SN-07 CHROMOPHORE: AN ANTHRACYCLINE ANTIBIOTIC FROM THE MACROMOLECULAR ANTIBIOTIC SN-07

Sir:

As reported in our previous paper[1], SN-07, a novel macromolecular antibiotic containing nucleic acid and a chromophore, was found in the culture broth of *Actinomadura roseoviolacea* var. *miuraensis* nov. var. Subsequently we showed that the chromophore could be isolated from SN-07 by nuclease P1 digestion and that it was essential for biological activity. It was difficult to prepare the nucleic acid free chromophore from SN-07 because of its instability during the enzyme digestion process. So we looked for an antibiotic, with an equivalent chromophore in the mycelial cake of above microorganism. Here we report on the isolation, physico-chemical and biological properties of this antibiotic (**I**). **I** was an anthracycline antibiotic and identical with the SN-07 chromophore by ^{1}H NMR, HPLC and TLC analyses.

The fermentation medium used was oatmeal 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 jar fermentor containing 120 liters of the medium at 32°C for 6 days. The fermentation broth was centrifuged and the mycelial cake (6.5 kg) was extracted two times with acetone and filtered off. The acetone extract was concentrated to a small volume *in vacuo* and then extracted three times with $CHCl_3$. The organic layer was washed with H_2O, dried over anhydrous Na_2SO_4 and concentrated to a small volume. An excess of *n*-hexane was added and the precipitate obtained was dissolved in MeOH. The MeOH solution was then centrifuged and the supernatant was subjected to silica gel column chromatography (2.8×32 cm). The column was washed with $CHCl_3$ (3.5 liters) and then eluted with same volume of $CHCl_3$ - MeOH (100:1). Further purification was achieved by repeated Sephadex LH-20 column chromatography (2.8× 120 cm) with MeOH to give **I** as a dark red powder (9.2 mg). **I** gave a single peak on analytical HPLC (column: YMC R-ODS-5 (4.6 × 250 mm), solvent: CH_3CN - 0.1 M NaH_2PO_4 (4:6), flow rate: 1 ml/minute, detector: UV (220 nm)).

Physico-chemical properties of **I** are as follows: $C_{33}H_{37}NO_{12}$; field desorption mass spectra (FD-MS) *m/z* 639 (M^+), 662 $(M+Na)^+$; mp 129~132°C (dec); $[\alpha]_D^{27}$ −270° (*c* 0.0318, $CHCl_3$); *Anal* Calcd for $C_{33}H_{37}NO_{12} \cdot H_2O$: C 60.27, H 5.94, N 2.13, found: C 59.72, H 5.88, N 2.21; IR ν_{max} (KBr) cm^{-1} 1720

Fig. 1. IR spectrum of SN-07 chromophore (KBr)

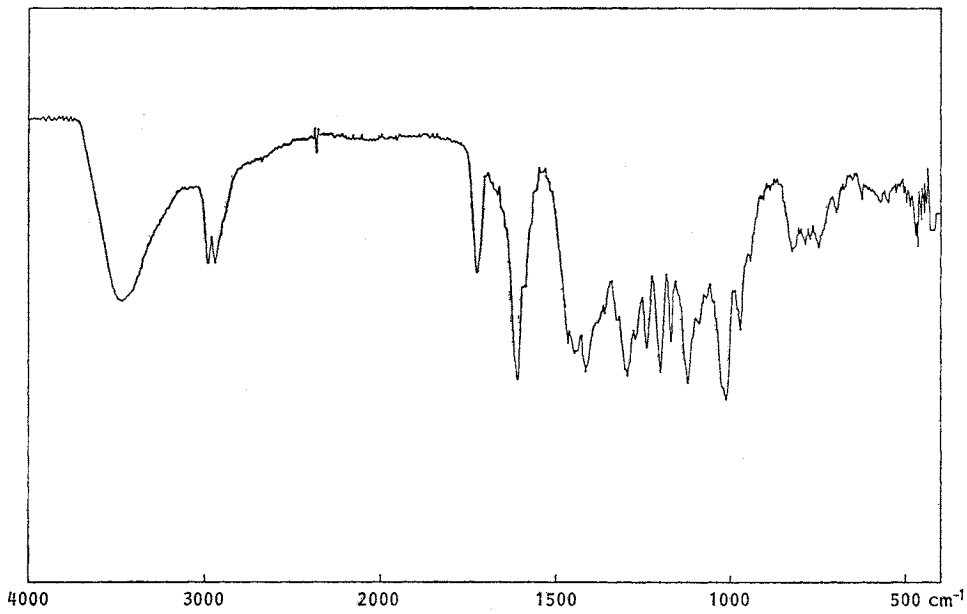


Fig. 2. ^{1}H NMR spectrum of SN-07 chromophore (400 MHz, $CDCl_3$).

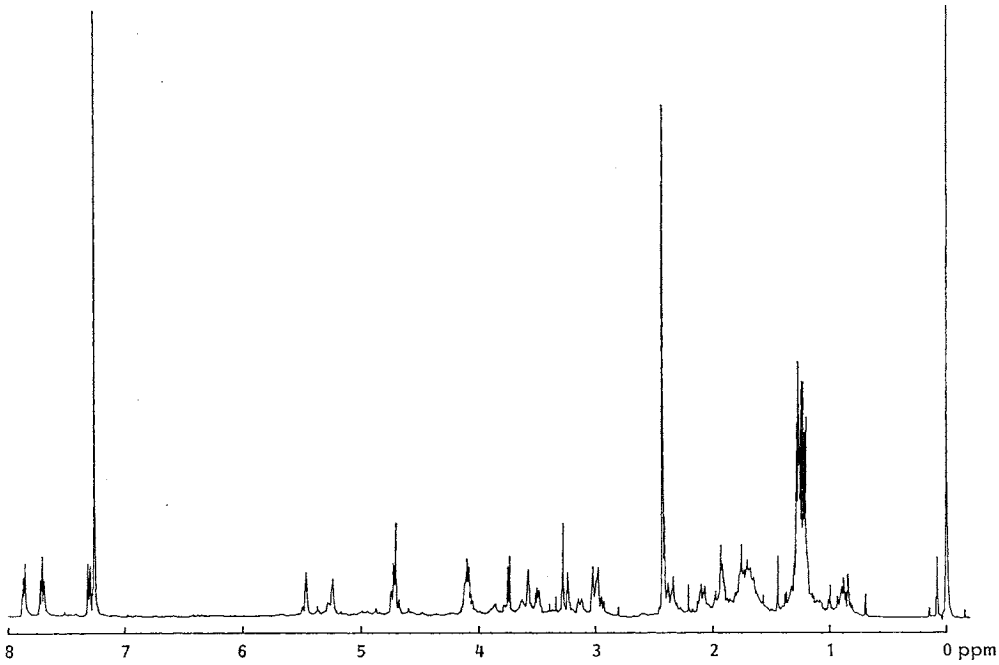
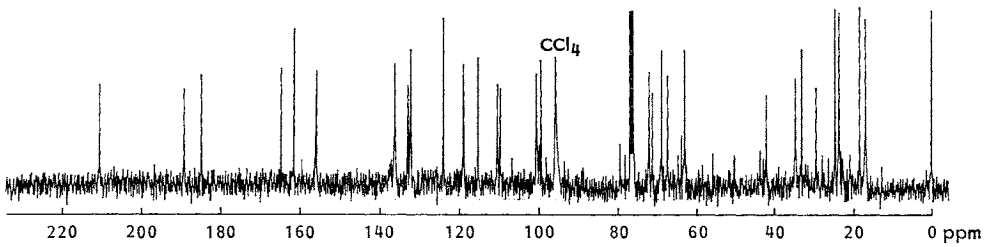


Fig. 3. ^{13}C NMR spectrum of SN-07 chromophore (100 MHz, $CDCl_3$).



(carboxyl), 1600 (C=O, quinone), 1410, 1290, 1230, 1200 (phenolic OH), 1160, 1120, 1010 (Fig. 1); UV $\lambda_{max}^{90\%\ MeOH}$ nm ($E_{1cm}^{1\%}$) 234 (632), 254 (470), 292 (172), 465 (312), 491 (394), 524 (288).

^{1}H and ^{13}C NMR spectra of **I** in $CDCl_3$ are shown in Figs. 2 and 3. The ^{1}H NMR spectrum of **I** was similar to that of carminomycin III[2~4] (rubeomycin A_1)[5] except for 6″-H, 5″-H and 4′-H. That is, the 6″-H signal of carminomycin III (δ 3.53 (1H, dd, J=12.4 and 9.6 Hz, 6″-H_A), 3.42 (1H, dd, J=12.4 and 4.0 Hz, 6″-H_B)) were disappeared in **I**, instead, one proton signal (δ 3.75 (1H, d, J=8.3 Hz, 6″-H)) appeared. And that, the signals at δ 3.80 (1H, m, 5″-H) and δ 3.93 (1H, br s, 4′-H) of carminomycin III were shifted to a higher field by about 0.3 ppm, that is, δ 3.50 (1H, m, 5″-H) and δ 3.58 (1H, br s, 4′-H) in **I**, respectively (Fig. 2). The ^{13}C NMR spectrum of **I** was also similar to that of carminomycin III, especially the aglycone moiety which was identical in that of carminomycin III. But the C-3′ signal (δ 45.9, CH) and C-6″ signal (δ 66.7, CH_2) of carminomycin III were shifted to a significantly lower field (Fig. 3).

On acid hydrolysis with 0.1 N HCl at 90°C for 60 minutes, **I** afforded the carminomycinone[2,6] identified by electron impact mass spectra (EI-MS), ^{1}H NMR and TLC analyses (EI-MS m/z 384 (M^+), 348 ($M-2H_2O)^+$). And the presence of carminomycin I[2,6] was confirmed by the above analyses on the hydrolysis of **I** by 0.4 N HCl at room temp for 2 hours. On analytical

Table 1. Antibacterial activity of SN-07 chromophore (agar dilution method).

Test organism	MIC (μg/ml)
Escherichia coli AB 1157	1.56
E. coli BE 1186	<0.003
Salmonella typhimurium TV 119	3.13
S. typhimurium SL 1102	0.1
Bacillus subtilis rec$^+$	0.2
B. subtilis rec$^-$	0.024
Staphylococcus aureus IFO 12732	0.39
Micrococcus luteus IFO 12708	0.024

HPLC, a retention time of I (8.8 minutes) is different from that of carminomycins I (5.5 minutes), II (9.6 minutes) and III (11.3 minutes), but it is same as that of barminomycin I[7,8]. From these results, I was an anthracycline antibiotic that was different from the known carminomycin-type antibiotics in the acetal moiety. A more detailed study on the structure is now under investigation.

During the isolation and purification of I, we found that it was chemically and structurally unstable, and decomposed. This chemical alteration occurred much more readily in the presence of $CHCl_3$, especially at acidic pH.

I was active against KB (IC_{50}=0.00005 μg/ml) and HeLa (IC_{50}=0.00011 μg/ml) cell cultures. MIC for some bacteria are shown in Table 1.

Recently, new anthracycline antibiotics, barminomycins I and II have been reported. Physico-chemical and biological properties of I are identical with those of barminomycin I. We have not identified barminomycin II as the SN-07 chromophore yet because of its small quantity.

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