A DEGRADATION STUDY OF VERMISPORIN AND DETERMINATION OF ITS ABSOLUTE CONFIGURATION

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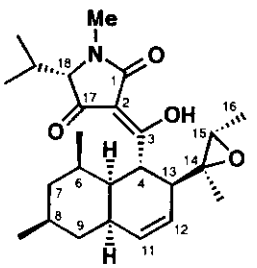
Abstract- The absolute configuration of vermisporin (**1**) was determined by X-Ray crystallography of the degradation product (**2**), and the decalin derivative (**3**) was efficiently prepared from **1**.

Vermisporin (**1**) is a new tetramic acid antibiotic isolated from the culture broth of *Ophiobolus vermisporus*. [1] It showed excellent antimicrobial activity against gram-positive bacteria and anaerobic species such as *Staphylococcus aureus*, *Bacteroides fraragilis*, *Clostridium perfringens*, and *Clostridium difficile* (MIC 0.12～2 μg/mL).[1,2] The structure and relative stereochemistry were elucidated as shown in **1** on the basis of spectroscopic studies and X-Ray crystallography,[1] 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 vermisporin (**1**) to the decaline derivative, including isolation of an unusual dichloride intermediate, and determination of the absolute configuration of **1**.



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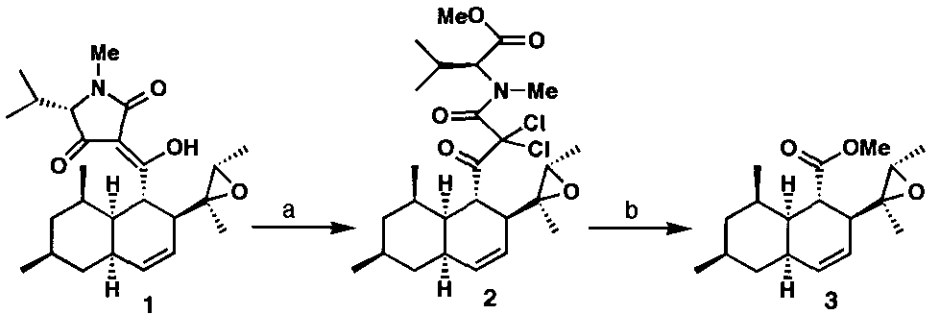
In order to obtain a compound corresponding to the decaline moiety of **1**, we tried oxidative cleavage of the tetramic acid part. Treatment of **1** with sodium hypochlorite and 1M NaOH in MeOH at room temperature for 8 h afforded, interestingly, unexpected dichloride (**2**) in 72% yield. Although 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,[3] oxidation of **1** provided the oxidative intermediate (**2**) and further hydrolysis of **2** to the decaline 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,[4] with other tetramic acid antibiotics.[5] 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.

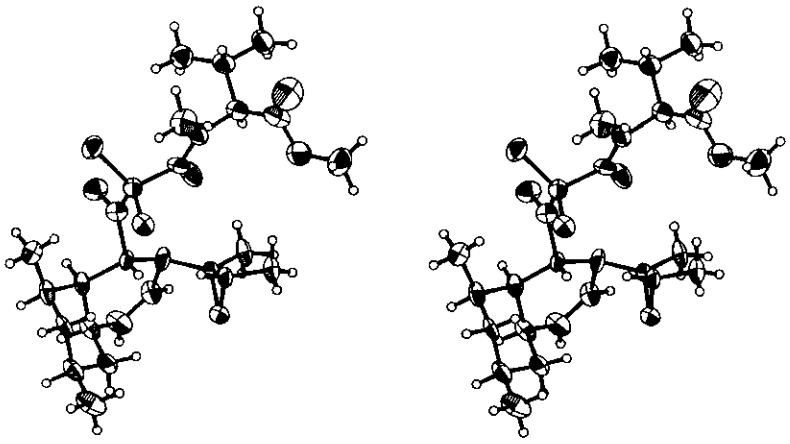


Figure 1. Stereoview of X-Ray structure of **2**.

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.[5]

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 $CDCl_3$, 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_2SO_3 (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_2O and then the resulting mixture was extracted with AcOEt. The organic layer was dried over Na_2SO_4, 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 ℃ (from n-hexane, prisms); $[\alpha]^{24}_{D}$ −18.5° (c 0.87, PhH); IR (KBr) 2290, 1730, 1635, 1445, 1200 cm^{-1}; ^1H NMR δ 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^+); HRMS (EI) calcd for $C_{26}H_{39}NO_5Cl_2$ (M^+) 515.2205, found 515.2096.

X–Ray crystal data of 2. $C_{26}H_{39}NO_5Cl_2$: crystal dimension 0.2×0.2×0.1 mm, monoclinic, space group $P2_1$, a=10.456(2) Å, b=10.226(2) Å, c=12.945(2) Å, β=92.35(1)°, V=1382.9(4) $Å^3$, Z=2, D_{calcd}=1.240gcm^{-3}, R=0.067, R_W=0.084. The data was collected on a Rigaku AFC5R diffractometer with graphite monochromated Cu-Kα radiation (λ=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_2O and

then the resulting mixture was extracted with AcOEt. The organic layer was dried over Na_2SO_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: $[\alpha]^{25}_D$ −22.3° (c 1.95, PhH); IR (neat) 2920, 1730, 1155 cm^{-1}; ^1H NMR δ 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 $C_{18}H_{29}O_3$ (MH^+) 293.2118, found 293.2120.

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