OXAZINOMYCIN PRODUCED BY A *PSEUDOMONAS* SPECIES

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(Received for publication January 5, 1984)

Isolation of oxazinomycin (minimycin) from streptomycete fermentations has been reported previously[1~5] and the antibiotic structure has been confirmed synthetically[6] to be 5-β-D-ribofuranosyl-1,3-oxazine-2,4-dione (**1**). Oxazinomycin is one of few examples of *C*-nucleoside antibiotics isolated from natural sources.[4,7] We now report the first isolation of **1** from a bacterial culture.

The producing organism, SC 11,793, was isolated from a lichen on a birch tree. It is a yellow-pigmented, oxidative Gram-negative polar flagellate rod which shares some features common to both pseudomonads and xanthomonads. One striking characteristic of SC 11,793 is its extreme sensitivity to sodium chloride, its growth being markedly reduced at 0.1% and totally inhibited at 0.5%. Failure of SC 11,793 to grow on a NaCl-free version of standard maintenance medium for xanthomonads (glucose - yeast extract - $CaCO_3$) suggests that SC 11,793 is not a xanthomonad. Furthermore, SC 11,793 closely resembles the *Pseudomonas paucimobilis* species described by HOLMES[8]. On the basis of direct comparison (partially presented in Table 1), SC 11,793 is assigned to be a strain of *Pseudomonas paucimobilis*.

Production and isolation of the antibiotic were followed by disc diffusion assays against *Staphylococcus aureus*. For the purpose of isolation, fermentations were carried out at 25°C for 24~28 hours on a rotary shaker (300 rpm; 5 cm stroke) in 500-ml Erlenmeyer flasks each containing 100 ml of the following sterilized medium: 0.4% yeast extract, 1.0% malt extract, and 0.4% dextrose in distilled water. The filtered broth from 100 flasks (approximately 9 liters) was equilibrated with Dowex 1-X4 (Cl^- form) resin (400 g) and the sorbed antibiotic was eluted with 1.0 M acetic acid (1 liter). The dried eluate (8.9 g) was washed with small portions of ethanol (totaling 50 ml) and the soluble material (2.1 g, dried) chromatographed on MCI Gel CHP20P resin (H_2O) to give a purified antibiotic fraction (240 mg). Silica gel flash chromatography ($CHCl_3$ - MeOH, 4: 1) of this fraction afforded 90 mg of EM5429 as a white solid.

Table 1. Direct comparison of SC 11,793 and *Pseudomonas paucimobilis*.

Characteristics	SC 11,793	*P. paucimobilis*[a]
Cytochrome oxidase	−	+(0.92)[b]
Gelatinase	−	−
DNase	−	−[c]
β-Galactosidase	+	+
Lysine decarboxylase	−	−
Ornithine decarboxylase	−	−
Arginine decarboxylase	−	−
Esculin hydrolysis	+	+
Poly β-hydroxybutyrate deposition	+	+
Nitrate reduction	−	−
Growth 41°C	−	−
Acid from glucose	+	+
maltose	+	+
lactose	+	+
cellobiose	+	+
sucrose	−	+
xylose	+	+
mannitol	+	−
sorbitol	+	−

[a] ATCC 29837.

[b] According to the API 20E (Analytab Products Inc.) computer identification system, 92% of *P. paucimobilis* strains gave a positive response for this test in their data base (API Species 4 (1): 13, 1980; See Table 3). In the Minitek Differentiation System (BBL), 88% of the strains were cytochrome oxidase positive. Thus, SC 11,793 could belong to the 8% to 12% cytochrome oxidase residual.

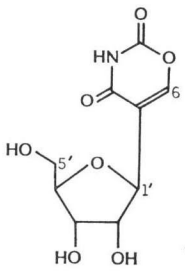
[c] HOLMES *et al.*[8] reported that 29/29 strains were DNase positive. Our test with their type strain, ATCC 29837, gave a negative response, in accordance with the API and BBL Minitek system reports.

The melting point, elemental analysis and mass spectroscopic data for the weakly acidic EM5429 solid were consistent with published data for **1**. Direct comparison of NMR spectra and optical rotations indicated that pure EM5429 and authentic oxazinomycin (Kaken Chemical Company, Ltd., Japan), are identical in all respects including

Table 2. Physical properties of EM5429 and oxazinomycin.

Property		EM5429	Oxazinomycin
$[\alpha]_D^{25}$ (c 1.0, H_2O)[a]		+26°	+18°
^1H NMR, 400 MHz (D_2O)	H_6	7.83 (d, 0.9)[a]	7.82 (d, 0.9)[a]
δ ppm (J, Hz)[b]	$H_{1'}$	4.73 (dd, 0.9, 4.9)	4.71 (d, 0.6, 4.9)
	$H_{2'}$	4.31 (dd, 5.0, 5.0)	4.30 (dd, 5.0, 5.0)
	$H_{3'}$	4.17 (dd, 5.5, 5.8)	4.15 (dd, 5.5, 6.1)
	$H_{4'}$	4.04 (m)	4.03 (m)
	$H_{5a'}$	3.87 (dd, 3.0, 12.5)	3.86 (dd, 3.1, 12.5)
	$H_{5b'}$	3.74 (dd, 4.8, 12.5)	3.73 (dd, 5.2, 12.5)
^{13}C NMR, 100 MHz (D_2O)	C-2	150.6	150.8[c]
δ (ppm)	C-4	163.9	163.9
	C-5	114.9	114.9
	C-6	155.2	155.5
	C-1′	78.9	78.8
	C-2′	74.3	74.3
	C-3′	71.5	71.4
	C-4′	83.9	83.9
	C-5′	62.7	62.2

[a] Authentic oxazinomycin and EM5429 spectra determined on the same instrument.
[b] ±0.3 Hz.
[c] Data obtained from ref 9.



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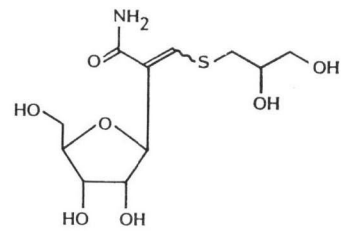

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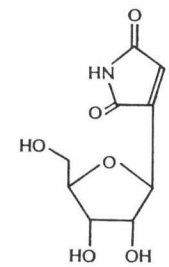

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absolute configuration (Table 2).

Routine mass spectrometric analysis of **1** provided protonated molecular ions (M+H 246 m/z) by chemical (CI, H_2O) and fast atom bombardment (tetramethylene sulfone) ionization techniques. However, when **1** was prepared for FABMS in a thioglycerol matrix, gas evolution from the mixture was immediately evident. FAB analysis of the residual thioglycerol solution suggested that **1** had undergone MICHAEL addition and decarboxylation to yield decomposition product **2**. Similar reactivity with thiols has been reported[7] for the maleimide *C*-nucleoside antibiotic showdomycin (**3**) and might be relevant to the antibiotic activity of such sulfhydryl trapping agents.

Acknowledgments

We wish to thank Dr. S. E. UNGER for mass spectral measurements, Dr. M. A. PORUBCAN for NMR spectral data, W. H. TREJO for taxonomical contributions, L. D. DEAN for initial characterization data and P. A. PRINCIPE for fermentation studies.

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