VOL. 52, NO. 3, MAR. 1999 THE JOURNAL OF ANTIBIOTICS pp. 231 — 234

Structure of NG-061, a Novel Potentiator of Nerve Growth Factor (NGF) Isolated from *Penicillium minioluteum* F-4627

RENUKA BHANDARI, TADASHI EGUCHI,† AKIKO SEKINE,†
YUJI OHASHI† and KATSUMI KAKINUMA*

Department of Chemistry, and Department of Chemistry and Materials Science†, Tokyo Institute of Technology, O-okayama, Meguro-ku, Tokyo 152-8551, Japan

MAYUMI ITO and KAZUTOSHI MIZOUE

Research Laboratories, Taisho Pharmaceutical Co., Ltd., 1-403, Yoshino-cho, Omiya-shi, Saitama 330-8530, Japan

(Received for publication December 15, 1998)

The structure of NG-061, a new potentiator of nerve growth factor (NGF) isolated from *Penicillium minioluteum* F-4627, was determined by spectroscopic analysis and X-ray diffraction method to be phenylacetic acid 2-(2-methoxy-4-oxocyclohexa-2,4-dienylidene)-hydrazide.

During the course of our screening program for low molecular weight natural products being able to potentiate and/or mimic neurotrophic effect of NGF, a novel fungal metabolite designated as NG-061, was isolated from the fermentation broth of *Penicillium minioluteum* F-4627[1]. In this paper, we wish to report the structure elucidation of NG-061 (**1**) by spectroscopic analysis and X-ray diffraction method.

Results and Discussion

The physico-chemical properties of **1** are summarized in Table 1. **1** was isolated as pale yellow crystals, and was found to have molecular formula as $C_{15}H_{14}N_2O_3$ based on the molecular ion peak observed at *m/z* 270 in EIMS and HR-EIMS. The prominent base peak of *m/z* 91 indicated the presence of a mono-alkyl substituted benzene ring in **1**. The UV spectrum of **1** was observed with two maxima at 207 and 340 nm in methanol. The IR spectrum of **1** showed a strong peak at 3438~3100 cm^{-1} due to a OH and/or NH group and two strong absorptions at 1680 and 1640 cm^{-1}, suggesting the existence of a conjugated ketone carbonyl and an amide moiety, respectively.

The ^1H and ^{13}C NMR spectra of **1** indicated that **1** existed in two forms in solution probably due to conformational change or tautomerization, and the ratio of two forms varied depending on the solvent; 1) *ca.* 4:1 in $CDCl_3$, 2) 8:1~10:1 in dimethyl sulfoxide-d_6, and 3) 1:1 in pyridine-d_5. The ^1H and ^{13}C NMR data in $CDCl_3$ solution were summarized in Table 2. The ^1H NMR spectrum showed 14 hydrogens, which is consistent with its molecular formula. The signal at δ_H 3.89 for 3H and the broad signal for 2H at δ_H 4.12 were straightforwardly assigned to a methoxy group and a methylene group, respectively, from ^1H and ^{13}C NMR data, and HMQC spectrum. The singlet at δ_H 5.86 was assigned to

Fig. 1. Structure of NG-061.

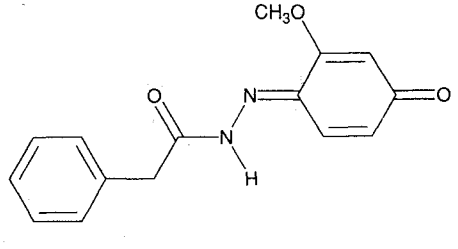


Table 1. Physico-chemical properties of NG-061.

Appearance	Pale yellow crystal
Melting point	189-190°C
EI-MS *m/z* (rel. intensity, %)	270 (95, M^+), 179 (8), 153 (17), 152 (15), 125 (29), 91 (100)
HR-EIMS (*m/z*)	
Found	270.1002
Calcd. for $C_{15}H_{14}N_2O_3$	270.1004
Molecular formula	$C_{15}H_{14}N_2O_3$
UV λ_{max} nm (ε) in methanol	207 (15,100), 340 (30,300)
IR ν_{max} cm^{-1} (KBr)	3438, 3111, 1680, 1640, 1569, 1543

Table 2. ^{13}C and ^{1}H NMR data of NG-061[a].

Carbon No.	δ_C	δ_H
1	38.7	4.12 (2H, broad s)
2	56.2	3.89 (3H, s)
3	106.9	5.86 (1H, s)
4	127.1	7.28 (1H, m)
5	128.5	6.34 (1H, d, J = 10.0 Hz)
6	128.6 (2C)	7.3 (2H, m)
7	129.4 (2C)	7.3 (2H, m)
8	132.8	
9	133.8	
10	138.6	7.07 (1H, d, J = 10.0 Hz)
11	159.9	
12	174.0	
13	186.5	
		11.34 (1H, N-H)

[a] The data were reported for the major peaks of NG-061 in $CDCl_3$.

an olefinic proton, which showed a cross peak with the carbon at δ_C 106.9 in the HMQC spectrum. The doublet at δ_H 6.34 was also due to an olefinic proton, which in turn coupled with the proton at δ_H 7.07 by $J=10$ Hz. The multiplet (5H) observed at δ_H 7.28~7.30 were correlated to carbon signals at δ_C 127.1, 128.6, and 129.4 in the HMQC spectrum, indicating the presence of a monosubstituted benzene ring. An exchangeable broad signal was observed at δ_H 12.40, which can be assigned to an amide proton.

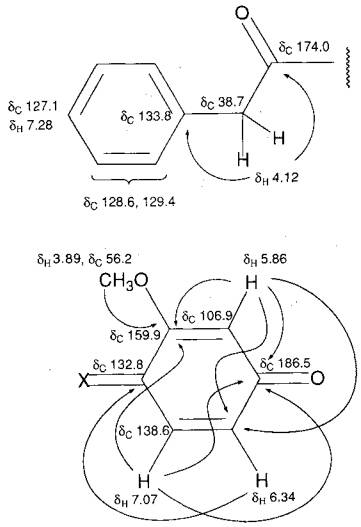
The HMBC experiments confirmed the heteronuclear connectivities in **1** which are shown in Figure 2. The correlations of the methylene protons at δ_H 3.89 with a carbonyl carbon at δ_C 174.0 and the phenyl carbon indicated the presence of a phenylacetyl group. Other correlations from the olefinic protons at δ_H 5.86, 6.34,

and 7.07 suggested that the structure of **1** is composed of cyclohexadienone moiety.

The remained structure of **1** was the linkage between the phenylacetyl group and cyclohexadienone group. Since additional information was not available from the NMR spectra, the X-ray crystallographic analysis was persued. Fortunately, **1** formed suitable needle crystals for X-ray analysis by recrystallization from CH_3CN. As shown in Figure 3, the ORTEP drawing clearly showed an acyl hydrazone structure in **1**, and finally, the structure of **1** was unambiguously determined to be phenylacetic acid 2-(2-methoxy-4-oxocyclohexa-2,4-dienylidene)hydrazide. It is conceivable from this structure that two forms of **1** in solution may exist due to the *S-cis* to *S-trans* isomerization of the amide group or tautomerization in the hydrazide moiety.

It is the first report to our knowledge that a phenylacetic acid (4-oxo-iminoquinone)hydrazide derivative such as **1** has been isolated from natural source as a bioactive product. There are only a few reports on phenyl acetic acid hydrazide derivatives. 1,4-Naphtoquinones-4-aryl(aroyl)hydrazones were synthesized as possible antituberculous agents[2] from a lead compound of 3-hydroxy-2-methyl-1,4-napthoquinone with antituberculous activity isolated from acetone extract of Tubercle bacilli. On the development of the method for spectrophotometric determination of hydrazides was detected phenylacetic acid 2-(2,3-dichloro-4-oxo-4H-naphthalene-1-ylidene)hydrazide as a reaction product[3]. To confirm whether **1** is a microbial metabolite or an artifact, we examined the time course of fermentation by extracting the culture broth, and by analyzing the extract with HPLC. The production of **1** gradually increased along with the progress of fermentation and reached to a plateau after 48 hours (data not shown). Therefore, it appears that **1** was produced as a metabolite produced by *Penicillium minioluteum* F-4627.

Fig. 2. HMBC connectivities of NG-061.

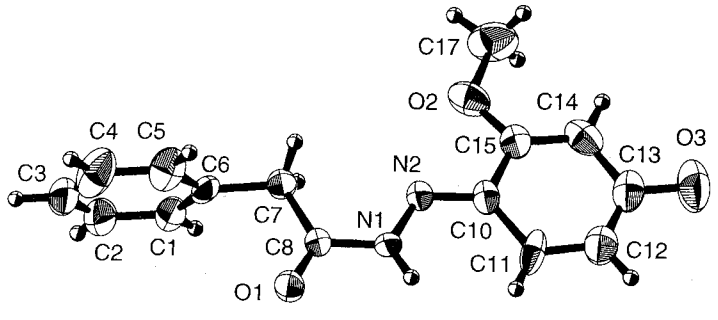


Experimental

General

Melting point was determined with a Yazawa micromelting point apparatus BY-1. IR spectrum was recorded on a Horiba FT-710 Fourier-transform Infrared spectrometer. UV spectrum was obtained with a Shimadzu UV-160A UV-Visible recording spectrophotometer. Mass spectra was determined with a JEOL

Fig. 3. ORTEP[8] drawing of NG-061 showing the atomic labelling.



50% Probability thermal ellipsoids for non-hydrogen atoms are shown.
H atoms are shown as small spheres of arbitrary radii.

JMS-AX505 HA Mass spectrometer. NMR spectra were measured on a Bruker DRX 500 spectrometer.

X-ray Crystalography

Crystal data: Monoclinic, a=26.5053(12), b=6.5972 (3), c=17.2633(4) Å, b=117.261(4)°, V=2683.4(2) Å^3, Z=8, space group C2/c, Dc=1.338 Mg m^{-3}, μ=0.087 mm^{-1}. Data collection: The X-ray analysis was carried out by Siemens Smart CCD diffractometer[5] with graphite monochromated Mo-$K\alpha$ radiation (λ=0.71073 Å) using pale yellow needle crystal, 0.28×0.09×0.07 mm at −50°C with low temperature apparatus. A total of 6354 reflections measured, of which 1936 are unique. Data were corrected for Lorentz and polarization effects. Semi-empirical absorption correction[6] was applied. Structure analysis and refinement: The crystal structure was solved by the direct methods with the program SIR-92[7], and refined by full-matrix least-squares on F^2 values using SHELXL-97[8]. Non-hydrogen atoms were refined with anisotropic temperature factors. Hydrogen atoms were included at calculated positions and refined in the riding mode except one bonding to the N atom, which was refined without constraint. The final R and wR2 for 1163 reflections with $I > 2\sigma(I)$ were 0.10 and 0.28, respectively and S=1.05. In the final refinement (Δ/σ) max became 0.001. Although there were two significant residual peaks, 0.8~0.9e Å^{-3}, they were at meaningless positions even if the disordered model was taken into account.

References

1) Ito, M.; N. Sakai, K. Ito, F. Mizobe, K. Hanada, K. Mizoue, R. Bhadari, T. Eguchi & K. Kakinuma: A novel fungal metabolite NG-061 enhances and mimics neurotrophic effect of nerve growth factor (NGF) on neurite outgrowth in PC12 Cells. J. Antibiotics 52: 224~230, 1999
2) Roushdi, I. M.; E. S. Ibrahim & N. S. Habib: Synthesis of 1,4-naphthoquinone-4-aryl(aroyl)hydrazones of potential antimicrobial activity. Pharmazie 31: 856~859, 1976
3) Plaizier, J. A.; J. G. van Damme & R. E. de Neve: Spectrophotometric determination of hydazides with 2,3-dichloro-1,4-naphtoquinone. Anal. Chem. 48: 1536~1538, 1976
4) Johnson, C. K.: ORTEP II. Report ORNL-5138. Oak Ridge National Laboratory, Tennessee, USA, 1976
5) Siemens: SMART. Area Detector Control Software. Siemens Analytical X-ray Instruments Inc., Madison, Wisconsin, USA, 1996
6) Sheldrick, G. M.: SADABS. Siemens Area Detector Absorption Correction Software. University of Göttingen, Germany, 1996
7) Altomare, A.; G. Cascarano, C. Giacovazzo, A. Guagliardi, M. C. Burla, G. Polidori & M. Camalli: SIR92—a program for automatic solution of crystal structures by direct methods. J. Appl. Cryst. 276: 435~457, 1994
8) Sheldrick, G. M.: SHELXL-97 Program fot the Refinement of Crystal Structures, University of Göttingen, Germany, 1997