

# Nigerasperones A~C, New Monomeric and Dimeric Naphtho- $\gamma$ -pyrones from a Marine Alga-derived Endophytic Fungus *Aspergillus niger* EN-13

Yi Zhang, Xiao-Ming Li, Bin-Gui Wang

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**Abstract** Three new naphtho- $\gamma$ -pyrones, 5-hydroxy-6,8-dimethoxy-2-hydroxymethyl-4*H*-naphtho[2,3-*b*]pyran-4-one (**1**, nigerasperone A), 3,3'-dihydro-2,2',5,5'-tetrahydroxy-8,8',10,10'-tetramethoxy-2,2'-dimethyl-(6',9-bi-4*H*-naphtho[1,2-*b*]pyran)-4,4'-dione (**2**, nigerasperone B), and 3'-hydro-2',5,5',8-tetrahydroxy-6,6',8'-trimethoxy-2,2'-dimethyl-(7,10'-bi-4*H*-naphtho[2,3-*b*]pyran)-4,4'-dione (**3**, nigerasperone C), together with nine related known compounds were characterized from *Aspergillus niger* EN-13, an endophytic fungus isolated from the marine brown alga *Colpomenia sinuosa*. Their structures were elucidated by detailed analysis of spectroscopic data and by comparison with literature reports. In the cytotoxic assay, these compounds did not show remarkable inhibitory effects against A549 and SMMC-7721 tumor cell lines. However, **3** and several known compounds showed weak antifungal activity against *Candida albicans* and moderate activity on DPPH scavenging.

**Keywords** *Colpomenia sinuosa*, algal endophytic fungus, *Aspergillus niger*, naphtho- $\gamma$ -pyrone

## Introduction

The secondary metabolites of the fungal strains of the genus *Aspergillus*, both terrestrial and marine origin, have been extensively studied. Like other members in this genus, marine isolates of *Aspergillus niger* have been proven to be prolific source of new secondary metabolites unknown from those of the terrestrial ones. For example, some isolates of *A. niger* from marine sponges and ascidians produced novel metabolites including asperazine, aspernigrins A and B, yanuthones A~E, nafuredin, and so on [1~4]. In our investigation on bioactive metabolites of marine-derived microorganisms, we isolated a pure strain, morphologically identified as *A. niger* EN-13, from the inner tissue of the brown alga *Colpomenia sinuosa* that was collected along the Qingdao coastline. The crude extract of the culture broth showed moderate cytotoxic activity against SMMC-7721 tumor cell line. During the course of chromatographic separation of the methanol and ethyl acetate extracts from the mycelium and broth of the fungus, three new naphtho- $\gamma$ -pyrones, which were named as nigerasperones A~C (**1**~**3**), along with nine related known compounds, aurasperones A (**4**) and B (**5**) [5], dianhydroaurasperone C (**6**) [6], asperpyrones A (**7**) and C (**8**) [7], and fonsecinones A~D (**9**~**12**) [5], were isolated and identified (Fig. 1). We wish to report here the isolation, structural elucidation, and bioassay of these metabolites from *A. niger* EN-13.

X.-M. Li (Corresponding author), B.-G. Wang (Corresponding author), Y. Zhang: Key Laboratory of Experimental Marine Biology, Institute of Oceanology, Chinese Academy of Sciences, Nanhai Road 7, Qingdao 266071, PR China, E-mail: wangbg@ms.qdio.ac.cn

Y. Zhang: Graduate School of Chinese Academy of Sciences, Beijing 100049, PR China

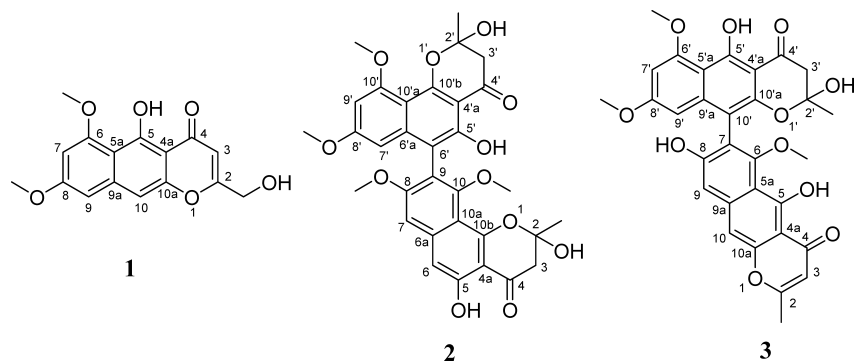


Fig. 1 Structures of nigerasperones A~C (1~3).

## Materials and Methods

### General Experimental Procedure

Melting points were measured using a SGW X-4 micro-melting point apparatus and uncorrected. Optical rotations were obtained on a JASCO P-1020 digital polarimeter. UV spectra were recorded on PuXi TU-1810 UV-visible spectrophotometer. IR spectra were taken on a NICOLET 510P FT-IR spectrometer in KBr discs. NMR spectra were recorded on a Bruker Avance 500 (500 MHz for  $^1\text{H}$  and 125 MHz for  $^{13}\text{C}$ ) spectrometer using TMS as internal standard and chemical shifts were recorded as  $\delta$  values. Mass spectra were measured on VG Auto Spec 3000 mass spectrometer. Semipreparative HPLC was performed on Dionex HPLC system equipped with P680 HPLC pump, UVD 340U UV-visible photodiode-array detector, and  $\text{C}_{18}$  column ( $5\ \mu\text{m}$ ,  $8.0\times 250\ \text{mm}$ ). Silica gel (200~300 mesh and  $\text{GF}_{254}$ ) for column chromatography (CC) and preparative thin-layer chromatography (P-TLC) were the products of Qingdao Haiyang Chemical Co., Qingdao, China. ODS was the product of Merck Corporation. Sephadex LH-20 was purchased from Pharmacia Corporation. Chloramphenicol, amphotericin B, DPPH ( $\alpha,\alpha$ -diphenyl- $\beta$ -picrylhydrazyl), and BHT (butylated hydroxytoluene) were purchased from Sigma Corporation.

### Microorganism

Endophytic fungus *A. niger* EN-13 was isolated from the tissue of surface-sterilized marine brown alga *C. sinuosa* that was collected at the Qingdao coastline, China. The fungus was morphologically identified as *A. niger* by one of the authors (Y.Z.) and was preserved in China Center for Type Culture Collection with access number CCTCC AF 206004.

Strains for antimicrobial assay, *Escherichia coli* (ATCC 9637), *Staphylococcus aureus* (ATCC 29213), *Candida*

*albicans* (ATCC 10231), and *A. niger* (ATCC 6275), were obtained from the stock cultures maintained at the School of Medicine and Pharmacology, the Ocean University of China, Qingdao, China.

### Fermentation

A small spoon of spores growing on malt agar slant was inoculated onto malt agar plate and cultured at  $28^\circ\text{C}$  for 7 days. Then a piece (size:  $4\ \text{cm}^2$ ) of mycelium was inoculated into 1000-ml Erlenmeyer flasks each containing 300 ml of culture medium composed of glucose 2.0%, peptone 0.5%, malt extract 0.3%, yeast extract 0.3%, and artificial sea salt 2.44%. The pH was adjusted to 7.4 before autoclaving. Static fermentation was carried out at  $25^\circ\text{C}$  for 30 days.

### Extraction and Isolation

The culture broth (30 liters) was filtered through absorbent cotton to separate the mycelia and supernatant. Air-dried mycelia were smashed and extracted with 3 liters of methanol to afford an extract. The supernatant was concentrated *in vacuo* to about 4 liters, and then extracted three times with ethyl acetate to give an ethyl acetate extract. Since the two extracts displayed similar TLC and HPLC profiles, so they were combined and evaporated under reduced pressure, resulting in a crude extract (42.0 g).

This extract was first chromatographed on a silica gel (200~300 mesh) column by eluting stepwise from chloroform to methanol. The chloroform-methanol (80:1) fraction (6.13 g) was subsequently subjected to a silica gel column eluted in gradient ratios with petroleum ether-acetone and chloroform-methanol consecutively. This afforded three yellowish fractions including fractions I (880 mg, petroleum ether-acetone, 2:1), II (808 mg, petroleum ether-acetone 1.5:1), and III (1.05 g, chloroform-methanol, 30:1). Fraction I was applied on

**Table 1** Physico-chemical properties of **1**~**3**

	<b>1</b>	<b>2</b>	<b>3</b>
Appearance	Yellow powder	Yellow powder	Yellow powder
Melting point	193~195°C	169~171°C	222~224°C
$[\alpha]_D^{20}$	—	-15.3° (c 0.27, MeOH)	-11.5° (c 0.34, MeOH)
Molecular formula	C <sub>16</sub> H <sub>14</sub> O <sub>6</sub>	C <sub>32</sub> H <sub>30</sub> O <sub>12</sub>	C <sub>31</sub> H <sub>26</sub> O <sub>11</sub>
Molecular weight	302	606	574
HR-ESI-MS <i>m/z</i>	[M+Na] <sup>+</sup>	[M+Na] <sup>+</sup>	[M+H] <sup>+</sup>
Calcd	325.0688	629.1634	575.1553
Found	325.0681	629.1642	575.1534
UVλ <sub>max</sub> <sup>MeOH</sup> nm (log ε)	210 (sh, 4.35), 226 (4.48), 255 (sh, 4.36), 276 (4.61), 325 (3.76), 400 (3.95)	236 (4.79), 281 (4.80), 316 (sh, 4.30), 380 (3.88)	232 (4.33), 282 (4.63), 329 (3.82), 403 (3.80)
IR ν <sub>max</sub> <sup>KBr</sup> cm <sup>-1</sup>	3427, 2926, 2855, 1659, 1629, 1582, 1439, 1254, 1210, 1168	3433, 2934, 2844, 1660, 1613, 1405, 1238, 1165	3430, 2935, 2850, 1650, 1619, 1424, 1262, 1165
NMR data	Table 2	Table 3	Table 4

preparative TLC (pTLC) (silica gel, chloroform - methanol, 40 : 1) and ODS column (MeOH) to give **1** (1.8 mg); separation of other eluates of fraction I produced **4** (62 mg), **9** (75 mg), and **10** (39 mg), by repeated pTLC and Sephadex LH-20 gel chromatography (chloroform - methanol, 1 : 1). Fraction II gave two subfractions after a CC separation on silica gel (petroleum ether - acetone, 2 : 1). One subfraction was further purified by semi-preparative HPLC (ODS, methanol - water, 65 : 35) and consecutive pTLC to give **8** (3 mg), and the other subfraction gave **11** (20 mg) by using of CC on ODS (methanol - water, 85 : 15) and Sephadex LH-20. Through consecutive CC on silica gel (petroleum ether - acetone, 18 : 1; chloroform - methanol, 80 : 1, respectively), Sephadex LH-20 and two times of pTLC (dichloromethane - methanol, 40 : 1 and 20 : 1, respectively), **2** (20 mg) was obtained from fraction III; Compounds **3** (30 mg), **5** (120 mg), **6** (32 mg), **7** (47 mg), and **12** (28 mg) were also purified from fraction III by repeated CC on silica gel, ODS column, and Sephadex LH-20.

### Biological Assay

*In vitro* cytotoxic assay to tumor cell lines SMMC-7721 (hepatocellular carcinoma cells) and A549 (human lung epithelial cells) was performed following the SRB method [8]. Antimicrobial assay was performed by using of the well diffusion method [9]. Chloramphenicol and amphotericin B were used as antibacterial and antifungal controls, respectively. The DPPH radical scavenging activity was determined by the method as previously report [10].

## Results and Discussion

### Physico-chemical Properties

Physico-chemical properties of **1**~**3** are summarized in Table 1. All of them showed the mutual absorptions at 220~240, 275~285, 380~410 nm in the UV spectra. In addition, the presence of hydroxyl and conjugated carbonyl groups in each of the molecules were indicated by the absorption bands at 3425~3435 and 1650~1660 cm<sup>-1</sup> in the IR spectra, respectively.

### Structure Determination

**1** was obtained as yellow powder. Its molecular formula was determined as C<sub>16</sub>H<sub>14</sub>O<sub>6</sub> by HR-ESI-MS, which was in agreement with the <sup>1</sup>H and <sup>13</sup>C NMR data (Table 2), indicating 10 degrees of unsaturation. The IR spectrum suggested the presence of hydroxyl (3427 cm<sup>-1</sup>) and conjugated carbonyl (1659 cm<sup>-1</sup>) groups in the molecule. The <sup>13</sup>C NMR and DEPT spectra indicated the presence of 16 carbons in the molecule including one hydroxymethyl group, two methoxy groups, four *sp*<sup>2</sup> methines, and nine quaternary *sp*<sup>2</sup> carbons (one of which was carbonyl), indicating that the compound to be tricyclic. The <sup>1</sup>H NMR spectrum showed signals for one hydroxymethyl ( $\delta_H$  4.54, 2H, d, *J*=5.1 Hz, -CH<sub>2</sub>OH;  $\delta_H$  4.96, 1H, br s, -CH<sub>2</sub>OH), two methoxyls ( $\delta_H$  3.91, 3.93), two *meta*-coupled aromatic protons ( $\delta_H$  6.44, br s;  $\delta_H$  6.80, d, *J*=0.8 Hz), two singlet aromatic or olefinic protons ( $\delta_H$  6.28, s and  $\delta_H$  7.07, s), and one phenolic hydroxyl proton ( $\delta_H$  14.84), which was obviously an intramolecular hydrogen-bonded and

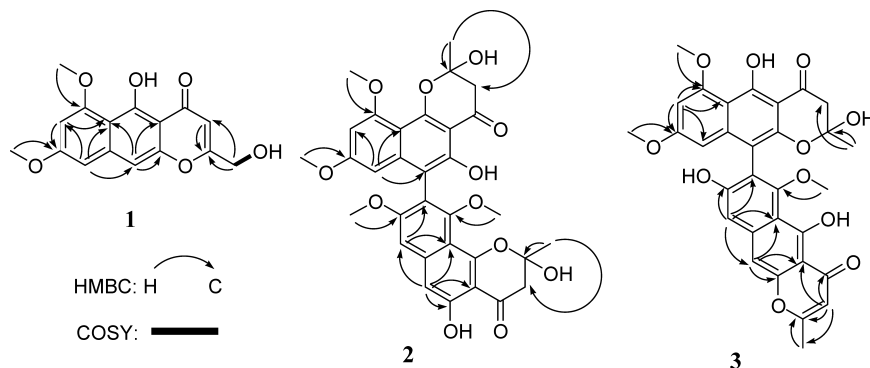
characteristic of linear naphtho- $\gamma$ -pyrone structural unit [5, 7]. Detailed analysis of the NMR data suggested that **1** was a linear naphtho- $\gamma$ -pyrone monomer, structurally related to the reported rubrofusarin B [11]. However, the 2-CH<sub>3</sub> group in rubrofusarin B was replaced by a 2-CH<sub>2</sub>OH group in **1**. This was indicated by the fact that the carbon signal for 2-CH<sub>3</sub> of rubrofusarin B at  $\delta_C$  20.4 disappeared while a hydroxymethyl signal at  $\delta_C$  61.3 for **1** was observed in the <sup>13</sup>C NMR spectrum. Also in the <sup>1</sup>H NMR spectrum the signal for the singlet methyl group in rubrofusarin B was replaced by a hydroxymethyl group ( $\delta_H$  4.54, 2H, -CH<sub>2</sub>OH and  $\delta_H$  4.96, 1H, -CH<sub>2</sub>OH) in **1**. This deduction was further confirmed by the observed HMBC correlations between the hydroxymethyl proton ( $\delta_H$  4.54) and C-2 ( $\delta_C$

171.9) and C-3 ( $\delta_C$  105.2) (Fig. 2). Based on the above evidences, the structure of **1** was determined as 5-hydroxy-6,8-dimethoxy-2-hydroxymethyl-4*H*-naphtho[2,3-*b*]pyran-4-one, which was named as nigerasperone A.

**2** was also obtained as yellow powder. Its molecular formula C<sub>32</sub>H<sub>30</sub>O<sub>12</sub> was determined by HR-ESI-MS and <sup>1</sup>H and <sup>13</sup>C NMR data (Table 3), indicating 18 degrees of unsaturation. The IR spectrum suggested the presence of hydroxyl (3433 cm<sup>-1</sup>) and conjugated carbonyl (1660 cm<sup>-1</sup>) groups. The <sup>13</sup>C NMR and DEPT spectra displayed the presence of 32 carbons including two methyls, four methoxys, two *sp*<sup>3</sup> methylenes, four *sp*<sup>2</sup> methines, two *sp*<sup>3</sup> quaternary carbons, and 18 quaternary *sp*<sup>2</sup> carbons (two of which were carbonyls), indicating that the compound to be hexacyclic. The <sup>1</sup>H NMR spectrum showed signals for two singlet methyls ( $\delta_H$  1.49, 6H), four methoxys ( $\delta_H$  3.59, 3.59, 3.74, and 3.94), two *meta*-coupled aromatic protons ( $\delta_H$  6.21, d, *J*=1.8 Hz and  $\delta_H$  6.41, d, *J*=1.8 Hz), two singlet aromatic protons ( $\delta_H$  6.86 and 7.07), two pairs of protons of two methylenes ( $\delta_H$  2.80, 2H, d, *J*=17.3 Hz and  $\delta_H$  3.07, 2H, d, *J*=17.3 Hz), and two phenolic hydroxyl protons ( $\delta_H$  12.86, 2H), which were obviously intramolecularly hydrogen-bonded and characteristic of angular naphtho- $\gamma$ -pyrone structural unit [5, 7]. All these characters indicated that **2** was an asymmetric dimer of two angular naphtho- $\gamma$ -pyrone monomers. A survey of the literature revealed that **2** exhibited highly similar NMR data with that reported for asperpyrone B [7] except for the differences in the chemical shifts of C-2 (C-2'), C-3 (C-3'), C-4 (C-4'), and 2-CH<sub>3</sub> (2'-CH<sub>3</sub>). The oxygenated olefinic carbons C-2 ( $\delta_C$  166.8) and C-2' ( $\delta_C$  166.5) in asperpyrone B were replaced by two *sp*<sup>3</sup> quaternary carbons ( $\delta_C$  101.1) in **2**, each of which was obviously connected to two oxygen atoms. Meanwhile, the olefinic carbons C-3 ( $\delta_C$  110.6) and C-3' ( $\delta_C$  110.3) in asperpyrone B were replaced by two saturated methylenes ( $\delta_C$  48.6, C-3 and C-3') in **2**. In addition, the carbon signals for the carbonyl groups C-4 and C-4' shifted downfield from  $\delta_C$  182.9 and 183.0 (in

**Table 2** <sup>1</sup>H and <sup>13</sup>C NMR data of **1** (acetone-*d*<sub>6</sub>, TMS,  $\delta$  in ppm)

No	$\delta_H$ (J in Hz)	$\delta_C$
2		171.9
3	6.28 (1H, s)	105.2
4		185.2
4a		105.1
5		162.0
5a		109.0
6		162.0
7	6.44 (1H, br s)	98.0
8		163.0
9	6.80 (1H, d, 0.8)	98.8
9a		140.5
10	7.07 (1H, s)	101.6
10a		154.0
2-CH <sub>2</sub> OH	4.54 (2H, d, 5.1)	61.3
2-CH <sub>2</sub> OH	4.96 (1H, br s)	
5-OH	14.84 (1H, s)	
6-OCH <sub>3</sub>	3.93 (3H, s)	56.2
8-OCH <sub>3</sub>	3.91 (3H, s)	55.8



**Fig. 2** Selected HMBC and COSY correlations of **1**~**3**.

**Table 3**  $^1\text{H}$  and  $^{13}\text{C}$  NMR data of **2** (acetone- $d_6$ , TMS,  $\delta$  in ppm)

No	$\delta_{\text{H}}$ (J in Hz)	$\delta_{\text{C}}$	No	$\delta_{\text{H}}$ (J in Hz)	$\delta_{\text{C}}$
2		101.1	2'		101.1
3	2.80 (1H, d, 17.3)	48.6	3'	2.80 (1H, d, 17.3)	48.6
	3.07 (1H, d, 17.3)			3.07 (1H, d, 17.3)	
4		199.0	4'		199.0
4a		109.0	4'a		109.4
5		157.0	5'		152.5
6	6.86 (1H, s)	105.4	6'		108.2
6a		141.6	6'a		143.9
7	7.07 (1H, s)	106.4	7'	6.21 (1H, d, 1.8)	98.4
8		162.0	8'		163.0
9		119.0	9'	6.41 (1H, d, 1.8)	96.6
10		158.0	10'		162.0
10a		108.0	10'a		106.4
10b		157.0	10'b		156.2
2-CH <sub>3</sub>	1.49 (3H, br s)	28.0	2'-CH <sub>3</sub>	1.49 (3H, br s)	28.0
5-OH	12.86 (1H, s)		5'-OH	12.86 (1H, s)	
8-OCH <sub>3</sub>	3.74 (3H, s)	56.4	8'-OCH <sub>3</sub>	3.59 (3H, s)	61.6
10-OCH <sub>3</sub>	3.59 (3H, s)	55.5	10'-OCH <sub>3</sub>	3.94 (3H, s)	56.3

asperpyrone B) to 199.0 (in **2**) because of the lacking of conjugated double bonds in C-2 and C-2'. Furthermore, the carbon signals of 2-CH<sub>3</sub> and 2'-CH<sub>3</sub> shifted to downfield from  $\delta_{\text{C}}$  20.6 as in asperpyrone B to  $\delta_{\text{C}}$  28.0 in **2**, while the proton signals of the 2-CH<sub>3</sub> and 2'-CH<sub>3</sub> shifted upfield from  $\delta_{\text{H}}$  2.46 and 2.53 to  $\delta_{\text{H}}$  1.49. All these changes revealed that both the C-2 and C-2' double bonds in asperpyrone B were hydrated in **2**. Thus, the structure of **2** was determined as 3,3'-dihydro-2,2',5,5'-tetrahydroxy-8,8',10,10'-tetramethoxy-2,2'-dimethyl-(6',9-bi-4*H*-naphtho[1,2-*b*]pyran)-4,4'-dione, which was named as nigerasperone B. Similar to reported 2, 3 (or 2', 3')-hydrated naphtho- $\gamma$ -pyrones [5, 6, 12~16], the configurations at the chiral centers C-2 and C-2' for **2** remained unknown.

**3**, also obtained as yellow powder, was determined to have the molecular formula to be C<sub>31</sub>H<sub>26</sub>O<sub>11</sub> by HR-ESI-MS and  $^1\text{H}$  and  $^{13}\text{C}$  NMR data (Table 4), indicating 19 degrees of unsaturation. The IR spectrum suggested the presence of hydroxyl (3430 cm<sup>-1</sup>) and conjugated carbonyl (1650 cm<sup>-1</sup>) groups. The  $^{13}\text{C}$  NMR and DEPT spectra displayed 31 carbon signals including two methyls, three methoxyls, one *sp*<sup>3</sup> methylene, five *sp*<sup>2</sup> methines, one *sp*<sup>3</sup> quaternary carbon, and 19 quaternary *sp*<sup>2</sup> carbons (two of which were carbonyls), indicating the compound to be hexacyclic. The  $^1\text{H}$  NMR spectrum showed signals for two singlet methyls ( $\delta_{\text{H}}$  1.54 and 2.43), three methoxyls ( $\delta_{\text{H}}$  3.56, 3.59, and 3.96), two *meta*-coupled aromatic protons

( $\delta_{\text{H}}$  6.20 and 6.43, d, *J*=1.8 Hz for each), three singlet aromatic or olefinic protons ( $\delta_{\text{H}}$  6.10, 7.12 and 7.13), a pair of protons of a methylene ( $\delta_{\text{H}}$  2.80 and 3.07, d, *J*=17.3 Hz for each), two intramolecularly hydrogen-bonded phenolic hydroxyl protons ( $\delta_{\text{H}}$  14.44 and 15.03) characteristic of two linear naphtho- $\gamma$ -pyrone structural units, and a lonely phenolic hydroxyl proton ( $\delta_{\text{H}}$  12.00) [5, 7]. All these signals indicated that **3** was an asymmetric dimer of two linear naphtho- $\gamma$ -pyrone monomers, one of which was hydrated at C-2' double bond as suggested by the presence of a *sp*<sup>3</sup> methene ( $\delta_{\text{H}}$  2.80, 3.07, d, *J*=17.3 Hz;  $\delta_{\text{C}}$  48.6) and a *sp*<sup>3</sup> quaternary carbon ( $\delta_{\text{C}}$  101.3). The second monomer should be a linear naphtho- $\gamma$ -pyrone unit with an 8-OH instead of an 8-OCH<sub>3</sub>, as revealed by the absence of the characteristic signal for 8-OCH<sub>3</sub> (usually showing a 3-proton singlet at  $\delta_{\text{H}}$  3.7~3.8 and a carbon signal around  $\delta_{\text{C}}$  56) and the emergence of the lonely phenolic hydroxyl proton ( $\delta_{\text{H}}$  12.00) instead. Detailed comparison of the NMR data between **3** and those reported for fonsecinone B [5] showed fairly consistent characteristics except for the substitution of 8-OCH<sub>3</sub> ( $\delta_{\text{H}}$  3.80) in fonsecinone B by 8-OH ( $\delta_{\text{H}}$  12.00) in **3**. From the above evidences discussed, the chemical structure of **3** was elucidated as 3'-hydroxy-2',5,5',8-tetrahydroxy-6,6',8'-trimethoxy-2,2'-dimethyl-(7,10'-bi-4*H*-naphtho[2,3-*b*]pyran)-4,4'-dione, which was named as nigerasperone C.

The structures of **4**~**12** were unambiguously elucidated by the analysis of MS and 1D and 2D NMR spectral data

**Table 4**  $^1\text{H}$  and  $^{13}\text{C}$  NMR data of **3** (acetone- $d_6$ , TMS,  $\delta$  in ppm)

No	$\delta_{\text{H}}$ (J in Hz)	$\delta_{\text{C}}$	No	$\delta_{\text{H}}$ (J in Hz)	$\delta_{\text{C}}$
2		169.3	2'		101.3
3	6.10 (1H, s)	107.5	3'	2.80 (1H, d, 17.3) 3.07 (1H, d, 17.3)	48.6
4		185.3	4'		198.9
4a		104.6	4'a		104.6
5		166.1	5'		163.1
5a		111.8	5'a		108.0
6		159.5	6'		163.1
7		119.2	7'	6.43 (1H, d, 1.8)	96.6
8		163.1	8'		163.4
9	7.12 (1H, s)	106.3	9'	6.20 (1H, d, 1.8)	98.6
9a		141.5	9'a		143.9
10	7.13 (1H, s)	101.2	10'		105.9
10a		153.8	10'a		153.8
2-CH <sub>3</sub>	2.43 (3H, s)	20.6	2'-CH <sub>3</sub>	1.54 (3H, s)	28.0
5-OH	14.44 (1H, s)		5'-OH	15.03 (1H, s)	
6-OCH <sub>3</sub>	3.56 (3H, s)	62.0	6'-OCH <sub>3</sub>	3.96 (3H, s)	56.4
8-OH	12.00 (1H, br s)		8'-OCH <sub>3</sub>	3.59 (3H, s)	55.5

and by comparison with literature reported values [5~7].

### Biological Activities

The *in vitro* cytotoxic activity assay revealed that none of these compounds showed significant inhibitory activity ( $\text{IC}_{50} > 10 \mu\text{g/ml}$ ) against the proliferation of tumour cell lines SMMC-7721 and A549.

**3**, **5**, **8**, and **9** exhibited weak inhibitory activity against fungus *C. albicans* in the antimicrobial assay. Compared with the clear inhibition zone (i.d. 12 mm) of the positive control amphotericin B, **3**, **5**, **8**, and **9** also show visible inhibition zones with the diameter of 9, 10, 14, and 9 mm, respectively. Nevertheless, their inhibition zones were fainter. So they were judged to be weakly active.

In the DPPH radical scavenging assay, **3**, **5**, **10**, and **12** showed moderate activity with scavenging ratios of 41.6%, 48.1%, 13.2%, and 37.5%, respectively, at a concentration 50  $\mu\text{g/ml}$ , compared to 80.4% of the positive control, BHT.

Naphtho- $\gamma$ -pyrones have been mainly reported from fungi of the genera *Fusarium* and *Aspergillus* as well as from some plants [5, 17~19]. Compounds of this family were reported to exhibit highly diverse bioactivities, including anticancer, antibacterial, antifungal, anti-signal transduction, antiallergic, and inhibitory activities to HMG-CoA reductase, xanthine oxidase and Taq DNA polymerase [7, 20, 21], revealing their potential prospect for further research and application. In the present report, *A. niger* EN-13 showed high structural diversity of naphtho- $\gamma$ -pyrone

derivatives.

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