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# BENZYL ISOTHIOCYANATE OF PAPAYA FRUIT*

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**Abstract**—The amounts of benzyl isothiocyanate in macerated papaya fruit pulp and seeds were determined by GLC. The concentration of benzyl isothiocyanate decreased in the pulp but increased in the seeds as maturity of the fruit progressed. Free benzyl isothiocyanate was detected in both the wax layer and the vapor which emanated from intact green papayas. This implies that benzyl isothiocyanate was a normal metabolite in green immature papaya.

## INTRODUCTION

BENZYL isothiocyanate (BITC) is found in enzymic hydrolysates of extracts from various plant families; namely, *Cruciferae*, *Moringaceae*, *Capparidaceae*, *Tropaeolaceae*, *Caricaceae*, *Gyrostemonaceae* and *Salvadoraceae*.[1] The presence of BITC in papaya (*Carica papaya* L.) seeds was confirmed by Ettlinger and Hodgkins,[2] concentrations as high as 2000 to 5000 ppm were found in macerated dry seed preparations.

Considerable work has been reported on the identification of various isothiocyanates in different plants but relatively little attention[3,4] has been given to the distribution of these compounds among various tissues through different stages of growth. In the past decade, gas–liquid chromatography (GLC) has been used for the analysis of isothiocyanates. Youngs and Wetter[5] determined quantities of the major isothiocyanates in 5–20 mg of rapeseed with solvent extraction followed by GLC. If proper procedures are used, GLC should be a highly effective tool for quantitative studies of volatile isothiocyanates from plant materials. In the present work, the change of concentration of BITC in papaya pulp and seeds of different maturities was examined by this procedure.

Isothiocyanates are generally considered to be liberated from their parent glucosinolates by the enzyme myrosinase upon injury of plant tissues. There is no direct experimental evidence to indicate the existence of free isothiocyanates in intact plant tissues and efforts have been made in this study to determine whether BITC is a normal metabolite in green papaya fruit.

## RESULTS

Benzyl isothiocyanate was isolated and purified from the papaya seeds or pulp by GLC. Identification was based on matching infrared (i.r.) spectra and retention times on three GLC columns (OV17, SE30 and 5% DC200 + 7·5% QF-1) with the authentic compound.

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[1] M. G. ETTLINGER and A. KJAER, *Recent Advances in Phytochemistry* (edited by T. J. MABRY, R. E. ALSTON and V. C. RUNECKLES), Vol. I, p. 125, Appleton-Century-Crofts, New York (1968).
R. GMELN and A. KJAER, *Phytochem.* **9**, 601 (1970).

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

[3] A. KJAER, *Progr. Chem. Org. Nat. Prod.* **18**, 122 (1960).

[4] E. JOSEFSSON, *Phytochem.* **6**, 1617 (1967).

[5] C. G. YOUNGS and L. R. WETTER, *J. Am. Oil Chemists' Soc.* **44**, 551 (1967).

*Quantitative Measurement of BITC*

The quantity of BITC in the pulp or seeds of papaya fruit was determined by comparing the peak heights on gas chromatograms with a standard curve. Concentrations of BITC in a representative set of papayas are shown in Table 1. The papayas were collected from the same tree at the same time, but of different weights and presumably representative of different stages of development. The concentration of BITC increases in seeds and decreases in the pulp during growth, and the seeds at all stages have higher concentrations of BITC than the pulp. When the papaya fruit is fully ripe, the seeds usually contained more than 500 times the concentration of BITC in the pulp.

Incubation of macerated green papaya pulp results in a 2–5-fold increase of free BITC, but repeated tests with fresh seeds at all stages of development have failed to show a similar increase.

*Detection of Free BITC in the Wax Layer and Vapor Phase*

The cuticle wax of carefully selected uninjured green papaya was stripped by briefly dipping the fruit in ethyl ether. After removal of the ether, benzyl isothiocyanate was found as a minor component. When the vapor expelled from green, intact papayas was trapped by adsorption on activated charcoal, trace amounts of BITC were also detected. A comparison of gas chromatograms of BITC in the above mentioned samples with a chromatogram of authentic compound is shown in Fig. 1. No quantitative measurements were attempted in these experiments because of the minute amounts of BITC in these preparations.

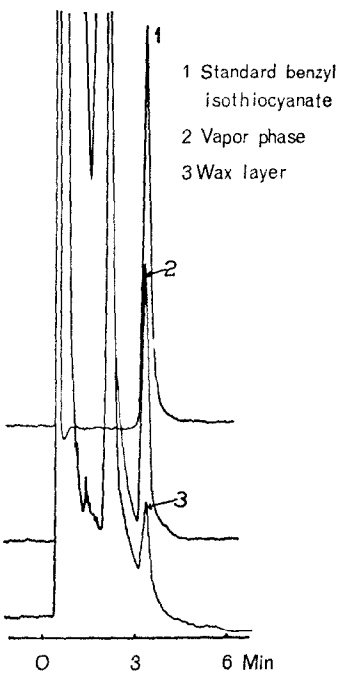



FIG. 1. GLC CHROMATOGRAMS OF FREE BENZYL ISOTHIOCYANATE IN WAX LAYER AND VAPOR PHASE OF GREEN PAPAYA FRUIT.

MT 220 Gas Chromatograph with a flame photometric detector. 6 × 1/4 in. glass column, 5% DC 200 + 7·5% QF − 1 on 80–100 mesh Gaschrom Q; Oven temp. 163°; flow rate $N_2$, 80 ml/min; $H_2$ 140 ml/min; $O_2$, 40 ml/min.

TABLE 1. THE CONTENT OF BENZYL ISOTHIOCYANATE IN PAPAYA FRUIT PULP AND SEEDS OF VARIOUS STAGES OF MATURITY

| Weight of the green immature papaya fruit* (g) | Benzyl isothiocyanate in pulp (ppm) | | Benzyl isothiocyanate in seed (ppm) |
|---|---|---|---|
| | unincubated | incubated† | incubated† |
| 32 | 86 | 291 | —‡ |
| 187 | 74 | 251 | 1830 |
| 384 | 52 | 126 | 1920 |
| 789 | 12 | 65 | 2520 |
| Ripened papaya | 4 | 4 | 2910 |

* Samples were collected from the same tree at the same time and presumably representative of different stages of maturity.
† Incubated at room temperature for 1 hr.
‡ Insufficient for quantitative analysis.

*Fungistatic Properties of BITC*

Gas chromatographically pure BITC was used to determine the inhibitory effect on the sporangia of *Phytophthora parasitica* Dast. Neither direct nor indirect germination[6] was observed when sporangia were placed in a 5 ppm aqueous solution of BITC. Detailed studies on the fungistatic effect of this compound will be reported in separate papers.

## DISCUSSION

Various techniques have been employed to estimate the amount of isothiocyanates in plant tissues.[1] The method used in this study has the advantage of good recovery (80%), and a relatively short time of analysis; benzyl isothiocyanate from a 2-g seed sample can be quantitatively analyzed with a standard deviation of $\pm 4\%$.

Sweep co-distillation, to eliminate the non-volatile plant materials, was found necessary before the sample was subjected to GLC to preserve the quality of the GLC columns for reproducible results. This technique is used commonly in pesticide residue research to clean up samples for GLC analysis.[7]

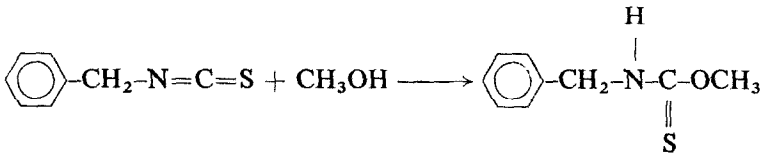
The increase in BITC concentration upon incubation of macerated pulp (Table 1) is a clear indication of a myrosinase-like activity. The absence of a similar effect in the seeds suggests an extremely high enzyme activity which causes almost instantaneous reaction upon cell rupture despite the low temperature of operation. The data from the incubated pulp and seed samples in Table 1 represent the potential of BITC production; however, the values for the unincubated pulp samples do not necessarily represent the actual amount of free BITC in the intact tissue. Saarivirta and Virtanen[8] have reported that in the seed preparation of *Lepidium sativum*, the glucosinolate of BITC was completely hydrolyzed at $+1°$ to $+2°$ within 5 min. The immediate enzymatic hydrolysis of the thioglucoside upon cell disruption would raise the difficulty of confirming the presence of free BITC in intact plant tissue. In this study, trace amounts of extra-cellular BITC were detected in the wax layer

[6] M. ARAGAKI, R. D. MOBLY and R. B. HINE, *Mycologia* **59**, 93 (1967).
[7] R. W. STORHERR and R. R. WATTS, *J. Assoc. Offic. Anal. Chem.* **48**, 1154 (1965).
[8] M. SAARIVIRTA and A. I. VIRTANEN, *Acta Chem. Scand.* **17**, 4 (1963).

and the vapor emanating from intact green papaya fruit by GLC. This implies that BITC is a normal metabolite of green papaya, although the amount present and its biochemical significance remain to be determined.

*P. parasitica* is the most important fungal pathogen of papaya in Hawaii. Typical symptoms of the disease in affected orchards consist of chlorosis, premature defoliation, stunting and severe root rot. Hine *et al.*[9] reported that mature, uninjured papaya fruit was susceptible when inoculated with zoospores of *P. parasitica* whereas immature, uninjured fruit was resistant. The effect of papain on this pathogen was also studied with the conclusion that the role of papain in the defensive mechanism of papaya fruit against this fungus was probably minor. Benzyl isothiocyanate has the greatest antimicrobial activities of all the mustard oils investigated.[10] The growth of *Staphylococcus* and *Penicillium glaucum* are inhibited at a concentration of 1–2 ppm. The concentration of BITC was found high in macerated green immature papaya pulp and low in the yellow, ripe sample. On the premise that BITC has a role in the defense mechanism of papaya, this finding is in accordance with the report that ripened papaya was more susceptible to *P. parasitica* invasion.

When BITC was extracted from papaya seeds with methanol, another sulfur containing compound in addition to BITC was invariably obtained. TLC and infrared spectrometry confirmed its identity as the methanol adduct of BITC. This compound was not present when the seeds were extracted with ethyl ether. Cairns[11] identified *N*-benzyl-thionocarbamic acid methyl ester[12] (Caricacin) in methanol extracts of papaya seeds and reported it to be a plant growth inhibitor formed by the reaction of methanol with an unknown precursor. Based on the known chemical reactivities of isothiocyanates, Caricacin is probably formed by the following reaction:



This ability of isothiocyanates, including BITC, to form adducts with nucleophiles (e.g. $RO^-$, $RS^-$, $R_2N^-$) provides the interesting possibility of producing phytotoxic thiocarbamates, dithiocarbamates, or thioureas from decomposing plant residues. Accumulation of these compounds in soil might have toxic effects on the plant itself.

## EXPERIMENTAL

*Identification of BITC in Papaya Pulp and Seeds*

60 g of dry mature papaya seeds were ground to powder (*ca.* 20 mesh) and extracted with 3 × 100 ml $Et_2O$. The combined $Et_2O$ extracts were concentrated in a flash evaporator. The oily residue was dissolved in 100 ml of *n*-hexane and extracted with 80, 60 and 40 ml portions of $CH_3CN$. The $CH_3CN$ phases were combined and washed twice with 50 ml of *n*-hexane. Benzyl isothiocyanate which has a relatively high polarity remained in the $CH_3CN$ phase. Evaporation of the $CH_3CN$ gave a crude preparation of BITC (*ca.* 0·4 g). Additional purification was performed with a Varian 1800 Gas Chromatograph equipped with a Carle

[9] R. B. HINE, M. ARAGAKI and J. TOKUNAGA, *Phytopathology* **55**, 1223 (1965).
[10] A. I. VIRTANEN, *Angew. Chem.* **1**, 303 (1962).
[11] T. M. CAIRNS, Ph.D. Dissertation, Isolation and Identification of Caricacin, A Plant Growth Inhibitor in the Methanolic Extract of Carica Papaya L. Univ. of Calif., Riverside, Calif. (1968). *Dissertation Abstr.* **29**, 2689B (1969).
[12] A. A. BURROWS and L. HUNTER, *J. Chem. Soc.* 4118 (1952).

Micro Detector System (Carle Instrument, Inc., Fullerton, California) containing 2·5 mm × 3 m stainless steel column packed with 3% OV17 on 80–100 mesh Chromosorb G, AW-DMCS solid support. The column temperature was 190° and the flow rate of the helium carrier gas was 25 ml/min. The effluent was collected in a glass capillary tubing and its i.r. spectrum was obtained from a thin liquid film on a Beckman IR-8 Spectrophotometer. Benzyl isothiocyanate from the papaya pulp was also isolated and identified. The procedure used was similar to that of the quantitative determination except that 500 g of sample was used.

Authentic BITC purchased from K & K Laboratories, Inc., Hollywood, California, U.S.A., was purified by the GLC procedure described above.

*Quantitative Determination of BITC in Papaya Pulp and Seeds*

Papaya fruit of different sizes were collected from the Waimanalo Experimental Farm, University of Hawaii, in the summer of 1969. The fruit was chilled in an ice bath and 10 g of pulp with the epidermis was cut into small pieces and homogenized with 15 ml $H_2O$ in an Omni Mixer (Ivan Sorvall, Inc., Norwalk, Connecticut, U.S.A.) at high speed for 2 min. The temperature of the macerate was kept close to 0° during this procedure. 15 ml of $CHOl_3$ was added and thoroughly mixed with the aqueous slurry for another 2 min. The mixture was centrifuged at 1850 *g* for 10 min and a known volume of the bottom $CHCl_3$ layer was transferred to a 10 ml concentrator tube, evaporated to 1 ml, and injected into a sweep co-distiller (Kontes Glass Co., Vineland, New Jersey, U.S.A.). The $N_2$ flow rate was 300 ml/min and the oven temperature was 165°. The collected effluent was adjusted to a concentration suitable for GLC quantitation. The GLC system was that used for isolation of the compound except that a flame ionization detector was employed instead of the Carle Micro Detector. The flow rate of $N_2$ was 25 ml/min; $H_2$ 25 ml/min and air 300 ml/min.

Incubated samples were prepared by homogenizing the pulp or seeds with 15 ml of water and the slurry was left at room temperature for 1 hr prior to chloroform extraction.

*Detection of BITC from Wax Layer*

Green, immature papayas with no observable injuries on their surfaces were carefully selected from the tree. Twelve fruits were subsequently dipped for 1 min into a 600 ml beaker containing 300 ml of freshly distilled $Et_2O$. The dry extract ($MgSO_4$) was evaporated to dryness, and the residue partitioned between hexane and $CH_3CN$. The $CH_3CN$ phase was concentrated to a small volume (*ca.* 0·05 ml) for GLC analysis.

*Detection of BITC in Vapor Phase*

12 green, uninjured papayas were enclosed in a glass jar. The peduncles where the papayas were detached from the tree were sealed with paraffin wax (m.p. 46°) to prevent the release of BITC from the cut surfaces. A stream of air was pumped into the jar through a filter containing 5 g of activated charcoal (8–12 mesh, Matheson, Coleman and Bell, Los Angeles, California) at a rate of 60 ml/min. The vapor swept out from the jar was trapped by a short column of 2·5 g of the same activated charcoal. After 3 days of continuous collection, the charcoal trap was eluted with 10 ml of $Et_2O$, the eluate was dehydrated and concentrated to approximately 0·05 ml for GLC analysis.

A MT 220 Gas Chromatograph (TRACOR, Inc., Austin, Texas, U.S.A.) equipped with a sulfur flame photometric detector[13] was used to detect the trace amount of BITC present in the wax layer and vapor phase. The GLC conditions are cited in the legend of Fig. 1.

*Acknowledgements*—The author wishes to thank Drs. Minoru Aragaki and John W. Hylin for their helpful discussion.

[13] S. S. Brady and J. E. Chaney, *J. Gas Chromatog.* **4**, 42 (1966).