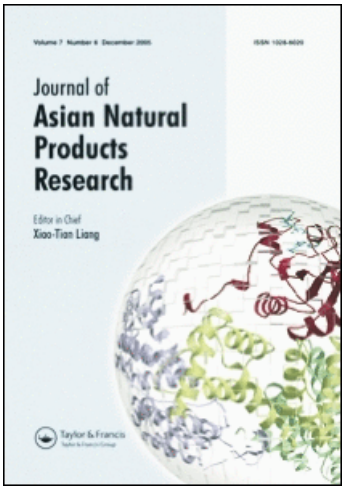

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A new C_{21} steroidal glycoside from *Cynanchum inamoenum* (Maxim.) Loes

Li-Qin Wang[a]; Yue-Mao Shen[a]; Jiang-Miao Hu[a]; Jun Zhou[a]
[a] State Key Laboratory of Phytochemistry and Plant Resources in West China, Kunming Institute of Botany, Kunming, China

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A new C_{21} steroidal glycoside from *Cynanchum inamoenum* (Maxim.) Loes

Li-Qin Wang, Yue-Mao Shen, Jiang-Miao Hu and Jun Zhou*

State Key Laboratory of Phytochemistry and Plant Resources in West China, Kunming Institute of Botany, Kunming, China

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A new C_{21} steroidal glycoside, 5β,6β-epoxy-glaucogenin C-3-*O*-β-D-glucopyranosyl-(1 → 4)-β-D-glucopyranosyl-(1 → 4)-β-D-cymaropyranosyl-(1 → 4)-β-D-oleandropyranosyl-(1 → 4)-β-D-oleandropyranoside, named inamoside D (**1**), was isolated from the MeOH extract of the roots of *Cynanchum inamoenum* (Maxim.) Loes (Asclepiadaceae). In addition, five known compounds, including 7-demethoxytylophorine (**2**), dehydrodiconiferyl alcohol γ′-*O*-β-D-glucopyranoside (**3**), β-D-fructofuranosyl-(2 → 1)-α-D-(6-*O*-sinapoyl)-glucopyranoside (**4**), neohancoside C (**5**), and cuchiloside (**6**), have also been isolated. The structure of **1** was determined by 1D- and 2D-NMR spectroscopies. All the compounds were isolated from this plant for the first time.

Keywords: *Cynanchum inamoenum* (Maxim.) Loes; Asclepiadaceae; inamoside D; steroidal glycoside

1. Introduction

Cynanchum inamoenum (Maxim.) Loes (Asclepiadaceae), widely distributed in China, is used as a folk medicine to treat different diseases such as scrofula, rupture, scabies, and internal fever [1]. As part of our ongoing investigations on *C. inamoenum*, a new C_{21} steroidal glycoside, named inamoside D (**1**), was obtained from the MeOH extract, along with 7-demethoxytylophorine (**2**) [2], dehydrodiconiferyl alcohol γ′-*O*-β-D-glucopyranoside (**3**) [3], β-D-fructofuranosyl-(2 → 1)-α-D-(6-*O*-sinapoyl)-glucopyranoside (**4**) [4], neohancoside C (**5**) [5], and cuchiloside (**6**) [6]. Their structures were determined by spectroscopic analyses (Figure 1).

2. Results and discussion

Compound **1** was obtained as a colorless amorphous solid; its molecular formula was determined as $C_{54}H_{84}O_{25}$ by the HR-ESI-MS (*m*/*z* 1131.5201 $[M - H]^-$, calcd 1131.5223) and ^1H and ^{13}C NMR data, suggesting 13 degrees of unsaturation. A careful comparison of the ^1H and ^{13}C NMR data of the aglycone with those of glaucogenin C [7] allowed the establishment of aglycone of **1** as 5,6-epoxy-glaucogenin C, because of the absence of two olefinic carbon signals at δ 141.5 (quarterly carbon) and 119.7 (secondary carbon), and the appearance of two oxygen-substituted carbon signals at δ 62.9 (quarterly carbon) and 63.2 (secondary carbon). The HMBC correlations were noted from H-6 to three carbons, δ 29.6 (C-7), 38.3 (C-8), and 62.9 (C-5), and from CH_3-19 to three carbons, δ 37.2 (C-10), 53.2 (C-9), and 62.9 (C-5), confirming the above assumption. Meanwhile, the ROESY correlation (Figure 2) between H-6 and H-7α, and H-6 and H-4α showed that H-6 was α-oriented. Thus, the

*Corresponding author. Email: jzhou@mail.kib.ac.cn

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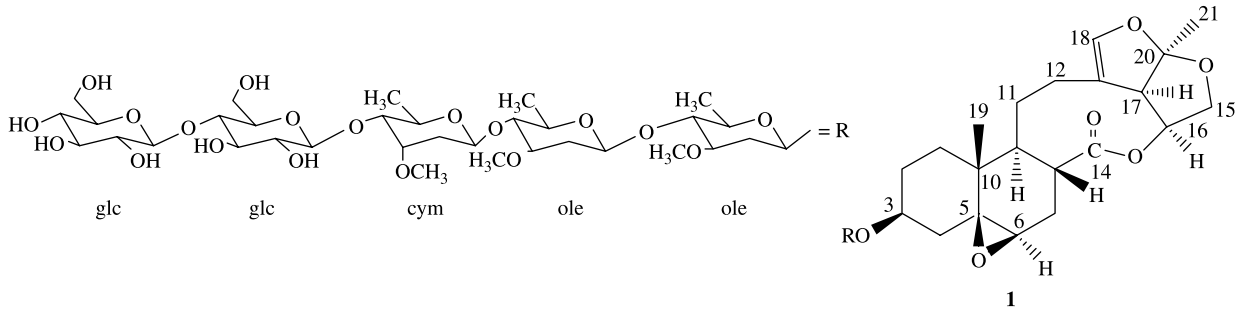

1

Figure 1. The structures of compound **1**.

aglycone of **1** was established as 5β,6β-epoxy-glaucogenin C. This kind of aglycone had not been reported in the literature.

In the ^{1}H and ^{13}C NMR spectra, five anomeric protons at δ_H 5.16 (1H, d, 7.8 Hz), 5.06 (1H, d, 7.8 Hz), 4.89 (1H, d, 10.1 Hz), 4.74 (1H, d, 9.2 Hz), 5.23 (1H, d, 9.6 Hz), and their corresponding anomeric carbons at δ_C 104.9, 104.3, 100.1, 102.0, and 96.9 were observed. From the coupling constants of the anomeric protons, five sugars of β-linkage were revealed. The HMQC–TOCSY experiment allowed the sequential assignments of all carbon resonances (Table 1) within each sugar residue, starting from the well-isolated anomeric proton signals. Comparing with the literature data [8] allowed the identification of the sugars as β-D-oleandropyranosyl, β-D-cymaropyranosyl, and β-D-glucopyranosyl. And acid hydrolysis of **1** furnished ole, cym, and glc, which was detected by TLC comparison with authentic samples. The inter-sugar linkages was decided, as shown in Figure 2, by the HMBC experiment, which showed distinct cross-peaks of correlation between H-1$''''''$ of glucose (δ_H 5.16) and C-4$''''$

Table 1. ^{1}H (500 MHz) and ^{13}C NMR (125 MHz) spectral data of compound **1** (δ, ppm; J, Hz, in C_5D_5N).

	1 δ_H	**1** δ_c		**1** δ_H	**1** δ_c		**1** δ_H	**1** δ_c
1α	1.21 dd 12.8, 12.3 Hz	35.8 t		β-D-ole	β-D-ole	5$'''$	4.21 m	69.1 d
1β	1.91 m							
2α	1.65 m		1′	5.23 d, 9.6 Hz	96.9 d	6$'''$	1.47 3H	18.8 q
2β	2.09 m	30.2 t					d 6.0 Hz	
3α	4.00 m	75.0 d	2′a	1.76 m	37.7 t	OMe	3.58 s, 3H	58.9 q
			2′e	2.51 m				
4α	1.76 m		3′	3.64 m	79.7 d		β-D-glc	
4β	2.19 t, 12.4 Hz	39.3 t						
5	\	62.9 s	4′	3.72 m	83.6 d	1$''''$	5.06 d, 7.8 Hz	104.3 d
6α	3.20 s	63.2 d	5′	3.53 m	72.1 d	2$''''$	4.00 m	75.3 d
7α	2.31 m		6′	1.72 d	18.9 q	3$''''$	4.22 m	78.3 d
7β	2.26 m	29.6 t		3H, 5.9 Hz				
8	2.65 m	38.3 d	OMe	3.51 s, 3H	57.4 q	4$''''$	4.27 m	81.5 d
9	1.02 dd 10.7, 10.8 Hz	53.2 d		β-D-ole	β-D-ole	5$''''$	4.27 m	76.5 d
10	\	37.2 s	1″	4.74 d, 9.2 Hz	102.0 d	6$''''$	4.49 m	
							4.27 m	62.3 t
11α	1.31 m		2″a	1.76 m	37.6 t		β-D-glc	
11β	2.65 m	23.9 t	2″e	2.51 m				
12α	1.91 m	29.9 t	3″	3.53 m	79.1 d	1$'''''$	5.16 d, 7.8 Hz	104.9 d
12β	1.31 m							
13	\	114.3 s	4″	3.46 m	82.8 d	2$'''''$	4.00 m	75.0 d
14	\	174.9 s	5″	3.64 m	71.7 d	3$'''''$	4.00 m	78.5 d
15α	4.24 m		6″	1.43 d, 3H, 5.5 Hz	18.8 q	4$'''''$	4.16 t, 9.2 Hz	71.6 d
15β	3.96 m	67.8 t						
16	5.42 ddd 8.3, 8.3, 8.3 Hz	75.6 d	OMe	3.48 s, 3H	57.4 q	5$'''''$	4.24 m	76.8 d
17	3.47 m	56.2 d		β-D-cym	β-D-cym	6$'''''$	4.55 br d, 9.6 Hz	62.5 t
							4.24 m	
18	6.49 s	143.9 d	1$'''$	4.89 d, 10.1 Hz	100.1d			
19	0.94 s, 3H	15.8 q	2$'''$a	1.91 m				
			2$'''$e	2.31 m	37.1 t			
20	\	118.6 s	3$'''$	4.06 m	77.9 d			
21	1.54 s, 3H	24.9 q	4$'''$	3.53 m	83.5 d			

ole, oleandropyranosyl; cym, cymaropyranosyl; glc, glucopyranosyl.

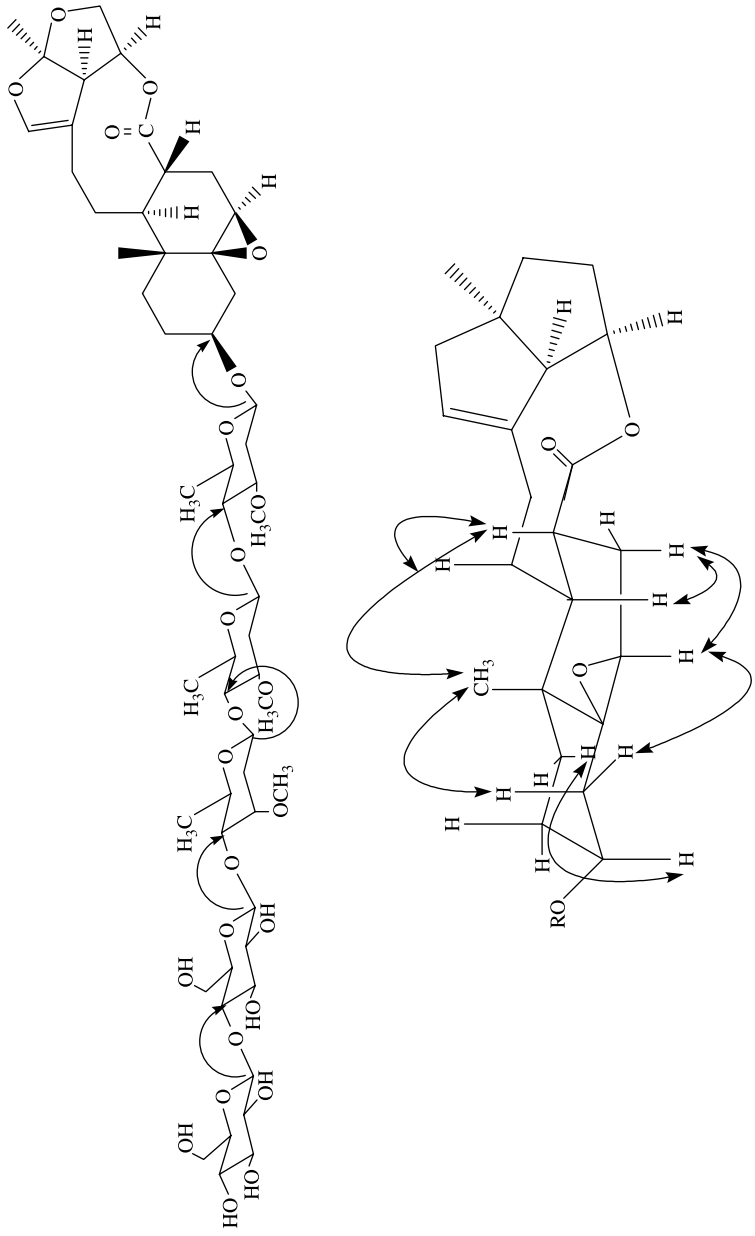

Figure 2. Key HMBC and ROESY correlations of **1**.

of glucose (δ_C 81.5); H-1$''''$ of glucose (δ_H 5.06) and C-4$'''$ of cymarose (δ_C 83.5); between H-1$'''$ of cymarose (δ_H 4.89) and C-4$''$ of oleandrose (δ_C 82.8); between H-1$''$ of oleandrose (δ_H 4.74) and C-4$'$ of oleandrose (δ_C 83.6); and between H-1$'$ of oleandrose (δ_H 5.23) and C-3 (δ_C 75.0). In addition, a ^{13}C NMR spectral comparison of **1** with that of glaucogenin C [7] revealed glycosidation shifts in the resonance of C-2 (−2.1 ppm), C-3 (+4.9 ppm), and C-4 (−3.7 ppm) of the aglycone moiety. These data supported that the sugar moiety was located only at the C-3 position of the aglycone.

The structures of all other compounds were determined by comparisons with that in the literature.

3. Experimental

3.1 General experimental procedures

The melting points were measured on X-4 micromelting apparatus and are uncorrected. Optical rotations were measured on SEPA-300 polarimeter. NMR spectra were recorded on Bruker AV-400 and Bruker DRX-500 spectrometers with TMS as an internal standard. The multiplicity of ^{13}C NMR was determined as DEPT. MS data were obtained on a VG Autospec-3000 spectrometer.

3.2 Plant material

The materials were collected in 2002 from Tai mountain in Shandong province, China, and identified by Wang Yong in Shandong Senior Technicians School of Chinese Traditional Medicines where a voucher specimen (No. yc02100811wch) has been deposited.

3.3 Extraction and isolation

The air-dried and powdered roots of *C. inamoenum* (Maxim.) Loes (3.7 kg) were extracted with MeOH (three times) under reflux to give a crude extract. After concentration of the combined extracts, the resulting gummy material was suspended in water and then partitioned with $CHCl_3$ to afford $CHCl_3$ and aqueous residues (110 and 60 g, respectively). The $CHCl_3$ residue was subjected to column chromatography over Si gel and eluted with $CHCl_3$–CH_3OH (9:1) to give three fractions. The third fraction was repeatedly subjected to column chromatography over Si gel, Sephadex LH-20, and RP-18 to afford compound **1** (33 mg). Compound **2** (290 mg) was afforded from the first fraction by repeated recrystallization. The aqueous residues was repeatedly subjected to column chromatography over Si gel, Sephadex LH-20, and RP-18 to afford compounds **3** (36 mg), **4** (46 mg), **5** (50 mg), and **6** (50 mg).

Inamoside D (**1**), $C_{54}H_{84}O_{25}$, colorless amorphous solid (CH_3OH–$CHCl_3$), mp 122–126°C, $[\alpha]_D^{16.3}$ +12.95 (*c* 0.193, CH_3OH), negative FAB-MS *m/z* (%): 1132 $[M]^-$ (41), 970 $[M\text{-glc}]^-$ (5); HR-ESI-MS *m/z* 1131.5201 ($[M-H]^-$) (calcd for $C_{54}H_{84}O_{25}$, 1131.5223); ^1H and ^{13}C NMR spectral data: see Table 1.

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