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FUNGITOXIC ACTIVITY OF COMPOUNDS ISOLATED FROM LICHENS

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ABSTRACT

Lichens collected in Mato Grosso do Sul, Brazil, were analysed to their secondary metabolites. The compounds isolated were tested against the phytopathogenic fungus Cladosporium sphaerospermum, using a bioautography test. Diffractaic acid, atranorin/chloroatranorin, usnic acid and the technical artifact product ethyl orselinate inhibited the fungus growth.

RESUMO

Liquens coletados em Mato Grosso do Sul, Brasil, foram analisados quanto aos seus metabólitos secundários. Os compostos isolados foram testados quanto a atividade de inibição de crescimento do fungo fitopatogênico Cladosporium sphaerospermum usando a técnica de bioautografia. Atranorina/Cloroatranorina, ácido difractáico, ácido úsnico e o produto de artefato de técnica orselinato de etila inibiram o crescimento do fungo.

KEYWORDS : lichens, fungitoxic activity, *Cladosporium sphaerospermum*, secondary metabolites.

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INTRODUCTION

The lichens like many plants, were used since antiquity as drugs or sources of them[1]. These organisms produce secondary metabolites and many of them are known for presenting biological and/or phaarmacological activities, such as, antimicrobial[2-11], anti-tumor[12-15], antioxidant[16], analgesic and antipyretic[17] and others.

In spite of these properties, the search of new biological and pharmacological activities of these compounds remains important. The search of new antifungal substances has received special attention due to the few drugs existent and which, besides, are of limited efficacy in the treatment of systemic mycoses[18].

During our studies of lichen specimens we have isolated several compounds belonging to the classes of depsides, depsidones, dibenzofurans, and bis-xanthones which have been tested for their biological activities. This paper, describes the results of fungitoxic activities shown by some of these compounds. Evaluation of this activity has been determined by a bioautography method, using spores from the phytopathogenic fungus *Cladosporium sphaerospermum* Penzig.

EXPERIMENTAL

General Experimental Procedures

All melting points are uncorrected. The IR spectra were recorded in KBr with a Perkin Elmer 783 spectrometer. The ^{1}H-nmr spectra were measured at 300 MHz and ^{13}C-nmr at 75 MHz using a Brucker DPX-300 spectrometer. Spectra were measured in $CDCl_3$ and CD_3COCD_3, with chemical shifts reported in δ values (ppm) (using TMS as the internal standard) and coupling constants in Hz. Mass spectra (MS) were obtained from the Analytical Center of the Federal University of Paraíba, João Pessoa, PB, Brazil. Thin-layer chromatography (TLC) was performed on silica gel 60 using the solvent systems a) benzene : dioxane : acetic acid, 90: 25: 4 v/v/v; b) toluene : ethyl acetate : acetic acid, 6 : 4 : 1 v/v/v; and c) toluene : chloroform, 1 : 1 v/v. Spots were visuallized with $MeOH/H_2SO_4$ 10% and *p*-anisaldehyde/H_2SO_4.

Plant Material

The lichens *Parmotrema tinctorum* (Nyl.)Hale, *Ramalina continentalis* Malme, and *Usnea subcavata* Motika were collected in the vilage of Palmeiras and Piraputanga in Mato Grosso do Sul. A voucher specimen of each lichen is deposited in the herbarium of the Chemistry Department of the Federal University of Mato Grosso do Sul, Campo Grande, Mato Grosso do Sul, Brazil.

Extraction and Isolation

The powder of each lichen (10.0 to 80.0 grams) was extracted in a Soxhlet apparatus with benzene and then with acetone or with chloroform only. The extracts were concentrated under reduced pressure on a rotaevaporator. Concentrated benzene and chloroform extracts obtained from *P. tinctorum* and *R. continentalis* were treated with ethanol several times to remove pigments. After this treatment, the residues were recrystallized from $CHCl_3$. Fractionation of the benzene extract from *U. subcavata* was by chromatography on a silica gel column with pure solvents and mixtures of hexane, dichloromethane, chloroform, acetone and methanol. The structures of compounds isolated and purified were elucidated by spectral analysis (IR, ^{1}H-nmr, ^{13}C-nmr, and MS).

Biossays

Stock solutions of pure compounds were prepared at 500 μg/mL in an appropriate solvent and were diluted to 250, 50 and 5 μg/mL. 20 μL of each solution corresponding to 10, 5, 1 and 0.1 μg were applied on a plate of silica gel (Merck). The plates were sprayed with a spore suspension of *Cladosporium sphaerospermum* in glucose and saline solution and incubated for 48 hours in the dark in a moist chamber at 30° C[19]. The fungitoxic activity was evaluated by comparison of the size (in mm) of the inhibition zones of fungus growth.

Fungus culture

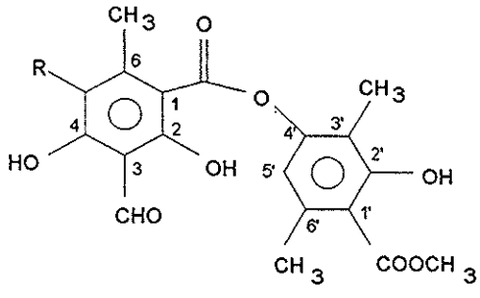
The fungus *Cladosporium sphaerospermum* Penzig was cultured in PDA (potato, dextrose, agar) medium at 28°C in the dark to obtain adequate sporulation. The maintenance of the innoculum was done according to the procedure described by Figueiredo and Pimentel[20].

RESULTS AND DISCUSSION

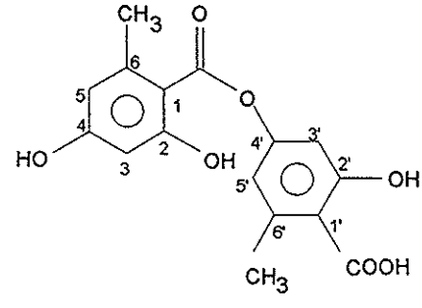
The melting points, IR, ^{1}H- and ^{13}C-nmr of the crystalline residue obtained from the benzene extract of *P. tinctorum* were identical to literature data for atranorin [21,22]. However the TLC of this residue in toluene-chloroform 1:1 v/v showed two components; possibly this fraction is a mixture of depsides atranorin (I) *[methyl (3-formyl-2,4-dihydroxy-6-methylbenzoyloxy-4' 2'-hydroxy-3',6'-dimethylbenzoate)]* and 5-chloroatranorin (II) *[methyl (3-formyl-2,4-dihydroxy-5-chloro-6-methylbenzoyloxy-4' 2'-hydroxy-3',6'-dimethylbenzoate)]*. The residue obtained from the acetone extract of this lichen was dissolved in diethyl ether and treated with $NaHCO_3/H_2SO_4$ [23]. By this procedure were isolated two compounds (III) and (IV). The IR, ^{1}H- and ^{13}C- nmr and mass spectra of these compounds correspond to those reported for the depside lecanoric acid (III)[23,24] *(2,4-dihydroxy-6-methylbenzoyloxy-4' 2'-hydroxy-6'-methylbenzoic acid)* and ethyl orsellinate (IV)[25] *(ethyl 2,4-dihydroxy-6-methylbenzoate)*. The last compound has not been cited for *P. tinctorum* and may have resulted from a technical artifact. The chloroform extract obtained from *Ramalina continentalis* after removal of pigments by treatment with ethanol, was recrystallized from $CHCl_3$ (mp 202-

203°C). It gave the same chromatographic behaviour as usnic acid (V) *[1,3(2H,9bH)-dibenzofurandione-2,6-diacetyl-7,9-dihydroxy-8,9b-dimethyl]* , and the IR and ^{1}H- nmr spectra were identical to literature values[21].

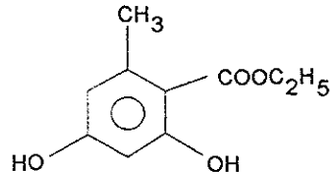
From *Usnea subcavata* we isolated usnic acid and the depside diffractaic acid (VI) *(2,4-dimethoxy-3,6-dimethylbenzoyloxy-4' 2'-hydroxy-3',6'-dimethylbenzoic acid).* These compounds were obtained by fractionation of the benzene extract by chromatography on silica gel column. The structural elucidation of the diffractaic acid was carried out by comparison of spectral data (IR, ^{1}H- and ^{13}C- nmr) with literature values[26, 27] and with the spectral data of an authentic sample of diffractaic acid.



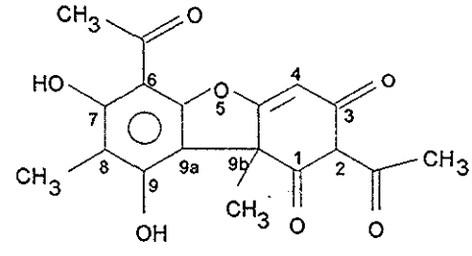
R = H Atranorin (I)
R = Cl 5-Chloroatranorin(II)



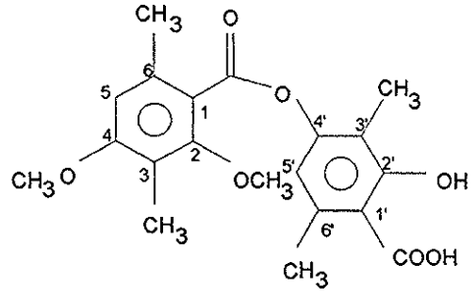
Lecanoric acid (III)



Ethyl orsellinate (IV)



Usnic acid (V)



Diffractaic acid (VI)

The results of the bioautography test using the fungus *Cladosporium sphaerospermum* (Table I), showed that atranorin/chloroatranorin and usnic acid are active in concentrations up to 1 μg. Lecanoric acid was not active at the concentrations tested. Ethyl orsellinate presented an inhibition zone larger that the other compounds at a concentration of 10 μg, however it was inactive in concentration of 1.0 μg . Although

Table 1. Activities of Compounds Isolated from Lichens Against *Cladosporium sphaerospermum*.

Compounds concentration (μg)	Atranorin (I) / Chloroatranorin (II)	Lecanoric acid (III)	Ethyl orsellinate (IV)	Usnic acid (V)	Diffractaic acid (VI)
	inhibition halo diameter (mm)				
10	11.0	0.0	15.0	8.0	4.0
5	9.0	0.0	8.0	8.0	3.0
1	8.0	0.0	0.0	7.0	0.0
0.1	0.0	0.0	0.0	0.0	0.0

the lichen compounds and phenolic derivatives are known to have activities against several microorganisms, the results here presented about the fungitoxic activities of lichenic compounds, and by the technical artifact product, ethyl orsellinate, suggest the need of additional investigations in order to evaluate the use of these compounds, or their derivatives, in the treatment of infections caused by these microorganisms.

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