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Artigo

Antimicrobial activity of methyl australate from *Ganoderma australe*

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RESUMO: "Atividade antimicrobiana do australato de metila de *Ganoderma australe*". Do fungo brasileiro *Ganoderma australe* foram isolados o ácido austrálico e o novo composto australado de metila, além de outros 9 compostos já conhecidos. Tanto o australado de metila quanto o ácido correpondente foram ativos contra fungos e bactérias Gram-positivas, sendo o éster metílico ainda ativo contra bactérias Gram-negativas.

Unitermos: *Ganoderma australe*, Ganodermataceae, atividade antimicrobiana, australato de metila.

ABSTRACT: In addition to nine known steroidal compounds, australic acid and the new methyl australate have been isolated from the Brazilian fungus *Ganoderma australe.* Both methyl australate and its corresponding acid were shown to be active against fungi and Gram-positive bacteria, the methyl ester being also active against Gram-negative bacteria.

Keywords: *Ganoderma australe*, Ganodermataceae, antimicrobial activity, methyl australate.

INTRODUCTION

Ganodermataceae is characterized by basidiospores with inner ornamented walls and outer smooth and hyaline surfaces. The family comprehends the *Amauroderma* Murril, *Elfvingia* Karsten, *Ganoderma* Karsten, *Haddowia* Stayaert, *Humphreya* Stayaert and *Magoderma* Stayaert genera, all featuring rigid basidiomata with different colour as red, white, yellow and purple (Loguercio-Leite et al., 2003; Kirk et al., 2001; Jong; Birmingham, 1992). Among the approximately 250 species belonging to the *Ganoderma* genus, *G. lucidum* (Curt.:Fr.) P. Karst. is the most studied one because of its great medicinal and commercial interest (Moncalvo; Ryvarden, 1997; Barbosa-Filho et al., 2006). From this species more than one hundred triterpenoids have been described and many of them have been attributed some biological activity (Ma et al., 2002; Kikuchi et al., 1985; Chen; Yu 1999; Wasser; Weis 1999; Min et al., 2001).

This paper deals with the antifungal and antibacterial activity of australic acid and the new methyl australate isolated from *Ganoderma australe*.

MATERIAL AND METHODS

Fungal specimen

Ganoderma australe (Fr.) Pat. was collected in Içara, Santa Catarina, Southern Brazil in 1999. The mushroom was identified by Dr. C. Loguercio-Leite, Department of Botany, Federal University of Santa Catarina, Brazil. A voucher of the basidioma was deposited at the Herbarium FLOR of the same University, under the cipher FLOR 11914.

Extraction and isolation

Ground dried basidioma (285 g) was extracted with MeOH for 10 days. After the solvent being evaporated, the residue was suspended in MeOH:H_2O (9:1) and partitioned *vs* hexane (4.3 g), $CHCl_3$ (6.7 g) and EtOAc (1.2 g). The $CHCl_3$ extract was purified on silica gel column eluted with EtOAc affording five fractions: Fr-1 (1.3 g), Fr-2 (2.2 g), Fr-3 (0.4 g), Fr-4 (0.8 g) and Fr-5 (1.2 g). Extended column chromatography from Fr-1 (SiO_2; a: EtOAc-hexane, 9:1; b: $CHCl_3$) yielded 5α-ergost-7-en-3β-ol (20 mg), the compound **1** (50 mg) (Fig. 1), 5α-ergost-7,22-dien-3β-ol (60 mg), 5,8-epidioxy-5α,8α-ergost-6,22-dien-3β-ol (16 mg), australic acid (**1a**, 50 mg) and a mixture of applanoxidic acid C and F (60 mg). Purification (SiO2; $CHCl_3$-CH_3OH, 98:2 and 95:5) of Fr-2 gave applanoxidic acid F (290 mg), C (220 mg), G (930 mg), A (310 mg) and H (55 mg). After

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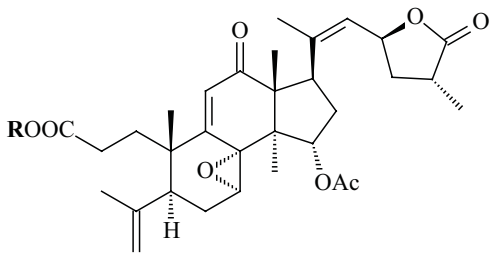
methylation with ethereal diazomethane the residue Fr-4 was purified on column chromatography with a gradient mixture of EtOAc hexane to give the methyl esters of applanoxidic acid F (35 mg), C (20 mg), G (75 mg), D (40 mg), H (32 mg) and A (113 mg). Fractions Fr-3 (a mixture of applanoxidic acids) and Fr-5 were not further processed during the present study. The known compounds, including australic acid, were identified by comparison of the NMR data with those reported in the literature (Chairul et al., 1991; Chairul et al., 1994; Yoshikawa et al., 2002; León et al., 2003).

Methyl australate (**1**): Oil. ^{1}H NMR (300 MHz, $CDCl_3$), δ: 5.97 (s, H-11), 5.54 (d, J=8.0 Hz; H-22), 5.39 (dd, J=9.6, 7.2 Hz; H-15), 5.30 (m, H-23), 4.94, 4.72 (brs, H-28), 4.04 (d, J=4.1 Hz; H-7), 3.66 (s, OMe), 3.24 (t, J=9.5; H-17), 2.87 (dd, J=12.7, 9.6 Hz; H-5), 2.74 (dd, J=15.0, 7.3 Hz; H-25), 2.05 (s, Me Ac), 1.92 (s, Me-21), 1.73 (s, Me-29), 1.30 (d, J= 7.3 Hz; Me-27), 1.21 (s, Me-30), 1.03 (s, Me-18), 0.98 (s, Me-19). ^{13}C NMR (75 MHz, $CDCl_3$), δ: 201.3 (s, C-12), 180.0 (s, C-26), 173.9 (s, C-3), 170.5 (s, O-CO, Ac), 163.1 (s, C-9), 144.3 (s, C-4), 140.2 (s, C-20), 129.8 (d, C-11), 126.2 (d, C-22), 115.7 (t, C-28), 75.1 (d, C-23), 71.5 (d, C-15), 66.2 (d, C-8), 61.1 (d, C-7), 58.8 (d, C-13), 51.7 (OMe), 51.5 (s, C-14), 44.2 (d,C-17), 43.9 (d, C-5), 43.7 (s, C-10), 37.1 (t, C-24), 36.4 (t, C-16), 34.3 (d, C-25), 29.4 (t, C-2), 31.8 (t, C-1), 27.1 (t, C-6), 23.4 (q, Me-19), 23.3 (q, Me-29), 21.1 (q, Me, Ac), 19.1 (q, Me-21), 17.9 (q, Me-18), 15.7 (q, Me-27), 15.1 (q, Me-30).



R

1 Me
1a H

Figure 1. Methyl australate and australic acid isolated from *Ganoderma australe.*

Antimicrobial activity

The minimal inhibitory concentration (MIC) of compound **1** and australic acid were determinated by a microdilution method. The test organisms used in this study were the fungi: *Microsporum canis* (Bodin) MIP 200501 (MIP, Departamento de Microbiologia e Parasitologia, UFSC), *Trichophyton mentagrophytes* (Blanchard) MIP 200503 and one no filamentous *Candida albicans* ATCC 14053 (American Type Culture Collection, Rockiville, MD), and four bacterial species: *Bacillus cereus* ATCC 11778, *Echerichia coli* ATCC 25922, *Pseudomonas aeruginosa* ATCC 27853 and *Staphylococcus aureus* ATCC 25923. Both compounds **1** and **1a** were dissolved in dimethyl sulfoxide (DMSO) and diluted (2.0 to 0.0312 mg/mL) in nutrient broth and Mueller-Hinton broth for tests with fungal and bacterial species, respectively. 100 µL from each dilution were poured in one of the 96 wells of a sterilized microplate as well as sterility controls (last dilution of each compound). Nutrient broth or Mueller-Hinton broth and pure DMSO were used as a growth control. Each test and growth controls well was inoculated with 5 µL of fungal inoculum (10^5 CFU/mL) or bacterial inoculum (10^6 CFU/ml). All experiments were performed in duplicates and the microdilution plates were incubated at 35 °C for 72 h (fungi) or 36 °C for 18 h (bacteria). The MIC, *i.e.* the lowest concentration of each substance at which no growth occurred, was first determined by reading the optical density (Elisa reader, CLX800-BIOTEK Instruments). The bacterial growth was confirmed by a change yellow to purple in the well mixture, after the addition (20 µL to each well) of an alcoholic solution (0.5 mg/mL) of 2-(4-iodophenyl)-3-(4-nitrophenyl)-5-phenyltetrazolium chloride (INT, Sigma).

RESULTS AND DISCUSSION

The comparison of ^{1}H and ^{13}C NMR spectral

Table 1. Antimicrobial activity of the compounds australic acid and methyl australate.

Compounds Microorganisms	Australic acid	Methyl australate	Ampicillin	Tetracycline	Fluconazole
Escherichia coli	>2.0[a]	2.0	0.00015	0.0013	NT
Pseudomonas aeruginosa	>2.0	2.0	0.1	0.0125	NT
Bacillus cereus	0.25	0.25	0.00312	NT	NT
Staphylococcus aureus	1.0	2.0	0.00015	0.00015	NT
Candida albicans	2.0	2.0	NT[b]	NT	0.0001
Microsporum canis	1.0	2.0	NT	NT	0.00125
Trichophytom mentagrophytes	1.0	2.0	NT	NT	0.0006

[a]MIC minimum inhibitory concentration expressed in mg/mL
[b]NT not teste

data of **1** and **1a** revealed the first to be the methyl ester of the second one, as suggested by the signals at 3.66 ppm in the proton spectrum and 51.7 in the carbon spectrum. As a confirmation, treatment of australic acid **1a** with diazomethane in Et_2O solution (2 h at room temperature) gave **1**.

The antibacterial and antifungal activities of the nine known compounds have already been reported, except for australic acid (Smania et al., 1999; Gerber et al., 2000, Smania et al., 2003; Smania et al., 2006). The antimicrobial activity of the australic acid and its methyl ester **1** are shown on table 1. The two compounds were active against the three tested fungal species. However, australic acid was more active against the filamentous fungi, while the ester exhibited the same MIC for the three species. Conversely, both **1** and **1a** showed activity against Gram-positive bacteria, with identical prominent MIC values (0.25 mg/mL) vs *B. cereus*. By contrast, the methyl ester was also active against Gram-negative bacteria. Likely, the increase of lipophilicity, due to the methylation of the carboxyl group, facilitate the transport of this substance through outer membrane of the Gram-negative cell, once it is formed by lipoproteins, lipopolysaccharides, and phospholipids. The values found in the microrganisms-test for the reference compounds, tetracycline, ampicillin and fluconazol, were in accordance with those reported in the literature (Pfaller et al., 1995; Traub; Leonhard, 1995).

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