Ethyl *m*-Hydroxycarbanilate Carbanilate (EP-475) Metabolism in Sugar Beets[1]

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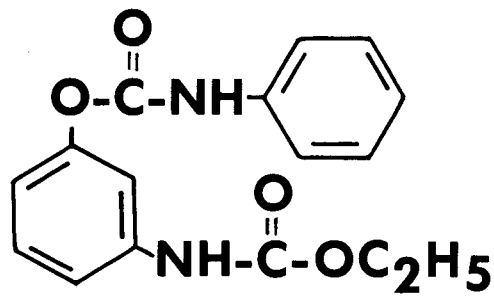
Ethyl m-hydroxycarbanilate carbanilate, or EP-475, is a broad spectrum postemergence herbicide for the control of weeds infesting sugar beets and red beets. EP-475 is particularly effective for the control of redroot pigweed (Amaranthus retroflexus), a property not shared by phenmedipham, a closely related carbanilate analog (1). Sonawane and Knowles (2) studied the metabolism of radiolabeled EP-475 in white rats. The herbicide was rapidly metabolized following oral administration to rats; hydrolysis and conjugation were the most important reactions for EP-475 in vivo. This paper reports a study of the metabolism of radiocarbon labeled EP-475 in sugar beets.

MATERIALS AND METHODS

EP-475 (shown below) uniformly labeled with radiocarbon in the m-aminophenol moiety (specific activity 1.95 mCi/mmole) was provided by NOR-AM Agricultural Products, Inc., Woodstock, Illinois. NOR-AM also provided nonradioactive samples of EP-475, ethyl-N-(3-hydroxyphenyl)-carbamate (EHPC), m-aminophenol (AP), m-aminophenyl acetate (APA) and 3'-hydroxyacetanilide (HA). A sample of an emulsifiable concentrate formulation of EP-475 (16.7% active ingredient) was also made available.



Thin-layer chromatography (TLC) was used to separate and to tentatively identify certain of the radioactive components isolated from EP-475-treated sugar beet leaves. The adsorbent was silica gel GF_{254} (500 microns), Brinkmann Instruments, Inc., Westbury, N. Y. The chromatograms were developed with benzene: ethanol (9:1) (1); average R_f values were 0.66 for EP-475, 0.35

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for EHPC, 0.22 for AP, 0.50 for APA, and 0.18 for HA. Additional techniques for TLC, radioautography, and liquid scintillation counting have been described (2).

Sugar beets, Beta vulgaris (L), variety HH-10 were germinated and grown in 5-gallon plastic pails under greenhouse conditions. In October 1971, sugar beet plants in the 4-leaf stage weighing approximately 0.5 g each were treated with EP-475. The treating solution was prepared as follows. To 78.8 mg of the nonradioactive EP-475 formulation were added 2.0 mg of radiocarbon labeled EP-475, and the mixture was diluted to a final volume of 18.2 ml with distilled water. Each plant received via a microsyringe a total of 182 µl of the treating solution divided equally among the 4 leaves. Thus, each plant received approximately 146,000 counts/min of EP-475-^{14}C.

At posttreatment periods of 0, 5, 10, 15, 30, 60, and 90 days 6 plants were analyzed in 2 groups of 3 as follows. The leaves were removed, combined, and placed in a beaker containing 150 ml of chloroform. The beaker was shaken for 10 min, and the entire procedure was repeated. The leaf rinses were combined, filtered, and dried over anhydrous sodium sulfate. The chloroform rinse was concentrated to a volume of 5 ml on the rotary evaporator. The total radioactivity in the concentrated leaf rinse was determined by radioassay of duplicate 100-µl aliquots. The remaining leaf rinse was further concentrated, and subjected to TLC and radioautography. The various zones on the thin-layer plate were radioassayed. The nature and relative amount of the various chloroform-soluble components in the leaf rinse were then calculated.

The leaf tissue remaining after the chloroform rinses was homogenized in 20 ml of methanol, filtered, and rehomogenized in methanol. The methanol extracts were combined and evaporated on the rotary evaporator until only the water remained. The aqueous fraction was adjusted to a volume of 5 ml with distilled water (pH 6.5), and it was extracted 3 times with 20-ml aliquots of ethyl acetate. The ethyl acetate extracts were combined, and concentrated to a volume of 5 ml. The total radioactivity in the ethyl acetate and aqueous fractions was then ascertained.

The roots were cut into small pieces and were dried along with the leaf residue in an oven at 60°C. These dried tissues were ground into a powder with a mortar and pestle, and duplicate 100-mg aliquots were combusted in oxygen purged flasks (2). Following radioassay of the trapped $^{14}CO_2$ the total radioactivity in the roots and leaf residue was calculated.

RESULTS AND DISCUSSION

Table 1 gives the results of fractionation of sugar beet plants treated with EP-475. The majority of the radioactive material was recovered in the chloroform leaf rinse throughout the 90-day period. These materials in the leaf rinse decreased from a

maximum of 98.2% at zero time to 39.3% by 90 days posttreatment (Table 1). When the chloroform-soluble radioactive components from the leaf rinse were subjected to TLC it was found that the parent compound, EP-475, decreased from 96.6% of the organosoluble radioactivity at zero time to 27.1% at 90 days (Table 2). The major EP-475 metabolite was EHPC which increased from only 3.2% at zero time to 49.4% by 90 days. Levels of AP and of unidentified material at the TLC origin also generally increased during the experimental period (Table 2).

TABLE 1
Results of fractionation of sugar beet seedlings treated with EP-475

	% Applied EP-475-^{14}C equivalents at indicated days						
Fraction	0	5	10	15	30	60	90
Leaf rinse	98.2	92.7	73.3	61.6	43.7	41.5	39.3
Leaf extract							
Organic fraction	<0.1	2.0	3.3	5.0	5.3	7.3	16.2
Aqueous fraction	<0.1	<0.1	1.5	1.8	3.4	2.2	3.9
Leaf residue	<0.1	<0.1	1.1	4.2	7.2	5.8	4.7
Roots	<0.1	<0.1	0.3	0.2	0.7	3.8	3.1
Total recovery of radiocarbon, %	98.2	94.7	79.5	72.8	60.3	60.6	67.2

TABLE 2
Nature and relative concentration of chloroform-soluble radioactive materials recovered from the leaf rinse of plants treated with EP-475

	% Chloroform-soluble radioactive material at indicated days						
Compound	0	5	10	15	30	60	90
EP-475	96.6	87.6	75.2	66.1	46.0	46.7	27.1
EHPC	3.2	8.9	16.2	25.2	35.9	39.8	49.4
AP	<0.1	2.4	4.4	3.7	9.7	6.2	11.5
HA	<0.1	<0.1	<0.1	<0.1	0.3	0.4	0.3
Unknown (R_f 0.52)	0.2	0.2	0.6	0.8	0.8	0.3	0.8
Origin	<0.1	0.9	3.6	4.2	7.3	6.6	10.9

The maximum level of EP-475 equivalents in the organic and aqueous fractions of the leaf extract (Table 1) was 16.2% and 3.9%, respectively, at 90 days. Combustion analysis of the leaf tissue remaining after solvent extraction indicated the presence of some unextractable radioactive material (Table 1). There was slight movement of EP-475 equivalents from the leaves into the roots; the maximum level of radioactivity in the roots was 3.8% and occurred 60 days posttreatment (Table 1). The total recovery of radiocarbon ranged from 98.2% at zero time to 60.3% at the 30-day sampling interval (Table 1). This decrease in recovery was probably due in part to volatility of certain of the radioactive components during the experimental period.

CONCLUSIONS

The results of these experiments indicate that EP-475 was very slowly absorbed and translocated to a limited extent when applied topically to the leaves of sugar beet plants. Appreciable degradation of the compound was also evident. Only 10.7% of the applied dose chromatographed as EP-475 by 90 days after treatment. The hydroxyphenylcarbamate EHPC was the major EP-475 metabolite though significant amounts of AP were also present.

LITERATURE CITED

1. JENNY, N. A. NOR-AM Agricultural Products, Inc. Private communication (1971).

2. SONAWANE, B. R. and KNOWLES, C. O. Pest. Biochem. Physiol. In press (1971).