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MASS-SPECTROMETRIC STUDY OF THE TRANSESTERIFICATION OF O,N^{α}-BIS(TRIFLUOROACETYL) *n*-BUTYL ESTERS OF SOME HYDROXYLATED AMINO ACIDS INTO THEIR O-CARBETHOXY, N^{α}-TRIFLUOROACETYL, *n*-BUTYL ESTER DERIVATIVES DURING THE N^{τ}-CARBETHOXY DERIVATIZATION OF HISTIDINE

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SUMMARY

The transesterification of O-TFA, N^{α}-TFA, *n*-butyl ester derivatives of some hydroxylated amino acids was studied by gas—liquid chromatography and combined gas—liquid chromatography—mass spectrometry. Changes in elution patterns and fragmentation of the two different O-derivatives are discussed.

INTRODUCTION

In 1974, Moodie [1] successfully resolved the problem of the gas—liquid chromatography (GLC) of histidine by derivatizing the N^{τ} [2] of the imidazole ring of histidine with ethoxyformic anhydride (EFA) and according to the same paper the GLC peak of tyrosyl-O,N^{α}-bis(trifluoroacetyl) *n*-butyl ester (O-TFA,TAB) showed a remarkable diminution.

In our laboratory, the same phenomenon was observed not only for tyrosine but also for some other hydroxylated amino acids during the derivatization of histidine with EFA. In this paper we report investigations on these reactions undergone by four hydroxylated amino acids (threonine, serine, hydroxyproline and tyrosine) using GLC and combined gas—liquid chromatography—mass spectrometry (GLC—MS).

EXPERIMENTAL

Preparation of O-trifluoroacetyl, TAB derivatives (O-TFA,TAB)

The derivatization was carried out according to the procedures developed by Gehrke and co-workers [3—5] and adapted in our laboratory [6]. Into a 10-ml PTFE-lined screw-capped tube was delivered 50 μl of a 10 m*M* solution in 0.1 *N* HCl of threonine, serine, hydroxyproline and tyrosine with N^{ϵ}-monomethyllysine (MML) as internal standard. After evaporation to dryness under nitrogen at 70°, 1 ml of anhydrous *n*-butanol (Merck, Darmstadt, G.F.R.) was added, through which a stream of dry hydrogen chloride was bubbled according to the procedure already described [6].

The esterification was performed for 20 min in a sand-bath maintained at 110°. After the mixture was brought to dryness under a nitrogen stream at 70°, 400 μl of a mixture of trifluoroacetic anhydride (Merck) and dichloromethane (Merck) (1:9, v/v) were added and acylation was carried out at 110° for 60 min after tightening the screw-cap securely.

Preparation of O-carbethoxy, TAB derivatives (O-CEO,TAB)

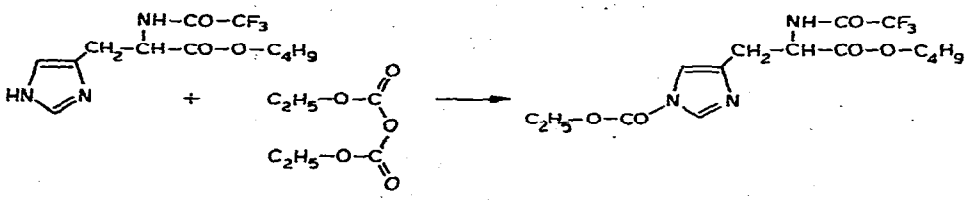
EFA derivatization was then performed immediately. The O-TFA,TAB derivatives obtained were dried under a nitrogen stream at room temperature, showing a drop of temperature due to the volatility of the solution. Then 400 μl of a mixture of EFA (Bayer, Leverkusen, G.F.R.) and dichloromethane (1:500, v/v) was added to the tube, which, after tightening the screw-cap, was heated at 125° for 60 min.

GLC and GLC—MS analysis

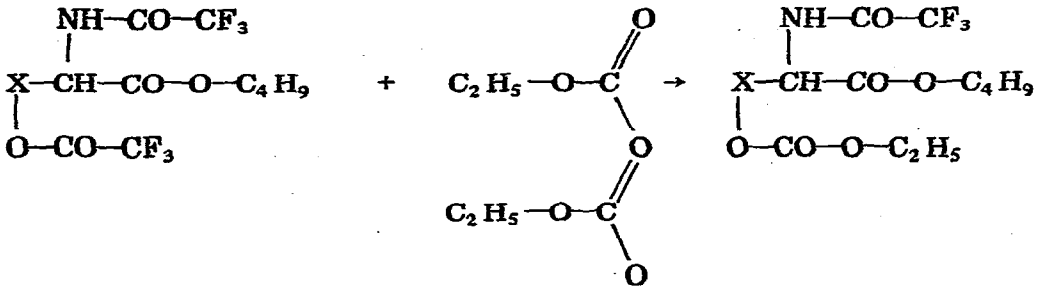
Gas-phase analysis of all derivatives was performed immediately. GLC analyses were carried out with a Packard model 419 gas chromatograph equipped with flame ionization detectors on a 3 m × 3 mm I.D. glass column packed with 1% OV-17 impregnated on Supelcoport. Flow-rates of hydrogen, air and nitrogen were 25, 250 and 30 ml/min, respectively. Temperatures were 205° at the injection port and 225° at the detector. The column temperature was initially held at 85° for 5 min and programmed at a rate of 2°/min. GLC—MS analysis was carried out with an LKB model 9000 gas chromatograph—mass spectrometer. Helium was used as the carrier gas and all the mass spectra were obtained at 28 eV. Other operating parameters were: injection port at 210°, molecular separator at 280°, ion source at 290°, accelerating voltage 3.5 kV, and trap current 60 μA. Mass spectra were recorded by an oscillograph recorder. L-amino acids or DL-amino acids were purchased from Calbiochem and used as received.

RESULTS AND DISCUSSION

In the case of TAB, histidine the underivatized form of imidazole NH is preponderant [1], therefore the reaction with EFA may written as

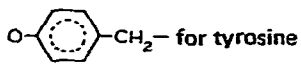


Therefore, the transesterification of O-TFA,TAB to O-CEO,TAB should correspond to the reaction scheme



O—TFA,TAB EFA O—CEO,TAB

where X— is CH_2— for serine, CH_3—CH— for threonine and



For hydroxyproline, the transesterification occurs in the same way.

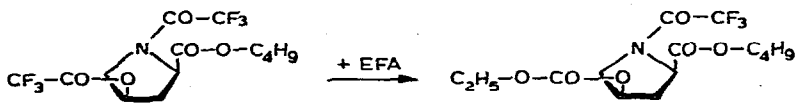


Fig. 1a shows the elution pattern of all amino acids as TAB derivatives when histidine was not eluted. Fig. 1b shows the elution of the same TAB derivatives after reaction with the EFA reagent. Four new peaks appeared (A, B, C, D) together with histidine. These peaks have been identified by mass spectrometry as O-CEO,TAB derivatives of, respectively, threonine, serine, hydroxyproline and tyrosine. When EFA derivatization of amino-acid TAB derivatives was performed with old reagent, the histidine peak was always half that when derivatization was carried out with fresh reagent.

These four amino acids were then studied together as shown in Fig. 2. Fig. 2a gives the elution pattern of O-TFA,TAB derivatives. The peaks in the chromatogram in Fig. 2b were again identified by GLC—MS, as given in Figs. 3—6, and also by comparing the retention times of individually derivatized and chromatographed amino acids. After transesterification to O-CEO, TAB, the retention times were systematically increased by about 12 min, which is equivalent to an increase of about 24° in elution temperature with respect

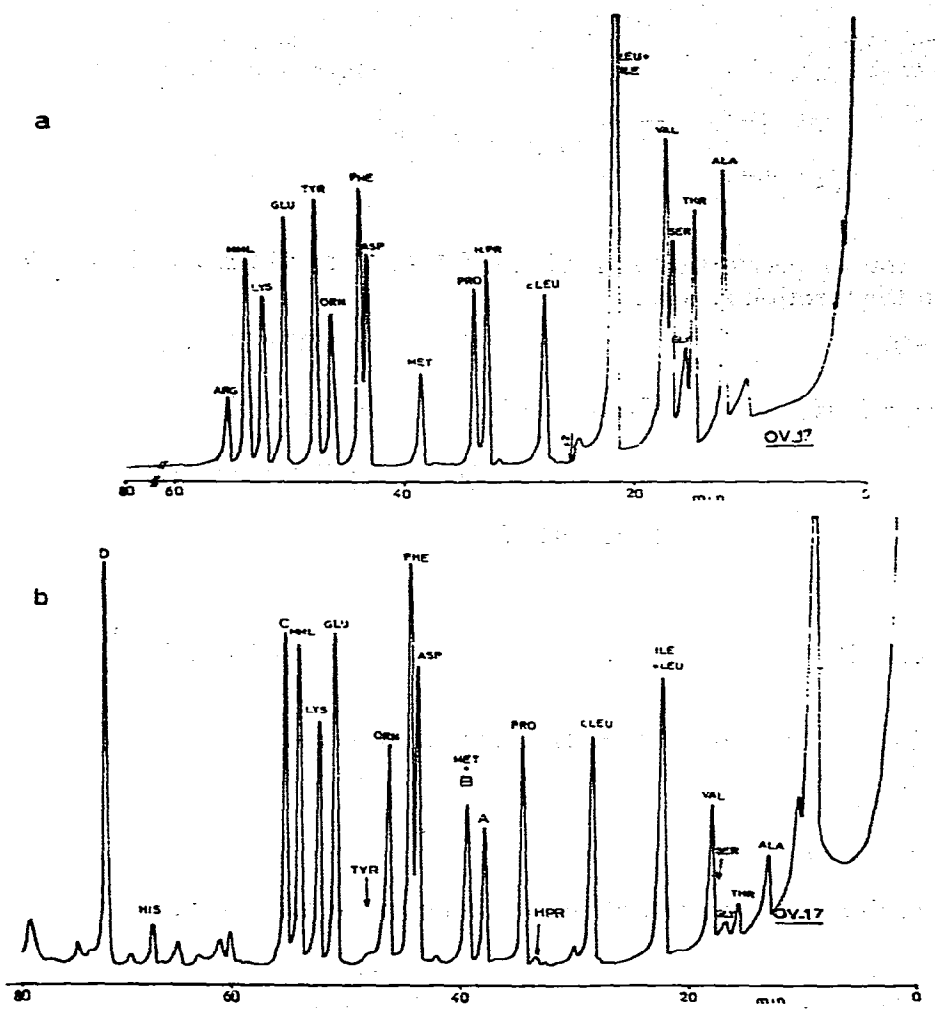


Fig. 1. GLC of 20 reference amino acids on an OV-17 column (cLeu, cycloleucine; MML, N^{ϵ}, monomethyllysine). (a) Chromatogram of the O-TFA,TAB amino-acid derivatives. (b) Chromatogram of the same derivatives treated by a three-week-old EFA mixture.

to that of O-TFA derivatives (Fig. 2a and b and Table I).

As can be seen in Table II, the transesterification yield was in the order tyrosine > serine > hydroxyproline > threonine.

Darbre and Blau [7] investigated the stability of O-TFA amino-acid derivatives by following their progressive hydrolysis in methylethyl ketone containing 5% water. Our results coincide with their hydrolysis order. It can therefore be said that the transesterification relates to ease of hydrolysis.

The fragmentations of O-TFA,TAB and O-CEO,TAB derivatives are shown in Table III and can be interpreted as follows.

(1) For the fragment M—101 there is a difference of 24 a.m.u. which is due to the difference of mass between O-TFA and O-CEO derivatives.

(2) In the case of serine and threonine, we have observed some common fragments and some other specific fragments for the O-CEO compounds.

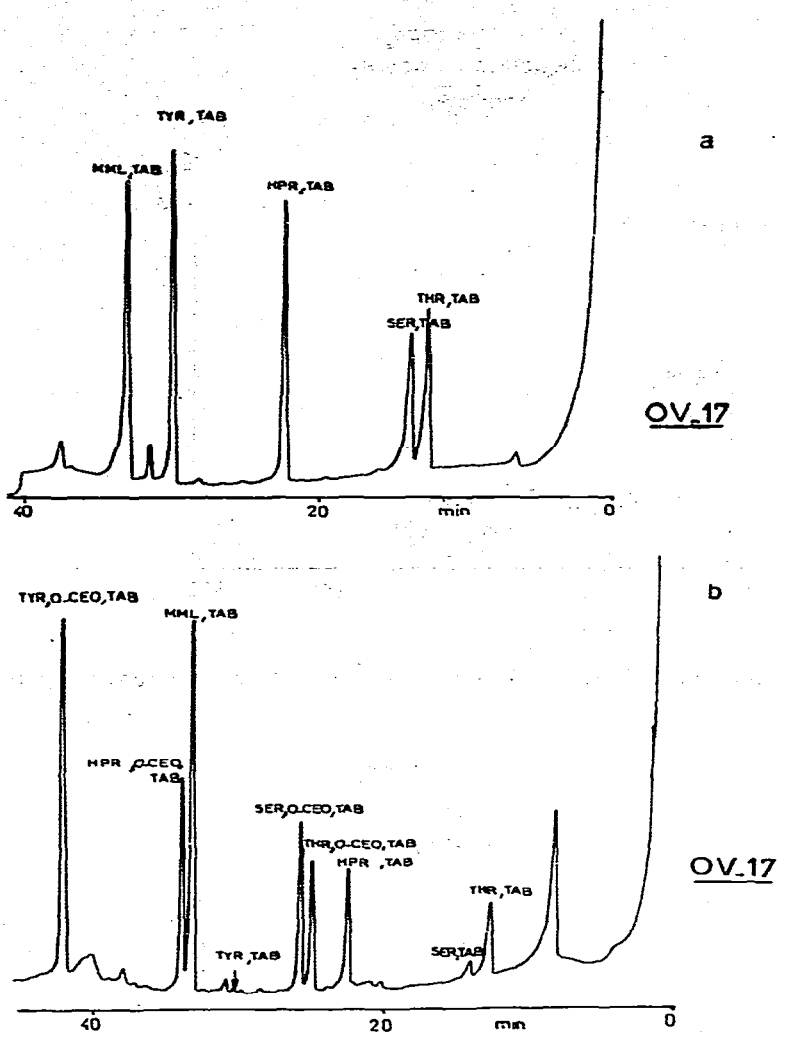


Fig. 2. Transesterification of four hydrolated L-amino acids: serine, threonine, hydroxyproline and tyrosine derivatives. (a) Chromatogram of the four amino acids in the form of O-TFA,TAB. (b) Chromatogram of the same derivatized amino acids after reaction with EFA. The new four peaks were identified as threonine O-CEO,TAB, serine O-CEO,TAB, hydroxyproline O-CEO,TAB and tyrosine O-CEO,TAB.

Both hydroxyproline O-TFA,TAB and O-CEO,TAB give the same fragments by eliminating $COOC_4H_9$ or $O{-}CO{-}CF_3$ and $O{-}COOC_2H_5$, respectively. The fact that tyrosine derivatives do not have fragments in common at relatively high mass ($m/e > 200$) can be explained by preferential fragmentation in the hydrocarbon side chain of the benzene ring.

(3) Fragment 113, lost during electron-impact ionization of O-TFA,TAB derivatives, has two possible structures: $NH_2{-}CO{-}CF_3$ or $O{-}CO{-}CF_3$. But with O-CEO,TAB derivatives the deleted fragment has $m/e = 89$, corresponding to $O{-}COO{-}C_2H_5$. Thus we may assign the $O{-}CO{-}CF_3$ structure to the fragment 113 of the O—TFA,TAB derivatives. Consequently we dem-

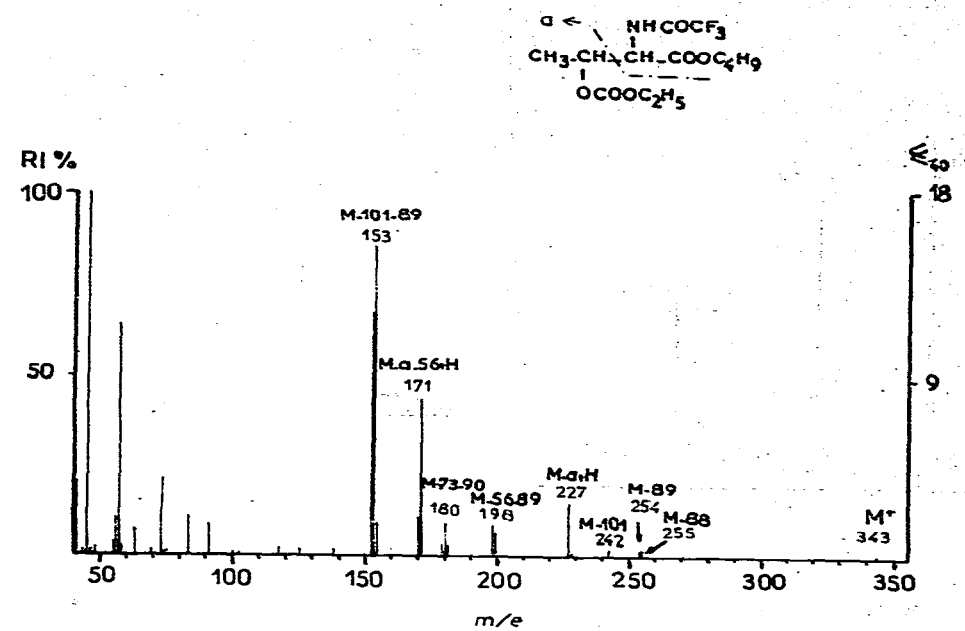


Fig. 3. Mass spectrum of threonine O-CEO, TAB derivative. See text for experimental conditions.

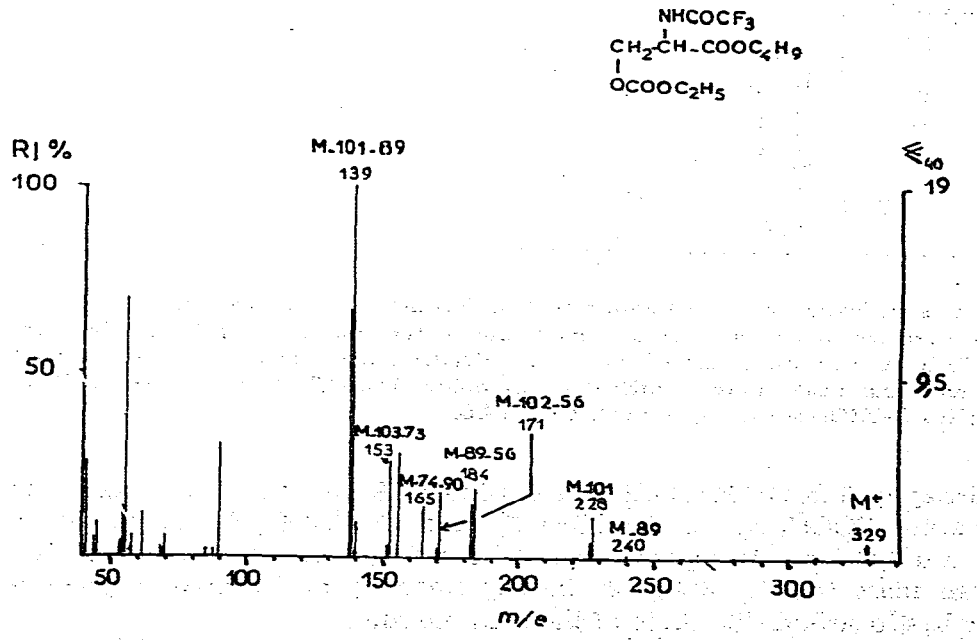


Fig. 4. Mass spectrum of serine O-CEO,TAB derivative, as in Fig. 3.

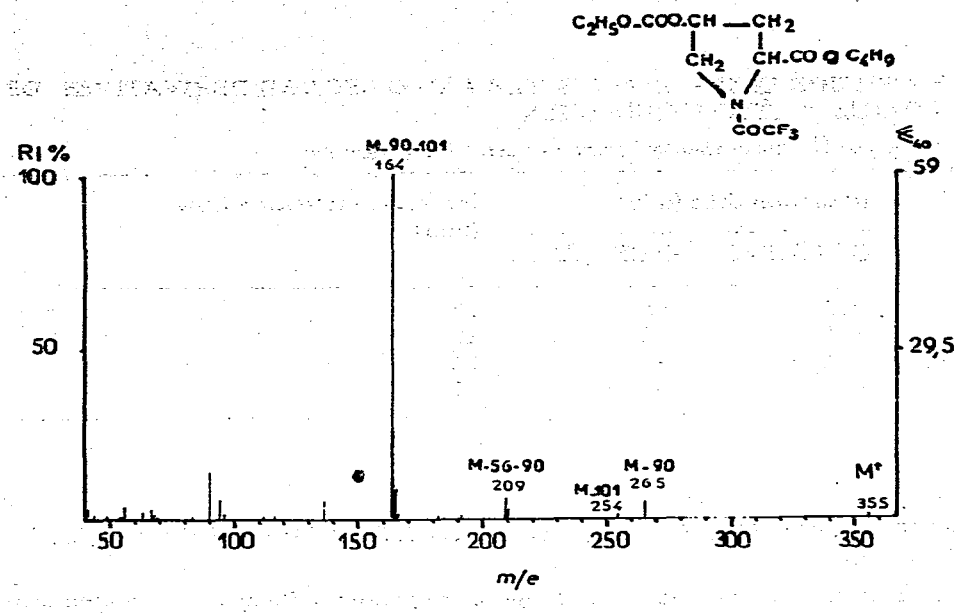


Fig. 5. Mass spectrum of hydroxyproline O-CEO,TAB derivatives, as in Fig. 3.

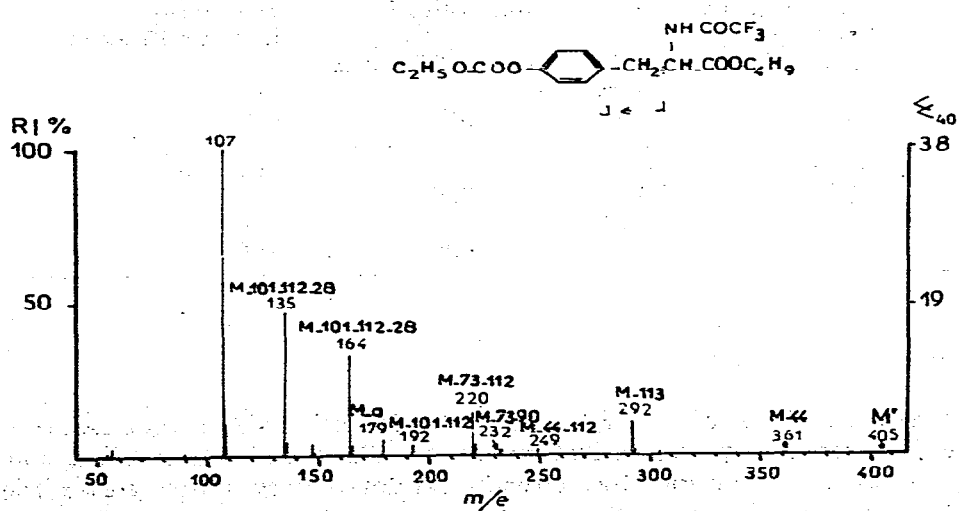


Fig. 6. Mass spectrum of tyrosine O-CEO,TAB derivatives, as in Fig. 3.

onstrate a fragmentation which corroborates well the fragmentation mechanism proposed by Gelpi et al. [8] and recently by Padieu et al. [9]. During electron-impact ionization the bond between alcoholic oxygen and the aliphatic hydrocarbon chain is more labile than that between the NH—CO—CF_3 group and the amino acid C^{α}, whereas the bond between the benzene ring of tyrosine and the phenolic oxygen is more stable than that between the NH—CO—CF_3 group and C^{α}.

TABLE I

RELATIVE RETENTION TIMES OF O-TFA,TAB AND O-CEO,TAB DERIVATIVES OF THE FOUR HYDROXYLATED AMINO ACIDS

Values with respect to N^{ε}-monomethyllysine as zero-time reference.

	Retention time (min)		Increase in retention time (min)
	O-TFA,TAB	O-CEO,TAB	
Threonine	−21	−8	13
Serine	−20	−8	12
Hydroxyproline	−11	+1	12
Tyrosine	−3	+9	12

TABLE II

TRANSESTERIFICATION FOR THE RELATIVE LEVELS OF THE FOUR HYDROXYLATED AMINO ACIDS

Values calculated as follows: in columns 1, 2 and 3, response coefficient R_c = amino acid peak height/MML peak height; in column 4, percentage transesterification = (value column 1 − value column2)/value column 1.

	Before reaction with EFA	After reaction with EFA		Percentage transesterification
	O-TFA,TAB	O-TFA,TAB remaining	O-CEO,TAB	
	(1)	(2)	(3)	(4)
Threonine	0.59	0.19	0.35	68
Serine	0.51	0.04	0.47	92
Hydroxyproline	1.05	0.31	0.58	70
Tyrosine	1.31		0.97	100

CONCLUSION

The transesterification of O-TFA,TAB into O-CEO,TAB, a side-reaction during the N^{τ}-carbethoxy derivatization of histidine and other imidazole compounds such as histamine, has already rendered very much easier the interpretation of mass spectra of some hydroxylated amino acids. Furthermore, this reaction may have a possible use for the determination of free hydroxyl groups in compounds other than amino acids.

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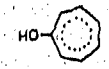
TABLE III

MAIN FRAGMENTS OBTAINED BY ELECTRON-IMPACT IONIZATION GLC–MS

O-TFA,TAB and O-CEO,TAB *derivatives of:* (a) serine and threonine, (b) tyrosine and hydroxyproline. See text for experimental conditions.

Fragments	Serine O-TFA,TAB M^+ 353		Serine O-CEO,TAB M^+ 329		Fragments	Threonine O-TFA,TAB M^+ 367		Threonine O-CEO,TAB M^+ 343	
	m/e	RI (%)	*m/e*	RI (%)		*m/e*	RI (%)	*m/e*	RI (%)
M–101 M–$COOC_4H_9$	252	1.7	228	10.5	M–101 M–$COOC_4H_9$	266	3.4	242	2.3
M–113–56 M–$CF_3COO–C_4H_8$	184	2.7			M–140 M–CH_2=$CHOCOCF_3$	227	3.3		
M–89–56 M–$C_2H_5OCOO–C_4H_8$			184	18.6	M–116 M–CH_2=$CHOCOOC_2H_5$			227	14.8
M–114–74 M–$CF_3COOH–C_4H_9OH$	165	4.2			M–113–56 M–$CF_3COO–C_4H_8$	198	4.8		
M–90–74 M–$C_2H_5OCOOH–C_4H_9OH$			165	12.8	M–89–56 M–$C_2H_5OCOO–C_4H_8$			198	6.7
M–113–101 M–$CF_3COO–COOC_4H_9$	139	57.3			M–114–73 M–$CF_3COOH–C_4H_9O$	180	7.6		
M–89–101 M–$C_2H_5OCOO–COOC_4H_9$			139	100	M–90–73 M–$C_2H_5OCOOH–C_4H_9O$			180	9.6
C_4H_9	57	100	57	3	M–140–56 M–CH_2=$CHOCOCF_3–C_4H_8$	171	12		
M–89 M–C_2H_5OCOO			240	1.8	M–116–56 M–CH_2=$CHOCOOC_2H_5–C_4H_8$			171	45
M–102–56 M–$CH\,OCOOC_2H_5–C_4H_8$			171	17	M–113–101 M–$CF_3COO–COOC_4H_9$	153	100		
M–103–73 M–$CH_2\,OCOOC_2H_5–C_4H_9O$			153	25.6	M–89–101 M–$C_2H_5OCOO–COOC_4H_9$			153	85.2
					M–88 M–C_2H_4OCOO			255	1.9
					M–89 M–C_2H_5OCOO			254	1.9
					C_2H_5O			45	100

TABLE III (continued)

Fragments	Tyrosine O-TFA,TAB M⁺ 429		Tyrosine O-carbethoxy,TAB M⁺ 405		Fragments	Hydroxyproline O-TFA,TAB M⁺ 379		Hydroxyproline O-carbethoxy,TAB M⁺ 355	
	m/e	RI (%)	*m/e*	RI (%)		*m/e*	RI (%)	*m/e*	RI (%)
M	429	0.1	405	0.1	M	379	0.1	355	0.04
M−101 M−$COOC_4H_9$	328	9.9	304	1.2	M−101 M−$COOC_4H_9$	278	16.0	254	0.2
M−113 M−NH_2COCF_3	316	34.1	292	11	M−114 M−CF_3COOH	265	2.5		
M−113−73 M−NH_2COCF_3−C_4H_9O	243	14.3			M−90 M−C_2H_3OCOOH			265	4.7
M−112−101 M−$NHCOCF_3$−$COOC_4H_9$	216	6.5	192	3.7	M−114−56 M−CF_3COOH−C_4H_8	209	1.6		
M−226 $NHCOCF_3$ M−HC−$COOC_4H_9$	203	100	179	6.3	M−90−56 M−C_2H_3OCOOH−C_4H_8			209	5.7
	107	14.3	107	100	M−114−101 M−CF_3COOH−$COOC_4H_9$	164	100		
					M−90−101 M−C_2H_3OCOOH−$COOC_4H_9$			164	100
M−112−73 M−$NHCOCF_3$−C_4H_9O			220	14.6					
M−44 M−C_2H_4O			361	0.3					
M−90−73 M−C_2H_3OCOOH−C_4H_9O			232	2.0					
M−112−44 M−$NHCOCF_3$−C_4H_8O			249	2.6					

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