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Structures of Phenazostatins A and B, Neuronal Cell Protecting Substances of Microbial Origin

Bong-Sik Yun, In-Ja Ryoo, Won-Gon Kim, Jong-Pyong Kim, Hiroyuki Koshino[1], Haruo Seto[2] and Ick-Dong Yoo*

Korea Research Institute of Bioscience and Biotechnology, KIST, P.O. Box 115, Yusong, Taejon 305-600, Korea
[1]The Institute of Physical and Chemical Research (RIKEN), Hirosawa 2-1, Wako, Saitama, 351-01, Japan
[2]Institute of Molecular and Cellular Biosciences, The University of Tokyo, Bunkyo-ku, Tokyo 113, Japan

Abstract : Phenazostatins A (**1**) and B (**2**) were isolated from the culture broth of *Streptomyces* sp. 833 as neuronal cell protecting substances. These compounds were established as a member of the phenazine class of antibiotics on the basis of various spectroscopic analyses.

L-Glutamic acid, an excitatory amino acid, mediates neuronal degeneration in hippocampus through the generation of oxygen radicals after brain-ischemic attack[1,2]. It has been suggested that free radical scavengers may prevent neuronal cell death caused by L-glutamate[3]. In the course of our screening for substances to protect neuronal cells from the L-glutamate toxicity by using neuronal hybridoma N18-RE-105 cells, we isolated two phenazine compounds, phenazostatins A (**1**)[4] and B (**2**), together with methyl saphenate[5,6]. This paper describes the isolation and structural determination of **1** and **2** (Fig. 1).

A hexane extract of the broth filtrate (2 liters) of *Streptomyces* sp. 833 was purified by SiO_2 and Sephadex LH-20 column chromatographies followed by an ODS column chromatography eluted with 80% MeOH to give **1** (0.8 mg) and **2** (2.0 mg).

The molecular formula of **1** was established as $C_{28}H_{20}N_4O_3$ by HR-FAB mass spectroscopy (*m/z* 461.1626 $(M+H)^+$ +1.2mmu) in combination with ^1H and ^{13}C NMR data. The UV absorption maxima at 252 and 365 nm in MeOH together with the presence of several nitrogen-conjugated ^{13}C resonances between 140 and 145 ppm suggested that **1** was a typical phenazine compound. The IR absorption at 1730 cm^{-1} and the ^{13}C signal at 167.2 ppm revealed the presence of an ester group in **1**. Two *ABX* proton spin systems (8.31, 7.92

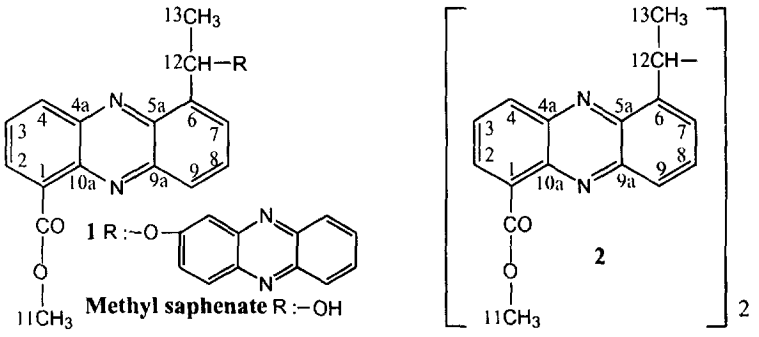

Figure 1. Structures of phenazostatins A (**1**) and B (**2**).

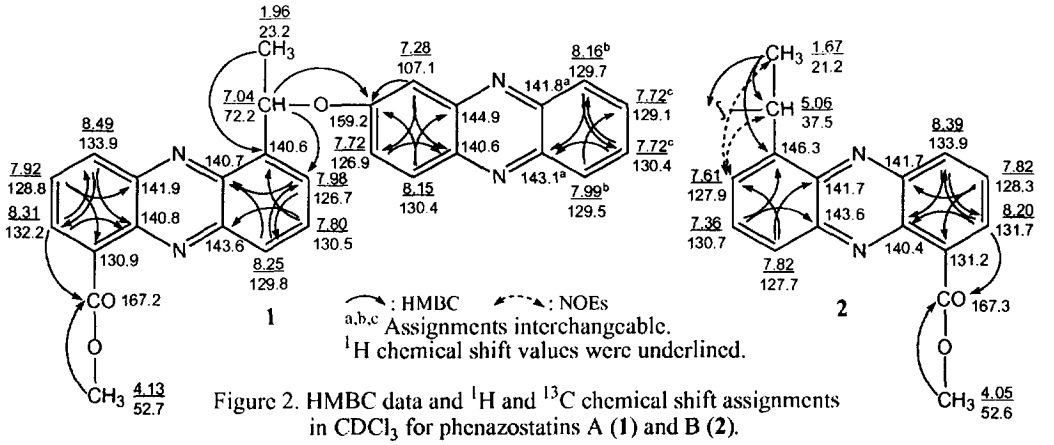

Figure 2. HMBC data and ^{1}H and ^{13}C chemical shift assignments in $CDCl_3$ for phenazostatins A (**1**) and B (**2**).

and 8.49 ppm, and 7.98, 7.80 and 8.25 ppm) revealed that **1** had 1,6- and/or 1,9-disubstituted phenazine moieties. Since ^{1}H and ^{13}C chemical shifts of **1** were in good agreement with those of methyl saphenate[7], the structure of **1** was concluded to be a 1,6-disubstituted phenazine derivative. The structure of **1** was finally assigned by the HMBC data as shown in Fig. 2. It may be important to note that 1,6-dicarboxylic phenazines are ubiquitous among microbial metabolites[6].

Compound **2** was previously reported by Umezawa *et al.*[8] as an inhibitor of phosphodiesterase, but structural elucidation including stereochemistry remains still to be unequivocally solved. The molecular formula of **2** was determined to be $C_{32}H_{26}N_4O_4$ by HR-FAB mass spectroscopy (*m/z* 531.2040 $(M+H)^+$ +0.6mmu). The UV and IR spectra of **2** were closely related to those of **1**, implying that **2** was also a phenazine compound. The ^{1}H NMR spectrum revealed only 13 protons, while only 16 carbons were observed in the ^{13}C NMR spectrum, indicating that **2** was a symmetric dimer. The ^{1}H and ^{13}C chemical shifts of **2** are completely assigned by the HMBC data as shown in Fig. 2. The 1,6-disubstituted phenazine moiety for **2** was determined by comparing the ^{13}C chemical shifts of **2** with those of methyl saphenate. Compounds 1 and 2 showing no optical rotation exist in nature as mixtures of enantiomers as suggested by Floss *et al.*[6] and more than three stereoisomers[9], respectively. The biological activities of 1 and 2 are now under investigation.

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4. Compound **1**. Mp 220-225 °C ; UV λ_{max} nm (ε) in MeOH : 252 (86,000), 365 (18,000) ; IR (KBr) : 1730, 1440, 1270, 1190, 1030, 760 cm^{-1} ; $[\alpha]_D$ = 0°(*c* =0.035, $CDCl_3$); HRFAB-MS : *m/z* 461.1626 (MH^+), $C_{28}H_{20}N_4O_3$ requires 461.1614. For compound **2**, see ref. 8.
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7. ^{13}C chemical shifts of methyl saphenate in $CDCl_3$+CD_3OD (1:1): 168.1 (1-CO), 145.2 (6), 144.2 (9a), 142.2 (4a), 141.8 (5a), 141.1 (10a), 134.4 (4), 133.2 (2), 132.0 (8), 131.4 (1), 129.7 (3), 129.2 (9), 127.2 (7), 66.3 (12), 53.0 (11), 24.8 (13).
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9. NMR spectra of **2** showed the broad ^{1}H signals at 7, 8, 12 and 13 positions and two ^{13}C peaks with low intensity for 7-CH. The HPLC analysis of **2** revealed three peaks, which were not resolved well.

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