DOI 10.1007/s10600-022-03663-4
Chemistry of Natural Compounds, Vol. 58, No. 2, March, 2022

A NEW TRITERPENE FROM *Metadina trichotoma*

Bin-Bin Fan,[1,2] Jia-Xiang Liu,[1,2] Hai-Jun Hu,[2,3] and Yu-Mei Zhang[1*]

*One new triterpene, 6α-hydroxyilexosapogenin A (**1**), and nine known compounds were isolated from* Metadina trichotoma. *Their structures were mainly elucidated by NMR and MS spectra. As evidenced by a glucose concentration assay, compound **2** can stimulate glucose uptake in 3T3-L1 adipocytes and is the first pyrocincholic acid type triterpenoid that shows promoting glucose uptake activity.*

Keywords: *Metadina trichotoma*, triterpenoid, 3T3-L1 adipocytes, glucose uptake.

Metadina trichotoma (Zoll. & Moritzi) Bakh. f. is a unique species in the genus *Metadina* and is mainly distributed in Southwest China, Vietnam, Malaysia, Myanmar, and India. As a stable, beautiful, insect- and corrosion-resistant high-quality wood, *M. trichotoma* is widely used in furniture, carving materials, and interior decoration materials [1]. The main characteristic chemical constituents of *M. trichotoma* are pentacyclic triterpenoids and their glycosides [2]. According to the literatures, pentacyclic triterpenoids mainly show antivirus [3], antitumor [4], anti-inflammatory, and hypoglycemic activities [5, 6]. In our previous investigations, the bioactive constituents responsible for cytotoxicity, adipogenesis, and lipid metabolism [2, 7] were reported from this species. In this paper, the isolation of one new triterpene, nine known compounds, and their ability to stimulate glucose uptake in 3T3-L1 adipocytes is reported.

Compound **1** was obtained as a white powder. It was found to possess the molecular formula $C_{30}H_{48}O_6$ by HR-ESI-MS *m*/*z* 527.3340 $[M + Na]^+$, which was confirmed by ^{13}C and DEPT NMR spectra. The ^{13}C NMR spectra of compound **1** showed the presence of six Me, nine CH_2, and seven CH, as well as eight quaternary carbon atoms. These data indicated that compound **1** has an oleanolic acid skeleton.

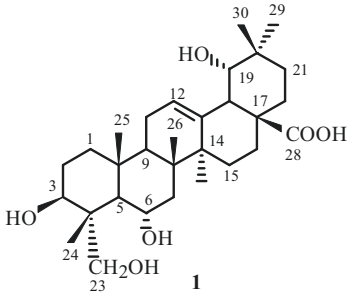
Compared with ilexosapogenin A, only one more hydroxy occurred in compound **1** [8]. In the $^1H–^1H$ COSY spectrum, the cross-peaks between δ_H 1.17 (H-5) and 3.99 (H-6), 3.99 (H-6), and 1.56 (H-7) indicated the presence of a -CH-CH(OH)-CH_2- fragment. In the HMBC spectrum, δ_H 1.56 (H-7) correlated with δ_C 41.7 (C-8) and 19.1 (C-26); δ_H 3.99 (H-6) showed cross-peaks with δ_C 56.6 (C-5), 45.2 (C-7), and 40.0 (C-10); δ_H 1.17 (H-5) showed long correlations with δ_C 74.7 (C-3), 44.4 (C-4), and 40.0 (C-10), which indicated that the hydroxy group was located at C-6 in compound **1**. In the ROESY spectrum, the ROESY correlations of δ_H 3.99 (H-6) with 1.03 (H-25) and 0.86 (H-26) suggested that the proton atom took the same β-orientation as H-25 and H-26, and the hydroxy was α-oriented at C-6. Hence, compound **1** was identified as 3β,6α,19α,23-tetrahydroxyolean-12-en-28-oic acid and named 6α-hydroxyilexosapogenin A.

Careful analysis of the 1D NMR and MS spectral data resulted in the identifications of the other nine known compounds as pyrocincholic acid 3β-*O*-β-D-quinovopyranosyl-28-*O*-β-D-glucopyranoside (**2**) [9], quinovic acid (**3**) [10], berchemolide (**4**) [11], syrinfaresinol-*O*-β-D-glucopyroside (**5**) [12], *E*-palmityl-4-hydroxycinnamic acid (**6**) [13], deacetylasperulosidic acid methyl ester (**7**) [14], 6-*O*-methyldeacetylasperulosidic acid methyl ester (**8**) [15], geniposide (**9**) [16], and scoponin (**10**) [17] respectively.

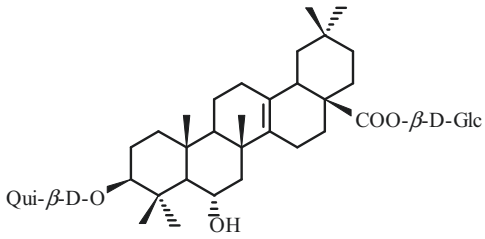
1) Key Laboratory of Tropical Plant Resources and Sustainable Use, Xishuangbanna Tropical Botanical Garden, Chinese Academy of Sciences, 650223, Kunming, P. R. China, e-mail: zymei@xtbg.ac.cn; 2) University of Chinese Academy of Sciences, 100049, Beijing, P. R. China; 3) State Key Laboratory of Phytochemistry and Plant Resources in West China, Kunming Institute of Botany, Chinese Academy of Sciences, 650204, Kunming, P. R. China. Published in *Khimiya Prirodnykh Soedinenii*, No. 2, March–April, 2022, pp. 258–260. Original article submitted January 15, 2021.

TABLE 1. ^{1}H (500 MHz) and ^{13}C (125 MHz) NMR Data for **1** (CD_3OD, δ, ppm, J/Hz)

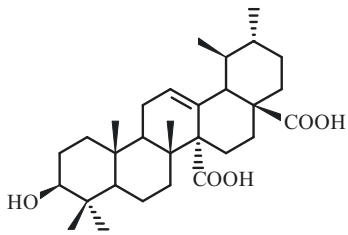
C atom	δ_H	δ_C	HMBC
1	1.56 (1H, overlapped), 1.04 (1H, overlapped)	39.3	C-2, 3, 10, 25
2	2.30 (1H, m), 1.69 (1H, m)	27.5	C-4, 10
3	3.40 (1H, dd, J = 10.8, 4.7)	74.7	C-1, 2, 4, 23, 24
4	–	44.4	–
5	1.17 (1H, d, J = 10.7)	56.6	C-3, 4, 6, 7, 10, 25
6	3.99 (1H, m)	68.3	C-5, 7, 10
7	1.56 (2H, overlapped)	45.2	C-5, 8, 26
8	–	41.7	–
9	1.76 (1H, overlapped)	48.7	C-11, 25, 26
10	–	40.0	–
11	1.98 (2H, m)	25.0	C-8, 9, 10, 12, 13
12	5.35 (1H, m)	125.1	C-9, 11, 14, 18
13	–	144.4	–
14	–	42.9	–
15	1.56 (1H, overlapped), 1.04 (1H, overlapped)	29.7	C-13, 14, 17
16	1.62 (2H, overlapped)	28.7	C-14, 18, 22, 28
17	–	46.8	–
18	3.04 (1H, s)	45.2	C-13, 14, 19, 28
19	3.24 (1H, d, J = 3.8)	82.5	C-13, 17, 20, 29
20	–	36.2	–
21	1.76 (1H, overlapped), 1.04 (1H, overlapped)	29.6	C-17, 20, 29, 30
22	1.76 (1H, overlapped), 1.56 (1H, overlapped)	34.2	C-18, 20, 28
23	3.74 (1H, d, J = 10.9), 3.69 (1H, d, J = 10.9)	70.3	C-3, 5, 24
24	0.93 (3H, s)	13.1	C-3, 5, 23
25	1.03 (3H, s)	17.0	C-1, 5, 9
26	0.86 (3H, s)	19.1	C-7, 8, 9, 14
27	1.35 (3H, s)	25.2	C-8, 13, 14, 15
28	–	182.4	–
29	0.96 (3H, s)	28.8	C-19, 21, 30
30	0.99 (3H, s)	25.2	C-19, 21, 29



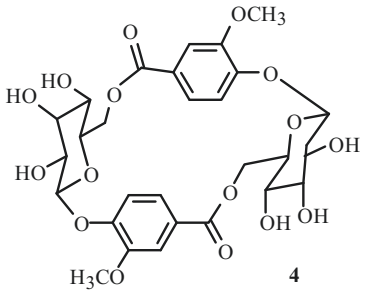
1



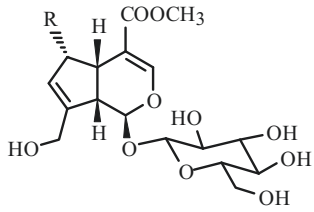
2



3



4



7 - 9

7: R = OH; **8:** R = OCH_3; **9:** R = H

Except compound **2**, all the other nine compounds were isolated from this plant for the first time. Bioactive assays on compounds **1**–**3**, **6**, **7**, and **9** indicated that only compound **2** could significantly stimulate glucose uptake in 3T3-L1 adipocytes (compound **2**: the uptake rate to 45.4%, *c* = 13.3 μM; positive drug insulin: the uptake rate to 51.3%, *c* = 10 nM).

Compound **2** was the first pyrocincholic acid type triterpenoid which could stimulate glucose uptake in 3T3-L1 adipocytes and might be a potential candidate for diabetes treatment.

EXPERIMENTAL

General. NMR spectra were recorded on a Bruker AM-400 imstrument, a DRX-500 NMR instrument, and s Avance III 600 MHz and AV-800 MHz spectrometers with TMS as internal standard. MS data were obtained on a VG AutoSpec-3000 spectrometer. HR-ESI-MS data were acquired using an Agilent G6230 spectrometer. UV data were obtained using a Shimadzu UV2401PC spectrophotometer. IR data were recorded on a NiCOLET iS10 spectrophotometer. Optical rotation data were obtained using an Autopol VI spectrometer.

Plant Material. Branches and leaves of *Metadina trichotoma* (Zoll. & Moritzi) Bakh. f. were collected from Xishuangbanna, Yunnan Province, People′s Republic of China, in October 2015. It was identified by Ms. Chun-Fen Xiao at Xishuangbannna Tropic Botanical Garden, the Chinese Academy of Sciences.

Extraction and Isolation. The powdered air-dried branches and leaves (21 kg) of *M. trichotoma* were extracted with 90% methanol three times at room temperature and then concentrated under reduced pressure. The concentrated methanol extract (1.07 kg) was dissolved in hot water and extracted with petroleum ether, EtOAc, and *n*-BuOH to afford the petroleum ether fraction (55 g), EtOAc fraction (155 g), *n*-BuOH fraction (355 g), and aqueous fraction (480 g). The EtOAc faction (155 g) was subjected to silica gel column chromatography with chloroform–methanol (30:1, 15:1, 9:1, 4:1, 1:1, 0:1) to give six fractions (Frs. 1–6). Fraction 3 was separated with silica gel column chromatography and Sephadex LH-20 to afford compound **6** (6 mg). Fraction 4 was separated with silica gel column chromatography and Sephadex LH-20 to afford compound **2** (56 mg). The *n*-BuOH fraction (355 g) was subjected to silica gel column chromatography with chloroform–methanol (15:1, 9:1, 4:1, 1:1) to give four fractions (Frs. A–D). Fraction A was separated with silica gel column chromatography and Sephadex LH-20 to afford compound **1** (20 mg). Fraction B was separated with silica gel column chromatography and Sephadex LH-20 to afford compounds **3** (3 mg) and **5** (2 mg). Fraction C was separated with silica gel column chromatography and Sephadex LH-20 to afford **4** (4 mg). Fraction D was separated with silica gel column chromatography and Sephadex LH-20 to afford compounds **7** (4 mg), **8** (4 mg), **9** (16 mg), and **10** (2 mg).

6α-Hydroxyilexosapogenin A (1), white powder, $C_{30}H_{48}O_6$, $[\alpha]_D^{20}$+52.1° (*c* 0.02, MeOH). UV/Vis (CH_3OH, λ_{max}, nm) (log ε): 206 (4.29). IR (KBr, ν, cm^{-1}): 3442, 2930, 1687, 1637, 1384, 1075, 1045. ^1H and ^{13}C NMR data, see Table 1. HR-ESI-MS *m/z* 527.3340 $[M + Na]^+$ (calcd 527.3340).

Glucose Uptake Activities. 3T3-L1 cells were cultured at 37°C in Dulbecco′s modified Eagle′s medium (DMEM)/high glucose containing 10% calf serum (CS) and 1% penicillin/streptomycin (P/S) in an atmosphere of 10% CO_2. Cell differentiation was induced as in previous reports [18, 19]. The assay for glucose uptake was done as previously described with minor modifications [20]. Differentiated 3T3-L1 adipocytes plated into 96-well plates were pre-incubated with DMEM/high glucose containing 10% FBS and 1% P/S overnight and then incubated with various concentrations of **2** in DMEM/low glucose containing 10% FBS, 1% P/S, and 0.2% bovine serum albumin (BSA) for 24 h. The medium was collected and its glucose concentrations were determined by the glucose oxidase method using the Glucose Kit. The amount of glucose uptake was calculated from the glucose concentrations of blank wells subtracting the remaining glucose in the cell-plated wells.

ACKNOWLEDGMENT

This investigation was supported by grants from the Institute of Drug Innovation of the Chinese Academy of Sciences (Grant No. CASIMM0520181002), and the Special Project for Environmental Protection in Yunnan Province (Grant No. E1YN051K).

Binbin Fan and Jiaxiang Liu contributed equally to this work.

REFERENCES

1. Delectis Florae Reipublicae Popularis Sinicae Agendae, Academiae Sinicae Edita, *Flora Reipublicae Popularis Sinicae*, **71**, 268 (1999).
2. Y. M. Zhang, N. H. Tan, H. Q. Huang, and G. Z. Zeng, *Z. Naturforsch. C*, **62**, 745 (2007).
3. J. Cinatal, B. Morgenstern, G. Bauer, P. Chandra, H. Rabenau, and H. W. Doerr, *Lancet*, **361**, 045 (2003).
4. F. Guan, Q. Wang, M. Wang, Y. Shan, Y. Chen, M. Yin, Y. Zhao, X. Feng, F. Liu, and J. Zhang, *Molecules*, **20**, 6419 (2015).
5. P. Bernard, T. Scior, B. Didier, M. Hibert, and J. Y. Berthon, *Phytochemistry*, **58**, 865 (2001).
6. V. Ramachandran and R. Saravanan, *J. Funct. Foods*, **5**, 1077 (2013).
7. L. Peng, Y. Lu, Y. Xu, J. Hu, F. Wang, Y. Zhang, and W. Xiong, *Nat. Prod. Bioprospect.*, **7**, 225 (2017).
8. K. Amimoto, K. Yoshikawa, and S. Arihara, *Chem. Pharm. Bull.*, **41**, 77 (1993).
9. A. Rumbero-Sanchez and P. Vazquez, *Phytochemistry*, **30**, 311 (1991).
10. T. Yoshioka, K. Murakami, K. Ido, M. Hanaki, K. Yamaguchi, S. Midorikawa, H. Gunji, and K. Irie, *J. Nat. Prod.*, **79**, 2521 (2016).
11. D. Peng, X. Lin, L. Jiang, J. Huang, G. Zeng, X. Deng, and Y. Zhou, *Nat. Prod. Res.*, **33**, 427 (2018).
12. H. Kobayashi, H. Karasawa, T. Miyase, and S. Fukushima, *Chem. Pharm. Bull.*, **32**, 1452 (1985).
13. M. Dudas, M. Vilkova, T. Beres, M. Repcak, and P. Martonfi, *Nat. Prod. Commun.*, **11**, 837 (2016).
14. M. Ono, N. C. Ishimatsu, H. Yoshimitsu, R. Tsuchihashi, M. Okawa, J. Kinjo, T. Ikeda, and T. Nohara, *Chem. Pharm. Bull.*, **55**, 632 (2007).
15. K. Machida, E. Takehara, H. Kobayashi, and M. Kikuchi, *Chem. Pharm. Bull*., **51**, 1417 (2003).
16. M. Ono, M. Ueno, C. Masuoka, T. Ikeda, and T. Nohara, *Chem. Pharm. Bull.*, **53**, 1342 (2005).
17. M. Kuroyanagi, M. Shiotsu, T. Ebihara, H. Kawai, A. Ueno, and S. Fukushima, *Chem. Pharm. Bull.*, **34**, 4012 (1986).
18. R. Govers, A. C. Coster, and D. E. James, *Mol. Cell. Biol*., **24**, 6456 (2004).
19. S. C. Frost and M. D. Lane, *J. Biol. Chem*., **260**, 2646 (1985).
20. L. Zhou, Y. Yang, X. Wang, S. Liu, W. Shang, G. Yuan, F. Li, J. Tang, M. Chen, and J. Chen, *Metabolism,* **56**, 405 (2007).