Journal of Natural Products
Vol. 58, No. 11, pp. 1683-1688, November 1995

BIOACTIVE GUANIDINE ALKALOIDS FROM *PTEROGYNE NITENS*

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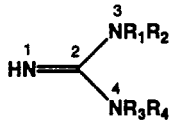
ABSTRACT.—Bioactivity-guided fractionation of a methanolic $CHCl_3$ extract of the leaves of *Pterogyne nitens* afforded the known guanidine alkaloid pterogynidine [**2**] and three new guanidine alkaloids, nitensidines A [**3**], B [**4**], and C [**5**], all of which exhibited selective activity towards the DNA repair-deficient yeast mutant RS 321 (IC_{12}=9.3–20.0 μg/ml); **3**, **4**, and **5** were moderately cytotoxic to CHO Aux B_1 cells (IC_{50}=8.5–13.0 μg/ml).

A number of unusual guanidine alkaloids displaying a broad spectrum of biological activities are known from marine organisms (1,2). In the plant kingdom, guanidine alkaloids are restricted to the families Euphorbiaceae and Leguminosae (3–7). The South American legume, *Pterogyne nitens* Tul., growing in Argentina has been reported previously to contain two terpenoid guanidine alkaloids, pterogynine [**1**] (3) and pterogynidine [**2**] (5).

As part of our search for natural product-based anticancer agents (8) we have investigated the methanolic $CHCl_3$ extract of the leaves of *Pterogyne nitens* collected in Brazil. This extract exhibited a positive response in our mechanism-based bioassay (8,9). Bioactivity-guided fractionation of this extract afforded pterogynidine [**2**] and three new guanidine alkaloids named nitensidines A [**3**], B [**4**], and C [**5**]. Also isolated were a biologically inactive flavonoid, apigenin (4′,5,7-trihydroxyflavone) and the flavonoid glycoside, pedalin [**6**]. In this paper we report the isolation, structure elucidation, and ^{13}C-nmr assignments of the bioactive alkaloids **2**–**5** and the ^{13}C-nmr spectral assignment of **6**.



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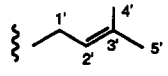

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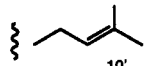

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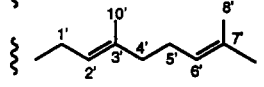

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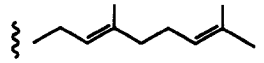

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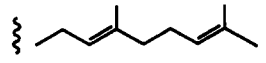

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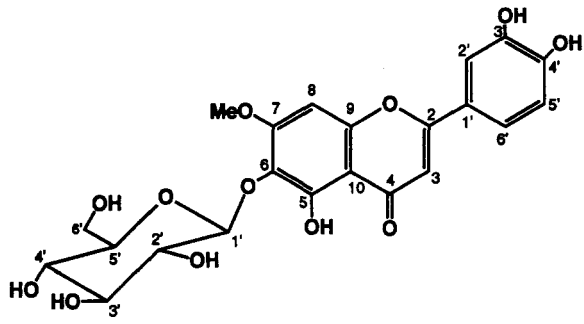

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RESULTS AND DISCUSSION

The dried and powdered leaves of *P. nitens* were extracted with hexane followed by a mixture of $CHCl_3$-MeOH (2:1). Evaporation gave an extract which showed preferential inhibition of the DNA repair-deficient yeast (*Saccharomyces cerevisiae*) strain RS 321 over the repair-proficient wild-type strain RAD+ (8). Bioactivity-guided fractionation of this extract by extraction with $CHCl_3$, with the $CHCl_3$-soluble fraction then being subjected to conventional acid extraction followed by basification of the acidic extract, followed by sequential extraction with $CHCl_3$ and EtOAc and evaporation of the combined organic extracts, afforded a bioactive alkaloidal fraction. Repeated chromatography of this alkaloid mixture over Si gel yielded four compounds, obtained as colorless oils. The structures were determined as guanidine alkaloids **2–5** with the aid of spectroscopic data, as described below.

The least polar alkaloid had spectral (ms, ^{1}H-nmr) data identical to those reported for pterogynidine [**2**] (5). The ^{13}C-nmr spectrum, assigned with the help of DEPT and ^{1}H-^{13}C HETCOR experiments (Table 1), further supported the proposed structure. This is the first instance in which ^{13}C-nmr spectral assignments have been made for this alkaloid. Two out of the three new alkaloids, **3** and **4**, had the same molecular formula, $C_{21}H_{37}N_3$, as deduced from their hreims and ^{1}H- and ^{13}C-nmr spectra. The presence of geranyl and guanidine moieties in both alkaloids was apparent from their ^{1}H- and ^{13}C-nmr spectra (Table 1). The ^{1}H-nmr spectra of **3** and **4** were almost superimposable in the high-field region. In the low-field region **3** had a D_2O-exchangeable broad triplet (2H) at δ 7.60 which was replaced in **4** by a D_2O-exchangeable broad singlet (2H) at δ 7.65. Both compounds had signals in their ^{13}C-nmr spectra due to three methyl, three methylene, two sp^2 methine, and three sp^2 quaternary carbons. Based on the above data and analysis of DQCOSY and ^{1}H-^{13}C HETCOR spectra, nitensidines A and B were assigned the structures **3** and **4**, respectively.

Nitensidine C [**5**], $C_{23}H_{41}N_3$ (hreims), exhibited ^{1}H- and ^{13}C-nmr spectral characteristics similar to those of nitensidine A [**3**]. However, the broad 2H triplet in the low-field region of the ^{1}H-nmr spectrum of **3** was absent in **5**, and was replaced by a singlet (6H, 2×CH_3) at δ 2.19. The ^{13}C-nmr spectrum of **5** resembled those of nitensidines A [**3**] and B [**4**], with the major difference being the presence of an additional Me resonance at δ 39.41 (Table 1). Both ^{13}C- and ^{1}H-nmr chemical shifts indicated the presence of two identically located *N*-Me groups in nitensidine C, indicating this alkaloid to be either the *N*-3,*N*-4 dimethylated derivative of nitensidine A, or the *N*-4,*N*-4 dimethylated derivative of nitensidine B. A choice between these two structures was made on the basis of the HMBC spectrum of the alkaloid (Figure 1), which showed in particular a correlation between the *N*-methyl protons and the C-1′ of the geranyl moiety, leading to the assignment of structure **5** for nitensidine C.

TABLE 1. ^{1}H- and ^{13}C-Nmr Data for the Alkaloids **2–5** in $CDCl_3$ (chemical shifts in ppm from internal TMS).

Position	δ_H[a]				δ_C[b]			
	2	**3**	**4**	**5**	**2**	**3**	**4**	**5**
1	7.60 br s	7.58 br s	7.65 br s	7.48 br s	—	—	—	—
2	—	—	—	—	158.0 s	157.7 s	158.3 s	157.7 s
3	7.68 br s	7.60 br t (2.1)	8.85 br s	—	—	—	—	—
4	7.68 br s	7.60 br t (2.1)	—	—	—	—	—	—
1′	3.79 br s	3.52 dd (5.8, 2.1)	3.74 d (5.8)	3.78 d (2.1)	39.7 t	39.3 t	39.4 t	39.7 t
2′	5.03 t (7.1)	5.25 t[d] (5.8)	5.19 dt (5.8, 1.3)	5.15 t (2.1)	123.7 d	117.7 d	118.8 d	118.2 d
3′	—	—	—	—	132.0 s	141.6 s	141.2 s	141.6 s
4′	1.59 s[c]	2.10 m[e]	2.05 m[f]	2.10 m[h]	17.7 q	39.2 t	39.4 t	39.4 t
5′	1.59 s[c]	2.10 m[e]	2.05 m[f]	2.10 m[h]	25.2 q	26.1 t	26.2 t	26.2 t
6′	—	5.25[d] t (5.6)	5.00 t (5.6)	5.25 t (5.6)	—	123.3 d	123.4 d	123.5 d
7′	—	—	—	—	—	132.0 s	132.2 s	132.1 s
8′	—	1.66 s	1.69 s[g]	1.68 s	—	17.7 q	17.7 q	17.8 q
9′	—	1.69 s	1.69 s[g]	1.66 s	—	25.5 q	25.7 q	25.7 q
10′	—	1.60 s	1.59 s	1.60 s	—	16.3 q	16.4 q	16.4 q
Me-N	—	—	—	2.19 s	—	—	—	39.4 s

[a]At 400 MHz; assignments made by a combination of DQCOSY, HETCOR, and HMBC; *J* in Hz in parentheses.
[b]At 100 MHz; multiplicities determined by DEPT; assignments made by a combination of HETCOR and HMBC.
[c–h]Overlapping signals.

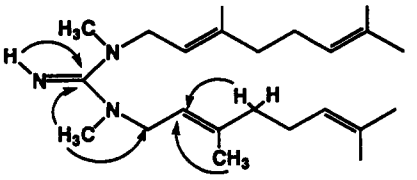

FIGURE 1. Key HMBC correlations observed for nitensidine C [**5**].

The biologically inactive $CHCl_3$-insoluble fraction of the methanolic $CHCl_3$ extract of *P. nitens* (see above) was extracted with MeOH, evaporated, and separated by reversed-phase tlc to afford apigenin and pedalin [**6**]. Pedalin has been isolated previously from *Sesamum indicum* and its structure elucidated on the basis of ^{1}H-nmr and ms data and comparison of its aglycone with a synthetic sample (11). A literature search revealed the absence of ^{13}C-nmr spectral data for **6**. Therefore, complete assignment of the ^{13}C-nmr spectrum for this compound was undertaken and achieved with the aid of DEPT, ^{1}H-^{13}C HETCOR, and HMBC experiments. The ^{13}C-nmr assignments for pedalin [**6**], along with ^{1}H-nmr spectral assignments and observed HMBC correlations, are presented in Table 2.

The guanidine alkaloids **2–5** were tested using our genetically engineered strains of the yeast *Saccharomyces cerevisiae* for mechanism-based anticancer activity (8–10), and

TABLE 2. ^{1}H- and ^{13}C-Nmr Spectral Data for Pedalin [**6**] in DMSO-d_6 (chemical shifts are in ppm from internal TMS).

Position	δ_H[a]	δ_C[b]	HMBC
1	—	—	—
2	—	164.1 s	H-2′, H-6′, H-3
3	6.59 s	102.6 d	—
4	—	182.0 s	H-3
5	—	152.5 s	—
6	—	125.2 s	H-8, H-2, OH-12
7	—	158.4 s	H-8, OMe
8	6.73 s	91.4 d	H-8
9	—	151.6 s	H-8
10	—	104.8 s	H-3, H-8, OH-12
1′	—	121.4 s	H-3, H-2′, H-6′
2′	7.53 d (1.5)	113.4 d	H-2′, H-6′
3′	—	145.7 s	H-2, H-5
4′	—	149.7 s	H-2′, H-5′, H-6′
5′	7.28 d (8.0)	115.8 d	H-6′, H-5′
6′	7.45 dd (8.0, 1.5)	118.9 d	H-2′, H-5′
1″	5.04 d (8.0)	102.1 d	H-2″, H-3″
2″	4.14 t (8.5)	76.5 d	H-3″, H-5″
3″	4.25 m	77.0 d	—
4″	4.96 m	69.9 d	—
5″	4.26 m	74.1 d	—
6″	3.56 dd (10.4, 2.8) 3.61 dd (10.4, 4.0)	60.9 t	H-6″, H-5″, H-4″
OMe	3.89 s	56.2 q	—

[a]At 400 MHz; assignments made by a combination of DQCOSY, HETCOR, and HMBC; *J* in Hz in parentheses.

[b]At 100 MHz; multiplicities determined by DEPT; assignments made by a combination of HETCOR and HMBC.

were found to be active only in the mutant strain RS 321, suggesting the potential of these alkaloids as DNA-modifying agents. The new alkaloids **3–5** were also tested for cytotoxicity using the Chinese hamster ovary auxiliary B1 cell line, and nitensidines A [**3**], B [**4**], and C [**5**] showed moderate activity. The bioassay data for these alkaloids are presented in Table 3. This is the first report of the DNA-modifying activity of guanidine alkaloids.

TABLE 3. Bioactivity Data of the Guanidine Alkaloids **2–5** and Two Standards.[a]

Compound	Organism or Cell Line[b]				
	RS 321	RS 167N (rad6)	RS 322YK (rad52Y)	RS 188N (RAD+)	CHO-Aux-B1
2	8.5	>1000	>1000	>1000	NT[c]
3	6.0	>8000	>1000	>1000	13.0
4	10.0	>8000	>500	>1000	9.3
5	13.0	>8000	>8000	>8000	20
Camptothecin	—	8.7	0.6	—	0.016
Streptonigrin	—	2.4	0.4	—	—

[a]Results are expressed as IC_{12} (RS 321, RS 167N, RS 322YK, RS 188N) (μg/ml) or IC_{50} (CHO-Aux-B1) (μg/ml) values.

[b]CHO-Aux-B1, Chinese hamster ovary auxilliary B1 cell line.

[c]Not tested.

It is noteworthy that all eleven guanidine alkaloids isolated thus far from terrestrial sources belong to the terpenoid class, and that the majority of these alkaloids contain isopentenyl substituents. In some, these isopentenyl groups have undergone cyclization to produce hexahydroimidazo-pyrimidines such as those found in *Alchornea javanensis* (4). Cyclized guanidine alkaloids, probably of sesquiterpene origin, are known from *Milletia laurentii* (6,7). This work, however, constitutes the first report of the natural occurrence of monoterpenoid (geranyl) derivatives of guanidine. The restricted occurrence of guanidine alkaloids in the Euphorbiaceae and Leguminosae may be of chemotaxonomic significance.

EXPERIMENTAL

GENERAL EXPERIMENTAL METHODS.—Ir spectra were obtained on a Perkin-Elmer model 1600 spectrometer. The ^{1}H- and ^{13}C-nmr spectra were recorded on a Varian Unity 400 spectrometer at 400 and 100.57 MHz, respectively, with TMS as internal standard. DQ-COSY, DEPT, ^{1}H-^{13}C HETCOR, and HMBC nmr experiments were performed on this same nmr spectrometer, using standard Varian pulse sequences. Mass spectra were taken on a VG 7070 E-HF instrument. Cc was performed using Merck Si gel G60 (230–400 mesh), prep. tlc with Si gel GF_{254} plates (Analtech, 250 μm, 20×20 cm) and reversed-phase prep. tlc with Whatman PLKC 18F linear K reversed-phase (250 μm, 20×20 cm) plates. Prep. tlc plates were sprayed with iodoplatinate spray reagent at the edges and bands were marked, scraped off, and extracted with EtOAc.

PLANT MATERIAL.—The leaves of *Pterogyne nitens* were collected by Dr. Claudia M.M. Young from Sao Paulo Botanic Gardens, Brazil, in December 1993. Voucher specimens are preserved by Dr. Young in the Herbarium of Sao Paulo Botanic Gardens. Extracts for biological screening were prepared from dry pulverized leaves through sequential solvent extraction with hexanes and a mixture of $CHCl_3$-MeOH (2:1).

EXTRACTION AND ISOLATION.—Dried and pulverized leaves of *P. nitens* (2.0 kg) were sequentially extracted with hexanes and a mixture of MeOH-$CHCl_3$ (2:1). The latter extract (2.5 g) was repeatedly extracted with cold $CHCl_3$ to obtain $CHCl_3$-soluble and -insoluble (1.5 g) fractions. The $CHCl_3$-insoluble residue was extracted with cold MeOH and the MeOH-soluble fraction (0.06 g) was submitted to reversed-phase tlc (eluent, MeOH-H_2O, 80:20) furnishing apigenin (12) and pedalin [**6**] (13). These were identified by comparison of their uv, ms, and ^{1}H-nmr spectral data with those reported. The above $CHCl_3$-soluble

fraction was extracted with aqueous HCl (2%). The acidic aqueous phase was treated with aqueous NH_3 to pH 9 and extracted sequentially with $CHCl_3$ and EtOAc. These extracts were combined and evaporated to yield the crude alkaloidal fraction (0.057 g) that was subjected to cc over Si gel using $CHCl_3$/MeOH mixtures (ranging from 20 to 30% MeOH in $CHCl_3$) to afford the pure alkaloids as colorless liquids, **2** (3.1 mg), **3** (3.7 mg), **4** (1.9 mg), and **5** (2.0 mg).

Pterogynidine [**2**].—Identified by comparison of ir, ms, and ^{1}H-nmr data with those reported (5). For ^{1}H- and ^{13}C-nmr spectral data, see Table 1.

Nitensidine A [**3**].—Ir (film) ν max 1655, 1615, 1000, 840 cm^{-1}; hreims *m/z* [M^+-1] 330.3069 ($C_{21}H_{37}N_3$ requires 330.2987) (5), 315 (3), 264 (10), 196 (50), 126 (40), 69 (100), 55 (60); for ^{1}H- and ^{13}C-nmr spectral data, see Table 1.

Nitensidine B [**4**].—Ir (film) ν max 1650, 1620, 1000, 845 cm^{-1}; hreims *m/z* [M^+-1] 330.3000 ($C_{21}H_{37}N_3$ requires 330.2987) (10), 315 (5), 290 (3), 264 (15), 196 (40), 126 (80), 81 (90), 69 (100); for ^{1}H- and ^{13}C-nmr spectral data, see Table 1.

Nitensidine C [**5**].—Ir (film) ν max 1655, 1620, 1000, 845 cm^{-1}; eims *m/z* [M^+] 359; for ^{1}H- and ^{13}C-nmr spectral data, see Table 1.

BIOLOGICAL TESTING.—The experimental methods utilized in the screening procedure have been described elsewhere (8,9). The IC_{12} values refer to the concentration in μg/ml required to produce a zone of inhibition of 12 mm diameter around a 100-μl well during a 48 h incubation period at 37°. The cytotoxicity assays were performed by Dr. F.L. McCabe at SmithKline Beecham Pharmaceuticals, Philadelphia, using the CHO B-1 cell line. The methanolic $CHCl_3$ extract of *P. nitens* showed selective activity against the mutant yeast strain RS 321, with an IC_{12} value of 950 μg/ml.

ACKNOWLEDGMENTS

This work was funded by a National Cooperative Drug Discovery Group grant (No. 1 U0I 50771) awarded to the University of Virginia (Dr. S.M. Hecht, Principal Investigator). Dr. Bolzani's visit to Virginia Polytechnic Institute and State University was supported by a FAPESP fellowship. We thank Dr. C.M.M. Young, Sao Paulo Botanic Gardens, for identification and collection of plant material, Dr. F.L. McCabe, SmithKline Beecham Pharmaceuticals, Philadelphia, for the cytotoxicity assays, and Mr. Kim Harich, Virginia Polytechnic Institute and State University, for mass spectral measurements.

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Received 22 May 1995