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Structure Determination of a Triterpene Saponin Extracted from the Barks of *Pseudolarix kaempferi* by 2D NMR

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Absract: : A triterpene saponin was extracted from the barks of *Pseudorix kaempferi* for the first time, and determined to be dammar-24 (25)-ene-3β, 6α, 12β, 20 (S)-tetraol-20-*O*-β-D-pyranoglucoside on the basis of NMR analysis. The chemical shifts of this compound were completely assigned using 2D NMR spectroscopy.

Key words: NMR, chemical shift, triterpene saponin, pyranoglucoside, *Pseudorix kaempferi* Gord

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Introduction

The root and trunk barks of *Pseudorix kaempferi* Gord. (Pinacese), known as 'Tu-Jin-Pi' in traditional Chinese medicine, has been widely used in China for treatment of skin diseases caused by fungal infections. Previously isolated classes of constituents were diterpenes[1~5] and triterpenoids[6~17]. As a part of our chemical studies on the aqueous part of the *Pseusorix kaempferi*, we have investigated the triterpene saponin in barks of this plant collected in Jiangsu Province, China. The present paper describes the

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isolation and structure elucidation of a saponin named dammar-24 (25)-ene-3β, 6α, 12β, 20(S)-tetraol-20-*O*-β-D-pyranoglucoside (compound **1**) by 2D NMR spectral method. This compound was isolated from Pseudorix genus for the first time.

1 Experimental

Plant material: the plant was collected in Jiangsu Province of China. The specimen was botanically identified by vice research fellow Shen Jingui (Shanghai Institute of Materia Medica, China).

1.1 Instruments

^{1}H NMR (500 MHz, MeOH-d_4) and ^{13}C NMR (125 MHz, MeOH-d_4) spectra were recorded on a Bruker ARX-500 spectrometer with TMS as an internal standard and 2D NMR (500 MHz, MeOH-d_4) was recorded at standard conditions. ESI-MASS spectrum was recorded on a Q-Tof-micro mass spectrometer.

1.2 Extraction and isolation

The dried root bark (10 kg) of *P. kaempferi* was extracted with EtOH (70%) under reflux. After removal of the ethanol *in vacuum*, the aqueous solution was filtered. The filtrate was then chromatographied sequentially over HP-20 macroporous resin column eluting gradiently with C_2H_5OH-H_2O, MCI gel column, HW-40F gel column and ODS gel column eluting with CH_3OH-H_2O to afford compound **1**.

2 Results and Discussion

Compound **1** was obtained as an amorphous powder and gave positive reaction to the Liebermann-Burchard and Molish tests for triterpene saponin. The molecular formula $C_{36}H_{62}O_9$ was determined on the basis of ESI (m/z 661.4[M^+Na]$^+$) together with ^{1}H and ^{13}C NMR spectral data (Table 1). A quick inspection of the ^{1}H and ^{13}C NMR spectra of the compound readily indicated the presence of a monosaccharide unit through easily identifiable signals of anomeric proton and carbon. The ^{13}C and DEPT NMR spectral data revealed the presence of the remaining 30 signals, which is indicative of a triterpene moiety.

The full assignments of proton and carbon signals were based on analysis of 1D NMR and 2D NMR spectra (Table 1). The ^{1}H NMR spectrum of compound 1 showed eight methyl singlets at δ_H 0.95, 0.95, 0.95, 1.08, 1.28, 1.34, 1.61 and 1.67, together with one olefinic proton at δ_H 5.10 (1H, br. s). In addition, the ^{13}C NMR and DEPT spectra showed eight methyl signals, two olefinic carbon signals at δ_C 126.1 (C-24) and 132.6 (C-25). In ^{1}H-^{1}H COSY spectrum, one olenfinic proton at δ_H 5.10 (H-24) was correlated with two methyl signals at δ_H 1.61 (3H, s, H-26) and 1.67 (3H, s, H-27) and a methane proton at δ_H 2.06 (2H, m, H-23), which showed correlation (^{1}H-^{1}H COSY) with δ_H 1.60 (1H, m, H-22a) and 1.79 (1H, m, H-22b). In HMBC

spectrum, the olenfinic proton at δ_H 5.10 (H-24) was correlated with δ_C 36.9 (C-22), 24.5 (C-23), 18.2 (C-26) and 26.2 (C-27). Moreover, in the HMBC spectrum a methyl proton signal at δ_H 1.34 (3H, s, H-21) showed long-range correlation with the signals at δ_C 53.4 (C-17), 85.2 (C-20) and 36.9 (C-22). Thus, the partial structure of the side chain in compound **1** could be elucidated as in Chart 1. Other partial structures of 1 were elucidated from the HMBC spectrum, including a geminal dimethyl groups (C_{29}-C_4-C_{30}) and three methyl groups attached to three quaternary carbons (C_{19}-C_{10}, C_{18}-C_8, C_{28}-C_{14}). The whole structure of the aglycone was deduced by ^{1}H-^{1}H COSY and HMBC and the detailed interrelations between ^{1}H-^{1}H and ^{1}H-^{13}C are shown in Chart 2 and Table 1. Apparently, according to its NMR spectral characteristics, the skeleton of compound **1** is a dammar-type triterpene. The detailed analysis of the interrelations between ^{1}H and ^{13}C for the triterpene allowed the signal assignments. The evidence above together with the comparison of chemical shifts[18], enabled the identification of the triterpene aglycone in compound 1 as dammar-24 (25)-ene-3β, 6α,12β, 20 (S)-tetraol.

The anomeric proton (δ_H 4.60) and an anomeric carbon signal (δ_C 98.6) indicated the presence of a monosaccharide unit in compound **1** and the ^{13}C chemical shifts at δ_C 98.6, 75.7, 78.5, 71.5, 78.2 and 62.8 indicated that it is a pyranoglucoside[19]. H-1, H-2 vicinal coupling constant between 7 and 8 Hz showed this sugar occurred in compound **1** as the β-anomer in C1 configuration. The sugar linkage was determined on the basis of HMBC experiment. A cross peak of ^{1}H-^{13}C long-range correlation was observed between the proton signal at δ_H 4.60 (H-1′) and the carbon signal at δ_C 85.2 (C-20), indicating that the sugar linked at C-20. Therefore, compound **1**, isolated from *Pseudolarix* genus for the first time, was determined to be dammar-24 (25)-ene-3β, 6α, 12β, 20 (*S*)- tetraol-20-*O*-β-D-pyranoglucoside.

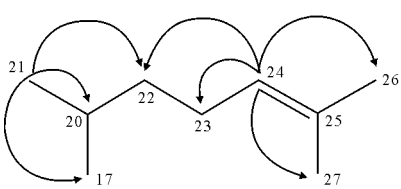

Chart 1. The piece A of compound **1**

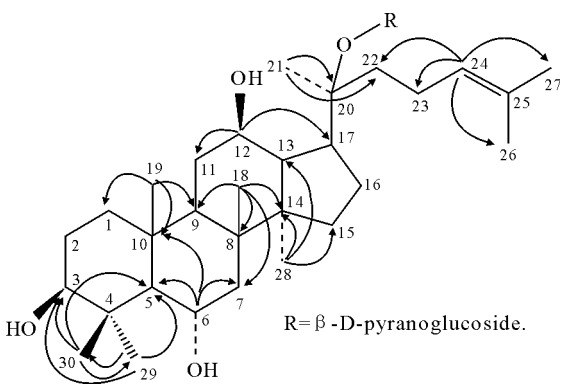

Chart 2. The structure and key HMBC of compound **1**

Table 1 NMR data of compound 1 (in MeOH-d_4)

Position	HMQC		COSY	HMBC
	δ_C	δ_H		
1	40.4 (t)	a 1.04 (1H, m)	H-1b, H-2	C-2, C-3, C-5, C-9, C-10, C-19
		b 1.70 (1H, m)	H-1a, H-2	
2	28.0 (t)	1.65 (2H, m)	H-1a, H-1b, H-3	/
3	79.8 (d)	3.14 (1H, m)	H-2	C-2, C-4, C-29, C-30
4	40.8 (s)			
5	62.4 (d)	0.90(1H, d, J=10.6)	H-6	C-1, C-3, C-4, C-6, C-7, C-9, C-10
6	69.2 (d)	4.02(1H,J_1=3.5,J_2=10.6)	H-5, H-7a, H-7b	C-5, C-7, C-10
7	47.5 (t)	a 1.55 (1H, m)	H-6, H-7b	C-5, C-6, C-8, C-9, C-14
		b 1.64 (1H, m)	H-6, H-7a	
8	42.3 (s)			
9	50.8 (d)	1.47 (1H, dd)	H-11a, H-11b	C-5, C-8, C-10, C-11, C-12, C-14, C-18, C-19
10	40.4 (s)			
11	31.2 (t)	a 1.18 (1H, m)	H-9, H-11b, H-12	C-8, C-9, C-10, C-12, C-13
		b 1.86 (1H, m)	H-9, H-11a, H-12	
12V	72.1 (d)	3.67 (1H, m)	H-11a, H-11b, H-13	C-11, C-17, C-28
13	49.7 (d)	1.74 (1H, m)	H-12, H-17	C-12, C-14, C-17, C-20
14	51.6 (s)			
15	31.4 (t)	a 1.06 (1H, m)	H-15b, H-16a, H-16b	C-14, C-28
		b 1.60 (1H, m)	H-15a, H-16a, H-16b	
16	27.5 (t)	a 1.40 (1H, m)	H-15a, H-15a, H-16b	C-15, C-17
		b 1.95 (1H, m)	H-15a, H-15b, H-16a	
17	53.4 (d)	2.28 (1H, m)	H-13, H-16a, H-16b	C-12, C-13, C-20, C-22
18	18.0 (q)	1.08 (3H, s)		C-7, C-8, C-9, C-14
19	18.0 (q)	0.95 (3H, s)		C-1, C-9, C-10
20	85.2 (s)			
21	23.1 (q)	1.34 (3H, s)		C-17, C-20, C-22
22	36.9 (t)	a 1.60 (1H, m)	H-22b, H-23	C-17, C-20, C-21, C-23, C-24
		b 1.79 (1H, m)	H-22a, H-23	
23	24.5 (t)	2.06 (2H, m)	H-22a, H-22b	C-22, C-24, C-25
24	126.1 (d)	5.10 (1H, brs)	H-23, H-26, H-27	C-22, C-23, C-26, C-27
25	132.6 (s)			
26	18.2 (q)	1.61 (3H, s)		C-24, C-25, C-27
27	26.2 (q)	1.67 (3H, s)		C-24, C-25, C-26

续表 1

Continuation of the Table 1

Position	HMQC		COSY	HMBC
	δ_C	δ_H		
28	17.6 (q)	0.95 (3H, s)		C-8, C-13, C-14, C-15
29	31.8 (q)	0.95 (3H, s)		C-3, C-4, C-5, C-30
30	16.4 (q)	1.28 (3H, s)		C-3, C-4, C-5, C-29
glcA-1′	98.6 (d)	4.60 (1H, d, J=7.7)	H-2′	C-20, C-5′
2′	75.7 (d)	3.08 (1H, m)	H-1′, H-3′	C-1′, C-3′, C-4′
3′	78.5 (d)	3.35 (1H, m)	H-2′, H-4′	C-1′, C-2′, C-4′
4′	71.5 (d)	3.30 (1H, m)	H-3′, H-5′	C-2′, C-3′, C-5′, C-6′
5′	78.2 (d)	3.20 (1H, m)	H-4′, H-6a′, H-6b′	C-1′, C-3′, C-4′, C-6′
6′	62.8 (t)	a 3.63 (1H, m)	H-5′, H-6b′	C-4′, C-5′
		b 3.78 (1H, m)	H-5′, H-6a′	

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2D NMR 对金钱松皮中的一个三萜皂苷进行结构解析

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摘要: 通过核磁共振谱并结合文献对一个三萜皂苷 dammar-24 (25)-ene-3β, 6α, 12β, 20(*S*)-tetraol-20-*O*-β-D- pyranoglucoside 进行结构解析. 通过 2D NMR (^{1}H-^{1}H COSY、HMQC、HMBC) 对其进行了 NMR 全归属.

关键词: NMR; 全归属; 2D NMR; 三萜皂苷; dammar-24 (25)-ene-3β, 6α, 12β, 20(*S*)-tetraol-20-*O*-β-D- pyranoglucoside; 金钱松

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