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AN ELECTRON-CAPTURE GAS CHROMATOGRAPHIC METHOD FOR THE DETERMINATION OF SUBSTITUTED UREA AND CARBAMATE HERBICIDES AS 2,4-DINITROPHENYL DERIVATIVES OF THEIR AMINE MOIETIES*

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SUMMARY

A method is described for the determination of residues of those substituted urea and carbamate herbicides which hydrolyze to give an aromatic amine. The 2,4-dinitrophenyl derivatives of the amines, which are amenable to gas chromatography and possess strong electron-capturing properties, are prepared by a novel procedure involving reaction on a silica gel chromatoplate. The application of this method to the determination of herbicides in river waters is described.

INTRODUCTION

Substituted ureas and carbamates containing an aromatic amine moiety have found wide application as herbicides, and the detection and identification of traces of these materials in soils, plants and river waters are of interest in the general context of environmental pollution by pesticide residues.

Phenyl ureas are thermally unstable and during gas chromatography breakdown frequently occurs with liberation of the component amines[1,2]. By taking special precautions, however, these compounds can be gas chromatographed directly[3,4], although the electron-capture response in some cases is rather low and the separation of similar ureas is poor. The phenyl-substituted carbamates are more stable and can be gas chromatographed directly under less stringent conditions[5], but in the absence of a simple nitrogen-specific detector characterization is difficult, as many other pesticides and natural coextractives elute in the same retention time range and give an electron-capture response. In general, therefore, the characteristics of these two groups of compounds do not make direct gas chromatography the preferred mode of analysis, and most methods cited in the literature rely on a preliminary hydrolysis stage followed by colorimetric detection of the liberated amine after diazotisation and coupling[6–9]. The azo dyes formed from the various amines can be separated to yield some qualita-

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tive information[7] but the procedure is complicated and time consuming. In addition, as several compounds can give rise to the same aromatic amine, a separation of the herbicides prior to hydrolysis is necessary for qualitative analysis. An alternative identification technique, involving the gas chromatographic detection of derivatives formed by the bromination of the amines[10], has been reported as being difficult to use in practice[11].

This paper describes a qualitative and quantitative analytical procedure applicable to those herbicides which can be hydrolysed to yield an aromatic amine. The 2,4-dinitrophenyl derivative of the amine is prepared by reacting it with 1-fluoro-2,4-dinitrobenzene on a silica gel chromatoplate. The derivative is then eluted from the adsorbent and identified and determined by electron-capture gas chromatography. Gas chromatographic conditions are described for the separation and detection of the derivatives of all the amines of interest. With samples low in coextractives, *e.g.* river water extracts, the herbicides can be separated, hydrolysed and reacted with 1-fluoro-2,4-dinitrobenzene on a single chromatoplate.

EXPERIMENTAL

Hydrolysis conditions

The hydrolysis of substituted ureas and carbamates has generally been achieved by an acid or alkaline digestion procedure. It has been shown that the reaction could be effected on silica gel by spraying with a hydriodic acid reagent and heating at 180° after covering the sprayed layer with a second glass plate[12]. This technique was tried and the yield of aromatic amine determined by scraping off the adsorbent, eluting the amine and preparing a derivative amenable to gas chromatography[13]. The variable yields of amine obtained indicated that this hydrolysis procedure could not easily be made quantitative and this was attributed to breakdown of the aromatic amine by further reaction with the hydriodic acid reagent as previously observed[12]. A hydrochloric acid spray was tried and although the yields of amine were lower the procedure appeared promising. Improved yields were obtained when the amine reagent 1-fluoro-2,4-dinitrobenzene was sprayed on the plate together with the hydrochloric acid, the nitrophenylation presumably enhancing the hydrolysis of the urea or carbamate by removing free amine from the reaction mixture as it is formed.

Amine derivative formation

To attain the detection levels necessary for a residue method, an amine derivative amenable to gas chromatography with electron-capture detection was required. In the course of this work we investigated two reagents which had been previously used for the detection of amines at low levels—heptafluorobutyric anhydride[14] and 1-fluoro-2,4-dinitrobenzene[13]. Aromatic amine derivatives showing the desired properties were produced with both reagents but heptafluorobutyric anhydride was finally rejected as it was prone to hydrolysis by atmospheric moisture and was more expensive than the alternative reagent. In a recent study the reagents 4-chloro-α,α,α-trifluoro-3,5-dinitrotoluene and α,α,α,4-tetrafluoro-3-nitrotoluene have been shown[15] to yield amine derivatives with improved characteristics over those given by 1-fluoro-2,4-dinitrobenzene; this information was not available at the time we carried out this work, but it seems probable that these reagents could well be used to improve the sensitivity of the

described method, although both are appreciably more expensive than 1-fluoro-2,4-dinitrobenzene.

Pure 2,4-dinitrophenyl derivatives of the amines of interest were prepared as analytical standards using the following procedure: Approximately 0.2 g of the amine was dissolved in a small volume of water containing five drops of concentrated hydrochloric acid. 25 ml of an aqueous disodium tetraborate solution (2.5% w/v) and 10 ml of a solution of 1-fluoro-2,4-dinitrobenzene in 1,4-dioxane (4% w/v) were added and the mixture heated in a water bath at 60° for several hours until an oil or a fine precipitate was produced; additional heating was often necessary to promote solidification of the oils. 10 ml of 2 *N* sodium hydroxide was added to the reaction mixture to hydrolyze excess reagent and the heating was continued for 1 h. The derivatives were filtered off in a Hirsch funnel and washed with 0.1 *N* sodium carbonate solution until the washings were colourless. The derivatives after drying at 120° gave the melting points shown in Table II. Solutions for gas chromatography were prepared by dissolving the compounds in acetone.

Thin-layer chromatography

The thin-layer chromatographic separation of substituted urea and carbamate herbicides has been studied by several workers and many solvent systems described [16,17]. The main requirement of a solvent system for our work was that it should separate those compounds which hydrolyze to give the same aromatic amine. No one solvent system was found capable of achieving this and recourse to two systems was necessary. Table I shows the R_F values of the compounds on silica gel chromatoplates with the two solvent systems found to be most useful.

Gas chromatography

To our knowledge the previous gas chromatographic use of 2,4-dinitrophenyl derivatives of amines has been confined to the detection of low molecular weight ali-

TABLE I

THIN-LAYER SEPARATION OF UREA AND CARBAMATE HERBICIDES

R_F values on 250 μ Silica Gel G plates 20 × 20 cm. Solvent developed for 10 cm in unlined tanks.

Herbicide	*Amine produced*	R_F *value (solvent 1)*[a]	R_F *value (solvent 2)*[b]
Barban	3-Chloroaniline	0.95	0.33
Chlorbufam	3-Chloroaniline	0.95	0.49
Chlorpropham	3-Chloroaniline	0.95	0.65
Monolinuron	4-Chloroaniline	0.73	0.30
Monuron	4-Chloroaniline	0.21	0.09
Diuron	3,4-Dichloroaniline	0.25	0.12
Linuron	3,4-Dichloroaniline	0.78	0.30
Propham	Aniline	0.91	0.53
Fenuron	Aniline	0.18	0.11
Metobromuron	4-Bromoaniline	0.73	0.30

[a]Solvent 1 : chloroform.
[b]Solvent 2 : hexane–acetone (5 : 1).

phatic amines[13]. The derivatives formed with aromatic amines are of relatively low volatility, but with a suitable stationary phase at low loading, and with a moderately high column temperature we have been able to obtain adequate gas chromatographic separations. Of the several stationary phases examined the silicone GE-XE 60 gave the best separations, and Fig. 1 shows a typical chromatogram. The derivatives of 3- and 4-chloroaniline are incompletely resolved, but can be distinguished, and all the other amine derivatives are adequately separated.

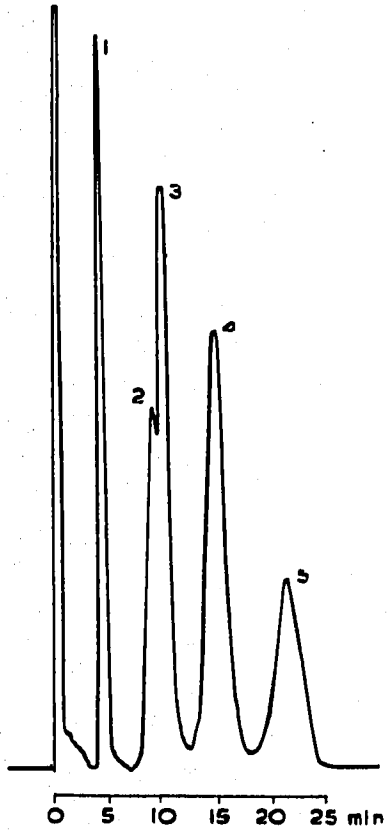

Fig. 1. Gas chromatographic separation of 2,4-dinitrophenyl derivatives of aromatic amines. (1) aniline DNP derivative, 3 ng; (2) 3-chloroaniline DNP derivative, 4.8 ng; (3) 4-chloroaniline DNP derivative, 4 ng; (4) 4-bromoaniline DNP derivative, 4.8 ng; (5) 3,4-dichloroaniline DNP derivative, 5 ng. Gas chromatographic conditions as in Table II.

Being aromatic nitro compounds the derivatives show strong electron-capturing properties facilitating their detection at nanogram levels. Table II shows the gas chromatographic retention times, sensitivities and melting points of the derivatives. The equipment used in this work was based on a Griffin & George chromatographic oven model TI/250 and a simple electron-capture detector described elsewhere[18].

Extraction and clean-up of samples

In the course of this work it became apparent that two approaches to herbicide analysis were necessary as the process of on-plate hydrolysis and derivative formation could not be applied in the presence of large quantities of coextractives. Samples such as surface waters can be analyzed directly by extracting with chloroform and subsequently separating, hydrolyzing and dinitrophenylating the extract on the plate.

With soil and plant samples, however, we were unable to develop a satisfactory procedure for separating the unchanged herbicides from coextractives and with such samples a preliminary hydrolysis is necessary, followed by a clean-up utilizing the basic properties of the amines produced. Where such treatment is given to a sample the amount of qualitative information derived is limited as herbicides yielding the same amine cannot be distinguished from one another.

PROCEDURE

Waters

Extract 1 l of water with three 50 ml portions of chloroform and dry the extracts by passage down a column containing granular anhydrous sodium sulphate. Evaporate the solution to small volume on a steam bath using a Kuderna-Danish evaporator fitted with a 10 ml pear-shaped flask. Reduce the volume still further by heating the flask after replacing the Kuderna-Danish evaporator with a micro Snyder column[10], and finally reduce the volume still further with a gentle stream of air. Spot the residual solution, together with herbicide standards (5–10 μg) on to 20 × 20 cm thin-layer plate coated with a 250 μ layer of Silica Gel G (Merck). Develop the plate in an unlined tank using either chloroform, or hexane–acetone (5:1) as the mobile phase and allow the solvent to rise some 10 cm from the origin. Remove the plate for spraying as described later.

Soils and plant material

Decompose the sample using the well established digestion–distillation procedure[6], or alternatively extract the herbicide by macerating a 50 g sample with three 100 ml portions of acetone, centrifuging the suspension after each maceration, and decanting off the supernatant liquid. Add the combined acetone extracts to 750 ml of a 2.5% aqueous sodium sulphate solution and extract with three 100 ml portions of chloroform. Dry and evaporate the combined extracts as described above. Add 2 ml of glacial acetic acid to the residue in the pear-shaped flask and warm on a steam bath to effect solution. Add 5 ml of 60% hydrochloric acid and boil under reflux for 2 h. Wash the condenser down with 5 ml of water and transfer the solution to a 100 ml separator. Dissolve about 4 g of anhydrous Na_2SO_4 in the solution and extract with three 25 ml portions of chloroform to remove unwanted coextractives. Discard the chloroform extracts. Add 15 ml of 5 *N* sodium hydroxide to the aqueous layer and extract with three 25 ml portions of chloroform to remove the amine present. Dry and evaporate the combined chloroform extracts as described above, but to prevent volatilization of the amine add 10 μl of glacial acetic acid before removing the final traces of solvent with a stream of air. Spot the residue on to a silica gel chromatoplate together with amine standards and spray the plate as described below.

Spray technique

Spray the thin-layer plates containing the separated herbicides or the free amines with about 7 ml of hydrochloric acid (5% v/v) and then spray with a similar quantity of a 1-fluoro-2,4-dinitrobenzene solution (4% w/v in acetone). Clip a clean glass plate over the gel surface with four spring clips (*e.g.* 14 cm "bulldog" clips) ensuring that the cover plate is held sufficiently tight to prevent spot migration during the heat-

TABLE II

GAS CHROMATOGRAPHIC PROPERTIES AND MELTING POINTS OF 2,4-DINITROPHENYL DERIVATIVES OF AROMATIC AMINES

Amine	*Melting point (°C) of the 2,4-DNP derivative*	*Retention time (min) on GE-XE 60 column*[a]	*Detection limit (ng) under same condition*
Aniline	153–154	3.6	0.2
3-Chloroaniline	182–183	8.4	0.3
4-Chloroaniline	162–163	9.5	0.5
4-Bromoaniline	152–153	14.3	1.0
3,4-Dichloroaniline	185–186	20.8	1.5

[a]1.0% GE-XE 60 and 0.1% Epikote 1001 on Chromosorb G AW/DMCS coated, 60–80 mesh, at 215°. Column: glass, 140 cm in length, 1.5 mm I.D. Carrier gas: nitrogen. Flow rate: 180 ml/min.

ing stage. Stand the plate vertically in an oven at 190° for 40 min. Remove the plate from the oven and when cool remove the cover plate. Scrape off the silica gel layer in the areas of interest (see Table I for R_F values) which are frequently characterised by the presence of a bright yellow coloration. Transfer the gel samples to a short glass column plugged with a cotton wool pad and elute the 2,4-dinitrophenyl derivative by passing two 5 ml portions of acetone down the column. Adjust the combined eluate volume to give a suitable derivative concentration (about 1 μg/ml of the derivative) and inject a 5 μl aliquot on to a gas chromatographic column such as described in Table II. Compare the retention times of unknown derivative peaks with those given by standards to obtain qualitative information, and derive quantitative information by plotting a peak height concentration curve using herbicide or amine standards treated as described above. Corrections for extraction losses should be made.

TABLE III

YIELDS OF 2,4-DINITROPHENYL DERIVATIVES OF AMINES OBTAINED BY HYDROLYSIS AND REACTION OF HERBICIDES ON SILICA GEL THIN-LAYER PLATES

Herbicide	*Mean yield of derivative*[a] (%)	*Mean deviation* (%)	*Mean deviation on single plate* (%)
Barban	65	6	4
Chlorbufam	72	7	3
Chlorpropham	76	6	6
Diuron	87	4	4
Fenuron	54	3	2
Linuron	72	6	2
Metobromuron	71	2	1
Monolinuron	68	11	4
Monuron	88	9	3
Propham	69	3	1

[a]Mean yield obtained by reaction of 15 separate 10 μg amounts of herbicide (3 spots on each of 5 plates) and calculated as the percentage of the theoretical yield assuming the herbicides to be 100% pure.

TABLE IV

DETERMINATION OF HERBICIDES IN WATER

Herbicide	*% extracted by chloroform from aqueous solution at 0.01 p.p.m.*	*% recovery from spiked samples at 0.01 p.p.m. (corrected for extraction losses)*
Barban	91	97, 105, 99
Chlorbufam	87	98, 100, 95
Chlorpropham	81	94, 98, 101
Diuron	77	99, 102, 105
Fenuron	83	97, 100, 104
Linuron	77	102, 99, 92
Metobromuron	80	104, 101, 98
Monolinuron	83	110, 98, 98
Monuron	80	98, 96, 96
Propham	96	99, 104, 100

RESULTS

The mean yield of derivative obtained by hydrolysis and dinitrophenylation of 10 μg amounts of herbicides using the on-plate reaction technique described are shown in Table III, together with the spread of results on a series of plates and on a single plate. The standard curves, obtained by reacting different quantities of herbicides as described and plotting the gas chromatographic peak height as a function of concentration, were linear over the range 1–20 μg. The yields obtained with free amines were usually 90% of theory and again linear calibration curves were derived.

Table IV shows the recovery data for a series of herbicides added to glass-distilled water at the 0.01 p.p.m. level. Extraction into the chloroform phase was incomplete and the observed levels were corrected to compensate for the extraction losses which are indicated in the first column. Analogous results were obtained with river waters although the degree of extraction attained was influenced by the composition of the water, being lower than with glass-distilled water.

Equally good results were obtained with samples of plants and soil where the clean-up procedure described above was used. Again, however, correction for extraction losses was necessary as the amines produced during the hydrolysis stage were incompletely extracted using the described procedure; the recoveries for aniline, 4-bromo-, 4-chloro-, 3-chloro- and 3,4-dichloroaniline were 66, 59, 64, 59 and 31% respectively.

DISCUSSION

The technique of on-plate dinitrophenylation is a rapid and convenient method of derivative formation. Although the yield of derivative is variable, Table III shows that less variation is encountered for reactions carried out on the same plate. The results obtained on a single plate, such as those shown in Table IV, are sufficiently accurate to be acceptable in a residue method. The described procedure can be used to determine herbicides in river waters at levels down to about 0.001 p.p.m. and in soil and plant material to 0.02–0.05 p.p.m.

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