THIN LAYER CHROMATOGRAPHY OF SUBSTITUTED NITROANILINES AND RELATED COMPOUNDS

C. L. KESWANI AND D. J. WEBER
Department of Biology, University of Houston, Houston, Texas (U.S.A.)
(Received January 30th, 1967)

INTRODUCTION

Substituted nitroanilines are used extensively as intermediates in the chemical industry for the production of dyes and related compounds. Certain of these nitroanilines are also used to control fungi. CLARK AND HAMS[1] tested forty-eight substituted nitroanilines for their fungicidal activity and eventually developed 2,6-dichloro-4-nitroaniline as a commercial fungicide commonly known as Allisan or Botran. In the production of substituted nitroanilines and in the determination of fungicide residue in plants, it is important to determine the impurities present and to detect possible breakdown products of the original compound. Most of the residue analyses of 2,6-dichloro-4-nitroaniline have been made using the colorimetric method of KILGORE, CHENG AND OGAWA[2]. While this method is simple and sensitive, it is not specific for 2,6-dichloro-4-nitroaniline since it reacts with contaminants such as 2-chloro-4-nitroaniline[3]. BECKMAN AND BEVENUE[4] used gas chromatography to detect 2,6-dichloro-4-nitroaniline and recently CHENG AND KILGORE[5] increased the sensitivity of this method by using an electron capture detector. While gas chromatography is the method of choice in residue analysis, it does not always resolve closely-related nitroanilines. A perusal of the literature indicated that there were few papers on the separation of nitroanilines. MARCINKIEWICZ AND GREEN[6], and LARSEN AND HARVEY[7] were able to separate some nitroanilines using paper chromatography. Unsubstituted nitroanilines were separated by BUCHOWSK AND PAWLOWSKI[8] to a limited extent using column chromatography. WAKSMUNDZKI *et al.*[9] were able to separate some unsubstituted nitroanilines with the use of thin layer chromatography. However, for substituted nitroanilines there does not appear to be available a suitable method for resolving closely related compounds.

In our investigations on the metabolism of 2,6-dichloro-4-nitroaniline by microorganisms, a technique was needed that would separate closely related nitroanilines. In this paper we are reporting a thin layer chromatographic method using Silica Gel G with purified *n*-hexane and acetone (ratio 3:1) that will resolve closely related substituted nitroanilines.

EXPERIMENTAL

Preparation of chromoplates

Chromoplates, 250 μ in thickness, were prepared by spreading Silica Gel G

suspended in distilled water (2:1) on glass plates according to the methods of STAHL[10]. The plates were allowed to dry at room temperature and then activated at 100° for one hour just before use. Substituted nitroanilines dissolved in acetone were applied to the chromoplate 2.5 cm from the lower edge and 2.5 cm apart from one another. Each spot contained 0.05 mg of sample. The chromoplates were equilibrated with the solvent system in the developing chamber for 15 min and then lowered into the solvent system consisting of purified *n*-hexane and acetone in a ratio of 3 to 1 (v/v). This solvent system was found to be the most satisfactory of the several systems tested. The plates were removed from the chamber when the solvent front was approximately 2 cm from the top. The position of the solvent front was marked and the R_F values of the various compounds determined. Poor separation of substituted nitroanilines was obtained with the hexane–acetone system when aluminum oxide with plaster of paris as a binder was used as coating on the chromoplates.

Visualization of substituted nitroanilines

Several of the nitroanilines were yellow in color and could be detected visually without further treatment. Other substituted nitroanilines and related compounds were colorless and had to be treated for identification. Three different methods were used to visualize the compounds.

1. Diazotization. A common method[11] of detecting phenols on paper chromatograms consists of spraying with diazotized *p*-nitroaniline which couples with the phenol to produce a colored compound (the reaction is shown in A of Fig. 1). This method was modified[3] in the following manner.

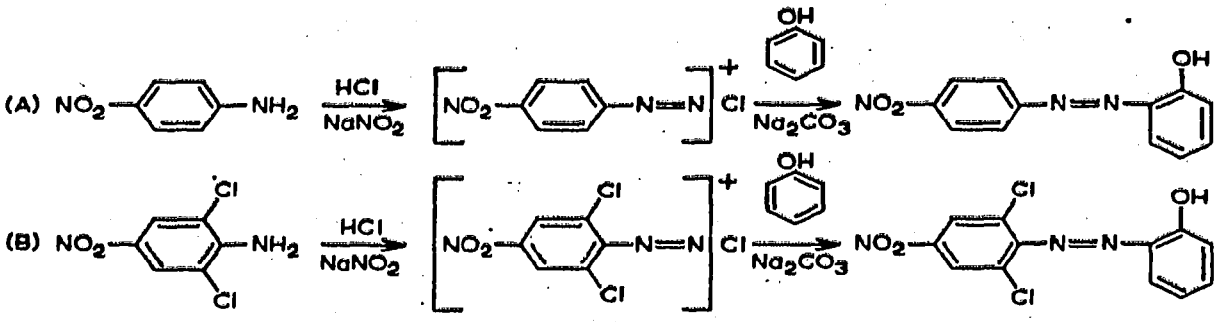

Fig. 1. Diazotization reactions of (A) nitroaniline and (B) 2,6-dichloro-4-nitroaniline.

The substituted nitroanilines were diazotized on the thin layer chromoplates by spraying in succession with 5 % $NaNO_2$ in 1 *N* HCl, with 5 % aqueous phenol solution, and finally with an aqueous solution of 7 % sodium carbonate. A colored compound is produced. (See B in Fig. 1.) This method will detect compounds having an amine group, *e.g.* 3,5-dichloroaniline. GROVES AND CHOUGH[12] modified this method by using N-(1-naphthyl)-ethylenediamine to couple with diazotized 2,6-dichloro-4-nitroaniline.

2. α-Naphthol. The procedure[13] consists of spraying the chromoplates with 5 % α-naphthol in methanol. The reagent will detect compounds in which there is no amine group, such as 2,6-dichloro-4-nitroanisole, and compounds where substitution has been made on the nitrogen of the amine group, such as 2,6-dichloro-4-nitroacetanilide. The color of the compounds after the treatment is yellow with a light pink background.

TABLE I

R_F AND COLOR REACTIONS OF NITROANILINES AND RELATED COMPOUNDS

	Compound	R_F	Structure	Normal color[a]	Color after diazotization[b]	Color after $FeCl_3$, $K_4[Fe(CN)_6]$[c]	Color after α-naphthol[d]
1	*p*-Nitroaniline	0.17		yellow	dark brown	green	orange
2	*m*-Nitroaniline	0.25		yellow	dark yellow	grey	orange
3	*o*-Nitroaniline	0.38		yellow	orange	green	yellow
4	2-Chloro-4-nitroaniline	0.28		yellow	light brown	green	yellow
5	2,6-Dichloroaniline	0.81		colorless	yellow	colorless	colorless
6	3,5-Dichloroaniline	0.43		colorless	yellow	yellow	yellow
7	2,6-Dichloro-4-nitroaniline	0.42		yellow	yellow	yellow	yellow
8	2,6-Dichloro-4-nitro-phenol	0.034		yellow	yellow	yellow	yellow
9	2,6-Dichloro-4-nitrophenyl acetate	0.69		yellow	yellow	yellow	yellow
10	2,6-Dichloro-N-methyl-4-nitroaniline	0.61		yellow	yellow	green	yellow
11	2,6-Dichloro-N,N-dimethyl-4-nitroaniline	0.87		yellow	yellow	yellow	yellow
12	2,6-Dichloro-4-nitroacetanilide	0.20		colorless	colorless	colorless	yellow
13	2,6-Dichloro-4-nitroanisole	0.92		colorless	colorless	colorless	yellow
14	4,6-Dichloro-2-nitroaniline	0.63		yellow	yellow	yellow	yellow
15	4-Nitro-2-aminotoluene	0.29		yellow	yellow	yellow	yellow
16	*p*-Nitro-*o*-aminophenol	0.10		brown	grey	grey	purple
17	2,4-Dinitroaniline	0.18		yellow	yellow	green	yellow
18	2-Nitro-4-chloroaniline	0.38		yellow	yellow	yellow	orange

[a] Color of compound on Silica Gel G plate.
[b] Color of compound after diazotization.
[c] Color of compound after spraying with 0.1 *M* ferric chloride and 0.1 *M* potassium ferricyanide.
[d] Color of compound after spraying with 5% α-naphthol in methanol.

This reagent will detect compounds that cannot be diazotized by nitrous acid and compounds that lack an amine group.

3. Ferric chloride. The chromoplates were sprayed with equal amounts of 0.1 *M* aqueous ferric chloride and 0.1 *M* aqueous potassium ferricyanide. This technique[13] was useful because of the change in color that developed with certain substituted nitroanilines.

RESULTS AND DISCUSSION

Various substituted nitroanilines and related compounds were separated on Silica Gel G plates using the hexane–acetone system. The structural formulas, R_F values, and color reactions of the compounds are shown in Table I. With this method it was possible to separate clearly *p*-nitroaniline, *m*-nitroaniline and *o*-nitroanilines; 2,6-dichloro-4-nitroaniline was easily separated from the 2-chloro-4-nitroaniline and other closely related 2,6-dichloro-4-nitro compounds. The amount of diffusion of the compounds during separation was small and no tailing was observed. A line drawing of the chromoplates is shown in Fig. 2 illustrating the separation obtained. The procedure will be useful in determining the purity of compounds such as 2,6-dichloro-4-nitroaniline, and in investigations determining the metabolism of substituted nitroanilines.

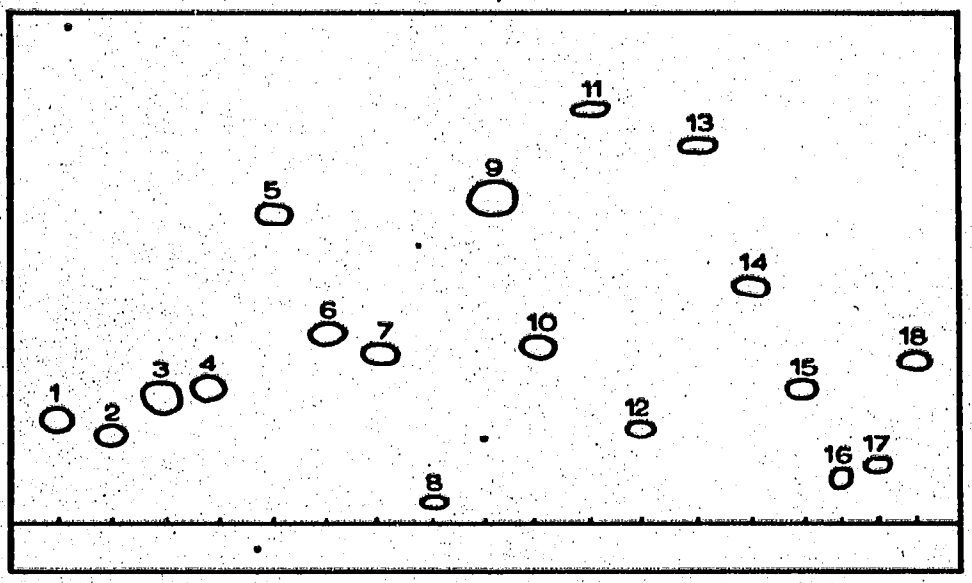

Fig. 2. Line drawing of the separation of substituted nitroanilines and related compounds on Silica Gel G in *n*-hexane–acetone (3:1) solvent system. For name of compound refer to number in Table I.

ACKNOWLEDGEMENTS

We gratefully acknowledge the contribution of chemicals for this study from Boots Pure Drug Co. Ltd., Nottingham, England; The Upjohn Co., Kalamazoo, Mich., and Allied Chemical, Morristown, N.J.

The financial support for this research was provided by USDA, Crops Protection Research Branch, Grant No. 12-14-100-3062(34).

SUMMARY

A thin layer chromatography system has been developed to separate substituted nitroanilines and related compounds. The solvent system consists of purified *n*-hexane and acetone (ratio 3:1). The absorbant used was Silica Gel G. Colorless compounds were detected by spraying in succession with 5 % $NaNO_2$ in 1 *N* HCl, 5 % aqueous phenol, and 7 % aqueous sodium carbonate, or with 0.1 *M* ferric chloride and 0.1 *M* potassium ferricyanide[13], or with 5 % α-naphthol in methanol[13]. R_F values of substituted nitroanilines and related compounds are listed.

REFERENCES

1 N. G. CLARK AND A. F. HAMS, *J. Sci. Food Agr.*, 11 (1961) 751.
2 W. W. KILGORE, K. W. CHENG AND J. M. OGAWA, *J. Agr. Food Chem.*, 10 (1962) 399.
3 D. J. WEBER, *Doctoral dissertation*, Univ. of California, Davis, 1963.
4 H. BECKMAN AND A. BEVENUE, *J. Food Sci.*, 27 (1962) 602.
5 K. W. CHENG AND W. W. KILGORE, *J. Food Sci.*, 31 (1966) 259.
6 S. MARCINKIEWICZ AND J. GREEN, *J. Chromatog.*, 10 (1963) 366.
7 J. E. LARSEN AND S. H. HARVEY, *Chem. Ind. (London)*, (1954) 45.
8 H. BUCHOWSKI AND W. PAWLOWSKI, *Chem. Anal. (Warsaw)*, 8 (1949) 965.
9 A. WAKSMUNDZKI, J. ROZYTO AND J. OSCIK, *Chem. Anal. (Warsaw)*, 8 (1963) 965.
10 E. STAHL, *Thin Layer Chromatography*, Academic Press, New York, 1965.
11 R. J. BLOCK, E. L. DURRUM AND G. ZWEIG, *Paper Chromatography and Paper Electrophoresis*, Academic Press, New York, 1958.
12 K. GROVES AND K. S. CHOUGH, *J. Agr. Food Chem.*, 14 (1966) 669.
13 M. GILLIO-TOS, S. A. PREVITERA AND A. VIMERCATI, *J. Chromatog.*, 13 (1964) 571.