Full Papers

New Protopine and Benzyltetrahydroprotoberberine Alkaloids from *Aristolochia constricta* and Their Activity on Isolated Guinea-Pig Ileum

Luca Rastrelli, Anna Capasso, Cosimo Pizza, and Nunziatina De Tommasi*

Dipartimento di Scienze Farmaceutiche, Facoltà di Farmacia, Università di Salerno, Piazza V. Emanuele 9-84084, Penta di Fisciano (SA), Italy

Ludovico Sorrentino

Dipartimento di Farmacologia Sperimentale, Università di Napoli "Federico II", via D. Montesano 49, 80131 Napoli, Italy

Received October 31, 1996[⊗]

Five new protopine-type alkaloids, 3,5-di-*O*-methylconstrictosine (**1**), 5,6-dihydro-3,5-di-*O*-methylconstrictosine (**2**), 5,6-dihydroconstrictosine (**3**), constrictosine (**4**), 3-*O*-methylconstrictosine (**5**), and a novel 8-benzylberberine-type alkaloid, (−)-8β-(4′-hydroxybenzyl)-2,3-dimethoxyberbin-10-ol (**6**) were isolated from the aerial parts of *Aristolochia constricta*. Their structures were elucidated by physical and spectroscopic data. The results of our pharmacological experiments indicated that MeOH extract, its partially purified fraction **VI** and the protopine derivatives constrictosine **1**−**5**, significantly reduced, in a dose dependent manner, the electrical, acetylcholine, and histamine contractions of the isolated guinea-pig ileum.

Aristolochia constricta (Mutis ex H.B.K.) is a medicinal plant found in Ecuador and widely distributed in South America. It belongs to the Aristolochiaceae in which aristolochic acids and aristolactams are the known main components.[1,2] Although the aerial parts of *A. constricta* are empirically used in folk medicine as an antispasmodic,[3] an emmenagogue, and against snake bites,[4] there are no data in the literature on the possible pharmacological effects exerted by extracts, fractions, and pure compounds isolated and purified from this plant.

In our continuing search for new bioactive metabolites from South American species, we have investigated the effects of extracts, partially purified fractions, and pure compounds obtained from *A. constricta* on the electrical (ECI)-, acetylcholine (Ach)-, and histamine (Hist)-induced contractions of the isolated guinea-pig ileum. Bioassay-directed fractionation of the extracts led to the isolation of five new protopine-type alkaloids (**1**−**5**) as compounds responsible, at least partially, for the observed antispasmodic activity and of a novel 8-benzylberberine-type alkaloid (**6**), that not showed activity on the induced contractions of the isolated guinea-pig ileum.

Results and Discussion

The aerial parts of *A. constricta* were successively extracted with light petroleum ether, $CHCl_3$, $CHCl_3-$MeOH (9:1), and MeOH. Each extract was tested on the electrically induced contractions of guinea-pig ileum. The administration of vehicle control DMSO up to 100 μL did not affect the guinea-pig activity (data not shown). Both $CHCl_3-$MeOH (9:1) and MeOH extracts at the concentrations used (125, 250, and 500 μg/mL) dose-dependently reduced the ECI (Figure 1a). The inhibition began 2−4 min after extract administration, it was enhanced with time and lasted for the entire recording period (15 min). Both petroleum and $CHCl_3$ extracts from 300 to 1000 μg/mL did not induce signifi-

---

* To whom all correspondence should be addressed. Phone: 0039-89-968954. FAX: 0039-89-968937.
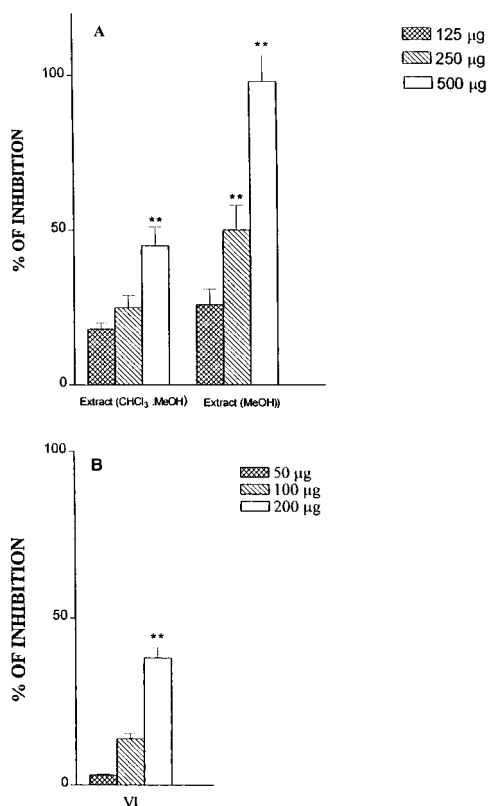⊗ Abstract published in *Advance ACS Abstracts,* September 15, 1997.

Figure 1. (**A**) Dose-related inhibition by CHCl₃—MeOH (9:1) and MeOH extracts from *A. constricta* on the ECI of guinea-pig ileum. (**B**) Dose-related inhibition of partially purified fraction VI on the ECI of guinea-pig ileum.

Table 1. IC₅₀ Values and Confidence Limits of Extracts, Partially Purified Fraction **VI**, and Pure Compounds **1–5** from *Aristolochia constricta* on ECI and Ach- and Hist-induced Contractions of Guinea-pig Ileum

	IC$_{50}$ values	confidence limits (lower—upper)
	Effect on ECI	
CHCl₃—MeOH extract	681.7 μg/mL	515.7—711.3
MeOH extract	196.3 μg/mL	160.3—272.1
fraction **VI**	220.3 μg/mL	180.3—265.4 M
compound **1**	6.6×10^{-5} M	4.8×10^{-5}—8.9×10^{-5} M
compound **2**	1.9×10^{-5} M	1.4×10^{-5}—2.5×10^{-5} M
compound **3**	2.1×10^{-5} M	1.6×10^{-5}—2.6×10^{-5} M
compound **4**	6.6×10^{-5} M	4.6×10^{-5}—9.4×10^{-5} M
compound **5**	8.6×10^{-5} M	5.8×10^{-5}—1.3×10^{-5} M
	Effect on Ach-Induced Contractions	
compound **1**	5.8×10^{-5} M	4.3×10^{-5}—7.8×10^{-5} M
compound **2**	2.0×10^{-5} M	1.5×10^{-5}—2.6×10^{-5} M
compound **3**	2.6×10^{-5} M	1.4×10^{-5}—2.8×10^{-5} M
compound **4**	7.5×10^{-5} M	5.1×10^{-5}—1.1×10^{-5} M
compound **5**	8.5×10^{-5} M	6.0×10^{-5}—1.2×10^{-5} M
	Effect on Hist-Induced Contractions	
compound **1**	6.8×10^{-5} M	4.9×10^{-5}—9.3×10^{-5} M
compound **2**	2.2×10^{-5} M	1.5×10^{-5}—3.1×10^{-5} M
compound **3**	2.6×10^{-5} M	1.8×10^{-5}—3.9×10^{-5} M
compound **4**	5.1×10^{-5} M	3.7×10^{-5}—6.9×10^{-5} M
compound **5**	5.9×10^{-5} M	4.2×10^{-5}—8.2×10^{-5} M

cant alterations of the ECI (data not shown). Table 1 shows the IC₅₀s calculated for the active extracts.

As the MeOH extract was more active in inhibiting the ECI contractions, it was submitted to a further purificaton by Sephadex LH-20 column with MeOH as eluent, and seven main fractions were collected and tested on the ECI at concentrations of 50, 100, and 200 μg/mL. Fractions **I–V** and **VII** did not show inhibitory

activity on the ECI (data not shown), whereas fraction **VI** was able to reduce significantly the ECI only at the higher concentrations used (200 μg/mL) (Figure 1b). Also in this case, the inhibition appeared 2−4 min after administration, and it was enhanced with time and lasted for the duration of the recording period (15 min). Table 1 shows the IC₅₀ calculated for the active fractions.

By means of reversed-phase HPLC, compounds **1−6** were isolated from fraction **VI**. Five of these compounds, at concentrations of 2.5×10^{-5}, 5×10^{-5}, 10^{-4} M, exerted a significant activity on the ECI. The relative order of potency was: compound **6** inactive; compound **1** < compound **5** < compound **4** < compounds **2** and **3** as indicated by their IC₅₀s (Table 1). Compounds **1−5** also reduced significanly both Ach- and Hist-induced contractions of isolated guinea-pig ileum (Table 1). These data appear to support that the inhibitory activity of the MeOH extract and of fraction **VI** may be due to the presence of constrictosine alkaloids. Reports of protopine-type alkaloids possessing both anticholinergic and antihistaminic properties[5] further confirm this hypothesis.

1	R = CH₃, R₁ = CH₃	**2**	R = CH₃, R₁ = CH₃
4	R = H, R₁ = H	**3**	R = H, R₁ = H
5	R = CH₃, R₁ = H		

6
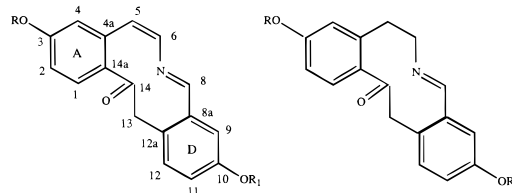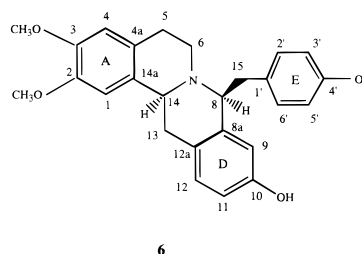
The structural elucidation of compounds **1−6** proceeded as follows.

The molecular formulas of compounds **1−6** (C₁₉H₁₇O₃N for **1**, C₁₉H₁₉O₃N for **2**, C₁₇H₁₅O₃N for **3**, C₁₇H₁₃O₃N for **4**, C₁₈H₁₅O₃N for **5**, C₂₆H₂₇O₄N for **6**) were determined from EIMS, ¹³C (Table 3), and ¹³C-DEPT NMR analysis.

The 500 MHz ¹H NMR spectrum of **1** (Table 2) exhibited the presence of 17 protons, each of which was identified with the help of ¹H−¹H COSY spectrum. Two signals (3H, s) for −OMe protons resonated at δ 3.85 and 3.87. Signals at δ 7.28 (1H, d, *J* = 8.60 Hz, H-1), δ 6.90 (1H, dd, *J* = 8.60, 2.45 Hz, H-2), δ 8.05 (1H, d, *J* = 2.45 Hz, H-4) and at δ 7.16 (1H, d, *J* = 2.50 Hz, H-9), δ 6.86 (1H, dd, *J* = 8.70, 2.50 Hz, H-11), δ 7.15 (1H, d, *J* = 8.70 Hz, H-12) suggested a structure containing two trisubstituted aromatic rings. An uncoupled proton at δ 8.77 (1H, s) could be assigned to the C-8 proton, whereas two coupled olefinic protons at δ 8.04 and 8.30 (both d, *J* = 5.12 Hz) were assigned to the C-5 and C-6 protons, respectively. A HETCOR (¹H−¹³C direct chemi-

Table 2. ^{1}H-NMR Data for Compounds **1–5** (CD$_3$OD)^a

proton	1	2	3	4	5
H-1	7.28 d (8.60)	7.22 d (8.70)	7.21 d (8.75)	7.25 d (8.60)	7.28 d (8.60)
H-2	6.90 dd (8.60, 2.45)	6.88 dd (8.70, 2.45)	6.73 dd (8.75, 2.44)	6.67 dd (8.60, 2.40)	6.90 dd (8.60, 2.45)
H-4	8.05 d (2.45)	7.91 (2.45)	7.75 d (2.44)	7.93 d (2.40)	8.05 d (2.45)
H-5	8.04 d (5.12)	2.86 t (8.38)	2.86 t (8.40)	8.06 d (5.18)	8.01 d (5.14)
H-6	8.30 d (5.12)	3.95 t (8.38)	3.90 t (8.40)	8.80 d (5.18)	8.28 d (5.14)
H-8	8.77 s	8.10 s	8.06 s	8.78 s	8.73 s
H-9	7.16 d (2.50)	7.12 d (2.50)	6.94 d (2.50)	7.45 d (2.40)	7.45 d (2.45)
H-11	6.86 dd (2.50, 8.70)	6.83 dd (2.50, 8.70)	6.75 dd (2.50, 8.70)	7.44 dd (2.40, 8.75)	7.42 dd (2.45, 8.70)
H-12	7.15 d (8.70)	7.15 d (8.70)	7.17 d (8.70)	7.48 (8.75)	7.48 d (8.70)
H-13	4.10 br s	4.08 br s	4.08 br s	4.10 br s	4.10 br s
OCH$_3$	3.85 s	3.85			3.83 s
OCH$_3$	3.87 s	3.88			

^{a} J values are in parentheses and reported in Hz; chemical shifts are given in δ units.

Table 3. ^{13}C-NMR Data for Compounds **1–5** (CD$_3$OD)^a

carbon	1	2	3	4	5
C-1	111.50	113.80	113.50	113.45	111.65
C-2	112.00	111.80	113.50	113.90	112.20
C-3	158.75	158.00	154.75	154.15	159.00
C-4	106.65	106.10	107.86	107.86	106.80
C-4a	132.75	132.75	132.75	132.38	132.85
C-5	116.85	21.00	19.95	118.60	116.90
C-6	137.80	50.10	50.00	137.95	137.85
C-8	140.00	139.10	139.10	139.40	140.10
C-8a	126.60	126.59	126.59	115.70	115.75
C-9	102.90	102.90	103.70	106.90	107.00
C-10	156.10	156.10	152.07	154.15	154.30
C-11	113.00	113.00	114.00	113.89	113.90
C-12	114.55	114.50	116.50	119.85	120.00
C-12a	122.75	128.73	128.73	122.45	122.55
C-13	50.00	50.00	50.00	50.00	49.00
C-14	185.05	185.00	184.44	189.30	189.30
C-14a	129.70	128.73	128.73	129.55	129.70
OCH$_3$	57.10	57.15			57.20
OCH$_3$	57.00	57.05			

^{a} Assignments were confirmed by ^{1}H–^{1}H COSY and ^{1}H–^{13}C HETCOR experiments.

cal shift correlation spectroscopy) correlated each hydrogen signal to the corresponding carbon signal and allowed the assignment of all resonances (Table 3). Protopine alkaloids are usually reported as C-2 substituted on the basis of biogenetic considerations. The unusual absence of C-2 substitution in **1** required further spectroscopic evidences including NOESY and COLOC techniques to establish this pattern clearly. The NOESY spectrum spatially interconnects the 3-OMe to H-4 and H-2; H-4 to H-5; H-6 to H-8; H-8 to H-9; 10-OMe to H-9 and H-11. The presence of methoxyl groups at C-3 and C-10 positions was confirmed by COLOC data, which indicated that C-3 is coupled to a methoxy singlet (δ 3.85) and to H-1; C-4 to both H-2 and H-5; C-5 to H-4; C-14 to H-1; C-14a to H-2. Moreover the COLOC spectrum showed the coupling of C-8 to H-6 and H-9; H-8 to C-6, C-9, and C-12a; C-13 to H-12; H-13 to C-14a, C-8a, and C-12. Compound **1** is a new natural product and is named 3,5-di-O-methylconstrictosine. The ^{1}H- and ^{13}C-NMR assignments are listed (Tables 2 and 3).

The EIMS of **2** gave a molecular ion at m/z 309 which was 2 mass units higher than that of **1**. The ^{13}C-NMR and ^{13}C-DEPT NMR experiments of **2** revealed 19 carbons consisting of 5 sp^3 and 14 sp^2 carbons. The ^{1}H-NMR (Table 2) spectrum showed signals due to six aromatic protons, two methoxy groups, one olefine proton, and three sp^3 methylenes. From the ^{1}H–^{1}H COSY data, a spin system was assigned to a segment $-CH_2-CH_2-$ (C-5–C-6), which was connected with H-8 through the nitrogen atom (N-7) by the ^{1}H-^{13}C long-range couplings observed in HMBC spectrum of **2** (cross

peaks: H-8/C-6, H$_2$-6/C-8). The chemical shifts of C-6 and H$_2$-6 coincided with the fact that C-6 was adjacent to a nitrogen atom. The connection with aromatic ring A was suggested by the HMBC connectivities for H$_2$-5/C-4, H$_2$-5/C-4a, H$_2$-6/C-4a, H-1/C-14, H-2/C-14a, while the aromatic ring D was connected by the HMBC correlation for H-8/C9, H-8/C-12a, H-12/C-13, H$_2$-13/C-8a. Furthermore, the HMBC data provided evidence for the position of substituents (HMBC cross peaks: OMe/C-3, OMe/C-10). The NOESY spectrum confirmed the C-3 and C-10 substitution. From all these data the structure of compound **2**, named 5,6-dihydro-3,5-di-O-methylconstrictosine, was assigned as reported.

5,6-Dihydro constrictosine (**3**) and constrictosine (**4**) differed from **2** and **1**, respectively, only in the substitution of the methoxy groups at C-3 and C-10 with a phenolic functions, as suggested by the absence of the signals at δ 3.85 and 3.87 for **3** and at δ 3.85 and 3.88 for **4** in the ^{1}H-NMR spectra, and at δ 57.10 and 57.00 for **3** and at δ 57.15 and 57.05 for **4** in the ^{13}C-NMR spectra, and by some small $\Delta\delta$ differences in the carbon resonances of ring A and D. Therefore, the structures of 5,6-dihydroconstrictosine (**3**) and constrictosine (**4**) were assigned as presented.

The NMR data of compound **5** were very similar to those of **1**, with the main differences being the absence of the signal due to one methoxyl group and the chemical shifts of one aromatic ring. The position of the methoxyl group on ring A was determined by homonuclear NOE experiments. On irradiation of methoxy protons (3H, s, δ 3.83) a significant NOE was observed at H-4 (1H, d, δ 8.05) and at H-2 (1H, dd, δ 6.90), while irradiation of H-9 (1H, d, δ 7.45) caused an appreciable NOE only at H-8 (1H, s, δ 8.73). These observations clearly revealed that the methoxyl group was located on C-3 and the hydroxy group is, therefore, on C-10. From all these data the structure of **5** was concluded to be 3-O-methylconstrictosine.

The ^{13}C-NMR and ^{13}C-DEPT NMR spectra of **6** revealed 26 carbons consisting of 8 sp^3 and 18 sp^2 ones, the latter of which corresponded to three aromatic rings. The ^{1}H-NMR spectrum of **6** (Table 4) revealed signals due to nine aromatic protons, two methoxyl groups, two sp^3 methines, and four sp^3 methylenes, each of which was identified with the help of ^{1}H–^{1}H COSY spectrum. The assignments of all protonated carbons were accomplished by interpretation of the HETCOR spectrum (Table 4). Particularly, the ^{1}H–^{1}H COSY spectrum of **6** revealed spin systems due to three segments [one AA'BB' (C-5–C-6) and two ABX patterns (C-8–C-15 and C-13–C-14)]. These three segments were connected

Table 4. ¹H- and ¹³C-NMR Data for Compound **6** (CD₃OD)[a]

position	¹³C	¹H	HMBC correlation ¹H
1	112.50	6.65 s	H-14
2	145.80		OMe, H-4
3	147.00		OMe, H-1
4	112.60	6.60 s	H-5
4a	130.05		H-6
5	30.15	2.70 t (8.0)	
6	49.00	3.38 t (8.0)	H-8, H-14
8	69.05	4.22 br t (7.0)	H-6
8a	127.10		H₂-13
9	108.10	7.50 d (2.0)	H-8
10	154.20		
11	114.35	7.40 dd (2.0, 7.5)	
12	119.90	7.42 d (7.5)	H₂-13
12a	133.05		H-8
13 (α)	35.05	2.60 dd, (12.5, 11.0)	
13 (β)		2.55 dd (12.5, 5.0)	
14	51.45	4.46 dd (11.0, 5.0)	H₂-6
14a	125.70		H-5, H₂-13
15	41.50	3.78 m	
1′	132.00		H-8
2′	131.10	6.90 br d (8.5)	H-15
3′	116.10	6.70 br d (8.5)	
4′	158.05		
5′	116.10	6.70 br d (8.5)	
6′	131.10	6.90 br d (8.5)	H-15
OCH₃	57.00	3.80 s	
OCH₃	57.15	3.82 s	

[a] Assignments were confirmed by ¹H–¹H COSY and ¹H–¹³C HETCOR experiments. *J* values are in parentheses and reported in Hz; chemical shifts are given in δ units.

with one another through the nitrogen atom (N-7) by the ¹H–¹³C long-range couplings observed in HMBC spectrum of **6** (cross peaks: H-8/C-6, H-14/C-6, H₂6/C-8, H₂6/C-14 and H-8/C-9). These segments were shown to be further connected with three aromatic rings as follows. The connection with the aromatic ring A was suggested by HMBC connectivities for H₂5/C-4, H₂6/C-4a, H-14/C-1, H₂-5/C-14a, H₂-13/C-14a, while the aromatic ring D was connected by the HMBC correlation for H₂-13/C-8a, H₂-13/C-12, H-8/C-9, and H-8/C-12a. The third aromatic ring E was attached to C-15 position (HMBC cross peaks: H₂-15/C-2′). From these results a benzyltetrahydroprotoberberine ring system was deduced for **6**. On the three aromatic rings were attached nine hydrogens, two hydroxyl groups, and two methoxy groups. The sp² carbon signals bearing these substituents were clearly distinguished by ¹³C chemical shifts as well as HMBC spectrum. Particularly, the HMBC correlations from the methoxy protons to the carbons bearing the methoxy groups were useful to discriminate methoxy-bearing carbons from hydroxy-bearing carbons. Furthermore, the HMBC data (Table 4) provided evidence for the position of substituents.

It is reported that if rings B and C of the tetrahydroprotoberberine alkaloids assume a half-chair conformation, they exist in the equilibrium of one B/C *trans*-quinolizidine (I *trans*) and two B/C *cis*-quinolizidine systems (II *cis* and III *cis*) [6, 7] (Figure 2). The three systems are called conformers although the nitrogen configuration in *trans*-quinolizidine is opposite to that of *cis*-quinolizidine. Previous ¹H-NMR literature data for *cis* and *trans* junction showed that for B,C-*trans* junction, H-14 and H-8 resonated in CD₃OD both at ~ 3.6 ± 0.2 ppm, whereas for the *cis* junction, a ~ 0.7 ppm downfield shift was recorded (~ 4.3 ± 0.2 ppm). Moreover, as reported by R. Suau,[7] in the ¹³C-NMR spectrum the *trans*-quinolizidine conformation is associated with
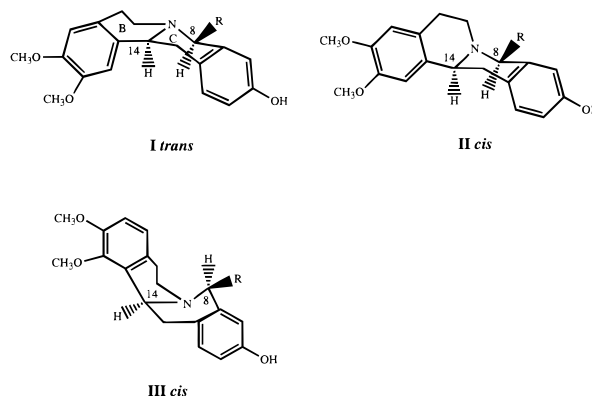
Figure 2. B/C-*trans* and B/C-*cis* forms of compound **6**.

a lowfield C-14 in contrast with the analogous signal of its conformer (Δδ ~ 9 ppm). Resonances at δ 4.22 and δ 4.46 respectively, for H-8 and H-14 in the ¹H-NMR spectrum and at 51.4 ppm for C-14 in the ¹³C-NMR spectrum of compound **6**, indicated a B/C *cis* conformation (Table 4). Moreover, the chemical shift, the multiplicity, and the coupling-constant value of H-14 (1H, dd, *J* = 11, 5 Hz) were consistent for an axial proton. Irradiation of this proton yielded a strong NOE at H-8 as expected for a II *cis* conformation of compound **6** (Figure 2). These observation also indicated the relative configuration at C-8 with an equatorial benzyl group. Therefore, to compound **6** was attributed the structure (−)-8β-[4′-hydroxybenzyl]-2,3-dimethoxy berbin-10-ol. 8-Benzylberberine alkaloids have been isolated previously from other natural sources,[9] the novelty of our compound **6** resides in the different substitution patterns of rings A (methoxy group at C-2 and C-3) and D (hydroxy group at C-10), which have never been reported, as far as we know, in the literature.

Experimental Section

General Experimental Procedures. The EIMS spectra were obtained from a VG-PROSPEC mass spectrometer (70 eV). Optical rotations were measured on a Perkin-Elmer 192 polarimeter equipped with a sodium lamp (589 nm) and a 10-cm microcell. IR spectra were performed with a Bruker IFS-48 spectophotometer; UV spectra were obtained from a Beckman DU 670 spectophotometer. ¹H-NMR, ¹³C-NMR, DEPT, and various 2D NMR (¹H–¹H COSY, ¹H–¹³C COSY, COLOC, NOESY, and HMBC) spectra were obtained in CD₃OD using a Bruker AMX-500 spectrometer, equipped with a Bruker X-32 computer, the UXNMR software package was used for NMR experiments. HPLC separations were performed on a Waters 590 series pumping system with a Waters R401 refractive index detector equipped with a Waters μ-Bondapak C18 column.

Plant Material. The aerial parts of *Aristolochia constricta* were collected in August 1994, in Chimborazo region, Ecuador. A specimen was authenticated by Dr. Carlos Donoso. A voucher sample is deposited at the Herbarium of Escuela Superior Politecnica del Chimborazo, Riobamba, Ecuador.

Extraction and Isolation. The powdered, dried, aerial parts (500 g) were defatted with petroleum ether (6 g) and CHCl₃ (7 g) in a Soxhlet apparatus and extracted successively at room temperature with CHCl₃–MeOH (9:1) (8 g) and MeOH (24 g). The most active MeOH extract (12 g) was partitioned between *n*-BuOH

and H_2O to afford an *n*-BuOH-soluble portion (7.0 g), which was chromatographed on a Sephadex LH-20 column using MeOH as eluent and collected fractions of 8 mL. Each the obtained fractions was combined according to TLC (Si gel, *n*-BuOH−HOAc−H_2O, 60:15:25) to give seven main fractions **I−VII**. Final separations of fraction **VI**, positive to Dragendorff's reagent on TLC, was achieved by reversed-phase HPLC on a C18 μ-Bondapak column (30 cm × 7.8 mm, flow rate 2.5 mL min^{-1}) using MeOH−H_2O (45:55) to yield pure compounds **1** (25.0 mg, t_R = 18 min), **2** (17.7 mg, t_R = 20 min), **3** (20.8 mg, t_R = 14 min), **4** (25.5 mg, t_R = 10 min), **5** (27.8 mg, t_R = 13 min), and **6** (19.4 mg, t_R = 23 min).

3,5-Di-*O*-methylconstrictosine (1): isolated as an amorphous yellow solid; $C_{19}H_{17}O_3N$; mp 189−195 °C; UV λ_{max} (MeOH) (log ϵ) 302 (4.72), 282 (4.96), 256 (4.36), 224 (4.12); IR (KBr) 3420 (OH), 1680 (C=O) cm^{-1}; EIMS *m/z* 307 [M]$^+$, 276, 133, 174; ^{1}H-NMR data, see Table 2; ^{13}C-NMR data, see Table 3.

5,6-Dihydro-3,5-di-*O*-methylconstrictosine (2): Isolated as an amorphous yellow solid; $C_{19}H_{19}O_3N$; mp 186−192 °C; UV λ_{max} (MeOH) (log ϵ) 282 (4.82), 256 (4.40); IR (KBr) 3420 (OH), 1680 (C=O) cm^{-1}; EIMS *m/z* 309 [M]$^+$, 278, 133, 176; ^{1}H-NMR data, see Table 2; ^{13}C NMR data, see Table 3.

5,6-Dihydroconstrictosine (3): isolated as an amorphous yellow solid; $C_{17}H_{15}O_3N$; mp 187−192 °C; UV λ_{max} (MeOH) (log ϵ) 282 (3.28), 256 (2.36); IR (KBr) 3420 (OH), 1680 (C=O) cm^{-1}; EIMS *m/z* 281 [M]$^+$, 264, 119, 162; ^{1}H-NMR data, see Table 2; ^{13}C-NMR data, see Table 3.

Constrictosine (4): isolated as an amorphous yellow solid; $C_{17}H_{13}O_3N$; mp 200−243 °C; UV λ_{max} (MeOH) (log ϵ) 302 (3.86), 282 (4.06), 256 (3.24), 224 (3.10); IR (KBr) 3420 (OH), 1680 (C=O) cm^{-1}; EIMS *m/z* 279 [M]$^+$, 262, 119, 160; ^{1}H-NMR data, see Table 2; ^{13}C-NMR data, see Table 3.

3-*O*-Methylconstrictosine (5): isolated as an amorphous yellow solid; $C_{18}H_{15}O_3N$; mp 215−239 °C; UV λ_{max} (MeOH) (log ϵ) 302 (3.92), 282 (4.16), 256 (3.38), 224 (3.32); IR (KBr) 3420 (OH), 1680 (C=O) cm^{-1}; EIMS *m/z* 293 [M]$^+$, 276, 119, 176; ^{1}H-NMR data, see Table 2; ^{13}C-NMR data, see Table 3.

(−)-8β-(4′-Hydroxybenzyl)-2,3-dimethoxyberbin-10-ol (6): isolated as an amorphous yellow solid; $C_{26}H_{27}O_4N$; mp 150−162 °C; [α]$^{25}_D$ −65.3 (*c* 1, MeOH). UV λ_{max} (MeOH) (log ϵ) 290 (4.36); IR (KBr) 3386 (OH), 1606 (C=O) cm^{-1}; EIMS *m/z* 417 [M]$^+$, 310 [M − $CH_2C_6H_4OH$]$^+$, 192 ($C_{11}H_{14}O_2N$), 107; ^{1}H-NMR and ^{13}C-NMR data, see Table 4.

Transmurally Stimulated Guinea-Pig Ileum Test. Male Charles River guinea pigs (180−200 g) were used for all experiments. The animals were housed in colony cages (four guinea pigs each) under standard light (light on from 7.00 a.m. to 7.00 p.m.), temperature (22 ± −1 °C), and room humidity (60% ± 10%) conditions for at least 1 week before the experimental sessions. Sections of guinea-pig ileum were prepared as described previously.[10] Pieces of ileum, 2−3 cm long, were set up in a 10-mL organ bath containing Tyrode solution with 5% CO_2 in 95% oxygen; the solution was maintained at 37 °C. The whole ileum preparation was placed between platinum electrodes and connected to a 85/2/50 MARB Stimulator (MARB, Pistoia, Italy). A force-displacement transducer and unirecord model polygraph was used for measurement of isotonic contraction (Ugo Basile, Italy). A resting tension of 0.5 *g* was applied. After a 30-min equilibration period, the preparation was stimulated with 0.5-ms pulses delivered transmurally at a frequency duration of 10 s at supramaximal voltage (25 V). Under these conditions, the preparation showed a contraction mean of 60 ± 0.57 mm. The inhibition of ileal contractions by drugs was expressed as the percentage of basal value (mean ± SEM). Ach- and Hist-induced contractions were performed as previously described.[11] Regression methods used for statistical analysis and critical significance was set at $p < 0.05$. IC$_{50}$ values were calculated according to the method of Litchfield and Wilcoxon.

Biological Experimental Procedures. The extracts [petroleum ether, CHCl$_3$, CHCl$_3$-MeOH (9:1), MeOH] were dissolved in dimethyl sulfoxide (DMSO, Merck), whereas both the partially purified fractions and pure compounds were dissolved in distilled H_2O. In preliminary experiments, the administration of DMSO up to 100 μL was added to the organ bath to verify whether this vehicle alone induced changes in the control contraction of the preparation. After these experiments, the effects of the extracts, partially purified fractions, and pure compounds on the ECI of guinea-pig ileum (*n* = 9) were investigated: petroleum ether, CHCl$_3$, CHCl$_3$−MeOH (9:1), MeOH extracts at concentrations of 125, 250, 500 μg/mL organ bath, 15 min contact period; partially purified fractions **I−VII** at concentration of 50, 100, 200 μg/mL organ bath, 15-min contact period; pure compound at concentration of 2.5 × 10^{-5}, 5 × 10^{-5}, 10^{-4} M organ bath, 15-min contact period.

References and Notes

(1) Hong, L.; Sakagami, Y.; Marumo, S.; Xinmin, C. *Phytochemistry* **1994**, *37*, 237.
(2) Lajide, L.; Escoubas, P.; Mizutani, K. *J. Agric. Food Chem.* **1993**, *41*, 669.
(3) Branch, L. C.; Do Silva, M. F. *Acta Amazonica* **1983**, *13* , 737.
(4) Velasco, J. *Historia del Rein de Quito, La Historia Natura*; Empresa Editoria El Comercio: Quito, Ecuador, 1946; Vol. 1.
(5) Ustunes, L.; Laekeman, G. M.; Gözler, B.; Vlietink, A. J.; Özer, A.; Herman A. G. *J. Nat. Prod.* **1988**, *51*, 1021.
(6) Iwasa, K.; Sugiura M.; Takao N. *J. Org. Chem.* **1982**, *47*, 4275.
(7) Suau, R.; Silva, M. V.; Valpuesta M. *Tetrahedron* **1990**, *46*, 4421.
(8) Xavier Lopes, L. M. *Phytochemistry* **1992**, *31*, 4005.
(9) Okpako, D. T.; Taiwo, Y. O. O. *Br. J. Pharmacol.* **1984**, *82*, 577.
(10) Capasso, A.; Pinto, A.; Mascolo, N.; Autore, G.; Capasso, F. *Phytotherapy Res.* **1991**, *5*, 85.