Chemical Constituents from the Roots of *Spathelia excelsa* and their Antiprotozoal Activity

Wagner A. dos Santos Moreira,* Maria da Paz Lima,* Antonio Gilberto Ferreira, Izabel C. Piloto Ferreira a and Celso V. Nakamura a

aCoordenação de Pesquisas em Produtos Naturais, Instituto Nacional de Pesquisas da Amazônia, CP 478, 69011-970 Manaus-AM, Brazil

bDepartamento de Química, Universidade Federal de São Carlos, CP 676, 13565 São Carlos-SP, Brazil

cDepartamento de Análises Clínicas, Universidade Estadual de Maringá, 87020-900 Maringá-PR, Brazil

A investigação fitoquímica das raízes de *Spathelia excelsa* levou ao isolamento das cromonas 10(2,3-epóxi-3-metilbutanil)spatheliacromeno e 10(2,3-dihidroxi-3-metilbutanil) metoxyspatheliacromeno (5-metoxyspatheliacromeno); o limonóide desacetilspathelin e epímero C-21 do protolimonóide 3β-angeloloirox-7α,24,25-trihidroxi-14,18-cicloapotirucal-21-hemiacetal; os alcalóides 7,8-dimetoxiflindersina, casimiroina e N-metil-4,7,8-trimetoxiquinolina-2(1H)-ona, além da mistura de β-sitosterol e stigmasterol. Nos ensaios biológicos sobre a forma promastigota de *Leishmania braziliensis*, desacetilspathelin apresentou atividade moderada e sobre a forma epimastigota de *Trypanosoma cruzi*, 10(2,3-epóxi-3-metilbutanil)spatheliacromeno apresentou atividade forte (IC50 = 11 µg mL−1).

Keywords: Rutaceae, Spathelia, Trypanosoma cruzi, Leishmania braziliensis

**Introduction**

*Spathelia* L. is a monotypic genus in the subfamily Spatheloideae (Rutaceae), that comprises about 15 species distributed in the Bahamas, Cuba, Jamaica and northern South America.1 Species of *Spathelia* resembles palm tree reaching up to 20 m, what distinguished them from other genus of Rutaceae. *Spathelia excelsa* (Krause) Cowan & Brizicky (sin. *Sohnreyia excelsa* K.), a hapaxant tree,2 is found only in the Brazilian Amazon. Quinolone alkaloids, limonoids, flavonoid and coumarin have been reported as constituents of *S. excelsa* leaves.3,4

In this paper we describe the isolation and structural identification of eight compounds from roots of this plant, and evaluate the trypanocidal and leishmanicidal activities of compounds 10(2,3-epoxy-3-methylbutanyl) spatheliachromen, deacetylspathelin, 3β-angeloloyx-7α,24,25-trihydroxy-14,18-cycloapotirucall-21-hemiacetal and casimiroin.

**Results and Discussion**

Chemical composition of the extracts

Investigation of the n-hexane and dichromethane combined extracts from *S. excelsa* roots yielded a mixture of...
Chemical Constituents from the Roots of *Spathelia excelsa* and their Antiprotozoal Activity

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Steroids (β-sitosterol and stigmasterol, 1), pyranochromones (2, 8), limonoid (3), protolimonoid (5), and alkaloids, pyranoquinolin-2(1\text{H})-one (4), and quinolin-2(1\text{H})-one (6, 7).

The $^1$H NMR spectrum (Table 1) of compounds 2 and 8 showed signals consistent with the presence of system 2,2-dimethylpyran, singlets for geminal methyls at $\delta$ 1.46 and 1.48 of 2, $\delta$ 1.47 and 1.50 of 8; a pair of doublets ($J$ 10 Hz) assigned to the olefinic hydrogen [2, $\delta$ 5.61 (H-3), 6.72 (H-4)] and [8, $\delta$ 5.72 (H-3), 6.72 (H-4)]. The signals of methyl doublet at $\delta$ 2.36 (2) and 2.29 (8), associated with hydrogen quartet at $\delta$ 6.02 ($J$ 0.72 Hz) 2, 5.98 ($J$ 0.8 Hz) 8, characterized the 2-methyl-\(\gamma\)-pyrone moiety of the chromone.

Chemical shifts of carbon in the PENDANT of 2 and $^{13}$C NMR of 8 (Table 1) were compared to those of *spatheliabischromen* (8a)5. In chromone 2, the group 2',3'-epoxy-3'-methylbutanyl attached to C-10 was determined by the presence of epoxy, which was confirmed by shifts at $\delta$ 59.1 (C) and 63.2 (CH). The HSQC experiment showed a correlation of this latter signal with hydrogen at $\delta$ 2.95. In the HBMC experiment (Table 2), we observed correlations between methylene hydrogen ($\delta$ 2.83 and 3.08) and carbons at $\delta$ 63.2 ($J$), 59.1, 155.1, 157.1 ($J$) and 103.3 ($J$). Chromone 2 is thus 10 (2,3-epoxy-3-methylbutanyl) spatheliachromen, reported for first time in the literature.

Spectral data ($^1$H and $^{13}$C NMR) comparison established that 8 was distinguished from 8a by the presence of methoxy group ($\delta$ 3.85 → 62.8, by HSQC) attached to C-5. Correlations observed in the HMBC experiment between methoxyl hydrogen and carbon at $\delta$ 153.8 (C-5), olefinic hydrogen $\delta$ 6.72 (H-4) with 153.8 (C-5), confirmed the position of this methoxy group at C-5, also showed the linearity of the pyranochromone 8 (5-methoxyspatheliabischromen).

The $^1$H NMR spectrum of 3 showed signals indicative of furan ring at $\delta$ 7.64 (dt), 6.51 (ddd) and 7.57 (m); five singlets in region $\delta$ 1.27-0.70 relative to the methyl groups. The singlets at $\delta$ 4.08 assigned to the H-15 protons are typical for limonoids with ring 14,15-epoxy D lactone. Data $^{13}$C NMR (Table 3) showed similarities with those reported for deacetylspathelin.3,6

The $^1$H NMR of compound 5 presented signals for oxymethine hydrogens at $\delta$ 4.84 (dd, H-3), 3.94 (sl, H-7), 4.52 (sl, H-23) and 3.62 (sl, H-24), hydrogen attached to a carbon hemiacetal at $\delta$ 5.34 (sl, H-21). $^{13}$C NMR spectrum compared with those of 5a7 and 5b8 indicated that protolimonoid 5 (Table 3) presents a mixture of epimers in C-23 and substitution angeloyl to C-3. The angelate group was evidenced by signals olefinic hydrogen at $\delta$ 6.01 (dd, J 7.2, 1.4, H-3'), methyls at $\delta$ 1.96 (dq, J 7.2, 1.4, Me-4') and $\delta$ 1.67 (s, Me-5'). HSQC
showed correlations of hydrogen at δ 4.84 and 3.94 with carbons at δ 81.0 (C-3) and 74.2 (C-7), respectively. In the HMBC experiment, the observed correlations of angelate group were of hydrogen at δ 4.84 (H-3), with carbon at δ 167.7 (C-1', 3J); hydrogen at δ 6.01 (H-3'), with carbons at δ 15.9 (Me-4', 2J) and 21.0 (Me-5', 3J); hydrogen at δ 1.96 (Me-4'), with carbons at δ 129.0 (C-2', 2J) and 167.7 (C-1', 3J).

Table 4 shows spectral data of 1H and 13C NMR of alkaloids 4, 6 and 7. The 1H NMR spectrum of 4 presented hydrogen assigned to the pyran ring at δ 5.44 and 6.68 (J =10 Hz), two methyls at δ 1.56, and aromatic hydrogens at δ 6.84 and 7.58 (J = 8.8 Hz). The carbon chemical shifts of 4 were compared with 8-methoxyflindersine9 and identified as 7,8-dimethoxyflindersine. Compound 6 was identified as casimiroin by comparing their spectral data (1H and 13C NMR) with those in the literature.10 The HSQC experiment showed the correlations between methylenedioxy hydrogens (δ 6.06, 2H) and carbon at δ 101.2. The HMBC correlations (Table 5) showed that there is no change in the position of the methylenedioxy group. The 1H and 13C NMR spectra of compound 7 were very similar to those of 6, except in substitution for C-7 and C-8. In the HSQC, the methyls at δ 3.96 and 3.78 correlated with carbons at δ 56.6 and 61.7, respectively. The HMBC experiment showed correlations which indicated the position of methoxyl groups at C-7 and C-8.

### Table 1. 1H (400 MHz) and 13C (100 MHz) chemical shifts for chromones 2 and 8, in CDCl3

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<th>No.</th>
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<td>3</td>
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### Table 2. HMBC (400/100 MHz) assignments for chromones 2 and 8, in CDCl3

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<td>1'</td>
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### Table 3. 13C (100 MHz) chemical shifts for limonoid 3 and protolimonoid 5, in (CD3)2CO

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<td>3'</td>
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<td>14.6</td>
<td>5'</td>
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Chemical Constituents from the Roots of *Spathelia excelsa* and their Antiprotozoal Activity

**Antiprotozoal activity evaluation**

In this study, the activity of compounds 2, 3, 5 and 6 against promastigote forms of *Leishmania braziliensis* was evaluated in different doses. In a concentration of 320 µg mL⁻¹, deacetylspathelin (3) and casimiroin (6) showed 45.6% and 42.5% inhibition, respectively, activities considered moderate. The chromone 2 had inhibition of 14.7% at 320 µg mL⁻¹, and protolimonoid (5) was not able to inhibit the parasite.

In the examination against epimastigotes forms of *Trypanosoma cruzi*, chromone 2 with IC₅₀ = 11 µg mL⁻¹ was the most active compound in the present study. The protolimonoid 5 showed moderate activity, 40.5% inhibition at 100 µg mL⁻¹ (Table 6).

Although this species presented botanical characteristics that differ from other of the Rutaceae, the compounds identified in this study are typical to this family. The compounds 2 and 8 are pyranochromones prenylated at C-10, a metabolite characteristic of *Spathelia*. Deacetylspathelin (3) is a characteristic limonoid of Rutaceae, A- and D-ring seco. Protolimonoids, as 5, which undergo apo-euphol rearrangement and contain a C-13, C-14 cyclopropane ring (glabretal-type compounds), has been encountered in families of Rutales (Meliaceae, Simaroubaceae and Rutaceae). Alkaloids 4, 6 and 7 are derivatives of anthranilic acid, a type that occurs widely in Rutaceae.

The results of assays against protozoan parasites showed significant activity in this study, mainly as trypanocidal. Further laboratory and clinical studies of the active compounds are required in order to understand their antiprotozoal principles.

**Experimental**

**General experimental procedures**

IR spectra were obtained in a Perkim Elmer Spectrum-2000 apparatus using KBr pellet samples. NMR spectra were measured in a Bruker DRX 400 apparatus;
chemical shifts (δ) were expressed in ppm, and coupling constants (J) in Hertz; TMS was used as internal standard. Low resolution EIMS was recorded on 2100, Varian. Column chromatography (CC) was performed with silica gel 60 (Merck, 70-230 and 230-400 mesh), cellulose (Merck), and sephadex LH-20 (Sigma). Analytical thin layer chromatography (TLC) was carried out with Merck Kieselgel 60 F254 (0.25 mm) plates.

Plant material

Spathelia excelsa was collected in the Adolpho Ducke Forest Reserve, Amazonas, Brazil, and a voucher (4227) was deposited in the Herbarium of the Instituto Nacional de Pesquisas da Amazônia (INPA), Manaus, AM.

Extraction and isolation of compounds

Dried and powdered roots of S. excelsa (735 g) were macerated at room temperature with n-hexane, then with CH₂Cl₂ and finally with MeOH. The hexane and CH₂Cl₂ combined extracts (14.9 g) were fractionated over silica gel (70-230 mesh; h x Φ = 23.0 x 4.5 cm), eluted with hexane, hexane:CH₂Cl₂ (9:1), CH₂Cl₂ and MeOH, to yield thirty fractions. The combined fractions 15-17 (RSE-8), 24-27 (RSE-9) and 28-30 (RSE-10) were rechromatographed. Fraction RSE-8 (2.5 g) was fractionated over silica gel (70-230 mesh; h x Φ = 16.0 x 4.5 cm), eluted with CH₂Cl₂:MeOH (1-10%), yielding twenty two subfractions. Subfractions 5-12 yielded a mixture of β-sitosterol, stigmasterol (1; 23.7 mg). Through column chromatography over sephadex LH-20 (h x Φ = 27 x 2 cm), eluted with CH₂Cl₂, CH₂Cl₂:acetone (2-100%), yielded compound 3 (33.3 mg).

Fraction RSE-9 (766 mg) was chromatographed on silica gel (230-400 mesh; h x Φ = 30 x 3 cm), eluted with hexane, hexane:EtOAc and EtOAc, eluted with EtOAc from the subfractions 13-20, after column chromatography over silica gel (230-400 mesh; h x Φ = 27 x 2 cm), eluted with CH₂Cl₂, CH₂Cl₂:EtOH (99:1), compound 2 (14 mg) was obtained.

RSE-9 (766 mg) was chromatographed on silica gel (230-400 mesh; h x Φ = 30 x 3 cm), eluted with hexane, hexane:CH₂Cl₂, CH₂Cl₂:acetone (1-10%), further recrystallized with hexane:acetone, eluted with hexane:acetone drops, producing compound 3 (68.8 mg) was again obtained from RSE-10A after cleaning with hexane and acetone drops. Compound 4 (21 mg) was obtained from RSE-10B, through column chromatography over silica gel (230-400 mesh), eluted with CH₂Cl₂, and CH₂Cl₂:acetone, after purification with hexane:acetone drops. Subfractions RSE-10C and RSE-10D yielded precipitates. These precipitates, which were purified with hexane:acetone drops, yielded compounds 5 (20.5 mg) and 6 (26.7 mg), respectively. Subfraction RSE-10E subjected to column chromatography over sephadex LH-20 (h x Φ = 25 x 2 cm), eluted with EtOAc, then subjected to column chromatography over silica gel (230-400 mesh), eluted with CH₂Cl₂, CH₂Cl₂:acetone (1-50%), yielded compound 7 (52 mg).

Compound 3 (68.8 mg) was again obtained from RSE-10A after cleaning with hexane and acetone drops. Compound 4 (21 mg) was obtained from RSE-10B, through column chromatography over silica gel (230-400 mesh), eluted with CH₂Cl₂, and CH₂Cl₂:acetone, after purification with hexane:acetone drops. Subfractions RSE-10C and RSE-10D yielded precipitates. These precipitates, which were purified with hexane:acetone drops, yielded compounds 5 (20.5 mg) and 6 (26.7 mg), respectively. Subfraction RSE-10E subjected to column chromatography over sephadex LH-20 (h x Φ = 25 x 2 cm), eluted with EtOAc, then subjected to column chromatography over silica gel (230-400 mesh), eluted with CH₂Cl₂, CH₂Cl₂:acetone (2-100%), yielded compound 8 (5 mg).

10-(2,3-Epoxy-3-methylbutanoyl)spatheliachromen (2)

Amorphous solid, mp 98.5-100º; IR ν max/cm⁻¹: 1663 (C=O), 1631(C=C), 1584, 1646, 764 (aromatic C=C); [M]⁺ m/z 342 (C₂₂H₂₂O₂). 1H NMR(400 MHz, CDCl3); see Table 1; COSY (400 MHz, CDCl₃), δ 6.72-5.61, 3.08-2.95, 3.08-2.83, 2.95-2.83; PENDANT (100 MHz,CDCl₃); see Table 1; HSQC (400/100 MHz, CDCl₃); see Discussion; HMBC (400/100 MHz, CDCl₃); see Table 2.

Deacetylspathelin (3)

Amorphous solid, mp 3458 (OH), 1740 (ester), 1715 (ketone). 1H NMR [400 MHz (CD₃)₂CO]: δ 7.64 (1H, dt, 1.6 e 0.8 Hz, H-21), 7.57 (1H, m H-23), 6.51 (1H, ddd, 1.8, 0.8, 0.3 Hz, H-22), 6.40 (1H, d, 12.4 Hz, H-1), 5.89 (1H, d, 12.4 Hz, H-2), 5.52 (1H, s, H-17), 4.99 (1H, d, 3.95 Hz, H-7), 4.39 (1H, d, 3.95 Hz, OH), 4.08 (1H, s, H-15), 3.69 (3H, s, OMe), 3.13 (3H, s, H-18), 1.27 (3H, s, H-19), 1.24 (3H, s, H-11), 1.94 (2H, m, H-12), 0.70 (3H, H-30). COSY [400 MHz, (CD₃)₂CO]: δ 6.40 (H-1) → δ 5.89 (H-2), δ 7.64 (H-21) → δ 6.51 (H-22), δ 7.57 (H-23) → H-22 (δ 6.51). 13C NMR [100 MHz, (CD₃)₂CO]; see Table 3.

7,8-Dimethoxyflindersin (4)

Amorphous solid; 1H NMR [400 MHz, CDCl₃]; see Table 4; 13C NMR (100MHz, CDCl₃); see Table 4.

3β-Angeloyloxy-7α,24,25-trihydroxy-21,23-oxide-14,18-cycloapotirucall-21-hemiacetal (5)

Amorphous solid, mp 208.9-210.9º; 1H NMR (400 MHz, C₅D₅N); see Discussion; 13C NMR (100 MHz, C₅D₅N); see Table 5.
Table 3; HSQC (400/100 MHz, C<sub>5</sub>D<sub>5</sub>N): see Discussion; HMBC (400/100 MHz, C<sub>5</sub>D<sub>5</sub>N): see Discussion.

**Casimiroin (6)**  
Amorphous solid; 1H NMR (400 MHz, CDCl<sub>3</sub>); see Table 4; 13C NMR (100 MHz, CDCl<sub>3</sub>); see Table 4; HSQC (400/100 MHz, CDCl<sub>3</sub>); see Discussion; HMBC (400/100 MHz, CDCl<sub>3</sub>); see Table 5.

**N-methyl-4,7,8-trimethoxyquinolin-2(1H)-one (7)**  
Amorphous solid; 1H NMR (400 MHz, CDCl<sub>3</sub>); see Table 4; 13C NMR (100 MHz, CDCl<sub>3</sub>); see Table 4; HSQC (400/100 MHz, CDCl<sub>3</sub>); see Discussion; HMBC (400/100 MHz, CDCl<sub>3</sub>); see Table 5.

10(2,3-Dihydroxy-3-methylbutanyl)-methoxyspatheliacromen (5-methoxyspatheliabromen) (8)  
1H NMR (400 MHz, CDCl<sub>3</sub>); see Table 1; 13C NMR (100 MHz, CDCl<sub>3</sub>); see Table 1; HSQC (400/100 MHz, CDCl<sub>3</sub>); see Discussion; HMBC (400/100 MHz, CDCl<sub>3</sub>); see Table 2.

**Leishmanicidal activity in vitro**

*L. braziliensis* promastigotes (LTB0016) were grown to a concentration of 4 x 10<sup>6</sup> cells mL<sup>-1</sup> in Schneider’s Drosophila medium, supplemented with 10% bovine fetal serum. The compounds (2, 3, 5 and 6) were added to the promastigote cultures (4 x 10<sup>6</sup> mL<sup>-1</sup>) at concentrations of 320 to 0.125 µg mL<sup>-1</sup>, solubilized in DMSO (the highest concentration used was 1.6%, v/v) and incubated at 25 ºC. After 24 h of incubation, the surviving parasites were counted in a Neubauer’s chamber and compared with controls which only had DMSO. All tests were done in triplicate, and pentamidine isethionate (Eurofarma) was used as reference drug. The LD<sub>50</sub>/24 values were determined by linear regression analysis from this inhibition percentage, using statistic error limits up to 10%.

**Trypanocidal activity in vitro**

Epimastigote forms of *T. cruzi* Y strain were grown in liver infusion tryptose (LIT) medium supplemented with 10% inactivated bovine fetal serum and assayed with different concentrations of compounds (2, 3, 5 and 6) which were dissolved in DMSO. Next, 1x10<sup>6</sup> protozoa per mL was introduced into 24-well plate each well containing 1 mL of diluted compound. Cell growth was determined by counting the parasites with a Neubauer haemocytometer after incubation for 96 h at 28 ºC. Assays were performed in duplicate.

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**Supplementary Information**

Supplementary data is available free of charge at http://jbcs.sbq.org.br, as PDF file.

**References**


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Chemical Constituents from the Roots of *Spathelia excelsa* and their Antiprotozoal Activity

Wagner A. dos Santos Moreira,a Maria da Paz Lima,*a Antonio Gilberto Ferreira,b Izabel C. Piloto Ferreirac and Celso V. Nakamuraa

*aCoordenação de Pesquisas em Produtos Naturais, Instituto Nacional de Pesquisas da Amazônia, CP 478, 69011-970 Manaus-AM, Brazil

bDepartamento de Química, Universidade Federal de São Carlos, CP 676, 13565 São Carlos-SP, Brazil

cDepartamento de Análises Clínicas, Universidade Estadual de Maringá, 87020-900 Maringá-PR, Brazil

Figure S1. ¹H NMR spectrum (400 MHz, CDCl₃) of 2.

*e-mail: mdapaz@inpa.gov.br
Figure S2. 1H-1H COSY spectrum (400 MHz, CDCl₃) of 2.

Figure S3. 13C Pendant spectrum (100 MHz, CDCl₃) of 2.
Figure S4. HSQC spectrum (400/100 MHz, CDCl₃) of 2.

Figure S5. HMBC spectrum (400/100 MHz, CDCl₃) of 2.
Figure S6. $^1$H NMR spectrum [400 MHz (CD$_3$)$_2$CO] of 3.

Figure S7. $^1$H-$^1$H COSY spectrum [400 MHz (CD$_3$)$_2$CO] of 3.
Figure S8. $^{13}$C NMR spectrum (100 MHz, CD$_3$CO) of 3.

Figure S9. $^1$H NMR spectrum (400 MHz, CDCl$_3$) of 4.
Figure S10. $^{13}$C NMR spectrum (100 MHz, CDCl$_3$) of 4.

Figure S11. $^1$H NMR spectrum (400 MHz, C$_5$D$_5$N) of 5.
Figure S12. $^1$H NMR spectrum (100 MHz, C$_5$D$_5$N) of 5.

Figure S13. $^1$H NMR spectrum (400 MHz, CDCl$_3$) of 6.
Figure S14. $^{13}$C NMR spectrum (100 MHz, CDCl$_3$) of 6.

Figure S15. HSQC spectrum (400/100 MHz, CDCl$_3$) of 6.
Figure S16. HMBC spectrum (400/100 MHz, CDCl$_3$) of 6.

Figure S17. $^1$H NMR spectrum (400 MHz, CDCl$_3$) of 7.
Chemical Constituents from the Roots of *Spathelia excelsa* and their Antiprotozoal Activity

**Figure S18.** $^{13}$C NMR spectrum (100 MHz, CDCl$_3$) of 7.

**Figure S19.** HSQC spectrum (400/100 MHz, CDCl$_3$) of 7.
**Figure S20.** HMBC spectrum (400/100 MHz, CDCl₃) of 7.

**Figure S21.** ¹H NMR spectrum (400 MHz, CDCl₃) of 8.
Figure S22. $^{13}$C NMR spectrum (100 MHz, CDCl$_3$) of 8.

Figure S23. HSQC spectrum (400/100 MHz, CDCl$_3$) of 8.
Figure S24. HMBC spectrum (400/100 MHz, CDCl$_3$) of 8.