

CHROM. 4628

A SPRAY METHOD FOR THE FLUORESCENT DETECTION OF SULPHUR-CONTAINING ORGANIC COMPOUNDS*

P. E. BELLIVEAU, V. MALLET AND R. W. FREI**

Department of Chemistry, Dalhousie University, Halifax, Nova Scotia (Canada)

(Received January 2nd, 1970)

SUMMARY

The *in situ* fluorescent analysis of a group of sulphur-containing pesticides is discussed. A wide choice of spray reagents is given and the uses of this method for many other systems, including selenium-containing compounds, is suggested. Visual detection limits ranging from 0.1 to 0.3 μg per spot are reported for the majority of pesticides investigated, and the technique appears suitable for quantitative work.

INTRODUCTION

The use of fluorometry for the *in situ* detection and determination of compounds on thin-layer chromatograms is relatively new. SAWICKI *et al.*¹ have done a considerable amount of work in this area in connection with air pollutants; *e.g.*, polynuclear hydrocarbons, which exhibit native fluorescence. For nonfluorescing compounds it is possible to make fluorescent derivatives which are then separated, or to spray with a suitable reagent directly on the chromatogram. A prominent example of the first approach is the analysis of amino acids as dansyl derivatives², which has also led to the development of a useful quantitative method. Spraying procedures for rendering certain inorganic or organic compounds fluorescent have been discussed in several papers, particularly in connection with clinical analysis³.

The application of *in situ* fluorescent detection in the field of pesticide analysis is uncommon. BALLSCHMITTER AND TÖLG⁴ suggested a number of fluorescence indicators in connection with chromatography of organohalogen compounds, which may also have some use in pesticide work. ABBOTT *et al.*⁵ have reported on the use of sprays for the detection of carbamates and related compounds on thin-layer chromatograms. Some of the spots were fluorescent and a semi-quantitative estimation was carried out by measuring the spot areas. RAGAB⁶ has recently published a method for the direct fluorescent detection of organothiophosphorous pesticides, using bromination and spraying separately with ferric chloride, followed by a chelating reagent solution.

* Presented at the Pittsburgh Conference on Analytical Chemistry, Cleveland, Ohio, U.S.A., March, 1970.

** To whom correspondence is to be addressed.

Similar procedures were suggested earlier in paper chromatography, *e.g.* by GORDON *et al.*⁷ for phosphate esters. No work in the pesticide field has yet been published on the quantitative measurement of the fluorescence directly on chromatograms with fluorometers or spectrofluorometers.

In this study a method which enables the detection of a large number of pesticides on thin-layer chromatograms by rendering the spots fluorescent is suggested. Compared to the method proposed by RAGAB⁶, this one is simpler in the sense that it can easily be standardised for quantitative analysis. It has also improved sensitivity and is useful for a wider range of compounds than suggested by RAGAB.

The bromination step is no deterrent to the development of a quantitative method since similar procedures using gas treatment of the plates have been developed successfully before⁸.

EXPERIMENTAL

Reagents

The metal solutions were prepared from reagent-grade metal chlorides ($\text{FeCl}_3 \cdot 6\text{H}_2\text{O}$, ZnCl_2 , $\text{MnCl}_2 \cdot 4\text{H}_2\text{O}$, $\text{NiCl}_2 \cdot 6\text{H}_2\text{O}$, $\text{CuCl}_2 \cdot 2\text{H}_2\text{O}$, $\text{MgCl}_2 \cdot 6\text{H}_2\text{O}$) by dissolving 0.1 g of the salt in 1 ml of 1 *N* HCl and diluting to 100 ml with 80 % ethanol. Solutions (0.05 %, w/v) of the chelating agents were made of the reagents: pyridine-2-aldehyde-2-pyridylhydrazone (PAPH), pyridine-2-aldehyde-2-quinolyhydrazone (PAQH), quinoline-2-aldehyde-2-quinolyhydrazone (QAQH), flavonol, and 8-hydroxyquinoline, and 0.005 % (w/v) of morin in 2-butanone. PAPH, PAQH, and QAQH were prepared in our laboratories as described previously⁹. The morin (Fluka A.G., Buchs SG, Switzerland) and the 8-hydroxyquinoline (Distillation Products Inc. (Eastman Kodak Co.), Rochester, N.Y.) were commercial samples. The tests were carried out on silica gel layers. Substances were spotted with a 10- μl Hamilton microsyringe.

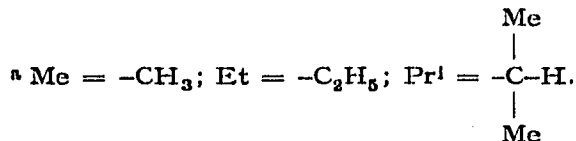
For the bromination procedure, a 10 % solution of bromine in carbon tetrachloride was used in a chromatographic tank and the chromatoplates were exposed to the bromine vapours for periods of time ranging from 30 sec to several minutes (see Table I). After bromination the plates were allowed to remain in air for at least 2 min for the elimination of excess bromine. Prior to spraying, the solutions containing the metal ions and chelating agents were combined in certain ratios (see Table III). For example, in the case of morin and iron(III) chloride a 1:1 ratio is recommended for optimum performance. The spraying was done with compressed air followed by drying of the plates in a stream of warm air for 2 min. The plates were then observed under an UV lamp at long wavelength.

RESULTS AND DISCUSSION

A group of pesticides and herbicides was tested with this procedure, using a morin- FeCl_3 mixture. Table I shows some of the compounds with positive reaction and their visual detection limits on silica gel layers. Other sulphur-containing compounds which reacted only under special conditions or not at all are shown in Table II. From these data it is obvious that contrary to previous reports⁶ this reaction is not specific for organothiophosphorous pesticides, since it is also applicable to sulphur-containing triazines and other phosphorus-free pesticides. No reaction is observed with sul-

TABLE I
PESTICIDES WITH POSITIVE REACTION

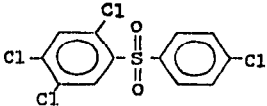
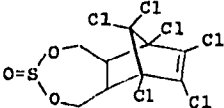
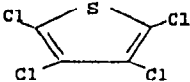
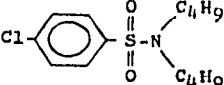
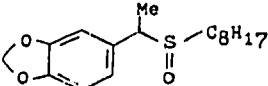
Pesticide (trade name)	Structure ^a	Time of exposure to bromine	Limit of detection (μg)	Colour of fluorescence
Parathion		30 sec	0.2	greenish yellow
Proban		30 sec	0.2	greenish yellow
Metron		30 sec	0.3	greenish yellow
Baytex		30 sec	0.3	greenish yellow
Imidan		90 sec	0.3	greenish yellow
Mesurool		3 min	0.3	bluish white
Prometryn		3 min	0.3	blue
Simetryn		5 min	0.3	blue
Ametryn		15 min	0.3	blue



phoxides (Table II). This would indicate that the oxidation state of the sulphur is critical. From visual observation it appears that the reaction already fails at the bromination step, since with successful bromination the formation of a brown spot or ring is noted. The reaction is positive with sulphide ions or elemental sulphur, but

TABLE II

PESTICIDES REACTING UNDER SPECIAL CONDITIONS OR NOT AT ALL

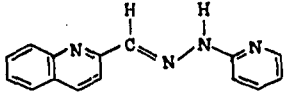
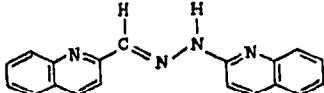
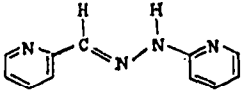

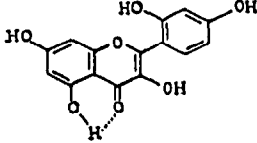
<i>Pesticide (trade name)</i>	<i>Structure</i>	<i>Observation</i>
Tedion		No reaction
Thiodan		Faint spot at 10 μg, but could be due to impurities
Penthene		Reaction occurs if NaOH is added
Warf		No reaction
Sulphoxide		Red-coloured spot is observed

doubtful in the case of SO_3^{2-} and negative for SO_4^{2-} . If the sulphur is bonded to phosphorus in the form ($\rightarrow\text{P}=\text{S}$), the bromination step appears to be completed in a few seconds; with sulphur-containing triazines on the other hand, several minutes of bromination are required (Table I). Sulphur-free triazines such as simeton and simazine, which have also been investigated, do not react at all.

We can conclude that the method is particularly suitable for organothiophosphorous compounds, but not specific. It is not even specific for sulphur, since a positive result was also obtained with a compound containing selenium. A typical example is N,N-dimethylselenourea, $(\text{CH}_3)_2\text{N}-(\text{C}=\text{Se})-\text{NH}_2$, for which a positive reaction was observed.

In the second phase of this investigation one pesticide, parathion, was chosen to study variation possibilities with the metal and chelate solutions. Table III shows some of the possible chelating agent to metal combinations with optimum ratios for the spray mixtures. Detection limits for parathion on silica gel under these various conditions have also been given. The volume ratios of the solutions are critical, since otherwise a fluorescent background is obtained and the sensitivity drops drastically. Little can be said at this time about the possible reaction mechanism in this detection method, but it seems that the metal ion must form a complex with the chelating agent that is nonfluorescent; otherwise a fluorescing background is obtained on the plate. It also is apparent that the optimum chelating agent to metal ratio, even though it is ob-

TABLE III
VARIATION OF CHELATING AGENT *vs.* METAL ION

Chelating agent	Structure	Metal ion	Ratio	Detection limit (μg)
PAQH		Mn ²⁺ Fe ³⁺ Cu ²⁺	1:2 1:5 5:1	0.1 0.1 0.1
QAQH		Mn ²⁺ Fe ³⁺ Cu ²⁺	1:4 1:7 5:1	0.1 0.1 0.1
PAPH		Mn ²⁺	1:6	0.1
8-Hydroxyquinoline		Mn ²⁺ Fe ³⁺ Mg ²⁺	1:2 1:3 1:4	0.3 0.3 0.3
Morin		Mn ²⁺ Fe ³⁺ Ni ²⁺ Zn ²⁺ Mg ²⁺	1:1 or 1:2 1:1 1:2 1:2 1:4	0.2 0.2 0.2 0.2 0.2

tained experimentally, is related to the stability of the corresponding metal chelate. For example, with the chelates of the substituted hydrazones PAQH and QAQH the order of complex stabilities is $\text{Cu}^{2+} > \text{Mn}^{2+} > \text{Fe}^{3+}$ (Table III). In both cases the ratios are shifting from a high to a low concentration of chelating agent in the same order, reflecting the different affinities of the metals for the chelating agent.

The dependence of ratios on chelate stability was further demonstrated when PAPH, PAQH and QAQH were used in conjunction with the Mn²⁺ ion. With a ratio of chelating agent to metal solution kept constant at 1:2, PAQH showed the best sensitivity, followed by QAQH and then by PAPH. This again corresponds to the known stability constants of the Mn²⁺ chelates¹⁰ which show that PAQH forms the most and PAPH the least stable complex.

CONCLUSIONS

It is obvious that the detection method discussed in this paper is not restricted to the compounds in Table I nor to the chelating agents and metal ions in Table III. Once the mechanism of the reaction is better understood (this is currently under investigation), optimum conditions can be chosen which will further improve the sensitivity, background, and stability of the fluorescent spots. Under the conditions

used the spots remain stable for a few hours. The applicability of this method to many sulphur-containing organic compounds, particularly of the P-S bond type, makes this technique valuable in pesticide work, but other groups of compounds such as pharmaceuticals or selenium and sulphur-containing air and water pollutants could be investigated to advantage. Preliminary experiments indicate that this method is useful for quantitative or semi-quantitative *in situ* fluorescence analysis of pesticides. *In situ* fluorescence spectroscopic techniques could be as sensitive and reliable as gas chromatography⁸. They are particularly useful for compounds that cannot be analyzed by gas chromatography because of their low volatility. In addition they provide a useful alternate approach to check GLC data.

ACKNOWLEDGEMENTS

This work was supported by grants from the National Research Council, the Canada Department of Agriculture, and the Canada Department of Energy, Mines and Resources. We thank Dr. O. HUTZINGER for his interest and helpful discussions.

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