Hydrolysis of Azadirachtin in Buffered and Natural Waters

Sunny Y. Szeto† and Michael T. Wan*,‡

Pacific Agriculture Research Centre, Agriculture and Agri-Food Canada, 6660 N.W. Marine Drive, Vancouver, British Columbia, Canada V6T 1X2, and Environment Canada, Conservation and Protection, Environmental Protection, Pacific and Yukon Region, 224 West Esplanade Avenue, North Vancouver, British Columbia, Canada V7M 3H7

The hydrolysis of azadirachtin was studied in several aqueous buffers of pH 4.1−8.1 and in four natural waters (pH 6.2, 7.3, 8.0, and 8.1) at 20−45 °C. Depending on the pH, several unidentified conversion products were detected in the incubated solutions. Azadirachtin hydrolyzed readily at 35 °C, and its disappearance followed simple pseudo-first-order kinetics. The rate constants ranged from 2.48×10^{-3} to 67.7×10^{-3} h^{-1} and were faster in basic than in acidic pH. On the basis of calculations from the Arrhenius plot, the energy of activation and the frequency factor *A* for the hydrolysis of azadirachtin at pH 7.0 were 103 kJ mol^{-1} and 2.51×10^{15} h^{-1}, respectively. On the basis of rate constants, azadirachtin appeared to be more susceptible to hydrolysis than synthetic organophosphates, e.g., chlorpyrifos, diazinon, malathion, parathion, and Ronnel, or carbamates, e.g., carbaryl and propoxur. All of these insecticides are currently used extensively for pest control. Accordingly, azadirachtin is expected to be nonpersistent in water.

Keywords: *Azadirachtin; hydrolysis; kinetics; aqueous solutions*

INTRODUCTION

Azadirachtin (AZA) is a botanical bioactive agent found only in the seed kernel of the neem tree, *Azadirachta indica* A. Juss (National Research Council, 1992; Tewari, 1992). Unlike synthetic chemical insecticides, which are mostly contact neurotoxins, AZA is a selective compound affecting the endocrine system of insects as well as an antifeedant (Mordue and Blackwell, 1993). Because of this selectivity and its rapid degradation (Barnaby *et al*., 1989; Ley *et al*., 1993), AZA is considered to be less damaging than synthetic insecticides to the environment and to pose a much smaller threat to nontarget organisms, including humans, via food residues, surface and ground water contamination, or accidental exposure (Koul *et al*., 1989; Isman, 1993; Quarles, 1994). An understanding of the kinetics of hydrolysis of AZA over normal pH ranges in the aquatic environment is needed to predict the stability of water-based spray mix and the fate of this compound in wetland areas. Although AZA in ethanol solvents has been reported to be relatively more stable at lower than higher pH (Larson, 1989), to date its kinetics of hydrolysis in aqueous solutions has not been investigated under conditions of different pH values, temperatures, and water types. The objective of this study was to determine the kinetics of hydrolysis of AZA in several buffers and four natural waters under controlled laboratory conditions, and the results are reported here.

EXPERIMENTAL PROCEDURES

Reagents. AZA (>95% pure) was purchased from Sigma Chemical Co. (St. Louis, MO). Acetonitrile was of HPLC grade (Burdick & Jackson Laboratories, Inc., Muskegon, MI), and all other chemicals were of reagent grade (Fisher Scientific Co., Fair Lawn, NJ). Four natural waters of pristine origin were used in this study: Port Coquitlam Park Creek, Kanaka Creek, Cultus Lake, and Sleese Creek; these are located about 45, 75, 100 and 130 km east of Vancouver, BC, Canada, respectively.

Incubation of AZA in Aqueous Buffers and Natural Waters. Buffered solutions of 0.05 M at pH 4.1, 4.5, 5.0, 5.5, 6.0, 6.6, 7.0, 7.5, and 8.0 were prepared with sterilized, deionized water according to the method of Dawson *et al*. (1969). Aliquots of 500 μL of a stock solution of AZA at 1900 μg/mL in acetonitrile were mixed thoroughly with the aqueous buffers and natural waters to produce final concentrations of AZA at 19 μg/mL. The controls were similarly prepared with acetonitrile to monitor any UV response that was produced during incubation in the absence of AZA. Aliquots of about 0.5 mL of the solutions were transferred to brown ampules and sealed under nitrogen. To determine the kinetics of hydrolyis as described previously by Szeto (1993), 300 sealed ampules were incubated at 35 °C in a water bath in darkness; those of pH 7.0 were also incubated in darkness at 25, 30, 40, and 45 °C.

Determination of AZA by High-Pressure Liquid Chromatography (HPLC). The method of Sundaram and Curry (1993) was modified for determination of AZA concentrations in the incubated solutions at various intervals by HPLC with a Varian Model 5000 high-pressure liquid chromatograph equipped with a Hewlett-Packard Model 1040A high-speed spectrophotometric detector. The operating parameters were as follows: column, Burdick & Jackson high ligand density OC5 octyl 5 μm, 4.6 (i.d.) × 250 mm; mobile solvent system, 35% acetonitrile and 65% 0.05 M phosphate buffer of pH 2.4, isocratic at 1 mL/min; UV detector wavelength, 210 ± 2 nm. Aliquots of 20 μL of the incubated solutions from the ampules were injected directly into the high-pressure liquid chromatograph for determination of AZA.

Quantifications of AZA were based on an external standard. Detector response was calibrated for each analysis with an aqueous standard solution containing 19 μg/mL of AZA prepared in the same manner as the incubated solutions and calculated from average peak areas of these external standards, which were injected before and after each sample.

RESULTS AND DISCUSSION

Conversion Products of AZA. When AZA was hydrolyzed in aqueous buffers and natural waters at 35 °C in the range of pH 4.1−8.1, several unidentified

* Author to whom correspondence should be addressed.
† Agriculture and Agri-Food Canada.
‡ Environment Canada.

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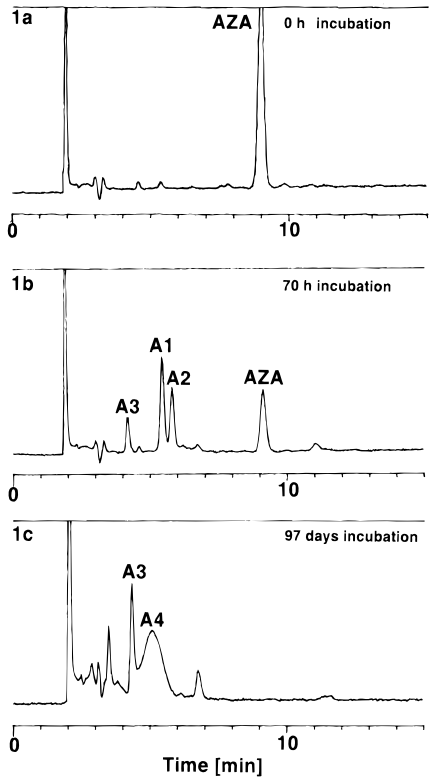

Figure 1. High-pressure liquid chromatograms of UV absorption at 210 nm of a 20-μL aliquot of 19 μg/mL of AZA in water from upper Kanaka Creek (pH 7.3) at 35 °C: (a) at 0 h of incubation; (b) after 70 h of incubation; (c) after 97 days of incubation.

conversion products were detected in the incubated solutions in the ampules by HPLC. The molecular structures of these products were not determined. The conversion products varied depending on the pH of the incubated solution, indicating that different mechanisms were involved in the hydrolysis of AZA at different pH values. Similar conversion products were detected by HPLC in the buffers and natural waters, having the same pH. Using water from upper Kanaka Creek (pH 7.3) as an example, four major conversion products, A1, A2, A3, and A4 were detected in the incubated solution at various time intervals (Figure 1). As AZA gradually disappeared, A1 was the first conversion product to appear in the incubated solution, followed by A2, A3, and A4. A1 and A2 disappeared as rapidly as AZA, whereas A3 and A4 persisted in the incubated solution for at least 97 days. The UV−visible spectra of AZA, A1, A2, A3, and A4 were identical, suggesting that the basic chromophore structure of AZA remained unchanged in all of the conversion products. Under the chromatographic conditions described, A1, A2, and A3 were chromatographed properly but not A4, as indicated by its extremely broad peak. None of the conversion products detected by HPLC in the incubated solutions in the range of pH 4.1−5.5 were chromatographed properly as shown by their broad peaks. However, their retention times were different from the retention time of A4 under identical chromatographic conditions. All of these conversion products in the range of pH 4.1−8.1 either became impossible to chromatograph or were not retained by the HPLC column when the mobile solvent system changed from 35% acetonitrile and 65% 0.05 M phosphate buffer of pH 2.4 to 35% acetonitrile and 65% water. This observation suggests

Table 1. Kinetic Data of Hydrolysis of AZA at 35 °C in 0.05 M Phosphate-Buffered Solutions and Natural Waters

medium	pH	no. of readings	corrln coeff	k_{obsd},[a] × 10^{-3} h^{-1}	half-life,[b] h
buffer	4.1	12	0.9954	2.48	279
buffer	4.5	13	0.9977	2.29	303
buffer	5.0	16	0.9960	2.52	275
buffer	5.5	13	0.9969	3.02	230
buffer	6.0	18	0.9946	3.37	206
buffer	6.6	11	0.9974	4.75	146
buffer	7.0	11	0.9983	12.0	57.8
buffer	7.5	10	0.9982	22.5	30.8
buffer	8.0	8	0.9934	58.0	12.0
Port Coquitlam Park Creek	6.2	21	0.9983	2.71	256
Kanaka Creek	7.3	10	0.9973	15.8	43.9
Cultus Lake	8.0	8	0.9980	67.7	10.2
Sleese Creek	8.1	8	0.9982	48.8	14.2

[a] K_{obsd} = pseudo-first-order rate constant. [b] Half-life was calculated from the corresponding k_{obsd}.

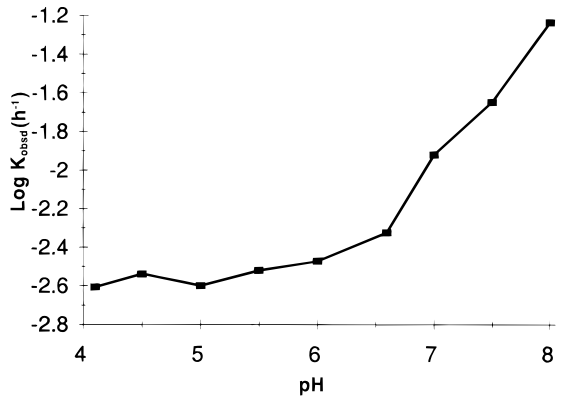

Figure 2. Profile of log k_{obsd} vs pH for the hydrolysis of AZA at 35 °C in 0.05 M phosphate-buffered solutions.

that the conversion products were more polar than AZA and contained a functional group, or groups, that were readily ionized in water. To determine the identities of these conversion products and to evaluate their potential impact on the aquatic environment, more research on their molecular structures would be needed.

Disappearance of AZA from Buffered Solutions and Natural Waters. In the range of pH 4.1−8.0, which inludes most natural waters everywhere, AZA hydrolyzed readily. All data for the disappearance of AZA at 35 °C in buffered solutions of this pH range and in four natural waters of pH 6.2, 7.3, 8.0, and 8.1 followed simple pseudo-first-order kinetics (Table 1). Depending on the pH, the rate of disappearance varied, being more rapid at basic than at acidic pH (Figure 2). The rates of disappearance in the natural waters were in general agreement with those observed in 0.05 M buffered solutions of similar pH. Any minor differences may be attributed to buffer catalysis in the buffered solutions and metal ion catalysis in the natural waters (Perdue and Wolfe, 1983; Mortland and Raman, 1967; Blanchet and St. George, 1982).

The pH rate profile (Figure 2) suggests that the hydrolysis of AZA at acidic, neutral, and basic pH involved different mechanisms. By a least-squares method a linear regression was calculated as follows: log k_{obsd} = 0.7183(pH) − 6.9971 (where n = 4, r = 0.996*, significant at p = 0.05) for pH 6.6−8.0. The calculated linear regression for pH 4.1−6.0, however, was log k_{obsd} = 0.0590(pH) − 2.8430 (where n =5, r = 0.812, not significant at p = 0.05). Furthermore, the fact that different conversion products were detected by HPLC

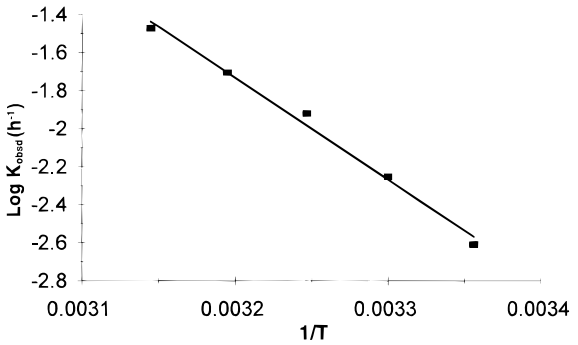

Figure 3. Arrhenius plot of log k_{obsd} vs $1/T$ for the hydrolysis of AZA at pH 7.0.

Table 2. Kinetic Data of Hydrolysis of AZA in 0.05 M Phosphate Buffer of pH 7.0 at Five Temperatures

temp, °C	k_{obsd},[a] × 10^{-3} h^{-1}	half-life, h	no. of readings	corrln coeff
25	2.46	282	13	0.9942
30	5.58	124	12	0.9965
35	12.0	57.8	11	0.9983
40	19.7	35.2	8	0.9978
45	33.8	20.5	9	0.9985

in the incubated buffered solutions and in the natural waters, having acidic or basic pH, provides evidence to support this hypothesis.

To study the influence of temperature on the rate of hydrolysis, the rate constants at 25, 30, 35, 40, and 45 °C were determined for the hydrolysis of AZA in 0.05 M phosphate buffer of pH 7.0. The Arrhenius plot was established from the kinetic data generated (Table 2). The linear regression of the Arrhenius plot of log k_{obsd} vs $1/T$ (Figure 3) was as follows: $\log k_{obsd} = -5368(1/T) + 15.4$ (where $n = 5$, $r = 0.9956$**, significant at $p = 0.01$). The energy of activation and the frequency factor A for the hydrolysis of AZA at pH 7.0 were calculated to be 103 kJ mol^{-1} and 2.51×10^{15} h^{-1}, respectively.

The susceptibility of AZA to hydrolysis was compared with that of the synthetic organophosphate insecticides chlorpyrifos, diazinon, malathion, parathion, and Ronnel, and the carbamates carbaryl and propoxur, all of which are used extensively for insect pest control in agriculture. At 35 °C, the rate of hydrolysis of AZA in 0.05 M phosphate buffer of pH 7.0 (12.0×10^{-3} h^{-1}, Table 1) was from 1 to 2 orders of magnitude faster than those of diazinon [$(7.2 \pm 1.2) \times 10^{-4}$ h^{-1}] and Ronnel [$(1.2 \pm 0.1) \times 10^{-3}$ h^{-1}] in distilled water as reported by Wolfe *et al*. (1983). At 25 °C, the hydrolysis rate for AZA in 0.05 M phosphate buffer of pH 7.0 (2.46×10^{-3} h^{-1}; Table 2) was almost 1 order of magnitude faster than that of chlorpyrifos ($3.7 \pm 0.5 \times 10^{-4}$ h^{-1}) in the pH range of 4−7.5 (Macalady and Wolfe, 1983). Using the Arrhenius plot (Figure 3), the hydrolysis rate at 20 °C for AZA in 0.05 M phosphate buffer of pH 7.0 was calculated to be 1.2×10^{-3} h^{-1}. This rate was of the same order of magnitude but slower than those of carbaryl in pH 7 (2.76×10^{-3} h^{-1}) and propoxur in pH 8 (1.8×10^{-3} h^{-1}) (Aly and El-Dib, 1972) and almost 1 order of magnitude faster than that of parathion in pH 7.4 (2.66×10^{-4} h^{-1}) (Gomaa and Faust, 1972). Since AZA hydrolyzed more rapidly in basic than in acidic pH (Figure 1), the hydrolysis rate for AZA at 20 °C in pH 7.4 and 8 would be faster than the calculated rate of 1.2×10^{-3} h^{-1} in pH 7.0. When compared to malathion, AZA appeared to be as readily degraded in natural water. The calculated half-lives at 35 °C in waters from Port Coquitlam Park Creek (256 h, pH 6.2) and Sleese Creek (14.2 h, pH 8.1) (Table 1) were comparable to those of malathion at 28 °C in water from Indian River (418 h, pH 6; 39.6 h, pH 8.16) (Wang and Hoffman, 1991). It appears that we can expect AZA to be hydrolyzed as readily as carbaryl, chlorpyrifos, diazinon, malathion, parathion, propoxur, and Ronnel, which are generally regarded as nonpersistent in the aquatic environment.

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