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Mass Spectrometric Investigation of Some α-Trifluoromethyl-α-amino Acids

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The fast atom bombardment-induced mass spectrometric behaviour of four fluorinated α-amino acids was studied in detail with the aid of metastable ion studies and accurate mass measurements. Comparison with the behaviour of unfluorinated analogues suggests that the presence of fluorine makes the carbonylic oxygen the most prone to protonation. This hypothesis was confirmed by AM1 semiempirical calculations which indicate that the carbonylic oxygen of the carboxyl group exhibits, in the case of fluorinated compounds only, the highest proton affinity.

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INTRODUCTION

Fluorinated analogues of naturally occurring, biologically active compounds often exhibit unique physiological properties.[1] A number of fluorine-containing amino acids have been synthesized and studied as potential therapeutic agents.[2] Among them, α-mono, di- and trifluoromethylamino acids have been found to display enzyme-inactivating features, acting as inhibitors of the parent amino acid decarboxylases and racemases (which are both pyridoxal phosphate-dependent enzymes). They are extremely selective and have great practical value also in elucidating physiological roles of specific enzymes.[3] α-Trifluoromethylamino acids have also been synthesized and incorporated into small peptides. The polarization effect of fluorine exerts a great influence on functional groups situated nearby. The α-trifluoromethyl group decreases the basicity of the amino group by about four pK_b units, so that activation of the amino group for peptide bond formation is very difficult to achieve. A much smaller effect is exerted on the pK_a of the carboxylic acid (1.1–0.6 units).[4]

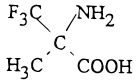
Pursuing our interest in the mass spectrometric (MS) behaviour of fluorine-containing compounds,[5–11] it was of interest to study the protonation of three α-trifluoromethyl-α-amino acids (**1–3**) and α-difluoromethylalanine (**4**) (all recently synthesized in enantiomerically pure form by using the fluorinated sulphinyl chiron route[12]), and to compare their behaviour with that of the corresponding α-methyl-α-amino acids (**5–7**), as obtained by fast atom bombardment (FAB) experiments.

EXPERIMENTAL

Compounds **1–4** were synthesized according to the literature.[12] Compounds **5** and **7** were purchased from Aldrich (Milan, Italy) and **6** from Sigma (Milan, Italy).

All mass spectrometric measurements were performed on a VG ZAB 2F mass spectrometer (VG, Altrincham, UK) operating under positive ion FAB conditions (8 keV xenon atoms bombarding glycerol solutions of the samples).

Metastable ion studies were performed by means of mass-analysed ion kinetic energy (MIKE) spectrometry.[13]



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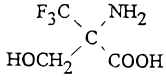

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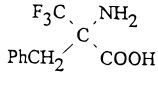

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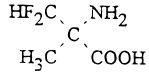

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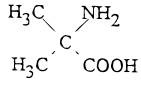

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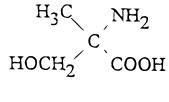

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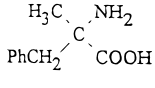

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Accurate mass measurements were achieved by the peak matching technique at 5000 resolving power (10% valley definition).

AM1 calculations were performed by means of the Hyperchem software package.

RESULTS AND DISCUSSION

The FAB mass spectra of **1–7**, obtained by dissolving the samples in glycerol at comparable concentrations, are reported in Table 1.

It must be emphasized that, contrary to what is usually reported for FABMS of α-amino acids,[14] in the present case hydrochloric acid was not added to the glycerol solutions of the samples. Therefore, cations of type **a**:

$$H_3\overset{+}{N}\text{—CHR—COO}^- \xrightarrow[\text{solution}]{\text{HCl}} H_3\overset{+}{N}\text{—CHR—COOH}$$

a

are not preformed in solution, whereas their formation can occur in the gas phase following the FAB mechanism suggested by Hiraoka and Kebarle.[15] This hypothesis is advanced on the basis of the following considerations:

i. The dielectric constant of glycerol ($\varepsilon = 46.5$) is lower than that of water ($\varepsilon = 78.8$). From the qualitative point of view, this value indicates a ΔpK_a value (defined as $\Delta pK_a = pK_{a\,(water)} - pK_{a\,(glycerol)}$) in the range from -3.0 to -4.0. Hence the non-ionized form of the amino acids will be favoured in glycerol solutions.[16]

ii. Glycine, known to exhibit zwitterions in the aqueous and solid phase, has been proved to exist as a non-ionic molecule in the gas phase.[17]

iii. The gas-phase acidity of haloacetic acids is F < Cl < Br, the opposite of what is observed in aqueous solution.

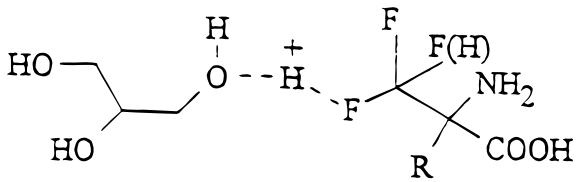
From the above considerations, it can be hypothesized that the compounds under study are represented in the gas phase by a population of species, among which the non-ionic ones represent a substantial share.

It is interesting that all the fluorinated compounds **1–4** exhibit abundant ions due to clustering of the molecules with protonated glycerol. The same ionic species is completely absent in the case of the unfluorinated analogues **5–7**. Furthermore, the relative abundance of the $[M + \text{glycerol} + H]^+$ complex is higher in the case of **1** (51%) and decreases from **2** to **4**. This behaviour could be explained by the formation of a 'proton-bound dimer'[18] in which the proton is shared by a hydroxyl group of glycerol and a fluorine atom (see structure **k**).

The lower abundances of this ion observed for **2–4** could be explained by (i) the presence of the CH_2OH substituent for **2** which could partially deactivate the above-reported effect of the α-trifluoromethyl group on functional groups situated nearby; (ii) steric hindrance for **3**; and (iii) the lower electronegativity of the CHF_2 group for **4**.

The FAB mass spectrum of α-trifluoromethylalanine (compound **1**) shows only a few fragment ions at *m/z* 112, 111 and 46, whereas in the MIKE spectrum of MH^+ only the first two ionic species are detected. The primary loss of NH_3, typical of the mass spectrometric behaviour of α-amino acids under FAB ionization conditions,[14] is in this case completely suppressed. It is interesting that the [111]/[112] abundance ratio is 0.45 under FAB conditions and ~1 in the MIKE spectrum (see Table 2), indicating that the formation of ions at *m/z* 112 is favoured from ions at higher internal energy content. Accurate mass measurements performed on these two ions gave values of 112.0358 (±0.005) and 111.0288 (±0.005), in agreement with the elemental formulae $C_3H_5NF_3$ (calculated 112.0373) and $C_3H_4NF_3$ (calculated 111.0295), respectively. They correspond to losses, from the protonated molecule, of CH_2O_2 and $CH_3O_2^{\cdot}$, respectively.

The behaviour, under the same experimental conditions, of the non-fluorinated analogue, i.e. α-methylalanine (**5**), is very different. In fact, as shown by the data reported in Table 1, primary losses of $CHO_2^{\cdot}$ and CH_2O_2 occur, giving rise to fragment ions at *m/z*



k

Table 1. Positive-ion FAB mass spectra of compounds 1–7 (*m/z* with relative abundances (%) in parentheses)

Ionic species	1	2	3	4	5	6	7
$[M + \text{glycerol} + H]^+$	251 (51)	267 (21)	327 (10)	233 (10)			
$[MH]^+$	158 (100)	174 (100)	234 (100)	140 (100)	104 (100)	120 (90)	180 (100)
$[MH - NH_3]^+$							163 (2)
$[MH - H_2O]^+$		156 (9)				102 (5)	135 (9)
$[MH - CH_2OH]^+$		142 (1)					
$[MH - 2H_2O]^+$		138 (3)					134 (38)
$[MH - CHO_2]^{+\cdot}$		129 (3)			59 (8)	75 (100)	
$[MH - CH_2O_2]^+$	112 (100)	128 (25)		94 (48)	58 (20)	74 (15)	
$[MH - CH_3O_2]^{+\cdot}$	111 (45)	127 (2)	187 (65)	93 (18)			
$[MH - CH_2OH - F]^+$		123 (2)					
$[MH - CHO_2 - HF]^{+\cdot}$		109 (7)					

Table 2. MIKE spectra of FAB-generated MH^+ of compounds 1–7 (m/z with absolute abundances (%) in parentheses)

Ionic species	1	2	3	4	5	6	7
$[MH - NH_3]^+$							163 (27)
$[MH - H_2O]^+$		156 (46)	216 (11)			102 (23)	
$[MH - CH_2OH]^+$		142 (1)				88 (3)	135 (36)
$[MH - 2H_2O]^+$		138 (2)				84 (5)	134 (37)
$[MH - CHO_2]^{+\cdot}$		129 (15)			59 (42)	75 (27)	
$[MH - CH_2O_2]^+$	112 (51)	128 (17)		94 (55)	58 (58)	74 (29)	
$[MH - CH_3O_2]^{+\cdot}$	111 (49)	127 (15)	187 (89)	93 (45)			
$[MH - CH_2OH - F]^+$		123 (1)					
$[MH - CHO_2 - HF]^{+\cdot}$		109 (3)					
						57 (7)	
						42 (3)	
						28 (3)	

59 and 58, of the same abundance in the MIKE spectrum. Further fragment ions are detected at m/z 45 ($COOH^+$) and 87 ($[MH - NH_3]^+$).

These differences could in principle be well explained by the change in total charge density, due to the presence of the CF_3 group in **1**, and by the consequent changes in the relative basicity of the different groups present in the molecules. Such changes would reflect on the activation of different protonation sites. In order to verify this aspect, we first performed AM1 calculations on the neutral molecules of the unfluorinated compound **5** and of the fluorinated analogue **1**. Whereas in **5** the highest charge density is on the nitrogen atom, the presence of a CF_3 group leads to the highest charge density on the carbonyl oxygen of **1**, as evidenced in Fig. 1, in which the charge distribution is presented on the main molecular plane. In order to obtain evidence more than 'pictorial,' the heats of formation of neutral molecules and of the even-electron cations originating from protonation on the nitrogen atom, the carbonyl oxygen and the hydroxyl oxygen were calculated for both **1** and **5**. From these data, reported in Fig. 2, it follows that for **1** the site exhibiting the highest proton affinity (**PA**) is the oxygen of the carboxylic group ($PA = 226.5$ kcal mol^{-1} (1 kcal = 4.184 kJ)), whereas for the unfluorinated compound **5** it is the amino group ($PA = 212.72$ kcal mol^{-1}).

Consequently, for the protonated molecule of **1**, the structure reported in Scheme 1, in which the protonation has occurred on the carbonyl oxygen, can be proposed. From this structure, CH_2O_2 loss originates from the heterolytic cleavage of the C—COOH bond whereas the loss of $CH_3O_2^{\cdot}$ involves an H rearrangement which leads to ion **a** reported in Scheme 1. However, for such ionic species, structure **b** cannot be excluded.

The same two processes seem to take place in the case of the protonated molecule of α-methylalanine, in which, on the basis of PA data, the protonation has reasonably occurred on the amino group. They originate from C—COOH bond cleavage with and without H rearrangement, but in this case they consist of CH_2O_2 and $CO_2H^{\cdot}$ losses.

Compound **4**, bearing a CF_2H group, should in principle exhibit a behaviour intermediate with respect to those of **1** and **5**. Both FAB and MIKE experiments

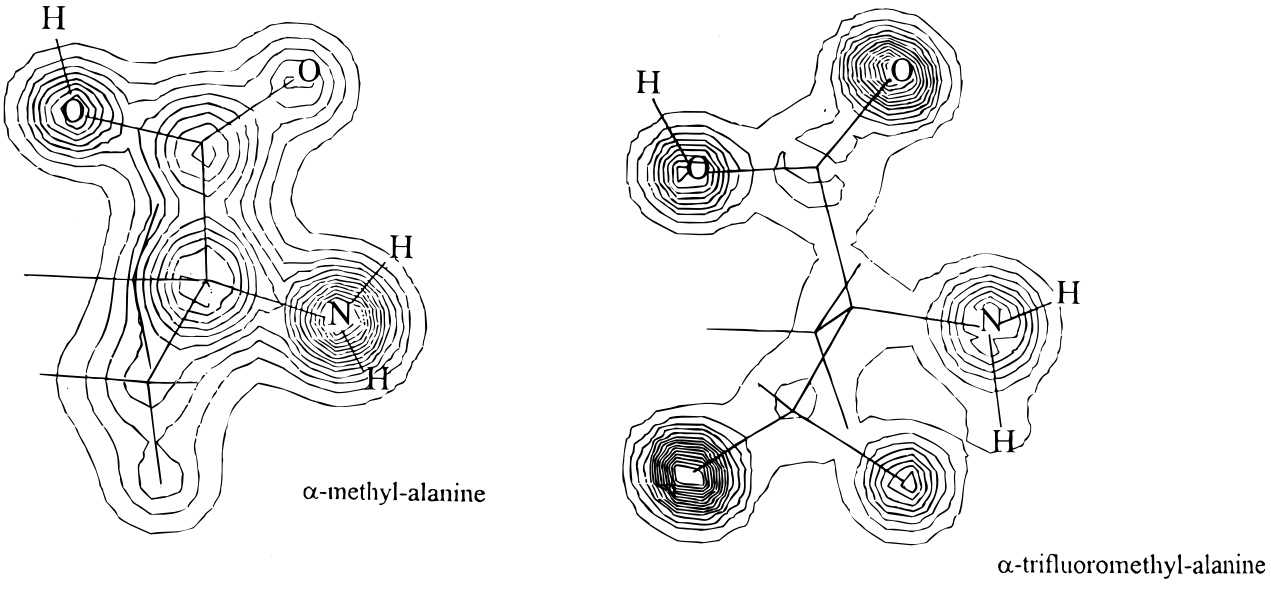


Figure 1. Total charge density distribution of compounds **1** and **4**, as obtained by AM1 calculations using Hyperchem software.

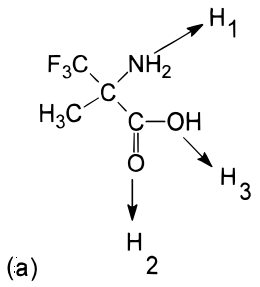


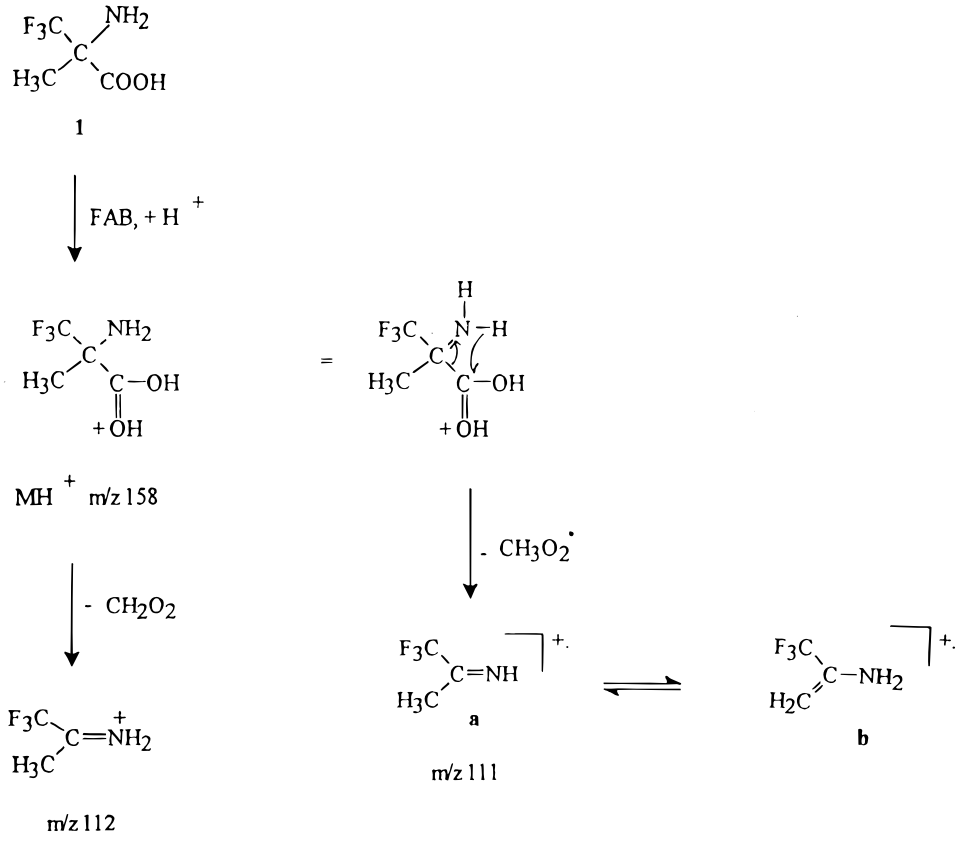
Figure 2. *PA* values calculated for the different functional groups of compounds (a) **1** and (b) **4**.

show that it behaves analogously to **1**: also in this case, primary losses of CH_2O_2 and $CH_3O_2^{\cdot}$ are observed from MH^+, leading to ions at *m/z* 94 and 93, suggesting that protonation has occurred on the carbonyl oxygen. It is interesting that losses of both radicals and neutral species related to the carboxyl group are observed, even though the even-electron rule[19] would indicate the former as being highly unfavoured.

A further interesting point is evidenced by the kinetic energy values relating to the $CHO_2^{\cdot}$, CH_2O_2 and $CH_3O_2^{\cdot}$ losses observed in the MIKE spectra of **1**, **4** and **5**, summarized in Table 3. It is worth noting that for all the compounds the decomposition process related to the highest $T_{1/2}$ values is that due to the loss of neutral CH_2O_2 species, to which the structure of formic acid can be reasonably assigned. Whereas for **1** and **4** the

Table 3. Kinetic energy release values (meV) related to the losses of $CHO_2^{\cdot}$, CH_2O_2 and $CH_3O_2^{\cdot}$ from the protonated molecules of 1, 4 and 5

Process	$T_{1/2}$ (meV) 1	4	5
$MH - CO_2H^{\cdot}$	—	—	148
$MH - CH_2O_2$	390	354	769
$MH - CH_3O_2^{\cdot}$	85	116	—



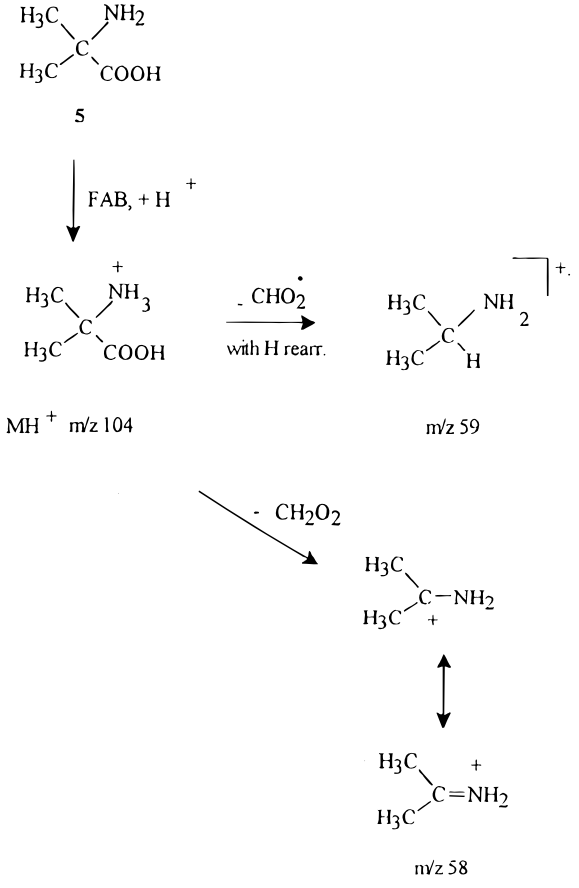
Scheme 1.

$T_{1/2}$ values related to this process are very similar, in the case of the unfluorinated compound it is about doubled.

The high $T_{1/2}$ values could be related either to high activation energies of the decomposition reactions or to the high stability of product ions. In our opinion, the latter appears to be the most reasonable hypothesis for the highly stable reaction products (formic acid and quaternary nitrogen cation), in agreement with the even-electron rule. The highest $T_{1/2}$ values observed for **5** could be justified either by the requirement for a higher activation energy or, more probably, to the highest stability of the isobutyliminium cation with respect to $CH_3(CF_3)C{=}NH_2^+$. The lower $T_{1/2}$ value (148 meV) associated with the loss of $CHO_2^{\cdot}$ from MH^+ of **5** is in agreement with a process requiring a simple bond cleavage.

The low kinetic energy release values related to the loss of $CH_3O_2^{\cdot}$ observed for both **1** and **4** are more unexpected. Even considering the molecular species protonated on the carbonyl oxygen, the $CH_3O_2^{\cdot}$ loss still requires a further rearrangement. The rearranged hydrogen could arise either from CH_3 or NH_2 groups. However, the MIKE spectrum of ions at *m/z* 111, reported in Fig. 3, shows an easy loss of a methyl radical, suggesting that such a group is still present in the product ion, and hence the H rearrangement process involves the NH_2 group.

Compound **2** at first sight behaves similarly to **1**, although some peculiar fragmentation pathways became detectable. As can be seen in Table 1 and Scheme 3, losses of CH_2O_2 and $CH_3O_2^{\cdot}$ are still present, suggesting that also for this compound the



Scheme 2.

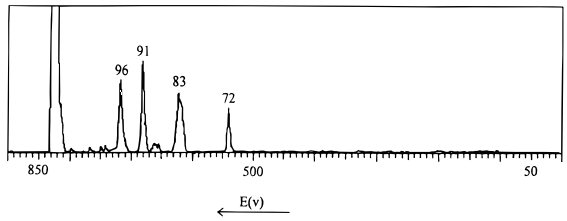
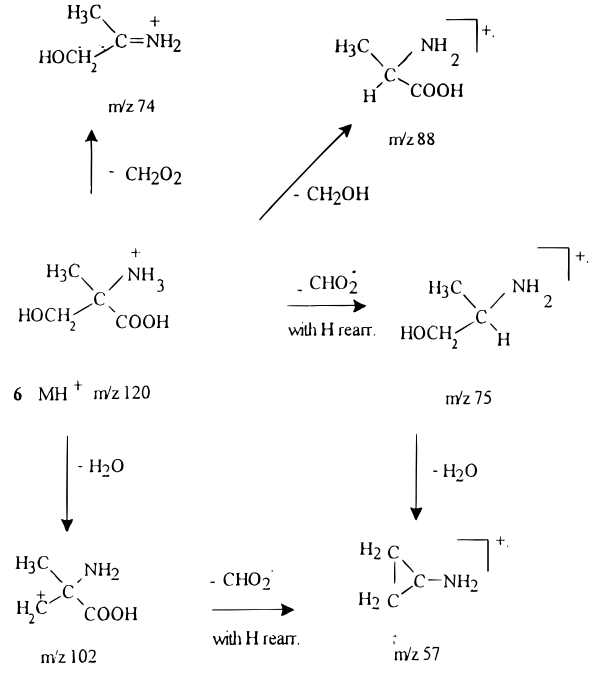


Figure 3. MIKE spectrum of ions at *m/z* 111 generated by positive-ion FAB of **I**.

protonation on the carboxyl group occurs. However, the loss of $CHO_2^{\cdot}$ (observed for the unfluorinated analogue **6**, see Table 1) is also present, suggesting that, in the case of **2** an at least partial protonation on the amino group has taken place. This behaviour can be justified by the presence of the hydroxymethyl group that partially neutralizes the deactivating effect of CF_3 on the nitrogen atom. The other observed primary fragmentation pathways of protonated molecules of **2** are those already observed for **6**. Only two new secondary fragmentation channels are activated, due to HF loss from the $[MH{-}COOH]^+$ species and F loss from the $[MH{-}CH_2OH]^{+\cdot}$ species.

Compound **3**, bearing a benzyl substituent, exhibits a behaviour strongly different from that of **1** and **2**. First, the losses of $CHO_2^{\cdot}$ and $CH_2O_2^{\cdot}$ observed for **1** and **2** are in this case completely absent under either FAB or MIKE conditions. The primary loss of $CH_3O_2^{\cdot}$ is the only one present. This could be due to the presence of the benzyl substituent, which would favour the formation, through $CH_3O_2^{\cdot}$ loss, of bicyclic structure(s). The presence of CF_3 not only inhibits the $CHO_2^{\cdot}$ and CH_2O_2 losses observed for the unfluorinated analogue **7**, but also deactivates the primary NH_3 loss, typical of protonated α-amino acids. The above data, together with the primary water loss, support the contention that for **3** the protonation has occurred on the carboxyl oxygen.



Scheme 3.

In conclusion, the above data, together with AM1 semiempirical calculations, indicate that the substitution of a CH_3 group with a CF_3 group strongly modifies the total electron density of α-amino acid neutral molecules. This reflects a change in proton affinities of the common functional groups present in the fluorinated and unfluorinated molecules, which in turn reflects FAB-activated protonation occurring on different sites.

REFERENCES

1. R. Filler, Y. Kobayashi and L. M. Yagupolskii (Eds), *Biomedical Aspects of Fluorine Chemistry* Elsevier, Amsterdam (1993).
2. V. P. Kukhar and V. A. Soloshonok (Eds), *Fluorine Containing Amino Acids*. Wiley, New York (1994).
3. R. B. Silverman, K. A. Bichler and A. J. Leon, *J. Am. Chem. Soc*. **118**, 1214, 1253 (1996); J. Kollonitsch, in *Biomedicinal Aspects of Fluorine Chemistry*, edited by R. Filler and Y. Kobayashi, p. 93. Kodansha, Tokyo (1982).
4. N. Sewald and K. Burger, in *Fluorine Containing Amino Acids*, edited by V. P. Kukhar and V. A. Soloshonok, p. 139. Wiley, New York (1994).
5. D. Favretto, P. Traldi, P. Bravo and F. Viani, *Rapid Commun. Mass Spectrom*. **5**, 72 (1991).
6. D. Favretto, P. Traldi, E. Celon and G. Resnati, *Org. Mass Spectrom*. **28**, 1179 (1993).
7. D. D. DesMarteau, G. Resnati, D. Favretto and P. Traldi, *Org. Mass Spectrom*. **27**, 204 (1992).
8. S. Catinella, D. Favretto, P. Traldi, P. Bravo and F. Viani, *Org. Mass Spectrom*. **27**, 179 (1992).
9. D. Favretto, M. D'Alpaos, P. Traldi, J. P. Bégué, D. Bonnet-Delpon and M. H. Rock, *Rapid Commun. Mass Spectrom*. **9**, 1376 (1995).
10. D. Favretto, P. Traldi, P. F. Bravo, F. Viani, J. Tamas, G. Czira and A. Somogyi, *Rapid Commun. Mass Spectrom*. **9**, 1127 (1995).
11. D. Favretto, P. Traldi, G. Resnati and V. A. Soloshonok, *J. Fluorine Chem*. **80**, 41 (1996).
12. P. Bravo, S. Capelli, S. V. Meille, F. Viani, M. Zanda, V. P. Kukhar and V. A. Soloshonok, *Tetrahedron: Asymmetry* **5**, 2009 (1994); P. Bravo, F. Viani, M. Zanda and V. A. Soloshonok, *Gazz. Chim. Ital*. **125**, 149 (1995); P. Bravo, F. Viani, M. Zanda, N. Fokina, V. P. Kukhar, V. A. Soloshonok, O. V. Shishkin and Y. T. Struchov, *Gazz. Chim. Ital*. in press.
13. R. G. Cooks, J. H. Beynon, R. M. Caprioli and G. R. Lester, *Metastable Ions*. Elsevier, Amsterdam (1973).
14. W. Kulik and W. Heerma, *Biol. Mass Spectrom*. **15**, 419 (1988).
15. K. Hiraoka and P. Kebarle, *J. Am. Chem. Soc*. **99**, 360 (1977).
16. C. Reichardt, *Solvents and Solvent Effects in Organic Chemistry*. VCH, Weinheim (1988).
17. M. J. Locke and R. T McIver, *J. Am. Chem. Soc*. **105**, 4226 (1983).
18. A. McLuckey, D. Cameron and R. G. Cooks, *J. Am. Chem. Soc*. **103**, 1313 (1981); B. D. Nourse and R. G. Cooks, *Int. J. Mass Spectrom. Ion Processes* **106**, 249 (1991).
19. M. Karni and A. Mandelbaum, *Org. Mass Spectrom*. **15**, 53 (1980).