This article was downloaded by: On: 22 January 2011 Access details: Access Details: Free Access Publisher Taylor & Francis Informa Ltd Registered in England and Wales Registered Number: 1072954 Registered office: Mortimer House, 37-41 Mortimer Street, London W1T 3JH, UK



To cite this Article Wang, Li-Qin , Shen, Yue-Mao , Hu, Jiang-Miao and Zhou, Jun(2008) 'A new C₂₁ steroidal glycoside from *Cynanchum inamoenum* (Maxim.) Loes', Journal of Asian Natural Products Research, 10: 9, 867 – 871 **To link to this Article: DOI:** 10.1080/10286020802144693 **URL:** http://dx.doi.org/10.1080/10286020802144693

PLEASE SCROLL DOWN FOR ARTICLE

Full terms and conditions of use: http://www.informaworld.com/terms-and-conditions-of-access.pdf

This article may be used for research, teaching and private study purposes. Any substantial or systematic reproduction, re-distribution, re-selling, loan or sub-licensing, systematic supply or distribution in any form to anyone is expressly forbidden.

The publisher does not give any warranty express or implied or make any representation that the contents will be complete or accurate or up to date. The accuracy of any instructions, formulae and drug doses should be independently verified with primary sources. The publisher shall not be liable for any loss, actions, claims, proceedings, demand or costs or damages whatsoever or howsoever caused arising directly or indirectly in connection with or arising out of the use of this material.



A new C₂₁ 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

(Received 8 October 2007; final version received 3 April 2008)

A new C₂₁ steroidal glycoside, 5 β ,6 β -epoxy-glaucogenin C-3-O- β -D-glucopyranosyl-(1 \rightarrow 4)- β -D-glucopyranosyl-(1 \rightarrow 4)- β -D-cymaropyranosyl-(1 \rightarrow 4)- β -D-oleandropyranosyl-(1 \rightarrow 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 \rightarrow 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 C₂₁ steroidal glycoside, named inamoside D (1), was obtained from the MeOH extract, along with 7-demethoxytylophorine (2) [2], dehydrodiconiferyl alcohol γ' -*O*- β -Dglucopyranoside (3) [3], β -D-fructofuranosyl-(2 \rightarrow 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 C54H84O25 by the HR-ESI-MS $(m/z \ 1131.5201 \ [M - H]^{-}, \text{ calcd } 1131.5223)$ and ¹H and ¹³C NMR data, suggesting 13 degrees of unsaturation. A careful comparison of the ¹H and ¹³C 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 CH₃-19 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-7 α , and H-6 and H-4 α showed that H-6 was α -oriented. Thus, the

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



L.-Q. Wang et al.

٥

15

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 ¹H and ¹³C NMR spectra, five anomeric protons at $\delta_{\rm H}$ 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 $\delta_{\rm C}$ 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 -Dcymaropyranosyl, 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 ($\delta_{\rm H}$ 5.16) and C-4^{////}

Table 1. ¹H (500 MHz) and ¹³C NMR (125 MHz) spectral data of compound 1 (δ , ppm; *J*, Hz, in C₅D₅N).

$ \begin{array}{c c c c c c c c c c c c c c c c c c c $					-		-		
$ \begin{array}{c ccccccccccccccccccccccccccccccccccc$		$rac{1}{\delta_{ m H}}$	$1 \over \delta_{\rm c}$		$rac{1}{\delta_{ m H}}$	$egin{array}{c} 1 \ \delta_{ m c} \end{array}$		$rac{1}{\delta_{ m H}}$	$\frac{1}{\delta_{\rm c}}$
$ \begin{array}{cccccccccccccccccccccccccccccccccccc$	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
$ \begin{array}{cccccccccccccccccccccccccccccccccccc$	1β	1.91 m							
2β2.09 m30.2 tdddddf.0 mf.0 m	2α	1.65 m		1'	5.23 d, 9.6 Hz	96.9 d	6‴	1.47 3H	18.8 g
$ \begin{array}{cccccccccccccccccccccccccccccccccccc$	2β	2.09 m	30.2 t		,			d 6.0 Hz	1
$\begin{array}{cccccccccccccccccccccccccccccccccccc$	3α	4.00 m	75.0 d	2′a	1.76 m	37.7 t	OMe	3.58 s, 3H	58.9 q
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 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 3.51 s, 3H 57.4 q 4'''' 4.27 m 81.5 d 9 1.02 dd 53.2 d β-D-ole β-D-ole 5'''' 4.27 m 62.3 t 10.7, 10.8 Hz 10 37.2 s 1'' 4.74 d, 9.2 Hz 102.0 d 6'''' 4.49 m 12α 1.31 m 2''a 1.76 m 37.6 t β-D-glc 4.27 m 62.3 t 13 1.91 m 29.9 t 3'' 3.53 m 79.1 d 1'''' 4.00 m 75.0 d 14 1.31 m 2''a 1.76 m 3.64 m 71.7 d 3''''' 4.00 m 78.5 d </td <td></td> <td></td> <td>2′e</td> <td>2.51 m</td> <td></td> <td></td> <td>,</td> <td>1</td>				2′e	2.51 m			,	1
$\begin{array}{cccccccccccccccccccccccccccccccccccc$	4α	1.76 m		3′	3.64 m	79.7 d		β-D-glc	
$\begin{array}{cccccccccccccccccccccccccccccccccccc$	4β	2.19 t. 12.4 Hz	39.3 t					F 0	
$ \begin{array}{cccccccccccccccccccccccccccccccccccc$	5	1	62.9 s	4′	3.72 m	83.6 d	1''''	5.06 d, 7.8 Hz	104.3 d
$ \begin{array}{cccccccccccccccccccccccccccccccccccc$	6α	3.20 s	63.2 d	5′	3.53 m	72.1 d	2''''	4.00 m	75.3 d
7β 2.26 m29.6 t3H, 5.9 Hz3H, 4.9 Hz82.65 m38.3 dOMe3.51 s, 3H $57.4 q$ $4''''$ $4.27 m$ $81.5 d$ 91.02 dd $53.2 d$ β -D-ole β -D-ole $5''''$ $4.27 m$ $76.5 d$ 10 \vee $37.2 s$ $1''$ $4.74 d$, $9.2 Hz$ $102.0 d$ $6''''$ $4.49 m$ 10 \vee $37.2 s$ $1''$ $4.74 d$, $9.2 Hz$ $102.0 d$ $6''''$ $4.49 m$ 10 \vee $37.2 s$ $1''$ $4.74 d$, $9.2 Hz$ $102.0 d$ $6''''$ $4.49 m$ 10 \vee $37.2 s$ $1''$ $4.74 d$, $9.2 Hz$ $102.0 d$ $6'''''$ $4.49 m$ 10 \vee $37.2 s$ $1''$ $4.74 d$, $9.2 Hz$ $102.0 d$ $6'''''$ $4.49 m$ 10 \vee $37.2 s$ $1''$ $4.74 d$, $9.2 Hz$ $102.0 d$ $6'''''$ $4.27 m$ $62.3 t$ 11α $1.31 m$ $2''a$ $1.76 m$ $37.6 t$ β -D-glc 112α $191 m$ $2.99 t$ $3''$ $3.53 m$ $79.1 d$ $1''''$ $4.00 m$ $75.0 d$ 12α $1.91 m$ $29.9 t$ $3''$ $3.64 m$ $71.7 d$ $3''''$ $4.00 m$ $75.0 d$ $13 \vee$ $114.3 s$ $4''$ $3.46 m$ $71.7 d$ $3''''$ $4.00 m$ $76.8 d$ 15α $4.24 m$ $6''$ $1.43 d$, $3H$, $5.5 Hz$ $18.8 q$ $4'''''$ $4.24 m$ $62.5 t$ 15α $4.9 d$ $62.2 d$ β -D-cym β -D-cym 6	7α	2.31 m		6'	1.72 d	18.9 g	3////	4.22 m	78.3 d
82.65 m38.3 dOMe3.51 s, 3H $57.4 q$ $4'''$ $4.27 m$ $81.5 d$ 91.02 dd $53.2 d$ β -D-ole β -D-ole β -D-ole $5'''$ $4.27 m$ $76.5 d$ 10 \vee $37.2 s$ $1''$ $4.74 d$, $9.2 Hz$ $102.0 d$ $6'''$ $4.49 m$ $4.27 m$ $62.3 t$ 10 \vee $37.2 s$ $1''$ $4.74 d$, $9.2 Hz$ $102.0 d$ $6'''$ $4.49 m$ $4.27 m$ $62.3 t$ 11 α $1.31 m$ $2''a$ $1.76 m$ $37.6 t$ β -D-glc $6'''$ $4.27 m$ $62.3 t$ 12 α $1.91 m$ $29.9 t$ $3''$ $3.53 m$ $79.1 d$ $1''''$ $5.16 d$, $7.8 Hz$ $104.9 d$ 12 α $1.91 m$ $29.9 t$ $3''$ $3.64 m$ $71.7 d$ $3''''$ $4.00 m$ $75.0 d$ 13 \vee $114.3 s$ $4''$ $3.46 m$ $82.8 d$ $2'''''$ $4.00 m$ $75.0 d$ 14 \vee $174.9 s$ $5''$ $3.64 m$ $71.7 d$ $3'''''$ $4.00 m$ $78.5 d$ 15 α $4.24 m$ $6''$ $1.43 d$, $3H$, $5.5 Hz$ $18.8 q$ $4'''''$ $4.16 t$, $9.2 Hz$ $71.6 d$ 15 β $3.96 m$ $67.8 t$ $6.2 d$ β -D-cym β -D-cym $6''''''$ $4.55 br d$, $9.6 Hz$ $62.5 t$ 16 $5.42 ddd$ $75.6 d$ Me $3.48 s$, $3H$ $57.4 q$ $5'''''$ $4.24 m$ $4.24 m$ 18 $6.49 s$ $143.9 d$ $1'''$ $4.89 d$, $10.1 Hz$ $100.1d$	7B	2.26 m	29.6 t		3H. 5.9 Hz	1			
9 1.02 dd 53.2 d β -D-ole β -D-ole 5'''' 4.27 m 76.5 d 10.7, 10.8 Hz 77.2 s 1'' 4.74 d, 9.2 Hz 102.0 d 6''' 4.49 m 4.27 m 62.3 t 11 α 1.31 m 2''a 1.76 m 37.6 t β -D-glc 11 β 2.65 m 23.9 t 2''e 2.51 m 29.9 t 3'' 3.53 m 79.1 d 1'''' 5.16 d, 7.8 Hz 104.9 d 12 β 1.31 m 114.3 s 4'' 3.46 m 82.8 d 2'''' 4.00 m 75.0 d 14 \vee 174.9 s 5'' 3.64 m 71.7 d 3'''' 4.00 m 78.5 d 15 α 4.24 m 6'' 1.43 d, 3H, 5.5 Hz 18.8 q 4'''' 4.16 t, 9.2 Hz 71.6 d 15 β 3.96 m 67.8 t 16 5.42 ddd 75.6 d OMe 3.48 s, 3H 57.4 q 5'''''' 4.24 m 76.8 d 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 8.3, 8.3, 8.3 Hz 143.9 d 1''' 4.89 d, 10.1 Hz 100.1 d 19 0.94 s, 3H 15.8 q 2'''' 4.06 m 77.9 d 21 1.54 s, 3H 24.9 q 4''' 3.53 m 83.5 d	8	2.65 m	38.3 d	OMe	3.51 s. 3H	57.4 a	4''''	4.27 m	81.5 d
$\begin{array}{cccccccccccccccccccccccccccccccccccc$	9	1.02 dd	53.2 d		B-D-ole	β-p-ole	5''''	4.27 m	76.5 d
$ \begin{array}{cccccccccccccccccccccccccccccccccccc$	-	10.7. 10.8 Hz			P	la			
$ \begin{array}{cccccccccccccccccccccccccccccccccccc$	10		37.2 s	1″	4.74 d, 9.2 Hz	102.0 d	6''''	4.49 m 4.27 m	62.3 t
11β2.65 m23.9 t2"e2.51 m101.1 tp F get12α1.91 m29.9 t3"3.53 m79.1 d1""'5.16 d, 7.8 Hz104.9 d12β1.31 m114.3 s4"3.46 m82.8 d2""'4.00 m75.0 d13\114.3 s4"3.64 m71.7 d3""'4.00 m75.0 d14\174.9 s5"3.64 m71.7 d3""'4.00 m78.5 d15α4.24 m6"1.43 d, 3H, 5.5 Hz18.8 q4""'4.16 t, 9.2 Hz71.6 d15β3.96 m67.8 t60Me3.48 s, 3H57.4 q5""'4.24 m76.8 d165.42 ddd75.6 dOMe3.48 s, 3H57.4 q5""'4.24 m76.8 d173.47 m56.2 dβ-D-cymβ-D-cym6""'4.55 br d, 9.6 Hz62.5 t186.49 s143.9 d1"'4.89 d, 10.1 Hz100.1d4.24 m190.94 s, 3H15.8 q2""a1.91 m2"'e2.31 m37.1 t20\118.6 s3"'4.06 m77.9 d43.5 d4.24 m211.54 s, 3H24.9 q4"'3.53 m83.5 d4.35 d	11α	1.31 m		2″a	1.76 m	37.6 t		B-D-glc	02.0 0
$\begin{array}{cccccccccccccccccccccccccccccccccccc$	11B	2.65 m	23.9 t	2″e	2.51 m	01101		p 5 810	
$\begin{array}{cccccccccccccccccccccccccccccccccccc$	12α	1.91 m	29.9 t	3//	3.53 m	79.1 d	1////	5.16 d. 7.8 Hz	104.9 d
$\begin{array}{cccccccccccccccccccccccccccccccccccc$	12B	1.31 m	_> t	2		//// u	-	0110 a, 110 112	10.1.7 0
$ \begin{array}{cccccccccccccccccccccccccccccccccccc$	13	\	114.3 s	4″	3.46 m	82.8 d	2'''''	4.00 m	75.0 d
$ \begin{array}{cccccccccccccccccccccccccccccccccccc$	14	Ň	174.9 s	5″	3.64 m	71.7 d	3'''''	4.00 m	78.5 d
$ \begin{array}{cccccccccccccccccccccccccccccccccccc$	15α	4.24 m	17 112 0	6″	1.43 d. 3H. 5.5 Hz	18.8 g	4'''''	4.16 t. 9.2 Hz	71.6 d
$ \begin{array}{cccccccccccccccccccccccccccccccccccc$	15ß	3.96 m	67.8 t	0	11.10 0, 011, 010 112	1010 4			, 110 u
$\begin{array}{cccccccccccccccccccccccccccccccccccc$	16	5.42 ddd	75.6 d	OMe	3.48 s. 3H	57.4 a	5////	4.24 m	76.8 d
$\begin{array}{cccccccccccccccccccccccccccccccccccc$	10	8.3. 8.3. 8.3 Hz	7010 G	01110	0110 0, 011	ern q	U		, o.o u
$\begin{array}{cccccccccccccccccccccccccccccccccccc$	17	3.47 m	56.2 d		β-D-cym	β-D-cym	6'''''	4.55 br d, 9.6 Hz	62.5 t
$\begin{array}{cccccccccccccccccccccccccccccccccccc$								4.24 m	
$\begin{array}{cccccccccccccccccccccccccccccccccccc$	18	6.49 s	143.9 d	1‴	4.89 d, 10.1 Hz	100.1d			
$\begin{array}{cccccccccccccccccccccccccccccccccccc$	19	0.94 s, 3H	15.8 q	2‴a	1.91 m				
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				2‴e	2.31 m	37.1 t			
21 1.54 s, 3H 24.9 q 4 ^{III} 3.53 m 83.5 d	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_R 83.6); and between H-1[/] of oleandrose (δ_H 5.23) and C-3 (δ_C 75.0). In addition, a ¹³C 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 ¹³C 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 CHCl₃ to

afford CHCl₃ and aqueous residues (110 and 60 g, respectively). The CHCl₃ residue was subjected to column chromatography over Si gel and eluted with CHCl₃–CH₃OH (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 (CH₃OH–CHCl₃), mp 122–126°C, $[\alpha]_D^{16.3}$ +12.95 (*c* 0.193, CH₃OH), negative FAB-MS *m/z* (%): 1132 [M]⁻ (41), 970 [M-glc]⁻ (5); HR-ESI-MS *m/z* 1131.5201 ([M – H]⁻) (calcd for C₅₄H₈₄O₂₅, 1131.5223); ¹H and ¹³C NMR spectral data: see Table 1.

Acknowledgements

We would like to thank Professor Jianhua Wang at the Laboratory of Medicinal Plant, Agronomy College, Shandong Agriculture University, for his kind help, and the members of analytical group in the State Key Laboratory of Phytochemistry and Plant Resources in West China, Kunming Institute of Botany, for measuring the spectral data.

References

- R.Z. Fang, M.G. Gilbert, W.D. Stevens, B.T. Li, in *Flora of China* (Science Press/Missouri Botanical Garden Press, Beijing/St Louis, 1995), Vol. 16, p. 217.
- [2] X. Li, J.P. Peng, and M. Onda, *Heterocycles*, 29, 1797 (1989).
- [3] F. Yoshizawa, T. Deyama, and N. Takizawa, *Chem. Pharm. Bull.* 38, 1927 (1990).
- [4] Y. Ikeya, K. Sugama, and M. Okada, *Chem. Pharm. Bull.* **39**, 2600 (1991).
- [5] H.X. Lou, X. Li, and M. Onda, J. Nat. Prod. 56, 1437 (1993).
- [6] N.G. Bisset and A.K. Choudhury, *Phytochemistry* **28**, 1553 (1989).
- [7] Z.X. Zhang, J. Zhou, K. Hayashi, and H. Mitsuhashi, *Chem. Pharm. Bull.* 33, 1507 (1985).
- [8] A.I. Hamed, M.G. Sheded, and C. Pizza, *Phytochemistry* 65, 975 (2004).