Metabolism of AC 206,784 Herbicide
[2-Chloro-*N*-(2,3-dimethylphenyl)-*N*-(1-methylethyl)acetamide] in Soybean Plants

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The metabolic fate of AC 206,784 [2-chloro-*N*-(2,3-dimethylphenyl)-*N*-(1-methylethyl)acetamide], an acetanilide herbicide, in soybean plants grown in soil treated with [^{14}C]AC 206,784 was investigated. The major metabolic pathways involved substitution of the 2-chloroacetamide moiety with the oxamic acid moiety, resulting in the formation of *N*-isopropyl-2,3-dimethyloxanilic acid (metabolite III) and oxidation of the 3-methyl group on the benzene ring of metabolite III to form 3-(hydroxymethyl)-*N*-isopropyl-2-methyloxanilic acid (metabolite IV). The metabolites were, in turn, conjugated to form polar products. Incubation of soybean seedlings in hydroponic solutions of AC 206,784 and metabolite III indicated that the plant absorbed AC 206,784 and readily converted it to polar conjugates, but it lacked the ability to convert it to metabolite III, and this conversion occurred in the soil. However, the soybean plant easily absorbed metabolite III from the water solution and further metabolized it to metabolite IV.

AC 206,784 [2-chloro-*N*-(2,3-dimethylphenyl)-*N*-(1-methylethyl)acetamide] is a selective herbicide for the control of annual grasses and is applied preplant incorporated (PPI) or preemergence (PE). There are a limited number of references in the literature regarding the uptake and metabolism of α-chloroacetamide herbicides. Jaworski (1964) and Smith et al. (1966) studied the metabolism of CDAA and other ^{14}C-labeled α-chloroacetamides and reported that soybean seeds and plants readily absorbed these herbicides from the treated soil and rapidly metabolized them, yielding glycolic acid as the major product. Jaworski (1969) also reported that different species of plants irrespective of their resistance or susceptibility to the α-chloroacetamide herbicides have the ability to metabolize these compounds, but the metabolism is much faster in the resistant plants such as soybean and corn.

Hydrolysis of the 2-chloro moiety of α-chloroacetamides is an important metabolic reaction in certain plant species. Lamoureux et al. (1971) studied the metabolism of 2-chloro-*N*-isopropylacetanilide (propachlor) and found evidence that one of the pathways for metabolism of α-chloroacetamide herbicides in higher plants involved glutathione conjugation.

This investigation of the metabolic fate of [^{14}C]AC 206,784 was initiated to determine the nature of major metabolites in the soybean plant to aid the toxicologist in evaluating their significance as crop residues.

MATERIALS AND METHODS

Radiolabeled Material. AC 206,784 and metabolite III (*N*-isopropyl-2,3-dimethyloxanilic acid) labeled with carbon-14 in the 2,3-dimethyl positions of the xylidine moiety and AC 206,784 labeled with carbon-13 in the 3-methyl position of the xylidine moiety were prepared by Dr. M. W. Bullock of American Cyanamid Co., Princeton, NJ.

Treatment of Field Plots with [^{14}C]AC 206,784. Equal amounts of [^{14}C]AC 206,784 and [^{13}C]AC 206,784 were mixed to achieve a final amount of 400 mg of AC 206,784 of the specific activity of 13 μCi/mg. The 50:50 mixture of the two isotopes was used to obtain doublet ion peaks in the mass spectra of AC 206,784 or its metabolites.

These doublets provide a means for distinguishing peaks due to the metabolites from the single peaks of nonlabeled naturally occurring compounds that are always present, even after extensive purification. Appropriate quantity of the AC 206,784 mixture [4 lb of active ingredient (a.i.)/acre] was transferred into a 6-oz. Brinkman sprayer bottle and a 4E formulation was prepared and mixed with 20 mL of distilled water. The preparation was evenly sprayed to cover an area of 3 ft × 3 ft in an experimental plot. One-half hour after spraying, the soil was raked 3 in. deep. One day after the treatment, soybean seeds (Adelphi variety) were sown and covered with soil. This method of herbicide application simulated PPI (preplant incorporated) treatment.

Sample Processing. Plants were harvested by cutting the stems 1–2 in. above the ground level at 1, 2, 3, and 5 months after sowing the seeds. For extraction of [^{14}C]AC 206,784 derived radioactivity from the plant tissues, 1- and 2-month-old soybean plants were homogenized as whole plants. Pods were separated from the foliage of 3- and 5-month-old soybean plants. Beans were also separated from the hull of mature (5-month-old) plants, and each tissue was analyzed separately.

For extraction of radioactivity from foliage and pod samples, the extraction scheme shown in Figure 1 was used. The sample was extracted with methanol (10 mL/g of tissue) followed by methanol containing 2% HCl. Combined extracts were concentrated and analyzed by TLC to determine relative proportions of metabolites.

In the case of soybean seed samples, because of the presence of oils in the seed, different extraction procedures were employed. The seeds were allowed to stand in dry ice for 1 h and ground to powder in a coffee grinder. Radioactivity from the powdered sample was extracted with successive series of solvent mixture (10 mL/g of seeds). These included methanol–chloroform–methanol (1:1 v/v), methanol–ammonium hydroxide (98:2 v/v), and methanol–hydrochloric acid (98:2 v/v). Combined extracts were concentrated, and the residue was partitioned between *n*-hexane and acetonitrile. The acetonitrile phase was concentrated and analyzed by TLC.

Chromatography. Thin-layer chromatography was performed on commercial precoated silica gel plates (Merck F-254). Development of chromatograms was accomplished in one or two solvent mixtures which were (A) nitromethane–chloroform (50:50 v/v) and (B) benzene–methanol–acetic acid (50:50:1 v/v/v). The radioactive

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                                   Plant Sample

                                        |
                                        |

       Radioactivity extracted with methanol followed by methanol containing HCl (2% v/v)

                                        |
            _____|_____
           |                                                         |
    Methanol Extract                                               Cake
           |
    _____|_____
   |                        |
Methanol evaporated, residue      Analyzed
partitioned between water and     by TLC
ethyl acetate
           |
     _____|_____
    |                                                                  |
Aqueous Phase I                                          Ethyl Acetate Phase I
    |                                                                  |
    |                                           _____|_____
    |                                          |                                                      |
Hydrolyzed with 3N HCl and              Analyzed                       Ethyl acetate evaporated
partitioned between ethyl               by TLC                         and residue dissolved in
acetate and water solution                                             water
    |                                                                              |
  __|_____                                         Sephadex G-10 Column
 |                           |                                                      |
Aqueous              Ethyl Acetate                                     Sephadex LH-20 Column
Phase II             Phase II                                                       |
 |                           |                                                   HPLC
 |                           |                                                      |
Adjustedd             Analyzed                                         Purified Metabolites isolated
pH at 10.0            by TLC                                                        |
 |                                                          _____|_____
 |_____                             |                                        |
 |                           |                        Derivatized                            Analyzed
Aqueous              Chloroform Phase                   III & IV                             by GC-MS
Phase III                    |                              |                                  I & II
                        Analyzed                        Analyzed
                        by TLC                          by GC-MS
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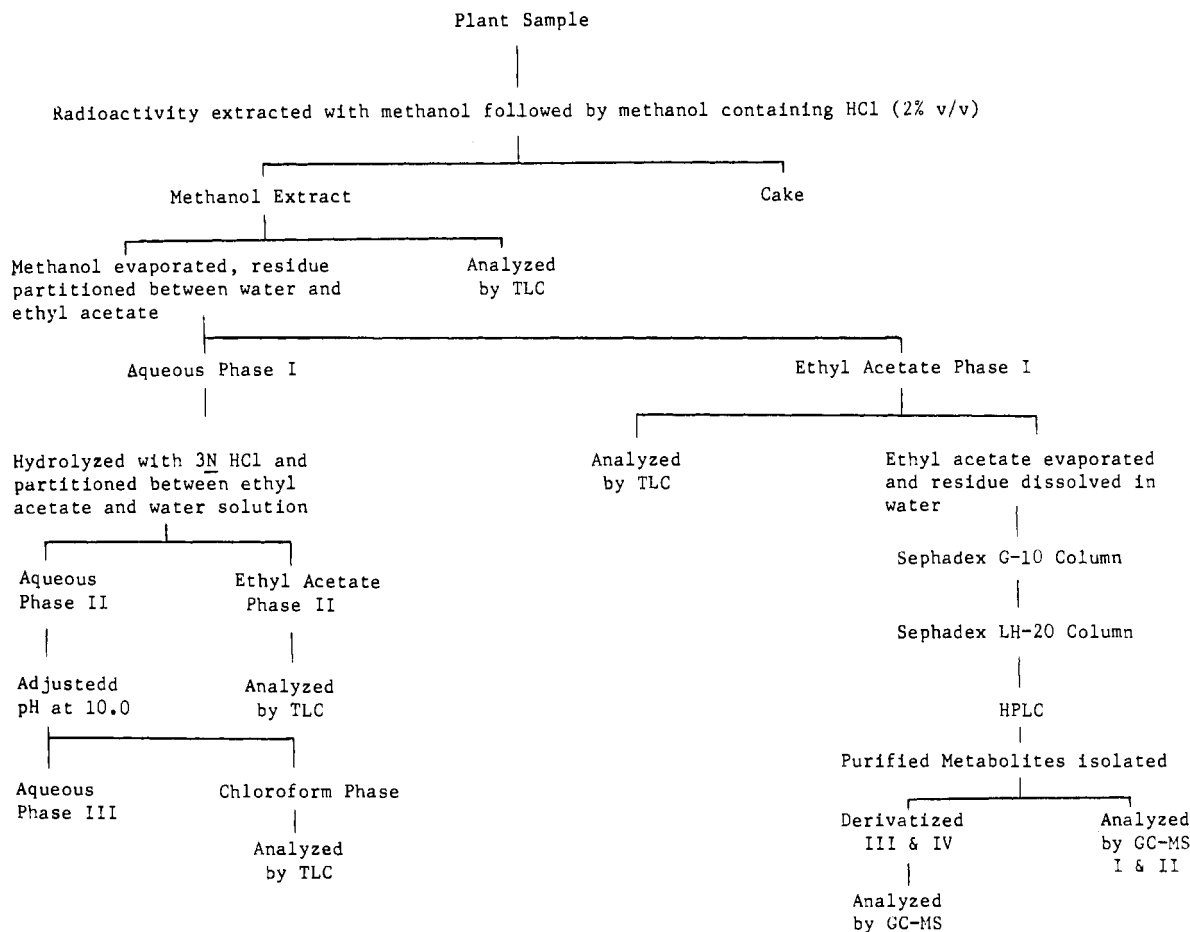
**Figure 1.** Flow diagram of the procedures used for isolation of metabolites from the soybean plant.

extracts obtained from the plant tissues were spotted on thin-layer plates along with standard reference nonradiolabeled AC 206,784 and authentic metabolites and developed by the two-dimensional system. Radiolabeled compounds were visualized in a Birchover Instrument radiochromatogram spark chamber (Model 986-010) or by radioautography on Kodak SB-54 single-coated blue-sensitive medical X-ray film (Eastman Kodak Co., Rochester, NY). The nonradiolabled reference compounds were visualized with ultraviolet light. Radioactive areas from the TLC plates were assayed by using the method of Ku et al. (1978). In this method, an Intertechnique Multi-Mat system consisting of a Model SL 300 scintillation spectrometer and a Microdata 1600 computer (Intertechnique, Westwood, NJ) was used. Aquasol-2 (New England Nuclear, Boston, MA) was used as the scintillation cocktail. For confirmation of the identity of the nonpolar metabolites by gas–liquid chromatography, a Tracor 550 gas chromatograph (Tracor Instruments, Austin, TX) was used. The gas chromatograph was equipped with a flame ionization detector and a Packard Model 894 radioactivity monitor for simultaneous detection of the radioactive metabolite from the plant as well as the nonradioactive standard when injected as a mixture. The GC was equipped with a 1 m × 4 mm i.d. glass column packed with 10% OV-101 on 80–100-mesh Gas-Chrom Q. GC conditions were as follows: helium carrier gas, 20 mL/min; column inlet temperature, 240 °C; column temperature, 220 °C.

Isolation of Major Metabolities. For isolation of major metabolites plant extract was partitioned between water and ethyl acetate, and the ethyl acetate phase was concentrated and purified by gel filtration chromatography. This was accomplished by elution from a 1 m long

(2.5-cm i.d.) chromatographic column packed with Sephadex G-10 gel (Pharmacia Fine Chemicals AB, Uppsala, Sweden) using distilled water as the eluting solvent and an elution rate of 1.5 mL/min. The eluted metabolites were further purified on a second chromatographic column, this one (1 m long, 1.5-cm i.d.) packed with Sephadex LH-20 gel. Methanol was used as the eluting solvent for this column and elution rate was 2.0 mL/min. The metabolites eluting from these columns were then purified by a liquid chromatograph (Model 7000, Micromeritics Instrument Corp., Norcross, GA) equipped with an O.D.S. M-9 preparative column. Acetonitrile (5%) in water was used as the solvent in this analysis, and the flow rate was maintained at 1.5 mL/min. The identity of major metabolites [metabolite III and metabolite IV, 3-(hydroxymethyl)-*N*-isopropyl-2-methyloxanilic acid] was confirmed by derivatizing the metabolites into their methyl esters (*N*-methyl-*N'*-nitro-*N*-nitrosoguanidine in 5% KOH) and cochromatography of the derivatives with the synthetic compounds by TLC as well as GC. Identity of the metabolites or their derivatives was also verified by GC mass spectral analysis. The CIMS analysis of the purified metabolites or their derivatives was performed by using a computerized Finnigan Model 4023 gas chromatograph–mass spectrometer (GC–MS) (Finnigan Corp., Sunnyvale, CA). Methane was used as the ionizing gas.

The aqueous phase was refluxed in 3 N HCl for 0.5 h to hydrolyze various conjugates. The solution was adjusted to pH 2.0 and the radioactivity extracted with ethyl acetate and analyzed by TLC. The aqueous phase II was adjusted at pH 10 and extracted with chloroform. The chloroform phase was analyzed by TLC.

Uptake of [^{14}C]AC 206,784 and [^{14}C]Metabolite III from Aqueous Solution by Hydroponically Grown
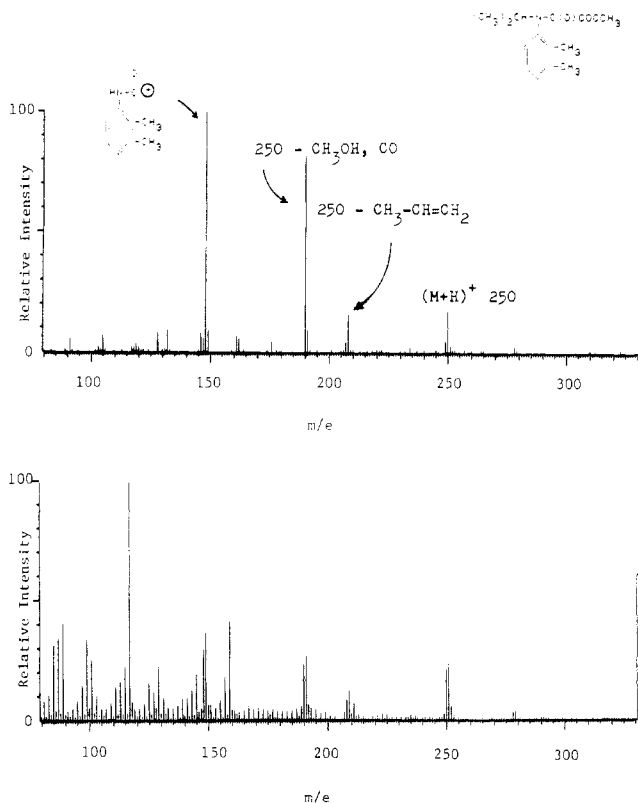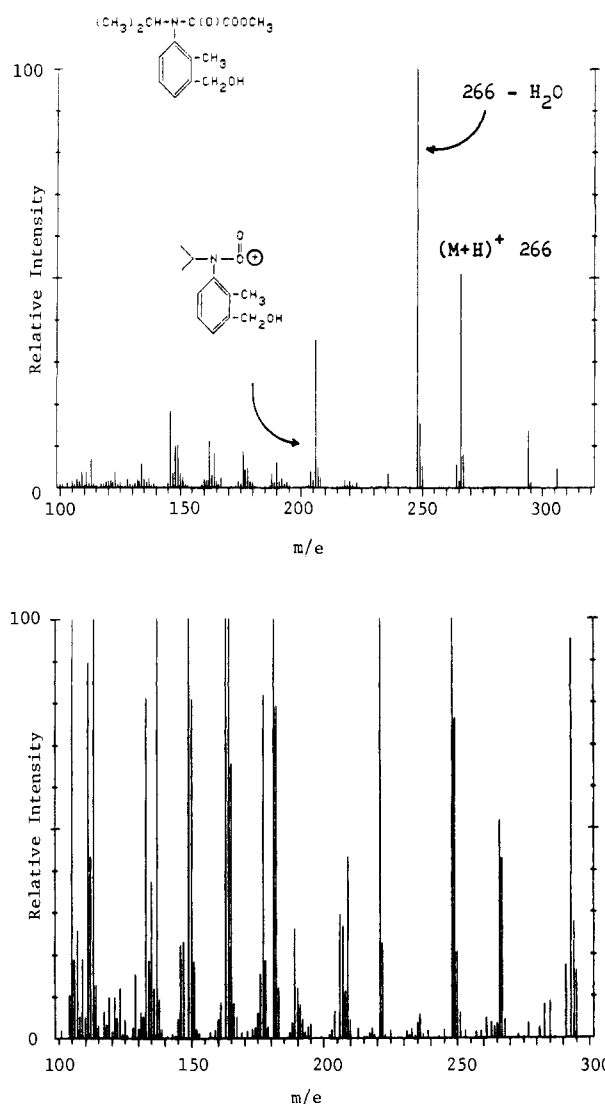
Figure 2. CIMS spectra of methylated metabolite III. Authentic standard (top) and methylated plant metabolite (bottom).

Plants. Two-week-old soybean seedlings which had been grown in a mixture of perlite and vermiculite (50:50) were taken out of the pots, their roots were washed, and the seedlings were placed in flasks containing aqueous solutions of [^{14}C]AC 206,784 (1.5 ppm) with a specific activity of 26 μCi/mg or ^{14}C-labeled metabolite III (10 ppm) with a specific activity of 0.888 μCi/mg. The roots of the seedlings were dipped into the radioactive solutions, while the aerial parts stayed outside the flask. The plants were allowed to absorb AC 206,784 for 7 days and metabolite III for 8 days. The foliage was separated from the roots and the radioactivity extracted from the foliage with methanol. The methanol solution was analyzed by TLC.

RESULTS AND DISCUSSION

Identification and Characterization of AC 206,784 Derived Radioactivity in Plants. In Table I, data related to the distribution of metabolites of AC 206,784 identified and characterized in various tissues of soybean plants at the indicated time intervals are presented.

Metabolite I [N-(2,3-dimethylphenyl)-N-isopropyl-2-(methylsulfinyl)acetamide] and its sulfone analogue, metabolite II [N-(2,3-dimethylphenyl)-N-isopropyl-2-(methylsulfonyl)acetamide] were present in small quantities in the immature plant. Compound I was found in measurable quantities only in the 1-month-old plant. Compound II, however was consistantly found in the extracts of immature plants, although not exceeding 2% of the total AC 206,784 derived radioactivity. In the 3-month-old plant, this metabolite was found in measurable amounts (1.7%) only in the foliage and not in the pods. No residues of this metabolite were detected in the tissues of mature plants. Chemical ionization mass spectrometry (CIMS) of the isolated I indicated the expected parent ion, (M + H)$^+$ ion, at m/e 268 and 269 which is consistent with the synthetic compound. Similarly, CIMS of the isolated II indicated the presence of the parent ion, (M + H)$^+$ ion, at m/e 284 and 285.

Figure 3. CIMS spectra of methylated metabolite IV. Authentic standard (top) and methylated plant metabolite (bottom).

Metabolites III and IV were the major metabolites of AC 206,784 and were found in all plant tissues at all time intervals. CIMS of the methylated III confirmed the presence of the expected (M + H)$^+$ ion at m/e 250 and 251 which is consistent with that of the standard (Figure 2). Both spectra show prominent fragment ions at m/e 208 (m/e 250 – CH$_3$CH=CH$_2$), 190 (m/e 250 – CH$_3$OH, CO) and 148 (m/e 250 – CH$_3$OH, CO, CH$_3$CH=CH$_2$). Similarly, methylated IV yielded a CIMS with the expected (M + H)$^+$ ion at m/e 266 and 267 which is consistent with that for the standard (Figure 3). Major fragment ions in these spectra are at m/e 248 (m/e 266–H$_2$O) and 206 (m/e 266–CH$_3$OH, CO). The relative proportion of the two metabolites changed as the plant grew older. Thus, in the 1-month-old plant, III was the most abundant metabolite, consistuting 56% of the total AC 206,784 derived radioactivity in the plant. In the 2-month-old and older plants, except for the mature seed, IV was present in larger proportions than III. However, in the mature soybean seed, III was present in twice as much quantity as IV. Lee (1979) has shown in studies related to the metabolism of AC 206,784 in the soil that III was always present in larger quantities than IV. This indicated that after having been taken up by the plant, substantial quantities of III are converted to IV within the plant.

Conversion of Metabolite III to Metabolite IV by the Soybean Plant. Soybean plants were kept in hy-

Table I. Relative Concentration of AC 206,784 Metabolites in Different Parts of the Soybean Plant at Various Intervals after Treatment of Soil with AC 206,784[a]

metabolite	1.0 month, foliage	2.0 months, foliage	3.0 months foliage	3.0 months pods	5.0 months foliage	5.0 months pod hull	5.0 months beans
			% concn of recovered radioact				
I	0.93						
II	1.90	1.95	1.73				
III	56.10	32.14	21.89	38.13	18.09	15.51	38.50
IV	19.02	34.33	32.90	39.84	36.24	38.70	17.29
V and conjugates	15.03	20.87	30.87	13.24	21.32	14.70	17.74
VI (unknown)	2.95	6.73	8.41		12.21		
unidentified compounds	1.93[b]		1.60[b]	4.99[b]			2.32[c]
total extractable radioact	97.86	96.02	97.40	96.20	87.86	68.91	75.85
unextracted radioact	2.14	3.98	2.60	3.80	12.14	31.09	24.15

[a] Treatment of Princeton soil with [^{14}C]AC 206,784 (specific activity 13.0 μCi/mg) at a 4 lb of a.i./acre rate. [b] Two unidentified minor metabolites on the TLC plate. [c] Radioactivity partitioned into the *n*-hexane phase during sample processing.
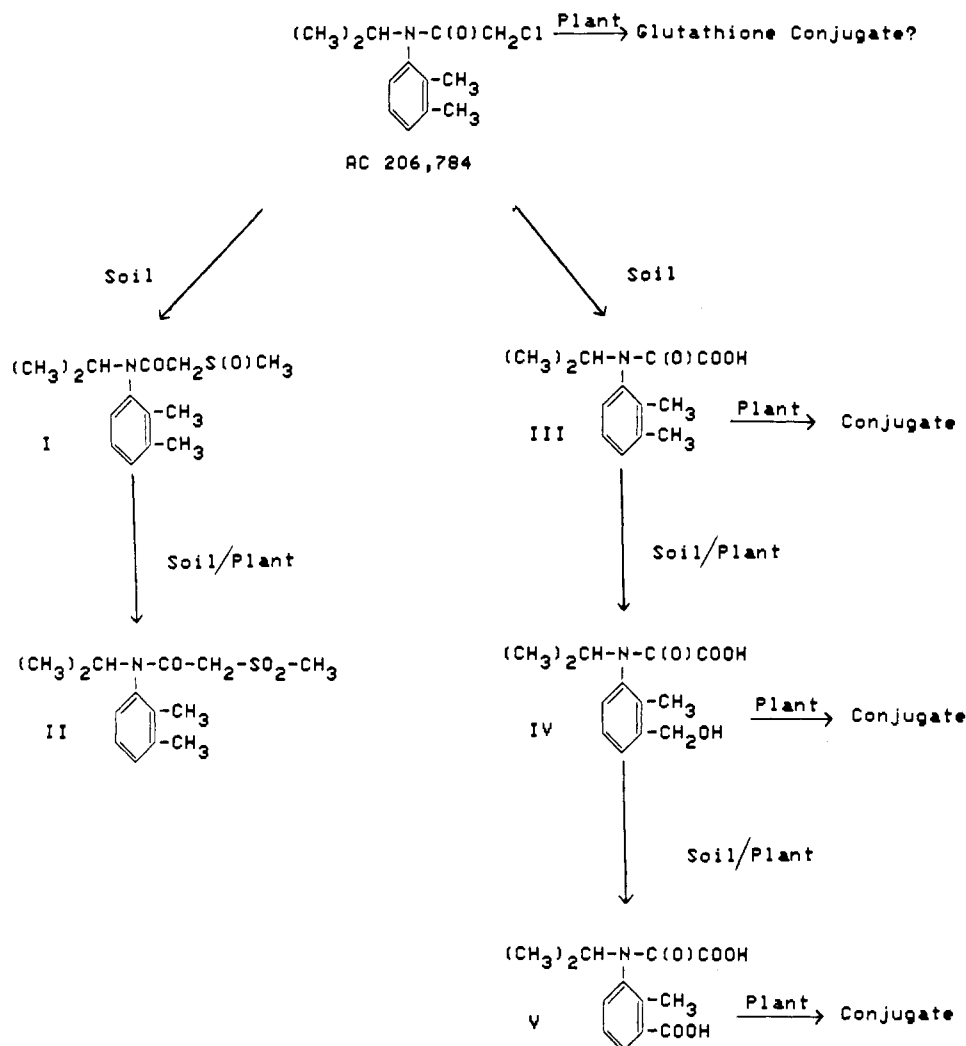

Figure 4. Proposed pathway for the metabolism of AC 206,784 in the soybean plant.

droponic solution containing 10 ppm of ^{14}C-labeled compound III for 8 days. a radioautograph of the whole plant showed uniform distribution of radioactivity in the plant. Residues in the plants were 26 ppm, and 98.2% of the radioactivity was extractable with methanol–acidic methanol. TLC of the extractable radioactivity showed six radiocomponents—three nonpolar and three polar. The three nonpolar components comprised 8.9% of the extracted radioactivity in the foliage. These metabolites were not identified. The polar ones were compound III (52.7%),

IV (30.8%), and a spot representing uncharacterized material, possibly conjugates of metabolites III and IV (5.8%). The results of this experiment showed that within 8 days about half of III taken up by the soybean plants had been converted to other compounds, mostly to IV. Thus, it suggests that metabolite IV is formed not only in soil but also within the plant tissue itself. When roots of soybean seedlings were immersed in an aqueous solution of AC 206,784 and the radioactivity from the foliage (5.45 ppm) was extracted (99.9% extractable) and analyzed by TLC,

Table II. Distribution of Radioactivity following Hydrolysis in 3 N HCl of the Aqueous Phase from Three-Month-Old Soybean Plants Grown in Soil Treated with [¹⁴C]AC 206,784

period of incubation, h	% distribution in indicated phase following adjustment of indicated pH		
	ethyl acetate (pH 2.0)[a]	chloroform (pH 10)[b]	aqueous (pH 10)
0.25	64.44	6.38	29.18
0.50	60.28	8.90	30.82
1.00	58.76	9.58	31.66
5.50	51.71	27.41	20.88

[a] Thin-layer chromatography of ethyl acetate solution showed the presence of metabolite III (46.05%), metabolite IV (24.07%), and six minor compounds (30.00%), none of which was more than 8.60%. [b] Thin-layer chromatography of chloroform solution showed the presence of N-isopropyl-2,3-xylidene (41.99%), 3-(isopropylamino)-2-methylbenzyl alcohol (54.26%), and 3-(isopropylamino)-o-toluic acid (3.75%).

the extractable material showed no trace of AC 206,784. Instead there were three radioactive areas, all three polar in nature. These products might be comprised of the gluthathione conjugatge and γ-glutamylcysteine conjugate of AC 206,784. Such a pathway for the metabolism of α-chloroacetamide herbicides, including propachlor which is structurally similar to AC 206,784 in higher plants, has been suggested by Frear and Swanson (1970) and Lamoureux et al. (1971). The radioautographs did not show any trace of III or IV. It indicates that the soybean plants lack the ability to convert AC 206,784 to its metabolite III and that this conversion occurs in the soil by microorganisms. Once the soil microorganisms convert AC 206,784 to III, the plant readily absorbs it and further converts it to IV and conjugated products. Metabolite V (3-carboxyl-N-isopropyl-2-methyloxanilic acid) was not isolated on the TLC plates since it moved with the conjugated metabolites. However, acid hydrolysis of the aqueous phase released free 3-(isopropylamino)-o-toluic acid in addition to other compounds, indicating the presence of V in free or conjugated form.

Compound VI was found in all soybean foliage samples at different stages of plant growth. However, it was not present in the soybean pods and seeds. It was present in small quantities in the young plants but as the plants grew, its concentration increased somewhat, reaching a maximum (12.2% of total radioactivity) in the foliage of the harvest plants. The structure of this metabolite was not determined, but its TLC behavior indicated that it is a polar compound. It could not be methylated when heated with BF₃–methanol up to 110 °C for 2.5 h; instead, a nonpolar product was formed probably due to thermal decomposition. GC–MS of this product showed a fragment ion at m/e 192, 193 which could be for N-(2,3-dimethylphenyl)-N-isopropylformamide.

Acid Hydrolysis. TLC of the aqueous phase I from soybean extract (Figure 1) showed that it contained mostly polar materials and III and IV were not present in significant quantities. This phase was hydrolyzed with 3 N HCl and the results are shown in Table II. A major part of the radioactivity (68.3–79.1%) was organosoluble. Most of the radioactivity (51.7–64.4%) partitioned at pH 2.0 into

ethyl acetate (phase III) with the remainder going into chloroform phase after adjustment of the pH to 10. TLC analysis of the ethyl acetate phase II showed metabolite III (46.0%), metabolite IV (24.0%), and six smaller radiocomponents (30.0%), none of which exceeded 8.6% of the radioactivity in the ethyl acetate phase II. TLC of the chloroform phase showed that major components in this solution were N-isopropyl-2,3-xylidene (42.0%), 3-(isopropylamino)-2-methylbenzyl alcohol (54.3%), and 3-(isopropylamino)-o-toluic acid (3.7%) which probably resulted from the hydrolysis of III, IV, and V, respectively. Thus, it appears that polar materials consist primarily of conjugates of metabolites III and IV, with some free or conjugated V. An increase in the period of hydrolysis from 0.25 to 1.0 h with dilute HCl did not seem to release significantly more free compounds (Table II). On the contrary, prolonged hydrolysis for 5.5 h resulted in the cleavage of the C–N bond, releasing increased amounts of the secondary amines mentioned above.

CONCLUSIONS

A proposed scheme for the degradation of AC 206,784 in soybean plant grown in soil treated with ¹⁴C-labeled compounds is summarized in Figure 4. Studies on the metabolism of the compound in soil showed that AC 206,784 was rapidly metabolized in soil (Lee, 1979). Analysis of soybean plants grown in soil treated with AC 206,784 showed III and IV as the two major metabolites. Other metabolites isolated from these plants were I, II, V, and an unidentified polar metabolite. Metabolites III, IV, and V were also present in conjugated forms. No effort was made to determine the nature of conjugation.

Experiments in which young soybean plants were exposed to III under hydroponic conditions showed that this compound was absorbed by the root system and rapidly metabolized to IV. From the metabolism profile, it appeared that III was taken up by the plants from the soil and oxidized to IV as well as conjugated. Some of the IV was, in turn, conjugated and some of it further oxidized to V which was also conjugated.

LITERATURE CITED

Frear, D. S.; Swanson, H. R. Phytochemistry 1970, 9, 2123–2132.
Jaworski, Ernest G. J. Agric. Food Chem. 1964, 12, 33–37.
Jaworski, Ernest G. J. Agric. Food Chem. 1969, 17, 165–170.
Ku, C. C.; Kapoor, I. P.; Rosen, J. D. J. Agric. Food Chem. 1978, 26, 1352–1357.
Lamoureux, G. L.; Stafford, L. E.; Tanaka, F. S. J. Agric. Food Chem. 1971, 19, 346–350.
Lee, P. W., American Cyanamid Company, unpublished data, 1979.
Smith, G. R.; Clark, A. P.; Jaworski, E. G. "Abstracts of Papers", 152nd National Meeting of the American Chemical Society, New York, NY, 1966; American Chemical Society: Washington, DC, 1966.