Tetrodecamycin and Dihydrotetrodecamycin, New Antimicrobial Antibiotics against *Pasteurella piscicida* Produced by *Streptomyces nashvillensis* MJ885-mF8

II. Structure Determination

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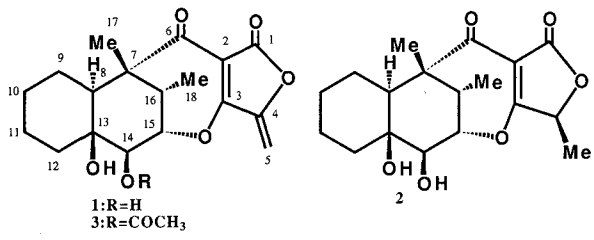
Novel antimicrobial antibiotics against *Pasteurella piscicida*, tetrodecamycin (**1**) and weakly active dihydrotetrodecamycin (**2**) were isolated from a culture broth of *Streptomyces nashvillensis* MJ885-mF8. The planar structure of **1** was determined to be 2-acyl-4-ylidene tetronic acid alkyl ether containing decaline ring by various NMR spectral data of **1** and its acetyl derivative (**3**). The structure of **2** was elucidated by comparison with the spectral data of **1** and confirmed by catalytic reduction of **1** into **2**. The X-ray crystallography of **2** showed the relative stereochemistry. Their absolute configurations were determined by using modified Mosher's method.

Pseudotuberculosis caused by *Pasteurella piscicida* is one of the most widely distributed bacterial diseases of cultured yellowtail, *Seriola quinqueradiata*.[1] Prevention of this disease is therefore considered as an important aspect for the successful culture of yellowtail. During our screening program on this aspect, we have isolated new antibiotics, tetrodecamycin (**1**) and dihydrotetrodecamycin (**2**) from a culture broth of *Streptomyces nashvillensis* MJ885-mF8. In the preceding paper[2], we have described the taxonomy, fermentation, isolation, characterization and biological activities of **1** and **2**. In this paper, we describe details of the structural elucidation of **1** and **2**.

Results

The physico-chemical properties of tetrodecamycin (**1**) and dihydrotetrodecamycin (**2**) were described in the previous paper[2]. The color reactions of **1** and **2** were positive to iodine and molybdophosphoric acid-sulfuric acid reagent and negative to $FeCl_3$, ninhydrin and Rydon-Smith reagents. The UV spectra of **1** and **2** in MeOH showed a characteristic absorption maximum at 271 nm and 249 nm, respectively. The IR spectrum of **1** suggested the presence of hydroxyl group (3460 cm^{-1}) and γ-lactone (1780 cm^{-1}) and the similar value of **2** (3530 and 1760 cm^{-1}) suggested the presence of the same functional groups as **1**.

Fig. 1. The structures of tetrodecamycin (**1**), dihydrotetrodecamycin (**2**) and acetyl derivative (**3**).



Structure of Tetrodecamycin

The molecular formula of tetrodecamycin (**1**) was determined to be $C_{18}H_{22}O_6$ on the basis of HRFAB-MS data. This molecular formula was supported by ^1H and ^{13}C NMR spectral data of **1** (Table 1). The ^{13}C NMR spectral data of **1** showed 17 signals but two signals overlapped at 164.64 ppm. All one bond connections between ^1H and ^{13}C were interpreted by DEPT and heteronuclear multiple quantum coherence (HMQC)[3] experiments of **1** (Table 1).

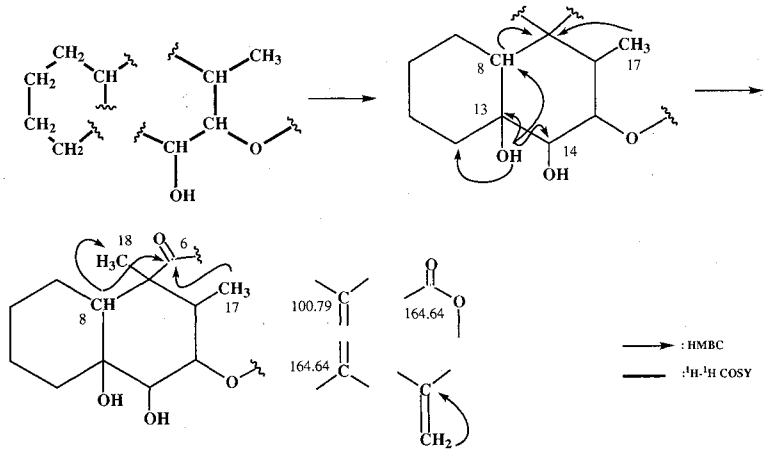
Two partial structures in Fig. 2 were substantiated by interpretation of the ^1H-^1H COSY spectrum. The ^1H-^{13}C long range coupling in the heteronuclear multiple bond correlation (HMBC)[4] experiment of **1** connected two partial structures and fixed positions of three sub-

Table 1. ^{13}C NMR data (125 MHz) and ^{1}H NMR data (500 MHz) of tetrodecamycin (**1**), dihydrotetrodecamycin (**2**) and acetyl derivative (**3**).

Position	Tetrodecamycin ($CDCl_3$)		Dihydrotetrodecamycin (CD_3OD)		14-O-Acetyltetrodecamycin (**3**) ($CDCl_3$ + 5 drops of benzene-d_6)	
	^{13}C	^{1}H (J=Hz)	^{13}C	^{1}H (J=Hz)	^{13}C	^{1}H (J=Hz)
1	164.64		171.49		164.42	
2	100.79		100.97		101.48	
3	164.64		183.07		164.13	
4	148.39		75.40	4.96(1H, q, J=6.7)	148.31	
5	96.40	5.36 (1H, d, J=2.5), 5.26 (1H, d, J=2.5)	17.93	1.48 (3H, d, J=6.7)	96.70	5.35 (1H, d, J=2.7) 5.18 (1H, d, J=2.7)
6	194.53		197.57		194.05	
7	53.10		54.23		52.80	
8	42.84	1.34 (1H, dd, J=4.2, 12.2)	44.30	1.41 (1H, dd, J=4.3, 12.2)	42.90	1.37 (1H, br dd, J=3.4, 13.1)
9	23.52	1.60 (1H, m)*, 1.50 (1H, m)	24.76	1.65 (1H, dq, J=3.7, 12.8), 1.34 (1H, m)	23.17	1.57 (1H, dq, J=3.7, 12.8), 1.48 (1H, m)
10	25.73	1.79 (1H, m) 1.12 (1H, tq, J=3.4, 13.4)	27.21	1.74 (1H, m), 1.15 (1H, m)	25.75	1.70 (1H, m), 1.03 (1H, m)
11	20.87	1.60 (1H, m)*, 1.43 (1H, tq, J=3.4, 13.4)	21.96	1.54 (1H, tq, J=3.7, 13.1), 1.47 (1H, m)	20.42	1.42 (2H, m),
12	39.70	1.25 (1H, dt, J=4.3, 14), 2.08 (1H, br dq, J=2.4, 14)	40.11	2.07 (1H, m), 1.19 (1H, m)	38.97	1.63 (1H, m), 1.08 (1H, m)
13	69.01		69.56		68.86	
14	78.57	3.62 (1H, d, J=7.0)	79.94	3.52 (1H, s)	80.28	4.65 (1H, d, J=1.2)
15	92.00	4.81 (1H, dd, J=0.5, 3.1)	94.87	4.82 (1H, dd, J=0.9, 3.4)	89.17	4.60 (1H, dd, J=1.2, 4.0)
16	32.72	2.66 (1H, dq, J=3.1, 7.4)	33.69	2.80 (1H, dq, J=3.4, 7.6)	33.17	2.82 (1H, dq, J=4.0, 7.3)
17	17.56	1.25 (3H, s)	17.93	1.20 (3H, s)	17.47	1.24 (3H, s)
18	13.60	1.01 (3H, d, J=7.4)	13.51	0.95 (3H, d, J=7.6)	13.05	0.91 (3H, d, J=7.3)
13-OH		2.07 (1H, s)				
14-OH		3.15 (1H, d, J=7.0)				
CO					169.42	
Me					20.72	2.09 (3H, s)

Chemical shifts in ppm from TMS as an internal standard.
* It is overlapped by H_2O.

Fig. 2. Partial structures of **1** from ^{1}H-^{1}H COSY and HMBC experiments.



stituents; a tertiary hydroxyl group (13-OH), a methyl group (17-CH_3) and a carbonyl group (C-6). These data established a substituted decaline residue. The presence of an exo-methylene group (δ_H 5.36 and 5.26) was also indicated by the HMBC spectrum.

The remaining chromophore part consisting of a carbonyl group, the exo-methylene group (above mentioned), a fully substituted olefine group (C-2 (δ_C 100.79) and C-3 (δ_C 164.64)) and an ester group (C-1, δ_C 164.64) could not be assigned a structure due to the overlapping signals of C-1 and C-3 at 164.64 ppm.

This problem was resolved by an acetyl derivative (**3**), which was obtained by the reaction of **1** with acetic anhydride in pyridine. The ^{13}C NMR spectrum of **3** in $CDCl_3$ containing small amount of C_6D_6 indicated 20 signals without overlapping. The partial structure of **3** was suggested by long range couplings from both the exo-methylene protons of 5-H_2 (δ_H 5.35 and 5.18) and a signal of 15-H (δ_H 4.60) to an oxygen bearing sp^2 carbon of C-3 (δ_C 164.13) as shown in Fig. 3.

The remaining framework consisting of a ester group (C-1 (δ_C 164.42)), a conjugated diene and a carbonyl carbon C-6 (δ_C 194.05) was constructed by the consideration of the chemical shift of C-2. The chemical shift of this sp^2 carbon, 100.79 ppm, could be only explained by the existence of an acyl tetronic acid alkyl ether structure as seen in gregatin B[5] (Fig. 4). This result was further supported by the IR spectrum (γ-lactone; 1780 cm^{-1})[6] and the UV spectrum (2-acyl-4-ylidene tetronic acid alkyl ether; 271 nm)[7]. From these data, the planar structure of tetrodecamycin was established.

Fig. 3. HMBC correlation of 14-O-acetyltetrodecamycin (**3**).

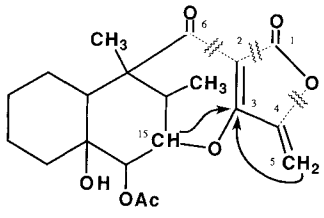
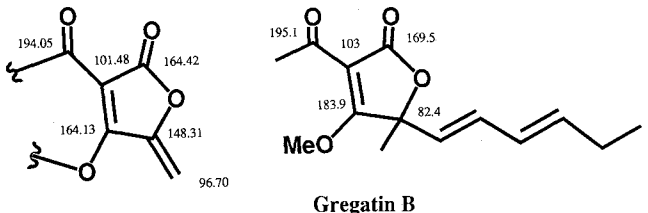


Fig. 4. ^{13}C NMR data of **3** and gregatin B.



3 Gregatin B

Structure of Dihydrotetrodecamycin

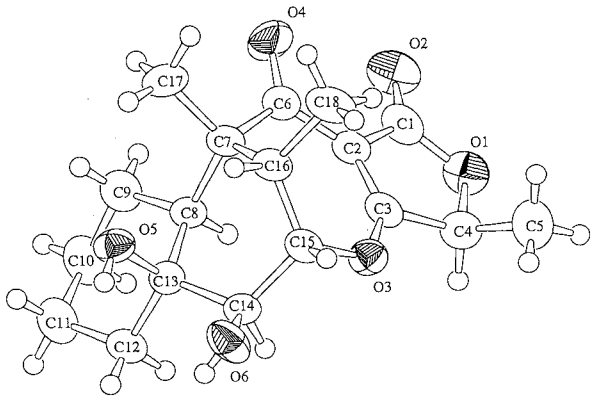
The molecular formula of dihydrotetrodecamycin (**2**) was determined to be $C_{18}H_{24}O_6$ on the basis of HRFAB-MS data and was supported by the ^1H and ^{13}C NMR spectral data (Table 1). The ^1H and ^{13}C NMR data of **2** were similar to those of **1** but the signals arising from the exo-methylene part were lacking. Instead of the exo-methylene group, one methyl and one methine group were observed in the ^1H and ^{13}C NMR spectrum of **2**. Additionally, a shift to shorter wave length (249 nm) of the UV absorption maximum of **2** compared with the value (271 nm) of **1** suggested partial reduction of the chromophore part. From these spectroscopic data, the structure of **2** was deduced to be 4,5-dihydrotetrodecamycin.

Relative Stereochemistry

Among tetrodecamycin (**1**), dihydrotetrodecamycin (**2**) and 14-O-acetyl-tetrodecamycin (**3**), **2** was recrystallized from a $CHCl_3$ solution to give colorless prismatic crystals. A crystal which had approximate dimensions of $0.22 \times 0.30 \times 0.35$ mm was chosen for the X-ray analysis and the stereochemistry was studied. As a result, the relative stereochemistry of **2** was determined. The ORTEP drawing of **2** was shown in Fig. 5.[8] Crystal data were summerized in Table 2.

The relative stereochemistry of **1** was proved by conversion of **1** into **2**. The compound **1** was hydrogenated in the presence of 10% palladium-carbon in EtOH to give two epimers, which were separated by HPLC (YMC-Pack, ODS-A, 150×4.6 mm) using 30% aqueous MeOH. The ^1H NMR data and specific rotation of one epimer were the same as those of **2**. Therefore,

Fig. 5. Molecular structure of dihydrotetrodecamycin.

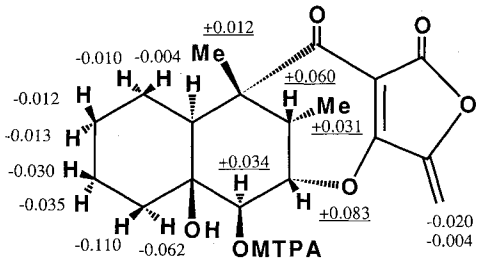


The thermal ellipsoids were given at 50% probability.

Table 2. Crystal data of **2**.

Emprical formula	$C_{18}H_{24}O_6 \cdot 1/10H_2O$
Formula weight	338.19
Crystal system	Hexagonal
Lattice parameters:	a = 16.788 (2) Å
	c = 11.742 (2) Å
	V = 2865.9 (4) Å
Space group	$P6_1$
Dcalc.	1.18 g/cm^3
μ(CuKα)	7.32 cm^{-1}

Fig. 6. $\Delta\delta$ values in MTPA esters **4** and **5**.



the relative stereochemistry of **2** was concluded to have the same stereochemistry as **1**.

Absolute Stereochemistry

Tetrodecamycin (**1**) or dihydrotetrodecamycin (**2**) has two hydroxyl groups in the molecule, one of which is a secondary hydroxyl group having the equatorial conformation. Therefore, we applied modified Mosher's method[9] to determine the absolute stereochemistry of **1**. Compound **1** was treated with (*S*)-/(*R*)-2-methoxy-2-phenyl-2-(trifluoromethyl)acetic acid (MTPA) chlorides in CH_2Cl_2 in the presence of triethylamine and 4-dimethylaminopyridine to give 14-(*R*)/(*S*)-MTPA ester (**4**/**5**), respectively. By analyzing ^{1}H-chemical shift differences between the (*R*) and (*S*)-MTPA esters ($\Delta\delta = \delta(S) - \delta(R)$) illustrated in Fig. 6, the configuration of C-14 was concluded to be *R*. Therefore, the absolute structure was established to be 7*R*, 8*S*, 13*S*, 14*R*, 15*S* and 16*S* for **1** and 4*R*, 7*R*, 8*S*, 13*S*, 14*R*, 15*S* and 16*S* for **2**.

Experimental

General

UV absorption spectra were measured with a Hitachi U-3210 spectrophotometer. IR absorption spectra were obtained with a Hitachi I-5020 FT-IR and Hitachi 260-30 spectrometer. FAB-MS and HRFAB-MS were obtained on a JEOL JMS-SX102 mass spectrometer. ^{1}H and ^{13}C NMR spectra were recorded on a JEOL JNM-GX400 and JNM-A-500 spectrometer. Chemical shift is given in ppm using TMS as an internal standard. Optical rotations were taken by a Perkin-Elmer 241 polarimeter using a micro-cell (light path 10 cm).

Preparation of 14-Acetyltetrodecamycin (3)

Acetic anhydride (2 ml) was poured into a solution of tetrodecamycin (**1**) (30.5 mg, 0.091 mmol) in pyridine (2 ml) at 0°C. The reaction mixture was stirred at room temperature for 18 hours. After evaporation under reduced pressure, the residue was purified by preparative TLC (Merck, Kiesel gel 60F_{254}) using toluene-acetone (2:1) mixture to give **3** (31.6 mg, 92%) as white powder.

The ^{1}H and ^{13}C NMR data were showed in Table 1.

FAB-MS *m/z* 377 $(M+H)^+$, 375 $(M-H)^-$; IR ν_{max} (KBr) cm^{-1} 3460, 2940, 1785, 1750, 1670, 1590, 1435, 1280, 1230; UV λ_{max} nm (log ε) 270 (14500), $\lambda_{max}^{0.01\ N\ MeOH}$ nm (log ε) 271 (14800) $\lambda_{max}^{0.01\ N\ NaOH\text{-}MeOH}$ nm (log ε) 252 (9430); $[\alpha]_D^{22}$ −23° (*c* 0.45, MeOH); Rf 0.68 (silica gel TLC Merck, Kiesel gel 60F_{254}, $CHCl_3$-MeOH 10:1).

Catalytic Reduction of Tetrodecamycin (1)

In hydrogen atmosphere, a solution of **1** (15 mg) in EtOH (1 ml) was hydrogenated at room temperature for 5 hours in the presence of catalytic amount of 10% palladium-carbon. After filtration, the crude mixture was purified by preparative TLC (Merck, Kiesel gel 60F_{254}) using $CHCl_3$-MeOH (10:1) mixture to give the two epimers (10 mg) as white powder. Furthermore, this epimeric mixture was separated by HPLC (YMC-Pack, ODS-A, 150 × 4.6 mm) using 30% aqueous MeOH to provide synthetic **2** (1.2 mg). The ^{1}H NMR data of synthetic **2** were identified with those of the natural compound. The specific rotation ($[\alpha]_D^{26}$ +79° (*c* 0.12, MeOH)) of synthetic **2** was as same as the value ($[\alpha]_D^{26}$ +78° (*c* 0.52, MeOH)) of the natural compound.

X-Ray Crystallography

The crystal of **2** was recrystallized from a $CHCl_3$ solution. A colorless prism crystal having approximate dimensions of 0.22 × 0.30 × 0.35 mm was mounted on a glass fiber. All measurements were made on a Rigaku AFC7R diffractometer with graphite monochromated Cu-Kα radiation and a rotating anode generator. Crystal data are shown in Table 2. Of the 3490 reflections which were collected, 1903 were unique. No decay correction was applied. An empirical absorption correction using the program DIFABS[10] was applied which resulted in transmission factors ranging from 0.38 to 1.77. The structure was solved by direct method (SHELXS86[11]) and expanded using Fourier techniques (DIRDIF92[12]). The non-hydrogen atoms were refined anisotropically. Hydrogen atoms were included but not refined. The final cycle of full-matrix least-squares refinement was based on 1650 observed reflections ($I > 2.00\sigma$ (I)) and 216 variable parameters and converged with unweighted agreement factors of R = 0.060 and Rw = 0.065. The maximum and minimum peaks on the final difference Fourier map corresponded to 0.23 and $-0.18e^-/A^3$,

respectively. All calculations were performed using the teXsan crystallographic software package of Molecular Structure Corporation.

Preparation of (*S*)-MTPA Ester (**4**)

Commercially available (*R*)-MTPA chloride (8.4 μl, 0.05 mmol) was added to a solution of **1** (10 mg, 0.03 mmol) in CH_2Cl_2 (1 ml) in the presence of triethylamine (8.4 ml, 0.06 mmol) and catalytic amount of 4-dimethylaminopyridine at room temperature. The reaction mixture was stirred for 18 hours, and diluted with CH_2Cl_2 and then was washed with water. The organic layer was dried over with Na_2SO_4 and evaporated under reduced pressure. The residue was purified by preparative TLC (Merck, Kiesel gel $60F_{254}$) using toluene-AcOEt (1:1) mixture to give **4** (6.5 mg, 40%).

FAB-MS *m/z* 551 $(M+H)^+$; IR ν_{max} (KBr) cm^{-1} 2950, 1790, 1760, 1680, 1620, 1595, 1180; ^1H NMR ($CDCl_3$) δ 7.55 (2H, m), 7.48 (3H, m), 5.449 (1H, d, J=3.0 Hz), 5.317 (1H, d, J=3.0 Hz), 4.843 (1H, d, J=1.2 Hz), 4.717 (1H, dd, J=1.2, 4.0 Hz), 3.596 (3H, d, J=0.9 Hz), 2.918 (1H, dq, J=4.0, 7.3 Hz), 1.760 (1H, m), 1.616 (1H, dq, J=3.4, 12.8 Hz), 1.535 (1H, m), 1.490 (1H, m), 1.475 (1H, m), 1.431 (1H, ddd, J=1.8, 4.0, 12.6 Hz), 1.380 (1H, m), 1.237 (3H, s), 1.171 (1H, dt, J=4.6, 13.4 Hz), 1.082 (1H, tq, J=3.7, 13.1 Hz), 1.018 (3H, d, J=7.3 Hz); ^{13}C NMR ($CDCl_3$) δ 193.9, 165.7, 164.3, 163.9, 148.1, 131.5, 130.2, 128.8, 127.1, 123.0 (q), 101.7, 97.2, 86.3, 84.3 (q), 82.4, 69.1, 55.6, 52.8, 42.9, 38.8, 33.8, 25.7, 23.1, 20.3, 17.4, 13.1.

Preparation of (*R*)-MTPA Ester (**5**)

The (*R*)-MTPA ester (**5**) (7.1 mg, 56%) was obtained from **1** (10 mg, 0.03 mmol) and (*S*)-MTPA chloride (8.4 ml, 0.05 mmol) by a similar procedure for **4**.

FAB-MS *m/z* 551 $(M+H)^+$; IR ν_{max} (KBr) cm^{-1} 2950, 1790, 1760 (sh), 1680, 1620, 1590, 1180; ^1H NMR ($CDCl_3$) δ 7.53 (2H, m), 7.48 (3H, m), 5.469 (1H, d, J=2.7 Hz), 5.321 (1H, d, J=2.7 Hz), 4.809 (1H, d, J=1.2 Hz), 4.634 (1H, dd, J=1.2, 4.0 Hz), 3.536 (3H, d, J=0.9 Hz), 2.858 (1H, dq, J=4.0, 7.6 Hz), 1.772 (1H, m), 1.620 (1H, m), 1.645 (1H, m), 1.50 (1H, m), 1.51 (1H, m), 1.430 (1H, ddd, J=2.4, 4.0, 12.5 Hz), 1.41 (1H, m), 1.225 (3H, s), 1.233 (1H, dt, J=4.6, 13.4 Hz), 1.095 (1H, tq, J=3.7, 12.8 Hz), 0.987 (3H, d, J=7.6 Hz); ^{13}C NMR ($CDCl_3$) δ 193.9, 165.9, 164.3, 163.9, 148.1, 131.1, 130.3, 128.9, 127.4, 123.0 (q), 101.6, 97.1, 88.32, 85.0 (q), 82.7, 68.9, 55.4, 52.8, 42.9, 39.1, 33.1, 25.7, 23.1, 20.4, 17.4, 13.1.

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