SYNTHESIS AND ANTIBACTERIAL ACTIVITY OF 9-*O*-[(2-METHOXYETHOXY)METHYL]-OXIMES OF TYLOSIN AND DEMYCAROSYLTYLOSIN

CATHERINE RUGGERI,
MARIA DE LOS A. LABORDE,†
AIMÉE DESSINGES,† LI MING,†
ALAIN OLESKER† and GABOR LUKACS†

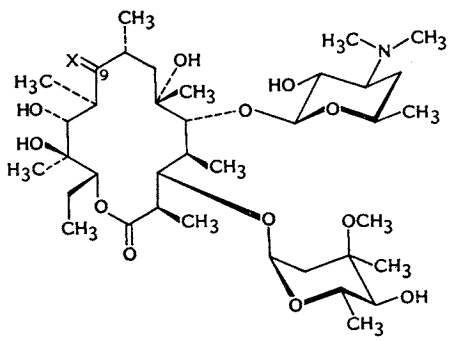
Institut de Recherche Internationale Servier,
27 Rue du Pont,
92202 Neuilly-sur-Seine, France
†Institut de Chimie des Substances
Naturelles du C.N.R.S.,
91198 Gif-sur-Yvette Cedex, France

(Received for publication November 30, 1988)

The rapid inactivation of erythromycin (Ery: **1**) in gastric medium has led researchers at Roussel-Uclaf to develop roxithromycin (**2**) which contains at position C-9 an oxime whose hydroxyl is etherified by the (2-methoxyethoxy)methyl (MEM) group. The new semisynthetic macrolide exhibits satisfactory pharmacokinetics while retaining the antibacterial *in vitro* spectrum of erythromycin.[1~4] We were interested to examine the influence of similar chemical modifications on the antibacterial properties of 16-membered macrolides and report here results related to tylosin (**3**).

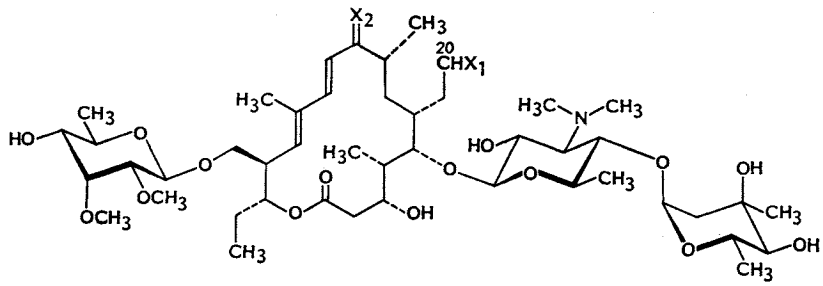
Synthesis

Upon treatment with hydroxylamine hydrochloride, the C-20 dimethylacetal of tylosin (**4**): $[\alpha]_D^{22}$ −51° (*c* 1.0, $CHCl_3$); MS *m/z* 961 (M^+); *Anal* Calcd for $C_{48}H_{83}NO_{18}$: C 59.93, H 8.64, N 1.46. Found: C 60.01, H 8.79, N 1.52, prepared as described for another 16-membered macrolide,[5] was readily transformed into a mixture of C-9 *syn* and *anti* oximes **5**: MS *m/z* 977 (MH^+); ^{13}C NMR δ 174.7 (C-1 major (maj)), 174.1 (C-1 minor (min)), 161.3 (C-9 maj), 158.4 (C-9 min), 103.6 (C-1′), 101.0 (C-1‴), 96.1 (C-1″); *Anal* Calcd for $C_{48}H_{84}N_2O_{18}$: C 59.02, H 8.61, N 2.87. Found: C 59.89, H 8.74, N 3.00. In the case of the erythromycin B oxime the major isomer was shown to have the *E*-stereochemistry as a result of an intramolecular hydrogen bond between the nitrogen atom and the C-11 hydroxy group.[6] In the absence of such a free hydroxy group in the environment of the oxime of the tylosin derivative **5**, no attempt was made to elucidate the stereochemistry of the predominating isomer (4:1). Chemoselective etherification of the oxime hydroxy groups of **5** was performed in THF solution in the presence of 1.1 equivalent of sodium hydride and 1.2 equivalent of MEM chloride affording, after flash chromatography with the solvent mixture CH_2Cl_2 - MeOH - NH_4OH (20 : 1 : 0.05), the 9-MEM oximes of tylosin C-20 dimethylacetal **6** (52%), (MS *m/z* 1,065 (MH^+); ^{13}C NMR δ 174.2 (C-1 maj), 174.7 (C-1 min), 162.2 (C-9 maj), 159.1 (C-9 min), 103.3 (C-1′), 101.2 (C-1‴), 96.5 (C-1″); *Anal* Calcd for $C_{52}H_{92}N_2O_{20}$: C 58.64, H 8.65, N 2.63. Found: C 58.71, H 8.49; N 2.72) as a colorless powder.

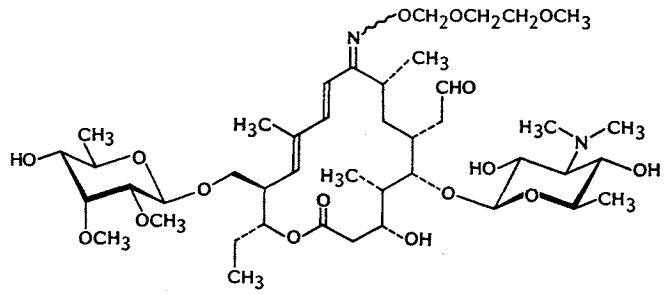


1 X=O
2 X=$NOCH_2OCH_2CH_2OCH_3$

Liberation of the aldehyde group from the dimethylacetal **6** appeared to be a delicate matter in view of the great sensitivity, under acidic conditions, of the glycosidic linkage between the mycaminose and mycarose residues. The most appropriate conditions required the use of the solvent mixture acetonitrile - water (1 : 1) and 3.5 equivalents of difluoroacetic acid at room temperature for about 40 hours. After neutralizing the excess of acid by slow addition to the mixture of 7 equivalents of triethylamine, standard workup furnished a crude residue which was separated to its constituents by flash chromatography using CH_2Cl_2 - MeOH - NH_4OH (20 : 1 : 0.05). Pure **7**, MS *m/z* 1,019 (MH^+) (15%) (Rf 0.65) and the corresponding demycarosyl derivative **8**, MS *m/z* 875 (MH^+) (26%) (Rf 0.50) were thus obtained as mixtures of *syn* and *anti* ether oximes. Characteristic ^{13}C NMR signals were exhibited for the major isomers in $CDCl_3$ solution: **7** 202.8 (C-20), 174.6 (C-1), 158.1 (C-9),



3 $X_1=X_2=O$
4 $X_1=(OCH_3)_2$ $X_2=O$
5 $X_1=(OCH_3)_2$ $X_2=NOH$
6 $X_1=(OCH_3)_2$ $X_2=NOCH_2OCH_2CH_2OCH_3$
7 $X_1=O$ $X_2=NOCH_2OCH_2CH_2OCH_3$



8

Table 1. Antibacterial activity.

Organism (number)	MIC_{80} (μg/ml)					
	MEM oxime demycarosyl Tyl (**8**)	MEM oxime Tyl (**7**)	Tyl	Ery	Jos	Spi
Staphylococcus aureus Ery-susceptible (29 strains)	2	2	2	0.25	1	4
S. aureus Ery-resistant (22 strains)	2	2	2	≧256	2	8
Streptococcus D (*Enterococcus*) Ery-susceptible (13 strains)	1	2	2	0.5	2	1

144.3 (C-11), 136.9 (C-13), 136.0 (C-12), 114.3 (C-10), 104.6 (C-1′), 101.3 (C-1‴), 98.0 (O-CH_2-O), 96.6 (C-1″) and in case of **8**: 202.9 (C-20), 174.5 (C-1), 158.0 (C-9), 144.2 (C-11), 136.6 (C-13), 135.9 (C-12), 114.3 (C-10), 104.8 (C-1′), 101.2 (C-1‴), 97.9 (O-CH_2-O).

For **7**, *Anal* Calcd for $C_{50}H_{86}N_2O_{19}$: C 58.94, H 8.45, N 2.75. Found: C 59.06, H 8.37, N 2.77. For **8**, *Anal* Calcd for $C_{43}H_{74}N_2O_{16}$: C 59.03, H 8.47, N 3.20. Found: C 58.91, H 8.61, N 3.13.

Antibacterial Activity

Susceptibility tests were performed by the serial 2-fold agar (Mueller-Hinton) dilution method in accordance with National Committee for Clinical Laboratory Standards. The MICs were determined for test compounds in com-

parison with tylosin (Tyl), Ery, josamycin (Jos), spiramycin (Spi). The antibiotics were dissolved according to the manufacturer's instructions and further diluted in the buffered agar medium. The bacterial inoculum was prepared to obtain, by multiple-replication, a deposit of 10^4 cfu per spot on the agar. The MIC_{90} was defined as the lowest concentration of drug which inhibited any visible growth for 90% of the strains. The bacteria tested were Gram-positive strains obtained from recent clinical specimen.

The MIC_{90} of MEM oximes **7** and **8** and reference macrolides are presented in Table 1. The antibacterial activity of MEM oximes **7** and **8** was lower than that of Ery, the most powerful macrolide antibiotic, but higher than that of Spi and very similar to that of Tyl and Jos.

Acknowledgment

The authors are indebted to Dr. G. ADAM, Institut de Recherche Internationale, Servier, for suggesting to carry out this research work and for helpful discussions and to Professor A. THABAUT, Hopital Begin, St-Mandé, France for performing the antimicrobial susceptibility tests.

References

1) CHANTOT, J. F.; J. C. GASC, S. GOUIN D'AMBRIÈRES & A. LUTZ: New ether oxime derivatives of erythromycin A: Preparation and antibacterial activities. Pregram and Abstracts of the 23rd Intersci. Conf. on Antimicrob. Agents Chemother., No. 447, p. 165, Las Vegas, Oct. 24~26, 1983
2) JONES, R. N.; A. L. BARRY & C. THORNSBERRY: In vitro evaluation of three new macrolide antimicrobial agents, RU28965, UR29065, RU-29702, and comparisons with other orally administered drugs. Antimicrob. Agents Chemother. 24: 209~215, 1983
3) BARLAM, T. & H. C. NEU: In vitro comparison of the activity of RU 28965, a new macrolide, with that of erythromycin against aerobic and anaerobic bacteria. Antimicrob. Agents Chemother. 25: 529~531, 1984
4) CHANTOT, J.-F.; A. BRYSKIER & J.-C. GASC: Antibacterial activity of roxithromycin: A laboratory evaluation. J. Antibiotics 39: 660~668, 1986
5) FREIBERG, L. A.; R. S. EGAN & W. H. WASHBURN: The synthesis of 9-epi-leucomycin A_3. The revised configurational assignment of C-9 in natural leucomycin A_3. J. Org. Chem. 39: 2474~2475, 1975
6) EGAN, R. S.; L. A. FREIBERG & W. H. WASHBURN: Configuration of 9-imino derivatives of erythromycin. J. Org. Chem. 39: 2492~2494, 1974