BAUMYCIN ANALOGS ISOLATED FROM *ACTINOMADURA* SP.

Sir:

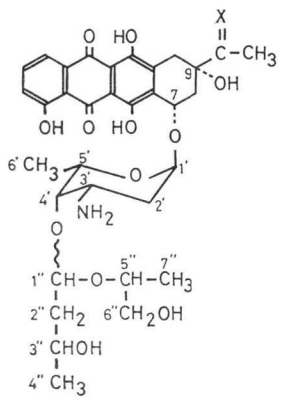
In continuing screening for antitumor anthracycline antibiotics, we have recently isolated *Actinomadura* sp. D326 from a soil sample collected in Japan (Atsugi city, Kanagawa). This isolate produced mainly four active components related to the baumycins[1,2]. These components were determined to be 4-hydroxybaumycinol A2 (**I**), 4-hydroxybaumycinol A1 (**II**), 4-hydroxybaumycin A2 (**III**) and 4-hydroxybaumycin A1 (**IV**).

The strain D326 was cultivated at 28°C for 6 days on a rotary shaker in 500-ml Erlenmeyer flasks containing 50 ml of the medium as previously described[3]. After centrifuging the cultured broth (70 liters), red pigments in the mycelial residue were extracted with 5 liters of acetone, followed by concentration *in vacuo* to a quarter volume and re-extraction twice with 1.5 liters of chloroform. The chloroform phase was concentrated yielding a red oily material, and the residual pigments were subjected to Sephadex LH-20 ($\phi 4 \times 40$ cm) column chromatography and eluted with a chloroform - methanol mixture (1 : 2, v/v). The first pigment band eluted containing glycosidic compounds and the second band containing non-glycosidic compounds were combined separately each and concentrated to dryness. The former (glycosides) was separated into three components by preparative chromatography on silica gel plate (60 PF_{254}, E. Merck) using a chloroform - methanol - aqueous ammonia (100: 15: 0.2, v/v/v) system. Each component was extracted from the scraped off silica gel with the same solvent mixture and was further purified by rechromatography on silica gel plates using a chloroform - methanol - acetic acid (100: 15: 0.5, v/v/v) system. The second glycosidic component was further separated into two subcomponents. The four glycosidic compounds **I**, **II**, **III** and **IV** thus obtained were separately dissolved in 30 ml of 0.1 M acetate buffer (pH 3.0), washed with toluene and extracted with chloroform after adjusting the pH to 7.0 with $NaHCO_3$. The chloroform layers were dried over anhydrous Na_2SO_4 and concentrated to a small volume and the pure pigments were obtained as dark red powders by precipitating with 8 volumes of *n*-hexane. These yielded as follows: **I**, 50 mg; **II**, 40 mg; **III**, 211 mg and **IV**, 60 mg.

From the second band, a non-glycosidic pigment was obtained by column chromatography on silica gel (Wakogel C-200, $\phi 2 \times 8.5$ cm) using chloroform and recrystallized from benzene to yield red needle crystals (307 mg; m.p. 207°C; *m/z* 428 (M^+); ν_{max}^{KBr} cm^{-1}: 1730, 1600), which was identified as ε-rhodomycinone[4].

For the structural elucidation of the glycosidic pigments, acid hydrolysis with 0.1 N HCl at 85°C for 30 minutes was performed. The aglycones which precipitated were recovered by filtration, recrystallized from acetone and identified by their IR, UV, mass and PMR spectra. The aqueous layer was used to define sugars by thin-layer chromatography according to the method described previously[3]. The results showed that compounds **I** and **II** contained 13-dihydrocarminomycinone and daunosamine, and **III** and **IV** had carminomycinone and daunosamine. Mild hydrolysis with 1% H_2SO_4 at 30°C for 30 minutes converted **I** and **II** to 13-dihydrocarminomycin[5], and **III** and **IV** to carminomycin[6], suggesting that these compounds have an additional moiety linked to daunosamine. Therefore, detailed analysis of their sugar moieties were achieved by hydrogenolysis with $Pd/BaSO_4$ according to the method employed for the baumycins[2]. Hydrogenolysis of **I** and **II** gave 7-deoxy-13-dihydrocarminomycinone, and 7-deoxycarminomycinone was obtained from **III** and **IV**. The sugar moiety obtained from **I**, **II**, **III** and **IV** showed the same Rf value as those from baumycin A1 and A2 on silica gel TLC, using the solvent system of chloroform - methanol (1: 2, v/v) and *n*-butanol - acetic acid - water (4: 1: 1, v/v/v). The sugar moiety obtained by hydrogenolysis was further acetylated with acetic anhydride and pyridine and compared with those from baumycins A1 and A2 which are stereoisomers of 4′-substituted daunomycin[2]. The spectral data indicated that the sugar moiety from **I** and **III** [PMR in $CDCl_3$, δ in ppm: 1.2 ~ (9H, $CH_3 \times 3$), 2.0 ~ (12H, Ac × 4), 1.7 ~ (4H, CH_2-2, -2′), 3.4 ~ (1H, CH-4), 3.4 ~ 4.1 (4H, CH-3, 5, 5′, 6), 4.5 ~ (1H, CH-1′), 5.3 ~ (1H, CH-3′), 5.6 ~ (1/2H, CH-1-β), 6.1 ~ (1/2H, CH-1-α), 6.5 (1H, NH)] was the same as that from baumycin A2 (4′-epimer A), and the sugar from **II** and **IV** [PMR in $CDCl_3$, δ in ppm: 1.2 ~ (9H, $CH_3 \times 3$), 2.0 ~ (12H, Ac × 4), 1.7 ~ (4H, CH_2-2, 2′), 3.3 ~ (1H, CH-4), 3.6 ~ 4.2 (4H, CH-3,5,5′,6), 4.6 (1H, CH-1′), 5.3 ~ (1H, CH-3′), 5.7 ~ (1/2H, CH-1-β), 6.2 (1/2H, CH-1-α), 6.7 ~

Fig. 1. Structure of D326 complex.



	Aglycone	Sugar
D326 **I**	X=OH, H	4'-epimer A
D326 **II**	X=OH, H	4'-epimer B
D326 **III**	X=O	4'-epimer A
D326 **IV**	X=O	4'-epimer B

(1H, NH)] was identical to that from baumycin A1 (4'-epimer B).

From the facts mentioned above, the structures of **I**, **II**, **III** and **IV** were proposed as shown in Fig. 1. The physicochemical properties of these compounds are as follows:

I: m.p. 149~153°C, $[\alpha]_D^{23}$ +72° (*c* 0.02, $CHCl_3$ - MeOH (1: 1)), ν_{max}^{KBr} cm^{-1}: 1600, 1010, $\lambda_{max}^{90\%\ MeOH}$ nm ($E_{1cm}^{1\%}$): 235 (482), 254 (384), 294 (111), 492 (189), 526 (138), 575 (21).

II: m.p. 148~152°C, $[\alpha]_D^{23}$ −34° (*c* 0.02, $CHCl_3$ - MeOH (1: 1)), ν_{max}^{KBr} cm^{-1}: 1595, 1005, $\lambda_{max}^{90\%\ MeOH}$ nm ($E_{1cm}^{1\%}$): 235 (551), 255 (431), 295 (120), 492 (223), 527 (159), 575 (16).

III: m.p. 167~171°C, $[\alpha]_D^{23}$ −1° (*c* 0.02, $CHCl_3$ - MeOH (1: 1)), ν_{max}^{KBr} cm^{-1}: 1710, 1600, 1010, $\lambda_{max}^{90\%\ MeOH}$ nm ($E_{1cm}^{1\%}$): 235 (581), 255 (430), 294 (125), 492 (221), 527 (155), 575 (15).

IV: m.p. 165~169°C, $[\alpha]_D^{23}$ +48° (*c* 0.02, $CHCl_3$ - MeOH (1: 1)), ν_{max}^{KBr} cm^{-1}: 1710, 1595, 1005, $\lambda_{max}^{90\%\ MeOH}$ nm ($E_{1cm}^{1\%}$): 235 (574), 255 (423), 292 (128), 492 (223), 526 (156), 575 (18).

The Rf values of these compounds are also shown in Table 1.

Compounds **III** and **IV** are identical to carminomycins II and III, which were recently isolated from *Actinomadura carminata*[7], as shown by the good agreement in their NMR spectra and other analytical data. Compounds **I** and **II** are 4-hydroxybaumycinols A2 and A1, respectively, which are produced by reduction of **III** and **IV** at the C-13 position.

Table 1. Rf Values of D326 complex.

Solvent systems	1	2	3
Compound **I**	0.23	0.03	0.07
II	0.48	0.07	0.14
III	0.44	0.10	0.16
IV	0.72	0.17	0.26
ε-Rhodomycinone	0.80	0.76	0.80

Solvent systems:
1; chloroform - methanol - aqueous ammonia (100 : 15 : 0.2, v/v/v).
2; chloroform - methanol - acetic acid (100 : 15 : 0.5, v/v/v).
3; benzene - chloroform - methanol (3 : 7 : 3, v/v/v).

Table 2. Antitumor activity of D326 complex against cultured L1210 leukemia cells.

Compounds	IC_{50} (μg/ml)		
	Growth	Biosynthesis on	
		DNA	RNA
I	0.006	0.90	0.73
II	0.025	1.30	0.97
III	0.015	0.92	0.41
IV	0.005	0.19	0.09
Carminomycin I	0.005	0.20	0.29

For the determination of cytotoxicity, the log. of the cell survivors on day 2 was plotted *versus* drug concentration and IC_{50} (50% inhibition concentration) values were estimated.

The effects of the D326 complex on the growth and biosynthesis of DNA and RNA of cultured L1210 leukemia cells were examined as summarized in Table 2. **I** and **IV** inhibited the growth of L1210 cells as strongly as carminomycin I, which exhibits stronger cytotoxicity than adriamycin or daunomycin (IC_{50}: approximately 0.01 μg/ml). **II** and **III** exhibited less cytotoxicity than **I** and **IV**. These baumycin analogs showed an equal inhibition of DNA and RNA synthesis. Preliminary *in vivo* experiments indicated that the antitumor activity of the D326 complex did not exceed that of carminomycin I in the i.p.-i.p. system against L1210 leukemia in mice.

Acknowledgements

The authors wish to thank Prof. H. UMEZAWA, Dr. T. TAKEUCHI and Dr. H. NAGANAWA, Institute of Microbial Chemistry, Tokyo, for their valuable discussions and for NMR and mass spectral analyses.

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(Received March 13, 1981)

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