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ANALYSIS OF AMINO ACIDS BY GAS–LIQUID CHROMATOGRAPHY AS *tert.*-BUTYLDIMETHYLSILYL DERIVATIVES

PREPARATION OF DERIVATIVES IN A SINGLE REACTION*

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SUMMARY

The effect of temperature, solvent and reagents on the formation of the N(O)-dimethyl-*tert.*-butylsilyl derivatives of proteic amino acids has been studied. Quantitative silylation is achieved using dimethyl-*tert.*-butylsilyltrifluoroacetamide with 1% *tert.*-butyldimethylchlorosilane in dimethylformamide by heating at 75°C for 30 min. Two peaks were obtained for arginine under these conditions. However, most of the standard proteic amino acids can be assayed. The N(O)-dimethyl-*tert.*-butylsilyl derivatives of the proteic amino acids have been analysed by gas chromatography–mass spectrometry using the methane chemical ionization mode. The spectral data are presented and have been used to confirm the structures of the amino acid derivatives.

INTRODUCTION

The procedures most commonly used to derivatise amino acids for analysis by gas–liquid chromatography (GLC) are based on the formation of a carboxylic acid alkyl ester followed by perfluoroacylation of the remaining functional groups. A third reaction is usually necessary for quantitation of histidine. Analyses based on such protocols are now routine[1] and give results having a precision and accuracy comparable to other procedures[2]. In addition, the resolution obtainable using capillary columns is unmatched by any other method and is a distinct advantage for the analysis of complex physiological samples.

A perfluoroalkyl amino acid alkyl ester derivative can be prepared in less than an hour and the reaction time per sample is not significant when samples are prepared in batches. Nevertheless, it is always desirable to minimise sample preparation time and to simplify the derivatisation procedure. Ideally, derivatives of all the standard

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amino acids would be quantitatively formed in a single, rapid reaction under mild conditions.

There has been a continuing effort to develop a single reaction procedure for preparing volatile amino acid derivatives. The most promising of these was trimethylsilylation, commonly abbreviated as silylation, which was first applied to amino acids by Ruhlmann and Giesecke[3]. A fine example of the early studies on this subject is the work of Gehrke and co-workers[4,5] establishing the kinetics of amino acid trimethyl ester formation and demonstrating the application of the procedure to the analysis of a variety of biological samples. A significant advantage of the silylation procedure was that the reaction could be performed in a single vial with no transfer, evaporation or addition of other reagents being required.

The early literature on this subject has been covered in an excellent review by Hušek and Macek[6] and the more recent material has been reviewed by Frank and Jaeger[7].

The initial promise of the silylation approach has not been realised in practice, largely because of a long reaction time (2.5 h), the formation of multiple derivatives of some amino acids under the basic conditions and elevated temperature (150°C) required for the silylation of arginine, and the sensitivity of the derivatives to water. In addition, the application to physiological samples was limited by the coelution of arginine and ornithine. Whatever the reason, silylation has been used relatively rarely for assaying a few specific amino acids [*e.g.* ref. 8] and has certainly not been commonly used to assay the total amino acid profile of a sample.

The earlier studies suffered the limitations of the reagents then available. As new silylation reagents are developed[9], it is appropriate to assess these reagents for their applicability to the derivatisation of amino acids. One such reagent is dimethyl-*tert.*-butylsilyltrifluoro acetamide (MTBSTFA)[10]. This reagent forms dimethyl-*tert.*-butylsilyl (DMBS) derivatives, which appear to be particularly stable. However, there is little record of its application to the analysis of amino acids. MTBSTFA has been used to form volatile derivatives of physiologic compounds found in plasma for investigation by gas chromatography–mass spectrometry (GC–MS)[11]. This study included a few amino acids, specifically alanine, leucine, histidine, 1-methylhistidine and 3-methylhistidine.

In earlier reports, we described the use of MTBSTFA to prepare derivatives of asparagine, glutamine and pyroglutamic, aspartic and glutamic acids[12,13] and illustrated the analysis of these compounds in physiological samples. In the course of those investigations, it was evident that other standard proteic amino acids present in the samples were being derivatised and resolved from the amides which were the compounds of primary interest. We now report our progress to date using MTBSTFA to prepare volatile derivatives of the standard proteic amino acids for analysis by GLC.

EXPERIMENTAL

Reagents

MTBSTFA was obtained from Pierce (Rockford, IL, U.S.A.) and redistilled before use to minimise interfering reagent peaks. The fraction distilling in the range 168–170°C was collected. Molar quantities of the reagent were calculated on the basis

of a density of 1.121 g/ml[10]. *tert.*-Butyldimethylchlorosilane (TBDMSCl) was obtained from Fluka, (Buchs, Switzerland). All other reagents and solvents were of analytical grade.

Derivatization

A TECAM, Model DB-3H, Driblock heater was used to heat samples during derivative preparation.

The standard amino acid mixture (10 μl of a solution containing 25 μmol/ml) was dispensed into a 1-ml Reacti-Vial (Pierce) and excess solvent was evaporated at 35°C using a stream of dry nitrogen (100–200 ml/min). A molar equivalent of octadecane in benzene was added and the solvent evaporated. MTBSTFA, TBDMSCl, catalyst and solvent(s) were added and the vial was heated. The proportions of the reagents and solvents, and the heating temperatures and times are indicated in the text. After cooling, an appropriate aliquot of the reaction mixture was injected directly in the gas chromatograph.

Chromatography

All chromatography was performed using a Varian Vista 6000 gas chromatograph equipped with a capillary injection system and dual flame ionization detectors. The column, a 15 m × 0.25 mm I.D. SPB-1 fused-silica capillary column (Supelco, Oakville, Canada), was operated in the split mode (1:20). The carrier gas (helium) flow was 60 cm/s. The detector and injector temperatures were maintained at 290°C. Data were acquired and processed using a Hewlett Packard Model 3354 Laboratory Data system. The one and three letter codes for amino acids are in accord with IUPAC-IUB recommendations[14].

Mass spectrometry

Mass spectra of the amino acid derivatives were obtained using a Finnigan Model 3300 mass spectrometer operated in the positive chemical ionization (CI) mode using methane as the reagent gas.

RESULTS AND DISCUSSION

Chromatography

A typical chromatogram illustrating the resolution of the DMBS amino acid derivatives is shown in Fig. 1. All the components are resolved sufficiently to permit accurate quantitation and, with one exception, a single peak was formed for each amino acid. The exception, arginine, formed two derivatives, the first eluting just after glutamic acid and the second eluting between lysine and histidine. These are labeled arginine-1 and arginine-2 respectively and will be referred to thus hereafter. Unlike amino acid perfluoroacyl ester derivatives, the histidine derivative formed a stable compound which chromatographed with no significant evidence of peak tailing.

Derivatization

Typically, the coefficient of variation ($n = 3$) of the relative molar responses (RMRs) was less than 3% for all except histidine (6%), cystine (3.5%), arginine-1 (16%) and argine-2 (9%).

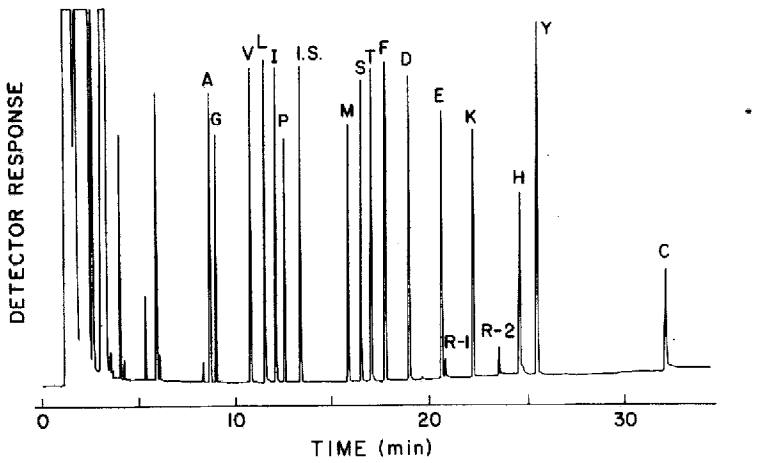

Fig. 1. Chromatogram illustrating the chromatography of the *tert.*-butyldimethylsilyl derivatives of proteic amino acid. A fused-silica capillary column (SPB-1, 15 m × 0.025 mm I.D.), operated in the split mode (1:20) was programmed at 6°C/min from 80°C to 260°C. Detector and injector temperatures were maintained at 290°C. The letters represent the standard single-letter convention for the amino acids. I.S. = Internal standard.

Effect of solvent and catalysts

The effect of various solvents and reagents on the formation of DMBS amino acid derivatives at 75°C for 30 min is shown in Table I. TBDMSCl was used at a concentration of 1.0%[11] as an adjunct in forming DMBS derivatives with amino acids having an active hydrogen[15,16]. The RMRs of arginine, lysine, serine and threonine suggest incomplete derivatization when MTBSTFA is used with TBDMSCl. The RMRs of the other amino acids range from 0.63 for glycine to 1.25 for tyrosine (experiment 1). Also, two peaks were obtained for arginine.

Conducting the reaction in dimethylformamide (DMF) but without TBDMSCl, reduced the RMRs of glycine (17.5%), isoleucine (17%), proline (21%), and lysine (20.7%), and increased the RMRs of arginine-1 and arginine-2 by more than 200%, of threonine by more than 130% and of leucine, phenylalanine and serine by more than 20%. The magnitude of the increase in the response of the arginine peaks is no doubt exaggerated because of the large error in quantitating the very small peaks obtained in experiment 1.

The further addition of TBDMSCl (experiment 3) increased the RMRs of most of the amino acids relative to the values obtained in experiment 1. These increases were particularly significant for arginine-1 (240%), arginine-2 (216%), threonine (115%), serine (42%) and lysine (41%). Only the RMRs of proline (9.5%) and isoleucine (14%) decreased. More importantly, the decreases observed for the RMRs of glycine, lysine and histidine were counteracted.

When pyridine was either substituted for, or added to a reaction mixture containing DMF (experiments 4–6), the RMR of proline was significantly reduced. Adding a basic catalyst reduced the RMR of serine (experiment 5). It was also evident from these experiments that DMF was required for optimal derivatisation of threonine.

In general, the conditions producing optimal overall derivatisation of all the

TABLE I

EFFECT OF SOLVENT AND REAGENTS ON THE RELATIVE MOLAR RESPONSES OF *tert.*-BUTYLDIMETHYLSILYL AMINO ACID DERIVATIVES

The molar responses are expressed relative to octadecane.

Amino acid	*Experiment**					
	1	*2*	*3*	*4*	*5*	*6*
Alanine	0.74	0.72	0.77	0.74	0.76	0.63
Glycine	0.63	0.52	0.67	0.65	0.68	0.51
Valine	0.68	0.87	0.89	0.82	0.83	0.83
Leucine	0.78	0.97	0.97	0.96	0.95	0.93
Isoleucine	1.10	0.91	0.95	1.25	1.40	0.86
Proline	0.84	0.66	0.76	0.58	0.57	0.42
Methionine	0.75	0.87	0.91	0.84	0.85	0.85
Serine	0.69	0.91	0.98	0.82	0.57	0.78
Threonine	0.43	1.01	0.92	0.41	0.23	0.94
Phenylalanine	0.86	1.05	1.06	1.02	1.05	0.97
Aspartic	0.94	1.06	1.07	1.03	1.05	0.99
Glutamic	0.94	0.94	0.92	0.93	0.96	0.86
Arginine-1	0.05	0.16	0.17	0.06	0.06	0.20
Lysine	0.58	0.46	0.82	0.72	0.86	0.37
Arginine-2	0.06	0.20	0.19	0.08	0.07	0.17
Histidine	0.92	0.77	0.88	0.81	0.92	0.72
Tyrosine	1.25	1.30	1.31	1.27	1.31	1.13
Cystine	1.03	1.14	1.02	1.10	1.10	1.05

* Experimental conditions: All reaction mixtures were heated at 75°C for 30 min. (1) 40 μl MTBSTFA, 1%TBDMSCl; (2) 30 μl MTBSTFA, 10 μl dimethylformamide; (3) 30 μl MTBSTFA, 10 μl dimethylformamide, 1 μl TBDMSCl; (4) 30 μl MTBSTFA, 10 μl pyridine, 1% TBDMSCl; (5) 30 μl MTBSTFA, 10 μl pyridine, 1% TBDMSCl, 1.5 μl triethylamine; (6) 30 μl MTBSTFA, 10 μl dimethylformamide, 10 μl pyridine, 1% TBDMSCl.

amino acids, and thus most suitable for an assay system, were those of experiment 3.

Two peaks were obtained for arginine under all of the above experimental conditions. The combined response for the arginine peaks was considerably greater in DMF than in pyridine but the maximum combined RMR observed (0.36) is still less than optimal for precisely assaying arginine. However, the temperature of the set of reactions described in Table I is optimal for the derivatisation of glutamine and asparagine[12,13]. Thus, it is possible under these conditions to derivatise in a single reaction and assay in a single GC analysis, asparagine, glutamine and all the standard proteic amino acids except arginine. Arginine may also be assayed but the RMRs of the two derivatives would require to be added and the precision would be considerably less than for the other amino acids.

Effect of reaction temperature

The effect of derivatization temperature on the RMRs of amino acid DMBS derivatives after heating for 30 min in 30 μl MTBSTFA, 10 μl DMF and 1% TBDMSCl is shown in Table II. For some of the amino acids, a temperature of 50°C

TABLE II

EFFECT OF TEMPERATURE ON THE RELATIVE MOLAR RESPONSES OF *tert.*-BUTYLDIMETHYLSILYL AMINO ACID DERIVATIVES

The molar responses are expressed relative to octadecane.

Amino acid	*Temperature (°C)**				
	50	*75*	*100*	*125*	*150*
Alanine	0.75	0.77	0.71	0.70	0.58
Glycine	0.61	0.67	0.38	0.30	0.04
Valine	0.87	0.89	0.91	0.88	0.91
Leucine	0.94	0.97	0.92	0.93	0.93
Isoleucine	1.07	0.95	0.95	0.97	0.99
Proline	0.74	0.76	0.58	0.61	0.30
Methionine	0.92	0.91	0.72	0.70	0.25
Serine	0.91	0.98	1.01	1.02	1.08
Threonine	0.59	0.92	0.65	0.64	0.69
Phenylalanine	1.05	1.06	1.05	1.04	0.98
Aspartic	1.07	1.07	1.05	1.05	0.99
Glutamic	0.89	0.92	0.87	0.85	0.78
Arginine-1	0.21	0.17	0.16	0.17	0.23
Lysine	0.74	0.82	0.50	0.39	0.07
Arginine-2	0.26	0.19	0.18	0.13	0.12
Histidine	0.77	0.88	0.45	0.49	0.06
Tyrosine	1.30	1.31	1.30	1.25	1.15
Cystine	0.94	1.02	0.76	0.81	0.28

* Experimental conditions are: 30 µl MTBSTFA + 10 µl dimethylformamide + 1.0% TBDMSCl, heated for 30 min.

was inadequate for complete derivative formation as indicated by the increase in RMRs after increasing the reaction temperature to 75°C. However, an increase in temperature to 100°C resulted in a decreased response for many amino acids and this decrease was accentuated by further increases in temperature to 125°C and 150°C. This trend was particularly significant for the basic amino acids lysine and histidine and for glycine, proline, methionine and cystine. The optimal overall responses were obtained at 75°C.

Arginine

The effect of different reaction conditions on the formation of the arginine derivatives is shown in Table III. The temperature was maintained at 150°C, because earlier reports on the silylation of amino acids indicated that an elevated temperature was required for quantitative derivatisation of arginine[4,5]. Heating for 30 and 45 min using 20 µl DMF, 25 µl MTBSTFA and 1% TBDMSCl produced significantly larger responses than when only 10 µl DMF was used. Although the responses increased when the reaction mixture was heated for the longer time, the proportion of the earlier eluting derivative remained at about 30% of the total amount of arginine derivatives formed. Increasing the amount of TBDMSCl to 2% did not have a significantly beneficial effect on total derivative formation or on the ratio of the two

TABLE III

EFFECT OF REACTION CONDITIONS ON THE FORMATION OF ARGININE *tert.*-BUTYLDIMETHYLSILYL DERIVATIVES AT 150°C

The molar responses are expressed relative to octadecane.

*Experiment**	*Reaction time (min)*	*Arginine-1*	*Arginine-2*	*Total*
1	30	0.23	0.12	0.35
2	30	0.29	0.66	0.95
3	45	0.33	0.74	1.07
4	30	0.29	0.74	1.03
5	45	0.10	0.51	0.61
6	30	0.42	0.28	0.72

* Experimental conditions are: (1) 30 μl MTBSTFA + 1.0% TBDMSCl + 10 μl DMF; (2) 25 μl MTBSTFA + 1.5% TBDMSCl + 20 μl DMF; (3) 25 μl MTBSTFA + 1.5% TBDMSCl + 20 μl DMF; (4) 25 μl MTBSTFA + 2.0% TBDMSCl + 20 μl DMF; (5) 25 μl MTBSTFA + 5.0% TBDMSCl + 20 μl DMF; (6) 25 μl MTBSTFA + 2.0% TBDMSCl + 20 μl DMF.

components. A further increase to 5% reduced the proportion of the arginine-1 peak but the total was also significantly reduced. A change of solvent to acetonitrile reversed the proportion of the peaks so that the arginine-1 peak was the larger but, again, the total was reduced compared with the conditions described above. In short, no set of conditions was established under which one derivative was formed for arginine.

Given that the DMBS derivatives have been used to form stable derivatives of asparagine and glutamine[12,13], and that, under the same reaction conditions, most of the standard proteic amino acids can also be assayed, further study of the derivatisation of arginine is justified.

Stability

The stability of the derivatives was tested over a 60-h period. Most of the values were substantially unchanged but the basic amino acids and cystine were significantly reduced after 40 h and the derivatives of histidine and cystine decreased consistently over the period studied. After 60 h, the RMRs of histidine and cystine had decreased to 80% of their initial values and that of lysine to 90% of the initial value. For many derivatives, there was a very slight increase during the first 17 h suggesting that, even at freezer temperature, the reaction proceeded slowly.

Mass spectra

To simplify the tabulation of mass spectral data, fragments have been rationalised as being derived from the molecular ion rather than the protonated quasi-molecular ion, and the charge designation has been omitted. For the same reason, interpretations of the fragments do not include all the isobaric ions nor do they necessarily apply for all the ions in a particular row of data, especially when comparing an amide with its corresponding acid.

In general the CI spectra of the DMBS derivatives of the proteic amino acids

are, like the electron impact (EI) spectra[11], relatively simple. The predominant ions reflect losses of part or all of the DMBS moiety with or without the associated functional group. Loss of the side chain, whether derivatised or not, produces a fragment at *m/z* 302 which constitutes the derivatised fundamental amino acid moiety. This ion is prominent for the aliphatic amino acids, alanine, valine, isoleucine and leucine, but is most likely due to loss of fragments from the *tert.*-butyl moiety of the derivatising group. Only for aspartic acid, threonine, phenylalanine, tyrosine and perhaps histidine is the ion *m/z* 302 sufficiently intense to act as a reliable indicator of the presence of the amino acid structure.

The complementary side chain fragment is not generally intense. However, it is a useful diagnostic ion for histidine (*m/z* 195; 20%), methionine (*m/z* 75; 21%), threonine (*m/z* 160; 11%), and tyrosine (*m/z* 221; 35%).

The mass spectral fragmentation patterns of the DMBS derivatives or the aliphatic amino acids are presented in Table IV. The protonated molecular ion is the base peak for alanine, glycine and valine. Other predominant ions in the spectra represent the loss of CH_3 and C_4H_9, the latter being the base peak for leucine. The loss of the silylated carboxylic acid group is represented by ions of low (7.5%, ala-

TABLE IV

METHANE CHEMICAL IONISATION FRAGMENTATION PATTERNS OF *tert.*-BUTYLDIMETHYLSILYL DERIVATIVES OF ALIPHATIC AMINO ACIDS

Ion	*Amino acid*									
	Gly		*Ala*		*Val*		*Ile*		*Leu*	
	m/z	%	*m/z*	%	*m/z*	%	*m/z*	%	*m/z*	%
M + C_3H_5	344	2.1	358	2.6	386	1.1	400	0.9	400	1.3
M + C_2H_5	332	14.0	346	8.5	374	4.5	388	3.9	388	6.8
M + H	304	100.0	318	100.0	346	100.0	360	93.8	360	95.2
M	303	–	317	–	345	–	359	–	359	–
M – CH_3	288	81.5	302	89.5	330	95.6	344	100.0	344	82.5
M – 3[CH_3] + 2H	260	2.8	274	6.2	302	8.6	316	9.3	316	4.3
M – C_4H_9	246	63.7	260	72.2	288	76.8	302	96.8	302	100.0
M – C_4H_7 – CH_3 – CH_3	218	10.2	232	10.0	260	8.2	274	8.9	274	10.7
M – C_4H_9 – C_4H_8	190	12.0	204	9.9	232	8.2	246	8.1	246	11.3
M – NHDMBS + H	174	6.0	188	6.0	216	3.5	–	–	–	–
M – NHDMBS – CH_3 + H	159	2.2	–	–	201	2.5	215	3.2	215	4.1
M – COODMBS	144	7.5	158	13.8	186	28.4	200	39.0	200	33.3
M – C_4H_9 – DMBS + 2H	–	–	–	–	–	–	189	4.4	189	3.8
M – C_4H_9 – DMBS + H	132	5.8	146	2.0	174	3.2	188	3.7	188	8.7
M – ODMBS – C_4H_9 + 2H	117	3.2	131	3.7	159	0.5	173	2.0	173	2.3
M – DMBS – COODMBS + 2H	–	–	–	–	–	–	86	12.2	86	11.9
M – side chain	302	2.4	302	85.5	302	8.6	302	96.8	302	100.0
DMBS	115	6.0	115	3.8	115	4.1	115	6.4	115	1.7
$OHSiCH_3CH_3$	75	2.0	75	4.1	75	8.7	75	4.6	75	8.4
$NHSiCH_3CH_3$	73	18.8	73	15.4	73	10.8	73	17.4	73	20.1

nine) to moderate (33.3%, leucine) intensity. Attributing specific fragments to the silylated amino group is difficult, but such ions would appear to be of generally low intensity. Distinguishing between N-silylation and O-silylation is difficult, especially in CI spectra, thus identifying these moieties in an unknown compound can also be difficult. In such circumstances, an odd molecular weight can be helpful but not always definitive if there is more than one nitrogen in the molecule.

The fragmentation patterns of aspartic, glutamic and pyroglutamic acids, and aspargine and glutamine are shown in Table V. The spectra of the DMBS derivatives of glutamine, asparagine and pyroglutamic acid have been described previously[12,13]. The molecular mass was readily identified in all cases by a moderately intense protonated molecular ion plus the typical methane CI adduct ions. Ions characteristic of the presence of two silylated carboxylic acid groups are not particularly intense and are difficult to identify unambiguously at unit mass resolution since the loss of the silylated amino group along with an additional proton corresponds to the iden-

TABLE V

METHANE CHEMICAL IONISATION FRAGMENTATION PATTERNS OF *tert.*-BUTYLDIMETHYLSILYL DERIVATIVES OF ASPARTIC, GLUTAMIC AND PYROGLUTAMIC ACIDS AND ASPARAGINE AND GLUTAMINE

Ion	*Amino acid*									
	Asp		*Asn*		*Glu*		*Gln*		*pGlu*	
	m/z	%	*m/z*	%	*m/z*	%	*m/z*	%	*m/z*	%
M + C_3H_5	–	–	–	–	530	<1.0	–	–	398	6.1
M + C_2H_5	515	<1.0	–	–	519	2.1	518	1.7	386	18.1
M + H	476	73.4	475	33.4	490	81.8	489	34.2	358	100.0
M	475	–	474	–	489	–	488	–	357	–
M – CH_3	460	39.4	459	44.6	474	63.5	473	25.0	342	46.3
M – C_4H_9	418	58.6	417	42.4	432	75.6	431	19.2	300	37.3
M – C_4H_9 – C_4H_8	362	16.9	361	3.6	376	6.2	375	10.2	244	12.8
M – ODMBS + 2H	346	6.0	–	–	–	–	–	–	228	2.4
M – ODMBS	–	–	343	6.9	358	24.6	357	23.7	–	–
M – ODMBS – CH_3 – H	328	<1.0	–	–	342	8.9	341	8.8	–	–
M – COODMBS	316	9.0	–	–	330	7.3	329	4.1	198	3.8
M – C_4H_9 – DMBS + H	304	14.3	–	–	318	3.0	317	<1.0	186	1.7
M – COODMBS – CH_3 + H	302	100.0	–	–	–	–	–	–	–	–
M – C_4H_9 – ODMBS – H	286	27.7	–	–	300	2.7	289	<1.0	–	–
M – DMBS – DMBS – H	244	15.9	–	–	258	<1.0	258	2.4	–	–
M – DMBS – NHDMBS	–	–	–	–	244	16.9	243	30.8	–	–
M – ODMBS – ODMBS + 2H	215	9.8	–	–	229	9.9	228	8.4	–	–
M – 2[COODMBS] + 2H	159	27.4	158	20.7	–	–	–	–	–	–
M – 2[COODMBS] – H	–	–	–	–	172	6.2	171	8.2	–	–
H – side chain – DMBS	302	100.0	302	7.0	302	3.0	302	1.2	–	–
Side chain + DMBS	173	3.1	172	–	187	8.4	186	9.1	–	–
DMBS	115	28.5	115	24.0	115	47.0	115	47.6	115	5.6
$OHSiCH_3CH_3$	75	19.5	75	100.0	75	100.0	75	100.0	75	14.0
$NHSiCH_3CH_3$	73	60.9	–	–	73	85.6	73	64.0	–	–

tical mass. These losses appear to be represented by the ions *m/z* 159 (27.4%) and *m/z* 172 (6.2%) for aspartic and glutamic acids respectively. Clearly, the corresponding ions for asparagine and glutamine must involve either the primary amino group or the amide group. Ions representing the combined loss of C_4H_9 and ODMBS, and two DMBS moieties were considerably more intense for aspartic acid than for glutamic acid. Also, the loss of COODMBS + CH_3 − H (*m/z* 302) represented the base peak for aspartic acid but the corresponding ions were either very weak or not detected for the other compounds.

The spectra of the DMBS derivatives of serine, threonine, proline and methionine are relatively simple (Table VI). The molecular mass is clearly indicated by the adduct series of ions with the exception of serine for which an intense M + 2H (*m/z* 449) was obtained. The loss of CH_3 either from the molecular ion or the protonated molecular ion represented the base peak for serine and threonine. The loss of the *tert.*-butyl moiety (C_4H_9) along with charge retention on the residual molecule, produced intense ions (44.5–91.5%) for all the compounds indicated. Loss of the silylated side chain produced a moderately intense ion for threonine (21.3%) but not for serine (2.8%). Loss of the methionine side chain was not favoured since only a very weak ion (3.6%) was observed at *m/z* 302. The compact structure of proline resulted in a base peak at *m/z* 184 resulting from loss of the silylated carboxylic acid group.

TABLE VI

METHANE CHEMICAL IONISATION FRAGMENTATION PATTERNS OF THE *tert.*-BUTYLDIMETHYLSILYL DERIVATIVES OF SERINE, THREONINE, PROLINE AND METHOIONINE

Ion	*Amino acid*							
	Ser		*Thr*		*Pro*		*Met*	
	m/z	%	*m/z*	%	*m/z*	%	*m/z*	%
M + C_3H_5	488	1.2	502	<1.0	–	–	418	1.4
M + C_2H_5	476	4.8	490	1.7	372	1.0	406	5.3
M + H	–	–	462	59.2	344	95.7	378	100.0
M	447	–	461	–	343	–	377	–
M − CH_3 + H	–	–	447	100.0	–	–	–	–
M − CH_3	432	100.0	–	–	328	86.0	362	48.8
M − C_4H_9 − CH_3 + C_2H_5	404	5.9	418	7.0	300	8.3	–	–
M − SCH_3	–	–	–	–	–	–	330	12.6
M − C_4H_9	390	91.5	404	79.8	286	94.2	320	44.5
M − C_4H_9 − CH_3 − CH_3 + 2H	362	7.6	376	5.4	258	12.5	292	4.8
M − C_4H_9 − C_4H_8	334	13.2	348	11.3	230	15.0	264	11.0
M − COODMBS	288	10.2	302	21.3	184	100.0	218	11.5
M − DMBS − NHDMBS	202	3.8	216	3.8	98	5.4	132	4.2
M − (side chain + DMBS)	302	2.8	302	21.3	–	–	302	3.6
Side chain + DMBS + H	146	2.8	160	10.7	–	–	–	–
DMBS	115	22.8	115	19.7	115	7.8	115	10.6
$OHSiCH_3CH_3$	75	31.7	75	42.7	75	10.5	75	20.7
$NHSiCH_3CH_3$	73	56.7	73	77.1	73	23.4	73	27.3

The molecular masses of the DMBS derivatives of the aromatic amino acids phenylalanine and tyrosine were readily identified (Table VII). The most intense ions represented loss of the *tert.*-butyl moiety or fragments thereof such as the loss of the methyl group. Ions representing loss of the side-chain moiety, with or without a DMBS group, were readily detected. Charge retention was favoured for the residual molecule as reflected in the intensity of the ions at *m/z* 302.

TABLE VII

METHANE CHEMICAL IONISATION FRAGMENTATION PATTERNS OF *tert.*-BUTYLDIMETHYLSILYL DERIVATIVES OF PHENYLALANINE AND TYROSINE

Ion	*Amino acid*			
	Phe		*Tyr*	
	m/z	%	*m/z*	%
M + C_3H_5	434	2.5	564	< 1.0
M + C_2H_5	422	12.1	552	7.1
M + 2H	–	–	525	63.2
M + H	394	100.0	–	–
M	393	–	523	–
M – CH_3	378	86.4	509	59.4
M + C_4H_9 – CH_3 + C_2H_5	350	5.6	480	–
M – C_4H_9	336	74.1	466	63.4
M – C_4H_9 – 2[CH_3] + 2H	308	9.7	438	4.9
M – C_4H_9 – C_4H_8	280	16.3	410	8.8
M – ODMBS + 2H	264	4.3	394	5.2
M – COODMBS	243	19.4	364	7.2
M – DMBS – COODMBS	120	13.5	250	7.6
M – (side chain + DMBS)	302	65.5	302	74.6
Side chain + DMBS	–	–	221	34.5
Side chain	91	12.4	107	4.3
DMBS	115	10.5	115	41.0
$OHSiCH_3CH_3$	75	39.3	75	–
$NHSiCH_3CH_3$	73	28.1	73	100.0

The mass spectrum of arginine presented in Table VIII represents the arginine-2 peak. A molecular mass of 499 is indicated but is is problable that this represents loss of a silylated amino group to form a stable neutral fragment which subsequently forms the observed series of adduct ions. This interpretation is hypothetical, but the alternative interpretation of the loss of NH_3 from a tri-silylated derivative of mass 516 seems less likely. Given the mass of *m/z* 516 for the arginine-1 peak (spectrum not shown in Table VIII but virtually identical to that of arginine-2), and the relative retention times of the two peaks, it seems likely that the arginine-1 peak is tri-silylated and the arginine-2 peak is tetra-silylated.

In other respects, the spectrum of arginine-2 contained moderately intense ions (24–37.6%) representing loss of all or parts of the silylated carboxylic acid group.

The derivatives of lysine and histidine (Table VIII) had the expected molecular masses and intense ions reflecting the loss of CH_3 and C_4H_9. No strong indication of the loss of the silylated carboxylic acid was observed for histidine but ions at *m/z* 341 (12.3%; M − ODMBS − CH_3 − H) and *m/z* 329 (16.2%; M − COODMBS) were observed for lysine (not shown in table). Loss of the silylated side chain was not favoured for any of the basic amino acids but the silylated side chain fragments did produce an intense ion for histidine *m/z* 197; 52.7%).

TABLE VIII

METHANE CHEMICAL IONISATION FRAGMENTATION PATTERNS OF *tert.*-BUTYLDIMETHYLSILYL DERIVATIVES OF ARGININE, LYSINE AND HISTIDINE

Ion	*Amino acid*					
	Arg		*Lys*		*His*	
	m/z	%	*m/z*	%	*m/z*	%
M + C_3H_5	540	–	529	1.0	538	1.0
M + C_2H_5	528	2.3	517	2.7	526	6.0
M + H	500	42.7	489	71.5	498	64.8
M	499	–	488	–	497	–
M − CH_3	484	35.4	473	48.6	482	57.8
M − C_4H_9	422	37.6	431	47.8	441	63.2
M − C_4H_9 − C_4H_8	386	2.6	375	3.6	384	8.5
M − HNDBMS + 2H	371	1.0	360	3.6	369	7.6
M − COODMBS + H	341	24.0	–	–	339	7.5
M − C_4H_8 − DMBS	328	17.5	317	2.5	326	3.2
M − NHDMBS − C_4H_9 − C_4H_9	258	7.4	256	2.4	–	–
M − (side chain + DMBS)	302	2.1	302	5.7	302	9.9
Side chain + DMBS + 2H	–	–	–	–	197	52.7
Side chain + DMBS	197	3.9	188	5.0	195	19.8
Side chain + 2H	86	8.0	74	84.7	83	25.6
Side chain	84	8.1	72	–	81	–
DMBS	115	24.7	115	49.5	115	16.5
$OHSiCH_3CH_3$	75	37.4	75	37.2	75	24.0
$NHSiCH_3CH_3$	73	100.0	73	100.0	73	100.0

The expected molecular mass of 696 was not observed for DMBS cystine and only ions of low intensity were observed above *m/z* 350. These were at *m/z* 639 (M − C_4H_9: <1%) and at *m/z* 378 (M − 2 × COODMBS; 2%). Cleavage of the S–S bond was represented by *m/z* 348 (7.9%) and by *m/z* 350 (M/2 + 2H; 11.8%). The presence of sulfur was indicated by the loss of 32 a.m.u. from the former to produce *m/z* 316 (9.9%), and by *m/z* 202 (M/2 − S − DMBS + H; 19.4%) and *m/z* 187 (M/2 − S − NDMBS; 20.5%). These ions could equally well be represented as being derived from *m/z* 350.

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