THE STRUCTURE OF SN-07 CHROMOPHORE

Sir:

In addition to producing the macromolecular antibiotic SN-07[1], *Actinomadura roseoviolacea* var. *miuraensis* nov. var., also biosynthesizes many anthracycline antibiotics. We have isolated one of these anthracycline antibiotics and identified it as the chromophore obtained from SN-07[2] (I). It was also shown that I was identical to barminomycin I[3,4]. In this paper, we wish to report on its proposed structure, a carminomycin analogue differing in the acetal moiety.

Acid hydrolysis of I with 0.4 N HCl (room temp, 2 hours) gave carminomycin I[5,6] identified by direct comparison with an authentic sample using ^{1}H NMR, HPLC, TLC and field desorption mass spectrometry (FD-MS). The authentic sample was prepared from carminomycin III[5,7,8] (kindly provided as rubeomycin A_1[9] by Ishihara Sangyo Kaisha, Ltd.) by the same acid hydrolysis. The molecular formula of I was determined to be $C_{33}H_{37}NO_{12}$ (MW 639) on the basis of FD-MS and elementary analysis. The aglycone moiety of I was identical with that of carminomycin III. However C-3′ and C-6″ signals of I were shifted to a significantly lower field. In addition the methylene signal of C-6″ changed to the methine signal. The ^{1}H NMR spectrum in $CDCl_3$ of I was almost identical with that of carminomycin III, but as in the case of ^{13}C NMR, the signal at δ 3.80 (1H, m, 5″-H) of carminomycin III were shifted to a higher field by about 0.3 ppm. Moreover, two double-doublet protons at δ 3.53 (1H, dd, J=12.4 and 8.9 Hz, 6″-H_A) and δ 3.42 (1H, dd, J=12.4 and 2.2 Hz, 6″-H_B) changed to one doublet proton at δ 3.75 (1H, d, J=8.3 Hz, 6″-H) in I (Table 1).

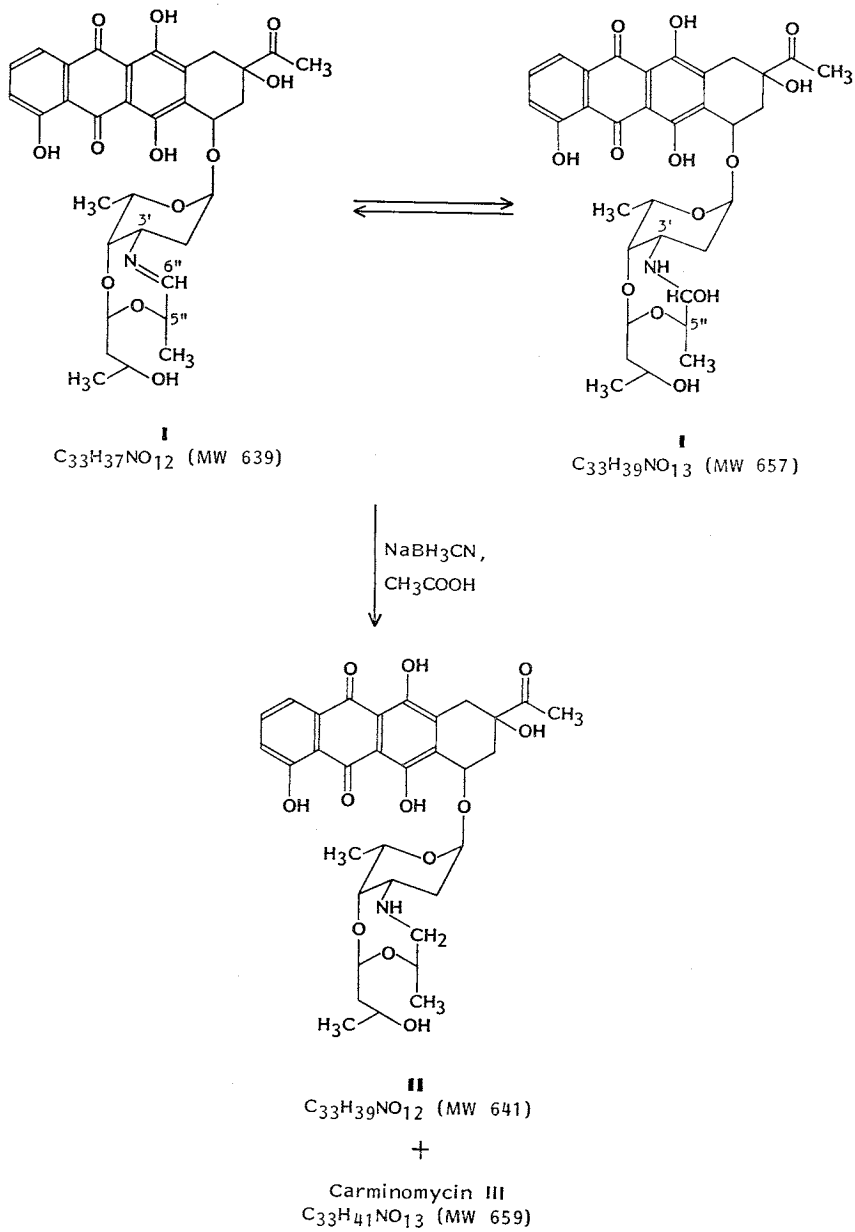
The two-dimensional (2D) proton-proton shift correlation spectrum (COSY) of I in $CDCl_3$ showed that the signal at δ 3.75 (6″-H) coupled with the signal at δ 3.50 (1H, m, 5″-H) and the signal at δ 3.50 (5″-H) coupled with the signal at δ 1.20 (3H, d, J=6.1 Hz, 7″-CH_3). We have found that the ^{1}H NMR spectrum of I in $CDCl_3$

Table 1. ^{1}H NMR chemical shifts of I, II and carminomycin III.

Proton	I (δ) ppm	II (δ) ppm	Carminomycin III (δ) ppm	Remarks
1-H	7.87 (dd)	7.90 (dd)	7.90 (dd)	Aglycone moiety
2-H	7.71 (dd)	7.72 (dd)	7.72 (dd)	
3-H	7.31 (dd)	7.33 (dd)	7.33 (dd)	
7-H	5.24 (br s)	5.28 (br s)	5.24 (br s)	
8-H_{eq}	2.36 (br d)	2.35 (br d)	2.30 (br d)	
8-H_{ax}	2.09 (dd)	2.09 (dd)	2.08 (dd)	
10-H_{eq}	3.26 (br d)	3.27 (dd)	3.25 (dd)	
10-H_{ax}	3.00 (d)	3.02 (d)	3.00 (d)	
$COCH_3$	2.43 (s)	2.42 (s)	2.42 (s)	
1′-H	5.46 (br d)	5.45 (br d)	5.45 (br d)	Daunosamine
2′-H_2	~1.72 (m)	~1.82 (m)	~1.70 (m)	moiety
3′-H	3.13 (m)	2.86 (m)	3.01 (m)	
4′-H	3.58 (br s)	3.56 (br s)	3.93 (br s)	
5′-H	4.10 (m)	4.11 (m)	4.12 (m)	
6′-H_3	1.27 (d)	1.29 (d)	1.32 (d)	
NH	—	4.74 (br s)	—	
1″-H	4.73 (dd)	4.68 (dd)	4.74 (dd)	Acetal moiety
2″-H_2	~1.92 (m)	~1.94 (m)	~1.85 (m)	
3″-H	4.10 (m)	4.11 (m)	4.17 (m)	
4″-H_3	1.23 (d)	1.24 (d)	1.23 (d)	
5″-H	3.50 (m)	3.81 (m)	3.80 (m)	
6″-H_A	3.75 (d)	2.79 (dd)	3.53 (dd)	
6″-H_B	—	2.67 (dd)	3.42 (dd)	
7″-H_3	1.20 (d)	1.11 (d)	1.07 (d)	

Spectra were measured in $CDCl_3$ using TMS as an internal reference (400 MHz).

Fig. 1. The structures of I and II.



gradually changed during measurement. The signal at δ 3.75 (6″-H) was gradually disappeared, instead the new signal at δ 7.42 (d, J=4.0 Hz) was appeared. The 2D proton-carbon shift correlation spectrum of I in $CDCl_3$ showed that a new observed signal at δ 7.42 in ^1H NMR correlated to a signal at δ 164.8 (C-6″) in ^{13}C NMR. The NMR spectra of I suggested that it was in the carbinolamine form at first and was converted to the imine form as shown in pyrrolo[1,4]-benzodiazepine antitumor antibiotics (*e.g.* neothramycin[10]) and saframycins[11]. Therefore we deduced that I had the interconvertible structure between imine and carbinolamine forms through C-3′ and C-6″.

Reduction of I with sodium cyanoborohydride ($NaBH_3CN$) (0°C, MeOH - 1 N CH_3COOH, 2:1, 15 minutes)[12] gave carminomycin III and an unknown reduction product (II)[3]. Physicochemical properties of II are as follows:

Table 2. Antibacterial activity of **I**, **II** and carminomycin III (agar dilution method).

Test organism	MIC (μg/ml)		
	I	**II**	Carminomycin III
Escherichia coli AB 1157	1.56	12.5	>25
E. coli BE 1186	<0.003	0.10	0.78
Salmonella typhimurium TV 119	3.13	25	>25
S. typhimurium SL 1102	0.10	0.78	1.56
Bacillus subtilis (*rec*$^+$)	0.20	0.78	1.56
B. subtilis (*rec*$^-$)	0.024	0.39	1.56
Staphylococcus aureus IFO 12732	0.39	1.56	6.25
Micrococcus luteus IFO 12708	0.024	0.39	1.56

Table 3. Cytotoxicity of **I**, **II** and carminomycin III against cultured KB and HeLa cells.

Test cell cultures	IC_{50} (μg/ml)		
	I	**II**	Carminomycin III
KB	0.00005	0.00054	0.0009
HeLa	0.00011	0.0018	0.0027

$C_{33}H_{39}NO_{12}$, FD-MS *m/z* 642 $(M+H)^+$, *m/z* 664 $(M+Na)^+$; mp 138~143°C (dec); $[\alpha]_D^{24}$ +145° (*c* 0.040, $CHCl_3$); IR ν_{max} (KBr) cm^{-1} 1710 (C=O), 1600 (C=O, quinone), 1410, 1290, 1240, 1200 (phenolic OH), 1160, 1120, 1020, 1000; UV $\lambda_{max}^{90\%\ MeOH}$ nm ($E_{1cm}^{1\%}$) 234 (533), 254 (386), 292 (111), 492 (206), 526 (134).

The ^1H NMR spectrum of **II** showed that 6″-H were shifted to a higher field by about 0.7 ppm (δ 2.79 (1H, dd, J=14.7 and 5.5 Hz, 6″-H_A), δ 2.67 (1H, dd, J=14.7 and 9.8 Hz, 6″-H_B)) in comparison with carminomycin III and one NH proton appeared (δ 4.74, br s) (Table 1). The ^{13}C NMR spectrum of **II** showed that C-3′ and C-6″ signals shifted to δ 51.6 (CH) and δ 52.8 (CH_2).

From these results, we proposed the structures of **I** and **II** as shown in Fig. 1. The structure of **I** is the same as that proposed for barminomycin I[3,4]. It was considered to be in equilibrium with the carbinolamine (equiv to aldehyde) and the imine forms in some conditions. For example the C-6″ signal (δ 164.8) disappeared in CD_3OD and the optical rotation $[\alpha]_D$ was also changed under these conditions. It remains to be determined that why the $[\alpha]_D$ of barminomycin I was reported as positive (+235°, *c* 0.017, $CHCl_3$)[3] in contrast to the negative value for that of **I** (−270°, *c* 0.0318, $CHCl_3$)[2].

A comparison of the biological activities of **I**, **II** and carminomycin III is shown in Tables 2 and 3. **I** had the strongest activity against bacteria and cell cultures. Possibly this higher activity is related to the fact that **I** has two DNA reactive sites. One is the aglycone moiety that can intercalate with DNA and the other is the carbinolamine (or chemical equiv) that can bind to DNA covalently in the same way as pyrrolo[1, 4]-benzodiazepine antitumor antibiotics[13]. Further studies on the mode of binding of **I** to DNA and the binding site are in progress.

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References

1) KIKUCHI, Y.; M. NIWANO, N. YAJIMA, G. NAKAMURA & N. MIYATA: A novel macro-

molecular antibiotic, SN-07. Taxonomy of producing organism, isolation, characterization and biological activities. J. Antibiotics 38: 1670~1676, 1985

2) KIMURA, K.; S. NAKAYAMA, T. KOYAMA, S. SHIMADA, N. KAWAGUCHI, N. MIYATA, Y. TAKESHITA & G. KAWANISHI: SN-07 chromophore: An anthracycline antibiotic from the macromolecular antibiotic SN-07. J. Antibiotics 40: 1353~1355, 1987
3) UMEZAWA, H.; T. TAKEUCHI, T. SAWA, H. NAGANAWA, M. HAMADA, Y. TAKAHASHI, M. IMOTO, A. ODAGAWA & T. UCHIDA (IMC): Anthracycline complex (Barminomycins I and II). Jpn. Kokai 97('87), Jan. 6, 1987
4) UMEZAWA, H.: New antitumor agents produced by microorganisms. *In* Recent Advances in Chemotherapy. Anticancer Section 1. *Ed.*, J. ISHIGAMI, pp. 3~19, University of Tokyo Press, Tokyo, 1985
5) BRAZHNIKOVA, M. G.; V. B. ZBARSKY, V. I. PONOMARENKO & N. P. POTAPOVA: Physical and chemical characteristics and structure of carminomycin, a new antitumor antibiotic. J. Antibiotics 27: 254~259, 1974
6) BRAZHNIKOVA, M. G.; V. B. ZBARSKY, D. TRESSELT & K. ECKARDT: Stereochemistry of the antibiotic carminomycin. J. Antibiotics 29: 469~471, 1976
7) ZBARSKY, V. B.; N. P. POTAPOVA, M. G. BRAZHNIKOVA, B. V. ROZYNOV, L. A. SIBELDINA & N. F. SEPETOV: Structure of carminomycin II and III. Antibiotiki 25: 488~492, 1980
8) MATSUZAWA, Y.; A. YOSHIMOTO, K. KOUNO & T. OKI: Baumycin analogs isolated from *Actinomadura* sp. J. Antibiotics 34: 774~776, 1981
9) OGAWA, Y.; H. SUGI, N. FUJIKAWA & H. MORI: Rubeomycin, a new anthracycline antibiotic complex. I. Taxonomy of producing organism, isolation, characterization and biological activities of rubeomycin A, A_1, B and B_1. J. Antibiotics 34: 938~950, 1981
10) MIYAMOTO, M.; S. KONDO, H. NAGANAWA, K. MAEDA, M. OHNO & H. UMEZAWA: Structure and synthesis of neothramycin. J. Antibiotics 30: 340~343, 1977
11) LOWN, J. W.; A. V. JOSHUA & J. S. LEE: Molecular mechanisms of binding and single-strand scission of deoxyribonucleic acid by the antitumor antibiotics saframycins A and C. Biochemistry 21: 419~428, 1982
12) BORCH, R. F.; M. D. BERNSTEIN & H. D. DURST: The cyanohydridoborate anion as a selective reducing agent. J. Am. Chem. Soc. 93: 2897~2904, 1971
13) HURLEY, L. H.: Pyrrolo(1,4)benzodiazepine antitumor antibiotics. Comparative aspects of anthramycin, tomaymycin and sibiromycin. J. Antibiotics 30: 349~370, 1977