

CHROM. 3641

## Use of hydriodic acid in the detection of pesticides after thin-layer chromatography

The use of thin-layer chromatography in the separation of pesticide residues has attracted much attention and many chromogenic reagents have been used in their detection. FINOCCHIARO AND BENSON<sup>1</sup> have extensively studied reagents applicable to the detection of carbamates and ureas, and ABBOTT *et al.*<sup>2</sup> describe several reagents not studied by the former authors. Chromogenic reagents used for the detection of organophosphorus pesticides have been recently reviewed<sup>3-5</sup>.

Many of the chromogenic reagents described are non-specific and are of limited value in practical analysis unless the samples examined are of known pesticide history or have been selectively extracted and cleaned-up. In this laboratory, where samples of unknown history are frequently encountered, we have been studying spray reagents with the object of improving specificity. Our approach to the problem has been to carry out hydrolysis reactions on the thin-layer plate and to use specific or semi-specific colour reactions of the breakdown products produced as an aid in the characterisation of the parent pesticides. COHA<sup>6</sup> has recently applied a similar technique to the detection of the pesticide dimefox. This paper describes techniques capable of detecting and partially characterising carbamate, thiocarbamate, substituted ureas and some nitrogen containing organophosphorus pesticides at  $\mu\text{g}$  levels after on-plate hydrolysis to amines.

### Experimental

*Choice of hydrolyzing procedure.* Mixtures of the various compounds, each at about 10  $\mu\text{g}$ , were separated on silica gel thin-layer plates using suitable solvent systems. The plates were then sprayed with a variety of acid and alkaline reagents capable of effecting hydrolysis. The degree of hydrolysis attained was assessed by subsequent spraying with a ninhydrin solution.

Using the screening procedure described, the most effective spray was found to be a hydriodic acid reagent previously recommended by BARNEY<sup>7</sup> for the hydrolysis of organophosphorus compounds. The most complete hydrolysis was achieved when a second glass plate was clipped over the sprayed plate, followed by heating in an oven, at 180°, for about 30 min; a technique we have previously found useful in the hydrolysis of organophosphorus pesticides<sup>8</sup>.

### Method

*Reagents.* Spray 1: 25 ml of hydriodic acid, s.g. 1.7, 25 ml of glacial acetic acid and 50 ml of water. The solution is stable for several weeks.

Spray 2: 5 g of sodium nitrite dissolved in 100 ml of 0.2 N hydrochloric acid. Prepare the solution just before use.

Spray 3: 5 g of  $\alpha$ -naphthol dissolved in 100 ml of methanol. The solution is stable for several days if refrigerated.

Spray 4: 3 g of ninhydrin dissolved in 95 ml absolute ethanol and 5 ml of glacial acetic acid. The solution is stable for several days.

### Procedure

The mixture to be separated is divided and the separate portions spotted on to a 20 × 20 cm thin-layer plate coated with a 250 μ layer of Silica Gel G (Merck). The plate is developed in a suitable solvent system, removed from the tank and the solvent allowed to evaporate. The plate is sprayed with spray 1, a clean glass plate clipped over the sprayed surface and the whole heated in an oven at 180° for 30 min. (The plate should be allowed to stand in a vertical plane to assist heat flow over its surface). The plate is removed from the oven and the cover plate unclipped avoiding the iodine fumes evolved. When cool, one portion of the plate is sprayed with spray 2 and gently warmed (*e.g.* on the surface of an oven) until dry. A pink spot on spraying with spray 3 indicates the presence of an aromatic amine. The second portion of the plate is sprayed with spray 4 and heated in an oven at 120° for 20 min. The formation of mauve or reddish brown spots indicates the presence of aromatic or aliphatic amines.

TABLE I

$R_F$  VALUES, COLOURS AND DETECTION LIMITS OF CARBAMATES, UREAS AND ORGANOPHOSPHORUS PESTICIDES AFTER HYDROLYSIS WITH HYDRIODIC ACID

Solvent 1: hexane-acetone 5:1; solvent 2: chloroform-acetone 9:1.

Pesticide*	$R_F$ solvent 1	$R_F$ solvent 2	Colour with ninhydrin spray	Detection limit μg	Colour** with diazotising spray	Detection limit μg
Arprocarb	0.24	0.83	brownish red	0.5	—	
Barban	0.33	0.96	pink	5.0	orange-pink	1.0
Butacarb	0.50	0.92	brownish red	0.5	—	
Carbaryl	0.18	0.80	brownish red	0.5	—	
Chlorbufam	0.49	0.92	mauve	2.0	orange-pink	0.5
Chlorpropham	0.65	0.97	mauve	2.0	orange-pink	0.5
Di-allate	0.92	0.95	orange	1.0	—	
EPTC	0.92	0.97	mauve	1.0	—	
Propham	0.53	0.92	mauve	1.0	pink-red	0.5
Tri-allate	0.91	0.95	orange	1.0	—	
Chloroxuron	0.08	0.63	brownish red	0.5	red-mauve	0.5
Cycluron	0.10	0.63	pink	1.0	—	
Diuron	0.12	0.63	brownish red	0.5	orange-pink	0.5
Fenuron	0.11	0.53	brownish red	0.5	pink-red	0.5
Linuron	0.30	0.89	mauve	0.5	orange-pink	0.5
Monuron	0.09	0.48	pink	0.5	pink	0.5
Crufomate	0.06	0.43	brown-red	0.5	—	
Dimefox	0.08	0.44	brown-red	0.2	—	
Dimethoate	0.05	0.37	brown-red	1.0	—	
Ethoate methyl	0.07	0.61	pink	1.0	—	
Fenitrothion	0.49	0.91	mauve	1.0	pink-mauve	1.0
Formothion	0.15	0.75	brownish red	2.0	—	
Mecarbam	0.42	0.95	pink	5.0	—	
Menazon	0.0	0.02	pink	1.0	—	
Morphothion	0.06	0.49	mauve	0.5	—	
Parathion	0.57	0.91	mauve	1.0	pink-mauve	2.0
Phosphamidon	0.04	0.34	brownish red	5.0	—	
Schradan	0.0	0.02	brownish red	0.1	—	
Thionazin	0.45	0.92	brownish red	1.0	—	
Vamidothion	0.01	0.16	brownish red	1.0	—	

\* B.S.I. approved names used where applicable.

\*\* —: Indicates no reaction.

### Results

Table I shows the  $R_F$  values in two solvent systems, detection limits and characteristic colours obtained for a variety of carbamates, substituted ureas and organophosphorus pesticides after hydrolysis with hydriodic acid. The oxygen analogues of dimethoate, parathion, and thionazin gave analogous colour reactions to the parent compounds. The following nitrogenous compounds failed to give colour reactions under the described conditions.

Triazines: ametryne, atrazine, desmetryne, GS 14260, methoprotryne, prometryne and simazine.

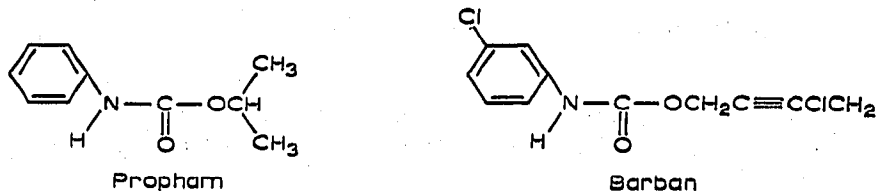
Uracils: bromacil, isocil and lenacil.

Organophosphorus compounds: azinphos ethyl, azinphos methyl, diazinon, phosalone and pyrimithate.

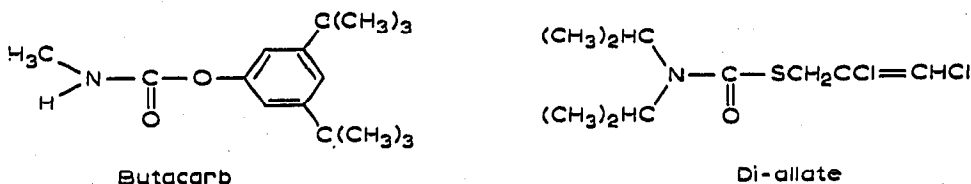
### Discussion

The results in Table I, together with our previous work<sup>8</sup> in which hydriodic acid was found to hydrolyze most organophosphorus pesticides to orthophosphate on silica gel layers, indicates the utility of this reagent. In practice we have found the reagent to have the following disadvantages.

(a) The breakdown with hydriodic acid is apparently a stepwise process and some of the detectable breakdown products are not necessarily the ultimate products of the reaction. Thus aromatic amines derived, for example, from carbamates such as propham or barban, and detectable with the chromogenic reagents described, will



further decompose to yield products which are not detectable. An optimum heating time at 180° of about 20–30 min was found for such compounds with additional heating leading to a reduction in sensitivity. Carbamates such as butacarb or di-allate

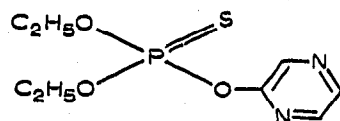


which decompose to aliphatic amines were not subject to such critical sensitivity–time relationships and heating times > 30 min led to enhanced sensitivity.

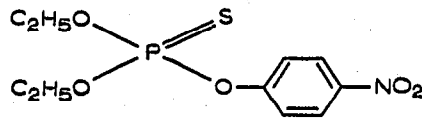
(b) During the heating process iodine fumes are evolved which can react with co-extractives present on the thin-layer plate to give dark areas obscuring any subsequent colour reaction. Where such interference is encountered a suitable clean-up procedure is essential. In our experience river water extracts rarely require clean-up.

Subject to the limitations described we have found the technique useful and reproducible for the thin-layer detection of small quantities of the compounds listed. The nature of the breakdown products produced *i.e.* whether aliphatic or aromatic

amine, the ninhydrin colour (which is frequently characteristic), together with the  $R_F$  data all assist in the characterisation of the parent compound. It is possible to distinguish organophosphorus pesticides from carbamates and ureas by use of the phosphate—specific spray described elsewhere<sup>6</sup>. In the interpretation of the thin-layer data caution is necessary as amine formation may not be due merely to simple hydrolysis but may involve ring cleavage or the reduction of nitro groups as is the case with thionazin and parathion respectively.



Thionazin



Parathion

A particularly useful feature of the spray described is the high sensitivity to the compounds dimefox and schradan, organophosphorus pesticides which are frequently difficult to detect by other procedures.

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