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Note

The use of amino acids as stationary phases in gas chromatography

II. Polyglutamate

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In Part I[1] we reported the separation of fatty acids, organic acids, amino acids and alcohols on monosodium L-glutamate or L-glutamic acid coated on to sodium chloride (commercial name Aji-shio, Ajinomoto Co., Kawasaki, Japan) as the column packing.

In this paper is reported the separation of hydrocarbons,, alcohols, ketones, fatty acids, organic acids, isomers of toluidine, amino acids and the resolution of racemic amino acids using poly-γ-methyl-D-glutamate (D-PMG) or poly-γ-ethyl-L-glutamate (L-PEG), which are used as artificial leather (commercial name Aji coate, Ajinomoto Co.). Also reported are the characterization and classification of D-PMG as the stationary phase by measuring the retention volumes of three different polar solutes on each commercial stationary phase[2].

EXPERIMENTAL

Apparatus and conditions

An F & M Model 402 gas chromatograph equipped with dual flame ionization detectors linked with a Honeywell recorder was used. U-shaped glass columns of dimensions 5.5 ft. × 1/4 in. O.D. were used, packed with 2.5% D-PMG or L-PEG on 80–100 mesh Diasolid M (Nihon Chromato Co., Tokyo, Japan), 10% D-PMG (or L-PEG) on 60–80 mesh Diatoport S (Hewlett-Packard, Avondale, Pa., U.S.A.) and 1% D-PMG or L-PEG on 100–120 mesh Supercoport (Supelco, Bellefonte, Pa., U.S.A.). Helium was used as the carrier gas at a flow-rate of 60 ml/min. A 3-μl portion of the sample was injected into the gas chromatograph.

Reagents and materials

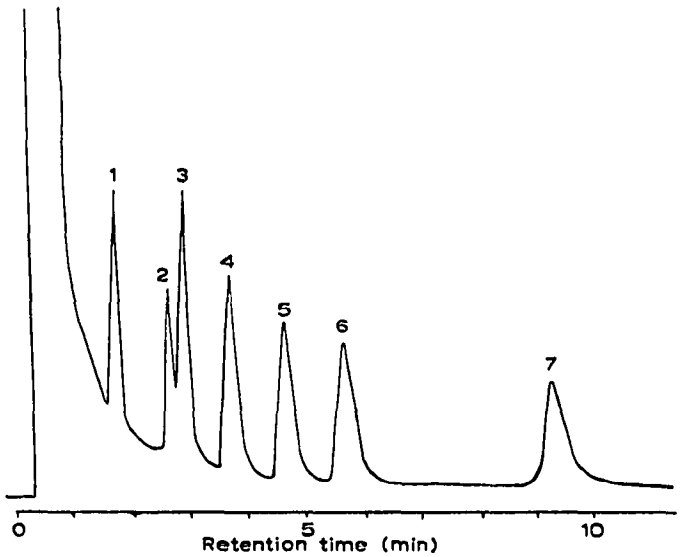
All solvents used in this study were of reagent grade. Fatty acid methyl esters, organic acid methyl esters, alcohols, hydrocarbons and ketones were purchased from Tokyo Kasei (Tokyo, Japan) and used without further purification. Amino acids were obtained from Ajinomoto. Hypovial and trifluoroacetic anhydride were purchased from Pierce (Rockford, Ill., U.S.A.).

Preparation of amino acid derivatives. N-Trifluoroacetyl amino acid *n*-butyl esters were prepared according to the method of Roach *et al.*[3] N-Trifluoroacetyl-L-prolyl amino acid methyl esters were prepared according to the method described elsewhere[4].

RESULTS

Hydrocarbons, alcohols, ketones, and organic acid methyl esters could be separated on D-PMG. Although the isomers of *o*-, *m*- and *p*-toluidine were separated on D-PMG, the isomers of xylene were not separated completely. Typical gas chromatograms are shown in Figs. 1 and 2.

The separation of saturated and unsaturated fatty acid methyl esters were



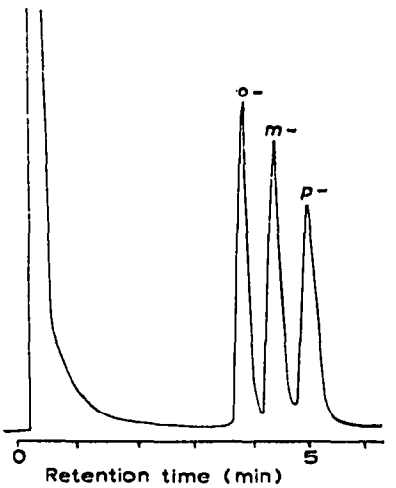


Fig. 1. Gas chromatogram of organic acid dimethyl esters at 150°. Peaks: 1 = oxalate; 2 = malonate; 3 = fumarate; 4 = succinate; 5 = maleate; 6 = glutarate; 7 = adipate.

Fig. 2. Gas chromatogram of toluidines at 150°.

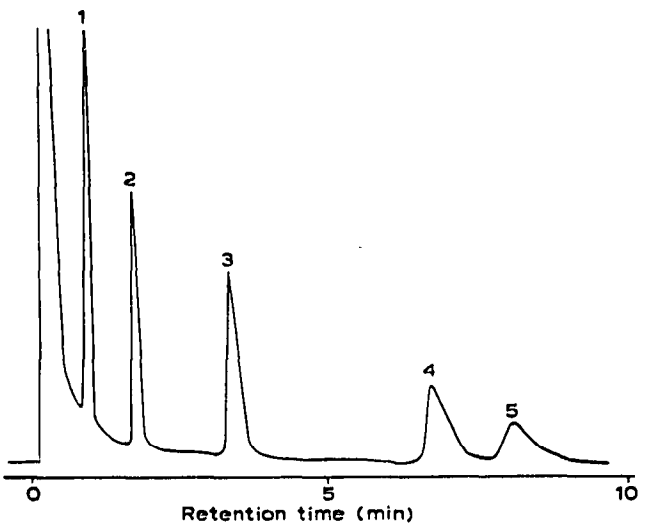


Fig. 3. Gas chromatogram of fatty acid methyl esters at 210°. Peaks: 1 = laurate; 2 = myristate; 3 = palmitate; 4 = stearate; 5 = linolenate.

studied. Although the peaks of methyl stearate (C_{18}) and oleate (Δ^1-C_{18}) overlapped, C_{18} (or Δ^1-C_{18}), linolate (Δ^2-C_{18}) and linolenate (Δ^3-C_{18}) could be separated on D-PMG. A typical gas chromatogram is shown in Fig. 3.

As amino acids are not sufficiently volatile to permit direct analysis, they must be converted into suitable volatile derivatives prior to gas chromatography. In this study, the amino acids were converted into N-trifluoroacetyl *n*-butyl esters and separated on D-PMG. Alanine, valine, isoleucine, glycine, threonine and proline were investigated. A gas chromatogram of the amino acid derivatives is shown in Fig. 4.

The resolution of racemic amino acids by gas chromatography has been carried out by many investigators. Two different methods have been adopted: (1) derivatization of the enantiomeric amino acids with optically active reagents to form diastereoisomers, which are chromatographed on optically inactive stationary phases[5–8]; and (2) derivatization of the enantiomeric amino acids with optically inactive reagents and chromatography of the derivatives on optically active stationary phases[9–11]. As D-PMG and L-PEG are optically active amino acid polymers, the author investigated the resolution of racemic amino acids, which were converted into N-trifluoroacetyl isopropyl esters on packed columns or capillary columns coated with D-PMG or L-PEG. The racemic amino acids could not be resolved.

The author also studied the resolution of racemic amino acids that were converted into N-trifluoroacetyl-L-prolyl amino acid methyl esters on D-PMG or L-PEG. Alanine, valine and leucine were not separated; aspartic acid, glutamic acid, isoleucine and phenylalanine were not separated completely; and proline, methionine and threonine could be separated on both stationary phases. The separation of three amino acids is shown in Fig. 5.

As can be seen in Fig. 5, D-amino acids always had shorter retention times than L-amino acids on both stationary phases. The order of elution of racemic amino acids

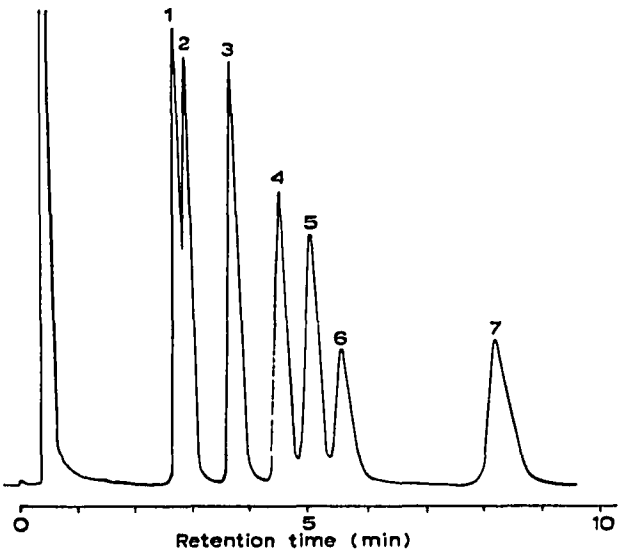


Fig. 4. Gas chromatogram of N-trifluoroacetyl amino acid *n*-butyl esters at 180°. Peaks: 1 = alanine; 2 = valine; 3 = isoleucine; 4 = leucine; 5 = glycine; 6 = threonine; 7 = proline.

seemed to be influenced by the L- or D-configuration of amino acids, and not by the optical activity of the stationary phases.

The author studied the classification of D-PMG by measuring the retention volumes of three compounds on each commercial stationary phase according to the method of Brown[2]. The retention volumes of the three compounds, *n*-heptane (b.p. 98.4°), *sec.*-butanol (b.p.100°) and diethyl ketone (b.p. 101.5°), were measured on a number of commercial stationary phases. The classification of the stationary phases is shown in Fig. 6.

It can be seen in Fig. 6 that D-PMG has similar properties to polyethylene glycol adipate and is closer to the diethyl ketone apex than polyethylene glycol adipate.

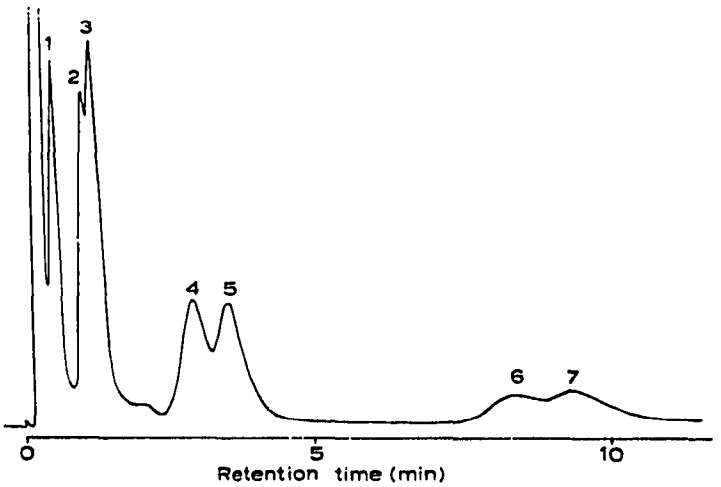


Fig. 5. Gas chromatogram of N-trifluoroacetyl L-prolyl amino acid methyl esters on 1% D-PMG at 210°. Peaks: 1 = unknown; 2 = D-isoleucine; 3 = L-isoleucine; 4 = D-proline; 5 = L-proline; 6 = D-glutamic acid; 7 = L-glutamic acid.

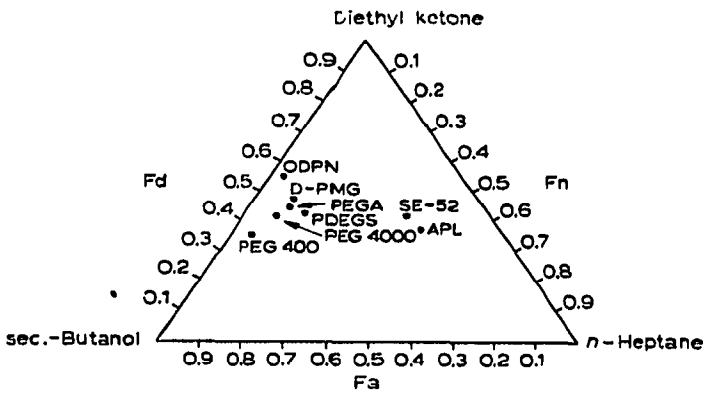


Fig. 6. Classification of stationary phases. APL = Apiezon L; PEGA = polyethylene glycol adipate; PDEGS = polydiethylene glycol succinate; PEG = polyethylene glycol; ODPN = β,β'-oxydipropionitrile; SE-52 = silicone; D-PMG = poly-γ-methyl-D-glutamate. For definition of Fa, Fd and Fn, see ref. 2.

D-PMG and L-PEG are stable at high temperatures (up to 230°), which permits their use in the separation of high-boiling compounds without the risk of decomposition.

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