THE APPLICATION OF THE IODINE – AZIDE REACTION IN THIN-LAYER CHROMATOGRAPHY STUDIES OF PESTICIDE PREPARATIONS

By

T. Cserháti* and F. Őrsi

Department of Biochemistry and Food Chemistry, Technical University Budapest

Received October 3, 1981

Presented by Prof. Dr. R. Lásztity

Introduction

With chemicals being used in wider and wider areas for plant protection, toxicological and ecological aspects become increasingly important. The formulation analysis of pesticides cannot be limited nowadays to the selective determination of the active agent: the identification and quantitative determination of impurities is becoming equally significant. The process is of particular importance with phosphoric ester derivatives, since they are more ore less toxic to human beings. Gas chromatographic analysis (GC) of these active agents has been developed many years ago [1]. However, the technique could not supplant thin-layer chromatography (TLC) [2] for the following reasons:

a) thermally unstable phosphoric ester derivatives cannot be analyzed by GC;

b) if flame ionization detectors are used, one cannot state whether the peaks indicating impurities correspond to presumably toxic side products of the active agents, or to other, non-toxic components of the formulation;

c) a substantial part of the decomposition products formed in hydrolysis or oxidation processes are non-volatile. Consequently, GC methods can only be used when combined with additional processes to yield volatile products;

d) the TLC technique, using non-destructive, selective detection processes, allows to isolate, in a much simpler manner as compared to traditional GC, volatile and non-volatile organic phosphoric ester derivatives in amounts in the order of milligrams for further studies of structure identification and toxic properties.

It is well known that P=S and C=S bonds have a catalytic effect on the reaction taking place between iodine and sodium azide [3]. This phenomenon has long been in use for the detection by TLC of active agents of pesticides containing the above bonds [5, 6]. TLC determination of organic phosphoric ester derivatives are usually performed on silica gel layers [7—13], less

* Research Institute for Plant Protection

frequently on aluminium oxide [14], Florisil [15] or polyamide [16, 17] layers. It is barely understandable why so little attention was pair to reverse-phase TLC separation, since R_f values obtained by this technique characterize the lipophilic nature of the compound and are hence of great significance in optimum molecular design [18]. The objective of our study was to find the range of applicability of the iodine — azide reaction in TLC analysis of phosphoric ester-based commercial pesticides, including the determination of both the active agent and the impurities.

Experimental

Materials and apparatus

The constitutional formulae of the phosphoric ester derivatives studied are presented in Fig. 1.

For adsorption TLC, plates 20×20 cm, thickness 0.25 mm were from Kieselgel G (manufacturer: REANAL) prepared. For reverse-phase TLC, the plates were prepared by impregnation with a solution of paraffine oil in *n*-hexane (dimensions of the plates were similar to the above).

A Telechrom Video-densitometer (manufacturer: CHINOIN) was utilized for quantitative determination.

Methods

Stock solutions of the pesticides containing the compounds listed in Fig. 1 in acetone were prepared, with concentrations of 2 mg active agent per cm^3 acetone. In adsorption TLC the solvent used was dichloromethane; in reverse-phase TLC the solvent was a 1:1 or 3:1 mixture of methanol and water. The active agents were applied in spots, in amounts of 2. . .40 μg. The plates were then dried in air, sprayed with a freshly prepared 1:1 mixture of 1 mol/l sodium azide and 1 mol/l iodins solutions, and subsequently, when — due to the catalytic effect of the P=S bond — the spoots became visible as white spots in the yellow background, the plates were sprayed with a 1% aqueous starch solution. The area of the white spots in the violet background was then measured instrumentally, and the equation best describing the relationship area *vs.* amount of active agent was selected. Since it is known that for homologue sequences of related compounds a relationship exists between their adsorptivity and lipophility, we attempted to calculate linear correlations between R_f values measured in adsorption and reverse-phase TLC.

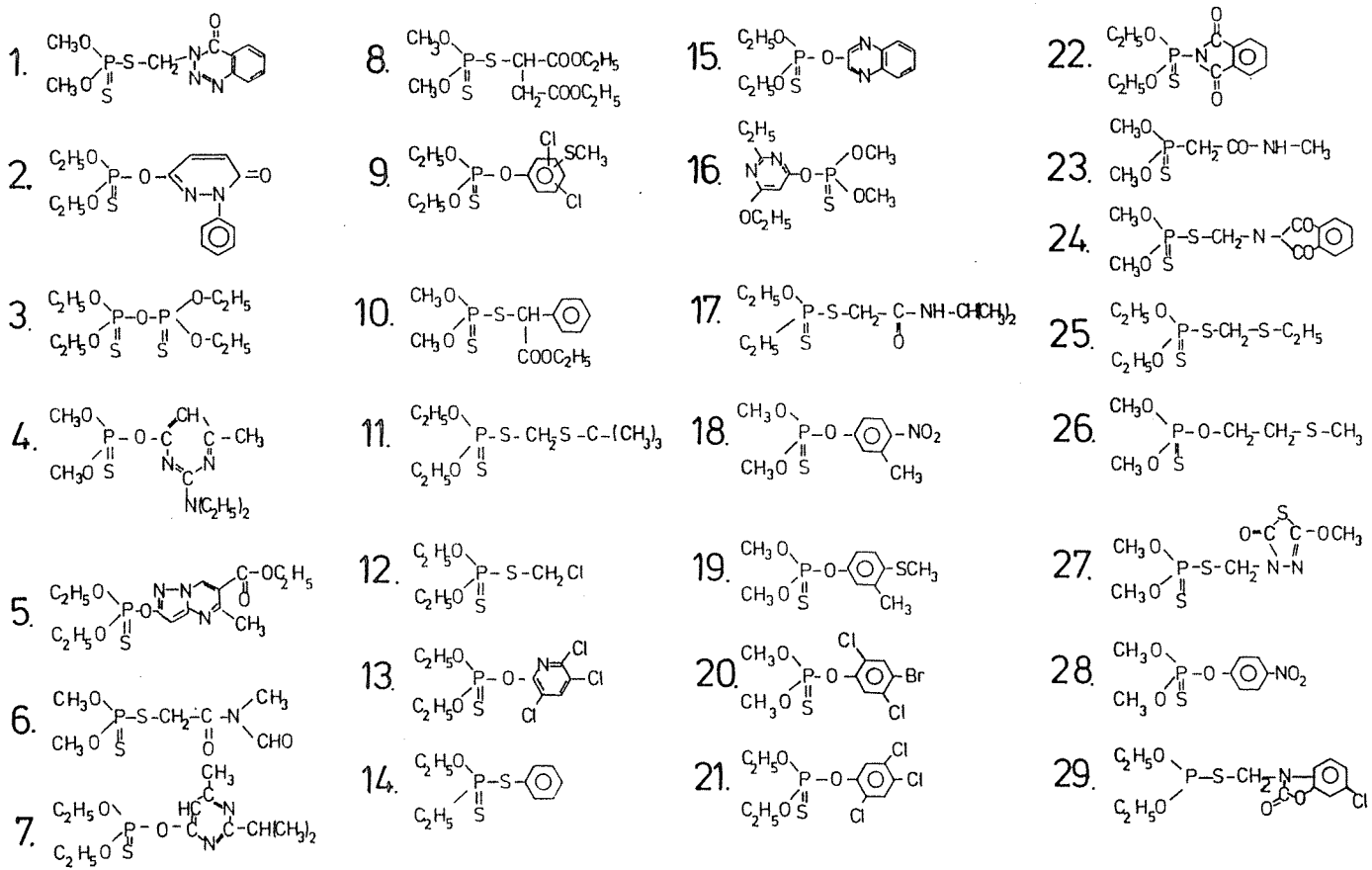

Fig. 1. The constitutional formulas of the thionphosphoric ester derivatives studied

Results and discussion

The R_f values of the phosphoric ester derivatives studied are presented in Table 1.

Table 1

R_f values thionphosphoric esters

No. of compound	I Silica gel, dichloromethane	II Reverse-phase, methanol : water 1 : 1	III Reverse-phase, methanol : water 3 : 1
1	0.59	0.34	
2	0.22	0.19	
3	0.92	0.15	
4	0.78	0.12	
5	0.48	0.07	
6	0.08	0.88	
7	0.49	0.13	
8	0.55	0.22	
9	0.07	0.03	
10	0.72	0.13	
11	0.87	0.05	
12	0.87		0.39
13	0.97		0.20
14	0.98		0.49
15	0.95	0.14	0.66
16	0.60	0.16	0.64
17	0.62	0.36	0.80
18	0.89	0.10	0.66
19	0.90		0.64
20	0.07		0.42
21	0.07		0.23
22	0.71		0.76
23	0.08		0.84
24	0.68	0.95	0.92
25	0.92	0.09	0.57
26	0.93		0.45
27	0.61		0.86
28	0.78		0.73
29	0.72		0.37

The following observations were made in the performation of the iodine—azide reaction:

The compounds containing several higly electronaffine substituents (compounds 9, 13, 20 and 21) do not catalyze the reaction in our experimental conditions. By heating the plates, the spots can be made visible; however, the background also becomes much paler. Thus the relative error of instrumental measurement will increase, so that these compounds cannot reliably be determined quantitatively by the iodine—azide reaction.

On the other hand, compounds with groups that will readily adsorb iodine (compounds 1, 2, 5, 7, 15 and 16), after spraying with the iodine—azide solution, will develop brown spots instead of white spots in the yellow background. However, in contrast to the compounds of the previous group, this difficulty can be surmounted by spraying the plates after to 100 °C, since at higher temperatures the extent of adsorption will decrease, while the rate of the catalytic reaction will increase.

The developed plates prepared for calibration purposes to compounds 8 and 24 are presented in Fig. 2.

The visual detection limit of the compounds varies largely from compound to compound, owing to the differences in the substituents (0.5. . .10 μg). Since the sensitivity of the Telechrom Videodensitometer is lower than that of the human eye, and the reproducibility of measurement decreases dramatically below 10^4 digital signals (Fig. 3), it is expedient to work in the substance range of 10 to 40 μg for quantitative determinations.

We attempted to approach the visibly non-linear relationship spot area *vs.* amount of substance (Fig. 4) with logarithmic and log-log functions. The relationships calculated for compound 8 are as follows (y = amount of sub-

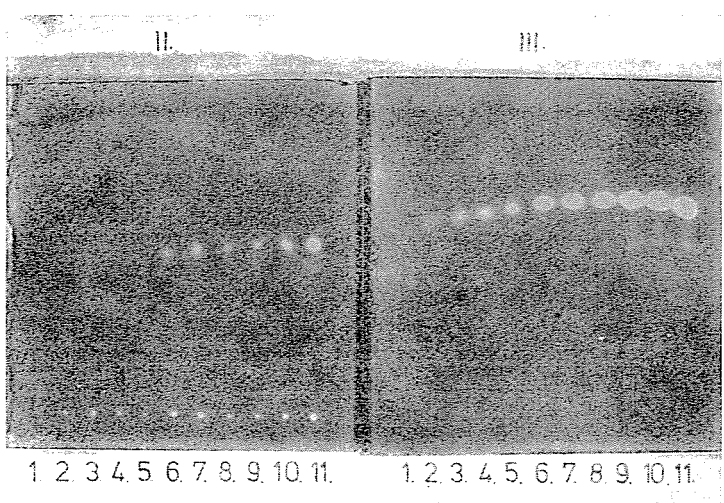

Fig. 2. Determination of thionphosphoric esters. Adsorption TLC, solvent CH_2Cl_2. Compound 8 on plate II, compound 24 on plate III. In both cases: 1—2 μg; 2—6 μg; 3, 4, 5—10 μg 6, 7, 8—20 μg; 9—30 μg; 10—40 μg; 11—50 μg

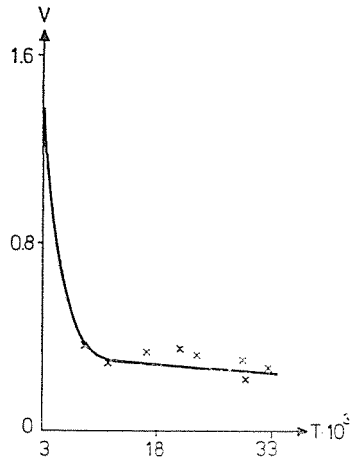

Fig. 3. Dependence of the reproducibility of videodensitometric area measurements (V) on area size (T)

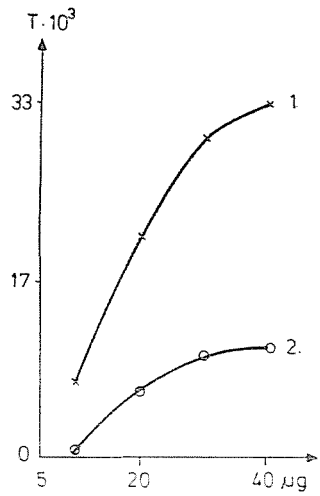

Fig. 4. Relationship between the amount of substance applied (μg) and spot area (T). Curve 1 — compound 8; curve 2 — compound 24

stance applied, μg, x = digital value of area, $n = 8$, $r_{99\%} = 0.8343$, $r_{99.9\%} = 0.9249$):

$$\mu g = -71.40 + 21.34 \log x \qquad r = 0.5751$$
$$\log \mu g = -1.20 + 0.59 \log x \qquad r = 0.8815$$
$$\log \mu g = 0{,}86 + 2.10 \cdot 10^{-5} x \qquad r = 0{,}9697$$

The correlation between the logarithm of amount of substance applied and the area of the spot being linear at a reliability level of 99.9%, the above

calibration curve allows a quantitative determination of the substances with satisfactory accuracy.

The equations describing the relationship lipophility *vs.* adsorptivity (the Roman numerals indicate the solvent systems in Table I)

$$R_{fI} = 0.69 - 0.30 \cdot R_{fII} \qquad n = 17 \qquad r = 0.2899$$
$$R_{fI} = 0.71 - 0.03 \cdot R_{fIII} \qquad n = 18 \qquad r = 0.0242$$

indicate that there is no correlation between adsorptivity and lipophility in the group of compounds studied.

The utilization of the method to detect impurities will be demonstrated by the following two examples. The separation of compounds 12 and 23, resp. and their decomposition products is presented in Figs 5 and Fig. 6. (In the experiments aiming to separate impurities, the solvents were changed as compared to the other runs.)

It may be seen from the figures that the method is eminently suitable for the separation and detection of decomposition products containing the P=S bond, even at semi-preparative amounts of the substance.

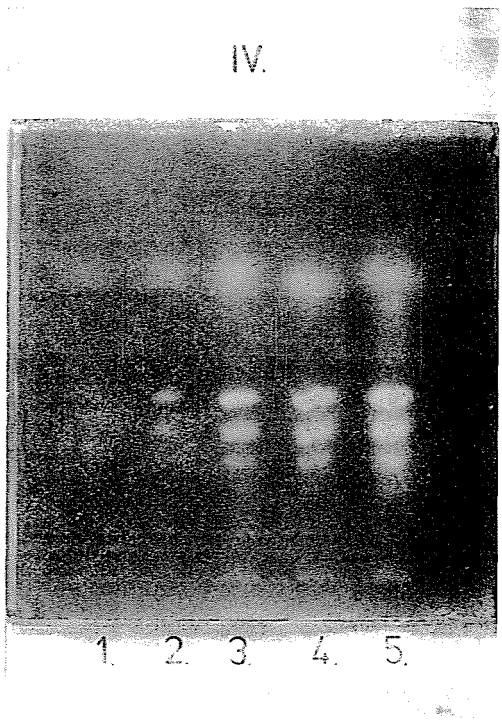

Fig. 5. Separation of compound 12 and its impurities. Reverse-phase TLC, methanol: water 4:1. 1 — 10 μg; 2—20 μg; 3—40 μg; 4—60 μg; 5—80 μg

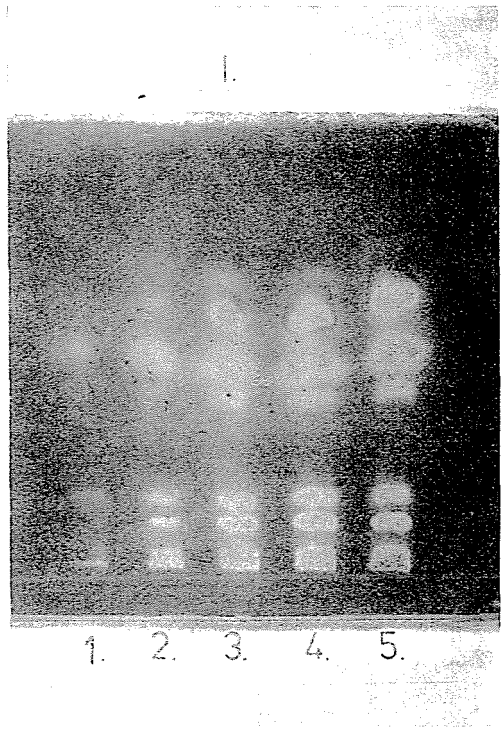

Fig. 6. Separation of compound 23 and its impurities. Adsorption TLC, benzene: acetone 9:1. 1—50 μg; 2—100 μg; 3—200 μg; 4—300 μg; 5—400 μg

Summary

The analytical applicability of the iodine-azide reaction catalyzed by the P=S bond was studied in thin-layer chromatographic detection and quantitative determination of thionphosphoric esters contained in 29 commercial pesticides. It was found that in the case of highly electronaffine substituents the sensitivity of the reaction is very low and hence it is unsuitable for quantitative determination. With substituents readily adsorbing iodine, it is expedient to carry out the reaction at elevated temperatures. The reaction was found to be suited for non-destructive quantitative determination of thionphosphoric esters and their decomposition products.

References

1. ZWEIG, G.: Analytical Methods for Pesticides and Plant Growth Regulators, Vol. VI, p. 191—231. New York—San Francisco—London, Academic Press, 1974.
2. ZWEIG, G.: Analytical Methods for Pesticides and Plant Growth Regulators, Vol. VII, p. 3—89. New York—San Francisco—London, Academic Press, 1974.
3. JACIMIRSZKIJ: A kémia analízis kinetikus módszere (The Kinetic method of Chemical analysis). Budapest, Műszaki Könyvkiadó, 1966.
4. SVÁB, J.: Biometriai módszerek a kutatásban (Biometric Methods in Research). Mezõ gazdasági Kiadó, Budapest, 1973.
5. ZWEIG, G.: Handbook of Chromatography, Vol. II, p. 151. USA, CRC Press, 1972.

6. STAHL, E.: Dünnschichtchromatographie, p. 506. Berlin—Göttingen—Heidelberg, Springer Verlag, 1962.
7. ABBOTT, D. C.—BURBRIDGE, A. S.—THOMSON, J.—WEBB, K. S.: Analyst. **92**, 171 (1967).
8. BRUKMAN, H.—WINTERLIN, W.: Bull. Environ. Contam. Toxicol. **1**, 78 (1966).
9. MENDOZA, C. E.—WALES, P. J.—BRAY, D. F.: Analyst. **93**, 689 (1968).
10. FISCHER, R.—KLINGELHOFFER, W.: Pflanzenschutz Ber. **27**, 165 (1961).
11. WALKER, K. C.—BEROZA, M.: JAOAC, **46**, 250 (1963).
12. BAUMLER, J.—RIPPSTEIN, S.: Helv. Chim. Acta, **44**, 1162 (1961).
13. GUTH, J. A.: Pflanzenschutz Ber. **35**, 138 (1967).
14. RAMASAMY, M. O.: Analyst, **94**, 1078 (1969).
15. HAMILTON, D. J.—SIMPSON, B. J.: J. Chromatogr. **39**, 186 (1969).
16. NAGASAWA, K.—YOSHIDOME, H.: J. Chromatogr. **39**, 282 (1969).
17. WANG, R. T.—CHOU, S. S.: J. Chromatogr. **42**, 416 (1969).
18. ANDREW, J.—STUPER, W. E.—BRUGGER, P.: Computer Assisted Studies of Chemical Structure and Biological Function. New York, Wiley, 1979.

Dr. Tibor CSERHÁTI Research Institute for Plant Protection
Dr. Ferenc ŐRSI H-1521 Budapest