THE ACTIVITY OF 4″-ACYLATED TYLOSIN DERIVATIVES AGAINST MACROLIDE-RESISTANT GRAM-POSITIVE BACTERIA

Sir:

In the screening program for microbial transformation of 16-membered macrolide antibiotics, *Streptomyces thermotolerans* ATCC 11416[1] was found to acylate the hydroxyl group at the 3- and 4″-positions of 16-membered macrolide antibiotics including tylosin (**1**).

This newly developed process can acylate not only tylosin but also other macrolide antibiotics such as leucomycin. In this paper, we report eight acylated derivatives[2] of tylosin. That is, 4″-butyryltylosin (**2**), 4″-isovaleryltylosin (**3**), 3-acetyltylosin (**4**), 3-acetyl-4″-butyryltylosin (**5**), 3-acetyl-4″-isovaleryltylosin (**6**), 3-propionyltylosin (**7**), 3-propionyl-4″-butyryltylosin (**8**) and 3-propionyl-4″-isovaleryltylosin (**9**). Details on the mechanisms of microbial transformation and the structural study of products will be reported in near future. Structures of the new acylated derivatives (**2**)~(**9**) are shown in Fig. 1 together with that of tylosin[3] (**1**).

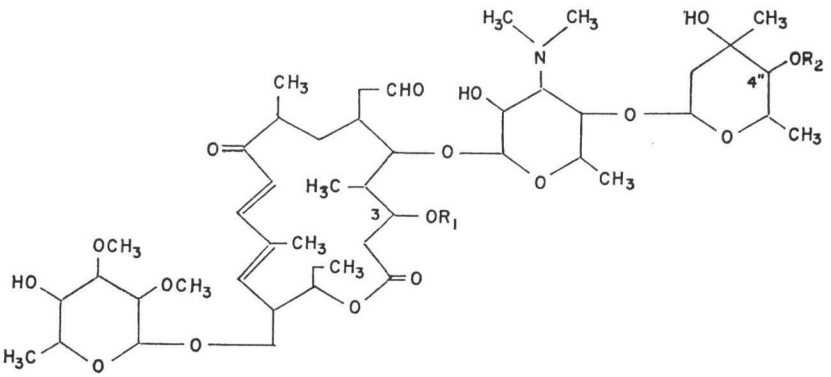
This communication deals with the role of the 4″-acyl group of acylated derivatives of tylosin in the antibacterial activity displayed against resistant strains.

The antibacterial spectra of the new tylosin derivatives are shown in Table 1 and Table 2 in comparison with other macrolides. The MIC (minimum inhibitory concentration; μg/ml) values against a variety of microorganisms were determined by the agar dilution method where BHI (brain heart infusion) medium containing 10% horse blood was used and the inoculum size was 10^6 cells per ml.

The strains listed in Table 1 are Gram-negative bacteria and macrolide-sensitive Gram-positive bacteria. The growth of the Gram-positive bacteria, but not of the Gram-negative bacteria was inhibited by the eight acylated derivatives of tylosin (**2**)~(**9**) as well as other macrolide antibiotics, *i.e.*, tylosin (16-membered macrolide antibiotic and the parent compound), erythromycin[4] (14-membered macrolide antibiotic) and leucomycin A_3[5] (16-membered macrolide antibiotic).

It is well known that the acyl group at the 3- and 4″-positions of 16-membered macrolide

Fig. 1. Structures of new tylosin derivatives acylated by microbial transformation.



Compound		R_1	R_2
4″-Butyryltylosin	(**2**)	H	$COCH_2CH_2CH_3$
4″-Isovaleryltylosin	(**3**)	H	$COCH_2CH(CH_3)_2$
3-Acetyltylosin	(**4**)	$COCH_3$	H
3-Acetyl-4″-butyryltylosin	(**5**)	$COCH_3$	$COCH_2CH_2CH_3$
3-Acetyl-4″-isovaleryltylosin	(**6**)	$COCH_3$	$COCH_2CH(CH_3)_2$
3-Propionyltylosin	(**7**)	$COCH_2CH_3$	H
3-Propionyl-4″-butyryltylosin	(**8**)	$COCH_2CH_3$	$COCH_2CH_2CH_3$
3-Propionyl-4″-isovaleryltylosin	(**9**)	$COCH_2CH_3$	$COCH_2CH(CH_3)_2$
Tylosin (substrate)	(**1**)	H	H

Table 1. Antibacterial activity of new acylated tylosin derivatives with reference to known macrolides (MIC: μg/ml).

Test microorganism	Compound										
	(2)	(3)	(4)	(5)	(6)	(7)	(8)	(9)	(1)	EM	LM-A_3
Escherichia coli NIHJ	100	100	200	100	100	200	200	200	200	>200	>200
Pseudomonas fluorescens NIHJ B 254	>200	>200	>200	>200	>200	>200	>200	>200	>200	>200	>200
Pseudomonas aeruginosa IAM 1133	>200	>200	>200	>200	>200	>200	>200	>200	>200	>200	>200
Staphylococcus aureus Smith	0.39	0.39	0.39	0.39	0.39	0.39	0.39	0.39	0.39	0.19	0.39
Staphylococcus aureus FDA 209P	0.39	0.39	0.39	0.39	0.39	0.39	0.39	0.39	0.39	0.19	0.39
Bacillus cereus ATCC 9634	0.39	0.39	0.78	0.78	0.78	0.78	0.78	0.78	0.78	0.39	0.39
Bacillus subtilis ATCC 6633	0.39	0.39	0.39	0.78	0.39	0.39	0.78	0.39	0.39	0.39	0.39
Sarcina lutea ATCC 9341	0.19	0.19	0.19	0.19	0.19	0.19	0.19	0.19	0.19	0.19	0.19
Mycobacterium smegmatis ATCC 607	3.13	3.13	3.13	3.13	3.13	3.13	3.13	3.13	1.56	0.39	0.78
Streptococcus pyogenes NY-5	0.39	0.39	0.39	0.39	0.39	0.39	0.39	0.39	0.39	0.19	0.39

MIC was determined by the agar dilution method in BHI medium containing 10% horse blood.
Compound: **(2)** 4″-butyryltylosin, **(3)** 4″-isovaleryltylosin, **(4)** 3-acetyltylosin, **(5)** 3-acetyl-4″-butyryltylosin, **(6)** 3-acetyl-4″-isovaleryltylosin, **(7)** 3-propionyltylosin, **(8)** 3-propionyl-4″-butyryltylosin, **(9)** 3-propionyl-4″-isovaleryltylosin, **(1)** tylosin, EM: erythromycin, LM-A_3: leucomycin A_3.

Table 2. Antibacterial activity of new acylated tylosin derivatives with reference to known macrolide antibiotics against macrolide-resistant bacteria (MIC: μg/ml).

Test microorganism	Compound											
	(2)	(3)	(4)	(5)	(6)	(7)	(8)	(9)	(1)	EM	LM-A_3	LM-U
*Staphylococcus aureus**1 MS 8710	50	50	>200	50	25	>200	50	25	>200	>200	>200	>200
*Staphylococcus aureus**2 MS 9610	100	100	>200	100	100	>200	200	100	>200	>200	>200	>200
*Staphylococcus aureus**3 MS 9931	12.5	12.5	100	25	12.5			25	200	>200	>200	>200
*Staphylococcus aureus**3 MS 9937	12.5	12.5	100	25	12.5			25	100	200	200	200
*Streptococcus pyogenes**4 MH 604	12.5	12.5	200	25	12.5			25	>200	>200	>200	>200
*Streptococcus pyogenes**4 MH 749	12.5	12.5	200	25	12.5			25	>200	>200	>200	>200
*Streptococcus pyogenes**4 MH 771	3.1	3.1	100	12.5	3.1	100	3.1	3.1	>200	>200	>200	>200

MIC was determined by the agar dilution method in BHI medium containing 10% horse blood.
Compound: **(2)** 4″-butyryltylosin, **(3)** 4″-isovaleryltylosin, **(4)** 3-acetyltylosin, **(5)** 3-acetyl-4″-butyryltylosin, **(6)** 3-acetyl-4″-isovaleryltylosin, **(7)** 3-propionyltylosin, **(8)** 3-propionyl-4″-butyryltylosin, **(9)** 3-propionyl-4″-isovaleryltylosin, **(1)** tylosin, EM: erythromycin, LM-A_3: leucomycin A_3, LM-U: leucomycin U.
Resistance pattern: *1 PC-G·OTC·CBM-A, *2 PC-G·CBM-A, *3 OTC·CBM-A, *4 PC-G·CEX·OTC·CBM-A (PC-G: benzylpenicillin, CEX: cephalexin, OTC: oxytetracycline, CBM-A: carbomycin A).

antibiotics is important for their antimicrobial activity. For example, ŌMURA *et al.*[6] reported that leucomycins having a hydroxyl group at the 3-position were generally more active than those having an acetyl group at the same position, and that the antibacterial activity of 4″-acyl leucomycins increased in parallel with the size of the 4″-acyl group.

Tylosin and its acylated derivatives have similar antibacterial activity against macrolide-sensitive Gram-positive bacteria. That is, introduction of an acyl group into the 3- and/or 4″-position(s) of tylosin did not increase the antibacterial activity against the macrolide-sensitive bacteria (Table 1). In contrast, the macrolide-resistant strains of *Staphylococcus* and *Streptococcus* displayed increased sensitivity to the new acylated tylosin derivatives (Table 2) (in a separate test, *Staphylococcus aureus* strains MS 8710 and MS 9610 were found resistant even at a concentration of 1,000 μg/ml to tylosin, erythromycin and leucomycins A_3 and U), and the 4″-acylation increased the antibacterial activity to a greater extent than the 3-acylation. For example, 4″-isovaleryltylosin (**3**) shows a stronger antimicrobial activity than 3-acetyltylosin (**4**) and tylosin (**1**). Thus one may conclude that the 4″-acyl group plays a significant role in the antibacterial activity of tylosin derivatives against macrolide-resistant bacteria in this series. But, in leucomycins, the 4″-acyl group seems to have no important effect on the antimicrobial activity with regard to resistant-bacteria: leucomycin U[7] (3-acetyl-4″-hydroxylleucomycin) is as inactive as leucomycin A_3 (3-acetyl-4″-isovalerylleucomycin). It is therefore probable that tylosin in itself has a latent specific activity against macrolide-resistant bacteria which is expressed by 4″-acylation.

ROKURO OKAMOTO
HIDEO NOMURA
MASAMI TSUCHIYA
HIROSHI TSUNEKAWA
TSUMORU FUKUMOTO
TAIJI INUI
TSUTOMU SAWA*
TOMIO TAKEUCHI*
HAMAO UMEZAWA*

Central Research Laboratories,
Sanraku-Ocean Co., Ltd., 4–9–1, Johnan,
Fujisawa, Japan
*Institute of Microbial Chemistry,
Kamiosaki, Shinagawa-ku, Tokyo, Japan

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