NOVEL ANTIFUNGAL ANTIBIOTICS MANIWAMYCINS A AND B

II. STRUCTURE DETERMINATION

YOSHIO TAKAHASHI, MASAHITO NAKAYAMA, ISAMU WATANABE, TAKEO DEUSHI, HIROYUKI ISHIWATA, MASAMI SHIRATSUCHI and GENJI OTANI

Tokyo Research Laboratories, Kowa Co., Ltd.,
Higashimurayama, Tokyo 189, Japan

(Received for publication June 24, 1989)

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; calcd for $C_{10}H_{18}N_2O_2$ 198.1369) the molecular formula of maniwamycin A was established as $C_{10}H_{18}N_2O_2$. 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-BH872α.[4]

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

Assignment	Maniwamycin A		Maniwamycin B	
	^1H	^{13}C	^1H	^{13}C
C-1	2.10 s	27.1 q	1.06 d (6.5)	19.2 q
C-2	—	205.0 s	3.69~3.80 m	67.5 d
C-3	4.52 q (7.5)	65.1 d	3.99 quintet (6.5)	60.5 d
C-4	1.37 d (7.5)	14.5 q	0.99 d (6.5)	11.6 q
C-1′	7.23 dd (14.0, 1.0)	136.9 d	7.08 d (14.0)	138.1 d
C-2′	6.90 dt (14.0, 7.5)	135.3 d	6.87 dt (14.0, 7.5)	133.7 d
C-3′	2.22 br q (7.5)	27.3 t	2.20 q (7.5)	27.2 t
C-4′	1.27~1.48 m	29.8[b] t	1.24~1.47 m	29.9[b] t
C-5′		21.6[b] t		21.7[b] t
C-6′	0.89 t (7.0)	13.6 q	0.89 t (7.0)	13.6 q

[a] ppm and *J*(Hz) in parentheses.
[b] Tentative assignments.

Fig. 1. ^{1}H-^{13}C COSY NMR spectrum of maniwamycin A (DMSO-d_6).

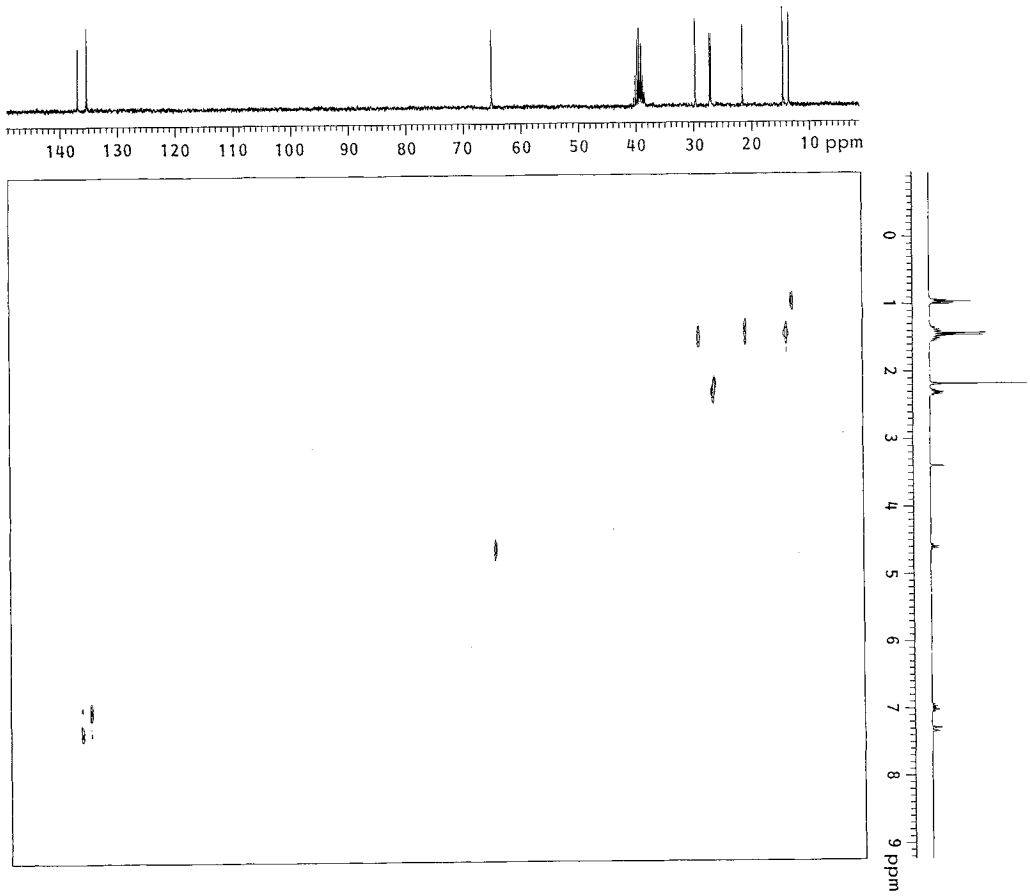
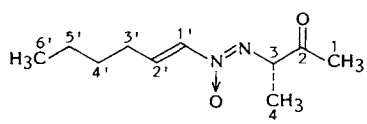
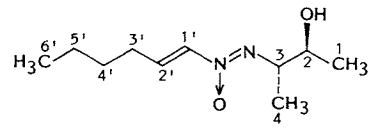


Fig. 2. The structures of maniwamycins A and B.



Maniwamycin A



Maniwamycin B

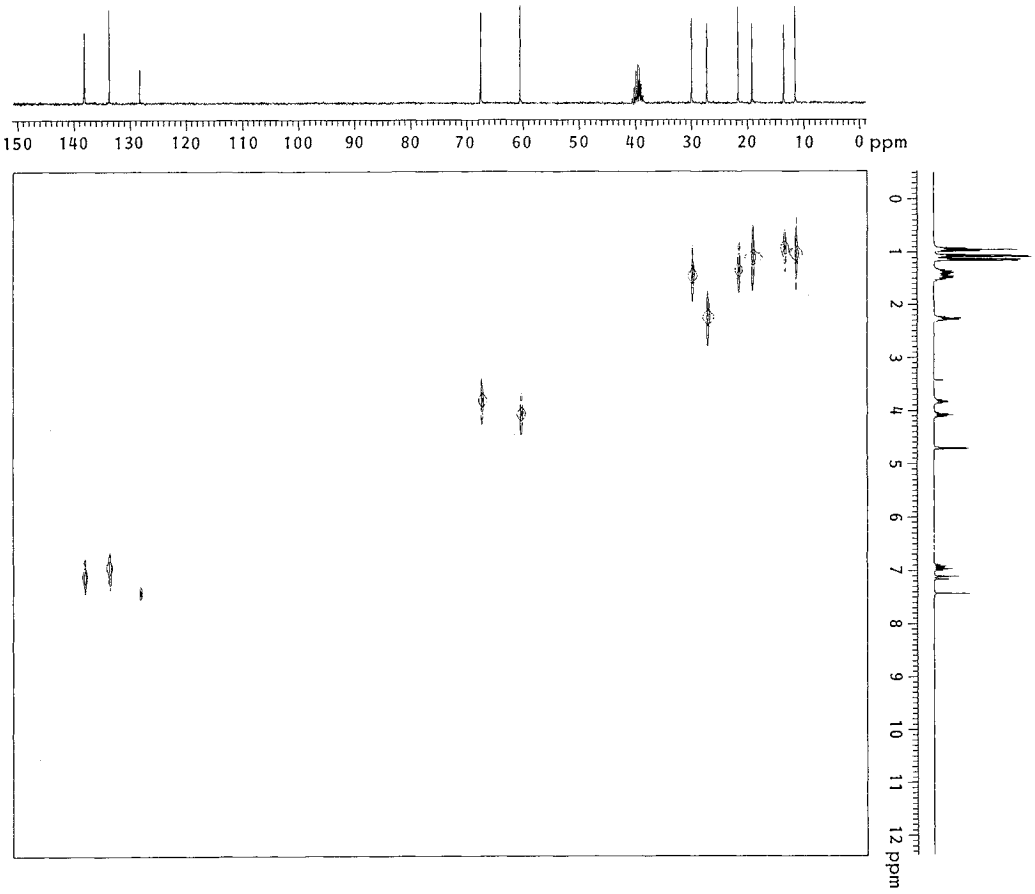
From the above described data, we propose that the structure of maniwamycin A is *Z*-(3*S*)-2-oxobutane-3-*NNO*-azoxy-1′-(*E*-1′-hexene) as shown in Fig. 2.

Structure of Maniwamycin B

The results of FAB-MS and HREI-MS of maniwamycin B showed the formula of this antibiotic as $C_{10}H_{20}N_2O_2$ (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*-(2*S*,3*S*)-2-hydroxybutane-3-*NNO*-azoxy-1′-(*E*-1′-hexene)

Fig. 3. ^{1}H-^{13}C COSY NMR spectrum of maniwamycin B (DMSO-d_6).



as shown in Fig. 2.

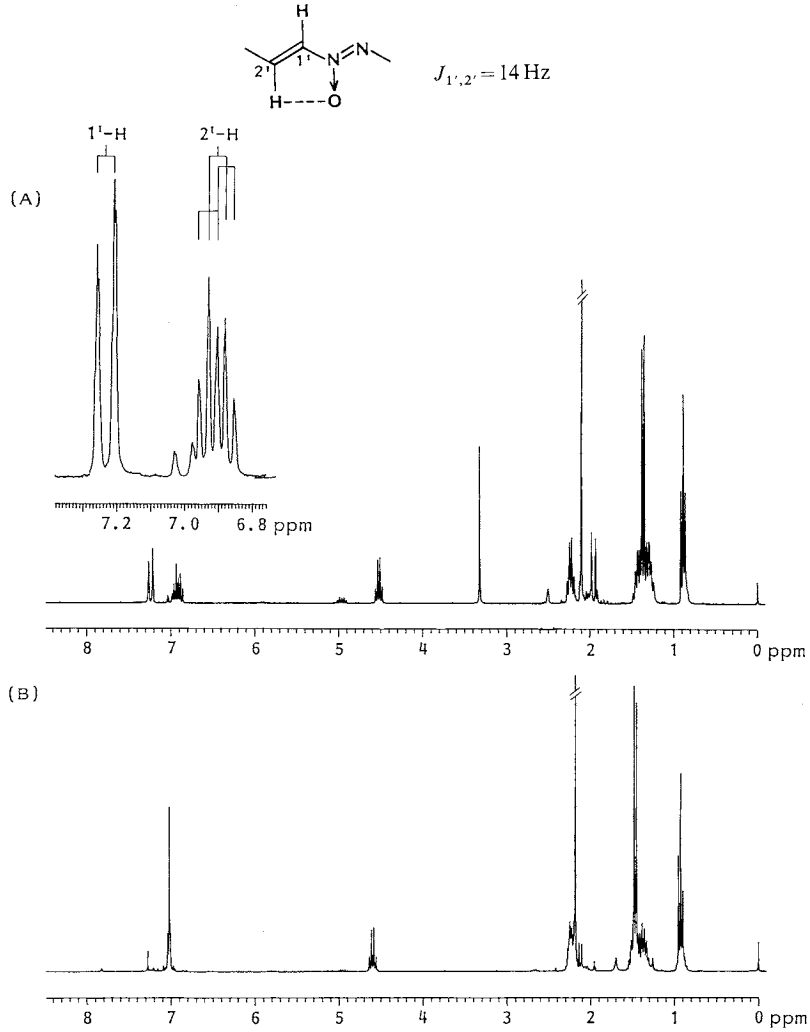
Geometrical and Stereochemical Studies

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 $CDCl_3$,[1] 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 (λ_{max}^{EtOH} nm (ε) 221 (5,440)) of the reduced derivative (H_2-Rh - Al_2O_3) of maniwamycin A[2] and from biogenetic point of view of other known naturally occurring azoxy compounds. STEVENS *et al.*,[6] cleaved the azoxy linkage of elaiomycin by hydrogenolysis over platinum dioxide in acetic acid to get D-threonine. Under similar conditions maniwamycin B was cleaved to get (2*S*,3*S*)-3-amino-2-butanol which was isolated as the benzyloxy-carbonyl derivative.

Only four antibiotics elaiomycin,[5] LL-BH872α,[4] valanimycin[7] and jietacins[8] 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*-α,β-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: $[\alpha]_D^{22}$ $-78.6°$ (*c* 0.5, $CHCl_3$); IR $\nu_{max}^{CHCl_3}$ cm^{-1} 2920, 1717, 1490; UV λ_{max}^{EtOH} nm (ε) 221 (5,440); ^1H NMR (270 MHz, $CDCl_3$) δ 0.89 (3H, t, J=6.5 Hz, 6'-CH_3), 1.26~1.41 (6H, m, 3', 4', 5'-CH_2), 1.42 (3H, d, J=7.0 Hz, 4-CH_3), 1.92~2.06 (2H, m, 2'-CH_2), 2.18 (3H, s, 1-CH_3), 4.22 (2H, t, J=7.0 Hz, 1'-CH_2), 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 to give crude material (*ca.* 1 g). This crude material was treated with diazomethane solution in ether to remove residual acetic acid. The resultant etherial solution was concentrated under reduced pressure and the residue was dissolved in anhydrous dichloromethane (5 ml). To this solution, triethylamine (1.0 ml, 7.2 mmol) and benzyl chloroformate (1.0 ml, 7.0 mmol) were added dropwise at 0°C. The reaction mixture was stirred for 30 minutes and then poured into water (20 ml) and extracted with ether (75 ml × 2). The extract was washed with 1 N hydrochloric acid (10 ml), saturated aqueous sodium bicarbonate (10 ml) and brine (10 ml), and dried over anhydrous sodium sulfate. After concentration under reduced pressure, the residual material was chromatographed on silica gel using chloroform as eluent. Further purification by preparative TLC (silica gel, $CHCl_3$-MeOH (9 : 1)) gave (2*S*,3*S*)-*N*-Cbz-3-amino-2-butanol (103 mg, 20%) as a colorless oil: $[\alpha]_D^{22}$ $+7.6°$ (*c* 1.0, $CHCl_3$); IR $\nu_{max}^{CCl_4}$ cm^{-1} 3430, 1719 (s), 1498; ^1H NMR (270 MHz, $CDCl_3$) δ 1.18 (3H, d, J=6.5 Hz, 1-CH_3 or 4-CH_3), 1.20 (2H, d, J=6.5 Hz, 1-CH_3 or 4-CH_3), 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, $C_6H_5CH_2OCO$), 7.35 (5H, m, C_6H_5).

Anal Calcd for $C_{12}H_{17}NO_3$: 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 (2*S*,3*S*)-*N*-Cbz-3-amino-2-butanol synthesized from *N*-Cbz-D-threonine.

Acknowledgments

We express our gratitude to Mr. T. OHSHIMA for NMR and to Mr. M. YONEDA for MS.

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