Structure–Activity Relationships for *S*-Ethyl *N*,*N*-Dipropylthiocarbamate (EPTC) Antidotes in Corn

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Thirty-one analogues of *N*,*N*-diallyl-2,2-dichloroacetamide (R-25788) were synthesized and examined for activity as antidotes for EPTC (*S*-ethyl *N*,*N*-dipropylthiocarbamate) in corn grown in quartz sand nutrient culture. Amide analogues of R-25788 with branched acid chains or only one N-substituted alkyl group had little activity. The effects of the number of chlorine substituents in the acid chain, of varying the degree of saturation and the chain length in the amide chains, and of replacing the chlorines of the acid chain by oxygen were all examined. It was established that N,N-disubstituted amides structurally very similar to EPTC were very active as antidotes for EPTC in the soil-free bioassay system. It is possible that these molecules may compete for the sites of action with the more toxic herbicide molecules, thereby reducing herbicide injury.

N,*N*-Diallyl-2,2-dichloroacetamide (R-25788) is a physiologically selective antidote for preventing EPTC (*S*-ethyl *N*,*N*-dipropylthiocarbamate) injury to corn without reducing the activity of this herbicide in other plant species (Chang et al., 1972; Rains and Fletchall, 1971). In addition, it protects corn from many herbicides other than EPTC (Chang et al., 1973); these include other thiocarbamates, a dithiocarbamate, a carbamate, an urea, and an amide.

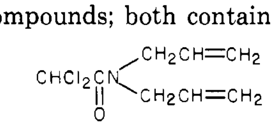
There is as yet no clear consensus among researchers on the most probable mechanism for the activity of thiocarbamate herbicides in most plant species; neither are the mechanisms involved in antidoting the action of these herbicides in plants understood. Inhibition of some types of RNA synthesis has been reported for EPTC and molinate (*S*-ethyl hexahydro-1*H*-azepine-1-carbothioate) (Chen et al., 1968). These workers established that synthesis of soluble RNA in barnyard grass was inhibited by molinate, and that this effect could be reversed by treatment with equimolar concentrations of gibberellic acid. Best and Schrieber (1972) reported that EPTC reduced the levels of ribosomal RNA (r-RNA), DNA-like RNA (D-RNA), and tenaciously bound RNA (TB-RNA) in corn. Treatment with 2,4-D alone increased all of these RNA's. Application of 2,4-D together with EPTC reversed the EPTC inhibition of all RNA types except r-RNA. Other investigators have reported that EPTC inhibited fatty acid synthesis in isolated chloroplasts (Wilkinson and Smith, 1975) and that this effect is prevented by the R-25788 antidote.

Another effect observed in EPTC-injured corn plants was a stimulation of peroxidase activity (Harvey et al., 1975). The stimulation of peroxidase activity was less when the corn was treated with EPTC together with the antidote, R-25788, while reduced peroxidase activity was observed in corn treated with R-25788 alone. However, it was not possible to determine if effects on peroxidase were specifically related to the toxic effects of EPTC. Chang et al. (1974) and later Lay and Casida (1976) examined the effects of R-25788 on EPTC uptake, translocation, and metabolism in corn. In these studies it was shown that R-25788 gave significant protection to corn even when applied alone to corn seedlings pretreated with EPTC. When R-25788 and [^{14}C]EPTC were applied simultaneously in the root medium of corn seedlings, root uptake, translocation, metabolism, and emanation of $^{14}CO_2$ or [^{14}C]EPTC vapors were all greater in R-25788-treated plants. The increased rates of these processes in R-25788 protected plants paralleled increased growth and were not different on a per unit plant weight basis. Thus, cause and effect relationships were difficult to elucidate; but clearly R-25788 does not protect corn by preventing EPTC uptake or translocation.

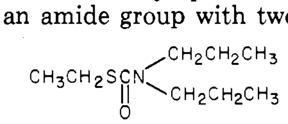
Most recently, Lay and Casida (1975, 1976) have established that thiocarbamate herbicides are converted to sulfoxide metabolites in corn and other plants and that it may be the sulfoxide rather than the parent thiocarbamate that is most directly phytotoxic. They proposed that the sulfoxide causes toxic effects by carbamoylating important thiol compounds such as glutathione (GSH) or coenzyme A (CoA-SH). They believe that R-25788 may protect corn by inducing greater levels of GSH and of the enzyme glutathione S-transferase. Thus the enzyme catalyzes the detoxification of EPTC by GSH, while sufficient GSH still remains to participate in the normal metabolic processes.

The object of the present study has not been to elucidate the mode of action for R-25788 and other antidotes for EPTC in plants. Rather, an attempt is made to make a more precise comparison of the activity of various amide antidotes for thiocarbamate herbicides in corn. This has potential practical application and will also provide structure–activity data with which any proposed mechanism of action must be compatible.

EPTC and R-25788 are structurally quite similar compounds; both contain an amide group with two N-



R-25788 antidote



EPTC herbicide

substituted three-carbon chains. EPTC possesses sulfur in the acid chain, and it is the sulfur which is assumed (Lay and Casida, 1975, 1976) to be essential for herbicidal activity. Instead of this sulfur, R-25788 contains two chlorines at essentially the same position in the acid chain. The importance of chemical similarities between amide antidotes and EPTC is the primary focus of this study.

MATERIALS AND METHODS

Synthesis of Amide Antidote Analogues. The antidote analogues were synthesized from appropriate acid chlorides and amines. Equimolar quantities of the amine and pyridine were dissolved in chloroform. To the stirred, cooled (ice bath) solution was added, dropwise, an equimolar amount of the acid chloride. The mixture was

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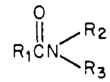
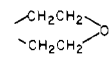
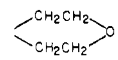
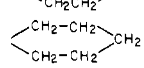
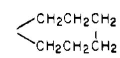
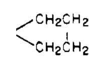
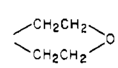
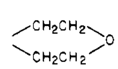
Table I. Comparative Activity of 31 Different Amides as Antidotes for EPTC in Corn Grown in Quartz Sand Nutrient Culture



Structures of amide antidotes applied with EPTC			Activity rating as an antidote for EPTC[a]
R_1	R_2	R_3	
$CHCl_2$-	-$CH_2CH_2CH_3$-	-$CH_2CH_2CH_3$	Very good
$CHCl_2$-			Very good
$CHCl_2$-	-$CH_2CH{=}CH_2$	-$CH_2CH{=}CH_2$	Very good
CH_2Cl-			Very good
$CHCl_2$-			Very good
$CHCl_2$-	-CH_2CH_3	-CH_2CH_3	Good
$CHCl_2$-	-$CH_2C{\equiv}CH$	-$CH_2C{\equiv}CH$	Good
$CHCl_2$-			Good
$CHCl_2$			Good
CH_3CH_2-O-	-$CH_2CH{=}CH_2$	-$CH_2CH{=}CH_2$	Good
CH_3CH_2-	-$CH_2CH_2CH_3$	-$CH_2CH_2CH_3$	Good
CH_3-	-$CH_2CH{=}CH_2$	-$CH_2CH{=}CH_2$	Moderate
CCl_3-	-$CH_2CH{=}CH_2$	-$CH_2CH{=}CH_2$	Moderate
CH_3CHCl-	-$CH_2CH{=}CH_2$	-$CH_2CH{=}CH_2$	Moderate
$CHCl_2$-	-$CH_2CH_2CH_2CH_3$	-$CH_2CH_2CH_2CH_3$	Moderate
$CHCl_2$-	-$CH_2CH(CH_3)_2$	-$CH_2CH(CH_3)_2$	Moderate
$CHCl_2$-	-CH_2CH_3	-$CH_2CH_2CH_2CH_3$	Moderate
CH_3-	-$CH_2CH_2CH_3$	-$CH_2CH_2CH_3$	Moderate
CH_2Cl-	-$CH_2CH_2CH_3$	-$CH_2CH_2CH_3$	Moderate
$CH_3CH_2CH_2$-	-$CH_2CH{=}CH_2$	-$CH_2CH{=}CH_2$	Moderate
CH_3-			Slight
$(CH_3)_2CH$-	-$CH_2CH{=}CH_2$	-$CH_2CH{=}CH_2$	Slight
CCl_3-	-$CH_2CH_2CH_3$	-$CH_2CH_2CH_3$	Slight
$(CH_3)_2CH$-	-CH_2CH_3	-CH_2CH_3	Slight
$(CH_3)_2CH$-	-$CH_2CH_2CH_3$	-$CH_2CH_2CH_3$	Slight
$CH_3CH_2CH_2$-	-$CH_2CH_2CH_3$	-$CH_2CH_2CH_3$	Slight
CH_3CH_2-	-$CH_2CH{=}CH_2$	-$CH_2CH{=}CH_2$	Slight
CH_3CHCl-	-$CH_2CH{=}CH_2$	-$CH_2CH{=}CH_2$	Slight
$CHCl_2O$	-H	-$CH_2CH{=}CH_2$	Slight
$CHCl_2O$	-H	-$CH_2CH_2CH_3$	Slight
CCl_3			Slight

[a] Very good, good, moderate, and slight activity as antidotes for EPTC in corn corresponds to average reductions in EPTC injury of >60, 40–59, 20–39, and <20%, respectively, in all experiments conducted.

protected from the atmosphere by a calcium chloride drying tube. When the addition was complete, stirring continued for approximately 20 min, after which the mixture was heated to reflux for 30 min to ensure that the reaction was complete.

The organic phase was washed with dilute acid, dilute base, and water, dried ($CaCl_2$) and evaporated. Vacuum distillation at approximately 2 Torr afforded the amides, usually as colorless oils. Overall yields were typically 50–60%. Products were characterized by infrared (C=O), NMR, and mass spectrometry, and where appropriate, by elemental analysis. The NMR spectra were all complicated due to hindered C–N rotation, and sharp resonances were not observed at room temperature (see Supplementary Material Available paragraph).

Quartz Sand Nutrient Culture Bioassay. Corn (*Zea mays* L. United Hybrid 106, Stewart 2501, Golden Beaver) seeds were germinated in petri dishes on moistened filter papers for 2 days in an incubator at 24 °C. When the radicle on the seed was approximately 1.5–2 cm, the seedlings were transferred to quartz sand nutrient culture in separate styrofoam cups. The seedlings were transplanted into a depth of 4.5 cm of No. 10 grade silica sand in a 9 × 7 cm cup and were covered with another 2 cm of quartz sand. The bottom of the cup containing the quartz sand was perforated with 16–20 small holes and then placed in a lower cup. A plastic stake was placed between the two cups to allow air passage. A runoff-hole punctured the outside cup at the 40 mL mark. The plants were maintained in a growth room with a 16-h photoperiod with 4600 lux light intensity at 24 °C and an 8-h dark period at 20 °C. Two days after transplanting, when the plants were just in the two-leaf stage and approximately 6 cm in height, the initial nutrient solution was drained out and the treatments applied. Appropriate combinations of EPTC and antidote solutions were added simultaneously in 20 mL volumes of nutrient solution to provide for the following treatments: EPTC 0 + antidote 0, EPTC 0 + antidote 10^{-4} M, EPTC 10^{-4} M + antidote 0, and EPTC 10^{-4} M + antidote 10^{-4} M in 40 mL half-strength Hoag-

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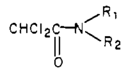
Table II. Comparative Activity of Dichloroacetamides Varying in Length and Saturation of Amide Chains as Antidotes to EPTC in Corn Grown in Quartz Sand Nutrient Culture

Antidotes added to EPTC



Amide chains: R_1	R_2	Corn injury (% reduction in shoot dry weight)[a]
Saturation		
$CH_3CH_2CH_2$-	$CH_3CH_2CH_2$-	5 d
CH_2=$CHCH_2$-	CH_2=$CHCH_2$-	14 c
CH≡CH-CH_2	CH≡$CHCH_2$-	26 b
Chain length		
CH_3CH_2-	CH_3CH_2-	46 ab
$CH_3CH_2CH_2$-	$CH_3CH_2CH_2$-	5 d
$CH_3CH_2CH_2CH_2$-	$CH_3CH_2CH_2CH_2$-	31 bc
CH_3CH_2-	$CH_3CH_2CH_2CH_2$-	55 a
EPTC alone (control)		45 ab

[a] EPTC and antidotes were applied simultaneously at equimolar concentrations of 10^{-4} M in quartz sand nutrient culture to 4-day germinated corn seedlings. Plants were harvested 11 days after treatment. Means followed by unlike letters significantly differ (0.05 level, ANOV and Duncan's Multiple Range Test).

land's nutrient solution. At 4 days after transplanting, the treatments were repeated. The plants were maintained with half-strength Hoagland's nutrient solution until harvested, 10–11 days after transplanting. Weights of the corn shoots were obtained after oven drying.

All individual experiments were conducted at least three times with four replicates for each treatment. Data were subjected to analysis of variance and Duncan's Multiple Range Tests to facilitate statistical comparisons of antidote activity.

RESULTS AND DISCUSSION

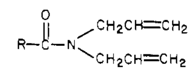
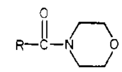
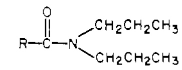
Over 30 amide analogues were synthesized and examined in preliminary bioassays as antidotes for reducing EPTC injury to corn. Most of these compounds exhibited moderate to high activity as EPTC antidotes (Table I). Some changes in structure compared with R-25788 always resulted in loss of activity as antidotes for EPTC. These included replacing the dichloromethyl group of R-25788 with any of the following groups: CCl_3-, $(CH_3)_2CH$-, $CH_3CH_2CH_2$-, CH_3CH_2-, or CH_3CHCl. N-Monosubstituted amides were also significantly less active than their N,N-disubstituted analogues.

Among dichloroacetamides varying in the structure of the amide side chains, *N,N*-dipropyl-2,2-dichloroacetamide was more active than the *N,N*-dipropenyl (R-25788) or *N,N*-dipropynyl compounds (Table II). For analogues with saturated amide side chains, three carbon atoms in the chains gave the maximum antidote effectiveness. Thus the *N,N*-diethyl-, *N,N*-dibutyl-, and *N*-ethyl-*N*-butyldichloroacetamides were all inferior antidotes to the *N,N*-dipropyl compound. However, linking the two side chains into a cyclic structure did not lower antidote effectiveness, the dichloroacetyl derivatives of piperidine, hexamethyleneimine, and morpholine all being satisfactory antidotes (Table I).

When *N,N*-diallyl acetamides with zero, one, two, or three chlorine substitutions on the 2 carbon of the acid chain were compared (Table III), the monochloro (CDAA) or dichloro (R-25788) were equally high in activity for reducing EPTC injury. Only slight reduction in EPTC injury was achieved with either the methyl [$CH_3CON(CH_2CH{=}CH_2)_2$] or trichloro [$CCl_3CON(CH_2CH{=}CH_2)_2$] analogues (Table III). When the corresponding series of *N,N*-dipropylacetamides were compared, the dichloro analogue [$CHCl_2CON(CH_2CH_2CH_3)_2$] was clearly the most active of the series as an antidote for EPTC; this was true also in the morpholine series (Table III).

Table III. Influence of Degree of Acid Chain Chlorination on the Activity of *N,N*-Dipropyl, *N,N*-Diallyl, and *N*-Morpholinyl Acetamides as Antidotes for EPTC in Corn Grown in Quartz Sand Nutrient Culture

EPTC injury to corn (% reduction in shoot dry wt) in the presence or absence of various acetamide antidotes[a]

R	 *N,N*-diallyl acetamides	 *N*-morpholinyl acetamides	 *N,N*-dipropyl acetamides
CH_3-	51 b	70 a	51 ab
CH_2Cl-	27 c	40 c	43 b
$CHCl_2$-	27 c	20 d	26 c
CCl_3-	52 b	53 b	49 b
EPTC alone	64 a	46 bc	59 a

[a] Antidotes and EPTC were applied at equimolar (10^{-4} M) concentrations to 5-day germinated corn seedlings in quartz sand nutrient culture. Plants were harvested 11 days after treatment. Means (four replications) followed by unlike letters significantly differ (0.05 level, ANOV and Duncan's Multiple Range Test).

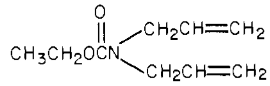
Table IV. Comparative Activity of Various *N,N*-Diallyl Amides with Different Acid Chains as Antidotes for EPTC in Corn Grown in Quartz Sand Nutrient Culture

Antidotes added to EPTC
$ROC(O)N(CH_2CH{=}CH_2)_2$

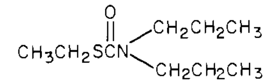
R	Corn injury (% reduction in shoot dry weight)[a]
CH_3-	46 b
CH_3CH_2-	43 b
$CH_3CH_2CH_2$-	49 ab
CH_3CH_2O-	26 d
Control, EPTC alone	56 a

[a] Antidotes and EPTC were applied at equimolar (10^{-4} M) concentrations in nutrient solution to 4-day germinated corn seedlings in quartz sand. Plants were harvested 11 days after treatment. Means (four replicates) followed by unlike letters significantly differ (0.05 level, ANOV and Duncan's Multiple Range Test).

N,N-Diallyl amides varying in numbers of carbons in the acid chain (CH_3CO-, CH_3CH_2CO-, or $CH_3CH_2CH_2CO$-) were only slightly active as EPTC antidotes (Table IV). However, introducing an electronegative oxygen into the chain as in ethyl *N,N*-diallylcarbamate significantly increased antidotal activity for EPTC in corn. It is remarkable how similar in structure this latter compound is to EPTC.



Ethyl *N,N*-diallylcarbamate (antidote)



EPTC (herbicide)

Many analogues of R-25788 have been examined as antidotes for EPTC in corn by other investigators. In the patent (Pallos et al., 1972) for these compounds, hundreds of analogues, including most of those in this study, were examined. Pallos et al. (1975) also reported on the activity of 17 different mono- and dichloroacetamides as EPTC antidotes for corn. In these previous studies, the bioassays were conducted in EPTC-treated soil with antidote-treated corn seeds. The antidotes gave essentially complete protection in most cases, thus few structure–activity relationships were evident. Lay and Casida (1976) examined at least 30 different dichloroacetamides for activity in increasing GSH-transferase and GSH levels in corn roots. They also used a soil bioassay with antidote-treated seeds and reported a general but not precise correlation between these effects and activity as antidotes.

In this study we attempted to use concentrations of EPTC that would result in severe corn injury and concentrations of antidotes that would result in often significant but rarely complete elimination of that injury. We felt it was extremely important to eliminate soil sorption phenomena so that differences in antidote activity would be strictly "plant related". With this approach, our experiments established that molecules structurally similar to EPTC are active as antidotes for this herbicide in corn. Moreover, analogues that are even more structurally similar to EPTC are often more active as antidotes. Thus among dichloroacetamides, the *N,N*-dialkyl chains of the amide are of optimal effectiveness when each contains three carbon atoms, as does EPTC. Also, in our soil-free tests, the saturated three-carbon chain (identical with EPTC) is superior to the unsaturated system used in the commercial antidote, R-25788. Antidote activity is promoted by the presence of electronegative groups in the "acid" chain of the amide: chloroacetyl, dichloroacetyl, and ethoxy-carbonyl groups being especially effective. It is possible that these electronegative groups cause the acid chain to be similar to the S-C_2H_5 group of EPTC, while lacking the toxic sulfur functionality.

While structural similarity to EPTC may not be a requirement for antidote activity, we have shown that many similar compounds are active and analogues which are more similar to EPTC have even higher antidote activity. We suggest that the soil-free bioassay used in this work has been advantageous in revealing this structure–activity relationship and that it may not have been evident to other investigators who employed bioassays in soil. In soil, differences in antidote activity are likely to reflect comparative differences in availability of antidote and/or herbicide to the plant, combined with any differences in activity at the site(s) of action within the plant.

In light of the data presented here, in the search for antidotes for other herbicides, investigators may find the examination of compounds structurally similar to the herbicide to be a fruitful first approach. Initial bioassays could well be some type of soil-free system. Later bioassays in soil could reveal structural changes which are necessary to make the antidote sufficiently available for plant uptake.

ACKNOWLEDGMENT

The technical assistance of Elise Pelly is gratefully acknowledged.

Supplementary Material Available: A listing of data from infrared (C═O), NMR, mass spectrometry, and elemental analyses used to characterize the antidotes and analogues (4 pages). Ordering information is given on any current masthead page.

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