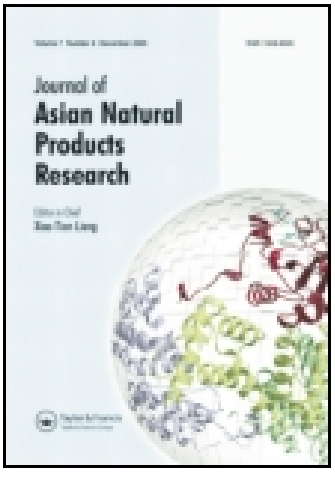

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Three new limonoids from Azadirachta indica

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Three new limonoids, azadiraindins E–G (**1**–**3**, respectively), together with six known analogs, were isolated from the fresh fruit coats of *Azadirachta indica*. The structures of these compounds were elucidated by spectroscopic methods (IR, MS, HR-ESI-MS, 1D NMR, and 2D NMR).

Keywords: Meliaceae; *Azadirachta indica*; limonoids; azadiraindins E–G

1. Introduction

Plants of the economically and medicinally important genus *Azadirachta* are known to be a rich source of limonoids, which have been found to possess various beneficial pharmacological effects [1–7]. In order to develop environment-friendly biopesticide, *Azadirachta indica* has been introduced and also planted on a large scale since 2000s in Yunnan province, China, which has become a growing base of botanical pesticides. In previous work, some new compounds were isolated from the branches and leaves of this plant [8,9]. To search for more new bioactive compounds, we examined the fresh fruit coats of *A. indica*, which led to the isolation of three new limonoids, along with six known ones (Figure 1). This work deals with the isolation and structural elucidation of these new compounds.

2. Results and discussion

Azadiraindin E (**1**) was isolated as colorless crystals (MeOH). The molecular formula of **1** was established to be $C_{28}H_{34}O_7$ on the basis of HR-ESI-MS at *m/z* 483.2382 $[M + H]^+$, with 12 degrees of unsaturation. Its IR spectrum showed absorptions at 1747, 1730, and 1665 cm^{-1} for carbonyl functions. The ^1H NMR spectrum (Table 1) showed the presence of five methyls (δ_H 1.05, 1.07, 1.08, 1.21, 1.22), one acetyl methyl (δ_H 2.03), and a pair of AB doublets [δ_H 5.89, 7.18 (d, $J = 10.2$ Hz)]. The ^{13}C NMR spectrum displayed 28 carbon signals which were classified by a DEPT experiment into 6 methyls, 4 methylenes (including an oxygenated one), 8 methines (including three olefinic ones), and 10 quaternary C atoms (including two ketones and two ester carbonyls). Comparison of the 1D and 2D NMR data with limonoids reported in the literature suggested that **1** had a skeleton similar to that of epoxyazadiradione (**4**) [10], with an 1-en-3-one system in ring A, an acetate group linked to C-7, and an oxirane ring bridging at C-14 and C-15. The difference between **1** and **4** was the signals of the furan ring, and the NMR signals of furan ring were in good accordance with those of ceramicine D [11]. The above deduction was supported by the presence of α,β-unsaturated ketone group at C-20 (δ_C 126.8), C-21 (δ_C 174.1), C-22 (δ_C 150.9) and the HMBC corre-

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lations from H-17 (δ_H 3.86) to C-20, C-22, C-23 (δ_C 71.2), from H-23 (δ_H 4.92) to C-20, C-21, and C-22, and from H-22 (δ_H 7.67) to C-20, C-21, and C-23.

The molecular formula of azadiraindin F **(2)** was inferred as $C_{29}H_{38}O_8$ by HR-ESI-MS and NMR data. ^1H, ^{13}C NMR, and DEPT experiments showed signals for 7 methyls (including one acetyl methyl and one oxy-methyl), 3 methylenes, 10 methines, and 9 quaternary carbons. Moreover, its NMR spectra were similar to those of azadiradione (**6**) [12] except for the signals of the furan ring group. In HMBC spectrum, the correlations from H-17 (δ_H 2.64) to C-16 (δ_C 208.7), C-13 (δ_C 47.7), C-22 (δ_C 74.7), from H-20 (δ_H 3.14) to C-22, C-21 (δ_C 175.1), C-23 (δ_C 108.9), from H-22 (δ_H 4.12) to C-20, C-21, C-23, from H-23 (δ_H 5.31) to C-16, C-20 (δ_C 40.7), C-21, C-OCH$_3$ (δ_C 58.0), and from H-OCH$_3$ (δ_H 3.52) to C-23, together with the spin systems of H-22/H-20, H-23 in the $^1H-^1H$ COSY spectrum, suggested that the furan ring group in **2** possessed the ester carbonyl, 22-OH, 23-OCH$_3$. The configurations at C-22 and C-23 were not determined by ROESY correlations.

Azadiraindin G (**3**) was isolated as a white amorphous powder, with a molecular formula of $C_{29}H_{36}O_9$ as determined by HR-ESI-MS, showing a quasimolecular ion at m/z 551.2263 $[M + Na]^+$. The ^1H NMR and ^{13}C NMR data of **3** (Table 1) were similar to those of gedunin **(8)** [7], and the difference still was the furan ring signals. The HMBC correlations were noted from H-17 (δ_H 5.57) to C-20 (δ_C 134.2), C-22 (δ_C 148.4), C-23 (δ_C 102.6), from H-22 (s, δ_H 7.24) to C-20, C-21 (δ_C 166.8), C-23, and from H-23 (s, δ_H 5.79) to C-20, C-21, C-22. These HMBC correlations, coupled with the chemical shift of C-20, C-21, C-22, C-23, demonstrated that

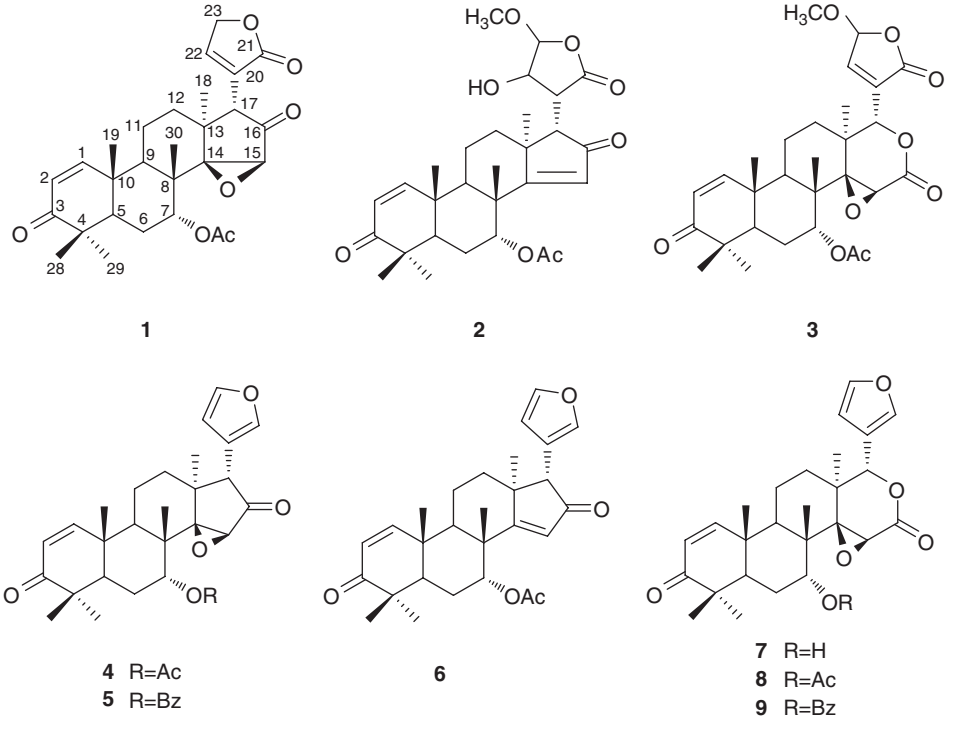

Figure 1. The structures of compounds **1**–**9**.

Table 1. ^{1}H and ^{13}C NMR spectral data of compounds **1**–**3** (δ in ppm, J in Hz, in $CDCl_3$).

	1[a]		**2**[b]		**3**[b]	
No	δ_C	δ_H	δ_C	δ_H	δ_C	δ_H
1	157.5 (d)	7.18 (d, $J = 10.2$)	156.6 (d)	7.16 (d, $J = 10.2$)	156.9 (d)	7.10 (d, $J = 10.2$)
2	125.7 (d)	5.89 (d, $J = 10.2$)	126.0 (d)	5.91 (d, $J = 10.2$)	126.0 (d)	5.87 (d, $J = 10.2$)
3	204.2 (s)		204.0 (s)		204.0 (s)	
4	43.2 (s)		44.1 (s)		42.6 (s)	
5	46.7 (d)	2.15–2.18 (m)	46.1 (d)	2.22–2.25 (m)	46.0 (d)	2.16–2.18 (m)
6	24.2 (t)	1.85–1.87 (m)	23.5 (t)	1.88–1.90 (m)	23.1 (t)	1.80–1.81 (m)
				1.95–1.98 (m)		1.92–1.94 (m)
7	73.8 (d)	4.72–4.73 (m)	73.7 (d)	5.35–5.36 (m)	73.1 (d)	4.52–4.54 (m)
8	44.2 (s)		45.3 (s)		44.0 (s)	
9	39.6 (d)	2.55–2.58 (m)	37.7 (d)	2.62–2.66 (m)	39.4 (d)	2.42–2.45 (m)
10	39.8 (s)		39.9 (s)		40.0 (s)	
11	16.0 (t)	1.91–1.92 (m) 1.96–1.99 (m)	15.6 (t)	1.87–1.88 (m)	14.8 (t)	1.86–1.87 (m)
				2.14–2.17 (m)		1.97–1.99 (m)
12	29.2 (t)	1.83–1.85 (m)	31.0 (t)	1.66–1.69 (m)	25.4 (t)	1.46–1.47 (m)
		2.37–2.41 (m)		2.05–2.07 (m)		2.04–2.07 (m)
13	42.7 (s)		47.7 (s)		39.3 (s)	
14	72.2 (s)		197.9 (s)		69.6 (s)	
15	56.8 (d)	3.42 (s)	123.7 (d)	5.97 (s)	56.7 (d)	3.50 (s)
16	207.2 (s)		208.7 (s)		168.9 (s)	
17	50.2 (d)	3.86 (s)	56.9 (d)	2.64–2.65 (m)	75.9 (d)	5.57 (s)
18	25.0 (q)	1.05 (s)	23.2 (q)	1.53 (s)	17.2 (q)	1.25 (s)
19	19.8 (q)	1.21 (s)	19.1 (q)	1.27 (s)	19.8 (q)	1.25 (s)
20	126.8 (s)		40.7 (d)	3.13–3.14 (m)	134.2 (s)	
21	174.1 (s)		175.1 (s)		166.8 (s)	
22	150.9 (d)	7.67 (d, $J = 1.2$)	74.7 (d)	4.11–4.14 (m)	148.4 (d)	7.24 (s)
23	71.2 (t)	4.92 (d, $J = 1.2$)	108.9 (d)	5.31 (s)	102.6 (d)	5.79 (s)
28	21.0 (q)	1.08 (s)	21.0 (q)	1.10 (s)	21.2 (q)	1.08 (s)
29	27.0 (q)	1.07 (s)	26.9 (q)	1.11 (s)	27.1 (q)	1.07 (s)
30	19.4 (q)	1.22 (s)	26.8 (q)	1.30 (s)	18.3 (q)	1.16 (s)

(*Continued*)

Table 1 – *continued*

No	**1**[a]		**2**[b]		**3**[b]	
	δ_C	δ_H	δ_C	δ_H	δ_C	δ_H
7—COCH$_3$	169.6 (s)		169.6 (s)		169.8 (s)	
7-COCH$_3$	21.3 (q)	2.03 (s)	21.3 (q)	1.98 (s)	21.0 (q)	2.10 (s)
OCH$_3$			58.0 (q)	3.52 (s)	58.0 (q)	3.63 (s)

[a] At 400 and 100 MHz.
[b] At 600 and 150 MHz.

the furan ring contained a ketone group at C-21, a double bond at C-20, C-22, and an hemiketal hydroxyl at C-23. The relative configurations at C-22 and C-23 were remained undetermined just by ROESY spectrum.

The known compounds were identified as epoxyazadiradione (**4**) [10], 7-deacetyl-7-benzoylepoxyazadiradione (**5**) [3,10], azadiradione (**6**) [12], 7-deacetyl-gedunin (**7**) [13], gedunin (**8**) [7], and 7-deacetyl-7-benzoylgedunin (**9**) [10] by comparison of their spectroscopic data with the literature values.

3. Experimental

3.1 General experimental procedures

Melting point was determined with an XRC-1 micromelting apparatus (Sichuan University, Sichuan, China). Optical rotations were measured with a Horiba SEPA-300 polarimeter (Horiba, Kyoto, Japan). UV spectra were detected on a Shimadzu UV-2401A spectrophotometer (Shimadzu Corporation, Kyoto, Japan). IR spectra were recorded on a Shimadzu IR-450 instrument by using KBr pellets (Shimadzu Corporation, Kyoto, Japan). NMR spectra were measured on a Bruker AV-400, DRX-500, or DRX-600 instrument (Bruker, ZŰrich, Switzerland) with TMS as the internal standard. HR-ESI-MS data were recorded on a VG Autospec-3000 spectrometer (VG, Manchester, England). Silica gel (200–300 mesh, Qingdao Marine Chemical, Inc., Qingdao, China), Lichroprep RP-18 (40–63 μm, Merck, Darmstadt, Germany), DIAION HP-20 (Mitsubishi, Kyoto, Japan), and Sephadex LH-20 (Amersham Pharmacia Biotech, Uppsala, Sweden) were used for column chromatography. Semi-preparative HPLC was performed on an agilent 1100 apparatus (Agilent, Santa Clara, CA, USA). Fractions were monitored by TLC (Qingdao Marine Chemical, Inc., Qingdao, China) and visualized by spraying with 10% H_2SO_4 in EtOH followed by heating.

3.2 Plant material

The fresh fruit coats of *A. indica* were collected at Yuanmou, Yunnan Province, China, in June 2010. The sample was identified by Prof. Hua Peng of the Kunming Institute of Botany, and a voucher specimen (KIB 20100618) has been deposited at the State Key Laboratory of Phytochemistry and Plant Resources in West China, Kunming Institute of Botany, Chinese Academy of Sciences.

3.3 Extraction and isolation

The fresh fruit coats of *A. indica* (11.0 kg) were extracted with MeOH (3 × 25 L, each for 2 days) at room temperature and concentrated *in vacuo* to give a crude extract (1060 g), which was partitioned between EtOAc and H_2O. The EtOAc part (440 g) was chromatographed on a DIAION HP-20 column (75–150 μm, 10 × 140 cm, 2000 g), eluted with a MeOH–H_2O mixed-solvent system (40:60, 60:40, 80:20, 100:0) to afford fractions A–D. Fraction B (160 g) was chromatographed on RP-18-gel column chromatography (500 g, 8 cm × 100 cm, MeOH–H_2O, 6:4 → 9:1) to afford subfractions B1–4. Fraction B1 (13 g) was chromatographed on RP-18-gel column chromatography (200 g, 4 cm × 50 cm, MeOH–H_2O, 5:5 → 6:4) to afford **4** (10.8 g). Fraction B2 (36 g) was chromatographed on RP-18-gel column chromatography (200 g, 4 cm × 50 cm, MeOH–H_2O, 6:4 → 7:3), and then purified over Sephadex LH-20 column (200 g, 2 cm × 200 cm, MeOH) to afford **5** (6 mg), **7** (15 mg), **8** (60 mg), and **9** (80 mg). Fraction C (120 g) was submitted over RP-18-gel column chromatography (400 g, 4 cm × 50 cm, MeOH–H_2O, 60:40 → 75:25), and then purified by repeated chromatography on silica gel (500 g, 6 cm × 100 cm, $CHCl_3$:MeOH, 8:1 → 6:1), to afford **1** (60 mg) and **6** (3.2 g). Fraction D (60 g) was submitted over repeated chromatography on silica gel, then separated by C_{18} (MeOH–H_2O 6:4 → 8:2) followed by semi-preparative HPLC (MeOH–H_2O; 65:35 → 80:20, 1 ml/min, detection at 225 nm) to obtain **2** (8 mg, R_t = 18.4 min) and **3** (5 mg, R_t = 21.2 min).

3.3.1 Azadiraindin E (*1*)

Colorless needle. M.p.: 186–187°C. $[\alpha]_D^{24}$ + 78.4 (*c* 0.08, MeOH). UV (MeOH) λ_{max} (log ε): 235 (4.39) nm. IR (KBr) ν_{max}: 3440, 2964, 1747, 1730, 1665 cm^{-1}. For ^1H and ^{13}C NMR spectral data, see Table 1. HR-ESI-MS *m/z*: 483.2382 $[M + H]^+$ (calcd for $C_{28}H_{35}O_7$, 483.2383).

3.3.2 Azadiraindin F (*2*)

White powder. $[\alpha]_D^{24}$ −156.1 (*c* 0.11, $CHCl_3$). UV (MeOH) λ_{max} (log ε): 241 (4.24) nm. IR (KBr) ν_{max}: 3441, 2949, 1782, 1735, 1671, 1241 cm^{-1}. For ^1H and ^{13}C NMR spectral data, see Table 1. ESI-MS: *m/z* 537 $[M + Na]^+$. HR-ESI-MS: *m/z* 537.2480$[M + Na]^+$ (calcd for $C_{29}H_{38}O_8Na$, 537.2464).

3.3.3 Azadiraindin G (*3*)

White powder. $[\alpha]_D^{24}$ + 65.4 (*c* 0.09, $CHCl_3$). UV (MeOH) λ_{max} (log ε): 241 (3.71) nm. IR (KBr) ν_{max}: 3441, 2936, 1748, 1731, 1665, 1646, 1246 cm^{-1}. For ^1H and ^{13}C NMR spectral data, see Table 1. ESI-MS: *m/z* 529 $[M + H]^+$. HR-ESI-MS: *m/z* 551.2263$[M + Na]^+$ (calcd for $C_{29}H_{36}O_9Na$, 551.2257).

Acknowledgments

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