Communications to the editor

BIOCONVERSION AND BIOSYNTHESIS OF 16-MEMBERED MACROLIDE ANTIBIOTICS XV. FINAL STEPS IN THE BIOSYNTHESIS OF LEUCOMYCINS

Sir:

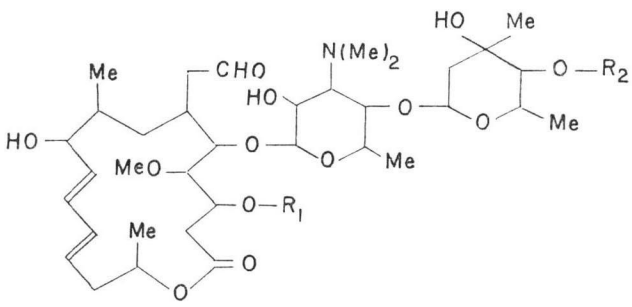
In previous studies on the bioconversion and biosynthesis of leucomycins using the enzyme inhibitor, cerulenin[1], it was found that leucomycin A_1 (LM-A_1) was readily acetylated to LM-A_3 by resting cells of *Streptomyces kitasatoensis* No. 66-14-3, one of the mutant strains which produces LM-A_1 and A_3 as main products[2,3]. The acetylation of the hydroxyl group at C-3 on the lactone ring seemed to occur at the final step of biosynthesis[2]. This paper deals with the confirmation of this process by examining the conversion of LM-V which has the hydroxyl groups at the C-3 and 4″ positions, to LM-A_3 in which the two hydroxyl groups are acylated.

Strain No. 66-14-3 was cultured at 27°C for 36 hours in a 30 liters jar fermentor containing 20 liters of a medium composed of 2.0% glucose, 0.5% meat extract, 0.5% peptone, 0.3% yeast extract, 0.5% NaCl, and 0.3% $CaCO_3$ (pH adjusted to 7.0 prior to sterilization). The mycelium was harvested by centrifugation, washed twice with saline and then 20 g of the wet mycelium was suspended in 100 ml of a medium composed of 2.0% glucose and 0.5% NaCl which was contained in a 500-ml SAKAGUCHI flask. To this cell suspension was added 100 mg of leucomycin together with 4 mg of cerulenin which inhibits leucomycin biosynthesis by the washed cells. LM-V, U, A_1 and A_3 were used for the experiments. The suspension was incubated at 27°C, and the bioconversion was examined at 1-hour intervals for 5 hours by the following procedures: The incubation mixture was extracted twice with 100 ml of ethyl acetate, and the solvent layer was concentrated *in vacuo*. The extracted material was analyzed by silica-gel thin-layer chromatography (Kieselgel 60, F_{254}, Merck, developed with chloroform - methanol - 1.5 N ammonia, 2:1:1) or by alumina thin-layer chromatography (Alumina oxide, Merck, developed with ethyl acetate).

The leucomycins were quantitatively detected by a dual-wavelength chromatogram scanner (Shimadzu Seisakusho Co., Ltd., model CS-910) at 232 nm. Each component was isolated by preparative thin-layer chromatography and identified with authentic samples of leucomycins by comparing the Rf values and by mass spectrometry. The conversion ratios of the leucomycins were calculated as the LM-Fr group (3-OH, 4″-OH or Acyl) and the LM-Ac group (3-O-Acetyl, 4″-OH or Acyl) which were clearly separated on alumina thin-layer chromatography[4], or as individual components by silica-gel thin-layer chromatography using the Rf values of the individual leucomycins.

Leucomycin V was converted to the LM-Fr group (A_1, A_5, A_7 and A_9) and LM-Ac group (U, A_3, A_4 and A_8) as shown in Fig. 2-a. The residual amount of LM-V was measured by silica-gel thin-layer chromatography. The conversion of LM-V to the LM-Fr group was more pre-

Fig. 1. Structure of leucomycins.



LM	R_1	R_2
LM-Fr A_1	H	$COCH_2CH(CH_3)_2$
A_5	H	$COCH_2CH_2CH_3$
A_7	H	$COCH_2CH_3$
A_9	H	$COCH_3$
V	H	H
LM-Ac A_3	$COCH_3$	$COCH_2CH(CH_3)_2$
A_4	$COCH_3$	$COCH_2CH_2CH_3$
A_6	$COCH_3$	$COCH_2CH_3$
A_8	$COCH_3$	$COCH_3$
U	$COCH_3$	H

Fig. 2. Bioconversion of leucomycins in the resting cells.

The resting cells were incubated with leucomycins (1 mg/ml) and cerulenin (40 μg/ml) at 27°C. The bioconversion was followed by silica-gel or alumina thin-layer chromatography and measured quantitatively by the dual wavelength chromatogram scanner at 232 nm.

* LM-V was excluded from LM-Fr. ** LM-U was excluded from LM-Ac.

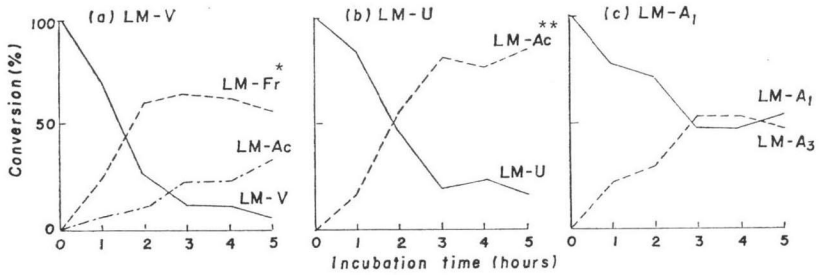
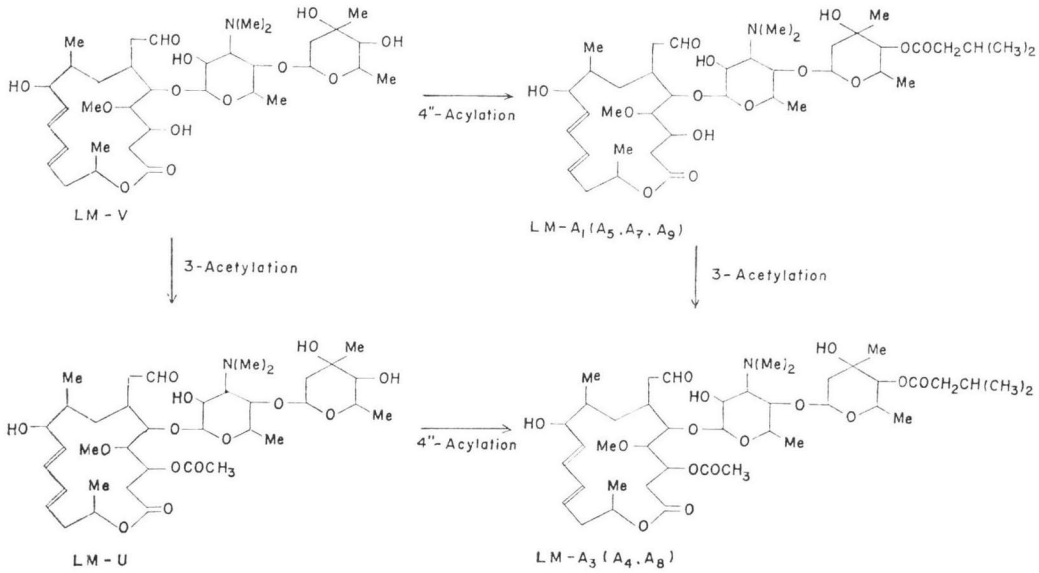


Fig. 3. Plausible biosynthetic pathways from LM-V to LM-A_3.



dominant than that to the LM-Ac group. LM-U was rapidly converted to the LM-Ac group including LM-A_3, A_4 and A_8 (Fig. 2-b). This indicates that the acylation at the 4″-position of mycarose is still predominant whether or not the 3-position of the lactone ring was acetylated. LM-A_1 also was converted to LM-A_3 (Fig. 2-c), while no reverse conversion was observed during any incubation period. Taking into consideration these results, two plausible pathways for the acylation of LM-V to LM-A_3 were considered as illustrated in Fig. 3. In contrast to the rapid conversion of LM-U to the LM-Ac group, the conversion of LM-V to the LM-Ac group was comparatively slow. The acetylation of LM-V to LM-U seemed to be a rate-limiting reaction, and this suggested that the formation of the LM-Ac group, as shown in Fig. 2-a, was mainly from LM-V *via* the LM-Fr group. From these results it was concluded that the acylation of the hydroxyl group at C-4″ on mycarose precedes the acetylation of the hydroxyl group at C-3 on the lactone ring, and that LM-A_3 is mainly derived from LM-V *via* LM-A_1.

From biosynthetic studies of the spiramycins, we have pointed out that the acylation of the hydroxyl group at C-3 on the lactone ring takes place as the final step[5,6]. As the acylating

enzyme of a spiramycin-producing strain of *Streptomyces ambofaciens* KA-1028 has a broad substrate specificity, LM-V was also acylated to LM-U by this enzyme[6]. This transformation seemed to be analogous with that by the leucomycin-producing strain. By contrast, FURUMAI *et al.*[7] have reported from biosynthetic studies of the platenomycins that the acylation of the hydroxyl group at C-3 takes place before the linking of the mycaminose moiety to the lactone ring, and that the acylation of the hydroxyl group at C-4'' on mycarose is the final step of the biosynthetic process. Although the acylating enzyme in the leucomycin-producing strain has not been investigated yet *in vitro*, the comparative studies of these enzymes will give us interesting information not only for making biosynthetic correlations among these macrolide antibiotics, but also for genetic correlations with these antibiotic-producing strains.

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