Chemical Constituents from *Bombacopsis glabra* (Pasq.) A. Robyns: Complete $^1$H and $^{13}$C NMR Assignments and X Ray Structure of 5-Hydroxy-3,6,7,8,4'-pentamethoxyflavone

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Do extrato hexânico da casca do caule de *Bombacopsis glabra* (Bombacaceae) foram isolados a flavona 5-hidroxi-3,6,7,8,4'-pentametoxiflavona (1) e os triterpenos lupenona, 9,19-ciclonanost-23-eno-3β,25-diol (2), (24R)-9,19-ciclonanost-25-eno-3β,24-diol (3) e (24S)-9,19-ciclonanost-25-eno-3β,24-diol (4). As estruturas foram determinadas por espectroscopia de RMN de $^1$C e de $^1$H (1D e 2D) e EM, e ainda, por comparação com dados da literatura para os triterpenos. A confirmação inequívoca da estrutura da flavona 1 foi realizada por um estudo de difração de raios X. As cinco substâncias foram isoladas pela primeira vez de espécies de Bombacaceae.

The flavone 5-hydroxy-3,6,7,8,4'-pentamethoxyflavone (1) and the triterpenes lupenone, 9,19-cycloanost-23-ene-3β,25-diol (2), (24R)-9,19-cycloanost-25-ene-3β,24-diol (3) and (24S)-9,19-cycloanost-25-ene-3β,24-diol (4) were isolated from the hexane extract of the stem bark of *Bombacopsis glabra* (Bombacaceae). The structures were determined by $^{13}$C and $^1$H NMR (1D and 2D) and mass spectrometry, and by comparison with literature data for triterpenes. The structure of the flavone 1 was unambiguously confirmed by a X-ray diffraction study. The five substances were isolated for the first time from Bombacaceae species.

**Keywords:** *Bombacopsis glabra*, Bombacaceae, triterpenes, 5-hydroxy-3,6,7,8,4'-pentamethoxyflavone, X-ray diffraction study

**Introduction**

The Bombacaceae family comprises 28 genera and 200 species.1 Despite the economic significance of *Ceiba* and *Ochroma* species as sources of kapok and balsa wood, respectively, little is known about the chemistry of plants from this family.2,3 As part of our program of study of the Brazilian flora, we have carried out the first investigation of the chemical constituents of the bark from *Bombacopsis glabra* (Pasq.) A. Robyns.

*B. glabra* [syn. *Pachira glabra* pasq., *Pachira macrocarpa* (Schlecht. Et Cham.) Walp., *Bombax glabrum* (Pasq.) A. Robyns and *Bombax aquaticum* (Aubl.) Schum] is a plant native to the Brazilian Atlantic forest and can be found from the State of Pernambuco in the northeast to Rio de Janeiro in the southeast.4

This study led to the isolation of the polyoxygenated flavone (1) and of the triterpenes lupenone, 9,19-cycloanost-23-ene-3β,24-diol (2), (24R)-9,19-cycloanost-25-ene-3β,24-diol (3) and (24S)-9,19-cycloanost-25-ene-3β,24-diol (4). The five compounds are reported for the first time in plants from Bombacaceae family. Compound 1 is structurally similar to flavones with citotoxic5,6 and insect antifeedant properties.7 The structure elucidation of the isolated compounds was performed by spectroscopic analysis and comparison with literature data. The unambiguous assignments of $^1$H and $^{13}$C NMR data of 1, 2 and 3 was performed by $^1$H-H-COSY, HMQC, HMBC and nOe difference NMR experiments. The structure of 1 was also confirmed by X-ray crystallography.

**Results and Discussion**

Although compound 1 has been previously synthesised8 and isolated from *Stachys aegyptiaca*8 but a
full $^1$H NMR assignment have not been made. In the present study, all $^1$H and $^13$C shifts were unambiguously and directly assigned by HMQC, HMBC and nOe difference experiments.

The $^2$J$_{HC}$ and $^3$J$_{HC}$ couplings by HMBC are shown in Figure 1. Also, $^2$H- $^1$H nOe enhancements were seen from H2',6' to 13-CH$_3$ (1.3 %) and 15-CH$_3$ (1.0 %) and from 12-CH$_3$ to the almost overlapping resonances of 11-CH$_3$ and/or 13-CH$_3$ (2.3 % in total). These enhancements defined the structure unambiguously, with only trivial reliance on shift data. The complete assignments are listed in Table 1.

Table 1. $^1$H and $^13$C NMR (CDCl$_3$) data for compound 1

<table>
<thead>
<tr>
<th>Atom</th>
<th>$\delta$</th>
<th>$\delta_{3J}$(H-1H couplings)</th>
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<tr>
<td>2</td>
<td>154.2</td>
<td>—</td>
</tr>
<tr>
<td>3</td>
<td>136.6</td>
<td>—</td>
</tr>
<tr>
<td>4</td>
<td>177.3</td>
<td>—</td>
</tr>
<tr>
<td>5</td>
<td>147.2</td>
<td>—</td>
</tr>
<tr>
<td>6</td>
<td>134.2</td>
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<td>150.9</td>
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<td>8</td>
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<td>9</td>
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<td>60.2</td>
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<tr>
<td>14</td>
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<tr>
<td>15</td>
<td>58.1</td>
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</tr>
<tr>
<td>1'</td>
<td>120.9</td>
<td>—</td>
</tr>
<tr>
<td>2', 6'</td>
<td>128.3</td>
<td>8.17d(J=9.0 Hz)</td>
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<tr>
<td>3', 5'</td>
<td>112.3</td>
<td>7.05 d(J=9.0 Hz)</td>
</tr>
<tr>
<td>4'</td>
<td>159.9</td>
<td>—</td>
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<tr>
<td>OH</td>
<td></td>
<td>12.44s</td>
</tr>
</tbody>
</table>

Figure 1. Important HMBC couplings in compound 1

Although pre-irradiation at $\delta$ 8.17 (H2'+H6') resulted in a nOe enhancement at 15-CH$_3$ (1.0%), compatible with the crystal structure [15-CH$_3$ - H2'; 3.2 Å], the enhancement observed at H13 (1.3%) cannot be explained from the crystal structure [13-CH$_3$ - H6' 4.2 Å]. This implies that in solution the C8-O6 bond has alternative conformations to bring 13-CH$_3$ closer to H6'.

An intramolecular hydrogen bond exists between O3 and O2 [O3 - O2 2.572(4) Å; H3 - O2 1.61(9) Å; O3-H3 1.02(9) Å; O3-H3 - O2 156(8)]. This is consistent with the absence of nOe enhancement at 11-CH$_3$ upon irradiation of H3 (Figure 2).
Compound 2 showed thirty resonances signals in the $^{13}$C NMR spectrum (6 C, 7 CH, 10 CH$_2$, and 7 CH$_3$) and the signals at $\delta$ 79.3 and 71.2 suggested the presence of two hydroxyl groups. The EI mass spectrum presented a peak at $m/z$ 424 [M-18, loss of water from the molecular ion]. These data were consistent with the molecular formula C$_{30}$H$_{50}$O$_2$. The $^1$H NMR spectrum of compound 2 showed, besides other signals, resonances for cyclopropane methylene ($\delta$ 0.54 and 0.31, 1H, d, each), seven methyls and two olefinic hydrogens ($\delta$ 5.59, m) suggesting a cycloartenol type triterpene. The detailed analysis of the $^1$H and $^{13}$C NMR spectra led us to propose the structure of 9,19-cyclolanost-23-ene-3β,25-diol. The 23Z isomer has been previously isolated and also the 3-acetate of this compound, having a E double bond. As the signals of H-23 and H-24 appeared as a multiplet centered at $\delta$ 5.59, a direct assignment of the double bond geometry was difficult, but the data were similar to those reported by Greca et al. for the Z isomer. Using two-dimensional NMR techniques (COSY, HMQC and HMBC), it was possible to assign unequivocal the $^1$H and $^{13}$C NMR spectra. Some of the $^{13}$C NMR assignments made by us differ slightly from those given by Greca et al., but were in full agreement with the data reported by Kamisato et al. Although compound 2 was identified many years ago, only now a complete unambiguous assignment of the $^1$H NMR spectrum has been achieved.

Compound 3 was isolated as a white solid and its $^1$H NMR and $^{13}$C NMR spectra were similar to those of compound 2. The EI mass spectrum showed a peak at $m/z$ 442, corresponding to the molecular formula C$_{30}$H$_{50}$O$_2$, suggesting that 3 was an isomer of 2. The major differences were relative to the signals corresponding to the side chain. In the $^1$H NMR spectrum, resonances at $\delta$ 4.95 (m) and another at $\delta$ 4.82 (m) were observed, consistent with the presence of a =CH$_2$ group, and this was confirmed by the signals at $\delta$ 147.36 (C-25) and 111.31 (C-26) in the $^{13}$C NMR spectrum. Furthermore, the location of the double bond between C-25 and C-26 was proposed, due to the absence of Me-26 signal at $\delta$ 1.7, when compared with corresponding signal in the $^1$H NMR spectrum of the compound 2, and also by the coupling observed in the $^1$H/$^1$H-COSY spectrum between olefine hydrogen (H-26 and H-26') and Me-27.

The chemical shift at high frequency observed for H-24 ($\delta$ 4.0, m), when compared with H-3, suggested that the hydroxyl group should be placed next to the double bond (at C-24). This position was further confirmed by couplings observed in the $^1$H/$^1$H-COSY spectrum for the side chain hydrogens: 21-CH$_3$ and H-20; H-20 and H-22/H-22'; H-22/H-22' and H-23/H-23'; and finally, between H-23/H-23' and H-24.

Compounds 4 and 3 were obtained as a mixture. The duplicity of most of the signals of the side chain, observed in the $^{13}$C NMR spectrum of the mixture, and the coincidence of the other signals, suggested that they are epimers at C-24. By comparison of the $^{13}$C chemical shifts of compounds 3 and 4 with literature data, they were identified as (24R)-9,19-cyclolanost-25-ene-3β,24-diol and (24S)-9,19-cyclolanost-25-ene-3β,24-diol, respectively. The complete assignments of the $^{13}$C NMR spectra of compounds 2, 3 and 4 are shown in Table 2.

**Experimental**

**General experimental procedures**

Melting points were determined on a MQAPF-301 apparatus and are uncorrected. The $^1$H and $^{13}$C NMR spectra were obtained on a Bruker DPX 400 (at 400 MHz and 100.6 MHz, respectively) and DPX 300 (at 300 MHz and 75 MHz, respectively) instruments. HMQC and HMBC experiments were optimized for $^1$J$_{CH}$ 145 Hz and $^2$ and $^3$J$_{CH}$ 7.7 Hz, respectively. Mass spectra were obtained on a V.G. Analytical ZAB-IF spectrometer, at 70 eV. Infrared spectra were registered on a Perkin Elmer FTIR PARAGON 1000 spectrophotometer. X-ray crystallographic data were collected on a Siemens SMART CCD area-detector diffractometer. UV data were obtained on a Varian CARY 50 spectrophotometer. Column chromatography and TLC plates were prepared using silica gel Merck.

**Plant material**

Bombacopsis glabra (Pasq.) A. Robyns was collected from Jequié region, Bahia State, Brazil and identified by Dr. J. Semir (Universidade Estadual de Campinas). A voucher specimen is deposited at the Herbarium UEC – UNICAMP.

**Extraction and isolation of chemical constituents**

The air dried stem bark of B. glabra (850.0 g) was ground and extracted with hexane (2.5 L) and ethanol (2.5 L) successively. The hexane extract was concentrated under reduced pressure to leave a brown residue (24.0 g). This gave 13 fractions following flash column chromatography on silica gel, eluting with hexane/ethanol acetate (9:1 to pure ethyl acetate). Fraction 5 (0.6 g) was purified by chromatography using the same solvent system to yield compound 1 (7.0 mg) and other eight subfractions. Chromatographic separation of subfraction 5.4, using hexane/ethyl acetate (7:3), yielded compound 3 (6.0 mg)
and a mixture of compounds 3 and 4 (8.0 mg). Fraction 2 (7.3 g) was chromatographed and eluted with hexane/ethyl acetate (20:1) to yield lupenone (1.0 g). Fraction 6 was dissolved in ethyl acetate. Compound 2 (10.0 mg), insoluble in this solvent, was separated by filtration.

5-Hydroxy-3,6,7,8,4′-pentamethoxyflavone (1)

Mp 120-121 °C (MeOH); UV λ<sub>max</sub>/nm (MeOH) 280 and 333; EIMS m/z 388 (M⁺, 79%), 373 (M⁺ - CH₃·, 100); ¹H (300 MHz, CDCl₃) and ¹³C (75 MHz, CDCl₃) NMR data, Table 1.

Crystal structure determination of compound 1

Crystals of 1 suitable for single crystal x-ray diffraction studies were obtained as orange plates by recrystallization from methanol. Data were collected using a Siemens SMART CCD area-detector diffractometer. A multi-scan absorption correction was applied using SADABS. The structure was solved by direct methods and refined by full-matrix least-squares on F² for all data using SHELXL 97. Hydrogen atoms were added at calculated positions and refined using a riding model. Anisotropic displacement displacement parameters were used for all non-H atoms.

Crystal data: C<sub>20</sub>H<sub>20</sub>O<sub>8</sub>; M = 388.36, triclinic, a = 9.2047(9), b = 9.4799(10), c = 11.8322(12) Å, α = 98.115(4), β = 99.396(4), γ = 113.659(4)°, U = 908.28(16) Å<sup>3</sup>, T = 180(2) K, λ(Mo-K) = 0.71073 Å, space group P<sub>1</sub>, Z = 2, µ(Mo-K) = 0.111 mm<sup>-1</sup>, crystal size 0.30 x 0.20 x 0.08 mm, 4760 reflections measured (2.41 < θ < 25.2°), 3203 unique (R<sub>int</sub> = 0.0308). The final values of R<sub>1</sub> [I > 2σ(I)] and wR<sub>2</sub> (all data) are 0.075 and 0.199, respectively; the largest difference peak and hole are 0.301 and -0.381 eÅ<sup>-3</sup>.

9,19-Cyclolanost-23-ene-3β,25-diol (2)

Colorless crystal, mp 205-206 °C (lit.¹⁸ mp 200-204 °C); IR ν<sub>max</sub>/cm<sup>-1</sup> 3336, 2966, 2929, 1377, 1151 and 971 (KBr); EIMS m/z 424 ([M – H₂O]⁺, 5%), 270 ([M – C₈H₁₇O – H₂O – Me]⁺, 100); complete assignment of ¹H and ¹³C NMR (CDCl₃, 400 and 100.6 MHz, respectively) are presented in Table 2.

(24R)-9,19-Cyclolanost-25-ene-3β,24-diol (3)

Colorless crystal, mp 177-179 °C; EIMS m/z 442 (M⁺, 20%), 424 ([M – H₂O]⁺, 100), 409 ([M – H₂O – Me]⁺, 93), 302 ([M – C₈H₁₇O – H₂O]⁺, 95); ¹H NMR (400 MHz, CDCl₃) δ 4.95 (m, H-26), 4.82 (m, H-26′), 4.0 (m, H-24), 3.3 (m, H-3), 1.7 (s, CH₃-27) 0.95 (s, CH₃-28 and CH₃-30), 0.88 (d, J = 6.9 Hz, CH₃-21), 0.86 (s, CH₃-18) 0.80 (s, CH₃-29), 0.54 (d, J = 4.0 Hz, H-19) and 0.32 (d, J = 4.0 Hz, H-19′); ¹³C NMR data are presented in Table 2.

Acknowledgments

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Supplementary Material

Crystallographic data (excluding structural factors) for the structure in the paper have been deposited with the Cambridge Crystallographic Data Center as supplementary publication number CCDC 154734. Copies of the data can be obtained, free of charge, on application to CCDC, 12 Union Road, Cambridge CB2 1EZ, UK (fax:+44 1223 336033 or e-mail: deposit@ccdc.cam.ac.uk).

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