http://dx.doi.org/10.5935/0103-5053.20130104
J. Braz. Chem. Soc., Vol. 24, No. 5, 880-883, 2013.
Printed in Brazil - ©2013 Sociedade Brasileira de Química
0103 - 5053 $6.00+0.00

Short Report

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

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DgCr22.1b, um isolado endofítico de *Talaromyces* sp., foi coletado na Floresta Amazônica a partir da planta medicinal *Duguetia stelechantha*. Do fracionamento do extrato metanólico de sua massa micelial, foi isolada uma nova xantona dimérica talaroxantona. A estrutura deste novo composto foi elucidada com base em análises espectroscópicas incluindo experimentos de ressonância magnética nuclear (RMN) 2D.

DgCr22.1b, an endophytic isolate of *Talaromyces* sp., was collected in the Amazonian Rainforest from the medicinal 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, *Talaromyes* 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 xanthones,[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 variecolor*[9] and the antibacterial blennolides from *Blennoria* sp.[10] Xanthones found in fungi are always found as tetrahydroxanthone group of dimers and heterodimers.[5] Many of these xanthone dimers from fungi displayed several biological activities such as antibacterial, antifungal and anticancer.[7] 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

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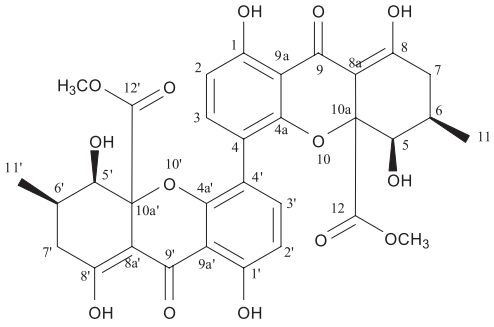

Figure 1. Structure of talaroxanthone.

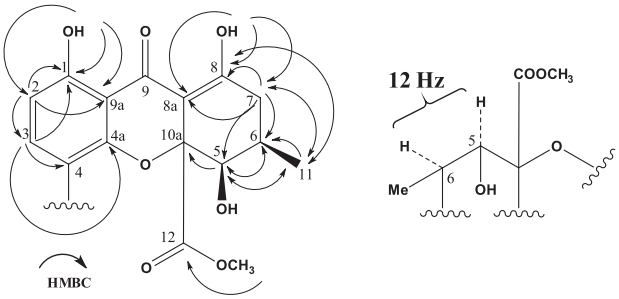

Figure 2. Key long-range correlations observed in the HMBC and NOESY of compound **1**.

symmetric homodimer structure of this metabolite. Both the presence of a conjugated carbonyl (δ_C 187.0) bound to two adjacent hydroxyl groups at δ_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-^{13}C NMR correlation experiments, suggested the presence of a tetrahydroxanthone ring as shown in Figure 2. The orientation of the methylene hydrogen at δ_H 2.27 (H-7α) (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 δ_H 2.71 (H-7β) (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 (δ_H 3.75) along with the methine hydrogen at H-6 (δ_H 2.42) was observed by the coupling constant $J_{5,6}$ 12 Hz previously reported for the eumitrin A_2.[12] The comparison of the ^{13}C NMR resonances for the C-5 (δ_C 76.4) with other xanthones presenting the hydroxyl group at C-5 with pseudoequatorial orientation such as blennolide B (δ_C 77.0, $\Delta\delta$ = +0.4)[10] and archexanthone A (δ_C 75.9, $\Delta\delta$ = –0.5)[13] presented close chemical shifts, whereas xanthones with pseudoaxial hydroxyl groups at C-5 have lower chemical shifts, secalonic acid B (δ_C 71.4, $\Delta\delta$ = – 5.0)[10] and blennolide A (δ_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 δ_H 6.60 (H-2) and δ_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 ^{13}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 $-CH_2OCCH_3$ groups at C-10a and C-10a' and an acetoxy group at C-5a. Talaroxanthone is related to the secalonic acids, also called ergochromes,[5] 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 *z*-gradient operating at 400 MHz for ^{1}H and 100 MHz for ^{13}C. The chemical shifts were referenced to the solvent peak $CDCl_3$ at δ 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 F_{254} 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 H_2O 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 sterile water for external cleaning, then fragments were immersed in 70% alcohol, later in 3% hypochlorite solution and finally in sterile water.[18] 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.[19] 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.[18] 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_2 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, t_R 5 min).

Talaroxanthone (**1**): yellow needles; mp 214-216 °C; $[\alpha]_D^{25}$ +98 ($CHCl_3$; *c* 0.0027); UV λ_{max}/nm (log ε) 222 (4.45), 255 (4.26) and 334 (4.60); IR (KBr) ν_{max}/cm^{-1} 1744, 1615, 1586, 1470, 1366, 1223, 1044, 897, 830; HRESIMS *m/z* 639.1636 $[M + H]^+$; calculated 639.1636; product ions (CID, 15 eV) 621.1507 $[M + H–H_2O]^+$, 589.1324 $[M + H–MeOH]^+$, 579.1443 $[M + H–COOCH_3]^+$, 561.1329 $[M + H–COOCH_3–H_2O]^+$, 543.1218 $[M + H–COOCH_3–2H_2O]^+$, 288.2895 [M + H monomer – CO]$^+$; ^{1}H and ^{13}C NMR data are given in Table 1.

Table 1. ^{1}H and ^{13}C NMR spectral data[a] for compound **1**

Position	**1**	
	δ_H (*J*)[b]	δ_C
1		161.2
2	6.60 (d, 8.7 Hz), 1H	109.8
3	8.04 (d, 8.7 Hz), 1H	141.3
4		114.8
4a		155.1
5	3.75 (d, 12 Hz), 1H	76.4
6	2.42 (m)	28.9
7β	2.71 (dd, 6.4 and 19.4 Hz), 1H	35.9
7α	2.27 (dd, 10.4 and 19.4 Hz), 1H	–
8		176.5
8a		100.9
9		187.0
9a		106.4
10		
10a		84.3
11	1.10 (d, 6.4 Hz), 3H	17.7
12		169.9
12-OCH_3	3.77 (s), 3H	52.9
1-OH	11.41 (s), 1H	
8-OH	13.66 (s), 1H	

[a]Experiments were carried out at 400 MHz for ^{1}H and 100 MHz for ^{13}C in $CDCl_3$ and TMS as internal reference (δ 0.00 ppm); [b]assignments confirmed by DEPT-135, HSQC, HMBC and NOESY experiments

Supplementary Information

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

Acknowledgments

This work was funded by grants from the Conselho Nacional de Desenvolvimento Científico e Tecnológico (CNPq), the Coordenação de Aperfeiçoamento de Pessoal de Nível Superior (CAPES) and the Fundação de Amparo a Pesquisa do Estado do Amazonas (FAPEAM).

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Submitted: June 26, 2012
Published online: April 30, 2013

FAPESP has sponsored the publication of this article.