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STRUCTURES OF NEOPOLYOXINS A, B, AND C.

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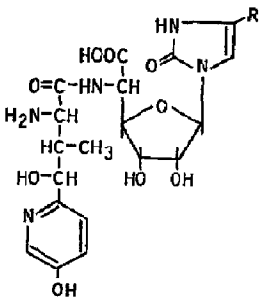
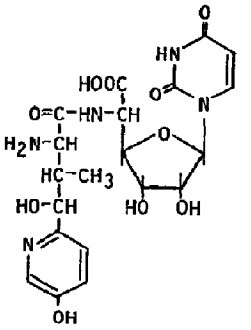
Summary: The absolute structures of fungal chitin synthetase inhibitors, neopolyoxins A, B, and C were determined as 1, 2, and 3 respectively on the basis of chemical and spectroscopic evidence.

A newly-isolated strain of streptomycete, *Streptomyces cacaoi* subsp. *asoensis*, was found to produce potent inhibitors of fungal cell wall chitin synthetase. Three compounds have been isolated and found to be different from all other members of the polyoxin family.[1] They were named neopolyoxins A, B, and C and their properties were described in a separate paper.[2] We wish to report here the elucidation of the absolute structures of these antibiotics.

Neopolyoxin A (1) ($C_{20}H_{25}N_5O_{10}$, d.p. >204°C) is an amphoteric compound and has characteristic absorption maxima in the UV spectrum: $\lambda_{max}^{H_2O}$ 215(sh), 283 nm, $\lambda_{max}^{0.05N\text{-}HCl}$ 223(sh), 287 nm, $\lambda_{max}^{0.05N\text{-}NaOH}$ 241, 304 nm; $\delta_H^{D_2O}$ 9.20 ppm(-CHO); $\delta_C^{D_2O}$ 180.4 ppm(-CHO)). It was hydrolyzed by leucine aminopeptidase to afford a nucleoside (4) and an amino acid (7). The nucleoside (4) ($C_{10}H_{13}N_3O_7$, d.p. >190°C, $\lambda_{max}^{0.05N\text{-}HCl}$ 286 nm, $\lambda_{max}^{0.05N\text{-}NaOH}$ 309 nm, $\delta_H^{3\%DCl}$ 9.21 ppm(-CHO),



1 : R = CHO
2 : R = COOH



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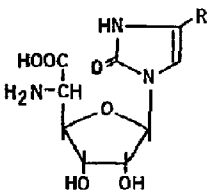
$\delta_C^{3\%DCl}$ 181.1 ppm(-CHO)) was oxidized with silver oxide to give the corresponding carboxylic acid (5): $C_{10}H_{13}N_3O_8$, m.p. 205-210°C, $\lambda_{max}^{H_2O}$ 252 nm, $\lambda_{max}^{0.05N\text{-}HCl}$ 263 nm, $\lambda_{max}^{0.05N\text{-}NaOH}$ 256 nm, $[\theta]_{252}$=-4,767 (H_2O).

Fox and his co-workers reported chemical transformation of uridine into imidazoline nucleoside.[3] Uracil polyoxin C (6), which is the nucleoside moiety of polyoxin L,[1] was treated with bromine followed by sodium bicarbonate. The UV, IR, and CD spectra of the crystalline product was identical with 5. Since the absolute configuration of 6 is firmly established,[1] this is an unambiguous proof for the absolute configuration of 4, 1-β-(5'-amino-5'-deoxy-D-allofuranosyl uronic acid)-2-oxo-4-imidazoline-4-carbaldehyde.[4]



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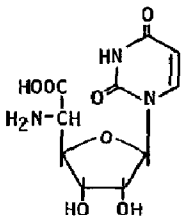
5 : R = COOH



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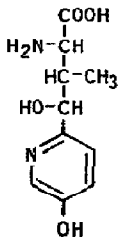

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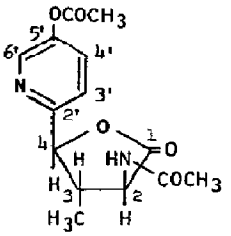

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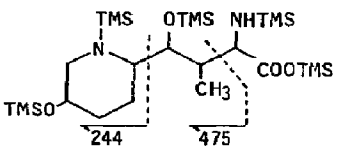
The structure of the isolated side chain amino acid (7) ($C_{10}H_{14}N_2O_4$, m.p. 206-210°C) is suggested to be 2-amino-4-hydroxy-4-(5-hydroxy-2-pyridyl)-3-methylbutyric acid by the UV, mass, ^1H, and ^{13}C NMR spectra: $\lambda_{max}^{H_2O}$ 219, 256, 279, 317 nm, $\lambda_{max}^{0.05N\text{-}HCl}$ 205, 228, 289 nm, $\lambda_{max}^{0.05N\text{-}NaOH}$ 242, 302 nm; M^+ for the O-tris(trimethylsilyl) derivative, m/z 442(0.13%, rel. int.), 427.1932 (2.3), 296.1533(21), 269.1259(100), 235.1231(78); $\delta_H^{3\%DCl}$ 1.20(3H, d), 2.84(1H, m), 4.18(1H, d), 5.20(1H, d), 7.93(1H, d), 8.07(1H, dd), 8.31(1H, d) ppm; $\delta_C^{3\%DCl}$ 12.3(q), 39.4(d), 54.4(d), 72.0(d), 126.4(d), 129.3(d), 132.9(d), 146.2(s), 155.8(s), 170.7(s) ppm. Treatment of 7 with acetic anhydride in methanol yielded a crystalline γ-lactone diacetate (8): m.p. 206-208°C; M^+, m/z 292.1023 (m/z calcd. for $C_{14}H_{16}N_2O_5$, 292.1058); λ_{max}^{MeOH} 265, 270(sh) nm; υ_{max}^{KBr} 1780, 1760, 1655 cm^{-1}; $\delta_H^{CDCl_3}$ 1.27(3H, d), 2.07(3H, s), 2.34(3H, s), 2.53(1H, m), 4.56(1H, dd), 5.03(1H,d), 6.18(1H, d), 7.48(2H), 8.33(1H). The NOE of 8 in $CDCl_3$ solution (20 mM) at 23°C was measured from the difference spectrum recorded on a 270 MHz NMR spectrometer.[5] Observed NOE enhancements of each proton signal upon irradiation of specific protons are listed in Table 1. Trans con-

Table 1. NOE Enhancements of 8

irr.	δ_H	observed NOE (%)					
		C_2-H	C_2-NH	C_3-H	C_3-CH_3	C_4-H	$H_{3'}$, $H_{4'}$
C_2-H	4.56	--	1.5	3.5	1.0	3.2	0
C_2-NH	6.18	4.4	--	4.4	0	0	0
C_3-H	2.53	2.0	5.1	--	1.0	1.8	0.8
C_3-CH_3	1.27	7.2	0	7.7	--	5.5	0
C_4-H	5.03	4.3	0	2.4	1.0	--	1.6
$H_{3'}$, $H_{4'}$	7.48	0	0	2.0	0	3.5	--



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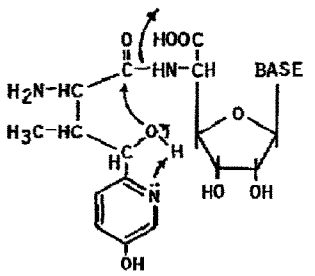
figuration for the substituents at C-2, C-3, and C-4 in 8 was established from the follwing experimental results. On irradiation of methyl protons (C_3-C<u>H</u>$_3$), C_2-<u>H</u> and C_4-<u>H</u> were enhanced (by 7.2 and 5.5%, respectively) but C_2-N<u>H</u> was not enhanced. Similarly, on irradiation of C_2-N<u>H</u>, C_3-<u>H</u> was enhanced (4.4%) but C_4-<u>H</u> or C_3-C<u>H</u>$_3$ were not enhanced. Converse NOE enhancements were observed between C_2-<u>H</u> and C_4-<u>H</u>. Furthermore, C_3-<u>H</u> was enhanced on irradiation of $C_{3'}$-<u>H</u> and $C_{4'}$-<u>H</u> of the pyridine ring.

Amino acid 7 was hydrogenated over 5% rhodium on alumina to obtain a diastereomeric mixture of hexahydro derivative after extensive purification by HPLC. For characterization, the purified product was trimethylsilylated and analyzed by gas chromatography-mass spectrometry. Several peaks were detected by gas chromatography. Two main peaks[6] gave the same mass spectrum for the penta(trimethylsilyl) derivatives: m/z 592(M^+), 577(M^+-CH_3), 502(M^+-TMSOH), 412(M^+-2TMSOH). Two intense ions at m/z 475 and 244 supported the structure 9. The observed positive CD band ($[\theta]_{214}$= +4,090, in 0.5N HCl) of the hydrogenated amino acid proved the L (i.e. S) configuration. Therefore, configuration of 7 has been determined as (2S, 3S, 4R).

Since deamination of 1 with nitrous acid followed by alkaline hydrolysis afforded 4 but not 7, the side chain amino group of 7 is unsubstituted in 1, Therefore, absolute structure 1 is proposed for neopolyoxin A.

Neopolyoxin B (2) ($C_{20}H_{25}N_5O_{11}$, d.p. >192°C, $\lambda_{max}^{H_2O}$ 215(sh), 252, 278(sh), 315 nm, $\lambda_{max}^{0.05N\text{-}HCl}$ 227(sh), 266, 285(sh) nm, $\lambda_{max}^{0.05N\text{-}NaOH}$ 243, 298 nm) is also an amphoteric compound. Enzymatic hydrolysis of 2 gave two products, a side chain amino acid identical with 7, and a nucleoside (5) ($C_{10}H_{13}N_3O_8$, m.p. 212°C; $\lambda_{max}^{H_2O}$ 252 nm, $\lambda_{max}^{0.05N\text{-}HCl}$ 263 nm, $\lambda_{max}^{0.05N\text{-}NaOH}$ 256 nm; M^+ for hexa(trimethylsilyl) derivative, m/z 735.3058(19%), 618.2696(60%, M^+-6'-CO_2SiMe_3), 273.1019(30%, base + 2H)[7]). The nucleoside was found to be identical with the imidazoline nucleoside (5) obtained by chemical transformation of 4 or 6. It incorporated 4 atoms of ^{18}O when treated with 1N HCl in 90% $H_2{}^{18}O$(6 hrs, 70°C), in accord with the structure proposed. Nitrous acid treatment followed by hydrolysis revealed the peptide sequence leading to the absolute structure of neopolyoxin B shown as 2.

Neopolyoxin C (3) ($C_{20}H_{25}N_5O_{10}$,1/2H_2O, d.p. >194°C, $\lambda_{max}^{H_2O}$ 260, 315 nm, $\lambda_{max}^{0.05N\text{-}HCl}$ 227(sh), 261, 285 nm, $\lambda_{max}^{0.05N\text{-}NaOH}$ 243, 301 nm) was hydrolyzed with 0.5N sodium hydroxide to give uracil, uracil polyoxin C (6), and an amino acid (7). Peptide sequence was determined similarly by nitrous acid treatment followed by alkaline hydrolysis. Thus, the absolute structure of neopolyoxin C was elucidated as 3.



The unusual instability of the neopolyoxin peptide bond at acidic pH can be explained by neighboring group participation of the γ-hydroxyl and nitrogen of the pyridine ring of this particular side chain amino acid as illustrated.

Since we have shown that the pyrimidine nucleoside of the polyoxins is biosynthesized by condensation of uridine and phosphoenolpyruvate[8], it should be reasonable to presume that the imidazoline ring

is formed from the uracil moiety at the nucleoside level by a ring contraction reaction similar to that of the chemical transformation.

Recently, Zähner and his co-workers reported the structures of nikkomycins X and Z.[9] Although no stereochemical assignments were made in their paper[10], their planar structures correspond to those of neopolyoxins A (1) and C (3), respectively. Nevertheless, they stated that the sugar moiety of nikkomycin was *not* identical with that of the polyoxins.[11]

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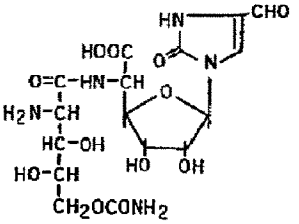
References and Notes

(1) K.Isono, K.Asahi, and S.Suzuki, J. Am. Chem. Soc., 91, 7490 (1969).

(2) K.Kobinata, M.Uramoto, M.Nishii, H.Kusakabe, G.Nakamura, and K.Isono, Agric. Biol. Chem., 44, 1709 (1980).

(3) B.A.Otter, E.A.Falco, and J.J.Fox, J. Org. Chem., 34, 2636 (1969).

(4) This nucleoside was identical with the nucleoside obtained from polyoxin N (M.Uramoto, J.Uzawa, S.Suzuki, K.Isono, J.G.Liehr, and J.A.McCloskey, Nucleic Acids Research, Special Publication, 5, s327 (1978)). Therefore, the structure of polyoxin N must be revised as follows:



(5) The sample was degassed 3 times by the freeze-thaw method and sealed in a NMR tube under N_2 gas. NOE's were determined by applying a 20's low power saturating pulse at the appropriate peak position, followed by a high power 90° observing pulse after 0.2-s waiting time. Off-resonance control spectra were measured in the same way, except that the presaturating pulse was perturbed. On-resonance and off-resonance spectra were the sum of 128 scans.

(6) Other minor peaks were identified as 4-deoxy isomers: M^+ for the tetra(trimethylsilyl) derivative, m/z 504, 489(M^+-CH_3), 387($M^+-CO_2SiMe_3$), 244.

(7) K.Isono, P.F.Crain, T.J.Odiorne, J.A.McCloskey, and R.J.Suhadolnik, J. Am. Chem. Soc., 95, 5788 (1973).

(8) (a) K.Isono, T.Sato, K.Hirasawa, S.Funayama, and S.Suzuki, J. Am. Chem. Soc.,100, 3937, (1978). (b) T.Sato, K.Hirasawa, J.Uzawa, T.Inaba, and K.Isono, Tetrahedron Lett., 1979, 3441.

(9) H.Hagenmaier, A.Keckeisen, H.Zähner, and W.A.König, Liebigs Ann. Chem., 1979, 1494 (1979).

(10) Glycosyl position of nikkomycin X is also not determined in their paper.[9]

(11) U.Dähn, H.Hagenmaier, H.Höhne, W.A.König, G.Wolf, and H.Zähner, Arch. Microbiol., 107, 143 (1976).

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