NOTES

BIOSYNTHESIS OF DAUNOMYCINONE FROM AKLAVINONE AND ε-RHODOMYCINONE

AKIHIRO YOSHIMOTO and TOSHIKAZU OKI
Central Research Laboratories, Sanraku-Ocean Co. Ltd., Johnan, Fujisawa, Kanagawa, Japan
HAMAO UMEZAWA
Institute of Microbial Chemistry, Kamiosaki, Shinagawa-ku, Tokyo, Japan

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In the microbial conversion of anthracyclinones by baumycin-negative mutant of *Streptomyces coeruleorubidus* ME130-A4, baumycins (4′-substituted daunomycins[1]) and other parental metabolites[2] were found to be produced from exogenous aklavinone or ε-rhodomycinone in the fermentation medium. Thus, these anthracyclinones or their derivatives have been shown to be the biosynthetic precursor of daunomycinone. In this paper, we report the bioconversion of [9-^{14}C]- and [16-^{14}C]-labeled aklavinones to daunomycinone by a baumycin-negative mutant *via* ε-rhodomycinone.

A baumycin-negative mutant (strain 1U-222)[3] of *S. coeruleorubidus* ME130-A4, that failed to synthesize any anthracyclic pigments but was able to produce the aminosugar daunosamine, was used for the bioconversion of aklavinone. *Streptomyces galilaeus* MA144-M1, strain 3AR-33, which was a variant yielding only aklavinone, was used for preparation of labeled aklavinone.

[9-^{14}C]- and [16-^{14}C]-akalvinones were prepared as follows: The strain 3AR-33 was incubated with shaking in a 500-ml Erlenmeyer flask containing 50 ml of the following 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% $ZnSO_4 \cdot 7H_2O$ in tap water, pH 7.4. After 24-hour cultivation 50 μCi of [1-^{14}C]-sodium propionate or 50μCi of [methyl-^{14}C]-L-methionine was added to the medium, and incubation was resumed for an additional 15 hours until maximum production of aklavinone was achieved. The culture broth obtained from two flasks was shaken vigorously with an equal volume of $CHCl_3$ - MeOH (2: 1) mixture. The $CHCl_3$ phase was evaporated to dryness *in vacuo*, and the yellowish residue (21.3 mg) was dissolved in a small amount of $CHCl_3$ and chromatographed on a silica gel column (Wako gel C-200, 1.5 × 15 cm) which was developed with $CHCl_3$ - MeOH (50: 1). Effluent from the regions of yellow pigment was fractionated by 3 ml followed by detection of aklavinone by silica gel thin-layer chromatography using $CHCl_3$ - MeOH (50: 1). Fractions containing aklavinone were combined and evaporated to dryness *in vacuo*. The amount of aklavinone was determined by molecular absorbance (13072) at 430 nm in MeOH. [9-^{14}C]-Aklavinone (3.4 mg) and [16-^{14}C]-aklavinone (3.2 mg) with specific activities of 22.3 and 43.0 cpm/nmole, respectively, were obtained.

For the bioconversion of ^{14}C-aklavinones to daunomycinone glycosides, strain 1U-222 was shaken for 40 hours in a 500-ml Erlenmeyer flask containing 100 ml of the seed medium: 1% soluble starch, 1% glucose, 1% soybean meal, 0.3% NaCl, 0.1% K_2HPO_4, 0.1% $MgSO_4 \cdot 7H_2O$, pH 7.4. This culture (1 ml) was inoculated into a 500-ml Erlenmeyer flask containing the fermentation medium described above and incubated at 28°C for 90 hours on a rotary shaker (220 rpm). Thereafter, 2 μmoles of [9-^{14}C]-aklavinone (21.4 cpm/nmole) or [16-^{14}C]-aklavinone (29.0 cpm/nmole) in 1 ml of MeOH was added, and the organism was reincubated for 40 hours. The culture broth was then extracted with a $CHCl_3$ - MeOH (1: 1) mixture, and the $CHCl_3$ layer was evaporated to dryness. The orange residue was found to contain aklavinone, ε-rhodomycinone, daunomycin and baumycins by thin-layer chromatography in comparison with authentic samples. Hydrolysis with 0.3 N HCl at 85°C for 30 minutes yielded the aglycones which were extracted into $CHCl_3$. The $CHCl_3$ extract was concentrated and then chromatographed on a thin-layer plate (Kiesel gel F_{254}, E. Merck & Co.) with $CHCl_3$ - MeOH (30: 1). The regions corresponding to daunomycinone (Rf value, 0.58) and to a mixture of aklavinone and ε-rhodomycinone (Rf value, 0.40) were scraped off and the compounds were eluted with a $CHCl_3$ - MeOH (10: 1) mixture. The latter two substances were separated by

Table 1. Bioconversion of ^{14}C-labeled aklavinone to daunomycinone and ε-rhodomycinone by a baumycin-negative mutant of *S. coeruleorubidus* ME 130-A 4, strain 1 U-222.

Substrate added	Specific activity	Product formed	Specific activity	Conversion (%)
	cpm/nmole		cpm/nmole	
[9-^{14}C]-Aklavinone	21.4	Daunomycinone	21.6	101.2
		ε-Rhodomycinone	20.7	97.4
[16-^{14}C]-Aklavinone	29.0	Daunomycinone	1.3	4.4
		ε-Rhodomycinone	27.6	91.5

thin-layer chromatography with benzene-acetone-methanol (100: 10: 1). Aklavinone (Rf value, 0.27) and ε-rhodomycinone (Rf value, 0.32) were scraped off and the respective compounds were eluted with $CHCl_3$ - MeOH (10: 1). After drying, daunomycinone and ε-rhodomycinone were dissolved in a small amount of MeOH; the concentration was measured by absorbance at 495 nm (molecular absorbance: daunomycinone=12151; ε-rhodomycinone=12870). The results of the bioconversion study with the labeled aklavinones are shown in Table 1. [9-^{14}C]-Aklavinone was converted to daunomycinone and ε-rhodomycinone without loss of specific radioactivity, while the radioactivity of [16-^{14}C]-aklavinone was not incorporated into daunomycinone. By contrast the radiolabel of the latter compound was incorporated into ε-rhodomycinone without significant loss. These results indicate that the aklavinone nucleus is incorporated into the daunomycinone skeleton and that aklavinone is metabolized to daunomycinone *via* ε-rhodomycinone. Loss of radioactivity at the C-16 position during the bioconversion to daunomycinone suggests that a 10-decarbomethoxylation is catalyzed by an enzyme present in the daunomycin-producing strain. From these results a possible biosynthetic pathway from ^{14}C-labeled aklavinone to daunomycinone can be proposed as illustrated in Fig. 1. Additional support for this route is provided by the finding that in the bioconversion of 1-hydroxy-ε-rhodomycinone (ε-isorhodomycinone) with *S. coeruleorubidus* ME130-A4, strain 1U-222, 1-hydroxy-daunomycin was produced.[4]

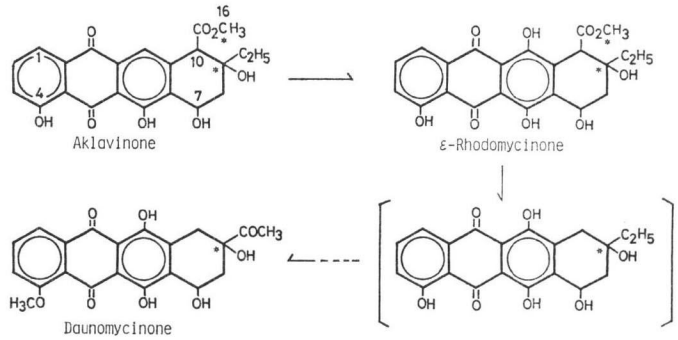
The experimental results described above suggest that aklavinone or its demethyl derivative is the biosynthetic precursor of daunomycinone. The difference in the present results from those reported by BLUMAUEROVA *et al.*[5] may be attributed to a difference in the mutants employed. Thus mutant used by them may lack an enzyme which hydrolyzes the ester bond in aklavinone.

References

1) TAKAHASHI, Y.; H. NAGANAWA, T. TAKEUCHI, H. UMEZAWA, T. KOMIYAMA, T. OKI & T. INUI: The structures of baumycins A1, A2, B1, B2,

Fig. 1. A possible biosynthetic pathway for synthesis of daunomycinone from ^{14}C-aklavinone by *S. coeruleorubidus*.

Symbol (*) represents the position of the ^{14}C-labeled carbon.



C1 and C2. J. Antibiotics 30: 622~624, 1977

2) KOMIYAMA, T.; Y. MATSUZAWA, T. OKI, T. INUI, Y. TAKAHASHI, H. NAGANAWA, T. TAKEUCHI & H. UMEZAWA: Baumycins, new antitumor antibiotics related to daunomycin. J. Antibiotics 30: 619~621, 1977

3) YOSHIMOTO, A.; T. OKI, T. TAKEUCHI & H. UMEZAWA: Microbial conversion of anthracyclinones to daunomycin by blocked mutants of *Streptomyces coeruleorubidus*. J. Antibiotics 33: 1158~1166, 1980

4) YOSHIMOTO, A.; Y. MATSUZAWA, T. OKI, H. NAGANAWA, T. TAKEUCHI & H. UMEZAWA: Microbial conversion of ε-pyrromycinone and ε-isorhodomycinone to 1-hydroxy-13-dihydrodaunomycin and N-formyl-1-hydroxy-13-dihydrodaunomycin and their bioactivities. J. Antibiotics 33: 1150~1157, 1980

5) BLUMAUEROVA, M.; E. KRALOVCOVA, D. HOSTALEK & Z. VANEK: Intra- and interspecific cosynthetic activity of mutants of *Streptomyces coeruleorubidus* and *Streptomyces galilaeus* impaired in the biosynthesis of anthracyclines. Folia Microbiol. 24: 128~135, 1979