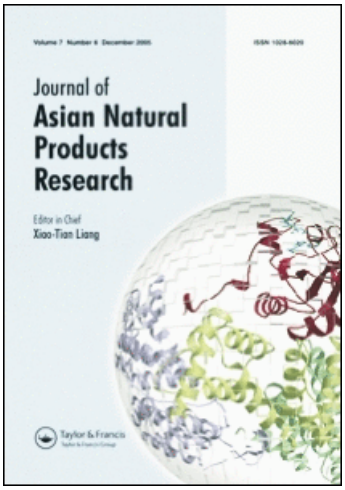

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Five nitro-phenyl compounds from the South China Sea mangrove fungus

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A novel nitro-phenyl glucoside (**1**) was isolated from mangrove endophytic fungus (fungus B60), collected from the Shenzhen mangrove *Acanthus ilicifolius* linn. Four related nitro-phenyl compounds (**2**–**5**) were also obtained, which were isolated for the first time as natural products. Their structures were established on the basis of NMR spectroscopic, mass spectrometric data and some chemical transformations. In the preliminary bioassay, compound **1** had a slight inhibitory effect on α-glucosidase with an IC_{50} of 160.3 μM.

Keywords: Fungus; Nitro-phenyl; α-Glucosidase; Cytotoxicity

1. Introduction

In the search for new bioactive natural products from marine organisms, increasing attention is being given to microorganisms such as bacteria, fungi and actinomycetes. We have embarked on the study of the metabolites of marine fungus and actinomycetes from the South China Sea and obtained several novel and bioactive secondary metabolites [1–5]. In this paper, we report one new and four related known nitro-phenyl compounds. To our knowledge, compounds **2**, **3**, **4** and **5** have not been reported previously as natural products. There were some reports on nitro compounds isolated from different organisms, but aromatic nitro compounds are quite rare in nature [6]. Most nitro compounds showed bioactivities, for example, the nitro aryl compound Aureothin found in the mycelia of several actinomycetes possessed antitumour, antifungal, and pesticidal activities [7]; Lajollamycin, the nitro-tetraene spiro-β-lactone-γ-lactam antibiotic isolated from the marine actinomycete *Streptomyces nodosus*. [8]; and Lobophorin B, the new anti-inflammatory macrolide produced from a marine bacterium [9].

In the preliminary bioassay, compound **1** possessed a slight inhibitory effect on α-glucosidase with an IC_{50} of 160.3 μM, while it was not cytotoxic to the Hep-2 cell line, and did not exhibit antibacterial and antifungal activities in a standard disc assay (100 μg/disc).

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2. Results and discussion

The ethyl acetate extract of a fermentation broth of the fungus (fungus B60) was repeatedly chromatographed on silica gel column using a gradient elution from petroleum to ethyl acetate to obtain compound **1** from the 100% ethyl acetate/petroleum fraction in an isolated yield of 0.2 mg/L. Compound **1** (figure 1) was obtained as an optically active colourless amorphous solid. The UV spectrum of **1** had a maximum absorption at 328 nm ($\log \varepsilon = 3.40$). A molecular formula of $C_{14}H_{19}NO_8$ was determined by HR-EI-MS at *m/z* 352.0986 $[M + Na]^+$ indicating 6 degrees of unsaturation.

The ^{13}C NMR spectrum for **1** (see table 1) revealed 14 carbon signals, attributable to $1 \times CH_3$, $2 \times CH_2$, $8 \times CH$, and $3 \times C$ in the DEPT spectrum. Considering the molecular formula and IR data (3341 cm^{-1}) for **1**, the four remaining protons should be present as hydroxyl group. Peracetylation of **1** with Ac_2O/pyridine gave the tetraacetyl derivative (**1a**), which supported the presence of four OH groups. Initial analysis of NMR data (see table 1) showed signals for the presence of a hexose residue (anomeric carbon δ_H 4.96, d, $J = 4.0$ Hz, δ_C 99.77), an aromatic ABX system [δ_H 8.03 (dd, $J = 8.5$, 2.5 Hz), 8.32 (d, $J = 2.5$ Hz), 7.42 (d, $J = 8.5$ Hz)], and an oxygen-substituted CH_2 (δ_H 4.64 and 4.87, δ_C 67.29). The resulting substructure encompassed all of the required hydrogens and carbons, and six of the oxygens, leaving only one nitrogen atom and two oxygen atoms unaccounted for. Since five out of six

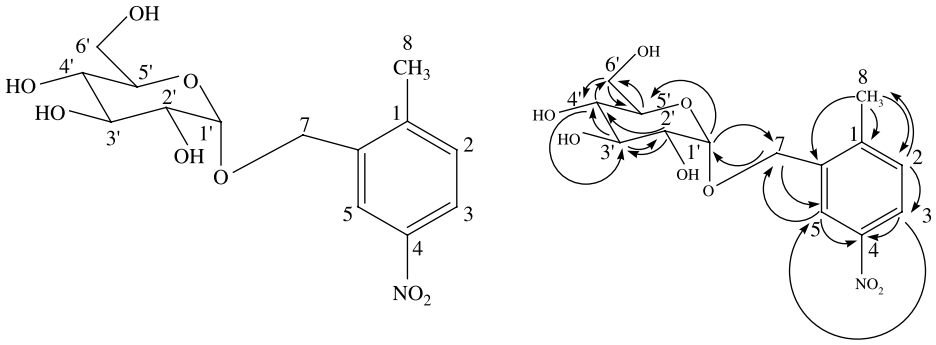

Figure 1. The structure and Key HMBC correlations of **1**.

Table 1. ^1H NMR and ^{13}C NMR data of **1** (δ in ppm, J in Hz).

C no.	*^{13}C NMR (DEPT)*	*^1H NMR*	*0*	*HMBC*
1	145.56			
2	131.79 CH	7.42 (d, 8.5)	H-3, H-8	C-3, C-4, C-6, C-8
3	123.06 CH	8.03 (dd, 8.5, 2.5)	H-2, H-5	C-4, C-5
4	147.33			
5	123.44 CH	8.32 (d, 2.5)	H-3, H-8	C-3, C-4, C-7
6	139.26			
7	67.29 CH_2	4.64 (d, 13.0)	H-8	C-1, C-2, C-5, C-6
		4.87 (d, 13.0)	H-8	
8	19.19 CH_3	2.45 (s)	H-2, H-5, H-7	C-1, C-2, C-6
1′	99.77 CH	4.96 (d, 4.0)	H-2′	C-7, C-5′
2′	73.31 CH	3.53 (dd, 9.5, 4.0)	H-1′, H-3′	C-3′, C-4′
3′	75.02 CH	3.80 (dd, 9.5, 9.5)	H-2′, H-4′	C-2′
4′	71.71 CH	3.45 (dd, 9.5, 9.0)	H-3′, H-5′	C-2′, C-3′, C-5′, C-6′
5′	73.85 CH	3.63 (m)	H-4′, H-6′	C-3′, H-6′
6′	62.65 CH_2	3.70 (dd, 11.5, 5.0)	H-5′	C-4′, C-5′
		3.81 (dd, 11.5, 3.0)	H-5′	

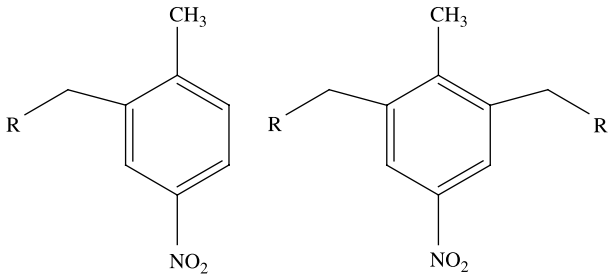

Figure 2. The structure of **2–5**.

unsaturation degrees were accounted for, compound **1** was inferred to contain a nitro group. The presence of the nitro moiety in **1** was further confirmed by IR absorptions characteristic for the $-NO_2$ group (N=O stretching) at 1524.7 cm^{-1} and 1344.7 cm^{-1}.

In the ^{13}C NMR spectrum the carbon resonances at δ 62.65 (CH_2), 73.85 (CH), 71.71 (CH), 75.02 (CH), 73.31 (CH), 99.77 (CH) revealed the presence of a glucosyl group. HMBC correlations for a proton of the hemiacetal (δ 4.96) to C-7 and C-5′ showed that the glucoside unit was attached to C-7. The chemical shift, coupling constant [δ 4.96 (1H, d, $J = 4.0$ Hz, H-1′) in the ^1H NMR spectrum, and the ^{13}C NMR data [δ 99.77 (CH, C-1′ of glc)] indicated an α-configuration for the glucosyl unit [10].

Two nitro compounds **2** and **3** (figure 2) were also isolated from the fungus extract. As far as we know, these two compounds have not been reported previously as natural products. They were only synthetic compounds [11,12].

Interestingly, we also isolated two related nitro-phenyl compounds **4** and **5** (figure 2) from another mangrove fungus (fungus B77), collected from the South China Sea coast. These two compounds also have not been reported previously as natural products. Compound **2** could easily be converted to compound **4** using $SOCl_2$.

In the preliminary bioassay, compounds **1**, **2** and **3** displayed no activity against *Candida albicans* (ATCC 10231), *Fusarium oxysporum*, *Staphylococcus aureus* (ATCC27154) and *Escherichia coli* (ATCC 25922) in a standard disc assays (100 μg/disc). Compounds **1** and **2** were investigated for their activity against the hepG2 cell line and were found to be weakly active. Compound **1** had a little inhibitory effect on α-glucosidase with an IC_{50} of 160.3 μM, while compound **1a** had no inhibitory effect on it.

From the biogenetic point of view, compound **1** may be derived from the related compound **2** by the glucosyl transferase. To date, not much is known about the biosynthesis of nitro aromatic natural products. Recently, studies on the biosynthesis of aureothin have revealed that an unprecedented type of *N*-oxygenase, AurF, is responsible for the oxidation of *p*-aminobenzoate to the corresponding nitro compound [6].

3. Experimental

3.1 General experimental procedures

The ^1H NMR and ^{13}C NMR data were recorded on a Varian INOVA-500 (499.77 and 125.68 MHz) NMR spectrometer or a Varian Mercury-Plus 300 (300.13 and 75.47 MHz)

NMR spectrometer with Me_4Si as the internal standard. Mass spectrum was obtained on a VG-ZABHS mass spectrometer. IR spectrum was measured on a Bruker Vector 22 spectrophotometer. UV spectrum was measured on a Shimadzu UV-2501PC spectrophotometer. Melting point was determined on an X-4 micro-melting point apparatus and is uncorrected. Optical rotation was measured on a Schmidt + Haensch Polartronic HH W5 polarimeter. Chromatography was carried out on silica gel column (200–300 mesh; Qingdao Haiyang Chemicals).

3.2 Fungus material

Two strains of the fungi (no. B60 and no. B77) were all isolated from the Shenzhen mangrove *Acanthus ilicifolius* linn. and were stored at the Department of Applied Chemistry, Zhongshan University, Guangzhou, China. Starter cultures were maintained on cornmeal seawater agar. Plugs of agar supporting mycelia growth were cut and transferred aseptically to a 250-ml Erlenmeyer flask containing 100 ml of liquid medium (glucose 1%, peptone 0.2%, yeast extract 0.1%, NaCl 0.25%). The flask was incubated at 28°C on a rotary shaker for 3 days. The mycelium was aseptically transferred to 500 ml Erlenmeyer flasks containing culture liquid (300 ml). The flasks were then incubated at 30°C with shaking for 15 days.

3.3 Extraction and isolation

The cultures (100 L) were filtered through cheesecloth. The filtrate was concentrated to 2 L below 60°C and extracted several times by shaking with twofold volumes of ethyl acetate. The combined extracts were chromatographed repeatedly on silica gel column using gradient elution from petroleum ether to ethyl acetate to obtain compounds **3**, **2** and **1** from the 10%, 20% and 100% ethyl acetate/petroleum, respectively.

Determination of the yields of the three compounds in the crude extract was limited by the complexity of the mixture, but based on the actual isolated amounts. The three compounds **1**–**3** are produced in yields of approximately 0.2, 10.0 and 2.0 mg/L, respectively.

3.4 Conversion of compound 1 to 1a

Compound **1** (7.0 mg) was dissolved in 1.0 ml of pyridine, 1.0 ml of acetic anhydride was then added, and the solution was allowed to stir at room temperature for 48 h. The solvent and excess reagents were removed with a high-vacuum pump. Purification by preparative TLC gave **1a** (8.5 mg, 80.4% yield): an amorphous solid. $[\alpha]_D^{22}$ + 122.5 (*c* 0.08, acetone). ^{1}H NMR (300 MHz, $CDCl_3$): δ 7.32 (H-2, d, 8.4), 8.07 (H-3, dd, J = 8.1, 2.0), 8.20 (H-5, d, J = 2.0), 4.58 (H-7, d, J = 12.9), 2.43 (H-8, s), 5.13 (H-1, d, J = 3.9), 4.92 (H-2, dd, J = 3.6, 10.2), 5.52 (H-3, dd, J = 9.9, 9.6), 5.09 (H-4, dd, J = 3.9, 9.9), 4.14 (H-5, m), 4.07 (H-6, dd, J = 12.0, 2.4), 4.29 (H-6, dd, J = 12.0, 4.5), 2.02 (CH_3), 2.03 (H_3), 2.09 (CH_3), 2.13 (CH_3). ^{13}C NMR (75 MHz, $CDCl_3$): δ 170.40, 169.97, 169.88, 169.37, 146.37, 144.04, 136.25, 131.09, 123.06, 122.91, 95.22, 70.62, 70.08, 68.51, 67.80, 66.87, 61.92, 20.81, 20.72, 20.65, 20.61, 19.13. UV (acetone): λ_{max}/nm 211 (ε 2.45), 325 (ε 1.43). LC MS: 525 $[M + NH_4]^+$.

3.5 Conversion of compound 2 to compound 4

Compound **2** (10. 0 mg) was dissolved in 1.0 ml of acetone, and 0.5 ml of pyridine, 100 μl of $SOCl_2$ was then added slowly, and the solution was allowed to stir at room temperature for

10 h. Typical work-up yielded one pure product with the following spectral data for **4** (8.2 mg, 73.5% yield): ^{1}H NMR (300 MHz, $CDCl_3$): δ 8.19 (1H), 8.09 (1H), 7.36 (1H), 4.63 (2H), 2.52 (3H).

3.6 Acidic hydrolysis of 1

A solution of **1** (3 mg) and 10% HCl was refluxed for 4 h. The mixture was diluted with H_2O and extracted with $CHCl_3$, and the $CHCl_3$ layer was evaporated to dryness. The residue was recrystallised in ethyl acetate to afford compound **2**. A sample of the aqueous layer was neutralised with 1 N NaOH. The sugar was directly analysed by TLC. D-Glucose was identified on comparison with authentic sample [18].

3.6.1 Compound 1. Amorphous solid. mp 119–121°C; $[\alpha]_D^{22}$ +122.06 (*c* 0.136, acetone) IR (KBr): ν_{max} 3341, 2925, 2893, 1609, 1590, 1524, 1344, 1031, 739, 609 cm^{-1}; UV (Acetone): λ_{max} 211 nm ($\log \varepsilon = 2.62$), 328 ($\log \varepsilon = 3.40$). ^{1}H NMR, ^{13}C NMR, and 2D NMR, see table 1; ESI-MS (negative): *m/z* 328 $[M—H]^-$, 657 $[2M—H]^-$. HRESI-MS: *m/z* 352.0986 $[M + Na]^+$ (calcd for $C_{14}H_{19}NO_8Na$, 352.1008).

3.6.2 Compound 2. Colourless solid. ^{1}H NMR (300 MHz, $CDCl_3$): δ 8.26 (1H), 8.01 (1H), 7.26 (1H), 4.76 (2H), 2.40 (3H). ^{13}C NMR (75 MHz, $CDCl_3$): δ 146.4, 143.3, 140.3, 130.7, 122.2, 121.4, 62.3, 18.9. IR (KBr): ν_{max} 3243.3, 2922.4, 2854.9, 1516.3, 1344.6, 1090.3, 1041.6, 739.7, 636.2 cm^{-1}. EI-MS: *m/z* 167 $[M]^+$.

3.6.3 Compound 3. Colourless solid. ^{1}H NMR (300 MHz, Acetone-d_6): δ 8.23 (2H), 4.76 (4H), 2.28 (3H). ^{13}C NMR (75 MHz, Acetone-d_6): δ 151.6 (C), 147.6 (2C), 145.9 (C), 124.8 (2CH), 66.9 ($2CH_2$), 18.7 (CH_3). IR (KBr): ν_{max} 3267.3, 2910.3, 2854.9, 1516.8, 1524, 1344.1, 1094.2, 745.6, 676.8 cm^{-1}. EI-MS: *m/z* 197 $[M]^+$.

3.6.4 Compound 4. ^{1}H NMR (300 MHz, $CDCl_3$): δ 8.19 (1H), 8.09 (1H), 7.36 (1H), 4.63 (2H), 2.52 (3H). ^{13}C NMR (75 MHz, $CDCl_3$): δ 146.61, 145.12, 137.20, 131.78, 124.77, 123.81, 43.72, 19.48. EI-MS: *m/z* 185 $[M]^+$, 187 $[M + 2]^+$.

3.6.5 Compound 5. ^{1}H NMR (300 MHz, $CDCl_3$): δ 8.19 (2H), 4.67 (4H), 2.56 (3H). ^{13}C NMR (75 MHz, $CDCl_3$): δ 145.70 (1C), 144.19 (C), 138.18 (2CH), 43.75 ($2CH_2$), 15.02 (CH_3). EI-MS: *m/z* 233 $[M]^+$, 235 $[M + 2]^+$, 237 $[M + 4]^+$.

3.7 Bioassays

The antibacterial activities of compounds **1**, **2** and **3** were tested by a usual procedure [13]. The cytotoxicity against Hep-2 cells was evaluated by MTT colorimetric assay [14]. The α-glycosidase activities were tested by the UV method [15–17].

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