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5-HYDROXYMETHYLBLASTICIDIN S AND BLASTICIDIN S FROM
STREPTOMYCES SETONII CULTURE A83094

STEPHEN H. LARSEN, DONNIS M. BERRY,
JONATHAN W. PASCHAL and JAMES M. GILLIAM

Lilly Research Laboratories,
Eli Lilly and Company,
Lilly Corporate Center,
Indianapolis, Indiana 46285, U.S.A.

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In our preceding paper we described the isolation and structure elucidation of antibiotic A83094A (16-deethylindanomycin) from the biomass of Streptomyces setonii. The culture filtrate from a fermentation of this same organism contains two broad-spectrum antibiotics which were isolated and determined to be 5-hydroxymethylblasticidin S (A83094B) and blasticidin S (A83094C) as shown in Fig. 1.

The flow diagram for the isolation of 5-hydroxymethylblasticidin S and blasticidin S as a complex is presented in Fig. 2. The antibiotic levels at each step were determined both by disc plate assay vs. Salmonella gallinarum and by HPLC. HPLC assays were run on a yBondapak C18 column (3.9 x 300 mm) using a mobile phase of CH3CN - H2O (4 : 96) containing 1 % NH4OAc (w/v) and UV detection at 225 nm.

Separation of the complex into individual antibiotics was accomplished using semi-preparative HPLC. The reversed phase chromatography system consisted of a M6000 pump, yBondapak C18 column (9.8 x 300 mm), Model 490 variable wavelength detector, U6K manual injector with 2.0 ml loop (all supplied by Millipore/Waters, Milford, Mass., U.S.A.) and mobile phase (as described above for analytical HPLC) pumped at 4 ml/minute. Eight separate chromatographic runs were performed as follows: 20 µl aqueous sample, containing 2 mg antibiotic mixture, was applied. 16 ml of eluate was discarded; then fractions were collected at 20-second intervals and assayed by HPLC. Fractions containing >95 % of each component were combined and lyophilized. Each lyophile was dissolved in 1.0 ml water and desalted by injection on a µBondapak C18 column (3.9 x 300 mm). After washing the column with 20 ml water, the antibiotic was eluted with CH3CN - H2O (50 : 50). The CH3CN was removed under reduced pressure and the remaining solution lyophilized to provide samples for physico-chemical analyses.

5-Hydroxymethylblasticidin S (A83094B) is a water soluble compound: MP >225°C (char); UV λmax (H2O, pH 7) 272 nm. The molecular weight of 452 (C18H28N8O6) was determined on the basis of fast atom bombardment (FAB)-MS data which displayed a M+H+, m/z 453, a 30-dalton increase in comparison to blasticidin S2). The monosodiated ion at m/z 475 was also seen.

The 1H NMR of A83094B (500 MHz, DMSO-d6) was compared to authentic blasticidin S and indicates that they are structurally similar (see Table 1). The differences are in the loss of 5-H in A83094B and the addition of a hydroxymethylene at δH 4.21. A nuclear Overhauser effect (NOE) is observed between the new methylene and 6-H. The presence of a hydroxymethylene is consistent with the mass spectral data. Based on recently published data, the structure of A83094B is similar to Sch 366052).

Fig. 1. The chemical structures of A83094B, A83094C and Sch 36605.
Fig. 2. Purification of antibiotic complex.

Culture filtrate (25 liters)
   Diaion HP-20 column chromatography (6 × 31 cm)
   eluted with 25% MeOH
Lyophilized active fractions (8.7 g)
   triturated with MeOH
MeOH triturant (3.6 g)
   precipitated with acetone
Precipitate dried in vacuo (1.53 g)
   Diaion HP-20SS column chromatography (5 × 8 cm)
   eluted with 5% MeOH and 10% MeOH
Concentrated active fractions (710 mg)
   Amberlite IRC-50 column chromatography (2.5 × 4 cm)
   eluted with 0.005 N HCl
   eluted with 0.005 N HCl - MeOH (4 : 1)
Lyophilized active fractions (257 mg)
   Sephadex LH-20 column chromatography (8.5 × 44 cm)
   eluted with MeOH
Concentrated active fractions (175 mg)
   Waters Assoc. SEP-PAK C18 cartridge
   eluted with H₂O
Mixture of 5-hydroxymethylblasticidin S and
   blasticidin S (110 mg)

<table>
<thead>
<tr>
<th>Position</th>
<th>A83094B</th>
<th>Blasticidin S</th>
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<tbody>
<tr>
<td>11'-H</td>
<td>3.52, 3.15</td>
<td>3.51, 3.18</td>
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<tr>
<td>10'-H</td>
<td>1.78, 1.36</td>
<td>1.80, 1.45</td>
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<tr>
<td>9'-H</td>
<td>2.87</td>
<td>2.96</td>
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<tr>
<td>8'-H</td>
<td>2.22, 2.07</td>
<td>2.24, 2.14</td>
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<tr>
<td>4'-H</td>
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<td>4.54</td>
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<tr>
<td>3'-H</td>
<td>5.93b</td>
<td>5.93b</td>
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<tr>
<td>2'-H</td>
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<tr>
<td>5-CH₃OH</td>
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<tr>
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</table>

a, b Assignments may be reversed.

Acknowledgment

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