SYNTHESES AND ANTIMICROBIAL ACTIVITIES OF 9-*O*-ACYL DERIVATIVES OF TYLOSIN AND DEMYCAROSYLTYLOSIN

Sir:

In the course of our studies[1~3] on the structure-activity relationship of tylosin (**1**), which has a potent antimicrobial activity against Gram-positive bacteria and mycoplasma, some 9-*O*-acyl derivatives of **1** and demycarosyltylosin (**8**) were found to possess *in vitro* activity comparable to **1**. Further, 9-*O*-propionyl-9-dihydrotylosin, among the derivatives, exhibited excellent therapeutic effect in mice infected with *Streptococcus pyogenes*. In this communication we describe the syntheses of 9-*O*-acyl derivatives of **1** and **8** and their antimicrobial activities.

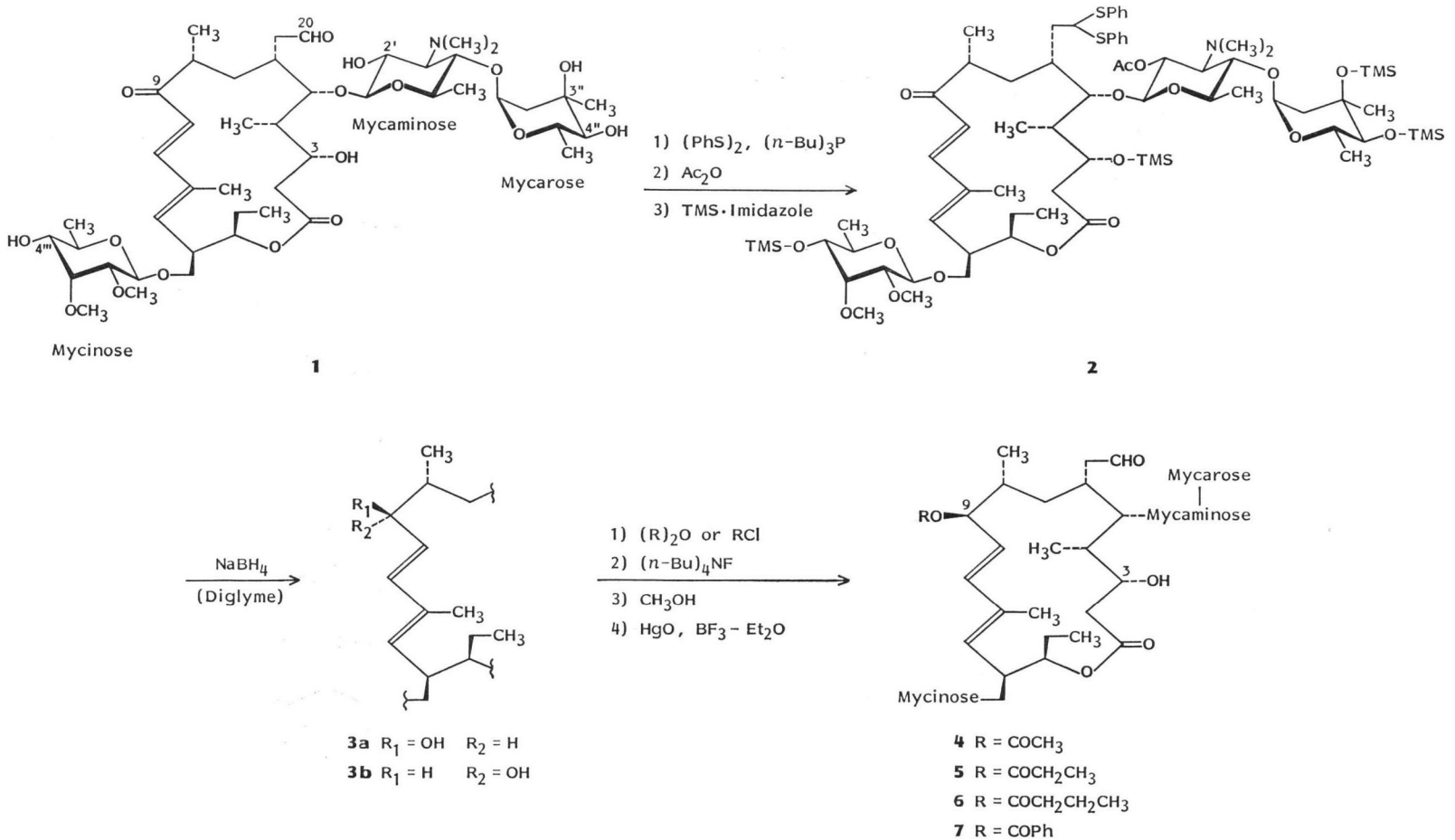
Proper protection of the formyl group at C-20 and several hydroxyl groups in **1** must be performed before the reduction of the carbonyl group at C-9 and subsequent acylation of the hydroxyl group at C-9. At first the formyl group was protected as a dithioacetal[3] as shown in Scheme 1. Acetylation of the 2′-hydroxyl group of the mycaminose moiety with acetic anhydride without external base, followed by silylation of the remaining hydroxyl groups with *N*-trimethylsilylimidazole in piperidine, afforded a fully protected compound, 2′-*O*-acetyl-3,3″,4″,4‴-tetra-*O*-trimethylsilyltylosin diphenyl dithioacetal (**2**): $[\alpha]_D^{23}$ −66° (*c* 1, MeOH); EI(electron impact)-MS *m/z* 1,447 (M^+) in 74% yield. The reduction of the carbonyl group at C-9 in **2** with sodium borohydride in diglyme afforded a mixture of isomeric allylic alcohols, **3a** and **3b** (3:1) in 64% yield. The use of protic solvents resulted in hydrolysis of silyl ethers. The configuration at C-9 of both epimers **3a** and **3b** could be assigned as 9*S* and 9*R*-isomers, respectively, from the coupling constants (**3a**; $J_{9,10}$=4.0 Hz and **3b**; $J_{9,10}$=9.0 Hz) between H-9 and H-10[4,5]. Acylation of the major allylic alcohol **3a** with acid anhydrides such as acetic, propionic and butyric anhydride, or benzoyl chloride in pyridine, followed by the successive deprotections: (1) Removal of four *O*-trimethylsilyl groups at 3,3″,4″ and 4‴ positions by treatment with tetrabutylammonium fluoride in tetrahydrofuran; (2) removal of 2′-*O*-acetyl group by methanolysis at 50°C; (3) hydrolysis of dithioacetal group by treatment with mercury oxide (red) and boron trifluoride etherate in 15% aq tetrahydrofuran[3], afforded 9-*O*-acyl derivatives, **4**~**7** [(9*S*)-9-*O*-acetyl-9-dihydrotylosin (**4**): $[\alpha]_D^{23}$ −64° (*c* 1, MeOH); ^{1}H NMR δ 2.06 (9-OCOCH$_3$) and 5.74 ($J_{9,10}$=3.0 Hz and $J_{10,11}$=16.5 Hz, H-10), (9*S*)-9-*O*-propionyl-9-dihydrotylosin (**5**): $[\alpha]_D^{23}$ −80° (*c* 1, MeOH); ^{1}H NMR δ 5.76 ($J_{9,10}$=4.0 Hz and $J_{10,11}$=16.0 Hz, H-10), (9*S*)-9-*O*-butyryl-9-dihydrotylosin (**6**): $[\alpha]_D^{23}$ −72° (*c* 1, MeOH); ^{1}H NMR δ 5.76 ($J_{9,10}$=4.5 Hz and $J_{10,11}$=16.0 Hz, H-10), (9*S*)-9-*O*-benzoyl-9-dihydrotylosin (**7**): $[\alpha]_D^{23}$ −47° (*c* 1, MeOH); ^{1}H NMR δ 5.92 ($J_{9,10}$=4.0 Hz and $J_{10,11}$=16.0 Hz, H-10).

On the other hand, 9-*O*-acyl derivatives of 9-dihydrodemycarosyltylosin were also prepared from **8** which was obtained by acidic hydrolysis of **1**, as shown in Scheme 2, in a similar manner as the synthesis of 9-*O*-acyl-9-dihydrotylosins. Acetylation of demycarosyltylosin dimethylacetal (**9**) with acetic anhydride in chloroform, followed by silylation with *N*-trimethylsilylimidazole in piperidine, provided 2′,4′-di-*O*-acetyl-3,4″-di-*O*-trimethylsilyldemycarosyltylosin (**10**): EI-MS *m/z* 1,045 (M^+); ^{1}H NMR δ 0.10, 0.17 (3-OSi(CH$_3$)$_3$ and 4″-OSi(CH$_3$)$_3$, respectively), 2.05 (2′-OCOCH$_3$ and 4′-OCOCH$_3$), 3.20 (20-(OCH$_3$)$_2$). Sodium borohydride reduction of **10** followed by acylation with acid anhydride (or acyl chloride) and then removal of *O*-trimethylsilyl and acetyl groups afforded (9*S*)-9-*O*-acyl-9-dihydrodemycarosyltylosin (**12**~**15**), [9-*O*-acetyl-9-dihydrodemycarosyltylosin (**12**): $[\alpha]_D^{28}$ −47° (*c* 1, MeOH); EI-MS *m/z* 797 (M^+−18), 9-*O*-propionyl-9-dihydrodemycarosyltylosin (**13**): $[\alpha]_D^{28}$ −53° (*c* 1, MeOH); EI-MS *m/z* 639 (M^+−mycaminose), 9-*O*-butyryl-9-dihydrodemycarosyltylosin (**14**): $[\alpha]_D^{28}$ −52° (*c* 1, MeOH); EI-MS *m/z* 653 (M^+−mycaminose), 9-*O*-benzoyl-9-dihydrodemycarosyltylosin (**15**): $[\alpha]_D^{21}$ −38° (*c* 1, MeOH); EI-MS *m/z* 756 (M^+−OCOC$_6$H$_5$)].

The *in vitro* antimicrobial activities of the 9-*O*-acyl derivatives of **1** and **8** together with 9-epimers, **3a** and **3b** are shown in Table 1. The configurational difference of the hydroxyl group at C-9 in **3a** and **3b** did not affect antimicrobial activity. The antimicrobial activities of 9-*O*-acyl derivatives were almost the same as those of the corresponding parent compounds **1** and **8**.

The *in vivo* activities of 9-*O*-acyl derivatives was tested in mice infected with *S. pyogenes* C-203 and the results are shown in Table 2.

Scheme 1.



TMS: Trimethylsilyl.

Scheme 2.

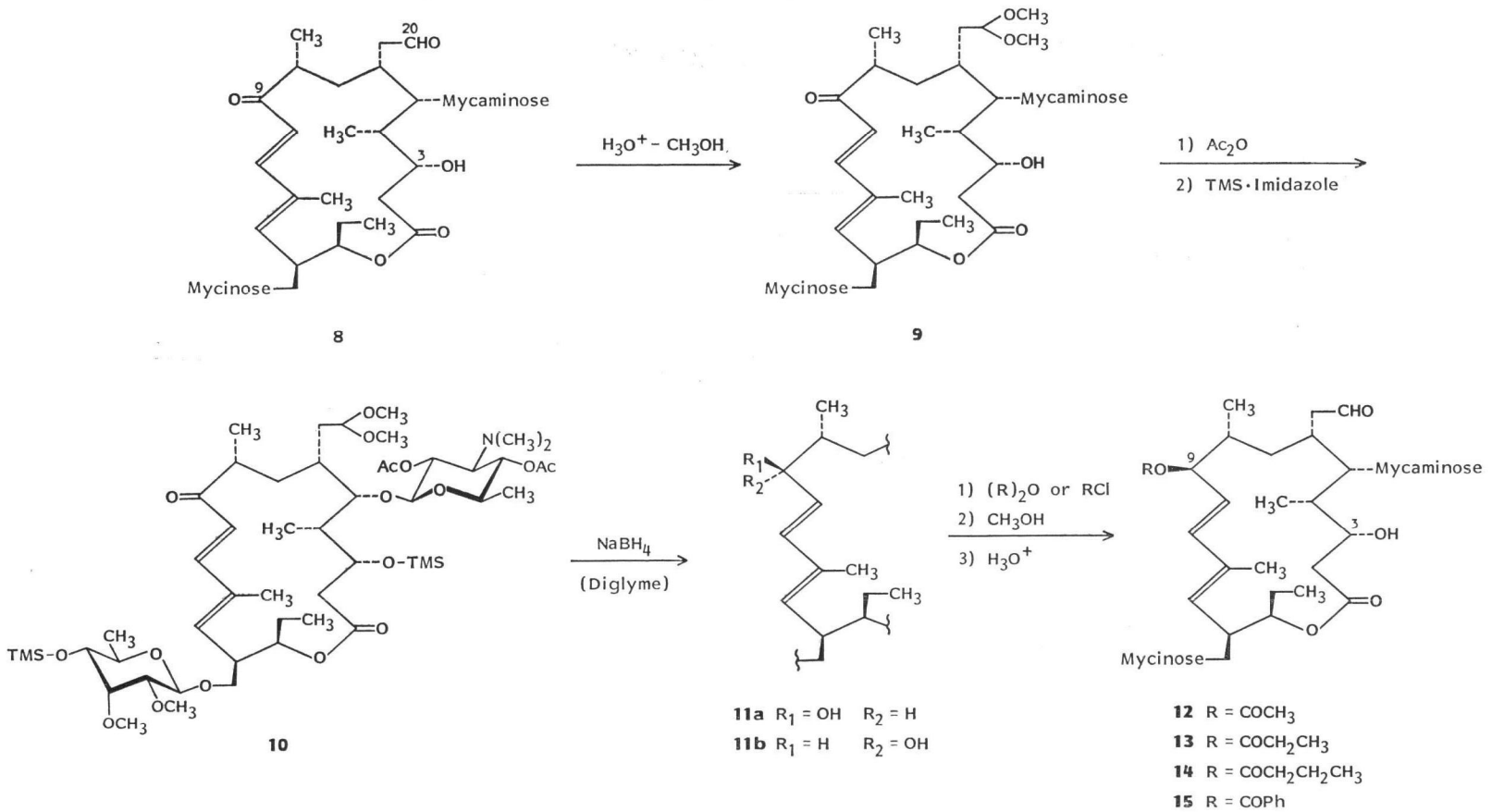

CH3
20
CHO
9
O
Mycaminose
H3C
3
OH
CH3
CH3
O
O
Mycinose
8
H3O+ - CH3OH
OCH3
OCH3
1) Ac2O
2) TMS·Imidazole
9
N(CH3)2
AcO
OAc
O-TMS
TMS-O
10
NaBH4
(Diglyme)
R1
R2
11a R1 = OH R2 = H
11b R1 = H R2 = OH
1) (R)2O or RCl
2) CH3OH
3) H3O+
RO
12 R = COCH3
13 R = COCH2CH3
14 R = COCH2CH2CH3
15 R = COPh

Table 1. Antimicrobial activities of tylosin (**1**), 9-dihydrotylosins (**3a** and **3b**), demycarosyltylosin (**8**) and their 9-*O*-acyl derivatives.

Test organisms	MIC (μg/ml)					
	1	**3a**	**3b**	**4**	**5**	**6**
Staphylococcus aureus ATCC 6538P	0.78	1.56	1.56	1.56	0.78	1.56
Bacillus subtilis ATCC 6633	0.20	0.39	0.39	0.20	0.20	0.39
B. cereus IFO 3001	0.39	0.78	0.78	0.78	0.78	0.78
Micrococcus luteus ATCC 9341	<0.10	<0.10	<0.10	<0.10	<0.10	<0.10
Escherichia coli NIHJ	100	>100	>100	>100	>100	>100
Klebsiella pneumoniae PCI 602	25	25	25	100	>100	100

Test organisms	MIC (μg/ml)					
	7	**8**	**12**	**13**	**14**	**15**
Staphylococcus aureus ATCC 6538P	0.78	3.12	1.56	1.56	1.56	1.56
Bacillus subtilis ATCC 6633	0.20	3.12	1.56	1.56	1.56	1.56
B. cereus IFO 3001	0.78	1.56	0.78	0.78	1.56	1.56
Micrococcus luteus ATCC 9341	<0.10	<0.20	0.20	0.20	0.20	0.20
Escherichia coli NIHJ	>100	>100	>100	>100	>100	>100
Klebsiella pneumoniae PCI 602	>100	>100	>100	>100	>100	>100

Test medium: Nutrient agar (Eiken).
Incubated at 37°C for 18~20 hours.

Table 2. *In vivo* mouse test of tylosin (**1**), demycarosyltylosin (**8**) and their 9-*O*-acyl derivatives.

Compound	ED_{50} (mg/kg) vs. *Streptococcus pyogenes* C-203 infection in mice	
	sc	po
1	1.0	50
5	2.5	12.5
7	>10	70.7
8	0.8	80
12	1.9	29
13	5.4	39
14	5.4	41
15	>10	65

Compounds **5** and **7** were less active than **1** subcutaneously. However, the ED_{50} value of compound **5** by oral administration was superior by about four times to that of **1**. These results suggest that the size of the alkyl chain in *O*-acyl group at C-9 has little effect on the *in vivo* activity. On the other hand, the ED_{50} values of 9-*O*-acyl derivatives of **8** were lower than that of **8** in oral administration, but higher subcutaneously.

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