オリゴペプチドの部分的加水分解(1)

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Partial Hydrolysis of Oligopeptide, I.

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

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In the sequencial analysis of oligopeptide by GLC[1] (and also GLC/MS[2]) method it is desirable that after its partial hydrolysis all the possible and reasonable fragments are observed in such a harmonious amount ratio in the chromatogram as can be clearly detected and identified. In this paper we selected two tetrapeptides, Z–Val–Ileu–Phe–Pro–OMe (**1**), which had once[3] been reported to be the part of the bitter peptide obtained from the partial hydrolysates of casein, and Z–Phe–Pro–Val–Ileu–OMe (**3**), which was the rearranged one of **1** in view of comparing the both, as model substances and investigated the separation patterns of their partial hydrolysates in GLC.

Partial Hydrolysis of Both the Tetrapeptides

Both tetrapeptides, **1** and **3**, were treated with a conc. HCl solution in concentration of 1mg/ml at 50°C for the time interval cited in Table I and II, respectively. The hydrolysates were converted to N–Tfa–methyl ester derivatives and then subjected to GLC–analysis in the same manner as described in the previous papers.[1,4] The individual peaks on each fractogram were identified to the respective fragments in comparison with the t_R–values of the standard Tfa–methyl ester derivatives (Table I and II).

Results and Discussions

In the case of the compound (**1**), as can be seen from Fig. 1 and Table I, besides all the possible fragments being deteced, several undesirable peaks were observed as fairly large peaks in all stages of hydrolysis. The height ratio of each peak for

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Abbreviation : DCCD, dicyclohexylcarbodi-imide ; HOBt, N-hydroxybenzotriazole ; GLC, gas liquid chromatography ; MS, mass spectrometry ; Tfa, trifluoroacetyl ; Z, carbobenzoxy. Unless otherwise described, L-optical isomer of the amino acid can be assumed throughout, except Gly.

Table I. t_R-Values of the Peaks and Their Assignment to the Corresponding Fragment Sequences in GLC-Analysis[a] of the Partial Hydrolysates of **1** (relative peak height ratios of each peak were presented in parenthesis).

Hydrolysates[b]	Hy^1-50_{23}		Hy^1-50_{47}		Hy^1-50_{72}		Hy^1-50_{90}		Hy^1-50_{120}		Assignment to fragment sequences	t_R [Min] of standard comp.
Peak No.	t_R [Min]	Ratio [%]	t_R [Min]	Ratio [%]	t_R [Min]	Ratio [%]	t_R [Min]	Ratio [%]	t_R [Min]	Ratio [%]		
1	4.7	(4)	4.4	(7)	4.2	(26)	4.3	(32)	4.0	(46)	Val -Ileu[6]	4.5
2[c]	14.2	(15)	13.6	(5)	13.7	(4)	14.1	(1)	13.3	(1)		
3	—	(0)	19.0	(1)	18.4	(>1)	18.0	(3)	17.5	(3)	Ileu-Phe[6]	18.5
4[c]	—	(0)	21.3	(10)	21.6	(16)	21.2	(13)	20.5	(13)		
5	26.2	(13)	25.4	(5)	—	(1)	—	(0)	—	(0)	Phe-Pro	26.3[d]
6[c]	33.2	(24)	32.2	(15)	33.0	(5)	32.6	(5)	32.0	(4)		
7[c]	36.4	(11)	35.7	(7)	—	(>1)	35.9	(2)	—	(0)		
8	40.0	(14)	39.5	(25)	40.0	(40)	39.5	(33)	38.6	(30)	Val-Ileu-Phe (**4**)	39.9
9	45.2	(2)	44.6	(1)	45.5	(1)	44.8	(2)	44.6	(1)	Ileu-Phe-Pro (**5**)	45.8
10[c]	54.0	(8)	53.2	(15)	54.2	(1)	53.4	(2)	—	(0)		
11	74.0	(10)	71.3	(10)	72.0	(6)	71.4	(8)	69.0	(2)	Val-Ileu-Phe-Pro (**8**)	75.8

a) Apparatus : a Hitachi Perkin-Elmer 063, column OV-17 (5%), 1m × 3mm, oven temp. 160-260°/2°/min programming and then isothermal, injection temp. 300°, carrier gas N_2 0.95-1.05 Kg/cm² at the inlet, FI-detector, H_2 0.6 Kg/cm² and air 1.5Kg/cm²,

b) For example, Hy^1-50_{23} means the hydrolysates which was obtained by treating the compound **1** at 50° for 23hr.

c) Unknown and undesirable peaks

d) In this experiment, Tfa-Phe-Pro-OMe was not isolated as pure substance, but the neutral fraction from the reaction mixture was injected. It showed a single peak. Cf. Lit.[6]

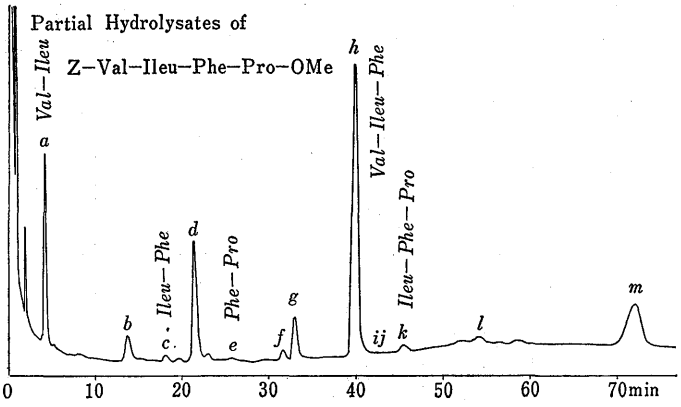

Fig. 1. Gas Chromatogram of a Mixture of Tfa–Peptide Methyl Esters obtained by Derivatization of an Acid Hydrolysate (12N HCl, 50°C, 3 days) of the Compound (**1**). (Oven temp. 160–260°/2°/min programming, other GLC conditions, cf. in Table I).

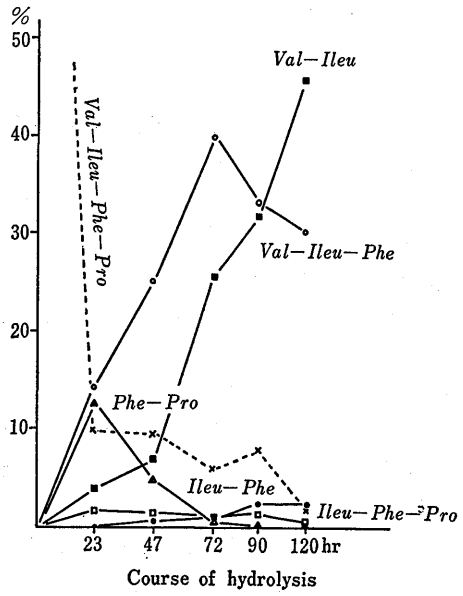

Fig. 2. Relation between the Relative Peak Height of the Respective Fragment and the Course of Hydrolysis of Z-Val–Ileu–Phe–Pro–OMe (**1**)

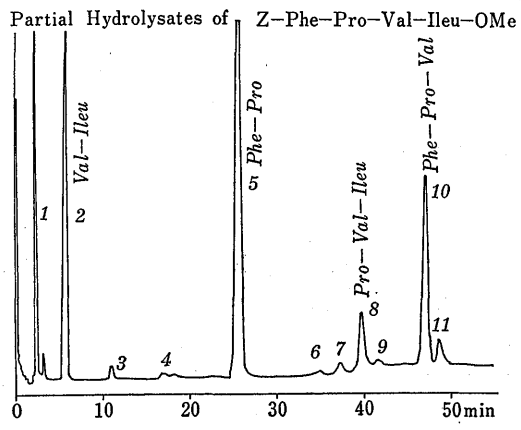

Fig. 3. Gas Chromatogram of a Mixture of Tfa–Peptide Methyl Esters obtained by Derivatization of an Acid Hydrolysate (12N HCl, 50°C, 90hr) of the Compound (**3**). (Oven temp. 150–260°/2°/min programming, other GLC conditions, s. in Table I).

the total sum of all peaks in each chromatogram was estimated in Table I shown in a parenthesis and the values of the reasonable peaks were plotted on a graph (Fig. 2.). As can be seen from this Fig, Val–Ileu and Val–Ileu–Phe were always

Table II. t_R-Values of the Peaks and Their Assignment to the Corresponding Fragment Sequences in GLC-Analysis[a] of the Partial Hydrolysates of **3** (Relative peak height ratio of each peak for the sum of all ones is given in parenthesis)

Partial hydrolysates / Peak No.	Hy^3-50_{90}[b]		Assignment to fragment sequence	t_R [Min] of stand. substance
	t_R [Min]	Ratio [%]		
1	5.9	(29)	Val-Ileu[6]	5.8
2	10.7	(1)	Pro-Val (**10**)	11.0
3[c]	17-18	(1)		
4	23.5	(47)	Phe-Pro[d]	25.8
5[c]	35-37	(1)		
6	36.8	(4)	Pro-Val-Ileu (**7**)	39.7
7[c]	42	(1)		
8	48.0	(16)	Phe-Pro-Val (**6**)	47.2
9[c]	49	(2)		

a), b), c) and d), s. the respective footnote in Table I.

detectable as large peaks and this tendency increased as the hydrolysis proceeded. On the other hand, in the case of the compound (**3**), although only one stage at 90hr hydrolysis was analyzed as shown in Table II and Fig. 3, not only Val-Ileu but also Phe-Pro were detected as large peaks of dipeptide and furthermore the latter was larger than the former. It means apparently that Phe-Pro was produced in the more amount than Val-Ileu, for Phe-Pro with the larger t_R-value must be observed as relatively lower peak than Val-Ileu with the smaller t_R-value. Phe-Pro-Val was also produced in a relatively large amount. From these data it will be at least clear that Val-Ileu is always detectable, but that Phe-Pro is stable only when it is not located in the C-terminal region in the sequence,[7] although it is not simple to follow the relation between the increase and decrease of each fragment during the course of hydrolysis exactly. In any case, it may be concluded that the condition of hydrolysis under which only the reasonable peaks can be observed in the average ratio of a peak height is not easily found due to the presumably complicated cyclic intermediates.

We have attempted a detailed study of the time course involved in the hope that the peak height of the standard Tfa-derivatives might be practically independent on the t_R-values. It was revealed, however, that their peak heights decreased with the increase of t_R-values, as can be seen in Fig. 4, but that this tendency was not regular for all the compounds. The relation factor of the amount to the peak area of the respective standard Tfa-derivative was also not always the same for all the compounds.

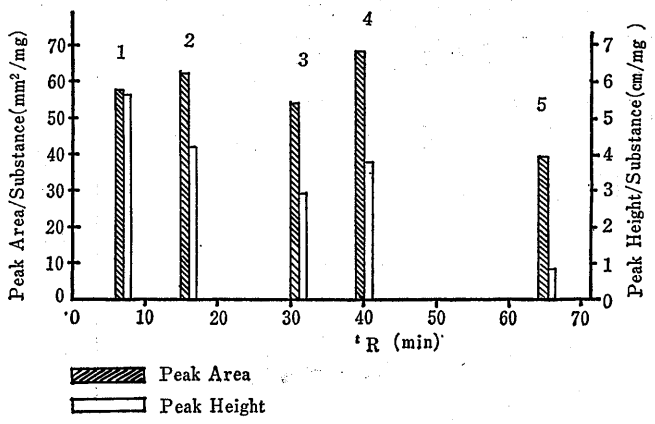

Fig. 4. Relation between the Peak Height Ratios or Peak Area Ratios and t_R-Values of some Tfa-derivatives.
1. Tfa-Pro-Val-, 2. -Ileu-Phe-, 3. -Pro-Val-Ileu-, 4. -Phe-Pro-Val- and 5. -Val-Ileu-Phe-OMe. (Each 3.00 mg of each substance was weighted and they were mixed. The mixture was disolved in 0.5ml MeOH+0.5ml AcOEt and 2μl was injected to GLC. 160-260°/2°/min programming condition. Apparatus and other conditions, cf. Table I.)

Syntheses of the Two Tetrapeptides and Some Standard Tfa-Methyl Ester Derivatives

The two tetrapeptides, **1** and **3**, were synthesized according to the strategy shown in Fig. 5. Z was used for amino protection, removed by the action of 25% HBr/AcOH. The amino component was liberated by the addition of triethyl amine. Coupling was throughout effected by means of DCCD/HOBt method.[5] Tfa-derivatives were synthesized from the corresponding Z-derivatives in the same manner as described in the previous paper.[6] Both Z-tetrapeptide methyl esters, **1** and **3**, and all synthesized Tfa-tripeptide methyl esters, such as Tfa-Val-Ileu-Phe-(**4**), -Ileu-Phe-Pro-(**5**), -Phe-Pro-Val-(**6**), -Pro-Val-Ileu-(**7**) and -Val-Ileu-Phe-Pro-OMe (**8**) were obtained as crystalline substance.

EXPERIMENTAL SECTION

The general experimental methods and procedures were essentially the same and the used apparatus was also the same as described in the previous paper.[4] All melting points were not corrected. TLC: CMA (=chloroform/methanol/acetic acid (760 : 40 : 24 Vol.)). Visualization, I_2 or acridine.[4]

1. Syntheses of the compounds, **1** - **9** : **1**, **2** and **3** were prepared according to the strategy shown in Fig. 5. Z-derivatives were synthesized from two components se-

Table III. Yield and Properties of the Synthesized Compounds, **1–9**

No.	Synthesized compounds	Purification[a]	Yield[b] [%]	Mp [°C]	Rf in TLC /CMA	$[\alpha]_D^{25}$[c] [°]	Molecular formula (molecular weight)	Elemental analysis C	H	N
1.	Z-Val/Ileu-Phe-Pro-OMe (**1**)	A	76	114–116	0.75	−71.4	$C_{34}H_{46}O_7N_4$ (622.8)	Calcd. 65.58	7.45	8.99
								Found 65.34	7.33	9.20
2.	Z-Pro/Val-Ileu-OMe (**2**)	A	69	149–151	0.69	−89.4	$C_{25}H_{37}O_6N_3$ (475.6)	63.14	7.84	8.84
								62.94	7.93	8.98
3.	Z-Phe/Pro-Val-Ileu-OMe (**3**)	A	57	70–75	0.69	−77.9	$C_{34}H_{46}O_7N_4$ (622.8)	65.58	7.45	8.99
								65.68	7.73	8.74
4.	Tfa-Val-Ileu-Phe-OMe (**4**)[d]	B	—	232–233	0.83	—	$C_{23}H_{32}O_5N_3F_3$ (487.5)	56.67	6.62	8.62
								56.64	6.71	8.77
5.	Tfa-Ileu-Phe-Pro-OMe (**5**)	A	30	195–197	0.83	−60.0	$C_{23}H_{30}O_5N_3F_3$ (485.5)	56.90	6.23	8.66
								57.18	6.35	8.41
6.	Tfa-Phe-Pro-Val-OMe (**6**)	A	36	113–114.5	0.90	−51.4	$C_{22}H_{28}O_5N_3F_3$ (471.5)	56.06	5.99	8.91
								56.22	6.04	8.84
7.	Tfa-Pro-Val-Ileu-OMe (**7**)	A	60	199–200	0.70	−91.6	$C_{19}H_{30}O_5N_3F_3$ (437.5)	52.17	6.91	9.61
								51.97	6.69	9.55
8.	Tfa-Val-Ileu-Phe-Pro-OMe(**8**)	A, C	94	201–203	0.75	—	$C_{28}H_{39}O_6N_4F_3$ (584.6)	57.52	6.72	9.58
								57.73	6.85	9.31
9.	Tfa-Pro-Val-OMe (**9**)[e]	A	61	81–83.5	0.85	−80.0	$C_{13}H_{19}O_4N_2F_3$ (324.3)	48.15	5.91	8.64
								48.26	5.86	8.78

a) A, crystallization from AcOEt/n-hexane ; B, sublimation in vacuo; C, preparative TLC separation.

b) It was concerned to the condensation reaction between the both components separated with the prime in the case of Z-derivatives and to the corresponding Z-derivatives in the cases of Tfa-compounds.

c) It was measured in MeOH (c=1.0) with an error within ±0.8°.

d) The starting material, Z-Val-Ileu-Phe-OMe, was obtained by condensing Z-Val-OH with H-Ileu-Phe-OMe only in a low yield (DCCD/HOBt : 15%, Mixed anhydride with EtOCOCl : 20%, mp 192–3°, but not enough analytically pure).

e) In the previous paper[6] it was obtained only as an oil.

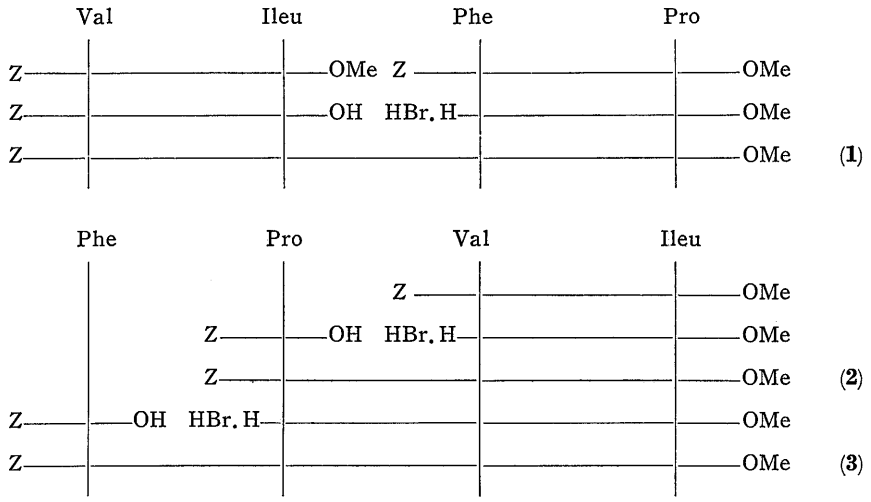

Fig. 5. Strategy for the syntheses of the tetrapeptides

parated by the prime in Table III by condensing them by DCCD/HOBt method.[5] Tfa-derivatives were prepared from the corresponding Z-derivatives by treating with 25% HBr/AcOH, followed by transesterification with methyl trifluoroacetate.[6] The data were summarized in the Table (Table III).

2. Partial hydrolysis of tetrapeptides, **1** *and* **3**: The compound (**1**) (80mg) was treated with 80ml of conc. HCl solution at 50°C and after each time interval cited in Table I each aliquot (15 ml) of the solution was sampled. It was evaporated to dryness in vacuo. **3** was also hydrolyzed analogously. The hydrolysates were converted to Tfa-methyl ester derivatives and then analyzed by GLC in the same manner as described in the previous paper.[4] GLC conditions were footnoted under Table I. The data were summarized in Table I and II.

REFERENCES

1) F. Weygand, B. Kolb, A. Prox, M. A. Tilak und I. Tomida, *Hoppe Seyler's Z. physiol. Chem.*, ***322***, 38 (1960).
2) H. Nau, *Angew. Chem.*, ***88***, 74 (1976).
3) T. Matoba, C. Nagayasu, R. Hayashi and T. Hata, *Agric. Biol. Chem.*, ***33***, 1662 (1969). cf. N. Minamiura, Y. Matsumura, J. Fukumoto and T. Yamamoto, *Agric. Biol. Chem.*, ***36***, 588 (1972).
4) I. Tomida and H. Ukisu, *Agric. Biol. Chem.*, ***44***, 1941 (1980).
5) W. König und R. Geiger, *Chem. Ber.*, ***103***, 788 (1970).
6) I. Tomida, J. Ohashi, T. Tokuda and M. Nakajima, *Nippon-Nogei-Kagaku-Kaishi*, ***39***, 378, 391 (1965).
7) Cf. The *Organic Chemistry of Peptides*, by Harry D. Law (1970 John Wiley & Sons Ltd) p. 12~.

摘　　要

オリゴペプチドの部分的加水分解　I

——2つのテトラ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つのテトラペプチド，Z–Val–Ileu–Phe–Pro–OMe 及び Z–Phe–Pro–Val–Ileu–OMe を合成し，塩酸による加水分解を行なった。そして加水分解物をN－トリフルオロアセチル－メチルエステルに導き，ガスクロマトグラフで分離し，両者の分離パターンを比較した。また各々に特有なジ－及びトリペプチドフラグメントを確認し，前者のテトラペプチドについては加水分解過程中の各フラグメントの消長についても観察した。そしてこの方法によりそれぞれのアミノ酸配列を確認しうることを示した。

なお，これらのテトラペプチドのうちの1つは，かつてカゼインの部分的加水分解物中の苦味ペプチドのシーケンスの一部であると報ぜられたものであるが，本論文は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の方法によって迅速に区別，確認，さらに決定さえされることを示したものである。