Chemistry of Natural Compounds, Vol. 45, No. 2, 2009

A NEW TRITERPENE GLYCOSIDE FROM *Premna microphylla*

Zha-Jun Zhan*, Lan Tang, and Wei-Guang Shan

UDC 547.918:547.926

*A new triterpene glycoside, namely 28-*O*-α-L-rhamnopyranosyl (1→2)-β-D-glucopyranoside tormentic acid ester, was isolated from the leaves of* Premna microphylla*, together with two known triterpenes, i.e., arjunolic acid (**2**) and hyptatic acid A (**3**). Its structure was established by mass-spectrometric and spectroscopic methods, especially 2D NMR techniques. This is the first report of the isolation of triterpenes from this plant.*

Key words: *Premna microphylla*, triterpene glycoside, tormentic acid glycoside.

The plant *Premna microphylla* Turez., distributed mainly in the southern part of China, is a shrub whose leaves are applied to treat skin cuts and infections, malaria, dysentery, headaches, and viper bites in Chinese traditional medicine [1]. The previous studies on the chemical constituents of this plant resulted in the isolation of glyceroglycolipid and ceramide [2]. In the current research, a new triterpene glycoside, named 28-*O*-*α*-L-rhamnopyranosyl (l→2)-*β*-D-glucopyranoside tormentic acid ester (**1**), together with arjunolic acid (**2**) and hyptatic acid A (**3**), was isolated from the leaves of *Premna microphylla*. Its structure was established by mass-spectrometric and spectroscopic analyses, especially 2D NMR techniques (^{1}H–^{1}H COSY, HMQC, HMBC, and NOESY).

Compound **1** was isolated as a white amorphous powder. Its molecular formula was identified as $C_{42}H_{68}O_{14}$ by HR-ESI-MS at *m/z* 819.4514 (calcd. for $[M + Na]^+$ 819.4507), indicating the presence of nine degrees of unsaturation. IR spectrum showed the presence of hydroxyl and ester carbonyl in **1** at 3427 and 1724 cm^{-1}, respectively. Forty-two carbon signals comprising eight methyls, nine methylenes, seventeen methines, and eight quaternary carbons were evident from its ^{13}C NMR and DEPT spectra, in which the following functionalities of one ester carbonyl (δ 178.8, C-28), one trisubstituted double bond (δ 129.9, 139.9), and two anomeric carbons (δ 95.5, 101.7) were distinguishable. The ^{1}H NMR spectrum of **1** showed signals for eight methyl protons (Table 1), in which two secondary methyl proton signals at δ 1.26 (d, J = 6.2) and 0.92 (d, J = 6.6) were ascribed to methyl signal of the rhamnose and methyl signal on ring E of triterpene, respectively. One carbonyl group and one double bond accounted for two degrees of unsaturation, and the remaining seven degrees of unsaturation were assumed to be due to the presence of seven cycles in **1**. The ^{1}H and ^{13}C NMR spectra of **1** showed the presence of two sugar moieties and a triterpene aglycone with an ursolic-type skeleton (C-12 and C-13 at 129.9 and 139.9, respectively) [3, 4]. The 2*α*,3*β*-OH substitution of this skeleton was evident from the chemical shift and the J value of two protons ascribable to C-3 (δ 3.55, d, J = 10.1 Hz) and to C-2 (δ 3.93, ddd, J = 10.1, 12.7 and 3.3 Hz), and was confirmed by the ^{1}H–^{1}H COSY and HMBC spectra. The rhamnosyl and glucosyl moieties could be distinguished in the structure of **1** on the basis of ^{1}H and ^{13}C NMR spectra, which was confirmed by the fragmentation of positive ESI-MS at *m/z* 331 $[Glc + Rha + Na]^+$. The large coupling constant of H-1′ at δ 5.37 (1H, d, J = 7.5 Hz) showed the *β*-configuration of the glucose, and the anomeric configuration of the rhamnose was determined as *α* from the ^{13}C NMR chemical shifts of C-3 and C-5 of the rhamnosyl moiety [5].

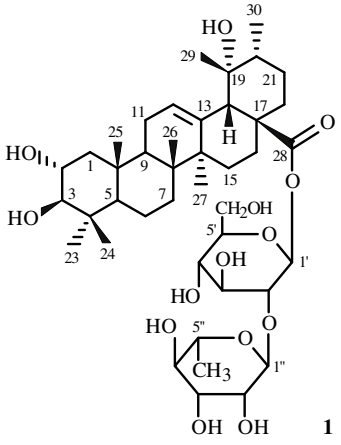
Analysis of the ^{1}H, ^{13}C NMR, and HMQC spectra enabled us to allot all the protons to their bonded carbons. The ^{1}H NMR signal at δ 2.49 was assigned to H-18 from the HMBC correlation of H-18/C-13, C-12, and C-28. The hydroxyl group at C-19 was indicated by HMBC cross peaks of H-18/C-19, H-18/20, and H-30/C-19. The HMBC cross peak of H-1′/C-28 suggested that the glucose was linked to the C-28 position of aglycone. The signal at δ 5.40 showed correlation with carbon at δ 77.0, which indicated that the rhamnosyl moiety was linked at C-2′ of glucose. Thus, the planar structure of **1** was confirmed.

College of Pharmaceutical Science, Zhejiang University of Technology, Hangzhou 310014, P. R. China, e-mail: zzjjzz1207@hotmail.com. Published in Khimiya Prirodnykh Soedinenii, No. 2, pp. 173–174, March–April, 2009. Original article submitted October 23, 2007.

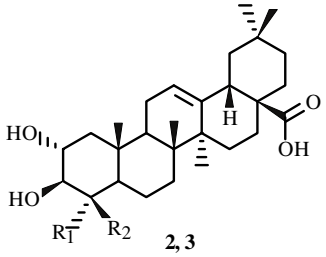
0009-3130/09/4502-0197

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

C atom	δ_H	δ_C	C atom	δ_H	δ_C
1	1.60 (1H, m); 1.40 (1H, m)	43.2	22	1.72 (2H, m)	38.6
2	3.93 (1H, ddd, J = 12.7, 10.1, 3.3)	67.5	23	0.87 (3H, s)	22.8
3	3.55 (1H, d, J = 10.1)	80.4	24	0.99 (3H, s)	29.6
4	-	39.8	25	1.00 (3H, s)	17.3
5	1.26 (1H, m)	49.9	26	0.77 (3H, s)	18.1
6	1.28 (2H, m)	19.6	27	1.36 (3H, s)	24.9
7	1.55 (1H, m); 1.43 (1H, m)	34.5	28	-	178.8
8	-	41.8	29	1.20 (3H, s)	27.4
9	2.00 (1H, m)	49.7	30	0.92 (3H, d, J = 6.6)	16.9
10	-	39.7	1′	5.37 (1H, d, J = 7.5)	95.5
11	1.98 (2H, m)	25.1	2′	3.81 (1H, m)	77.0
12	5.32 (1H, d, J = 2.9)	129.9	3′	3.58 (1H, m)	79.7
13	-	139.9	4′	3.66 (1H, m)	71.7
14	-	43.0	5′	3.35 (1H, m)	78.6
15	1.73 (1H, m); 1.00 (1H, m)	30.2	6′	3.77 (1H, m)	62.8
16	1.73 (1H, m); 1.25 (1H, m)	27.3	1″	5.40 (1H, d, J = 1.8)	101.7
17	-	48.8	2″	3.41 (1H, m)	72.2
18	2.49 (1H, s)	55.5	3″	3.90 (1H, m)	72.5
19	-	74.0	4″	3.58 (1H, m)	73.9
20	1.84 (1H, m)	42.8	5″	3.39 (1H, m)	70.6
21	2.62 (1H, dt, J = 13.5, 4.3); 1.55 (1H, m)	26.9	6″	1.26 (3H, d, J = 6.2)	18.5



1



2, 3

2: $R_1 = CH_2OH, R_2 = CH_3$
3: $R_1 = CH_3, R_2 = CH_2OH$

The relative stereochemistry of **1** was deduced by comparison of the spectral data with that of tormentic acid [6], especially the chemical shifts and coupling constants of protons, which was further confirmed by the NOESY correlation. In the NOESY spectrum, the H-18 showed interaction with the signals at δ 1.20 (H-29) and 1.84 (H-9), which indicated that the hydroxyl at C-19 has α-orientation. On the basis of all of the above information, the structure of **1** was formulated.

The structures of the two known triterpenes that were also isolated from *P. microphylla*, *i.e.,* arjunolic acid (**2**) [7] and hyptatic acid A (**3**) [8], were corroborated by comparison of their spectroscopic data with those reported in the literature.

EXPERIMENTAL

General Experimental Procedures. All solvents used were of analytical grade (Shanghai Chemical Plant, Shanghai, P. R. China). Silica gel (230–400 mesh), and MCI CHP20P gel (75–150 μ; Mitsubishi Chemical Industries Ltd.) were used for column chromatography (CC). Pre-coated silica gel GF_{254} plates (Qingdao Haiyang Chemical Plant) were used for thin-layer-chromatography (TLC). Optical rotations were determined on a Perkin-Elmer 341 polarimeter. IR spectra were recorded on a Perkin-Elmer 577 spectrometer with KBr disks. NMR spectra were measured on a Bruker AM-400 spectrometer with TMS as internal standard. ESIMS was recorded on a Finnigan LCQ^{DECA} mass spectrometer.

Plant Material. The leaves of *P. microphylla* were collected from Zhejiang province, P. R. China, in June, 2006, and authenticated by Prof. Yong-Hong Zhang of the Fujian Medical University, P. R. China. A voucher specimen (No.200607014P) was deposited at the Zhejiang University of Technology.

Extraction and Purification. The powdered leaves of *P. micorphylla* (1.2 kg) were percolated with aq. 95% EtOH. After solvent removal, the crude extract (72 g) was suspended in H_2O (2 L) and extracted with EtOAc (each 5 × 400 mL) to afford fraction E (43 g). Fraction E was applied to a column of MCI gel CHP20P eluted with MeOH–H_2O (1:9–9:1) to give two fractions 1 and 2. Fraction 1 eluted with MeOH–H_2O (4:6) (1.2 g) was subjected to silica gel column chromatography eluted with $CHCl_3$–MeOH (4:1) to yield **1** (7 mg). Fraction 2 eluted with MeOH–H_2O (8:2) (2.4 g) was subjected to silica gel column chromatography eluted with EtOAc-MeOH-HCOOH (10:1:0.1) to afford **2** (172 mg) and **3** (96 mg).

Compound 1: white amorphous powder, $[\alpha]_D^{20}$ +106° (*c* 0.70, MeOH); IR (KBr): 3427, 1724, 1637, 1456, 1385, 1074 cm^{-1}; Positive ESIMS *m/z*: 819 $[M+Na]^+$, 511 [M - Glc - Rha + Na]$^+$, 331 [Glc + Rha + Na]$^+$; Negative ESIMS *m/z*: 795 $[M–H]^–$; Positive HR-ESI-MS *m/z*: 819.4514 $[M+Na]^+$ (calcd. for $C_{42}H_{68}O_{14}$ 819.4507). ^1H and ^{13}C NMR data: see Table 1.

ACKNOWLEDGMENT

The financial support of the Open Foundation of Pharmaceutical Key Disciplines, Zhejiang province, P. R. China, is gratefully acknowledged.

REFERENCES

1. Jiangsu New Medical College, *Dictionary of Chinese Herb Medicine*, Shanghai Scientific and Technologic Press, Shanghai (1986), p. 2573
2. Z. J. Zhan and J. M. Yue*, Lipids*, **38**, 1299 (2003).
3. D. G. Durham, X. Liu, and R. M. E. Richards, *Phytochemistry*, **36**, 1469 (1994).
4. N. De Tommasi, F. De Simone, C. Pizza, N. Mahmood, P. Moore, C. Conti, N. Orsi, and M. Stein, *J. Nat. Prod.*, **55**, 1067 (1992).
5. R. Kasai, M. Okihara, J. Asakawa, K. Mizutani, and O. Tanaka, *Tetrahedron*, **35**, 1427 (1979).
6. M. H. Yang, G. Blunden, M. J. O′Neill, and J. A. Lewis, *Planta Med.*, **58**, 227 (1992).
7. A. P. Kundu and S. B. Mahato, *Phytochemistry*, **32**, 999 (1993).
8. T. Yamagishi, D. C. Zhang, and J. J. Chang, *Phytochemistry*, **27**, 3213 (1988).