NOTES

MICROBIAL TRANSFORMATION OF LEUCOMYCIN A_5

KARTAR SINGH and SUMANAS RAKHIT
Ayerst Research Laboratories
Montreal, Quebec, Canada

(Received for publication February 25, 1978)

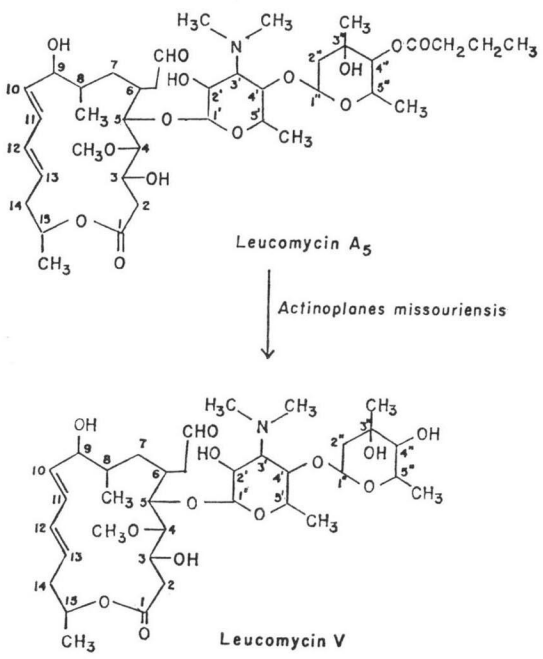
In an earlier paper from our laboratory[1], antimicrobial activities of various leucomycin (kitasamycin) congeners were compared. In continuation of this work, we tested a large number of microorganisms for their ability to transform leucomycin. Of these, *Actinoplanes missouriensis** (IMRU-824, AY B-866) was found to be the most effective in transforming leucomycin and was used for the present study on transformation of leucomycin A_5.

Leucomycin A_5 conversion was followed by thin-layer chromatography on silica gel, Merck F-254 plates using chloroform – methanol – acetic acid – water (158: 22: 16: 4). The plates were sprayed with 10% H_2SO_4 and heated for 10~15 minutes at 120°C.

The organism was grown in a medium containing 3% glycerol and 3% Special X soy flour (Archer Daniels Midland Co., Minneapolis) for 48~72 hours at 30°C on a rotary shaker; leucomycin was added and incubation continued for an additional 48~72 hours. Alternatively, after 48~72 hours of growth, the cells were collected by centrifugation, washed twice with water and once with 0.02 M phosphate buffer, pH 7.2. They were suspended in the original volume of this buffer; leucomycin was added, and the mixture incubated at 30°C on a rotary shaker. In preliminary experiments, we found that leucomycin-transforming activity of the cells was greatly enhanced if the cells were induced with 10 μg/ml demycarosyl 3-deacetyl leucomycin for 12~24 hours. Using induced cells, 1 mg/ml of leucomycin A_5 was completely transformed in 48~72 hours into one main, more polar product. Demycarosyl 3-deacetyl leucomycin could not be replaced by mycarose, leucomycin complex or the individual components A_1, A_3 or A_5 as an inducer and was routinely used at 10 μg/ml.

A preparative scale conversion was carried out with leucomycin A_5. Ten 2-liter Erlenmeyer flasks, each containing 400 ml of medium, were inoculated with *A. missouriensis* from agar slants and incubated on a rotary shaker at 30°C for 66 hours. To each flask, 4 mg of demycarosyl 3-deacetyl leucomycin (dissolved in water and filtered through a Millipore 0.45 μm filter) were added. After 24 hours of further incubation, the cells were collected and washed by centrifugation. The washed cells were suspended in 4 liters of 0.02 M phosphate buffer, pH 7.2, and 4 g leucomycin A_5 dissolved in 40 ml of acetone were added. The suspension was distributed into ten 2-liter Erlenmeyer flasks, and the flasks incubated at 30°C on a rotary shaker. After an additional 66 hours of incubation, the reaction mixture was cooled to 5~10°C, adjusted to pH 5.0 with 1 N HCl, and filtered through a bed of Celite. The filtrate was adjusted to pH 8.5 with 1 M Na_2CO_3 and extracted twice with 2 liters of ethylacetate. The combined solvent extracts were washed with 400 ml of water, dried with anhydrous Na_2SO_4

Fig. 1. Transformation of leucomycin A_5



* This organism was obtained from Dr. RUTH GORDON, Institute of Microbiology, Rutgers University, New Brunswick, N.J., U.S.A.

and evaporated to dryness to yield 2.5 g of a product with weak antimicrobial activity (Table 1).

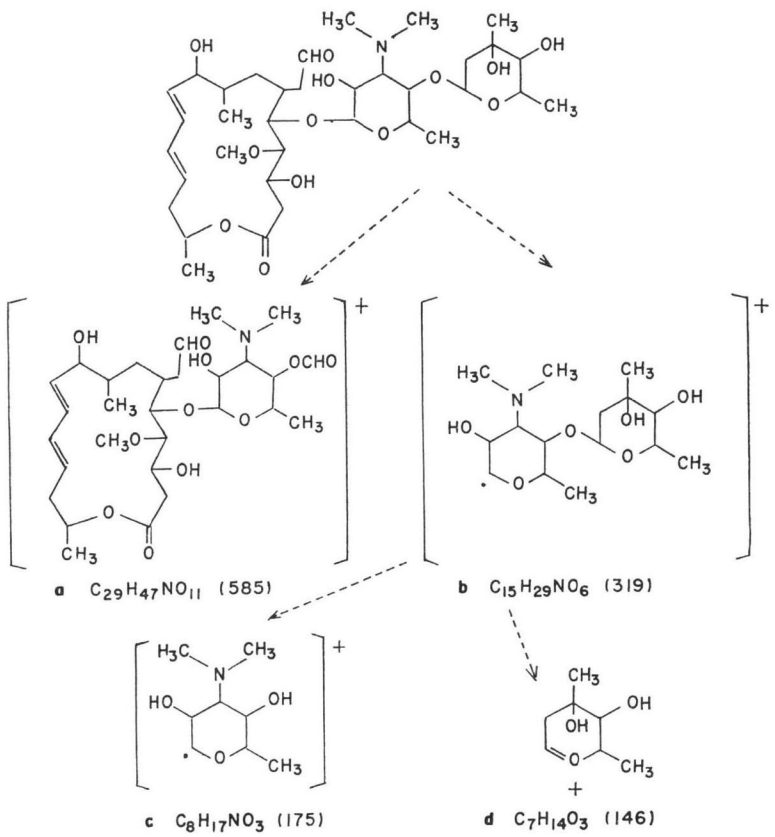
The product was further purified by chromatography over Al_2O_3 (activity III), with 30% ethyl acetate in benzene to afford 1.8 g of pure transformation product, m.p. 68~70°C, λ_{max}^{MeOH} 232 nm ($E_{1cm}^{1\%}$ 305); NMR $\delta CDCl_3$, C<u>H</u>O, 9.6; OC<u>H</u>$_3$, 3.5; N(C<u>H</u>$_3$)$_2$ 2.5; no acyl bands. From the NMR and the UV spectra, it was indicated that this product contained the parent diene chromophore and the aldehydic and mycaminose moieties were unchanged. The mass spectrum (MS) of the product showed a weak molecular ion peak characteristic of other macrolides at *m/e* 701 indicating the loss of the butyryl side chain on the mycarose moiety[2]. The appearance of other fragments (Fig. 2) at *m/e* 585 ($C_{29}H_{47}NO_{11}$) *a*, 319 ($C_{15}H_{29}NO_6$) for *b*, 175 ($C_8H_{17}NO_3$) for *c* and 146 ($C_7H_{14}O_3$) for *d* indicates the transformation product is deacyl leucomycin A_5 (leucomycin V)[3]. The product was acetylated by acetic anhydride and pyridine at 50°C for 16 hours and purified by chromatography over silica gel (30% acetone in benzene). The identity of the acetylated product with diacetyl leucomycin A_5 pre-

Table 1. Minimum inhibitory concentration (MIC)* of leucomycin A_5 and its transformation product

Substrate and product	Minimum inhibitory concentration (μg/ml)		
	Staphylococcus pyogenes pen S	*Staphylococcus pyogenes* pen R	*Streptococcus faecalis*
Leucomycin A_5	0.8	3.2	0.8
Transformation product of leucomycin A_5	50	100	12.5

* MIC was determined by the usual serial, two-fold, tube dilution method in nutrient broth.

Fig. 2. Plausible fragmentation of deacylated leucomycin A_5



pared similary from leucomycin A_8[2] was established by comparing their NMR and mass spectra, which further confirms the identity of the transformation product.

Acknowledgements

We are grateful to Dr. D. KLUEPFEL for supplying us with various leucomycins, and to Dr. G. SCHILLING and his associates for the spectral analyses. Technical assistance of Mrs. SHEILA SUN and Mrs. ANGELE RICHARD is gratefully acknowledged.

References

1) RAKHIT, S. & K. SINGH: Structure activity relationship in sixteen membered macrolide antibiotics. J. Antibiotics 27: 221~224, 1974
2) INOUYE, S.; T. TSURUOKA, T. SHOMURA, S. OMOTO & T. NIIDA: Studies on antibiotic SF-837, a new antibiotic. II. Chemical structure of antibiotic SF-837. J. Antibiotics 24: 460~475, 1971
3) ŌMURA, S. & A. NAKAGAWA: Chemical and biological studies on 16-membered macrolide antibiotics. J. Antibiotics 28: 401~433, 1975