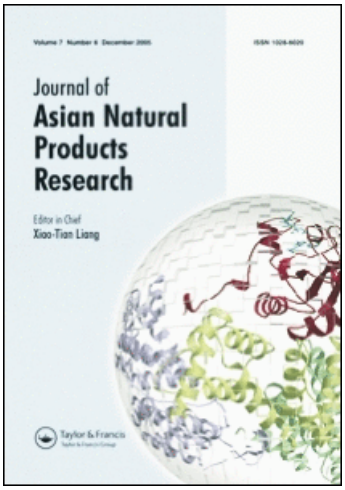

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BOWDICHINE, A NEW DIAZA-ADAMANTANE ALKALOID FROM *BOWDICHIA VIRGILIOIDES*

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A novel alkaloid named bowdichine (**1**), only the third with a diaza-adamantane skeleton containing an unusual *N*-acetyl enamine moiety, and the known alkaloids acosmine (**2**), ormosanine (**3**) and podopetaline (**4**) have been isolated from the stem bark of *Bowdichia virgilioides*. The structures were elucidated on the basis of spectral data, mainly 1D and 2D NMR of the diaza-adamantane alkaloids **1** and **2**.

Keywords: *Bowdichia virgilioides*; Fabaceae; Alkaloids; Bowdichine; Acosmine; Ormosanine

INTRODUCTION

Bowdichia virgilioides Kunt (Fabaceae) is a tree popularly known in Brazil as "sucupira". It occurs in the Atlantic Forest and its seeds are used in Brazilian folk medicine to treat rheumatism, arthritis, and skin diseases [1,2] while the bark is employed for chronic diarrhea and purification of blood [3]. Previous chemical investigation has resulted in the isolation of flavonoids [4–6], benzofuranoids [7], essential oil [8], triterpenoids [7,9,10] and alkaloids [9–11].

In this paper we report a novel diaza-adamantane alkaloid, named bowdichine (**1**), together with the known acosmine (**2**), ormosanine (**3**) and podopetaline (**4**), isolated from the stem bark of *Bowdichia virgilioides* (Fig. 1). The structures were elucidated on the basis of spectral data, mainly 1D and 2D NMR of the diaza-adamantane alkaloids **1** and **2**. Compound **1** is only the third known alkaloid with a diaza-adamantane skeleton containing an unusual

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N-acetyl enamine moiety. The other two alkaloids acosmine (**2**) and acosmine acetate of this new group were previously isolated from the seeds of *Acosmium panamense*, a tall tree from the same family, originally from Central America [12].

RESULTS AND DISCUSSION

The known alkaloids acosmine (**2**), ormosanine (**3**) and podopetaline (**4**) were identified on the basis of spectral data involving comparison with values reported in the literature [9,12,13].

Bowdichine (**1**) had its structure elucidated on the basis of ^{1}H NMR (1D and 2D ^{1}H–^{1}H COSY), ^{13}C NMR (HBBD and DEPT) and heteronuclear 2D-shift-correlated (^{1}H–^{13}C COSY-$^{n}J_{CH}$, $n = 1$, HMQC; $n = 2$ and 3, HMBC) spectra, which were also used to complete ^{1}H and ^{13}C chemical shift assignments unambiguously (Tables I and II). The NMR spectral analysis of **1** was facilitated by comparison with the spectroscopic data of **2** and values published in the literature for acosmine (**2**) and acosmine acetate [12].

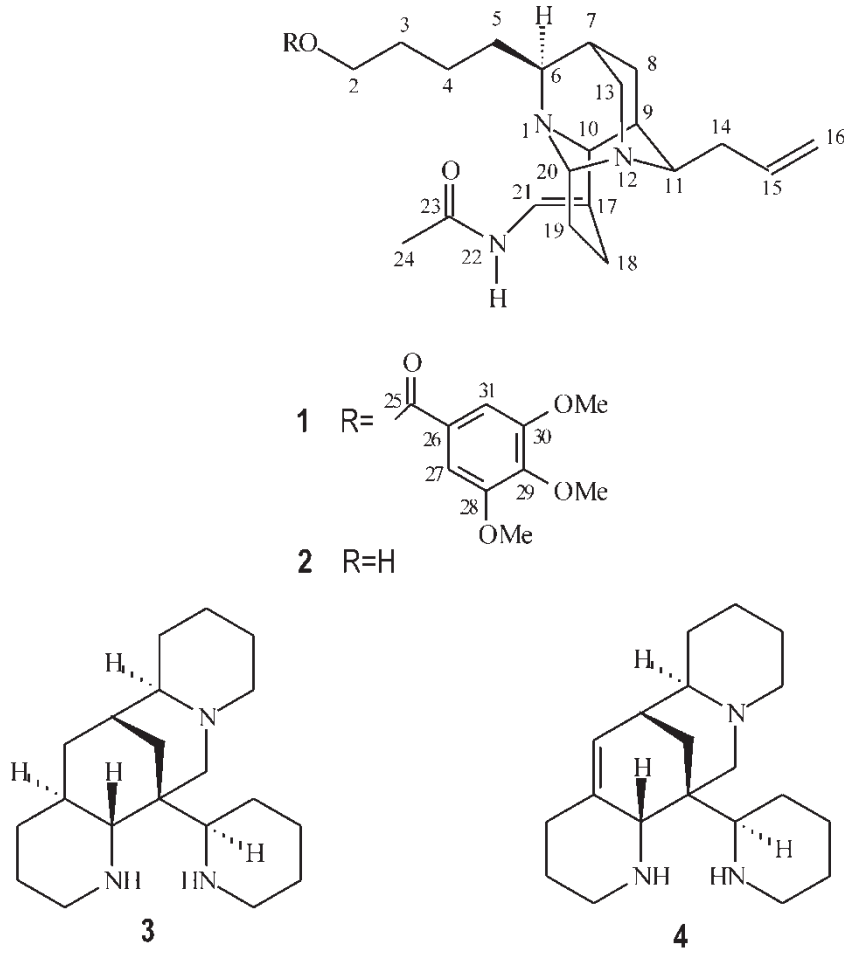

FIGURE 1 Structures of compounds **1**–**4**.

The major difference between the diaza-adamantane alkaloids **1** and **2** was attributed to the presence of a 3,4,5-trimethoxybenzoyloxy group linked at CH_2-2 of **1** [2-*O*-(3,4,5-trimethoxybenzoyl)acosmine]. The presence of this group was recognized by ^{1}H NMR (δ_H 7.24, s, H-27 and H-31 and 3.86, s, MeO-28, MeO-29 and MeO-30), ^{13}C NMR [δ_C 166.42 (C-25), 153.12 (C-28 and C-30), 141.36 (C-29), 61.08 (MeO-29, sterically hindered) and 56.46 (MeO-28 and MeO-30)]. The assignment of these ^{1}H and ^{13}C chemical shifts was confirmed by HMQC and HMBC spectra (Tables I and II). The location of the 3,4,5-trimethoxybenzoyloxy group at CH_2-2 of **1** was confirmed by ^{1}H (δ_H 4.28, t, $J = 6.6$ Hz) and ^{13}C (δ_C 65.21) chemical shifts compared with δ_H 3.55 (t, $J = 6.0$ Hz, 2H-2) and δ_C 61.71 (CH_2-2) revealed by ^{1}H and ^{13}C NMR spectra of **2**, along with the presence of cross-peaks corresponding to heteronuclear long-range couplings ($^3J_{CH}$) between C-25 (δ_C 166.42) and both 2H-2 (δ_H 4.28, t, $J = 6.6$ Hz) and 2H-27,31 (δ_H 7.24, s) observed in the HMBC spectrum (Tables I and II).

The NOESY spectrum of **1** in $CDCl_3$ showed cross-peaks assigned to dipolar interaction (spatial proximity) of H-10 (δ_H 3.82) with H-6 (δ_H 2.92), H-8α (δ_H 1.85), H-9 (δ_H 1.39) and H-21 (δ_H 6.62), H-11 (δ_H 3.49) with H-18β (δ_H 2.45), H-19β (δ_H 1.85) and H-9 (δ_H 1.39), H-13α (δ_H 3.32) with H-20 (δ_H 4.56), H-7 (δ_H 1.26) and H-8β (δ_H 2.38). These results, confirmed by additional NOESY spectrum obtained in C_6D_6 as solvent, were used to establish the relative stereochemistry shown in structure **1**.

Thus, the NMR spectral data (Tables I and II) allowed us to establish the structure of the new diaza-adamantane alkaloid, named bowdichine, as **1**. Comparison of the NMR spectral data of this alkaloid recorded in $CDCl_3$ and C_6D_6 as solvents are summarized in Table II, allowing us to evaluate the modifications in the ^{1}H and ^{13}C chemical shifts caused by solvent effect.

EXPERIMENTAL

General Experimental Procedures

^{1}H NMR (500 MHz) and ^{13}C NMR (125 MHz) were recorded at room temperature with a Bruker NMR spectrometer (DRX 500) with an inverse multinuclear 5 mm probe head equipped with a shielded gradient coil. The spectra were recorded in $CDCl_3$, and the solvent signals (7.27 and 77.0 ppm, respectively) were used as reference. The chemical shifts (δ) are given in ppm, and the coupling constant (J) in Hz. All programs used for performing the 2D NMR experiments are part of the Bruker library. UV spectra (MeOH) were measured on a Specord UV–VIS Jena, and IR spectra (KBr) on a Perkin–Elmer 467. Alumina was used for column chromatography. Si gel 60 PF_{254}, was used to purify the alkaloid by preparative TLC (1.25 mm thick, 20×20 cm Si gel plates). Analytical TLC was performed on precoated Si gel F_{254} plates. After development the dried plates were examined under short-wave (254 nm) or long-wave (366 nm) UV light and sprayed with Dragendorff's reagent. All solvents used were analytical grade.

Plant Material

Bowdichia virgilioides Kunt was collected near the city of Santa Rita, Paraiba, Brazil in January 2000. A voucher specimen has been deposited in the Herbarium of the Laboratório de Tecnologia Farmacêutica of the Universidade Federal da Paraíba under the number AGRA 086 (JPB).

TABLE I NMR spectral data for diaza-adamantane alkaloids **1** and **2**, including values reported in the literature [12] for acosmine (**2**, in parenthesis), in $CDCl_3$*

	1				***2***	
	$^1H-^{13}C$ HMQC-$^1J_{CH}$		$^1H-^{13}C$ HMBC-$^nJ_{CH}$		$^1H-^{13}C$ COSY-$^1J_{CH}$	
	δ_C	δ_H	$^2J_{CH}$	$^3J_{CH}$	δ_C	δ_H
C						
17	121.03	–	H-10; H-18b		120.89 (120.8)	–
23	167.27	–	HN-22; 3H-24		167.95 (167.4)	–
25	166.42	–		2H-2; 2H-27,31	–	–
26	125.65	–	2H-27,31		–	–
28,30	153.12	–	2H-27,31	MeO-28/MeO-30	–	–
29	141.36			2H-27,31; MeO-29	–	–
CH						
6	65.31	2.92 (t, 7.0)	H-5b	H-7; 2H-13	64.67 (64.9)	2.92 (t, 6.5)
7	26.56	1.26 (br s)	H-6; 2H-13		25.94 (26.2)	1.14 (sl)
9	35.57	1.39 (br s)	H-8a; H-10, H-11	H-14a, H-15	34.89 (35.2)	1.30 (sl)
10	65.17	3.82 (s)		2H-13; H-20; H-21	64.75 (64.8)	3.75 (sl)
11	52.88	3.49 (t, 7.3)	2H-14	H-10; 2H-13; H-15; H-20	52.38 (52.4)	3.38 (t, 7.0)
15	135.59	5.63 (m)	2H-14	H-11	135.02 (135.4)	5.65 (m)
20	68.54	4.56 (d, 7.9)		H-6; H-10; 2H-13	67.98 (68.0)	4.98 (d, 7.5)
21	117.19	6.62 (d, 10.2)		H-10	117.18 (117.0)	6.61 (d, 10.2)
27,31	107.11	7.24 (s)			–	–
CH_2						
2	65.21	4.28 (t, 6.6)			61.71 (62.1)	3.55 (t, 6.0)
3	29.01	1.75	2H-2		32.14 (32.3)	1.39
4	23.68	1.45	2H-3; 2H-5	2H-2; H-6	22.99 (23.0)	1.50
5	30.76	1.80	H-6	2H-3; H-7	30.18 (30.4)	1.75
		1.68				1.50
8	31.77	2.38, H-8ax		H-6; H-11; 2H-13	31.10 (31.4)	2.32
		1.85, H-8eq				1.85
13	46.75	3.32 (s)		H-6; H-11	46.10 (46.4)	3.04 (sl)
14	35.71	2.50		2H-16	35.21 (35.5)	2.32
		2.35				
16	116.71	5.05 (d, 18.3)		2H-14	116.48 (116.3)	5.06 (d, 16.3)
		5.02 (d, 11.1)				5.00 (d, 10.0)
18	20.11	2.45	2H-19	H-20; H-21	19.61 (19.8)	2.40

TABLE I – *continued*

	1				***2***	
	$^1H-^{13}C$ HMQC-$^1J_{CH}$		$^1H-^{13}C$ HMBC-$^nJ_{CH}$		$^1H-^{13}C$ COSY-$^1J_{CH}$	
	δ_C	δ_H	$^2J_{CH}$	$^3J_{CH}$	δ_C	δ_H
19	26.75	2.30 2.25 1.85	H-18b; H-20		26.19 (26.4)	2.29 1.80
CH_3						
24	23.53	2.05 (s)			22.81 (23.1)	2.03 (s)
MeO-28	56.46	3.86 (s)			–	–
MeO-29	61.08	3.86 (s)			–	–
MeO–30	56.46	3.86 (s)			–	–

* Number of hydrogen atoms bound to carbon atoms deduced by comparative analysis of HBBD- and DEPT-^{13}C NMR spectra. Superimposed ^1H signals are described without multiplicity and chemical shifts were deduced by $^1H-^{13}C$ COSY-$^nJ_{CH}$ ($n = 1$, HMQC; $n = 2$ and 3, HMBC) NMR spectra. Homonuclear 2D $^1H-^1H$ COSY spectrum and $\{^1H\}-^1H$ NOE difference spectra were also used in these assignments. Chemical shifts (δ_H) and coupling constants (J, in parenthesis) of hydrogen atoms were deduced from 1D ^1H NMR spectrum.

TABLE II Comparison of NMR spectral data for diaza-adamantane alkaloid **1** in C_6D_6 and $CDCl_3$*

	C_6D_6				*$CDCl_3$*	
	HMQC		*HMBC*		*HMQC*	*HMBC*
	δ_C	δ_H	$^2J_{CH}$	$^3J_{CH}$	δ_C	δ_H
C						
17	119.86	–	H-10		121.03	–
23	166.58	–	3H-24	H-21	167.27	–
25	166.52	–		2H-2; 2H-27,31	166.42	–
26	125.83	–	2H-27,31		125.65	–
28, 30	153.73		2H-27,31	MeO-28/MeO-30	153.12	–
29	143.63			2H-27,31; MeO-29	141.36	
CH						
6	64.52	2.75 (t, 6.9)			65.31	2.92 (t, 7.0)
7	27.01	0.87 (s)			26.56	1.26 (br s)
9	35.32	1.22 (s)	H-11	H-14a	35.57	1.39 (br s)
10	64.43	3.75 (s)		H-20; H-21	65.17	3.82 (s)
11	52.77	3.67 (t, 6.7)	2H-14	H-13a; H-10; H-20	52.88	3.49 (t, 7.3)
15	135.35	5.82 (m)	2H-14	H-11	135.59	5.63 (m)
20	68.99	4.61 (d, 7.3)		H-6; H-10; H-13b	68.54	4.56 (d, 7.9)
21	117.91	6.99 (d, 10.1)		H-10	117.19	6.62 (d, 10.2)
27, 31	107.90	7.62 (s)	–	–	107.11	7.24 (s)
CH_2						
2	64.98	4.41 (t, 6.6)			65.21	4.28 (t, 6.6)
3	28.98	1.73	2H-2		29.01	1.75
4	23.35	1.52	H-5a	2H-2	23.68	1.45
		1.38				
5	30.07	1.89	H-6		30.76	1.80
		1.38				1.68
8	31.16	2.06	H-7		31.77	2.38, H-8ax
		1.54				1.85, H-8eq
13	46.23	3.23 (d, 13.7)		H-6; H-10	46.75	3.32 (s)
		3.14 (d)				
14	35.01	2.59 (m)	H-11; H-15	2H-16	35.71	2.50
		2.35				2.35
16	116.35	5.12 (d, 17.6)		2H-14	116.71	5.05 (d, 18.3)
		5.08 (d, 10.3)				5.02 (d, 11.1)
18	19.82	2.40		H-21	20.11	2.45
		2.28				2.30
19	26.37	2.25			26.75	2.25
		2.02				1.85
CH_3						
24	22.63	1.83 (s)			23.53	2.05 (s)
MeO-28	55.86	3.50 (s)			56.46	3.86 (s)
MeO-29	60.33	3.87 (s)			61.08	3.86 (s)
MeO-30	55.86	3.50 (s)			56.46	3.86 (s)

* Number of hydrogen atoms bound to carbon atoms deduced by comparative analysis of HBBD- and DEPT-^{13}C NMR spectra. Superimposed ^{1}H signals are described without multiplicity and chemical shifts were deduced by ^{1}H–^{13}C COSY-$^nJ_{CH}$ ($n = 1$, HMQC; $n = 2$ and 3, HMBC) NMR spectra. Homonuclear 2D ^{1}H–^{1}H COSY spectrum and {^{1}H}–^{1}H NOE difference spectra were also used in these assignments. Chemical shifts (δ_H) and coupling constants (J, in parenthesis) of hydrogen atoms were deduced from the 1D ^{1}H NMR spectrum.

Extraction and Isolation

Air-dried and powdered stem bark (5 kg) of *B. virgilioides* was extracted with 95% ethanol and the extract was concentrated under reduced pressure to yield a brown viscous raw material (500 g). This crude ethanolic extract (CEE) was redisolved in 1500 mL of MeOH–H_2O (1:1) and extracted with hexane (2 × 500 mL). To the defatted MeOH–H_2O extract was

added NH_4OH (250 mL) with stirring, at approximately 5°C. The alkaline solution was then extracted with $CHCl_3$ (3×500 mL) and the extract concentrated (25 g) and treated with a solution of 3% HCl (250 mL) and then filtered over Celite. The acidic phase was alkalinized with NH_4OH and extracted again with $CHCl_3$. The organic fraction was concentrated under vacuum, furnishing 9 g of the total alkaloid fraction (TAF). To the TAF was added acetone (100 mL) and the resultant solution was kept in a freezer for 24 h, leading to the formation of a whitish precipitate later identified as a mixture of the alkaloids ormosanine and podopetaline. The acetone-soluble part was chromatographed over an aluminium oxide column and eluted with $CHCl_3$–MeOH mixtures of increasing polarity. Fractions 5–8, on evaporation of solvent, gave an amorphous unresolved mixture of ormosanine and podopetaline (1:1) (1 g). Fraction 44–45 was repeatedly purified over silica gel PTLC using $CHCl_3$–MeOH (95:5) as eluent to yield pure bowdichine (16 mg) and acosmine (7 mg).

Bowdichine (**1**): Viscous oil. Yield: 0.00032% relative to plant material. UV λ_{max} (MeOH) (nm): 225, 250, 298; IR ν_{max} (KBr film) (cm^{-1}): 3280, 3150, 3040, 2920, 1690, 1640, 1570, 1490, 1410, 1350, 1210; ^1H and ^{13}C NMR data are listed in Tables I (in $CDCl_3$) and II (in C_6D_6 and $CDCl_3$).

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