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TWO NEW TETRACYCLIC TRITERPENOIDS FROM THE FRESH BARK OF *Ailanthus altissima*

Tian-Hui Zhu,[1] Wei-Na Zhang,[2] Ying-Ying Gao,[1] Zhen Pei,[1] Li-Kang Zhao,[1] Tao Jiang,[3*] Yi-Bing Wu,[1*] Zhi-Yu Ni[4*]

*Two new triterpenoids (**1**, **2**) were isolated from the fresh bark of* Ailanthus altissima. *The structures were elucidated on the basis of spectroscopic analysis including 1D and 2D NMR techniques and high-resolution mass spectrometry.*

Keywords: *Ailanthus altissima*, bark, triterpenoid, isolation, structure assignment.

Ailanthus altissima (Mill.) Swingle belongs to the *Ailanthus* genus of the Simaroubaceae family. It is commonly known as tree of heaven in China. The barks of the plant have been used as a traditional Chinese medicine for many years and the bioassay investigation has shown that the extract of this plant exhibited anti-inflammatory, fumigant toxicity, and phytotoxic activities [1]. In the previous study, we found a new triterpenoid in the fresh bark of *Ailanthus altissima* [2]. In this study, another two new tetracyclic triterpenoids were isolated from the same resource.

Compound **1** was obtained as white amorphous powder, exhibited a $[M + Na]^+$ peak in the HR-ESI-MS at *m/z* 495.3448, which matched the molecular formula of $C_{30}H_{48}O_4Na$ (calcd 495.3450). There are seven degrees of unsaturation. In the ^{13}C NMR spectra (Table 1), together with HSQC and DEPT experiments, 30 carbon resonances were resolved and they were further sorted to eight Me, seven CH_2, six CH groups, and nine quaternary C atoms. One of the implied seven unsaturation degrees was attributed to the carbonyl group because the ^{13}C NMR spectrum contained a low-field signal resonating at δ 216.5. In addition, two carbon-carbon double bonds at δ 118.4, 141.4, 145.7, and 116.1 were observed. No further sp^2- or sp-hybridized carbon atoms implied that the remaining four degrees were a tetracyclic molecule.

The ^1H NMR spectrum of **1** displayed signals of seven tertiary Me groups, two of which are at δ 1.13 (s) and 1.06 (s) and mutually correlated in the HMBC experiment. Both exhibited long-range correlations with the C=O group at δ 216.5, a quaternary carbon at δ 47.8, and a tertiary carbon group at δ 50.1. According to this, a ketone group was located at C-3.

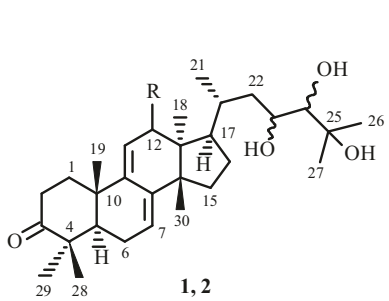
The HMBC technique showed the placement of the double bonds (Fig. 1): δ_H 1.16 (Me-19) correlated with δ_C 145.7 (C-9) and δ_H 0.88 (Me-30) correlated with δ_C 141.4 (C-8). Besides, there are two double bonds and HMBC at δ_H 5.23 (H-11) related to δ_C 36.1 (C-10), δ 38.3 (C-12), and δ_C 44.1 (C-13). Therefore, the two carbon-carbon double bonds are between C-7, C-8 and C-9, C-11.

In addition to an oxygen atom in a ketone group, the remaining three oxygen atoms in the molecular formula must be hydroxyl groups; this conjecture was in accordance with observed chemical shifts in the ^1H NMR (δ 4.12, 3.17) and ^{13}C NMR (δ 69.6, 74.9, and 73.3) spectra. ^1H and ^{13}C NMR spectral data demonstrated that one quaternary and two tertiary carbons were substituted by hydroxyl groups. The 23-OH, 24-OH, and 25-OH substituents of the side chain were established by the ^1H–^1H COSY cross-peaks.

1) School of Pharmaceutical Sciences, Hebei Medical University, Shijiazhuang, 050017, Hebei Province, P. R. China, e-mail: wuyibing@hebmu.edu.cn; wybjean@126.com; 2) Hebei Institute of Dermatology, Shijiazhuang, 050000, Hebei Province, P. R. China; 3) The Fourth Hospital of Hebei Medical University and Hebei Cancer Hospital, Shijiazhuang, Hebei Province, 050000, P. R. China, e-mail: hdjt1975@163.com; 4) School of Basic Medical Sciences, Hebei University, Baoding, 071002, Hebei Province, P. R. China, e-mail: 949192098@qq.com. Published in *Khimiya Prirodnykh Soedinenii*, No. 1, January–February, 2023, pp. 64–66. Original article submitted September 14, 2021.

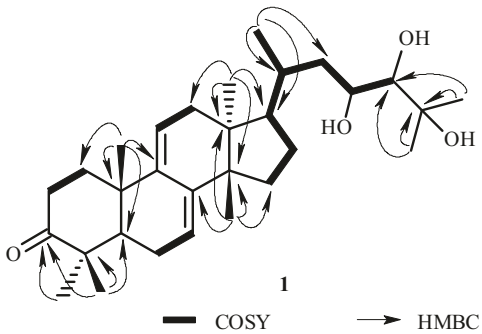
TABLE 1. ^{1}H (700 MHz) and ^{13}C (175 MHz) NMR Data of Compounds **1** and **2** ($CDCl_3$, δ, ppm, J/Hz)

C atom	**1**		**2**	
	δ_H	δ_C	δ_H	δ_C
1	2.13 (m); 1.82 (m)	36.9	2.19 (m); 1.84 (m)	35.6
2	2.83 (td, J = 14.8, 5.2) 2.32 (dt, J = 13.9, 2.7)	34.9	2.84 (td, J = 14.7, 5.3) 2.35 (m)	34.5
3	–	216.5	–	215.7
4	–	47.8	–	47.5
5	1.68 (m)	50.1	1.65 (m)	49.7
6	2.21 (m); 2.09 (m)	24.2	2.22 (m); 2.16 (m)	24.1
7	5.38 (br.s)	118.4	5.49 (br.s)	120.9
8	–	141.4	–	140.6
9	–	145.7	–	145.3
10	–	36.1	–	36.9
11	5.23 (br.s)	116.1	5.41 (d, J = 5.2)	117.0
12	2.17 (2H, m)	38.3	4.06 (d, J = 4.6)	73.7
13	–	44.1	–	48.5
14	–	49.6	–	47.3
15	1.65 (m); 1.33 (m)	31.1	1.70 (m); 1.40 (m)	32.1
16	2.06 (m); 1.35 (m)	28.2	2.02 (m); 1.34 (m)	28.1
17	1.61 (m)	51.7	2.20 (m)	44.4
18	0.63 (s)	16.1	0.62 (s)	16.5
19	1.16 (s)	19.9	1.23 (s)	19.2
20	1.41(m)	33.8	1.45 (m)	33.4
21	0.95 (d, J = 6.4)	19.0	1.06 (d, J = 6.4)	18.1
22	1.88 (m); 1.20 (m)	40.6	1.93 (m); 1.30 (m)	40.3
23	4.12 (m)	69.6	4.15 (m)	69.3
24	3.17 (s)	74.9	3.18 (s)	74.7
25	–	73.3	–	75.2
26	1.33 (s)	27.5	1.32 (s)	27.2
27	1.31 (s)	26.2	1.31 (s)	26.0
28	1.13 (s)	22.2	1.14 (s)	21.9
29	1.06 (s)	24.5	1.05 (s)	24.1
30	0.88 (s)	23.1	1.07 (s)	25.0



1, 2

1: R = H; **2:** R = *β*OH



1

COSY HMBC

Fig. 1. Chemical structures of **1** and **2**, key ^{1}H–^{1}H COSY and HMBC correlations of **1**.

The NOESY experiment provided the relative configuration of the tetracyclic core. The correlations between H-2a (δ 2.83)/Me-19 and H-2a (δ 2.83)/Me-28 (δ 1.13) indicated that the Me-19 was upward equatorial. Me-19 and Me-30 had strong NOE, indicating that these were *cis* di-axial oriented. Me-30 and Me-18 had no NOE, suggesting that Me-18 might be downward equatorial. The NOESY correlation Me-18/Me-21 was observed, leading to (*S*)-configuration at C-20. Me-19/H-5 and Me-30/H-17 had no NOE, indicating that H-5 and H-17 were downward equatorial. The structure of **1** was very similar to ganodermanondiol [3], except for the hydroxyl group at C-23.

By comparing the ^{1}H and ^{13}C NMR chemical shifts of the side chain of 1 [δ_H 4.12 (1H, m, H-23), 3.17 (1H, s, H-24); δ_C 69.6 (C-23), 74.9 (C-24)] with those of (20*S*,23*R*,24*S*)-7α,23,24,25-tetrahydroxyapotirucallan-3-one [4] [δ_H 4.17 (1H, dd, J = 8.1, 5.1 Hz, H-23), 3.96 (1H, s, H-24); δ_C 69.6 (C-23), 75.2 (C-24)], we cannot get the configurational assignment of C-23 and C-24.

Compound **2** had a similar structure to **1**, except for the 12-OH. The chemical shift at δ_H 4.06 (1H, d, H-12) and the correlation with δ_C 145.3 (C-9), 117.0 (C-11), 73.7 (C-12), 48.5 (C-13) showed that there is a hydroxyl group at C-12. The NOE between H-12/Me-21 and H-12/Me-18 indicated that the 12-OH had a different direction than the Me-18 and Me-21; we infer that the 12-OH is upward equatorial.

EXPERIMENTAL

General. Column chromatography (CC): silica gel (200–300 mesh); TLC: silica gel GF_{254} (Qingdao Marine Chemical Factory, China); visualization under UV light (254 and 365 nm) and by spraying with a solution of 10% (v/v) H_2SO_4 in EtOH, followed by heating at 110°C; Sephadex LH-20 (Pharmacia), and ODS gel (50 mm; Merck). HPLC: Shimadzu LC-6AD equipped with an SPD-10A detector, with reversed-phase (RP) C_{18} column (YMC-Pack, ODS-A; 20 × 250 mm, 5 mm). ^{1}H and ^{13}C NMR spectra on a NEO-700 machine were taken at 700 MHz and 175 MHz, resp. in $CDCl_3$ with chemical shift (δ) in ppm as referenced to a residual solvent peak ($CHCl_3$) taken as an internal standard at 7.26 ppm in ^{1}H NMR and 77.0 ppm in ^{13}C NMR. Full assignments of the proton and carbon signals were secured with the help of ^{1}H–^{1}H COSY, HSQC, and HMBC spectra. ESI-MS: Agilent-1100 LC/MSD trap mass spectrometer. HR-ESI-MS: Agilent Technologies 6250 Accurate-Mass Q-TOF LC/MS spectrometer.

Plant Material. The plant, collected in April 2017 in Anguo, Hebei Province, China, was kindly authenticated by Prof. J. H. Wang. A voucher specimen (2017-4-29-TH) is avaible for inspection at the Herbarium of the Department of Medicinal Natural Product Chemistry, School of Pharmaceutical Sciences, Hebei Medical University.

Extraction and Isolation. The fresh bark of *Ailanthus altissima* (20 kg) was cut into smaller pieces and extracted exhaustively by 95% EtOH at room temperature three times. The solvent was removed under vacuum to give a crude extract (705.9 g), which was then subjected to extraction successively by petroleum ether (PE), CH_2Cl_2, and EtOAc for three times, respectively. The crude CH_2Cl_2 extract (143.2 mg) was loaded onto a silica gel column eluting with a PE–EtOAc–MeOH gradient (5:1:0–1:1:0–0:1:0–0:0:1). The elution of No.1 to No. 76 (each 500 mL) was combined into 14 fractions (Frs. 1–14) on the basis of their TLC profiles. Fraction 4 (17.5 g) was applied to a silica gel column and eluted with a CH_2Cl_2–acetone (40:1–10:1–1:1) gradiently to give 9 fractions denoted Subfrs. 1–9. Subfraction 9 was purified by preparative reversed phase HPLC (MeOH–H_2O, 6:4–9:1; flow rate, 4.0 mL/min; 220 nm; t_R = 12.59 min) to afford **1** (1.23 mg). Fraction 10 (3.9 g) was applied to a silica gel column and eluted with a CH_2Cl_2–acetone (10:1–5:1–0:1) gradiently to yield 5 fractions denoted Subfrs. 1–5. Subfraction 5 was purified by preparative reversed phase HPLC (MeOH–H_2O, 6:4–9:1; flow rate, 4.0 mL/min; 220 nm; t_R = 11.46 min) to afford **2** (4.52 mg).

23-Hydroxyganodermanondiol (1), white powder. ^{1}H and ^{13}C NMR data, see Table 1. HR-ESI-MS *m/z* 495.3448 $[M + Na]^+$ (calcd for $C_{30}H_{48}O_4Na$, 495.3450).

12β,23-Dihydroxyganodermanondiol (2), white powder. ^{1}H and ^{13}C NMR data, see Table 1. HR-ESI-MS *m/z* 511.3402 $[M + Na]^+$ (calcd for $C_{30}H_{48}O_5Na$, 511.3399).

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