NEW ANTHRACYCLINE ANTIBIOTIC, 2-HYDROXYACLACINOMYCIN A

Sir:

In the course of biosynthetic study of anthracycline antibiotics1~6), microbial glycosidation of biologically inactive anthracyclonones to active anthracyclines led to the creation of new analogs with higher therapeutic index. In this communication, we describe the microbial glycosidation of 2-hydroxyaklavinone6), which was produced by a mutant strain ANR-58 derived from aclacinomycin-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 composition: Glucose 1%, soluble starch 1.5%, soya bean meal (Ajinomoto Co., Ltd.) 2%, K2HPO4 0.1%, MgSO4·7H2O 0.1%, CuSO4·5H2O 0.0007%, FeSO4·7H2O 0.0001%, MnCl2·4H2O 0.0008% and ZnSO4·7H2O 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-hydroxyaklavinone (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 complete 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 (F254 plate E. Merck Co.), and developed with chloroform - methanol - aqueous ammonia (50:10:0.5, v/v/v) mixture. After drying, the spots corresponding to 2-hydroxyaclacinomycin A (I) (Rf=0.51) and residual 2-hydroxyaklavinone (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 centrifuged 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 Sephadox LH-20 column (40x5 cm) and eluted with methanol. Initial yellow fractions were purified by the repeated preparative layer (Kieselgel 60PF254, E. Merck Co.) chromatography using two different solvent systems chloroform - methanol - 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 concentrated, dissolved in 20 ml of 0.1 m acetate buffer (pH 3.5) and washed with toluene. The aqueous layer was neutralized with sodium bicarbonate 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 CDCl3 (100 MHz).
Physicochemical properties of I are as follows:
m.p. 165~167°C; IR (KBr) cm⁻¹: 1735, 1675, 1620, 1610, 1010; λmax 90% MeOH nm (ε1%1cm): 222 (375), 256 (235), 295 (207), 450 (110); λmax 0.1 N NaOH-90% MeOH nm (ε1%1cm): 240s (370), 297 (250), 330s (196), 540 (143); [α]D +42.3° (c 0.04, MeOH); Anal. calcd. for C₄₂H₅₃NO₁₆ (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 double 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 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. 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 inhibited preferentially RNA synthesis. The IC₅₀ 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 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 (LD₅₀, about 50 mg/kg, i.p.) was lower than aclacinomycin A.

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