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Amino Acids and Peptides. VI.[1,2] Curtius Rearrangement of Acyl Amino Acid and Peptide Azides and Reactivity of the Isocyanates

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Studies on the rate of Curtius rearrangement of acyl amino acid and peptide azides were carried out by means of IR (infrared) spectrophotometry at 25°. It was found that Z–Gly–N_3 and Z–Pro–N_3 were more stable than the other acyl amino acid azides. The reactivity of isocyanates derived from azides with side chain functional groups of various amino acids or additives was also studied. It was found that isocyanates thus obtained were decomposed in the presence of triethylamine, its hydrochloride or 1-hydroxybenzotriazole at 25°.

Keywords——acyl amino acid azides; Curtius rearrangement; IR spectra; reactivity of isocyanate; side chain functional group; additives

One of the commonly used peptide formation procedures is the azide method developed by Curtius[4] and modified by Honzle and Rudinger.[5] The great advantage of this method is that racemization is minimized and the main disadvantage is the tendency of the azide to rearrange to the isocyanate, resulting in the formation of a urea derivative instead of a peptide during the peptide synthesis.[6,7] For fragment condensation by the azide method, two strategies are currently adopted, one being azide coupling between two large peptides and the other being acylation of the amino component of a relatively large peptide with a large excess of relatively small peptide azides.[8] In the latter method, unreacted excess azide components can be removed by washing with organic solvent or by column chromatography, but it is inevitable that the azide components rearrange to the corresponding isocyanates during the isolation of the desired peptide. It is well known that isocyanate reacts with primary or secondary amine,[9] substituted guanidine hydrochloride,[10] indole nitrogen[11] and alcohols and phenols.[12] Therefore, it is of interest to investigate the reactivity of isocyanate formed from an acyl amino acid or peptide azide with the functional groups of amino acid residues.

This report deals with the rate of rearrangement of various acyl amino acid and peptide azides to the corresponding isocyanates at 25°, and with the reactivity of the resulting isocyanates. The rate studies on the rearrangement were carried out by measuring the infrared (IR) band of ν_{CON_3} in the region of 2130—2180 cm^{-1} and ν_{NCO} at 2270 cm^{-1}.[7] As shown in

1) Part V: Y. Okada, S. Iguchi, M. Mimura, and M. Yagyu, *Chem. Pharm. Bull.*, **28**, 1320 (1980).
2) 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). Amino acids, peptides and their derivatives mentioned in this paper are of the L-configuration except in the case of glycine.
3) Location: *Ikawadani-machi, Tarumi-ku, Kobe, 673, Japan.*
4) Th. Curtius, *Ber.*, **35**, 3226 (1902).
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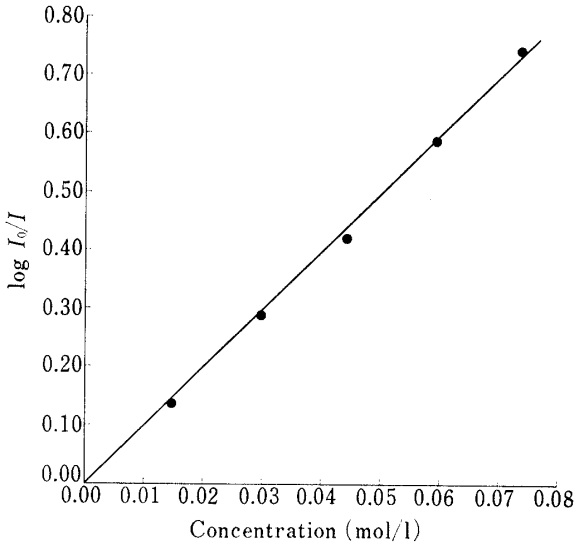

Fig. 1. Plot of log I_0/I *vs.* the Concentration of 1-Benzyloxycarbonylaminoethyl Isocyanate at Room Temperature (25°)

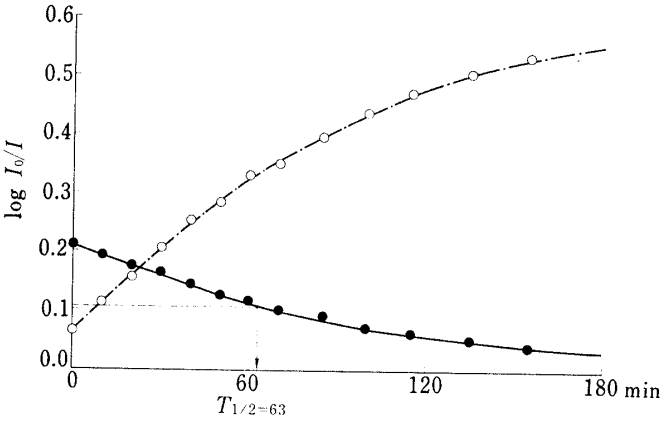

Fig. 2. The Rate of Curtius Rearrangement of Z–Ala–N_3 at 25°

—●—: azide (2140 cm^{-1}).
----○----: isocyanate (2250 cm^{-1}).

Fig. 1, a straight line relationship between log I_0/I and the concentration of 1-benzyloxycarbonylaminoethyl isocyanate (I) (rearranged product from Z–Ala–N_3) was obtained. This showed that the concentration of isocyanate was proportional to log I_0/I. A proportional relation between log I_0/I and the concentration of azide was also reported previously.[13]

Benzyloxycarbonyl (Z)-amino acid hydrazides or Z-peptide hydrazides were converted to the corresponding azides in the usual manner, and these were extracted with $CHCl_3$. The decrease in absorbance of ν_{N_3} and the increase in absorbance of ν_{NCO} were measured without isolation to determine the rate of the Curtius rearrangement.

In the course of the rearrangement, the decrease in absorbance of ν_{N_3} at 2140 cm^{-1} and the increase in absorbance of ν_{NCO} at 2250 cm^{-1} were observed. The half-life of azide in $CHCl_3$ was determined as illustrated in Fig. 2. The half-lives of Z-amino acid azides (amino acid=Ala, His, Ile, Phe, Ser, Thr, Leu and Tyr) were 20—120 min but Z–Gly–N_3 and Z–Pro–N_3 were more stable than the above amino acid azides at 25° (half-lives: 610 min and 420 min respectively), as summarized in Table I. It was also found that Z–Ser–Pro–N_3[14] and Z–Ala–Pro–N_3 were fairly stable at 25° (half-lives: 450 min).

Table I. Rate of Curtius Rearrangement of N-Protected Amino Acid Azide (25°)

Amino acid	Half-life of azide (min)
Alanine	63
	14 (at 40°)
Glycine	610
Histidine	115
Isoleucine	22
Leucine	34
Proline	420
Phenylalanine	31
Serine	119
Threonine	37
Tyrosine	56

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In this study, we found that no absorbance of ν_{NCO} appeared in the case of Z–Ser–N_3 or Z–Thr–N_3 even though the absorbance due to the azide of these derivatives decreased, and that the rate of formation of isocyanate from Z–amino acid azide (amino acid=Ala, His, Ile, Leu, Phe, Tyr, Gly and Pro) was proportional to the rate of decrease of the corresponding azide, as expected. This was due to the fact that intramolecular reaction of isocyanate with the hydroxyl group of serine or threonine occurred immediately to form the corresponding azlactone.[15,16] Further, the Curtius rearrangement of Z-peptide azides, Z–Phe–Ser–N_3[14]

Table II. Effects of Amino Acid Derivatives on 1-Benzyloxycarbonylaminoethyl Isocyanate (I)

Amino acid derivative	Temperature (°C)															
	25								40							
	Time (hr)								Time (hr)							
	0		1		2		3		0		1		2		3	
	Mol. 1	eq. 3	Mol. 1	eq. 3	Mol. 1	eq. 3	Mol. 1	eq. 3	Mol. 1	eq. 3	Mol. 1	eq. 3	Mol. 1	eq. 3	Mol. 1	eq. 3
None	100	100	100	100	100	100	100	100	100	100	100	100	100	100	100	100
Z–Arg–OH·HCl	111	—	103	—	97	—	111	—	108	—	99	—	81	—	95	—
Z–Arg–OH	113	—	103	—	115	—	108	—	108	—	115	—	98	—	103	—
Z–Arg(NO_2)–OH	111	99	102	96	104	79	105	82	108	84	118	98	107	82	107	75
Z–Ser–OMe	97	96	103	100	78	89	88	92	87	83	85	80	80	67	67	69
Z–Thr–OMe	95	102	93	104	86	100	88	101	97	95	95	93	102	87	91	82
Z–His–OMe	103	97	87	85	99	94	97	92	99	92	98	93	95	95	93	93
Z–Tyr–OMe	91	88	102	97	95	94	97	92	90	100	100	100	100	94	79	79
Z–Trp–OMe	96	78	90	69	100	62	93	56	83	76	75	38	42	28	36	20

The time of adding one or three equivalents of amino acid derivative to the isocyanate solution was taken as zero time. The amount of isocyanate which remained was determined by measuring the absorbance of ν_{NCO} as a function of time. Data are expressed as a ratio to the amount of isocyanate in the solution to which no amino acid derivative had been added.

Table III. Effects of Additives on 1-Benzyloxycarbonylaminoethyl Isocyanate (I)

Additive	Time (min)				
	0	10	60	120	
None	100	100	100	100	
Water	100	98	102	95	
CH_3COOH	100	77	80	78	
Phenol	100	98	98	96	
Cresol	100	96	96	102	
Pentachlorophenol	100	102	97	92	
Hydroquinone	100	97	103	101	
Resorcinol	100	98	93	93	
2,4-Dinitrophenol	100	100	98	98	
8-Hydroxyquinoline	100	100	96	100	
1-Hydroxybenzotriazole	100	77	49	34	
	100	54	34	22	(at 40°)
N–Hydroxysuccinimide	100	93	89	80	
	100	84	66	54	(at 40°)
Et_3N	100	0	0	0	
Et_3N·HCl	100	54	14	0	

The time of adding one equivalent of additive to the isocyanate solution was taken as zero time. The amount of isocyanate which remained was determined by measuring the absorbance of ν_{NCO} as a function of time. Data are expressed as a ratio to the amount of isocyanate in the solution to which no additive had been added.

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and Z–Pro–Ser–N_3, was studied. No absorbance of ν_{NCO} was observed, even though the absorbance of ν_{N_3} at 2140 cm^{-1} decreased. From the chloroform solution of these azides, solid materials were obtained and identified as azlactone derivatives formed from the starting peptide azides. Hence, it was deduced that a large excess of N-protected peptide azide which had a serine or threonine residue at the C-terminus, would rearrange to the corresponding isocyanate, followed by immediate formation of the azlactone derivative without any intermolecular reaction with side chain functional groups of constituent amino acid residues in the peptide.

Next, the reactivity of isocyanate with side chain functional groups of various amino acids was studied. Z–Ala–N_3 in $CHCl_3$ solution was stored for 3 hr at room temperature (25°) to form the corresponding isocyanate (I). Next, one equivalent or 3 equivalents of amino acid derivative was added at 25° or 40°. The concentration of isocyanate which remained in the solution was determined by measurement of ν_{NCO} at 2250 cm^{-1}, and the results are summarized in Table II.

It was found that the concentration of isocyanate decreased markedly when mixed with tryptophan derivative, although it is not known yet whether the indole moiety of tryptophan reacted with isocyanate or catalyzed isocyanate polymerization.

The effects of additives on isocyanate at 25° were also studied. After addition of one equivalent of various additives, ν_{NCO} at 2250 cm^{-1} was followed as a function of time. The results are summarized in Table III. 1-Hydroxybenzotriazole, triethylamine and its hydrochloride affected isocyanate markedly, decreasing the absorbance of ν_{NCO} at 25°, and N-hydroxysuccinimide also affected it at 40°. It was concluded that isocyanate derived from excess azide during peptide synthesis might be decomposed in the presence of triethylamine, its hydrochloride or 1-hydroxybenzotriazole at 25°. Therefore, the reaction of isocyanate with side chain functional groups of amino acid residues in the peptide might be avoided in the presence of these reagents.

Experimental

All melting points were determined using a Yamato melting point apparatus (model MP-21) and are uncorrected. Optical rotations were taken with an automatic polarimeter (model DIP-180, Japan Spectroscopic Co. Ltd.). IR spectra were measured on a Hitachi 260-30 spectrophotometer.

General Procedure for Studies of the Rate of Rearrangement of Z-Amino Acid Azides——Z-amino acid hydrazide (3 mmol) was dissolved in 1 N HCl (10 ml) cooled with ice-salt. $NaNO_2$ (0.4 g) in water (1.0 ml) was added to the cold solution with stirring. After 5 min, the azide was extracted with $CHCl_3$ (15 ml). The extract was washed with 5% $NaHCO_3$ and water and dried over Na_2SO_4. The time of transferring the azide solution from a cold room (4°) to the laboratory (25°) was taken as zero time. The IR absorption of the samples from 2500 to 2000 cm^{-1} was examined *versus* pure solvent ($CHCl_3$) as a function of time.

Z-Pro-Ser-OMe——H–Ser–OMe (prepared from 15.1 g of H–Ser–OMe·HCl and 13.6 ml of triethylamine) and Z–Pro–ONp (35.9 g) were dissolved in dioxane (100 ml) and stirred at room temperature overnight. After removal of the solvent, the residue was extracted with AcOEt. The extract was washed with 1 N HCl, 5% Na_2CO_3 and water, dried over Na_2SO_4 and concentrated. Petroleum ether was added to the residue to afford a solid mass, which was collected by filtration and recrystallized from AcOEt; yield 22.3 g (75.4%), mp 89—90°, $[\alpha]_D^{25}$ −55.8° (c=1.0, MeOH). *Anal.* Calcd for $C_{17}H_{22}N_2O_6$: C, 58.3; H, 6.32; N, 8.0. Found: C, 58.6; H, 6.39; N, 8.0.

Z-Pro-Ser-NHNH$_2$——Hydrazine hydrate (80%, 0.34 ml) was added to a solution of Z–Pro–Ser–OMe (1.0 g) in MeOH (15 ml). The solution was kept at room temperature overnight. Crystalline material was collected by filtration and washed with MeOH and ether; yield 0.80 g (80%), mp 179—182°, $[\alpha]_D^{25}$ −35.9° (c=1.0, DMF). *Anal.* Calcd for $C_{16}H_{22}N_4O_5$: C, 54.8; H, 6.32; N, 15.9. Found: C, 55.1; H, 6.31; N, 15.7.

4-(Z-Phe-amino)oxazolidone-2——Z–Phe–Ser–N_3 was prepared in a cold room (4°) as follows. Z–Phe–Ser–$NHNH_2$ (0.4 g) was dissolved in 1 N HCl (8 ml) and cooled with ice-salt. $NaNO_2$ (0.1 g) was added to the cold solution and the whole was stirred for 5 min. The azide was extracted with $CHCl_3$ (15 ml), which was washed with 5% $NaHCO_3$ and water, and dried over Na_2SO_4. The solution was kept at room temperature (25°) for 3 hr. A white precipitate that formed was collected by filtration; yield 0.2 g (53%), mp 199—201°, $[\alpha]_D^{25}$ −16.7° (c=0.5, DMF). *Anal.* Calcd for $C_{20}H_{21}N_3O_5 \cdot 1/2H_2O$: C, 61.2; H, 5.65; N, 10.7. Found: C, 61.5; H, 5.62; N, 10.7.

4-(Z-Pro-amino)oxazolidone-2——Z–Pro–Ser–N_3 was prepared in a cold room (4°) as follows. Z–Pro–Ser–$NHNH_2$ (0.35 g) was dissolved in 1 N HCl (3 ml) and cooled with ice-salt. $NaNO_2$ (0.1 g) in H_2O (0.5 ml) was added to the cold solution with stirring. After 5 min, the azide was extracted with $CHCl_3$ (15 ml), and the extract was washed with 5% $NaHCO_3$ and water, then dried over Na_2SO_4. The solution was kept at room temperature (25°) overnight and then concentrated. Petroleum ether was added to the residue to form a white precipitate, which was collected by filtration and recrystallized from AcOEt; yield 0.09 g (27%), mp 193—195°, $[\alpha]_D^{25}$ −47.4° (c=0.8, DMF). *Anal.* Calcd for $C_{16}H_{19}N_3O_5$: C, 57.6; H, 5.74; N, 12.6. Found: C, 57.6; H, 5.47; N, 12.5.

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Studies on the Constituents of Asclepiadaceae Plants. XLVIII.[1] 5α,6α-Epoxycaudatin, a New Polyoxypregnane Derivative from *Cynanchum caudatum* Max.

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A new polyoxypregnane derivative, 5α,6α-epoxycaudatin, was isolated from *Cynanchum caudatum* Max. and its structure was elucidated on the bases of physical data and chemical reaction. This compound is a probable intermediate in the biosynthesis of 5,6-glycolic compounds. Kidjoranin was also isolated from this plant for the first time.

Keywords——5α,6α-epoxycaudatin; polyoxypregnane; Asclepiadaceae; *Cynanchum caudatum* Max.; kidjoranin; epoxidation; 5,6-glycolpregnane; 5α,6α-epoxycynanchogenin

The structures of several polyoxypregnane derivatives from *Cynanchum caudatum* Max. (Asclepiadaceae) were reported in our previous paper.[3] From the same crude aglycone mixture, cynanchogenin (II),[4] caudatin (III),[5] penupogenin (VI),[6,7] 20-O-cinnamoylsarcostin (VII),[3] ikemagenol derivatives (VIII) and (IX),[3] compound I and compound V were isolated. The spectral data and *Rf* values on thin-layer chromatography (TLC) of compounds (I) and (V) were similar to those of penupogenin and caudatin, respectively. Compound (I), mp 146—149°, was found to be identical with kidjoranin[6] by comparison of the spectral data and mixed mp (145—150°) with those of an authetic sample.

The object of the present paper is to report the structure elucidation of a new compound, V. V showed the following properties: mp 215—219.5° $[\alpha]_D$ −30° (c=0.23, MeOH), molecular

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