

5-HYDROXYMETHYLBlasticidin S
AND Blasticidin S FROM
STREPTOMYCES SETONII
CULTURE A83094

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In our preceding paper¹⁾ 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 μ Bondapak C18 column (3.9 \times 300 mm) using a mobile phase of CH₃CN - H₂O (4 : 96) containing 1% NH₄OAc (w/v) and UV detection at 225 nm.

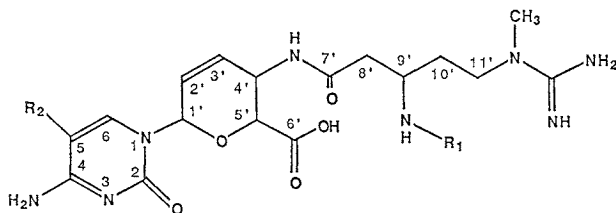
Separation of the complex into individual antibiotics was accomplished using semi-preparative HPLC. The reversed phase chromatography system consisted of a M6000 pump, μ Bondapak C18 column (9.8 \times 300 mm), Model 490 variable wavelength detector, U6K man-

ual 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 μ l 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 μ Bondapak C18 column (3.9 \times 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 λ_{max} (H₂O, pH 7) 272 nm. The molecular weight of 452 (C₁₈H₂₈N₈O₈) 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 S^{2,3)}. The monosodium 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 δ_H 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 36605⁴⁾.

Fig. 1. The chemical structures of A83094B, A83094C and Sch 36605.



5-Hydroxymethylblasticidin S (A83094B)
Blasticidin S (A83094C)
Sch 36605

R₁=H R₂=CH₂OH
R₁=H R₂=H
R₁=Leucine R₂=CH₂OH

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 1. ¹H NMR data of A83094B and blasticidin S in DMSO-*d*₆.

Position	A83094B	Blasticidin S
11'-H	3.52, 3.15	3.51, 3.18
10'-H	1.78, 1.36	1.80, 1.45
9'-H	2.87	2.96
8'-H	2.22, 2.07	2.24, 2.14
4'-H	4.54	4.54
3'-H	5.93 ^a	5.93 ^b
2'-H	5.65 ^a	5.68 ^b
1'-H	6.33	6.32
5'-H	3.80	3.81
6-H	7.30	7.37
5-H	—	5.76
5-CH ₂ OH	4.21	—
11'-NCH ₃	2.87	2.87

^{a, b} Assignments may be reversed.

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