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New isocoumarins, naphthoquinones, and a cleistanthane-type diterpene from *Nectria pseudotrichia* 120-1NP

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ABSTRACT

Four new compounds, namely, nectriapyrones A (**2**) and B (**3**), nectriaquinone B (**5**), and zythiostromic acid C (**8**), were isolated from the brown rice culture of *Nectria pseudotrichia* 120-1NP together with four known compounds (**1**, **4**, **6**, and **7**). To the best of our knowledge, this is the first report of **4** from a natural source. Their structures were determined on the basis of 1D/2D-NMR spectroscopy and HRESITOFMS data. In addition, the absolute configuration of secondary alcohols in **8** were determined using modified Mosher's ester method. All isolated compounds were evaluated for their antimicrobials activity, phytotoxicity, and cytotoxicity.

1. Introduction

Endophytes have been attracting much attention in the field of natural products chemistry because of their sustainability to biosynthesized diverse structure and bioactive molecules [1,2]. Most plants in natural ecosystems live in symbiosis with fungal endophytes, which include species with various symbiotic and ecological functions that can greatly affect the environmental adaptation of their host [3]. In particular, Indonesia houses biodiversity hotspots and vast rainforest, which offer a wide plant and fungal diversity [4]. This biological diversity is reflected in a structural diversity representing a promising source of new bioactive compounds. We have previously reported that the fungal strain *N. pseudotrichia* 120-1NP, isolated from an Indonesian plant, produce cytotoxic compounds named nectrianolins A-C [5].

In our continuing search for fungal metabolites, we reinvestigated the EtOAc extract of this strain, leading to the identification of two new isocoumarins, namely, nectriapyrones A (**2**) and B (**3**), one new naphthoquinones, namely, nectriaquinone B (**5**), and the new cleistanthane-type diterpene, zythiostromic acid C (**8**), along with four known compounds, 3,4-dimethyl-6,8-dihydroxyisocoumarin (**1**), nectriaquinone A (**4**), herbarin (**6**) and *O*-methylherbarin (**7**). Herein, we report the isolation, structure determination, and biological activities of these compounds.

2. Experimental

2.1. General experimental procedures

Column chromatography were conducted on silica gel 60 (*Kanto Chemical Co.*, Inc., Japan) and ODS (*Fuji Silysia*, Japan). Flash Chromatography was conducted using Büchi Flash Chromatography C-601 (*Büchi,* Switzerland) and packed column Biotage® SNAP Ultra (*Biotage*, Sweden) 10 g (25 μm). Thin layer chromatography (TLC) was carried out on precoated silica gel 60 F_{254} plates (*Merck*, Germany), and spot were detected by spraying with 1% vanillin in H_2SO_4 followed by heating, or by UV irradiation. Melting points were measured using a Micro Melting Point Apparatus Yanaco 2300 (*Yanagimoto,* Japan). Optical rotations were measured using a Horiba SEPA-300 polarimeter (*Horiba,* Japan). IR and UV–vis spectra were respectively recorded with Horiba FT710 (*Horiba*, Japan) and Shimadzu UV-1800 spectrometer (*Shimadzu*, Japan). Mass spectra were obtained with a Synapt G2 (*Water Corporation*, USA) and JEOL HX110 mass spectrometer (*JEOL*, Japan). NMR data were recorded on JEOL ECZ-600 spectrometer (*JEOL*, Japan) at 600 MHz for ^{1}H and 150 MHz for ^{13}C. Chemical shift are given on a δ (ppm) scale with TMS as an internal standard. ^{1}H, ^{13}C, DEPT, COSY, HMQC, and HMBC spectra were recorded using standard JEOL pulse sequences.

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2.2. Fungal material and fermentation

The fungal strain 120-1NP was isolated from healthy stem of *Gliricidia sepium* collected in the Wanagama forest of Universitas Gadjah Mada (southern latitude 7°53′37″, east longitude 110°32′49″), Yogyakarta, Indonesia. This strain was identified as *N. pseudotrichia* using DNA analysis of the 18S rRNA regions and was deposited in GenBank: LC317048. This fungus was deposited at our laboratory in the Faculty of Agriculture, Yamagata University. *N. pseudotrichia* 120-1NP was cultivated on sterilized brown rice (3660 g) at 25 °C for four weeks.

2.3. Extraction and isolation

The moldy media were extracted using MeOH, and the MeOH extract was concentrated. The resulting aqueous concentrate was partitioned into EtOAc. The purification of the EtOAc layer was guided by intense characteristic coloration with vanillin-sulfuric acid solution on TLC plates. The EtOAc layer (19.74 g) was chromatographed on a silica gel column using 10% stepwise of *n*-hexane-EtOAc (100:0–0:100, each 300 mL), then a mixture of EtOAc-MeOH (50:50, 300 mL), and finally MeOH (300 mL) to give 13 fractions (Fr. 1–1 to 1–13). Fraction 1–5 (655 mg) was further chromatographed on silica gel column using 10% stepwise of $CHCl_3$-EtOAc (100:0–0:100, each 100 mL) to afford 11 fractions (Fr. 1-5-1 to 1-5-11). Fraction 1-5-3 which was contained amorphous powder was filtered and the residue washed using MeOH to yield **3** (37 mg). In each 1–6 and 1–7 fractions contained amorphous powder which then was filtered and the residues washed using MeOH to obtained **1** (15 mg) and **2** (28 mg), respectively. Fraction 1–8 to 1–10 were combined (4.32 g) and was chromatographed on silica gel using 10% stepwise of $CHCl_3$-EtOAc (100:0–0:100, each 200 mL) to afford 11 fractions (Fr. 1-8-1 to 1-8-11). Fraction 1-8-5 to 1-8-7 were combined (503 mg) and was subjected on ODS using 10% stepwise of H_2O and MeOH (100:0–0:100, each 50 mL) to afford 11 fractions (1-8-5-1 to 1-8-5-11). Fraction 1-8-5-5 to 1-8-5-7 were combined (184 mg) and then was subjected to flash chromatography using eluent hexane-EtOAc (1:1) to afford **4** (19 mg), **5** (59 mg), **6** (10 mg), and **7** (5 mg). Fraction 1-8-8 (41 mg) was purified using ODS column with eluent system H_2O-MeOH (3,7) to afford **8** (28 mg).

2.3.1. Nectriapyrone A (2)

6,8-Dihydroxy-3,4,7-trimethylisocoumarin: white amorphous powder; mp 195–198 °C; UV (MeOH) λ_{max} (log ε) 328 (3.4), 277 (3.5) nm; IR (KBr) ν_{max} 3436, 1635, 1396, 1018 cm^{-1}; ^1H NMR (600 MHz, pyridine-d_5) and ^{13}C NMR (150 MHz, pyridine-d_5) data, see Table 1; HRESITOFMS (positive-ion mode) *m/z* 243.0628 $([M + Na]^+$, calcd for $C_{12}H_{12}NaO_4$, 243.0633).

Table 1
^1H NMR (600 MHz) and ^{13}C NMR (150 MHz) spectroscopic data for **2** and **3**.

Pos.	**2** (pyridine-d_5)		**3** ($CDCl_3$)	
	δ_C, type	δ_H, mult. (*J* in Hz)	δ_C, type	δ_H, mult. (*J* in Hz)
1	166.8, C		166.5, C	
3	149.0, C		149.3, C	
4	108.7, C		108.7, C	
4a	138.0, C		138.2, C	
5	100.1, CH	6.64, s	94.6, CH	6.34, s
6	165.1, C		164.6, C	
7	110.8, C		111.9, C	
8	161.9, C		160.4, C	
8a	99.0, C		100.1, C	
4-Me	11.9, CH_3	1.82, s	12.6, CH_3	2.11, s
3-Me	16.7, CH_3	2.02, s	17.3, CH_3	2.29, s
7-Me	8.5, CH_3	2.53, s	7.9, CH_3	2.14, s
6-OMe			55.8, O-CH_3	3.93, s

Table 2
^1H NMR (600 MHz) and ^{13}C NMR (150 MHz) spectroscopic data for **4** and **5**.

Pos.	**4** ($CDCl_3$)		**5** ($CDCl_3$)	
	δ_C, type	δ_H, mult. (*J* in Hz)	δ_C, type	δ_H, mult. (*J* in Hz)
1	182.6, C		183.2, C	
2	147.7, C		147.5, C	
3	137.4, C		141.2, C	
4	184.1, C		185.6, C	
4a	135.4, C		135.7, C	
5	102.8, CH	7.22, d (2.1)	103.1, CH	7.02, d (2.1)
6	164.1, C		164.2, C	
7	103.8, CH	6.70, d (2.8)	103.5, CH	6.49, d (2.1)
8	161.5, C		161.4, C	
8a	114.4, C		114.2, C	
1′	41.4, CH_2	3.73, s	36.9, CH_2	a 2.68, dd (13.2, 8.4); b 2.72, dd (13.2, 4.2)
2′	203.5, C		67.5, CH	4.04, m
3′	29.9, CH_3	2.28, s	23.9, CH_3	1.27, d (6.2)
4′	13.3, CH_3	2.08, s	13.6, CH_3	2.10, s
8-OMe	55.6, O-CH_3	3.92, s	55.2, O-CH_3	3.82, s
6-OMe	56.2, O-CH_3	3.95, s	56.3, O-CH_3	3.84, s

2.3.2. Nectriapyrone B (3)

*8-Hydroxy-6-*methoxy-*3,4,7-trimethylisocoumarin*: white amorphous powder; mp 172–175 °C; UV (MeOH) λ_{max} (log ε) 333 (3.8), 279 (3.9) nm; IR (KBr) ν_{max} 3421, 1646, 1137, 1018 cm^{-1}; ^1H NMR (600 MHz, $CDCl_3$) and ^{13}C NMR (150 MHz, $CDCl_3$) data, see Table 1; HRESITOFMS (positive-ion mode) *m/z* 257.0784 $([M + Na]^+$, calcd for $C_{13}H_{14}NaO_4$, 257.0789).

2.3.3. Nectriaquinone A (4)

3-(2-Oxopropyl)-6,8-dimethoxy-2-methyl-1,4-napthoquinone: yellow amorphous powder; mp 161–162 °C; UV (MeOH) λ_{max} (log ε) 404 (3.4), 265 (4.1), 214 (4.1) nm; IR (KBr) ν_{max} 1697, 1650, 1519, 1160 cm^{-1}; ^1H NMR (600 MHz, $CDCl_3$) and ^{13}C NMR (150 MHz, $CDCl_3$) data, see Table 2; HRESITOFMS (positive ion mode) *m/z* 311.0895 $([M + Na]^+$, calcd for $C_{16}H_{17}NaO_5$, 311.0895).

2.3.4. Nectriaquinone B (5)

3-(2-(S)-Hydroxypropyl)-6,8-dimethoxy-2-methyl-1,4-napthoquinone: yellow amorphous powder; mp 132–136 °C; $[\alpha]_D^{20}$ + 110.0 (*c* 0.01, CH_2Cl_2), +72.0 (*c* 0.1, CH_2Cl_2); UV (MeOH) λ_{max} (log ε) 402 (3.2), 266 (4.1), 219 (4.1) nm; IR (KBr) ν_{max} 3421, 1697, 1650, 1519, 1018 cm^{-1}; ^1H NMR (600 MHz, $CDCl_3$) and ^{13}C NMR (150 MHz, $CDCl_3$) data, see Table 2; HRESITOFMS (positive ion mode) *m/z* 313.1056 $([M + Na]^+$, calcd for $C_{16}H_{18}NaO_5$, 313.1052).

2.3.5. Zythiostromic acid C (8)

3α,5α,7β,8β-Tetrahydroxycleistanth-13(17),15-dien-18-oic acid: colorless oil; $[\alpha]_D^{20}$ + 168.0 (*c* 0.1, MeOH); UV (MeOH) λ_{max} (log ε) 211 (4.0) nm; IR (KBr) ν_{max} 3432, 1631, 1396, 1025 cm^{-1}; ^1H NMR (600 MHz, CD_3OD) and ^{13}C NMR (150 MHz, CD_3OD) data, see Table 3; HRESITOFMS (positive ion mode) *m/z* 389.1938 $([M + Na]^+$, calcd for $C_{20}H_{30}NaO_6$, 389.1940).

2.4. Preparation of the MTPA ester derivatives for 8

To **8** (1.0 mg) in dry pyridine was added (*R*)-(−)-α-methoxy-α-(trifluoromethyl)phenylacetyl chloride (MTPACl, 10 μL), the mixture was stirred at room temperature for 24 h. Purification by column chromatography was done on silica gel using *n*-hexane: EtOAc (10% stepwise, each 4 mL) eluent system to afford the mono-(*S*)-MTPA ester (**8a**, 0.7 mg) and di-(*S*)-MTPA ester (**8c**, 0.3 mg). Compound **8** (1.0 mg) was treated with (*S*)-(+)-α-methoxy-α-(trifluoromethyl)phenylacetyl chloride (MTPACl, 10 μL) in the same procedure to afford the mono-