NEW ANTITUMOR ANTIBIOTICS, ALCACINOMYCINS A AND B

Sir:

In the study of antitumor antibiotics new members of anthracycline group, aclacinomycins A and B, have been isolated from Streptomyces sp. No. MA144-MI (ATCC 31133) which was classified as S. galilaeus.

Aclacinomycins were produced by the shaking culture of MA144-MI strain at 28°C for 4 days in a medium containing 1% glucose, 1% potato starch, 1.5% soybean meal ("Prorich", Ajinomoto Co.), 0.1% KH₂PO₄, 0.1% MgSO₄·7H₂O, 0.3% NaCl, 0.007% CuSO₄·5H₂O, 0.0001% FeSO₄·7H₂O, 0.0008% MnCl₂·4H₂O and 0.0002% ZnSO₄·7H₂O, pH 7.0. Total aclacinomycin was assayed by a disc plate method against Bacillus subtilis or Micrococcus flavus, and aclacinomycins A and B were separately determined by a silica gel thin-layer chromatography using chloroform-methanol (20:1) and by reading the optical density of spots with Rf 0.36 and 0.71 at 430 nm using a Shimazu dual-wave length TLC scanner, Model CS-900. In the case of testing the cultured broth, aclacinomycins were extracted with chloroform or a chloroform-methanol mixture (1:1) before the thin-layer chromatography and were developed with acetone or chloroform-methanol mixture (20:1).

In an example, the cultured broth (pH 7.4) which contained 32 mcg/ml of aclacinomycin A and 23 mcg/ml of aclacinomycin B was adjusted to pH 4.5 and filtered. Aclacinomycins were extracted from the mycelia and the filtrate at pH 6.8 with acetone and ethyl acetate, respectively, and the active extracts were concentrated in vacuo and filtered. Aclacinomycins were precipitated by addition of n-hexane to the concentrated solution. Further purification of A and B was accomplished by a column chromatography of Column-Lite (Fuji Chemical Ind. Co., 30-60 mesh) developed with a chloroform-methanol mixture (1:1). Red fractions containing aclacinomycins A and B were evaporated to dryness in vacuo, and dissolved in a small amount of chloroform. After adding the 0.01 M phosphate buffer (pH 7.2) containing 1 mM EDTA to each aclacinomycin A and B solution and shaking vigorously to remove the residual metal ions, the chloroform phase was washed twice by shaking with water, dried over anhydrous sodium sulfate, and concentrated in vacuo. Thus, pure aclacinomycins A and B were obtained as yellow microcrystalline powders by addition of n-hexane to the concentrated solution. The yields were 10 to 20% from the cultured broth.

Physicochemical properties of aclacinomycins A and B are as follows:

Aclacinomycin A: m.p. 129-135°C under decomposition; [α]₂₄D +29° (c 1.0, CHCl₃); anal. calcd. for C₄₂H₅₄O₁₅N: C 62.05, H 6.69, O 29.52, N 1.72; found: C 62.37, H 6.67, O

Fig. 1. Ultraviolet and visible light absorption spectra of aclacinomycin A.

\[ E_{\text{max}}^{1%} \]

- in methanol
- in 0.1N HCl - methanol
- in 0.1N NaOH - methanol
29.38, N 1.82. The ultraviolet and visible light absorption spectra and the IR spectrum in KBr are shown in Figs. 1, 2.

Aclacinomycin B: m.p. 135–145°C under decomposition; $[\alpha]_D^{25} +3^\circ$ (c 1.0, CHCl$_3$); anal. calcd. for C$_{42}$H$_{52}$O$_{15}$N: C 62.21, H 6.46, O 29.60, N 1.73; found: C 61.87, H 6.29, O 29.80, N 1.89. The ultraviolet and visible light absorption spectra and the IR spectrum in KBr are shown in Figs. 3, 4.
Aclacinomycins A and B are soluble in chloroform and ethyl acetate, and moderately soluble in methanol, ethanol, dioxane, benzene, acetone, acidic water and pyridine while sparingly soluble or insoluble in ethyl ether, n-hexane, cyclohexane and petroleum ether. The solution of aclacinomycins A and B in conc. HCl is yellow, that in conc. H$_2$SO$_4$ intensely reddish brown to reddish purple. Addition of alkali to aqueous solutions of the antibiotics gives an intense reddish purple color. Alcoholic magnesium acetate yields a purplish red color. On thin-layer chromatography using silica gel 60F-254 (E. Merck), the antibiotic gives a single spot at Rf 0.36 for A and 0.71 for B with chloroform - methanol (20:1), and at Rf 0.15 for A and 0.49 for B with acetone - n-hexane (1:1), respectively.

Acid hydrolysis of aclacinomycins A and B (25 mg each) with 2 ml of 0.3 N H$_2$SO$_4$ for 3 hours at 85°C yielded the aglycone, aklavincnone, in the form of orange-yellow needles in good yield (16 mg and 14 mg): m.p. 171-174°C; anal. calcd. for C$_{22}$H$_{20}$O$_8$: C 64.08, H 4.86; found: C 64.19, H 5.11. m/e 412. The IR spectrum in KBr, the ultraviolet and visible light absorption spectra and the fragment ion peaks of the mass spectrum were identical with those of aklavincnone, A and B possess three sugars corresponding to rhodosamine, 2-deoxyfucose and cinerulose which were detected in the hydrolysate of an authentic cinerubin A. On partial hydrolysis of aclacinomycin A in methanol containing 5% HCl for 2 hours at room temperature, the antibiotic gave the methylated disaccharide and 1-deoxypyrromycin: orange-yellow crystals [α]$_D$ +216° (c 1.0, CHCl$_3$); anal. calcd. for C$_{30}$H$_{35}$O$_{11}$N: C 63.25, H 6.19, O 28.08, N 2.45; found: C 62.44, H 6.26, O 28.08, N 2.38. It solidified at 121-127°C and then melted at 230-235°C. The NMR and IR spectra and the ultraviolet and visible light absorption spectra showed that this compound is 1-deoxypyrromycin by the comparison with those of pyrromycin. After neutralizing and evaporating the acid hydrolysate, the residue was extracted twice with chloroform and water. By evaporation and distillation of the aqueous layer, the methyl disaccharide of aclacinomycin A crystallized. The IR and NMR spectra and melting point of the sugar coincided with those of the methyl disaccharide obtained from cinerubin A. On the other hand, when aclacinomycin B was hydrolyzed under the same condition, 1-deoxypyrromycin and a methylated disaccharide were obtained. This methyl disaccharide, which was crystallized from acetone-cyclohexane following the chloroform extraction of the acid hydrolysate and the silica gel column chromatography, was identified with the sugar moiety of cinerubin B by the IR, NMR spectra and melting point. Thus, it was determined that aclacinomycins belong to the group of aklavincnone glycosides including aklavin, requinomycin and galirubins, and have the structures shown below.

Aklavin and requinomycin are different from aclacinomycin in the sugar moiety, molecular formula and optical rotation. The most similar to aclacinomycins are galirubins. ECKARDT$^{31}$ reported the isolation of galirubins and galirubinones from the mycelia of S. galilaeus: galirubin A (ε-pyrromycinone glycoside), galirubin B (aklavinone glycoside), galirubinone C (ζ-pyrromycinone), and galirubinone D (7-deoxyaklavinone). A sample of a galirubin mixture supplied by ECKARDT gave several spots on silica gel thin-layer chromatography using acetone or chloroform - methanol (20:1). The main component, an orange-red spot, was identified as cirerubin A by co-
chromatography with an authentic cinerubin A. A faint yellow spot which was detected just above the orange-red spot in acetone showed the same Rf value as aclacinomycin A. However, it was not certain whether this minor component is galirubin B, since direct comparison with an authentic galirubin B was not possible and two or three other yellow spots were detected in the galirubin mixture. In respect of the sugar moiety, moreover, ECKARDT reported the presence of two sugar spots in the acid hydrolysate of galirubin B by paper chromatography. Therefore, aclacinomycins A and B having three sugar moieties should be new members of the anthracyclic group.

Aclacinomycins exhibited inhibition against L 1210 leukemia in BDF1 mice, and A was stronger. When 1.5 mg/kg/day of aclacinomycin A was injected intraperitoneally once daily for 10 days, the survival period was 300% of the control. A slight decrease of body weight appeared in a dose of 4 mg/kg/day. Aclacinomycins inhibited the growth of cultured L 1210 cells (ID$_{50}$ at 0.12 mcg/ml of A, and at 0.24 mcg/ml of B) and vaccinia virus in HeLa cells. Fifty per cent inhibition of RNA synthesis was caused at 0.1 mcg/ml of A and 0.2 mcg/ml of B and at 0.5 mcg/ml of 1-deoxypyrromycin. The LD$_{50}$'s of aclacinomycins A and B in mice were 22.6 mg/kg and 13.7 mg/kg by a single intraperitoneal injection, and 33.7 mg/kg and 16.4 mg/kg by a single intravenous injection, respectively. In addition, ECG change in hamster produced by adriamycin at 3 mg/kg, single i.p., was not exhibited by aclacinomycin A at 50 mg/kg, single i.p. The antimicrobial spectra of the antibiotics tested by the broth dilution method are shown in Table 1.

Acknowledgement
The authors are grateful to Dr. J. GELZER, CIBA-GEIGY Ltd., for a supply of cinerubin A, and to Dr. K. ECKARDT, Zentralinstitut für Mikrobiologie und Experimentelle Therapie, Akademie der Wissenschaften der DDR., for a supply of galirubin mixture.
Central Research Laboratory of Sanraku-Ocean Co., Ltd., Jonan, Fujisawa, Kanagawa, Japan

HAMAO UMEZAWA
MASAAKI ISHIZUKA
HIROSHI NAGANAWA
HIROYUKI SUDA
MASA HAMADA
TOMIO TAKEUCHI

Institute of Microbial Chemistry,
Kamiosaki, Shinagawa-ku, Tokyo, Japan
(Received June 4, 1975)

References