BIOSYNTHESIS OF MYCINAMICINS BY A BLOCKED MUTANT OF *MICROMONOSPORA GRISEORUBIDA*

Sir:

Mycinamicin II (**2**) is a 16-membered macrolide antibiotic produced by *Micromonospora griseorubida*, which has strong antibacterial activity against Gram-positive bacteria[1]. Cultivation of *M. griseorubida* causes accumulation of other macrolide compounds in addition to mycinamicin II (**2**)[2~4]. These are structurally classified as basic compounds (mycinamicins) or neutral compounds (5-*O*-dedesosaminyl-mycinamicins), considered to be biosynthetic intermediates of mycinamicin II (**2**).

During biosynthetic studies on mycinamicins, we isolated a blocked mutant C-34-10 of a high mycinamicin II (**2**) producing industrial strain, which produces potential intermediate for formation of protomycinolide IV (**9**)[5]. Bioconversion experiments using blocked mutants are often useful for the approach to exploring the biosynthetic pathway. Biosynthesis of tylosin was extensively studied by ŌMURA *et al.*[6] and Eli Lilly Company's researchers[7]. Latter group investigated that tylosin blocked mutants which produce no macrolide compounds could make tylosin if provided with tylactone or other macrolide intermediates of tylosin. We were interested in the biosynthetic pathway of mycinamicin II (**2**) from the view point of producing organism-metabolite relationship. In this communication, we present the results of a detailed analysis of bioconversion efficiencies of many potential intermediates to mycinamicin II (**2**). The data provided definitive information about the biosynthetic pathway from protomycinolide IV (**9**) to mycinamicin II (**2**).

The culture for microbial conversion of biosynthetic intermediates of mycinamicin II (**2**) was carried out at 28°C in a shaken 150-ml Erlenmeyer flask containing 20 ml of a seed medium (dextrin 1.0%, glucose 1.0% casamino acids 0.5%, yeast extract 0.5%, $CaCO_3$ 0.1%, pH 7.0). Two ml of this culture was then inoculated into 150-ml Erlenmeyer flask containing 20 ml of medium (dextrin 7.0%, glucose 0.5%, cotton meal 2.5%, soybean meal 0.5%, $CaCO_3$ 0.5%, $MgSO_4 \cdot 7H_2O$ 0.4%, K_2HPO_4 0.1%, $CoCl_2 \cdot 6H_2O$ 0.0002%). After 48 hours, biosynthetic intermediates of mycinamicin II (**2**) were separately added to the flask and cultivation was continued for an additional 120 hours. Since the strain C-34-10 itself does not produce any detectable amount of macrolide, the fate of non-labeled compounds added to this strain under antibiotic fermentation conditions can be readily monitored by HPLC procedure described previously[4].

Table 1 summarizes the results of bioconversion experiments with fifteen potential intermediates in mycinamicin II (**2**) biosynthesis. Mycinamicins III (**3**), IV (**4**), V (**5**), VI (**6**), VII (**7**) and VIII (**8**) were converted to mycinamicin II (**2**) more efficiently, and were converted to mycinamicin I (**1**) in substantial

Table 1. Bioconversion patterns of mycinamicin II-like compounds.

Compound	Bioconversion efficiencies (%)[a]				
	1	**2**	**5**	**12**	**15**
Mycinamicin I (**1**)	66.1	3.7	—	—	—
Mycinamicin II (**2**)	—	92.8	—	—	—
Mycinamicin III (**3**)	7.8	61.9	—	—	—
Mycinamicin IV (**4**)	13.6	83.0	—	—	—
Mycinamicin V (**5**)	—	70.2	8.6	—	—
Mycinamicin VI (**6**)	10.7	66.8	—	—	—
Mycinamicin VII (**7**)	9.4	54.0	—	—	—
Mycinamicin VIII (**8**)	8.8	55.8	—	—	—
Protomycinolide IV (**9**)	3.7	13.7	—	3.4	34.9
Mycinolide IV (**10**)	5.7	25.4	—	6.2	50.3
5-*O*-Dedesosaminyl-mycinamicin I (**11**)	17.5	5.0	—	22.1	—
5-*O*-Dedesosaminyl-mycinamicin II (**12**)	—	45.2	—	13.2	—
5-*O*-Dedesosaminyl-mycinamicin IV (**14**)	10.0	8.1	—	18.8	56.7
5-*O*-Dedesosaminyl-mycinamicin V (**15**)	—	41.7	3.9	7.1	35.7
5-*O*-Dedesosaminyl-mycinamicin VI (**16**)	9.0	19.6	—	10.8	57.9

[a] The percentages were based upon recovered mycinamicin II-like compounds.
—: Not detected.

Fig. 1. The preferred biosynthetic pathway of mycinamicin II (**2**).

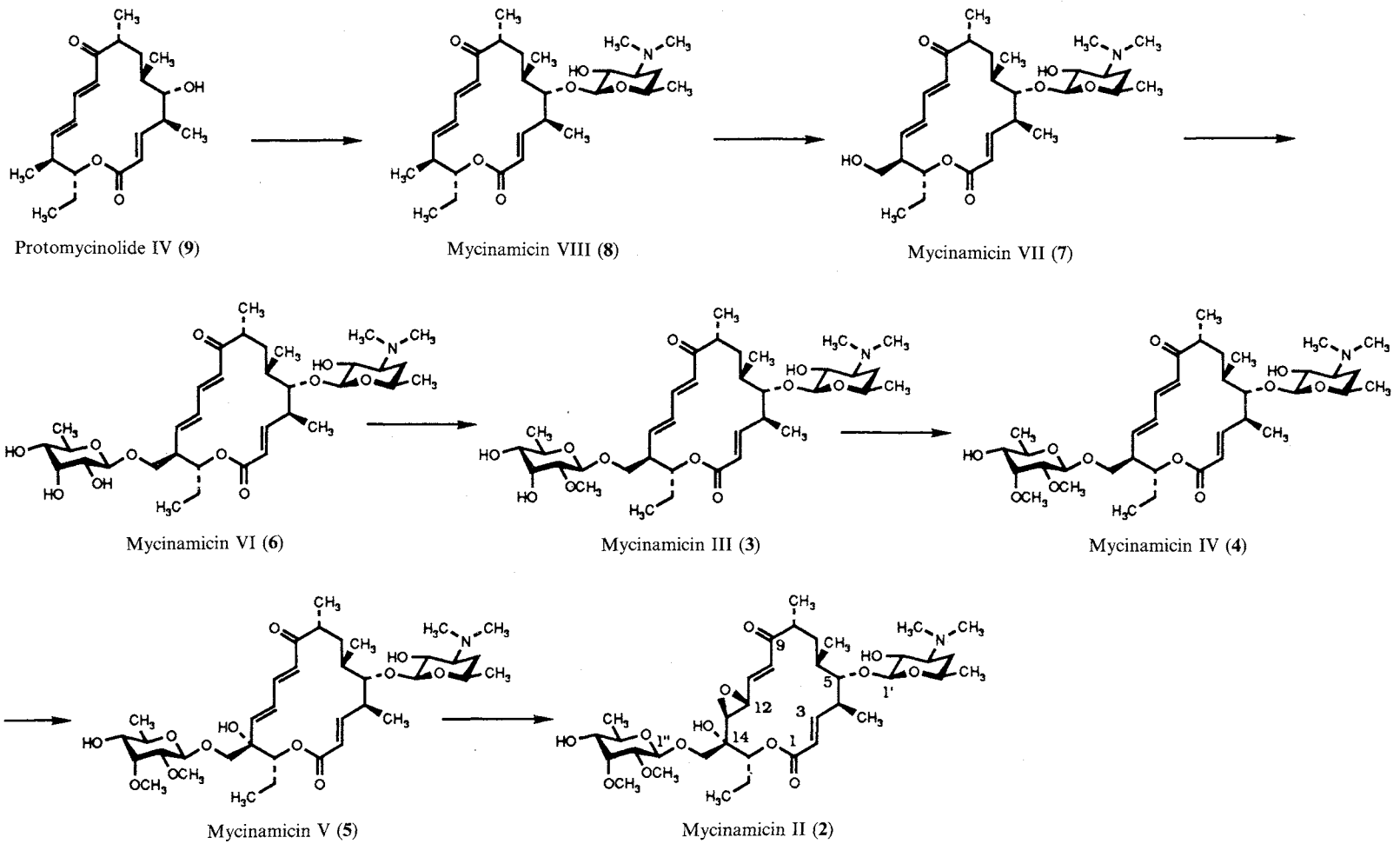
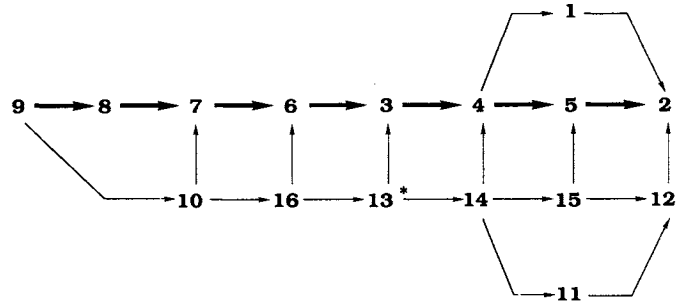


Fig. 2. The scheme of network metabolism of mycinamicin II (**2**) biosynthesis.



* Compound **13** is 5-*O*-dedesosaminyl-mycinamicin III, which barely exists in fermentation broth of *Micromonospora griseorubida*. In the present study, bioconversion of **13** was not tested.

quantities. High conversion efficiencies to **2** support the interpretation that basic pathway as shown Fig. 1 appears to be normal metabolism. When **1** was added to the strain C-34-10, only 3.7% was converted to **2**, whereas the majority remained unreacted. We suggest that **1** is shunt metabolite, since the hydroxylation reaction at the C-14 position must occur before the epoxidation reaction at the C-12～C-13 position. Protomycinolide IV (**9**) is a 16-membered lactone with the fundamental carbon skeleton of mycinamicin aglycones, and may be the first macrolide intermediate. Compound **9**, mycinolide IV (**10**), 5-*O*-dedesosaminyl-mycinamicins IV (**14**) and VI (**16**) were converted to mycinamicin II (**2**) less efficiently, and were also converted to 5-*O*-dedesosaminyl-mycinamicins II (**12**) and V (**15**). Compound **12** was converted to **2** efficiently, while **15** was converted to not only **2** and **12** but also **5**. Fig. 2 shows a scheme of mycinamicin II (**2**) biosynthetic pathway. Although biosynthesis from **9** to **2** takes the formation of network pathway, the bioconversion efficiency results suggest that normal metabolism follows the direct (heavy arrow) pathway.

When biosynthetic pathway for mycinamicin II (**2**) is compared to that of tylosin[7], some differences appear. Oxidation reactions in mycinamicin II (**2**) biosynthesis occur after addition of neutral sugar and 2″- and 3″-*O*-methylation reactions. Although neutral compounds pathway of mycinamicin II (**2**) biosynthesis appears to be shunt metabolism, there is no correspondence pathway known in tylosin biosynthesis.

Acknowledgments

We are grateful to Dr. M. HAYASHI for providing mycinamicin II intermediates and Miss T. SUGIYAMA for technical assitance.

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(Received March 30, 1990)

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