NOTES

ERYTHRONOLIDE A GLYCOSIDATION TO ERYTHROMYCIN A BY A BLOCKED MUTANT OF *STREPTOMYCES ERYTHRAEUS*

ROBERTO SPAGNOLI* and
LUCIANO TOSCANO**

*Department of Microbiology
**Department of Chemistry
Pierrel S.p.A Research Laboratories
Via Degli Artigianelli, 10, 20159 Milan, Italy

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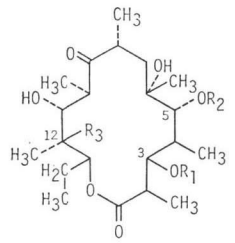
The commonly accepted biosynthetic pathway[1] leading to erythromycin A (I) requires that substrate for sugars (L-mycarose and D-desosamine) attachment should be erythronolide B (IV). Once both sugars are linked to the aglycone, giving rise to erythromycin D, C-12 hydroxylation would occur resulting in erythromycin C production. Erythromycin C is further converted to erythromycin A by *O*-methylation of the neutral sugar (at C-3″). According to this scheme, erythronolide A (III) is not considered to be a biosynthetic intermediate. This view is supported by the fact that neither erythronolide A nor its neutral monoglycoside were ever detected and isolated in the fermentation broths of normal strains or blocked mutants of *Streptomyces erythraeus*. On the contrary the corresponding 12-deoxy compounds were found[2,3]. We investigated whether permeation barriers or strict substrate specificity could prevent conversion of erythronolide A to erythromycin A in *Streptomyces erythraeus*. Bioconversion studies using blocked mutants are often useful for this purpose, since it is known that other modified lactones, related to erythronolide B, often accept L-mycarose, giving rise to novel monoglycosides which, with a few exceptions[1,4], usually do not serve as acceptors for D-desosamine[5~7].

During our investigations, we attempted to feed erythronolide A, obtained by glycoside cleavage reaction on erythromycin A[8], to a suitable early blocked mutant of a high erythromycin producing industrial strain, incapable of synthesizing the antibiotic *de novo*, but able to convert biosynthetic precursors to erythromycin. The strain Pierrel LMC 1648 (registered as ATCC 31772) was derived from *Streptomyces erythraeus* Pierrel LMC 1056, an erythromycin producer, by UV irradiation. It was selected as a blocked mutant by the agar-disc method[9], and its ability to convert erythronolide B to erythromycin A (I) and erythromycin B (II) in variable amounts was assessed by preliminary shake flask trials.

The culture for microbial conversion of erythronolide A was prepared as follows: strain ATCC 31772 was inoculated into 100 ml of seed medium consisting of 3.0% sucrose, 0.8% corn steep solids, 0.9% soybean oil, 0.2% ammonium sulfate, 0.7% calcium carbonate, in a 500-ml Erlenmeyer flask. Following incubation for 2 days at 33°C under shaking at 220 rpm, 1.5 ml of the seed culture were inoculated into 30 ml of a medium containing 3.0% corn dextrins, 4.0% raw corn starch, 3.0% soybean meal, 2.0% soybean oil, 0.2% ammonium sulfate, 0.6% calcium carbonate, in a 250-ml Erlenmeyer flask at 33°C for 120 hours. To the culture was added 500 μg/ml of crystalline erythronolide A 24 hours after the start of fermentation. Erythronolide A was detectable in the culture filtrate up to 16 hours after the addition. It disappeared with rapid conversion to erythromycin A alone, as proven by the HPLC procedure of TSUJI *et*

Chart 1. Structures of erythromycins A (I), B (II), and erythronolides A (III), B (IV).



I: R_1=Cladinosyl, R_2=Desosaminyl, R_3=OH
II: R_1=Cladinosyl, R_2=Desosaminyl, R_3=H
III: R_1=R_2=H, R_3=OH
IV: R_1=R_2=R_3=H

al.[10], which was modified as below. Lichrosorb RP8 10 μm stainless steel column, 250×4.6 mm i.d., and UV detector at 210 nm were used. Flow rate of the mobile phase was 2.0 ml/minute, and the column was operated at 40°C. The mobile phase used for erythronolide A determination consisted of acetonitrile - 0.01 M phosphate pH 7.0, 40: 60. Retention time was 2.5 minutes. For erythromycin A determination a different mobile phase was used consisting of acetonitrile - 0.01 M phosphate pH 7.0, 64: 36. Retention time was 8.5 minutes.

Erythromycin A was isolated from the clarified filtered broth by ethyl acetate extraction at pH 7.0, and purified by column partition chromatography on silica gel[11].

An analytical dihydrate sample was prepared by successive crystallization from chloroform and from water at 50°C: mp 200~203°C[12] (the crystals were covered with silicone oil and heated in a Kofler apparatus; transformation to an anhydrate form was observed at 110~135°C); $[\alpha]_D^{20}$ −65° (*c* 1.0, methanol)[13]; λ_{max} 288 nm (ε 29.7)[14]. IR and ^{13}C NMR spectra were identical to corresponding spectra of an authentic sample of erythromycin A.

In conclusion these results provide good evidence that erythronolide A could be an intermediate of erythromycin A biosynthesis. Neither permeation barriers nor steric hindrance oppose the conversion of erythronolide A to erythromycin A under the conditions employed. Failure to obtain blocked mutants accumulating erythronolide A or 3-*O*-mycarosylerythronolide A, might possibly be explained by an inducible nature of C-12 hydroxylase formation (which would require the presence of erythromycin D to be expressed), or, alternatively, by an "assembly-line" arrangement of the concerned biosynthetic enzymes, the hydroxylase coming after both glycosidases in the sequence. Besides, we cannot exclude that, at least for some strains, a pathway other than the commonly accepted one could lead to formation of erythromycin A.

It is well known that the C-12 hydroxyl group enhances the biological activity of erythromycin[15]; correspondingly, the results presented here also point to the possibility of using certain lactones of erythronolide A type for the biological synthesis of new antibiotics by the *Streptomyces erythraeus* ATCC 31772.

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