NEW ANTHRACYCLINE ANTIBIOTIC, 2-HYDROXYACLACINOMYCIN A

Sir:

In the course of biosynthetic study of anthra-cycline antibiotics[1~6], microbial glycosidation of biologically inactive anthracyclinones to active anthracyclines led to the creation of new analogs with higher therapeutic index. In this communi-cation, we describe the microbial glycosidation of 2-hydroxyaklavinone[6], which was produced by a mutant strain ANR-58 derived from aclacino-mycin-producing *Streptomyces galilaeus* MA144-M1, by an aclacinomycin-negative mutant strain KE303 and the preliminary characterization of new anthracycline 2-hydroxyaclacinomycin A.

The strain KE303 was cultured in 500-ml flasks containing 50 ml medium of the following com-position: Glucose 1%, soluble starch 1.5%, soybean meal(Ajinomoto Co., Ltd.) 2%, K_2HPO_4 0.1%, $MgSO_4 \cdot 7H_2O$ 0.1%, $CuSO_4 \cdot 5H_2O$ 0.0007%, $FeSO_4 \cdot 7H_2O$ 0.0001%, $MnCl_2 \cdot 4H_2O$ 0.0008% and $ZnSO_4 \cdot 7H_2O$ 0.0002%, pH 7.4. The cultivation was carried out for 17 hours on a rotary shaker at 210 rpm at 28°C, and then 0.5 ml of a methanol solution of 2-hydroxy-aklavinone (2 mg/ml) was added to each flask at the final concentration of 20 μg/ml, and the cultivation was continued for 24 hours to com-plete the glycosidation. Dark yellow antibiotics were extracted from the cultured broth with a solvent mixture of chloroform - methanol (3: 2,

v/v), concentrated to dryness, dissolved in a small amount of chloroform, spotted onto silica gel thin-layer (F_{254} plate E. Merck Co.), and develop-ed with chloroform - methanol - aqueous am-monia (50: 10: 0.5, v/v/v) mixture. After drying, the spots corresponding to 2-hydroxyaclacino-mycin A (I) (Rf=0.51) and residual 2-hydroxy-aklavinone (II) (Rf=0.30) were determined by a Shimadzu chromatoscanner model CS-910. The conversion rate of II to I was over 90%.

Fifty liters of the cultured broth were centri-fuged to harvest the mycelium, and the product was extracted from the mycelium with 8 liters of acetone, concentrated, and re-extracted with 3 liters of chloroform. After concentrated to dryness, the residue was subjected to Sephadex LH-20 column (40×5 cm) and eluted with methanol. Initial yellow fractions were purified by the repeated preparative layer (Kieselgel 60PF$_{254}$, E. Merck Co.) chromatography using two different solvent systems chloroform - metha-nol - aqueous ammonia (50: 10: 0.3, v/v/v) and chloroform - methanol - acetic acid (80: 10: 0.5, v/v/v) mixtures. The pigment extract was con-centrated, dissolved in 20 ml of 0.1 M acetate buffer (pH 3.5) and washed with toluene. The aqueous layer was neutralized with sodium bicar-bonate and extracted with chloroform. To the concentrate was added excess *n*-hexane to form orange precipitate, and 293 mg of pure I were obtained by filtration and drying of the precipitate *in vacuo*.

Fig. 1. PMR spectrum of 2-hydroxyaclacinomycin A in CDCl$_3$ (100 MHz).
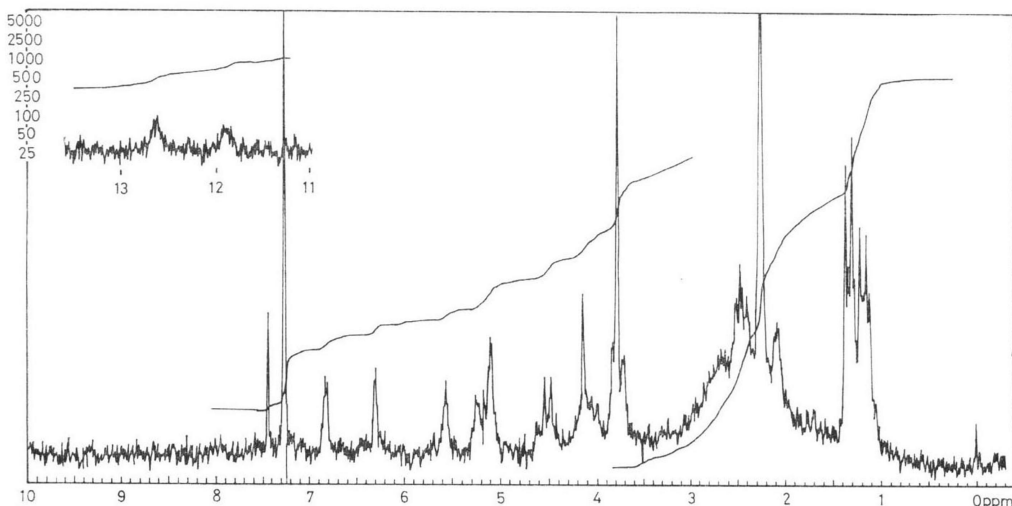
Physicochemical properties of **I** are as follows: m.p. $165 \sim 167°C$; IR (KBr) cm^{-1}: 1735, 1675, 1620, 1610, 1010; $\lambda_{max}^{90\% \ MeOH}$ nm (E$_{1cm}^{1\%}$): 222 (375), 256 (235), 295 (207), 450 (110); $\lambda_{max}^{0.1N \ NaOH-90\% \ MeOH}$ nm (E$_{1cm}^{1\%}$): 240s (370), 297 (250), 330s (196), 540 (143); $[\alpha]_D^{23}$ +42.3° (c 0.04, MeOH); *Anal.* calcd. for $C_{42}H_{53}NO_{16}$ (MW. 827.9): C 60.93, H 6.45, N 1.69; found: C 60.27, H 6.20, N 1.64%.

The molecular formula of **I** suggested that the antibiotic possesses one additional oxygen atom in comparison with aclacinomycin A. The PMR spectrum of **I** (Fig. 1) has a strong resemblance to that of aclacinomycin A in the high and middle fields. In the anomeric region, three signals at δ 5.56, 5.24 and 5.10 corresponding to four protons were found and assigned to C-1′, C-7, C-1″ and C-1‴ protons, respectively. This indicated that **I** had a trisaccharide moiety. Two doublet peaks at δ 6.83 and 6.31 with small coupling constant (2.0 Hz) were considered to be aromatic protons in *meta* positions at C-1 and C-3. Thus, it assigned one additional phenolic hydroxyl to C-2. Treatment of **I** in 0.1 N hydrochloric acid at 85°C for 30 minutes gave an aglycone and sugar moieties. The aglycone moiety was identified as **II**, which was added to the fermentation medium, by direct comparison of its melting point, Rf value and mass spectra. Sugar moieties were identified as rhodosamine, 2-deoxyfucose and cinerulose A on silica gel thin-layer as detected in the acid hydrolysate of aclacinomycin A. The partial methanolysis of the antibiotic in 0.01 N methanolic hydrogen chloride-acetone at room temperature for 45 minutes resulted in rhodosaminyl-2-hydroxyaklavinone, and methyl-L-cinerulosyl-2-hydroxy-L-fucoside[5] which was identified by direct comparison with the color development and Rf value of the authentic sample obtained by methanolysis of aclacinomycin A. In order to determine the site of glycosidic linkage, **I** was reductively cleaved by hydrogenolysis on palladium catalyst, and the product gave a yellow aglycone on TLC using benzene - acetone (5: 1, v/v) with the Rf value of 0.39 shown to be 2-hydroxy-7-deoxyaklavinone by comparison of its physicochemical properties with those of authentic compound 58C[4]. Thus, the structure of **I** was deduced to be cinerulosyl-2-deoxyfucosyl - rhodosaminyl- 2 -hydroxyaklavinone, as shown in Fig. 2.

The antibiotic **I** showed a marked cytotoxicity against cultured L1210 leukemia cells and inhi-

Fig. 2. Structure of 2-hydroxyaclacinomycin A.
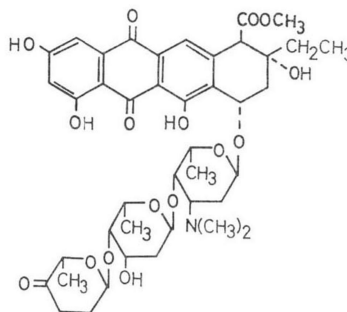
bited preferentially RNA synthesis. The IC$_{50}$ values for RNA and DNA synthesis were 0.10 and 0.95 μg/ml, respectively. The antibiotic **I** exhibited strong inhibition against L1210 leukemia in CDF$_1$ mice. When 6 mg/kg/day of **I** was administered intraperitoneally once daily for 10 days, the increase of life span (ILS %) was 118 and effective dose range exhibiting over 45% ILS was 0.5 to 8 mg/kg/day, which was much wider than aclacinomycin A. Acute toxicity of **I** (LD$_{50}$, about 50 mg/kg, i.p.) was lower than aclacinomycin A.

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References

1) OKI, T.; A. YOSHIMOTO, Y. MATSUZAWA, T. TAKEUCHI & H. UMEZAWA: Biosynthesis of anthracycline antibiotics by *Streptomyces galilaeus*. I. Glycosidation of various anthracyclinones by an aclacinomycin-negative mutant and biosynthesis of aclacinomycins from aklavinone. J. Antibiotics 33: 1331~1340, 1980

2) OKI, T.; Y. MATSUZAWA, K. KIYOSHIMA, A. YOSHIMOTO, H. NAGANAWA, T. TAKEUCHI & H. UMEZAWA: New anthracyclines, feudo-

mycins, produced by the mutant from *Strepto-myces coeruleorubidus* ME130-A4. J. Antibiotics 34: 783~790, 1981

3) YOSHIMOTO, A.; T. OKI, T. TAKEUCHI & H. UMEZAWA: Microbial conversion of anthra-cyclinones to daunomycin by blocked mutants of *Streptomyces coeruleorubidus*. J. Antibiotics 33: 1158~1166, 1980

4) MATSUZAWA, Y.; A. YOSHIMOTO, N. SHIBAMOTO, H. TOBE, T. OKI, H. NAGANAWA, T. TAKEUCHI & H. UMEZAWA: New anthracycline meta-bolites from mutant strains of *Streptomyces galilaeus* MA144-M1. II. Structure of 2-hydro-xyaklavinone and new aklavinone glycosides.

J. Antibiotics 34 (8): 1981 (in press)

5) MATSUZAWA, Y.; A. YOSHIMOTO, T. OKI, H. NAGANAWA, T. TAKEUCHI & H. UMEZAWA: Biosynthesis of anthracycline antibiotics by *Streptomyces galilaeus*. II. Structure of new anthracycline antibiotics obtained by microbial glycosidation and biological activity. J. Anti-biotics 33: 1341~1347, 1980

6) YOSHIMOTO, A.; Y. MATSUZAWA, T. OKI, T. TAKEUCHI & H. UMEZAWA: New anthracycline metabolites from mutant strains of *Streptomyces galilaeus* MA144-M1. I. Isolation and charac-terization of various blocked mutants. J. Anti-biotics 34 (8): 1981 (in press)