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# Bioactive hydroanthraquinones from endophytic fungus *Nigrospora* sp. BCC 47789

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ABSTRACT

A new hydroanthraquinone, nigrosporone A (**1**), and a new naturally occurring nigrosporone B (**2**), together with eleven known compounds, were isolated from an endophytic fungus, *Nigrospora* sp. BCC 47789. The structure of nigrosporone A was confirmed by X-ray crystallographic analysis. The absolute configurations of nigrosporone B were determined by the modified Mosher's method. Nigrosporone B exhibited a broad range of biological activities including antimalarial, antitubercular, antibacterial and cytotoxic activities, while nigrosporone A and Fusaquinon A showed only cytotoxic activity.

## 1. Introduction

Endophytic fungi are of great interest for many researchers due to their ability to produce a variety of bioactive compounds that have potential for use in medicinal and agricultural applications (Kumar et al., 2014; Strobel and Daisy, 2003). Endophytes in the genus *Nigrospora* have been reported to be rich sources of secondary metabolites that show various biological activities, for example, antibacterial nigrosporins (Tanaka et al., 1997) and anthraquinone derivatives (Yang et al., 2012), antifungal griseofulvins (Zhao et al., 2012), plant growth-inhibiting nigrosporides (Harwooda et al., 1995), phomalactones (Kim et al., 2001) and phytotoxic lactones (Fukushima et al., 1998). As part of our research programme on the discovery of bioactive compounds from Thai microorganisms, we came across the crude extract of endophytic fungus, *Nigrospora* sp. BCC 47789, exhibiting antiplasmodial (*Plasmodium falciparum*, K1 strain, $IC_{50}$ 5.82 μg/ml), antimycobacterial (*Mycobacterium tuberculosis* H37Ra, MIC 50 μg/ml) and cytotoxic activities against Vero cells ($IC_{50}$ 9.53 μg/ml). Therefore, the chemical constituents from the crude extract of this fungus were investigated. The study led to the isolation of a new hydroanthraquinone, nigrosporone A (**1**), and a new naturally isolated nigrosporone B (**2**), shown in Fig. 1, along with eleven known compounds. Details regarding isolation, structure elucidation, and biological activities of these compounds are presented herein.

## 2. Results and discussion

Nigrosporone A (**1**), obtained as red needles, has the molecular formula of $C_{15}H_{18}O_6$ as deduced from the molecular ion peak at *m/z* 317.0994 $[M+Na]^+$ by HRESIMS data. The $^1H$, $^{13}C$ and HSQC spectroscopic data revealed the presence of one 1,2,3,4-tetrasubstituted benzene ring, one carbonyl, five hydroxyls, one methyl, two methylenes, four methine groups (including two oxygenated ones), and one oxygenated quaternary carbon. The downfield hydroxyl proton at $\delta_H$ 12.03 indicated H-bonding with a carbonyl, which corresponded to the low absorption frequency of a conjugated carbonyl group at 1640 $cm^{-1}$ in the IR spectrum. The COSY spectrum showed correlations between H-6/H-7 and OH-3/H-3/H-4/H-4a/H-9a/H-1/H-9/OH-9 while the HMBC spectrum showed correlations from H-1 to C-3/C-4a/C-9, H-4 to C-2, H-4a to C-10, H-6 to C-8/C-10a, H-7 to C-5/C-8a, $CH_3$-2 to C-1/C-2, OH-2 to C-2/C-3, OH-3 to C-2/C-3/C-4, OH-5 to C-5/C-6/C-10a, OH-8 to C-7/C-8, and OH-9 to C-8a/C-9/C-9a (Fig. 2). This information suggested the structural similarity of nigrosporone A with a co-metabolite, 9α-hydroxydihydrodesoxybostrycin (Sommart et al., 2008). The only difference between these two compounds was the missing methoxy group at C-7 in nigrosporone A. The *trans*-diaxial relationship of H-4a/H-9a/H-9 was established by the large coupling constants between H-4a and H-9a ($J$ = 12.4 Hz) and H-9a and H-9 ($J$ = 9.8 Hz), respectively. The NOESY correlations between H-4a, H-1$\beta$, H-3, and $CH_3$ indicated that these protons were cofacial (Fig. 2). The large coupling constant

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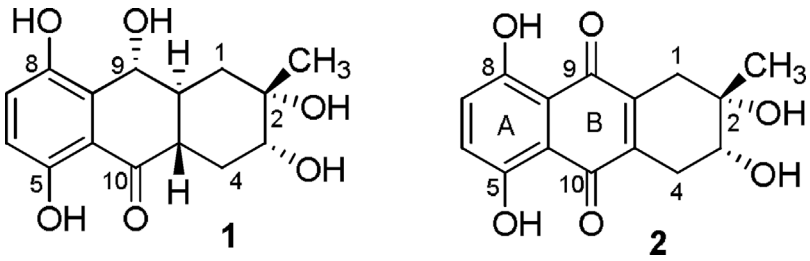


**Fig. 1.** Chemical structures of compounds **1** and **2**.

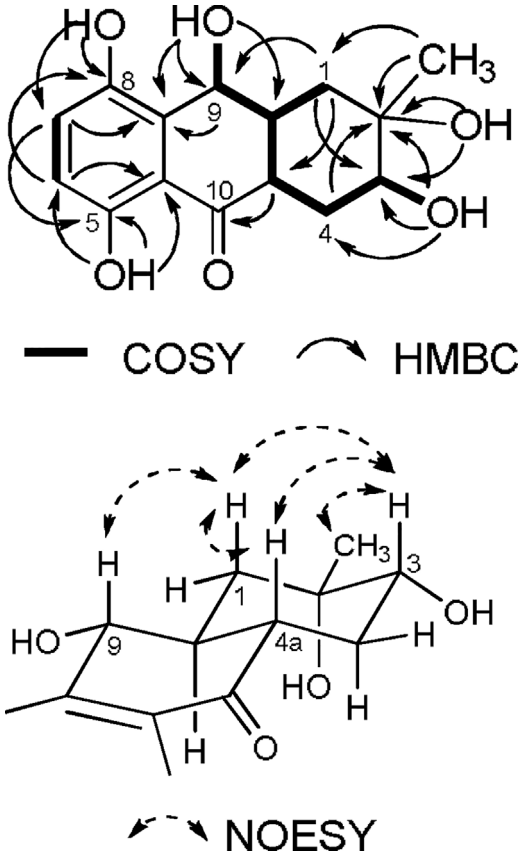


**Fig. 2.** Selected COSY, HMBC, and NOESY correlations of compound **1**.

between H-3 and H-4α ($J$ = 11.7 Hz) supported the axial orientation of H-3. The absolute configurations at C-2, C-3, C-4a, C-9, and C-9a are proposed to be the same as those of the known co-metabolite 9α-hydroxydihydrodesoxybostrycin (Sommart et al., 2008). The structure of nigrosporone A (**1**) was further confirmed by the X-ray diffraction analysis (Fig. 3).

Compound **2** with the molecular formula, $C_{15}H_{14}O_6$, from HRESIMS, showing the molecular ion peak at $m/z$ 313.0697 $[M+Na]^+$ in combination with $^{13}C$ NMR spectroscopy, was obtained as a red solid. The $^1H$ NMR spectrum showed the presence of two aromatic protons, one methine proton, two methylenes, one methyl, two H-bonding aromatic hydroxyls and two aliphatic hydroxyl groups. The similar chemical shifts for C-6/C-7, C-5/C-8, C-8a/C-10a, C-9/C-10, and C-4a/C-9a suggested a symmetrical structural feature in ring AB. The correlation between H-3 and H-4 in the COSY spectrum together with the HMBC correlations from H-1 to C-3/C-4a/C-9/$CH_3$, H-3 to C-4a/$CH_3$, H-4 to C-2/C-9a/C-10, H-6 to C-8/C-10a, H-7 to C-5/C-8a, and $CH_3$ to C-1/C-2/C-3 established the structural features of compound **2**, as depicted in Fig. 1. The large coupling constant between H-4α and H-3 ($J$ = 8.0 Hz) indicated the axial orientation of H-3. The intense NOESY cross-peak between H-3 and 2-$CH_3$ indicated their *cis*-relationship. The absolute configuration was determined by the modified Mosher's method (Ohtani et al., 1991). The Δδ-values of the (*S*)- and (*R*)-MTPA esters, **2a** and **2b** (Fig. 4) indicated 3*R* configuration. Thus, the absolute configuration of **2** was established to be 2*S*,3*R*. Although compound **2** has previously been synthesized (Rösner et al., 1978), it was isolated for the first time from natural source and was named as nigrosporone B.

The eleven known compounds were identified as 9α-hydroxydihydrodesoxybostrycin (Sommart et al., 2008), fusaranthraquinone (Trisuwan et al., 2010), 9α-hydroxyhalorosellinia A (Sommart et al., 2008), nigrosporin B (Tanaka et al., 1997), 4-deoxybostrycin (Noda et al., 1970; Wang et al., 2013), Fusaquinon A (Chen et al., 2008), austrocortinin (Archard et al., 1985), griseofulvin (MacMillan, 1953), dechlorogriseofulvin (Jarvis et al., 1996), 7-hydroxy-3-(2-hydroxypropyl)-5-methyl-isochromen-1-one (Wang et al., 2012), and (3*R*,5*R*)-harzialactone A (Amagata et al., 1998; Mereyala and Gadikota, 1999; Mereyala et al., 2000) by analysis of their spectroscopic data, including NMR and MS, together with the specific rotations, which were identical in all respects to those reported in the literature.

Since the biological activity of nigrosporone B (**2**) and Fusaquinon A have not been reported, nigrosporones A and B (**1** and **2**) and

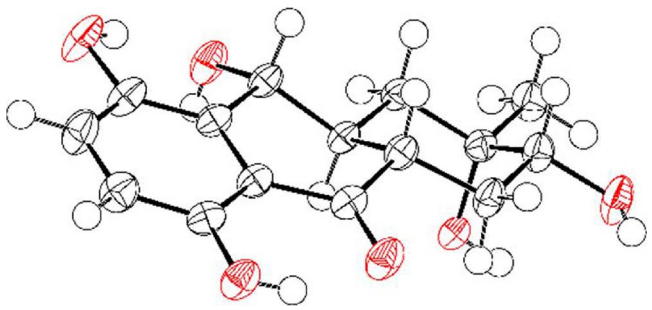

**Fig. 3.** X-ray crystal structure of compound **1**.

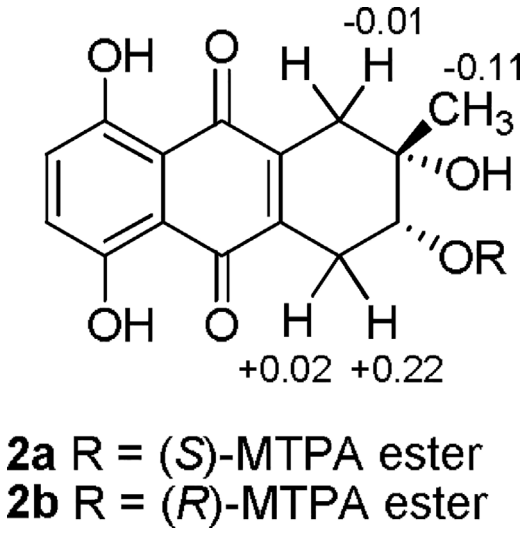


**Fig. 4.** Δδ-Values ($\delta_S$- $\delta_R$) of (*S*)- and (*R*)-MTPA esters **2a** and **2b**.