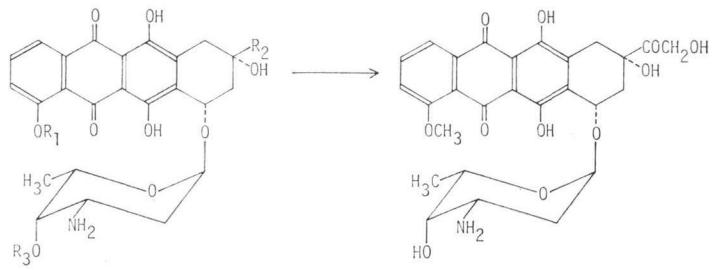
MICROBIAL CONVERSION OF DAUNOMYCIN, CARMINOMYCIN I AND FEUDOMYCIN A TO ADRIAMYCIN

Sir:

In the studies on biosynthesis of anthracycline antibiotics, we isolated from *Streptomyces peucetius* subsp. *caesius* ATCC27952 a blocked mutant which is unable to produce adriamycin (ADM), but can efficiently convert daunomycin (DM), 13-dihydrodaunomycin (DDM), carminomycin I (CM) and feudomycin A (FM)[1] to ADM. The microbial reduction of glycosidic bond at C-7 position and side chain carbonyl group at C-13 in anthracycline antibiotics is well known[2~6], but the oxygenation at C-14 position in DM, DDM, CM and FM has not yet been reported. We found that the mutant strain 2N-267 (ATCC 31847) which was derived from *S. peucetius* subsp. *caesius* by treatment with *N*-methyl-*N'*-nitro-*N*-nitrosoguanidine, was capable of converting DM, DDM, CM and FM to ADM, as shown in Table 1.

The mycelium for microbial conversion was prepared as follows: strain 2N-267 was inoculated into 100 ml of the medium consisting of 1.5% soluble starch, 1% glucose, 1% soy bean meal, 0.1% yeast extract, 0.1% K_2HPO_4, 0.1% $MgSO_4 \cdot 7H_2O$, 0.3% NaCl, 0.0007% $CuSO_4 \cdot 5H_2O$, 0.0001% $FeSO_4 \cdot 7H_2O$, 0.0008% $MnCl_2 \cdot 4H_2O$ and 0.0002% $ZnSO_4 \cdot 7H_2O$, pH 7.4, in a 500-ml Erlenmeyer flask. One milliliter of the seed culture, incubated for 2 days at 28°C under shaking at 210 rpm, was inoculated into 30 ml of the medium contained in a 250-ml Erlenmeyer flask. Mycelial conversion was carried out by cultivation at 28°C for 4 days under shaking (230 rpm)

Table 1. Bioconversion of various anthracyclines by *Streptomyces peucetius* subsp. *caesius* mutant 2N-267.



	Substrates	R_1	R_2	R_3	Products
Glycosides	Daunomycin	CH_3	$COCH_3$	H	Adriamycin (ADM), Dihydrodaunomycin (DDM)
	Dihydrodaunomycin	CH_3	$CHOHCH_3$	H	ADM, DM
	4'-*O*-THP-daunomycin	CH_3	$COCH_3$		4'-*O*-THP-DDM
	Feudomycin A	CH_3	CH_2CH_3	H	ADM, DM, DDM
	Carminomycin I	H	$COCH_3$	H	ADM, DM, DDM
	Feudomycin B	CH_3	CH_2COCH_3	H	No change
	Baumycin A1	CH_3	$COCH_3$		No change
	4-Hydroxybaumycin A1	H	$COCH_3$	〃	No change
Aglycones	Daunomycinone	CH_3	$COCH_3$	No sugar	Dihydrodaunomycinone
	Dihydrodaunomycinone	CH_3	$CHOHCH_3$	〃	No change
	Feudomycinone A	CH_3	CH_2CH_3	〃	No change
	Adriamycinone	CH_3	$COCH_2OH$	〃	Dihydroadriamycinone

in a medium consisting of 5% sucrose, 2% soy bean meal, 0.2% yeast extract, 0.2% corn steep liquor, 0.1% K_2HPO_4, 0.05% $MgSO_4 \cdot 7H_2O$, 0.2% $CaCO_3$, 0.001% $CuSO_4 \cdot 5H_2O$, 0.0002% $FeSO_4 \cdot 7H_2O$, 0.0013% $MnCl_2 \cdot H_2O$, and 0.0003% $ZnSO_4 \cdot 7H_2O$; pH 7.4. The mycelium, harvested from the cultured broth (1 liter) of strain 2N-267, was treated with lytic enzyme, washed with 5 mM $MgCl_2$ and suspended in 5 mM $MgCl_2$ (1 liter). The mycelial suspension (30 ml) was placed in a 250-ml Erlenmeyer flask, and DM, DDM, CM or FM was added at the final concentration of 10 μg/ml. After shaking, aerobically for 24 hours, 5 ml of the aliquot of the reaction mixture was extracted twice with 5 ml of methanol - chloroform mixture (2: 3, v/v) and the combined extracts were evaporated to dryness. The residue was dissolved in 200 μl of chloroform, and a portion of the solution was subjected to thin-layer chromatography (Kieselgel $60F_{254}$, E. Merck & Co.) using the solvent system $CHCl_3$ - methanol - AcOH - H_2O (75: 25: 5: 5 in volume). The other portion was hydrolyzed in 1 ml of 0.3 N HCl at 85°C for 30 minutes and added to 200 μl of toluene. Aglycones in the toluene layer were examined by TLC using the solvent system benzene - acetone - formic acid(100: 30: 1 in volume). The products on TLC were quantitated by a Shimadzu-TLC scanner model CS-910. ADM and adriamycinone were identified by direct TLC comparison with authentic samples using several solvent systems. Fifteen to 20 mg of ADM was extracted and purified by preparative LC from the reaction mixture, in which 125 mg each of DM, DDM, CM or FM was used as a substrate. ADM produced was also identified by IR, PMR and CMR.

Aeration was required for conversion of DM, DDM, CM or FM to ADM. Incubation without shaking did not produce ADM, but reduced substrates to 7-deoxyaglycones, such as 7-deoxydaunomycinone, 7-deoxycarminomycinone, 7-deoxyfeudomycinone *etc.* The optimal pH in 0.1 M tris-HCl buffer, incubation time and temperature were 8.5, 20~24 hours and 28~30°C, respectively. The best substrate concentration of DM, DDM, CM and FM for conversion was 10 μg/ml. One to 10 mM of $MgCl_2$ and 0.1 to 0.5 mg/ml of lytic enzyme No. 2 (Kyowa Hakko Kogyo, Tokyo) were optimal for treatment of mycelia. The addition of 5 mM of $CaCl_2$, NaCl and KCl, 0.1 mM of dithiothreitol and 2-mercaptoethanol, 1 mM of NADPH, NADH and NAD^+ were not effective for the conversion reaction. The maximum conversion rate was 60% of substrate when the incubation was performed under the optimum conditions described above. On the other hand, only 20% conversion was obtained in the absence of $MgCl_2$ and lytic enzyme; and both treatments ($MgCl_2$ and the lytic enzyme) increased the conversion rate 3-fold. It was suggested that the effect of $MgCl_2$ and the lytic enzyme was due to the formation and stabilization of spheroplasts[7] and the increased transport of substrates into the mycelium. Feudomycin B, baumycin Al, 4-hydroxybaumycin Al, 4′-*O*-tetrahydropyranyldaunomycin, daunomycinone, feudomycinone, carminomycinone and dihydrodaunomycinone were not converted to ADM and adriamycinone, as shown in Table 1.

The hydroxyl group at C-4 of CM was converted to the methoxyl group. The carbon at C-13 of DDM and FM was oxidized to the carbonyl group. These facts indicate the presence of specific enzymes for the conversion of DM, DDM, CM and FM to ADM.

As previously reported[8,9], we found that DM was biosynthesized from aklavinone or ε-rhodomycinone in *S. coeruleorubidus*, and that FM and CM were converted to DM, while daunomycinone and related aglycones were not glycosidated. We propose a biosynthetic pathway where DM is produced by 10-demethoxycarbonylation, 4-*O*-methylation and oxidation at C-13 of the intermediate glycosides *via* ε-rhodomycinone from aklavinone. The direct glycosidation of adriamycinone has not been observed in *S. peucetius* subsp. *caesius* and *S. coeruleorubidus*, but DM and related glycosides were oxidized to ADM.

Toshikazu Oki
Yukio Takatsuki
Hiroyasu Tobe
Akihiro Yoshimoto
Central Research Laboratories,
Sanraku-Ocean Co., Ltd.
Fujisawa, Kanagawa 251, Japan

Tomio Takeuchi
Hamao Umezawa
Institute of Microbial Chemistry,
14–23 Kamiosaki 3-chome,
Sinagawa-ku, Tokyo 141, Japan

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