Degradation of the Triazinone Herbicide Metamitron by *Arthrobacter* sp. DSM 20389

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The herbicide metamitron (I) is degraded by *Arthrobacter* sp. DSM 20389 by hydrolytic cleavage of the amide bond in the triazinone ring. Benzoylformic acid acetylhydrazone (III) and benzoylformic acid (IV) could be identified as metabolites. Small amounts of desaminometamitron (V) and 3-methyl-6-phenyl-2,3-dihydro-1,2,4,5-tetrazine-2-carboxylic acid (XI) were also found. Besides these compounds, 3-methyl-6-phenyl-1,2,4,5-tetrazine (VI), its 4′-hydroxy derivative VII, and 4-(benzalimino)-3-methyl-6-phenyl-1,2,4-triazin-5(4*H*)-one (VIII), 4-(benzalimino)-3-methyl-5-phenyl-1,2,4-triazole (IX), and dibenzalhydrazine (X) as products of combined microbial and abiotic reaction sequences were determined.

Metamitron [3-methyl-4-amino-6-phenyl-1,2,4-triazin-5(4*H*)-one (I); trademark Goltix] is a comparatively new selective pre- and postemergence herbicide for weed control in sugar beet crops. It is degraded to desaminometamitron (V) in both soil and plants (Jarczyk, 1976; Schmidt and Fedtke, 1977) and by different soil microorganisms (Engelhardt and Wallnöfer, 1978). Resting cells of "chloridazon-degrading" bacteria metabolize metamitron in analogy to chloridazon via "2,3-dihydroxymetamitron", resulting in 3-methyl-4-amino-1,2,4-triazin-5(4*H*)-one, a compound which is not further degraded. Under abiotic conditions, 3-methyl-6-phenyl-1,2,4,5-tetrazine (VI) was found as the main transformation product (Blecher et al., 1979).

Recently, we selected *Arthrobacter* sp. DSM 20389 from a variety of different soil bacteria investigated for degradation of metamitron because of its ability to decompose the herbicide within 2 weeks of incubation and to form considerably high amounts of 3-methyl-6-phenyl-1,2,4,5-tetrazine in the presence of light. The present study describes the pathways of metamitron degradation used by this bacterium with particular emphasis on the structure elucidation of metabolites and intermediates formed.

MATERIALS AND METHODS

Chemicals. [U-*phenyl*-^{14}C]Metamitron (specific activity 77 μCi/mg: radiochemical purity about 99%) as well as an unlabeled sample and some potential degradation products like methyl benzoylformate acetylhydrazone (III), 3-methyl-6-phenyl-1,2,4-triazin-5(4*H*)-one (V), 4-(benzalimino)-3-methyl-6-phenyl-1,2,4-triazin-5(4*H*)-one (VIII), 4-(benzalimino)-3-methyl-5-phenyl-1,2,4-triazole (IX), methyl benzoylformate (IV), and dibenzalhydrazine (X) were kindly provided by Bayer AG, Sparte Pharma, Isotopeninstitut, Wuppertal, West Germany. Benzoylformic acid (IV) was purchased from EGA Chemie, Steinheim, West Germany. All other chemicals used are commercially available compounds.

Thin-Layer Chromatography (TLC). TLC was performed on silica gel plates (150 g/LS 254; 0.1-mm thickness; Merck, Darmstadt, West Germany) by using the following solvent systems: (A) toluene–dioxane–acetic acid (90:25:4 v/v/v); (B) ethyl acetate–hexane (1:3 v/v); (C) toluene–acetic acid (9:1 v/v); (D) chloroform–toluene (9:1 v/v); (E) ethyl acetate–chloroform–acetic acid (12:8:1 v/v/v).

Organism and Growth Conditions. Stock cultures of *Arthrobacter* sp. DSM 20389 were maintained on Standard I agar (Merck, Darmstadt, West Germany) slopes at 4 °C. Liquid cultures were grown in 100-mL Erlenmeyer flasks containing 25 mL of Hegeman's (1966) mineral base modified by addition of 0.02% yeast extract, about 0.40 mmole/L [^{14}C]metamitron (specific activity 0.02 μCi/mg) and 1 g/L disodium succinate as the carbon source. The medium was adjusted to pH 7.2. The inocula were from 24-h cultures in Standard I bouillon (Merck, Darmstadt). Cultures were incubated on a rotary shaker (New Brunswick G 10) at 28 °C and 150 rpm. The flasks were kept in daylight or in the dark. For large-scale preparations of metabolites, cultivation was performed by using 2-L Erlenmeyer flasks filled with 1 L of medium containing 0.80 mmol of the unlabeled herbicide.

Determination of the Degradation Products. The bacteria were separated from the culture medium by centrifugation at 5000*g*. The supernatant was extracted twice with 25-mL portions of ethyl acetate (extract 1), acidified with 1 mL of 4 N HCl, and extracted again twice with 25 mL of ethyl acetate (extract 2). Both organic layers were dried (Na_2SO_4) and concentrated by evaporation. The compounds of extract 1 were separated on TLC plates with solvent system A. Extract 2 was treated first with diazomethane as described by Eistert et al. (1968). The methylated metabolites were then separated by successive TLC in solvent systems B and A.

The aqueous layer was neutralized immediately after the extraction procedure with solid $NaHCO_3$ and concentrated to dryness with a Büchi rotavapor (bath temperature not exceeding 45 °C). The resultant solid residue was treated 3 times with 15-mL portions of methanol, and the combined extracts were reduced to a small volume, streaked onto TLC plates, and developed in solvent system E. In some cases the cleanup procedure was modified by drying the neutral aqueous layer right after preparing extract 1.

The radioactive areas found were removed from the plates, and the compounds were each eluted several times from the silica gel with a total of 20 mL of methanol. After evaporation of the solvent, the R_f values of the remaining residues were compared with those of reference substances in further TLC tests.

Analytical Techniques. Growth of the bacteria was assayed by measuring the turbidity of the cultures at 578 nm with a Zeiss Model DM 4 spectrophotometer.

Radioactivities in the culture media, ethyl acetate extracts, aqueous layers, and eluates of radioactive TLC areas were determined by counting in a Beckman liquid scintillation counter, Type LS 200 (München, West Germany). Radioactivities on TLC plates were recorded with a Frie-

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Table I. Distribution of Radioactivity (Related to 100% Initial Radioactivity) after 14 Days of Incubation of *Arthrobacter* sp. DSM 20389 in the Dark

compound	% radioactivity	
	cultures	noninoculated controls
3-methyl-4-amino-6-phenyl-1,2,4-triazin-5(4*H*)-one (I)	2	54
methyl benzoylformate acetylhydrazone (III)[b]	3.9	
methyl benzoylformate (IV)[b]	34	2.4
3-methyl-6-phenyl-1,2,4-triazin-5(4*H*)-one (V)	0.3	3
3-methyl-6-phenyl-1,2,4,5-tetrazine (VI)	10.4	0.5
3-methyl-6-(4-hydroxyphenyl)-1,2,4,5-tetrazine (VII)	tr[a]	
4-(benzalimino)-3-methyl-6-phenyl-1,2,4-triazin-5(4*H*)-one (VIII)	0.3	1.2
4-(benzalimino)-3-methyl-5-phenyl-1,2,4-triazole (IX)	0.7	
dibenzalhydrazine (X)	4.6	
2-(methoxycarbonyl)-3-methyl-6-phenyl-2,3-dihydrotetrazine (XI)[b]	0.5	
compound XII[b]	21	

[a] Traces. [b] Compounds after methylation with diazomethane.

seke chromatogram scanner, Type LB 2733 (Karlsruhe-Durlach, West Germany).

Melting points were determined by using a Kofler hot stage (Reichert, Austria).

Electron-impact mass spectra (EI-MS) were recorded on a Varian MAT CH 7 under the conditions of automatic evaporation of the sample material by electronic control of the total ion current (Hillig et al., 1979). Field-desorption mass spectra (FD-MS) were measured after application of a solution of the compound on wires with carbon whiskers, on a Varian MAT CH 5. High-resolution mass spectra (HR-MS) were obtained from a Varian MAT CH 5D by peak matching. ^{1}H nuclear magnetic resonance (^{1}H NMR) spectra were measured on a Bruker WP Fourier transform spectrometer and ^{13}C nuclear magnetic resonance (^{13}C NMR) spectra on a Bruker WM 360 Fourier transform instrument. Fourier transform infrared (FT-IR) absorption spectra were obtained by using a Nicolet FT-IR spectrometer, 7199.

Isolation of Metabolites. Metabolites were isolated from twelve 1-L cultures harvested after a 14-day incubation period. The cells were separated by centrifugation at 5000*g*. Ethyl acetate extracts of the culture media and the methanol extracts of the dried aqueous layers were prepared as described above for the radioactive experiments. Compounds V and IX were isolated from extract 1 after repreated TLC in solvent systems A and E and compounds VIII and VI after TLC in systems A and B. The dried extract 2 and the dried aqueous layer were first treated with diazomethane (Eistert et al., 1968). After methylation, the metabolites of extract 2 were isolated by repeated TLC processes in solvent systems A, B, C, and D. The methylated metabolite of the aqueous layer was obtained after TLC in solvent system B.

RESULTS

Degradation of Metamitron by *Arthrobacter* sp. DSM 20389 in the Presence of Light. During growth of *Arthrobacter* sp. DSM 20389 on 1 g/L disodium succinate and 0.40 mmol/L metamitron in the presence of light, the cultures developed a deep purple-red color within 2–3 weeks of incubation. After 12 days of incubation, about 54% of the radioactivity was extractable with ethyl acetate, whereas 40% remained in the aqueous layer. Besides a number of different minor metabolites, ethyl acetate extracts contained 12% metamitron and 7.7% of compound VI, identified as 3-methyl-6-phenyl-1,2,4,5-tetrazine. After an incubation period of 19 days, radioactivities of ethyl acetate extracts increased to about 68%, whereas those of the remaining aqueous layers were reduced to 26% of the starting activity. In these extracts about 7% of the total activity was metamitron, whereas 30% was transformed to compound VI. This amount did not increase during further incubation.

Since the amounts of compound VI in noninoculated controls never exceeded 4% of the starting radioactivity in the presence of light, the high portion of compound VI formed must be due predominantly to an enzymatic attack on the metamitron molecule rather than to chemical destruction alone, as shown by Blecher et al. (1979). For the formation of compound VI, these authors suggested a mechanism including a hydrolytic opening of the metamitron molecule between N-4 and C-5, followed by a CO_2 split off and formation of the tetrazine ring.

Degradation of Metamitron by *Arthrobacter* sp. DSM 20389 Grown without Light. When grown in the absence of light, cultures of *Arthrobacter* sp. DSM 20389 turned yellowish and only small amounts of compound VI were found in ethyl acetate extracts. Since it was suggested that the absence of light prevented the formation of the tetrazine ring but not the hydrolytic cleavage of the metamitron molecule, the cultures were kept in the dark during incubation for the isolation of further degradation products.

During growth of the bacterium on 1 g of disodium succinate, 0.40 mmol/L metamitron was metabolized almost completely within 14 days. About 26% of the radioactivity was extractable with ethyl acetate from the neutral medium (extract 1), 28% was extractable after acidification (extract 2), and 35% remained in the aqueous layer. The radioactivity of extract 1 consisted of residual metamitron and the metabolites V–IX. Extract 2 contained the compounds III, VI, and X–XII, whereas in the aqueous layer only the metabolite IV was present. The distribution of radioactivities (related to 100% starting activity) of a typical run after 14 days of incubation and that of a noninoculated control are summarized in Table I.

Degradation of Metamitron under Abiotic Conditions. After 14 days of incubation in noninoculated (sterile) controls, about 74% of the radioactivity applied was extractable with ethyl acetate without acidification of the medium and 5% was extractable after acidification, with 7% remaining in the aqueous layer. Besides small amounts of different degradation products, 54% of the starting activity was identified as metamitron (Table I). In the ^{14}C balance, about 25% was lost during the incubation and isolation procedures.

Identification of Metabolites. The metabolites III–XII (formed in the dark) shown in Table II were identified by comparing their mass spectra with those recorded for the reference substances. From extract 1, five compounds (V–IX) could be isolated and recorded mass spectrome-

trically. During the TLC cleanup of extract 1, one purple-red zone was most significant, from which three metabolites, VI, VII, and VIII, were separated.

For metabolite VI a molecular ion at m/e 172 was detected which indicates the loss of CO and 2 H in contrast to the parent molecule metamitron. The base peak at m/e 103 is assigned to a benzonitrile fragment. Its mass spectrum as well as the red color demonstrates the structure of 3-methyl-6-phenyl-1,2,4,5-tetrazine which has been described recently by Blecher et al. (1979). Compound VII, the second red-colored metabolite, showed a molecular ion at m/e 188.0706 in the HR-MS which is in good agreement with the calculated molecular weight of 188.0689 ($C_9H_8N_4O$). The base peak at m/e 119 is assigned to the hydroxybenzonitrile ion. The ^1H NMR spectrum indicates, besides a CH_3 group (3.03 ppm; s), an AB system of aromatic protons which suggests a C-4 substitution in the phenyl moiety. Compound VII therefore is designated as 3-methyl-6-(4-hydroxyphenyl)-1,2,4,5-tetrazine.

The HR-MS of compound VIII exhibited a molecular ion at m/e 290.1178 which gives a formula of $C_{17}H_{14}N_4O$ (M_r calcd 290.1168). In comparison to metamitron ($C_{10}H_{10}N_4O$), this means an introduction of C_7H_4 which is explained by condensation of metamitron with benzaldehyde resulting in 4-(benzalimino)-3-methyl-6-phenyl-1,2,4-triazin-5(4*H*)-one. The structure was confirmed by comparison of its mass spectrum with that of the authentic material.

From extract 1, two additional, more polar products, V and IX, were isolated. The EI-MS of V showed a molecular ion at m/e 187 and fragmentation ions at m/e 104 (base peak) and m/e 42 which are assigned to benzonitrile and the acetonitrile cation. The mass spectrum was identical with that of desaminometamitron. The HR-MS of compound IX exhibited the molecular ion at m/e 262.1229 which gives the formula $C_{16}H_{14}N_4$ (M_r calcd 262.1218). IX differs from compound VIII only by the loss of CO. The proposed triazole structure of compound IX was confirmed with that of authentic material.

The formation of the tetrazine structure of VI is most probably due to hydrolytic ring cleavage of metamitron. The resulting compound II (Figure 1) is easily transformed to compound VI in the culture solution in the presence of light and oxygen during the cleanup procedures. So that this reaction could be avoided, extract 2 was dried, dissolved in methanol, and treated with diazomethane. After separation by TLC, compound X and the methylated compounds III, XI, XII, as well as IV and VI in very small amounts, were obtained.

From the HR-MS of the methylated compound III, the formula $C_{11}H_{12}N_2O_3$ was calculated. The base peak consisting of $C_9H_9N_2O$ results from a CO_2CH_3 group being split off from the molecule. The fragment at m/e 177 indicates the cleavage of a methyl group from III. In the ^1H NMR spectrum (60 MHz; $CDCl_3$; Me_4Si) of III, two singlets at 2.37 and 2.42 ppm (methyl groups), two additional singlets at 3.87 and 3.92 ppm (methoxy groups), a multiplet of aromatic protons, and two broad bands at 8.4 and 11.6 ppm were detected. These results are explained by the existence of a syn–anti mixture of the isomers of methyl benzoylformate acetylhydrazone which are confirmed by the results of the ^{13}C NMR spectrum (90.52 MHz). The final elucidation of the proposed structure was obtained by comparison of the ^1H NMR and EI mass spectrum of compound III with that of the synthetic anti isomer.

The structure of compound X was confirmed by comparison of the EI-MS with authentic dibenzalhydrazine.

Table II. Physical Data of the Metabolites of Metamitron

compound	R_f in solvent A	R_f in solvent B	R_f in solvent E	mp, °C	EI mass spectrum (rel intensity) M^+	m/e	m/e	m/e
3-methyl-4-amino-6-phenyl-1,2,4-triazin-5(4*H*)-one (I)	0.25	0.0	0.52					
methyl benzoylformate acetylhydrazone (III)[b]	0.53	0.70	0.65	n.d.	220 (3)	161 (100)	119 (37)	77 (24)
methyl benzoylformate (IV)[b]	0.84	0.80	0.90	liquid at 20 °C	164 (2)	151 (8)	105 (100)	77 (60)
3-methyl-6-phenyl-1,2,4-triazin-5(4*H*)-one (V)	0.05	0.0	0.20	259	187 (50)	104 (100)	42 (83)	
3-methyl-6-phenyl-1,2,4,5-tetrazine (VI)	0.76	0.79	0.98	72-74	172 (9)	103 (100)	76 (28)	50 (10)
3-methyl 6-(4-hydroxyphenyl)-1,2,4,5-tetrazine (VII)		n.d.[a]		190	188 (9)	109 (100)	91 (18)	64 (21)
4-(benzalimino)-3-methyl-6-phenyl-1,2,4-triazin-5(4*H*)-one (VIII)	0.60	0.0	n.d.	172–4	290 (13)	187 (46)	118 (100)	90 (90)
4-(benzalimino)-3-methyl-5-phenyl-1,2,4-triazole (IX)	0.15	0.0	0.35	127	262 (100)	185 (16)	159 (11)	104 (19)
dibenzalhydrazine (X)	0.90	0.88	0.88	n.d.	208 (42)	207 (41)	131 (100)	104 (40)
2-(methoxycarbonyl)-3-methyl-6-phenyl-2,3-dihydrotetrazine (XI)[b]		n.d.		n.d.	232 (2)	145 (34)	104 (100)	42 (60)
compound XII[b]	0.65	0.24	0.93	n.d.	246 (0)	103 (100)	76 (44)	

[a] n.d. = not determined. [b] Compounds after methylation with diazomethane.

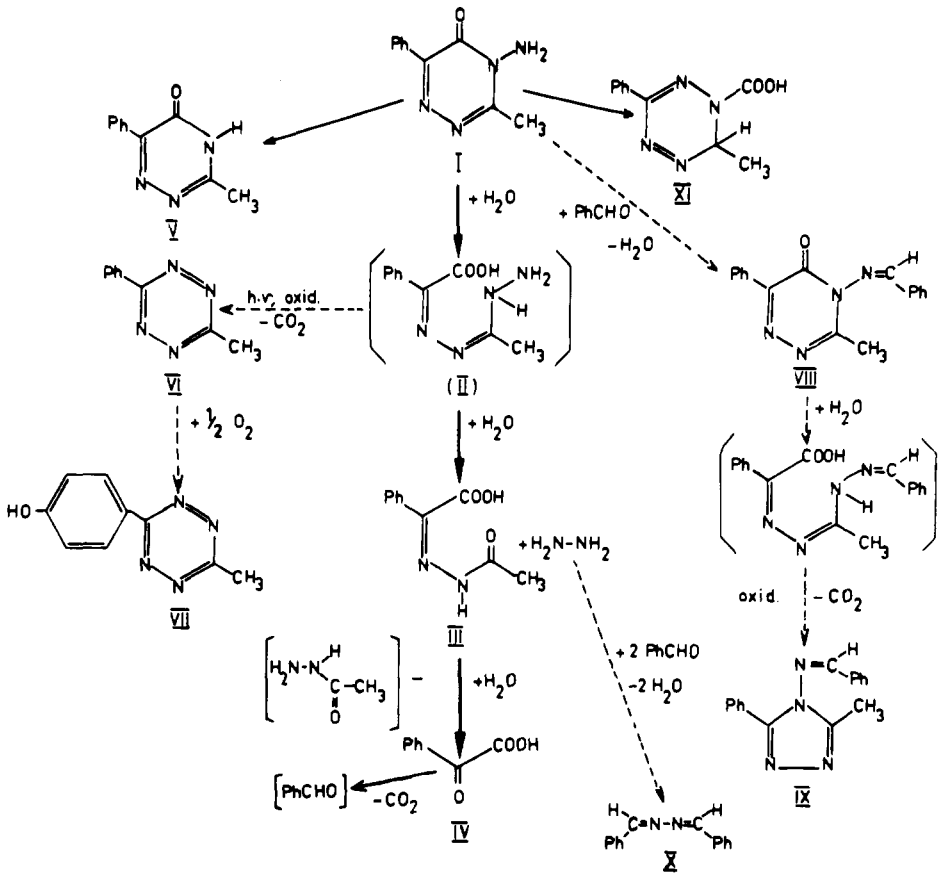

Figure 1. Proposed pathways for the degradation of metamitron (I) by *Arthrobacter* sp. DSM 20389. Ph = phenyl; arrows = microbial degradation pathways; dashed arrows = abiotic degradation pathways. Metabolites III, IV, and XI were identified after methylation with diazomethane.

From the HR-MS of the methylated metabolite XI, a molecular ion at m/e 232.0960 with a formula of $C_{11}H_{12}N_4O_2$ was calculated which indicates a formal addition of CH_2O to the starting compound metamitron. The additional carbon is obviously introduced as a methylene group during the reaction with diazomethane, whereas the oxygen could be incorporated by the addition of water. Furthermore, the removal of 2 H leads to compound XI. The fragment ion at m/e 145.0766 ($M^+ - N_2 - CO_2CH_3$) as well as the singlet at 3.75 ppm in the ^1H NMR spectrum (60 MHz; $CDCl_3$; Me_4Si) confirms the presence of a carboxymethyl group. Furthermore, aromatic protons and a CH_3CH group [2.03 (d) and 5.63 (q) ppm; J_{HH} = 7 Hz] were detected. Besides this, the data obtained with an additional ^{13}C NMR spectrum were also in accordance with the postulated chemical structure of compound XI (Figure 1).

The chemical structure of the methylated compound XII could not be elucidated because of impurities. From its FD-MS, a molecular ion at m/e 246 was identified. The ^1H NMR spectrum showed, besides aromatic protons, singlets for three methyl groups at 2.33, 3.53, and 3.58 ppm, respectively. The singlets at 3.53 and 3.58 ppm probably may be caused by methoxycarbonyl groups.

Since compound IV could be isolated from the neutral aqueous layer in much higher yields (about 32% of the starting radioactivity) than from the acidified medium (about 9% of the starting activity), it is suggested that it is partially destroyed after acidification, forming benzaldehyde. The mass spectrum of the methylated compound IV which was isolated predominantly from the neutral aqueous layer after treatment with diazomethane showed a molecular ion at m/e 164 and a main fragment at m/e 105 ($C_6H_5CO^+$) in the EI-MS. These data and the excellent agreement of the FT-IR spectrum of IV with that obtained by Sadtler Research Laboratories, Inc., No. 28946h, confirmed the chemical structure of methyl benzoylformate.

From the structure of these different metabolites, the pathway shown in Figure 1 is proposed for the degradation of metamitron by *Arthrobacter* sp. DSM 20389.

DISCUSSION

The results obtained in this study clearly show that *Arthrobacter* sp. DSM 20389 is able to open the triazinone ring of metamitron hydrolytically between N-4 and C-5, thus forming the hypothetic molecule II (Figure 1) as intermediate which could not be isolated under the given conditions. Compound III represent a hydrolysis product of this intermediate II. Further hydrolyzation of the imino group of III most probably yields benzoylformic acid (IV) and hydrazine. A possible alternative degradation pathway should be considered too: The hypothetic intermediate II may be cleaved directly to benzoylformic acid and acetamidrazone, a metabolite which could not be determined radiochemically, since the starting compound metamitron was only labeled in the phenyl moiety of the molecule in this study. Besides compounds III and IV, the radioactive intermediate XII was isolated after methylation with diazomethane. Structure elucidation failed until now although its molecular ion could be determined at m/e 246. Nevertheless, a methoxycarbonyl group in XII only can be derived from a ring-opened intermediate similar to II. Two more degradation products of metamitron were found in minor quantities, desaminometamitron (V), the main degradation product of metamitron in soil, and a dihydrotetrazine (XI) which may be one precursor of the tetrazine VI. The formation of XI is assumed by an ox-

idative cleavage of the C-5–C-6 single bond in I followed by a cyclization reaction. It is notable that some minor metabolites could be isolated in the bacterial culture medium, formed by secondary reactions with metabolites of metamitron: Compound VIII is the formal condensation product of metamitron and benzaldehyde derived from metabolite IV by decarboxylation. Compound VIII may be cleaved hydrolytically, decarboxylated, and oxidized to the triazole IX prior to ring contraction (Figure 1). Dibenzalhydrazine (X) results from the reaction of hydrazine and benzaldehyde which are formed enzymatically during the herbicide degradation as discussed above.

In the presence of light, the hypothetic intermediate II is converted to the tetrazine derivative (VI) after decarboxylation and oxidation. Traces of compound VI are oxidized further to the 4'-hydroxyphenyl compound VII. The comparatively high amounts of compound VI (Table I), even in experiments run in the dark, are most probably due to aboitic secondary reactions during the extraction and cleanup procedures.

The reason why tetrazine could usually not be found in metamitron-treated soils may be that the herbicide is too rapidly transformed to its desamino analogue (V) either by the action of UV light at the soil surface or by various soil fungi and bacteria (Engelhardt and Wallnöfer, 1978). Desaminometamitron with its three nitrogen atoms, however, cannot be transformed to a tetrazine derivative.

Within soil, microbial degradation of metamitron certainly proceeds to desaminometamitron as well as to the hypothetic ring fission product II which is decomposed further to products similar to those identified in this study. The formation of compound VI seems most unlikely because of the absence of light; also, the chemical structure of II makes its binding to soil organic matter most probable.

Whether metamitron is degraded in the environment by the same reaction sequence as shown by the in vitro studies must be confirmed by further experiments.

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Mechanism of Cholinesterase Inhibition by Methamidophos

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[*O-methyl*-^{14}C]Methamidophos and [*S-methyl*-^{14}C]methamidophos were synthesized and used to determine the nature of the phosphorylating moiety in the inhibition of electric eel acetylcholinesterase by methamidophos. Gel filtration chromatography served to separate the phosphorylated enzyme from excess labeled inhibitors. The results revealed that the methylthio–phosphorus linkage is cleaved during the phosphorylation process.

Although methamidophos (*O,S*-dimethyl phosphoramidothioate) is one of our most important insecticides and has been in agricultural use for a number of years, relatively little is known about its mechanism of action. For example, methamidophos is a relatively poor anticholinesterase yet is highly toxic to animals which die of typical cholinergic symptoms of poisoning (Quistad et al., 1970). Further, in its inhibition of acetylcholinesterase, it is not known whether the P–O or P–S linkage in methamidophos is cleaved during the inhibition process. While cleavage of the P–S linkage in the inhibition reaction had been previously suggested (Quistad et al., 1970), subsequent work revealed that the P–O linkage was also highly labile and either P–S or P–O cleavage occurred during alkaline hydrolysis, depending on the solvent system employed (Fahmy et al., 1972).

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This report is concerned with a study designed to determine the nature of the phosphorylating moiety when electric eel acetylcholinesterase (EEAChE) is inhibited by methamidophos.

MATERIALS AND METHODS

^{14}C-Labeled Methamidophos. [*O-methyl*-^{14}C]Methamidophos was prepared according to Lubkowitz et al. (1974). [^{14}C]Methanol (specific activity 55 mCi/mmol; 0.58 mg; ICN) diluted with nonlabeled methanol (1.74 mg) was added to 22 mg (0.141 mmol) of *S*-methyl phosphorothioic dichloride (Hilgetag et al., 1960) in 15 mL of dichloromethane at 0 °C. After being stirred for 30 min, the solution was saturated with dry ammonia and then filtered to remove ammonium chloride. The resulting [*O-methyl*-^{14}C]methamidophos was purified by preparative thin-layer chromatography (TLC) using silica gel G-1000 plates (Analtech, Newark, DE) with acetone–dichloromethane (1:1 v/v) as the developing solvent. The area between R_f 0.3 and R_f 0.45 was scraped and eluted with

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