Cladinose Analogues of Sixteen-membered Macrolide Antibiotics

V. Preparation of Unsubstituted L-Cladinose Analogues: Effect of Methylation of a 3'-Hydroxyl Group on the Bioactivity

Keiichi Ajito, Akira Shimizu, Seiji Shibahara, Osamu Harra, Ken-ichi Kurihara, Minako Araake, Kazuyo Tohyama, Shinji Miyadoh, Shoji Omoto and Shigeharu Inouye

Pharmaceutical Research Center, Meiji Seika Kaisha, LTD., Morooka-cho, Kohoku-ku, Yokohama 222, Japan

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Sixteen-membered macrolide antibiotics1) have been used in clinic because of their efficacy and safety. The design and synthesis of 16-membered macrolide derivatives considering deacylation2) at a neutral sugar moiety in vivo would be an important approach to generate conceptually effective analogues in clinical use. As part of our program in this area we have recently designed and synthesized 4-O-alkyl-L-cladinose analogues of Ieucomycins3~5), and their efficacy was demonstrated both in vitro and in vivo6). In this communication we wish to report the preparation and potency of unsubstituted L-cladinose analogues (4~6) of sixteen-membered macrolides, which were designed based on a structure of a neutral sugar in erythromycin. The reported compounds (4~6) exhibited dramatically enhanced activity in vitro in comparison with mycarose-type counterparts (Fig. 1). The greatly improved protective effects in vivo7) of 1 and its 9-O-acetyl derivative 8 (Fig. 2) could be explained with the unexpectedly improved in vitro activity of 4 determined as their major metabolite.

There are structural differences in neutral sugar moieties between sixteen- and fourteen-membered macrolide antibiotics. Although 14-membered macrolides possess mainly either cladinose or mycarose at the C-3 position, only mycarose is attached to the C-4' position in 16-membered macrolides. Since a sixteen-membered macrolide having an unsubstituted α-L-cladinosyl residue has not been reported, studies of the compounds 4~6 would be challenging.

Effective transformations of 1 to the desired L-cladinose analogues (4~6) were done by appropriate microbial transformations8) (Scheme 1). Oxidation of an allylic alcohol of 1 affording a dienone (29), 80% yield, MP 108°C, [α]D7 -30° (c 0.6, MeOH), FD-MS m/z 826 (M+H)+. A carbomycin B-type analogue of 2 (3'-O-methylcarbomycin B) has been synthesized by Tatsuta et al.9) via glycosylation as a leading research. On the other hand, regioselective removal of a 3-O-propionyl group of 1 was achieved with an efficient biotransformation10) using Phialophora sp. PF108311), furnishing a 3-OH derivative (3, 28% yield), MP 111~113°C, [α]D7 -79° (c 1.0, MeOH), EI-MS m/z 771 (M)+, 1H NMR (400MHz, CDCl3) δ 3.79 (1H, brd, J=11.0Hz, 3-H) accompanied with recovered 1 (16%). Useful cleavage of the 3-O-acetyl group in leucomycin family by synthetic chemistry has not been reported.

Next, these 4-O-acyl-L-cladinose derivatives (1~3) were converted to the corresponding unsubstituted L-cladinose analogues (4~6) respectively, using Mucor spinscoes IAM 607112) or Paecilomyces sp. PF11089). All new compounds provided satisfactory spectroscopic data (Table 1). One explanation concerning these incomplete conversion yields might involve steric hindrance of a 3'-β-<9-methyl group of 1~3. Bioconversion of a 4-O-acetyl-L-mycarose derivative to the unsubstituted-L-mycarose analogue was proceeded more efficiently13). This difficulty about 4'-de-O-propionylation by biological medium of 1~3 might suggest that a 4'-<9-acyl group of these cladionose analogues would be hardly cleaved by metabolism in vivo in comparison with that of the L-mycarose counterparts.

Antibacterial activities in vitro of the novel unsubstituted z-L-cladinosyl derivatives (4~6), compared with those of sixteen-membered macrolide antibiotics possessing 1-myacrose, 4'-de-O-propionylleucomycin A1 13) (7), DOP14) and leucomycin V15) (LM-V) (Fig. 1), are shown in Table 2. As judged from MIC values, these cladinose analogues exhibited about four to sixteen times...
Scheme 1. Transformations of compound 1 to unsubstituted L-cladinose analogues (4 - 6).

Table 1. Physico-chemical properties of 4 - 6.

<table>
<thead>
<tr>
<th>Compound</th>
<th>MP (°C)</th>
<th>EI-MS m/z</th>
<th>[a]D (°) (c 1.0, MeOH)</th>
<th>*H NMR (400MHz, CDCl3)</th>
</tr>
</thead>
<tbody>
<tr>
<td>(4)</td>
<td>120 - 122°</td>
<td>111</td>
<td>-56°</td>
<td>0.92 (IH, br, d, 7-H), 0.98 (3H, d, 19-H), 1.16 (3H, d, 6'-H), 1.22 (3H, t, 3-OOCOCH2C6H5), 1.22 (3H, s, 3'-CH3), 1.23 (3H, d, 6'-H), 1.26 (3H, d, 16-H), 1.34 (1H, br, tt, 7-H), 1.57 (IH, dd, 2”-Hax), 1.89 (IH, m, 8-H), 2.15 (1H, dt, 14-H), 2.23 (1H, d, 2”-Heq), 2.24 (1H, br d, 2-H), 2.32 (1H, br dd, 17-H), 2.39 (1H, t, 5’-H), 2.46 (1H, br dt, 14-H), 2.51 and 2.65 (each 1H, 2 x dq, 3-OOCOCH2C6H5), 2.55 (6H, s, 3’-N(CH3)2), 2.76 (1H, dd, 2-H), 2.86 (1H, br dd, 17-H), 3.01 (1H, br dt, 4’-H), 3.21 (1H, dd, 2-H), 3.22 (1H, s, 3’-OCH3), 3.58 (3H, s, 4-OCH3), 3.88 (3H, br dd, 5’-H), 4.07 (1H, dd, 9-H), 4.18 (1H, dq, 5”-H), 4.51 (1H, d, 1’-H), 4.89 (1H, d, 1’-H), 5.03 (1H, dd, 15-H), 5.14 (1H, br d, 3-H), 5.61 (1H, dd, 10-H), 5.79 (1H, ddd, 13-H), 6.08 (1H, br dd, 12-H), 6.67 (1H, br dd, 11-H), 9.63 (1H, br s, 18-H).</td>
</tr>
<tr>
<td>(5)</td>
<td>121 - 124°</td>
<td>769</td>
<td>-27°</td>
<td>1.14 (3H, t, 3-OOCOCH2C6H5), 1.16 (3H, d, 6'-H), 1.20 (3H, d, 19-H), 1.22 (3H, s, 3’-CH3), 1.23 (3H, d, 6'-H), 1.29 (3H, d, 16-H), 1.49 (1H, br t, 7-H), 1.57 (1H, dd, 2”-Hax), 1.64 (1H, dt, 7-H), 1.78 (1H, br t, 6-H), 2.24 (1H, d, 2”-Heq), 2.26 (1H, br d, 2-H), 2.58 (6H, br s, 3’-N(CH3)2), 2.75 (1H, br dd, 17-H), 2.78 (1H, dd, 2-H), 3.01 (1H, ddd, 13-H), 3.22 (1H, d, 1’-H), 3.45 (1H, t, 4’-H), 3.60 (3H, s, 4-OCH3), 3.89 (1H, dq, 5’-H), 4.15 (1H, br dd, 5’-H), 4.60 (1H, d, 9-H), 4.89 (1H, dq, 15-H), 4.89 (1H, d, 1’-H), 5.09 (1H, br dd, 3-H), 6.22 (2H, m, 12-H, 13-H), 6.34 (1H, d, 10-H), 7.38 (1H, dd, 11-H), 9.63 (1H, br s, 18-H).</td>
</tr>
<tr>
<td>(6)</td>
<td>117 - 122°</td>
<td>715</td>
<td>-74°</td>
<td>0.99 (3H, d, 19-H), 1.19 (3H, d, 6'-H), 1.22 (3H, s, 3’-CH3), 1.23 (3H, d, 6'-H), 1.31 (3H, d, 16-H), 1.57 (1H, dd, 2”-Hax), 1.60 (1H, br d, 7-H), 1.91 (1H, m, 8-H), 2.12 (1H, dt, 14-H), 2.23 (1H, d, 2-H), 2.24 (1H, d, 2”-Heq), 2.34 (1H, br dd, 17-H), 2.40 (1H, t, 5’-H), 2.51 (1H, br dt, 14-H), 2.56 (6H, s, 3’-N(CH3)2), 2.70 (1H, dd, 2-H), 2.88 (1H, br dd, 17-H), 3.01 (1H, br t, 4’-H), 3.10 (1H, br d, 4-H), 3.22 (1H, s, 3’-OCH3), 3.23 (1H, dd, 2-H), 3.26 (1H, dq, 5’-H), 3.44 (1H, t, 4’-H), 3.55 (3H, s, 4-OCH3), 3.80 (1H, br d, 3-H), 4.11 (1H, dd, 9-H), 4.11 (1H, br d, 5-H), 4.18 (1H, dq, 5’-H), 4.58 (1H, d, 1’-H), 4.89 (1H, d, 1’-H), 5.29 (1H, dq, 15-H), 5.61 (1H, ddd, 13-H), 5.69 (1H, dd, 10-H), 6.04 (1H, br dd, 12-H), 6.27 (1H, dd, 11-H), 9.80 (1H, br s, 18-H).</td>
</tr>
</tbody>
</table>
higher activity than their counterparts respectively (for example 4 vs. 7). This is the first report about 16-membered macrolides having unsubstituted cladinose at the C-4' position, since it has been difficult to predict enhancement of in vitro activity by introducing a methyl group into the 3''-OH.

A neutral sugar moiety of natural sixteen-membered macrolide antibiotics and their acylated derivatives is converted to unsubstituted mycarose by metabolism in general. As reported partly in our previous paper, however, compound 1 and its 9-O-acetyl derivative (8) were mainly metabolized to an active metabolite 4 in mice. Actually, compound 4 was detected as a main metabolite in urine after oral administration of 8 in mice. Moreover, incubation of 8 with human liver S9 fraction gave 4 as one of major metabolites. These observations explained that the 4-O-acyl-L-cladinose analogues, compound 1, 3 and their derivatives, for example 8, could exhibit dramatically improved efficacy in vivo (In leucomycin family, compounds having an sp³ carbon at the C-9 position exhibited superior efficacy in vivo⁴,¹¹⁰ than those having an sp² carbon like compound 2).

In conclusion, a series of sixteen-membered macrolides possessing an unsubstituted α-L-cladinose moiety were prepared via appropriate biotransformations. They showed antibacterial activity four to sixteen-fold more potent in vitro than the counterparts with the α-L-mycarose moiety. The clarified preliminary metabolic pathway of 1 and 3 and the described potency of their metabolites (4 and 6) open the way for eventually new metabolically-programmed sixteen-membered macrolide antibiotics.

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