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Structure of Kaitocephalin, A Novel Glutamate Receptor Antagonist Produced by *Eupenicillium shearii*

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Abstract: Kaitocephalin was isolated from *Eupenicillium shearii* as a glutamate receptor antagonist, which protected chick telencephalic neurons as well as rat hippocampal neurons from kainate toxicity. It consists of a pyrrolidine moiety with tricarboxylic acids and a dichlorohydroxybenzoate substructure as shown in Figure 1. © 1997 Elsevier Science Ltd.

During cerebral ischemia and subsequent reperfusion injury, neuronal degeneration is caused by the excitatory amino acid, L-glutamic acid, which acts as a neurotransmitter in the major part of brain.[1,2] The ionotropic L-glutamate receptors, which are considered to play an important role in neuronal cell death, are mainly classified into two types; NMDA (*N*-methyl-D-aspartic acid) and AMPA/KA (α-amino-3-hydroxy-5-methyl-4-isoxazole propionic acid/kainate) receptors. Since the AMPA/KA receptor antagonist 2,3-dihydroxy-6-nitro-7-sulfamoyl-benzo(F)quinoxaline (NBQX) was reported to protect neuronal cells from ischemia injury even administered after an ischemic attack[3], AMPA/KA receptor antagonists are expected to be effective to treat ischemia-reperfusion injury such as stroke.

In the course of our screening for substances that protect chick primary telencephalic neurons from kainate toxicity[4], we have isolated a potent kainate-toxicity suppressor designated kaitocephalin (**1**)[5] as a white powder from *Eupenicillium shearii* PF1191. Here we describe the isolation, structure elucidation and brief biological activities of **1**.

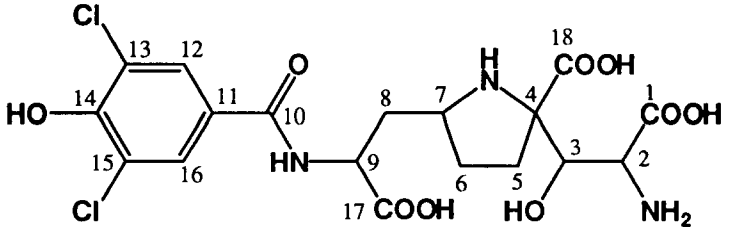

Figure 1. Structure of kaitocephalin (**1**)

[§] The first two authors contributed equally to this work.

The kaitocephalin producing organism, identified as *Eupenicillium shearii* PF1191 was cultivated in a solid medium consisting of wet rice and soybean meal for 14 days. The whole culture (10 kg) was extracted with 67% aqueous acetone (10 L). After concentration *in vacuo*, the residual aqueous layer was adsorbed on active charcoal and the active material was eluted with 50% acetone. The active residue was applied to a Toyopearl HW-40F column and eluted with 60% MeOH. The active eluate was chromatographed on an ODS column (ODS-SS-1020T, Senshu Scientific Co.) with 20% MeOH. Finally, **1** (9.2 mg) was obtained by HPLC using a PEGASIL ODS column (Senshu-Pak, 20 ϕ x 250 mm) developed with 5% MeOH containing 20 mM diethylamine-CO_2 (pH 7.0).

The molecular formula of **1** was established as $C_{18}H_{21}N_3O_9Cl_2$ by HRFAB-MS [$(M+H)^+$, *m/z* 494.0757 (+2.4 mmu error)]. IR absorptions at 3410 cm^{-1} and 1640 cm^{-1} implied the presence of exchangeable protons and an amide function, respectively. One dimensional ^1H and ^{13}C NMR spectral data[6] and the phase-sensitive DQF spectral data revealed the two proton spin systems, from 5-H (2.28, 2.01 ppm) to 9-H (4.35 ppm) through 6-H (2.12, 1.61 ppm), 7-H (3.70 ppm) and 8-H (2.41, 2.06 ppm), and between 2-H (4.16 ppm) and 3-H (4.41 ppm). The ^1H NMR spectrum taken in the presence of H_2O enabled to observe an exchangeable amide proton at 8.20 ppm (9-NH, doublet), which had been suggested by the IR spectrum and was coupled to 9-H. Furthermore, ^1H-^{13}C long-range couplings between C-4 (77.1 ppm) and 2-H, 3-H, 5-H and 6-H in the HMBC spectra of **1** proved the relationship between these two sequences through a quaternary carbon C-4 as shown in Figure 2.

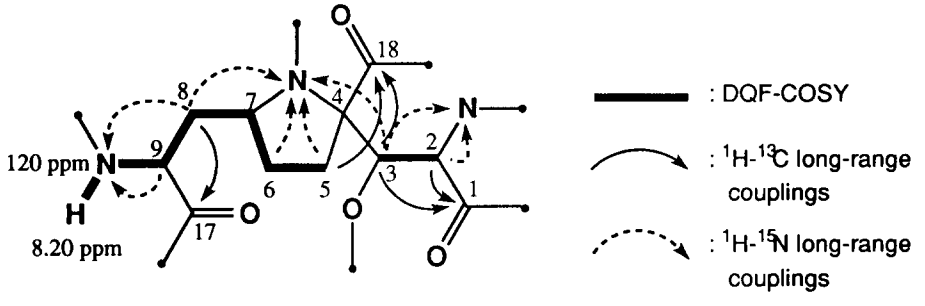

Figure 2. Substructure including the pyrrolidine moiety of **1** and relevant long-range couplings.

Detailed analysis of long-range couplings observed in the ^1H-^{13}C HMBC and ^1H-^{15}N HMBC (^{15}N-HMBC) revealed the substructure of **1** including the pyrrolidine moiety. Thus, 8-H and 9-H were long-range coupled to a carbonyl carbon C-17 (177.8 ppm) and 9-N (120 ppm), the latter ^{15}N chemical shift being characteristic for amide nitrogens[7]. Furthermore, 8-H was long-range coupled to an amine nitrogen 4-N (64 ppm) which in turn long-range coupled to 3-H, 5-H and 6-H. These results suggested that C-4, C-5 (32.7 ppm), C-6 (30.4 ppm), C-7 (59.7 ppm) and 4-N constructed a pyrrolidine skeleton as shown in Figure 2. Likewise, long-range couplings from 3-H and 5-H to a carbonyl carbon C-18 (175.0 ppm) linked C-18 to the C-4 position, whose ^{13}C chemical shift was compared to that of the analogous carbon in lactacystin[8] (81.5 ppm). In addition, 2-H and 3-H were long-range coupled to a carbonyl carbon C-1 (171.3 ppm) and an amino nitrogen 2-N (40 ppm). The ^{13}C chemical shift of C-3 (71.4 ppm) suggested it to be oxygenated. These data are ascribed to the substructure including the pyrrolidine as shown in Figure 2.

Another substructure was determined as follows. In the HMBC spectrum of **1**, two equivalent aromatic protons 12,16-H (7.62 ppm) were long-range coupled to a carbonyl carbon C-10 (168.2 ppm), aromatic carbons C-12,16 (128.6 ppm) and C-13,15 (123.0 ppm), and C-14 (153.8 ppm) which was ascribed to an oxygenated sp^2 carbon based on its ^{13}C chemical shift. The presence of two chlorine atoms in this benzene ring was elucidated by detecting a fragment ion peak at *m/z* 189 derived from the molecular ion in the MS-MS spectrum. The substitution pattern of this benzoate moiety (Figure 3) was corroborated by similar ^{13}C chemical shifts of the corresponding unit in complestatin[9]. Finally, a long-range coupling from 9-H to the amide carbonyl carbon C-10 revealed the structure of **1** as shown in Figure 3. The molecular formula of **1** proved that the presence of three carboxylic acids at C-1, C-17 and C-18, an amine 2-NH_2, a hydroxyl group 3-OH and a phenolic hydroxyl function 14-OH as shown in Figure 1.

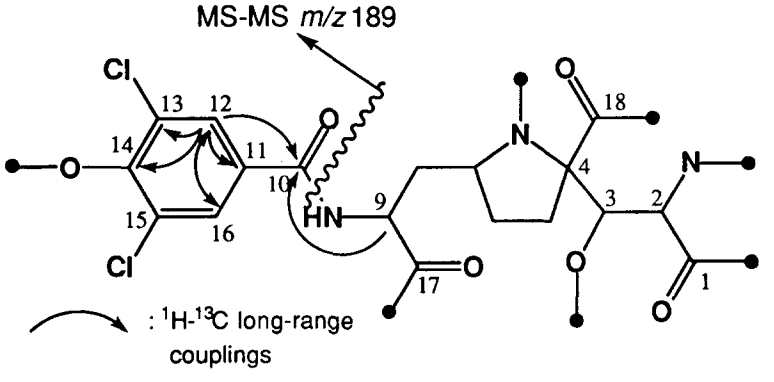

Figure 3. Dichlorohydroxybenzoate moiety and its connectivity to the substructure containing the pyrrolidine ring.

The stereochemical studies of five chiral carbons C-2, C-3, C-4, C-7 and C-9 in **1** are now under way.

Kaitocephalin protected chick primary telencephalic and rat hippocampal neurons from kainate toxicity at 500 μM with EC_{50} values 0.68 μM and 2.4 μM, respectively, without showing any cytotoxic effect. Although a well known AMPA/KA antagonist CNQX with a quinoxalinedione skeleton effectively protected chick primary telencephalic neurons from kainate toxicity with EC_{50} value 0.53 μM, it exhibited cytotoxicity against chick primary telencephalic and rat hippocampal neurons at the concentrations of 20 μM and 2 μM, respectively. Kaitocephalin also protected chick primary telencephalic and rat hippocampal neurons from AMPA/cyclothiazide (500 μM/50 μM) toxicity with EC_{50} values 0.6 and 0.4 μM, respectively.

Kaitocephalin is the first AMPA/KA antagonist from nature and consisting of a quite different skeleton from other known AMPA/KA antagonists. Further studies on detailed biological activities of **1** will be reported elsewhere.

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5. Mp. 235-238℃ (dec.): $[\alpha]_D^{21}$ = - 31° (*c* 0.7, H_2O): UV absorption λ_{max} nm (ε); 217 (18,200), 297 (8,200) in H_2O; 214 (23,200), 255 (6,700) in 0.01 N HCl-H_2O: IR ν_{max} (KBr) cm^{-1}; 3410, 1640, 1560, 1485, 1385
6. The NMR data for **1** are as follows: ^{1}H-NMR (δ_H, D_2O at 500 MHz): 7.62 (s, 12,16-H, 3H), 4.41 (brs, 3-H), 4.35 (dd, *J* = 8.0, 6.0 Hz, 9-H), 4.16 (brs, 2-H), 3.70 (m, 7-H), 2.41 (ddd, *J* = 14.5, 7.0, 6.0 Hz, 8-H), 2.28 (ddd, *J* = 14.0, 6.0, 2.0 Hz, 5-H), 2.12 (m, 6-H), 2.06 (m, 8-H), 2.01 (m, 5-H), 1.61 (m, 6-H), An exchangeable proton was observed at 8.20 ppm (d) in H_2O/D_2O = 9 : 1. ^{13}C-NMR (δ_C, D_2O at 125 MHz): 177.8 (C-17), 175.0 (C-18), 171.3 (C-1), 168.2 (C-10), 153.8 (C-14), 128.6 (C-12,16), 125.5 (C-11), 123.0 (C-13,15), 77.1 (C-4), 71.4 (C-3), 59.7 (C-7), 56.2 (C-2), 54.2 (C-9), 35.6 (C-8), 32.7 (C-5), 30.4 (C-6).
7. These ^{15}N chemical shifts were determined indirectly from the ^{15}N-HMBC spectrum by using formamide (112 ppm) as an external standard.
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