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Monoterpene and pregnane glucosides from *Solenostemma argel*

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Abstract

From the aerial parts of *Solenostemma argel*, two monoterpene glucosides have been isolated and identified as 6,7-dihydroxy-dihydrolinalool 3-*O*-β-glucopyranoside and 6,7-dihydroxy-dihydrolinalool 7-*O*-β- glucopyranoside. A pregnane glucoside was also isolated and assigned as pregn-5-ene-3,14-β-dihydroxy-7,20-dione 3-*O*-β-glucopyranoside together with the known compounds benzyl alcohol *O*-β-apiofuranosyl-(1 → 6)-β- glucopyranoside, 2-phenylethyl *O*-α-arabinopyranosyl-(1 → 6)-β-glucopyranoside, astragalin and kaempferol-3-*O*-α-rhamnopyranosyl-(1 → 2)-β-glucopyranoside.

Keywords: *Solenostemma argel*; Asclepiadaceae; Monoterpene; Preganane glucosides

1. Introduction

Solenostemma argel Hayne (Asclepiadaceae) is a wild perennial plant commonly growing in the eastern desert and along the Nile in south Egypt (Tackholm, 1974). The leaves of the plant are used as adulterant of Senna (Mahran & Saber, 1964). In Egyptian folk medicine, the plant is used as a purgative, antipyretic, expectorant, antispasmodic, and in cases of bile congestion (Hocking, 1955). In addition, the plant is used in herbal mixtures for the treatment of viral B and C hepatitis, as an immunostimulant, and in the treatment of hypercholesterolemia (Shawkat, 1997). In preliminary investigations of the plant, two crystalline substances (Mahran, Wabha & Saber, 1967), as well as kaempferol (Khaled & Novak, 1974), quercetin, rutin and an unknown saponin (El-Fishawy, 1976) have been isolated. In addition, the antibacterial activity of some compounds isolated from the stems was also reported (Tharib, El-Migriab & Veitch, 1986). The present study deals with the isolation and structure elucidation of new monoterpene and pregnane glucosides, together with four known phenolic glycosides from the aerial parts of the plant.

2. Results and discussion

The ethanolic extract of the leaves and stems of *S. argel* was fractionated successively with *n*-hexane, chloroform and *n*-butanol. The latter was repeatedly chromatographed (silica gel, LiChroprep RP-18, then HPLC) to afford seven compounds (**1**–**7**).

The molecular formula of compound **1** was determined as $C_{16}H_{30}O_8$ from HR-FAB mass spectrometry. Inspection of the ^{13}C-NMR spectrum of **1** (Table 1) revealed the presence of one β-glucopyranosyl unit in addition to 10 carbon signals for the aglycone. DEPT experiments indicated the presence of three methyl groups (δ 25.4, 25.7 and 25.9), three methylenes (δ 25.9, 37.0 and 114.0), two methines (δ 78.5 and 145.3), as well as two quaternary carbons (δ 72.8 and 79.9) in the aglycone moiety. The chemical shifts of the agly-

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cone carbons were very similar to those reported for 3,7-dimethyl-1-octene-3,6,7-triol (6,7-dihydroxydihydrolinalool) previously isolated from *Cunila spicata* (Manns, 1995). However, the downfield shift of C-3 from δ 73.2 (Manns, 1995) to δ 79.9, together with the upfield shifts of C-10 and C-4 from δ 28.1 and 39.0 (Manns, 1995) to δ 25.4 and 37.0, respectively, established the attachment of the glucosyl unit to C-3 of the aglycone (Bradbury & Jenkins, 1984). Moreover, the ^{1}H-NMR spectrum of compound **1** provided further confirmation, from the signals at δ 6.35 (*dd*, $J_{2,1} = 17.8$ Hz and $J_{2,1'} = 11.2$ Hz), δ 5.26 (*d*, $J_{1,2} = 17.8$ Hz), δ 5.11 (*d*, $J_{1',2} = 11.2$ Hz) for H-2, 1 and 1′, respectively. The three singlets at δ 1.46, 1.44 and 1.43 were clearly due to the three methyl groups. Furthermore, the β configuration of the glucosyl unit was determined from the coupling constant (7.8 Hz) of its anomeric proton signal at δ 4.94 in the ^{1}H-NMR spectrum (Agrawal, 1992). The assignment was substantiated by negative FAB mass spectral analysis which exhibited a quasi-molecular ion peak at *m/z* 349 [M-H]$^-$ and another significant peak at *m/z* 187 [M-Glc]$^-$. Consequently, the structure of compound **1** was assigned as 6,7-dihydroxy-dihydrolinalool 3-*O*-β-glucopyranoside.

HR-FAB mass spectral analysis of compound **2** showed the same molecular formula as **1** ($C_{16}H_{30}O_8$). At the same time, ^{1}H-NMR and negative FAB mass spectra of **2** were very similar to those of **1**. Comparison of the ^{13}C-NMR spectrum of **2** with that of **1** revealed the upfield shift of C-3 (δ 72.5) together with the downfield shifts of C-4 and 10 (δ 40.9 and 28.8, respectively) indicating that the β-glucosyl unit is located in a different position. The downfield shift of C-7 (δ 80.5), in addition to the upfield shifts of C-6, 8 and 9 (δ 76.0, 22.8 and 22.4, respectively), established the attachment of the sugar moiety to C-7 (Bradbury & Jenkins, 1984). Additionally, the negative FAB mass spectral analysis of **2** exhibited a quasi-molecular ion peak at *m/z* 349 [M-H]$^-$ and a significant peak at *m/z* 187 [M − Glc]$^-$. Therefore, the structure of compound **2** was assigned as 6,7-dihydroxy-dihydrolinalool 7-*O*-β-glucopyranoside.

Table 1
^{13}C-NMR spectral data of compounds **1**–**3** (100 MHz, pyridine-d_5)

C	**1**	**2**	C	**3**	C	**3**
1	114.0	111.1	1	38.9	18	16.5
2	145.3	146.9	2	30.2	19	17.7
3	79.9	72.5	3	77.7	20	212.6
4	37.0	40.9	4	35.8[a]	21	31.2
5	25.9	26.6	5	170.9	*β-Glc*	
6	78.5	76.0	6	123.6	1	102.1
7	72.8	80.5	7	198.4	2	75.3
8	25.9	22.8	8	41.5	3	78.5[a]
9	25.7	22.4	9	48.4	4	72.2
10	25.4	28.8	10	38.9	5	78.3[a]
			11	21.1	6	63.2
			12	34.3[a]		
β-Glc						
1	99.6	97.4	13	48.2		
2	75.3	75.3	14	82.1		
3	78.6	78.6[a]	15	33.5		
4	71.9	71.5	16	27.0		
5	78.6	78.2[a]	17	60.5		
6	63.3	62.6				

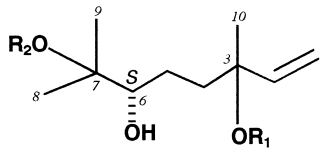
[a] Values may be interchanged in each column.

The absolute configuration at C-6 of the aglycones of compounds **1** and **2** was determined as *S* by modified Horeau's method (Brooks & Gilbert, 1973). The absolute configuration at C-3 was already reported as β-hydroxy and α-methyl by Manns (1995). However, nothing was mentioned about the procedure that was used to solve this problem. We consider that the reported NMR spectral data were inadequate to determine the absolute configuration at C-3.

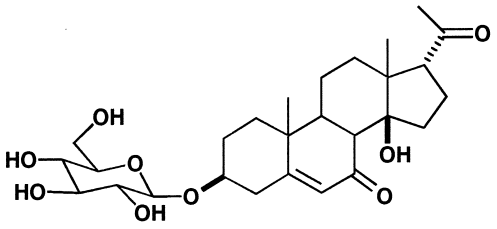
The molecular formula of compound **3** was determined as $C_{27}H_{40}O_9$ from the HR-FAB mass spectral analysis. The UV spectrum of **3** showed a maximum absorption characteristic of an α,β-unsaturated ketone (λ_{max} 239 nm) (Silverstein, Bassler & Morrill, 1981). Analysis of the ^{13}C-NMR spectrum revealed the presence of one β-glucopyranosyl unit as well as a C-21 pregnane skeleton (Table 1) (Deepak, Srivastav & Khare, 1997). DEPT experiments indicated that the aglycone consists of three methyl groups, seven methylenes, five methines and six quaternary carbons. The chemical shifts of the carbon signals of the aglycone were very similar to those reported for carumbelloside II by Lin et al. (1994). However, the downfield shift of C-7 from δ 27.9 (Lin et al., 1994) to δ 198.4 indicated the presence of an additional carbonyl group at C-7, in addition to the one C-20 (δ 212.6). The position of the C-7 carbonyl group was confirmed from the downfield shift of C-5 (δ 170.9) (Abe & Yamauchi, 1992), as well as from the singlet at δ 5.78 (H-6) in the ^{1}H-NMR spectrum. Moreover, the latter showed three singlets at δ 2.2, 1.2 and 0.98 for Me-21, 18 and 19, respectively. The doublet at δ 2.6 (J = 8.7 Hz) was assigned to H-17β due to the coupling with H-16α (δ 1.6, *m*) (Luo, Lin, Cordell, Xue & Johnson, 1993). The β configuration of the glucopyranosyl unit was determined from the coupling constant (J = 7.6 Hz) of its anomeric proton doublet at δ 5.1 in the ^{1}H-NMR spectrum. Attachment of the sugar moiety to C-3 of the aglycone was apparent from the downfield shift of C-3 (δ 77.7), together with the upfield shifts of C-2 and C-4 (δ 30.2 and 35.8, respectively) in the ^{13}C-NMR spectrum (Bradbury & Jenkins, 1984). Furthermore, the structure elucidation was confirmed from the positive FAB mass analysis which exhibited a quasi-molecular ion peak at *m/z* 509 [M + H]$^+$ and a significant peak at *m/z* 347 [M + H − Glc]$^+$. Conse-

quently, the structure of compound **3** was assigned as pregn-5-ene-3,14-β-dihydroxy-7,20-dione 3-*O*-β-glucopyranoside.

^{1}H- and ^{13}C-NMR spectral data of compound **4** were coincident with those of benzyl alcohol *O*-β-apiofuranosyl-(1 → 6)-β-glucopyranoside previously isolated from *Epimedium grandiflorum* (Miyase, Ueno, Takizawa, Kobayashi & Oguchi, 1988). The data of compound **5** were superimposable with those of 2-phenylethyl *O*-α-arabinopyranosyl-(1 → 6)-β-glucopyranoside isolated from *Rhodiola sacra* (Yoshikawa et al., 1997). Compounds **6** and **7** have been assigned as astragalin and kaempferol-3-*O*-α-rhamnopyranosyl-(1 → 2)-β-glucopyranoside by comparison of their NMR spectral data with those reported in the literature (Harborne & Mabry, 1982). This is the first report of the isolation of compounds **4**–**7** from *S. argel*.



	R_1	R_2
1	β-Glc	H
2	H	β-Glc



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3. Experimental

3.1. General

NMR spectra were recorded in pyridine-d_5 using a JEOL JNM A-400 spectrometer (400 MHz for ^{1}H-NMR and 100 MHz for ^{13}C-NMR) with TMS as internal standard. The mass spectra were recorded using a JEOL JMS-SX 102 spectrometer. Optical rotations were measured with a Union PM-1 digital polarimeter. Preparative HPLC was carried out on columns of ODS (150 × 20 mm i.d., YMC) and polyamine II (250 × 20 mm i.d., YMC) with a Tosoh refraction index (RI-8) detector. The flow rate was 6 ml/min. TLC was carried out on precoated silica gel plates (kieselgel 60 F_{254}, Merck). For CC, silica gel G (E. Merck), LiChroprep RP-18 (40–63 mm, Merck) and Diaion HP-20 (Mitsubishi Chem. Ind. Co. Ltd) were used. GLC was carried out on a Shimadzu GC-8A gas chromatograph using the following conditions: dual FID, N_2 as a carrier gas (1.2 kg/cm^2), Neutra Bond-1 column (25 m × 0.25 mm), isothermal column temperature 200°C and injection temperature 210°C. The solvent systems were: (I) $CHCl_3$–MeOH (8 : 2–6 : 4), (II) 40–70% MeOH, (III) 50% MeOH, (IV) 90% MeCN, (V) CH_2Cl_2–MeOH–H_2O (8 : 2 : 0.2), (VI) CH_2Cl_2–MeOH–H_2O (7.5 : 2.5 : 0.2), (VII) EtOAc–MeOH–H_2O (7.5 : 2.5 : 0.2), (VIII) EtOAc–MeOH–H_2O (7 : 3 : 0.3) and (IX) MeCN. The spray reagent used was 10% H_2SO_4 in ethanol.

3.2. Plant material

The plant material was collected in April, 1997 from the Halayeb and Shalatin area of the Eastern Desert of Egypt. The identity of the plant was confirmed by Prof. A. Fayed, Department of Botany and Plant Taxonomy, Faculty of Science, Assiut University. A voucher sample is kept in the Herbarium of the Faculty of Pharmacy, Assiut University, Egypt.

3.3. Extraction and isolation

The dried leaves and stems (2 kg) of *S. argel* were extracted with EtOH. After removal of the solvent by evaporation, the residue was extracted with *n*-hexane, $CHCl_3$ and *n*-BuOH, successively. The *n*-BuOH extract was subjected to a column of silica gel (system I) affording five fractions. Fraction two was chromatographed on RP-18 using system II followed by repeated prep. HPLC on ODS and polyamine columns (systems III and IV, respectively) to provide compounds **1**–**7**.

3.3.1. Compound 1

6,7-Dihydroxy-dihydrolinalool 3-*O*-β- glucopyranoside. White powder (40 mg), $[\alpha]_D^{20}$ −7.8° (MeOH, *c* 4.0, MeOH), R_f 0.42 (system V). ^{1}H-NMR spectral data (pyridine-d_5): δ 6.35 (1H, *dd*, J = 17.8, 11.2 Hz, H-2), 5.26 (1H, *d*, J = 17.8 Hz, H-1), 5.11 (1H, *d*, J = 11.2 Hz, H-1′), 4.94 (1H, *d*, J = 7.8 Hz, H-1 Glc), 1.8–2.8 (4H, *m*, H-4, 5) and 1.46, 1.44, 1.43 (9H, each *s*, Me-8, 9, 10). Negative HR-FAB mass; Found: 349.1895 [M-H]$^-$ ($C_{16}H_{29}O_8$ requires 349.1862).

3.3.2. Compound 2

6,7-Dihydroxy-dihydrolinalool 7-*O*-β- glucopyranoside. White powder (80 mg), $[\alpha]_D^{20}$ −11.1°(MeOH, *c* 8.0). R_f 0.45 (system V). ^{1}H-NMR spectral data (pyridine-d_5): δ 6.12 (1H, *dd*, J = 17.3, 10.8 Hz, H-2), 5.50 (1H, *dd*, J = 17.3, 2.0 Hz, H-1), 5.07 (1H, *dd*, J =

10.8, 2.0 Hz, H-1′), 5.06 (1H, *d*, *J* = 7.3 Hz, H-1 Glc), 1.7–2.6 (4H, *m*, H-4, 5) and 1.44, 1.43, 1.40 (9H, each *s*, Me-8, 9, 10). Negative HR-FAB mass; Found: 349.1895 $[M-H]^-$ ($C_{16}H_{29}O_8$ requires 349.1862).

3.3.3. Compound 3

Pregn-5-ene-3,14-β-dihydroxy-7,20-dione 3-*O*-β-glucopyranoside. White powder (22 mg), $[\alpha]_D^{20}$ −3.6° (MeOH, *c* 0.06), R_f 0.25 (system VI). UV λ_{max}^{MeOH} nm (log ε) 239 (1.79). ^{1}H-NMR spectral data (pyridine-d_5): δ 5.78 (1H, *s*, H-6), 5.10 (1H, *d*, *J* = 7.6 Hz, H-1 Glc), 3.8 (1H, *m* H-3*a*), 2.6 (1H, *d*, *J* = 8.7 Hz, H-17*b*), 2.2 (3H, *s*, Me-21), 1.8 (1H, *m*, H-16*β*), 1.6 (1H, *m*, H-16α), 1.20 (3H, *s*, Me-18), 0.98 (3H, *s*, Me-19). Negative HR-FAB mass; Found: 507.2599 $[M-H]^-$ ($C_{27}H_{39}O_9$ requires 507.2594).

3.3.4. Compound 4

Benzyl alcohol *O*-β-apiofuranosyl-(1 → 6)-β-glucopyranoside. White powder (13 mg), R_f 0.30 (system VII).

3.3.5. Compound 5

2-Phenylethyl *O*-α-arabinopyranosyl-(1 → 6)-β-glucopyranoside. White powder (30 mg), R_f 0.42 (system VII).

3.3.6. Compound 6

Kaempferol-3-*O*-β-glucopyranoside (astragalin). Yellow powder (800 mg), R_f 0.45 (system VIII).

3.3.7. Compound 7

Kaempferol-3-*O*-α-rhamnopyranosyl-(1 → 2)-β-glucopyranoside. Yellow powder (180 mg), R_f 0.30 (system VIII).

3.4. Enzymatic hydrolysis of compounds *1* and *2*

Compounds **1** and **2** (20 mg) were each dissolved in 0.5 ml MeOH. A solution of crude hesperidinase (100 mg) in 20 ml H_2O was added. After stirring at 37°C overnight, the mixture was extracted with diethyl ether. The diethyl ether extracts were evaporated and the residues were purified by HPLC (polyamine column, system IX) to afford the aglycones **1a** (3 mg) and **2a** (5 mg).

3.5. Modified Horeau's reaction

To solutions of **1a** and **2a** (3 mg each) in dry pyridine, (±)-2-phenylbutyric acid anhydride (12 μl) was added and kept in a sealed vial at 40°C for 1.5 h. In each case, a parallel reaction was carried out with cyclohexanol. (+)-(*R*)-α-phenylethylamine (12 μl) was added and mixed thoroughly by agitation for 30 min. The mixtures were dried by N_2 gas and the residues were dissolved in EtOAc and finally subjected to GLC analysis. The increments of the percentage area representing (−)-(*R*)-α-phenylbutyric acid were +6.1% (**1a**) and +5.8% (**2a**).

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