Chromatographic identification of carbonyl compounds VII. Thin-layer chromatographic resolution of mixtures of keto acid methyl ester 2,4-dinitrophenylhydrazones*

Numerous solvent mixtures and adsorbents have been utilised in attempts to develop a method for the separation of 2,4-dinitrophenylhydrazone mixtures of keto acid methyl esters on thin layers, so that each component occurs in only one spot. The method developed has already been reported briefly in a preliminary communication[1], and is discussed below in greater detail, with consideration being given to the possible interference of the reagent with identification of the components, and an examination is made of the completeness of isolation of the components from aqueous ethanol.

Experimental

Treatment and dissolution of keto acid methyl ester hydrazones. Two hundredths of a millimole of each of the pure hydrazones of pyruvic, 2-oxobutyric, 2-oxoisovaleric, 2-oxoisocaproic, 2-oxo-3-methylvaleric, levulinic, 2-oxoglutaric and oxalacetic acid methyl esters were weighed and dissolved in a 10-ml volume of dioxan (for chromatography, E. Merck AG) and their mixture R containing 0.02 mmole of each component was likewise dissolved in 10 ml of dioxan.

A mixture of the same keto acid hydrazones, isolated from 4 l of the 8 wt. % aqueous ethanol solution by adsorption on carbon and selective elution (first the aldehyde hydrazones and then the keto acid hydrazones) from the latter (Part I), was treated with methanol containing hydrogen chloride to liberate the acid hydrazones from their pyridinium salts. After evaporation of the solvent, the residue was esterified with diazomethane in diethyl ether at 0° (*cf.* Part V). The resulting ester mixture, designated M, was isolated and dissolved in 10 ml of dioxan.

Thin-layer chromatography and staining of the spots. A 4-μl volume of the dioxan solution of each keto acid methyl ester hydrazone, 4 μl of the solution containing their mixture R and two different volumes, 4 and 40 μl, of dinitrophenylhydrazine solution (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. Equal volumes (4, 2, 1 and 0.5 μl) of the solutions of the mixtures M and R were applied side by side to a second activated thin layer of Silica Gel HF_{254}. The plates were equilibrated in the vapour of the chromatographic solvent mixture composed of 18 volumes of petroleum ether (boiling range 60–80°, British Drug Houses Ltd.), 2 volumes of pyridine (Baker Analyzed Reagent, J. T. Baker Chemical Co.) and 1 volume of diethyl oxalate (purum, Fluka AG) in a closed chamber for half an hour before the run. The running time was 2 h in the closed chamber and then 2 h in the open chamber. The chromatograms were first photographed and then sprayed with a mixture containing 3 volumes of petroleum ether, 1 volume of pyridine and 1 volume of epichlorohydrin (purum, Fluka AG) and kept in a closed chamber above the same solvent mixture for one hour, after which the chromatograms were photographed again.

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* For Parts I–VI of this series, see *J. Chromatog.*, 27 (1967) 374, 380, 384; 28 (1967) 253, 259, 263.

Results and discussion

Thin-layer chromatograms of the hydrazones of the keto acid methyl esters. Fig. 1 shows the thin-layer chromatogram of the individual pure 2,4-dinitrophenylhydrazones of the keto acid methyl esters, their mixture R and dinitrophenylhydrazine. The photograph was taken one hour after the chromatogram had been sprayed with the (3:1:1) petroleum ether–pyridine–epichlorohydrin mixture. The spots that had turned red were encircled by continuous lines and those that had remained yellow by

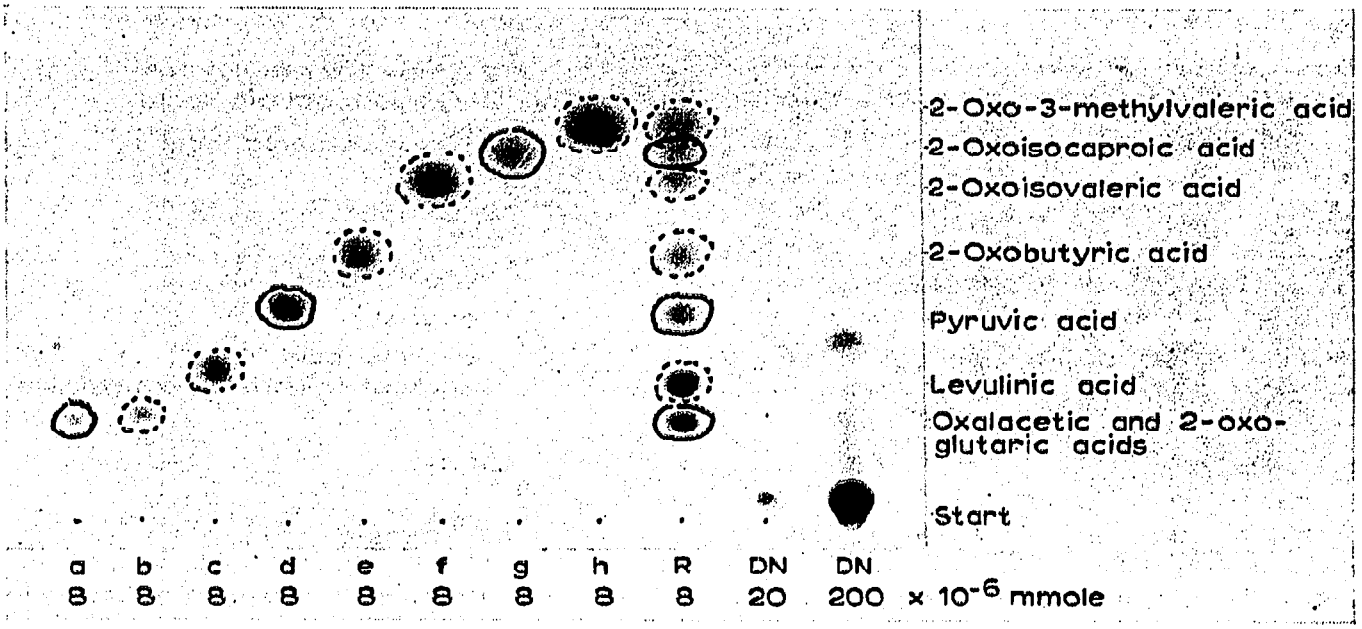

Fig. 1. Thin-layer chromatogram of pure 2,4-dinitrophenylhydrazones of oxalacetic (a); 2-oxoglutaric (b); levulinic (c); pyruvic (d); 2-oxobutyric (e); 2-oxoisovaleric (f); 2-oxoisocaproic (g); 2-oxo-3-methylvaleric (h) acid methyl esters; their mixture (R) and 2,4-dinitrophenylhydrazine (DN). The chromatographic solvent was petroleum ether–pyridine–diethyl oxalate (18:2:1) and adsorbent Silica Gel HF_{254}. Spots that became reddish within one hour after spraying the plate with a (3:1:1) mixture of petroleum ether–pyridine–epichlorohydrin are encircled by continuous lines, and spots which remained yellow by broken lines.

broken lines. Even the latter spots, except the spot of the levulinic acid derivative, gradually turned red during the course of several hours. It is possible, by this method, to identify the isomers 2-oxoisocaproic acid and 2-oxo-3-methylvaleric acid owing to the different rates of staining of their hydrazones; the derivative of the former changes colour more rapidly than that of the latter, although their R_F values differ only slightly. Each keto acid methyl ester hydrazone gave only one spot when the petroleum ether–pyridine–diethyl oxalate (18:2:1) mixture was used as chromatographic solvent, but some of the hydrazones gave two successively migrating spots of stereoisomers when the ratio of the last two solvent components was varied. The spots of the dinitrophenylhydrazones of monomethyl 2-oxoglutarate and monomethyl oxalacetate overlap, but the spots of the other keto acid ester hydrazones are satisfactorily resolved.

Fig. 2 shows a similarly produced thin-layer chromatogram of a mixture M of keto acid methyl ester hydrazones isolated from the 8 wt. % aqueous ethanol solution and of the reference mixture R.

The amounts of the mixtures applied to the thin layer were varied similarly, and the intensities of the corresponding spots should have been equal if the adsorption and elution had been quantitative. There is no sign of a spot due to levulinic acid in the chromatogram of the mixture M. The possible existence of oxalacetic acid in small quantities among other keto acids cannot be determined by this method because monomethyl oxalacetate hydrazone migrates at the same rate as monomethyl

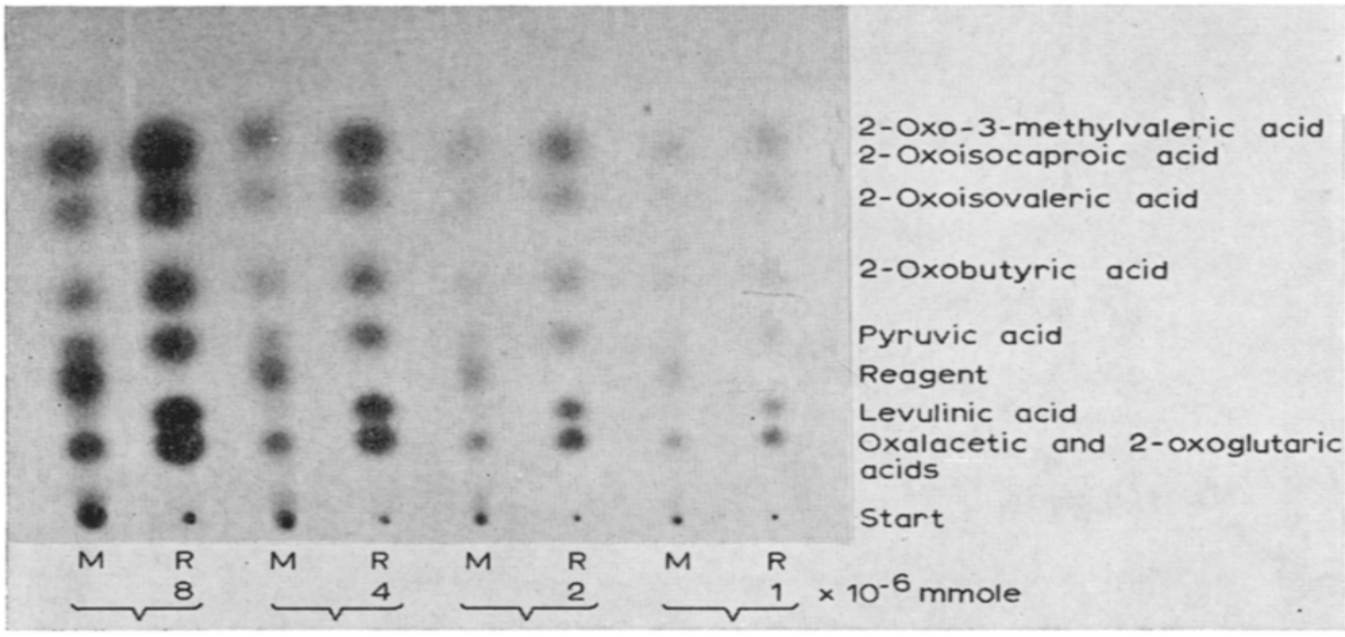

Fig. 2. Thin-layer chromatogram of the mixture M of 2,4-dinitrophenylhydrazones of keto acid methyl esters prepared by esterification with diazomethane of keto acid hydrazones isolated by adsorption on carbon from aqueous ethanol and elution from the carbon, and of the reference mixture R of pure keto acid methyl ester hydrazones. The chromatographic solvent was petroleum ether–pyridine–diethyl oxalate (18 : 2 : 1) and adsorbent Silica Gel HF_{254}. This thin-layer chromatogram was photographed before spraying.

2-oxoglutarate hydrazone. All the keto acid methyl ester hydrazones in the mixture M, except those of levulinic acid and oxalacetic acid, could be identified on the basis of their locations and different rates of staining of the spots. One additional spot due to dinitrophenylhydrazine is seen in the chromatogram of the mixture M (*cf.* Fig 1). The reagent, however, does not interfere with the identification of individual components.

Research Laboratories of the State Alcohol Monopoly (Alko), Helsinki (Finland)

P. RONKAINEN

1 P. RONKAINEN, *J. Chromatog.*, 20 (1965) 403.

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