

**Epostatins, New Inhibitor of
Dipeptidyl Peptidase II, Produced
by *Streptomyces* sp. MJ995-OF5**

II. Structure Elucidation

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(Received for publication November 21, 1997)

During the course of screening fermentation broths for new inhibitors of dipeptidyl peptidase II (EC 3.4.14.2), we discovered epostatins ($C_{23}H_{33}N_3O_5$) from *Streptomyces* sp. MJ995-OF5. The taxonomy of producing strain, fermentation, isolation, physico-chemical properties and biological properties have been described in the preceding paper¹. Herein we report on the structure determination of epostatins (Fig. 1) on the basis of spectroscopic studies.

The molecular weight of epostatins was measured by FAB-MS (m/z 432 [$M+H^+$], m/z 430 [$M-H^-$]), and the molecular formula of $C_{23}H_{33}N_3O_5$ was determined by interpretation of the HRFAB-MS, NMR spectral analyses and elemental analysis¹. The structure of epostatins was elucidated on the basis of 1D and 2D NMR experiments in $DMSO-d_6$.

In the 1H NMR spectrum, one methyl, eight methylene and four methine, five olefinic and three of five exchangeable protons were observed. The ^{13}C NMR spectrum showed twenty-three carbon signals, and analysis of the DEPT experiment revealed that the ^{13}C NMR signals consisted of fourteen sp^3 including one quaternary carbon, six sp^2 and three carbonyl carbons. Signals of six olefinic carbons indicated the presence of three double bonds in epostatins. These and the three

carbonyl carbons account for six degrees of unsaturation, so the remaining three degrees of unsaturation should be due to the presence of three rings in the molecule. The carbons bearing protons were assigned by the Heteronuclear Single Quantum Coherence (HSQC) experiment. The 1H and ^{13}C NMR data for epostatins are summarized in Table 1.

Analysis of the 1H - 1H COSY experiment revealed

Table 1. 1H and ^{13}C NMR data for epostatins in $DMSO-d_6$.

Position	δ_C^a ppm (multiplicity)	δ_H^b ppm (multiplicity, J in Hz)
1a	62.4 (d)	3.30 (1H, d, 2.1)
2	35.0 (d)	1.90 (1H, m)
3	45.4 (t)	2.76 (1H, dd, 5.8, 12.5), 2.09 (1H, m)
4a	62.0 (d)	3.77 (1H, br s)
5	139.9 (d)	6.35 (1H, dt, 1.9, 6.6)
6	131.0 (d)	7.03 (1H, d, 6.6)
7	148.3 (s)	—
7a	65.5 (s)	—
8	116.2 (d)	5.75 (1H, d, 12.1)
9	134.8 (d)	7.37 (1H, dd, 12.1, 15.0)
10	124.5 (d)	6.07 (1H, d, 15.0)
11	165.1 (s)	—
1'	—	8.27 (1H, d, 7.9)
2'	51.8 (d)	4.22 (1H, ddd, 8.9, 8.1, 1.9)
3'	27.0 (t)	1.97 (1H, m), 1.75 (1H, m)
4'	31.4 (t)	2.11 (2H, m)
5'	173.4 (s)	—
6'	—	7.29 (1H, br s), 6.75 (1H, br s)
7'	173.5 (s)	—
1''	29.0 (t)	1.27 (1H, m), 1.23 (1H, m)
2''	26.0 (t) ^c	1.36 (2H, m)
3''	29.7 (t) ^c	1.36 (2H, m)
4''	31.2 (t)	1.27 (2H, m)
5''	22.1 (t)	1.27 (2H, m)
6''	14.0 (q)	0.86 (3H, t, 7.0)

^a 125 MHz.

^b 500 MHz.

^c These assignments are interchangeable.

Fig. 1. Planar structure of epostatins.

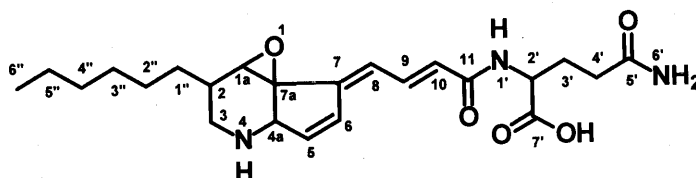
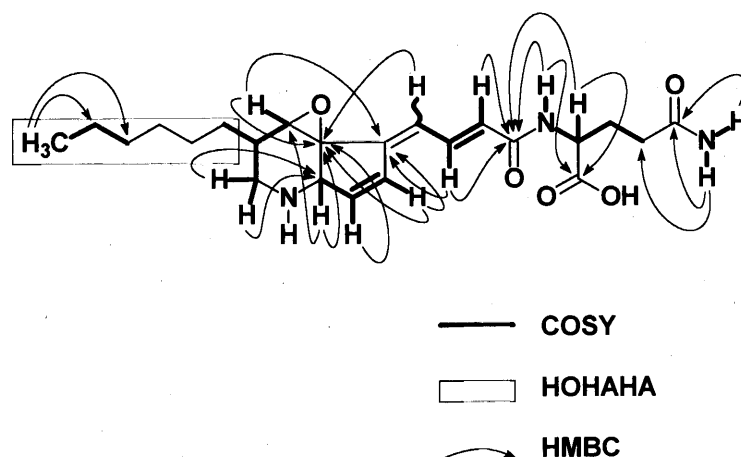


Fig. 2. ^1H - ^1H COSY, HOHAHA and HMBC correlations for epostatatin.

six partial structures (Fig. 2): C-1a~C-3, C-4a~C-6, C-8~C-10, N-1'~C-4', C-4''~C-6'' and one amide (N-6'). Analysis of the Homonuclear Hartmann-Hahn (HOHAHA) experiment revealed the presence of a C6 unit at position C-2 in the molecule, which was assigned to be a *n*-hexyl group (C''-6~C''-1).

The connectivities of these partial structures were elucidated by the Heteronuclear Multiple Bond Correlation (HMBC) experiment. The methine proton at δ 3.77 (H-4a) was coupled to two carbons at δ 62.4 (C-1a) and δ 65.5 (C-7a). C-1a and C-7a could be oxygenated aliphatic carbons according to their chemical shifts. These data indicated that an epoxide ring was formed by C-1a and C-7a. Methylene protons at δ 2.76 (H-3a) and δ 2.09 (H-3b) showed long-range coupling to a carbon at δ 62.0 (C-4a). Both carbons at δ 45.4 (C-3) and δ 62.0 (C-4a) seemed to link a nitrogen (N-4) on the basis of their ^{13}C chemical shifts. The above observation suggested that epostatatin contained a piperidine ring fusing with the epoxide ring. Long-range couplings from protons at δ 6.35 (H-5), δ 7.03 (H-6) and δ 5.75 (H-8) to the carbon C-7a, and from protons at δ 3.30 (H-1a), δ 7.03 (H-6) and δ 7.37 (H-9) to a carbon at δ 148.3 (C-7) revealed the presence of a cyclopentene ring which was fused with the piperidine ring. These spectral analyses showed that epostatatin was closely similar to the known skeleton, dihydroabikoviromycin^{2~4)} (dihydro-latumcidin).

An amide proton at δ 6.75 (H-6'a) showed long-range couplings to a carbonyl carbon at δ 173.4 (C-5') and a methylene carbon at δ 31.4 (C-4'). An amide proton at δ 8.27 (H-1') and a methine proton at δ 4.22 (H-2') had long-range couplings to a carbonyl carbon at δ 173.5

(C-7'). These data indicated the presence of a glutamine moiety.

Long-range couplings from the amide proton (H-1'), the methine proton (H-2') and two olefinic methine protons at δ 7.37 (H-9) and δ 6.07 (H-10) to a carbonyl carbon at δ 165.1 (C-11) revealed the linkage between the skeleton and the glutamine moiety. The structure of epostatatin in Fig.1 was supported by the detection of L-glutamic acid from acid hydrolysate of epostatatin (6N HCl, 110°C, 22 hours) by HPLC on CROWN PAK CR (+) (0.2% perchloric acid, pH 1.2).

The geometry of the double bond at C-9 was determined to be *9E* by the coupling constant, $J_{9,10} = 15.0$ Hz. Other stereochemistry of epostatatin remains to be determined. Thus, epostatatin was determined to be *N* α -[4-(2-hexyl-1a,2,3,4,4a,7-hexahydro-cyclopent[*b*]oxireno[*c*]-pyrid-7-ylidene)-2-butenoyl]-L-glutamine.

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