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The Absolute Stereochemistry of Micropeptin 90

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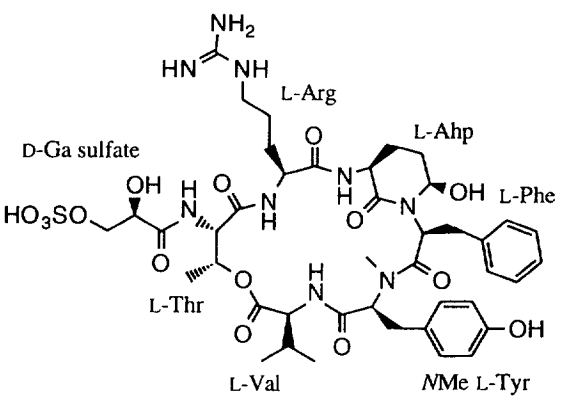
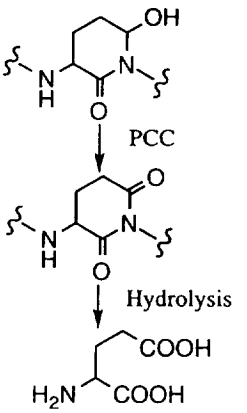
Abstract: The isolation and structure elucidation of micropeptin 90, a cyclic depsipeptide from the cultured cyanobacterium *Microcystis aeruginosa* (NIES-90) was previously described.[1] Its absolute stereochemistry was elucidated to be 1 on the basis of chemical degradation and NOESY spectrum.

The cyclic depsipeptides containing Ahp (3-amino-6-hydroxy-2-piperidone) unit have frequently been found in the constituents of the freshwater-blooming cyanobacteria.[2] The absolute chemistry of the Ahp unit has only been ambiguously described on the basis of inference from NMR characteristics and its derivation from L-Glu, in cyanopeptolins.[2c] Recently, A90720A containing Ahp unit, was also isolated from the terrestrial cyanobacterium *Microchate laktakensis* and its absolute stereochemistry was determined by the single crystal X-ray diffraction of the bovine trypsin-A90720A complex.[3] Here we first report the absolute stereochemistry of Ahp of micropeptin 90 (**1**) on the basis of chemical degradation and NOESY spectrum.



1



Scheme 1

The stereochemistry of usual amino acids[4] was determined as all L by the HPLC analysis of the L-Marfey's reagent derivatives of the acid hydrolyzate (Marfey's method).[5] The stereochemistry of *N*-MeTyr was also determined as L by the HPLC analysis of both L- and D-Marfey's reagent derivatives.[6]

The stereochemistry of the Ga sulfate (glyceric acid 3-*O*-sulfate)[7] was determined to be D by chiral GC analysis of *O*-heptafluorobutyryl isopropyl ester derivative. The stereochemistry of Ahp[8] was deduced as

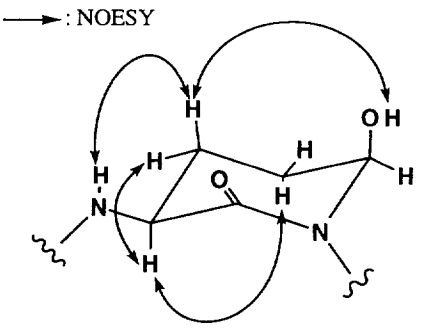


Fig 1. The relative stereochemistry of Ahp in micropeptin 90

follows. PCC oxidation of **1** (Scheme 1) followed by hydrolysis with HCl afforded glutamic acid which was proved to be L-form from the HPLC analysis of the L-Marfey's reagent derivatives (Marfey's method). The relative stereochemistry of Ahp of **1** was decided as in Fig. 1 by NOESY correlations (Ahp NH/H-4a, H-4a/OH, H-4b/H-3 and H-3/H-5). Therefore, the stereochemistry of Ahp was decided to be (3S, 6R)-3-amino-6-hydroxy-2-piperidone (L-Ahp). This absolute stereochemistry was coincident with those of cyanopeptolins and A90720A.

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6. To the acid hydrolyzate of a 100 μg portion of the peptide, 50 μL of 1-fluoro-2,4-dinitrophenyl-5-L-alanine amide in acetone (L-FDAA) (10 mg/mL) and 100 μL of 1 M $NaHCO_3$ were added, and the mixture was kept at 80 °C for 3 min. To the reaction mixture, 50 μL of 2 N HCl and 300 μL of 50% MeCN were added and the reaction mixture was analyzed by reversed-phase ODS-HPLC: column Cosmosil MS (Nacalai tesque) (4.6 x 250 mm); gradient elution from H_2O/TFA (100:0.1) to MeCN/H_2O/TFA (60:40:0.1) in 60 min; UV (340 nm). Retention times of the standard amino acids (min): D-Arg (32.2), L-Arg (33.6), L-Thr (34.8), D-Thr (38.0), L-Val (45.6), D-Val (49.6), L-Phe (50.0), D-Phe (52.8). Retention times of the amino acids of micropeptin 90 (min): Arg (33.4), Thr (34.8), Val (45.2), Phe (50.0).
   N-Me L-Tyr was derivatized with D- and L-FDAA as described above, respectively.[4] The derivatives were analyzed by reversed-phase ODS-HPLC: column Cosmosil MS (4.6 x 250 mm); mobile phase MeCN/H_2O/TFA (23:77:0.1); UV (340 nm). Retention times of standards (min): *N*-Me L-Tyr-L-FDAA (39.2), *N*-Me L-Tyr-D-FDAA (40.8). Retention time of *N*-MeTyr-L-FDAA in the acid hydrolyzate of micropeptin 90 (min): 39.2.
7. A hundred μg of micropeptin 90 was dissolved in 0.5 mL of 6 N HCl and heated at 110 °C for 16 h. The reaction mixture was evaporated in a stream of nitrogen, dissolved in 10% HCl in *i*-PrOH (200 μL), and heated at 100 °C for 30 min. The product was evaporated, dissolved in heptafluoro-*n*-butyric anhydride (100 μL) and CH_2Cl_2 (100 μL), reacted at 100 °C for 10 min, and evaporated. The residue was dissolved in CH_2Cl_2 and analyzed by GC with Chirasil-L-Val (Chrompak) column. The oven temperature was maintained at 40 °C for 3 min and raised to 70 °C at 4 °C/min, which was maintained for 25 min. Retention times for glyceric acids (min): D-glyceric acid (18.84), L-glyceric acid (19.07). Retention time of a derivative of the acid hydrolyzate of micropeptin 90 (min): 18.77.
8. Micropeptin 90 (2 mg) was dissolved in CH_2Cl_2 (2 mL), and then 2.2 mg of PCC (pyridinium chlorochromate) in CH_2Cl_2 (1 mL) was added to the solution with stirring at room temperature. After stirring for 4 h, diethylether (3 mL) and an excess amount of anhydrous $MgSO_4$ were added, and the reaction mixture was stirred at room temperature for 20 min. After filteration, the solution was evaporated and dissolved in 6 N HCl (0.2 mL), and heated at 110 °C for 6 h to yield Glu. Formation of Glu was confirmed by amino acid analysis.
   To this hydrolyzate, 50 μL of L-FDAA in acetone (10 mg/mL) and 10 μL of 1 M $NaHCO_3$ were added, and the mixture was kept at 80 °C for 3 min. To the reaction mixture, 50 μL of 2 N HCl and 200 μL of 50% MeCN were added and the reaction mixture was analyzed by ODS-HPLC: column Cosmosil MS (4.6 x 250 mm); gradient elution from H_2O/TFA (100:0.1) to MeCN/H_2O/TFA (60:40:0.1) in 60 min; UV (340 nm). Retention times of the standard amino acids (min): L-Glu (39.4), D-Glu (40.4). Retention time of a derivative derived from micropeptin 90 (min): 39.4.

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