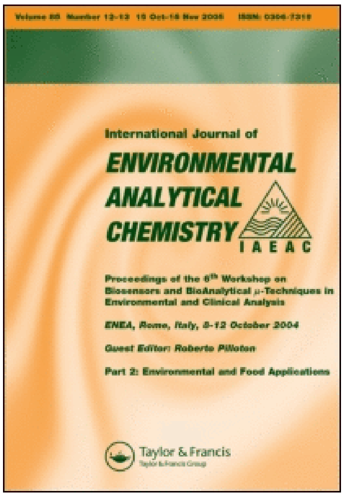

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Derivatization and GLC Determination of the Sterically Hindered 2,6-Difluorobenzoic Acid in Pond Water

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A gas-liquid chromatographic procedure has been developed for the analysis of 2,6-difluorobenzoic acid at picogram levels from fortified pond water. Residues from fortified pond water were extracted using ethyl acetate and derivatized with pentafluorobenzyl bromide. The resulting pentafluorobenzyl ester derivative proved to be highly sensitive to EC-GLC detection. At a signal to noise ratio of 3:1 at maximum sensitivity, the limit of detection for the derivative was 1.6 picograms per injection.

Recoveries from fortified pond water for 2,6-difluorobenzoic acid at the 1.00, 0.10, and 0.02 mg/L levels were 105, 105, 102%, respectively. Advantages of the method were short reaction time, facile removal of excess reagent by evaporation to *dryness*, no column cleanup required at any of the fortification levels studied, low toxicity of reagents, and no explosive hazard *cf.* preparation of the methyl ester derivative using diazomethane.

INTRODUCTION

2,6-Difluorobenzoic acid (2,6-DFBA) is a metabolite of the insect growth regulator diflubenzuron [1-(2,6-difluorobenzoyl)-3-(4-chlorphenyl) urea][1]. It is often desirable to be able to analyze for insecticide metabolites, especially if they are of toxic concern. In addition, 2,6-DFBA may form conjugates with sulfate, sugars, glucuronide and phosphate esters, for example, which may be more toxic than 2,6-DFBA or diflubenzuron themselves.[2]

Because of these toxicological concerns regarding 2,6-DFBA and the fact that no analytical method has been reported for its determination at residue levels, a rapid, sensitive EC-GLC method was developed for its analysis from fortified pond water. An interesting problem which had to be overcome in the preparation of a derivative was the combined effects of steric hindrance, resonance, and polar (inductive, field) effects due to the 2,6-difluoro substituents on the ring.

Hyman[3] reported minimal conversion of 2,3,6-trichlorobenzoic acid to its methyl ester using tetramethylammonium hydroxide and explained this phenomenon by noting that steric hindrance of the COOH was probable. It is well known that di-ortho-substituted benzoic acids are resistant to all but the strongest methylating agents.

Norris and Bearse[4] reported that the reaction of 2,6-dichlorobenzoic acid with thionyl chloride resulted in amongst the lowest velocity constants of a series of substituted benzoic acids, and that it was the presence of the ortho substituents that greatly reduced reactivity.

Metcalf *et al.*[1], reported that the ortho groups of 2,6-DFBA may deactivate the benzoic acid to glycine conjugation. Chau and Terry[5] studied the derivatization of 2,3,6-trichlorobenzoic acid with BCl_3/2-chloroethanol in various solvents, but conversion to the 2-chloroethyl derivative was minimal. They found, however, that reaction with dicyclohexyl carbodiimide/2-chloroethanol yielded higher recoveries of the 2-chlorethyl derivative. This reaction was investigated with 2,6-DFBA and although a peak was obtained that was not present in the reagent blank, its identity as a 2-chloroethyl derivative could not be confirmed by GLC-MS.

This paper describes the development of a method for the analysis of 2,6-DFBA from fortified pond water as its pentafluorobenzyl ester.

MATERIALS AND METHODS

Reagents and Standards

1. *Sodium sulfate*

Reagent grade, anhydrous sodium sulfate was extracted with hexane in a Soxhlet apparatus to remove organic contaminants, and washed with ethyl acetate prior to use.

2. *Derivatization reagent*

Pentafluorobenzyl bromide (PFBBr) was obtained from Aldrich Chemical Company. PFBA reagent was prepared as a 1% (V/V) solution by dissolving 1 mL reagent in 100 mL acetone in an aluminium foil covered volumetric flask. (*Caution:* PFBBr is a strong lachrymator.)

3. *Sodium carbonate* (30% *w/v*)

Prepared by dissolving 30 g of anhydrous sodium carbonate (Macco, Reagent -A.C.S.) in 100 mL distilled water.

4. *Sulfuric acid* (10% *v/v*)

Prepared by adding 100 mL of distilled water to 10 mL of concentrated sulfuric acid.

5. *Solvents*

All solvents used were of distilled in glass quality obtained from Caledon Chemical Co.

6. *Pond water*

Taken from artificial ponds (prepared by J. Madder of the Department of Entomology) at the Glenlea Research Station, Manitoba, and stored in a glass container until used.

7. *Analytical standard*

2-6-Difluorobenzoic acid (2,6-DFBA) was supplied by Thompson Hayward Chemical Co., Kansas City, U.S.A. and recrystallized from methanol (m.p. of 158°C, 159°C reported). Its structure was confirmed by direct inlet probe-mass spectrometry (70 ev ionization potential) using a Finnigan Model 1015 mass spectrometer. The standard solution was prepared in ethyl acetate and appropriate dilutions made as required.

Apparatus

1. *Glassware*

Treated prior to use with Dri-film (Pierce Co.) (15% in toluene), a silanizing reagent, to prevent adsorption of residues.

2. *Gas-Liquid Chromatography* (*GLC*).

A Varian model 2440, equipped with a tritium foil electron-capture detector was used. Operating conditions: temperatures (°C): injector 179, column 130 or 150, detector 182. Nitrogen. carrier-gas flow rate: 40 mL/min.

3. *GLC column.*

Pyrex (1.7 m × 2 mm id) packed with 5% W/W OV-101 on 100-120 mesh Chromosorb W (AW) DMCS.

4. *Gas-Liquid Chromatography—Mass Spectrometry* (*GLC-MS*).

A Du-Pont Dimaspec GC-MS was equipped with the column described in 3. Operating conditions: temperature (°C): injector 180, column 150 or temperature program at 50-150 at 8°/min, jet separator 220, source 220; ionization potential 70 ev. Helium carrier gas flow rate: 10 mL/min.

2,6-DFBA in fortified pond water

1. *Fortification of pond water.*

50 Millilitre samples of pond water were transferred to a 125 mL glass stoppered separatory funnel and the water spiked with *ca.* 0.02, 0.10 and

1.00 mg/L of 2,6-difluorobenzoic acid. 2.7 Millilitres or less of the standard ethyl acetate solution was added in each case and the system allowed to equilibrate for 1 h. "Spiking" was done in duplicate.

2. *Extraction and derivatization of* 2,6-*DFBA*.

After the equilibration period described above, and pH of the water was adjusted to 2 (indicator paper) with 10% H_2SO_4 (V/V), 2.5 mL 5% sodium chloride was added and the water extracted with 4 × 10 mL ethyl acetate. Each extraction was filtered through anhydrous sodium sulfate on Whatman glass fiber filter paper into a 100 mL round bottom flask.

The combined extract was taken to *ca.* 0.7 mL on a rotary evaporator at 30°C. The sample was transferred to a 5 mL graduated centrifuge tube with the aid of 3 × 0.5 mL ethyl acetate. The extract was then evaporated to dryness at 30°C under a low stream of nitrogen. Approximately 200 μL (about 2 drops) of 1% (V/V) pentafluorobenzyl bromide reagent, 10 μL 30% Na_2CO_3 and 1 mL acetone were added to the dry residue. The tube was stoppered and maintained at 50°C for 30 min in a water bath followed by evaporation of the contents to *dryness* under nitrogen. The residue was dissolved in hexane and made to an appropriate volume for EC-GLC analysis. No further cleanup was required at any of the fortification levels studied. Quantitation utilized the external standard method and peak height measurements. The structure of the derivatized product was confirmed by GLC-MS and direct inlet probe-MS as the pentafluorobenzyl ester.

RESULTS AND DISCUSSION

Initial attempts to derivatize 2,6-difluorobenzoic acid (2,6-DFBA) with various derivatization reagents proved to be fruitless. This observation was not surprising since it was anticipated that the *o*-fluoro groups would sterically hinder the derivatization of the carboxyl group, and that conjugation and the polar effects would deactivate 2,6-DFBA toward derivatization.

The use of diazomethane has proven most successful for preparation of a methyl ester derivative of di-ortho-halo benzoic acids[6-9]. The reaction of diazomethane with 2,6-DFBA was tried and resulted in formation of the methyl ester (identified by GLC-MS); however, sensitivity was too low for use of this derivative at subnanogram levels.

Attention was then turned to the derivatization reagent pentafluorobenzylbromide (1-bromomethyl-2,3,4,5,6-pentafluorobenzene). Its reaction had been reported with 2,3,6-trichlorobenzoic acid (2,3,6-TCBA) resulting in a symmetrical peak (*via* EC-GLC) and good sensitivity (0.1 ng gave *ca.* 40%

full-scale deflection, column temperature 200°[10]. Several other investigators reported the successful derivatization of herbicidal acids[11-13], the phenolic hydrolysis products of *N*-methylcarbamates[14,15], organic acids [16,17], and phenols and mercaptans[16,17].

Pentafluorobenzylation of 2,6-DFBA proved to be a straightforward reaction. The reaction conditions described by Chau and Terry[10] for 2,3,6-trichlorobenzoic acid were modified for 2,6-DFBA resulting in an EC-GLC (column temperature, 130°) peak with good symmetry and excellent sensitivity; 0.5 ng gave a 23% full scale deflection at attenuation 64. To shorten the methodology for the reaction, the following modifications were made giving the same peak (retention time = 25.6 min) and sensitivity. 10 Microlitres of 30% Na_2CO_3 was used in place of 30 μL K_2CO_3 (to decrease co-extracted interferences); the sample was reacted at 50° for 30 min (cf. 5 h at room temperature) and evaporated to dryness under nitrogen after reaction (*cf.* the benzene extraction of an aqueous layer and drying of benzene over anhydrous sodium sulfate used by Chau and Terry[10]). EC-GLC of the reaction product gave a single, symmetrical peak with a retention time of 25.6 min (column temperature: 130°). Confirmation of this peak by GLC-MS and DIP-MS yielded spectra showing a strong parent ion (M+) of m/e 338 and fragmentation ions of m/e 181 ($-C_7H_3O_2F_2$) and m/e 141 ($-C_7H_2OF_5$).

Recovery data at three levels from fortified pond water were 105, 105, 102% at the 1.03. 0.10 and 0.02 mg/L levels of fortification, respectively. The described procedure has several advantages over alternative methods, for instance, short reaction time, facile removal of excess reagent by evaporation to *dryness*, no column cleanup required at the fortification levels studied, low toxicity of reagents, and no explosive hazard compared to the procedure using diasomethane to form the methyl ester. The linear range of the electron capture detector to the pentafluorobenzyl ester of 2,6-DFBA standard was 0.002-1.0 ng.

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