Communications to the editer

NEW ANTITUMOR ANTIBIOTICS: 13-METHYLACLACINOMYCIN A AND ITS DERIVATIVES

Sir:

We have previously reported that *Streptomyces galilaeus* MA144-M1 produces 21 anthracycline antibiotics[1~5]. In this paper, we will report the isolation of an additional anthracycline antibiotic, 13-methylaclacinomycin A having 13-methylaklavinone as the aglycone, from the same cultured broth.

Cultured broth of *Streptomyces galilaeus* MA144-M1 (ATCC 31133) was extracted with toluene and the yellow pigments in the toluene layer were transferred to acetate buffer (0.2 M, pH 3.5), and re-extracted with toluene at pH 7.0. The toluene layer was dried over anhydrous sodium sulfate, concentrated under reduced pressure, and subjected to Prep LC System 500 (Prepak Silica) high performance liquid chromatography (Waters Associates Inc.) with 2% isopropanol in toluene. From one of pigment peaks 13-methylaclacinomycin A was obtained as a yellow powder by pouring the eluate into 10 volumes of *n*-hexane.

The physicochemical properties of 13-methylaclacinomycin A are as follows: mp 160.5°C; IR (KBr disc) cm^{-1}; 1730, 1670, 1620, 1010; λ_{max}^{MeOH} nm ($E_{1cm}^{1\%}$); 229 (531), 258 (310), 289 (121), 430 (158); $[\alpha]_D^{20}$ 1.96° (*c* 0.93, $CHCl_3$); elemental *anal.* calcd. for $C_{43}H_{55}NO_{15}$, C 62.53, H 6.71, N 1.70; found C 62.43, H 6.65, N 1.98; ^{1}H-NMR ($CDCl_3$) δ in ppm, 0.9~1.4 (m, 15H, methyls), 2.15 (s, 6H, dimethylamino at C-3′), 3.65 (s, 3H, methoxyl), 12.0 and 12.6 (phenolic OH).

A 50 mg sample of 13-methylaclacinomycin A was dissolved in 5 ml of 0.1 N HCl, heated at 85°C for 30 minutes, neutralized with silver carbonate, and extracted with chloroform. From the chloroform layer 15 mg of 13-methylaklavinone was obtained as a yellow powder after purification by HPLC (silica gel). Recrystallization from benzene yielded 10 mg of orange-yellow

Fig. 1. ^{1}H-NMR spectrum of 13-methylaklavinone.

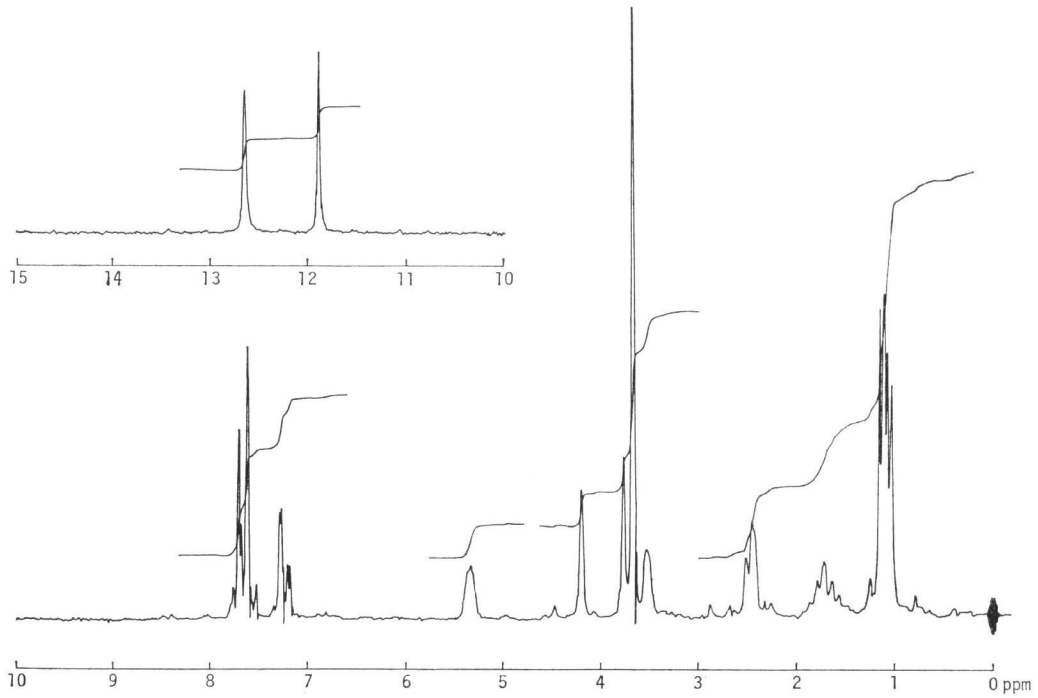
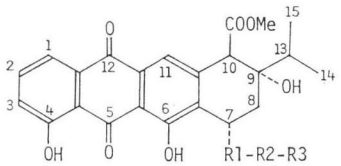
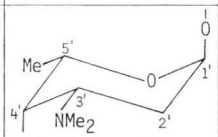
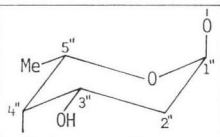
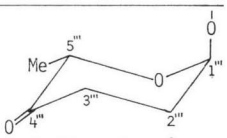
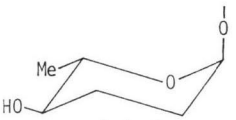
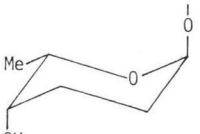


Table 1. The structures of the 13-methylaclacinomycins.



Compounds	R1	R2	R3
13-Methylaclacinomycin A	 L-Rhodosamine	 2-Deoxy-L-fucose	 L-Cinerulose A
13-Methylaclacinomycin M	〃	〃	 L-Amicetose
13-Methylaclacinomycin N	〃	〃	 L-Rhodinose
13-Methylaclacinomycin S	〃	〃	OH
13-Methylaklavin	〃	OH	
13-Methylaklavinone	OH		

needles. The aqueous layer was concentrated under reduced pressure, spotted on silica gel thin-layer (60 F_{254} Merck Co.) and developed with *n*-butanol - acetic acid - water (4: 1: 1). L-Rhodosamine, 2-deoxy-L-fucose and L-cinerulose were identified by comparison with authentic samples prepared by hydrolysis of aclacinomycin A.

The structure of 13-methylaklavinone was determined by comparing its spectral data (^{1}H-NMR, IR, MS) with those of aklavinone[6,7]. It is obvious from the ^{1}H-NMR spectrum shown in Fig. 1 that this aglycone has an isopropyl group at the C-9 position. The multiplet peaks at δ 1.5~1.9 (1H) assigned to the methine proton at C-13 position were coupled with the double doublets at δ 1.0~1.3 (6H) assigned to the two methyl groups (14-CH_3 and 15-CH_3). The structure of 13-methylaklavinone was also supported by mass spectroscopy showing *m/z* 426 (M^+). The properties of 13-methylaklavinone are as follows: mp 216°C; IR(KBr disc) cm^{-1}; 1730, 1670, 1620; elemental *anal.* calcd. for $C_{23}H_{22}O_8$, C 64.78, H 5.20; found C 64.64, H 5.18; ^{1}H-NMR ($CDCl_3$) δ in ppm, 1.0~1.3 (dd, 6H, methyls at C-14 and C-15), 1.5~1.9 (m, 1H, methine at C-13), 2.4~2.6 (m, 2H, methylene), 3.5 (s, 1H, alcoholic OH at C-7), 3.7 (s, 3H, methoxyl), 3.8 (s, 1H, methine at C-10), 5.35 (s, 1H, methine at C-7), 7.15~7.35 (m, 1H, methine at C-3), 11.9 and 12.6 (phenolic OH).

Methanolysis of 13-methylaclacinomycin A gave 13-methylaklavin and a methyl disaccharide.

To a solution of 100 mg of 13-methylaclacinomycin A in 20 ml of dry acetone - methanol (10: 1) was added 2 ml of 0.15 N dry HCl - MeOH. The mixture was allowed to stand for 10 minutes at room temperature and neutralized with silver carbonate. After filtration, the filtrate was evaporated under reduced pressure and subjected to HPLC (silica gel) with benzene - ethyl acetate (65: 35) followed by chloroform - methanol - aqueous ammonia (100: 10: 1). From the former eluate 24 mg of a colorless oily substance was obtained and identified as methyl-2-deoxy-L-fucosyl-L-cinerulose by comparing its spectral data with those of an authentic sample obtained from aclacinomycin A. From the latter eluate with the latter solvent 30 mg of 13-methylaklavin was obtained after purification by HPLC (silica gel) with chloroform - methanol (10: 1). The properties of 13-methylaklavin are as follows: mp 142.5°C; IR (KBr disc) cm^{-1}; 1730, 1670, 1620, 1020; elemental *anal.* calcd. for $C_{31}H_{37}NO_{10}$, C 63.80, H 6.39, N 2.40; found C 63.52, H 6.32, N 2.37; ^{1}H-NMR ($CDCl_3$) δ in ppm, 2.3 (s, 6H, dimethylamino at C-3'). The hydrolysis of 13-methylaklavin in 0.1 N HCl for 30 minutes at 85°C gave 13-methylaklavinone and L-rhodosamine.

The reduction of the carbonyl group of L-cinerulose of 13-methylaclacinomycin A with sodium borohydride gave 13-methylaclacinomycin M and 13-methylaclacinomycin N, containing L-amicetose or L-rhodinose respectively. To a solution of 2.9 g of 13-methylaclacinomycin A in 150 ml of toluene was added a solution of 63 mg of sodium borohydride in 8 ml of ethanol with stirring. The mixture was allowed to react with stirring for 2 minutes at room temperature and thereafter was shaken with 600 ml of acetate buffer (0.2 M, pH 4.5) - chloroform (1: 1). The chloroform layer was washed with water, dried over anhydrous sodium sulfate, evaporated under reduced pressure and precipitated with *n*-hexane to give 2.8 g of yellow powder. This powder contained 13-methylaclacinomycin M (67%) and N(26%), which were separated by HPLC (silica gel) with chloroform - methanol (30: 1). The properties of 13-methylaclacinomycin M are as follows: mp 165°C; $[\alpha]_D^{20}$ 53° (*c* 1.01, $CHCl_3$); elemental *anal.* calcd. for $C_{43}H_{57}NO_{15}$, C 62.38, H 6.94, N 1.69, found C 62.20, H 6.76, N 1.84. 13-Methylaclacinomycin N as follows: mp 164.5°C; $[\alpha]_D^{20}$ 45.7° (*c* 1.00, $CHCl_3$); elemental *anal.* calcd. for $C_{43}H_{57}NO_{15}$, C 62.38, H 6.94, N 1.69; found C 62.45, H 6.86, N 1.91.

Table 2. 50% Inhibitory concentration (IC_{50}) of 13-methylaclacinomycin A and its derivatives on DNA and RNA synthesis and growth of cultured L-1210 cells.

Compound	IC_{50} [mcg/ml]		
	Growth (on day 2)	DNA synthesis	RNA synthesis
13-Methylaclacinomycin A	0.015	0.46	0.04
〃 M	0.012	0.41	0.036
〃 N	0.016	0.68	0.04
〃 S	0.014	0.31	0.052
13-Methylaklavin	0.16	1.15	0.29

13-Methylaclacinomycin M(or N) was hydrolyzed in 0.1 N HCl for 70 minutes at room temperature to give 13-methylaclacinomycin S and L-amicetose (or L-rhodinose). The properties of 13-methylaclacinomycin S are as follows: mp 165.5°C; $[\alpha]_D^{20}$ 90° (*c* 1.01, $CHCl_3$); elemental *anal.* calcd. for $C_{37}H_{47}NO_{13}$, C 62.26, H 6.64, N 1.96; found C 62.43, H 6.59, N 2.12.

13-Methylaclacinomycin S was hydrolyzed in 0.1 N HCl for 30 minutes at 85°C to give 13-methylaklavinone, 2-deoxy-L-fucose and L-rhodosamine.

The processes for the determination of the compounds described above are summarized in Table 1.

13-Methylaclacinomycin A and its chemically derived analogues strongly inhibited DNA and RNA synthesis and growth of cultured L-1210 cells as summarized in Table 2. 13-Methylaclacinomycin A was similar to aclacinomycin A in activity against leukemia L-1210 in CDF_1 mice. When 5 mg/kg/day was injected intraperitoneally once daily for 10 days, the increase of life span (ILS %) was 75. The acute toxicity of 13-methylaclacinomycin A was lower than that of aclacinomycin A.

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