



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

MACIA C.S. DE ALMEIDA¹, LUCIANA G.S. SOUZA¹, DANIELE A. FERREIRA¹, FRANCISCO C.L. PINTO¹, DÉBORA R. DE OLIVEIRA², GILVANDETE M.P. SANTIAGO³, FRANCISCO J.Q. MONTE¹, RAIMUNDO BRAZ-FILHO^{2,4} and TELMA L.G. DE LEMOS¹

¹Departamento de Química Orgânica e Inorgânica, Centro de Ciências, Universidade Federal do Ceará, Av. Mister Hull, s/n, Pici, 60021-970 Fortaleza, CE, Brazil

²Departamento de Química, Instituto de Ciências Exatas, Universidade Federal Rural do Rio de Janeiro, Rodovia BR 465, Km 07, s/n, Zona Rural, 23890-000 Seropédica, RJ, Brazil

³Departamento de Farmácia, Universidade Federal do Ceará, Rua Capitão Francisco Pedro, 1210, Porangabuçu, 60451-970 Fortaleza, CE, Brazil

⁴Setor de Química de Produtos Naturais, Universidade Estadual do Norte Fluminense Darcy Ribeiro, Av. Alberto Lamego, 2000, Parque Califórnia, 28013-600 Campos dos Goytacazes, RJ, Brazil

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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 ¹H and ¹³C 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 distributed 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**).

Correspondence to: Francisco José Queiroz Monte
E-mail: fmonte@dqoi.ufc.br

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, Kortensniemi 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 CD₃OD 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 a 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 μ M). 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 F₂₅₄, 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, CH₂Cl₂, 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 μ L from combined fractions (F 60-69, 93.6 mg), was further subjected to reversed-phase chromatography, using H₂O-MeOH (9 : 1) as mobile phase in a 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, CH₂Cl₂, 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 CH₂Cl₂ 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 CHCl₃. An third portion of the EtOH extract (5.0 g) was dissolved in H₂O/MeOH (50:50 v/v) and partitioned between hexane (3x100 mL), CH₂Cl₂ (3x100 mL) and EtOAc (3x100 mL). After evaporation of the organic phases were obtained: 875 mg (F-hexane), 494 mg (F-CH₂Cl₂) 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 μ L from combined fractions (F 80-92, 190.0 mg), was further subjected to reversed-phase chromatography, using H₂O-MeOH (9 : 1) as mobile phase in a 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 (ν_{\max} 3419 cm^{-1}), ester CO (ν_{\max} 1733 cm^{-1}) and C = C (ν_{\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 $C_8H_8O_4$ for **1**.

Comparative analysis of the $\{^1H\}$ - and DEPT 135° NMR spectra allowed to recognize signals corresponding six methines (three sp^2 at δ_C 113.3, 121.0 and 143.0 and three sp^3 oxygenated at δ_C 70.1, 72.8 and 83.5) and two non hydrogenated (olefinic at δ_C 163.3 and carbonyl at δ_C 176.7) carbon atoms (Table I). The signals at δ_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 1H NMR spectrum of compound **1** showed virtually two sets of signals: δ_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 δ_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 $HO^6CH-^5CH=^4CH-$ and $-O-^8CH-^7CH(OH)-^6CHOH$ in **1**, that were confirmed by 2D HSQC NMR spectral data revealing heteronuclear correlation via one ($^1J_{CH}$) involving the CH-6 (δ_C 70.1/ δ_H 4.52), CH-5 (δ_C 143.0/ δ_H 6.16), CH-4 (δ_C 121.0/ δ_H 6.63), CH-8 (δ_C 83.5/ δ_H 5.11) and CH-7 (δ_C 72.8/ δ_H 4.49), along with additional transversal peak corresponding to CH-2 (δ_C 113.3/ δ_H 5.89), shown in Table I. Thus, the chemical shifts δ_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 (δ_C 176.7). The broad singlet signals at δ_H 5.11, 4.52 and 4.49 were correlated to the carbon atoms signals at δ_C 83.5, 70.1 and 72.8, respectively, in the HSQC NMR spectrum.

TABLE I
 1H - and ^{13}C NMR chemical shifts assignments **1 and **5** (CD_3OD , 500 MHz). Chemical shifts in δ (ppm), coupling constants (J) in Hz (in parenthesis)^a.**

Position	1			5 (Griffonilide)	
	δ_C	δ_H (J in Hz)	HMBC (J_{H-C})	δ_C	δ_H (J in Hz)
1	176.7	-		175.8	-
2	113.3	5.89 (s)	C1, C3, C4, C8	112.5	5.89 (d, ~2.0)
3	163.3	-		164.7	-
4	121.0	6.63 (dd, 10.0, 2.5)	C3, C6, C8	120.6	6.62 (dd, 9.5, 2.5)
5	143.0	6.16 (d, 10.0)	C3, C7	144.2	6.27 (dd, 9.5, 1.9)
6	70.1	4.52 (br s)		73.6	4.33 (dt, 7.6, 2.5, 1.9)
7	72.8	4.49 (br s)	C3, C5, C8	80.0	3.53 (dd, 10.8, 7.6)
8	83.5	5.11 (br s)	C2, C3	85.1	4.90 (dd, 10.8, 1.9)

^aNumber of hydrogens bound to carbon atoms deduced by comparative analysis of the $\{^1H\}$ - and DEPT- ^{13}C NMR spectra. Chemical shifts and coupling constants (J) obtained from 1D 1H NMR spectrum. The HSQC, HMBC and 1H - 1H -COSY spectra were also used to 1H and ^{13}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 (δ_{H} 5.89) showed correlation with C-1 (δ_{C} 176.7, $^2J_{\text{CH}}$), C-3 (δ_{C} 163.3, $^2J_{\text{CH}}$) and CH-8 (δ_{C} 83.5, $^3J_{\text{CH}}$) and the H-8 (δ_{H} 5.11) with δ_{C} C-3 (δ_{C} 163.3, $^2J_{\text{CH}}$) and CH-2 (δ_{C} 113.3, $^3J_{\text{CH}}$), in agreement with the presence of an α,β -unsaturated lactone. Further, the olefinic hydrogen H-4 (δ_{H} 6.63) revealed correlation with the carbons C-3 (δ_{C} 163.3, $^2J_{\text{CH}}$) and CH-8 (δ_{C} 83.5, $^3J_{\text{CH}}$), while the other olefinic H-5 (δ_{H} 6.16) showed heteronuclear coupling with C-3 (δ_{C} 163.3, $^3J_{\text{CH}}$). 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**, (6*R*,7*S*,7*aS*)-7,7*a*-dihydro-6,7-dihydroxybenzofuran-2(6*H*)-one] (Wu et al. 1979) led to elucidation of **1** [(6*R*,7*R*,7*aS*)-7,7*a*-dihydro-6,7-dihydroxybenzofuran-2(6*H*)-one], a new 7-*epi*-griffonilide (Figure 1), which is being reported for the first time in the literature.

Complete and unambiguous ^1H - and ^{13}C -NMR chemical shifts assignments of **1** based on ^1H - ^1H COSY, ^1H - ^{13}C COSY- nJ_{CH} ($n=1$, HSQC; $n=2$ and 3, HMBC), and ^1H - ^1H NOESY were summarized in Table I.

Compound **2** was obtained as an orange resin with solubility in methanol, $[\alpha]_D^{22} + 129.31$ (c 0.1, MeOH). The FT-IR spectrum of **2** was very similar to **1**, showing absorption bands characteristic for

OH (ν_{max} 3395 cm^{-1}), ester CO (ν_{max} 1737 cm^{-1}) and C = C (ν_{max} 1633 cm^{-1}). In the HREIMS spectrum (positive mode), peaks at m/z 169.0504 ($[\text{M} + \text{H}]^+$, calc 169.0501) and m/z 191.0320 ($[\text{M} + \text{Na}]^+$, calc 191.0320) were consistent with a molecular formula $\text{C}_8\text{H}_8\text{O}_4$ for **2**, a isomer of **1**.

Comparative analysis of the $\{^1\text{H}\}$ - and DEPT 135° NMR spectra of **2** allowed to recognize signals corresponding six methines [three sp^2 at δ_{C} 113.4 (CH-2), 123.4 (CH-4) and 139.8 (CH-5) and three sp^3 oxygenated at δ_{C} 68.7 (CH-6), 74.5 (CH-7) and 83.5 (CH-8)] and two sp^2 no hydrogenated at δ_{C} 164.5 (C-3) and at δ_{C} 176.7 (C-1, conjugated carbonyl) carbon atoms (Table II). Thus, analysis of the ^1H and ^{13}C NMR spectra data of **2** confirmed that it was closely relate to **1** (and to **5**). In fact, the ^1H and ^{13}C -NMR feature were virtually identical except for the small changes in chemical shifts (^1H and ^{13}C) 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 δ_{H} 4.49 of **1** to a double doublets at δ_{H} 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 $\text{HO}^6\text{CH}^5\text{CH}=\text{CH}^4\text{CH}^3$ - and $-\text{O}^8\text{CH}^7\text{CH}(\text{OH})^6\text{CHOH}$ in **2**, that were confirmed by 2D HSQC NMR experiment by cross peaks corresponding to heteronuclear interactions involving CH-6 (δ_{C} 68.7/ δ_{H} 4.40), CH-5 (δ_{C} 139.8/ δ_{H} 6.42) and CH-4 (δ_{C} 123.4/ δ_{H} 6.70) and CH-8 (δ_{C} 83.5/ δ_{H} 5.23) and CH-7 (δ_{C} 74.5/ δ_{H} 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 (δ_{H} 4.40) and H-8 (δ_{H} 5.23) hydrogen of **2**. The diaxial spin-spin interaction between H-7 (δ_{H} 3.64) and H-8 (δ_{H} 5.23) was de-

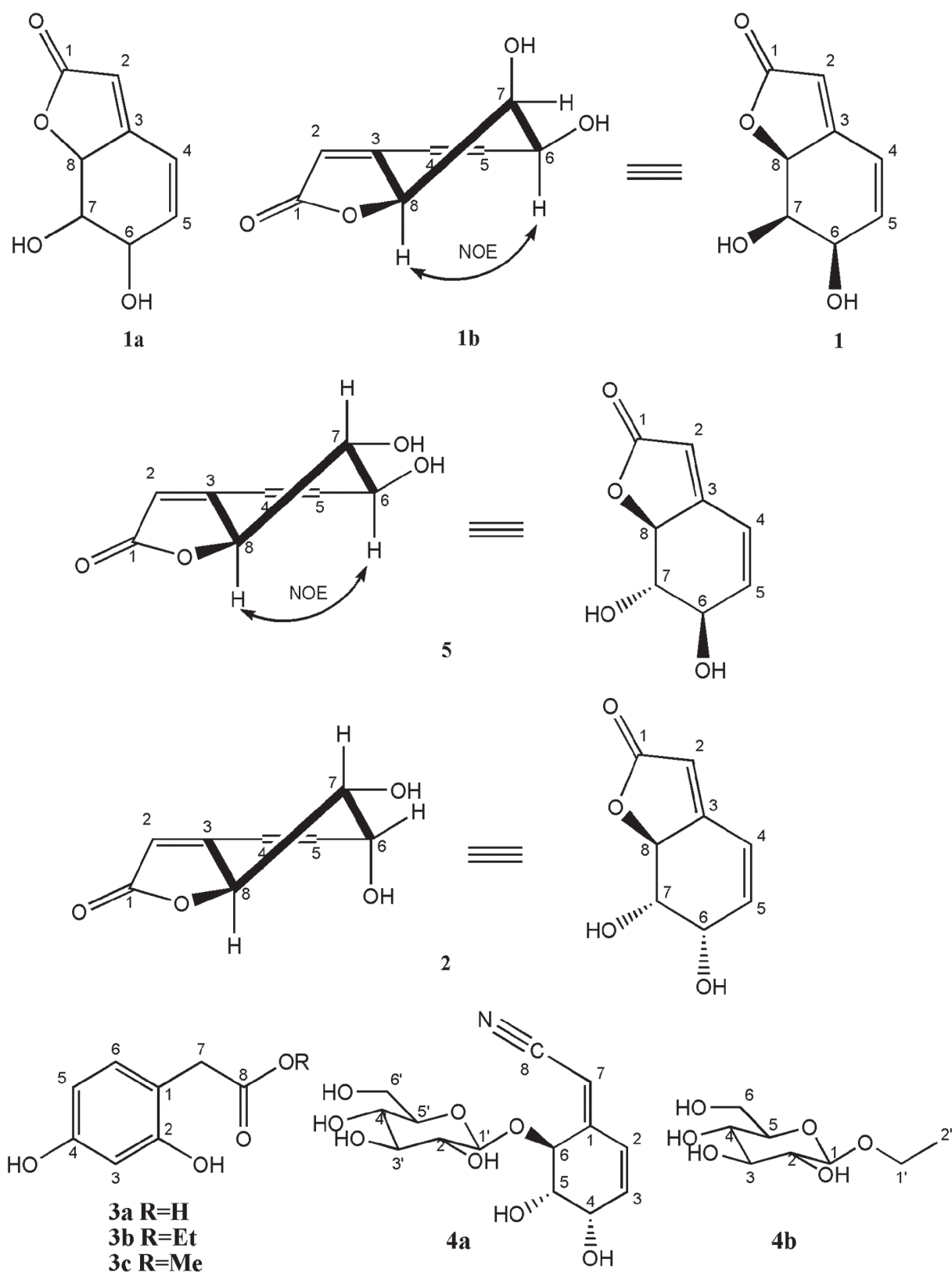


Figure 1 - Chemical structures of compounds 1-4 isolates from *B. pentandra*.

TABLE II
¹H- and ¹³C NMR chemical shifts assignments **2** and Dasycarponilide (CD₃OD, 500 MHz). Chemical shifts in δ (ppm), coupling constants (*J*) in Hz (in parenthesis)^a.

Position	2			Dasycarponilide	
	δ _C	δ _H (<i>J</i> in Hz)	HMBC (<i>J</i> _{H-C})	δ _C	δ _H (<i>J</i> in Hz)
1	176.7	-		175.8	-
2	113.4	5.90 (d, 1.8)	C1, C3, C4, C8	113.3	5.90 (d, 1.9)
3	164.5	-		164.3	-
4	123.4	6.70 (d, 9.6)	C2, C3, C5, C6, C8	123.3	6.69 (d, 9.5)
5	139.8	6.42 (dd, 9.6, 5.5)	C3, C4, C6, C7	139.7	6.41 (dd, 9.5, 5.4)
6	68.7	4.40 (dd, 5.3, 4.2)	C4, C5, C7, C8	68.7	4.39 (dd, 5.4, 4.1)
7	74.5	3.64 (dd, 10.4, 4.2)	C3, C8	74.4	3.63 (dd, 10.5, 4.1)
8	83.5	5.23 (dd, 10.4, 1.7)	C3, C7	83.4	5.22 (dd, 10.5, 1.9)

^aNumber of hydrogens bound to carbon atoms deduced by comparative analysis of the {¹H}-and DEPT-¹³C NMR spectra. Chemical shifts and coupling constants (*J*) obtained from 1D ¹H NMR spectrum. The HSQC, HMBC and ¹H-¹H-COSY spectra were also used to ¹H and ¹³C chemical shift assignments.

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 δ_H 4.40 (H-6, dd, *J*_{6eq-5} = 5.3 and *J*_{6eq-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**, (6*R*,7*S*,7*aS*)-7,7*a*-dihydro-6,7-dihydroxybenzofuran-2(6*H*)-one] (Wu et al. 1979) and dasycarponilide (Wu et al. 1979), led to elucidation of **2** [(6*S*,7*S*,7*aS*)-7,7*a*-dihydro-6,7-dihydroxybenzofuran-2(6*H*)-one], known as dasycarponilide (Figure 1), being first reported in genus *Bauhinia*.

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

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

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REFERENCES

- ALMEIDA MCS, SOUZA LGS, FERREIRA DA, MONTE FJQ, BRAZ-FILHO R AND LEMOS TLG. 2015. Chemical composition of the essential oil and fixed oil *Bauhinia pentandra* (Bong.) D. Dietr. Phcog Mag 11: 362-364.
- CECHINEL FILHO V. 2009. Chemical composition and biological potential of plants from the genus *Bauhinia*. Phytother Res 23: 1347-1354.
- DUARTE-ALMEIDA JM, NEGRI G AND SALATINO A. 2004. Volatile oils in leaves of *Bauhinia* (Fabaceae Caesalpinioideae). Biochem Syst Ecol 32: 747-753.
- KORTESNIEMI M, SINKKONEN J, YANG B AND KALLIO H. 2014. ¹H NMR spectroscopy reveals the effect of genotype and growth conditions on composition of sea buckthorn (*Hippophaë rhamnoides* L.) berries. Food Chem 147: 138-146.
- MAIA NETO M, ANDRADE NETO M, BRAZ-FILHO R, LIMA MAS AND SILVEIRA ER. 2008. Flavonoids and alkaloids from leaves of *Bauhinia unguolata* L. Biochem Syst Ecol 36: 227-229.
- NAHAR L, RUSSELL WR, MIDDLETON M, SHOEB M AND SARKER SD. 2005. Antioxidant phenylacetic acid derivatives from the seeds of *Ilex aquifolium*. Acta Pharm 55: 187-193.

- SALATINO A, BLATT CTT, SANTOS DYAC AND VAZ AMSF. 1999. Foliar flavonoids of nine species of *Bauhinia*. *Rev Bras Bot* 22: 17-20.
- SILVA KL AND CECHINEL FILHO V. 2002. Plantas do gênero *Bauhinia*: composição química e potencial farmacológico. *Quím Nova* 25: 449-454.
- SILVA TMS, LINS ACS, SARMENTO-FILHA MJ, RAMOS CS, AGRA MF AND CAMARA CA. 2013. Riachin, a new cyanoglucoside from *Bauhinia pentandra* and its antioxidant activity. *Chem Nat Compd* 49: 685-690.
- SILVERSTEIN RM AND WEBSTER FX. 2000. Identificação espectrométrica de compostos orgânicos, 6ª ed., Rio de Janeiro: Livros Técnicos e Científicos, 460 p.
- WU J, FAIRCHILD EH, BEAL JL, TOMIMATSU T AND DOSKOTCH RW. 1979. Lithospermoside and dasycarponin, cyanoglucosides from *Thalictrum*. *J Nat Prod* 42: 500-511.

