Communications to the Editor

TETRODECAMYCIN, A NEW ANTIMICROBIAL ANTIBIOTIC FROM *Streptomyces*

Sir:

In the course of our screening program for novel antimicrobial antibiotics against *Pasteurella piscicida*, we found that a strain of *Streptomyces* sp. MJ885-mF8 isolated from a soil sample collected at Suginami-ku, Tokyo, Japan, produces a new antimicrobial antibiotic, tetrodecamycin (**1**). In this paper we report the production, isolation, physicochemical properties and biological properties of **1**.

A slant culture of the tetrodecamycin-producing organism was inoculated into a 500-ml Erlenmeyer flask containing 110 ml of a seed medium consisting of galactose 2.0%, dextrin 2.0%, Bacto-Soytone (Difco) 1.0%, corn steep liquor 0.5%, glycerol 1.0%, $(NH_4)_2SO_4$ 0.2% and $CaCO_3$ 0.2% (adjusted to pH 7.4 before sterilization). The inoculated medium was incubated at 27°C for 24 hours on a rotary shaker. Two ml of the seed culture was then transferred to each of 500-ml Erlenmeyer flasks containing 110 ml of the same medium (6 liters). The fermentation was carried out at 27°C for 48 hours on a rotary shaker.

The culture broth was centrifuged to separate the mycelial cake and supernatant. The mycelial cake was extracted with acetone (2 liters), which was filtered and concentrated *in vacuo* to an aqueous solution. The solution was diluted to 2 liters with water and then extracted with ethyl acetate (2 liters). The supernatant (5.8 liters) was extracted with ethyl acetate (5.8 liters). The organic layers were combined and concentrated to dryness under reduced pressure. The dried residue (1.4 g) was dissolved in methanol (20 ml) and the solution was washed with *n*-hexane (20 ml), and concentrated to dryness *in vacuo*. The residue (1.2 g) was chromatographed on a silica gel (50 g) using mixture of toluene-acetone (20:1, 15:1, 10:1, 7:1, and 5:1). The fractions which gave Rf 0.46 on silica gel TLC (chloroform-methanol, 10:1) were collected and concentrated under reduced pressure to give a colorless oil (146 mg) of pure tetrodecamycin (**1**).

Physico-chemical properties of tetrodecamycin (**1**) are summarized in Table 1. The antibiotic is soluble in methanol, ethyl acetate, acetone, acetonitrile, slightly soluble in chloroform and insoluble in *n*-hexane and water. The molecular formula $C_{18}H_{22}O_6$ was determined by HRFAB-MS. The IR spectrum of **1** showed an absorption at 1780 cm^{-1} due to an unsaturated lactone. The UV spectral data at 271 nm (ε 12,500) indicated the presence of an acyl tetronic acid alkyl ether[1]. The substance gave positive color reaction to molybdophosphoric acid-sulfuric acid reagent and negative to $FeCl_3$, Ninhydrin and Rydon-Smith reagents.

The structure of tetrodecamycin (**1**) (Fig. 1) was determined by NMR spectral analysis of **1** and its acetyl derivative (**2**). The ^1H and ^{13}C NMR data of **1** and **2** are shown in Table 2. Tetrodecamycin is a new type of antibiotic related to the acyl tetronic acids. Details of the structure determination of **1**

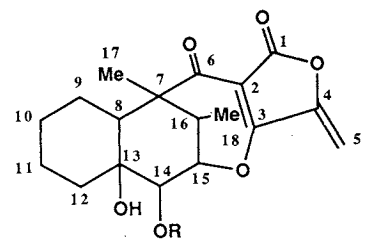
Table 1. Physico-chemical properties of tetrodecamycin (**1**).

Appearance	Colorless oil
Nature	Neutral
Molecular formula	$C_{18}H_{22}O_6$
FAB-MS (*m/z*)	$(M+H)^+$ 335
	$(M-H)^-$ 333
HRFAB-MS (*m/z*)	
Calcd:	335.1459 (as $C_{18}H_{23}O_6$)
Found:	335.1477 $(M+H)^+$
UV λ_{max} (ε) in	
MeOH	271 (12,500)
0.01 N NaOH-MeOH	252 (8,230)
0.01 N HCl-MeOH	270 (12,600)
IR ν_{max}^{KBr} cm^{-1}	3460, 2930, 1780, 1670, 1590, 1440, 1280
$[\alpha]_D^{23}$	−6° (*c* 0.5, MeOH)
Rf	0.47[a]
	0.66[b]

[a] Silica gel TLC (Merck Art. No. 5715) chloroform-methanol (10:1).

[b] Silica gel TLC (Merck Art. No. 5715) toluene-acetone (1:1).

Fig. 1.



1 R=H: Tetrodecamycin
2 R=Ac

Table 2. ^{13}C NMR data (125 MHz) and ^{1}H NMR data (500 MHz) of tetrodecamycin (**1**) and acetyl derivative (**2**) in $CDCl_3$[a].

Position	1		2[b]	
	^{13}C	^{1}H	^{13}C	^{1}H
1	164.64		164.42	
2	100.79		101.48	
3	164.64		164.13	
4	148.39		148.31	
5	96.40	5.36 (1H, d, J=2.5 Hz), 5.26 (1H, d, J=2.5 Hz)	96.70	5.35 (1H, d, J=2.7 Hz), 5.18 (1H, d, J=2.7 Hz)
6	194.53		194.05	
7	53.10		52.80	
8	42.84	1.34 (1H, dd, J=4.2, 12.2 Hz)	42.90	1.37 (1H, br dd, J=3.4, 13.1 Hz)
9	23.52	1.60 (1H, m)[c], 1.50 (1H, m)	23.17	1.57 (1H, dq, J=3.7, 12.8 Hz), 1.48 (1H, m)
10	25.73	1.79 (1H, m), 1.12 (1H, tq, J=3.4, 13.4 Hz)	25.75	1.70 (1H, m), 1.03 (1H, m)
11	20.87	1.60 (1H, m)[c], 1.43 (1H, tq, J=3.4, 13.4 Hz)	20.42	1.42 (2H, m)
12	39.70	1.25 (1H, dt, J=4.3, 14 Hz), 2.08 (1H, br dq, J=2.4, 14 Hz)	38.97	1.63 (1H, m), 1.08 (1H, m)
13	69.01		68.86	
14	78.57	3.62 (1H, d, J=7.0 Hz)	80.28	4.65 (1H, d, J=1.2 Hz)
15	92.00	4.81 (1H, dd, J=0.5, 3.1 Hz)	89.17	4.60 (1H, dd, J=1.2, 4.0 Hz)
16	32.72	2.66 (1H, dq, J=3.1, 7.3 Hz)	33.17	2.82 (1H, dq, J=4.0, 7.3 Hz)
17	17.56	1.25 (3H, s)	17.47	1.24 (3H, s)
18	13.60	1.01 (3H, d, J=7.3 Hz)	13.05	0.91 (3H, d, J=7.3 Hz)
13-OH		2.07 (1H, s)		
14-OH		3.15 (1H, d, J=7.0 Hz)		
CO			169.42	
Me			20.73	2.09 (3H, s)

[a] Chemical shifts in ppm from TMS as an internal standard.
[b] Add 5 drops of benzene-d_6.
[c] It is overlapped by DHO.

Table 3. The antimicrobial activities of tetrodecamycin (**1**).

Test organism	MIC (μg/ml)	Test organism	MIC (μg/ml)
Staphylococcus aureus FDA 209P	6.25	*Pseudomonas aeruginosa* A3	>50
S. aureus Smith	12.5	*Mycobacterium smegmatis* ATCC607	>100
S. aureus MS9610	12.5	*Candida tropicalis* F-1	>100
S. aureus MRSA No. 5	12.5	*C. albicans* 3147	>100
S. aureus MRSA No. 17	12.5	*Saccharomyces cerevisiae* F-7	>100
Micrococcus luteus FDA16	12.5	*Xanthomonas oryzae*	12.5
Bacillus anthracis	6.25	*Aeromonas punctata* IAM1646	50
B. subtilis PCI 219	12.5	*A. salmonecida* ATCC 14174	50
Escherichia coli NIHJ	50	*Aeromonas* sp (KT444)	12.5
E. coli K-12	>100	*Pasteurella piscicida* sp. 6395	1.56
Proteus mirabilis IFM OM-9	25	*Pa. piscicida* sp. 6356	6.25

will be reported later.

The antimicrobial activities of **1** are measured on agar as shown in Table 3 and **1** shows especially potent inhibitory activity against *Pasteurella piscicida*. Tetrodecamycin did not show any toxicity in mice at a dose of 100 mg/kg when administered intraperitoneally.

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(Received November 12, 1993)

Reference

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