7-epi-griffonilide, a new lactone from Bauhinia pentandra: complete $^1$H and $^{13}$C chemical shift assignments

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ABSTRACT

A new lactone, 7-epi-griffonilide (1), and six known compounds, 2, 3a – 3c, 4a and 4b, were isolated from the leaves of Bauhinia pentandra (Fabaceae). The structures elucidation of 1 and 2 were based on detailed 2D NMR techniques and spectral comparison with related compounds, leading to complete assignment of the $^1$H and $^{13}$C NMR spectra.

Key words: Bauhinia pentandra, Fabaceae, cyanoglucoside, lactones, RMN.

INTRODUCTION

In the course of our continuing search for natural products from medicinal plants, we have investigated the leaves of Bauhinia pentandra (Bong.) D. Dietr. B. pentandra is widely distribute in Northeast Brazil where is known as “mororó” and used in folk medicine. The genus Bauhinia contains many species of plants with medicinal interest (Silva and Cechinel Filho 2002). It consists of about 300 species, distributed in most tropical countries, including Africa, Asia and America (Cechinel Filho 2009). Previous phytochemical studies with plants from Bauhinia genus report the presence of lactones, flavonoids, terpenoids, steroids, triterpenes, tannins, quinones and alkaloids (Silva and Cechinel Filho 2002, Maia Neto et al. 2008), while studies from B. pentandra reported the chemical composition of the essential and fatty oils (Duarte-Almeida et al. 2004, Almeida et al. 2015), as well as the isolation of flavonoids (Salatino et al. 1999) and cyanoglucosides (Silva et al. 2013). In this paper, we report the isolation and structure elucidation of lactones (1 and 2), phenylacetic derivatives (3a, 3b and 3c) and a mixture of cyanoglucoside (4a) and glucopyranoside (4b).
Compound 1 is new, and 2, 3a, 3b, 3c, 4a e 4b has not been reported previously in the Bauhinia genus. The structural assignments and relative stereochemistry of 1 and 2 were based on detailed 2D NMR spectroscopy, while 3a, 3b, 3c, 4a e 4b were identified by comparison with NMR spectral data from literature (Wu et al. 1979, Nahar et al. 2005, Kortesniemi et al. 2014).

MATERIALS AND METHODS

GENERAL EXPERIMENTAL PROCEDURES

Optical rotations were measured on a Jasco Polarimeter, Model P-2000, operating at a wavelength of 589 nm and 20°C. NMR spectra were recorded in CD$_3$OD solutions on Bruker DPX-500 spectrometer (equipped with the standard Bruker software) with chemical shifts reported in δ units (ppm) relative to TMS as internal standard. HRMS were performed on Bruker (model mocroTOF) mass spectrometer equipped with an ESI ion source. HPLC analysis was performed on Shimadzu chromatograph, model LC-20AT, equipped with two high pressure pumps and UV-Vis detector, model SPD-M20A. Semi-preparative reversed-phase chromatography was carried out on a Phenomenex column (C18, 250 x 10 mm, 10 µM). The solvents used were deionized water and methanol with spectral grade; detections in the range of 200 to 400 nm. CC was performed on Merck Silica gel 60 F$_{254}$ and TLC on Merck Silica gel 60 plates (0.25 mm).

PLANT MATERIAL

Bauhinia pentandra leaves were collected in Medicinal Plant Garden, Universidade Federal do Ceará (UFC), Ceará, Brazil. A voucher specimen No. 53444, identified by Dr. Edson Paula Nunes, was deposited at the Herbarium Prisco Bezerra, Departamento de Biologia (UFC).

EXTRACTION AND ISOLATION

The dried and pulverized leaves of B. pentandra (507.0 g) were extracted at temperature room with EtOH which gave a residue (80.7 g) after solvent evaporation under reduced pressure. A part of this extract (20.0 g) was prefractionated by CC on Silica gel under reduced pressure eluted successively with hexane, CH$_2$Cl$_2$, EtOAc and MeOH. The fraction eluted with EtOAc (1.97 g) was further chromatographed over a column of Silica gel with solvents of increasing polarity from hexane through EtOAc to MeOH. A total of 125 fractions were collected and combined based on their TLC patterns; 200 µL from combined fractions (F 60-69, 93.6 mg), was further subjected to reversed-phase chromatography, using H$_2$O-MeOH (9:1) as mobile phase in an isocratic system, flow rate 4.0 mL/min and monitored by HPLC semi-preparative column to yield 1 (15.5 mg, $t_R$ 7.15 min) and 2 (10.7 mg, $t_R$ 7.67 min). Another portion of the EtOH extract (12.7 g) was prefractionated by CC on Silica gel eluted successively with hexane, CH$_2$Cl$_2$, EtOAc and MeOH. F-EtOAc fraction after evaporation of the solvent afforded 448.0 mg. Part of this fraction (72.4 mg) was further chromatographed on Silica gel column using solvents of increasing polarity from CH$_2$Cl$_2$ through EtOAc to MeOH to obtain a solid dark brown viscous consisting of the mixture of compounds 3a, 3b and 3c (13.4 mg), soluble in CHCl$_3$. An third portion of the EtOH extract (5.0 g) was dissolved in H$_2$O/MeOH (50:50 v/v) and partitioned between hexane (3x100 mL), CH$_2$Cl$_2$ (3x100 mL) and EtOAc (3x100 mL). After evaporation of the organic phases were obtained: 875 mg (F-hexane), 494 mg (F-CH$_2$Cl$_2$) and 331 mg (F-EtOAc). F-EtOAc fraction (331 mg) was rechromatographed on Silica gel column eluting, successively with hexano, AcOEt and MeOH. A total of 92 fractions were collected and combined based on their TLC patterns; 200 µL from combined fractions (F 80-92, 190.0 mg), was further subjected to reversed-phase chromatography, using H$_2$O-MeOH (9:1) as mobile phase in an isocratic system, flow rate 3.0 mL/min and monitored by HPLC semi-preparative C18 column (Phenomenex) to
yield a resin dark brown consisting of the mixture of compounds 4a and 4b (9.3 mg, \( t_R \) 16 min), soluble in MeOH.

**RESULTS AND DISCUSSION**

Compound 1 was obtained as an orange resin soluble in methanol, \([\alpha]_D^{22} - 3.11 \) (c 0.14, MeOH). The FT-IR spectrum showed absorption bands characteristic for OH (\( \nu_{\text{max}} \) 3419 cm\(^{-1}\)), ester CO (\( \nu_{\text{max}} \) 1733 cm\(^{-1}\)) and C = C (\( \nu_{\text{max}} \) 1633 cm\(^{-1}\)). In the HREIMS spectrum (positive mode), peaks at \( m/z \) 169.0498 ([M + H\(^+\), calc 169.0501) and \( m/z \) 191.0315 ([M + Na\(^+\), calc 191.0320) were consistent with a molecular formula C\(_8\)H\(_8\)O\(_4\) for 1.

Comparative analysis of the \( \{^1\text{H}\}\) and DEPT 135° NMR spectra allowed to recognize signals corresponding six methines (three sp\(^2\) at \( \delta_c \) 113.3, 121.0 and 143.0 and three sp\(^3\) oxygenated at \( \delta_c \) 70.1, 72.8 and 83.5) and two no hydrogenated (olefinic at \( \delta_c \) 163.3 and carbonyl at \( \delta_c \) 176.7) carbons atoms (Table I). The signals at \( \delta_c \) 176.7 and 83.5 were used to characterize the presence of a five-membered lactone ring (Silverstein and Webster 2000). These assignments were consistent with the HREIMS empirical formula, supporting the presence of two hydroxyl groups and five degrees of unsaturation/ring. Thus, NMR data suggested 1 as a bicyclic molecule.

The \( ^1\text{H} \) NMR spectrum of compound 1 showed virtually two sets of signals: \( \delta_H \) 6.63 (dd, 10.0 and 2.5 Hz), 6.16 (d, 10.0 Hz) and 5.89 (s), attributed to the olefinic hydrogen atoms H-4, H-5 and H-2, respectively, and \( \delta_H \) 5.11 (br s, H-8), 4.52 (br s, H-6) and 4.49 (br s, H-7) corresponding to the methinic hydrogens attached to oxygenated carbons. The 2D COSY NMR experiment revealed spins interactions systems attributed to the presence of HO\(^-\)CH-\(^5\)CH=\(^4\)CH- and –O-\(^8\)CH-\(^7\)CH(OH)-\(^6\)CHOH in 1, that were confirmed by 2D HSQC NMR spectral data revealing heteronuclear correlation via one \( \{^1\text{J}_{\text{CH}}\} \) involving the CH-6 \( \delta_c \) 70.1/\( \delta_H \) 4.52), CH-5 \( \delta_c \) 143.0/\( \delta_H \) 6.16), CH-4 \( \delta_c \) 121.0/ \( \delta_H \) 6.63), CH-8 \( \delta_c \) 83.5/ \( \delta_H \) 5.11) and CH-7 \( \delta_c \) 72.8/ \( \delta_H \) 4.49), along with additional transversal peak corresponding to CH-2 \( \delta_c \) 113.3/ \( \delta_H \) 5.89), shown in Table I. Thus, the chemical shifts \( \delta_H \) 6.63, 6.16 and 5.89 indicate that the double bonds are conjugated with each other, and these in turn with the carbonyl group \( \delta_c \) 176.7). The broad singlet signals at \( \delta_H \) 5.11, 4.52 and 4.49 were correlated to the carbon atoms signals at \( \delta_c \) 83.5, 70.1 and 72.8, respectively, in the HSQC NMR spectrum.

**TABLE I**

<table>
<thead>
<tr>
<th>Position</th>
<th>( \delta_c ) (ppm)</th>
<th>( \delta_H ) (Hz)</th>
<th>HMBC (( \delta_{\text{hm}} ))</th>
<th>Position</th>
<th>( \delta_c ) (ppm)</th>
<th>( \delta_H ) (Hz)</th>
</tr>
</thead>
<tbody>
<tr>
<td>1</td>
<td>176.7</td>
<td>-</td>
<td></td>
<td>1</td>
<td>175.8</td>
<td>-</td>
</tr>
<tr>
<td>2</td>
<td>113.3</td>
<td>5.89 (s)</td>
<td>C1, C3, C4, C8</td>
<td>2</td>
<td>112.5</td>
<td>5.89 (d, ~2.0)</td>
</tr>
<tr>
<td>3</td>
<td>163.3</td>
<td>-</td>
<td></td>
<td>3</td>
<td>164.7</td>
<td>-</td>
</tr>
<tr>
<td>4</td>
<td>121.0</td>
<td>6.63 (dd, 10.0, 2.5)</td>
<td>C3, C6, C8</td>
<td>4</td>
<td>120.6</td>
<td>6.62 (dd, 9.5, 2.5)</td>
</tr>
<tr>
<td>5</td>
<td>143.0</td>
<td>6.16 (d, 10.0)</td>
<td>C3, C7</td>
<td>5</td>
<td>144.2</td>
<td>6.27 (dd, 9.5, 1.9)</td>
</tr>
<tr>
<td>6</td>
<td>70.1</td>
<td>4.52 (br s)</td>
<td></td>
<td>6</td>
<td>73.6</td>
<td>4.33 (dt, 7.6, 2.5, 1.9)</td>
</tr>
<tr>
<td>7</td>
<td>72.8</td>
<td>4.49 (br s)</td>
<td>C3, C5, C8</td>
<td>7</td>
<td>80.0</td>
<td>3.53 (dd, 10.8, 7.6)</td>
</tr>
<tr>
<td>8</td>
<td>83.5</td>
<td>5.11 (br s)</td>
<td>C2, C3</td>
<td>8</td>
<td>85.1</td>
<td>4.90 (dd, 10.8, 1.9)</td>
</tr>
</tbody>
</table>

\(^{a}\)Number of hydrogens bound to carbon atoms deduced by comparative analysis of the \( \{^1\text{H}\}\) and DEPT-\(^{13}\text{C}\)NMR spectra. Chemical shifts and coupling constants \( (J) \) obtained from 1D \(^1\text{H}\) NMR spectrum. The HSQC, HMBC and \(^1\text{H}^{-1}\text{H}\)-COSY spectra were also used to \(^1\text{H}\) and \(^{13}\text{C}\) chemical shift assignments.
The data were used to postulate the constitutional structure 1a, which was confirmed by correlations exhibited by the HMBC NMR spectrum. Importantly, the olefinic hydrogen H-2 (δH 5.89) showed correlation with C-1 (δc 176.7, 2JCH) and C-3 (δc 163.3, 3JCH) and CH-8 (δc 83.5, 3JCH) and the H-8 (δH 5.11) with δc C-3 (δc 163.3, 2JCH) and CH-2 (δc 113.3, 3JCH), in agreement with the presence of an α,β-unsaturated lactone. Further, the olefinic hydrogen H-4 (δH 6.63) revealed correlation with the carbons C-3 (δc 163.3, 2JCH) and CH-8 (δc 83.5, 3JCH), while the other olefinic H-5 (δH 6.16) showed heteronuclear coupling with C-3 (δc 163.3, 3JCH). The latter correlations, in turn, are in agreement with a lactone ring joint with a cyclohexene ring (1a).

The relative stereochemistry of 1a was determined from the coupling constants of relevant hydrogens and from the observed 1H-1H NOESY. Thus, the signals corresponding to hydrogens H-6, H-7 and H-8 as broad singlets are consistent with the absence of diaxial interactions (1b). In agreement with these observations, the NOESY spectrum of 1 showed cross-peak assigned to dipolar interaction (spatial proximity) between H-6 and H-8 (1b).

These evidences, as well as the comparison with literature data reported for griffonilide [5, (6R,7S,7aS)-7,7a-dihydro-6,7-dihydroxybenzofuran-2(6H)-one] (Wu et al. 1979) led to elucidation of the structural formula C8H8O4 for 2, a isomer of 1.

Comparative analysis of the {1H}- and DEPT 135° NMR spectra of 2 allowed to recognize signals corresponding six methines [three sp2 at δc 113.4 (CH-2), 123.4 (CH-4) and 139.8 (CH-5) and three sp3 oxygenated at δc 68.7 (CH-6), 74.5 (CH-7) and 83.5 (CH-8)] and two sp2 no hydrogenated at δc 164.5 (C-3) and at δc 176.7 (C-1, conjugated carbonyl) carbon atoms (Table II). Thus, analysis of the 1H and 13C NMR spectra data of 2 confirmed that it was closely relate to 1 (and to 5). In fact, the 1H and 13C-NMR feature were virtually identical except for the small changes in chemical shifts (1H and 13C) and more pronounced changes in the multiplicity and the coupling constants in the case of hydrogen atoms (Table II).

The main difference in the 1H NMR spectrum of 1 and 2 consisted in the signal for H-7, which had changed from a broad singlet at δH 4.49 of 1 to a double doublets at δH 3.64 (J = 10.0 and 4.2 Hz) of 2, indicating diaxial (J = 10.0 Hz) interaction with H-8. The 2D COSY NMR experiment also revealed spin systems compatible with the presence of the HO-CH⁺-CH=–CH- and –O-CH⁺-CH(OH)-⁺CHOH in 2, that were confirmed by 2D HSQC NMR experiment by cross peaks corresponding to heteronuclear interactions involving CH-6 (δc 68.7/δH 4.40), CH-5 (δc 139.8/δH 6.42) and CH-4 (δc 123.4/δH 6.70) and CH-8 (δc 83.5/δH 5.23) and CH-7 (δc 74.5/δH 3.64), summarized in Table II.

The relative stereochemistry of 2 was deduced from their mutual coupling constants of relevant hydrogens and from dipolar interaction revealed by 1H-1H NOESY. Unlike 1 was not observed signal noe between H-6 (δH 4.40) and H-8 (δH 5.23) hydrogen of 2. The diaxial spin-spin interaction between H-7 (δH 3.64) and H-8 (δH 5.23) was de-
Figure 1 - Chemical structures of compounds 1-4 isolates from B. pentandra.
duced from the large coupling constant \(J = 10.4\) Hz) covering these hydrogen atoms. The values of the coupling constants \(J\) observed in the signal at \(\delta_H 4.40\) (H-6, dd, \(J_{6\text{eq}-5} = 5.3\) and \(J_{6\text{eq}-7} = 4.2\) Hz) suggested absence of diaxial coupling, as expected for the proposed stereochemistry.

These evidences, as well as the comparison with literature data reported for griffonilide \([5, (6R,7S,7aS)-7,7a\text{-dihydro}-6,7\text{-dihydroxybenzofuran}-2(6\text{H})\text{-one}]\) (Wu et al. 1979) and dasycarponilide (Wu et al. 1979), led to elucidation of \([6S,7S,7aS)-7,7a\text{-dihydro-6,7\text{-dihydroxybenzofuran}-2(6\text{H})\text{-one}],\) known as dasycarponilide (Figure 1), being first reported in genus Bauhinia.

Complete and unambiguous \(^1\)H- and \(^{13}\)C-NMR chemical shifts based on assignment of 2 based on \(^1\)H – \(^1\)H COSY, \(^1\)H – \(^{13}\)C COSY, \(^1\)H–\(^1\)H-COSY (n=1, HSQC; n=2 and 3, HMBC), and \(^1\)H – \(^1\)H NOESY were summarized in Table II.

The compounds 3 and 4 (Figure 1) were identified by analysis of the spectral data obtained main by 1D and 2D NMR and mass spectra involving comparison with values described in the literature to 3 and 4 (Kortesnimi et al. 2014, Wu et al. 1979).

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7-epi-GRIFFONILIDE, A NEW LACTONE FROM B. pentandra


