

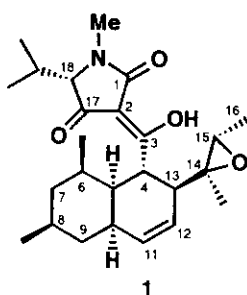
## A DEGRADATION STUDY OF VERMISPORIN AND DETERMINATION OF ITS ABSOLUTE CONFIGURATION

Nobuto Minowa\*<sup>†</sup>, Yoshio Kodama<sup>†</sup>, Kenzo Harimaya<sup>†</sup>, and Takashi Mikawa<sup>‡</sup>

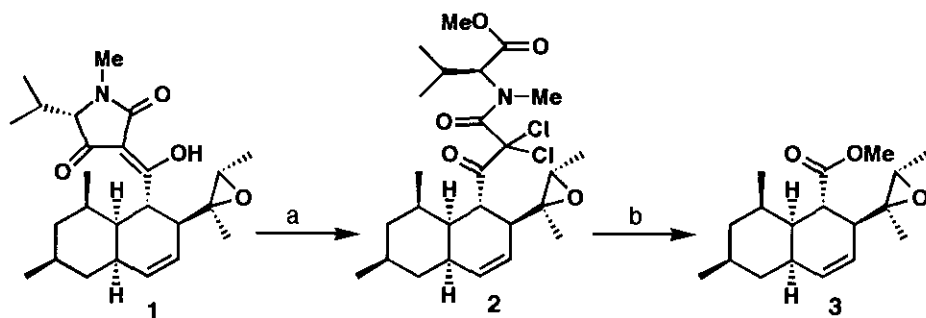
<sup>†</sup> Pharmaceutical Research Center, Meiji Seika Kaisha Ltd., 760 Morooka-cho, Kohoku-ku, Yokohama 222-8567, Japan, <sup>‡</sup>Yokohama Research Center, Mitsubishi Chemical Corporation, 1000 Kamoshida-cho, Aoba-ku, Yokohama 227-8502, Japan

**Abstract-** The absolute configuration of vermispোরিন (**1**) was determined by X-Ray crystallography of the degradation product (**2**), and the decalin derivative (**3**) was efficiently prepared from **1**.

Vermispোরিন (**1**) is a new tetramic acid antibiotic isolated from the culture broth of *Ophiobolus vermispোরinus*.<sup>1</sup> It showed excellent antimicrobial activity against gram-positive bacteria and anaerobic species such as *Staphylococcus aureus*, *Bacteroides fragilis*, *Clostridium perfringens*, and *Clostridium difficile* (MIC 0.12–2 µg/mL).<sup>1,2</sup> The structure and relative stereochemistry were elucidated as shown in **1** on the basis of spectroscopic studies and X-Ray crystallography,<sup>1</sup> while the absolute configuration of **1** remained undefined. In order to determine the absolute configuration and also to obtain the decaline derivative which is necessary to synthesize analogues, we have undertaken a degradation study of **1**. In this paper, we describe a degradation of vermispোরিন (**1**) to the decaline derivative, including isolation of an unusual dichloride intermediate, and determination of the absolute configuration of **1**.

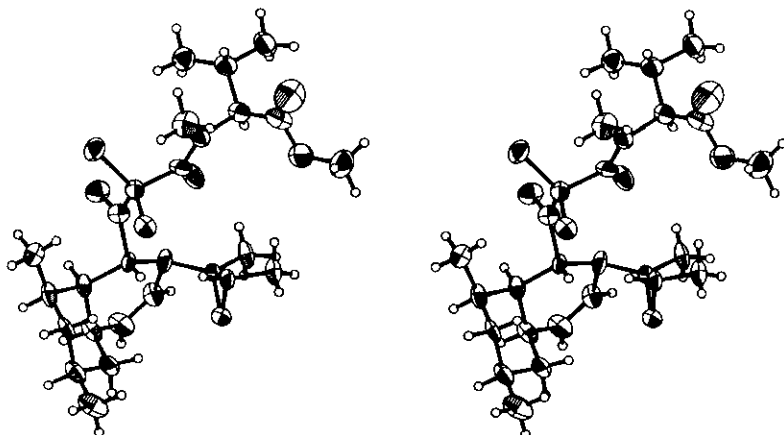


In order to obtain a compound corresponding to the decaline moiety of **1**, we tried oxidative cleavage of the tetramic acid part. Treatment of **1** with sodium hypochlorite and 1M NaOH in MeOH at room temperature for 8 h afforded, interestingly, unexpected dichloride (**2**) in 72% yield. Although **1**, 3-



**Scheme 1.** Reagents and Conditions: (a) NaOCl, 1M NaOH, MeOH, rt, 72%;  
(b) NaOMe, MeOH, rt, 68%.

diketones are usually oxidized by sodium hypochlorite to carboxyl groups,<sup>3</sup> oxidation of **1** provided the oxidative intermediate (**2**) and further hydrolysis of **2** to the decalin derivative did not proceed. This unusual result is presumably due to the steric hindrance at the C-3 carbonyl group of **2**. The structure of **2**, including its absolute stereochemistry, was determined by X-Ray crystallographic analysis as shown in Figure 1; thus, the absolute configuration of **1** was established as shown in **1**. It was found that the C-18 carbon of **1** has the same stereochemistry (*S*-configuration), which would be originated from L-amino acids,<sup>4</sup> with other tetramic acid antibiotics.<sup>5</sup> Further cleavage of the ketone part in the dichloride (**2**) was successfully performed by using sodium methoxide to afford the desired decalin derivative (**3**) in 68% yield.



**Figure 1.** Stereoview of X-Ray structure of **2**.

In summary, the absolute configuration of vermisporin was determined *via* degradation to dichloride (**2**) and the decaline derivative (**3**) was efficiently prepared. This procedure for determination of the absolute configuration of **1** would be applicable to other naturally occurring tetramic acids.<sup>5</sup>

## EXPERIMENTAL

Melting points were taken on a Mitamura micro melting point apparatus and are uncorrected. IR spectra were recorded on Jasco A-202 spectrometer. Optical rotation were measured with a Perkin-Elmer 241 polarimeter. NMR spectra were recorded on JEOL GX-400 spectrometer in CDCl<sub>3</sub>, with tetramethylsilane as the internal standard. Mass spectra were obtained with a Hitachi M-80B spectrometer. Column chromatography was performed by using Wakogel C-200 or C-300.

**Dichloride (2).** To a solution of vermisporin (**1**) (123 mg, 0.296 mmol) in MeOH (6 mL) at rt was slowly added 1M NaOH (0.5 mL), followed by NaOCl solution (available chlorine 8.5–13.5%, 1.5 mL). The mixture was stirred at rt for 8 h. Then 1M aqueous Na<sub>2</sub>SO<sub>3</sub> (2 mL) was added and the mixture was neutralized by addition of 1M HCl. After removal of the solvent, the residue was diluted with H<sub>2</sub>O and then the resulting mixture was extracted with AcOEt. The organic layer was dried over Na<sub>2</sub>SO<sub>4</sub>, filtered and concentrated. The crude product was purified by chromatography on silica gel (n-hexane : AcOEt=12 : 1) to afford 109 mg (72 %) of the dichloride (**2**) as a white solid: mp 140–141 °C (from n-hexane, prisms); [ $\alpha$ ]<sub>D</sub><sup>24</sup> –18.5° (c 0.87, PhH); IR (KBr) 2290, 1730, 1635, 1445, 1200 cm<sup>-1</sup>; <sup>1</sup>H NMR  $\delta$  0.82 (3H, d, *J*=7.6 Hz), 0.91 (6H, d, *J*=6.5 Hz), 1.00 (3H, d, *J*=6.5 Hz), 1.19 (3H, d, *J*=5.3 Hz), 1.22 (3H, s), 0.84–1.90 (6H, m), 2.08–2.58 (4H, m), 2.85 (1H, q, *J*=5.3 Hz), 3.42 (3H, br s), 3.73 (3H, s), 3.95 (1H, dd, *J*=11.3, 6.7 Hz), 4.67 (1H, d, *J*=10.7 Hz), 5.43 (1H, dd, *J*=9.9, 3.8 Hz), 5.95 (1H, ddd, *J*=9.9, 6.5, 1.9 Hz); FDMS (*m/z*) 515 (M<sup>+</sup>); HRMS (EI) calcd for C<sub>26</sub>H<sub>39</sub>NO<sub>5</sub>Cl<sub>2</sub> (M<sup>+</sup>) 515.2205, found 515.2096.

**X-Ray crystal data of 2.** C<sub>26</sub>H<sub>39</sub>NO<sub>5</sub>Cl<sub>2</sub>: crystal dimension 0.2×0.2×0.1 mm, monoclinic, space group P2<sub>1</sub>, a=10.456(2) Å, b=10.226(2) Å, c=12.945(2) Å,  $\beta$ =92.35(1)°, V=1382.9(4) Å<sup>3</sup>, Z=2, D<sub>calcd</sub>=1.240gcm<sup>-3</sup>, R=0.067, R<sub>w</sub>=0.084. The data was collected on a Rigaku AFC5R diffractometer with graphite monochromated Cu-K  $\alpha$  radiation ( $\lambda$ =1.54178 Å). Atomic coordinates, bond lengths and angles, and thermal parameters have been deposited at the Cambridge Crystallographic Data Centre, 12 Union Road, Cambridge, CB2 1EZ, UK.

**Methyl Ester (3).** To a solution of the dichloride (**2**) (80.4 mg, 0.156 mmol) in MeOH (4.5 mL) at rt was added a solution of 28 % NaOMe in MeOH (0.8 mL). The mixture was stirred at rt for 17 h and neutralized by addition of 1M HCl. After removal of the solvent, the residue was diluted with H<sub>2</sub>O and

then the resulting mixture was extracted with AcOEt. The organic layer was dried over  $\text{Na}_2\text{SO}_4$ , filtered and concentrated. The crude product was purified by chromatography on silica gel (n-hexane : AcOEt=12 : 1) to afford 31.0 mg (68 %) of the ester (**3**) as a colorless oil:  $[\alpha]_D^{25} -22.3^\circ$  (c 1.95, PhH); IR (neat) 2920, 1730, 1155  $\text{cm}^{-1}$ ;  $^1\text{H NMR}$   $\delta$  0.87 (3H, d,  $J=7.4$  Hz), 0.93 (3H, d,  $J=6.4$  Hz), 1.17 (3H, s), 1.28 (3H, d,  $J=5.6$  Hz), 0.73-1.81 (6H, m), 2.05-2.13 (2H, m), 2.28 (1H, dd,  $J=10.3$ , 1.8 Hz), 2.82 (1H, q,  $J=5.6$  Hz), 2.86-2.89 (1H, m), 3.69 (3H, s), 5.32 (1H, dd,  $J=9.9$ , 2.2 Hz), 5.78 (1H, ddd,  $J=9.9$ , 5.1, 2.8 Hz); EIMS ( $m/z$ ) 292 ( $M^+$ ), 260, 215, 189, 161; HRMS (FAB) calcd for  $\text{C}_{18}\text{H}_{29}\text{O}_3$  ( $MH^+$ ) 293.2118, found 293.2120.

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### REFERENCES

1. (a) T. Mikawa, N. Chiba, H. Ohkishi, Y. Sato, S. Miyadoh, and M. Sezaki, *Jpn Kokai JP* 0240329 (*Chem. Abstr.*, 1990, **113**, 126593r); (b) N. Yoshikawa, T. Mikawa, H. Ohkishi, Y. Sato, Y. Takeuchi, and S. Miyadoh, *J. Jpn. Society for Bioscience Biotechnology and Agrochemistry*, 1991, **65**, 317(2Cp5).
2. N. X. Chin and H. C. Neu, *Eur. J. Clin. Microbiol. Infect. Dis.*, 1992, **11**, 755.
3. W. T. Smith and G. L. McLeod, *Org. Synth. Coll. Vol. IV*, 1963, 345.
4. (a) C. E. Sticking and R. J. Townsend, *Biochem. J.*, 1961, **78**, 412; (b) N. J. Phillips, J. T. Goodwin, A. Fraiman, R. J. Cole, and D. G. Lynn, *J. Am. Chem. Soc.*, 1989, **111**, 8223.
5. B. J. L. Royles, *Chem. Rev.*, 1995, **95**, 1981.

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