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# Lipoperoxidation and Cyclooxygenase Enzyme Inhibitory Piperidine Alkaloids from *Cassia spectabilis* Green Fruits

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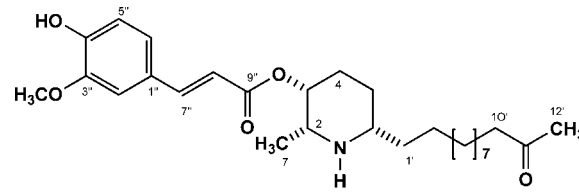
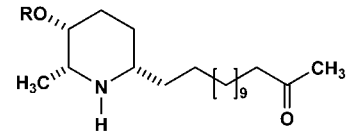
Phytochemical work in the search for bioactive metabolites from the methanolic extract of *Senna spectabilis* green fruits led to the isolation of a new piperidine alkaloid, (+)-3-*O*-feruloylcassine (**1**), in addition to the known (−)-spectaline (**2**) and (−)-3- *O*-acetylspectaline (**3**). The isolates were submitted to in vitro evaluation of lipoperoxidation (LPO) and cyclooxygenase enzymes (COX-1 and -2) inhibitory properties and showed moderate antioxidant activities (40–70%) at 100 ppm when compared to commercial standards BHT and vitamin E and moderate inhibition of COX-1 (ca. 40%) and marginal inhibition of COX-2 enzymes (<10%) at 100 ppm when compared to nonsteroidal anti-inflammatory drugs (NSAIDs) aspirin, rofecoxib, and celecoxib, respectively.

The chemotaxonomy of widely occurring *Senna* and *Cassia* (Fabaceae) species has been the subject of extensive studies, leading to reclassification of some species, including *Cassia spectabilis.*[1] Both of these genera exhibit a broad spectrum of pharmacological properties and ethnomedical uses, especially those associated with inflammation.[2−6] In Brazil, the tea of their crushed leaves is used to treat throat inflammation and diarrhea.[7] Therefore, many plants belonging to *Senna* and *Cassia* species have been or are being studied for their anti-inflammatory botanical extracts or for the isolation of new metabolites with unique chemical structures, mode of action, selectivity, and/or effectiveness. Extracts of species from both genera have been investigated for anti-inflammatory activity using in vitro and in vivo assays. For example, *Cassia italica* is one of the most studied species due to its analgesic, antipyretic, laxative, and antitumor properties.[8] Its ethanolic extract decreased the severity of rat paw edema in a potency comparable to acetylsalycilic acid and inhibited prostaglandin biosynthesis by rat peritoneal leucocytes.[9] An extract of *Senna dariensis* has been used against snakebite and was highly active in the inhibition of the hemorrhagic effect of the venom from *Bothrops atrox*,[10] whereas *Senna neglecta* and *Senna alata* have been used in southern Brazil and Thailand, respectively, to treat inflammation linked to microbial infection.[11,12] *Cassia tora* and *C. angustifolia* leaf extracts have also shown anti-inflammatory properties using in vivo models with histamine-induced rat hind paw edema and 12-*O*-tetradecanoylphorbol-13-acetate (TPA)-induced mouse ear edema, respectively, whereas *C. fistula* leaves methanolic extract was evaluated in in vitro models and efficiently protected free radical-induced lipid peroxidation in model membranes and also inhibited the 5-lipoxygenase-mediated peroxidation of arachidonic acid.[13−15]

As part of our ongoing research on active metabolites in plants of Cerrado and Atlantic Rain Forest of São Paulo State (Brazil), we have recently reported the isolation of several new piperidine alkaloids including (−)-spectaline (**2**), (−)-3-*O*-acetylspectaline (**3**), and (+)-spectaline from leaves, flowers, and ripe fruits of *Senna spectabilis*, which showed anti-inflammatory and acetylcholinesterase inhibitory properties.[16−18] Herein we report the isolation and structure elucidation of a new piperidine alkaloid, (+)-3-*O*-feruloylcassine (**1**), from unripe fruits of *Senna spectabilis* ((DC.) Irwin & Barneby), along with the identification of the known alkaloids **2** and **3**. In addition, we also report their free radical scavenging potential toward DPPH, lipoperoxidation (LPO), and cyclooxygenases-1 and -2 (COX-1 and COX-2) inhibitory activities.




**1**




**2** R = H
**3** R = Ac

Compound **1** was isolated as a pale yellow solid (mp 7–74 °C). HRESIMS and elemental analysis of **1** indicated the molecular formula $C_{28}H_{43}NO_5$, with eight degrees of unsaturation. Its IR spectrum suggested the presence of secondary amine (3413, 1514 $cm^{-1}$), ketone (1710 $cm^{-1}$), and olefinic (3050, 1629, 1529 $cm^{-1}$) moieties. The $^1H$ NMR spectroscopic signals for two vicinal olefinic methines at $\delta$ 7.68 (d, $J = 15.5$ Hz, H-7″) and $\delta$ 6.36 (d, $J = 15.5$ Hz, H-8″) with a *trans* relative configuration, three aromatic methines at $\delta$ 7.06 (brs, H-2″), $\delta$ 7.00 (d, $J = 8.5$ Hz, H-6″), and $\delta$ 6.82 (d, $J = 8.5$ Hz, H-5″), and three methoxyl protons at $\delta$ 3.81 (s, H-10″) suggested a feruloyl moiety in compound **1**. This spectrum also gave signals for one oxymethine hydrogen at $\delta$ 4.99 (brs, H-3), three methyl protons at $\delta$ 1.19 (m, H-7), and two methines at $\delta$ 3.14 (m, H-2) and 2.80 (m, H-6). The two multiplets at $\delta$ 1.62 and 1.48, integrated for four protons, were assigned to $H\text{-}4_{eq}/H\text{-}5_{eq}$ and $H\text{-}4_{ax}/H\text{-}5_{ax}$, respectively, on the basis of correlations observed in the $^1H-^1H$ COSY spectrum. Signals for methylene protons at $\delta$ 1.48 (m, H-1′), 1.16–1.21 (m, H-2′−H-9′), and 2.34 (t, 7.5 Hz, H-10′) and methyl protons at $\delta$ 2.06 (s, H-12′) clearly indicated a long saturated side chain as previously observed in compounds **2** and **3**.[16] The $^{13}C$ NMR and DEPT-135° spectra showed signals for three methine carbons at $\delta$ 69.0, 56.9, and 54.4,

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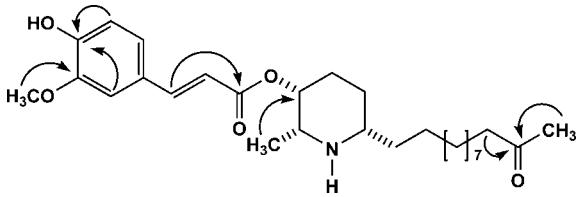


**Figure 1.** Selected HMBC (H→C) correlations for compound **1**.

methylene carbons at $\delta$ 29.1–29.7, and one methyl carbon at $\delta$ 16.9, which strongly supported the 2,6-disubstituted-piperidin-3-ol ring similar to those reported for (−)-spectaline (**2**) and (−)-3-*O*-acetylspectaline (**3**)[16] (Figure 1).

The joint analysis of the $^{13}$C NMR, $^{1}$H NMR, and HMBC spectra evidenced signals for a carbonyl carbon at $\delta$ 209.6, correlating to methylene protons at $\delta$ 2.34 and to methyl protons at $\delta$ 2.06, which were assigned to a terminal methyl ketone subunit on the side chain at C-6. The carboxyl signal at $\delta$ 166.7 correlated to the olefinic methine at $\delta$ 7.68 in the HMBC spectrum and suggested an $\alpha,\beta$-unsaturated conjugation between the piperidine unit and the 1,3,4-trisubstituted aromatic ring. The downfield value of the methine carbon at $\delta$ 69.0, when compared to that in (−)-spectaline ($\delta$ 67.9), and its correlation with the H-7 methyl protons led us to propose a conjugated ester at C-3 on the piperidine ring. The HMBC correlations observed for the methoxy protons at $\delta$ 3.81 to the quaternary carbon at $\delta$ 147.0 and for the aromatic methines H-2″ ($\delta$ 7.06), H-5″ ($\delta$ 6.82) to the carbon at $\delta$ 148.3 evidenced the presence of aromatic methoxy and hydroxy substituents at C-3″ and C-4″ (Figure 1). The NOESY 1D experiment indicated the position of methoxy at C-3″ and hydroxy at C-4″ on the basis of the sole spatial correlation of the methoxy protons and H-2″. All of these assignments were confirmed by additional HMBC correlations and led us to propose the structure of 2-methyl-3-feruloyl-6-(dodecyl-11′-one)piperidine for compound **1**, which was confirmed by the molecular ion peak at *m*/*z* 474.3198 in its HRESIMS. The relative configurations at C-2, C-3, and C-6 were established by comparison of the coupling constants observed for **1** with those published for **2** and **3**[16,19] and NOESY 1D experiments. The irradiation of the H-2 signal confirmed its relative spatial correlations to H-3, H-4$_{ax}$, H-6, and H-7 protons as *cis*. Therefore, the structure of **1** was assigned as (+)-2-methyl-3-feruloyl-6-(dodecyl-11′-one)piperidine.

The free radical scavenging activity of compounds **1**–**3** was evaluated spectrophotometrically by using the stable free radical DPPH and measuring the decrease in absorption at 517 nm. Compounds **2** and **3** were weakly active in this assay and exhibited absorbance variation lower than 10% at 60 ppm. However, compound **1** showed moderate activity ($\%\Delta A = 40\%$ at 60 ppm) when compared to the standard rutin ($\%\Delta A = 92\%$ at 60 ppm), which may be associated with the presence of a feruloyl moiety in the structure of **1**.

The lipoperoxidation inhibitory activity of the isolates was evaluated by measurement of the fluorescence decay after adding $Fe^{2+}$ to initiate the oxidation of large unilamellar vesicles. The alkaloids were tested at 100 ppm, the extract was tested at 250 ppm, and commercial antioxidants BHT, BHA, and vitamin E were used as standard reference compounds at 1.9, 2.2, and 4.3 ppm, respectively. Alkaloids **2** and **3** exhibited moderate inhibition (ca. 75% after 21 min, Figure 2) of the liposome peroxidation. Alkaloid **1** showed only 50% inhibition of the LPO, despite the presence of a feruloyl moiety in its structure, which is normally associated with an increase in antioxidant activity. This behavior might be at least partly explained by the possible chelation of $Fe^{2+}$ ions by compounds **2** and **3**, in contrast to the poor ability for $Fe^{2+}$ chelation of compound **1**. The use of AAPH as the free radical initiator, instead of $Fe^{2+}$, confirmed this observation by showing a similar trend in lipid peroxidation inhibitory activity (ca. 50%) by compounds **1**–**3**.

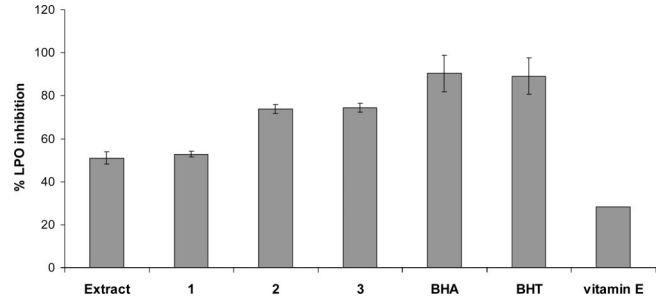


**Figure 2.** Antioxidant activities of MeOH extract (250 ppm) and piperidine alkaloids **1**−**3** (100 ppm). The positive controls BHA, BHT, and α-tocopherol were tested at final concentrations of 1.8, 2.2, and 4.31 ppm, respectively. Vertical bars represent the standard deviation of each triplicate experiment.

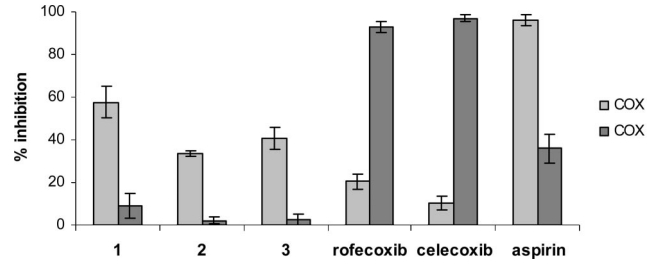


**Figure 3.** COX-1 and COX-2 inhibitory activities of piperidine alkaloids **1**−**3**. Positive controls rofecoxib, celecoxib, and aspirin were tested at final concentrations of 1.7, 1.7, and 108 ppm, respectively, in order to obtain inhibition at ≥50%. Vertical bars represent standard deviation from duplicate experiments.

Inhibition of COX-1 and -2 enzyme activity of compounds **1**−**3** was evaluated by polarographic measurement of the oxygen uptake during the COX-mediated conversion of arachidonic acid into prostaglandins. Each alkaloid was tested at 100 ppm and compared to standard reference compounds rofecoxib, celecoxib, and aspirin, which were tested at 1.7, 1.7, and 108 ppm, respectively. Alkaloids **1**−**3** inhibited COX-1 enzyme by 33, 40, and 58%, respectively. However, the inhibitory activities of COX-2 enzyme by these compounds were marginal (Figure 3).

In summary, we have isolated three alkaloids (**1**–**3**) from the green fruits of *S. spectabilis*, which showed weak to moderate free radical scavenging activity toward DPPH and moderate lipoperoxidation inhibitory properties. In addition, these compounds displayed moderate anti-inflammatory activities, especially toward COX-1. Additional research is needed to further explain the anecdotal medicinal claims of this fruit and its potential to be considered as a medicinal or dietary supplement.

## Experimental Section

**General Experimental Procedures.** Optical rotations were measured on a Perkin-Elmer polarimeter model 341 using a sodium lamp (589 nm) at 20 °C. IR spectra were recorded on a Perkin-Elmer 1725X FT spectrometer with KBr pellets. $^{1}$H and $^{13}$C NMR spectra were recorded on a Varian Unit 500 spectrometer at 500 and 125 MHz, respectively, with $CDCl_3$ as solvent and TMS as internal standard; gCOSY, gHMQC, gHMBC, NOESY, and DEPT NMR experiments were performed in the same spectrometer, using standard Varian pulse sequences. High-resolution mass spectra were measured on a Q-TOF Micromass spectrometer, using the ESI mode and MeOH−$H_2O$ (1:1) as solvent (cone voltage 25 V). $Al_2O_3$ grade I, type WN-3 and silica gel (200–400 mesh) were used in column chromatography and TLC; visualization of TLC plates was made by spraying with iodochloroplatinate reagent (Merck) and Dragendorff's reagent.

**Plant Material.** Plant material was collected in February 2004 from a specimen of *Senna spectabilis* cultivated in the Chemistry Institute at Sao Paulo State University (Araraquara-SP). A voucher (SP 370917) was deposited at the herbarium of São Paulo Botanic Garden, São Paulo-SP, Brazil.

**Extraction and Isolation.** Green fruits of *S. spectabilis* (8 kg) were ground and extracted with EtOH (5 × 5 L). Evaporation of solvent yielded 676 g of a crude extract (8.5%). The extract was analyzed by TLC (silica gel, $CHCl_3$−MeOH−$NH_4OH$, 36:3:1) to confirm the presence of alkaloids by spraying with Dragendorff and iodochloroplatinate reagents. This extract (9 g) was then suspended in MeOH−$H_2O$, 4:1, the insoluble residue was removed by filtration, and the remaining solution was partitioned successively with hexanes, $CH_2Cl_2$, EtOAc, and *n*-BuOH. Analysis of the organic fractions by TLC revealed that most of the alkaloidal constituents were concentrated in the $CH_2Cl_2$ fraction (5.7 g). An aliquot of the $CH_2Cl_2$ fraction (1 g) was then fractionated by neutral alumina column chromatography (hexanes/$Et_2O$ and $Et_2O$/MeOH gradients) to give 30 fractions. Fractions 1–3 (30.4 mg) were further purified by neutral alumina column chromatography ($CH_2Cl_2$−MeOH, 99:1) and gave compounds **2** (3.4 mg) and **3** (9 mg). Fractions 28–30 (402.6 mg) showed strong UV absorption at 254 nm. An aliquot (200 mg) of this mixture was purified by silica gel column chromatography (EtOAc−MeOH−TEA, 90:9.9:0.1) to give alkaloid **1** (20.2 mg).

**(+)-2-Methyl-3-feruloyl-6-(dodecyl-11′-one)piperidine (1):** pale yellow oil; $[\alpha]^{20}_D$ +2.9 (*c* 1.00, $CHCl_3$); UV (MeOH) $\lambda_{max}$ (log $\varepsilon$) 326 (4.25), 236 (4.03) nm; IR (KBr) $\upsilon_{max}$ 3413, 3050, 2927, 2854, 1710, 1629, 1596, 1515, 1452, 1373 $cm^{-1}$; $^1H$ NMR ($CDCl_3$, 500 MHz) $\delta$ 7.68 (1H, d, $J$ = 15.5 Hz, H-8″), 7.06 (1H, br s, H-2″), 7.00 (1H, brd, $J$ = 8.5 Hz, H-6″), 6.82 (1H, d, $J$ = 8.5 Hz, H-5″), 6.36 (1H, d, $J$ = 15.5 Hz, H-7″), 4.99 (1H, br s, H-3), 3.81 (3H, s, $OCH_3$), 3.14 (1H, m, H-2), 2.80 (1H, m, H-6), 2.34 (2H, t, $J$ = 7.5 Hz, H-10′), 2.06 (3H, s, H-12′), 1.62 (2H, m, H-$4_{eq}$/H-$5_{eq}$), 1.48 (6H, m, H-$4_{ax}$/H-$5_{ax}$/H-1′/H-9′), 1.21–1.16 (14H, brs, H-2′-H-8′), 1.19 (3H, m, H-7); $^{13}C$ NMR ($CDCl_3$, 125 MHz) $\delta$ 209.6 (C, C-11′), 166.7 (C, CO feruloyl moiety), 148.3 (C, C-4″), 147.0 (C, C-3″), 145.7 (CH, C-8″), 126.8 (C, C-1″), 123.3 (CH, C-6″), 114.9 (CH, C-7″ and C-5″), 109.6 (CH, C-2″), 69.0 (CH, C-3), 56.9 (CH, C-6), 55.9 ($CH_3$, $OCH_3$), 54.4 (CH, C-2), 43.8 ($CH_2$, C-10′), 34.9 ($CH_2$, C-1′), 29.1–29.7 ($CH_2$, C-4, C-2′−C-8′), 25.6 ($CH_2$, C-5), 23.8 ($CH_2$, C-9′), 16.9 ($CH_3$, C-7); HRESIMS *m*/*z* $[M + H]^+$ 474.3198 (calcd for $C_{28}H_{44}NO_5$ 474.3219).

**Determination of Radical-Scavenging Activity.** 2,2-Diphenylpicrylhydrazyl (DPPH) was used as a stable radical in methanol as per the published procedure.[20] Rutin was used as a reference compound.

**Lipid Peroxidation Assay.** This antioxidant bioassay was conducted, as per the published procedure from our laboratory, by analysis of a model liposome oxidation using fluorescence spectroscopy.[21]

**Cyclooxygenase Enzymes (COX-1 and -2) Inhibitory Assay.** The COX-1 enzyme inhibitory assay was conducted with an enzyme preparation from ram seminal vesicles, and inhibition of COX-2 was performed by using a preparation from insect cell lysate cloned with human PGHS-2 enzyme as per the published procedure from our laboratory.[22,23]

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**Supporting Information Available:** $^1H$ NMR, $^{13}C$ NMR, DEPT, g-COSY, g-HMBC, g-HMQC, and NOESY of (+)-2-methyl-3-feruloyl-6-(dodecyl-11′-one)piperidine are available free of charge via the Internet at http://pubs.acs.org.

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