MICROBIAL *O*-PHOSPHORYLATION OF MACROLIDE ANTIBIOTICS

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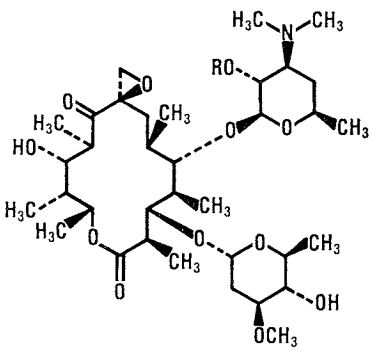
Crude enzyme preparations of *Streptomyces coelicolor* (Müller), UC 5240 in the presence of ATP and Mg^{2+} catalyzed the conversion of oleandomycin, tylosin, leucomycin A_3 and a mixture of spiramycins I, II and III to their antibiotically inactive 2′-*O*-phosphates.[1] Under identical conditions, erythromycin was converted to anhydroerythromycin-2′-*O*-phosphate.[1] As *O*-phosphorylation of aminocyclitols, lincosaminides and nucleosides is a well-known mechanism of antibiotic inactivation,[2] *O*-phosphorylation of macrolides was investigated as a transformation of potential significance. Such an *O*-phosphorylating enzyme could protect macrolide producing microorganisms during antibiotic biosynthesis or could be a mechanism of macrolide resistance in pathogens.

The data reported here concerns the conversion of oleandomycin and a mixture of spiramycins I, II and III to their 2′-*O*-phosphates by *S. coelicolor* in fermentation (Fig. 1). Such an investigation was considered to be an important step in establishing the physiological significance of these *O*-phosphorylations. The antibacterial activities of these macrolide-2′-*O*-phosphates were also investigated.

Fermentation Conditions for Conversion

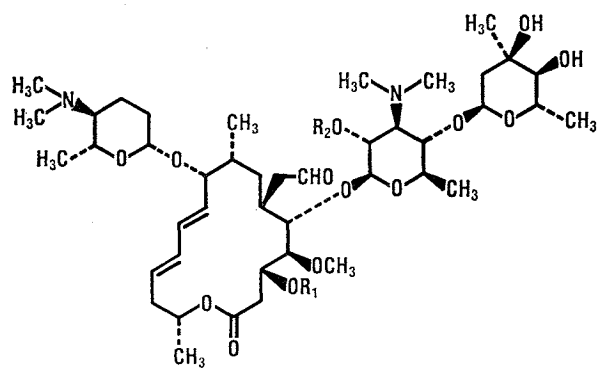
S. coelicolor, UC 5240 (NRRL 3532) was stored and maintained on sterile soils in the culture collection of The Upjohn Company. The organism was inoculated into a seed medium (GS-7) which contained Cerelose (C. P. C. International) and Pharmamedia (Procter and Gamble) each added at 25 g/liter of tap water. The medium was adjusted to pH 7.2 with NH_4OH and was autoclaved for 30 minutes. The inoculated 100 ml volumes of GS-7 were shaken in wide-mouth 500-ml fermentation flasks at 250 rpm for 48 hours at 28°C. The mature seed cultures were used as the source of inoculum (5% seed rate) for the fermentation medium. The latter was a modification of a medium reported by COATS and ARGOUDELIS[3] and contained glucose 20 g, NZ-Amine B (Sheffield Chemicals) 5 g, yeast extract 2.5 g, $NaNO_3$ 1.5 g and $FeSO_4$ 10 mg per liter of deionized water. After formulation the medium was adjusted to

Fig. 1. Structures of macrolide antibiotics and 2′-*O*-phosphates (**1a**, oleandomycin; **1b**, oleandomycin-2′-*O*-phosphate; **2a**, spiramycins I, II, III; **2b**, spiramycins I, II, III-2′-*O*-phosphates).



1a R=H

1b R=$\overset{O}{\overset{\|}{P}}(OH)_2$



2a R_1=H, $COCH_3$, $COCH_2CH_3$; R_2=H

2b R_1=H, $COCH_3$, $COCH_2CH_3$; R_2=$\overset{O}{\overset{\|}{P}}(OH)_2$

See correction[4] concerning stereochemical configurations of macrolide structures published by WILEY *et al.*, 1987.[1]

Table 1. FAB-MS data for macrolide 2′-*O*-phosphates produced by *Streptomyces coelicolor*.

Macrolide 2′-*O*-phosphate	Formula	MW	Observed mass fragments (m/z)[a]
Oleandomycin	$C_{35}H_{62}NO_{15}P$	767	766 $(M-H)^-$, 704, 542, 254, 236, 209
Spiramycin I[b]	$C_{43}H_{75}N_2O_{17}P$	922	977 $(M-H)^-$, 963 $(M-H)^-$, 903, 414, 396[c]
Spiramycin II[b]	$C_{45}H_{77}N_2O_{18}P$	964	
Spiramycin III[b]	$C_{46}H_{79}N_2O_{18}P$	978	

[a] The mass units reported here also were observed in the FAB-MS of the previously described macrolide *O*-phosphates.[1]

[b] Spiramycins I, II and III were converted in one experiment as a mixture.

[c] The 977 ion is the predominant peak in this spectrum; whereas, the 921 peak was predominant in the spectrum of the enzymically produced spiramycin *O*-phosphates.[1]

Table 2. Bacterial growth inhibition by macrolide antibiotics and macrolide-2′-*O*-phosphates.

Test organism	Zone of growth inhibition (mm)			
	O	OP	S	SP
Bacillus subtilis UC 564	27	11	23	14
Clostridium perfringens UC 6509	19	0	18	9
Micrococcus luteus UC 130	32	16	26	12
Mycobacterium avium UC 159	16	7	20	9
Staphylococcus aureus UC 80	17	10	11	0
S. epidermidis UC 719	26	10	19	7
Streptococcus faecalis UC 241	21	10	17	7
S. pyogenes UC 6055	36	21	31	20
Bacteroides fragilis UC 6513	37	18	32	20
Bordetella bronchiseptica UC 6481	12	0	0	0
Escherichia coli UC 51	0	0	0	0
Haemophilus influenzae UC 6483	16	0	11	0
Klebsiella pneumoniae UC 57	16	0	15	0
Neisseria gonorrhoeae UC 3065	28	0	24	10
Pasteurella haemolytica UC 6531	13	0	11	0
Pseudomonas aeruginosa UC 95	0	0	0	0
Rhodopseudomonas spheroides UC 3238	13	0	17	0

Compounds tested at a concentration of 1 mg/ml using 6.35 mm paper discs and seeded agar trays.
O: Oleandomycin, OP: oleandomycin-2′-*O*-phosphate, S: spiramycin, SP: spiramycin-2′-*O*-phosphate.

pH 7.2 with NH_4OH and was sterilized by autoclaving. The medium was employed in the manner described for GS-7. After the initial 24 hours of growth in this medium, sterile, aqueous oleandomycin or spiramycin mixture was added aseptically to final concentrations ranging between 30 and 100 μg/ml. The fermentations were continued for an additional period of 3 days using the conditions described. These fermentations were the source of the macrolide-2′-*O*-phosphates.

Isolation and Characterization of Conversion Products

In the cases of both oleandomycin-2′-*O*-phosphate and the spiramycins I, II and III-2′-*O*-phosphates isolation and characterization were performed generally as described in the Chemical Procedures Section of our previous publication.[1] Mass spectral characterization of the isolated compounds is presented in Table 1. Fast atom bombardment (FAB)-MS peak matching of the fragments arising from the sugars identified those fragments containing the phosphorous atom and showed that they also were derived from the amino sugars.[1]

Biological Characterization

The MICs of the spiramycin mixture, oleandomycin and their 2′-*O*-phosphates vs. *S. coelicolor*

were determined by growth inhibition. Suspensions of *S. coelicolor* were inoculated into 5 ml volumes of the fermentation medium contained in 25 ml fermentation flasks. The sterilized macrolides were added aseptically before inoculation at final concentrations ranging between 7.5 and 1,000 μg/ml. Growth inhibition was determined after incubation (250 rpm, 28°C) of *S. coelicolor* for 24 hours in the presence of the macrolides. The MIC values obtained for both antibiotics were 25 μg/ml, while the conversion products were inhibitory only at concentrations >500 μg/ml. Because of the activity of these antibiotics vs. freshly inoculated *S. coelicolor*, it was necessary to add them after 24 hours of growth in the conversion experiments.

Bacterial growth inhibition by the two macrolide antibiotics and their 2′-*O*-phosphates is shown in Table 2. Aqueous solutions of oleandomycin, the spiramycin mixture and their 2′-*O*-phosphates were prepared at a concentration of 1 mg/ml. Paper assay discs (Schleicher and Schuell, No. 740-E, 6.35 mm) were saturated with these solutions and were applied to agar trays seeded with the microorganisms listed in Table 2. After application of the macrolides, the trays were incubated over night at either 32 or 37°C. The degrees of growth inhibition observed are listed in Table 2.

Data presented in Table 2, as well as the MIC values vs. *S. coelicolor* for these compounds show substantial reduction of antimicrobial activity following the conversion of macrolide antibiotics to their 2′-*O*-phosphates. This inactivation would appear to be a potential means of microbial resistance to macrolides. In addition, data reported here and in our previous publication[1] suggest that *O*-phosphorylation of other macrolide antibiotics containing the same type of amino sugar could be brought about by *S. coelicolor*.

Acknowledgment

The authors wish to dedicate this publication to the memories of Drs. PAUL F. WILEY and ALEXANDER D. ARGOUDELIS.

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