COMMUNICATIONS TO THE EDITOR

A Novel Neuronal Cell Protecting Substance, Espicufolin, Produced by *Streptomyces* sp. cu39

Sir:

It is well accepted that the excitatory amino acid, L-glutamic acid, which acts as a neurotransmitter in the major part of brain, induces neuronal cell death following brain ischemic attack[1]. L-Glutamic acid is also considered to generate oxygen radicals through a variety of intracellular cascades in such events[2]. In the course of our screening for suppressors of glutamate-toxicity to ameliorate and/or overcome brain ischemic injury using neuronal hybridoma N18-RE-105 cells as an *in vitro* model, we isolated espicufolin (**1**, Fig. 1). In this paper, we report the fermentation, isolation and structure determination of **1**.

The espicufolin producing organism, identified as *Streptomyces* sp. cu39, was cultivated in the medium consisting of glucose 2.5%, soybean meal 1.5%, dried yeast 0.2% and $CaCO_3$ 0.4% at 27°C for 5 days. The mycelial acetone extract was concentrated to a small volume and the aqueous residue was extracted with EtOAc. The solvent layer was dried over Na_2SO_4 and concentrated to give an oily residue. This material was applied to a silica gel column packed with $CHCl_3$ - MeOH (50 : 1) and eluted with the same solvent system. The combined active eluate was then subjected to preparative silica gel TLC developed with $CHCl_3$ - MeOH (50 : 1). Further purification by a Sephadex LH-20 column ($CHCl_3$ - MeOH, 1 : 1) gave a crude sample of **1**. Finally, **1** was purified by HPLC using a PEGASIL ODS column (Senshu-Pak, i.d. 20 × 250 mm, 85% MeOH) as a yellow powder.

The physico-chemical properties of **1** are summarized in Table 1. The molecular formula of **1** was determined as $C_{22}H_{18}O_6$ by high resolution FAB-MS. The UV and visible spectra of **1** revealed the presence of a chromophore identical with those of pluramycin A[3] and neopluramycin[4]. The ^1H and ^{13}C NMR spectral data are shown in Table 2. The structure of **1** was elucidated as follows.

The correlation between 8-H (7.67 ppm), 9-H (7.76 ppm) and 10-H (7.37 ppm) confirmed by DQF-COSY showed the presence of a 1,2,3-trisubstituted benzene ring. An aromatic proton 8-H showed long range couplings to quinone carbonyl carbons C-7 (181.5 ppm) and C-12 (187.0 ppm). The phenolic hydroxyl proton 11-OH (12.68 ppm), hydrogen-bonded to a carbonyl at a *peri* position, was long-range coupled to aromatic carbons C-10 (124.7 ppm), C-11 (161.3 ppm), and C-11a (116.7 ppm). In addition to this naphtoquinone substructure, an aromatic proton 6-H (8.47 ppm) was long-range coupled to aromatic carbons C-4a, C-12a, C-12b, and the quinone carbonyl carbons C-7 and C-12, which were in turn long-range coupled to the aromatic proton 8-H. Furthermore, a hydroxy methyl proton 13-H (5.13 ppm) was long-range coupled to C-4a (124.0 ppm), C-5 (153.3 ppm), C-6 (118.7 ppm), and C-6a (136.0 ppm). These results showed the presence of an anthraquinone moiety in **1**. Other ^1H-^{13}C long range couplings revealed by D-HMBC experiments[5] are shown in Fig. 2.

Another substructure, a butyl γ-pyrone moiety was revealed as follows. The sequence from a methyl proton

Table 1. Physico-chemical properties of espicufolin.

Appearance	Yellow powder
MP	184 ~ 186 °C
$[\alpha]_D^{21}$	- 2.5 ° (*c* 0.02, MeOH)
Molecular formula	$C_{22}H_{18}O_6$
HRFAB-MS (*m/z*)	
Found	379.1198 $(M+H)^+$
Calcd	379.1182
UV λ_{max}^{MeOH} nm (ε)	208 (15,400), 239 (28,100) 266 (13,500), 406 (4,700)
$\lambda_{max}^{MeOH+NaOH}$ nm (ε)	240 (24,100), 328 (4,500) 517 (2,500)
IR ν_{max} (KBr) cm^{-1}	3490, 1675 (sh), 1650, 1585, 1460, 1275, 1220

Table 2. ^{13}C and ^1H NMR chemical shifts of espicufolin in DMSO-d_6.

No.	δ_C	δ_H	No.	δ_C	δ_H
2	172.5		11	161.3	
3	110.6	6.35	11a	116.7	
4	178.3		12	187.0	
4a	124.0		12a	119.7	
5	153.3		12b	155.6	
6	118.7	8.47	13	62.2	5.13
6a	136.0		14	38.0	2.78
7	181.5		15	26.7	1.89 1.73
7a	132.1		16	11.4	0.92
8	118.7	7.67	17	17.6	1.37
9	136.6	7.76	11-OH		12.68
10	124.7	7.37	13-OH		5.66

Fig. 1. Structure of espicufolin.

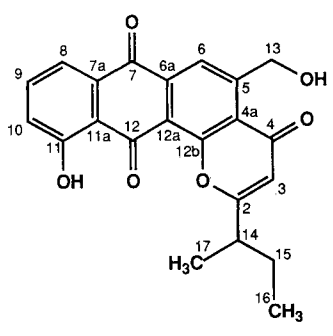
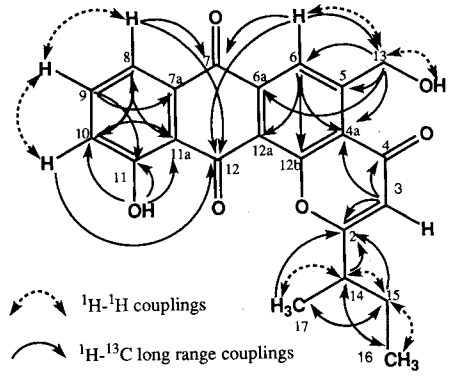


Fig. 2. ^{1}H-^{1}H and ^{1}H-^{13}C connectivities of espicufolin as revealed by DQF-COSY and HMBC experiments.



16-H (0.92 ppm) to a methyl proton 17-H (1.37 ppm) through methylene protons 15-H (1.73, 1.89 ppm) and a methine proton 14-H (2.78 ppm) was confirmed by DQF-COSY. In addition, this methine proton 14-H was long-range coupled to C-2 (172.5 ppm), which was also long-range coupled to the methyl protons 17-H, the methylene protons 15-H and an olefinic proton 3-H (6.35 ppm). Furthermore, long range couplings from the olefinic proton 3-H to a carbonyl carbon C-4 (178.3 ppm) and C-4a were observed (Fig. 2). These relations and IR absorptions at 1675 (sh), 1650 and 1585 cm^{-1}, and UV and visible spectral data suggested the presence of a γ-pyrone moiety. According to their ^{13}C chemical shifts, C-2 and C-12b were assignable to oxygenated sp^2 carbons. Therefore, C-2 and C-12b had to be connected by an ether bond and the γ-pyrone moiety was fused to the anthraquinone structure in **1**. Consequently, the structure of **1** was determined as shown in Fig. 1

In the evaluation system we employed[6,7], **1** suppressed the toxicity of L-glutamate in N18-RE-105 cells with EC_{50} value 40 nM. Since the L-glutamate toxicity in N18-RE-105 cells was thought to be caused by glutathione depletion[8], we assessed buthionine sulfoximine (BSO) toxicity. Espicufolin, however, did not suppress the BSO toxicity, which is considered to involve oxygen radicals. This result strongly suggests that the mode of action of espicufolin is not related to antioxidative activity. Further investigation on its biological activity is now under way.

Acknowledgements

This work was supported in part by a Grant-in Aid for Scientific Research (C) to K.S.

JUNG-SIK KIM
KAZUO SHIN-YA
JUN EISHIMA
KAZUO FURIHATA†
HARUO SETO

Institute of Molecular and Cellular Biosciences,
The University of Tokyo,
† Division of Agriculture and Agricultural Life Sciences,
The University of Tokyo,
Bunkyo-ku, Tokyo 113, Japan

(Received May 23, 1996)

References

1) CHOI, D. W.: Cerebral hypoxia: some approaches and unanswered questions. J. Neurosci. 10: 2493 ~ 2501, 1990
2) COYLE, J. T. & P. PUTTFARCKEN: Oxidative stress, glutamate, and neurodegenerative disorders. Science 262: 689~695, 1993
3) MAEDA, K.; T. TAKEUCHI, K. NITTA, K. YAGISHITA, R. UTAHARA, T. OSATO, M. UEDA, S. KONDO, Y. OKAMI & H. UMEZAWA: A new antitumor substance, pluramycin. J. Antibiotics, Ser A 9: 75~81, 1956
4) KONDO, S.; T. WAKASHIRO, M. HAMADA, T. TAKEUCHI & H. UMEZAWA: Isolation and characterization of a new antibiotic, neopluramycin. J. Antibiotics 23: 354~359, 1970
5) FURIHATA, K. & H. SETO: Decoupled HMBC (D-HMBC), an improved technique of HMBC. Tetrahedron Lett. 36: 2817~2820, 1995
6) 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
7) 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
8) 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