



## Three new C<sub>21</sub> steroidal glycosides from the roots of *Cynanchum inamoenum*

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Received 29 March 2007

### Abstract

Three new C<sub>21</sub> steroidal glycosides named inamoside E (1), inamoside F (2) and inamoside G (3) were isolated from the roots of *Cynanchum inamoenum* (Maxim.) Loes. Their structures were determined by spectroscopic analysis, especially by 1D and 2D NMR experiments.

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**Keywords:** *Cynanchum inamoenum* (Maxim.) Loes; Asclepiadaceae; Inamoside E, F, G

*Cynanchum inamoenum* (Maxim.) Loes (Asclepiadaceae), widely distributed in China, is used as folk medicine to treat many diseases, such as scrofula, rupture, scabies, and internal fever [1]. In continuation of our studies on the plants of *Cynanchum* genus, the roots (3.7 kg) of *C. inamoenum* collected in Mountain Tai were extracted with MeOH. The MeOH extract was partitioned between chloroform and water, the chloroform part was repeatedly chromatographed over silica gel, RP-18 and Sephadex LH-20 to afford three new compounds, and their structures were determined by physicochemical and spectroscopic analysis, especially by 1D and 2D NMR spectroscopy.

Compound 1 was obtained as pale yellow amorphous powder. Its molecular formula was determined as C<sub>47</sub>H<sub>72</sub>O<sub>19</sub> (*m/z* 939.4575 [M–H], calcd. 939.4539) by its HRFABMS and <sup>13</sup>C NMR DEPT spectrum. The <sup>1</sup>H NMR spectrum of 1 showed two methyl signals of the aglycone moiety at δ<sub>H</sub> 0.85 (s, 3H, H-19), 1.54 (s, 3H, H-21), one olefinic proton signal at δ<sub>H</sub> 5.41 (m, 1H, 6-H), one olefinic deshielded proton at δ<sub>H</sub> 6.49 (s, 1H, H-18) assigned to the proton on the trisubstituted double bond, three protons adjacent to oxygen at δ<sub>H</sub> 3.95 (m, 1H, 15-βH), 4.29 (m, 1H, 15-αH), 5.45 (m, 1H, 16-H), and one hydroxy-methine protons at δ<sub>H</sub> 3.76 (3-H). All of these data were consistent with those of glaucogenin C [2]. The <sup>1</sup>H NMR spectrum of 1 showed three secondary methyl and two methoxyl methyl signals of deoxysugars, and four anomeric proton signals at δ 5.10 (d, 1H, 7.8Hz), 4.73 (d, 1H, 8.3Hz), 5.51 (d, 1H, 8.8Hz), 4.82 (d, 1H, 9.8Hz) indicating the presence of four sugar moieties with four β-linkages. Comparing the <sup>13</sup>C NMR spectral data with those of glaucogenin C [2] found that the chemical shifts of 1 are different from those of glaucogenin C [2] at C-2 (–2.3 ppm), C-3 (+7.9 ppm), C-4 (–2.7 ppm) due to glycosidation shifts, therefore the sugar moiety was linked to the C-3 hydroxyl group of the aglycone. Having as a starting point the anomeric proton, the

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Table 1  
The  $^{13}\text{C}$  NMR (125 MHz) data of 1, 2 and 3 in  $\text{C}_5\text{D}_5\text{N}$

	1	2	3
1	36.5	36.6	36.6
	30.1	30.2	30.1
3	77.6	77.7	77.5
4	39.1	39.2	39.0
5	140.6	140.7	140.6
6	120.6	120.6	120.5
7	28.5	28.6	28.4
8	40.7	40.8	40.8
9	53.3	53.4	53.2
10	38.7	38.7	38.7
11	23.9	24.1	23.9
12	29.9	30.1	30.1
13	114.4	114.5	114.4
14	175.5	175.6	175.5
15	67.8	67.9	67.8
16	75.6	75.7	75.7
17	56.2	56.3	56.2
18	143.9	143.6	143.9
19	17.9	18.0	17.9
20	118.6	118.7	118.5
21	24.8	24.9	24.8

	1 $\beta$ -D-Ole	2 $\beta$ -D-Ole	3 $\beta$ -D-3-Demethyl-
1'	98.2	98.2	98.3
2'	38.0	38.0	40.2
3'	79.1	79.3	70.3
4'	83.2	83.2	88.6
5'	71.8	71.8	70.9
6'	18.9	18.6	18.3
OMe	57.2	57.3	-

	1 $\beta$ -D-Digit	2 $\beta$ -D-Digit	3 $\beta$ -D-Digit
1''	98.6	98.7	99.9
2''	39.1	38.8	38.2
3''	68.8	69.1	67.6
4''	83.4	81.6	80.6
5''	67.7	68.0	69.3
6''	18.6	18.4	18.2

	1 $\beta$ -D-Ole	2 $\alpha$ -L-Cym	3 $\alpha$ -L-Cym
1'''	101.4	98.5	98.5
2'''	37.2	32.6	32.4
3'''	79.3	73.5	73.4
4'''	83.0	77.6	77.7
5'''	72.0	65.9	66.4
6'''	18.8	18.9	18.8
OMe	57.5	57.5	57.1

	1 $\beta$ -D-Glc	2 $\beta$ -D-Glc	3 $\beta$ -D-Glc
1''''	104.5	101.8	101.9
2''''	75.6	75.3	75.3
3''''	78.3	78.5	78.5
4''''	72.0	71.8	71.8

Table 1 (Continued)

	1 β-D-Glc	2 β-D-Glc	3 β-D-Glc
5 <sup>'''</sup>	78.6	78.6	78.5
6 <sup>'''</sup>	63.0	70.3	70.1
	1	2 β-D-Glc	3 β-D-Glc
1 <sup>''''</sup>	–	105.5	105.5
2 <sup>''''</sup>	–	75.2	75.3
3 <sup>''''</sup>	–	78.6	78.6
4 <sup>''''</sup>	–	71.8	71.9
5 <sup>''''</sup>	–	77.9	77.9
6 <sup>''''</sup>	–	62.8	62.8

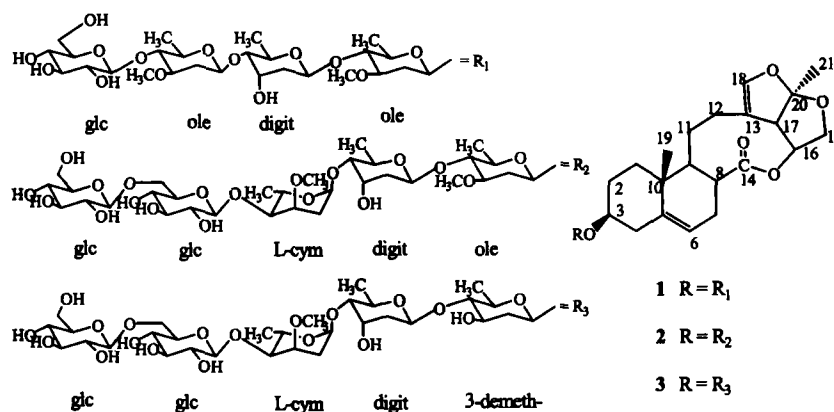
3-Demethyl-: 3-demethyl-2-deoxy-thevetopyranosyl; ole: oleandropyranosyl; digit: digitoxopyranosyl; cym: cymaropyranosyl; glc: glucopyranosyl.

<sup>1</sup>H-<sup>1</sup>H COSY, HMQC, HMBC and HMQC-TOCSY spectra, coupling constants allowed the complete assignments of chemical shifts and the identification of sugars moieties as β-D-glucopyranosyl, β-D-oleandropyranosyl and β-D-digitoxopyranosyl to be made (Table 1). The inter-sugar linkages were determined by HMBC correlations between H-1<sup>''''</sup> of glucose (δ 5.10) and C-4<sup>'''</sup> of oleandrose (δ 83.0); H-1<sup>'''</sup> of oleandrose (δ 4.73) and C-4<sup>''</sup> of digitoxose (δ 83.4); H-1<sup>''</sup> of digitoxose (δ 5.51) and C-4<sup>'</sup> of oleandrose (δ 83.2); and H-1<sup>'</sup> of oleandrose (δ 4.81) and C-3 of the aglycone (δ 77.6).

On the foregoing evidence, the structure of **1** was deduced to be glucogenin C 3-*O*-β-D-glucopyranosyl-(1 → 4)-β-D-oleandropyranosyl-(1 → 4)-β-D-digitoxopyranosyl-(1 → 4)-β-D-oleandropyranoside (Fig. 1), named inamoside E.

Compound **2** was obtained as pale yellow amorphous powder. Its molecular formula was determined as C<sub>53</sub>H<sub>82</sub>O<sub>24</sub> (*m/z* 1101.5085 ([M-H]<sup>-</sup>, calcd. 1101.5117) by its HRFABMS. The <sup>1</sup>H NMR spectrum of **2** showed three secondary methyl and two methoxyl methyl signals of deoxysugars, and five anomeric proton signals at δ 5.21 (d, 1H, 9.0 Hz), 4.99 (d, 1H, 7.7 Hz), 4.96 (s, 1H), 5.41 (d, 1H, 8.3 Hz), 4.85 (d, 1H, 10.2 Hz) indicating the presence of five sugar moieties with four β-linkages and one sugar moiety with one α-linkage. And the <sup>13</sup>C NMR spectral data were compared with those of cynatratoside D [2] and the glycosidation shift was found at C-6<sup>'''</sup> (+7.5 ppm) of β-D-glucopyranose, to which there was another β-D-glucopyranose (i.e. the terminal β-D-glucopyranose) was linked. This conclusion was further confirmed by the 2D NMR (HMQC, HMBC and HMQC-TOCSY).

Therefore the structure of **2** was deduced to be glucogenin C 3-*O*-β-D-glucopyranosyl-(1 → 6)-β-D-glucopyranosyl-(1 → 4)-α-L-cymaropyranosyl-(1 → 4)-β-D-digitoxopyranosyl-(1 → 4)-β-D-oleandropyranoside (Fig. 1), named inamoside F.

Fig. 1. The structures of compounds **1**, **2**, and **3**.

Compound **3** was obtained as pale yellow amorphous powder. Its molecular formula was determined as  $C_{52}H_{80}O_{24}$  ( $m/z$  1087.4953  $[M-H]^-$ , calcd. 1087.4961) by its HRFABMS. The  $^1H$  NMR spectrum of **3** showed three secondary methyl and one methoxyl methyl signals of deoxysugars, and five anomeric proton signals at  $\delta$  5.22 (d, 1H, 7.7 Hz), 4.98 (d, 1H, 7.7 Hz), 4.92 (s, 1H), 5.28 (d, 1H, 9.8 Hz), 4.88 (d, 1H, 9.4 Hz) indicating the presence of four sugar moieties with four  $\beta$ -linkages and one sugar moiety with one  $\alpha$ -linkage. The structure of **3** corresponded to one replaced the inner  $\beta$ -D-oleandrose of **2** with  $\beta$ -D-3-de-methyl-2-deoxy-thevetose by comparison the spectral data with those of compound **2**. The  $\beta$ -D-3-demethyl-2-deoxythevetose could be determined by comparison its NMR spectral data (Table 1) with those in the literature [3]. The HMQC, HMBC and HMQC-TOCSY experiments also confirmed that the conclusion was reasonable.

On the foregoing evidence, the structure of **3** was deduced to be glaucogenin C 3-O- $\beta$ -D-glucopyranosyl-(1  $\rightarrow$  6)- $\beta$ -D-glucopyranosyl-(1  $\rightarrow$  4)- $\alpha$ -L-cymaropyranosyl-(1  $\rightarrow$  4)- $\beta$ -D-digitoxopyranosyl-(1  $\rightarrow$  4)- $\beta$ -D-3-demethyl-2-deoxythevetopyranoside (Fig. 1), named inamoside G [4].

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- [4] The partial  $^1H$  NMR (500MHz) data of **1**, **2** and **3** ( $\delta$  in ppm,  $J$  in Hz, in  $C_5D_5N$ ) Compound **1**:  $\delta$  0.85 (s, 3H, H-19), 1.54 (s, 3H, H-21), 6.49 (s, 1H, H-18), 5.41 (m, 1H, H-6), 1.47 (d, 3H, 5.9 Hz, H-6'  $\beta$ -D-ole), 3.46 (s, 3H, H-3'-OMe  $\beta$ -D-ole), 1.43 (d, 3H, 5.4 Hz, H-6''  $\beta$ -D-digit), 1.65 (d, 3H, 5.9 Hz, H-6'''  $\beta$ -L-ole), 3.51 (s, 3H, H-3'''-OMe  $\beta$ -D-ole), 4.82 (d, 1H, 9.8 Hz, H-1'  $\beta$ -D-ole), 5.51 (d, 1H, 8.8 Hz, H-1''  $\beta$ -D-digit), 4.73 (d, 1H, 8.3 Hz, H-1'''  $\beta$ -D-ole), 5.10 (d, 1H, 7.8 Hz, H-1''''  $\beta$ -D-glc); Compound **2**:  $\delta$  0.84 (s, 3H, H-19), 1.54 (s, 3H, H-21), 6.51 (s, 1H, H-18), 5.43 (m, 1H, H-6), 1.45 (overlap, 3H, H-6'  $\beta$ -D-ole), 3.55 (s, 3H, H-3'-OMe  $\beta$ -D-ole), 1.35 (d, 3H, 5.1 Hz, H-6''  $\beta$ -D-digit), 1.45 (3H, overlap, H-6'''  $\alpha$ -L-cym), 3.45 (s, 3H, H-3'''-OMe  $\alpha$ -L-cym), 4.85 (d, 1H, 10.2 Hz, H-1'  $\beta$ -D-ole), 5.41 (d, 1H, 8.3 Hz, H-1''  $\beta$ -D-digit), 4.96 (s, 1H, H-1'''  $\alpha$ -L-cym), 4.99 (d, 1H, 7.7 Hz, H-1''''  $\beta$ -D-glc), 5.21 (d, 1H, 9.0 Hz, H-1'''''  $\beta$ -D-glc); Compound **3**:  $\delta$  0.83 (s, 3H, H-19), 1.53 (s, 3H, H-21), 6.48 (s, 1H, H-18), 5.39 (m, 1H, H-6), 1.38 (d, 3H, 6 Hz, H-6'  $\beta$ -D-3-demethyl-), 1.27 (d, 3H, 6 Hz, H-6''  $\beta$ -D-digit), 1.44 (d, 3H, 6.5 Hz, H-6'''  $\alpha$ -L-cym), 3.53 (s, 3H, H-3'''-OMe  $\alpha$ -L-cym), 4.88 (d, 1H, 9.4 Hz, H-1'  $\beta$ -D-3-demethyl-), 5.28 (d, 1H, 9.8 Hz, H-1''  $\beta$ -D-digit), 4.92 (s, 1H, H-1'''  $\alpha$ -L-cym), 4.98 (d, 1H, 7.7 Hz, H-1''''  $\beta$ -D-glc), 5.22 (d, 1H, 9.0 Hz, H-1'''''  $\beta$ -D-glc).