STRUCTURES OF DNACIN A_1 AND B_1, NEW NAPHTHYRIDINOMYCIN-TYPE ANTITUMOR ANTIBIOTICS

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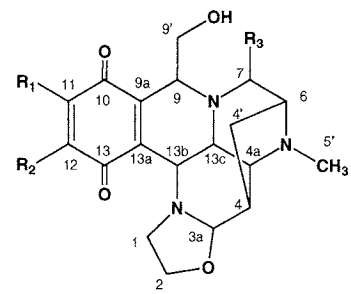
Dnacin A_1 and B_1 were revealed to be new naphthyridinomycin-type antitumor antibiotics with formulae of $C_{20}H_{23}N_5O_4$ and $C_{19}H_{24}N_4O_5$, respectively. The gross structure of dnacin A_1 was elucidated by the spectroscopic analyses. Conversion of dnacin B_1 into A_1 by treatment with potassium cyanide indicated the presence of an α-carbinolamine moiety in dnacin B_1. The relative stereochemistry of dnacins was clarified by analysis of the NOESY spectra.

Dnacin A_1 (**1**) and B1 (**2**) are benzoquinoid antibiotics which were isolated from the culture broth of *Actinosynnema pretiosum* C-14482 during our screening system using an Hfr strain of *E. coli* and which show strong activity against Gram-positive, Gram-negative, and acid-fast bacteria.[1~3] They also show antitumor activity by binding to DNA and indeed prolong the life-span of mice with leukemia P388.[4] In addition, we recently found that they inhibit cdc25B phosphatase which is expressed at high levels in some cancer cells.[5] In this report, we describe the structures of dnacins.

Results and Discussion

Physico-chemical properties of **1** and **2** are summarized in Table 1. In our previous studies, the molecular formulae of dnacins were not ascertained. However, the molecular ion peak at *m/z* 397 in the FD-MS spectrum, number of carbons in the ^{13}C NMR spectrum, and elemental analysis revealed that the molecular formula of **1** is $C_{20}H_{23}N_5O_4$. In the case of **2**, although only the dehydrate peak was observed at *m/z* 370 in the FD-MS spectrum, the molecular formula was determined to be $C_{19}H_{24}N_4O_5$.

Fig. 1. Structures of dnacins and naphthyridinomycins.



	R_1	R_2	R_3
Dnacin A_1 (**1**)	NH_2	H	CN
Dnacin B_1 (**2**)	NH_2	H	OH
Cyanonaphthyridinomycin	OCH_3	CH_3	CN
Naphthyridinomycin	OCH_3	CH_3	OH

Dnacin A_1 (**1**) has IR absorption bands at 1650, 1625, and 1600 cm^{-1}, and UV absorption maxima at 213, 281, and 496 nm in MeOH, indicating the presence of an aminobenzoquinone moiety.[2] The ^1H and ^{13}C NMR spectral data of **1** and **2** are shown in Table 2. The assignments of all the signals were accomplished by 2D NMR techniques including ^1H-^1H COSY, ^{13}C-^1H COSY, COLOC, and

Table 1. Physico-chemical properties of dnacin A_1 (**1**) and B_1 (**2**).

	1	**2**
Appearance	Dark red needles	Dark red needles
MP	>300°C (dec)	>300°C (dec)
$[\alpha]_D^{20}$ (*c* 0.06, $CHCl_3$)	+125°	+50°
FD-MS *m/z*	397 (M^+)	370 ($M^+ - H_2O$)
Molecular formula	$C_{20}H_{23}N_5O_4 \cdot 0.5H_2O$	$C_{19}H_{24}N_4O_5 \cdot 1.2H_2O$
Analysis (%)	Found Calcd	Found Calcd
	C: 59.10 59.10	C: 55.61 55.66
	H: 5.70 5.95	H: 6.31 6.49
	N: 17.14 17.23	N: 13.64 13.66
UV (MeOH) λ nm (ε)	213 (22,300), 281 (9,000), 496 (2,100)	213 (24,300), 283 (9,300), 496 (2,100)
IR (KBr) ν cm^{-1}	3430, 1680, 1650, 1625, 1600	3580, 3420, 3175, 1685, 1650, 1610

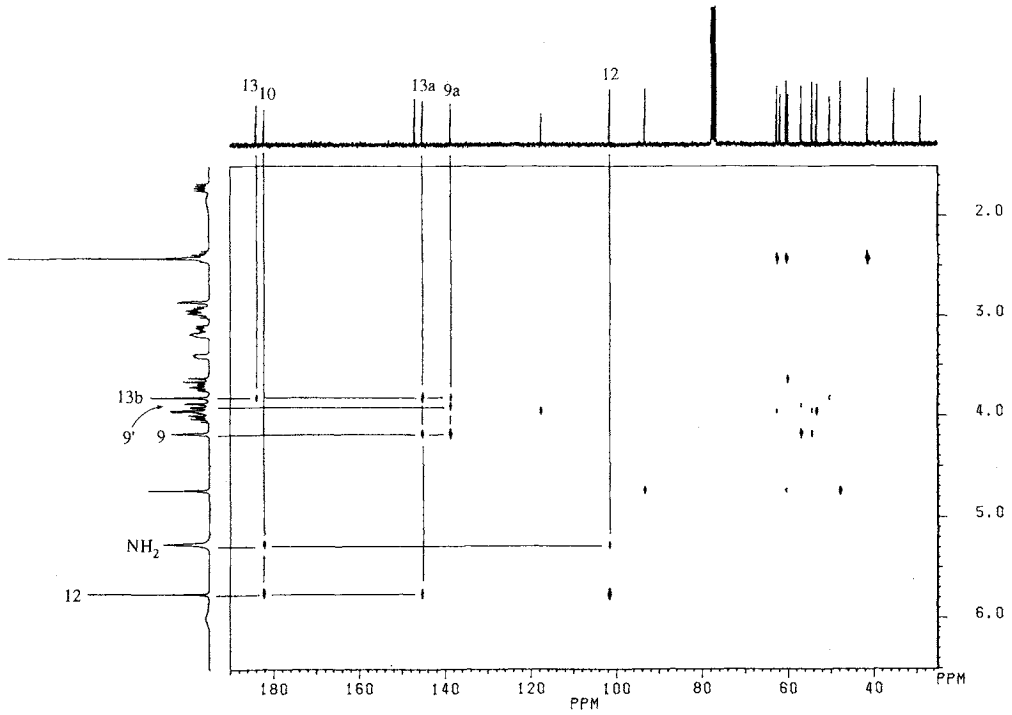
Table 2. ^1H and ^{13}C NMR spectral data of dnacin A_1 (**1**) and B_1 (**2**).

Position	**1**[a]		**2**[b]	
	^{13}C	^1H (*J*=Hz)	^{13}C	^1H (*J*=Hz)
1	50.1	2.95 m, 3.12 ddd (2.5, 7.2, 12.4)	50.9	2.92 dt (12.6, 9.5), 3.10 ddd (2.5, 7.2, 12.6)
2	61.6	3.71 ddd (2.5, 7.2, 8.8), 4.01 dt (9.6, 7.2)	62.8	3.70 ddd (2.5, 7.2, 9.5), 3.96 dt (9.5, 7.2)
3a	93.1	4.73 s	94.8	4.71 s
4	35.1	2.95 m	35.2	2.87 dt (12.5, 6.4)
4′	29.0	1.71 dd (6.8, 13.3), 2.40 dt (6.8, 13.3)	29.6	1.54 dd (6.4, 12.5), 2.36 dt (7.1, 12.5)
4a	60.2	3.18 m	61.5	3.13 m
5′	41.3	2.42 s (3H)	41.0	2.37 s (3H)
6	62.4	3.39 m	61.8	3.44 m
7	54.2	3.94 d (3.4)	89.3	4.17 d (3.6)
9	56.7	4.18 br s	54.4	4.44 br s
9′	59.8	3.65 dd (1.0, 11.4), 3.89 dd (2.5, 11.4)	60.7	3.49 dd (1.4, 10.9), 4.03 dd (2.7, 10.9)
9a	138.5		140.3	
10	182.0		183.8	
11	146.9		150.8	
12	101.4	5.77 s	100.1	5.69 s
13	183.8		185.3	
13a	145.1		146.9	
13b	47.6	3.81 s	49.1	3.74 s
13c	53.1	2.85 d (2.9)	53.3	2.98 d (3.1)
CN	117.4			
NH_2		5.25 br s (2H)		
OH		5.98 br		

[a] In $CDCl_3$.
[b] In CD_3OD.

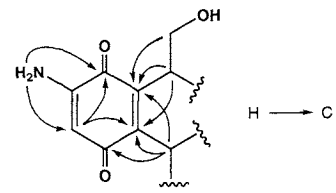
NOESY. Comparison of the ^{13}C NMR spectra of **1** and **2** revealed a couple of differences. The resonance at δ 54.2 in **1** was shifted to δ 89.3 in **2**, and the signal at δ 117.4 in **1** was not observed in **2**. From the chemical shifts, we supposed that the signal at δ 117.4 in **1** is attributable to a cyano carbon, nevertheless no absorption band was observed around 2300 cm^{-1} in the IR spectrum of **1**, and the signal at δ

Fig. 2. COLOC spectrum of dnacin A_1 (**1**).



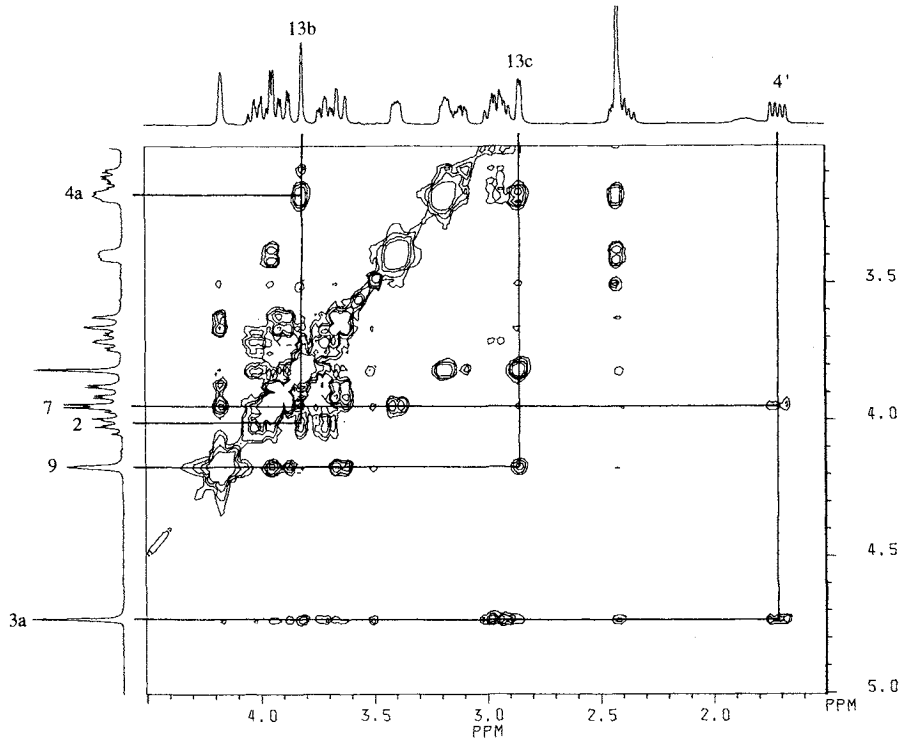
89.3 was assigned to an α-carbinolamine carbon (–N–CH–OH). These considerations were confirmed by the transformation of **2** into **1** by treatment with potassium cyanide in MeOH. Incorporation of a cyano group into an α-carbinolamine moiety has also been reported in the case of naphthiridinomycin-type antibiotics.[6~12] The ^{13}C NMR data of **1** was very similar to that of cyanonaphthyridinomycin[13], except for the benzoquinone moiety. The position of the amino group on the quinone ring was determined by a COLOC experiment (Figs. 2 and 3). The 9'-H signal at δ 3.89 had a cross peak with the signal at δ 138.5 which was assignable to C-9a. On the other hand, the aromatic proton signal at δ 5.77 had a cross peak not with the signal at δ 138.5 but with the signal at δ 145.1 which was assignable to C-13a. These observation clarified the presence of an amino substituent at C-11. Therefore, the gross structures of **1** and **2** were elucidated to be as shown in Fig. 1.

Fig. 3. ^{13}C-^1H long range pattern of dnacin A_1 (**1**).



The NOESY spectrum of **1** gave important informations about stereochemistry (Figs. 4 and 5). The signal at δ 1.71 (4'-H) showed cross peaks with the signals of 3a-H and 7-H, indicating that these protons are on the same side as the bridge at C-4'. On the other hand, the singlet signal at δ 3.81 (13b-H) showed cross peaks with the signals of 4a-H and 2-H (δ 4.01), and the signal at δ 2.85 (13c-H) had a cross peak with the signal of 9-H. These findings indicated that 13b-H, 13c-H, 4a-H, and 9-H are on the opposite side of C-4'. The relative stereochemistry of **1** and **2** revealed here is same as that of other naphthyridino-

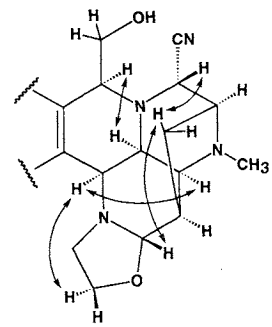
Fig. 4. NOESY spectrum of dnacin A_1 (**1**).



mycin-type antibiotics.

Cyanocycline A[7~10] (cyanonaphthyridinomycin)[8], B, and C[11] and naphthyridinomycin[6,7] have 11-methoxy and 12-methyl substituents, while naphtocyanidine[11] (cyanocycline F)[10] and SF-1739 HP[12] have 11-hydroxy and 12-methyl substituents. Dnacins A_1 (**1**) and B_1 (**2**) which have an 11-amino substituent are new members of this group.

Fig. 5. NOE network of dnacin A_1 (**1**).



Experimental

NMR Spectroscopy

NMR spectra were recorded on a Bruker AC-300 instrument (^{1}H, 300 MHz; ^{13}C, 75 MHz) at 24°C: Chemical shifts (δ) are reported in ppm downfield from TMS, and 0.1 M solutions were used. All NMR experiments were performed using standard programs of the Bruker library. The COLOC spectra were obtained from a 256 × 4 K data matrix. Parameters were optimized for J_{CH} = 7.1 Hz, and the conditions were as follows: number of scans, 80; total measuring time, 13.5 hours. The NOESY spectra were obtained from a 256 × 1 K data matrix. The mixing time was set to 1 second, and the conditions were as follows: number of scans, 32; total measuring time, 13 hours.

Conversion of **2** into **1**

Acetic acid (20 ml, 0.35 mmol) and potassium cyanide (13 mg, 0.20 mmol) were added to a solution of **2** (40 mg, 0.10 mmol) in MeOH (2.0 ml), and the reaction mixture was stirred for 20 minutes at room

temperature. After concentration, the residue obtained was suspended in water and extracted with $CHCl_3$ at pH 8.0. The organic layer was concentrated and applied to a column of silica gel. Elution with $CHCl_3$-MeOH (50:1 and 25:1) followed by concentration gave **1** as dark red crystals (28 mg, 68%).

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