# PRODUCTION OF MYCAROSYL PROTYLONOLIDE BY A MYCAMINOSE IDIOTROPH FROM THE TYLOSIN-PRODUCING STRAIN *STREPTOMYCES FRADIAE* KA-427*

Sir:

We have reported the isolation of a mutant, strain No. 261, which produces a 16-membered lactone named protylonolide (**1**), by the treatment of the tylosin-producing strain *Streptomyces fradiae* KA-427[1] with *N*-methyl-*N'*-nitro-*N*-nitrosoguanidine. This communication deals with the isolation of another new metabolite, named mycarosyl protylonolide (**2**), which is an interesting intermediate in tylosin biosynthesis, from the culture filtrate of mutant No. 261. We also provide evidence that mutant No. 261 is an idiotroph of mycaminose.

A seed culture was prepared by shake culture of *S. fradiae* KA-427 No. 261 for 3 days at 27°C. The production medium contained 1.0% glucose, 2.0% starch, 0.5% peptone, 0.5% yeast extract, 0.3% L-asparagine, and 0.4% $CaCO_3$ (pH 7.4). The seed culture (inoculum size; 1%) was transferred into a 50-liter jar fermentor containing 35 liters of the production medium, and then incubated at 27°C with aeration. Maximum production of **2** was attained at 3 days. Metabolite **2** was extracted with benzene from the culture filtrate. The extract was concentrated to dryness to afford a crude material. The material was purified by high performance liquid chromatography (solvent; chloroform - methanol, 70: 1), followed by preparative thin-layer chromatography (solvent; chloroform - methanol, 30: 1) on silica gel to provide mycarosyl protylonolide (**2**) as a white powder. The compound was crystallized from a mixture of benzene and cyclohexane to give colorless needles, mp. 163°C, $[\alpha]_D^{24}$ −23.4° (*c* 1.0, $CHCl_3$).

The molecular formula of **2** was established to be $C_{30}H_{50}O_8$ by mass spectrum ($M^+$, *m/e* 538.3519), elemental analysis (C, 65.03; H, 9.05%) and $^{13}C$-NMR spectral analysis. The UV absorption maximum at 283 nm (ε, 17,580) indicates the existence of α, β, γ, δ-unsaturated ketone. The hydrolysis of **2** with 1 N HCl-methanol gave its aglycone and a neutral sugar moiety. The aglycone moiety was identified as protylonolide, which was coproduced in the fermentation broth, by comparison of both IR and mass spectra. With regard to the neutral sugar, **2** was evidenced to be identical with α,β-*O*-methylmycaroside obtained by methanolysis of tylosin (**4**).

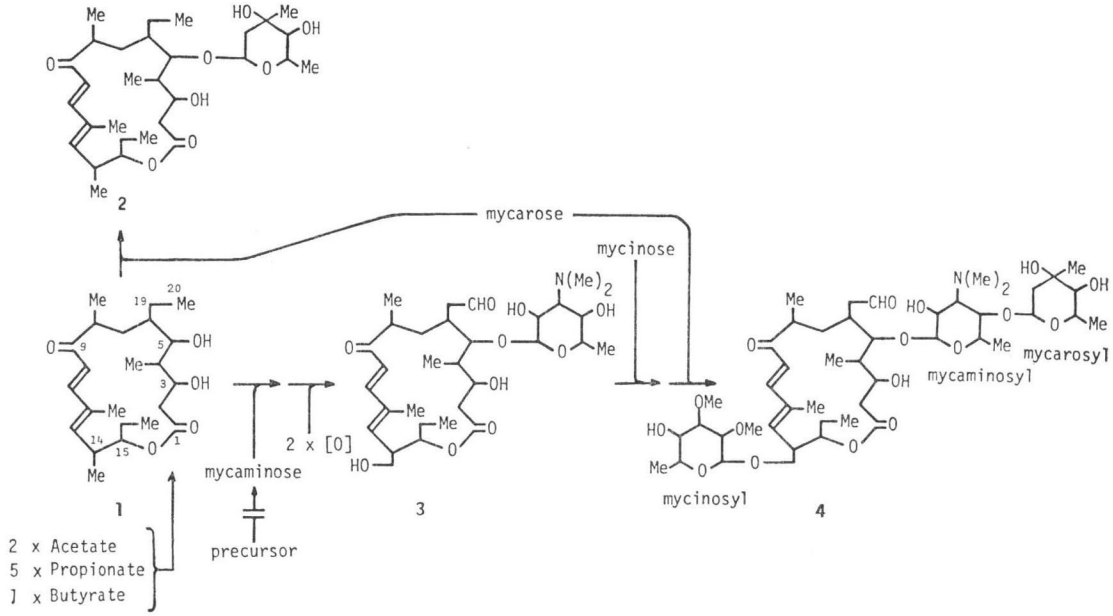
The assignment[2] as to whether the mycarose portion is attached to the oxygen atom at C-3 or C-5 on the lactone ring was carried out by comparison of the $^{13}C$ shift value of a lactone ester carbonyl at C-1 of **2** with those of the related compounds. The ester carbonyl in **4** (δ 173.9), cirramycin $A_1$(δ 173.5) or leucomycin $A_5$ (δ 173.5), in which the hydroxyl group is placed in C-3, was observed at about 4 ppm lower field than that of leucomycin $A_3$ (δ 169.9) or spiramycin II (δ 169.9) which have an acetoxyl group at the same position. Observation of the ester carbonyl at δ 174.2 in **2** led to the conclusion that the mycarose portion must be attached to the oxygen atom at C-5 on the lactone ring. The α glycosidic linkage of mycarose was evidenced from observation[3] of anomeric coupling constant $J_{^{13}C-^1H}$ value of 166.5 Hz at δ 100.1 Thus, the structure of **2** could be assigned to α-mycarosyl protylonolide as shown in Chart 1.

Metabolites related to **4** (others than **1** and **2**) were not detected in the culture filtrate of mutant No. 261. The mutant was capable of transforming mycaminosyl tylonolide (**3**), which was obtained by acidic hydrolysis of **4**, to **4**. These evidences suggest that mutant No. 261 would be an idiotroph which requires mycaminose for the biosynthesis of **4**. We therefore examined the biosynthesis of **4** by strain No. 261 under supplementation with mycaminose. To the 2-day culture of the mutant mycaminose (100 μg/ml) was added, and incubation continued for 2 days at 27°C with aeration. The compound isolated from the culture filtrate possessed antimicrobial activity, and was identified as **4** by silica gel TLC, IR and mass spectroscopies. From these results, it was concluded that the mutant is a mycaminose idiotroph. The biosyntheses of compounds **1** and **2** and the bioconversion of **3** to **4** by the mutant are shown in Chart 1.

FURUMAI *et al.*[4] have reported that the oxidation of the ethyl group at C-6 to a formylmethyl group takes place after glycosidation with mycaminose at the C-5 position on the lactone ring,

* Bioconversion and biosynthesis of 16-membered macrolide antibiotics. XVIII. Part XVII of this series appears in: S. ŌMURA, C. KITAO and N. SADAKANE, J. Antibiotics 33: 911~912, 1980

Chart 1. Biosyntheses of protylonolide (**1**) and mycarosyl protylonolide (**2**) and bioconversion of mycaminosyl tylonolide (**3**) to tylosin (**4**) by *S. fradiae* KA-427 No. 261 (mycaminose idiotroph).




plateno lide, an intermediate in the biosynthesis of platenomycin. The isolation of **2** from the mycaminose idiotroph provides important evidence in considering some properties of the enzymes which catalyze glycosidation of mycarose to the aglycone and oxidation of the C-19 methyl group. The former enzyme might be toler ant and the latter very specific for the substrate. Therefore, mycarosyl protylonolide, in which mycarose is attached to the C-5 hydroxyl, would be rejected by the enzyme which catalyzed subsequent oxidation of C-19 methyl group to aldehyde group. This is not in contradistinction to the biosynthetic pathway proposed by the authors[5] from the results of the biosynthetic study of **4** using the antibiotic cerulenin, a specific inhibitor of fatty acid and polyketide biosyntheses.

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