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Short communication

Spermidine alkaloid from *Banara parviflora*

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ABSTRACT

Banara parviflora (A. Gray) Benth. is a species that belongs to Salicaceae family and is native to Southern Brazil. Fractionation of the ethanolic extract from *Banara parviflora* leaves afforded, a new compound identified as N^1,N^8-dibenzoylspermidinyl-N^4-acetamide, a spermidine alkaloid. The structure was elucidated by spectroscopic methods (IR, MS, ^{1}H, ^{13}C and 2-D NMR).

Introduction

The Salicaceae family has a cosmopolitan distribution, including about 50 genera and 1000 species. Most genera of Salicaceae occurring in Brazil comprise only a few species and have a restricted distribution, except for the species of the genus *Casearia* and *Banara* (Souza and Lorenzi, 2005). The genus *Banara* Aubl. was originally described by Aublet in 1775 and was initially classified as Flacourtiaceae (Sobral et al., 2006). In Chase et al. (2002) approximately two-thirds of all Flacourtiaceae genera, including *Banara* Aubl., were included in the Salicaceae family based on morphological and molecular studies (Chase et al., 2002; Mosaddik et al., 2007). The genus comprises about 64 species, mainly distributed in South America. The species *Banara parviflora* (A. Gray) Benth. is popularly known in Rio Grande do Sul as "farinha-seca", meaning "dry flour" (Sobral et al., 2006). Other species of Flacourtiaceae now classified as Salicaceae family, presents mainly phenylpropanoids, sesquiterpenes and clerodane diterpenes in their constitution (Vieira-Júnior et al., 2011; Xia et al., 2015). Otherwise, we did not detect diterpenes compounds in the investigation of *B. parviflora*. Among the scarce chemical studies to the genus *Banara*, there is one screening study that indicates the presence of a cyanogenic glycoside from *B. parviflora* (Spencer and Seigler, 1985).

This paper describes the isolation and identification of the flavone orientin and also the isolation and structural elucidation of a new derivative of spermidine from *B. parviflora* leaves and contributes with complete spectral data to characterize this kind of alkaloid.

Materials and methods

The infrared spectra (IR) were obtained in an IR Prestige-21 FTIR-8400 S (Shimadzu) using KBr. The NMR spectra were obtained on an apparatus Bruker Avance 2 (^{1}H: 500 MHz, ^{13}C: 125 MHz) in $CDCl_3$. Chemical shifts are given in δ (ppm) using TMS as internal standard. The 2D experiments (HSQC, HMBC, COSY, NOESY) were performed using standard Bruker pulse sequences. High-resolution APPI (MS and MS2) mass spectra were obtained on a Bruker spectrometer MicrOTOF-Q II, in positive ion mode. During the isolation procedures, chromatographic separations were performed on silica gel 60 columns (0.04–0.063 mm, 0.063–0.2 mm), activated charcoal (Nuclear®) and gel permeation on molecular Sephadex LH-20 (GE Healthcare®). For TLC analysis, silica gel 60 F256 (Merck®) plates were used. The substances were detected by ultraviolet absorption at 254 and 366 nm, and also by spraying with Dragendorff reagent and diphenylboryloxy-ethylamine 1% in methanol (Natural Reagent). For HPLC analysis, a SCL-10A Shimadzu chromatograph was used, consisting of a LC-10AD pump and NT-10AV ultraviolet detector. HPLC grade methanol, acetonitrile and acetic acid were provided by MTedia®. The purified water Milli-Q (Millipore®) was also used as mobile phase. A Shimpack preparative HPLC column (ODS 10 mm, 250 mm × 21.2 mm) was used for the separations and 0.45 μm Nylon membranes

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(Millipore®) for the filtration of the mobile phase and samples. The flavonoid orientin (3′,4′,5,7-tetrahydroxyflavone-8-glucoside, ≥98.0%) purchased from Extrasynthèse was used as standard.

The plant material (*B. parviflora* (A. Gray) Benth., Salicaceae) was identified by one of us (SALB) and was collected in January of 2009 in Taquarí, Rio Grande do Sul. Voucher specimens (ICN 189972) were deposited at the Herbarium of Department of Botany, UFRGS.

The fresh leaves (400 g) were ground and extracted with ethanol 96 °C for seven days at room temperature. The extract was filtered and concentrated on a rotary evaporator at temperatures below 40 °C resulting in the crude ethanol extract of the leaves of *B. parviflora* (15 g). The dried ethanol extract (15 g) was fractionated by Si gel vacuum liquid chromatography, using a step gradient from hexane to ethyl acetate and then ethyl acetate to methanol as mobile phase, 200 ml of each fraction. Eight fractions were obtained. The last fraction H, which had the greatest yield (9 g), was mostly composed of chlorophylls and was used to perform further fractionation on activated charcoal and silica (1:1) using 200 ml of methanol and methanol:acetonitrile (50:50, v/v) as mobile phase. The methanol fraction yielded 234 mg while the methanol:acetronitrile fraction yielded 45 mg. The methanol fraction (234 mg) was permeated through Sephadex LH-20 in methanol resulting mainly in two fractions (PS20-25: 8.2 mg and PS60: 4.6 mg) that were subsequently purified by preparative HPLC (mobile phase:acetonitrile:methanol:Milli-Q water (30:30:40, v/v/v), flow rate 3 ml/min, detection at 230 nm) resulting in substances **1** and **2**. The yield of the substance **1** in the leaves was 0.004% (w/w) of the dried leaves.

Results and discussion

As an initial approach to the chemical composition of the leaves, the ethanol crude extract was first analyzed by TLC, using different spray reagents as Dragendorff, Ehrlich and Natural Reagent and two major compounds were detected. The fractions and substances were analyzed by thin layer chromatography to monitor the isolation. The orientin standard was used in both tests, TLC and HPLC (injected in the same conditions with the substance **2**) to confirm the identity of the compound **2**. The substance **1** was positive to Dragendorff reagent. The substance **2** was obtained as yellowish powder and showed an UV spectrum similar to flavonoid derivatives. Afterwards, it was identified as orientin by UV, TLC and HPLC comparison with an authentic sample.

Substance **1** was obtained in the form of a white viscous oil that after successive purification procedures was elucidated by spectroscopic analysis (UV, IR, MS and NMR). The ultraviolet maximum absorption was observed at 232 nm and 276 nm (methanol). IR spectrum showed a characteristic absorption in 3410 v cm^{-1} (>NH) and at 1643 and 1635 v cm^{-1} (>C═O) suggesting the presence of an aromatic amide derivative. The atmospheric pressure photoionization (APPI) high resolution mass spectrum exhibited a $[M+H]^+$ ion peak at *m/z* 396.22623, therefore a molecular formula $C_{23}H_{29}N_3O_3$ was deduced, in accordance with the NMR data.

The ^{13}C NMR spectrum and the DEPT experiments revealed the presence of 23 carbon atoms, three of which are carbonyls (δ_C 171.8, 168.0 and 167.6), 12 carbons correspond to aromatic rings, while the remaining carbons correspond to seven CH_2 and only one CH_3 group. This methyl group was attributed to a singlet methyl resonance at δ_H 2.13. Some signals of the ^1H NMR spectrum, namely δ_H 6.85 and δ_H 7.92 (broad *dd*), showed no correlations in the HSQC-DEPT 135 spectrum and were assigned to NH protons, taking into account the information obtained by IR and MS. The COSY spectrum clearly showed correlations between the above mentioned amide protons with the aliphatic methylene hydrogens at δ_H 3.47 and 3.37. These methylene hydrogens and the other CH_2 hydrogens could be sequentially assigned by COSY correlations. Two chains could be distinguished, one with 3 carbons and the other with 4 carbons separated by a —NH— group, characterizing a spermidine derivative (Table 1).

Table 1
^{13}C and ^1H NMR data of compound **1** (^1H, 500 MHz; ^{13}C, 125 MHz; $CDCl_3$, ppm).

Position	**1**	
	δ_C [a]	δ_H [b] (multiplicity, J in Hz)
1	36.2 (CH_2)	3.37 (m, 6.0; 6.8)
2	27.1 (CH_2)	1.78 (m, 6.2; 6.8)
3	42.5 (CH_2)	3.46 (dd, 6.2)
5	48.4 (CH_2)	3.32 (dd, 7.1)
6	26.0 (CH_2)	1.68 (m)
7	27.0 (CH_2)	1.65 (m)
8	39.1 (CH_2)	3.47 (m)
9	171.8 (C)	–
10	21.3 (CH_3)	2.13 (s)
^{8}C-NH	–	6.85 (dd, 6.0; 7.2)
^{1}C-NH	–	7.93 (dd, 6.1; 7.1)
1′	167.6 (C)	–
2′	134.2 (C)	–
3′; 7′	127.0 (CH)	7.87 (dd, 7.1; 1.7)
4′; 6′	128.5 (CH)	7.43 (dd, 7.1; 1.7)
5′	131.3 (CH)	7.48 (m, 7.1)
1″	168.0 (C)	–
2″	134.3 (C)	–
3″; 7″	126.8 (CH)	7.77 (dd, 7.2; 1.5)
4″; 6″	128.5 (CH)	7.43 (dd, 7.2; 1.5)
5″	131.5 (CH)	7.50 (m, 7.2)

[a] 125 MHz, $CDCl_3$.
[b] 500 MHz, $CDCl_3$.

Detailed examination of the HMBC correlations allowed establishing the connectivity as shown in Fig. 1, mainly the hydrogens of both monosubstituted aromatic rings and their correlations. Thus, the high-resolution mass spectra and its fragmentation pattern (Scheme 1), together with the NMR (^1H, ^{13}C and 2-D NMR) data allowed us to propose the structure of **1** as N^1,N^8-dibenzoylspermidinyl-N^4-acetamide.

This is the first report of the occurrence of this substance in the literature, however, a similar structure, N^1,N^8-dibenzoylspermidine, was reported by Alemayehu et al. (1988) as a constituent of *Cassia floribunda* Cav., Fabaceae. The additional acetamide could be included in the structure by an acetylation using acetyl-CoA (Dewick, 2002). The ^1H and ^{13}C NMR data of the compound N^1,N^8-dibenzoylspermidine, provided in the literature, were compared to the substance **1** data, here characterized, and the similarity of the signals allowed us to confirm the structure elucidation. Other derivatives of spermidine have also been reported from other plants, such as N^1,N^{10}-di-dihydrocaffeoylspermidine from extracts of *Iochroma cyaneum*, Solanaceae, and a feruloylspermidine of *Corylus avellana* L., Betulaceae (Meurer et al., 1986; Sattar

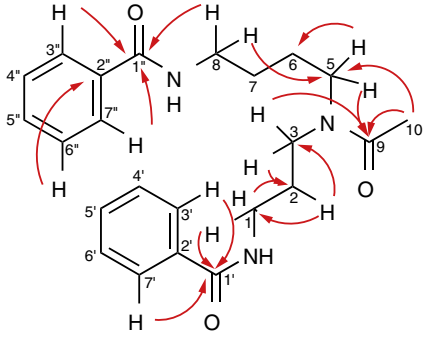
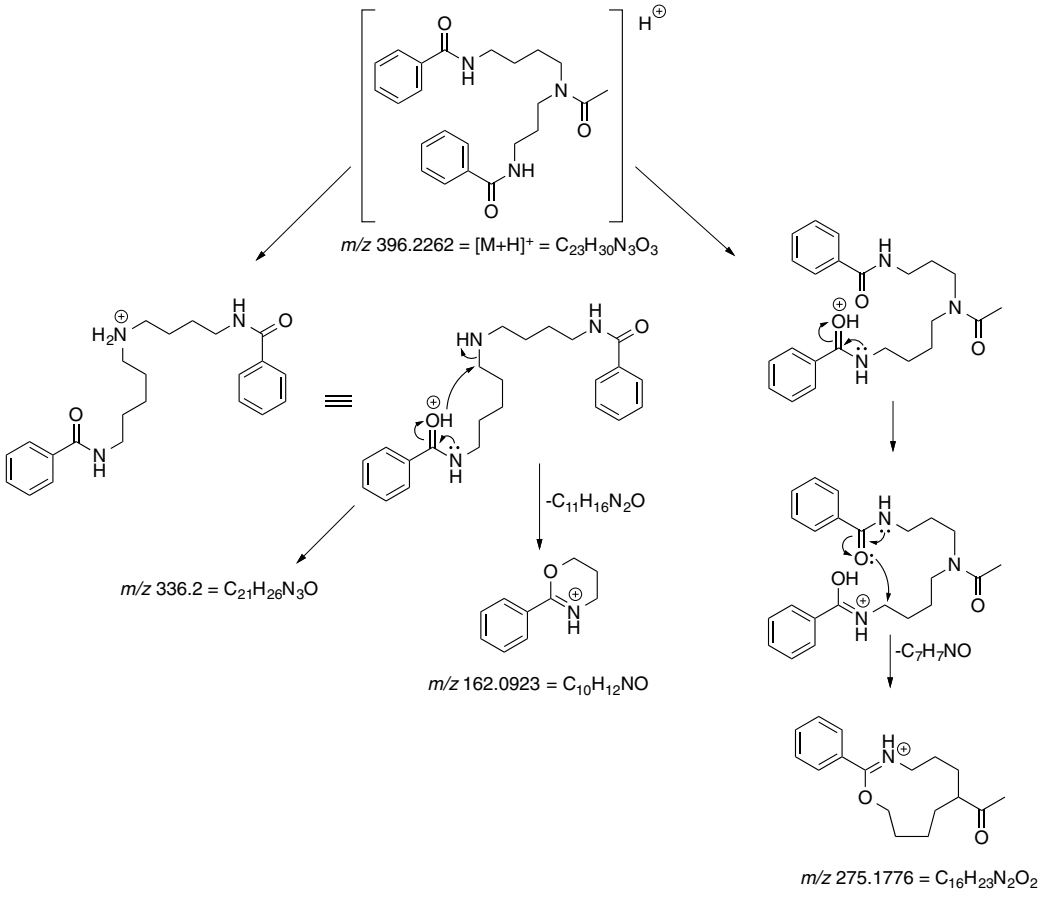


Fig. 1. H–C correlations by HMBC experiments for substance **1**.



Scheme 1. Proposed fragmentation pattern and the main fragment ions in high resolution MS/MS spectrum of the compound **1**.

et al., 1990) as well a siderephore (iron transport compound), parabactin A and its derivatives, from *Micrococcus denitrificans* and also excreted by the soil bacterium *Paracoccus denitrificans*, featuring iron chelator properties, this substance acts like a transport system to assimilate iron in the microorganism with a high affinity to the ferric L-parabactin receptor in membranes (Bergeron et al., 1980, 1988). Additionally, a siderophore also very similar to the substance **1**, agrobactin was identified as a constituent of *Agrobacterium tumefaciens* (Ong et al., 1979). Considering the fact that the siderophores are iron transport compounds and commonly they are found only in microorganisms and in some graminaceous plants, there is a possibility of the substance **1** had been isolated from some endophytic microorganisms (Ahmed and Holmström, 2014).

According to the methodologies described by Bianco et al. (2013), the antimicrobial (against *Staphylococcus aureus* and *Enterococcus faecalis*), antiprotozoal (*Leishmania braziliensis* and *Trypanosoma cruzi*) and antiviral (Herpes Simplex Virus type 1) activities were evaluated for the substance **1**, which did not show activity in this models, at the concentration tested.

***N*^{1},*N*^{8}-dibenzoylspermidinyl-*N*^{4}-acetamide (1)**: white oil, R_f: 0.45 (ethyl acetate, water and formic acid 90:5:5). IR (KBr): 3410, 1643, 1635, 1456, 1309, 615 cm^{-1}. UV/vis λ_{max} (MeOH): 232 nm; 273.3 nm; 526.3 nm. ^1H and ^{13}C NMR: Table 1. APPI MS: *m/z* 396.22623 ($[M+H]^+$ (100), (calc. for $C_{23}H_{30}N_3O_3^+$ 396.22817); MS/MS experiment (CE 15 eV, Argon) on $[M+H]^+$ *m/z* 396.23: *m/z* 354.21752 $[M+H\text{-}CH_2CO]^+$ (calc. for $C_{21}H_{28}N_3O_2$ 354.21760); *m/z* 336.20720 $[M+H\text{-}CH_2CO\text{-}H_2O]^+$, (calc. for $C_{21}H_{26}N_3O$ 336.20704); *m/z* 275.17766 $[M+H\text{-}C_7H_7NO]^+$ (calc. for $C_{16}H_{23}N_2O_2$ 275.17540); *m/z* 233.16654 [*m/z* 275-CH_2CO]$^+$ (calc. for $C_{14}H_{21}N_2O$ 233.16484) and *m/z* 162.09237 (calc. for $C_{10}H_{12}NO$ 162.09134).

Authors' contributions

MIGM contributed with laboratory work, structural elucidation, analysis of the data and writing the manuscript. LAZ contributed with the extraction and isolation of the compounds from *B. parviflora*. SALB suggested the investigation, contributed in collecting the plant, identification and herbarium deposit. JAP, MSBC and GMC participated in the structural elucidation and spectroscopic analysis and to critical reading the manuscript. EPS supervised the work, collected the plant, and contributed writing the manuscript. All the authors have read the final manuscript and approved the submission.

Conflicts of interest

The authors declare no conflicts of interest.

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