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Note

Detection of volatile fatty and dicarboxylic acids on alkaline pherograms with Mills' reagent

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The separation of the title acids by high-voltage paper electrophoresis in alkaline medium has been demonstrated by Gross[1,2]. A solution of the volatile salt, ammonium carbonate, was used as electrolyte, and the separated acids were located on dried pherograms as their ammonium salts using a solution of an acid–base indicator, bromophenol blue or bromocresol purple, as spray reagent.

Sensitive detection of the acids by this method depends on complete removal of the background electrolyte, and Gross accomplished this by heating the papers at 80°C for 10 min.

An alternative detection reagent which does not require the removal of the electrolyte (with consequent risk of loss of some of the more volatile fatty acids) is the manganous sulphate–potassium permanganate–sulphuric acid mixture described by Mills[3]. With this reagent, the choice of electrolyte is not restricted to volatile salts, and sodium carbonate and sodium tetraborate buffers may also be used.

EXPERIMENTAL

Materials

The acids were pure commercial preparations. They were prepared for use as 0.2 *M* solutions in water either in free form or as their sodium salts.

Electrolyte

Sodium hydrogen carbonate–sodium carbonate solution (pH 9.2) containing 6.72 g sodium hydrogen carbonate and 1.06 g anhydrous sodium carbonate in 1 l of water.

Spray reagent[3]

This is prepared as two stock solutions: (a) 0.1% (w/v) $MnSO_4 \cdot 4H_2O$ in aqueous 1 *M* sulphuric acid, and (b) 0.5% potassium permanganate in water. Four volumes of (a) are mixed with one volume of (b) immediately before use.

Apparatus and procedure

The enclosed-strip paper electrophoresis apparatus and procedure are de-

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scribed in detail elsewhere[4]. Whatman No. 4 paper was used and the test solutions were applied across the middle of paper strips by means of a platinum wire loop delivering 0.5 μl. Caffeine was used as marker for zero migration and the 4-nitrobenzenesulphonate ion as the standard of anionic rate from which M_N values were calculated for the relative mobilities of the experimental acids. For convenience, 10^2 M_N values are recorded in Table I.

Electrophoresis was allowed to proceed for 40 min at about 25 V cm^{-1}, the temperature on the paper strips being maintained at 20°C. The papers were then heated at 100°C until just dry and examined under a Hanovia Chromatolite UV lamp to reveal the marker compounds. The papers were sprayed with the freshly-mixed reagent and, without drying, laid flat on a glass plate at room temp. Within 2–3 min, the purple background changed to brown and the experimental acids were simultaneously located as violet spots. These spots were visible for only about one minute before they were obscured by the uniform deposition of brown manganese dioxide. A permanent record was obtained by stopping the reaction while the spots were still visible by dipping the papers into dilute sodium carbonate solution. They were washed thoroughly and dried, the acids then being seen as pale buff spots against the brown ground. Alternatively, a record was made by outlining the spots as they appeared with a waterproof marker ink, neutralising and drying the papers as convenient later.

RESULTS AND DISCUSSION

The Guyard reaction, by which manganous ions reduce permanganate to manganese dioxide, was adapted by Mills[3] for the detection on paper of organic compounds that are oxidisable by permanganate. Phenols and phenolic acids[5], aromatic amines, reducing sugars[3], α-hydroxy acids[5] and olefinic compounds[6], including unsaturated acids, all react more or less rapidly with the spray reagent, bleaching the permanganate and thus appearing as white or pale yellow spots against the purple permanganate background which, after an induction period of a few minutes during which the Guyard reaction takes place, changes to brown as manganese dioxide is precipitated on the fibres of the paper.

The saturated fatty acids and dicarboxylic acids higher in the series than malonic do not react with permanganate and it was not expected that they would be detected in this way. It was found, however, that the Guyard reaction is retarded in their presence and that when the general deposition of manganese dioxide occurs on the paper background, the acids are visible as coloured spots where the deposition is delayed.

Some inorganic ions, notably phosphate and fluoride, are known to retard the Guyard reaction, probably by complexing with and thus stabilising one of the reaction intermediates, the manganic [Mn(III)] ion[7,8]. Phosphate and fluoride therefore also give coloured spots. In fact, as little as 0.1 μg of fluoride is detectable in this way. All spots, for these inorganic ions and for the carboxylic acids alike, are of the same distinctive shade of violet, and it seems likely that the acids are acting in a similar way to the inorganic ions to interfere with the course of the Guyard reaction. Spots of formic acid containing 0.25 μmole are required for easy detection, but all other acids tested were readily detected in 0.1 μmolar quantities after electrophoresis under the standard conditions.

The relative mobilities of the homologous series of fatty acids, C_1–C_7, expressed as 10^2 M_N values as defined in the Experimental section, are given in Table I, as numerical data for this series were not recorded by Gross[1].

Values for the mobilities of the dicarboxylic acids at various levels of pH have been recorded previously[2,9–12]; present values measured in carbonate electrolyte at pH 9.2 are included in Table I for comparison. Oxalic and malonic acids are detectable as permanganate-reactive compounds, not because they retard the Guyard reaction. Oxalic acid reacts in a uniquely characteristic way on contact with the spray reagent. It gives an immediate pale yellow spot on the purple ground, but the spot does not persist; it soon becomes brown, even before the background deposition of manganese dioxide begins. It was noted previously[5] that the mobilities of a series of aliphatic hydroxy acid anions measured in borate buffer were all significantly lower than those in carbonate buffer of the same pH and sodium ion concentration. It is now confirmed that carboxylic acids in general run more slowly in the presence of

TABLE I

RELATIVE MOBILITIES OF ACIDS IN CARBONATE-BICARBONATE ELECTROLYTE (pH 9.2)

Acids were detected with Mills' reagent[3] after paper electrophoresis at 25 V cm^{-1} and 20°C for 40 min. M_N values express mobilities relative to the 4-nitrobenzensulphonate ion of absolute mobility 9.3 cm/h · kV.

Acid	10^2 M_N
Fatty	
Formic	184
Acetic	139
Propionic	125
Butyric	111
Isobutyric	112
Valeric	101
Hexanoic	95
Heptanoic	90
Dicarboxylic	
Oxalic	209
Malonic	180
Methylmalonic	163
Succinic	161
Glutaric	151
Adipic	143
Pimelic	138
Suberic	131
Miscellaneous	
Pivalic	99
Benzoic*	104
Monomethyl succinate	108
Phthalic*	144
Tricarballylic	169
Phosphate (HPO_4)	165
Fluoride	201

* Also detectable on dried pherograms as "absorbing" spots under an UV lamp.

borate. The mobilities of all the acids used in the present study were similarly reduced in 0.05 *M* sodium tetraborate buffer, by an average of 14% of the corresponding values in the carbonate electrolyte as given in Table I.

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