

Synthesis and Configurational Analysis of *dl*-Myxoviromycin

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dl-Myxoviromycin and its configurational isomer (epimer) were synthesized by a series of reactions shown in Chart 1. The *cis* configuration was proposed for myxoviromycin from the configurational analysis.

Myxoviromycin is an antibiotic, isolated by Kuroya and Ishida^{2,3)} in 1956 from cultured broth of streptomyces, and remarkable for its antiviral activities. On the other hand, Umezawa and Nakamura^{4,5)} reported an antibiotic named amidinomycin and proposed the planar structure of N-(2-amidinoethyl)-3-aminocyclopentanecarboxamide (I) for amidinomycin, and later the identity of both antibiotics was confirmed.⁶⁾

In this paper, a total synthesis of *dl*-myxoviromycin and its epimeric isomer was reported and also their configurations were discussed.

The reactions involved in this total synthesis were shown in Chart 1.

The starting material, *cis*-3-aminocyclopentanecarboxylic acid (III) was obtained by the catalytic hydrogenation of 3-hydroxyiminocyclopentanecarboxylic acid (II) according to the modified Nakamura's method.⁵⁾

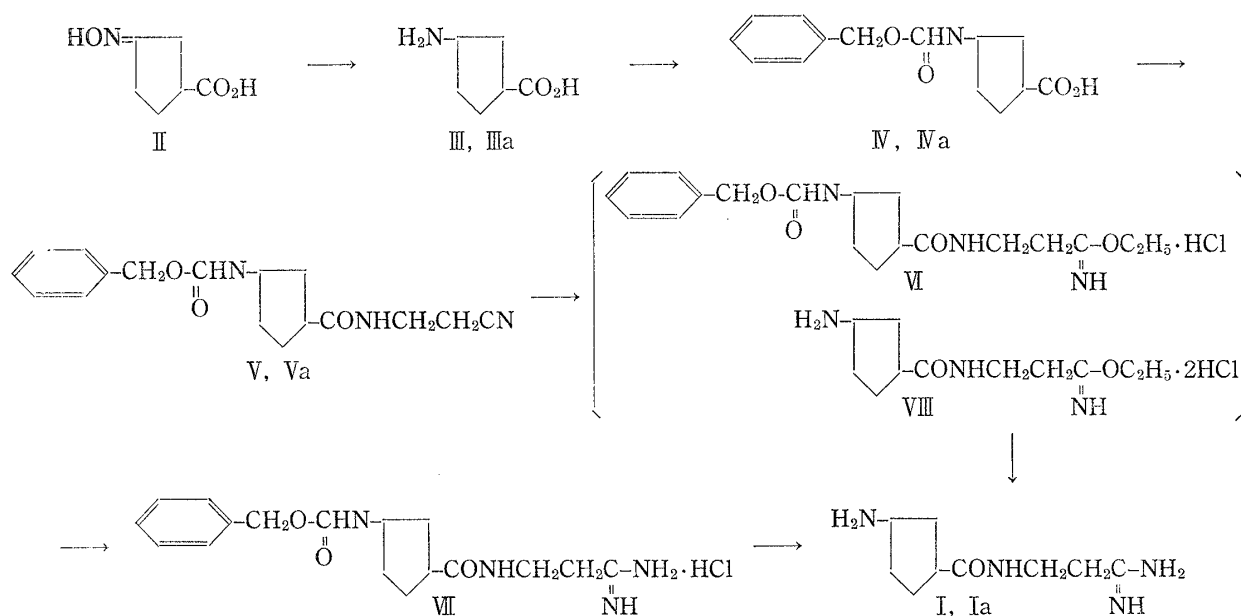


Chart 1

1) Location: 278, Kasugade-cho, Konohana-ku, Osaka.

2) M. Kuroya, K. Kikuchi, N. Kobayashi, K. Matsumoto, T. Chiba, S. Matsuura, and Y. Hinuma, *Jap. J. Microbiol.*, **1**, 85 (1957).

3) N. Ishida, M. Kuroya, J. Shōji, and K. Katagiri, *J. Antibiotics* (Japan), **14**, Ser. A, 165 (1961).

4) S. Nakamura, K. Karasawa, N. Tanaka, H. Yonehara, and H. Umezawa, *J. Antibiotics* (Japan), **14**, Ser. A, 103 (1961).

5) S. Nakamura, *Chem. Pharm. Bull.* (Tokyo), **9**, 641 (1961).

6) S. Nakamura, H. Umezawa, and N. Ishida, *J. Antibiotics* (Japan), **14**, Ser. A, 163 (1961).

The amino acid (III) was benzyloxycarbonylated with benzyl chloroformate and aqueous sodium hydroxide, giving the benzyloxycarbonyl amino acid (IV), which was chlorinated with thionyl chloride in benzene, followed by the condensation with 3-aminopropionitrile in ether to afford N-(2-cyanoethyl)-3-benzyloxycarbonylaminocyclopentanecarboxamide (V), and its infrared and nuclear magnetic resonance spectrum supported its structure.

The cyanoethylamide (V) was treated with dry hydrogen chloride and ethanol in benzene at about 5°. The amorphous powder obtained, was treated with alcoholic ammonia to afford a sirupy material, which was proved to be a mixture composed of N-(2-amidinoethyl)-3-benzyloxycarbonylaminocyclopentanecarboxamidehydrochloride (VII) and its de-benzyloxycarbonylated product corresponding to I.

Separation of the benzyloxycarbonylated amidine (VII) from the de-benzyloxycarbonylated one could be accomplished with an adsorption chromatography on an active carbon column. The de-benzyloxycarbonylated amidine could be easily eluted by water, which was proved to be I by conversion to the sulfate.³⁾ Subsequently, VII could be eluted by 50% aqueous methanol which was characterized as the picrate.

Such de-benzyloxycarbonylation might have occurred in the alcoholysis process rather than in the amidination, because the same type of de-benzyloxycarbonylation was known,⁷⁾ and its ratio seemed to depend on the amount of hydrogen chloride. The alcoholysis product was, therefore, assumed to be a mixture of the benzyloxycarbonylated imino ether (VI) and its de-benzyloxycarbonylated one (VIII).

The crude benzyloxycarbonylated amidine (VII) contaminated by I was advantageously hydrogenated catalytically with palladium-charcoal in dilute acetic acid, giving the crude amine (I), which was adsorbed on the ion-exchange resin column (IRC-50, sodium type) and developed with dilute sulfuric acid.³⁾ At first, a very small amount of by-product was eluted, which gave a positive reaction with sodium nitroprusside reagent (white pink). Then, the sulfate was obtained as colorless needles which gave a positive reaction with ninhydrin (violet) and sodium nitroprusside reagent⁸⁾ (red).

The sulfate did not show a definite melting point, but gradually decomposed at about 285° (in sealed tube) like myxoviomycin and had no optical activity. The infrared spectrum of this sulfate was in agreement with that of myxoviomycin as shown in Fig. 1. From these data described above with the satisfactory elemental analysis and its biological activities,⁹⁾ the synthetic compound I was proved to be the racemic myxoviomycin.

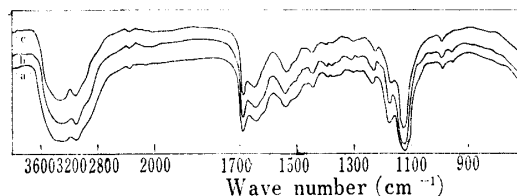


Fig. 1. Infrared Spectra (in 0.25% KBr disk) of (a) Myxoviomycine-sulfate, (b) *dl*-N-(2-Amidinoethyl)-3-aminocyclopentanecarboxamide (I), -sulfate (c) Its Epimer (Ia)-sulfate

The structure of the by-product detected by the ion-exchange chromatography was assumed to be an amide type derivative (IX), because the *R_f* value of this by-product by paper chromatography¹⁰⁾ was the same as that of the product obtained by the reductive de-benzyloxycarbonylation of N-(2-carbamoyl-ethyl)-3-benzyloxycarbonylaminocyclopentanecarboxamide (X). X was isolated when V was alcoholized at about 30°.

Therefore, the by-product (IX) might have been formed *via* the route shown in Chart 2.

In the procedure from II to III, it seems probable that the *trans* amino acid (IIIa) was produced as well as the *cis* amino acid (III), by analogy with the case of hydrogenation of

7) G. Hilgetag, H. Paul, J. Gunter, and M. Witt, *Chem. Ber.*, **97**, 704 (1964).

8) C.J. Weber, *J. Biol. Chem.*, **78**, 465 (1928).

9) Unpublished.

10) *R_f* value was 0.33–0.35 with the developing solvent (BuOH: AcOH: Pyridine: H₂O=4:1:1:2) on Tōyō Rōshi No. 50.

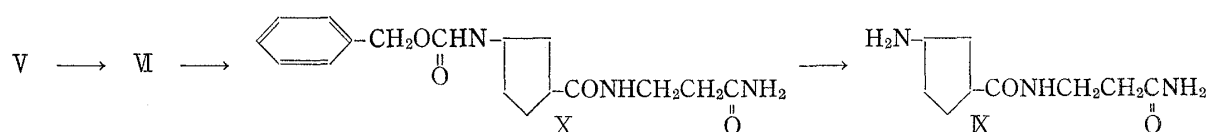


Chart 2

hydroxyiminocyclohexanecarboxylic acid,¹¹) but the isolation of this epimer (IIIa) was unsuccessful.

However, the epimer (Va) corresponding to V could be isolated as follows.

The crude amino acid product obtained by hydrogenation of II, was benzyloxycarbonylated and amidated without purification, giving an epimeric mixture of the cyanoethylamide (V+Va).

Recrystallization of the crude cyanoethylamide from ethyl acetate gave V as a main product. Then, the mother liquor was chromatographed on a silica-gel column with benzene and benzene-ethyl acetate to separate three components; an oily material, the needles (V), mp 139°, and colorless needles (Va), mp 126—127°, in this order. But the definite separation of V and Va was rather difficult and therefore, both components (V and Va) were eluted overlapping each other to a large extent, giving the mixed needles (mp about 100—120°). Va was considered to be the epimer of V, because the infrared and the nuclear magnetic resonance spectrum were very similar to those of V.

Meanwhile, the oily material changed to a crystalline mass on standing *in vacuo*, whose structure was suggested to be N-benzyloxycarbonyl-lactam derivative (XI) as shown in Chart 3 from its spectral data and a similar lactamization.¹²)

By the same procedure as described in the synthesis of I, Va was converted to the amidine (Ia), the epimer of I.

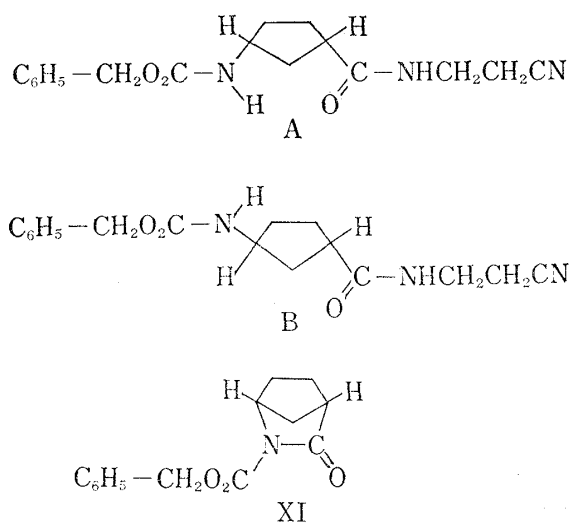


Chart 3

The sulfate (Ia) was obtained as needles having no definite melting point, but gradually decomposed at about 290° like myxoviromycin. The infrared spectrum of Ia (Fig. 1) was almost identical with those of I and myxoviromycin except for a little differences of the absorption bands (near 1470 cm⁻¹, near 1030 cm⁻¹ and 955 cm⁻¹) and from this result I was confirmed to have the same configuration as myxoviromycin. I and Ia were quite alike in their structure, but significant differences between them were observed biologically.⁹)

The configurational analysis of V by comparing with Va was attempted in order to determine the configuration of myxoviromycin,

which was considered to be the same as that of V.

Since an appreciable amount of the lactam derivative was also separated as by-product in the procedure from III to V, the *cis* form is more probable for V, because XI was considered to have derived from the *cis* configuration. But another possibility had to be considered, in which the *cis* form might be occurred by epimerization of the *trans* form and then XI might be formed, if the *cis* form is more stable than the *trans* form. However, such possibility would be rare because Va could hardly be isolated in the same procedure.

11) W. Schneider and R. Dillman, *Chem. Ber.*, **96**, 2377 (1963).

12) F.R. Hewgill and P.R. Jefferies, *J. Chem. Soc.*, 2769 (1955).

In the next place the infrared absorption of V and Va were examined. The key bands of these compounds measured in several conditions are illustrated in Fig. 2 and listed in Table I.¹³⁾

In the measurement in chloroform solution, these compounds showed two NH bands at 3460 cm^{-1} and 3360 cm^{-1} , and the former band would be assigned to the vibrations of the NH in the free state and the latter to that involved in the hydrogen bonding, from the fact that the relative intensity (3360 cm^{-1} to 3460 cm^{-1}) of two bands was changeable on dilution of the solution.¹⁴⁾ Moreover, it was found that this relative intensity of V was stronger than that of Va in every present experiment, and V showed an appreciable absorption of the band (3360 cm^{-1}) even in such a dilute solution as that the band of Va almost disappeared (about 0.0064 mole/liter). Furthermore, the two carbonyl bands of V (especially acid amide one) were found at lower frequencies than those of Va.

From these facts, V is considered to be capable of forming hydrogen bonds even in a solution so dilute that the usual intermolecular hydrogen bonding would have disappeared.

These results would be compatible with the assumption of the *cis* form for V. The *cis* configuration (A) shown in Chart 3 would be able to form either the intramolecular hydrogen bonding as *cis*-cyclohexane-1, 3-diol¹⁵⁾ or the strong intermolecular hydrogen bonding between two molecules in the ring dimer as δ -valerolactam.¹⁶⁾

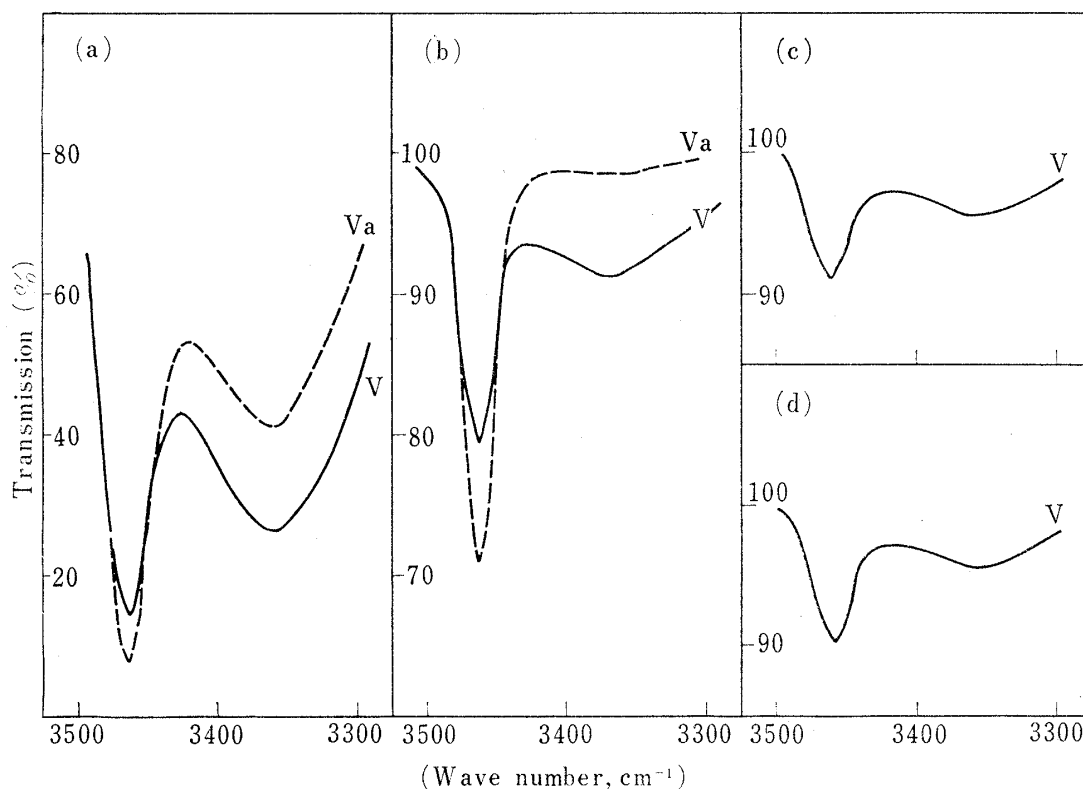


Fig. 2. Infrared Absorption Curves of N-(2-Cyanoethyl)-3-benzyloxycarbonylamino-cyclopentanecarboxamide (V), (solid line) and Its Epimer (Va), (broken line): (a) 0.064 mole/liter (Path length, 1 mm), (b) 0.0064 mole/liter (1 mm), (c) $0.00076\text{ mole/liter}$ (5 mm), (d) 0.0038 mole/liter (1 mm)

13) All the spectra were measured by the spectrophotometer, IR-27G (Shimadzu Co., Ltd.) at the temperature-regulated room (20°) except those of Fig. 4(c) and Fig. 4(d) which were measured by the spectrophotometer, DS-402G (Japan Spectroscopic Co., Ltd.) at the temperature-regulated room (22°).

14) S. Mizushima, T. Shimanouchi, S. Nagakura, K. Kuratani, M. Tsuboi, H. Baba, and O. Fujioka, *J. Am. Chem. Soc.*, **72**, 3493 (1950).

15) L.P. Kuhn, *J. Am. Chem. Soc.*, **74**, 2492 (1952).

16) M. Tsuboi, *Bull. Chem. Soc. Japan*, **22**, 215 (1949).

TABLE I. Infrared Absorption of N-(2-Cyanoethyl)-3-benzyloxycarbonyl-aminocyclopentanecarboxamide (V) and Its Epimer (Va) in CHCl_3 or Nujol (wave number, cm^{-1})

	Concentration in CHCl_3	Free NH	Hydrogen- bonded NH	Urethane CO	Acid amide CO
V	0.0032 mole/liter	3460	3360	1713	1674
	0.0064 mole/liter	3460	3360	1713	1674
	in Nujol		3335	1682	1652
Va	0.0032 mole/liter	3460		1720	1681
	0.0064 mole/liter	3460		1721, 1718	1681
	0.0128 mole/liter	3460	3360	1721, 1713	1681
	in Nujol		3335	1680	1650

In the *trans* configuration (B), on the other hand, it seems difficult to expect such special hydrogen bondings.

Moreover, the intramolecular hydrogen bonding is considered more probable than the ring dimer type hydrogen bonding, since the relative intensity measured in the concentration of 0.0038 mole/liter was found almost equal to that of 0.00076 mole/liter as shown in Fig. 2.

In the nuclear magnetic resonance spectra of V and Va measured in deuteriochloroform solution, some significant differences were observed.¹⁷⁾ The chemical shifts of two types of NH protons measured in several concentrations are listed in Table II. Of these two signals, the triplet one (found at lower magnetic field) was assigned to the proton of the acid amide NH, and the doublet one (found at higher magnetic field) to the proton of the urethane NH by comparing their spectra with that of N-methyl-N-(2-cyanoethyl)-3-benzyloxycarbonyl-aminocyclopentanecarboxamide (XII).

The signal of the urethane NH proton of V was always found at lower magnetic field than that of Va in every present experiment, if compared in the same concentrations. From the shifts to higher magnetic field on dilution, the intermolecular hydrogen bonding was considered to take place in such concentration as the present experiments were made. But from the tendency of the shifts, this significant difference between V and Va seems to be maintained even at such a dilution that the usual intermolecular hydrogen bonding would have disappeared.

In the nuclear magnetic resonance spectra, the magnetic anisotropy effect of the carbonyl group has to be considered. Inspection of the molecular model indicated the possibility in which the urethane NH proton of the *cis* form (A) and the methyne proton on 3-position of

TABLE II. Nuclear Magnetic Resonance: Chemical Shifts of NH Protons of N-(2-Cyanoethyl)-3-benzyloxycarbonylaminocyclopentanecarboxamide (V), Its Epimer (Va) and N-Methyl-N-(2-Cyanoethyl)-3-benzyloxycarbonyl-amino cyclopentanecarboxamide (XII) in CDCl_3 (ppm to TMS)

Concentration in CDCl_3	V		Va		XII Urethane NH ^{a)}	Difference of Urethane NH $\delta(\text{V}-\text{Va})$
	Urethane NH ^{a)}	Acid amide NH ^{b)}	Urethane NH ^{a)}	Acid amide NH ^{b)}		
0.10 mole/liter	5.88	6.25	4.88	6.18		1.00
0.19 mole/liter	5.92	6.40	5.00	6.38	5.90	0.92
0.38 mole/liter	6.05	6.65	5.20	6.60		0.85

a) doublet

b) triplet (these signals were not sharp)

17) All NMR spectra were measured by the spectrometer, A-60 (Varian Co., Ltd.) at 60 Mc and all chemical shifts are given in the value, ppm (δ) from TMS.

the *trans* form (B) would be deshielded by the effect of the acid amide carbonyl group like the case of α,β -olefinic acid ester.¹⁸⁾

But from the fact that the methyne proton on 3-position of Va showed the signal at the same chemical shift (4.15 ppm)¹⁹⁾ as that of V, the contribution of such an effect is considered to be small. Therefore, the difference of the urethane NH protons between V and Va might be mainly due to the intramolecular hydrogen bonding.

On the basis of these data, the *cis* configuration is proposed for V, and therefore myxoviomycin and the present synthetic *dl*-myxoviomycin are considered to have the *cis* configuration.

Experimental²⁰⁾

***cis*-3-Aminocyclopentanecarboxylic Acid (III)**—Six grams of 3-hydroxyiminocyclopentanecarboxylic acid²¹⁾ was dissolved in 150 ml of warm MeOH and hydrogenated with Adams catalyst. After filtration, evaporation of the solvent left a sirupy residue. This residue must be an epimeric mixture of *cis* and *trans*-amino acid, but paper chromatography with the developing solvent (BuOH: AcOH: Pyridine: H₂O = 4:1:1:2) showed only one spot (*R_f* value, 0.63–0.65). This residue was purified by adsorption chromatography on an active carbon column with H₂O. The eluate was fractionated and each fraction was checked by the ninhydrin test. The residue obtained by concentrating the positive fractions was recrystallized from 95% EtOH to give 2.0 g of III in colorless prisms (40%), which showed the same melting point (241–242° with decomp.) and *R_f* value (0.41, HCl salt type) as those of the literature.⁵⁾

***cis*-3-Benzylloxycarbonylaminocyclopentanecarboxylic Acid (IV)**—To a stirred mixture of 1.6 g of III, 3.15 g of benzyl chloroformate and 20 ml of H₂O was added dropwise a solution of 0.95 g of NaOH in 10 ml of H₂O, care being taken not to make the reaction mixture too strongly basic. The resultant aq. solution, after removal of the excess benzyl chloroformate with benzene, was acidified with dil. HCl and allowed to stand overnight in a refrigerator to give 2.95 g (94%) of colorless needles (IV), mp 79–81°. *Anal.* Calcd. for C₁₄H₁₇O₄N: C, 63.89; H, 6.50; N, 5.32. Found: C, 63.90; H, 6.22; N, 5.53.

***cis*-N-(2-Cyanoethyl)-3-benzylloxycarbonylaminocyclopentanecarboxamide (V)**—A mixture of 0.50 g of IV and 0.50 g of freshly distilled SOCl₂ in 5 ml of abs. benzene was refluxed for 1 hr with a trace of pyridine. After removal of the solvent, the residue (acid chloride) was dissolved in 10 ml of ether and treated with 0.30 g of 3-aminopropionitrile. Evaporation of the solvent and discharging the resultant residue onto cooled H₂O gave 0.48 g of crude, powdery (V), mp 122–130°. Repeating recrystallization from AcOEt gave 0.29 g of colorless needles (V), mp 137–139°. *Anal.* Calcd. for C₁₇H₂₁O₃N₃: C, 64.74; H, 76.1; N, 13.33. Found: C, 64.89; H, 6.88; N, 13.36. NMR_{60°C}^{CDCl₃} ppm from TMS: *ca.* 1.85 (six protons, multiplet—ring methylene), 2.57 (two protons, triplet—methylene adjacent to nitrile), 3.45 (two protons, quartet—methylene adjacent to acid amide), 5.07 (two protons, singlet—benzyl methylene), 7.30 (five protons—phenyl ring).

The mother liquor of V was chromatographed on a silica-gel column with benzene and benzene-AcOEt to separate 0.09 g (*ca.* 19%) of oily material and 0.07 g of colorless needles. The oily material was proved to be the pre-substance of the N-benzylloxycarbonyl-lactam derivative (XI) described below, and the needles, mainly V from its IR spectrum.

Separation of N-Benzylloxycarbonyl-lactam Derivative (XI) and *trans*-N-(2-Cyanoethyl)-3-benzylloxycarbonylaminocyclopentanecarboxamide (Va)—Recrystallization of 1.75 g of the crude cyanoethylamide obtained from the hydrogenated material (an epimeric mixture of III and IIIa) gave 0.7 g of V. The mother liquor of V was chromatographed on silica-gel (20 g) with benzene and benzene-AcOEt (gradually raising the ratio of AcOEt to benzene). The eluate was fractionated, and the residue obtained by concentrating each fraction was checked by means of thin-layer chromatography and their melting points. At first, 0.25 g of oily material was obtained, and this oily material changed to 0.23 g of crystalline mass of the N-benzylloxycarbonyl-lactam derivative (XI), namely, N-benzylloxycarbonyl-3,6-methano-2-piperidone. *Anal.* Calcd. for C₁₄H₁₆O₃N: C, 68.55; H, 6.16; N, 5.71. Found: C, 68.68; H, 6.21; N, 5.48. IR_{max}^{Nujol} cm⁻¹: 1805 (lactam CO), 1690 (urethane CO). NMR_{60°C}^{CDCl₃} ppm from TMS: *ca.* 1.8 (six protons—ring methylene), 2.75

18) L.M. Jackman, "Application of Nuclear Magnetic Resonance Spectroscopy in Organic Chemistry," p. 121, Pergamon Press Ltd., London.

19) The signal of the other methyne proton on 1-position of Va (2.7 ppm) was found at a little lower magnetic field than that of V (about 2.6 ppm). These signals were assigned by comparing with the chemical shift (about 2.4 ppm) of the methyne proton of N-(2-cyanoethyl)-cyclopentanecarboxamide which had been prepared by condensation of cyclopentanecarboxylic acid chloride and 3-aminopropionitrile, colorless needles, mp 71.5–72.5°.

20) All melting points are uncorrected and all compounds which have the asymmetric carbons are *dl*-forms.

21) F.W. Kay and W.H. Perkin, *J. Chem. Soc.*, 89, 1640 (1906).

(one proton-methyne adjacent to carbonyl), 4.52 (one proton-methyne adjacent to nitrogen), 5.20 (two protons, singlet-benzyl methylene), *ca.* 7.30 (five protons-phenyl ring).

The IR and NMR spectra of the oily material were not identical with those of XI, although they were alike each other.

Next, 0.12 g of the pure needles of V was obtained and successively 0.4 g of mixed needles and then 0.25 g of crude needles (mp 120–123°) of Va were obtained. Recrystallization from AcOEt gave Va (mp 126–127°). *Anal.* Calcd. for $C_{17}H_{21}O_3N_3$: C, 64.74; H, 6.71; N, 13.33. Found: C, 64.80; H, 6.79; N, 13.18.

Alcoholysis of V—To a mixture of 1.65 g of V and 1.8 g of abs. EtOH in 80 ml of abs. benzene was introduced 1.6 g (about 8 mole equiv.) of dry HCl gas at about 5°. After standing overnight in a refrigerator, a heavy oil produced was separated by decantation from the benzene layer. An amorphous powder (VI + VIII) was obtained by drying the oil *in vacuo*. A small amount of colorless needles (*ca.* 15 mg), however, was isolated by keeping the benzene solution in a refrigerator for a week, which was considered to be the benzyloxycarbonylated imino ether (VI), namely, N-(2-imidoethoxyethyl)-3-benzyloxycarbonylaminocyclopentanecarboxamidehydrochloride, mp 166°. These needles gave a positive silver nitrate test. *Anal.* Calcd. for $C_{19}H_{27}O_4N_3 \cdot HCl$: N, 10.56. Found: N, 10.06.

Amidination of a Mixture of VI and VIII and Separation of *cis*-N-(2-Amidinoethyl)-3-benzyloxycarbonylaminocyclopentanecarboxamide (VII)—To the foregoing amorphous powder of alcoholysis product obtained from 1.6 g of V, was added 1.5 g of ammonia dissolved in 15 ml of abs. EtOH. The mixture was stirred for 3 hr and allowed to stand overnight. Evaporation of the solvent left a sirupy residue of a mixture of I-hydrochloride and VII-hydrochloride. This amidinated material was submitted to the reductive de-benzyloxycarbonylation.

But 2.0 g of another amidinated material which had been obtained from 2.1 g of V as described above except that about 16 mole equiv. of dry HCl gas was introduced in the alcoholysis process, was chromatographed on 12.5 g of active carbon (Wako Chemicals Co.). The eluate was separated into 20 ml of fractions and each fraction was checked by ninhydrin and sodium nitroprusside reagent. Thus, 0.75 g of the de-benzyloxycarbonylated amidine corresponding to I was obtained.

Then, 0.25 g (*ca.* 10%) of VII-hydrochloride was obtained. This substance was positive against silver nitrate and sodium nitroprusside (orange) but insensitive toward ninhydrin reagent, and was crystallized as the picrate, yellow needles, mp 217–218°. *Anal.* Calcd. for $C_{17}H_{24}O_3N_4 \cdot C_6H_3O_7N_3$: C, 49.19; H, 4.84; N, 17.46. Found: C, 49.02; H, 5.02; N, 17.90.

***cis*-N-(2-Amidinoethyl)-3-aminocyclopentanecarboxamide (I) and Detection of *cis*-N-(2-Carbamoylethyl)-3-amino-cyclopentanecarboxamide (IX)**—In 50 ml of H_2O containing 0.3 g of AcOH was dissolved the foregoing amidinated material, and then hydrogenated with 0.5 g of 5% Pd-C. About 60 ml of H_2 was absorbed (calculated amount based on VII: about 120 ml). After removal of the catalyst and the solvent, the sirupy residue was converted to the sulfate according to the ion-exchange resin method reported by Ishida³⁾ except that ninhydrin and sodium nitroprusside reagent were used for checking. Thus, IX was detected, and then I-sulfate, contaminated with Na_2SO_4 was obtained. After recrystallization from H_2O and MeOH, 0.71 g of crystalline I-sulfate was obtained. *Anal.* Calcd. for $C_9H_{18}ON_4 \cdot H_2SO_4$: C, 36.47; H, 6.80; N, 18.90. Found: C, 36.30; H, 7.17; N, 18.57.

***trans*-N-(2-Amidinoethyl)-3-aminocyclopentanecarboxamide (Ia)**—By treating 0.705 g of Va in the same procedure as described above for the *cis* series, 0.60 g of crude powdery Ia-sulfate was obtained. By recrystallization, 0.31 g of crystalline Ia-sulfate was obtained. Ia-sulfate was more hygroscopic than I. *Anal.* Calcd. for $C_9H_{18}ON_4 \cdot H_2SO_4$: C, 36.47; H, 6.80; N, 18.90. Found: C, 35.96; H, 6.83; N, 18.56.

Isolation of *cis*-N-(2-Carbamoylethyl)-3-benzyloxycarbonylaminocyclopentanecarboxamide (X)—To a mixture of 0.8 g of V and 1.0 g of abs. EtOH in 200 ml of abs. ether, was introduced dry HCl gas at about 5°, most of V remained unchanged and, after adding 50 ml $CHCl_3$ to the reaction mixture, introduction of HCl gas was continued at about 30°. The resulting semi-solid was isolated and treated with ethanolic ammonia, and allowed to stand overnight in a refrigerator. By filtration, 0.15 of resulting precipitate was isolated. Recrystallization from aq. EtOH gave X, mp 209–210°. *Anal.* Calcd. for $C_{17}H_{23}O_4N_3$: C, 61.27; H, 6.96; N, 12.60. Found: C, 60.97; H, 7.24; N, 12.90. IR $_{max}^{Nujol}$ cm^{-1} : 3400, 3330, 3200 (NH), 1680 (urethane CO), 1650, 1630 (acid amide CO).

***cis*-N-Methyl-N-(2-cyanoethyl)-3-benzyloxycarbonylaminocyclopentanecarboxamide (XII)**—XII was prepared in a manner similar to the experiment for V except that 3-methylaminopropionitrile was used instead of 3-aminopropionitrile. XII was obtained as colorless needles, mp 66–69. IR $_{max}^{Nujol}$ cm^{-1} : 3330 (NH), 2240 (CN), 1680 (urethane CO), 1635 (acid amide CO). NMR $_{MCl_3}^{CDCl_3}$ ppm from TMS: in comparison with the NMR spectrum of V, one of the NH signal disappeared and a singlet peak (3.17, NCH_3) appeared.

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