Phytochemistry, 1972, Vol. 11, pp. 2531 to 2533. Pergamon Press. Printed in England.

SHORT COMMUNICATION

BENZYL ISOTHIOCYANATE IN THE CARICACEAE*

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(*Received* 21 *September* 1971)

Key Word Index—*Carica*; *Jarilla*; *Jacaratia*; Caricaceae; chemotaxonomy; benzyl isothiocyanate; content.

Abstract—GLC quantitation of benzyl isothiocyanate (BITC) in 1–2 macerated seeds of Caricaceae is described. In *Carica* and *Jarilla* genera, the content of this compound ranges from 1·37 to 1·96%. In seeds of *Jacaratia*, however, only 2–4 ppm of BITC was found. The striking quantitative differences suggest the possible use of BITC content as a chemotaxonomic criterion.

INTRODUCTION

CARICACEAE is a family of dicotyledonous angiosperms made up of four genera: *Carica*, *Jacaratia*, *Jarilla* and *Cylicomorpha*. Badillo[1] considers that there are 30 valid species: 21 in *Carica*, 2 in *Cylicomorpha*, 6 in *Jacaratia* and 1 in the genus of *Jarilla*. In the early literature, the genus *Jacaratia* was often not differentiated from *Carica*; for example, *Jacaratia mexicana* was classified as *Carica mexicana* as recently as 1961.[2] Lack of agreement in taxonomy and excessive synonomy in Caricaceae prompted this study of benzyl isothiocyanate (BITC) distribution in the different genera.

Distribution of isothiocyanates in the plant kingdom has been reviewed.[3,4] Gmelin and Kjær[5] recently reported benzylglucosinolate, the precursor of BITC, to be the only glucoside in *Carica* and *Jarilla*, suggesting that this compound could be characteristic of the family. It was pointed out, however, that more species should be tested to verify this theory. GLC quantitation of BITC in *Carica papaya* L. has been reported by Tang.[6] A simplified experimental procedure is described and the significance of quantitative differences of BITC in *Carica*, *Jacaratia* and *Jarilla* are discussed.

RESULTS AND DISCUSSION

BITC is invariably the dominant volatile component in macerated embryos and endosperms of mature *Carica* and *Jarilla* seeds. This compound can be quantitatively determined with a GLC equipped with flame-ionization detectors.[6] Table 1 indicates that levels of BITC

* Journal series No. 1363 of the Hawaii Agricultural Experiment Station.

[1] V. M. BADILLO, *Agronomia Tropical* **17**, 245 (1967).
[2] L. O. WILLIAMS, *Fieldiana (Bot.)* **29**, 368 (1961).
[3] A. KJÆR, *Chemical Plant Taxonomy* (edited by T. SWAIN), p. 453, Academic Press, London (1963).
[4] A. KJÆR, *Comparative Phytochemistry* (edited by T. SWAIN), p. 187, Academic Press, London (1966).
[5] R. GMELIN and A. KJÆR, *Phytochem.* **9**, 591 (1970).
[6] C. S. TANG, *Phytochem.* **10**, 17 (1971).

in macerated embryos and endosperms of *Carica* seeds (wet wt. basis) ranged from 1·96% in *C. papaya* L. to 1·37% in *C. pubescens*; in *Jarilla heterophylla*, 1·76% of BITC was found. However, in the 3 available species of the genus *Jacaratia*, BITC was not detected using the same experimental procedure.

TABLE 1. CONTENTS OF BENZYL ISOTHIOCYANATE IN MACERATED EMBRYO AND ENDOSPERM OF CARICACEAE

Plant species	Concn of benzylisothiocyanate (%)*
Carica cauliflora	1·72
C. goudotiana	1·84
C. horovitziana	1·86
C. monoica	1·85
C. papaya	1·96
C. pubescens	1·37
Jarilla heterophylla	1·76
Jacaratia corumbensis	0†
J. mexicana	0
J. spinosa	0

* Concentration based on the wet wt of embryo and endosperm. Standard deviation within ±10%.

† Benzyl isothiocyanate was not found with the GLC-flame ionization detection system. With the GLC-flame photometric detection system, 2–4 ppm of BITC in all *Jacaratia* samples were estimated.

Jacaratia samples were subsequently examined on a GLC equipped with a flame-photometric detector, which responds selectively to sulfur-containing compounds.[7] Gas chromatograms indicate that BITC was indeed produced in amounts ranging from 2 to 4 ppm. An unknown GLC peak with a retention time of 2·5 min was also found in all 3 species of *Jacaratia*, but not in *Carica* and *Jarilla* samples, suggesting possible existence of a volatile sulfur compound in addition to BITC in *Jacaratia*.

The contents of isothiocyanates of individual plants are probably influenced by environmental variations, including availability of sulfur and nitrogen as nutrients.[8] As a consequence, differentiation of *Carica* and *Jarilla* based on BITC contents is not practical because both genera possess similarly high levels. The very low trace amount of BITC in *Jacaratia*, approx. 1/6000th of that of *Carica* or *Jarilla*, however, suggests that the difference is of genetic rather than environmental origin and may therefore be a useful chemotaxonomic parameter.

From the present study, it is clear that *J. mexicana* contains only trace amount of BITC, which supports its classification as a species of *Jacaratia* rather than of *Carica*.

EXPERIMENTAL

Seed materials. Mature seeds of the following species of the family Caricaceae were obtained from the collection of the Department of Horticulture, University of Hawaii: *Carica cauliflora* Jacq. Pl.; *C. goudotiana* (Tr. et Planch.) Solms; *C. horovitziana* Badillo; *C. monoica* Desf.; *C. papaya* L.; *C. pubescens* Lenne et Koch; *Jacaratia corumbensis* Kuntze; *J. mexicana* A. DC.; *J. spinosa* (Aubl.) A. DC.; and *Jarilla heterophylla* (erv.

[7] S. S. BRADY and J. E. CHANEY, *J. Gas Chromatogr.* **4**, 42 (1966).
[8] E. JOSEFSSON, *J. Sci. Food Agric.* **21**, 98 (1970).

ex La Llave) Rusby (syn. *J. chocola*). The seed samples were collected in the wild from Central and South America except that of the *C. papaya*, which was obtained from a local source. All seeds were still viable, having been stored below 4° for less than 2 yr.

Sample preparation. One or two seeds were used in each analysis. Seed coats were removed and the embryo and endosperm weighed immediately on an analytical balance. These kernels were then homogenized thoroughly in 1 ml of citrate–phosphate buffer, pH 6·4, with a Duall tissue grinder. The sample was then transferred to a culture tube with a Teflon lined cap. 1 ml of crude myrosinase solution[9] was used to rinse the tissue grinder followed by another rinse with 1 ml of the same citrate–phosphate buffer and the rinses were added to the culture tube. The combined mixture was incubated at room temp. for 1 hr and then extracted by adding 6 ml of redistilled $CHCl_3$ and shaking vigorously. The two phases were separated in a low speed centrifuge and the lower phase was used for GLC quantitation without any further 'cleanup' process.[6]

For the *Jacaratia* samples, about 10 seeds with seed coats removed were homogenized with 3 ml of the citrate–phosphate buffer. The homogenate was then combined with 1 ml of crude myrosinase solution and 1 ml of buffer solution. Samples were incubated at room temp. for 1 hr. 20 ml $CHCl_3$ was used for extraction and the lower $CHCl_3$ layer was concentrated under a jet of N_2 gas to 0·1 ml for GLC analysis. In a control experiment, 20 ml of 10 ppm BITC–$CHCl_3$ solution was reduced to 0·1 ml by a jet of N_2 gas and then the volume was restored to 20 ml. GLC analysis of this sample indicated no significant change in its original concentration. Due to the high sensitivity of the flame photometric detector to all volatile sulfur compounds, extreme care was taken to avoid contamination.

GLC. Quantitative analyses of BITC in $CHCl_3$ extracts of *Carica* and *Jarilla* samples were performed on a Varian 1800 Gas Chromatograph equipped with dual flame ionization detectors containing 2·5 mm × 3 m stainless steel column packed with 3% OV-17 on 80–100 mesh Chromosorb G, AW-DMCS solid support. The column temp. was 190° and the flow rate of N_2 was 25 ml/min; H_2, 25 ml/min; and air, 300 ml/min. The peak height of each sample was measured and the quantity of BITC in the original calculated by means of a standard curve prepared from authentic BITC. Average values for duplicate experiments are listed in Table 1. For the *Jacaratia* species tested, the concentrated $CHCl_3$ extract was injected on a Bendix 2500 Gas Chromatograph equipped with a Tracor Flame Photometric Detector and a 6 mm × 2 m glass column containing the packing material previously described. The column temperature was 175°, flow rates; N_2 was 40 ml/min; H_2, 180 ml/min; air, 300 ml/min. The amounts of BITC in *Jacaratia* samples were estimated by comparison with the amount of authentic BITC required to give a peak height equal to that of the sample. This practice was adopted due to the non-linear response of the flame photometric detector to sulfur containing compounds in the range used in this experiment.

Acknowledgement—The authors are grateful to the partial support of the present investigation by the Biomedical Science Support, PHS No. 5, FR07026-4, 1969, University of Hawaii.

[9] I. Tsuruo, M. Yoshida and T. Hata, *Agric. Biol. Chem.* **31**, 18 (1967).