IN VITRO AND *IN VIVO* EVALUATION OF C-20- AND C-23-MODIFIED DERIVATIVES OF TYLOSIN AGAINST VETERINARY PATHOGENS

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Three series of semi-synthetic derivatives of tylosin-related macrolides were evaluated for utility in veterinary medicine. 23-Modified derivatives of 5-*O*-mycaminosyltylonolide (OMT) possessed potent activity *in vitro* against species of *Pasteurella* and *Mycoplasma*. An experimental infection in chicks caused by *Pasteurella multocida* was utilized to evaluate efficacy; several of these derivatives of OMT effectively treated the infection when given subcutaneously, but none were effective after oral administration in drinking water. Macrolides retaining the 4′-*O*-mycarosyl moiety (tylosin, DMT) had relatively poor activity against *Pasteurella in vitro*. Certain 20-modified derivatives of desmycosin demonstrated good oral bioavailability in chicks and a lead compound with oral efficacy in the *Pasteurella* infection model was discovered.

Tylosin is a valuable antibiotic in veterinary medicine.[1] The isolation of a large number of biosynthetic intermediates and shunt metabolites (Scheme 1) from various biosynthetically-blocked mutant strains of *Streptomyces fradiae*[2~4] prompted an extensive evaluation of their potential application in veterinary areas. In this paper, we report the results of our initial survey of activity against several important veterinary pathogens for several series of semisynthetic derivatives of tylosin-related macrolides.

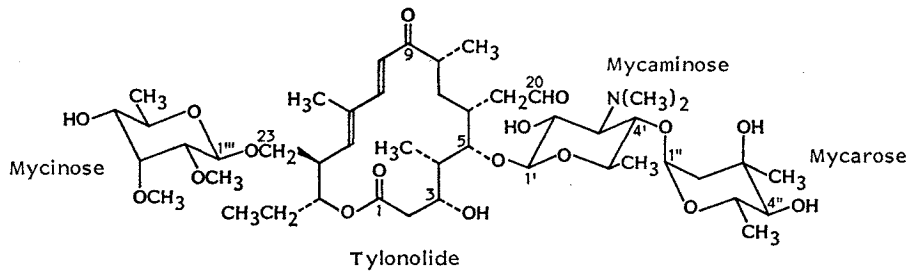
Results and Discussion

C-23 Derivatives of DMT and OMT: *In Vitro* Activity

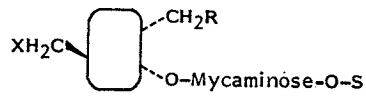
The synthesis of a large number of 23-*O*-acyl derivatives of 23-demycinosyltylosin (DMT) and 5-*O*-mycaminosyltylonolide (OMT) encompassing a wide variety of acyl substituents has been reported.[5,6] These ester derivatives generally possessed excellent *in vitro* antimicrobial activity against Gram-positive bacteria and were often more potent than their respective parent compounds (DMT and OMT). Unfortunately, these derivatives were less efficacious against experimental infections in rodents and, in particular, exhibited no significant *in vivo* activity after oral administration.[5] Similar conclusions were drawn from the evaluation of an extensive series of 23-modified derivatives of OMT involving substituents at C-23 other than esters, such as ethers, thioethers, halides and amines.[7~9] As was found for the ester derivatives, these C-23 non-ester derivatives treated experimental infections in rodents poorly after oral administration.[7]

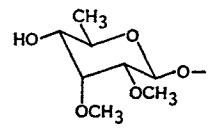
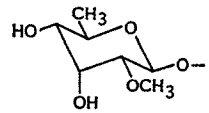
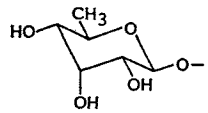
Species of the genera *Mycoplasma* and *Pasteurella* are often responsible for serious infections of the respiratory tract in animals raised for food.[1] Representative 23-modified derivatives of OMT possessed excellent activity *in vitro* against selected species of *Mycoplasma* and *Pasteurella*, with MICs

Scheme 1. Tylosin-related macrolides available as starting materials for chemical modification.



Tylosin



Compound name		X	R
S=Mycarosyl	S=H		
Tylosin	Desmycosin		CHO
Macrocin	Lactenocin		CHO
DOMM	DOML		CHO
DMT	OMT	OH	CHO
DMOT	DOMT	H	CHO
DODMT	DOOMT	OH	CH_3
DODMOT	DODOMT	H	CH_3

well within the therapeutically useful range (Table 1). The activity of most derivatives was generally better than that of OMT itself; the exceptions were those compounds containing very polar (23-NH_2, **17**) or long lipophilic (23-*O*-octanoyl, **8**) substituents, in accord with previous results against species of Staphylococci and Streptococci.[5,7]

An analogous evaluation of representative 23-modified derivatives of DMT revealed excellent activity against *Mycoplasma* species, but poorer activity against *Pasteurella* species (Table 2). The 23-modified non-ester derivatives (**27**~**30**) were prepared from DMT by S_N2 displacement reactions on 23-*O*-(trifluoromethanesulfonyl)-DMT (see Experimental section). These *in vitro* results indicated that improvements in the activity of tylosin against *Pasteurella* species would most likely occur with those macrolides lacking the saccharide mycarose. This is consistent with other reports indicating an increase of *in vitro* activity against Gram-negative bacteria for the demycarosyl series of macrolides.[3,5,8]

Table 1. *In vitro* activity of derivatives of OMT against veterinary pathogens.

Compound	Substituent on C-23	MIC (μg/ml)						
		S.a.	P.m. (b)	P.m. (t)	P.h.	M.g.	M.s.	M.h.
OMT	OH	1.56	1.56	3.12	6.25	0.39	0.78	3.12
1	OAc	0.39	1.56	1.56	3.12	0.05	0.10	0.78
2	$O(COC_6H_{11})$	0.20	1.56	0.78	1.56	<0.05	0.20	1.56
3	O(PhAc)	0.39	0.78	0.78	1.56	0.05	0.05	0.20
4	O(PhOAc)	0.39	0.78	1.56	1.56	<0.05	NT	1.56
5	O(PhGly)	3.12	6.25	3.12	6.25	0.20	0.39	6.25
6	OBz	0.39	1.56	0.78	3.12	<0.05	0.39	0.39
7	O(Piv)	1.56	3.12	3.12	3.12	0.10	<0.05	0.10
8	O(Oct)	0.78	6.25	6.25	NT	<0.05	0.10	6.25
9	$O(COOCH_3)$	1.56	3.12	1.56	6.25	0.05	0.20	0.78
10	OTs	0.20	3.12	1.56	3.12	0.05	0.10	0.78
11	$O(PO(OPh)_2)$	0.39	6.25	6.25	12.5	0.78	0.78	0.78
12	Br	0.78	3.12	3.12	6.25	0.20	0.78	0.78
13	F	1.56	3.12	3.12	6.25	0.10	0.39	0.78
DOMT	H	1.56	0.78	0.78	3.12	<0.02	0.39	0.39
14	OPh	0.39	1.56	0.78	3.12	<0.05	0.39	0.20
15	SPh	0.39	1.56	1.56	3.12	0.10	0.39	12.5
16	N_3	0.78	1.56	3.12	6.25	0.39	0.39	3.12
17	NH_2	12.5	6.25	6.25	12.5	0.78	0.78	12.5
18	NH(PhAc)	0.78	3.12	3.12	12.5	1.56	0.20	50.0
19	$N(CH_2)_7$	0.39	0.78	0.78	1.56	<0.05	0.10	0.78
20	PerH-Quin	1.56	0.78	0.78	0.78	0.02	0.05	0.39
21	6-Azabicyclo	1.56	0.78	1.56	1.56	0.05	0.05	1.56
22	3-Azabicyclo	0.78	0.78	0.78	0.39	<0.05	<0.05	0.39

Organisms: S.a.; *Staphylococcus aureus* 19C, P.m. (b); *Pasteurella multocida* (bovine isolate) 17E, P.m. (t); *Pasteurella multocida* (turkey isolate) 60A, P.h.; *Pasteurella haemolytica* 23C, M.g.; *Mycoplasma gallisepticum* 29C, M.s.; *Mycoplasma synoviae* 40A, M.h.; *Mycoplasma hyorhinis* 29E.

Abbreviations: Ac; acetyl, COC_6H_{11}; cyclohexylcarbonyl, PhAc; phenylacetyl, PhOAc; phenoxyacetyl, PhGly; phenylglycyl, Bz; benzoyl, Piv; pivaloyl, Oct; octanoyl, Ts; tosyl, Ph; phenyl, PerH-Quin; decahydroquinolinyl, 6-Azabicyclo; 1,3,3-trimethyl-6-aza[3.2.1]bicyclooctyl, 3-Azabicyclo; 3-aza[3.2.2]-bicyclononyl, NT; not tested.

Table 2. *In vitro* activity of derivatives of DMT and tylosin against veterinary pathogens.

Compound	Substituent on C-23	MIC (μg/ml)						
		S.a.	P.m. (b)	P.m. (t)	P.h.	M.g.	M.s.	M.h.
Tylosin	O(mycinosyl)	1.56	12.5	25	50	0.39	0.10	1.56
Macrocin	O(demethyl-mycinosyl)	1.56	12.5	25	50	0.78	0.20	3.12
DOMM	O(6-deoxy-allosyl)	6.25	25	25	50	1.56	1.56	12.5
DMT	OH	0.78	6.25	6.25	12.5	0.39	0.78	1.56
23	OPr	0.78	6.25	25	50	0.39	NT	3.12
24	$O(COC_6H_{11})$	0.78	3.12	6.25	25.0	0.20	0.39	1.56
25	O(PhAc)	0.78	1.56	3.12	12.5	<0.05	<0.05	1.56
26	$O(COOCH_3)$	1.56	6.25	12.5	25.0	0.10	0.10	1.56
DMOT	H	0.39	3.12	6.25	12.5	<0.05	0.39	0.78
27	SPh	0.39	6.25	6.25	25.0	<0.05	0.20	0.78
28	N_3	1.56	6.25	6.25	25.0	0.20	0.78	NT
29	NH_2	6.25	6.25	12.5	12.5	1.56	0.78	25
30	NH(PhAc)	1.56	12.5	25.0	25.0	0.10	0.39	1.56

Abbreviations: See Table 1 and Scheme 1.

C-23 Derivatives of DMT and OMT: *In Vivo* Activity

Initially, the evaluation of macrolides for efficacy against *Pasteurella* infections was performed in mice. OMT, given subcutaneously, successfully treated such infected mice (Table 3). This result was subsequently extended to the successful treatment of pneumonia caused by *Pasteurella* in both pigs and calves, in which OMT was given by injection.[10] However, attempts to treat infected mice by oral administration of OMT and its derivatives gave irreproducible results, probably due to the relatively poor and erratic oral absorption of this class of compounds in rodents.

The use of tylosin in poultry for the treatment of chronic respiratory disease due to *Mycoplasma* is well-known. As an alternative to testing in mice, an experimental *Pasteurella* infection in one-day-old chicks was established for

Table 3. Treatment of pasteurellosis in mice with OMT.

Compound	Dose (sc)	Number of deaths/ number treated
OMT	50 mg/kg×2	0/10
	50 mg/kg×1	0/10
	25 mg/kg×2	0/10
	25 mg/kg×1	7/10
	10 mg/kg×2	1/10
	10 mg/kg×1	7/10
	1 mg/kg×2	7/10
Tylosin tartrate	50 mg/kg×2	9/10
Non-medicated	—	10/10

Table 4. Efficacy of macrolide derivatives against experimental infections in chicks caused by *Pasteurella multocida*.

Compound[a]	sc		po		Compound[a]	sc		po	
	Dose (mg/kg ×2)	Mortality[b]	Dose (g/liter)[c]	Mortality[b]		Dose (mg/kg ×2)	Mortality[b]	Dose (g/liter)[c]	Mortality[b]
OMT	30	0	1.32	3	**13**	30	4	—	—
	15	0	0.53	10	DOMT	30	6	—	—
	7.5	4	0.26	10	**14**	30	10	—	—
1	30	1	0.53	9	**15**	30	10	—	—
	15	4			**16**	30	10	—	—
	7.5	10			**17**	30	0	0.53	10
2	30	1	0.53	10	**18**	30	6	—	—
	15	1			**19**	30	4	—	—
	7.5	9			**20**	30	6	—	—
3	30	0	0.53	10	**21**	30	10	—	—
	15	3			**22**	30	10	—	—
	7.5	9			Tylosin	30	8	0.53	8
4	30	0	0.53	10	Macrocin	30	9	—	—
	15	2			DMT	30	8	—	—
	7.5	8			**23**	60	6	—	—
5	30	0	0.53	10	**24**	30	10	—	—
6	30	8	—	—	**25**	30	10	—	—
7	30	0	0.53	10	**26**	30	10	—	—
8	30	1	0.53	5	DMOT	30	10	0.53	9
9	30	0	0.53	9	**27**	30	9	—	—
10	30	8	—	—	**28**	30	10	—	—
11	30	10	—	—	**29**	30	6	—	—
12	30	7	—	—	**30**	30	10	—	—

[a] Compound numbers: See Tables 1 and 2.
[b] Deaths within group of 10 treated chicks.
[c] Compound administered in drinking water.
—: Not tested due to poor parenteral activity.

Table 5. Efficacy of macrolide derivatives against experimental infections in chicks caused by *Mycoplasma gallisepticum*.

Compound[a]	Number of doses[b]	Mortality	Air sac lesions	Antibodies to *M. gallisepticum*
OMT	1	3/10	10/10	7/7
	4	0/10	0/10	1/10
1	1	0/10	4/10	4/10
3	1	1/10	8/10	7/9
4	1	4/10	10/10	6/6
DMT	1	0/10	7/10	7/10
	4	0/10	0/10	0/10
25	1	0/10	5/10	6/10
26	1	1/10	5/10	6/9
	4	0/10	0/10	0/10
DMOT	1	0/10	8/10	8/10
27	1	0/10	7/10	8/10
	4	0/10	3/10	7/10
28	1	0/10	3/10	5/10
	4	0/10	0/10	0/10
30	1	1/10	8/10	8/9
	4	0/10	0/10	3/10
Desmycosin	1	1/10	6/10	5/9
Tylosin	1	0/10	0/10	0/10
Non-medicated	—	2/10	10/10	8/8
Non-infected	—	0/10	0/10	0/10

[a] Compound numbers: See Tables 1 and 2.
[b] Compounds given by sc injection at 60 mg/kg (2 mg/chick).

evaluation of macrolide derivatives by both parenteral and oral administration. Measurable blood levels were obtained for tylosin after oral dosing at 50 and 100 mg/kg (see below), demonstrating its bioavailability by the oral route. In addition, our evaluation of derivatives against this model *Pasteurella* infection in chicks yielded results which were more consistent and reproducible than those obtained from the murine model. Consequently, all further evaluation of *in vivo* activity vs. *Pasteurella* was conducted using the experimental infection in chicks.

The efficacy of selected 23-modified derivatives of DMT and OMT against our model *Pasteurella* infection in chicks is recorded in Table 4. OMT and several of its ester derivatives gave complete protection when administered subcutaneously at 30 mg/kg. However, most of the non-ester derivatives were much less efficacious after parenteral dosing, suggesting that the ester derivatives might be hydrolyzed back to OMT *in vivo*. A more detailed pharmacokinetic and metabolic study would be necessary to answer this question. As anticipated from their reduced *in vivo* activity, the derivatives of DMT and tylosin were less effective *in vivo*, indicating that the demycarosyl series of macrolides were the more promising compounds against this Gram-negative bacterium. Unfortunately, there was little indication of efficacy after oral dosing with any of these compounds.

Representative derivatives of OMT and DMT were also tested *in vivo* against an experimental infection in chicks caused by *Mycoplasma gallisepticum* (Table 5). However, none of the derivatives treated as well as tylosin when given by subcutaneous injection. The degree of treatment was improved if the derivatives were given four times rather than once, but in spite of excellent MICs, none of the derivatives appeared superior to tylosin *in vivo*.

C-20 Derivatives of Tylosin and Desmycosin: *In Vitro* Activity

During the course of the evaluation described above, another series of macrolide derivatives was discovered which was orally efficacious against experimental infections in mice and yielded higher blood levels after oral administration to rodents. These derivatives were modified at the C-20 aldehyde group of tylosin and desmycosin by a wide variety of different functional groups.[11] *In vitro* evaluation of these compounds against veterinary pathogens indicated that many of the derivatives of desmycosin had only moderate activity against *Pasteurella* species (Table 6). However, a few derivatives (**37**, **43** and **46**) showed somewhat lower MIC values than those obtained for the parent macrolide (desmycosin).

Analogous C-20 derivatives of tylosin were inactive against *Pasteurella* species, in accord with the previous conclusion that the 4′-*O*-mycarosyl series had poor *in vitro* activity against this Gram-negative bacterium. Most of the 20-modified derivatives of tylosin and desmycosin showed relatively good *in vitro* activity against *M. gallisepticum* and *Mycoplasma synoviae* but poor activity against *Mycoplasma hyorhinis*; in contrast, their parent macrolides (tylosin and desmycosin) had good activity against all three species. Unfortunately, no correlations could be established concerning *in vitro* activity between Gram-positive bacteria (Staphylococci and Streptococci), *Pasteurella* species and *Mycoplasma* species.

Table 6. *In vitro* activity of derivatives of desmycosin against veterinary pathogens.

Compound	Substituent on C-19	MIC (μg/ml)						
		S.a.	P.m. (b)	P.m. (t)	P.h.	M.g.	M.s.	M.h.
Desmycosin	CHO	1.56	12.5	6.25	25	0.78	0.20	3.12
31	CH_2OH	3.12	12.5	25	25	3.12	0.39	50
32	CH_2OPh	0.39	12.5	12.5	12.5	0.39	1.56	25
33	$CH_2OPh(3\text{-}N(CH_3)_2)$	6.25	25	12.5	25	1.56	1.56	25
34	$CH_2OPh(4\text{-}CH_2N(CH_2)_7)$	1.56	12.5	12.5	25	0.39	0.20	25
35	CH_2O(3-pyridyl)	1.56	25	25	50	0.78	3.12	50
36	CH_2OAc	3.12	25	25	25	1.56	1.56	50
37	CH_2O(PhOAc)	0.39	6.25	6.25	6.25	<0.05	1.56	50
38	CH_2OTs	0.78	25	25	25	0.10	3.12	50
39	CH_2OCH_3	0.78	25	25	25	0.39	0.39	50
40	CH_2Br	0.78	12.5	12.5	12.5	0.39	12.5	>50
41	CH_2Cl	0.78	12.5	6.25	12.5	0.39	NT	50
42	CH_2(phthalimido)	0.78	25	12.5	12.5	0.78	0.78	50
43	CH_2N_3	0.39	6.25	6.25	12.5	0.39	NT	50
44	CH_2NH_2	12.5	>50	>50	>50	6.25	25	>50
45	CH_2NH(PhOAc)	0.78	12.5	25	12.5	<0.05	1.56	50
46	$CH_2N(CH_2)_7$	0.39	3.12	6.25	1.56	0.10	0.20	25
Lactenocin	CHO	3.12	12.5	6.25	25	1.56	0.78	12.5
47	CH_2OH	6.25	50	50	50	12.5	NT	50
48	CH_2OPh	0.39	25	12.5	25	0.78	6.25	50
49	CH_2I	0.78	25	25	25	3.12	1.56	50
50	CH_3	0.78	25	25	25	1.56	NT	50
Tylosin	CHO	1.56	12.5	25	50	0.39	0.10	1.56
51	CH_2OH	6.25	>50	>50	>50	1.56	3.12	50
52	CH_2OPh	3.12	>50	>50	>50	1.56	NT	50
53	CH_2Cl	3.12	>50	>50	>50	0.78	NT	50
54	CH_2(phthalimido)	6.25	>50	>50	>50	3.12	6.25	>50

Abbreviations: See Table 1.

Table 7. Peripheral plasma levels of macrolide derivatives in chicks after oral administration.

Compound[a]	Dose (mg/kg)	Concentration (μg/ml)					
		0.25 hour	0.5 hour	1 hour	2 hours	4 hours	6 hours
Tylosin	100	0.3	0.7	2.8	1.7	0	0
	50	0	0.2	2.1	0.6	0.4	—
52	100	3.3	5.5	4.6	4.4	4.9	—
	50	1.5	3.5	2.5	2.2	1.3	—
53	50	1.5	2.2	1.3	1.1	0	—
54	100	26.0	23.5	24.6	15.2	10.2	1.2
	50	4.3	5.3	4.5	2.0	1.7	—
Desmycosin	100	0	0	0.1	4.4	—	—
32	100	6.8	6.5	9.8	11.0	8.0	4.7
	50	7.2	2.6	1.7	3.4	0.5	—
42	100	20.5	16.3	34.2	14.0	11.0	3.7
	50	5.2	4.5	2.6	5.1	1.1	—
45	100	2.6	6.6	1.5	2.5	0.3	0.1
46	100	3.5	2.4	3.5	3.2	1.8	1.8
OMT	100	0	0	0	0	0	0
3	100	0	0	0	—	—	—

[a] Compound numbers: See Tables 1 and 6.

The most promising lead compound in this series (see below) was the heptamethyleneamino derivative **46**, which incorporated a substituent reported to confer excellent *in vitro* activity when attached to OMT at the C-23 position.[8] The activity of this particular derivative of OMT (**19**) has been shown in Tables 1 and 4. Incorporation of this tertiary amino group into desmycosin at C-20 *via* reductive amination methodology yielded the first example of a macrolide derivative with potentially useful activity against *Pasteurella* species.[12,13] Reductive amination derivatives of tylosin and desmycosin have also been reported by ŌMURA's group;[14,15] however, their *in vitro* activity against *Pasteurella* did not translate into oral efficacy against our model *Pasteurella* infection in chicks.[16]

C-20 Derivatives of Tylosin and Desmycosin: *In Vivo* Activity

Following oral administration of tylosin at either 100 or 50 mg/kg to chicks, measurable peripheral plasma levels were obtained, demonstrating better oral bioavailability of tylosin in chicks as compared to mice or rats.[11] Furthermore, a substantial increase in plasma concentration and duration occurred with the 20-modified derivatives of tylosin and desmycosin as compared to the parent macrolides (Table 7). This result extended our previous report of this effect in rodents for the series of aldehyde-modified derivatives.[11] In contrast, neither OMT nor its 23-phenylacetyl derivative (**3**) gave measurable plasma levels in chicks following an oral dose of 100 mg/kg; their lack of measurable plasma levels after oral dosing is most likely responsible for their lack of oral efficacy in the chick protection test.

Although desmycosin, 20-dihydrodesmycosin (**31**) and lactenocin protected chicks against *Pasteurella* infections when administered subcutaneously at 30 mg/kg×2, many 20-modified derivatives of desmycosin did not protect at this dose (Table 8), despite comparable MIC values against this bacterium. Other considerations such as pharmacokinetics, tissue distribution, the nature of the infection and the mechanism of antibacterial inhibition must be important factors which are not understood at this time. However, in contrast to the majority of derivatives tested, the heptamethyleneamino derivative (**46**) gave complete protection at subcutaneous doses of both 30 and 15 mg/kg×2. Even

Table 8. Efficacy of derivatives of desmycosin against experimental infections in chicks caused by *Pasteurella multocida*.

Compound[a]	sc		po		Compound[a]	sc		po	
	Dose (mg/kg ×2)	Mortality[b]	Dose (g/liter)[c]	Mortality[b]		Dose (mg/kg ×2)	Mortality[b]	Dose (g/liter)[c]	Mortality[b]
Desmycosin	30	0	0.53	8	**39**	30	10	—	—
	15	4	0.26	8	**40**	30	7	—	—
	7.5	10	0.13	10	**41**	30	7	0.53	10
31	30	0	0.53	5	**42**	30	10	—	—
32	30	10	0.53	10	**43**	30	9	—	—
33	30	10	—	—	**45**	30	8	—	—
34	30	10	—	—	**46**	30	0	0.53	1
35	30	9	—	—		15	0	0.26	3
36	30	5	—	—		7.5	5	0.13	8
37	30	8	—	—		3.8	10	0.07	10
38	30	10	—	—	Lactenocin	30	0	0.53	7

[a] Compound numbers: See Table 6.
[b] Deaths within group of 10 treated chicks.
[c] Compound administered in drinking water.
—: Not tested due to poor parenteral activity.

Table 9. Efficacy of derivatives of desmycosin against experimental infections in chicks caused by *Mycoplasma gallisepticum*.

Compound[a]	Dose (g/liter)[b]	Mortality	Air sac lesions	Antibodies to *M. gallisepticum*
32	0.13	0/10	2/10	10/10
37	0.13	1/10	5/10	7/9
38	0.13	0/10	4/10	9/10
43	0.13	0/10	7/10	9/10
46	0.53	0/10	0/10	2/10
	0.13	0/10	1/10	5/10
Tylosin	0.13	0/10	2/10	6/10
Non-medicated	—	1/10	10/10	9/9
Non-infected	—	0/10	0/10	0/10

[a] Compound numbers: See Table 6.
[b] Given by gavage twice per day, 5 total doses, in a quantity equivalent to a daily consumption of 20 ml of drinking water.

more unexpectedly, this derivative (**46**) afforded protection against *Pasteurella* infection when administered to chicks in their drinking water at a dosage of 0.53 g/liter. Furthermore, both the parenteral and oral efficacy showed a good dose response. These favorable results represented the first positive lead in our search for a new, orally effective macrolide derivative with activity against both *Pasteurella multocida* and *Pasteurella haemolytica*. The extension of these results to the synthesis and identification of tilmicosin (EL-870)[17] as a therapeutic agent for treatment of pneumonia in swine and calves will be reported in forthcoming publications.[13,16]

Evaluation of representative 20-modified derivatives of desmycosin against an experimental infection due to *M. gallisepticum* in chicks also indicated efficacy after oral administration in this particular model (Table 9). Again, the reductive amination derivative **46** appeared to give somewhat better efficacy than other 20-modified derivatives of desmycosin, even though all of the compounds

had good *in vitro* activity against *M. gallisepticum*. In this experiment, **46** was comparable to tylosin, indicating that further evaluation and structure-activity studies within the series of reductive amination products of desmycosin were warranted.[13,16]

Experimental

Materials and Methods

^{1}H NMR spectra were measured in $CDCl_3$ solution on a Bruker WH-360 or Jeol FX90A NMR spectrometer; chemical shifts are given in ppm from internal TMS. Field desorption mass spectra (FD-MS) were obtained on a Varian-MAT 731 spectrometer using carbon dendrite emitters. UV spectra were measured in 95% ethanol solution on a Cary 219 spectrometer. IR spectra were recorded in chloroform solution on a Nicolet MX-1 FT-IR spectrometer. Optical rotations were measured on a Perkin-Elmer Model 241 polarimeter. MP's were taken on a Mel-temp apparatus and are uncorrected. TLC was performed using E. Merck plates of Silica gel 60 with a fluorescent indicator (F-254); visualization was effected by UV light. Product purification was carried out by chromatography on silica gel, using either flash chromatography techniques[18] (E. Merck grade 60 Silica gel) or a Waters Model 500 Prep LC system.

Antibiotic susceptibility data were obtained by microtiter procedures. Determination of *in vivo* activity against *Pasteurella* infections was conducted in 1-day-old chicks, in which compounds were administered parenterally or orally after challenge of the chicks with *P. multocida* (0.1 ml of a 10^4-dilution of a 20-hour Tryptose broth culture of an avian *P. multocida* given subcutaneously). In these tests, unless indicated otherwise, all non-medicated infected chicks died within 24 hours of *Pasteurella* challenge. For parenteral administration, the compounds were administered by subcutaneous injection at the specified dosage, either 1 (if single injection) or 1 and 4 hours post-challenge of the chicks with *P. multocida*. Testing for oral efficacy was conducted by dissolution of the compounds in the chicks' drinking water, provided *ad libitum*. *In vivo* activity against *Mycoplasma* was determined after infections were induced in chicks by injecting 0.2 ml of a broth culture of *M. gallisepticum* into the abdominal air sac of 2- to 3-day-old chicks. The compounds were administered by gavage five times at a dose equivalent to 0.13 g/liter (on the day of challenge once prior to and once following the challenge, two times on the next day and once on the third day). Twenty-one days post-infection, a blood sample was taken to measure antibodies to *M. gallisepticum* and the chicks were sacrificed. The presence or absence of air sac lesions was recorded.

Peripheral plasma levels were determined by microbiological assay using *Micrococcus luteus* seeded in Difco Antibiotic Media No. 1. Zone sizes were measured with a Fisher Zone Reader and antibiotic concentrations were calculated from the standard curve for the appropriate compound. Concentrations represent an average value from 6 chicks per time period.

23-Azido-23-deoxy-DMT (28)

A solution of DMT (15.0 g, 20.2 mmol) and *sym*-collidine (5.8 ml, 43.7 mmol) in dichloromethane (250 ml) was cooled to −78°C under argon and treated dropwise with trifluoromethanesulfonic anhydride (5.9 ml, 34.5 mmol) and then with lithium azide (2.975 g, 60.7 mmol). The cooling bath was removed and after 30 minutes, the reaction mixture was diluted with acetonitrile to bring the lithium azide into solution. After 2 hours, the reaction mixture was evaporated to dryness, and the residue was dissolved in dichloromethane. This solution was extracted with saturated sodium bicarbonate solution, dried (sodium sulfate), filtered and evaporated. The crude product was purified by silica gel chromatography, eluting with a linear gradient of dichloromethane (4 liters) and methanol - dichloromethane (3 : 22, 4 liters) to give 10.37 g (67%) of 23-azido-23-deoxy-DMT: MP 231 ~ 237°C (softened ~170°C); IR ($CHCl_3$) cm^{-1} 2103 (N_3), 1721, 1690; UV λ_{max}^{EtOH} nm (ε) 281 (20,500); FD-MS *m/z* 767 (MH^+); elemental *Anal* found C 59.24, H 8.36, N 7.07, calcd for $C_{38}H_{62}N_4O_{12}$: C 59.51, H 8.15, N 7.31.

23-Amino-23-deoxy-DMT (29)

A solution of 23-azido-23-deoxy-DMT (7.98 g, 10.4 mmol), triphenylphosphine (2.86 g, 10.9

mmol) and water (0.25 ml, 13.9 mmol) in distilled THF (200 ml) was stirred at room temperature for 4 days.[19] The reaction mixture was evaporated to give a glassy solid which was partitioned between ethyl acetate and 0.1 M acetic acid solution. The aqueous layer was separated, washed with ethyl acetate and carefully poured into saturated sodium bicarbonate solution. The resulting mixture was extracted with dichloromethane, dried, filtered and evaporated to give 7.5 g (97.5%) of 23-amino-23-deoxy-DMT: MP 210~218°C (softened ~190°C); $[\alpha]_D^{25}$ +6.0° (*c* 1.0, MeOH); IR ($CHCl_3$) cm^{-1} 1725, 1675; UV λ_{max}^{EtOH} nm (ε) 283 (20,700); *pKa* (66% DMF) 7.2 ($N(CH_3)_2$), 8.9 (NH_2); fast atom bombardment mass spectrum (FAB-MS) *m/z* 724 (M^+-16); elemental *Anal* found C 61.38, H 8.42, N 3.78, calcd for $C_{38}H_{64}N_2O_{12}$: C 61.60, H 8.71, N 3.78.

23-(Phenylacetyl)amino-23-deoxy-DMT (**30**)

A solution of 23-amino-23-deoxy-DMT (1.48 g, 2.0 mmol) in 10% aqueous acetone (50 ml) was treated with *N*-(phenylacetyl)oxysuccinimide (446 mg, 2.0 mmol) and stirred at room temperature for 2 hours. After the addition of a few drops of methanol, the reaction mixture was evaporated to an aqueous solution which was extracted with dichloromethane. The organic layer was separated and extracted with saturated sodium bicarbonate solution, dried, filtered and evaporated. The residual glassy solid was purified by flash chromatography on silica gel, eluting with a linear gradient of dichloromethane (750 ml) and methanol - dichloromethane (1 : 4, 750 ml) to yield 1.07 g (62%) of 23-(phenylacetyl)amino-23-deoxy-DMT: MP 153~159°C (softened ~135°C); $[\alpha]_D^{25}$ +28.7° (*c* 1.0, MeOH); ^1H NMR ($CDCl_3$) δ 3.53 (2H, s, CH_2Ph), 7.15~7.30 (5H, m, phenyl); IR ($CHCl_3$) cm^{-1} 1721, 1685; UV λ_{max}^{EtOH} nm (ε) 281 (20,000), end absorption; FD-MS *m/z* 859 (MH^+); elemental *Anal* found C 64.14, H 8.39, N 3.25, calcd for $C_{46}H_{70}N_2O_{13}$: C 64.31, H 8.21, N 3.26.

23-Phenylthio-23-deoxy-DMT (**27**)

A solution of DMT (10.0 g, 13.5 mmol) and *sym*-collidine (3.6 ml, 27.3 mmol) in dichloromethane (200 ml) was cooled to −78°C under argon and treated dropwise with trifluoromethanesulfonic anhydride (3.4 ml, 20.2 mmol). Ten minutes after the addition was completed, the reaction mixture was warmed to −25°C, and thiophenol (2.08 ml, 20.2 mmol) was added. The cooling bath was removed, and the reaction was allowed to come to room temperature over a 1-hour period. The reaction solution was extracted with saturated sodium bicarbonate solution, dried, filtered and evaporated. The residue was dissolved in cyclohexane and again evaporated to give a crude product. This product was purified by silica gel chromatography, eluting with a linear gradient of dichloromethane (4 liters) and methanol - dichloromethane (5 : 95, 4 liters) to give 1.4 g (13%) of 23-phenylthio-23-deoxy-DMT: MP 139~145°C; $[\alpha]_D^{25}$ +72.0° (*c* 1.0, MeOH); ^1H NMR ($CDCl_3$) δ 7.23~7.44 (5H, m, phenyl); IR ($CHCl_3$) cm^{-1} 1721, 1690; UV λ_{max}^{EtOH} nm (ε) 282 (21,800), 255 (sh), 212 (sh); FD-MS *m/z* 834 (MH^+); elemental *Anal* found C 63.28, H 8.09, N 1.68, S 3.92, calcd for $C_{44}H_{67}NO_{12}S$: C 63.36, H 8.10, N 1.68, S 3.84.

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