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Short Report

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

Vanderlúcia F. Paula*[a], Luiz C. A . Barbosa[b], William Errington[c], Oliver W. Howarth[c] and Mariluze P. Cruz[a]

[a]*Departamento de Química e Exatas, Universidade Estadual do Sudoeste da Bahia, 45200-000, Jequié - BA, Brazil*

[b]*Departamento de Química, Universidade Federal de Viçosa, 36571-000, Viçosa - MG, Brazil*

[c]*Department of Chemistry, University of Warwick, Coventry - CV4 7AL, UK*

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-ciclolanost-23-eno-3β,25-diol (**2**), (24*R*)-9,19-ciclolanost-25-eno-3β,24-diol (**3**) e (24*S*)-9,19-ciclolanost-25-eno-3β,24-diol (**4**). As estruturas foram determinadas por espectroscopia de RMN de ^{13}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-cyclolanost-23-ene-3β,25-diol (**2**), (24*R*)-9,19-cyclolanost-25-ene-3β,24-diol (**3**) and (24*S*)-9,19-cyclolanost-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.

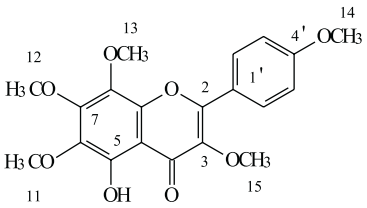
B. glabra [syn. *Pachira glabla* pasq., *Pachira macrocarpa* (Schlecht. Et Cham.) Walp., *Bombax glabrum* (Pasq.) A. Robyns and *Bombax aquaticum* (Aubl.) Schum] is a plant native to the Brazilian Atlantic forest and can be found from the State of Pernambuco in the northeast to Rio de Janeiro in the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

This study led to the isolation of the polyoxygenated flavone (**1**) and of the triterpenes lupenone , 9,19-cyclolanost-23-ene-3β,24-diol (**2**), (24*R*)-9,19-cyclolanost-25-ene-3β,24-diol (**3**) and (24*S*)-9,19-cyclolanost-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 citotoxic[5,6] 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 $^{1}H^{1}H$-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 synthesised[8] and isolated from *Stachys aegyptiaca*[9] but a

* e-mail: vfpaula@uesb.br



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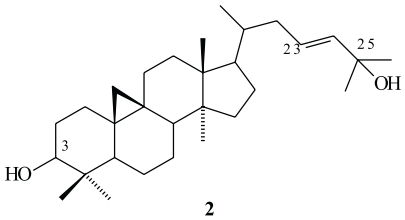

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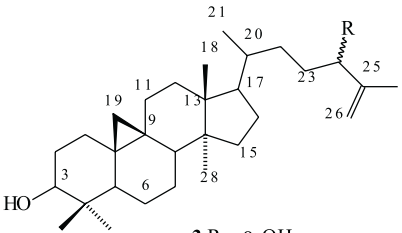

3 R = β-OH
4 R = α-OH

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, ^{1}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**

Atom	δ_X	δ_{XH} (*^{1}H-^{1}H couplings*)
2	154.2	—
3	136.6	—
4	177.3	—
5	147.2	—
6	134.2	—
7	150.9	—
8	130.9	—
9	143.0	—
10	105.5	—
11	59.2	3.96s
12	59.8	4.12s
13	60.2	3.96s
14	53.5	3.92s
15	58.1	3.88s
1'	120.9	—
2', 6'	128.3	8.17d(*J*=9.0 Hz)
3', 5'	112.3	7.05 d(*J*=9.0 Hz)
4'	159.9	—
OH	—	12.44s

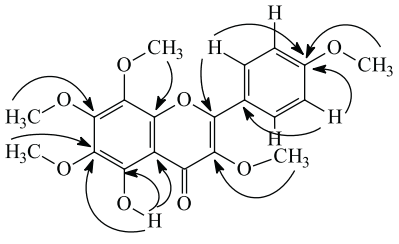

Figure 1. Important HMBC couplings in compound **1**

The crystal structure of **1** (Figure 2) confirmed the substitution pattern deduced from NMR. The benzopyran ring (C) is essentially coplanar with ring A. However, the methoxyphenyl ring (B) is twisted 23º from the plane of ring C. A similar result has been found for other flavones.[10] The methoxy group lies in the plane of the phenyl ring. This is in agreement with Bolt and Bauch[11] where the methyl groups of -methoxynaphthalene derivatives lie in the plane of the aromatic ring, *syn* to Cα. However, the methoxy groups at carbons C3, C6, C7 and C8 are not coplanar with ring A, their torsion angles being 110.8(4)º, 66.7(6)º, -62.1(5)º and -74.0(5)º respectively, presumably because of steric hindrance. In contrast, one of the methyl groups in the slightly less hindered 8-hydroxy-1,2,3-trimethoxy-benzo[*a*]anthracene-7,12-dione, lies in the plane of the aromatic ring.[12]

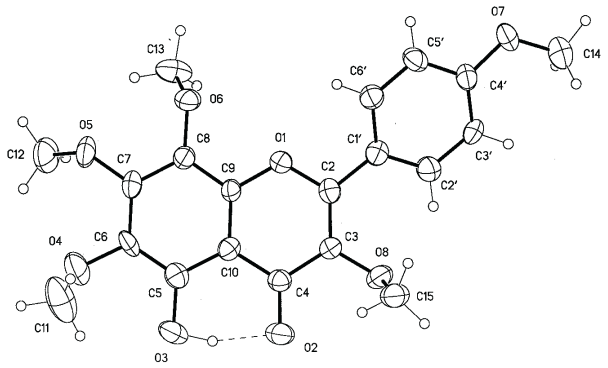

Figure 2. A computer generated drawing of the crystalline structure of compound **1**

Although pre-irradiation at δ 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 δ 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 ^1H NMR spectrum of compound **2** showed, besides other signals, resonances for cyclopropane methylene (δ 0.54 and 0.31, 1H, d, each), seven methyls and two olefinic hydrogens (δ 5.59, m) suggesting a cycloartenol type triterpene. The detailed analysis of the ^1H and ^{13}C NMR spectra led us to propose the structure of 9,19-cyclolanost-23-ene-3β,25-diol. The 23*Z* isomer has been previously isolated[13] and also the 3-acetate of this compound, having a *E* double bond.[14] As the signals of H-23 and H-24 appeared as a multiplet centered at δ 5.59, a direct assignment of the double bond geometry was difficult, but the data were similar to those reported by Greca *et al*[13] for the *Z* isomer. Using two-dimensional NMR techniques (COSY, HMQC and HMBC), it was possible to assign unequivocal the ^1H and ^{13}C NMR spectra. Some of the ^{13}C NMR assignments made by us differ slightly from those given by Greca *et al*.,[13] but were in full agreement with the data reported by Kamisato *et al*.[15] Although compound **2** was identified many years ago, only now a complete unambiguous assignment of the ^1H NMR spectrum has been achieved.

Compound **3** was isolated as a white solid and its ^1H NMR and ^{13}C NMR spectra were similar to those of compound **2**. The EIMS 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 ^1H NMR spectrum, resonances at δ 4.95 (m) and another at δ 4.82 (m) were observed, consistent with the presence of a $=CH_2$ group, and this was confirmed by the signals at δ 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 δ 1.7, when compared with corresponding signal in the ^1H NMR spectrum of the compound **2**, and also by the coupling observed in the $^1H^1H$-COSY spectrum between olefine hydrogen (H-26 and H-26') and Me-27.

The chemical shift at high frequency observed for H-24 (δ 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 $^1H^1H$-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,[14] they were identified as (24*R*)-9,19-cyclolanost-25-ene-3β,24-diol and (24*S*)-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 ^1H 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 $^1J_{CH}$ 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/ethyl 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_{max}/nm (MeOH) 280 and 333; EIMS *m/z* 388 ($M^{+\bullet}$, 79%), 373 ($M^{+\bullet}$ - $CH_3^{\cdot}$, 100); ^1H (300 MHz, $CDCl_3$) and ^{13}C (75 MHz, $CDCl_3$) 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.[16] The structure was solved by direct methods and refined by full-matrix least-squares on F^2 for all dara using SHELXL 97.[17] 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: $C_{20}H_{20}O_8$; 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^3, T = 180(2) K, λ = 0.71073 Å, space group P, Z = 2, μ(Mo-K) = 0.111 mm^{-1}, crystal size 0.30 x 0.20 x 0.08 mm, 4760 reflections measured ($2.41 < \theta < 25.2^o$), 3203 unique (Rint = 0.0308). The final values of R1 [$I > 2\sigma(I)$] and wR2 (all data) are 0.075 and 0.199, respectively; the largest difference peak and hole are 0.301 and -0.381 eÅ^{-3}.

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

Colorless crystal, mp 205-206 °C (lit.[18] mp 200-204 °C); IR ν_{max}/cm^{-1} 3336, 2966, 2929, 1377, 1151 and 971 (KBr); EIMS *m/z* 424 ($[M - H_2O]^{+\bullet}$, 5%), 270 ($[M - C_9H_{17}O - H_2O - Me]^+$, 100); complete assignment of ^1H and ^{13}C NMR ($CDCl_3$, 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 ($M^{+\bullet}$, 20%), 424 ($[M - H_2O]^{+\bullet}$, 100), 409 ($[M - H_2O - Me]^+$, 93), 302 ($[M - C_8H_{17}O - H_2O]^+$, 95); ^1H NMR (400 MHz, $CDCl_3$) δ 4.95 (m, H-26), 4.82 (m, H-26'), 4.0 (m, H-24), 3.3 (m, H-3), 1.7 (s, CH_3-27) 0.95 (s, CH_3-28 and CH_3-30), 0,88 (d, *J* 6.9 Hz, CH_3-21), 0.86 (s, CH_3-18) 0.80 (s, CH_3-29), 0.54 (d, *J* 4.0 Hz, H-19) and 0.32 (d, *J* 4.0 Hz, H-19'); ^{13}C NMR data are presented in Table 2.

Table 2. ^{13}C NMR assignment for compounds **2**, **3** and **4,** and ^1H assignment for compound **2**, assigned via COSY, HMBC, HMQC and selected nOe-difference spectra

C	2		3	3 and 4
	^{13}C	^1H (mult, *J*/Hz)		(mixture)
1	32.4	1.54(a)#, 1.23(e)	31.8[c]	32.1[d]
2	30.8	1.75(e), 1.57(a)	30.3	30.6
3	79.3	3.24(a)	78.7	79.1
4	40.9	—	40.4	40.7
5	47.5	1.28(a)	47.0	47.3
6	21.5	1.59(e), 0.78(a)	21.0	21.3
7	26.5	1.32(e), 1.07(a)	25.9[a]	26.2[b]
8	48.4	1.50(a)	47.9	48.2
9	20.4	—	19.9	20.2
10	26.4	—	26.0[a]	26.3[b]
11	26.9	1.99, 1.10	26.3	26.7
12	33.2	1.59, 1.3	32.8	33.1
13	45.5	—	45.2	45.5
14	49.2	—	48.7	49.0
15	36.0	1.28	35.4	35.8
16	28.5	1.89, 1.29	28.0	28.4 and 28.3
17	52.4	1.56	52.5	52.7
18	18.5	0.97	18.0	18.6
19	30.3	0.31 (d, *J*=4.0), 0.54 (d, *J*=4.0)	29.8	30.1
20	36.8	1.45	35.8	36.2 and 36.1
21	18.7	0.86 (d, *J*=7.1)	17.9	18.3
22	39.4	2.2, 1.7	31.9[c]	32.2[d]
23	126.0	5.59	31.4	31.7 and 31.9
24	139.8	5.59	76.3	76.6 and 76.8
25	71.2	—	147.4	147.7 and 148.0
26	30.4	1.30	111.3	111.6 and 111.1
27	30.3	1.30	17.1	17.8 and 17.4
28	19.7	0.88	19.2	19.5
29	25.8	0.97	25.3	25.7
30	14.4	0.80	13.9	14.2

[a,b,c,d] The assignments with same letter can be changed.
(a) = axial e (e) = equatorial

*(24S)-9,19-Cyclolanost-25-ene-3β,24-diol (**4**)*

^1H NMR (300 MHz, $CDCl_3$) data were similar to those of the compound **3**. ^{13}C NMR (75 MHz, $CDCl_3$) data are presented in Table 2.

Ackowledgments

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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 apllication 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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