (3H, s, H-12) and 1.24 (3H, s, H-11)] were observed (Supplemental Figure S11). The \textsuperscript{13}C NMR and DEPT135 spectra indicated the presence of 11 carbons, including four quaternary [δC 182.0 (C-1), 174.7 (C-8), 89.5 (C-6), and 42.8 (C-2)], one methine group [δC 113.3(C-9)], four methylene groups [δC

![Figure 1. Structures of compounds 1 and 2.](image1)

![Figure 2. Key HMBC and \textsuperscript{1}H-\textsuperscript{1}H COSY correlations of 1 and 2.](image2)

![Figure 3. Key NOESY correlations of 2.](image3)

70.9 (C-10), 40.9 (C-5), 36.8 (C-3), and 19.9 (C-4)] and two methyl groups [δC 20.0 (C-11) and 24.8 (C-12)] (Supplemental Figure S12 and S13). These spectroscopic features suggested that the structure of 2 was very similar to that of 3,9-dihydroxy dihydroactinidiolide.\textsuperscript{12} The obvious difference was that hydroxy group at C-4 of 3,9-dihydroxy dihydroactinidiolide was replaced by a proton in 2. The NOESY experiment showed the correlation between H-10 and H-12, confirming that H-10 and H-12 had a β-configuration (Figure 3 and Supplemental Figure S17). Therefore, compound 2 was characterized as chaenomelesterpenoid B (Figure 1).

The protective effects of the compounds were tested against corticosterone-induced damage in PC-12 cells using real-time cellular analysis (RTCA). Compounds 1 and 2 significantly improved cell viability and corticosterone-induced damage in PC-12 cells with EC\textsubscript{50} values of 15.7 and 12.6 µM, respectively.

**Experimental Part**

**Materials**

NMR spectra (including 1D and 2D) were recorded on a Bruker Avance III 500 MHz spectrometer (500 MHz for \textsuperscript{1}H-NMR and 125 MHz for \textsuperscript{13}C-NMR), optical rotations on an APIV (Rudolph Research Analytical), and IR spectra on a Nicolet iS10 Microscope Spectrometer (Thermo Fisher Scientific). HR-ESI-MS were obtained on a Bruker maXis HD mass spectrometer and UV spectra on a Shimadzu UV-2401PC apparatus. Preparative HPLC was conducted using a Saipuruisi LC-50 instrument with an UV200 detector and YMC-Pack ODS-A column (250 × 20 mm, 5 µm and 250 × 10 mm, 5 µm). Column chromatography was performed using Diaion HP-20 (Mitsubishi Chemical Corporation), Toyopearl HW-40, MCI gel CHP-20 (TOSOH Corporation), Sephadex LH-20 (Amersham Pharmacia Biotech AB), LiChroprep RP-18 gel (Merck, Darmstadt), and silica gel (Marine Chemical Industry). For TLC, self-made silica gel G plates (Qingdao Marine Chemical Industry) were used. All of the chemical reagents were supplied by Beijing Chemical Plant and Tianjin No. 3 Reagent Plant. RTCA was measured with an xCELLLigence RTCA System (Acea Biosciences, Inc.).
Plant materials

The fruits of *Chaenomeles sinensis* (Thouin) Koehne were collected in November 2016 from Fangcheng County, Nanyang City, Henan Province, China. The plants were identified by Professor Chengming Dong of Henan University of Chinese Medicine. A voucher specimen (No. 20171101) has been deposited in the Department of Natural Medicinal Chemistry, School of Pharmacy, Henan University of Chinese Medicine, Zhengzhou, China.

Isolation

The fruits of *C. sinensis* (30.0 kg) were extracted thrice with 50% acetone-water using a tissue crushing extraction method. The filtrate was evaporated under vacuum to obtain the extract (2.67 kg), which was then precipitated using 80% ethanol (10 L × 5); the liquid supernatant was concentrated in a vacuum evaporator to yield the gross extract, which was resuspended in H2O (2.5 L). The extract was passed through a Diaion HP-20 macroporous resin column and eluted with 0%, 10%, 20%, 30%, 40%, 50%, 70%, and 95% EtOH-H2O successively to obtain eight fractions (A–H). Fr.C (39.3 g) was chromatographed on Toyopearl HW-40C eluting with water, and gradually decreasing the polarity with MeOH to yield 5 fractions (C1–C5). Fr.C3 was separated by Toyopearl HW-40C CC by eluting with MeOH-H2O (50:50) to yield 4 fractions (C3-1–C3-4). Fr. 3-2 (150.0 mg) was subjected to ODS CC eluting with MeOH-H2O (0:100–40:100) to generate 4 fractions (Fr. C3-2-1–Fr. C3-2-6). Fr. C3-2-5 was purified by semi-preparative HPLC (Saipuruisi LC-50) (MeCN-H2O, 18:82, v/v) to afford compound 1 (5.3 mg, tR = 41.33 minutes). Fr. C3-4 was purified by semi-preparative HPLC (MeCN-H2O, 10:90, v/v) to afford compound 2 (0.8 mg, tR = 130.85 minutes).

Acid-Catalyzed Hydrolysis of Compounds

Compound 1 (1 mg) was treated with 2 mol/L aqueous HCl (2 ml) (sealed flask, heating in water bath, 80 °C, 3 hours). Then the acidic aqueous mixture was dried, H2O (2 ml) was added, and the mixture extracted with EtOAc (3 × 2 ml). The aqueous layer was subjected to chiral-phase HPLC analysis under the following conditions. The sugars of the hydrolysis were separated and detected using a CHIRALPAK AD-H column (250 × 4.6 mm) using n-hexane:EtOH:TFA (750:250:0.25) as the mobile phase (0.5 mL/min–1) and an evaporative light scattering detector (ELSD). Identification of D-glucose was carried out by comparison of its retention time with that of authentic samples (tR = 18.3 minutes; D-glucose).

Biological Assay

PC-12 cells were cultured in RPMI 1640 medium containing 10% FBS, 50 units/mL penicillin, and 50 µg/mL streptomycin and penicillin in a humidified atmosphere at 37 °C in 5% CO2. The RTCA assay was performed for 60 hours using an xCELLigence RTCA system. Background impedance signals were measured with 100
with a target density of $2 \times 10^4$ cells in $100 \mu L$ medium per well. The cells were seeded into 16-well E-plates and incubated in medium. The cells were digested with 0.25% trypsin to obtain a single-cell suspension. The cells were cultured for 4 days, and the medium was changed every 15 minutes until the end of the experiment. After plating, impedance was routinely recorded at 15 minutes intervals. One day after seeding, the cells in 16-well E-plates were treated with test compounds 1 and 2 at 5 different concentrations (0.1, 1, 10, 50, and 100 µM) and corticosterone (500 µM). All the incubations were performed with a final solvent concentration of 0.1% DMSO. After compound administration, impedance was measured every 5 minutes for the following time points, and afterward every 15 minutes until the end of the experiment.15,16

### Conclusions

A new sesquiterpenoid, chaenomelesterpenoid A (1), and a new norisoprenoid, chaenomelesterpenoid B (2), were isolated from *Chaenomeles* fruits. The two compounds significantly improved cell viability and corticosterone-induced damage in PC-12 cells with EC$_{50}$ values of 15.7 and 12.6 µM, respectively. Our study provides useful information on the chemistry of *C. sinensis* (Thouin) Koehne.

### Declaration of Conflicting Interests

The author(s) declared no potential conflicts of interest with respect to the research, authorship, and/or publication of this article.

### Funding

The author(s) disclosed receipt of the following financial support for the research, authorship, and/or publication of this article: The National Key Research and Development Project (2017YFC1702800), the Central Government Guide Local Science and Technology Development Funds (14104349), the Key Scientific Research Project of Institutions of Higher Learning in Henan (21B360003), and the Special Project of Scientific Research on Traditional Chinese Medicine in Henan (20-21ZY2151) supported this study.

### Supplemental Material

Supplemental material for this article is available online.

### References

1. Zhang R, Zhan S, Li S, et al. Anti-hyperuricemic and nephro-protective effects of extracts from *Chaenomeles sinensis* (Thouin) Koehne in hyperuricemic mice. Food Funct. 2018;9(11):5778-5790. doi:10.1039/C8FO01480A
2. Zhang LH, Xu HD, Li SF. Effects of micronization on properties of *Chaenomeles sinensis* (Thouin) Koehne fruit powder. Innovative Food Science & Emerging Technologies. 2009;10(4):633-637. doi:10.1016/j.ifset.2009.05.010
3. Cha K-J, Song C-S, Lee J-S, et al. *Chaenomeles sinensis* Koehne extract suppresses the development of atopic dermatitis-like lesions by regulating cytokine and filaggrin expression in NC/Nga mice. Int J Med Sci. 2019;16(12):1604-1613. doi:10.7150/ijms.37854
4. Sanchez S, Sanchez S, Seo S-Y. Antidiabetic and antiacetylcholinesterase effects of ethyl acetate fraction of *Chaenomeles sinensis* (Thouin) Koehne fruits in streptozotocin-induced diabetic rats. Exp Toxicol Pathol. 2013;65(1-2):55-60. doi:10.1016/j.etp.2011.05.010
5. Yin ZH, Zhao C, Zhang JJ, et al. Research progress on chemical constituents and pharmacological activities of *Chaenomeles sinensis*. Chin J Exp Tradit Med Form. 2017;23(9):234-242. doi:10.13422/j.cnki.syfjx.2017090221
6. Feng WS, Zhang ZG, Li M, et al. Chaenomelesalkaloid A, a new alkaloid from the fruits of *Chaenomeles sinensis* (Thouin) Koehne. Acta Pharm Sin. 2018;53(6):976-979. doi:10.16438/j.0513-4870.2018-0211
7. Bokern M, Heuer S, Wray V, et al. Ferulic acid conjugates and betacyanins from cell cultures of *Beta vulgaris*. Phytochemistry. 1991;30(10):3261-3265. doi:10.1016/0031-9422(91)83189-R
8. Tian L, YJ L, SW L, et al. Chemical constituents of *Syringa oblata* leaves. Chin J Exp Tradit Med Form. 2013;19(01):144-147. doi:10.13422/j.cnki.syfjx.2013.01.051
9. Li HB, Yu Y, Wang ZZ, et al. Research on chemical constituents from Re-Du-Ning injection (III); *Chin Tradit Herbal Drugs*. 2016;47(10):1643-1649. doi:10.7501/j.issn.0253-2670.2016.10.002
10. Li M, Zeng M, Zhang Z, et al. Uridine derivatives from the seeds of *Lepidium apetalum* wildl. and their estrogenic effects. Phytochemistry. 2018;155:45-52. doi:10.1016/j.phytochem.2018.07.013
11. Shi JY, Zhang ZG, Li M, et al. Phenolic constituents from the fruit of *Chaenomeles sinensis*. Chin Pharm J. 2020;55(20):1666-1672. doi:10.11669/cpj.2020.20003
12. Bai M, Cai Y, Wu S-Y, et al. A new norisoprenoid from the leaves of *Ficus pumila*. Nat Prod Res. 2019;33(9):1292-1297. doi:10.1080/14786419.2018.1471077

### Table 2. NMR Spectral Data of Compound 2 (In CD$_3$OD).

| No. | $\delta_{H}$ | $\delta_{C}$ | No. | $\delta_{H}$ | $\delta_{C}$ |
|-----|-------------|-------------|-----|-------------|-------------|
| 1   | 182.0       |             | 6   | 89.5        |             |
| 2   | 42.8        |             | 8   | 174.7       |             |
| 3   | 1.65 (1H, m) | 36.8        | 9   | 5.80 (1H, s) | 113.3       |
|     | 1.35 (1H, m) | 5.3         | 10  | 3.57 (1H, d, J = 11.0 Hz) | 70.9    |
|     |             | 3.55 (1H, d, J = 11.0 Hz) |         |             |             |
| 4   | 1.80 (2H, m) | 19.9        | 11  | 1.56 (3H, s) | 20.0        |
| 5   | 2.24 (1H, d, J = 6.0, 12.5) | 40.9 | 12  | 1.24 (3H, s) | 24.8    |
|     | 1.43 (1H, d, J = 5.3, 12.5) |             |     |             |             |

### ORCID ID

Meng Li https://orcid.org/0000-0002-9633-9257

### Supplemental Material

Supplemental material for this article is available online.
13. Feng W, Lv Y, Zheng X, Zhang Y, Cao Y, Pei Y. A new megastigmae from fresh roots of *Rehmannia glutinosa*. *Acta Pharm Sin B*. 2013;3(5):333-336. doi:10.1016/j.apsb.2013.07.001

14. Lopes JF, Gaspar EMSM. Simultaneous chromatographic separation of enantiomers, anomers and structural isomers of some biologically relevant monosaccharides. *J Chromatogr A*. 2008;1188(1):34-42. doi:10.1016/j.chroma.2007.12.016

15. Li G, Wu K, Li YH, et al. Evaluation of hypoxia/reoxygenation injury of H9c2 myocardial cells and intervention effect of notoginsenoside R1 based on RTCA technology. *Chin Pharmacol Bull*. 2019;35(3):436-440. doi:10.3969/j.issn.1001-1978.2019.03.027

16. He XY, Chen JL, Xiang H, et al. Comparative study of the corticosterone and glutamate induced PC12 cells depression model by $^1$H NMR metabolomics. *Yao Xue Xue Bao*. 2017;52(2):245-252. doi:10.16438/j.0513-4870.2016-0543