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Reduced perylenequinone derivatives from an endophytic *Alternaria* sp. isolated from *Pinus ponderosa*

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Dedicated to Prof. Norman R. Farnsworth, for his pioneering work in phytochemistry and pharmacognosy, by transforming pharmacognosy from descriptive medical botany and mycology into the dynamic chemistry- and biology-based multidisciplinary science that it is today.

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ABSTRACT

The consecutive solvent extraction of endophytic *Alternaria* sp. (DC401) isolated from *Pinus ponderosa* followed by chromatographic techniques led to the isolation of five perylenequinone compounds and one dihydronaphthaquinone derivative, which include three new perylenequinones (**1**–**3**). The compounds were identified as 6-methoxy-3,6a,7,10-tetrahydroxy-4,9-dioxo-4,5,6,6a,6b,7,8,9-octahydroperylene (**1**), 3,6a,9,10-tetrahydroxy-7,8-epoxy-4-oxo-4,5,6,6a,6b,7,8,9-octahydroperylene (**2**), 6-methoxy-3,6a,9,10-tetrahydroxy-7,8-epoxy-4-oxo-4,5,6,6a,6b,7,8,9-octahydroperylene (**3**), 3,6a,7,10-tetrahydroxy-4,9-dioxo-4,5,6,6a,6b,7,8,9-octahydroperylene (altertoxin I) (**4**), 3,6a,7,10-tetrahydroxy-4,9-dioxo-4,6a,6b,7,8,9-hexahydroperylene (dehydroaltertoxin I) (**5**), and 7-chloroscytalone (**6**). Structure of compounds **1**–**6** was determined on the basis of detailed spectroscopic analysis, as well as by comparison with literature reports. The antileismanial, antimicrobial, antimalarial and in vitro cytotoxic activities of compounds **1**–**6** were evaluated.

1. Introduction

Perylenequinone natural products are generally dark colored pigments isolated from diverse sources. They comprise a family of natural products characterized by an oxidized pentacyclic core represented by parent perylenequinones. As such, the unique structural, biological and photochemical properties of perylenequinones have been extensively studied (Mulrooney et al., 2010, 2012). In this article, we report the isolation of five perylenequinone compounds as 6-methoxy-3,6a,7,10-tetrahydroxy-4,9-dioxo-4,5,6,6a,6b,7,8,9-octahydroperylene (**1**), 3,6a,9,10-tetrahydroxy-7, 8-epoxy-4-oxo-4,5,6,6a,6b,7,8,9-octahydroperylene (**2**), 6-methoxy-droperylene (**3**), 3,6a,7,10-tetrahydroxy-4,9-dioxo-4,5,6,6a,6b,7, 8,9-octahydroperylene (altertoxin I) (**4**), 3,6a,7,10-tetrahydroxy-4,9-dioxo-4,6a,6b,7,8,9-hexahydroperylene (dehydroaltertoxin I) (**5**), and one dihydronaphthaquinone 7-chloroscytalone (**6**), from an endophytic *Alternaria* sp. isolated from *P. ponderosa*. The compounds **1**–**3** are the first report of reduced perylenequinones. The isolated metabolites were screened for antileismanial, antimicrobial, antimalarial and in vitro cytotoxicity activities.

2. Results and discussion

2.1. Structural elucidation

The ESI-MS of compound **1** (Fig. 1) showed molecular ion peak at *m/z* 383.3540 $[M+H]^+$ in the positive ion mode, collaborating with molecular formula $C_{21}H_{18}O_7H$, supported by ^{13}C NMR

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experiment and also by equivalence with altertoxin I (Stinson et al., 1982). DEPT experiment revealed the presence of one methyl as methoxyl, two methylenes and seven methines. As the molecule is symmetrical its structural elucidation is illustrated through fragments A–D constituted here.

Fragment A: the ^{1}H NMR revealed two pairs of ortho-coupled aromatic protons at δ_H 7.08 (d, J = 8.8), 7.05 (d, J = 8.8) and δ_H 6.89 (d, J = 8.8), 7.79 (d, J = 8.8). Correlation of signals between δ_H 7.08 (d, J = 8.8) and δ_H 7.79 (d, J = 8.8) in NOESY spectrum suggested the presence of biphenyl moiety. The spectrum also exhibited two chelated phenolic hydroxyl protons, assigned to C-3 and C-10. Consequently it was necessary for the two carbonyl groups to be located at C-4 and C-9, respectively. Similar arguments help us to assign the other aromatic carbons and protons (A).

Fragment B: the ^{1}H NMR spectrum contained an A_2X spin system on the basis of resonances at δ_H 3.35 (dd, 16.2, 1.9), 3.13 (dd, 16.0, 10.0) and δ_H 4.64 supported the partial structure as –CH_2–CH–, as the NOESY interaction between methoxyl δ_H 3.40 (s) with δ_H 3.35 (dd) extending the structure to be as –CH_2–CH–OCH_3.

Fragment C: the ^{1}H NMR chemical shift values at δ_H 3.51 (d, 9.5) and δ_H 4.64 (m), is indicative of the fact of the presence of *trans* orientation of protons at C-6a and C-7, which in term supported the argument α and β orientation of hydroxyls at C-6a and C-7, respectively. So part structure was assigned as –C(OH)–CH–CHOH–.

Fragment D: all these findings are consistent with the partial structure D. The remaining quaternary carbon atom (C-6a, δ_C 71.4) which becomes tertiary hydroxyl, must be connected to C-6, C-6a and C-3b, leading the structure 1. The magnitude of the coupling constants between H-6, assumed as β and H_2-5 protons (J = 16 Hz) well accounts for a preferred half-chair conformation of cyclohexenone ring with C_6–OCH_3 equatorial position. As the NOESY experiment did not reveal any correlation between H-6b and H-7 protons indicating their *trans*-orientation, consequently no interaction between hydroxyls at C-7 and C-6a revealed their β and α orientation, respectively. Thus the structure of **1** was established as 6-methoxy-3,6a,7,10-tetrahydroxy-4,9-dioxo-4,5,6,6a,6b,7,8,9-octahydroperylene (Fig. 1).

The compound **2** depicted molecular ion peak at m/z 353.3271 $[M+H]^+$ in positive ESI-MS. The ^{13}C NMR showed 20 resonances for 20 carbons, which in DEPT experiment revealed two methylenes and 8 methines. The lone signal at δ_C 206.8 in ^{13}C NMR spectra suggested a carbonyl. The four aromatic proton doublets at δ_H 8.04, 7.01, 6.86 and 7.59 were assigned to aromatic protons two found each in southern and northern part of the molecule. The signals at δ_C 69.2 and 62.0 in ^{13}C NMR were assigned to carbinol carbons. The presence of β-epoxy proton signals at δ_H 3.86 (d, J = 3.7) and δ_H 3.56 (d, J = 3.5) indicating its *cis*-orientation. The structure elucidation of **2** was carried out in the same way as that of **1** by fragmenting it. The proton at H-6b and H-9 should be axial (β) in orientation as depicted by their coupling constants (J = 4.2 and J = 3.2 Hz), respectively, for H-6b and H-9. The presence of methylene was suggested to be in cyclohexenone ring as shown by correlation between δ_H/δ_C 2.39/206.8 in HMBC spectrum (Fig. 2). Based on the above evidence and by comparing data with that of compound **1** (Fig. 1), structure 2 was assigned to compound **2** and was elucidated to be 3,6a,9,10-tetrahydroxy-7,8-epoxy-4-oxo-4,5,6,6a,6b,7,8,9-octahydroperylene (Fig. 1).

The compound **3** showed the molecular ion peak at 383.3536 $[M+H]^+$, in agreement with the molecular formula $C_{21}H_{18}O_7H$. The ^{13}C NMR depicted C-21 carbon skeleton while as DEPT showed nine methines, one methylene and one methyl as methoxyl. By comparing the data of **3** with **2** it showed only the additional sight of methoxyl (δ_H/δ_C 3.48/57.2). Orientation of proton at C-6b is trans-axial arranged because by comparing coupling constants of **2** and **3**. These deductions were also supported by the fact that no NOESY correlations were obtained between H-6b and H-9/H-6. Based on the above evidence it was apparent that OH-6a and epoxide at C7–C8 separated by three bonds were gauche arranged in **3**, so the upfield shift of the C-6a carbon signal (δ_C 41.3) in the ^{13}C NMR spectrum of **3** should be attributed to a γ-gauche effect (Gao et al., 2009) (Fig. 3). Thus the structure of **3** was elucidated as 6-methoxy-3,6a,9,10-tetrahydroxy-7,8-epoxy-4-oxo-4,5,6,6a,6b,7, 8,9-octahydroperylene (Fig. 1). The compounds **4**, **5** and **6** were elucidated as 3,6a,7,10-tetrahydroxy-4,9-dioxo-4,5,6,6a,6b,7,8,9-octahydroperylene (altertoxin I) (Stinson et al., 1982; Gao et al., 2009),