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Structure of a New Microbial Metabolite, Neuchromenin

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Abstract: The structure of a new microbial metabolite, neuchromenin, was elucidated to be as shown in Fig. 1 by NMR spectral analysis including a variety of two-dimensional techniques. Neuchromenin was found to possess a novel pyranochromene skeleton. Copyright © 1996 Elsevier Science Ltd

Nerve growth factor (NGF) is a neurotrophic factor essential for survival and functioning of nerve cells and can induce neurite outgrowth of PC12 rat pheochromocytoma cells.[1,2] In the course of our screening for bioactive microbial metabolites, a novel substance designated neuchromenin (**1**) was isolated from the culture broth of *Eupenicillium javanicum* var. *meloforme* PF1181.[3] Neuchromenin induced neurite outgrowth of PC12 cells at concentrations of 2.5~10 μg/ml. We report here the isolation and structural elucidation of **1**.

The acetone extract of a 2-liter portion of the mycelium was partitioned between EtOAc and water. The organic layer was chromatographed on a silica gel column with $CHCl_3$-MeOH (50 : 1). The active eluate was subjected to ODS column chromatography with MeOH-50 mM disodium citrate (1 : 1). The active fraction was further purified on a Sephadex LH-20 column to give a yellow powder of neuchromenin (**1**, 10 mg).[4]

The molecular formula of **1** was established to be $C_{13}H_{12}O_5$ by HRFAB-MS (*m/z* 249.0782, MH^+, +1.9 mmu error). The ^{13}C NMR spectrum confirmed the presence of thirteen carbons, which were classified into one ketone carbonyl carbon, eight aromatic or olefinic carbons and four aliphatic carbons. The HMQC spectrum established all one-bond ^1H-^{13}C connectivities as summarized in Table 1.

Table 1. ^{13}C (125 MHz) and ^1H (500 MHz) NMR Spectral Data for Neuchromenin in CD_3OD.

	δ_C	δ_H (J = Hz)		δ_C	δ_H (J = Hz)
2	77.1* d	4.73** m	7	104.3 d	6.36 s
3	43.5 t	2.59 dd (17.0, 13.0)	8	153.3 s	
		2.52 dd (17.0, 4.0)	9	141.8 s	
4	191.0 s		10	110.9 d	7.02 s
4a	102.4 s		10a	109.2 s	
5	64.0 t	5.04 d (12.0)	10b	166.0 s	
		4.75 d (12.0)	11	20.6 q	1.56 d (6.0)
6a	155.2 s				

*,**Chemical shifts are given in ppm using TMS as internal standard.

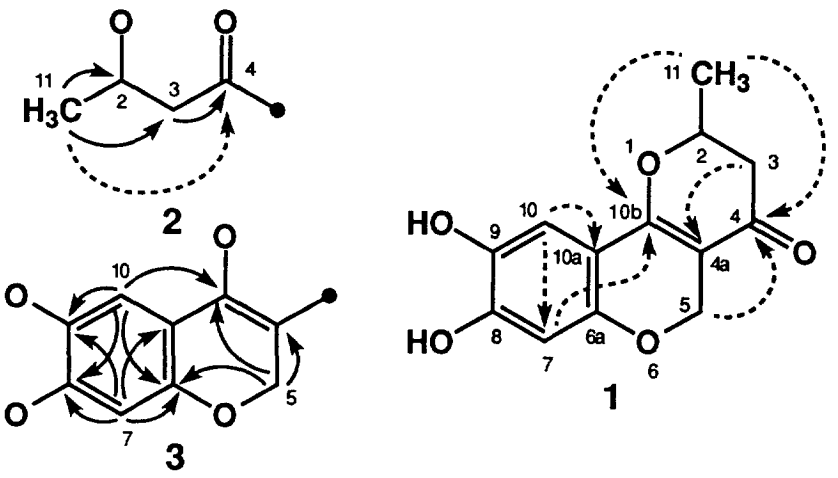

Fig. 1. ^{1}H-^{13}C long-range correlations derived from HMBC (solid arrows) and PFG HMBC (dashed arrows).

The ^{1}H NMR data showed a proton spin network representing 2-H, 3-H and 11-H as shown in Fig. 1. The HMBC spectrum displayed a ^{1}H-^{13}C long-range coupling between 3-H and C-4 to construct a partial structure **2**. Both of two singlet aromatic protons (δ 7.02 and 6.36) revealed long-range correlations to C-6a, C-8 and C-9, and were required to be in a 1,2,4,5-tetrasubstituted benzene ring. An isolated methylene (δ 5.04 and 4.75) showed ^{1}H-^{13}C long-range couplings to C-6a and the remaining two olefinic carbons (C-4a and C-10b). C-10b was also coupled with 10-H, indicating the presence of a chromene ring (partial structure **3**). Additional ^{1}H-^{13}C couplings derived from a pulse-field gradient (PFG) HMBC experiment[5] connected partial structures **2** and **3** to establish the total structure of **1** as shown in Fig. 1.

Neuchromenin (**1**) has a novel structure containing a 2*H*,5*H*-pyrano[3,2-*c*][1]benzopyran skeleton. Such examples have been reported as rare plant metabolites including bothrioclinin[6] and triptispinocoumarin.[7] The unique structural feature combined with its neurotrophic activity makes it a good candidate for treating patients with nerve diseases. Further studies on the biological activities are in progress.

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REFERENCES AND NOTES

1. Barde, Y. A. *Neuron* **1989**, 2, 1525-1534.
2. Hirao, T.; Tsuge, N.; Imai, S.; Shin-ya, K. & Seto, H. *J. Antibiot.* **1995**, 48, 1494-1496.
3. The culture has been deposited with the National Institute of Bioscience and Human-Technology, Agency of Industrial Science and Technology, Japan, with the accession number FERM P-15635.
4. MP: 195~200℃ (dec.). IR (KBr) νmax: 3480, 1615, 1560 cm^{-1}. UV λmax nm (ε): 223 (8,100), 256 (12,200), 309 (9,700) and 387 (14,700) in MeOH; 273 (8,900), 324 (7,000) and 423 (29,500) in 0.01N NaOH-MeOH. $[\alpha]_D^{20}$ -520° (*c* 0.1, MeOH).
5. Hurd, R. E. & John, B. K. *J. Magn. Reson.* **1991**, 91, 648-653.
6. Bohlmann, F. & Zdero, C. *Phytochem.* **1977**, 16, 1261-1263.
7. Bittner, M.; Jakupovic, J.; Bohlmann, F.; Grenz, M. & Silva, M. *Phytochem.* **1988**, 27, 3263-3266.

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