GAS CHROMATOGRAPHY OF VOLATILE AMINO ACID DERIVATIVES

II. LEUCINE, CYSTEINE, PROLINE, HYDROXYPROLINE, METHIONINE, PHENYLALANINE, ASPARTIC ACID AND GLUTAMIC ACID

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INTRODUCTION

In the previous paper[1] we reported on a variety of stationary phases which were screened for their possible application in the separation of the trifluoroacetyl (TFA) amino acid *n*-amyl esters of the seven most volatile amino acids. The present communication gives a similar set of data for the next seven amino acids, using the same volatile derivatives. For the reasons stated in the previous paper the separations were again carried out under isothermal conditions, but the number of phases screened is smaller, because some of those tried in the previous survey were not sufficiently stable at the higher temperatures required for these compounds.

MATERIALS AND METHODS

Apparatus

A D6 chromatograph (Griffin and George Ltd., Alperton, Middlesex) with a Martin gas-density balance detector was used. Columns consisted of two stainless steel tubes of 5 mm internal diameter connected at their lower end by a stainless steel capillary U-tube. Packed length was 182 cm. Nitrogen (99.9 % "White spot", British Oxygen Co., Wembley, Middlesex) was used as carrier gas.

Preparation of columns

Silocel C22 firebrick (L. Light & Co. Ltd., Colnbrook, Bucks.) was crushed, graded and deactivated, coated with stationary phase and packed as described previously[1].

Stationary phases

The following stationary phases were obtained from F & M Scientific Europa N.V., Leidsestraat 67, Amsterdam: F-50 (methyl chlorophenyl silicone), SE-52 (phenyl methyl silicone elastomer), SE-54 (phenyl vinyl methyl silicone elastomer), XE-60 (cyanoethyl silicone elastomer), BDS (1,4-butanediol succinic acid polyester), castorwax, celanese ester No. 9 (tripelargonate) and Versamid 900 (polyamide from dimerised linoleic acid and ethylene diamine). MS type silicones were obtained from

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Hopkin & Williams, Chadwell Heath, Essex. The following phases were obtained from Applied Science Laboratories Inc., State College, Pa., U.S.A. through the courtesy of Dr. F. A. VANDENHEUVEL of the Canada Department of Agriculture, Ottawa: Hi-Eff-8B (cyclohexanedimethanol succinic acid polyester), ECN:SS-S and EGCN:SS-S (ethylene glycol succinate–cyanoethyl silicone copolymers), and EGSS-X (ethylene glycol succinate–methyl silicone copolymer). DEGA (diethylene glycol adipate), DEGS (diethylene glycol succinate), PEG-S (polyethylene glycol succinate) and PPS (polypropylene succinate) were obtained from Griffin & George Ltd., M & B (dimethyl polysiloxane) was obtained from May & Baker Ltd., Dagenham, Essex, QF-1 (fluorosilicone fluids FS 1265, 10,000 cs) from Midland Silicones Ltd., Barry, Glamorgan, SE 30 (dimethyl silicone elastomer) from I.C.I. Ltd., Stevenston, Ayrshire, PEG-A (polyethylene glycol adipate) from W.G. Pye & Co., P.O. Box 60, Cambridge, DSPO (Duo Seal Pump Oil) from T.J. Sas & Son Ltd., Vernon Place, Holborn, W.C.1., PEG 20M (polyethylene glycol) from Union Carbide Ltd., Grafton St., London, W.1., and PEG-L (polyethylene glycol lauryl ether) from Honeywill & Stein Ltd., Mayfair Place, London, W.1. We are indebted to Advita Ltd., Wellington Avenue, Walton-on-Thames, for generous gifts of Admul 19 (polyglycerol ester of mixed fatty acids) and Admul S57 (polyglycerol ester of hardened tallow fatty acids).

The method of FARQUHAR *et al.*[2] was used to prepare the following polyesters: BDA (1,4-butanediol adipate), NPGS (*neo*-pentyl glycol succinate), NPGG (*neo*-pentyl glycol glutarate), NPG Seb (*neo*-pentyl glycol sebacate), and EAA (ethanolamine adipate).

Preparation of derivatives

The N-TFA amino acid *n*-amyl esters were made by the method described previously[1]. With cysteine the N,S-bis TFA and with hydroxyproline the O,N-bis TFA derivatives were obtained. With aspartic and glutamic acids the di-*n*-amyl esters were formed.

RESULTS AND DISCUSSION

In Tables I, II and III retention times relative to aspartic acid are given for seven amino acids and bicumyl, which is a suitable standard for this group.

Catalytic decomposition effects were noted for some of the phases: the derivatives affected were those of cysteine and of hydroxyproline. Usually the phases concerned had been found to have a catalytic effect on the decomposition of the threonine and serine derivatives. The stability of the N-TFA, O-TFA and S-TFA derivatives is being investigated more fully.

On most phases tested the order of emergence of the derivatives is proline, methionine, phenylalanine, aspartic acid and glutamic acid. However, the order of emergence of the cysteine, hydroxyproline and proline peaks shows considerable variation, although these three always appear before the methionine peak. As previously noted[1], XE-60, which is regarded as more polar than the other silicone type phases, shows a closer resemblance to the phases in Table II than to those in Table I. EAA (Table III) is noteworthy because aspartic acid precedes methionine as well as phenylalanine in the order of emergence from the column.

There is a remarkable difference between the retention times of aspartic and

TABLE I

RELATIVE RETENTION DATA FOR SILICONE TYPE STATIONARY PHASES

The figures are the retention times relative to the aspartic acid derivative taken as 1.00. The actual retention time in min for this derivative is given in brackets. All packings were prepared with 5% w/w of liquid phase to support. Gas flow: 38 ml N_2/min; column temperature: 190°.

Liquid phase	*N-TFA, n-amyl ester*	*N,S-bis TFA, n-amyl ester*	*N,O-bis TFA, n-amyl ester*	*N-TFA, n-amyl ester*			*N-TFA, di-n-amyl ester*		*Bi-cumyl*	*HETP (mm) Asp peak*
	Leu	*CySH*	*Hypro*	*Pro*	*Met*	*Phe*	*Asp*	*Glu*		
MS 115	0.24	0.29	0.38	0.35	0.54	0.78	(25.8)	1.65	0.88	0.8
MS 200	0.22	0.28	0.36	0.34	0.51	0.76	(19.6)	1.68	0.84	2.1
MS 550	0.19	0.22	0.34	0.35	0.49	0.72	(13.4)	1.69	0.81	1.3
MS 710	0.15	0.19	0.30	0.39	0.53	0.80	(28.3)	1.87	1.10	0.5
M & B	0.21	0.25	0.33	0.34	0.50	0.74	(12.8)	1.64	0.84	3.0
F-50	0.21	0.26	0.33	0.32	0.52	0.80	(23.0)	1.71	0.90	0.7
QF-1	0.22	0.36	0.63	0.43	0.57	0.68	(26.3)	1.83	0.26	1.0
SE-30	0.21	0.27	0.35	0.36	0.52	0.71	(23.0)	1.63	0.86	1.2
SE-52*	0.16	0.22	0.32	0.30	0.47	0.73	(32.4)	1.82	0.78	1.0
SE-54*	0.15	0.20	0.31	0.31	0.46	0.71	(42.8)	1.80	0.76	1.3
XE-60	0.17	0.40	0.52	0.34	0.73	0.82	(34.2)	1.97	0.29	1.0

* Column temperature: 170°.

TABLE II

RELATIVE RETENTION DATA FOR POLYESTER TYPE PHASES

The figures are the retention times relative to the aspartic acid derivative taken as 1.00. The actual retention time in min for this derivative is given in brackets. All packings were prepared with 5%w/w of liquid phase to support. Gas flow: 38 ml N_2/min; column temperature: 190°.

Liquid phase	*N-TFA, n-amyl ester*	*N,S-bis TFA, n-amyl ester*	*N,O-bis TFA, n-amyl ester*	*N-TFA, n-amyl ester*			*N-TFA, di-n-amyl ester*		*Bi-cumyl*	*HETP (mm) Asp peak*
	Leu	*CySH*	*Hypro*	*Pro*	*Met*	*Phe*	*Asp*	*Glu*		
BDA	0.14	0.35	0.37	0.29	0.78	1.02	(49.0)	2.45	0.54	0.9
BDS	0.15	0.36	0.40	0.31	0.73	0.93	(32.6)	1.99	0.48	0.9
Hi-Eff-8 B	0.14	—	—	0.30	0.70	0.94	(44.0)	2.10	0.61	0.9
DEGA	0.19	—	—	0.62	0.89	1.16	(39.3)	2.01	0.52	0.7
DEGS	0.15	—	—	0.37	0.81	1.03	(25.6)	1.99	0.50	0.7
NPGS	0.15	0.38	0.44	0.26	0.66	0.83	(49.3)	2.03	0.37	1.3
NPGG	0.14	—	0.41	0.28	0.72	0.91	(67.0)	2.16	0.42	0.9
NPG Seb	0.15	0.32	0.34	0.26	0.65	0.85	(44.0)	2.09	0.55	0.8
PEG-A	0.14	0.34	0.40	0.31	0.75	0.97	(47.8)	2.06	0.49	1.0
PEG-S	0.13	—	—	0.30	0.62	0.84	(56.5)	1.90	0.51	0.7
PPS	0.14	—	—	0.25	0.69	0.84	(68.5)	2.02	0.53	1.6

— = compound applied but gave no peak.

TABLE III

RELATIVE RETENTION DATA FOR MISCELLANEOUS TYPES OF STATIONARY PHASES

The figures are the retention times relative to the aspartic acid derivative taken as 1.00. The actual retention time in min for this derivative is given in brackets. All packings were prepared with 5% w/w of liquid phase to support. Gas flow: 38 ml N_2/min; column temperature: 190°.

Liquid phase	*N-TFA, n-amyl ester*	*N,S-bis TFA, n-amyl ester*	*N,O-bis TFA, n-amyl ester*	*N-TFA, n-amyl ester*			*N-TFA, di-n-amyl ester*		*Bi-cumyl*	*HETP (mm) Asp peak*
	Leu	*CySH*	*Hypro*	*Pro*	*Met*	*Phe*	*Asp*	*Glu*		
Admul 19	0.14	—	—	0.27	0.53	0.80	(55.5)	2.00	0.72	1.5
Admul S57	0.14	—	—	0.27	0.55	0.78	(50.0)	2.02	0.77	1.0
Castorwax	0.15	0.24	0.27	0.26	0.54	0.79	(55.2)	2.00	0.85	0.9
Celanese ester	0.14	0.28	0.31	0.22	0.54	0.77	(86.0)	2.07	0.60	1.1
DSPO	0.17	0.20	0.25	0.29	0.48	0.78	(36.0)	1.49	1.51	1.1
EAA*	0.12	—	—	0.32	1.18	1.25	(19.0)	2.26	0.38	1.7
ECNSS-S*	0.12	—	—	0.31	0.83	0.99	(74.2)	2.26	0.31	0.8
EGCNSS-S*	0.12	—	—	0.30	0.81	0.96	(69.5)	2.27	0.30	1.2
EGSS-X*	0.12	—	—	0.32	0.83	1.08	(53.2)	2.23	0.46	1.7
EGS	0.15	0.24	0.28	0.25	0.52	0.79	(58.2)	1.97	0.89	0.5
PEG 20M	0.12	—	—	0.30	0.62	0.82	(39.5)	1.67	0.58	2.3
PEG-L	0.12	—	—	0.30	0.62	0.85	(57.0)	1.96	0.63	0.5
Versamid 900	0.17	—	—	0.30	0.73	0.90	(39.2)	2.12	0.63	1.5

* Column temperature: 170°.
— = compound applied but gave no peak.

glutamic acids in view of the fact that there is a difference of only $-CH_2-$ between the molecules of the derivatives. A similar difference occurs between valine and leucine[1]. On the other hand, the proline and hydroxyproline derivatives differ by an O-TFA group but show only slight differences in retention times.

The inclusion of values for the leucine derivative makes it possible to relate the sequence on any phase of the amino acids in this investigation with that obtained with the previous group of amino acid derivatives[1]. It is important that the last peak

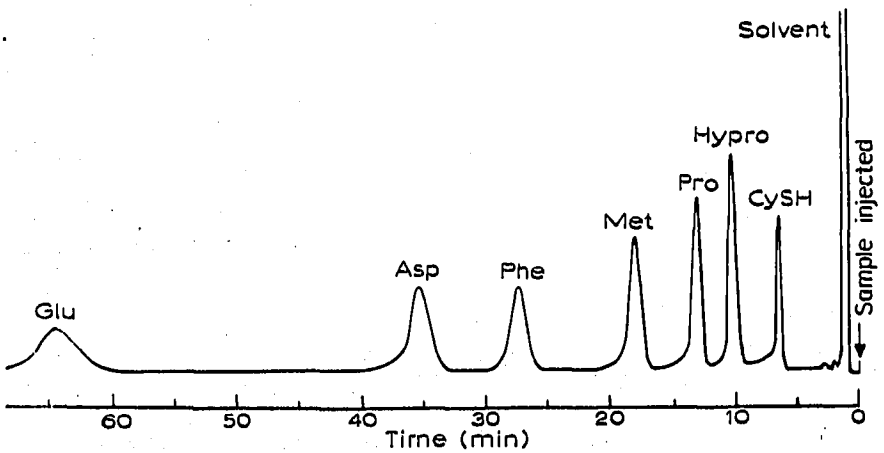

Fig. 1. Separation of a mixture of 7 trifluoroacetylated amino acid amyl esters, on Silocel C22 coated with 5% MS710 at 185°. Nitrogen flow rate: 38 ml/min.

of the first series will come off before the first peak of the second series. With every phase in Tables I, II and III the leucine peak emerges before that of any of the other seven amino acids; however, with DSPO (Table III) and some of the phases in Table I, some interference occurs as the leucine peak is close to the cysteine peak.

Of the phases investigated, only MS710 is capable of completely separating the seven derivatives (see Fig. 1). The next best phase is XE60, which, however, shows incomplete resolution of the methionine and phenylalanine peaks (Table I).

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SUMMARY

Data are presented for the relative retention times of the volatile derivatives prepared from leucine, cysteine, proline, hydroxyproline, methionine, phenylalanine, aspartic acid and glutamic acid on 35 different stationary phases. The best resolution of these eight amino acids is obtained on Silicone MS710.

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