Communications to the Editor

STUDIES ON THE ISOTETRACENONE ANTIBIOTICS

I. CAPOAMYCIN, A NEW ANTITUMOR ANTIBIOTIC

Sir:

During the course of our screening program for new antitumor antibiotics, the cultured broth of an organism No. 23-41 showed antitumor activity and was found to contain a new antibiotic which we named capoamycin.

Strain No. 23-41 was isolated from a soil sample collected at Fujioka, Gunma, Japan. Taxonomic studies were carried out in accordance with the method adopted by the International Streptomyces Project (ISP). Microscopical and cultural observation and cell wall analysis of No. 23-41 indicated that this strain belongs to the genus *Streptomyces*. Based on the cultural characteristics and physiological properties, it was identified as a strain of *Streptomyces capoamus*.

No. 23-41 was cultivated on a rotary shaker at 27°C for 3 days in 500-ml Erlenmeyer flasks containing 100 ml of a medium consisting of glucose 2.5%, soybean meal 1.5%, dry yeast 0.2% and calcium carbonate 0.4%. The cultured broth (1 liter) was filtered with the aid of Celite and the mycelial cake was extracted with acetone. After the extract was concentrated *in vacuo*, the residue was partitioned between chloroform and water. The organic solvent layer was evaporated to a small volume and then 6 volumes of *n*-hexane were added. The supernatant was concentrated to dryness to give crude capoamycin. This crude material was chromatographed on a Toyopearl HW40F column. Development of the column with methanol gave a yellow band which was collected and concentrated *in vacuo* to give an orange yellow powder (142 mg) of pure capoamycin.

Capoamycin shows no definite melting point and begins to decompose at around 70°C. The physico-chemical properties of capoamycin are as follows: $[\alpha]_D^{21}$ +209° (*c* 0.1, acetone); *Anal* Calcd for $C_{35}H_{38}O_{10}$: C 67.95, H 6.19, O 25.86; Found: C 68.03, H 6.26, O 25.71; UV λ_{max}^{MeOH} nm ($E_{1cm}^{1\%}$), 235 (704), 257 (782), 305 (139) and 442 (100); FAB-MS *m/z* 724 (M + diethanolamine + H)$^+$. The IR spectrum (KBr) indicates the presence of hydroxyl groups (3420 cm^{-1}), an unsaturated ester carbonyl group (1710 cm^{-1}), an unsaturated ketone carbonyl group in a 6-membered ring (1690 cm^{-1}), a non-chelated quinone carbonyl (1670 cm^{-1}, sh) and a chelated quinone carbonyl group (1638 cm^{-1}).

The ^{1}H NMR spectrum in $CDCl_3$ (Fig. 2) shows the following signals: δ 12.25 (s, 8-OH), 7.84 (d, 10-H, J=7.6 Hz), 7.58 (d, 11-H, J=7.6), 6.91 (d, 6-H, J=10.0), 6.43 (d, 5-H, J=10.0), 6.22 (br s, 2-H), 2.76 (d, 4-Ha, J=18.8), 2.51 (d, 4-Hb, J=18.8) and 1.94 (s, 3-CH_3) ascribed to a modified benz[a]anthraquinone chromophore; δ 4.87 (dd, 1′-H, J=11.2, 1.6), 4.64 (dd, 4′-H, J=9.4, 9.0), 3.97 (ddd, 3′-H, J=11.0, 9.0, 5.3), 3.66 (dq, 5′-H, J=9.4, 6.0), 2.57 (ddd, 2′-Ha, J=11.3, 5.3, 1.6), 1.51 (ddd, 2′-Hb, J=11.3, 11.2, 11.0) and 1.30 (d, 6′-H_3, J=6.0) assignable to a β-olivoside moiety; δ 7.33 (m, 3″-H), 6.19 (m, 4″-H and 5″-H), 5.87 (d, 2″-H, J=15.4), 2.19 (dt, 6″-H_2, J=5.4, 7.1), 1.44 (tt, 7″-H_2, J=7.3, 7.1), 1.31 (m, 8″-H_2 and 9″-H_2) and 0.90 (t, 10″-H_3, J=7.4) due to a 2,4-decadienoic acid side chain.

The chromophore moiety of capoamycin was

Fig. 1. Structure of capoamycin.

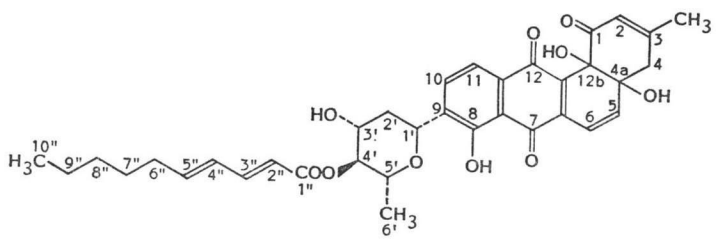

Fig. 2. 400 MHz ^1H NMR spectrum of capoamycin in $CDCl_3$.

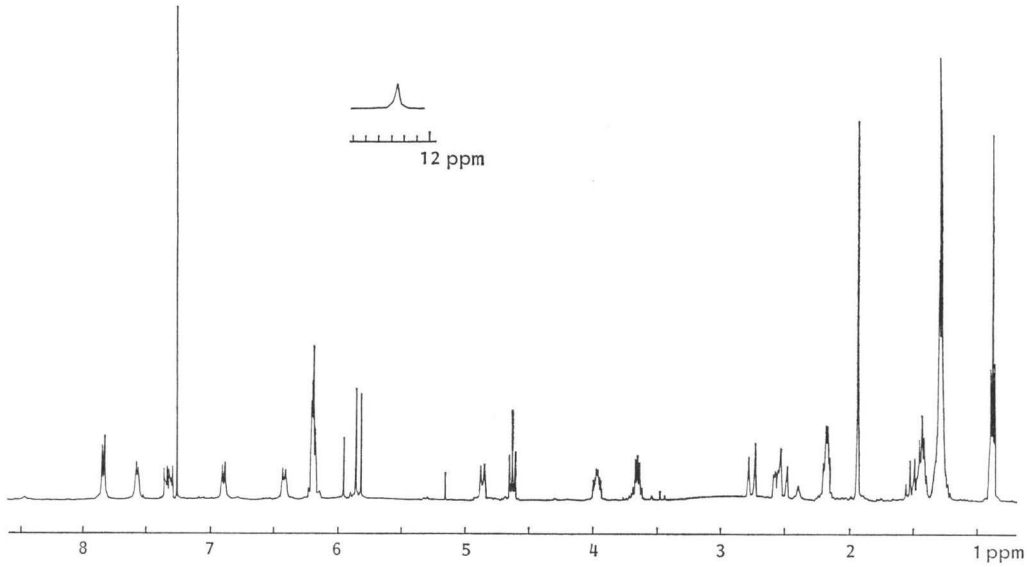

Table 1. Effect of capoamycin on the growth and induction of phagocytic activity of M1 cells.

Dose (ng/ml)	Phagocytic cells (%)	Number of cells (10^5 cells/ml)
0	1	12.1
10	1	15.6
20	13	11.8
40	15	9.9
80	25	7.6
160	33	3.6
320	44	0.8

M1 cells at 2×10^5 cells/ml were incubated with various concentration of capoamycin for 72 hours and then their phagocytic activities were examined. Cell growth was determined from the cell number after trypan blue stained cells had been excluded.

Table 2. Antitumor activity of capoamycin against Ehrlich mouse ascites carcinoma.

Dose (mg/kg/day)	T/C* (%)	Toxicity**
1.25	104	0/5
2.5	102	0/5
5	159	0/5
10	121	1/5

Treatment schedule: day 1, 5 (ip).

* The ratio of mean survival time of the treated group divided by that of the control group.

** Mortality due to toxicity of the antibiotic.

Table 3. Antimicrobial spectrum of capoamycin.

Organism	MIC (μg/ml)
Staphylococcus aureus FDA 209P	12.5
Bacillus subtilis ATCC 6633	12.5
B. cereus IAM 1729	12.5
Escherichia coli NIHJ JC-2	>100
Klebsiella pneumoniae PCI-602	>100
Salmonella typhimurium IID 971	>100
Serratia marcescens IAM 1184	>100
Pseudomonas aeruginosa NCTC 10490	>100
Saccharomyces cerevisiae ATCC 9763	12.5
Candida albicans No. Yu 1200	12.5
Aspergillus fumigatus IFO 4400	100
Penicillium chrysogenum ATCC 10002	1.56
Trichophyton mentagrophytes	12.5

suggested to consist of the tetracyclic system shown in Fig. 1 by ^{13}C NMR analysis. Based on long range selective proton decoupling experiments, the following signals were assigned: δ 195.7 (s, C-1), 187.3 (s, C-7), 182.6 (s, C-12), 157.5 (s, C-3 and C-8), 143.3 (d, C-5), 138.6 (s, C-12a), 138.4 (s, C-6a), 137.4 (s, C-9), 133.2 (d, C-10), 130.5 (s, C-11a), 124.4 (d, C-2), 119.8 (d, C-6), 119.5 (d, C-11), 113.7 (s, C-7a), 75.6 (s, C-4a and C-12b), 41.7 (t, C-4) and 24.3 (q, 3-CH_3). From these data and the nuclear Overhauser effect observed between 4-H_2 and 5-H, the structure of the chromophore was

defined as shown in Fig. 1.

The long range coupling between 10-H and 1′-H indicates that the olivose residue is connected to the C-9 position through a C-glycosidic linkage.

Hydrolysis of capoamycin with 0.1 N NaOH at room temp gave (*E,E*)-2,4-decadienoic acid [$C_{10}H_{16}O_2$; MS, *m/z* 182 (M^+, measured as a methyl ester)]. An acylation shift was observed on H-4′ in comparison with the ^1H NMR spectrum of aquayamycin,[1] which is a related compound possessing a 2,3-dihydro-3-hydroxy chromophore and no acid side chain.

Thus, it is concluded that the structure of capoamycin is as shown in Fig. 1 with uncertainity about the stereochemistry of the ring juncture and the absolute configuration.

On the basis of the structural similarity of capoamycin and the kerriamycins[2] to aquayamycin, P-1894 B[3] and the sakyomicins[4] *etc.*, which contain the modified benz[a]anthraquinone nucleus in common, we wish to propose the generic name "isotetracenone antibiotics" to cover the compounds belonging to this family. The structural studies will be reported in full in due course.

It is of interest that capoamycin showed differentiation-inducing activity on mouse myeloid leukemia cells (M1).[5] The effect on the induction of phagocytic activity of M1 cells is summarized in Table 1. Table 2 shows the effect of capoamycin on mouse Ehrlich ascites carcinoma. Intraperitoneal injections of capoamycin on days 1 and 5 caused prolongation of the life spans of treated mice. The antimicrobial activity of capoamycin is shown in Table 3. The LD_{50} for capoamycin in mice was approximately 15 mg/kg (ip).

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