

A New Dammarane-type Triterpene with PTP1B Inhibitory Activity from *Gynostemma pentaphyllum*

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Gynostemma pentaphyllum (Thunb.) Makino (Cucurbitaceae), a perennial liana herb, is widely cultivated in China, Japan and Korea. It has been traditionally used as medicinal herbs for treatment of cardiovascular disease, hyperlipidaemia, hypertension, hepatitis, diabetes and cancer,¹ and the biologically active components are believed to be Saponins.² Previous phytochemical studies on *G. pentaphyllum* have been revealed the presence of saponins as well as carotenoids,⁴ flavonoids,⁵ chlorophylls,⁶ lignin,⁷ and polysaccharides.⁸ During the course of our searching for protein tyrosine phosphatase 1B (PTP1B) inhibitors from natural sources, *G. pentaphyllum* was investigated. From the extract of the root of this plant, a new dammarane-type triterpene named 22(*S*)-3-oxodammar-20,24-dien-26,22-lactone (**1**) was isolated, together with six known compounds 24-(*Z*)-3-oxodammar-20(21),24-dien-27-oic acid (**2**), 25-methoxy-5 α -dammar-20-en-3 β ,24-diol (**3**), 24(*S*)-25-epoxy-5 α -protost-20,25-dien-3-one (**4**), (20*S*,23*S*)-3 β ,20-dihydroxydammarane-24-ene-21-oic acid-21,23-lactone (**5**), 20(*S*)-dammarane-25(26)-ene-3 β ,12 β ,20-triol (**6**), (20*S*,24*S*)-dammarane-25(26)-ene-3 β ,12 β ,20,24-tetrol (**7**) (Fig. 1). Herein we reported the isolation, structural elucidation of a new dammarane-type triterpene and evaluation of PTP1B inhibitory activity about these isolates.

Results and Discussion

Compound **1** was obtained as white solid. Its molecular formula was determined as C₃₀H₄₅O₃ by HRESIMS. The ¹H NMR spectrum of **1** showed the presence of six methyl singlets at δ 0.91, 0.92, 0.97, 1.02, 1.06, 1.91, an oxymethine signal as a doublet of doublets at δ 4.75 (J = 12.4, 3.6 Hz), a exomethylene singlets at δ 5.22 and 5.25, and another olefinic signal at δ 6.59 as a broad triplet (J = 6.4 Hz) (Table 1). These data indicated that the structure of **1** might contain terminal olefinic and tri-substituted olefinic groups, which also supported by the presence of four sp² signals (δ 149.4, 139.4, 128.6 and 113.7) in ¹³C NMR spectrum of **1** (Table 1). Moreover, the ¹³C NMR spectrum displayed signals attributed to an oxymethine carbon (δ 81.1), an ester carbonyl group

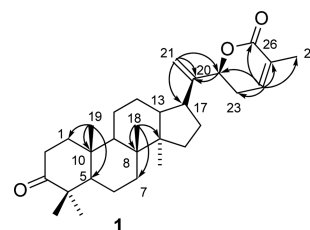


Figure 2. Key HMBC correlations of compound **1**.

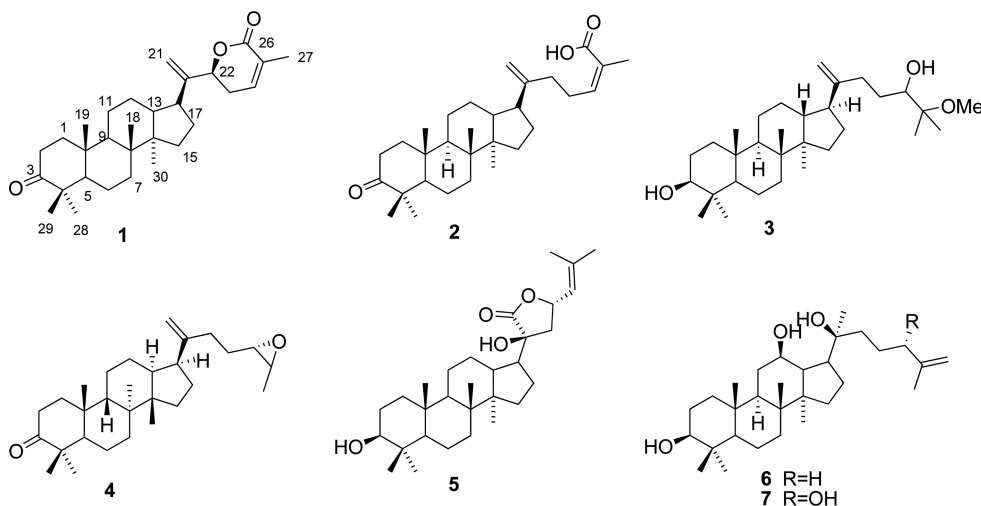


Figure 1. Structures of compounds **1**-**7**.

(δ 166.3) and a ketone carbonyl group (δ 218.3). In addition, six methyls, ten methylenes, six methines, and eight quaternary carbons were determined by display of its DEPT-135 data. All the above observations and chemical shifts suggested that **1** could be a dammarane-type triterpene. In particular, the ^1H and ^{13}C NMR spectra of **1** were in close agreement with those of 24(*E/Z*)-3-oxodammara-20,24-dien-26-al, except for the side-chain moiety.⁹ The presence of an α,β -unsaturated- δ -lactone moiety was established by observation of the HMBC spectrum data (Fig. 2), which showed long-range correlations from δ 4.75 (H-22) to δ 149.4 (C-20) and 113.7 (C-21), from δ 6.59 (H-24) to δ 81.1 (C-22), 29.2 (C-23), 128.6 (C-25), and 166.3 (C-26), from δ 5.22, 5.25 (H-21) to δ 149.4 (C-20) and 81.1 (C-22). The relative stereochemistry of the basic ring moiety was assigned by comparison of chemical shifts with related dammarane-type triterpenes.¹⁰ The δ -lactone moiety was confirmed the 22*S* configuration on the basis of a negative Cotton effect at 257 nm ($\Delta\epsilon$ -16.9) in the CD spectrum.¹¹ Therefore, the structure

Table 1. ^1H (400 MHz) and ^{13}C NMR (100 MHz) spectroscopic data of **1** and **2** (CDCl_3 , δ , ppm, *J*/Hz)

Pos.	1		2	
	δ_{H} (mult, <i>J</i> in Hz) ^a	δ_{C} ^a	δ_{H} (mult, <i>J</i> in Hz)	δ_{C}
1	1.40, m, 1.89, m	40.1	1.43, m, 1.90, m	40.1
2	2.32, m, 2.46, m	34.3	2.42, m, 2.48, m	34.3
3		218.3		218.5
4		47.7		47.6
5	1.34, m	55.6	1.37, m	55.5
6	1.45, m, 1.54, m	19.9	1.45, m, 1.53, m	19.9
7	1.29, m, 1.54, m	34.6	1.29, m, 1.55, m	34.5
8		41.0		40.9
9	1.36, m	50.5	1.39, m	50.4
10		37.1		37.0
11	1.24, m, 1.53, m	22.7	1.23, m, 1.23, m	22.7
12	1.24, m, 1.62, m	25.3	1.24, m, 1.71, m	25.2
13	2.11, m	45.1	2.04, m	45.1
14		50.0		50.1
15	1.24, m, 1.57, m	33.4	1.22, m, 1.55, m	33.2
16	1.71, m, 1.94, m	30.5	1.80, m	28.4
17	2.96, m	40.2	2.64, m	44.0
18	0.97, s	15.7	0.96, s	15.7
19	0.92, s	16.2	0.91, s	16.2
20		149.4		151.4
21	5.22, s, 5.25, s	113.7	4.88, s, 4.93, s	109.9
22	4.75, dd (12.4, 3.6)	81.1	2.06, m, 2.17, m	38.1
23	2.31, m, 2.53, m	29.2	2.64, m	28.9
24	6.59, br t (6.4)	139.4	6.07, br dd (7.6, 7.2)	146.5
25		128.6		126.4
26		166.3		172.9
27	1.91, s	17.2	1.90, br s	20.7
28	1.06, s	26.9	1.06, s	26.9
29	1.02, s	21.2	1.02, s	21.2
30	0.91, s	17.1	0.88, s	16.9

^aChemical shifts in ppm relative to TMS; coupling constants (*J*) in Hz.

of **1** was determined as 22(*S*)-3-oxodammara-20,24-dien-26,22-lactone.

The known compounds **2-7** were identified as 24-(*Z*)-3-oxodammara-20(21),24-dien-27-oic acid (**2**),¹² 25-methoxy-5 α -dammar-20-en-3 β ,24-diol (**3**),¹³ 24(*S*)-25-epoxy-5 α -protost-20,25-dien-3-one (**4**),¹³ (2*S*,23*S*)-3 β ,20-dihydroxyl-dammara-24-ene-21-oic acid-21,23-lactone (**5**),¹⁴ 20(*S*)-dammarane-25(26)-ene-3 β ,12 β ,20-triol (**6**),¹⁵ (2*S*,24*S*)-dammarane-25(26)-ene-3 β ,12 β ,20,24-tetrol (**7**)¹⁵ based on the NMR data.

All the isolates were assayed their inhibitory activity against PTP1B using an *in vitro* assay (Table 2), and RK-682 was used as positive control.¹⁶ As shown in Table 2, compounds **1**, **2** and **4** showed potential inhibitory activities of PTP1B with IC_{50} values ranging from 13.2 ± 1.9 to 19.2 ± 2.1 μM , while the remaining compounds displayed moderate effects. The structure-activity relationships of **1-5** against PTP1B indicated that the presence of a hydroxyl group located at C-3 might be responsible for the decrease of inhibitory activity of these compounds. Among the above isolates, compounds **3-4** were reported for the first time from this plant.

Experimental

General Experimental Procedures. UV spectra were taken in MeOH using a Shimadzu spectrophotometer. Nuclear magnetic resonance (NMR) spectra were obtained from a Varian Unity Inova 400 MHz spectrometer using TMS as the internal standard. All accurate mass experiments were performed on a Micromass QTOF (Micromass, UK) mass spectrometer. Column chromatography was conducted using silica gel 60, Sephadex LH-20 and RP-18 for thin-layer chromatography, precoated TLC silica gel 60 F₂₅₄ plates from Merck were used. HPLC runs were carried out using a Shimadzu System LC-10AD pump equipped with a model SPD-10Avp UV detector, and an Optima Pak[®] C₁₈ column (10 \times 250 mm, 10 mm particle size, RS Tech Korea).

Plant Material. The root of *G. pentaphyllum* was collected in Xuzhou, Jiangsu province, People's Republic of China, and authenticated by Professor Gao Li (College of Pharmacy, Yanbian University). A voucher specimen of the plant (No. 20101006) was deposited at the College of Pharmacy, Beihua

Table 2. PTP1B inhibitory activity of compounds **1-7**

Compounds	IC_{50} (μM) ^a
1	13.2 ± 1.9
2	15.2 ± 1.1
3	21.9 ± 2.3
4	19.2 ± 2.1
5	23.3 ± 1.7
6	29.1 ± 2.2
7	20.5 ± 0.7
RK-682 ^b	4.5 ± 0.5

^a IC_{50} values were determined by regression analyses and expressed as mean \pm SD of three replicates. ^bPositive control.¹⁶

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Extraction and Isolation. The root (5.0 kg) of *G. pentaphyllum* was extracted with MeOH at room temperature for 2 weeks and the solution was concentrated to obtain a crude extract. This extract was suspended in H₂O, partitioned successively with CHCl₃ and EtOAc, and then the organic solvents were removed. A portion of the CHCl₃-soluble fraction (10.0 g) was chromatographed over a silica gel column using a gradient of CHCl₃-MeOH (from 70:1, 50:1, 20:1 to 10:1), and was separated into 10 fractions (Fr.D1-Fr.D10). Fr.D4 (CHCl₃-MeOH 10:1, 1.0 g) was chromatographed over silica gel, eluted with a stepwise gradient of *n*-hexane-EtOAc (from 20:1, 19:1 to 0:1) to afford 10 sub-fractions (Fr.D4.1-Fr.D4.10). Purification of Fr.D4.4 (110.0 mg) by semipreparative HPLC using an isocratic solvent system of 95% MeCN in H₂O over 60 min to yield compounds **1** (4.4 mg) and **2** (6.4 mg). The EtOAc extract (50.7 g) was subjected to silica gel CC and eluted with a gradient of CH₂Cl₂/MeOH (25:1, 20:1, 15:1, 10:1, to 5:1) to yield 5 fractions (Fr.E1-E5). Fr.E2 (709.0 mg) was purified by preparative HPLC using an isocratic solvent system of 75% MeCN in H₂O over 30 min followed by 80% MeCN in H₂O over 70 min to obtain compounds **5** (5.9 mg) and **6** (5.1 mg). Fr.E3 (2.2 g) was subjected to an RP-18 column and was eluted with MeOH-H₂O (1:1, 2:1, to 10:1) to yield six fractions (Fr.E3.1-Fr.E3.6). The most active fraction, Fr.E3.5 (571.0 mg), was further separated by a silica gel column eluted with CHCl₃-MeOH (40:1, 35:1, to 10:1) to yield 7 subfractions (Fr.E3.5.1-Fr.E3.5.7). Fr.E3.5.3 was purified by preparative HPLC using an isocratic solvent system of 50% MeCN in H₂O over 50 min obtain compound **3** (3.4 mg). Fr.E3.5.5 was separated by HPLC, using a gradient of 40-50% MeCN in H₂O as the mobile phase to produce compounds **7** (3.1 mg) and **4** (4.9 mg).

Compound 1: White solid; [α]_D²⁰ (c, 0.08, CHCl₃); ¹H NMR (400 MHz, CDCl₃) and ¹³C NMR data (100 MHz, CDCl₃) spectral data see Table 1; HRESIMS *m/z* 453.3363 [M + H]⁺ (Δ -1.5 mmu, calcd for C₃₀H₄₅O₃).

PTP1B Assay. The enzyme activity was measured using *p*-nitrophenyl phosphate (*p*NPP) as described previously.¹⁶ To Each 96 well (final volume: 100 μ L) was added 2 mM *p*NPP and PTP1B (0.05-0.1 μ g) in a buffer containing 50 mM citrate (pH 6.0), 0.1 M NaCl, 1 mM EDTA, and 1 mM dithiothreitol (DTT), with or without test compounds. Following incubation at 37 °C for 30 min, the reaction was

terminated with 1 M NaOH. The amount of produced *p*-nitrophenol was estimated by measuring the absorbance at 405 nm.

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Supporting Information. The NMR spectral data of compound **1** are available as Supporting Information.

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