Talaroxanthone, a Novel Xanthone Dimer from the Endophytic Fungus Talaromyces sp. Associated with Duguetia stelechantha (Diels) R. E. Fries

Hector H. F. Koolen,*a,b Laís S. Menezes,* Mayane P. Souza,* Felipe M. A. Silva,* Fabiana G. O. Almeida,* Antonia Q. L. de Souza,† Angelita Nepel,* Andersson Barison,* Flávio Henrique da Silva,† Danilo Elton Evangelista* and Afonso D. L. de Souza*a

*Departamento de Química, Universidade Federal do Amazonas, 69077-000 Manaus-AM, Brazil
bInstituto de Química, Universidade Estadual de Campinas, 13084-971 Campinas-SP, Brazil
†Escola Superior de Ciências da Saúde, Universidade do Estado do Amazonas, 69065-001 Manaus-AM, Brazil

DgCr22.1b, a novel xanthone dimer, was isolated from the Amazonian Rainforest plant Duguetia stelechantha. From the fractionation of the methanolic mycelial extract, a new xanthone dimer talaroxanthone was isolated. The structure of this new compound was elucidated based on spectroscopic analyses including 2D nuclear magnetic resonance (NMR) experiments.

Keywords: Duguetia stelechantha, endophytic fungi, Talaromyces sp., xanthone

Introduction

Fungi from the genus Talaromyces are known to be a rich and reliable source of biologically active and/or chemically novel compounds.1 So far, almost all secondary metabolites from this telemorphic state of the genus Penicillium appear to be of polyketide origin.2-4 Endophytic organisms have been fructuous producers of new xanthenes,5,6 as illustrated by the antibacterial and antifungal microsphaeropsones from Microsphaeropsis spp.,7 the antimalarial phomoxanthones A and B from Phomopsis sp.,8 the cytotoxic tajixanthone from Emericella variecolor9 and the antibacterial blennolides from Blennoria sp.10 Xanthenes found in fungi are always found as tetrahydroxanthone group of dimers and heterodimers.9 Many of these xanthone dimers from fungi displayed several biological activities such as antibacterial, antifungal and anticancer. Therefore, endophytic fungi are considered to be a promising resource for new lead compounds in drug development. Our research group focuses on the discovery of new structural and bioactive secondary metabolites from endophytic fungi that have been isolated from Amazonian endemic plants.11 This work describes the chemical investigation of Talaromyces sp., an endophytic fungus strain from the Amazonian Rainforest plant Duguetia stelechantha (Annonaceae), which resulted in the identification of a new xanthone dimer, talaroxanthone (Figure 1).

Results and Discussion

Talaroxanthone (Figure 1) was isolated as a yellow powder from the extract of mycelia from flask cultures of Talaromyces sp. DgCr22.1b. The molecular formula C_{32}H_{30}O_{14} was established by high-resolution mass spectrometry (HRMS). The ^{13}C nuclear magnetic resonance (NMR) spectrum shows 16 signals, indicating the
symmetric homodimer structure of this metabolite. Both the presence of a conjugated carbonyl ($\delta_C 187.0$) bound to two adjacent hydroxyl groups at $\delta_H 11.41$ and $13.66$ ppm (characteristic shifts of a keto-enol structure), as well as the analysis of the long-range (HMBC (Heteronuclear Multiple-Bond Correlation Spectroscopy)) $^1$H–$^1$C NMR correlation experiments, suggested the presence of a tetrahydroxanthone ring as shown in Figure 2. The orientation of the methylene hydrogen at $\delta_H 2.27$ (H-7$\alpha$) (dd, $J 10.4, 19.4$ Hz) was suggested by the vicinal coupling constant at $J_{6,7} 10.4$ Hz, indicating a pseudoaxial orientation, and consequently the pseudoequatorial orientation of the hydrogens at $\delta_H 2.71$ (H-7$\beta$) (dd, $J 6.4, 19.4$ Hz) by the absence of this coupling constant. A trans diaxial conformation (Figure 2) between the carbinolic hydrogen at H-5 ($\delta_H 3.75$) along with the methine hydrogen at H-6 ($\delta_H 2.42$) was observed by the coupling constant $J_{5,6} 12$ Hz previously reported for the eumitrin A.$^1$2 The comparison of the $^1$C NMR resonances for the C-5 ($\delta_C 76.4$) with other xanthones presenting the hydroxyl group at C-5 with pseudoequatorial orientation such as blennolide B ($\delta_C 77.0$, $\Delta\delta = +0.4$)$^{10}$ and archexanthone A ($\delta_C 75.9$, $\Delta\delta = -0.5$)$^{13}$ presented close chemical shifts, whereas xanthones with pseudoaquilinidic hydroxyl groups at C-5 have lower chemical shifts, secalonic acid B ($\delta_C 71.4$, $\Delta\delta = -5.0$)$^{10}$ and blennolide A ($\delta_C 71.3$, $\Delta\delta = -5.1$).$^{10}$ These comparisons led to the establishment of the orientations of the hydroxyl group at C-5 and the methyl connected to C-6 as pseudoequatorial (Figure 2).

The aromatic hydrogens at $\delta_H 6.60$ (H-2) and $\delta_H 8.04$ ppm (H-3) were assigned as being ortho to each other due to their coupling constants ($J_{2,3} 8.7$ Hz) and COSY (correlation spectroscopy) data. The observed chemical shift for H-3 is different than the observed for phomoxanthone A,$^8$ but any other connection between the aromatic rings was dismissed by the observed data. The symmetry observed by $^1$H and $^1$C NMR and confirmed by HRMS/MS indicates that this molecule is a homodimer connected at C4-C4', which is rare in fungal metabolites.$^8$ This connectivity was also confirmed by HMBC correlations of the hydroxyl group at C-1. Talaroxanthone differs from the phomoxanthones at positions C-10a, C-10a' and C-5, presenting as substituents methylacetate at C-10a and C-10a' and a hydroxyl at C-5. The Phomopsis xanthones, on the other hand, contain –CH$_2$OCCH$_3$ groups at C-10a and C-10a' and an acetoxy group at C-5a. Talaroxanthone is related to the secalonic acids, also called ergochromes,$^3$ which are also tetrahydroxanthones, but with a C2–C2' linkage pattern,$^{14}$ usually found in compounds from the Claviceps,$^{15}$ Penicillium,$^{16}$ Pyrenochaeta and Aspergillus genera.$^{17}$

**Conclusions**

The potential of endophytic fungi as sources of novel substances was demonstrated by the isolation of a new xanthone dimer named talaroxanthone from the mycelial mass of the endophytic fungus *Talaromyces DgCr22.1b*. This compound is responsible for the yellow pigmentation observed for this strain. To the best of our knowledge, compound 1 is the first example of a xanthone homodimer related to the secalonic acids with C4–C4' linkage along with the presence of methylacetate groups at the positions C10a and C10a' differently from the previous isolated phomoxanthones.

**Experimental**

**General procedures**

1D and 2D NMR experiments were recorded on a Bruker AVANCE 400 spectrometer operating at 9.4 T, equipped with a 5 mm multinuclear direct detection probe with $\mu$-gradient operating at 400 MHz for $^1$H and 100 MHz for $^1$C. The chemical shifts were referenced to the solvent peak CDCl$_3$ at $\delta 7.27$ and 77.0 ppm, respectively. High resolution electrospray ionization mass spectrometry (HRESIMS) measurements were recorded on a Waters Synapt HDMS instrument with quadrupole time-of-flight (QTOF) geometry. Optical rotations were recorded on a Jasco P-1020 polarimeter. Fourier transform infrared
(FTIR) spectra were recorded on a Bomem MB102 spectrophotometer. A Shimadzu LC-6AD pump equipped with a Shimadzu SPD-10AV UV detector and a Rheodyne injector was used for high pressure liquid chromatography (HPLC). Silica gel 60 (70-230 mesh) was used for column chromatography, while silica gel 60 F254 was used for analytical (0.25 mm) thin layer chromatography (TLC). A Phenomenex Phenyl Hexyl 5 µm preparative column (10 mm × 250 mm) was used for the HPLC separations. All solvents used for chromatography and mass spectrometry were from Tedia (HPLC grade), and H2O was ultrapure (Milli-Q, Millipore). All microbiological culture media were purchased from Biosystems.

Fungal material

The roots of Duguetia stelechantha (Diels) R. E. Fries were collected in the experimental farm of the Universidade Federal do Amazonas, Amazonas State, Brazil. The plant material was washed with detergent and sterilized water for external cleaning, then fragments were immersed in 70% alcohol, later in 3% hypochlorite solution and finally in sterile water. After this process, plant fragments were inoculated in Petri dishes containing ISP2-agar medium and incubated for five days. The fragments containing hyphae of fungi were subsequently transferred to test tubes with the same medium and incubated at 26 °C for 30 days. After this period, the mitosporic strain was purified by the Tween technique. The isolated strain was identified according to traditional morphological criteria and sequencing of the fungus ITS-1 to ITS-2 rDNA and compared with sequences from the GenBank. A voucher was deposited in the fungal collection of the GEMMA group of the Universidade Federal do Amazonas under the code DgCr22.1b.

Production and isolation

An isolated culture of the strain DgCr22.1b was grown on ISP, broth medium (International Streptomyces Project 2) at 25 °C for 19 days, into 39 × 1 L Erlenmeyer flasks each containing 300 mL of medium. The mycelia were separated from the broth and extracted with EtOAc 1:1 MeOH (1 L, 2 days), the solvent was evaporated under reduced pressure and provided an red solid gum (30.2 g). The crude extract was subjected to column chromatography (silica gel, 4 × 20 cm) with EtOAc as eluent. A yellow pigment was eluted after ca. 500 mL elution with EtOAc. This process was repeated twice to yield a yellow gum (2.1 g). This yellow gum was subjected to another column chromatography (silica gel, 2.5 × 30 cm) eluted with a gradient of hexane and EtOAc (9:1, 0:1) to give 17 main fractions. The fractions 11-13 (1.9, 104 mg) were subjected to semi-preparative HPLC using isocratic MeOH as the eluent at a flow rate of 8.7 mL min−1 to obtain 1 (70.6 mg, tR 5 min).

Talaroxanthone (1): yellow needles; mp 214-216 °C; [α]D25 +98 (CHCl3; c 0.0027); UV λmax nm (log ε) 222 (4.45), 255 (4.26) and 334 (4.60); IR (KBr) vmax cm−1 1744, 1615, 1586, 1470, 1223, 1044, 897, 830; HRESIMS m/z 639.1636 [M + H]+; calculated 639.1636; product ions (CID, 15 eV) 621.1507 [M + H−H2O]+, 589.1324 [M + H−MeOH]+, 579.1443 [M + H−COOCH3]+, 561.1329 [M + H−COOCH3−H2O]−, 543.1218 [M + H−COOCH3−2H2O]−, 288.2895 [M + H monomer − CO]+; 1H and 13C NMR data are given in Table 1.

Table 1. 1H and 13C NMR spectral data for compound 1

<table>
<thead>
<tr>
<th>Position</th>
<th>δH (J)6</th>
<th>δC</th>
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<tbody>
<tr>
<td>1</td>
<td>161.2</td>
<td></td>
</tr>
<tr>
<td>2</td>
<td>6.60 (d, 8.7 Hz), 1H</td>
<td>109.8</td>
</tr>
<tr>
<td>3</td>
<td>8.04 (d, 8.7 Hz), 1H</td>
<td>141.3</td>
</tr>
<tr>
<td>4</td>
<td>114.8</td>
<td></td>
</tr>
<tr>
<td>4a</td>
<td>155.1</td>
<td></td>
</tr>
<tr>
<td>5</td>
<td>3.75 (d, 12 Hz), 1H</td>
<td>76.4</td>
</tr>
<tr>
<td>6</td>
<td>2.42 (m)</td>
<td>28.9</td>
</tr>
<tr>
<td>7β</td>
<td>2.71 (dd, 6.4 and 19.4 Hz), 1H</td>
<td>35.9</td>
</tr>
<tr>
<td>7α</td>
<td>2.27 (dd, 10.4 and 19.4 Hz), 1H</td>
<td>–</td>
</tr>
<tr>
<td>8</td>
<td>176.5</td>
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</tr>
<tr>
<td>8a</td>
<td>100.9</td>
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</tr>
<tr>
<td>9</td>
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<tr>
<td>9a</td>
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<td>1.10 (d, 6.4 Hz), 3H</td>
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<tr>
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<tr>
<td>8-OH</td>
<td>13.66 (s), 1H</td>
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</table>

Experiments were carried out at 400 MHz for 1H and 100 MHz for 13C in CDCl3, and TMS as internal reference (δ 0.00 ppm); assignments confirmed by DEPT-135, HSQC, HMBC and NOESY experiments.

Supplementary Information

Supplementary information (1H, 13C, COSY, HMBC, HSQC, NOESY spectra and MS data) are available free of charge at http://jbcs.sbq.org.br as PDF file.
Acknowledgments

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