THIN-LAYER CHROMATOGRAPHY OF 2,4-DINITROPHENYLHYDRAZONES OF AROMATIC ALDEHYDES AND KETONES

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INTRODUCTION

Study of the products of degradation reactions of aromatic constituents of wood has emphasized the advantages of a rapid method for the analysis and identification of aldehydes and ketones. In this field, the formation and identification of derivatives, generally 2,4-dinitrophenylhydrazones (2,4-DNPH), is necessary.

Many authors have already published papers on the qualitative analysis of carbonyl compounds by paper chromatography both on untreated and on treated paper[1-5]. The aim of the present research was to find a rapid method of separation and identification of aromatic 2,4-DNPH's. Thin-layer chromatography was investigated as this technique offers the advantages of speed of operation and has proved of value in the separation of 2,4-DNPH's[6,7].

EXPERIMENTAL AND RESULTS

Preparation of the chromatoplates

Glass plates (20 × 20 cm² and 6.5 × 20 cm²) were prepared from Silica Gel G (Merck) according to STAHL by mixing 1 part of dry powder with 2.7 parts of distilled water containing 0.5 g/l of soluble starch and 10 mg/l of sodium fluorescein. The slurry was spread on the glass plates by means of a glass rod according to the manner described by LEES AND DE MURIA[8]. The plates were dried in air for 30 min, activated at 105° for 30 min, and then allowed to cool (30 min) before applying the spots of standard solutions. The starch content of the silica gel coating only has a binding effect. A series of experiments with silica gel layers, containing different amounts of starch, showed that the separation of 2,4-DNPH's was not affected.

The presence of sodium fluorescein in the thin layer, which does not affect the R_F values of 2,4-DNPH's, enabled the substances to be located under ultraviolet light as dark spots on a yellow background.

A slurry prepared with a ratio of dry powder/water of 1:2.7 was more suitable than the usual one (1:2) for the application of a uniform layer of silica gel by the glass rod technique.

A silica gel coating of thickness of 0.5–0.6 mm was obtained by sticking three layers of adhesive tape to the opposite edges of the plate. This thickness was chosen after a series of experiments which showed that a very thin layer enhanced imperfections in the coating while a thicker one gave a poor chromatographic separation.

Variations of the thin layer thickness between 0.5 and 0.6 mm are not critical, as can be seen from Table I. The R_F values are, within certain limits, independent of the variations of layer thickness.

TABLE I

REPRODUCIBILITY OF R_F AND R_v VALUES OF ALDEHYDE 2,4-DINITROPHENYLHYDRAZONES

2,4-DNPH of	R_F		R_v	
	Average value	*S.D.*	*Average value*	*S.D.*
Vanillin	0.23	±0.013	0.71	±0.019
Veratraldehyde	0.33	±0.014	1.00	—
Anisaldehyde	0.49	±0.020	1.48	±0.012
Benzaldehyde	0.60	±0.024	1.81	±0.013

Development of chromatograms

One μl of each of the several solutions of standard 2,4-DNPH's, in chloroform or ethyl acetate, were spotted on the plates 2 cm from the base. The loading of each 2,4-DNPH spot varied from 0.1 μg to 1 μg; if the load is progressively increased beyond 1 μg, tailing rapidly becomes bad enough to spoil the chromatogram. The spotted plates were air-dried and developed, in a closed Shandon tank, at room temperature, in ethyl acetate–ligroine (75–120°) (1:2) by the ascending technique until the solvent was 14 cm above the spots (35–40 min). The spots of the 2,4-DNPH's were visible as such, but the exposure of the solvent-freed plates to ammonia vapours increased the colour intensity. The examination of the developed chromatoplates in ultraviolet light gave an even greater sensitivity, even traces of 2,4-DNPH being detectable in this way. As can be seen from the photograph in ultraviolet light, the proposed technique gives sharp round spots with a satisfactory sensitivity (0.1 μg) (Fig. 1).

Reproducibility of R_F values

The above R_F values vary with the loading of the spot, the mode of activation of the plates, the temperature and the equilibrium conditions of the chromatographic tank.

It is generally agreed that the R_F reproducibility can be considered satisfactory within the limits ± 0.05. It was determined in this case in a series of 10 experiments using plates of 6.5 × 20 cm²; these experiments were completely independent as far as the coating preparation and activation of the plates and the chromatographic tank saturation are concerned. The results are given in Table I (S.D. stands for standard deviation). It was found that better reproducibility can be obtained by recording the data in terms of the movement of a standard substance rather than of that of the solvent. The 2,4-DNPH of veratraldehyde was found useful for the purpose and the data are given in terms of R_v, defined as:

$$R_v = \frac{\text{distance in mm from the starting point to the substance}}{\text{distance in mm from the starting point to veratraldehyde 2,4-DNPH}}.$$

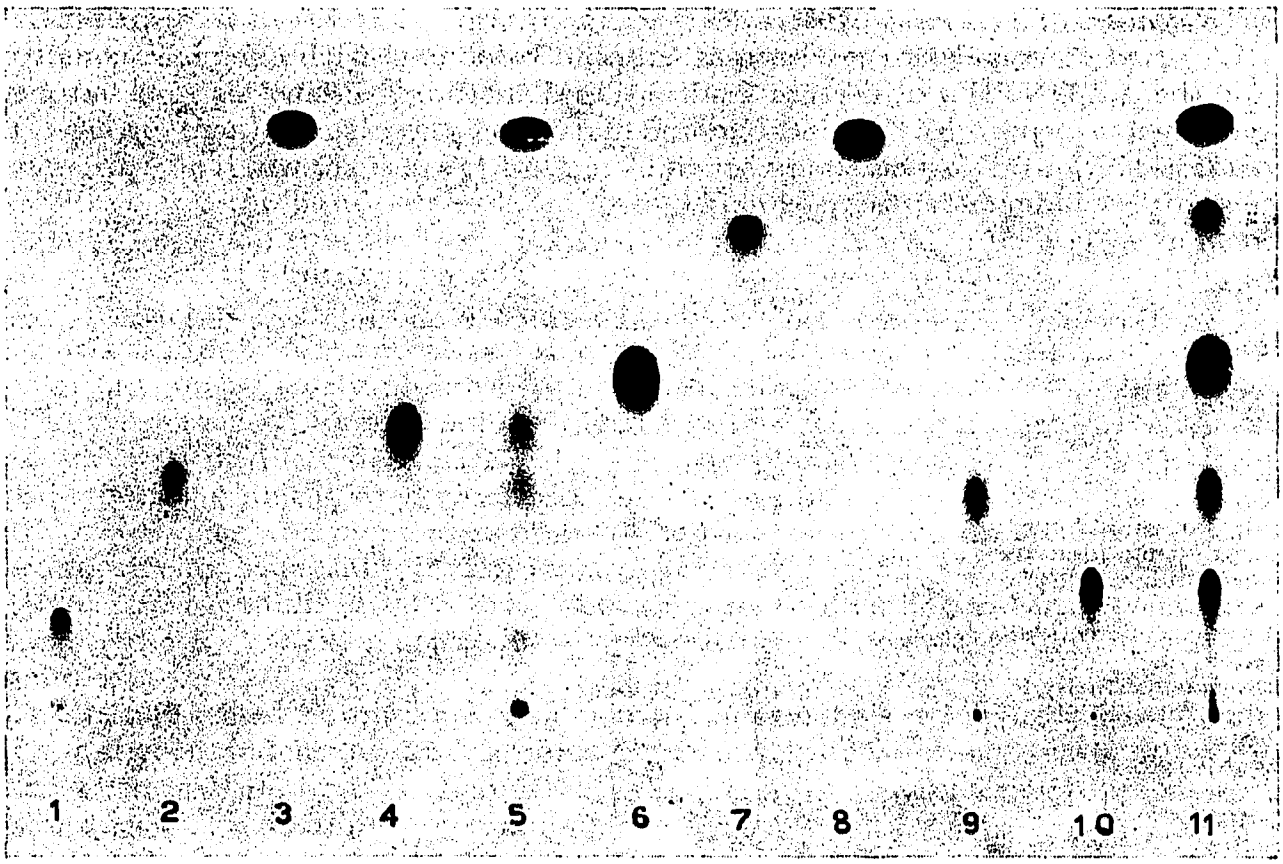

Fig. 1. Photograph in U.V. light of a chromatogram of aldehyde 2,4-dinitrophenylhydrazones. 1 = syringaldehyde; 2 = vanillin; 3 = cinnamaldehyde; 4 = *p*-hydroxybenzaldehyde; 5 = mixture of 1, 2, 3 and 4; 6 = veratraldehyde; 7 = salicylaldehyde; 8 = benzaldehyde; 9 = 2,4-dihydroxybenzaldehyde; 10 = sinapaldehyde; 11 = mixture of 6, 7, 8, 9 and 10.

The standard must be run on each plate alongside the sample to be tested.

A higher activation temperature (120°) of the thin layer or a rise of chromatographic tank temperature cause an increase of the R_F of the 2,4-DNPH spots while the R_v values are comparable to those obtained by the standard procedure; these experiments confirm the usefulness of R_v values (Table II).

TABLE II

R_F AND R_v VALUES OF ALDEHYDE 2,4-DINITROPHENYLHYDRAZONES AT DIFFERENT ACTIVATION AND ELUTION TEMPERATURES

2,4-DNPH of	R_F			R_v		
	Standard conditions	*Elution temp. 50°*	*Activation temp. 120°*	*Standard conditions*	*Elution temp. 50°*	*Activation temp. 120°*
Vanillin	0.23	0.26	0.27	0.71	0.70	0.71
Veratraldehyde	0.33	0.37	0.38	1.00	1.00	1.00
Anisaldehyde	0.49	0.55	0.56	1.48	1.47	1.46
Benzaldehyde	0.60	0.66	0.69	1.81	1.78	1.79

R_F and R_v values and chemical structure

We tried to correlate the chromatographic behaviour with chemical constitution for the 2,4-DNPH's of certain hydroxy and methoxy aldehydes and ketones, especially with respect to the number and nature of these substituent groups. In Table III the R_F and R_v values of several 2,4-DNPH's are shown. The values are the mean of six independent experiments.

TABLE III

R_F AND R_v VALUES OF 2,4-DINITROPHENYLHYDRAZONES OF SOME AROMATIC ALDEHYDES AND KETONES

2,4-DNPH of	R_F	R_v
Benzaldehyde	0.60	1.77
Salicylaldehyde	0.48	1.41
m-Hydroxybenzaldehyde	0.32	0.94
p-Hydroxybenzaldehyde	0.30	0.88
Protocatechuic aldehyde	0.02	0.06
2,4-Dihydroxybenzaldehyde	0.23	0.68
2,5-Dihydroxybenzaldehyde	0.21	0.62
Anisaldehyde	0.48	1.41
o-Methoxybenzaldehyde	0.49	1.44
m-Methoxybenzaldehyde	0.50	1.47
Vanillin	0.23	0.68
Syringaldehyde	0.05	0.15
3-Ethoxy-4-hydroxybenzaldehyde	0.32	0.94
Isovanillin	0.23	0.68
o-Vanillin	0.32	0.94
Veratraldehyde	0.34	1.00
2,4-Dimethoxybenzaldehyde	0.41	1.21
3,5-Dimethoxybenzaldehyde	0.47	1.38
2,5-Dimethoxybenzaldehyde	0.45	1.33
2,3-Dimethoxybenzaldehyde	0.47	1.38
p-Ethoxybenzaldehyde	0.53	1.56
Acetylvanillin	0.33	0.97
4-Ethoxy-3-methoxybenzaldehyde	0.42	1.24
Cinnamaldehyde	0.60	1.77
p-Coumaraldehyde	0.33	0.97
Coniferaldehyde	0.27	0.80
Sinapaldehyde	0.15	0.44
p-Hydroxybenzylacetone	0.32	0.94
p-Methoxybenzylacetone	0.49	1.44
Acetovanillone	0.23	0.68
Acetosyringone	0.00	0.00
2,4-Dihydroxyacetophenone	0.26	0.76

From the data in Table III, it can be seen that the presence and the number of hydroxyl groups in a compound has a pronounced effect upon the R_F values. This is due to the fact that OH groups in the molecule give rise to the possibility of bonding with the absorbent silica gel coating.

The 2,4-DNPH's containing two hydroxyl groups have in general lower R_F values than the monohydroxylated ones. The *ortho* position of the two hydroxyl groups causes a strong absorption on silica gel. The *meta* and *para* positions of the two OH groups do not affect the R_F values. Methylation of hydroxyl groups causes an increase in R_F values, and in the case of the 2,4-DNPH's of monomethoxy-aldehydes

the position of the methoxy group does not affect the R_F values. For the 2,4-DNPH's of dimethoxy-aldehydes it seems that the position of the methoxy groups with respect to each other influences the R_F values.

The choice of the 2,4-DNPH of veratraldehyde as reference substance was suggested by the fact that its R_F is a limiting value between the hydroxylated 2,4-DNPH's and the non-hydroxylated ones. If $R_v > 1$, the molecule of the 2,4-DNPH does not contain free hydroxyl groups, while $R_v < 1$ would be a clear indication of the presence of hydroxyl groups.

The 2,4-DNPH of *o*-hydroxybenzaldehyde is an exception to this rule. The hydroxyl group in the *ortho* position to the aldehyde group markedly decreases the polarity; consequently, the absorption on the silica gel through the phenolic hydroxyl group is decreased by internal hydrogen bonding. In support of this, the R_F value of *o*-hydroxybenzaldehyde 2,4-DNPH is comparable with that of the 2,4-DNPH of the corresponding methylated aldehyde.

However, the phenomenon can be influenced by additional substitution in the aromatic ring, especially if this occurs *ortho* to either the hydrazone or the hydroxy group (see *o*-vanillin 2,4-DNPH). The conjugated double bond in the side chain increases the R_F values in comparison to the corresponding aldehydes without a double bond.

The present chromatographic method does not solve the problem of distinguishing between aldehydic and ketonic functions but, combined with other physical and chemical methods, it may give valuable information concerning the chemical structure of the examined compounds.

SUMMARY

Separations of 2,4-dinitrophenylhydrazones of aromatic aldehydes and ketones were achieved on thin layers of silica gel G (according to STAHL) using ethyl acetate–ligroine (1:2).

The use of a comparative method, in which veratraldehyde 2,4-dinitrophenylhydrazone is chromatographed alongside the unknown substances, gives a clear indication of the presence in the molecule of hydroxyl groups.

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