BIOCONVERSION AND BIOSYNTHESIS OF 16-MEMBERED MACROLIDE ANTIBIOTIC, TYLOSIN, USING ENZYME INHIBITOR: CERULENIN

Sir:

The antibiotic cerulenin[1] is a specific inhibitor of what is called "condensing enzyme" which is involved in the condensing step between acyl-ACP and malonyl-ACP in the biosynthesis of fatty acids. It also inhibits the biosynthesis of substances such as leucomycin[2], 6-methylsalicylic acid[3], candicidin[4] and flavone[5], which are presumably derived *via* "polyketide". It was thus expected that cerulenin might offer us an effective devise to explore the biosynthesis of macrolide antibiotics as an alternative to blocked mutants which have been conventionally utilized to study the biosynthesis of macrolides[6,7] and tetracycline[8].

Taking advantage of such salient inhibitory property of cerulenin, we have studied the final steps of the biosynthetic pathway of tylosin (**1**) and relomycin (**2**), the dihydro derivative of **1**, which is accumulated toward the end of fermentative production of **1**[9]. It has been established that the aldehyde group of 16-membered macrolide[10] originates from the methyl group of butyrate. However, the question as to whether the hydroxyl group of **2** is generated during the oxidation process from the methyl group into the aldehyde group or it is formed through the reduction of the aldehyde precursor after oxidation has remained unknown. So, we examined how the mutual conversion of **1** and **2** is accomplished in the biosynthetic processes.

The producing strain *Streptomyces fradiae* C–373, which provides both **1** and **2**, was cultured at 27°C using a 500-ml Sakaguchi flask containing 100 ml of culture medium composed of lactose 2.0%, Pharmamedia 4.0%, $MgSO_4 \cdot 7H_2O$ 0.5%, KCl 0.5%, NaCl 0.5%, $CaCO_3$ 0.75% and tap water (pH 7.5). At the beginning of fermentation, 20 mcg/ml of cerulenin was added to the culture broth to prevent the formation of antibiotics and after that the same amount of cerulenin was added every 24 hours. After incubation for 48 hours or 168 hours, 100 mcg/ml of **1** and its related compounds obtained by acid hydrolysis of **1** or **2** (See Fig. 1), were added to the culture, and then the incubation was continued for additional 24 hours. The product in the culture broth was extracted with benzene and the concentrated extract was subjected to thin-layer chromatography (Silica gel G, the bottom layer of a mixture of chloroform - methanol - 3% aq. ammonia=2:1:1 as a developer). The components on the chromatogram were then quantitatively analysed on a Shimadzu double-beam chromatogram scanner (model CS–910) at 282 nm where the maximum UV absorption of **1** and its related compounds occurs. The transformed **1** and **2** were estimated from the ratio of the peak area to the total peak area of the potential metabolites. Similarly, the relative ratio of **1** to **2** or **2** to **1** was also estimated and shown in parenthesis (%) in Table 1.

When mycaminosyl tylonolide **4** was added to 48-hour culture, 23.6% of **4** was converted to **1**. However, the conversion of **4** to **2** was only 2.6%

Fig. 1. Structures of tylosin and its related compounds

		R_1	R_2	R_3
Tylosin	**1**	CHO	Mycarosyl	Mycinosyl
Demycarosyl tylosin	**3**	CHO	H	Mycinosyl
Mycaminosyl tylonolide	**4**	CHO	H	H
Relomycin	**2**	CH_2OH	Mycarosyl	Mycinosyl
Demycarosyl relomycin	**5**	CH_2OH	H	Mycinosyl
Mycaminosyl relonolide	**6**	CH_2OH	H	H

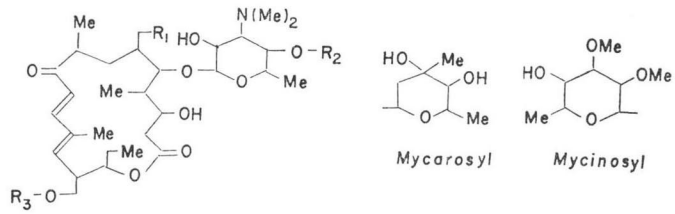

Table 1. The conversion of tylosin and its related compounds into tylosin and relomycin by *S. fradiae* under the inhibited conditions of lactone synthesis by cerulenin.

Compound added		Tylosin **1**		Relomycin **2**	
		48 hours	168 hours	48 hours	168 hours
Mycaminosyl tylonolide,	**4**	23.6[a](90.2)[b]	14.8 (41.6)	2.6 (9.8)	20.8 (58.4)
Demycarosyl tylosin,	**3**	21.3 (74.9)	15.2 (39.3)	7.2 (25.1)	23.5 (60.7)
Mycaminosyl relonolide,	**6**	9.3 (22.8)	4.6 (11.3)	31.4 (77.2)	35.7 (88.7)
Demycarosyl relomycin,	**5**	11.0 (27.4)	3.5 (8.2)	29.2 (72.6)	39.3 (91.8)
Tylosin,	**1**	80.0 (89.3)	65.6 (86.3)	9.6 (10.7)	10.4 (13.7)
Relomycin,	**2**	0 (0)	0 (0)	81.8 (100)	66.7 (100)
None[c]		0 (0)	0 (0)	0 (0)	0 (0)
Control[d]		59.7 (82.8)	8.9 (34.3)	12.4 (17.2)	17.0 (65.7)

[a] Conversion ratio (%) of each compounds was calculated from total peak area detected with Shimadzu double-beam chromatoscanner (model CS-910) at 282 nm.
[b] Relative ratio of **1** or **2** to **2** or **1** (%).
[c] No compounds added.
[d] No cerulenin and compounds added.

in the same culture broth. The relative ratio of **1** to **2** was 90.2%: that of **2** to **1** was 9.8%, suggesting that the cells in the early stage has a rapid activity of glycoside bond formation. On the other hand, in the 168-hour culture the relative ratio of **1** and **2** changed drastically: **1** to **2** 41.6%, **2** to **1** 58.4%. This indicates that the reduction of aldehyde group into hydroxyl group takes place in this later stage besides the glycosidation. Similarly, when demycarosyl tylosin **3** was added to the 48-hour culture, its conversion to **1** was better than that to **2**, and also in the 168-hour culture **2** was again predominantly produced. The similar trends were observed when mycaminosyl relonolide **6** or demycarosyl relomycin **5** was added to the 48-hour or 168-hour culture; the conversion to **2** seems much more predominant than that to **1** because of rapid glycosidation. However, it is obvious from the table that the relative amount of **1** was decreased in the 168-hour culture, and the percentage of **2**, on the contrary, was increased under the same conditions. In contrast, addition of **1** or **2** to the 48-hour or 168-hour culture demonstrated minor mutual conversion or complete absence of mutual conversion between these compounds.

These results disclose that; (1) the conversion of alcohol to aldehyde is predominant in the early stage of incubation, (2) in the later stage, however, reduction of aldehyde to alcohol is predominant. In the recent paper by SENO *et al.*[11] it is reported that **2** is derived directly from **1**, however, the present results indicate that the redox-reaction of alcohol and aldehyde, is most likely to precede the binding of mycarose.

Results from the present investigation also point out that cerulenin can be successfully applied to biosynthetic studies of compounds produced *via* polyketide in place of previously employed methods involving blocked mutants or radio isotopes.

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