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Further Bioactive Piperidine Alkaloids from the Flowers and Green Fruits of *Cassia spectabilis*

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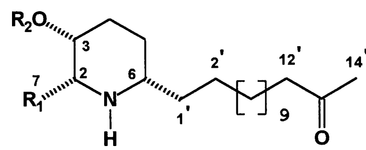
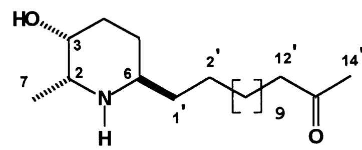
The flowers of *Cassia spectabilis* yielded three new piperidine alkaloids, (−)-3-*O*-acetylspectaline (**1**), (−)-7-hydroxyspectaline (**2**), and iso-6-spectaline (**3**), together with the known (−)-spectaline (**4**). The green fruits of this plant were also investigated, resulting in the isolation of **1** and **4**. Their structures were elucidated using a combination of multidimensional NMR and MS techniques, and relative stereochemistries were established by NOESY correlations and analysis of coupling constants. The DNA-damaging activity of these compounds was evaluated using a mutant yeast, *Saccharomyces cerevisiae*, assay.

Cassia is a major genus of the Leguminosae, comprising about 600 species, some widely distributed throughout the world. In Brazil, *Cassia* plants are recognized as ornamental due to the beauty of their yellow blossoms.[1] Several species of *Cassia* have been reported to accumulate phenolic compounds with diverse biological and pharmacological properties,[2] and these are widely used in traditional medicine for their antimicrobial, laxative, antiulcerogenic, analgesic, and antiinflammatory properties.[2,3] A few 2,6-disubstituted-3-piperidinols with alkyl long chains have been isolated from C. *spectabilis*,[4,5] *C. excelsa*,[6,7] *C. carnaval*,[8] and *C. leptophylla*.[9] In our previous investigation on the chemical composition of the $CHCl_3$/MeOH (2:1) extract of the leaves of *C. leptophylla*, we reported the occurrence of eight new piperidine alkaloids, four that were DNA-damaging active and four that were inactive.[9] This species was mistakenly identified at that time and now has been identified as *Cassia spectabilis* (DC.) Irwin et Barn (Leguminosae). As part of our ongoing search for new bioactive metabolites from plants of Cerrado and Atlantic Forest of the State of São Paulo, we have isolated three new piperidine alkaloids, (−)-3-*O*-acetylspectaline (**1**), (−)-7-hydroxyspectaline (**2**), and iso-6-spectaline (**3**), from the methanolic extract of the flowers and **1** from the green fruits of *C. spectabilis,* along of the known bioactive alkaloid (−)-spectaline (**4**). Herein, we present the isolation, structure elucidation, and inhibitory activity of these alkaloids on mutant yeast strains of *Saccharomyces cerevisiae.*

(−)-3-*O*-Acetylspectaline (**1**) was isolated as a pale yellow powder. HRESIMS and elemental analysis of **1** indicated a molecular formula $C_{22}H_{41}NO_3$, with three unsaturation degrees. Its IR spectrum showed the presence of secondary amine (3341, 1550 cm^{-1}), ester carbonyl (1730 cm^{-1}), and ketone (1715 cm^{-1}) functional groups. The ^{1}H NMR spectrum of **1** showed one hydroxymethine at δ 4.75 (br s, H-3), two methines at δ 2.53 (m, H-6) and 2.82 (dq, J = 2.0, 6.5 Hz, H-2), three methyl peaks at δ 1.00 (d, J = 6.50 Hz, H-7), 2.06 (s, H-14′), 2.00 (s, acetyl), and several methylene



1 R_1 = CH_3; R_2 = Ac
2 R_1 = CH_2OH; R_2 = H
4 R_1 = CH_3; R_2 = H



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protons at δ 1.20−1.22 (br s, H-4′-H-9′). The ^{13}C NMR spectrum showed the presence of three methine carbons at δ 70.4, 56.5, and 53.8, which, when analyzed together with the signals at δ 209.3, 29.1−29.2, and 18.5, strongly suggested a 2,6-disubstituted-3-piperidinol ring, similar to those reported for (−)-spectaline (**4**),[9] previously isolated from *C. spectabilis*. The downfield value observed for hydroxymethine C-3 (δ 70.4) when compared with this carbon in (−)-spectaline (δ 67.9), together with the signals at δ 170.9 and 21.3, clearly indicated that an acetyl group was located at this carbon and allowed us to conclude that **1** is a natural acetyl derivative of (−)-spectaline. From the TOCSY ^{1}H−^{1}H correlations, it was possible to confirm the sequences of H-7/H-2/H-3/H-4/H-5/H-6/H-1′ protons. These assignments were confirmed by HMBC data that allowed unambiguous identification of the atoms in the piperidine ring and joining of the alkyl long chain to C-6 (Figure 1, Supporting Information). 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 **4** and NOESY correlations (Figure 2, Supporting Information). Compound **1** was hydrolyzed under neutral conditions with $(MeO)_2Mg$,[10,11] and the reaction product was identified as (−)-spectaline (**4**).[9] The absolute stereochemistry of **4** has been determined by total synthesis,[12] and the absolute configurations at C-2, C-3, and C-6 for alkaloid **1** were assigned as *R*, *R*, and *S*, respectively.

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Table 1. DNA-Damaging Activity of Piperidine Alkaloids **1**−**4**[a,b]

sample	strains of *Saccharomyces cerevisiae* (μg/mL) RS 188N (rad +)	RS 322YK (rad 322Y)	RS 321N
1	150	26	32
4	125	16	17
camptothecin[c]	200	5	
streptonigrin[c]			4

[a] Compounds **2** and **3** were inactive. [b] Results are expressed as IC_{12} (μg/mL). [c] Positive control substances.

Compound **2** was obtained as colorless oil, with a molecular formula of $C_{20}H_{39}NO_3$ indicated by the molecular ion peak at *m/z* 342.3072 in its HRESIMS. The IR absorption bands for a secondary amine (3340, 1550 cm^{-1}), ketone function (1725 cm^{-1}), and a strong peak for a hydroxyl group (3450 cm^{-1}) were similar to those of **4**.[9] Comparison of the ^{1}H and ^{13}C NMR data of **2** with those of **4** suggested that compound **2** was very similar to **4** except for the data related to the piperidine ring. The ^{13}C data of **2** showed the same resonances observed for **4** with the exception of the values for C-2 (61.1), C-3 (66.2), C-6 (56.9), and C-7 (64.7), indicating that the C-7 methyl group in **2** is a hydroxymethylene group. The significant shielding observed for C-3 (66.2) when compared to **4** could be explained by the γ-effect of the C-7 hydroxymethylene (Figure 2, Supporting Information). With this molecular feature, the OH group at C-3 prefers the axial orientation, permitting an intramolecular H-bond between the hydroxyl at C-7 and the lone pair of electrons on N.[4,13] These findings were further supported by ^{1}H NMR spectra, which showed signals at δ 3.66 (1H, dd, J = 6.5, 11.0 Hz) and 3.74 (1H, dd, J = 4.5, 11.0 Hz) attributable to hydrogens at C-7. The structure of **2** was also strongly supported by HMBC (Figure 1, Supporting Information), ^{1}H−^{1}H COSY, and TOCSY correlations. The stereocenters C-2, C-3, and C-6 in **2** were also assigned as *R*, *R*, and *S*, respectively, by a combination of NMR spectroscopy, molecular modeling, and a comparative analysis based on the known configuration of **4**.

Alkaloid **3** was assigned the molecular formula $C_{20}H_{40}NO_2$ on the basis of the HRESIMS. The ^{1}H and ^{13}C NMR spectral data, however, were quite different from those observed for **4**, mainly the data related to the piperidine ring. Detailed analysis of ^{1}H and ^{13}C chemical shift values closely matched those of iso-6-carnavaline[5] and leptophylline B,[9] except for the values attributed to the long chain, which was similar to those found for **4**. By NOESY spectra, no correlation was observed between H-2 and H-6, as expected due to the changing of configuration at C-6 (Figure 2, Supporting Information). Additionally, interpretation of COSY, HMQC, and HMBC spectra confirmed that **4** was iso-6-spectaline.

Biological activity data for alkaloids **1**−**4** were obtained using mechanism-based yeast mutant bioassays[14] and are summarized in Table 1. These data show that besides (−)-spectaline (**4**),[9] already reported to exhibit some effect on DNA, only alkaloid **1** showed activity, which was comparable to that observed for **4**.

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 125.67 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 ESI mode and $MeOH/H_2O$ (1:1) as solvent (cone voltage 30 V). Al_2O_3 grade I, type WN-3 was used for column chromatography, TLC, and preparative TLC; visualization of TLC plates was made by spraying with iodochloroplatinate reagent (Merck) and Dragendorff's reagent.

Plant Material. The flowers and fruits of *C. spectabilis* were collected in May and December 1999 from a specimen cultivated at the Botanical Garden of the state of São Paulo, Brazil. A voucher specimen (SILVA-193) has been deposited in the herbarium of the Botanic Garden.

Bioassays. Identical to those reported earlier.[14]

Extraction and Isolation. The ethanolic extract of the flowers was concentrated, redissolved in $MeOH/H_2O$ (8:2), and partitioned successively with hexanes, CH_2Cl_2, EtOAc, and *n*-BuOH. The CH_2Cl_2-soluble fraction was concentrated, yielding 39 g of crude material. This was redissolved in 100 mL of CH_2Cl_2 and extracted with aqueous 40% HCl (3 × 50 mL); the combined aqueous fractions were adjusted to pH 9 using concentrated NH_4OH. The resulting aqueous basic solution was exhaustively extracted with CH_2Cl_2, dried over anhydrous $MgSO_4$, and concentrated, furnishing 9 g of a crude alkaloidal fraction. The crude alkaloidal portion was further chromatographed on a neutral Al_2O_3 column with a gradient mixture of $EtOH/CHCl_3$/hexanes as eluent, affording piperidine alkaloids (−)-spectaline (**4**) (4.82 g) and **1** (151 mg) and a complex alkaloid mixture. Further purification of this mixture on preparative TLC, using CH_2Cl_2/*n*-hexane/EtOH (6.5:3.0:0.5) as eluent, afforded (−)-7-hydroxyspectaline (**2**) (5.5 mg) and iso-6-spectaline (**3**) (20 mg).

The fruit extract was partitioned successively with hexanes, CH_2Cl_2, EtOAc, and *n*-BuOH. The CH_2Cl_2 fraction obtained from the partition procedures was extracted with aqueous 40% HCl (3 × 50 mL) and then adjusted to pH 9 with NH_4OH. The aqueous basic solution was exhaustively extracted with CH_2Cl_2 as indicated above for the isolation of alkaloids from flowers. Purification of this alkaloidal mixture by preparative TLC afforded **4** (3.0 mg) and **1** (4.2 mg) as the major constituents.

(−)-3-*O*-Acetylspectaline (1): pale yellow amorphous solid; mp 36.7−39.7 °C; $[\alpha]_D^{20}$ −16° (*c* 0.19, CH_2Cl_2); IR (KBr) ν_{max} 3341, 3350, 2925, 2852, 1730, 1715, 1550, 1452, 1370 cm^{-1}; ^{1}H NMR ($CDCl_3$, 500 MHz) δ 4.75 (1H, br s, H-3), 2.82 (1H, br d, J = 6.5, 2.0 Hz, H-2), 2.53 (1H, m, H-6), 2.34 (2H, t, J = 7.5 Hz, H-12′), 2.06 (3H, s, H-14′), 2.00 (3H, s, $COCH_3$), 1.98 (1H, ddd, J = 11.5, 6.0, 4.5 Hz, H-4a), 1.52 (1H, m, H-5a), 1.50 (2H, m, H-10′), 1.41 (1H, dddt, J = 11.5, 6.0, 3.2 Hz, H-4b), 1.36 (3H, m, H-5b, H-1′), 1.26 (2H, m, H-2′), 1.25 (2H, m, H-10′), 1.23 (2H, m, H-3′), 1.20−1.22 (12H, br s, H-4′ − H-9′), 1.00 (3H, d, J = 6.5 Hz, H-7); ^{13}C NMR ($CDCl_3$, 125 MHz) δ 209.3 (C, C-13′), 170.9 (C, $COCH_3$), 70.4 (CH, C-3), 56.5 (CH, C-6), 53.8 (CH, C-2), 43.7 (CH_2, C-12′), 36.9 (CH_2, C-1′), 29.8 (CH_3, C-14′), 29.7 (CH_2, C-4), 29.5 (CH_2, C-10′), 29.4 (CH_2, C-3′), 29.1−29.2 (CH_2, C-4′−C-9′), 26.8 (CH_2, C-5), 25.9 (CH_2, C-2′), 23.8 (CH_2, C-11′), 21.3 (CH_3, $COCH_3$), 18.5 (CH_3, C-7); HREIMS *m/z* [M + H]$^+$ 368.3163 (calcd for $C_{22}H_{42}NO_3$ 368.3165).

(−)-7-Hydroxyspectaline (2): colorless oil, $[\alpha]_D^{20}$ −7° (*c* 0.04, CH_2Cl_2); IR (KBr) ν_{max} 3450, 3340, 2922, 1725, 1550, 1440 cm^{-1}; ^{1}H NMR ($CDCl_3$, 500 MHz) δ 3.76 (1H, br s, H-3), 3.74 (1H, dd, J = 11.0, 4.5 Hz, H-7), 3.66 (1H, dd, J = 11.0, 6.5 Hz, H-7), 2.70 (1H, dq, J = 7.5, 2.0 Hz, H-2), 2.52 (1H, m, H-6), 2.40 (2H, t, J = 7.5 Hz, H-12′), 2.06 (3H, s, H-14′), 1.96 (1H, ddd, J = 12.5, 6.5, 6.0 Hz, H-4a), 1.60 (1H, dddt, J = 12.5, 6.5, 3.0 Hz, H-4b), 1.50 (1H, m, H-5a), 1.46 (2H, m, H-1′), 1.36 (1H, m, H-5b), 1.26 (2H, m, H-2′), 1.25 (2H, m, H-11′), 1.23 (2H, m, H-10′), 1.19−1.20 (14H, br s, H-3′−H-9′); ^{13}C NMR ($CDCl_3$, 125 MHz) δ 209.4 (C, C-13′), 66.2 (CH, C-3), 64.7 (CH_2, C-7), 61.1 (CH, C-2), 56.9 (CH, C-6), 43.8 (CH_2, C-12′), 36.8 (CH_2, C-1′), 31.8 (CH_2, C-4), 29.8 (CH_3, C-14′), 29.7 (CH_2, C-3′), 29.6 (CH_2, C-10′), 29.2−29.4 (CH_2, C-4′−C-9′), 26.3 (CH_2, C-5), 25.8 (CH_2, C-2′), 23.9 (CH_2, C-11′); HREIMS m/z $[M + H]^+$ 342.3072 (calcd for $C_{20}H_{40}NO_3$ 342.3008).

Iso-6-spectaline (3): yellow amorphous solid, mp 116.7−119.6 °C, $[\alpha]_D^{20}$ −7° (c 0.31, CH_2Cl_2); IR (KBr) ν_{max} 3348, 2825, 1725, 1560, 1370 cm^{-1}; ^1H NMR ($CDCl_3$, 500 MHz) δ 3.66 (1H, br s, H-3), 3.02 (1H, ddd, $J = 7.2$, 5.5, 5.0 Hz, H-6), 2.98 (1H, br d, $J = 6.5$, 2.5 Hz, H-2), 2.35 (2H, t, $J = 7.5$ Hz, H-12′), 2.06 (3H, s, H-14′), 1.95 (1H, ddd, $J = 13.0$, 10.8, 4.5 Hz, H-4a), 1.50 (1H, ddd, $J = 13.0$, 6.6, 5.5 Hz, H-5a), 1.49 (1H, ddd, $J = 10.8$, 6.6, 5.0 Hz, H-4b), 1.46 (2H, dt, $J = 11.0$, 7.2 Hz, H-1′), 1.36 (1H, br dd, $J = 13.0$, 5.0 Hz, H-5b), 1.26 (3H, d, $J = 6.5$ Hz, H-7), 1.25 (4H, m, H-10′, H-11′), 1.23 (2H, m, H-3′), 1.20−1.22 (12H, br s, H-4′ − H-9′); ^{13}C NMR ($CDCl_3$, 125 MHz) δ 209.4 (C, C-13′), 66.6 (CH, C-3), 58.3 (CH, C-2), 57.6 (CH, C-6), 43.8 (CH_2, C-12′), 34.7 (CH_2, C-1′), 31.2 (CH_2, C-4), 29.8 (CH_2, C-14′), 29.5 (CH_2, C-3′), 29.3 (CH_2, C-10′), 29.1−29.2 (CH_2, C-4′−C-9′), 27.6 (CH_2, C-2′), 25.6 (CH_2, C-5), 23.8 (CH_2, C-11′), 15.9 (CH_3, C-7); HREIMS m/z $[M + H]^+$ 326.3463 (calcd for $C_{20}H_{40}NO_2$ 326.3059).

(−)-Spectaline (4): pale white amorphous solid; mp 48.9−52.5 °C, $[\alpha]^{20}_D$ −12° (c 0.14, CH_2Cl_2); all physical and spectroscopic data were in good agreement with those published in the literature.[9]

Hydrolysis of 3-*O*-Acetylspectaline. To a solution of **1** (32 mg, 0.082 mmol) in dry MeOH (1.7 mL) was added 4 g of activated magnesium metal. After an exothermic initiation of the reaction, it was kept under N_2 atmosphere and agitated at room temperature for 8 h. CCD analysis then indicated a less polar product had been formed. More Mg metal (2 g) was added, and the reaction was continued for another 48 h. After total conversion, the reaction was completed by the addition of 0.5 mL of aqueous 2 N HCl followed by extraction with CH_2Cl_2. The combined organic extracts were dried over anhydrous $MgSO_4$ and concentrated, affording 28.3 mg of a solid product, mp 52.6−54.9 °C, $[\alpha]_D^{20}$ −9° (c 0.11, CH_2Cl_2), identified as (−)-spectaline (**4**).[9]

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Supporting Information Available: Figure 1, HMBC correlations, and NOESY correlations for alkaloids **1** and **2**, and Figure 2 showing configurations and Newman projections for alkaloids **1**−**4** are available free of charge via the Internet at http://pubs.acs.org.

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