ISSN: 0975-8585

Research Journal of Pharmaceutical, Biological and Chemical Sciences

5-Alkylresorcinols From *Roupala montana* Aubl. Wood Residues And Their Leishmanicidal And Antifungal Activities.

Daniela Roque de Sousa Rodrigues[1], Welem de Oliveira Santiago[1], Henrique Cativo dos Santos[1], Claudete Catanhede do Nascimento[2], João Vicente Braga de Souza[3], Ana Claúdia Alves Cortez[3], Bruno Bezerra Jensen[4], Claudia Dantas Comandolli-Wyrepkowski[4], Antonia Maria Ramos Franco[4], and Maria da Paz Lima[1]*.

[1]Laboratório de Química de Produtos Naturais, Instituto Nacional de Pesquisas da Amazônia, Manaus, 69067-375, Amazonas, Brazil.
[2]Laboratório de Tecnologia da Madeira, Instituto Nacional de Pesquisas da Amazônia, Manaus, 69067-375, Amazonas, Brazil.
[3]Laboratório de Micologia Médica, Instituto Nacional de Pesquisas da Amazônia, Manaus, 69067-375, Amazonas, Brazil.
[4]Laboratório de Leishmaniose e Doenças de Chagas, Instituto Nacional de Pesquisas da Amazônia, Manaus, 69067-375, Amazonas, Brazil.

ABSTRACT

Phytochemical studies of the Proteaceae family show the predominance of 5-alkylresorcinol derivatives metabolites with pharmacological activities. In this paper, fractionation of the methanolic extract of *Roupala montana* (Aubl) wood residues provided the resorcinol derivatives bilabol 15:1 **(1)**, ethyldehydrogravipane (**2**), 5-[14"-1',3'-dihydroxyphenyl)-*cis*-tetradec-8'-ene-1yl] resorcinol (**3**), dehydrogravipane (**4**) and bis-norstriatol (**5**). Compound **2** showed potent activity against the fungi *Cryptococcus neoformans* and *C. gattii* with a MIC of 5 and 10 μg.mL^{-1}, respectively, and also inhibited the promastigote form of *Leishmania amazonensis* (IC_{50} = 47.0 μg.mL^{-1}). This study was an opportunity for the increase of knowledge of its secondary metabolism as well as to find active principles of an Amazonian specimen of *R. montana*.

Keywords: Proteaceae, *Leishmania amazonenses, Cryptococcus neoformans*, *C. gattii, Leishmania amazonensis*

https://doi.org/10.33887/rjpbcs/2022.13.3.20

Corresponding author

ISSN: 0975-8585

INTRODUCTION

Proteaceae is an archaic family dating back approximately 96-85 million years [1] and comprises 80 genera and over 1700 species [2], whose diversity in Brazil is focused on the Atlantic Forest and the Amazon Rainforest [3]. Phytochemical studies show the predominance of 5-alkylresorcinol metabolites of the family which are glucosides of 5-alkylresorcinols from *Grevillea* [4]; bis-5-alkylresorcinols derivatives with a 20-membered skeleton (cyclophane-type) from *Kermadecia* [5-7) and *Grevillea* [8]; and compounds with a saturated or unsaturated polymethylene long-chain connecting two resorcinol residues identified from species of *Grevillea* [8-12], *Panopsis* [13] and *Heliciopsis* [14]. Some of these derivatives showed important pharmacological activities [5-8].

Roupala montana (Aubl) is known as "*Brazilian leopardwood*" due to the mottled grain on the radial face of the wood. One phytochemical study of the aerial parts of the species that were collected in the southeast of Brazil revealed the glycosylated flavonoids quercetin and isorhamnetin [15]. In this paper, we identified for the first time the resorcinol derivatives from the wood residues of an Amazonian specimen of *R. montana* and evaluated its activity against *Leishmania amazonensis* and the fungi *Candida albicans, Cryptococcus neoformans* and *Cryptococcus gattii*.

MATERIALS AND METHODS

General experimental procedures

NMR spectra were measured in a spectrometer (Bruker Fourier-300) with TMS as the internal standard. LC-HRMS measurements were obtained using a mass spectrometer (MicroTOF-QII, Bruker Daltonics) connected to a liquid chromotagraph (Prominence UFLC Shimadzu). Chromatographic separations were carried out on silica gel 60 (Merck), microcrystalline cellulose (Merck) and Sephadex LH-20 (Sigma). Analytical TLC was performed with silica gel 60 F254 (0.25 mm) pre-coated aluminum sheets (Merck) and spots were visualized using UV light (254 and 365 nm), and by spraying with vanillin-sulphuric acid.

Woody residues and extraction

Wood residues of *R. montana* obtained from a forest management area of National Institute of Amazonian Research (INPA) were identified through macroscopic comparisons with standard samples cataloged and incorporated into à xiloteque of Wood Anatomy and Identification Laboratory. Samples of the evaluated wood (2 x 2 x 3 cm blocks) are kept at the Wood Technology Laboratory. The largest residues were evaluated for their technological properties, and the smallest residues were used for phytochemical studies. Extraction was performed by macerating the residue samples with hexane, followed by methanol.

Chromatographic fractionation of methanolic extract

The fractionation of the methanolic extract (80.1 g) in the silica gel column (70-230 mesh), and eluted with hexane, hex-EtOAc (2-30%), yielded sixteen fractions. The grouped fractions 6-7 (RMM-6; 648 mg), 9 (RMM-9; 6.49 mg) and 16-17 (RMM-16; 4.84 g) were subjected to a new chromatographic fractionations. The fractionation of RMM-6 in a silica gel column (230-400 mesh) eluted with hex-EtOAc (2-50%), was followed by a Sephadex LH-20 column eluted with MeOH to yield compound **1** (294 mg). RMM-9 was subjected to a column of Sephadex LH-20, eluted in MeOH, subfractions 3 and 5 were subjected to new fractionations. Subfraction 3 was fractionated in a silica gel column (230-400 mesh) and eluted with hex:acetone (8:2) followed by a microcrystalline cellulose column and eluted with hexane-purified compound **2** (17 mg). Fractionation of subfraction 5 was carried out in a silica gel column (230-400 mesh) and eluted with CH_2Cl_2:EtOAc (8:2), which provided the compounds **3** (35 mg) and **4** (10 mg). RMM-16 was subjected to a Sephadex LH-20 column and eluted with MeOH. The pigment-free subfractions were fractionated in a column of silica gel column (230-400 mesh) and eluted with CH_2Cl_2:EtOAc (98:2-8:2) and yielded compound **5** (366 mg).

Spectroscopic data of compounds

Bilobol 15:1 **(1)**. HRESIMS m/z 319.2624 $[M + H]^+$. ^1H NMR (300 MHz, Acetone-d6, J/Hz): 8.06 (s, OH), 6.18 (d, J = 1.8 Hz, H-4, 6), 6.17 (d, J = 1.8 Hz, H-2), 5.34 (m, H-8', 9'), 2.46 (t, J = 7.4 Hz, H-1'), 2.04 (m, H-7', 10'), 1.55 (m, H-2'), 1.31 (m, H-3'), 1.30 (m, H-4', 5', 6', 11', 12', H-13'), 0.87 (t, J = 6.8 Hz, H-15'). ^{13}C NMR (75 MHz, Acetone-d6): 158.39 (C-1, 3), 144.92 (C-5), 129.66, 129.59 (C-8', C-9'), 106.76 (C-4 and C-6), 100.00 (C-2), 35.70 (C-1'), 31.74 (C-13'), 31.19 (C-2'), 29.57-29.07 (C-3', 4', 5', 6', 11', 12'), 26.87 (C-7', C-10'), 22.44 (C-14'), 13.47 (C-15'). HSQC and HMBC (Acetone-d6): Text.

Methyldehydrogravipane (**2**). ESIMS m/z 423.17 $[M-H]^-$. ^1H NMR (300 MHz, Acetone-d6, J/Hz): 8.29 (s, OH, C-1,3), 6.91 (s, OH, C-1',3'), 6.42 (d, J = 2.2 Hz, H-4), 6.34 (d, J = 2.2 Hz, H-2). 6.29 (sl, H-4', 6'), 5.34 (m, H-8", 9"), 3.61 (s, OCH_3), 2.53 (t, J = 6.8 Hz, H-1''), 2.31(t, J = 6.5 Hz, H-14"), 1.96 (m, H-7", 10"), 1.88 (m, H-12"), 1.86 (m, H-6"), 1.61 (m, H-2"), 1.52-1.29 (m, H- 4", 5",11'), 1.49 (m, H-13"), 1.29 (m, H-3"). ^{13}C NMR (75 MHz, Acetone-d6): 159.52 (C-1), 158.21 (C-3), 155.64 (C-1', 3'), 145.66 (C-5), 142.29 (C-5'), 129.73, 129.51 (C-8", 9"), 111.66 (C-6), 108.88 (C-2'), 107.74 (C-4), 107.04 (C-4',6'), 97.01(C-2), 54.74 (OCH_3), 34.96 (C-1''), 33.50 (C-14"), 31.24 (C-13"), 29.94 (C-2"), 29.73-27.55 (C-4", 5", 11'), 27.62 (C-12"), 26.43 (C-3"), 26.49 (C-7", 10"), 25.9 (C-6"). HSQC and HMBC: Text.

5-[14"-1',3'-Dihydroxyphenyl)-*cis*-tetradec-8'-ene-1yl]resorcinol (**3**). ESIMS m/z 413.20 $[M + H]^+$. ^1H NMR (300 MHz, Acetone-d6, J/Hz): 8.51 (s, OH C-3,3'), 8.25 (s, OH C-1,1'), 6.34 (t, J = 1.0 Hz, H-2, 2'), 6.29 (d, J = 2.0 Hz, H-4,6), 6.26 (d, J = 2.0 Hz, H-4', 6'), 5.35 (m, H-8", 9"), 2.49 (t, J = 6.5, H-1", 14"), 2.01 (m, H-2"), 1.99 (m, H-3", 6", 12"), 1.57 (m, H-7", 10", 13"), 1.52-1.26 (m, H-4", 5", 11"). ^{13}C NMR (75 MHz, Acetone-d6): 159.96 (C-1,3), 158.46 (C-1',3'), 144.51 (C-5,5'), 129.95, 129.55 (C- 8" ,9"), 108.53 (C-4,6), 107.03 (C-4',6'), 101.53 (C-2,2'), 35.11 (C-1'', 14"), 30.26 (C- 7", 10", 13"), 29.73-27.38 (C- 4", 5",11'), 27,03 (C-2"), 25.70 (C-3", 6", 12"). HSQC and HMBC: Text.

Dehydrogravipane (**4**). ESIMS m/z 409.42 $[M-H]^-$. ^1H NMR (300 MHz, Acetone-d6, J/Hz): 8.16 (s, OH, C-3), 7.17 (s, OH, C-1), 7.13 (s, OH, C-1',3'), 6.34 (d , J = 2.3 Hz, H-4), 6.31 (sl, H-4', 6'), 6.27 (d , J = 2.3 Hz, H-2), 5.31 (m, H-8", 9"), 2.53 (t, J = 7.5 Hz, H-1''), 2.30 t (t, J = 7.5 Hz, H-14"), 1.91 (m, H-7", 10"), 1.89 (m, H-6"), 1.88 (m, H-12"), 1.87 (m, H-2"), 1.65 (m, H-13"), 1.28 (m, H-3"), 1.52-1.27 (m, H-4", 5",11'). ^{13}C NMR (75 MHz, Acetone-d6): 156.10 (C-1), 158.06 (C-3), 156.10 (C-1'), 155.10 (C-3'), 145.60 (C-5), 143.05 (C-5'), 129.76, 129.47 (C-8", 9"), 109.84 (C-6), 107.54 (C-2'), 107.23 (C-4, 4', 6'), 100.39 (C-2), 35.00 (C-1''), 33.64 (C-14"), 31.25 (C-13"), 29.90 (C-12"), 29.72-27.55 (C-4", 5", 11"), 27.93 (C-2"), 26.46 (C-7", 10"), 26.43 (C-3"), 25.60 (C-6"). HSQC and HMBC: Text.

Bis-norstriatol (**5**). ESIMS m/z 415.2823 $[M + H]^+$. ^1H NMR (300 MHz, Acetone-d6, J/Hz): 8.15 (s, OH), 6.18 (d, J = 2.0 Hz, H-4, 6, 4', 6'), 6.16 (d, J =1.8 Hz, H-2, 2'), 2.45 (t, J = 7.41 Hz, H-1", 14"), 2.05 m (H-3", 12"), 1.55 m (H-2", 13"), 1.30-1.28 m (H-4" to H-11"). ^{13}C NMR (75 MHz, Acetone-d6): 158.39 (C-1, 3, 1', 3'), 144.94 (C-5, 5'), 106.76 (C-4, 6, 4',6'), 99.99 (C-2, 2'), 35.69 (C-1", 14"), 31.20 (C-2", 13"), 29.37 (C-3", 12"), 29.74-28.20 (C-4" to C-11"). HSQC and HMBC.

Antifungal assay

Antifungal susceptibility was performed using *Cryptococcus neoformans* (ATCC 90112), *Cryptococcus gattii* (ATCC 32269) and *Candida albicans* (ATCC 6019) obtained from the culture collection at the National Institute of Amazonian Research, Manaus, Amazonas, Brazil. Minimum inhibitory concentration (MIC) assays were performed using the broth microdilution method, as described by the Clinical and Laboratory Standards Institute [16]. Amphotericin B and Fluconazole were used as the antifungal standards.

Leishmanicidal assay

The effects of the 5-alkylresorcinols derivatives were evaluated using promastigote forms of *L. amazonensis* (MHO/BR/2009/IM5584) obtained from the National Institute of Amazonian Research, Manaus, Amazonas, Brazil. The bioassays were performed in 96-well plates using promastigote forms as described by Fumarola et al. [17]. Pentamidine isethionate (Pentacarinat®) was used as a positive control. Assays were carried out in triplicate and repeated twice.

ISSN: 0975-8585

RESULTS AND DISCUSSION

Identification of compounds 1–5

The ^{1}H NMR spectrum of compound **1 (**Figure 1)showed signals of three aromatic hydrogens at δ 6.17 (1H) and 6.18 (2H) with coupling constants of 1.8 Hz, as well as signals of a monounsaturated aliphatic chain with olefinic hydrogens at δ 5.34, methylenes at δ 2.46-1.30 and methyl at δ 0.87. The ^{13}C NMR spectrum, together with the DEPT, indicated the presence of aromatic, olefin and alkane carbons. Methylene groups from the side chain had their chemical shifts of ^{1}H and ^{13}C attributed based on HSQC and HMBC experiments. Thus, **1** is a (*Z*)-pentadecenyl resorcinol known as bilobol (15:1), which is an alkylresorcinol that is relatively rare in nature, and its main source is *Ginkgo biloba* [18] – one of the most famous medicinal plants in the world.

The ^{1}H NMR spectrum of **2** showed signals of aromatic *m*-coupled hydrogens at δ 6.42 (H-4) and 6.34 (H-2) and symmetrical hydrogens at δ 6.29 (H-4' and H-6'); signals for long-chain methylenes (δ 2.56-1.25), and olefinic hydrogens (δ 5.34), in addition to the signal of a methoxy group. The ^{13}C NMR spectrum and DEPT indicated the presence of aromatics, olefins, methylenes and methoxyl. The HMBC correlations between δ 2.56 (H-1") and δ 142.29 (H-5') and 107.04 (H-4' and H-6'), as well as between δ 2.31 (H-14") and δ 145.66 (C-5), 111.66 (C-6) and 107.74 (C-4), shows the connectivity of resorcinols to the long chain. The NMR spectra data in 1D (^{1}H, ^{13}C and DEPT) and 2 D (HSQC and HMBC) of compound **4** were similar to that of **2** except for the absence of methoxyl. The HMBC experiment showed the correlation between the hydroxyl hydrogen (δ 8.16) positioned at C-1 and the carbons at δ 158.06 (C-3), 107.23 (C-4) and 100.39 (C-2). Compounds **2** and **4** were identified as methyldehydrograviphane and dehydrograviphane, respectively reported from *Gravillea robusta* [8].

The NMR data of **3** suggested an *n*-alkylresorcinol with an unsaturated polymethylene chain connecting two resorcinol residues identified as 5-[14"-1',3'-dihydroxyphenyl)-*cis*-tetradec-8'-ene-1yl]resorcinol. The NMR data of **5** were similar to **3,** though with a saturated polymethylene chain compatible for bis-norstriatol, and both compounds have been previously isolated from *Gravillea robusta* [9].

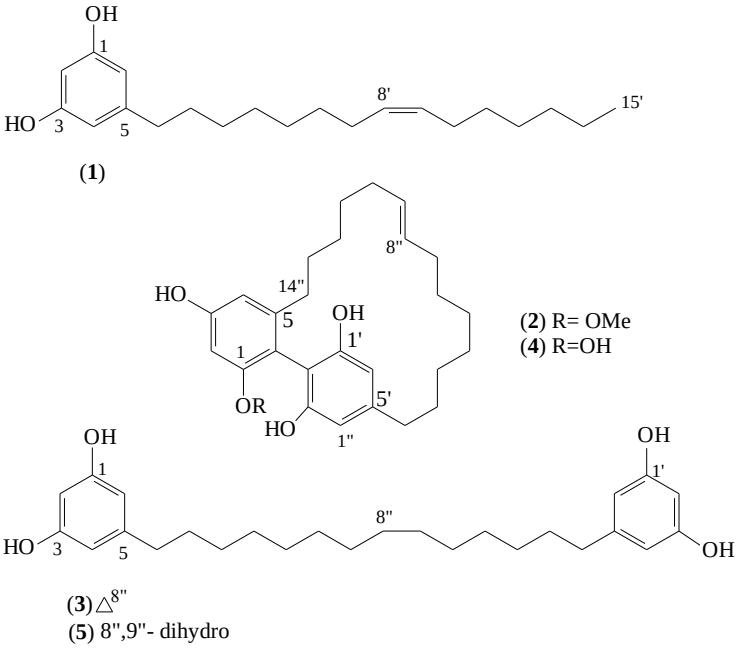

Figure 1: Structures of the 5-alkylresorcinols 1–5.

Leishmanicidal and antifungal activities of compounds

Bilobol 15:1 (**1**) and methyldehydrograviphane (**2**) showed potent activity against the fungi *Cryptococcus neoformans* and *C. gattii* (Table 1). The structure of molecule **2,** containing bisresorcinol connected by a long cyclic unsaturated polyemethylene chain, was much more active for the two fungal

species with a MIC of 5 and 10 µg.mL^{-1}, respectively. This molecule was also the most active molecule (IC_{50} = 47.0 µg.mL^{-1}) for the promastigote form of *Leishmania amazonensis* (Table 2). The other alkylresorcinols identified from *R. montana* have moderate leishmanicidal activity, based on the classification profile of leishmanicidal activity [19]. The presence of the methoxyl group on the aromatic ring may be an explanation for the greater activity exhibited by compound **2,** which by being more lipophilic, contributed to greater affinity with the membranes of the fungi and protozoa. The polymethylene chain containing unsaturation also seems to contribute to leishmanicidal activity because the saturated compound **5** was the least active among the tested alkyresorcinols.

Table 1: MIC (µg.mL^{-1}) of compounds 1 and 2 against fungal strains

Compounds	*Candida albicans*	*Cryptococcus neoformans*	*Cryptococcus gattii*
1	>320	40	20
2	>320	5	10
Fluconazole	0.125	4	4
Anfotericin B	0.5	0.065	0.250

Table 2: Effect of the alkyresorcinols 1-5 against *Leishmania amazonensis*

Compounds	IC_{50} (µg.mL^{-1})
1	67.9
2	47.0
3	72.0
4	67.9
5	89.0
Pentamidine isethionate	< 0.63

CONCLUSION

Phytochemical and biological research on wood residues from *Roupala montana* resulted in the identification of 5-alkylresorcinol derivatives with leishmanicidal and antifungal activities. Thus, this study was an opportunity for the increase of knowledge of its secondary metabolism as well as to find active principles of an Amazonian specimen of *R. montana*.

ACKNOWLEDGMENTS

The authors are grateful for the support from the Fundação de Amparo à Pesquisa Estado do Amazonas (FAPEAM), Grant No 01.02.016301.03412/2021-78.

REFERENCES

[1] Anderson CL, Bremer K, Friis EM. American Journal of Botany 2005; 92(10): 1737-1748.
[2] Weston PH, Barker NP. Telopea 2006; 11(3): 314-344
[3] Prance GT, Pirani JR. [accessed 2021, May 28]. http://floradobrasil.jbrj.gov.br/reflora/listaBrasil.
[4] Yamashita Y, Matsunami K, Otsuka H, Shinzato T, Yoshio Takeda Y. Phytochemistry 2008; 69(15): 2749-2752.
[5] Beniddir MA, Simonin A-L, Martin, MT, Dumontet V, Poullain C, Gueritte F, Litaudon M. Phytochemistry Letters 2010; 3(2): 75-78.
[6] Khazem M, Jolly C. Gaslonde T, Massip S, Negrier P, Espeau P, Michel S, Litaudon M. European Journal of Organic Chemistry 2018; 42: 5819-5822

[7] Jolly C, Thoison O, Martin TM, Dumontet V, Gilbert A, Pfeiffer B, Leonce S, Sevent T, Gueritte F, Litaudon M. Phytochemistry 2008; 69(2): 533-540.
[8] Chuang TH, Wu PL. Cytotoxic 5-alkylresorcinol metabolites from the leaves of *Grevillea robusta*. Journal of Natural Products 2007; 70(2):319-323.
[9] Ahmed AS, Nakamura N, Meselhy MR, Makhboul MA, El-Emary N, Hattori M. Phenolic constituents from *Grevillea robusta. Phytochemistry* 2000; 53(1):149-154
[10] Wang H, Leach DN, Thomas MC, Blanksby SJ, Forster PI, Waterman PG. Natural Product Communications 2008; 3(1):57-64.
[11] Wang H, Leach DN, Thomas MC, Blanksby SJ, Forster PI, Waterman PG. Natural Product Communications 2009; 4(7): 951-958.
[12] Wang H, Leach DN, Thomas MC, Blanksby SJ, Forster PI, Waterman PG. Helvetica Chimica Acta 2011; 94(10): 1812-1819.
[13] Deng J-Z, Starck, SR, Hecht SM. Journal of Natural Products 1999; 62(3): 477-480
[14] Giang PM, Thao DT, Nga NT, Trung BV, Anh DH, Viet PH. Pharmaceutical and Chemical Journal 2019, 53(7): 628-634.
[15] Oliveira PF, Acésio NO, Leandro LF, Cunha NL, Uchôa CJM, Januário AH, Tavares DC. Drug and Chemical Toxicology 2014; 37(1): 93-99.
[16] CLSI Reference method for broth dilution antifungal susceptibility testing of yeasts: Approved standard, Clinical and Laboratory Standards Institute. *Pennsylvania* (USA): NCCLS (3rd Ed.), 2008.
[17] Fumarola L, Spinelli R, Brandonisio O. Research in Microbiology 2004; 155(4): 224-230.
[18] Wang D, Song X, Yan H, Guo M, Fu R, Jiang H, Zhua H, Wang X. RSC Advances 2018; 60(8): 34321-34330.
[19] Osorio E, Arango GJ, Jiménez N, Alzate F, Ruiz G, Gutiérrez D, Paco, MA, Giménez A, Robledo S. Journal of Ethnopharmacology 2007; 111(3): 630-635.