N-Oxidation and Cleavage of the Amino Acid Derived Herbicide Glyphosate and Anilino Acid of the Insecticide Fluvalinate

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Glyphosate and the anilino acid of fluvalinate undergo nitrogen–carbon bond cleavage on reaction with *m*-chloroperoxybenzoic acid, leading ultimately to many of the same products formed on their metabolism and environmental degradation. N-Oxidation, dehydration of the hydroxylamines, and hydrolysis of the imines are plausible mechanisms to explain the oxidative conversion of glyphosate to (aminomethyl)phosphonic acid, glycine, and formaldehyde and of the fulvalinate anilino acid to 2-chloro-4-(trifluoromethyl)aniline. 2-Chloro-4-(trifluoromethyl)-*N*-isobutyranilide is a significant product of secondary oxidation of the intermediate imine(s). Methylation of the carboxylic acid group decreases product formation via decarboxylation reactions. Glyphosate undergoes little or no oxidation in rats or a liver homogenate preparation, but the fluvalinate anilino acid is converted to an intermediate imine and 2-chloro-4-(trifluoromethyl)aniline on incubation with the liver system.

Glyphosate herbicide and fluvalinate insecticide contain glycine and valine substituents, respectively. These pesticides are degraded by metabolic cleavage of either *N*-methylene bond of glyphosate (Rueppel et al., 1977; Jacob et al., 1985; Coupland, 1985) and metabolic or photochemical cleavage of the *N*-alkyl bond of fluvalinate (Quistad and Staiger, 1984; Quistad et al., 1982, 1983) to their respective primary amines. These cleavage reactions might involve oxidation at the nitrogen or at the carbon α to the nitrogen. Cleavage of the carbon–phosphorus bond by radical processes has also been suggested (Cordeiro et al., 1986). Since cleavage of secondary amines through hydroxylamine intermediates is well recognized (Lindeke and Cho, 1982; Damani, 1982) and many monooxygenase reactions involve peroxide species (Nordblom et al., 1976), peracid oxidation might be a suitable biomimetic model for oxidative attack at nitrogen leading to cleavage (Casida and Ruzo, 1986). This investigation therefore considers the mechanism of oxidative cleavage of these two amino acid derived pesticides using *m*-chloroperoxybenzoic acid (MCPBA) as a possible biomimetic model.

MATERIALS AND METHODS

Spectroscopy. Nuclear magnetic resonance (NMR) spectroscopy for samples dissolved in acetonitrile-d_3, deuterium oxide (D_2O), or combinations thereof utilized a Bruker WM-300 instrument operated at 300 MHz (^1H), 75.5 MHz (^{13}C), 282 MHz (^{19}F), or 121.4 MHz (^{31}P). Chemical shifts (δ) are referenced back to tetramethylsilane at 0.0 ppm ($\delta(^1H)$ (CH_3CN) 1.93; $\delta(^{13}C)$ (CH_3CN) 1.3). Values for δ in ^{19}F and ^{31}P spectra are referenced to fluorotrichloromethane and trimethyl phosphate, respectively, and coupling constants (J) are given in hertz (Hz). Glyphosate derivatives were quantitated by NMR with 3-(trimethylsilyl)propionic acid as the internal standard.

Mass spectrometry (MS) was accomplished with a Hewlett-Packard 5985B system by chemical ionization (CI, 230 eV) with methane as the reactant gas (0.8 Torr), electron impact (EI, 70 eV), or fast atom bombardment (FAB, xenon, glycerol matrix). Samples were introduced either with heating directly (60–150 °C) or by gas chromatography (GC) with a Hewlett-Packard 5840A instrument. GC utilized a 10-m high-performance capillary methyl silicone fused silica column operated at 80–240 °C (20 °C/min). All CI-MS analyses reported gave the corresponding M + 29 and M + 41 signals at approximately 15 and 5% of the quasi-molecular ion (MH^+) intensity. In some cases product yields were estimated from the total MS ion current.

Chromatography. Thin-layer chromatography (TLC) of glyphosate derivatives was performed on 0.25-mm-thickness DEAE cellulose plates developed with ethanol (65 mL), water (35 mL), 15 N ammonium hydroxide (2.5 mL), 17 N acetic acid (2 mL), and trichloroacetic acid (3.5 g) with ninhydrin for product visualization (Sprankle et al., 1978). TLC of fluvalinate derivatives was carried out on 0.25-mm- (qualitative) or 0.5-mm- (preparative) thickness silica gel 60 F_{254} chromatoplates developed with solvent systems as indicated. Fluvalinate derivatives were quantitated by GC using a Hewlett-Packard 5830A instrument equipped with an electron capture detector and a 5% OV 101 (2 m × 2 mm (i.d.)) glass column operated at 135 °C.

Model Compounds and Standards. These chemicals were obtained from the following sources: glyphosate and glyphosine, $[(HO)_2P(O)CH_2]_2NCH_2CO_2H$, from Chem Services (West Chester, PA); MCPBA (70% pure, determined by titration with sodium thiosulfate), sarcosine ($CH_3NHCH_2CO_2H$), formaldehyde, glycine, and phosphoric acid from Aldrich (Milwaukee, WI); methylphosphonic and (hydroxymethyl)phosphonic acids from Alpha (Danvers, MA); (aminomethyl)phosphonic acid (AMPA) from Sigma (St. Louis, MO). Methyl glycinate was prepared by treatment of glycine with diazomethane in methanol.

Glyphosate and Its Derivatives. Table I shows the NMR characteristics of glyphosate and its derivatives. [^{14}C]Glyphosate was labeled at the phosphonomethyl carbon with a specific activity of 30 mCi/mmol and was purified by TLC before use. The methyl ester of glyphosate was obtained on esterification of glyphosate with methanol containing 1% HCl at reflux for 24 h followed by solvent evaporation and dissolving the residue in 20% acetonitrile-d_3–D_2O.

Anilino Acid and Its Derivatives. GC–MS characteristics of these compounds are given in Table II. The methyl ester was obtained by reacting the anilino acid with diazomethane. 2-Chloro-4-(trifluoromethyl)aniline was prepared by reaction of 4-(trifluoromethyl)aniline (Aldrich) with equimolar *N*-chlorosuccinimide (Anderson et al., 1985) in carbon tetrachloride and isolation by TLC (hex-

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Table I. NMR Characteristics of Glyphosate and Its Derivatives

compound	resonance of indicated nuclei,[a] δ
	Glyphosate and Its Oxidation Products
glyphosate	^{1}H 3.11 (d, CH_2P, J = 13), 3.83 (s, CH_2CO); ^{13}C 166.3(s, C=O), 44.8 (d, CH_2P, J = 137), 50.0 (s, CH_2CO); ^{31}P 5.50
glycine	^{1}H 3.62 (s, CH_2); ^{13}C 41.2 (s, CH_2), 171.2 (s, C=O)
AMPA	^{1}H 2.9 (d, CH_2, J = 13); ^{13}C 37.0 (d, J = 142); ^{31}P 7.72
formaldehyde hydrate	^{1}H 4.67 [s, $CH_2(OH)_2$]; ^{13}C 82.6
phosphoric acid	^{31}P -3.00
	Comparison Compounds
(carboxymethyl)glyphosate	^{1}H 3.14 (d, CH_2P, J = 13), 3.73 (s, OCH_3), 3.99 (s, CH_2CO); ^{31}P 5.30
methyl glycinate	^{1}H 3.72 (s, OCH_3), 3.78 (s, CH_2)
glyphosine	^{1}H 3.56 (d, 2 CH_2P), 4.31 (s, CH_2CO); ^{13}C 169.0 (s, C=O), 57.0 (s, CH_2), 53.4 (d, CH_2P, J = 135); ^{31}P 4.02
glyphosine N-oxide	^{1}H 4.19 (d, 2 CH_2P), 4.74 (s, CH_2CO); ^{13}C 166.5 (s, C=O), 64.5 (s, CH_2), 62.5 (d, CH_2P, J = 128); ^{31}P 1.95
sarcosine	^{1}H 2.59 (s, CH_3N), 3.46 (s, CH_2CO)
methylamine	^{1}H 2.45
methylphosphonic acid	^{31}P 26.30
(hydroxymethyl)phosphonic acid	^{31}P 18.44
glyoxylic acid monohydrate	^{1}H 5.18 (s)

[a] All spectra in 20% acetonitrile-d_3–D_2O solutions.

Table II. GC–MS Characteristics of the Fluvalinate Anilino Acid and Its Derivatives

compound[a]	ret time (R_t)	ions m/z (intensity)
anilino acid	8.3	CI 296 (MH^+, 75), 276 (M - F, 100); EI 295 (M^+, 10), 250 (89), 206 (100)
imine E	3.7	CI 250 (MH^+, 100), 230 (M - F, 70); EI 249 (M^+, 20), 234 (M - CH_3, 30), 214 (M - Cl, 40), 206 (M - C_3H_7, 100)
aniline	2.9	CI 196 (MH^+, 50), 176 (M - F, 100); EI 195 (M^+, 100), 176 (M - F, 72)
nitrobenzene	2.5	CI 226 (MH^+, 100), 206 (M - F, 11), 176 (41); EI 225 (M^+, 74), 179 (M - NO_2, 100)
oxaziridine	5.9	CI 266 (MH^+, 100), 246 (M - F, 29), 224 (15)
isobutyranilide	6.1	CI 266 (MH^+, 70), 246 (M - F, 100); EI 265 (M^+, 22), 230 (M - Cl, 35), 195 (100)
azoxyaniline dimer	9.2	CI 405 (70), 403 (MH^+, 100), 385 (50), 383 (M - F, 70), 369 (45), 367 (M - Cl, 40)
anilino acid esters and their imine D analogues		
methyl ester	8.0	CI 310 (MH^+, 75), 290 (M - F, 100); EI 309 (M^+, 7), 250 (100), 206 (45)
imine D of methyl ester	6.8	CI 308 (MH,+, 100), 288 (M - F, 90); EI 307 (M^+, 8), 250 (30), 248 (100)
fluvalinate	16.0	CI 503 (MH^+, 20), 483 (M - F, 100), 250 (45), 210 (50)
imine D of fluvalinate	12.7	CI 501 (MH^+, 25), 481 (M - F, 90), 210 (100)

[a] Designations refer to structures in Figure 2.

ane:acetone = 5:2, R_f 0.33) in 78% yield; ^{19}F NMR (CD_3CN) δ -60.4. 2-Chloro-4-(trifluoromethyl)nitrobenzene was prepared by reaction of the aniline with MCPBA (3 equiv) in dichloromethane and isolation by TLC (hexane:acetone = 5:2, R_f 0.5); ^{19}F NMR (CD_3CN) δ -62.6. 2-Chloro-4-(trifluoromethyl)phenylisopropylimine (imine E) was obtained as the major product (~40%) from reaction of the aniline with equivalent isobutyraldehyde in benzene containing 0.01% p-toluenesulfonic acid over freshly dried molecular sieves. This reaction gives several unidentified minor products whose yield increases with time as monitored by ^{19}F NMR. The imine does not survive attempted chromatographic isolation and was thus only characterized by GC–MS.

Chemical Oxidations. Oxidations were monitored by NMR and/or MS and the products characterized by comparison with authentic standards. Glyphosate, glyphosine, AMPA, glyphosate methyl ester, and glyoxylic, methylphosphonic and (hydroxymethyl)phosphonic acids (0.035 mmol) were treated with MCPBA (0.5–2 equiv) in D_2O containing 0–40% acetonitrile-d_3 (optimally 20%) at 25 °C, resulting in complete reaction within several hours as monitored by ^{1}H NMR. The anilino acid (0.034 mmol) was dissolved in acetonitrile-d_3 containing 0–20% D_2O, warmed to 40 °C, equivalent MCPBA was added, and after 1–16 h dimethyl sulfoxide (Me_2SO) was added to quench the MCPBA; when the reaction was not quenched, artifact peaks were observed in GC–MS and GC–EC. The reaction mixtures used for quantitation by GC–EC and GC–MS were treated with diazomethane to generate the stable methyl esters. Experiments utilizing [^{18}O]MCPBA (Wagner and Rastetter, 1983) were carried out as with the unlabeled material. Where appropriate, chemical reactions were buffered with citrate and phosphate (pH 5, 7.4) and carbonate (pH 9).

Oxidations of the methyl ester of the anilino acid, fluvalinate, isobutyranilide, and the aniline were carried out in acetonitrile in a manner analogous to that of the anilino acid itself.

Biological Assays. Glyphosate (10 μg) and [^{14}C]glyphosate (400 000 dpm) were incubated for 1 h with the 9000g supernatant fraction from rat liver homogenate (1 mL) in 0.05 M phosphate buffer (pH 7.4) at 37 °C with and without added NADPH (1 mg). Protein was precipitated by addition of 3% trichloroacetic acid and centrifugation, and aliquots (50 μL) of the soluble portion were examined for glyphosate derivatives by TLC and autoradiography. The anilino acid (25 μg) was incubated with the same rat liver system and then extracted twice with ether (2 mL) for analysis of the ether-soluble products by GC–MS [EI and CI with selected ion monitoring (SIM)]. Glyphosate (25 mg) and [^{14}C]glyphosate (125 000 dpm) were administered intraperitoneally to a rat with a bile cannula, and the urine and bile collected over a period of 24 h were analyzed by ^{31}P NMR and liquid scintillation counting (LSC). Hydrogen peroxide (0.88 μmol) was added to a solution of glyphosate (1.62 μmol) and horseradish peroxidase (Sigma) (0.1 mg) in buffered D_2O (1 mL, 0.01

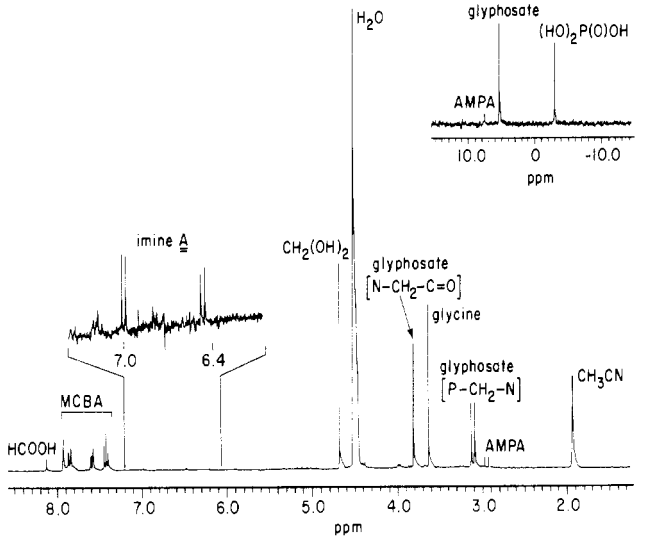

Figure 1. ^1H and ^{31}P (inset) NMR spectra of the products from reaction of glyphosate and MCPBA (0.7 equiv) in D_2O containing 20% acetonitrile-d_3 for 3 h at 25 °C. The ^1H region of δ 6.0–7.2 is expanded to reveal signals tentatively assigned to imine A.

M phosphate, pD 6), and the reaction at 37 °C was monitored by ^{31}P and ^1H NMR.

Possible binding of MCPBA-activated glyphosate (equimolar mixture incubated for up to 4 h) to bovine serum albumin (BSA) was examined in reaction mixtures consisting of the activated [^{14}C]glyphosate (20 μg in an 8-μL aliquot, 64 000 dpm) and BSA (100 μg) in water (1 mL) incubated for 30 min at 37 °C. Protein was precipitated with bovine γ-globulin (0.1% w/v in 0.1 M phosphate, pH 7.4) and poly(ethylene glycol) (25%) (Cuatrecasas, 1972), the mixture was cooled (0 °C) for 15 min, and the protein collected on Millipore cellulose acetate filters was subjected to LSC.

Mutagenesis was assayed with the Ames procedure using *Salmonella typhimurium* strain TA 100 (Maron and Ames, 1983) for glyphosate, the anilino acid, and mixtures generated from these compounds on peracid treatment.

RESULTS

Products of Glyphosate Oxidation with MCPBA in D_2O–Acetonitrile-d_3 (Figures 1 and 2). Glyphosate reacts stoichiometrically with 0.7 equiv of MCPBA in D_2O–acetonitrile-d_3 (4:1) within 3 h at 25 °C to yield phosphoric acid and AMPA detected by ^{31}P NMR and glycine, formaldehyde (hydrated), and formic acid (minor) revealed by ^1H NMR (Figure 1). Two weak sets of ^1H NMR doublets at 6–7 ppm, the characteristic region for imines (Gordon and Ford, 1972), may correspond to the syn and anti forms of glyphosate imine A, a proposal supported by FAB examination of the reaction mixture at an early stage of conversion that reveals a signal at m/z 167 appropriate for this imine or another dehydration product of the glyphosate hydroxylamine.

Glyoxylic, methylphosphonic, and (hydroxymethyl)-phosphonic acids are not detected by NMR, and neither these compounds nor AMPA react with MCPBA under these conditions to form either phosphoric acid or one-carbon units.

Rates of Glyphosate Oxidation with Equimolar MCPBA in Various Solvents. The reaction in D_2O–acetonitrile-d_3 (4:1) proceeds in two distinct phases. In the first few hours little reaction occurs and the solution remains clear whereas in the second phase the oxidation is rapid and 3-chlorobenzoic acid (MCBA) precipitates. The reaction rate is enhanced on increasing the acetonitrile concentration or reaction temperature without detectable effect on the product distribution. A pronounced isotope effect is observed on substituting nondeuteriated solvents for deuteriated solvents with a 5-fold increase in the oxidation rate (^{31}P NMR). Both the rate and product distribution are sensitive to changes in the pD of the reaction. The rate increases with increasing pD (5.0, 7.4, 9.0) for reactions run in buffered D_2O–acetonitrile-d_3 (4:1); the reaction is complete in 15 min at pD 9.0 vs. 3 h for normal reaction conditions (pD 2.0). As the reaction rate increases, the major products are phosphoric acid, glycine, and formaldehyde; no AMPA and formic acid are detected.

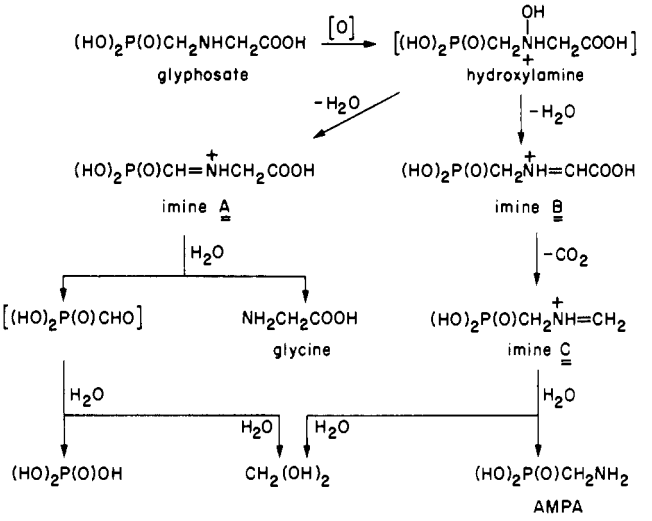

Figure 2. Oxidation of glyphosate with MCPBA, indicating designations of products.

Cyanide (2–10 equiv) added to the reaction mixture increases the reaction rate by 5- to 10-fold without changing the normal product distribution. At 25 equiv of potassium cyanide the rate decreases and numerous unidentified products are evident by ^1H NMR. Use of a two-phase system involving the standard aqueous solutions stirred over chloroform yields the expected products in the absence of cyanide, but in its presence (2 equiv) again a multitude of unidentified products are detected.

Lack of Reactivity of Glyphosate and Oxidized Glyphosate in Other Systems. No products are detected by ^1H NMR on attempted reaction of glyphosate with Fenton's reagent (hydroxyl radical) (Walling and Kato, 1971) or with the standard horseradish peroxidase–hydrogen peroxide system in D_2O solution. [^{14}C]Glyphosate was recovered unchanged on incubation with rat liver homogenate under standard conditions.

Glyphosate alone and preincubated with equimolar MCPBA failed to bind to bovine serum albumin and to show significant mutagenesis (<0.01 revertant/μg) in the *S. typhimurium* TA 100 Ames assay. [^{14}C]Glyphosate administered ip to a rat with a bile cannula is excreted unchanged in the urine to the extent of 84% of the administered dose within 24 h based on direct spotting of the urine for TLC and direct ^{31}P NMR detection of glyphosate in the urine. No labeled (LSC) or unlabeled glyphosate (^{31}P NMR) is detected in the bile over the same period.

Oxidation of the Methyl Ester of Glyphosate, Glyphosine, and Sarcosine with Equimolar MCPBA in D_2O–Acetonitrile-d_3. Oxidation of the methyl ester of glyphosate proceeds at about the same rate as that of glyphosate, but the product distribution differs since cleavage of the carbon–nitrogen bond appears to take place only in the phosphonomethylamino substituent, leading to phosphoric acid, methyl glycinate, and hydrated formaldehyde (Table III).

Table III. Product Distribution on Peracid Oxidation of Glyphosate and Its Methyl Ester

product	yield,[a] % free acid	yield,[a] % carboxymethyl
glycine (or methyl glycinate)	41 ± 3	69 ± 2
AMPA	8 ± 1	ND
$CH_2(OH)_2$	48 ± 3	30 ± 2
HCOOH	4 ± 1	0.8 ± 0.6

[a] Yields as percentages of ^1H NMR integrations confirmed by addition of standard AMPA, glycine, and methyl glycinate. Reactions carried out to completion under identical conditions with 0.7 equiv of MCPBA, resulting in loss of ~70% of the glyphosate and (carboxymethyl)glyphosate.

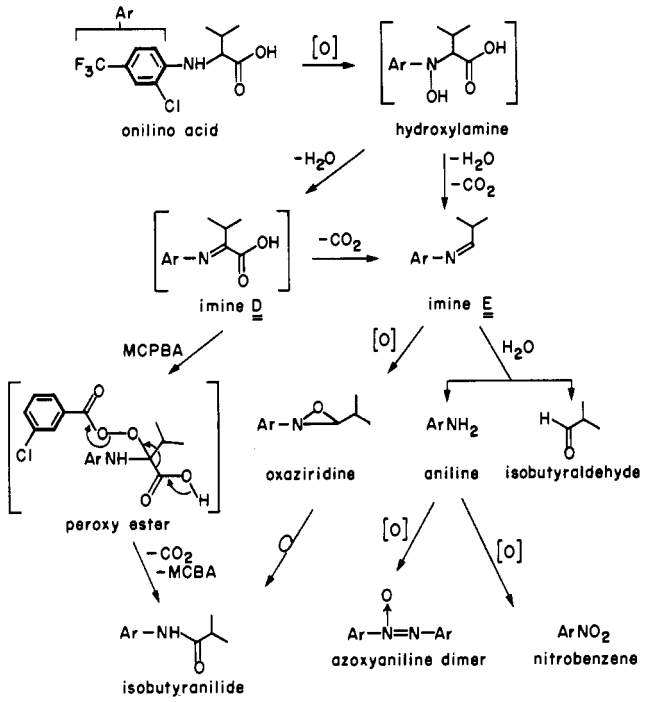

Figure 3. Oxidation of the anilino acid moiety of fluvalinate with MCPBA, indicating designations of products.

Glyphosine under these oxidation conditions yields a single major product with NMR spectral features consistent with those anticipated for the corresponding *N*-oxide whereas sarcosine yields methylamine and formaldehyde (Table I).

Products from Oxidation of the Fluvalinate Acid Moiety and Related Compounds with Equimolar MCPBA in D_2O–Acetonitrile-d_3 (Figure 3). The anilino acid does not react at an appreciable rate with equimolar MCPBA in acetonitrile-d_3 at 25 °C, but within 16 h at 40 °C the reaction is complete with 30% conversion of the starting material. The reaction mixture develops a light yellow color and decomposes on attempted TLC. Detection was with iodine vapor or the (nitrobenzyl)-pyridine reagent (Hammock et al., 1974).

The two major products characterized by GC–MS (Table II) and NMR are the corresponding nitrobenzene (~40%) and the isobutyranilide (~50%) [^1H NMR δ 1.18 (d, 2 CH_3), 2.72 (m, CH) and 7.59 (d), 7.76 (s), and 8.36 (d) (aromatics); ^{19}F NMR δ −61.6] isolated by TLC (hexane:acetone = 5:2, R_f 0.41) of the reaction mixture. When [^{18}O]MCPBA (Wagner and Rastetter, 1983) is used, the nitrobenzene and isobutyranilide formed have the expected 2-amu mass enhancement, establishing that the introduced oxygen originates from MCPBA and not from water. Additional minor products are isobutyraldehyde and the aniline (~5%). GC–MS (Table II) of the reaction mixture reveals two products in <5% yield, tentatively identified as the oxaziridine and imine E derivatives. Attempted isolation of the imine by TLC was unsuccessful.

When the reaction is carried out in D_2O–acetonitrile-d_3 (1:5 or 1:10), the product mixture is simplified because the hydrolysis of the intermediate imines appears to be favored over their oxidation by MCPBA. ^1H NMR reveals isobutyraldehyde [^1H NMR δ 1.00 (d, 2 CH_3), 2.41 (m, CH), 9.53 (s, CHO)] but no multiplet corresponding to the isobutyranilide. GC–MS confirms that the major product (80%) is the nitrobenzene with only a trace amount of amide present. Lesser amounts of aniline (15%) and azoxyaniline dimer (5%) are also noted.

Addition of tri-*tert*-butylphenol (TTBP) (10 equiv) to the MCPBA reaction mixture as a radical inhibitor only reduced the reaction rate by ~5%. Attempts to trap a putative carbinolamine intermediate with trimethylsilyl chloride or acetyl chloride did not produce identifiable products.

Peracid oxidation slowly converts the methyl ester of the anilino acid to the methyl derivative corresponding to imine D (Table II). The reaction develops a purple color, and trace amounts of the aniline, nitroso derivative, nitrobenzene, and azoxyaniline dimer (GC–MS) are detected. The imine is hydrolyzed to the aniline on addition of hydrochloric acid. The insecticide fluvalinate is oxidized at a negligible rate with only trace amounts of the corresponding imine D derivative detected after 16 h. The isobutyranilide does not react with MCPBA under these conditions.

Reactions of the Anilino Acid of Fluvalinate in Biological Systems. The anilino acid is converted to the aniline and imine E on incubation with the supernatant fraction of rat liver homogenate in the presence and absence of added NADPH based on GC–MS–SIM (EI or CI). The aniline itself does not undergo detectable metabolism under the same conditions.

The anilino acid alone or pretreated with MCPBA (0.5–2.5 equiv) does not show significant mutagenic activity (<0.4 revertant/μg; Ames TA 100 assay).

DISCUSSION

The products obtained on MCPBA oxidation of glyphosate and of the anilino acid of fluvalinate originate from nitrogen–carbon bond cleavage (Figures 2 and 3). These transformations can be envisaged as occurring via N-hydroxylation or oxidation of an α-carbon, processes ultimately yielding the same products. Attempts to alter the reaction rate of the anilino acid by addition of the radical inhibitor TTBP were unsuccessful, suggesting that α-carbon oxidation is not involved. The most plausible mechanism involves oxidation at nitrogen to form the hydroxylamine, which on dehydration to the imine and subsequent hydrolysis yields the observed products. Support for nitrogen as the susceptible site of oxidation comes from our observation that glyphosine is oxidized by MCPBA to its *N*-oxide and sarcosine to methylamine and formaldehyde and by previous observations on peracid oxidation of tertiary amine analogues of glyphosate (Franz, 1985).

The imine intermediates of glyphosate and of the anilino acid are detected, but in only low yields relative to the end products, such as the primary amines, as expected from their instability. Cyanide was not useful in trapping the glyphosate imine intermediates. The glyphosate products indicate a preference in dehydration of its hydroxylamine toward conjugation with the phosphate rather than the carboxylate group; i.e., a ratio of 6:1 is obtained for formation of glycine vs. AMPA. The observed high kinetic

isotope effect establishes the importance of hydrogen-bond-breaking events as rate-determining steps in glyphosate oxidation. Methylation of the carboxylic acid group decreases product formation via decarboxylation reactions and sufficiently stabilizes the methylated derivative of imine D for spectral examination. These results suggest the importance of decarboxylation in imine formation toward the carboxylate group of glyphosate and the anilino acid.

The anilino acid reacts with MCPBA in aqueous acetonitrile to form the aniline and isobutyraldehyde by hydrolysis of imine E, which arises through dehydration/decarboxylation reactions of the hydroxylamine. An alternative mechanism, with imine D as a discrete intermediate, is less likely due to the observed resistance to oxidation of the methyl ester of the anilino acid. In acetonitrile only, the MCPBA oxidation proceeds slowly and the product distribution reflects extensive secondary oxidation, forming nitrobenzene and isobutyranilide. The intermediate nitrosobenzene and hydroxylamino derivative are not detected, but the azoxyaniline dimer must originate from such species. Two routes are depicted in Figure 3 for isobutyranilide formation, the first via imine D and the α-[(chlorobenzoyl)peroxy]anilino acid and the second via the oxaziridine. In the first route MCPBA adds to the imino carbon of imine D to form the peroxy ester that can undergo internal nucleophilic attack by nitrogen to yield an oxaziridine (not shown) or decomposition by decarboxylation to the amide. Formation of oxaziridines from imines may involve either (a) concerted electrophilic attack of the peroxy acid on the imine via a three-membered cyclic intermediate state or (b) a two-stage reaction involving the imino carbon (Plesnicar, 1983). Process b is suggested with the anilino acid since the decreased nucleophilicity of nitrogen would favor the decarboxylation route. Alternatively, the isobutyranilide can arise from the oxaziridine in a rearrangement similar to that previously observed (Duhamel et al., 1985) on silica gel. The extent to which this rearrangement may involve secondary degradation processes (thermal, GC, TLC) is not known.

This study uses peracid oxidation as a possible model for reactions of biological relevance. The products from peracid oxidation are generally those formed biologically in this and previous studies. Thus, glyphosate is metabolized by soil microorganisms or in plants to carbon dioxide and AMPA (Nomura and Hilton, 1977; Rueppel et al., 1977; Coupland, 1985) and glycine and one-carbon fragments (Jacob et al., 1985). The anilino acid is formed from fluvalinate in plant and animal systems and is subsequently degraded to the aniline (Quistad and Staiger, 1984; Quistad et al., 1982, 1983). The presumed hydroxylamine and imine intermediates are not of sufficient stability for detection in in vivo systems. Glyphosate is not readily metabolized in the in vivo and in vitro biological systems examined here, possibly in part because of its low lipophilicity, consistent with the lack of tissue residues in animal feeding studies (Atkinson, 1985). Microsomal oxidation of the anilino acid gives imine E and the aniline. Decarboxylation of the anilino acid to the isobutyranilide parallels the products but not necessarily the mechanism of known enzymatic reactions, i.e. the oxidative decarboxylation of L-lysine to 5-aminonorvaleramide by L-lysine oxygenase (Takeda and Hayaishi, 1966) and the first reaction in the oxidative decarboxylation of tryptophan to serotonin by tryptophan 5-monooxygenase (Ichiyama et al., 1970).

Metabolic cleavage of the nitrogen–carbon bond of glyphosate and the anilino acid of fluvalinate probably involves sequential N-oxidation, dehydration of the hydroxylamine, and hydrolysis of the imine as in the peracid model systems examined here.

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