An Azafluorenone Alkaloid and a Megastigmane from *Unonopsis lindmanii* (Annonaceae)

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O alcalóide azafluorenona 5,8-dimetóxi-7-hidróxi-1-metil-4-azafluoren-9-one e o megastigmano (–)-(5R*, 6S*)-megastigman-3-one-10,7-olide foram isolados das partes aéreas de *Unonopsis lindmanii* (Annonaceae), juntamente com os compostos conhecidos (3S*, 5S*, 8R*)-3,5-dihidróxi-megastigma-6,7-dien-9-one (grasshopper ketone), *N*-trans-feruloiltiramina, (–)-anonaine, (–)-asimilobina, liriodenina e (–)-siringaresinol. Este é o primeiro relato da presença de megastigmanos em Annonaceae. As estruturas dos compostos foram elucidadas com base em dados espectroscópicos.

The azafluorenone alkaloid 5,8-dimethoxy-7-hydroxy-1-methyl-4-azafluoren-9-one and the megastigman (–)-(5R*, 6S*)-megastigman-3-one-10,7-olide were isolated from aerial parts of *Unonopsis lindmanii* (Annonaceae), along with the known compounds (3S*, 5S*, 8R*)-3,5-dihydroxy-megastigma-6,7-dien-9-one (grasshopper ketone), *N*-trans-feruloyltyramine, (–)-anonaine, (–)-asimilobine, liriodenine and (–)-siringaresinol. This is the first description of the presence of megastigmanes in Annonaceae. The structures of the compounds were elucidated based on spectroscopic data.

**Keywords:** *Unonopsis lindmanii*, Annonaceae, alkaloids, azafluorenone, megastigmanes

**Introduction**

Annonaceae is one of the largest families of the Magnoliide subclass, with approximately 135 genera and 2300 species, mostly pantropical. In Brazil, Annonaceae comprises about 26 genera and 260 species and has a great significance in the Brazilian vegetation. 1,2 Although the occurrence of different types of alkaloids has been frequently described, compounds with unusual skeletons, such as acetogenins,3,4 polyacetylenes,5 cyclopeptides,6,7 styryl lactones,8-10 indolidinoids11 and monoterpene glucosides12 have been recently reported, most of them showing biological activities as anticancer,3,13,14 antimicrobial,11,15 cytotoxic,10,16 antinflammatory7,17 and antiprotozoal.18

As part of our research on the chemistry of Annonaceae species,6,19,22 the isolation and structural elucidation of two new compounds from the aerial parts of *Unonopsis lindmanii* R. E. Fries (R. E. Fries) are discussed: the azafluorenone alkaloid 5,8-dimethoxy-7-hydroxy-1-methyl-4-azafluoren-9-one (1) and the megastigman (–)-(5R*, 6S*)-megastigman-3-one-10,7-olide (2) (Figure 1). *U. lindmanii* is a medium-sized tree, which is widely distributed in the Central-Western region of Brazil occurring mainly in riparian forest and
carrado. The chemical composition of *Unonopsis* genus was not widely investigated, and previous studies revealed the presence of some aporphines, bisaporphines, phenantrenes and azafluorenone alkaloids\(^{18,22,24,25}\) and polycarpol.\(^{26}\) The present phytochemical study of *U. lindmanii* also led to the isolation and identification of the known compounds grasshopper ketone,\(^{27}\) liriodenine,\(^{28}\) \((-\text{-}))-anonaine,\(^{29}\) \((-\text{-}))-asimilobine,\(^{29}\) \((-\text{-}))-siringaresynol\(^{30}\) and *N*-trans-feruloyltyramine.\(^{31}\) The structural elucidation of the compounds was established on the basis of spectroscopic techniques.

**Results and Discussion**

The ethanolic extract of the heartwood of *U. lindmanii* was chromatographed on a silica gel column and then on a Sephadex LH-20 column to afford compound 1. The positive HRESIMS of 1 revealed a pseudo-molecular ion at \(m/z 272.0957 \text{ [M + H]}\), consistent with the molecular formula \(C_{13}H_{15}NO_5\). The presence of a carbonyl group was demonstrated by an IR band at 1649 cm\(^{-1}\). The \(^1\)H nuclear magnetic resonance (NMR) spectrum of 1 (Table 1) showed three signals of aromatic hydrogens at \(\delta_C 6.91\) (s, H-6), 6.86 (d, \(J = 5.2\) Hz, H-2) and 8.23 (d, \(J = 5.2\) Hz, H-3), two methoxy groups at \(\delta_C 3.92 \text{ (s, 8-OCH}_3\text{)}\) and \(4.01 \text{ (s, 5-OCH}_3\text{)}\), and a signal of a methyl bonded to an aromatic ring at \(\delta_C 2.59 \text{ (s, 1-CH}_3\text{)}\). The \(^13\)C NMR spectrum of 1 (Table 1) contained fifteen signals attributed to a conjugated carbonyl (\(\delta_C 190.7\)), three aromatic protonated sp\(^2\) carbons bound to hydrogens, eight sp\(^2\) aromatic carbons without hydrogens attached, two methoxy groups and one methyl. Atomic connectivity was established using COSY, one-bond (HSQC), long-range (HMBC) \(^1\)H-\(^13\)C NMR correlation experiments and nuclear Overhauser effect spectroscopy (NOESY) (Figure 2). The \(^13\)C NMR spectrum of 1 showed a signal for only one methoxyl group attached to ortho-disubstituted carbon at \(\delta_C 61.1\), which was correlated in HSQC with the \(^1\)H NMR signal at \(\delta_H 4.01\) and in HMBC with the signal at \(\delta_C 142.5\). These correlations imply that the aromatic hydrogen at \(\delta_C 6.91\) must not be vicinal to this methoxyl group. The second methoxyl signal at \(\delta_C 3.92\) showed one-bond correlation with the \(^13\)C NMR signal at \(\delta_C 56.5\) and long-range correlation with the signal at \(\delta_C 156.4\). In the HMBC experiment, the aromatic hydrogen at \(\delta_C 6.91\) showed strong correlations with the carbons at \(\delta_C 119.2\) and \(156.4\), suggesting two possible structures for this compound: 5,8-dimethoxy-7-hydroxy or 5,8-dimethoxy-6-hydroxy. A comparison between the \(^1\)H NMR chemical shifts with the 5,8-dimethoxy-6-hydroxy-1-methyl-azafluorenone (kinabaline)\(^{32}\) indicated that H-7 displays a higher field resonance (\(\delta_C 6.34\)) when compared to that (\(\delta_C 6.91\)) in compound 1. This evidence allowed the assignment of the resonance at \(\delta_C 61.1\) to 8-OME, \(\delta_C 142.5\) to C-8, \(\delta_C 56.5\) to 5-OME, \(\delta_C 156.4\) to C-5, \(\delta_C 147.2\) to C-7 and \(\delta_C 119.2\) to C-4b. Therefore, the most likely structure for the new alkaloid must be 5,8-dimethoxy-7-hydroxy-1-methyl-4-azafluoren-9-one. This structure received further support from reduction of 1, which yielded 1a.

The compound 1a was obtained via reduction of 1 with NaBH\(_4\). In the \(^1\)H NMR spectrum of 1a (Table 1), the presence of a signal at \(\delta_C 5.70\) (s, H-9) was consistent

<table>
<thead>
<tr>
<th>Position</th>
<th>(\delta_C), type</th>
<th>(\delta_H) (m, J/Hz)</th>
<th>(\delta_C), type</th>
<th>(\delta_H) (m, J/Hz)</th>
</tr>
</thead>
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<td>149.2, C</td>
<td>147.7, C</td>
<td>1a</td>
<td>149.2, C</td>
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<tr>
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<td>7.15 (d, 5.0)</td>
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<td>146.6, CH</td>
<td>8.30 (d, 5.0)</td>
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<tr>
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<td>149.5, C</td>
<td>4b</td>
<td>119.2, C</td>
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<td>158.3, C</td>
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<td>6.97 (s)</td>
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<td>8</td>
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<td>137.7, C</td>
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<td>56.9, CH</td>
<td>5-OCH(_3)</td>
<td>3.91 (s)</td>
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<tr>
<td>8-OCH(_3)</td>
<td>61.1, CH</td>
<td>61.0, CH</td>
<td>8-OCH(_3)</td>
<td>3.82 (s)</td>
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</table>

![Figure 1. Chemical structures of compounds 1, 1a and 2.](image1.png)

![Figure 2. HMBC (H \(\leftrightarrow\) C) correlations of 1 and 1a.](image2.png)
with the expected product. The HMBC spectrum of 1a showed hydrogens of the methyl attached to C-1 correlating strongly with carbons at \( \delta_c 147.7, 123.9 \) and 139.0 assigned to C-1, C-2 and C-9a, respectively. In this same experiment, the hydrogen H-9 correlates strongly with two carbons, one C-9a (\( \delta_c 139.0 \), as defined previously) and the other at \( \delta_c 144.1 \), corresponding to the carbon in equivalent position to C-9a, i.e., C-8a. Moreover, it was observed that the hydrogen \( \delta_h 6.97 \) (H-6) correlates strongly with the carbons at \( \delta_c 116.6 \) (C-4b) and 137.7 (C-8), which were located in meta position in relation to H-6, but that did not correlate with the carbon at \( \delta_c 144.1 \) (C-8a). This observation allowed us to determine the correct position of hydrogen H-6 (\( \delta_h 6.97 \)). Consequently, the structure of 1a corresponds to that displayed in Figure 1.

Compound 2 was isolated as a brownish-yellow amorphous solid from the CHCl₃ leaf extract, after chromatographic steps using silica gel column. From HRESIMS spectrum of 2, a pseudo-molecular ion peak at \( m/z 225.1551 \) [M + H]⁺ was obtained, corresponding to the molecular formula \( C_{13}H_{20}O_5 \). In the \(^1\)H NMR spectrum of 2 (Table 2), two methyl singlets were observed at \( \delta_h 0.76 \) and 1.03 (H-11 and H-12, respectively), and a doublet at \( \delta_h 1.04 \) (J \( = 6.4 \) Hz) was attributed to the methyl H-13 hydrogen. The signal at \( \delta_h 2.05 \) (dd, J 13.4 and 2.0 Hz) was assigned to H-2, \( \text{equatorial} \), with geminal coupling constant of 13.4 Hz and a \( ^J_{\text{equatorial-equatorial}} \) coupling of 2.0 Hz with the H-4 \( \text{axial-equatorial} \), appearing as a double doublet, while the H-2 \( \text{axial} \) appeared as a doublet at \( \delta_h 2.25 \) (J 13.4 Hz) with geminal coupling constant of 13.4 Hz. The signals at \( \delta_h 2.00 \) (J 11.2 Hz) and 2.29 (dd, J 11.2 and 2.0 Hz) were assigned to the methylene hydrogens H-4 \( \text{axial-equatorial} \), respectively, supported by HSQC spectrum. The analysis of the \(^13\)C NMR spectrum revealed the presence of 13 carbons, suggesting a megastigmane skeleton. The signals at \( \delta_c 211.0 \) and 176.3 were attributed to the carbonyl at C-3 and the carboxyl at C-10, the last signal indicating a possible lactone ring. A signal at \( \delta_c 71.9 \) was assigned to C-7, that one at \( \delta_c 52.1 \) to C-6, and the signals at \( \delta_c 20.6, 29.9 \) and 20.9 assigned to the three methyl carbons C-11, C-12 and C-13, respectively. Correlations between C-3 and H-2 and H-4 were visualized in the HMBC spectrum, as well as \( ^J_{\text{axial-equatorial}} \) correlations between C-10 and H-7. The C-13 signal at \( \delta_c 20.9 \), which had its position confirmed through HMBC and HSQC experiments, was consistent with the equatorial position of this group, in comparison with spectral data from previously described analogues.\(^{33}\) In the NOESY experiment, some important correlations were observed between H-11 (\( \delta_h 0.76 \)), which is in axial position, and H-5 (\( \delta_h 1.79 \)), confirming the configuration at position 5; between H-11 and H-8 (\( \delta_h 1.62 \)) and between H-5 and H-7 (\( \delta_h 4.14 \)), indicating the position of the lactone ring (Figure 3). These assignments were checked by COSY, HSQC and HMBC analyses, and the relative configuration was based on correlations in the NOESY experiment, confirming the structure of 2 as (–)-(5R*, 6S*)-megastigman-3-one-10,7-olide.

Table 2. NMR spectroscopic data for compound 2 (300 MHz \(^1\)H and 75 MHz \(^13\)C, CDCl₃)

<table>
<thead>
<tr>
<th>Position</th>
<th>( \delta_c ), type</th>
<th>( \delta_h ) (m, J/Hz)</th>
<th>HMBC (C ( \rightarrow ) H)</th>
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<td>H-2/H-11/H-12</td>
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<td>1.05 (d, 6.2)</td>
<td>H-6</td>
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<td>3</td>
<td>211.0, C</td>
<td>1.79 (m)</td>
<td>H-13</td>
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<td>4</td>
<td>50.1, CH₂</td>
<td>2.00 (d, 11.2); 2.29 (dd, 11.2; 2.0)</td>
<td>H-6/H-11/H-12/H-4</td>
</tr>
<tr>
<td>5</td>
<td>36.3, CH₃</td>
<td>1.05 (d, 6.2)</td>
<td>H-12</td>
</tr>
<tr>
<td>6</td>
<td>52.1, CH₂</td>
<td>1.79 (m)</td>
<td>H-13</td>
</tr>
<tr>
<td>7</td>
<td>71.9, CH</td>
<td>4.14 (m)</td>
<td>H-7</td>
</tr>
<tr>
<td>8</td>
<td>24.1, CH₂</td>
<td>1.62 (m)</td>
<td>H-7</td>
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<td>9</td>
<td>36.5, CH₂</td>
<td>1.85 (m)</td>
<td>H-12</td>
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<td>10</td>
<td>176.3, C</td>
<td>0.76 (s)</td>
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<td>11</td>
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<tr>
<td>13</td>
<td>20.9, CH₃</td>
<td>1.04 (d, 6.4)</td>
<td>H-4</td>
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</table>

Figure 3. Selected NOESY correlations of 2.

Conclusions

Although alkaloids are a common theme in Annonaceae, the occurrence of azafluorenones is rare and restricted to this family. Some hypotheses for the biosynthesis of these alkaloids are based on different sources, some suggesting their origin from oxaphopinhic alkaloids, supported by the co-occurrence of these compounds.\(^{34}\) Other authors indicated a possible route from a polyketide pathway\(^{34}\) or from a shikimic acid intermediate bound to a glutamic acid unit as a base to the skeleton of these molecules.\(^{35}\) Despite several proposals, the biosynthetic pathway to the formation of azafluorenones remains unknown. This study
contributed to the expansion of the chemical characterization of the *Unonopsis* genus since the compounds grasshopper ketone, (−)-syringaresinol and *N*-trans-feruloyltyramine are being described for the first time in *Unonopsis*. To the best of our knowledge, this is the first time that the presence of megastigmanes in Annonaceae is described, indicating the importance of continuing the investigation of this family as a source of novel molecules.

**Experimental**

**General procedures**

Silica gel (70-230 mesh, Merck) and Sephadex LH-20 (Amersham Biosciences) were used for column chromatography (CC), whereas silica gel 60 GF$_{254}$ was employed for analytical (0.50 mm) and preparative (1.0 mm) thin layer chromatography (TLC). The $^1$H and $^13$C NMR spectra were obtained at 300 and 75 MHz, respectively, on a Bruker DPX-300 spectrometer using CDCl$_3$ (Aldrich) and acetone-$d_6$ (Aldrich) as solvents and tetramethylsilane (TMS) as an internal standard. The IR spectra were obtained on a Perkin-Elmer 783 spectrometer and the specific optical rotations on a Perkin-Elmer 341 MS polarimeter. Mass spectra were obtained in Agilent Ultra Q-TOF mass spectrometer with electrospray ionization.

**Plant material**

Heartwood and leaves of *Unonopsis lindmanii* were collected in March 2005 in Pantanal (Mato Grosso do Sul, Brazil) and identified by Dr. Renato Mello Silva (University of São Paulo, Brazil). A voucher specimen (No. 4730) was deposited in the Herbarium GC/MS (Universidade Federal do Mato Grosso do Sul, Brazil) and identified by Dr. Renato Mello Silva (University of São Paulo, Brazil). A voucher specimen (No. 4730) was deposited in the Herbarium GC/MS (Universidade Federal do Mato Grosso do Sul, Brazil).

**Extraction and isolation**

Dried heartwood (2.9 kg) was subjected to maceration in ethanol for 7 days, yielding 28.5 g of ethanolic extract. The extract was dried under reduced pressure and then resuspended in MeOH, resulting in a precipitate and a supernatant. The composition of the supernatant was essentially sugars, and the precipitate (6.0 g) was submitted to column chromatography on silica gel with a gradient of polarity hexane-ethyl acetate-methanol, yielding fractions A-D. Fraction A was a mixture of β-sitosterol and stigmasterol (40.3 mg). Fraction B (38 mg) was re-chromatographed on a Sephadex LH-20 column, using ethyl acetate as solvent, yielding the alkaloid 5,8-dimethoxy-7-hydroxy-1-methyl-4-azafluoren-9-one (1) (6.3 mg). Fraction C (248.2 mg) was submitted to CC on Sephadex LH-20 using CH$_2$Cl$_2$:MeOH (1:1) as eluent, yielding the lignan (−)-syringaresinol (38.8 mg) and *N*-trans-feruloyltyramine (35.4 mg). Fraction D (23.5 mg), containing the alkaloids (−)-anonaine (6.0 mg) and (−)-asimilobine (3.6 mg), was fractionated by CC Sephadex LH-20 using CH$_2$Cl$_2$:MeOH (1:1) as eluent. Dried leaves (950 g) of *U. lindmanii* were extracted in CHCl$_3$, in basic medium (10% NH$_4$OH, pH 9) under constant stirring for 5 days, yielding 21.5 g of crude extract. This extract was partitioned using 5% HCl and CHCl$_3$. The pH value of the acidic aqueous fraction was adjusted to 9 with NH$_4$OH and then extracted with CHCl$_3$. The chloroform phases were concentrated under reduced pressure, yielding 1.6 g from the chloroform extract. This extract was submitted to CC on silica, yielding megastigmanes (−)(5R*, 6S* )-megastigman-3-one-10,7-olide (2) (7.8 mg) and grasshopper ketone (3) (10.6 mg), and the alkaloids lirodenine (5.7 mg) and (−)-asimilobine (13.3 mg).

**5,8-Dimethoxy-7-hydroxy-1-methyl-4-azafluoren-9-one (1):** yellow amorphous solid; IR (KBr) $\tilde{\nu}_{\text{max}}$/cm$^{-1}$ 3422, 2917, 2850, 1680, 1464, 1246, 1117, 1036. For $^1$H and $^13$C NMR data, see Table 1; HRESIMS $m/z$ (rel. int.) 272.0957 [M + H]$^+$ (11) (C$_{13}$H$_{13}$NO$_3$ [M + H]$^+$ calc. 272.27596), 239.06 (43), 211.06 (100), 183.07 (18), 155.12 (7); LRESIMS (rel. int.) $m/z 272.09$ [M + H]$^+$ (11), 239 (43), 211 (100), 183 (18), 155 (7).

5,8-Dimethoxy-7-hydroxy-1-methyl-4-azafluoren-9-ol (1a): NaBH$_4$ (1.4 mg) was added to a solution of 1 (5.3 mg) in isopropanol (1.5 mL), and the mixture was stirred at room temperature for 1 h. After completion, the reaction was quenched with H$_2$O and extracted with 3 × 5 mL of CH$_2$Cl$_2$. Layers were separated and washed with H$_2$O. Anhydrous Na$_2$SO$_4$ was added to the organic fraction, filtered, concentrated to dryness, and purified by preparative TLC on silica gel developed in CH$_2$Cl$_2$, to produce 3.2 mg (yield 57.1%) of the reduced product as a pale yellow amorphous solid, which was identified as 1a by NMR analysis (Table 1).

(5R*, 6S*)-Megastigman-3-one-10,7-olide (2): brownish-yellow amorphous solid; $[\alpha]_{D}^{20} = -54.34$ (c 0.0024, CHCl$_3$); IR (KBr) $\tilde{\nu}_{\text{max}}$/cm$^{-1}$ 3391, 2931, 2851, 1655, 1456, 1122, 1047; $^1$H and $^13$C NMR data (see Table 2); HRESIMS $m/z$ (rel. int.) 225.1551 [M + H]$^+$ (100) (C$_{15}$H$_{21}$NO$_3$ [M + H]$^+$ calc. 225.30404), 248.13 (24), 236.13 (34), 222.14 (30); LRESIMS (rel. int.) $m/z 225$ [M + H]$^+$ (100), 248 (24), 236 (34), 222 (30).
Supplementary Information

Supplementary information (1H NMR and 13C NMR spectra for compounds 1, 1a and 2) is available free of charge at http://jbc.ssbq.org.br as PDF file.

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