ISOLATION AND CHARACTERIZATION OF 10-DEOXYMETHYLNOLIDE PRODUCED BY *Streptomyces venezuelae*

Sir:

Methymycin (V) and neomethymycin (VI) are macrolide antibiotics co-produced by fermenting cultures of *Streptomyces venezuelae* ATCC 15439. Methynolide (II), neomethynolide (III)\(^1\) and YC-17 (IV)\(^2\) have all been reported as biosynthetic precursors of V and VI. In previous work we demonstrated intact incorporation of isotopically labeled advanced precursors into V and VI thus providing further confirmation for the processive chain elongation mechanism proposed for macrolide biosynthesis.\(^3\) In this paper, we report the isolation and characterization of 10-deoxymethynolide (I), the parent aglycone of this series of metabolites II ~ VI.

I is accumulated in liquid cultures of *S. venezuelae* grown in the presence of xanthotoxin (8-methoxypsoralen), a reported inhibitor of monooxygenase activity.\(^4\) A concentrated spore suspension (0.1 ml) of *S. venezuelae* was used to inoculate 100 ml SCM medium consisting of soluble starch 15 g, Soytone 20 g, CaCl\(_2\) 0.1 g, yeast extract 1.5 g and MOPS 10.5 g per 1 liter nanopure H\(_2\)O, pH 7.2. The culture was grown at 27°C on a rotary shaker at 250 rpm for 48 hours. Two 2.8-liter baffled flasks containing 2 x 500 ml SCM media each had 108 mg (0.5 mmol) xanthotoxin in 1 ml DMSO added after autoclaving and were inoculated with 2 x 15 ml 48-hour seed culture. These vegetative cultures were then grown under the same conditions for 46 hours. The cultures were harvested by centrifugation at 10,000 x g for 10 minutes and the supernatant pH adjusted to 9.5 with KOH. The broth was extracted with 5 x 100 ml CHCl\(_3\). Emulsions were clarified with Celite. Drying (MgSO\(_4\)) and concentration *in vacuo* provided 223 mg oil. The mycelia were extracted with 2 x 100 ml acetone, the acetone removed by rotovapor, and the yellow concentrate extracted with 3 x 75 ml CHCl\(_3\). Drying (MgSO\(_4\)) followed by concentration *in vacuo* provided 75 mg of recovered xanthotoxin.

The crude supernatant extract was purified by flash chromatography on silica gel. Elution with a gradient of 0 ~ 20% (v/v) MeOH in CHCl\(_3\) provided 8 mg each of I, V and VI. Only minor amounts of other unidentified metabolites had accumulated in the broth. A total of 150 mg xanthotoxin was recovered from both the mycelia and broth. This experiment was repeated with a 10-liter culture of *S. venezuelae* grown in the presence of 2 mM xanthotoxin in a 14-liter New Brunswick fermentor to obtain 250 mg I. Cultures of *S. venezuelae* grown under the same conditions in the absence of xanthotoxin typically produced 50 ~ 75 mg/liter of both V and VI with no significant accumulation of other macrolides.

Characterization of I, [\(\alpha\)]\(_{D}\)\(^{25} + 67.75°\) (c 8.0 mg/ml, CHCl\(_3\)), UV \(\lambda_{max}^{MeOH}\) nm (\(\varepsilon\)) 226 (9100), IR \(v_{max}\) (neat) cm\(^{-1}\) 3464, 2970, 2937, 2879, 1726, 1687, 1628, 1459, 1383, 1325, CI-MS (NH\(_4^+\)) Calcd for C\(_{17}\)H\(_{28}\)O\(_4\) 297.2066 (M\(+\)H\(^+\))\(^+\), Found 297.2071 (M\(+\)H\(^+\))\(^+\). \(^1\)H NMR and \(^13\)C NMR (Table 1), confirmed the structure to be that of 10-deoxymethynolide (I). All NMR assignments were confirmed by \(^1\)H-\(^1\)H-COSY, \(^1\)H,\(^13\)C-HETCOR, INEPT, and J Resolved experiments (data not shown). Assignments of relative stereochemistry are based on comparison of chemical shifts and coupling constants with \(^1\)H NMR spectra of V and VI.

Scheme 1. Methymycin and related metabolites of *Streptomyces venezuelae* ATCC 15439.

<table>
<thead>
<tr>
<th></th>
<th>R(_1)</th>
<th>R(_2)</th>
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<tbody>
<tr>
<td>I</td>
<td>10-Deoxymethynolide</td>
<td>–H</td>
</tr>
<tr>
<td>II</td>
<td>Methynolide</td>
<td>–H</td>
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<tr>
<td>III</td>
<td>Neomethynolide</td>
<td>–OH</td>
</tr>
<tr>
<td>IV</td>
<td>YC-17</td>
<td>–H</td>
</tr>
<tr>
<td>V</td>
<td>Methymycin</td>
<td>–H</td>
</tr>
<tr>
<td>VI</td>
<td>Neomethymycin</td>
<td>–OH</td>
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Table 1. $^1$H NMR (400 MHz, CDCl$_3$) and $^{13}$C NMR (100 MHz, CDCl$_3$) data of 10-deoxymethynolide (I).

<table>
<thead>
<tr>
<th>$^{13}$C</th>
<th>$\delta$ (ppm)</th>
<th>m</th>
<th>$^1$H</th>
<th>$\delta$ (ppm)</th>
<th>m</th>
<th>$J$</th>
<th>Hz</th>
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<tbody>
<tr>
<td>C-1</td>
<td>174.7</td>
<td>CO$_2$</td>
<td>2-H</td>
<td>2.6</td>
<td>dq</td>
<td>$J_{2,3}$</td>
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<tr>
<td>C-2</td>
<td>43.4</td>
<td>CH</td>
<td>3-H</td>
<td>3.55</td>
<td>d</td>
<td>$J_{3,4}$</td>
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<tr>
<td>C-3</td>
<td>78.2</td>
<td>CH</td>
<td>4-H</td>
<td>1.27$^a$</td>
<td>m</td>
<td>$J_{4,5a}$</td>
<td>*</td>
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<tr>
<td>C-4</td>
<td>33.22</td>
<td>CH</td>
<td>5-Ha</td>
<td>1.65</td>
<td>m</td>
<td>$J_{4,5b}$</td>
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<tr>
<td>C-5</td>
<td>33.24</td>
<td>CH$_2$</td>
<td>5-Hb</td>
<td>1.32$^a$</td>
<td>m</td>
<td>$J_{4,15}$</td>
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<tr>
<td>C-6</td>
<td>45.1</td>
<td>CH</td>
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<td>2.53</td>
<td>ddq</td>
<td>$J_{5a,5b}$</td>
<td>*</td>
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<tr>
<td>C-7</td>
<td>204.9</td>
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<td>8-H</td>
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<td>15-H</td>
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<td>16-H</td>
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<td>$J_{12a,12b}$</td>
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<tr>
<td>C-17</td>
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<td>17-H</td>
<td>1.12</td>
<td>d</td>
<td>$J_{12ab,13}$</td>
<td>7.43</td>
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All shifts relative to TMS.
Asterisks indicate that the coupling constant could not be determined due to non-first order effects.
$^a,b$ These values may be interchanged.

I proved difficult to crystallize and was therefore derivatized as the 3,5-dinitrobenzoate ester (I-DNB) by stirring 23 mg I and an approximate five-fold excess of 3,5-dinitrobenzoyl chloride (freshly recrystallized from pentane) in 1ml 50% (v/v) pyridine (dried over KOH) in CH$_2$Cl$_2$ (distilled from P$_2$O$_5$) with a catalytic amount of DMAP for 48 hours at room temperature. The reaction mixture was then diluted with 1ml CH$_2$Cl$_2$ and washed with 1 x 1 ml 5% (v/v) HCl followed by 1 x 1 ml saturated NaHCO$_3$. The organic phase was dried over MgSO$_4$ and concentrated to 32 mg crude orange solid. Preparative TLC purification (20 x 20 cm x 1 mm silica, 4% (v/v) MeOH in CHC$_2$H$_4$) provided 25 mg (66%) white amorphous solid, mp 225~228°C; EI-MS Caled for C$_{24}$H$_{30}$N$_2$O$_9$, Found 490.1951. The $^1$H NMR spectrum of I-DNB (CDCl$_3$, 400 MHz) was consistent with the expected structure of the derivative.

Thus growing S. venezuelae in the presence of xanthotoxin provides 10-deoxymethynolide. This material may serve as a valuable standard in enzymatic studies of the methymycin polyketide synthase currently in progress in our laboratory.

Acknowledgment

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References