Indole Alkaloids from the Tunicate *Aplidium meridianum*

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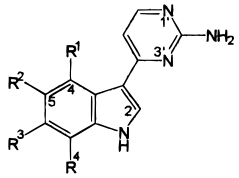
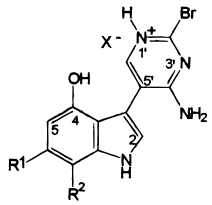
Five new indole alkaloids, meridianins A−E (**1**−**5**), have been isolated from the tunicate *Aplidium meridianum*, which was collected at a depth of 100 m near the South Georgia Islands, and their structures were elucidated by spectroscopic techniques. Compounds **2**−**5** showed cytotoxicity toward murine tumor cell lines.

Marine invertebrates are a very important source of antitumor secondary metabolites. Among these, 3-substituted indoles have frequently been isolated, especially from tunicates and sponges. The substituent at position 3 of indoles is often an additional heterocyclic ring,[1−5] with recent examples including the didemnimides from the tunicate *Didemnum conchyliatum*,[6] alboinon from *Dendrodoa grossularia*,[7] and the sponge metabolites psammopemmins A−C (**6**−**8**).[8] As part of our ongoing study of bioactive compounds isolated from South Atlantic invertebrates, we have examined the constituents of the tunicate *Aplidium meridianum* (Sluiter, 1906[9,10]) (family Polyclinidae) collected near the South Georgia Islands. In this paper we report the isolation and structure elucidation of five novel indole alkaloids, meridianins A−E (**1**−**5**). All these compounds have a brominated and/or hydroxylated indole nucleus with a 2-aminopyrimidine substitutent at C-3.



1 R1=OH; R2=R3=R4=H
2 R1=OH; R2=R4=H; R3=Br
3 R1=R3=R4=H; R2=Br
4 R1=R2=R4=H; R3=Br
5 R1=OH; R2=R3=H; R4=Br



6 R1= H; R2=H
7 R1=H; R2=Br
8 R1=Br; R2=H

The HRMS of compound **1** gave the molecular formula $C_{12}H_{10}N_4O$, indicative of 10 degrees of unsaturation, while the NMR data suggested the presence of two heteroaromatic moieties. The ^{1}H NMR signals (Table 1) at δ 8.20 (d, 1H, J = 1.2 Hz) and δ 11.71 (br s, 1H), together with the UV spectrum of **1**, indicated the presence of a 3-substituted indole. The characteristic ^{1}H NMR coupling pattern [δ 6.36 (dd, 1H, J = 7.1, 0.7 Hz), δ 6.78 (dd, 1H, J = 7.5, 0.7 Hz) and δ 6.96 (dd, 1H, J = 7.1, 7.5 Hz)] suggested the presence of a hydroxyl group either at C-4 or C-7. A sharp downfield signal at δ 13.55 (s, 1H, exchangeable with D_2O) confirmed the presence of a deshielded hydroxyl group. This was consistent with substitution at C-4, which was further supported by correlations observed in a COLOC spectrum and comparison with reference data.[8] With this substructure established, we could then determine by subtraction the formula of the fragment at C-3 as $C_4H_4N_3$. This formula, together with ^{1}H NMR signals at δ 7.09 (d, 1H, J = 5.4 Hz), δ 8.10 (d, 1H, J = 5.4 Hz), and δ 6.69 (s, 2H, exchangeable with D_2O), suggested the presence of either a 4′-substituted 2-aminopyrimidine or 2-aminopyridazine ring. The ^{13}C NMR downfield signals (Table 2) at δ 161.9 (C), δ 160.6 (C), and δ 158.5 (CH) favored the former structure. This was confirmed by interpetration of the COLOC spectrum and comparison with literature data.[11] Assignment of all protonated carbons was obtained by selective heteronuclear decoupling experiments. Unambiguous assignment of the C-2′/C-4′and C-3/C-3a pairs was achieved through a gated decoupling experiment (DMSO-d_6 −D_2O). In this spectrum, C-2′ appeared as a clean doublet (J = 12.2 Hz) due to the large H-6′/C-2′ ^3J coupling, resulting from a nitrogen atom in the coupling path. On the other hand, the signal for C-4′consisted of a doublet of doublets (J = 4.0; 2.7 Hz) due to the smaller ^2J and ^3J couplings with H-5′and H-6′.[12] In the case of the C-3/C-3a pair, the C-3a multiplet showed three large ^3J couplings with H-2, H-5, and H-7, while C-3 appeared as a doublet due to the large ^2J (8 Hz) coupling with H-2 typical of indoles.

The HRMS of compounds **2**−**5** contained the correct clusters of peaks for the molecular formulas $C_{12}H_9N_4OBr$, $C_{12}H_9N_4Br$, and $C_{12}H_9N_4Br$, $C_{12}H_9N_4OBr$, respectively, indicating that all of these compounds also have 10 degrees of unsaturation. The NMR data of **2**−**5**

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Table 1. ^{1}H NMR Spectral Data [$(CD_3)_2SO$, 200 MHz] for Compounds 1−5[a]

	compound				
proton	**1**	**2**	**3**	**4**	**5**
N1−H	11.71 (brs)	11.99 (s)	11.85 (brs)	11.76 (brs)	11.86 (brs)
2	8.20 (d; 1.2)	8.32 (d; 2.8)	8.24 (d; 2.5)	8.21 (d; 2.6)	8.29 (brs)
4			8.75 (brd; 1.8)	8.55 (d; 8.4)	
4-OH	13.55 (s)	13.92 (s)			13.89 (s)
5	6.36 (dd; 7.1, 0.7)	6.54 (d; 1.5)		7.24 (dd; 8.4, 1.8)	6.37 (d; 8.3)
6	6.96 (dd; 7.1, 7.5)		7.28 (dd; 8.4, 1.8)		7.19 (d; 8.3)
7	6.78 (dd; 7.5, 0.7)	7.02 (d; 1.5)	7.41 (d; 8.4)	7.63 (d; 1.8)	
2′-NH_2	6.69 (s; 2H)	7.02 (s; 2H)	6.48 (s; 2H)	6.43 (s; 2H)	6.79 (s; 2H)
5′	7.09 (d; 5.4)	7.18 (d; 5.5)	7.00 (d; 5.5)	7.00 (d; 5.1)	7.23 (d; 5.4)
6′	8.10 (d; 5.4)	8.17 (d; 5.5)	8.11 (d; 5.5)	8.12 (d; 5.1)	8.19 (d; 5.4)

[a] δ in ppm, J in Hz.

Table 2. ^{13}C NMR Spectral Data for Compounds **1**−**5** [δ in ppm, $(CD_3)_2SO$]

	compound				
carbon	**1**	**2**	**3**	**4**	**5**
2	128.5	129.9	129.6	129.2	129.2
3	113.8	113.7	113.3	114.8	116.1
3a	114.5	114.0	127.1	124.5	115.2
4	152.1	153.0	124.6	124.3	152.0
5	105.6	108.8	113.4	123.1	107.3
6	124.4	116.7	124.7	113.9	126.7
7	102.4	105.3	113.9	114.5	92.6
7a	139.4	139.7	135.9	138.0	136.9
2′	161.9	160.7[a]	163.6[b]	163.6[c]	160.2[d]
4′	160.6	160.8[a]	162.3[b]	162.3[c]	161.8[d]
5′	104.5	104.6	105.4	105.4	104.8
6′	158.5	157.1	157.2	157.2	159.0

[a−d] Values marked with the same letter may be interchangeable.

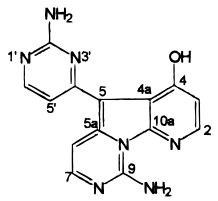
indicated the presence of a 2-aminopyrimidine ring similar to that of **1**, while the coupling patterns of the indole protons clearly established their structural differences. The molecular formula of **2** indicated the presence of bromine and hydroxyl substituents in the indole ring. The ^{1}H NMR spectrum of **2** [δ 6.54 (d, J = 1.5 Hz), δ 7.02 (d, J = 1.5 Hz)] showed that the indole protons were *meta* coupled, while their upfield chemical shifts were in accordance with the presence of a hydroxyl substituent. A sharp downfield signal at δ 13.92 (s, 1H, exchangeable with D_2O) located the hydroxyl group at C-4. The chemical shifts of all the carbon atoms of **2** were assigned from a COLOC experiment and comparison with literature data.[8]

The typical ^{1}H NMR AMX coupling pattern of **3** [δ 7.28 (dd, J = 8.4, 1.8 Hz), δ 7.41 (d, J = 8.4 Hz), δ 8.75 (br d, J = 1.8 Hz)] placed the bromine substituent either at C-5 or C-6. However, the downfield shift of the H-4 signal, which appeared as a *meta* doublet, unequivocally positioned the bromine at C-5.

HRMS indicated that **3** and **4** were isomers. The ^{1}H NMR spectrum of **4** showed an AMX system with the downfield H-4 signal as an *ortho* doublet (J = 8.4 Hz), thus establishing that the bromine substituent was located at C-6.

The molecular formula of **5** indicated the presence of bromine and hydroxyl substituents in the indole ring. A downfield signal at δ 13.89 (sharp s), together with two *ortho*-coupled protons [δ 6.37 (d, J = 8.3 Hz) and δ 7.19 (d, J = 8.3 Hz)], suggested the presence of a hydroxyl group at C-4 and a bromine either at C-7 or C-5. The presence of NOE correlations in a phase-sensitive NOESY experiment between 4-OH/H-5′ and 4-OH/H-5 confirmed that the bromine group was at C-7. Assignment of the ^{13}C NMR chemical shifts of compound **5** (see Table 2) was completed with the help of COLOC, DEPT, and heteronuclear irradiation spectra.

Meridianins A (**1**), B (**2**), and E (**5**) and psammopemmins A−C (**6**−**8**)[8] have a similar indole nucleus substitution pattern, but the latter compounds have a 5′-substituted 4′-amino 2′-bromopyrimidine moiety as the additional heterocycle at C-3. On the other hand, variolin B (**9**) isolated from the sponge *Kirkpatrickia varialosa*[11] has a 2-aminopyrimidine ring on a 3-substituted azaindole, but differs from the meridianins in the presence of an additional heterocyclic ring. Compounds **2**−**5** showed cytotoxicity toward LMM3 (murine mamarian adenocarcinoma cell line) with IC_{50} values of 11.4 μM for compound **2**, 9.3 μM for compound **3**, 33.9 μM for compound **4**, and 11.1 μM for compound **5**.



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Experimental Section

General Experimental Procedures. UV and IR spectra were recorded on a Hewlett−Packard model 8451 A diode array spectrophotometer and a Nicolet Magna-IR model 550 spectrometer, respectively. NMR spectra (δ ppm, J in Hz) were obtained on a Bruker AC-200 spectrometer. HREIMS were determined with a VG-ZAB-SEQ instrument at 70 eV. HPLC separations were carried out using a Thermo Separations pump and UV detector, a Shodex RI-71 detector, and a YMC RP-18 (20 × 250 mm) column.

Animal Material. The green tunicate *Aplidium meridianum*[9,10] was collected by trawling at a depth of 100 m near the South Georgia Islands and stored at −20 °C until analyzed. Taxonomic classification was carried out by one of us (M. T.). *Aplidium meridianum* forms very soft, dome-shaped green colonies with a maximun diameter of 7 cm. A voucher specimen is deposited at the Cátedra de Anatomía Comparada, Facultad de Ciencias Exactas, Físicas y Naturales, Universidad Nacional de Córdoba, Córdoba, Argentina.

Extraction and Isolation. The frozen tunicate (900 g) was triturated and extracted three times with EtOH (2 L). This extract was taken to dryness under reduced pressure to yield a yellow residue that was flash-

chromatographed on reversed-phase Si using an H_2O−MeOH gradient. The fraction eluted with MeOH−H_2O (50:50) (1.5 g) was chromatographed on Sephadex LH-20 (4 × 80 cm column, MeOH) to yield meridianin A (26 mg). The fraction eluted from reversed-phase with MeOH−H_2O (80:20) (1.7 g) was similarly chromatographed on Sephadex LH-20, and 24 fractions were collected. Fraction 23 gave pure meridianin B (**2**) (18.7 mg); fractions 17 and 18 were pooled and further separated by HPLC using H_2O−MeCN (70:30) as eluent to yield pure meridianins C (**3**) (12 mg) and D (**4**) (16 mg). Fraction 19 was purified by HPLC using MeOH−H_2O (78:22) to give pure meridianin E (**5**) (37 mg).

Meridianin A (1): recrystallized from MeOH−H_2O as yellow needles; mp 164−168 °C; UV (CH_3Cl) λ_{max} (log ϵ) 248 (3.68), 356 (3.58) nm; IR (KBr) ν_{max} 3437, 3351, 3200, 2924, 1647, 1605, 1533, 1469, 1326, 820, 721 cm^{-1}; ^1H NMR and ^{13}C NMR see Tables 1 and 2, respectively; HREIMS *m/z* 226.0857 (calcd for $C_{12}H_{10}N_4O$, 226.0855).

Meridianin B (2): recrystallized from EtOAc as a yellow powder; mp 190 °C (dec); UV (CH_3Cl) λ_{max} (log ϵ) 246 (3.87), 354 (3.71) nm; IR (KBr) ν_{max} 3452, 3357, 3226, 2919, 1634, 1590, 1532, 1466, 1429, 1327, 1225, 816 cm^{-1}; ^1H NMR and ^{13}C NMR see Tables 1 and 2, respectively; HREIMS *m/z* 303.9959 (calcd for $C_{12}H_9N_4OBr$, 303.9960).

Meridianin C (3): recrystallized from MeOH−H_2O as a yellow powder; mp 103−106 °C; UV (CH_3Cl) λ_{max} (log ϵ) 244 (4.06), 324 (4.10) nm; IR (KBr) ν_{max} 3399, 3326, 3190, 2917, 1666, 1586, 1514, 1450, 1169, 880, 808, 784, 664 cm^{-1}; ^1H NMR and ^{13}C NMR see Tables 1 and 2, respectively; HREIMS *m/z* 288.0008 (calcd for $C_{12}H_9N_4Br$, 288.0011).

Meridianin D (4): recrystallized from EtOAc−MeOH as a yellow powder; mp 218−221 °C; UV (CH_3Cl) λ_{max} (log ϵ) 240 (4.17), 324 (4.14) nm; IR (KBr) ν_{max} 3432, 3325, 3263, 3176, 2925, 1663, 1573, 1516, 1449, 891, 818 cm^{-1}; ^1H NMR and ^{13}C NMR see Tables 1 and 2, respectively; HREIMS *m/z* 288.0007 (calcd for $C_{12}H_9N_4Br$, 288.0011).

Meridianin E (5): recrystallized from MeOH−H_2O as yellow crystals; mp 172−175 °C; UV (MeOH) λ_{max} (log ϵ) 224 (4.20), 358 (3.85) nm; IR (KBr) ν_{max} 3387, 3335, 3226, 2927, 1634, 1590, 1538, 1392, 1225, 802, 721 cm^{-1}; ^1H NMR and ^{13}C NMR see Tables 1 and 2, respectively; HREIMS *m/z* 303.9960 (calcd for $C_{12}H_9N_4OBr$, 303.9960).

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