

## Determination of the Absolute Configuration of Synerazol

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Synerazol (**1**) was originally isolated as an antifungal antibiotic from *Aspergillus fumigatus*<sup>1</sup>. **1** possesses an epoxy functionality instead of the 1,2-dihydroxyl group in pseurotin A (**2**), the first identified member of this class of secondary metabolites. While the absolute configuration of **2** was determined by X-ray diffraction<sup>2</sup>, the stereochemistry of **1** remains to be solved. We recently found that fluorinated analogs of synerazol, produced by the precursor-directed biosynthesis using fluorophenylalanines, show potent antiangiogenic activity<sup>3</sup>. Since pseurotin analogs do not show such activities, the epoxy moiety is considered important.

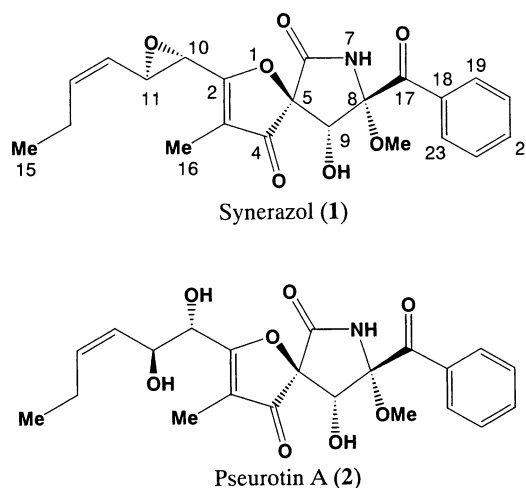
*A. fumigatus* TP-F0182 produces both **1** and **2** in a liquid fermentation<sup>3</sup>. **1** and **2** were confirmed to be synerazol and pseurotin A, respectively, by comparing the UV, optical rotation, FAB-MS and <sup>1</sup>H and <sup>13</sup>C NMR data<sup>1,2,4</sup>. **1** is liable to react with a water molecule in acidic and neutral aqueous medium to yield a diol compound whose chromatographic behavior is identical with that of **2**. It was thus assumed that **1** and **2** are derived from the same biosynthetic precursor and therefore have a common stereochemical character. We herein report the determination of the absolute configuration of **1** in two parts: (1) determination of the stereochemistry in the right half of the molecule by spectroscopic comparison with **2**; (2) determination of the absolute configuration of the epoxy moiety by using the modified Mosher's MTPA method<sup>5</sup>.

Based on the assumption, the epoxy ring of **1** was cleaved in aqueous tetrahydrofuran in the presence of *p*-toluenesulfonic acid (*p*-TsOH). <sup>1</sup>H and <sup>13</sup>C NMR spectra of the hydrolysate **3** were in good accordance with those of **2** and, in the NMR spectrum of the equimolar mixture of **2** and **3**, splittings of proton and carbon signals due to the heterologous mixture were not observed. Therefore the

relative configuration of **3** is identical with **2** and thus the configuration of the spiro ring system of synerazol is identical with that of **2**. Next, the optical rotations of **2** and **3** were compared. The  $[\alpha]_D$  value of **3** was +85.3 (*c* 0.28, CHCl<sub>3</sub>) whereas that of **2** was +87.2 (*c* 0.47, CHCl<sub>3</sub>). Hence the absolute configuration of **1** was established except for its epoxide moiety.

To determine the absolute configuration at C-10 and C-11, the epoxy ring was opened and the modified Mosher's method was applied to the generated secondary alcohol. Prior to the epoxide cleavage, 9-OH was protected with acetyl group by the treatment of **1** with acetic anhydride in pyridine, which was associated with the acetylation of 7-NH group to give **4**. To circumvent the generation of two secondary hydroxyl groups, methanol was used for the epoxy opening. **4** was reacted with methanol in the presence of *p*-TsOH to give **5**. The cleavage of the epoxy ring exclusively occurred at C-11, which was confirmed by the detection of <sup>1</sup>H-<sup>1</sup>H couplings between 10-OH (d,  $J_{10-OH,10}$ =6.3 Hz), 10-H (t,  $J_{10,10-OH} \sim J_{10,11}$ =6.1 Hz) and 11-H (ddd,  $J_{10,11}$ =6.1 Hz). To determine the absolute configuration at C-10, (*R*)- and (*S*)-MTPA esters (**6a** and **6b**) were prepared from **5** and their <sup>1</sup>H NMR chemical shifts were compared. The negative  $\Delta\delta$  values were arranged on the left side of the MTPA plane and the positive values except for 9-OAc and 19-H protons could be seen on the right side of the MTPA plane, indicating 10-*S* configuration (Fig. 3). The absolute configuration of

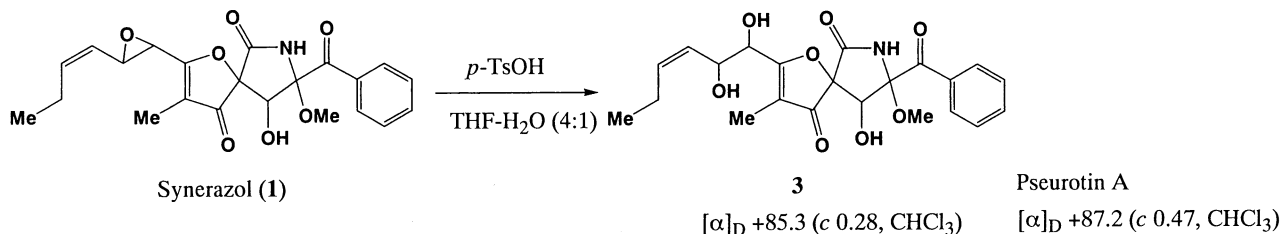
Fig. 1. Structures of synerazol and pseurotin A.



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Fig. 2. Acid-catalyzed epoxide opening and determination of the absolute configuration of **1**.

## (i) Determination of the absolute configuration of the spiro ring system



## (ii) Determination of the absolute configuration of the epoxide

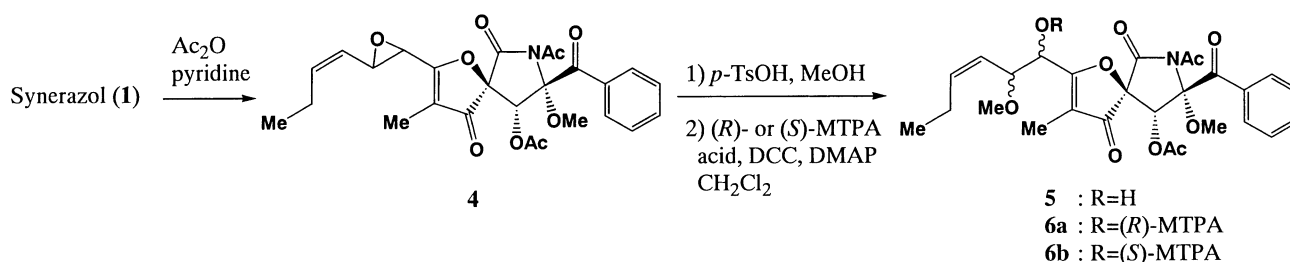
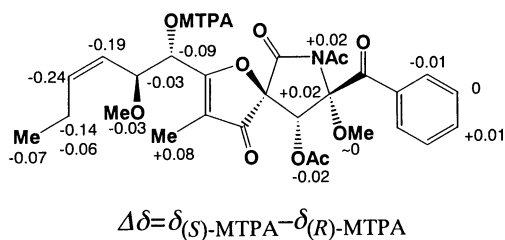


Fig. 3. Application of the modified Mosher's method.



synerazol was hence defined as **1** in Fig. 1.

## Experimental

### General

All NMR experiments were performed on a JEOL JNM-LA400 NMR spectrometer in CDCl<sub>3</sub> with TMS as an internal standard. The MS spectra were measured on a JEOL JMS AX500 spectrometer. Optical rotations were measured on a Horiba SEPA-300 polarimeter.

### Hydrolysis of Synerazol

Synerazol (8.0 mg, 19 μmol,  $[\alpha]_D^{18} +21.6$  (c 0.46, CHCl<sub>3</sub>)) was dissolved in a solution of *p*-toluenesulfonic acid monohydrate (5 mg, 26 μmol) in THF-H<sub>2</sub>O (4:1, 5 ml), and the mixture was allowed to stand for 24 hours at 30°C. Then, the reaction mixture was diluted with water, extracted with EtOAc, washed with saturated NaHCO<sub>3</sub> solution, dried over MgSO<sub>4</sub>, filtered and concentrated *in vacuo*. The residue was purified by preparative TLC [*n*-hexane/EtOAc (1:2)] to give **3** (4.4 mg, 54% yield).

**3**:  $[\alpha]_D^{24} +85.3$  (c 0.28, CHCl<sub>3</sub>).

Pseurotin A (**2**):  $[\alpha]_D^{24} +87.2$  (c 0.47, CHCl<sub>3</sub>),  $[\alpha]_D^{26} -3.0$  (c 0.5, MeOH).

### Acetylation of Synerazol

To a solution of synerazol (100 mg, 0.24 mmol) in dry pyridine (2 ml) was added acetic anhydride (1 ml) at room temperature. After 18 hours, the reaction mixture was poured onto ice-water and extracted with EtOAc. The organic layer was successively washed with saturated CuSO<sub>4</sub> solution, saturated NaHCO<sub>3</sub> solution and brine, dried over MgSO<sub>4</sub>, filtered and concentrated *in vacuo*. The residue was chromatographed on a column of silica gel [*n*-hexane/EtOAc (10:1~4:1)] to give 7,9-*N,O*-diacetyl-synerazol **4** (72 mg, 60%).

**4:**  $[\alpha]_D^{26} +1.8$  (*c* 1.0, CHCl<sub>3</sub>); <sup>1</sup>H NMR:  $\delta$  1.09 (3H, t, *J*=7.6 Hz, 15-H), 1.85 (3H, s, 16-H), 2.09 (3H, s, 9-OCOCH<sub>3</sub>), 2.30 (1H, ddqd, *J*=1.4, 7.6, 7.6 and 14.6 Hz, 14-H), 2.36 (1H, ddqd, *J*=1.2, 7.6, 7.6 and 14.6 Hz, 14-H), 2.56 (3H, s, 7-NCOCH<sub>3</sub>), 3.63 (3H, s, 8-OCH<sub>3</sub>), 3.79 (1H, d, *J*=2.0 Hz, 10-H), 4.17 (1H, dd, *J*=1.7 and 8.6 Hz, 11-H), 5.11 (1H, tdd, *J*=1.5, 9.0 and 10.5 Hz, 12-H), 5.59 (1H, s, 9-H), 5.90 (1H, td, *J*=7.6 and 11.0 Hz, 13-H), 7.40 (2H, t, *J*=8.1 Hz, 20 and 22-H), 7.51 (1H, tt, *J*=1.2 and 7.3 Hz, 21-H), 8.01 (2H, dd, *J*=1.2 and 6.8 Hz, 19 and 23-H); <sup>13</sup>C NMR:  $\delta$  5.19 (16-C), 14.05 (15-C), 20.18 (9-OCOCH<sub>3</sub>), 21.32 (14-C), 25.38 (7-NCOCH<sub>3</sub>), 52.53 (10-C), 53.82 (8-OCH<sub>3</sub>), 55.05 (11-C), 75.01 (9-C), 88.61 (5-C), 93.75 (8-C), 113.91 (3-C), 123.48 (12-C), 127.74 (20 and 22-C), 130.44 (19 and 23-C), 132.51 (21-C), 135.78 (18-C), 141.54 (13-C), 165.10 (6-C), 169.05 (9-OCOCH<sub>3</sub>), 170.32 (7-NCOCH<sub>3</sub>), 181.36 (2-C), 194.94 (4-C), 197.53 (17-C); EI-MS *m/z* 497 M<sup>+</sup>; HREI-MS *m/z* 497.1718 M<sup>+</sup> (calcd *m/z* 497.1686 for C<sub>26</sub>H<sub>27</sub>O<sub>9</sub>N).

#### Acid-catalyzed Epoxide Opening with Methanol of 7,9-*N,O*-Diacetylsynerazol (**4**)

7,9-*N,O*-Diacetylsynerazol **4** (70 mg, 0.14 mmol) was dissolved in a solution of *p*-toluenesulfonic acid monohydrate (6 mg, 0.003 mmol) in MeOH (6 ml) and the solution was kept at 4°C for 18 hours. The reaction mixture was diluted with water, extracted with EtOAc, washed with saturated NaHCO<sub>3</sub> solution and brine, dried over MgSO<sub>4</sub>, filtered and concentrated *in vacuo*. The residue was chromatographed on a column of silica gel (*n*-hexane/EtOAc 10:1~3:1) to give 7,9-*N,O*-diacetyl-11-*O*-methylpseurotin A **5** (34 mg, 45%).

**5:**  $[\alpha]_D^{26} -52.9$  (*c* 0.49, CHCl<sub>3</sub>); <sup>1</sup>H NMR:  $\delta$  1.01 (3H, t, *J*=7.6 Hz, 15-H), 1.75 (3H, s, 16-H), 2.08 (3H, s, 9-OCOCH<sub>3</sub>), 2.08 (1H, ddqd, *J*=1.5, 7.3, 7.6 and 14.6 Hz, 14-H), 2.22 (1H, ddqd, *J*=1.3, 7.3, 7.6 and 14.6 Hz, 14-H), 2.57 (3H, s, 7-NCOCH<sub>3</sub>), 2.78 (1H, d, *J*=6.3 Hz, 10-OH), 3.35 (3H, s, 11-OCH<sub>3</sub>), 3.63 (3H, s, 8-OCH<sub>3</sub>), 4.36 (1H, ddd, *J*=0.8, 6.1 and 9.5 Hz, 11-H), 4.69 (1H, t, *J*=6.1 Hz, 10-H), 5.33 (1H, tdd, *J*=1.7, 9.5 and 11.0 Hz, 12-H), 5.62 (1H, s, 9-H), 5.85 (1H, td, *J*=7.7 and 11.0 Hz, 13-H), 7.40 (2H, t, *J*=7.6 Hz, 20 and 22-H), 7.52 (1H, tt, *J*=1.2 and 7.3 Hz, 21-H), 8.01 (2H, dd, *J*=1.5 and 7.0 Hz, 19 and 23-H); <sup>13</sup>C NMR:  $\delta$  5.69 (16-C), 14.23 (15-C), 20.18 (9-OCOCH<sub>3</sub>), 21.29 (14-C), 25.40 (7-NCOCH<sub>3</sub>), 53.84 (8-OCH<sub>3</sub>), 56.57 (11-OCH<sub>3</sub>), 70.04 (10-C), 75.13 (9-C), 77.11 (11-C), 88.32 (5-C), 93.63 (8-C), 112.88 (3-C), 124.13 (12-C), 127.75 (20 and 22-C), 130.42 (19 and 23-C), 132.49 (21-C), 135.91 (18-C), 139.95 (13-C), 165.05 (6-C), 169.19 (9-OCOCH<sub>3</sub>), 170.49 (7-NCOCH<sub>3</sub>), 185.27 (2-C), 195.68

(4-C), 197.83 (17-C); FAB-MS *m/z* 530 (M+H)<sup>+</sup>, 552 (M+Na)<sup>+</sup>; HRFAB-MS *m/z* 530.2019 (M+H)<sup>+</sup> (calcd *m/z* 530.2026 for C<sub>27</sub>H<sub>32</sub>O<sub>10</sub>N).

#### (*R*)- and (*S*)- $\alpha$ -Methoxy- $\alpha$ -(trifluoromethyl)phenylacetyl (MTPA) Esters of Compound **5**

A solution of (*R*)-MTPA acid (20.4 mg, 87  $\mu$ mol), dicyclohexylcarbodiimide (26 mg, 126  $\mu$ mol) and 4-dimethylaminopyridine (10.6 mg, 87  $\mu$ mol) in dry CH<sub>2</sub>Cl<sub>2</sub> (2 ml) was added to **5** (15 mg, 28  $\mu$ mol). The mixture was allowed to stand for 18 hours at room temperature. Then, the reaction mixture was loaded on a column of silica gel and chromatographed with the eluent of *n*-hexane/EtOAc (10:1~5:1) to afford 8.9 mg of (*R*)-MTPA ester **6a**. In the same manner as described above, **5** (15 mg) was reacted with (*S*)-MTPA acid (20.4 mg) to give 7.6 mg of (*S*)-MTPA ester **6b**.

**6a:** <sup>1</sup>H NMR:  $\delta$  0.924 (3H, t, *J*=7.6 Hz, 15-H), 1.762 (3H, s, 16-H), 2.041 (3H, s, 9-OCOCH<sub>3</sub>), 1.933 (1H, ddqd, *J*=1.6, 7.6, 7.6 and 14.6 Hz, 14-H), 2.147 (1H, ddqd, *J*=1.4, 7.6, 7.6 and 14.6 Hz, 14-H), 2.558 (3H, s, 7-NCOCH<sub>3</sub>), 3.332 (3H, s, 11-OCH<sub>3</sub>), 3.432 (3H, s, CH<sub>3</sub>O- of MTPA), 3.611 (3H, s, 8-OCH<sub>3</sub>), 4.497 (1H, t, *J*=9.0 Hz, 11-H), 5.250 (1H, tdd, *J*=1.5, 9.5 and 11.0 Hz, 12-H), 5.539 (1H, s, 9-H), 5.725 (1H, d, *J*=8.5 Hz, 10-H), 5.823 (1H, td, *J*=7.8 and 11.0 Hz, 13-H), 7.33~7.45 (5H, m, C<sub>6</sub>H<sub>5</sub>- of MTPA), 7.406 (2H, t, *J*=8.1 Hz, 20 and 22-H), 7.517 (1H, tt, *J*=1.2 and 7.3 Hz, 21-H), 8.001 (2H, dd, *J*=1.2 and 7.3 Hz, 19 and 23-H).

**6b:** <sup>1</sup>H NMR:  $\delta$  0.859 (3H, t, *J*=7.6 Hz, 15-H), 1.762 (3H, s, 16-H), 2.023 (3H, s, 9-OCOCH<sub>3</sub>), 1.796 (1H, ddqd, *J*=1.5, 7.3, 7.6 and 14.6 Hz, 14-H), 2.091 (1H, ddqd, *J*=1.3, 7.3, 7.6 and 14.6 Hz, 14-H), 2.573 (3H, s, 7-NCOCH<sub>3</sub>), 3.304 (3H, s, 11-OCH<sub>3</sub>), 3.452 (3H, s, CH<sub>3</sub>O- of MTPA), 3.612 (3H, s, 8-OCH<sub>3</sub>), 4.465 (1H, t, *J*=9.1 Hz, 11-H), 5.060 (1H, tdd, *J*=1.5, 9.4 and 11.0 Hz, 12-H), 5.554 (1H, s, 9-H), 5.581 (1H, td, *J*=7.7 and 11.0 Hz, 13-H), 5.635 (1H, d, *J*=8.8 Hz, 10-H), 7.3~7.43 (5H, m, C<sub>6</sub>H<sub>5</sub>- of MTPA), 7.410 (2H, t, *J*=7.8 Hz, 20 and 22-H), 7.524 (1H, tt, *J*=1.2 and 7.3 Hz, 21-H), 7.997 (2H, dd, *J*=1.2 and 7.3 Hz, 19 and 23-H).

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