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THE CHEMISTRY AND ANALYSIS OF DIFLUBENZURON

AND ITS MAJOR METABOLITES

by

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ABSTRACT

A new gas-liquid chromatographic (GLC) procedure has been developed for the analysis of diflubenzuron, 1-(4-chlorophenyl)-3-(2,6-difluorobenzoyl) urea (Dimilin, TH 6040, PH 6040) and its major metabolites: 4-chloroaniline, 4-chlorophenylurea, and 2,6-difluorobenzoic acid in pond water. The residues from fortified pond water were extracted using ethyl acetate, and following derivatization, cleaned up on a micro Florisil column. Diflubenzuron and 4-chlorophenylurea were determined as their cleavage products, N-trifluoroacetyl-4-chloroaniline; 4-chloroaniline as its N-trifluoroacetyl derivative, and 2,6-difluorobenzoic acid as its O-pentafluorobenzyl derivative.

A 50% full scale deflection (fsd) was obtained with 0.03 ng of the N-trifluoroacetyl and pentafluorobenzyl derivatives. Recoveries from fortified unfiltered pond water for diflubenzuron, 4-chloroaniline, 4-chlorophenylurea and 2,6-difluorobenzoic acid at the 1.00, 0.10, and 0.02 mg/L levels were: 91.09, 75.02, 63.29%; 52.87, 22.60, 5.77%; 98.87, 84.10, 83.00%; 105.40, 105.44, and 101.94% respectively. Diflubenzuron and 4-chlorophenylurea were found to undergo cleavage during reaction with trifluoroacetic and heptafluorobutyric anhydrides. The products, N-monoperfluoroacyl-2,6-difluorobenzamide (from diflubenzuron) and N-monoperfluoroacyl-4-chloroaniline (from diflubenzuron and 4-chlorophenylurea) were identified as occurring during derivatization and not from thermal degradation of the 1- or 3-N-monoperfluoroacyl derivatives of diflubenzuron or 4-chlorophenylurea in the GLC injection port or column. Elucidation of a novel in vitro cleavage reaction and a proposed degradation scheme is presented based on EC-GLC, TC-GLC, TLC, GLC-MS and direct inlet probe-MS analysis.

The pentafluorobenzyl ester derivative of 2,6-difluorobenzoic acid was identified by GC-MS and proved to be a highly sensitive and stable derivative. 2,6-difluorobenzoic acid was extracted from acidified pond water (pH2) and reacted with pentafluorobenzyl bromide after conversion to the sodium salt of the acid.

Recoveries from filtered pond water for diflubenzuron were 94.10 and 89.30% at the 0.020 and 0.060 mg/L levels, respectively. 4-chloroaniline recoveries (filtered pond water) were 60.10 and 74.62% at the 0.02 and 0.10 mg/L fortification levels respectively. It is postulated that diflubenzuron and 4-chloroaniline are immobilized by adsorption, for example, to particulate organic matter in the pond water since recovery data from unfiltered pond water followed the linear form of the Freundlich equation.

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I INTRODUCTION

Diffubenzuron, 1-(4-chlorophenyl)-3-(2,6-difluorobenzoyl) urea (Dimilin, TH6040, PH6040) is a broad spectrum insecticide-larvicide (Thompson-Hayward Chemical Co; 1974) with a very low mammalian toxicity (acute oral LD₅₀ mice technical >4640 mg/kg). It is an insect growth regulator which interferes with the formation of insect cuticle (Post et al; 1974) when applied during the larval stages of many classes of insects. Extensive use is anticipated in the control of many domestic, agricultural and forest insects, such as mosquitoes, lepidopterous soybean insects, cutworms, grasshoppers, tussock moth, and spruce budworms.

Following application of diflubenzuron, residues may be present in aquatic systems via direct treatment of water or by runoff from treated areas into lakes, streams and ponds. Diflubenzuron is degraded in the environment to four major metabolites or degradation products. They are: 4-chloroaniline, 4-chlorophenylurea, 2,6-difluorobenzoic acid and 2,6-difluorobenzamide (Metcalf et al; 1975; Ruzo et al; 1974). A rapid, sensitive and reliable method is therefore required to enable analysis of diflubenzuron and its major metabolites in studies of their persistence and fate in aquatic systems. Since most laboratories rely on GLC for routine pesticide analysis, it was desirable to develop a sensitive GLC method for diflubenzuron and its major metabolites.

The purpose of this investigation was to study the chemistry and analysis of diflubenzuron and its metabolites from the environmental substrate pond water. The objectives of this study were as follows:

- (1) To develop a gas-liquid chromatographic method for the analysis of diflubenzuron and its metabolites 4-chloroaniline, 4-chlorophenylurea and 2,6-difluorobenzoic acid from fortified pond water.

(2) To elucidate the nature of the chemical reactions involved with diflubenzuron, 4-chloroaniline, and 4-chlorophenylurea in the presence of perfluoroanhydrides and 2,6-difluorobenzoic acid with pentafluorobenzyl bromide.

(3) To note the effect of pond water particulate matter on adsorption of diflubenzuron and 4-chloroaniline.

II. LITERATURE REVIEW

Current Methodology for the Analysis of Diflubenzuron and its Major Metabolites.

Methodology for the analysis of diflubenzuron and its major metabolites may be described according to the following techniques and instrumentation used for separation and quantitation: Thin-layer chromatography (TLC), high-pressure (or performance) liquid chromatography (HPLC) and gas-liquid chromatography (GLC). Of some 122 papers published on diflubenzuron only 12 of these describe methodology for analysis; the remaining 110 papers being concerned with toxicological aspects. In addition, only 4 of the 12 methodology papers provide for analysis of one or more of the metabolites or degradation products of diflubenzuron.

(a) Thin-Layer Chromatography:

Metcalf et al. (1975) studied the degradation and environmental fate of diflubenzuron in several systems: a model ecosystem, soil, microorganisms (Pseudomonas putida), sheep liver microsomes, and photodegradation using three radiolabelled versions of diflubenzuron (2,6-difluorobenzoyl ($^{14}\text{C}=\text{O}$ label), p-chlorophenyl (^{14}C -ring-U) and a 2,6-difluorobenzoyl (^3H -ring) version. Extracts of the organisms or photodegradative reactions, analytical standards of diflubenzuron, and suspected metabolites or degradation products were subjected to TLC on fluorescent silica gel (E. Merck GF-254). The plates were developed in benzene-dioxane-acetic acid (90:30:1 by volume) and visualized by radioautography on no-screen x-ray film. No indication is given as to the method of quantitation but it was presumably accomplished by use of liquid scintillation spectrometry or a radiochromatogram scanner of

the TLC spots.

Maini and Desio (1976) analyzed diflubenzuron in the feces of the orchard insect Zeuzera pyrina using TLC. The extracts and analytical standard were spotted on silica gel G plates and developed in chloroform-ethyl-acetate-methanol (100:2:1). Detection with a AgNO_3 reagent and UV light was used. The minimum amount of diflubenzuron detected was 1 μg . Quantitation was based on spotting a series of standard concentrations and comparison with the unknown on the basis of Rf value and spot intensity.

(b) High Pressure Liquid Chromatography:

Corley et al. (1974) developed a method for the analysis of residues of diflubenzuron and its degradation products, 4-chloroaniline, and 2,6-difluorobenzamide from cows' milk using HPLC (coupled to an ultraviolet detector (UV)) for separation and detection. Recoveries were essentially quantitative for diflubenzuron (0.1-1.0 mg/L) while recoveries were not reported for the two degradation products. The lowest detectable level (2x noise) for diflubenzuron was 10 ng at 254 nm and a flow rate of 0.2 ml/min. 2,6-Difluorobenzamide was well separated from diflubenzuron ($R_T=44$ and 15.6 min respectively). However, 4-chloroaniline at a retention time (R_T) of 10.2 min chromatographed with a tailing solvent peak.

Oehler and Holman (1975) reported an analytical method for the analysis of diflubenzuron from bovine manure at residue levels using UV₂₅₄-HPLC. A Florisil cleanup was also required prior to high pressure liquid chromatography. Recoveries ranged from 92 to 106% for manure fortified between 0.5 and 2.0 ppm. 100 Ng injected gave a 13% full scale deflection (fsd).

Several internal publications from Thompson-Hayward Chemical Co. (1975) followed much the same methodology as Oehler and Holman (1975). A number of different substrates were used for fortification with diflubenzuron and determination of recoveries employed UV-HPLC. At lower fortification levels (0.01 ppm) a Florisil cleanup was required prior to HPLC for water samples and Florisil or Florisil-alumina-silica gel for soil and forest samples (litter and foliage) and fish, eggs, milk or bovine tissues, respectively. The lower detection limit at 254 nm was 5 ng. Recoveries from water averaged 86% at fortification levels of 0.01 to 0.1 ppm, from soil and forest samples 90.7 and 80%, and from bovine tissues, fish, milk and eggs 90, 87.8, 85.8 and 79.7%, respectively. Duplicate analysis were not generally reproducible since variations of 10 and often 20% were reported. A final internal publication describes a UV-HPLC method for determination of active ingredient in technical Dimilin and 25% wettable powder; however, no detection limit, or recoveries were reported.

During an investigation to determine whether diflubenzuron accumulated in bovine tissues, manure or milk, Miller et al. (1976) followed the procedure of Corley et al. (1974) previously outlined. One modification was the use of a UV wavelength setting of 220 nm permitting a lower detectability limit of 5 ng cf 10 ng at 254 nm. When diflubenzuron was added to untreated feces at levels of 0.1, 0.5, and 1.0 ppm, recoveries averaged 94%, while recoveries from fat and muscle averaged 93 and 94%, respectively. The lower limit of detection was 0.1 ppm. Also fat and muscle tissues at 5 ppm were analyzed by the method of Oehler and Holman (1975) resulting in recoveries of 89 and 94%, respectively.

Finally, and most recently, Schaefer and Dupras, Jr. (1976)

reported a study on the persistence and stability of diflubenzuron and 4-chlorophenylurea in tap and field waters. UV₂₅₄-HPLC was utilized for all quantitative analysis. No pre-column cleanup was used for any of the water samples (tap, pasture, pond, dairy drain, and sewage). Recoveries were generally high (90-100%) from tap, pasture and pond water and much lower for dairy drain and sewage waters (30-86%) at 0.01 to 0.10 mg/L levels. Their lower recoveries were attributed to the waters having a greater content of organic matter and hence loss through adsorption. Recoveries of 4-chlorophenylurea from tap water were 99, 100 and 95% at 0.1, 0.05 and 0.01 mg/L, respectively. No recoveries were reported for 4-chlorophenylurea from the other waters and the effects of particulate matter adsorption were not described. They found it was possible to estimate diflubenzuron concentrations to a detection limit of 0.001 ppm or 0.6 ng (minimum peak was twice background). Retention times were 5 and 7 min for diflubenzuron and 4-chlorophenylurea, respectively.

Although UV-HPLC has received considerable attention as an instrument for the separation and detection of diflubenzuron, 4-chloroaniline, and 4-chlorophenylurea, methodology based on GLC is favored for three main reasons:

(1) Much smaller sample sizes and extraction volumes can be used, e.g., 600 ml water samples/600 ml extraction solvent (Schaefer and Dupras, Jr., 1976) cf 50 ml water sample and 40 ml extraction solvent (Worobey & Webster, 1977) which reduces time (reduction of extraction volume to dryness) and cost.

(2) HPLC instrumentation is not yet commonly found in many pesticide monitoring and regulatory laboratories.

(3) HPLC detectors presently in use (UV) are inherently less sensitive than the electron capture (EC) detector commonly used with GLC.

(c) Gas-Liquid Chromatography:

Ruzo et al. (1974) reported the direct GLC of one of the major photodegradative/metabolic products of diflubenzuron, 2,6 difluorobenzamide. Using flame-ionization detection, and a 6 ft X 1/8 in i.d. glass column packed with 4% SE-30 (column temperature 170 degrees centigrade) a retention time of 2.3 minutes was reported. They did not describe detection limits since their study was basically qualitative.

The first GLC method for diflubenzuron residues was published by Lawrence and Sundaram (1976). The paper reports a GLC method for diflubenzuron after the compound is converted to a derivative amenable to GLC. Derivative formation for GLC has been found necessary since at normal GLC temperatures and on columns commonly used by pesticide residue analysts, chromatography was unsuccessful (Corley et al. 1974). The gas-liquid chromatographic analysis of diflubenzuron was carried out after the insecticide was converted to its N,N'-dimethyl analog (N-methyl-N-(4-chlorophenyl)-N'-methyl-N'-(2,6-difluorobenzoyl) urea). The methylation was accomplished in dimethyl sulphoxide (DMSO) with sodium hydride and methyl iodide. The derivative was thermally stable and chromatographed as a single peak on 3% OV-1 at 235°C. The sensitivity of the product to electrolytic-conductivity detection was approximately 50 ng for 50% full-scale deflection in the nitrogen mode and 70 ng for the same response in the chloride (reductive) mode. About 0.25 ng was required for 50% full-scale deflection by electron-capture detection. A method of extraction was described for diflubenzuron residues from spruce

foliage and as little as 0.2-0.5 ppm could be detected using the Coulson detector (nitrogen mode). Recoveries of diflubenzuron from fortified spruce foliage were generally greater than 80% above the 1.0 ppm fortification level. No information was given regarding electron-capture detection of foliage extracts.

Most recently Worobey and Webster (1977) described a GLC procedure developed for the analysis of diflubenzuron in pond water. Diflubenzuron residues were reacted with trifluoroacetic anhydride to produce a cleavage product, N-trifluoroacetyl-4-chloroaniline, and quantitated using electron capture detection. The derivative was found to be thermally stable and a 50% full scale deflection was obtained with 0.03 ng injected on a 5% OV-101 column at 122 degrees centigrade. Recoveries from fortified unfiltered pond water were 65.7, 72.8, 82.0 and 90.8% at the 0.020, 0.059, 0.197 and 0.788 ppm (mg/L) levels, respectively. Recoveries from fortified filtered pond water were 94.1 and 89.3% at the 0.020 and 0.060 mg/L levels, respectively. Trifluoroacetylation was chosen to provide a thermally stable derivative with increased volatility and enhanced electron-capturing properties.

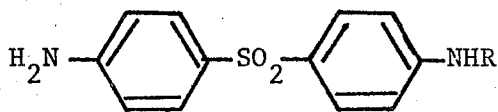
Another useful characteristic of the trifluoroacetyl derivative was that after derivatization the reaction mixture may be evaporated to dryness (to remove excess trifluoroacetic anhydride) without losses and this itself acted as a cleanup since the more volatile interferences were lost through evaporation while the derivative was retained.

In-Vitro Degradation of Pesticides and Drugs During Derivatization With Perfluoroanhydrides.

The use of derivatization techniques in the GLC of pesticides

or drugs is now widely accepted. The functional group transformations are usually straight-forward reactions carried out prior to injection into the chromatograph and often produce more thermally stable and detector-sensitive derivatives of the intact parent compounds. Perfluoroacylation of insecticidal carbamates (Magallona, 1975; Cochrane, 1975) and herbicidal ureas (Cochrane, 1975; Cochrane and Purkayastha, 1973; Saunders and Vanatta, 1974; Lawrence and Ryan, 1977; Ryan and Lawrence, 1977) as intact derivatives of the parent compounds have been previously reported and reviewed. With certain carbamates and ureas, however, perfluoroanhydrides may form unforeseen derivatives of the parent compound.

Vandenheuvel *et al.* (1973) investigated the reaction of heptafluorobutyric anhydride with a monosubstituted urea drug and a substituted carbamate drug. Need for an assay procedure to quantitate p-ureido-p'-amino-diphenyl sulfone (Ia) led them to consideration of



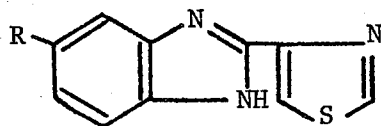
Ia, R = CONH₂

Ib, R = H

hydrolysis of Ia to p,p'-diaminodiphenylsulfone (Ib) and conversion of this compound to a derivative suitable for electron-capture detection. They found that the reaction of Ib (hydrolysis product) with heptafluorobutyric anhydride resulted in the corresponding diheptafluorobutyramide. However, when Ia was reacted with heptafluorobutyric anhydride the product possessed the same retention time on the GLC as the authentic diheptafluorobutyramide of Ib. GLC-mass spectrometry demonstrated in both cases that the compound eluted from the GLC column was the same, namely, the diheptafluorobutyramide

of Ib. This remarkable conversion was not an "on column" alteration, since the product isolated from the reaction mixture possessed the same infrared and direct probe mass spectra as the diheptafluorobutyramide of Ib.

A somewhat related in-vitro cleavage reaction followed by concomitant conversion to a heptafluorobutyryl derivative was observed to occur during the reaction of the drug cambendazole (IIa)

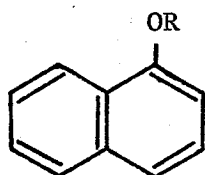


IIa, R = $(\text{H}_3\text{C})_2\text{CHCONH}$

IIb, R = NH_2

The reaction of IIa with heptafluorobutyric anhydride resulted in the formation of a mixture of mono and diheptafluorobutyramides of IIb. They then went on to prove that the formation of the mono and diheptafluorobutyramides of IIb did, in fact, occur as the result of an in-vitro cleavage reaction followed by subsequent conversion to these products.

Kalifa and Mumma (1972) and Mumma and Kalifa (1972) reported that N-hydroxymethylcarbaryl (IIIa) when reacted with trifluoroacetic



IIIa, R = CONHCH_2OH

IIIb, R = COCF_3

IIIc, R = $\text{COF}_2\text{CF}_2\text{CF}_3$

or heptafluorobutyric anhydride yielded only the corresponding perfluoroacyl derivative of 1-naphthol (IIIb or c).

Adsorption of Diflubenzuron and 4-Chloroaniline.

Many excellent reviews of pesticide adsorption have been published (Bailey and White, 1964, 1970; Weber, 1971; S.C.I. Monograph, 1970). Adsorption of some phenylurea herbicides (Weber, 1971) on clays was postulated to occur by way of hydrogen bonds between the carbonyl oxygen atoms of the phenylureas and the water of hydration surrounding metal cations on the clay surface or through ion-dipole bonds between the carbonyl groups and the metal cations. Hance, (1969) suggested that the lack of any significant pH effect on the adsorption of some phenylureas was evidence that the compounds did not ionize and were probably adsorbed by physical Van der Waals forces.

Bailey et al. (1968) listed and discussed several possible mechanisms for phenylurea and aniline adsorption by clays and suggested that one or more may occur simultaneously, depending on the nature of the functional groups and the acidity of the system. Aniline was the most strongly adsorbed compound studied.

Stevenson (1972) presents a more recent review on adsorption of phenylurea herbicides by soils and especially organic matter. He lists and discusses Van der Waals, hydrogen-bonding, and ligand exchange as mechanisms for their adsorption.

(a) Adsorption of Chloroaniline Residues:

Bartha and Hsu (1976) reported that the bulk of chloroaniline residues derived from phenylamide herbicides are immobilized in spontaneous interactions with soil. Bailey et al. (1968) reported that of 50 μ moles aniline applied to a neutral and an acidic montmorillonite adsorbent, 21.4 and 39.0 μ moles were adsorbed based on their conditions of fortification and recovery. Bartha (1971) found that

4-chloroaniline fortified soil was bound to the soil more at 5 ppm than at 500 ppm (i.e. 77.4% cf 53.2%). This greater retention at lower levels is reflected in the extractable residues; 10.8% at 5 ppm and 40.3 % at 500 ppm. Hsu and Bartha (1974) reported that binding of 4-chloroaniline to sterile soil or purified humic acid was very rapid and at a 5 ppm level of fortification binding went to near completion (80%) within 27 hours. In addition, at 6 and 60 min, residues were bound at 25 and 55%, respectively.

(b) Adsorption of Diflubenzuron Residues:

Schaefer and Dupras, Jr. (1976) studied recoveries of diflubenzuron (at 0.10, 0.05 and 0.01 mg/L levels) from tap, pasture, pond, dairy drain and sewage lagoon waters. Reduced recoveries occurred in water having a greater content of organic matter, e.g., dairy drain and sewage water, presumably through adsorption. They also investigated recoveries of diflubenzuron (0.1 mg/L level) from tap water containing different amounts of straw (0-3 g/60.0 ml tap water) and held for 24 and 48 hrs at 21°C in the dark. The recoveries diminished as the amount of straw or the holding time increased. Extraction of the straw after 18 hr exposure showed that diflubenzuron was present and they concluded that the losses were partly, if not largely, due to adsorption. Carringer et al. (1975) studied the adsorption of diflubenzuron and other pesticides onto soil organic matter and concluded that adsorption appeared to be inversely related to the water solubility of the compound; they reported a water solubility of 0.3 ppm for diflubenzuron.

Finally, Worobey and Webster (1977) reported that diflubenzuron was very likely adsorbed by pond water organic matter. A plot of percent

recovery of diflubenuron from unfiltered pond water versus mg diflubenuron/L (fortification level) resulted in a curve, but when plotted as log concentration (mg/L) versus percent recovery, a straight line relationship was obtained. This was recognized as being similar to the linear form of the Freundlich equation (Khan, 1974): $X = KC^n$; where X = amount of adsorbate taken up by a unit mass of the adsorbent, C = equilibrium concentration in solution, n = slope of the line, K = intercept of the isotherm. Analysis of their data by using the Freundlich equation gave a linear relationship. A value of n (0.710) was less than unity, indicating a convex, or L-type of isotherm (Weber, 1970). Apparently, this type of isotherm may arise due to a minimum competition of solvent for sites of the adsorbing surface or when the solid has a high affinity for the solute. Values of n that are less than unity have been reported for adsorption of various herbicides on humic acid, whereas montmorillonite gave a value of n greater than unity, indicating a concave or S-type isotherm (Weber, 1970).