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A NEW COUMARIN FROM *Zanthoxylum nitidum*

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*One new coumarin compound, isopranferin (**1**), along with six known compounds **2–7**, were isolated from* Zanthoxylum nitidum*. Their structures were determined on the basis of spectral data including 1D and 2D NMR and HR-EI-MS. Compounds **2–4**, **6**, and **7** were isolated from this plant for the first time. The* in vitro *cytotoxicity of compounds **1–7** to RAW264.7 cells, THP-1 cells, and Caco-2 cells was firstly tested by 3-(4,5-dimethylthiazol-2-yl)-2,5-diphenyltetrazolium bromide (MTT) methods.*

Keywords: *Zanthoxylum nitidum*, coumarin, isopranferin, cytotoxicity.

Zanthoxylum nitidum (Roxb.) DC. is a valuable traditional Chinese medicine distributed in the south of China. Chinese people utilize the roots of *Z. nitidum* as its medicinal part and call it 'liang-mian-zhen'. *Z. nitidum* was found to exhibit cytotoxic [1], antibacterial [2], antiviral and antifungal [3], analgesic [4], antioxidant [5], and anti-inflammatory [6] activities. Thus, the potential bioactivities of *Z. nitidum* promted us to investigate its chemical constituents.

Many alkaloids [3, 7], coumarins [2], benzenoids [6, 8], steroids [8], and their derivatives have been previously reported in *Z. nitidum*. As part of our ongoing search for bioactive secondary metabolites from Chinese tropical medicinal plants, a careful investigation on the chemical constituents of roots of *Z. nitidum* led to the isolation and identification of one new coumarin, isopranferin (**1**), together with six known compounds, palmitic acid (**2**) toddaculine (**3**), 6-(3′-methyl-1′,3′-butadienyl)-5,7-dimethoxycoumarin (**4**), toddalolactone (**5**), toddalenone (**6**), and mexoticin (**7**).

Compound **1** was isolated as a white amorphous powder. The molecular formula was determined as $C_{19}H_{24}O_6$ on the basis of the $[M]^+$ peak in the HR-EI-MS spectrum at *m/z* 348.1572. The UV spectrum showed absorption maxima at 204.50 and 327.00 nm, while the IR spectrum suggested the presence of a conjugated aromatic bond (1612.26 cm^{-1}).

A group of signals at δ 7.88 (1H, d, J = 10.0 Hz) and 6.25 (1H, d, J = 10.0 Hz) in the ^1H NMR spectrum (Table 1) suggested the presence of a coumarin nucleus. The ^1H NMR and ^{13}C NMR spectra of **1** were similar to those of pranferin [9, 10]. A major difference was in the signal of δ 63.6 (5-OCH_3). Moreover, the acetonide group in pranferin is located at C-8. The ^{13}C NMR spectrum showed 19 signals, sorted by DEPT experiments into six CH_3, one CH_2, four CH, and eight quaternary C. Based on comparison of the NMR spectral data with those reported in the literature, the signals of three *O*-bearing C at δ 106.6 (C-3′), 80.5 (C-4′), and 81.5 (C-2′) of the acetonide group were consistent with the coumarin group [9]. The HMBC show the correlation signal from δ 3.00 (dd, J = 15.0, 10.0 Hz, Hb-1′) to δ 161.7 (C-7), 156.1 (C-5), 117.5 (C-6), and 81.5 (C-2′), which indicated that the acetonide group was linked to the coumarin group through C-1′–C-6 (Fig. 1). The COSY showed that signal at δ 7.88 (d, J = 10.0 Hz, H-4) was correlated to 6.25 (d, J = 10.0 Hz, H-3), and the signal at δ 4.03 (dd, J = 10.0, 5.0 Hz, H-2′) was correlated to 3.00, 2.68 (2H-1′). The ROESY showed that the signal at δ 6.64 (s, H-8) was correlated to 3.90 (s, 7-OCH_3). Thus, the structure of **1** was determined as isopranferin.

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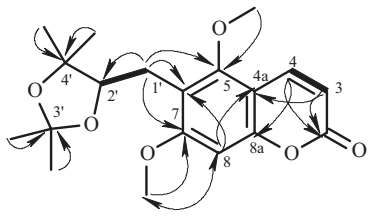
TABLE 1. ^{13}C and ^{1}H NMR Chemical Shift Data of Compound **1** and Pranferin ($CDCl_3$, δ, ppm, J/Hz)

C atom	**1** (500 MHz)		Pranferin (300 MHz)	
	δ_H	δ_C	δ_H	δ_C
2	–	161.3	–	161.0
3	6.25 (d, J = 10.0)	112.5	6.25 (d, J = 9.5)	113.1
4	7.88 (d, J = 10.0)	139.2	7.63 (d, J = 9.5)	143.8
5	–	156.1	7.35 (d, J = 8.6)	127.1
6	–	117.5	6.87 (d, J = 8.6)	107.6
7	–	161.7	–	153.3
8	6.64 (s)	95.5	–	114.9
4a	–	107.4	–	113.0
8a	–	155.1	–	160.7
2′	4.03 (dd, J = 10.0, 5.0)	81.5	4.10 (dd, J = 8.0, 4.7)	81.9
3′	–	106.6	–	106.6
4′	–	80.5	–	80.5
Ha-1′	2.68 (dd, J = 15.0, 5.0)	24.1	2.94 (dd, J = 13.6, 4.7)	23.2
Hb-1′	3.00 (dd, J = 15.0, 10.0)		3.22 (dd, J = 13.6, 8.0)	
3′-Me	1.41 (s)	28.6	1.44 (s)	28.7
3′-Me	1.25 (s)	27.0	1.31 (s)	27.0
4′-Me	1.24 (s)	25.8	1.27 (s)	25.8
4′-Me	1.24 (s)	22.9	1.21 (s)	22.8
5-OCH_3	3.92 (s)	63.6	–	
7-OCH_3	3.90 (s)	56.2	3.94 (s)	56.2



1

COSY → HMBC ↔ ROESY

Fig. 1. Key COSY, HMBC, and ROESY correlations of compound **1**.

The six known compounds isolated were identified as palmitic acid (**2**) [11], toddaculine (**3**) [12], 6-(3′-methyl-1′,3′-butadienyl)-5,7-dimethoxycoumarin (**4**) [13], toddalolactone (**5**) [14], toddalenone (**6**) [15], and mexoticin (**7**) [16] by comparison with spectroscopic data in the literature.

The *in vitro* cytotoxicity of compounds **1**–**7** were tested by 3-(4,5-dimethylthiazol-2-yl)-2,5-diphenyltetrazolium bromide (MTT) methods. Compared with the blank control group, compound **3** showed significant cytotoxicity to RAW264.7 cells THP-1 cells, and Caco-2 cells at 80 μM. Furthermore, the cytotoxicity of compound **3** at a concentration of 40 μM in THP-1 cells was significant. In addition, compound **4** showed significant cytotoxicity against Caco-2 cells at a concentration of 10–80 μM (see Table 2).

EXPERIMENTAL

General. Optical rotations were taken on a Jasco DIP-370 digital polarimeter. UV spectra were measured on a Shimadzu-UV-2401A spectrophotometer with methanol as solvent. Infrared (IR) spectra were recorded on a Bio-Rad-FTS-135 spectrometer in KBr pellets. 1D and 2D NMR spectra were obtained on a Bruker-DRX-500 spectrometer with chemical shifts recorded in δ (ppm) using tetramethylsilane (TMS) as internal standard, while the coupling constants (J) were given in hertz.

TABLE 2. *In vitro* Cytotoxicity of Compounds **1**–**7**

Group	Concentration, μM	Cell Type					
		THP-1		Raw264.7		Caco-2	
		Cell viability, %	SD	Cell viability, %	SD	Cell viability, %	SD
Blank		100.00	3.88	100.00	6.02	100.00	6.43
Taxol	5	76.09	1.63	75.00	2.36	66.24	0.50
1	10	101.75	1.04	102.86	2.32	115.29	0.79
	20	101.90	5.32	112.72	1.59	118.84	5.98
	40	106.78	2.18	108.08	3.89	110.56	4.16
	80	90.75	1.92	108.40	2.57	99.07	2.30
2	10	100.54	5.54	101.17	3.31	105.29	3.04
	20	104.26	5.92	112.22	1.08	109.12	2.16
	40	97.05	7.88	103.76	1.27	105.53	6.75
	80	99.64	8.22	105.77	3.00	107.97	7.77
3	10	89.78	12.10	102.61	2.51	98.41	8.11
	20	88.50	4.63	103.62	2.73	95.19	3.97
	40	81.89	3.35	99.13	3.66	94.71	2.63
	80	76.02	8.04	87.09	2.16	85.42	2.66
4	10	100.91	7.10	102.92	1.26	77.36	4.26
	20	108.94	4.25	110.30	0.80	73.61	2.10
	40	104.85	2.98	112.38	1.78	72.36	1.93
	80	106.61	3.74	116.01	2.74	76.15	4.02
5	10	96.14	0.96	102.37	2.12	98.85	8.74
	20	102.54	6.56	108.34	5.88	99.68	5.50
	40	102.70	5.63	105.35	1.16	101.38	8.74
	80	100.38	2.95	108.97	8.68	93.78	4.09
6	10	109.67	2.39	114.63	4.23	109.01	3.15
	20	105.39	3.30	124.44	0.62	115.77	7.76
	40	111.93	1.73	116.34	4.62	101.34	4.43
	80	114.51	3.22	101.54	7.02	95.44	2.50
7	10	93.76	2.14	103.35	4.22	101.03	2.55
	20	99.84	2.98	106.31	3.03	104.87	2.85
	40	100.41	1.49	106.17	1.23	105.36	5.70
	80	98.71	1.44	116.20	3.68	105.42	5.34

Mass spectra were obtained on a MS Waters AutoSpec Premier P776 mass spectrometer (EI-MS) and a Micro Q-TOF MS (HR-EI-MS) spectrometer. Column chromatography was run on silica gel (200–300 mesh; 10–40 mm) (Qingdao Marine Chemical Inc., P.R. China), RP-18 gel (40–63 mm) (Merck), and Sephadex LH-20 (Pharmacia). Fractions were monitored by thin-layer chromatography (TLC), and spots were visualized by heating silica gel plates sprayed with 10% H_2SO_4–H_2O.

Plant Material. The roots of *Zanthoxylum nitidum* were collected from the Chinese herbal medicine market, Yunnan, P. R. China, in March 2018 and authenticated by Prof. Qi-Shi Song, Xishuangbanna Tropical Botanical Garden. A voucher specimen (No. 20180303) was deposited at the Research Group on Ethnomedicine of Xishuangbanna Tropical Botanical Garden, Chinese Academy of Sciences.

Extraction and Isolation. The air-dried and powdered roots of *Zanthoxylum nitidum* (100 kg) were extracted with 90% aqueous ethanol and filtered at room temperature. The filtrate was concentrated and extracted with ethyl acetate, then extracted by BuOH. The ethyl acetate extract (7 kg) was subjected to silica gel column chromatography eluted with a MeOH–$CHCl_3$ from 0:1 up to 1:1 (by increasing MeOH and reducing $CHCl_3$) to generate seven fractions (Frs.1–7). All subfractions were collected and combined by TLC monitoring. Fraction 1 (3780 g) was further chromatographed over silica gel using acetone–petroleum ether from 1:20 up to 1:1 (by increasing acetone and reducing petroleum ether) to generate five subfractions (Subfrs. 1–5). Compounds **2** (4.00 g) and **3** (3.40 g) were isolated by recrystallization from Subfr. 1. Compounds **1** (1.05 g) and **4** (3.22 g) were isolated by recrystallization from Subfr. 3. Compounds **5–7** (> 4.00 g) were obtained from Subfr. 2 in a similar fashion.

Cytotoxicity Assays. Compounds **1–7** were dissolved with DMSO to a stock concentration of 40 mM and then diluted to the required concentrations with the medium. The cytotoxicity of compounds **1–7** against three cell lines, RAW 264.7 cells, THP-1 cells, and Caco-2 cells, was measured. Briefly, cells were placed in 96-well plates for 12 h and continuously exposed to different concentrations (80, 40, 20, and 10 μM) of the compounds for 48 h, with Taxol (Sigma, USA) as the positive control. Inhibition rates of cell proliferation after compound treatment were determined by the MTT method [17].

Isopranferin (5,7-dimethoxy-6-(3′,3′,4′,4′-tetramethyl-[2′,4′]dioxolan-1′-ylmethyl)-chromen-2-one) (1), white amorphous powder, $[\alpha]_D^{24.9}$ –8.8325° (*c* 0.0044 mg/mL, MeOH). UV (MeOH, λ_{max}, nm) (log ε): 204.50 (2.1292), 327.00 (1.6426). IR (neat, ν_{max}, cm^{-1}): 2973.39, 1733.59, 1612.26, 1385.96, 1207.64, 1140.91, 1090.07, 833.03. For ^1H (500 MHz, $CDCl_3$) and ^{13}C NMR (125MHz, $CDCl_3$), see Table 1. HR-EI-MS *m/z* 348.1572 $[M]^+$ (calcd for $C_{19}H_{24}O_6$, 348.1573).

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