Studies on Polyoxins, Antifungal Antibiotics. XIII.[1] The Structure of Polyoxins[2]

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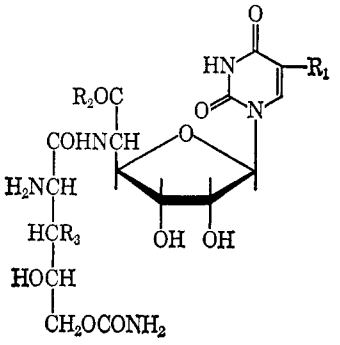
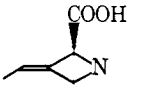
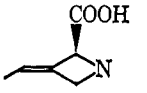
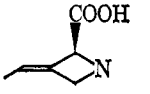
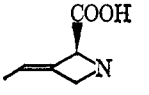
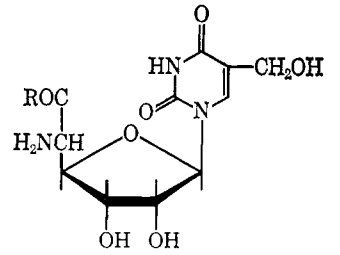
Abstract: Polyoxins A–L were found to be a new class of peptide nucleosides having the structures shown in Scheme I. Hydrolytic degradation of polyoxins afforded three groups of unusual α-L-amino acids, *i.e.*, 1-(5′-amino-5′-deoxy-β-D-allofuranuronosyl)-5-hydroxymethyluracil (polyoxin C) or its three nucleobase analogs, 5-*O*-carbamoyl-2-amino-2-deoxy-L-xylonic acid or its 3-deoxy analog, and 3-ethylidene-L-azetidine-2-carboxylic acid. These structures were established on chemical and spectroscopic evidence. 5′-Aminofuranuronoside common to all polyoxins constitutes a nucleoside with α-amino acid nature. Sequence analysis showed polyoxins are di- or tripeptide of these moieties. The structures so elucidated embody all the interesting chemical features of polyoxins. The hypothetical biochemical significance of the structure was also discussed.

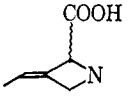
As a result of our screening program for antibiotics active against a phytopathogenic fungus, *Pellicularia filamentosa f. sasakii*, that causes the sheath blight disease of rice plant, the polyoxin complex was discovered in the culture broths of *Streptomyces cacaoi* var. *asoensis* and proved to be an excellent agricultural fungicide of wide use. A comprehensive series of papers describing our work[4-8] on the isolation and characterization of the components of the polyoxin complex have been reported. Twelve polyoxins designated alphabetically from A to L, suggesting their close structural relations were described. This paper gives the details of the structure elucidation of all polyoxins

Scheme I



Polyoxin	R_1	R_2	R_3
1a, A	CH_2OH		OH
b, B	CH_2OH	HO	OH
d, D	COOH	HO	OH
e, E	COOH	HO	H
f, F	COOH		OH
g, G	CH_2OH	HO	H
h, H	CH_3		OH
j, J	CH_3	HO	OH
k, K	H		OH
l, L	H	HO	OH



	R	
1′c, C	HO	
i, I		

(1) Part XII: K. Isono and S. Suzuki, *Agr. Biol. Chem.* (Tokyo), **32**, 1046 (1968).

(2) A preliminary account of this work appeared in parts: (a) K. Isono and S. Suzuki, *Agr. Biol. Chem.* (Tokyo), **30**, 813 (1966); (b) *ibid.*, **30**, 815 (1966); (c) K. Isono and S. Suzuki, *Tetrahedron Lett.*, 203 (1968); (d) *ibid.*, 1133 (1968); (e) K. Asahi, T. Sakurai, K. Isono, and S. Suzuki, *Agr. Biol. Chem.* (Tokyo) **32**, 1046 (1968); (f) ref 1.

(3) To whom inquiries should be addressed.

(4) S. Suzuki, K. Isono, J. Nagatsu, T. Mizutani, K. Kawashima, and T. Mizuno, *J. Antibiot.* (Tokyo), **A18**, 131 (1965).

(5) K. Isono J. Nagatsu, Y. Kawashima, and S. Suzuki, *Agr. Biol. Chem.* (Tokyo), **29**, 848 (1965).

(6) S. Suzuki, K. Isono, J. Nagatsu, Y. Kawashima, K. Yamagata, K. Sasaki, and K. Hashimoto, *ibid.*, **30**, 817 (1966).

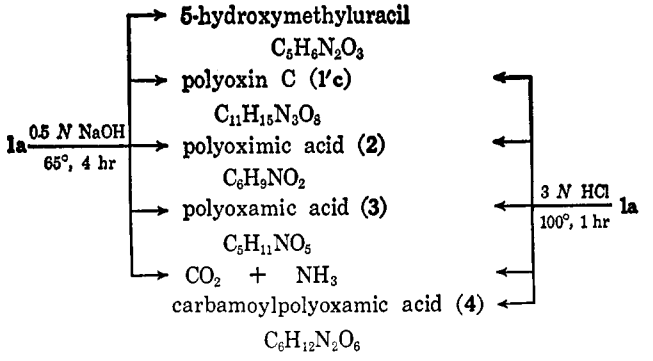
(7) K. Isono, J. Nagatsu, K. Kobinata, K. Sasaki, and S. Suzuki, *ibid.*, **31**, 190 (1967).

(8) K. Isono, K. Kobinata, and S. Suzuki, *ibid.*, **32**, 792 (1968).

depicted in Scheme I, and the biochemical significance of their unique structures.

Hydrolysis of Polyoxin A. The structure study started with polyoxin A, a crystalline main component of the polyoxin complex. Controlled alkaline hydrolysis of polyoxin A (**1a**), $C_{23}H_{32}N_6O_{14}$, with 0.5 *N* sodium hydroxide afforded four degradation products evolving 1 equiv each of ammonia and carbon dioxide (Scheme II). The first was identified by uv and ir spec-

Scheme II



troscopy as 5-hydroxymethyluracil which constituted the chromophoric group of the parent antibiotic. The second compound, which also absorbed in the uv region was found to be identical with polyoxin C (**1'c**), which was isolated as one of the components of the polyoxin complex. The remaining two compounds were new amino acids and designated polyoximic acid (**2**) and polyoxamic acid (**3**), respectively. To avoid possible racemization during the mild alkali treatment, acid hydrolysis of polyoxin A was attempted by refluxing **1a** with 3 *N* hydrochloric acid. In this case, **1'c**, **2**, and **3** were obtained in relatively low yield but they were identical with those obtained by alkaline hydrolysis. An additional amino acid was also obtained and designated carbamoylpolyoxamic acid (**4**).

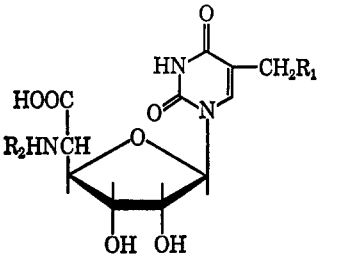
Polyoxin C (1'c). Analyses indicated **1'c** was an amphoteric compound with pK_a' values of 2.4, 8.1, and 9.5, having the formula $C_{11}H_{15}N_3O_8 \cdot H_2O$. A crystalline hydrobromide and two sulfonamides, an *N*-tosylate (**5**), and an *N*-brosylate (**6**) were prepared. Prolonged hydrolysis of **1'c** with 3 *N* hydrochloric acid gave 5-hydroxymethyluracil, which was also obtained as a result of overoxidation when **1'c** was oxidized with excess sodium metaperiodate at room temperature. The uv spectra **1'c** are of characteristic of N-1-substituted uracil derivatives as shown in Table I.

Table I. Ultraviolet Absorption Spectra of Uracil Derivatives

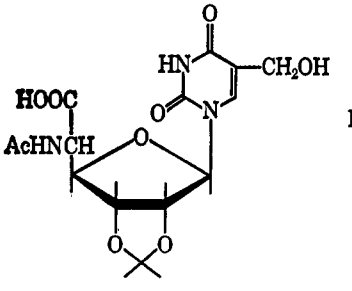
Compd	Uv max, mμ (ε) HCl (pH 1–2)	Uv max, mμ (ε) NaOH (pH 12–13)
Polyoxin C	262 (9410)	264 (7320)
5-Hydroxymethyluridine[a]	264 (9450)	263.5 (6950)
5-Methyluridine[a]	266.5 (9300)	266.5 (7090)
5-Hydroxymethyldeoxyuridine[a]	264 (9600)	264 (7030)
Thymidine[b]	267 (9650)	267 (7380)
1-Methyluracil[b]		265 (7020)
3-Methyluracil[b]		282.5 (10,700)

[a] Data of R. E. Cline, R. M. Fink, and K. Fink, *J. Amer. Chem. Soc.*, **81**, 2521 (1959). [b] Data of D. Shugar and J. J. Fox, *Biochim. Biophys. Acta*, **9**, 199, 369 (1952).

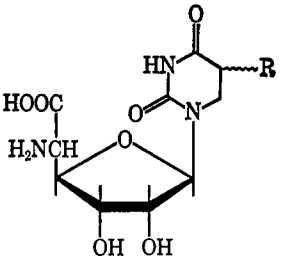
N-Acetylation of **1'c** was effected with acetic anhydride and sodium acetate in aqueous solution at room temperature, affording *N*-acetylpolyoxin C (**7**) as an amorphous powder homogeneous by tlc. It consumed 1 equiv of periodate rapidly, and formed an isopropylidene derivative (**9**) by treatment with acetone



1'c, R_1 = OH; R_2 = H
5, R_1 = OH; R_2 = *p*-$CH_3C_6H_4SO_2$
6, R_1 = OH; R_2 = *p*-$BrC_6H_4SO_2$
7, R_1 = OH; R_2 = Ac
8, R_1 = H; R_2 = Ac

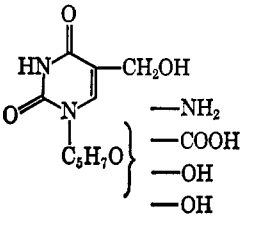


9



10, R = CH_3
11, R = CH_2OH

and sulfuric acid. Paper electrophoresis showed **1'c** had an isoelectric point near pH 4. The pK_a' of 9.5 was assigned to the uracil moiety, and the pK_a' of 8.1 to a primary amino group (1.00 equiv mole of Van Slyke amino nitrogen was estimated). The pK_a' of 2.4 was ascribed to an acidic group, most probably an α-substituted carboxylic acid. The ir spectrum of **1'c** justified the presence of carboxylate and ammonium groups. The above data suggested the following partial formula for **1'c**.



Hydrogenolysis of the allylic oxygen of **7** over platinum, afforded the corresponding deoxy derivative **8**, the pmr spectrum of which in DMSO-d_6 gave significant information about the amino position and the ring size of the sugar moiety. The pmr spectrum with assignment is given in Figure 1. The quartet at δ 4.69 was assigned to a proton on the carbon having an acetamido group, because on a spin-decoupling experiment with the doublet of NH proton at δ 8.40 (J = 8.0 Hz), it became a *doublet* with a coupling constant of 5.5 Hz. A similar doublet was obtained when a drop of D O was added to exchange the NH proton of an acetamido group. This strongly suggested that the acetamido

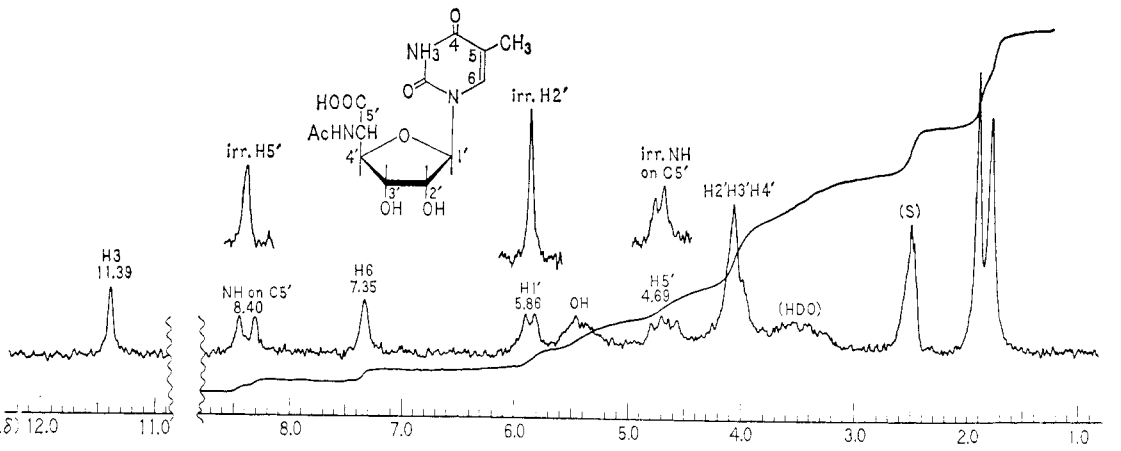


Figure 1. Pmr spectrum of *N*-acetylpolyoxin C (**8**) in DMSO-d_6; internal standard, TMS.

group is attached to C-5′. It is clear that the acetamido group cannot be on C-2′ because of lack of coupling with an anomeric proton. A periodate oxidation experiment proved conclusively the presence of an *cis*-glycol grouping at C-2′ and C-3′. Only the C-4′ position as the point of attachment of the acetamido function needs to be considered. In this case, only the pyranoside is possible and the splitting pattern of the C-4′ proton is reasonably expected to be a quartet either the proton is *cis* or *trans* to adjacent two protons, unless the pyranose is flexible and considerably distorted from the normal chair form, as reported in the case of the limited number of substituent groups.[9] The C-5′ position for amino group was also supported by the depressed sensitivity to ninhydrin when treated with cupric nitrate.[10] Since **1′c** has a primary amino group, the furanose structure must be postulated. The doublet at δ 5.86 was assigned to the anomeric proton, the splitting[11a] (5.0 Hz) of which was diagnostic of a furanoside.[11b] The allyl methyl on C-5 appeared as a somewhat broad singlet at δ 1.80 which was assumed to be subjected to an allylic coupling with the olefinic proton at δ 7.35. The broad signal corresponding to two protons at δ 5.1–5.7 was exchanged with D_2O and assigned to two hydroxyl groups. The low-field singlet at δ 11.39 was also exchangeable with D_2O and was assigned to the NH proton of uracil. The unresolved multiplet centered at δ 4.09 was ascribed consequently to the protons on C-2′, C-3′, and C-4′.

Hydrogenolysis of **1′c** over 5% rhodium on alumina[12] yielded C-5 epimers of dihydrodeoxypolyoxin C (**10**) and dihydropolyoxin C (**11**) in a 6:1:0.8 ratio. However, attempts to obtain an intact aminouronic acid by hydrolysis were unsuccessful nor could an aminohexose be obtained after reduction of the carboxyl group to an alcohol.

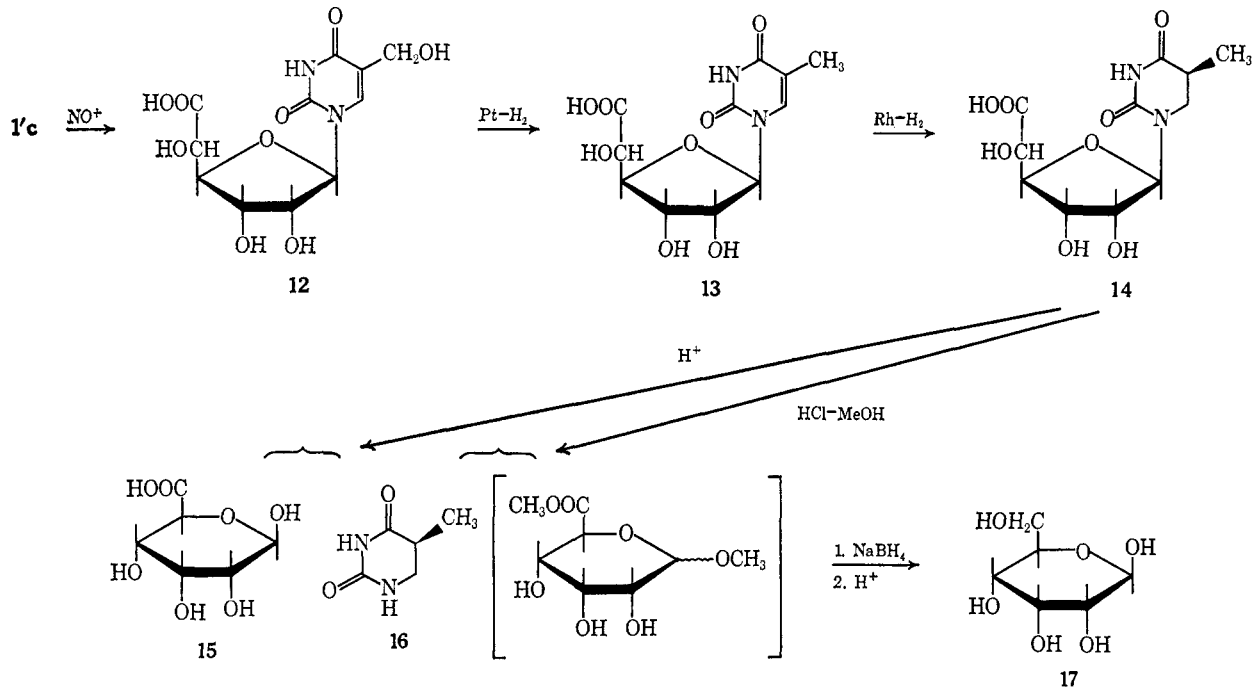
It is well known that strong neighboring-group participation of the carboxyl group of α-amino acids results in retention of configuration on deamination with nitrous acid. **1′c** was deaminated smoothly to give compound **12** in good yield, which was hydrogenated over platinum to remove the allylic oxygen to give **13**. Further hydrogenation of **13** over 5% rhodium on alumina gave the 5,6-dihydro compound (**14**) (Scheme III). In contrast to **1′c**, hydrogenation of **13** proceeded highly stereoselectively and one of the C-5 epimers was obtained exclusively. A similar result has recently been reported with the hydrogenation of thymidine.[13] Dowex 50W hydrolysis of **14** afforded crystalline (−)-dihydrothymine[14] (**16**) and a syrup of a hexuronic acid (**15**), which was characterized as a crystalline brucinate, but it was not identical with the known hexuronic acids, glucuronic, galacturonic, mannuronic,[15] altruronic,[16] and taluronic,[17] on tlc.

To convert the hexuronic acid moiety to hexose, methanolysis of **14** was attempted. After separation on a cellulose column from dihydrothymine, the methyl glycoside mixture[18] was treated with sodium borohydride followed by Dowex 50W hydrolysis, and afforded a crystalline hexose (**17**), $[\alpha]^{20}D$ +4° → +15°, which was identified unambiguously as β-D-allose by tlc and X-ray powder diffraction.[19,20]

Confirmation of the C-5′ configuration was made from ORD. The N-dithiocarbethoxy[21] derivative of 2′,3′-*O*-isopropylidenedeoxypolyoxin C (**18**) was conveniently adopted because the n → π* transition near 330 mμ of the dithiocarbethoxy group is completely free from other chromophoric groups of the molecule. It was obtained as an amorphous but analytically pure powder, uv max (MeOH) 254, 330 mμ (ε 18,900, 160), which showed the positive Cotton effect at 359 mμ ([φ] +1400) as confirmed by positive CD maximum at 343 mμ ([θ] +1320) (Figure 2) indicating D sugar, *i.e.*, L-amino acid configuration, consistent with formation

(9) E. E. Leutzinger, W. A. Bowles, R. K. Robins, and L. B. Townsend, *J. Amer. Chem. Soc.*, **90**, 127 (1968).
(10) P. O. Larsen and A. Kjaer, *Biochim. Biophys. Acta*, **38**, 148 (1960).
(11) (a) L. D. Hall, *Advan. Carbohydrate Chem.*, **19**, 59 (1964); (b) C. D. Jardetzky and O. Jardetzky, *J. Amer. Chem. Soc.*, **82**, 222 (1960); C. D. Jardetzky, *ibid.*, **82**, 229 (1960).
(12) W. E. Cohn and D. G. Doherty, *ibid.*, **78**, 2863 (1956).
(13) Y. Kondo and B. Witkop, *ibid.*, **90**, 764 (1968).
(14) K. Balenović and N. Bregant, *Croat. Chim. Acta*, **32**, 193 (1960).
(15) R. Whisler and J. N. BeMiller, *Methods Carbohydrate Chem.*, **2**, 35 (1963).
(16) F. G. Fisher and H. Schmidt, *Chem. Ber.*, **92**, 2184 (1959).
(17) J. R. Siddiqui and C. B. Purves, *Can. J. Chem.*, **41**, 382 (1963). We wish to thank Dr. J. R. Siddiqui, Food Research Institute, Central Experimental Farm, Ottawa, for a precious sample of brucine taluronate.
(18) Both anomeric pyranosides and furanosides were to be expected.
(19) M. L. Wolfrom, J. N. Schumacher, H. S. Isbell, and F. L. Humoller, *J. Amer. Chem. Soc.*, **76**, 5816 (1954).
(20) We thank Professor M. Nakajima, University of Kyoto, for a generous gift of synthetic D-allose.
(21) B. Sjöberg, A. Fredga, and C. Djerassi, *J. Amer. Chem. Soc.*, **81**, 5002 (1959); C. Djerassi, K. Undheim, R. C. Sheppard, W. G. Terry, and B. Sjöberg, *Acta Chem. Scand.*, **15**, 903 (1961); C. Djerassi, H. Wolf, and E. Bunnenberg, *J. Amer. Chem. Soc.*, **84**, 4552 (1962).

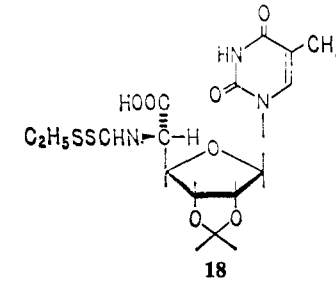
Scheme III



of D-allose with retention of C-5′ configuration on deamination.

The assignment of anomeric configuration has recently been established or substantiated for numerous α and β anomers of pyrimidine and purine nucleosides and nucleotides on the basis of the sign of the Cotton effect.[22] A positive Cotton effect **1′c** near 280 mμ in water should indicate the β configuration, if the *anti* conformation analogous to the normal pyrimidine β-nucleosides is assumed. This conformation is considered to be highly probable because **1′c** has a uracil ring and a 5′-substituted D-ribofuranose ring. Substitution at C-5′ is considered not to affect the conformation very much because Ulbricht's rule covered many 5′-mononucleotides. Some modification on 5′-amino and carboxyl was attempted. Deaminodeoxypolyoxin C (**13**) and its methyl ester (**19**) as well as *N*-acetylpolyoxin C (**7**) showed the same positive sign of Cotton effect and no large difference was observed in their amplitudes, indicating that C-5′ substitution did not make a large alteration in the uracil ring–furanose ring conformation[23] (Figure 3).

Alternative proof for the β configuration was obtained from pmr spectroscopy. No safe conclusion could be drawn from the splitting of the anomeric proton (4.9 Hz) of **7**, regarding *cis* or *trans* orientation of C-1′ and C-2′ protons. However, if the furanose ring was constrained by fusoin with 2′,3′-*O*-isopropylidene ring as in **9**, the splitting was considerably reduced to give a value of 2.0 Hz. This suggests a *trans* relation of C-1′ and C-2′ protons, *i.e.*, anomeric β configuration. Direct assignment of the configuration of ribose nucleosides can be made when the splitting of an anomeric proton is low enough[24] and reduced splittings of 2′,3′-*O*-isopropylidene derivatives or 2′,3′-cyclic phosphates were characteristic for β-nucleosides.[25] It should be



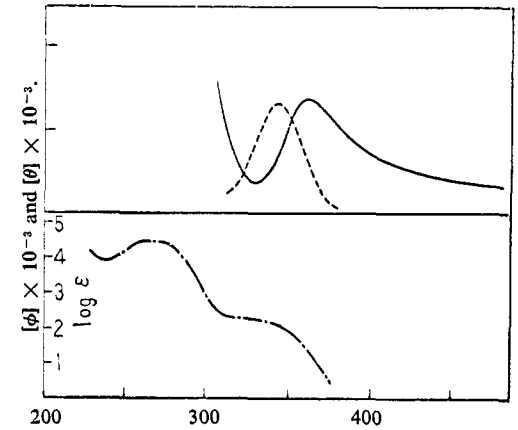


Figure 2. ORD (——), CD (– – – –), and uv (— · —) curves of *N*-dithiocarbethoxy-2′,3′-*O*-isopropylidenedeoxypolyoxin C (**18**) in methanol.

(22) T. L. V. Ulbricht, J. P. Jennings, P. M. Scopes, and W. Klyne, *Tetrahedron Lett.*, 695 (1964); T. R. Emerson and T. L. V. Ulbricht, *Chem. Ind.* (London), 2129 (1964); T. L. V. Ulbricht, T. R. Emerson, and R. J. Swan, *Biochem. Biophys. Res. Commun.*, **19**, 643 (1965); T. R. Emerson, R. J. Swan, and T. L. V. Ulbricht, *ibid.*, **22**, 505 (1966); T. L. V. Ulbricht, *Tetrahedron Lett.*, 1561 (1966); T. R. Emerson, R. J. Swan, and T. L. V. Ulbricht, *Biochemistry*, **6**, 843 (1967); I. Frič, J. Šmejkal, and J. Farkás, *Tetrahedron Lett.*, 75 (1966).

(23) The X-ray crystallographic analysis of *N*-brosylpolyoxin C showed this derivative also had *anti* conformation in the crystal structure as will be shown in the next section.

(24) R. U. Lemieux and J. W. Lown, *Can. J. Chem.*, **41**, 889 (1962); L. Goldman and J. W. Marsico, *J. Med. Chem.*, **6**, 413 (1963).

(25) N. J. Leonard and R. A. Laursen, *J. Amer. Chem. Soc.*, **85**, 2026 (1963); T. Nishimura, B. Shimizu, and I. Iwai, *Chem. Pharm. Bull.* (Tokyo), **12**, 1471 (1964).

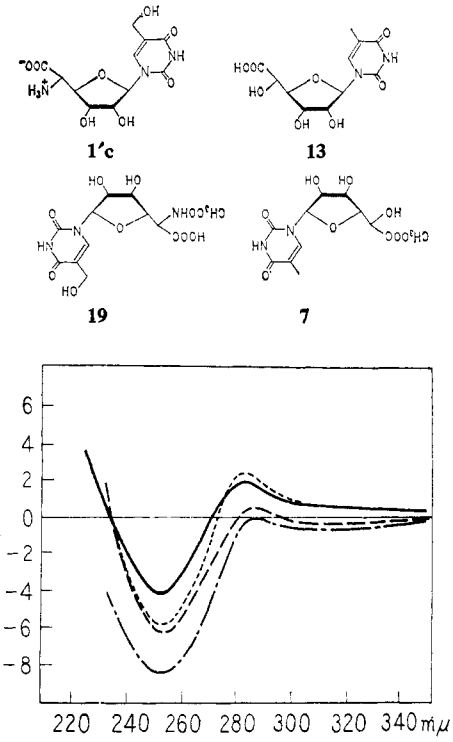
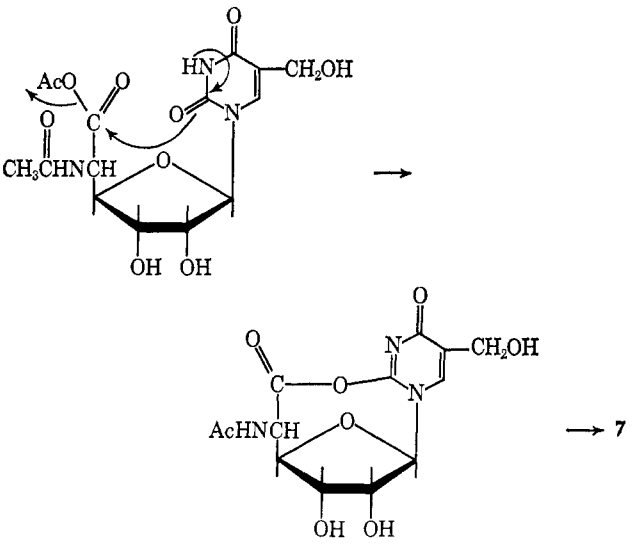


Figure 3. ORD curves of polyoxin C (**1'c**) (——), deaminodeoxypolyoxin C (**8**) (— — —), methyl deaminodeoxypolyoxinate (**19**) (- - -), and *N*-acetylpolyoxin C (**7**) (------).

noted that the splitting of an anomeric proton of **1'c** (4.8 Hz) and of **7** (4.9 Hz) is consistent with a normally puckered C-2′ *endo*-ribose nucleotide conformation.[26,27] The structure of **1'c** was thus unambiguously established as 1-(5′-amino-5′-deoxy-β-D-allofuranuronosyl)-5-hydroxymethyluracil.

It is well known that α-amino acids will undergo racemization during acetylation with excess acetic anhydride *via* an oxazolone intermediate.[28] **1'c** was acetylated with excess acetic anhydride and sodium acetate in D_2O. Analytically pure **7** thus obtained showed the quartet at δ 4.69 ascribed to the C-5′ proton indicating that no racemization occurred during acetylation. Tritium labeling reaction for *N*-acetylamino acid[29] *via* oxazolone formation in ^3H_2O was applied for **7**, also giving negative result. Moreover, acid hydrolysis of **7** regenerated **1'c** exclusively, and no diastereomer. A possible explanation for this retention of C-5′ configuration is as follows. The C-2 carbonyl of 5-hydroxymethyluracil participates with the formation of an oxazolone. An active lactone thus formed would easily regenerate the original compound, **7**.

Crystal Analysis of *N*-Brosylpolyoxin C.[30] Because of the importance of the structure of polyoxin C, crystal analysis of *N*-brosylpolyoxin C was undertaken to verify the structure proposed in the preceding section.



A three-dimensional X-ray structure analysis was carried out on *N*-brosylpolyoxin C monohydrate (**6**). The crystals are monoclinic with cell constants a = 20.61, b = 5.61, c = 9.24 Å, and β = 101.0°. The space group is $P2_1$ and there are two molecules per unit cell. Multiple-film equiinclination Weissenberg data were taken with Cu Kα radiation for reciprocal levels *h0l* through *h4l*. The intensities of 543 independent reflections were estimated by use of a microphotometer. Intensities were corrected for Lorentz and polarization factors. No correction was made for absorption.

The coordinates of the bromine and the sulfur atoms were estimated from a Patterson synthesis and used to calculate the structure factors for a Fourier synthesis. Four successive Fourier and one ΔF syntheses gave the positions of all 33 nonhydrogen atoms. R factor, $\Sigma||F_o| - |F_c||/\Sigma|F_o|$, at this point was 24.0%. At this stage, refinement by the method of least squares was initiated. Several cycles of least-squares refinement dropped R to 0.10. The geometry of the molecule is illustrated in Figure 4.

N-Brosylpolyoxin C and the crystal water are connected by the formation of two hydrogen bonds with a O–O distance of approximately 2.6 Å. The plane of the furanose ring and the plane of the pyrimidine ring have a dihedral angle of 69.2°, which is comparable to the values in cytidine 3′-phosphate (62°),[31] 5-fluoro-2′-deoxy-β-uridine (71.9°),[32] calcium thymidylate (75°),[33] and cytidine (75°).[34] The torsion angle, as defined by Donohue and Trueblood,[35] is −51°, which is representative *anti* conformation analogous to the usual pyrimidine nucleosides and nucleotides. The furanose ring exists in the C-2′ *endo* conformation. Four atoms in the furanose ring (C-1′–O–C-4′–C-3′) are coplanar and C-2′ is displaced approximately 0.7 Å away from the plane.

Polyoximic Acid (2). Mild alkaline hydrolysis of **1a** afforded a racemic **2**. The same compound was obtained by acid hydrolysis which also showed no optical activity. It gave a characteristic yellow color with ninhydrin. It was an amphoteric compound having the

(26) C. D. Jardetzky, *J. Amer. Chem. Soc.*, **84**, 62 (1962).
(27) The X-ray analysis study on *N*-brosylpolyoxin C justified the C-2′ *endo* conformation as shown in the succeeding section.
(28) J. W. Cornforth in "Heterocyclic Compounds," Vol. V, John Wiley & Sons, Inc., New York, N. Y., 1957, p 340.
(29) H. Matsuo, Y. Fujimoto, and T. Tatsuno, *Tetrahedron Lett.*, 3465 (1965); H. Matsuo, Y. Fujimoto, and T. Tatsuno, *Biochem. Biophys. Res. Commun.*, **22**, 69 (1966).
(30) The details of the experimental data will be submitted elsewhere. We are indebted to Dr. T. Sakurai of this institute for his guidance in this section.
(31) J. Kraut and L. H. Jensen, *Acta Cryst.*, **16**, 79 (1963).
(32) D. R. Harris and W. M. Macintyre, *Biophys. J.*, **4**, 203 (1964).
(33) K. N. Trueblood, P. Horn, and V. Luzzati, *Acta Cryst.*, **14**, 965 (1961).
(34) S. Furberg, *ibid.*, **3**, 325 (1950).
(35) J. Donohue and K. N. Trueblood *J. Mol. Biol.*, **2**, 363 (1960).

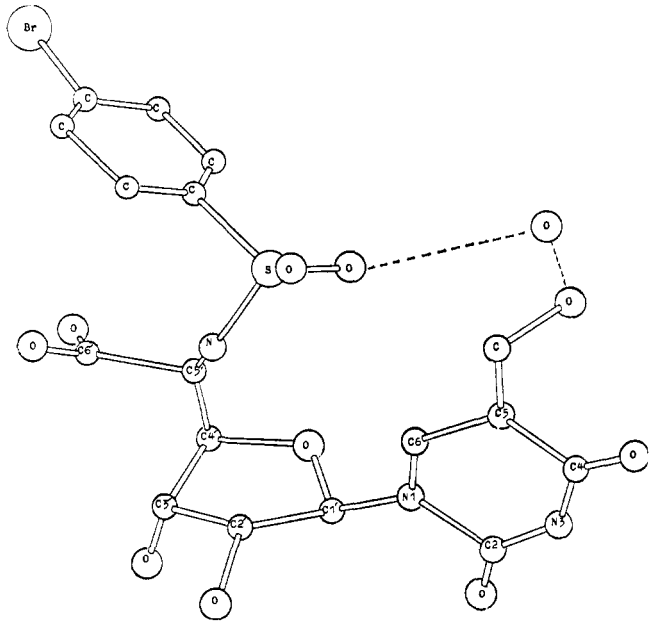


Figure 4. A perspective drawing of the *N*-brosylpolyoxin C molecule viewed along the *b* axis.

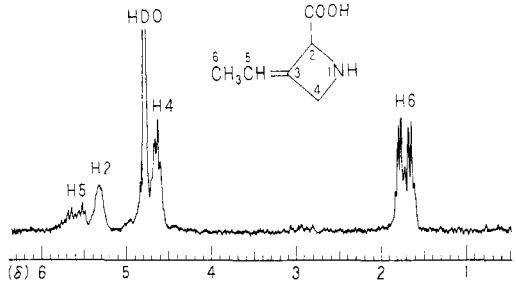


Figure 5. Pmr spectrum of polyoximic acid (**2**) in D_2O; internal standard, DSS.

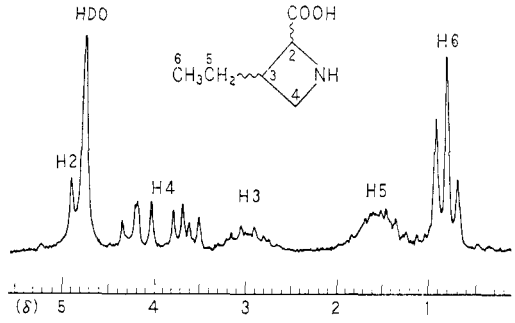


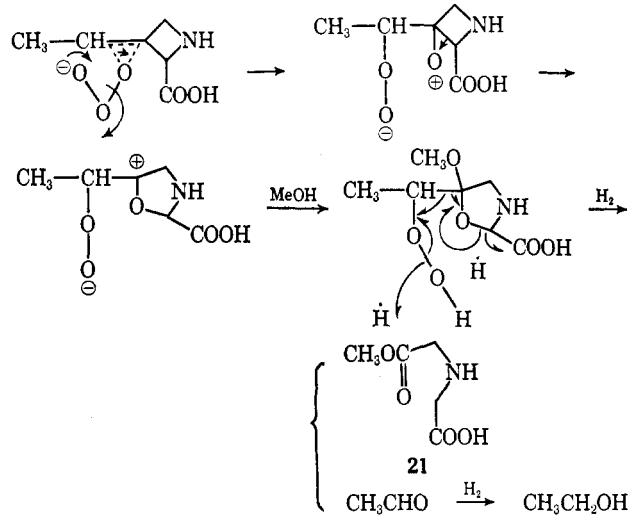
Figure 6. Pmr spectrum of racemic dihydropolyoximic acid (**20**) in D_2O; internal standard, DSS.

pK_a' values of 2.5 and 9.8, attributable to the α-amino acid. The ir spectrum showed the characteristic bands of carboxylate and immonium (3100–2200, 1600, and 1385 cm^{-1}) as well as a trisubstituted olefin (810 cm^{-1}). Hydrogenation of **2** over palladium consumed 1 equiv mole of hydrogen to afford a dihydro compound (**20**), which lacked a band due to olefin in the ir spectrum. Slightly increased pK_a' values (2.7 and 10.1) suggested the presence of a β,γ-double bond, which was also consistent with the facile racemization during hydrolysis. This compound gave a single spot on ppc and tlc which indicated it to be an enantiomeric mixture rather than a mixture of diastereomers. The above data suggested that **2** is a cyclic imino acid with a double bond in the β position.

The pmr spectrum of **2** in D_2O is shown with assignment in Figure 5. The signals were coupled finely with allylic or homoallylic protons.[36] Spin-decoupling experiment showed that an allylic C-6 methyl group at δ 1.72 was coupled with an olefinic proton at C-5 (J = *ca.* 7.5 Hz), indicating the presence of an ethylidene group. Since no large coupling was observed between C-2 methine at δ 5.32 and C-4 methylene centered at δ 4.65, only one structure for **2** is possible. The pmr spectrum of **20** supported this structure (Figure 6). The C-4 methylene protons (δ 3.69, 4.21) of this compound showed an octet of an AB part of ABX pattern as the result of decreased equivalency by double-bond saturation.

Reductive ozonolysis was attempted to obtain a four-membered cyclic ketone by treatment of **2** with ozone in cold methanol. Subsequent hydrogenation over palladium unexpectedly afforded iminodiacetic acid half methyl ester (**21**) in a good yield, absorbing 2 equiv of hydrogen, which was identified as iminodiacetic acid after hydrolysis. A possible reaction mechanism is proposed in Scheme IV. Migration of the α-carbon as a carbanion is highly probable in the second zwitterionic stage, because of the inductive effect of imino and carboxyl groups as well as strain of a four-membered azetidine ring.

Scheme IV



Optically active dihydropolyoximic acid (**20**), $[\alpha]^{20}D$ $-94.6°$ (*c* 1.5, H_2O), was obtained by hydrolysis of the hydrogenated compound of the parent antibiotic, dihydrodeoxypolyoxin A (**22**). Clearly, the allylic oxygen was hydrogenolyzed and double-bond saturation in this case might also proceed stereoselectively as in the case of racemic **2**, because tlc showed a single spot identical with racemic **20**. The *N*-dithiocarbethoxy derivative[21] (**23**) showed a positive Cotton effect at 353 mμ ([ϕ] -230 pk) in methanol as substantiated by the positive CD maximum at 330 mμ ([θ] $+2000$), indicating the L configuration for the α-carbon[37] (Figure 7).

(36) S. Sternhell, *Rev. Pure Appl. Chem.*, **14**, 15 (1964), and references cited therein.

(37) We prepared the N-dithiocarbethoxy derivative of L-azetidine-2-carboxylic acid, which showed a similar optical behavior: $[\phi]_{349}$

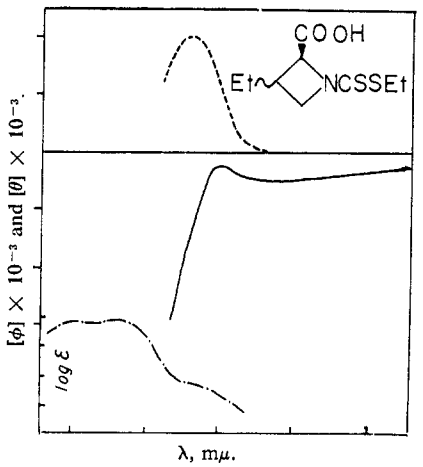


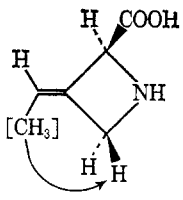
Figure 7. ORD (——), CD (– – – –), and uv (— · —) curves of *N*-dithiocarbethoxy-(−)-dihydropolyoximic acid (**23**) in methanol.

Recently, intramolecular NOE technique[38] was successfully applied to *cis* or *trans* assignment of ethylidene side chain in quinuclidine[39] and dehydrovoachalotine.[40] Irradiation of C-6 methyl protons of **2** (10% solution in D_2O) resulted in relative increase of the integrated intensity of C-4 methylene protons to the sum of signal intensities of C-2 proton and C-5 proton as shown in Table II. Positive enhancement of the integrated intensity of the C-4 methylene protons reached an average of 22% leaving the intensity of the sum of C-2 and C-5 protons unaltered.

Table II. NOE of Polyoximic Acid

	Area signal intensity (in arbitrary units)[a]		%
	$H_2 + H_5$	H_4	increase
Normal	100	125 ± 3[b]	
CH_3 irradiated 1[c]	100	155 ± 1	24.0
2	100	153 ± 3	22.4
3	100	151 ± 3	20.8

[a] Each datum is the average of three different runs. [b] The larger value than the theoretical (100) is considered to come from the overlapping with HDO signal. [c] Variable frequency power (1, weak; 2, medium; 3, strong).



On the basis of above discussion, the structure of 3-ethylidene-L-azetidine-2-carboxylic acid (**2**) was assigned to polyoximic acid, in which the ethylidene group is *trans* with respect to the carboxyl group.

Polyoxamic Acid (3) and Carbamoylpolyoxamic Acid (4). **3** was first obtained by alkaline hydrolysis of **1a** as a crystalline monohydrate, $C_5H_{11}NO_5 \cdot H_2O$, having the pK_a' values of 2.9 and 8.7. Its ir spectrum showed the bands for carboxylate, ammonium, and hydroxyl groups. The amino position was suggested to be α by the paper chromatographic detection of a reducing spot ascribed to a tetrose[41] and the insusceptibility to ninhydrin when treated with cupric nitrate.[10] Unambiguous proof of the α-amino structure was obtained by acetylation of **3** in methanol to yield *N*-acetyl-γ-lactone (**24**), ir ($CHCl_3$) 1787 cm^{-1}, which was not oxidized by periodate. Moreover, subsequent exhaustive acetylation of **3** or **24** with acetic anhydride and pyridine resulted in elimination of the β-acetoxy group, affording a five-membered unsaturated lactone (**25**), uv max (MeOH) 243 mμ (ε 8300), ir (CCl_4) 1777 and 1753 cm^{-1}. Reduction of **24** with 5% sodium amalgam[42,43] gave the known 2-acetamido-2-deoxy-α-L-xylose[44] (**26**), $[\alpha]^{20}D$ $-45° \rightarrow -6.4°$, in good yield. The identity with the corresponding synthetic D-xylose derivative[45] was confirmed by X-ray powder diffraction.

Acid hydrolysis of **1a** with 3 *N* hydrochloric acid afforded a new crystalline amino acid **4**, together with **3**, which were separable on cellulose chromatography with 75% aqueous phenol. The presence of a carbamoyl group was indicated by an absorption at 1705 cm^{-1} and a positive *p*-(*N*,*N*-dimethylamino)benzaldehyde test.[46] On mild alkaline and prolonged acid hydrolyses, it gave 1 equiv each of ammonia and carbon dioxide, affording **3** (Scheme V). The position of the carbamoyl group was presumed to be at C-5′, because **1a** consumed 3 equiv of periodate and yielded a five-membered unsaturated lactone (**27**) [uv max (MeOH) 243 mμ (ε 8800); ir ($CHCl_3$) 1765, 1740, 1714 cm^{-1}] on acetylation with acetic anhydride and pyridine. Retention of the α-L configuration during alkaline hydrolysis was proved by obtaining **3** by acid hydrolysis, and both **3** and **4** showed positive Cotton effect either in aqueous solution or in 0.5 *N* hydrochloric acid, ascribing to $n \rightarrow \pi^*$ transition of the carboxyl group.[47] This is consistent with the L-*xylo* configuration. Thus, structure **4** as 5-*O*-carbamoyl-2-amino-2-deoxy-L-xylonic acid is firmly established.

Amino Acid Sequence and the Structure of Polyoxin A. Three degradation products, the structures of which were elucidated as above, are all unusual α-L-amino acids, and the pK_a' of **1a** (3.0, 7.3, 9.6) would correspond

−480 pk, $[\theta]_{330}$ +2600. As the L configuration of this amino acid was established chemically by Fowden, this confirms our assignment of polyoximic acid. We thank Professor L. Fowden, University College, London, for a precious sample and kind information. Both free amino acids showed a peak at 225 mμ in 0.5 *N* hydrochloric acid ascribed to the carboxyl $n \rightarrow \pi^*$ transition. In aqueous solution, however, both showed a trough at 205 mμ, which is considered to correspond to a negative CD maximum at 193 mμ of L-proline and L-hydroxyproline; *cf.* M. Legrand and R. Vienett, *Bull. Soc. Chim. Fr.*, 679 (1965).

(38) F. A. L. Anet and A. J. R. Bourn, *J. Amer. Chem. Soc.*, **87**, 5250 (1965), and references cited therein.

(39) J. C. Nouls, G. Van Binst, and R. H. Martin, *Tetrahedron Lett.*, 4065 (1967).

(40) J. C. Nouls, P. Wollast, J. C. Breakman, G. Van Binst, J. Pecker, and R. H. Martin, *ibid.*, 2731 (1968).

(41) P. J. Stoffyn and R. W. Jeanloz, *Arch. Biochem. Biophys.*, **52**, 373 (1954).

(42) F. H. Humoller, *Methods Carbohydrate Chem.*, **1**, 103 (1962); H. S. Isbell, *ibid.*, **1**, 135 (1962).

(43) W. B. Benfrow, Jr., and C. R. Hauser, "Organic Syntheses," Coll. Vol. II, John Wiley and Sons, Inc., New York, N. Y., 1943, p 607; A. F. Holleman, *ibid.*, Coll. Vol. I, 1956, p 554.

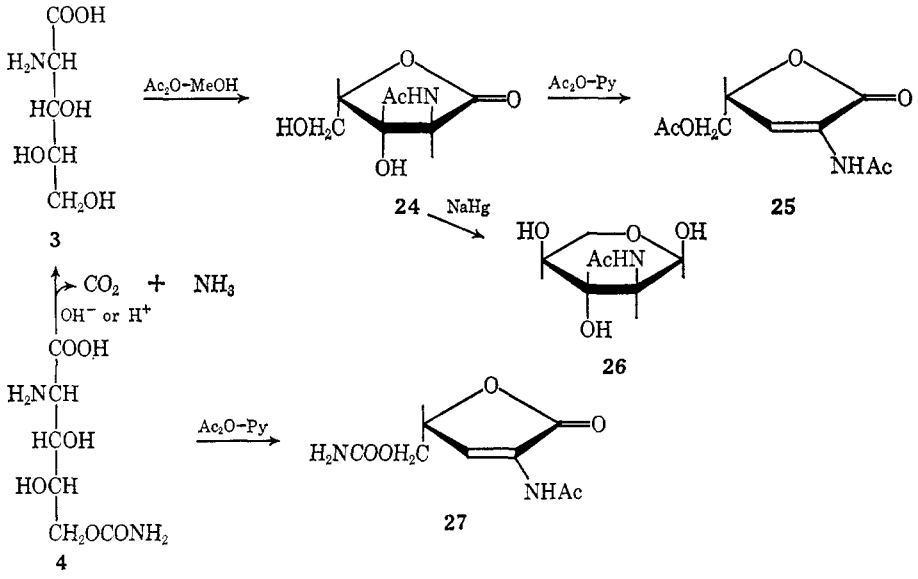
(44) M. L. Wolfrom, D. Horton, and A. Böckmann, *Chem. Ind.* (London), 41 (1963).

(45) M. L. Wolfrom and K. Anno, *J. Amer. Chem. Soc.*, **75**, 1038 (1953). We thank Professor M. L. Wolfrom, The Ohio State University, for the kind gift of 2-acetamido-2-deoxy-D-xylose.

(46) R. M. Fink, R. E. Cline, C. McGaughey, and K. Fink, *Anal. Chem.*, **28**, 4 (1956).

(47) J. P. Jennings, W. Klyne, and P. M. Scopes, *J. Chem. Soc.*, 294 (1965); A. Kjaer, W. Klyne, P. M. Scopes, and D. R. Sparrow, *Acta Chem. Scand.*, **18**, 2412 (1964).

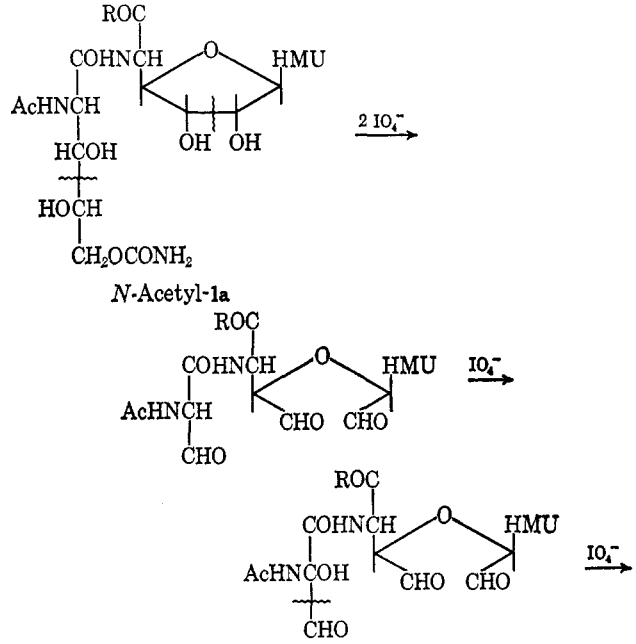
Scheme V



to carboxyl, amino, and uracil groups, respectively. N-Terminal determination was performed by the deamination method. **1a** was deaminated with nitrous acid giving an amorphous product, which showed two spots on tlc. Each compound gave **1'c** and **2** but not **3** on tlc after alkaline hydrolysis, indicating that the N terminal is **3**. The partial hydrolysis product (**1'i**) with the formula $C_{17}H_{22}N_4O_9$, $pK_a' = 2.9, 6.1, 9.3$, was isolated in low yield on mild alkaline hydrolysis. The molecular constituents were found to be **1'c** and **2** by hydrolysis. **1'i** gave 0.72 equiv of Van Slyke amino nitrogen, indicating that the N terminal is **1'c**. Further, **1'i** was subjected similarly to deamination followed by hydrolysis, giving **2** but not **1'c**. This compound was later proved to be identical with polyoxin I[48] (*vide infra*).

Thus, the amino acid sequence was established and the structure **1a**, obtained by uniting with amide linkages these three amino acid moieties, embodies all the features of polyoxin A. The uv spectra (Table III) are characteristic of *N*-1-substituted uracil derivatives.[49] The pK_a' values of 3.0, 7.3, and 9.6 are reasonably assigned to the carboxyl, amino, and uracil groups, respectively. The splitting of the anomeric proton, 5.5 Hz, in the pmr spectrum suggests the furanose structure. Polyoxin A gave a positive *p*-(*N*,*N*-dimethylamino)-benzaldehyde test[46] indicating the presence of a carbamoyl group. A rapid equivalent mole estimation of Van Slyke amino group corresponds to the primary amino group and a second slow equivalent mole indicated the carbamoyl group. Polyoxin A consumed 3 equiv of periodate rapidly, consistent with the assigned structure. *N*-Acetylpolyoxin A lacked the pK_a' corresponding to 7.3 and consumed 4 equiv of periodate. This was somewhat unexpected but the active hydrogen of an aldehyde formed by primary oxidation could suffer further oxidation to give a hydroxyl derivative, which underwent succeeding cleavage (Scheme VI). Although the amide II band in the ir spectrum of **1'a** is obscured by the broad carboxylate–carbonyl band, the ir spectrum of the hydrochloride clearly showed a

Scheme VI



HMU = hydroxymethyluracil

R = polyoximic acid

shoulder at 1550 cm^{-1}, justifying the presence of a secondary amide group (Figure 8).

It should be of particular interest to account for the unusual lability of the glycosyl bond of **1a** to alkali. Susceptibility of the peptide α-hydrogen to alkali is known to cause racemization. Because of the situation of the α-hydrogen on C-5' of the furanoside in this case, racemization at C-5' could be the cause of spontaneous hydrolysis of the nucleoside as follows.

Although epimerized uronic acids at C-4' and C-5' were not identified, the argument is supported by the facts that polyoxin B (**1b**) which has free C-6' carboxyl (*vide infra*) afforded very little 5-hydroxymethyluracil on alkaline hydrolysis, and **1'c** was very stable to alkali. Obviously, the carboxylate ion prevented the dissociation of the α-hydrogen and the amino group is less electron withdrawing than the acylamino group. Thus, racemization at C-5' is reflected to the spontaneous libera-

(48) The C_{19} formula of polyoxin I previously presented[7] should be revised.

(49) (a) Reference in Table I, footnote *a*; (b) references in Table I, footnote *b*.

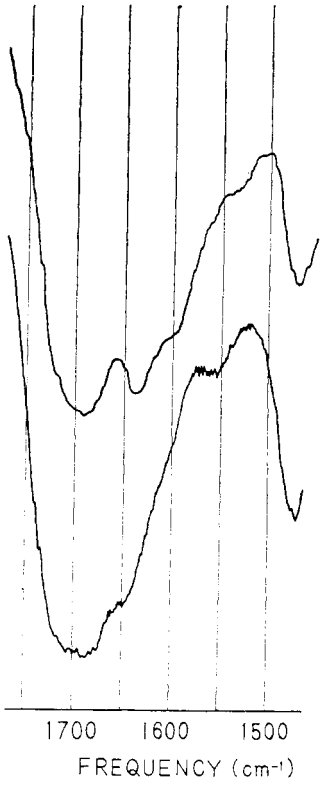
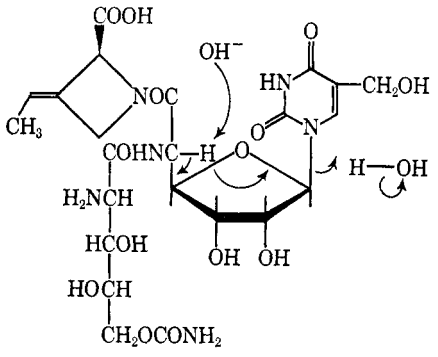


Figure 8. Carbonyl region of ir of polyoxin A (upper) and the hydrochloride (lower) in KBr.

tion of 5-hydroxymethyluracil and the fact that no C-5′ epimer of **1′c** was obtained by alkaline hydrolysis suggested the elimination of aglycone should be preponderant to the competing reprotonation reaction at C-5′.

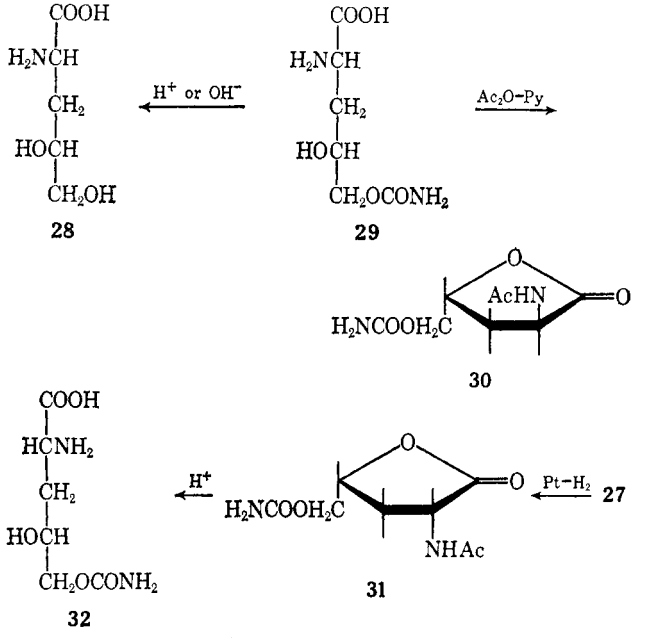


Analogous mechanisms were postulated for the alkaline hydrolysis of *S*-adenosylmethionine[50] and the cyanide-catalyzed decomposition of vitamin B_{12}.[51]

Other Polyoxins. The close structural relation of polyoxins has been suggested in the previous papers of this series[2c,7] on the basis of alkaline hydrolysis. Some of the properties of polyoxins and the molecular constituents are summarized in Table III. There are four modifications in the chromophore: 5-hydroxymethyluracil, uracil-5-carboxylic acid, thymine, and uracil. Polyoxins are divided roughly into two groups, one having **2** and negative rotation, and the other having no **2** and positive rotation. Deoxypolyoxamic acid (**28**) was designated for a new amino acid obtained from hydrolysis of polyoxins E and G instead of **3**.

A. Deoxypolyoxamic Acid (28) and Carbamoyldeoxypolyoxamic Acid (29). Alkaline hydrolysis of polyoxin E or G yielded a colorless syrup of a new amino acid, which underwent partial crystallization on desiccation. It analyzed for $C_5H_{11}NO_4$ having the pK_a' values of 3.0 and 9.0, and an α-amino group was presumed in a similar way to **3**. The crystalline carbamoyl compound (**29**) was obtained similarly by acid hydrolysis together with **28**, which were separated on a cellulose column with 75% phenol. Beside the bands characteristic for carboxylate and ammonium, **29** showed a band at 1703 cm^{-1} ascribed to a carbamoyl group in the ir spectrum. A positive Cotton effect at 222 mμ ([ϕ] +2000 pk) in 0.5 *N* hydrochloric acid indicated the α-L configuration.[47] On mild alkaline or prolonged acid hydrolysis, **29** gave **24** (Scheme VII).

Scheme VII



28 consumed 1 equiv of periodate/mole affording 0.7 equiv of formaldehyde, whereas **29** consumed no periodate, indicating strongly the 3-deoxy-5-*O*-carbamoyl structure. On acetylation with acetic anhydride and pyridine, **29** yielded a crystalline *N*-acetyl-γ-lactone (**30**): mp 188–191°; ir ($CHCl_3$) 1782, 1735, 1680 cm^{-1}. To obtain the 3-deoxy derivative of **4**, the unsaturated lactone (**27**) obtained by acetylation of **4** was hydrogenated over platinum, yielding *exclusively* one of the two diastereomers **31**: mp 174–175°; ir ($CHCl_3$) 1783, 1737, 1683 cm^{-1}. Compounds **30** and **31** were not identical though they have similar ir and pmr spectra. **31** was hydrolyzed with 1 *N* hydrochloric acid to give a free carbamoylamino acid (**32**), which showed a negative Cotton effect at 222 mμ ([ϕ] −2160 tr) in 0.5 *N* hydrochloric acid, indicating α-D configuration. This was just the opposite sign and almost the same molecular rotation as that of **29**. Since **31** has the α-D-*threo* configuration, the α-L-*erythro* configuration should be assigned to **29** and it is reasonable that the stereoselective hydrogenation yielded a *cis* compound (**31**) exclusively. From these data, the structure of 2-amino-2,3-dideoxy-5-*O*-carbamoyl-L-xylonic acid is proposed for **29**.

(50) W. Frank, J. Wieczorkowski, N. A. Hughes, and J. Baddiley, *Proc. Chem. Soc.*, 449 (1961); J. Baddiley, W. Frank, N. A. Hughes, and J. Wieczorkowski, *J. Chem. Soc.*, 1999 (1962).
(51) A. W. Johnson and N. Shaw, *Proc. Chem. Soc.*, 447 (1961).

Table III. Properties and Molecular Constituents of Polyoxins

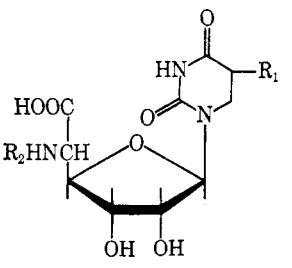
Polyoxin	Formula	pK_a' I	II	III	IV	$[\alpha]^{20}D$, deg (c 1, H_2O)	Uv max, mμ (log ε) 0.05 *N* HCl	0.05 *N* NaOH	Chromophore	Polyoximic acid (2)	Polyoxamic acid (3)	Deoxypolyoxamic acid (28)
A	$C_{23}H_{32}N_6O_{14}$	3.0		7.3	9.6	−30	262 (3.94)	264 (3.80)	5-Hydroxymethyluracil	+	+	−
B	$C_{17}H_{25}N_5O_{13}$	3.0		6.9	9.4	+34	262 (3.94)	264 (3.82)	5-Hydroxymethyluracil	−	+	−
C	$C_{11}H_{15}N_3O_8$	2.4		8.1	9.5	+11	262 (3.97)	264 (3.87)	5-Hydroxymethyluracil	−	−	−
D	$C_{17}H_{23}N_5O_{14}$	2.6	3.7	7.3	9.4	+30	276 (4.05)	271 (3.85)	Uracil-5-carboxylic acid	−	+	−
E	$C_{17}H_{23}N_5O_{13}$	2.8	3.9	7.4	9.3	+19	276 (4.00)	271 (3.81)	Uracil-5-carboxylic acid	−	−	+
F	$C_{23}H_{30}N_6O_{15}$	2.7	3.9	7.2	9.3	−18	276 (4.06)	271 (3.87)	Uracil-5-carboxylic acid	+	+	−
G	$C_{17}H_{25}N_5O_{12}$	3.2		7.3	9.3	+37	262 (3.92)	264 (3.82)	5-Hydroxymethyluracil	−	−	+
H	$C_{23}H_{32}N_6O_{13}$	3.3		7.2	9.4	−38	265 (3.88)	266 (3.79)	Thymine	+	+	−
I	$C_{17}H_{22}N_4O_9$	2.7		6.1	9.5	−25	262 (3.94)	264 (3.78)	5-Hydroxymethyluracil	+	−	−
J	$C_{17}H_{25}N_5O_{12}$	3.0		7.1	9.9	+32	264 (3.91)	267 (3.81)	Thymine	−	+	−
K	$C_{22}H_{30}N_6O_{13}$	3.0		7.2	9.3	−17	259 (3.95)	262 (3.86)	Uracil	+	+	−
L	$C_{16}H_{23}N_5O_{12}$	3.0		7.0	9.4	+34	259 (3.96)	262 (3.85)	Uracil	−	+	−

B. Aminourono Nucleosides. Alkaline hydrolysis of a variety of polyoxins afforded the three modifications of nucleosides corresponding to polyoxin C as shown in Table IV. These were designated tentatively as polyoxin C acid (**33**), thymine-polyoxin C (**34**), and uracil-polyoxin C (**35**). Interconversion of these nucleosides was attempted to verify their structure.

Table IV. Aminourono Nucleosides Obtained by Hydrolysis of Polyoxins

Polyoxin	Aminourono nucleoside
A	Polyoxin C (**1'c**)
B	Polyoxin C (**1'c**)
D	Polyoxin C acid (**33**)
E	Polyoxin C acid (**33**)
F	Polyoxin C acid (**33**)
G	Polyoxin C (**1'c**)
H	Thymine-polyoxin C (**34**)
I	Polyoxin C (**1'c**)
J	Thymine-polyoxin C
K	Uracil-polyoxin C (**35**)
L	Uracil-polyoxin C (**35**)

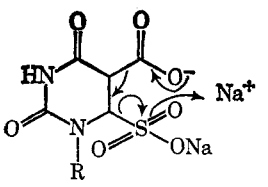
N-Acetylpolyoxin C (**7**) was oxidized catalytically over platinum in an oxygen stream[49a] to give a mixture of an aldehyde (**36**) and an acid (**37**), which was further oxidized with silver oxide followed by deacylation to give a crystalline nucleoside, $[\alpha]^{23}D$ +15.0° (*c* 0.774, 1 *N* HCl) identical with **33**. This is unambiguous proof of the structure of 1-(5'-amino-5'-deoxy-β-D-allofuranuronosyl)uracil-5-carboxylic acid for **33**. Hydrogenolysis of **1'c** over platinum afforded deoxy compound, the ir spectrum and specific rotation of which were identical with those of **34**. Thus, the structure of 1-(5'-amino-5'-deoxy-β-D-allofuranuronosyl)thymine is proposed for **34**. Decarboxylation[52] of **33** was attempted. Refluxing of **33** in 3 *N* hydrochloric acid for a prolonged time afforded uracil as a main product together with an unreacted material. Careful examination of paper electrophoresis at pH 7.0 showed, however, a ninhydrin-positive spot slowly migrating to the anode. The hydrolysate was passed through an Amberlite CG-4B column. Preparative tlc of the effluent afforded a small amount of chromatographically pure compound, which showed the uv spectra characteristic to **35** and was identical with it in tlc and paper electrophoresis. Confirmation was made from the pmr spectrum of the C-3', C-4', and C-5' regions (δ 4.0–5.0) of **35**, which showed the signal pattern virtually identical with that of **34** (Figure 9). Absolute configuration was deduced from a similar pattern of an optical rotatory dispersion curve of **35** ($[\phi]_{280}$ +2340 pk, $[\phi]_{253}$ −5300 tr) to that of **1'c**. Thus, the structure of 1-(5'-amino-5'-deoxy-β-D-allofuranuronosyl)uracil is proposed for **35**.



33, R_1 = COOH; R_2 = H
34, R_1 = CH_3; R_2 = H
35, R_1 = H; R_2 = H
36, R_1 = CHO; R_2 = Ac
37, R_1 = COOH; R_2 = Ac

C. Structure of Polyoxins. Periodate oxidation data for each of the polyoxins are summarized in Table V. Polyoxins having polyoxamic acid consumed 3 equiv of periodate rapidly, whereas polyoxins E and G having deoxypolyoxamic acid consumed 1 equiv of periodate. Polyoxin I (**1'i**) showed anomalously high consumption of periodate liberating 5-hydroxymethyluracil as in the case of **1'c**, suggesting it had a free amino group in the aminouronic acid moiety. **1'i**

(52) When the sodium salt of **33** was treated with excess sodium bisulfite in aqueous solution at 50°, crystalline **35** was obtained in fairly good yield. The same reaction of sodium uracil-5-carboxylate afforded uracil. A possible mechanism for this facile decarboxylation reaction is as follows. The intermediate i formed by the nucleophilic



i

addition of bisulfite to C-6 would probably undergo decarboxylation and desulfonation. Details of this reaction and its application to the transformation of the parent antibiotics are under study.

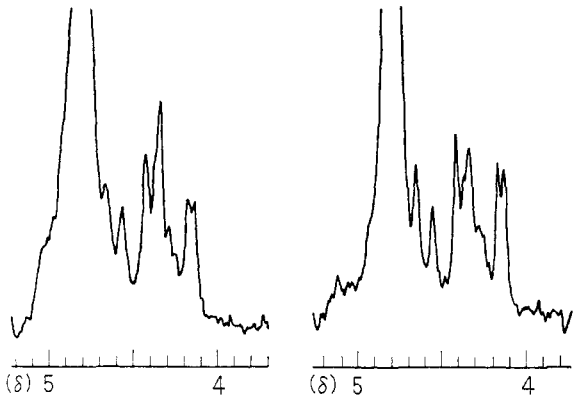
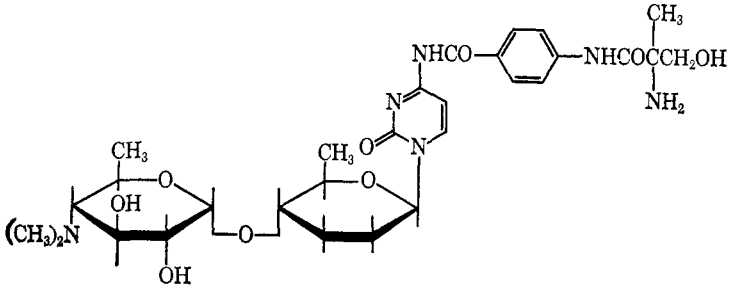


Figure 9. Accumulated pmr spectra of uracil-polyoxin C (**35**) (left) and deoxypolyoxin C (**34**) (right) in D_2O; internal standard, DSS.

was identical with the partial alkaline hydrolysis product of polyoxin A (*vide supra*). The easy racemization of polyoximic acid moiety with alkali is considered to yield a diastereomeric mixture. Indeed, the unexpected splittings of an olefinic proton signal and of an anomeric proton signal indicated that the **1'i** obtained is a diastereomeric mixture. From this fact, it is highly probable that **1'i** is a partial degradation product of polyoxin A in the course of isolation process. The same might be the case for polyoxin C, which is considered to come from polyoxin B or G. The pK_a' values of polyoxins are listed in Table II. The pK_a^{I} (2.4–3.0) is ascribed to carboxyl and the pK_a^{II} (3.7–3.9) to allylic carboxyl common to polyoxins D, E, and F. The pK_a^{III} is assigned to amino group, having a value of 7.2–7.4 except for polyoxins C and I. The pK_a^{IV} (9.2–9.6) is common to all polyoxins and is attributed to a uracil group. Although no sequence analysis was presumed for each polyoxin, the above data suggested that they had a common amino acid sequence. Thus, the structure of polyoxins was established as listed in Scheme I. As would be expected, hydrogenation of polyoxin B and polyoxin H afforded polyoxin J and dihydrodeoxypolyoxin A (**22**), respectively.

All the known pyrimidine nucleoside antibiotics [amicetin (**38**) and its two homologs,[53] gougerotin[54] (**39**) and blasticidin S[55] (**40**)] have common structural features; they are all cytosine nucleosides with 4-amino-4-deoxyhexose derivatives. Polyoxins are the first example of uracil nucleoside antibiotics and the 5-aminouronic acid structure is unknown either synthetically or in nature.[56] Polyoximic acid is a rare example of azetidine in nature, although L-azetidine-2-carboxylic acid was discovered in *Liliaceae* and *Agavaceae*.[57] Moreover, it is striking that the constituents of polyoxins are all α-L-amino acid. Thus, polyoxins are nucleosides which are simultaneously peptides of unusual α-L-amino acids.



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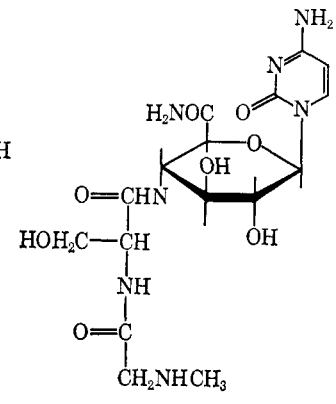

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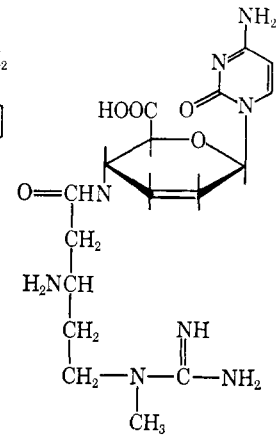

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Biochemical Aspects. Sasaki, *et al.*,[58] suggested that the site of action of polyoxins was related to cell-wall chitin biosynthesis.[59] This is consistent with the *swelling out* phenomenon of the fungal cell when treated with polyoxins[60] and the highly specific activity of polyoxins. The minimum structure common to all active

Table V. Periodate Oxidation of Polyoxins

Polyoxin	Periodate consumed, equiv — Time, hr: 0.25	1	3	6	24	48
A	2.57	2.64	2.64	2.71	2.81	3.02
B	2.84	2.86	2.88	2.92	3.02	
C		1.27	1.95	2.48	4.88	5.48
D	2.90	2.93	2.93	2.95	3.08	
E	1.09	1.11		1.25		
F	2.53	2.55	2.61	2.68	2.81	
G	1.07	1.09	1.13	1.17	1.39	
H	2.42	2.44	2.51	2.59	2.84	
I	1.72	2.11	3.03	3.91	4.98	5.10
J	2.74	2.76	2.79	2.83	2.93	
K	2.59	2.62	2.64	2.68	2.81	
L	2.60	2.62	2.70	2.73	2.99	

(53) T. H. Haskell, *J. Amer. Chem. Soc.*, **80**, 747 (1958).

(54) H. Iwasaki, *Yakugaku Zasshi*, **82**, 1358 (1962); J. J. Fox, Y. Kuwada, K. A. Watanabe, T. Ueda, and E. B. Whipple, *Antimicrobial Agents Chemotherapy*, 518 (1965); J. J. Fox, Y. Kuwada, and K. A. Watanabe, *Tetrahedron Lett.*, 6029 (1968).

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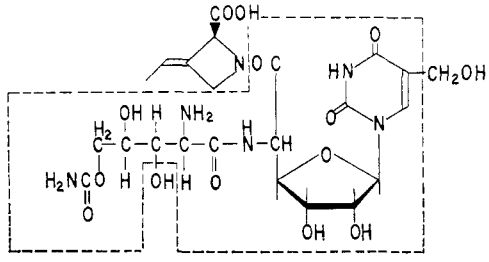
(56) To our knowledge, an antibiotic, nojirimycin, is the only example of 5-amino-5-deoxy sugar occurring in nature, whose structure was proposed as D-glucopiperidinose; *cf.* S. Inoue, T. Tsuruoka, and T. Niida, *J. Antibiot.* (Tokyo), **A19**, 288 (1966); S. Inoue, T. Tsuruoka, and T. Niida, *Tetrahedron*, **23**, 2125 (1968).

(57) L. Fowden, *Biochem. J.*, **64**, 323 (1956); L. Fowden and M. Bryant, *ibid.*, **70**, 626 (1958).

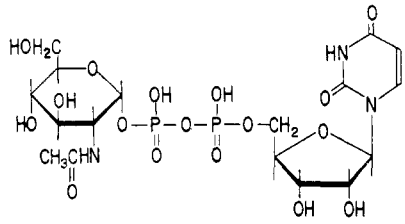
(58) S. Sasaki, N. Ohta, I. Yamaguchi, S. Kuroda, and T. Misato, *Nippon Nogei Kagaku Kaishi*, **42**, 633 (1968).

(59) According to a recent private communication from Dr. E. Cabib of the National Institutes of Health, polyoxin A is a powerful inhibitor of yeast chitin synthetase, although it showed no effect on growth of yeast.

(60) J. Eguchi, S. Sasaki, N. Ohta, T. Akashiba, T. Tsuchiyama, and S. Suzuki, *Nippon Shokubutsu Byōri Gakkaiho*, **34**, 280 (1968).



1a



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polyoxins can be depicted by the dotted line in **1a**. It should be interesting and noteworthy to point out the gross structural similarity of this structure to uridine diphosphate *N*-acetylglucosamine (**41**), believed to be an active form of *N*-acetylglucosamine for chitin synthetase.[61] Indeed, the mode of action of all the hitherto known aminoacyl nucleoside antibiotics such as puromycin,[62] amicetin,[63] gougerotin,[64] and blasticidin S[65] is related to inhibition of protein synthesis.[66] In this respect, polyoxins are considered to be a new class of peptide nucleoside antibiotics.

Experimental Section[67]

Melting points were taken on a Yanagimoto micro melting point apparatus and are uncorrected. Uv spectra were run on a Cary 14 recording spectrophotometer and ir spectra on a Perkin-Elmer 521 grating infrared spectrophotometer. Optical rotations were determined with a Perkin-Elmer 141 polarimeter. All pmr spectra were run on a JNM-C60 nmr spectrometer and spin decoupling was accomplished with a JNM-SD-20B spin decoupler. Chemical shifts were measured to an internal standard, TMS or DSS, and are recorded as δ values. Coupling constants were expressed in Hz. NOE experiment was made by a JNM-C-60H nmr spectrometer with JNM-SD-30 spin decoupler. ORD and CD curves were run on a Model ORD/UV-5 spectropolarimeter (Japan Spectroscopic Co. Ltd.). All X-ray powder measurements were taken by a Debye–Scherrrer powder camera (Rigaku Denki Lab.) (Cu Kα, 1.5418 Å, Ni filter, camera diameter 114.6 mm) and recorded on a Sakura X-ray film, type N, and intensities were estimated visually. Tlc were developed on Avicel SF microcrystalline cellulose. Nucleosides were detected under ultraviolet ray (254 mμ). Polyols were generally detected with periodate–benzidine spray reagent[68] and amino acids were detected with ninhydrin spray reagent. The following solvent systems were utilized: A, butanol–acetic acid–water (4:1:2); B, 75% aqueous phenol; C, butanol–pyridine–water (2:2:1); D, propanol–pyridine–acetic acid–water (15:10:3:10); E, butanol saturated with water; F, ethyl acetate–acetic acid–water (3:1:3). Avicel was employed for cellulose column chromatography. Paper electrophoresis was carried out in 0.05 *M* phosphate buffer for 2 hr at 350 V. Samples for elemental microanalysis were dried over P_2O_5 at 100° in small portions in a nitrogen stream for 2 hr just before analysis unless otherwise stated.

Alkaline Hydrolysis of Polyoxin A. A solution of 2.6 g of **1a** in 50 ml of 0.5 *N* NaOH was heated in a water bath at 65° for 4 hr. The hydrolysate was passed through a column of 30 ml of Amberlite IRC-50 (H). The effluent and washings were combined and evaporated to dryness giving 2.3 g of a residue. It was dissolved in a small amount of pyridine–acetic acid buffer of pH 3.1[69] and placed on a column of 650 ml of Dowex 50W-X8 (100–200 mesh) buffered with the same buffer. Chromatography was run with the same buffer and fractionated into 15-ml portions.

A. Polyoxin C (1'c). Fractions 25–42 were combined, concentrated to a small volume, and added with about 100 ml of ethanol. Precipitate was collected and recrystallized from 5 ml of water, yielding 70 mg of **1'c** as crystals: mp 260–267° dec, $[\alpha]^{24}D$ +11.2° (*c* 0.5, H_2O); ir (KBr) 1060, 1405, 1420, 1480, 1620, 1690, 3060, 3330 cm^{-1}. A sample was dried at 56° for analysis.

Anal. Calcd for $C_{11}H_{15}N_3O_8 \cdot H_2O$: C, 39.40; H, 5.11; N, 12.53; Van Slyke-N, 4.41 (one). Found: C, 39.83; H, 4.78; N, 12.39; Van Slyke-N, 4.38.

A small sample was dried at 100° for 2 hr.

Anal. Calcd for $C_{11}H_{15}N_3O_8$: C, 41.64; H, 4.77; N, 13.25. Found: C, 41.40; H, 4.64; N, 12.74.

B. 5-Hydroxymethyluracil. The filtrate was concentrated and the residue was recrystallized from 5 ml of water to 207 mg of 5-hydroxymethyluracil: uv max (0.05 *N* HCl) 261 mμ (ϵ 8000), (0.05 *N* NaOH) 286 mμ (ϵ 7400). The ir spectrum in KBr was identical with a synthetic sample.[70]

C. Polyoximic Acid (2). Fractions 50–77 including two ninhydrin-positive substances were combined, evaporated to dryness, and subjected to cellulose column chromatography using the solvent system A. The first fraction which showed a yellow ninhydrin test was recrystallized from a small volume of methanol, affording 134 mg of **2** as colorless crystals: mp 158–160° dec; $[\alpha]^{20}D$ 0° (*c* 2, H_2O); ir (KBr) 811, 1383, 1605, 2200–3000, 3110 cm^{-1}.

Anal. Calcd for $C_6H_9NO_2$: C, 56.68; H, 7.14; N, 11.02. Found: C, 57.18; H, 6.33; N, 10.83.

D. Polyoxamic Acid (3). The second fraction which showed a violet ninhydrin test was crystallized from a small volume of aqueous ethanol affording 95 mg of **3** as colorless needles: mp 171–173° dec; $[\alpha]^{23}D$ +2.8°; $[\alpha]^{23}_{365}$ +23° (*c* 1.02, H_2O). A sample was dried at room temperature.

Anal. Calcd for $C_5H_{11}NO_5 \cdot H_2O$: C, 32.79; H, 7.15; N, 7.65. Found: C, 33.24; H, 7.01; N, 7.75.

A small sample was dried at 100° for 2 hr.

Anal. Calcd for $C_5H_{11}NO_5$: C, 36.36; H, 6.71; N, 8.48. Found: C, 36.26; H, 6.95; N, 8.48.

Acid Hydrolysis of Polyoxin A. A solution of 2.8 g of **1a** in 15 ml of 3 *N* HCl was refluxed for 1 hr. The hydrolysate was passed through a column of 40 ml of Amberlite CG-4B (OH). The effluent and washings were combined, evaporated to dryness, and subjected to column chromatography on 500 ml of Dowex 50W-X8 (100–200 mesh) buffered with pyridine–acetic acid buffer (pH 3.1). Similar treatment as in the case of alkaline hydrolysis afforded 40 mg of **1'c**, mp 257–264° dec, and 75 mg of **2**, mp 158–160° dec, $[\alpha]^{20}D$ +1.9°, $[\alpha]^{20}_{365}$ +2.5° (*c* 1, H_2O). These compounds showed ir spectra identical with those of the compounds obtained by alkaline hydrolysis. The polyoxamic acid fraction showed two spots on tlc with 75% phenol. It was subjected to a cellulose chromatography with 75% phenol. The first fraction, after treatment with ether to remove phenol, was crystallized from aqueous ethanol to 65 mg of **4** as colorless plates, mp 226–232° dec, $[\alpha]^{22}D$ +1.3°, $[\alpha]^{22}_{365}$ +22.2° (*c* 1.04, H_2O). It gave positive *p*-(N,N-dimethylamino)benzaldehyde test (yellow).

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(66) For a review, see J. J. Fox, K. A. Watanabe, and A. Bloch, *Progr. Nucleic Acid Res. Mol. Biol.*, **5**, 294 (1966).

(67) We thank Dr. H. Honma and staff for elemental microanalyses and Mr. J. Uzawa for pmr spectral determinations. We are indebted to Dr. S. Hayashi and Mr. N. Esumi of the Japan Electron Optics Lab. Co. Ltd. for the NOE experiment.

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(70) J. H. Burckhalter, R. J. Seiwald, and H. C. Scarborough, *J. Amer. Chem. Soc.*, **82**, 991 (1960).

Anal. Calcd for $C_6H_{12}N_2O_6$: C, 43.62; H, 5.51; N, 13.46. Found: C, 34.65; H, 5.70; N, 13.13.

The second fraction was treated similarly, affording 21 mg of **3** as colorless needles, mp 166–170° dec, $[\alpha]^{22}D$ +1.5°, $[\alpha]^{22}_{365}$ +20.6° (*c* 1.04, H_2O), the ir spectrum of which was identical with that of the compound obtained by alkaline hydrolysis.

Polyoxin I (1'i) from Alkaline Hydrolysis of Polyoxin A. During the purification of a large-scale alkaline hydrolysate of **1a** (20 g, 0.5 *N* NaOH, 65°, 4 hr) on Dowex 50W chromatography, a uv-, ninhydrin-, and periodate-positive minor product was eluted out following the polyoxamic acid fraction. It showed a little higher R_f value than **1'a**. This fraction (250 mg) was further purified by cellulose chromatography with butanol–acetic acid–water (4:1:1) and butanol–pyridine–water (2:1:1). The pure compound was obtained as 60 mg of colorless amorphous power from water–ethanol–ether: $[\alpha]^{23}D$ −27.0°; uv max (0.05 *N* HCl) 262 mμ (ε 9120), (0.05 *N* NaOH) 264 mμ (ε 6480); pK_a' = 2.7, 6.1, 9.5.

Anal. Calcd for $C_{17}H_{22}N_4O_{17}$: C, 47.88; H, 5.20; N, 13.14; Van Slyke N, 3.33 (one). Found: C, 47.35; H, 5.21; N, 12.83; Van Slyke N, 2.40.

The pmr and ir spectra as well as tlc were identical with those of polyoxin I.[7,48]

Dihydrodeoxypolyoxin A (22). A solution of 124 mg of **1a** in 10 ml of H_2O was hydrogenated over platinum (from 19.7 mg of PtO_2) at atmospheric pressure and at room temperature. Approximately 2 equiv of hydrogen was absorbed. The reaction solution was filtered to remove platinum, concentrated to a small volume and precipitated with ethanol–ether. The precipitate was purified on a cellulose chromatography with butanol–acetic acid–water (4:1:1). Crystallization was not successful but the purified powder of **22** was homogeneous on tlc, mp 180–220° dec. It was biologically active.

Anal. Calcd for $C_{23}H_{34}N_6O_{13}$: C, 45.84; H, 5.69; N, 13.95. Found: C, 45.38; H, 5.68; N, 13.68.

***N*-Acetylpolyoxin A.** To a suspension of 130 mg of **1a** in 20 ml of methanol was added 30 drops of acetic anhydride. On standing at room temperature with occasional shaking, it became clear after 40 min. Six hours later, water was added to the reaction mixture and the whole was evaporated to dryness. The residue was chromatographed on cellulose with butanol–acetic acid–water (4:1:1). *N*-Acetylpolyoxin A was obtained as amorphous powder from water–ethanol–ether: 85 mg; ir (KBr) 1525 cm^{-1}; pK_a' = 3.4, 9.2. It showed a single spot on tlc. It was positive to the periodate–benzidine test but negative to the ninhydrin test; periodate oxidation: 3.06 (1 hr), 3.34 (2 hr), 3.76 (6 hr), and 3.77 equiv (24 hr).

Anal. Calcd for $C_{25}H_{34}N_6O_{15}$: C, 45.59; H, 5.20; N, 12.76. Found: C, 45.80; H, 4.89; N, 12.34.

Polyoxin C Hydrobromide. A solution of 20 mg of **1'c** in 0.3 ml of a 1:5 mixture of HBr-saturated methanol and methanol was placed in a refrigerator for 1 hr. Crystals appeared, were collected by filtration, washed thoroughly with methanol, and dried over KOH; mp 220–225° dec; ir (KBr) 1668, 1705, 2500–3200, 3330 cm^{-1}.

Anal. Calcd for $C_{11}H_{16}N_3O_8Br$: C, 33.18; H, 4.05; N, 10.55; Br, 20.07. Found: C, 32.75; H, 4.40; N, 9.83; Br, 18.06.

***N*-*p*-Toluenesulfonylpolyoxin C (5).** To a suspension of 110 mg of **1'c** in 1.33 ml of 0.5 *N* NaOH was added 63 mg of pulverized *p*-toluenesulfonyl chloride. The suspension was shaken for 2 days at room temperature. The crystalline slurry was added with 20 ml of water and acidified with 0.75 ml of 1 *N* HCl. A white precipitate was collected by filtration and recrystallized from aqueous ethanol to give 93 mg of crystalline **5**, mp 250–262° dec.

Anal. Calcd for $C_{18}H_{21}N_3O_{10}S$: C, 45.86; H, 4.49; N, 8.91; S, 6.80. Found: C, 45.50; H, 4.69; N, 8.84; S, 6.62.

***N*-*p*-Bromobenzenesulfonylpolyoxin C (6).** A solution of 110 mg of **1'c** in 1.33 ml of 0.5 *N* NaOH was treated with 94 mg of *p*-bromobenzenesulfonyl chloride in a similar way as the foregoing. Compound **6** was obtained as 107 mg of colorless needles, mp 220–260° dec. It was dried at room temperature over P_2O_5.

Anal. Calcd for $C_{17}H_{18}N_3O_{10}BrS \cdot H_2O$: C, 36.83; H, 3.64; N, 7.58; Br, 14.42; S, 5.78. Found: C, 36.86; H, 3.84; N, 7.68; Br, 14.40; S, 5.44.

***N*-Acetylpolyoxin C (7).** To a solution of 500 mg of **1'c** and 250 mg of sodium acetate in 100 ml of water was added 2.2 ml of acetic anhydride and the solution was allowed to stand overnight at room temperature. The solution was passed through a column of 10 ml of Dowex 50W-X8 (H). The effluent and washings were combined, and evaporated to dryness, and purified on a cellulose column with the solvent system A. A main fraction was crystallized from aqueous ethanol to 440 mg of **7** as a crystalline powder: mp 210–220° dec; ir (KBr) 1550, 1690 (broad) cm^{-1}.

Anal. Calcd for $C_{13}H_{17}N_3O_9$: C, 43.46; H, 4.77; N, 11.70. Found: C, 43.11; H, 4.78; N, 11.54.

***N*-Acetyldeoxypolyoxin C (8).** A solution of 150 mg of **7** in 20 ml of water was hydrogenated over platinum (from 30 mg of PtO_2) at atmospheric pressure and room temperature. About 1 equiv of hydrogen was absorbed in 2 hr. The catalyst was filtered off, the filtrate was concentrated to dryness, and the residue was purified on cellulose chromatography with butanol–acetic acid–water (4:1:1). The main fraction was concentrated and triturated with ethanol, affording 106 mg of **8** as a crystalline powder, mp 224–226° dec.

Anal. Calcd for $C_{13}H_{17}N_3O_8$: C, 45.48; H, 4.99; N, 12.24. Found: C, 45.14; H, 4.96; N, 12.46.

***N*-Acetyl-2',3'-*O*-isopropylidenepolyoxin C (9).** To a suspension of 150 mg of **7** in 10 ml of acetone was added 0.1 ml of concentrated H_2SO_4. The suspension was shaken for 8 hr at room temperature, until in solution. After standing overnight, the solution was added with excess anhydrous Na_2CO_3 and shaken for 3 hr. The salt was filtered off and the filtrate was evaporated to dryness. The residue (130 mg) was purified on a cellulose column with butanol–acetic acid–water (4:1:1), affording 35 mg of analytically pure powder of **9**. It was negative to the ninhydrin and periodate–benzidine tests; pmr (DMSO-d_6) δ 1.29 (s, 3, CH_3), 1.49 (s, 3, CH_3), 1.89 (s, 3, CH_3CO), 5.88 (d, 1, 1'-H, $J_{1',2'}$ = 2.0 Hz), 7.55 (s, 1, 6-*H*), 8.32 (d, 1, AcN*H*, *J* = 9.0 Hz).

Anal. Calcd for $C_{16}H_{21}N_3O_9$: C, 48.12; H, 5.30; N, 10.52. Found: C, 47.25; H, 5.35; N, 10.66.

Rhodium Hydrogenation of Polyoxin C (1'c). A solution of 1.19 g of **1'c** in 200 ml of water was hydrogenated over 286 mg of 5% rhodium on alumina[71] at 3.5 kg/cm^2 pressure for 5 hr. The catalyst was filtered off and the filtrate was concentrated to 15 ml. On cooling, 780 mg of crystalline **10** precipitated out, which on recrystallization from 50 ml of water afforded 600 mg of the pure compound, mp 186–187° dec. A small sample was dried at 100° for 4 hr for analysis.

Anal. Calcd for $C_{11}H_{17}N_3O_7$: C, 43.56; H, 5.65; N, 13.86. Found: C, 43.26; H, 5.71; N, 13.47.

The mother liquor was chromatographed on cellulose. From the first fraction, 142 mg of the C-5 epimer of **10** was obtained as crystals, mp 234–236° dec.

Anal. Calcd for $C_{11}H_{17}N_3O_7$: C, 43.56; H, 5.65; N, 13.86. Found: C, 43.38; H, 5.69; N, 13.24.

From the second fraction, 114 mg of one of the C-5 epimers of **11** was obtained after recrystallization, mp 208–213° dec.

Anal. Calcd for $C_{11}H_{17}N_3O_8$: C, 41.38; H, 5.37; N, 13.16. Found: C, 41.37; H, 6.31; N, 13.16.

Deaminopolyoxin C (12). A solution of 690 mg of **1'c** in a mixture of 200 ml of water and 10.5 ml of acetic acid was ice-cooled below 5° and added dropwise with an ice-cooled solution of 690 mg of sodium nitrite in 40 ml of water over a period of 30 min, and left to stand overnight in a refrigerator. The reaction solution was passed through a column of 25 ml of Dowex 50W-X8 (H). The effluent and washings were concentrated carefully *in vacuo* at low temperature to a thick syrup, which was triturated with small volume of ethanol to give 560 mg of crystalline **12**. A sample recrystallized from aqueous ethanol showed mp 250–260° dec.

Anal. Calcd for $C_{11}H_{14}N_2O_9$: C, 41.51; H, 4.43; N, 8.80. Found: C, 41.74; H, 4.47; N, 8.70.

Deoxypolyoxin C (34). A solution of 750 mg of **1'c** in 150 ml of water was hydrogenated over platinum (from 50 mg of PtO_2) at atmospheric pressure and at room temperature. Approximately 1 equiv of hydrogen was taken in 2 hr. The catalyst was filtered off and the filtrate was concentrated to 50 ml. When cooled, 620 mg of crystalline **34** was obtained: mp 240–244° dec, $[\alpha]^{22}D$ +8.7°, $[\alpha]^{22}_{365}$ +34.6° (*c* 0.208, H_2O).

Anal. Calcd for $C_{11}H_{15}N_3O_7$: C, 43.85; H, 5.02; N, 13.94. Found: C, 43.49; H, 4.99; N, 13.98.

Deaminodeoxypolyoxin C (13). To an ice-cooled solution of 300 mg of **34** in a mixture of 50 ml of water and 5 ml of acetic acid was added dropwise an ice-cooled solution of 300 mg of sodium nitrite in 10 ml of water during 30 min and the resulting solution was left to stand overnight in a refrigerator. The reaction mixture was passed through a column of 10 ml of Dowex 50W-X8 (H). The effluent and washings were concentrated carefully *in vacuo* at low temperature to a syrup, which crystallized on triturating with a

(71) Purchased from the Nippon Engelhard Co., Ltd., Tokyo.

small volume of ethanol, affording 265 mg of **13**, mp 231–233° dec; $[\alpha]^{22}D$ −48° (*c* 0.622, H_2O); ir (KBr) 1685, 1773 cm^{-1}.

Anal. Calcd for $C_{11}H_{14}N_2O_8 \cdot 0.5C_2H_5OH$: C, 44.31; H, 5.27; N, 8.61. Found: C, 44.12; H, 5.08; N, 8.40.

The solvated ethanol could not be removed on drying at 100°, the presence of which was demonstrated in the pmr spectrum (DMSO-d_6), δ 1.10 (t), 3.44 (q).

Deaminodihydrodeoxypolyoxin C (14). A solution of 1.15 g of **12** in 100 ml of water was hydrogenated over platinum (from 100 mg of PtO_2) at atmospheric pressure and at room temperature. After the reaction was completed (about 1 equiv of hydrogen was absorbed), the catalyst was filtered off and the filtrate was added with 300 mg of 5% rhodium on alumina and hydrogenated at 4 kg/cm^2 pressure at room temperature for 4 hr. The catalyst was filtered off, and the filtrate was evaporated to dryness and subjected to cellulose column chromatography with butanol–acetic acid–water (4:1:1). **14** was crystallized from water: 760 mg; mp 211–213° dec; ir (KBr) 1676, 1715, 1731 cm^{-1}; uv, no absorption; pmr (DMSO-d_6) δ 1.08 (d, 3, CH_3, J = 6.2 Hz), 5.75 (d, 1, 1′-H, $J_{1',2'}$ = 6.2 Hz).

Anal. Calcd for $C_{11}H_{16}N_2O_8$: C, 43.42; H, 5.30; N, 9.21. Found: C, 43.43; H, 5.17; N, 8.94.

Hydrolysis of Deaminodihydrodeoxypolyoxin C (14). To a solution of 178 mg of **14** in 20 ml of water was added 4 ml of Dowex 50W-X8, (H, 100–200 mesh), the mixture was refluxed for 4 hr. The resin was filtered off, the filtrate (pH 2) was neutralized with 3 drops of 1:9 NH_4OH and concentrated to a small volume. Crystals that appeared on cooling were collected to 27 mg of (−)-dihydrothymine (**16**), mp 267–268° dec, $[\alpha]^{23}D$ −9.2° (*c* 0.229, H_2O). Its identification with the synthetic (±)-dihydrothymine was made from the pmr spectra. The mother liquor was purified on a cellulose chromatography with the solvent system A. Fractions positive to the aniline hydrogen phthalate test were collected and decolorized with carbon from aqueous solution, affording 55 mg of alluronic acid (**15**) as a colorless syrup. It was positive to the 2,4-dinitrophenylhydrazine and aniline hydrogen phthalate tests but negative to the ferric hydroxamate test.

Compound **15** *(50 mg)* was dissolved in 20 ml of water and 140 mg of brucine tetrahydrate was added. After refluxing for 10 min, the solution was cooled and extracted three times with chloroform to remove excess brucine. The aqueous layer was concentrated to a small volume and added with a small amount of ethanol, affording 69 mg of the brucine salt as crude crystals. It was recrystallized from water: mp 197–199° dec; $[\alpha]^{24}D$ −21.6° (*c* 1.044 H_2O); X-ray powder (Cu Kα) 7.50 (s), 7.14 (s), 6.71 (s), 6.22 (vs), 5.79 (w), 5.28 (w), 4.98 (m), 4.63 (vw), 4.46 (s), 4.21 (vw), 4.02 (vs), 3.80 (vs), 3.58 (m), 3.45 (vw), 3.36 (m), 3.16 (m).

Anal. Calcd for $C_{29}H_{36}N_2O_{11}$: C, 59.17; H, 6.17; N, 4.76. Found: C, 58.85; H, 6.18; N, 4.81.

Methanolysis of Deaminodihydrodeoxypolyoxin C (14). *Compound* **14** *(750 mg) dried at 100° was dissolved in 5 ml of methanolic* HCl and the solution was refluxed for 1.5 hr. The yellow solution was evaporated to dryness and dried over NaOH for 1 hr. The brown residue was applied on a cellulose column developed with butanol–acetic acid–water (4:1:1). From the first fraction, 130 mg of (−)-dihydrothymine, $[\alpha]^{23}D$ −8.0° (*c* 0.575, pyridine), was obtained. From the second fraction positive to the periodate–benzidine test, 145 mg of the methyl glycoside was obtained as a syrup. It showed a major periodate-positive spot on tlc and paper electrophoresis, slightly contaminated with few other periodate-positive spots.

β-D-Allose (17). The syrupy methyl glycoside (145 mg) was dissolved in 5 ml of water and added dropwise with a solution of $NaBH_4$ in 4 ml of water over 5 min. After standing overnight at room temperature, the reaction solution was slightly acidified (pH 4) with 5% acetic acid and passed through 4 ml of Dowex 50W-X8 (H). The effluent and washings were combined and evaporated to dryness. Methanol was added to the residue and evaporation was repeated four times. The residue was dissolved in water, passed through 3 ml of Amberlite CG-4G (OH) and the effluent and washings were evaporated to dryness, affording 44 mg of colorless residue. It was dissolved in 5 ml of water and added with 1 ml of Dowex 50W-X8 (H, 100–200 mesh). After refluxing for 3 hr, the resin was filtered off and the filtrate was passed through 0.5 ml of Amberlite CG-4B (OH). The effluent and washings were combined and evaporated to dryness, affording 26 mg of a colorless syrup. It was further purified by cellulose chromatography with butanol–acetic acid–water (4:1:1). From the fraction positive to an aniline hydrogen phthalate test, 3.5 mg of **17** was obtained as colorless needles, which were recrystallized from aqueous ethanol: mp 140–141°; $[\alpha]^{20}D$ +4° → +15° (5 min → 1.5 hr, equilibrium) (*c* 0.112, H_2O); X-ray powder (CuKα), 7.20 (m), 5.44 (m), 4.60 (vs 2), 4.17 (s), 4.00 (vw 1), 3.59 (m), 3.29 (w), 3.13 (m), 2.88 (m), 2.71 (m), 2.63 (w), 2.57 (w), 2.47 (vw), 3.40 (m), 2.36 (m). These values correspond to those of one of the lower melting (mp 128°) dimorphous forms of β-D-allose reported by Wolfrom, *et al.*[19] The same mobility was obtained with a synthetic D-allose on tlc with seven kinds of solvent systems.

***N*-Dithiocarbethoxy-2′,3′-*O*-isopropylidenedeoxypolyoxin C (18).** To a suspension of 165 mg of finely powdered **34** in 200 ml of acetone was added 0.5 ml of concentrated H_2SO_4. After shaking overnight at room temperature, 3 g of anhydrous Na_2CO_3 was added to the clear solution and the mixture was shaken for 7 hr. Salts were filtered off, and the solution was concentrated to a small volume and precipitated with ether, yielding 78 mg of isopropylidene derivative as a white powder. It gave a single uv spot on tlc and was negative to the periodate–benzidine test. This powder (68.2 mg, 0.2 mmol) was dissolved in a mixture of 3.6 ml of H_2O and 0.4 ml of 1 *N* KOH and added with 0.05 ml of CS_2 and 1 ml of dioxane. After shaking the mixture for 2 hr at room temperature, 0.1 ml of bromoethane was added and stirred for additional 2 hr. The reaction mixture was acidified with 1 *N* HCl and extracted three times with ethyl acetate. On evaporation of the solvent layer, 35 mg of **18** was obtained as a colorless powder: pmr (DMSO-d_6) δ 1.36 (t, 3, CH_2CH_3, J = 7.5 Hz), 1.38 (s, 3, CH_3), 1.59 (s, 3, CH_3), 1.95 (s, 3, 5-CH_3), 3.29 (q, 2, CH_2CH_3), 7.12 (s, 1, 6-*H*), 7.97 (d, 1, AcN*H*, J = 8.0 Hz).

Anal. Calcd for $C_{17}H_{23}N_3O_7S_2$: N, 9.43; S, 14.39. Found: N, 9.15; S, 12.89.

Methyl Deaminodeoxypolyoxinate C (19). Ten milligrams of **13** was refluxed for 1 hr in 1 ml of methanolic HCl. Methanol was evaporated *in vacuo* and the residue was dried over KOH. The crystalline residue was recrystallized from a small volume of ethanol, yielding 5 mg of **19**: mp 190–197°; ir (KBr) 1686, 1733 cm^{-1}.

Anal. Calcd for $C_{12}H_{16}N_2O_8$: C, 45.57; H, 5.10; N, 8.86. Found: C, 45.79; H, 5.24; N, 8.39.

(±)-Dihydropolyoximic Acid (20). A solution of 51 mg of **2** in 10 ml of methanol was hydrogenated over 10 mg of palladium. The catalyst was filtered off and the filtrate was evaporated to dryness. Crystallization from ethanol afforded 28 mg of **20**: mp 201° dec; ir (KBr) 1395, 1615, 2200–3200, 3400 cm^{-1}; pK_a' = 2.7, 10.1.

Anal. Calcd for $C_6H_{11}NO_2$: C, 55.79; H, 8.58; N, 10.85. Found: C, 55.48; H, 8.67; N, 10.70.

(−)-Dihydropolyoximic Acid (20). A solution of 500 mg of **22** in 10 ml of 0.5 *N* NaOH was heated in a bath at 65° for 4 hr. The hydrolysate was passed through 6 ml of Amberlite XE-64 (H). The effluent and washings were combined and evaporated to dryness, and the residue was chromatographed over a Dowex 50W-X8 column with pyridine–acetic acid buffer (pH 3.1). Fractions giving a brownish color to ninhydrin were collected and further purified by cellulose chromatography with the solvent system A. Crystallization was effected from ethanol, affording 18 mg of **20**: mp 183–193° dec; $[\alpha]^{20}D$ −94.6° (*c* 1.5, H_2O); ir (KBr) 1415, 1605, 2200–3600 cm^{-1} (different from (±)-dihydro compound).

Anal. Calcd for $C_6H_{11}NO_2$: C, 55.79; H, 8.58; N, 10.85. Found: C, 55.29; H, 8.69; N, 10.75.

Compound **22** (312 mg) was refluxed with 4 ml of 3 *N* HCl for 8 hr. The hydrolysate was purified similarly, affording 9.5 mg of **20**, which was fully identical with a sample obtained by alkaline hydrolysis described above.

Ozonolysis of Polyoximic Acid (2). To a chilled solution of 350 mg of **2** in a Dry Ice–acetone bath, ozone was passed through for 45 min. Immediately after the end of that time, the reaction solution was hydrogenated over 130 mg of palladium at atmospheric pressure at room temperature. Approximately 2 equiv of hydrogen was absorbed in 3 hr. Catalyst was removed by filtration and on evaporating methanol, 261 mg of **21** was obtained. It was recrystallized from methanol–ethanol: mp 159–161°; pK_a' = 2.5, 7.4; pmr (D_2O) δ 3.73 (s, 2, CH_2), 4.05 (s, 2, CH_2), 3.83 (s, 3, CH_3).

Anal. Calcd for $C_5H_9NO_4$: C, 40.81; H, 6.17; N, 9.52. Found: C, 41.22; H, 6.39; N, 9.67.

A solution of 30 mg of **21** in 2 ml of 0.5 *N* $Ba(OH)_2$ was heated at 70° in a bath for 2 hr. The hydrolysate was acidified with 0.1 *N* H_2SO_4 and the precipitated $BaCO_3$ was filtered off. The filtrate was concentrated to small volume and added with ethanol, yielding 3 mg of the crystalline sulfate. It gave an identical ir spectrum with an authentic sample of iminodiacetic acid sulfate: ir (KBr) 850, 888, 1035, 1095, 1175, 1245, 1325, 1420, 1620, 1740, 2300–3100, 3560 cm^{-1}.

Dithiocarbethoxy-(−)-dihydropolyoximic Acid (23). To a solution of 7.2 mg of (−)-**20** in 0.12 ml of 1 *N* KOH was added 0.05 ml of CS_2. After shaking for 1.5 hr at room temperature, 0.05 ml of bromoethane was added and the solution was shaken for additional 1.5 hr. The reaction mixture was evacuated to remove excess bromoethane. A small volume of water was added and the solution was acidified with 1 *N* HCl. The turbid solution thus obtained was extracted three times with ethyl acetate. On evaporation of the solvent, 10.8 mg of **23** was obtained as a colorless syrup: uv max (MeOH) 253, 279, 330 mμ (ε 7200, 8400, 54). It showed a single uv-positive spot on tlc and was used for the ORD measurement without further purification (Figure 2).

***N*-Acetylpolyoxaminolactone (24).** To a suspension of 786 mg of **3** in 20 ml of methanol was added 1.5 ml of acetic anhydride. After 2 hr standing at room temperature with occasional shaking, the solution became clear. After additional standing overnight at room temperature, 3 ml of water was added to the reaction mixture, which was concentrated to give a syrup. It was crystallized from ethanol to 400 mg of **24**: mp 150–152°; ir ($CHCl_3$) 1655, 1787 cm^{-1}.

Anal. Calcd for $C_7H_{11}NO_5$: C, 44.44; H, 5.86; N, 7.41. Found: C, 44.62; H, 6.03; N, 7.42.

***N*,*O*^{5}-Diacetyl-3-deoxy-2,3-didehydropolyoxaminolactone (25).** A suspension of 300 mg of finely powdered **3** in a mixture of 20 ml of pyridine and 15 ml of acetic anhydride was shaken for 2 days at room temperature, the clear solution was added with small volume of water and evaporated to dryness. The crystalline residue was recrystallized from aqueous ethanol to afford 120 mg of **25**: mp 160–161°; uv max (MeOH) 243 mμ (ε 8300); ir (CCl_4) 1664, 1719, 1753, 1777 cm^{-1}; pmr ($CDCl_3$) δ 2.11 (s, 3, CH_3COO), 2.23 (s, 3, CH_3CONH), 4.19, 4.47 (o, 2, CH_2), 5.31 (m, 1, CH), 7.46 (d, 1, $=CH-$, J = 2.4 Hz), 7.91 (broad, 1, AcNH).

Anal. Calcd for $C_9H_{11}NO_5$: C, 50.70; H, 5.20; N, 6.57. Found: C, 50.54; H, 5.00; N; 6.40.

2-Acetamido-2-deoxy-4-α-xylose (26). To an ice-cooled solution of 1.38 g of oxalic acid dihydrate and 1.42 g of sodium oxalate in 30 ml of water was added 90 mg of **24** and then 5 g of 5% sodium amalgam was added at once under vigorous agitation. Stirring was continued for 30 min, the reaction mixture (pH ~3), after being neutralized to pH 6.0 with 1 *N* NaOH, was concentrated to a small volume and added with 50 ml of methanol, and the sodium oxalate that precipitated was filtered off. Methanol was evaporated and the residue was dissolved in water and passed through columns of 2 ml of Dowex 50W-X8 (H) and 3 ml of Amberlite CG-4B(OH). The effluent and washings were combined and concentrated to a small volume, affording 35 mg of **26** as colorless needles. It was recrystallized from aqueous ethanol: mp 190–193° dec; $[\alpha]^{20}D$ −45° → −6.4° (3 min → 95 min, equilibrium) (*c* 1.08, H_2O); X-ray powder (Cu Kα) 6.83 (vs, 2), 5.04 (m), 4.65 (w), 4.25 (vs, 1), 3.93 (s), 3.78 (s), 3.50 (s) 3.30 (vw), 3.15 (vw), 3.05 (s).

Anal. Calcd for $C_7H_{13}NO_5$ (191.18): C, 43.97; H, 6.85; N, 7.33. Found: C, 43.84; H, 6.81; N, 7.27.

It was positive to periodate–benzidine, aniline hydrogen phthalate, ammoniacal silver nitrate, and Elson–Morgan tests.

***N*-Acetyl-3-deoxy-2,3-didehydro-*O*-carbamoylpolyoxaminolactone (27).** A suspension of finely powdered **4** in 10 ml of pyridine was shaken for 3 days at room temperature. The reaction mixture was filtered and 67 mg of the unreacted material was recovered. The filtrate was added with ice, evaporated to dryness and the crystalline residue was recrystallized from ethanol, affording 165 mg of **27**: mp 209° dec, uv max (MeOH) 243 mμ (ε 8800); ir ($CHCl_3$) 1662, 1714, 1740, 1765 cm^{-1}.

Anal. Calcd for $C_8H_{10}N_2O_6$: C, 44.86; H, 4.71; N, 13.08. Found: C, 44.89; H, 4.72; N, 13.17.

From the mother liquor, 83 mg of the *N*,*O*-diacetyl compound was obtained on recrystallization. It showed a double melting point, 166–170° and 203–206°, indicating elimination of an acetoxy group.

Anal. Calcd for $C_{10}H_{14}N_2O_7$: C, 43.80; H, 5.15. Found: C, 44.38; H, 5.30.

On heating for 1 hr in pyridine on a boiling-water bath, it gave **27**.

Deoxypolyoxamic Acid (28). Crude polyoxin G (1 g) was dissolved in 50 ml of 0.5 *N* NaOH and heated in a boiling-water bath for 2 hr. The hydrolysate was passed through 20 ml of Amberlite IRC-50(H), the effluent was evaporated to dryness, and the residue was chromatographed on Dowex 50W-X8 buffered with pyridine–acetic acid (pH 3.1). A ninhydrin- and periodate–benzidine-positive fraction was further purified by cellulose chromatography with the solvent system A. Compound **28** was obtained as a colorless syrup (60 mg), which crystallized partially on drying: $[\alpha]^{25}D$ +11° (*c* 2, H_2O); pK_a' = 3.0, 9.1; ir (KBr) 1410, 1500, 1590, 1630, 2500–3400 cm^{-1}.

Anal. Calcd for $C_5H_{11}NO_4$: C, 40.26; H, 7.43; N, 9.39. Found: C, 40.18; H, 6.87; N, 8.80.

A few milligrams of polyoxin E was hydrolyzed similarly and **28** was detected on tlc.

Carbamoyldeoxypolyoxamic Acid (29). A mixture of polyoxins, D, E, and F (20 g) was dissolved in 300 ml of 1 *N* HCl and heated in a boiling-water bath for 1 hr. The hydrolysate was passed through 300 ml of Amberlite IR-4B (OH) and 50 ml of Amberlite IRC-50 (H). The effluent and washings were combined and evaporated to dryness, affording about 3 g of a residue, which was submitted to Dowex 50W chromatography with pyridine–acetic acid buffer (pH 3.1). Compounds **2**, **28** + **29**, and **3** + **4** were eluted out in this order, with good separation. The second fraction (**28** + **29**) was purified by cellulose column chromatography with 75% aqueous phenol. Compound **28** eluted first, was treated with ether, and crystallized from aqueous ethanol, affording 145 mg of colorless plates: mp 215–216° dec; $[\alpha]^{23}D$ +5.8°; $[\alpha]^{22}_{365}$ +22.9° (*c* 1.04, H_2O); ir (KBr) 1403, 1425, 1633, 1703, 2100, 2500–3500 cm^{-1}.

Anal. Calcd for $C_6H_{12}N_2O_5$: C, 37.50; H, 6.29; N, 14.58. Found: C, 37.59; H, 6.30; N, 14.22.

The second fraction afforded a syrup of **28**, which was identical on tlc with deoxypolyoxamic acid obtained by alkaline hydrolysis of polyoxins E and G. Small samples of pure polyoxins E and G were hydrolyzed and purified similarly and **29** was identified on tlc with crystals obtained as above.

***N*-Acetylcarbamoyldeoxypolyoxaminolactone (30).** Carbamoyldeoxypolyoxamic acid (**29**), a suspension of 110 mg of finely powdered **29** in a mixture of 10 ml of pyridine and 2 ml of acetic anhydride was shaken overnight at room temperature. The reaction mixture was filtered and concentrated and the residue crystallized from ethanol, affording 110 mg of **30**: mp 188–191°; ir ($CHCl_3$) 1680, 1735, 1782 cm^{-1}.

Anal. Calcd for $C_8H_{12}N_2O_5$: C, 44.44; H, 5.60; N, 12.96. Found: C, 44.12; H, 5.72; N, 12.92.

Catalytic Hydrogenation of *N*-Acetyl-3-deoxy-2,3-dehydro-5-*O*-carbamoylpolyoxaminolactone (27). A solution of 146 mg of **27** in 30 ml of water was hydrogenated over platinum (from 40 mg of PtO_2) for 4 hr at atmospheric pressure and at room temperature. Approximately 1.3 equiv of hydrogen was absorbed. The catalyst was removed by filtration, the filtrate was evaporated to dryness, and the residue was recrystallized from ethanol, affording 96 mg of the dihydro compound **31**: mp 174–175°; ir ($CHCl_3$) 1683, 1737, 1784 cm^{-1}.

Anal. Calcd for $C_8H_{12}N_2O_5$ (216.19): C, 44.44; H, 5.60; N, 12.96. Found: C, 44.26; H, 5.57; N, 12.70.

2-Amino-2,3-dideoxy-5-*O*-carbamoyl-L-lyxonic Acid (32). A solution of 20 mg of **31** in 2 ml of 1 *N* HCl was refluxed for 1 hr. The hydrolysate was passed through 2 ml of Amberlite CG-4B (OH) and evaporated to dryness, and the residue was purified by preparative tlc with 75% aqueous phenol. The main ninhydrin-positive fraction was recrystallized twice from aqueous ethanol, affording 3.7 mg of **32**, mp 200–205° (dec), $[\phi]_{222}$ −2160 tr (0.5 *N* HCl).

Anal. Calcd for $C_6H_{12}N_2O_5$: C, 37.50; H, 6.29; N, 14.58. Found: C, 37.95; H, 6.32; N, 14.22.

It was positive to ninhydrin and *p*-(N,N-dimethylamino)benzaldehyde tests but negative to the periodate–benzidine test.

Oxidation of *N*-Acetylpolyoxin C (7). PtO_2 (250 mg) was hydrogenated in 20 ml of water at 3.3 kg/cm^2 for 1 hr. After oxygen was bubbled through this suspension for 30 min, 100 mg of **7** was added and oxygen bubbling was continued overnight. The catalyst was filtered off and the filtrate was concentrated to a syrup, which showed a main *o*-dianisidine-positive spot and a minor spot identical with **37** (see the next part) on paper electrophoresis. The syrup was dissolved in 20 ml of water which contained 130 mg of Na_2CO_3, and about 300 mg of freshly prepared wet Ag_2O was added to it. After shaking for 2 hr at 70°, an *o*-dianisidine-positive spot disappeared almost completely. The reaction mixture was filtered and passed through 25 ml of Dowex 50W-X8 (H). The black solution thus obtained was acidified with 1 *N* HCl and a black precipitate was filtered off. The colorless filtrate was evaporated to dryness. The residue was dissolved in 2 ml of 3 *N* HCl and heated on a boiling bath for 1 hr. The hydrolysate was dried over NaOH. The residue was purified on a cellulose column with the solvent system A. From the main fraction, 38 mg of crystalline **33** was obtained after recrystallization from water: $[\alpha]^{23}D$ +15.0°, $[\alpha]^{23}_{365}$

+46.8° (*c* 0.774, 1 *N* HCl). The ir spectrum in KBr was identical with that of polyoxin C acid.

Polyoxin C Acid (33). Polyoxin D (**1d**) (150 mg) was hydrolyzed with 6 ml of 0.5 *N* NaOH at 65° for 4 hr. The hydrolysate was passed through 10 ml of Amberlite IRC-50 (H). The effluent was evaporated to dryness and the residue was chromatographed on Dowex 50W-X8 with pyridine–acetic acid buffer (pH 3.1). From the uv and ninhydrin-positive fraction, crystals were obtained, which were pulverized and extracted with ether overnight to remove uracil-5-carboxylic acid. The residue was recrystallized from water affording 50 mg of **33**: mp 240–260° dec; $[\alpha]^{23}D$ +2.5° (*c* 0.24, H_2O); $[\alpha]^{23}D$ +14.6° (*c* 1.15, 1 *N* HCl); uv max (0.05 *N* HCl) 220 mμ (ε 10,400), 275 mμ (ε 11,700), (0.05 *N* NaOH) 270 mμ (ε 7100). A sample was dried at room temperature.

Anal. Calcd for $C_{11}H_{13}N_3O_9 \cdot 0.5H_2O$: C, 38.83; H, 4.15; N, 12.35. Found: C, 38.58; H, 4.01; N, 12.21.

A small sample was dried at 100° for 2 hr before analysis.

Anal. Calcd for $C_{11}H_{13}N_3O_9$: C, 39.88; H, 3.96; N, 12.69. Found: C, 40.13; H, 4.16; N, 12.36.

Small samples of polyoxins E and F were hydrolyzed similarly and **33** was detected on tlc.

The *N*-acetyl derivative was prepared in a similar way to *N*-acetylpolyoxin C as a homogeneous white powder: mp 150–180° dec; pmr (DMSO-d_6) δ 1.94 (s, 3, CH_3), 4.09 (m, 3, 2′, 3′, and 4′ H's), 4.72 (q, 1, 5′-H, J = 8.4 and 4.6 Hz), 5.83 (d, 1, 1′-H, $J_{1',2'}$ = 3.7 Hz), 2.34 (s, 1, 5-H), 2.36 (d, 1, AcN*H*, J = 8.4 Hz). On acid hydrolysis (3 *N* HCl, 100° 1 hr) it gave polyoxin C acid (**33**), which was identified by the ir spectrum.

Thymine-polyoxin C (34). Polyoxin H (**1h**) (100 mg) was hydrolyzed in 2 ml of 0.5 *N* NaOH at 65° for 4 hr. The hydrolysate was passed through 3 ml of Amberlite XE-64 (H) and the effluent was evaporated to dryness. The residue was purified by preparative tlc with the solvent systems A and C. From uv and ninhydrin-positive fraction, 3 mg of crystalline **34** was obtained: mp 235–240° dec; $[\alpha]^{22}D$ +7°, $[\alpha]^{22}_{365}$ +37° (*c* 0.046, H_2O). The ir spectrum was identical with that of deoxypolyoxin C.

A few milligrams of polyoxin J was hydrolyzed similarly and **34** was identified on tlc.

Uracil-polyoxin C (35). Polyoxin K (**1k**) (250 mg) was treated with 10 ml of 0.5 *N* NaOH at 65° for 4 hr. The hydrolysate was passed through a column of Amberlite XE-64 (H), the effluent was concentrated, and the residue was submitted to a cellulose chromatography with the solvent system A. The uv and ninhydrin-positive fraction was further purified by preparative tlc with the solvent system B. On crystallization from aqueous ethanol, 4.3 mg of crystalline **35** was obtained: mp 240–247° dec; $[\alpha]^{22}D$ +15.8 (c 0.205, H_2O); $[\phi]_{280}$ +2340 pk $[\phi]_{253}$ −5300 tr (H_2O); ux max (0.05 *N* HCl) 258 mμ (ε 9460), (0.05 *N* NaOH) 262 mμ (ε 7310).

Anal. Calcd for $C_{10}H_{13}N_3O_7$: C, 41.81; H, 4.56; N, 14.63. Found: C, 41.52; H, 4.60; N, 14.65.

Polyoxin L (**1l**) (20 mg) was hydrolyzed similarly and the hydrolysate was purified on preparative tlc with the solvent systems A and B. On crystallization, about 1 mg of crystalline **35** was obtained. The ir spectrum was identical with that of the compound obtained from polyoxin K (**1k**).

Decarboxylation of Polyoxin C Acid (33). A solution of 1 g of **33** in 4 ml of 3 *N* HCl was refluxed overnight. The reaction mixture was passed through 20 ml of Amberlite CG-4B (OH) and 3 ml of Amberlite XE-64 (H). The effluent was concentrated to a small volume and precipitated with ethanol–ether. The residue thus obtained was purified by preparative tlc with the solvent system A. The ninhydrin- and uv-positive fraction was collected. The purified material thus obtained [uv max (0.05 *N* HCl) 259 mμ, (0.05 *N* NaOH) 262 mμ] was identical with **35** on tlc. Acid elution from Amberlite CG-4B recovered 380 mg of the unreacted material.

Hydrogenolysis of Polyoxin B. Polyoxin B (**1b**) (50 mg) was hydrogenated over platinum (from 10 mg of PtO_2) in 10 ml of water at atmospheric pressure and at room temperature. Approximately 1 equiv of hydrogen was taken up in 3 hr. After the catalyst was filtered off, the filtrate was evaporated to dryness and the residue was chromatographed on cellulose with the solvent system A. Deoxypolyoxin B was obtained as a homogeneous powder from water–ethanol–ether, $[\alpha]^{23}D$ +30.6° (*c* 1.03, H_2O). It showed the same R_f value with polyoxin J (**1j**) on tlc. A small sample was dried at 110° for 4 hr before analysis.

Anal. Calcd for $C_{17}H_{25}N_5O_{12}$: C, 41.55; H, 5.13; N, 14.25. Found: C, 41.62; H, 5.19; N, 13.85.

Hydrogenation of Polyoxin H. Polyoxin H (**1h**) (40 mg) was hydrogenated over platinum (from 8 mg of PtO_2) in 10 ml of water at atmospheric pressure and at room temperature. The catalyst was filtered off and the filtrate was evaporated to dryness. The residue was chromatographed on cellulose with butanol–acetic acid–water (4:1:1), then purified on preparative tlc with 75% phenol. The homogeneous powder thus obtained showed the identical pmr spectrum in D_2O and R_f values on tlc with those of **22**.

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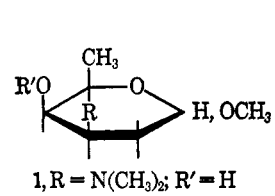
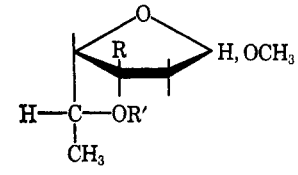
Communications to the Editor

The Megalomicins. I. D-Rhodosamine, a New Dimethylamino Sugar

Sir:

D-Rhodosamine, a new dimethylamino sugar isolated from megalomicin A, B, C_1, and C_2, a new family of macrolide antibiotics elaborated by *Micromonospora megalomicea* sp. n.,[1] has been shown to be 2,3,6-trideoxy-3-dimethylamino-D-*lyxo*-hexopyranose.[2] Methanolysis of megalomicin A gave anomeric mixtures of the 1-*O*-methyl pyranoside **1** and furanoside **2** forms





1, R = $N(CH_3)_2$; R′ = H
3, R = O⟵$N(CH_3)_2$; R′ = H
4, R = $N(CH_3)_2$; R′ = CH_3CO
6, R = R′ = H

2, R = $N(CH_3)_2$; R′ = H
8, R = $N(CH_3)_2$; R′ = CH_3CO
9, R = O⟵$N(CH_3)_2$; R′ = H
11, R = R′ = H
12, R = H; R′ = CH_3

of D-rhodosamine.

(1) (a) H. Reimann, R. S. Jaret, and A. K. Mallams, paper presented at the 8th Interscience Conference on Antimicrobial Agents and Chemotherapy, American Society for Microbiology, New York, N. Y., Oct 21–23, 1968, Abstracts, p 4; (b) M. J. Weinstein, G. H. Wagman, J. Marquez, G. Luedemann, E. Oden, and J. A. Waitz, ref 1a, p 4; (c) M. J. Weinstein, G. H. Wagman, J. A. Marquez, R. T. Testa, E. Oden, and J. A. Waitz, *J. Antibiotics* (Tokyo), *Ser. A*, **22**, 253 (1969); (d) J. A. Marquez, A. Murawski, G. H. Wagman, R. S. Jaret, and H. Reimann, *ibid.*, **22**, 259 (1969); (e) J. A. Waitz, E. L. Moss, Jr., E. Oden, and M. J. Weinstein, *ibid.*, **22**, 265 (1969).

(2) L-Rhodosamine occurs in a number of antibiotics and in particular in the rhodomycins [H. Brockmann, E. Spohler, and T. Waehneldt, *Chem. Ber.*, **96**, 2925 (1963)].