CHROM. 5188

AN INVESTIGATION OF FLAVONES AS FLUOROGENIC SPRAY REAGENTS FOR ORGANIC COMPOUNDS ON A CELLULOSE MATRIX*

PART II. DETECTION OF PESTICIDES

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(First received September 16th, 1970; revised manuscript received December 1st, 1970)

SUMMARY

Several classes of pesticides such as carbamates, *s*-triazines, organophosphates and chlorinated hydrocarbons have been tested. Yellow fluorescent spots were observed on cellulose layers sprayed with fisetin. The visual detection limits obtained for these compounds with this new fluorogenic method range between 0.01 to 0.1 μg. The method was also extended to herbicides and fungicides of a variety of chemical structures and conclusions were drawn as to the type of fluorescence phenomenon observed.

Some functional groups such as nitro and possibly amino, and molecules with a quinoid type of structure were found to quench the fluorescence of the spray reagent; while others, such as carboxylic, cyano and methoxy groups, do not.

INTRODUCTION

A new method for the determination of organic compounds and pesticides in particular by *in situ* fluorescence was described in a preceding paper[1]. Baygon was utilized as the test substance in that study. The pesticide was spotted on cellulose layers and sprayed with a 0.05 % solution of a flavone in isopropanol producing bright yellow fluorescent spots. The flavones utilized were flavonol, fisetin and robinetin, all 3-hydroxy derivatives with the 5-position not substituted.

In this study, it was intended to assess the usefulness of this method to a wide range of pesticide systems such as *s*-triazines, organophosphates, carbamates, chlorinated hydrocarbons and others, including compounds with herbicidal and fungicidal properties.

The object of the screening program was to observe the behavior of these compounds with varying structural features and functional groups when sprayed with the particular flavone, in order to learn more about the applications and limitations of this new fluorogenic technique.

* Presented in part at the CIC-ACS Joint Chemical Conference in Toronto, May 1970.

EXPERIMENTAL

For a more detailed description, see the preceding paper[1].

GENERAL PROCEDURE

All the pesticide solutions prepared were 1000 mg/l in methylene chloride. Dilution series (1 to 0.01 μg/l) were prepared for the carbamates, organophosphates, *s*-triazines, and chlorinated hydrocarbons, and spotted with a Hamilton microsyringe whereby a spot diameter of approx. 2 mm was obtained. The plates were sprayed with a 0.05 % solution of fisetin in isopropanol and the resulting fluorescence observed under long wavelength UV light.

The other pesticides, *i.e.* those listed under fungicides, herbicides and miscellaneous compounds were assessed by comparing visually the fluorescence given by 1 μl of solution (1 μg) with that given by the same concentration of Landrin (detection limit, 0.01 μg/spot) and *p,p'*-DDT (detection limit, 0.1 μg/spot).

The carbamates and some organophosphates, *i.e.*, Proban and Imidan were recrystallized prior to use.

RESULTS AND DISCUSSION

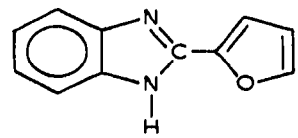
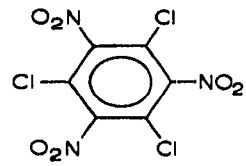
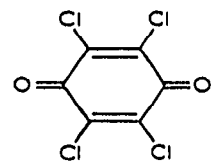
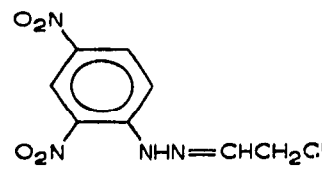
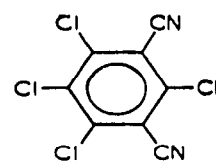
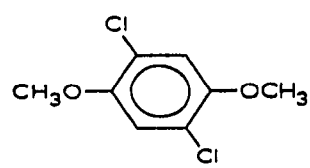
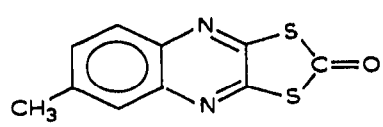
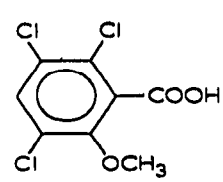
In the preceding paper[1] a particular flavone such as fisetin was sprayed on a cellulose matrix spotted with the carbamate Baygon. The pesticide was believed to increase the polarity of the medium and since flavones are more fluorescent in a more polar medium, fluorescence enhancement of the flavone in the spot area was said to occur. Therefore in the absence of interferences such as chemical reactions, quenching effects, etc., a wide range of compounds could be expected to produce this fluorescence enhancement effect as long as they are sufficiently polar to increase the polarity of the medium.

The method is applicable to carbamates other than Baygon. Barban, Bux, CIPC, IPC, Landrin, Matacil, Mesurol, Pirimicarb, Sevin, Swep and Zectran were tested and detection limits of 0.06–0.01 μg were found. For Matacil and Zectran a brown fluorescence was observed instead of the usual bright yellow, although there was no noticeable change in the fluorescence spectra. Both have a N,N-dimethylamino group which is not present in the other carbamates tested. A number of organophosphorus insecticides (Dyfonate, Ethion, Malathion, Methyl trithion, Proban) were investigated. The visual detection limits (0.04–0.02 μg) were comparable to those obtained for the carbamates. However, Parathion and Imidan quench the fluorescence. This may be attributed to Parathion having a nitro group and Imidan an enedione type of structure, both of which are known to quench fluorescence because of their tendency to reabsorb the energy emitted in the fluorescence process[2].

Fluorescence enhancement was also observed with *s*-triazines. The following compounds were investigated: Ametryne, Atrazine, Dyrene, Prometryne, Prometone, Propazine, Simazine, and Trietazine. The thiomethoxy triazines gave a brownish-yellow fluorescence but not as dark as that given by Zectran or Matacil mentioned earlier. That given by the methoxy triazines was slightly brownish while the chloro triazines showed the usual yellow fluorescence. Visual detection limits (0.04–0.1 μg) were somewhat lower than for carbamates and organophosphorous pesticides.

TABLE I

FUNGICIDES

Pesticide	*Structure*	*Observation*[a]
Bay 33172		V.S.Q.
Bulbosan		S.Q.
Chloranil		V.S.Q.
Chloroacetaldehyde-2,4-dinitrophenylhydrazone		V.S.Q.
Daconil 2787		(—)
Demosan		(—)
Morestan[b]		Q
Tricamba		(+)

[a] V.S.Q. = very strong quenching; S.Q. = strong quenching; Q = quenching; (—) = no fluorescence at the 1.0 μg level; (+) = slight fluorescence at the 1.0 μg level.

[b] Fluorescent in the original state.

The method is also suitable for chlorinated hydrocarbons but with reduced sensitivities (~ 0.1 μg) due to their lower polarities. Yellow fluorescence was observed in all cases. Some of the compounds tested were: Heptachlor, Dieldrin, Endrin, Lindane and isomers, DDT, DDE, DDD, and also a mixture of polychlorinated biphenyls.

Some common fungicides have been listed in Table I. It can be observed that compounds having nitro groups, such as Bulbosan and chloroacetaldehyde-2,4-dinitrophenylhydrazone, quench the fluorescence. The same observation was made for chloranil which possesses a quinoid type of structure. Bay 33172 also shows some very strong quenching properties but there are no apparent reasons for that.

Daconil 2787 and Demosan do not show any fluorescence or quenching at that concentration level (1 μg per spot). An inspection of the structure of Demosan reveals that this compound would have a low polarity and consequently would be expected to show only little fluorescence.

Morestan, which is also used as insecticide and miticide, is highly fluorescent in the native state but when sprayed with the flavone, light quenching results. Structurally this compound has a carbonyl group in direct conjugation with two sulfur atoms capable of electron delocalization (see structure I) and as was observed earlier for Imidan, the aromatic ketone type of structure quenches the fluorescence.

(I)

Tricamba, a benzoic acid derivative, shows some fluorescence at the 1.0 μg level. Thus the –COOH and $-OCH_3$ groups do not seem to have any appreciable quenching effects.

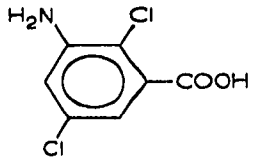
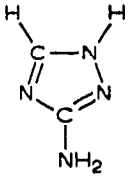
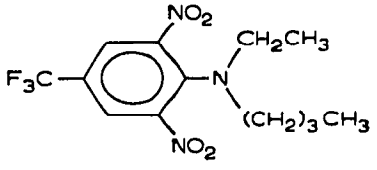
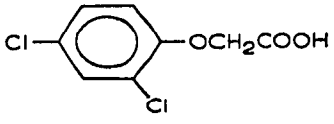
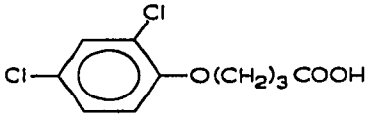
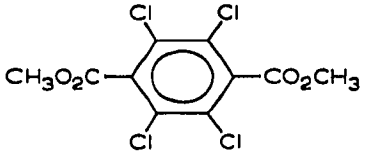
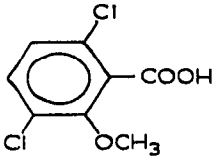
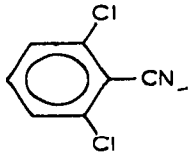
Testing was carried out with a series of common herbicides, other than triazines, of varying structural composition (Table II). Amiben is also a benzoic acid derivative but contrary to Tricamba it contains an amino group and it is slightly quenched. Other benzoic acid derivatives studied are Dicamba and TCB. Both of them are slightly fluorescent. Amitrole shows initially a slight fluorescence but after a short time the spot exhibits quenching. Here again we have the presence of an amino group in the molecule.

A series of phenoxy type herbicides, 2,4-D, 2,4-DB, MCPA, Silvex, and 2,4,5-T, was included in the study. All of them are fluorescent at the 1.0 μg level. This supports the previous assumption that the –COOH group does not have any fluorescence quenching effects toward the spray. Other herbicides containing nitro groups such as Benefin, DNBP, and Trifluralin were also studied. As expected all of them quench the fluorescence.

Dichlobenil and Diphenatrile, which have a cyano group in common, fluoresce quite well at the 1.0 μg level. Ioxynil is highly fluorescent hence suggesting that substituted iodines probably enhance the fluorescence. On the other hand a similar compound, Bromoxynil (not shown in the table), which contains bromine instead of iodine was found to be barely fluorescent.

TABLE II

HERBICIDES

Pesticide	*Structure*	*Observation*[a]
Amiben		Q
Amitrole		(+) → Q
Benefin		V.S.Q.
2,4-D		(+)
2,4-DB		(+ +)
DCPA		(+)
Dicamba		(+)
Dichlobenil		(+)

(*Continued on p. 74*)

TABLE II (*continued*)

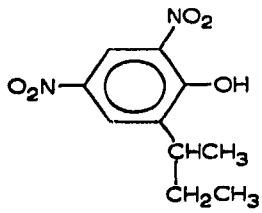
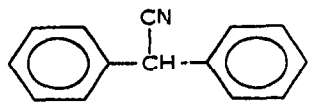
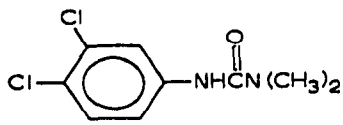
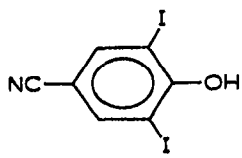
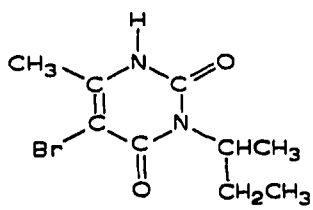
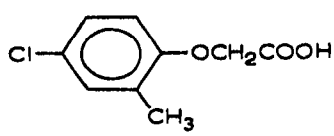
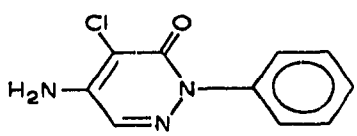
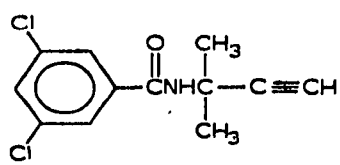
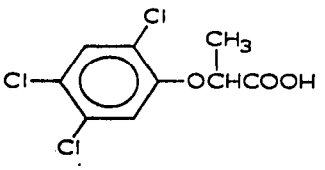
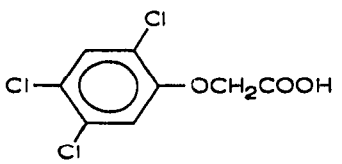
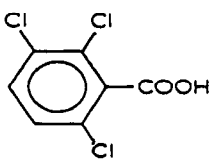
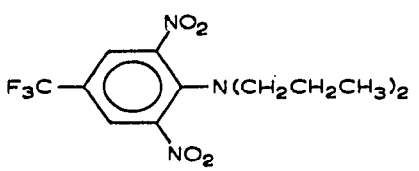
Pesticide	*Structure*	*Observation*[a]
DNBP		S.Q.
Diphenatrile		(+)
Diuron		(+++)
Ioxynil		(+++)
Isocil		(+++)
MCPA		(+)
Pyrazon[b]		(+)
RH-315		(+++)

TABLE II (*continued*)

Pesticide	*Structure*	*Observation*[a]
Silvex		(+)
2,4,5-T		(+)
TCB		(+)
Trifluralin		V.S.Q.

[a] (++) = as fluorescent as *p,p'*-DDT at the 1.0 μg level; (+++) = as fluorescent as Landrin at the 1.0 μg level.
[b] Fluorescent in the original state.

Very strong fluorescence is observed at the 1.0 μg level for Diuron, a phenyl urea, and for RH-315, which is an amide. This behavior was expected because of their similarity with carbamates. Pyrazon which is highly fluorescent in the native state does not yield very stable fluorescence after spraying.

Table III presents a few additional compounds. Quenching is observed for both Binapacryl and Menazon. In the former it can be attributed to the presence of nitro groups and in the latter to amino groups in the aromatic nucleus.

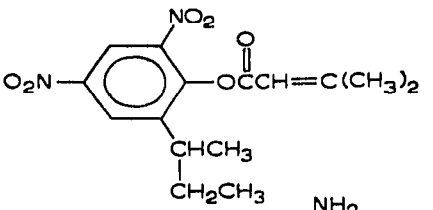
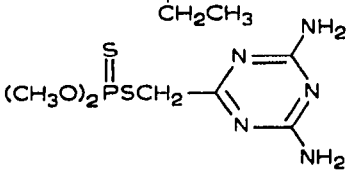
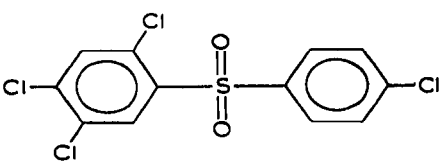
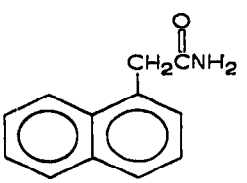
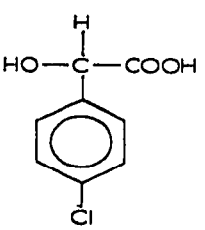
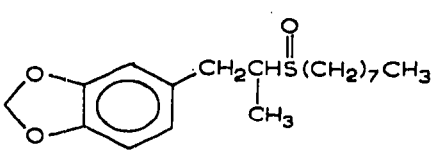
To investigate the feasibility of this detection method after actual chromatographic separation, one microgram each of Landrin, Ametryne, and Proban were spotted on a cellulose plate treated with mineral oil, and eluted in 50 % acetone, as in the previous study[1]. All three spots were easily discernable when the plate was sprayed with the fisetin solution and no interferences from the developing solvents were observed.

CONCLUSIONS

Even though this study is by no means exhaustive, it has been demonstrated that fluorescence enhancement of the flavone can be applied to the detection of a

TABLE III

MISCELLANEOUS COMPOUNDS

Pesticide[a]	*Structure*	*Observation*[b]
Binapacryl (M)		V.S.Q.
Menazon (M)		Q
Tetradifon (M)		(+++)
1-Naphthalene acetamide (PGR)		(+++)
p-Chloromandelic acid (PGR)		(+)
Sulfoxide (Sinergist)		(++++)

[a] M = miticide; PGR = plant growth regulator.
[b] (++++) = more fluorescent than Landrin at the 1.0 μg level.

large variety of pesticides or other organic compounds, for that matter. The fact that some pesticides quench the fluorescence is no detriment since it only renders the method more selective. Certain functional groups such as nitro, and aromatic compounds possessing a quinoid type of structure, including aromatic ketones, diketones, and amines, are shown to be responsible for the fluorescence quenching.

Benzoic acids, phenoxy type compounds, amides, phenylureas, and nitrile de-

rivatives, on the other hand, do not exhibit quenching properties. On the contrary, they seem to enhance the fluorescence.

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.

REFERENCES

1 V. MALLET AND R. W. FREI, *J. Chromatogr.*, 54 (1971) 251.
2 D. M. HERCULES, *Fluorescence and Phosphorescence Analysis*, Interscience Publishers, 1966.