Brominated Indole Alkaloids from the Marine Tunicate Pseudodistoma arborescens

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BROMINATED INDOLE ALKALOIDS FROM THE MARINE TUNICATE *PSEUDODISTOMA ARBORESCENS*

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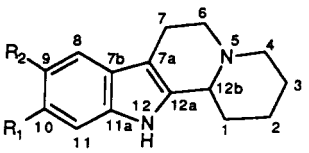
ABSTRACT.—Chemical investigation of the cytotoxic CH_2Cl_2-soluble extract of the marine tunicate *Pseudodistoma arborescens* led to the isolation of four brominated indole alkaloids, arborescidines A [**1**], B [**2**], C [**3**], and D [**4**], which were characterized by their spectral data, especially 2D nmr. Only arborescidine D [**4**] showed moderate activity (IC_{50} 3 μg/ml) in vitro against the growth of KB human buccal carbinoma cells.

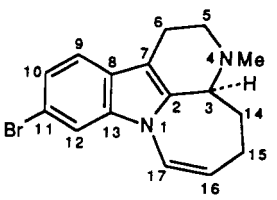
Our screening of New Caledonian marine organisms in the KB cytotoxicity assay revealed activity in the CH_2Cl_2-soluble extract from the tunicate *Pseudodistoma arborescens* Millar (Polyclinidae) (100% activity at 10 μg/ml). Further investigations showed that the activity was almost completely located in the acidic H_2O-soluble fraction and led to the isolation of four brominated indole alkaloids, arborescidines A [**1**], B [**2**], C [**3**], and D [**4**].

Cytotoxic alkaloids of a completely different kind have been previously reported from two other species of the *Pseudodistoma* genus, *Pseudodistoma kanoko* (1) and *Pseudodistoma novaezelandiae* (2). They were characterized by the presence of primary amine functions and a linear unsaturated chain, which could be linked to a piperidine ring.

The EtOH extract of the lyophilized organism was partitioned between CH_2Cl_2 and H_2O. The alkaloids contained in the CH_2Cl_2 phase were separated by chromatography on Si gel and elution with a mixture of CH_2Cl_2 and increasing amounts of MeOH. This led to the direct isolation of arborescidines A [**1**], B [**2**], and C [**3**]; aborescidine D [**4**] was isolated from the more polar fractions by repeated chromatography on Si gel using various solvent systems.



1 R_1=Br, R_2=H
5 R_1=R_2=H
6 R_1=H, R_2=Br



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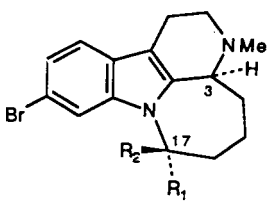
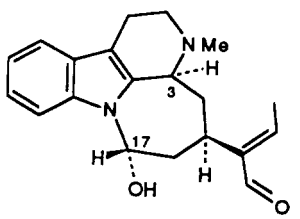


3 R_1=OH, R_2=H
4 R_1=H, R_2=OH



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The major alkaloid arborescidine A [**1**] crystallized from MeOH, mp 202°, $[\alpha]_D$ −85°. The uv spectrum exhibited two maxima at 232 and 285 nm (log ε 4.24 and 3.62) typical of an indole chromophore. Mass spectral analysis revealed that the compound was monobrominated. The molecular formula $C_{15}H_{17}{}^{81}BrN_2$ was established from the molecular peak at *m/z* 306 together with ^{13}C-nmr methods. The ^{1}H-nmr spectrum displayed a deuterium-exchangeable signal at δ 7.76, which was assigned to the indole NH. In the aromatic region, the characteristic pattern for indole alkaloids having a substituent meta or para to the NH group was observed between δ 7.42 and δ 7.00. All other signals were similar to those previously described for an alkaloid extracted from *Dracontomelum mangiferum*, which was identified as 1,2,3,4,6,7,12,12b-octahydroindolo[2,3*a*]quinolizine [**5**] (3,4). Thus, the structure of arborescidine A was concluded to be **1**. This was further supported by the characteristic ms fragment at *m/z* 248 (5,6), which corresponded to the brominated tetrahydro β-carboline moiety.

2D nmr studies, COSY, HMQC, and HMBC (Table 1) confirmed this structural assignment. The position of the bromine at C-10 was deduced from the ^3J-correlation between H-8 (d, $J = 8.5$) and C-7a observed in the HMBC spectrum. Furthermore, the chemical shifts of the aromatic protons were different from those reported for eudistomidin B (7) and woodinine (8), where the bromine was attached at position para to the indole NH. The regioisomer **6** (*R*-isomer) with a bromine atom at C-9 has been synthesized (9) and differs from **1** by its melting point, but no precise nmr data were reported.

Arborescidine B [**2**] was obtained as an amorphous oil, $[\alpha]_D$ +70°. The uv spectrum showed three maxima at 231, 258, and 288 nm (log ε 4.45, 4.32, 3.95), which could correspond to an indole alkaloid possessing an additional chromophore. The molecular formula $C_{16}H_{17}BrN_2$ was deduced from the mass spectrum, showing an $[M]^+$ peak at *m/z* 318 for ^{81}Br, and the ^{13}C-nmr spectrum. The ^{1}H nmr contained the same typical pattern of the aromatic protons as for arborescidine A. The NH signal was missing, but the spectrum exhibited a singlet (3H) at δ 2.58 assigned to a methyl group most probably attached to N-4, because its upfield position ruled out the possiblity of attachment to the less basic N-1. In addition, two signals at δ 5.13 and δ 6.86 were assigned to a double bond located α to nitrogen, as indicated from the chemical shift of the more downfield proton. All other 1D ^{1}H-nmr data and the 1D ^{13}C-nmr spectrum (Table 1) were in accordance with structure **2** for arborescidine B. This structure was fully supported by COSY, HMQC, and HMBC experiments. The HMBC spectrum (Table 1) indicated that the bromine was attached to C-11 (cross peak H-9/C-7) as in arborescidine A. The cross peaks H-5/*N*Me and H-14/*N*Me further confirmed the position of the *N*-methyl group, and the cross peak H-17/C-2 assured the position of the double bond.

Arborescidine C [**3**] crystallized from MeOH, mp 172–173°, $[\alpha]_D$ +3°. The uv spectrum showed two maxima at 230 and 280 nm (log ε 3.89 and 3.56). The molecular formula $C_{16}H_{19}BrN_2O$ was deduced from the $[M]^+$ peak at *m/z* 336 for ^{81}Br and the ^{13}C nmr. This spectrum and the ^{1}H-nmr spectrum were similar to those of arborescidine B except that the olefinic signals were absent. Instead, the ^{1}H nmr exhibited a double doublet ($J = 4$ Hz, $J' = 1$ Hz) assigned to an equatorial hydrogen geminal to the OH group of a carbinolamine (δ 77.2 in the ^{13}C nmr), and the other signals supported structure **3** for arborescidine C. This was further supported by 2D experiments (COSY, HMQC, HMBC) (Table 2). The nOe's observed between H-17 and H-12 in the NOESY spectrum and between OH-17 and H-3 in an nOe difference experiment (DMSO-d_6) confirmed the relative 3*SR*,17*SR* configuration in the most probable chair-like conformation for the seven membered ring. Akagerine [**7**], an alkaloid previously

TABLE 1. ^{13}C-nmr (62.5 MHz) and ^{1}H-nmr (400 MHz) Data for Arborescidines A [**1**] and B [**2**].[a]

Position	**1**			Position	**2**		
	δC	δH (*J*, Hz)	HMBC		δC	δH (*J*, Hz)	HMBC
1	29.8	2.03 m	C-3,C-2	2	138.1		
		1.73 m					
2	24.3	1.86 m		3	62.2	3.43 brd (10)	C-2,C-7,C-14
		1.46 m					C-15,*N*Me
3	25.7	1.73 m		5	52.4	3.16 m	C-3,C-6,C-7,*N*Me
		1.53 m	C-2			2.75 m	
4	55.7	3.00 m	C-7a	6	20.2	2.93	C-5,C-7
		2.36 m	C-12b				
6	53.3	3.03 m	C-7a	7	109.1		
		2.63 m	C-7a,C-12a,C-12b				
7	21.5	3.00 m	C-7a	8	125.7		
		2.60 m	C-7a,C-12a,C-12b				
7a	108.3			9	119.2	7.33 d (8.5)	C-7,C-8,C-11,C-13
7b	126.5			10	123.2	7.25 dd (8.5,1.5)	C-8,C-12
8	119.3	7.30 d (8.5)	C-7a,C-7b,C-10,C-11a	11	115.3		
9	122.6	7.16 dd (8.5,1.5)	C-7b,C-11	12	112.3	7.50 d (1.5)	
10	114.6			13	137.0		
11	113.6	7.42 d (1.5)	C-7b,C-8,C-10	14	29.7	2.40 m	C-3,C-15,*N*Me
						1.75 m	C-3,C-15
11a	136.9			15	27.7	2.58 m	
						2.48 m	C-3
12		7.67 s	C-7a,C-7b,C-12a	16	111.1	5.13 m	
12a	136.0			17	121.6	6.86 dt (10.2)	C-2,C-12,C-15
12b	60.1	3.18 brd (11)	C-12a	NMe	42.0	2.58 s	
NH		7.76 brs					

[a]$CDCl_3$; ^{1}H and ^{13}C chemical shifts based on COSY and HMQC experiments.

TABLE 2. ^{13}C-nmr (62.5 MHz) and ^{1}H-nmr (400 MHz) Data for Arborescidines C [3] and D [4].[a]

Position	3			4		
	δC	δH (*J*, Hz)	HMBC	δC	δH (*J*, Hz)	HMBC
2	137.5			137.6		
3	61.2	3.71 brd (11)	C-2,C-15,*N*Me	61.7	3.13 brd (11)	C-2,C-14,C-15
5	50.2	2.96 m	C-3,C-6,C-7,*N*Me	50.6	2.90 m	C-3,C-6,C-7,*N*Me
		2.68 m			2.30 m	
6	19.5	2.68 m		19.5	2.60 m	C-3,C-5,C-7
					2.58 m	
7	108.0			109.4		
8	125.2			125.4		
9	119.1	7.27 d (8.5)	C-7,C-11,C-13	120.0	7.17 d (8.5)	C-7,C-11,C-13
10	122.1	7.10 dd (8.5,1.5)	C-8,C-12	123.2	7.30 dd (8.5,1.5)	C-8,C-12
11	114.5			115.2		
12	111.6	7.40 d (1.5)		111.3	6.63 d (1.5)	
13	136.9			137.0		
14	32.1	2.23 m		32.9	2.30 m	
		1.43 m	C-2,C-3,C-15		1.43 m	C-2,C-3,C-15
15	20.0	2.12 m		21.1	2.10 m	C-14
		1.80 m			1.90 m	
16	34.2	2.23 m		34.2	1.90 m	
		1.58 m			1.60 m	C-15
17	77.2	6.00 dd (4,1)[b]	C-2,C-5	80.0	5.65 brd (4)	
NMe	42.1	2.45 s		42.3	2.30 s	
OH		6.40 d (4)[c]				

[a]$CDCl_3$; ^{1}H and ^{13}C chemical shifts based on COSY and HMQC experiments.
[b]6.26 brt ($J = 4$) in DMSO-d_6.
[c]DMSO-d_6.

extracted from *Strychnos usambarensis*, was assigned such a conformation by X-ray determination (10).

Arborescidine D [**4**] was obtained as an amorphous solid, $[\alpha]_D$ −8°. Spectral data indicated that **4** was the alcohol epimeric to arborescidine C [**3**]. The ^{13}C-nmr spectra of both compounds were quite similar except for the oxymethine peak at δ 80.0 in **4**. Some differences were observed in chemical shift in the ^{1}H nmr, especially for H-3, H-17, and H-12 (Table 2). H-17 (br d, $J = 4$) was again equatorial as seen from the small coupling constants and an nOe cross peak H-12/H-17. These observations could only be explained by a boat-like conformation of the seven-membered ring combined with a change in the electronic effect induced by the carbinolamine, which resulted in a strong upfield shift of H-12 at δ 6.63 (Table 2).

The cd spectrum of **1** showed a positive Cotton effect at 280 nm, indicating an *S* configuration at C-12b (11). The same configuration at C-3 was tentatively assigned to alkaloids **2**–**4**, based on biosynthetic arguments, but this could not be clearly deduced from cd measurements, since no general studies have been made for this type of indole alkaloids. A positive Cotton effect at 256, 296, and 260 nm was observed for compounds **2**, **3**, and **4**, respectively.

Among the four alkaloids **1**–**4** isolated from *P. arborescens*, arborescidine D [**4**] was the only one that showed moderate activity (IC_{50} 3 μg/ml) in the KB cytotoxicity test.

EXPERIMENTAL

GENERAL EXPERIMENTAL PROCEDURES.—Melting points (uncorrected) were determined on a micro hot-stage apparatus. Optical rotations at 20° were taken on a Perkin-Elmer 241 polarimeter. Spectra were recorded on: (uv) Shimadzu UV-161 uv-visible spectrophotometer; (ir) Nicolet 205 FT-IR spectrometer; [eims (70 eV)] Kratos MS 50; (nmr) Bruker AC 250 (^{13}C spectra), AC 400 (^{1}H and 2D spectra); (cd) Jobin Yvon Mark 5. Uv and cd spectra were recorded in MeOH. Cc was performed using Si gel Merck H60, and tlc with Si gel 60 F_{254}. Visualization was by viewing under uv light and spraying with Dragendorff's reagent followed by 50% H_2SO_4.

ANIMAL MATERIAL.—The tunicate *P. arborescens* was collected in March 1990 on the North-East Barrier reef of New Caledonia using SCUBA, under the auspices of the CNRS-ORSTOM program "Substances Marines d'Intérêt Biologique" (SMIB). Samples (ref. UA 350) were identified by Mrs. F. Monniot of the Museum d'Histoire Naturelle de Paris, France and conserved at ORSTOM, Nouméa, New Caledonia.

EXTRACTION AND PURIFICATION.—The freeze-dried animal material (350 g) was extracted with 80% EtOH (1×) and then with EtOH (3×) at room temperature. The pooled extracts were concentrated in vacuo (200 ml). H_2O (700 ml) was added, and the mixture was extracted with CH_2Cl_2. The organic layer was concentrated to dryness. The residue (3.4 g) was dissolved in Et_2O and extracted with 10% HCl until a negative Mayer test was obtained. Removal of Et_2O from the pooled organic fraction gave an inactive extract (2.76 g). The acidic medium was basified with NH_4OH and partitioned with CH_2Cl_2. Concentration of the pooled CH_2Cl_2 fractions gave the active extract (crude alkaloids, 0.43 g). The alkaloids were fractionated by cc eluted with CH_2Cl_2, followed by increasing concentrations of MeOH in CH_2Cl_2. Elution with CH_2Cl_2-MeOH (9:1) led to the isolation of pure **1** (10 mg) and **2** (156 mg). Elution with CH_2Cl_2-MeOH (98:2) yielded **3**, which was purified by recrystallization from MeOH (30 mg). Further elution with CH_2Cl_2-MeOH (98:5) provided a mixture (90 mg) showing activity on KB cells, which was purified by two successive cc on Si gel by eluting first with EtOAc–2-butanone (5:3) containing increasing amounts of HCO_2H-H_2O (1:1) and then with a mixture of CH_2Cl_2/MeOH containing increasing amounts of MeOH. Arborescidine D [**4**] was eluted with EtOAc–2-butanone–HCO_2H–H_2O (5:3:1:1) from the first column (still impure) and CH_2Cl_2-MeOH (98:5) from the second (9 mg).

Arborescidine A [**1**].—Mp 202° (MeOH); $[\alpha]_D$ −85° ($CHCl_3$, $c = 1$); uv λ max 232 (log ε 4.24), 285 nm (log ε 3.62) nm; ir ν max (Nujol) cm^{-1} 3400, 3170, 1616, 1582; eims *m/z* (% rel. int.) 306 (70), 305 (100), 304 (71), 303 (94), 248 (52), 246 (50); ^{1}H and ^{13}C nmr see Table 1; cd λ ext 280 nm (Δε +0.61). *Anal.* calcd for $C_{15}H_{17}BrN_2$: C 59.02, H 5.62, Br 26.18, N 9.18; found C 58.80, H 5.55, Br 26.02, N 9.12.

Arborescidine B [**2**].—$[\alpha]_D$ +70° ($CHCl_3$, $c = 0.6$); uv 231 (log ε 4.45), 258 (log ε 4.32), 288 (log ε

3.95) nm; ir ν max (film) cm^{-1} 3350, 2851, 2798, 1660, 1600; eims *m/z* (% rel. int.) 318 (73), 317 (73), 316 (78), 315 (57), 275 (90), 273 (100); ^1H and ^{13}C nmr see Table 1; cd λ ext 232 ($\Delta\epsilon$ −4.8) and 256 ($\Delta\epsilon$ +12.4).

Arborescidine C [**3**].—Mp 172–173° (MeOH), $[\alpha]_D$ +3° ($CHCl_3$, *c* = 1); uv λ max 230 (log ϵ 3.89), 280 (log ϵ 3.56) nm; ir ν max (film) cm^{-1} 3300, 2851, 2798, 1600; eims *m/z* (% rel. int.) 336 (22), 335 (15), 334 (24), 333 (12), 265 (100), 263 (78); ^1H and ^{13}C nmr see Table 2; cd λ ext 274 ($\Delta\epsilon$ −1) and 296 ($\Delta\epsilon$ +0.56). *Anal.* calcd for $C_{16}H_{19}BrN_2O$: C 57.32, H 5.71, Br 23.84, N 8.36, O 4.77; found C 57.13, H 5.60, Br 23.84, N 8.25, O 4.97.

Arborescidine D [**4**].—$[\alpha]_D$ −8° ($CHCl_3$, *c* = 0.5); uv λ max 231 (log ϵ 4.47), 285 (log ϵ 3.91) nm; ir ν max (film) cm^{-1} 3300, 2851, 2798, 1600; eims *m/z* (% rel. int.) 336 (14), 335 (44), 334 (14), 333 (44), 275 (90), 273 (100); ^1H and ^{13}C nmr see Table 2; cd λ ext 242 ($\Delta\epsilon$ −1.6), 260 ($\Delta\epsilon$ −0.05), 278 ($\Delta\epsilon$ −0.13).

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