GAS CHROMATOGRAPHY OF VOLATILE AMINO ACID DERIVATIVES

III. ASPARTIC ACID, LYSINE, ORNITHINE, TRYPTOPHAN AND TYROSINE

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INTRODUCTION

The preparation and gas chromatography of volatile derivatives of many of the common amino acids found in proteins were studied previously[1,2], and data were presented for the retention times of trifluoroacetyl amino acid *n*-amyl esters on a variety of stationary phases prepared, packed and used under standardized conditions. The conversion of lysine, tryptophan and tyrosine to the corresponding volatile derivatives has now been studied. For comparative purposes the non-protein amino acid ornithine has been included, particularly in view of the finding that arginine is partially converted to ornithine during the preparation of the trifluoroacetylated methyl ester[3].

MATERIALS AND METHODS

Apparatus

A D6 Chromatograph fitted with gas density balance detector (Griffin & George Ltd., Alperton, Middlesex) was used. Nitrogen (99.9% "White spot", British Oxygen Co., Wembley, Middlesex and "High-purity oxygen-free" Air Products Ltd., London, N.18) was used as carrier gas.

Columns

The data in the tables were obtained with 182 cm × 0.5 cm internal diameter stainless steel U-tube columns previously used[1]. The coating of the supports and the filling of the columns was carried out as previously[1].

Stationary phases

These were obtained from various sources over a period of time, but these materials are now generally available through suppliers of gas chromatography equipment. Silicones RTV-60, RTV-108, SF-1034, SF-1066 and SF-1080 were obtained from General Electric (U.S.A.).

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Preparation of derivatives

The N-trifluoroacetyl(TFA) amino acid *n*-amyl esters were prepared by the methods previously described[1]. With tyrosine the O,N-bis-TFA and with lysine, ornithine and tryptophan the N,N'-bis-TFA derivatives were obtained. Some modifications were introduced owing to the poor yields obtained. Lysine fails to dissolve in the amyl alcohol with HCl bubbling continuously through the mixture even after 2 h at 120°. The resulting yield is very low. This difficulty was overcome by first dissolving the lysine in anhydrous trifluoroacetic acid before adding the amyl alcohol[4]. This also improves the yield with ornithine. The trifluoroacetylation of tryptophan amyl ester hydrochloride in pure trifluoroacetic anhydride at room temperature for increasing periods of time leads to a progressive darkening of colour from brown to

TABLE I

RELATIVE RETENTION DATA

The figures are the retention times relative to the aspartic acid derivative taken as 1.00. The actual retention time in minutes for this derivative is given in brackets. Gas flow: 30 ml N_2/min; column temperature: 190°.

Liquid phase		*% w/w of phase*	*O,N-Bis-TFA n-amyl ester*	*N-TFA di-n-amyl ester*	*N,N'-Bis TFA n-amyl ester*			*H.E.T.P (mm) Asp peak*
			Tyr	*Asp*	*Orn*	*Lys*	*Try*	
Silicone type phases								
1	MS-115	5	1.12	(24.6)	0.77	1.18	3.34	0.8
2	MS-200	5	1.08	(21.4)	0.80	1.17	2.74	1.4
3	MS-550	5	1.17	(11.2)	0.84	1.47	3.70	2.4
4	DC-50	5	1.07	(22.3)	0.72	1.21	3.80	1.0
5	MS-71	5	0.94	(30.0)	0.78	1.30	3.78	0.7
6	SE-30	5	1.09	(28.8)	0.78	1.15	3.32	1.0
7	SE-52	5	1.06	(17.0)	0.83	1.25	3.58	1.0
8	SE-54	5	1.00	(12.5)	0.80	1.24	3.39	1.5
9	M & B	5	1.09	(19.2)	0.79	1.20	3.55	1.1
10	F-44	5	1.10	(23.0)	0.79	1.21	3.85	1.4
11	SF-1080	5	1.03	(33.8)	0.71	1.31	4.33	0.6
12	RTV-60	5	1.10	(10.8)	0.81	1.23	3.08	1.1
13	RTV-108	5	1.06	(15.0)	0.77	1.20	3.48	1.0
14	JXR[a]	5	1.11	(19.9)	0.73	1.20	3.56	0.8
15	F-50	5	1.08	(22.1)	0.73	1.13	3.48	1.6
16	QF-1 (FS-1265)	5	1.08	(24.2)	2.24	3.84	4.45	0.9
17	DC-LSX-3-0295	5	1.68	(20.4)	2.28	3.04	4.01	1.2
18	XE-60	5	1.91	(43.4)	4.79	0.87	6.90	0.8
Non-silicone type phases								
19	DSPO	5	1.08	(41.7)	1.03	1.89	5.27	0.9
20	Castorwax	2	1.32	(16.6)	1.04	2.55	5.81	2.1
21	Celanese ester	2	1.03	(29.1)	2.20	3.26	6.28	0.8
22	BDA	2	1.81	(18.2)	4.85	0.46	7.94	1.2
23	BDS	2	1.71	(11.5)	4.70	0.75	7.11	1.5
24	NPGS	2	1.93	(21.1)	4.89	7.01	7.96	0.8
25	NPGSeb	2	1.69	(27.5)	3.64	5.16	7.56	0.6
26	PEG-A	2	1.44	(20.5)	3.32	4.59	6.10	0.5

[a] Column temperature 180°.

indigo. LAMKIN AND GEHRKE[7] carry out all trifluoroacetylation with 10% trifluoroacetic anhydride in methylene chloride for 2 h at room temperature. We have used this method successfully with the tryptophan derivative and we have also found it to work equally well with other amino acids. WEYGAND AND GEIGER[4] refer to the instability of tryptophan in trifluoroacetic acid.

RESULTS AND DISCUSSION

Table I gives the retention times of the derivatives of tyrosine, ornithine, lysine and tryptophan relative to aspartic acid, so that they may be related to other amino acids previously reported[1,2]. On the silicone-type phases ornithine comes off the column before aspartic acid (relative figures 0.71 to 0.84) except in the cases of QF-1, DC-LSX and XE-60. The figures for QF-1 and DC-LSX are closely similar, as might be expected because of their chemical similarity. XE-60 stands out as the most polar of these phases and as reported before[1,2] behaves more like a polyester-type phase. Lysine and tyrosine have similar retention times, except on the three phases mentioned. MS-710 is unique in that tyrosine appears before aspartic acid. Tryptophan always shows a long retention time: this reflects the low volatility of this derivative. The group of non-silicone stationary phases are rather varied chemically but may be subdivided into three as indicated in Table I. Castorwax and DSPO exhibit behaviour that is only slightly more polar than most of the silicones, as shown by the retention data for lysine. Celanese ester behaves essentially the same as QF-1 and DC-LSX. Finally, the polyesters are much more polar than most of the silicones.

Previously the retention time of the glycine derivative relative to the leucine derivative was held to be indicative of the relative polarity of a stationary phase[1] (see also ROHRSCHNEIDER[6]): in this case the retention time for the lysine derivative

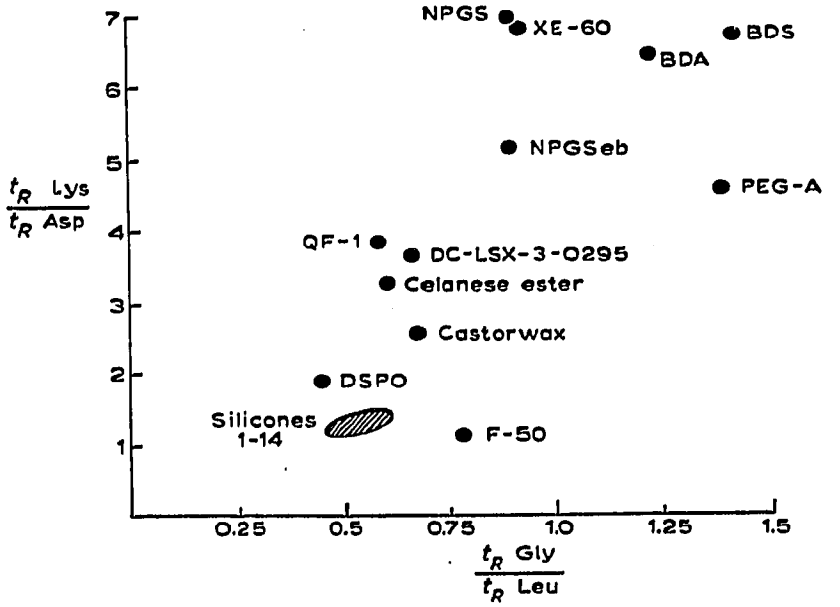

Fig. 1. Ratio of relative retention times of the trifluoroacetylated *n*-amyl esters of lysine to aspartic acid plotted against the ratio for glycine to leucine on 26 different stationary phases. The lysine and aspartic acid derivatives were separated with a column temperature of 190°; gas flow: 38 ml/min. The glycine and leucine derivatives were separated with a column temperature of 150°; gas flow: 38 ml/min. The silicones 1–14 are listed in Table I.

relative to that for aspartic acid shows a similar relationship. Fig. 1 shows this on a graph of the ratios of the retention times of glycine to leucine plotted against the ratios of lysine to aspartic acid. Either of these criteria used as a measure of the polarity of the stationary phase gives broadly the same sequence. However, one cannot use the polarity as more than a guide to the choice of stationary phase, since the retention times of any mixture of compounds also depend on other specific chemical interactions.

The only phases which will completely resolve the amino acids listed in Table I are the silicones QF-1 and DC-LSX and the non-silicones Castorwax, Celanese ester, BDA, NPGSeb and PEG-A. However, if glutamic acid and phenylalanine are present, then only Castorwax and NPGSeb are suitable. A further difficulty is that under the same isothermal conditions it is desirable to separate the amino acids cysteine, proline, hydroxyproline, methionine, phenylalanine, aspartic acid, glutamic acid, tyrosine, ornithine, lysine and tryptophan in a single run, and in order to achieve this it has been found necessary to resort to mixed phases. Results with these mixed stationary phases will be presented in a further communication.

TABLE II

RELATIVE RETENTION DATA

The figures are the retention times relative to the leucine derivative taken as 1.00. The actual retention time in minutes for this derivative is given in brackets. All packings were prepared to contain 5 % of liquid phase. Gas flow: 38 ml N_2/min; column temperature: 150°.

Liquid phase	*N-TFA amino acid n-amyl ester*					*O,N-Bis TFA n-amyl ester*		*Biphenyl*	*Bibenzyl*	*H.E.T.P. (mm) Leu peak*
	Ala	*Val*	*Gly*	*Ileu*	*Leu*	*Thr*	*Ser*			
DC-560	0.44	0.71	0.50	1.04	(19.5)	0.57	0.63	0.76	1.41	1.3
DC-LSX-3-0295	0.55	0.75	0.67	0.99	(17.1)	0.91	1.14	0.33	0.49	0.8
F-44	0.41	0.70	0.49	1.06	(20.0)	0.59	0.63	0.59	1.35	0.9
JXR[a]	0.43	0.71	0.47	1.01	(16.3)	0.61	0.61	0.71	1.32	1.1
RTV-60	0.45	0.72	0.47	0.99	(9.6)	0.58	0.66	0.75	1.30	1.2
RTV-108	0.47	0.72	0.53	1.00	(14.7)	0.58	0.67	0.72	1.27	0.8
SE-52	0.44	0.72	0.53	0.98	(10.8)	0.62	0.70	0.81	1.45	1.5
SE-54	0.46	0.72	0.56	1.02	(13.4)	0.58	0.65	0.76	1.40	1.7
SF-1034	0.58	0.59	1.37	0.79[b]	(36.0)	0.49	—	0.99	1.74	0.7
SF-1066	0.56	0.58	1.22	0.81[b]	(31.8)	0.54	—	0.87	1.53	0.6
SF-1080	0.44	0.69	0.55	1.04	(19.5)	0.50	0.64	1.08	2.05	1.1
XF-1105	0.46	0.65	0.59	0.99[b]	(32.2)	0.72	1.00	0.49	0.92	0.5
XF-1150	0.61	0.61	1.19	0.78[b]	(33.7)	0.81	1.68	0.48	0.76	0.5
Antarox Co-990	0.74	0.56	2.15	0.71[b]	(17.8)	—[c]	—	1.38	2.14	0.6
Castorwax	0.42	0.60	0.68	0.87[b]	(42.0)	0.48	0.76	0.80	1.51	0.8
Celanese ester	0.40	0.57	0.62	0.85	(60.5)	0.60	0.98	0.65	1.21	1.3
DEGS	0.72	0.61	1.70	0.77	(14.5)	—	—	1.14	1.64	0.8
ECNSS-S[a]	0.62	0.56	1.53	0.69[b]	(38.1)	—	—	0.60	0.96	1.2
Ucon-50-HB-2000	0.61	0.58	1.50	0.82	(21.0)	—	—	1.21	2.08	0.6
PEG-S	0.63	0.54	1.69	0.72	(35.0)	—	—	0.94	1.46	0.7

[a] Column temperature 140°.
[b] Shows partial resolution of alloiso- and isoleucine.
[c] — = compound applied but gave no peak.

APPENDIX

Tables II and III contain supplementary data for the derivatives studied earlier[1,2], on stationary phases which were not available at the time. It was felt to be useful for these data to be extended to new materials so that direct comparisons can be made. Of the phases in Tables II and III XF-1105 and XF-1150 were of special interest because of the high efficiencies obtained. However, they cannot be used above 150°. Antarox CO-990 is the most polar of the phases we have so far investigated.

TABLE III

RELATIVE RETENTION DATA

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

Liquid phase	*N-TFA n-amyl ester*	*S,N-Bis TFA n-amyl ester*	*O,N-Bis TFA n-amyl ester*	*N-TFA n-amyl ester*			*N-TFA di-n-amyl ester*		*Bicumyl*	*H.E.T.P. (mm) Asp peak*
	Leu	*CySH*	*HyPro*	*Pro*	*Met*	*Phe*	*Asp*	*Glu*		
DC-560	0.19	0.20	0.33	0.33	0.50	0.75	(26.0)	1.70	0.84	1.1
DC-LSX-3-0295	0.21	0.29	0.65	0.45	0.59	0.70	(18.8)	1.80	0.28	0.9
F-44	0.20	0.24	0.33	0.33	0.51	0.77	(24.7)	1.72	0.88	0.8
JXR[a]	0.20	0.24	0.33	0.32	0.49	0.78	(20.5)	1.71	0.81	0.8
RTV-60	0.21	0.28	0.37	0.39	0.55	0.79	(11.7)	1.64	0.86	1.3
RTV-108	0.23	0.27	0.36	0.37	0.54	0.78	(18.5)	1.65	0.83	0.6
SF-1034	0.14	—[b]	—	0.33	0.58	0.82	(52.2)	1.92	0.70	0.7
SF-1066	0.13	—	—	0.29	0.56	0.81	(55.5)	1.87	0.59	0.6
SF-1080	0.17	0.21	0.32	0.35	0.52	0.79	(31.5)	1.80	1.11	1.1
Antarox CO-990	0.13	—	—	0.35	0.70	0.95	(26.5)	1.93	0.68	0.6
DEGS	0.15	—	—	0.37	0.81	1.03	(25.6)	1.99	0.50	0.6
Ucon-50-HB-2000	0.14	—	—	0.32	0.59	0.87	(28.0)	1.89	0.66	0.9
PEG-S	0.13	—	—	0.30	0.62	0.84	(56.5)	1.89	0.51	0.6

[a] Column temperature: 180°.
[b] — = Compound applied but gave no peak.

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SUMMARY

Data are presented for the relative retention times of the volatile derivatives derived from aspartic acid, lysine, ornithine, tryptophan and tyrosine. Earlier work on many of the common protein amino acids has been extended by the inclusion of data for a further twenty stationary phases.

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