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IN VITRO DEGRADATION OF DIFLUBENZURON (DIMILIN) DURING DERIVATIZATION WITH PERFLUOROANHYDRIDES*

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SUMMARY

Diflubenzuron (1-(4-chlorophenyl)-3-(2,6-difluorobenzoyl)urea) and one of its metabolites, 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 the intact diflubenzuron in the gas–liquid chromatography (GLC) injection port or on the GLC column. Elucidation of an *in vitro* cleavage reaction and a proposed degradation scheme is presented based on electron capture–GLC, thin-layer chromatography, GLC–mass spectrometry and direct-inlet probe mass spectrometric analysis.

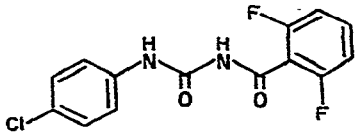
INTRODUCTION

Use of derivatization techniques in the gas–liquid chromatography (GLC) of pesticides is now widely accepted. The functional group transformations are usually straightforward 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[1,2] and herbicidal ureas[2–5] have been previously reported and reviewed.

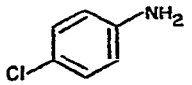
With certain carbamates and ureas, however, perfluoroanhydrides may form unforeseen derivatives of the parent compound. Khalifa and Mumma[6] reported that reaction of N-hydroxymethylcarbaryl with trifluoroacetic or heptafluorobutyric anhydrides resulted in the formation of only the corresponding perfluoroacyl derivative of 1-naphthol. VandenHeuvel *et al.*[7] investigated the reaction of heptafluorobutyric anhydride with a mono-substituted urea drug and a substituted carbamate drug. In both cases they showed that the urea and carbamate moieties were cleaved

* Contribution No. 11, Pesticide Research Laboratory.

during the reaction with concomitant conversion to the corresponding N- and O-heptafluorobutyramide derivatives.



I



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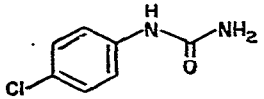
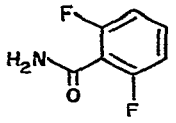


II



IV

Diflubenzuron, I (Dimilin, 1-(4-chlorophenyl)-3-(2,6-difluorobenzoyl) urea), a new growth regulating insecticide, is thermally unstable to temperatures normally used in GLC as previously reported[8]. The data in this report was obtained during an attempt to develop a GLC method for the simultaneous determination of diflubenzuron and three of its metabolites, 4-chlorophenylurea (II), 4-chloroaniline (III) and 2,6-difluorobenzamide (IV), at residue levels. The use of trifluoroacetic or heptafluorobutyric anhydrides as derivatization reagents to produce more volatile, thermally stable and/or electron-capture sensitive derivatives resulted in the formation of unexpected reaction products for diflubenzuron and 4-chlorophenylurea. The formation of these unexpected products were confirmed to be the results of an *in vitro* cleavage reaction and not of GLC thermal degradation of N-monoperfluoroacyl derivatives of the parent ureas.

EXPERIMENTAL

Materials and reagents

All solvents were of glass-distilled residue-free grade. Trifluoro and heptafluorobutyric anhydrides were obtained from Aldrich, Milwaukee, Wisc., U.S.A. Diflubenzuron, 4-chlorophenyl urea, 4-chloroaniline and 2,6-difluorobenzamide were supplied by Thompson Hayward (Kansas City, Kan., U.S.A.). Each was recrystallized from benzene and its identify was confirmed by mass spectrometry. Standard solutions of each compound containing 100 ng/μl were prepared in ethyl acetate.

Gas–liquid chromatography

All columns [electron capture–GLC (GLC–ECD) and GLC–mass spectrometry (MS)] were of Pyrex and packed with 5% OV-101 Chromosorb W AW DMCS, 100–120 mesh. Dimensions used are reported with results.

Electron-capture detection

A Varian 2440 gas chromatograph equipped with a tritium foil detector was

used; operating conditions: injector 180°, column 150°, detector 200°; nitrogen carrier gas flow-rate was 40 ml/min.

Mass-spectrometry

Mass spectra were obtained with either a DuPont Dimaspec or Finnigan 1015 mass spectrometer using either GLC–MS or direct-inlet probe (DIP) techniques. GLC–MS and DIP–MS conditions are given in the legends to tables and figures.

Thin-layer chromatography (TLC)

TLC was carried out on 0.75 mm thick silica gel G plates (20 × 20 cm, pH 7) and developed in chloroform–ethylacetate–acetic acid (85:10:15). Visualization was obtained using a very light spray of 0.01 % fluorescene in ethanol, followed by ultraviolet detection of spots. Elution of spots was carried out using ethyl acetate.

Preparation of derivatives

Diflubenzuron, 4-chlorophenylurea, 4-chloroaniline, or 2,6-difluorobenzamide (10–100 μg/0.2 ml ethyl acetate in a 5-ml graduated glass-stoppered centrifuge tube) was treated with trifluoroacetic or heptafluorobutyric anhydride (0.2 ml) at 50° for 30 min in a water bath. The reaction mixture was then evaporated just to dryness under nitrogen and the residue taken up in an appropriate volume of ethyl acetate for analysis.

RESULTS AND DISCUSSION

In an attempt to extend the trifluoroacetic anhydride reaction previously reported for I[9] to three of its metabolites, II, III and IV, it was found that all three metabolites reacted producing single highly sensitive peaks, products IIC and IIIA having identical retention times by GLC–ECD (Fig. 1). Reaction of diflubenzuron (I) with trifluoroacetic anhydride yielded two peaks, IB having the same retention time as reaction product IVA, and IC corresponding in retention time to reaction products IIC and IIIA (Fig. 1). The use of several GLC columns of various polarities under a variety of column temperatures and flow-rates failed to resolve peaks IC, IIC, and IIIA.

As mentioned earlier the *in vitro* cleavage of certain ureas and carbamates in the presence of perfluoroanhydride derivatization reagents (trifluoroacetic and heptafluorobutyric anhydrides) has been demonstrated[6,7]. Thus it was necessary to elucidate in this instance whether the reaction products of I and II were the result of on-column degradation or *in vitro* cleavage.

The first approach was to identify all reaction products of I to IV via the reaction with trifluoroacetic anhydride using GLC–MS. The total ion monitor chromatograms (Fig. 2) revealed four peaks from the reaction of I with trifluoroacetic anhydride having parent ions at *m/e* 153 (IA), 253 (IB), 223 (IC), and 157 (ID). II yielded three products with parent ions at *m/e* 153 (IIA), 127 (IIB), and 223 (IIC); III, one product with *m/e* 223 (IIIA); and IV, two products, with *m/e* 253 (IVA) and 157 (IVB). Table I lists fragmentation ions and their relative abundances for each of their products. The total ion monitor chromatograms indicated that ID and IVB

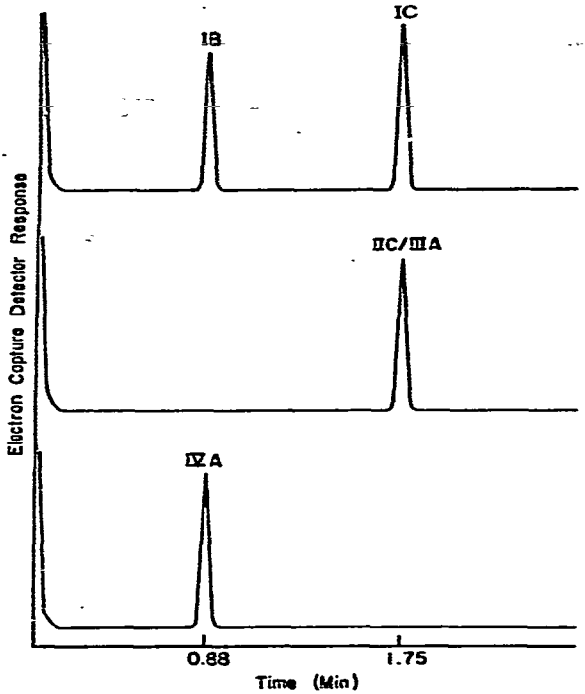


Fig. 1. Gas chromatograms of products of the reaction of I, II, III and IV with trifluoroacetic anhydride using electron capture detection. (Reaction of II or III yields the same product and therefore the same chromatogram). Column conditions: 1.7 m × 2 mm I.D. glass column; 5% OV-101 on Chromosorb W AW DMCS, 100–120 mesh; column temperature = 150°.

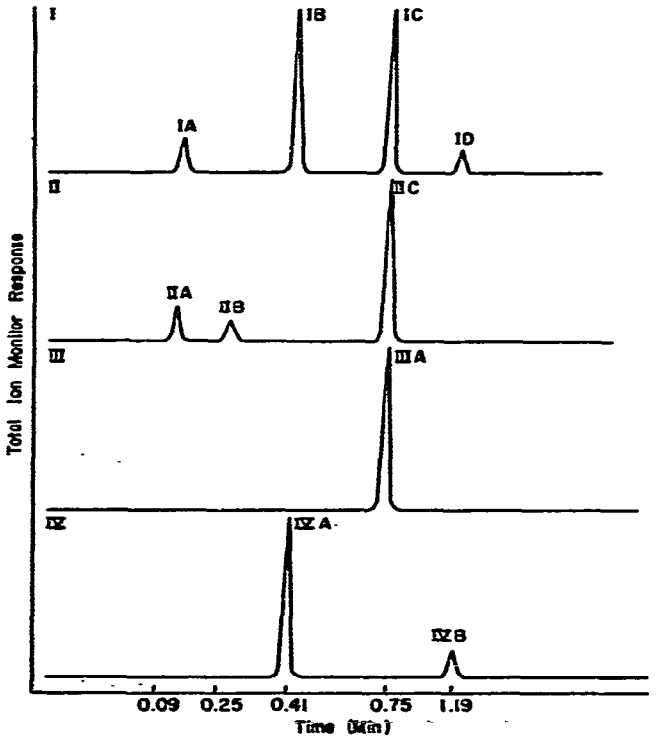


Fig. 2. GLC–MS total ion monitor chromatograms of trifluoroacetic anhydride reaction products from I, II, III and IV. GLC–MS conditions: 5% OV-101, 1.6 m × 2 mm I.D. glass column; ionization potential = 70 eV; temp. prog. = 50–150° at 8°/min.

TABLE I

THE MASS SPECTRA OF TRIFLUOROACETYL DERIVATIVES OF DIFLUBENZURON AND METABOLITES FROM FIG. 2

Ions of less than 3% intensity or less than *m/e* 100 are not included. P = parent ion.

TIM peak (m/e)	*Compounds*									
	I				*II*			*III*	*IV*	
	A	*B*	*C*	*D*	*A*	*B*	*C*	*A*	*A*	*B*
253		23.1P							34.2P	
225			33.0				33.3	33.6		
223			100.0P				100.0P	100.0P		
157				40.0P						60.5P
156			13.1				9.6	10.7		
155	33.3				40.5					
154			39.8				30.6	32.8		
153	100.0P				100.0P					
141		100.0		100.0					100.0	100.0
139		3.1							4.14	
129						45.8				
128			33.5				24.7	27.1		
127	17.8				18.9	100.0P				
126			47.7				34.6	37.3		
125	53.3				51.4					
113		22.3	5.7	50.0			3.4	3.5	32.9	31.6
111			14.2				10.2	10.7		

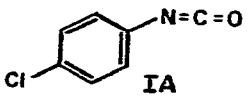
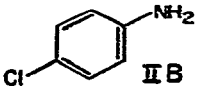
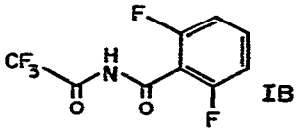
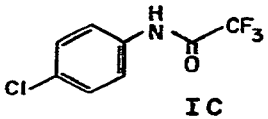
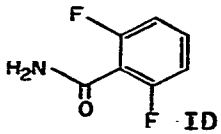
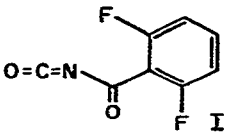
(*m/e* 157), IA and IIA (*m/e* 153), and IIB (*m/e* 127) are minor components. Structural assignments for all parent ions are shown in Table II.

The fact that degradation of I and II had occurred during trifluoroacetylation was shown by TLC of the reaction products of compounds I–IV. Products from I and II yielded spots IC and IIC with the same R_F (0.90) as product IIIA (Fig. 3). Elution and GLC–MS of these spots showed single chromatographic peaks for each with parent ions in each case at *m/e* 223. DIP–MS analysis at ambient temperature of the same material showed only *m/e* 223. It was evident that product IVA, the imide derivative, detected by GLC–MS with parent ion at *m/e* 253, had decomposed on the TLC plate since no IV A could be detected from the TLC separation of derivatized IV; the only compound detected had a parent ion at *m/e* 157 (IVB). In addition, the area from I (ID) with the same R_F (0.43) as spot IVB showed a parent ion at *m/e* 157. The fact that no IVA or IB could be isolated by TLC is to be expected since asymmetrical imides are very sensitive to hydrolysis in much the same way as asymmetrical anhydrides, and the TLC plate was not activated. The GLC–MS data for all TLC spots are listed in Table III.

Finally, as shown in Table IV, DIP–MS of the entire trifluoroacetic anhydride reaction product from I showed very minor peaks at *m/e* 406 (N-1- or N-3-trifluoroacetyl derivative of I) and *m/e* 502 N,N-1,3-ditrifluoroacetyl derivative of I). Intense parent ions at *m/e* 253, 223, and a strong peak at *m/e* 183 corresponding to 2,6-difluorobenzoylisocyanate were also evident. The product with parent *m/e* 183 was not detected by GLC–MS of the reaction products of I and therefore may arise in an ion-

TABLE II

PRODUCTS FROM THE TRIFLUOROACETYLATION REACTION OF COMPOUNDS I, II, III AND IV DETECTED BY GLC–MS, AND/OR DIP–MS

Parent m/e	*I*	*II*	*III*	*IV*
153,155	 IA	IIA	–	–
127,129	—	 IIB	–	–
253	 IB	–	–	IVA
223,225	 IC	IIC	IIIA	–
157	 ID	–	–	IVB
183*	 IE	–	–	–

* Product IE seen only on DIP–MS of reaction product of I.

molecule reaction in the mass spectrometer, or as a product of the *in vitro* degradation reaction which does not elute from the GLC or elutes with a very long retention time. Ambient DIP–MS (7 eV) analysis showed very strong *m/e* 253 and 223 confirming these ions as parent ions of the *in vitro* reaction products. The fact that the intensity of the *m/e* 406 peak was much less than *m/e* 253 or 223 was taken to indicate that it was not the parent ion for peaks at *m/e* 253 and 223. Previous mass spectra of substituted phenylureas have shown strong parent ions[11,12]. In addition, the fact that the *m/e* 406 peak was not present at 5 eV indicates that it was unlikely to be a parent ion. Fragmentation of either the mono- or the di-trifluoroacetyl derivatives (*m/e* 502) could be expected to yield daughter ions with even mass numbers, *i.e.*, 252 and 222 respectively, unless a hydrogen atom rearrangement occurred. (There are none available to be rearranged in the di-derivative and it is unlikely that an N–H would be

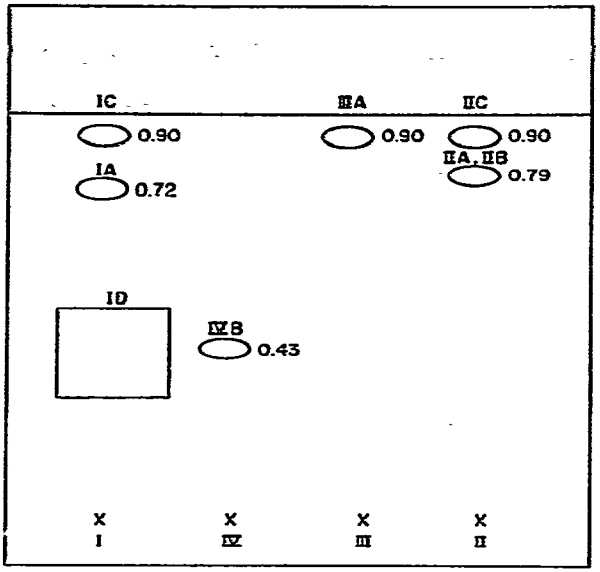


Fig. 3. TLC of diflubenzuron (I) and its metabolites II, III, IV after reaction with trifluoroacetic anhydride.

TABLE III

THE MASS SPECTRA OF TLC ELUATES FROM FIG. 3

Ions of less than 3% intensity or less than *m/e* 100 are not reported. Ionization potential = 70 eV, GLC–MS, 5% OV-101, column temperature, 150°.

TIM peak (m/e)	*Eluate*							
	IVB	*IA*	*ID*	*IC*	*IIIA*	*IIC*	*IIA*	*IIB*
253								
225				33.3	33.3	33.3		
223				100.0P	100.0P	100.0P		
157	60.5P		41.8P					
156				4.2	4.2	3.7		
155		27.3					35.0	
154				12.5	12.5	11.0		
153		100.0P					100.0P	
141	100.0		100.0					
129								42.9
128				11.7	11.7	7.4		
127		9.1					15	100.0P
126				15.0	15.0	11.0		
125		36.4					35	
113	31.6		20.9					
111								

rearranged easily.) Thus it was concluded that *m/e* 253 and 223 were parent ions corresponding to products of an *in vitro* cleavage during the trifluoroacetylation step.

With the trifluoroacetic anhydride reagent, the imide peak (*m/e* 253), IB, increased in intensity (GLC–ECD) with time and temperature of the reaction. How-

TABLE IV

THE DIRECT-INLET PROBE MASS SPECTRA OF THE TRIFLUOROACETYL DERIVATIVES OF DIFLUBENZURON (I)

Ion intensity reported as millimeters peak height. Mass spectrometer source temperature, 150°. IP = ionization potential.

m/e	*DuPont, IP = 70 eV*	*Finnigan, IP = 70 eV*	*Finnigan, IP = 7 eV*	*Finnigan, IP = 5 eV*
504	—	2	4	2
502	—	5	12	5
408	—	4.5	—	—
406	0.5	13	3	—
253	200	2350	950	150
225	440	1900	3100	400
223	1290	5800	9000	1200
183	240	3100	5100	500

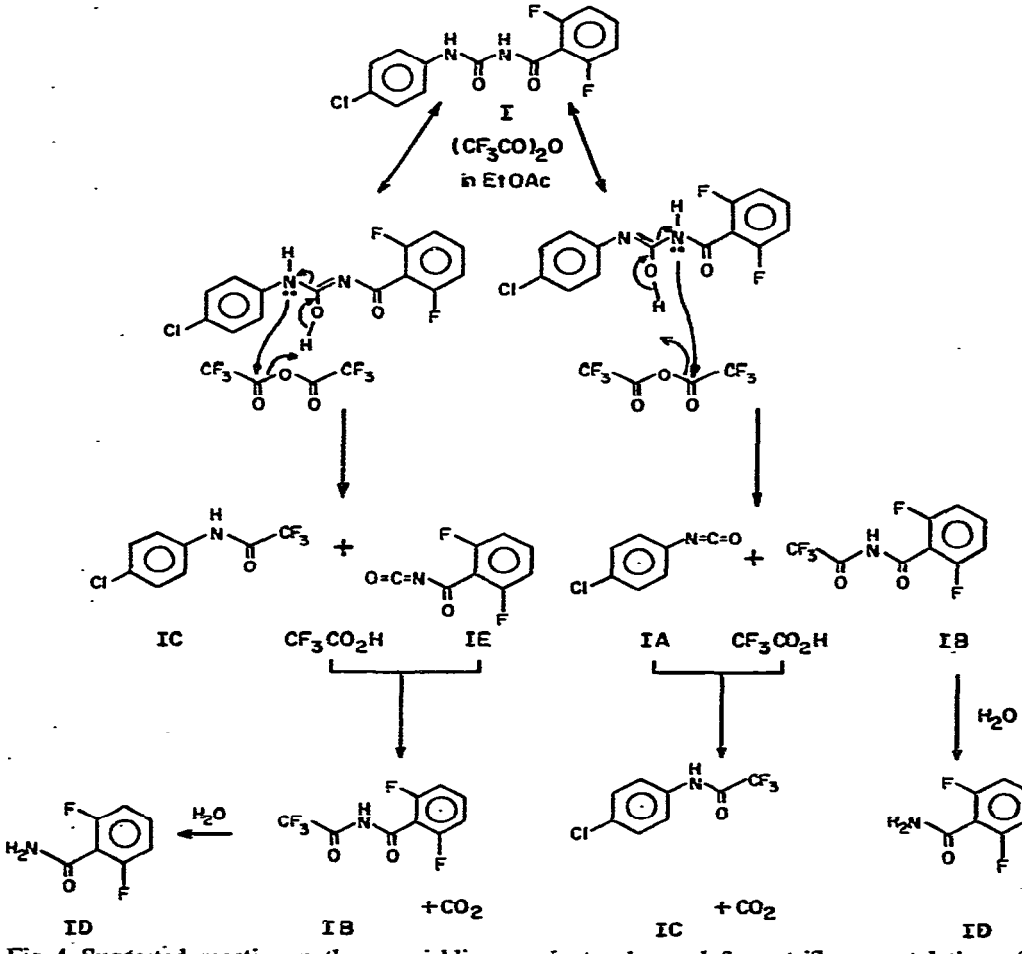


Fig. 4. Suggested reaction pathways yielding products observed from trifluoroacetylation of diflubenzeron (I).

ever, it decreased with prolonged evaporation to dryness, and on standing in ethyl acetate. No effect was observed whether 20 or 200 μl of the derivatization reagents were used.

The importance of a careful investigation into the chemistry occurring during a derivatization step and subsequent GLC is evident. Indeed, a better understanding of these phenomena may lead to a more simplified and/or rational approach to analysis. For example, knowledge that the urea moiety of I and II is cleaved during perfluoroacylation with concomitant conversion of the cleavage products to perfluoroamides eliminates the necessity for a separate hydrolysis step. By this method I, and its metabolites, II and III can be simultaneously analyzed for as the mono-N-perfluoramide derivative of III. The method published by us for analysis of diflubenzuron in pond water as its N-trifluoroacetyl derivative[9], in fact proceeds via this pathway. Hydrolysis of herbicidal phenyl ureas to a substituted aniline product or carbamates to the corresponding phenol followed by derivatization is commonly used as an approach to residue analysis by GLC[3].

Thus, when dealing with derivatization techniques, one must be alert to the possibility of the unexpected, during both the GLC and the derivatization steps.

ACKNOWLEDGEMENT

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