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# Gas chromatographic–mass spectrometric analysis of products arising from pyrolysis of amino acids in the presence of hexamethyldisilazane

Giuseppe Chiavari[a,*], Daniele Fabbri[a,b], Silvia Prati[b]

[a]*Dipartimento di Chimica "G. Ciamician", Università di Bologna, Via Selmi 2, 40126 Bologna, Italy*
[b]*Laboratorio di Chimica Ambientale, Università di Bologna, Via Marconi 2, 48100 Ravenna, Italy*

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**Abstract**

α-Amino acids were pyrolysed at 600°C in the presence of hexamethyldisilazane (HMDS) and the formed volatile products were analysed on line by gas chromatography–mass spectrometry (GC–MS). Glycine, alanine, valine, leucine, isoleucine, norleucine, methionine, phenylalanine yielded principally the trimethylsilyl (TMS) ester of the parent amino acid. TMS esters of carboxylic acids arising from reductive deamination were observed for serine, threonine and aspartic acid. Decarboxylation resulted in the formation of amines which represented abundant products released from tyrosine, cysteine and methionine. Cyclic compounds arising from the condensation of two amino acids were revealed as characteristic products of glycine, alanine, serine, proline and hydroxyproline. Degradation products of the side chain were released at relatively high levels from tryptophane, tyrosine and hystidine. Since each amino acid produced a characteristic distribution of TMS products, in-situ pyrolysis/silylation with HMDS may find application as a screening technique for the detection of amino acids and related materials in complex matrices. The potentiality of the procedure was tested on a dipeptide (Tyr–Leu). 

*Keywords:* Pyrolysis; Amino acids; Hexamethyldisilazane

## 1. Introduction

Analytical pyrolysis is widely utilised for the chemical characterisation of complex organic materials of natural origin [1]. Information on structural components of the analysed material is inferred from the occurrence of diagnostic compounds (markers) in the pyrolysate. The study of the thermal behaviour of low-molecular-mass model compounds is relevant for the identification of useful markers for analytical purposes and the elucidation of the structural relationships existing between the precursor and the evolved pyrolysis products.

α-Amino acids are important constitutive units or starting components of a variety of organic macromolecules, such as proteins, melanines, humic substances. The pyrolytic behaviour of common amino acids has been investigated in detail and the principal thermal degradation products have been identified by means of pyrolysis in combination with gas chromatography and mass spectrometry (Py–GC–MS) [2–5]. However, the analytical application of simple

*Corresponding author. Tel.: +39-51-2099-533; fax: +39-51-2099-456.

*E-mail address:* chiavari@ciam.unibo.it (G. Chiavari).

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pyrolysis is limited by the fact that potentially diagnostic fragments bearing polar functional groups (e.g., COOH, NH, OH) could escape detection because of thermal instability, high retention and low volatility. The GC–MS analysis of polar compounds is improved by the use of proper derivatising reagents mixed with the sample prior to pyrolysis (reactive pyrolysis). Several methylating agents have been used in reactive pyrolysis [6], among them tetramethylammonium hydroxide (TMAH) [7–10], tetramethylammonium acetate (TMAAc) [11] and trimethylsulfonium hydroxide [12]. TMAH has the benefit to associate the methylating properties of the tetramethylammonium cation with the hydrolytic activity of the hydroxide ion. The behaviour of amino acids when pyrolysed in the presence of TMAH has been recently investigated [8] and applications for the study of complex organic materials, such as bacteria [9] and humic substances [10], have been described.

Recent studies have shown that pyrolysis in combination with silylating reagents is a promising technique for the analysis of complex organic materials, such as lignins [13] and lipids [14]. It has been shown that pyrolysis of triglicerydes mixed with hexamethyldisilazane (HMDS) results in the formation of trimethylsilyl (TMS) esters of fatty acids whose distribution is informative for the identification of binders in painting layers [14]. It is expected that other classes of organic compounds might give silylated diagnostic products when pyrolysed with HMDS. Such a possibility is investigated in this work for amino acids.

## 2. Experimental

Amino acids (racemic modifications) pure HMDS and the dipeptide Tyr–Leu (Tyr is the amino terminal) were from Aldrich.

Pyrolysis experiments were carried out at 600°C for 10 s at the maximum heating rate with a CDS Pyroprobe 1000 heated filament pyrolyser (Chemical Data System, Oxford, USA) coupled to a Varian 3400 gas chromatograph and a Saturn II ion trap mass spectrometer (Varian Analytical Instruments, Walnut Creek, CA, USA).

A Supelco SPB5 capillary column (30 m×0.32 mm I.D., 0.25 μm film thickness) was used with a temperature programme from 50°C (held for 10 min) to 300°C (held for 5 min) at 5°C $min^{-1}$ with helium as carrier gas. Temperatures of split/splitless injector (split mode) and Py–GC interface were kept at 250°C and 150°C, respectively. Mass spectra were recorded at 1 scan $sec^{-1}$ under electron impact at 70 eV, mass range 45 to 300 $m/z$. Samples (less than 0.1 mg) were inserted into a pre-pyrolysed quartz capillary tube and added with 5 μl of HMDS prior to pyrolysis.

## 3. Results and discussion

Table 1 lists GC retention data, mass spectral characteristics and proposed structural assignments for the principal products released from each amino acid following pyrolysis in the presence of HMDS. Typical MS chromatograms are reported in Figs. 1–3 for leucine, serine and tyrosine, respectively. The structure of some of the principal silylated products identified in the chromatograms are presented in Fig. 4. Tentative identification of the compounds was accomplished by comparison with NIST 92 mass spectral data and from the interpretation of the fragmentation pattern of the mass spectrum. The match between experimental and literature mass spectra was complicated by the presence of additional peaks corresponding to adducts whose formation was favoured by the relatively high pressure of the ion trap MS. For instance, the experimental mass spectrum of the principal pyrolysis/silylation product of aspartic acid, that is the diTMS ester of butenedioic acid, exhibited two peaks at $m/z$ 333 and 407 which were attributed to molecule–ion adducts between the parent compound ($M_r$ 260) and the intense fragment ions at $m/z$ 73 and 147. The $[M+1]^+$ ion associated with the protonated molecule was often present as an intense signal in the mass spectrum registered in the correspondence of the maximum of chromatographic peak of several compounds.

The chromatograms of all the analysed amino acids were characterised by the presence of a peak eluting at 11.6 min (not reported in Table 1) whose mass spectrum contained ions at $m/z$ 73, 117, 147

Table 1
Principal products released from pyrolysis of amino acids in the presence of HMDS

| Amino acid[a] | Peak number[b] | Ions ($m/z$)[c] | Assigned structure ($M_r$) |
|---|---|---|---|
| Ala | **2** | 75, 116, 162, 190, **234** | Alanine, TMS ester (161) |
| | 7 | **70**, 100, 130, 172, 188, 260 | – |
| | 11 | 73, **116**, 147, 190, 218 | Alanine, diTMS (233) |
| | 35 | 73, 195, 210, **269**, 284 | 2,5-diTMSoxy-3,6-dimethylpyrazine (284) |
| Arg | **4** | 45, 73, 100, **171**, 189 | Silylated product |
| | 23 | 56, **69**, 115, 171 | 3-Amino-2-piperidone (114) |
| | 51 | 138, **151**, 180 | – |
| Asp | 26 | 73, **147**, 171, 245 | Butenedioic acid (Z), diTMS ester (260) |
| | 28 | 73, **147**, 173, 243 | Butanedioic acid, diTMS ester (262) |
| | **29** | 73, 75, 147, **245** (333, 407) | Butenedioic acid (E), diTMS ester (260) |
| | 43 | **73**, 147, 307, 335 | Aspartic acid, triTMS (349) |
| Cys | **31** | 75, 85, **102**, 158, 176, 204 (220) | 3-Thioetheneamine, diTMS (219) |
| Gly | **1** | **73**, 75, 114, 147, 176 | Glycine, TMS ester (147) |
| | 12 | **73**, 102, 147, 176, 204 | Glycine, diTMS (219) |
| | 33 | 73, 167, 182, **241**, 256 | 2,5-diTMSoxypyrazine (256) |
| | 34 | 73, 75, 196, **255**, 270 | 2,5-diTMSoxy-3-methylpyrazine (270) |
| His | 5 | 67, **68** | Imidazole (68) |
| | **8** | 54, **81**, 82 | 3-Methylpyrazole (82) |
| Hyp | 6 | 68, 73, **116**, 144, 160 | Silylated product |
| | 30 | 53, 67, **80**, 117, 146 | 1,1′-Methylene bis 1H-pyrrole (146) |
| | 41 | **68**, 73, 75, 158, 269 | TMSoxy, TMS proline (275) |
| | **55** | 65, 93, 103, 130, **186** | Diketodipirrole (186) |
| | 62 | 73, 158, 212, 356, **370** | Silylated 2,5-diketopiperazine derivative |
| | 63 | 73, 158, 212, 356, **370** | (5H,10H-dipyrrole[1,2,a:1′,2′-d]pyrazine-5,10-dione octahydro-2,7-diTMSoxy) (370) |
| Ile | 17 | 69, **89**, 204 | Isoleucine, TMS ester (203) |
| | 25 | 73, **158**, 218, 232, 260 | Isoleucine, diTMS ester (275) |
| Leu | **15** | 73, 75, **86**, 170, 188 | Leucine, TMS ester (203) |
| | 21 | 73, 102, **158**, 232, 260 | Leucine, diTMS (275) |
| Met | 3 | 58, 89, 105, 106,106 144, 162 | 3-Methylthiopropylamine (105) |
| | **38** | 56, 61, 73, 104, 178, **221** | Methionine, TMS ester (221) |
| | 44 | 73, 128, **176**, 250, 293 | Methionine, diTMS (293) |
| Nor | **18** | 73, 75, **86**, 170, 188 | Norleucine, TMS ester (203) |
| | 27 | 45, 73, **158**, 232, 260 | Norlecine, diTMS (275) |
| Phe | 46 | **91**, 182 | Bibenzyl (1,2-diphenylethane) (182) |
| | **50** | 73, 77, 91, 120, 146 (238) | Phenylalanine, TMS ester |
| | 53 | 73, 147, 192, 218, 266 | Phenylalanine, diTMS |
| | 57 | 73, 332, **346** | Silylated product |
| Pro | **58** | 70, 110, 138, 166, **194** (235) | 2,5-Diketopiperazine derivative (194); (5H,10H-dipyrrole[1,2,a:1′,2′-d]pyrazine-5,10-dione octahydro) |

Table 1. Continued

| Amino acid[a] | Peak number[b] | Ions ($m/z$)[c] | Assigned structure ($M_r$) |
|---|---|---|---|
| Ser | 14 | 73, **147**, 177, 219 | 3-TMSoxypropanoic acid, TMS ester (234) |
| | **36** | 73, 195, 210, **169**, 284 | 2,5-diTMSoxy-3,6-dimethylpyrazine (284) |
| | 49 | 73, 101, 116, **167**, 211, 299 (327, 393) | Silylated product |
| Thr | 16 | 73, **147**, 191, 233 | 3-TMSoxybutanoic acid, TMS ester (248) |
| | **24** | **73**, 117, 130, 147, 219 | Threonine, diTMS (263) |
| | 32 | 68, 109, **123**, 137, 152 | – |
| | 54 | **73**, 115, 130, 195, 253 | Silylated product |
| Trp | 22 | 63, 89, 117 | Indole (117) |
| | **37** | 77, 130, 131 | Methylindole (131) |
| | 40 | 73, **174**, 189 | *N*-TMS indole (189) |
| | 47 | 73, 139, 188, **203** | *N*-TMS, 3-methylindole (203) |
| | 56 | 77, 103, **130**, 160 | 2-(3-Indole)ethylamine (160) |
| Tyr | 9 | 73, 77, 95, **151**, 166 | Phenol, TMS ether (166) |
| | 13 | 73, 91, 135, **165**, 180 (345) | 4-Methylphenol, TMS ether (180) |
| | 20 | 73, 91, 151, **177**, 192 | 4-Ethenylphenol, TMS ether (192) |
| | 39 | 51, 77, **108**, (138) | 2-(4-Hydroxyphenyl)ethylamine (137) |
| | **42** | 45, 73, 165, **180**, 193 | 2-(4-TMSoxyphenyl)ethylamine (209) |
| Val | **10** | 55, 72, 146, 156, **190** (244, 345) | Valine, TMS ester (189) |
| | 19 | 45, 73, 100, **144**, 218 | Valine, diTMS (261) |

[a] Abbreviated name of the amino acid (listed in alphabetical order).
[b] Peaks numbered with increasing retention times; values in bold indicate the most abundant peak in the chromatogram.
[c] Values in bold indicate the base peak. Values in parentheses are attributed to adducts.

(base peak), 191 and 219 (plus ions at $m/z$ 307 and 381 probably corresponding to adducts of the molecule ($M_r$ 234) with the ions 73 and 147, respectively). Although it was identified as TMSoxy propanoic acid TMS ester by the library with a good fit (80% purity, 92% fit), it was believed to be a condensation product of HMDS ($C_8H_{26}N_2Si_3$) on the ground of its ubiquity. In fact, it was also detected as a principal pyrolysis/silylation product of lipids [14].

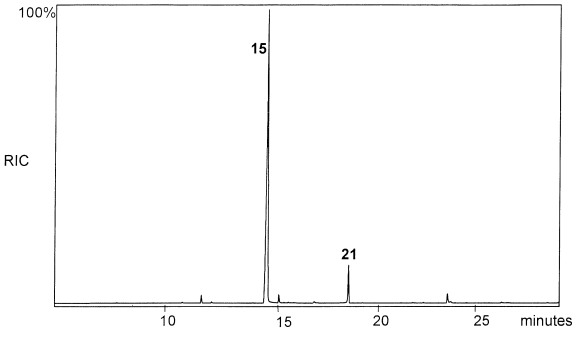


Fig. 1. Reconstructed ion chromatogram (RIC) obtained from the pyrolysis of leucine in the presence of HMDS. Peak numbers refer to Table 1.

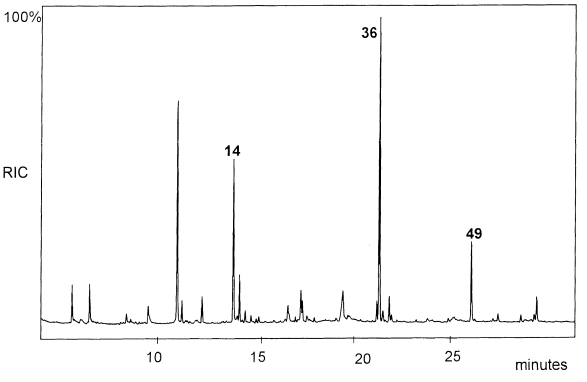


Fig. 2. Reconstructed ion chromatogram (RIC) obtained from the pyrolysis of serine in the presence of HMDS. Peak numbers refer to Table 1.

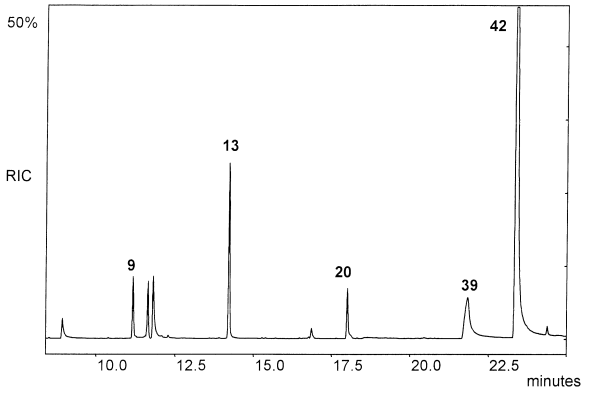


Fig. 3. Reconstructed ion chromatogram (RIC) obtained from the pyrolysis of tyrosine in the presence of HMDS. Peak numbers refer to Table 1.

### *3.1. Silylated amino acids and condensation products*

Several amino acids, in particular those with an alkyl side chain, produced the corresponding TMS derivatives upon pyrolysis with HMDS. The principal product was generally the TMS ester.

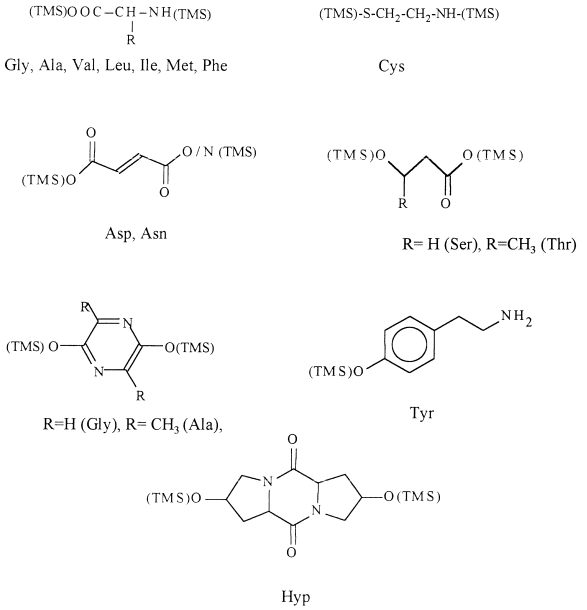


Fig. 4. Characteristic pyrolysis/silylation products of α-amino acids.

Besides silylated intact amino acids, the occurrence of cyclic dimers was observed for simple amino acids with no-bulky side chains. Diketopiperazines (DKPs) are known to be important thermal condensation products of amino acids and related compounds, which are formed from the attack of the amino group to the carbonyl carbon of an other amino acid followed by cyclisation [2,3]. The formation of DKPs is favoured by the presence of TMAH and in this case methylated DKPs are produced [8]. Proline and hydroxyproline, in which the nitrogen is part of a pyrrolidine ring, form very stable cyclic structures, and their corresponding DKPs have been observed as principal pyrolysis products [3]. The 2,5-diketopiperazine derivative of proline was also observed as the dominant peak in the pyrolysis of proline with HMDS. This compound cannot be found as a TMS derivative lacking groups containing active hydrogens in its structure capable to be silylated. On the other hand, the DKP of hydroxyproline contains two hydroxy groups which can be silylated and, as expected, pyrolysis/silylation of hydroxyproline produced the corresponding TMSoxy DKPs (Fig. 4). The existence of two peaks displaying the same mass spectra was explained by the occurrence of two diastereomers differing for the relative position of the hydroxy groups.

Glycine and alanine gave rise to TMS forms of condensation products when pyrolysed with HMDS, their mass spectra, however, were not consistent with the corresponding silylated DKPs. The $M_r$ of products was two mass units less than the corresponding DKP derivatives, and were tentatively identified as pyrazine derivatives (Fig. 5). Pyrazines are known to be formed from self-condensation of α-aminocarbonylic compounds, with dehydration (loss of two water molecules) and oxidation (formal loss of $H_2$). When pyrolysed with HMDS, glycine and alanine yielded 2,5-TMSoxypyrazine and 3,6-dimethyl-2,5-TMSoxypyrazine, respectively. Serine also gave 3,6-

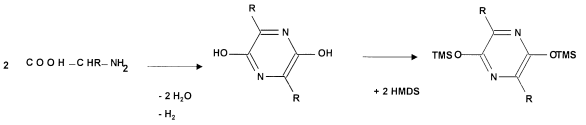


Fig. 5. Formation of pyrazine derivatives.

dimethyl-2,5-TMSoxypyrazine, probably as a consequence of the loss of the hydroxy group from the side chain.

### 3.2. Decarboxylation and deamination products

*N*-TMS amines formally derived from the loss of $CO_2$ were found to be important pyrolysis/silylation products of some amino acids. Silylated 2-(4-hydroxyphenyl)ethylamine was the principal product released from tyrosine. Notably, (4-hydroxyphenyl)ethylamine has been reported to be a major pyrolysis products of tyrosine [3]. Decarboxylation seems to be an important process also for sulfur-containing amino acids, cysteine and methionine.

Reductive deamination (formal loss of the NH group) or ammonia elimination afforded carboxylic acids found as TMS esters. Pyrolysis/silylation of aspartic acid took place with the formation of the TMS esters of butanedioic acid, *cis*-butenedioic acid (maleic acid) and *trans*-butenedioic acid (fumaric acid). Interestingly, the diTMS ester of fumaric acid was the most abundant pyrolysis/silylation product of aspartic acid. This could be due to the relative acidity of the hydrogen in α to the carboxy group of the side chain which favours the elimination of $NH_3$. Serine produced preferentially the saturated carboxylic acid formally arising from reductive deamination, that is the TMS ester/ether of 3-hydroxypropanoic acid. Only low levels of the TMS ester of benzenepropanoic acid were observed in the chromatogram obtained from pyrolysis/silylation of phenylalanine.

### 3.3. Side chain products

Degradation products lacking both the original amino and carboxy group while preserving the side chain moiety were observed for aromatic amino acids. GC–MS traces obtained from pyrolysis of tyrosine with HMDS exhibited several phenolic products. Phenol and 4-methylphenol were also observed in normal pyrolysis, in addition pyrolysis/silylation yielded the TMS ethers of 4-vinylphenol, 4-methylphenol and phenol. Phenylalanine afforded bibenzyl (1,2-diphenylethane) a dimerisation product also observed in conventional pyrolysis. GC traces of tryptophane were dominated by indoles, with low levels of indole TMS derivatives.

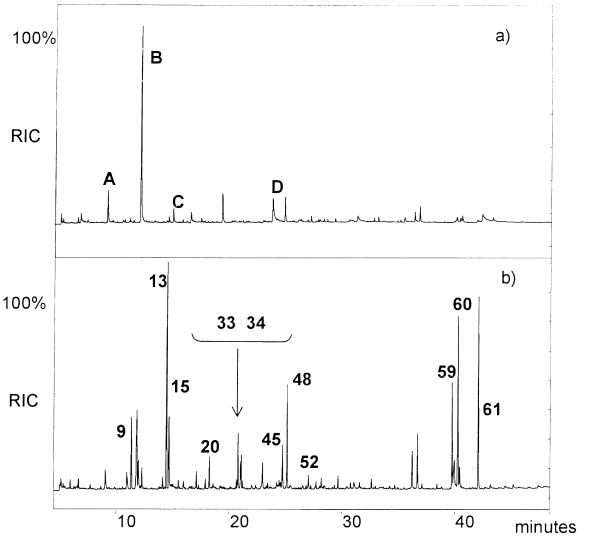


Fig. 6. Reconstructed ion chromatogram (RIC) obtained from the pyrolysis of the dipeptide Tyr–Leu (a) without HMDS (A= phenol, B=*p*-cresol, C=4-ethylphenol, D=4-hydroxybenzeneacetonitrile and (b) with HMDS. Peak numbers refer to Tables 1 and 2.

### 3.4. Analysis of dipeptide

Fig. 6 reports the GC–MS traces obtained from the pyrolysis of Tyr–Leu (a) without and (b) with HMDS. The products obtained from the pyrolysis/silylation are listed in Table 2. In conventional pyrolysis only products arising from the aromatic amino acid tyrosine were detected (phenol and its derivatives), while products indicative of the aliphatic amino acid were lacking. Conversely, a leucine marker (the TMS ester of leucine) was clearly detected when pyrolysis was performed with HMDS. Moreover the presence of leucine is confirmed by peak number 48 tentatively identified as the pyrazine derivative with an isobutyl substituent.

The occurrence of the tyrosine residue in the sample was evidenced by a series of TMS derivatives of phenol, most of them already observed from the analysis of the pure amino acid (Table 1).

## 4. Conclusions

The results of this study show that HMDS exhibits valuable characteristics as in-situ derivatisation re-

Table 2
Principal products released from pyrolysis of the dipeptide Tyr–Leu with HMDS

| Peak number | Ions ($m/z$) | Assigned structure ($M_r$) |
|---|---|---|
| 9 | 73, 77, 95, **151**, 166 | Phenol, TMS ether (166) |
| **13** | 73, 91, 135, **165**, 180 | 4-Methylphenol, TMS ether (180) |
| 15 | 73, 75, **86**, 170, 188 | Leucine, TMS ester (203) |
| 20 | 73, 91, 151, **177**, 192 | 4-Ethenylphenol, TMS ether (192) |
| 33 | 73, 167, 182, **241**, 256 | 2,5-DiTMSoxypyrazine (256) |
| 34 | 73, 75, 196, **255**, 270 | 2,5-DiTMSoxy-3-methylpyrazine (270) |
| 45 | 116, **190**, 205 | (4-TMSoxyphenyl)acetonitrile(205) |
| 48 | **270**, 297, 312 | 2,5-DiTMSoxy-3-isobutylpyrazine (312) |
| 52 | 73, **179**, 219 | Tyrosine *O*-TMS-, TMS ester (325) |
| 59 | **73**, 433, 448 | Silylated compound |
| 60 | 73, **179**, 358 | Silylated compound |
| 61 | **73**, 448, 476, 490 | Silylated compound |

agent for analytical pyrolysis of very polar compounds, such as amino acids. When pyrolysed with HMDS, each amino acid yielded distinctive silylated products. A selection of the principal pyrolysis/silylation products characteristic of most of the amino acids investigated in this study is presented in Fig. 4. The effectiveness of these products as markers for the detection of amino acid derivatives was preliminary tested by the analysis of a dipeptide containing an aromatic (Tyr) and an aliphatic residue (Leu). Markers of both amino acids were detected by pyrolysis/silylation, while the occurrence of the aliphatic residue was not evidenced by conventional pyrolysis.

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