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

II. NEW ANTHRACYCLINE METABOLITES PRODUCED BY A BLOCKED MUTANT STRAIN RPM-5

AKIHIRO YOSHIMOTO,† SHIZUKA FUJII, OSAMU JOHDO, KATSURO KUBO, HIROSHI NISHIDA and ROKURO OKAMOTO

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

TOMIO TAKEUCHI

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

(Received for publication July 8, 1992)

A daunorubicin-blocked mutant strain RPM-5 derived from a new baumycin-producing *Streptomyces* sp. D788 accumulated a major precursor metabolite D788-1 (10-carboxy-13-deoxocarminomycin) and nine minor metabolites in the culture broth. Five among them were new with a substituent at C-10 or the altered side chains at C-9. Isolation, purification and identification of all anthracycline metabolites produced by strain RPM-5 are described with their antitumor activities against L1210 cells.

New anthracycline metabolites produced by biosynthetically blocked mutants are important sources in search for more potent antitumor activity of anthracycline antibiotics and for the synthesis of anthracycline compounds with improved antitumor efficacy. Isolation of a variety of blocked mutants is also indispensable to reveal a biosynthetic route in antibiotic production.

In preceding paper,[1] we have reported the isolation of many blocked mutants from a baumycin (4′-substituted daunorubicin)-producing *Streptomyces* sp. D788. This paper deals with details of the anthracycline production by one of their blocked mutant, strain RPM-5. All detectable anthracycline metabolites accumulated in this culture broth were isolated, identified and quantified for their yields. Ten anthracycline components were obtained including a major metabolite D788-1 (10-carboxy-13-deoxocarminomycin), which has been isolated as a water-soluble anthracycline from a daunorubicin beer.[2] Eighty five % or more of a total yield of the anthracyclines was D788-1 and the remainder were shared by five new and four known minor components. Accordingly, it was proved that strain RPM-5 was a blocked mutant which resulted in a major accumulation of D788-1 by the genetic blockage in a biosynthetic step of 10-decarboxylation.

All the anthracycline metabolites are also evaluated for their antitumor activities *in vitro* and *in vivo* on L1210 cells and discussed from a viewpoint of daunorubicin biosynthesis.

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

Materials and Methods

Microbial Strain

The isolation of blocked mutant strain RPM-5 was described in a preceding paper.[1] The strain was 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.

Fermentation and Extraction

The seed culture of strain RPM-5 was grown using 500-ml Erlenmeyer flasks containing 100 ml each of seed medium: Soybean meal 1%, glucose 0.5%, soluble starch 0.5%, yeast extract 0.1%, NaCl 0.1%, K_2HPO_4 0.1%, $MgSO_4 \cdot 7H_2O$ 0.1% in tap water (pH 7.4 before autoclave). Cultivation was carried out at 30°C for 2 days on a rotary shaker (200 rpm). Fermentation was then performed using a 30-liter jar containing 15 liters of a production medium.[1] The seed culture (750 ml) was added to the jar and 130-hour cultivation was done at 28°C with agitation at 450 rpm and aeration of 15 liters/minute to give a maximum yield. The fermentation broth was separated into supernatant fluid and mycelia by filtration after adjustment of pH to 1.7 with 6 N H_2SO_4. The pigmented products were extracted from the mycelial cake with 80% acetone (pH 2.3). The extract was concentrated *in vacuo* and the aqueous concentrate was combined with the above filtrate. The resulting solution was adjusted to pH 2.3 with 4 N NaOH and passed through a Diaion HP-20 column (750 ml) at SV 4 to adsorb the pigments. After washing with water, the column was eluted with 50% acetone (pH 2.3, with HCl). The pigmented eluate was pooled and concentrated *in vacuo*. The concentrate was adjusted to pH 8.5 with 4 N NaOH, and then extracted with $CHCl_3$. The $CHCl_3$ layer was washed with water, dried over anhydrous Na_2SO_4 and evaporated to a small volume. The crude anthracycline complex (1.18 g) was precipitated by addition of an excess of *n*-hexane (extract A).

On the other hand, the aqueous layer after the $CHCl_3$ extraction was adjusted to pH 2.5 with 6 N HCl and extracted with *n*-BuOH. The extract thus obtained was evaporated to dryness to yield 6.44 g of crude anthracycline complex (extract B).

HPLC and TLC

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 phases were 30% CH_3CN (pH 2.0, with H_3PO_4) and 45% CH_3CN in 10 mM 10-camphorsulfonic acid (pH 4.2). Flow rate was 1.0 ml/minute. Samples were dissolved in the mobile phase or diluted with it and the 10 μl was injected. Detection was done at 254 nm using a UV detector (UVILOG-5 III A) (Oyo-Bunko Kiki Co., Ltd.).

Analytical TLC was carried out using Silica gel plate F_{254} (Merck Co.). Solvent systems were $CHCl_3$-MeOH-H_2O-aq NH_3-AcOH (120:50:5:1:1) for check on the isolation and purification process, and *n*-BuOH-AcOH-H_2O (4:1:1) for sugar identification. The sugar was detected by spraying TLC with *p*-anisaldehyde-H_2SO_4 in 95% EtOH and heating at 90°C for 15 minutes. Daunosamine gave a sky blue spot with a Rf value of 0.25.

Preparative TLC was also carried out using Silica gel plate PF_{254} (Merck Co.). Solvent systems used were $CHCl_3$-MeOH-H_2O-AcOH-aq NH_3 (125:55:5.5:0.9:1.1) and $CHCl_3$-MeOH-H_2O-AcOH (120:25:6:14).

Antitumor Activity *In Vitro*

Inhibitory effects on growth and nucleic acid synthesis in murine leukemia L1210 cell culture were determined according to the method as described previously.[3]

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 spectra were recorded with a Jeol GX-400 spectrometer at 400 MHz 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 Hitachi M-80H spectrometer. Specific rotations were determined on a Jasco DIP-181 Digital Polarimeter.

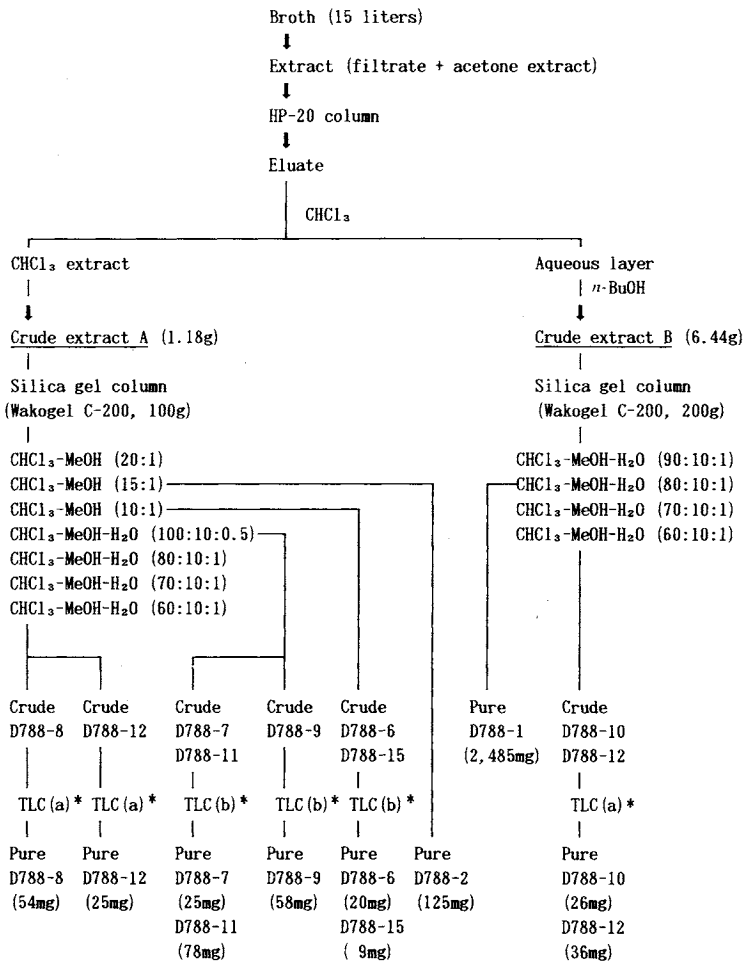
Results

Isolation and Purification

HPLC analysis revealed that crude extract A contained eight components: D788-2, D788-6, D788-7, D788-8, D788-9, D788-11, D788-12 and D788-15, and crude extract B had three components: D788-1, D788-10 with concomitant D788-12. The D788-serial names have been used for anthracycline products obtained from the culture broths of *Streptomyces* sp. D788 and its mutant isolates.

They were isolated and purified from the crude extracts A (1.18 g) and B (6.44 g) by column chromatography on silica gel (Wakogel C-200) and further by a preparative TLC on Silica gel PF_{254} plate as shown in Fig. 1. Pure D788-1 and D788-2 were obtained directly by column chromatography,

Fig. 1. Isolation and purification



* Silica gel PF_{254} plate.
Solvent: (a) $CHCl_3$-MeOH-H_2O-AcOH-aq NH_3 (120:55:5.5:0.9:1.1).
(b) $CHCl_3$-MeOH-H_2O-AcOH (120:25:6:14).

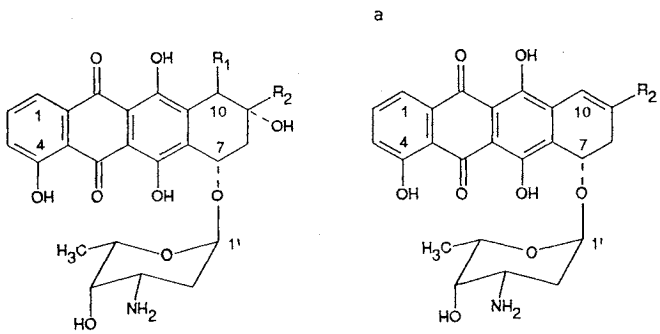
but the others were partially purified. Further purification was carried out by alternate TLC using two solvents. The TLC band containing each component completely separated was scraped off and eluted with $CHCl_3$-MeOH-H_2O (40:10:1). Each eluate was evaporated to dryness. All components thus obtained, except for D788-1 and D788-10, were dissolved in 0.1 M AcOH buffer (pH 3.0). After washed with $CHCl_3$ and adjusted pH to 8.5 with 1 N NaOH, the solution was extracted with $CHCl_3$. The $CHCl_3$ layer was washed with saturated saline and dried over anhydrous Na_2SO_4 and concentrated *in vacuo*. The addition of an excess of *n*-hexane gave all red powders. Whereas, D788-1 and D788-10 were dissolved in 1% $NaHCO_3$ and washed with $CHCl_3$. After pH adjustment to 2.5 with HCl, D788-1 solution were extracted with *n*-BuOH and D788-10 solution with $CHCl_3$. The solvent layers were evaporated to a small volume and an excess of *n*-hexane was added to precipitate pure compound.

All the compounds, structurally elucidated as described below, are summarized in Fig. 2.

Structural Determination

In total acid hydrolysis (0.1 N HCl, 85°C, 30 minutes) followed by TLC analysis, all these compounds gave reddish aglycones and a sugar. The sugar was identified as daunosamine by comparing Rf value (0.25) and spot color (sky blue) on TLC with authentic sample. The aglycones obtained from D788-6, D788-7 and D788-12 were ε-rhodomycinone (ε-RMN), β-RMN and 13-dihydrocarminomycinone, respectively, by TLC comparison with the authentic samples, and by mass[4] and ^1H and ^{13}C NMR spectra.[4~7] D788-1 and D788-10 were soluble in alkaline water and showed the presence of acidic group. ^1H NMR data evidenced that D788-1, D788-2, D788-7, D788-11 and D788-12 were known compounds

Fig. 2. Anthracycline metabolites produced by strain RPM-5.



Compound	Structure		Identified as	Remarks
	R_1	R_2		
D788-1	COOH	CH_2CH_3	10-Carboxy-13-deoxocarminomycin	Known[2]
D788-2[a]	—	CH_2CH_3	9,10-Anhydro-13-deoxocarminomycin	Known[8]
D788-6	$COOCH_3$	CH_2CH_3	10-Methoxycarbonyl-13-deoxocarminomycin	New
D788-7	OH	CH_2CH_3	10-Hydroxy-13-deoxocarminomycin (oxaunomycin)	Known[9]
D788-8[a]	—	$CH(OH)CH_3$	9,10-Anhydro-13-dihydrocarminomycin	New
D788-9	$COOCH_3$	CH_2COCH_3	10-Methoxycarbonyl-4-*O*-demethylfeudomycin B	New
D788-10	COOH	CH_3	10-Carboxy-4-*O*-demethylfeudomycin C	New
D788-11	H	CH_2CH_3	13-Deoxocarminomycin	Known[10]
D788-12	H	$CH(OH)CH_3$	13-Dihydrocarminomycin	Known[5]
D788-15	$COOCH_3$	CH_3	10-Methoxycarbonyl-4-*O*-demethylfeudomycin C	New

[a] With the above structure a.

Table 1. Physico-chemical properties of new compounds produced by strain RPM-5.

Compound	D788-6	D788-8	D788-9	D788-10	D788-15
Melting point (°C)	138~140 (dec)	126~128 (dec)	175~180 (dec)	174~176 (dec)	180~184 (dec)
$[\alpha]_D$ (0.01, MeOH)	+165°	+309°	+62°	+200°	+250°
UV and Vis:	206 (331),	206 (609),	206 (307),	209 (362),	234 (639),
λ_{max} nm ($E^{1\%}$)	235 (647),	227 (429),	235 (671),	226 (371),	254 (378),
in 90% MeOH	255 (403),	269 (619),	255 (428),	257 (329),	292 (131),
	290 (141),	494 (283),	291 (153),	497 (169)	492 (228)
	492 (229)	515 (300)	493 (226),		
			526 (146)		
IR (KBr) cm^{-1}	1720, 1590,	1600, 1460,	1725, 1600,	1710, 1600,	1732, 1605,
	1450, 1420,	1400, 1370,	1450, 1430,	1450, 1420,	1456, 1433,
	1390, 1280,	1285, 1250,	1400, 1290,	1390, 1280,	1402, 1290,
	1230, 1190,	1200, 1170,	1240, 1190,	1230, 1190,	1238, 1196,
	1160, 1115,	1110, 1015,	1165, 1110,	1160, 1115,	1165, 1123,
	1000, 980	980	1005, 980	1010, 980	1015, 986
FAB-MS	558	498	586	530	544
m/z $(M+H)^+$					
Molecular formula	$C_{28}H_{31}NO_{11}$	$C_{26}H_{27}NO_9$	$C_{29}H_{31}NO_{12}$	$C_{26}H_{27}NO_{11}$	$C_{27}H_{29}NO_{11}$
HPLC (Rt: minute)[a]	8.60	6.90	5.99	3.20	6.33
HPLC (Rt: minute)[b]	26.25	—	13.01	—	14.71
TLC (Rf)[c]	0.52	0.36	0.51	0.16	0.46

[a] 45% CH_3CN in 0.01 M $CmSO_3H$ (pH 4.3).
[b] 30% CH_3CN in dil H_3PO_4 (pH 2.0).
[c] $CHCl_3$-MeOH-H_2O-AcOH-aq NH_3 (120:55:5.5:0.9:1.1).

Table 2. ^1H NMR chemical shift assignments for new compounds produced by strain RPM-5.

Proton	D788-6 $CDCl_3$	D788-8 $CDCl_3+CD_3OD$	D788-9 $CDCl_3$	D788-10 CD_3OD	D788-15 $CDCl_3$
Aglycone moiety					
1-H	7.75 d (7.3)	7.86 d (7.7)	7.87 d (7.3)	7.75 br s	7.87 d (7.3)
2-H	7.62 t (8.1)	7.68 t (7.7)	7.70 t (8.1)	7.69 br s	7.70 t (7.3)
3-H	7.22 d (8.8)	7.29 d (7.7)	7.31 d (8.1)	7.25 br s	7.31 d (8.1)
7-H	5.21 br d (2.2)	5.28 br s	5.15 br d (2.9)	5.10 br d	5.25 br s
8-Ha	2.36 d (15.4)	2.80 d (18.7)	2.59 d (14.7)	2.37 dd (15.0, 4.3)	2.34 dd (14.7, 3.7)
8-Hb	2.20 dd (14.7, 3.7)	2.53 br dd	2.22 dd (14.7, 4.4)	2.23 d (15.0)	2.23 d (14.7)
10-H	4.25 s	7.08 br d (1.1)	4.40 s	4.17 s	4.26 s
9-CH_3	—	—	—	1.51 s	1.42 s
9-$\underline{CH}(OH)CH_3$	—	4.49 q (6.2)	—	—	—
9-$\underline{CH}(OH)\underline{CH_3}$	—	1.42 d (6.6)	—	—	—
9-$\underline{CH_2}CH_3$	1.85 m (7.3)	—	—	—	—
	1.45 m (7.3)				
9-$CH_2\underline{CH_3}$	1.12 t (7.5)	—	—	—	—
9-$\underline{CH_2}$-CO-CH_3	—	—	3.10 d (16.1)	—	—
			2.44 d (15.4)	—	
9-CH_2-CO-$\underline{CH_3}$	—	—	2.27 s	—	—
10-$COO\underline{CH_3}$	3.72 s	—	3.72 s	—	3.73 s
Daunosamine moiety					
1'-H	5.45 br d (2.2)	5.28 br s	5.46 br s	5.50 br s	5.48 br d (2.9)
2'-Ha	1.7~1.9	1.72 td (12.8, 3.7)	1.7~1.8	2.06 br t	1.78 td (13.2, 3.7)
2'-Hb		1.55 dd (13.2, 4.4)		1.95 br d	1.68 dd (13.2, 5.1)
3'-H	3.10 m	3.12 br d	3.15 m	3.60 br d (15.0)	3.10 br d (9.5)
4'-H	3.48 br s	3.47 br s	3.47 br s	3.67 br s	3.47 br s
5'-H	4.14 q (6.6)	4.01 q (6.2)	4.16 q (6.6)	4.30 q (6.2)	4.12 q (6.6)
6'-CH_3	1.34 d (6.6)	1.31 d (6.6)	1.36 d (6.6)	1.30 d (6.6)	1.35 d (6.6)

which were identified as 10-carboxy-13-deoxocarminomycin,[2] akrobomycin,[8] oxaunomycin,[9] 13-deoxocarminomycin[10] and 13-dihydrocarminomycin,[5] respectively. The other products D788-6, D788-8, D788-9, D788-10 and D788-15 were new. Their physico-chemical properties are shown in Table 1. Their UV and Vis spectra in 90% MeOH solution were similar to each other and the λ_{max} were observed all around 235, 255 and 495 nm. IR spectra (KBr) indicated that D788-6, D788-9 and D788-15 had an ester carbonyl (1720~1730 cm^{-1}) and D788-10 had a carbonyl group (1710 cm^{-1}). D788-8 had no peak at a similar wave length.

Their ^1H and ^{13}C NMR chemical shift assignments are shown in Tables 2 and 3, respectively. ^1H and ^{13}C chemical shifts of D788-6, D788-15, D788-9 and D788-10 were almost identical to each other except for those of the substituents at C-9 and C-10. The ^{13}C NMR chemical shifts of the aglycone

Table 3. ^{13}C NMR chemical shift assignments for new compounds produced by strain RPM-5.

Carbon	D788-6 $CDCl_3$	D788-8 $CDCl_3+CD_3OD$	D788-9 $CDCl_3$	D788-10 CD_3OD	D788-15 $CDCl_3$
Aglycone moiety					
1	119.50	119.41	119.61	120.51	119.73
2	136.91	136.58	137.03	138.37	137.16
3	124.70	124.51	124.86	125.78	124.95
4	162.42	162.26	162.73	163.63	162.82
4a	115.77	116.21	116.12	117.09	116.16
5	190.37	189.78	190.86	192.08	190.93
5a	111.35	112.23	111.55	112.52	111.73
6	156.74[a]	155.92	156.74[a]	157.53[a]	156.83[a]
6a	135.87	134.64	135.93	136.87[a]	135.79
7	70.97	64.49	70.35[a]	72.30	70.66
8	33.22	30.98	35.54	38.38	36.63
9	71.11	149.30	70.43[a]	69.22	69.43
10	52.04	112.23	51.53	53.95	52.81
10a	135.28	130.17	134.50	137.13[a]	135.24
11	156.79[a]	154.82	157.05[a]	158.00[a]	156.92[a]
11a	111.00	111.03	111.31	112.25	111.38
12	185.78	186.37	186.20	187.46	186.29
12a	133.13	133.44	133.48	134.63	133.53
9-CH_3	—	—	—	28.63	27.81
9-$\underline{CH_2}CH_3$	32.19	—	—	—	—
9-$\underline{CH_2}CH_3$	6.70	—	—	—	—
9-$\underline{CH}(OH)CH_3$	—	70.35	—	—	—
9-$\underline{CH}(OH)CH_3$	—	21.28	—	—	—
9-$\underline{CH_2}COCH_3$	—	—	50.86	—	—
9-$CH_2\underline{C}OCH_3$	—	—	207.68	—	—
9-CH_2COCH_3	—	—	32.27	—	—
10-COOH	—	—	—	173.89	—
10-$\underline{C}OOCH_3$	171.24	—	170.67	—	171.54
10-$COOCH_3$	52.35	—	52.35	—	52.50
Daunosamine moiety					
1′	101.60	97.63	101.52	101.44	101.33
2′	32.62	32.42	32.51	29.50	32.28
3′	46.26	46.22	46.38	49[b,c]	46.34
4′	70.51	69.74	70.75	67.93[c]	70.49
5′	67.14	66.80	69.01	67.93[c]	67.15
6′	16.85	16.79	16.94	16.91	16.95

[a] Similar values may be interchanged.
[b] The value is obscure because of solvent interference.
[c] Determined by ^1H-^{13}C COSY.

moiety of D788-6 were almost the same as those of ε-RMN though the chemical shift of C-7 shifted to a lower field because of the sugar linkage. The methoxycarbonyl signal at C-10 was also observed with D788-15 and D788-9. The methyl proton (δ 1.4~1.5, singlet) which displayed long range connectivities to C-8, C-9 and C-10 was observed with D788-15 and D788-10. The chemical shifts of the side chain at C-9 of D788-9 were the same as those detected in sulfurmycins,[11] indicating that C-9 was substituted with an acetonyl group. The carbonyl carbon of D788-10 was shifted to a lower field (δ 173.89) as seen with D788-1,[2] indicating the presence of a carboxyl group at C-10. Concerning D788-8, one additional proton (δ 7.08, br d, 10-H) observed in an olefin field, and a lower-field chemical shift (δ 4.49, q) of methine proton and a doublet signal at δ 1.42, correlated to the methine proton, showed that D788-8 was a 9,10-anhydroanthracycline as akrobomycin[8] and the adjacent carbon to C-9 was oxidized to possess a hydroxyl group. The chemical shifts of sugar moieties of all these compounds agreed with those of daunosamine moiety in daunorubicin. The small coupling constants ($J<2.9$ Hz) of all anomeric protons in these compounds indicated the configurations of the glycosidic bonds were α.

FAB-MS analysis supported their molecular formula which were given by ^{1}H and ^{13}C NMR analyses. These results proved new products to be 10-methoxycarbonyl-13-deoxocarminomycin (D788-6), 9,10-anhydro-13-dihydrocarminomycin (D788-8), 10-methoxycarbonyl-4-*O*-demethylfeudomycin B (D788-9), 10-carboxy-4-*O*-demethylfeududomycin C (D788-10) and 10-methoxycarbonyl-4-*O*-demethylfeudomycin C (D788-15).

Antitumor Activity *In Vitro*

Inhibitory activities of all anthracycline metabolites produced by strain RPM-5 on the growth and nucleic acid synthesis of murine leukemia L1210 cell culture was examined and the results are shown in Table 4. As already reported, D788-7 had an intensely potent cell growth inhibitory activity and a high

Table 4. Inhibitory activities of new compounds produced by strain RPM-5 against growth and nucleic acid synthesis of murine leukemia L1210 cell culture.[a]

Product	IC_{50} (μg/ml)		
	Growth	DNA	RNA
D788-1 (10-Carboxy-13-deoxocarminomycin)	0.03	>5.0	>5.0
D788-2 (9,10-Anhydro-13-deoxocarminomycin)	0.18	2.80	1.30
D788-6 (10-Methoxycarbonyl-13-deoxocarminomycin)	0.25	2.60	1.30
D788-7 (10-Hydroxy-13-deoxocarminomycin)	0.0003	0.29	0.25
D788-8 (9,10-Anhydro-13-dihydrocarminomycin)	0.22	3.40	1.58
D788-9 (10-Methoxycarbonyl-4-*O*-demethylfeudomycin B)	0.22	2.90	2.59
D788-10 (10-Carboxy-4-*O*-demethylfeudomycin C)	0.08	>5.0	>5.0
D788-11 (13-Deoxocarminomycin)	0.03	1.30	1.30
D788-12 (13-Dihydrocarminomycin)	0.06	1.20	1.20
D788-15 (10-Methoxycarbonyl-4-*O*-demethylfeudomycin C)	0.10	>5.0	>5.0
Daunorubicin[b]	0.02	0.42	0.16

[a] For the growth inhibition test, L1210 cell culture (5×10^4 cells/ml) were exposed for 48 hours to the drugs and the viable cells were counted by Coulter counter. In the inhibition test for nucleic acid syntheses, L1210 cell cultures (8×10^5 cells/ml) with supplemented ^{14}C-labeled uridine or thymidine (0.05 μCi/ml) were exposed for 60 minutes to the drugs and the incorporation of the radioisotopes into acid insoluble material was measured. Details of the method was previously described.[3]

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

[b] Reference compound.

antitumor effect of about 200 (T/C %) against mice bearing L1210 cells under ip-ip treatment with daily ten doses (optimum dose: 0.06 mg/kg).[9] D788-1, D788-11 and D788-12 were as active as daunorubicin against L1210 cell culture. However, the activities of all the new compounds were very weak and their antitumor effects *in vivo* were also less than 120 (T/C %) even in elevating dose more than 10 mg/kg.

Discussion

A HPLC of direct acetone extract from RPM-5 culture broth revealed only one major peak (corresponding to D788-1) and three minor peaks (corresponding to D788-6, D788-11 and D788-12). From their peak areas it was determined that about 95% of total anthracycline yields was D788-1 and the remaining 5% shared by the other components. This was also confirmed by a practical product isolation from 15-liter culture broth. A yield of D788-1 was about 2.5 g and total yields of the other nine components was about 0.45 g. These findings clearly show that strain RPM-5 is a biosynthetic mutant which has a genetic blockage at a biosynthetic step of 10-decarboxylation from D788-1 to a 10-decarboxy precursor metabolite in daunorubicin production. The nine minor components were accumulated as either by-products (or degraded compounds) or precursor metabolites. D788-2 was a chemically degraded product from D788-1 with acetone and identified as akrobomycin.[8] It seems that D788-7 (oxaunomycin),[9] and D788-8 were also chemical by-products while D788-9, D788-10 and D788-15 were biosynthetic by-products. D788-6, D788-11 and D788-12 are some precursor metabolites in daunorubicin biosynthesis. We have isolated a blocked mutant 58NR-58 whose major product is D788-6.[1] Details of this mutant will be described in the following paper.[12] We consider that D788-6 is the precursor of D788-1 and D788-1 the precursor of D788-11 in the biosynthetic pathway of daunorubicin. CASSINELLI *et al.* have reported that a mutant of daunorubicin-producing *Streptomyces peucetius* var *caecius* produces 13-deoxocarminomycin (D788-11). However, it is not shown whether it is a major or a minor product. Further evidence for the precursor activity of D788-11 would still need the isolation of a blocked mutant which accumulates it as a major product.

There were not biologically active compounds with a good antitumor effect among strain RPM-5 products except for D788-7. The marked antitumor activity *in vitro* and *in vivo* of D788-7 has been previously clarified.[9] The bioactivity of D788-1 was very interesting since it is the first anthracycline of acidic and water soluble property. However, it had only a little antitumor effect against L1210 cells although it exhibited a strong cell-growth inhibition activity comparative to that of daunorubicin. However, it is a important chemical since it was found that a photochemical treatment of D788-1 was a very efficient way for D788-7 production.[13]

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