

The separation and staining of methyl esters of the 2,4-dinitrophenylhydrazones of keto acids on thin layers of silica gel

Aliphatic keto acids that are of interest as metabolites can be chromatographed as their 2,4-dinitrophenylhydrazones on thin layers^{1,2}. They can also be analysed by treating their dinitrophenylhydrazones with diazomethane followed by liberation of the methyl esters by oxidation with ozone and resolution of these esters by gas chromatography³. The purpose of this study was to develop a method of thin-layer chromatography for separating the methyl esters of the dinitrophenylhydrazones of the keto acids and staining them for identification. The keto acids studied were oxalacetic acid, α -ketoglutaric acid, levulinic acid, pyruvic acid, α -ketobutyric acid, α -ketoisovaleric acid, α -ketoisocaproic acid and α -keto- β -methylvaleric acid. Attempts to separate the dinitrophenylhydrazones of the last two acids on thin layers were not successful^{1,2}.

Materials and methods

α -Keto- β -methylvaleric acid was prepared by condensing *sec.*-butyl-magnesium chloride and diethyl oxalate and saponifying the resulting ester^{4,5}. The other keto acids were commercial products. Their conversion to dinitrophenylhydrazones and esters was carried out as described previously^{1,3}. The thin-layer adsorbent was Silica gel HF₂₅₄ according to Stahl from E. Merck A.G., Darmstadt. Four micrograms of each acid dissolved in dioxan was added separately and in a mixture to a layer of Silica gel activated by heating. The plate was chromatographed, first in a closed chamber and then in an open chamber, for a period of about 4 h with a solvent mixture composed of 18 vol. of petroleum ether (60-80°C, B.D.H.), 1 vol. of diethyl

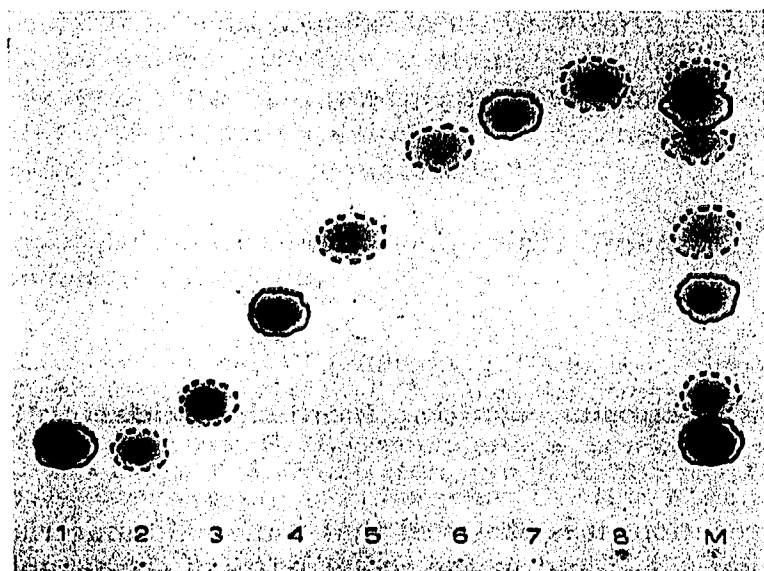


Fig. 1. Thin-layer chromatogram of the methyl esters of dinitrophenylhydrazones of keto acids. Spots that turned red within one hour after the chromatogram was sprayed with the staining mixture are encircled with a continuous line and the spots that remained yellow with a broken line. The acids whose dinitrophenylhydrazones were converted to methyl esters for the chromatography were (1) oxalacetic acid, (2) α -ketoglutaric acid, (3) levulinic acid, (4) pyruvic acid, (5) α -ketobutyric acid, (6) α -ketoisovaleric acid, (7) α -ketoisocaproic acid, and (8) α -keto- β -methylvaleric acid. M denotes the mixture of the derivatives of the eight keto acids.

oxalate (purum, Fluka A.G.) and 2 vol. of pyridine ("Baker analyzed", Reagent, J. T. Baker Chemical Co.). After the chromatographic run, the plate was sprayed with a 3:1:1 mixture of petroleum ether, pyridine and epichlorohydrin (purum, Fluka A.G.) and stored exposed to vapours from the same solvent mixture in a closed chamber for about 1 h, after which it was photographed.

Results

Each keto acid gave rise to one spot when the chromatogram was run as described. The derivatives of oxalacetic and α -ketoglutaric acids migrated at the same rate, but the other components were satisfactorily separated. All the spots remained the same colour, yellow, for some time after they were sprayed. The first to change its colour to red was the derivative of oxalacetic acid, followed by the derivatives of pyruvic and α -ketoisocaproic acids. The chromatogram in Fig. 1 was photographed 1 h after being sprayed; the red-coloured spots are encircled by a continuous line, and the yellow spots by a broken line. All the components turned red within several hours except the derivative of levulinic acid which was the last to change. The structural isomers α -ketoisocaproic acid and α -keto- β -methylvaleric acid, whose dinitrophenylhydrazones are difficult to separate by thin-layer chromatography^{1,2}, can be identified in the form of the methyl esters of their dinitrophenylhydrazones on a thin layer on the basis of their different rates of staining despite the small difference in the R_F values.

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Thin-layer chromatography of tarry deposits resulting from the oxidation of aromatic hydrocarbons

In the study of certain gaseous reactions it is observed that some solid material is deposited on the walls of the reaction vessel and in the cooler parts of the tubing connecting the reactor to the rest of the apparatus. Typical examples of reactions forming these deposits are the pyrolysis of hydrocarbons¹ and their derivatives², and the oxidation of aromatic hydrocarbons³. The deposits generally account for only a small proportion of the reactants, and it is usual to regard them as the products of a relatively unimportant side-reaction.

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