Short Communication

Neopolyoxins A, B, and C, New
Chitin Synthetase InhibitorsKimie KOBINATA, Masakazu URAMOTO,
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In the course of the screening for inhibitors of fungal cell wall biosynthesis, a newly isolated streptomycete, *Streptomyces cacaoi* subsp. *asoensis* was found to produce chitin synthetase inhibitors which are different from the polyoxins.¹⁾ They were isolated and named as the neopolyoxins. We wish to describe here isolation and characterization of neopolyoxins A, B, and C. The structures have been elucidated (Fig. 1), which will be reported in a separate paper.²⁾

The strain was cultured at 28°C for 160 hours in jar fermentors containing 18 liters each of a medium: glucose (1%), soluble starch

FIG. 1. The Structures of the Neopolyoxins.

(9%), soybean flour (2%), dried yeast (4%), NaNO₃ (0.2%), and K₂HPO₄ (0.2%). Fermentation broth was adjusted to pH 5.0 with 10% HCl and filtered. The filtrate (36 liters) was passed through a column of Dowex 50W X-8 (H) and the antibiotics were eluted with 0.6 N NH₄OH. After neutralization, it was passed through a charcoal column, which was washed with water and eluted with 40% acetone. The eluate was concentrated *in vacuo* to a small volume. By addition of ethanol and ether, 15 g of crude powder of the neopolyoxins was obtained. It was further purified by Dowex 50W chromatography using 0.1 N pyridine-acetic acid (pH 4.0→5.0). A fraction

TABLE I. PHYSICO-CHEMICAL PROPERTIES OF NEOPOLYOXINS

	Neopolyoxin A	Neopolyoxin B	Neopolyoxin C
mp (dec.)	204~207°C	192~196°C	194~197°C
Formula	C ₂₀ H ₂₅ N ₅ O ₁₀	C ₂₀ H ₂₅ N ₅ O ₁₁	C ₂₀ H ₂₅ N ₅ O ₁₀ ·1/2H ₂ O
Calc.	C, 48.46; H, 5.10; N, 14.13;	C, 46.94; H, 5.13; N, 13.69;	C, 47.63; H, 5.20; N, 13.89;
Found	C, 48.91; H, 5.02; N, 13.96;	C, 46.65; H, 5.43; N, 13.70;	C, 47.32; H, 5.09; N, 13.61;
pKa'	2.9, 4.3, 7.3, 8.7, 10.3	2.4, 3.0, 4.4, 7.7, 9.1, 11.1	2.7, 4.1, 7.1, 8.5, 9.7
Titre equiv.	515	530	537
[α] _D ²¹	+24.3° (c 0.325, H ₂ O)	+19.0° (c 0.722, H ₂ O)	+49.5° (c 1.00, H ₂ O)
UV; λ _{max} mμ (E _{1cm} ^{1%})			
In H ₂ O	215sh (303), 283 (324)	215sh (277), 252 (163), 278sh (77), 315 (11)	260 (203), 315 (13)
In 0.05 N HCl	223sh (187), 287 (384)	227sh (133), 266 (171), 285sh (140)	227sh (164), 261 (194), 285sh (145)
In 0.05 N NaOH	241 (320), 304 (286)	243 (277), 298 (72)	243 (338), 301 (84)
IR: ν _{max} ^{KBr} (cm ⁻¹)	3400, 1705, 1655, 1610, 1500, 1434, 1403, 1280, 1195, 1116, 1055, 1010, 730	3100~3400, 1685, 1620, 1580, 1498, 1415, 1280, 1120, 1060, 990, 740	3100~3400, 1710, 1670, 1610, 1490, 1465, 1388, 1270, 1110, 1050, 810, 640, 550

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FIG. 2. UV Absorption Spectra of the Neopolyoxins.

Left, neopolyoxin A; middle, neopolyoxin B; right, neopolyoxin C.

—, in H_2O ; ----, in 0.05 N HCl; — · —, in 0.05 N NaOH.

TABLE II. ANTIFUNGAL ACTIVITY OF NEOPOLYOXINS

Conventional agar dilution method was employed using a potato-sucrose medium.

Test organism	Minimal inhibitory concentration (mcg/ml)		
	Neopolyoxin A	Neopolyoxin B	Neopolyoxin C
<i>Glomerella cingulata</i>	3.12	> 100	6.25
<i>Botrytis cinerea</i>	0.1	6.25	—
<i>Cochliobolus miyabeanus</i>	0.05	50	0.1
<i>Alternaria mali</i>	3.12	> 100	3.12
<i>Alternaria mali</i> (polyoxin resistant)	> 100	> 100	> 100
<i>Colletotrichum lagenarium</i>	6.25	100	3.12
<i>Pyricularia oryzae</i>	< 0.05	1.56	0.05
<i>Pellicularia sasakii</i>	3.12	25	1.6
<i>Sclerotinia cinerea</i>	1.6	> 100	3.12
<i>Candida albicans</i>	0.4	> 100	0.8

eluted with a buffer of pH 4.5 contained neopolyoxin B, which was further purified by Avicel cellulose chromatography ($n\text{-BuOH-MeOH-H}_2\text{O}$, 4:1:0.5→4:1:1.5). Neopolyoxin B (175 mg) was obtained as purified white powder from ethanol-ether. A fraction eluted with a buffer of pH 5.0 contained neopolyoxins A and C. They were separated by Dowex 50W chromatography using 0.2N pyridine-formic acid buffer (pH 3.4→4.8). After similar purification by Avicel

chromatography, neopolyoxin A (190 mg) and neopolyoxin C (290 mg) were obtained as white powder.

Neopolyoxins A, B, and C are all amphoteric compounds and unstable in acid or alkaline conditions. Their physico-chemical properties are summarized in Table I. They have characteristic UV absorption spectra (Fig. 2) and gave positive ninhydrin, periodate-benzidine, and ferric chloride tests.

Biological activity of the neopolyoxins is

similar to that of the polyoxins. ID_{50} to chitin synthetase from *Pyricularia oryzae* is: A 0.83, B 41.7, and C 1.7 mcg/ml. Antifungal activity is summarized in Table II. Cross-resistance with the polyoxins was observed with polyoxin-resistant *Alternaria mali*. The antibiotics showed low toxicity to mice ($LD_0 > 250$ mg/kg by intravenous injection).

Structural similarity of neopolyoxins A and C with nikkomycins X and Z³⁾ is discussed in a separate paper.²⁾

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