NOTES

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

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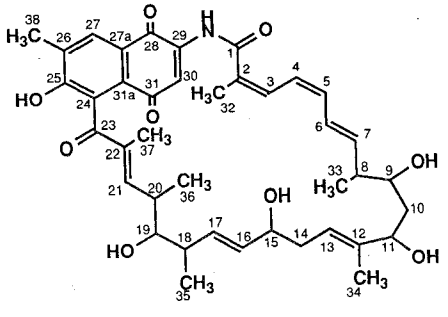
The neuronal degeneration which results from cerebral ischemia is thought to be due to an overexcretion of the excitatory amino acid, L-glutamic acid, which acts as a neurotransmitter in the major part of brain[1]. Brain ischemia injury may be expected to be overcome by L-glutamate toxicity suppressors. In the course of our screening for substances that protect neuronal hybridoma N18-RE-105[2] 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 $CoCl_2 \cdot 6H_2O$ 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_2SO_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_3CN containing 20 mM Na-phosphate buffer (pH 7.0).

Fig. 1. Structure of naphthomycinol (**1**).



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 ^1H and ^{13}C NMR spectral data are shown in Table 2. The structure of **1** was elucidated as follows.

The ^1H 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 oxymethine. The phase-sensitive DQF-COSY spectrum of

Table 1. Physico-chemical properties of naphthomycinol.

Appearance	Reddish brown powder
MP	167~172°C
$[\alpha]_D^{19}$	−31.12° (*c* 0.50, MeOH)
Molecular formula	$C_{40}H_{49}NO_9$
HRFAB-MS (*m/z*)	
Found	710.3276 $(M+Na)^+$
Calcd	710.3305
UV λ_{max}^{MeOH} nm (ε)	233 (31,700), 273 (32,700)
	282 (33,000), 307 (32,700)
$\lambda_{max}^{MeOH+NaOH}$ nm (ε)	238 (33,500), 270 (sh, 29,100)
	283 (sh, 32,300), 304 (36,400)
	551 (3,000)
IR ν_{max} (KBr) cm^{-1}	3450, 2940, 1660, 1500, 1340

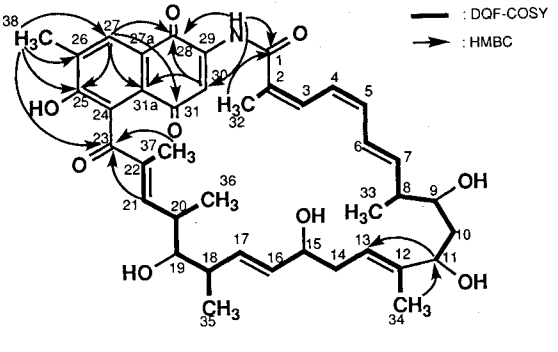
Table 2. ^{13}C and ^1H NMR chemical shifts of naphthomycinol in $CDCl_3$.

No.	δ_C	δ_H	No.	δ_C	δ_H
1	168.5		22	138.2	
2	127.8		23	202.3	
3	135.9	6.80	24	122.0	
4	124.5	6.47	25	161.3	
5	134.2	6.17	26	132.1	
6	126.6	6.50	27	131.5	7.94
7	140.3	5.56	27a	119.4	
8	47.1	2.19	28	179.8	
9	75.2	3.53	29	138.0	
10	42.7	1.65 1.80	30	119.1	7.52
11	77.9	4.05	31	186.2	
12	138.1		31a	135.5	
13	123.9	5.29	32	20.7	2.13
14	34.9	2.04	33	17.3	1.16
15	72.5	3.98	34	11.3	1.57
16	137.8	5.52	35	15.8	0.94
17	133.0	5.37	36	10.7	0.80
18	41.3	2.07	37	12.7	2.00
19	76.2	3.05	38	16.4	2.36
20	33.6	2.68	25-OH		9.60
21	146.0	5.84	NH		8.53

1 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-^{13}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[12] 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 ($J_{4,5} = 11.0$, $J_{6,7} = 15.0$ Hz) in naphthomycinol, proved that C(4)=C(5) and C(6)=C(7) have *Z*- and *E*-configuration, respectively. According to the ^{13}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 stereochemistries 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 ($J_{16,17} = 15.0$ 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.

Fig. 2. ^{1}H-^{1}H and ^{1}H-^{13}C connectivities of **1** as revealed by DQF-COSY and HMBC experiments.



In the evaluation system we employed[13,14], **1** 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[15], we assessed buthionine sulfoximine (BSO) toxicity which directly inhibits gliutathione 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 antioxidative activity. Detailed investigations on other biological activities are now under way.

Acknowledgments

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References

1) Choi, D. W.: Glutamate neurotoxicity and diseases of the nervous system. Neuron 1: 623~624, 1988
2) Malouf, A. T.; R. L. Schnaar & J. T. Coyle: Characterization of glutamic acid neurotransmitter binding site on neuroblastoma hybrid cells. J. Biol. Chem. 20: 12756~12762, 1984
3) Shin-ya, K.; M. Tanaka, K. Furihata, Y. Hayakawa & H. Seto: Structure of carquinostatin A, a novel neuronal cell protecting substance produced by *Streptomyces exfoliatus*. Tetrahedron Lett. 34: 4943~4944, 1993
4) Shin-ya, K.; S. Shimizu, T. Kunigami, K. Furihata, K. Furihata, Y. Hayakawa & H. Seto: A new neuronal cell protecting substance, lavanduquinocin, produced by *Streptomyces viridochromogenes*. J. Antibiotics 48: 574~578, 1995
5) Shin-ya, K.; S. Shimizu, T. Kunigami, K. Furihata, Y. Hayakawa & H. Seto: Novel neuronal cell protecting substances, aestivophoenins A and B, produced by *Streptomyces purpeofuscus*. J. Antibiotics 48: 1378~1381, 1995
6) Kunigami, T.; K. Shin-ya, K. Furihata, K. Furihata, Y. Hayakawa & H. Seto: A novel neuronal cell protecting substance, 4-demethoxymichigazone, produced by *Streptomyces halstedii*. J. Antibiotics 49: 312~313, 1996
7) Williams, T. H.: Naphthomycin, a novel ansa macrocyclic antimetabolite: Proton NMR spectra and structure elucidation using lanthanide shift reagent. J. Antibiotics 28: 85~86, 1975
8) Keller-Schierlein, W.; M. Meyer, L. Cellai, S. Cerrini, D. Lamba, A. Serge, W. Fedeli & M. Brufani: Metabolites of microorganisms 229, absolute configuration of naphthomycin A determined by X-ray analysis and chemical degradation. J. Antibiotics 37: 1357~1361, 1984
9) Keller-Schierlein, W.; M. Meyer, A. Zeeck, M. Damberg, R. Machinek, H. Zahner & G. Lazar: Isolation and structural elucidation of naphthomycins B and C. J. Antibiotics 36: 484~492, 1983
10) Meyer, M.; W. Keller-Schierlein, S. Megahed, H.

ZAHNER & A. SEGRE: Metabolites of microorganisms. sulfur-containing ansa compounds of the naphthomycin type. Helv. Chim. Acta 69: 1356~1364, 1986

11) MUKHOPADHYAY, T.; C. M. M. FRANCO, G. C. S. REDDY, B. N. GANGULI & H. W. FEHLHABER: A new ansamycin antibiotic, naphthomycin H from a *Streptomyces* species Y-83,40369. J. Antibiotics 38: 948~951, 1985

12) FURIHATA, K. & H. SETO: Decoupled HMBC (D-HMBC), an improved technique of HMBC. Tetrahedron Lett. 36: 2817~2820, 1995

13) MIYAMOTO, M.; T. H. MURPHY, R. L. SCHNAAR & J. T. COYLE: Antioxidants protect against glutamate-induced cytotoxicity in a neuronal cell line. J. Pharmacol. Exp. Ther. 250: 1132~1140, 1989

14) MURPHY, T. H.; M. MIYAMOTO, A. SASTRE, R. L. SCHNAAR & J. T. COYLE: Glutamate toxicity in a neuronal cell line involves inhibition of cystine transport leading to oxidative stress. Neuron 2: 1547~1558, 1989

15) KANE, D. J.; T. A. SARAFIAN, R. ANTON, H. HAHN, E. B. GRALLA, J. S. VALENTINE, T. ORD & D. E. BREDESEN: Bcl-2 inhibition of neural death: Decreased generation of reactive oxygen species. Science 262: 1274~1277, 1993