PRODUCTION OF NEW ANTHRACYCLINE ANTIBIOTICS 1-HYDROXY-OXAUNOMYCIN AND 6-DEOXYOXAUNOMYCIN BY LIMITED BIOSYNTHETIC CONVERSION USING A DAUNORUBICIN-NEGATIVE MUTANT

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A limited biosynthetic conversion of some known anthracyclinones using a specific daunorubicin-nonproducing mutant provided four new anthracycline antibiotics: 1-Hydroxy-10-methoxycarbonyl-13-deoxocarminomycin; 1-hydroxy-13-deoxocarminomycin; 1-hydroxyoxaunomycin and 6-deoxyoxaunomycin. Their isolation and purification from bioconversion broth, structural determination and antitumor activities against leukemic L1210 cells are described.

Microbial conversion with anthracycline-nonproducing mutants is a useful means for obtaining new hybrid anthracyclines. We have obtained a number of new hybrid anthracyclines by bioconversion of natural anthracyclinones using biosynthetically blocked mutants of aclarubicin-producing *Streptomyces galilaeus* MA144 M1 and daunorubicin-producing *Streptomyces coeruleorubidus* ME-130 A4.[1~6]

Recently we have found an intensely potent anthracycline antibiotic oxaunomycin (7-*O*-daunosaminyl β-rhodomycinone) in the culture broth of a blocked mutant of a new baumycin-producing *Streptomyces* sp. D788.[7] Since it was 30-fold more cytotoxic against L1210 cell culture than its *N*-dimethyl derivative betaclamycin T (7-*O*-rhodosaminyl β-rhodomycinone), our attention was directed to the 7-*O*-daunosaminyl derivatives of other rhodomycinone aglycones such as β-isorhodomycinone (β-isoRMN) and α-citromycinone (α-CTN). Their corresponding 7-*O*-rhodosaminyl derivatives such as obelmycin A[8] and yellamycin A[9] have been isolated and evaluated biologically by us, but their 7-*O*-daunosaminyl derivatives have not been yet obtained.

In this paper we describe production of the target 7-*O*-daunosaminyl hybrid anthracyclines 1-hydroxyoxaunomycin and 6-deoxyoxaunomycin by bioconversion of β-isoRMN and α-CTN using a specific daunorubicin (DRN)-nonproducing mutant.

Materials and Methods

Microbial Strain

A bioconversion strain used for 7-*O*-daunosaminylation was a DRN-nonproducing mutant strain

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OXA-4 which was derived from new baumycin-producing *Streptomyces* sp. D788.[10] This strain was capable of daunosaminylating a precursor aglycone although at least two steps of DRN biosynthesis were impaired. It was grown on YS slant agar (yeast extract 0.3%, soluble starch 1% and agar 1.5%, pH 7.5) and stored at 5°C until use.

Media and Seed Culture

Medium for seed culture was soluble starch 0.5%, glucose 0.5%, soybean meal 1.0%, yeast extract 0.1%, K_2HPO_4 0.1%, $MgSO_4 \cdot 7H_2O$ 0.1% and NaCl 0.3% in tap water (pH 7.5) (seed medium). Slant culture was inoculated into a 500-ml Erlenmeyer flask containing 100 ml of seed medium and cultivation was carried out at 30°C for 2 days on a rotary shaker (200 rpm). The seed culture was inoculated 5% into conversion medium which consisted of soluble starch 4.0%, maltose 2.0%, dry yeast 2.0%, soybean meal 2.0%, yeast extract 0.2%, NaCl 0.1%, $MgSO_4 \cdot 7H_2O$ 0.1%, $CaCO_3$ 0.2%, $CuSO_4 \cdot 5H_2O$ 0.0007%, $FeSO_4 \cdot 7H_2O$ 0.0001%, $MnSO_4 \cdot 4H_2O$ 0.0008% and $ZnSO_4 \cdot 7H_2O$ 0.0002% in tap water (pH 7.4).

Microbial Conversion

Preliminary biosynthetic conversion of natural aglycones was examined with 250-ml Erlenmeyer flasks containing 30 ml each of conversion medium. Cultivation was carried out at 30°C on a rotary shaker (200 rpm). After 3 days, a methanol solution of substrate aglycone (2 mg/ml) was added to the culture to give a final concentration of 30 μg/ml and the cultivation was continued for 4 additional days. A sample of 2 ml was withdrawn at an appropriate time during a period of bioconversion and 2 ml each of acetone and $CHCl_3$ added. After agitation, the mixture was centrifuged and the solvent layer was evaporated to dryness. The residual pigments were analyzed by TLC or HPLC.

Rhodomycinone (RMN)-type aglycones such as ε-RMN, β-RMN, ε-isoRMN, β-isoRMN and α-CTN, and aklavinone (AKN) and ε-pyrromycinone (PMN) were used in these bioconversion experiments. All of the former RMN-type aglycones used were prepared from acid-treated culture broths of *Streptomyces violaceus* A262 and its blocked mutants.[8,9] AKN and ε-PMN were prepared by acid hydrolysis of *Streptomyces galilaeus* products, MA144 A1 and A2, respectively.[11]

A large-scale bioconversion for obtaining new hybrid anthracyclines was performed using four 30-liter jar fermenter containing 15 liters of conversion medium. Cultivation was carried out at 28°C with agitation of 350 rpm and aeration of 15 liters/minute. Substrate aglycone (ε-isoRMN, β-isoRMN or α-CTN) was fed to give a final concentration of 40 μg/ml by addition of 150 ml of the methanol solution after 3 days and the cultivation was continued for 4 additional days. The conversion broth was harvested and centrifuged. Products were extracted from the mycelial pellet with 5 liters of acetone. The acetone extract was evaporated to about a half volume, adjusted to pH 3.5 with 6 N HCl and washed with $CHCl_3$. The aqueous layer was then adjusted to pH 8.5 with 4 N NaOH and extracted with $CHCl_3$. The extract was evaporated to a small volume and an excess of ethyl ether was added to precipitate crude product.

Acid Hydrolysis

Samples (about 1 mg) in 0.1 N HCl were heated at 80°C for 30 minutes and the component sugar and aglycone were determined by TLC as previously described.[8]

TLC and HPLC

Silica gel F_{254} plates (Merck Co.) were used in analytical TLC. Solvents used were $CHCl_3$ - MeOH - HCOOH (40 : 10 : 1) and $CHCl_3$ - MeOH - aq NH_3 (50 : 10 : 1) for bioconversion tests and checks on process of isolation and purification, and *n*-BuOH - AcOH - H_2O (4 : 1 : 1) for sugar analysis and $CHCl_3$ - MeOH (20 : 1) for aglycone analysis. Preparative TLC was performed using Silica gel PF_{254} plates (Merck Co.) with a solvent of $CHCl_3$ - MeOH - H_2O - AcOH - aq NH_3 (120 : 50 : 5 : 1 : 1). HPLC was carried out on a Hitachi 655 liquid chromatographic apparatus with a reverse phase analytical column, A312 (ODS) (6 × 150 mm) (Yamamura Chemical Laboratories Co.). Mobile phase used was 35% CH_3CN (pH 2.0, adjusted with concd H_3PO_4) and was run at a flow rate of 1.0 ml/minute. Samples were dissolved in or diluted with the mobile phase and a 10 μl aliquot was injected. Detection was at 436 nm for α-CTN bioconversion and at 495 nm for the other bioconversion using a visible light detector (SPD-6AV: Shimadzu Corporation).

General

Melting points were determined on a Kofler hotstage microscope. UV spectra were determined on a Hitachi EPS 3T and IR spectra (KBr pellet) on a Hitachi EPI-GS spectrophotometer. ^{1}H and ^{13}C NMR were recorded with a Jeol GX-400 spectrometer at 400 and 100 MHz, respectively. Chemical shifts are expressed in δ value (ppm) with TMS as an internal reference and coupling constants are given in J (Hz). Mass spectra were recorded with a Hitachi M-80H or a Jeol JMS SX102A spectrometer. Specific rotations were determined on a Jasco DIP-181 Digital Polarimeter.

Results

Bioconversion of Some Natural Aglycones with Strain OXA-4

Streptomyces sp. D788 strain OXA-4 used for bioconversion was derived as a pigment-less nonproducing mutant from a DRN-blocked mutant strain RPM-5, which was damaged at a 10-decarboxylation step of precursor metabolite D788-1 (10-carboxy-13-deoxocarminomycin) in the DRN biosynthesis so that it accumulated D788-1 as a major metabolite with some minor metabolites (or by-products), D788-6 (10-methoxycarbonyl-13-deoxocarminomycin), D788-7 (10-hydroxy-13-deoxocarminomycin; oxaunomycin) and D788-11 (13-deoxocarminomycin) (dCM) in the culture broth.[12] Thus, the capability of producing a pigmented aglycone molecule was lost in this strain, but that of daunosaminylating a precursor aglycone was still maintained with some limited biosynthetic modification activities on the aglycone moiety.

The bioconversion of several aglycones fed in growing culture of strain OXA-4 was tested and the results are shown in Table 1. Aglycones tested were AKN, ε-PMN, ε-RMN, ε-isoRMN, β-RMN and β-isoRMN, and were fed to a 3-day old growing culture. After additional 4-day cultivation, the conversion cultures were harvested and products were extracted with organic solvents and assayed by TLC and HPLC. Bioconversion of AKN and ε-RMN gave the same three products **1a**～**1c** when examined by TLC. Further TLC and HPLC analyses revealed that **1a**～**1c** were identical to the anthracycline metabolite produced by parent strain RPM-5: D788-6, D788-11 and D788-7, respectively. These results were predictable since AKN and ε-RMN are precursor aglycones in DRN biosynthesis.[13] By analogy it was expected that bioconversion of ε-PMN (1-hydroxy-AKN) or ε-isoRMN (1-hydroxy-ε-RMN) with strain OXA-4 would produce the corresponding 1-hydroxy analogs. In fact, feeding of these aglycones also gave both the same three products **2a**～**2c** whose Rf values on TLC were parallel to those of **1a**～**1c**, respectively, with an acidic solvent. HPLC behaviors were also similar for both groups. However, their spot colors on TLC were red for **1a**～**1c** but purple for **2a**～**2c**. Among them, **2c** was produced in very poor yield, but it was found by HPLC and TLC analyses that **2c** was identical to **4a**, which was obtained by the β-isoRMN bioconversion as described below.

On the other hand, the bioconversion of β-RMN, β-isoRMN or α-CTN which has a hydroxy group at C-10 in place of a methoxycarbonyl group gave only one product in either case. The product from β-RMN, **3a**, showed a red color spot on TLC. Single products **4a** (purple) and **5a** (yellow) were obtained from β-isoRMN (purple) and α-CTN (yellow), respectively. **3a** was found to be identical to **1c** (D788-7; 7-*O*-daunosaminyl-β-RMN) in complete accordance with their behaviors on TLC and HPLC. This showed that bioconversion of β-RMN, but not ε-RMN or AKN, with strain OXA-4 was stopped at a daunosaminylation step without any further biosynthetic modification of the aglycone. This bioconversion manner suggested that **4a** and **5a** were also 7-*O*-daunosaminyl-β-isoRMN and 7-*O*-daunosaminyl-α-CTN,

Table 1. Bioconversion of some anthracyclinones by a daunorubicin-nonproducing mutant strain OXA-4 of *Streptomyces* sp. D788.

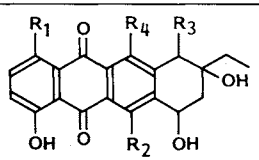
Substrate aglycone[a]	Structure[b]				Product					Remarks
						TLC[c]				
						Spot color	Rf (1)	Rf (2)	HPLC[d] Rt (minutes)	Name
	R_1	R_2	R_3	R_4						
1. AKN or	H	OH	$COOCH_3$	H	**1a**	Red	0.23	0.21	13.20	D788-6
ε-RMN	H	OH	$COOCH_3$	OH	**1b**	Red	0.19	0.19	18.64	D788-11 (13-deoxycarminomycin)
					1c	Red	0.16	0.15	4.56	D788-7 (oxaunomycin)
2. ε-PMN or	OH	OH	$COOCH_3$	H	**2a**	Purple	0.23	0.19	13.91	1-Hydroxy-D788-6
ε-IsoRMN	OH	OH	$COOCH_3$	OH	**2b**	Purple	0.19	0.17	20.59	1-Hydroxy-D788-11
					2c	Purple	0.16	0.10	4.65	1-Hydroxy-D788-7
3. β-RMN	H	OH	OH	OH	**3a**	Red	0.16	0.15	4.56	D788-7
4. β-IsoRMN	OH	OH	OH	OH	**4a**	Purple	0.16	0.10	4.65	1-Hydroxy-D788-7 (1-hydroxyoxaunomycin)
5. α-CTN	H	H	OH	OH	**5a**	Yellow	0.20	0.09	9.85	6-Deoxy-D788-7 (6-deoxyoxaunomycin)

[a] AKN: aklavinone; PMN: pyrromycinone; RMN: rhodomycinone; CTN: citromycinone.

[c] (1) $CHCl_3$ - MeOH - HCOOH (40 : 10 : 1).
(2) $CHCl_3$ - MeOH - aq NH_3 (40 : 10 : 0.2).

[d] 35% CH_3CN (pH 2.0, adjusted with concd H_3PO_4). Flow rate: 1.0 ml/minute (40°C).

[b]



respectively.

Isolation and Identification of New Compounds

To obtain the new compounds **2a**, **2b**, **4a** and **5a**, a large scale-bioconversion was carried out using a 30-liter jar fermenter containing 15 liters of the growing culture. The amount of added substrate aglycone (ε-isoRMN, β-isoRMN or α-CTN) was 600 mg. The bioconversion was stopped after 4-day cultivation. The broth was centrifuged and the mycelial pellets were extracted with acetone. The products were recovered finally as crude powder from the acetone extract and then purified according to the same procedure as shown in Fig. 1. As a result, pure **2a** (6.9 mg) and **2b** (3.3 mg) with trace amount of **2c** were obtained by bioconversion of ε-isoRMN, and pure **4a** (42 mg) and **5a** (58 mg) by bioconversion of β-isoRMN and α-CTN, respectively.

TLC analysis of aglycone and sugar after acid hydrolysis revealed that aglycones of **2a**, **4a** and **5a** were ε-isoRMN, β-isoRMN and α-CTN, respectively, by comparison with Rf values of authentic samples, and that the sugars were all L-daunosamine in terms of Rf value (0.35) and a spot color of white blue. The aglycone of **2b** was new and was considered to be 1-hydroxy-13-deoxocarminomycinone from a similarity in the biosynthetic conversion with ε-RMN. Their physico-chemical properties are shown in Table 2. In mass spectra, their molecular ion peaks coincided with the calculated molecular weights of the proposed molecular formulas. UV and IR spectra of **2a**, **4a** and **5a** were almost identical to those of their substrate aglycones, but the IR spectrum of **2b** indicated the absence of ester carbonyl group (1725 cm^{-1}) which is present in the substrate ε-isoRMN. Their ^{1}H and ^{13}C NMR chemical shift assignments are shown in Tables 3 and 4, respectively. ^{1}H and ^{13}C NMR chemical shifts of **2a**, **4a** and **5a** revealed that they were the daunosaminyl body of ε-isoRMN, β-isoRMN and α-CTN, respectively. ^{1}H and ^{13}C NMR chemical shifts of **2b** lacked those assigned for a carbomethoxy group at C-10 as compared with those of **2a**, indicating that **2b** is decarbomethoxy derivative of **2a**. The sugar linkage sites were all determined to be at C-7 since their chemical shifts at C-7 in ^{13}C NMR shifted to lower field about 6~9 ppm compared with those of their corresponding aglycones.[8,9] The sugar linkage site of **5a** was further confirmed by a technique of gated long range selective proton decoupling in which a long range coupling between 1'-H and C-7 was observed. The small coupling constants ($J = \sim 4$ Hz) of their anomeric protons indicate that the configuration of the glycosidic bonds are α. Thus, these new compounds were found to be 1-hydroxy-D788-6 (**2a**), 1-hydroxy-D788-11 (**2b**), 1-hydroxy-D788-7 (**4a**) and 6-deoxy-D788-7 (**5a**) as illustrated in Fig. 2.

Fig. 1. Isolation and purification from crude extract.

Crude powder

LH-20 column (40 × 350 mm)
eluent: $CHCl_3$ - MeOH (1 : 2)

Major pigmental fraction

silica gel column (Wako gel C-200, 100 g)
eluent:
$CHCl_3$ - MeOH (100 : 1) 500 ml
$CHCl_3$ - MeOH (50 : 1) 500 ml
$CHCl_3$ - MeOH (10 : 1) 1,000 ml
$CHCl_3$ - MeOH - H_2O (80 : 10 : 5) 1,500 ml
$CHCl_3$ - MeOH - H_2O (50 : 10 : 2) 1,000 ml

Partially purified fraction

Preparative TLC (Silica gel PF_{254})
solvent: $CHCl_3$ - MeOH - H_2O - AcOH - aq NH_3 (120 : 50 : 5 : 1 : 1)

Purified fraction

dissolved in 1% AcOH
washed with toluene (twice)
adjusted to pH 8.0 with satd $NaHCO_3$
extracted with $CHCl_3$
washed with H_2O
dried over Na_2SO_4
evaporated
precipitated with ethyl ether

Pure powder

Table 2. Physico-chemical properties of new compounds produced by bioconversion with strain OXA-4.

Compound	**2a**	**2b**
Appearance	Red-violet powder	Red-violet powder
Melting point (°C)	217~220	170~173
$[\alpha]_D^{23}$	+13° (*c* 0.002, MeOH)	+43° (*c* 0.002, MeOH)
UV and VIS:		
λ_{max} nm ($E_{1cm}^{1\%}$) in 90% MeOH	204 (sh, 208), 241 (748), 299 (109), 491 (sh, 171), 521 (287), 548 (276), 560 (286)	205 (sh, 204), 241 (693), 299 (109), 489 (sh, 176), 521 (296), 547 (278), 559 (299)
IR (KBr) cm^{-1}	3400, 2925, 1725, 1590, 1450, 1425, 1395, 1300, 1250, 1185, 1170, 1120, 1005, 980, 795	3400, 2930, 1585, 1450, 1400, 1300, 1250, 1185, 1010, 980, 795
MS *m/z*	574 $(M+H)^+$ (FAB-MS)	516 $(M+H)^+$ (FAB-MS)
Molecular formula	$C_{28}H_{31}NO_{12}$	$C_{26}H_{29}NO_{10}$
Identified as	1-Hydroxy-D788-6	1-Hydroxy-D788-11

Compound	**4a**	**5a**
Appearance	Red-violet powder	Yellow powder
Melting point (°C)	249~253	144~147
$[\alpha]_D^{23}$	+398° (*c* 0.004, $CHCl_3$)	+137° (*c* 0.02, $CHCl_3$)
UV and VIS:		
λ_{max} nm ($E_{1cm}^{1\%}$) in 90% MeOH	203 (345), 241 (871), 297 (134), 419 (sh, 208), 523 (340), 550 (322), 562 (342)	204 (429), 230 (793), 257 (530), 290 (sh, 182), 435 (246)
IR (KBr) cm^{-1}	3400, 2920, 1590, 1460, 1405, 1305, 1260, 1190, 1015, 985, 800	3400, 2920, 1620, 1600, 1580, 1450, 1370, 1325, 1255, 1110, 1010, 980, 900
MS *m/z*	531 (M^+) (FD-MS)	500 $(M+H)^+$ (FD-MS)
Molecular formula	$C_{26}H_{29}NO_{11}$	$C_{26}H_{29}NO_9$
Identified as	1-Hydroxy-D788-7	6-Deoxy-D788-7

Antitumor Activity *In Vitro* and *In Vivo*

In vitro antitumor effect of new compounds was examined by assaying their inhibitory activities against the cell growth and nucleic acid synthesis of cultured L1210 cells. The corresponding 1-dehydroxy or *N*-dimethyl analogs were also tested for comparison. The results are shown in Table 5. Growth inhibitory activities of **4a** and **5a** were very strong, but those of **2a** and **2b** were moderate. Activity comparison with their *N*-dimethyl analogs showed that loss of *N*-dimethyl group caused a significant decrease in the growth inhibitory activity. As reported previously[7] and also cited in Table 5, one exception was observed between **3a** (D788-7) and its *N*-dimethyl analog (betaclamycin T). Concerning the inhibitory activity on nucleic acid synthesis, these new compounds exhibited a greater effect on inhibition of DNA synthesis than RNA synthesis as compared with their *N*-dimethyl derivatives. Especially, **2b** and **5a** inhibited DNA synthesis more greatly than RNA synthesis.

Antitumor effects *in vivo* of **4a** and **5a** were examined against mice ip inoculated with leukemic L1210 cells. The drugs were administered daily ip from day 1 to day 10. **4a** and **5a** had a maximum antitumor activity of 188 and 158% (T/C) at optimum doses of 0.32 and 0.08 mg/kg/day, respectively. In a similar experiment, *N*-dimethyl derivatives of **4a** and **5a** (obelmycin A and yellamycin A) and oxaunomycin exhibited a T/C % of 155, 169 and 200 at optimum doses of 2.5, 0.39 and 0.06 mg/kg/day, respectively.

Table 3. ^{1}H NMR chemical shift assignments for new compounds obtained by bioconversion with strain OXA-4.

Proton	**2a** (1-hydroxy-D788-6)	**2b** (1-hydroxy-D788-11)	**4a** (1-hydroxy-D788-7)	**5a** (6-deoxy-D788-7)
Aglycone moiety				
1-H	—	—	—	7.82 d (8.0)
2-H	7.31 s	7.16 s	7.41 s	7.69 t (8.0)
3-H				7.32 d (8.0)
6-H	—	—	—	7.78 s (8.0)
7-H	5.22 br d	5.05 br s	5.34 br s	4.93 br s
8-Ha	2.33 d (14.7)	2.32 d (14.7)	2.23 d (16.0)	2.29 dd (16.0, 4.0)
8-Hb	2.22 dd (15.4, 4.4)	Obscure	2.15 dd (16.0, 4.0)	2.18 d (16.0)
10-H (or $-CH_2$)	4.27 s	3.15 d (19.4) (Ha) 2.50 d (18.7) (Hb)	4.83 s	4.91 s
13-Ha	1.8～1.9	1.6 m～	1.82 m (8.0)	1.85 q (8.0)
13-Hb	1.47 m (7.3)		1.74 m (8.0)	1.72 q (8.0)
14-CH_3	1.12 t (7.3)	1.08 t (7.3)	1.10 t (8.0)	1.11 t (8.0)
16-OCH_3	3.72 s	—	—	—
Daunosamine moiety				
1'-H	5.50 br s	5.44 br s	5.45 br s	5.29 d (4.0)
2'-Ha	1.8～2.05	1.6 m～	1.7 m～	1.85 t (12.0)
2'-Hb				1.70 dd (12.0, 4.0)
3'-H	3.29 br d (11.0)	3.45 br	2.93 br d	3.07 m
4'-H	3.72	3.83 br s	3.46 s	3.49 br s
5'-H	4.13 q (6.6)	4.17 br	4.10 q (7.0)	4.08 q (7.0)
6'-CH_3	1.32 d (6.6)	1.32 d (4.8)	1.33 d (7.0)	1.34 d (7.0)

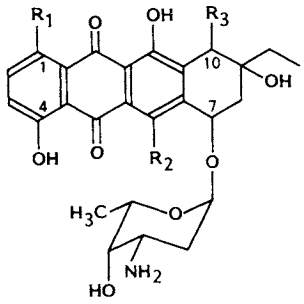
Spectra were measured in $CDCl_3$ - CD_3OD (20 : 1) for **2a**, **4a**, **5a** and $CDCl_3$ - CD_3OD (10 : 1) for **2b** at 400 MHz. Chemical shifts are expressed in δ (ppm) from internal TMS. Coupling constants in parenthesis are given in J (Hz).

Table 4. ^{13}C NMR chemical shift assignments for new compounds obtained by bioconversion with strain OXA-4.

Carbon	**2a** (1-hydroxy-D788-6)	**2b** (1-hydroxy-D788-11)	**4a** (1-hydroxy-D788-7)	**5a** (6-deoxy-D788-7)
Aglycone moiety				
1	157.63*	157.39	157.82	119.68
2	129.50*	129.29*	129.65	137.12
3	129.44*	129.09*	129.65	125.17
4	157.57*	157.39	157.82	162.70
4a	112.64	112.67*	113.03	116.21
5	189.25	188.81	189.61	187.68
5a	111.76	111.27	112.48*	132.09
6	156.48*	156.49*	156.67*	120.38
6a	135.38	134.56	135.02	143.48
7	70.90	70.44	70.97*	73.66
8	33.42	36.99	33.14	33.81
9	70.96	69.18	72.31	72.64
10	51.77	36.15	66.12	66.56
10a	134.56	137.64	138.37	134.23
11	155.83*	156.05*	156.95*	162.37
11a	111.49	110.83	112.00*	115.38
12	189.25	188.81	189.61	187.86
12a	112.64	112.64*	113.03	133.34
13	29.58	35.15	30.27	30.29
14	6.55	7.30	6.45	6.56
15	171.29	—	—	—
16	52.36	—	—	—
Sugar moiety				
1'	100.40	99.66	101.64	98.90
2'	32.21	28.91	32.53	33.52
3'	46.87	47.17	46.48	46.42
4'	70.96	66.83	70.52*	70.57
5'	66.98	66.47	67.89	68.02
6'	16.36	16.41	16.77	16.89

* Values may be interchanged between similar values.
Spectra were measured in $CDCl_3$ - CD_3OD (20 : 1) for **2a**, **4a**, **5a** and $CDCl_3$ - CD_3OD (10 : 1) for **2b**.

Fig. 2. Structure of hybrid anthracycline antibiotics obtained by bioconversion with strain OXA-4.



Compound	Structure		
	R_1	R_2	R_3
1a (D788-6)	H	OH	$COOCH_3$
1b (D788-11)	H	OH	H
2a (1-hydroxy-D788-6)	OH	OH	$COOCH_3$
2b (1-hydroxy-D788-11)	OH	OH	H
3a (oxaunomycin)	H	OH	OH
4a (1-hydroxyoxaunomycin)	OH	OH	OH
5a (6-deoxyoxaunomycin)	H	H	OH

Table 5. Antitumor activities *in vitro* of new compounds **2a**, **2b**, **4a** and **5a**, and the related compounds against L1210 leukemia cells.

Compound	IC_{50} (μg/ml)[a]			DNA/RNA
	Cell growth	DNA Synthesis	RNA synthesis	
1a (D788-6)	0.25	2.60	1.30	2.0
1b (D788-11)	0.03	1.30	1.30	1.0
2a (1-hydroxy-D788-6)	0.22	2.20	1.10	2.0
2b (1-hydroxy-D788-11)	0.11	0.64	1.20	0.5
3a (oxaunomycin)	0.0005	0.31	0.30	1.0
4a (1-hydroxyoxaunomycin)	0.005	1.00	0.78	1.3
5a (6-deoxyoxaunomycin)	0.006	0.55	4.00	0.1
N-Dimethyl **1a** (epelmycin D)	0.006	0.95	0.23	4.1
N-Dimethyl **3a** (betaclamycin T)	0.01	0.21	0.06	1.5
N-Dimethyl **4a** (obelmycin A)	0.001	0.58	0.14	4.1
N-Dimethyl **5a** (yellamycin A)	0.007	0.28	0.23	1.2

[a] *In vitro* antitumor activities against cultured L1210 cells were assayed according to the same method as previously described.[1)]

Discussion

The intensely potent antitumor activity of oxaunomycin (7-*O*-daunosaminyl-β-RMN) led us to obtain other similar RMN-type anthracyclines such as 7-*O*-daunosaminyl-β-isoRMN and 7-*O*-daunosaminyl-α-CTN. For their preparation we tried to apply a bioconversion method using a DRN-nonproducing mutant. We have found that a pigment-less nonproducing mutant of DRN-producing *Streptomyces coeruleorubidus* converted the exogenously added ε-RMN and AKN to DRN in the growing culture.[12)] This bioconversion included a daunosaminylation of the added aglycone and further biosynthetic modifications on the aglycone level toward DRN. Therefore, use of bioconvertant which had a limited or no activity on the biosynthetic modification of aglycone moiety was necessary to achieve selective daunosaminylation. For this purpose, we employed a DRN-nonproducing mutant strain OXA-4 which was derived from a newly isolated baumycin producer. This strain had genetic blockages at the biosynthetic steps of decaketide formation and 10-decarboxylation of a precursor metabolite D788-1. As shown in Table 1, it was found that this strain produced only 7-*O*-daunosaminyl-β-RMN from β-RMN without production of any other metabolites. Thus, we were able to obtain 7-*O*-daunosaminyl-β-isoRMN (**4a**) and -α-CTN (**5a**) by bioconversion of β-isoRMN and α-CTN with strain OXA-4. As we have found that the glucosidation of α-CTN, but not β-isoRMN, with an aclarubicin-nonproducing mutant of *Streptomyces galilaeus* takes place at a C-10 hydroxyl group,[6)] it is interesting that the glycosidation with strain OXA-4 occurs at a C-7 hydroxyl group.

1-Hydroxyoxaunomycin (**4a**) and 6-deoxyoxaunomycin (**5a**) thus obtained exerted their optimum

antitumor activities *in vivo* at a 5-fold or more lower dose as compared with their *N*-dimethyl derivatives, obelmycin A and yellamycin A, respectively. However, the potent antitumor activities seen *in vitro* and *in vivo* with oxaunomycin were not observed with these new 1-hydroxy and 6-deoxy analogs.

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