Communications to the Editor

NEW POTENT ANTHRACYCLINES, BARMINOMYCINS I AND II

Sir:

We have previously reported on baumycins A1, A2, B1 and B2[1], which are daunorubicin analogues having unique acetal moieties at 4′ position. In a continuing search for new antitumor antibiotics, extremely potent anthracyclines, which attach a new type of 4′-*O*-substituent, were isolated from a carminomycin-producing strain designated MG463-yF4, and named barminomycins I (**1**)[2] and II (**2**). The culture broth of this strain exhibited a significant activity against L1210 leukemia and B16 melanoma cells in mice. In this broth five components of anthracycline-glycosides were detected, but three of them, carminomycins I, II and III[3], were less potent than a mixture of the anthracyclines in the broth. Accordingly we attempted to isolate two others. In this communication, the production, isolation and characterization of anthracyclines **1** and **2** are reported.

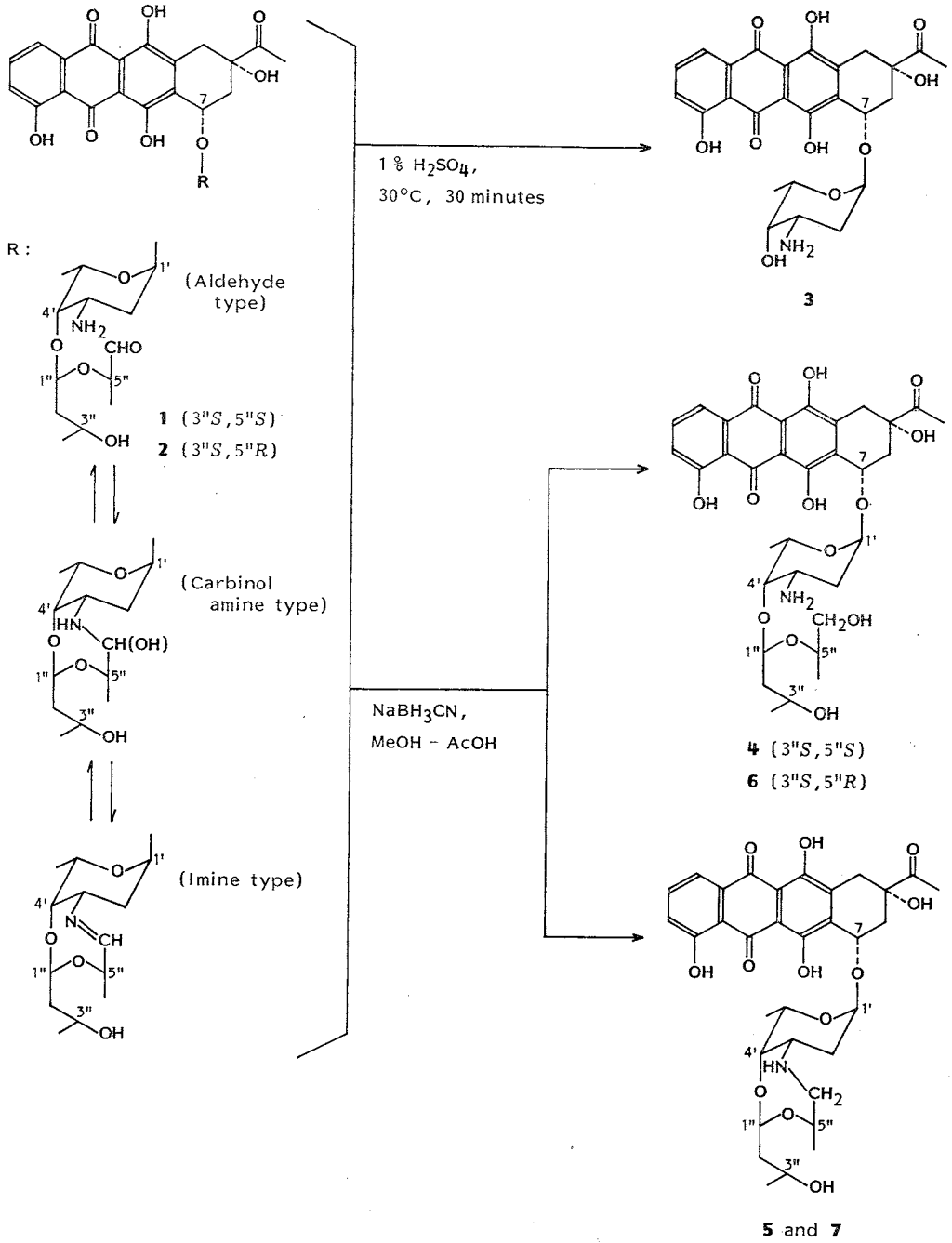
The strain MG463-yF4 was isolated from a soil sample collected in Nangoku-shi, Kochi, Japan, and the taxonomic studies showed that the strain belongs to *Actinomadura roseoviolacea* or *Actinomadura carminata*. It was deposited with the Fermentation Research Institute, Agency of Industrial Science and Technology and was given the accession number, FERM-P7352[4].

The strain was cultured at 27°C for 48 hours in a 30-liter jar fermentor under agitation at 150 rpm and aeration at a rate of 15 liters/minute in a medium consisting of glycerol 3%, fish meal 2%, and $CaCO_3$ 0.2%, pH 7.4 before sterilization. After filtration of the cultured broth (15 liters), red pigments in the filtrate were adsorbed on a column of Diaion HP-20. The column was washed successively with water and 50% MeOH, and the active material was eluted with 100% MeOH. The eluates were concd to dryness *in vacuo*. The dried residue was dissolved in a small amount of $CHCl_3$ and the solution was subjected to a column packed with 50 g of silicic acid. After washing with a mixture of $CHCl_3$ - MeOH (100:1), the active fraction was eluted with $CHCl_3$ - MeOH (10:1), concd to a small volume, and spotted on silicic acid thin-layer plates, which were developed with $CHCl_3$ - MeOH - AcOH - H_2O (70:10:1:1). The bands corresponding to **1** (Rf 0.39) and **2** (Rf 0.33) were scraped off, and the antibiotics were extracted with $CHCl_3$ - MeOH (2:1). After drying, they were further purified by HPLC on Nucleosil $5C_{18}$ using a mixture of CH_3CN - 0.2% phosphoric acid (40:60) as an eluant. Active fractions thus obtained were extracted with $CHCl_3$ and concd *in vacuo*. Thus, 3.1 mg of barminomycin I (**1**) and 3.1 mg of barminomycin II (**2**) were obtained.

Barminomycins I (**1**) and II (**2**) gave the same molecular ion peaks at *m/z* 639 $(M)^+$ (imine type) in field desorption mass spectra (FD-MS), and at *m/z* 658 $(M+H)^+$ (aldehyde type and/or carbinol amine type) in secondary ion mass spectra (SI-MS). Mild acid hydrolysis with 1% sulfic acid at 30°C for 30 minutes converted both **1** and **2** to carminomycin I (**3**)[3], which were identified by direct comparison of their Rf values on silica gel TLC, specific rotations, mass spectra and ^1H NMR spectra. These results suggested **1** and **2** have the same partial structure, 7-*O*-daunosaminyl-carminomycin. Since **1** and **2** were labile in the various solvents used for NMR and gave complicated ^1H NMR spectra, it was difficult to define their structures by their NMR spectra. Therefore reduction with $NaBH_3CN$ in a mixture of MeOH and 1 N AcOH (2:1) was performed. Reduction of **1** produced red pigments **4** and **5**, These were extracted with $CHCl_3$, and purified by silica gel TLC with $CHCl_3$ - MeOH (10:1). Compound **4** was confirmed to be carminomycin III[5] (identical with 4-hydroxybaumycin A1†,[6] or rubeomycin A_1[7]) by the ^1H NMR spectrum, FD-MS (*m/z* 660 $(M+H)^+$; molecular formula: $C_{33}H_{41}NO_{13}$), and specific rotation ($[\alpha]_D^{27}$ +178° (*c* 0.02, $CHCl_3$); documented as +170.4° (*c* 0.053, $CHCl_3$)[7]). The ^{13}C NMR spectrum of compound **5** showed 33 carbon signals, which was fairly close to that of rubeomycin A_1[7] except for the signals corresponding to C-3′ (δ 51.6)

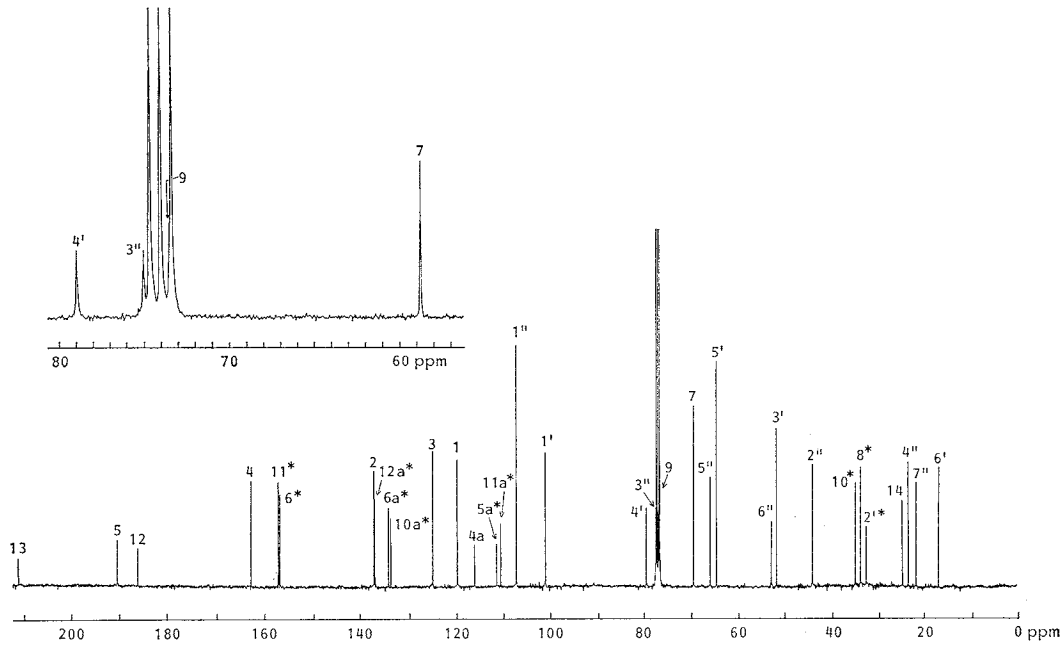
† The name "4-demethylbaumycins" is recommended for these compounds.

Fig. 1. Structures of anthracyclines **1**, **2**, **3**, **4**, **5**, **6** and **7**.



and C-6″ (δ 52.8) and C-7″ (δ 21.9) (Fig. 2). As shown in Table 1, the ^{1}H NMR spectrum of **5** was also similar to that of **4** but for higher field shifts of 3′-H (δ 2.89) and 6″-H (δ 2.69 and δ 2.80). The molecular formula of **5** was determined to be $C_{33}H_{39}NO_{12}$ on the basis of FD-MS (*m/z* 642 $(M+H)^+$). From these results, the structure of **5** was determined as shown in Fig. 1. Likewise, compounds **6** and **7** were obtained by the reduction of **2** with $NaBH_3CN$, and **6** was shown to be identical with carminomycin II[5] (identical with 4-hydroxybaumycin

Fig. 2. ^{13}C NMR spectrum of compound **5** (100 MHz, in $CDCl_3$).



* Similar values may be interchanged.

Table 1. ^1H NMR spectral data (ppm)[a] of **4**, **5**, **6** and **7**.

Proton	4	5	6	7
1-H	7.87 dd	7.88 dd	7.77 dd	7.88 dd
2-H	7.71 dd	7.71 dd	7.67 dd	7.71 dd
3-H	7.31 dd	7.32 dd	7.26 dd	7.32 dd
7-H	5.20 br s	5.28 dd	5.18 br s	5.28 dd
8-H_{ax}	2.08 dd	2.09 dd	2.09 dd	2.09 dd
8-H_{eq}	2.29 ddd	2.35 ddd	2.31 ddd	2.35 ddd
10-H_{ax}	2.98 d	3.01 d	2.94 d	3.01 d
10-H_{eq}	3.23 dd	3.26 dd	3.20 dd	3.25 dd
$COCH_3$	2.42 s	2.42 s	2.41 s	2.42 s
1′-H	5.44 dd	5.45 dd	5.48 dd	5.49 dd
2′-H_{ax}	1.77 m	1.64 m	1.8～2.0 m	～1.6 m
2′-H_{eq}	1.85 m	1.85 m	1.8～2.0 m	1.6～1.9 m
3′-H	3.09 m	2.89 m	3.29 m	3.06 m
4′-H	3.93 br s	3.57 br s	3.80 m	3.90 br s
5′-H	4.14 dq	4.11 m	4.14 dq	4.12 dq
6′-H	1.32 d	1.28 d	1.30 d	1.30 d
NH	—	4.71 br s	—	4.69 br s
1″-H	4.74 t	4.68 t	4.85 dd	4.97 dd
2″-H_a	1.7～1.9 m	1.9～2.0 m	1.7～1.9 m	1.80 ddd
2″-H_b	1.7～1.9 m	1.9～2.0 m	1.7～1.9 m	1.98 ddd
3″-H	4.17 m	4.13 ddq	4.23 m	4.03 ddq
4″-H	1.23 d	1.24 d	1.20 d	1.25 d
5″-H	3.80 ddq	3.82 ddq	～3.80 m	3.87 m
6″-H_a	3.41 br s	2.69 ddd	3.54 m	2.50 m
6″-H_b	3.51 dd	2.80 ddd	3.54 m	3.04 m
7″-H	1.06 d	1.11 d	1.17 d	1.12 d

[a] Measured on $CDCl_3$ at 400 MHz with TMS as an internal reference. Chemical shift assignments were made on the basis of decoupling experiments.

—: Signals could not be observed.

A2[†,6] or rubeomycin A[7]) by direct comparison of its Rf value on TLC, ^{1}H NMR and FD-MS (*m/z* 660 $(M+H)^+$). Compound **7** gave the same molecular ion peak as **5** in FD-MS, and the ^{1}H NMR spectrum was also similar to **6** except for higher field shifts of 3′-H (δ 3.06) and 6″-H (δ 2.50 and δ 3.04) (Table 1). Thus, **7** was confirmed to have the same planar structure as **5**. Because these compounds (**5** and **7**) were products of an intramolecular BORCH's reductive alkylation[8], parental compounds **1** and **2** should have a CHO group at 5″ position. The other reductive products, **4** and **6** were determined to have (3″*S*,5″*S*) and (3″*S*,5″*R*)-configuration[9], respectively. So, **1** and **2** should have (3″*S*,5″*S*) and (3″*S*,5″*R*)-configuration as depicted in Fig. 1. Additionally, three types, aldehyde type, carbinol amine type and imine type, were proposed for the structures of **1** and **2**, which will explain the complexity of their ^{1}H NMR spectra.

Table 2. Rf values and retention times of **1**, **2**, **3**, **4**, **5**, **6** and **7**.

Compound	Rf value[a]	Retention time[b] (minutes)
Barminomycin I (**1**)	0.42 (leading)	7.0
Barminomycin II (**2**)	0.35	8.5
Carminomycin I (**3**)	0.15	5.0
Carminomycin III (**4**)	0.32	8.1
5	0.34	7.6
Carminomycin II (**6**)	0.25	7.7
7	0.40	7.2

[a] Solvent system: $CHCl_3$ - MeOH - AcOH - H_2O (60 : 10 : 1 : 1).

[b] Compounds were analyzed using a reversed phase column (Nucleosil $5C_{18}$, 4.6×250 mm) and a mobile phase of CH_3CN - 0.2% phosphoric acid (40 : 60). The flow rate was 1.5 ml/minute into a 254 nm-detector.

The physico-chemical properties of **1**, **2**, **5** and **7** are as follows:

1: UV $\lambda_{max}^{0.1N\ HCl-90\%MeOH}$ nm ($E_{1cm}^{1\%}$) 233 (712), 253 (505), 291 (150), 491 (275), 525 (180); IR ($CHCl_3$) cm^{-1} 1720, 1610, 1420, 1290, 1120, 1010; FD-MS *m/z* 639.

2: UV $\lambda_{max}^{0.1N\ HCl-90\%MeOH}$ nm ($E_{1cm}^{1\%}$) 233 (702), 253 (499), 291 (145), 491 (270), 525 (176); IR ($CHCl_3$) cm^{-1} 1720, 1610, 1420, 1290, 1120, 1010; FD-MS *m/z* 639.

5: MP 129~134°C; $[\alpha]_D^{27}$ +294° (*c* 0.043, $CHCl_3$); UV $\lambda_{max}^{0.1N\ HCl-90\%MeOH}$ nm ($E_{1cm}^{1\%}$) 233 (666), 253 (489), 290 (145), 491 (272), 525 (179); IR (KBr) cm^{-1} 3450, 1720, 1610, 1420, 1300, 1210, 1120, 1030; FD-MS *m/z* 642 $(M+H)^+$.

7: MP 124~132°C; $[\alpha]_D^{27}$ +187° (*c* 0.023, $CHCl_3$): UV $\lambda_{max}^{0.1N\ HCl-90\%MeOH}$ nm ($E_{1cm}^{1\%}$) 233 (663), 253 (486), 290 (148), 490 (270), 525 (177); IR (KBr) cm^{-1} 3450, 1720, 1610, 1420, 1300, 1200, 1120, 1030; FD-MS *m/z* 642 $(M+H)^+$.

Their Rf values on silica gel TLC and retention times for HPLC are summerlized in Table 2.

The antimicrobial activity of barminomycin I and its reductive compounds was examined by agar dilution method, and the results are

Table 3. MICs of barminomycin I and its reductive compounds.

Test organism	MIC (μg/ml)		
	Barminomycin I (**1**)	Carminomycin III (**4**)	**5**
Staphylococcus aureus FDA 209P	0.2	3.12	0.78
S. aureus Smith	0.2	3.12	0.78
S. aureus MS8710	0.2	1.56	1.56
S. aureus MS9610	0.2	1.56	1.56
Micrococcus lysodeikticus IFO 3333	0.2	1.56	0.78
Bacillus subtilis PCI 219	<0.1	1.56	0.39
Escherichia coli NIHJ	3.12	>50	50
E. coli K-12	0.78	>50	6.25
E. coli BE1121	<0.1	1.56	0.2
E. coli BE1186	<0.1	1.56	0.2
Klebsiella pneumoniae PCI 602	0.39	50	6.25
Serratia marcescens	0.78	25	3.12
Proteus vulgaris OX19	3.12	>50	>50
Pseudomonas aeruginosa A3	1.56	>50	12.5
Mycobacterium smegmatis ATCC 607	0.78	6.25	6.25

† See footnote on p. 407.

Table 4. Effect of anthracyclines **1**, **2**, **3**, **4**, **5**, **6** and **7**, and doxorubicin on the growth of P388 leukemia cells.

Compound	IC_{50} (μg/ml)
Barminomycin I (**1**)	~0.00001
Barminomycin II (**2**)	~0.00002
Carminomycin I (**3**)	0.002
Carminomycin III (**4**)	0.006
5	0.0015
Carminomycin II (**6**)	0.010
7	0.002
Doxorubicin	0.013

IC_{50} values were determined on day 2 culture.

shown in Table 3. The cytotoxic activity of barminomycins, carminomycins and doxorubicin against P388 leukemia cells was compared. The results show that barminomycins I and II have more than 100-time high potency than other anthracyclines (Table 4). In preliminary *in vivo* experiments, treatment of barminomycins I and II (0.39~1.5 μg/kg/day, days 0 to 10, ip) showed remarkable prolongation (T/C 140~500%) of the survival period of CDF_1 mice bearing L1210 leukemia. The doses exhibiting toxicity against tumor bearing mice for barminomycins were about 1,000-time less than those for carminomycins. In the paper reported by OGAWA *et al.*[7], rubeomycin A_1 (carminomycin III) was described to be about 10-time potent than rubeomycin A (carminomycin II), but purified carminomycin III was not so potent. The rubeomycin A_1 used might have been contaminated by barminomycins.

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