Search for Antifungal Compounds from the Wood of Durable Tropical Trees

Alice M. S. Rodrigues,[†,‡] Phellipe N. E. T. Theodoro,[‡] Véronique Eparvier,[†] Charlie Basset,[†,‡] Maria R. R. Silva,[§] Jacques Beauchêne,[⊥] Laila S. Espíndola,*[,†,‡] and Didier Stien*[,†]

CNRS, UMR ECOFOG, Université des Antilles et de la Guyane, 97300 Cayenne, France, Laboratório de Farmacognosia, Universidade de Brasília, Brasília, Brazil, Instituto de Patologia Tropical e Saúde Pública, Universidade Federal de Goiás, Goiânia, Brazil, and CIRAD, UMR ECOFOG, BP 709, 97387 Kourou, France

Received March 4, 2010

Research on antifungal compounds from the durable wood from French Guiana Amazonian forest trees highlights the correlation between the activity of their extracts against wood-rotting fungi and human pathogens. The fractionation of an ethyl acetate extract of *Sextonia rubra* wood led to the isolation of rubrenolide (**1**) and rubrynolide (**2**). The potential of compounds **1** and **2** is described through the evaluation of their activity against 16 pathogenic fungi and their cytotoxicity toward NIH-3T3 mammalian fibroblast cells.

Infectious diseases, particularly those affecting the skin and mucosal membranes, are a serious problem worldwide, especially in tropical and subtropical developing countries. Several of these infections are caused by fungi, among which dermatophytes and yeasts are the most frequent.[1] In the past decade, the number of immunosuppressed patients has increased dramatically. Such patients frequently develop opportunistic systemic and superficial mycoses such as aspergillosis, candidiasis, and filamentous fungi infections.[2−4] Today, the drugs available for these diseases suffer from a number of drawbacks, such as low potency, poor solubility, the emergence of resistant strains, and toxicity.[5,6] These factors demonstrate that novel antifungal compounds with greater efficacy are urgently needed.

In the Amazonian forest of French Guiana, some woody species are commercialized for residential construction and other outdoor applications because of their excellent resistance to decay. Technological data demonstrating the durability of woods have been reported for many species, including Amazonian species.[7] Complete data are available from our group, and partial data may be accessed through the Mariwenn[8] (http://ecofog.cirad.fr/Mariwenn/) and Tropix (http://tropix.cirad.fr/) databases. The objective of the present work was to evaluate whether or not commercial waste from durable wood in French Guiana contains antifungal compounds potentially useful against dermatophytes and yeasts. This type of strategy may increase the value of forest-based residues and could lead to a sustainable and rational exploitation of metabolites of therapeutic value, thus preserving biodiversity and protecting its potential biotechnological and medical arsenal.

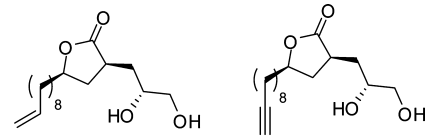
Fourteen species (Table S1, Supporting Information) showed activity against the wood-rotting fungi *Pycnoporus sanguineus* and *Gloeophyllum trabeum*. Among them, nine species were selected, and their minimal inhibitory concentrations (MIC values, in μg/mL) have been measured for dermatophytes and yeasts (Table S2, Supporting Information).

One of the most potent MIC values (both 3.9 μg/mL) was obtained from the ethyl acetate extract of *Sextonia rubra* (Mez) van der Werff (Lauraceae) sapwood and heartwood against *C. albicans* ATCC 10231. In addition, the ethyl acetate extract of *S. rubra* sapwood and heartwood showed activity against *C. glabrata* LMGO 44 (7.81 μg/mL), *C. krusei* LMGO 174 (15.62 μg/mL), *M. gypseum* LMGO 10 (15.62 μg/mL), and *M. canis* LMGO 22 (125 μg/mL).

Table 1. Antifungal Activity (MIC, μg/mL) of Rubrenolide (**1**) and Rubrynolide (**2**)

strain	**1**	**2**	itraconazole	fluconazole
Microsporum gypseum LMGO 10	16	32	0.25	16
Microsporum gypseum LMGO 533	256	32	0.975	64
Microsporum canis LMGO 22	128	64	16	>64
Microsporum canis LMGO 02	16	32	16	>64
Tricophyton rubrum LMGO 4218	16	32	0.5	32
Tricophyton rubrum LMGO 06	8	32	0.125	4
Tricophyton rubrum LMGO 08	8	32	0.125	8
Tricophyton mentagrophytes LMGO 1931	64	>256	0.5	4
Tricophyton mentagrophytes LMGO 09	8	32	0.25	>64
Candida albicans ATCC 10231	64	128	0.5	1
Candida albicans LMGO 102	16	32	>16	>64
Candida parapsilosis ATCC 22019	32	128	0.25	4
Candida parapsilosis LMGO 05	32	128	0.25	4
Candida glabrata LMGO 44	4	32	0.5	8
Candida krusei LMGO 174	64	64	16	>64
Cryptococcus gattii LMGO L1	256	128	2	16

S. rubra was chosen initially for the isolation of active compounds. The fractionation of *S. rubra* sapwood and heartwood ethyl acetate extract allowed the known lactones rubrenolide (**1**) and rubrynolide (**2**) to be isolated.[9−11] The biological activities of these compounds have not been reported in the literature. These compounds were tested against nine strains of dermatophytes and seven yeasts and displayed activities ranging from 4 to 256 μg/mL (Table 1). These two lactones comprise a very high proportion of the ethyl acetate extract.



1 **2**

Finally, we sought to determine the cytotoxicity of rubrenolide (**1**) and rubrynolide (**2**) in NIH-3T3 mammalian fibroblasts cells. Both **1** and **2** displayed rather low cytotoxicities, with IC_{50} values > 100 μg/mL in each case. The present work suggests that rubrenolide (**1**) has some potential as a lead compound in the search of new antifungal agents. In addition, its very high potency against dermatophytes should encourage the use of *in vivo* bioassays under topical administration, thereby limiting the risk of side effects related to its possible general toxicity.

* To whom correspondence should be addressed. Tel: +594 594 29 75 17. Fax: +594 594 28 47 86. E-mail: didier.stien@guyane.cnrs.fr (D.S.). Tel: +55 61 3307 3046. Fax: +55 61 3107 1943. E-mail: darvenne@unb.br (L.S.E.).

† CNRS Cayenne.

‡ Universidade de Brasília.

§ Universidade Federal de Goiás.

⊥ CIRAD, Kourou.

10.1021/np1001412 © 2010 American Chemical Society and American Society of Pharmacognosy
Published on Web 10/01/2010

Experimental Section

General Experimental Procedure. Optical rotations were measured on a Jasco P-1010 polarimeter. NMR spectra were recorded on a Varian 400MR spectrometer equipped with a 5 mm Auto X PGF $^{1}H/^{15}N-^{31}P$ inverse detection probe. NMR spectra were recorded at 400 MHz for ^{1}H and at 100.6 MHz for ^{13}C. Electrospray ionization high-resolution mass spectra were measured on a quadrupole-time-of-flight instrument (UltrOTOF-Q, Bruker Daltonics, Billerica, MA). Solvents were distilled prior to extraction and chromatography.

Plant Material. Plant materials (stem bark and wood) from 15 durable wood species belonging to eight families were collected from commercial forest waste in July 2007 at Régina, French Guiana. When possible, sapwood and heartwood were separated, and for wide trunks, internal and external heartwoods were separated as well. All species are well known and used in the building industry because of their exceptional durability against wood-rotting fungi (i.e., with natural durabilities against fungi rated 1 to 3 on a scale of 5, class 1 being the most durable). Botanists Marie-Françoise Prévost and Sophie Gonzalez performed botanical identifications at the French Guiana Herbarium (CAY), where a voucher specimen of each plant has been deposited (Table S1, Supporting Information).

Extraction and Isolation. Plant material was dried at room temperature and ground into powder. Then, 200 g from each sample was extracted three times at room temperature for 48 h with ethyl acetate (3 × 500 mL) and then twice for 48 h with methanol (2 × 500 mL). After filtration, fractions from each solvent were combined and concentrated to dryness under reduced pressure at a temperature below 30 °C. Crude extracts (Table S1, Supporting Information) were tested against the xylofagous fungi *Pycnoporus sanguineus* L. (Murrill) 270 (CTFT) and *Gloeophyllum trabeum* (Pers.) Murrill BAM Ebw 109 in a qualitative agar well perforation bioassay.[12]

The ethyl acetate extract from *S. rubra* sapwood + heartwood (extraction yield 4.2%, 2.00 g) was purified by column chromatography (EtOAc, then MeOH). The ethyl acetate eluate was evaporated and triturated with hexane. The insoluble portion was collected by filtration and dried under vacuum. NMR spectroscopy demonstrated that this fraction contained a mixture of two known compounds, rubrenolide (**1**) and rubrynolide (**2**) (1:1, 700 mg, 1.5% from the wood).[9−11] This material retained biological activity in repeated assays, whereas no activity was detected in the other fractions. Separation of the two compounds was achieved according to the procedure described by Thijs and Zwanenburg,[11] and NMR, HRMS, and optical rotation measurements allowed the confirmation of structural assignments by comparison with literature data.

Rubrenolide (1): white solid, MW 298.4, $[\alpha]^{24.8}_{D}$ +35 (*c* 0.23; $CHCl_3$) [lit.[9] $[\alpha]^{22}_{D}$ +21 ($CHCl_3$)]; HREIMS *m*/*z* $[M + H]^+$ 299.2215 (calcd for $C_{17}H_{31}O_4^+$, 299.2217).

Rubrynolide (2): white solid, MW 296.4, $[\alpha]^{24.5}_{D}$ +29 (*c* 0.2; $CHCl_3$) [lit.[9] $[\alpha]^{22}_{D}$ +21 ($CHCl_3$)]; HREIMS *m*/*z* $[M + H]^+$ 297.2079 (calcd for $C_{17}H_{29}O_4^+$, 297.2060).

Biological Testing. Fungi. The species of human pathogenic microorganisms used in this study were filamentous dermatophytes and yeasts. The former are *Microsporum gypseum* (*M.g.* LMGO 10 and *M.g.* LMGO 533), *Microsporum canis* (*M.c.* LMGO 02 and *M.c.* LMGO 22), *Trichophyton rubrum* (*T.r.* LMGO 06, *T.r.* LMGO 08 and *T.r.* LMGO 4218), and *Trichophyton mentagrophytes* (*T.m.* LMGO 09 and *T.m.* LMGO 1931). Among the latter are the following: *Candida albicans* (*C.a.* ATCC 10231 and *C.a.* LMGO 102), *Candida parapsilosis* (*C.p.* ATCC 22019 and *C.p.* LMGO 05), *Candida glabrata* (*C.g.* LMGO 44), *Candida krusei* (*C.k.* LMGO 174), and *Cryptococcus gattii* (*C.g.* LMGO L1). LMGO (Laboratório de Micologia de Goiás) strains are clinical isolates from patients at the Federal University of Goiás Hospital. Strains were maintained on potato dextrose agar. All strains were cultured onto an appropriate new agar plate at 28 °C for 2 days (yeasts) or 5 days (filamentous fungi) prior to any antimicrobial test.

Antimicrobial Assay by Microdilution Method. The standard microdilution test as described by the Clinical and Laboratory Standards Institute guidelines (M27-A2 and M38-A) was used to determine minimal inhibition concentrations (MIC) against dermatophyte fungi and yeasts.[12] Crude extracts and pure compounds were tested at concentrations ranging from 500 to 0.98 μg/mL and from 256 to 0.5 μg/mL, respectively. Fluconazole and itraconazole were used as positive controls and were tested at concentrations of 64 to 0.125 μg/mL and 16 to 0.031 μg/mL, respectively. The microplates were incubated at 32 °C, and results were observed after 5 days for filamentous fungi and 2 days for yeasts. The MIC values reported in Table S2 (Supporting Information) refer to the lowest concentration preventing visible growth in the wells. All assays were conducted in triplicate.

Cytotoxicity Evaluation on Mammalian Cells. Cytotoxicity was evaluated over a concentration range of 300 to 9.4 μg/mL for NIH-3T3 cells.[13]

Acknowledgment. The authors gratefully acknowledge the Programme Amazonie du CNRS (France), CAPES, CNPq, and FAPDF (Brazil) for financial support, as well as E. Louisanna (INRA, UMR Ecofog) for her kind help with this program.

Supporting Information Available: Selected species and extraction yields, minimal inhibitory concentrations of active plant extracts against dermatophytes and yeasts, and ^{1}H and ^{13}C NMR spectra of compounds **1** and **2**. This material is available free of charge via the Internet at http://pubs.acs.org.

References and Notes

(1) Hay, R. J. *Clin. Dermatol.* **2006**, *24*, 201–212.
(2) Gabardi, S. D.; Kubiak, W.; Chandraker, A. K.; Tullius, S. G. *Transplant Int.* **2007**, *20*, 993–1015.
(3) Nucci, M.; Marr, K. A. *Clin. Infect. Dis.* **2005**, *41*, 521–526.
(4) Pfaller, M. A.; Pappas, P. G.; Wingard, J. R. *Clin. Infect. Dis.* **2006**, *43*, S3–S14.
(5) Martinez-Rossi, N. M.; Peres, N. T. A.; Rossi, A. *Mycopathologia* **2008**, *166*, 369–383.
(6) Sanglard, D.; Odds, F. C. *Lancet Infect. Dis.* **2002**, *2*, 73–85.
(7) Scheffer, T. C.; Morrell, J. J. *Natural Durability of Wood: a Worldwide Checklist of Species*; Research Contribution 22, Forest Research Laboratory, Oregon State University: Corvallis, OR, 1998.
(8) Ollivier, M.; Baraloto, C.; Marcon, E. *Ann. For. Sci.* **2007**, *64*, 781–786.
(9) Franca, N. C.; Gottlieb, O. R.; Coxon, D. T. *Phytochemistry* **1977**, *16*, 257–262.
(10) Fujioka, H.; Ohba, Y.; Hirose, H.; Nakahara, K.; Murai, K.; Kita, Y. *Tetrahedron* **2008**, *64*, 4233–4245.
(11) Thijs, L.; Zwanenburg, B. *Tetrahedron* **2004**, *60*, 5237–5252.
(12) Melo e Silva, F.; de Paula, J. E.; Espindola, L. S. *Mycoses* **2009**, *52*, 511–517.
(13) Albernaz, L. C.; Paula, J. E.; Romero, G. A. S.; Grellier, P.; Silva, M. R. R.; Mambu, L.; Espindola, L. S. *J. Ethnopharmacol.* **2010**, *131*, 116–121.

NP1001412