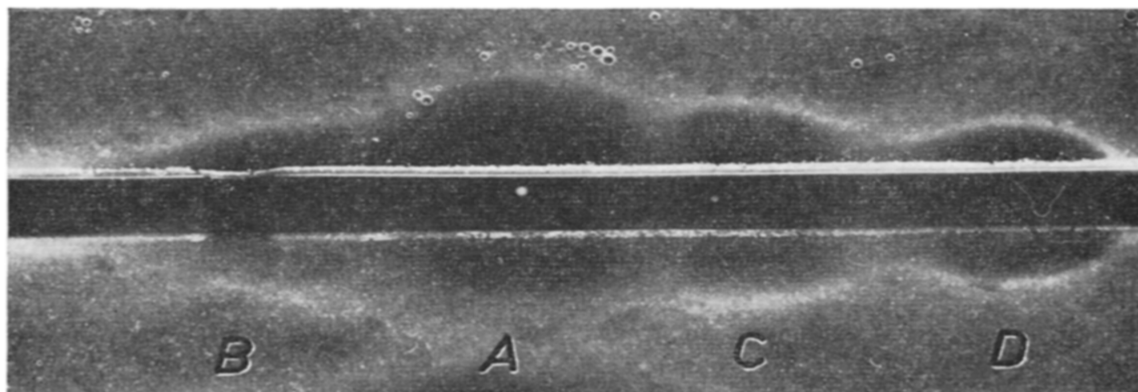

Fig. 1. Die extrazellulären Desoxyribonukleasen des Stammes S 84 von *Streptococcus pyogenes.*

S 84 (Typ 3). Dieser Stamm entlässt in das Nährmedium vier verschiedene Desoxyribonukleasen, welche sich nicht nur elektrophoretisch sondern auch in anderen Eigenschaften unterscheiden. Drei von ihnen können mit den von WANNAMAKER[2] beschriebenen Enzymen A, B und C identifiziert werden. Das vierte entspricht wahrscheinlich dem von AYOUB UND WANNAMAKER[4] erwähnten Enzym und soll deshalb Enzym D genannt werden.

Mit dem Rest des Elektrophoresestreifens kann man zusätzliche Untersuchungen anstellen (z.B. Bestimmung des pH-Optimums und anderer Fermenteigenschaften). Hierzu wird der Streifen in zentimeterbreite Fraktionen zerlegt und diese tiefgefroren. Die nach dem Auftauen abgeschiedene Flüssigkeit (evtl. Zugabe eines kleinen Volumens Puffer) enthält das Ferment.

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Eingegangen den 21. Januar 1964

J. Chromatog., 15 (1964) 107–108

The analysis of straight-chain (n-C_1–C_9) carboxylic acids by a thin-layer chromatographic method

Straight chain carboxylic acids of low molecular weight are frequently encountered as by-products of biological and chemical processes. The identification of individual acids of this type can be difficult, particularly as they often occur, or are recovered, in dilute aqueous solution. Straight chain carboxylic acids have been analysed by paper chromatography[1–8]. A thin-layer chromatographic method has been described[9] for which it is shown that for the carboxylic acids of even carbon numbers 4 to 18 and

odd carbon numbers 5 to 11 there is a linear relationship between log R_F and the total carbon number of the acids. However, a good separation of the acids of lower molecular weight was not obtained. Another thin-layer chromatographic method is described[10] for the separation of formic, acetic, pyruvic and lactic acids. We have developed a much improved separation by thin-layer chromatography of the straight chain carboxylic acids (n-C_1–C_9) in dilute aqueous solutions.

Experimental and results

Development of a suitable solvent system. The majority of the solvents described in the literature[1–10] for the analysis of low molecular weight fatty acids by paper and thin-layer chromatography are mixtures of a lower alcohol, water and ammonia. Preferably the acids are separated as their salts, *e.g.* salts of the acid with morpholine, ethylamine, ammonia, etc., or as derivatives, *e.g.* hydroxamic acids.

The separation of n-C_1 to C_9 carboxylic acids was examined (both the free acids and the ammonium salts being used) on thin-layer plates of neutral silica gel with various solvent systems including those previously employed by other authors[1–10]. It was found, however, that the best separation was obtained with the solvent system methyl acetate–ammonia 2.5 % vol. aq. (95:5, v/v) and this was adopted for the subsequent investigations.

Spray reagents. Many of the common acid–base indicators were examined as spray reagents for locating the acid zones on the chromatoplate. Alcoholic methyl red was found to be the most suitable.

The separation procedure. The separation of the acids is carried out in the usual way, a 20 cm × 20 cm plate covered with silica gel being used. When the solvent front has reached a line on the chromatoplate line indicating the limit of travel, the plate is removed from the solvent tank and placed in an oven at 105° for 2–3 min to evaporate the solvents. After cooling, the plate is replaced in the tank and again developed with the solvent. Finally the plate is sprayed with alcoholic methyl red

TABLE I

R_F VALUES OF LOW MOLECULAR WEIGHT, STRAIGHT CHAIN CARBOXYLIC ACIDS (AND OF SOME BRANCHED CHAIN ACIDS FOR REFERENCE PURPOSES)

Acid	R_F		
	After double run in fresh solvent	*After double run in solvent aged for 24 h*	*After single run in solvent aged for 24 h*
Formic	0.05	0.07	0.03
Acetic	0.10	0.13	0.06
Propionic	0.15	0.30	0.15
n-Butyric	0.24	0.40	0.22
n-Valeric	0.39	0.50	0.30
n-Hexanoic	0.52	0.57	0.34
n-Heptanoic	0.55	0.60	0.39
n-Octanoic	0.58	0.66	0.43
n-Nonanoic	0.61	0.69	0.45
Trimethylacetic	0.57	0.71	0.47
α-Methylbutyric (*dl*)	0.39	0.65	0.39
β-Methylbutyric	0.34	0.53	0.31
Isobutyric	0.27	0.57	0.32

solution and heated in the oven at 105° until the acids appear as dark red spots on an orange background. The R_F values of the acids are recorded in Table I.

The effect of solvent aging. If the developing solvent was allowed to stand for 24 h in a stoppered flask before use, the R_F values were greatly increased so that only a single development was required in order to obtain a satisfactory separation. Results obtained with the aged solvent are also shown in Table I. Because the R_F values of the acids depend upon the age of the solvent it is clearly necessary with unknown mixtures to run the reference acids at the same time.

Applicability of the method to steam distillates. Since straight chain carboxylic acids of low molecular weight are often isolated from oil products by steam distillation the application of the method to such distillates was investigated. Analysis of steam distillates obtained from a blend of C_1–C_6 acids clearly indicated that as the distillation proceeded the concentrations of the higher molecular weight acids in the distillate became less. Thus, the combination of steam distillation and thin-layer chromatography promises to be a quick and simple way of separating and identifying the straight chain carboxylic acids of low molecular weight encountered in the study of chemical and biological processes.

Discussion

Excellent separation of the n-C_1 to C_9 carboxylic acids is obtained either by a single development with the aged solvent mixture methyl acetate–aqueous ammonia or by a double development with fresh solvent. The acids are separated as their ammonium salts, the excess ammonia being driven off by heating the plate after development so that the acids can be located as compact red zones when sprayed with methyl red solution. The separation takes about half an hour for a single development so that the complete analysis including the time taken to prepare the plate requires one hour. The method is very sensitive and it is estimated that 5 μg of an acid can be detected.

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Received December 2nd, 1963