http://dx.doi.org/10.5935/0103-5053.20160112

J. Braz. Chem. Soc., Vol. 27, No. 12, 2203-2209, 2016.
Printed in Brazil - ©2016 Sociedade Brasileira de Química
0103 - 5053 $6.00+0.00

Article

Bioactive Indole Alkaloids from *Croton echioides*

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Bioguided fractionation of a hydroethanolic extract from the stem bark of *Croton echioides* Baill. led to isolation of the new indole alkaloid *N-trans*-feruloyl-3,5-dihydroxyindolin-2-one as a stereoisomeric mixture and the known alkaloids *N-trans-p*-coumaroyl-tryptamine, *N-trans-p*-coumaroyl-5-hydroxytryptamine, *N-trans*-4-methoxy-cinnamoyl-5-hydroxytryptamine, *N-trans*-feruloyl-5-hydroxytryptamine (moschamine), from the ethyl acetate fraction. The flavonoids 3-*o*-methyl kaempferol, 3-*o*-methyl quercetin, 3,7-di-*o*-methyl quercetin and 3,3'-di-*o*-methyl quercetin, together with the benzoic acid derivatives 4-hydroxybenzoic acid, 4-hydroxy-3-methoxybenzoic acid and 4-hydroxy-3,5-dimethoxybenzoic acid were also isolated. Alkaloids and the flavonoids showed strong antioxidant properties *in vitro* radical scavenging assay (2,2-diphenyl-1-picrylhydrazyl, DPPH), with IC_{50} values ranging from 9.2 to 17.5 µmol L^{-1}, lower than those of the positive control Trolox (IC_{50} = 17.9 µmol L^{-1}). Alkaloids showed cytotoxic activity against the HCT-116 human cancer cell line, with IC_{50} values ranging from 86.8 to 210.7 µmol L^{-1}. Compound *N-trans*-4-methoxy-cinnamoyl-5-hydroxytryptamine was the most active, with an IC_{50} value of 86.8 µmol L^{-1}.

Keywords: *Croton echioides*, Euphorbiaceae, indole alkaloid, cytotoxic activity, antioxidant activity

Introduction

Plants of the genus *Croton* are well known for their many therapeutic uses, including anti-inflammatory, antiulcer, antitumor, antioxidant and cytotoxic activity.[1-3] This genus is a rich source of special metabolites, mainly clerodane diterpenes, flavonoids, and different classes of alkaloids.[4] Alkaloids related to benzylisoquinolines, such as morphinandienones and tetrahydroprotoberberine alkaloids are the most frequent class found in members of the genus *Croton*.[5] Glutarimide alkaloids and a new class of sesquiterpene guaiane-type alkaloids have recently been reported from *Croton* species.[6]

Croton L. is the second-largest genus in the family Euphorbiaceae, with about 1,200 species, 350 of which occur in Brazil.[5] *Croton echioides* Baill., popularly known as "quebra-faca", "caatinga branca", "velame" and "canela-de-velho", is a small native tree found in the North-eastern region of Brazil.[7,8] The stem bark of this plant has been widely marketed as an aphrodisiac and tonic, as a substitute for the roots of Amazon Marapuama, *Ptychopetalum olacoides* Benth. (Olacaceae).[9] To our knowledge, no chemical or biological study of this species has been reported.

In this paper, we describe the isolation and structure identification of a new indole alkaloid identified as *N-trans*-feruloyl-3,5-dihydroxyindolin-2-one (**1**) together with the known alkaloids *N-trans-p*-coumaroyl-tryptamine (**2**),[10] *N-trans-p*-coumaroyl-5-hydroxytryptamine (**3**),[11] *N-trans*-4-methoxy-cinnamoyl-5-hydroxytryptamine (**4**)[12] and *N-trans*-feruloyl-5-hydroxytryptamine (moschamine) (**5**),[13] the flavonoids 3-*o*-methyl kaempferol (**6**),[14] 3-*o*-methyl quercetin (**7**),[14] 3,7-di-*o*-methyl quercetin (**8**)[15] and 3,3'-di-*o*-methyl quercetin (**9**),[16] and benzoic acid derivatives 4-hydroxybenzoic acid (**10**), 4-hydroxy-

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In memorian of Prof Gentil José Vidotti.

3-methoxybenzoic acid (**11**) and 4-hydroxy-3,5-dimethoxybenzoic acid (**12**)[17] from the stem bark of *Croton echioides*. The *in vitro* cytotoxicity against the HCT-116 human cancer cell line was determined for the isolated alkaloids **1-5** and the free radical (2,2-diphenyl-1-picrylhydrazyl, DPPH) scavenging activities were also determined for the compounds **1**-**12**.

Experimental

General experimental procedures

Infrared (IR) spectra were recorded on a Bomem MB-Series spectrometer (Bomem, Quebec, CA, USA) in a 400 to 4000 cm^{-1} spectral region using KBr matrix. Shimadzu UV-1650-PC spectrometer (Shimadzu, Kyoto, Japan) was used for UV-Vis determinations. Optical rotation was measured on a JASCO P-2000 polarimeter (Jasco, Tokyo, Japan) in MeOH. High-resolution electrospray ionization mass spectrometry (HR-ESIMS) were recorded on a Bruker-Daltonics MicroTof (Bruker-Daltonics, Bremen, Germany), and the ESIMS were recorded on a Thermo Scientific Orbitrap LTQ XL spectrometer (Thermo Fisher System, San Jose, CA, USA) performed at University of Münster, Germany. Nuclear magnetic resonance (NMR) spectra were recorded on a Varian Mercury Plus 300 MHz spectrometer (7.02 T) (Varian, Palo Alto, CA, USA) operated at 75 MHz for ^{13}C and 300 MHz for ^{1}H and 2D NMR, using deuterated MeOH as solvent and tetramethylsilane (TMS) as internal standard. Medium pressure liquid chromatography (MPLC) separation was performed using a Waters 6000A (Waters, Milford, Massachusetts, USA) pump with a manual sample injection valve 10 mL loop coupled with different columns (450 × 10 mm internal diameter (i.d.); 340 × 30 mm i.d.; 300 × 50 mm i.d.) loaded with Sepra C18-E 50 µm (Phenomenex, Torrance, CA, USA) as the stationary phase. The MPLC system is equipped with a Pharmacia-Biotech FRAC-200 (Amersham Biosciences KK, Tokyo, Japan) fraction collector. Preparative counter-current chromatography was performed using a high-speed counter-current chromatography (HSCCC) (P. C. Inc., Potomac, USA) chromatograph equipped with a polytetrafluoroethylene (PTFE) column (2.5 mm i.d.; 200 mL total capacity) coupled to a Waters Mod. 6000A (Waters, Milford, Massachusetts, USA) pump and a manual sample injection valve with a 10 mL loop. Column chromatography was carried out on Sephadex LH 20 (Pharmacia Fine Chemicals Inc., New Market, NJ, USA). Pharmacia-Biotech FRAC-200 fraction collector (Amersham Biosciences KK, Tokyo, Japan) was used for isolations procedures. Thin-layer chromatography (TLC) was performed on precoated silica gel aluminum sheets (Kieselgel 60 F254, 0.20 mm, Merck, Darmstadt, Germany). Fractions and pure compounds were monitored by TLC, and spots were revealed by heating (110 °C) the plates sprayed with a 2% vanillin in chloride acid/EtOH (1:2) solution.

HSCCC separations

The elution systems used in HSCCC separations were optimized by determination of the partition coefficient (Kp) of target compounds in a series of biphasic liquid systems by UV-Vis determinations. Sets of biphasic solvent systems composed of hexane, petroleum ether, ethyl acetate, methanol, ethanol and water was examined at different arrangements and volume ratios. The biphasic liquid system was prepared by adding portions of the solvents in a separatory funnel and, after the equilibrium was reached, 1 mL of each phase (upper and lower) were added in a test tube. After that, 1 mg of the solute was introduced. The test tube was shaken manually until equilibrium was reached. The system was equilibrated for at least 1 h and the concentration of the solute in the upper and the lower phase was analyzed by UV-Vis. The Kp was calculated as the ratio of the solute concentration in the upper and the lower phase. For the samples purification process, the most suitable solvent system (H_2O/EtOH/EtOAc/hexane; 2.5:1:1.5:1.5 v/v) was prepared and thoroughly equilibrated in a separatory funnel at room temperature and the two phases were separated shortly before use. The sample solution was prepared by dissolving the crude sample in 10 mL of the stationary lower phase of the solvent system. The multilayer coiled column was first entirely filled with the lower phase. The upper mobile phase was then pumped into the inlet of the column on tail to head option at the flow rate of 1.0 mL min^{-1}, while the apparatus was run at 800 rpm. After hydrodynamic equilibrium was reached, a sample was injected. The eluent was continuously monitored by TLC and the fractions were collected according to the chromatographic profile. After target compounds were eluted, the HSCCC apparatus was stopped and the column contents were fractionated.

Plant material

The aerial parts of flowering plants of *Croton echioides* Baill. were collected in Lagoa Real County, Bahia State, Brazil, in November 2008, and identified by Prof Daniela Santos Carneiro-Torres. A voucher specimen (No. HUEFS 139049) was deposited in the herbarium of the State University of Feira de Santana, Bahia State, Brazil.

Extraction and isolation

Air-dried and ground stem bark (7.6 kg) of *C. echioides* were extracted with EtOH/H_2O (70%, 76 L) through an Ultra-Turrax Ika Works UTC 115/KT (Ika Works, Wilmington, NC, USA) at room temperature for 48 h, subdivided into 5 min of Ultra-Turrax turbo extraction followed by 2 h of rest, successively. The crude extract was filtered, the solvent was evaporated under reduced pressure, and the residue was lyophilized to give the crude extract (383 g, yield 5.04%). Part of the crude extract (300 g) was suspended in water (2.5 L) and partitioned with hexane, CH_2Cl_2, EtOAc and BuOH (2.5 L) to give the fractions respectively termed HF (62.6 g, yield 20.9%), DF (44.2 g, yield 14.7%), ethyl acetate fraction (EAF) (23.5 g, yield 7.8%) and BF (53.6 g, yield 17.9%). The EAF was subjected to chromatography column over Sephadex LH-20 (460 × 55 mm i.d.) eluting with a gradient of EtOH/H_2O, MeOH, Me_2CO/H_2O and AcOH/H_2O to give 26 fractions (EAF-1 to EAF-26). Fraction EAF-9 (2.43 g, yield 10.3%) was separated by preparative MPLC (column 300 × 50 mm i.d., MeOH/H_2O 1:1 v/v, 3 mL min^{-1}) to give four subfractions (EAF-9.1 to EAF-9.4). Subfraction AF-9.2 (190 mg, yield 7.8%) was purified by HSCCC using H_2O/EtOH/EtOAc/hexane (2.5:1:1.5:1.5 v/v, 1 mL min^{-1}), to yield **1** (47.2 mg, yield 24.8%), 4-hydroxy-3-methoxybenzoic acid (35.0 mg, yield 18.4%) and 4-hydroxy-3,5-dimethoxybenzoic acid (15.0 mg, yield 7.9%). Subfraction EAF-9.3 (241 mg, yield 9.9%) was purified by HSCCC in the same conditions as AF-9.2 to provide 4-hydroxybenzoic acid (5.0 mg, yield 2.1%). The EAF-17 (1.04 g, yield 4.4%) fraction was subjected to MPLC in a C-18 column (340 × 30 mm i.d., MeOH/H_2O, 3:7 v/v, 2 mL min^{-1}), to give two subfractions (AF-17.1 and AF-17.2). Subfractions EAF-17.1 (106 mg, yield 10.2%) and AF-17.2 (60 mg, yield 5.8%) were purified by MPLC in a C-18 column (450 × 10 mm i.d., MeOH/H_2O, 1:1 v/v, 1.5 mL min^{-1}) to yield **4** (5.0 mg, yield 8.3%) and **5** (13.4 mg, yield 22.3%). Purification of the EAF-18 fraction (398.8 mg, yield 1.7%) by MPLC in a C-18 column (340 × 30 mm i.d., MeOH/H_2O, 7:3 v/v, 2 mL min^{-1}), afforded **8** (28.4 mg, yield 7.1%) and **9** (25.2 mg, yield 6.3%). Purification of EAF-19 (283 mg, yield 1.20%) by MPLC (340 × 30 mm i.d., EtOH/H_2O, 3:7 to 9:1 v/v, 2 mL min^{-1}) provided **6** (10.5 mg, yield 3.7%). Subfraction EAF-19.1 (65.0 mg, yield 23.0%) was purified by MPLC (450 × 10 mm i.d., MeOH/H_2O, 5:5 v/v, 1.5 mL min^{-1}) to provide **3** (51.6 mg, yield 79.4%). Purification of EAF-20 (220.6 mg, yield 0.9%) by MPLC (340 × 30 mm i.d., MeOH/H_2O, 7:3 v/v, 2 mL min^{-1}) afforded compounds **7** (78.2 mg, yield 35.4%) and **2** (16.2 mg, yield 7.3%).

N-trans-Feruloyl-3,5-dihydroxyindolin-2-one (**1**)

Amorphous gray solid; mp: 156-158 °C; $[\alpha]_D^{25} = 0°$ (*c* 0.25, MeOH); IR (KBr) ν_{max} / cm^{-1} 3390, 1711, 1650, 1600, 1208; NMR data see Table 1; HR-ESIMS, positive ion mode, *m/z* 407.1220 $[M + Na]^+$; calcd. for $C_{20}H_{20}N_2O_6$: 407.1219.

N-trans-p-Coumaroyl-tryptamine (**2**)

Amorphous yellow solid; NMR data were in agreement with literature values;[10] HR-ESIMS, positive ion mode, *m/z* 329.1257 $[M + Na]^+$; calcd. for $C_{19}H_{18}N_2O_2$: 329.1266.

N-trans-p-Coumaroyl-5-hydroxytryptamine (**3**)

Amorphous gray solid; NMR data were in agreement with literature values;[11] HR-ESIMS, positive ion mode, *m/z* 345.1212 $[M + Na]^+$; calcd. for $C_{19}H_{18}N_2O_3$: 345.1215.

N-trans-4-Methoxy-cinnamoyl-5-hydroxytryptamine (**4**)

Amorphous gray solid; mp 160-162 °C; ^1H NMR (300 MHz, CD_3OD) δ 7.47 (d, 1H, *J* 16.2 Hz, H-15), 7.45 (d, 2H, *J* 8.4 Hz, H-17, H-21), 7.15 (d, 1H, *J* 8.7Hz, H-7), 7.02 (s, 1H, H-2), 6.95 (d, 1 H, *J* 2.4 Hz, H-4), 6.93 (d, 2H, *J* 9.0 Hz, H-18, H-20), 6.65 (dd, 1H, *J* 8.7, 2.4 Hz, H-6), 6.44 (d, 1H, *J* 15.6 Hz, H-14), 3.81 (s, 3H, OCH_3), 3.56 (t, 2H, *J* 7.2 Hz, H-11), 2.92 (t, 2H, *J* 7.2 Hz, H-10); ^{13}C NMR (75 MHz, CD_3OD) 168.0 (C, C-13), 161.3 (C, C-19), 149.8 (C, C-5), 140.3 (CH, C-15), 131.9 (C, C-8), 129.3 (CH, C-17, C-21), 128.2 (C, C-9), 127.7 (C, C-16), 123.2 (C, C-2), 118.2 (CH, C-14), 114.1 (CH, C-18, C-20), 111.6 (CH, C-7), 111.3 (C, C-3), 111.2 (CH, C-6), 102.4 (CH, C-4), 54.7 (CH_3, OCH_3), 40.3 (CH_2, C-11), 25.2 (CH_2, C-10); ESIMS, positive ion mode, *m/z* 359.5 $[M + H]^+$; calcd. for $C_{20}H_{19}N_2O_3$: 337.5.

N-trans-Feruloyl-5-hydroxytryptamine (**5**)

Amorphous gray solid; NMR data were in agreement with literature values;[11] HR-ESIMS, positive ion mode, *m/z* 375.1310 $[M + Na]^+$; calcd. for $C_{20}H_{20}N_2O_4$: 375.1321.

Cytotoxic assays

The cytotoxicity activity against HCT-116 cells was determined by *in vitro* sulforhodamine B test (SRB-test).[18] HCT-116 cell lines, originated from a human colorectal carcinoma, were cultured and maintained in Dulbecco's modified Eagle's medium (DMEM, Gibco, Grand Island, NY, USA) supplemented with 10% heat-inactivated fetal bovine serum (FBS, Gibco) and 50 µg mL^{-1} gentamycin, in incubator set at 37 °C, 5% CO_2 and 95% relative humidity. The cells were expanded when monolayer reached confluence after 3. After reaching 80% confluence, cells were digested by using 0.25% trypsin (Gibco) containing 1 mmol EDTA (ethylenediamine tetraacetic acid). The cells were seeded in 96-well tissue plates (TPP-Techno Plastic Products, Trasadingen, Switzerland) at a density of 8 × 10^5 cell mL^{-1} in 100 µL of medium for 24 h in the CO_2 incubator. The sample solutions with different concentrations (3.12, 6.25, 12.5, 25, 50 and 100 µg mL^{-1}) were prepared and added to the culture media. After incubation for 48 h, the cell monolayers were washed with 100 µL phosphate buffered saline (PBS) sterile, fixed with trichloroacetic acid and stained for 30 min with SRB (7.16 mmol L^{-1}) (Sigma Chemical Co., St. Louis, MO, USA) in acetic acid solution. The dye was removed by four washes with acetic acid solution (175 mmol L^{-1}), and protein-bound dye was extracted with 10 mmol L^{-1} unbuffered tris base [tris(hydroxymethyl)aminomethane] for determination of optical density in a computer-interfaced, 96-well microtiter plate reader (Power Wave XS, BIO-TEK, Winooski, VT, USA). The IC_{50} values were determined from a percentage of cells relative to untreated control and the half-life of the HTC cells in the presence and absence of each tested sample was estimated as the time point at which the cell viability decreased to 50% in relation to the beginning of the test. All the tests were performed in triplicate.

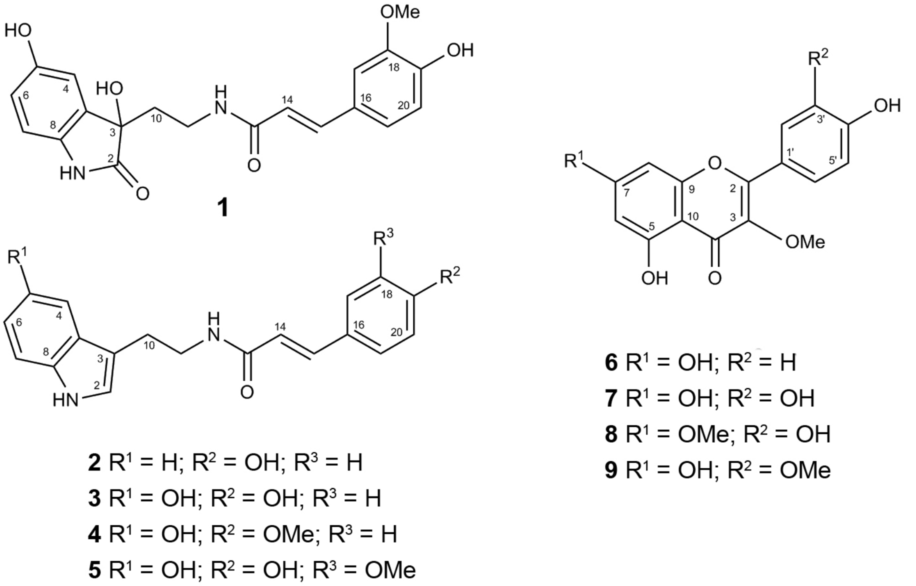
DPPH assay

The free radical scavenging activity was determined by *in vitro* DPPH (2,2-diphenyl-1-picrylhydrazyl) assay.[19] Sample stock solutions (1.0 mg mL^{-1}) of analyte and Trolox (positive control) were diluted to final concentrations of 250, 125, 50, 25, 10 and 5 µmol L^{-1}, in methanol. 1 mL of a 0.3 mmol L^{-1} DPPH solution in methanol was added to 2.5 mL of sample solutions of different concentrations, and allowed to react at room temperature. After 30 min the absorbance values were measured at 518 nm and converted into the percentage antioxidant activity. 1 mL of methanol DPPH solution 0.3 mmol L^{-1} plus methanol (2.5 mL) was used as a negative control. The IC_{50} values were calculated by linear regression. All the tests were performed in triplicate.

Results and Discussion

The ethanol-aqueous extract of stem bark of *C. echioides* was successively partitioned into hexane, dichloromethane, ethyl acetate and butanol. The resulting fractions were subjected to free radical DPPH scavenging assay, which demonstrated that the ethyl acetate fraction was the most active, with IC_{50} = 48.9 ± 0.2 µg mL^{-1}. In order to isolate bioactive compounds, this fraction was submitted to fractionation on a chromatographic column and the subfractions were also assayed in the DPPH. From that results, the most active subfractions were purified by chromatographic methods affording indolinone and *N*-substituted tryptamine alkaloid type compounds (**1**-**5**) which chemical structures are shown in Figure 1. In addition, were isolated flavonoids derived from kaempferol and quercetin (**6**-**9**), as well was benzoic acid derivatives (**10**-**12**). The structures of the isolated compounds were elucidated by analysis of their NMR spectroscopic data (including NMR 2D) and HR-ESIMS, and by comparison with those reported.

Compound **1** was isolated as a stereoisomeric mixture at C-3 position due to the absence of optical rotation



1

2 R^1 = H; R^2 = OH; R^3 = H
3 R^1 = OH; R^2 = OH; R^3 = H
4 R^1 = OH; R^2 = OMe; R^3 = H
5 R^1 = OH; R^2 = OH; R^3 = OMe

6 R^1 = OH; R^2 = H
7 R^1 = OH; R^2 = OH
8 R^1 = OMe; R^2 = OH
9 R^1 = OH; R^2 = OMe

Figure 1. Bioactive compounds isolated of *Croton echioides.*

deviation. The IR spectrum showed mainly two strong bands at 1711 and 1650 cm^{-1} corresponding to the carbonyl stretching vibrations of (γ) lactam and NHCO, respectively. A broad band was found at 3390 cm^{-1} due to N–H and OH stretching. The positive HR-ESIMS spectrum gave the ion peak at *m/z* 407.1220 [M + Na]$^+$ (calculated 407.1219), corresponding to the molecular formula $C_{20}H_{20}N_2O_6$. The NMR spectrum showed signals for oxindole and *trans*-feruloyl moieties which were confirmed by ^{1}H and ^{13}C NMR data assignments (Table 1). Specifically, from the ^{1}H NMR spectra three aromatic hydrogens at δ 6.87 (d, *J* 2.1 Hz, H-4), 6.68 (dd, *J* 8.4 and 2.1 Hz, H-6) and 6.72 (d, *J* 8.4 Hz, H-7), which evidenced the presence of the 1,3-dihydro-3,5-dihydroxy-2*H*-indol-2-one moiety substituted at C-3 position by one aminoethyl unit at 3.26 (m, H-11) and δ 2.13 (t, *J* 7.8 Hz, H-10). This was confirmed by the signals for a carbonyl group at δ 181.7 (C-2), a quaternary carbon at δ 77.0 (C-3), together with the signals for the aromatic ring at 154.8 (C-5), 134.6 (C-9), 133.7 (C-8), 116.7 (C-6), 113.0 (C-4) and 112.0 (C-7), and for an aminoethyl group at δ 38.2 (C-10) and 35.7 (C-11), in the ^{13}C NMR spectra. The presence of a *trans*-feruloyl group was evidenced by the signals for the aromatic system at δ_H/δ_C 7.09 (d, *J* 1.8 Hz, H-17)/ 111.6 (C-17), 6.78 (d, *J* 8.4 Hz, H-20)/116.5 (C-20) and δ_H/δ_C 6.99 (dd, *J* 8.4 and 1.8 Hz, H-21)/123.2 (C-21), a *trans*-olefin system at δ_H/δ_C 7.36 (d, *J* 15.6 Hz, H-15)/142.0 (C-15) and 6.32 (d, *J* 15.6 Hz, H-14)/118.7 (C-14), and for a methoxy group at δ_H/δ_C 3.87 (3H, s)/56.4. The signals of the carbonyl group and aromatic carbons of the *trans*-feruloyl moiety were observed at δ_C 169.2 (C-13), and at δ_C 149.8 (C-19), 149.3 (C-18) and 128.3 (C-16), respectively. The linkage of the moieties *trans*-feruloyl and 1,3-dihydro-3,5-dihydroxy-2*H*-indol-2-one was confirmed by the ^3J correlation of the H-15 (δ 7.36) and H-11 (δ 3.26) signals with the carbon carbonyl group at δ 169.2 (C-13) in the HMBC spectra. In addition, it was observed an HMBC correlation between the methylene hydrogens at δ 2.13 (H-10) and the carbonyl and quaternary carbons at C-2 and C-3, respectively. These data supported the structure determination of **1** as *N-trans*-feruloyl-3,5-dihydroxyindolin-2-one.

Compound **4** has previously been synthesized, but no complete NMR assignments were reported.[12] Here, we report its isolation and characterization from a natural source.

The alkaloids (**1-5**), flavonoids (**6-9**) and benzoic acid derivatives (**10-12**) were evaluated for their antioxidant activity by the free scavenging DPPH method. The results for compounds **1-5**, expressed by IC$_{50}$, are shown in Table 2. Compounds **3-5** and the flavonoids **7** and **8** showed prominent antioxidant properties, with IC$_{50}$ values ranging from 9.2 to 17.5 µmol L^{-1}, lower than those of the positive control Trolox (IC$_{50}$ = 17.9 µmol L^{-1}). Comparison of the IC$_{50}$ value of compounds **2** (203.7 µmol L^{-1}) and **3** (10.7 µmol L^{-1}) showed that the hydroxy group at the C-5 of the tryptamine moiety is fundamental for the antioxidant activity of these indole alkaloids.

The cytotoxic activity of isolated indole alkaloids (**1-5**) was tested *in vitro* against the HCT-116 human cancer cell line, and the results are expressed in Table 2, with IC$_{50}$ values ranging from 86.8 to 210.7 µmol L^{-1}. Compound **4** was the most bioactive, with an IC$_{50}$ value of 86.8 µmol L^{-1}. According to the texted protocol, the alkaloid **1** was not bioactive (IC$_{50}$ > 250 µmol L^{-1}), which suggests that the indole unit present in alkaloids (**2-5**) is important for the cytotoxic activity.

Table 1. ^{1}H, ^{13}C NMR and HMBC data of the compound **1** in CD_3OD

Position	δ_H mult. (*J* in Hz) / ppm	δ_C / ppm	HMBC
2		181.7	H-10
3		77.0	H-10, H-11
4	6.87, d (2.1)	113.0	
5		154.8	H-4, H-6, H-7
6	6.68, dd (8.4, 2.1)	116.7	H-4
7	6.72, d (8.4)	112.0	
8		133.7	H-7
9		134.6	H-4, H-10
10	2.13, t (7.8)	38.2	H-11
11	3.26, m	35.7	H-10
13		169.2	H-11, H-14, H-15
14	6.32, d (15.6)	118.7	H-15
15	7.36, d (15.6)	142.0	H-17, H-21
16		128.3	H-14, H-15, H-20
17	7.09, d (1.8)	111.6	H-15, H-21
18		149.3	$-OCH_3$
19		149.8	H-17, H-20, H-21
20	6.78, d (8.4)	116.5	
21	6.99, dd (8.4, 1.8)	123.2	H-15, H-17
$-OCH_3$	3.87 (s)	56.4	

HMBC: heteronuclear multiple bond correlation.

Table 2. DPPH antioxidant activity and *in vitro* cytotoxicity against HCT-116 cells of compounds **1-5**

Compound	Antioxidant activity IC$_{50}$ / (µmol L^{-1})	Cytotoxicity IC$_{50}$ / (µmol L^{-1})
1	30.0 ± 0.7	n.a.
2	203.7 ± 25.3	144.3 ± 28.6
3	10.7 ± 0.4	210.7 ± 1.4
4	17.5 ± 0.4	86.8 ± 9.7
5	14.5 ± 0.4	142.1 ± 60.5
Trolox	17.9 ± 1.1	–

n.a. = not active (IC$_{50}$ > 250 µmol L^{-1}).

To our knowledge, this is the first report of indolinone alkaloid from a species of *Croton*, which is an important contribution to chemotaxonomic knowledge of this genus. Concerning the reported activities for the known compounds, the alkaloids have mainly antioxidant and tyronase inhibitory activities.[20-22] Also, anti-inflammatory activity (compounds **2** and **3**),[23] growth-promoting activity for fibroblasts (compound **3**)[24,25] and *in vitro* cytotoxic activity against the CaCo2 colon cancer cells (compound **5**)[21] were previously reported. This is the first report on the cytotoxicity of compounds **1-5** against the HCT-116 human cancer cell line.

Conclusions

The present report is the first chemical study of *C. echioides* Baill., despite this plant species being widely marketed as a substitution to the traditional Amazon Marapuama, *Ptychopetalum olacoides*. Five indole alkaloids were isolated and characterized by NMR data. One of them (*N-trans*-feruloyl-3,5-dihydroxyindolin-2-one) is a new natural product from *Croton* species. Their antioxidant and cytotoxicity activity were reported together with other phenolic compound types. These results represent a relevant contribution to the chemical and biological knowledge of this vegetal species and can support its correct and safe medicinal uses.

Supplementary Information

The NMR spectra of compounds **1** to **5** are available free of charge at http://jbcs.sbq.org.br as a PDF file.

Acknowledgments

This work was supported by the Brazilian granting agencies CNPq, CAPES/Proap, INCT_if, FINEP and Fundação Araucária. The authors are grateful to Prof Janet W. Reid, JWR Associates, Trumansburg, New York, for the English language revision.

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Submitted: November 18, 2015

FAPESP has sponsored the publication of this article.