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GAS CHROMATOGRAPHY–MASS SPECTROMETRY OF THE N(O)-HEPTAFLUOROBUTYRYL ISOBUTYL ESTERS OF THE PROTEIN AMINO ACIDS USING ELECTRON IMPACT IONISATION*

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SUMMARY

The N(O)-heptafluorobutyryl isobutyl esters of the protein amino acids have been analysed by combined gas chromatography–mass spectrometry using electron impact ionisation. The spectral data have been used to confirm the structures of the amino acid derivatives.

INTRODUCTION

There have been numerous developments in the analysis of amino acids by gas–liquid chromatography of appropriate derivatives and these have been reviewed in detail by Hušek and Macek[1]. However, there have been relatively few studies in which gas chromatography (GC) has been combined with mass spectrometry (MS) to confirm the identity and structure of the derivatives formed. Gelpi *et al.*[2] used GC–MS to study the N(O)-trifluoroacetyl (TFA) *n*-butyl esters of most of the protein amino acids and proposed mechanisms for the fragmentation of these derivatives. Felker and Bandurski[3] used GC–MS for quantitative analysis of the protein amino acids as their N(O)-heptafluorobutyryl (HFB) isoamyl esters.

The separation of the protein amino acids as their N(O)-HFB isobutyl esters has been reported from this laboratory[4,5]. These amino acid derivatives have been studied using GC–MS (a) to confirm their identity and structure; (b) to provide a spectral basis for further study of non-protein acids in GC separations of physiological samples. We now present the mass spectral data.

EXPERIMENTAL

The N(O)-HFB isobutyl amino acid esters were prepared and chromatographed as previously described[4,5].

GC–MS was performed using a Finnigan Model 3300 gas chromatograph–

* NRCC No. 15662.

mass spectrometer. The separator oven and transfer line were maintained at 240°. The ioniser voltage was 70 eV. Spectra were recorded at a rate of 2 sec per scan. Accumulation of spectra and manipulations of data such as background subtraction were performed using a Finnigan Model 6000 MS data system.

RESULTS AND DISCUSSION

A reconstructed gas chromatogram illustrating the separation of most of the protein amino acids is shown in Fig. 1. The separation is excellent and enabled good spectra of the individual amino acids to be obtained.

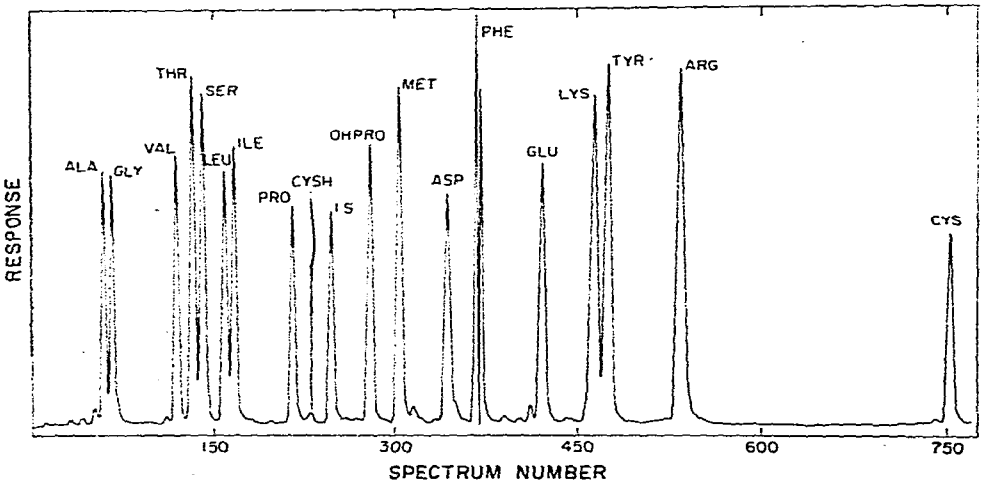

Fig. 1. Reconstructed gas chromatogram illustrating the separation of N(O)-HFB isobutyl amino acids. Detector saturation occurred in the phenylalanine peak. Cysteine was not present in the sample shown but was chromatographed separately.

The 70-eV electron-impact MS fragmentation patterns of the aliphatic amino acids glycine, alanine, valine, leucine and isoleucine are shown in Table I. The spectra had several characteristic features such as the perfluoroalkane series and ions representing the loss of C_4H_7 (M − 55), C_4H_9O (M − 73) and C_4H_9OCO (M − 101) from the ester group. The perfluoroalkane ions were generally much more prominent in these spectra than in the spectra of the corresponding isoamyl esters[3] with the exception of the quantity of the CF_3 ion obtained for isoleucine.

Ions corresponding to M − 149 (glycine, m/e = 178, 7%; alanine, m/e = 192, 5.9%), M − 199 (glycine, m/e = 128, 9.0%; alanine, m/e = 142, 3.3%), M − 219 and M − 249 (Table I) were present in significant amounts only in the spectra of glycine and alanine. Also the ion m/e = 226 (M − 101) was much more intense than the corresponding ions in the spectra of N-TFA *n*-butyl glycine[2] and N-HFB isoamyl glycine[3] (hereafter referred to for simplicity as the *n*-butyl and isoamyl amino acid; for the same reason, the charge designation has been omitted).

The valine spectrum contained two ions (M − 116, m/e = 253, 11.2%; M − 155, m/e = 214, 16.1%) constituting less than 0.4% of the base ion in the spectra of all the other aliphatic amino acids. These two ions should, therefore, be

TABLE I

ALIPHATIC AMINO ACID 70-eV FRAGMENTATION PATTERNS

Ion	*Amino acid*									
	Gly		*Ala*		*Val*		*Leu*		*Ile*	
	m/e	%	*m/e*	%	*m/e*	%	*m/e*	%	*m/e*	%
Molecular ion	327	—	341	—	369	—	383	—	383	—
Base ion	56	100.0	240	100.0	54	100.0	68	100.0	56	100.0
M − C_4H_7	272	1.7	286	0.7	314	0.3	328	0.3	328	0.3
M − OC_4H_9	254	—	268	0.4	296	0.1	310	0.1	310	0.1
M − $OCOC_4H_9$	226	66.1	240	100.0	268	49.1	282	13.2	282	21.5
M − (C_3F_7+H_2)	206	5.4	220	4.6	248	2.2	262	1.0	262	1.0
M − ($C_5H_8O_2$+C_3H_7)	—	—	—	—	226	4.9	240	36.7	240	0.3
M − ($C_5H_9O_2$+C_4H_8)	170	1.6	—	—	212	0.8	226	5.7	226	9.8
M − C_3F_7	158	0.5	172	0.3	—	—	214	13.4	214	13.6
M − (C_2F_5+$OCOC_4H_8$)	108	17.1	122	16.0	150	1.5	164	0.2	164	0.2
M − 249*	78	13.1	92	9.8	120	0.3	134	0.3	134	0.7
M − (C_3F_7CO+C_4H_8)	74	14.1	88	14.4	116	8.3	130	6.8	130	7.5
M − (C_3F_7CO+OC_4H_8)	58	40.7	72	19.3	100	24.1	114	8.5	114	21.6
C_4H_7	55	89.6	55	47.8	55	95.2	55	49.8	55	70.7
C_4H_8	56	100.0	56	77.1	56	99.3	56	64.9	56	100.0
C_4H_9	57	91.4	57	93.1	57	96.6	57	98.6	57	88.1
CF_3	69	68.0	69	72.5	69	43.1	69	96.1	69	58.2
CF_3CF_2	119	7.5	119	7.5	119	5.4	119	3.5	119	3.6
$CF_3CF_2CF_2$	169	39.8	169	27.4	169	8.7	169	13.1	169	15.1

* The structure of these fragments is uncertain. The ion masses indicate homology and could correspond to the formulae C_2H_5NOF and C_3H_7NOF for Gly and Ala, respectively, suggesting that some rearrangement has occurred.

useful for diagnostic purposes. These ions were also observed in the isoamyl ester spectrum but at a slightly lower intensity (*m/e* = 253, 6.7%; *m/e* = 214, 8.4%).

Losses of 143, 157 and 169 were characteristic of the spectra of leucine and isoleucine and only small amounts of these ions were observed for glycine, alanine and valine. The ion (M − 143) was much more prominent in the leucine spectrum (36.7%) than in the spectrum of isoleucine (0.3%) indicating a preferred loss of the secondary carbon (*m/e* = 43) along with $C_5H_8O_2$ in leucine. A similar observation was made for the isoamyl esters[3]. However, loss of the butoxy group (C_4H_9O, M = 73) along with the amino acid side chain (C_4H_9, M = 57) gave a more intense M − 130 ion for isoleucine (19.3%) than for leucine (4.5%) suggesting easier cleavage of the bond between the α and β carbons in isoleucine; a similar effect was also observed for the isoamyl esters[3]. The ions corresponding to the combined loss of CF_3 and C_4H_9 (M − 126) noted for the isoamyl esters were not observed for the isobutyl esters. Alternatively, the loss of 55 (M − C_4H_7) observed for the isobutyl (Table I) and *n*-butyl esters[2] would correspond to a loss of C_5H_9 (M = 69) if a similar mechanism operated in the fragmentation of the isoamyl esters. Thus, the loss of 126 from the isoamyl ester could represent the ion M − (C_5H_9 + C_4H_9). The corresponding ion in the isobutyl ester spectrum would then be M − (C_4H_7 + C_4H_9), (M − 112). The intensities of these ions were: leucine 1.4% and isoleucine 5.4%. Considering the loss of C_4H_7 observed for the *n*-butyl and isobutyl esters of leucine, it seems reasonable

that an analogous loss would occur for the isoamyl ester. Thus the loss of 69 from isoamyl leucine and isoleucine may represent loss of an ester fragment rather than CF_3[3]. This possibility is also suggested by the absence of ions corresponding to M — 69 in the spectra of the isobutyl esters. This suggestion was further supported by the presence of an ion corresponding to M — 69 in the spectrum of N-acetyl isoamyl leucine, in which the ion could not be attributed to a loss of CF_3.

TABLE II

70-eV FRAGMENTATION PATTERNS OF N(O)-HFB ISOBUTYL ESTERS OF SERINE, THREONINE, HYDROXYPROLINE, PROLINE AND PIPECOLIC ACID

Ion	*Compound*									
	Ser		*Thr*		*OH-Pro*		*Pro*		*Pip*	
	m/e	%	*m/e*	%	*m/e*	%	*m/e*	%	*m/e*	%
Molecular ion	553	—	567	—	579	—	367	0.3	381	0.1
Base ion	55	100.0	56	100.0	264	100.0	266	100.0	67	100.0
M — C_4H_7	498	0.2	512	0.2	524	0.2	312	0.1	326	—
M — OC_4H_9	480	0.1	494	0.1	506	0.1	294	0.1	308	0.2
M — $OCOC_4H_9$	452	0.6	466	0.7	478	1.7	266	100.0	280	51.9
M — $OCOC_3F_7$	340	0.1	354	0.2	366	0.2	—	—	—	—
M — (COC_3F_7 + C_4H_8)	300	0.2	314	0.1	—	—	—	—	—	—
M — (COC_3F_7 + OC_4H_8)	284	2.1	298	1.2	310	1.0	98	4.6	112	5.4
M — ($NHCOC_3F_7$ + $OCOC_4H_9$ + H)	239	44.9	253	46.0	265	13.4	53	14.6	67	100.0
M — ($OCOC_3F_7$ + $OCOC_4H_9$ + H)	238	49.5	252	43.6	264	100.0	52	6.9	66	41.2
M — (C_3F_7 + C_2F_5 + C_4H_7)	210	21.2	224	1.5	236	0.1				
C_4H_7	55	100.0	55	99.4	55	43.4	55	17.3	55	90.5
C_4H_9	57	67.8	57	97.8	57	91.6	57	28.9	57	37.3
CF_3	69	92.2	69	98.7	69	79.1	69	95.6	69	35.3
CF_3CF_2	119	20.0	119	12.1	119	7.8	119	6.5	119	3.4
$CF_3CF_2CF_2$	169	54.9	169	60.8	169	33.5	169	38.4	169	12.7

The 70-eV electron-impact mass fragmentation patterns of the N(O)-HFB isobutyl esters of serine, threonine, hydroxyproline, proline and the chromatographic internal standard pipecolic acid are shown in Table II. The hydroxy amino acids showed characteristic losses of 55, 73 and 101 although the corresponding ions were much less intense than in the spectra of the aliphatic amino acids. Although the intensity of the ions is low, the loss of the O-acyl group (M — 213) appeared to be characteristic of the hydroxy amino acids. The significance of this loss was enhanced by the intensity of the ion M — 314 (M — $C_3F_7CO_2$ — $C_4H_9CO_2$). The isobutyl serine and threonine spectra were generally similar to the *n*-butyl and isoamyl spectra except for having a more intense ion corresponding to $C_3F_7CONHCCH_2$ (*m/e* = 238, 49.5%) for serine and $C_3F_7CONHC_3H_4$ (*m/e* = 252; 43.6%) for threonine. Also the serine spectrum contained an ion of *m/e* = 210 (21.2%) present only in small amounts in the spectra of threonine and hydroxyproline. This ion, therefore, distinguishes serine from the other hydroxy amino acids.

The spectra of isobutyl proline and pipecolic acid also contained ions charac-

TABLE III

DICARBOXYLIC AND BASIC AMINO ACID 70-eV FRAGMENTATION PATTERNS

Ion	*Amino acid*									
	Asp		*Glu*		*Lys*		*His*		*Arg*	
	m/e	%	*m/e*	%	*m/e*	%	*m/e*	%	*m/e*	%
Molecular ion	441	—	455	—	594	0.1	407	—	818	—
Base ion	55	100.0	55	100.0	67	100.0	81	100.0	70	100.0
M − C_4H_7	386	0.2	400	—	539	0.1	352	—	763	—
M − OC_4H_9	368	—	382	0.2	521	0.5	334	—	745	0.2
M − $OCOC_4H_9$	340	2.5	354	0.6	493	0.7	306	23.8	717	0.7
M − (OC_4H_9+C_4H_8)	312	2.2	326	1.7						
M − ($OCOC_4H_9$+C_4H_8)	284	10.8	298	8.2						
M − ($OCOC_4H_9$+ OC_4H_{10})	266	5.6	280	10.8						
M − ($OCOC_4H_9$+ $OCOC_4H_9$)	239	22.5	253	5.9						
M − ($OCOC_4H_9$+ $OCOC_4H_{10}$)	238	5.8	252	30.8						
M − ($C_3F_7CONH_2$+ $OCOC_4H_9$)	127	2.8	141	0.5	280	50.5			504	6.4
C_4H_9	57	93.6	57	96.8	57	96.7	57	11.0	57	97.4
$C_4H_5N_2$ (His)*	81	—	81	—	81	13.2	81	100.0	81	9.3
$C_4H_6N_2$ (His)*	82	6.2	82	11.6	82	25.6	82	28.8	82	7.5
C_4H_7NO (Glu)*	85	1.8	85	96.2	85	12.7	85	—	85	4.1
C_6H_8N(Lys); $C_5H_6N_2$(His)	94	0.4	94	0.5	94	11.2	94	8.4	94	8.4
$C_4H_5NO_2$ (Asp)	99	23.5	99	3.5	99	8.9	99	—	99	18.2
$C_5H_7N_3$ (His)	109	0.1	109	2.8	109	10.1	109	14.7	109	9.0
C_6H_8NO (Lys)	110	0.8	110	3.4	110	10.9	110	5.0	110	5.0
CF_3	69	41.9	69	27.6	69	55.3	69	33.8	69	95.5
CF_3CF_2	119	2.9	119	2.1	119	4.1	119	7.1	119	12.4
$CF_3CF_2CF_2$	169	7.4	169	5.8	169	21.8	169	6.0	169	28.9

* The assignment applies only to the amino acid indicated in parentheses; the structure of the fragments would obviously be different for the other amino acids.

teristic of the loss of ester fragments with the M − 101 ion being the parent ion. Ions corresponding to the diagnostic ions at *m/e* = 139 and 96 noted for the *n*-butyl ester of proline[2] were observed at *m/e* = 239 (1.5%) and *m/e* 96 (6.0%) but at a slightly greater intensity despite the large ionisation energy used in this study. The homologue of the cyclic ketene ion (*m/e* = 96) was observed for pipecolic acid (*m/e* = 110, 4.2%) but no ion was detected at *m/e* = 239.

The 70-eV fragmentation patterns of the N(O)-HFB isobutyl esters of the dicarboxylic amino acids (Table III) are characterised by the perfluoroalkane series, by the loss of ester fragments and by the presence of significant amounts of ions (M − 129, M − 175, M − 202 and M − 203) either absent or having very low intensity in the spectra of the amino acids described earlier. The aspartic acid spectrum also contained a significantly more intense ion at *m/e* = 99 (23.5%; $C_4H_4NO_2$) and at *m/e* = 88 (7.6%; $C_3H_6NO_2$; less than 0.5% for the other spectra in Table III). The glutamic acid spectrum contained a significantly more intense ion at *m/e* = 85 (96.2%;

C_4H_6NO). In general, the spectra of the isobutyl esters of aspartic acid and glutamic acid were similar to the spectra of the *n*-butyl and isoamyl esters.

Fragmentation of the basic amino acids was also characterised by small amounts of ions indicating losses of ester fragments and by the perfluoroalkane series of ions (Table III). The ion $m/e = 306$ (M − 101) in the histidine spectrum was more intense than in the spectra of lysine and arginine but the ion $m/e = 57$ (C_4H_9) was much less intense. Other ions apparently characteristic of the fragmentation of the basic amino acids were $m/e = 81$ and $m/e = 94$. The ion $m/e = 138$ was more intense in the histidine (4.2%) and arginine (8.5%) spectra than in the spectra of lysine or the dicarboxylic amino acids where it amounted to less than 1% of the base ion. The arginine spectrum also contained an ion $m/e = 95$ at an intensity of 16% whereas in the histidine spectrum, its intensity was less than 1% but for lysine it amounted to 4%.

The spectrum of the histidine derivative was also obtained using an ioniser voltage of 35 eV. The main difference was the detection of the molecular ion $m/e = 407$ confirming the identity of the product as the mono-HFB isobutyl ester. Furthermore, co-injection with acetic anhydride confirmed the formation of the N-acetyl, N-HFB isobutyl ester ($m/e = 449$). Significantly more intense ions corresponding to loss of ester fragments (*e.g.*, $m/e = 306$, 54.2%) were recorded when the spectrum was obtained using the solid probe injection system but the base ion was unchanged ($m/e = 81$) suggesting that the ester bond is more readily cleaved in the isobutyl ester than in the *n*-butyl ester[2]. Ions at $m/e = 341$ (M − 66, 3.1%), $m/e = 345$ (M − 62, 7.3%) and $m/e = 355$ (M − 52, 2.6%) were detected using this latter technique but corresponding ions were not obtained for either the *n*-butyl[2] or the isoamyl esters[3]. The (M − 66) ion was not detected in other spectra and thus must derive from the loss of the imidazole ring which has itself lost a proton. The ion $m/e = 345$ corresponds to the loss of C_3H_7 + one fluorine atom and the ion $m/e = 355$ to the loss of C_3H_2N suggesting rearrangement of the imidazole moiety with retention of NH in the amino acid side chain. Ions at $m/e = 221$ (M − 186; 23%) and $m/e = 281$ (M − 126; 11.5%) were also detected by solid probe injection but not via the chromatographic column. It is suggested that these ions correspond to the loss of (CF_3CF_2 + $C_3N_2H_3$) and (CF_3 + C_4H_9), respectively.

The spectra of the derivatives of the aromatic amino acids phenylalanine, tyrosine and tryptophan were characterised by small amounts of ions indicative of the loss of ester fragments, by the perfluoroalkane series of ions and by ions peculiar to the aromatic amino acids (Table IV). The fragmentation of the N-acylated isobutyl ester of phenylalanine was, in some respects, very similar to that of the *n*-butyl ester. For instance, the ion $m/e = 148$ (M − $C_3F_7CONH_2$ − C_4H_8) was very intense (99.4% *vs.* 100% for the *n*-butyl ester) whereas the corresponding ion in the isoamyl ester spectrum was not nearly so intense (51.5%). In other respects, however, the isobutyl spectrum resembled the isoamyl ester spectrum. For example, the ion $m/e = 91$ was very intense (98.7% *vs.* 100% for the isoamyl ester) and the ion $m/e = 204$ (M − $C_3F_7CONH_2$) at 7% was, like the corresponding isoamyl spectrum ion (17.4%) much less intense than the corresponding isobutyl spectrum ion (95%). This latter difference between ion intensities probably results from an ionisation energy of 20 eV being used to fragment the *n*-butyl esters. The phenylalanine isobutyl ester spectrum also differed from the spectra of the *n*-butyl and isoamyl esters in the presence of significantly more intense ions at $m/e = 131$ (M − $C_3F_7CONH_2$ −

TABLE IV

AROMATIC AMINO ACID 70-eV FRAGMENTATION PATTERNS

Ion	*Amino acid*					
	Phe		*Tyr*		*Tyr*	
	m/e	%	*m/e*	%	*m/e*	%
Molecular ion	417	—	629	—	652	2.8
Base ion	147	100.0	57	100.0	326	100.0
$M - OC_4H_9$	344	0.1	556	0.1	579	0.3
$M - OCOC_4H_9$	316	2.6	528	5.6	551	3.6
$M - OCOC_3F_7$	204	7.0	416	2.0	439	14.6
$M - (OCOC_3F_7 + OC_4H_8)$	148	99.4	360	35.5	383	17.5
$M - (OCOC_3F_7 + OCOC_4H_9)$	103	89.2	315	2.7	338	1.3
C_4H_7	55	16.0	55	41.2	55	0.6
C_4H_9	57	42.0	57	100.0	57	3.6
C_5H_5	65	27.2	65	5.7		
C_5H_{10}	70	2.4	70	1.4	70	0.3
C_6H_5	77	26.8	77	42.8	77	1.7
C_6H_6	78	12.0	78	47.6	78	0.7
$C_6H_4CH_2$ or C_6H_5CH	90	99.8	90	46.9	90	0.2
$C_6H_5CH_2$	91	98.7	91	34.7	91	0.3
$C_6H_4C_2HNCH_2$	129	6.5	129	7.2	129	56.0
$C_6H_5CHCHCO$	131	63.6	131	9.8	131	5.0
CF_3	69	24.6	69	94.1	69	10.0
CF_3CF_2	119	60.9	119	29.7	119	2.1
$CF_3CF_2CF_2$	169	4.3	169	37.8	169	18.9

OC_4H_9) and $m/e = 104$ ($ArCH = CH_2$). The ion $m/e = 119$ was also significantly more intense but this ion cannot be assigned unambiguously in the N-HFB esters since it could represent either C_2F_5 or ($M - C_4H_9O_2C - C_3F_7CO$).

The spectrum of N-HFB tyrosine was generally similar to the spectra of the *n*-butyl and isoamyl derivatives. Significant differences arose for ions ($M - C_3F_7CONH_2$) and the aromatic ions $ArCH_2$. The aromatic ring in the isobutyl ester appeared to lose the O-acyl group more readily than the *n*-butyl and isoamyl esters since the ions $m/e = 90$ (C_7H_6) and $m/e = 91$ (C_7H_7) are much more intense. Nevertheless, like the isoamyl ester of tyrosine, charge retention appeared to be preferentially on the non-aromatic part of the molecule since the ion C_4H_9 was the base ion.

The spectrum of diacyl isobutyl tryptophan was generally similar to the spectra of the *n*-butyl and isoamyl derivatives. However, an intense ion at $m/e = 129$ (C_9H_7N, 56%) corresponding to cleavage of the amino acid side chain and loss of the acyl group from the indole N was not reported for the other two derivatives. When N-HFB isobutyl tryptophan was chromatographed small amounts of six components were detected in addition to the main tryptophan peak but none of these was monoacyl tryptophan (Fig. 2). The major of these other components (spectrum 630) had a parent ion identical to the main tryptophan peak, intense ions at $m/e = 326$ and 129 indicating the presence of the acylated and deacylated indole nucleus, respectively, and ions characteristic of the isobutyl ester. However, this component was not identified.

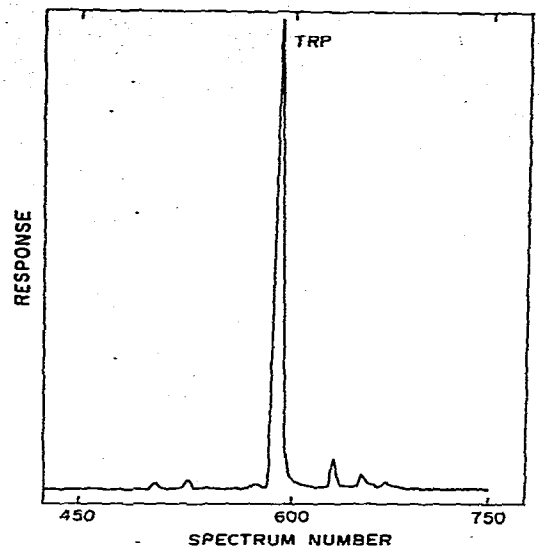

Fig. 2. Reconstructed gas chromatogram illustrating the chromatography of N-HFB isobutyl tryptophan.

TABLE V

70-eV FRAGMENTATION PATTERNS OF SULPHUR-CONTAINING AMINO ACIDS

Ion	*Amino acid*					
	Met		*Cysh*		*Cys*	
	m/e	%	*m/e*	%	*m/e*	%
Molecular ion	401	0.5	569	—	744	0.2
Base ion	75	100.0	169	100.0	57	100.0
M − C_4H_7	346	0.1				
M − OC_4H_9	328	0.4				
M − $OCOC_4H_9$	300	0.3	468	15.3		
M − (C_4H_7+$CH_3SCH_2CH_2$)	271	7.4				
M − ($OCOC_4H_9$+SCH_3)	253	14.8				
$C_3F_7CONHCHCH_2CO_2$			283	17.7	283	35.8
$C_3F_7CONHCCHCO$			265	32.3	265	87.7
$C_3F_7CONHCHCH_2$			240	61.3	240	3.7
$C_3F_7CONHCHCH$			238	53.2	238	91.4
$OCSCH_2CH(NHCO)COH$			159	32.3	159	2.5
$H_3CSCH_2CHCHCO_2$ or $OCSCH_2CHCO_2$	131	14.2	131	9.7	131	2.2
S–CH_2–$CHCO_2$	103	4.6	103	30.6	103	24.1
C_4H_9	57	96.8	57	64.5	57	100.0
$CH_3SCH_2CH_2$	75	100.0	75	1.6	75	9.3
$SCHCHCO$	86	9.8	86	3.2	86	11.6
CF_3	69	30.9	69	67.7	69	32.1
CF_3CF_2	119	3.4	119	14.5	119	4.1
$CF_3CF_2CF_2$	169	8.1	169	100.0	169	8.4

The mass spectrum of N-HFB isobutyl methionine (Table V), in addition to traces of ions indicating loss of ester fragments, contained ions characteristic of the methionyl side chain. Except for having a different base ion ($m/e = 75$; $CH_3SCH_2CH_2$) and the intensity of ions at $m/e = 131$ (14.2%; M $-$ CH_3SCH_2CH $-$ C_4H_9), $m/e = 86$ (9.8%; $CH_2CH_2CH_2CO_2$), $m/e = 74$ (55.4%; CH_3SCH_2CH) and $m/e = 61$ (99.2%; CH_2SCH_3) the spectrum corresponded closely to the spectrum of the isoamyl ester.

The mass spectrum of N,S-HFB isobutyl cysteine contained a relatively intense ester loss ion at $m/e = 468$ (15.3%; M $-$ $OCOC_4H_9$) and differed from the *n*-butyl and isoamyl spectra in having intense ions at $m/e = 283$ (17.7%; M $-$ C_3F_7COS $-$ C_4H_9), $m/e = 265$ (32.5%; $C_3F_7CONHC_3OH$) and $m/e = 238$ (53.2%; $C_3F_7CONHCHCH$). The spectrum of cystine was very similar to that of cysteine. The paucity of fragments of m/e greater than 283 demonstrated very easy cleavage of the S–S bond. The main differences from the cysteine spectrum were the intensities of the ions at $m/e = 283$ (35.8%; $C_3F_7CONHC_3H_3O_2$), $m/e = 265$ (87.7%; $C_3F_7CONHC_3OH$) $m/e = 240$ (3.7%; $C_3F_7CONHCH_2CH_2$) and $m/e = 159$ (2.5%; M/2 $-$ $C_3F_7CONH_2$). The major difference from the spectra of the corresponding *n*-butyl and isoamyl esters was the negligible quantity of fragments greater than M/2 and the greater intensity of the ions smaller than M/2. This difference would be expected for the comparison with the *n*-butyl esters which were examined using a lower ionising voltage (20 eV) but not necessarily for the isoamyl esters where the more standard ioniser voltage of 70 eV was used.

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