The use of amino acids as stationary phase in gas chromatography

Part 1. Monosodium L-glutamate

The analysis of amino acids is an essential part of peptide and protein studies. Not only is it of importance for the determination of the overall composition of these biological materials but it has acquired added importance in studies of amino acid sequence in peptide chains. Present available methods in sequence studies, such as paper chromatography, paper electrophoresis and ion exchange chromatography are all time-consuming procedures. On the contrary, gas chromatographic analysis, which needs neither a large amount of sample nor a long operating time, might be useful in studies on proteins. Several attempts to employ gas-liquid chromatography for amino acids have been made[1-7].

JOHNSON AND MEISTER[8] have recently reported on the chromatography of a number of N-acetylamino acid *n*-amyl esters using 2- to 8-foot column packed with Chromosorb W coated with 0.5% to 5% polyethylene glycol (Carbowax 1540 or 6000). We investigated the gas chromatographic analysis of N-acetylamino acid *n*-amyl esters on 2- to 3-meter column packed with Carbowax 1540 coated onto Chromosorb according to JOHNSON *et al.*, and have found that this method was unfortunately time-consuming for the elution, and moreover, it gave unsatisfactory resolution of these derivates.

In the present note, the unique application of an optically active amino acid as selective stationary phase is described for the rapid and effective separation of amino acid derivatives. 10%-monosodium L-glutamate coated onto sodium chloride (commercial name, "Aji-Shio", Ajinomoto Co.,) was found to give satisfactory results. Furthermore, we are trying to extend the application of optically active amino acids to the gas chromatographic resolution of optical antipodes.

Apparatus

A Perkin-Elmer Model 154-D gas chromatograph equipped with a hydrogen flame-ionization detector was used. The carrier gas was nitrogen. A 50 cm to 2 m U-shaped stainless-steel column $^1/_4$ in. external diam. was packed with monosodium L-glutamate coated onto sodium chloride, or with monosodium L-glutamate or L-glutamic acid when used without a support medium.

Materials

N-acetylamino acid esters were prepared with HCl–alcohol and acetic anhydride. Derivatives were analysed by infrared spectroscopy. Fatty acids and fatty acid methyl esters made by Tokyo Kasei Co. were used without further purification.

Results

The gas chromatograms of some amino acid derivatives and fatty acids on "Aji-Shio" are given in Figs. 1 and 2, respectively. All solutes were eluted very rapidly and moreover, the resolution of each peak was very high. In the above experiments a very wide range of particle sizes of commercial "Aji-Shio" was used. In Figs. 3 and 4 (the chromatograms of amino acid derivatives and fatty acid methyl esters, respectively), sieved "Aji-Shio" (50–100 mesh) was used. As can be seen from Figs. 1–4, the reten-

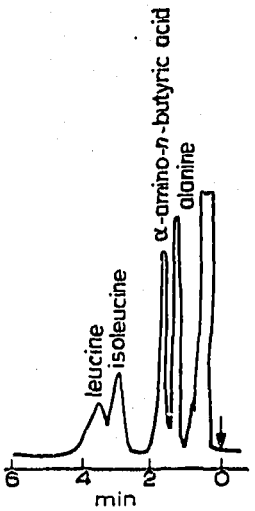

Fig. 1. Chromatogram of N-acetyl-*n*-amyl esters of 4 amino acids on a 2 m column packed with commercial "Aji-Shio" (10% monosodium glutamate coated on to NaCl). Temperature: 159°; carrier gas: nitrogen; Flow rate: 66ml/min.

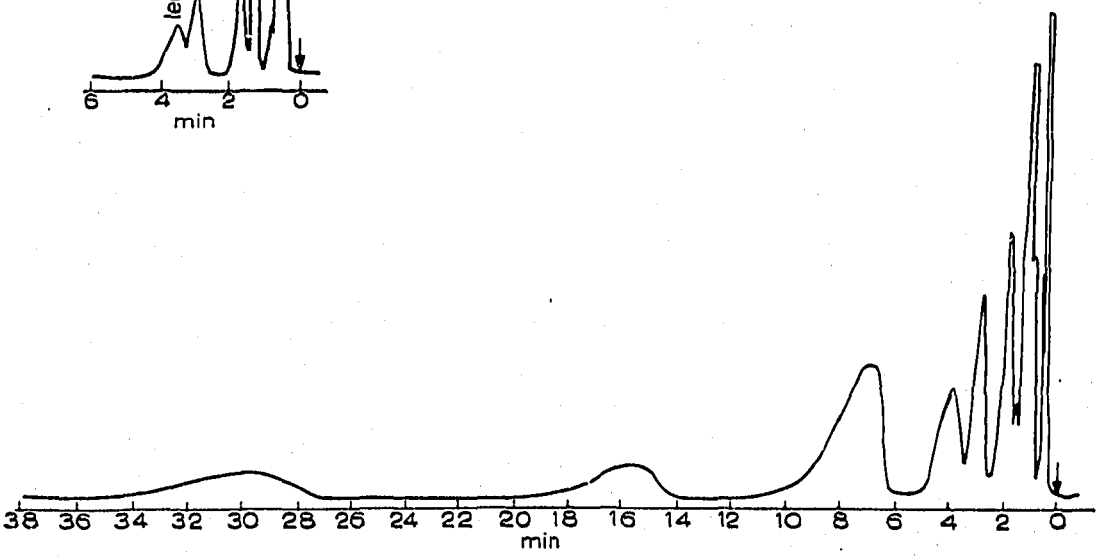

Fig. 2. Chromatogram of a mixture of C_1–C_8 acids on commercial "Aji-Shio". Column length: 1 m; Temperature: 134°; carrier gas: nitrogen; Flow rate: 48 ml/min.

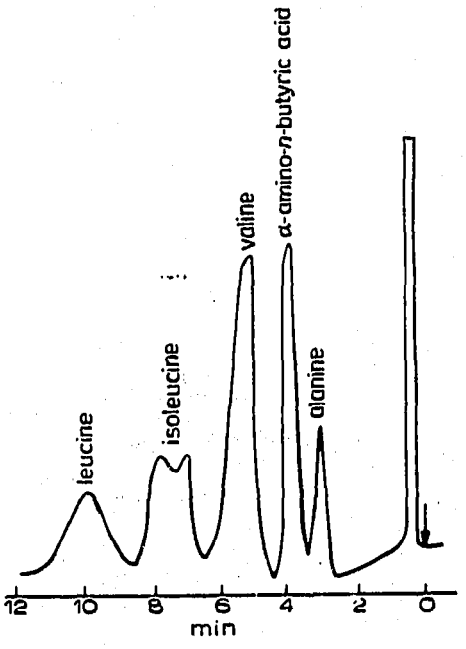

Fig. 3. Separation of N-acetyl-*n*-amyl esters of 5 amino acids on a 2 m column containing 10% monosodium glutamate on NaCl (50–100 mesh) at 155° and 45 ml of N_2 per minute.

Fig. 4. Separation of methyl esters of several fatty acids at 155° and 53 ml of N_2 per minute on a 2 m column containing 10% monosodium glutamate on NaCl (50–100 mesh).

Fig. 5. Chromatogram of N-acetyl-*n*-amyl esters of 4 amino acids on a 50 cm column packed with monosodium glutamate (50-100 mesh); no NaCl used as support. Temperature: 159°; Flow rate: 40 ml/min of N_2.

Fig. 6. Chromatogram of a mixture of C_1–C_8 acids on a 50 cm column packed with nonosodium glutamate (50-100 mesh); no NaCl used as a support. Temperature: 143°; Flow rate: N_2 53 ml/min.

Fig. 7. Chromatogram of methyl esters of fatty acids on a 50 cm column packed with glutamic acid (50-100 mesh) at 145° and 53 ml/min of N_2.

tion times of all components were very short and were the same for sieved "Aji-Shio" as for the unsieved material, but the sieved material gave higher resolution. DL-Isoleucine showed two incompletely resolved peaks. We confirmed that these two peaks correspond to D-alloisoleucine and L-isoleucine, respectively.

The chromatograms of some derivatives of amino acids and fatty acids on 50-100 mesh monosodium L-glutamate without support are shown in Figs. 5 and 6; this gave short retention times but poor resolution. It has been suggested that a satisfactory resolution could be obtained under suitable chromatographic conditions of column length, column temperature, particle size, etc. In Fig. 7, a chromatogram of fatty acid methyl esters on 50–100 mesh L-glutamic acid without a support, the same result was obtained as that when monosodium L-glutamate was used.

Hitherto, for the complete separation of active-amyl alcohol and iso-amyl alcohol a very long column was required and then the retention time was long. It was found that "Aji-Shio" is useful for the separation of these alcohols as is shown in Fig. 8.

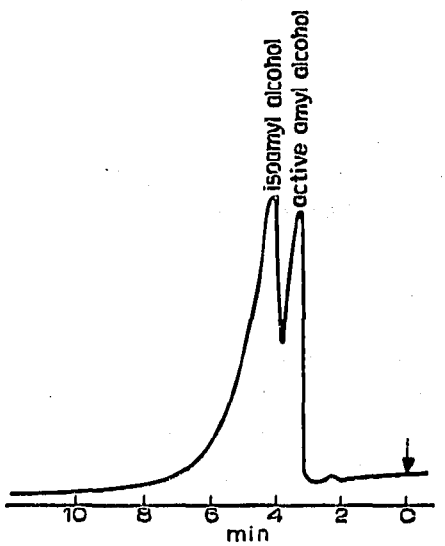

Fig. 8. Separation of active-amyl alcohol and iso-amyl alcohol on a 1 m column containing 10% monosodium glutamate on NaCl (50–100 mesh) at 48° and 48 ml/min of N_2.

Monosodium L-glutamate is stable at high column temperatures (up to 210°) and this fact permits its use in the separation of high-boiling compounds without the danger of decomposition of the stationary phase.

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