A NEW ANTHRACYCLINE ANTIBIOTIC SN-706

Sir:

In addition to the macromolecular antibiotic SN-07[1], *Actinomadura roseoviolacea* var. *miuraensis* nov. var. (strain 07) produced many anthracycline antibiotics such as carminomycins and SN-07 chromophore[2,3]. Here we report on the isolation, physico-chemical, biological properties and structure determination of one of these antibiotics, designated SN-706, and identified as a carminomycin analog with a different acetal moiety.

The fermentation medium consisted of oatmeal 40 g, soluble starch 40 g, K_2HPO_4 7 g, $NaH_2PO_4 \cdot 2H_2O$ 3 g, $MgSO_4 \cdot 7H_2O$ 1 g, $FeSO_4 \cdot 7H_2O$ 1 g in 1 liter deionized water and the pH was adjusted to 7.0. The fermentation was carried out in a 200-liter fermentor containing 120 liters of the medium at 32°C for 4 days. After centrifuging the fermentation broth, the red pigments in the mycelial cake (5 kg) were extracted three times with acetone. The acetone extract was concentrated to a small volume (2 liters) *in vacuo* and then extracted three times with 2 liters of $CHCl_3$ at pH 8.6. The $CHCl_3$ layer was concentrated to a small volume and an excess of *n*-hexane was added. The precipitate obtained (1 g) was subjected to silica gel column chromatography. The column was washed with $CHCl_3$ - MeOH (100 : 1) and SN-706 was eluted from the column with $CHCl_3$ - MeOH (100 : 5). Further purification was achieved by preparative TLC (Silica gel, E. Merck, $CHCl_3$ - MeOH - AcOH, 20 : 5 : 1). A red band (Rf 0.68) was scraped off and eluted with MeOH. The MeOH extract was evaporated *in vacuo* and the residue dissolved in $CHCl_3$. An excess of *n*-hexane was added to precipitate SN-706 as a reddish brown powder (18 mg). SN-706 gave a single peak on analytical HPLC, YMC R-ODS-5 (4.6×250 mm), CH_3CN - 0.1 M NaH_2PO_4 (40 : 60), 1.5 ml/minute, UV at 220 nm; retention time: 9.1 minutes (carminomycin I: 4.4, carminomycin II: 7.5, carminomycin III: 8.4, SN-07 chromophores: 6.9). MP 161~163°C (dec); $[\alpha]_D^{27}$ +124.7° (*c* 0.055, $CHCl_3$); field desorption mass spectra (FD-MS) *m/z* 716 $(M+H)^+$: *Anal* calcd for $C_{36}H_{45}NO_{14}$: C 60.41, H 6.29, N 1.96, found: C 60.24, H 6.34, N 1.93;

Fig. 1. ^{13}C NMR spectrum of SN-706 (25 MHz, $CDCl_3$).

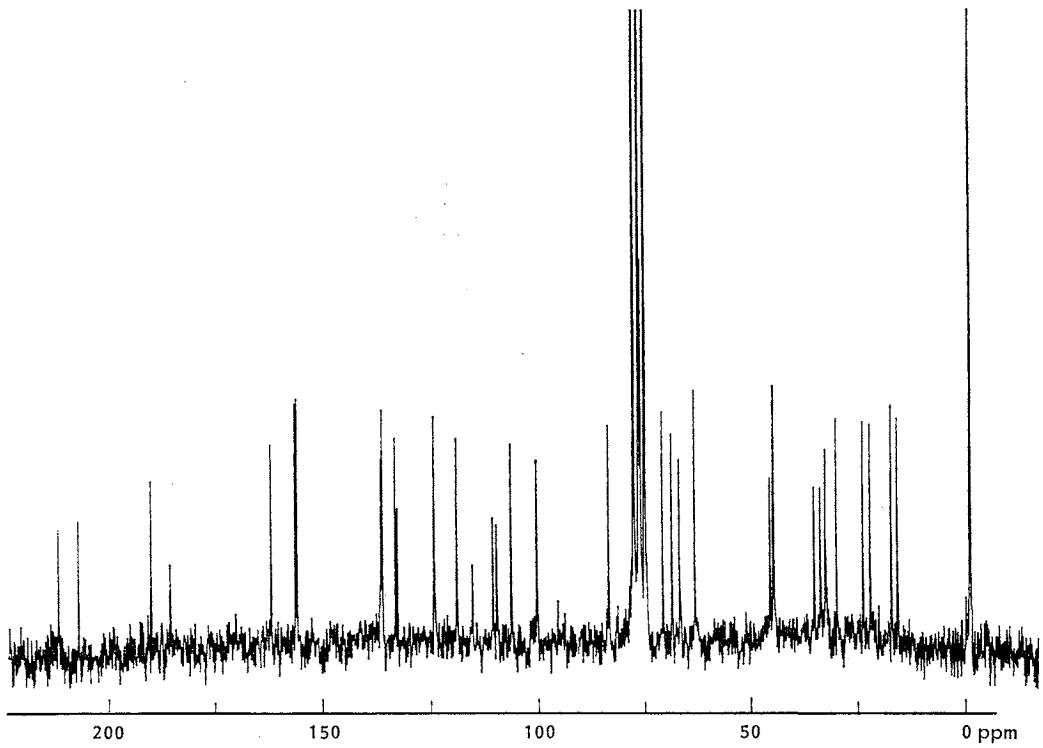


Table 1. ^{13}C NMR chemical shifts of SN-706 and carminomycin III.

Carbon No.	SN-706[a] (δ)	Carminomycin III[b] (δ)	Remarks
C-1	119.6	119.5	Aglycone moiety
C-2	137.0	136.9	
C-3	124.7	124.7	
C-4	162.5	162.4	
C-4a	116.0	115.7	
C-5	186.1	185.7	
C-5a	(111.2)	(111.0)	
C-6a	(156.8)	(156.7)	
C-6	(133.7)	(133.9)	
C-7	69.5	69.5	
C-8	36.2	(35.4)	
C-9	76.6	76.5	
C-10	34.8	(34.9)	
C-10a	(133.3)	(133.1)	
C-11	(156.4)	(156.5)	
C-11a	(110.3)	(110.2)	
C-12	190.5	190.2	
C-12a	136.7	136.8	
C-13	211.9	211.7	
C-14	24.9	24.7	
C-1′	100.9	100.8	Daunosamine moiety
C-2′	31.1	33.4	
C-3′	46.5	45.9	
C-4′	84.1	81.5	
C-5′	64.1	64.1	
C-6′	16.9	16.9	
C-1″	106.9	106.4	Acetal moiety
C-2″	45.6	45.7	
C-3″	67.7	67.7	
C-4″	23.2	23.3	
C-5″	75.7	75.6	
C-6″	71.6	66.7	
C-7″	45.6	—	
C-8″	207.3	—	
C-9″	33.6	—	
C-10″	18.4	(7″) 18.0	

TMS was used as an internal reference. Similar value in parentheses may be interchanged.

[a] 25 MHz ($CDCl_3$), this tentative assignment is given on the basis of ^1H-^{13}C COSY.

[b] 22.5 MHz ($CDCl_3$), this tentative assignment is given on the basis of the value of ref 6.

IR ν_{max}^{KBr} cm^{-1} 3460, 1715 (C=O), 1600 (C=O, quinone), 1590, 1415, 1290, 1235, 1200 (phenolic OH), 1165, 1120, 1010; UV λ_{max}^{MeOH} nm ($\varepsilon \times 10^{-4}$) 235 (3.35), 254 (2.54), 294 (0.74), 493 (1.30), 527 (0.97), 578 (0.24).

Acid hydrolysis of SN-706 with 0.4 N HCl (room temperature, 2 hours) gave carminomycin I[4,5] identified by direct comparison with an authentic sample using ^1H NMR, HPLC, and FD-MS. The authentic sample of carminomycin I was prepared from carminomycin III[4,6,7] (kindly provided as rubeomycin A_1[8] by Ishihara Sangyo Kaisha, Ltd.) by the same acid hydrolysis.

The ^{13}C NMR spectrum of SN-706 indicated the presence of 36 carbons and was identical with that of carminomycin III in the signals of their corresponding carbons, except for the presence of the following three additional carbons: δ 33.6 (CH_3), 45.6 (CH_2), 207.3 (C=O). In addition, the C-6″ signal of SN-706 was observed as CH (δ 71.6) compared to CH_2 (δ 66.7) of carminomycin III (Fig. 1 and Table 1).

Fig. 2. ^1H NMR spectrum of SN-706 (500 MHz, $CDCl_3$).

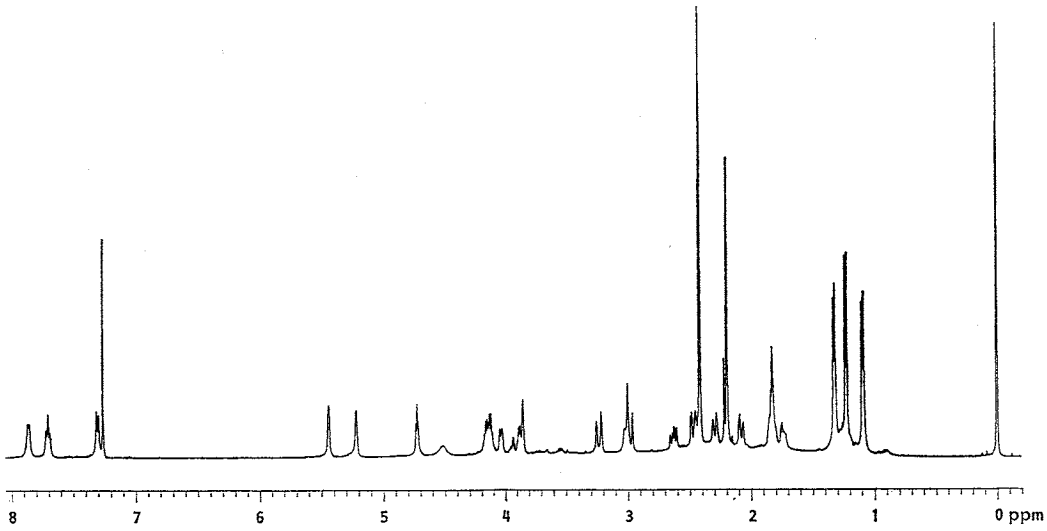
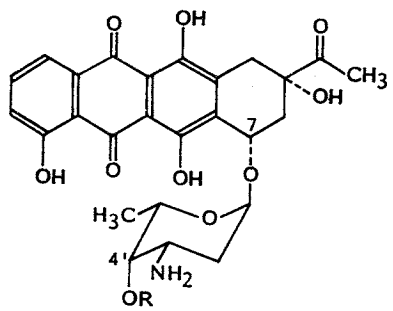
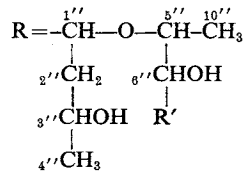


Fig. 3. The structures of SN-706 and related compounds.



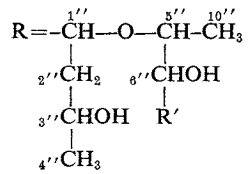
Carminomycin I R=H

Carminomycins II and III (II: 5″-*R*, rubeomycin A, D326III; III: 5″-*S*, rubeomycin A_1, D326IV)



R′=H

SN-706



The ^1H NMR spectrum of SN-706 was identical with carminomycin III except for the presence of following additional signals: δ 2.63 (dd, J=15.4 and 9.6 Hz, 7″-H_a), 2.47 (dd, J=15.4 and 3.6 Hz, 7″-H_b), 2.19 (s, 9″-H_3). The 6″-H methylene signal of carminomycin III (δ

Table 2. Antibacterial activity of SN-706.

Test organism	MIC (μg/ml)
Escherichia coli BE 1186	0.4
Salmonella typhimurium IFO 13245	25
S. typhimurium TA 1535	0.8
Bacillus subtilis rec$^+$	0.8
B. subtilis rec$^-$	0.2
Staphylococcus aureus IFO 12732	1.6
Micrococcus luteus IFO 12708	0.4

Agar dilution method.

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 a methine signal and shifted to lower field by about 0.5~0.6 ppm (δ 4.04, 1H, m, 6″-H) in SN-706 (Fig. 2). The shift to lower field of 6″-CH indicated substitution at C-6″ of the acetal moiety.

The two-dimensional proton-proton shift correlation spectrum (COSY) of SN-706 showed that 6″-CH was coupled with 7″-CH_2. The 7″-CH_2 chemical shifts (^{1}H: δ 2.63 and 2.47, ^{13}C: δ 45.6) suggested C-8″ to be a carbonyl group. Therefore we proposed the structure of SN-706 as a carminomycin analog substituted with CH_2COCH_3 at C-6″ as shown in Fig. 3.

SN-706 was active against KB (IC_{50} 0.54 ng/ml) and HeLa (IC_{50} 0.94 ng/ml) cells in culture. MIC values for some bacteria are shown in Table 2. SN-706 prolonged the survival period of BDF_1 mice to which P388 leukemia cells were ip inoculated. The antitumor activity expressed as increased life span (ILS) was 65.9% (ip injection, 1~9 days) at a dose of 20 μg/kg.

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