Phytochemistry 70 (2009) 554–557

Contents lists available at ScienceDirect

Phytochemistry

journal homepage: www.elsevier.com/locate/phytochem

Pyrone derivatives from the marine-derived fungus *Nigrospora* sp. PSU-F18

Kongkiat Trisuwan [a], Vatcharin Rukachaisirikul [a,*], Yaowapa Sukpondma [a], Sita Preedanon [b], Souwalak Phongpaichit [b], Jariya Sakayaroj [c]

[a] Department of Chemistry and Center of Excellence for Innovation in Chemistry, Faculty of Science, Prince of Songkla University, Hat Yai, Songkhla 90112, Thailand
[b] Natural Products Research Center and Department of Microbiology, Faculty of Science, Prince of Songkla University, Hat Yai, Songkhla 90112, Thailand
[c] National Center for Genetic Engineering and Biotechnology (BIOTEC), Thailand Science Park, Klong Luang, Pathumthani 12120, Thailand

ARTICLE INFO

Article history:
Received 30 September 2008
Received in revised form 29 November 2008
Available online 23 February 2009

Keywords:
Nigrospora sp.
Marine-derived fungus
Pyrones
Antibacterial activity

ABSTRACT

Pyrones, named nigrosporapyrones A–D (**1–4**), and five known compounds were isolated from the marine-derived fungus *Nigrospora* PSU-F18. Their structures were elucidated on the basis of spectroscopic evidence. The antibacterial activity against the standard *Staphylococcus aureus* ATCC 25923 and methicillin-resistant *S. aureus* was evaluated.

1. Introduction

The genus *Nigrospora* has been recognized as plant endophyte and also as a rich source of biologically active secondary metabolites, including herbicidal lactones (Fukushima et al., 1998), antibacterial nigrosporins (Tanaka et al., 1997), phytoxin epoxyexserohilone (Cutler et al., 1991) and antibiotic griseofulvin (Furuya et al., 1967). We recently reported the isolation and the antibacterial activity of metabolites isolated from the first marine-derived fungus *Nigrospora* sp. (Trisuwan et al., 2008). We described herein investigation of the second marine-derived fungus *Nigrospora* sp. PSU-F18 of which the ethyl acetate extract from the culture broth exhibited interesting antibacterial activity against standard *Staphylococcus aureus* ATCC 25923 (SA) and a clinical isolate of methicillin-resistant *S. aureus* (MRSA). Investigation of the broth extract led to isolation and structural elucidation of four new pyrones, nigrosporapyrones A–D (**1–4**) together with five known compounds, solanapyrone A (**5**) (Alam et al., 1989), (+)-phomalactone (**6**) (Yang et al., 1997), 5-(*S*)-[1-(1(*S*)-hydroxybut-2-enyl)]-dihydrofuran-2-one (**7**) (Fukushima et al., 1998), musacin F (**8**) (Grabley et al., 1994) and tyrosol (**9**) (Rasser et al., 2000).

2. Results and discussion

The marine-derived fungus *Nigrospora* sp. PSU-F18 was isolated from the sea fan (*Annella* sp.). As neither conidia nor spores were observed, this fungus was identified based on the analyses of the partial large subunit (LSU) and the ribosomal internal transcribed spacer (ITS) regions of their rDNA gene. Its LSU sequence (EU852533) matched with the closely related sequence of *Nigrospora* sp. AY234934 with 100% bootstrap support. Moreover, its ITS sequence (EU714386) was well placed in the major *Nigrospora* clade comprising *Nigrospora* sp. (AM262341, EU714382, EF589888, AM921707, EF564154) and *Nigrospora oryzae* (EU427044, EU529995, EU529994, EU529993, DQ219433, EU196745) with high statistical support (98%) and sequence similarity between 94.7% and 95.8%. The fungus PSU-F18 was then identified as *Nigrospora* sp.

The culture broth of this fungus grown in potato dextrose broth at room temperature for 4 weeks was extracted with EtOAc. The crude extract obtained was subjected to chemical investigation, leading to the isolation of compounds **1–9**. Six of them were pyrone derivatives (**1–6**), including four solanapyrone derivatives (**1–3** and **5**). Their antibacterial activity against SA and MRSA was evaluated.

Nigrosporapyrone A (**1**) was obtained as a colorless gum with the molecular formula $C_{18}H_{22}O_5$ from HREIMS. The UV spectrum displayed maximum absorption bands at λ_{max} 228, 273 and 320 nm while the IR spectrum showed absorption bands for hydroxyl (3390 cm^{-1}), conjugated ester carbonyl (1719 cm^{-1}) and conjugated aldehyde carbonyl (1698 cm^{-1}) groups. The ^1H and ^{13}C NMR spectra (Table 1) displayed characteristic signals for a decalin skeleton with a C3–C4 double bond, a hydroxyl group at C-6 (δ 73.6) and a methyl group attached at C-2 (δ 35.1). The ^1H–^1H COSY correlations supported the presence of the decalin moiety. The following HMBC correlations (Table 2), H_3-16 (δ 0.98, d,

* Corresponding author. Tel.: +66 74 288 435; fax: +66 74 212 918.
E-mail address: vatcharin.r@psu.ac.th (V. Rukachaisirikul).

0031-9422/$ - see front matter © 2009 Elsevier Ltd. All rights reserved.
doi:10.1016/j.phytochem.2009.01.008

Table 1
^{1}H and ^{13}C NMR data of **1–3**.

Position	**1**		**2**		**3**	
	δ_H, mult. (J in Hz)	δ_C, mult.	δ_H, mult. (J in Hz)	δ_C, mult.	δ_H, mult. (J in Hz)	δ_C, mult.
1	2.33, m	49.0, CH	2.24, dd (12.0, 9.9)	48.5, CH	2.29, m	46.8, CH
2	2.65, m	35.1, CH	2.63, m	34.6, CH	2.59, m	34.7, CH
3	5.63, d (9.9)	132.1, CH	5.62, d (10.2)	132.5, CH	5.49, dt (10.0, 1.5)	131.0, CH
4	6.06, ddd (9.9, 5.1, 2.4)	128.5, CH	6.04, ddd (10.2, 4.8, 2.4)	128.3, CH	5.64, m	131.2, CH
5	2.06, m	44.1, CH	2.02, m	44.2, CH	2.59, m	30.4, CH
6	3.50, td (10.2, 4.5)	73.6, CH	3.46, m	73.8, CH	a: 1.81, dm (14.0) b: 1.36, td (14.0, 2.5)	36.1, CH_2
7	a: 2.00, m b: 1.28, m	35.4, CH_2	a: 1.96, m b: 1.30, m	35.4, CH_2	4.10, m	65.6, CH
8	1.40, m	27.9, CH_2	1.46, m	27.9, CH_2	1.55, m	27.7, CH_2
9	a: 1.63, m b: 1.40, m	20.1, CH_2	a: 1.46, m b: 1.32, m	20.1, CH_2	a: 1.93, tm (13.0) b: 1.28, m	21.5, CH_2
10	2.37, m	36.8, CH	2.41, m	36.3, CH	2.29, m	35.3, CH
11		175.5, qC		175.0, qC		175.0, qC
12	6.14, s	95.9, CH	5.97, s	96.1, CH	5.98, s	95.6, CH
13		173.5, qC		171.5, qC		172.5, qC
14		102.0, qC		94.9, qC		94.9, qC
15		162.2, qC		160.5, qC		160.6, qC
16	0.98, d (7.2)	20.2, CH_3	0.98, d (6.9)	20.2, CH_3	0.97, d (7.0)	20.3, CH_3
17	10.15, s	186.6, CH	9.98, s	191.3, CH	10.00, s	191.4, CH
18	4.08, s	57.8, CH_3	3.54, q (5.4)	44.9, CH_2	3.52, q (5.5)	44.8, CH_2
18-NH			10.85, brs		10.87, brt (1.5)	
19			3.89, t (5.4)	61.1, CH_2	3.90, t (5.5)	61.2, CH_2

Table 2
Selected HMBC correlations of **1–3**.

Position	**1**	**2**	**3**
H-1	C-5, C-11, C-12, C-16	C-2, C-5, C-10, C-11, C-12, C-16	C-10, C-11, C-16
H-2	C-3, C-16		C-1
H-3	C-1, C-2, C-5, C-16	C-1, C-2, C-5, C-16	C-1, C-2, C-5, C-16
H-4	C-2, C-5, C-10	C-2, C-5, C-10	C-3
H-5	C-1, C-3, C-4, C-6, C-10	C-1, C-3, C-4, C-6, C-10	C-1
H-6	C-4, C-8		C-5
H-7	C-5, C-6, C-8, C-9	C-6, C-8, C-9	
H-8	C-6, C-10	C-7, C-9	
H-9	C-5, C-8, C-10	C-7, C-8	C-1
H-10	C-2, C-5, C-8	C-1, C-5	C-1
H-12	C-1, C-11, C-13, C-14, C-17	C-1, C-11, C-13, C-14, C-17	C-1, C-13, C-14
H-16	C-1, C-2, C-3	C-1, C-2, C-3	C-1, C-2, C-3
H-17	C-13, C-14, C-15	C-12, C-14, C-15	C-13, C-14, C-15
H-18	C-13	C-13, C-19	C-19
H-19		C-18	C-18

J = 7.2 Hz)/C-1 (δ 49.0), C-2 and C-3 (δ 132.1); H-6 (δ 3.50, td, J = 10.2 and 4.5 Hz)/C-4 (δ 128.5), supported the assigned location of the double bond, hydroxyl and methyl groups. In addition, the ^{1}H and ^{13}C NMR spectra of **1** established the presence of a pyrone ring with methoxyl and the formyl groups at C-13 (δ 173.5) and C-14 (δ 102.0), respectively. The presence of this unit was confirmed by the chemical shifts and following HMBC correlations: H-12 (δ 6.14, s)/C-11 (δ 175.5), C-13 and C-14; H-17 (δ 10.15, s)/C-13, C-14 and C-15 (δ 162.2); H_3-18 (δ 4.08, s)/C-13. The linkage between C-1 of the decalin unit and C-11 of the pyrone ring was established according to the HMBC correlations of H-1 (δ 2.33, m) of the decalin unit with C-11 and C-12 (δ 95.9) of the pyrone ring. Consequently, nigrosporapyrone A had the structure **1** (Fig. 1) that differed from **5** in the presence of a hydroxyl group attached at C-6. The relative configuration was established by the following NOEDIFF results (Fig. 2). Irradiation of H-2 (δ 2.65, m) affected H-10 (δ 2.37, m) and H-12, indicating their location at the same side of the molecule and pseudoaxial orientations for H-2 and H-10. When H-5 was irradiated, the signal intensity of H-10 was enhanced, thus suggesting a *cis* ring fusion of the decalin moiety. The large coupling constant (10.2 Hz) observed between H-5 and H-6 established axial orientations for both protons. These data permitted assignment of the relative configuration for **1** at all chiral centers except for C-6 identical to that of **5**.

Nigrosporapyrone B (**2**) with the molecular formula $C_{19}H_{25}NO_5$ from HREIMS was obtained as a colorless gum. The UV and IR spectra were almost identical to those of **1**. Its ^{1}H NMR spectrum (Table 1) was similar to that of **1** except for the replacement of the methoxyl group in **1** with signals for an aminohydroxyethyl moiety [δ 10.85 (br s, 18-NH), 3.89 (t, J = 5.4 Hz, H_2-19) and 3.54 (q, J = 5.4 Hz, H_2-18)]. The attachment of this unit at C-13 (δ 171.5) was confirmed by a HMBC correlation of H_2-18 with C-13 (Table 2). Consequently, nigrosporapyrone B (**2**) was identified as an aminoalcohol derivative of **1**.

Nigrosporapyrone C (**3**) was obtained as a colorless gum with the molecular formula identical to that of **2**. Its UV, IR and ^{1}H NMR spectra were almost identical to those of **2**. Furthermore, compounds **2** and **3** consisted of the same number and types of carbons. The differences were found in the ^{1}H–^{1}H COSY spectrum. A hydroxymethine proton (δ 4.10, m, H-7) was correlated with H_2-6 [δ 1.81 (dm, J = 14.0 Hz, H_a-6) and 1.36 (td, J = 14.0 and 2.5 Hz, H_b-6)] and H_2-8 (δ 1.55, m), but not with H-5 as found in **2**. These

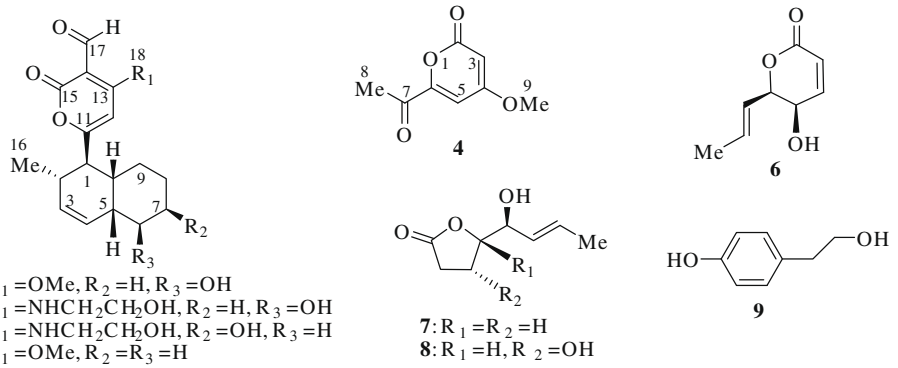

Fig. 1. Structures of compounds **1–9**.

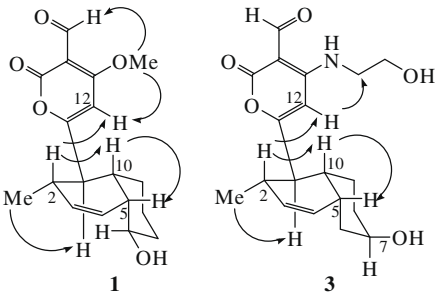

Fig. 2. Selected NOEDIFF results of compounds **1** and **3**.

data established the attachment of the hydroxymethine proton at C-7 (δ 65.6), not at C-6. Irradiation of H-5 (δ 2.59, m) affected signal intensity of H-10 (δ 2.29, m), but not H-7, in the NOEDIFF experiment (Fig. 2), established a *cis* ring fusion of the decalin skeleton and the presence of 7-OH at an axial orientation. The appearance of H-5 at much lower field and that of C-5 at much higher field than those observed in compounds **1** and **2** due to Van der Waals repulsion (a 1,3-diaxial interaction) supported the assigned location of 7-OH. Thus, nigrosporapyrone C (**3**) differed from **2** in the location of the hydroxyl group in the decalin skeleton.

Nigrosporapyrone D (**4**) with the molecular formula $C_8H_8O_4$ from HREIMS was obtained as a colorless gum. It UV spectrum with maximum absorption bands at λ_{max} 229, 254 and 304 nm indicated that **4** had a conjugated pyrone chromophore (Lee et al., 1995). The IR spectrum showed absorption bands for a conjugated ester carbonyl (1706 cm^{-1}) and a conjugated ketone carbonyl (1698 cm^{-1}) groups. Its ^1H NMR spectrum was similar to that of pestalopyrone (Lee et al., 1995) except that signals for a 1-methyl-1-propenyl unit of pestalopyrone were replaced with a methyl signal of an acetyl group (δ 2.53, s, H_3-8). The presence of the acetyl substituent was supported by signals of ketone carbonyl and methyl carbons at δ 192.0 and 26.1, respectively, in the ^{13}C NMR spectrum. The attachment of this unit at C-6 (δ 154.5) was confirmed by a HMBC correlation of H_3-8 with C-6. Consequently, nigrosporapyrone D (**4**) was assigned as a new pyrone derivative.

All isolated compounds, except for compounds **2**, **3** and **9** which were not obtained in sufficient amount, were tested for antibacterial activity against SA and MRSA. Among them, **1** exhibited the best activity with a MIC value of 128 μg/mL, while the others were inactive with MIC values of >128 μg/mL against both strains. This is the second report on the antibacterial activity against SA of solanapyrone analogues as solanapyrones J and K isolated from an unidentified fungus have recently been found to show this activity (Schmidt et al., 2007).

3. Concluding remarks

Analogues of the solanapyrones have been previously isolated from the fungus *Alternaria solani* (Ichihara et al., 1983 and Oikawa et al., 1998), *Ascochyta rabiei* (Alam et al., 1989) and two unidentified fungi (Jenkins et al., 1998 and Schmidt et al., 2007). Thus, this is the first report on the isolation of solanapyrone derivatives from the fungus *Nigrospora sp*. Biosynthetic investigations previously established that the carbon skeleton was constructed *via* a polyketide pathway (Oikawa et al. 1989). In addition, the formation of the decalin skeleton involved an intramolecular Diels-Alder reaction of a polyketide-derived pyrone (Oikawa et al., 1994 and 1995). The replacement of the methoxyl group with the 2-aminohydroxyethyl group would then occur at the later stage (Oikawa et al., 1998).

4. Experimental

4.1. General

Optical rotations were measured on a JASCO P-1020 polarimeter. Infrared spectra (IR) were recorded on a Perkin–Elmer 783 FTS 165 FT-IR spectrometer. Ultraviolet (UV) absorption spectra were measured in MeOH on a Shimadzu UV-160A spectrophotometer. ^1H and ^{13}C NMR spectra were recorded in $CDCl_3$ on a 300 MHz Bruker FTNMR Ultra Shield spectrometer. Mass spectra were obtained on a MAT 95 XL mass spectrometer (Thermofinnigan). Thin-layer chromatography (TLC) and precoated TLC (PTLC) were performed on silica gel GF_{256} (Merck). Column chromatography (CC) was carried out on silica gel (Merck) type 100 (70–230 Mesh ASTM) with a gradient system of EtOAc-light petroleum, on Sephadex LH-20 with MeOH or on reversed phase silica gel C-18 with a gradient system of MeOH-H_2O.

4.2. Fungal material

The marine-derived fungus PSU-F18 was isolated from sea fan (*Annella* sp.) collected near Similan Islands, Thailand, in 2006. This fungus was deposited as *Nigrospora* sp. PSU-F18 (GenBank accession numbers EU852533 and EU714386) at the Department of Microbiology, Faculty of Science, Prince of Songkla University and the National Center for Genetic Engineering and Biotechnology (BIOTEC) Culture Collection, Thailand (BCC number 28630).

4.3. Fermentation and isolation

The marine-derived fungus *Nigrospora* sp. PSU-F18 was grown on potato dextrose agar at 25 °C for 5 days. Three pieces (0.5×0.5 cm^2) of mycelial agar plugs were inoculated into 500 mL Erlenmeyer flasks containing 300 mL of potato dextrose

broth at room temperature for 4 weeks. The culture (15 L) was filtered to give the filtrate and mycelia. The filtrate (5 × 3 L) was extracted three times with EtOAc (3 × 1 L) to afford a broth extract (4.4 g) as a brown gum after evaporation to dryness under reduced pressure. The crude extract was fractionated by CC over Sephadex LH-20 to give three fractions (A–C). Fraction B (4.0 g) was purified by silica gel CC to give eight fractions (B1–B8). Fraction B2 (13.0 mg) contained **4** (2.5 mg). Fraction B4 (420.9 mg) was separated by CC over silica gel to afford four fractions. The second and third fractions contained **5** (10.7 mg) and **7** (240.9 mg), respectively. Fraction B5 (32.0 mg) was further separated by CC over Sephadex LH-20 to give **1** (8.0 mg) and **6** (8.3 mg). Fraction B6 (53.4 mg) was subjected to CC over reversed phase silica gel to yield three fractions. The second fraction (35.2 mg) upon purification on CC over reversed phase silica gel gave **2** (1.8 mg), **8** (2.8 mg) and **9** (1.3 mg). Fraction B7 (43.0 mg) was purified by CC over reverse phase silica gel to give **3** (1.8 mg).

4.4. Nigrosporapyrone A (*1*)

Colorless gum; $[\alpha]_D^{29}$ −254 (*c* 0.10, $CHCl_3$); UV λ_{max}^{MeOH} nm ($\log\varepsilon$): 228 (3.75), 273 (3.84), 320 (2.65); FT-IR ν_{max}^{neat} cm^{-1}: 3390, 1719, 1698; For ^1H NMR and ^{13}C NMR ($CDCl_3$) spectra, see Table 1; EIMS *m/z* (% relative intensity): 318 (9), 290 (100), 153 (97), 71 (62); HREIMS *m/z* 318.1463 $[M]^+$ (calcd for $C_{18}H_{22}O_5$ 318.1467).

4.5. Nigrosporapyrone B (*2*)

Colorless gum; $[\alpha]_D^{29}$ −232 (*c* 0.10, $CHCl_3$); UV λ_{max}^{MeOH} nm ($\log\varepsilon$): 237 (4.43), 280 (4.10), 316 (4.02); FT-IR ν_{max}^{neat} cm^{-1}: 3390, 1719, 1692; For ^1H NMR and ^{13}C NMR ($CDCl_3$) spectra, see Table 1; EIMS *m/z* (% relative intensity): 347 (59), 257 (37), 110 (100), 105 (11); HREIMS *m/z* 347.1731 $[M]^+$ (calcd for $C_{19}H_{25}NO_5$, 347.1733).

4.6. Nigrosporapyrone C (*3*)

Colorless gum; $[\alpha]_D^{29}$ −202 (*c* 0.10, $CHCl_3$); UV λ_{max}^{MeOH} nm ($\log\varepsilon$): 235 (3.85), 281 (3.41), 314 (3.42); FT-IR ν_{max}^{neat} cm^{-1}: 3395, 1706, 1689; For ^1H NMR and ^{13}C NMR ($CDCl_3$) spectra, see Table 1; EIMS *m/z* (% relative intensity): 347 (24), 257 (35), 110 (100), 105 (31); HREIMS *m/z* 347.1735 $[M]^+$ (calcd for $C_{19}H_{25}NO_5$, 347.1733).

4.7. Nigrosporapyrone D (*4*)

Colorless gum; UV λ_{max}^{MeOH} nm ($\log\varepsilon$): 229 (3.19), 254 (2.32), 304 (2.63); FT-IR ν_{max}^{neat} cm^{-1}: 1706, 1698; ^1H NMR ($CDCl_3$, 300 MHz); δ 6.67 (1H, *d*, *J* = 2.4 Hz, H-5), 5.72 (1H, *d*, *J* = 2.4 Hz, H-3), 3.87 (3H, *s*, H-9), 2.53 (3H, *s*, H-8), ^{13}C NMR ($CDCl_3$, 75 MHz); δ 192.0 (C, C-7), 169.8 (C, C-4), 161.5 (C, C-2), 154.5 (C, C-6), 103.9 (CH, C-5), 93.2 (CH, C-2), 56.0 (CH_3, C-9), 26.1 (CH_3, C-8); EIMS *m/z* (% relative intensity): 168 (77), 125 (100), 69 (90); HREIMS *m/z* 168.0421 $[M]^+$ (calcd for $C_8H_8O_4$, 168.0423).

4.8. Antibacterial activity testing

MICs were determined by the agar microdilution method (Lorian, 1996). The test substances were dissolved in DMSO (Merck, Germany). Serial 2-fold dilutions of the test substances were mixed with melted Mueller-Hinton agar (Difco) in the ratio of 1:100 in microtiter plates with flat-bottomed wells (Nunc, Germany). Final concentration of the test substances in agar ranged from 0.03 to 128 μg/mL. SA and MRSA were used as test strain. Inoculum suspensions (10 μL) were spotted on agar-filled wells. The inoculated plates were incubated at 35 °C for 18 h. MICs were recorded by reading the lowest substance concentration that inhibited visible growth. Vancomycin, a positive control drug, exhibited a MIC value of 1 μg/mL. Growth controls were performed on the agar containing DMSO.

Acknowledgments

V. Rukachaisirikul thanks the Commission on Higher Education and the Thailand Research Fund for the TRF Senior Research Scholar (Grant No. RTA5180007). The Center of Excellence for Innovation in Chemistry (PERCH-CIC) and Prince of Songkla University, are gratefully acknowledged for partial support. Finally, K. Trisuwan thanks the Royal Golden Jubilee Ph. D. Program (Grant No. PHD/0109/2550) of the Thailand Research Fund for a scholarship.

References

Alam, S.S., Bilton, J.N., Slawin, A.M.Z., Williams, D.J., Sheppard, R.N., Strange, R.N., 1989. Chickpea blight: production of the phytotoxins solanapyrones A and C by *Ascochyta rabiei*. Phytochemistry 28, 2627–2630.

Cutler, H.G., Hoogsteen, K., Littrell, R.H., Arison, B.H., 1991. Epoxyexserohilone, a novel metabolite from *Nigrospora sphaerica*. Agric. Biol. Chem. 55, 2037–2042.

Fukushima, T., Tanaka, M., Gohbara, M., Fujimori, T., 1998. Phytotoxicity of three lactones from *Nigrospora sacchari*. Phytochemistry 48, 625–630.

Furuya, K., Enokita, R., Shirasaka, M., 1967. Antibiotics from fungi. II: new griseofulvin producer, *Nigrospora oryzae*. Ann. Sankyo Res. Lab. 19, 91–95.

Grabley, S., Thiericke, R., Wink, J., Helsberg, M., Schmidt, K., Burkhardt, K., Zeeck, A., Schneider, A., Fiedler, H-P., 1994. DE Patent 43 03 684 A1.

Ichihara, A., Tazaki, H., Sakamura, S., 1983. Solanapyrones A, B and C, phytotoxic metabolites from the fungus *Alternaria solani*. Tetrahedron Lett. 24, 5373–5376.

Jenkins, K.M., Toske, S.G., Jensen, P.R., Fenical, W., 1998. Solanapyrones E–G, antialgal metabolites produced by a marine fungus. Phytochemistry 49, 2299–2304.

Lee, J.C., Yang, X., Schwartz, M., Strobel, G., Clardy, J., 1995. The relationship between an endangered North American tree and an endophytic fungus. Chem. Biol. 2, 721–727.

Lorian, V., 1996. Antibiotics in Laboratory Medicine, 4th ed. William and Wilkins, Baltimore. pp. 28–32.

Oikawa, H., Yokota, T., Abe, T., Ichihara, A., Sakamura, S., Yoshizawa, Y., Vederas, J.C., 1989. Biosynthesis of solanapyrone A, a phytotoxin of *Alternaria solani*. J. Chem. Soc. Chem. Commun., 1282–1284.

Oikawa, H., Suzuki, Y., Naya, A., Katayama, K., Ichihara, A., 1994. First direct evidence in biological Diels-Alder reaction of incorporation of diene-dienophile precursors in biosynthesis of solanapyrones. J. Am. Chem. Soc. 116, 3605–3606.

Oikawa, H., Katayama, K., Suzuki, Y., Ichihara, A., 1995. Enzymatic activity catalyzing *exo*-selective Diels-Alder reaction in solanapyrone biosynthesis. J. Chem. Soc. Chem. Commun., 1321–1322.

Oikawa, H., Yokota, T., Sakano, C., Suzuki, Y., Naya, A., Ichihara, A., 1998. Solanapyrones, phytotoxins produced by *Alternaria solani*: biosynthesis and isolation of minor components. Biosci. Biotechnol. Biochem. 62, 2016–2022.

Rasser, F., Anke, T., Sterner, O., 2000. Secondary metabolites from a *Gloeophyllum* sp. Phytochemistry 54, 511–516.

Schmidt, L.E., Gloer, J.B., Wicklow, D.T., 2007. Solanapyrone analogues from a Hawaiian fungicolous fungus. J. Nat. Prod. 70, 1317–1320.

Tanaka, M., Fukushima, T., Tsujino, Y., Fujimori, T., 1997. Nigrosporins A and B, new phytotoxic and antibacterial metabolites produced by a fungus *Nigrospora oryzae*. Biosci. Biotechnol. Biochem. 61, 1848–1852.

Trisuwan, K., Rukachaisirikul, V., Sukpondma, Y., Preedanon, S., Phongpaichit, S., Rungjindamai, N., Sakayaroj, J., 2008. Epoxydons and a pyrone from the marine-derived fungus *Nigrospora* sp. PSU-F5. J. Nat. Prod. 71, 1323–1326.

Yang, Z.-C., Jiang, X.-B., Wang, Z.-M., Zhou, W.-S., 1997. Total synthesis of (+)-asperlin, (+)-acetylphomalactone and (5S,6S,7R,8S)-asperlin based on the kinetic resolution of 2-furylmethanols. J. Chem. Soc., Perkin Trans. 1, 317–321.