NOVEL ANTIFUNGAL ANTIBIOTICS MANIWAMYCINS A AND B
II. STRUCTURE DETERMINATION

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The structures of the new azoxy antibiotics maniwamycins A and B have been determined by
means of spectral analyses and chemical studies.

Maniwamycins A and B are new antifungal antibiotics produced by a Streptomyces sp. In the preceding
paper, we reported the taxonomy of the producing strain, fermentation, isolation, physico-chemical
properties and biological properties of these antibiotics.1) In this paper, we describe the structure
determination of maniwamycins A and B.

Structure of Maniwamycin A

The fast atom bombardment (FAB)-MS of maniwamycin A revealed an ion peak at m/z 199 (M + H). Through the high-resolution electron impact (HREI)-MS of the ion peak at m/z 198 (found 198.1363; calc’d for C_{10}H_{18}N_{2}O_{2} 198.1369) the molecular formula of maniwamycin A was established as C_{10}H_{18}N_{2}O_{2}. The UV absorption maximum at 235 nm (ε 12,400) and the characteristic band near 1500 cm^{-1} in the IR spectrum implied the presence of an azoxy group. \(^{13}C\) NMR data (Table 1) showed the presence of three C-methyls, three methylenes, one sp\(^{3}\) methine, two olefinic methines and one carbonyl carbon. Assignment of the signals for C-1', C-2' and C-3' were confirmed by the \(^1H-^{13}C\) correlation spectroscopy (COSY) spectrum (Fig. 1). The position of the oxygen atom in the azoxy group was determined to be on the olefin group side by the proton chemical shift (δ 4.52) of the sp\(^{3}\) methine adjacent to the azoxy group\(^{2-4}\) and by the UV absorption maximum.\(^{2}\) This is similar to elaiomycin\(^{5}\) and LL-BH872x.\(^{4}\)

Table 1. \(^1H\) and \(^{13}C\) NMR data\(^*\) of maniwamycins A and B (DMSO-\(d_6\)).

<table>
<thead>
<tr>
<th>Assignment</th>
<th>(^1H)</th>
<th>(^{13}C)</th>
<th>(^1H)</th>
<th>(^{13}C)</th>
</tr>
</thead>
<tbody>
<tr>
<td>C-1</td>
<td>2.10 s</td>
<td>27.1 q</td>
<td>1.06 d (6.5)</td>
<td>19.2 q</td>
</tr>
<tr>
<td>C-2</td>
<td>—</td>
<td>205.0 s</td>
<td>3.69~3.80 m</td>
<td>67.5 d</td>
</tr>
<tr>
<td>C-3</td>
<td>4.52 q (7.5)</td>
<td>65.1 d</td>
<td>3.99 quintet (6.5)</td>
<td>60.5 d</td>
</tr>
<tr>
<td>C-4</td>
<td>1.37 d (7.5)</td>
<td>14.5 q</td>
<td>0.99 d (6.5)</td>
<td>11.6 q</td>
</tr>
<tr>
<td>C-1'</td>
<td>7.23 dd (14.0, 1.0)</td>
<td>136.9 d</td>
<td>7.08 d (14.0)</td>
<td>138.1 d</td>
</tr>
<tr>
<td>C-2'</td>
<td>6.90 dt (14.0, 7.5)</td>
<td>135.3 d</td>
<td>6.87 dt (14.0, 7.5)</td>
<td>133.7 d</td>
</tr>
<tr>
<td>C-3'</td>
<td>2.22 br q (7.5)</td>
<td>27.3 t</td>
<td>2.20 q (7.5)</td>
<td>27.2 t</td>
</tr>
<tr>
<td>C-4'</td>
<td>1.27~1.48 m</td>
<td>29.8(^b) t</td>
<td>1.24~1.47 m</td>
<td>29.9(^b) t</td>
</tr>
<tr>
<td>C-5'</td>
<td>0.89 t (7.0)</td>
<td>21.6(^b) t</td>
<td>0.89 t (7.0)</td>
<td>21.7(^b) t</td>
</tr>
<tr>
<td>C-6'</td>
<td>0.89 t (7.0)</td>
<td>13.6 q</td>
<td>13.6 q</td>
<td></td>
</tr>
</tbody>
</table>

\(^*\) ppm and J(Hz) in parentheses.
\(^{b}\) Tentative assignments.
From the above described data, we propose that the structure of maniwamycin A is Z-(3S)-2-oxobutane-3-NNO-azoxy-1'-(E-1'-hexene) as shown in Fig. 2.

**Structure of Maniwamycin B**

The results of FAB-MS and HREI-MS of maniwamycin B showed the formula of this antibiotic as C_{10}H_{20}N_{2}O_{2} (MW 200).\(^1\) \(^{13}\)C NMR data in Table 1 showed that it is constructed of ten carbon atoms classified to three C-methyls, three methylenes, two sp\(^3\) methines and two olefinic methines. Assignment of the signals for C-1, C-4, C-1', C-2' and C-3' were confirmed by the \(^1\)H-\(^{13}\)C COSY spectrum (Fig. 3). The IR spectrum\(^1\) and \(^1\)H and \(^{13}\)C NMR spectra indicated that maniwamycin B is a dihydro-compound of maniwamycin A at C-2.

Thus, the structure of maniwamycin B is Z-(2S,3S')-2-hydroxybutane-3-NNO-azoxy-1'-(E-1'-hexene)
as shown in Fig. 2.

Geometrical and Stereochemical Studies

The oxidation of maniwamycin B with pyridinium chlorochromate afforded maniwamycin A. This suggests the same geometry of the olefin and the azoxy moiety, and the same configuration of C-3 in these antibiotics.

In the $^1$H NMR spectra of maniwamycins A and B in CDCl$_3$, the signals of the olefin overlapped each other (δ 7.0). When the $^1$H NMR spectrum of maniwamycin A was measured in DMSO-d$_6$, the signals of the olefin were less overlapped as shown in Fig. 4. The coupling constant was able to read from the spectrum; $J_{1,2} = 14$ Hz. Therefore, the olefin geometry was determined as the E configuration. It was supposed that the azoxy geometry was trans as regards carbon substituents by the UV absorption maximum ($\lambda_{max}$ nm (ε) 221 (5,440)) of the reduced derivative (H$_2$-Rh・Al$_2$O$_3$) of maniwamycin A and from biogenetic point of view of other known naturally occurring azoxy compounds. Stevens et al., cleaved the azoxy linkage of elaimycin by hydrogenolysis over platinum dioxide in acetic acid to get d-threonine. Under similar conditions maniwamycin B was cleaved to get (2S,3S)-3-amino-2-butanol which was isolated as the benzylxoy-carbonyl derivative.

Only four antibiotics elaimycin, EL-BH872x, valanimycin and jietacins have been reported to
Fig. 4. $^1$H NMR spectra of maniwamycin A in DMSO-$d_6$ (A) and CDCl$_3$ (B).

contain an azoxy moiety. Maniwamycins A and B are new compounds of this class and the first natural compounds possessing a trans-$\alpha,\beta$-unsaturated azoxy chromophore.

**Experimental**

**General**

UV spectra were recorded on a Beckman DU-65 spectrophotometer. IR spectra were obtained using a Shimadzu IR-435 spectrophotometer. MS spectra were measured on a Jeol JMS-D300 mass spectrometer. Optical rotations were measured with a Jasco DIP-4 digital polarimeter. $^1$H and $^{13}$C NMR spectra were recorded on a Jeol GSX 270 spectrometer. The chemical shifts in DMSO-$d_6$ and CDCl$_3$ refer to an internal standard of TMS (0 ppm).

**Oxidation of Maniwamycin B**

Maniwamycin B (62 mg, 0.31 mmol) was dissolved in anhydrous dichloromethane (1.0 ml) with stirring, and treated with powdered molecular sieves 4A (60 mg) and pyridinium chlorochromate (200 mg, 0.93 mmol) under nitrogen at room temperature. After 10 hours, ether (5 ml) was added and the mixture was stirred
for 10 minutes. The organic layer was passed through a short pad of Florisil and evaporated under reduced pressure. Chromatography of the residue on silica gel using ether-n-hexane (1:5) as eluent gave maniwamycin A (24 mg, 40%), as a colorless oil, which was identical with the authentic product obtained from the fermentation in all respects.

Hydrogenation of Maniwamycin A

To a solution of maniwamycin A (56 mg, 0.28 mmol) in methanol (2.0 ml) was added 5% rhodium on aluminum oxide (25 mg). The mixture was stirred under hydrogen (1 atm) at room temperature for 1.5 hours. Ether (5.0 ml) was added, the suspension was filtered through Celite and the filtrate was evaporated under reduced pressure. The residue was purified by preparative TLC (silica gel, ether-n-hexane (1:1)) to give the reduced maniwamycin A (15 mg, 26%) as a colorless oil: [α]D2 -78.6° (c 0.5, CHCl3); IR νmax cm⁻¹ 2920, 1717, 1490; UV λmax nm (ε) 221 (5,440); 1H NMR (270 MHz, CDCl3) δ 0.89 (3H, t, J=6.5Hz, 6'-CH3), 1.26-1.41 (6H, m, 3', 4', 5'-CH2), 1.42 (3H, d, J=7.0 Hz, 4-CH3), 1.92-2.06 (2H, m, 2'-CH2), 2.18 (3H, s, 1-CH3), 4.22 (2H, t, J=7.0Hz, 1'-CH2), 4.49 (1H, q, J=7.0 Hz, 3-CH).

Hydrogenolysis of Maniwamycin B

The solution of maniwamycin B (467 mg, 2.33 mmol) in acetic acid (2.5 ml) with platinum dioxide (106 mg) was stirred under hydrogen (1 atm) at room temperature. After 5 hours, further platinum dioxide (97 mg) was added and the solution was stirred again under the same conditions for 16 hours. The mixture was filtered and the catalyst was washed with acetic acid. The combined filtrate was concentrated under reduced pressure, the residue was dissolved in anhydrous dichloromethane (5 ml), and treated with diazomethane solution in ether to remove residual acetic acid. The resultant ethereal solution was concentrated under reduced pressure and the residue was chromatographed on silica gel using chloroform as eluent. Further purification by preparative TLC (silica gel, CHCl3-MeOH (9:1)) gave (2S,3S)-N-Cbz-3-amino-2-butanol (103 mg, 20%) as a colorless oil: [α]D2 +7.6° (c 1.0, CHCl3); IR νmax cm⁻¹ 3430, 1719 (s), 1498; 1H NMR (270 MHz, CDCl3) δ 1.18 (3H, d, J=6.5Hz, 1-CH3 or 4-CH3), 1.20 (2H, d, J=6.5 Hz, 1-CH3 or 4-CH3), 1.94 (1H, br s, OH), 3.58-3.78 (2H, m, 2-CH and 3-CH), 4.90 (1H, br s, CbzNH), 5.11 (2H, s, C₆H₅CH₂OCO), 7.35 (5H, m, C₆H₅).

Analyzed Calcd for C₁₂H₁₇NO₃: C 64.55, H 7.67, N 6.27.

Found: C 65.16, H 8.29, N 5.66.

This obtained compound was identical with the authentic (2S,3S)-N-Cbz-3-amino-2-butanol synthesized from N-Cbz-D-threonine.

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References

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