ANTHRACYCLINE METABOLITES FROM BAUMYCIN-PRODUCING *Streptomyces* sp. D788

I. ISOLATION OF ANTIBIOTIC-BLOCKED MUTANTS AND THEIR CHARACTERIZATION

AKIHIRO YOSHIMOTO[†,*], OSAMU JOHDO, SHIZUKA FUJII, KATSUROU KUBO, HIROSHI NISHIDA and ROKURO OKAMOTO

Central Research Laboratories, Mercian Corporation, 4-9-1 Johnan, Fujisawa 251, Japan

TOMIO TAKEUCHI

Institute of Microbial Chemistry, 3-14-23 Kamiosaki, Shinagawa-ku, Tokyo 141, Japan

(Received for publication February 15, 1992)

Biosynthetically blocked mutants were obtained from a baumycin-producing *Streptomyces* sp. D788 newly isolated from soil. The first mutant isolated was a baumycin-negative but daunorubicin-accumulating mutant with a loss of 4'-substitution activity, from which all other blocked mutants were successively derived. These included a known 11-deoxydaunorubicin-producing mutant and several new types of mutants which produced mainly 10-carboxy-13-deoxocarminomycin, 10-methoxycarbonyl-13-deoxocarminomycin, their 11-deoxy derivatives or a precursor aglycone, respectively.

In this paper, all the anthracycline components produced by the parent strain and its two known blocked mutants, a daunorubicin producer and a 11-deoxydaunorubicin producer, are also determined by HPLC and five new components are isolated. Cytotoxicities *in vitro* of all the components against L1210 cell culture are also described.

In a continuing search for new anthracycline compounds of microbial origin, our attention has been focussed on isolating biosynthetically blocked mutants producing new anthracycline metabolites from a microorganism producing a known anthracycline. Recently we reported the isolation of such blocked mutants or variants from a rhodomycin type of anthracycline producer *Streptomyces violaceus* A262 and their production of about twenty new anthracycline analogs.[1] A classical anthracycline antibiotic daunorubicin was known to be produced by several *Streptomyces* strains. However, there have been a few reports concerning blocked mutants which accumulated new anthracycline metabolites. CASSINELLI *et al.* isolated two blocked mutants from *Streptomyces peucetius* var. *caesius*, which produced 11-deoxydaunorubicin[2] and 13-deoxocarminomycin.[3] PANDEY *et al.*[4] isolated a mutant from an unidentified *Streptomyces* strain that produced a unique aglycone antibiotic called maggiemycin. We also isolated a blocked mutant which produced feudomycins with a variation in the side chain at C-9 from *Streptomyces coeruleorubidus*.[5] A blocked mutant accumulating ε-rhodomycinone has also been reported by several groups.[2,4~6] However, there has been no detailed study about the range of compounds that blocked mutants accumulate in their culture broth.

† Present address: Laboratory for Microbial Biochemistry, Faculty of Biological Science, Hiroshima University, 1-4-4 Kagamiyama, Higashi-Hiroshima 724, Japan.

* Corresponding author.

In this paper we deal with the isolation of new and known types of antibiotic-blocked mutants derived from a baumycin-producing *Streptomyces* sp. D788 and also provide details of the detectable anthracycline components produced by the parent strain and its two known blocked mutants (a daunorubicin-producing mutant strain G1-1 and a 11-deoxydaunorubicin-producing mutant strain KL-330). As a result, five new anthracycline compounds were obtained, their structures defined and their antitumor activities *in vitro* assessed.

Details of the novel blocked mutants and their products will be described separately.[7,8]

Materials and Methods

Microbial Strain

An unspeciated *Streptomyces* sp. D788 was isolated as a baumycin (BM) producer from a soil collected in Fujisawa, from which various antibiotic-blocked mutants were artificially derived as described below. The parent and mutant strains were grown on YS agar (yeast extract 0.3%, soluble starch 1.0% and agar 1.5%, pH 7.2) and stored at 5°C until use.

Media

YS agar as described above was used as a plate medium for mutant isolation. Seed cultures were grown in a medium containing 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). The medium used for the antibiotic production test contained soluble starch 4.0%, dry yeast 2.5%, yeast extract 0.2%, NaCl 0.2%, $CaCO_3$ 0.3%, $CuSO_4 \cdot 7H_2O$ 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 8.0 before autoclave) (production medium).

Mutagenesis Treatment

N-Methyl-*N'*-nitro-*N*-nitrosoguanidine (NTG) was used as a mutagen. Microbial slants were grown on YS agar at 28°C for 10 days and the spores from one slant culture were collected and suspended by mild sonication in 5 ml of 0.1 M Tris-HCl buffer (pH 8.0) containing 0.1% Tween 100. The suspension was filtered through a small glass tube packed with sterilized defatted cotton and NTG was added at a final concentration of 1 mg/ml to the filtrate (about 10^9 spores/ml). NTG treatment was carried out for 45 minutes at room temperature in the dark to give a killing of more than 80%. The spore suspension thus treated was diluted serially 10-fold with 0.9% saline, 0.1 ml aliquots were plated on YS agar and incubated at 28°C for 7 days. The colonies were tested for antibiotic production as described below.

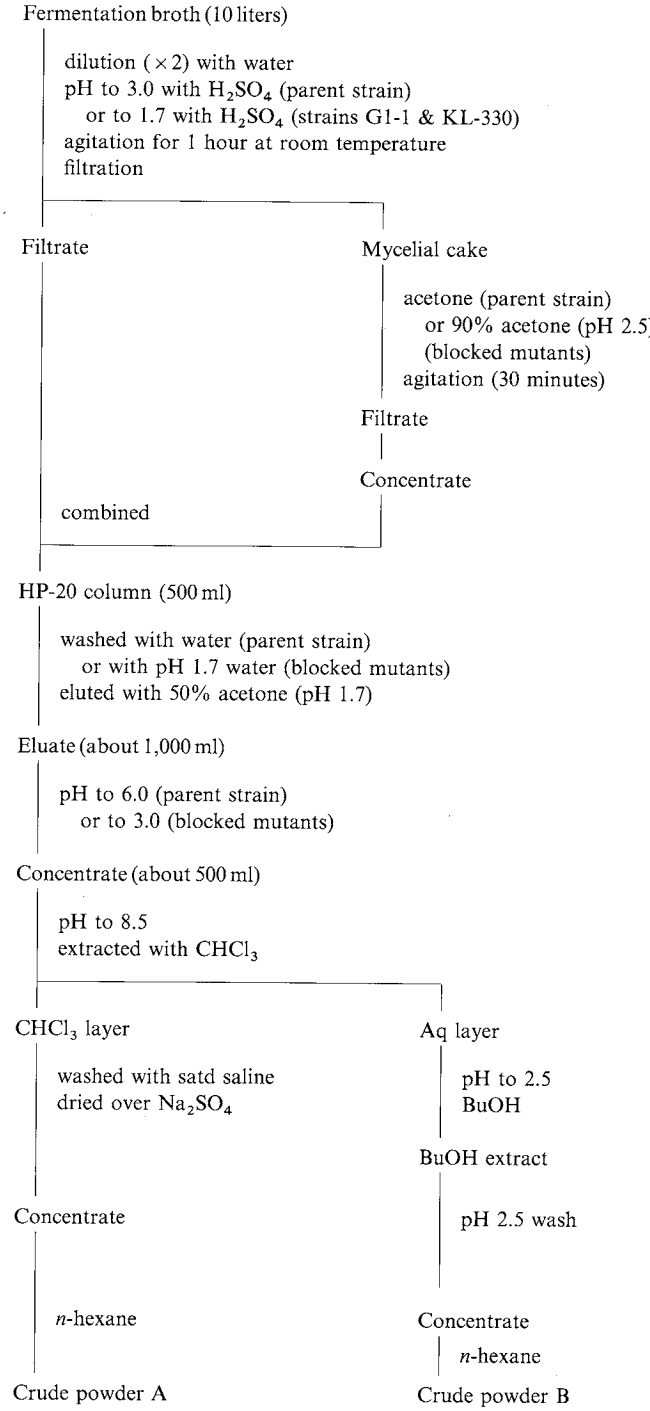
Screening for Antibiotic-Blocked Mutants

The colonies obtained above were inoculated into small glass tubes containing 4 ml each of the seed medium and cultivated on a reciprocal shaker at 28°C for 2 days, and the entire culture was added to a 250-ml Erlenmeyer flask containing 30 ml of the production medium. These flasks were incubated at 30°C for 5 days on a rotary shaker (200 rpm). To screen for daunorubicin (DRN)-producing mutant from the parental BM producer, 2 ml of the test culture and 1 ml of 1 M Tris-HCl (pH 8.5) were mixed and then 2 ml each of acetone and chloroform were added. After agitation, the mixture was centrifuged and the solvent layer evaporated to dryness. The residual pigments were dissolved in 0.1 ml of $CHCl_3$-MeOH (15 : 1) and 20 μl was spotted on a Silica gel F_{254} plate (Merck Co.) which was developed with the solvent $CHCl_3$-MeOH-H_2O-aq NH_3-AcOH (120 : 50 : 5 : 1 : 1). Thus, a colony producing a new spot of DRN (Rf value: 0.32) without any spots of BMs (Rf values >0.53) could be identified. In a subsequent round of mutagenesis of DRN-producing mutant, 1 ml of the test cultures was added to 1 ml of 1 M sodium citrate-citric acid buffer (pH 3.5) and 2 ml of acetone in a small glass tube. After mixing and standing for one hour at room temperature, the mixture was centrifuged and the supernatant (20 μl) subjected to TLC in the same manner as described above. Thus, the colonies showing new spots other than DRN or yellow spots could be identified.

Fermentation and Product Isolation

A large scale fermentation for product isolation was carried out using 200-ml Erlenmeyer fasks containing 50 ml each of production medium (10-liter total). All seed cultures were grown at 30°C for 2~3 days in a 500-ml Erlenmeyer flask containing 50 ml of seed medium and inoculated 4% into a fermentation flask. The cultivation was carried out at 30°C for 5 days. Crude product isolation from the

Fig. 1. Preparation of crude extracts from fermentation broth.



fermentation broth was carried out as shown in Fig. 1. Thus, anthracyclines produced by the parent strain were recovered as a crude powder A and those produced by strains G1-1 and KL-330 as two separate crude powders A and B.

Antitumor Activity *In Vitro*

Inhibitory effects of the products on growth and nucleic acid synthesis in murine L1210 leukemia cell culture were examined as previously described.[9]

HPLC

HPLC was performed on a Hitachi 655 liquid chromatographic apparatus with a reverse phase analytical column, A312 (ODS) (6 × 150 mm) (Yamamura Chemical Laboratories Co., Ltd.). Solvents used as mobile phase were 35% CH_3CN in pH 2.0 water (with H_3PO_4) and 35%, 40% and 45% CH_3CN in 0.01 M 10-camphorsulfonic acid ($CmSO_4$, pH 4.2). Flow rate was 1.0 ml/minute unless otherwise noted. Samples were dissolved in the mobile phase and 10 μl aliquotes injected. Detection was conducted at 254 nm using a UV detector (UVILOG-5III A, Oyo Bunko Kiki Co., Ltd.). In the HPLC assay, all products except BM were estimated as μg DRN or 11-deoxy DRN (for 11-deoxy analogs) for convenience using DRN and 11-deoxy DRN as standards.

General

MP's 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. ^1H and ^{13}C NMR were recorded with a Jeol GX-400 spectrometer at 400 MHz and 100 MHz, respectively. Chemical shifts are expressed in δ values (ppm) with TMS as an internal reference and coupling constants are givenin J (Hz). Mass spectra were recorded with a Hitachi M-80H spectrometer. Specific rotations were determined on a Jasco DIP-181 Digital Polarimeter.

Results and Discussion

Isolation of Blocked Mutants from *Streptomyces* sp. D788

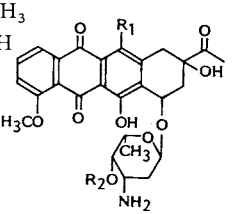
Streptomyces sp. D788 is a BM producer, as described later, and produced major BMs A1 and A2 and minor C1 (*N*-formyl DRN) which have been previously identified as anthracycline products of *S. coeruleorubidus*.[10,11] No measurable DRN was observed in the culture broth. We attempted the isolation of various antibiotic-blocked mutants from this BM producer by mutagenesis with NTG and obtained five groups of mutants which were blocked in a different step of anthracycline biosynthesis. The results are shown in Table 1. The first blocked mutant target was a BM-negative but DRN-producing strain with a biosynthetic blockage in 4'-substitution. After several attempts, we were able to obtain a DRN-producing strain G1-1 from parent strain D788. Further mutations of strain G1-1 gave a unique blocked mutant 1S-238 together with a DNR-negative but ε-rhodomycinone (RMN)-producing mutant (3F-940), a yellow pigmented mutant (6S-2181) producing 11-deoxy DRN and related compounds, seven antibiotic-nonproducing mutants and also yield-improved strains (CR-3, 6S-2117 and 7S-2527) of the DRN producer. The same type of mutant (RPM-5) as strain 1S-238 was also derived from an other DRN-producer CR-3 and was found to produce a water-soluble anthracycline D788-1 (10-carboxy-13-deoxocarminomycin)[12] as a major product. Thus, these mutants were blocked in a 10-decarboxylation step of DRN biosynthesis. 11-Deoxy DRN-producer KL-330 was also isolated from another DRN producer 6S-2117 and had a block in 11-oxidation. A new blocked mutant 4L-660, which was derived from a DRN-producer 7S-2527, produced new components D788-5 (10-methoxycarbonyl-13-deoxo DRN) and D788-6 (4-*O*-demethyl D788-5).[7] This type of mutation was also observed in the DRN producer mutant KL-58 (58NR-58 and -66 isolated) and a deesterification step from 10-methoxycarbonyl (D788-5) to 10-carboxyl (D788-1) appeared to

Table 1. Summary of antibiotic-blocked mutants derived from a baumycin-producing *Streptomyces* sp. D788.

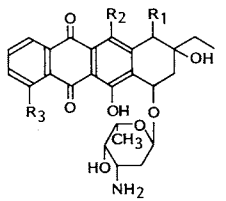
Group	Mutant strain[a]		Major product	Loss of possible biosynthetic step	Remarks
D788	—		Baumycin (BM)	—	
I	G1-1 (D788)	CR-3 (G1-1)	Daunorubicin (DRN)	4′-Substitution	
	6S-2117 (G1-1)	7S-2527 (G1-1)			
	KL-58 (6S-2117)	GH-1373 (KL-58)			
	AF2-2 (GH-1373)				
	F-36 (AF2-2)		13-Dihydro DRN		
II	RPM-5 (CR-3)	1S-238 (1T-2181)	D788-1 (10-carboxy-13-deoxocarminomycin)	10-Decarboxylation	
	IE1-78 (RPM-5)	OXA-1878 (IE1-78)			
III	4L-660 (7S-2527)		D788-5 (10-methoxycarbonyl-13-deoxo DRN)	15-Demethylation	
	58NR-58 (KL58)				
IV	KL-330 (6S-2117)	6S-2181 (G1-1)	11-Deoxy DRN	11-Oxidation	
	YXA-338 (OXA-1878)		11-Deoxy D788-1	11-Oxidation 11-Decarboxylation	
	YDK-18 (58NR-58)		11-Deoxy D788-5	11-Oxidation 15-Demethylation	
V			Aglycone:	7-*O*-Daunosaminylation	
	3F-940 (G1-1)	3FM-90 (CR-3)	ε-Rhodomycinone (RMN)	—	
	YM-28 (KL-330)		Aklavinone (AKN)	11-Oxidation	
VI	3F-773 (G-1)	10F-3397 (G1-1)	[b] None but able to biotransform	Decaketide formation	
	6S-2245 (G1-1)	Banji-2 (G1-1)			
	7CS-2165 (CR-3)	3L-445 (7S-2575)			
	8S-2895 (G1-1)		[c] None and unable to biotransform		
	OXA-4 (IE1-78)		[b] None but able to biotransform		
	OXA-8 (IE1-78)		[c] None and unable to biotransform		
	DKN-1 (58NR-58)		[b] None but able to biotransform		
	YM-190 (KL-330)		[b] None but able to biotransform		
	NYDK-12 (YDK-18)		[b] None but able to biotransform		

	R_1	R_2
BM A1 & A2	OH	X
DRN	OH	H
11-deoxy DRN	H	H

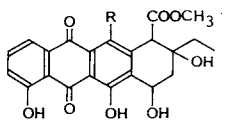
X = –CH(CH$_2$CHOHCH$_3$)–O–CH(CH$_2$OH)–CH$_3$



	R_1	R_2	R_3
D788-1	COOH	OH	OH
11-deoxy D788-1	COOH	H	OH
D788-5	$COOCH_3$	OH	OCH_3
11-deoxy D788-5	$COOCH_3$	H	OCH_3



	R
ε-RMN	OH
AKN	H



[a] Strain in parenthesis shows the parent strain from which the mutant was derived.
[b] Capable of producing the parental anthracyclines from exogenously added AKN.
[c] Incapable of producing the parental anthracyclines from exogeneously added AKN.

be impaired. A 11-deoxy D788-5-producing mutant YDK-18 and D788-5-nonproducing mutant DKN-1 were then derived by NTG mutagenesis of strain 58NR-58. An aklavinone (AKN)-producing mutant YM-28 and 11-deoxy DRN-nonproducing mutant YM-190 were also isolated from the 11-deoxy DRN producer KL-330. Strain AF2-2 was a DRN producer which accumulated only DRN without any minor components. Mutant F-36 was derived from the DRN producer and gave a favorable accumulation of 13-dihydro DRN.

The nonproducing strains derived from each of above blocked mutant were tested for their biosynthetic transformation ability of precursor AKN to glycosidic anthracyclines. We have demonstrated with another DRN producer *Streptomyces coeruleorubidus* ME130-A4 that AKN is precursor in the biosynthesis of DRN.[13] Thus, AKN in MeOH was added to the 4 day-cultivation broth of the nonproducing mutants (final concentration 30 μg/ml) and the incubation was continued for further 2 days. Culture broths were assayed for their anthracycline products. Among 14 strains tested, 12 strains exhibited the ability of biosynthetic transformation of exogenously added AKN to glycosidic anthracyclines which were produced by their parental strains. Two strains 8S-2895 and OXA-8, however, showed no biotransformation capability. It seems that the former nonproducing mutants have a blockage in an early biosynthetic step of polyketide assembly but not in the glycosidation and further biosynthetic conversions. Therefore, these are very useful since the limited and preferred bioconversion of substrate anthracycline was possible by their selective use. Thus, new hybrid 1-hydroxy anthracyclines were obtained by the limited bioconversion using strain OXA-4[14] and the selective 4-*O*-methylation of some anthracyclines was achieved by bioconversion using strain DKN-1.[15]

HPLC Analysis of Anthracyclines Produced by Parent and Known Blocked Mutant Strains

From the aspect of anthracycline biosynthesis and search for new anthracycline components, all the detectable anthracycline components produced were determined by HPLC analysis of the crude extracts obtained as shown in Fig. 1. Strains examined were parent *Streptomyces* sp. D788 and its blocked mutants, a DRN producer G1-1, its variant F-36, a 11-deoxy DRN producer KL-330 and aglycone producers 3F-940 and YM-28.

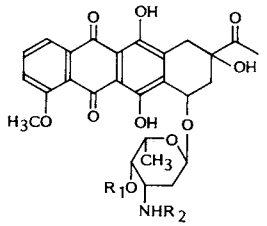
The results are summarized in Table 2. *Streptomyces* sp. D788 was found to produce BMs A1 and A2 (A1 epimer) with minor BM C1 (*N*-formyl DRN) and an unidentifed component. About 70% and 25% of the anthracyclines produced were BMs A2 and A1, respectively. Yields of the minor components were as low as 1%. No measurable DRN was accumulated in the culture broth, and BMs B1 and B2 and *N*-acetyl DRN (produced by BM-producing *Streptomyces coeruleorubidus* ME130-A4[10]) were not detected. Strain G1-1 was found to be a 4′-substitution-less mutant, it did not produce BMs. The major product was DRN with a total yield of about 60%. Nine minor components, including D788-1 (17%), doxorubicin (DOX) (4%), 13-dihydro DRN (7%), 13-dihydrocarminomycin (1%) and 4′-*O*-daunosaminyl DRN (2%), were detected. D788-14 (4-*O*-demethyl feudomycin B) and D788-13 (9,10-anhydro DRN) were new compounds although their yields were also lower than 2% total. Strain KL-330 derived from DRN-producing mutant 6S-2117 seemed to have an additional defect in 11-oxidation so that it produced a series of 11-deoxy compounds parallel to compounds produced by strain G1-1. D788-3 (10-carboxy-11-deoxy-13-dexocarminomycin) and D788-4 (9,10-anhydro-11-deoxy-13-deoxocarminomycin) were new compounds. However, this strain showed a preferential production of D788-3

Table 2. Summary of anthracyclines produced by *Streptomyces* sp. D788 and its blocked mutant strains G1-1, F-36, KL-330, 3F-940 and YM-28.

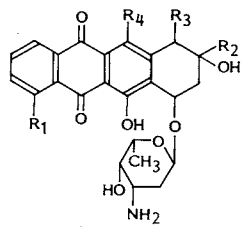
Microbial strain	Series name	Product	Structure					HPLC (Rt: minutes)	Yield		Notice	Reference
			Basal[a]	R_1	R_2	R_3	R_4		ratio	%		
Parent		Baumycin A1	A	X*	H	—	—	14.36[b]	0.35	25	Known	11)
		Baumycin A2	A	X*	H	—	—	13.92[b]	1.00	72	Known	11)
		Baumycin C1	A	H	CHO	—	—	11.62[b]	0.02	1	Known	11)
		Baumycin Y	—	—	—	—	—	16.74[b]	0.02	1	Unknown	
G1-1		Daunorubicin (DRN)	B	OCH_3	$COCH_3$	H	OH	9.87[c] 6.92[d]	1.00	57	Known	
		Doxorubicin	B	OCH_3	$COCH_2OH$	H	OH	5.28[c] —	0.08	4	Known	
		13-Dihydrodaunorubicin	B	OCH_3	$CH(OH)CH_3$	H	OH	6.19[c] 4.76[d]	0.13	7	Known	
		13-Dihydrocarminomycin	B	OH	$CH(OH)CH_3$	H	OH	— 6.22[d]	<0.01	1	Known	
	D788-1	10-Carboxy-13-deoxocarminomycin	B	OH	CH_2CH_3	COOH	OH	4.44[c] —	0.35	17	Known	12)
	D788-2	9,10-Anhydro-13-deoxocarminomycin	C	OH	CH_2CH_3	H	OH	— 14.50[d]	0.11	6	Known	16)
	D788-21	Feudomycin (FM) D	B	OCH_3	CH_3	OH	OH	4.01[c]	0.06	3	New	
	D788-18	4′-*O*-Daunosaminyl DRN	A	DaN**	H	—	—	9.62[c]	0.03	2	Known	17)
	D788-14	4-*O*-Demethyl FM B	B	OH	CH_2COCH_3	H	OH	9.16[c]	0.02	1	New	
	D788-13	9,10-Anhydro DRN	C	OCH_3	$COCH_3$	H	OH	7.61[f]	<0.01	1	New	
F-36		Daunorubicin						5.20[f]	0.3	23		
		13-Dihydrodaunorubicin						3.95[f]	1.0	77		
KL-330		11-Deoxy DRN	B	OCH_3	$COCH_3$	H	H	5.84[e]	0.47	29	Known	2)
	D788-3	11-Deoxy-D788-1	B	OH	CH_2CH_3	COOH	H	4.20[e]	1.00	61	New	
	D788-4	11-Deoxy-D788-2	C	OH	CH_2CH_3	H	H	16.72[e]	0.09	5	New	
		11-Deoxy DOX	B	OCH_3	$COCH_2OH$	H	H	7.22[e]	0.04	2	Known	2)
		11-Deoxy-D788-11	B	OH	CH_2CH_3	H	H	4.60[e]	0.04	2	Known	18)
3F-940		ε-Rhodomycinone	—					[0.65][g,***]	1.00	100		
YM-28		Aklavinone	—					[0.67][g,***]	1.00	100		

[a]

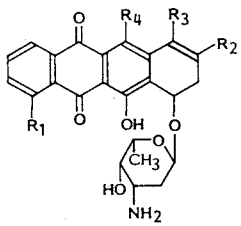
A



B



C



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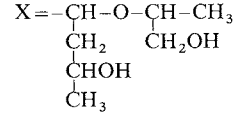

** DaN: daunosamine

HPLC

[b] 35% CH_3CN (pH 2.0 with H_3PO_4); flow rate: 1.2 ml/minute.
[c] 35% CH_3CN in 0.01 M $CmSO_4$; flow rate: 1.0 ml/minute.
[d] 40% CH_3CN in 0.01 M $CmSO_4$; flow rate: 1.0 ml/minute.
[e] 45% CH_3CN in 0.01 M $CmSO_4$; flow rate: 1.0 ml/minute.
[f] 35% CH_3CN (pH 2.0 with H_3PO_4); flow rate: 1.0 ml/minute.

*** TLC

[g] Rf value on Silica gel plate F_{254} (solvent: $CHCl_3$ - MeOH (20 : 1))

(total 61%) over 11-deoxy DRN (29%). Strain F-36 was a 13-dihydro DRN producer in that about 80% of the anthracyclines produced was 13-dihydro DRN and the other 20% was DRN. The accumulation of any other related components was not observed in this strain. Mutant strains 3F-994 and YM-28 produced only ε-RMN and AKN, respectively and no other anthracycline spots were detected when their broth extracts were analyzed by TLC.

Isolation and Identification of Anthracycline Components

Details of isolation and structural identification of the anthracyclines produced by parent strain (*Streptomyces* sp. D788) and blocked mutant strains G1-1 and KL-330 are as follows.

Parent Strain

The crude powder A (669 mg) obtained according to the method described in Fig. 1 was purified by column chromatography on a silica gel (42 mm i.d.: Wakogel C-200, 100 g) which was eluted with $CHCl_3$ - MeOH (100 : 1 ~ 5 : 1) mixture in a stepwise gradient. Early fractions contained unkown compounds, those containing BM A1 and those containing BM A2 were collected separately and evaporated to dryness *in vacuo*. The later two were dissolved in 1% acetic acid and washed with toluene. The aqueous layers were adjusted to pH 8.0 with 1 N NaOH and extracted with $CHCl_3$. After drying over anhydrous Na_2SO_4, the $CHCl_3$ layers were evaporated and an excess of *n*-hexane was added to precipitate pure BMs A1 (39 mg) and A2 (156 mg). Early fractions contained unknown compounds were purified by TLC using Silica gel plate PF_{254} (Merck Co.) with a $CHCl_3$ - MeOH (4 : 1) mixture as a developing solvent. Two bands thus separated were scraped off and extracted with $CHCl_3$ - MeOH (2 : 1). The extracts were evaporated to dryness followed by acidic extraction and toluene washing as described above and gave pure powders by adding an excess of *n*-hexane. One of them was BM C1 (3 mg) and the other (4.2 mg) was unidentified. BMs A1 and A2 showed the same molecular ion peak *m/z* 674 $(M+H)^+$ in FAB-MS spectrum (molecular formula: $C_{34}H_{43}NO_{13}$). BM C1 gave a molecular ion peak *m/z* 556 $(M+H)^+$ in FDA-MS (molecular formula: $C_{28}H_{29}NO_{11}$). ^1H and ^{13}C NMR analyses proved that these compounds were BMs A1, A2 and C1 by comparison with authentic samples.[11]

Strain G1-1

The crude powder A (985 mg) obtained from 10-liter fermentation broth was chromatographed on a silica gel column (30 mm i.d.; Wakogel C-200, 100 g) with a stepwise gradient consisting of the following solvent systems: $CHCl_3$ - MeOH - H_2O (20 : 1 : 0, 15 : 1 : 0, 10 : 1 : 0, 5 : 1 : 0.1). Fractions (10 ml each) were monitored by TLC and those containing mainly D788-2 (akrobomycin[16]), D788-13 (new), D788-14 (new), D788-18 (4′-*O*-daunosaminyl DRN)[17] and DRN (eluted in this order) were pooled, separately. DOX, 13-dihydro DRN and FM D (new) were not well separated and fractions containing them were mixed. After evaporation to dryness, all these fractions, except for those containing DRN, were purified by TLC on Silica gel PF_{254} using developing solvents as follows: $CHCl_3$ - MeOH (10 : 1) for D788-2 and D788-13; $CHCl_3$ - MeOH - aq NH_3 (45 : 10 : 1) for D788-14 and -18; $CHCl_3$ - MeOH - H_2O - AcOH (75 : 25 : 5 : 5) for the other mixture. DRN fractions were more than 97% pure by HPLC assay and pure DRN powder (349 mg) was obtained after evaporation and precipitation with *n*-hexane. Yields of the other minor compounds were very low because of purification losses (DOX, 18 mg; 13-dihydro DRN, 26 mg; 13-dihydrocarminomycin, 3.5 mg; D788-2, 25 mg; D788-21, 6.2 mg; D788-18, 5.1 mg; D788-14, 3.1 mg;

Table 3. Physico-chemical properties of new compounds produced by strains G1-1 and KL-330.

	Strain G1-1			Strain KL-330	
	D788-13 (9,10-anhydro daunorubicin)	D788-14 (4-*O*-demethyl feudomycin B)	D788-21 (feudomycin D)	D788-3 (11-deoxy D788-1)	D788-4 (11-deoxy D788-2)
Appearance	Red-brown powder	Red-brown powder	Red-brown powder	Yellow powder	Yellow powder
Melting point (°C)	155~160 (dec)	159~160 (dec)	160~162 (dec)	186~187 (dec)	109~113 (dec)
$[\alpha]_D$ (*c* 0.01, $CHCl_3$)	+258°	+156°	+230°	+112°	+294°
UV and VIS:					
λ_{max} nm ($E^{1\%}_{1cm}$) in 90% MeOH	234 (813), 254 (461), 293 (160), 492 (293)	235 (615), 253 (423), 289 (165), 475 (208), 496 (218), 531 (127)	235 (620), 252 (487), 292 (114), 500 (186), 535 (222)	206 (399), 228 (594), 260 (406), 432 (205)	223 (501), 260 (489), 294 (510), 450 (309)
IR (KBr) cm^{-1}	1660, 1615, 1580, 1445, 1410, 1355, 1290, 1252, 1110, 1050, 1010, 980	1700, 1615, 1580, 1410, 1290, 1110, 1010	1615, 1585, 1400, 1280, 1010, 980	1700, 1670, 1620, 1380, 1290, 1010, 980, 760	1670, 1645, 1620, 1590, 1470, 1385, 1290, 1015, 980, 760
FAB-MS *m/z* $(M+H)^+$	510	528	516	528	466
Molecular formula	$C_{27}H_{27}NO_9$	$C_{27}H_{29}NO_{10}$	$C_{26}H_{29}NO_{10}$	$C_{27}H_{29}NO_{10}$	$C_{26}H_{27}NO_7$

Table 4. ^{1}H NMR chemical shifts for new compounds produced by strains G1-1 and KL-330.

Proton	Strain G1-1			Strain KL-330	
	D788-13 (9,10-anhydro daunorubicin) in $CDCl_3$	D788-14 (4-*O*-demethyl feudomycin B) in $CDCl_3$	D788-21 (feudomycin D) in $CDCl_3$	D-788-3 (11-deoxy D788-1) in CD_3OD	D788-4 (11-deoxy D788-2) in $CDCl_3$
Aglycone moiety					
1-H	8.01 d (8.1)	7.82 d (8.0)	8.02 d (8.0)	7.76 dd (7.7, 2.0)	7.82 d (6.6)
2-H	7.78 t (8.1)	7.66 d (8.0)	7.56 t (8.0)	7.73 t (7.7)	7.66 t (7.3)
3-H	7.39 d (8.8)	7.27 d (8.0)	7.32 d (8.0)	7.31 dd (7.7, 1.8)	7.29 d (7.3)
4-OCH_3	4.08 s	—	4.08 s	—	—
7-H	5.37 dd (5.1, 1.5)	5.15 m	5.16 m	5.14 d (4.8)	5.28 br d
8-Ha	3.47 dd (19.1, 1.5)	2.69 d (16.0)	2.18 m	2.56 dd (15.0, 5.1)	2.71 d (17.6)
8-Hb	2.37 ddd (19.1, 5.1, 2.9)	2.02 dd (16.0, 4.0)		2.30 d (15.0)	2.56 dd (19.1, 3.7)
10-Ha	7.94 d (2.9)	3.30 dd (19.0, 1.5)	4.81 s	4.02	6.42 br s
10-Hb	—	2.65 d (19.0)	—	—	—
11-H	—	—	—	7.66 s	7.59 s
9-CH_3	—	—	1.47 s	—	—
9-$COCH_3$	2.54 s	—	—	—	—
9-CH_2CH_3	—	—	—	1.83 m (7.3), 1.64 m (7.3)	2.19 q (7.3)
9-CH_2CH_3	—	—	—	1.12 t (7.3)	1.19 t (7.3)
9-CH_2COCH_3	—	3.04 d (16.0), 2.40 d (16.0)	—	—	—
9-CH_2COCH_3	—	2.30 s	—	—	—
Daunosamine moiety					
1′-H	5.30 d (3.7)	5.46 br s	5.17 br s	5.49 d (2.9)	5.27 d (3.7)
2′-Ha	1.61 ddd (13.2, 12.5, 3.7)	1.5～1.9 m	1.5～1.9 m	2.06 td (12.5, 3.7)	1.64 td (13.2, 3.7)
2′-Hb	1.46 dd (13.2, 5.1)			1.92 dd (12.5, 4.4)	1.52 dd (13.2, 5.1)
3′-H	3.05 ddd (12.5, 5.1, 2.9)	2.9～3.2 m	3.0～3.3 m	3.58 br d	3.15 ddd (12.5, 5.1, 2.9)
4′-H	3.37 br s	3.49 br s	3.52 br s	3.66 br s	3.42 br s
5′-H	3.90 q (6.6)	4.16 q (6.6)	4.08 q (6.5)	4.27 q (6.6)	3.99 q (6.6)
6′-CH_3	1.33 d (6.6)	1.36 d (6.6)	1.35 d (6.5)	1.30 d (6.6)	1.34 d (6.6)

Chemical shifts are expressed by δ (ppm) from internal TMS. Coupling constants in parenthesis are given in J (Hz).

D788-13, 3.3 mg). The crude powder B (217 mg) was purified by a silica gel column chromatography (20 mm i.d.; Wakogel C-200, 50 g) with the solvent $CHCl_3$ - MeOH - H_2O - AcOH (40 : 10 : 1 : 0.1). D788-1[12] containing fractions were pooled and evaporated to dryness. D788-1 was dissolved in 1% $NaHCO_3$, washed and extracted again with *n*-BuOH at pH 2.5. After drying, the purified D788-1 (122 mg) was obtained as crystals from MeOH - toluene. All the known compounds isolated were identified by ^1H and ^{13}C NMR and FDA-MS analyses. D788-13 (9,10-anhydro DRN), D788-14 (4-*O*-demethyl FM B) and D788-21 (FM D) were new compounds and their physico-chemical properties as shown in Table 3, and their ^1H and ^{13}C NMR assignments in Tables 4 and 5, respectively.

Strain KL-330

The crude powder A (963 mg) was chromatographed on a silica gel column (20 mm i.d.; Wakogel C-200,

Table 5. ^{13}C NMR chemical shifts for new compounds produced by strains G1-1 and KL-330.

Carbon	Strain G1-1			Strain KL-330		Remarks
	D788-13 (9,10-anhydro daunorubicin) in $CDCl_3$	D788-14 (4-*O*-demethyl feudomycin B) in $CDCl_3$	D788-21 (feudomycin D) in $CDCl_3$	D788-3 (11-deoxy D788-1) in CD_3OD	D788-4 (11-deoxy D788-2) in $CDCl_3$	
1	119.79	119.51	119.62	120.90	119.91	Aglycone moiety
2	135.79	136.93	137.08	138.62	136.81	
3	118.62	124.72	124.55	125.73	124.62	
4	161.15	162.68	162.40	163.62	162.44	
4a	120.96	116.12	116.02	117.02	116.06	
5	186.90*	190.69	189.91	193.92	191.95	
5a	114.47*	111.11*	111.24	115.70	114.25	
6	155.10	157.17*	156.43*	163.19	160.71	
6a	134.33	135.23	135.48	132.53	133.47*	
7	64.63	70.46*	70.42	72.66	64.98	
8	27.89	34.51	35.58	35.83	34.89	
9	138.62	70.20	70.08	71.91	148.75	
10	127.19	36.55	66.62	58.68	119.84	
10a	130.23	138.28	135.20	144.78	143.30	
11	154.55	156.97*	156.51*	121.47	118.62	
11a	112.47*	110.68*	111.25	133.97	125.92	
12	186.72*	186.25*	186.06	182.44	181.92	
12a	135.35	133.47	133.32	134.82	133.65*	
9-CH_3	—	—	27.78	—	—	
9-$COCH_3$	198.19	—	—	—	—	
9-$COCH_3$	25.66	—	—	—	—	
9-CH_2CH_3	—	—	—	33.38	30.67	
9-CH_2CH_3	—	—	—	7.18	11.58	
9-CH_2COCH_3	—	50.66	—	—	—	
9-CH_2COCH_3	—	207.28	—	—	—	
9-CH_2COCH_3	—	32.22	—	—	—	
10-COOH	—	—	—	173.85	—	
4-OCH_3	56.73	—	56.53	—	—	
1′	98.41	101.23	99.96	101.52	97.92	Daunosamine moiety
2′	33.03	32.47	31.82	29.53	33.07	
3′	46.10	46.30	46.23	48.68	46.19	
4′	71.10	70.52*	69.64	67.93	71.00	
5′	66.55	66.84	67.28	67.93	66.33	
6′	16.95	16.89	16.70	16.96	17.10	

* Similar values may be interchanged.

100 g) with the solvent $CHCl_3$-MeOH-H_2O (80:10:0.5). By TLC monitoring, the fractions containing 11-deoxy D788-2 (new), 11-deoxy DRN[2], 11-deoxy DOX[2] and 11-deoxy D788-11[18], respectively, were pooled and evaporated to dryness. The 11-deoxy DRN portion was rechromatographed on a silica gel column with the same solvent and the pure compound (218 mg) was obtained. Further purification of the other three was carried out by TLC on a Silica gel PF_{254} plate with the solvent $CHCl_3$-MeOH-AcOH (100:10:1). Yields of 11-deoxy D788-2, 11-deoxy DOX and 11-deoxy D788-11 were 125 mg, 15.2 mg and 9.8 mg, respectively. The crude powder B (585 mg) was also purified by a column chromatography (25 mm i.d.; Wakogel C-200, 100 g) using solvents as follows: $CHCl_3$-MeOH-H_2O (100:10:0.5, 60:10:0.5). The pure fractions containing 11-deoxy D788-1 (new) were pooled and concentrated. Final purification was carried out by extraction with 1% $NaHCO_3$, washing with $CHCl_3$ and reextraction with EtOAc after adjustment to pH 2.5 with 4 N HCl. The yield was 394 mg. Physico-chemical properties of new compounds, 11-deoxy D788-1 and -2, are also shown in Table 3 and the ^1H NMR and ^{13}C NMR data in Tables 4 and 5, respectively.

Cytotoxicities

All of the anthracyclines produced by the parent and the blocked mutant strains G1-1 and KL-330 were tested for their cytotoxicities against murine leukemic L1210 cell culture and the results are shown in Table 6. It was found that BM A2, a major product of the parent organism, had the strongest growth inhibitory effect on L1210 cell culture. BM A1 was less active than A2, but exhibited a moderate growth

Table 6. Inhibitory activities of anthracyclines produced by parent, G1-1 and KL-330 strains on the growth and nucleic acid synthesis of murine leukemia L1210 cells.

Strain	Product	IC_{50} (μg/ml)		
		Growth	DNA	RNA
Parent	Baumycin A1	0.015	1.15	0.38
	Baumycin A2	0.005	1.30	0.48
	Baumycin C1	>1.0	>5.0	>5.0
G1-1	Daunorubicin (DRN)	0.02	0.42	0.16
	Doxorubicin (DOX)	0.02	1.40	0.55
	13-Dihydrodaunorubicin	0.17	1.90	1.60
	13-Dihydrocarminomycin	0.06	1.25	1.20
	D788-1 (10-carboxy-13-deoxocarminomycin)	0.027	>5.0	>5.0
	D788-2 (9,10-anhydro-13-deoxocarminomycin)	0.18	2.80	1.30
	Feudomycin B	0.83	1.50	0.80
	Feudomycin D	0.15	>5.0	>5.0
	4′-*O*-Daunosaminyl DRN	0.58	4.00	1.70
	D788-13 (9,10-anhydro DRN)	0.49	>5.0	>5.0
	D788-14 (4-*O*-demethyl feudomycin B)	0.33	1.63	0.57
KL-330	11-Deoxy DRN	>1.0	2.60	1.60
	11-Deoxy DOX	>1.0	>5.0	>5.0
	D788-3 (11-deoxy D788-1)	1.0	1.98	1.90
	D788-4 (11-deoxy D788-2)	1.00	2.37	1.06
	11-Deoxy D788-11	0.15	1.20	1.20

For the growth inhibition test, the L1210 cell cultures (5×10^4/ml) were exposed for 48 hours to drugs and viable cells were counted by coulter counter. In the inhibition test for nucleic acid synthesis, the L1210 cell cultures (8×10^5/ml) were exposed for 60 minutes to drugs with supplemented ^{14}C-labeled uridine or thymidine (0.05 μg Ci/ml), and the incorporation of the radioisotopes into acid insoluble material was measured.

IC_{50} values are expressed as drug concentration required to inhibit by 50% control of the growth and DNA and RNA syntheses of L1210 cell culture.

inhibition as well as 4'-desubstituted A2 (DRN) and DOX. D788-1 also had a significant growth inhibitory effect. However, the other minor compounds produced by strain G1-1 were all only weakly active against L1210 cell growth. The 11-deoxy series of compounds produced by KL-330 showed a marked loss of the growth inhibitory effect in comparison with the corresponding 11-hydroxy compounds. Five new anthracycline components also had very weak for antitumor activities *in vitro* and *in vivo* (data not shown).

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