NEW ANTHRACYCLINE ANTIBIOTICS OBTAINED BY MICROBIAL GLYCOSIDATION OF β-ISORHODOMYCINONE AND α_2-RHODOMYCINONE

Sir:

In an attempt to obtain new anthracycline antibiotics, we have studied the production of hybrid anthracyclines by the biosynthetic glycosidation of natural or semisynthetic anthracyclinones using antibiotic-negative mutants of aclacinomycin-producing *Streptomyces galilaeus* MA144-M1[1] and baumycin-producing *Streptomyces coeruleorubidus* ME130-A4[2]. Thus far we have been successful in preparing 12 hybrid anthracyclines by this bioconversion technique: 2-hydroxyaclacinomycin A[3], CG5～CG9 compounds[4], 4-methoxyaclacinomycin A[4], trisarubicinol[5] and 1-hydroxydaunorubicinol[6].

In this paper we describe the production and preliminary characterization of two additional anthracycline antibiotics, CG10 (**I**) and CG11 (**II**), by the microbial glycosidation of β-isorhodomycinone and α_2-rhodomycinone using the aclacinomycin-negative mutant strain KE303 of *S. galilaeus* MA144-M1[7]. Compounds **I** and **II** were identified as 7-*O*-(cinerulosyl-2-deoxyfucosylrhodosaminyl)-β-isorhodomycinone and 10-*O*-(cinerulosyl-2-deoxyfucosylrhodosaminyl)-α_2-rhodomycinone, respectively. They exhibited a potent growth inhibition against cultured leukemia L1210 cells.

The aglycones β-isorhodomycinone and α_2-rhodomycinone used as the glycosidation substrates were obtained by acid hydrolysis of the anthracycline mixture extracted from the cultured broth of strain IU-17 which was derived from the roseorubicin-producing *Streptomyces roseoviolaceus* A529[8].

S. galilaeus mutant strain KE303 was inoculated into a 500-ml Erlenmeyer flask containing 100 ml of seed medium (0.3% yeast extract and 1% soluble starch, pH 7.2) and cultured at 28°C for 2 days on a rotary shaker (210 rpm). The seed culture (1 ml) thus prepared was added to a 500-ml Erlenmeyer flask containing 50 ml of the fermentation medium which consisted of 1.5% soluble starch, 1% glucose, 2% soybean meal, 0.1% K_2HPO_4, 0.1% $MgSO_4 \cdot 7H_2O$, 0.3% NaCl, 0.0007% $CuSO_4 \cdot 5H_2O$, 0.0001% $FeSO_4 \cdot 7H_2O$, 0.0008% $MnCl_2 \cdot 4H_2O$ and 0.0002% $ZnSO_4 \cdot 7H_2O$, pH 7.4. Fermentation was carried out at 28°C on a rotary shaker (210 rpm). After 24 hours, 0.5 ml of a methanolic solution of substrate aglycone (2 mg/ml) was added to a flask and the cultivation was continued for another 40 hours to effect the glycosidation. In this way, 600 mg of each aglycone was glycosidated with a total of 30 liters of strain KE303 culture.

The procedure for isolation and purification of the glycosidation products from the conversion broth was same in both cases. The mycelia from all 30 liters of conversion broth was collected by centrifugation. The glycosidation products were extracted from this mycelia with 6 liters of acetone. The acetone extract was concentrated to one-fourth volume by evaporation and extracted twice with 2 liters of chloroform. After evaporation of the chloroform layer, the pigmented residue was dissolved in about 80 ml of methanol and the insoluble materials were removed by centrifugation. The supernatant fluid was placed on a Sephadex LH-20 column (ϕ 4×40 cm) which was eluted with methanol. The fractions containing the first pigment to elute were pooled and evaporated to dryness. The resulting residue was then chromatographed on preparative silica gel plates (E. Merck, 60 PF_{254}) using $CHCl_3$ - MeOH (20: 1) as solvent. A main pigment band corresponding to **I** or **II** was scraped off and extracted with $CHCl_3$ - MeOH (10: 1). The extract was evaporated to dryness and the residue was dissolved in 30 ml of 0.1 M acetate buffer (pH 3.2). This solution was washed with 15 ml of toluene, neutralized with sodium bicarbonate and extracted with $CHCl_3$. The chloroform layer was dried over Na_2SO_4 and evaporated to a small volume. An excess of *n*-hexane was added to it to precipitate **I** or **II**. Thus, pure powders **I** and **II** were obtained in yield of 54 mg and 48 mg, respectively. Physico-chemical properties of **I** and **II** are as follows:

I: Red powder; mp 147～151°C; IR (KBr) cm^{-1} 3400, 2920, 1720, 1660, 1590, 1400, 1370, 1300, 1240; $\lambda_{max}^{90\%\ MeOH}$ nm ($E_{1cm}^{1\%}$) 235 (308), 255 sh (216), 285 sh (113), 495 (92), 525 (101), 548 (63); *Anal* Calcd for $C_{40}H_{51}NO_{16}$ (MW 801.24): C 59.92, H 6.36, N 1.74; found: C 59.52, H 6.13, N 1.65.

II: Orange powder; mp 138～145°C; IR (KBr) cm^{-1} 3400, 2930, 1730, 1600, 1460, 1400,

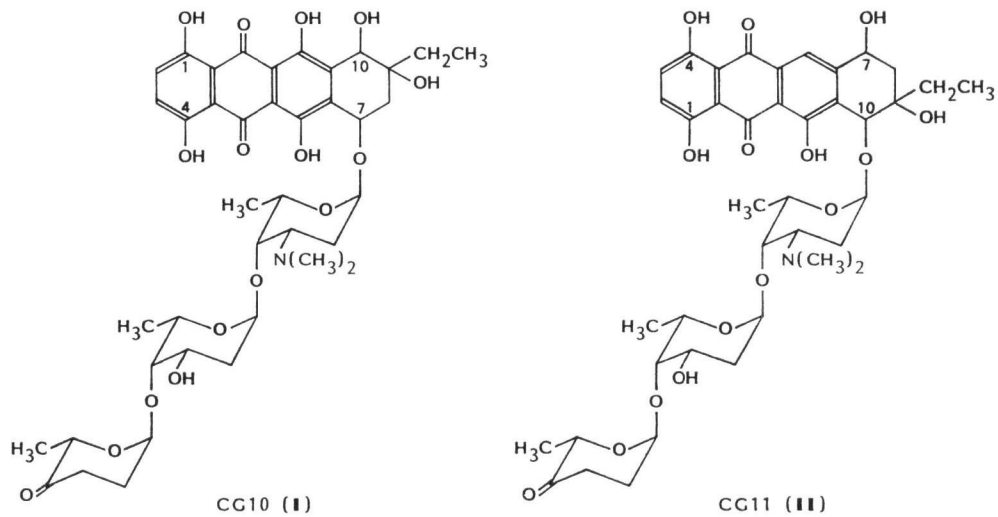
Table 1. Chemical shift-assignments of ^1H NMR spectra of **I** and **II**.

Assignment	**I** (δ) ppm	**II** (δ) ppm	Aclacinomycin A (δ) ppm*	Remarks
1-H	—	—	7.79 (dd)	Aglycone moiety
2-H	>7.27 (s)	>7.27 (s)	7.69	
3-H			7.22	
6-H	—	8.05 (s)	—	
7-H	5.17 (b)	5.05 (b)	5.28 (b)	
8-CH_2	2.4 (m)	2.4 (m)	2.4 (m)	
10-H	4.90 (m)	5.00 (bs)	4.90 (bs)	
13-CH_2	1.7 (m)	1.7 (m)	1.7 (m)	
14-CH_3	1.11 (t)	1.14 (t)	1.09 (t)	
16-OCH_3	—	—	3.70 (s)	
1′-H	5.50 (b)	5.38 (b)	5.25 (b)	Rhodosamine moiety
2′-H	1.85 (m)	1.85 (m)	1.9 (m)	
3′-H	2.4 (m)	2.4 (m)	2.4 (m)	
3′-$N(CH_3)_2$	2.15 (s)	2.20 (s)	2.17 (s)	
4′-H	3.75 (bs)	3.70 (bs)	3.78 (bs)	
5′-H	4.55 (q)	4.55 (q)	4.56 (q)	
6′-CH_3	1.16 (d)	1.16 (d)	1.16 (d)	
1″-H	5.03 (b)	4.90 (b)	5.03 (b)	2-Deoxyfucose moiety
2″-CH_2	1.90 (m)	1.92 (m)	1.90 (m)	
3″-H	4.1 (m)	4.02 (m)	4.1 (m)	
4″-H	3.65 (bs)	3.62 (bs)	3.7 (bs)	
5″-H	4.02 (q)	4.02 (q)	4.02 (q)	
6″-H	1.25 (d)	1.28 (d)	1.29 (d)	
1‴-H	5.08 (t)	5.03 (t)	5.07 (t)	Cinerulose moiety
2‴-CH_2	2.0 (m)	2.0 (m)	2.0 (m)	
3‴-CH_2	2.4 (m)	2.4 (m)	2.4 (m)	
5‴-H	4.45 (q)	4.45 (q)	4.50 (q)	
6‴-H	1.35 (d)	1.35 (d)	1.33 (d)	

* Data cited from ref 10.

Spectra were measured in $CDCl_3$ using TMS as the internal reference.

Fig. 1. Structure CG10 (**I**) and CG11 (**II**).



1280; $\lambda_{max}^{90\%\ MeOH}$ nm ($E_{1cm}^{1\%}$) 239 (495), 256 (265), 290 (106), 492 (132), 512 (102), 526 (84); *Anal* calcd for $C_{40}H_{51}NO_{15}$ (MW 785.25): C 61.46, H 6.49, N 1.78; found: C 61.30, H 6.41, N 1.83.

Acid hydrolysis (0.1 N HCl, 85°C, 30 minutes) followed by silica gel TLC analysis[9,10] revealed that the sugar moieties of **I** and **II** consisted of rhodosamine, 2-deoxyfucose and cinerulose as in aclacinomycin A. Also, their aglycones were found to be identical to those which were added as glycosidation substrates (β-isorhodomycinone for **I** and α_2-rhodomycinone for **II**).

^{1}H NMR spectra of **I** and **II** (Table 1) supported their identification as cinerulosyl-2-deoxyfucosylrhodosaminyl-β-isorhodomycinone and -α_2-rhodomycinone, respectively, by direct comparison with that of aclacinomycin A. The sequence of the sugar moiety was determined to be same as that of aclacinomycin A by partial methanolysis in 0.01 N methanolic hydrogen chloride - acetone mixture according to the procedure previously described[9].

The site for the trisaccharide linkage on the aglycone is probably the hydroxyl group at C-7 for **I** and C-10 for **II** since in the ^{13}C NMR spectra the signals assigned to C-7 of **I** and C-10 of **II** shift down to δ 70.4 and 70.2, respectively, as compared with that (δ 62.5) to C-7 of β-isorhodomycinone and that (δ 66.0) to C-10 of α_2-rhodomycinone. Their sites were also supported by comparison on ^{1}H NMR spectra of the aglycones obtained hydrolytically from acetylated compounds (**I** and **II**) with acetylated aglycones (β-isorhodomycinone and α_2-rhodomycinone).

The results showed that CG10 (**I**) and CG11 (**II**) were 7-*O*-(cinerulosyl-2-deoxyfucosyl-rhodosaminyl)-β-isorhodomycinone and 10-*O*-(cinerulosyl-2-deoxyfucosylrhodosaminyl)-α_2-rhodomycinone, respectively, which have chemical structure as shown in Fig. 1.

The bioactivities of **I** and **II** were examined by their effects on cultured leukemia L1210 cells. The concentration required to inhibit growth, and the syntheses of DNA and RNA by 50% (IC_{50}) were 0.003, 0.62, and 0.056 μg/ml, respectively, for **I** and 0.89, 1.70 and 0.85 μg/ml, respectively, for **II**. **I** was more active against L1210 leukemia cells than adriamycin, daunomycin and aclacinomycin A (IC_{50}: 0.02, 0.02 and 0.03 μg/ml, respectively), while **II** was a weakly active compound. **I** inhibited RNA synthesis more strongly than it did DNA synthesis while **II** inhibited both syntheses to a similar extent.

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