Phytochemistry, 1968, Vol. 7, pp. 913 to 920. Pergamon Press. Printed in England

THE METABOLISM OF 3,4-DICHLOROPROPIONANILIDE IN PLANTS. PARTIAL PURIFICATION AND PROPERTIES OF AN ARYL ACYLAMIDASE FROM RICE*

D. S. FREAR and G. G. STILL

Crops Research Division, Agricultural Research Service, U.S. Department of Agriculture, Metabolism and Radiation Research Laboratory, Fargo, North Dakota

(*Received* 3 *January* 1968)

Abstract—An aryl acylamidase (aryl-acylamine amidohydrolase, EC 3.5.1.a) from rice, which hydrolyzes 3,4-dichloropropionanilide, has been partially purified and characterized. The distribution of the enzyme in rice and barnyard grass tissues was determined. The enzyme displayed a broad specificity for chlorinated ring-substituted propionanilide analogs, but was specific for 3,4-dichloropropionanilide when compared with several alkyl substituted analogs. The enzyme was inhibited by sulfhydryl reagents and was strongly inhibited at $1{\cdot}0 \times 10^{-6}$ M by the insecticidal carbamates 1-naphthyl methylcarbamate, 4-benzothienyl methylcarbamate, 2-chloro-4,5-xylyl methylcarbamate, 2,4,5-trimethylphenyl methylcarbamate, 3,4,5-trimethylphenyl methylcarbamate and 4-(methylthiol) 3,5-xylyl methylcarbamate. The partially purified enzyme had a broad pH optimum between 7·5 and 7·9, with an apparent K_m of $2{\cdot}93 \times 10^{-3}$ M for 3,4-dichloropropionanilide. The K_i for 1-naphthyl methylcarbamate was $1{\cdot}51 \times 10^{-8}$ M with 3,4-dichloropropionanilide as the substrate. The significance of the enzyme distribution in the resistant and susceptible species and the inhibition of the rice enzyme by insecticidal carbamates is discussed.

INTRODUCTION

PROPANIL (3,4-dichloropropionanilide) is used in post-emergence foliar applications as a selective herbicide for the control of barnyard grass (*Echinochloa* spp.) and other annual weeds commonly occurring in rice (*Oryza sativa* L.). Recent reports by Unger *et al.*,[1] McRae *et al.*[2] and Still[3] have shown that 3,4-dichloropropionanilide is rapidly hydrolyzed to 3,4-dichloroaniline and propionic acid in rice seedlings.

Bowling *et al.*[4] and McRae *et al.*[2] have reported that certain insecticidal carbamates and organophosphates produce synergistic effects in rice seedlings when applied with propanil.

This paper describes the preparation and properties of a particulate aryl acylamidase (aryl acylamine amidohydrolase, EC 3.5.1.a) from rice which hydrolyzes 3,4-dichloropropionanilide and its analogs. The distribution of the enzyme was studied in resistant and susceptible species (rice and barnyard grass) and the effect of several carbamate and organophosphate insecticides on enzyme activity was determined.

RESULTS AND DISCUSSION

Distribution of a Propanil Hydrolyzing Aryl Acylamidase in Rice and Barnyard Grass Tissues

Table 1 shows the results of an enzyme distribution study. The enzyme was present in all the tissues studied. Rice leaves, however, were found to contain sixty times more enzyme

* Use of trade names is for the purpose of identification and does not constitute endorsement by the U.S. Department of Agriculture.

[1] V. H. UNGER, D. H. MCRAE and H. F. WILSON, *Weed Soc. Abs.* 86 (1964).
[2] D. H. MCRAE, R. Y. YIH and H. F. WILSON, *Weed Soc. Abs.* 87 (1964).
[3] G. G. STILL, *Weed Soc. Abs.* 64 (1967).
[4] C. C. BOWLING and H. R. HUDGINS, *Weeds* 14, 94 (1966).

units than barnyard grass leaves. Rice roots and barnyard grass roots contained the same amount of enzyme activity, but this was only 3 per cent of the enzyme units found in rice leaf. This striking difference in enzyme distribution may be of considerable significance in determining the relative phytotoxicity of propanil to these two plant species, particularly since post-emergence foliar application of propanil is generally used in the control of barnyard grass in rice.[2]

TABLE 1. DISTRIBUTION OF A PROPANIL HYDROLYZING ARYL ACYLAMIDASE IN RICE AND BARNYARD GRASS TISSUES

Tissue*	Units†/gm (fresh wt.)	Total units	Specific activity‡
Rice leaves	314·4	6288	9·8
Barnyard grass leaves	5·2	103	0·5
Rice roots	5·7	226	2·2
Barnyard grass roots	5·8	230	2·1

* Plants were grown 19 days in one-half strength Hoagland's nutrient solution with a 12 hr photoperiod at 1600 ft-c, 21–32° and 75 per cent relative humidity. Assays were made on fresh, crude, cell-free extracts from 20 g of leaf and 40 g of root tissue.

† An enzyme unit is the amount of enzyme required to catalyze the hydrolysis of 1 mμmole of propanil per hour under the assay conditions used.

‡ Specific activity is the number of enzyme units per mg of protein.

Partial Purification of a Propanil Hydrolyzing Aryl Acylamidase from Rice

The results of a typical purification experiment are shown in Table 2 where a purification of 1·85 fold was obtained with better than a 100 per cent recovery. A small molecular weight endogenous inhibitor appears to be removed on gel filtration as indicated by the apparent increase in enzyme recovery. Similar results were observed in dialysis experiments with either 30,000 *g* supernatant or 0–50 per cent $(NH_4)_2SO_4$ fractions.

TABLE 2. PURIFICATION OF RICE LEAF ARYL ACYLAMIDASE

Fraction	Volume (ml)	Protein (mg/ml)	Units*/ml	Total units	Specific activity (units/mg protein)	Recovery (%)	Purification (fold)
75,000 *g* supernatant	164	6·7	33·4	5478	4·99	100	—
0–50% $(NH_4)_2SO_4$	25	30·7	205·0	5125	6·68	94	1·34
Sephadex G-50	49	13·0	120·0	5880	9·23	107	1·85

* One unit = 1 mμmole 3,4-dichloroaniline/hr.

The enzyme activity of rice leaf homogenates and extracts of rice leaf acetone powders can be sedimented under several different gravitational fields and appear to be associated with cell membrane fragments of different sizes. After centrifugation at 150,000 *g* for 2 hr, only 20 per cent of the crude cell-free aryl acylamidase activity of rice leaf acetone powder extracts remains in the supernatant. The greatest enzyme activity appears to be associated with particles that can be sedimented between 30,000 *g* for 30 min and 150,000 *g* for 2 hr.

Particulate aryl acylamidase enzymes have also been observed in rat liver and kidney preparations by Williams and Jacobson,[5] Mahadevan and Tappel,[6] and in chicken kidney preparations by Nimmo-Smith.[7]

Attempts to solubilize the enzyme by sonication and with detergents for further purification have been ineffective and often result in considerable enzyme inactivation. Similar results were obtained by Nimmo-Smith[7] with chicken kidney preparations.

Stability

The lyophilized Sephadex G-50 eluate can be stored for several months under vacuum at −15° with little loss of activity. The Sephadex G-50 eluate was stable for 24 hr at 4° and could be frozen in buffer for 5 to 6 days with no loss of activity. Repeated freezing and thawing resulted in a slight increase in enzyme activity. Heating the enzyme in a boiling water bath for 10 min resulted in complete inactivation.

Optimum pH

The pH optimum for 3,4-dichloropropionanilide hydrolysis by partially purified rice aryl acylamidase was found to be between 7·5 and 7·9 as shown in Fig. 1. This optimum pH is similar to that observed by Mahadevan and Tappel[6] and Nimmo-Smith[7] with aryl acylamidases from rat and chicken kidney preparations.

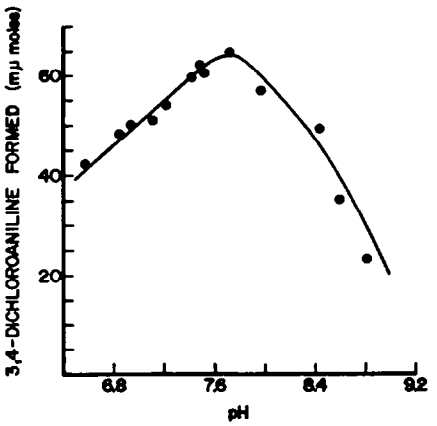

FIG. 1. OPTIMUM pH OF RICE LEAF ARYL ACYLAMIDASE.

The complete reaction mixture contained 250 μmoles buffer, 0·40 μmole cysteine, 1 μmole 3,4-dichloropropionanilide, 2·8 mg of Sephadex G-50 enzyme, and distilled water to a final volume of 2·0 ml. The reaction was incubated for 3 hr at 25°. Controls were placed in a boiling water bath for 10 min and cooled to room temperature before the addition of substrate. Phosphate (—⊙—); Bicine (—●—).

Inhibition and Activation Studies

As shown in Table 3, several thiol inhibitors were found to be very effective in inhibiting the aryl acylamidase enzyme, while no inhibition was observed with NaCN, EDTA, NaN_3 and Na_2HAsO_4 at the 1 mM level. Rat liver, rat kidney, and chicken kidney aryl acylamidases have also been shown to be strongly inhibited by various sulfhydryl reagents.[6,7] Enzyme

[5] C. H. WILLIAMS and K. H. JACOBSON, *Toxicol. Appl. Pharmacol.* **9**, 495 (1966).
[6] S. MAHADEVAN and A. L. TAPPEL, *J. Biol. Chem.* **242**, 2369 (1967).
[7] R. H. NIMMO-SMITH, *Biochem. J.* **75**, 284 (1960).

TABLE 3. INHIBITION OF RICE ARYL ACYLAMIDASE BY THIOL REAGENTS

Inhibitor	Concentration (mM)	Inhibition (%)
Iodosobenzoate	0·5	74
Na_2AsO_2	1·0	74
$HgCl_2$	0·1	74
$CuSO_4$	0·2	59
p-Chloromercuribenzoate	0·5	50
p-Benzoquinone	0·5	47
N-ethylmaleimide	0·5	44
Iodoacetate	0·5	18

The complete reaction mixture contained 250 μmoles phosphate buffer, pH 7·5, 1·0 μmole 3,4-dichloropropionanilide, inhibitor, 2·1 mg Sephadex G-50 enzyme, and distilled water to a final volume of 2·0 ml. The inhibitors were incubated for 15 min at 25° with enzyme, buffer and distilled water before the addition of the substrate. The reaction was then incubated for 3 hr at 25°. Controls were placed in a boiling water bath for 10 min and cooled to room temperature before the addition of inhibitor and substrate.

TABLE 4. INHIBITION OF RICE ARYL ACYLAMIDASE BY INSECTICIDAL CARBAMATES AND ORGANOPHOSPHATES

Inhibitor	Concentration (μM)	Inhibition (%)
4-Benzothienyl methylcarbamate (MCA-600)	1·0	92
	0·1	87
1-Naphthyl methylcarbamate (Carbaryl)	1·0	90
	0·1	76
4-(Methylthiol)-3,5-xylyl-methylcarbamate (Mesurol)	1·0	76
	0·1	66
3,4,5-Trimethylphenyl methylcarbamate (SD-8530)	1·0	76
	0·1	58
2-Chloro-4,5-xylyl methylcarbamate (Banol)	1·0	76
	0·1	53
2,4,5-Trimethylphenyl methylcarbamate (SD-8786)	1·0	71
	0·1	55
Diethyl mercaptosuccinate, S-ester with *O,O*-dimethyl phosphorodithioate (Malathion)	10·0	15
	1·0	4
O,O-Diethyl-*O*-(4-nitrophenyl) phosphorothioate (Parathion)	10·0	30
	1·0	10
Mixture of *O,O*-diethyl *S*-(and *O*)-2-(ethylthio) ethyl phosphorothioates (Demeton)	1·0	6
O,O-Dimethyl *S*-4-oxo-1,2,3-benzotriazin-3(4*H*)-ylmethyl phosphorodithioate (Guthion)	1·0	9
O-Ethyl *O*-(4-nitrophenyl) phenylphosphonothioate (EPN)	1·0	2

The complete reaction mixture contained 250 μmoles phosphate buffer, pH 7·5, 0·40 μmole cysteine, 1 μmole 3,4-dichloropropionanilide, inhibitor, 2·0 mg Sephadex G-50 enzyme, and distilled water to a final volume of 2·0 ml. The inhibitors were incubated for 15 min at 25° with enzyme, buffer, cysteine and distilled water before the addition of the substrate. The reaction was then incubated 3 hr at 25°. Controls were placed in a boiling water bath for 10 min and cooled to room temperature before the addition of inhibitor and substrate.

activity was not affected by Mg^{2+} or Mn^{2+} but was inhibited about 30 per cent by Ni^{2+}, Co^{2+}, Zn^{2+}, or Fe^{3+} at the 1 mM level. Nimmo-Smith[7] also observed inhibition of chicken kidney aryl acylamidase preparations by Ni^{2+} and Co^{2+}. Addition of the following reducing agents; 3-mercaptoethanol, dithiothreitol, reduced glutathione and cysteine at the 1 mM level, resulted in no significant increase in enzyme activity.

Inhibition of the enzyme by several insecticidal carbamates and organophosphates is shown in Table 4. The inhibition by the insecticidal carbamates appeared to be competitive as the inhibition was reversed by increasing the substrate concentration. At 1·0 and 0·1 μM concentrations the organophosphate insecticides showed little inhibition. These results do not agree with the work of Williams and Jacobson[5] who found that 3,4-dichloropropionanilide hydrolysis by an aryl acylamidase from female rat liver was inhibited to about the same extent with either 4-benzothienyl methylcarbamate (MCA 600) or *O,O*-diethyl-*O*-(4-nitrophenyl) phosphorothioate (parathion).

Even though the organophosphate insecticides were generally found to be less effective inhibitors of rice aryl acylamidase than the carbamate insecticides, they are apparently still active enough to produce synergistic effects in rice seedlings according to Bowling and Hudgins[4] and McRae *et al.*[2]

Substrate Specificity

The effect of ring substitution on aryl acylamidase activity is shown in Table 5. The enzyme was found to exhibit little specificity for monochloro and dichlorosubstituted propionanilides with 3,4-dichloropropionanilide being only 42 per cent as reactive as 2,3-dichloropropionanilide. The effect of mono-substituted chloropropionanilides on enzyme activity appear to be opposite those observed by Nimmo-Smith[7] with a particulate chicken kidney aryl acylamidase and mono-substituted chloroacetanilide substrates.

TABLE 5. SUBSTRATE SPECIFICITY OF RICE ARYL ACYLAMIDASE: THE EFFECT OF CHLORINE RING SUBSTITUTION

Substrate	Relative activity (%)
2,3-Dichloropropionanilide	100
2,4-Dichloropropionanilide	84
o-Chloropropionanilide	60
m-Chloropropionanilide	42
3,4-Dichloropropionanilide	42
3,5-Dichloropropionanilide	30
2,5-Dichloropropionanilide	27
p-Chloropropionanilide	21
2,6-Dichloropropionanilide	1

The complete reaction mixture contained 250 μmoles phosphate buffer, pH 7·5, 0·40 μmole cysteine, 1 μmole of substrate, 2·3 mg Sephadex G-50 enzyme, and distilled water to a final volume of 2·0 ml. The reaction was incubated for 3 hr at 25° and controls were placed in a boiling water bath for 10 min and cooled to room temperature before the addition of substrate.

Various alkyl analogs of 3,4-dichloroanilide resulted in the substrate specificity shown in Table 6. Alkyl branching at either α or β to the carbonyl carbon resulted in almost

complete loss of activity. Variation in the carbon chain length showed that 3,4-dichloropropionanilide was the preferred substrate. A similar substrate preference for the straight 3 carbon alkyl group was reported by Nimmo-Smith[7] with particulate aryl acylamidase from chicken kidney.

TABLE 6. SUBSTRATE SPECIFICITY OF RICE ARYL ACYLAMIDASE: THE EFFECT OF VARIOUS 3,4-DICHLOROANILIDE ALKYL ANALOGS

Substrate	Relative acitivity
3,4-Dichloroacetanilide	59
3,4-Dichloropropionanilide	100
3,4-Dichlorobutyranilide	32
3,4-Dichlorovaleranilide	39
3,4-Dichloro-2-methyl propionanilide	2
3,4-Dichloro-2-methyl acrylanilide	0
3,4-Dichloro-3-methyl butyranilide	0

The complete reaction mixture contained 250 μmoles phosphate buffer, pH 7·5, 0·40 μmole cysteine, 1 μmole of substrate, 2·0 mg Sephadex G-50 enzyme, and distilled water to a final volume of 2·0 ml. The reaction was incubated for 3 hr at 25° and controls were placed in a boiling water bath for 10 min and cooled to room temperature before the addition of substrate.

This enzyme will also hydrolyze benzoyl arginine 4-nitroanilide when assayed according to the method of Erlanger, Kokowsky and Cohen.[8] The enzyme is specific for the anilide linkage since 3-(4′-chlorophenyl)-1,1-dimethylurea (monuron), 3-(4′-chlorophenyl)-1-methylurea, and isopropyl N-(3-chlorophenyl) carbamate (CIPC) were not hydrolyzed to 3- or 4-chloroaniline under standard assay conditions.

Kinetic Studies

A double reciprocal plot of initial velocities at several concentrations of 3,4-dichloropropionanilide gave a straight line. The apparent K_m for 3,4-dichloropropionanilide was found to be $2{\cdot}93 \times 10^{-3}$ M when the kinetic data were processed on an IBM 1620 computer using the Fortran program of Cleland.[9,10] The apparent K_i for 1-naphthyl methylcarbamate (carbaryl) was similarly calculated to be $1{\cdot}51 \times 10^{-8}$ M.

The very low K_i value for 1-naphthyl methylcarbamate, and the strong inhibition observed with other insecticidal carbamates shown in Table 4, may explain the strong synergistic effects observed in rice fields between 3,4-dichloropropionanilide and various insecticidal carbamates.[2,4] Apparently, the insecticidal carbamates are potent inhibitors of 3,4-dichloropropionanilide hydrolysis and block its normal detoxication pathway in rice.

MATERIALS AND METHODS

Preparation of Acetone Powders

Rice (*Oryza sativa* L. var. Nato) seedlings were grown in the greenhouse in one-half strength Hoagland's nutrient solution. Leaves from 1-month-old seedlings were harvested,

[8] B. F. ERLANGER, N. KOKOWSKY and W. COHEN, *Arch. Biochim. Biophys.* **95**, 271 (1961).
[9] W. W. CLELAND, *Nature* **198**, 463 (1963).
[10] W. W. CLELAND, in *Advances in Enzymology* (edited by F. F. NORD), Vol. 29, p. 1. Interscience, New York (1967).

cut into 2 to 4 cm sections and frozen with liquid nitrogen in preparation for lyophilization. After lyophilization, the dry leaf sections were ground in a Wiley cutting mill to pass through a 30-mesh screen. The resulting rice leaf powder was then extracted three times with 10 vols. of acetone at $-15°$, filtered, air dried at 4° and stored under vacuum over conc. H_2SO_4 at 4°.

Enzyme Extraction and Partial Purification

Ten g of rice leaf acetone powder were extracted with 20 vols. of 0·1 M phosphate buffer at pH 7·5 with 1×10^{-3} M cysteine. The resulting slurry was strained through cheese-cloth and centrifuged at $75{,}000 \times g$ for 60 min. The supernatant from this centrifugation was fractionated between 0 and 50 per cent saturation by addition of crystalline enzyme grade $(NH_4)_2SO_4$. The protein precipitate at 50 per cent saturation was collected by centrifugation at 10,000 *g* for 10 min and dissolved in 25 ml of 0·1 M phosphate buffer, pH 7·5, containing 1×10^{-3} M cysteine.

The dissolved 0–50 per cent $(NH_4)_2SO_4$ fraction was layered on top of a $2{\cdot}5 \times 38$ cm Sephadex G-50 column equilibrated with 0·05 M phosphate buffer, pH 7·5, containing 0·2 M NaCl. The enzyme was then eluted with approximately 1·5 void volumes of the equilibrating buffer at a flow rate of 1 ml per min. The absorbance at 254 nm was measured to estimate the distribution of protein in eluate fractions. After elution from the Sephadex G-50 column, the enzyme was lyophilized in 20-ml ampules, sealed under vacuum and stored at $-15°$. All steps in the extraction and purification of the enzyme were carried out at 0–4°.

Enzyme Assays

The enzyme assay was based on the rate of aniline formation. The standard reaction mixture contained 250 μmoles of K phosphate buffer, pH 7·5, 0·40 μmole cysteine, 1 μmole of substrate, enzyme and distilled water to a final volume of 2·0 ml. The reaction was initiated by the addition of substrate and incubated at 25°. Controls were heated in a boiling water bath for 10 min and cooled to room temperature prior to the addition of substrate. The reaction was stopped by the addition of 5·0 ml of acid solution (8 volumes of 1 N HCl and 1 volume of glacial acetic acid). All enzyme purification, pH optimum, inhibitor and kinetic studies were carried out with 3,4-dichloropropionanilide as the substrate. Under the conditions of the assay, enzyme activity was linear with time and proportional to protein concentration.

The enzyme unit (U) was defined as the amount of enzyme required for the hydrolysis of 1 mμmole of acyl anilide per hour under the conditions of the assay.

Protein was determined either by the method of Lowry *et al.*[11] or by a micro Kjeldahl Gunning-Dyer procedure[12] with crystalline bovine serum albumin as the standard.

Quantitative Determination of Anilines

After addition of the acid mixture to stop the reaction, the precipitated proteins were centrifuged at $1000 \times g$ for 5 min and a 5·0 ml aliquot of the supernatant taken for analysis. 0·5 ml of fresh 1 per cent $NaNO_2$ was added with mixing. 10 min later, 1·0 ml of 10 per cent ammonium sulfamate and 3 ml of methanol were added with mixing to remove excess nitrite. The resultant diazonium salt of aniline was allowed to stand 10 min and then coupled with

[11] O. H. LOWRY, N. J. ROSEBROUGH, A. L. FARR and R. J. RANDALL, *J. Biol. Chem.* **193**, 265 (1951).
[12] R. BALLENTINE, in *Methods in Enzymology* (edited by S. P. COLOWICK and N. O. KAPLAN), Vol. III, p. 987. Academic Press, New York (1957).

0·5 ml of 1 per cent N-naphthylethylenediamine di-HCl-ide. The solution was filtered through a 1·2 μ membrane filter and read at the appropriate wavelength of maximum absorption. With this modified Riden and Hopkins[13] procedure, quantitative results were obtained by comparison with standard curves of the anilines used. Chloroaniline standards for colorimetric determinations were purified by recrystallization, sublimation, or preparative thin-layer chromatography and found to be homogeneous on thin-layer chromatography or gas chromatography.[14, 15]

Reagents

Ring substituted and alkyl substituted anilides used as substrates were synthesized by the procedure of Huffman and Allen.[16] After recrystallization, each compound was found to be homogeneous on TLC, exhibited a melting point within 1–2° of the reported value, and gave an i.r. spectra which was consistent with the desired structure.

Sephadex G-50 was purchased from Pharmacia Fine Chemicals. Benzoyl arginine 4-nitroanilide was obtained from Nutritional Biochemicals Corporation, Cleveland, Ohio. Bicine [N,N-bis(2-hydroxyethyl)glycine] was purchased from Calbiochem, Los Angeles, California. The 3-(4′-chlorophenyl)-1-methylurea used was synthesized by Dr. R. E. Kadunce.

High purity pesticides were generously provided as follows: 4-benzothienyl methylcarbamate (MCA 600) from Mobil Chemical Company, Metuchen, New Jersey; 1-naphthyl methylcarbamate (carbaryl) from Union Carbide Company, New York; 4-(methylthiol)-3,5-xylyl methylcarbamate (mesurol), *O,O*-dimethyl *S*-4-oxo-1,2,3-benzotriazin-3(4*H*)-ylmethyl phosphorodithioate (guthion), and a mixture of *O,O*-diethyl *S*-(and *O*)-2-(ethylthio) ethyl phosphorothioates (demeton) from Chemagro Corporation, Kansas City, Missouri; 2,4,5-trimethylphenyl methylcarbamate (SD-8786) and 3,4,5-trimethylphenyl methylcarbamate (SD-8530) from Shell Chemical Company, New York, New York; 2-chloro-4,5-xylyl methylcarbamate (banol) from Upjohn Company, Kalamazoo, Michigan; diethyl mercaptosuccinate *S*-ester with *O,O*-dimethyl phosphorodithioate (malathion) from American Cyanamid Company, Princeton, New Jersey; *O,O*-diethyl *O*-(4′-nitrophenyl) phosphorothioate (parathion) from Monsanto Company, St. Louis, Missouri; 3-(4′-chlorophenyl)-1,1-dimethylurea (monuron) and *O*-ethyl *O*-(4′-nitrophenyl) phenylphosphonothioate (EPN) from E. I. duPont de Nemours and Company, Wilmington, Delaware; isopropyl N-(3-chlorophenyl) carbamate (CIPC) from Pittsburgh Plate Glass Company, Pittsburgh, Pennsylvania; 3′,4′-dichloropropionanilide (propanil) from Rohm and Haas Company, Philadelphia, Pennsylvania; N-(3,4-dichlorophenyl) acrylamide (dicryl) from Niagara Chemical Division, FMC Corporation, Middleport, New York.

Acknowledgements—The authors wish to thank Miss Sharon Smylie, Mr. Eugene Mansager and Mrs. Agnes Hindemith for their technical assistance during the course of this work. The authors also wish to thank Dr. Fred S. Tanaka for the synthesis of the 3,4-dichloroanilide alkyl analogs and Dr. Roy J. Smith for the barnyard grass and rice seed.

[13] J. R. RIDEN and T. R. HOPKINS, *J. Agr. Food Chem.* **9**, 47 (1961).
[14] K. J. BOMBAUGH, *Anal. Chem.* **37**, 72 (1965).
[15] H. G. HENKEL, *J. Gas Chromatogr.* **3**, 320 (1965).
[16] C. W. HUFFMAN and S. E. ALLEN, *J. Agr. Food Chem.* **8**, 298 (1960).