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GAS CHROMATOGRAPHY AND MASS SPECTROMETRY OF N-TRIFLUOROACETYL *n*-BUTYL ESTERS OF ALKYLATED TYROSINES AND LYSINES

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SUMMARY

The gas chromatographic behaviour of three O-*n*-alkyltyrosines, four N^{ε}-*n*-alkyllysines and three N^{ε},N^{ε}-di-*n*-alkyllysines was studied as their N-trifluoroacetyl *n*-butyl ester (BTFA) derivatives on columns of OV-17 and Dexsil 300 GC. The retention indices and some other retention parameters are discussed in relation to the chemical structures of the amino acids. Mass fragmentation patterns of the BTFA derivatives of these amino acids obtained by gas chromatography–mass spectrometry with electron impact ionization are also described.

INTRODUCTION

Gehrke and co-workers[1,2] established a gas chromatographic (GC) method of analysis of protein amino acids as their N(O)-trifluoroacetyl *n*-butyl ester (BTFA) derivatives. Since then GC techniques have widely been used for the analysis of protein amino acids as well as unusual amino acids from various sources, after conversion into BTFA derivatives or other N(O)-trifluoroacyl esters.

We have been engaged in the utilization of the GC method for the analysis of unusual amino acids present in the hydrolyzates of chemically modified proteins[3–8]. These amino acids originate from the chemical reactions of the reactive functional groups in the protein side chains. We are interested in finding a correlation between the GC retention characteristics and the chemical structures of the amino acids, in the expectation that, once established, this would be useful for the identification of unknown peaks in the chromatograms of complex amino acid mixtures such as the hydrolyzates of chemically modified proteins, when authentic samples are not available.

In a previous paper[3], the GC characteristics of various S-substituted cysteines were studied as their BTFA derivatives. The present investigation concerns the GC characteristics of O-alkyltyrosines, N^{ε}-alkyllysines and N^{ε},N^{ε}-dialkyllysines as their BTFA derivatives and the comparison with the GC characteristics of the BTFA derivatives of S-alkylcysteines. Cysteine, tyrosine and lysine residues in proteins are

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susceptible to alkylation with various reagents. Lysine residues may be alkylated to give N^{ε}-alkyllysine and/or $N^{\varepsilon},N^{\varepsilon}$-dialkyllysine residues, depending on the reaction conditions employed. The alkyl–hetero atom bonds in the alkylated amino acid residues are more or less stable under the acid hydrolysis conditions, and the alkylated amino acids should be found in the hydrolyzates of alkylated proteins.

EXPERIMENTAL

Amino acids

O-Methyl-L-tyrosine[9], O-ethyl-L-tyrosine hydrochloride[10] and O-*n*-propyl-L-tyrosine hydrochloride[10] were prepared from L-tyrosine. N^{ε}-Methyl-L-lysine was a gift of Professor G. Ebert, University of Marburg/Lahn, G.F.R. N^{ε}-Ethyl-DL-lysine[11] was obtained by the reaction of 5-δ-bromobutylhydantoin with ethylamine, followed by alkaline hydrolysis and was purified in the form of the hydrochloride, m.p. 210°C (decomp.). N^{ε}-*n*-Propyl-DL-lysine, m.p. 205.5°C (decomp.), N^{ε}-*n*-butyl-DL-lysine hydrobromide, m.p. 225°C (decomp.), and $N^{\varepsilon},N^{\varepsilon}$-dimethyl-DL-lysine dioxalate, m.p. 143–144°C (decomp.), were prepared in a similar way. $N^{\varepsilon},N^{\varepsilon}$-Diethyl-DL-lysine and $N^{\varepsilon},N^{\varepsilon}$-di-*n*-propyl-DL-lysine were obtained as oils when the corresponding hydantoins were hydrolyzed. They could not be crystallized. Oily oxalates were used for conversion into BTFA derivatives.

Other amino acids used were as described previously[3,4].

Reagents

Ethyl acetate of guaranteed reagent grade (Wako, Tokyo, Japan) was purified according to Hurd and Strong[12], and heptafluorobutyric anhydride of the same grade (Tokyo Chemical, Tokyo, Japan) was used without further purification. Other reagents used were as described previously[3].

Derivatization

The conversion of amino acids into their BTFA derivatives was carried out according to Kaiser *et al.*[2]. N(O)-Heptafluorobutyryl *n*-butyl esters (BHFB) were prepared by reaction of the *n*-butyl esters of the amino acid hydrochlorides[2] in a similar manner to the preparation of N(O)-heptafluorobutyryl isobutyl esters[13]. In both BTFA and BHFB preparations, the concentration of each amino acid in the final acylation mixture was *ca.* 0.25 μg/μl.

Gas chromatography

A Shimadzu Model GC-4BMPF dual-column gas chromatograph, equipped with hydrogen flame ionization detectors (FID) and a linear temperature programmer, was used. One of the two glass columns, 1 m × 0.3 cm I.D., was packed with 1.5% (w/w) OV-17 on acid-washed and heat-treated high-performance Chromosorb G (80–100 mesh), and the other with 1.5% (w/w) Dexsil 300 GC on the same quality Chromosorb G. Both column packings were purchased from Nihon Chromato (Tokyo, Japan). The carrier gas (N_2) flow-rate was 70 ml/min. Other chromatographic conditions were as given previously[3].

Gas chromatography–mass spectrometry (GC–MS)

The GC–MS measurements were carried out on a Shimadzu-LKB Model GC-

MS 9000 gas chromatograph–mass spectrometer combined with a Shimadzu Model GC-MS PAC 300 on-line data processing system.

GC–MS retention indices were determined from the total ion current (TIC) chromatogram obtained at 20 eV with a glass column (1 m × 0.3 cm I.D.) packed with the same quality OV-17 packings used for GC. The temperature programme was the same as that used for the determination of GC retention indices. The flow-rate of the carrier gas (He) was 30 ml/min. In most cases, BTFA-amino acids were injected with appropriate pairs of even carbon-number *n*-paraffins, as in the case of the GC determination. Reproducibility of the retention times was generally good so that, in some cases, BTFA-amino acids and *n*-paraffins were injected separately in two successive chromatographic runs and the retention indices were calculated from the retention times thus determined separately.

Mass spectra were determined on the OV-17 column described above or on columns packed with other OV-17 packings: 2% (w/w) on Chromosorb W HP (80–100 mesh) and 2% (w/w) on Gas-Chrom Q (80–100 mesh), both purchased from Wako. They were obtained at 70 eV with an accelerating potential of 3.5 kV. The ion source was maintained at 290°C in most cases and the separator was kept at 280°C or at a temperature 10°C lower than the ion-source temperature. The column temperature was programmed at a rate of 5°C/min from 140°C to 210°C from 160°C to 210°C. The usual amount of sample injected was 1 μl.

RESULTS AND DISCUSSION

The abbreviations of the amino acids used are listed in Table I. O-Alkyltyrosines, N^{ε}-alkyllysines and $N^{\varepsilon},N^{\varepsilon}$-dialkyllysines were synthesized in this laboratory, except for NML. OET, DEL and DPL could not be obtained in pure form; however,

TABLE I

ABBREVIATIONS USED FOR AMINO ACIDS

Class		No.	Name	Abbreviation
(A)	O-Alkyltyrosines	1	O-Methyltyrosine	OMT
		2	O-Ethyltyrosine	OET
		3	O-*n*-Propyltyrosine	OPT
(B)	N^{ε}-Alkyllysines	4	N^{ε}-Methyllysine	NML
		5	N^{ε}-Ethyllysine	NEL
		6	N^{ε}-*n*-Propyllysine	NPL
		7	N^{ε}-*n*-Butyllysine	NBL
(C)	$N^{\varepsilon},N^{\varepsilon}$-Dialkyllysines	8	$N^{\varepsilon},N^{\varepsilon}$-Dimethyllysine	DML
		9	$N^{\varepsilon},N^{\varepsilon}$-Diethyllysine	DEL
		10	$N^{\varepsilon},N^{\varepsilon}$-Di-*n*-propyllysine	DPL
(D)	S-Alkylcysteines	11	S-Methylcysteine	SMC
		12	S-Ethylcysteine	SEC
		13	S-*n*-Propylcysteine	SPC
		14	S-*n*-Butylcysteine	SBC
(E)	Others	15	Cysteine	CySH
		16	Lysine	Lys
		17	Norleucine	Norleu
		18	Phenylalanine	Phe
		19	Tyrosine	Tyr

their GC peaks were clearly distinguished from the peaks of impurities, and the GC–MS analysis confirmed that the main chromatographic peaks observed were those of the expected amino acids.

GC retention

The GC retention data of the BTFA-amino acids were recorded on two thermally stable liquid phases, OV-17 and Dexsil 300 GC, by the linear temperature programming method used previously[3]. The Kovát's retention indices, I^{14}, were determined as previously[3], in order to investigate the correlation between the amino acid structure and the GC retention behaviour. The retention indices of BTFA derivatives of O-alkyltyrosines and N^{ε}-alkyllysines were fairly reproducible. However, those of the BTFA derivatives of DML and DEL were significantly variable, especially on polar OV-17.

Table II lists the retention indices of BTFA-amino acids determined in this work as well as those reported earlier. The difference, ΔI, between the two retention indices determined on the stationary phases of different polarity is also given. The ΔI values of BTFA derivatives of three O-alkyltyrosines range between 116 and 142, larger than the ΔI value of BTFA-Phe. The ΔI values of BTFA derivatives of four N^{ε}-alkyllysines range between 24 and 51, slightly smaller than those of BTFA derivatives of α,ω-diaminomonocarboxylic acids[3].

Fig. 1 shows the good linear relationships between the retention index and the molecular weight for BTFA derivatives of O-alkyltyrosines and N^{ε}-alkyllysines. It was assumed previously[3] that the retention index of a BTFA-amino acid was the sum

TABLE II

RETENTION INDICES OF BTFA-AMINO ACIDS

Class	*No.*	*Amino acid*	*Retention index, I*				*Ref.*
			OV-17 by GC	*OV-17 by GC–MS*	*Dexsil 300 GC by GC*	*Difference, ΔI, by GC*	
(A)	1	OMT	2118	2118	1986	132	
	2	OET	2181	2164	2039	142	
	3	OPT	2246	2245	2130	116	
(B)	4	NML	2030	2055	1991	39	
	5	NEL	2103	2074	2052	51	
	6	NPL	2141	2115	2117	24	
	7	NBL	2217	2200	2190	27	
(C)	8	DML	1911	1930	1909	2	
	9	DEL	1952	2018	1932	20	
	10	DPL	2063	2046	2048	15	
(D)	11	SMC	1627	–	1530	97	3
	12	SEC	1680	–	1595	85	3
	13	SPC	1752	–	1670	82	3
	14	SBC	1835	–	1757	78	3
(E)	15	CySH	1499	–	1470	29	
	16	Lys	2000	2000	1930	70	3
	17	Norleu	1478	–	1452	26	3
	18	Phe	1852	–	1746	106	
	19	Tyr	1910	1907	1870	40	

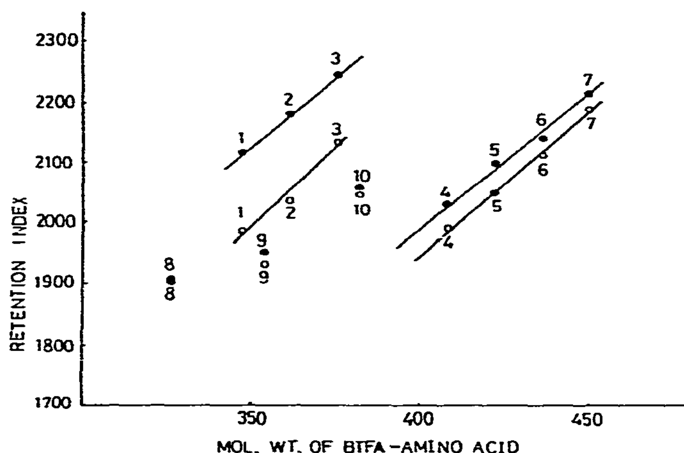


Fig. 1. Retention indices of BTFA derivatives of O-alkyltyrosines, N$^{\varepsilon}$-alkyllysines and N$^{\varepsilon}$,N$^{\varepsilon}$-dialkyllysines on OV-17 (●) and Dexsil 300 GC (○) *versus* their molecular weights. Compounds are numbered as in Table I.

of the group retention indices, i, of the individual structural units, and an attempt was made to predict the retention indices of BTFA-amino acids from their chemical structures. From the plots of the retention indices *versus* molecular weights, group retention indices, i_C, of a methylene of the O- or N$^{\varepsilon}$-alkyl groups and of a $C_4H_9OCOCH(NHCOCF_3)CH_2C_6H_4O$–group, i_R, can be calculated (see Table III). The difference between i_R and the retention index of BTFA-Phe corresponds to the group retention index, i_O, of ether oxygen. The i_O values are about two thirds of the group retention indices of thioether sulphur[3] found for BTFA derivatives of S-substituted cysteines.

By definition, the retention indices of neighbouring members of *n*-paraffins differ by 100. The observed values of i_C are apparently lower than 100, as is the case for BTFA derivatives of S-alkylcysteines[3]. The values observed for BTFA derivatives of N$^{\varepsilon}$-alkyllysines are much lower than the group retention indices of the methylene unit in BTFA-Lys (140 on either OV-17 or Dexsil 300 GC)[3]. From the plots of the retention indices *versus* molecular weights, the group retention index, $i_{R'}$, of a

TABLE III

GROUP RETENTION INDICES, i

Class		*OV-17*	*Dexsil 300 GC*	*Ref.*
(A)	i_C	64	73	
	i_R	2053	1907	
	i_O	201	161	
(B)	i_C	63	67	
	$i_{R'}$	1965	1915	
	$i_{\varepsilon\text{-NTFA}}$	487	463	
(D)	i_C	77	81	3
	i_S	297	239	3

$C_4H_9OCOCH(NHCOCF_3)(CH_2)_4NCOCF_3$ group is obtained. Then, the difference between $i_{R'}$ and the retention index of BTFA-Norleu is assumed to be the group retention index, $i_{t\text{-NTFA}}$, of a tertiary $NCOCF_3$ group. The observed $i_{t\text{-NTFA}}$ values in Table III are larger than the group retention indices for a $NHCOCF_3$ group observed for BTFA derivatives of α,ω-diaminomonocarboxylic acids[3].

As stated earlier, the retention indices of BTFA derivatives of DML and DEL varied greatly with slight changes in the column conditions. The retention indices of these compounds (Table II) are the lowest of those determined on different occasions. The retention indices of BTFA derivatives of $N^{\varepsilon},N^{\varepsilon}$-dialkyllysines (Table II) increase with increasing molecular weight It should be mentioned, however, that in another set of determinations the retention indices of BTFA-DML and BTFA-DEL were unusually high (2270 and 2200, respectively) on OV-17. The GC peak of BTFA-DML was broad with significant tailing. It seems that BTFA-DML with a tertiary amino group shielded by the small methyl group interacts most with the column packings in this particular case.

The GC–MS retention indices of BTFA derivatives of amino acids were determined from the total ion current (TIC) chromatograms on OV-17. The temperature programme and the column packings used were the same as those for GC, but the column size and the carrier gas flow-rate were different (see Experimental). The GC–MS retention indices were nearly equal to those determined by GC (see Table II). The shape of the peak of BTFA-DML in the TIC chromatogram was as

TABLE IV

DIFFERENCE, ΔMe, BETWEEN EFFECTIVE AND ACTUAL MOLECULAR WEIGHTS OF BTFA-AMINO ACIDS

Class	*No.*	*Amino acid*	*Mol. wt. of BTFA-amino acid*	*ΔMe*		*Ref.*
				OV-17	*Dexsil 300 GC*	
(A)	1	OMT	347.3	− 48.2	− 66.7	
	2	OET	361.4	− 53.5	− 73.4	
	3	OPT	375.4	− 58.4	− 74.6	
(B)	4	NML	408.3	−121.6	−127.0	
	5	NEL	422.4	−125.4	−132.6	
	6	NPL	436.4	−134.1	−137.5	
	7	NBL	450.4	−137.4	−141.2	
(C)	8	DML	326.4	− 56.3	− 56.6	
	9	DEL	354.4	− 78.6	− 81.4	
	10	DPL	382.5	− 91.1	− 93.2	
(D)	11	SMC	287.3	− 57.1	− 70.7	3
	12	SEC	301.3	− 63.7	− 75.6	3
	13	SPC	315.4	− 67.6	− 79.1	3
	14	SBC	329.4	− 69.9	− 80.9	3
(E)	15	CySH	369.3	−157.0	−161.1	
	16	Lys	394.3	−111.8	−121.6	3
	17	Norleu	283.3	− 74.0	− 77.6	3
	18	Phe	317.3	− 55.5	− 70.4	
	19	Tyr	429.3	−159.4	−165.0	

sharp as other BTFA-amino acids, and the observed retention index compared well with the lowest retention index observed by GC.

ΔMe of Evans and Smith

From Kováts' retention index, Evans and Smith[15,16] proposed another retention parameter, ΔMe. Table IV gives the ΔMe values of BTFA-amino acids calculated from their retention indices on OV-17 and Dexsil 300 GC. It was reported[16,17] that ΔMe was virtually constant throughout a homologus series, except for a few initial homologues. The ΔMe values of BTFA derivatives of O-alkyltyrosines range between −48 and −58 on OV-17 and −67 and −75 on Dexsil 300 GC. These values are in agreement with the ΔMe values of BTFA-Phe and very close to the reported ΔMe values of BTFA derivatives of monoaminomonocarboxylic acids[3]. Thus, ether oxygen has little influence on the ΔMe value as in the case[3] of thioether sulphur.

In previous work[3] it was shown that the second CF_3CONH group in BTFA derivatives of α,ω-diaminomonocarboxylic acids has a significant effect on the ΔMe value. The ΔMe values of BTFA derivatives of $N^ε$-alkyllysines are nearly equal to those of BTFA derivatives, of α,ω-diaminomonocarboxylic acids. The ΔMe value of BTFA-DPL lies between those of BTFA derivatives of monoaminomonocarboxylic acids and α,ω-diaminomonocarboxylic acids. The absolute ΔMe values of BTFA-DML are much lower than those of BTFA-DPL on both liquid phases.

Finally, retention indices of BTFA derivatives of O-alkyltyrosines, $N^ε$-alkyllysines and S-alkylcysteines are compared (Table V). The retention index of an S-alkylcysteine and that of the corresponding $N^ε$-alkyllysine differ on average by 339 and 450 on OV-17 and Dexsil 300 GC, respectively. The lysine derivative is eluted before the corresponding O-alkyltyrosine on OV-17, while the two are eluted at the same time on Dexsil 300 GC. $N^ε,N^ε$-Dialkyllysines have shorter retention times than the corresponding $N^ε$-monoalkyllysines on both liquid phases.

Table V also shows the difference in the retention indices of BTFA derivatives of CySH, Lys and Tyr. The difference in the retention indices of BTFA-Lys and BTFA-Tyr is much different from that observed for the BTFA derivatives of $N^ε$-

TABLE V

COMPARISON OF RETENTION INDICES OF BTFA DERIVATIVES OF O-ALKYLTYROSINES, $N^ε$-ALKYLLYSINES AND S-ALKYLCYSTEINES

Retention indices: I_{AX} of a class (A) amino acid with an alkyl substituent, X; I_{BX} of a class (B) amino acid with an alkyl substituent, X; I_{DX} of a class (D) amino acid with an alkyl substituent, X.

Alkyl substituent, X	$I_{AX} - I_{BX}$		$I_{BX} - I_{DX}$		$I_{AX} - I_{BX}$	
	OV-17	*Dexsil 300 GC*	*OV-17*	*Dexsil 300 GC*	*OV-17*	*Dexsil 300 GC*
H*	−90	−60	501	460	411	400
CH_3	88	− 5	403	461	491	456
C_2H_5	78	−13	423	457	501	444
n-C_3H_7	105	13	389	447	494	460
n-C_4H_9	–	–	382	433	–	–

* The actual substituent in the BTFA derivative is a CF_3CO group.

alkyllysines and O-alkyltyrosines. Similarly, the difference in retention indices of BTFA-CySH and BTFA-Tyr is also different from that of BTFA derivatives of S-alkylcysteines and O-alkyltyrosines. Thus, the contribution of the CF_3CO group to the retention index is dependent on the atom to which it is bound.

FID molar response

FID molar response is an additive property of the structural features, and the contribution of a structural unit to the molar response is usually expressed as the effective carbon number (ecn)[17]. The effective carbon number of a molecule (ECN) is a sum of the ecn values of the individual structural units. The ecn value of a carbon atom in an alkyl or an aryl group is 1.0 and that of an oxygen atom in ether is -1.0[17]. In previous work[3], the ecn values of $-CO_2-$ (ester) and $-NHCOCF_3$ were estimated to be -0.5 and -0.7, respectively, for BTFA-amino acids. The last value was assumed to be applicable to the trifluoroacetamide groups in BTFA derivatives of N^ε-alkyllysines. By use of these values, the relative molar response (RMR) to BTFA-Glu was calculated.

The RMR values of BTFA derivatives of O-alkyltyrosines and N^ε-alkyllysines available in pure form were determined on OV-17 and Dexsil 300 GC as described previously[3]. Table VI shows that the values observed on either column agree satisfactorily with each other, and the RMR values calculated from the ECN values compare well with the observed values. Considering the results of Islam and Darbre[18] and of Felt and Hušek[19] for different types of volatile derivatives of amino acids, it seems that the ecn values of perfluorocarboxamide groups are influenced by the FID apparatus used and by the operating conditions.

TABLE VI

FID MOLAR RESPONSES (RMR) OF BTFA-AMINO ACIDS RELATIVE TO BTFA-GLUTAMIC ACID

Class	*No.*	*Amino acid*	*RMR calc.*		*RMR found*	
			ECN	*RMR*	*OV-17*	*Dexsil 300 GC*
(A)	1	OMT	10.8	1.16	1.17	1.18
	3	OPT	12.8	1.38	1.26	1.31
(B)	4	NML	8.1	0.87	0.94	0.91
	5	NEL	9.1	0.98	0.99	1.05
	6	NPL	10.1	1.09	1.05	1.11
	7	NBL	11.1	1.19	1.14	1.27

Gas chromatography–mass spectrometry

The GC–MS of BTFA derivatives of 19 protein amino acids has been studied by Gelpi *et al.*[20] by electron impact ionization at 20 eV. Leimer *et al.*[21] reported the GC–MS of BTFA derivatives of 49 amino acids by electron impact ionization at 70 eV. In this work, the mass spectra of BTFA derivatives of O-alkyltyrosines, N^ε-alkyllysines and $N^\varepsilon,N^\varepsilon$-dialkyllysines were taken at 70 eV. Some of the assignments made in this work for the fragment ions must be considered as tentative since no

TABLE VII

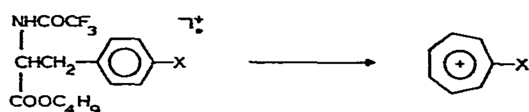
MASS FRAGMENTATION PATTERNS OF BTFA DERIVATIVES OF O-ALKYLTYROSINES

r.a. = Relative abundance.

Ion	*BTFA-Phe*		*BTFA-Tyr*		*BTFA-OMT*		*BTFA-OET*		*BTFA-OPT*	
	m/z	*r.a.*	*m/z*	*r.a.*	*m/z*	*r.a.*	*m/z*	*r.a.*	*m/z*	*r.a.*
Molecular ion (M)	317	0.5	429	0.2	347	1	361	2	375	3
M − C_4H_9OCO	216	13	328	9	246	2	260	2	274	3
M − CF_3CONH_2	204	39	316	31	234	9	248	9	262	11
M − CF_3CONH_2 − C_4H_8	148	80	260	93	178	5	192	4	206	2
M − CF_3CONH_2 − C_4H_9O	131	15	243	13	161	2	175	1	189	0.4
M − C_4H_9OCO − CF_3CO	119	10	231	5	149	1	163	1	177	0.4
M − C_4H_9OCO − CF_3CONH_2	103	18	215	7	133	3	147	2	161	0.3
M − $C_4H_9OCOCHNHCOCF_3$	91	100	203	100	121	100	135	100	149	100
$C_2H_2C_6H_4OH$	–	–	119	2	119	1	119	3	119	6
$CH_2C_6H_4OH$	–	–	107	4	107	1	107	48	107	95
$CH_2C_6H_5$	91	100	91	4	91	2	91	4	91	5
C_6H_5	77	7	77	3	77	3	77	4	77	4

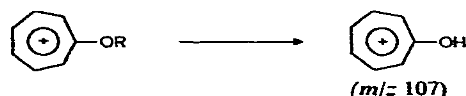
attempt was made to determine the elemental composition by high resolution measurements and there are several possible structures of different elemental compositions for some of the fragment ions. The ion formulae given in Tables VII–IX do not necessarily indicate the exact position of some of the hydrogen atoms and the charge designation is omitted for simplicity. The ions of m/z < 50 are also omitted.

Table VII compares the mass fragmentation patterns of BTFA derivatives of Phe, Tyr and O-alkyltyrosines. The fragmentation patterns of the BTFA derivatives of the five aromatic amino acids are very similar. The most significant ions in the high m/z region are (M − CF_3CONH_2) ions. The base peaks are (M − $C_4H_9OCOCHNHCOCF_3$) ions, which are actually stable tropylium ions:



X = H, $OCOCF_3$ or OR (R = alkyl group)

In the case of BTFA derivatives of O-alkyltyrosines, ions of m/z 107 and 119 are formed. The relative abundance of the ion of m/z 107 increases significantly with increasing carbon number of the O-alkyl group:



(m/z 107)

Characteristic ions for the BTFA derivatives of all the aromatic amino acids are $CH_2C_6H_5$ and C_6H_5, although their relative abundances are low.

The mass fragmentation patterns of BTFA-Phe and BTFA-Tyr given in Table VII are generally similar to those reported by Leimer *et al.*[21]. However, the fragmen-

TABLE VIII

MASS FRAGMENTATION PATTERNS OF BTFA DERIVATIVES OF N^{ϵ}-ALKYLLYSINES

r.a. = Relative abundance.

Ion	*BTFA-Lys*		*BTFA-NML*		*BTFA-NEL*		*BTFA-NPL*		*BTFA-NBL*	
	m/z	*r.a.*	*m/z*	*r.a.*	*m/z*	*r.a.*	*m/z*	*r.a.*	*m/z*	*r.a.*
Molecular ion (M)	394	1	408	3	422	2	436	2	450	3
M − C_4H_9OH	320	5	334	41	348	17	362	38	376	14
M − CF_3CO	297	–	331	6	325	6	339	13	353	6
M − C_4H_9OCO	293	5	307	23	321	11	335	24	349	12
M − $CF_3-C_4H_{10}OCO-H$	195	2	209	10	223	6	237	9	251	4
$(CF_3CON)C_8H_{17}$	–	–	–	–	–	–	–	–	224	23
$(CF_3CON)C_7H_{15}$	–	–	–	–	–	–	210	41	210	9
$(CF_3CON)C_6H_{13}$	–	–	–	–	196	22	196	19	196	2
$(CF_3CON)C_5H_{11}$	182	1	182	20	182	11	182	3	182	50
$(CF_3CON)C_5H_9$	180	100	180	56	180	41	180	86	180	57
$(CF_3CON)C_4H_9$	168	4	168	10	168	3	168	100	168	4
$(CF_3CON)C_3H_7$	154	1	154	4	154	100	154	5	154	2
$(CF_3CON)C_3H_5$	152	4	152	9	152	9	152	13	152	8
$(CF_3CON)C_2H_5$	140	3	140	100	140	7	140	35	140	100
$(CF_3CON)CH_3$	126	12	126	5	126	40	126	49	126	12
CF_3	69	8	69	24	69	12	69	14	69	10
C_4H_9	57	12	57	18	57	18	57	27	57	41

tation pattern of BTFA-OMT in the table is much different from that reported previously[21]. The mass spectrum of BTFA-OMT did not change significantly when the ion-source temperature was lowered to 270°C and/or the amount of sample injected was reduced to one tenth*. The mass spectrum of the N(O)-heptafluorobutyryl *n*-butyl ester (BHFB) derivative of OMT was taken and compared with that of BTFA-OMT. The two fragmentation patterns were very similar.

Table VIII compares the mass fragmentation patterns of BTFA derivatives of Lys and N^{ϵ}-alkyllysines. The abundant ions in the high *m/z* region are (M −

TABLE IX

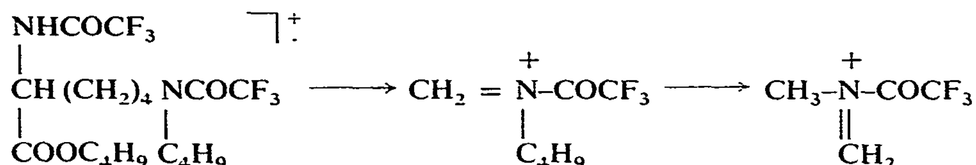
MASS FRAGMENTATION PATTERNS OF BTFA DERIVATIVES OF $N^{\epsilon},N^{\epsilon}$-DIALKYLLYSINES

n = 1 for DML, n = 2 for DEL and n = 3 for DPL. r.a. = Relative abundance.

Ion	*BTFA-DML*		*BTFA-DEL*		*BTFA-DPL*	
	m/z	*r.a.*	*m/z*	*r.a.*	*m/z*	*r.a.*
Molecular ion (M)	326	3	354	2	382	2
M − H	325	0.6	353	1	381	2
M − $C_{n-1}H_{2n-1}$	325	0.6	339	8	353	100
M − C_4H_9OCO	225	5	253	5	281	10
$(C_nH_{2n+1})_2NCH_2$	58	100	86	100	114	98

* In the case of BTFA derivatives of S-substituted cysteines such as S-β-aminoethylcysteine, the fragmentation patterns changed considerably on varying the ion-source temperature and the amount of sample injected[22].

C_4H_9OH) and (M − C_4H_9OCO). The ion of $CF_3CONC_5H_9$ (*m/z* 180) is formed abundantly for all the BTFA derivatives and is the base peak for BTFA-Lys. The ions of *m/z* 126, 140, 154, 168 and 182 are found for all the BTFA derivatives of $N^ε$-alkyllysines. There are isomeric structures for these ions except for that of *m/z* 126. The ions of $CF_3CON(C_nH_{2n+1})CH_2$ [n = 1 for BTFA-NML (*m/z* 140), n = 2 for BTFA-NEL (*m/z* 154), n = 3 for BTFA-NPL (*m/z* 168) and n = 4 for BTFA-NBL (*m/z* 182)] are formed abundantly for BTFA derivatives of $N^ε$-alkyllysines and are base peaks except for BTFA-NBL. The base peak ion for BTFA-NBL is $CF_3CON(CH_3)CH_2$ (*m/z* 140) formed from $CF_3CON(C_4H_9)CH_2$:



The ions of $CF_3CON(C_nH_{2n+1})(CH_2)_4$ (*m/z* 182 for BTFA-NML, *m/z* 196 for BTFA-NEL, *m/z* 210 for BTFA-NPL and *m/z* 224 for BTFA-NBL) are formed abundantly. Other significant ions for all the BTFA derivatives include C_5H_7 (*m/z* 67).

The mass fragmentation pattern of BTFA-Lys given in Table VIII is generally similar to that reported by Leimer *et al.*[21], however, that of BTFA-NML is different from that reported by these authors. The mass fragmentation pattern of BHFB-NML was similar to that of BTFA-NML.

Table IX shows the mass fragmentation patterns of BTFA derivatives of $N^ε,N^ε$-dialkyllysines. The ions of $(C_nH_{2n-1})NCH_2$, where n = 1 for BTFA-DML, n = 2 for BTFA-DEL and n = 3 for BTFA-DPL, are formed abundantly and are the base peaks except for BTFA-DPL, for which the ion of (M − C_2H_5) is the most abundantly formed. The mass fragmentation pattern of BHFB-DPL was very similar to that of BTFA-DPL.

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