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Active polyketides isolated from *Penicillium herquei*

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ABSTRACT

In this work we are reporting the isolation by classical methods of chromatography of six polyketides from *Penicillium herquei*. The compounds citreorosein (1), emodin (2), janthinone (3), citrinin (4), citrinin H1 (5) and dicitrinol (6) were identified by spectral methods of 1D and 2D NMR and MS. Compounds 1, 2 and 3 were tested against promastigotes forms of *Leishmania brasiliensis* and 1 and 2 were also assayed against *Escherichia coli*, *Pseudomonas aeruginosa* and *Bacillus subtilis* and showed good activity.

Key words: polyketides, biological assay, *P. herquei*.

INTRODUCTION

The search for substances with useful biological activities to man is one of the most studied fields in science as a whole. There is always the need to renew the arsenal of active compounds due to the parasites acquire resistance to drugs already on the market, as well as the emergence of new diseases.

Fungi are good producers of secondary metabolites, many with useful biological activity (Petrini et al. 1992, Jarvis and Miller 1996, Li et al. 1998, Stierle et al. 1995). Endophytic fungi often produce substances that can help the host plant to fight infestations by other fungi, bacteria and viruses, even assisting in its development (Shittu et al. 2009, Hamayun et al. 2009). Since

such substances can also be useful for people, it is therefore of great importance to study the biological activities of substances produced by endophytic fungi.

In this paper we report the isolation of six polyketides citreorosein (1), emodin (2), janthinone (3), citrinin (4), citrinin H1 (5) and dicitrinol (6) (Figure 1). Compounds 1, 2 and 3 were tested against promastigote forms of *Leishmania brasiliensis* and 1 and 2 were also assayed for antimicrobial activities.

MATERIALS AND METHODS

GENERAL PROCEDURES

The IR spectrum was measured in BOMEN MB-102 spectrophotometer in KBr pellet. APCIMS data were acquired in negative mode using a MICROMASS

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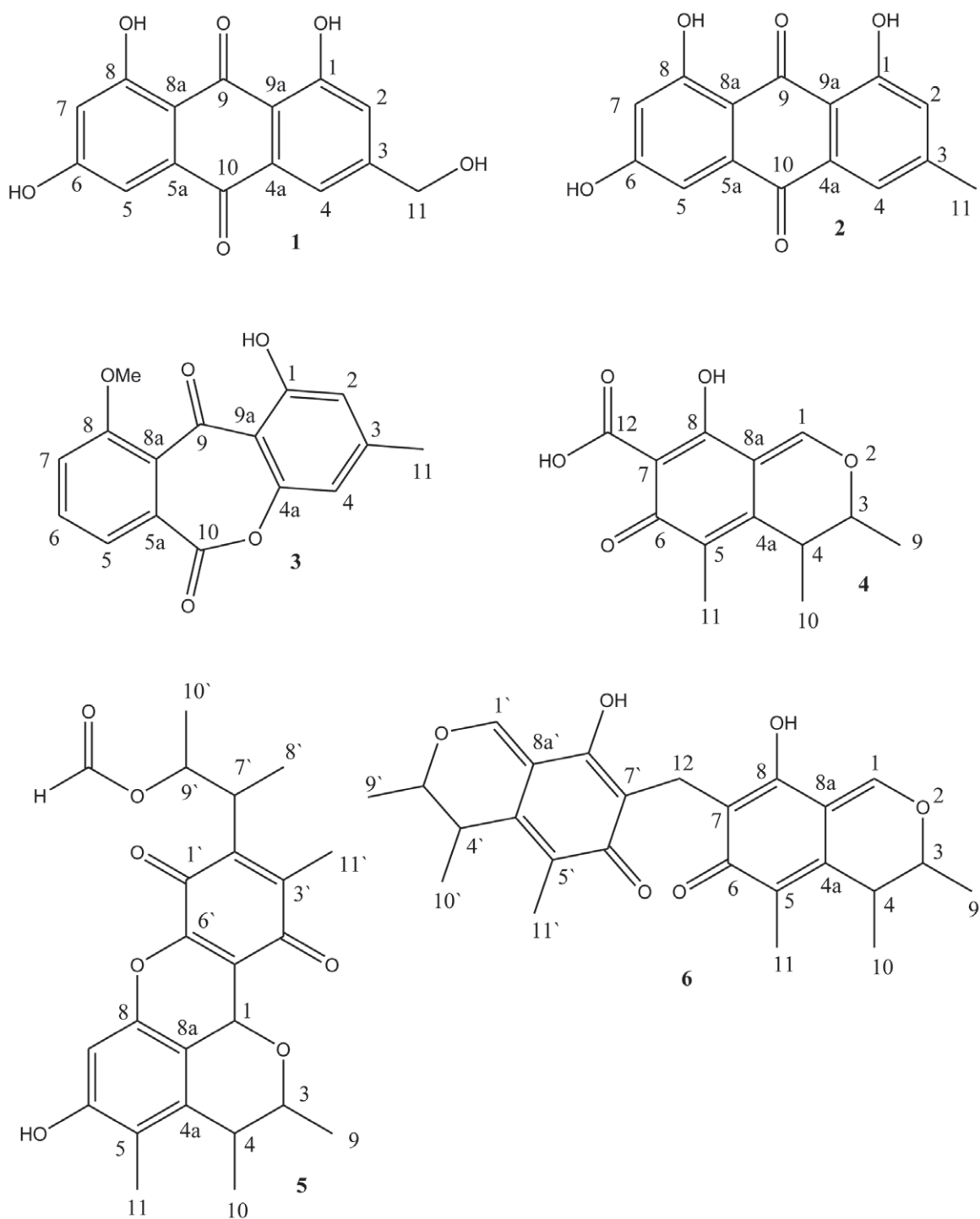


Figure 1 - polyketides isolated from *P. herquei*.

QUATTRO-LC instrument (Waters, Manchester – United Kingdom) equipped with an ESI/APCI ion source "Z-spray" type. ^1H and ^{13}C NMR experiments

were obtained in a BRUKER DRX-400 spectrometer (Bruker Daltonics, Germany) in CDCl_3 with TMS as internal standard.

MICROORGANISM

P. herquei belong to the collection of the Laboratório de Bioquímica Micromolecular de Microorganismos, Departamento de Química - Universidade Federal de São Carlos and it is identified by the number LaBioMi 019. This collection contains isolates from *Melia azedarach* (Santos et al. 2003).

CULTURE OF *P. herquei* in Rice and Polyketides Isolation

Forty-five Erlenmeyer flasks (500 mL) containing 90 g rice ("Uncle's Been's®") and 75 mL distilled water per flask were autoclaved for 45 min at 121°C. Small cubes of PDA medium containing mycelium of *P. herquei* were added in 42 Erlenmeyer flasks under sterile condition. Three flasks were used as control. After 20 days of growth at 25°C the biomass obtained was macerated with dichloromethane, ethyl acetate and methanol. The dichloromethane solution was evaporated under reduced pressure, producing a yellowish residue (24.2 g). Part of this residue (10.0 g) was subjected to a low-pressure silica gel CC eluted with *n*-hexane, ethyl acetate and methanol gradient. The medium polarity fractions eluted with ethyl acetate were repeatedly chromatographed on silica gel CC eluted with *n*-hexane, acetone and methanol gradient. Finally, they provide the polyketides citreorosein (1), emodin (2), janthinone (3), citrinin (4), citrinin H1 (5) and dicitrinol (6).

LEISHMANICIDAL ASSAY

Promastigote forms of *Leishmania viannia braziliensis* MHOM/BR1987/M11272 were grown at 25°C in Schneider's Drosophila medium supplemented with 10% fetal calf serum (FCS). Cells were collected at logarithmic phase, resuspended in fresh medium, counted in Neubauer chamber and the concentration adjusted to 4×10^6 cells/mL. The test was conducted in vitro. Substances were added at 320 µg/mL to 0.125 µg/mL solubilized in DMSO

and incubated at 25°C for 24 h. After this period, surviving parasites were counted in Neubauer chamber and compared with controls. Pentamidine isethionate (Eurofarma®) was used as positive control drug and DMSO as negative control. The LD50/24 was determined by linear regression analysis of the inhibition percentage with 10% statistical error.

ANTIBACTERIAL BIOASSAY

Microorganisms' susceptibility to the polyketides test were determined by microbroth dilution assay as recommended by the Subcommittee on Antifungal Susceptibility Testing of the US National Committee for Clinical Laboratory Standards (NCCLS 1997).

RESULTS AND DISCUSSION

POLYKETIDES IDENTIFICATION

Compounds 1, 2 and 3 were identified by 1D and 2D NMR and MS spectral analyses in comparison with the literature and showed total similarity to the polyketides citreorosein (1), emodin (2) and janthinone (3) (Fujimoto et al. 2004, Cohen and Towers 1995, Marinho et al. 2005). Substances 4, 5 and 6 1D and 2D NMR and MS data are consistent with citrinin, citrinin H1 and dicitrinol, previously isolated by our team from *P. janthinellum* (Marinho and Rodrigues Filho 2011).

LEISHMANICIDAL TEST

The substance 2 inhibited 50% of parasites at 320 µg/mL showing a promising result. However, substances 1 and 3 inhibited about 20% and 22% of parasites at 320 µg/mL, respectively. It seems that the methyl hydroxylation in the substance 1 reduced the activity in more than 50%. Results obtained in leishmanicidal tests with substances 1, 2 and 3 suggest that positions C-3 and C-6 must be reduced and oxidized, respectively, for there being activity, since when both positions are oxidized or reduced there is significant decrease in the activity presented.

ANTIMICROBIAL ACTIVITY

The antibacterial activity of citreorosein (1) was examined in the presence of *E. coli*, *P. aeruginosa* and *B. subtilis*. Results were compared with those obtained for emodin (2) under the same conditions (Table I). In general, citreorosein is less active than emodin, except against *E. coli*, which stops growing in a medium containing 31.25 µg/mL of 1.

TABLE I
Bacteria growth behavior in the presence of compounds 1 and 2 at different concentrations.

Concentration c [µg/mL] of test compounds	<i>E. coli</i>		<i>P. aeruginosa</i>		<i>B. subtilis</i>	
	1	2	1	2	1	2
500	=	=	=	=	=	=
250	=	+	=	=	-	=
125	=	+	=	=	+	-
62.50	=	+	=	=	+	-
31.25	=	+	+	-	+	-
15.63	+	+	+	-	+	+
7.81	+	+	+	-	+	+

= bactericidal effect, - bacteriostatic effect, + no active.

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RESUMO

Neste trabalho estamos relatando o isolamento por métodos clássicos de cromatografia de seis policetídeos de *Penicillium herquei*. Os compostos citreoseina (1), emodina (2), janthinona (3), citrinina (4), citrinina H1(5) e dicitrinol (6) foram identificados por métodos

espectrais de RMN 1D e 2D e EM. Os compostos 1, 2 e 3 foram testados contra formas promastigotas de *Leishmania brasiliensis* e 1 e 2 também foram ensaiados contra a *Escherichia coli*, *Pseudomonas aeruginosa* e *Bacillus subtilis* e mostraram boa atividade.

Palavras-chave: policetídeos, ensaio biológico, *P. herquei*.

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