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GAS–LIQUID CHROMATOGRAPHIC BEHAVIOUR OF N-TRIFLUOROACETYL *n*-BUTYL ESTERS OF VARIOUS S-SUBSTITUTED CYSTEINES

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SUMMARY

The gas–liquid chromatographic behaviour of four S-*n*-alkyl-, three S-ω-carboxyalkyl- and S-β-aminoethylcysteines, lanthionine, cystine, S,S′-methylene- and S,S′-ethylenebiscysteines, and some homocysteine analogues, as their N-trifluoroacetyl *n*-butyl esters, was studied on columns of OV-17 and Dexsil 300 GC and compared with the behaviour of similar non-sulphur-containing amino acids. The retention indices and some other parameters are discussed in relation to the structure of the amino acids.

Relative molar responses were determined and an empirical additive method for calculating relative molar responses from the structural feature is described.

INTRODUCTION

During the course of studies on the chemical treatment of wool fibres, the need for a convenient and reliable method for the quantitative analysis of artifact amino acids which may be produced by reactions of reactive side-groups of wool proteins during the treatment has become apparent.

Gas chromatography is one of the most efficient quantitative analytical methods for analyzing mixtures of very similar compounds. Gehrke *et al.*[1] developed a quantitative gas–liquid chromatographic (GLC) method for the twenty natural protein amino acids and other non-protein amino acids. Essentially, this method consists in procedures for the nearly quantitative conversion of the amino acids into their N-trifluoroacetyl *n*-butyl esters (BTFA) and the temperature-programmed GLC of the mixture of the BTFA derivatives with a dual-column system of EGA and OV-17, the former being used for the separation of most BTFA-amino acids and the latter for the separation of less volatile BTFA-amino acids such as BTFA-arginine and -cystine. More recently, Gehrke and Takeda[2] reported a single-column separation of the twenty protein amino acids as their BTFA derivatives with Apiezon M.

This paper describes the GLC behaviour of eleven S-substituted cysteines in addition to some related amino acids as their BTFA derivatives on OV-17 and Dexsil

300 GC, and the correlation between the Kovàts retention indices and the structures of the amino acids is discussed. A method for the calculation of flame ionization detector (FID) responses of sulphur-containing amino acids is also presented. S-Substituted cysteine residues are produced when wool fibres are treated with thiol-blocking reagents for the purpose of cysteine analysis or the elimination of cysteine functions or when reduced wool fibres are treated with alkylating reagents.

EXPERIMENTAL

Amino acids

S-Methylcysteine[3] (SMC), S-ethylcysteine[3] (SEC), S-*n*-propylcysteine[4] (SPC), S-*n*-butylcysteine[4] (SBC), S-carboxymethylcysteine[4] (SCMC), S-β-carboxyethylcysteine[5] (SCEC), S-γ-carboxypropylcysteine[4] (SCPC), S-carboxymethylhomocysteine[4] (SCMHC), S-β-carboxyethylhomocysteine[4] (SCEHC), S-γ-carboxypropylhomocysteine[4] (SCPHC), S-β-aminoethylcysteine (SAEC) hydrochloride[6] and S,S'-ethylenebiscysteine[7] (EBC) were prepared from L-cysteine or L-methionine. All of these amino acids were purified by recrystallization from ethanol–water. The elemental analyses of the amino acids were as follows.

SMC: calculated for $C_4H_9O_2NS$: C, 35.54%; H, 6.71%; N, 10.36%. Found: C, 32.93%; H, 6.88%; N, 10.92%.

SEC: calculated for $C_5H_{11}O_2NS$: C, 40.25%; H, 7.43%; N, 9.39%. Found: C, 40.11%; H, 7.35%; N, 9.10%.

SPC: calculated for $C_6H_{13}O_2NS$: C, 44.15%; H, 8.03%; N, 8.58%. Found: C, 44.28%; H, 8.29%; N, 8.37%.

SBC: calculated for $C_7H_{15}O_2NS$: C, 47.43%; H, 8.53%; N, 7.89%. Found: C, 47.16%; H, 9.00%; N, 7.91%.

SCMC: calculated for $C_5H_9O_4NS$: C, 33.51%; H, 5.06%; N, 7.82%. Found: C, 33.10%; H, 4.70%; N, 7.44%.

SCEC: calculated for $C_6H_{11}O_4NS$: C, 37.30%; H, 5.74%; N, 7.25%. Found: C, 36.95%; H, 5.95%; N, 7.04%.

SCPC: calculated for $C_7H_{13}O_4NS$: C, 40.57%; H, 6.32%; N, 6.76%. Found: C, 39.81%; H, 6.15%; N, 6.71%.

SCMHC: calculated for $C_6H_{11}O_4NS$: C, 37.30%; H, 5.74%; N, 7.25%. Found: C, 35.65%; H, 5.75%; N, 6.92%.

SCEHC: calculated for $C_7H_{13}O_4NS$: C, 40.57%; H, 6.32%; N, 6.76%. Found: C, 39.71%; H, 6.13%; N, 6.65%.

SCPHC: calculated for $C_8H_{15}O_4NS$: C, 43.38%; H, 6.78%; N, 6.33%. Found: C, 42.97%; H, 6.80%; N, 6.45%.

SAEC·HCl: calculated for $C_5H_{13}O_2ClN_2S$: C, 29.81%; H, 6.48%; N, 13.96%. Found: C, 29.84%; H, 6.69%; N, 13.68%.

EBC: calculated for $C_8H_{16}O_4NS$: C, 35.77%; H, 5.96%; N, 10.45%. Found: C, 35.31%; H, 5.62%; N, 10.76%.

DL-Norvaline, DL-norleucine, DL-2-aminooctanoic acid, DL-ethionine, DL-2-aminopimelic acid, L-2,4-diaminobutyric acid hydrochloride and 2,6-diaminopimelic acid (D,D-, L,L- and *meso*-forms) were of guaranteed reagent grade purchased from Tokyo Kasei Kogyo (Tokyo, Japan). DL-Alanine, L-methionine, L-aspartic acid, L-glutamic acid, L-ornithine hydrochloride, L-lysine hydrochloride and L-cystine were

of guaranteed reagent grade from Nippon Rikagaku Yakuhin (Tokyo, Japan). DL-Lanthionine, DL-homocystine and L-djenkolic acid were of special reagent grade from Wako Junyaku Kogyo (Osaka, Japan).

Reagents

Methanol and methylene chloride of guaranteed reagent grade were dried over molecular sieves. *n*-Butanol and trifluoroacetic anhydride of guaranteed reagent grade were used without further purification.

A mixture of hydrochloric acid and methanol was prepared as follows. Hydrogen chloride gas, generated by the slow addition of concentrated hydrochloric acid to concentrated sulphuric acid, was dried over concentrated sulphuric acid, washed with anhydrous methanol and absorbed in anhydrous methanol to give a 5.25% (w/w) hydrochloric acid–methanol mixture. A 5.25% (w/w) hydrochloric acid–*n*-butanol mixture was prepared in a similar manner.

Derivatization

The conversion of the amino acids into their N-trifluoroacetyl *n*-butyl esters was carried out at the macro level (10–50 mg of total amino acids) according to Gehrke *et al.*[1].

Apparatus

A Shimadzu Model GC-4APF dual-column gas chromatograph, equipped with hydrogen FIDs and a linear temperature programmer, was used.

Gas–liquid chromatographic conditions

One of two glass columns, 1 m long × 3 mm I.D., was packed with 1.5% (w/w) of OV-17 on 80–100 mesh acid-washed and heat-treated high-performance Chromosorb G and the other with 1.5% (w/w) of Dexsil 300 GC on the same quality Chromosorb G, both column packings being purchased from Nishio Kogyo (Tokyo, Japan). The columns were arranged parallel to each other in the dual-column system. The temperature programme most frequently used began with an initial temperature of 160°, increasing at the rate of 8°/min to a final temperature of 280°. The injection port and detector temperatures were 250 and 280°, respectively. Flow-rates of the carrier gases employed were 70 ml/min for nitrogen, 50 ml/min for hydrogen and 1100 ml/min for air.

Determination of retention indices

The *n*-paraffins used for the determination of retention indices were pairs of hydrocarbons selected from C_{10}, C_{12}, C_{14}, C_{16}, C_{18}, C_{20}, C_{24}, C_{28}, C_{32} and C_{36} *n*-paraffins obtained from Applied Science Labs., State College, Pa., U.S.A.

Determination of FID relative molar response values

FID molar responses of the amino acids as their N-trifluoroacetyl *n*-butyl esters were determined from the gas chromatograms from a mixture of glutamic acid (reference) and amino acids whose peaks did not overlap. The peak area ratio was obtained by cutting out the peaks from the chromatographic paper and weighing them. Three independent determinations were made to obtain average relative molar response (RMR) values.

RESULTS AND DISCUSSION

Amino acids and stationary phases

The classification, names and abbreviations of the α-amino acids used in this study are listed in Table I. Eighteen sulphur-containing amino acids studied, most of which are S-substituted cysteines and homocysteines, are classified as (A) monoaminomonocarboxylic acids (S-alkylcysteines and S-alkylhomocysteines), (B) monoaminodicarboxylic acids (S-ω-carboxyalkylcysteines and S-ω-carboxyalkylhomocysteines), (C) diaminomonocarboxylic acid (S-β-aminoethylcysteine) and (D) diaminodicarboxylic acids (cystine, homocystine, lanthionine and biscysteines). The GLC behaviour

TABLE I

AMINO ACIDS INVESTIGATED

Class of amino acid	*No.*	*Name*	*Abbreviation*
(A) Monoaminomonocarboxylic			
(i) Non-sulphur-containing	1	Alanine	ALA
	2	Norvaline	NORVAL
	3	Norleucine	NORLEU
	4	2-Amino-*n*-octanoic acid	AOA
(ii) S-Alkylcysteines	5	S-Methylcysteine	SMC
	6	S-Ethylcysteine	SEC
	7	S-*n*-Propylcysteine	SPC
	8	S-*n*-Butylcysteine	SBC
(iii) S-Alkylhomocysteines	9	Methionine	MET
	10	Ethionine	ETH
(B) Monoaminodicarboxylic			
(i) Non-sulphur-containing	11	Aspartic acid	ASP
	12	Glutamic acid	GLU
	13	2-Aminopimelic acid	APA
(ii) S-Carboxyalkylcysteines	14	S-Carboxymethylcysteine	SCMC
	15	S-β-Carboxyethylcysteine	SCEC
	16	S-γ-Carboxypropylcysteine	SCPC
(iii) S-Carboxyalkylhomocysteines	17	S-Carboxymethylhomocysteine	SCMHC
	18	S-β-Carboxyethylhomocysteine	SCEHC
	19	S-γ-Carboxypropylhomocysteine	SCPHC
(C) Diaminomonocarboxylic			
(i) Non-sulphur-containing	20	2,4-Diaminobutyric acid	DABA
	21	Ornithine	ORN
	22	Lysine	LYS
(ii) S-Aminoalkylcysteines	23	S-β-Aminoethylcysteine	SAEC
(D) Diaminodicarboxylic			
(i) Non-sulphur-containing	24	2,6-Diaminopimelic acid	DAPA
(ii) Sulphur-containing	25	Lanthionine	LAN
	26	Cystine	CYS
	27	Homocystine	HOMOCYS
(Biscysteines)	28	S,S′-Methylenebiscysteine (djenkolic acid)	MBC
	29	S,S′-Ethylenebiscysteine	EBC

of eleven non-sulphur-containing amino acids of similar homologous series (from type A to type D) was also studied for comparison.

The most widely used liquid phases for the GLC of BTFA-amino acids are EGA for lower-boiling BTFA derivatives and OV-17 for higher-boiling derivatives. As we were interested in high-temperature-resistant stationary phases for the analysis of amino acids of high molecular weights from chemically modified wool fibres, we investigated two high-temperature-resistant materials, OV-17 and Dexil 300 GC, the latter being a linear carborane dimethylsiloxane polymer[8], available from (Analabs). In general, carborane polymers have high thermal stability and the maximum operating temperature of Dexsil 300 GC is claimed to be 500°, which is much higher than that of OV-17 (300°)[9]. Rohrschneider constants[10,11] reported for Dexsil 300 GC indicate that the characteristics of Dexsil 300 GC as the stationary phase are similar to those of OV-3 (ref. 12), that is, less polar than OV-17, as shown in Table II. Recently, Pollock[13] reported the resolution of N-trifluoroacetyl (+)-2-butyl esters of DL-amino acids by capillary gas chromatography with Dexsil 400 GC (some methyl groups of the carborane dimethylsiloxane polymer are replaced with phenyl groups).

Retention characteristics

Table III shows the retention temperatures of BTFA-amino acids measured with linear temperature programming from 100° at the rate of 8°/min. Homocystine and lanthionine were reported to give no peak on EGA after standard BTFA derivatization treatments and the possibility of difficulty in the derivatization was also mentioned[14]. In this work, both amino acids were found to give distinct peaks on both OV-17 and Dexsil 300 GC. Arginine, histidine and cystine were reported to give peaks on OV-17 but not on EGA as their BTFA derivatives. The amino acids that gave the highest retention temperatures of those tested were homocystine and S,S′-ethylenebis-cysteine.

It was noted that all of the amino acids were eluted at a lower temperature on OV-17 than on Dexsil 300 GC. Retention temperatures or retention times vary with alteration in the operating conditions and the column preparations. To discuss the

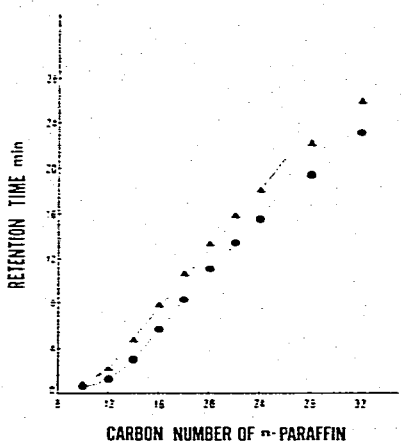


Fig. 1. Retention times of *n*-paraffins on OV-17 (●) and Dexsil 300 GC (▲) *versus* their carbon numbers.

TABLE II

ROHRSCHNEIDER CONSTANTS OF OV-17 AND DEXSIL 300 GC

Stationary phase	*Phenyl substitution (%)*	*Rohrschneider constants*				
		X	*Y*	*Z*	*U*	*S*
OV-1	0	0.16	0.20	0.50	0.85	0.48
OV-3	10	0.42	0.81	0.85	1.52	0.89
OV-17	50	1.30	1.66	1.79	2.83	2.47
Dexsil 300 GC	0	0.43	0.64	1.11	1.51	1.01

TABLE III

RETENTION TEMPERATURES OF BTFA-AMINO ACIDS

Class	*No.*	*Amino acid*	*Retention temperature* (°C)*	
			OV-17	*Dexsil 300 GC*
A-i	1	ALA	114.7	119.1
	2	NORVAL	124.8	134.6
	3	NORLEU	132.9	144.0
	4	AOA	151.0	164.6
A-ii	5	SMC	148.4	153.0
	6	SEC	153.7	160.8
	7	SPC	161.8	169.2
	8	SBC	170.1	179.6
A-iii	9	MET	160.8	169.2
	10	ETH	167.0	176.2
B-i	11	ASP	169.8	177.2
	12	GLU	184.6	192.8
	13	APA	204.2	214.4
B-ii	14	SCMC	208.5	215.0
	15	SCEC	218.1	225.6
	16	SCPC	227.0	235.4
B-iii	17	SCMHC	220.0	225.6
	18	SCEHC	230.0	237.8
	19	SCPHC	237.6	247.2
C-i	20	DABA	156.2	166.9
	21	ORN	176.6	184.4
	22	LYS	188.2	198.8
C-ii	23	SAEC	200.2	205.8
D-i	24	DAPA	212.2	230.2
D-ii	25	LAN	221.6	235.4
	26	CYS	240.6	254.4
	27	HOMOCYS	260.6	276.4
	28	MBC	253.8	266.8
	29	EBC	263.2	276.4

* Operating conditions: initial temperature, 100 °; temperature-programming rate, 8 °/min.

correlation of the amino acid structures with their GLC behaviour, the Kováts[15] retention indices (I) were determined for pairs of *n*-paraffins according to the equations for isothermal[15] and temperature-programmed[16] GLC. The retention temperatures of the C_{10}–C_{36} *n*-paraffins used for the determinations in the linear temperature-programmed GLC with an initial temperature of 100° and a programming rate of 8°/min on OV-17 and on Dexsil 300 GC are plotted against numbers of carbon atoms in the *n*-paraffins in Fig. 1. The curvature observed for low-boiling paraffins is considered to result from the initial temperature in the programme being too high at the relatively low flow-rate used[17]. A curvature was also observed for high-boiling paraffins.

The retention index of a compound corresponds to 100 times the number of carbon atoms in a hypothetical *n*-paraffin which is supposed to have the same retention time as the solute under the same GLC operating conditions. The retention index was reported to vary only slightly with alteration in the operating conditions[16-18]. Retention indices of S-methyl-, S-*n*-propyl- and S-β-carboxyethylcysteines determined under various operating conditions are given in Tables IV and V, and show that the retention index does not vary much under a variety of operating conditions. Hence the retention index can be regarded as a constant that depends only on the column packing material.

TABLE IV

RETENTION INDICES OF SMC AND SPC ON OV-17 UNDER VARIOUS OPERATING CONDITIONS

Operating conditions		*Retention index*	
Initial temperature (°C)	*Programming rate (°C/min)*	*SMC*	*SPC*
80	8	1621	1746
100	Isothermal	1630	1762
100	8	1627	1752

TABLE V

RETENTION INDEX OF SCEC UNDER VARIOUS OPERATING CONDITIONS

Operating conditions		*Retention index*	
Initial temperature (°C)	*Programming rate (°C/min)*	*OV-17*	*Dexsil 300 GC*
100	8	2337	2200
125	8	2343	2200
140	6	2341	2200
170	Isothermal	2345	
180	Isothermal	2342	2200
190	Isothermal	2338	

Table VI gives the retention indices of sulphur-containing and non-sulphur-containing amino acids on OV-17 and Dexsil 300 GC determined by temperature-programmed GLC. The difference between the two retention indices (ΔI) determined on the stationary phases of different polarity is also given in Table VI. All of the re-

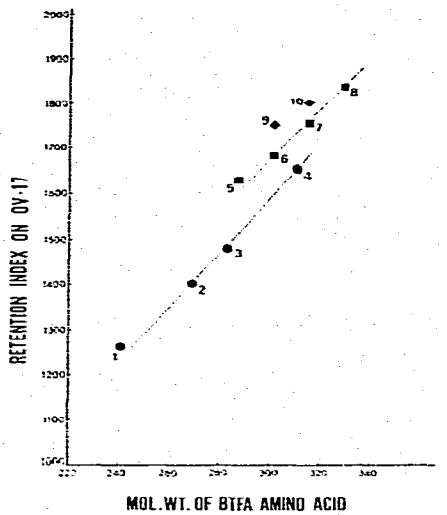
TABLE VI

RETENTION INDICES OF BTFA-AMINO ACIDS

Class	*No.*	*Amino acid*	*Retention index*		*Difference (ΔI)*
			OV-17	*Dexsil 300 GC*	
A-i	1	ALA	1261	1218	43
	2	NORVAL	1400	1368	32
	3	NORLEU	1478	1452	26
	4	AOA	1651	1628	23
A-ii	5	SMC	1627	1530	97
	6	SEC	1680	1595	85
	7	SPC	1752	1670	82
	8	SBC	1835	1757	78
A-iii	9	MET	1747	1670	77
	10	ETH	1800	1729	71
B-i	11	ASP	1835	1734	101
	12	GLU	1977	1873	104
	13	APA	2170	2086	84
B-ii	14	SCMC	2225	2092	133
	15	SCEC	2334	2200	134
	16	SCPC	2436	2307	129
B-iii	17	SCMHC	2348	2200	148
	18	SCEHC	2466	2310	156
	19	SCPHC	2577	2427	150
C-i	20	DABA	1701	1648	53
	21	ORN	1878	1800	78
	22	LYS	2000	1930	70
C-ii	23	SAEC	2117	2000	117
D-i	24	DAPA	2257	2243	14
D-ii	25	LAN	2374	2307	67
	26	CYS	2612	2524	88
	27	HOMOCYS	2865	2800	65
	28	MBC	2788	2667	121
	29	EBC	2902	2800	102

tention indices on OV-17 were greater than the corresponding retention indices on Dexsil 300 GC, which confirms that Dexsil 300 GC was less polar than OV-17.

It was reported that the ΔI values of homologous series of compounds were nearly equal to each other[15]. The results in Table VI indicate that the ΔI values of sulphur-containing amino acids are always different from and higher than those of the corresponding non-sulphur-containing amino acids. Thus the ΔI values of four S-alkylcysteines ranged between 78 and 97 and those of two S-alkylhomocysteines (methionine and ethionine) between 71 and 77, while those of four corresponding non-sulphur-containing monoaminomonocarboxylic acids ranged between 23 and 43. Similarly, the ΔI values of three S-carboxyalkylcysteines were between 129 and 134, those of three S-carboxyalkylhomocysteines were between 148 and 156, while those of three non-sulphur-containing monoaminodicarboxylic acids were between 84 and 104. From these ΔI values, it can be seen that S-substituted cysteines and very similar



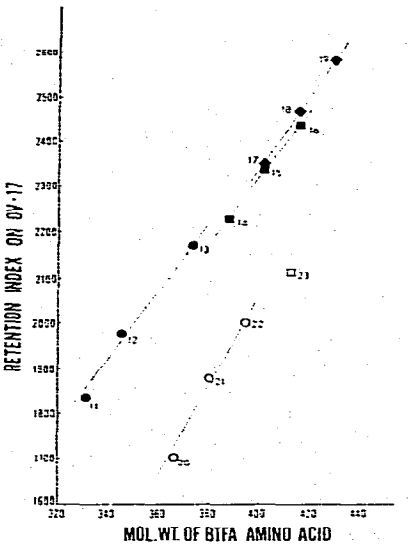
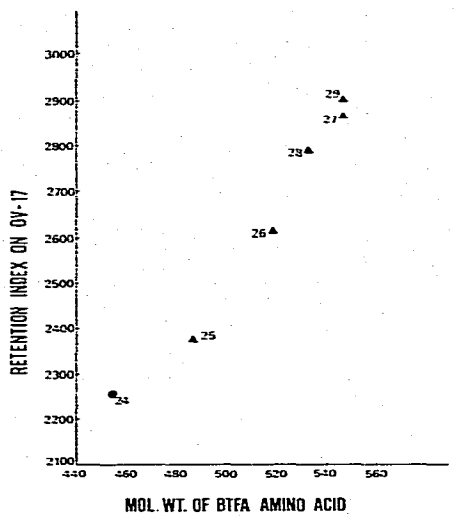


Fig. 2. Retention indices of BTFA-amino acids on OV-17 *versus* their molecular weights. ●, Non-sulphur-containing monoaminomonocarboxylic acids; ■, S-alkylcysteines; ◆, S-alkylhomocysteines.

Fig. 3. Retention indices of BTFA-amino acids on OV-17 *versus* their molecular weights. ●, Non-sulphur-containing monoaminodicarboxylic acids; ■, S-carboxyalkylcysteines; ◆, S-carboxyalkylhomocysteines; , non-sulphur-containing diaminomonocarboxylic acids; , S-*β*-aminoethylcysteine.



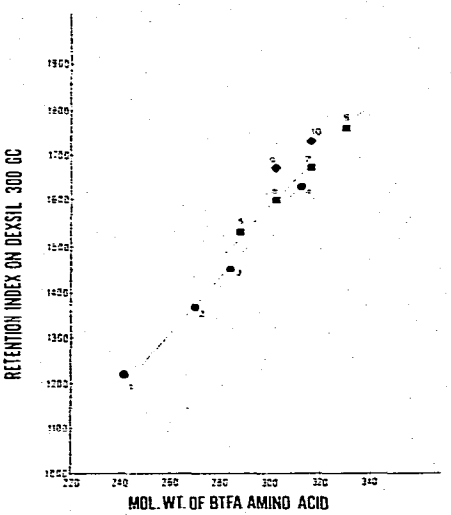


Fig. 4. Retention indices of BTFA-amino acids on OV-17 *versus* their molecular weights. ●, Diaminopimelic acid; ▲, sulphur-containing diaminodicarboxylic acids.

Fig. 5. Retention indices of BTFA-amino acids on Dexsil 300 GC *versus* their molecular weights. ●, Non-sulphur-containing monoaminomonocarboxylic acids; ■, S-alkylcysteines; ◆, S-alkylhomocysteines.

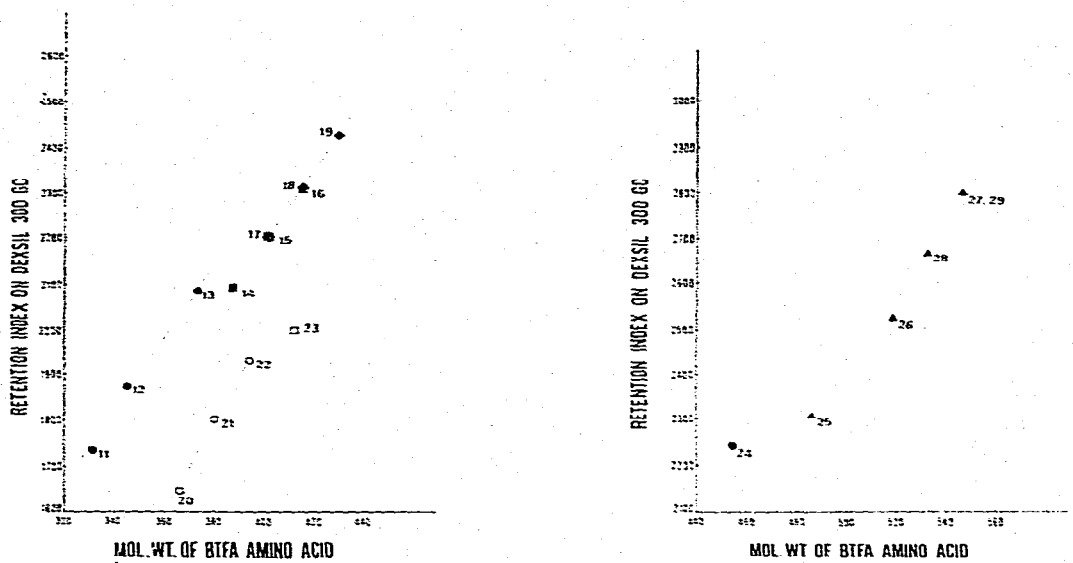


Fig. 6. Retention indices of BTFA-amino acids on Dexsil 300 GC *versus* their molecular weights. ●, Non-sulphur-containing monoaminodicarboxylic acids; ■, S-carboxyalkylcysteines; ◆, S-carboxyalkylhomocysteines; , non-sulphur-containing diaminomonocarboxylic acids; , S-*β*-aminoethylcysteine.

Fig. 7. Retention indices of BTFA-amino acids on Dexsil 300 GC *versus* their molecular weights. ●, Diaminopimelic acid; ▲, sulphur-containing diaminodicarboxylic acids.

S-substituted homocysteines belong to different homologous series of compounds in their GLC behaviour. From a comparison of the *I* values, it seems that cystine and homocystine may be regarded as homologues and are apparently different from biscysteines.

Retention indices are plotted against molecular weights of the BTFA-amino acids in Figs. 2–7. It is well known that the plots of retention indices of homologous series of compounds lie on a straight line except for a few initial members[19]. To a first approximation, the retention indices of monoaminomonocarboxylic acids and of monoaminodicarboxylic acids lie on a straight band region, regardless of the presence or absence of sulphur, while the retention values of diaminomonocarboxylic acids and diaminodicarboxylic acids apparently lie on different lines. However, the plots of retention indices of monoaminomono- and -dicarboxylic acids of non-sulphur-containing, cysteine, and homocysteine series gave different lines for each homologous series in most instances. This result can be the basis of the GLC separation of a pair of very similar structural isomers such as methionine and S-ethylcysteine. Table VII shows the differences in the retention indices for five pairs of structural isomers. In several instances, the separation of structural isomers could not be achieved on Dexsil 300 GC.

The identification of unknown peaks in gas chromatograms can be assisted if the retention index of a compound can be predicted from its structural formula. It was of interest to ascertain whether the observed retention indices for BTFA-amino acids could be divided into individual additive contributions of methylene groups and of other functional groups. As discussed earlier, the plot of retention index *versus* molecular weight is linear for a homologous series, but not parallel to the plots for other homolo-

TABLE VII

DIFFERENCE BETWEEN RETENTION INDICES OF STRUCTURAL ISOMERS

Structural isomers		*Difference between retention indices ($I_1 - I_2$)*	
Compound 1	*Compound 2*	*OV-17*	*Dexsil 300 GC*
MET	SEC	67	75
ETH	SPC	48	59
SCMHC	SCEC	14	0
SCEHC	SCPC	30	3
HOMOCYS	EBC	−37	0

gous series (see Figs. 2–7). Hence an additive group retention index of a methylene group (i_C) can be assumed for a homologous series, but this value cannot necessarily be applied to other series of compounds.

The contributions of a methylene group calculated for each homologous series obtained from the slope of the plot of the retention index *versus* molecular weight are shown in Table VIII. By definition, the retention index of an *n*-paraffin of carbon number z is $100z$, and retention indices of neighbouring members of homologous series of various types of compounds are known to differ by about 100, in general. The observed i_C values of BTFA-amino acids were found to range between 77 and 140 (except for the methionine–ethionine series). The i_C values observed for BTFA-S-alkylcysteines on either OV-17 or Dexsil 300 GC were approximately equal to those of non-sulphur-containing BTFA monoaminomonocarboxylic acids and were lower than 100. The i_C values for BTFA-S-alkylhomocysteines calculated from the retention indices of methionine and ethionine were 53 on OV-17 and 59 on Dexsil 300 GC and were apparently too low. Methionine is the first member of the homologous series and probably shows anomalously higher retention index than would otherwise be expected from the retention indices of the higher homologues.

Strictly, i_C values obtained for S-substituted cysteines and homocysteines from

TABLE VIII

METHYLENE GROUP RETENTION INDICES (i_C) AND SUMMED CONTRIBUTIONS (i_R) OF OTHER GROUPS TO RETENTION INDICES

Amino acid series	i_C		R^*	i_R	
	OV-17	*Dexsil 300 GC*		*OV-17*	*Dexsil 300 GC*
A-i**	83	86	AA	1152	1112
A-ii**	77	81	AA – S	1449	1351
B-i**	112	113	AA – BE	1722	1634
B-ii**	105	107	AA – S – BE	2015	1879
B-iii**	115	113	AA – S – BE	2003	1862
C-i**	140	140	AA – TFA	1460	1370
CYS-HOMOCYS***	126	138	2(AA – S)	2361	2248
MBC-EBC***	114	133	2(AA – S)	2446	2268

* AA = n-$C_4H_9OCO(CF_3CONH)CH$–; S = –S–; BE = n-C_4H_9OCO–; TFA = CF_3CONH–.
** i_C obtained from the slope of the retention index *versus* molecular weight graph.
*** i_C obtained by the difference between two retention indices.

the slopes should be applied only to the methylene groups in S-substituting alkyl groups. The methylene group retention index for BTFA-S-carboxyalkylcysteines was nearly equal to that of the corresponding homocysteine derivatives. Therefore, the same i_C value was assumed to be applicable to the methylene groups of cysteine and homocysteine moieties. It was also assumed, for convenience, that the group retention index of a terminal methyl group was equal to the group retention index of a methylene group.

The i_C values for sulphur-containing BTFA-monoaminodicarboxylic acids were approximately equal to those for non-sulphur-containing monoaminodicarboxylic acids, and were about 110. The i_C values for non-sulphur-containing BTFA-diaminomonocarboxylic acids were found to be much higher (140). The i_C values of sulphur-containing BTFA-diaminodicarboxylic acids were estimated from the differences between the retention indices of homocystine and cystine and of S,S'-ethylene- and S,S'-methylenebiscysteines. The calculated values from either pair were nearly equal to each other and were higher than 100.

On the assumption of an additive function of the structural features of a molecule, the sum of the contributions of groups other than methylene groups (i_R) can also be calculated from the relationship between the retention index and molecular weight (see also Table VIII). From the non-sulphur-containing monoaminomonocarboxylic acid series, the group retention index (i_{AA}) of an n-$C_3H_9OCO(CF_3CONH)CH-$ (α-amino acid) group was obtained. Then, the group retention index of a sulphur atom (i_S) was obtained by comparison of the i_R values of S-alkylcysteines and non-sulphur-containing monoaminomonocarboxylic acids, on the assumption that the i_{AA} values for S-alkylcysteines were equal to those for non-sulphur-containing monoaminomonocarboxylic acids. The i_S values were also obtained by comparison of the i_R values of non-sulphur-containing monoaminodicarboxylic acids with those of S-carboxyalkylcysteines or S-carboxyalkylhomocysteines on a similar assumption. Table IX shows the i_S values thus obtained, indicating that the same i_S value can be used for different types of sulphur-containing amino acids, except for diaminodicarboxylic acids.

TABLE IX

SULPHUR GROUP RETENTION INDICES (i_S)

Amino acids used for estimation of i_S	i_S	
	OV-17	*Dexsil 300 GC*
A-i and A-ii*	297	239
B-i and B-ii*	293	245
B-i and B-iii*	281	228
LAN and CYS**	238	217
DAP and MBC**	266	212

* i_S obtained as the difference between two i_R values.
** i_S obtained as the difference between two retention indices.

If it is assumed that the i_{AA} values of monoaminomonocarboxylic acids are applicable to monoaminodicarboxylic acids, one can calculate the contribution (i_{BE}) of

TABLE X

GROUP RETENTION INDICES OF n-C_4H_9OCO– (i_{BE}) AND CF_3CONH– (i_{TFA}) GROUPS

Amino acids used for estimation	*Group retention index*		
	i	*OV-17*	*Dexsil 300 GC*
B-i and A-i	i_{BE}	570	522
B-ii and A-ii		566	528
B-iii and A-ii		554	511
C-i and A-i	i_{TFA}	308	258

an n-C_4H_9OCO– (butyl ester) group. Table X indicates that the calculated i_{BE} values agreed with each other fairly well. Table X also indicates the group index (i_{TFA}) of an CF_3CONH– (trifluoroacetamino) group calculated in a similar manner.

The i_{AA} values were 1152 and 1112 for OV-17 and Dexsil 300 GC, respectively, while the sum of the i_{BE} (the average value of i_{BE} obtained for three types of monoamino-dicarboxylic acids) and i_{TFA} values was 871 and 778 for OV-17 and Dexsil 300 GC, respectively. The difference between i_{AA} and the sum of i_{BE} and i_{TFA} was 281 and 334 for OV-17 and Dexsil 300 GC, respectively, and this difference seemed much higher than expected for the retention index of an α-carbon atom in the amino acid group. It was considered that there was no additivity of the component group retention indices in this instance owing to the branched structure of the α-amino acid group.

The sum of the i_S and i_{AA} values obtained for sulphur-containing diamino-dicarboxylic acids was much smaller than that obtained from the retention indices of S-alkylcysteines (Table VIII). The i_S value for diaminodicarboxylic acids was obtained from the data mentioned above, and was slightly lower than that for other types of amino acids, and the i_{AA} value for diaminodicarboxylic acids was calculated from the i_C and i_S values for diaminodicarboxylic acids. The i_{AA} value thus obtained was much smaller than that for other types (A, B and C) of amino acids.

Now, the retention index of a BTFA-amino acid can be calculated from the following equation:

$$I = \Sigma n_X \cdot i_X$$

where n_X is the number of X groups in a molecule and i_X is the group retention index of the group X. The retention indices of ethionine, S-β-aminoethylcysteine and di-

TABLE XI

COMPARISON OF OBSERVED AND CALCULATED RETENTION INDICES

Amino acid	*Stationary phase*	*Group retention index used*				*Retention index*	
		i_C	i_{AA}	i_S	i_{TFA}	*Calculated*	*Found*
ETH	OV-17	80	1152	290		1762	1800
	Dexsil 300 GC	84	1112	237		1685	1729
SAEC	OV-17	140	1152	290	308	2170	2117
	Dexsil 300 GC	140	1112	237	258	2027	2000
DAPA	OV-17	120	949			2258	2257
	Dexsil 300 GC	136	914			2236	2243

aminopimelic acid, which were not used for the calculation of any group retention index, were calculated and compared with the observed retention indices. The group retention indices used for the calculation (also shown in Table XI) were average values of the appropriate group retention indices observed. The results in Table XI demonstrate that the retention indices calculated from the group retention indices agree fairly well with the observed values.

ΔMe of Evans and Smith[20–22]

The correlation of retention data with chemical structure can also be discussed in terms of another parameter, the ΔMe factor of Evans and Smith[20–22]. This parameter is defined as the difference between the effective molecular weight (Me) and the actual molecular weight (M) of a solute

$$\Delta Me = Me - M$$

TABLE XII

DIFFERENCES BETWEEN EFFECTIVE MOLECULAR WEIGHTS AND ACTUAL MOLECULAR WEIGHTS (ΔMe) OF BTFA-AMINO ACIDS

Class	*No.*	*Amino acid*	*Mol. wt. of BTFA-amino acid*	ΔMe	
				OV-17	*Dexsil 300 GC*
A-i	1	ALA	241.2	−62.5	−68.3
	2	NORVAL	269.3	−70.9	−75.4
	3	NORLEU	283.3	−74.0	−77.6
	4	AOA	311.3	−77.7	−80.9
A-ii	5	SMC	287.3	−57.1	−70.7
	6	SEC	301.3	−63.7	−75.6
	7	SPC	315.4	−67.6	−79.1
	8	SBC	329.4	−69.9	−80.9
A-iii	9	MET	301.3	−54.3	−65.3
	10	ETH	315.4	−60.9	−70.9
B-i	11	ASP	331.3	−71.9	−86.1
	12	GLU	345.3	−66.0	−80.6
	13	APA	373.3	−66.9	−78.7
B-ii	14	SCMC	387.4	−73.3	−92.0
	15	SCEC	401.4	−72.1	−90.9
	16	SCPC	415.5	−71.8	−89.9
B-iii	17	SCMHC	401.4	−70.1	−90.9
	18	SCEHC	415.5	−67.6	−89.5
	19	SCPHC	429.4	−66.0	−87.1
C-i	20	DABA	366.3	−125.7	−133.1
	21	ORN	380.3	−114.9	−125.8
	22	LYS	394.3	−111.8	−121.6
C-ii	23	SAEC	412.3	−113.3	−129.7
D-i	24	DAPA	454.5	−135.9	−137.9
D-ii	25	LAN	486.4	−151.4	−160.8
	26	CYS	518.5	−150.1	−162.4
	27	HOMOCYS	546.5	−142.6	−151.7
	28	MBC	532.5	−139.6	−156.4
	29	EBC	546.5	−137.5	−151.8

where the effective molecular weight is the molecular weight of a hypothetical *n*-paraffin which is supposed to have the same retention time as the solute, and thus is related to the Kovàts retention index (I) as follows:

$$Me = 14.026 \cdot \frac{I}{100} + 2.016$$

Table XII gives the ΔMe values of BTFA-amino acids calculated from their retention indices on OV-17 and Dexsil 300 GC. All of the ΔMe values obtained were negative, which suggests a shielding effect of trifluoroacetyl groups. It was reported[21,22] that ΔMe was virtually constant throughout a homologous series except for a few initial homologues. The ΔMe values of monoaminomonocarboxylic acids, except for methionine, on Dexsil 300 GC ranged between −68 and −81, regardless of the presence or absence of sulphur. The ΔMe values of sulphur-containing monoaminomonocarboxylic acids on OV-17 were only slightly lower than those of the non-sulphur-containing analogues. The ΔMe values of monoaminodicarboxylic acids ranged between −66 and −73 and between −81 and −92 on OV-17 and Dexsil 300 GC, respectively. The ΔMe values of diaminomonocarboxylic acids ranged between −112 and −126 and between −122 and −133 on OV-17 and Dexsil 300 GC, respectively. Hence the addition of a second carboxyl group changed ΔMe only slightly, while the addition of a second amino group on the terminal carbon atom altered ΔMe significantly.

The ΔMe values of sulphur-containing diaminodicarboxylic acids ranged between −138 and −151 and between −152 and −162 on OV-17 and Dexsil 300 GC, respectively, and the absolute values were higher than those of the diaminomonocarboxylic acids. The absolute ΔMe value of 2,6-diaminopimelic acid on Dexsil 300 GC was apparently lower than those of the corresponding sulphur-containing analogues. It is interesting that ΔMe is not very sensitive to small variations in structural features, in contrast to ΔI.

Molar response

The FID molar responses of BTFA-amino acids relative to that of BTFA-glutamic acid are listed in Table XIII. The RMR values of some amino acids, taken from the literature (recalculated if necessary), are collected in Table XIV. The RMR values observed on OV-17 and Dexsil 300 GC agreed with each other within 10% for most amino acids. The RMR values of S-methylcysteine and S-carboxymethylhomocysteine were not determined as the elemental analyses of these two amino acids were not satisfactory, although the gas chromatogram showed a single peak except for solvent peaks in each instance. The RMR values of S,S′-methylene- and S,S′-ethylenebiscysteines on OV-17 were not determined because the baseline drift was extensive. Gehrke *et al.*[1] reported that RMR values varied slightly from one stationary phase to another. The RMR values of cystine and methionine given in the literature varied considerably from one report to another. The RMR value of aminooctanoic acid in the literature did not agree with the values obtained in this work.

The preparation of pure artifact amino acids for authentic samples usually involves tedious and time-consuming work. If the RMR can be estimated from the chemical formula of a given amino acid, then the quantitative determination of the artifact amino acid in chemically modified wool can be greatly facilitated. In many instances, it is considered that the FID molar response is an additive property of the structural

TABLE XIII

FID MOLAR RESPONSES (RMR) OF BTFA-AMINO ACIDS RELATIVE TO BTFA-GLUTAMIC ACID OBSERVED IN THIS WORK

Class	*No.*	*Amino acid*	*RMR calculated*				*RMR found*	
			According to literature[25]		*This work*		*OV-17*	*Dexsil 300 GC*
			ECN	*RMR*	*ECN*	*RMR*		
A	1	ALA	5.3	0.51	4.8	0.52	0.51	0.48
	2	NORVAL	7.3	0.70	6.8	0.73	0.74	0.70
	3	NORLEU	8.3	0.80	7.8	0.84	0.87	0.87
	4	AOA	10.3	0.99	9.8	1.05	1.10	1.14
	5	SMC	6.3	0.68	4.9	0.53		
	6	SEC	7.3	0.70	5.9	0.63	0.71	0.69
	7	SPC	8.3	0.80	6.9	0.74	0.83	0.83
	8	SBC	9.3	0.89	7.9	0.85	0.90	0.92
	9	MET	7.3	0.70	5.9	0.63	0.66	0.64
	10	ETH	8.3	0.80	6.9	0.74	0.83	0.83
B	11	ASP	9.4	0.90	8.3	0.89	0.89	0.93
	12	GLU	10.4	1.00	9.3	1.00	1.00	1.00
	13	APA	12.4	1.19	11.3	1.22	1.09	1.11
	14	SCMC	10.4	1.00	8.4	0.90	0.87	0.86
	15	SCEC	11.4	1.10	9.4	1.01	0.96	1.00
	16	SCPC	12.4	1.19	10.4	1.12	1.04	1.15
	17	SCMHC	11.4	1.10	9.4	1.01		
	18	SCEHC	12.4	1.19	10.4	1.12	1.05	1.12
	19	SCPHC	13.4	1.29	11.4	1.23	0.98	1.08
C	20	DABA	6.7	0.64	5.1	0.56	0.56	0.61
	21	ORN	7.7	0.74	6.1	0.66	0.69	0.69
	22	LYS	8.7	0.81	7.1	0.76	0.72	0.80
	23	SAEC	7.7	0.74	5.2	0.56	0.48	0.52
D	24	DAPA			10.8	1.14	1.00	1.07
	25	LAN			8.7	0.94	0.87	0.91
	26	CYS			7.8	0.84	0.78	0.78
	27	HOMOCYS			9.8	1.05	0.50	0.63
	28	MBC			8.8	0.95		0.86
	29	EBC			9.8	1.05		1.03

features and the contribution of a structural unit to the molar response is usually expressed as the effective carbon number (ECN). The ECN of a molecule is a sum of the ECN values of structural units in the molecule. Sometimes, the ECN of a molecule is expressed as the carbon number of the molecule minus the sum of non-effective carbon numbers (Non-ECN) of structural units in the molecule. Thus, Non-ECN is the carbon number minus ECN.

Islam and Darbre[25] recently reported RMR values for trifluoroacetyl methyl esters (MTFA) of 29 amino acids on polar mixed silicone stationary phases by linear temperature-programmed GLC. Non-ECN values determined for monoaminomonocarboxylic acids (A-i type), monoaminodicarboxylic acids (B-i type), and diaminomonocarboxylic acids (C-i type) are 3.7, 4.6 and 5.3, respectively. These Non-ECN values indicate that the effect of the CF_3CONH-group was not additive: CF_3CONH-attached to the α-carbon had a higher Non-ECN value than a second CF_3CONH-

TABLE XIV

FID MOLAR RESPONSES (RMR) OF BTFA-AMINO ACIDS RELATIVE TO BTFA-GLUTAMIC ACID GIVEN IN THE LITERATURE

No.	*Amino acid*	*RMR*				
		Ref. 23	*Ref. 1*	*Ref. 24*	*Ref. 14*	*Ref. 2*
1	ALA	0.53*	0.48***	0.52***	0.53***	0.53§§
2	NORVAL		0.75**		0.58***	
3	NORLEU		0.83**		0.85***	
4	AOA				0.82***	
9	MET	0.70*	0.54***	0.56***	0.55***	0.67§§
11	ASP	0.92*	0.85***	0.91***	0.90***	0.94§§
12	GLU	1.00*	1.00***	1.00***	1.00***	1.00§§
14	SCMC				0.87***	
21	ORN		0.69***		0.76***	0.58§§
22	LYS	0.86*	0.72***	0.86***	0.86***	0.76§§
26	CYS	0.41*	0.79§	0.94§		0.38§§

* On 1.00% (w/w) neopentyl glycol succinate on Gas-Chrom A.
** On 5% (w/w) DC-550 on Chromosorb W AW.
*** On 0.325% (w/w) ethylene glycol adipate on HT Chromosorb G AW or Chromosorb W AW.
§ On 1.50% (w/w) OV-17 on HP Chromosorb G.
§§ On 10% (w/w) Apiezon M on HP Chromosorb W.

group attached to the terminal carbon atom. It was pointed out[25] that the presence of the thioether bond did not alter the ECN, by comparison of the responses of MTFA-methionine and non-sulphur-containing amino acids. It was also stated that the Non-ECN values obtained could be applied to the molar response values reported previously[20] at the same laboratory for N-trifluoroacetyl methyl, *n*-butyl and *n*-pentyl esters of five amino acids.

The Non-ECN values of Islam and Darbre[25] were applied to the BTFA-amino acids (except for BTFA-diaminodicarboxylic acids) studied in this work and RMR values (relative to glutamic acid) were calculated. The results shown in Table XIII indicate that the calculated RMR values agree fairly well with the observed values for sulphur-containing and non-sulphur-containing monoaminomonocarboxylic acids and non-sulphur-containing monoaminodicarboxylic and diaminomonocarboxylic acids, but a discrepancy was obvious for other types of amino acids. It seemed that a positive Non-ECN value of a sulphur atom should be taken for monoaminodicarboxylic and diaminomonocarboxylic acids in order to obtain comparable calculated RMR values. The response of sulphur compounds was reported to be considerably influenced by the design of the detector and by the operating conditions[27].

In this paper, an empirical and completely additive method for the calculation of ECN values of sulphur-containing and non-sulphur-containing amino acids is proposed in which the ECN values for –COO–, $-NHCOCF_3$ and –S– groups are assumed to be −0.5, −0.7 and −0.9, respectively. These values are within the range of reported ECN values for each structural unit[27–29]. The ECN and RMR values calculated on this basis are also recorded in Table XIII for comparison. The calculated RMR values compare well with the observed values, except for S-carboxypropylhomocysteine and homocystine. The observed RMR value of homocystine is apparently too low and the extensive degradation of the BTFA derivative in the chromatographic columns or the

incomplete recovery of the BTFA derivative in the derivatization seems to occur. It is interesting to note that cystathionine did not give any peak under normal reaction and chromatographic conditions. The validity of the ECN values given in this work should be checked further by comparing calculated and observed RMR values for other amino acids.

CONCLUSION

Linear temperature-programmed GLC of S-*n*-alkyl-, S-*ω*-carboxyalkyl- and S-*β*-aminoethylcysteines, lanthionine, cystine, S,S'-alkylenebiscysteines and some homocysteine analogues, as well as similar non-sulphur-containing amino acids, as their N-trifluoroacetyl *n*-butyl esters, was studied on OV-17 and the less polar and more thermally stable Dexsil 300 GC, the temperature being programmed from 100° at the rate of 8°/min.

The Kovàts retention indices and ΔI values as well as Evans and Smith[21, 22] ΔMe values were determined. The retention index on OV-17 was always higher than the corresponding retention index on Dexsil 300 GC and the difference (ΔI) was constant for each homologous series. The plot of the retention index against molecular weight was linear for a homologous series, except for the first member, but was not parallel to the plots for other homologous series.

An attempt was made to calculate the retention index on an assumption of an additive property of contributions of the structural features to the retention index. The contribution of a methylene group to the retention index varied with the type of amino acid, ranging from 80 for monoaminomonocarboxylic acids to 140 for diaminomonocarboxylic acids on OV-17 and from 84 for monoaminomonocarboxylic acids to 140 for diaminomonocarboxylic acids on Dexsil 300 GC. The contribution of a sulphur atom to the retention index was 290 on OV-17 and 237 on Dexsil 300 GC for the amino acids except for diaminodicarboxylic acids. The contribution of an *n*-C_4H_9OCO-$(CF_3CONH)CH$– group was 1152 on OV-17 and 1112 on Dexsil 300 GC for the amino acids except for diaminodicarboxylic acids. The contribution of this group in diaminodicarboxylic acids was 949 on OV-17 and 914 on Dexsil 300 GC. The contribution of a second *n*-C_4H_9OCO– group on the terminal carbon atom was 563 on OV-17 and 520 on Dexsil 300 GC, and that of a second CF_3CONH– group on the terminal carbon atom was 308 on OV-17 and 258 on Dexsil 300 GC.

All of the ΔMe values were negative, which suggests that only slight interactions between the amino acid derivatives and the stationary phases occur. The ΔMe value was nearly constant for both sulphur-containing and non-sulphur-containing monoaminomono- and -dicarboxylic acids.

The effective carbon numbers of a CF_3CONH– group, a C_4H_9OCO– group and a sulphur atom were assumed to be −0.7, 3.5 and −0.9, respectively, in the calculation of the FID response. The calculated relative molar responses by assuming a complete additive property compared well with the observed values.

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