respect to the content of any given amino acid, one or two fruits were much higher (1.5 or more times) than all the others. The surprisingly high levels of aspartic acid in mamey sapote, glycine in tucuma, and hydroxyproline in sapodilla were confirmed by ion-exchange analyses. The very high hydroxyproline content in sapodilla suggests a high level of hydroxyproline-containing proteins (e.g., extensin), which could be present in the cell walls (Lamport and Miller, 1971; Trowell, 1977). This suggestion is supported by the report (Venkataraman and Reithel, 1958) that sapodilla contains oligosaccharides, often associated with such proteins. Overall, glutamic and aspartic acids were most frequently found in larger quantities than the other amino acids. The alanine content was outstanding in mango and longan, as was the proline content of sapodilla and loquat and the serine content of mamey sapote. The quantities (in milligrams) of the nonrequired amino acids supplied by 100 g of fruit are shown in Table VI. The contrast of the highest value (e.g., Asp 532 mg) compared with the range of other values (Asp 32–219 mg) is readily apparent.

CONCLUSION

Certain tropical fruits contain from 10 to 26% of the minimum daily requirement of most essential amino acids per 100-g serving of natural fresh fruit. Tucuma palm fruit, in particular, contains a large quantity of all the required amino acids except the sulfur amino acids (and possibly tryptophan which was not determined).

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Isolation and Identification of the Major Polar Metabolites of Methidathion in Tomatoes

Bruce J. Simoneaux,* Gus Martin, James E. Cassidy, and Daniel P. Ryskiewich

Tomatoes treated topically with [^{14}C]methidathion equivalent to 10 ppm in the whole fruit were found to produce two major polar metabolites. One was characterized by chromatographic techniques as demonomethyl methidathion. The second metabolite was identified by mass spectrometry as a cysteine conjugate. Confirmation of the postulated structure for the cysteine conjugate was done by cochromatography with a synthesized standard using several thin-layer chromatographic and electrophoretic systems. A method for synthesis of this conjugate is reported. The presence of these metabolites in other crops is discussed.

Methidathion, *S*-[5-methoxy-2-oxo-1,3,4-thiadiazol-3-(2*H*)-yl] *O*,*O*-dimethyl phosphorodithioate, is an organophosphorus insecticide widely used on citrus, tobacco, alfalfa, cotton, sorghum, and several other crops. Its metabolism in higher plants has been investigated by several workers (Bull, 1968; Cassidy et al., 1969; Dupuis et al., 1971).

Demonomethyl methidathion was postulated to be one of the polar metabolites present in cotton by Bull (1968)

Agricultural Division, CIBA-GEIGY Corporation, Greensboro, North Carolina 27409.

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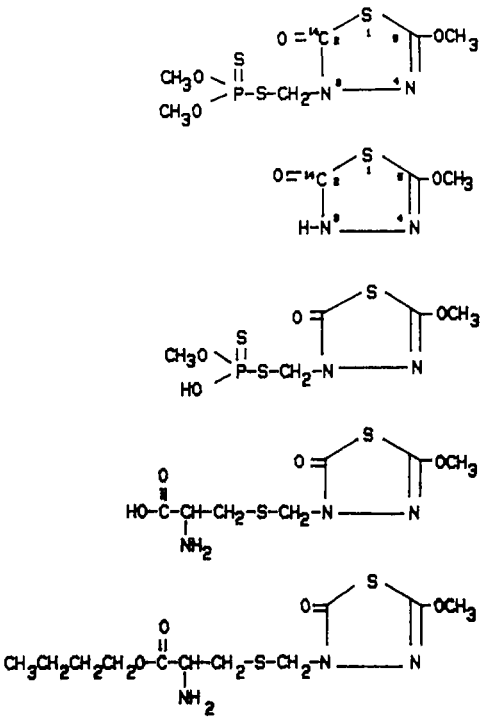

Figure 1. Chemical structures of methidathion and related compounds. [2Δ-^{14}C] denotes the heterocyclic position of ^{14}C labeling.

and in beans and alfalfa by Dupuis et al. (1971). More recently, Chopade et al. (1980) have investigated the metabolism of methidathion in tomato fruit as a function of treatment level and postharvest interval. They have characterized two major polar metabolites to be demonomethyl methidathion [*S*-[[5-methoxy-2-oxo-1,3,4-thiadiazol-3(2*H*)-yl]methyl] *O*-methyl phosphorodithioate] and a cysteine conjugate [*S*-[[5-methoxy-2-oxo-1,3,4-thiadiazol-3(2*H*)-yl]methyl]-L-cysteine].

The objectives of this investigation were to identify a major polar metabolite of methidathion found in tomato fruit by using mass spectrometry and to confirm its identification by synthesis. The results from this study were compared with those from other investigators.

EXPERIMENTAL SECTION

Chemicals. Structures for methidathion and related compounds are given in Figure 1. [2-^{14}C]Methidathion (14.0 μCi/mg) labeled in the carbonyl position of the thiadiazole ring was prepared by the reaction of [2-^{14}C]RH with the potassium salt of *O,O*-dimethyl phosphorodithioic acid as outlined by Rüfenacht (1968). [2-^{14}C]RH was prepared by the reaction of thiocarbazinic acid O-methyl ester with [^{14}C]phosgene according to Cassidy et al. (1969).

The sodium salt of demonomethyl methidathion was prepared by the reaction of methidathion with sodium thiophenol in ethanol at 60 °C for 5 h.

The cysteine conjugate [*S*-[[5-methoxy-2-oxo-1,3,4-thiadiazol-3(2*H*)-yl]-methyl]-L-cysteine] was prepared by the reaction of 3-(chloromethyl)-5-methoxy-1,3,4-thiadiazol-2(3*H*)-one with L-cysteine hydrochloride hydrate in 2 parts of triethylamine and 1 part of *p*-dioxane–water (4:1) at reflux temperatures for 16 h.

The butyl ester of the cysteine conjugate was prepared according to the procedure of Kaiser et al. (1974); 1.5 N hydrogen chloride in butanol and the cysteine conjugate were heated in a sealed glass tube for 2 h at 100 °C.

Treatment of Tomatoes. A stock solution containing 0.4 mg/mL [2-^{14}C]methidathion was prepared in ethanol. Ten partially ripe tomatoes purchased in a store were surface drenched to runoff with 20 mL of the stock solution. The average weight of each tomato was 300 g. The tomatoes were allowed to air dry and were placed in a laboratory hood under Gro-Lux fluorescent lights with a 12-h light and 12-h dark cycle for 7 days.

Sample Preparation and Extraction. The treated tomatoes were cut into quarters and blended in a Waring blender for 5 min with powdered dry ice to produce a homogeneous sample. A 500-g subsample of the tomato puree was extracted for 5 min in a Waring blender with 1500 mL of acetone–water (9:1 v/v). The resultant suspension was filtered by suction through Whatman No. 1 filter paper in a porcelain Buchner funnel. Aliquots of the filtrate were added directly to the scintillation fluid (Aquasol) for subsequent measurement of total radioactivity by liquid scintillation counting. Subsamples of the original blended puree and the extraction residue were combusted for total radioactivity measurement using a Harvey biological material oxidizer. The extract was concentrated to remove the acetone by using a Büchi Rotovapor R evaporator. The concentrate was diluted to 1000-mL total volume with water and partitioned twice with equal volumes of chloroform. Aliquots of the chloroform and aqueous phases were radioassayed by direct scintillation counting in Aquasol.

Thin-Layer Chromatography (TLC). Aliquots of the chloroform and aqueous phases were spotted along with appropriate standards on silica gel GF plates (Analtech) and developed in one or more of the following solvent systems: (A) methyl ethyl ketone–acetic acid–water (3:1:1); (B) acetonitrile–water (2:1); (C) methylene chloride–methanol (8:2); (D) chloroform–methanol–acetic acid (60:40:1); (E) acetonitrile–water–NH_4OH (40:9:1); (F) methyl ethyl ketone–acetic acid–water (5:1:1). The thickness of the plates was always 0.25 mm unless specified otherwise. Radiolabeled regions were identified by autoradiography and by spark chamber pictures (Birchover Instruments, Ltd.). Unlabaled standards were visualized by UV fluorescence quenching or with ninhydrin spray reagent (E. Merck).

Electrophoresis. Whatman No. 1 paper (19 × 76 cm) was used as the buffer support. Different buffer systems

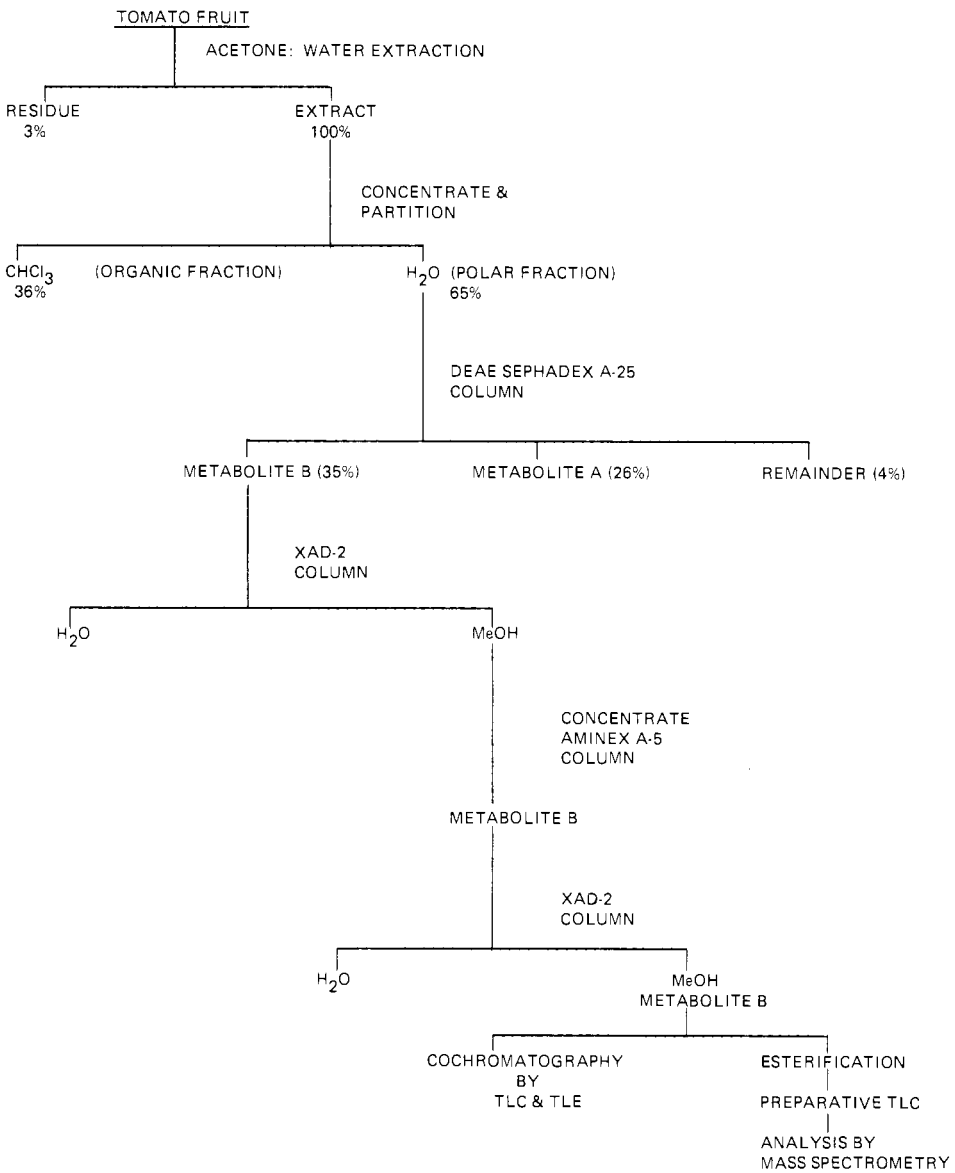

Figure 2. Isolation and purification scheme for metabolite B in tomato fruit.

used were as follows: (G) formic acid–acetic acid–water (5:15:100) to give pH 2; (H) sodium borate (0.016 N) and potassium dihydrogen phosphate (0.043 N) in water to give pH 9. A Savant electrophoresis instrument was operated at 5 °C for 30 min at 4000 V and 100 mA current for each separation. RBY reference dye (Gelman Instrument Co.) was spotted on each chromatogram to determine the quality of each separation. Radiolabeled regions were visualized by spark chamber pictures (Birchover Instruments, Ltd.). The cysteine conjugate standard was visualized with ninhydrin spray reagent (E. Merck).

Analytical Separation of Polar Metabolites. An aliquot of the aqueous phase following chloroform partitioning was applied to a 1.5 cm i.d. × 25 cm DEAE-Sephadex A-25 column similar to that described by Paulson et al. (1972) in order to quantitate individual polar metabolites A and B (Table I). The sample was eluted with a linear KBr gradient (250 mL of water in chamber 1 and 250 mL of 1 N KBr in chamber 2). Fractions (5 mL) were collected and aliquots (0.1 mL) analyzed by direct liquid scintillation counting (Aquasol).

Purification of Polar Metabolite B. The aqueous phase obtained following chloroform partitioning of the tomatoe fruit extract (Figure 2) was applied in two equal portions to two 4.5 × 32 cm columns packed with DEAE-Sephadex A-25. The columns were washed with water until a major peak had eluted (metabolite B). Metabolite A was acidic in nature and remained on the column. Enough final buffer (1 N KBr) was added to elute metabolite A from the column. The aqueous fractions containing metabolite B were combined and concentrated. The concentrate containing Metabolite B was applied to a 4 × 40 cm column packed with XAD-2 resin (Rohm and Haas) and washed with an additional volume of water to

Table I. Distribution of ^{14}C in Tomato Extracts after Treatment of Fruit with [2-^{14}C]Methidathion for 7 Days

fraction	% of total radioactivity
organic soluble radioactivity	
methidathion	32
unknowns	3
subtotal:	35
polar soluble radioactivity	
metabolite A	26
metabolite B	35
unknowns	4
subtotal:	65
nonextractable radioactivity	3
total:	103

Table II. Mobilities of the Major Metabolites of Methidathion in Several TLC and TLE Systems

TLC system	R_f metabolite A	R_f metabolite B
A	0.82	0.68
B	0.71	0.51
C	0.45	0.17
D	0.69	0.41
E	0.73	0.36

TLE system	mobility, cm/h, of metabolite B
G	−6.5
H	+5.0

elute from the column coextracted sugars and salts. Methanol was used to remove the adsorbed radioactivity from the column.

The methanol fraction was concentrated to dryness and applied in starting buffer to a 1.5 × 15 cm Aminex A-5 column. The sample was eluted with a linear salt gradient, 250 mL of 0.1 N ammonium formate at pH 4 (starting buffer) in chamber 1 and 250 mL of 1.0 N ammonium formate at pH 6 in chamber 2. Fractions (5 mL) were collected and aliquots (0.1 mL) analyzed by direct scintillation counting (Aquasol). The initial radioactivity eluted from the column was not further characterized. The predominant radioactivity in the basic peak was desalted on a XAD-2 column as previously described in this section. The radioactive methanolic fraction from this column was subjected to cochromatography with the synthesized cysteine conjugate in the five TLC and two TLE systems listed in the previously discussed sections.

A portion of the methanol fraction was esterified with 1.5 N HCl in 1-butanol according to the procedure of Kaiser et al. (1974). The esterified sample was lyophilized to dryness and redissolved in 1.4 mL of methanol. This solution was spotted on a methanol-washed 250-μm silica gel plate (Analtech) and developed by using solvent system F. The radioactive zone was visualized by a spark chamber picture and scraped from the plate. The radioactivity was eluted from the silica gel by using boiling ethyl acetate several times. The ethyl acetate was concentrated to 1.0 mL prior to mass spectral analysis of the *n*-butyl ester of metabolite B.

Mass Spectral (MS) Analysis. Approximately 1 μg of the purified *n*-butyl ester of the isolated material (metabolite B) and the *n*-butyl ester of the synthesized cysteine conjugate was subjected to mass spectral analysis by direct probe insertion in a Finnigan Model 3100 D instrument using the chemical ionization mode.

Radioassay. All radioactive measurements were done with a Beckman LS-255 scintillation counter. Samples were corrected for background counts and corrected for quenching by external standardization.

RESULTS AND DISCUSSION

Metabolite Distribution. Tomato fruit treated with radiolabeled methidathion by an ethanol solution surface drench contained 9.94 ppm equivalent to [2-^{14}C]methidathion. Ethanol was chosen as the solvent for methidathion treatment to ensure uniform coverage of the tomato fruit with chemical. Because of the short time duration before complete evaporation of the solvent had occurred, it was not felt that any plant enzymes or membranes were seriously disrupted. Most of the radioactivity was extractable (Table I) with only small amounts of radioactivity found in the nonextractable residue (3%). Methidathion was extensively metabolized to polar metabolites (65%) in a 7-day incubation period. Radioautograms of a typical TLC separation of aqueous soluble radioactivity (solvent system A) show the presence of two polar metabolites. Metabolite A (26%) and metabolite B (35%) together accounted for 61% of the total tomato radioactivity (Table I). The radioactivity associated with the organic fraction was found to be mostly methidathion (32%).

Metabolite Characterization. Comparison by cochromatography on TLC plates and retention volumes on DEAE-Sephadex A-25 columns to a synthesized standard show metabolite A to be demonomethyl methidathion.

Spraying of the developed chromatograms with ninhydrin resulted in a positive spot with metabolite B. The net electrophoretic migration of metabolite B at buffer pH 2 and 9 showed definite zwitterionic character (Table II). These data indicated that metabolite B was an amino acid conjugate.

Metabolite Identification. The *n*-butyl esters of metabolite B and the synthesized conjugate were prepared as previously described. Mass spectral data was obtained on ~1 μg of each derivative by using direct probe insertion in the CI mode with methane reagent gas. Fragmentation patterns for both standard and metabolite B derivatives showed ions at M + 1, M + 29, and M + 41 corresponding to *m/e* values of 322, 350, and 362. Other ions detected in both samples were at *m/e* values of 190, 145, 133, and 85. The presence of fragments at *m/e* 145 and 85 is consistent with the thiadiazolyl ring portion of the molecule remaining unchanged. For further verification of this structure, comparisons by cochromatography of the isolated metabolite B and the synthesized cysteine conjugate were done with various TLC systems (A–E) and TLE systems (G and H). In all seven systems, metabolite B and the standard cochromatographed.

The spectral data presented in this paper and successful synthesis of a cysteine conjugate standard have confirmed the characterization of this metabolite in tomato fruit by Chopade et al. (1980). They treated tomato fruit with several postulated intermediates and concluded that demonomethyl methidathion was the major precursor in the formation of the cysteine conjugate. Even though the intermediate compounds shown in their pathway for metabolism of methidathion by tomato fruit could not be identified, these are the usual pathways postulated for formation of cysteine conjugates in plants (Hutson, 1976).

A major polar metabolite of alfalfa (lower zone) described by Cassidy et al. (1969) using TLC system E appears to be the cysteine conjugate identified in this paper on tomatoes. Another polar metabolite (upper zone) in alfalfa degraded to a zone that was postulated to be demonomethyl methidathion. On the basis of all published work, many agriculturally important crops appear to share a common metabolic pathway for methidathion.

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