CHROMATOGRAPHIC IDENTIFICATION OF CARBONYL COMPOUNDS

II. THIN-LAYER CHROMATOGRAPHY OF BISHYDRAZONES OF DICARBONYL COMPOUNDS*

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Many of the methods utilizing thin-layer chromatography of 2,4-dinitrophenylhydrazones of carbonyl compounds have been developed for the analysis of aliphatic aldehydes and ketones[1–8], although the separation of aromatic aldehydes[2,4], unsaturated carbonyl compounds[2,7], dicarbonyl compounds[4,6,9–12], keto acids[13–15] and hydroxycarbonyl compounds[16] has also been studied. A solvent system previously developed in our laboratory for the thin-layer chromatography of glyoxal and methylglyoxal bishydrazones[9] was used in this investigation to separate the bishydrazones of these two compounds and diacetyl. Instead of silica gel containing gypsum, a silica gel without gypsum was employed as the adsorbent because diacetyl was more easily separated on the latter. Two parallel chromatograms were run. In one the individual hydrazones, a mixture of the latter, and the reagent were resolved side by side. The purpose was to investigate the resolution of the mixture and the interference of the reagent in the identification of the components. In another chromatographic run, a mixture of bishydrazones (M) isolated by precipitation from ethanol–water solution (see Part I, ref. 17) and a mixture of pure bishydrazones (R) were resolved side by side. The purpose was to compare how completely the bishydrazones had precipitated from the aqueous ethanol.

EXPERIMENTAL

Dissolution of bishydrazones

Two hundredths of a millimole of each of the pure bis[(2,4-dinitrophenyl)-hydrazones] of glyoxal, methylglyoxal and diacetyl were weighed and a further mixture (R) containing 0.02 mmole of each was prepared by weighing. Each of the weighed individual bishydrazones and their mixture R were dissolved in 200-ml volumes of pyridine ("Baker Analyzed" Reagent, J. T. Baker Chemical Co.). A bishydrazone mixture (M) was isolated by precipitation from 4 l of the 8 wt. % aqueous ethanol; this also contained, besides the above mentioned three dicarbonyl compounds, aldehydes and keto acids (see Part I, ref. 17—there being 0.02 mmole of each component). The mixture (M) was also dissolved in 200 ml of pyridine.

* For Part I of this series, see ref. 17.

Thin-layer chromatography

A 2-μl volume of the pyridine solution of each reference bis[(2,4-dinitrophenyl)-hydrazone], 2 μl of the pyridine solution containing their mixture R and 4 μl of a solution of dinitrophenylhydrazine (1 mg/ml) were applied to an activated thin layer of Silica Gel HF_{254} (for thin-layer chromatography, E. Merck AG). The activation was carried out by heating at 120° for half an hour. To another plate covered by a thin layer of activated Silica Gel HF_{254} were applied side by side different volumes (4, 2, 1, 0.5 and 0.2 μl) of the pyridine solutions of the mixtures M and R. The two plates were held in the vapour above the chromatographic solvent mixture in a closed chromatographic chamber for half an hour. The solvent mixture was composed of 34 volumes of benzene (for chromatography, E. Merck AG), five volumes of petroleum ether (boiling range 60–80°, British Drug Houses Ltd.) and one volume of ethyl acetate (for chromatography, E. Merck AG). The running time was 1 h.

RESULTS AND DISCUSSION

Thin-layer chromatograms of the bishydrazones

The thin-layer chromatogram of the pure bis[(2,4-dinitrophenyl)hydrazones] of glyoxal, methylglyoxal and diacetyl, their mixture R and 2,4-dinitrophenylhydrazine is shown in Fig. 1. The reagent is seen to have given rise to several spots of which two are clearly more distinct than the others. One of the two dominating spots migrated a greater and the other a lesser distance than the spots of the bishydrazones, and only one diffuse spot occurred in the range where the spots of the bishydrazones were located. This last-mentioned spot may interfere with the identification of the bishydrazones if the precipitate contains about one hundred times as much reagent as bishydrazones. This is hardly possible in practice, for the precipitated hydrazones

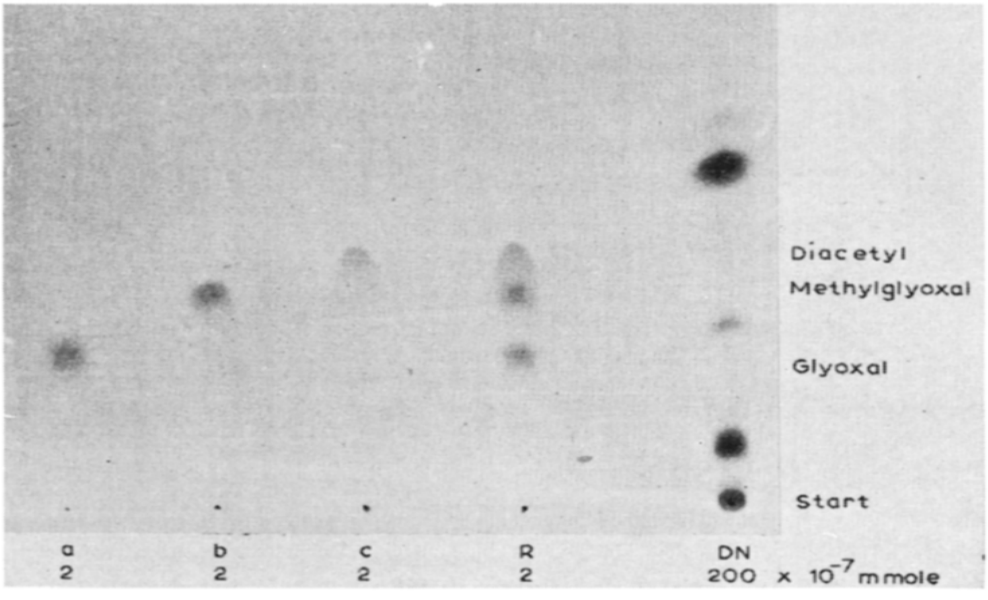

Fig. 1. Thin-layer chromatogram of pure bis[(2,4-dinitrophenyl)hydrazones] of glyoxal (a); methylglyoxal (b); diacetyl (c); their mixture (R) and 2,4-dinitrophenylhydrazine (DN). The chromatographic solvent was benzene–petroleum ether–ethyl acetate (34:5:1) and adsorbent Silica Gel HF_{254}.

normally do not contain appreciable amounts of the reagent. In addition, the bishydrazones are so sparingly soluble in ethanol[18] that they can be washed free of the reagent and any possible coprecipitated monohydrazones after precipitation and filtration.

The chromatogram in Fig. 2 was obtained when the bishydrazone mixture M isolated by precipitation from 8 wt. % aqueous ethanol (see Part I, ref. 17) and the reference mixture R were separated side by side. If the precipitation of bishydrazones from aqueous ethanol had been quantitative, the spots of corresponding components of the two mixtures should have been equal in size and intensity. The amounts

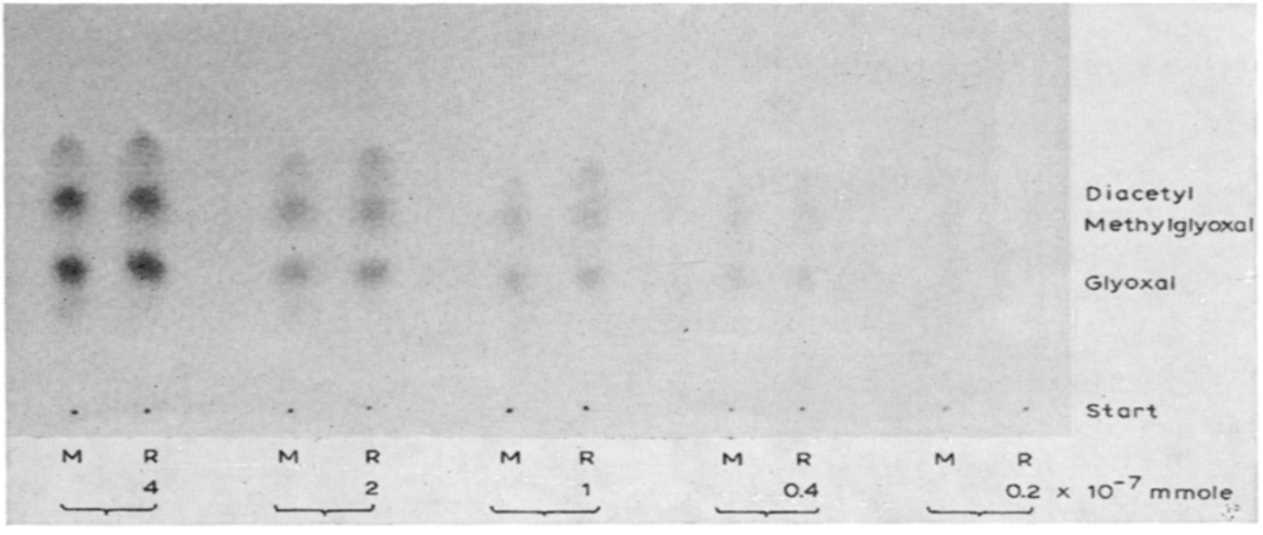

Fig. 2. Thin-layer chromatogram of the bis[(2,4-dinitrophenyl)hydrazones] of mixture M isolated from aqueous ethanol by precipitation and of the pure bishydrazones of reference mixture R. The chromatographic solvent was benzene–petroleum ether–ethyl acetate (34:5:1) and adsorbent Silica Gel HF_{254}.

of both mixtures applied to the thin layer were varied similarly to reveal possible differences in the intensities and sizes of the spots with decreasing sample size. As judged from the intensities of the spots, glyoxal and methylglyoxal were precipitated almost completely as their bishydrazones. The intensities of the spots of diacetyl bishydrazone differed slightly and hence this derivative was not precipitated as completely as the other two dicarbonyl compounds. The quantities of the compounds in the spots can, of course, be determined accurately spectrophotometrically after scraping off the adsorbent containing the spots and eluting the hydrazones from the latter. To avoid tailing, the amounts of bishydrazones applied to thin layers should be only about one tenth of the amounts of monohydrazones that are applied when the latter are analysed by thin-layer and paper chromatography[10].

The solution from which the bishydrazone mixture M was precipitated contained also aldehydes and keto acids (see Part I, ref. 17), 0.02 mmole of each component. The aldehyde hydrazones migrate in this case faster on the thin-layer chromatogram than the bishydrazones, and the keto acid hydrazones remain on the starting line. Since no spots due to aldehyde or keto acid hydrazones are seen in the chromatogram (Fig. 2) they do not precipitate from so dilute a solution.

SUMMARY

The resolution of a mixture of glyoxal, methylglyoxal and diacetyl bis[(2,4-dinitrophenyl)hydrazones] and the possible interference of 2,4-dinitrophenylhydrazine in their chromatography has been studied on silica gel thin layers. The completeness of the precipitation of dicarbonyl compounds as their bishydrazones from aqueous ethanol has been investigated by comparing the intensities of the spots of the components in the chromatogram with the spots of a mixture of pure bishydrazones used as the reference standard.

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