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Chinese Chemical Letters 20 (2009) 820–822
CHINESE CHEMICAL LETTERS
www.elsevier.com/locate/cclet

A new spirostanol saponin from the rhizomes of *Paris mairei*

Xiao Xiao Liu [a], Lei Wang [b], Ting Ting Zhang [a], Qiang Wang [a,*]

[a] *Department of Chinese Materia Medica Analysis, China Pharmaceutical University, Nanjing 210038, China*
[b] *Department of Phytochemistry, China Pharmaceutical University, Nanjing 210038, China*

Received 17 November 2008

Abstract

A new spirostanol steroidal saponin, named maireioside A (**1**), together with three known steroidal saponins, hypoglaucin G (**2**), parisaponin I (**3**), and diosgenin-3-*O*-α-L-rhamnopyranosyl (1→4)-[α-L-rhamnopyranosyl (1→2)]-β-D-glucopyranoside (**4**), were isolated from the rhizomes of *Paris mairei*. The structure elucidation was accomplished by 1D and 2D NMR methods, HR-ESI–MS, and hydrolysis.

Keywords: Spirostanol saponin; *Paris mairei*; Maireioside A

Plants of the genus *Paris* (Trilliaceae) are widely used in traditional Chinese medicine for the treatment of injuries, fractures and hemorrhage, such as *Paris polyphylla* Smith var. *chinensis* and *P. polyphylla* Smith var. *yunnanensis* [1]. There are many reports regarding the isolation of steroid saponins from the rhizomes of these plants [2–4]. However, the chemical constituents of *P. mairei* Lévl, which is distributed in southwestern China, have not been investigated. As part of a systematic examination of the bioactive constituents from plants of the genus *Paris* [5–7], we herein describe the isolation and structure elucidation of a new steroidal saponin, named maireioside A (**1**) (Fig. 1), as well as three known steroidal saponins **2**–**4**.

The rhizomes of *P. mairei* were collected in May 2005, in Yunnan province of China, and were authenticated by Prof. Qiang Wang (China Pharmaceutical University). A voucher specimen (MAL020) was deposited in the Herbarium of the Department of Pharmacognosy, China Pharmaceutical University, China.

The 80% ethanol extract of the dry raw material (15 kg) was concentrated and suspended in H_2O, then partitioned by *n*-butanol. The *n*-butanol extract was sequentially submitted to column chromatographies on macroporous resin, silica gel, and ODS, leading to the isolation of compounds **1** (9 mg), **2** (12 mg), **3** (10 mg) and **4** (16 mg).

Maireioside A (**1**) was obtained as a colorless amorphous powder. $[\alpha]_D^{25}$ −64.2 (*c* 0.07, MeOH). Positive results from both Liebermann–Burchard and Molish reactions indicated that **1** could be a steroidal glycoside. The HR-ESI–MS of **1** exhibited a quasi-molecular ion $[M+HCOO]^-$ at *m/z* 1109.4967 (calcd. 1109.5021), consistent with the molecular formula $C_{50}H_{80}O_{24}$. The IR spectrum of **1** showed characteristic absorptions for hydroxyl groups (3450 cm^{-1}) and glycosidic linkages (1041 cm^{-1}). The carbon and proton signals of **1** in the NMR spectra were

* Corresponding author.
E-mail address: qwang49@126.com (Q. Wang).

1001-8417/$ – see front matter
doi:10.1016/j.cclet.2009.03.008

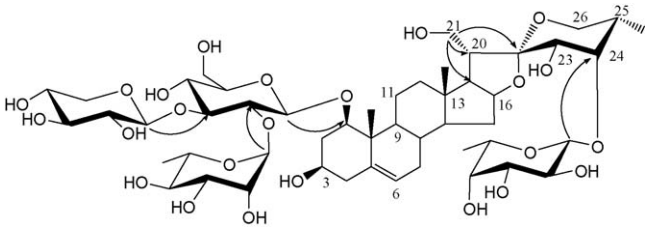

Fig. 1. The chemical structure and key HMBC correlations of **1**.

assigned by extensive techniques including HSQC, HMBC, ^{1}H–^{1}H COSY, ROESY and TOCSY. The ^{1}H NMR of the aglycone portion showed signals for three methyl groups at δ_H 1.12 (s, 3H, Me-18), 1.38 (s, 3H, Me-19), 1.09 (d, 3H, J = 6.8 Hz, Me-27) and an olefinic proton at δ_H 5.54 (d, 1H, J = 5.8 Hz). The ^{13}C NMR and DEPT spectra exhibited 50 carbon signals, 27 of which were attributed to the aglycone part. The ^{1}H NMR data and a quaternary carbon signal at δ_C 111.8 suggested **1** to be a spirostanol glycoside [8]. Comparison of the ^{1}H and ^{13}C NMR data (Table 1) of compound **1** with those of trikamsteroside E [9] indicated the aglycone of **1** was (1β,3β,23S,24S,25S)-spirost-5-ene-1,3,21,23,24-pentol. The coupling constants of proton signals due to H-1 (δ_H 3.87 (dd, 1H, J = 3.9, 12.0 Hz)), as well as the ROESY correlations between H-1 (δ_H 3.87) and H-3 (δ_H 3.72) and H-9 (δ_H 1.63), confirmed the α-configuration of H-1 and H-3. As a natural occurring product with the skeleton of spirost-5-ene, the configurations at C-20 and C-22 of **1** were determined to be R and S [10]. Furthermore, the ROESY correlations between H-23 (δ_H 4.33) and H-20 (δ_H 3.35) and H-25 (δ_H 1.98), as well as the large coupling constants between H-26α and H-25 (J = 11.2 Hz), suggested the configurations at C-23 and C-25 to be S. The configuration of C-24 (S) could also be deduced from the small coupling

Table 1
^{13}C and ^{1}H NMR data of compound **1** (δ in ppm, J in Hz).

Position	δ_C [a]	δ_H [b]	Position	δ_C [a]	δ_H [b]
1	84.0	3.87 dd (3.9, 12.0)	glu		
2	37.7	2.34 m, 2.63 m	1	99.9	4.79 d (7.8)
3	68.1	3.72 m	2	76.5	4.12 m
4	43.8	2.52 dd (5.0, 12.7), 2.65 m	3	88.5	4.03 m
5	139.5	–	4	70.3	3.76 m
6	124.7	5.54 d (5.8)	5	77.7	3.76 m
7	31.9	1.41 m, 1.72 m	6	63.3	4.15 m, 4.43 br d (10.1)
8	33.2	1.45 m	rha		
9	50.3	1.63 m	1	101.7	6.38 s
10	42.8	–	2	72.5	4.54 m
11	24.1	1.62 m, 2.83 m	3	72.5	4.76 m
12	40.4	1.45 m, 1.95 br d (12.4)	4	74.2	4.27 dd (9.4, 9.4)
13	41.1	–	5	69.6	4.78 m
14	57.1	1.18 m	6	19.2	1.70 d (6.1)
15	32.6	1.53 m, 1.88 m	xyl		
16	83.3	4.61 m	1	105.3	4.92 d (7.6)
17	58.2	2.03 dd (6.3, 8.4)	2	74.8	3.96 dd (7.9, 7.9)
18	17.1	1.12 s	3	78.4	4.05 m
19	15.1	1.38 s	4	70.7	4.09 m
20	46.2	3.35 ddd (6.6, 6.6, 6.6)	5	67.3	3.66 m, 4.25 m
21	62.6	4.05 m, 4.20 m	fuc		
22	111.8	–	1	103.5	5.72 d (8.2)
23	71.3	4.33 br s	2	71.0	4.57 m
24	81.7	4.21 m	3	73.5	4.75 m
25	35.3	1.98 m	4	73.5	4.08 m
26	61.7	3.40 dd (4.5, 10.8), 4.02 dd (11.2, 11.2)	5	70.2	4.52 m
27	13.2	1.09 d (6.8)	6	17.0	1.51 d (6.5)

[a] Measured at 125 MHz in pyridine-d_5.
[b] Measured at 500 MHz in pyridine-d_5.

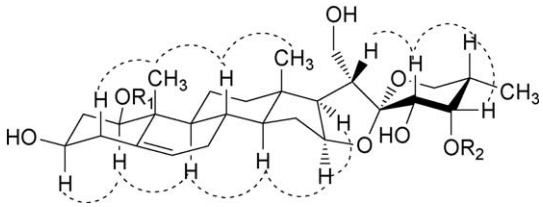

Fig. 2. Key ROESY correlations of the aglycone moiety of **1**.

constants between H-23 and H-24 ($J < 3$ Hz), together with the ROESY cross-peak between H-24 (δ_H 4.21) and H-25 (δ_H 1.98), and the absence of NOE correlation between H-24 and H-26α (δ_H 4.02) (Fig. 2).

The anomeric carbon resonances at δ_C 99.9, 101.7, 103.5 and 105.3 revealed the presence of four sugar residues, correlating with the corresponding protons at δ_H 4.79 (d, 1H, J = 7.8 Hz), 6.38 (s, 1H), 5.72 (d, 1H, J = 8.2 Hz) and 4.92 (d, 1H, J = 7.6 Hz) in the HSQC spectrum. Acid hydrolysis of **1** with 2 mol/L HCl gave D-glucose, D-xylose, L-rhamnose and L-fucose, which were confirmed by TLC comparison with the authentic samples and the reaction with L-cysteine methyl ester hydrochloride followed by GC analysis (the standard D- and L-sugars were subjected to the same reaction). Analysis of COSY, HMBC, ROESY and TOCSY data of **1** allowed the assignment of the ^{1}H and ^{13}C NMR signals of β-D-xylopyranosyl, β-D-glucopyranosyl, α-L-rhamnopyranosyl and β-L-fucopyranosyl units (Table 1). The linkage positions and sequence of the sugar moieties could be assigned by the HMBC experiment. Hence, in the HMBC spectrum, the correlations H-1(Xyl)/C-3(Glc), H-1(Rha)/C-2(Glc), H-1(Glc)/C-1(aglycone) and H-1(Fuc)/C-24(aglycone) revealed that the glycosidic chain of α-L-rhamnopyranosyl-(1 → 2)-[β-D-xylopyranosyl-(1 → 3)]-β-D-glucopyranosyl [8] was attached to C-1 and the β-L-fucopyranosyl unit was assigned to C-24 of the aglycone. Thus, compound **1** was elucidated as (1β,3β,23S,24S,25S)-spirost-5-ene-1,3,21,23,24-pentol 1-*O*-[α-L-rhamnopyranosyl-(1 → 2)-[β-D-xylopyranosyl-(1 → 3)]-β-D-glucopyranosyl]-24-*O*-β-L-fucopyranoside, named maireioside A.

It is noteworthy that the aglycone of maireioside A was seldom found in nature. To the best of our knowledge, this kind of aglycone has only previously been isolated from *Trillium kamtschaticum* [9].

The three known steroidal saponins, hypoglaucin G (**2**) [11], parisaponin I (**3**) [12], and diosgenin-3-*O*-α-L-rhamnopyranosyl (1 → 4)-[α-L-rhamnopyranosyl (1 → 2)]-β-D-glucopyranoside (**4**) [13], were also isolated and identified on the basis of their physical and spectroscopic data.

Acknowledgments

We appreciate the kind help of the Research Institute of Alpine Economic Plant of Yunnan Agriculture Science Institute and Prof. Li Chun Yuan who made the plant material collection.

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