CHROM. 3940

Purification of N-trifluoroacetyl amino acid *n*-butyl esters for analysis by gas chromatography

GEHRKE AND STALLING[1] have developed a gas chromatographic method for the analysis of N-trifluoroacetyl *n*-butyl (TAB) derivatives of the 20 amino acids commonly found in proteins. Although all the TAB derivatives may be easily prepared in an impure form, many are oils which cannot be crystallised or are unstable during distillation. The impure preparations frequently contain small amounts of unreacted amino acids which produce multiple peaks during chromatography. In some cases these peaks are eluted slowly and interfere with the resolution of the derivatives of other amino acids. This type of contamination becomes important when radioactive TAB amino acids are eluted and trapped at the end of the column for the measurement of radioactivity, since minor contaminants of high specific activity will obscure TAB amino acids of low radioactivity.

This paper describes a method developed to purify radioactive TAB amino acids using silica gel column chromatography before gas–liquid chromatography (GLC). The incompletely reacted amino acids which are more polar than the TAB amino acids may be separated by elution with organic solvents of various polarities.

Experimental

Silicic acid (British Drug Houses, Poole, England) was soaked overnight in 10 *N* hydrochloric acid, washed with water, ethanol and chloroform and heated overnight at 120°. The silicic acid was then mixed with a light petroleum (b.p. 60–80°) and poured into a 20 × 1 cm column. The TAB derivatives were dissolved in 100–200 μl of dichloromethane and applied to the top of the column, which was then eluted successively with mixtures of 1%, 10% and 50% (v/v) ether in light petroleum, then ether and finally with methanol. Radioactivity in the eluted fractions was measured, using a

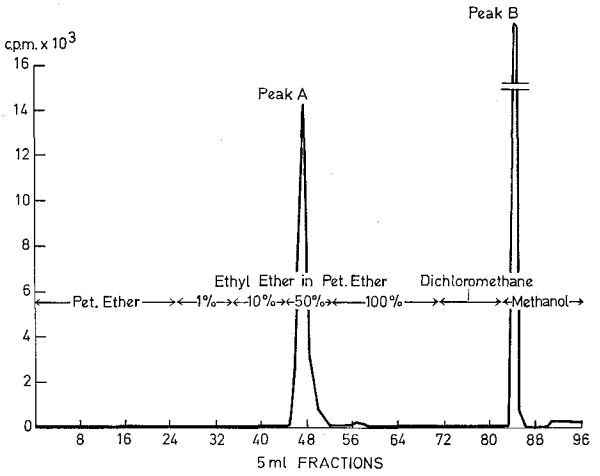

Fig. 1. Distribution of radioactivity in fractions obtained by chromatography on silica gel of the TAB derivative of [U-^{14}C]-L-alanine.

Tri-Carb scintillation counter 314EX, in a toluene phosphor (efficiency 70%) and the radioactive fractions were analysed by GLC. As an example the preparation and the purification of the TAB derivative of [U-^{14}C]-L-alanine is described.

[U-^{14}C]-L-Alanine (sp. act. 2.2 $\times$ 10^6 d.p.m./μmole) was converted to the TAB derivative by the procedure described by GEHRKE AND STALLING[1] and the excess reagents evaporated at a temperature below 60° on a rotary evaporator. The product was fractionated on a silicic acid column by treatment with the solvents indicated (Fig. 1). Samples were analysed for radioactivity and representative fractions were examined by GLC at 140° using the conditions of BLAU AND DARBRE[2].

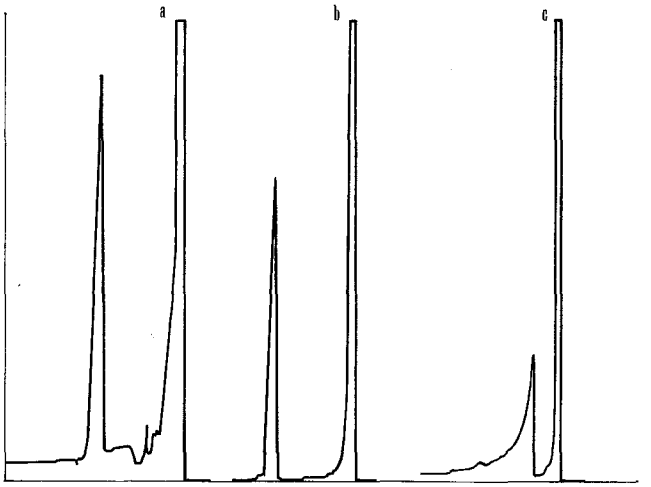

Fig. 2. (a) Gas–liquid chromatography pattern of the unpurified TAB derivative of [U-^{14}C]-L-alanine. (b) Gas–liquid chromatography pattern of the TAB derivative of [U-^{14}C]-L-alanine after purification by silica gel chromatography (this corresponds to peak A in Fig. 1). (c) Gas–liquid chromatography pattern of the methanol eluate (peak B, Fig. 1) by silica gel purification. This behaved similarly to the butyl ester of L-alanine.

Results

Without purification by silica gel chromatography two major and several minor components were detectable by GLC (Fig. 2a). The two major components were separated by silica gel chromatography and gave single radioactive peaks (Figs. 2b and c) in the gas chromatograph, the second compound eluted with methanol from the silica gel column having the same chromatographic behaviour as the *n*-butyl ester of alanine.

Other TAB amino acids may be similarly prepared as well as the *n*-butyl ester of mono- and di-trifluoroacetyl derivatives of the hydroxy amino acids.

Discussion

This method has been used mainly to obtain pure TAB amino acids from impure preparations from tissues and is particularly useful where GLC is used in conjunction with measurements of radioactivity in the GLC eluent since the specific activity of small amounts of purified derivatives can be measured under conditions where quantitation cannot be achieved.

With extracts containing radioactive amino acids and prepared from the livers of mice injected with [U-^{14}C]-glucose the procedure was simplified. The impure mixture of TAB amino acids was applied to the silica gel column, which was washed with 100 ml of light petroleum (b.p. 60–80°), and then the TAB derivatives were eluted with 50 ml of diethyl ether. Also, standard TAB amino acids may become contaminated during preparation and storage but may be readily purified before use by silica gel chromatography.

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Amino acid micro determination with a standard single-column analyzer

In investigations of protein structure the amount of material available for quantitative amino acid analysis and degradation as well as the rate of analysis per day if often a limiting factor. Therefore, it was advantageous to have a single-column system available for the determination of all protein constituent amino acids from one sample, as was described first by PIEZ AND MORRIS[1]. Instruments based on this principle are commercially available (Phoenix Precision Instrument Co., Philadelphia, Pa; Technicon Instrument Co., Ltd., Hanworth Lane, Chertsey, Surrey). Previously, for a complete analysis at least 0.1 μmole of sample had to be applied to the top of a column 0.6 cm (or more) in internal diameter, though only part (30% or less) of the resolved material was used for quantitative detection by the analytical system. BYFIELD[2] described the use of a column 0.3 × 120 cm for the usual 22-hour chromatogram, which permitted sub-micro determination in connection with a voltage amplifier attached to the flow cell colorimeter.

In this paper a similar set of modifications for a standard model of an amino