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27-Nor-triterpenoid glycosides from *Mitragyna inermis*

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Abstract

From the bark of *Mitragyna inermis*, two 27-nor-triterpenoid glycosides, named inermiside I (**1**) and II (**2**), were isolated and their structures determined based on extensive 2D-NMR and MS spectral analysis as 6-deoxy-β-D-glucopyranosyl-[3-*O*-β-D-glucopyranosyl-(1→6)-β-D-glucopyranosyl]-pyrocincholate and 6-deoxy-β-D-glucopyranosyl-pyrocincholate, respectively. In addition, the known quinovic acid (**6**), 3-*O*-[β-D-glucopyranosyl-(1→4)-α-L-rhamnopyranosyl]-quinovoic acid (**3**),β-D-glucopyranosyl-[3-*O*-(β-D-glucopyranosyl)]-quinoviate (**4**) and cytotoxic 3-*O*-(β-D-6-deoxy-glucopyranosyl)-quinovic acid (**5**) were also isolated.

Keywords: *Mitragyna inermis*; Rubiaceae; Pyrocincholic acid; Quinovic acid; Triterpenoid saponins; Cytotoxic activity

1. Introduction

Mitragyna inermis (Willd.) O. Kuntze (Rubiaceae) is a shrub growing in West Africa (Ivory Coast, Ghana, Mali and Senegal). The leaves of the plant are used in folk medicine by the native people for the indication of hepatic disorders (Toure et al., 1996). A number of indole alkaloids were previously reported from this plant (Shellard and Sarpong, 1969, 1970; Shellard et al., 1971), while scant phytochemical work has been done on the non-alkaloidal constituents (Bishay et al., 1988). In the present paper, we report the isolation and structure elucidation of two triterpenoid saponins inermiside I (**1**) and inermiside II (**2**) by means of 1D- and 2D-NMR spectroscopic techniques including DEPT, HMQC and HMBC. These were shown to possess an uncommon 27-nor-triterpenoid aglycone which has been only reported isolated from *Adina rubella* (He et al., 1996) and *Isertia haenkeana* (Rumbero and Vazquez, 1991) so far. Cytotoxic bioassay directed-fractionation led to the isolation of 3-*O*-(β-D-6-deoxy-glucopyranosyl)-quinovic acid (**5**) as the active component responsible for the cytotoxicity exhibited by the extract of *M. inermis*.

2. Results and discussion

A MeOH extract of the bark of *M. inermis* was shown to be cytotoxic against a panel of human cancer cell lines and applied to a silica gel column chromatography using $CHCl_3$, EtOAc and MeOH as eluent to afford three fractions (Fr. I–III). These three fractions' cytotoxic activities against HL-60, Bel-7402, 293 and KB cell lines were evaluated in vitro. The only cytotoxic Fr. I (Bel-7402 cell inhibition $>50\%$ at a concentration of 10^2 μg/ml) was subjected to further chromatographic purification over silica gel, Sephadex LH-20 and ODS resulting in the isolation of the cytotoxic compound 3-*O*-(β-D-6-deoxy-glucopyranosyl)-quinovic acid (**5**) which exhibited 43.01 and 91.74% growth inhibition of Hela cell at the concentration of 10^2 and 10^3 μg/ml, respectively. A new compound inerside II (**2**) and the known quinovic acid (**6**) (Pöllmann et al., 1997) were also isolated from the same fraction. The previously reported cytotoxic quinovic acid (Raffauf et al., 1978) showed no cytotoxic activity in our assay. From the other two non-cytotoxic fractions, a new saponin inermiside I (**1**) and two known 3-*O*-[β-D-glucopyranosyl-(1→4)-α-L-rhamnopyranosyl]-quinovoic acid (**3**) (Fang et al., 1995) as well asβ-D-glucopyranosyl-[3-*O*-(β-D-glucopyranosyl)]-quinoviate (**4**) (Fan and He, 1997) were obtained.

Compound **1** was obtained as colorless needles. Its molecular formula was deduced as $C_{47}H_{76}O_{17}$ by ^{13}C

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NMR DEPT and HRFABMS in which a sodiated molecular ion $[M+Na]^+$ at m/z 935.4977 was detected. The ^{13}C NMR spectrum revealed 18 carbon signals for the glycone portion and 29 carbon signals for the aglycone portion including two quaternary olefinic signals (δ 130.44, δ 137.03) and one carboxyl carbon (δ 176.86). A comparison between the ^{13}C NMR spectral data of 1 and rubelloside C (He et al., 1996) (Table 1) revealed that the carbon signals of the aglycone of the two molecules were almost identical, suggesting the aglycone was pyrocincholic acid with two glycosidic linkages at C-3 and C-28, respectively, which is consistent with the observed downfield shift in C-3 by ca. 10.05 ppm and an upfield shift in C-28 (−6.48 ppm) with respect to the corresponding signals in pyrocincholic acid (Jimeno et al., 1995). The ^1H NMR spectrum (Table 2) of **1** indicated the presence of three sugar subunits: one 6-deoxy-D-glucose and two glucose units. In the HMQC spectrum three anomeric proton signals δ 6.32 (*d*, $J=8.0$ Hz), δ 4.94 (*d*, $J=7.7$ Hz), δ 5.10 (*d*, $J=7.7$ Hz) showed correlation with three anomeric carbon signals at δ 95.78, 106.88, 105.43, respectively. All three anomeric protons appear as doublet signals with coupling constants around 8.0 Hz, diagnostic of the axial orientations for all the three monosaccharide moieties. Proof of attachment of the inner glucose residue to C-3 of the aglycone was shown by the correlation of the anomeric proton at δ 4.94 with the signal at 89.11 in the HMBC spectrum. The 1→6 interglycosidic linkage with the second glucose moiety was deduced from the correlation in the HMBC experiment of H-1″ signal (δ 5.10) with the C-6′ at δ 69.63. The upfield shift of the 6-deoxy-D-glucose anomeric signal (C-1‴, δ 95.78 in ^{13}C NMR) indicated the esterifying unit of C-28 aglycone, as shown by the HMBC correlation between H-1‴ (δ 6.32) and C-28 (δ 95.78). The assignment of the 6-deoxy-D-glucose has been compared with the ^{13}C NMR spectral data of the reference (Rumbero-Sanchez and Vazquez, 1991). The proton signals of sugar moieties were assigned from the HMQC and HMBC spectrum. From these results, the structure of **1** was established as 6-deoxy-β-D-glucopyranosyl-[3-*O*-β-D-glucopyranosyl-(1→6)-β-D-glucopyranosyl]-pyrocincholate.

Compound **2** was obtained as colorless needles whose molecular formula $C_{35}H_{56}O_7$ was determined by ^{13}C NMR DEPT and HRFAB MS (m/z 611.3919 $[M+Na]^+$). Its ^1H and ^{13}C NMR spectra (Tables 1 and 2)

Table 1
^{13}C NMR spectral data of compounds **1** and **2** (125 MHz, C_5D_5N)

C	**1**[a]	**2**	[b]	DEPT		**1**	**2**
		Sapogenol	moiety			Sugar	moiety
1	38.62	38.18	38.7	CH_2		glc-	6-deoxy-glc-
2	26.97	26.61	27.0	CH_2	1′	106.88	106.59
3	89.11	88.71	89.2	CH	2′	74.17	75.76
4	39.80	39.41	40.0	C	3′	78.54	78.18
5	55.91	55.46	56.1	CH	4′	71.13	76.69
6	18.87	18.48	18.9	CH_2	5′	78.07	72.54
7	39.75	39.32	39.8	CH_2	6′	69.63	18.66
8	38.19	37.70	38.1	C		glc-	
9	56.65	56.18	56.7	CH	1″	105.43	
10	37.34	36.93	37.4	C	2″	75.31	
11	18.21	17.84	18.3	CH_2	3″	78.58	
12	32.19	31.94	32.3	CH_2	4″	71.68	
13	130.44	130.44	130.7	C	5″	78.87	
14	137.03	136.59	137.1	C	6″	62.81	
15	21.13	20.94	21.3	CH_2		6-deoxy-glc-	
16	24.25	24.10	24.4	CH_2	1‴	95.78	
17	45.83	44.99	45.3	C	2‴	76.11	
18	39.62	39.61	39.8	CH	3‴	78.54	
19	41.60	41.43	41.8	CH_2	4‴	77.04	
20	30.72	30.57	30.9	C	5‴	72.89	
21	34.47	34.34	34.8	CH_2	6‴	18.99	
22	31.38	31.50	31.8	CH_2			
23	28.31	27.92	28.3	Me			
24	16.83	16.46	16.7	Me			
25	16.83	16.39	16.7	Me			
26	21.01	20.54	20.3	Me			
27	–	–	–	–			
28	176.86	180.04	180.0	C			
29	32.49	32.30	32.6	Me			
30	25.16	25.10	25.3	Me			

[a] 100 Hz, C_5D_5N.
[b] From the literature (He et al., 1996).

Table 2
^1H NMR spectral data of compounds **1** and **2** (500 MHz, C_5D_5N)

H	**1**[a]	**2**	[b]
H-3	3.50 *dd* (4.3, 11.7)	3.42 *dd* (4.0, 11.5)	3.31 *dd* (4.2, 11.6)
H-18	2.86 *d* (3.7, 11.8)	2.87 *dd* (4.0, 12.0)	2.85 *dd* (3.9, 11.7)
Me-23	1.40 *s*	1.33 *s*	1.30 *s*
Me-24	1.05 s	0.95 *s*	1.10 *s*
Me-25	0.92 *s*	0.77 *s*	0.77 *s*
Me-26	1.22 *s*	0.98 *s*	0.95 *s*
Me-29	0.99 *s*	0.98 *s*	0.99 *s*
Me-30	0.99 *s*	1.00 *s*	1.00 *s*
H-1′	4.94 *d* (7.7)	4.88 *d* (8.0)	
H-2′	4.20 overlap	4.05 *t* (8.5)	
H-3′	4.27 *t* (4.6)	4.16 *t* (8.5)	
H-4′	4.40 overlap	3.75 *t* (9.0)	
H-5′	4.18 overlap	3.83 *m*	
H-6′	4.79 *d* (10.0)		
	4.43 overlap	1.67 *d* (6.0)	
H-1″	5.10 *d* (7.7)		
H-2″	4.11 *t* (8.2)		
H-3″	3.96 *t* (4.0)		
H-4″	4.27 overlap		
H-5″	4.25 overlap		
H-6″	4.56 *d* (2.4, 11.8)		
	4.43 overlap		
H-1‴	6.32 *d* (8.0)		
H-2‴	4.11 *t* (8.2)		
H-3‴	4.27 *t* (4.6)		
H-4‴	3.81 *t* (8.8)		
H-5‴	3.89 *m*		
H-6‴	1.74 *d* (6.0)		

[a] 400 Hz, C_5D_5N.
[b] From the literature (He et al., 1996).

showed that compound **2** has one sugar moiety. In the ^{1}H NMR spectrum, there are characteristic signals of a nor-triterpenoid structure (He et al., 1996), which has no olefinic proton resonances. The ^{13}C NMR spectrum of the aglycone showed 29 carbon signals including two quaternary olefinic carbons (δ 130.44, δ 136.59) and one carboxyl carbon (δ 180.04). A comparison of the ^{13}C NMR spectra of **2** and **1**, revealed its aglycone carbon signals were almost identical except for the chemical shift of the carboxylic carbon of **2** which was 180.04 ppm compared to 176.86 ppm in **1**. This results from the deglycosidation of the carboxylic group. This indicated that the aglycone of **2** was pyrocincholic acid with a sugar moiety attached to C-3. The ^{1}H and ^{13}C NMR spectroscopic signals of the sugar moiety of **2** were very similar to data of 6-deoxy-D-glucose (Rumbero-Sanchez and Vazquez, 1991). Thus **2** was established as 6-deoxy-β-D-glucopyranosyl-pyrocincholate. Compound **2** has previously been obtained as a partial hydrolysis product of a saponin from *I. haenkeana* (Jimeno et al., 1995).

This is the first report of 27-nor-triterpenoid glycosides and compounds **3–5** from the genus Mitragyna.

3. Experimental

3.1. General

Mps: uncorr. IR: Impact-410 (nicolet). FAB MS and ESI MS were in PE-Mariner and HP5989A mass spectrometer in the positive ion mode. HR FAB MS were obtained on a MAT-90 instrument (Finnigan-MAT, Bremen, Germany) equipped with Microvip data system. The ^{1}H and ^{13}C NMR spectra of **1–6** were recorded on Bruker ACF-400 and ACF-500 spectrometers, respectively, all with TMS ($\delta=0$) as int. standard and pyridine-d_5 as solvent. The DEPT experiments were carried out with $\theta=45$, 90, 135° used in ^{13}C NMR spectra.

3.2. Plant material

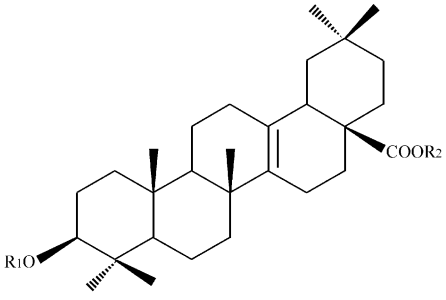
Bark of *M. inermis* was obtained from the National Arboretum of Mali in 1998 and identified by Professor Yu Bo-yang. A voucher specimen (98-08-16) of the plant is deposited at the Herbarium of China Pharmaceutical University.

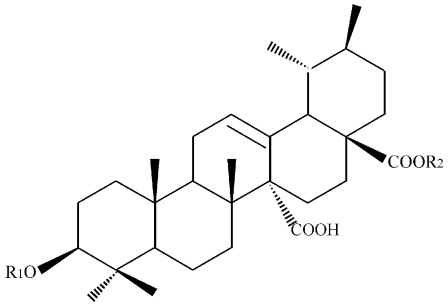
3.3. Extraction and isolation

Dried bark (2.5 kg) was extracted with MeOH, and 76 g residue was obtained. The residue was chromatographed on a silica gel column with $CHCl_3$, EtOAc and MeOH as eluent to give three fractions I–III. These fractions were evaluated for anti-tumor activity. Fr. I showed anti-tumor activity. The first two fractions eluted with a PE–EtOAc gradient yielded four different compounds. The former Fr. I purified by repeated CC afforded **2** (8 mg), **5** (2.36 g) and **6** (105 mg), while Fr. II purified on Sephadex LH-20 with MeOH yielded compound **3** (170 mg) and **4** (278 mg). Fr. III was chromatographed on a silica gel column with $CHCl_3$–MeOH and then on an ODS column with MeOH–H_2O (3:2) to obtain compound **1** (40 mg).

3.4. Compound *1*

Needles (MeOH–H_2O: 3–2), $C_{47}H_{76}O_{17}$, mp 226–228 °C, $[\alpha]_D^{25}$ −32.52 (MeOH; *c* 1). IRõ$_{max}$ cm^{-1}: 3424, 2942, 1748, 1453, 1174, 1071. ESIMS: *m*/*z* 935 $[M+Na]^+$. HRFABMS *m*/*z*: calc. for $C_{47}H_{76}NaO_{17}$: 935.4980; found: 935.4977 $[M+Na]^+$. For ^{13}C and ^{1}H NMR spectral analysis, see Tables 1 and 2.





Compound	R_1	R_2
1.	glc″-(1→6)glc′-	6-deoxy-D-glc‴-
2.	6-deoxy-D-glc	H
Pyrocincholic acid	H	H

	R_1	R_2
3.	glc-rha	H
4.	glc	glc
5.	6-deoxy-D-glc	H
6.	H	H

3.5. Compound **2**

Needles (MeOH), $C_{35}H_{56}O_7$, mp 211–213 °C, $[\alpha]_D^{25}$ −0.125 (MeOH; *c* 0.001). FABMS: *m/z* 611 $[M+Na]^+$, 589, 425, 409, 379, 287. HRFABMS *m/z*: calc. for $C_{35}H_{56}NaO_7$: 611.3924; found: 611.3919 $[M+Na]^+$. For ^{13}C and ^1H NMR spectral analysis, see Tables 1 and 2.

3.6. Cytotoxic activity

Hela (human carcinoma of the cervix) cell lines were grown as a monolayer in Dulbecco's modified Eagle's medium (DMEM, Gibco), supplemented with 10% newborn calf serum (Gibco), and 1% of penicillin–streptomycin mixture (10 000 UI/ml). The cells were maintained at 37 °C in 5% CO_2 and 90% humidity. Cytotoxicity was assessed using the colorimetric MTT reduction assay.

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