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ISOTHIOCYANATE-PRODUCING GLUCOSIDES IN SPECIES OF CAPPARIDACEAE*

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Abstract—A series of 38 botanically authenticated species of *Capparidaceae* have been analyzed by paper chromatography for their content of isothiocyanate-producing glucosides. The distribution pattern (Table 1) indicates the predominance within this family of glucocapparin and glucocleomin. The taxonomic significance and possible biogenesis of the glucosides are discussed.

INTRODUCTION

THE family Capparidaceae contains almost one thousand species divided into about 45 genera distributed through tropical and subtropical regions of both hemispheres.[1] Though widely reputed as remedies in native medicine, members of the caper family have received only scant attention chemically.

Early references[2] to unknown pungent principles in seeds of *Cleome viscosa* L. and *Gynandropsis pentaphylla* DC. were resolved when it was demonstrated in this laboratory some years ago that seed samples of several caper species contained a glucoside, glucocapparin, which gives methyl isothiocyanate on enzymic hydrolysis;[3] subsequently glucocapparin was isolated from seeds of *Cleome spinosa* Jacq. (syn. *C. pungens* Willd.).[4] Paper chromatography showed that glucocapparin was present in growing parts of *Capparis spinosa* L.[5,6] and in seeds of *Cleome pungens* Willd.[7,8]

During continued studies in this laboratory on the distribution of isothiocyanate-producing glucosides in *Capparidaceae*, two additional representatives were structurally elucidated. The minor constituent, glucocleomin, accompanying glucocapparin in many species,[3,7] was identified as a glucoside containing a 2-hydroxy-2-methylbutyl side-chain and yielding (−)-5-ethyl-5-methyl-2-oxazolidinethione on enzymic hydrolysis.[9] Another constituent, glucocapangulin, was found in the South-American species *Capparis angulata* Ruiz *et* Pav. and was shown to possess a 4-oxoheptyl side-chain.[10] In the course of analyses of about 40 other species by paper chromatography the distribution pattern of these and other glucosides of the same structural type have been determined. It is the purpose of this paper to present the results obtained together with some comments on the biogenesis and possible chemotaxonomic significance of this well-defined group comprising about 40

* Part XLV of a series of papers on naturally derived isothiocyanates. Part XLIV: *Acta Chem. Scand.* **16**, 783 (1962).

[1] F. PAX and K. HOFFMANN, in *Die natürlichen Pflanzenfamilien*. Edited by A. ENGLER and K. PRANTL. Vol. 17b, p. 146. Duncker and Humblot, Berlin (1936).
[2] G. DRAGENDORFF, *Die Heilpflanzen der verschiedenen Völker und Zeiten*, p. 260. F. Enke, Stuttgart (1898).
[3] A. KJÆR, R. GMELIN and I. LARSEN, *Acta Chem. Scand.* **9**, 857 (1955).
[4] A. KJÆR and R. GMELIN, *Acta Chem. Scand.* **10**, 335 (1956).
[5] P. DELAVEAU, *Bull. soc. botan. France* **105**, 224 (1958).
[6] A. KJÆR and R. GMELIN, Unpublished results (1955).
[7] O.-E. SCHULTZ and W. WAGNER, *Z. Naturforsch.* **11b**, 73 (1956).
[8] W. WAGNER, *Papierchromatographische Analyse der Senfölglucoside, präparative Darstellung ihrer Acetylderivate und ein Beitrag zu ihrer allgemeinen Struktur*. Dissertation, Univ. Tübingen (1956).
[9] A. KJÆR and H. THOMSEN, *Acta Chem. Scand.* **16**, 591 (1962).
[10] A. KJÆR and H. THOMSEN, *Acta Chem. Scand.* **14**, 1226 (1960).

individual compounds,[11] equally interesting with regard to their chemistry, biogenesis and botanical distribution.

RESULTS AND DISCUSSION

From the results in Table 1 it appears that glucocapparin is a widely distributed constituent of species of the caper family, frequently accompanied by glucocleomin. This

TABLE 1

Species	No.	Plant parts*	Gluco-capparin	Gluco-cleomin	Other glucosides†	Evidence‡	Reference
Boscia Fischeri Pax	1	W	+			PG	
Capparis angulata Ruiz *et* Pav.	2	S			Gcgl	I	(10)
C. cartilagenea	3	W			Gpt+Gchl	PGT	
C. galeata Fresen.	4	W(L)			Gpt+Gchl	PGT	
C. inermis Forsk.	5	S	+			PG	
C. Mitchellii Lindbl.	6	L			Gpt+Gchl	PGT,I	
C. nobilis	7	L	+			PGT	
C. ovalifolia Ruiz *et* Pav.	8	S	+			PGT	
C. quiniflora DC.	9	L	+			PGT	
C. rupestris Sibth. *et* Sm.	10	L	+			PG	(6)
C. salicifolia Griseb.	11	S			Gcsl	PG,I	(22)
C. spinosa L.	12	S(L)	+	+		PGT	(6)
C. Tuereckheimii Donn. Smith	13	S	+			PG	
C. Tweediana Eichl.	14	R	+			PG	
Cleome arabica L.	15	S	+	+		PG	(3)
C. arborea Bss.	16	S	+	+		PG	(3)
C. gigantea L.	17	S	+	+		PG	(3)
C. graveolens (Raf.) Schultes	18	S	+	+		PGT	(3)
C. integrifolia Torr. *et* Gray	19	S	+	+		PG	
C. machycarpa Raf.	20	S	+	+		PG	
C. monophylla L.	21	S	+		Gx	PG	(3)
C. ornithopodioides L.	22	S	+	+		PG	
C. pilosa Benth.	23	S	+	+		PG	
C. rosea Vahl	24	S	+			PGT	
C. speciosissima Deppe.	25	S		+		PG	(3)
C. spinosa Jacq.	26	S	—	+		I	(4, 9)
C. trachysperma (Torr. *et* Gray) Pax *et* K. Hoffm.	27	S	—	—		PG	(3)
C. viscosa L.	28	S	+	—		PG	(3)
Crataeva Roxburghii R. Br.	29	B(L)	+			PGT,I	(23)
C. Tapia L.	30	S	+	+		PGT	
Gynandropsis gynandra (L.) Briq.	31	S	+			PG	(3)
G. speciosa (H.B.K.) DC.	32	S	+			PG	
Maerua aethiopica (Fenzl.) Oliv.	33	S(R,L)	+			PGT	
M. Hoehnelii Schweinf.	34	B(R,L)	+	(+)		PG	
M. pubescens (Klotzsch) Gilg	35	B(L)				PG	
Ritchiea Albersii Gilg	36	B(R)	+			PGT	
Thylachium africanum Lour.	37	R	+			PGT	
T. Thomasii Gilg	38	B(R,L)	+			PG	

* The following abbreviations are used: B, bark; L, leaves; R, root; S, seeds; W, wood. Symbols in parentheses indicate that the corresponding plant parts have been separately analysed with the same results.

† The following symbols are used: Gcgl, glucocapangulin;[10] Gpt, glucoputranjivin;[11] Gchl, glucocochlearin;[11] Gcsl, glucocappasalin plus unknown glucoside;[22] Gx, unknown glucoside.

‡ PG indicates: paper chromatographic analyses of glucosides; PGT indicates: paper chromatography of thiourea-derivatives of isothiocyanates; I indicates: isolation of glucoside or thiourea.

[11] A. KJÆR, *Fortschr. Chem. Org. Naturstoffe* **18**, 122 (1960).
[22] A. KJAER and H. THOMSEN, *Acta Chem. Scand.* **16**, 2065 (1962).
[23] A. KJÆR and H. THOMSEN, *Acta Chem. Scand.* **16**, 783 (1962).

chemical pattern is well in line with the commonly accepted phylogenetic alliance of *Capparidaceae* with families such as *Cruciferae, Moringaceae* and *Resedaceae*, all of which contain isothiocyanate-producing glucosides and myrosinase cells. As recently pointed out by Hegnauer,[12] this distribution of structurally similar glucosides, as well as of myrosinase cells, is contrary to the recent proposal by Hutchinson[13] that *Capparidaceae* and *Moringaceae* on the one hand and *Papaveraceae, Cruciferae* and *Resedaceae* on the other should be of fundamentally different ancestry. The occurrence of this characteristic type of glucosides in all families of the order *Rhoeadales* (*sensu* Wettstein) except *Papaveraceae* supports the opinion expressed by Takhtajan[14] that the derivation of *Papaveraceae* is entirely different from that of *Capparidaceae, Cruciferae, Moringaceae* and *Resedaceae*, which together constitute the natural order *Capparidales*. It should not be forgotten, however, that the application of a single chemical criterion as here to taxonomic discussions calls for caution, and that the faculty to produce even a rather unique structural type of compound is only one of numerous abilities of a given species. However, Hegnauer[13] provided further support for Takhtajan's order *Capparidales* by parallel considerations of the contents of fatty acids and sinapine in the various families discussed.

It appears striking and may be taxonomically significant that glucocapparin and glucocleomin, the dominant caper constituents, have not been encountered with certainty in any of about 400 cruciferous species analyzed thus far in this laboratory.* Surprisingly, glucocleomin was recently discovered, together with other glucosides, in seed kernels of the Indian tree *Putranjiva Roxburghii* Wall. (*Euphorbiaceae*).[15]

The appearance in a few caper species (Nos. 3, 4 and 6) of glucoputranjivin and glucocochlearin, containing an isopropyl and optically active *sec*-butyl side-chain, respectively, parallels the simultaneous occurrence of these two glucosides in several crucifers and suggests a common biogenetic pathway, probably involving intermediates in the metabolism of valine and isoleucine.[15] However, the number of caper species in the present investigation is too limited to permit any evaluation of the possible taxonomical significance of these branched chain glucosides. It is noteworthy that neither the latter nor the two long, unbranched side-chain glucosides in the South-American *Capparis* species (Nos. 2 and 11) are accompanied by glucocapparin or glucocleomin in any of the species studied.

Biogenetically all glucosides of the caper family seem derivable from acetate units, either obviously as in glucocapparin and glucocapangulin, or, more implicitly, in the branched side-chain glucosides and glucocappasalin. It may be significant in this connexion that flower buds of *Capparis spinosa* L. are one of the earliest known sources of rutin[16] (*cf.* also [17]), now known to be biosynthesized from cinnamic acid and acetate equivalents.[18] The consistently high fat contents of seeds of the caper family is presumably another reflection of a lively acetate-condensing activity. It is hoped that continued investigations will clarify details in the biosynthetic mechanisms operating in the production of constituents of the caper family. (See note added in proof at end of paper.)

* S. B. BAILEY, M. L. BAZINET, J. L. DRISCOLL and A. I. MCCARTHY, *J. Food. Sci.* **26**, 163 (1961) list, without experimental details, methyl isothiocyanate as a trace constituent of fresh cabbage.

[12] R. HEGNAUER, *Planta Medica* **9**, 37 (1961).
[13] J. HUTCHINSON, *The Families of Flowering Plants*, Vol. 1, University Press, Oxford (1959).
[14] A. TAKHTAJAN, *Die Evolution der Angiospermen*. Gustav Fischer Verlag, Jena (1959).
[15] A. KJÆR and P. FRIIS, *Acta Chem. Scand.* **16**, 936 (1962).
[16] ROCHLFDER and HLASIWETS, *Liebigs Ann. Chem.* **82**, 197 (1852).
[17] S. KANTHAMANI, C. R. NARAYANAN and K. VENKATARAMAN, *J. Sci. Ind. Research (India)* **19B**, 409 (1960).
[18] H. GRISEBACH and W. D. OLLIS, *Experientia* **17**, 4 (1961).

EXPERIMENTAL

Botanical Materials

The collection of the species listed in Table 1 presented considerable difficulties and was rendered possible only through the good offices of several individuals and institutions. It is believed that the material presented in Table 1 is essentially correct as to botanical identity. Most species were collected in the field by botanists and their identity confirmed using herbarium material, when necessary by specialists. In several cases the same species were obtained under synonymous names; such duplications have been omitted from Table 1.

Seed material was ground in a mortar before extraction whilst leaf, bark and root material was disintegrated in 70% methanol using a Waring Blendor.

Analyses

Paper chromatography of the isothiocyanate-producing glucosides in 70% methanol extracts of disintegrated plant materials was performed essentially as described by Schultz and Wagner.[7,8] Schleicher and Schüll paper No. 2043b was used for descending one-dimensional chromatograms in both solvent systems employed, viz. (*i*) butanol : ethanol : water (4 : 1 : 4) and (ii) butanol : pyridine : water (6 : 4 : 3). The chromatograms were sprayed with ammoniacal silver nitrate (0·2 g $AgNO_3$ and 5 ml conc. NH_3, diluted with methanol to 100 ml), heated for 3–5 min at 120°, sprayed again in the same way, and finally dipped through nitric acid (1 *N*). After thorough washing of the paper with water the glucosides appeared as gray-violet spots on a yellowish background. Glucotropaeolin and other appropriate authentic glucosides were co-chromatographed as reference compounds.

In several cases the glucoside pattern was confirmed by paper chromatography of the thioureas derived from the isothiocyanates produced by enzymic hydrolysis of the parent glucosides. The enzymic fission was performed on the original glucoside extracts, after these had been freed of methanol, by adding citrate buffer to pH 5·5, a trace of ascorbic acid,[19] and a cell-free solution of myrosinase.[20] The isothiocyanates produced were steam-distilled or extracted with ether and converted into thioureas by reaction with methanolic ammonia. Paper chromatography of the thioureas was performed on Whatman paper No. 1 according to the procedure earlier described from this laboratory.[21] For thioureas with too low R_{Ph}-values[21] in water-saturated chloroform, the upper layer of butanol : ethanol : water (4 : 1 : 4) was used as the mobile phase. Appropriate synthetic thioureas served as references on the chromatograms.

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[19] M. G. ETTLINGER, G. P. DATEO, Jr., B. W. HARRISON, T. J. MABRY and C. P. THOMPSON, *Proc. Natl. Acad. Sci. Wash.* **47**, 1875 (1961).
[20] C. NEUBERG and J. WAGNER, *Biochem. Z.* **174**, 457 (1926).
[21] A. KJÆR and K. RUBINSTEIN, *Acta Chem. Scand.* **7**, 528 (1953).

Added in proof—L. C. MATAS (*Farmacognosia* (Madrid) **20**, 307 (1960)) recently provided evidence for the presence of the crucifer *Matthiola tristis* (L.) R. Br.