

**4''-O-(4-METHOXYPHENYL)-
ACETYLTYLOSIN, A NEW
MACROLIDE DERIVATIVE OF
THERAPEUTIC IMPORTANCE**

Sir:

In the continuing search for macrolide derivatives which are useful in clinical treatment of macrolide-resistant pathogens^{1,2}, a series of tylosin derivatives have been synthesized and microbiologically evaluated³⁻⁵. In spite of good antibacterial activity *in vitro*, they were found to be poorly effective in the treatment of experimentally infected animals. Subsequent enzymological examination revealed that hepatic esterase was the major agent responsible for the rapid decrease in the blood level of acylated macrolide derivatives, leading to the poor therapeutic efficacy in animal experiments. Based on these findings, a macrolide derivative which is not only active against a variety of macrolide-resistant clinical isolates, but also resistant to hepatic esterase has been sought for among new and old macrolide derivatives. This

paper describes the synthesis and biological evaluation of 4''-O-(4-methoxyphenyl)acetyltirosin (code name: IMC-XV) (Fig. 1).

4''-O-(4-Methoxyphenyl)acetyltirosin was synthesized as follows¹; after protection of the 2'-hydroxyl group by acetylation, tylosin was first chloroacetylated at the 4'''-OH and then (4-methoxyphenyl)acetylated at 4''-OH. The resulting tri-*O*-acyl product was deacylated at the 2' and 4''' positions to give 4''-O-(4-methoxyphenyl)acetyltirosin. The overall yield of the new derivative was 24% from the starting macrolide. The chemical structure of this compound was confirmed by spectrometric methods. In brief, the 4''-O-(4-methoxyphenyl)acetylation was evidenced by the lowfield shifts of 4''-H by 1.64 ppm and of C-4'' by 2.3 ppm and the high-field shift of C-5'' by 2.9 ppm in the ¹H and ¹³C NMR spectra. The molecular weight was determined to be 1,064 by mass spectrometry.

The comparative antibacterial spectra of 4''-O-(4-methoxyphenyl)acetyltirosin and the reference macrolides are shown in Table 1. It is apparent that the 4''-O-(4-methoxyphenyl)acetylation is

Fig. 1. Structure of 4''-O-(4-methoxyphenyl)acetyltirosin.

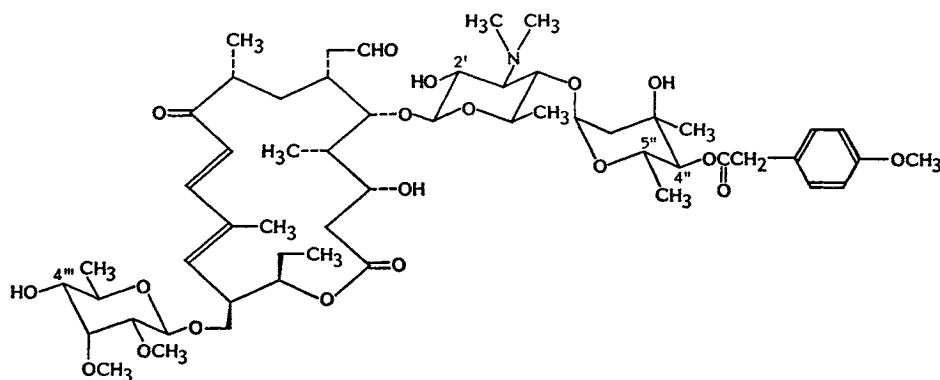


Table 1. Antibacterial activity (MIC in $\mu\text{g/ml}$).

Bacterium	IMC-XV	Tylosin	Josamycin	Erythromycin
<i>Bacillus subtilis</i> ATCC 6633	1.6	0.8	0.2	0.05
<i>Micrococcus luteus</i> ATCC 9341	0.4	0.2	0.1	0.05
<i>Staphylococcus aureus</i> FDA 209P	0.8	0.8	1.6	0.4
<i>S. aureus</i> Smith	1.6	0.8	1.6	0.2
<i>S. aureus</i> EMf	0.8	6.3	100	>100
<i>S. aureus</i> MS8710 (M)*	25	>100	>100	>100
<i>S. aureus</i> MS9610 (R)**	6.3	>100	>100	>100
<i>S. aureus</i> MS9937 (M)*	3.1	>100	>100	>100

* Membrane permeability-dependent type.

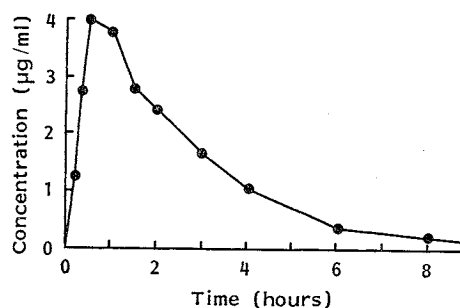
** Ribosome-dependent type.

Table 2. Anti-mycoplasmal activity (MIC in $\mu\text{g/ml}$).

<i>Mycoplasma</i>	IMC-XV	Tylosin	Josamycin	Erythromycin
<i>Mycoplasma pneumoniae</i> Mac	<0.0002	<0.0002	0.006	<0.0002
<i>M. pulmonis</i> PG 22	0.05	0.39	12.5	>100
<i>M. gallisepticum</i> A-69	0.004	0.006	3.13	>100
<i>M. gallisepticum</i> E-103	<0.0002	0.78	12.5	>100
<i>M. gallisepticum</i> S-4A	0.0008	0.78	6.25	>100
<i>M. gallisepticum</i> TS-18	<0.0002	0.012	0.20	0.05

Fig. 2. Plasma level of IMC-XV in dogs after oral administration at a dose of 25 mg/kg (by HPLC analysis).

Mean ($n=3$).



effective to revive the antibacterial activity of the parent compound against macrolide-resistant isolates of the membrane permeability-dependent type and the ribosome-dependent type. The increased bulkiness of the new derivative has a negative influence on the specific antibacterial activity against macrolide-sensitive strains. Against *Micrococcus luteus* ATCC 9341, for example, the MIC value of the new derivative is 0.4 $\mu\text{g/ml}$, whereas the parent macrolide shows 0.2 $\mu\text{g/ml}$.

Table 2 shows the comparative anti-mycoplasmal activities of the new macrolide compound and the reference macrolides. Like the macrolide-resistant bacterial strains, the macrolide-resistant mycoplasmal strains become sensitive when the 4-methoxyphenylacetyl group is introduced at 4''.

The improved resistance of the new derivative to hepatic esterase was proved by *in vitro* enzymological assays (data not shown). Furthermore the *in vivo* stability of 4''-O-(4-methoxyphenyl)acetyltylosin was confirmed by oral administration to dogs (Fig. 2). Under similar conditions, the plasma level of josamycin was 2.3 $\mu\text{g/ml}$ at 30 minutes, 1.3 $\mu\text{g/ml}$ at 60 minutes and 0.7 $\mu\text{g/ml}$ at 120 minutes. The concentra-

tions of the new macrolide in blood samples were measured by HPLC using $\mu\text{Bondapak C}_{18}$ (eluting solvent system, $\text{CH}_3\text{CN} - 0.15 \text{ M CH}_3\text{COONH}_4 - \text{CH}_3\text{COOH}$, 3:2:0.15). The ED_{50} value of the new derivative (MIC 6.25 $\mu\text{g/ml}$) was determined to be 200 mg/kg by oral administration to mice experimentally infected by *Staphylococcus aureus* strain KS-54 (macrolide-resistant clinical isolate), while josamycin (MIC >100 $\mu\text{g/ml}$) was found to be ineffective at a dose of 600 mg/kg.

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