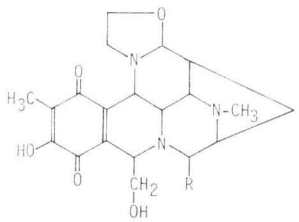
NEW SEMISYNTHETIC ANTITUMOR ANTIBIOTICS, SF-1739 HP AND NAPHTHOCYANIDINE

Sir:

In 1976, we reported the fermentation, isolation and biological properties of substance SF-1739[1]. It belongs to the indicator antibiotic group, which exhibit yellow color in acid and reddish purple in alkaline solution. The antibiotic exhibited high activity to bacteria and tumor cells, but is quite unstable, in particular to alkalis. The structure was not determined. Therefore, an attempt was made to produce more stable derivatives of substance SF-1739, with retention of bioactivity. We found that the desired derivatives, SF-1739 HP and naphthocyanidine, could be obtained by treatment of substance SF-1739 with mineral acid, and then by addition of cyanide anion. This treatment with acid involved the hydrolysis of a methoxyl group attached to benzoquinone.

The present communication describes the isolation, characterization and bioactivity of these semisynthetic antitumor antibiotics, SF-1739 HP and naphthocyanidine.

A culture of *Streptomyces griseoplanus* SF-1739 was fermented, as described previously[1]. The broth filtrate (50 liters) was adjusted to pH 6.0 and passed through a column of Amberlite IRC-50 (H^+, 1 liter). The antibiotic adsorbed was eluted with 0.2 N HCl (4.5 liters). The eluate was adjusted to pH 9.0, and extracted with ethyl acetate (2 liters). The organic layer was immediately re-extracted with 0.05 N HCl (500 ml), and the aqueous layer was concentrated. To the solution which contained substance SF-1739 was added an equal volume of conc. HCl, and the mixture was stood at room temperature for 2 days. The reaction mixture was adjusted to pH 7.0 with $NaHCO_3$, and concentrated to dryness. The residue was chromatographed on a silica gel column. The fraction eluted with $CHCl_3$ - MeOH (9: 1) afforded a semisynthetic antibiotic SF-1739 HP (**I**, 100 mg). A solution of **I** (100 mg) in methanol (10 ml) was added to 16 mg of potassium cyanide, and stood at room temperature for 30 minutes. The mixture was separated by preparative high performance liquid chromatography (Wako-gel C-300) developing with $CHCl_3$-MeOH (20: 1), and afforded the semisynthetic antibiotic naphthocyanidine (**II**, 60 mg).



SF-1739 HP (**I**) R = OH
Naphthocyanidine (**II**) R = CN

Compound **I** is a purple powder. It is soluble in water and methanol, but insoluble in chloroform, ethyl acetate and ethyl ether. It gives positive LEMIEUX and iodine reactions, but negative ninhydrin, MOLISCH and biuret reactions. It showed $[\alpha]_D^{25}$ +64° (*c* 0.5, MeOH); UV λ_{max}^{MeOH} nm ($E_{1cm}^{1\%}$) 275 (165), 330 (sh.); IR ν_{max}^{KBr} cm^{-1} 3400, 2950, 2900, 1650, 1530, 1390, 1240, 1180 and 1000; CMR (25 MHz, D_2O, ppm) 8.5, 28.7, 34.2, 40.5, 48.0, 50.0, 51.3, 53.4, 60.5, 60.6, 62.4, 66.5, 79.3, 93.8, 114.1, 137.9, 146.6, 169.2, 185.3 and 186.8.

Anal. Calcd. for $C_{20}H_{25}N_3O_6$:
C 59.54, H 6.26, N 10.41
Found: C 59.85, H 6.54, N 10.86

Fig. 1. PMR spectrum of **I** (100 MHz in D_2O).

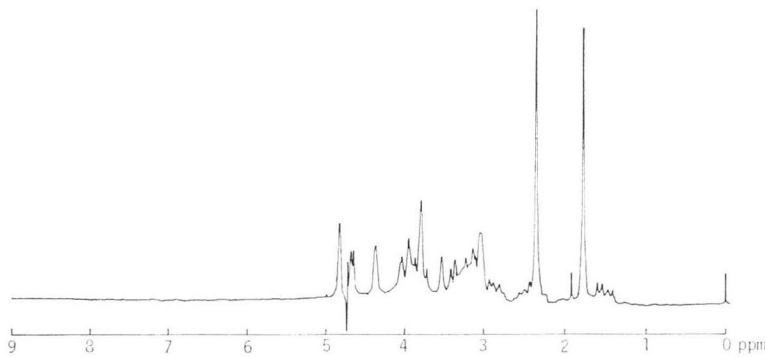


Fig. 2. PMR spectrum of **II** (100 MHz in D_2O).

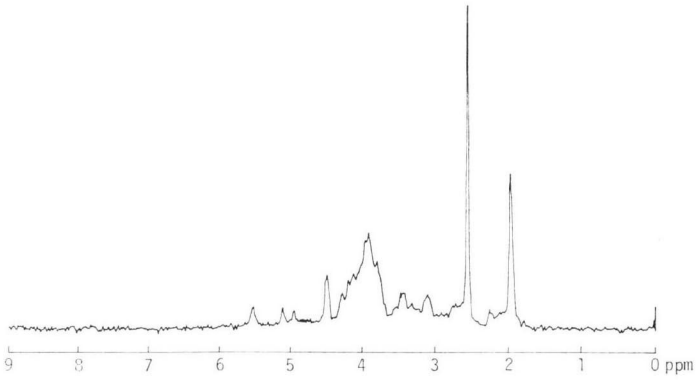
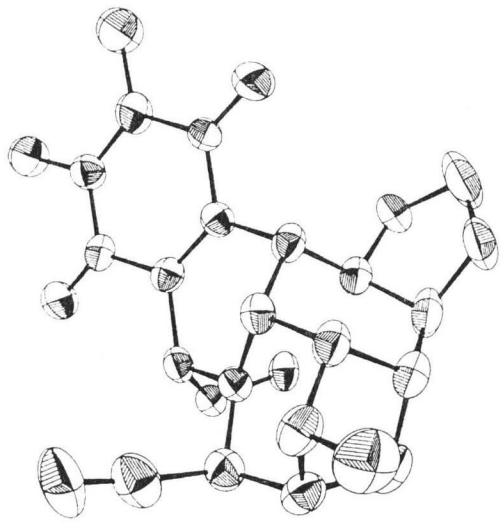


Fig. 3. Stereoscopic view of **II**.



The PMR spectrum of **I** is shown in Fig. 1.

Compound **II** is reddish yellow crystals. Its solubility and color reactions are similar to those of compound **I**. It showed $[\alpha]_D^{25}$ +76° (*c* 0.5, MeOH); UV λ_{max}^{MeOH} nm ($E_{1cm}^{1\%}$) 273 (300), 395 (32); IR ν_{max}^{KBr} cm^{-1} 3400, 2970, 2900, 2240, 1670, 1550, 1400, 1240, 1180 and 1050; CMR (25 MHz, $CDCl_3$, ppm) 8.1, 29.2, 35.3, 41.2, 48.1, 50.1, 53.5, 54.4, 56.6, 59.9, 60.6, 61.8, 62.7, 93.4, 113.8, 117.6, 136.6, 146.0, 164.3, 183.9 and 186.4.

Anal. Calcd. for $C_{21}H_{24}N_4O_5$:
C 61.45, H 5.41, N 13.64
Found: C 61.04, H 5.73, N 14.06

The PMR spectrum of **II** is shown in Fig. 2.

The presence of a cyanide group in **II** was indicated by the weak IR band at 2240 cm^{-1} and the signal at 117.6 ppm in its CMR spectrum[2]. The CMR spectra of both **I** and **II** revealed methyl groups at 8.5 and 8.1 ppm respectively (C-CH_3), 40.5 and 41.2 ppm respectively (N-CH_3) and six pairs of singlets between 113.8 and 186.8 ppm ascribed to the quaternary quinonoid carbons. Comparison of these CMR data with those of saframycin[2,3] indicated the presence of a 2-methyl-3-hydroxy-*p*-benzoquinone moiety in **I**

Table 1. The chemical stability and acute toxicity of substances SF-1739, SF-1739 HP (**I**) and naphthocyanidine (**II**).

Compound	Stability*	Toxicity** LD_{50} (i.v.)
SF-1739	5.7%	0.45 mg/kg
I	96.0%	2.0 mg/kg
II	98.0%	24.3 mg/kg

* The activity remaining after incubation of a 250 μg/ml solution in 0.1 M phosphate buffer (pH 7.0) at 28°C for 3 days was determined by a bioassay with *Bacillus subtilis*.
** JCL-ICR mice, male.

Table 2. Antibacterial spectra of substances SF-1739, SF-1739 HP (**I**) and naphthocyanidine (**II**).

Organism	MIC* (μg/ml)		
	SF-1739	**I**	**II**
Staphylococcus aureus 209P	0.09	0.20	0.20
Bacillus subtilis ATCC 6633	0.09	12.5	25
Escherichia coli NIHJ JC-2	0.78	6.25	6.25
Pseudomonas aeruginosa IFO 3080	3.13	3.13	6.25
Proteus vulgaris OX 19	1.56	0.78	0.78
Klebsiella pneumoniae PCI-602	0.78	1.56	3.13

* MIC values were determined by an agar-diffusion method with an inoculum of 10^6 cells/ml.

Table 3. Antitumor activities of SF-1739 HP (I) and naphthocyanidine (II) against leukemia P388.

Compound	Dose (mg/kg)	Survival days* (T/C)	ILS** (%)	60-days*** survivors
I	4	5.8 / 9.0	−35.6	0 / 5
	2	>42.2 / 9.0	>368.6	2 / 5
	1	19.8 / 9.0	120.0	0 / 5
	0.5	16.8 / 9.0	86.7	0 / 5
	0.25	16.8 / 9.0	86.7	0 / 5
	0.125	14.2 / 9.0	57.8	0 / 5
II	32	4.2 / 10.5	−60.0	0 / 5
	16	25.4 / 10.5	141.9	0 / 5
	8	29.8 / 10.5	183.8	0 / 5
	4	20.4 / 10.5	94.3	0 / 5
	2	16.6 / 10.5	58.1	0 / 5
	1	15.6 / 10.5	48.6	0 / 5

Animal: CDF_1 mice, male. Inoculum: 1.0×10^6 cells, i.p.. Treatment: Day 1~3, i.p.

* T/C=treated/control

** Increase of life span (%) was calculated from the average life span of the treated animals and that of the control animals.

*** Number of 60-days survivors/number of animals used.

and **II**. A signal at 79.3 ppm in **I**, which was assigned to the carbon of an α-carbinolamine[2], disappeared in **II**, while a new signal appeared at 117.6 ppm due to nitrile. This indicated that the hydroxyl group in **I** was replaced by cyano group in **II**. The complete structure of **II** was established by X-ray crystallographic study. The crystals of **II** were orthorhombic, space group $P2_12_12_1$, $a=13.800$ Å, $b=19.243$ Å, $c=9.272$ Å, $\alpha=\beta=\gamma=90^\circ$ and $Z=4$. Total reflection intensities of 1627 were measured with a Philips PW-1100 four-circle diffractometer. The stereoscopic view of **II** is shown in Fig. 3. From these results, the structures **I** and **II** were proposed for SF-1739 HP and naphthocyanidine.

The chemical stability, acute toxicity and antibacterial activity of these antibiotics, substance SF-1739, SF-1739 HP (**I**) and naphthocyanidine (**II**), are summarized in Tables 1 and 2. The antibacterial activities of **I** and **II** were retained in particular against Gram-negative bacteria, while considerable improvements in stability and acute toxicity were achieved in **I** and **II**, as compared with the parent antibiotic, substance SF-1739. The antitumor activities of **I** and **II** are shown in Table 3. Compounds **I** and **II** showed marked activity against leukemia P388 with the maximum ILS of >368.6% and 183.8%, respectively. Details of the structure determination and biological activity of the semisynthetic antibiotics, SF-1739 HP and naphthocyanidine, will be described in separate papers.

JIRO ITOH
SHOJI OMOTO
SHIGEHARU INOUYE
YOSHIO KODAMA
TAKASHI HISAMATSU
TARO NIIDA

Central Research Laboratories,
Meiji Seika Kaisha, Ltd.,
Kohoku-ku, Yokohama, Japan

YASUAKI OGAWA

Pharmaceutical Development Laboratories,
Meiji Seika Kaisha, Ltd.,
Horikawa-cho, Kawasaki, Japan

(Received January 7, 1982)

References

1) WATANABE, H.; T. SHOMURA, Y. OGAWA, Y. KONDO, K. OHBA, J. YOSHIDA, C. MORIYAMA, T. TSURUOKA, M. KOJIMA, S. INOUYE & T. NIIDA: Isolation and characterization of a new antibiotic, SF-1739 substance. Sci. Reports Meiji Seika Kaisha 16: 20~28, 1976

2) ARAI, T.; K. TAKAHASHI, K. ISHIGURO & K. YAZAWA: Increased production of saframycin A and isolation of saframycin S. J. Antibiotics 33: 951~960, 1980

3) ARAI, T.; K. TAKAHASHI, A. KUBO, S. NAKAHARA, S. SATO, K. AIBA & C. TAMURA: The structures of novel antibiotics, saframycin B and C. Tetrahedron Lett. 1979: 2355~2358, 1979