ed to follow the route shown in Chart 2. (I) also undergoes fission of the ring in alkali hydroxide solution at room temperature to form 2′-(hydroxyiminomethyl)formanilide (X).

In contrast to the foregoing reactions, application of hydrogen cyanide, sodium hydrogensulfite, and hydrazine, which have lower reactivity as anionoid reagents than those mentioned above, to (I) resulted in the formation of a corresponding 4-quinazolinecarbonitrile (XII), sodium 4-quinazolinesulfonate (XIII), and 4-hydrazinoquinazoline (XIV). These reagents did not react with the 2-position of (VIII) and only hydrazine reacted with (VIII) to form 4-hydrazinoquinazoline 1-oxide (XI). These experimental results indicate that the nucleophilic acitivity of 4-position in (I) is greater than that of 2-position in (VII).

It was thereby concluded that the polar effect of the N-oxide group in (I), together with the effect of ring-nitrogen and the fused benzene ring, markedly increases the nucleophilic activity of 4-position in the quinazoline ring system. On the other hand, stability of the ring has been markedly decreased and facile ring fission occurs between 2- and 3-positions.

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104. Shoshiro Nakamura : Structure of Amidinomycin.

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Amidinomycin[1] is a new antibiotic, obtained from cultured broth of a strain resembling *Streptomyces flavochromogenes* collected in Shizuoka Prefecture in 1958. It inhibits the growth of spore-bearing bacteria, such as *B. subtilis*, *B. megatherium*, and *B. anthracis*, at the concentration of 5～200 γ/cc. This new antibiotic was named amidinomycin, because of having an amidine radical in its structure. The free base and the hydrochloride are not obtained as pure crystals, but the reineckate,[1] $C_9H_{18}ON_4 \cdot 2H[Cr(NH_3)_2(SCN)_4]$, pink needles of m.p. 208～211°(decomp.), and the sulfate,[1] $C_9H_{18}ON_4 \cdot H_2SO_4$, colorless needles or prisms of m.p. 285～288°(decomp.), are obtained as pure crystals. The sulfate is optically active, $[\alpha]_D^{21}$ −3.9°(c=3, H_2O). Its ultraviolet and infrared absorption spectra are shown in Figs. 1 and 2.

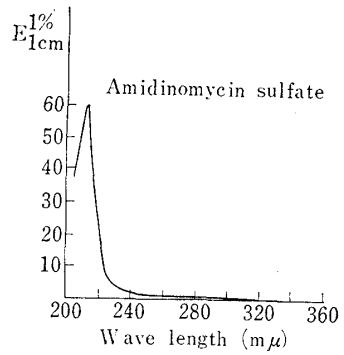

Fig. 1. Ultraviolet Absorption Spectrum of Amidinomycin Sulfate (in water)

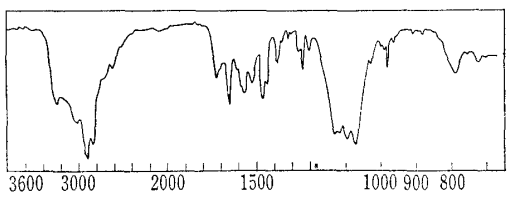

Fig. 2. Infrared Absorption Spectrum of Amidinomycin Sulfate (in Nujol)

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

Amidinomycin gives a new amino acid, $C_6H_{11}O_2N$, and a base, $C_3H_9N_3$, by acid hydrolysis, and the structure of N-(2-amidinoethyl)-3-aminocyclopentanecarboxamide is elucidated for amidinomycin from the following procedures.

Hydrolysis[2] of amidinomycin sulfate (I) in 6*N* hydrochloride at 120° for 3 hours gives three Ninhydrin-positive spots by paper chromatography with a solvent system of butanol-acetic acid-water (4:2:1). Their Rf values are 0.39~0.41 (pink-brown), 0.27~0.28 (violet), and 0.09 (yellow green). The reaction mixture is purified by partition chromatography on a cellulose column using the same solvent above described. A new amino acid, $C_6H_{11}O_2N$ (II) (0.87 mole), m.p. 250~251° (decomp.), is crystallized from the first Ninhydrin-positive fraction. Following the new amino acid, a trace of another amino acid is eluted and identified as β-alanine (VII) by paper chromatography. The concentration of last Ninhydrin-positive effluent gives a base $C_3H_9N_3 \cdot 2HCl$ (III) (0.75 mole), m.p. 165~167° (decomp.). It was proved, further, that β-alanine is the secondary hydrolyzed product of the base (III).

The above facts indicate that carboxyl radical of the amino acid (II) and the amino radical of the base (III) is conjugated with amide bond in amidinomycin.

The amino acid is optically active, $[\alpha]_D^{14}$ −14.7° (c=1, H_2O), and has no characteristic maximum in ultraviolet absorption spectrum. Its infrared spectrum is shown in Fig. 3 and the bands[3] attributable to most of the amino acids are observed at 2700, 2620, 2220, and 1330 cm^{-1}. This amino acid gives the following Rf values by paper chromatography with several kinds of solvents; 0.17 with butanol-pyridine-water (4:2:1), 0.85 with water-saturated phenol, and 0.56 with butanol-acetic acid-water (2:1:1). The carboxyl group of this amino acid can be methylated with diazomethane. The acetylated product, $C_8H_{13}O_3N$ (IV), white crystals of m.p. 144~146°, is obtained by the reaction with acetic anhydride and pyridine. In the infrared absorption spectrum of the acetylated product (IV), the bands[4] suggesting =NH radical at 3340 cm^{-1}, a carbonyl in secondary amide at 1610 cm^{-1}, and a secondary amide at 1560 and 1300 cm^{-1}, are indicated. As no double bond and no C-methyl group are shown, the presence of cyclopropane, cyclobutane, or cyclopentane is considered in this amino acid (II). However, the band[5] attributable to cyclopropane or cyclobutane is not detected in the infrared absorption spectrum. Thus, the presence of cyclopentane having an amino and a carboxyl groups is suggested for this amino acid. As this amino acid gives pK values[6] of 3.7 and 10.5, which are very similar to that of ψ-aminobutyric acid, the structure of 3-aminocyclopentanecarboxylic acid is the most probable for it.

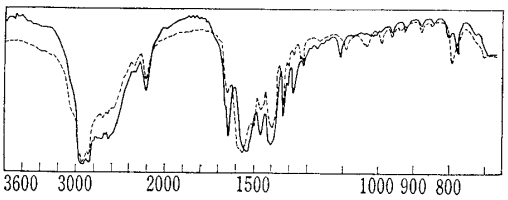

Fig. 3. Infrared Absorption Spectra (in Nujol)
——— Cyclopentaminin (II)
------ 3-Aminocyclopentanecarboxylic acid (IX)

3-Aminocyclopentanecarboxylic acid is a new compound and obtained by hydrogenation of 3-hydroxyiminocyclopentanecarboxylic acid[7] (VIII) in acetic acid with Adams catalyst. The synthesized 3-aminocyclopentanecarboxylic acid (IX), $C_6H_{11}O_2N$, white crystals of m.p. 240~241° (decomp.), always shows one spot and same Rf values as the amino acid (II)

2) S. Nakamura, K. Karasawa, H. Yonehara, N. Tanaka, H. Umezawa : J. Antibiotics (Japan), in press.
3) L. J. Bellamy : "The Infra-red Spectra of Complex Molecules," 200 (1954). Methuen & Co., London.
4) *Idem* : *Ibid.*, 175 (1954).
5) *Idem* : *Ibid.*, 13 (1954).
6) S. Mizushima, S. Akabori : "Chemistry of Proteins," **2**, 244 (1954). Kyōritsu Shuppan Co., Tokyo.
7) F. W. Kay, W. H. Perkin : J. Chem. Soc., **89**, 1640 (1906).

obtained from amidinomycin by paper chromatography with several kinds of solvents. The results of paper chromatography suggest its identity with that of synthesized one (IX) which seemed to be composed of either *cis*- or *trans*-form, but their infrared absorption spectra are rather different (Fig. 3). The synthesized product (IX) was acetylated and the acetyl derivative, $C_8H_{13}O_3N$ (X), white crystals of m.p. 141~143°, melted at 141~143° by admixture with the acetyl derivative (IV) of the natural amino acid, m.p. 144~146°. These acetylated products are not completely identical in their infrared absorption spectra. The bands at 1160, 980, and 940 cm^{-1} are observed in the natural (IV), but not in the synthesized one (X). These differences are considered to be due to the latter being a racemate. Further, both these acetyl compounds are methylated with diazomethane. The infrared absorption spectra of these methyl esters (V), (XI), were taken in chloroform and their identity was confirmed (Fig. 4).

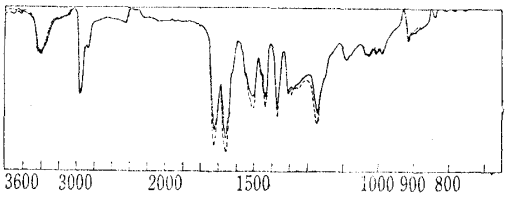

Fig. 4. Infrared Absorption Spectra (in $CHCl_3$)

——— Methyl ester of acetylcyclopentaminin (V)

------ Methyl ester of 3-acetamidocyclopentanecarboxylic acid (XI)

Thus, the structure of 3-aminocyclopentanecarboxylic acid is determined for this amino acid (II). This is the first example of isolation of this amino acid from natural sources and the acid was named cyclopentaminin after its structure.

Hydrochloride of the basic compound, $C_3H_9N_3 \cdot 2HCl$ (III), is optically inactive and the ultraviolet absorption spectrum is very similar to that acetamidine hydrochloride : $\lambda_{max}^{H_2O}$ mμ ($E_{1cm.}^{1\%}$) : 209 (136), 280 (1.6). The bands at 1690 and 1600 cm^{-1}, indicating the presence of an amidine group, are observed in its infrared absorption spectrum. The presence of an amidine group in this base is supported by its pK values (Table I). The amidine group is changeable to an acid amide, further, to carboxyl in alkaline solution, when the compound, $C_3H_9N_3 \cdot 2HCl$, is allowed to stand in a solution of *N* NaOH at room temperature for one hour. The spots corresponding to β-alanine (VII) and its acid amide (VI) are appear on paper chromatogram. The reaction mixture was boiled for 30 minutes and subjected to partition chromatography on a cellulose column with a solvent system of butanol-acetic acid-water (4:2:1). The compound, $C_3H_7O_2N$ (VII), colorless plates of m.p. 197° (decomp.), is obtained and identified as β-alanine by comparison with the infrared absorption spectrum of an authentic sample. Thus, the basic compound, $C_3H_9N_3$ (III) is confirmed to be 2-amidinoethylamine.

Table I. pK Values at 28°(in H_2O)

Substances	pK_1	pK_2
Amidinomycin Sulfate	9.6	>12
Cyclopentaminin	3.7	10.5
3-Aminocyclopentanecarboxylic acid	3.7	10.5
Acetylcyclopentaminin	4.3	—
2-Amidinoethylamine·2HCl	7.4	>12
4-Aminobutyric acid	3.8	10.3
Methyl ester of 4-aminobutyric acid	9.7	—
Acetamidine·HCl	>12	—

As mentioned already, amidinomycin gives 3-aminocyclopentanecarboxylic acid and 2-amidinoethylamine by acid hydrolysis, and three formulae (A), (B) and (C) are possible for the structure of the antibiotic.

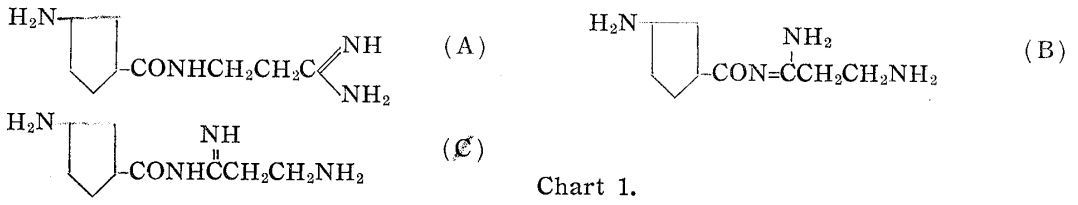

Chart 1.

Amidinomycin is a dibasic compound, having $pK_1=9.6$ and pK_2 higher than 12 (Table I). It should be tribasic, having $pK_1=7.4$, $pK_2=9.6$ and pK_3 higher than 12 in formula (B). In formula (C), pK_1 must be 7.4 and pK_2 must be 9.6. Then, the formula (A) is reasonable for amidinomycin and $pK_1=9.6$ must correspond with the free amino group of cyclopentaminin moiety and pK_2 higher than 12 must correspond to the free amidine group. Therefore, the structure of N-(2-amidinoethyl)-3-aminocyclopentanecarboxamide could be proposed for amidinomycin and there is no phenomenon against this structure.

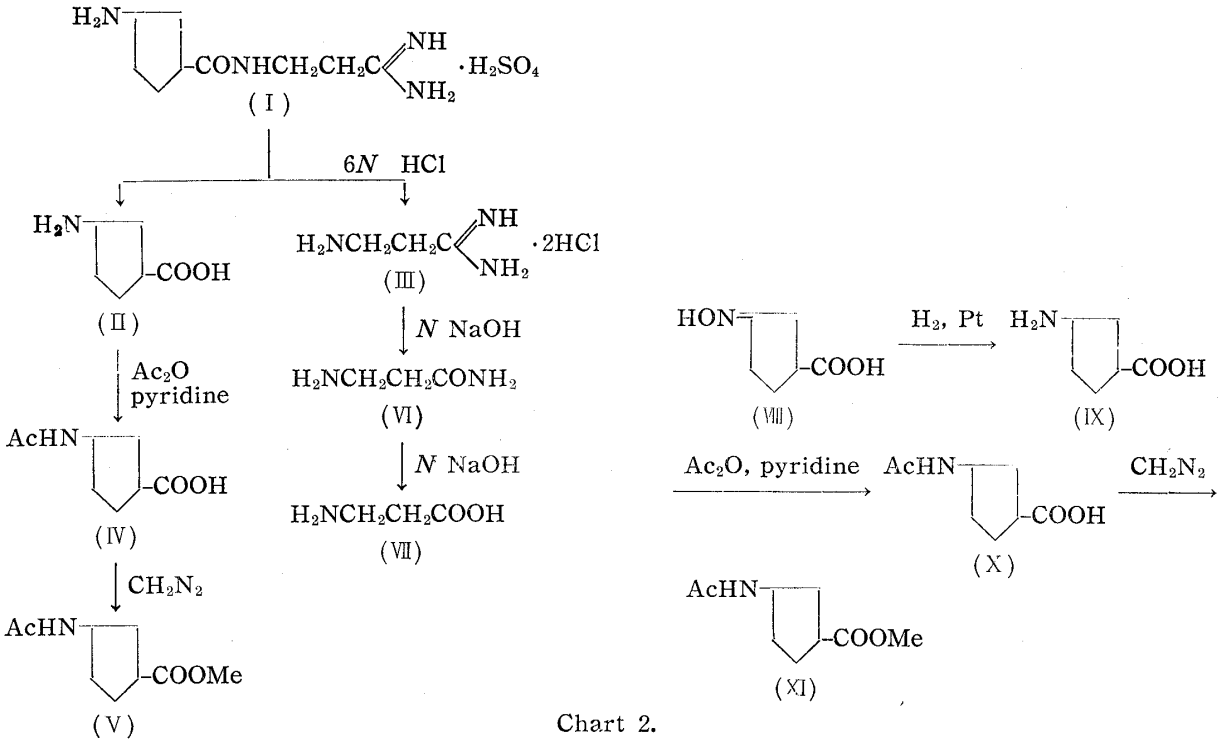

Chart 2.

Experimental

Hydrolysis of Amidinomycin Sulfate (I)——Two hundred and sixty mg. of amidinomycin sulfate was hydrolyzed with 6*N* HCl (4 cc.) for 3 hr. on an oil bath at 120°. The reaction mixture was evaporated *in vacuo* to remove HCl, the residue was dissolved in H_2O (20 cc.), and pH of the solution was adjusted to 7.0 by addition of Amberlite IR-4B. The filtrate was evaporated to dryness, the residue was fractionated by 20 cc. with partition chromatography on a cellulose column using a solvent system of BuOH-AcOH-H_2O (4:2:1).

The fraction Nos. 6~7 were conbined. After removal of the solvent, the dried material (98 mg.) was dissolved in MeOH with warming, added with Me_2CO, kept in a refrigerator, and white granular crystals (II) appeared. Sublimed at 170~190°, m.p. 248~251° (decomp.). *Anal.* Calcd. for $C_6H_{11}O_2N$ (Cyclopentaminin): C, 55,79; H, 8.58; N, 10.85. Found: C, 55.36; H, 8.56; N, 10.78.

The trace of amino acid (VII) obtained from the fraction No. 8 was identified as β-alanine by paper chromatography.

By concentration of the fraction Nos. 12~14 to 1/3 in volume, white plate crystals (III) precipitated and washed with Me_2CO, m.p. 165~167°. Yield, 105 mg. *Anal.* Calcd. for $C_3H_9N_3 \cdot 2HCl$: C, 22.51; H, 6.93; N, 26,26; Cl, 44.30. Found: C, 22.69; H, 6.92; N, 26.03; Cl, 44.47.

Acetylcyclopentaminin (IV)——Cyclopentaminin (II) (50 mg.) was dissolved in Ac_2O and pyridine with warming, allowed to stand overnight at room temperature, the solvent was removed, the residue was dissolved in Me_2CO, and Et_2O added. By keeping in refrigerator, colorless crystals (34 mg.) m.p. 144~146°, appeared. *Anal.* Calcd. for $C_8H_{13}O_3N$: C, 56.12; H, 7.65; N, 8.18. Found : C, 56.48; H, 7.74; N, 8.43.

Methyl Ester of Acetylcyclopentaminin (V)——Acetylcyclopentaminin (20 mg.) was dissolved in MeOH and Et_2O solution of diazomethane was added. After the solvent was removed, the residue was dried over $CaCl_2$ *in vacuo*. Colorless oil was obtained.

3-Oxocyclopentanecarboxylic Acid——The method of Kay and Perkin[7] was followed. Purified by distillation, m.p. 58~60°. *Anal.* Calcd. for $C_6H_8O_3$: C, 56.24; H, 6.29. Found : C, 56.27; H, 6.36.

3-Hydroxyiminocyclopentanecarboxylic Acid(VIII)[7]——The method of Kay and Perkin was followed. Recrystallization from MeOH and Et_2O gave white crystalline powder, m.p. 182~183° (decomp.). *Anal.* Calcd. for $C_6H_9O_3N$: C, 50.34; H, 6.34; N, 9.79. Found : C, 50.61; H, 6.45; N, 9.46.

3-Aminocyclopentanecarboxylic Acid(IV)——Four hundred and seventeen mg. of 3-hydroxyiminocyclopentanecarboxylic acid (VIII) was dissolved in 30 cc. of AcOH and hydrogenated with Adams catalyst. One hundred and thirty-five cc. of H_2 was absorbed in 2 hr. (calculated for 2 mole : 130.6 cc.). The reaction product was purified by partition chromatography on a cellulose column using a solvent of BuOH-AcOH-H_2O (4:2:1). Recrystallization from MeOH and Me_2CO gave white crystals (171 mg.) of 3-aminocyclopentanecarboxylic acid, m.p. 240~242° (decomp.). *Anal.* Calcd. for $C_6H_{11}O_2N$: C, 55.79; H, 8.58; N, 10.85. Found : C, 55.53; H, 8.55; N, 10.68.

3-Acetylaminocyclopentanecarboxylic Acid(X)——3-Aminocyclopentanecarboxylic acid (50 mg.) was acetylated with Ac_2O and pyridine. The reaction mixture, processed as described previously, afforded 28 mg. of white crystals, m.p. 141~143°. *Anal.* Calcd. for $C_8H_{13}O_3N$: C, 56.12; H, 7.65; N, 8.18. Found : C, 56.30; H, 7.68; N, 8.19.

Methyl Ester of 3-Acetylaminocyclopentanecarboxylic Acid(XI)——The procedure above-mentioned was followed. Colorless oil was obtained.

Hydrolysis of $C_3H_9N_3 \cdot 2HCl$ (III)——Sixty mg. of $C_3H_9N_3 \cdot 2HCl$ was hydrolyzed with *N* NaOH (10 cc.) on an oil bath at 120° for 30 min. After cool, the reaction mixture was neutralized by addition of Amberlite IRC-50 and the filtrate was evaporated to dryness *in vacuo*. The residue was applied to partition chromatography on a cellulose column, and eluted with a solvent of BuOH-AcOH-H_2O (4:2:1). The Ninhydrin-positive fractions were combined and the solvent was evaporated. The residue gave white plates (VII) by recrystallization from MeOH and Et_2O, m.p. 197° (decomp.). *Anal.* Calcd. for $C_3H_7O_2N$: C, 40.44; H, 7.92; N, 15.72. Found : C, 40.39; H, 7.92; N, 14.76.

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Summary

Acid hydrolysis of amidinomycin gives two Ninhydrin-positive compounds, a new amino acid, 3-aminocyclopentanecarboxylic acid, and a base, 2-amidinoethylamine. The name of cyclopentaminin is given for the new amino acid, and its characters and synthetic method are described. The structure of N-(2-amidinoethyl)-3-aminocyclopentanecarboxamide is proposed for amidinomycin.

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