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Note

Separation of keto acid 2,4-dinitrophenylhydrazones by thin-layer electrophoresis

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Numerous systems for the separation of keto acid 2,4-dinitrophenylhydrazones (DNPH's) by paper or thin-layer chromatography have been reported[1–3] and in most of these systems the dicarboxylic keto acid DNPH's show low mobilities relative to the monocarboxylic keto acid derivatives. In the course of current work on the degradation of phenolic compounds by soil microorganisms it became necessary to separate the DNPH of 3-oxoadipic acid from other keto acids and neutral carbonyl compounds without recourse to the customary solvent→alkali→reacidification→solvent extraction procedures for the isolation of keto acid DNPH's and it was visualised that such separations might be achieved by thin-layer electrophoresis (TLE). This paper reports the development of a simple technique for the separation of keto acid DNPH's by TLE on cellulose thin layers.

EXPERIMENTAL

Materials

DNPH derivatives of keto acids were prepared by the addition of a slight excess of 0.2% 2,4-dinitrophenylhydrazine in 2 *N* HCl to an aqueous solution of the keto acid and allowing the mixture to react at 25° for 30 min. The keto acid DNPH's were then extracted into a small quantity of ethyl acetate and stored at 4°.

Thin-layer plates for chromatography were prepared from Whatman CC41 or Macherey, Nagel & Co. MN300 cellulose powder. Previous experience had shown that the robust nature of cellulose thin layers made them preferable for TLE separations.

Thin-layer electrophoresis

Sample spots of keto acid DNPH's were applied to the dry cellulose layers as a row of spots 20 mm from the cathode end of the plate. The plates were held nearly vertical and then carefully and uniformly sprayed with buffer solution until they became translucent. Care was taken to avoid "flooding" and the risk of sample migration. Electrophoresis was carried out in a Camag Model 61-701 TLE cell (Camag, Muttenz, Switzerland) using a water-cooled platen and a potential gradient of 20 V/cm. (For a full description of the TLE procedure refer to Walker and Thompson[4].)

RESULTS AND DISCUSSION

Preliminary experiments to establish the optimum conditions for TLE separations of keto acid DNPH's showed that the monocarboxylic keto acids were poorly separated below pH 7.5, but that good separation could be obtained between pH 7.5 and 9.5. The procedure finally adopted used 0.02 *M* ammonium carbonate/

TABLE I

RELATIVE ELECTROPHORETIC MOBILITIES OF KETO ACID 2,4-DINITROPHENYLHYDRAZONES

Cellulose thin layers: 0.02 *M* ammonium carbonate/bicarbonate buffer pH 8.8; potential gradient 20 V/cm.

Keto acid	R_M *value*
Oxaloacetic	109
3-Oxoadipic	73
2-Oxoglutaric	100
Glyoxylic	59
Laevulinic	50
2-Oxobutyric	18
Phenylpyruvic	13*, 38
Pyruvic	35*, 55
2,4-Dinitrophenylhydrazine	0

* Faint spot.

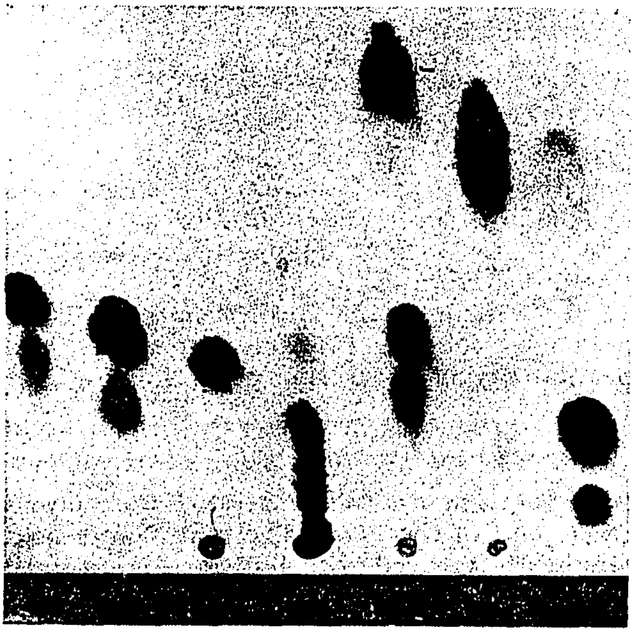

Fig. 1. Thin-layer pherograms of keto acid 2,4-dinitrophenylhydrazones (0.02 *M* ammonium carbonate/bicarbonate buffer, pH 8.8). The various keto acid DNPH's are identified as follows: Gly = glyoxylic; Pyr = pyruvic; Lev = laevulinic; αKB = 2-oxobutyric; OxAc = oxaloacetic; αKG = 2-oxoglutaric; Ph-pyr = phenylpyruvic.

bicarbonate buffer at pH 8.8 and under these conditions, with a potential gradient of 20 V/cm, good separations of keto acid DNPH's were achieved after 45 min. Experiments with mixed cellulose/silica gel thin layers did not yield as good separations as did those with cellulose only.

The results of these experiments are summarised in Table I whilst Fig. 1 shows the appearance of the final TLE plate: As with chromatographic separations, pyruvic acid DNPH was separated into its *syn* and *anti* isomers. Thus TLE appears to offer a useful alternative to established chromatographic procedures for keto acid DNPH's since it is rapid and has the advantage of readily differentiating mono- and dicarboxylic keto acids. By contrast with most chromatographic systems the latter show the highest R_M values under TLE so that combined TLE/TLC might be an advantageous system for two-dimensional separations of keto acid DNPH's. In the present work the authors have found the isopropanol–ammonia–water (20:1:2) solvent system[2,3] satisfactory for paper and thin-layer chromatography.

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