CHROMATOGRAPHIC IDENTIFICATION OF CARBONYL COMPOUNDS*

VI. THIN-LAYER CHROMATOGRAPHIC RESOLUTION OF MIXTURES OF KETO ACID 2,4-DINITROPHENYLHYDRAZONES

PENTTI RONKAINEN
Research Laboratories of the State Alcohol Monopoly (Alko), Helsinki (Finland)
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Solvent mixtures containing petroleum ether, ethyl formate and propionic acid, previously developed in this laboratory for the thin-layer chromatography of keto acid 2,4-dinitrophenylhydrazones[6,7], have now been thoroughly examined in preliminary experiments by varying the ratios of the solvent components, and using silica gel, cellulose, nylon and aluminium oxide as adsorbents. However, the best results were obtained by application of the method previously employed, after slight modification. This modification involved the use of a neutral silica gel suspension instead of one of acidified silica gel for preparing the thin layers on the plates, and was used in this study. The purpose was that of studying the possible interference of the reagent used in the identification of the components; the influence of the solvents in which the hydrazones are applied to the thin layers on the separation of stereoisomers of the hydrazones; and the completeness of the isolation of the components from aqueous ethanol.

EXPERIMENTAL

Dissolution of keto acid hydrazones

Two hundredths of a millimole of each of the pure 2,4-dinitrophenylhydrazones of pyruvic acid, 2-oxobutyric acid, 2-oxoisovaleric acid, 2-oxoisocaproic acid, 2-oxo-3-methylvaleric acid, levulinic acid, 2-oxoglutaric acid and oxalacetic acid were weighed, and a mixture R containing 0.02 mmole of each was prepared by weighing. The weighed individual hydrazones and their mixture R were dissolved in 10-ml volumes of dioxan (for chromatography, E. Merck AG). Also a pyridine ('Baker Analysed' Reagent, J. T. Baker Chemical Co.)–water (1:1, v/v) mixture was employed as solvent in place of dioxan. A mixture (M) of the same keto acid hydrazones isolated from 4 l of the 8 wt. % aqueous ethanol solution by adsorption on carbon and by selective elution (first the aldehyde hydrazones and then the keto acid hydrazones) from the latter (Part I) was dissolved in 10 ml of a (1:1, v/v) mixture of pyridine ('Baker Analysed' Reagent, J. T. Baker Chemical Co.) and distilled water. After samples of this solution had been taken for application to one plate, the remaining solution was evaporated to dryness in a Rotavapor and the residue M was dissolved

* For Parts I–V, see refs. 1–5.

in 10 ml of dioxan (for chromatography, E. Merck AG). A second mixture of hydrazones isolated by adsorption on carbon from the 4 l of 8 wt. % aqueous ethanol was eluted from the carbon with only the pyridine–water azeotropic mixture and contained besides the keto acid hydrazones, hydrazones of the other monocarbonyl compounds studied (Part I). This mixture M' was dissolved in 10 ml of a (1:1, v/v) pyridine–water mixture. Solutions containing only dinitrophenylhydrazine (1 mg/ml) in dioxan and in a (1:1, v/v) pyridine–water mixture were also prepared.

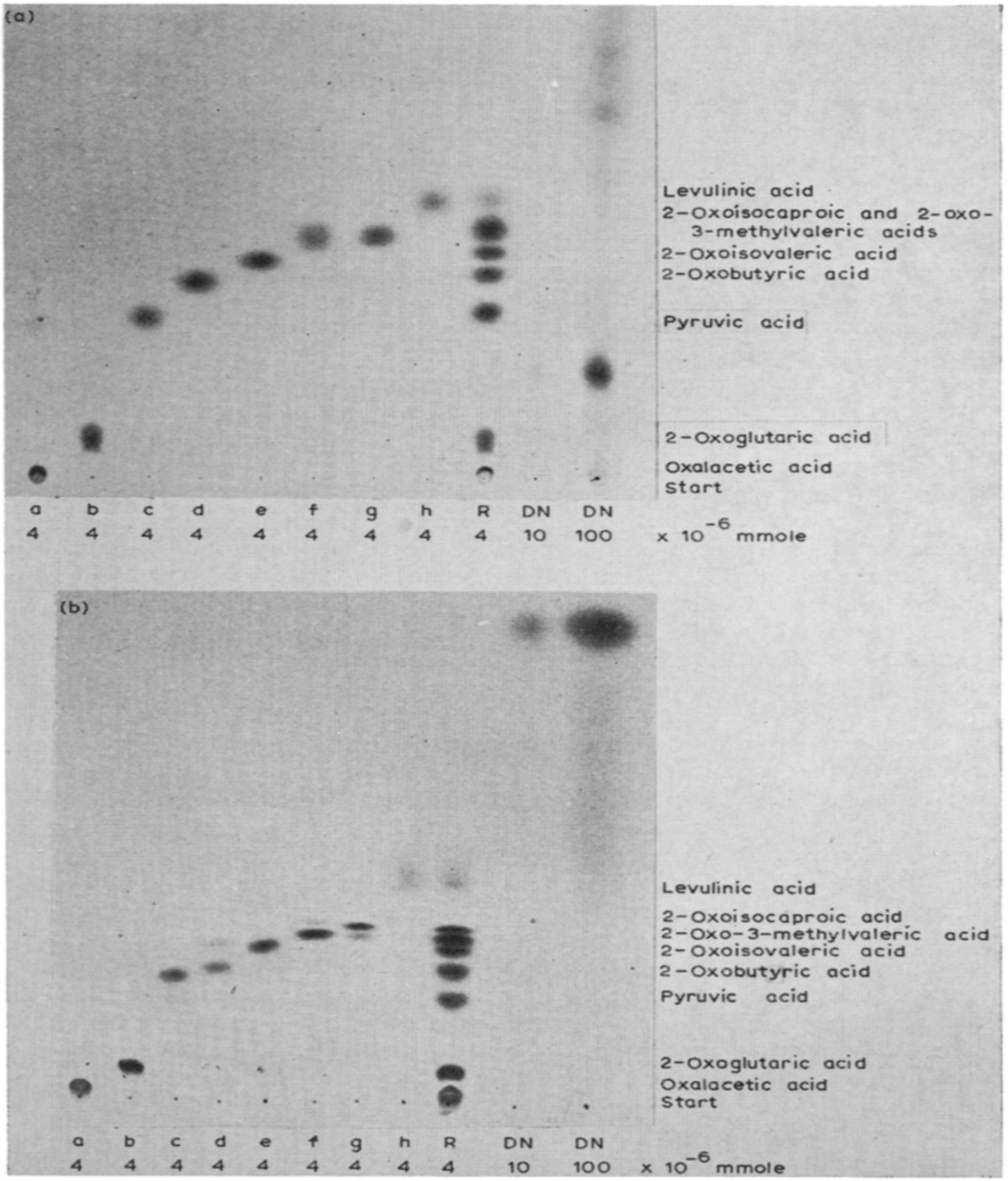
Thin-layer chromatography

A 2-μl volume of the dioxan solution of each keto acid hydrazone, 2 μl of the dioxan solution of the mixture R and two different volumes, 4 and 40 μl, of the dioxan solution of dinitrophenylhydrazine were applied to an activated thin layer of Silica Gel G (for thin-layer chromatography, E. Merck AG). The activation was carried out by heating at 120° for half an hour. The same compounds were applied to a second thin-layer plate, but in pyridine–water instead of in dioxan. To a third and fourth thin layer were applied 4, 2, 1, 0.5 and 0.2 μl of the solutions of the mixture M and the same volumes of the solutions of the reference mixture R, equal volumes of both mixtures side by side, in pyridine–water (Plate 3) and in dioxan (Plate 4). The mixtures M' and R were also applied to a thin layer side by side as above. The plates were equilibrated in the vapour above the chromatographic solvent in a closed chamber for half an hour before they were resolved with a solvent composed of 26 volumes of petroleum ether (boiling range 60–80°, British Drug Houses Ltd.), 14 volumes of ethyl formate (puriss., Fluka AG) and 3 volumes of propionic acid (puriss., Fluka AG). The running time was about 3 h.

RESULTS AND DISCUSSION

Thin-layer chromatograms of the keto acid hydrazones

Thin-layer chromatograms of pure 2,4-dinitrophenylhydrazones of keto acids, their mixture R and dinitrophenylhydrazine run with petroleum ether–ethyl formate–propionic acid (26:14:3) are shown in Fig. 1a and 1b. The solvent in which the dinitrophenylhydrazones, their mixture and the reagent were applied to the thin layer was dioxan in the former case (Fig. 1a) and a (1:1, v/v) pyridine–water mixture in the latter. In the former chromatogram each keto acid hydrazone gave only one spot. The dinitrophenylhydrazones of the structural isomers 2-oxoisocaproic acid and 2-oxo-3-methylvaleric acid migrated at the same rate. It has previously been found[6] that when the proportion of propionic acid in the chromatographic solvent is increased, or the propionic acid is replaced by formic acid, several keto acid hydrazones are resolved into two stereoisomeric forms which give two successive spots in the chromatogram. The two chromatograms (Fig. 1a and 1b) show that the resolution of the stereoisomers is not solely determined by the solvent mixture employed to develop the chromatograms, but also by the solvent in which the dinitrophenylhydrazones are applied to the thin layer. The hydrazones of the structural isomers 2-oxoisocaproic acid and 2-oxo-3-methylvaleric acid are seen to have separated in the chromatogram in Fig. 1b, but the smaller spots, to which they also give rise, will interfere with their identification if one of them is present in a much higher concentration than the other. Also the rate of migration of dinitrophenylhydrazine and



Figs. 1 a (upper) and 1 b (lower). Thin-layer chromatograms of pure 2,4-dinitrophenylhydrazones of oxalacetic acid (a), 2-oxoglutaric acid (b), pyruvic acid (c), 2-oxobutyric acid (d), 2-oxoisovaleric acid (e), 2-oxo-3-methylvaleric acid (f), 2-oxoisocaproic acid (g), levulinic acid (h), their mixture (R) and 2,4-dinitrophenylhydrazine (DN). The chromatographic solvent was petroleum ether–ethyl formate–propionic acid (26:14:3) and the adsorbent was Silica Gel G. The solvents in which the components, their mixture and the reagent were applied to the thin layers were dioxan (Fig. 1 a) and a (1:1, v/v) pyridine–water mixture (Fig. 1 b).

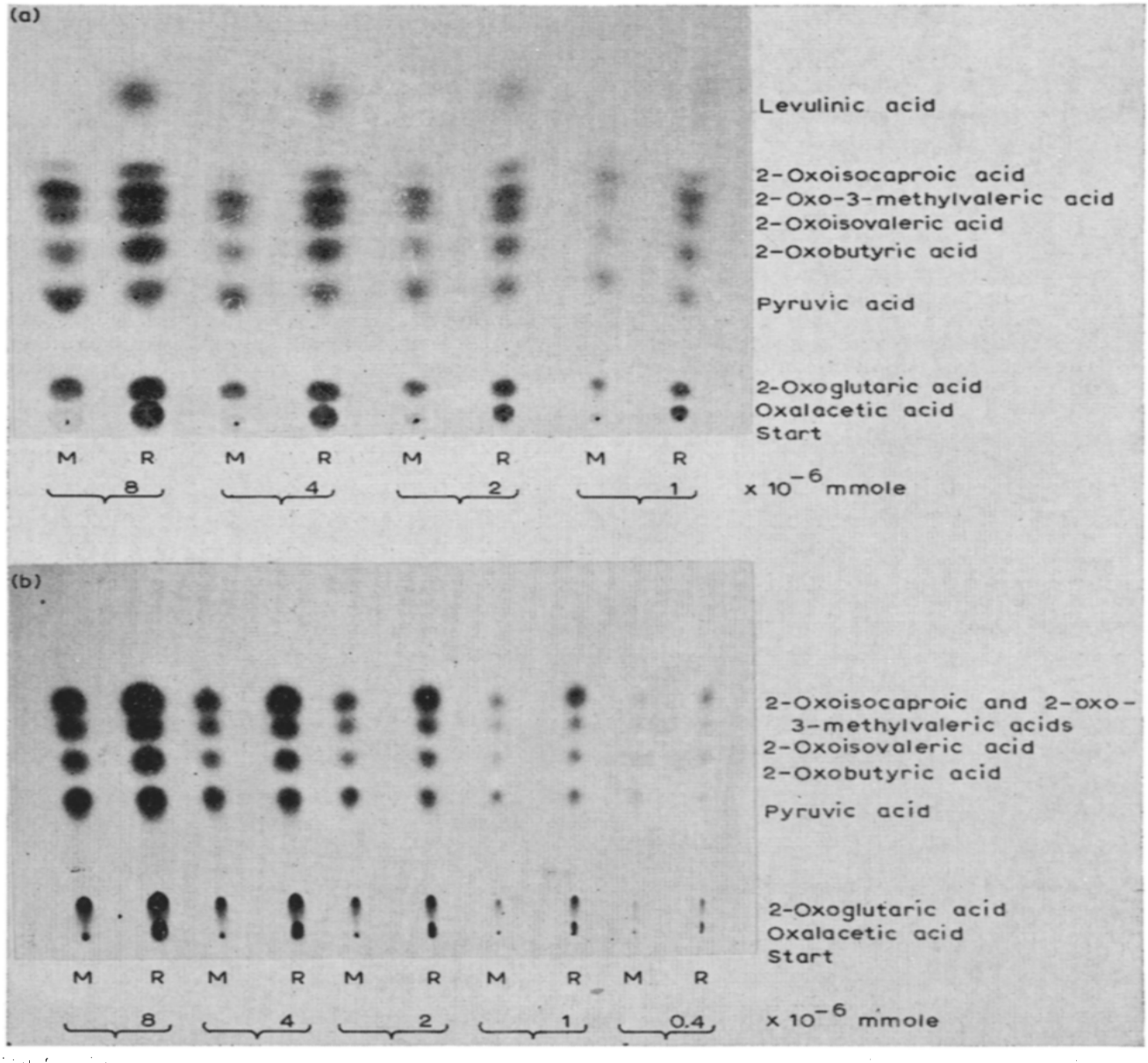
its distribution over several spots seems to depend on the solvent in which the compounds are applied to the thin layer. When the solvent is dioxan (Fig. 1a), part of the reagent migrates between pyruvic acid and 2-oxoglutaric acid hydrazones, although most of the reagent migrates before the hydrazones close behind the solvent front. When the solvent in which the compounds are applied to the thin layer is a pyridine–water mixture (Fig. 1b), all of the reagent migrates immediately behind the solvent front.

The thin-layer chromatograms in Figs. 2a and 2b are those of the keto acid hydrazone mixtures M and R. The mixture M was isolated by adsorption from 8 wt. % aqueous ethanol and R was the reference mixture. The mixtures were applied in the pyridine–water solution to the starting line on the thin-layer plate when the chromatogram of Fig. 2a was run, and in dioxan solution when the chromatogram of Fig. 2b was run. In both cases the chromatographic solvent was the petroleum ether–ethyl formate–propionic acid (26:14:3) mixture. The solvent for application of the mixtures M and R to a thin layer was exchanged by evaporating the solutions of the mixtures M and R remaining after samples of these mixtures in the pyridine–water solvent had been taken for application to one plate (Fig. 2a) to dryness in a Rotavapor and dissolving the residue in dioxan for application to the other thin-layer plate (Fig. 2b). The amounts of the mixtures applied to the thin layers were varied similarly and hence the intensities of the spots of each component should have been equal in the two parallel chromatograms if the isolation of the keto acid hydrazones by adsorption and elution had been complete. No spots due to levulinic acid hydrazone and only a weak spot due to oxalacetic acid hydrazone are seen in the chromatogram for mixture M in Fig. 2a; the other components were more completely isolated. No spot due to levulinic acid hydrazone is observed in the chromatogram for the reference mixture R in Fig. 2b either, and it must be concluded that the greater part of this compound must have decomposed when the solution of the mixture R in the pyridine–water mixture was evaporated to dryness in the Rotavapor. The decomposition products migrated close behind the solvent front. The decomposition of levulinic acid hydrazone in the evaporation stage also explains why the compound gave no spot in the chromatogram for the mixture M in Fig. 2a; this mixture was eluted with pyridine–water azeotrope from carbon and the effluent was evaporated to dryness in the Rotavapor. Experiments carried out with levulinic acid alone revealed that the low yield was not only due to the decomposition during the evaporation in the Rotavapor but also to the fact that its dinitrophenylhydrazone was less effectively eluted from carbon than the other keto acid hydrazones. The decomposition of levulinic acid hydrazone can be avoided to some extent by carrying out the evaporation of the solvent at a low pressure and by not evaporating the solution to complete dryness, but even then only traces of the levulinic acid hydrazone are found in the chromatogram.

The disappearance of oxalacetic acid hydrazone during the isolation process is due to the instability of this compound, which, as established in separate experiments, releases carbon dioxide and changes into the pyruvic acid derivative. This decarboxylation hence increases the intensity of the spot containing pyruvic acid hydrazone in the thin-layer chromatogram.

Fig. 2c shows a thin-layer chromatogram of the mixture M′ of aldehyde hydrazones and keto acid hydrazones isolated together by adsorption on carbon from

8 wt. % aqueous ethanol and elution from the carbon only with the pyridine–water azeotropic mixture (without prior elution of the aldehyde hydrazones with methyl formate and dichloromethane) and of the reference mixture R of keto acid hydrazones. In contrast to the experiments already described, all the carbonyl compounds, aldehydes, dicarbonyl compounds and keto acids mentioned previously (Part I) except pyruvic acid were initially added to the 8 wt. % aqueous ethanol, the purpose being to obtain information about the possible decarboxylation of oxalacetic acid. Both mixtures were applied to the thin layer in the pyridine–water (1:1, v/v) mixture.



Figs. 2a (upper) and 2b (lower). Thin-layer chromatogram of the mixture M of 2,4-dinitrophenylhydrazones of keto acids isolated by adsorption on carbon from aqueous ethanol and elution from the carbon, and that of the reference mixture R of pure keto acid hydrazones. The chromatographic solvent was petroleum ether–ethyl formate–propionic acid (26:14:3) and the adsorbent was Silica Gel G. The solvents in which the mixtures were applied to the thin layers were a (1:1, v/v) pyridine–water mixture (Fig. 2a) and dioxan (Fig. 2b).

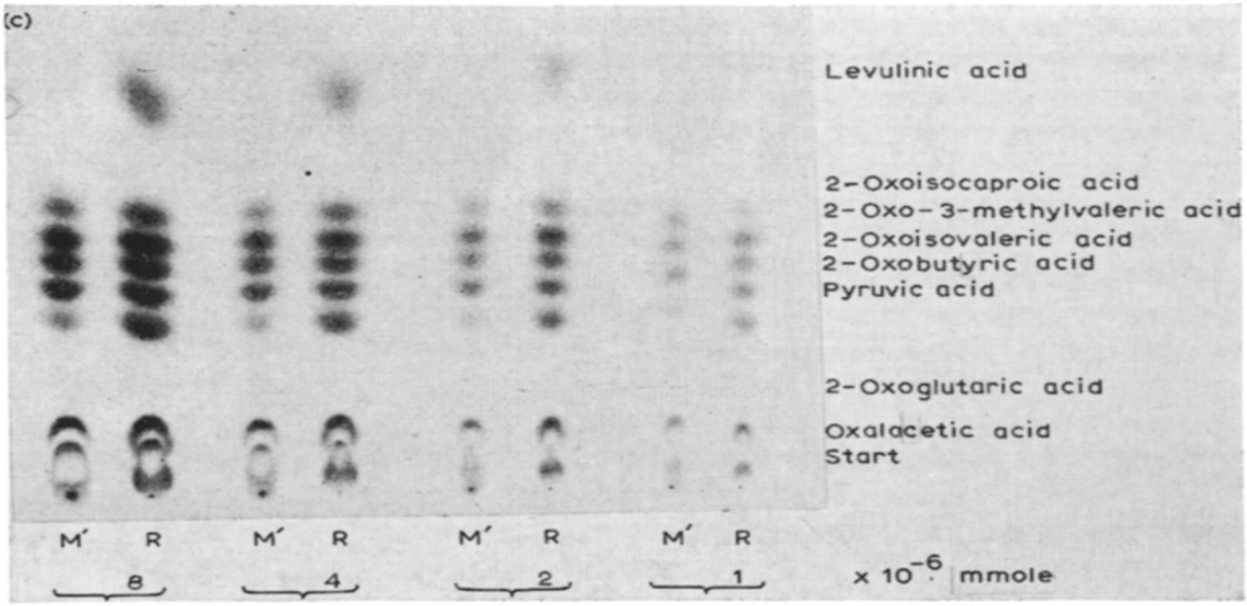


Fig. 2c. Thin-layer chromatogram of the mixture M′ of 2,4-dinitrophenylhydrazones of carbonyl compounds isolated by adsorption on carbon from aqueous ethanol and elution from the carbon with the pyridine–water azeotrope (without prior extraction of aldehyde hydrazones) and of the reference mixture R of pure keto acid hydrazones. The chromatographic solvent was petroleum ether–ethyl formate–propionic acid (26:14:3) and the adsorbent was Silica Gel G. The solvent in which the mixtures were applied to the thin layer was a (1:1, v/v) pyridine–water mixture.

The solvent employed in running the thin-layer chromatogram was the petroleum ether–ethyl formate–propionic acid (26:14:3) mixture used to obtain the chromatograms of Figs. 1a, 1b, 2a and 2b. When this chromatographic solvent mixture is used, the aldehyde hydrazones and dinitrophenylhydrazine do not interfere with the identification of the keto acid hydrazones as they all migrate as one group ahead of the keto acid hydrazones near the solvent front. The spots of the corresponding components in the mixtures M′ and R should have been equal in size and intensity. The presence of oxalacetic acid in mixture M′ is clearly evident, although the acid had undergone partial decarbcxylation as there are weak spots due to pyruvic acid hydrazone (which was not initially present) in the chromatogram of the mixture.

SUMMARY

A study has been made of the resolution of a mixture of pyruvic acid, 2-oxobutyric acid, 2-oxoisovaleric acid, 2-oxoisocaproic acid, 2-oxo-3-methylvaleric acid, levulinic acid, 2-oxoglutaric acid and oxalacetic acid 2,4-dinitrophenylhydrazones, and the possible interference of 2,4-dinitrophenylhydrazine in identification of the components, by the application of thin-layer chromatography. With pure keto acid hydrazones (mixture R) as reference standard, the completeness of the isolation by the adsorption and elution technique (Part I), of these keto acids as their hydrazones (mixture M) from aqueous ethanol was examined. The partial decomposition of oxalacetic acid hydrazone was also examined by making a comparison of the intensities of the spots of the components of both the test and reference mixtures in the chromatograms.

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