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Cardenolides and β-Sitosterol Glucoside from *Pergularia tomentosa* L.

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Abstract – The aerial parts of *Pergularia tomentosa* L. afforded three cardenolides, desglucouzarin, coroglaucigenin and uzarigenin, in addition to β-sitosterol glucoside. The isolated compounds were identified by physical and spectral means, including IR, UV, $[\alpha]_D$, 1D-, 2D-NMR and FAB-MS experiments. The cardenolides, ghalakinoside, calactin and pergularoside previously reported from roots, were also identified in the aerial parts.

Key words – *Pergularia tomentosa*, *Asclepiadacae*, cardenolides, desglucouzarin, coroglaucigenin, calactin, ghalakinoside, pergularoside, uzarigenin, β-sitosterol glucoside, NMR, FAB-MS.

Introduction

The milkweed family, *Asclepiadaceae,* comprises some 200 genera and 2500 species of perennial shrubs and herbs distributed through the tropics and temperate areas of the world. The family is reputed for cardenolides-containing plants, notably in the genera *Asclepias, Pergularia, Gomphocarpus* and *Calotropis* (Migahid, 1978; Selber *et al.*, 1984; Rizk, 1986). Some *Asclepias* spp. have found medicinal uses in the treatment of cancers, tumors and warts (Koike *et al.*, 1980).

The genus *Pergularia* is represented in Saudi Arabia by two species, *P. tomentosa* L. [*Daemia cordata* (Forsck) R. Br. ex. Schult] and *P. daemia* (*Forsck*) Choiv [= *Daemia extensa* (Jacq.) R.Br.] (Migahid, 1978; Rizk, 1986).

P. Tomentosa is reputed for diverse folk uses as an antirheumatic and in treatment of some skin diseases, as laxative, abortive and for treatment of asthma and bronchitis (Al-Said *et al.*, 1988a; 1989, references there in). In previous publications, the presence of ghalakinoside, calactin and pergularoside was reported in the roots of *P. tomentosa* (Al-Said *et al.*, 1988a; 1988b; Hifnawy *et al.*, 1990). Ghalakinoside, was identified as a cytotoxic cardiac glycoside (Al-Said *et al.*, 1988a) and a potential antitumor agent (Al-Said *et al.*, 1989).

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The present report deals with the cardenolides of the aerial parts of *P. tomentosa* and describes the isolation and identification of desglucouzarin, coroglaucigenin, and uzarigenin in addition to β-sitosterol glucoside. Ghalakinoside, calactin and pergularoside, previously reported in the roots have also been identified as the major cardiac glycosides in the aerial parts.

Experimental

General – Mp. (uncorr.); IR (KBr); UV (MeOH); ^{1}H-NMR, 600 MHz and ^{13}C-NMR, 150 MHz, TMS as int. standard; MS, positive FAB-MS (m/z); $[\alpha]_D$ $MeOH_3$; GC: column, BP5 (25 m×0.25 mm), inj. temp. 280°C, temp. programming at 200°C for 1 min., 200-300°C at rate 5°C/min and at 300°C for 1 min; detector, FID. TLC: Si gel 60 F_{254} plates; systems; $CHCl_3$-MeOH, 9:1 (S1); EtOAc-CH_3COCH_3, 4:1 (S2); EtOAc-MeOH-H_2O, 10:1:0.5 (S3), acetone-water 9:1 (S4); CH_3OH-H_2O, 6:4 (S5); spray reagents: Kedde's, *p*-anisaldehyde/ H_2SO_4 and $AgNO_3$ reagents.

Plant Material – The aerial parts and root bark of *P. tomentosa* were collected from Riyadh area in the flowering stage. The identity was established by Dr. Sultan-ul-Abdin, Faculty of Pharmacy, King Saud university. A voucher specimen was deposited in the herbarium of the Faculty of Pharmacy, King Saud University, Riyadh, Saudi Arabia.

Extraction – The powdered aerial parts of *P. tomentosa* (1400 g) were exhausted with MeOH

(Soxhlet). The methanolic extract was concentrated to about 200 ml and allowed to stand overnight. The solution was filtered and the residue was washed with cold MeOH. The filterate and combined washings were evaporated to leave a greenish dark brown residue (14 g). Root bark (50 g) were similarly treated for TLC comparison.

Isolation – The aerial parts extract (12 g) was fractionated on a Si gel column (type 60, 70-230 mesh, 400 g). Gradient elution was performed using $CHCl_3$ containing increasing amounts of MeOH and collecting 300 ml fractions. Four main combined fractions were collected, fr. **A** (4-10), fr. **B** (11-23), fr. **C** (24-42) and fr. **D** (52-59).

Fraction **C**, eluted with 5% MeOH/$CHCl_3$, afforded upon recrystalization from MeOH colourless rosette crystals (62.2 mg) of compound **1**. Fraction **D**, eluted with 10% MeOH/$CHCl_3$, afforded upon recrystalization from MeOH white needle crystals (190 mg) of compound **2**.

Fraction **B** (5% MeOH/$CHCl_3$) showed the presence of four Kedde's positive spots (three of them were minors). This fraction was decolourized by passing through a column of charcoal using MeOH as the eluent. The eluate was evaporated to leave 2.6 g residue. It was rechromatographed on a Si gel column using EtOAc-MeOH-H_2O (100:10:2.5) as the eluent and 100 ml fractions were collected. Compound **3** was separated from fractions 15-17, as spherical white crystals (58.5 mg) after recrystallization from acetone. Fraction **A** (eluted with 2%CH_3OH) contained a mixture of two Kedde's positive spots. It was subjected to rechromatography on Si gel column using a mixture of EtOAc-acetone as the eluent and 50 ml fractions were collected. Compound **4** was separated from fractions 16-24 (15% acetone) as colourless needle crystals (140 mg) and compound **5** (26 mg) was obtained from fractions 8-12 by crystallization from acetone.

Compound 1: Colourless rosette crystals (MeOH), mp. 278.9 °C; IR (KBr) ν_{max}: 3400, 2920, 1650, 1460, 1380, 1265, 1100-1000 (br) and 800 cm^{-1}; FAB^+-MS, 599 (M^++Na), 414 (M^+-hexose), 397 (M^+-hexose-OH), 255, 233; ^1H and ^{13}C-NMR: Table 1.

Compound 2: White needle crystals (MeOH), mp. 268 –9°C; $[\alpha]_D$ = –8.09 (C = 0.084%, MeOH); IR (KBr) ν_{max}: 3490, 3390, 2910, 1720, 1610, 1450, 1360, 1200, 950, 880 cm^{-1}; UV (CH_3OH): 218 nm.; FAB^+-MS: 629 (M^++1+glycerol), 559 (M^++Na), 537 (M^++1), 374 (M^+-hexose); 356 (M^+-hexose-H_2O); ^1H- and ^{13}C-NMR (Table 1);

Compound 3: Spherical white crystals (acetone) mp. 242-3°C; $[\alpha]_D$ = +10.7-(C = 0.084% CH_3OH); IR (KBr) ν_{max}: 3400 (br), 2900, 1780, 1700-1760 br., 1620, 1450, 1370 cm^{-1}; UV (MeOH): 218 nm; FAB^+-MS: 483 (M^++glycerol), 391 (M^++1), 369, 307, 289; ^1H- and ^{13}C-NMR (Table 1).

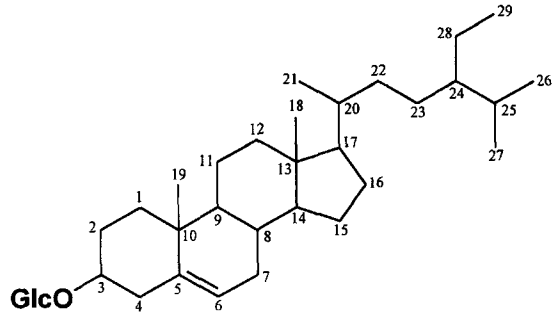
Compound 4: Colourless needle crystals (acetone), mp. 265-7°C, $[\alpha]_D$ = 63.6 (C = 0.82%, MeOH); IR (KBr) ν_{max}: 3500, 2900, 1820, 1780, 1760, 1620, 1480 cm^{-1}; UV (MeOH): 218 nm.

Compound 5: Amorphous, mp. 249-50°C; $[\alpha]_D$ = +14.6 (C = 0.82%, MeOH); IR (KBr) ν_{max}: 3400, 2900, 1780, 1730, 1620, 1450, 1360 cm^{-1}; UV (MeOH): 218 nm.

Discussion

TLC of the alcohol extract of both aerial parts and root bark against the previously reported compounds (systems S_1, S_3 and S4) revealed relatively semilar composition of the cardenolide content, though with different relative concentrations. The methanol extract of the aerial parts was subjected to a series of column chromatographic fractionation on Si gel. These afforded four cardenolides **2**, **3**, **4** and **5**, in addition to the non-cardenolide **1**.

Compound **1** analysed for $C_{35}H_{60}O_6$ as deduced from FAB^+-MS. It gave positive Liebermann-Burchard's test, Molish's test and negative reaction to Kedde's reagent. This indicated the non-cardenolide steroidal glycosidic nature of the compound. Acid hydrolysis of **1** revealed the presence of glucose as the sugar moiety and β-sitosterol as the aglycone (Sakamoto *et al.*, 1996). Analysis of the NMR data (Table 1) and MS fragmentation revealed similarity to β-sitosterol (De Rosa *et al.*, 1997; Kalinowski *et al.*, 1984;



β-Sitosterol glucoside (**1**)

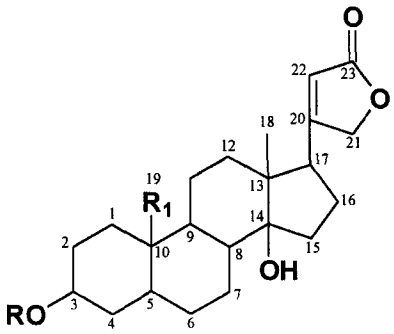
Table 1. ^{1}H- and ^{13}C-NMR data of the isolated compounds[1].

Compound No.	**1** (Pyr-d_5)		**2** (DMSO-d_6)		**3** (CD_3OD)	
	^{1}H-NMR[2]	^{13}C-NMR	^{1}H-NMR[2]	^{13}C-NMR	^{1}H-NMR[2]	^{13}C-NMR
1	0.98, 1.72 m	37.48 t	0.92, 1.75 m	36.67 t	1.77 , 2.3 m	32.62 t
2	1.4 m, 1.68 m	29.4 t	1.75m	29.07t	1.75m, 1.45m	32.44 t
3	3.8 m	78.6 d	3.05 m	76.41 d	3.55 m	71.70 d
4	2.45 t, 2.72 d (13.2)	39.06 t	1.14, 1.63 m	33.97 t	1.60, 1.45 m	39.08 t
5	–	140.8 s	0.95 m	43.77 t	1.2 d (10.99)	45.9 t
6	5.33 br.d (4.8)	121.9 d	1.13, 1.23 m	28.53 t	1.4 m, 1.5 m	29.40 t
7	1.45 m, 1.55 m	32.0 t	0.92, 1.94 m	27.27 d	1.12 m	28.73 d
8	1.50 m	32.1 d	1.42 td (2.93)	40.81 d	1.75 m	43.04 d
9	0.85 m	50.3 d	0.87 td (2.93)	49.41 s	1.00 m	51.48 s
10	–	36.4 s	–	35.46 d	–	40.38 d
11	0.90 m	20.4 t	1.18 m, 1.4 m	20.82 t	1.60 m	24.01 t
12	1.95 m, 1.90 m	39.4 t	1.3, 1.42 m	38.89 t	1.40 m	41.45 t
13	–	42.5 s	–	48.64 s	–	51.13 s
14	1.05 m	56.2 d	–	83.69 s	–	86.46 s
15	1.27 m	23.4 t	1.55t, 1.92t	32.18 t	1.6 m, 2.1 dd (1.02, 13.2)	33.36 t
16	1.65 m	28.5 t	1.75m, 1.96 m	26.38 t	1.1 m, 2.05 m	28.02 t
17	1.10 m	56.1 d	2.72 dd (9.6, 5.4)	50.15 d	2.81 dd (9.6, 6)	52.12 d
18	0.64 s	12.2 q	0.72 s	15.73 q	0.92 s	16.49 q
19	0.92 s	11.9 q	0.75 S	12.01 q	3.85, 3.7 dd (11.72, 2.46)	59.9 t
20	1.30 m	36.1 d	–	176.46 s	–	178.5 s
21	0.97 d (5.86)	18.9 q	4.88 m, 4.95 dd (1.47, 18.32)	73.22 t	4.91, 5.02dd (1.47, 16.45)	75.3 t
22	1.30 m	36.1 t	5.89 s	116.26 d	5.88 s	117.7 d
23	1.25 m	26.4 t	-	173.96 s	–	177.25 s
24	1.85 m	39.9 d	–	–	–	–
25	1.95 m	29.3 d	–	–	–	–
26	0.88 d (7.4)	19.1 q				
27	0.88 d (7.4)	19.2 q				
28	0.86 m	21.3 t				
29	1.05	19.4q				
1'	5.04 d [*J* 8.06Hz)	102.57 d	4.2 d (*J* 8.06 Hz)	100.68 d		
2'	4.06 m	75.34 d	2.85 m	73.51 d		
3'	3.96 m	78.50 d	3.10 m	76.80 d		
4'	4.28 m	71.68 d	3.01 m	70.15 d		
5'	3.94 m	78.o7 d	3.50 m	76.41 d		
6'	4.41 br., 4.55 m	62.80 t	3.35 m, 3.65 ddd (2, 5.4, 12)	61.15 t		

[1] δ value; [2] J values in Hz in brackets.

Patterson, 1984). The IR spectrum of compound **1** was superimposable on that of reference sample of β-sitosterol glucoside. Thus, compound **1** was identified as β-sitosterol-3-*O*-glucopyranoside.

Compound **2** gave positive reactions with Kedde's reagent and Molish's test indicating its cardenolide glycosidic nature. It analysed for $C_{29}H_{44}O_9$ as deduced from the FAB$^+$-MS. Other fragmentation pattern was similar to those reported for desglucouzarin (Koike *et al.*, 1980). ^{13}C-NMR (Table 1) proved the presence of butadiene lactone with a steroidal nucleus. Analysis of the chemical shift data of the NMR spectra of compound **2** (Table 1) indicated uzarigenin glycosylated at C-3, since upfield shifts were observed with C-2 and C-4 accompanied by a downfield shift of C-3 (Tori *et al.*, 1977; Yamauchi *et al.*, 1978). Analysis of HMBC spectrum revealed a correlation between H-22 (H_δ 5.89) and C-20 (C_δ 176.46), C-21 (C_δ 73.22), C-23 (C_δ 173.96); H-21 (H_δ 4.95) and C-20 (C_δ 176.46); H-18 (H_δ 0.72) and



R = Glc; $\mathbf{R_1}$ = CH_3 : Desglucouzarin (**2**)
R = H; $\mathbf{R_1}$ = CH_2OH : Coroglaucigenin (**3**)

C-12 (C_δ 38.89), C-14 (C_δ 83.69) and C-17 (C_δ 50.15); H-19 (H_δ 0.75) and C-1 (C_δ 36.67), C-9 (C_δ 49.4) and C-10 (C_δ 35.46). This confirmed the suggestion that **2** could be uzarigenin-3-hexoside. Acid hydrolysis of **2** revealed the presence of glucose (TLC-S5 and GC) and anhydrouzarigenin as the aglycone part (Koike *et al.*, 1980). Thus, compound **2** was identified as desglucouzarin (uzarigenin-3-*O*-glucopyranoside). Full ^{1}H- and ^{13}C-NMR data, as concluded from the CHSHF and HHCOSY spectra is presented in Table 1, since such data are not available in literature.

Compound **3** gave positive Kedde's reaction and negative Molish's test, indicating a cardenolide aglycone. Positive FAB-MS showed a molecular ion at *m/z* 391 corresponding to a molecular formula $C_{23}H_{34}O_5$. ^{13}C-NMR (Table 1) proved the presence of a butadiene lactone with a steroidal nucleus having a hydroxymethylene at C-19 and a β-hydroxyl at C-3 (El-Said *et al.*, 1988a). The other carbon signals indistinguishable from those reported for coroglaucigenin (Yamauchi *et al.*, 1978; Tori *et al.*, 1973). Analysis of HMBC experiment spectrum revealed a correlation between H-1 (H_δ 2.3) and C-3 (C_δ 71.70) and C-5 (C_δ 45.9); H-19 (H_δ 3.85) and C-1 (C_δ 32.62), and C-9 (C_δ 51.48); H-18 (H_δ 0.92) and C-12 (C_δ 41.45), C-14 (C_δ 86.46) and C-17 (C_δ 52.12); H-21 (H_δ 4.91 & H_δ 5.02) and C-20 (C_δ 178.53); H-22 (H_δ 5.88) and C-21 (C_δ 75.3), C-23 (C_δ 177.25). This further confirmed the identity of compound **3** as coroglaucigenin. Full ^{1}H-NMR data is presented in Table 1 since such data is not available in literature.

Compounds **4** and **5** were identified as calactin and uzarigenin, respectively, from TLC co-chromatography with reference samples, UV and $[\alpha]_D$ which were comparable with those reported for the same compounds (Bauer *et al.*, 1961; Al Said *et al.*, 1988b). The IR spectra of both isolated compounds were superimposable with those of authentic compounds. In addition to such compounds, ghalakinoside and pergularoside, previously reported in the roots were also detected in the last chromatographic fractions, (frs. 65-90) eluted with 15-20% CH_3OH, by TLC comparison with the previously isolated compounds (Al-Said *et al.*, 1988b; Hifnawy *et al.*, 1990).

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