CHROMATOGRAPHIC IDENTIFICATION OF CARBONYL COMPOUNDS

III. PAPER CHROMATOGRAPHIC RESOLUTION OF ALDEHYDE 2,4-DINITROPHENYLHYDRAZONE MIXTURES*

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(Received September 29th, 1966)

In the preliminary experiments, different paper chromatographic methods[1–4] were tested with a view to determining their ability to separate the 2,4-dinitrophenylhydrazones of aliphatic aldehydes. As the possible interference of the reagent, dinitrophenylhydrazine, in the identification of hydrazones had not previously been taken into account, particular attention was paid to its possible interference in evaluation of the different methods. The method of HORNER AND KIRMSE[2], as modified by PIHA *et al.*[4], proved to be the most appropriate one, and was used in this study. However, instead of the thin Whatman No. 1 and Whatman No. 4 papers used earlier[4,5] the thicker Whatman No. 3 filter paper was employed, as large samples could be applied to this paper without any loss of resolution. Two parallel chromatograms were run in the paper chromatographic analyses. In one of these runs, the individual aldehyde hydrazones, their mixture, and the reagent, were resolved side by side. The aim was to examine the resolution of the mixture and the possible interference of the reagent in identification of the components. In the other chromatographic run, a mixture of aldehyde hydrazones (M), isolated by adsorption on carbon from an ethanol–water solution, and subsequent elution from the carbon (see Part I, ref. 6) and a mixture of pure aldehyde hydrazones (R) were resolved side by side, with a view to comparing the completeness of the isolation of the aldehyde hydrazones from aqueous ethanol.

EXPERIMENTAL

Dissolution of aldehyde hydrazones

Two hundredths of a millimole of each of the pure 2,4-dinitrophenylhydrazones of acetaldehyde, propionaldehyde, butyraldehyde, isobutyraldehyde, valeraldehyde, isovaleraldehyde, 2-methylbutyraldehyde and furfural were weighed and a mixture R, containing 0.02 mmole of each, was prepared by weighing. The weighed hydrazones and their mixture R were dissolved in 10-ml volumes of methyl formate (purum, Fluka AG). A mixture of unprecipitated hydrazones isolated by adsorption on carbon from the 4 l of 8 wt. % aqueous ethanol from which the bishydrazones had been removed was eluted from the carbon (see Part I, ref. 6) and dissolved in 10 ml of methyl formate (purum, Fluka AG). This mixture was designated M.

* For Parts I and II of this series, see refs. 6 and 7.

Paper chromatography

A 4-μl volume of the methyl formate solution of each reference aldehyde 2,4-dinitrophenylhydrazone, 4 μl of a solution of their mixture R and two different volumes, 4 and 40 μl, of a solution of dinitrophenylhydrazine in methyl formate (1 mg/ml) were applied to a sheet of Whatman No. 3 paper. To another sheet of the same paper were applied, side by side, different volumes (4, 2, 1, 0.5 and 0.2 μl) of the solutions containing the mixtures M and R. The papers were dipped in a solution composed of equal volumes of dimethylformamide (for synthesis, E. Merck AG) and acetone (for chromatography, E. Merck AG), but the solvent was not allowed to rise to the starting line. The acetone was then allowed to evaporate by holding the paper sheets in air for about five minutes. The paper sheets were then allowed to become saturated with the vapour above a layer of cyclohexane (for chromatography, E. Merck AG) saturated with dimethylformamide in a closed chamber overnight. The latter solvent mixture was also used to run the chromatograms for 6–7 h at room temperature.

RESULTS AND DISCUSSION

Paper chromatograms of the aldehyde hydrazones

Paper chromatograms of the pure 2,4-dinitrophenylhydrazones of the aliphatic C_2–C_5 aldehydes and furfural, their mixture R and the reagent dinitrophenylhydrazine are shown in Fig. 1. The dinitrophenylhydrazone of furfural was separated into the *cis* and *trans* isomers (*cf.* refs. 1 and 5) and hence yielded two spots. Part of the reagent remained on the starting line and part was distributed among several spots. If the precipitate of the aldehyde hydrazones contains much excess reagent, the latter

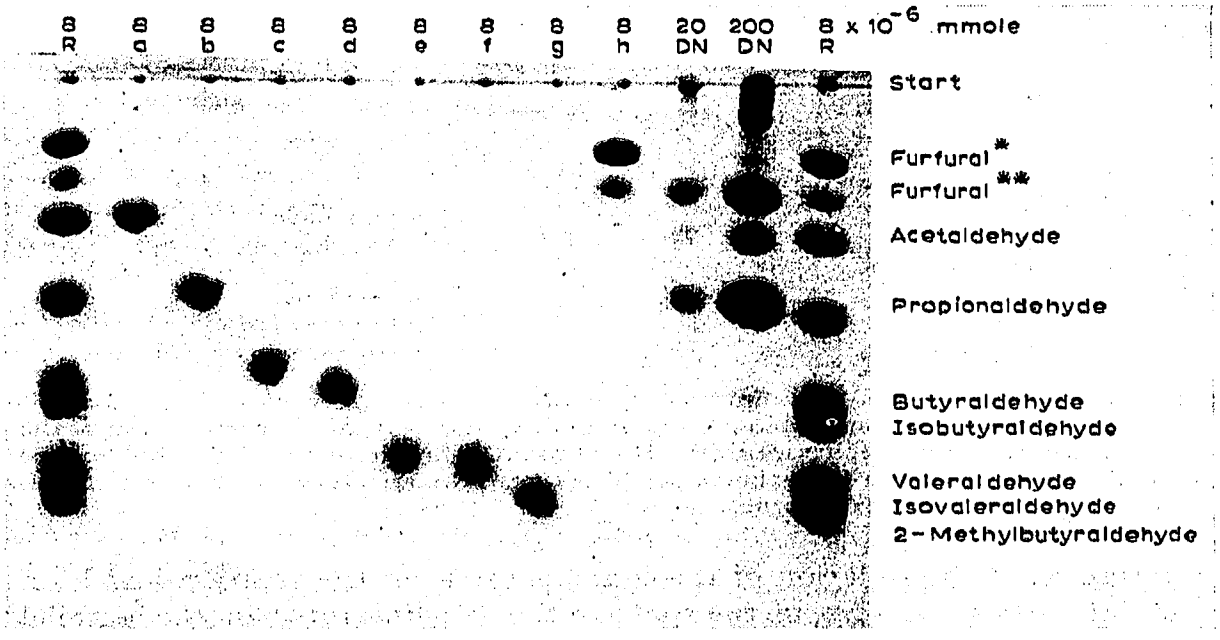

Fig. 1. Paper chromatogram of pure 2,4-dinitrophenylhydrazones of acetaldehyde (a); propionaldehyde (b); butyraldehyde (c); isobutyraldehyde (d); valeraldehyde (e); isovaleraldehyde (f); 2-methylbutyraldehyde (g); furfural (h); their mixture (R) and 2,4-dinitrophenylhydrazine (DN). The chromatographic solvent was cyclohexane saturated with dimethylformamide and the paper Whatman No. 3. **Trans* form of furfural hydrazone. ***Cis* form of furfural hydrazone.

may lead to errors in the identification of the hydrazones of propionaldehyde, furfural (*cis* isomer) and acetaldehyde. As already found previously in our laboratory[5], the *cis* isomer of furfural hydrazone migrates at the same rate as formaldehyde hydrazone, and errors may therefore occur in the identification of the latter when an excess of the reagent is present. The identification of furfural on the basis of the *trans* isomer of furfural hydrazone is not subject to interference by excess reagent. The spot of the *trans* form of furfural hydrazone is usually much more intensely coloured than that of the *cis* form and, in contrast to the other spots, which are yellow in colour, is orange yellow and hence readily identified. Dinitrophenylhydrazine does not interfere with the identification of the dinitrophenylhydrazones of butyraldehyde and valeraldehyde. On the other hand, the R_F values of the dinitrophenylhydrazones of the isomeric butyraldehydes differ so little from each other in paper chromatograms that the spots containing these derivatives are partly superimposed; the same applies to the dinitrophenylhydrazones of the isomeric valeraldehydes. Thus the method described only reveals the presence of the two groups of structural isomers.

The paper chromatogram in Fig. 2 shows the separation of the aldehyde hydrazones of mixture M isolated from 8 wt. % aqueous ethanol solution and that of the

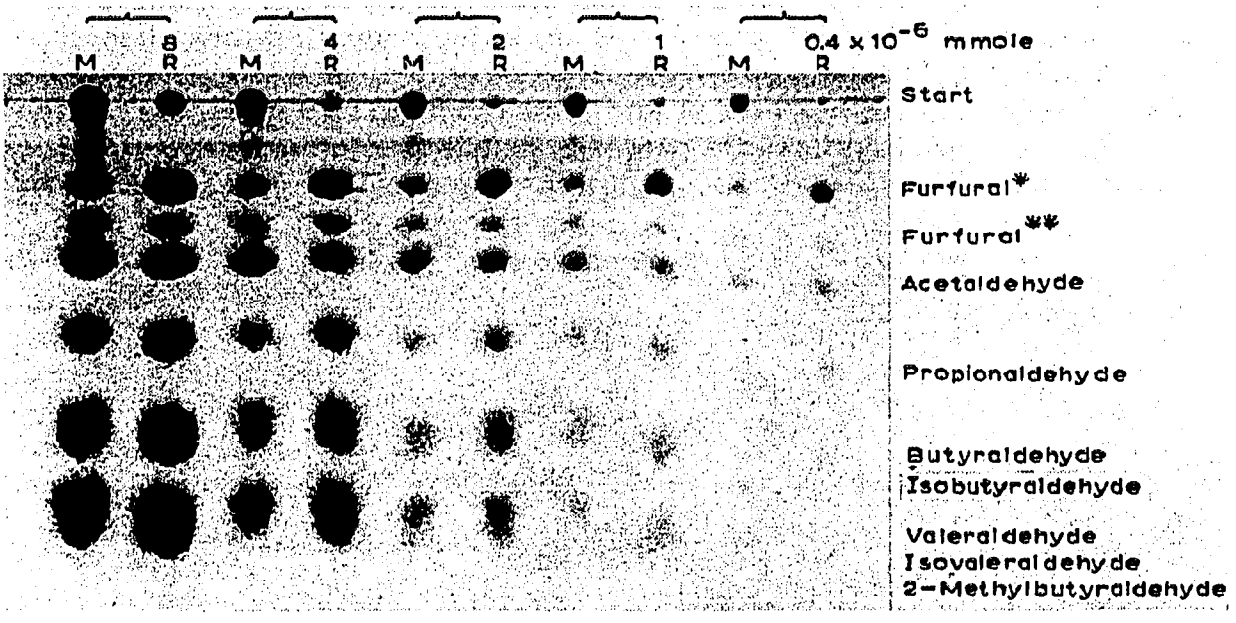

Fig. 2. Paper chromatogram of the 2,4-dinitrophenylhydrazones of aldehydes of mixture M isolated by adsorption on carbon from aqueous ethanol and by elution from the carbon, and of the pure aldehyde hydrazones of reference mixture R. The chromatographic solvent was cyclohexane saturated with dimethylformamide and the paper Whatman No. 3. **Trans* form of furfural hydrazone. ***Cis* form of furfural hydrazone.

reference mixture R. If the isolation of the components from the solution had been complete, the intensities and sizes of the spots of the two mixtures in the parallel rows should have been equal. The amounts of both mixtures applied to the paper were varied similarly to reveal possible differences in the intensities and sizes of the spots with decreasing sample size. The intensity of the spot of the *trans* form of furfural hydrazone is clearly weaker in the chromatogram of the mixture M than in

that of the reference mixture R. The isolation of the other components from the solutions seems to have been more complete. On the other hand, it should be noted, when examining the intensities of the spots in the chromatogram, that the mixture M included the greater part of the excess reagent which had been added to the solution, was adsorbed on carbon and eluted from the latter together with the aldehyde hydrazones, and hence the identification of the dinitrophenylhydrazones of acetaldehyde, propionaldehyde and furfural (*cis* form of furfural hydrazone) is less reliable owing to the interference of the excess reagent (Fig. 1).

It should also be noted that when the concentrations of the aldehydes in the solution under study are so high that they can be isolated by precipitation from the solution, the mixture of their dinitrophenylhydrazones contains very little of the reagent and hence paper chromatography can then be used to identify acetaldehyde and propionaldehyde[4,5] as well.

The solution from which the mixture (M) of aldehyde hydrazones had been isolated by the adsorption and elution technique (see Part I, ref. 6) also contained dicarbonyl compounds and keto acids, 0.02 mmole of each component. The dicarbonyl compounds were removed by precipitation as bishydrazones, and the aldehyde and keto acid hydrazones by adsorption on carbon. The aldehyde hydrazones were eluted from the carbon with methyl formate and dichloromethane successively. According to the isolation experiments (see Part I, ref. 6) the keto acid hydrazones do not elute from the carbon with the solvents mentioned, and thus do not interfere with the identification of aldehyde hydrazones.

SUMMARY

A paper chromatographic study has been made of the resolution of a mixture of acetaldehyde, propionaldehyde, butyraldehyde, isobutyraldehyde, valeraldehyde, isovaleraldehyde, 2-methylbutyraldehyde and furfural 2,4-dinitrophenylhydrazones, and the possible interference of 2,4-dinitrophenylhydrazine in identification of the components, was investigated.

Using pure aldehyde hydrazones (mixtures R) as a reference standard, an examination was made of the completeness of the isolation of these aldehyde hydrazones from aqueous ethanol (mixture M), by an adsorption and elution technique, by comparing the intensities of the spots of the components of both mixtures in the chromatogram.

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