BIOSYNTHESIS OF ANTHRACYCLINE ANTIBIOTICS BY *STREPTOMYCES GALILAEUS*

I. GLYCOSIDATION OF VARIOUS ANTHRACYCLINONES BY AN ACLACINOMYCIN-NEGATIVE MUTANT AND BIOSYNTHESIS OF ACLACINOMYCINS FROM AKLAVINONE

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An aclacinomycin-negative mutant strain KE303 which required aklavinone aglycone for the production of anthracycline antibiotics was derived from *Streptomyces galilaeus*, and employed for the glycosidation of various anthracyclinones. ε-, γ- and β-Rhodomycinones, ε-isorhodomycinone, ε- and β-pyrromycinones and chemically modified aklavinones were found to be glycosidated to the biologically active anthracyclines, when they were fed to the growing culture. However, the feeding of daunomycinone, 13-deoxydaunomycinone, adriamycinone and steffimycinone did not yield any glycoside. The bioconversion of presumptive precursor glycosides revealed that aclacinomycin A is biosynthesized by the step-wise glycosidation from aklavinone *via* aklavin and MA144 S1.

Microbial conversion of biologically inactive anthracyclinones to active anthracyclines is an important approach in the search for new anthracyclines with higher therapeutic index. We have isolated an aclacinomycin-negative mutant capable of producing anthracycline antibiotic aclacinomycins[1,2] by feeding aklavinone aglycone to the growing culture, and attempted to use it for the aim of obtaining new anthracyclines from various anthracyclinones.

The creation of biologically active anthracyclines by microbial conversion has not been reported yet, although some papers have dealt with the microbial conversions toward biologically less active compounds, such as reductive deglycosidation[3,4] or reduction of the side chain[3,5,6]. In this paper we describe the microbial glycosidation of natural and chemically modified anthracyclinones by an aclacinomycin-negative mutant of *Streptomyces galilaeus* MA144-M1 and the preliminary characterization of the new anthracyclines thus obtained. The biosynthetic pathway to aclacinomycin A by a step-wise glycosidation from aklavinone is also indicated.

Materials and Methods

Microorganism

Spores (5×10^8 cells/ml) of *Streptomyces galilaeus* MA144-M1, strain 6U-21 (aclacinomycin A producer)[1] were grown on YS agar (0.3% yeast extract, 1% soluble starch and 1.5% agar, pH 7.2) and the resultant cells were treated with N-methyl-N′-nitro-N-nitrosoguanidine at 1 mg/ml in 0.2 M Tris-HCl buffer (pH 8.5) under shaking at 28°C for 60 minutes (killing rate 99.85%), plated on YS agar after dilution, and incubated for 5 days at 28°C. About 400 colonies were cultured on YS slant and

tested for their ability to produce aclacinomycin A and related pigmental compounds by shaking for 3 days at 28°C in the fermentation medium as described below. The antibiotic-negative strains which had lost the ability to produce an acetone-extractable pigment in mycelium were thereafter examined for their capability in producing aclacinomycin A in media to which aklavinone was added. An aclacinomycin-negative mutant KE303 was thus selected and was used throughout the microbial conversion experiments in this paper.

Anthracyclinones and anthracyclines as precursors

Anthracyclinones (Table 1) were prepared by the acid hydrolysis of the corresponding anthracyclinone glycosides with 0.1 N HCl at 85°C for 30 minutes as follows: Aklavinone from aclacinomycins; 10-decarbomethoxyaklavinone and 4-O-methylaklavinone from 10-decarbomethoxyaclacinomycin A and 4-O-methylaclacinomycin A, respectively[7], ε-pyrromycinone from rhodirubins[8]; γ-rhodomycinone, β-rhodomycinone and β-pyrromycinone from an anthracycline mixture produced by *Actinomyces roseoviolaceus* A529 (IFO 13081)[9]; daunomycinone from baumycins[10,11]; adriamycinone from adriamycin (Farmitalia S. A, Milan); carminomycinone from carminomycin I (Bristol Laboratories, Syracuse). Steffimycinone was prepared by 128-hour refluxing of steffimycin (Upjohn Co., Kalamazoo) in 1 N HCl-MeOH[12]. 7-Deoxyaklavinone was produced by fermentation of *S. galilaeus* MA144-M1[2]. ε-Rhodomycinone and ε-isorhodomycinone were obtained from the cultured broth of blocked mutants of *S. coeruleorubidus* ME130-A4 and *A. roseoviolaceus* A529, respectively (unpublished data). 13-Deoxydaunomycinone was isolated from acid hydrolysate of the culture of a blocked mutant 4N-140 of *S. coeruleorubidus* ME130-A4 (unpublished data). Dihydrodaunomycinone, dihydroadriamycinone and their 7-deoxy compounds were prepared by microbial conversion according to the method of MARCHALL *et al.*[3].

MA144 S1, L1, N1 and aklavin (1-deoxypyrromycin) were aclacinomycin analogs[2]. MA144 U5 was a product of a blocked mutant 9U-653 of *S. galilaeus* MA144-M1[13]. MA144 KH was obtained by methanolysis of MA144 K1, which was prepared from aclacinomycin A by N, N-didemethylation with photochemical treatment[2]. These anthracyclines are listed in Table 2 with their chromatographic properties.

Microbial conversion

In experiments of the microbial conversion of anthracyclinones and anthracyclines, *S. galilaeus* MA144-M1, strain KE303 was inoculated into a 500-ml Erlenmeyer flask containing 100 ml of seed medium (1.5% soluble starch, 1% glucose, 1% soy bean meal, 0.1% yeast extract, 0.1% $K_2HPO_4 \cdot 7H_2O$, 0.3% NaCl) and cultured at 28°C for 2 days on a rotary shaker (210 rpm). The inoculum (1 ml) thus prepared was added to a 500-ml Erlenmeyer flask containing 50 ml of the fermentation medium: 1.5% soluble starch, 1% glucose, 3% soybean meal, 0.1% yeast extract, 0.1% K_2HPO_4, 0.1% $MgSO_4 \cdot 7H_2O$, 0.3% NaCl, 0.007% $CuSO_4 \cdot 5H_2O$, 0.001% $FeSO_4 \cdot 7H_2O$, 0.0008% $MnCl_2 \cdot 4H_2O$ and 0.0002% $ZnSO_4 \cdot 7H_2O$, pH 7.4. Fermentation was performed at 28°C on a rotary shaker (210 rpm). After 20-hour cultivation, one ml of a methanol solution of aglycone (500 μg/ml) was added to a flask and the cultivation was further continued for 24 hours. The preliminary test of microbial conversion was carried out using five Erlenmeyer flasks for each substrate aglycone. The culture broth (200 ml) thus prepared was mixed with 100 ml of the $CHCl_3$ - MeOH (3: 2, v/v) mixture by vigorously shaking. The $CHCl_3$ layer was evaporated to dryness, and the residue was chromatographed on preparative silica gel plates (60 PF_{254}, E. Merck Co.) using a solvent of $CHCl_3$ - MeOH (20: 1, v/v or 10: 1, v/v). The pigment bands on the chromatogram were scratched and extracted with the $CHCl_3$ - MeOH (5: 1, v/v) mixture. After evaporation the Rf values of the resulting compounds were compared with those of aglycones by TLC using a solvent of $CHCl_3$ - MeOH (20: 1, v/v) before and after acid hydrolysis, in order to see if they were glycosidated, or either unconverted or modified. Identification of the products thus produced was carried out by co-chromatography with authentic compounds in three solvent systems and mass spectrometry (Hitachi RMU-6 mass spectrometer) as shown in Table 1.

Isolation and purification of glycosidic conversion products

The microbial glycosidation was carried out using 250 flasks (total aglycone added: 125 mg) for each aglycone. The cultured broth thus obtained was centrifuged and the conversion products were

extracted from mycelial pellet with two liters of acetone. The acetone extract was concentrated *in vacuo* and extracted with one liter of chloroform. After concentration *in vacuo*, the crude pigment was dissolved in 40 ml of methanol and the insoluble material was centrifuged off. The supernatant was subjected to a Sephadex LH-20 column ($40 \times \phi$ 3.5 cm) chromatography and eluted with methanol. The eluate of the first pigment band containing the glycosidic products was pooled and concentrated *in vacuo* to dryness. The residue was then chromatographed on preparative silica gel plate using a solvent of $CHCl_3$ - MeOH (20: 1, v/v). The compound in a main band was eluted with the $CHCl_3$ - MeOH (5: 1, v/v) mixture and obtained as 20~60 mg powder by precipitation with excess of *n*-hexane after concentration. Further purification was performed by extraction with 0.1 M acetate buffer (pH 3.0) containing 2 mM EDTA from the toluene solution (10 ml). The aqueous layer was then extracted with about 20 ml of toluene after adjusting pH at 7.0 with 4 N NaOH. The toluene layer was washed with water and dried over anhydrous Na_2SO_4. The glycosidic product was precipitated by addition of excess *n*-hexane after concentration *in vacuo*. The microcrystalline powder yield from 125 mg of substrate aglycone was approximately 40 mg from aklavinone, 31 mg from 10-decarbomethoxyaklavinone, 24 mg from 4-O-methylaklavinone, 38 mg from ε-pyrromycinone, 12 mg from β-pyrromycinone, 27 mg from β-rhodomycinone, 10 mg from γ-rhodomycinone, 31 mg from ε-rhodomycinone and 12 mg from ε-isorhodomycinone.

Analyticals

Thin-layer chromatography was carried out on silica gel plate 60 F_{254} (E. Merck & Co.). The solvent systems used were: S1, $CHCl_3$ - MeOH (20: 1, v/v); S2, $CHCl_3$ - MeOH (10: 1, v/v); S3, $CHCl_3$ - MeOH - HCOOH (100: 10: 1, v/v/v); S4, benzene - acetone - HCOOH (100: 30: 1, v/v/v); S5, benzene - ethylacetate - MeOH - HCOOH - H_2O (5: 5: 1.5: 0.2, v/v/v/v); S6, $CHCl_3$ - MeOH - NH_4OH (90: 10: 0.2, v/v/v) and S7, benzene - ethylacetate - MeOH - 0.1 N HCl (5: 5: 1.5: 0.15, v/v/v/v).

Acid hydrolysis of the conversion product was performed with 0.1 N HCl at 85°C for 30 minutes. The hydrolysate was extracted with $CHCl_3$ to remove aglycone fraction. The aqueous layer was neutralized with $AgCO_3$ and the precipitate was centrifuged off. The supernatant was concentrated *in vacuo* and chromatographed on silica gel plate with a solvent of *n*-butanol - acetic acid - water (4: 1: 1, v/v/v). The sugars were detected by spraying *p*-anisaldehyde and identified by Rf value and color as described in previous paper[2].

Results

Microbial Conversion of Various Anthracyclinones

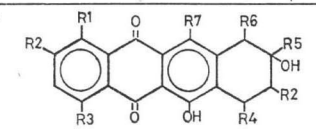
The microbial conversion of various anthracyclinones by mutant strain KE303 gave the results as shown in Table 1. This indicates that 10-decarbomethoxyaklavinone, 4-O-methylaklavinone, ε- and β-pyrromycinones, ε-, γ- and β-rhodomycinones, and ε-isorhodomycinone as well as aklavinone can be glycosidated, and these glycosides were confirmed by TLC analysis of the acid hydrolysates to be formed through the direct glycosidation of the aglycone added. On the other hand, no glycosidic product was obtained by the bioconversion of daunomycinone, 13-deoxydaunomycinone, adriamycinone and steffimycinone, whereas carminomycinone gave glycosidic products slightly. The unglycosidated aglycones were mostly reduced, that is, to 13-dihydrodaunomycinone and 7-deoxy-13-dihydrodaunomycinone from daunomycinone or to steffimycinol from steffimycinone. 7-Deoxyaklavinone and 13-deoxydaunomycinone was not significantly changed.

Anthracycline Glycosides Obtained by Microbial Conversion

In those cases where aglycones were converted to anthracycline glycosides by the microbial conversion with the mutant strain KE303, a large-scale feeding culture was carried out to obtain sufficient quantities of glycosidic products. Products CG1, 2, 3, 4, 5, 6, 7, 8 and 9 were isolated from aklavinone, 10-decarbomethoxyaklavinone, 4-O-methylaklavinone, ε-pyrromycinone, ε-rhodomycinone, ε-isorhodo-

Table 1. Bioconversion of various anthracyclinones by *S. galilaeus* MA144-M 1, strain KE 303, their structures and chromatographic properties.

Anthracyclinone	Structure*							Rf value**			*m/z* (M^+)	Conversion product	Rf value**	*m/z* (M^+)
	R1	R2	R3	R4	R5	R6	R7	S1	S4	S7			S1	
Aklavinone	H	H	OH	OH	CH_2CH_3	$COOCH_3$	H	0.67	0.67	0.81	412	glycoside (CG1)	0.49	
												〃	0.61	
10-Decarbomethoxyaklavinone	H	H	OH	OH	CH_2CH_3	H	H	0.55	0.51	0.75	354	〃 (CG2)	0.36	
4-O-Methylaklavinone	H	H	OCH_3	OH	CH_2CH_3	$COOCH_3$	H	0.64	0.43	0.70	426	〃 (CG3)	0.38	
7-Deoxyaklavinone	H	H	OH	H	CH_2CH_3	$COOCH_3$	H	0.70	0.72	0.80	396	unchanged	0.70	396
ε-Pyrromycinone	OH	H	OH	OH	CH_2CH_3	$COOCH_3$	H	0.65	0.62	0.78	428	glycoside (CG4)	0.48	
												〃	0.61	
ε-Rhodomycinone	H	H	OH	OH	CH_2CH_3	$COOCH_3$	OH	0.66	0.65	0.78	428	〃 (CG5)	0.53	
												〃	0.63	
ε-Isorhodomycinone	OH	H	OH	OH	CH_2CH_3	$COOCH_3$	OH	0.65	0.64	0.77	444	〃 (CG6)	0.51	
β-Rhodomycinone	H	H	OH	OH	CH_2CH_3	OH	OH	0.44	0.50	0.70	386	〃 (CG7)	0.28	
γ-Rhodomycinone	H	H	OH	H	CH_2CH_3	OH	OH	0.53	0.66	0.77	370	〃 (CG8)	0.31	
												〃	0.13	
β-Pyrromycinone	OH	H	OH	OH	CH_2CH_3	OH	H	0.32	0.42	0.65	386	〃 (CG9)	0.12	
Daunomycinone	H	H	OCH_3	OH	$COCH_3$	H	OH	0.59	0.32	0.66	398	dihydrodaunomycinone	0.25	400
												7-deoxydihydrodaunomycinone	0.33	384
Dihydrodaunomycinone	H	H	OCH_3	OH	$CHOHCH_3$	H	OH	0.25	0.11	0.43	400	〃	〃	
7-Deoxydihydrodaunomycinone	H	H	OCH_3	H	$CHOHCH_3$	H	OH	0.33	0.26	0.73	384	unchanged	0.33	384
13-Deoxydaunomycinone	H	H	OCH_3	OH	CH_2CH_3	H	OH	0.58	0.32	0.69	384	〃	0.58	384
Carminomycinone	H	H	OH	OH	$COCH_3$	H	OH	0.66	0.67	0.79	384	dihydrocarminomycinone	0.34	386
												glycoside (unidentified)	0.03	
Dihydrocarminomycinone	H	H	OH	OH	$CHOHCH_3$	H	OH	0.34	0.40	0.52	386	not tested		
Adriamycinone	H	H	OCH_3	OH	$COCH_2OH$	H	OH	0.32	0.16	0.52	414	dihydroadriamycinone	0.06	416
Dihydroadriamycinone	H	H	OCH_3	OH	$CHOHCH_2OH$	H	OH	0.06	0.02	0.28	416	not tested		
7-Deoxydihydroadriamycinone	H	H	OCH_3	H	$CHOHCH_2OH$	H	OH	0.33	0.26	0.73	400	〃		
Steffimycinone	H	OCH_3	OH	OH	CH_3	O	H	0.48	0.39	0.65	384	steffimycinol	0.38	386
Steffimycinol	H	OCH_3	OH	OH	CH_3	OH	H	0.38	0.28	0.60	386	not tested		

* 

** Solvent systems and thin-layer are described in the text.

mycinone, β-rhodomycinone, γ-rhodomycinone and β-pyrromycinone, respectively, by preparative silica gel thin-layer chromatography. Their physicochemical properties are shown in Table 3. It was found that products CG1 to 9 contain three types of sugars, L-rhodosamine, 2-deoxy-L-fucose and L-cinerulose A. In their UV and visible absorption spectra and the chromatographic behavior, CG1 and CG4 were identical to aclacinomycin A and cinerubin A, respectively, which are major anthracycline metabolites of the parental strain MA144-M1[2,13], and CG2 and CG3 also corresponded with 10-decarbomethoxy-aclacinomycin A and 4-O-methylaclacinomycin A, respectively[7]. Because of the similarity in the sugar components, it was suggested that the other products consist of the L-cinerulosyl-2-deoxy-L-fucosyl-L-rhodosaminyl group linked to the C-7 position of the substrate aglycone. Structures of CG5 to 8 are described in the following paper[14].

Microbial Conversion of Aclacinomycin Analogs

Details of the conversion of aklavinone and aclacinomycin analogs by the mutant strain KE303 were investigated by feeding cultivation at early and late stages, and the results are shown in Table 4. When the conversion of aklavinone was examined at an early stage (18-hour cultivation) aclacinomycin A (L-cinerulosyl-2-deoxy-L-fucosyl-L-rhodosaminyl aklavinone) was produced, and its production was

Table 2. Anthracyclines produced by the parent and mutant strains of *S. galilaeus* MA144-M 1 and their chemical derivatives.

Compound	Structure*		Rf values**			
	Aglycone	Sugar (R)	S 1	S 2	S 5	S 6
Fermentation product:						
Aclacinomycin A	aklavinone	-RhoN-deFuc-Cin A	0.49	0.68	0.32	0.80
〃 B	〃	-RhoN-deFuc=Cin B	0.61	0.80	0.31	0.82
MA 144 N 1	〃	-RhoN-deFuc-Rho	0.25	0.54	0.25	0.69
〃 L 1	〃	-meDauN-deFuc-Cin A	0.36	0.67	0.28	0.80
〃 U 5	〃	-deFuc-deFuc-Cin A	0.36	0.63	0.38	0.66
〃 S 1	〃	-RhoN-deFuc	0.17	0.45	0.22	0.55
Aklavin	〃	-RhoN	0.17	0.38	0.22	0.70
Chemical derivative:						
MA 144 K 1	aklavinone	-DauN-deFuc-Cin A	0.32	0.65	0.28	0.78
〃 KH	〃	-DauN	0.03	0.11	0.25	0.48

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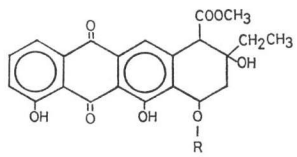
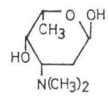
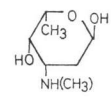


** Solvent systems are described in the text.

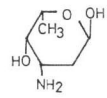
Sugar:



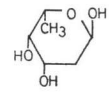
RhoN (Rhodosamine)



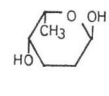
meDauN (N-Monomethyl daunosamine)



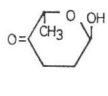
DauN (Daunosamine)



deFuc (2-Deoxyfucose)



Rho (Rhodinose)



Cin A (Cinerulose A)

Table 3. Physicochemical properties of anthracyclines produced by

Product / Analysis	CG1	CG2	CG3	CG4
Aglycone*	AKN	10-decarbomethoxy AKN	4-O-methyl AKN	ε-PMN
(*m/z*, M^+)	(412)	(354)	(426)	(428)
Molecular formula	$C_{42}H_{53}NO_{15}$	$C_{40}H_{51}NO_{13}$	$C_{43}H_{55}NO_{15}$	$C_{42}H_{53}NO_{16}$
Anal. Calcd.	C 62.14, H 6.58 N 1.73 %	C 63.73, H 6.82 N 1.86 %	C 62.54, H 6.71 N 1.71 %	C 61.01, H 6.34 N 1.69 %
Found	C 62.28, H 6.65 N 1.81 %	C 64.12, H 6.78 N 1.79 %	C 62.77, H 6.82 N 1.67 %	C 61.25, H 6.52 N 1.72 %
mp (°C)	144~146	139~141	145~148	190~193
Rf value S1**	0.49	0.36	0.38	0.48
(TLC) S2	0.68	0.63	0.67	0.71
S3	0.32	0.29	0.27	0.32
$\lambda_{nm}^{90\%\ MeOH}$ ($E_{1cm}^{1\%}$)	229 (530)	230 (687)	229 (527.5)	235 (580)
	258 (324.5)	260 (483.5)	258 (324)	258 (294.5)
	290s (127.5)	295 (212.5)	285s (157.5)	290 (116.5)
	433 (163)	433 (239.5)	418 (138.5)	395 (40)
			435s (127.5)	492 (175)
				510s (136.5)
				523s (115)
$\lambda_{nm\ 0.1\ N\ HCl}^{90\%\ MeOH-}$ ($E_{1cm}^{1\%}$)	229 (535)	230 (687)	229 (482.5)	234 (595)
	258 (325)	260 (488.5)	258 (305)	258 (595)
	290s (130)	295 (212.5)	285s (147)	290 (120)
	433 (167)	433 (240)	418 (131.5)	395 (41.5)
			435s (120)	485 (16.7)
				492 (175)
				510s (136.5)
				523s (115)
$\lambda_{nm\ 0.1\ N\ NaOH}^{90\%\ MeOH-}$ ($E_{1cm}^{1\%}$)	214 (1150)	217 (1230)	217 (571)	214 (1160)
	238 (485)	235 (570)	250 (390)	242 (623)
	286 (122.5)	287 (180)	280s (115)	285s (122.5)
	315s (80)	315s (108)	320 (77)	530s (165)
	523 (145)	520 (245)	515 (90)	565 (241.5)
				607 (204)
$\nu_{max}^{cm^{-1}}$ (KBr)	1740, 1680 1630, 1020	1730, 1670 1620, 1010	1735, 1675 1630, 1010	1740, 1600 1010
Identification	Aclacinomycin A	10-Decarbomethoxy aclacinomycin A	4-O-Methyl aclacinomycin A	Cinerubin A

* Abbreviation: AKN, aklavinone; PMN, pyrromycinone; RMN, rhodomycinone.

** Solvent systems are described in the text.

bioconversion of anthracyclinones in *S. galilaeus* MA144-M 1, strain KE 303.

CG5	CG6	CG7	CG8	CG9
ε-RMN	ε-isoRMN	β-RMN	γ-RMN	β-PMN
(428)	(444)	(386)	(370)	(386)
$C_{42}H_{53}NO_{16}$	$C_{42}H_{53}NO_{17}$	$C_{40}H_{51}NO_{15}$	$C_{40}H_{51}NO_{14}$	$C_{40}H_{51}NO_{15}$
C 61.01, H 6.34 N 1.69 %	C 59.78, H 6.33 N 1.66 %	C 61.08, H 6.54 N 1.78 %	C 62.35, H 6.68 N 1.82 %	C 61.08, H 6.54 N 1.78 %
C 60.84, H 6.33 N 1.60 %	C 58.97, H 6.23 N 1.68 %	C 61.14, H 6.47 N 1.83 %	C 61.87, H 6.52 N 1.74 %	C 61.53, H 6.38 N 1.68 %
160~162	162~164	163~165	143~145	128~135
0.53	0.51	0.30	0.31	0.12
0.72	0.73	0.60	0.61	0.35
0.31	0.30	0.25	0.27	0.19
235 (513)	240 (508)	235 (502)	236 (431)	234 (496)
255 (307)	295 (90)	252 (315)	254 (384)	256 (256)
292 (102)	490s (123)	292 (98)	295 (98)	290 (106)
492 (184)	521 (184)	495 (183)	495 (196)	490 (132)
527s (118)	547 (175)	528 (136)	528 (150)	512s (102)
585s (14)	560 (187)	580 (30)	560s (23)	526s (84)
	605 (48)			
235 (526)	240 (555)	234 (530)	236 (468)	234 (504)
255 (319)	295 (98)	254 (330)	254 (400)	251 (264)
292 (106)	490s (145)	290 (105)	295 (106)	290 (106)
492 (192)	521 (210)	495 (198)	495 (201)	492 (143)
527s (119)	547 (185)	528 (135)	528 (148)	514s (115)
	559 (194)	570s (14)	560s (30)	526s (93)
	605 (48)			
242 (555)	243 (585)	241 (595)	242 (591)	235 (433)
287 (99)	280s (95)	285 (107)	290 (108)	296 (88)
566 (223)	585 (229)	565 (220)	558 (222)	560 (141)
605 (194)	632 (261)	600s (175)	592 (200)	597 (127)
1730, 1600 1010	1730, 1590 1010	1730, 1600 1010	1730, 1600 1020	1730, 1595 1010
11-Hydroxyaclacinomycin A	11-Hydroxycinerubin A	10-Decarbomethoxy-10,11-dihydroxyaclacinomycin A	4‴-Dehydrorhodomycin Y	10-Decarbomethoxy-10-hydroxycinerubin A

accompanied with MA144 N1 (L-rhodinosyl-2-deoxy-L-fucosyl-L-rhodosaminyl aklavinone), MA144 L1 (L-cinerulosyl-2-deoxy-L-fucosyl-N-monomethyl-L-daunosaminyl aklavinone), MA-144 S1 (2-deoxy-L-fucosyl-L-rhodosaminyl aklavinone), aklavin (L-rhodosaminyl aklavinone) and 7-deoxyaklavinone. At a late stage (30-hour cultivation), only aclacinomycins A and B appeared. The time course of the production of aclacinomycin analogs from aklavinone during the feeding cultivation was similar to the productive pattern of the analogs in the fermentation of the parental strain 6U-21[1]. Similarly, aclacinomycin A, MA144 N1, L1 and S1 were produced from aklavin and aclacinomycin A, MA144 N1 and L1 from MA144 S1. The accumulation of aclacinomycin A also predominated in both cases. MA144 N1 was microbiologically converted to aclacinomycins A and B. A significant conversion was not observed with MA144 L1 and K1 (L-cinerulosyl-2-deoxy-L-fucosyl-L-daunosaminyl aklavinone) which are analogs having N,N-didemethylated L-rhodosamine moiety, and also with MA144 U5 (L-cinerulosyl-2-deoxy-L-fucosyl-2-deoxy-L-fucosyl-aklavinone) having no amino sugar. The feeding cultivation of MA144 KH (L-daunosaminyl aklavinone) produced MA144 K1 as the main product, and aclacinomycin A, MA144 S1 and aklavin as minor components.

Table 4. Bioconversion of aclacinomycin analogs by *S. galilaeus* MA144-M1, strain KE 303.

Experiment	Substrate	Product	%*
No. 1	Aklavinone	aclacinomycin A	24.2
		MA144 N1	4.2
		〃 L1	2.3
		〃 S1	10.3
		aklavin	10.5
		7-deoxyaklavinone	16.0
		substrate remained	32.0
	Aklavin	aclacinomycin A	41.9
		MA144 N1	4.4
		〃 L1	4.0
		〃 S1	27.0
		substrate remained	7.5
	MA144 S1	aclacinomycin A	48.1
		MA144 N1	8.2
		〃 L1	7.2
		substrate remained	36.7
No. 2	Aklavinone	aclacinomycin A	75.3
		〃 B	24.7
		substrate remained	0
	MA144 N1	aclacinomycin A	58.8
		〃 B	26.7
		substrate remained	13.5
	MA144 L1	unchanged	
	MA144 K1	〃	
	MA144 U5	〃	
	MA144 KH	aclacinomycin A	9.8
		MA144 K1	44.1
		〃 L1	2.2
		〃 S1	21.0
		aklavin	10.9
		substrate remained	12.0

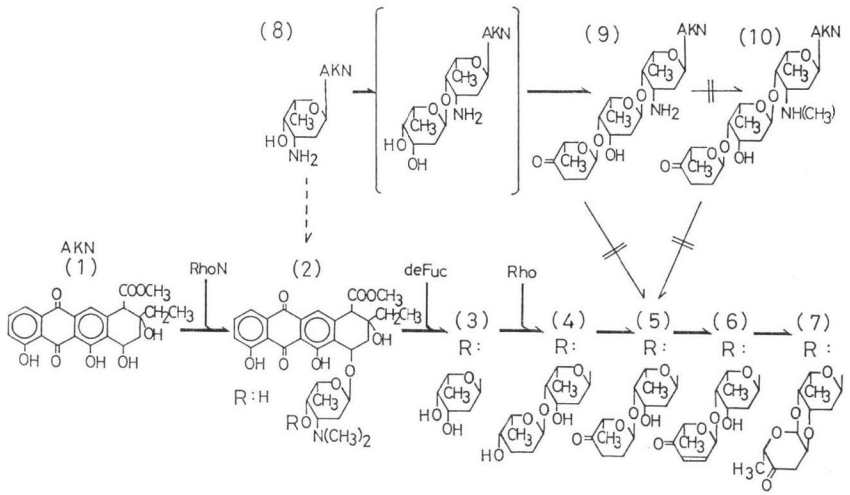
* % to total compounds recovered.

Conversion products were determined at 18 hours (experiment No. 1) and 30 hours (experiment No. 2) after addition of substrate.

Discussion

In experiments on the microbial conversion using an aclacinomycin-negative mutant KE303, which did not produce aklavinone but permitted the glycosidation of exogenous aklavinone, we found that besides aklavinone several other anthracyclinones were converted to biologically active glycosides. Thus, various anthracycline glycosides were obtained from the following anthracyclinones: aklavinone, ε- and β-pyrromycinones, ε-, γ- and β-rhodomycinones, ε-isorhodomycinone, 4-O-methylaklavinone and 10-decarbomethoxy-aklavinone, but no glycosides were produced from daunomycinone, 13-deoxydaunomycinone, adriamycinone and steffimycinone. This means that structural requirements are present in the glycosidation of anthracyclinones by *S. galilaeus* MA144-M1, strain KE303. The presence of a methoxyl group at C-4 and oxygen at C-13 (carbonyl group) affects the glycosidation. The carbo-

Fig. 1. Proposed biosynthetic pathway of aclacinomycins in *Streptomyces galilaeus* through bioconversion of some natural and chemical analogues.



(1) aklavinone, **(2)** aklavin, **(3)** MA144 S1, **(4)** MA144 N1, **(5)** aclacinomycin A, **(6)** aclacinomycin Y, **(7)** aclacinomycin B, **(8)** MA144 KH, **(9)** MA144 K1, **(10)** MA144 L1

AKN: aklavinone, RhoN: L-rhodosamine, deFuc: 2-deoxy-L-fucose, Rho: L-rhodinose

→: main path, - - →: by-pass, ⫲→: not proceeded.

methoxyl group at C-10 did not affect the glycosidation, since 10-decarbomethoxyaklavinone and β-rhodomycinone were good precursors. The multiple existence of hydroxyl groups at C-1, C-10, and C-11 in the anthracyclinone skeleton also did not affect glycosidation. 13-Deoxydaunomycinone, which has the ethyl group in place of the acetyl group at C-9 position, was not glycosidated, while 4-O-methylaklavinone was equally converted as aklavinone and carminomycinone, suggesting that the presence of both the methoxyl group at C-4 and oxygen at C-13 position produce a strict blockage of glycosidation of these anthracyclinones by *S. galilaeus* MA144-M1, strain KE303. We have elucidated that the glycosidation of either daunomycinone or 13-deoxydaunomycinone by an antibiotic-negative mutant of the daunomycin-producing *Streptomyces coeruleorubidus* did not occur, while aklavinone, ε-rhodomycinone and carminomycinone were converted to daunomycin[15]. It is interesting that the substrate specificity for glycosidation is similar between *S. galilaeus* and *S. coeruleorubidus*.

In experiments on the conversion of various anthracyclines, we could also demonstrate that MA144 N1 was biosynthesized from aklavinone by a step-wise glycosidation *via* aklavin and MA144 S1, as shown in Fig. 1. MA144 N1 was thereafter converted to aclacinomycins A and Y through the successive oxidation of the terminal sugar by a specific oxidoreductase, and aclacinomycin B was non-enzymatically derived from aclacinomycin Y[16]. The conversion of MA144 KH proceeded toward the predominant formation of MA144 K1, although a low accumulation of aclacinomycin A, MA144 S1 and aklavin occurred at the same time. However, MA144 K1 and L1 could not be converted to aclacinomycin A. These results indicate that aklavinone is directly glycosidated to aklavin containing L-rhodosamine, but not *via* N,N-didemethyl aklavin. MA144 L1 having N-monomethyl daunosamine is not an intermediate metabolite in the biosynthesis of aclacinomycin A, but a degradation product of the latter.

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