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Short Communication

Imidazolinones

POTENT INHIBITORS OF ACETOHYDROXYACID SYNTHASE

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ABSTRACT

The imidazolinones, a new chemical class of herbicides, were shown to be uncompetitive inhibitors of acetohydroxyacid synthase from corn. This is the first common enzyme in the biosynthetic pathway for valine, leucine, and isoleucine. The K_i for the imidazolinones tested ranged from 2 to 12 micromolar. These results may explain the mechanism of action of these new herbicides.

The imidazolinones are a new chemical class of herbicides discovered and being developed by American Cyanamid Company (see structures in Table I). These herbicides kill both monocotyledonous and dicotyledonous species, and selectivity is achieved by differential metabolism of the compounds to nonherbicidal forms (7, 9). Death of the whole plant can require several weeks. The meristematic tissues die first, followed by slow necrosis of the mature tissue.

The precise mechanism of action of many herbicides are unknown. The imidazolinones are somewhat unusual in that a biochemical pathway has been identified which is inhibited by these herbicides. The imidazolinones interfere with the biosynthesis of the branched chain amino acids valine, leucine, and isoleucine (10). Corn tissue grown in suspension culture and treated with AC 243,997 (2-[4-isopropyl-4-methyl-5-oxo-2-imidazolin-2-yl]nicotinic acid) showed changes in the free amino acid levels (10). Most of the amino acids either increased or remained constant, but the levels of valine, leucine, and isoleucine decreased. Exogenously supplying these three amino acids to corn tissue cultures and seedlings prevented the phytotoxic effects of AC 243,997 (10). All three of these amino acids were necessary for maximal protection to occur.

There are four enzymes that catalyze biosynthetic steps leading to valine, leucine, and isoleucine. Acetohydroxyacid synthase (EC 4.1.3.18) is the first enzyme in the biosynthesis of valine and leucine and the second enzyme in the biosynthesis of isoleucine. Acetohydroxyacid synthase is feedback inhibited cooperatively by valine and leucine in higher plants (4). Thus, it is a key controlling point for the levels of the branched chain amino acids and is a prime site for inhibition by a xenobiotic. The imidazolinones were found to be potent inhibitors of acetohydroxyacid synthase and this may explain why the levels of valine, leucine, and isoleucine decreased after treatment with the imidazolinones.

MATERIALS AND METHODS

Chemicals. Analytical grade imidazolinones were synthesized at the American Cyanamid Agricultural Research Center, Princeton, NJ. Acetoin was obtained from Eastman Kodak.

Plant Material. Corn (*Zea mays* L. Pioneer var 3547) seeds were planted in sand and watered with 0.2 mM $CaCl_2$. The plants were grown in darkness at 28°C for 3 d. The roots and shoots from the etiolated seedlings were used.

Acetohydroxyacid Synthase Assay. The procedure used was a modification of one used by B. J. Miflin (5). Roots and coleoptiles of the corn seedlings were homogenized in an extraction buffer (50 mM K-phosphate buffer (pH 7.5), 5 mM EDTA, 5 mM $MgCl_2$, 1 mM valine, 1 mM leucine). The homogenate was filtered through eight layers of cheese cloth and centrifuged at 14,000*g* for 15 min. The supernatant fraction was brought to 65% saturation with respect to $(NH_4)_2SO_4$ and allowed to stand 1 h on ice. Then the mixture was centrifuged at 14,000*g* for 15 min and the supernatant was discarded.

The $(NH_4)_2SO_4$ precipitated pellet was redissolved in 50 mM K-phosphate buffer (pH 7.5), 1 mM EDTA, and 0.1 M NaCl. This solution was passed through a Sephadex G-25 column equilibrated with the same buffer. The eluate containing the desalted protein was collected and used for the assay procedure.

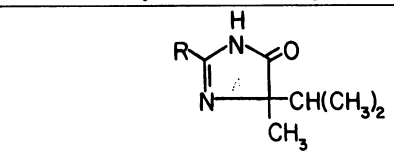
The assay solution contained 20 mM pyruvate, 0.32 mM thiamine pyrophosphate, 0.5 mM $MnSO_4$, 20 mM K-phosphate buffer (pH 7, 0.5 ml), and 0.4 ml of enzyme solution. Inhibitors were added in 0.1-ml aliquots to bring the final volume to 1 ml. This mixture was incubated at 37°C for 1 h after which time the reaction was stopped with 0.2 ml of 30% H_2SO_4. The reaction tubes were assayed for acetolactate by decarboxylation at 60°C for 15 min and subsequent measurement of the acetoin formed by the method of Westerfield (11). The imidazolinones were tested for inhibition of acetohydroxyacid synthase activity over a range of 4.6×10^{-8} to 1×10^{-4} M. The apparent K_i values were calculated using a Dixon Plot (8).

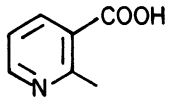
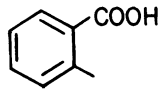
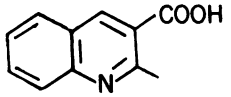
RESULTS AND DISCUSSION

Three different imidazolinones were compared for their inhibitory action on acetohydroxyacid synthase. All three were potent inhibitors of the enzyme (Table I). We have found that these imidazolinones also have excellent herbicidal activity and that their phytotoxic effects can be reversed by exogenous applications of valine, leucine, and isoleucine (10).

The inhibition of acetohydroxyacid synthase by AC 243,997 was uncompetitive as shown in the Hanes-Woolf plot (Fig. 1). This indicates that this imidazolinone binds to the enzyme-pyruvate complex. The acetohydroxyacid synthase we extracted from corn was cooperatively inhibited by valine and leucine. The

Table I. *K_i Values for Imidazolinones for Acetohydroxyacid Synthase Extracted from Corn Seedings*



AC Number	R	Ki (uM)
243,997		12
222,164		1.7
252,214		3.4

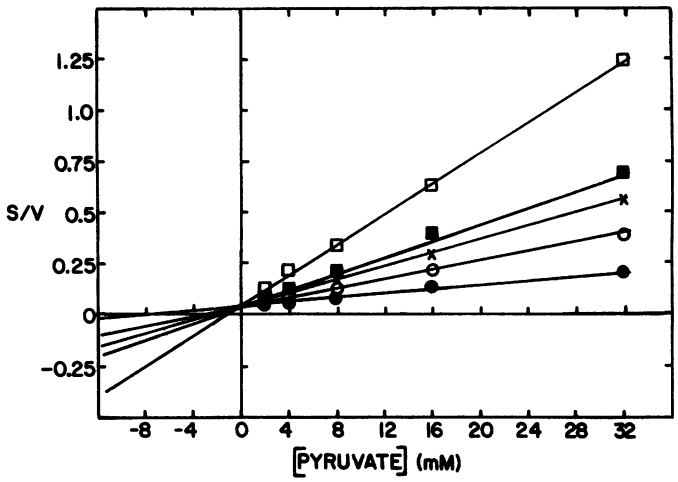


FIG. 1. Inhibition of acetohydroxyacid synthase by AC 243,997. Activities were measured in 20 mM K-phosphate buffer (pH 7.5) at 37°C with different concentrations of pyruvate. The levels of AC 243,997 used were 0 (●), 3.1 μM (○), 6.2 μM (×), 12.5 μM (■), 25 μM (□).

properties were similar to those described by Miflin (5) for acetohydroxyacid synthase extracted from barley shoots.

The inhibition of acetohydroxyacid synthase by the imidazolinones could explain the herbicidal effects of these compounds. These herbicides kill plants very slowly and the first symptoms appear in the meristematic tissue. In corn root tips, the level of the soluble proteins decreased within 24 h after application of AC 243,997 (9). Jensen (4) and Clowes (3) have shown that the rate of protein synthesis is highest in the root tip region. Furthermore, observations by Oaks (6) showed that in excised maize embryos an adequate supply of amino acids is required in addition to a carbohydrate for a normal increase in protein nitrogen. Oaks (6) also showed that inhibition of leucine synthesis by externally supplying this amino acid caused a change in the internal pool size of leucine in maize root tips. If the imidazolinones inhibit the synthesis of valine, leucine, and isoleucine *in vivo*, there may be a rapid decrease in the pool sizes of these amino acids. This could cause a decrease in protein synthesis. A lower rate of protein synthesis, in turn, could cause a slow down in the rate of cell division, and eventually death of the cell. Mature tissue has larger pools of amino acids as well as protein reserves that can be catabolized for amino acids. Thus, it should take longer for the phytotoxic effect of the imidazolinones to become apparent in mature tissue, as was observed.

The inhibition of amino acid synthesis is a relatively new mechanism of action of herbicides. Until recently, glyphosate was the only other herbicide that was known to interfere with amino acid biosynthesis. Glyphosate inhibits the biosynthesis of aromatic amino acids (1). The symptomatology of glyphosate has some similarities to the imidazolinones in that the meristematic tissue dies first followed by slow necrosis of the mature tissue. More recently, Chaleff and Mauvais (2) reported that acetohydroxyacid synthase is inhibited by the sulfonyl area herbicides. It appears that the sulfonyl ureas and the imidazolinones may have the same mechanism of action. The effectiveness of these types of herbicides indicates that amino acid biosynthesis is a target for the disruption of plant metabolism and could be a site of action of future herbicides.

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