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

Sir:

In the course of our studies1~3) 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 dithioacetal3) 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): [a]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 9S and 9R-isomers, respectively, from the coupling constants (3a; J9,10=4.0 Hz and 3b; J9,10=9.0 Hz) between H-9 and H-10. 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 methanolation at 50°C; (3) hydrolysis of dithioacetal group by treatment with mercury oxide (red) and boron trifluoride etherate in 15% aq tetrahydrofuran3), afforded 9-O-acyl derivatives, 4~7 ([9S]-9-O-acetyl-9-dihydrotylosin (4): [a]D 23 -64° (c 1, MeOH); 1H NMR δ 2.06 (9-OCOCH3) and 5.74 (J9,10=3.0 Hz and J10,11=16.5 Hz, H-10), (9S)-9-O-propionyl-9-dihydrotylosin (5): [a]D 23 -80° (c 1, MeOH); 1H NMR δ 5.76 (J9,10=4.0 Hz and J10,11=16.0 Hz, H-10), (9S)-9-O-butyryl-9-dihydrotylosin (6): [a]D 23 -72° (c 1, MeOH); 1H NMR δ 5.76 (J9,10=4.5 Hz and J10,11=16.0 Hz, H-10), (9S)-9-O-benzoyl-9-dihydrotylosin (7): [a]D 23 -47° (c 1, MeOH); 1H NMR δ 5.92 (J9,10=4.0 Hz and J10,11=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+) ; 1H NMR δ 0.10, 0.17 (3-OSi(CH3)3 and 4"'-OSi(CH3)3, respectively), 2.05 (2'-OCOCH3 and 4'-OCOCH3), 3.20 (20-(OCH3)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 (9S)-9-O-acyl-9-dihydrodemycarosyltylosin (12~ 15), [9-O - acetyl - 9 - dihydrodemycarosyltylosin (12): [a]D 23 -47° (c 1, MeOH); EI-MS m/z 797 (M+-mycaminose), 9-O-propionyl-9-dihydrodemycarosyltylosin (13): [a]D 23 -53° (c 1, MeOH); EI-MS m/z 639 (M+-mycaminose), 9-O-butyryl-9-dihydrodemycarosyltylosin (14): [a]D 23 -52° (c 1, MeOH); EI-MS m/z 653 (M+-mycaminose), 9-O-benzoyl-9-dihydrodemycarosyltylosin (15): [a]D 23 -38° (c 1, MeOH); EI-MS m/z 756 (M+-OCOC6H5)

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.

1) (PhS)$_2$, (n-Bu)$_3$P
2) A$_2$O
3) TMS-Imidazole

NaBH$_4$ (Diglyme)

1) (R)$_2$O or RCl
2) (n-Bu)$_4$NF
3) CH$_3$OH
4) HgO, BF$_3$ - Et$_2$O

TMS: Trimethylsilyl.
Scheme 2.

1) Ac₂O
2) TMS·Imidazole

Mycinose

H₂O⁺ - CH₃OH

Mycinose

11a R₁ = OH R₂ = H
11b R₁ = H R₂ = OH
12 R = COCH₃
13 R = COCH₂CH₃
14 R = COCH₂CH₂CH₃
15 R = COPh

Mycinose

(TMS-O)

(Diglyme)
Compounds 5 and 7 were less active than 1 subcutaneously. However, the ED50 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 ED50 values of 9-O-acyl derivatives of 8 were lower than that of 8 in oral administration, but higher subcutaneously.

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

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

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

<table>
<thead>
<tr>
<th>Compound</th>
<th>ED50 (mg/kg) vs. Streptococcus pyogenes C-203 infection in mice</th>
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<tr>
<td></td>
<td>sc</td>
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<tr>
<td>1</td>
<td>1.0</td>
</tr>
<tr>
<td>5</td>
<td>2.5</td>
</tr>
<tr>
<td>7</td>
<td>&gt;10</td>
</tr>
<tr>
<td>8</td>
<td>0.8</td>
</tr>
<tr>
<td>12</td>
<td>1.9</td>
</tr>
<tr>
<td>13</td>
<td>5.4</td>
</tr>
<tr>
<td>14</td>
<td>5.4</td>
</tr>
<tr>
<td>15</td>
<td>&gt;10</td>
</tr>
</tbody>
</table>

Compounds 5 and 7 were less active than 1 subcutaneously. However, the ED50 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 ED50 values of 9-O-acyl derivatives of 8 were lower than that of 8 in oral administration, but higher subcutaneously.

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References