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Absolute Stereochemistry Determination of 16-Methyloxazolomycin Produced by a *Streptomyces* sp.

Geonseek Ryu and Si-Kwan Kim*

Korea Ginseng & Tobacco Research Institute,
302 Shinseong-dong Yuseong-ku, Taejon 305-345, Korea

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16-Methyloxazolomycin (**1**) is an antibacterial, cytotoxic and antialgal triene containing a spiro β-lactone-γ-lactam system and an oxazole ring, produced by a *Streptomyces* sp. isolated from a soil sample collected in Taejon, Korea. In 1985 Uemura's group and Seto's group reported oxazolomycins[1,2] and curromycins,[3,4] produced by a *Streptomyces* sp. and *Streptomyces hygroscopicus*, respectively. More recently, we reported the production, isolation and structural elucidation of **1**.[5] Although extensive NMR studies enabled the assignment of the relative stereochemistry of some of the stereogenic centers, the configurations at carbons 3′, 4, 6 and 7 have hitherto remained unknown. We herein describe the determination of the absolute stereochemistry of **1** based on interpretation of NMR data and chemical transformation.

In the previous paper,[5] the stereogenic centers of the spiro β-lactone-γ-lactam system in **1** were determined as $2R$,* $3S$,* $15S$* and $16S$* by NOESY experiments.[6] First of all, to determine the relative configurations at carbons 4, 6 and 7, **1** was treated with 2 N AcOH in MeOH at room temperature for 2 days to yield methyl ester **2** (Table 1 and Fig. 1). Acidic treatment of **2** [camphor sulfonic acid (CSA) cat., $CHCl_3$/MeOH (9 : 1)] at room temperature for 8 hours gave the readily separated tetrahydropyran-γ-lactam spiro compounds **3a** and **3b** (36%) in a 1.6 to 1 ratio and a ratio of 4 : 1 (78%) was obtained when the same acidic treatment of **2** was prolonged for 72 hours. However, only two products **3a** and **3b** of the *anti*-diol type were afforded, while the alternative compounds **3a′** and **3b′** of the *syn*-diol type, which would be expected to have higher energy due to both electronic and steric effects, were not observed. The proposed structures were fully confirmed by ^{1}H NMR analysis of the **3a** (major) and **3b** (minor) components.

The coupling constants observed led to chair tetrahydropyran rings with a splitting pattern only consistent with an axially disposed H6 ($J_{6\sim7}>10$ Hz) in both isomers (Table 1). These configurational and conformational features were validated by the intracyclic NOE contacts H4-H6 and H5ax-H7 in both **3a** and **3b** (Fig. 2). Further, these rigid bicyclic systems exhibited long-range space contacts (H5ax-CH_3-2 and H7ax-H2 in **3a** and H4ax-H2 in **3b**), that allowed the assignment of the relative stereochemistry at the chiral carbons in tetrahydropyran rings of **3a** and **3b**. The $4S$,* $6R$,* $7R$*

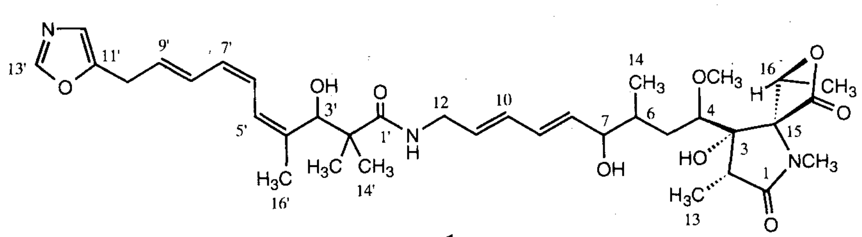

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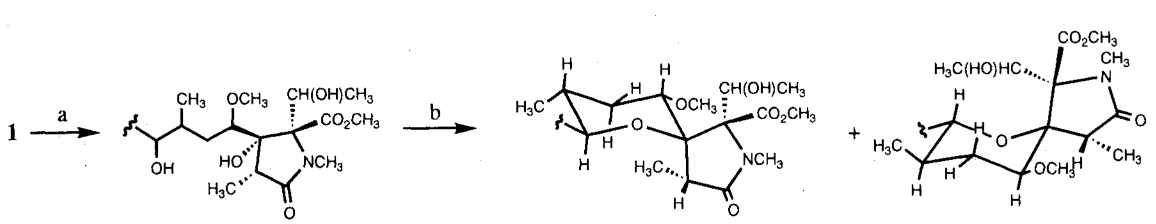
Fig. 1. Formation of spiro compounds **3a** and **3b** from 16-methyloxazolomycin (**1**).

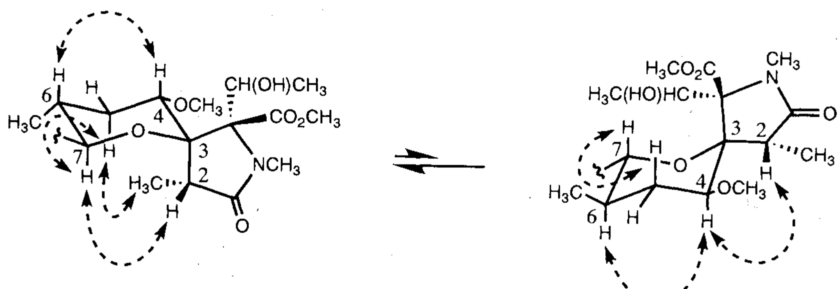


2 **3a** **3b**

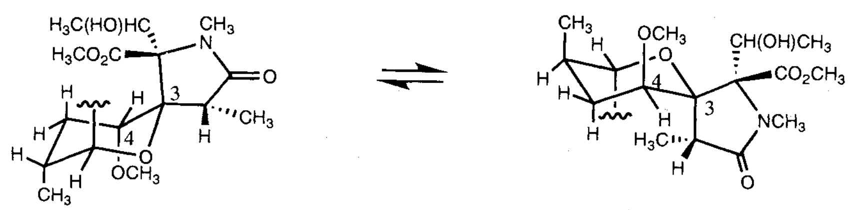
a) 2 N AcOH/MeOH, 48h, rt
b) CSA cat., $CHCl_3$/MeOH (9 : 1)

Fig. 2. Possible chair tetrahydropyran γ-lactam spiro compounds of the *anti*-3,4-diol type (**3a**, **3b**) or the *syn*-3,4-diol type (**3a′**, **3b′**).

The arrows indicate the NOEs observed for **3a** and **3b**.

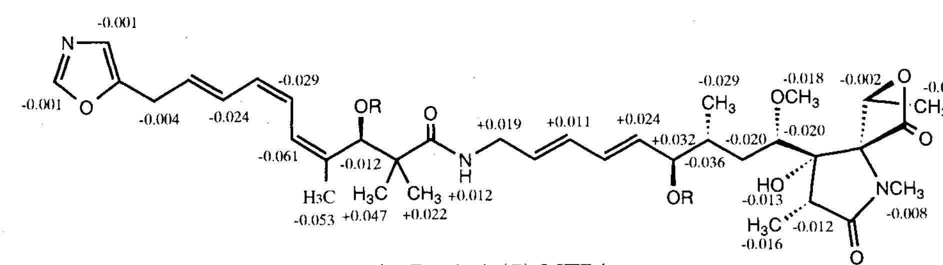


3a **3b**

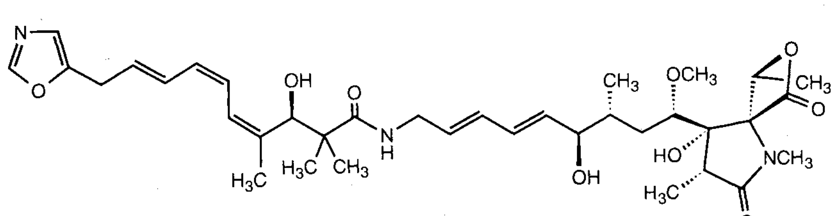


3a′ **3b′**

Fig. 3. Application of the modified Mosher method: $\Delta\delta = \delta_{(-)\text{-}(S)\text{-MTPA}} - \delta_{(+)\text{-}(R)\text{-MTPA}}$



4a R=(+)-(*R*)-MTPA
4b R=(−)-(*S*)-MTPA



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configuration of the natural antibiotic was thus deduced.

To determine the absolute configurations at carbons 3′ and 7 by the modified Mosher method,[7,8] the bis-(*R*)- and bis-(*S*)-MTPA esters (**4a, 4b**) were made. In the bis-MTPA esters, it can be seen that the positive and negative $\Delta\delta$ (δ_S–δ_R) values are well arranged on both sides of each of two carbinyl carbons (C-3′ and C-7) as shown in Fig. 3. The general tendency of $\Delta\delta$ values, which are all negative on the left side of the MTPA plane of the 3′-MTPA ester and negative, too, as expected, on the right side of the MTPA plane of the 7-MTPA ester indicated 3′*R*,7*R* configurations. Because the relative

Table 1. ^{1}H NMR Data of Compounds **1**, **2**, **3a** and **3b** in DMSO-d_6 at 400 MHz.

Position	1	2	3a	3b
2	2.34 (H, q, 6.6)	2.33 (H, q, 6.4)	2.36 (H, q, 6.2)	2.34 (H, q, 6.3)
4	3.38 (H, t, 6.9)	3.40 (H, t, 6.2)	3.52 (H, dd, 10.8, 4.6)	3.48 (H, dd, 10.6, 4.7)
5	1.95 (H, m)	1.97 (H, m)	1.98 (H, m)	2.17 (H, m)
	1.15 (H, m)	1.16 (H, m)	1.20 (H, br t, 10.3))	1.24 (H, br t, 10.3)
6	1.58 (H, m)	1.58 (H, m)	1.52 (H, m)	1.64 (H, m)
7	3.81 (H, m)	3.82 (H, m)	4.26 (H, d, 10.2)	4.08 (H, d, 10.3)
8	5.57 (H, dd, 16.8, 11.2)	5.43 (H, dd, 16.3, 10.8)	5.52 (H)[c]	5.42 (H, dd, 16.2, 10.2)
9	6.14 (H, dd, 11.2, 16.8)	5.98 (H)[a]	6.39 (H)[d]	6.32 (H)[e]
10	6.09 (H, dd, 14.3, 10.8)	6.02 (H, dd, 14.4, 10.2)	6.12 (H, dd, 14.6, 9.9)	6.08 (H, dd, 10.1, 14.6)
11	5.59 (H, m)	5.62 (H)[b]	5.55 (H)[c]	5.60 (H, m)
12	3.71 (2H, m)	3.73 (2H, m)	3.80 (2H, m)	3.84 (2H, m)
13	1.03 (3H, d, 6.6)	1.03 (3H, d, 6.8)	1.24 (3H, d, 6.2)	1.08 (3H, d, 6.3)
14	0.86 (3H, d, 6.2)	0.86 (3H, d, 6.2)	0.92 (3H, d, 6.2)	0.98 (3H, d, 6.3)
16	4.82 (H, q, 6.3)	3.88 (H, q, 6.5)	3.80 (3H, q, 6.4)	3.76 (3H, q, 6.6)
3′	4.59 (H, s)	4.56 (H, s)	4.58 (H, s)	4.56 (H, s)
5′	6.35 (H, d, 11.3)	6.32 (H, d, 11.4)	6.36 (H)[d]	6.82 (H, d, 11.2)
6′	6.27 (H, dd, 10.9, 11.3)	6.18 (H, dd, 11.4, 10.3)	6.24 (H, dd, 10.1, 11.3)	6.34 (H)[e]
7′	5.90 (H, dd, 11.5, 10.9)	5.95 (H)[a]	5.92 (H, dd, 11.5, 10.1)	5.92 (H, dd, 10.3, 11.6)
8′	6.70 (H, dd, 11.5, 14.5)	6.58 (H, dt, 14.4, 14.8)	6.81 (H, dd, 11.5, 14.3)	6.63 (H, dd, 11.6, 15.1)
9′	5.75 (H, dt, 14.5, 4.5)	5.60 (H)[b]	5.79 (H. dt, 14.3, 4.4)	5.80 (H, dt, 15.1, 4.6)
10′	3.52 (2H, br d, 7.0)	3.55 (2H, br d, 7.1)	3.56 (2H, d, 7.1)	3.58 (2H, d, 7.2)
12′	6.84 (H, s)	6.84 (H, s)	6.88 (H, s)	6.91 (H, s)
13′	8.14 (H, s)	8.14 (H, s)	8.20 (H, s)	8.18 (H, s)
14′	0.95 (3H, s)	0.95 (3H, s)	0.98 (3H, s)	0.99 (3H, s)
15′	1.10 (3H, s)	1.10 (3H, s)	1.08 (3H, s)	1.14 (3H, s)
16′	1.70 (3H, s)	1.70 (3H, s)	1.81 (3H, s)	1.86 (3H, s)
16-CH_3	1.69 (3H, d, 6.3)	1.69 (3H, d, 6.5)	1.76 (3H, d, 6.4)	1.74 (3H, d, 6.6)
NCH_3	2.77 (3H, s)	2.77 (3H, s)	2.86 (3H, s)	2.90 (3H, s)
OCH_3	3.15 (3H, s)	3.15 (3H, s)	3.40 (3H, s)	3.28 (3H, s)
3-OH	5.31 (H, s)	5.31(H, s)		
7-OH	4.82 (H, s)	4.82 (H, s)		
3′-OH	5.49 (H, s)	5.31 (H, s)		
16-OH				
CO_2CH_3		3.60 (3H, s)	3.48 (3H, s)	3.51 (3H, s)
NH	7.63 (H, br t, 5.9)	7.63 (H, br t, 6.0)	7.54 (H, br t, 5.6)	7.62 (H, br t, 5.5)

[a~e] Multiplicities were not assigned due to extensive overlap.

stereochemistry at other asymmetric centers has been assigned by NMR techniques described above, the absolute configurations of **1** are indicated as 2*R*, 3*S*, 4*S*, 6*R*, 15*S*, 16*S* rather than the opposite configurations. The complete stereostructure of 16-methyloxazolomycin as **5** was hence defined. 16-Methyloxazolomycin and oxazolomycin have identical configurations at all common, backbone stereocenters except for carbon 16.

Experimental

General

UV spectra were recorded on a Hitachi 330 spectrophotometer. Infrared spectra were measured with a JASCO FT/IR-5300 infrared spectrometer. ^{1}H and ^{13}C spectra were recorded on a Brucker ARX-400 NMR spectrometer at 400 and 100 MHz, respectively. FAB-MS and HRFAB-MS were measured with a JEOL JMX-SX 102 mass spectrometer and high resolution FAB-MS spectra were determined using a dual target inlet probe. Optical rotations were performed with a JASCO DIP-371 digital polarimeter. Thin layer chromatography was carried out on Merck silica gel 60 F_{254} plates, and MPLC and HPLC were carried out on a Waters 510 apparatus.

Isolation

The fermentations were carried out as described in the previous paper.[5] The whole broth (100 liters) was subjected to filtration. The mycelial cake was extracted twice with methanol/acetone (1 : 1, 10 liters), which was then removed from the extract by evaporation. The combined filtrates were passed through a Diaion HP-20 column, and washed with H_2O followed by MeOH. The MeOH eluate was partitioned between CH_2Cl_2 and 60% MeOH. The concentrated CH_2Cl_2 layer was fractionated by ODS flash chromatography using stepwise elution with 40, 60, 80, 100% MeOH. The active 80% MeOH fraction was gel-filtered on Sephadex LH-20 in *n*-hexane/CH_2Cl_2/MeOH (4 : 2 : 1) to give an active fraction. This fraction was further purified on a reversed-phase MPLC (ODS, 40~60 μm, 65% MeOH) column and repeated ODS HPLC with 63% MeOH containing 0.5 mM $NaClO_4$ to afford 16-methyloxazolomycin (**1**, 72 mg).

16-Methyloxazolomycin Methyl Ester (**2**)

60 mg of 16-Methyloxazolomycin (**1**, 0.089 mmol) was dissolved in 2 ml of methanol, and 3 drops of 2 N AcOH was added to the solution with stirring. Stirring was continued at room temperature for 48 hours. The solution was filtered, and the filtrate was concentrated with a rotary evaporator. The residues were purified by prepative TLC [$CHCl_3$/MeOH (95 : 5)] to give **2** (51 mg, 82%).

2: $[\alpha]_D^{23}$ +25 (*c* 1.02, MeOH), FAB-MS: *m/z* 724 $(M+Na)^+$, 702 $(M+H)^+$, HRFAB-MS *m/z* 702.3968 cacld. for $C_{37}H_{56}N_3O_{10}$, found: 702.3980, ^1H NMR data are shown in Table 1.

Spiro Compounds (**3a**, **3b**) from 16-Methyloxazolomycin Methyl Ester

30 mg of 16-Methyloxazolomycin methyl ester (**2**, 0.043 mmol) in chloroform - methanol (9 : 1, 2 ml) at room temperature was treated with camphor sulfonic acid (2 mg, 0.06 equiv.) for 72 hours. At this time, TLC indicated the formation of two faster migrating products and the reaction was quenched by addition of a few drops of pyridine and evaporated *in vacuo*. The residues were purified by prepative TLC with chloroform/methanol (95 : 5) to give first compound **3a** (18 mg, 61%) as pale yellow amorphous powder. The second isomer **3b** was then eluted (5.4 mg, 17%).

3a: $[\alpha]_D^{23}$ +102 (*c* 1.35, MeOH), FAB-MS: *m/z* 722 $(M+Na)^+$, 700 $(M+H)^+$, HRFAB-MS *m/z* 700.3811 cacld. for $C_{37}H_{54}N_3O_{10}$, found: 700.3818, ^1H NMR data are shown in Table 1.

3b: $[\alpha]_D^{23}$ −70 (*c* 0.94, MeOH), FAB-MS: *m/z* 722 $(M+Na)^+$, 700 $(M+H)^+$, HRFAB-MS *m/z* 700.3811 cacld. for $C_{37}H_{54}N_3O_{10}$, found: 700.3814 ^1H NMR data are shown in Table 1.

(+)-(*R*)- and (−)-(*S*)-α-Methoxy-α-(trifluoromethyl) phenylacetyl (MTPA) Ester (**4a**, **4b**) from 16-Methyloxazolomycin

To solution of 3.3 mg of **1** in 100 μl of dry pyridine was added 20 μl of (+)-(*R*)-MTPA chloride and 6.0 mg of 4-dimethylaminopyridine (DMAP). The mixture was allowed to stand under N_2 at room temperature for 6 hours. After the consumption of starting material was confirmed by TLC, 50 μl of H_2O, 100 μl of CH_2Cl_2, and 200 μl of MeOH were added. The solvents were removed under vacuum, and the residue was separated on prepative TLC [($CHCl_3$/MeOH (95 : 5)] to give 2.4 mg of (*R*)-MTPA ester **4a**. According to the same experimental procedure, 20 μl of (−)-(*S*)-MTPA chloride and 3.6 mg of **1** were reacted to obtain 2.1 mg of (−)-(*S*)-MTPA ester **4b**. The concentrations of the esters were adjusted to same concentration in DMSO-d_6, and ^1H NMR spectra were measured at 400 MHz.

4a: FAB-MS: *m/z* 1124 $(M+Na)^+$, 1102 $(M+H)^+$; ^1H NMR (DMSO-d_6): 8.331 (1H, s, H13′), 7.38~7.45 and 7.48~7.54 (10H, m, 2×ph-MTPA's), 7.242 (1H, t, 6.0, NH), 7.082 (1H, s, H12′), 6.893 (1H, dd, 10.5, 14.4, H8′), 6.713 (1H, d, 10.9, H5′), 6.50 (1H, dd, 11.0, 16.6, H9), 6.378 (1H, dd, 10.9, 11.6, H6′), 6.182 (1H, dd, 14.1, 11.0, H10), 6.012 (1H, dd, 16.6, 10.9, H8), 5.74~5.82 (3H overlapped, H9′, H7′ and H11), 5.186 (1H, s, 3-OH), 5.024 (1H, q, 6.2, H16), 4.883 (1H, s, H3′), 4.72 (3H, s, CH_3O-MTPA's), 4.24 (3H, s, CH_3O-MTPA's), 4.026 (1H, dd, 10.9, 4.3, H7), 3.695 (2H, m, H12), 3.476 (1H, t, 6.9, H4), 3.324 (3H, s, OCH_3), 3.193 (2H, d, 7.0, H10′), 2.842 (3H, s, NCH_3), 2.466 (1H, q, 6.4, H2), 2.024 (1H, m, H5), 1.832 (3H, d, 6.2, 16-CH_3), 1.686 (3H, s, H16′), 1.560 (1H, m, H6), 1.223 (1H, m, H5), 1.082 (3H, s, H15′), 0.980 (3H, d, 6.4, H13), 0.866 (3H, s, H14′), 0.774 (3H, d, 6.3, H14).

4b: FAB-MS: *m/z* 1124 $(M+Na)^+$, 1102 $(M+H)^+$; ^1H NMR (DMSO-d_6): 8.330 (1H, s, H13′), 7.38~7.50 and 7.54~7.62 (10H, m, 2×ph-MTPA's), 7.254 (1H, t, 6.0, NH), 7.081 (1H, s, H12′), 6.869 (1H, dd, 10.5, 14.4, H8′), 6.652 (1H, d, 10.9, H5′), 6.50 (1H, dd, 11.0, 16.6, H9), 6.349 (1H, dd, 10.9, 11.6, H6′), 6.193 (1H, dd, 14.1, 11.0, H10), 6.036 (1H, dd, 16.6, 10.9, H8), 5.74~5.82 (3H overlapped, H9′, H7′ and H11), 5.173 (1H, s, 3-OH), 5.022 (1H, q, 6.2, H16), 4.871 (1H, s, H3′), 4.73 (3H, s, CH_3O-MTPA's), 4.24 (3H, s, CH_3O-MTPA's), 4.058 (1H, dd, 10.9, 4.3, H7), 3.714 (2H, m, H12), 3.456 (1H, t, 6.9, H4), 3.306 (3H, s, OCH_3), 3.189 (2H, d, 7.0, H10′), 2.834 (3H, s, NCH_3), 2.454 (1H, q, 6.4, H2), 2.004 (1H, m, H5), 1.826 (3H, d, 6.2, 16-CH3), 1.633 (3H, s, H16′), 1.524 (1H, m, H6), 1.203 (1H, m, H5), 1.129 (3H, s, H15′), 0.964 (3H, d, 6.4, H13), 0.888 (3H, s, H14′), 0.745 (3H, d, 6.3, H14).

Acknowledgments

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