

A NEW LIMONOID FROM *Azadirachta indica*

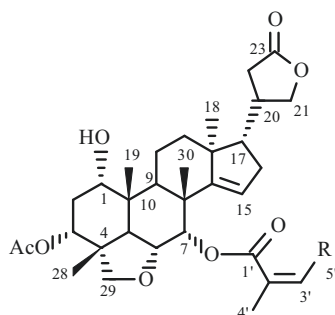
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A new limonoid, 3-acetoxy-7-(2'-methylpropenoyl)-vilasinin lactone (**1**), together with a known analogue, 3-acetoxy-7-tigloylvilasinin lactone (**2**), was isolated from the leaves and stems of *Azadirachta indica*. Their structures were elucidated based on spectral methods and by comparison with the literature.

**Keywords:** Meliaceae, *Azadirachta indica*, 3-acetoxy-7-(2'-methylpropenoyl)-vilasinin lactone.

*Azadirachta indica* A. Juss., also known as neem, is a tree in the family Meliaceae. In past studies, a variety of structurally interesting limonoids with various biological activities [1], such as antifeedant [2], insecticidal [3], anti-inflammatory [4], and cytotoxicity, [5] were discovered from this plant. As part of our ongoing phytochemical studies on Meliaceae medicinal plants [6, 7], a detail chemical investigation on the leaves and stems of *A. indica* was carried out. This led to the isolation of a new limonoid, 3-acetoxy-7-(2'-methylpropenoyl)-vilasinin lactone (**1**), along with a known compound, 3-acetoxy-7-tigloylvilasinin lactone (**2**), which were isolated by semipreparative HPLC. This work deals with the isolation and structural elucidation of the new compound.

Compound **1** was obtained as colorless crystals (MeOH). Its molecular formula was established to be C<sub>32</sub>H<sub>44</sub>O<sub>8</sub> by HR-ESI-MS  $m/z$  579.2927 [M + Na]<sup>+</sup> (calcd 579.2934), which indicated 11 degrees of unsaturation. The IR spectrum indicated the presence of OH (3520 cm<sup>-1</sup>) and carbonyl (1775, 1712 cm<sup>-1</sup>) groups. The <sup>1</sup>H NMR spectrum (Table 1) showed the presence of five methyls ( $\delta$  0.95, 1.01, 1.15, 1.17, 1.95), one acetate methyl ( $\delta_{\text{H}}$  2.05), one olefinic proton ( $\delta$  5.43), and two terminal olefinic protons [ $\delta$  6.09 and 5.52 (each, 1H, s)]. The <sup>13</sup>C NMR data in combination with analysis of DEPT and HSQC spectra displayed the presence of 32 carbon signals due to six methyls, eight methylenes (including two oxygenated ones and an olefinic one), nine methines (including four oxygenated ones), and nine quaternary C atoms (including three ester carbonyls and two olefinic ones).



**1, 2**

**1:** R = H; **2:** R = CH<sub>3</sub>

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TABLE 1. <sup>1</sup>H (400 MHz) and <sup>13</sup>C (100 MHz) NMR Data of Compounds **1** and **2** (CDCl<sub>3</sub>, δ, ppm, J/Hz)

C atom	<b>1</b>		<b>2</b>	
	δ <sub>C</sub>	δ <sub>H</sub>	δ <sub>C</sub>	δ <sub>H</sub>
1	71.8 (CH)	3.56 (d, J = 8.4)	71.9 (CH)	3.55 (m)
2	30.0 (CH <sub>2</sub> )	2.00 (m), 2.32 (m)	30.0 (CH <sub>2</sub> )	2.00 (m), 2.32 (m)
3	73.7 (CH)	5.10 (m)	73.7 (CH)	5.09 (m)
4	42.3 (C)	–	42.3 (C)	–
5	40.2 (CH)	2.40 (d, J = 12.4)	40.3 (CH)	2.38 (d, J = 12.2)
6	72.6 (CH)	4.19 (dd, J = 12.4, 2.8)	72.7 (CH)	4.17 (dd, J = 12.2, 2.6)
7	74.1 (CH)	5.61 (d, J = 2.8)	73.7 (CH)	5.62 (d, J = 2.6)
8	40.3 (C)	–	40.2 (C)	–
9	34.3 (CH)	2.79 (m)	34.4 (CH)	2.78 (m)
10	44.1 (C)	–	44.2 (C)	–
11	14.9 (CH <sub>2</sub> )	1.60 (m), 1.71 (m)	15.7 (CH <sub>2</sub> )	1.85 (m), 2.10 (m)
12	33.4 (CH <sub>2</sub> )	1.46 (m), 1.68 (m)	33.4 (CH <sub>2</sub> )	1.46 (m), 1.69 (m)
13	47.0 (C)	–	46.9 (C)	–
14	158.2 (C)	–	158.1 (C)	–
15	119.8 (CH)	5.43 (d, J = 1.6)	119.8 (CH)	5.42 (d, J = 1.5)
16	34.6 (CH <sub>2</sub> )	1.98, 2.14 (m)	34.6 (CH <sub>2</sub> )	1.92, 2.12 (m)
17	58.1 (CH)	1.70 (m)	58.1 (CH)	1.66 (m)
18	26.6 (CH <sub>3</sub> )	1.15 (s)	26.6 (CH <sub>3</sub> )	1.14 (s)
19	15.4 (CH <sub>3</sub> )	0.95 (s)	15.4 (CH <sub>3</sub> )	0.94 (s)
20	37.5 (CH)	2.68 (m)	37.5 (CH)	2.68 (m)
21	72.5 (CH <sub>2</sub> )	3.91, 4.44 (t, J = 8)	72.5 (CH <sub>2</sub> )	3.90 (t), 4.44 (t)
22	34.0 (CH <sub>2</sub> )	2.19 (m), 2.48 (m)	34.0 (CH <sub>2</sub> )	2.18 (m), 2.48 (m)
23	176.7 (C)	–	176.3 (C)	–
28	18.8 (CH <sub>3</sub> )	1.17 (s)	18.8 (CH <sub>3</sub> )	1.17 (s)
29	77.6 (CH <sub>2</sub> )	3.26, 3.50 (d, J = 7.6)	77.7 (CH <sub>2</sub> )	3.25, 3.48 (d, J = 7.5)
30	20.1 (CH <sub>3</sub> )	1.01 (s)	20.1 (CH <sub>3</sub> )	1.01 (s)
COCH <sub>3</sub>	169.1 (C)	–	169.0 (C)	–
COCH <sub>3</sub>	20.8 (CH <sub>3</sub> )	2.05 (s)	20.9 (CH <sub>3</sub> )	2.05 (s)
1'	165.9 (C)	–	166.6 (C)	–
2'	136.8 (C)	–	129.4 (C)	–
3'	124.7 (CH <sub>2</sub> )	6.09, 5.52 (s)	136.7 (CH)	6.76 (q, J = 7.0)
4'	12.1 (CH <sub>3</sub> )	1.95 (s)	18.4 (CH <sub>3</sub> )	1.83 (s)
5'	–	–	14.4 (CH <sub>3</sub> )	1.76(d, J = 7.0)

In the HMBC spectrum, cross-peaks of H-29 (δ 3.26, 3.50) to C-4 (δ 42.3), C-5 (δ 40.2), and C-6 (δ 72.6), and from H-5 (δ 2.39) to C-4, and C-6, indicated that a furan ring may be built up between C-29 and C-6, which was confirmed by the molecular formula and unsaturation degrees. The <sup>1</sup>H–<sup>1</sup>H COSY spectrum revealed the presence of four fragments: C-1/C-2/C-3, C-5/C-6/C-7, C-9/C-11/C-12, C-15/C-16/C-17/C-20/C-21/C-22. Comparison of the 1D and 2D NMR data with limonoids reported in the literature suggested that the structure of **1** was similar to 3-acetoxy-7-tigloylvilasinin lactone (**2**) [8], except that a tigloyl at C-7 in **2** was replaced by a 2'-methylpropenoyl group in **1**. This was supported by the presence of two terminal olefinic protons and a downfield methyl proton at H-4' (δ 1.95), along with HMBC spectrum correlations from H-3' (δ 3.86) to C-1' (δ 165.9), C-2' (δ 136.8), and C-4' (δ 18.4), from H-4' to C-2', and from H-7 (δ 5.61) to C-1'. Therefore, the chemical structure of **1** was assigned as 3-acetoxy-7-(2'-methylpropenoyl)-vilasinin lactone.

## EXPERIMENTAL

**General.** IR spectra were recorded on a Shimadzu IR-450 instrument using KBr pellets. NMR spectra were measured on a Bruker AV-400 instrument (Bruker, Zurich, Switzerland) with TMS as internal standard. HR-ESI-MS data were recorded on a VG Autospec-3000 spectrometer. Semipreparative HPLC was performed on Agilent 1100 liquid chromatograph with a ZORBAX SB-C18 column (250 × 9.4 mm). Silica gel (200–300 mesh, Qingdao Marine Chemical, Inc.), Lichroprep RP-18

(40–63  $\mu\text{m}$ , Merck), and DIAION HP-20 (Mitsubishi) were used for column chromatography. Fractions were monitored by TLC and visualized by spraying with 10%  $\text{H}_2\text{SO}_4$  in EtOH followed by heating.

**Plant Material.** The leaves and stems of *A. indica* were collected at Fengqing, Yunnan Province, China, in July 2011. The sample was identified by Prof. Hua Peng of the Kunming Institute of Botany, and a voucher specimen (KIB 20110720) has been deposited at the State Key Laboratory of Phytochemistry and Plant Resources in West China, Kunming Institute of Botany, Chinese Academy of Sciences.

**Extraction and Isolation.** The leaves and stems of *A. indica* (8.0 kg) were extracted with  $\text{Me}_2\text{CO}$  ( $3 \times 18$  L, each for 2 days) at room temperature and concentrated *in vacuo* to give a crude extract (850 g), which was partitioned between EtOAc and  $\text{H}_2\text{O}$ . The EtOAc part (290 g) was chromatographed on a silica gel column and eluted with petroleum ether (PE)– $\text{Me}_2\text{CO}$  (50:1, 20:1, 10:1, 4:1) to give four major fractions (Frs. 1–4). Fraction 4 (50 g) was submitted to DIAION HP-20 column chromatography (400 g,  $4 \times 50$  cm,  $\text{MeOH-H}_2\text{O}$ , 60:40 $\rightarrow$ 75:25), and then purified by repeated chromatography on silica gel (500 g,  $6 \times 100$  cm,  $\text{CHCl}_3\text{-MeOH}$ , 8:1 $\rightarrow$ 6:1) to afford a mixture of **1** and **2**. Separation of the mixture by semipreparative HPLC yielded **1** (12 mg,  $t_{\text{R}} = 15.2$ ) and **2** (35 mg,  $t_{\text{R}} = 16.0$ ) using  $\text{MeOH-H}_2\text{O}$  with the ratio of 65:35 at 2 mL/min.

**Compound 1.** Colorless needles,  $[\alpha]_{\text{D}}^{24} -32.6^\circ$  ( $c$  1.14,  $\text{CHCl}_3$ ). UV ( $\text{MeOH}$ ,  $\lambda_{\text{max}}$ , nm) ( $\log \epsilon$ ): 202 (2.89). IR (KBr,  $\nu_{\text{max}}$ ,  $\text{cm}^{-1}$ ): 3520, 2932, 1775, 1712, 1176. For  $^1\text{H}$  and  $^{13}\text{C}$  NMR data, see Table 1. ESI-MS  $m/z$  579  $[\text{M} + \text{Na}]^+$ ; HR-ESI-MS  $m/z$  579.2927 (calcd for  $\text{C}_{32}\text{H}_{44}\text{O}_8 \text{Na}$ , 579.2934).

The known compound was identified as 3-acetoxy-7-tigloylvilasinin lactone (**2**) by comparison of experimental data with those previously reported [8].

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