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A COMPARATIVE STUDY OF THE CARDENOLIDE CONTENT OF DIFFERENT ORGANS OF *GOMPHOCARPUS SINAICUS*

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Key Word Index—*Gomphocarpus sinaicus*; *Asclepias sinaica*; Asclepiadaceae; cardenolide pattern; 5,6-dehydrocalotropin.

Abstract—An examination of the various tissues of *Gomphocarpus sinaicus* revealed no remarkable differences in the cardenolide patterns of the plant organs (except seeds). 5,6-Dehydrocalotropin was found to be the main cardiac glycoside in all organs.

INTRODUCTION

Gomphocarpus sinaicus Boiss. is a toxic plant for man and animals, due to its high content of cardiac glycosides. It was reported that insects of several orders sequester cardenolides from their milk weed food plants, where the bitter nature and emetic properties of the stored compounds provide a means of protection for these insects against predators [1]. During plant collection, it has been noticed that the insects fed only on the roots and seeds by opening the pericarp to eat only the seeds of the plant. Therefore, it was considered interesting to investigate the cardenolide content of the different air-dried plant organs and to compare the contents of the same organ of the plant growing wild with that cultivated in the greenhouse. The cardenolide content in the freeze-dried leaves and stems of the cultivated plants was also studied.

RESULTS AND DISCUSSION

The results presented in Fig. 1, Fig. 2 and Table 1 show that 5,6-dehydrocalotropin (**8**) RR_t 0.84 [2] was the main cardenolide in all the investigated samples of plant organs (Fig. 2), except seeds which had another major peak (UK A, RR_t 0.67) of an unknown cardenolide, which was found mainly in seeds and was more polar than 5,6-dehydrocalotropin. The pericarp contained the largest amount of cardenolides (0.92%), followed by the leaf. The root and seed contained the least amount (about 0.3%). This explains why the insects feed only on seeds and roots of the plant.

The cardenolide patterns in the different plant organs (leaf, stem and pericarp) showed no remarkable difference. The concentration of the main glycoside, 5,6-dehydrocalotropin (about 0.3%) was nearly the same in all organs expect in the root (0.18%) which was nearly 60% of that found in other organs. The cardenolide

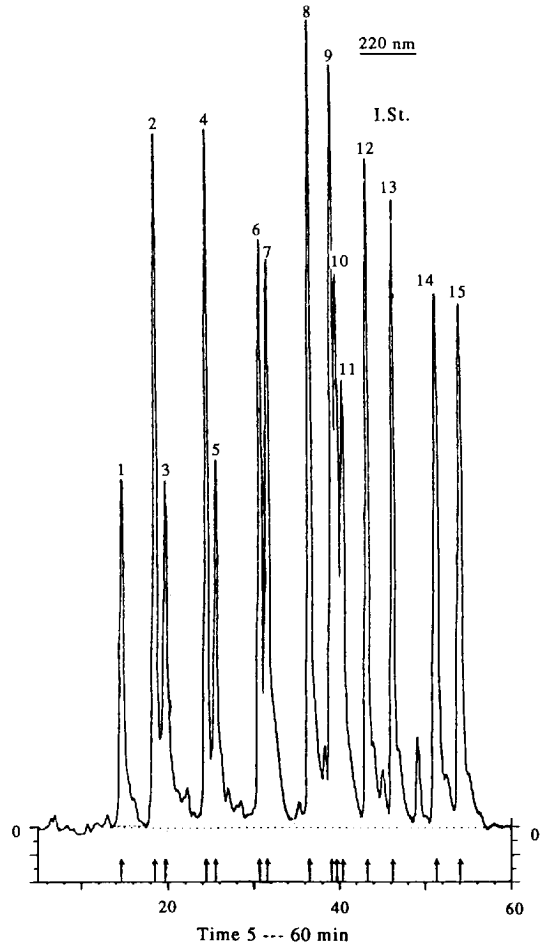

Fig. 1. Chromatogram of a mixture of isolated cardenolides from *G. sinaicus*. (1–15) = peaks of isolated cardenolides as indicated in Table 1, where a 20 μl aliquot of a sample solution containing 0.15–0.25 mg of each cardenolide in 2 ml methanol, was injected. HPLC conditions were as described in the text.

Table 1. The cardenolide content of different plant organs of *G. sinaicus* (mean value in mg 100 g^{-1} air-dried powdered drug)

Plant organ	Peak no. RR_t															
	(1) 0.34	(2) 0.43	(3) 0.46	(4) 0.56	(5) 0.59	UK A 0.67	(6) 0.71	(7) 0.73	UK B 0.76	(8) 0.84	(10)* 0.92	(13) 1.07	UK C 1.12	(14) 1.19	(15) 1.25	Total card.
Stem																
Egy.	10	12	7	12	—	17	8	45	26	323	87	13	21	21	28	629
MR.	—	—	8	21	12	—	7	89	32	315	89	–	22	9	—	604
Lyo.	—	—	9	24	10	12	—	98	27	306	88	—	18	8	12	612
Leaf																
Egy.	15	13	8	22		43	15	51	51	345	147	41	28	35	46	860
MR.	4	11	7	16	24	—	10	75	45	320	82	28	24	11	13	670
Lyo.	—	13	9	22	15	14	12	82	46	327	98	—	25	17	19	699
Pericarp																
Egy.	38	14	10	92	—	38	43	50	72	334	145	18	—	25	42	921
Root																
Egy.	—	—	7	—	—	65	19	32	—	184	8	8	–	—	22	345
Seed																
Egy.	—	—	—	—	—	275	15	—	—	26	—	–	—	—	—	316

Abbreviations: RR_t, relative retention time to digoxin; Egy., plant growing in Egypt; MR., plant cultivated in Marburg; Lyo., lyophilized organ from plant cultivated in Marburg.
(1)–(15); Peaks of the isolated cardenolides from *G. sinaicus* [2,4] arranged in order according to their elution from HPLC column (Fig. 1), using digoxin (12) as an internal standard.
(5), (6), (9) and (11): Peaks of isolated unidentified cardenolides.
UK A, B and C: Unknown cardenolides in the extracts of different plant organs.
(1), 16α-OH 5,6-Dehydrocalotropin; (2), 5,6-dehydrocalotropagenin; (3), 15 β-OH-5,6-dehydrocalotropin; (4), 15 β-OH-calotropin; (7), 3′-epiafroside; (8), 5,6-dehydrocalotropin; (10), calotropin; (13), frugoside-19-acetate; (14), xysmalogenin; (15), uzarigenin.
*This value is representative for peaks (9) and (10) together, because they eluted very near to each other.

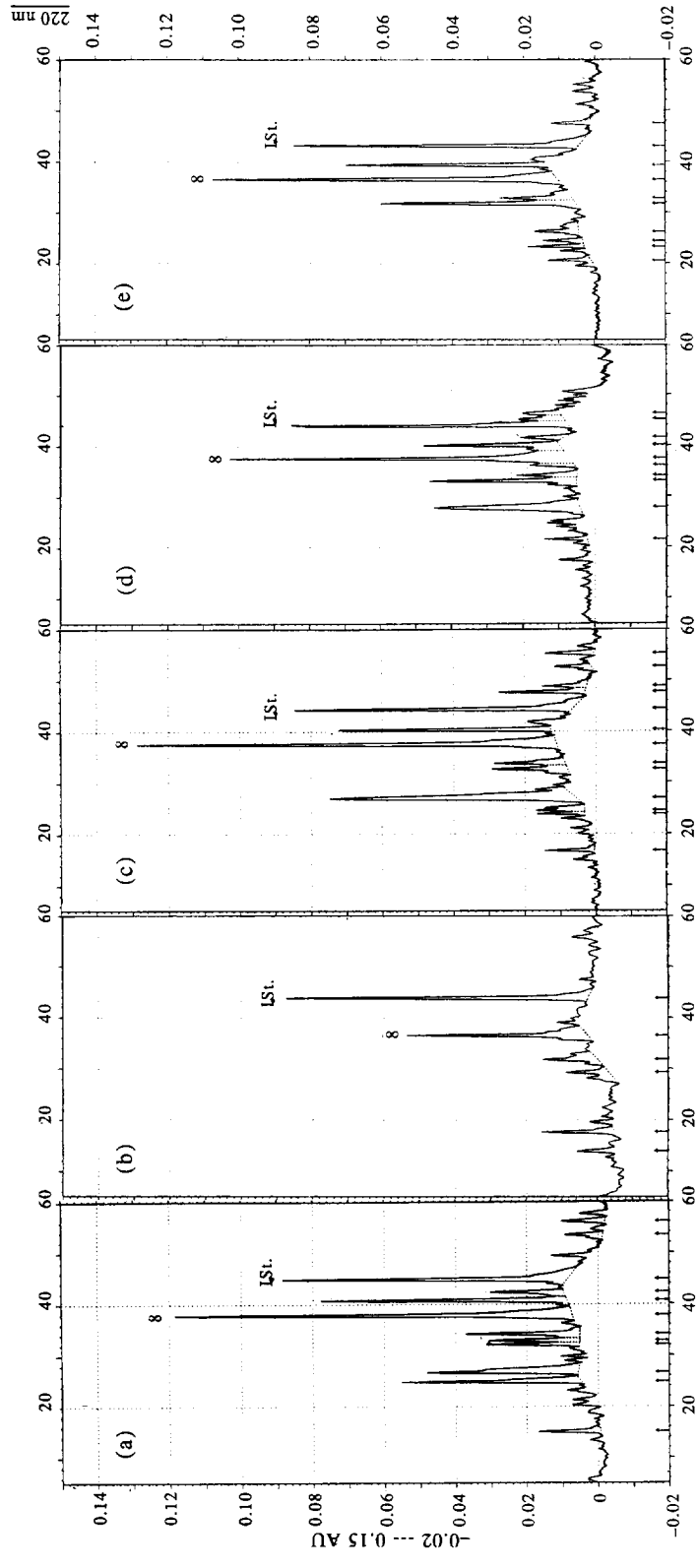

Fig. 2. HPLC chromatograms of the cardenolides in the extracts of different plant organs of *G. sinaicus* where the air-dried organs (a) pericarp Egy., (b) root Egy., (c) leaf Egy., (d) leaf MR. and the freeze-dried organ (e) leaf Lyo.

pattern in the stems and leaves of plants growing wild in Egypt (Stem. Egy. and Leaf Egy.) and that cultivated in the greenhouse in Marburg (Stem MR. and Leaf MR.), showed no significant difference. This indicates that the cardenolide composition could be genetically fixed [3]. The total cardenolide content in the wild plants (in Egypt) was slightly larger than that of the plants cultivated in the greenhouse.

No remarkable difference was noticed between the cardenolide pattern of the freeze-dried leaves and stems (Leaf Lyo. and stem Lyo.) and the respective air-dried plant organs (Leaf MR. and Stem MR.). This indicated the relative stability of most cardenolide constituents in these organs, which may be due to the doubly linked sugars [2, 4].

EXPERIMENTAL

Plant material. Samples of *G. sinaicus* Boiss. were collected from Sinai (Egypt) in May 1991 at the flowering and fruiting stage. While the cultivated plant was collected from the greenhouse in Marburg (Germany) in August 1992 at the same stage. The samples were air-dried at room temp. and then finely powdered. The freeze-dried plant organs were collected fresh from the greenhouse, lyophilized and then finely powdered.

Sample preparation for HPLC analysis [3, 5]. Each powdered organ (500 mg) was separately extracted by refluxing with 20 ml of 70% aq. MeOH on a boiling water bath for 10 min and then rapidly cooled to room temp. The extract was mixed well with 5 ml of 15% lead acetate soln. Disodium hydrogen phosphate soln (4%, 5 ml) was added and the mixt. was mixed again. It was then diluted with water to 50 ml and centrifuged for 5 min. An aliquot of 10 ml of the clear supernatant in each case was applied over an Extrelut 20-column (Merck). After 10 min the column was eluted with 80 ml of $CHCl_3$ (Lichrosolv). The eluate was evapd to dryness *in vacuo*. The residue was dissolved in 0.5 ml of MeOH (Lichrosolv) and transferred to 2 ml capacity measuring flask. Standard digoxin solution (1 ml, 0.25 mg ml^{-1}) was added as an int. standard. The soln was then completed with MeOH to 2 ml. This solution (20 μl) was used for HPLC analysis. This was carried out on a Lichrocart 125-4 cartridge column, filled with Lichrospher 100 RP18 (5 μm) (Merck), connected to two Waters 501 pumps, which were controlled by gradient control chromatography (Baseline 810), Waters 990 Photodiode Array detector which was operated at 220 mm and APCIII 990 integrator.

The cardenolides were eluted with MeCN–H_2O (10–35%) linear gradient in 60 min at a flow rate of 1 ml min^{-1} at 25°. Analysis of each sample was carried out twice, if there was deviation of the results by more than 5% of the mean value (standard deviation, %), a third analysis was carried out. The relative concentration of each compound was calculated relative to digoxin as an int. standard (I. St.) [6].

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