THE POLYOXINS: PYRIMIDINE NUCLEOSIDE PEPTIDE ANTIBIOTICS INHIBITING FUNGAL CELL WALL BIOSYNTHESIS

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1. Discovery

As early as 1950th, search for new antibiotics for agricultural use has started in Japan. Rice blast desease was a primary target of this research project, which is caused by infection of a pathogenic fungus, Piricularia oryzae. Thus a nucleoside antibiotic, blasticidin S and an aminoglycoside antibiotic, kasugamycin have been discovered and proved to be useful for prevention of this desease. In 1963, we initiated search for antibiotics which show preventive effect for sheath-blight desease of rice plant, which is another serious desease of rice plant that is caused by a pathogenic fungus, Pellicularia filamentosa f. sasakii. Screening was carried out by the in vivo pot test using growing rice plant inoculated with the pathogen. Very quickly, we obtained a polyoxin-producing streptomycete, which was designated as Streptomyces cacaoi var. asoensis.[1]

Since the antimicrobial spectrum of this antibiotic was very selective, being restricted to some species of phytopathogenic fungi, it was presumed to be a new type at the early stage of the research. It was later proved that the polyoxins are a new class of nucleoside peptide antibiotics having unique chemical and biological features. The antibiotics have been widely in practical use since 1966 as an excellent agricultural fungicide with no side-effect.

2. Fermentation and Isolation

For production of the polyoxins, Streptomyces cacaoi was fermented in organic media containing glucose, soluble starch, soybean flour, dry yeast and inorganic salts.[1] Fermentation was carried out in either flask, jar fermentor, or fermentation tank at 28°C.

The polyoxins are amphoteric compounds. The isolation procedure[1-5] is outlined in Fig. 1. All the polyoxins were adsorbed on Dowex 50W (H) from

Fig.1 Isolation procedure for polyoxins.

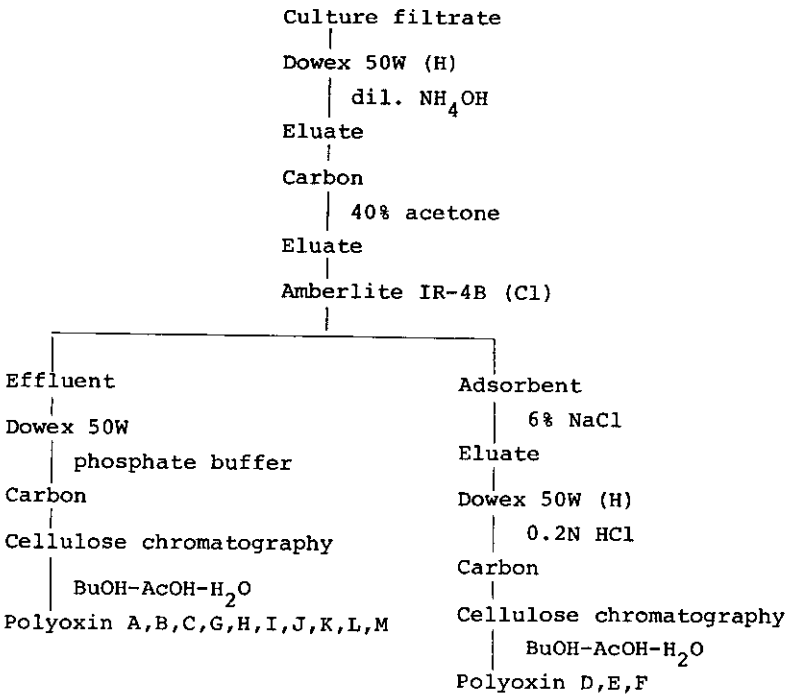


Table 1. Physicochemical properties of polyoxins.

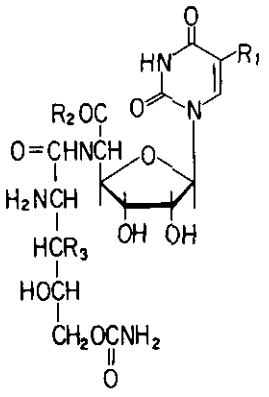
Polyoxin	Formula	pKa'	$[\alpha]_D^{20}$ (c=1,H_2O)	λmax nm (log ε)	
				0.05 N HCl	0.05 N NaOH
A	$C_{23}H_{32}N_6O_{14}$	3.0 - 7.3 9.6	-30°	262 (3.94)	264 (3.80)
B	$C_{17}H_{25}N_5O_{13}$	3.0 - 6.9 9.4	+34°	262 (3.94)	264 (3.82)
C	$C_{11}H_{15}N_3O_8$	2.4 - 8.1 9.5	+11°	262 (3.97)	264 (3.87)
D	$C_{17}H_{23}N_5O_{14}$	2.6 3.7 7.3 9.4	+30°	276 (4.05)	271 (3.85)
E	$C_{17}H_{23}N_5O_{13}$	2.8 3.9 7.4 9.3	+19°	276 (4.00)	271 (3.81)
F	$C_{23}H_{30}N_6O_{15}$	2.7 3.9 7.2 9.3	-18°	276 (4.06)	271 (3.87)
G	$C_{17}H_{25}N_5O_{12}$	3.2 - 7.3 9.3	+37°	262 (3.92)	264 (3.82)
H	$C_{23}H_{32}N_6O_{13}$	3.3 - 7.2 9.4	-38°	265 (3.88)	266 (3.79)
I	$C_{17}H_{22}N_4O_9$	2.7 - 6.2 9.3	-35°	262.5 (3.94)	264 (3.78)
J	$C_{17}H_{25}N_5O_{12}$	3.0 - 7.1 9.9	+31.7°	264 (3.91)	267 (3.81)
K	$C_{22}H_{30}N_6O_{13}$	3.0 - 7.2 9.3	-16.5°	259 (3.95)	262 (3.86)
L	$C_{16}H_{23}N_5O_{12}$	3.0 - 7.1 9.4	+34.4°	259 (3.96)	262 (3.85)
M	$C_{16}H_{23}N_5O_{11}$	3.0 - 7.4 9.5	+49.9°	259 (3.94)	261 (3.81)

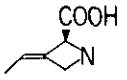
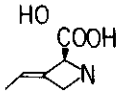
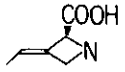
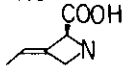
culture filtrates and were eluted with dilute ammonium hydroxide. Polyoxins D, E, and F which are more acidic, were able to be separated from other polyoxins by adsorption on Amberlite IR-4B (Cl). Final separation of each component polyoxin was achieved by repeated Avicel cellulose chromatographies using n-butanol-acetic acid-water as solvent. Polyoxins A, C, and H have been crystallized from aqueous ethanol. Other polyoxins were obtained as amorphous white powder. Physicochemical properties are summerized in Table 1. Polyoxins C and I are biologically inactive and were obtained only in minute amount. They are considered to be formed from other polyoxins by hydrolysis during isolation.

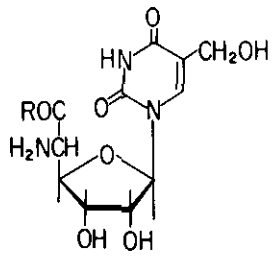
3. Structure Elucidation

For convenience, established structures of the polyoxins A-M are shown in Fig. 2. The structural study was performed first with polyoxin A (1), a main component of the polyoxins with combination of chemical and physical methods. Hydrolysis scheme is given in Fig. 3. Polyoxin A is composed from three

Fig. 2



	Polyoxin	R_1	R_2	R_3
1	A	CH_2OH		OH
2	B	CH_2OH	HO	OH
3	D	COOH	HO	OH
4	E	COOH	HO	H
5	F	COOH		OH
6	G	CH_2OH	HO	H
7	H	CH_3		OH
8	J	CH_3	HO	OH
9	K	H		OH
10	L	H	HO	OH
11	M	H	HO	H



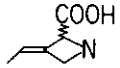
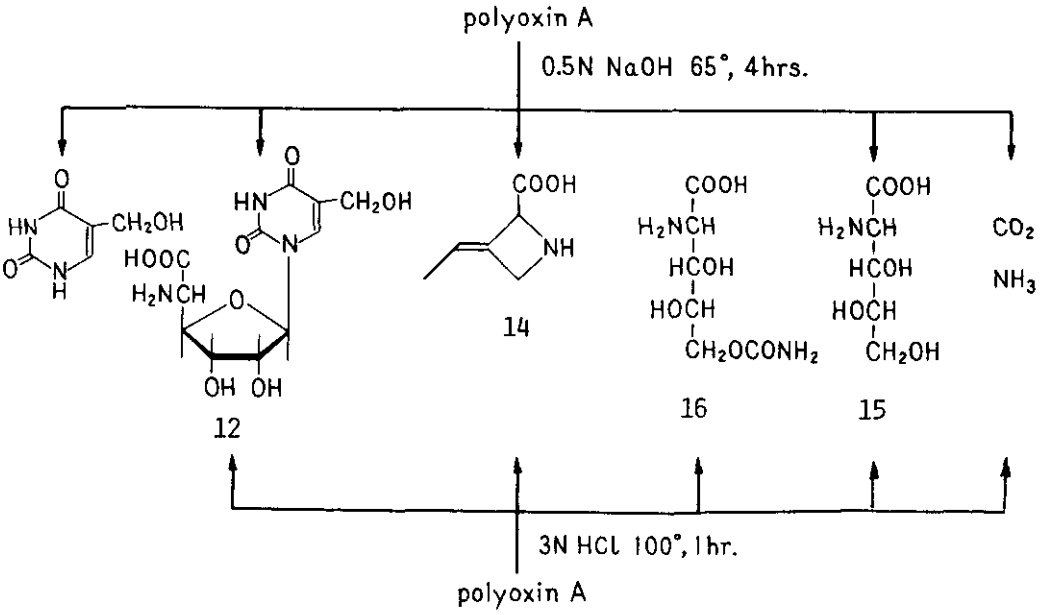
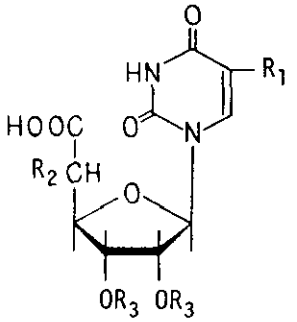
		R
12	C	HO
13	I	

Fig. 3. Hydrolysis of polyoxin A



unusual amino acids. One of which is polyoxin C (12) which was isolated before from the polyoxin complex.[2,3] The other two amino acids were designated as polyoximic acid (14) and polyoxamic acid (15).

Polyoxin C (12) was a key compound in determining the structure of the polyoxins since it constitutes a basic nucleoside skeleton common to all the members of the polyoxins. Prolonged acid hydrolysis and periodate oxidation gave 5-hydroxymethyluracil. Uv data showed 12 is an N-1 nucleoside. Hydrogenolysis of an N-acetyl derivative of polyoxin C (17) gave N-acetyldeoxypolyoxin C (18). ^{1}H-NMR spectrum of this compound in dimethylsulfoxide-d_6 showed H-5' as quartet

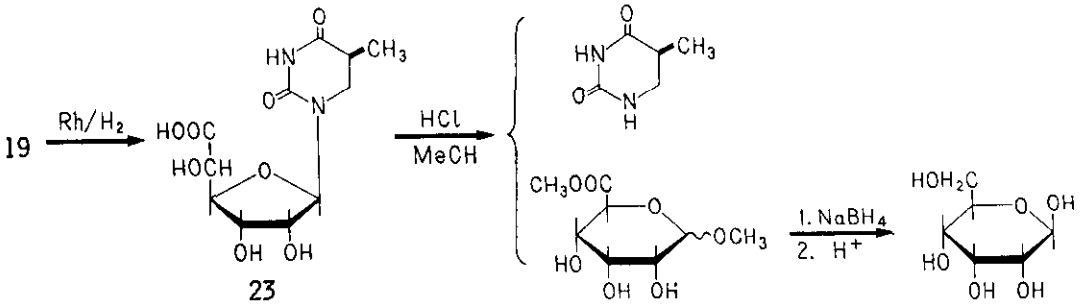


	R_1	R_2	R_3
12	CH_2OH	NH_2	H
17	CH_2OH	CH_3CONH	H
18	CH_3	CH_3CONH	H
19	CH_2OH	OH	H
20	CH_3	OH	H
21	CH_3	C_2H_5SSCNH	Ip
22	CH_2OH	p-$BrC_6H_4SO_2NH$	H

Ip: isopropylidene

(δ 4.69), which becomes doublet on spin-decoupling with -NHCOCH$_3$ (δ 8.40). This proves the position of the amino group is on carbon 5', subsequently the sugar must be a furanose. Configuration of the sugar was determined by obtaining D-allose by a series of reactions; i.e. deamination, hydrogenation (12→19→20→23). Methanolysis of 23 followed by hydride reduction and acid hydrolysis afforded D-allose (Fig. 5). Configuration at carbon-5' was supported by a circular dichroism spectrum of N-dithiocarbethoxy derivative ($[\theta]_{343}$ = +1320). The anomeric β-

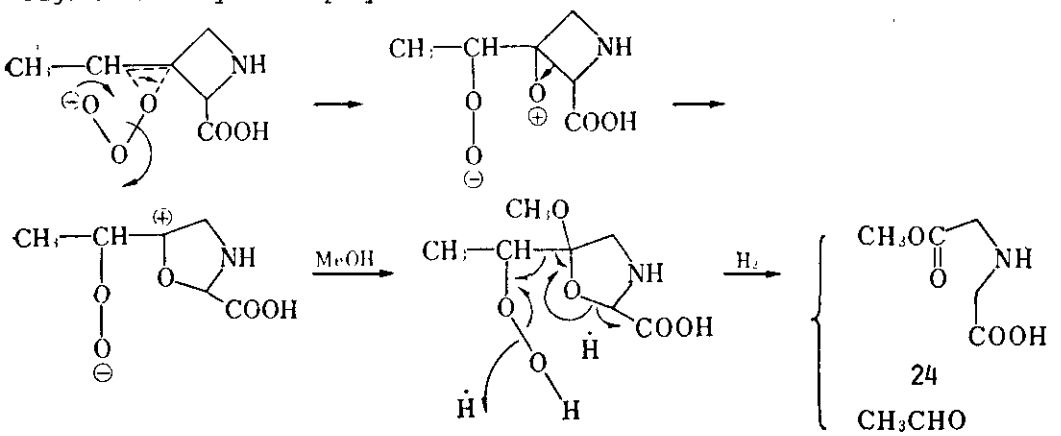
Fig. 5 Degradation of polyoxin C.



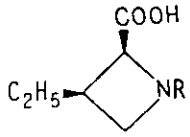
configuration was assigned on the basis of the positive Cotton effect near 280 nm and the reduced coupling constant of the anomeric proton ($J_{1',2'}$ = 2.0 Hz) on constraining by fusion with a 2',3'-O-isopropylidene ring. The established structure, 1-(5'-amino-5'-deoxy-β-D-allofuranosyluronic acid)-5-hydroxymethyluracil (12) was later confirmed by X-ray analysis of N-brosyl derivative (22).[11,12]

Polyoximic acid (14) is a rare example of naturally-occuring azetidine derivative. The structure, 3-ethylidene-L-azetidine-2-carboxylic acid was assigned on the basis of ^{1}H-NMR analysis and reductive ozonolysis in methanol which afforded iminoacetic acid half methyl ester (24) (Fig. 6). This amino acid

Fig. 6 Ozonolysis of polyoximic acid.



undergoes easy racemization either by acid or alkali. Optically active form was only obtainable as dihydro derivative (25), which was obtained by hydrolysis of hydrogenated polyoxin A. L-Configuration was assigned on the basis of positive Cotton effect of N-dithiocarbethoxy derivative (26). Double bond stereochemistry



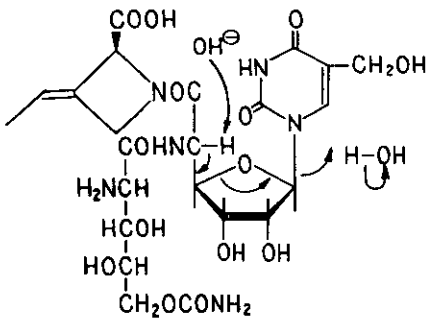
25 R = H

26 R = C_2H_5SSC

was assigned by nuclear Overhauser effect, in which 4-methylene signals showed 22% enhancement when methyl-protons were irradiated. Synthesis of this simple compound has never been reported.

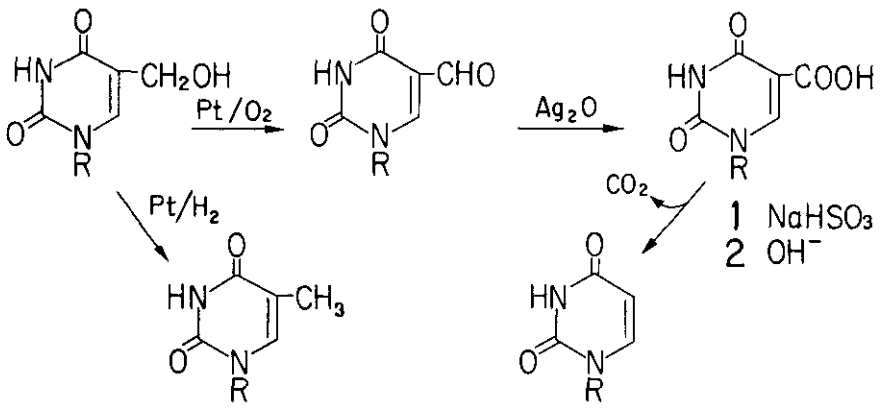
The structure of polyoxamic acid (15) was established as 2-amino-2-deoxy-L-xylonic acid. N-Acetylation followed by sodium amalgum reduction gave N-acetyl-L-xylosamine (26), which was an antipode of a synthetic compound.[12] In polyoxin A, the hydroxy group on carbon-5 is carbamoylated. Carbamoylpolyoxamic acid (16) was obtained by acid hydrolysis or enzymatic hydrolysis using leucine aminopeptidase. Sequence determination established the structure of polyoxin A as 1, which is constructed by uniting with amide linkage these three amino acids. It is to be noted that unusual lability of a nucleoside bond of 1 is explained by the mechanism initiated by abstraction of peptide-α-hydrogen (Fig. 7).

Fig. 7. Alkaline hydrolysis mechanism of polyoxin A.



By similar way, the structure of all the polyoxins are determined. Interconversion of the 5-substituted pyrimidine nucleosides was done as shown in Fig.8.

Fig. 8 Interconversion among the polyoxin nucleosides.

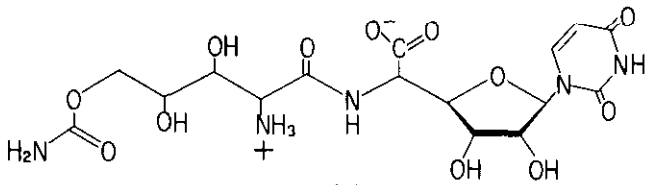


4. Mechanism of Action

As mentioned in Chapter 1, the biological activity of the polyoxins are very characteristic because of their specific action against phytopathogenic fungi and lack of activity against other microorganisms, plants, fish, and mammals. Sasaki et al[14] suggested that the site of action of the polyoxins was related to cell wall-chitin biosynthesis. Swelling of fungal cells was also observed when treated with polyoxins.[15]

On establishment of the structure of the polyoxins, we have pointed out[11] the gross structural similarity of the polyoxins to UDP-N-acetylglucosamine (27), a substrate for chitin synthetase (Fig. 9). Shortly after. Endo and Misato[16]

Fig. 9 Structural similarity of polyoxin L (10) and UDP-N-acetylglucosamine (27).



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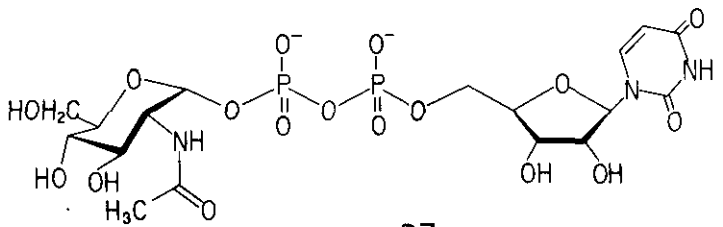

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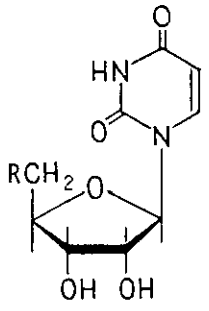
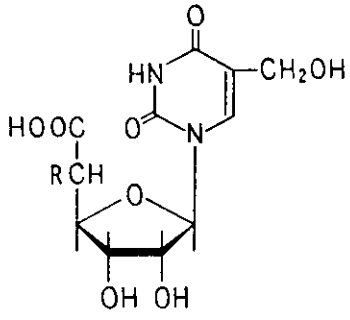
have shown that polyoxin D is indeed a strong competitive inhibitor for chitin synthetase from Neurospora crassa. Later on, it has been shown that chitin synthetases from various sources were all susceptible to polyoxins, including Saccharomyces,[17] Piricularia,[18] Alternaria,[19] Phycomyces,[20] Mucor,[21] Agaricus,[22] and Coprinus.[23] Endo et al[16,27] have also developed a simple procedure for the preparation of UDP-N-acetylglucosamine-^{14}C utilizing polyoxin D. This is based on the findings that UDP-N-acetylglucosamine accumulated in the presence of polyoxin D acts as afeed back inhibitor for the enzyme, fructose phosphate amino-transferase. It is especially interesting that chitin synthesis in insect tissue culture (American cockroach) was also shown to be inhibited by polyoxin D.[24] Polyoxin A was shown to be insecticidal when injected into the abdomen of grass-hoppers nymphs.[25] Difference in sensitivity among the species of fungi may be due to difference of the permeability barrier of cell membrane.[16,17] It was also shown that the resistant strain of Alternaria showed decreased uptake of poly-oxins.[26]

5. Structure-Activity Relationship

Polyoxins L and M are the smallest molecules among the biologically active polyoxins. This implies that C-terminal polyoximic acid and 5-substituents of uracil are not essential for the activity. A number of aminoacyl derivatives of 5'-amino-5'-deoxyuridine (28) have been synthesized.[28] However, none of them was active. In contrast, many of the aminoacyl derivatives of polyoxin C (29) (semisynthetic polyoxins) showed antifungal activity, indicating 6'-carboxyl is essential for the activity.[29] Regarding to the side-chain aminoacyl group, the α-L-aminoacyl group is absolutely required for the activity. None of the acyl derivatives, α-D-aminoacyl derivatives and β-aminoacyl derivatives were active.



28 R = aminoacyl

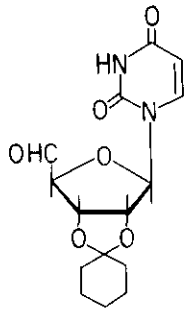


29 R = aminoacyl

ω-Substituents affect the activity. Polar groups such as ureido, carbamoyl, and carbamoyloxy increased the activity. However, ionic groups such as amino or carboxyl significantly decreased the activity. From these data, it was concluded that the ionic binding through the L-dipeptide structure, accompanied by the hydrogen bonding through the carbamoyloxy group play important roles for the reversible binding between the polyoxins and chitin synthetase.[29] In vitro activity of these compounds against chitin synthetase from Piricularia oryzae was also examined and discussed.[18] Importance of the ionized α-L-amino group was also concluded.

6. Chemical Synthesis

Two independent syntheses of the nucleoside skeleton of the polyoxin have been reported. Moffatt and his co-workers[30] utilized 2',3'-O-cyclohexylidene-uridine-5'-aldehyde (30) obtained by DMSO-DCC oxidation of the corresponding uridine for this synthesis. Cyanohydrin synthesis followed by hydrolysis gave epimeric hydroxyamides (31). After separation of epimers, β-D-taluronamide was tosylated. Displacement with azide, acid hydrolysis, and reduction of azide afforded uracil-polyoxin C (32a). This route may not be applied for preparation of purine analogs since it includes an acid hydrolysis step.



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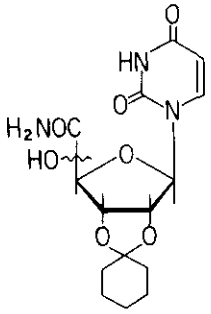

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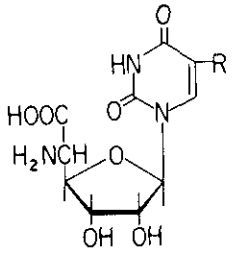
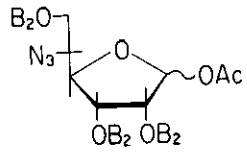


32a R=H
32b R=CH$_3$

Ohrui et al,[31] starting from glucose, introduced azido group into carbon-5 of allofuranose derivative after double inversions. After conversion to the sugar acetate (33), it was coupled with silylated thymine in the presence of $SnCl_4$. Chromic acid oxidation to uronic acid, followed by reduction of the azido group afforded thymine-polyoxin C (32b).



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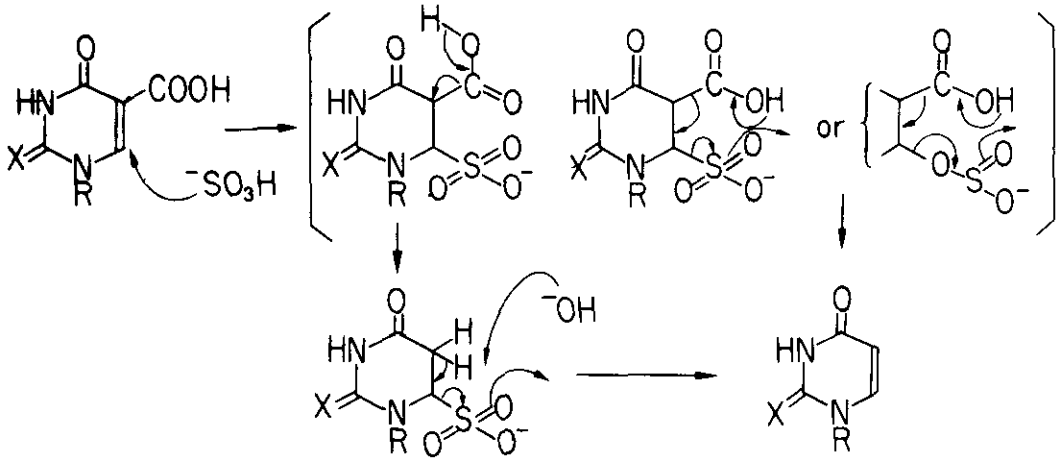
Kuzuhara et al[32,33] have also succeeded in the total synthesis of polyoxin J (8). In this synthesis, the side chain amino acid, carbamoylpolyoxamic acid (16) was synthesized from L-sorbose utilizing the carbons 3 - 5 chirality which were all inverted in the final product. Two independent syntheses of polyoxamic acid (15) were also reported.[34,35]

7. Chemical Modification

(1) Bisulfite-catalyzed decarboxylation[5,11] When polyoxins having 5-carboxyuracil base were treated with 2 — 3 equivalents of $NaHSO_3$ at 50°C (pH 4.0), they were easily converted quantitatively into two compounds. The one is the corresponding uracil derivative and the other is an epimeric mixture of 5,6-dihydro-6-sulfonate derivative (34). Obviously, nucleophilic addition of bisulfite first occured on carbon 6 of 5-carboxyuracil. Elimination of the sulfonate group by mild alkali treatment yielded the uracil group quantitatively. This reaction is comparable to the bisulfite-catalyzed hydrolytic deamination of cytosine derivatives, which were discovered by Hayatsu et al and Shapiro et al in the same year.[37] In our decarboxylation reaction, bisulfite concentration required is much lower. This reaction was very useful for interconversion of the polyoxins (polyoxins D,E,F⟶polyoxins L,M,K respectively). It was also extremely useful for the biosynthetic study of 5-substituted uracils of the polyoxins (vide infra) as well as ϕ-uridine.[38]

Fig. 10 Bisulfite-catalysed decarboxylation of 5-carboxyuracils.



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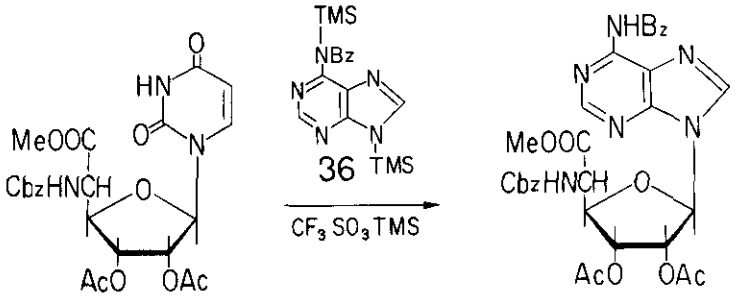
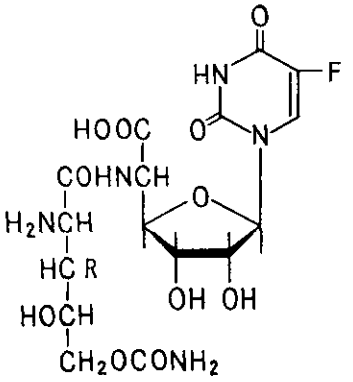
(2) Transglycosylation[39] Since the polyoxins can be regarded as nucleotide analogs, it was interesting to prepare an adenine analog of the polyoxin nucleoside, which may be regarded as an AMP analog. For this purpose, we developed *a direct transglycosylation reaction from pyrimidine to purine.* 3-Benzoyl-N$^{5'}$-carbobenzoxy-2',3'-di-O-acetyl methyl ester (35) was reacted with N^6-benzoyl-N^6, 9-bis(trimethylsilyl)adenine (36) in acetonitrile—dichloroethane in the presence of trimethylsilyl trifluoromethanesulfonate at the refluxing temperature. Adenine derivative (37) was obtained in 20% yield. After deprotection, 9-β-(5'-amino-5'-deoxy-D-allofuranosyl uronic acid)adenine was obtained in 50% yield. Unfortunately, this compound showed no biological activity. Some aminoacyl derivatives showed weak antifungal activity.[40]



8. Biosynthesis

(1) 5-Substituted pyrimidines[41] Since the polyoxins contained methyl, hydroxymethyl and carboxyl groups on carbon-5 of uracil, it was interesting to study the biosynthesis of these 5-substituted pyrimidines. *Biosynthetic* pathway of thymine of the polyoxins was shown to be independent of thymidylate synthetase. This new pathway was proposed on the basis of two experimental evidences. First, two known inhibitors of DNA synthesis (1-formylisoquinoline thiosemicarbazone and 5-fluoro-2'-deoxyuridine), when added to cultures of S. cacaoi, inhibited the synthesis of TMP from exogenously supplied uracil but did not inhibit the synthesis of thymine or hydroxymethyluracil of the polyoxins. Second, exogenously supplied thymine and hydroxymethyluracil were taken up by cells but were not incorporated into the polyoxins. The uracil in polyoxins K, L, and M could be the parent pyrimidine with one-carbon addition occuring at carbon-5 to form thymine and hydroxymethyluracil. Carbon-3 of serine but not the methyl group of methionine was a one-carbon precursor.

(2) Synthesis of aberrant polyoxins. S. cacaoi was shown to be able to synthesize unnatural polyoxins, as evidenced by the incorporation of 5-fluoro-, 5-bromo-, and 6-azauracils into polyoxins.[41] Two new polyoxin analogs have been synthesized when 5-fluorouracil is added to the cultures.[42] 5-Fluoropolyoxin L (38) and 5-fluoropolyoxin M (39) have been isolated and characterized.



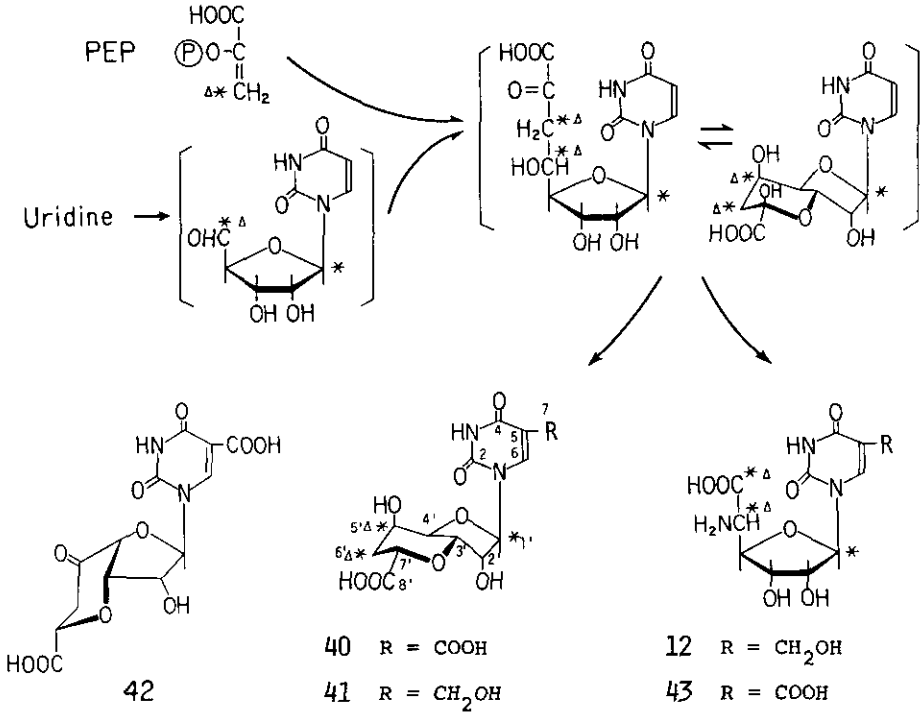
38 R = OH

39 R = H

There was a marked decrease in the molar ratio of thymine and hydroxymethyluracil compared to uracil. Apparently the unnatural 5-fluoropolyoxins inhibited the addition of the one-carbon unit to carbon-5 of uracil in the polyoxins. 5-Fluoropolyoxins showed inhibitory activity against E. coli and Staph. faecalis, which are not sensitive to the natural polyoxins.

(3) Nucleoside skeleton.[43,44] Biosynthetic study of the aminouronic acid moiety was a difficult problem because there was no practical carbon-to-carbon degradation method available except carbon-6'. This difficulty has been overcome utilizing ^{13}C-labeled glucose and ^{13}C-NMR analysis. [1-^{13}C]Glucose labeled 1'-,5'- and 6'-carbons and [6-^{13}C]glucose labeled 5'-and 6'-carbons. In addition, [U-^{14}C]uridine was incorporated into the polyoxin nucleoside and the ^{14}C distribution ratio between pyrimidine base and sugar was almost same to those of RNA-uridine and cytidine. No one-carbon precursor was incorporated, however, [3-^{14}C]glycerate specifically labeled carbon-6 (83% ^{14}C distribution). These experimental results strongly suggest the following conclusion. Uridine possibly via the 5'-aldehyde, reacts with phosphoenolpyruvate to give intermediate octofuranose uronic acid nucleoside (Fig. 11). Oxidative elimination of the terminal two carbons (C-7' and 8') followed by introduction of an amino group on carbon-5' by transamination would result in the formation of the polyoxin nucleoside. Additional proof was obtained by the isolation and structure

Fig. 11 Biosynthesis of the nucleoside skeleton of the polyoxins and octosyl acids. * Label from [1-^{13}C]glucose, Δ from [6-^{13}C]glucose. Incorporation stage of the one-carbon unit is not known.

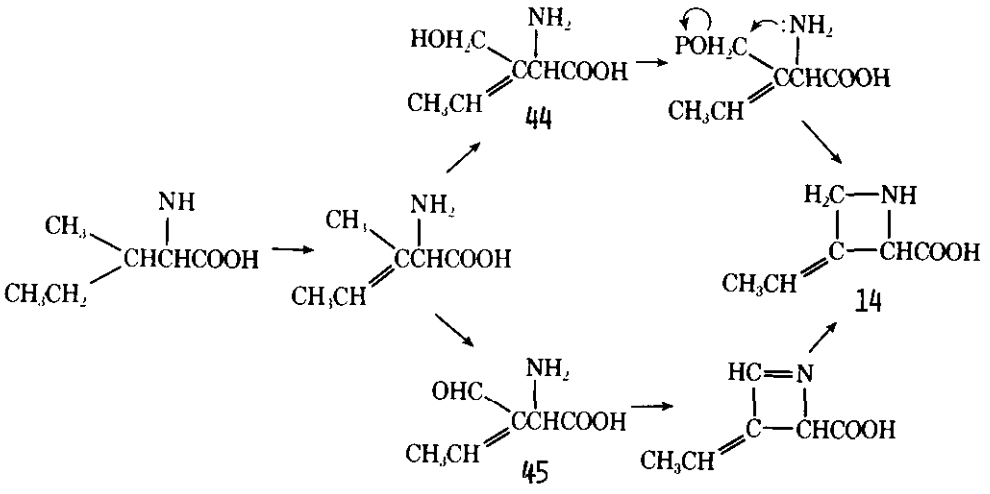


determination of shunt metabolites, octosyl acids A (40), B (41), and C (42). Labeling pattern by [1- and 6-^{13}C]glucose was shown to be identical with those of the polyoxin nucleosides (12, 43). Octosyl acids were biologically inactive but an adenine analog prepared by the transglycosylation reaction[46] (Chapter 7) was found to be a competitive inhibitor of cyclic AMP phosphodiesterase.[47] Antifungal pyrimidine nucleoside antibiotics, ezomycins[48] have the same sugar skeleton and are presumed to be biosynthesized by a similar way.

(3) Side-chain amino acid

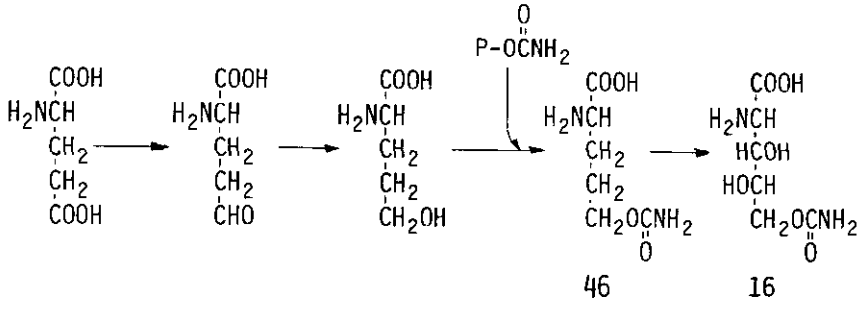
i) Polyoximic acid[49] (14). L-Isoleucine was proved to be a precursor for this azetidine carboxylic acid derivative. Possible biosynthetic pathway was proposed (Fig. 12), which includes dehydrogenation at β,γ-carbons, allylic oxidation followed by cyclization. It is to be noted that the two hypothetical intermediate amino acids, L-2-amino-3-hydroxymethyl-3-pentenoic acid (44) and L-2-amino-3-formyl-3-pentenoic acid (45) were reported to be isolated from Bankera fuligineoalba.[50]

Fig. 12. Proposed biosynthetic pathway for polyoximic acid.



ii) Carbamoylpolyoxamic acid (16).[51,52] L-Glutamate was proved to be a precursor of this aminoaldonic acid.[51] In vivo and in vitro experimental evidences were presented which support the biosynthetic pathway shown in Fig. 13. Carbamoyl phosphate was shown to be a carbamoyl donor to α-amino-δ-hydroxyvaleric acid.[52] The α-L-amino acid structure was proved to be incorporated intact into this amino acid using ^{14}C-, ^{3}H-, and ^{15}N-labeled compounds.[52,53]

Fig. 13 Biosynthetic pathway for carbamoylpolyoxamic acid.

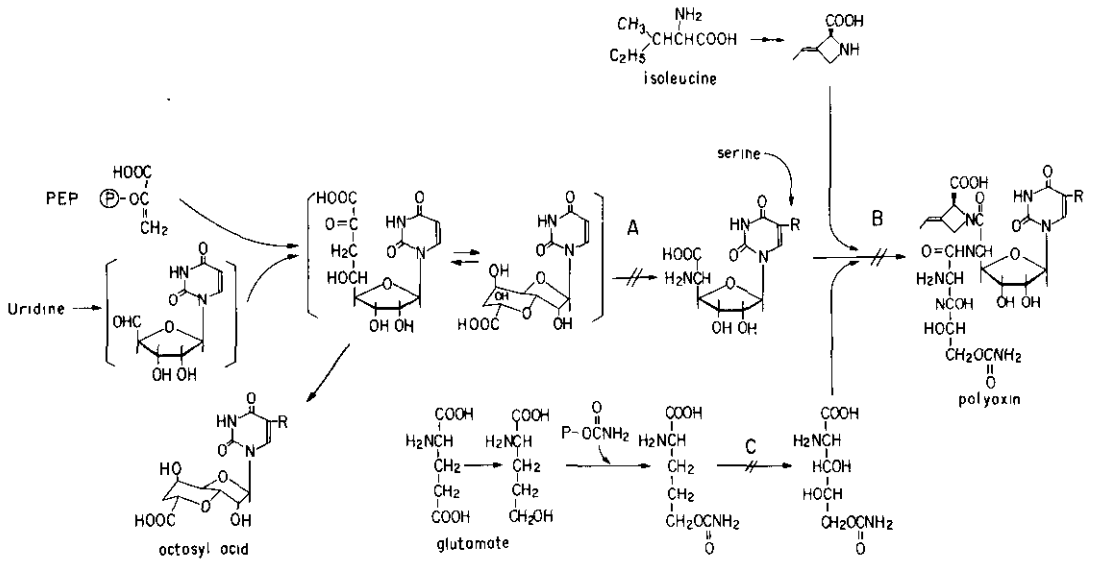


(4) The final step of the polyoxin biosynthesis.[54] Three polyoxin-non-producing mutants which accumulate UV-absorbing compounds were obtained by UV irradiation. Strain A accumulated octosyl acid A (40). Strain B accumulated octosyl acid A (40) and polyoxin C acid (43). Strain C accumulated, in addition, L-α-amino-δ-carbamoyloxyvalerate (46), polyoxin I (13), and its 5-carboxy analog as well as octosyl acid A 5'-phosphate. Characterization of these metabolites

led to the conclusion that the final step of the biosynthesis is the amide-bond formation among these hyper-modified amino acids and defective points of these mutants could be as shown in Fig. 14.

In conclusion, proposed biosynthetic pathways for the polyoxins and the octosyl acids are summerized in Fig. 14.

Fig. 14

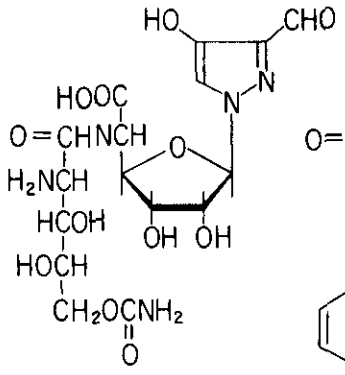


9. Polyoxin N[35] and the Neopolyoxins.[56]

Upon focusing our efforts to find new inhibitors for fungal cell wall biosynthesis, two new Streptomyces strains have been selected. Streptomyces piomogenus[55] was shown to produce polyoxins L and M. Apparently, this strain lacks enzymes for the biosynthesis of polyoximic acid and one-carbon addition onto carbon-5 of uracil. The third minor active component was isolated and designated as polyoxin N (47).[55] Unlike other polyoxins, it has 3-formyl-4-hydroxypyrazole instead of pyrimidine.

Another newly isolated Streptomyces cacaoi var. asoensis was found to produce neopolyoxins[56], which are distinctly differentiated from other polyoxins by UV absorption spectrum. Neopolyoxins A (48) and B (49) were isolated and characterized. Neopolyoxin A has the same nucleobase to that of polyoxin N and a new side-chain amino acid having a 3-hydroxypyridine ring. Neopolyoxin B has a new 2-oxo-4-imidazoline-4-carboxylic acid chromophore. Biosynthesis of this

base may be a biological equivalent of a chemical reaction reported by Fox and his co-workers[57], which transforms 5-halogenouracil nucleoside to the corresponding imidazoline derivative in one step by action of alkali.



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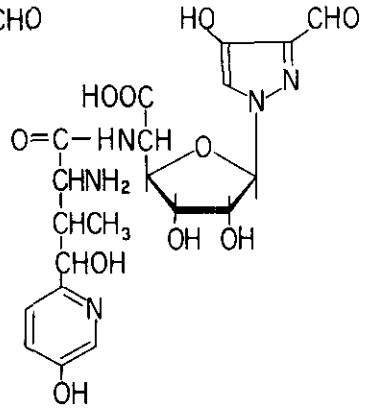

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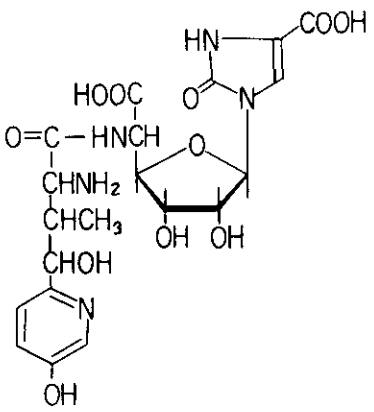

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Summary

The polyoxins are an unique group of pyrimidine nucleoside peptide antibiotics produced by Streptomyces cacaoi var. asoensis. The structure of all the members have been established and polyoxin J has been synthesized. The polyoxins inhibit competitively cell wall chitin biosynthesis of fungi. The selective and potent antifungal activity endowed usefulness as agricultural fungicide. The ionic L-dipeptide structure was shown to be essential for the activity. Two new reactions, facile decarboxylation and transglycosylation were developed for the chemical transformation of these antibiotics. Biosynthesis was extensively studied. Two side-chain amino acids are biosynthesized from L-isoleucine and L-glutamate. The nucleoside skeleton is formed by condensation of uridine and phosphoenolpyruvate and the subsequent modification. Carbon-3 of serine is a one-carbon donor for the 5-substituent group of pyrimidine. Aberrant 5-fluoropolyoxins were biosynthesized by addition of 5-fluorouracil to the culture. Shunt metabolites, octosyl acids were isolated and characterized. Directed screening for inhibitors of fungal cell wall biosynthesis has led to the discovery of polyoxin N and the neopolyoxins from newly isolated Streptomyces.

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