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New perylenequinone derivatives from the endophytic fungus *Alternaria tenuissima* SS77

Fernanda O. Chagas [a], Luís G. Dias [b], Mônica T. Pupo [a,*]

[a] Departamento de Ciências Farmacêuticas, Faculdade de Ciências Farmacêuticas de Ribeirão Preto, Universidade de São Paulo, Avenida do Café, s/n, 14040-903, Ribeirão Preto, SP, Brazil
[b] Departamento de Química, Faculdade de Filosofia, Ciências e Letras de Ribeirão Preto, Universidade de São Paulo, Avenida Bandeirantes, 3900, 14040-901, Ribeirão Preto, SP, Brazil

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ABSTRACT

Three new natural products, 1,4,6b,7,10-pentahydroxy-1,2,6b,7,8,12b-hexahydroperylene-3,9-dione (**1**), 1,4,9,12a-tetrahydroxy-12-methoxy-1,2,11,12,12a,12b-hexahydroperylene-3,10-dione (**2**) and 1,4,9-trihydroxy-1,2-dihydroperylene-3,10-dione (**3**), were obtained from cultures of the endophytic fungus *Alternaria tenuissima* SS77. Their chemical structures were determined by combined analysis of one- and two-dimensional NMR spectra, UV and ESIHRMS data. Compound **3** is likely an intermediate in the biosynthetic pathway of some perylenequinones produced by this fungus. A new hypothesis for the biosynthesis of partially reduced perylenequinones is suggested.

Introduction

Perylenequinones are secondary metabolites characterized by a conjugated aromatic pentacyclic dione; however, natural products with partially reduced perylene core have also been included in this class of compounds.[1] Perylenequinone derivatives are found in fungi, plants, insects (aphids), and animals (crinoids).[1–10] Different structural features may be attached to the perylenequinone backbone, making perylenequinone derivatives relatively diverse.

Most reported perylenequinones have fungal origin, and the simplest naturally occurring perylenequinone known, the 4,9-dihydroxyperylene-3,10-quinone (**a**), was isolated from the fungus *Daldinia concentrica* in 1956.[11] Cercosporin (**b**) is among the first of isolated mold perylenequinones, which was reported in 1957, even though its structure was only completely determined by 1972.[12–14] This compound was deeply studied mainly due its photoactivity.[9,10] Cercosporin and other similar compounds, such as hypocrellin A (**c**) and scutiaquinone A (**d**) contain carbon substituents attached to perylenequinone backbone[1,8–10] (Fig. S1).

Another group of mold perylenequinones, in which the parent natural product 4,9-dihydroxyperylene-3,10-quinone is included, does not contain carbon substituents and usually has a partially reduced perylene.[1,8] Many mycotoxins produced by *Alternaria* species, such as altertoxin II (**e**), stemphytriol (**f**), and alterlosin II (**g**) (Fig. S1), belong to this second group of mold perylenequinones,[15] some of which have been reported as phytotoxins[16,17] and others have gained importance over the years for causing food and feed contaminations, offering risks to human health due to their mutagenic effects.[18–21]

In plants, toxic effects of these perylenequinones may occur in the same way that of cercosporin, by generation of free radicals that damage host cells after photoactivation.[9] Mutagenicity observed in bacterial and mammal cells have been attributed to various mechanisms of action.[20,21] Particular structural features of different perylenequinone derivatives contribute to their photoactive and mutagenic effects, affording compounds with variable levels of bioactivity.

As part of our ongoing program for prospecting natural products from endophytic microorganisms, the fungus *Alternaria tenuissima* SS77 has been cultured in diverse growth conditions, including mixed microbial cultivation. *A. tenuissima* SS77 has produced polyketides belonging to perylenequinones class, such as stemphyperylenol (**h**), alterperylenol (**i**) and altertoxin I (**j**) (Fig. S1), in single and mixed cultures, and was able to remodel its secondary metabolism in mixed fermentation.[22]

In this Letter we report the identification of further perylenequinones produced by this fungus. The high structural similarity among those compounds made it possible to undoubtedly characterize them by NMR and UV. Furthermore, we have proposed that the biosynthetic pathways for all those perylenequinones more

* Corresponding author. Tel.: +55 16 3315 4710; fax: +55 16 3315 4178.
E-mail addresses: fer_ochagas@yahoo.com.br (F.O. Chagas), lgdias@ffclrp.usp.br (L.G. Dias), mtpupo@fcfrp.usp.br (M.T. Pupo).

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likely share the same precursor in early steps, which later has the perylene core reduced and undergoes peripheral oxidations, affording the final products.

Results and discussion

Compound **1** was obtained as a pale yellow powder. NMR spectra of **1** were acquired in CD_3OD and $CDCl_3$, and the combined analyses allowed the structural determination. Carbons were assigned from *g*HSQC and *g*HMBC spectra (CD_3OD) (Table S1). The ^1H NMR, *g*HSQC, and *g*HMBC spectra (Supplementary material) contained resonances for eighteen carbons in a five fused ring structure comprising two aromatic rings with a couple of aromatic *ortho* coupled hydrogens each [δ_H 6.99 (1H, d, J = 8.6 Hz, H-5), 8.16 (1H, d, J = 8.6 Hz, H-6), 6.89 (1H, d, J = 8.6 Hz, H-11), 8.20 (1H, d, J = 8.6 Hz, H-12); δ_C 117.4 (C-5), 136.6 (C-6), 115.7 (C-11), 137.1 (C-12)], two chelated phenolic hydroxyls [δ_H 12.09 (s, 4-OH), 12.23 (s, 10-OH); δ_C 161.1 (C-4), 162.0 (C-10)], five non-hydrogenated aromatic carbons [δ_C 144.0 (C-3b), 133.3 (C-6a), 115.8 (C-9a), 146.0 (C-9b), 133.9 (C-12a)], a carbonyl carbon [δ_C 204.4 (C-3)], two methylene groups [δ_H 3.09 (1H, dd, J = 12.4 Hz, 15.6 Hz, H-2α), 3.20 (1H, dd, J = 4.5 Hz, 15.6 Hz, H-2β), 3.29 (1H, dd, J = 12.4 Hz, 15.6 Hz, H-8α), 3.01 (1H, dd, J = 4.5 Hz, 15.6 Hz, H-8β); δ_C 48.0 (C-2), 43.9 (C-8)], two hydroxylated methine secondary carbons [δ_H 4.69 (1H, m, H-1), 2.07 (sl, 1-OH), 4.88 (1H, m, H-7), 2.62 (d, J = 6.3 Hz, 7-OH); δ_C 68.7 (C-1), 69.5 (C-7)], a methine tertiary carbon [δ_H 4.42 (1H, d, J = 9.8 Hz, H-12b); δ_C 45.3 (C-12b)], and a hydroxylated tertiary carbon [δ_H 2.71 (s, 6b-OH)]. Data from *g*HSQC and *g*HMBC spectra confirmed that each couple of aromatic hydrogen belongs to different phenol groups (rings A and E). The correlations showed the methylene group at position 2 was connected to hydroxylated methine secondary carbon C-1, which was linked to methine tertiary carbon C-12b, constituting part of the cyclohexenone ring B; additionally, the methylene group at position 8 was linked to the hydroxylated methine secondary carbon C-7, which together were part of ring D. The spectral data of **1** were similar to those of partially reduced perylenequinones previously isolated from *A. tenuissima* SS77.[22] In comparison to other analogues, compound **1** possessed five hydroxyls, including two chelated phenolic ones, and no double bond in any cyclohexenone rings. The positions of hydroxyl groups were determined by *g*HMBC correlations. The ESIHRMS data confirmed the molecular formula of **1** as $C_{20}H_{16}O_7$ by the presence of pseudo-molecular ion at m/z $[M-H]^-$ 367.08510 (calculated m/z 367.08233).

The partially reduced perylenequinones from *Alternaria* spp., and related genera, are divided into two sub-groups according to their structural scaffolds. These compounds exhibit biphenyl or dihydroanthracene frameworks according to relative position of both phenol groups of their structures[23,24] (Fig. 1).

Determining whether compound **1** has a biphenyl or a dihydroanthracene framework was not possible by *g*HMBC, since no key correlations were observed. However, the UV spectra of compounds presenting one or another scaffold are quite characteristic (Fig. 1). The main difference between UV spectra is the presence of a shoulder (sh) band at 285 nm in perylenequinones displaying dihydroanthracene framework, which is absent in perylenequinones with biphenyl framework. Besides, when compared to biphenyl perylenequinones, dihydroanthracene perylenequinones present a characteristic bathochromic shift in the 300–400 nm band. Also, dihydroanthracene perylenequinones display the maximum molar absorptivity (ε) at 258 nm, followed by 214 nm, 285 (sh) and 357 nm, while biphenyl perylenequinones have the maximum ε bellow 200 nm, followed by 215 nm, 260 and 342 nm, as shown in the UV spectra of stemphyperylenol (**h**) and alterperylenol (**i**) (Fig. 1). Therefore, the relative intensities between bands around 250 and 350 nm can be used to indicate the correct framework.

This is the first Letter correlating perylenequinone UV absorptions with their respective frameworks. These characteristic UV spectra were observed in all isolated reduced perylenequinones from *A. tenuissima* SS77 (Fig. S28) and also in previously reported compounds.[16,17,25–27] Thus, based on UV spectra, it was determined that compound **1** possesses a dihydroanthracene framework. Therefore, compound **1** is different from the previously reported stemphytriol (**f**) (Fig. S1), which possesses a biphenyl framework.[26]

The relative stereochemistry of **1** was established based on hydrogen coupling constants. The coupling constants of H-2α and H-12b hydrogens enabled presuming the spatial orientation of hydroxyl at C-1 and hydrogen H-12b. The coupling constants of 12.4 Hz and 9.8 Hz indicated that H-2α presents a *pseudo*-diaxial correlation to H-1, and H-1 presents a *pseudo*-diaxial correlation to H-12b, respectively. Thus, the spatial orientation of hydroxyl at C-1 and hydrogen H-12b were *pseudo*-equatorial and *pseudo*-axial, respectively. In the same way, by the coupling constants between H-7 and H-8α hydrogens (J = 12.4 Hz) that indicated a *pseudo*-diaxial correlation, the orientation of hydroxyl at C-7 was determined as *pseudo*-equatorial. Such as found in all structures of partially reduced perylenequinones, the hydroxyl at C-6b is suggested to be in *pseudo*-axial, likewise hydrogen H-12b, maintaining the relative planarity of the five fused ring structure. The relative configuration between C-12b and C-6b could not be determined. However, considering all isolated reduced perylenequinones, it was assumed that H-12b and OH-6b were in the same side of molecule. Then, the relative configurations of the stereogenic centers at position 1, 12b, 6b and 7 were *S**, *S**, *R** and *S**, respectively.

Compound **1** is very similar to stemphyperylenol (**h**) (Fig. S1), of which we have previously determined the absolute configuration of stereogenic centers as 1R, 12bR, 6bR, and 7R, by comparing experimental ECD with theoretical calculations.[22] However, due

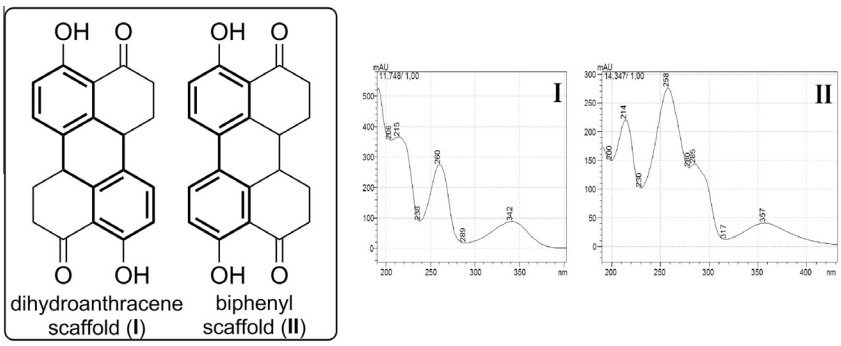

Figure 1. Basic structures of perylenequinones presenting dihydroanthracene (**I**) and biphenyl (**II**) scaffolds, and characteristic experimental UV spectra of both types of perylenequinones.