tion. Further investigation of the dealkylation products, namely the chlorinated and hydroxylated 4,6-diamino-s-triazines, was hindered by extraction and partition difficulties but is in progress.

ACKNOWLEDGMENT

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Atrazine Metabolism in Sorghum: Catabolism of the Glutathione Conjugate of Atrazine

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The major pathway of atrazine metabolism in intact sorghum was shown to involve the following steps: atrazine → *S*-(4-ethylamino-6-isopropylamino-*s*-triazinyl-2)glutathione (III) → γ-glutamyl-*S*-(4-ethylamino-6-isopropylamino-*s*-triazinyl-2)cysteine (IV) → *S*-(4-ethylamino-6-isopropylamino-*s*-triazinyl-2)cysteine (V) → *N*-(4-ethylamino-6-isopropylamino-*s*-triazinyl-2)cysteine (VI) → *N*-(4-ethylamino-6-isopropylamino-*s*-triazinyl-2)lanthionine (VII). From 40 to 87% of the atrazine entering sorghum through the roots was estimated to be metabolized *via* this pathway. Evidence indicated that atrazine can also be metabolized *via* this route after first undergoing N-dealkylation. The conversion of V to VI was shown to be a nonenzymatic rearrangement. This is the first reported occurrence of V, VII, 2-hydroxy-4-amino-6-isopropylamino-*s*-triazine (VIII), and 2-hydroxy-4,6-diamino-*s*-triazine (ammeline, XII) in sorghum.

Although three reaction types have been demonstrated in the metabolism of atrazine in higher plants, N-dealkylation, hydrolysis, and conjugation with glutathione, a clear picture has not yet emerged on the complete metabolism of atrazine in any tolerant plant species (Shimabukuro *et al.*, 1971). Dealkylation of the alkylamino side chains is a general pathway that occurs in many plants, but in tolerant species this pathway is generally in competition with faster reactions and simple dealkylated products such as 2-chloro-4-amino-6-isopropylamino-*s*-triazine (I) do not accumulate in high concentrations. Because of this, N-dealkylation may not appear to be of significance in short-term metabolic studies, even though terminal metabolites may have undergone N-dealkylation. Hydrolysis of the 2-chloro group is an important metabolic reaction in certain species such as corn, and in this species hydrolysis and dealkylation result in metabolites such as 2-hydroxy-4-amino-6-isopropylamino-*s*-triazine (VIII). However, even in corn, where two of the predominant reactions result in simple products, the chemical nature of the majority of the terminal residue is still uncertain. Most atrazine-tolerant species differ from corn in that displacement of the 2-chloro substituent by glutathione, rather than hydrolysis, is the first step in the primary route of metabolism (Lamoureux *et al.*, 1970; Lamoureux *et al.*, 1972; Thompson, 1972). Preliminary studies with atrazine metabolism in sorghum indicated that the glutathione and γ-glutamylcysteine conjugates of atrazine are transitory intermediates in this metabolic pathway. This report is primarily concerned with the further elucidation of the steps involved in the metabolism of atrazine *via* conjugation with glutathione. The structures of the metabolites discussed in this report are shown in Figure 8.

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EXPERIMENTAL SECTION

General Methods. Electron impact mass spectra were measured on a Varian MAT CH-5DF or on a Varian M-66 with a V-5550 console. Samples were introduced with a solid-sample probe which was heated from 25° to 250°, depending on the sample. The source temperature on both instruments was 180°. The methods used for monitoring ^{14}C, preparation of *S*-(4-ethylamino-6-isopropylamino-*s*-triazinyl-2)glutathione (III) and γ-glutamyl-*S*-(4-ethylamino-6-isopropylamino-*s*-triazinyl-2)cysteine (IV), and the preparation of thin-layer plates have been described previously (Lamoureux *et al.*, 1972). Silica gel thin-layer plates used for purification of samples for mass spectral analyses were washed with the solvent used for chromatography or with absolute methanol before chromatography. The following solvent systems were used (solvent ratios are expressed on a volume:volume basis): (A) 1-butanol–acetic acid–water (12:3:5); (B) ethanol–water (7:3); (C) benzene–acetic acid–water (60:40:3); (D) 88% phenol–water–28% ammonium hydroxide (100:20:0.3); (E) benzene–ethanol (7:3); (F) ethyl acetate–acetic acid–water (23:1:1); (G) ethanol–28% ammonium

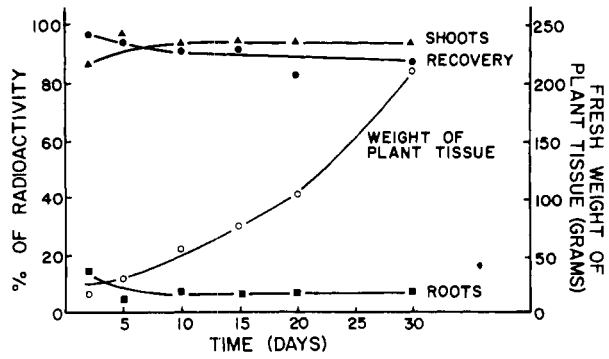

Figure 1. Percent distribution of radioactivity between the roots and shoots of sorghum as a function of time. The plants were root-treated with atrazine-^{14}C for 2 days and harvested periodically. The recovery indicated is the total percent recovery of all ^{14}C used in the experiment.

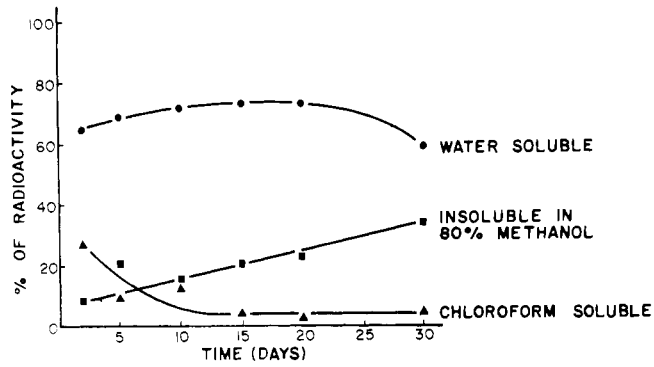

Figure 2. Percent distribution of the radioactivity in sorghum shoots into various solubility classes as a function of time. The sorghum was root-treated with atrazine-^{14}C for 2 days and harvested periodically.

hydroxide (7:3); (H) toluene-acetic acid-methyl ethyl ketone-methanol-water (10:2:80:5:6); (I) ethyl acetate-methyl ethyl ketone-formic acid-water (5:3:1:1); (K) chloroform-tetrahydrofuran-*tert*-butanol-water (10:6:50:14); (L) benzene-methanol (90:10); (M) benzene-ethyl formate-formic acid (75:24:1); (N) acetone-chloroform-acetic acid (30:50:15); (O) 2-propanol-28% ammonium hydroxide-water (8:1:1); (P) benzene-acetic acid (50:4).

Time Study. Sorghum (*Sorghum vulgare* Pers., N.D. 104) was germinated in vermiculite and grown in aerated ⅓-strength Hoagland's solution in a controlled environment chamber with a relative humidity of 40%, a 14-hr photoperiod (15,000 lm/m^2) at 28°, and a nyctoperiod at 25°. After the plants were 22 days old, they were divided into 12 lots of six plants/lot. Each lot was treated for 2 days with 50 ml of 4.17×10^{-6} *M* atrazine-^{14}C (9.42×10^5 dpm) in ⅓-strength Hoagland's solution. Two lots were harvested after the treatment period; the remaining lots were placed in fresh atrazine-free ⅓-strength solution after the roots of the plants had been rinsed with water to remove residual radioactive treating solution. These plants were harvested 5, 10, 15, 20, and 30 days after initiation of treatment. The roots and shoots of one lot from each time period were extracted separately with 5 ml of cold 80% aqueous methanol/g of fresh tissue. The extracts were concentrated and partitioned between water and chloroform. A 160,000-dpm aliquot of the aqueous phase of the shoot extract from each time period was then chromatographed on a column of AG 50W-X2 resin with a pyridine-acetate buffer gradient. An aliquot of the 200-ml eluate from each chromatogram, corresponding to III and VII in Figure 3, was concentrated and hydrolyzed in 0.5 ml of 6 *N* HCl at 50° for 20 hr. The hydrolysates were chromatographed by tlc with solvent A. All radioactive zones were scraped from the plates and counted by liquid scintillation techniques. The procedures used in extraction, partitioning, and column chromatography were those described by Lamoureux *et al.* (1972) for the quantitative comparison of metabolism. The radioactivity in the 80% methanol-insoluble residue from each extraction was determined by liquid scintillation counting after combustion in oxygen (Shimabukuro, 1967).

Three 50-ml aliquots of radioactive treating solution, identical to that used in the study, were inoculated by emersing sorghum roots in these solutions. After 10 min the roots were removed and the solutions were incubated under the conditions described in the time study. After 60 hr the solutions were partitioned against chloroform and the chloroform phases were examined by tlc in solvent P.

Treatment of Sorghum with Metabolites III, IV, and VII. Each of three 25-day-old sorghum seedlings was treated with a different atrazine metabolite by injecting

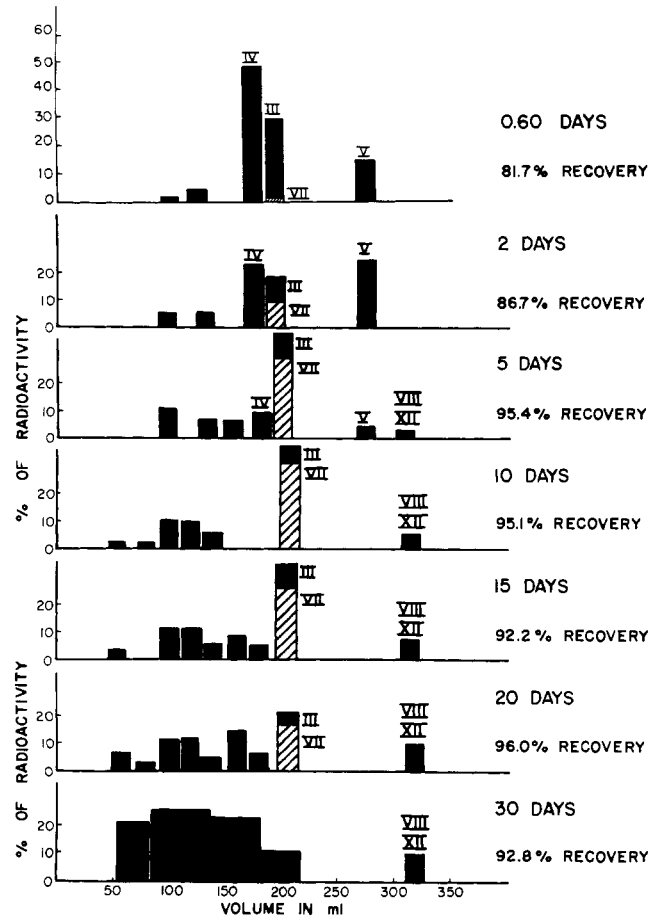

Figure 3. Chromatography of the aqueous extracts from sorghum shoots on columns of AG 50W-X2 eluted with pyridine-acetate buffer. The first group of plants was root-treated with atrazine-^{14}C for 0.60 days and harvested immediately after treatment. These plants were of identical size and age as the other plants, but were treated on a separate date. The remaining sets of plants were root-treated for 2 days and harvested periodically. The recoveries indicate the percent ^{14}C recovered during chromatography.

40 μl (200,000 dpm) of an aqueous solution of the metabolite into the stem of the plant. Seedlings treated with metabolites III and IV, respectively, were harvested 2 days after treatment; that which had been treated with VII was harvested 10 days after treatment. The extract from the foliar tissue of each seedling was partitioned with solvents and chromatographed on columns of AG 50W-X2. The resulting 200-ml eluate from each chromatogram was subsequently hydrolyzed and analyzed by tlc. The methods of

extraction, partitioning, hydrolysis, and chromatography were described in the previous paragraph.

Enzymatic Synthesis of *S*-(4-Ethylamino-6-isopropylamino-*s*-triazinyl-2)cysteine and Comparison with Metabolite V. γ-Glutamyltranspeptidase was prepared from hog kidney by the method of Orlowski and Meister (1965), as modified by Schwimmer (1971). Metabolite IV (1.0 μmol, 250,000 dpm) was incubated with 5 mg of γ-glutamyltranspeptidase for 1 hr at 37° under the conditions described in Table IV of the paper by Orlowski and Meister (1965). Immediately after treatment, the crude reaction mixture was derivatized with 2,4-dinitrofluorobenzene (Cowgill and Pardee, 1957), and the resulting ethyl acetate-soluble fraction was esterified with absolute methanol–HCl by method I of Greenstein and Winitz (1961). The product was purified for mass spectral analysis by tlc with solvents L (R_f 0.67), M (R_f 0.10), and ethyl ether (R_f 0.50). The final product was a bright yellow, radioactive substrate which absorbed uv light.

A second sample of IV (1.5 μmol, 375,000 dpm) was incubated with γ-glutamyltranspeptidase in a similar manner. After the reaction was complete, a 25,000-dpm aliquot was dissolved in 1 ml of 0.2 *M* pH 6.5 phosphate buffer and partitioned 3× against 1-ml portions of chloroform. The radioactivity in the chloroform and aqueous phases was measured by liquid scintillation. A second aliquot of the reaction mixture (50,000 dpm) was dissolved in 1 ml of 0.22 *M* pH 7.5 phosphate buffer and incubated at 37° for 16 hr. This solution was then adjusted to pH 6.5 with dilute HCl and partitioned with three equal volumes of chloroform. The chloroform phase and the aqueous phase were assayed for radioactivity. The chloroform phase was concentrated and chromatographed on a column of AG 50W-X2 in the manner previously described. A third aliquot from the γ-glutamyltranspeptidase reaction mixture (50,000 dpm) was chromatographed on a column of AG 50W-X2 resin without further treatment. The final aliquot (250,000 dpm) was incubated in 2 ml of phosphate buffer as above for 16 hr, adjusted to pH 6.5, and partitioned 3× against 20-ml portions of chloroform. The chloroform fraction (183,000 dpm) was esterified in 1 ml of absolute methanol by method I of Greenstein and Winitz (1961). The product was purified by tlc with solvent E, R_f 0.83. The ester was eluted from the gel with five 2-ml portions of absolute methanol (155,000 dpm) and analyzed by mass spectroscopy.

Aqueous extracts of V were prepared in the manner described for the 2-day harvest of the time study. The resulting aqueous extracts, obtained after partitioning with $CHCl_3$, were treated with 0.22 *M* pH 7.5 phosphate buffer, partitioned, and chromatographed on AG 50W-X2 under the conditions described in the previous section. Two-dimensional thin-layer chromatograms were developed with solvent C in the first direction and with solvent A in the second direction.

Purification of Metabolite VII. The crude methanolic extract containing VII was obtained as a byproduct from a previous study (Bakke *et al.*, 1972). The preparation of this extract is summarized below. Sorghum seedlings were root-treated in nutrient culture with 9 l. of ½-strength Hoagland's solution, which was 1.11×10^{-5} *M* with respect to atrazine-^{14}C (specific activity = 250,000 dpm/μmol). After 20 days in the nutrient solution, the foliar tissue from these plants (1911 g) was extracted with 80% aqueous methanol. The methanolic extract was concentrated to near dryness, dissolved in 500 ml of water, and partitioned 3× against 500-ml portions of chloroform. The insoluble residue remaining after the extraction contained 18.9×10^6 dpm, the aqueous extract contained 73.4×10^6 dpm, and the chloroform-soluble extract contained 13.2×10^6 dpm.

Aliquots of 13.9×10^6 dpm of the aqueous fraction (after partitioning) were concentrated to a heavy syrup, dissolved in 40 ml of pH 2.15 buffer, and applied to 2.5 × 105-cm columns of AG 50W-X2 (200–400 mesh) resin. The columns were kept at 15°, while the samples were eluted at 1 ml/min with a pyridine–acetate buffer gradient. The first chamber of the gradient device contained 700 ml of 0.2 *N* pH 3.1 buffer and the second and third chambers each contained 700 ml of 2.0 *N* pH 5.0 buffer. The preparation of the resin and buffers was described by Schroeder *et al.* (1962). The major radioactive peak corresponding to VII was eluted with 950–1060 ml of buffer. This peak contained an average of 5×10^6 dpm. The average recovery of ^{14}C from the column was 12.2×10^6 dpm (88.2%). The fraction containing VII was concentrated to dryness, dissolved in a minimum volume of water, and applied to a 1.75 × 105 cm column of AG 1-X2 (200–400 mesh) resin in the acetate form. This column was kept at 15° while samples were eluted with an acetic acid gradient at a rate of 0.6 ml/min. The gradient device contained 275 ml of water in each of the first two chambers, 275 ml of 0.50 *N* acetic acid in chamber 3, and 275 ml of 0.38 *N* acetic acid in chamber 4. The major fraction from this column was eluted after 650 ml of gradient solvent had been used. This fraction contained an average of 87% of the radioactivity eluted from the column. Total recoveries varied from 72 to 90%. The primary fraction was evaporated to dryness and the residue was extracted with one 5-ml portion and two 1-ml portions of absolute methanol. The methanolic extract was evaporated to dryness, dissolved in water, and chromatographed with water on a 1.75 × 110 cm column of Sephadex LH-20. The pure metabolite was eluted after 153 ml of solvent had been used. Recovery of radioactivity from this column was 92%. In some instances it was necessary to chromatograph the resulting product on a thin-layer plate with solvent A to achieve complete purification.

Hydrogenolysis of VII. Metabolite VII (0.53 μmol) was sealed in a small screw-cap test tube with 1 ml of absolute ethanol and a small excess of activated Raney nickel (Grace #28, W. R. Grace Co.). The suspension was stirred continuously, while the temperature was allowed to rise from 85° to 105° over a 20-hr period. After cooling, the supernatant was decanted and the catalyst was washed successively with 500-μl portions of hot water, hot methanol, and hot water. The supernatant and washes were combined and spotted on a thin-layer plate that had been prewashed with methanol. The chromatogram was developed with solvent F. The radioactive zones detected at R_f 0.19 (39%) and 0.58 (18%) were eluted from the gel with methanol. The product, with an R_f of 0.19, was further purified by tlc with methanol, R_f 0.77. Both products were transferred to capillary tubes for mass spectral analysis.

Treatment of VII with C-S Lyase. Metabolite VII was treated with L-cysteine C-S lyase of *Albizzia lophanta*, prepared by the method of Schwimmer and Guadagni (1968). The reaction mixture contained 2 μmol of VII (500,000 dpm), 0.02 μmol of pyridoxal-5-phosphate, and 1 mg of C-S lyase in 0.500 ml of 0.175 *M* pH 7.5 sodium phosphate buffer. A blank was prepared which was identical to the reaction mixture except for the absence of VII. After the reaction mixture and blank had been incubated for 16 hr at 37°, the reaction mixture was assayed against the blank for pyruvic acid by use of a lactic acid dehydrogenase–NADPH coupled reaction. The assay solution was 0.1 *M* with respect to pH 7.5 sodium acetate buffer. It contained 40 μl of the C-S lyase reaction mixture, 400 μmol of NADPH, and 125 μg of Beef Heart Type III LDH (Sigma) in a volume of 1.10 ml. The corresponding reference was prepared in a similar manner, except that 40 μl of the C-S lyase blank was used instead of the reaction mixture. A standard curve was prepared by using varying amounts of pyruvate in place of the C-S lyase reaction mixture. The difference in absorbance was measured at 340 nm after 45 min at 37°. A 400,000-dpm sample of the C-S lyase reac-

tion mixture from the first step was diluted to 1 ml and adjusted to pH 6 by addition of 0.1 *N* HCl. The solution was partitioned 5× against 1-ml volumes of chloroform. The combined chloroform fraction (339,000 dpm) was streaked onto a 20 × 20-cm thin-layer plate and chromatographed with solvent A. An intense uv quenching zone at R_f 0.6 was scraped from the plate and eluted with five 1-ml portions of methanol and five 1-ml portions of 50% aqueous methanol. Both eluates were combined (244,000 dpm). A 175,000-dpm sample was esterified in absolute methanol by the method described previously. The esterified product was streaked onto three 5 × 20 cm prewashed thin-layer plates and chromatographed with solvent E. The product at R_f 0.67 was eluted from the gel with five 0.5-ml portions of methanol (117,000 dpm) and subjected to mass spectral analysis. A 0.1-μmol sample of the purified product (25,000 dpm) was quantitatively analyzed for the presence of free thiol by the method of Ellman (1959).

Treatment of VII with Performic Acid. A 0.500-μmol sample of VII and a 0.500-μmol sample of cystine were treated with performic acid in a manner similar to that described by Schram *et al.* (1954). The resulting samples were analyzed for cysteic acid by tlc with solvent G. The samples were then hydrolyzed in 6 *N* HCl under a nitrogen atmosphere at 110° for 16 hr and the hydrolysates were analyzed for the presence of cysteic acid by paper chromatography with solvent D and by tlc with solvent B.

Derivatization of VII for Mass Spectral Analysis and Comparison. Aliquots of VII (0.50 μmol) were suspended in 1.5 ml of absolute methanol or absolute ethanol and treated by method I of Greenstein and Winitz (1961). The resulting esters were chromatographed on thin-layer plates with solvent B and eluted from the silica gel 3× with 0.5-ml portions of absolute methanol (average yield, 90%). The products were analyzed by mass spectroscopy. A 0.50-μmol sample of the diethyl ester of VII was reacted with 50 μl of trifluoroacetic anhydride in a sealed vial for 1 hr at room temperature. The excess anhydride was removed under a stream of nitrogen and the product was transferred to a capillary tube for mass spectral analysis.

Synthesis of *N*-(4-Ethylamino-6-isopropylamino-*s*-triazinyl-2)-meso-*L*-lanthionine Diethyl Ester. The diethyl ester dihydrochloride of *L*-meso-lanthionine (A grade, Calbiochem) was prepared by method I of Greenstein and Winitz (1961). The product from 100 mg of lanthionine was dried over calcium chloride and sodium hydroxide under 0.5 mm. The resulting residue was dissolved in 4 ml of absolute ethanol and treated with AG 1-X8 in the free base form, according to the procedure of Nicholls *et al.* (1963). The only modification in the procedure was to use absolute ethanol instead of absolute methanol. The resulting hydrochloride-free ester was concentrated to a heavy syrup (128.4 mg) and dissolved in 10 ml of absolute benzene to which had been added 54 mg (250,000 dpm) of uniformly ^{14}C-ring-labeled atrazine. The mixture was protected from the atmosphere and refluxed for 44 hr. After cooling, the benzene was removed and the precipitated product was dissolved in absolute ethanol. The ethanolic solution was streaked onto thin-layer plates and chromatographed with solvent A. A radioactive uv-absorbing ninhydrin positive product was detected at R_f 0.55; and a radioactive uv-absorbing ninhydrin negative product was detected at R_f 0.66. The product at R_f 0.55 was eluted from the gel with ethanol and chromatographed on a 1 × 100-cm LH-20 column developed with ethanol. The resulting product (96,000 dpm) was compared with the diethyl ester of VII.

Characterization of Metabolites VIII and XII. The 320-ml peak from the chromatogram of the 20-day extract from the time study (Figure 3) was used for characterization of VIII and XII. This fraction was chromatographed on AG 50W-X8 resin in a manner similar to that described by Plaisted and Thornton (1964) and Hurter (1966). The methods were modified in that the 1 × 17-cm column (jacketed at 60°) was packed with AG 50W-X8 resin (200–400 mesh), and 450 ml of 0.25 *N* and 450 ml of 3.0 *N* HCl, respectively, were used in chambers 1 and 2 of the gradient device. Two products were eluted from the column and compared to 2-hydroxy-4-amino-6-ethylamino-*s*-triazine, 2-hydroxy-4-amino-6-isopropylamino-*s*-triazine, hydroxyatrazine, and 2-hydroxy-4,6-diamino-*s*-triazine (ammeline) by thin-layer chromatography with solvents A and O, column chromatography on AG 50W-X2 with the pyridine–acetate buffer gradient system, and by column chromatography on AG 50W-X8 with the HCl gradient described.

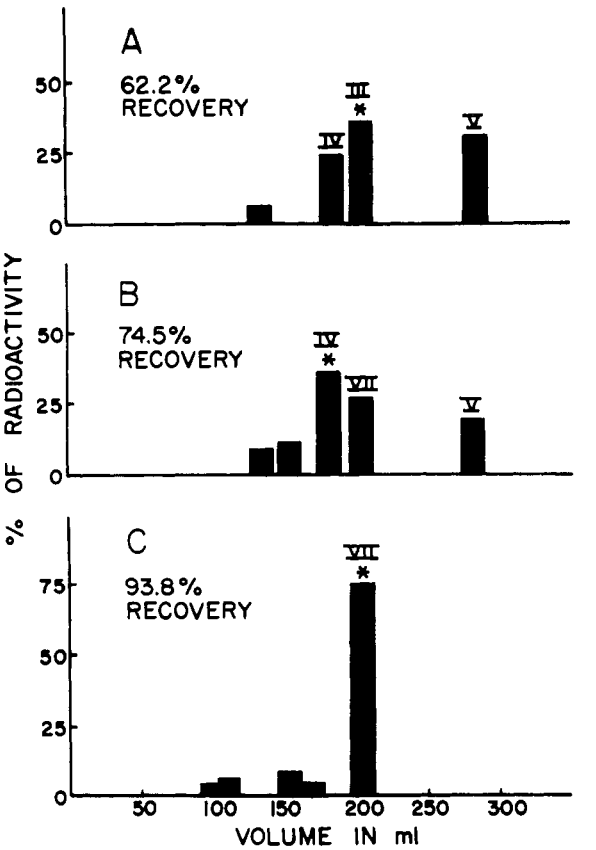

Figure 4. Chromatography of the aqueous extracts from sorghum seedlings treated with (A) III, (B) IV, and (C) VII. In each case 200,000 dpm of the indicated metabolite (*) in 40 μl was injected into the stem of a plant. Plants A and B were harvested 2 days after treatment, and plant C was harvested 10 days after treatment. The resulting extracts were chromatographed on AG 50W-X2 with the pyridine–acetate buffer gradient.

Treatment of Sorghum with Metabolite I. Metabolite I was prepared by isolation from treated pea plants as described by Shimabukuro *et al.* (1966). The sorghum seedlings were grown in nutrient solution under the conditions described in the time study. Three 20-day-old seedlings were treated as one lot with 50 ml of 5 × 10^{-6} *M* uniformly ^{14}C-ring-labeled metabolite I (377,800 dpm) in ⅓-strength Hoagland's solution. After 48 hr, the seedlings were removed from the solution, the roots were rinsed with water, the plants were placed in fresh ⅓-strength Hoagland's solution, and after an additional 72 hr the plants were harvested. The roots and shoots were extracted separately and the extracts were partitioned between chloroform and water. The aqueous phase of the extract from the shoots was chromatographed on AG 50W-X2 with the pyridine–acetate gradient as previously described. An aliquot of the primary fraction from the column (170-ml fraction) was hydrolyzed in 6 *N* HCl at 80° for 16 hr. The hydrolysate was compared by tlc in solvent

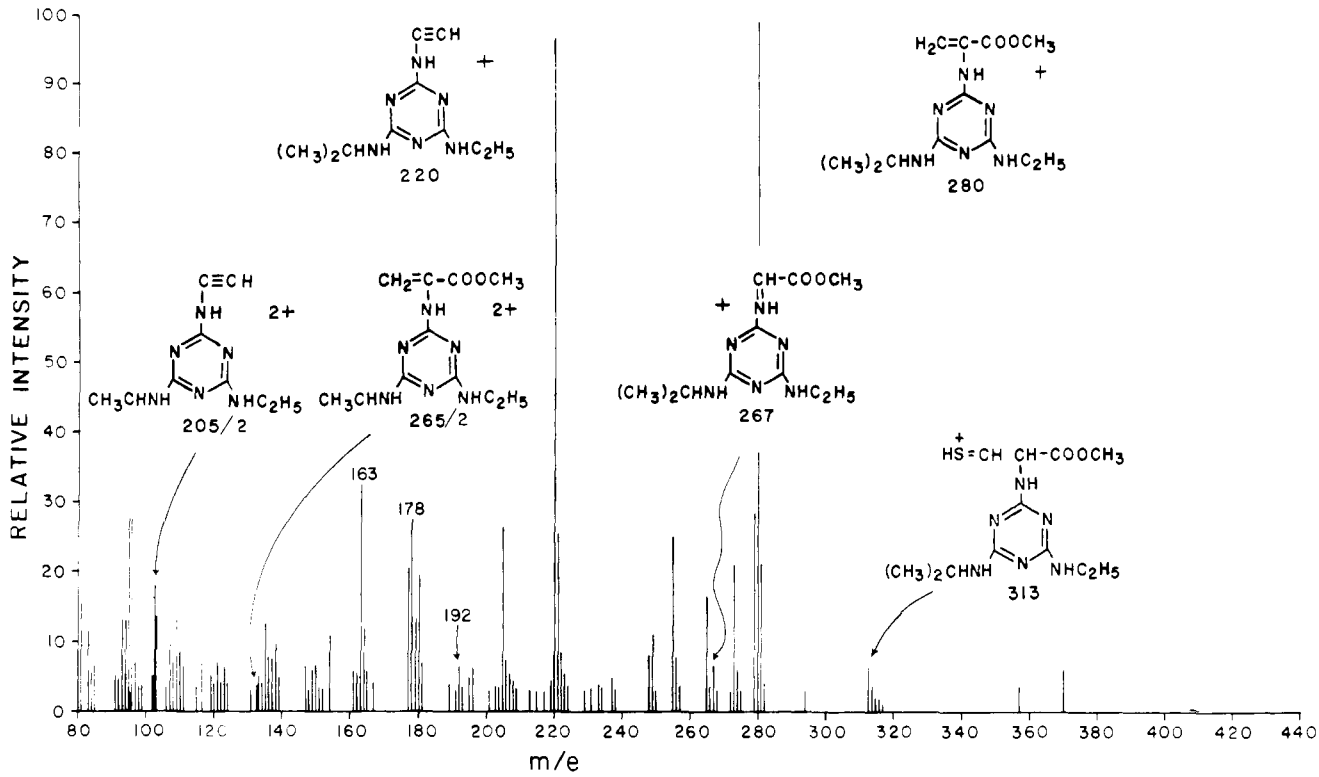

Figure 5. The electron impact mass spectrum of the dimethyl ester of the rearrangement product, obtained after treating IV with γ-glutamyltranspeptidase (this product is the disulfide dimer of metabolite VI, Figure 8).

A to hydroxyatrazine, 2-hydroxy-4-amino-6-isopropylamino-*s*-triazine, 2-hydroxy-4-amino-6-ethylamino-*s*-triazine (IV), ammeline, and the original 170-ml eluate.

RESULTS

Time Study. The translocation of atrazine-^{14}C from the roots to the shoots of sorghum occurred very rapidly and was nearly complete 3 days after the source of atrazine to the roots was removed (5-day harvest, Figure 1). The nearly static percent distribution of ^{14}C between the roots and shoots after 5 days indicated that the atrazine had become immobilized in the plant. The high levels of ^{14}C present in the shoots (93%) as compared to the roots (7%) indicated that the roots were not quantitatively important in the production of terminal atrazine metabolites. This agrees with a previous report in which one of the authors studied the metabolism of atrazine in sorghum over a 2-week period (Shimabukuro, 1967). Although a slow decrease in the total recovery of ^{14}C with time was observed, from 97% after 2 days to 86% after 30 days, this decrease probably resulted from a systematic analytical error related to the 14-fold increase in plant mass that was observed during this same period. Other possible explanations for this decrease in recovery, exudation of atrazine or its metabolites from the roots or shoots, metabolism of atrazine to $^{14}CO_2$, etc., would be expected to cause a shift in the percent distribution of ^{14}C between the roots and shoots. Since a shift was not observed, these explanations seem less likely than the one advanced.

The data in Figure 2 indicate that for periods up to 30 days, water-soluble metabolites represent the most quantitatively significant form of atrazine residue in sorghum. It is also evident that a product–precursor relationship exists between the atrazine residue in the 80% methanol-insoluble fraction and the water-soluble fraction. Extrapolation of these data indicates that these fractions would have contained equal amounts of atrazine residue after approximately 36 days. The ^{14}C in the chloroform-soluble fraction decreased rapidly during the first 10 days and reached a static concentration of about 6%. This agrees with a previous study in which the chloroform-soluble fraction reached a static concentration of 13% after 10 days (Shimabukuro, 1967).

The results obtained by chromatography of the aqueous extracts from sorghum shoots on AG 50W-X2 are shown in Figure 3. The treating solutions of atrazine in ⅓-strength Hoagland's solution which were inoculated with the bacteria present on sorghum roots did not produce any detectable breakdown of atrazine. After 60 hr, the average recovery of ^{14}C from these solutions was 100%. Of this, 98% was partitioned into chloroform, as compared to 99% for the zero time control. Thin-layer chromatographic examination of chloroform phases indicated that no modification of the atrazine-^{14}C had occurred. Thus, it was concluded that the radioactive products observed in the time study were the result of plant metabolism. Metabolites III (*S*-(4-ethylamino-6-isopropylamino-*s*-triazinyl-2)glutathione) and IV (γ-glutamyl-*S*-(4-ethylamino-6-isopropylamino-*s*-triazinyl-2)cysteine) were identified in a previous study (Lamoureux *et al.*, 1970). Although III was not resolved from VII by chromatography on AG 50W-X2, it was possible to differentiate between III and VII by selective acid hydrolysis of III to hydroxyatrazine. Hydroxyatrazine was then separated from VII by thin-layer chromatography and the relative amounts of the two products were determined by liquid scintillation spectrometry. The recovery of ^{14}C during chromatography of the 0.60-day and 2-day extracts averaged 84%, while the recovery of ^{14}C from the five remaining extracts averaged 94%. The lower recovery of ^{14}C from the first two chromatograms may have been caused by the presence of a higher concentration of V, which was shown to be labile under the chromatographic conditions employed. This is discussed in a later section. The loss of chromatographic resolution encountered with the 30-day extract may have been caused by overloading the column, the presence of unstable metabolites, or the presence of more metabolites than could be separated with the available number of theoretical plates in the chromatographic system.

It is apparent from Figure 3 that atrazine metabolism

in sorghum is a complex multistep process in which some of the metabolites such as III, IV, V, and VII are clearly transitory intermediates, while others, such as VIII, appear to be terminal products. Some product-precursor relationships were indicated by this study, but because of the multiplicity of products formed and the rates involved, it seemed desirable to obtain additional data concerning the reaction sequence in this pathway. For this reason, young sorghum seedlings were treated with pure III, IV, and VII by injecting these metabolites into the stems of sorghum seedlings. The seedlings treated with III and IV were harvested 48 hr after treatment. Because of the results of a preliminary study, the seedling treated with VII was not harvested until after 10 days. The recovery of ^{14}C from these seedlings was 76, 84, and 83%, respectively. The aqueous extracts contained 90 to 91% of the recovered radioactivity, but only in the case of VII was the ^{14}C in the insoluble residue determined. It contained 4.8% of the recovered radioactivity. The aqueous extracts from these seedlings were chromatographed on AG 50W-X2. The results in Figure 4A clearly established the conversion of III to IV as an important metabolic reaction, while the absence of III in seedlings treated with IV (Figure 4B) is proof that the reverse reaction is insignificant. A careful comparison of the first three chromatograms in Figure 3 and the chromatograms in Figure 4, A and B, reveals that IV is probably converted to V, which in turn is converted to VII. Figures 3 and 4C show that VII is slowly degraded to other products that have elution volumes of 110 to 170 ml. Although it is understood that other possibilities such as the direct conversion of atrazine to IV were not excluded by this study, the data in Figures 3 and 4 are consistent with the metabolic sequence: atrazine → III → IV → V → VII → other products.

Identification of Metabolite V. The results from the time study indicated that V was a key intermediate produced by the metabolism of IV. Although it did not appear that V would be a persistent residue in sorghum, the key position it occupies in the pathway made it desirable that its structure be determined.

When V was isolated by chromatography on AG 50W-X2 and rechromatographed on AG 50W-X2 under the same conditions, it did not elute in the original 275-ml fraction. Instead, several new radioactive products were detected. The major product had an elution volume of 375 ml, but the recovery of radioactivity during rechromatography was low and irreproducible. When an aqueous extract from sorghum that contained V as 25% of the radioactivity was incubated in pH 7.5 phosphate buffer for 16 hr at 37° and chromatographed on AG 50W-X2, metabolite V was not detected. After incubation at pH 7.5 for 16 hr and readjustment to pH 6.5, 15.1% of the radioactivity in the aqueous extract could be partitioned into chloroform. When this chloroform-soluble fraction was chromatographed on AG 50W-X2, 46% of the recovered radioactivity was in the 375-ml fraction, but total recovery of radioactivity from the column was only 33%.

Based on these preliminary data, V was hypothesized to be *S*-(4-ethylamino-6-isopropylamino-*s*-triazinyl-2)cysteine. An unambiguous synthesis of this compound was carried out by the action of γ-glutamyltranspeptidase on pure γ-glutamyl-*S*-(4-ethylamino-6-isopropylamino-*s*-triazinyl-2)cysteine (IV). (This is the third reaction in the primary sequence indicated in Figure 8.) The crude reaction product was treated with 2,4-dinitrofluorobenzene and methanol-HCl. Attempts to purify the resulting DNP methyl ester by the gas chromatographic method of Ikekawa *et al.* (1966) were not successful, and it was subsequently purified by thin-layer chromatography. Considerable decomposition of the derivative was encountered during purification. The mass spectrum of the yellow, radioactive derivative was characterized by a weak peak corresponding to the expected molecular ion at *m/e* 480, and intense peaks at *m/e* 424, 421, 267, 227, and 213. The ion at 424 was probably bis(4-ethylamino-6-isopropylamino-*s*-triazinyl-2)disulfide, formed by pyrolysis of the derivative on the sample probe which was heated to 200°. The ion at 421 corresponds to the expected loss of $COOCH_3$ from the parent ion, the ion at 267 to the fragment $CH_2CNH(DNP)COOCH_3$, the ion at 227 to 2-methylthio-4-ethylamino-6-isopropylamino-*s*-triazine, and the base peak at 213 to 2-mercapto-4-ethylamino-6-isopropylamino-*s*-triazine. The spectrum was consistent with the expected derivative *N*-(2,4-dinitrophenyl)-*S*-(4-ethylamino-6-isopropylamino-*s*-triazinyl-2)cysteine methyl ester. The original product of the enzymatic synthesis was concluded to be *S*-(4-ethylamino-6-isopropylamino-*s*-triazinyl-2)cysteine.

The *S*-cysteine conjugate from the enzymatic synthesis had a retention volume of 275 ml on AG 50W-X2, but attempts to purify this compound resulted in its rearrangement to a product with an elution volume of 375 ml. The mass spectrum of the dimethyl ester of the rearrangement product was obtained in the usual manner, and in addition, portions of this spectrum were scanned for the presence of decoupled metastable ions. The spectrum and the probable structures of the key ions are shown in Figure 5. Based on this spectrum, the derivative was concluded to be *N,N'*-bis(4-ethylamino-6-isopropylamino-*s*-triazinyl-2)cystine dimethyl ester. Although a parent ion (626) was not observed, the product was assumed to be the oxidized disulfide dimer because before esterification it did not produce a positive reaction for a free thiol with Ellman's Reagent. The ion at 313 was probably formed by cleavage of the disulfide bond. Metastable daughters of the ion, *m/e* 313, were detected at 280 and 267, indicating a common origin for these ions. The ion at 280 was the base peak in the spectrum and was probably formed by cleavage α to the disulfide bond. The much less intense ion at 267 was probably formed by cleavage β to the disulfide bond. The intense ion at 220 corresponds to the loss of $HCOOCH_3$ from the base peak. The ions at 205, 192, 178, and 163 were detected in the normal spectrum and as metastable daughters of the ion, *m/e* 220. Corresponding losses from *m/e* 280 were also detected but they were of much lower intensity and were not detected in the scan of metastable ions. These ions were probably formed from the ions at *m/e* 280 and 220 by loss of the neutral fragments CH_3, C_2H_4, C_3H_6, and C_3H_6NH from the ethylamino and isopropylamino side chains of the triazine moiety. The doubly charged ions at 102.5 and 132.5 correspond to the loss of CH_3 from doubly charged ions of mass 220 and 280. The presence of doubly charged ions corresponding to the loss of CH_3 from intense ion fragments has been detected in other spectra of 4,6-bis(alkylamino)-*s*-triazines (Lamoureux *et al.*, 1972). The ions at 102.5, 132.5, 163, 178, 192, and 205 indicate the presence of the 4-ethylamino-6-isopropylamino-*s*-triazinyl moiety in the ions at 220 and 280. This mass spectrum is very similar to the published spectrum of *N,N'*-bis(trifluoroacetyl)-*L*-cystine dimethyl ester (Harp and Gleason, 1971). In the spectrum of the trifluoroacetyl derivative, the base peak was produced by cleavage α to the disulfide bond, and intense peaks were observed for ions formed by loss of $HCOOCH_3$ from the base peak and by cleavage of the disulfide bond. The ion resulting from cleavage β to the disulfide bond was only 8% of base peak.

Based on these data, the rearrangement product from the enzymatic synthesis was concluded to be *N,N'*-bis(4-ethylamino-6-isopropylamino-*s*-triazinyl-2)cystine, the disulfide dimer of VI (Figure 8). This compound was subsequently identified as a chloroform-soluble metabolite of atrazine in sorghum (Shimabukuro *et al.*, 1973).

The properties of biosynthetic *S*-(4-ethylamino-6-isopropylamino-*s*-triazinyl-2)cysteine and the corresponding disulfide dimer of the *N*-cysteine conjugate are compared with V and its major rearrangement product in

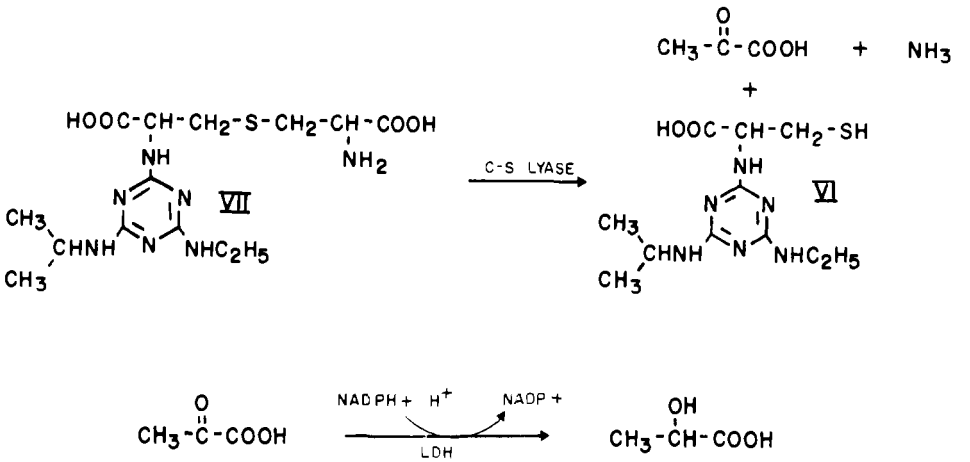

Figure 6. Treatment of *N*-(4-ethylamino-6-isopropylamino-*s*-triazinyl-2)lanthionine (VII) with C-S lyase, and subsequent analysis of the liberated pyruvic acid with a NADPH-lactic acid dehydrogenase-coupled reaction.

Table I. Comparison of the Properties of V with Synthetic *S*-(4-Ethylamino-6-isopropylamino-*s*-triazinyl-2)cysteine

Method of comparison	Metabolite V	Synthetic product
A. Chromatography on AG 50W-X2	Retention volume, 275 ml	Retention volume, 275 ml
B. Rechromatography of the isolated 275-ml fractions on AG 50W-X2	The major product eluted at 375 ml, but several minor peaks were detected, and the recovery of ^{14}C was low	Retention volume, 375 ml
C. Incubation in pH 7.5 buffer for 16 hr at 37°	V was quantitatively altered to other products, 60.4% of which were $CHCl_3$-soluble at pH 6.5. The major $CHCl_3$-soluble product had an elution volume of 375 ml on AG 50W-X2	The *S*-cysteine conjugate was quantitatively altered to a $CHCl_3$-soluble product, which had an elution volume of 375 ml on AG 50W-X2
D. Thin-layer chromatography	The primary rearrangement product of V and the rearrangement product of the *S*-cysteine conjugate cochromatographed on the two-dimensional thin-layer chromatographic system employed	

Table I. As a result of this comparison, metabolite V was concluded to be *S*-(4-ethylamino-6-isopropylamino-*s*-triazinyl-2)cysteine.

Isolation and Characterization of VII. Metabolite VII was purified from an aqueous extract of sorghum plants by chromatography on AG 50W-X2 resin, AG 1-X2 resin, and chromatography on LH-20. Analysis of the product on a Technicon amino acid analyzer and thin-layer chromatography in solvents A and B indicated that VII was radiochemically homogeneous and free from ninhydrin-reactive or ultraviolet-absorbing contaminants. Metabolite VII was not altered by incubation in 6 *N* HCl at 40° for 16 hr, indicating that it was not a sulfhydryl conjugate similar to III or IV. It also appeared to be more resistant to hydrogenolysis in the presence of Raney nickel than either III or IV. However, two radioactive products were liberated by hydrogenolysis of VII. The major product was obtained in a 39% yield and the minor product was obtained in an 18% yield. The major product was tentatively identified by mass spectroscopy as *N*-(4-ethylamino-6-isopropylamino-*s*-triazinyl-2)alanine and the minor product was tentatively identified as *N*-(4-ethylamino-6-isopropylamino-*s*-triazinyl-2)alanine methyl ester.

The enzyme, L-cysteine C-S lyase of *Albizzia lophanta*, is specific for the L-cysteine or L-cysteine sulfoxide moiety, unaltered except for substitution on the sulfur atom (Schwimmer and Kjaer, 1960). The products of the enzyme-catalyzed reaction are pyruvate, ammonia, and a free thiol. When two samples of VII were incubated with C-S lyase, a 92% (mol/mol) yield of nonradioactive pyruvate was obtained from each sample. The principal radioactive product from the reaction partitioned into chloroform in an 85% yield after the reaction medium had been adjusted to pH 6. A 0.1-μmol sample of the chloroform-soluble product was found to contain less than 0.005 μmol of free thiol when analyzed by the method of Ellman (1959). The reaction product cochromatographed with *N,N'*-bis(4-ethylamino-6-isopropylamino-*s*-triazinyl-2)cystine in a two-dimensional tlc system. The mass spectrum of the methyl ester of this product was identical to the mass spectrum of the methyl ester of *N,N'*-bis(4-ethylamino-6-isopropylamino-*s*-triazinyl-2)cystine discussed in the identification of V. These results indicated that VII was either *N*-(4-ethylamino-6-isopropylamino-*s*-triazinyl-2)cystine or *N*-(4-ethylamino-6-isopropylamino-*s*-triazinyl-2)lanthionine. The reaction of *N*-(4-ethylamino-6-isopropylamino-*s*-triazinyl-2)lanthionine with C-S lyase and the analysis of pyruvate are illustrated in Figure 6.

If VII was the conjugate of cystine, it would be expected to yield 1 μmol of cysteic acid/μmol of conjugate treated with performic acid, but the corresponding conjugate of lanthionine would not be expected to liberate cysteic acid in an appreciable yield. When VII was treated with performic acid, less than 0.05 μmol of cysteic acid was liberated/μmol of conjugate treated. These results indicated that VII was probably the lanthionine conjugate.

The methyl ester of VII was prepared, and the electron impact and chemical ionization mass spectra of the product were measured. These spectra and the probable structures of the principal ions are shown in Figure 7. The minor peaks at *m/e* 398 and 339 in the ei spectrum are probably due to the loss of ammonia and from loss of both ammonia and a carbethoxy group from the amino termi-

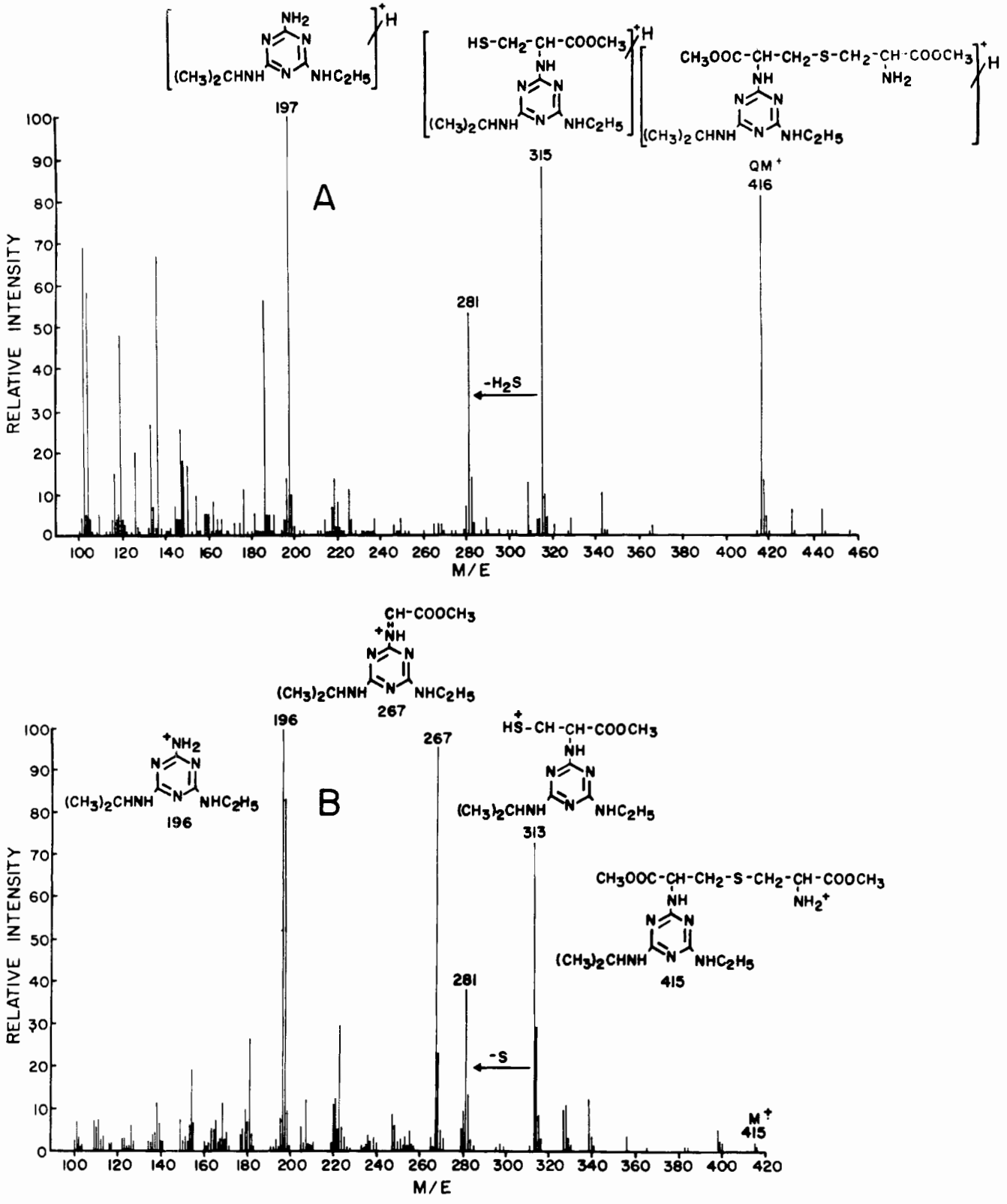

Figure 7. (A) The chemical ionization mass spectrum of the dimethyl ester of VII (obtained by use of methane gas). The chemical ionization mass spectrometry was performed by the Battelle Memorial Institute Mass Spectrometry Laboratory. (B) The electron impact mass spectrum of the dimethyl ester of VII.

nal lanthionine residue in a manner analogous to that described for lysine by Biemann (1962). The spectra are consistent with that of the proposed lanthionine conjugate. The mass spectra of the diethyl ester of VII and the diethyl ester mono-*N*-trifluoroacetyl derivative of VII were also consistent with the proposed structure.

Synthesis of *N*-(4-Ethylamino-6-isopropylamino-*s*-triazinyl-2)-meso-*L*-lanthionine Diethyl Ester and Comparison with the Diethyl Ester of VII. *N*-(4-Ethylamino-6-isopropylamino-*s*-triazinyl-2)-meso-*L*-lanthionine diethyl ester was synthesized by the general procedure of D'Alelio and Underwood (1943). The mass spectrum of the product was consistent with the expected structure and contained a weak molecular ion at *m/e* 443. The spectrum was nearly identical to that of the diethyl ester of VII. Both the diethyl ester of VII and the synthet-

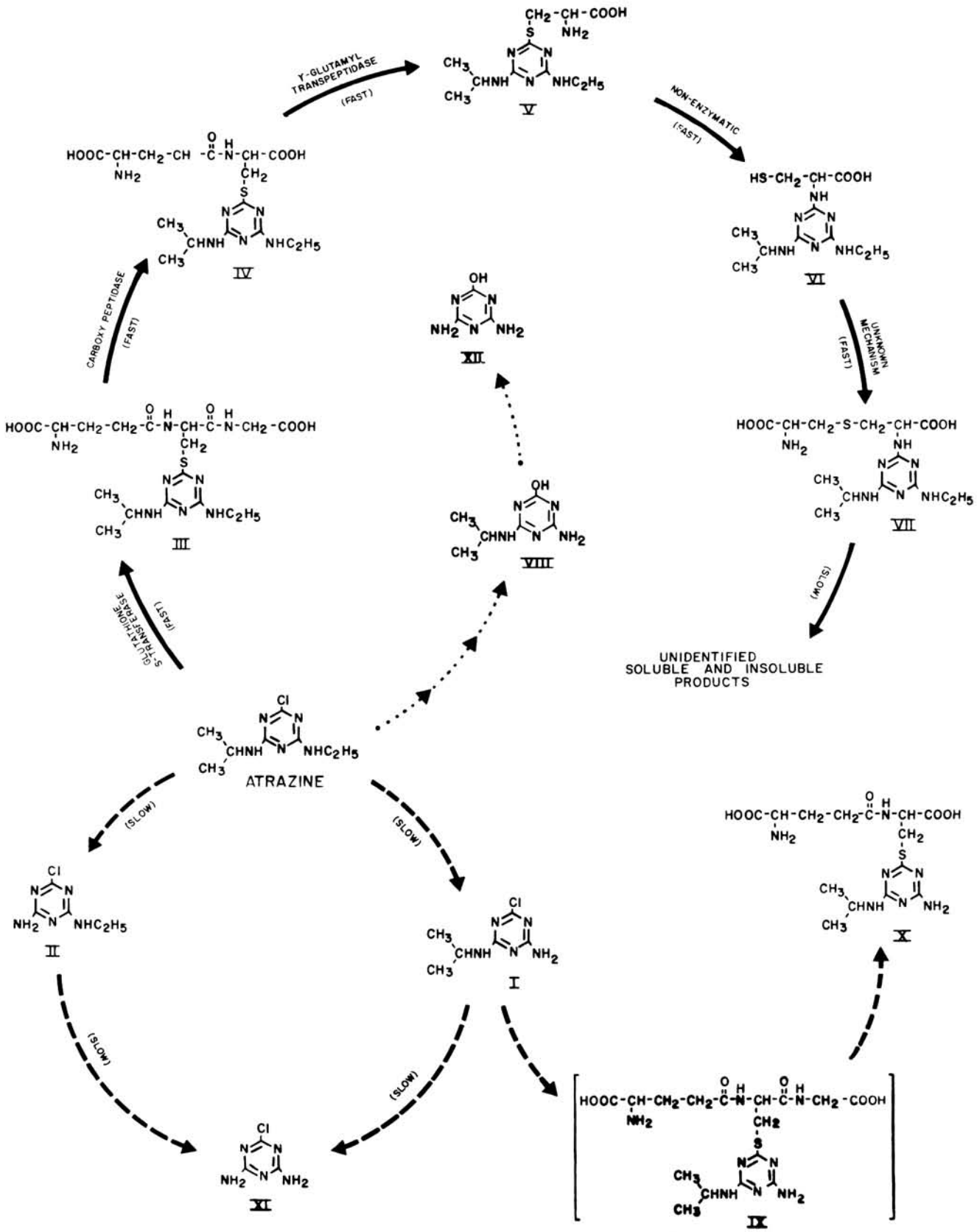

Figure 8. The metabolic pathway of atrazine in sorghum. The major pathway is indicated by bold arrows, the dashed arrows indicate minor pathways, and the dotted arrows indicate a hypothesized reaction(s). The structure in brackets was not indentified.

ic product chromatographed at the same R_f in five different tlc systems (Table II).

Metabolite VII was concluded to be *N*-(4-ethylamino-6-isopropylamino-*s*-triazinyl-2)lanthionine. This compound contains two asymmetric carbon atoms. The α-carbon atom adjacent to the primary amino group on the lanthionine moiety was shown to be in the L configuration on the basis of the C-S lyase reaction. However, the configuration on the α'-carbon atom of the lanthionine moiety was not determined.

Characterization of VIII and XII. The slow appearance and steady increase in the concentration of metabo-

Table II. Thin-Layer Chromatographic Comparison of the Diethyl Ester of VII with the Diethyl Ester of Synthetic *N*-(4-Ethylamino-6-isopropylamino-*s*-triazinyl-2)-meso-*L*-lanthionine

Solvent system	Diethyl ester of VII	N-(4-Ethylamino-6-isopropylamino-*s*-triazinyl-2)-meso-*L*-lanthionine diethyl ester
A	R_f 0.55	R_f 0.54
B	R_f 0.77	R_f 0.76
H	R_f 0.33 and 0.35	R_f 0.31 and 0.35
I	R_f 0.61	R_f 0.58
K	R_f 0.62	R_f 0.59

Table III. Distribution of ^{14}C in Sorghum Treated with ^{14}C-Labeled 2-Chloro-4-amino-6-isopropylamino-*s*-triazine (Compound I)

	Water-soluble	Chloroform-soluble
Shoots (5.7 g fresh wt)	77.6%	15.3%
Roots (6.4 g fresh wt)	6.0%	1.1%

Three sorghum plants were root-treated for 48 hr with 5×10^{-6} *M* uniformly ring-labeled 2-chloro-4-amino-6-isopropylamino-*s*-triazine. The plants were harvested and extracted 72 hr after the treatment. The plants absorbed 61% of the substrate. 87% of the radioactivity used in the experiment was recovered.

lites VIII and/or XII (Figure 3) indicated that they were terminal metabolites. Hydroxyatrazine, 2-hydroxy-4-amino-6-isopropylamino-*s*-triazine, and 2-hydroxy-4-amino-6-ethylamino-*s*-triazine chromatographed with the same elution volume as VIII and XII (320 ± 6 ml) on AG 50W-X2. However, when the 320-ml fraction from the 20-day harvest (Figure 3) was chromatographed on AG 50W-X8 with the HCl gradient, two products were eluted from the column in a yield of 92.2%. The major component VIII (66%) had the same retention volume as 2-hydroxy-4-amino-6-isopropylamino-*s*-triazine (378 ml) and the minor component XII (34%) had the same retention volume as ammeline (198 ml). Under these conditions, hydroxyatrazine had a retention volume of 666 ml, and 2-hydroxy-4-amino-6-ethylamino-*s*-triazine had a retention volume of 306 ml. Metabolites VIII and XII were further confirmed to be 2-hydroxy-4-amino-6-isopropylamino-*s*-triazine and ammeline, respectively, by co-thin-layer chromatography with solvents A and O. Both of these chromatographic systems were capable of resolving a mixture of the above mentioned *s*-triazine standards. The structures of metabolites VIII and XII are shown in Figure 8.

Compound I, the expected precursor of VIII, is rapidly metabolized to water-soluble compounds in intact sorghum, as indicated by the data in Table III. However, when the aqueous extract obtained from sorghum treated with I was chromatographed on AG 50W-X2, VIII was not detected. The primary metabolite had a retention volume of 170 ml and accounted for 30% of the ^{14}C eluted from the column. This is comparable to the elution volume of IV. When the metabolite with an elution volume of 170 ml was subjected to mild acid hydrolysis, 2-hydroxy-4-amino-6-isopropylamino-*s*-triazine was liberated. This is consistent with an earlier report (Lamoureux *et al.*, 1972) in which compounds thought to be 2-hydroxy-4-amino-6-alkylamino-*s*-triazines were detected as minor products from hydrolysis of "purified" γ-glutamylcysteine conjugates of five different 4,6-bis(alkylamino)-*s*-triazines that were obtained from excised sorghum leaves. In each of these hydrolysates, the 2-hydroxy-4,6-bis(alkylamino)-*s*-triazine corresponding to the parent herbicide was the major product.

DISCUSSION

The major pathway of atrazine metabolism in sorghum appears to be as indicated by the bold arrows in Figure 8. The first step, formation of the glutathione conjugate, was first demonstrated by Lamoureux *et al.* (1969, 1970). The reaction is catalyzed by a glutathione *S*-transferase enzyme which is present in the leaves of sorghum, corn, and other 2-chloro-4,6-bis(alkylamino)-*s*-triazine-resistant plant species (Frear and Swanson, 1970). Metabolite IV was identified by Lamoureux *et al.* (1969, 1970). The conversion of III to IV was indicated by the data in Figures 3 and 4. This reaction is typical of the type catalyzed by carboxypeptidase enzymes which have been found in higher plants (Visuri *et al.*, 1969; Wells, 1965). The conversion of IV to V was demonstrated *in vitro* using γ-glutamyltranspeptidase from hog kidney; however, this enzyme is also known to be present in at least some species of higher plants (Thompson *et al.*, 1964). This conversion is consistent with the data in Figures 3 and 4. The metabolic sequence, through the formation of V, closely resembles the mercapturic acid pathway found in mammals (Boyland and Chasseaud, 1969). One triazine, 2-chloro-4-(ethylamino)-6-(1-cyano-6-methylethylamino)-*s*-triazine, has been shown to be metabolized *via* the mercapturic acid pathway in the rat (Crayford and Hutson, 1972; Hutson *et al.*, 1970). To this point, the only difference between the mercapturic acid pathway and the pathway under investigation is the order in which the glutamyl and glycine residues are removed from the glutathione conjugate. The obligatory step in the mercapturic acid pathway, N-acetylation of the terminal amino residue of cysteine, was not detected in the metabolism of atrazine in sorghum. Instead of undergoing N-acetylation, V rearranges to VI. *In vitro* studies showed this nonenzymatic rearrangement to be nearly quantitative after 16 hr at 37° in pH 7.5 buffer. Metabolite VI was detected and isolated as the chloroform-soluble disulfide dimer in a concurrent study of the chloroform-soluble metabolites of atrazine in sorghum (Shimabukuro *et al.*, 1973). The rearrangement of the *S*-cysteine conjugate to the *N*-cysteine conjugate is analogous to the facile rearrangement of *S*-DNP-cysteine to *N*-DNP-cysteine, which occurs at near neutral pH (Hansen *et al.*, 1959). This rearrangement probably involves the formation of a five-membered cyclic intermediate. The difficulty encountered by Crayford and Hutson in the attempted synthesis of *S*-cysteine conjugates of triazines was attributed to this type of rearrangement (Crayford and Hutson, 1972).

Although the lanthionine conjugate of atrazine (VII) is probably formed from VI, the mechanism of this reaction was not determined. The authors felt that VII might be formed from serine and VI in a reaction catalyzed by a transulfurase in a manner analogous to the biosynthesis of cystathionine from homocysteine and serine. However, radioactive VII was not detected in sorghum plants treated with atrazine and U-^{14}C-labeled serine. Lanthionine can be formed either by the base-catalyzed hydrolysis of cystine (Sloane and Untch, 1966) or by the photochemical decomposition of cystine under acidic conditions (Asquith and Shah, 1971). Therefore the possibility that VII may be formed by condensation of VI with cysteine to form the mixed disulfide mono-*N*-(4-ethylamino-6-isopropylamino-*s*-triazinyl-2)cystine, which then expels one atom of sulfur by either of the above processes to yield the corresponding sulfide VII, should be considered.

Lanthionine is frequently associated with embryonic tissue, but the role of this amino acid seems to be uncertain. Sloane and Untch (1966) presented evidence that in chick embryo it is protein-bound, and they have suggested that it may function there as a cross-linking amino acid. This hypothesis is interesting because a high percent of the terminal residue of atrazine in sorghum is in the form of 80% methanol-insoluble compounds. Although it would

seem likely that the insoluble residue may be in the form of protein-bound materials, no direct evidence was obtained in this study that indicated the nature of this residue. Only 4.4% conversion of VII to insoluble residue was observed 10 days after treatment, and four water-soluble metabolites amounting to 22% of the dose were formed, indicating that if lanthionine is a precursor to the insoluble residue, the mechanism of incorporation may involve more than a single step.

N-Dealkylation of the ethylamino- and isopropylamino side chains, as indicated by the dashed lines in Figure 8, is a relatively slow reaction which competes with the major conjugation pathway. Compounds I and II, resulting from these slow reactions, were established as minor metabolites of atrazine in sorghum by Shimabukuro (1967); however, the presence of XI was only recently reported (Shimabukuro *et al.*, 1973). The results of this study indicate that at least one of these metabolites (I) is slowly converted to the corresponding γ-glutamylcysteine conjugate in a pathway that parallels the major glutathione conjugation pathway of atrazine. Metabolites VIII and XII (Figure 8), which were characterized in this report, are the 2-hydroxy analogs of I and XI. Cursory consideration of the structures of these metabolites and the previously demonstrated mode of formation of VIII and XII in corn would lead to the conclusion that in sorghum they are probably formed by hydrolysis of I and XI. Although this possibility was not completely eliminated by the results in this study, the fact that VIII was not detected in sorghum 5 days after treatment with I does open this hypothesis to serious question. Because of this, the mechanism of formation of VIII and XII is not precisely indicated in Figure 8.

It is apparent from the data presented that the major pathway indicated in Figure 8 is important in the metabolism and deposition of atrazine in sorghum. Although it was previously shown that this pathway operates almost to the complete exclusion of other pathways in excised sorghum leaves (Lamoureux *et al.*, 1972), quantitative evaluation of this pathway in root-treated intact plants is difficult because of translocation dynamics and contributions to metabolism *via* other pathways in the roots. The data in Figures 1–4 show that the minimum possible contribution of the major pathway to the metabolism of atrazine in sorghum is approximately 40%. If the unidentified metabolites in Figure 4C are assumed to be the same as those in Figure 3, and if the insoluble residue in the shoots is formed as a result of the major pathway indicated, the actual contribution of this pathway may approach 87%. Although VII was the last metabolite identified that could be attributed to the major pathway indicated, it was shown that this compound is slowly converted to other products. Further research will be necessary to determine the nature of the ensuing steps of metabolism.

ACKNOWLEDGMENT

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