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GLUCOSINOLATES OF EGYPTIAN *CAPPARIS* SPECIES

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Abstract—*Capparis ovata* var *palaestina* Zoh, *C spinosa* var *aegyptia* Boiss and *C spinosa* var *deserti* Zoh, were investigated for glucosinolates Glucoiberin, glucocapparin, sinigrin, glucocleomin, glucocapangulin, glucobrassicin and neoglucobrassicin, in addition to two others, were isolated Four of these *viz* glucoiberin, sinigrin, glucobrassicin and neoglucobrassicin were detected for the first time in *Capparis* species Comparative chromatographic analyses of the glucosinolates of the plants examined revealed qualitative differences

INTRODUCTION

AN EXTENSIVE study of the glucosinolates of many *Capparis* species, although not including any from Egypt, has been carried out [1-7] Glucocapparin represents the most common glucosinolate occurring in *Capparis* species Brown and Stuart[6] stated that dried leaves of *C flexuosa* L, collected in Jamaica contained both glucocapparin and gluconorcappasalin However, in a recent work, Gmelin and Kjaer[7] identified the glucosinolate of *C flexuosa*, collected in Columbia, as benzyl-glucosinolate, a common constituent of *Cruciferae* and other families,[8] this discrepancy, may be due to differences in the material collected in Columbia and Jamaica

According to Täckholm,[9] the genus *Capparis* is represented in Egypt by 3 species and 4 varieties, however a recent revision[10] recognizes 4 species and 2 varieties A study of the carbohydrates, lipids and flavonoids of the Egyptian *Capparis* species did not reveal any qualitative differences [11] This work aims to give a comparative picture of the glucosinolates of certain Egyptian *Capparis* species (*C ovata* var *palaestina* Zoh, *C spinosa* var. *aegyptia* (Lam) Boiss and *C spinosa* var *deserti* Zoh) to help in defining their debatable taxonomic status

RESULTS AND DISCUSSION

The procedures used for the preparation of the glucosinolates include lead acetate precipitation as well as purification on both alumina and cellulose columns.[12] Treatment of the 70% methanolic extract with activated charcoal, followed by removal of rutin,

✠ Deceased 30 January 1971

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[3] A KJAER and H THOMSEN, *Phytochem* **2**, 29 (1963)
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clarified the solution and resulted in a better separation of the glucosinolates by paper chromatography Though the three procedures gave the same qualitative picture, extraction of the plants with methanol (70%) proved to be simple and efficient

The results obtained from both paper and TLC techniques showed that paper chromatography using *n*-BuOH–EtOH–H_2O (4 1 4)[3] and applying the descending technique for 3 developments gave the best separation and the number of components detected were more than those detected by TLC using different solvents [13,14]

The 9 glucosinolates of the studied species (Fig 1) were isolated by preparative paper chromatography Some constituents viz glucoiberin, glucocapparin and glucobrassicin were isolated in sufficient amounts to permit adequate examination, while others were obtainable in minute amounts ond their identification was based on chromatographic data of the glucosinolates and the thiourea adducts of their corresponding *iso*thiocyanates

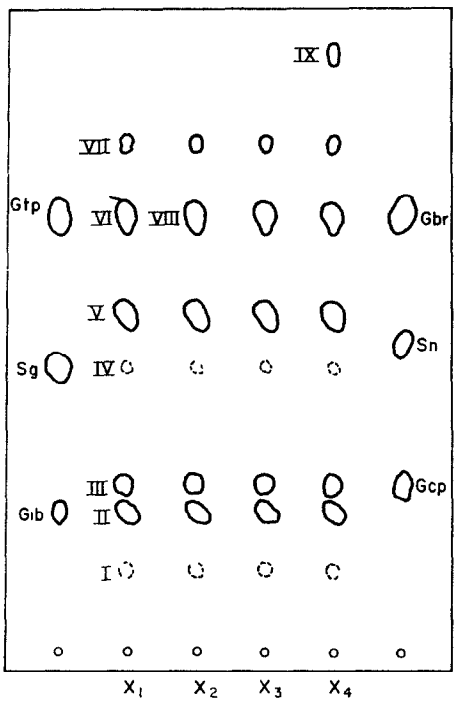

Fig 1 Descending paper chromatogram of the glucosinolates of *Capparis* species Solvent BuOH–EtOH–H_2O (4 1 4, 3 developments) Spraying reagent Ammoniacal $AgNO_3$-X 1, Glucosinolate mixture of *C ovata* var *palaestina* (New Valley) X 2, *C spinosa* var *aegyptia* (Wadi El-Rashrash) X 3, *C spinosa* var *aegyptia* (Wadi Hof) X 4, *C spinosa* var *deserti* (El-Sollum) Gib glucoiberin, Sg sinigrin, Gtp glucotropaeolin, Gcp glucocapparin, Sn sinalbin, Gbr glucobrassicin

Glucosinolate No I

It was obtained in small amounts and its identity was proved through the detection of glucose and sulphate ions in both its enzymic and acid hydrolysates as well as the detection of the liberated hydroxylamine in its acid hydrolysate

Glucosinolates Nos II and III

These components were identified as glucoiberin and glucocapparin The melting point of the glucosinolates and/or their tetraacetate derivatives as well as their R_B values (relative

[13] H Wagner, L Horhammer and H Nuffr, *Arzneimittel-Forsch* **15**, 433 (1965)
[14] E Stahl, *Dünnschicht-Chromatographie* Springer-Verlag, Berlin–Heidelberg–New York (1967)

TABLE 1 THE R_B VALUES OF THE ISOLATED GLUCOSINOLATES AND AVAILABLE AUTHENTIC REFERENCES

Glucosinolate	Paper Chromatography					TLC
	A	B	C	D	E	F
I	0 11	0 21	0 22	0 26	0 18	0 18
II	0 21	0 34	0 36	0 43	0 31	0 36
III	0 26	0 36	0 47	0 45	0 54	0 58
IV	0 57	0 61	0 59	0 75	0 73	0 72
V	0 63	0 72	0 85	0 74	0 83	0 80
VI	1 00	1 00	1 00	1 00	1 00	1 00
VII	1 01	1 05	1 04	1 08	1 72	1 72
VIII	1 00	0 81	0 94	0 95	0 64	
IX	1 23			1 27		
Glucotropaeolin	1 00	1 00	1 00	1 00	1 00	1 00
Glucoiberin	0 21	0 34	0 36	0 43	0 31	0 36
Glucocapparin	0 26	0 36	0 47	0 45	0 54	0 58
Sinigrin	0 57	0 61	0 59	0 75	0 73	0 72
Sinalbin	0 72	0 58	0 67	0 78	0 93	
Glucobrassicin	1 00	1 00	1 00	1 00	1 00	1 00

Solvents A, BuOH–EtOH–H_2O (4 1 4), B, BuOH–HOAc–H_2O (4 1 5), C, BuOH–HOAc–H_2O (4·1·3), D, (4 1 2), E, BuOH–pyridine–H_2O (6 4 3), F, BuOH–PrOH–HOAc–H_2O (3 1 1:1)

to glucotropaeolin) in different solvent systems were in accordance with those reported (Table 1) Moreover, myrosinase hydrolysis is followed by treatment of the corresponding *iso*thiocyanates with methanolic ammonia afforded the expected thiourea derivatives (iberin thiourea and methyl thiourea respectively)

Glucosinolate No IV

The component No. IV, obtained in small quantities, was identified as sinigrin by paper chromatography as well as by the preparation of the allyl thiourea.

Glucosinolate No V

It was identified as glucocleomin by paper chromatography (Table 1), and by enzymic hydrolysis and extraction of the nonvolatile *iso*thiocyanate (cleomin) with ether. The UV spectrum of the *iso*thiocyanate and the shift produced in the alkaline media agreed with those reported for cleomin

Glucosinolate No. VI

The component No. VI was identified as glucobrassicin by its R_f in different solvents, by Spring's reagent (which showed its indolic nature) as well as through the identification (TLC) of the two indole components, *viz* 3-hydroxymethylindole and 3,3′-diindolylmethane produced in its myrosinase hydrolysate The UV spectrum of the glucosinolate is identical with that of glucobrassicin The identity of the prepared tetramethyl ammonium (TMA) salt with an authentic sample (m p , m m p , IR) confirmed its identity

Glucosinolate No VII

The component No VII, obtained in small quantities, was identified as glucocapangulin by paper chromatography of the glucosinolate and its corresponding thiourea derivative.

Glucosinolate No VIII

The component No VIII of *C spinosa* var *aegyptia* and var. *deserti*, though had the same R_f and colour as glucobrassicin (VI) upon spraying with ammoniacal silver nitrate or copper sulphate, yet did not react with the specific indole reagent It was isolated by preparative paper chromatography and proved by enzymic and acid hydrolysis to be a glucosinolate of the normal type The failure of this glucosinolate to give the specific colour of indoles, the m p of its TMA salt exclude the possibility of being glucobrassicin

Glucosinolate No IX

This component is detected only in *C spinosa* var *deserti* and identified by paper chromatography as neoglucobrassicin

This is the first time that glucoiberin, sinigrin, glucobrassicin and neoglucobrassicin, have been found in the genus *Capparis*, though Sharaudolf[15] showed chromatographically the presence of two indole glucosinolates (glucobrassicin and neoglucobrassicin) in etiolated seedlings of *Cleome* species and *Gynandropsis* species (*Capparidaceae*) Gmelin and Kjaer[7] recently reported the presence of benzyl glucosinolate in some *Capparis* species and claimed this to be the first record of an aromatic glucosinolate in the family *Capparidaceae*

Of the seven glucosinolate components detected in *C ovata* var *palaestina* six viz glucosinolate No I, glucoiberin, glucocapparin, sinigrin, glucocleomin and glucocapangulin, were detected without exception in the other species studied Moreover, the detection of glucobrassicin in only *C ovata* var *palaestina* and neoglucobrassicin in only *C ovata* var *deserti*, as well as the absence of both indolic glucosinolates in *C spinosa* var *aegyptia* (collected from two localities), emphasizing the qualitative difference of the glucosinolates in the studied species, agreed with the latest taxonomic classification[10] of the Egyptian *Capparis* species, introducing a new species (*ovata*) and a new variety (*deserti*)

EXPERIMENTAL

Plant material Capparis ovata var *palaestina* Zoh was collected from Dakhla (New Valley), *C spinosa* var *aegyptia* (Lam) Boiss from two localities (Wadi Hof and Wadi El-Rashrash) and *C spinosa* var *deserti* Zoh from El-Sollum The plants were collected from its desert habitats by the end of the flowering season in March and April and were kindly authenticated by Dr M N Hadidy, Botany Dept , Faculty of Science, Cairo University

Preparation of the glucosinolates Method I—Lead method 100 g of the defatted powdered plants were extracted with MeOH and the extract concentrated *in vacuo* at 50° The remaining residue was taken with hot H_2O (200 ml), left at 0° for 24 hr, filtered from the precipitated resin, treated with 10% basic lead acetate, filtered and the pale yellow filtrate was acidified with dil H_2SO_4 and filtered The aq solution was neutralized ($BaCO_3$) and the filtrate concentrated *in vacuo* at 50° (20 ml)

Method II—Column chromatography 500 g of the fresh plant was chopped and extracted with boiling MeOH (containing $CaCO_3$) The extract was concentrated *in vacuo* at 50° to about 1 l left to stand at room temp overnight, filtered from the deposited precipitate and evaporated *in vacuo* 100 g of the greenish residue were chromatographed on acidic alumina (3 2 kg), the column was first washed with H_2O (5 l), then elution was fiected by 1% K_2SO_4 (5 l) The eluate was evaporated *in vacuo* to dryness and the residue was refluxed with MeOH 22 g of the residue (obtained after evaporation of the solvent) were rechromatographed on 700 g cellulose powder (Schleicher & Schul) and elution was carried out using the upper layer of the solvent system *n*-BuOH–EtOH–H_2O (4 1 3) The eluate was evaporated *in vacuo* at 50° and the residue was taken with H_2O (200 ml)

Method III 1 0 kg of the fresh plant was chopped and extracted with boiling MeOH (70%) The concentrated extract (1 l), after cooling and removing of the deposited substance, was diluted with MeOH to 2 l , and boiled with activated charcoal for 15 min The pale yellow filtrate was evaporated *in vacuo* and the residue was taken in hot H_2O (300 ml) filtered and left at 0° overnight The precipitated rutin[11] was filtered off and the filtrate containing the glucosinolates was concentrated (40 ml)

[15] H SCHRAUDOLF, *Experientia* **21**, 520 (1965)

Paper chromatography Whatmans No 1, using different solvent systems,[1,3, 16,17] was tried and detection of the glucosinolates was carried out by spraying with different reagents[4,18] (ammoniacal $AgNO_3$ $CuSO_4$, Sprinc's and Procházka's reagents)

Thin-layer chromatography Attempts to obtain better, or even the same, separation of the glucosinolate components than on paper, using silica gel G and different solvents[13,14] were unsuccessful. Visualization of the glucosinolates on TLC was achieved by ammoniacal $AgNO_3$ as well as trichloroacetic acid and $FeCl_3$–$K_3Fe(CN)_6$ [13]

Separation of the glucosinolates by preparative paper chromatography Sheets of Whatman No 3 MM were used with *n*-BuOH–EtOH–H_2O (4 1 4) for 3 developments each for 18 hr The chromatogram was then dried and the individual glucosinolates were cut out into strips according to the location of individual zones Each zone, obtained from about 60 chromatograms, was separately reduced to small pieces, extracted with MeOH–H_2O (30 70) and the extract concentrated *in vacuo* at 40°

Preparation of myrosinase Myrosinase was prepared by a modification of the procedure of Neuberg and Wagner[19] from *Sinapis alba* and its activity verified on sinigrin

Glucosinolate No I Attempts to crystallize out the small quantity, obtained, were unsuccessful

Acid hydrolysis of I 5 mg of I, was heated at 50° with 2 ml 20% HCl for 2 5 hr, then left at room temp overnight Glucose was detected by paper chromatography, the SO_4^{2-} by $BaCl_2$ and the liberated hydroxylamine by paper chromatography using MeOH–6 N HCl (7 3) as solvent system[20] and picryl chloride[18] as spraying reagent

Glucoiberin (II) The glucosinolate tetraacetate K salt was prepared in the usual manner (Ac_2O and pyridine at room temp for 48 hr) and had m p 145–147° (Lit [21] 145–147°)

Enzyme hydrolysis of II About 10 mg of II, dissolved in 5 ml H_2O, after adding few crystals of ascorbic acid and buffering at pH 5 6, was treated with 2 mg of the dry enzyme and left at room temp. for 18 hr Glucose and SO_4 were detected as mentioned above and the *iso*thiocyanate (iberin) was extracted with Et_2O

Iberin thiourea The Et_2O extract, after drying, gave a residue which was dissolved in 4 N methanolic NH_4OH and the mixture allowed to stand overnight The thiourea possesses the same R_f as iberin thiourea (R_f 0 44 *n*-BuOH–EtOH–H_2O (4 1 4),[22] using Grote's[23] and iodine azide starch[18] as spraying reagents)

Glucocapparin (III) The glucosinolate, after crystallization from MeOH, decomposed above 198° (Lit [21] above 198°) The prepared tetraacetate melted at 208–210° (Lit [21] 209–210°) *Methyl thiourea* The *iso*thiocyanate, obtained by the myrosinase hydrolysis, was steam distilled and the distillate was collected in ice-cold methanolic NH_4OH (4 N). The mixture, after leaving overnight, was evaporated to dryness and thiourea, after crystallization from acetone, melted at 121° (Lit [24] 121°) and had the same R_f (0 6)[22] and R_{pH} (related to phenylthiourea) (0 04 using $CHCl_3$ saturated with H_2O)[25] as methyl thiourea

Sinigrin (IV) The minute residue, obtained from the preparative paper chromatography, had the same R_f as sinigrin The thiourea derivative, prepared as mentioned above, had the same R_{pH} as allyl thiourea

Glucocleomin (V) The R_B value (0 63) in solvent A (Table 1) is the same as the reported value for glucocleomin in the same solvent [26] The *iso*thiocynate (cleomin), obtained by the myrosinase hydrolysis, after extraction with ether and purification on alumina[26] has λ_{max} 240 displaced by alkali to 231 nm (Lit [26] 240 displaced to 231 nm)

Glucobrassicin (VI) The TMA salt of VI, prepared in the usual manner[5] (30 mg in 100 ml H_2O, passed through a column of Amberlite IR-120 loaded with tetramethylammonium cation) had m.p and mixed m p 149°, and the IR spectrum was identical with that of authentic derivative *Hydrolysis of VI* 20 mg of VI, on hydrolysis with myrosinase at pH 7 afforded in addition to glucose and SO_4, two indole components (R_f 0 095 and 0 55, TLC Silica gel G, solvent $CHCl_3$ + 1% EtOH)[27] possessing the same R_f as the authentic 3-hydroxymethylindole and 3,3′ di-indolylmethane, the hydrolytic products of glucobrassicin under the same conditions

Glucocapangulin (VII). The R_f of VII, as well as of its corresponding thiourea derivative agreed with those of glucocangulin (R_{pH} 1 04, Ref 1, 1 04)

Glucosinolate VIII Glucosinolate VIII was not identified, its TMA salt melted at 194°

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Neoglucobrassicin (IX) Glucosinolate IX was identified by paper chromatography (R_f 0 42, Lit [28] 0 4–0 45, which lies between glucobrassicin and tryptophan)

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Key Word Index—Capparis, Capparidaceae, glucosinolates, chemotaxonomy