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Azinomycins A and B, New Antitumor Antibiotics. II. Chemical Structures

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The structures of azinomycins A and B, new antitumor antibiotics produced by a strain of *Streptomyces*, were determined on the basis of their spectral and chemical properties. The structures of three related metabolites coproduced with these antibiotics were also determined.

Keywords——antitumor antibiotic; azinomycin A; azinomycin B; chemical structure; ^{1}H-NMR; ^{13}C-NMR; FAB-MS

In the course of our screening program for new antitumor antibiotics, a Streptomycete identified as *Streptomyces griseofuscus* S42227 was found to produce two new compounds possessing good antitumor activity in mice, which we named azinomycins A (**1**) and B (**2**), and three biologically inactive compounds structurally related to **1** and **2** (**5**, **6** and **7**). A description of the producing organism, fermentation conditions, physico-chemical properties, antimicrobial and antitumor activities of azinomycins A and B will be published elsewhere.[1] In the present paper, the structure determination of these antibiotics and related metabolites will be discussed in detail.

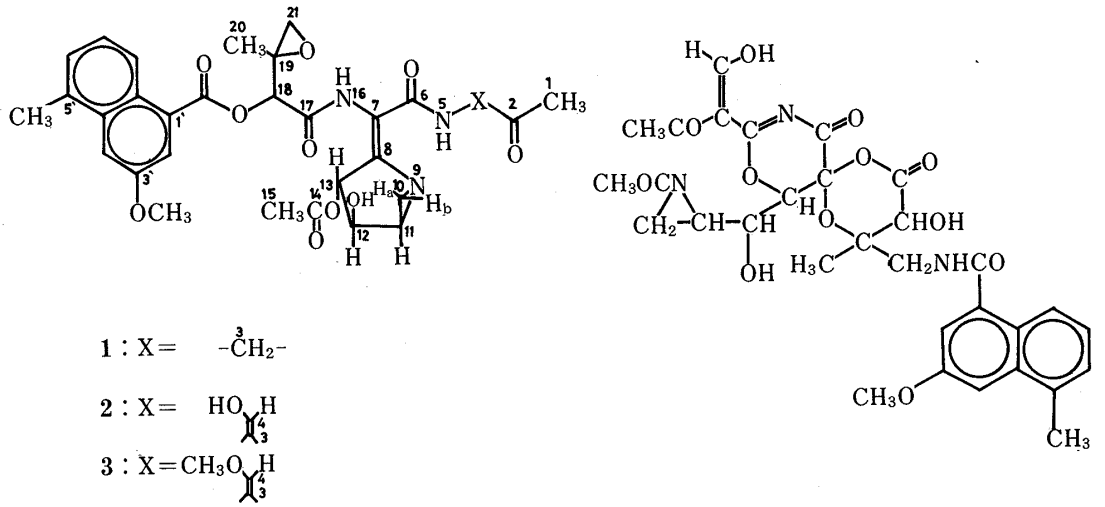
The molecular weights of **1** and **2** were determined by positive and negative ion fast atom bombardment mass spectrometry (FAB-MS) as 595 and 623, respectively. Elemental analysis, proton and carbon-13 nuclear magnetic resonance (^{1}H- and ^{13}C-NMR) and MS studies confirmed the molecular formulas of **1** and **2** as $C_{30}H_{33}N_3O_{10}$ and $C_{31}H_{33}N_3O_{11}$, respectively. The spectral data for **1** and **2**[1] were similar to those for carzinophilin (**4**),[2a] but the molecular formulas of azinomycins A and B were different from that of carzinophilin ($C_{31}H_{33}N_3O_{12}$).[2c]

The molecular formulas of **5**, **6** and **7** were determined by electron impact-mass spectra (EI-MS) and elemental analysis as $C_{18}H_{19}NO_5$, $C_{14}H_{14}O_3$ and $C_{13}H_{13}NO_2$, respectively. The ultraviolet (UV) spectra, especially those of **5** and **6**, were very similar to those of **1** and **2**, which suggested that **5** and **6** have the same chromophore as that of **1** and **2**. The ^{1}H- and ^{13}C-NMR data of **7** and its molecular formula suggested that **7** is a trisubstituted naphthalene derivative with methyl, methoxyl and carbamoyl substituents. When compared with published data, the spectral data for **7** were coincident with those for 3-methoxy-5-methyl-naphthalene-1-carboxamide,[2b] which was reported as a degradation product of carzinophilin. The ^{1}H-NMR data for **6** were very similar to the published data for **8**,[2b] except that the signal of an ester methyl group (δ 3.96) was present. Alkaline hydrolysis of **7** and methylation of the hydrolysate gave a methyl ester whose spectral data were coincident with those for **6**. Thus, **6** was determined to be methyl-3-methoxy-5-methyl-naphthalene-1-carboxylate. When the ^{1}H- and ^{13}C-NMR data for **5** were compared with those for **6** (Table II and Experimental), the presence of a 3-methoxy-5-methyl-naphthalene-1-carbonyl moiety in **5** was evident, and the remaining part of **5** ($C_5H_8NO_3$) was indicated to be connected to the naphthalene ring by an ester linkage. The oxymethine group in **5** (^{1}H-NMR: δ 5.20, s, ^{13}C-NMR: δ 75.9) must be attached to this naphthalene ring, and because the signal of this

methine group was a singlet, the oxymethine group must be connected to two quaternary carbons. Based on the data cited above and the following groups [a carbamoyl group (^{1}H-NMR: δ 6.14, 5.76, D_2O-exchangeable, ^{13}C-NMR: δ 168.7, IR: 1675 cm^{-1}), an oxirane ring (^{1}H-NMR: δ 3.00, d, J=4.3 Hz, δ 2.76, d, J=4.3 Hz, ^{13}C-NMR: δ 55.8, 53.3) and a methyl group (^{1}H-NMR: δ 1.52, ^{13}C-NMR: δ 17.6)], the structure of **5** was concluded to be as shown in Fig. 2.

Comparison of the ^{1}H- and ^{13}C-NMR data for **1** and **2** with those for **5**, **6** and **7** (Tables II and IV, and Experimental) showed that **1** and **2** also have the 3-methoxy-5-methylnaphthalene-1-carbonyl moiety as a partial structure. Positive ion FAB-MS of **1** and **2** showed a common fragment ion at m/z 199 corresponding to this partial structure (Table I). Further, comparison of the ^{1}H- and ^{13}C-NMR data for **1** and **2** with those for **5** showed that **1** and **2** have the structure of **5**, other than the carbamoyl group, as a partial structure.

Azinomycins A and B were acid-labile, and the decomposition of **2** proceeded more easily than that of **1**, presumably because an acidic hydroxyl group was present in **2**. Treatment of **2** with diazomethane gave the methyl derivative **3**, which was purified by column chromatography on silica gel. In the positive ion FAB-MS of **1**, **2** and **3**, $[M+H]^+$ ions were observed at m/z 596, 624 and 638, respectively. Besides the $[M+H]^+$ ions, common fragment ions were observed at m/z 523 and 199 among **1**, **2** and **3**, indicating the presence of the same partial

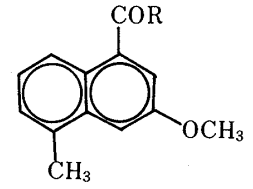


1 : X= $-\overset{3}{C}H_2-$

2 : X= HO, H (4, 3)

3 : X= CH_3O, H (4, 3)

Fig. 1



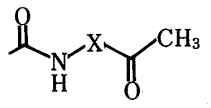
5 : R= $-OCH(CH_3)(CONH_2)$

6 : R= $-OCH_3$

7 : R= $-NH_2$

8 : R= $-OH$

Fig. 2

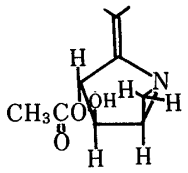


partial structure A

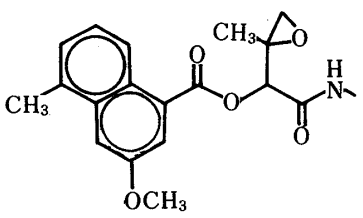
1 : X= $-CH_2-$

2 : X= HO, H

3 : X= CH_3O, H



partial structure B

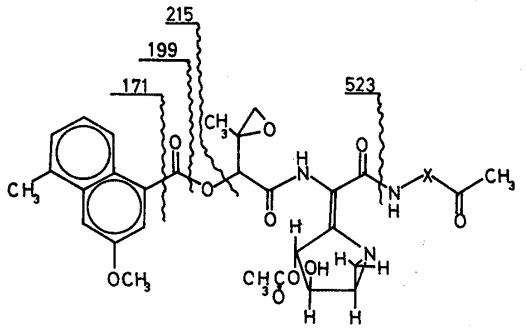


partial structure C

Fig. 3

TABLE I. Pseudomolecular Ions and Major Fragment Ions Observed in the Positive and Negative FAB-MS of **1**, **2** and **3**

	1	**2**	**3**
Positive ion FAB-MS	596 $[M+H]^+$	624 $[M+H]^+$	638 $[M+H]^+$
m/z	523	523	523
	199	199	199
Negative ion FAB-MS	594 $[M-H]^-$	622 $[M-H]^-$	636 $[M-H]^-$
m/z	535 $[M-CH_3COOH]^-$	562 $[M-H-CH_3COOH]^-$	576 $[M-H-CH_3COOH]^-$
	215	215	215
	171	171	171



structures in them (Table I). Comparison of the mass fragmentation of **2** with those of **1** and **3** suggested that the acidic hydroxyl group in **2** was present in the part corresponding to the fragment designated as [M-523].

In the ^{13}C-NMR spectra of **1**, **2** and **3**, signals due to 30, 31 and 32 carbons were observed, respectively. When the ^{13}C-NMR data for **2** were compared with those for **3** (Tables II and III), a signal due to the methoxyl group (δ 62.2) newly formed by methylation was observed in **3** and the signal of the C-4 carbon (δ 150.6) was shifted to lower field (δ 154.5) in **3**. Thus the acidic hydroxyl group in **2** was determined to be attached to the C-4 carbon.

The ^{13}C-NMR data for **1** and **3** were very similar, but different in part. While ^{13}C signals due to the carbons at C-1, C-2, C-6 and C-7 in **3** were observed at δ 25.6 (q), 193.8 (s), 161.4 (s) and 120.4 (s), respectively, those in **1** were observed at 27.2 (q), 202.6 (s), 163.2 (s) and 120.1 (s), respectively. The carbon at C-3 (δ 50.6, t) in **1** may correspond to one of the carbons at C-3 (δ 117.6, s) or C-4 (δ 154.5, d) in **3**. The chemical shifts of the other carbons in **1** and **3** were coincident with each other, so the difference of the structures of **1** and **3** may lie in the partial structure containing the carbons at C-1, C-2, C-3, and C-6 in **1**, and at C-1, C-2, C-3, C-4, C-4 OCH_3 and C-6 in **3** .

The ^{1}H-NMR data for **1** and **3** showed differences corresponding to those observed in the ^{13}C-NMR data for **1** and **3**. The methyl protons (H-1) in **1** and **3** were observed at δ 2.16 (s) and 2.23 (s), respectively. Based on the ^{13}C-NMR data for the C-1 and C-2 carbons, these methyl protons were assigned as acetyl methyl groups. The methylene protons at H-3 (δ 4.28, dd) were observed in **1** only, and were coupled with an amide proton (5-NH, δ 10.09, dd) in **1**. The carbon at C-6 was assumed to be the amide carbon, connected to the 5-NH group. Therefore, the partial structure A in **1** was derived as shown in Fig. 3. The methine proton at H-4 (δ 7.16, s), confirmed by a ^{1}H–^{13}C selective decoupling experiment, was observed in **3** only. The methoxy protons at δ 3.88 in **3** were assigned as the H-4 protons. The carbon at C-3 in **3** was assumed to form the double bond with the methine carbon at C-4. Nuclear Overhauser effect (NOE) enhancements were observed between the H-1 and H-4 protons and between the 4-OCH_3 and H-4 protons in **3** (Fig. 4). Furthermore, in view of the presence of the amide proton (δ 10.86, s) in **3**, the partial structure A for **3** was derived as shown in Fig. 3.

TABLE II. ^{13}C-NMR Data for **1**, **2**, **5**, **6**, **7** and **9**

Carbons	**1**	**2**	**5**	**6**	**7**[a]	**9**
C-1	27.2	23.9[b]				23.6
C-2	202.6	191.1[b]				195.4
C-3	50.6	118.4				117.6
C-4		150.6[b]				154.3
C-6	163.2	161.8				155.7
C-7	120.1	119.1				92.5
C-8	149.6	153.9				167.6
C-10	35.8	36.8				14.5
C-11	45.4	46.7				57.3
C-12	76.9	76.7				74.3
C-13	84.0	84.3				80.3
C-14	172.7	172.2				170.7
C-15	20.7	20.6				20.9
C-17	163.8	164.2	168.7			166.5
C-18	76.7	76.5	75.9			76.9
C-19	56.0	56.0	55.8			56.3
C-20	17.0	17.2	17.6			18.3
C-21	53.7	53.4	53.3			53.1
C-1′	128.4	128.1	128.3	129.6	133.6	127.8
C-1′ CO	165.7	165.5	165.6	167.8	173.3	166.0
C-1′ $COCH_3$				52.2		
C-2′	121.8	122.0	122.0	121.4	117.5	122.0
C-3′	156.0	155.9	155.9	156.0	156.6	155.9
C-3′ OCH_3	55.6	55.5	55.6	55.5	55.6	55.6
C-4′	108.6	108.5	108.5	108.1	105.7	108.8
C-4a′	133.1	133.1	133.2	133.1	133.6	133.2
C-5′	134.4	134.3	134.4	134.4	134.6	134.4
C-5′ CH_3	20.0	19.9	20.0	20.0	20.0	20.0
C-6′	127.7	127.7	127.8	127.5	128.0	127.7
C-7′	125.1	125.1	125.2	124.8	124.6	125.3
C-8′	123.9	123.7	123.8	124.0	123.7	123.9
C-8a′	126.9	126.8	126.9	126.9	125.9	127.1

a) Measured in $CDCl_3+CD_3OD$ (1 : 1 v/v) solution. *b*) Observed as broad signals.

The common fragment ion at *m/z* 523 in the positive FAB-MS of **1** and **3** can be explained in terms of cleavage of the amide bond between the C-6 carbonyl group and the 5-NH group.

To confirm the results described above, long-range selective decoupling (LSPD) experiments were carried out on **3**. On irradiation of the H-5 proton in **3**, the signals due to the C-3, C-4, C-6 and C-7 carbons were sharpened and the intensity increased (Fig. 5), which confirmed the partial structure A, and further suggested that the C-6 carbon in the partial structure A was connected to the C-7 carbon. The fact that the C-6 carbon was attached to the C-7 carbon was also suggested by comparison of the ^{13}C-NMR data for **1** and **3**.

LSPD experiments were also applied to establish the partial structure of **3** corresponding to the structure of **5**. On irradiation of the H-2′ or H-18 proton, the double doublet due to the 1′-CO carbon collapsed to a doublet. On irradiation of the H-18 or H-16 proton, the double doublet due to the C-17 carbon collapsed to a doublet. These results confirmed the assignment of the C-17 carbon and the presence of the partial structure C (Fig. 3). Further, the shape of the signal due to the C-8 carbon (δ 149.5) changed and the intensity of the signal due to the C-6 carbon increased on irradiation of the H-16 proton, suggesting that the partial structure C was connected to the partial structure A *via* the C-7 carbon, and that the carbon at C-7 was connected to the carbon at C-8.

TABLE III. ^{13}C-NMR Data for **3**[a)]

Carbons	[b)]	$^1J_{C,H}$ (Hz)	$^2J_{C,H}$, $^3J_{C,H}$ (Hz)
C-1	25.6 Q, s	128	
C-2	193.8 S, m		
C-3	117.6 S, d		^2J(C-3, H-4) 12.3
C-4	154.5 D, m	178	
C-4 OCH_3	62.2 Q, d	142	^2J(C-4 OCH_3, H-4) 6.0
C-6	161.4 S, s		
C-7	120.4 S, d		^3J(C-7, H-13) 3.2
C-8	149.5 S, m		
C-10	35.9 DD, d	172, 176	^3J(C-10, H-12) 8.0
C-11	45.3 D, m	182	
C-12	77.0 D, m	146	
C-13	84.0 D, dd	145	^2J(C-13, H-12) 5.0, ^3J(C-13, H-11) 5.0
C-14	172.8 S, dq		^2J(C-14, H-15) 7.0, ^3J(C-14, H-13) 2.8
C-15	20.7 Q, s	128	
C-17	163.8 S, dd		^2J(C-17, H-16) 2.8, ^2J(C-17, H-18) 2.8
C-18	76.6 D, m	152	
C-19	56.0 S, m		
C-20	17.2 Q, m		
C-21	53.5 T, m	178	
C-1′	128.4 S, d		^3J(C-1′, H-8′) 3.2
C-1′ CO	165.7 S, dd		^3J(C-1′ CO, H-18) 2.8, ^3J(C-1′ CO, H-2′) 6.3
C-2′	121.8 D, d	162	^3J(C-2′, H-4′) 5.3
C-3′	156.0 S, m		
C-3′ OCH_3	55.6 Q, s	145	
C-4′	108.7 D, d	155	^3J(C-4′, H-2′) 4.6
C-4a′	133.1 S, m		
C-5′	134.4 S, m		
C-5′ CH_3	20.0 Q, d	128	^3J(C-5′ CH_3, H-6′) 6.0
C-6′	127.8 D, m	158	
C-7′	125.1 D, s	158	
C-8′	123.9 D, m	162	
C-8a′	126.9 S, m		

a) Measured on a JEOL JNM GX 400 spectrometer. *b*) Capital and small letters in this column show splitting patterns due to one-bond and long-range ^{13}C–^{1}H couplings, respectively.

Partial structure B, the remaining part of **3** other than the partial structures A and C, consisted of $C_8H_9No_3$, which is a common partial structure for **1**, **2**, and **3**. Partial structure B contained a tetrasubstituted double bond that consisted of the C-7 and C-8 carbons. ^{1}H–^{1}H decoupling experiments and ^{13}C–^{1}H selective decoupling experiments on **3** showed the presence of a partial structure consisting of a methylene group (C-10: δ 35.9, H-10a: δ 2.25, d, H-10b: δ 2.51, d), a methine group (C-11: δ 45.3, H-11: δ 3.22, ddd), a hydroxymethine group (C-12: δ 77.0, H-12: δ 4.63, dd, 12-OH: δ 4.08, s) and an oxymethine group (C-13: δ 84.0, H-13: δ 55.5, d). LSPD experiments irradiating the H-13 (δ 5.55, d) or H-15 protons (δ2.18, s) of **3** showed that the acetyl group was attached to the C-13 carbon. The presence of the acetyl group in **3** was also suggested by the result of negative ion FAB-MS of **3**. Further, on irradiation of the H-13 proton, the doublet due to the C-7 carbon became a singlet, and the shape of the signal due to the C-8 carbon changed. Thus the C-13 carbon was shown to be connected to the C-7 or C-8 carbon. It was already shown that the C-7 carbon was attached to the N-16 nitrogen, C-6 carbon and C-8 carbon (double bond), so the C-13 carbon was confirmed to be connected to the C-8 carbon.

The catalytic hydrogenation of **3** with H_2 over PtO_2 gave a dihydro derivative **9**. The ^{1}H-

TABLE IV. ^{1}H-NMR Data for **1**, **2**, **3**, **9** and **10**

Protons	**1**[a]	**2**	**3**[a]	**9**	**10**
	δ, ppm J, Hz	δ, ppm J, Hz	δ, ppm J, Hz	δ, ppm J, Hz	δ, ppm J, Hz
1	2.20 s	2.24 s	2.24 s	2.08 s	2.10 s
3	4.28 dd 19.8, 5.1				
	4.28 dd 19.8, 5.1				
4		7.32 s	7.19 s	7.20 s	7.12 s
4 OH		12.40 br			
4 OCH_3			3.90 s	3.78 s	3.80 s
5	10.09 dd 5.1, 5.1	12.32 s	10.89 s	7.36 or 7.76	7.40
9 NH				7.36 or 7.76	7.40
10a	2.21 d 4.5	2.30	2.25 d 3.9		
10b	2.53 d 5.1	2.70	2.51 d 5.4		
10 CH_3				1.20 d 4.7	1.10 d 6.5
11	3.23 ddd 5.4, 5.1, 4.5[b]	3.36 m	3.22 ddd 5.8, 5.4, 3.9[b]	3.8	4.16 m
12	4.62 dd 5.4, 3.8	4.64 dd 4.8, 4.0	4.63 dd 5.8, 3.9	3.9	5.12 d 4.3
12 OH	3.98 s	3.96	4.08 s	3.9	
12 $OCOCH_3$					1.98 s
13	5.53 d 3.8	5.50 d 4.0	5.55 d 3.9	5.36 s	5.58 s
15	2.19 s	2.18 s	2.18 s	2.16 s	2.20 s
16	8.54 s	8.20 br	8.50 s	8.16 br	8.32 br
18	5.01 s	5.12 s	5.08 s	5.42 s	5.60 s
20	1.52 s	1.52 s	1.51 s	1.54 s	1.56 s
21a	2.84 d 4.4	2.80 d 4.3	2.80 d 4.3	2.70 d 4.3	2.72 d 4.3
21b	3.00 d 4.4	2.98 d 4.3	2.99 d 4.3	3.14 d 4.3	3.18 d 4.3
2′	7.93 d 2.6	7.94 d 2.9	7.94 d 2.4	8.02 d 2.9	7.96 d 2.9
3′ OCH_3	3.98 s	3.96 s	3.96 s	3.95 s	3.98 s
4′	7.48 d 2.6	7.46 d 2.9	7.46 d 2.4	7.48 d 2.9	7.50 d 2.9
5′ CH_3	2.67 s	2.66 s	2.66 s	2.64 s	2.67 s
6′	7.34 dd 5.9, 3.3	7.32	7.35 dd 7.4, 2.4	7.32	7.36
7′	7.35 dd 5.9, 5.9	7.32	7.36 dd 7.4, 7.4	7.32	7.36
8′	8.56 dd 5.9, 3.3	8.54 dd 7.0, 3.6	8.56 dd 7.4, 2.4	8.64 m	8.64 m

a) Measured at 400 MHz. *b*) $J_{11,12}$, $J_{10b,11}$ and $J_{10a,11}$, respectively.

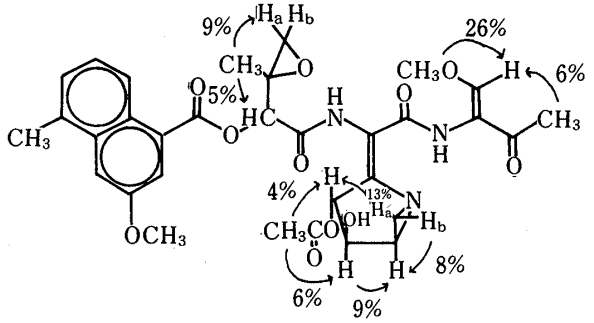


Fig. 4. ^{1}H NOEs for **3**

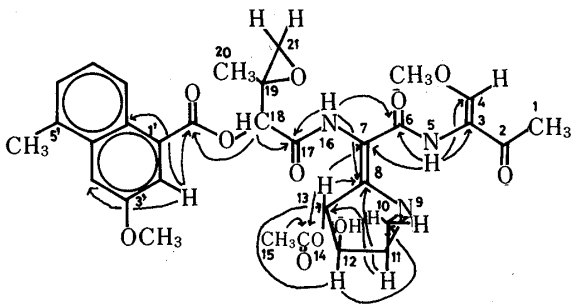
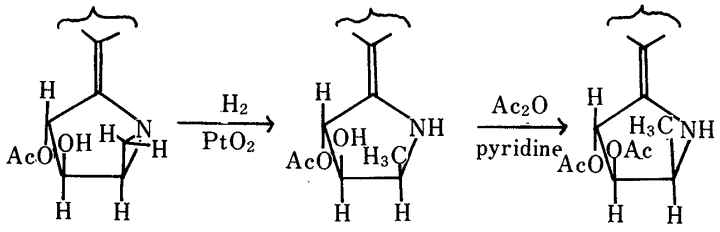


Fig. 5. The Results of LSPD Experiments for **3**



3 **9** **10**

Fig. 6

NMR of **9**: δ 1.20 (10-CH_3), 3.80—3.90 (H-11), 7.76 or 7.36 (9-NH), and ^{13}C-NMR of **9**: δ 14.5 (10-CH_3), 57.3 (C-11) showed that the 9-NH and 10-CH_3 groups were newly formed while the 10-CH_2 group in **3** had disappeared. Acetylation of **9** with acetic anhydride and pyridine gave a diacetate **10** (Fig. 6). The ^{1}H-NMR data for **10** [δ 4.16 (11-H), 5.12 (12-H), 1.98 (12-OAc)] showed that the 12-OH group in **9** was acetylated. These results showed the presence of an aziridine ring consisting of the N-9 nitrogen, C-10 carbon and C-11 carbon in partial structure B. In the LSPD experiment irradiating the H-11 proton in **3**, the shape of the signal due to the C-8 carbon changed, showing that the H-11 proton and C-8 carbon were separated by two or three bonds. Thus, the nitrogen at N-9 was shown to be connected to the carbon at C-8. Based on the above data, it was concluded that a 1-azabicyclo[3.1.0]hexane ring system was present in partial structure B.

The relative stereochemistry in the partial structure B was confirmed by the ^{1}H NOE experiments (Fig. 4). NOE enhancements were observed between the H-12 and H-11 protons (9%), H-15 and H-12 protons (6%), and H-15 and H-13 protons (4%), but not between the H-12 and H-13 protons. These data confirmed the relative configurations between the H-11 and H-12 protons (*cis*) and between the H-12 and H-13 protons (*trans*). NOE enhancements were also observed between the H-10a and H-13 protons (13%) and between the H-10b and H-11 protons (8%), which further confirmed the presence of the 1-azabicyclo[3.1.0]hexane ring system in the partial structure B. Thus, partial structure B, including the relative stereochemistry, was determined to be as depicted in Fig. 3.

The amide proton (5-NH) in **3** was observed at rather low field (δ 10.8) and that in **9** was observed at δ 7.76 or δ 7.36. This suggested that intramolecular hydrogen bonding was present between the 5-NH group and the nitrogen in the aziridine ring (N-9) in **3**.

All of the data and considerations described above led to the structures **1**, **2** and **3** as depicted in Fig. 1.

Experimental

General——All melting points were determined on a Yanagimoto micro melting point apparatus and are uncorrected. Infrared (IR) spectra were measured on a Hitachi 285 infrared spectrometer. UV spectra were recorded

on a Hitachi 200-20 spectrometer. FAB-MS were obtained on a JEOL JMS-DX303 mass spectrometer using 6 KeV xenon atom bombardment. Glycerol was used as the supporting matrix. EI-MS were also obtained on a JEOL JMS-DX303 mass spectrometer. NMR spectra were measured on a JEOL FX-90Q spectrometer or JEOL JNM GX-400 spectrometer, and unless otherwise stated, NMR spectra were measured at 90 MHz (^{1}H-NMR) or at 22.5 MHz (^{13}C-NMR) in $CDCl_3$. Optical rotations were measured with a JASCO DIP-4 digital polarimeter.

Materials——The $CHCl_3$ extract from the fermentation broth of *Streptomyces griseofuscus* S42227 was used for the isolation of **1** and **2**.[1] The $CHCl_3$ extract cited above was also used as the source material of **5**, **6** and **7**.

Isolation of Azinomycins (1 and 2) and Related Metabolites (5, 6 and 7)——The $CHCl_3$ extract from the fermentation broth (16 l) was concentrated to 30 ml, and diluted with *n*-hexane (300 ml). The resultant precipitate was collected by centrifugation and extracted with diethyl ether (50 ml) to afford the diethyl ether-soluble fraction and insoluble fraction. The diethyl ether-soluble fraction was concentrated and chromatographed on silica gel ($CHCl_3$–MeOH 50 : 1 v/v) to give azinomycin A (**1**, 20 mg) from EtOAc as colorless plates. The diethyl ether-insoluble fraction was extracted with $CHCl_3$ (50 ml), and *n*-hexane was added gradually to this solution. The precipitate initially obtained was discarded and that obtained thereafter was collected by centrifugation to give azinomycin B (**2**, 120 mg) as a white amorphous solid. MS, ^{13}C-NMR and ^{1}H-NMR data for **1** and **2** are shown in Tables I, II and IV, respectively.

The related substances present in the same fermentation broth were also extracted with $CHCl_3$. The $CHCl_3$ extract from the fermentation broth (200 l) was chromatographed on silica gel ($CHCl_3$ and $CHCl_3$–MeOH 50 : 1 v/v). Firstly **6**, then **5**, **1** and **7** were eluted in that order. Each fraction containing **6**, **5** and **7** was rechromatographed on silica gel using $CHCl_3$–*n*-hexane (1 : 2 v/v), $CHCl_3$–acetone (2 : 1 v/v) and $CHCl_3$–acetone (4 : 1 v/v), respectively, to give **6** (15 mg), **5** (18 mg) and **7** (42 mg).

5: Colorless needles from $CHCl_3$–*n*-hexane, mp 153—154 °C. $[\alpha]_D^{25}$ +48 (c=0.33, MeOH). IR (KBr): 1725, 1675 cm^{-1}. UV λ_{max}^{MeOH} nm (ε): 217 (46600), 245 (sh), 303 (3300), 343 (4900). ^{1}H-NMR: δ 1.52 (3H, s), 2.63 (3H, s), 2.76 (1H, d, J=4.3 Hz), 3.00 (1H, d, J=4.3 Hz), 5.20 (1H, s), 5.76 (1H, br), 6.14 (1H, br), 7.24—7.40 (2H), 7.42 (1H, d, J=2.9 Hz), 7.90 (1H, d, J=2.9 Hz), 8.62 (1H, dd, J=7.2, 5.4 Hz). ^{13}C-NMR see Table II. *Anal.* Calcd for $C_{18}H_{19}NO_5$: C, 65.64; H, 5.82; N, 4.25. Found: C, 65.80; H, 5.85; N, 4.23. EI-MS m/z: 329 (M^+).

6: Colorless needles from diethyl ether–*n*-hexane, mp 85—86 °C. IR (KBr): 1715 cm^{-1}. UV λ_{max}^{MeOH} nm (ε): 218 (70000), 245 (sh), 298 (6300), 340 (6800). ^{1}H-NMR δ: 2.63 (3H, s), 3.92 (3H, s), 3.96 (3H, s), 7.24—7.38 (2H), 7.40 (1H, d, J=2.7 Hz), 7.76 (1H, d, J=2.7 Hz), 8.56 (1H, dd, J=7.2, 4.7 Hz). ^{13}C-NMR see Table II. *Anal.* Calcd for $C_{14}H_{14}O_3$: C, 73.03; H, 6.13. Found: C, 73.23; H, 6.11. EI-MS m/z 230 (M^+). **6** was also obtained by chemical interconversion of **7**. A solution of **7** (15 mg) in MeOH and 10% aqueous NaOH (1 : 1 v/v, 20 ml) was refluxed for 20 h. Then the solution was acidified with dil. HCl and extracted with $CHCl_3$. Excess CH_2N_2 was added to this $CHCl_3$ solution. The product was purified by chromatography on silica gel ($CHCl_3$–*n*-hexane 1 : 2 v/v) to give the methyl ester (12 mg). The ^{1}H-NMR, IR and thin-layer chromatography (TLC) of this ester were identical with those of **6**.

7: Colorless needles from $CHCl_3$–*n*-hexane, mp 178—179 °C. IR (KBr): 1650 cm^{-1}. UV λ_{max}^{MeOH} nm (ε): 225 (50700), 280 (4900), 292 (4900), 322 (2800), 335 (3400). ^{1}H-NMR δ: 2.62 (3H, s), 3.92 (3H, s), 5.90 (2H, br), 7.24—7.40 (4H), 8.12 (1H, m). ^{13}C-NMR see Table II. *Anal.* Calcd for $C_{13}H_{13}NO_2$: C, 72.54; H, 6.09; N, 6.51. Found: C, 72.71; H, 6.16; N, 6.63. EI-MS m/z: 215 (M^+).

Methylation of 2——Excess diazomethane was added to a solution of **2** (60 mg) in $CHCl_3$ (3 ml) at room temperature. After 10 min, the solution was evaporated under reduced pressure and the residue was purified by chromatography on silica gel ($CHCl_3$–acetone 2 : 1 v/v) to give **3** (22 mg) as a white amorphous solid, UV λ_{max}^{MeOH} nm (ε): 217 (60000), 250 (31500), 344 (5400). ^{1}H-NMR see Table IV. ^{13}C-NMR see Table III.

Reduction of 3——A solution of **3** (40 mg) in tetrahydrofuran (THF) (5 ml) was hydrogenated in the presence of PtO_2 at room temperature for an hour. It was filtered and the filtrate was evaporated. The residue was purified by column chromatography on silica gel (EtOAc–acetone 2 : 1 v/v) to give **9** (16 mg) as a white amorphous solid, FAB-MS (positive) m/z: 640 $[M+H]^+$, 525, 309, 199. ^{1}H-NMR see Table IV. ^{13}C-NMR see Table II.

Acetylation of 9——A solution of **9** (15 mg) in 0.5 ml of acetic anhydride and 3 ml of pyridine was stirred at room temperature for an hour. The product was extracted with $CHCl_3$ and purified by column chromatography on silica gel (EtOAc–acetone 3 : 1 v/v) to give **10** (9 mg) as a white amorphous solid, FAB-MS (positive) m/z: 682 $[M+H]^+$, 567, 351, 199. ^{1}H-NMR see Table IV.

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References and Notes

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