

MICROBIAL CONVERSION OF ANTHRACYCLINONES
TO DAUNOMYCIN BY BLOCKED MUTANTS OF
STREPTOMYCES COERULEORUBIDUS

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Baumycin-negative mutants of *Streptomyces coeruleorubidus* ME130-A4 do not convert daunomycinone to daunomycin and baumycins. They biosynthesize daunomycin from aklavinone and ϵ -rhodomycinone, indicating the glycosidation of the aglycone before the synthesis of daunomycinone. The biosynthetic pathway from ϵ -rhodomycinone to daunomycin was further confirmed by studying the bioconversion of anthracyclines and anthracyclines, which are presumed to be intermediates.

Daunomycin which is produced by *Streptomyces coeruleorubidus*^{1,2)}, *Streptomyces peucetius*³⁾ and some other *Streptomyces* strains has been proven to be synthesized by the condensation of nine acetate units and a propionate unit followed by the formation of a hypothetical decaketide⁴⁾. Recently BLUMAUEROVA *et al.* reported their studies on anthracycline biogenesis by *S. coeruleorubidus* and *S. galilaeus*^{5,6)}. We have studied the microbial conversion of a number of anthracyclines (aglycones) by baumycin-negative mutants of *S. coeruleorubidus* ME130-A4 which had lost the ability to synthesize daunomycinone but still had the ability to produce daunosamine and found that aklavinone and ϵ -rhodomycinone were the precursors of the aglycone moiety of daunomycin⁷⁾. On the basis of these studies, the biosynthetic pathway from aklavinone to daunomycin was proposed by H. UMEZAWA⁸⁾ in 1977.

We have studied the microbial conversion of 15 anthracyclines, searching for precursor aglycones and intermediate glycosides leading to daunomycin, and have further defined the biosynthetic pathway from ϵ -rhodomycinone to daunomycin.

Materials and Methods

Isolation of baumycin-negative mutants

Spore cells of *S. coeruleorubidus* ME130-A4 from a week old culture grown on YS agar (0.3% yeast extract, 1% soluble starch and 1.5% agar, pH 7.0) were exposed to 1 mg/ml of N-methyl-N'-nitro-N-nitrosoguanidine in 0.2 M Tris-HCl (pH 8.5) with shaking at 28°C in the dark, or their suspensions in saline were irradiated by ultraviolet light up to about 0.5~1% survival. The subcultures thus obtained were grown on YS agar and tested for the production of baumycin and related anthracycline compounds by the fermentation method previously described.²⁾ Baumycin-negative mutants were thus selected and further examined for their ability to produce baumycins and daunomycin in a medium to which ϵ -rhodomycinone was added. As the result of this study, strains 1U-222, 1U-492 and 1N-372

were selected and used throughout the experiments described in this paper.

Media and cultural conditions

The fermentation media used were as follows:

- I. 4% sucrose, 2.5% soybean meal, 0.1% yeast extract, 0.25% NaCl, 0.32% CaCO₃, 0.0005% CuSO₄·7H₂O, 0.0005% MnCl₂·4H₂O and 0.0005% ZnSO₄·7H₂O, pH 7.4.
- II. 1.5% soluble starch, 1% glucose, 3% soybean meal, 0.1% yeast extract, 0.3% NaCl, 0.1% MgSO₄·7H₂O, 0.1% K₂HPO₄, 0.007% CuSO₄·5H₂O, 0.001% FeSO₄·7H₂O, 0.0008% MnCl₂·4H₂O and 0.002% ZnSO₄·7H₂O, pH 7.4.
- III. 2% glycerol, 1% glucose, 3% dry yeast, 0.1% MgSO₄·7H₂O, 0.3% NaCl, 0.1% K₂HPO₄, 0.1% CaCO₃, 0.0005% CuSO₄·5H₂O, 0.0005% MnCl₂·4H₂O and 0.0005% ZnSO₄·7H₂O, pH 7.0.
- IV. 3% glycerol, 1% soybean meal, 2% corn steep liquor, 0.1% yeast extract, 0.3% NaCl and 0.2% CaCO₃, pH 7.0.
- V. 6% glucose, 2.5% dry yeast, 0.2% NaCl, 0.1% K₂HPO₄, 0.1% MgSO₄·7H₂O, 0.2% CaCO₃, 0.0005% CuSO₄·7H₂O, 0.0005% MnCl₂·4H₂O and 0.0005% ZnSO₄·7H₂O, pH 7.2.

Cultivation was carried out in 500-ml Erlenmeyer flasks containing 50 ml medium at 28°C on a rotary shaker (220 rpm)

Anthracyclines and anthracyclines

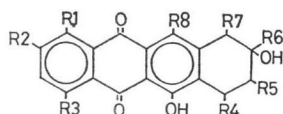
The following anthracyclines (Table 1) were prepared by acid hydrolysis of the corresponding anthracyclines with 0.1 N HCl at 85°C for 30 minutes: Aklavinone from aclacinomycin,^{9,10} 10-decarbomethoxyaklavinone and 4-O-methylaklavinone from 10-decarbomethoxyaclacinomycin A and 4-O-methylaclacinomycin A which were chemically derived from aclacinomycin A,¹¹ ε-pyromycinone from rhodirubins,¹² γ-rhodomyconone and β-rhodomyconone from mixture of rhodomycins produced

Table 1. Structure and Rf value of anthracyclines.

Compound	Structure*								Rf value**		
	R1	R2	R3	R4	R5	R6	R7	R8	S2	S3	S4
Aklavinone	H	H	OH	OH	H	CH ₂ CH ₃	COOCH ₃	H	0.67	0.81	0.67
2-Hydroxyaklavinone	H	OH	OH	OH	H	CH ₂ CH ₃	COOCH ₃	H	0.24	0.74	0.40
10-Decarbomethoxyaklavinone	H	H	OH	OH	H	CH ₂ CH ₃	H	H	0.55	0.75	0.51
4-O-Methylaklavinone	H	H	OCH ₃	OH	H	CH ₂ CH ₃	COOCH ₃	H	0.64	0.70	0.43
7-Deoxyaklavinone	H	H	OH	H	H	CH ₂ CH ₃	COOCH ₃	H	0.70	0.80	0.72
ε-Rhodomyconone	H	H	OH	OH	H	CH ₂ CH ₃	COOCH ₃	OH	0.66	0.78	0.65
γ-Rhodomyconone	H	H	OH	H	H	CH ₂ CH ₃	OH	OH	0.53	0.77	0.66
β-Rhodomyconone	H	H	OH	OH	H	CH ₂ CH ₃	OH	OH	0.44	0.70	0.50
ε-Pyromycinone	OH	H	OH	OH	H	CH ₂ CH ₃	COOCH ₃	H	0.65	0.78	0.62
ε-Isorhodomyconone	OH	H	OH	OH	H	CH ₂ CH ₃	COOCH ₃	OH	0.65	0.77	0.64
Daunomycinone	H	H	OCH ₃	OH	H	COCH ₃	H	OH	0.59	0.66	0.32
Dihydrodaunomycinone	H	H	OCH ₃	OH	H	CHOHCH ₃	H	OH	0.25	0.43	0.11
7-Deoxydihydrodaunomycinone	H	H	OCH ₃	H	H	CHOHCH ₃	H	OH	0.33	0.73	0.26
13-Deoxydaunomycinone	H	H	OCH ₃	OH	H	CH ₂ CH ₃	H	OH	0.58	0.69	0.32
Carminomycinone	H	H	OH	OH	H	COCH ₃	H	OH	0.66	0.79	0.67
Adriamycinone	H	H	OCH ₃	OH	H	COCH ₂ OH	H	OH	0.32	0.52	0.16
Dihydroadriamycinone	H	H	OCH ₃	OH	H	CHOHCH ₂ OH	H	OH	0.06	0.28	0.02
7-Deoxydihydroadriamycinone	H	H	OCH ₃	H	H	CHOHCH ₂ OH	H	OH	0.33	0.73	0.26
Steffimycinone	H	OCH ₃	OH	OH	OCH ₃	CH ₃	O	H	0.48	0.65	0.39

** Solvent systems used for thin-layer chromatography are described in the text.

*



by *Actinomyces roseoviolaceus* A529 (IFO 13081)¹³⁾, daunomycinone and adriamycinone from daunomycin and adriamycin (Farmitalia Carloerba), carminomycinone from carminomycin I (Bristol Lab.), and steffimycinone from steffimycin (Upjohn Co.). 7-Deoxyaklavinone¹⁰⁾ and 2-hydroxyaklavinone (unpublished data) were obtained by fermentation of *S. galilaeus* MA144-M1 and of its blocked mutant ANR-58, respectively. ϵ -Rhodomycinone and ϵ -isorhodomycinone were also directly produced by fermentation of a blocked mutant IU-85 derived from *S. coeruleorubidus* ME130-A4 and of a blocked mutant 2N-519 of *A. roseoviolaceus* A529 (IFO 13081), respectively. 13-Deoxydaunomycinone was prepared by acid hydrolysis of the fermentation products of a blocked mutant 4N-140 of *S. coeruleorubidus* ME130-A4. Dihydrodaunomycinone and dihydroadriamycinone and their 7-deoxy-derivatives were prepared by microbial conversion according to the method of MARSHALL *et al.*¹⁴⁾ MA144 KH (7-O-daunosaminylaklavinone)¹⁰⁾ and 11-hydroxy-aklavin (7-O-rhodosaminyl- ϵ -rhodomycinone) were obtained by methanolysis of MA144 K1 (7-O-[4'-(4''-O-cinerulosyl-2-deoxyfucosyl)-daunosaminyl]-aklavinone)¹⁰⁾, and of 11-hydroxyaclacinomycin A,¹⁵⁾ respectively.

Microbial conversion and extraction of conversion products

Unless otherwise stated, the strain IU-222 was cultured at 28°C for 48 hours in a shaken 500-ml Erlenmeyer flask containing 100 ml of a seed medium (1% soluble starch, 1% glucose, 1% soybean meal, 0.3% NaCl, 0.1% K₂HPO₄ and 0.1% MgSO₄·7H₂O, pH 7.4). One ml of this culture was then inoculated into a 500-ml Erlenmeyer flask containing 50 ml of medium I and the cultivation was carried out at 28°C on a rotary shaker (220 rpm). After 72 to 90 hours, 0.5 to 1 mg of an anthracyclinone in 1 ml MeOH was added to a flask and the cultivation was continued for an additional 40 hours. The conversion products were extracted by vigorous mixing with 40 ml of a CHCl₃-MeOH (1:1) mixture and the CHCl₃ extract was evaporated to dryness.

Thin-layer chromatographic identification and assay of conversion products

In order to examine the formation of the baumycins, an aliquot of each conversion product was chromatographed on a silica gel plate before and after acid hydrolysis (0.1 N HCl, 85°C, 30 minutes), and their chromatographic behavior was compared with parental metabolites obtained from the culture of *S. coeruleorubidus* ME130-A4. The aglycones were detected on the thin-layer chromatogram (TLC) by scanning at either 495 nm or 430 nm using a Shimadzu TLC scanner (model CS-910). The conversion products, if necessary, were purified by preparative thin-layer chromatography: the pigment bands were scraped from the silica gel thin-layer and extracted with a mixture of CHCl₃ - MeOH (5:1); the extract was washed with a half volume of 0.1 M Tris-HCl (pH 7.5) buffer and thereafter twice with water and evaporated to dryness.

For the identification of an aglycone and a sugar, about 0.3~1 mg of the purified compound was hydrolyzed in 2 ml of 0.1 N HCl at 85°C for 30 minutes. After the extraction of the resulting aglycone twice with 2 ml of CHCl₃, the aqueous layer was neutralized by the addition of AgCO₃, the precipitate was centrifuged off, the supernatant was concentrated, spotted on a silica gel thin-layer plate and compared with authentic daunosamine, rhodosamine, 2-deoxyfucose and rhodinosose. The plate was developed with *n*-BuOH - acetic acid - H₂O (4:1:1) and the sugar spots were detected by spraying with a *p*-anisaldehyde reagent¹⁶⁾. Rf values were 0.13 for rhodosamine, 0.42 for daunosamine, 0.54 for 2-deoxyfucose and 0.68 for rhodinosose. For analysis of the aglycones, the chloroform extract was chromatographed on silica gel thin-layer plates with three solvent systems (S2, S3 and S4) and compared with the substrate aglycone and various standard aglycones.

Thin-layer chromatography

The solvent systems used were as follows: S1, CHCl₃ - MeOH (10:1); S2, CHCl₃ - MeOH (20:1); S3, benzene - ethylacetate - MeOH - 0.1 N HCl (5:5:1.5:0.15); S4, benzene - acetone - formic acid (100:30:1); S5, CHCl₃ - MeOH - H₂O - acetic acid (80:20:2:0.2); S6, CHCl₃ - MeOH - H₂O - acetic acid (75:25:5:5); S7, CHCl₃ - MeOH - NH₄OH (90:10:0.2). Silica gel plates were kiesel gel 60 F₂₅₄ (E. Merck Co.) and preparative silica gel plates were prepared from kiesel gel 60 PF₂₅₄ (E. Merck Co.).

Results

Microbial Conversion of Various Anthracyclines by the Strain 1U-222

S. coeruleorubidus ME130-A4^{2,17)} produced 12 anthracycline compounds, as shown in Table 2. Among them the major products were baumycin A1 and A2. A blocked mutant 1U-222, which was

Table 2. Anthracycline compounds produced by *Streptomyces coeruleorubidus* ME130-A4.

Compound		Rf value*			
		S 1	S 5	S 6	S 7
Aglycone	ϵ -Rhodomycinone	0.78	0.86	0.95	0.82
	7-Deoxydihydrodaunomycinone	0.49	0.78	0.86	0.55
	Dihydrodaunomycinone	0.44	0.73	0.82	0.48
Glycoside	N-Acetyldaunomycin	0.45	0.70	0.80	0.43
	N-Formyldaunomycin	0.46	0.62	0.78	0.43
	N-Formyldihydrodaunomycin	0.21	0.50	0.73	0.31
	Baumycin A1	0.11	0.43	0.74	0.57
	" A2	0.03	0.36	0.72	0.43
	" B1	0.07	0.43	0.73	0.12
	" B2	0.02	0.31	0.66	0.03
	Daunomycin	0.01	0.27	0.59	0.18
Dihydrodaunomycin	0.00	0.15	0.43	0.06	

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

Table 3. Bioconversion of various anthracyclines by *S. coeruleorubidus* ME130-A4, mutant strain 1U-222.

Substrate aglycone	Conversion to glycoside	Product
Aklavinone	+	Baumycins, daunomycin & dihydrodaunomycin
2-Hydroxyaklavinone	-	Unchanged
10-Decarbomethoxyaklavinone	+	Baumycins, daunomycin & dihydrodaunomycin
7-Deoxyaklavinone	-	Unchanged
ϵ -Rhodomycinone	+	Baumycins, daunomycin & dihydrodaunomycin
γ -Rhodomycinone	-	Unidentified aglycone ^{a)}
β -Rhodomycinone	-	Unidentified glycosides ^{b)}
ϵ -Pyrromycinone	+	1-Hydroxybaumycins ^{c)}
ϵ -Isorhodomycinone	+	"
Daunomycinone	-	Dihydrodaunomycinone & 7-deoxydihydrodaunomycinone
Dihydrodaunomycinone	-	7-Deoxydihydrodaunomycinone
13-Deoxydaunomycinone	-	Unchanged
Carminomycinone	+	Baumycins, daunomycin & dihydrodaunomycin
Adriamycinone	-	Dihydroadriamycinone & 7-deoxydihydroadriamycinone
Steffimycinone	-	Steffimycinol

a) A modified aglycone with $R_{f-\text{rhodomycinone}}$ value of 0.42 in the S2 solvent.

b) Compounds with Rf values of 0.07 and 0.02 in the S2 solvent. They gave both a modified aglycone with $R_{\beta\text{-rhodomycinone}}$ value of 0.32 in the S2 solvent, and daunosamine by acid hydrolysis.

c) Two of them were identified as 1-hydroxydihydrodaunomycin and N-formyl-1-hydroxydihydrodaunomycin²⁰⁾.

incapable in producing any anthracyclic pigment, was examined to determine if any anthracycline could be glycosidated to baumycins, daunomycin or some other related glycosides. As shown in Table 3, aklavinone, 10-decarbomethoxyaklavinone, ϵ -rhodomycinone and carminomycinone were converted to daunomycin, baumycin and dihydrodaunomycin. Although daunomycinone, 13-deoxydaunomycinone, adriamycinone and their 13-dihydro-derivatives could not be glycosidated to form daunomycin or adriamycin, a reduction of the 13-carbonyl function and reductive glycosidic cleavage leading to removal of the 7-oxy function did take place. The conversion of 1-hydroxyanthracyclines (ϵ -pyrromycinone and ϵ -isorhodomycinone) by the mutant 1U-222 gave new anthracycline antibiotics which were identified as 1-hydroxy-13-dihydrodaunomycin and its N-formyl derivative¹⁸). β -Rhodomycinone was converted to unidentified glycosides which consisted of a modified aglycone and daunosamine. 2-Hydroxyaklavinone, 7-deoxyaklavinone and 4-O-methylaklavinone were not converted to any glycosides. These aglycones and γ -rhodomycinone were significantly modified, while steffimycinone was transformed to steffimycinol.

Conversion Products of Aklavinone, ϵ -Rhodomycinone, Carminomycinone and Daunomycinone

The nature of the bioconversion products from aklavinone, ϵ -rhodomycinone, carminomycinone and daunomycinone was investigated by silica gel TLC (Table 4). The feeding of the former three aglycones to the fermentation medium in which the strain 1U-222 was cultivated resulted mainly in 13-dihydrodaunomycinone, 7-deoxy-13-dihydrodaunomycinone and six anthracycline glycosides (baumycin A1, A2, B1 and B2, daunomycin and 13-dihydrodaunomycin). Moreover, ϵ -rhodomycinone was obtained as an additional conversion product of aklavinone. These conversion products were the same as the original fermentation products of the parental strain ME130-A4. The glycosidation rates (2~

Table 4. Bioconversion products produced by strain 1U-222 from aklavinone, ϵ -rhodomycinone, carminomycinone and daunomycinone.

Conversion product		Substrate (nmoles)			
		Aklavinone	ϵ -Rhodomycinone	Carminomycinone	Daunomycinone
Residual substrate	Aklavinone ϵ -Rhodomycinone Carminomycinone Daunomycinone	85.0(4.3)	97.1(4.2)	105.3(5.2)	168.7(8.4)
Product	ϵ -Rhodomycinone	866.3(43.3)			
	7-Deoxydihydrodaunomycinone	7.0(0.4)	8.0(0.4)	8.0(0.4)	30.5(1.5)
	Dihydrodaunomycinone	28.8(1.4)	80.0(4.0)	50.4(2.5)	983.5(49.2)
	Baumycin A1	76.4(3.8)	64.6(3.2)	38.3(1.9)	0
	" A2	102.0(5.1)	119.0(5.9)	83.0(4.2)	0
	" B1	30.8(1.6)	28.4(2.4)	trace	0
	" B2	41.0(2.0)	39.1(2.0)	21.2(1.1)	0
	Daunomycin	69.0(3.6)	41.6(2.1)	47.5(2.4)	0
	Dihydrodaunomycin	45.3(2.3)	25.2(1.3)	28.5(2.4)	0

Bioconversion of 2 μ moles substrate was carried out using an Erlenmeyer flask. (See methods) The mycelial extract was chromatographed on a silica gel plate with the solvents S4 and S5. Spots developed in the solvents S4 and S5 were determined for aglycones and glycosides, respectively, by a Shimadzu TLC scanner model CS-910. The values in parenthesis show % of products to substrate.

6%) of these aglycones were not significantly different from each another. In the case of daunomycinone, the bulk of the conversion products was dihydrodaunomycinone (49.2%) and the remainder was 7-deoxy-13-dihydrodaunomycinone (1.5%) and neither baumycins nor daunomycin was obtained.

Further Studies on the Failure to Observe Glycosidation of Daunomycinone

In order to confirm that *S. coeruleorubidus* ME130-A4 may be lacking in the ability to synthesize daunomycin or baumycins from daunomycinone, the conversion of daunomycinone was examined under various cultural conditions using various media and mutant strains. The effect of media on the daunomycinone conversion by 1U-222 is shown in Table 5. The media had an influence upon the antibiotic productivity. Medium I was most suitable for the antibiotic production by the parental strain, and the media III and VI did not support the production of any anthracycline compounds by the cultivation of a parental strain. As measured by acetone-extractable anthracycline compounds from mycelia detected by their absorptivity at 495 nm, the antibiotic productivity in the media II and IV was about 30% and 90% of that in the medium I, respectively. The conversion of aklavinone to the parental metabolites occurred only when it was added to the culture of 1U-222 grown in the antibiotic-producing media I, II and IV. In the case of daunomycinone, however, its conversion products were 13-dihydrodaunomycinone and 7-deoxy-13-dihydrodaunomycinone in all media, and none of glycosidic compounds was obtained.

Table 6 shows the conversion of daunomycinone by three bioconvertible baumycin-negative mutants which were independently isolated after treatment with N-methyl-N'-nitro-N-nitrosoguanidine

Table 5. Effect of cultural media on bioconversion of aklavinone and daunomycinone by a strain 1U-222.

Medium	Substrate	Conversion product (μ mole)			Antibiotic* productivity by the parent strain (%)
		Daunomycinone glycosides	Dihydro-daunomycinone	7-Deoxydihydro daunomycinone	
I	Aklavinone	0.68	+**	+	100
	Daunomycinone	0	1.64	0.21	
II	Aklavinone	0.43	+	+	30
	Daunomycinone	0	1.54	0.17	
III	Aklavinone	0	0	0	0
	Daunomycinone	0	1.19	0.11	
IV	Aklavinone	1.06	+	+	90
	Daunomycinone	0	1.64	0.21	
V	Aklavinone	0	0	0	0
	Daunomycinone	0	1.34	0.21	

Bioconversion of 3 μ moles aklavinone or daunomycinone in an Erlenmeyer flask was carried out by the cultivation of mutant strains 1U-222 in various media as described in the text. Conversion products were extracted by mixing with 40 ml of CHCl_3 -MeOH (1:1) and the CHCl_3 extract was chromatographed on silica gel plates with parental metabolites as references using solvents of S2 and S5. Daunomycinone glycosides were estimated as daunomycinone after acid hydrolysis. The amount of daunomycinone, dihydrodaunomycinone and 7-deoxydihydrodaunomycinone were determined by a TLC scanner.

* Antibiotic productivity by parent strain is shown by relative absorptivity value (%) at 495 nm of acetone-extractable anthracycline pigment from mycelia.

** minor (dihydrodaunomycinone + 7-deoxydihydrodaunomycinone).

Table 6. Bioconversion of daunomycinone and aklavinone by three baumycin-negative mutants.

Mutant strain	Substrate	Conversion product (μ mole)		
		Daunomycinone glycosides	Dihydro-daunomycinone	7-Deoxydihydro-daunomycinone
1N-372	Aklavinone	0.64	+	+
	Daunomycinone	0	1.28	0.10
1U-479	Aklavinone	0.70	+	+
	Daunomycinone	0	1.46	0.12
1N-222	Aklavinone	1.04	+	+
	Daunomycinone	0	1.96	0.16

Bioconversion and assay of conversion products were carried out as described in Table 5, except that the indicated mutant strains were cultivated in the medium IV for the bioconversion.

or UV of *S. coeruleorubidus* ME130-A4. The strains 1N-372 and 1U-479 as well as 1U-222 converted aklavinone and ϵ -rhodomycinone to baumycins and daunomycin, whereas glycosidation of daunomycinone to daunomycin was not observed. Further attempts to isolate mutants capable of producing daunomycin or baumycin from daunomycinone were not successful.

Conversion of Various Anthracyclines by Strain 1U-222

The possible bioconversions of aklavinone and ϵ -rhodomycinone monosaccharides to daunomycin, baumycins or their related daunomycinone glycosides by strain 1U-222 was studied (Table 7). No meaningful conversions of aklavin, MA144 KH and 11-hydroxyaklavin were observed under the bioconversion conditions where aklavinone was converted to baumycins or daunomycin. No daunomycinone glycosides were detected following their addition to the culture of 1U-222. They appeared to undergo deglycosidation, yielding modified aglycones. However, 13-deoxydaunomycin and carminomycin I gave daunomycin and baumycins as their conversion products. Daunomycin was further modified to baumycins and 13-dihydrodaunomycin.

Table 7. Bioconversion of anthracycline glycosides by a mutant strain 1U-222.

Substrate	Structure*	Conversion product
Aklavin	Aklavinone-RhoN	Unchanged
MA144 KH	Aklavinone-DauN	"
11-Hydroxyaklavin	ϵ -Rhodomycinone-RhoN	"
13-Deoxydaunomycin	13-Deoxydaunomycinone-DauN	Baumycins, daunomycin & dihydrodaunomycin
Carminomycin I	Carminomycinone-DauN	" " "
Daunomycin	Daunomycinone-DauN	Baumycins & dihydrodaunomycin

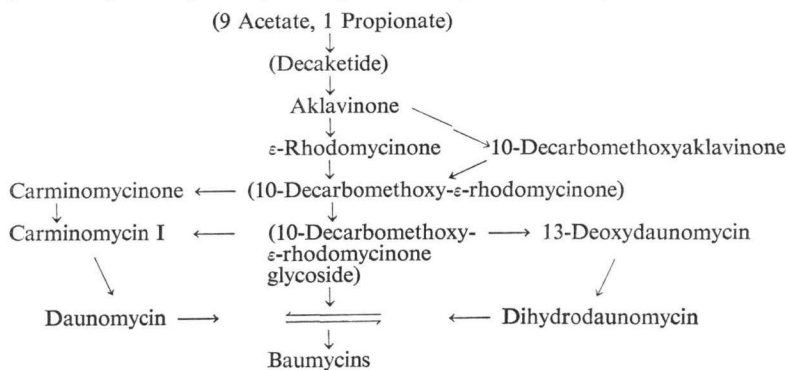
* RhoN: rhodosamine, DauN: daunosamine.

Discussion

In a study using mutational blocked mutants, we found that aklavinone and ϵ -rhodomycinone but neither daunomycinone nor 13-dihydrodaunomycinone are converted to parental anthracycline glycosides such as baumycins and daunomycin. This indicates that aklavinone and ϵ -rhodomycinone are the precursors for the aglycone moiety of daunomycin in its biosynthesis by *S. coeruleorubidus* ME130-A4. It is evident that aklavinone should be utilized *via* ϵ -rhodomycinone by 11-hydroxylation (probably by an oxidase action) for the biosynthesis of daunomycin, since the 1U-222 culture fed with aklavinone

yielded ϵ -rhodomycinone together with other parental metabolites. The addition of 1-hydroxy analogs of aklavinone and ϵ -rhodomycinone, *i.e.* ϵ -pyrromycinone and ϵ -isorhodomycinone, to fermentation media also gave 1-hydroxydaunomycinone glycosides as the conversion products, one of which was identified as 1-hydroxy-13-dihydrodaunomycin¹⁸⁾. As reported in another paper⁷⁾ describing the bio-conversion experiments with [9-¹⁴C] and [16-¹⁴C]-aklavinones, we have also demonstrated that the tetracyclic carbon skeleton of aklavinone is the source of daunomycinone⁷⁾. Therefore, it can be proposed that daunomycin is derived from aklavinone *via* ϵ -rhodomycinone⁸⁾. 10-Decarbomethoxyaklavinone and carminomycinone also exhibited positive precursor activity for the formation of daunomycin (Table 3). However, MA144 KH (7-O-daunosaminylaklavinone), aklavin (7-O-rhodosaminylaklavinone) and 11-hydroxyaklavin (7-O-rhodosaminyl- ϵ -rhodomycinone) could not be converted to the corresponding daunomycinone glycosides. This implies that ϵ -rhodomycinone is modified before glycosidation during its bio-conversion to daunomycin. It is clear that the glycosidation of the precursor aglycone by *S. coeruleorubidus* is strictly restricted by methylation of hydroxyl group at C-4 and partially by 13-oxidation of the ethyl group at C-9 to an acetyl group, since daunomycinone, 13-deoxydaunomycinone and adriamycinone could not be glycosidated. Recently we have found 13-deoxydaunomycin, which is a new intermediate metabolite in the biosynthesis of daunomycin, in the culture filtrate of a blocked mutant 4N-140 of *S. coeruleorubidus* ME130-A4 (unpublished data). From these results and on the basis of the evidence that the basal carbon skeletons of ϵ -pyrromycinone and daunomycinone are built by condensation of nine acetate units and one propionate unit^{4,19)}, the biosynthetic pathway leading to daunomycin in *S. coeruleorubidus* can be proposed as shown in Fig. 1.

Fig. 1. Proposed biosynthetic pathway leading to daunomycin and baumycins in *S. coeruleorubidus*.



Although BLUMAUEROVA *et al.*⁵⁾ inferred that the precursor aglycone of daunomycin should be daunomycinone itself rather than ϵ -rhodomycinone, our extensive efforts to produce daunomycin from daunomycinone were not successful.

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