Picloram Metabolism in Leafy Spurge: Isolation and Identification of Glucose and Gentiobiose Conjugates

D. Stuart Frear,* Harley R. Swanson, and Eugene R. Mansager

Picloram (4-amino-3,5,6-trichloropicolinic acid) metabolism was studied in rooted cuttings and excised leaves of leafy spurge (*Euphorbia esula* L.). [*carboxy*-^{14}C]Picloram was readily absorbed, translocated, and metabolized to polar products. Solvent partitioning, together with adsorption, ion-exchange, thin-layer, and high-performance liquid chromatography were used to isolate methanol-soluble metabolites from [^{14}C]picloram-treated leaves. Isolated metabolites were identified by qualitative analysis of hydrolysis products and by mass spectroscopic analysis (CI/MS) of methylated and/or acetylated derivatives. Minor acidic metabolites were identified as picloram *N*-glucoside conjugates. A major neutral metabolite fraction was identified as a mixture of isomeric picloram glucose esters, and a minor neutral metabolite fraction was identified as a mixture of isomeric picloram gentiobiose esters.

Leafy spurge (*Euphorbia esula* L.) is a serious weed problem in both the United States and Canada (Dunn, 1979; Moore and Frankton, 1969; Messersmith and Lym, 1983). As an aggressive deep-rooted perennial weed, leafy spurge grows under a variety of environmental conditions and is associated primarily with pastures, grazing lands, rights of way, parks, and nonagricultural land areas.

Available herbicide treatments for leafy spurge are limited, and repeated applications are often required for effective control (Lym and Messersmith, 1983). Picloram (4-amino-3,5,6-trichloropicolinic acid) is one of the most effective auxinic herbicides recommended for postemergence use in the selective control of leafy spurge (Lym and Messersmith, 1983, 1985). Unfortunately, detailed information concerning the behavior and metabolic fate of picloram in leafy spurge has not been reported.

Metabolism studies in a variety of sensitive and tolerant plants have shown that picloram forms water-soluble complexes that can be hydrolyzed to yield picloram (Hall and Vanden Born, 1988; Hallmên, 1974, 1975; Sharma and VandenBorn, 1973a; Hallmên and Eliasson, 1972; Eliasson and Hallmên, 1973; Kudaikina et al., 1981a). Recent picloram metabolism studies have identified acid-labile *N*-glucoside and alkali-labile glucose ester conjugates in sunflower and other plants (Chkanikov et al., 1983; Kudaikina et al., 1981a). Conjugates of picloram with mustard oils in radish and mustard plants also have been reported (Chkanikov et al., 1984).

The purpose of the present study was to isolate and identify the major metabolites of picloram in leafy spurge.

MATERIALS AND METHODS

Chemicals. Picloram and [*carboxy*-^{14}C]picloram (0.97 μCi/μmol) were provided by Dow Chemical Co. Based on TLC and HPLC analysis, chemical and radiochemical purities were greater than 99%.

Radioactivity Measurements. Quantitative ^{14}C measurements were made with Insta-Gel (Packard Instrument Co.) and a Beckman 6800 liquid scintillation spectrometer. Methanol-insoluble ^{14}C residues were determined by combustion analysis with a Packard Model 306 sample oxidizer. A Packard Model 7220 radiochromatogram scanner was used to detect separated ^{14}C zones on TLC plates.

Instrumentation. Mass spectra were obtained with a Varian MAT 112S spectrometer equipped with a combination EI/CI source and a S-200 data system. Samples were inserted with a solid sample probe. Ammonia was used as the ionizing gas for chemical ionization (CI) spectra.

Chromatography. Silica gel HF plates (Supelco, 250 or 500 μm) were used for thin-layer chromatography (TLC) of ^{14}C metabolites. Chromatograms were developed in paper-lined tanks with the following solvent systems: (a) CH_2Cl_2–MeOH–H_2O–HOAc (65:25:4:4, v/v); (b) CH_2Cl_2–MeOH–H_2O (65:25:4, v/v); (c) 2-butanone–HOAc–H_2O (10:1:1, v/v); (d) methyl acetate–2-propanol–H_2O (18:1:1, v/v); (e) ether; (f) 1-butanol–95% EtOH–NH_4OH (2:1:1, v/v); (g) 1-butanol–95% EtOH–H_2O (40:11:19, v/v). Separated metabolites were located with a Packard 7220 radiochromatogram scanner or by fluorescence quenching under UV light and eluted with CH_3CN–H_2O (7:3, v/v). Multiple solvent development was used for improved metabolite separation.

A high-performance liquid chromatography (HPLC) system (Waters Associates) with a 254-nm UV detector and a Model 1055 CAI radioactivity monitor was used for ^{14}C metabolite separation and quantitation. HPLC separations were performed on C_{18} Nova-Pak (4–5-μm) or Radial-Pak (10-μm) cartridges (Waters Associates) with 15-min linear gradient programs. The following gradients were used: (A) 0–60% CH_3CN in H_2O; (B) 15–60% CH_3CN in H_2O; (c) 20–80% CH_3CN in H_2O; (D) 5–80% MeOH in aqueous 1% HOAc. Nova-Pak cartridges were used with gradient B at a flow rate of 1.5 mL/min. All other gradients were used with Radial-Pak cartridges at flow rates of 2.0 mL/min.

A Varian Model 3700 gas chromatograph with FID and a glass column (180 cm × 2 mm (i.d.)) packed with 3% SP-2100 (Supelco) on 80/100-mesh Supelcoport was used for gas/liquid chromatographic (GLC) analysis of carbohydrate TMS derivatives. Injector and detector temperatures were at 250 °C. Isothermal oven temperatures were either 185 °C (glucose derivatives) or 245 °C (gentiobiose derivatives), and the helium gas flow rate was 30 cm^3/min.

Metabolite Derivatization. Acidic (fraction I) metabolites were methylated in MeOH–Et_2O (1:1, v/v) by reaction with an excess of CH_2N_2 at 4 °C for 16 h. Acetylated derivatives were prepared by reaction with an excess of acetic anhydride–pyridine (10:1, v/v) at 40 °C for 4 h. Excess reagents were removed with a stream of nitrogen.

Carbohydrate Analysis. Purified metabolites were hydrolyzed with 0.1 N HCl at 80 °C for 2 h or 1 N NH_4OH at 40 °C for 2 h. Hydrolysates were concentrated to dryness, reacted with a slight excess of Tri-Sil Z (Pierce) at 50 °C for 2 h, and analyzed by GLC. Reference standards were prepared in the same manner.

Plant Material and Treatments. Selected plant accessions of leafy spurge (*E. esula* L.) were propagated by stem or root cuttings placed in Vermiculite with nutrient solution (Blankendaal et al., 1972) or by root cuttings placed in soil moistened with tap water. Established cuttings were maintaned in an environmental growth chamber or the greenhouse. [^{14}C]Picloram treatments were made at room temperature with a 12-h photoperiod under cool white fluorescent light (50–60 μE m^2 s^{-1}).

Translocation and Metabolism Studies. In a preliminary short-term in vivo translocation and metabolism study, roots of a 6-week-old cutting (accession 1978-MI-001) were washed with distilled water and pulse-treated for 24 h with 1 mL of [^{14}C]pi-

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Table I. Translocation and Metabolism of Root-Absorbed [^{14}C]Picloram

tissue[a]	MeOH-insol, %	MeOH sol,[b] % metabolites (R_f 0.33, 0.80)	MeOH sol,[b] % picloram (R_f 0.59)
root	<1	3	2
stem	<1	4	12
leaves	1	23	54

[a] Six-week-old rooted cutting (accession 1978-MI-001). Tissues harvested 48 h after initiation of 24-h pulsed root treatment. [b] ^{14}C products separated by TLC (solvent g).

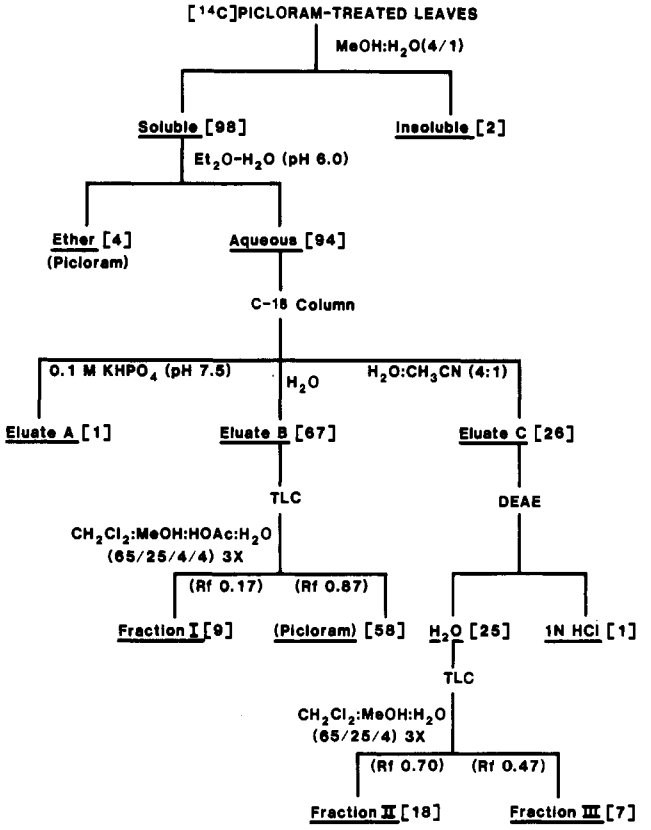

Figure 1. Extraction and separation of picloram metabolite fractions I–III. Brackets indicate percent ^{14}C distribution.

cloram solution (0.5 μCi; sp act. 0.97 μCi/μmol). Treated roots were rinsed with distilled water and maintained in distilled water for an additional 24 h before the tissues (roots, stem, leaves) were separated and extracted with MeOH–H_2O (4:1, v/v). Extracts were vacuum-filtered (10-μm LC Millipore), and the insoluble residue was analyzed for ^{14}C by combustion. Filtrates were taken to dryness (in vacuo 30 °C) and dissolved in 1 mL of MeOH–H_2O (4:1, v/v) for TLC separation and analysis of ^{14}C products (Table I).

Metabolite Extraction and Purification. Large-scale excised leaf treatments (accession 1978-MI-001) were used for metabolite isolation and identification. Approximately 500 leaves (25 g fw) were washed with distilled water for 30 min and treated via the cut ends with 10 mL of a pH 6.5 [^{14}C]picloram solution (2.38×10^{-3} M; 0.13 μCi/μmol). Distilled water was added as necessary during the treatment. After 72 h the treated tissues were weighed, frozen in liquid N_2, and stored at −12 °C until metabolites were extracted and identified.

Procedures for extraction and separation of three major metabolite fractions (I–III) are summarized in Figure 1. Treated leaf tissues (25 g fw) were homogenized for 3 min in an Omnimixer with 200 mL of MeOH–H_2O (4:1, v/v). The homogenate was vacuum-filtered (10-μm LC Millipore) and concentrated in vacuo (30 °C). The aqueous concentrate (15 mL) was adjusted to pH 6.0 and partitioned twice with equal volumes of ether. After ether extraction the aqueous phase was concentrated to a small volume (5 mL), adjusted to pH 7.5 with 1.0 M K_2HPO_4/KH_2PO_4 buffer,

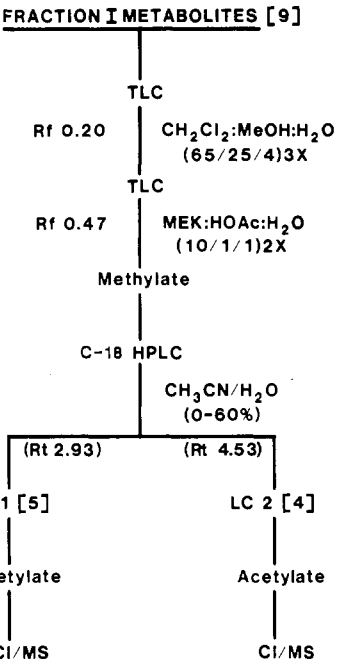

Figure 2. Isolation and identification of fraction I metabolites. Brackets indicate percent ^{14}C distribution.

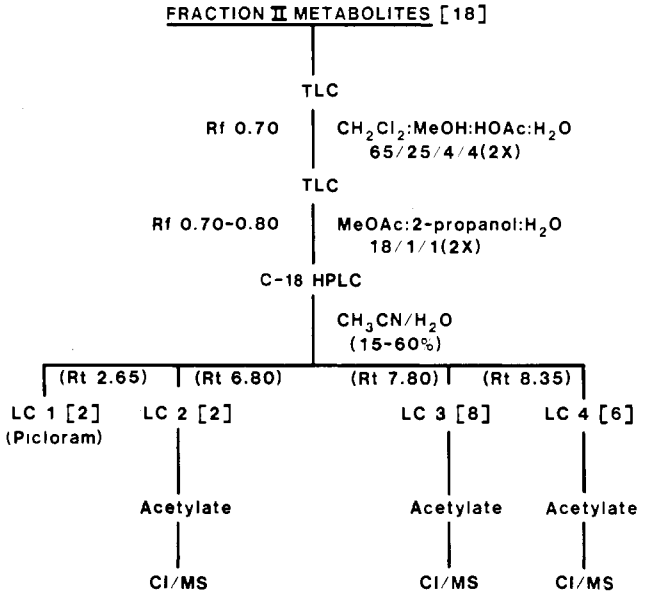

Figure 3. Isolation and identification of fraction II metabolites. Brackets indicate percent ^{14}C distribution.

and placed on a 2.5 × 6.0 cm C_{18} reversed-phase column equilibrated with 0.1 M K_2HPO_4/KH_2PO_4 buffer, pH 7.5. Elutates A–C were obtained by stepwise elution with 50 mL of 0.1 M K_2HPO_4/KH_2PO_4 buffer, pH 7.5, 75 mL of distilled water, and 50 mL of CH_3CN–H_2O (1:4, v/v), respectively. Eluate B metabolites (fraction I) were separated from unreacted picloram by TLC. Eluate C metabolites (fractions II and III) were purified by ion-exchange chromatography on a 1 × 5 cm DEAE Sephacel (Pharmacia) column and separated by TLC.

Fraction I metabolites were purified further by TLC, separated as methylated derivatives by HPLC, acetylated, and identified by CI/MS (Figure 2). Fraction II and III metabolites were purified separately by TLC, isolated by HPLC, and acetylated prior to identification by CI/MS (Figures 3 and 4).

RESULTS AND DISCUSSION

Root-absorbed [^{14}C]picloram was rapidly translocated to the foliar tissues and metabolized to polar products (Table I). Similar patterns of picloram root absorption, acropetal transport, and metabolism have been reported

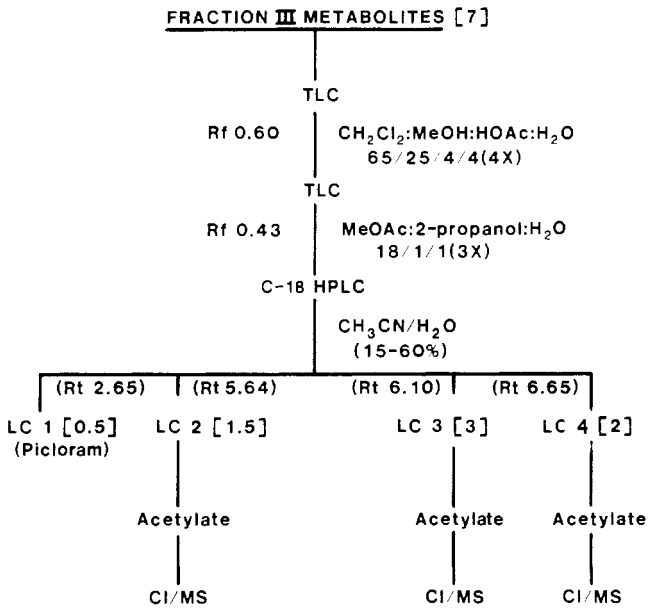

Figure 4. Isolation and identification of fraction III metabolites. Brackets indicate percent ^{14}C distribution.

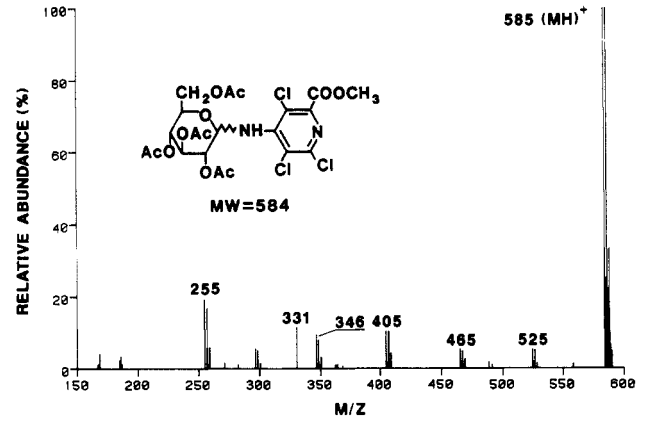

Figure 5. Mass spectrum of methylated and acetylated fraction I metabolite.

in other plant species (Hall and Vanden Born, 1988; Hallmén, 1975; Eliasson and Hallmén, 1973; Isensee et al., 1971; Gaudiel and Vanden Born, 1979; Sharma and Vanden Born, 1973a,b). Two days after the initiation of the pulsed root treatment, >90% of the absorbed ^{14}C was present in stem and leaf tissues; 30% was present as methanol-soluble metabolites; and only trace amounts were associated with the methanol-insoluble residue fraction.

Since leaf tissues were active sites of picloram metabolism, excised leaves were used for the isolation and identification of metabolites. Three major metabolite fractions (I–III) were extracted and separated from excised leaves 72 h after treatment with [^{14}C]picloram (Figure 1). These metabolite fractions represented 34% of the absorbed ^{14}C. Of the absorbed ^{14}C, 62% remained as picloram while only 2% was associated with the methanol-insoluble residue.

Acidic fraction I metabolites were retained on DEAE Sephacel. After TLC purification, fraction I metabolites were methylated and separated as two products (LC1 and LC2) by HPLC (Figure 2). Both products contained glucose as shown by acid hydrolysis of the methylated products and carbohydrate analysis (GLC) of the hydrolysates. When the methylated products were acetylated, both products gave identical chemical ionization mass spectra and were tentatively identified as *N*-glucoside conjugates. A typical mass spectrum is shown in Figure 5. A base peak protonated molecular ion with a characteristic chlorine

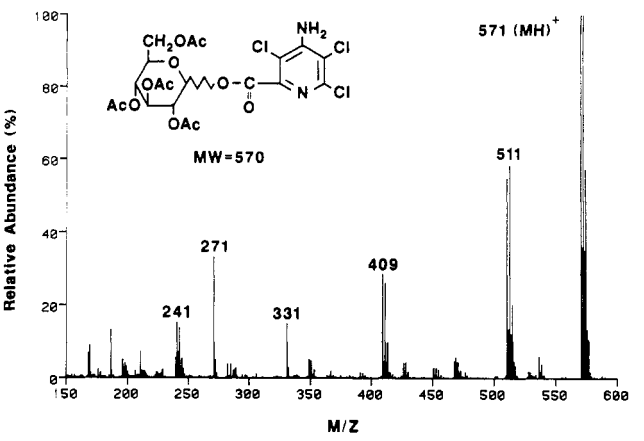

Figure 6. Mass spectrum of acetylated fraction II metabolite.

cluster was observed at m/z 585 together with a weak glucose tetraacetate ion at m/z 331. A protonated picloram methyl ester ion fragment was also present at m/z 255 together with a series of weak protonated ion fragments (MH^+ – 60 and – 59) at m/z 525, 465, 405, and 346. Differences in the chromatographic behavior of the two methylated *N*-glucoside metabolites (LC1 and LC2) are unexplained. An initial unstable malonated *N*-glucoside metabolite may have been decarboxylated to yield an acetylated *N*-glucoside during methylation and/or mass spectral analysis (Frear et al., 1985). Methylation conditions for fraction I metabolites were harsher than those normally used for malonated glucosides (Frear et al., 1983) because of difficulties in methylating the carboxyl group of picloram. Recent studies by Chkanikov et al. (1983) have also reported the isolation and mass spectroscopic identification of a picloram *N*-glucoside in sunflower.

Isolated fraction II metabolites were purified by TLC and separated by reversed-phase HPLC (Figure 3). The least polar ^{14}C product (LC1) was identified as [^{14}C]picloram by cochromatography (HPLC) and appeared to result from a partial breakdown of fraction II metabolites during purification. The more polar ^{14}C products (LC2, LC3, LC4) were acetylated and tentatively identified as isomeric glucose esters by mass spectroscopy. All three acetylated derivatives gave the same chemical ionization mass spectra. A typical mass spectrum is shown in Figure 6. A base peak protonated molecular ion with a characteristic chlorine isotope cluster was observed at m/z 571. Additional protonated ion fragments were also observed at m/z 511 (MH^+ – 60) and m/z 409 (MH^+ – 60, – 59, and – 43) as well as a weak picloram ion fragment at m/z 241. Characteristic glucose acetate ion fragments also were observed at m/z 331 and 271. Identification of isolated LC2, LC3, and LC4 products as isomeric glucose esters was supported by the chromatographic identification of glucose (GLC) and picloram amide [TLC, R_f 0.40, solvent system e; HPLC, R_t 3.27 min, gradient C] as ammonolysis products. Isomeric 2-*O*-, 4-*O*-, and 6-*O*-glucose esters of indole-3-acetic acid (IAA) have been isolated and identified in corn (Ehmann, 1974). Also, when isolated products LC3 or LC4 were rechromatographed [HPLC gradient B], additional products that corresponded to peaks LC2, LC3, and LC4 were formed. These data suggest that the isolated isomeric glucose esters of picloram (LC2, LC3, LC4) are unstable and subject to possible migration of the picloram moiety during chromatographic separation. Similar interconversions, presumably by acyl migration, have been reported previously with glucose and inositol esters of indole-3-acetic acid (Ehmann, 1974; Labarca et al., 1965; Chisnell, 1984). Glucose ester conjugation of picloram has

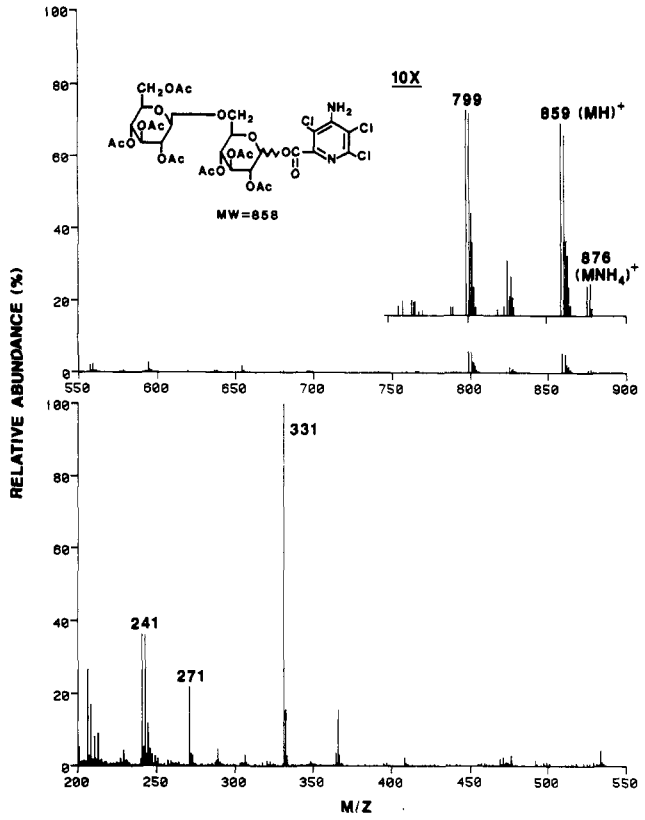

Figure 7. Mass spectrum of acetylated fraction III metabolite.

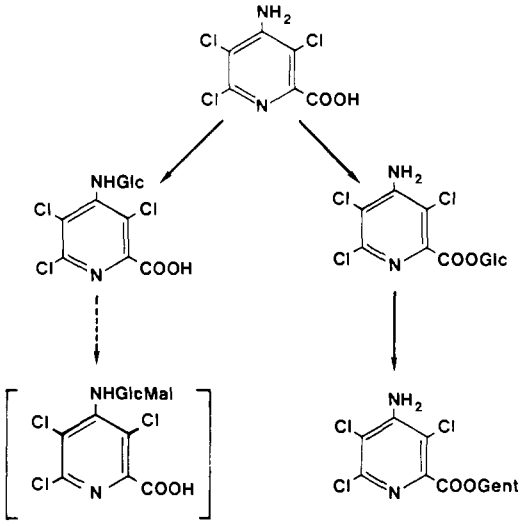

Figure 8. Proposed scheme of picloram metabolism in leafy spurge: Glc, glucose; Gent, gentiobiose; Mal, malonate. Proposed metabolite structure in brackets.

been reported in leaves of tolerant corn and sensitive bean (Kudaikina et al., 1981b).

Isolated fraction III metabolites were purified by TLC and separated by reversed-phase HPLC (Figure 4). A partial breakdown of fraction III metabolites to [^{14}C]picloram (LCI) was observed during purification. Separated fraction III products (LC2, LC3, LC4) yielded gentiobiose (GLC) and picloram amide (TLC, HPLC) upon ammonolysis and underwent rearrangement when rechromatographed [HPLC gradient B]. These data suggested that fraction III metabolites were isomeric gentiobiose esters of picloram. Identification was confirmed by mass spectroscopy of acetylated derivatives. All three acetylated derivatives gave the same chemical ionization mass spectra as shown in Figure 7. Weak pseudomolecular ions [$(MNH_4)^+$ and $(MH)^+$] were observed at m/z 876 and 859 together with a weak ion fragment at m/z 799 ($MH^+ - 60$) and a picloram ion fragment at m/z 241. Characteristic glucose acetate ion fragments also were observed at m/z 331 and 271.

In conclusion, picloram is metabolized rapidly to either *N*-glucoside or isomeric glucose and gentiobiose ester conjugates in leafy spurge (Figure 8).

ACKNOWLEDGMENT

We express our appreciation to C. H. Lamoureux and R. G. Zaylskie for their technical assistance in mass spectrometric measurements, to M. Blankendaal for greenhouse support, and to Dow Chemical Co. for providing needed radiochemicals and reference compounds.

Registry No. Picloram, 1918-02-1; picloram *N*-glucoside, 121787-98-2; picloram glucose ester, 77750-03-9.

LITERATURE CITED

Blankendaal, M.; Hodgson, R. H.; Davis, D. G.; Hoerauf, R. A.; Shimabukuro, R. H. *Growing plants without soil for experimental use*; ARS Miscellaneous Publication No. 1251; USDA: Washington, DC, 1972.

Chisnell, J. R. *Myo*-Inositol esters of indole-3-acetic acid are endogenous components of *Zea mays* L. shoot tissue. *Plant Physiol.* **1984**, *74*, 278–283.

Chkanikov, D. I.; Makeev, A. M.; Pavlova, N. N.; Nazarova, T. A. Formation of picloram N-glucoside in plants. *Fiziol. Rast.* **1983**, *30*(1), 95–101.

Chkanikov, D. I.; Pavlova, N. N.; Makeev, A. M.; Nazarova, T. A. Conjugates of picloram and 2,4-D with mustard oils in plants of the family *Cruciferae*. *Fiziol. Rast.* **1984**, *31*(2), 321–327.

Dunn, P. H. The distribution of leafy spurge (*Euphorbia esula*) and other weedy *Euphorbia* spp. in the United States. *Weed Sci.* **1979**, *27*, 509–516.

Ehmann, E. Identification of 2-O-(indole-3-acetyl)-D-glucopyranose, 4-O-(indole-3-acetyl)-D-glucopyranose and 6-O-(indole-3-acetyl)-D-glucopyranose from kernals of *Zea mays* by gas-liquid chromatography-mass spectrometry. *Carbohydr. Res.* **1974**, *34*, 99–114.

Eliasson, L.; Hallmén, U. Translocation and metabolism of picloram and 2,4-D in *Populus tremula*. *Physiol. Plant.* **1973**, *28*, 182–187.

Frear, D. S.; Mansager, E. R.; Swanson, H. R.; Tanaka, F. S. Metribuzin metabolism in tomato: isolation and identification of N-glucoside conjugates. *Pest. Biochem. Physiol.* **1983**, *19*, 270–281.

Frear, D. S.; Swanson, H. R.; Mansager, E. R. Alternate pathways of metribuzin metabolism in soybean: formation of N-glucoside and homoglutathione conjugates. *Pest. Biochem. Physiol.* **1985**, *23*, 56–65.

Gaudiel, R.; Vanden Born, W. H. Picloram translocation and redistribution in soybean plants following root uptake. *Pest. Biochem. Physiol.* **1979**, *11*, 129–134.

Hall, J. C.; Vanden Born, W. H. The absence of a role of absorption, translocation or metabolism in the selectivity of picloram and clopyralid in two plant species. *Weed Sci.* **1988**, *36*(1), 9–14.

Hallmén, U. Translocation and complex formation of picloram and 2,4-D in rape and sunflower. *Physiol. Plant.* **1974**, *32*, 78–83.

Hallmén, U. Translocation and complex formation of root-applied 2,4-D and picloram in susceptible and tolerant species. *Physiol. Plant.* **1975**, *34*, 266–272.

Hallmén, U.; Eliasson, L. Translocation and complex formation of picloram and 2,4-D in wheat seedlings. *Physiol. Plant.* **1972**, *28*, 143–149.

Isensee, A. R.; Jones, G. E.; Turner, B. C. Root absorption and translocation of picloram by oats and soybeans. *Weed Sci.* **1971**, *19*(6), 727–731.

Kudaikina, I. V.; Makeev, A. M.; Chkanikov, D. I. Picloram metabolism in certain plants. *Fiziol. Biokhim. Kulturn. Rast.* **1981a**, *13*(3), 306–309.

Kudaikina, I. V.; Makeev, A. M.; Chkanikov, D. I. 1-O-(4-amino-3,5,6-trichloropicolyl)-D-glycopyranoside as a product

of picloram metabolism in plants. *Fiziol. Rast.* **1981b**, *28*(2), 435–439.
Labarca, C.; Nicholls, P. B.; Bandurski, R. S. A partial characterization of indoleacetylinositols from *Zea mays*. *Biochem. Biophys. Res. Commun.* **1965**, *20*, 641–646.
Lym, R. G.; Messersmith, C. G. Control of leafy spurge with herbicides. *N.D. Farm Res.* **1983**, *40*(5), 16–19.
Lym, R. G.; Messersmith, C. G. Leafy spurge control with herbicides in North Dakota: 20-year summary. *J. Range Manage.* **1985**, *38*(2), 149–154.
Messersmith, C. G.; Lym, R. G. Distribution and economic impacts of leafy spurge in North Dakota. *N.D. Farm Res.* **1983**, *40*(5), 8–13.
Moore, R. J.; Frankton, C. *Euphorbia* × pseudo-*esula* (E *cyparissias* × E. *esula*) in Canada. *Can. Field Nat.* **1969**, *83*, 243–246.
Sharma, M. P.; Vanden Born, W. H. Uptake, cellular distribution and metabolism of ^{14}C-picloram by excised plant tissues. *Physiol. Plant.* **1973a**, *29*, 10–16.
Sharma, M. P.; Vanden Born, W. H. Fate of picloram in Canada thistle, soybean and barley. *Weed Sci.* **1973b**, *21*(4), 350–353.

Received for review September 26, 1988. Accepted April 3, 1989. Mention of a trademark or proprietary product does not constitute a guarantee or warranty of the product by the U.S. Department of Agriculture and does not imply its approval of the exclusion of other products that may also be suitable.

Metabolism of Norflurazon by Rats

Gary B. Quistad,* Annette L. Saunders, Wayne S. Skinner, Carol C. Reuter, and Katherine D. Collier

Within 4 days of dosage with 2 and 110 mg/kg of [4,5-^{14}C-*pyridazinyl*]norflurazon, rats excreted 19–28 and 65–80% of the applied radiocarbon in the urine and feces, respectively. Four days posttreatment, the residues remaining in the carcass were minimal (<1% applied dose). Norflurazon was thoroughly degraded as evidenced by the paucity of parent compound in excrement (≤2% of applied dose). Nine metabolites were identified from the urine and feces, arising predominantly by two major pathways: N-demethylation and reaction with glutathione. Although norflurazon was degraded to numerous metabolites, only one (sulfoxide **3**) contributed more than 10% of the applied radioactivity. The other identified metabolites each represented 1–2% of the applied dose.

Norflurazon is a fluorinated pyridazinone herbicide registered under the trade names Zorial, Solicam, and Evital. It is utilized as a preemergent herbicide to selectively control annual grasses and broadleaf weeds in numerous commercial crops. Norflurazon is employed also for noncrop uses such as at airports, storage areas, and rights of way. This herbicide is absorbed by the roots of germinating weeds and is translocated to the growing areas where, in susceptible species, it inhibits carotenoid biosynthesis, resulting in chlorophyll photodegradation (Sandmann and Böger, 1982). Norflurazon is also an inhibitor of fatty acid desaturation in both plants and rat liver cells (Hagve et al., 1985). When emerging from the treated soil, the weed seedlings are white, become necrotic, and soon die.

This work was undertaken to define the fate of norflurazon in a mammalian model, the rat. Although the metabolism of norflurazon was studied more than a decade ago in rats (Karapally, 1974), recent advances in analytical methodology and instrumentation allowed us to identify several new metabolites. We now report the metabolic fate of norflurazon in rats.

EXPERIMENTAL SECTION

Analytical Methods. Reversed-phase liquid chromatography (LC) was performed with Spectra-Physics instruments (Models 8000A and 8700): ultraviolet detection at 254 nm; 10-μm Li-Chrosorb RP-8 column, 25 × 0.46 cm; elution at 1.6 mL/min. The following solvent systems of acetonitrile or methanol and a constant 0.1% trifluoroacetic acid were utilized (all gradients linear): SS 1, gradient 15–30% acetonitrile over 5 min, isocratic at 30% for 15 min, gradient 30–40% over 5 min, isocratic at 40% for 5 min, gradient 40–90% over 5 min, hold at 90% for 5 min; SS 2, gradient 30–35% methanol over 5 min, isocratic at 35% for 15 min, gradient 35–50% over 15 min, gradient 50–90% over 5 min, hold at 90% for 5 min; SS 3, gradient 15–22% acetonitrile over 5 min, isocratic at 22% for 15 min, gradient 22–40% over 5 min, gradient 40–90% over 5 min, hold at 90% for 5 min. Analysis by thin-layer chromatography (TLC) employed precoated silica gel GF plates (Analtech, 1000 μm) with development in the following mobile phases: SS 4, ethyl acetate; SS 5, ethyl acetate–hexane, 4:1; SS 6, chloroform–methanol, 5:1.

Radiocarbon was quantified by liquid scintillation counting (LSC, Packard Tri-Carb Models 4430 and 2425C) and by radiochromatography scanning (Bioscan System 200-HP).

Urine and feces were each extracted with a Polytron homogenizer (Brinkmann). After methylation (CH_2N_2), selected urinary and fecal extract samples were assayed by TLC, which involved cospotting the samples with appropriate authentic standards (visualized under UV light by quenching of fluorescence) followed by radiochromatographic scanning. The TLC zones were then scraped and eluted (methanol), and the ^{14}C was quantified by LSC. The radicarbon in certain zones was analyzed further by LC with coinjection of an aliquot plus selected authentic standards and collection of timed eluate fractions followed by LSC. Unextractable ^{14}C residues were combusted to $^{14}CO_2$, which was then trapped in scintillation fluid (Biological oxidizer, Model OX-300; Carbon 14 Cocktail; Harvey Instrument Co.) and quantified by LSC.

Mass spectra were obtained with a Hewlett-Packard instrument (Model 5985A) in the electron-impact (EI), chemical ionization (CI with CH_4), and fast atom bombardment (FAB) modes.

Synthetic Standards. The following authentic standards were supplied by Sandoz Ltd.: 4-chloro-5-(methylamino)-2-[3-(trifluoromethyl)phenyl]-3(2*H*)-pyridazinone (norflurazon, **1**); 4-chloro-5-amino-2-[3-(trifluoromethyl)phenyl]-3(2*H*)-pyridazinone (desmethylnorflurazon, **2**).

Radiosynthesis. [4,5-^{14}C-*pyridazinyl*]Norflurazon (96.5% pure, 2.85 mCi/mmol specific activity) was obtained from Sandoz Ltd. Additional purification of the [^{14}C]norflurazon (to 98.8% purity) was effected at Zoecon (TLC, SS 4).

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0021-8561/89/1437-1412$01.50/0