A Novel Neuronal Cell Protecting Substance, Naphthomycinol, Produced by *Streptomyces* sp. PF7

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The neuronal degeneration which results from cerebral ischemia is thought to be due to an overexcretion of the excitatory amino acid, L-glutamic acid, which acts as a neurotransmitter in the major part of brain\(^1\). Brain ischemia injury may be expected to be overcome by L-glutamate toxicity suppressors. In the course of our screening for substances that protect neuronal hybridoma N18-RE-1052) cells from L-glutamate toxicity, we isolated carquinostatin A\(^3\), lavanduquinocin\(^4\), aestivophoenins A and B\(^5\), and 4-demethoxymichigazone\(^6\). Further investigation has resulted in the isolation of a novel neuronal cell protecting substance, naphthomycinol (1, Fig. 1). We report herein the fermentation, isolation and structure determination of 1.

The naphthomycinol producing organism, identified as *Streptomyces* sp. PF7, was cultivated in a 50-liter jar fermenter containing 30 liters of the medium consisting of dextrin 3.0%, glucose 0.3%, soybean meal 2.0%, CaCO\(_3\) 0.3% and CoCl\(_2\)-6H\(_2\)O 0.001% at 27°C for 3 days. The mycelial acetone extract was concentrated to a small volume. The aqueous residue was adjusted to pH 3 and extracted with EtOAc. The solvent layer was dried over Na\(_2\)SO\(_4\) and concentrated to give an oily residue. This material was washed with n-hexane and the remaining residue was applied to a silica gel column packed with CHCl\(_3\)-MeOH (7:1). The active eluate was then subjected to a Toyopearl HW-40F column and eluted with 100% MeOH. 1 was finally purified by HPLC using a PEGASIL ODS column (Senshu-Pak, 20 i.d. × 250 mm) developed with 30% CH\(_3\)CN containing 20 mM Na-phosphate buffer (pH 7.0).

The physico-chemical properties of 1 are summarized in Table 1. The molecular formula of 1 was established as C\(_{40}\)H\(_{49}\)NO\(_9\) by high-resolution FAB-MS. An IR absorption at 1660 cm\(^{-1}\) implied the presence of a quinone carbonyl function. The \(^1\)H and \(^{13}\)C NMR spectral data are shown in Table 2. The structure of 1 was elucidated as follows.

The \(^1\)H and \(^{13}\)C NMR spectral data indicate that the structure of 1 is very similar to those of naphthomycins\(^7\)–\(^{11}\) except for the presence of an additional oxygen atom. The phase-sensitive DQF-COSY spectrum of 1 showed the presence of a quinone carbonyl function. The IR spectrum showed a strong absorption at 1660 cm\(^{-1}\) indicating the presence of a quinone carbonyl function. The \(^1\)H and \(^{13}\)C NMR spectral data showed that the structure of 1 is very similar to those of naphthomycins\(^7\)–\(^{11}\) except for the presence of an additional oxygen atom.
I revealed the two proton spin systems from C-2 to C-11 and from C-12 to C-22 as shown in Fig. 2. The methyl protons 32-H (2.13 ppm), 34-H (1.57 ppm) and 37-H (2.00 ppm) were allylic coupled to the methine protons 3-H (6.80 ppm), 13-H (5.29 ppm) and 21-H (5.84 ppm), respectively. Furthermore, long range couplings from the singlet methyl proton 34-H to methine carbon C-11 (77.9 ppm, $\delta_{H}=4.05$ ppm) and C-13 (123.9 ppm) showed the linkage between the two proton spin systems (Fig. 2). Furthermore, the carbonyl carbon C-23 was long-range coupled to the methyl proton 37-H and an olefinic methine proton 21-H (5.84 ppm). In the naphthoquinone moiety, a methyl proton 38-H (2.36 ppm) was long-range coupled to aromatic carbons C-25 (161.3 ppm), C-26 (132.1 ppm) and C-27 (131.5 ppm). In addition, an aromatic proton 27-H showed $^1$H-$^1$C long-range couplings to aromatic carbons C-25 and C-31a (135.5 ppm), and quinone carbonyl carbons C-28 (179.8 ppm) and C-31 (186.2 ppm) in the HMBC spectrum as shown in Fig. 2. Another aromatic proton 30-H (7.52 ppm) was also long-range coupled to C-28 and C-31a. An amide proton (8.53 ppm) showed long-range couplings to C-28, C-30 (119.1 ppm) and an amide carbon C-1 (168.5 ppm), which was in turn long-range coupled to the singlet methyl proton 32-H. Furthermore, a D-HMBC experiment on the aromatic methyl proton 38-H revealed the connectivity between the ansa bridge and the nephthoquinone substructure through a carbonyl carbon C-23 (202.3 ppm) as shown in Fig. 2.

The proton coupling constants of the triene system ($J_{\alpha,5}=11.0$, $J_{\alpha,7}=15.0$ Hz) in naphthomycinol, proved that C(4)=C(5) and C(6)=C(7) have Z- and E-configuration, respectively. According to the $^1$C chemical shift of the allylic methyl carbon C-32 (20.7 ppm), the stereochemistry of C(2)=C(3) was deduced to be Z. The stereochemistry of C(12)=C(13) and C(21)=C(22) were determined both to be E on the basis of high-field chemical shifts for C-34 (11.3 ppm) and C-37 (12.7 ppm). The remaining olefinic bond C(16)=C(17) was concluded to have an E-configuration by the coupling constant ($J_{\alpha,17}=15.0$ Hz) as shown in the structure (Fig. 1). Naphthomycinol, a member of the naphthomycins series, is the first compound which has a hydroxyl function at C-11 so far reported.

In the evaluation system we employed, I decreased the L-glutamate toxicity in N18-RE-105 cells with EC$_{50}$ value 400 nM. Since the L-glutamate toxicity in N18-RE-105 cells was thought to be caused by glutathione depletion, we assessed buthionine sulfoximine (BSO) toxicity which directly inhibits glutathione synthesis. Antioxidants such as vitamin E suppress both the L-glutamate and the BSO toxicities in N18-RE-105 cells. Naphthomycinol, however, did not suppress the BSO toxicity. This result strongly suggests that the mode of action of naphthomycinol is not based on the antioxidantive activity. Detailed investigations on other biological activities are now under way.

Acknowledgments

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