THE MICROBIAL TRANSFORMATION OF TYLOSIN BY THE SPIRAMYCIN-PRODUCING STRAIN, *STREPTOMYCES AMBOFACIENS* KA-1028*

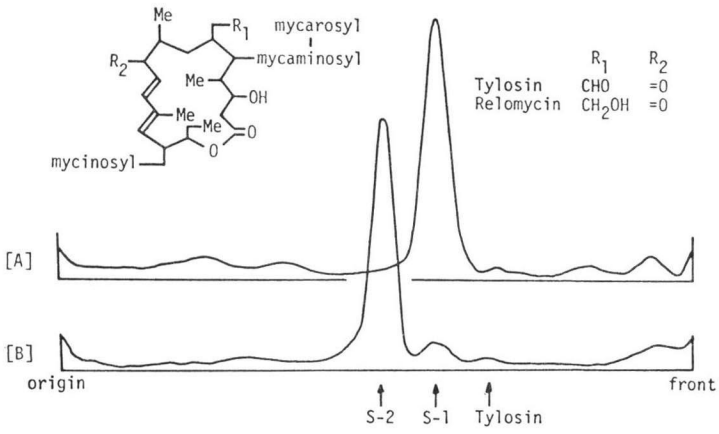
Sir:

In previous papers[1~4], we have pointed out the usefulness of cerulenin[5], a specific inhibitor of fatty acid and polyketide biosyntheses, in examining the biosynthesis of 16-membered macrolide antibiotics. Furthermore, the biosynthetic investigation suggested that the bioconversion using a microorganism which is a producer of an other macrolide antibiotic and in the presence of cerulenin seemed to be useful for producing new related macrolides. The present communication describes the microbial transformation of tylosin to a new compound by a spiramycin-producing strain, *Streptomyces ambofaciens* KA-1028, under conditions in which spiramycin biosynthesis is inhibited by cerulenin.

Strain KA-1028 was cultured in a 500-ml SAKAGUCHI flask containing 100 ml of spiramycin production medium composed of 1.0% glucose, 1.0% dried yeast, 0.1% $NaNO_3$, 0.5% NaCl and 1.0% $CaCO_3$ (adjusted to pH 7.5 with 2 N NaOH prior to autoclaving). In order to inhibit production of the spiramycins, 40 μg/ml of cerulenin was added to the medium at the beginning and every 24-hour intervals. After 48-hour cultivation at 27°C, 100 μg/ml of tylosin was added to the culture, and the cultivation was continued for additional 24 hours. The cultured broth was centrifuged to remove mycelia and the supernatant was extracted with benzene. The benzene layer was concentrated to dryness *in vacuo* and the residue was purified by silica gel TLC (Kieselgel 60 F_{254}, Merck) which was developed with $CHCl_3$-MeOH - 1.5 N NH_4OH (2: 1: 1, bottom layer) to give tylosin-related compounds. The microbial transformation of tylosin was monitored by a dual wavelength chromatogram scanner (Model CS-910, Shimadzu Seisakusho Co., Ltd.) scanned at 232 nm and 282 nm. As shown in Fig. 1, two major peaks, S-1 and S-2, were detected on the chromatogram. Both compounds were isolated by means of preparative silica gel TLC which was developed with the same solvent system described above. The compound S-1 showed a UV absorption maximum at 282 nm indicating the presence of $\alpha,\beta,\gamma,\delta$-unsaturated ketone. It was identified as 20-dihydrotylosin (relomycin), one of the major components produced by the tylosin-producing strain of *Streptomyces fradiae*[2], by comparison of silica gel TLC, IR and mass spectroscopies with those of authentic relomycin. The second compound, S-2, exhibited a UV ab-

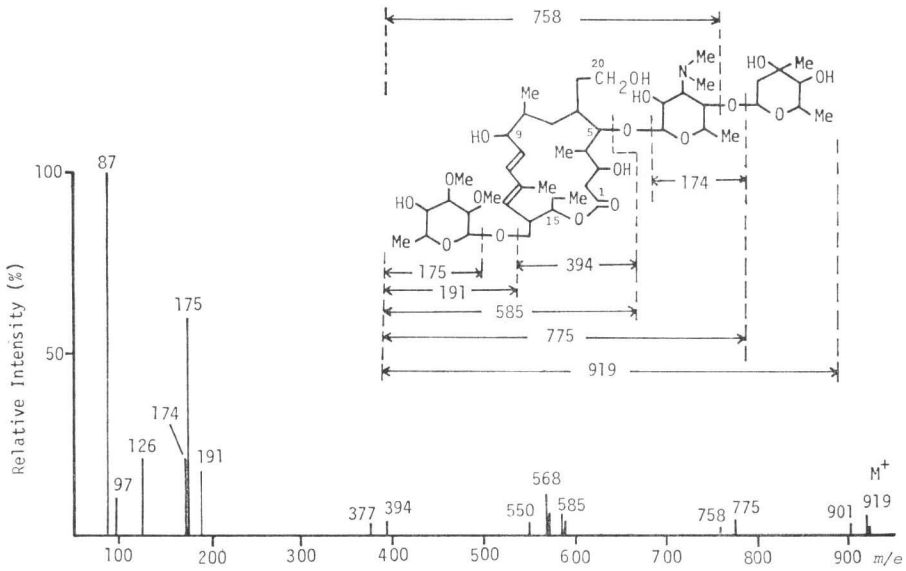
Fig. 1. Bioconversion of tylosin by spiramycin-producing *S. ambofaciens* KA-1028.

Tylosin was added to the cerulenin-supplemented culture of KA-1028. After extraction with benzene, the bioconversion was examined by silica gel TLC which was scanned [A]: at 282 nm, [B]: at 232 nm.



* Bioconversion and biosynthesis of 16-membered macrolide antibiotics. Part XVII. Part XVI of this series appears in: S. ŌMURA, C. KITAO & H. MATSUBARA, Chem. Pharm. Bull. 28: 1963~1965, 1980

Fig. 2. Mass spectrum of 9,20-tetrahydrotylosin.



sorption maximum at 231.5 nm (log ε=4.418 in MeOH), suggesting the presence of a conjugated diene corresponding to the leucomycin or spiramycin family of antibiotics. The infrared spectrum of compound S-2 showed a lack of bands at 2720 and 2820 cm^{-1}, characteristic of the aldehyde group at the C-20 position in tylosin. The compound S-2 was identified as 9,20-tetrahydrotylosin from the mass spectra; *m/e* 919 (M^+), 901 (M^+-H_2O), 775 (M^+—mycarosyl), 585 (M^+—mycarosylmycaminosyl), 394 (aglycone), and 175 (mycinosyl) as shown in Fig. 2.

9, 20-Tetrahydrotylosin showed weak antimicrobial activity against Gram-positive bacteria (1/15 potency of tylosin in MIC against *Sarcina lutea* PCI 1001), but no activity against Gram-negative bacteria.

It is of interest that *S. ambofaciens* KA-1028 has no ability to reduce the aldehyde group of its own antibiotic, spiramycin, under the same condition described above.

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