Pergamon 0031-9422(95)00940-X Phytochemistry, Vol. 42, No. 2, pp. 523–529, 1996

0031-9422/96 $15.00 + 0.00

# RE-INVESTIGATION OF THE CARDENOLIDE GLYCOSIDES FROM *GOMPHOCARPUS SINAICUS*

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(*Received* 25 *September* 1995)

**Key Word Index**—*Gomphocarpus sinaicus*; Asclepiadaceae; aerial parts; cardenolide glycoside; $\Delta^7$-cardenolides; $\Delta^5$-cardenolides.

**Abstract**—Nine cardenolide glycosides and one aglycone, including five new compounds, were isolated from the aerial parts of the milkweed, *Gomphocarpus sinaicus*. The elucidation of the structures and stereochemistry of the new glycosides was accomplished using FAB mass spectrometry, $^1$H NMR, $^{13}$C NMR, $^1$H–$^1$H COSY, $^1$H–$^{13}$C COSY, NOE and NOESY. The new compounds were identified as 7,8-dehydrocalotropin, 17$\alpha$-hydroxy-7,8-dehydrocalotropin, 19-acetylglucocoroglaucigenin, 19-acetylglucofrugoside and humistratagenin. Some of these results differ from those reported in three recent publications on the cardenolides of the same plant species.

## INTRODUCTION

Many genera of the milkweed family Asclepiadaceae, comprising some 2500 species, examined thus far appear to contain cardioactive chemicals known as cardenolides [1]. *Gomphocarpus sinaicus* Boiss. (syn. *Asclepias sinaica* Muschl.), known locally as Ghalquit ed-deeb or Hargel, is one of the wild plants growing in the sandy mountainous regions in South Sinai province, Egypt [2]. The stems of the title plant have been reported [3] to contain 5$\alpha$- and $\Delta^5$-cardenolide glycosides with doubly linked sugars which were identified as calotropin, 5,6-dehydrocalotropin, 3′-epiafroside, 14$\beta$,17$\alpha$-epoxy-5,6-dehydrocalotropin and 15$\beta$-hydroxycalotropin. In addition, two cardenolide aglycones have been isolated and identified as xysmalogenin and uzarigenin. Four additional cardenolide glycosides have been isolated [4] from the stems of *G. sinaicus*. Two of them proved to be new; their structures have been determined as 15$\beta$-hydroxy-5,6-dehydrocalotropin and frugoside-19-acetate. The other two cardenolides have been identified as 5,6-dehydrocalotropagenin and 16$\alpha$-hydroxy-5,6-dehydrocalotropin. A comparative study [5] of the cardenolide content of the different organs of *G. sinaicus* was carried out and 5,6-dehydrocalotropin was shown to be the main cardenolide glycoside in all organs; no remarkable differences in the cardenolide patterns of the plant organs except seeds were observed. In our re-investigation, the structures of the compounds thought to be 5,6-dehydrocalotropin and 5,6-dehydrocalotropagenin were revised to 7,8-dehydrocalotropin and 7,8-dehydrocalotropagenin, respectively. Also, four additional singly linked cardenolide glycosides, two of which are new compounds, were isolated.

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## RESULTS AND DISCUSSION

The chloroform-soluble fraction of a methanolic extract of the aerial parts of *G. sinaicus* afforded 10 compounds: nine cardenolide glycosides (**1–9**) and one cardenolide aglycone (**10**), of which five compounds were known [3, 6–8]. The identities of the known cardenolide glycosides calotropin (**1**), 3′-*O*-$\beta$-D-glucocalotropin (**2**), 3′-epiafroside (**3**) and desglucouzarin (**4**) were established by comparing them with authentic samples or with literature data [3, 6, 7, 9]. The molecular formula of compound **5** was considered to be $C_{29}H_{44}O_9$ based on its FAB mass spectrum. The aglycone moiety of **5** was identified as coroglaucigenin by comparing the $^1$H and $^{13}$C NMR data (Tables 1 and 2) with those reported for 4′-*O*-cellobiosylfrugoside, which had coroglaucigenin as the aglycone [6]. The NMR data for the sugar moiety of **5** were consistent with those reported for the D-allomethylosyl moiety in 12$\beta$-hydroxycoroglaucigenin-3-*O*-$\beta$-D-allomethyloside [6]. Additionally, the sugar was identified by capillary gas chromatographic comparison of its alditol acetate with an authentic sample. Thus, compound **5** was identified as the known cardenolide glycoside frugoside [8].

Compound **6** was identical (Tables 1 and 2) with a compound which has previously been isolated from *G.*

Table 1. $^1$H NMR spectral data for compounds **1**, **5-10** and **8a**

| H | **1*** | **5*** | **6*** | **6†** | **6†‡** | **6§** | Diacetylhumistratin [13]* | **7*** | **8*** | **8a*** | **9*** | **10*** |
|---|---|---|---|---|---|---|---|---|---|---|---|---|
| 1$\alpha$ | 1.15 *t* (12) | 0.85 *td* (13.5, 3) | 1.15 *t* (12)[a] | 0.95 *t* (12) | 0.94 *t* (12) | 1.07 | | 1.15 *t* (12) | 0.88 *td* (13.5, 3) | 0.85 *td* (13.5, 3) | 0.78 *td* (13.5, 3) | 1.52 *t* (12) |
| 1$\beta$ | 2.48 *dd* (12, 4.5) | 2.62 *dt* (13.5, 3) | 2.50 *dd* (12, 4.5) | 2.29 *dd* (12, 4.5) | 2.28 *dd* (12.5, 5) | 2.43 *dd* (13, 5) | 2.43 *dd* (13, 5) | 2.50 *dd* (12, 4.5) | 2.25 *dt* (13.5, 3) | 2.25 *dt* (13.5, 3) | 18 | 2.95 *dd* (12, 4.5) |
| 2$\beta$ | 4.45 *td* (12, 4.5) | | 4.85 hidden | 4.07 *td* (10.5, 4.5) | 4.07 *ddd* (4.4, 4.7) | 4.24 *td* (10.5, 4.5) | | | 1.67, 2.18 | 1.84, 2.18 | | 4.40 *ddd* (12, 9, 4.5) |
| 3$\alpha$ | 4.32 *m* | 4.06 *m* | 4.30 *ddd* (12, 10, 4.5) | 3.80 | 3.78 *ddd* (3.8, 4.1) | 3.93 *ddd* (12, 10, 4.5) | 3.9–4.2 *m* (H-2, H-3, H-5') | 4.28 *td* (12, 4.5) | 3.94 *m* | 4.02 *m* | 4.00 *m* | 3.85 *ddd* (12, 9, 4.5) |
| 4$\alpha$ | | | 1.85 *dt* (13, 4) | 1.68 *dt* (13.4) | | | | | | | | |
| 4$\beta$ | | | 1.35 *m* | 1.08 *q* (*ca* 13) | | | | | | | | |
| 5$\alpha$ | 1.73 *m* | | 1.72 *m*[b] | | | | | | | | | |
| 6 | | | | | | | | | | | | |
| 7 | | | 6.36 | 5.95 *m* | 5.95 *t* (2.5) | 6.08 | 5.73 *br s* | 6.44 | | | | 6.42 |
| 9$\alpha$ | | | 2.28 | | | | | | | | | |
| 11$\alpha$ | 1.57 *m* | | 1.55 *m* | | | | | | | | | |
| 11$\beta$ | 1.24[a] | | 1.17[a] | | | | | | | | | |
| 12$\alpha$ | 1.57 *m* | | 1.48 *m* | | | | | | | | | |
| 12$\beta$ | 1.27[a] | | 1.34 | | | | | | | | | |
| 15 | | | 1.85[b], 2.36 | | | | | | | | | |
| 16 | | | 2.15[c] | 2.04 *m* | 2.03 *m* | | | 2.42 *td* (12, 3), 2.74 *dt* (9) | | | | |
| 17 | 2.75 | 2.78 *dd* (9, 5) | 2.85 | 2.71 *m* | 2.70 *m* | 2.81 *m* | 2.81 *t*(5) | | 2.75 *dd* (9, 4.5) | 2.77 *m* | 2.75 *m* | 2.80 *m* |
| 18 | 0.88 *s* | 1.04 *s* | 0.85 *s* | 0.64 *s* | 0.63 *s* | 0.77 *s* | 0.78 *s* | 1.02 *s* | 1.02 *s* | 1.02 *s* | 1.03 *s* | 0.88 *s* |
| 19 | 9.99 *s* | 3.89 *d* (12), 4.06 *d* (12) | 9.86 *d* (1.5) | 9.60 *d* (1.5) | 9.59 *d* (1.25) | 9.73 | 9.71 *d* (1) | | 4.22 *d* (11), 4.47 *d* (11) | 4.22 *d* (12), 4.47 *d* (12) | 4.24 *d* (12), 4.45 *d* (12) | 10.00 *s* |
| 21 | 5.24 *dd* (18, 1.5), 4.98 *dd* (18, 1.5) | 5.26 *dd* (18, 1), 5.05 *dd* (18, 1) | 5.25 *dd* (18, 1.5), 5.05 *dd* (18, 1.5) | 4.88 *dd* (18, 1.5) 4.72 *dd* (18, 1.5) | 5.87 *dd* (18, 1.9), 4.71 *dd* (18, 1.9) | 4.91 *dd* (18, 1.5) 4.79 *dd* (18, 1.5) | 4.94 *dd* (18, 1.5) 4.80 *dd* (18, 1.5) | 5.65 *dd* (18, 1.5), 5.23 *dd* (18, 1.5) | 5.27 *dd* (18, 1.5), 5.02 *dd* (18, 1.5) | 5.26 *dd* (18, 1.5), 5.04 *dd* (18, 1.5) | 5.25 *dd* (18, 1.5), 5.24 *dd* (18, 1.5) | 5.25 *dd* (18, 1.5), 5.02 *dd* (18, 1.5) |
| 22 | 6.08 *br s* | 6.08 *br s* | 6.11 *br s* | 5.77 *br s* | 5.76 *br s* | 5.92 | 5.93 | 6.68 *br s* | 6.10 *br s* | 6.08 *br s* | 6.10 *br s* | 6.13 *br s* |
| OAc | | | | | | | 2.06 *s*, 2.13 *s* | | 1.97 *s* | 1.97 *s* | 1.96 *s* | |
| 1' | 5.00 *s* | 5.40 *d* (7.5) | 5.08 *s* | 4.36 *s* | 4.36 *s* | 4.58 *s* | 4.81 *s* | 5.08 *s* | 5.37 *d* (7.5) | 5.38 *d* (7.5) | 5.05 *d* (7.5) | |
| 2' | | 3.92 *dd* (7.5, 3) | | | | | | | 3.90 *dd* (8, 3) | 3.92 *dd* (7.5, 3) | 4.03 | |
| 3' | 4.12 *dd* (12, 4.5) | 4.68 *t* (3) | 4.12 *dd* (12, 4.5) | 3.50 *dd* (12, 4.5)[a] | 3.49 *dd* (11.7, 4.7) | *ca* 3.63[a] | 5.76 *t* (3) | 4.12 *dd* (12, 4.5) | 5.07 | 4.68 *t* (3) | 4.25 *t* (9) | |
| 4' | 2.02 *td* (12, 4.5), 2.12 *q* (12) | 3.68 *dd* (9.3) | 2.02 *td* (12, 4.5), 2.12 *q* (12)[c] | 1.62, 1.45 | 1.69 *m* | 1.83 *m* | | 2.02 *td* (12, 4.5), 2.12 *q* (12) | 3.82 *dd* (9, 3) | 3.68 *dd* (9, 3) | 4.24 *t* (9) | |
| 5' | 3.75 *m* | 4.36 *m* | 3.76 *m* | 3.50 *m*[a] | 3.49 *m* | 3.63[a] | | 3.75 *m* | 4.50 *m* | 4.36 *m* | 4.00 *m* | |
| 6' | 1.35 *d* (6) | 1.65 *d* (6) | 1.35 *d* (6) | 1.12 *d* (6) | 1.12 *d* (6) | 1.26 *d* (6) | 1.22 *d* (6) | 1.35 *d* (6) | 1.71 *d* (6) | 1.65 *d* (6) | 4.40 *dd* (12,45 H-6'a), (4.60 *dd* (12, 2 H-6'b) | |
| 1'' | | | | | | | | | 5.05 *d* (7.5) | | | |
| others | | | | | | | | | 4.35 *dd* (11, 5 H-6''a), 4.44 *dd* (11, 2 H-6''b) | | | |

*Spectra were measured in pyridine-$d_5$.

†Spectra were measured in $CDCl_3$–$CD_3OD$ (4:1).

‡Authentic spectrum in literature [3] calibrated to $H_3$-6' = 1.12 ppm, rearranged according to structure revision.

§Spectra were measured in $CDCl_3$.

Signals with the same superscript in the same column partially or completely overlapped.

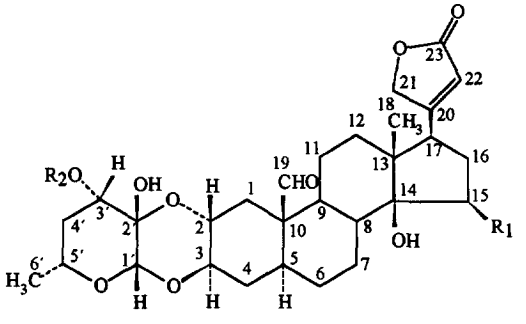
Table 2. $^{13}C$ NMR spectra data for compounds **1**, **5**–**10** and **8a**

| C | 1* | 5* | 6* | 6† | 6†‡ | 6§ | Diacetyl-humistratin [13]§ | 7* | 8* | 8a* | 9* | 10* |
|---|---|---|---|---|---|---|---|---|---|---|---|---|
| 1 | 36.5 | 32.6 | 35.5 | 34.6 | 34.9** | 34.9 | 34.7** | 35.4 | 32.4 | 32.4 | 32.3 | 39.7 |
| 2 | 69.3 | 30.7 | 68.9 | 68.4 | 68.3 | 68.9 | 70.6 | 69.0 | 30.2 | 30.2 | 30.1 | 72.7 |
| 3 | 72.4 | 77.4 | 72.0 | 71.4 | 71.7 | 71.6 | 70.8 | 72.0 | 76.7 | 76.7 | 76.7 | 75.5 |
| 4 | 33.9 | 35.4 | 33.7 | 32.9 | 32.2** | 33.1 | 32.9** | 33.8 | 35.1 | 35.0 | 35.0 | 37.8 |
| 5 | 42.5 | 44.8 | 38.8 | 38.6 | 38.9¶ | 39.2 | 39.5 | 38.9 | 44.5 | 44.5 | 44.4 | 38.6 |
| 6 | 27.9 | 28.8 | 29.6 | 29.1 | 29.5‖ | 29.4 | 29.4 | 29.6 | 28.5 | 28.6 | 28.6 | 29.8 |
| 7 | 27.9 | 28.1 | 120.9 | 120.7 | 121.0‖ | 121.1 | 121.0 | 120.7 | 27.8 | 27.8 | 27.8 | 120.9 |
| 8 | 43.4 | 42.3 | 140.7 | 139.3 | 139.6¶ | 139.7 | 139.7 | 141.6 | 42.2 | 42.2 | 42.2 | 140.8 |
| 9 | 48.7 | 50.7 | 44.9 | 44.4 | 44.6 | 44.7 | 44.5 | 45.2 | 49.9 | 49.9 | 50.1 | 44.8 |
| 10 | 52.9 | 39.9 | 52.3 | 52.1 | 52.4 | 52.2 | 52.2 | 52.3 | 38.6 | 38.7 | 38.6 | 52.6 |
| 11 | 22.2 | 23.4 | 23.7 | 23.1 | 23.4 | 23.3 | 23.2 | 23.8 | 22.7 | 22.7 | 22.7 | 23.9 |
| 12 | 39.2 | 40.5 | 38.8 | 38.9 | 39.1 | 39.1 | 39.2 | 32.3 | 39.9 | 39.9 | 40.0 | 38.9 |
| 13 | 49.7 | 50.3 | 50.8 | 50.1 | 50.3 | 50.5 | 50.6 | 54.5 | 50.1 | 50.1 | 50.1 | 50.8 |
| 14 | 84.0 | 84.8 | 84.4 | 84.2 | 84.6 | 85.0 | 85.0 | 85.0 | 84.6 | 84.6 | 84.6 | 84.4 |
| 15 | 32.5 | 33.1 | 39.6 | 38.7 | 39.0** | 38.8 | 38.8** | 39.6 | 33.1 | 33.1 | 33.1 | 39.6 |
| 16 | 27.1 | 27.3 | 27.9 | 27.4 | 27.7 | 27.8 | 27.8 | 38.3 | 27.1 | 27.2 | 27.3 | 27.9 |
| 17 | 51.2 | 51.5 | 50.7 | 50.3 | 50.6 | 50.1 | 50.1 | 85.5 | 51.4 | 51.4 | 51.5 | 50.7 |
| 18 | 15.8 | 16.3 | 16.1 | 15.5 | 15.8 | 15.9 | 15.9 | 15.7 | 16.1 | 16.1 | 16.1 | 16.1 |
| 19 | 207.9 | 59.1 | 206.7 | 206.8 | 207.0 | 205.9 | 205.6 | 206.7 | 62.1 | 62.1 | 62.1 | 207.4 |
| 20 | 175.5 | 176.1 | 175.3 | 175.3 | 175.4 | 174.3 | 174.2 | 180.7 | 175.8 | 175.8 | 175.8 | 175.4 |
| 21 | 73.6 | 73.7 | 73.6 | 73.7 | 73.9 | 73.5 | 73.4 | 73.9 | 73.7 | 73.7 | 73.7 | 73.7 |
| 22 | 117.8 | 117.6 | 117.9 | 117.3 | 117.7 | 118.1 | 118.0 | 117.7 | 117.7 | 117.7 | 117.7 | 117.9 |
| 23 | 174.3 | 174.5 | 174.3 | 175.3 | 175.3 | 173.8 | 173.8 | 174.5 | 174.4 | 174.5 | 174.4 | 174.4 |
| OAc | | | | | | | 168.6, 168.8 | | 170.7 | 170.8 | 170.7 | |
| | | | | | | | 20.8, 21.7 | | 20.9 | 20.9 | 20.9 | |
| 1′ | 97.3 | 99.6 | 97.3 | 95.6 | 95.9 | 95.6 | 93.2 | 97.3 | 99.9 | 99.6 | 102.3 | |
| 2′ | 92.7 | 72.6 | 92.7 | 91.1 | 91.3 | 91.1 | 95.4 | 92.7 | 72.1 | 72.6 | 75.3 | |
| 3′ | 73.8 | 72.9 | 73.9 | 72.7 | 73.0 | 73.2 | 70.4 | 73.9 | 72.4 | 72.9 | 78.6 | |
| 4′ | 39.9 | 74.4 | 39.9 | 38.1 | 38.4 | 38.8 | 35.0 | 39.9 | 83.5 | 74.4 | 71.9 | |
| 5′ | 68.5 | 70.4 | 68.5 | 68.1 | 68.3 | 68.1 | 66.6 | 68.5 | 68.8 | 70.4 | 78.6 | |
| 6′ | 21.5 | 18.8 | 21.5 | 20.6 | 21.0 | 21.1 | 20.9 | 21.5 | 18.5 | 18.8 | 63.0 | |
| 1″ | | | | | | | | | 106.3 | | | |
| 2″ | | | | | | | | | 75.2 | | | |
| 3″ | | | | | | | | | 78.3 | | | |
| 4″ | | | | | | | | | 71.7 | | | |
| 5″ | | | | | | | | | 78.2 | | | |
| 6″ | | | | | | | | | 62.6 | | | |

*Spectra were measured in pyridine-$d_5$.
†Spectra were measured in $CDCl_3$–$CD_3OD$ (4:1).
‡According to literature [3]; data for C-2 and C-3 taken from the authentic spectrum [18].
§Spectra were measured in $CDCl_3$.
‖,¶Rearranged according to structure revision.
**Rearranged according to signal assignments in our data.

*sinaicus* stems [3] and *Asclepias vestita* [10] and for which the structure of 5,6-dehydrocalotropin has been proposed [10]. Our NMR data, however, suggested that the structure should be revised to 7,8-dehydrocalotropin. The unusual position of the double bond at C-5 is solely based on the NMR parameter of the H-7 signal: for diacetylhumistratin (2′,3′-diacetyl-7,8-dehydrocalactin), H-7 is reported to resonate at $\delta$ 5.73 (in $CDCl_3$) and to possess a half-height width ($W_{1/2}$) of 14 Hz. The NMR spectra of **6**, as well as some other doubly linked cardenolide glycosides supposed to be 5,6-dehydrocardenolides [3, 10], show H-7 at $\delta$ 6.08 (in $CDCl_3$) with $W_{1/2}$ of *ca* 9 Hz. Since the structure of humistratin has been unequivocally determined by X-ray crystallography, the differences in the chemical shift and $W_{1/2}$ of H-7 have been taken as evidence that in **6** and similar compounds the unsaturation is not at C-7 [10]. The $^{13}C$ NMR data for diacetylhumistratin and **6** agree very well. For those parts of the aglycone that are not influenced by the different sugar moieties the chemical shifts deviate by not more than $\pm 0.3$ ppm (see Table 2). Although we cannot offer an explanation for the chemical shift difference of H-7, the close similarity of the $^{13}C$ NMR data for diacetylhumistratin and **6** does not support a different position of the double bond in the compounds. Furthermore, there is additional evidence that **6** is a 7,8-dehydrocardenolide. In our $^1H$ NMR spectrum (in $CDCl_3$–$CD_3OD$; 4:1),

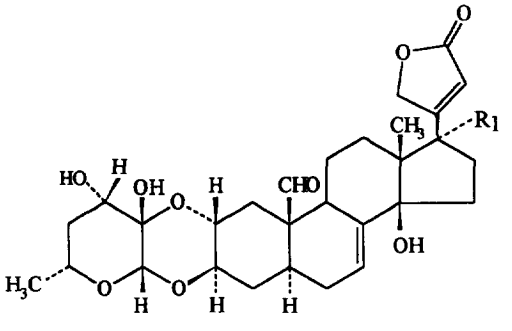
the signal for the axial H-4$\beta$ at $\delta$ 1.08 resonated as a quartet of $J = ca$ 13 Hz, suggesting a *trans*-diaxial coupling with a proton at C-5 in addition to its coupling with the axial H-3$\alpha$ and the geminal H-4$\alpha$. The H-4$\alpha$ signal at $\delta$ 1.68 was observed as a doublet of triplets of $J = 4$ and 13 Hz, revealing an equatorial–axial coupling with H-5$\alpha$ besides its equatorial–axial coupling with H-3$\alpha$ and the geminal coupling with H-4$\beta$. The presence of a proton at C-5 was further confirmed by selective decoupling experiments. Saturation of the H-3$\alpha$ signal at $\delta$ 3.80 caused the removal of one 4 Hz coupling from the signal of H-4$\alpha$ that now appeared as a doublet of doublets with $J_{4\alpha,5\alpha} = 4$ and $J_{4\alpha,4\beta} =$ 13 Hz. This saturation caused also the removal of one 13 Hz coupling from the signal of H-4$\beta$, which collapsed to a triplet with $J_{4\beta,4\alpha}$ and $J_{4\beta,5\alpha} = ca$ 13 Hz. Consequently, **6** could not possess a double bond at C-5. The whole sequence of coupling from H-1$\alpha,\beta$ via H-2$\beta$, H-3$\alpha$, H-4$\alpha,\beta$, H-5$\alpha$, H-6$\alpha,\beta$, H-7, H-9, H-11$\alpha,\beta$ to H-12$\alpha,\beta$ was observed in the $^1$H–$^1$H COSY spectrum (in pyridine-$d_5$), suggesting unsaturation at C-7. Several C-H long-range connectivities gave independent support for the suggested structure. The connectivities of C-5 to H-1$\beta$, C-9 to H-12$\beta$ and C-8 to H-11$\alpha$ confirmed the assignments of the corresponding carbon signals. It was also observed in a NOE measurement that irradiation at H-1$\alpha$ at $\delta$ 1.15 resulted in an enhancement of the peaks due to H-3$\alpha$, H-5$\alpha$ and H-9$\alpha$. From the above observations, **6** was determined to have unsaturation at C-7 and thus identified as 7,8-dehydrocalotropin. Since the NMR data for **6** are very similar to those for the other doubly linked cardenolides supposed to be 5,6-dehydroderivatives [3, 10], these compounds might also be 7,8-dehydrocardenolides.



**1** $R_1 = R_2 = H$

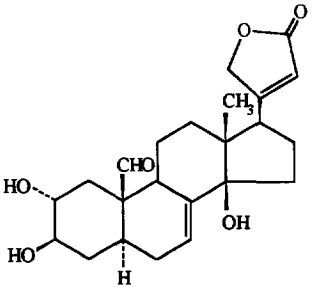
**2** $R_1 = H$, $R_2 =$ β-D-glc

**3** $R_1 = OH$, $R_2 = H$



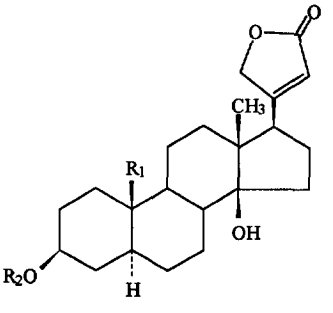
**6** $R_1 = H$

**7** $R_1 = OH$



**10**

The molecular formula of **7** was deduced to be $C_{29}H_{38}O_{10}$ by its FAB mass spectrum. Comparing the $^1$H and $^{13}$C NMR data for **7** with those for **6**, signals due to rings A, B and C of the steroidal framework and the sugar moiety were in good agreement with those of **6**. Proton signals due to the butenolide ring showed downfield shifts in comparison with those of **6** (H-22: +0.57 ppm; H-21a: +0.18 ppm; H-21b: +0.40 ppm). In the $^{13}$C NMR spectrum, the tertiary carbon signal for C-17 was shifted from $\delta$ 50.7 in **6** to 85.5 in **7**. The signals due to C-13, C-16 and C-20 were also shifted downfield (+3.7, +10.4 and +5.4 ppm, respectively) and that due to C-12 was shifted upfield (−6.5 ppm). Since these shifts were similar to those reported for 17-hydroxycardenolides [11, 12], compound **7** should be a 17-hydroxy-7,8-dehydrocalotropin. The orientation of the hydroxyl group was determined to be $\alpha$ by the correspondence of the chemical shifts of C-20 in **7** ($\delta$



**4** $R_1 = CH_3$, $R_2 =$ β-D-glc

**5** $R_1 = CH_2OH$, $R_2 =$ β-D-allomethylose

**8** $R_1 = CH_2OAc$, $R_2 =$ β-D-allomethylose (4← 1) β-D-glc

**8a** $R_1 = CH_2OAc$, $R_2 =$ β-D-allomethylose

**9** $R_1 = CH_2OAc$, $R_2 =$ β-D-glc

180.7) to 17$\alpha$-hydroxygomphoside ($\delta$ 181.1) and 17$\alpha$-hydroxycalactin ($\delta$ 180.8) and the significantly different chemical shift of C-20 in 17$\beta$-hydroxygomphoside ($\delta$ 172.8) [11]. Thus, compound **7** was determined to be the new compound 17$\alpha$-hydroxy-7,8-dehydrocalotropin.

The molecular formula of **8** was deduced to be $C_{37}H_{56}O_{15}$ based on its FAB mass spectrum, suggesting it to be a cardenolide bioside. In the $^1$H NMR spectrum, two anomeric protons were observed at $\delta$ 5.37 and 5.05, each as a 7.5 Hz doublet, suggesting the two sugars to be $\beta$-linked. A methyl signal that originated from the methyl group of a 6-deoxyhexose was also observed at $\delta$ 1.71 (*d*, $J = 6$ Hz). The $^{13}$C NMR data for the two sugar moieties were consistent with a terminal D-glucosyl and a 4-*O*-substituted D-allomethylose of which C-4 was shifted downfield in comparison to **5**. The sugar moieties were identified as glucose and allomethylose by capillary gas chromatographic comparison of their alditol acetates with authentic samples. Also, the fragment peaks in the negative FAB mass spectrum observed at *m/z* 577 $[M - \text{glucose} - H]^-$ and *m/z* 431 $[M - \text{glucose-allomethylose} - H]^-$ confirmed that glucose was the terminal sugar unit. In the $^1$H and $^{13}$C NMR spectra, **8** showed two carbon signals at $\delta$ 170.7 and 20.9 and a characteristic proton signal at $\delta$ 1.97 (3H, *s*), suggesting the existence of an acetoxyl group in this compound. The $^{13}$C NMR spectrum of **8** was similar to that of **5** in the aglycone moiety except for C-19, the signal of which was shifted downfield (+3.0 ppm) and for C-10, which was shifted upfield (−1.3 ppm). Thus, the acetoxyl group was considered to be attached to the C-19 position. This was confirmed by the NOESY spectrum where cross peaks were observed between the proton signal at $\delta$ 1.97 (acetyl protons) and each of the $CH_2$-19 at $\delta$ 4.47 and 4.22, $CH_3$-18 at $\delta$ 1.02 and one of the H-21 at $\delta$ 5.27. Furthermore, the monoglycoside 19-acetylfrugoside (**8a**), was obtained by the enzymic hydrolysis of **8**. Its NMR data were in good agreement with those reported in ref. [4]. Therefore, **8** was identifed as 19-acetylglucofrugoside.

The molecular formula of **9** ($C_{31}H_{46}O_{11}$) and the similarity of the NMR spectral data for the aglycone part with those for **8** (Tables 1 and 2) suggested the structure of a 19-acetylcoroglaucigenin monoglycoside. The sugar moiety was identified as glucose by the NMR spectrum and capillary gas chromatography of its alditol acetates. Thus, **9** was determined to be 19-acetylglucocoroglaucigenin.

Compound **10** was identical with the free cardenolide isolated from the stems of *G. sinaicus* [14] and supposed to be 5,6-dehydrocalotropagenin. The molecular formula and similarity of the NMR spectral data for **10** (Tables 1 and 2) compared to those for **6** suggested **10** to be the hitherto unknown parent genin of **6** and of humistratin [13] for which the name humistratagenin has been proposed [13].

The cardenolide content in *G. sinaicus* has been investigated by El-Askary *et al.* [5] using HPLC. Applying the same conditions on our plant extract and using **1** and **3** as standards, of the 11 compounds isolated previously, four compounds (**1**, **3**, **6** and **10**) are identical with the compounds isolated in this study. Compounds **2** and **7** revealed the same $RR_t$ (0.76 and 0.71, respectively) as two unidentified compounds cited in ref. [5]. Xysmalogenin, 15$\beta$-hydroxy-5,6-dehydrocalotropin, 16$\alpha$-hydroxy-5,6-dehydrocalotropin and 14,17-epoxy-5,6-dehydrocalotropin [3, 4] were not observed in our study. Uzarigenin and 19-acetylfrugoside were also not detected in our extract, but instead their glucosides, desglucouzarin (**4**) and 19-acetylglucofrugoside (**8**), respectively. The observed differences might be attributed to the difference in the time of collections in each case. In ref. [5] the plant was collected in May at the flowering and fruiting stage and in our study we collected the plant in October at the end of the flowering stage.

## EXPERIMENTAL

*Plant material.* Plant material used in this study was collected in October 1991 from South Sinai, Egypt, and was allowed to dry in the shade. It was identified at the St. Catherine Research Centre, Suez Canal University, Egypt, where voucher specimens of the plant are deposited.

*General.* $^1$H and $^{13}$C NMR spectra were measured with a Bruker AM 400 spectrometer operating at 400 and 100 MHz, respectively. All spectra were measured in pyridine-$d_5$ except those for **6** which were also measured in $CDCl_3$–$CD_3OD$ (4:1) and $CDCl_3$; standard: solvent peak. The chemical shifts are given in $\delta$ (ppm). Selective decoupling experiments were done in $CDCl_3$–$CD_3OD$ (4:1). Signal assignments for **1**, **5**, **6**, **8** and **9** were done based on 2D $^1$H–$^1$H COSY and $^{13}$C–$^1$H COSY. $^1$H–$^1$H connectivities were observed by COSY 45. $^{13}$C–$^1$H COSY was optimized for coupling of 150 Hz and L.R. $^{13}$C–$^1$H COSY was optimized for coupling of 8 Hz. 1D-NOE difference and 2D-NOESY 45 spectra were measured in pyridine-$d_5$. FAB-MS were recorded in the negative mode; matrix: glycerol. Optical rotations were recorded on a Perkin-Elmer 241 polarimeter at 26° in cells of path length 1 dm and vol. 1 ml. TLC was performed on precoated silica gel 60 $F_{254}$ plates (Merck) with $CHCl_3$–MeOH (4:1) (system A) and $CHCl_3$–MeOH (17:3) (system B). Cardenolides were visualized on TLC plates by spraying with Kedde reagent (1:1 mixt. of 5% 3,5-dinitrobenzoic acid in MeOH and 2 N KOH). TLC for sugars was also carried out on silica gel 60 plates with $CH_2Cl_2$–MeOH–HOAc–$H_2O$ (10:6:5:2) and the sugars were detected using thymol–$H_2SO_4$ reagent (0.5 g thymol was dissolved in 95 ml ethanol and 5 ml conc. $H_2SO_4$ added) followed by heating at 100° for 5–8 min. For CC, silica gel 60 (70–230 mesh) was used. Flash chromatography (FC) was performed under red. pres. (600–800 mbar) on a prepacked RP-18 column (25–40 $\mu$m). MPLC sepns were carried out on a home-packed Lichroprep C-18 column (15–25 $\mu$m; 500 × 26 mm) and run at

4.5 ml $min^{-1}$. The fractionation was monitored in parallel by UV detection (225 nm) and TLC. HPLC was performed on an Nucleosil 100 C-18 column (7 $\mu$m; 250 × 8 mm) and on Eurospher 100 (Si) (7 $\mu$m; 250 × 8 mm) using a Water Associates unit; detection: UV (225 nm). The solvent systems used for both MPLC and HPLC were: MeOH–$CH_3CN$–$H_2O$ (3:2:5) (system C); MeOH–$CH_3CN$–$H_2O$ (1:1:1.7) (system D); $CH_3CN$–$H_2O$ (1:3) (system E) and $CHCl_3$–MeOH–$H_2O$ (19:1:0.1) (system F). Systems C and D were optimized based on the PRISMA model using the personal computer program OPTISOLV [15, 16].

*Extraction and isolation.* The dried, ground aerial parts of *G. sinaicus* (5 kg) were percolated (3×) with MeOH and the extracts were concd to 1.5 l under red. pres. The concd extract was decanted from an insoluble wax (*ca* 50 g), diluted with $H_2O$ (3 l) and extracted with $CHCl_3$ (3 × 2 l). The wax was triturated with petrol (2 × 500 ml) and the residue was added to the $CHCl_3$ extract, which was then filtered under red. pres. through powdered charcoal (100 g). The charcoal was washed with $CHCl_3$ and the combined filtrates were concd under red. pres. to give a dark brown gum (35 g, 0.7%). The gum (15 g) in $CHCl_3$ was chromatographed on silica gel (300 g; 20 ml frs) and eluted with system B to give 5 frs, of which 3 responded to Kedde's reagent. The frs were controlled by TLC and grouped as follows: 480–1000 ml = I; 1720–2060 ml = II; 2080–4000 ml = III.

Fr. I (4 g) was again subjected to CC on silica gel (330 g, 15 ml frs) and eluted with system F to obtain the two Kedde-positive frs: 70–110 and 170–222. Fr. 70–110 (400 mg) was further purified using FC on RP-18 with 60% MeOH in $H_2O$. The eluate was concd under red. pres. and sepd by MPLC using system D to give 3 main frs: 6, 8 and 9. Frs 6 and 8 were subjected to HPLC on silica gel using system F to obtain **3** (12.6 mg) (FAB-MS, $^1H$ and $^{13}C$ NMR, [3]) and **6** (30 mg), respectively. Fr. 9 was obtained in a pure form and characterized as **1** (35.9 mg) (FAB-MS, $^1H$ and $^{13}C$ NMR, [9]). Fr. 170–220 (106 mg) was also purified by FC on RP-18 with 70% MeOH in $H_2O$. The concd eluate (68.5 mg) was subjected to HPLC on RP-18 using system E to give **7** (3.7 mg) and **10** (7.5 mg).

Fr. II (700 mg) was further subjected to CC on silica gel (70 g; 10 ml frs) and eluted with system A. Frs 20–75 were combined (561 mg) and purified by FC on RP-18 with 60% MeOH in $H_2O$. The concd eluate was then sepd by MPLC using system C to give 3 pure compounds: **2** (18.5 mg) (FAB-MS, $^1H$ and $^{13}C$ NMR, [6]), **4** (23.3 mg) (FAB-MS, $^1H$ and $^{13}C$ NMR, [7]) and **5** (18.3 mg); in addition to a fourth fr. which needed to be purified by HPLC on RP-18 using system C to obtain **9** (17.9 mg).

Fr. III (1100 mg) was chromatographed on silica gel (110 g; 8 ml frs) and eluted with system A. Frs 65–130 were combined (300 mg) and purified by FC on RP-18 with 60% MeOH in $H_2O$. The eluate was concd under red. pres. and the residue (202 mg) was sepd by MPLC using system C to give **8** (80 mg).

*Compound* **6**. Amorphous powder $[\alpha]_D^{26}$ +160 (MeOH; *c* 0.3). FAB-MS *m/z*: 529 $[M-H]^-$ and 401 $[M-\text{sugar}-H]^-$. $^1H$ and $^{13}C$ NMR: Tables 1 and 2.

*Compound* **7**. Amorphous powder $[\alpha]_D^{26}$ +60 (MeOH; *c* 0.2). FAB-MS *m/z*: 545 $[M-H]^-$. $^1H$ and $^{13}C$ NMR: Tables 1 and 2.

*Compound* **8**. Amorphous powder $[\alpha]_D^{26}$ +186.7 (MeOH; *c* 0.3). FAB-MS *m/z*: 739 $[M-H]^-$, 577 $[M-\text{glucose}-H]^-$ and 431 $[M-\text{glucose}-\text{allome}-\text{thylose}-H]^-$. $^1H$ and $^{13}C$ NMR: Tables 1 and 2.

*Compound* **9**. Amorphous powder $[\alpha]_D^{26}$ −30 (MeOH; *c* 0.3). FAB-MS *m/z*: 593 $[M-H]^-$. $^1H$ and $^{13}C$ NMR: Tables 1 and 2.

*Compound* **10**. Amorphous powder $[\alpha]_D^{26}$ + 55 (MeOH; *c* 0.12). FAB-MS *m/z*: 401 $[M-H]^-$. $^1H$ and $^{13}C$ NMR: Tables 1 and 2.

*Enzymic hydrolysis of* **2**, **4**, **8** *and* **9**. A soln of the glycoside (5–10 mg) in $H_2O$ was treated with 10–20 mg $\beta$-glucosidase (Roth, Karlsruhe) and the mixt. was stirred at 37° for 24 hr. The reaction mixt. was diluted with $H_2O$ (10 ml) and then filtered. The filtrate was shaken with $CHCl_3$ (3 × 10 ml) and the aq. layer was sepd and concd under red. pres. For **8**, the organic layer was concd to give **8a**, which was further subjected to acid hydrolysis.

*Acid hydrolysis of* **5**, **8a** *and peripalloside*. A 3–5 mg amount of the glycoside was treated with 1 ml of Killiani mixt. (3.5 ml conc. HOAc, 1.0 ml conc. HCl, 5.5 ml $H_2O$) at 100° for 3 hr. After cooling, the mixt. was diluted with $H_2O$ (5 ml) and extracted with $CHCl_3$ (3 × 5 ml). The aq. layer was then neutralized on a Dowex 1 ($OH^-$) column (10 × 1 cm) with $H_2O$ and the eluate was concd to give the free sugars.

*Identification of sugars using GC*. The sugars were determined as their alditol acetates, which were prepd according to ref. [17]. The sugars were first reduced with $NaBH_4$ soln (2 g $NaBH_4$ in 100 ml dry DMSO at 100°). Excess borohydride was destroyed by addition of 18 M HOAc. The alditols were then acetylated using $Ac_2O$ in 1-methyl-imidazole at room temp. for 10 min. The reaction mixt. was then diluted with $H_2O$ and extracted with $CH_2Cl_2$. The organic layer, which can be stored at −20° for several days, was analysed by capillary GC. GC analyses were carried out on WCOT Rtx 5 column (95% dimethyl-, 5% diphenyl-polysiloxan) 30 m × 0.32 mm, film thickness 0.25 $\mu$m; carrier gas: He, linear velocity 25 cm $sec^{-1}$; injector: 280°, split mode, split ratio: 75:1; detector: FID, 280°; operating conditions: 190° isothermal, injections of 1 $\mu$l. The retention times $R_t$ of the peaks were at 8.51 min (D-allomethylose) and 18.90 min (D-glucose).

*HPLC analysis of the cardenolides in the extract of* G. sinaicus. The samples were prepd according to ref. [5] and digoxin was used as int. standard. HPLC analysis was carried out on Eurospher 100-C18 (5 $\mu$m; 250 × 4 mm) and the cardenolides were eluted with $CH_3CN$–$H_2O$ (10–35% linear gradient in 60 min at a flow rate of 1 ml $min^{-1}$). UV detection at 220 nm [5]. A mixt. of the isolated cardenolides **1**–**10** was analysed where a 20 $\mu$l aliquot of a sample soln containing

0.15–0.25 mg of each cardenolide in 2 ml MeOH was injected.

*Acknowledgements*—Financial support by the DAAD, Bonn, Germany (to N. A.) is gratefully acknowledged. Thanks are due to Dr J. Wörth, Freiburg University, Germany, for recording the FAB-MS. We would like also to thank Prof. Dr B. Kopp, University of Vienna, Austria, for a sample of peripalloside, and Prof. Dr T. Yamauchi, Fukuoka University, Japan, for a reference sample of calotropin.

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