

# Transesterification of O, N -bistrifluoroacetyl, n-butyl esters of some hydroxylated amino acids into O-carbethoxy, N-trifluoroacetyl, n-butyl esters during N-carbethoxy derivatization of histidine and histamine.

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## Histidine 및 Histamine의 N-carbethoxy 유도체 형성시 수종의 수산기함유 아미노산의 치환 에스터화작용에 대하여

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### SUMMARY

The transesterification of some hydroxylated amino acids derivatives was studied by gas-liquid chromatography and combined gas-liquid chromatography-mass spectrometry and the fragmentation of these two different O-derivatives was discussed.

### INTRODUCTION

Recently Moodie (1) has successfully resolved the problem of histidine by derivatizing the N-trifluoroacetyl, n-butyl esters (TAB) with ethoxy formate anhydride(EFA). He mentioned in the same paper that tyrosine showed a remarkable diminution.

In our laboratory the same phenomenon was observed not only for tyrosine, but also for some other hydroxylated amino acids. In this paper we will report investigations on those reactions undergone by four hydroxylated amino acids(threonine, serine, hydroxyproline and tyrosine) by

gas-liquid chromatography (GLC) and combined gas-liquid chromatography-mass spectrometry(GLC-MS).

### EXPERIMENTS

#### a) Derivatization into TAB

The derivatization was carried out according to the procedures developed by Gehrke's group (4) and in our laboratory (2), (3). In a 10 ml teflon-lined screw-capped tube were taken 50  $\mu$ l of 10 mM solution of threonine, serine, hydroxyproline, tyrosine and  $\epsilon$ -N-monomethyl lysine(MML) (as internal standard) in 0.10N HCl. After drying them under nitrogen at 70°C, 1 ml of anhydrous

n-butanol was added, and through which a stream of dry hydrogen chloride was bubbled according to the method (2) at room temperature.

Ultrasonic homogenization and 60 minutes reheating were followed by the first 30 minutes reheating at 110°C. It was brought to dryness under a nitrogen stream at 70°C and 500  $\mu$ l aliquots of trifluoroacetic anhydride-dichloromethane mixture(1:9 V/V) were added to acylate it at 110°C for 60 minutes under secure tightening. GLC analysis or derivatization with EFA was immediately performed.

#### b) Derivatization into O-carbethoxy (O-CEX) derivative

The TAB derivative was evaporated below 10°C degree under a nitrogen stream, then 500  $\mu$ l of the EFA-dichloromethane mixture(1:500 V/V), "prepared about one month ago", were added in the tube which, in turn, after tightening hermetically was heated at 125°C for 60 minutes.

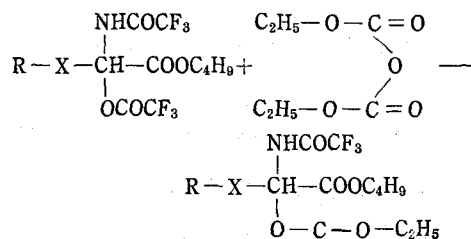
#### c) GLC and GLC-MS analysis

GLC analysis were carried out with Packard 419 equipped with flame ionization detector. A glass column of 3 m by 3 mm in diameter was filled with 1% OV-17 impregnated on Supelcoport. Inlet pressures of hydrogen, air and nitrogen were 1.00 atm, 1.25 atm and 2.00 atm respectively. The temperature of injection port was at 205°C and that of detector at 255°C. The column temperature was initially held at 85°C for 5 minutes, and programmed at 4°C/min to 225°C and then kept isothermal. Combined GLC-MS analysis were done with a LKB-9000. Helium was used as the carrier gas and all of the mass spectra were obtained at 28 eV. Other operating parameters were; injection port 210°C, molecular separator 280°C, ion source 200°C, accelerating voltage 3.5KV and trap current 60 $\mu$ A. Mass spectrum recording was obtained by means of an oscillog-recorder.

## RESULTS AND DISCUSSION

#### a) Reaction condition

The derivatization of O-CEX derivatives can be written as follows.



While the EFA-dichloromethane mixture was freshly prepared, the transesterification did not occur even in the presence of a trace amount of any possible catalyser: HCl, water,  $\text{NH}_4\text{OH}$ ,

$\text{C}_6\text{H}_5\text{S}^-$  or  $\text{C}_2\text{H}_5\text{O}^-$ . This reaction occurred only when the reaction mixture was kept at room temperature for about one month regardless of light. Though no study on the possible changes in the composition of the reaction mixture was undertaken, it is evident that a certain period of time is necessary to have a modification which leads to catalyze this reaction of transesterification.

#### b) GLC and GLC-MS analysis.

The peaks in the chromatograms in Fig. 1a and 1b were identified by massspectra as given in Fig. 2 to Fig. 5 as well as by comparing the retention times of the individually derivatized and chromatographed amino acids. After the transesterification into O-CEX derivatives, the retention times were systematically increased by about 40°C with respect to those of O-TFA ones(Fig. 1b and Table 1).

This phenomenon could be attributed to the  $-\text{O}-\overset{\text{O}}{\parallel}{\text{C}}-\text{C}_2\text{H}_5$ , where the fluorines were substituted by hydrogens and another oxygen atom was added.

As seen Table 2 the transesterification yield was in the following order: tyrosine>serine>hydroxyproline>threonine. Darbre and Blau (5) investigated the stability of O-TFA derivatives by following the progressive hydrolysis of some amino acids kept in a methyl-ethyl ketone solution containing 5% of water. Our results are coinciding with their hydrolysis order. Therefore it can be said that the transesterification depends on the

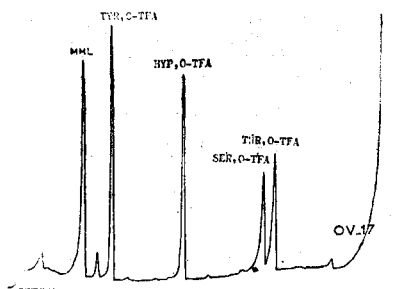


Fig. 1a. The chromatogram of the O-TFA derivatives of the four hydroxylated amino acids: Threonine, Serine, Hydroxyproline, Tyrosine

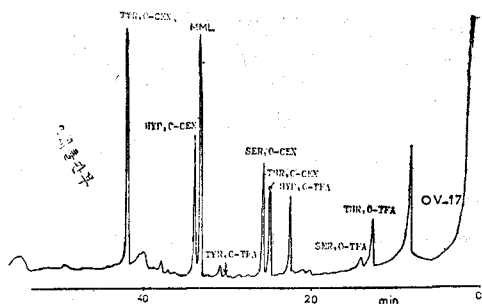


Fig. 1b. The chromatogram of the same derivatives after EFA. The new peaks were identified by GLC-MS as threonine, O-CEX, serine, O-CEX, hydroxyproline, O-CEX and tyrosine, O-CEX.

Table 1. Relative retention times of O-TFA and O-CEX with respect of MML for the four hydroxylated amino acids.

Derivatives Amino acids	O-TFA(mm)	O-CEX(mm)	Augmentation of retention time(mm)	Augmentation of retention temp.(°C)
Threonine	104	42	62	42
Serine	98	38	60	40
Hydroxyproline	55	-4	59	39
Tyrosine	16	-45	61	41

Table 2. Transesterification ratio of the two derivatives of the four amino acids

Derivatives Amino acids	*O-TFA before reaction of EFA	*After reaction of EFA		**Transesterification ratio (%)
		Remaining O-TFA	O-CEX	
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	0.00	0.97	100

\* Peak height of each amino acid/peak height of MML.

\*\* (Value of O-TFA before the reaction of EFA) - (Value of O-TFA after the reaction of EFA) / Value of O-TFA before the reaction of EFA

ease of hydrolysis.

The fragmentation of O-CEX and O-TFA derivatives is shown in Table 3. For fragment M-101 there is a difference of  $m/e=24$  which is due to

the mass difference between  $\text{—O—C(=O)—CF}_3$  and  $\text{—O—C(=O)—O—C}_2\text{H}_5$ . In the case of threonine and serine we have observed some common fragments

and some others specific to the O-CEX derivatives. Both hydroxyproline, O-TFA and O-CEX give

the same fragments by eliminating  $\text{—O—C(=O)—CF}_3$  and  $\text{—O—C(=O)—C}_2\text{H}_5$  respectively. The fact that tyrosine derivatives do not have the fragments in common at relatively high mass range ( $m/e > 200$ ) can be explained by the preferential fragmen-

**Table 3a.** Fragment comparison of O-TFA and O-CEX derivatives of tyrosine and hydroxyproline

Amino acids	Tyrosine O-TFA M <sup>+</sup> 429		Tyrosine O-CEX M <sup>+</sup> 405		Amino acids	Hydroxy-proline O-TFA M <sup>+</sup> 379		Hydroxyproline O-CEX M <sup>+</sup> 355	
	Fragments	m/e	RI %	m/e		RI %	Fragments	m/e	RI %
M	429	0.1	405	0.1	M	379	0.1	355	0.04
M-101 COOC <sub>4</sub> H <sub>9</sub>	328	9.9	304	1.2	M-101 COOC <sub>4</sub> H <sub>9</sub>	278	16.0	254	0.2
M-113 NH <sub>2</sub> COCF <sub>3</sub>	316	34.1	292	11	M-114 CF <sub>3</sub> COOH	265	2.5		
M-113-73 NH <sub>2</sub> COCF <sub>3</sub> , C <sub>4</sub> H <sub>9</sub> O	343	14.3			M-90 C <sub>2</sub> H <sub>5</sub> OCOOH			265	4.7
M-112-101 NHCOCF <sub>3</sub> , COOC <sub>4</sub> H <sub>9</sub>	216	6.5	192	3.7	M-114-56 CF <sub>3</sub> COOH, C <sub>4</sub> H <sub>9</sub>	209	1.6		
M-226 NHCOCF <sub>3</sub>   HC COOC <sub>4</sub> H <sub>9</sub>	203	100	179	6.3	M-90-56 C <sub>2</sub> H <sub>5</sub> OCOOH, C <sub>4</sub> H <sub>9</sub>			209	5.7
	107	143	107	100					
M-112-73 NHCOCF <sub>3</sub> , C <sub>4</sub> H <sub>9</sub> O			220	14.6	M-114-101 CF <sub>3</sub> COOH, COOC <sub>4</sub> H <sub>9</sub>	164	100		
M-44 C <sub>2</sub> H <sub>4</sub> O			361	0.3	M-90-101 C <sub>2</sub> H <sub>5</sub> OCOOH, COOC <sub>4</sub> H <sub>9</sub>			164	100
M-90-73 C <sub>2</sub> H <sub>5</sub> OCOOH, C <sub>4</sub> H <sub>9</sub> O			232	2.0					
M-112-44 NHCOCF <sub>3</sub> , C <sub>2</sub> H <sub>4</sub> O			249	2.6					

**Table 3b.** Fragment comparison of O-TFA and O-CEX derivatives of threonine and serine.

Amino acids	Serine O-TFA M <sup>+</sup> 353		Serine O-CEX M <sup>+</sup> 329		Amino acids	Threonine O-TFA M <sup>+</sup> 367		Threonine O-CEX M <sup>+</sup> 343	
	Fragments	m/e	RI %	m/e		RI %	Fragments	m/e	RI %
M-101 COOC <sub>4</sub> H <sub>9</sub>	252	1.7	228	10.5	M-101 COOC <sub>4</sub> H <sub>9</sub>	266	3.4	242	2.3
M-113-56 CF <sub>3</sub> COO, C <sub>4</sub> H <sub>9</sub>	184	2.7			M-140 CH <sub>2</sub> = CHOCOCF <sub>3</sub>	227	3.3		
M-89-56 C <sub>2</sub> H <sub>5</sub> OCOO			184	18.6	M-116 CH <sub>2</sub> = CHOCOOC <sub>2</sub> H <sub>5</sub>			227	14.8
M-114-74 CF <sub>3</sub> COOH, C <sub>4</sub> H <sub>9</sub> OH	165	4.2			M-113-56 CF <sub>3</sub> COO, C <sub>4</sub> H <sub>9</sub>	198	4.8		
M-90-74 C <sub>2</sub> H <sub>5</sub> OCOOH, C <sub>4</sub> H <sub>9</sub> OH			165	12.8	M-89-56 C <sub>2</sub> H <sub>5</sub> OCOO, C <sub>4</sub> H <sub>9</sub>			198	6.7
M-113-101 CF <sub>3</sub> COO, COOC <sub>4</sub> H <sub>9</sub>	139	57.3			M-114-73 CF <sub>3</sub> COOH, C <sub>4</sub> H <sub>9</sub> O	180	7.6		
M-89-101 C <sub>2</sub> H <sub>5</sub> OCOO, COOC <sub>4</sub> H <sub>9</sub>			139	100	M-90-73 C <sub>2</sub> H <sub>5</sub> OCOOH, C <sub>4</sub> H <sub>9</sub> O			180	9.6
C <sub>4</sub> H <sub>9</sub>	57	100	57	3	NHCOCF <sub>3</sub>   CH-CO	153	100	153	85.2

M-89 $C_2H_5OCO$			240	1.8	M-88 $C_2H_4OCO$		255	1.9
M-102-56 $CH_2-OCOOC_2H_4$ $C_4H_8$			171	17	M-89 $C_2H_5OCO$		254	1.9
M-103-73 $CH_2-OCOOC_2H_5$ $C_4H_8O$			153	25.6	$C_2H_5O$		45	100

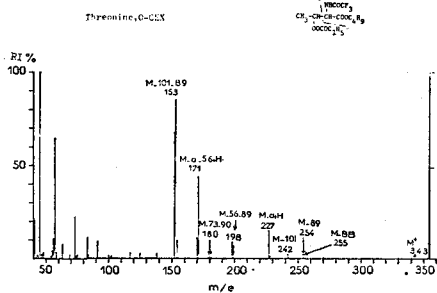
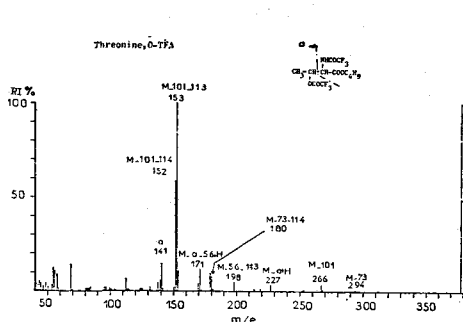


Fig. 2. Mass spectrum of threonine, O-TFA and threonine, O-CEX

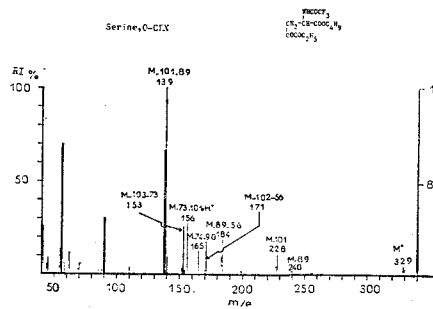
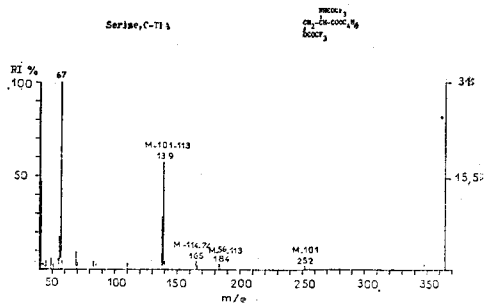


Fig. 3. Mass spectrum of serine, O-TFA and serine, O-CEX

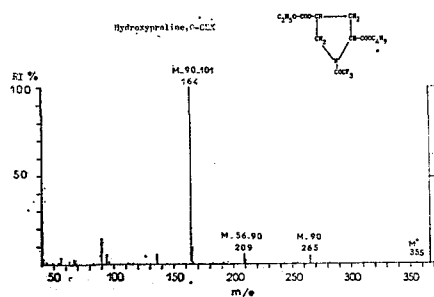
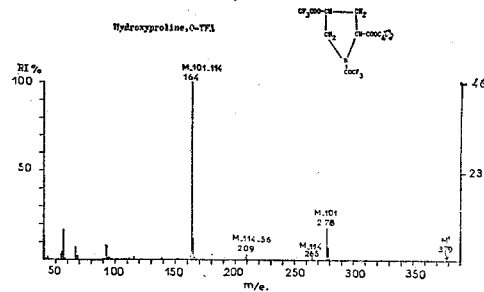


Fig. 4. Mass spectrum of hydroxyproline, O-TFA and hydroxyproline, O-CEX.

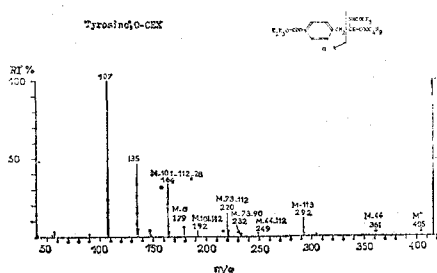
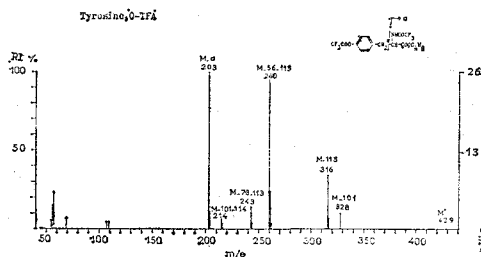


Fig. 5. Mass spectrum of tyrosine, O-TFA and tyrosine, O-CEX

tation in the side chain attached to the benzene ring. The interpretation of the ion  $m/e=M-113$  in O-TFA's is not always evident because of the two possible origins of that fragment:  $\text{NH}_2\text{COOF}_3$  and  $-\text{OCOCF}_3$ . But with O-CEX derivatives we were able to avoid that uncertainty and in consequence we could establish the true fragmentation. These results were well in agreement with the fragmentation mechanisms proposed by Gelpi and his colleagues (6).

### CONCLUSION

The transesterification of O-TFA into O-CEX derivatives, a side reaction during the derivatization for histidine or histamine quantification, already rendered very much easier the interpretation of mass spectra of some hydroxylated amino acids. On the other hand this reaction can have a possible utilization for the identification of free hydroxyl group not only in amino acids but in some other compounds.

### 적 요

가스 크로마토와 가스 크로마-토질량분석기에 의하여 수종의 수산기 함유 아미노산의 치환 에스터화반응을 고찰하고 이들의 fragmentation기작을 설명했다.

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