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

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SUMMARY

A gas–liquid chromatographic procedure is described which will separate and quantitate the seventeen amino acids typically found in protein acid hydrolyzates. If present, tryptophan, cysteine and carboxymethylcysteine can also be simultaneously assayed via this method. The amino acids, as their *tert.*-butyldimethylsilylated derivatives, are readily separable on SE-30 (wall-coated open-tubular) and OV-17 (bonded) capillary columns, as well as on packed gas–liquid chromatographic columns coated with the same liquid phases. Retention times and responses for all amino acids and internal standards are given. Mass spectral analysis of all *tert.*-butyldimethylsilylated amino acids is presented and displays a characteristic and unique [M − 57] fragment ion for each amino acid which often dominates the mass spectrum. Application of this method is demonstrated using four well characterized proteins.

INTRODUCTION

Over the past several years there have been extensive developments in the utilization of gas–liquid chromatography (GLC) for amino acid separation and analysis. General reviews of these methods have appeared in the literature and provide an excellent scope of the research in this area[1–3]. For GLC analysis, amino acids must first be derivatized so that a volatile compound is obtained. A major reason for the current diversity in experimental approaches to produce suitable volatile compounds is related to the fact that amino acids are multifunctional and possess basic, neutral and acidic moieties. Usually the *n*-butyl esters of the amino acid carboxylates are produced first, followed by the subsequent acylation of the remaining functions possessing active protons (*e.g.*, amines, hydroxyl groups and thiols) with trifluoroacetic anhydride to yield the respective N(O,S)-trifluoroacetyl (TFA) *n*-butyl ester[4–6]. Alternatively, the *n*-propyl[7] or isobutyl esters[8,9] of the amino acids can be synthesized followed by acylation with heptafluorobutyric anhydride. Most of these methods and some others[10–12] provide excellent separation and quantitation of amino

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acids on either packed or capillary columns employing either flame ionization or electron-capture GLC detectors.

Earlier investigations proposed the use of the trimethylsilyl (TMS) function as a means of producing a volatile amino acid derivative[13]. This was ideal in that it employed a single protective functionality to block all active protic sites on the amino acids while conferring the volatility needed for the amino acids to be analyzed by GLC. Also, a single derivatizing reagent was all that was required. While the derivatiatization method was relatively easy to perform, the resulting TMS derivatives were very unstable, easily hydrolyzed and produced, in practice, varying GLC results for many of the amino acids.

Recently, it has been reported that the *tert.*-butyldimethylsilyl (tBDMS) function is considerably more stable than the TMS group[14]. Furthermore, N-methyl-N-(*tert.*-butyldimethylsilyl)trifluoroacetamide (MTBSTFA) has been reported to be a very powerful tBDMS silyl donor capable of *tert.*-butyldimethylsilylating active protic functions (*i.e.*, hydroxyl, amino, carboxylic and thiol moieties)[15] and has been employed in the derivatization of arginine and glutamine for analysis by GLC[16]. The present investigation reports on an analytical method which employs the tBDMS derivatives of the amino acids for their separation and quantification, in a single GLC analysis. This includes the amino acids typically found in protein acid hydrolyzates, as well as tryptophan and carboxymethylcysteine. The hydrochloride salts of the amino acids, dissolved in dimethylformamide, are derivatized in a single step with MTBSTFA. As the tBDMS-amino acid derivatives, the neutral and acidic amino acids are stable for over 24 h and the basic amino acids are stable for 6 h. Separation of the tBDMS-amino acids is readily accomplished on a SE-30 wall-coated open-tubular (WCOT) capillary column with each tBDMS-amino acid displaying a single chromatographic peak, exception for arginine which showed two peaks. Chromatographic results are also given on a bonded OV-17 capillary and two packed GLC columns. Application of this procedure on four well characterized proteins is also presented. Lastly, as is commonly observed for many compounds possessing a tBDMS function when analyzed by mass spectrometry (MS)[15,17], the mass spectrum for each tBDMS-amino acid is relatively simple being dominated by a unique and unambiguous mass minus 57 [M − 57] fragment ion which for many of the amino acids serves as the base fragment ion.

EXPERIMENTAL

Materials

All amino acid standards were obtained from Pierce (Rockford, IL, U.S.A.) with the exception of carboxymethylcysteine which was purchased from Sigma (St. Louis, MO, U.S.A.). N,N-Dimethylformamide (DMF), acetonitrile and dimethyl sulfoxide (DMSO) were purchased from Aldrich (Milwaukee, WI, U.S.A.). All solvents were redistilled prior to use. N-Methyl-N-*tert.*-butyldimethylsilyltrifluoroacetamide (MTBSTFA), with and without 1% *tert.*-butyldimethylsilyl chloride (tBDMS-Cl), was either synthesized in this laboratory[15] or purchased from Regis (Morton Grove, IL, U.S.A.). Bovine testicular hyaluronidase (Type VI-S), bovine serum albumin (crystallized), and ribonuclease A from bovine pancrease (Type I-A, crystallized) were obtained from Sigma.

Gas chromatography

Packed-column GLC was performed using a Perkin-Elmer Sigma 3 instrument equipped with dual flame ionization detectors. The glass chromatographic columns were 6 ft. × 1/8 in. O.D. (1.8 mm I.D.), packed with either 3.0% SE-30 or 3.0% SP-2250 (Supelco, Bellefonte, PA, U.S.A.) on Supelcoport, 100–120 mesh. The nitrogen flow-rate was 18 ml/min, with injector and detector temperatures of 300°C. After an initial hold of 1 min at 110°C the column was temperature programmed at 5°C/min to 260°C. Direct capillary GLC analysis was performed with a Varian Model 3700, (Varian, Park Ridge, IL, U.S.A.) equipped with dual flame ionization detectors. Chromatographic columns employed were a 25 m × 0.32 mm I.D. fused-silica capillary column with 0.25-μm bonded methylphenyl (50%) silicone (Quadrex, New Haven, CT, U.S.A.) and a 12 m × 0.32 mm I.D. WCOT capillary column coated with 0.5-μm SE-30 (Varian). The helium flow-rate was 5 ml/min, with injector and detector temperatures of 300°C. After an initial hold of 2 min at 100°C the column was temperature programmed at 10°C/min to 280°C. Peak areas and retention times were recorded using a Shimadzu C-R3A Chromatopac integrator (Shimadzu, Columbia, MA, U.S.A.).

Mass spectrometry

Mass spectra were obtained on a Kratos MS 50 S mass spectrometer (Kratos, Urmston, Manchester, U.K.) interfaced with a Carlo Erba Model 4160 gas chromatograph. Mass spectra were recorded at 70 eV with an ionization current of 50 μA, a source temperature of 250°C, and a transfer temperature of 218°C.

Hydrolysis of proteins

Proteins (2 mg) and amino acid standards were each subjected to acid hydrolysis in 0.2 ml 6.0 *N* hydrochloric acid at 125°C for 24 h under a nitrogen atmosphere. Samples were then dried in 3.0-ml Reactivials (Pierce) under a stream of dry nitrogen gas, taken up in 0.25 ml of 95% ethanol, to which was then added 2.0 ml of benzene, and were again dried. This latter step was repeated twice.

Amino acid standard solution

The standard amino acid solution contained 3.3 μmol/ml of each amino acid in 0.05 *M* hydrochloric acid. Norleucine or α-aminobutyric acid was included at the same concentration as the internal standard. For GLC and GLC–MS analysis 0.1 ml aliquots of this stock solution were dried in Reactivials in the same manner as described above for protein hydrolyzates.

Derivatization of amino acids

Both the standard amino acids and protein hydrolyzates were derivatized by the following procedure. To the respective Reactivial, equipped with a small PTFE-coated stir bar and a PTFE-faced silicone septum, 10 μl of DMF were added to the sample to be derivatized. Then 250 μl of MTBSTFA, without tBDMS-Cl, were added and the vials were heated at 70°C for 25 min and allowed to stand at room temperature for 95 min. For packed and capillary column GLC analysis 1.5 and 0.1 μl were injected per analysis, respectively. In other experiments, DMSO, acetonitrile or no solvent was substituted for DMF. Additionally, MTBSTFA containing 1%

tBDMS-Cl, as a catalyst, was employed in several studies in lieu of MTBSTFA without the silyl chloride.

RESULTS AND DISCUSSION

Derivatization

With the exception of threonine and histidine, *tert.*-butyldimethylsilylation of all the amino acids, as their hydrochloride salts, dissolved in 10 μl of DMF was complete after the addition of 250 μl of MTBSTFA. *tert.*-Butyldimethylsilylation of threonine was found to be complete after 2 h at room temperature with this lag time apparently due to the steric interferences of placing a bulky tBDMS group on both the α-amino and β-hydroxy moieties of this amino acid. Heating of the sample had very little effect upon the rate of threonine derivatization though doubling the amount of DMF did reduce the time required for derivatization to 90 min. In contrast, the complete *tert.*-butyldimethylsilylation of histidine was achieved only by heating the sample at 70°C for 25 min. This is probably related to the fact that histidine possesses a β-imidazole moiety and it has been shown that silylated imidazole compounds are themselves excellent donors of the silyl function[18]. Heating of the sample apparently supplies the energy required to accelerate the transfer of the tBDMS group from the silyl donor *N*-methyl-*N*-trifluoroacetamide of MTBSTFA to the imidazole ring of histidine.

The use of MTBSTFA containing 1.0% tBDMS-Cl reduced the derivatization time required for threonine to 15 min and showed very little effect upon the neutral and acidic amino acids. In contrast, the basic amino acids arginine and histidine were completely absent when analyzed indicating that the silyl chloride interfered with their derivatization.

Substitution of acetonitrile or DMSO for DMF as the amino acid solvent resulted in incomplete derivatization for most amino acids and also a more protracted time period required for the complete derivatization of threonine. In addition, very poor results were achieved if the amino acids were *tert.*-butyldimethylsilylated directly with MTBSTFA in the absence of any organic solvent. This appeared to be closely related to the observation that most of the amino acids displayed very poor solubility in MTBSTFA.

Stability of the tBDMS-amino acids as a function of time

In order to study the stability of each tBDMS-amino acid derivative, an amino acid standard, in DMF, was *tert.*-butyldimethylsilylated by adding 250 μl of MTBSTFA, heated at 75°C for 25 min and allowed to stand at room temperature for 95 min. Aliquots of this standard were injected at 6-h-intervals for 24 h, with the results presented in Table I. As is shown, all the amino acids demonstrated very little degradation in the first 6-h interval following derivatization. This remained true for all the neutral, acidic, aromatic and thiol-containing amino acids during the 24-h duration. Notably, though, all the basic amino acids showed a steady decrease in concentration after the initial 6 h indicating a loss of the derivative. This degradation was especially marked for histidine and arginine with the loss being further enhanced if the DMF, used as the solvent for derivatization, was not of a high grade and kept dry.

TABLE I

STABILITY OF THE *tert.*-BUTYLDIMETHYLSILYL AMINO ACID DERIVATIVES

Data expressed as the mean relative weight response (RWR) of five different sample injections of each respective amino acid relative to the relative weight response of the internal standard γ-aminobutyric acid; [RWR = (amino acid/γ-aminobutyric acid)]. A 0.1-ml aliquot of a standard amino acid solution containing 3.3 μmol/ml of each amino acid was dried under nitrogen, taken up in 10 μl of DMF and derivatized with 250 μl of MTBSTFA. The sample was heated at 70°C for 25 min then allowed to stand at room temperature for 95 min. Aliquots (0.1 μl) were injected on a 12 m × 0.32 mm I.D., WCOT capillary column coated with 0.5 μm SE-30 at indicated time intervals. The helium flow-rate was 5 ml/min, with injector and detector temperatures of 300°C. After an initial hold of 2 min at 100°C the column was temperature programmed at 10°C/min to 280°C.

Amino acid	*Relative weight response*				
	0	*6 h*	*12 h*	*18 h*	*24 h*
Alanine	0.93	0.94	0.94	0.96	0.95
Glycine	0.95	0.93	0.91	0.91	0.87
Valine	0.95	0.96	0.94	0.94	0.96
Leucine	0.97	1.01	1.00	0.97	0.98
Isoleucine	0.97	0.95	0.94	0.95	0.93
α-Aminobutyric acid	1.02	0.96	0.94	0.92	0.90
Proline	0.95	0.92	0.90	0.88	0.85
Serine	1.37	1.42	1.41	1.44	1.39
Threonine	1.31	1.35	1.37	1.33	1.28
Methionine	1.06	1.00	0.98	0.97	0.95
Phenylalanine	1.16	1.09	1.14	1.20	1.20
Aspartate	1.22	1.31	1.26	1.22	1.26
Cysteine	0.99	1.01	0.97	0.96	0.97
Glutamate	1.29	1.18	1.20	1.22	1.17
Lysine	0.69	0.61	0.56	0.50	0.44
Arginine (I + II)	0.55	0.53	0.46	0.40	0.35
Tyrosine	1.01	1.10	0.99	0.97	0.95
Histidine	0.72	0.70	0.55	0.46	0.28
Tryptophan	0.91	0.87	0.84	0.84	0.80
Cystine	0.94	1.00	0.92	0.91	0.92

GLC separations

The GLC separation of the tBDMS derivatives of an amino acid standard mixture on a SE-30 (WCOT) fused-silica capillary column is presented in Fig. 1. As shown, each amino acid derivative displayed a single symmetrical chromatographic peak, with the exception of arginine which emerged from the column as two closely related peaks. Notably, each peak exhibited no significant peak tailing. Complete baseline separation was achieved for each amino acid in the standard mixture, which contained all the amino acids typically found in protein acid hydrolyzates, with the addition of tryptophan, carboxymethylcysteine, cysteine and the internal standard, α-aminobutyric acid. Fig. 2 shows the separation of the same amino acid mixture, with the addition of γ-aminobutyric acid, on a bonded OV-17 fused-silica capillary column. Except for lysine, tryptophan and cystine, which exhibited two peaks, each amino acid displayed a single peak and all peaks showed complete baseline separation. Though not shown, separation of the tBDMS-amino acids on columns packed

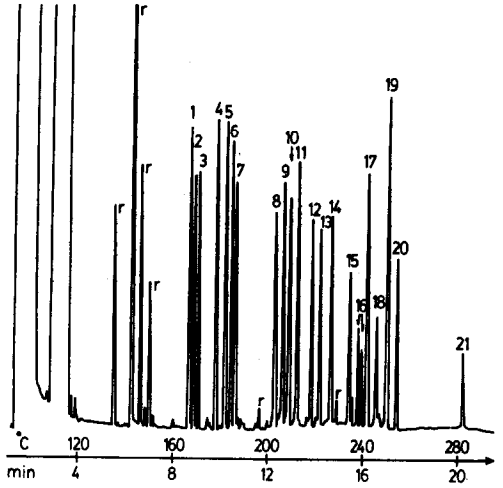

Fig. 1. Gas–liquid chromatogram of the tBDMS derivatives of eighteen protein amino acids in addition to cystine, carboxymethylcysteine and α-aminobutyric acid (internal standard) separated on a 12 m × 0.32 mm I.D., WCOT and GLC columns coated with 0.5 μm SE-30. Injected sample contained 5.0 nmol of each tBDMS-amino acid and internal standard. After an initial hold of 2 min at 100°C the column was temperature programmed at 10°C/min to 280°C. Helium was employed as the carrier gas at a flow-rate of 5 ml/min. Peaks: 1 = glycine; 2 = alanine; 3 = α-aminobutyric acid (internal standard); 4 = valine; 5 = leucine; 6 = isoleucine; 7 = proline; 8 = methionine; 9 = serine; 10 = threonine; 11 = phenylalanine; 12 = aspartic acid; 13 = cysteine; 14 = glutamate; 15 = lysine; 16 = arginine (two peaks); 17 = carboxymethylcysteine; 18 = histidine; 19 = tyrosine; 20 = tryptophan; 21 = cystine. Peaks labeled r were found to be present in the derivatizing reagent.

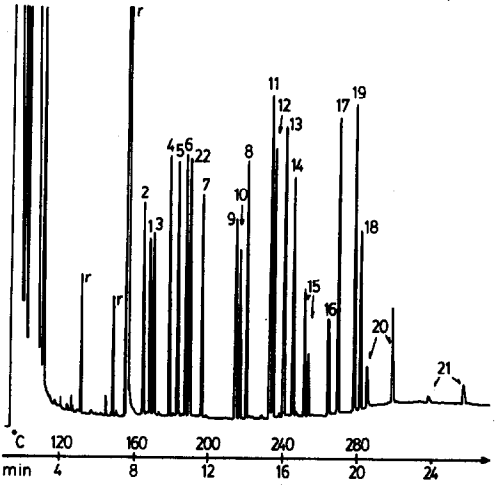

Fig. 2. Gas–liquid chromatogram of the tBDMS derivatives of eighteen protein amino acids, in addition to cystine, carboxymethylcysteine and two internal standards separated on a 25 m × 0.32 mm I.D., fused-silica capillary column with 0.25-μm bonded OV-17 (50% phenylmethyl silicone). Injected sample contained 5.0 nmol of each tBDMS-amino acid and internal standard. After an initial hold of 2 min at 100°C the column was temperature programmed at 10°C/min to 280°C. Helium was employed as the carrier gas at a flow-rate of 5 ml/min. Peaks: 1 = glycine; 2 = alanine; 3 = α-aminobutyric acid (internal standard); 4 = valine; 5 = leucine; 6 = isoleucine; 7 = proline; 8 = methionine; 9 = serine; 10 = threonine; 11 = phenylalanine; 12 = aspartic acid; 13 = cysteine; 14 = glutamate; 15 = lysine (two peaks); 16 = arginine; 17 = carboxymethylcysteine; 18 = histidine; 19 = tyrosine; 20 = tryptophan (two peaks); 21 = cystine (two peaks); 22 = γ-aminobutyric acid (alternate internal standard). Peaks labeled r were found to be present in the derivatizing reagent.

with 3% SE-30 and 3% SP-2250 (similar to OV-17) gave similar chromatographic results but did show less peak resolution than that achieved on capillary columns in addition to showing some loss of histidine and no observed peak for cystine.

Retention data

Retention times and relative retention times for each tBDMS-amino acid de-

TABLE II

RETENTION TIMES (RT) AND RELATIVE RETENTION TIMES (RRT) OF AMINO ACIDS AS THEIR *tert.*-BUTYLDIMETHYLSILYL DERIVATIVES BY PACKED AND CAPILLARY COLUMN GLC ANALYSIS

The 3.0% SE-30 and the 3.0% SP-2250 packed GLC columns were each 6 ft. × 1/8 in. O.D. (1.8 mm I.D.) glass columns, employing Supelcoport, 100–120 mesh, as the support. For both columns the nitrogen flow-rate was 18 ml/min, with injector and detector temperatures of 300°C. After an initial hold of 1 min at 110°C the columns were temperature programmed at 5°C/min to 260°C. A 25 m × 0.32 mm I.D., fused-silica capillary column with 0.25 μm bonded methylphenyl (50%) silicone and a 12 m × 0.32 mm I.D., WCOT capillary column coated with 0.5 μm SE-30 were used. For both columns the helium flow-rate was 5 ml/min, with injector and detector temperatures of 300°C. After an initial hold of 2 min at 100°C the columns were temperature programmed at 10°C/min to 280°C. The retention time is expressed in minutes; the relative retention time is relative to the indicated internal standard, marked with an asterisk.

Amino acid	*Packed column*				*Capillary column*			
	3% SE-30		*3% SP-2250*		*OV-17 (bonded)*		*SE-30 (WCOT)*	
	RT	*RRT*	*RT*	*RRT*	*RT*	*RRT*	*RT*	*RRT*
Alanine	7.69	0.90	9.15	0.68	8.48	0.91	6.94	0.92
Glycine	7.33	0.86	9.92	0.74	8.99	0.96	6.68	0.88
α-Aminobutyric acid	8.50	1.00*	9.92	0.74	9.37	1.00*	7.57	1.00*
Valine	9.63	1.13	11.39	0.84	9.90	1.06	8.16	1.08
Leucine	10.50	1.23	12.20	0.91	10.32	1.10	8.67	1.15
Isoleucine	11.08	1.30	12.98	0.96	10.77	1.15	9.02	1.19
Norleucine	11.48	1.35	13.46	1.00*	10.98	1.17	9.25	1.22
γ-Aminobutyric acid	11.48	1.35	14.55	1.08	11.47	1.22	9.25	1.22
Proline	11.48	1.35	14.55	1.08	11.64	1.24	9.25	1.22
Serine	16.12	1.90	18.19	1.35	13.48	1.44	11.87	1.57
Threonine	16.67	1.96	18.56	1.38	13.72	1.46	12.18	1.61
Methionine	15.29	1.80	19.33	1.44	14.05	1.50	11.42	1.51
Phenylalanine	17.42	2.05	21.75	1.62	15.34	1.64	12.61	1.67
Aspartate	18.92	2.23	22.14	1.64	15.47	1.65	13.40	1.77
Cysteine	19.69	2.32	23.21	1.72	16.06	1.71	13.90	1.84
Glutamate	20.90	2.46	24.37	1.81	16.58	1.77	14.50	1.92
Lysine I	22.78	2.68	25.58	1.90	17.90	1.91	15.51	2.05
Lysine II	–	–	26.21	1.94	17.41	1.86	–	–
Arginine I	24.28	2.86	28.30	2.10	18.45	1.97	16.32	2.16
Arginine II	–	–	–	–	–	–	20.52	2.71
Carboxymethylcysteine	24.48	2.88	29.08	2.16	18.98	2.02	16.65	2.20
Tyrosine	26.41	3.11	30.70	2.28	19.05	2.03	17.53	2.32
Histidine	25.43	2.99	31.15	2.31	19.30	2.06	16.95	2.24
Tryptophan I	26.41	3.11	33.23	2.47	20.11	2.15	17.53	2.32
Tryptophan II	–	–	36.78	2.73	22.10	2.36	–	–
Cystine I	–	–	–	–	23.89	2.55	21.69	2.87
Cystine II	–	–	–	–	25.72	2.75	–	–

rivative on 3% SE-30 and 3% SP-2250 packed GLC columns and on an OV-17 (bonded) and SE-30 (WCOT) capillary columns are given in Table II. As might be expected, the elution order of amino acids was the same for the packed column and the capillary column possessing the same liquid phase. Typically, the amino acids emerged from the non-polar SE-30 packed and capillary columns primarily in the order of increasing molecular weight of the amino acid derivatives. This is also true for the results achieved on the intermediate polarity columns (SP-2250 and OV-17) except for the reversal of the elution order of alanine and glycine, threonine and methionine, and tyrosine and histidine.

Quantitative aspects

Employing amino acid standards, a linear response curve in the range 1–100

TABLE III

RELATIVE WEIGHT RESPONSE (RWR) AND RELATIVE STANDARD DEVIATION (R.S.D.) OF THE *tert.*-BUTYLDIMETHYLSILYL AMINO ACIDS

Data expressed as the RWR and R.S.D. (%) of ten different sample injections of each respective amino acid relative to the internal standard α-aminobutyric acid on the SE-30 and to norleucine on the OV-17 capillary columns. Program: as described in Table II.

Amino acid	*SE-30 (WCOT) (n = 10)*		*OV-17 (bonded) (n = 10)*	
	RWR	*R.S.D. (%)*	*RWR*	*R.S.D. (%)*
Alanine	0.93	1.52	0.97	1.23
Glycine	0.95	1.65	0.94	1.54
α-Aminobutyric acid	(1.00)		–	
Valine	0.95	1.66	0.96	1.72
Leucine	0.97	1.57	1.01	1.59
Isoleucine	0.97	1.68	0.96	1.73
Norleucine	–		(1.00)	
γ-Aminobutyric acid	1.02	2.32	0.97	3.11
Proline	0.95	2.17	0.85	3.21
Serine	1.37	2.03	1.28	1.99
Threonine	1.31	2.14	1.24	2.57
Methionine	1.06	1.89	1.04	2.42
Phenylalanine	1.16	0.92	1.21	0.77
Aspartate	1.22	0.95	1.21	1.11
Cysteine	0.99	1.78	1.06	1.54
Glutamate	1.29	0.89	1.18	1.08
Lysine I	0.69	2.57	0.12	3.21*
Lysine II	–		0.57	
Arginine I	0.13	3.78*	0.58	3.70*
Arginine II	0.42		0.09	
Carboxymethylcysteine	1.13	2.01	1.09	1.56
Tyrosine	1.01	3.23	1.03	3.01
Histidine	0.72	4.73	0.56	4.85
Tryptophan I	0.91	3.67	0.23	3.33*
Tryptophan II	–		0.44	
Cystine I	0.94	2.23	0.12	2.12*
Cystine II	–		0.55	

* For amino acids displaying two chromatographic peaks the R.S.D. (%) was calculated using the combined RWRs of the peaks for that amino acid.

nmol was obtained for each neutral and acidic amino acid using a flame ionization detector. The basic amino acids lysine and arginine demonstrated a linear response curve in the range 2–150 nmol. Histidine displayed a linear response curve in the range 5–150 nmol. For the analysis of hydrolyzed biological samples a functional range of 6–100 nmol for each amino acid was typically used.

Table III shows the relative weight response and relative standard deviation of the tBDMS-amino acids with respect to the respective internal standard, *i.e.*, α-aminobutyric acid or norleucine, depending on the capillary column employed in the study. As is indicated by the data, excellent precision in the relative weight response of each amino acid is noted.

Mass spectrometry

All synthesized tBDMS-amino acids were each subjected to combined GLC–MS analysis. The results are presented in Table IV. All the tBDMS-amino acids produced the same general fragmentation pattern, as depicted in Fig. 3. Most tBDMS-amino acids yielded mass spectra that were dominated by a single unique [M − 57] fragment ion. As is typical of tBDMS derivatives, this fragment ion results from the elimination of one *tert.*-butyl function (*i.e.*, [M − $C(CH_3)_3$], Fig. 3) from the molecule. Even the exceptions to this [M − 57] fragment ion domination of the

TABLE IV

INTERPRETATION AND RELATIVE INTENSITIES OF THE MAJOR FRAGMENT IONS IN THE MASS SPECTRA OF THE *tert.*-BUTYLDIMETHYLSILYL DERIVATIVES OF AMINO ACIDS

Data expressed as *m/z* (% relative intensity) where the intensities of the fragment ions of each amino acid are given relative to the indicated base fragment ion, *i.e.* *m/z* (100), observed in the respective mass spectrum.

Amino acid	*m/z (relative intensities)**						
	M^+	*M − 15*	*M − 57*	*M − R*	*M − 85*	*M − 159*	*M − 302*
Glycine	303 (0.2)	288 (3.2)	246 (100)		218 (22)		
Alanine	317 (0.2)	302 (12.4)	260 (100)	[302]	232 (20)	158 (72)	
Valine	345 (0.5)	330 (1.8)	288 (100)	302 (58)	260 (35)	186 (56)	
Leucine	359 (0.2)	344 (1.5)	302 (100)	[302]	274 (20)	200 (54)	
Isoleucine	359 (0.3)	344 (2.1)	302 (100)	[302]	274 (23)	200 (67)	
Proline	343 (0.3)	328 (2.6)	266 (91)		258 (13)	184 (100)	
Serine	447 (0.2)	432 (4.6)	390 (100)	302 (24)	362 (21)	288 (30)	
Threonine (2)	347 (0.7)	332 (3.5)	290 (100)	302 (12)	262 (10)	188 (22)	
Threonine (3)	461 (0.8)	446 (5.4)	404 (98)	[302]	376 (18)	302 (100)	159 (11)
Methionine	377 (1.2)	362 (8.6)	320 (100)	302 (8)	292 (36)	218 (44)	75 (7)
Phenylalanine	393 (0.1)	378 (3.3)	336 (95)	302 (100)	308 (11)	234 (27)	91 (3)
Aspartate	475 (0.4)	460 (5.2)	418 (100)	302 (40)	390 (9)	316 (16)	173 (5)
Cysteine	463 (0.2)	448 (3.6)	406 (100)	302 (56)	378 (20)	304 (22)	
Glutamate	489 (0.7)	474 (9.2)	432 (100)	302 (12)	404 (15)	330 (21)	187 (2)
Lysine	488 (0.3)	473 (5.2)	431 (100)	302 (5)	403 (10)	329 (62)	
Arginine (4)	630 (0.4)	615 (3.2)	573 (100)	302 (7)	545 (12)	471 (21)	328 (11)
Tyrosine	523 (0.1)	508 (2.6)	466 (92)	302 (100)	438 (22)	364 (14)	221 (9)
Histidine	497 (0.6)	482 (1.6)	440 (100)	302 (42)	412 (7)	338 (51)	195 (4)
Tryptophan (2)	432 (1.2)	417 (4.8)	375 (100)	302 (90)	347 (11)	273 (10)	130 (6)
Cystine (4)	696 (0.4)	681 (5.5)	639 (100)		611 (13)	537 (5)	

* Scheme symbols correspond to the designated fragmention letters used in Fig. 3.

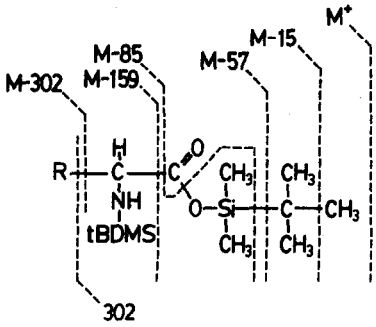

Fig. 3. General mass-spectral fragmentation scheme produced by the tBDMS derivatives of amino acids. Scheme fragmentation assignments are employed in Table IV for identification of the fragment ions seen for each amino acid derivative.

TABLE V

ANALYSIS OF PROTEIN AMINO ACIDS AS THEIR *tert.*-BUTYLDIMETHYLSILYL DERIVATIVES

Data expressed as mole percent with the analytical results reported in the literature presented within parentheses. Each protein (2 mg) was subjected to acid hydrolysis in 0.2 ml of 6.0 *N* hydrochloric acid at 125°C for 24 h under a nitrogen atmosphere. Samples were dried, taken up with 10 μl of DMF and derivatized with the addition of 250 μl of MTBSTFA. Samples were heated at 70°C for 25 min and then allowed to stand at room temperature for 95 min. Aliquots (0.1 μl each) were injected on a 12 m × 0.32 mm I.D., WCOT capillary column coated with 0.5 μm SE-30. The helium flow-rate was 5 ml/min, with injector and detector temperatures of 300°C. After an initial hold of 2 min at 100°C the column was temperature programmed at 10°C/min to 280°C.

Amino acid	*Bovine serum albumin**	*Hyaluronidase***	*Pepsinogen****	*Ribonuclease*§
Glycine	2.79 (2.83)	6.08 (6.14)	9.71 (9.68)	2.50 (2.42)
Alanine	8.07 (8.13)	5.62 (5.76)	4.53 (4.42)	9.54 (9.68)
Valine	6.21 (6.18)	7.21 (7.26)	6.90 (6.92)	7.21 (7.26)
Isoleucine	2.38 (2.30)	4.21 (4.18)	8.72 (8.76)	2.40 (2.42)
Leucine	10.66 (10.78)	9.01 (8.92)	6.84 (6.95)	1.66 (1.61)
Proline	5.10 (4.95)	6.10 (6.08)	4.22 (4.27)	3.15 (3.23)
Serine	4.55 (4.59)	8.34 (8.28)	13.68 (13.83)	11.87 (12.10)
Threonine	5.47 (5.65)	5.34 (5.42)	7.25 (7.39)	7.91 (8.06)
Methionine	0.77 (0.71)	1.09 (1.03)	1.00 (1.05)	3.29 (3.23)
Phenylalanine	4.63 (4.59)	4.11 (4.03)	4.26 (4.17)	2.34 (2.42)
Glutamate	13.34 (13.25)	8.27 (8.35)	9.01 (8.91)	9.59 (9.68)
Aspartate	9.31 (9.36)	11.34 (11.20)	11.02 (11.14)	12.21 (12.10)
half-Cystine	6.18 (6.18)	2.97 (3.06)	1.77 (1.63)	6.38 (6.45)
Lysine	9.38 (9.89)	6.01 (6.12)	2.12 (2.23)	7.88 (8.06)
Arginine	3.38 (3.89)	4.47 (4.61)	1.49 (1.62)	3.10 (3.23)
Tyrosine	3.30 (3.36)	4.62 (4.84)	4.76 (4.89)	4.10 (4.02)
Histidine	2.70 (3.00)	2.12 (2.19)	0.47 (0.55)	3.09 (3.23)

* Ref. 19.
** Ref. 20.
*** Ref. 21.
§ Ref. 22.

mass spectrum still possessed [M − 57] fragment ions of relative intensities that were greater than 90% of the base fragment ion. These exceptions were phenylalanine, tyrosine and proline. Phenylalanine and tyrosine presented mass spectra of which the [M − R], *i.e.* *m/z* 302 (Fig. 3), fragment ion served as the base ion. This probably indicates that the phenyl and the *p*-hydroxyphenyl moieties, that serve as the variable (R) groups of these amino acids, respectively, are slightly better leaving groups than the $C(CH_3)_3$ function of tBDMS. Proline showed a very prominent [M − 159] fragment ion representing the loss of O=C–O–tBDMS from the derivative resulting in the formation of the 2-(tBDMS)-pyrrolidine (*m/z* 184) fragment ion. Each amino acid displayed a weak molecular ion [M^+, less than 2% relative intensity] and a small [M − 15] fragment ion representing the loss of CH_3 from a tBDMS function (Fig. 3). Except for glycine, proline and cystine, the mass spectrum of each amino acid displayed a [M − R] fragment ion representing the loss of the variable (R) moiety of the amino acid. Some amino acids also showed a weak [M − 302] which represents the loss of HC(NH–tBDMS)COO–tBDMS from the variable (R) moiety of the respective amino acid (Fig. 3). The mass spectrum of each amino acid showed a [M − 85] fragment ion of moderate relative intensity indicating the loss of C=O and $C(CH_3)_3$. Lastly, except for glycine, the mass spectrum of each amino acid displayed a [M − 159] fragment ion suggesting the loss of COO–tBDMS from each amino acid.

Analysis of amino acids in protein acid hydrolyzates

Table V presents the results of analyzing the amino acids found in the acid hydrolyzates of four well characterized proteins using the *tert.*-butyldimethylsilyl derivatization method. As can be seen in Table V, for each protein the amino acid composition determined, via this method, was in excellent agreement with published amino acid data.

In conclusion, a method is described in which amino acids, that are typically found in the acid hydrolyzates of proteins, are derivatized to their respective tBDMS derivative and analyzed by GLC. The derivatives are readily made and have excellent packed- and capillary-column characteristics. In addition, each tBDMS-amino acid shows a unique, unambiguous [M − 57] fragment ion when analyzed by MS which allows for easy identification.

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