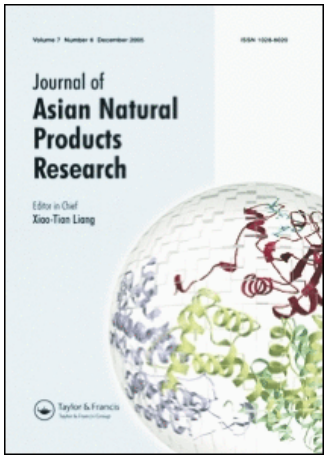

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A new anti-HIV lupane acid from *Gleditsia sinensis* Lam.

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A new lupane acid, 2β-carboxyl,3β-hydroxyl-norlupA (1)-20 (29)-en-28-oic acid (**1**), together with five known lupane acid derivatives (**2**–**6**), were isolated from the stings of *Gleditsia sinensis* Lam.. Their structures were elucidated on the basis of 1D and 2D NMR techniques. All these known compounds were isolated from this genus for the first time. The new compound **1** showed strong anti-HIV activity.

Keywords: *Gleditsia sinensis*; 2β-Carboxyl,3β-hydroxyl-norlupA(1)-20 (29)-en-28-oic acid; Lupane acid; Anti-HIV activity

1. Introduction

Gleditsia sinensis Lam., a perennial arbour, is distributed widely throughout China. Its stings, a traditional Chinese medicine, have been used for the treatment of apoplexy, exanthema and tinea corporis [1]. A number of flavonoids, triterpenoids and oligosaccharides from this genus have been reported [2,3].

Our studies on searching bioactive triterpenoids from the stings of *G. sinensis* led to the discovery of a new and five known lupane-type (or lupane-like-type) compounds, zizyberanalic acid **2** [4], betulic acid **3** [5], alphitolic acid **4** [6], 3-*O*-trans-*p*-coumaroyl alphitolic acid **5** [7] and 2-hydroxypyracrenic acid **6** [8]. In this paper, we describe the isolation and structure elucidation of the new compound, 2β-carboxyl,3β-hydroxyl-norlupA (1)-20 (29)-en-28-oic acid **1** (see figure 1).

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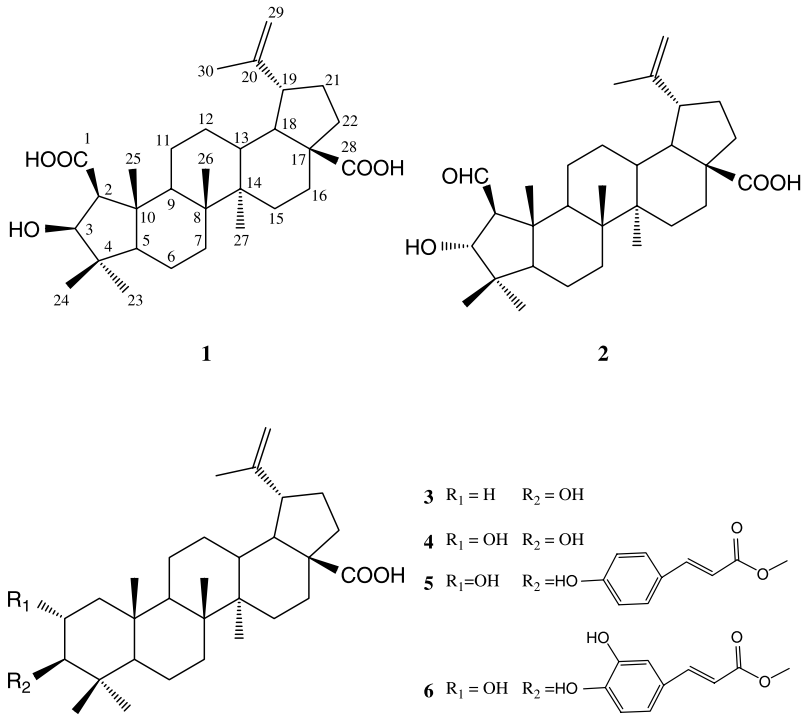

1 **2**

Figure 1. The structures of **1**–**6**.

2. Results and discussion

Compound **1** was obtained as colourless needles and analyzed for $C_{30}H_{46}O_5$ by HRESI-MS, which was consistent with its NMR data. Its IR spectrum exhibited absorption bands for hydroxyl (3432 cm^{-1}), carbonyl (1710 cm^{-1}) and olefinic groups (1641 cm^{-1} and 883 cm^{-1}). The ^1H NMR spectrum of **1** (table 1) revealed the presence of six methyl groups at δ 1.07 (s, 3H), 1.14 (s, 2 × 3H), 1.21 (s, 3H), 1.67 (s, 3H) and 1.74 (s, 3H), one proton of carbinol methine at δ 4.67 (d, $J = 7.4$ Hz, 1H), and two olefinic protons at δ 4.69 and 4.86 (s, each 1 H). Its ^{13}C NMR (DEPT) spectrum (table 1) displayed 30 carbon signals (6 × CH_3, 9 × CH_2, 7 × CH, 8 × C). The ^{13}C NMR (DEPT) spectrum also indicated the presence of two carboxylic groups at δ 175.7 (s) and 178.9 (s), one isopropenyl group at δ 151.2 (s), 110.1 (t), and one carbinol methine at δ 83.2 (d δ 83.2(d)). These revealed that the structure of **1** possessed the characteristics of lupane-type triterpenoid.

A careful comparison of the ^1H NMR and ^{13}C NMR data of **1** with those of ceanothic acid, 2α-carboxyl,3β-hydroxyl-norlupA (1)-20 (29)-en-28-oic acid [9] showed that the two compounds were very similar except for some differences in ring-A: C-2 and C-25 were shifted upfield to δ 63.2 and 15.0 respectively, and C-5 was downfield to δ 62.8 in the ^{13}C NMR spectrum of **1**; In the ^1H NMR spectrum, H-3 (δ 4.67, d, $J = 7.4$ Hz) and H-2 (δ_H 2.89, d, $J = 7.4$ Hz) each appeared as a doublet in **1** instead of each as singlet in ceanothic acid. These data indicated that **1** possessed the same structure as ceanothic acid except for the configuration of C-2 and C-3. Comparison of the coupling constants of H-3 ($J = 7.4$ Hz), with that reported in literature [10] strongly indicated that the stereochemistry of C-2 and C-3 should be 2β,3β-oriented. This relative configuration was confirmed by the ROESY

Table 1. NMR data of compound **1** (C_5D_5N).

Position	*^{13}C (DEPT)*	*^{1}H*	*^{1}H–^{1}H COSY*	*HMBC (H to C)*
1	175.7 (C)			
2	63.2 (CH)	2.89, d, 7.4	H-3	C-1/C-3/C-5/C-9/C-10/C-25
3	83.2 (CH)	4.67, d, 7.4	H-2	C-2/C-4/C-10/C-23
4	43.1 (C)			
5	62.8 (CH)	1.17, m	H-6	
6	18.5 (CH_2)	1.56, m;	H-5/H-7	
		1.46, m		
7	34.9 (CH_2)	1.41, m;	H-6	
		1.14, m		
8	42.0 (C)			
9	51.2 (CH)	1.81, m	H-11	C-8/C-10/C-11
10	48.2 (C)			
11	24.7 (CH_2)	2.00, m;	H-9/H-12	
		1.83, m		
12	25.9 (CH_2)	1.28, m	H-11/H-13	
13	38.6 (CH)	2.74, m	H-12/H-18	C-12/C-14/C-17/C-18
14	43.0 (C)			
15	30.6 (CH_2)	1.94, m;	H-16	
		1.23, m		
16	33.0 (CH_2)	2.61, m;	H-15	C-14/C-15/C-17/C-18/C-28
		1.58, m		
17	56.6 (C)			
18	49.9 (CH)	1.71, br s	H-13/H-19	C-13/C-17/C-29/C-28
19	47.9 (CH)	3.46, m	H-18/H-21	C-13/C-18/C-20/C-21/C-29/C-30
20	151.1 (C)			
21	31.3 (CH_2)	2.21, m;	H-19/H-22	C-17/C-18/C-19/C-20/C-22
		1.49, m		
22	37.7 (CH_2)	2.21, m;	H-21	C-17/C-18/C-21/C-28
		1.57, m		
23	32.2 (CH_3)	1.14, s		C-3/C-4/C-5/C-24
24	20.0 (CH_3)	1.20, s		C-3/C-4/C-5/C-23/-25
25	15.0 (CH_3)	1.07, s		C-2/C-9/C-10/C-24/C-26
26	17.0 (CH_3)	1.14, s		C-7/C-9/C-14/C-25
27	14.8 (CH_3)	1.67, s		C-13/C-14/C-15
28	178.9 (C)			
29	110.1 (CH_2)	4.86, br s		C-19/C-20/C-21/C-30
		4.69, br s		
30	19.5 (CH_3)	1.74, s		C-19/C-20/C-29

spectrum of **1**, in which the correlations between H-5α (δ 1.17, m) and H-2α (δ 2.89, d, $J = 7.4$ Hz), H-5α and H-3α (δ 4.67, d, $J = 7.4$ Hz) were observed clearly (see figure 2). Thus, the structure of **1** was elucidated as 2β-carboxyl,3β-hydroxyl-norlupA (1)-20 (29)-en-28-oic acid.

3. Experimental

3.1 General experimental procedures

Melting points were measured on YANACO-MP-52 apparatus and are uncorrected. Optical rotations were performed on a Horiba SEAP-300 polarimeter. IR spectra were taken on a Shimadzu IR-450 spectrometer with KBr pellets. NMR spectra were measured on Bruker AV-400 or DRX-500 spectrometers with TMS as internal standard.

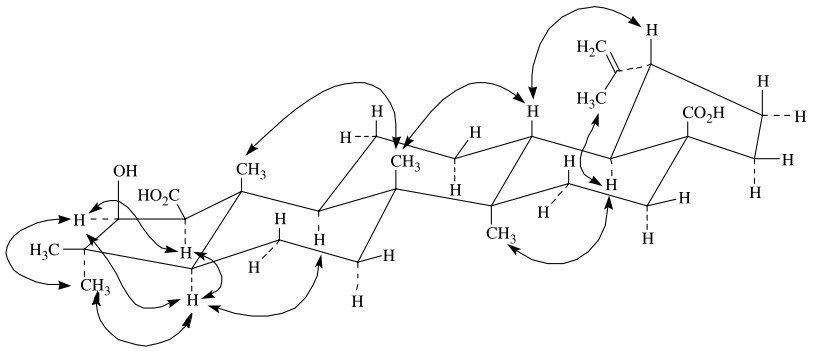

Figure 2. Key ROESY correlations of **1**.

3.2 Plant material

The stings of *Gleditsia sinensis* were purchased from the Chinese Herbal Market of Kunming, China. It was identified by Professor Wang Zongyu. A voucher specimen (No. 20031005) is deposited in the State Key Laboratory of Phytochemistry and Plant Resources in West China, Kunming Institute of Botany.

3.3 Extraction and isolation

The air-dried and milled stings of *G. sinensis* (15 kg) were extracted with 90% MeOH three times under reflux. After removal of the solvent *in vacuo*, the syrup (410 g) was suspended in water (1500 ml) and extracted with petroleum ether (3 × 1000 ml), EtOAc (3 × 1000 ml) and *n*-BuOH (3 × 1000 ml) successively. The EtOAc extract (140 g) was subjected to column chromatography on silica gel (200–300 mesh) and eluted with $CHCl_3$/Me_2CO (10:0, 9:1, 8:2, 7:3, 0:10) to afford five fractions [Frs. 1–5]. Fraction 3 (27 g) was further subjected to repeated chromatography (silica gel; 200–300 mesh) using a gradient system of $CHCl_3$/MeOH of increasing polarity (50:1 → 10:1) as eluent and purified over LH-20 eluting with Me_2CO to afford **1** (18 mg), **4** (386 mg) and **6** (72 mg). Fraction 2 (25 g) yielded **3** (1.1 g), **2** (112 mg) and **5** (48 mg).

3.4 Inhibition assay for the cytopathic effects of HIV-1

50 μl of 4×10^4 C8166 cells were seeded onto a microtitre plate containing 100 μl of various concentrations of compounds, and then 50 μl HIV-1_{IIIB} dilution with 200 $TCID_{50}$ (50% tissue culture infectious dose) of HIV-1_b stock solution was added. After mixing completely, it was incubated for 72 h at 37°C in a humidified atmosphere of 5% CO_2 without changing medium [11]. Each condition was performed in triplicate, AZT was the drug for positive control in each experiment. The syncytial cells were detected from five different fields under an inverted microscope (100 ×). The % inhibition of syncytial cell formation was calculated by percentage of syncytial cell number in compounds treated culture to that in infected control culture. The concentration of compounds reducing HIV-1 replication by 50% (EC_{50}) can be determined by dose response curve. The EC_{50} value of compound **1** ($EC_{50} < 0.064$ μg/ml) indicated strong anti-HIV activity.

3.4.1 2β-Carboxyl,3β-hydroxyl-norlupA (1)-20 (29)-en-28-oic acid (1). Colourless needles (MeOH); mp 324–327°C; $[\alpha]_D^{23}$ −16.3 (*c* 0.8, MeOH); IR bands (KBr): 3432, 2925, 2854, 1710, 1641, 1462, 1377, 1271, 1124, 1072, 883, 741 cm^{-1}; EI-MS: *m/z* 487 $[M + H]^+$(3%), 469 $[M + H - H_2O]^+$(5), 440 $[M - H_2O - CO]^+$ (11), 248 (RDA ion) (49), 219 (26), 203 (48), 189 (71), 175 (80), 161 (46), 147 (49), 133 (70), 121 (100); HRESI-MS: *m/z* 509.3232 $[M + Na]^+$ (calcd for $C_{30}H_{46}O_5Na$, 509.3242); ^1H NMR and ^{13}C NMR spectral data: see table 1.

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