Notes

MICROBIAL CONVERSION OF ANTHRACYCLINE ANTIBIOTICS III. GLYCOSIDATION OF NATURAL AND CHEMICALLY SYNTHESIZED ANTHRACYCLINE AGLYCONES

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Microbial glycosidation of anthracycline aglycones is a useful process to develop new anthracycline antibiotics. OKI *et al.*[1,2] reported several new anthracycline antibiotics prepared by glycosidation of naturally occurring anthracycline aglycones: 11-hydroxyaclacinomycin A, 11-hydroxycinerubin A, 10-decarbomethoxy-10,11-dihydroxyaclacinomycin A and 4‴-dehydrorhodomycin Y. We isolated a pigment-negative strain of *Streptomyces galilaeus* OBB-111-848, which was derived from an auramycins/sulfurmycins/aclacinomycins producer, OBB-111, and was capable of glycosidating anthracycline aglycones. In this report we describe microbial glycosidation of natural and chemically synthesized (racemic) anthracycline aglycones.

S. galilaeus OBB-111-848 was used all through this study. The medium used for glycosidation consisted of 2% glucose, 2% soluble starch, 1% Pharmamedia (Traders Oil Mill), 0.3% NaCl and 0.3% $CaCO_3$. 5 ml of the seed culture was inoculated into 100 ml of fresh medium and incubated at 27°C on a rotary shaker. 5 or 10 mg of an anthracycline aglycone dissolved in dimethyl sulfoxide was added after an appropriate incubation period and the flask was further incubated for glycosidation. Natural anthracycline aglycones (Table 1) were prepared by acid hydrolysis of the corresponding anthracycline with 0.1 N HCl at 95°C for 60 minutes: auramycinone from auramycins[3], sulfurmycinone from sulfurmycins[3], aklavinone from aclacinomycins[4], 1-hydroxyauramycinone from 1-hydroxyauramycins[5], 1-hydroxysulfurmycinone from 1-hydroxysulfurmycins[5], ε-pyrromycinone from cinerubins[6], aranciamycinone from aranciamycin[7] and daunomycinone from daunomycin[8] (Farmitalia S.A., Milan). 11-Hydroxyauramycinone and 11-hydroxysulfurmycinone were prepared by microbial conversion of auramycinone and sulfurmycinone, respectively, by *S. coeruleorubidus* ATCC 31276, a baumycins producer[9,10].

Chemically synthesized anthracycline aglycones, namely, Ro 22-7287, Ro 22-8035, Ro 22-8156, Ro 22-8190, Ro 22-8287, Ro 22-8507 and aklavinone[11] were prepared by Dr. M. USKOKOVIC, Hoffmann-La Roche, Nutley, U.S.A. Chemically synthesized aglycones are racemic.

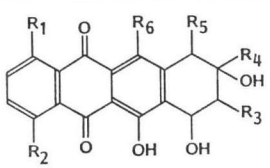
The natural anthracycline aglycones as listed in Table 1 were subjected to glycosidation by *S. galilaeus* OBB-111-848. 5 mg each of the aglycones was added to the culture of 100 ml and glycosidation proceeded for 3 days at 27°C on a rotary shaker. The whole culture was extracted with a solvent mixture of $CHCl_3$ and MeOH (1: 1). The chloroform layer was recovered, concentrated *in vacuo* and the concentrate was analyzed on TLC (Silica gel plate $60F_{254}$, E. Merck & Co.) developed by a solvent of toluene - MeOH (10: 1). Glycosidation products were compared with authentic samples with respect to Rf values and fragmentation patterns in mass spectrum.

Among the natural anthracycline aglycones, auramycinone, sulfurmycinone, aklavinone, 1-hydroxyauramycinone, 1-hydroxysulfurmycinone, ε-pyrromycinone, 11-hydroxyauramycinone and 11-hydroxysulfurmycinone were glycosidated, but aranciamycinone and daunomycinone were not, as shown in Table 1. This result indicates that hydroxyl group attached to anthraquinone did not affect the glycosidation ability of *S. galilaeus* OBB-111-848. The finding that daunomycinone was reduced to dihydrodaunomycinone by this strain was consistent with that observed by OKI *et al.*[1] using mutant strain KE303, which was a blocked mutant of *S. galilaeus* MA144-M1.

Six chemically synthesized aglycones with modifications at the saturated ring of aklavinone and racemic aklavinone were tested for glycosida-

Table 1. Bioconversion of natural anthracycline aglycones by *S. galilaeus* OBB-111-848.

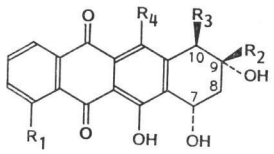
Aglycone	Structure*						Rf value**	Conversion product	Rf value**
	R_1	R_2	R_3	R_4	R_5	R_6			
Auramycinone	H	OH	H	CH_3	$COOCH_3$	H	0.35	7-Deoxyauramycinone	0.60
								Auramycin B	0.34
								Auramycin A	0.25
Sulfurmycinone	H	OH	H	CH_2COCH_3	$COOCH_3$	H	0.33	7-Deoxysulfurmycinone	0.75
								Sulfurmycin B	0.32
								Sulfurmycin A	0.21
Aklavinone	H	OH	H	CH_2CH_3	$COOCH_3$	H	0.51	7-Deoxyaklavinone	0.75
								Aclacinomycin B	0.40
								Aclacinomycin A	0.30
1-Hydroxyauramycinone	OH	OH	H	CH_3	$COOCH_3$	H	0.35	7-Deoxy-1-hydroxyauramycinone	0.60
								1-Hydroxyauramycin B	0.34
								1-Hydroxyauramycin A	0.25
1-Hydroxysulfurmycinone	OH	OH	H	CH_2COCH_3	$COOCH_3$	H	0.33	7-Deoxy-1-hydroxysulfurmycinone	0.75
								1-Hydroxysulfurmycin B	0.32
								1-Hydroxysulfurmycin A	0.21
ε-Pyrromycinone	OH	OH	H	CH_2CH_3	$COOCH_3$	H	0.51	ζ-Pyrromycinone	0.75
								Cinerubin B	0.40
								Cinerubin A	0.30
11-Hydroxyauramycinone	H	OH	H	CH_3	$COOCH_3$	OH	0.35	7-Deoxy-11-hydroxyauramycinone	0.60
								11-Hydroxyauramycin B	0.34
								11-Hydroxyauramycin A	0.25
11-Hydroxysulfurmycinone	H	OH	H	CH_2COCH_3	$COOCH_3$	OH	0.33	7-Deoxy-11-hydroxysulfurmycinone	0.75
								11-Hydroxysulfurmycin B	0.32
								11-Hydroxysulfurmycin A	0.21
Aranciamycinone	H	OH	OCH_3	CH_3	O	H	0.28	Aglycone (unidentified)	0.22
Daunomycinone	H	OCH_3	H	$COCH_3$	H	OH	0.31	13-Dihydrodaunomycinone	0.10

* 

** Solvent system: toluene - MeOH, 10: 1 (Silica gel plate, $60F_{254}$, Merck).

Table 2. Bioconversion of chemically synthesized anthracycline aglycones by *S. galilaeus* OBB-111-848.

Aglycone	Structure*				Rf value**	Conversion product	Rf value**
	R_1	R_2	R_3	R_4			
Ro 22-7287	H	CH_3	$OCOCH_3$	OH	0.32	Aglycone (unidentified)	0.22
Ro 22-8035	H	CH_3	$SCOCH_3$	OH	0.38	Aglycones (unidentified)	0.43, 0.33, 0.32, 0.27, 0.23, 0.18
Ro 22-8156	H	CH_3	Cl	OH	0.47	Aglycones (unidentified)	0.20, 0.17
Ro 22-8190	H	CH_3	OCH_3	OH	0.42	Unchanged	
Ro 22-8287	H	CH_3	9,10 Epoxy	OH	0.76	Aglycone (unidentified)	0.17
Ro 22-8507	H	CH_2CH_3	$OCOCH_3$	H	0.40	Glycoside (unidentified)	0.18
						Aglycones (unidentified)	0.28, 0.27
dl-Aklavinone	OH	CH_2CH_3	$COOCH_3$	H	0.51	Glycoside II	0.40
						Glycoside I	0.30

* 

** The same as in Table 1.

tion. 10 mg of each aglycone was added to the culture and products were analyzed after 3 days. No glycoside product was obtained in case of Ro 22-7287, Ro 22-8035, Ro 22-8156, Ro 22-8190 and Ro 22-8287, whereas Ro 22-8507 gave a minute quantity of a glycoside with the Rf value of 0.18 (Table 2). Racemic aklavinone was glycosidated to give two glycosides, designated glycosides I and II, leaving unglycosidated aglycone.

These results indicated that the functional group of R_2 and R_3 were related to the substrate specificity for glycosidation by *S. galilaeus* OBB-111-848.

As described above, racemic aklavinone yielded two glycosides besides an unglycosidated aglycone upon glycosidation. In order to determine their structures, especially stereochemistry of sugar attachment, the three compounds were isolated and characterized.

10 mg of racemic aklavinone was added to each of 10 flasks and glycosidated. The culture broth thus obtained was centrifuged at 8,000 rpm for 10 minutes to separate the cells from the supernatant. The cells were extracted with 1 liter of the solvent mixture of $CHCl_3$ and MeOH (1: 1), to which 500 ml of H_2O was added to separate $CHCl_3$ layer. The supernatant, on the other hand, was extracted with equal volume of $CHCl_3$. The extracts were combined, evaporated to dryness and dissolved in a small amount of $CHCl_3$. Purification was carried out by preparative TLC (Silica gel plate $60F_{254}$, E. Merck & Co., toluene - MeOH, 10: 1), whereby there were obtained 6 mg of glycoside I, 5.7 mg of glycoside II and 35 mg of residual aglycone.

Physico-chemical properties of these compounds are shown in Table 3. By acid hydrolysis, glycoside I released L-rhodosamine, 2-deoxy-L-fucose and cinerulose A and glycoside II gave L-rhodosamine, 2-deoxy-L-fucose and cinerulose B. From the $[\alpha]_D$ values, UV and visible absorption spectra and chromatographic behavior, glycosides I and II were identified as aclacinomycins A and B, respectively.

On the other hand, the unglycosidated aglycone was optically active showing the $[\alpha]_D$ value of $-119°$. $[\alpha]_D$ value of natural aklavinone which has 7*S*, 9*R*, 10*R* configuration was $+143°$. From this observation, it was concluded that *S. galilaeus* OBB-111-848 glycosidated racemic aklavinone stereoselectively, introducing sugars into only the natural (7*S*, 9*R*, 10*R*)-aklavinone.

Acknowledgment

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Table 3. Physico-chemical properties of conversion products.

Analysis	Product		
	Glycoside I	Glycoside II	Unglycosidated aglycone
Substrate	Racemic aklavinone	Racemic aklavinone	Racemic aklavinone
Molecular formula	$C_{42}H_{53}O_{15}N$	$C_{42}H_{51}O_{15}N$	$C_{22}H_{20}O_8$
MP	151°C	160°C	nd*
$[\alpha]_D$ (c 0.1, $CHCl_3$)	−10°	+5°	−119°
Rf value (TLC)			
$CHCl_3$ - MeOH, 19: 1	0.27	0.55	0.70
Toluene - MeOH, 9: 1	0.25	0.38	0.46
UV λ_{max} ($E^{1\%}_{1cm}$) in MeOH	229 (540), 258 (325), 290 (sh, 130), 433 (160)	229 (510), 258 (310), 290 (135), 433 (165)	229 (980), 258 (650), 290 (250), 433 (320)
IR $\nu^{cm^{-1}}_{max}$ (KBr)	1740, 1680, 1630, 1580	1730, 1680, 1620, 1580	1730, 1680, 1625, 1580
Identification	Aclacinomycin A	Aclacinomycin B	7*R*,9*S*,10*S*-Aklavinone

* not determined.

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