# HYBRID BIOSYNTHESIS AND ABSOLUTE CONFIGURATION OF MACROLIDE ANTIBIOTIC M-4365 $G_1$

Sir:

Mutasynthesis has been applied in hybrid biosynthesis of a veriety of antibiotics such as aminoglycosides, macrolides, and penicillins[1]. However it is time-consuming and troublesome to obtain an idiotroph suitable for the biosynthetic design of a new antibiotic. As an alternative to the idiotroph method, we have designed a simple method employing an eznyme inhibitor and antibiotic producer. Fig. 1 shows the comparison of mutasynthesis with the new method for hybrid biosynthesis. The enzyme inhibitor prevents the biosynthesis of intermediate B which combines with another intermediate A to form antibiotic AB. Compound B′, which is supplemented in the medium, and an analogue of intermediate B, is incorporated into a new antibiotic AB′. In this novel method, the inhibitor must be specific for an enzyme involved in the biosynthesis of an intermediate.

Cerulenin is a specific inhibitor of *de novo* fatty acid and "polyketide" biosyntheses[2]. We have studied the biosynthesis of antibiotics such as spiramycin[3], tylosin[4], leucomycin[5] and nanaomycin[6] with the aid of cerulenin. More recently, we reported on the biotransformation of the 16-membered macrolide, tylosin by a producer of spiramycin, which is another 16-membered macrolide, under culture conditions producing inhibition of the biosynthesis of its own antibiotic[7].

This communication reports the "hybrid" biosynthesis of desosaminyl protylonolide which is identified as antibiotic M-4365 $G_1$. A plane structure of M-4365 $G_1$, one of the components of antibiotic M-4365 (Fig. 2) from *Micromonospora capillata* NCRL 0940 reported by OKUDA *et al.*[8], consists of desosamine and protylonolide which is a metabolite of a blocked mutant obtained from a tylosin producer *Streptomyces fradiae* KA-427[10]. The absolute configuration of M-4365 $G_1$ can be identified either by comparing the aglycone obtained by the hydrolysis of M-4365 $G_1$ with the protylonolide whose absolute configuration has already been determined[11] or by comparing the properties of M-4365 $G_1$ itself with those of the desosaminyl protylonolide. We obtained $\alpha$-D-desosaminyl protylonolide, which was identified as M-4365 $G_1$, from protylonolide by the new hybrid bio-

Fig. 2. Structures of M-4365 $G_1$, $G_2$ and $G_3$.

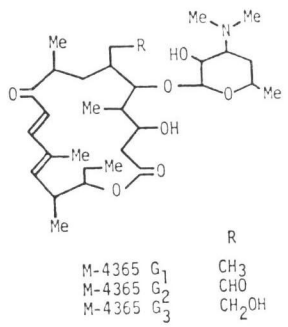
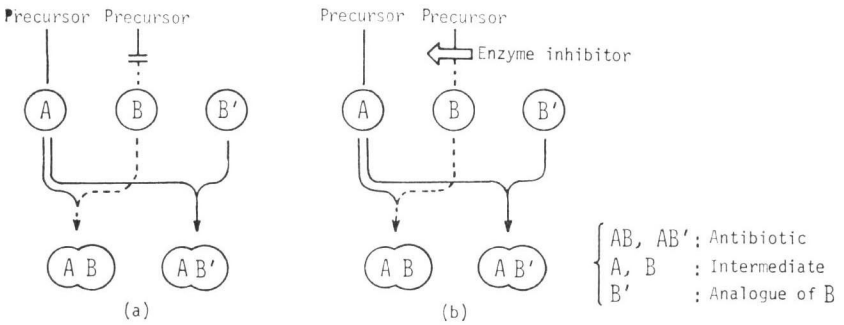



Fig. 1. Methods for hybrid biosynthesis.
(a) Mutasynthesis using B idiotroph.
(b) New hybrid biosynthesis using enzyme inhibitor and antibiotic producer.




* Bioconversion and biosynthesis of 16-membered macrolide antibiotics. XX. Part XIX of this series appears in: S. ŌMURA, Y. TANAKA, H. TANAKA, Y. TAKAHASHI & Y. IWAI: J. Antibiotics 33: 1568~1569, 1980.

Fig. 3. Hybrid biosynthesis of M-4365 $G_1$ from protylonolide by picromycin producer (*Streptomyces* sp. AM-4900) in the presence of cerulenin.

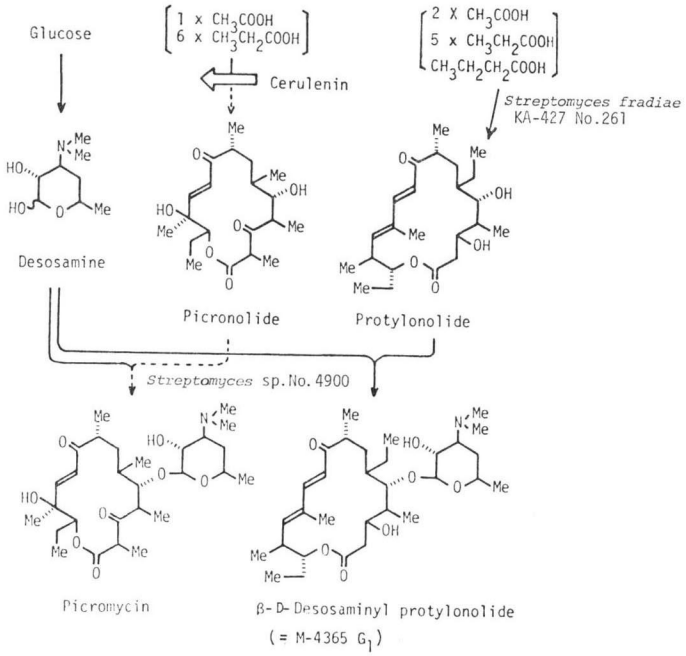
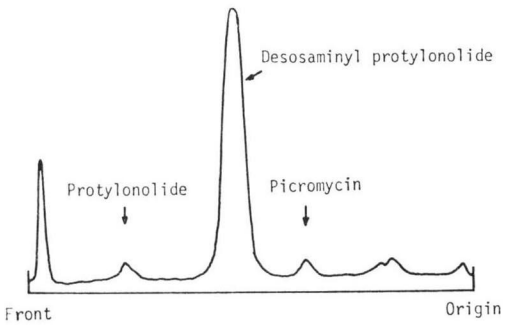



Fig. 4. Silica gel TLC of the extract in the hybrid biosynthesis of M-4365 $G_1$ from protylonolide.

Solvent system; $CHCl_3$ - MeOH - conc. $NH_3$= 20: 1: 0.01, detected by chromatogram scanner at 282 nm.




synthesis instead of hydrolysis of M-4365 $G_1$ to obtain the aglycone, since the glycosides of aminosugars generally resist acid hydrolysis.

A macrolide antibiotic, picromycin, is biosynthesized from two kinds of intermediates; picronolide, which is one of the polyketide compounds whose biosynthesis is inhibited by cerulenin, and the deoxysugar desosamine derived from glucose (Fig. 3)[12,13]. The capacity for the biosynthesis of and glycosidation with desosamine of a picromycin producer, *Streptomyces* sp. No. 4900 (a soil isolate), was tested in the hybrid biosynthesis of desosaminyl protylonolide.

A seed culture was prepared by shaking a culture of *Streptomyces* sp. No. 4900 in a medium (in %: glucose 2, meat extract 0.5, peptone 0.5, dried yeast 0.3, NaCl 0.5, $CaCO_3$ 0.3, pH 7.0) for 2 days at 27°C. The seed culture (inoculum size, 1%) was transferred into a 500-ml SAKAGUCHI flask containing 100 ml of a production medium (in %: oat meal 6.0, Pharmamedia 0.4, $CaCO_3$ 0.2, $CaSO_4 \cdot 2H_2O$ 0.1, $CaCl_2 \cdot 2H_2O$ 0.05, $ZnSO_4 \cdot 7H_2O$ 0.03, $MgSO_4 \cdot 7H_2O$ 0.01, $FeSO_4 \cdot 7H_2O$ 0.1, pH 7.5). To the culture medium 40 $\mu$g/ml of cerulenin was added at the beginning and every 24 hours thereafter to prevent the formation of picronolide. After 2 days of cultivation, 50 $\mu$g/ml of protylonolide was added and the culture was incubated for additional 4 days. The cultured broth was collected, centrifuged at 3,000 rpm for 10 minutes, extracted with benzene, and then the extract was concentrated *in vacuo*. A TLC analysis of the extract is shown as Fig. 4. The mixture of the products was subjected to silica gel column chromatography, eluting with the solvent system; chloroform - methanol - conc. ammonia (20: 1: 0.01),

and preparative silica gel TLC using the same solvent system. Thirty mg of a white powder of desosaminyl protylonolide was obtained from a 4-liter culture to which 200 mg of protylonolide was added. The physicochemical properties (Mass spectrum: $m/z$ 551 ($M^+$), 533 ($M^+-H_2O$), 377 (aglycone), and 174 (desosamine), $^1$H-NMR: $\delta$ 4.2 (1H, $J$=8.0 Hz, anomeric proton), $\delta$ 2.25 (6H, dimethylamino group)) indicated that desosamine was attached to protylonolide in glycosidic linkage. These results and CD data ($[\theta]_{225}$ $0.7\times10^{-2}$, $[\theta]_{285}$ $1.5\times10^{-3}$ and $[\theta]_{340}$ $0.6\times10^{-3}$) were identical to those of an authentic sample of M-4365 $G_1$ from *Micromonospora capillata* NCRL 0940. From the above results, it was concluded that the absolute configuration of the aglycone of antibiotic M-4365 G is identical with that of tylosin[11].

In this communication we have described a new simple method of "hybrid" biosynthesis, which is useful as an alternative to mutasynthesis. Although the "hybrid" biosynthesis of macrolide M-4365 $G_1$ was effectively carried out with the aid of the polyketide inhibitor, cerulenin, new specific enzyme inhibitors are desired to extend the method for the biosynthesis of other antibiotics.

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