

## AROMATIC AMINES AS SPRAY REAGENTS IN THE THIN-LAYER CHROMATOGRAPHY OF CHLORINATED ORGANIC PESTICIDES

V. M. ADAMOVIĆ

*Department of Sanitary Chemistry, Institute for Health Protection of the S.R. of Serbia, Belgrade (Yugoslavia)*

(Received December 13th, 1965)

### INTRODUCTION

Since thin-layer chromatography has been used for analyses of chlorinated pesticide residues<sup>1-4</sup>, it has been found that silver nitrate reagent, the reagent most frequently used in paper chromatography<sup>5-15</sup>, did not give satisfactory results: the background was dark, the spots with low concentrations were not sufficiently defined, etc. Other reagents such as methyl yellow<sup>16</sup>, indophenol blue<sup>17</sup>, or N,N-dimethyl-*p*-phenylenediamine hydrochloride in sodium ethoxide<sup>18</sup> were also not satisfactory for various reasons.

However, the "spot test" for amine compounds<sup>19</sup> can be modified (organochlorine pesticides being used instead of fluorescein chloride, and diphenylamine and zinc chloride as reagents) and can be used for the detection of chlorinated pesticides. LEMLEY<sup>20</sup> was one of the first to suggest the possibility of detecting DDT with zinc chloride, phenol and diphenylamine. CUETO<sup>21</sup> melted dieldrin with diphenylamine and zinc chloride at 205° and used the coloured compound so obtained for colorimetric determination of dieldrin. GRAUPNER<sup>22</sup> treated toxaphene in a similar way. MCKINLEY<sup>23</sup> described a procedure for the detection of captan by spraying the chromatogram with diphenylamine and zinc chloride in acetone and irradiating with U.V. light. BONDI<sup>24</sup> described the detection of aldrin, dieldrin, toxaphene and DDT by a "spot test" technique, but got a negative reaction for lindane. KATZ<sup>25</sup> used diphenylamine-zinc chloride and diphenylamine-iodine as spray reagents in thin-layer chromatography and after heating at 200° he obtained coloured compounds with DDT, methoxychlor, kelthane, captan and toxaphene.

All the above references are to reactions of chlorinated insecticides with diphenylamine in the presence of zinc chloride or iodine. We also used diphenylamine, but found<sup>26</sup> that DDT, lindane, aldrin, dieldrin, endrin, toxaphene, together with 2,4-D, 2,4,5-T, MCPA and MCPP, under the influence of U.V. light react with diphenylamine, even if zinc chloride or iodine are absent, to give characteristically coloured products.

Investigation of the behaviour of other aromatic amines showed that those compounds having a free or substituted amino-group react, under the above mentioned conditions, with chlorinated insecticides, giving more or less characteristically coloured reaction products.

## EXPERIMENTAL

*Reagents*

1. *Silica gel according to STAHL* ("Kemika", Zagreb). 30 g of adsorbent and 72 ml of water are shaken for 2 min in an Erlenmeyer flask with a ground stopper and then applied to glass plates by means of an applicator; the thickness of the layer is 0.25 mm. After air-drying the adsorbent is activated at a temperature of 120° for 30 min and then cooled in a desiccator over silica gel.

2. *Solvent*. Benzine fraction (b.p. 90 to 100°), purified by shaking with sulphuric acid (20 % SO<sub>3</sub>), water and a 2 % solution of sodium bicarbonate and again water. The solvent obtained in this way is dried over anhydrous sodium sulfate and distilled.

3. *Standard insecticide solutions*. 0.1 % solutions of recrystallized active material: *p,p'*-DDT, lindane, aldrin, dieldrin and endrin in ethyl acetate.

4. *Chromogenic reagents*. 0.5 % v/v or 0.1 % w/v ethanol solution of the following amine compounds freshly distilled or recrystallized: aniline; *o*-, *m*-, and *p*-toluidine; *o*-aminophenol; *p*-anisidine·HCl; *p*-nitroaniline; dimethylaniline; *p*-phenylenediamine; *p*-aminodimethylaniline; *p*-dimethylaminobenzaldehyde; benzidine; *o*-tolidine; 3,3'-diaminobenzidine; diphenylamine;  $\alpha$ -naphthylamine; N-1-naphthylethylenediamine·2 HCl; N-phenyl-1-naphthylamine.

All chromogenic reagents have to be freshly prepared before they are used.

## APPARATUS

The ultraviolet light source was an Analysen Quarzlampe, Hanau, 2536 Å.

## PROCEDURE

Known amounts of the standard insecticide solutions were spotted to the prepared plates from a 10  $\mu$ l micro-syringe, and after air-drying the plate was developed in a jar saturated with the vapor from the solvent. The solvent front was allowed to reach a previously marked line 15 cm from the origin and the plate was then removed. Development time was 40–50 min.

The plate was dried at room temperature, then sprayed with the chromogenic reagent and exposed to the U.V. light without a filter at a distance of 25 cm for 1–15 min, depending on the chromogenic reagent being used.

## RESULTS AND DISCUSSION

Before obtaining the results described in Table I we tested the amine compounds listed above under *Chromogenic reagents* under the conditions given by MCKINLEY<sup>23</sup> and KATZ<sup>25</sup>, but the results were unsatisfactory; the sensitivity was shown to be very low and some of the reactions failed completely. However, when we used diphenylamine in the form of a "spot test"<sup>20</sup>, we got sensitivities ranging in limits between 0.2 and 0.5  $\mu$ g. With some of the compounds which we have just described an even higher sensitivity could be achieved with the "spot test", and this will be the subject of a future publication.

As regards the stability of the colours obtained diphenylamine is particularly

TABLE I

COLOUR OF THE SPOTS FORMED AND DETECTABLE LIMITS IN MICROGRAMS

No.	Aromatic amine	Aldrin	<i>p-p'</i> -DDT	Lindane	Dieldrin	Endrin	Background colour
1	Aniline	yellow-brown (0.5-1)	yellow-brown (5)	yellow-brown (0.5-1)	yellow-brown (0.5-1)	yellow-brown (0.5-1)	bright-cream
2	<i>o</i> -Toluidine	reddish brown (1)	reddish brown (3-5)	reddish brown (0.5-1)	reddish brown (1)	reddish brown (1)	cream
3	<i>m</i> -Toluidine	reddish brown (1)	reddish brown (3)	reddish brown (0.5-1)	reddish brown (1)	reddish brown (1)	cream
4	<i>p</i> -Toluidine	yellowish brown (1)	reddish brown (3)	reddish brown (0.5)	yellowish brown (1)	yellowish brown (1)	bright-rose
5	<i>o</i> -Aminophenol	—	—	reddish brown (10)	reddish brown (10)	reddish brown (10)	cream
6	<i>p</i> -Anisidine·HCl	reddish brown (3-5)	violet (1)	reddish brown (3-5)	reddish brown (3-5)	reddish brown (3-5)	white (brown)
7	<i>p</i> -Nitroaniline	—	reddish brown (5-10)	—	—	—	yellow-green
8	Dimethylaniline	bright-green (0.5)	brown (1)	grey-violet (1)	bright-green (0.5)	bright-green (0.5)	white
9	<i>p</i> -Phenylenediamine	grey-olive green (0.5)	grey-olive green (0.5)	violet (0.5)	grey-olive green (0.5)	grey-olive green (0.5)	almost white
10	<i>p</i> -Aminodimethylaniline	rose (1)	rose (1)	rose (1)	rose (1)	rose (1)	dirty-red
11	<i>o</i> -Dimethylaminobenzaldehyde	orange (1)	yellow (1)	orange (1)	orange (1)	orange (1)	bright-yellow-green
12	Benzidine	yellow-green (0.5)	yellow-green (0.5)	blue-green (0.5)	yellow-green (0.5)	yellow-green (0.5)	bright-cream
13	<i>o</i> -Tolidine	green (0.5)	yellow-green (0.5)	blue (0.5)	green (0.5)	green (0.5)	white-grey
14	3,3'-Diaminobenzidine	bright brown (3)	bright-brown (2)	bright-brown (4)	bright-brown (3)	bright-brown (3)	yellowish green
15	Diphenylamine	emerald-green (0.5)	reddish brown (1-2)	dark-violet (0.5)	green-yellow (0.5)	green-yellow (0.5)	almost white
16	$\alpha$ -Naphthylamine	reddish brown (1)	reddish yellow (0.5)	reddish brown (0.5)	reddish brown (1)	reddish brown (1)	almost white
17	<i>N</i> -1-Naphthylethylenediamine·2 HCl	grey-blue (1-2)	grey-blue (2)	grey-blue (0.5)	grey-blue (1-2)	grey-blue (1-2)	bright-yellow
18	<i>N</i> -Phenyl-1-naphthylamine	grey-blue (5)	green (3)	violet (3)	grey-blue (5)	grey-blue (5)	bright-orange

outstanding, and it is possible to read the chromatogram even after 60 days, with only a slight darkening of the background. Such stability of the coloured spots was not obtainable with other amine compounds, but in their case it is possible to revive the colour by means of further U.V. irradiation (benzidine, *o*-tolidine, dimethylamine and others).

In addition to the data in Table I some additional information concerning each of the amines used is given below.

#### *Aniline*

The irradiation time necessary after spraying with this reagent is 5–7 min. DDT is more difficult to detect than the other insecticides (sensitivity limit 5  $\mu\text{g}$ ).

#### *o-, m-, and p-Tolidine*

All three isomers behave similarly. Apart from the DDT spot, the colour of which fades, the other spots remain stable even after several hours.

#### *o-Aminophenol*

As distinct from the other reagents, *o*-aminophenol is dissolved in petroleum ether. To produce any coloured spots it was necessary to irradiate for 15 min, but because of lack of sensitivity the reagent has no practical value.

#### *p-Aminodimethylaniline and p-nitroaniline*

These reagents are of no account either, as with the first an intensely coloured background is obtained, and the second does not possess the required sensitivity.

#### *p-Anisidine*

This reagent is only of use with DDT, with which it forms a violet-red spot on a white background after 30–60 sec irradiation. In order to develop the spots of the other insecticides, 5 min irradiation are required, but this darkens the background so that the spots are barely discernible.

#### *Dimethylaniline*

This has been shown to be a very sensitive reagent, and with it a certain amount of selectivity is possible in the following manner. The irradiation usually lasts 2–7 min, but if the plate is irradiated for only 2 min the aldrin, dieldrin and endrin spots are blue-green, DDT brown and lindane violet. On further irradiation only the colour of the DDT spots remains unchanged. Dimethylaniline reacts with chlorophenoxy acids as well, producing violet-coloured spots.

#### *p-Phenylenediamine*

Irradiation of the chromatogram for 30–60 sec is sufficient to produce a hardly noticeable background with well defined spots; the sensitivity is better than 0.5  $\mu\text{g}$ . If the irradiation is continued for 5 min the background becomes dirty-pink and the spots grey-blue, excepting lindane and toxaphene, which are coloured violet.

#### *p-Dimethylaminobenzaldehyde*

If the chromatogram sprayed with *p*-dimethylaminobenzaldehyde is irradiated

for 5–7 min, only a lemon-yellow spot due to DDT appears on the white background; after 15 min irradiation the background becomes more intensively coloured and the insecticide spots become orange. Under the unfiltered U.V. light the coloured spots become more noticeable.

#### *Benzidine and o-tolidine*

Both of these compounds behave similarly, and are equally sensitive and suitable for use as chromogenic reagents, not only for the insecticides mentioned but also for toxaphene and derivatives of chlorophenoxy acids. The chromatogram need only be irradiated for 5–10 sec to cause the appearance of coloured spots; further irradiation intensifies the colours but the background also becomes coloured. In daylight the colours change quickly, the background becomes brighter and the insecticide spots sandy-yellow; a subsequent brief irradiation produces the same effect. If the chromatogram is exposed to sunlight the same result is obtained after somewhat longer interval (several minutes).

#### *3,3'-Diamino-benzidine*

In contrast to benzidine and *o*-toluidine, 3,3'-diaminobenzidine cannot be used so successfully.

#### *Diphenylamine*

Diphenylamine is regularly used as a chromogenic reagent by us, not only for the cases when standard insecticide solutions are being examined but when impure extracts from cereals, vegetable, clover or milk are being investigated; residues of fat, waxes and other coloring pigments do not interfere. In addition to the insecticides mentioned toxaphene, MCPA, MCPP, 2,4-D and 2,4,5-T also react with diphenylamine; their spots are coloured grey after an irradiation of 5 min. The colour is very stable, making the identification of 0.5  $\mu\text{g}$  of insecticide possible even after 2 months. The same effect can be produced by sunlight.

#### *$\alpha$ -Naphthylamine*

It takes only 30 seconds to develop the coloured spots with  $\alpha$ -naphthylamine; if the irradiation is continued up to 2 min the background becomes rosy and the spots less discernible. The colours are unstable in daylight and after 5–10 min only the lindane spot is visible.

#### *N-1-Naphthylethylenediamine and N-phenyl-1-naphthylamine*

Both these give similarly coloured reaction products; the first reagent is somewhat more sensitive; the irradiation time is about 10 min.

#### CONCLUSION

Eighteen aromatic amines have been tested for their ability to identify chlorinated insecticides in thin-layer chromatography. Of these *o*-aminophenol, *p*-anisidine, *p*-nitroaniline, *p*-aminodimethylaniline and 3,3'-diaminobenzidine have no practical value; either their sensitivity is inadequate, they do not react at all, or the chromatogram background is too dark. The other amines can all be used practically as chromo-

genic reagents; dimethylaniline, *p*-phenylenediamine, benzidine, *o*-tolidine, diphenylamine and  $\alpha$ -naphthylamine are particularly useful.

#### ACKNOWLEDGEMENTS

The author wishes to express his thanks to the Institute for Health Protection of the S.R. of Serbia, Belgrade, for permission to publish this paper, to Prof. Dr. S. ŠIBALIĆ and Prof. Dr. A. DAMANSKI, Belgrade, for their interest and helpful suggestions and to Mrs. S. NAUMOVIĆ for the English translation.

The author is also indebted to the Shell International Petroleum Company Ltd., London, for recrystallized active samples of the insecticides mentioned.

#### SUMMARY

A simple and rapid procedure for identification of some chlorinated insecticides on thin-layer chromatograms by irradiating the sprayed chromatogram with U.V. light is described. Eighteen aromatic amines were used as chromogenic reagents; the following showed the highest sensitivity and, consequently, a certain practical value: dimethylaniline, *p*-phenylenediamine, benzidine, *o*-tolidine, diphenylamine and  $\alpha$ -naphthylamine.

#### REFERENCES

- 1 H. J. PETROWITZ AND J. G. PASTUSCHKA, *Chemiker Ztg.*, 85 (1961) 867.
- 2 D. C. ABBOT, H. EGAN AND J. THOMSON, *J. Chromatog.*, 16 (1964) 481.
- 3 K. C. WALKER AND M. BEROZA, *J. Assoc. Offic. Agr. Chemists*, 46 (1963) 250.
- 4 H. V. MORLEY AND M. CHIBA, *J. Assoc. Offic. Agr. Chemists*, 47 (1964) 306.
- 5 L. C. MITCHELL, *J. Assoc. Offic. Agr. Chemists*, 35 (1952) 920.
- 6 L. C. MITCHELL AND W. I. PATTERSON, *J. Assoc. Offic. Agr. Chemists*, 36 (1953) 553.
- 7 L. C. MITCHELL AND W. I. PATTERSON, *J. Assoc. Offic. Agr. Chemists*, 37 (1954) 996.
- 8 R. G. BRIDGES, A. HARRISON AND F. P. W. WINTERINGHAM, *Nature*, 177 (1956) 86.
- 9 L. C. MITCHELL, *J. Assoc. Offic. Agr. Chemists*, 40 (1957) 296.
- 10 L. C. MITCHELL, *J. Assoc. Offic. Agr. Chemists*, 41 (1958) 781.
- 11 L. C. MITCHELL, *J. Assoc. Offic. Agr. Chemists*, 44 (1961) 643.
- 12 L. C. MITCHELL, *J. Assoc. Offic. Agr. Chemists*, 45 (1962) 682.
- 13 P. A. MILLS, *J. Assoc. Offic. Agr. Chemists*, 42 (1959) 734.
- 14 P. A. MILLS, *J. Assoc. Offic. Agr. Chemists*, 44 (1961) 1.
- 15 P. A. MILLS, *J. Assoc. Offic. Agr. Chemists*, 44 (1961) 175.
- 16 L. F. KRZEMINSKI AND W. A. LANDMANN, *J. Chromatog.*, 10 (1963) 515.
- 17 S. O. GRAHAM, *Science*, 139 (1963) 835.
- 18 J. BÄUMLER AND S. RIPPSTEIN, *Helv. Chim. Acta*, 44 (1961) 1162.
- 19 F. FEIGL, *Spot Tests in Organic Analysis*, Elsevier, Amsterdam, 1960.
- 20 J. D. LEMLEY, *Mass. Inst. Technol. M.R. No. 176, Project A II.1* (1945).
- 21 C. CUETO, *J. Agr. Food Chem.*, 8 (1960) 273.
- 22 A. J. GRAUPNER AND C. L. DUNN, *J. Agr. Food Chem.*, 8 (1960) 286.
- 23 W. MCKINLEY AND S. J. GRAHAM, *J. Assoc. Offic. Agr. Chemists*, 43 (1960) 89.
- 24 A. BONDI, *Talanta*, 10 (1963) 679.
- 25 D. KATZ, *J. Chromatog.*, 15 (1964) 269.
- 26 V. M. ADAMOVIĆ, *Symp. Role Pesticides Fields Agr. Nutr.*, 1st, May 10th and 11th, 1965, Novi Sad, Yugoslavia.