Azicemicins A and B, New Antimicrobial Agents Produced by *Amycolatopsis*

II. Structure Determination

TOSHIO TSUCHIDA, RYUICHI SAWA, YOSHIKAZU TAKAHASHI, HIRONOBU IINUMA, TSUTOMU SAWA, HIROSHI NAGANAWA and TOMIO TAKEUCHI

Institute of Microbial Chemistry,
3-14-23 Kamiosaki, Shinagawa-ku, Tokyo 141, Japan

(Received for publication March 27, 1995)

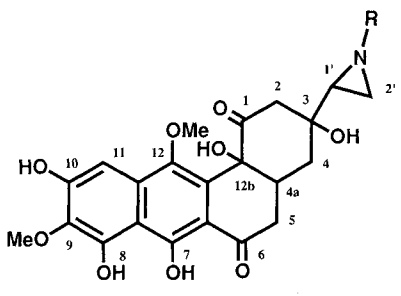
A new structural class of antibiotics, azicemicins A (**1**) and B (**2**) were isolated from the culture broth of *Amycolatopsis* sp. MJ126-NF4. Their structures were elucidated from their physico-chemical properties, various NMR experiments and chemical transformations and were shown to be 3-(1-methyl-2-aziridinyl)- and 3-(2-aziridinyl)-3,4-dihydro-3,7,8,10,12b-pentahydroxy-9,12-dimethoxybenz[a]anthracene-1,6(2H,5H)-dione, respectively.

In the course of our screening program for new antibiotics, we isolated antibiotics of a new type, azicemicins A (**1**) and B (**2**), from the fermentation broth of *Amycolatopsis* sp. MJ126-NF4. In the preceding paper[1], we have described the taxonomy and fermentation of the organism, and the isolation, characterization and biological activities of **1** and **2**. In this paper, we describe details of the structural elucidation of **1** and **2**.

Results

The physico-chemical properties of azicemicins A (**1**) and (**2**) were described in the previous paper[1]. Compounds **1** and **2** showed the same Rm value, 0.51 (L-alanine=1, 3500 V, 15minutes, formic acid - acetic acid - water=25:75:900, pH 1.8). The basicity of **1** and **2** suggested the presence of a nitrogen atom in their structure. The IR spectrum of **1** indicated the presence of hydroxyl, nonchelated carbonyl and chelated carbonyl groups at 3430, 1720 and 1630 cm^{-1}, respectively. The UV spectrum of **1** showed absorption maxima at 234, 277, 322, 355 and 419 nm. These absorption maxima were similar to those of the aureolic acid family such as the chromomycins[2,3]. The IR and UV spectrum of **2** were almost same as those of **1**.

Fig. 1. The structures of azicemicins A (**1**) and B (**2**).



1 R=Me
2 R=H

Structure of Azicemicin A

The molecular formula of azicemicin A (**1**) was determined to be $C_{23}H_{25}NO_9$ based on HRFAB-MS data and was supported by ^1H and ^{13}C NMR spectral data (Tables **1** and **2**). All one bond connections between ^1H and ^{13}C were elucidated by the ^1H-^{13}C COSY and DEPT experiments. The NMR data and the molecular formula indicated the presence of three methyls, four methylenes, two sp^3 methines and one sp^2 methine, eleven quaternary carbons, two carbonyl carbons and five hydroxyl groups. The partial structure **1c** (Scheme 1) was elucidated by the ^1H-^1H COSY and ^1H-^{13}C long range couplings in the heteronuclear multiple bond correlation (HMBC)[4] experiments. A methyl signal at δ_H 2.39 was assignable to an N-methyl group from its ^{13}C chemical shift of δ_C 46.9. The long range connectivities from the N-methyl protons to 1'-CH (δ_H 1.53, δ_C 44.0) and 2'-CH_2 (δ_H 1.35, 1.9, δ_C 31.7) which were coupled to each other suggested the presence of an aziridine ring. This result was confirmed by the large $^1J_{CH}$ values of C-1' ($^1J_{C\text{-}1',1'\text{-}H}$=166 Hz) and C-2' ($^1J_{C\text{-}2',2'\text{-}H}$=170 Hz) observed in the proton-coupled INEPT spectrum. The methylene protons 2'-H_2 of the aziridine ring were coupled to an oxygen bearing quaternary carbon C-3 (δ_C 70.8). The ^1H-^1H COSY spectral data showed the connectivity from 4-H_2 (δ_H 1.9) to 5-H_2 (δ_H 2.53, 3.49). The long range connectivities from 4-H_2 to C-1' and from 4a-H (δ_H 2.21) and 4-H_2 to C-3 constructed the partial structure, **1a**. The methylene protons 2-H_2 (δ_H 2.57, 2.95)

Table 1. ^{1}H NMR (400 MHz) of azicemicins A (**1**), B (**2**) and derivatives of **1** in $CDCl_3$[a].

Position	1	2	3	4	5[e]	6[f]
1						
2	2.57 (dd 2.0, 12.7)[b]	2.65 (d 12.2)	2.56 (dd 2.0, 12.0)	2.57 (dd 1.5, 12.0)	2.60 (dd 2.4, 12.2)	2.54 (dd 2.4, 12.2)
	2.95 (d 12.7)	3.00 (d 12.2)	2.96 (d 12.0)	2.95 (d 12.0)	3.02 (d 12.2)	2.98 (d 12.2)
3						
4	1.9 (m)[c]	1.9 (m)[c]	1.9 (m)	1.9 (m)	1.60 (t 13.7)	1.9 (m)
					1.99 (m)	
4a	2.21 (m)	2.20 (m)	2.23 (m)	2.24 (m)	2.47 (m)	2.19 (m)
5	2.53 (dd 2.0, 17.6)	2.51 (dd 2.4, 17.6)	2.55 (dd 2.0, 18.0)	2.55 (dd 1.5, 18.0)	5.42 (d 6.8)	2.46 (dd 2.4, 16.6)
	3.49 (dd 5.4, 17.6)	3.48 (dd 5.4, 17.6)	3.51 (dd 5.0, 18.0)	3.5 (d 18.0)		3.43 (dd 4.9, 16.6)
6						
6a						
7						
7a						
8						
9						
10						
11	6.93 (s)	6.94 (s)	7.09 (s)	6.85 (s)	6.84 (s)	6.86 (s)
11a						
12						
12a						
12b						
1′	1.53 (dd 3.4, 6.3)	2.24 (dd 3.4, 6.3)	1.53 (dd 3.0, 6.5)	1.51 (dd 3.0, 6.5)	1.52 (dd 3.4, 6.3)	d
2′	1.35 (d 6.3)	1.54 (d 3.4)	1.35 (d 6.5)	1.35 (d 6.5)	1.34 (d 6.3)	1.36 (d 6.3)
	1.9[c]	1.9[c]	1.84 (d 3.0)	1.88 (d 3.0)	1.88 (d 3.4)	1.89 (d 3.4)
N-Me	2.38 (s)		2.39 (s)	2.38 (s)	2.39 (s)	2.38 (s)
9-OMe	4.07 (s)	4.08 (s)	4.0[c]	3.97 (s)	3.97 (s)	3.97 (s) or 3.98 (s)
12-OMe	3.64 (s)	3.65 (s)	3.69 (s)	3.69 (s)	3.64 (s)	3.71 (s)
3-OH		3.49 (s)	3.06 (s)	3.04 (s)		
7-OH			15.1 (s)	16.43 (s)		
8-OH	10.2 (s)	10.2 (s)		9.97 (s)	9.71 (s)	
10-OH						
12b-OH	4.93 (s)	4.68 (s)	4.67 (s)	4.67 (s)	4.61 (s)	4.72 (s)
8-OMe			4.0[c]			
10-OMe			4.0[c]	4.00 (s)	3.97 (s)	3.97 (s) or 3.98 (s)

[a] Chemical shifts in ppm from TMS as an internal standard. [b] Coupling constants in J=Hz. [c] These signals were obscured by overlapping signals. [d] Overlapped with H_2O. [e] *tert*-Butyl 1.15 (s). [f] *tert*-Butyl 1.14 (s) and 1.18 (s).

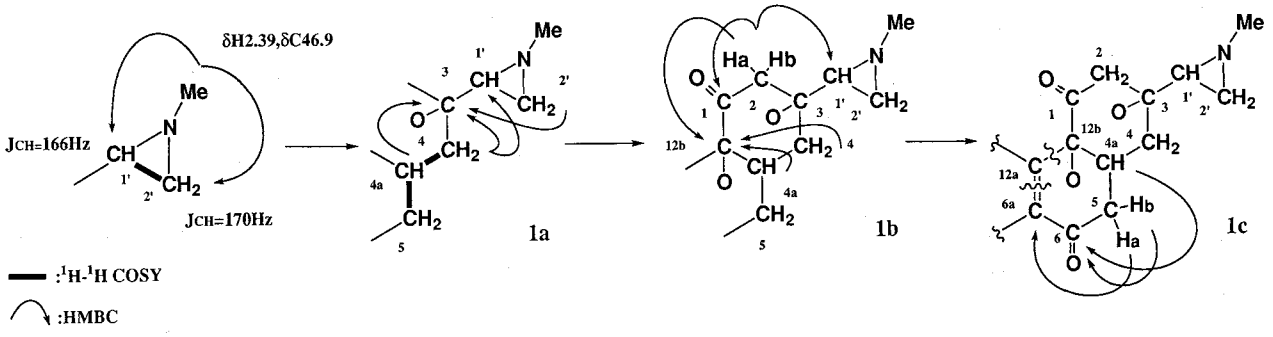
Table 2. ^{13}C NMR data (100 MHz) of azicemicins A (**1**), B (**2**) and methylether derivatives of **1** in $CDCl_3$[a].

Position	1	2	3	4	Position	1	2	3	4
1	206.5	206.5	206.7	206.6	11	98.3	98.3	98.4	98.3
2	47.2	47.3	47.2	47.2	11a	131.5	131.5	131.6	131.1
3	70.8	70.8	70.9	70.8	12	143.9	143.9	143.2	143.9
4	41.5	41.2	41.7	41.7	12a	123.9	123.9	125.0	124.1
4a	41.1	41.1	41.1	41.7	12b	75.5	75.6	76.0	75.7
5	37.5	37.4	38.5	37.2	1′	44.0	37.4	44.0	43.9
6	200.7	200.7	201.7	201.2	2′	31.7	33.4	31.7	31.7
6a	105.7	105.8	107.5	106.1	N-Me	46.9		46.9	46.9
7	164.6	164.6	162.2	163.6	9-OMe	60.9	61.0	[b]	60.9
7a	110.2	110.3	116.5	110.8	12-OMe	62.3	62.4	62.3	62.3
8	150.7	150.8	153.1	151.7	8-OMe			[b]	
9	133.5	133.5	143.6	135.7	10-OMe			[b]	56.1
10	154.2	154.2	157.1	158.1					

[a] Chemical shifts in ppm from TMS as an internal standard. [b] 56.1, 61.5, 62.3 unassigned.

were coupled to C-1′ and a carbonyl carbon C-1 (δ_C 206.5). The protons 4-H_2, 4a-H and 2-Ha (δ_H 2.57) were coupled to an oxygen bearing quaternary carbon C-12b (δ_C 75.5). These data suggested the presence of a cyclohexanone ring composed of C-1, C-2, C-3, C-4, C-4a and C-12b as shown in the partial structure, **1b**. On the other hand, 4a-H and 5-H_2 were correlated with a carbonyl carbon C-6 (δ_C 200.7), which showed the connectivity between C-5 (δ_C 37.5) and C-6. A signal at δ_H 2.53 (5-Ha) showed long range coupling to an aromatic ring carbon

Scheme 1.

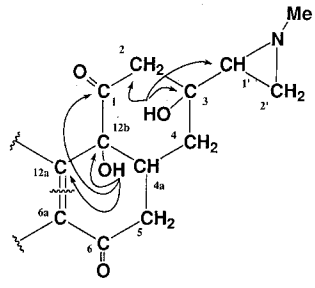


C-6a (δ_C 105.7). From these data, the partial structure **1c** was depicted.

No extension of the carbon frame in **1c** was made from the HMBC spectrum of **1** because of the absence of ^1H-^{13}C long range couplings from 12b-OH or 4a-H to C-12a. However, long range connectivities around C-12a and C-12b were found in the HMBC spectrum of dimethylether derivative (**3**) and methylether derivative (**4**), which were obtained by the reaction of **1** with diazomethane in methanol. The HMBC spectrum of **3** gave long range couplings from the two hydroxy (12b-OH and 3-OH) protons to their two- and/or three-bonds removed carbons (Fig. 2). The long range cross peaks between the hydroxy proton 12b-OH (δ_H 4.67) and C-1 (δ_C 206.7), C-12a (δ_C 125.0) and C-12b (δ_C 76.0) indicated that this hydroxyl group was attached to the quaternary carbon C-12b, and C-12b connected to C-12a. The other hydroxy proton at δ_H 3.06 (3-OH) showed couplings to C-2 (δ_C 47.2), C-3 (δ_C 70.9) and C-1′ (δ_C 44.0), and was attached to the quaternary carbon C-3. On the basis of the above data, all connectivities of the nonaromatic part of **1** were clarified as shown in Fig. 2.

The comparision of the UV absorption maxima of **1** with those of the chromomycins[2,3] suggested that the chromophore of **1** was a naphthalene derivative, 6,8,9-trihydroxy-1,2,3,4-tetrahydroanthracen-1-one. For the structure elucidation of this remaining naphthalene moiety including two methoxy groups and three hydroxyl groups, the following long range bond connectivities in the HMBC spectrum of **1** were applicable; i) from 11-H (δ_H 6.93) to C-7a (δ_C 110.2), C-9 (δ_C 133.5), C-10 (δ_C 154.2) and C-12 (δ_C 143.9), ii) from 12-OMe (δ_H 3.64) to C-12, iii) from 9-OMe (δ_H 4.07) to C-9, iv) from 8-OH (δ_H 10.2) to C-7a, C-8 (δ_C 150.7) and C-9.(Fig. 3, **1d**). Moreover, NOE was observed between the aromatic proton 11-H and 12-OMe (δ_H 3.64). From these data, two structures **1d** and **1e** were possible for **1** (Fig. 3).

Fig. 2. Partial structure of dimethyl ether derivative **3** elucidated from HMBC experiments.

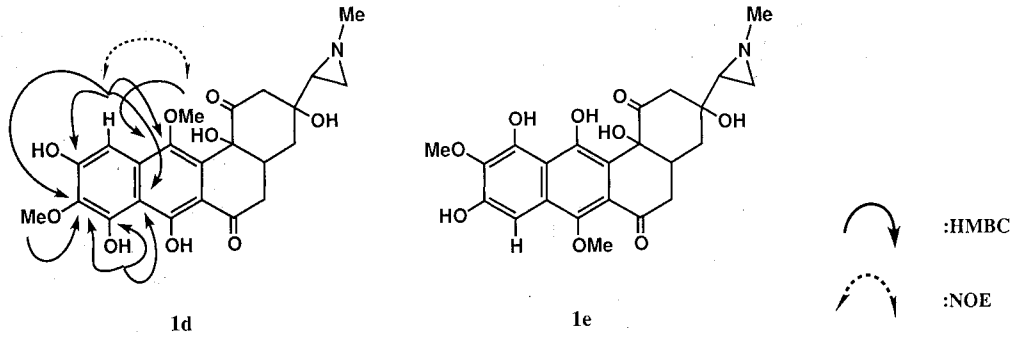


Although various NMR spectral data of **3** and **4** showed the positions of the newly introduced one or two methyl groups (Fig. 4), there were still two possibilities (**1d** or **1e**). To confirm the connectivity between C-6a and C-7 (C-12a and C-12), a 6,7-O-silyl derivative (**5**) was prepared from the methylether derivative (**4**). The methylether derivative (**4**) was treated with di-*tert*-butylsilyl bis(trifluoromethane-sulfonate) in the presence of 2,6-lutidine[5] in CH_2Cl_2 to afford the desired compound **5** and a 7,8-O-silyl derivative (**6**) (Fig. 5). The ^1H NMR spectral data of **5** and **6** are summarized in Table 1. The ^1H-^1H COSY spectrum of **5** clarified that a newly formed olefinic proton 5-H (δ_H 5.47) was connected to 4a-H (δ_H 2.47). The 5-H proton was revealed to be an olefinic proton of the silyl enol ether. Thus, the connectivity between C-6 and C-7 was established and the structure of azicemicin A (**1**) was established as shown in Fig. 1.

Structure of Azicemicin B

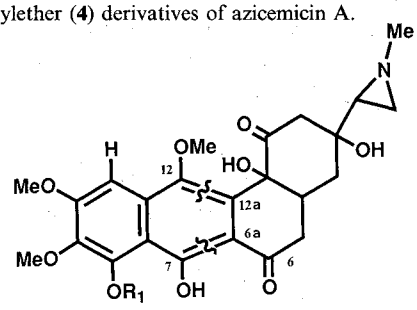
The molecular formula of azicemicin B (**2**) was determined to be $C_{22}H_{23}NO_9$ by HRFAB-MS data and was supported by ^1H and ^{13}C NMR spectral data. As summarized in Tables 1 and 2, ^1H and ^{13}C chemical shifts of **2** were similar to those of **1** except for the aziridine ring. No N-methyl group was observed in the

Fig. 3. Possible structures of naphthalene ring elucidated from NOE and HMBC experiments of **1**.



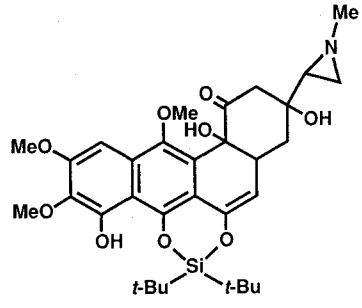
1d 1e

Fig. 4. Structures of 8,10-O-dimethylether (**3**) and 10-O-methylether (**4**) derivatives of azicemicin A.

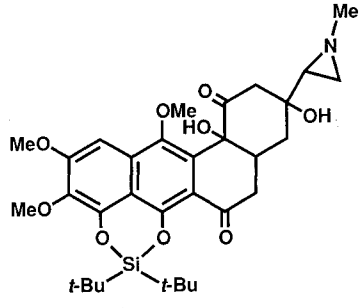


3 R_1=Me
4 R_1=H

Fig. 5. Structures of silyl derivatives **5** and **6**.



5



6

^{1}H and ^{13}C NMR spectra of **2**. These results suggested that the N-methyl group of aziridine ring in **1** was replaced by a hydrogen. For the structure confirmation, **2** was converted to **1** by a treatment with methyl iodide in methanol. TLC and ^{1}H and ^{13}C NMR data of synthetic **1** were identical with those of the natural product (**1**).

Discussion

The azicemicins are a new structural class that contain a chromomycin-like anthracenone moiety, 3,4-dihydro-3,7,8,10,12b-pentahydroxy-9,12-dimethoxy-benz[a]anthracene-1,6(2H,5H)-dione, and an aziridine moiety in their structure. The relative stereostructure of azicemicin A could not be defined by a NOESY experiment. Azicemicin B was the same relative stereostructure as azicemicin A since its N-methyl derivative is identical with natural azicemicin A.

Experimental

General

IR spectra were recorded with a Hitachi I-5020 and Hitachi 260-30 spectrometers. UV spectra were taken on a Hitachi U-3210 spectrometer. ^{1}H and ^{13}C NMR spectra were recorded on a JEOL JNM-GX400 spectrometer. Mass spectra were obtained with a JEOL JMS-SX102 spectrometer.

Preparation of 3 and 4

A small excess of ethereal diazomethane was added to a solution of **1** (28 mg, 0.061 mmol) in MeOH (5 ml) at 0°C, and then the mixture was stirred at room temperature for 8 hours. After evaporation of the solvent under reduced pressure, the residue was purified by preparative TLC (Merck, Kiesel gel 60F_{254}) developed with $CHCl_3$-90% aqueous MeOH (10:1) to give **3** (6.2 mg, 21%) and **4** (9.4 mg, 33%), respectively.

The ^{1}H and ^{13}C NMR data were showed in Tables 1 and 2.

3: FAB-MS *m/z* 488 $(M+H)^+$, HRFAB-MS; *m/z* 488.1914 $(M+H)^+$ (Calcd for $C_{25}H_{30}O_9N$ 488.1920); IR ν_{max} (KBr) cm^{-1} 3410, 1720, 1620, 1580, 1420, 1390; UV λ_{max}^{MeOH} nm (log ε) 234 (4.12), 272 sh (4.29), 280 (4.40),

304 (3.53), 316 (3.49), 386 (3.71), 402 (3.67)

4: HRFAB-MS; *m/z* 474.1751 $(M+H)^+$ (Calcd for $C_{24}H_{28}O_9N$ 474.1764)

IR ν_{max} (KBr) cm^{-1} 3410, 1720, 1630, 1520, 1420, 1390; UV λ_{max}^{MeOH} nm (log ε) 234 (4.11), 277 (4.31), 318 (3.54), 333 (3.38), 406 (3.80)

Preparation of **5** and **6**

Di-*tert*-butylsilyl bis(trifluoromethanesulfonate) (4.6 ml, 0.0125 mmol) and 2,6-lutidine (4.2 ml, 0.0377 mmol) were added to a solution of **4** (5.2 mg, 0.0144 mmol) in CH_2Cl_2 (0.3 ml) at 0°C. This solution was stirred at the same temperature for an hour, and then was diluted with CH_2Cl_2. The CH_2Cl_2 solution was washed with water and dried over with Na_2SO_4. After removal of the solvent under reduced pressure, the residue was purified by preparative TLC (Merck, Kiesel gel $60F_{254}$) which was developed with $CHCl_3$-MeOH (20:1) to give crude **5** and **6**. And then these were applied to a Sephadex LH-20 column (120 ml) and developed with MeOH to give **5** (2.1 mg, 31%) and **6** (1.5 mg, 21%), respectively.

The ^1H and ^{13}C NMR data were showed in Tables 1 and 2.

5: FAB-MS *m/z* 614 $(M+H)^+$, 612 $(M-H)^-$

6: FAB-MS *m/z* 614 $(M+H)^+$, 612 $(M-H)^-$

Conversion from **2** to **1**

Methyl iodide (0.155 ml, 2.49 mmol) was poured into a solution of **2** (11 mg, 0.0249 mmol) in MeOH (1 ml) at room temperature, and the reaction mixture was stirred at the same temperature for 4 hours. After evaporation *in vacuo*, the residue was purified by preparative TLC (Merck, Kiesel gel $60F_{254}$) using $CHCl_3$-90% aqueous MeOH (10:1) to give **1** (2.2 mg, 19%).

References

1) TSUCHIDA, T.; H. IINUMA, N. KINOSHITA, T. IKADA, T. SAWA, M. HAMADA & T. TAKEUCHI: Azicemicins A and B, a new antimicrobial agent produced by *Amycolatopsis* I. Taxonomy, fermentation, isolation, characterization and biological activities. J. Antibiotics 48: 217~221, 1995

2) MIYAMOTO, M.; K. MORITA, Y. KAWAMATSU, S. NOGUCHI, R. MARUMOTO. SASAI, A. NOHARA, Y. NAKADAIRA, Y. Y. LIN & K. NAKANISHI: The reactions of chromomycinone and derivatives. Tetrahedron 22: 2761~2783, 1966

3) FUNAYAMA, S.; M. ISHIBASHI, K. KOMIYAMA & S. ŌMURA: A new antibiotic, okicenone. II. Physico-chemical properties and structure elucidation. J. Antibiotics 44: 819~823, 1991

4) BAX, A. & M. F. SUMMERS: ^1H and ^{13}C assignments from sensitivity-enhanced detection of heteronuclear multiple-bond connectivity by 2D multiple quantum NMR. J. Am. Chem. Soc. 108: 2093~2094, 1986

4) COREY, E. J. & P. B. HOPKINS: Diisopropylsilyl ditriflate and di-*tert*-butylsilyl ditriflate: new reagents for the protections of diols. Tetrahedron Lett. 23: 4871~4874, 1982