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Oximidine III, a New Antitumor Antibiotic against Transformed Cells from *Pseudomonas* sp.

II. Structure Elucidation

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The structure of oximidine III, a new antitumor antibiotic against transformed cells from *Pseudomonas* sp. QN05727, was determined to be a benzolactone enamide containing an *O*-methyloxime moiety as shown in Fig. 1 by NMR spectral analysis including a variety of two-dimensional techniques.

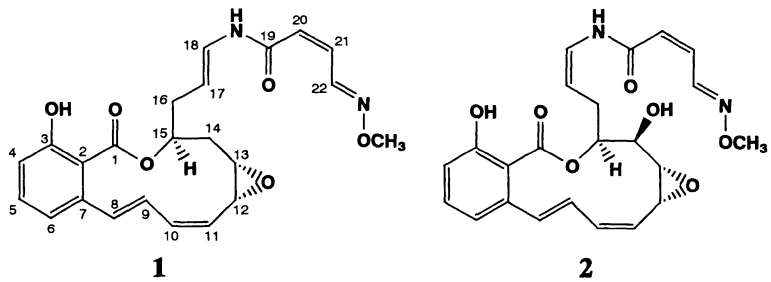
In the preceding paper[1], we have described the fermentation, isolation, physico-chemical properties and biological activity of oximidine III (**1**, Fig. 1), a new antitumor antibiotic against transformed cells, and the taxonomy of the producing organism, *Pseudomonas* sp. QN05727. We report here the structure elucidation of oximidine III (**1**).

Results

The molecular formula of **1** was established to be $C_{23}H_{24}N_2O_6$ by high-resolution FAB-MS. Although the ^1H NMR spectrum of **1** in acetone-d_6 at room temperature exhibited extensively broad signals, measurement at $-12°C$ sharpened these signals as shown in Fig. 2. The ^1H and ^{13}C NMR signals at $-12°C$ appeared as twin peaks in the area ratio of 1 : 1 (Fig. 2, Table 1), indicating the presence of conformational or tautomeric isomers in acetone-d_6.

COSY and heteronuclear multiple-quantum coherency (HMQC)[2] experiments revealed three spin networks for each isomer to generate partial structures **A**, **B** and **C** as shown in Fig. 3. The existence of an epoxide group at C-12 and C-13 was required by their ^{13}C and ^1H chemical shifts

Fig. 1. Structures of oximidines III (**1**) and I (**2**).



1 **2**

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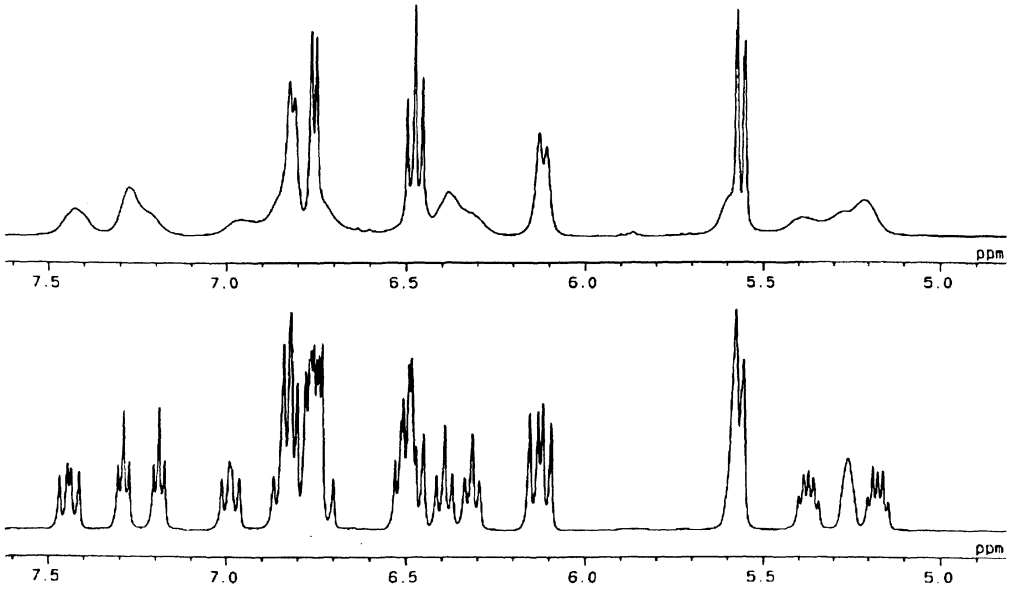
Fig. 2. ^{1}H NMR spectrum of **1** in acetone-d_6 at 22°C (top) and −12°C (bottom).



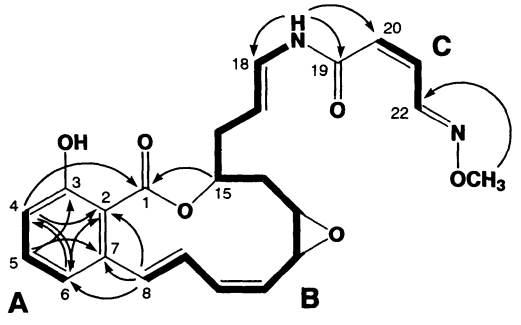
Bold lines show proton spin systems and arrows show ^{1}H-^{13}C long-range correlations.

Table 1. ^{13}C and ^{1}H NMR data for the two conformers of oximidine III in acetone-d_6 at −12°C.

	1a		**1b**	
No.	δ_C	δ_H (multiplicity, J = Hz)	δ_C	δ_H (multiplicity, J = Hz)
1	168.5		170.1	
2	123.5		113.6	
3	155.3		160.6	
4	115.2	6.84 (d, 8.0)	116.7	6.82 (d, 8.0)
5	130.4	7.19 (t, 8.0)	133.3	7.31 (t, 8.0)
6	119.7	6.73 (d, 8.0)	122.4	6.79 (d, 8.0)
7	139.2		140.7	
8	132.7	6.73 (d, 16.0)	130.1	6.49 (d, 16.0)
9	131.8	7.45 (dd, 16.0, 11.0)	134.7	7.45 (dd, 16.0, 11.0)
10	130.6	6.33 (dt, 1.0, 11.0)	133.0	6.41 (t, 11.0)
11	124.7	5.57 (d, 11.0)	126.6	5.59 (dd, 11.0, 2.5)
12	55.6	3.60 (dd, 3.5, 1.0)	58.2	3.63 (dd, 4.0, 2.5)
13	53.9	3.48 (dd, 9.0, 3.5)	56.4	3.30 (ddd, 10.0, 4.0, 4.0)
14	30.5	2.00 (dd, 16.0, 1.0)	30.0	1.99 (dd, 16.0, 4.0)
		1.86 (ddd, 16.0, 9.0, 4.0)		1.81 (ddd, 16.0, 10.0, 7.0)
15	72.2	5.25 (m)	73.6	5.60 (m)
16	33.1	2.77 (dt, 14.0, 7.0)	37.7	2.42 (dt, 14.0, 7.0)
		2.64 (dt, 14.0, 7.0)		2.35 (dt, 14.0, 7.0)
17	109.5	5.38 (dt, 14.0, 7.0)	108.1	5.18 (dt, 14.0, 7.0)
18	126.0	6.97 (dd, 14.0, 11.0)	126.3	6.83 (dd, 14.0, 11.0)
19	162.2		162.2	
20	125.8	6.10 (d, 11.5)	126.2	6.17 (d, 11.5)
21	134.9	6.49 (dd, 11.5, 10.5)	134.7	6.50 (dd, 11.5, 10.5)
22	148.2	9.07 (d, 10.5)	148.2	9.12 (d, 10.5)
OMe	62.3	3.80 (s)	62.3	3.80 (s)
18-NH		9.61 (d, 11.0)		9.60 (d, 11.0)

(Table 1). In one isomer (**1a**), partial structure **A** was extended to be a 2,3-disubstituted phenol based on a heteronuclear multiple-bond correlation (HMBC)[3] experiment, which exhibited long-range couplings from 4-H to C-2, from 5-H to C-3 and C-7, and from 6-H to C-2 (Fig. 3). Long-range correlations from 8-H to C-2, C-6, and C-7 established the connection of partial structures **A** and **B**. A carbonyl carbon (C-1) displayed a four-bond coupling to 4-H, indicating its substitution at C-2. A long-range coupling from 15-H to C-1 identified an ester linkage between C-1 and C-15 to construct a 12-membered lactone ring. Partial structures **B** and **C** were joined *via* an amide carbonyl (C-19) based on long-range couplings from 18-NH to C-18, C-19 and C-20 (Fig. 3). The terminal sp^2 methine (C-22) in partial structure **C** needed to be connected to the remaining nitrogen atom in an imine-type bond. A four-bond ^1H-^{13}C correlation from a methoxyl group to C-22 confirmed the presence of an *O*-methyloxime moiety. The geometrical configurations were determined to be 8*E*, 10*Z*, 17*E* and 20*Z* from their relevant proton coupling constants ($J_{8\text{-}9}$=16.0, $J_{10\text{-}11}$=11.0, $J_{17\text{-}18}$=14.0, $J_{20\text{-}21}$=11.5 Hz). A NOESY experiment revealed a weak NOE between 22-H and the methoxyl group, indicating *E* geometry for the oxime double bond. These results established the planar structure of isomer **1a** as shown in Fig. 3. The same planar structure was required for the other isomer (**1b**) by similar two-dimensional NMR correlations and similar coupling constants in the olefinic region (Table 1), suggesting that these two isomers are in conformational isomerism.

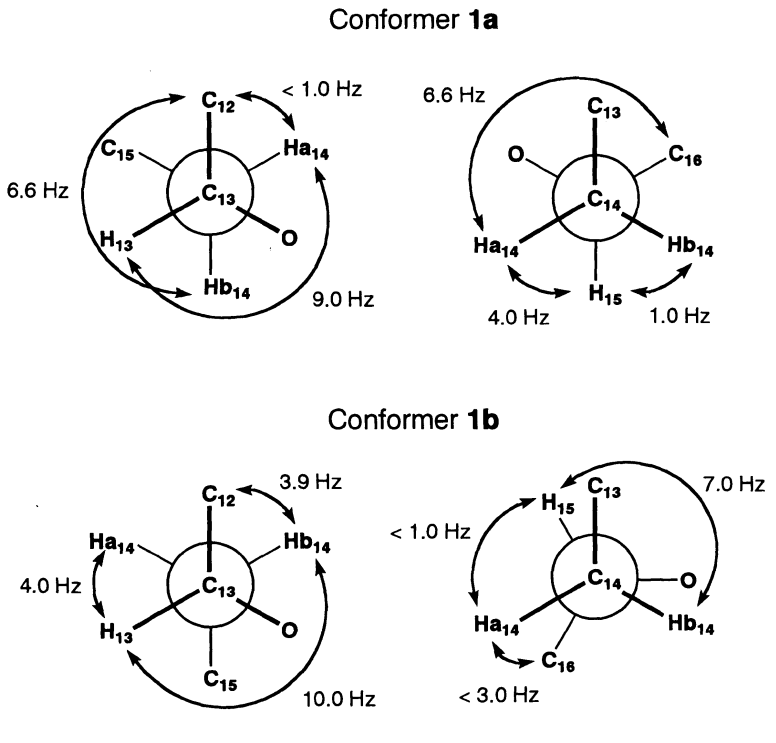
Fig. 3. COSY and HMBC data summary for conformer **1a**.



Bold lines show proton spin systems and arrows show ^1H-^{13}C long-range correlations.

The epoxide stereochemistry was identified as *cis* by a significant NOE and a large coupling constant observed between 12-H and 13-H in each conformer. The relative configurations of C-13 and C-15 were analyzed by ^1H-^1H and ^1H-^{13}C coupling constants[4] as shown in Fig. 4. In conformer **1a**, a large coupling constant (9.0 Hz) revealed

Fig. 4. ^1H-^1H and ^1H-^{13}C coupling constant analyses for conformers **1a** and **1b**.



an anti relationship between 13-H and 14-Ha. A *J*-resolved HMBC[5] experiment displayed a large three-bond coupling constant ($^3J_{H\text{-}C}$=6.6 Hz) between 14-Hb and C-12, indicating these atoms to be in an anti arrangement. Both of 14-H_2 exhibited small couplings (4.0 and 1.0 Hz) with 15-H and were required to be gauche to 15-H. An anti relationship between 14-Ha and C-16 ($^3J_{H\text{-}C}$=6.6 Hz) established the relative stereochemistry of conformer **1a** as shown in Fig. 4. In conformer **1b**, anti orientations between 13-H and 14-Hb and between 14-Ha and 13-O were determined from $J_{13H\text{-}14Hb}$=10.0 Hz and $^2J_{14Ha\text{-}C13}$=2.7 Hz. Atypical coupling constants on 15-H ($J_{14Ha\text{-}15H}$<1.0 Hz, $J_{14Hb\text{-}15H}$=7.0 Hz) revealed a dihedral angle of around 90° between 14-Ha and 15-H. A small two-bond ^1H-^{13}C coupling ($^2J_{14Ha\text{-}C15}$<3.0 Hz) indicated nearly an anti relationship between 14-Ha and 15-O. These data confirmed the same relative configurations of conformer **1b** as those of **1a** (Fig. 4).

Oximidine III (**1**) is a new benzolactone enamide structurally related to oximidine I (**2**, Fig. 1)[6]. The 3- to 8-fold higher activity of **1** than that of **2**[1] suggests the significant effect of a 14-hydroxyl group or a C-17 geometry. Studies on the absolute stereochemistry of **1** are in progress.

Experimental

Mass spectra were measured on a JEOL HX-110 spectrometer in the FAB mode using *m*-nitrobenzyl alcohol as matrix and polyethylene glycol as internal standard. NMR spectra were obtained on a JEOL JNM-A500 spectrometer with ^1H NMR at 500 MHz and with ^{13}C NMR at 125 MHz. Chemical shifts are given in ppm using TMS as internal standard. COSY, HMQC, HMBC, NOESY and *J*-resolved HMBC experiments were carried out at −12°C.

Acknowledgments

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