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GLUCOSINOLATES IN THE CARICACEAE

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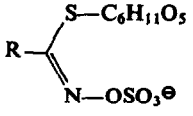
Abstract—Five species of the family Caricaceae were examined for glucosinolates. Like *Carica papaya* L., they all contained benzylglucosinolate (I, R = $C_6H_5CH_2$) as the sole thioglucoside.

INTRODUCTION

THE FAMILY Caricaceae, indigenous to tropical America and Africa, comprises about fifty species, usually arranged in four to five genera.[1] Apart from the species *Carica papaya* L., widely cultivated throughout the tropics, the family has attracted only scant chemical interest.[2]

In connexion with current studies on the distribution of glucosinolates (I) in the plant kingdom, we report the results of an examination of seeds of five species of the family Caricaceae for glucosinolates. Previously, Ettlinger and Hodgkins[3] demonstrated that seed of papaya (*C. papaya* L.), on maceration in water or an organic solvent, afforded benzyl isothiocyanate (II), the expected enzymic hydrolysis product of benzylglucosinolate (I, R = $C_6H_5CH_2$ (cf. e.g. Ref. 4).



(I)

$C_6H_5CH_2—N=C=S$

(II)

RESULTS AND DISCUSSION

Seeds, collected in the wild, of four *Carica* species, viz. *C. cauliflora* Jacq., *C. chilensis* (Planch.) Solms-Laub., *C. pennata* Heilb., and *C. quercifolia* (St. Hil.) Solms-Laub., and of one species of the genus *Jarilla* (syn. *Mocinna*), viz. *J. chocola* Standl. were subjected to analysis for glucosinolates by traditional methods (cf. e.g., Ref. 5). Paper chromatography, in two solvents, of methanolic extracts of the five seed specimens clearly showed the presence in all extracts of only one glucosinolate, indistinguishable, by its R_f, from authentic benzylglucosinolate.

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[1] H. HARMS, in *Die Natürlichen Pflanzenfamilien* (edited by A. ENGLER), 2 Aufl., Vol. **21**, pp. 510–522, W. Engelmann, Leipzig (1925).

[2] R. HEGNAUER, *Chemotaxonomie der Pflanzen*, Vol. III, pp. 373–377. Birkhäuser Verlag, Basel und Stuttgart (1964).

[3] M. G. ETTLINGER and J. E. HODGKINS, *J. Org. Chem.* **21**, 204 (1956).

[4] M. G. ETTLINGER and A. KJÆR, in *Recent Advances in Phytochemistry* (edited by T. J. MABRY, R. E. ALSTON and V. C. RUNECKLES), Vol. 1, p. 58, Appleton-Century-Crofts, New York (1968).

[5] A. KJÆR, *Fortschr. Chem. Org. Naturstoffe* **18**, 122 (1960).

From seeds of *C. cauliflora* and *C. pennata*, available to us in larger quantities, crystalline tetramethylammonium benzylglucosinolate was isolated and unequivocally identified by comparison with an authentic specimen.

On addition of a crude myrosinase preparation to suspensions in aqueous buffer of the defatted seed powders, all five developed the characteristic smell of benzyl isothiocyanate (II). In the case of *C. cauliflora* and *J. chocola* the liberated mustard oil was unambiguously identified as (II) by reaction with ammonia and identification of the reaction product as benzylthiourea.

The finding of benzylglucosinolate (I, $R = C_6H_5CH_2$) in five species of Caricaceae, additional to the previously studied *C. papaya* L.,[3] suggests that this thioglucoside may be characteristic for the family. More species will be needed, however, to substantiate the suggestion. Benzylglucosinolate is frequently encountered in species of the family Cruciferae,[4,5] and has been recently established also as a constituent of a member of the related Capparidaceae.[6] More interesting, however, is the frequent appearance of benzylglucosinolate in families carrying no obvious phylogenetic relationship to each other or to those constituting the order Rhoeadales. Such families, with reported occurrence of benzylglucosinolate, are: Moringaceae, Salvadoraceae, Tropaeolaceae, and, possibly, Gyrostemonaceae.[4]

EXPERIMENTAL

Plant Materials

The *Carica* seed material employed in the present investigation was collected in the wild by Dr. D. K. Cox in Mexico (*C. cauliflora* and *C. pennata*), and by Dr. J. P. Hjerting in Chile (*C. chilensis*) and Argentina (*C. quercifolia*). Seeds of *Jarilla chocola* were kindly placed at our disposal by Dr. I. A. Wolff, U.S. Dept of Agriculture, Northern Utilization Research and Development Division, Peoria, Illinois, U.S.A.

Paper Chromatographic Analysis

1-g portions of defatted seed powder were extracted by boiling with 70 per cent methanol (10 ml). 10-μl aliquots of the filtered extracts were chromatographed ascendingly on Schleicher and Schüll No. 2043b paper in (i) butanol:ethanol:water (4:1:4, upper phase), and (ii) butanol:pyridine:water (6:4:3), with tetramethyl benzylglucosinolate as a reference compound. The dried chromatograms were dipped into $AgNO_3$ in aqueous acetone [0·75 g, dissolved in water (10 ml), and diluted to 400 ml with acetone] and dried. They were then sprayed on both sides with 5 per cent NaOH in methanol. After 5 min, the glucosinolates appeared as dark-brown spots. The papers were washed in dilute HNO_3 and water, and finally dried at 100°. On all chromatograms one spot was observed, indistinguishable from that of benzylglucosinolate.

Isolation of Benzylglucosinolate

Seeds of *C. cauliflora* (100 g) were milled and defatted with two 1-l portions of petroleum ether. The seed powder was extracted with two 1-l portions of 70 per cent methanol, and the extract was concentrated *in vacuo* to a small volume. The residue was dissolved in water (500 ml), the solution filtered through Celite, and then passed slowly through a column containing anionotropic alumina (Woelm, 100 g). The column was rinsed with water (100 ml), and the glucosinolate was eluted with a 1 per cent solution of tetramethylammonium hydroxide (150 ml). After evaporation to dryness *in vacuo*, the residue was dissolved in hot 90 per cent ethanol, the solution filtered hot through Celite, and set aside for crystallization. After standing at +4° overnight the crystals were filtered off, washed with anhydrous ethanol and ether, and dried (270 mg). A pure specimen (210 mg) was produced after two subsequent recrystallizations from 90 per cent ethanol, m.p. 192–194° (uncorr., decomp.). The salt exhibited an i.r. spectrum coinciding with that of tetramethylammonium benzylglucosinolate.

The isolation of the same glucosinolate from seed of *C. pennata* proceeded in the same way.

Isothiocyanate from Seeds of Jarilla chocola

The residue from a methanolic extract of 25 g of powdered, defatted seeds of *J. chocola* was dissolved in a citrate buffer (200 ml, pH 6·4). The solution was filtered through Celite, and a crude myrosinase solution

[6] R. GMELIN and A. KJÆR, *Phytochem.* 9, 601 (1970).

(5 ml) and ascorbic acid (10 mg) were added. After 4 hr, the turbid reaction mixture was extracted with ether, the ether solution was washed twice with 1 N NaOH and water, and finally dried. Excess methanolic ammonia was added to the solution; after 20 hr at room temperature, the solution was taken to dryness, and the crystalline residue was recrystallized from dilute ethanol to give colourless crystals (67 mg), m.p. 161–162°, alone or in admixture with authentic 1-benzylthiourea. The i.r. spectrum was indistinguishable from that of authentic 1-benzylthiourea.

The production of 1-benzylthiourea from extracts of seed of *C. pennata* proceeded similarly.

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