of the starting materials were 60, 68, and 91%, respectively. These data show a higher reactivity of **3** compared with those of **14**, **15**, and **4**, and support that the mechanism of the reaction of **3** involves an interaction between the methyl group and the oxygen atom, on the formation of the radical **1**, in such a way as shown in Chart 2.[8]

References and Notes

1) The radicals **1** and **2** have been proposed to be the key intermediates in the acyloxy rearrangements of the *N*-oxides **3** and **4**.[2,3] While the presence of **1** is yet unlikely[3,4] in the reaction of **3** with acetic anhydride, **2** has been shown to be present in a similar reaction of **4**.[5]
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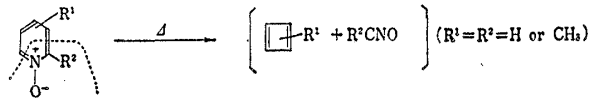
7) The *N*-oxide **3** underwent a complete decomposition at 650° giving aforementioned products, although the data of fvp of **3** at 650° are eliminated in Table. The data will be shown in a future paper.
8) A heterolytic cleavage of the N–O bond of **3** also may be undeniable for the formation of **5** (at least in part).
9) RH represents molecules which bear one or more hydrogen atoms. R· means each radical involved in the reaction system.
10) Several attempts to detect compounds expected from a decomposition as given by



were failed, as well as in fvp of **3** (even in run c).

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Synthesis of Stereoisomeric Suc-Tyr-Leu-Val-*p*NA and Their Properties as Substrate and Inhibitor for Human Spleen Fibrinolytic Proteinase (SFP)[1]

Stereoisomeric analogues of Suc–Tyr–Leu–Val–*p*NA were synthesized in the conventional manner and their properties as the substrate and/or the inhibitor against human spleen fibrinolytic proteinase (SFP) were tested. Suc–D–Tyr–L–Leu–L–Val–*p*NA (II) was hydrolyzed to release *p*-nitroaniline with K_{cat}/K_m value (3700), whereas K_{cat}/K_m value of Suc–L–Tyr–L–Leu–L–Val–*p*NA (I) was 22647. Suc–L–Tyr–D–Leu–D–Val–*p*NA (III) inhibited the hydrolytic activity of SFP towards both the peptide (I) and fibrin.

Keywords——Suc–Tyr–Leu–Val–*p*NA; stereoisomer; peptide synthesis; synthetic substrate; synthetic inhibitor; human spleen fibrinolytic proteinase; inhibition of fibrinolysis

A neutral proteinase (SFP) capable of degrading fibrin and fibrinogen was isolated and purified from human spleen tissue.[2] The most properties of this enzyme examined[2] are similar to those of elastase, especially to those of human leucocyte elastase which has attracted our interest because of its involvement in tissue destruction occurring in pulmonary emphysema.[3] Recently, we reported that newly synthesized chromogenic substrate, Suc–L–Tyr–L–Leu–L–Val–*p*NA (I), was specific for SFP.[4,5] The values of K_{cat}/K_m for hydrolysis of I by SFP and porcine pancreatic elastase were 22647 and 21 respectively, whereas those for hydrolysis of the most widely used peptide substrate for elastase, Suc–$(Ala)_3$–*p*NA[6,7] by SFP and porcine pancreatic elastase were 488 and 4000 respectively. With regard to human leucocyte elastase, it was reported that the substitusion of Ala by Val in position P_1 of Suc–$(Ala)_3$–*p*NA led to significantly specific substrates for that elastase.[8–10] From these results, it was deduced SFP and human leucocyte elastase would have similar substrate specificity. Therefore, the development of substrates and/or inhibitors for SFP would promise a possible application of them to human leucocyte elastase resulting in their diagnostic and therapeutic use. The interesting properties of stereoisomeric Z–$(Ala)_3$–OMe as substrates for SFP and porcine pancreatic elastase were also described previously.[11]

In this communication, we wish to report the synthesis of stereoisomeric Suc–Tyr–Leu–Val–*p*NA and their properties as the substrate and/or the inhibitor for human spleen fibrinolytic proteinase (SFP). Synthetic route to eight kinds of stereoisomeric tripeptide is illustrated in Fig. 1. Boc–Leu–OH and H–Val–*p*NA were coupled by DCC–HOBt method[12] to give Boc–Leu–Val–*p*NA. After removal of Boc group by HCl/dioxane, Boc–Tyr–OH was coupled with DCC–HOBt to afford Boc–Tyr–Leu–Val–*p*NA, which was treated with HCl/dioxane followed by succinylation with succinic anhydride to give desired stereoisomeric peptide deri-

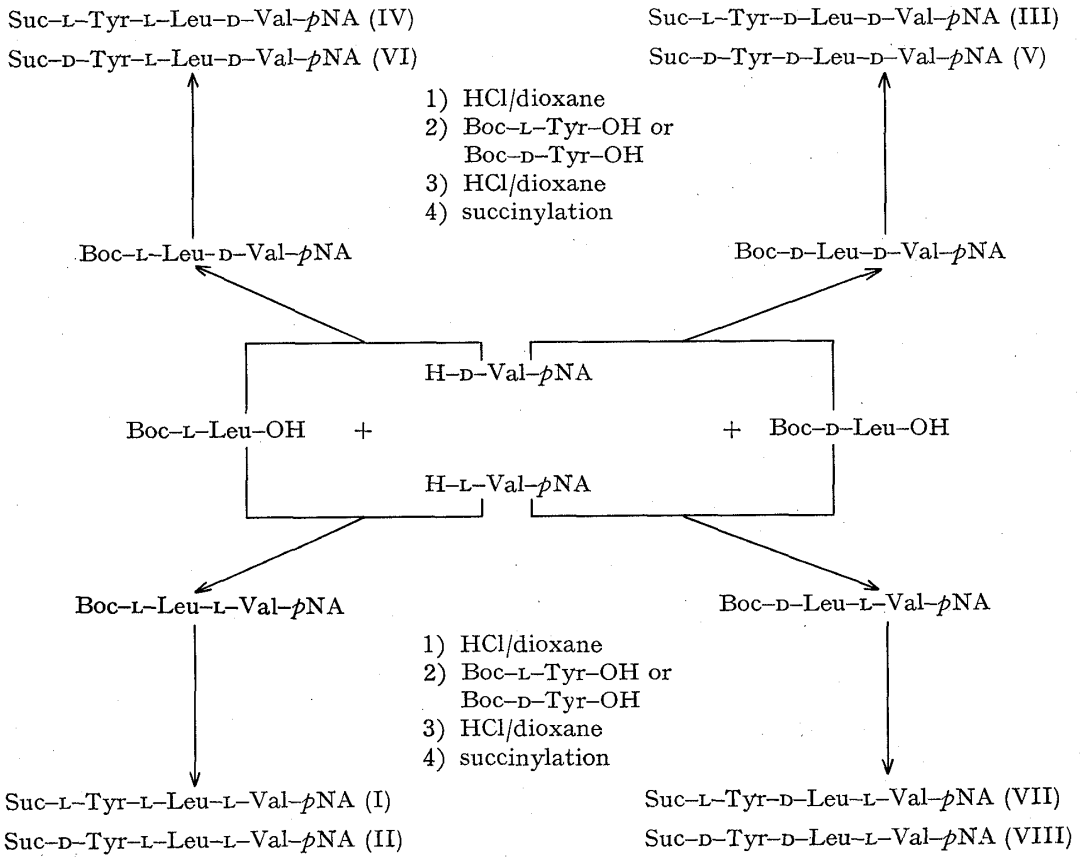

Fig. 1. Synthetic Scheme for the Stereoisomeric Suc–Tyr–Leu–Val–*p*NA

Table I. Results of Amino Acid Analysis and *Rf* Values of Suc–Tyr–Leu–Val–*p*NA

Compound No.		Amino acid ratios of acid hydrolysate (110°, 20 hr)			TLC	
		Tyr	Leu	Val (recovery, %)	Rf^1	Rf^2
I	L–L–L	0.95	0.98	1.00 (82)	0.38	0.48
II	D–L–L	0.80	1.00	1.00 (80)	0.35	0.46
III	L–D–D	0.94	0.98	1.00 (83)	0.35	0.46
IV	L–L–D	0.92	1.02	1.00 (82)	0.36	0.47
V	D–D–D	0.98	1.00	1.18 (85)	0.38	0.48
VI	D–L–D	1.09	0.99	1.00 (80)	0.31	0.46
VII	L–D–L	0.84	0.91	1.00 (85)	0.31	0.46
VIII	D–D–L	0.94	1.05	1.00 (86)	0.36	0.47

Thin-layer chromatography was performed on silica gel (Kieselgel G, Merck). Rf^1 and Rf^2 values refer to the systems of $CHCl_3$, MeOH and AcOH (90:8:2), and $CHCl_3$, MeOH and H_2O (8:3:1, lower phase), respectively.

Table II. Comparative Inhibitory Effect on Amidolysis of Suc–L–Tyr–L–Leu–L–Val–*p*NA by SFP

P_1 P_2 P_3	K_i(mM)	% inhibition[a)]
Suc–L–Tyr–D–Leu–D–Val–*p*NA (III)	0.12	54
Suc–L–Tyr–L–Leu–D–Val–*p*NA (IV)	0.19	44
Suc–D–Tyr–D–Leu–D–Val–*p*NA (V)	0.20	42
Suc–D–Tyr–L–Leu–D–Val–*p*NA (VI)	0.24	37
Suc–L–Tyr–D–Leu–L–Val–*p*NA (VII)	0.35	29
Suc–D–Tyr–D–Leu–L–Val–*p*NA (VIII)	0.94	6

a) The reaction mixture contained 0.4 mM of inhibitor, 0.3 mM of substrate, I and SFP.

Table III. Inhibitory Effect of Suc–L–Tyr–D–Leu–D–Val–*p*NA on Fibrinolysis by SFP

Case No.	Inhibitor concentration in fibrin plate (mM)			
	2.5	1.25	0.625	0
1	0	0	0	42
2	0	0	100	87
3	0	0	145	121
4	40	320	480	490

The figures in the table represent average values of lysis area (mm^2) by SFP.

vatives (Table I). They were homogeneous upon thin-layer chromatography on silica gel as shown in Table I.

The properties of these peptides as the substrate and/or the inhibitor for SFP were tested. The peptide, D–L–L (II), was hydrolyzed to release *p*-nitroaniline with lower K_{cat}/K_m value (3700) than that of L–L–L (I) (K_{cat}/K_m, 22647).[4,5] Other peptides, III, IV, V, VI, VII and VIII were not hydrolyzed by SFP. However, it was of interest that they exhibited an inhibitory effect on amidolytic activity of SFP towards I and II. The inhibitory effect is summarized in Table II. From the results, it can be seen that Suc–L–Tyr–D–Leu–D–Val–*p*NA (III) inhibited SFP activity towards I most effectively. The peptide (III) also exhibited a significant inhibitory effect on fibrinolytic activity of SFP in a dose-response manner as shown in Table III. The peptide (III) is useful for development of a new type of inhibitor for SFP and human leucocyte elastase.

References and Notes

1) Abbreviations used are those recommended by the IUPAC–IUB Commission on Biochemical Nomenclature: *Biochemistry*, **5**, 2485 (1966); *ibid.*, **6**, 362 (1967); *ibid.*, **11**, 1726 (1972). Z=benzyloxycarbonyl, Boc=*tert*-butyloxycarbonyl, *p*NA=*p*-nitroanilide, OMe=methyl ester, Suc=succinyl.
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Enzymatic Formation of 5-Fluorouracil from 1-(Tetrahydro-2-furanyl)-5-fluorouracil (Tegafur) in Human Tumor Tissues

1-(Tetrahydro-2-furanyl)-5-fluorouracil (Tegafur) was phosphorolyzed to form 5-fluorouracil (5-FU) by a soluble fraction of human lung cancer. The catalysis was suppressed in the presence of excess thymidine, but not in the presence of 1-(2′-deoxy-β-D-glucopyranosyl)thymine, an inhibitor of uridine phosphorylases. The cleavage of Tegafur to 5-FU was assumed to be catalyzed by a thymidine phosphorylase activity, which is greatly enhanced in the human tumor tissues, and to represent a possible activation mechanism of Tegafur.

Keywords——1-(tetrahydro-2-furanyl)-5-fluorouracil; 5-fluorouracil; human tumor; lung cancer; thymidine phosphorylase; thymidine; 1-(2′-deoxy-β-D-glucopyranosyl)-thymine

1-(Tetrahydro-2-furanyl)-5-fluorouracil (Tegafur, FT-207) has shown an antitumor activity with a spectrum of activity similar to 5-fluorouracil (5-FU) and is considered to be a chemical depot form of 5-FU.[1] Metabolites hydroxylated at the tetrahydrofuranyl moiety were found in the urine and the plasma after the administration of Tegafur.[2–4] The hydroxylated metabolites were formed *in vitro* by rat liver microsomes.[5] One of the metabolites, 1-(4-hydroxytetrahydro-2-furanyl)-5-fluorouracil, was converted to 5-FU, while Tegafur was not, by a horse liver thymidine phosphorylase preparation.[2] It has been believed that 5-FU is generated *in vivo* by hepatic metabolism involving cytochrome P-450.[6–9] Recently, Au