Communication to the Editor

A NOVEL NUCLEOSIDE ANTIBIOTIC, 5-FORMYLOXYMETHYLURIDINE

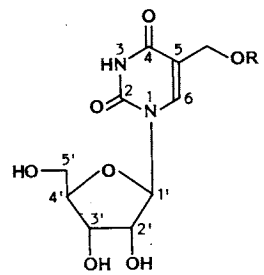
Sir:

During the course of a screening program for new antibiotics, we isolated a new nucleoside antibiotic **1** from the cultured broth of a bacteria BMI585-mF4 which was identified as *Serratia plymuthica*. This antibiotic showed the weak antibiotic activity against some Gram-negative bacteria.

A slant culture of producing strain BMI585-mF4 was inoculated into a 500-ml Erlenmeyer flask containing 100 ml of the same medium used for jar fermenter, and incubated for a day at 27°C on a rotary shaker. The cultured broth (300 ml) was transferred into a 30-liter jar fermenter with 12 liters of the production medium consisting of mannitol 2.0%, yeast extract 0.25%, $(NH_4)_2SO_4$ 0.5%, KCl 0.4%, K_2HPO_4 0.02% and $CaCO_3$ 0.4% at pH 7.4. The fermentation was carried out for 18 hours at 27°C under aeration of 12 liters/minute and agitation of 250 rpm. The cultured broth was centrifuged for 10 minutes, and the supernatant was adsorbed on a column of Diaion HP-20. The column was washed with water (2 liters) and the antibiotic was eluted with 50% aq methanol. The active eluate was concentrated under reduced pressure to dryness. The residue thus obtained was purified by chromatography on Silica gel 60 (Merck, Art. No. 7734, 40 g) developed with a mixture of chloroform, methanol and water (40 : 10 : 1).

The active fraction was subjected to Sephadex LH-20 column chromatography (200 ml) and eluted with methanol. The active eluate was then adsorbed on a column of Diaion CHP-20 (40 ml) and eluted by linear gradient system of water and methanol (0~20%). The active fraction was lyophilized to give 118 mg of white powder **1** (Fig. 1).

Fig. 1. Structures of **1** and 5-hydroxymethyluridine.



1 R=CHO
5-Hydroxymethyluridine R=H

Physico-chemical properties and NMR chemical shifts of **1** were listed in Tables 1 and 2. The ^1H and ^{13}C NMR spectra clearly showed that **1** had a pentofuranose moiety and existence of hydroxy methylene group (δ 4.99 and 59.9), one aromatic proton (δ 8.18 and 143.1) and formyl group (δ 8.17 and 164.2). Resemblance of the UV spectrum of **1** to that of polyoxins[1] showed that **1** had uracil moiety as a chromophore. And the chromophore was deduced to be 5-formyloxymethyluracil from above findings and long range coupling between the methylene protons (δ 4.99) and C-4, C-5, C-6 and formyl carbon in heteronuclear multiple-bond connectivity (HMBC) spectrum. Mild acid hydrolysis (0.01 M HCl, at room temperature) of **1** gave 5-hydroxymethyluridine[2] that was identified with an authentic sample by the optical rotation ($[\alpha]_D$

Table 1. Physico-chemical properties of **1**.

Appearance	White powder
SI-MS (*m/z*)	303 (MH^+)
FD-MS (*m/z*)	302 (M^+)
Molecular formula	$C_{11}H_{14}N_2O_8$
Optical rotation $[\alpha]_D$	−2.4° (*c* 0.5, H_2O)
UV $\lambda_{max}^{H_2O}$ (ε)	208 (9,700), 265 (9,800)
IR ν_{max} (KBr) cm^{-1}	3400, 3060, 2930, 2820, 1700, 1470, 1400, 1275, 1175, 1100, 1055, 910, 780, 760

Table 2. ^1H and ^{13}C Chemical shifts (δ ppm) of **1** in D_2O.

Assignment	Carbon (multiplicity)	Proton
4	165.6 s	
CHO	164.2 d	8.17 (1H, s)
2	152.1 s	
6	143.1 d	8.18 (1H, s)
5	109.5 s	
1′	90.3 d	5.92 (1H, d, J=4.0 Hz)
4′	84.8 d	4.15 (1H, td, J=3.0, 4.0, 5.5 Hz)
2′	74.7 d	4.35 (1H, dd, J=4.0, 5.5 Hz)
3′	69.8 d	4.24 (1H, t, J=5.5 Hz)
5′	61.1 t	3.69 (1H, dd, J=13.0, 3.0 Hz)
		3.84 (1H, dd, J=13.0, 4.0 Hz)
5-CH_2O	59.9 t	4.99 (2H, s)

Table 3. Antibacterial activity of **1**.

	MIC (μg/ml)
Staphylococcus aureus Smith	>100
Micrococcus luteus FDA 16	>100
Bacillus subtilis PCI 219	>100
Escherichia coli NIHJ	>100
E. coli K-12	100
E. coli K-12 ML 1629	25
E. coli BEM 11	50
E. coli BE 1121	<0.78
E. coli BE 1186	12.5
Shigella dysenteriae JS 11910	50
S. flexneri 4b JS 11811	50
S. sonnei JS 11746	50
Salmonella typhi T-63	>100
Proteus vulgaris OX 19	25
P. mirabilis IFM OM-9	>100
Aeromonas punctata IAM 1646	50
A. salmonicida ATCC 14174	25
Vibrio anguillarum NCMB-6	25

$-3.9°$ (c 0.5, H_2O)) and ^1H NMR spectrum. Thus, the structure of **1** was determined to be 5-formyloxymethyluridine.

MICs against various bacteria on Mueller-Hinton agar (Difco) and fungi on nutrient (contains 1% glucose) agar were determined and showed in Table 3. The compound was injected intravenously to mice and did not show any toxic sign at 50 mg/kg.

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