CHROM. 4681

AN ELECTRON-CAPTURE GAS CHROMATOGRAPHIC METHOD FOR THE DETERMINATION OF SOME CARBAMATE INSECTICIDES AS 2,4-DINITROPHENYL DERIVATIVES OF THEIR PHENOL MOIETIES*

I. C. COHEN, J. NORCUP, J. H A. RUZICKA AND B. B. WHEALS

Ministry of Technology, Laboratory of the Government Chemist, Cornwall House, Stamford Street, London, S.E.1. (Great Britain)

(Received February 26th, 1970)

SUMMARY

A method is described for the analysis of residues of insecticidal carbamates which involves hydrolysis to a phenol and concurrent formation of the corresponding electron-capturing 2,4-dinitrophenyl ether. Free phenols do not interfere. The application of this method to the determination of carbamates in river waters and vegetables is described.

INTRODUCTION

The introduction and widespread acceptance of the carbamate pesticides has led to a need for specific and sensitive methods for the analysis of their residues in soils, waters and foodstuffs. The carbamate esters used fall into two general classes, *viz.*

(i) R–NH–CO_2–Alkyl (herbicides) and

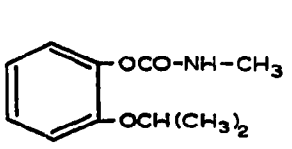
(ii) CH_3–NH–CO_2–R′ (insecticides),

where R and R′ are substituted aryl or heterocyclic ring systems.

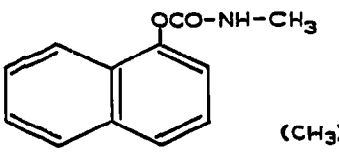
A procedure for the analysis of residues of herbicidal carbamates has been presented previously[1]. The current paper is concerned with the determination of four insecticidal carbamates, *viz.* propoxur, butacarb, carbaryl and methiocarb (a molluscicide).

There are several colorimetric[2,3] and TLC[4,5] methods available for the analysis of the insecticidal carbamates. The former procedures, which involve hydrolysis to the corresponding phenol followed by formation of a derivative amenable to spectrophotometric determination, have poor specificity. TLC methods, in which the intact carbamate or its phenol hydrolysis product are visualised, are prone to interference from co-extractives and require rigorous clean-up procedures. A GLC method incor-

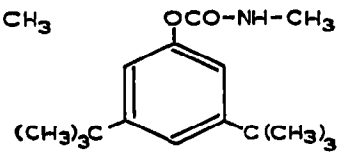
* Permission to publish has been given by the Government Chemist. Crown Copyright reserved.



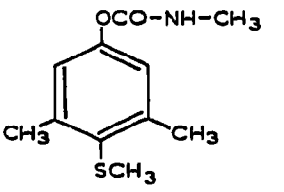
Propoxur
(Arprocarb)
(Unden)
(Bayer 39007)



Carbaryl
(Sevin)
(UC-7744)



Butacarb
(RD-14639)



Methiocarb
(Mesurol)
(Bayer 37344)
(H-321)

porating a sensitive and specific detector would be preferable[6], since it would give more precise quantitative data and be less susceptible to interference from co-extractives. Direct GLC is precluded because of the tendency of some free carbamates to on-column breakdown and the poor response to them of the detectors available. The detection limit using an electron-capture detector was 20–100 ng, while using a caesium bromide thermionic detector, with gas flow rates and the inter-electrode distance adjusted for maximum sensitivity to nitrogen, the detection limit was 50–100 ng.

Determination of traces of these carbamates by GLC therefore requires an indirect procedure which again generally involves hydrolysis to the phenol followed by derivative formation. The derivative must be amenable to GLC and should be able to be detected at the nanogram level. A method in which the esterified phenol is brominated[7] has only been applied to carbaryl; a sensitive method utilising the trichloroacetylated phenol[8] requires the laboratory preparation of an acetylating agent which, being susceptible to hydrolysis, is not very stable. The electron-capturing properties of halomethyldimethylsilyl phenol derivatives have not allowed estimation of less than 10 ng of carbamate[9]. We found dimethylthiophosphorylation[10] to give poor yields with the less acidic phenols; in addition the presence of excess reagent, which could not be removed, gave a large background current with the thermionic detector, making quantitative interpretation of the chromatograms difficult.

This paper describes a quantitative and qualitative method for the analysis of residues of insecticidal carbamates in the presence of free phenols. Hydrolysis of the carbamate to the phenol and formation of the corresponding 2,4-dinitrophenyl ether is effected concurrently. The derivative, extracted into hexane, is identified and determined by GLC with electron-capture detection.

EXPERIMENTAL

REINHEIMER *et al.* have described a procedure[11] which has been used for the preparation of 1-fluoro-2,4-dinitrobenzene (FDNB) derivatives of phenols[12]. A method for the analysis of residues of carbamates involving the reaction with FDNB of the phenols formed by alkaline hydrolysis of the insecticides seemed promising.

The yields of 2,4-dinitrophenyl ethers, obtained when the carbamates under review were heated in a buffer with a 1% (w/v) solution of FDNB in acetone, varied

markedly with changes in the volume of reagent and the pH of the buffer. However, 0.5 ml of FDNB solution and a phosphate buffer of pH 11.0 gave good and consistent results. At pH 11.0 amines do not react with FDNB, thus there was no interference from the amine hydrolysis products of the herbicidal carbamates or the allied substituted ureas such as propham and metobromuron.

Several organophosphorus pesticides, *e.g.* fenchlorphos, dichlofenthion and bromophos, can hydrolyse to phenols yielding FDNB derivatives with similar retention times to those derived from the carbamates. They did not interfere, however, presumably because of their failure to hydrolyse under such mild conditions.

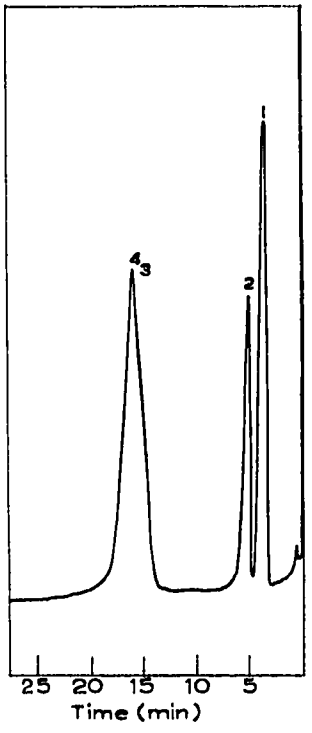


Fig. 1. Gas chromatographic separation of 2,4-dinitrophenyl derivatives of phenols. For GLC conditions, see Table I. 1 = 2-isopropoxyphenol DNP derivative, 2 = 3,5-di-*tert.*-butylphenol DNP derivative; 3 = 1-naphthol DNP derivative, 4 = 3,5-dimethyl-4-methylthiophenol DNP derivative.

Free phenols, especially those corresponding to the parent carbamate and which therefore may be present as breakdown products, are a potential source of interference. Their removal using extraction procedures based on the acidity of phenols did not prove satisfactory and hence elimination of the phenols by selective oxidation was tried. Of the several oxidants used, *i.e.* potassium permanganate, ferric chloride, potassium dichromate, nitric acid, hydrogen peroxide, ammonium persulphate and ceric sulphate, only the last removed phenols completely with no loss of any of the carbamates. Even with ceric sulphate, satisfactory results were only obtained if about 20% acetone was present in the aqueous reaction phase. Breakdown of the carbamates was then completely inhibited, while under the conditions of the procedure 500 μg of phenol gave a response equivalent to only 0.5 μg of the corresponding carbamate.

Gas chromatography

The dinitrophenyl ethers were gas chromatographed at 211°, a slightly lower temperature than used previously[1] for the 2,4-dinitrophenyl derivatives of aromatic amines; all other conditions were, however, the same. Good separation of the 2-isopropoxyphenyl, 3,5-di-*tert.*-butylphenyl and 1-naphthyl ethers was achieved, but the 1-naphthyl and the 3,5-dimethyl-4-methylthiophenyl ethers were not resolved (Fig. 1). Reaction of a mixture of these two ethers with peracetic acid resulted in total oxidation of the thio-linkage and allowed estimation of the individual derivatives by difference.

Extraction and clean-up

River water, after removal of free phenol, was analysed directly by chloroform extraction and derivative formation. Some sources of analytical grade chloroform required redistillation to prevent almost complete loss of the butacarb; even then recoveries of this compound were not very good. A comparison of the IR spectra of the redistilled and unredistilled chloroform did not indicate the presence of any impurities in the analytical grade reagent which could have caused the loss of butacarb.

Plant materials require a more rigorous clean-up. Hydrolysis of the carbamates followed by steam distillation gave very poor yields. Apart from this, a clean-up involving a preliminary hydrolysis was unacceptable since it would preclude differentiation between carbamates and free phenols. Most of the coextracted plant material could be removed by coagulation[13] and filtration. Free phenols were then oxidised and the carbamates extracted. Butacarb gave very poor yields, but since it is used mainly in sheepdips it is unlikely to be present in vegetable material.

PROCEDURE

Reagents

The following reagents were applied: Buffer solution pH 11.0; 8.2 ml of 0.1 *N* sodium hydroxide and 100 ml of 0.05 *M* disodium hydrogen phosphate diluted to 200 ml with deionised water. 1-Fluoro-2,4-dinitrobenzene solution; 1 g of FDNB dissolved in 100 ml of acetone. Coagulating solution; 1.25 g of ammonium chloride and 2.5 ml of orthophosphoric acid (s.g. 1.75) dissolved in 1 l of deionised water. Ceric sulphate solution; 20 mg of ceric sulphate dissolved in 20 ml of approximately 4 *N* sulphuric acid (prepare freshly as required). Hydrogen peroxide solution; 16 ml of 100 volumes hydrogen peroxide dissolved in 100 ml of glacial acetic acid. Redistilled chloroform. Redistilled hexane. Anhydrous sodium sulphate, granular.

Water

To 1 l of the sample of water, contained in a 2-l separating funnel, add the freshly prepared ceric sulphate solution. After 15 min dissolve 20 g of anhydrous sodium sulphate in the water and extract with three 50-ml portions of redistilled chloroform, shaking for 1 min for each extraction. Dry the extracts by passage through a column containing 15 g of granular anhydrous sodium sulphate. Combine the dried extracts and evaporate to a small volume in a Kuderna–Danish evaporator fitted with a 10-ml pear-shaped flask, and then reduce the volume still further using a micro-

Snyder column. Finally take to dryness in a gentle stream of air, hydrolyse and form the derivative as described below.

Vegetable material

Macerate 50 g of vegetable tissue for 2 min with each of three separate 100-ml portions of acetone; centrifuge after each operation. Add the combined supernatant extracts to 1 l of deionised water containing 20 g of anhydrous sodium sulphate and extract with three 50-ml portions of redistilled chloroform, shaking for 1 min for each extraction. Pass the extracts down a column containing 15 g of granular anhydrous sodium sulphate into a Kuderna–Danish evaporator. Reduce the volume to 5 ml, wash with chloroform into a 150-ml beaker and evaporate to dryness in a gentle stream of air. Redissolve the extracted vegetable matter in 50 ml of acetone and add 50 ml of coagulating solution. Cover with a watch glass and allow to stand overnight. Filter the coagulated solution, under vacuum, through a tightly packed filter paper pulp pad about 0.5 cm thick contained in a coarse sintered glass filter funnel. Wash the beaker with 30 ml of coagulating solution and pass the washings through the filter pad. Transfer the filtrate, which should be almost colourless, to a 250-ml separating funnel and add 20 ml of freshly prepared ceric sulphate solution. After 15 min, add 10 ml of propan-2-ol to minimise emulsion formation. Extract with redistilled chloroform and evaporate to dryness as described above for water samples.

Hydrolysis and derivative formation

Pipette 0.5 ml of 1% (w/v) FDNB in acetone into the pear-shaped flask containing the cleaned-up residue and add 10 ml of the buffer solution. Allow the mixture to react in a water bath at 50° for 30 min. Transfer the yellow reaction mixture to a 100-ml separator and shake for 1 min with 10 ml of redistilled hexane. Discard the aqueous layer and dry the hexane phase by passage through a column containing about 5 g of granular anhydrous sodium sulphate. (Any emulsion in the hexane layer may be dispersed by shaking with about 1 g of anhydrous sodium sulphate just prior to passing through the drying column.) Inject 5 μl of the hexane extract on to the GLC column described in Table I.

Analysis of a mixture of methiocarb and carbaryl

If peaks corresponding to methiocarb or carbaryl are obtained the following

TABLE I

GAS CHROMATOGRAPHIC PROPERTIES OF 2,4-DINITROPHENYL DERIVATIVES OF PHENOLS

Column: glass 140 cm in length 1.5 mm I.D., 1.0% GE-XE 60 and 0.1% Epikote 1001 on 60–80 mesh Chromosorb G AW/DMCS at 211°. Carrier gas: Nitrogen 180 ml/min

Phenol	*Retention time (min) on GE-XE 60 column*	*Detection limit (ng)*
2-Isopropoxyphenol	3.5	0.1
3,5-Di-*tert.*-butylphenol	5.5	0.2
1-Naphthol	16.2	0.2
3,5-Dimethyl-4-methylthiophenol	15.2	0.2

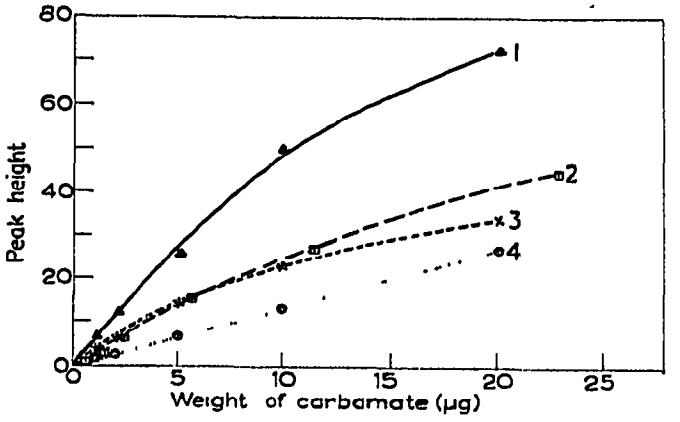


Fig. 2. Calibration curve, prepared by subjecting standard amounts of carbamate to hydrolysis and derivative formation. 1 = propoxur; 2 = methiocarb; 3 = butacarb; 4 = carbaryl.

procedure is adopted: Take 5 ml of the hexane extract to dryness in a gentle stream of air and add 3 ml of the hydrogen peroxide solution. Heat the mixture for 15 min at 50°. Wash into a 100-ml separating funnel with enough saturated sodium bicarbonate solution to give a neutral solution. Extract with 5 ml of redistilled hexane and dry by passage down a column containing 4 g of granular anhydrous sodium sulphate. Inject 5 μl of this solution on to the GLC column. The thiophenyl derivative will have been removed and any remaining peak will be due to carbaryl. Any methiocarb present may be estimated from the difference between the two chromatograms.

RESULTS

The calibration curves shown in Fig. 2 were plotted by subjecting standard amounts of the insecticides to hydrolysis and derivative formation. These curves were

TABLE II

RECOVERIES OF CARBAMATES FROM VARIOUS SPIKED SUBSTRATES

Sample	*Carbamate*	*Number of determinations*	*Mean recovery (%)*	*Relative mean deviation (%)*
Thames river water, 1 l spiked with 10 μg of carbamate	Propoxur	12	94	2.5
	Butacarb	6	41	15
	Methiocarb	6	97	4
	Carbaryl	6	63	5
Peas, 50 g spiked with 10 μg of carbamate	Propoxur	8	87	6
	Methiocarb	4	89	5
	Carbaryl	4	87	5
Lettuce, 50 g spiked with 10 μg of carbamate	Propoxur	8	96	7
	Methiocarb	4	97	4
	Carbaryl	4	82	0
Apple, 50 g spiked with 10 μg of carbamate	Propoxur	6	100	9
	Methiocarb	4	94	5
	Carbaryl	4	94	5

used in the calculation of the percentage recoveries (Table II) of carbamate from spiked samples of water, peas, apples and lettuce.

The water taken from the Thames in Central London gave several interfering peaks before reaction with ceric sulphate. The mean recovery of butacarb varied between 40% and 65% from day to day, but on any particular day the relative mean deviation was never greater than 15%. No reason could be found for the generally poor recoveries of butacarb nor for the daily fluctuations, which were not observed with the other three carbamates.

Incomplete coagulation occurred in the presence of 50 ml of acetone and a small amount of coextractive remained in solution resulting in a rather broad injection peak, which made measurement of the propoxur peak difficult. If the presence of this compound is indicated a more accurate analysis may be obtained by using 15 ml of acetone instead of 50 ml. The early peak due to coextractives is then greatly reduced with no reduction in the propoxur recovery; however, the recoveries of methiocarb and carbaryl are reduced.

The method described allows the analysis of insecticidal carbamates in river waters at levels down to about 0.005 p.p.m. and in plant material down to 0.1 p.p.m. The method for plant material was unsuccessful when applied to soil samples, and for these more stringent clean-up procedures would be required.

REFERENCES

1 I. C. COHEN AND B. B. WHEALS, *J. Chromatog.*, 43 (1969) 233.
2 D P. JOHNSON AND H. A. STANSBURY, *J. Agr. Food Chem.*, 13 (1965) 235.
3 W. R. BENSON AND J. M. FINICCHIARO, *J. Assoc. Offic. Agr. Chemists*, 49 (1966) 452.
4 N. J. PALMER AND W. R. BENSON, *J. Assoc. Offic. Agr. Chemists*, 51 (1968) 679.
5 J. M. FINICCHIARO AND W. R. BENSON, *J. Assoc. Offic. Agr. Chemists*, 48 (1965) 736.
6 General Referee Reports, *J. Assoc. Offic. Agr. Chemists*, 52 (1969) 266.
7 W. H. GUTERMANN AND D. J. LISK, *J. Agr. Food Chem* , 13 (1965) 48.
8 L. I. BUTLER AND L. M. MCDONOUGH, *J. Agr. Food Chem.*, 16 (1968) 403.
9 C. A. BACHE, L. E. ST. JOHN AND D J. LISK, *Anal. Chem.*, 40 (1968) 1241.
10 *Report of the Government Chemist*, Her Majesty's Stationary Office, London, 1968, p. 93.
11 J. D. REINHEIMER, J. P. DOUGLAS, H. LEISTER AND M. B. VOELKEL, *J. Org. Chem.*, 22 (1957) 1743
12 I. C. COHEN, J. NORCUP, J. H. A. RUZICKA AND B. B. WHEALS, *J. Chromatog* , 44 (1969) 251.
13 H. NIESSEN AND H. FREHSE, *Pflanzenschutz-Nachr "Bayer"*, 16 (1963) 205.