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Structure of Mescengricin, A Novel Neuronal Cell Protecting Substance Produced by *Streptomyces griseoflavus*

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Abstract: Mescengricin was isolated from *Streptomyces griseoflavus* 2853-SVS4 as a neuronal cell protecting substance. It possesses an α-carboline structure substituted by glycerol-ester and dihydropyrone residues as shown in Fig. 1. © 1997 Elsevier Science Ltd.

During cerebral ischemia and subsequent reperfusion injury, neuronal degeneration is mediated by excitotoxicity of the excitatory amino acid, L-glutamate, which acts as a neurotransmitter in the major part of the brain.[1,2] Thus, brain ischemia injury is expected to be overcome by compounds which suppress the excitotoxicity induced by L-glutamate.

In the course of our screening for substances that protect chick primary mescencephalic neuronal cells[3] from the L-glutamate toxicity, we have isolated mescengricin (**1**)[4] as a reddish brown powder from *Streptomyces griseoflavus*. Here we describe the fermentation, isolation and structure elucidation of **1**.

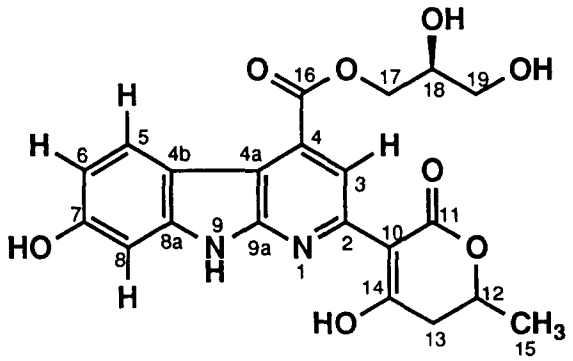

Figure 1. Structure of mescengricin (**1**)

The mescengricin producing organism, identified as *Streptomyces griseoflavus* 2853-SVS4 was cultured in a 50-liter jar fermenter containing 30 liters of the medium consisting of glycerol 2.0%, molasses 0.5%, casein 0.5%, polypeptone 0.1% and $CaCO_3$ 0.4% at 27°C for 3 days. Acetone extraction of the mycelium of the producing organism followed by EtOAc extraction gave a crude active material. After washing with *n*-hexane, this material was applied to a silica gel column packed and developed with $CHCl_3$-MeOH (7 : 1). The active eluate thus obtained was then applied to a Toyopearl HW-40F column and eluted with 100% MeOH. A pure

sample of **1** (3.3 mg) was finally obtained by HPLC using a PEGASIL ODS column (Senshu-Pak, 20 ø x 250 mm) developed with 60% MeOH.

The molecular formula of **1** was established as $C_{21}H_{20}N_2O_8$ by HRFAB-MS [(M+H)+, *m/z* 429.1324 (+2.6 mmu error)]. IR absorptions at 1730 cm^{-1} and 1300 cm^{-1} implied the presence of an ester function. One dimensional ^1H- and ^{13}C-NMR spectral data[5] together with the correlations revealed by phase-sensitive DQF-COSY proved the presence of a 1,2,4-trisubstituted benzene substructure and two spin systems from methyl protons 15-H (1.36 ppm) to methylene protons 13-H (2.58, 2.74 ppm) through a methine proton 12-H (4.56 ppm), and a glycerol moiety as shown in Fig. 2 and 3.

Since an aromatic proton 3-H (8.57 ppm) playing a key role for the structure elucidation was observed as a very broad signal, a few ^1H-^{13}C correlations were observed in the HMBC spectrum of **1**. This problem was overcome by preparation of a free acid derivative (**2**)[6] lacking the glycerol-ester side chain. The aromatic proton 3-H (8.67 ppm) of **2** was observed as a sharp signal together with three exchangeable proton signals (9.90, 12.40 and 18.10 ppm). Therefore, the structure determination was carried out by analyzing the NMR spectral data of **2**.

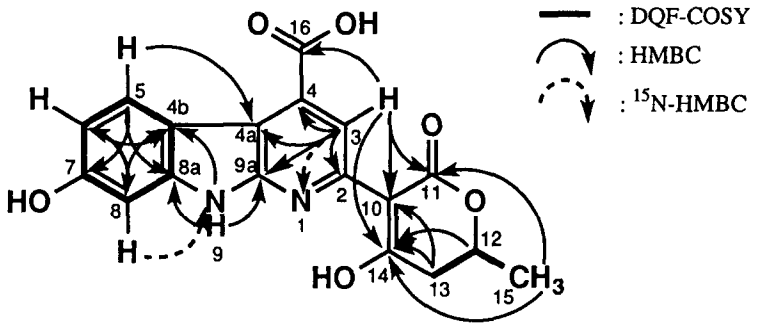

Figure 2. NMR analyses of **2** (in DMSO-d_6)

In the HMBC spectrum of **2**, long-range couplings were detected from the aromatic proton 3-H to quaternary carbons C-2 (112.4 ppm), C-4 (134.0 ppm) and C-4a (112.2 ppm). A long-range coupling from 3-H to a carbonyl carbon C-16 (167.6 ppm) revealed the lingkage of C-16 to C-4. Additional long-range couplings were observed from methylene protons 13-H (2.63, 2.79 ppm) to quaternary carbons C-10 (95.4 ppm) and to an oxygenated sp^2 carbon C-14 (181.8 ppm) which was long-range coupled to the methine proton 12-H (4.58 ppm), and from methyl protons 15-H (1.37 ppm) to an ester carbonyl carbon C-11 (165.7 ppm) and C-14. The ^{13}C chemical shifts of C-10, C-11 and C-14, and those correlations cited above established the γ-lactone moiety as shown in Fig. 2. Furthermore, long-range couplings from the aromatic proton 3-H to the quaternary carbons C-10, C-11 and C-14 in decoupled-HMBC (D-HMBC) experiment[7] revealed the position of the substitution of the γ-lactone moiety on the α-carboline structure.

In addition to these ^{1}H-^{13}C correlations, an ^{15}N-HMBC experiment proved a long-range coupling between 3-H and a pyridinium nitrogen N-1 (207 ppm). These results established a substituted pyridine moiety as shown in Fig. 2.

The connectivity between the trisubstituted benzene ring and the substituted pyridine moiety was determined as follows. In the D-HMBC spectrum of **2**, an exchangeable proton 9-NH (12.40 ppm) was long-range coupled to quaternary carbons C-4b (112.0 ppm), C-8a (141.0 ppm) and C-9a (146.3 ppm), the last one being long-range coupled through four-bonds to the aromatic proton 3-H. Furthermore, a long-range coupling between an aromatic proton 5-H (8.45 ppm) and C-4a was observed. In addition to these correlations, an aromatic proton 8-H (6.89 ppm) was long-range coupled to an amine nitrogen N-9 (122 ppm) in the ^{15}N-HMBC spectrum. The remaining substituent on C-7 was determined to be oxygen according to the ^{13}C chemical shift of C-7 (157.9 ppm). Thus, the structure consisting of an α-carboline chromophore was determined as shown in Fig. 2.

The glycerol moiety was located at the C-16 position by analyzing long-range couplings in the HMBC spectrum of **1** as shown in Fig. 3. Furthermore, NOE experiment on the methylene protons 17-H, which showed NOEs between 3-H and 5-H, also supported the structure of **1**.

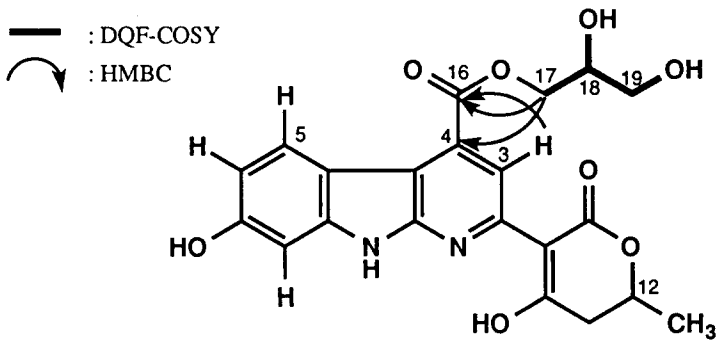

Figure 3. HMBC experiment of **1**

The stereochemistry at position C-18 of **1** was determined as follows. An acetonide derivative of **1** was prepared by treatment of **1** with 2,2-dimethoxypropane and pyridinium *p*-toluene-sulfonate in CH_2Cl_2. The reaction mixture was hydrolysed with 1N NaOH at room temperature for one hour to give a glycerol dimethylketal. The stereochemistry at C-18 was determined to be *S* by comparing the optical rotation value of the obtained glycerol dimethylketal $[\alpha]_D^{21}$=+9.8° (*c*=0.06, benzene) with that of D-(+)-glycerol dimethylketal (+10.8°, *c*=15.19).[8] The stereochemistry of C-12 remains to be established.

Mescengricin decreased the L-glutamate toxicity in chick primary mescencephalic neurons with an EC_{50} value 6.0 nM. When the antioxidative activity of **1** was examined following the experiment of L-glutamate toxicity in N18-RE-105 cells[9] where antioxidants such as vitamin E are known to suppress L-glutamate

toxicity[10-13], no activity was observed. Since the mechanism leading to L-glutamate toxicity in neuronal cells is not fully understood, investigating the mode of action of **1** may lead its clarification. Further detailed studies on biological activities of **1** are now under way.

In conclusion, mescengricin is the first natural product with an α-carboline chromophore. Accordingly unraveling its biosynthetic pathway should also be interesting.

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4. Mp. 247-249°C (dec.); $[\alpha]_D^{20} = -33°$ (*c* 0.04, MeOH); UV absorption in methanol at λ_{max} nm (ε); 210 (10,500), 256 (5,200), 284 (6,400) and 400 (4,500) in MeOH; 238 (10,000) and 410 (4,800) in 0.01 N NaOH-MeOH.
5. The NMR data for **1** are as follows: ^{1}H-NMR (δ_H, DMSO-d_6 at 500 MHz) : 8.57 (br.s, 3-H), 8.42 (dd, *J* = 9, 2 Hz, 5-H), 6.74 (dd, *J* = 9, 2 Hz, 6-H), 6.89 (d, *J* = 2 Hz, 8-H), 4.56 (m, 12-H), 2.58 (dd, *J* = 18, 2 Hz, 13-H), 2.74 (dd, *J* = 18, 12 Hz, 13-H), 1.36 (3H, d, *J* = 6 Hz, 15-H), 4.37 (ddd, *J* = 11, 11, 4 Hz, 17-H), 4.51 (ddd, *J* = 11, 9, 6 Hz, 17-H), 3.87 (m, 18-H), 5.08 (br.s, 18-OH), 3.48 (2H, m, 19-H), 4.76 (br.s, 19-OH). ^{13}C-NMR (δ_C, DMSO-d_6 at 125 MHz): 149.1 (C-2), 112.8 (C-3), 131.6 (C-4), 111.7 (C-4a), 111.9 (C-4b), 126.0 (C-5), 110.1 (C-6), 157.9 (C-7), 96.8 (C-8), 141.4 (C-8a), 147.4 (C-9a), 95.8 (C-10), 165.9 (C-11), 71.0 (C-12), 38.4 (C-13), 181.1 (C-14), 20.6 (C-15), 167.1 (C-16), 68.0 (C-17), 70.6 (C-18), 63.9 (C-19).
6. $C_{18}H_{14}N_2O_6$, HRFAB-MS [(M+H)$^+$, *m/z* 353.0800 (+2.6 mmu)]. The NMR data for **2** are as follows: ^{1}H-NMR (δ_H, DMSO-d_6 at 500 MHz) : 8.67 (s, 3-H), 8.45 (d, *J* = 9 Hz, 5-H), 6.75 (dd, *J* = 9, 2 Hz, 6-H), 9.90 (br. s, 7-OH), 6.89 (d, *J* = 2 Hz, 8-H), 12.40 (br. s, 9-NH), 4.58 (m, 12-H), 2.63 (dd, *J* = 17, 3 Hz, 13-H), 2.79 (dd, *J* = 17, 12 Hz, 13-H), 18.10 (br. s, 14-OH), 1.37 (3H, d, *J* = 6 Hz, 15-H). ^{13}C-NMR (δ_C, DMSO-d_6 at 125 MHz): 148.4 (C-2), 112.4 (C-3), 134.0 (C-4), 112.2 (C-4a), 112.0 (C-4b), 126.2 (C-5), 110.3 (C-6), 157.9 (C-7), 96.8 (C-8), 141.0 (C-8a), 146.3 (C-9a), 95.4 (C-10), 165.7 (C-11), 70.0 (C-12), 38.1 (C-13), 181.8(C-14), 20.2 (C-15), 167.6 (C-16).
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