MICROBIAL TRANSFORMATION OF AKLANONIC ACID, A POTENTIAL EARLY INTERMEDIATE IN THE BIOSYNTHESIS OF ANTHRACYCLINES

Sir:

Recently, we isolated a new pigment from fermentations of *Streptomyces* sp. ZIMET 43717[1]. The close structural relationship of this compound (aklanonic acid, **Ia**, Fig. 1) to anthracyclinones induced us to attempt its biotransformation to anthracyclines by daunorubicin-negative mutants derived from different daunorubicin producing organisms. The results of our experiments provided evidence that aklanonic acid very probably is an early intermediate in the biosynthetic pathway of anthracyclines.

Thus, aklanonic acid, its methyl ester, and aklanone, a chemical conversion product of aklanonic acid (Fig. 1)[1], were fed to fermentations of the mutant strains O_1P_7 and $1P_5$. Both mutant strains were isolated after *N*-methyl-*N'*-nitro-*N'*-nitrosoguanidine treatment of daunorubicin-producing *Streptomyces griseus* strains. Strain O_1P_7 was derived from IMET JA 5142. Strain $1P_5$ is a negative mutant of strain 1P which produces a mixture of new red and blue pigments[2]. The chemical structures of the two main red components designated 1P/I and 1P/II[3] are shown in Fig. 1.

Fermentations of the strains O_1P_7 and $1P_5$ were carried out as described[2] in 500-ml flasks each containing 80 ml of medium for 24 hours. Then 3 ml of a solution of substrate in methanol (1 mg/ml) were added to each flask and cultivation continued for 24 hours. Extracts of mycelium with acetone and chloroform (1:1) were purified by chromatography on KH_2PO_4 buffered silica gel (chloroform - acetone, 10:2). Identification of the biotransformation products was performed as indicated in Table 1. Glycosides extracted from the culture filtrate were hydrolyzed with 0.1 N HCl. Compound 1P/II was purified by column chromatography on silica gel with benzene.

The results are listed in Table 1. Aklanonic acid as well as its methyl ester were transformed by mutant O_1P_7 to give ε-rhodomycinone, 7-deoxy-ε-rhodomycinone and glycosides of dauno-

Fig. 1.

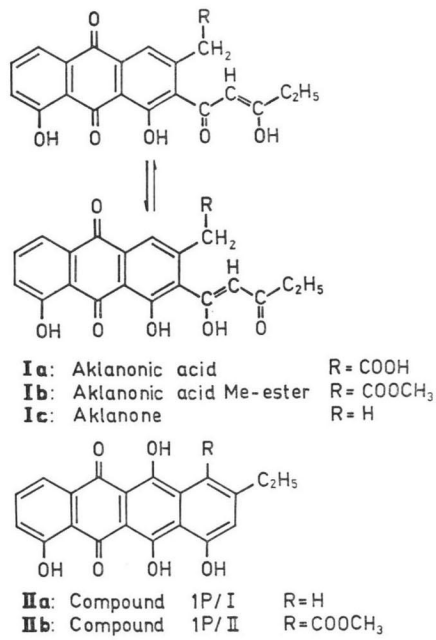

Table 1. Microbial conversion of aklanonic acid, aklanonic acid methyl ester and aklanone by anthracycline- and anthracyclinone-negative mutants.

Substrate	Blocked mutant	Conversion products
Aklanonic acid	O_1P_7	ε-Rhodomycinone[a,b,d], 7-deoxy-ε-rhodomycinone[d], daunomycinone[b,d,e]
Aklanonic acid methyl ester	O_1P_7	ε-Rhodomycinone[b,c,d]
Aklanone	O_1P_7	—
Aklanonic acid	$1P_5$	Compound 1P/II[b,c], blue pigments
Aklanonic acid methyl ester	$1P_5$	Compound 1P/II[b], blue pigments
Aklanone	$1P_5$	—

[a] Identified by ^1H NMR spectroscopy.
[b] Identified by TLC.
[c] Identified by IR spectroscopy.
[d] Identified by high resolution mass spectrometry.
[e] From hydrolysates of glycosides isolated from the culture filtrate. Glycosides not identified.

mycinone. Both precursors were converted by strain $1P_5$ to give compound 1P/II (Fig. 1) as well as blue and other pigments chromatographically identical with those found in the extracts of the parent strain 1P[2].

Feeding of mutants blocked in the biosynthesis of parent anthracyclines with presumptive intermediates is an useful method to study the biosynthetic sequences. On the basis of such experiments several biogenetic schemes have been advanced[4,5,6]. However, very little is known about the intermediates prior to the ring closure giving the tetracyclic carbon skeleton. Two early intermediates have been postulated[4]. Recently, several anthraquinone compounds related to aklanonic acid were isolated from mutants of *Streptomyces coeruleorubidus* and *Streptomyces galilaeus*, respectively[7,8,9], and the authors suggested that these compounds may play a role as precursors[4,9]. Our findings that aklanonic acid was transformed to ε-rhodomycinone and daunomycinone give strong support to these ideas. Furthermore, from the fact that aklanonic acid can serve as precursor for daunomycinone glycosides as well as for compound 1P/II, which requires different biogenetic steps, it appears that the anthraquinone intermediates at this early stage are polyvalent precursors. Probably the different tautomeric forms of aklanonic acid (Fig. 1, additional keto form not indicated) are not equally susceptible to the enzyme reactions leading to different routes of biogenesis (*e.g. via* different ways of ring closure). Aklanone was not accepted as a precursor which is in good accordance with the decaketide hypothesis.

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