Partial Hydrolysis of Oligopeptide, II.

——Partial Hydrolysis of Two Hexapeptides and Their Separation Patterns of Fragments in Gas Chromatography

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The purpose of this investigation was to extend the previously obtained phenomena[1] to the hexapeptides and test generality of this conventional method[2] in the field of the determination of the sequence of oligopeptides. Two hexapeptides, Z-Gly-Pro-Phe-Pro-Val-Ileu-OMe (**1**) and Z-Gly-Pro-Val-Ileu-Phe-Pro-OMe (**2**), were prepared and their partial hydrolysis was carried out to investigate their fragmentation patterns in GLC-analysis. The former compound (**1**) had once[2] been reported to be a bitter peptide obtained from the partial hydrolysates of casein. The latter compound (**2**) was selected as the another sequence to compare the both cases.

Partial Hydrolysis of Two Hexapeptides and Their Derivatization to N-Tfa-Methyl Ester Derivatives

Both the hexapeptides, **1** and **2**, were treated with conc. HCl solution in the concentration of 1mg/2ml at 40°C for 2 or 4 days. The respective partial hydrolysates were derived to N-Tfa-methyl ester derivatives and then subjected to the GLC-analysis in the same manner as described in the previous papers,[1,6] respectively.

Results and Discussion

As shown in Fig. 1 and 2 and in Table I and II, most of the possible and reasonable di- and tripeptide fragments were observed in the gas chromatogram of the respective hydrolysates. Their patterns of fragmentation were very different from each other, as could be expected. In the fractogram of the compound (**1**) the largest peak of tripeptide fragments was Pro-Val-Ileu (t_R=17min) and the next one Phe-Pro-Val (t_R=26min), while in the case of the compound (**2**) the largest one was Val-Ileu-Phe (t_R=23min) and the next one Ileu-Phe-Pro (t_R=28min). These

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Abbreviation : s. the previous paper[1]

Table I. t_R-Values of Synthesized Di- and Tripeptide Methyl Esters in GLC[a)]

Tfa-di- and -tripeptide methyl ester	t_R [Min]
Val-Ileu[b,d)]	1.0
Pro-Val[b,d)]	2.6
Ileu-Phe[d)]	6.4
Gly-Pro[b,d)]	7.1
Phe-Pro[b)]	8.6
Pro-Phe[d)]	12.5
Gly-Pro-Val (**11**)	15.5
Pro-Val-Ileu[c,b)]	18.6
Val-Ileu-Phe (**9**)	23.1
Phe-Pro-Val[c)]	25.6
Ileu-Phe-Pro[c)]	29.6
Gly-Pro-Phe (**5**)	30.0
Pro-Phe-Pro (**7**)	37.4

a) Apparatus : A Hitachi-Perkin Elmer 063, FI-Detector ; GLC conditions : oven temp. 190-260°/2°/min programming and then isothermal, carrier gas N_2 0.9 kg/cm² at the inlet, attenuation 1×256.
b) Common fragments to both hexapeptides, **1** and **2**.
c) Lit. 1) d) Lti. 5)

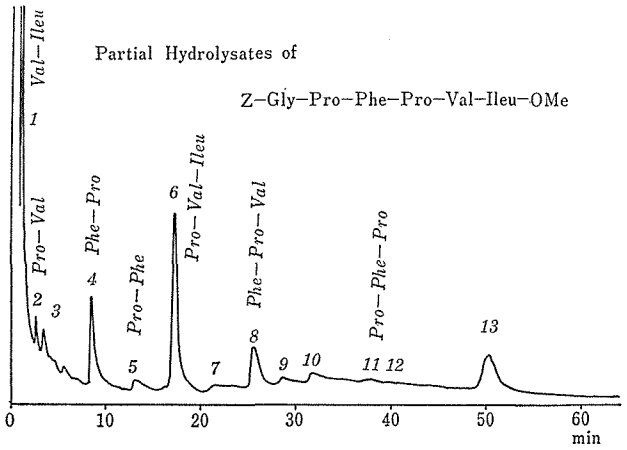

Fig. 1. Gas Chromatogram of a Mixture of Tfa-Peptide Methyl Esters obtained by Derivatization of an Acid Hydrolysate (12N HCl, 40°C 4 days) of the Compound (**1**). (Oven temp. 160-260°/2°/min programming, other GLC conditions, cf. in Table I).

data show that the both sequences can be clearly distinguished from each other in tripeptide peaks and we can even confirm the respective sequence if these tripeptides will be identified, and it seems reasonable to assume that such method can be

Table II. Assignment of Peaks in Gas Chromatogram of Derived Tfa-Peptide Methyl Esters of Partial Hydrolsates of both Hexapeptides, **1** and **2**, to the Expected Fragment Sequences.[a]

A. Hexapeptide (**1**)

Peak mark in Fig. 1	t_R [Min]	Assignment to sequences
1 (large)	1.0	Val-Ileu[b]
2 (small)	2.8	Pro-Val[b]
4 (large)	8.6	Phe-Pro
5 (small)	13.0	Pro-Phe
6 (large)	17.1	Pro-Val-Ileu[b]
8 (large)	25.6	Phe-Pro-Val
11 (very small)	38	Pro-Phe-Pro
		(Gly)-Pro-Phe-Pro-Val-Ileu

B. Hexapeptide (**2**)

Peak No. in Fig. 2	t_R [Min]	Assignment to sequences
a (large)	1.0	Val-Ileu[b]
b (large)	2.8	Pro-Val[b]
c (middle)	5.5	Ileu-Phe
d (middle)	6.9	Gly-Pro[b]
e (large)	8.5	Phe-Pro
i (large)	23.1	Val-Ileu-Phe
k (large)	27.8	Ileu-Phe-Pro
		Gly-Pro-Val-Ileu-Phe-Pro

a), b): cf. the respective footnote in Table I.

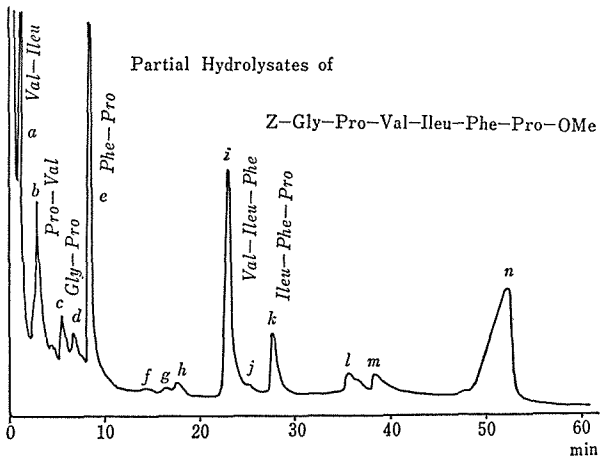

Fig. 2. Gas Chromatogram of a Mixture of Tfa-Peptide Methyl Esters obtained by Derivatization of an Acid Hydrolysate (12N HCl, 40°C, 4 days) of the Compound (**2**) (Oven temp. 160°-260°/2°/min programming, other GLC conditions, s. in Table I).

applied effectively for the confirmation of the oligopeptide sequence, as was pointed out previously.[1]

Syntheses of Two Hexapeptides and Some Standard Tfa-Tripeptide Methyl Ester Derivatives

Analogously to the previous paper[1] we have used Z for amino protection and DCCD/HOBt-method[4] for the peptide coupling. Tfa-derivatives were derived from the corresponding Z-derivatives.[5]

Z-Gly-Pro-Phe-Pro-Val-Ileu-OMe (**1**) was prepared by condensing Z-Gly-Pro-OH and H-Phe-Pro-Val-Ileu-OMe, which was obtained from the corresponding Z-derivative[1] by the action of 25% HBr/AcOH followed by treating with triethyl amine. Z-Gly-Pro-Val-Ileu-Phe-Pro-OMe (**2**) was also similarly prepared from Z-Gly-Pro-OH and H-Val-Ileu-Phe-Pro-OMe, which was obtained from the corresponding Z-derivative.[1] Both tetrapeptides were obtained as crystals. Before being purified enough analytically, the crude products of them were subjected to the partial hydrolysis.

Z-tripeptide derivatives, Z-Gly-Pro-Phe- (**3**), -Pro-Phe-Pro- (**5**), and -Gly-Pro-Val-OMe (**7**), were obtained only as an oily substance. Both Tfa-tripeptide derivatives, Tfa-Gly-Pro-Phe- (**4**) and -Gly-Pro-Val-OMe (**8**), were obtained as a crystalline substance, Tfa-Pro-Phe-Pro-OMe (**6**), however, only as an amorphos solid.

EXPERIMENTAL SECTION

The experimental methods and procedures were essentially the same and the used apparatus was also the same as described in the previous paper.[1] All melting points were uncorrected. TLC: CMA.[1] Visualization; A: acridine.

1. Z-Gly-Pro-OH: Z-Gly-Pro-OMe (oil, 2.60g, 8.12mM) was treated with a mixture of 1N NaOHaq solution (1.6eq, 12.8ml) and MeOH (43ml) under stirring overnight. The reaction mixture was evaporared in vacuo. Water (50ml) was added and the solution was acidified with conc. HCl. It was extracted twice with AcOEt. The organic layer was dried over anhydrous Na_2SO_4 and then evaporated to dryness to give a crude crystalline substance (2.64g, ~100%). Crystallization from AcOEt, 1.52g. mp 155–156° (Lit.[7] 155.5–157.5°) NMR ($CDCl_3$)δ: 1.8–2.2 ($-CH_2-CH_2-$ in Pro, 4H) 3.3–3.6 ($N-CH_2-$ in Pro, 2H) 3.8–4.0 ($-CH_2-$ in Gly, 2H) 4.2–4.5 (αCH in Pro, 1H) 5.07 (s, $-CH_2-$ in Z) 6.0 (b, NH in Gly, 1H) 7.25 (s, C_6H_5 in Z, 5H) 8.32 (s, COOH, 1H).

2. Z-Gly-Pro-Phe-Pro-Val-Ileu-OMe (**1**): Z-Gly-Pro-OH (0.47g, 1.53mM) and HBr·H-Phe-Pro-Val-Ileu-OMe, which was obtained from 0.62g (1mM) of the correspon-

Table III. Yield and Properties of the Prepared Z- and Tfa-Tripeptide Methyl Esters, **3**–**8**

No.	Prepared compounds	Purification[a] or separation	Yield[b] [%]	Mp [°C]	Rf in TLC /CMA[f]	$[\alpha]_D^{25}$[c]	Molecular formula (molecular weight)	Elemental analysis	C	H	N
1.	Z-Gly/Pro-Phe-OMe (**3**)	B	85	Oil[d]	0.84						
2.	Tfa-Gly-Pro-Phe-OMe (**4**)	A	82	121–123	0.74	−55.4	$C_{19}H_{22}O_5N_3F_3$ (429.4)	Calcd.	53.15	5.16	9.79
								Found	53.19	5.45	9.84
3.	Z-Pro-Phe/Pro-OMe (**5**)	B	~100	Oil[d]	0.90						
4.	Tfa-Pro-Phe-Pro-OMe (**6**)	C	78	Amorphous solid	0.77	−81.4	$C_{22}H_{26}O_5N_3F_3$ (469.5)		56.29	5.58	8.95
									56.45	5.78	8.71
5.	Z-Gly/Pro-Val-OMe (**7**)	B	95	Oil[d]							
6.	Tfa-Gly-Pro-Val-OMe (**8**)	A	57	124–127	0.76	−101	$C_{15}H_{22}O_5N_2F_3$ (381.4)		47.24	5.82	11.02
									46.98	5.98	10.97

a) A : Recrystallized from AcOEt/n-hexane B : Only by fractionation, and then subjected to decarbobenzoxylation followed by trifluoroacetylation. C : Separation by means of preparative TLC (solvent : CMA[e]).

b) It was concerned to the condensation reaction of the both components at the position shown with the prime in thec ase of Z-derivatives or to the trifluoroacetylating reacton of the corresponding Z-derivatives in the case of Tfa-derivatives.

c) It was measured in MeOH (c=1.0) with an error within ± 0.3°C, with an except at **8** (c=0.23).

d) These oily Z-derivatives were converted to Tfa-derivatives without characterization.

e) CMA : Developing solvent (chloroform (85)/MeOH (15)/AcOH (2)).

ding Z-derivative,[1] were condensed each other by DCCD/HOBt method in tetrahydrofuran. The neutral fraction was obtained from the reaction mixture. It was successively washed with $NaHCO_3$ aq, 2N HCl aq and NaCl aq solution. The obtained solid matter was recrystallized from AcOEt/n-hexane. A crystalline substance, 0.60g (77%). TLC: CMA/A, Rf=0.69, a single spot. mp ~140° (partly decomp.) -170° (decomp.) (no definite value). $[\alpha]_D^{22}$ −89.2° (c=1.0, MeOH). Found: C, 63.22; H, 7.91; N, 10.89. Calcd. for $C_{41}H_{56}N_6O_9$ (776.9): C, 63.38; H 7.27; N, 10.82%. It was subjected to hydrolysis without further purification.

3. Z-Gly-Pro-Val-Ileu-Phe-Pro-OMe (**2**): Z-Gly-Pro-OH (0.24g, 0.78mM) and H-Val-Ileu-Phe-Pro-OMe, which was obtained from the corresponding Z-derivative[1] (0.49g, 0.78mM), were condensed each other in the same manner as in **1**. The obtained neutral substance (0.51g, 84%) was crystallized from AcOEt/n-hexane. TLC/CMA/A, Rf=0.73, a single spot. 0.45g (74%). mp 110~185-187° (no definite value). $[\alpha]_D^{22}$ −92.8° (c=1.0, MeOH). Found : C, 63.38; H, 7.47; N, 10.82. Calcd. for $C_{41}H_{56}N_6O_9$ (776.9): C, 63 38; H, 7.27; N, 10.82%.

4. Syntheses of the compounds, **3-6**: The data were summarized in the following Table (Table III). Z-derivatives were synthesized by condensation of the both components at the position shown with the prime and Tfa-derivatives from the corresponding Z-derivatives in the same manner as described in the previous papers[1,5]

5. Partial hydrolysis of hexapeptides, **1** *and* **2**: Hexapeptide (10mg), **1** or **2**, were dissolved in conc. HCl (20ml) and warmed respectively at 40°C. Each aliquot (10ml) was sampled from the respective reaction mixture after 2 or 3 days. Each aliquot was evaporated to dryness in vacuo. Each residue was converted to Tfa-methyl ester derivatives and then analyzed by GLC in the same manner as described in the previous papers.[1,6] GLC conditions, cf. Table 1. The data were summarized in Table I and II.

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摘　　要

オリゴペプチドの部分的加水分解 II
——2つのヘキサペプチドの部分的加水分解と，それらの開裂片のガスクロマトグラフにおける分離パターンについて

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2つのヘキサペプチド，Z–Gly–Pro–Val–Ileu–Phe–Pro–OMe と Z–Gly–Pro–Phe–Pro–Val–Ileu–OMe を合成し，それぞれ部分的加水分解を行ない，それらの開裂ペプチドをガスクロマトグラフで分離した。そして各々に特有の2，3のトリペプチドのフラグメントを確認同定したが，このことから両者のシーケンスを区別確認しうることを示した。

本実験は前報における方法をさらに拡張して検討しようとしたものであり，上記に合成したヘキサペプチドのうちの1つは，かつてカゼインの部分的加水分解物中の苦味物質であると報ぜられたものであるが，このようなオリゴペプチドのシーケンスがガスクロマトグラフ法により迅速確実に確認区別しうることを示した。