NOTES

TRISARUBICINOL, NEW ANTITUMOR ANTHRACYCLINE ANTIBIOTIC

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As previously reported[1~4], an aclacinomycin-negative mutant strain KE303, which was isolated from *Streptomyces galilaeus* MA144-M1, is capable of producing new potent antitumor anthracycline antibiotics by the microbial conversion of biologically inactive anthracyclinones. In this communication, we describe the microbial glycosidation of carminomycinone and 13-dihydrocarminomycinone using strain KE303, and the characterization of 13-dihydrocarminomycinone trisaccharide, trisarubicinol (Fig. 1).

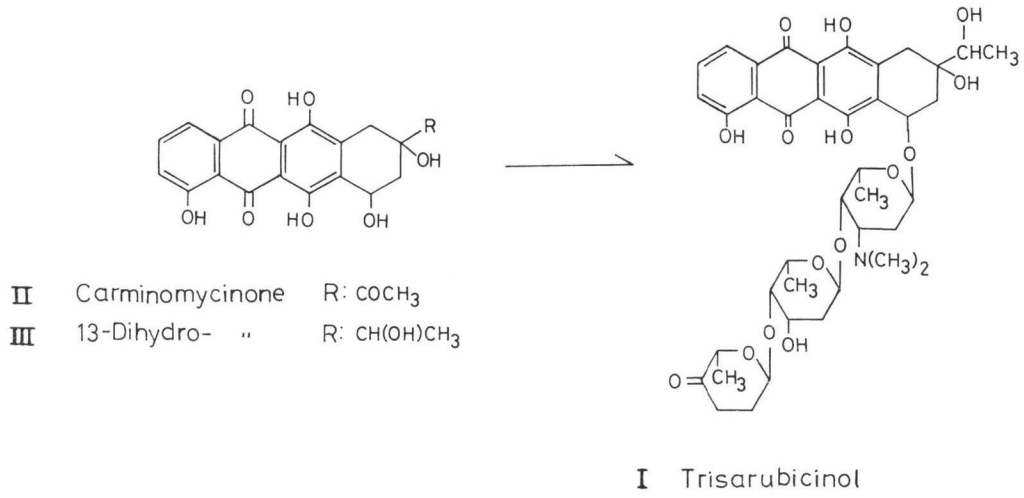
The strain KE303 was cultured in 500-ml flasks containing 50 ml medium of the following composition: Soluble starch 1.5 %, glucose 1 %, soy bean meal (Ajinomoto Co.) 2 %, yeast extract 0.2 %, NaCl 0.3 %, K_2HPO_4 0.1 %, $MgSO_4 \cdot 7H_2O$ 0.1 %, $CuSO_4 \cdot 5H_2O$ 0.0007 %, $FeSO_4 \cdot 7H_2O$ 0.0001 %, $MnCl_2 \cdot 4H_2O$ 0.0008 %, $ZnSO_4 \cdot 7H_2O$ 0.0002 %, pH 7.4. The cultivation was carried out for 17 hours at 28°C on a rotary shaker, and then 0.5 ml of methanol solution of carminomycinone (1 mg/ml) was added to each flask at the concentration of 10 μg/ml, and the cultivation was further continued for 24 hours to complete the glycosidation. The pigments were extracted from the cultured broth (5 ml) with a solvent mixture of chloroform - methanol (3: 2, v/v, 5 ml), and the chloroform layer was concentrated to dryness, dissolved in 0.2 ml of chloroform, spotted 20 μl onto silica gel thin-layer (F_{254} plate, E. Merck Co.) and developed with chloroform - methanol - aqueous ammonia (100: 10: 0.3, v/v/v) mixture. After drying, the spots corresponding to trisarubicinol (**I**) (Rf=0.79), residual carminomycinone (**II**) (Rf=0.67) and its reduced form, 13-dihydrocarminomycinone (**III**) (Rf=0.43) were determined by a Shimadzu chromatoscanner model CS-910. The conversion rate of **II** to **I** was about 30 %.

Fifty liters of the cultured broth were centrifuged to harvest the mycelium, and the pigments were extracted from the mycelium with 8 liters of acetone, concentrated to one-third volume, and re-extracted with 3 liters of chloroform. After concentration to dryness, the crude pigmented residue was dissolved in 100 ml of chloroform - methanol (1: 2, v/v), subjected to Sephadex LH-20 column, and eluted with the same solvent mixture. The initial red eluate was concentrated, dissolved in a small amount of chloroform, and chromatographed on preparative thin-layer (Kieselgel $60PF_{254}$, E. Merck Co.) using a chloroform - methanol (20: 1, v/v) mixture. The major band corresponding to **I** showing Rf at 0.2 was scraped off, and extracted with 200 ml of chloroform - methanol - aqueous ammonia (100: 15: 0.2, v/v/v) mixture. The extract was concentrated to dryness, dissolved in 0.1 M acetate buffer (pH 3.5) and washed with toluene. The aqueous layer was neutralized the pH to 7.0 with sodium bicarbonate and extracted with chloroform. The chloroform extract was dried over sodium sulfate and concentrated to 2.5 ml under reduced pressure. To the concentrate excess *n*-hexane was added to form a dark red precipitate, and 42 mg of pure trisarubicinol were obtained by filtration and drying of the precipitate *in vacuo*.

Physicochemical properties of **I** are as follows: m.p. 149~152°C; IR (KBr) cm^{-1}: 1720, 1600, 1290, 1005; $\lambda_{max}^{90\%MeOH}$ nm ($E_{1cm}^{1\%}$): 234(419), 254 (342), 292 (95), 465 (125), 493 (162), 514 (20), 526 (117), 575 (15); $\lambda_{max}^{0.1NNaOH\text{-}90\%MeOH}$ nm ($E_{1cm}^{1\%}$): 241 (482), 286 (92), 560 (181), 596 (154); $[\alpha]_D^{23}$ + 1.84° (*c* 0.8, $CHCl_3$); *Anal.* Calcd. for $C_{40}H_{51}NO_{15}$ (m.w. 785.84); C 61.14, H 6.54, N 1.78, O 30.54; Found: C 60.62, H 6.47, N 1.93%.

The PMR spectrum of **I** exhibited four signals in the anomeric region at δ 5.03, 5.07, 5.24 and 5.52 which were assigned to the C-1'', C-1''', C-7 and C-1' protons, respectively. This indicated that **I** had trisaccharide moiety. The signal at δ 2.39 showing the acetyl protons at C-9 of car-

Fig. 1. Microbial glycosidation of carminomycinone and 13-dihydrocarminomycinone by a mutant strain KE303 of *Streptomyces galilaeus* MA144-M1.



minomycinone, which was used as the substrate, was missing in the PMR spectrum of **I**.

The structure of aglycone moiety obtained by acid hydrolysis of **I** in 0.1 N HCl at 85°C for 30 minutes was identified as 13-dihydrocarminomycinone in direct comparison with the melting point, Rf value, IR and mass spectra of the authentic sample[5]. On the other hand, sugar moieties obtained from the above hydrolysate were identified to be composed of rhodosamine, 2-deoxyfucose and cinerulose A by silica gel TLC as detected in the acid hydrolysate of aclacinomycin A[6]. The sequence of sugar moiety was examined to be cinerulosyl-2-deoxyfucosyl-rhodosaminide by partial methanolysis in 0.01 N methanolic hydrogen chloride-acetone mixture at room temperature for 45 minutes, as previously described. The possible site for glycosidic linkage of 13-dihydrocarminomycinone should be the hydroxyl group at C-7 position. In the CMR spectrum of **I**, the signal at C-7 (δ 70.0) indicated that the glycosidic moiety linked to the C-7 position of 13-dihydrocarminomycinone.

The antibiotic **I** showed a marked antitumor activity against murine leukemia L1210 in CDF_1 mice. When 7.5 and 5.0 mg/kg/day of **I** was administered intraperitoneally once daily for 10 days, the increase of life span was 143 and 114 %, respectively. The concentrations required to inhibit synthesis by 50 % (IC_{50}) of DNA and RNA indicated that trisarubicinol was a more potent inhibitor of RNA synthesis than was carminomycin I, as shown in Table 1. The IC_{50} value of **I** for RNA synthesis was one-sixth that of DNA synthesis, while the IC_{50} of carminomycin I for RNA and DNA syntheses was about equal.

Table 1. Inhibition of the cell growth and macromolecular synthesis of cultured L1210 leukemia cells by trisarubicinol and carminomycin I.

Anthracycline	IC_{50}(μM)			Ratio
	Growth	DNA synthesis	RNA synthesis	$\frac{IC_{50}\ DNA}{IC_{50}\ RNA}$
Trisarubicinol	0.013	0.48	0.08	6.0
Carminomycin I	0.020	0.39	0.56	0.7

IC_{50} values were estimated by Probit analysis. Cytotoxicity was determined on the day 2 culture.

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