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CARDENOLIDE GLYCOSIDES FROM *GOMPHOCARPUS SINAICUS*

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Key Word Index—*Gomphocarpus sinaicus*; *Asclepias sinaica*; Asclepiadaceae; stems; cardenolide; calotropin.

Abstract—Two new cardenolide glycosides, one with a doubly-linked sugar, 15β-hydroxy-5,6-dehydrocalotropin and the other with a singly linked sugar, coroglaucigenin-3-(6-deoxy-β-D-allopyranoside)-19-acetate, were isolated from the chloroform extract of the stems of *Gomphocarpus sinaicus*. Two other known cardenolides, were also isolated and identified as 5,6-dehydrocalotropagenin and 16α-hydroxy-5,6-dehydrocalotropin. These compounds were isolated for the first time from *G. sinaicus*. Their identification was confirmed by 400 MHz ^{1}H and ^{13}C NMR, and EI mass spectral analysis.

INTRODUCTION

Gomphocarpus (*Asclepias*) species are known to contain mainly 5α-cardenolide glycosides, with a doubly-linked sugar [1–4], which are accompanied by Δ^5-cardenolides [4]. We have previously [5] mentioned the isolation and identification of five cardenolide glycosides and two genins from the stems of *Gomphocarpus sinaicus* Boiss. We report now the isolation and identification of other cardenolides from the stems of this plant.

RESULTS AND DISCUSSION

Continued fractionation of the chloroform extract of stems of *G. sinaicus* afforded four other cardenolides including two new cardenolide glycosides, one with a doubly-linked sugar (**8**) and the other with a singly-linked sugar (**11**). In addition two compounds (**9** and **10**), which were previously isolated from *Asclepias vestita* [4] were obtained. The identity of the known cardenolides was established by comparison of their spectral data with literature data. Also, we report in this paper the ^{13}C NMR spectrum for **9**. The identification of the new cardenolides was confirmed by spectral data (EI MS, ^{1}H and ^{13}C NMR) (Tables 1 and 2) as described below.

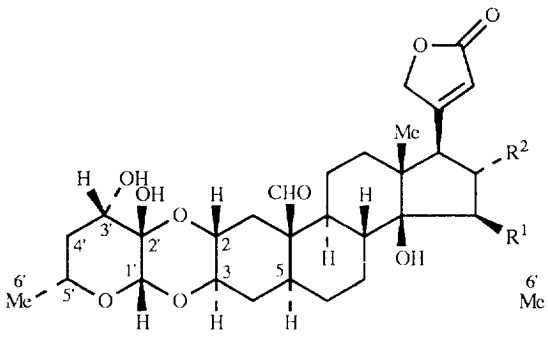
The ^{1}H NMR spectrum of **8** (Table 1) was very similar to that of 15-β-hydroxy-calotropin (**7**) [5] but showed the presence of signals at δ3.60 [*dd*, J = 4.7 (3′β, 4′β), 11.7 (3′β, 4′α) Hz], *s* at δ4.58 (H-1′β) and *m* at δ3.66 (H-5′β) [4]. This indicated that the 3′-OH is an α-hydroxyl group (calotropin derivatives) and the sugar moiety is similar to that of calotropin and 15β-hydroxy-calotropin. The presence of a signal at δ4.41 (*t*, J = 8.6, 8.7 Hz) [4], indicated the presence of a 15β-hydroxyl group. The presence of signals, near δ6 (*m*) for a vinyl proton at position C-6 and at δ9.78 (*d*, J = 1.38 Hz) for H-19, are diagnostic for the Δ^5-19-oxo function [4].

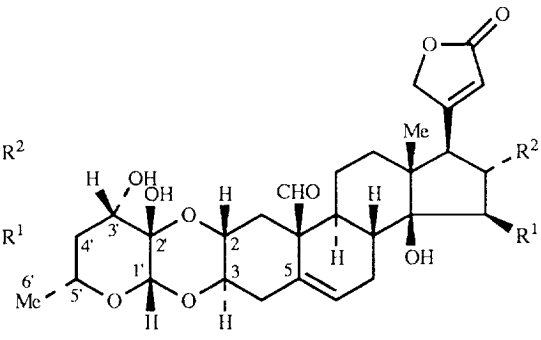
The ^{13}C NMR spectrum of **8** was very similar to that of 15β-hydroxy-calotropin (**7**), except for the presence of two olefinic carbons C-5 and C-6, which resonate downfield at δ_c 139.0 and 123.5, respectively, indicating the presence of Δ^5-double bond.

The mass spectrum of **8** showed that its genin ion (*m/z* 418) was 2 units less than that of 15β-hydroxy-calotropin (**7**) (*m/z* 420) [5] so, was defined as 15β-hydroxy-5,6-dehydrocalotropin.

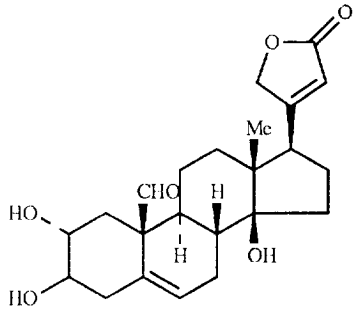
The ^{1}H NMR spectrum of **11** (Table 1) showed signal at δ2.04 (*s*), indicating the presence of an acetyl group [6]. Two signals at δ4.23 and 4.38 (*d*, J = 12 Hz), indicated the presence of 19-alcoholic protons which are deshielded by an acetyl group. This indicated the acetylation of the 19-hydroxy group [2, 3]. All the vicinal protons in the sugar were interlinked by mutual decoupling (as indicated by the ^{1}H–^{1}H COSY spectrum) and the magnitude of the vicinal coupling constants between protons (1′,2′ = 7.9 Hz; 2′,3′ = 3 Hz; 3′,4′ = 3 Hz; 4′,5′ = 9.5 Hz and 5′,6′ = 6.3 Hz) showed that the substituents at positions 2′,4′ and 5′ of the pyranoside were equatorial, while that at 3′ was axial. Also H-1′ resonated downfield at δ4.72 due to a 1-3 diaxial deshielding effect by an axial 3′β-hydroxy group [2, 4]. This confirmed that 3′ has a β-hydroxy group. The 7.9 Hz *trans*-diaxial coupling between the anomeric proton (H-1′) and the adjacent H-2′, indicated that 2′-hydroxy group was equatorial (α). It also confirmed that the sugar moiety was a β-glycoside [2, 6].

The ^{13}C NMR spectrum of **11** (Table 2) showed signals for acetyl group carbons at δ_c 172.9 and 21.0. A signal for (CH_2) at δ_c 63.3, confirmed the presence of the 19-hydroxy group, which was deshielded by an acetyl group. The

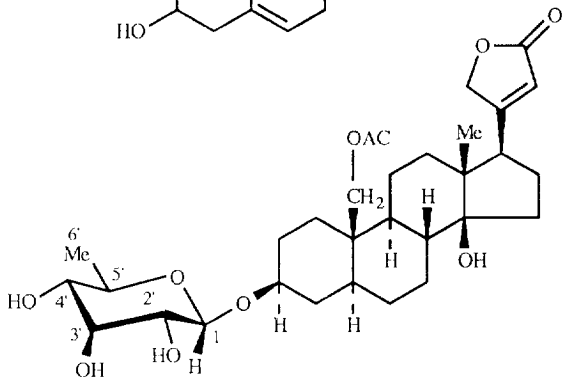




	R^1	R^2	
(3) Calotropin	H	H	(4) 5,6-Dehydro-calotropin
(7) 15β-OH-Calotropin	OH	H	(8) 15β-OH-5,6-Dehydro-calotropin
	H	OH	(10) 16α-OH-5,6-Dehydro-calotropin



(9) 5,6-Dehydro-calotropagenin



(11) Coroglaucigenin-3-(6-deoxy-β-D-allopyranoside)-19-acetate

absence of a signal for C-2′ as a quartet carbon near δ_c92 and the presence of a signal for C-1′ as a methine carbon at δ_c99.9 indicated that the sugar moiety was singly linked to the aglycone at the C-3 hydroxy group. The anomeric carbon C-1′ resonated at δ_c99.9 which indicated the presence of an adjacent equatorial 2′-hydroxy group [2].

The mass spectrum of **11** showed a fragmentation pattern of the genin which was in full agreement with that reported for the acetylated product of coroglaucigenin-3-β-D-glucoside [3]. The presence of a tertiary hydroxyl and acetoxyl groups in the aglycone residue was clearly marked by the appearance of the characteristic peaks at *m/z* 397, 355 and 337.

From all of the above data, **11** was identified as coroglaucigenin-3-(6-decxy-β-D-allopyranoside)-19-acetate (frugoside-19-acetate).

EXPERIMENTAL

Generally for instrumental procedures, plant material, extraction, fractionation and isolation see ref. [5], where the purified chloroform extract was fractionated by Vacuum Liquid Chromatographic (VLC) column on silica gel H (Merck). Elution was carried out by gradient addition of petrol, EtOAc and MeOH. Frs, each of 500 ml were collected. The eluted frs were classified according to TLC into 4 groups. Group A (3.1 g) eluted with 80% EtOAc–petrol. It was further fractionated by CC on silica gel G, eluted with 25–100% EtOAc–petrol, in frs each of 15 ml, then collected into 2 main frs: Fr. *a* (190 mg), see ref. [5], Fr. *b* (130 mg) eluted with 53% EtOAc–petrol. Analyt. HPLC using 25% MeCN–H_2O as mobile phase, showed 1 main peak R_t 24.10 (87.8%). This peak was sepd

Table 1. ^{1}H NMR (400 MHz) chemical shifts of **8–11** (δ, coupling constant J in Hz)

Proton	**8**	**9**	**10**	**11**	A	B
Hα-1	1.08 *t* (12)	0.95 *t* (12)	1.10 *t* (12)	1.09 *t* (12)	—	
Hβ-1	2.42 *dd* (5, 12)	2.47 *dd* (5, 12)	2.44 *dd* (5, 12)	—	—	
Hβ-2	4.20 *m*	3.68 *m*	4.23 *m*	1.39 *m*	—	
Hα-3	3.90 *m*	3.43 *m*	3.95 *m*	3.85 *m*	3.70 *m*	
H-6	6.05 *m*	6.10 *m*	6.09	—	—	
H-15	4.41 *t* (8.6, 8.7)	—	2.00 2.72 (2 *dd*)	—	—	
				1.87 *m*	—	
H-16	2.56 *m*	—	4.60 *m*	2.26 *m*		
Hα-17	2.83 *dd* (6.4, 9.5)	2.86 *m*	2.65 *d* (5)	2.82 *dd* (5, 9.5)	2.78 *dd*	
Hβ-18	0.81	0.80	0.76	0.89	0.92	
H-19	9.78 *d* (1.38)	9.75	9.75 *d* (3)	4.23 *d* 4.38 *d* (12.3, 12)	3.79 *dd* 3.90 *dd*	
H-21	4.94	4.94	4.82	4.95	4.80	
(2 *dd*)	5.06 (18.6, 1.6)	5.05 (18.6, 1.6)	4.91 (18.6, 1.6)	5.03 (18.6, 1.6)	4.98 (18.6, 1.6)	
H-22	5.95	5.95	5.98	5.89	5.88	
Hβ-1′	4.58	—	4.57	4.72 *d* (7.9)	4.72 *d* (8)	4.67 *d* (8)
Hα-2′	—		—	3.46 *dd* (3, 7.9)	3.42 *dd* (3, 8)	3.36 *dd* (3, 8)
H-3′	3.60 *dd* (4.7, 11.7)		3.67 *m*	4.02 *t* (3)	4.24 *m*	4.12 *t*
H-4′	1.73 *m*		1.84 *m*	3.15 *dd* (3, 9.5)	3.30 *dt* (3, 9.5)	3.24 *dd* (3, 9.5)
Hβ-5′	3.66 *m*		3.67 *m*	3.71 *dq* (6.3, 9.5)	3.70 *m*	3.67 *dq* (6, 9.5)
Hα-6′	1.23 *d* (6)	—	1.23 *d* (6)	1.23 *d* (6.3)	1.32 *d* (6)	1.27 *d* (6)
MeCO	—	—		2.04		
Solvent	CD_3OD (δ pm 4.75)				$CDCl_3$	$CDCl_3$—CD_3OD (10:1)

A = Frugoside [6].
B = 3(6-deoxy-β-allopyranosyl) in aspecioside [6, 7].

by prep. HPLC using 40% MeOH–H_2O, giving **11** (36 mg). Group B (5.95 g), see ref. [5]. Group C (3.10 g) frs were eluted with 100% EtOAc. Analyt. HPLC using 10–35% MeCN–H_2O (gradient linear in 60 min), showed 3 main peaks R_t 20.19 (7.5%), 25.10 (41.8%) and 26.49 (6.4%). These peaks were sepd by prep. HPLC using 30% MeOH–H_2O, to give **8** (16 mg), **7** (68 mg) [5] and an unknown compound (6 mg), respectively. Group D (2.13 g) frs were eluted with 1–5% MeOH–EtOAc. Analyt. HPLC using 10–35% MeCN–H_2O (as above), showed several peaks from which 2 peaks, R_t 18.53 (6.5%) and 14.67 (2.4%), were isolated by prep. HPLC using 32% MeOH–H_2O as mobile phase, to give **9** (28 mg) and **10** (2 mg), respectively.

15β-*Hydroxy*-5,6-*dehydrocalotropin* (**8**). $C_{29}H_{38}O_{10}$, mp 198–202°, EIMS *m/z* (rel. int.): 418 ($[G]^+$, 4.9), 400 ($[G-H_2O]^+$, 16.8), 382 ($[G-2H_2O]^+$, 19.2), 372 ($[G-H_2O-CO]^+$, 14.5), 354 ($[G-2H_2O-CO]^+$, 31.2), 129 ($[SH]^+$, 46), 128 ($[S]^+$, 46), 91 (base peak, 100).

5,6-*Dehydrocalotropagenin* (**9**). $C_{23}H_{30}O_6$, mp 205–208°. EIMS *m/z* rel. int. 402 ($[G]^+$, 1.5), 384 ($[G-H_2O]^+$, 35.6), 369 ($[G-H_2O-Me]^+$, 7.5), 91 (base peak, 100).

16α-*Hydroxy*-5,6-*dehydrocalotropin* (**10**). $C_{29}H_{38}O_{10}$, mp 155–158°. EIMS *m/z* (rel. int.) 418 ($[G]^+$, 1.0), 400 ($[G-H_2O]^+$, 0.9) 382, 366, 354 ($[G-2H_2O-CO]^+$, 3.4), 129 ($[SH]^+$, 16.4), 128 ($[S]^+$, 23), 44 (base peak, 100).

Frugoside-19-*acetate* (**11**). $C_{31}H_{46}O_{10}$, mp 148–152°. EIMS *m/z* (rel. int.) 578 ($[M]^+$, 0.31), 433 ($[GH]^+$, 2.1), 415 ($[GH-H_2O]^+$, 10.5), 397 ($[GH-2H_2O]^+$, 9.2), 355 ($[GH-H_2O-HOAc]^+$, 22.5), 337 ($[GH-2H_2O-HOAc]^+$, 32.4), 147 ($[SH]^+$, 30.4), 145 ($[S-H]^+$, base peak 100).

Table 2. ^{13}C NMR (100 MHz) chemical shifts (δ) of **8**, **9** and **11** compared with reported data

C	**8**	**9**	**11**	A	B
1	36.5	39.9*	35.7	33.0	
2	69.8	73.0	30.6	32.7	
3	73.0	75.8	78.9	70.7	
4	35.9	39.4	33.1	39.8	
5	139.0	141.0	45.7	45.2	
6	123.5	121.9	29.6	28.8	
7	30.5	30.4	28.5	28.1	
8	39.7	39.4	43.1	42.4	
9	45.5	45.5	50.8	50.8	
10	53.4	53.5	39.6	40.6	
11	24.3	24.5	23.5	23.4	
12	39.2	40.0*	41.1	39.8	
13	49.2	51.5	51.0	50.2	
14	84.0	85.8	86.2	84.4	
15	75.0	37.8	33.4	32.0	
16	34.3	28.6	28.0	27.3	
17	49.5	51.6	52.1	51.6	
18	17.0	16.4	16.4	16.3	
19	208.2	208.5	63.3	59.5	
20	177.1	177.9	178.3	176.2	
21	75.3	75.3	75.3	73.4	
22	118.3	118.0	117.8	117.6	
23	177.0	177.1	177.2	174.6	
1′	97.3		99.9		98.2
2′	92.7		72.9		69.6
3′	74.0		72.5		70.6
4′	39.6		74.4		72.6
5′	69.4		70.6		71.0
6′	21.4		18.2		17.6
C=O			172.9		
Me			21.0		
Solvent	CD_3OD	(δ ppm 49.0)		C_6D_5N	$CDCl_3$–CD_3OD (10:1)

*Signals within a vertical column may be reversed.
A = Coroglaucigenin [8].
B = 3(6-deoxy-β-allopyranosyl) in aspecioside [7].

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