Chemical Constituents of *Aristolochia constricta*: Antispasmodic Effects of Its Constituents in Guinea-Pig Ileum and Isolation of a Diterpeno−Lignan Hybrid

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Twenty constituents were isolated from the *n*-hexane and chloroform extracts of *Aristolochia constricta,* a plant whose aerial parts have been used empirically in folk medicine for various purposes. The inhibitory effects of these constituents on smooth muscle contraction in isolated guinea-pig ileum were studied in order to observe their antispasmodic effects. 3,4-Dibenzyldihydrofuran-type lignans [(−)-cubebin, (−)-hinokinin, and (−)-pluviatolide] and a kaurene-type diterpene [(−)-kaur-16-en-19-oic acid] were isolated as active principals. They inhibited electrically induced and acetylcholine-induced contraction in the isolated guinea-pig ileum. In addition, 9-*O*-[(−)-kaur-15-en-17-oxyl]cubebin was isolated as a new diterpeno−lignan hybrid, although this constituent did not exhibit antispasmodic activity.

Aristolochia constricta Griseb. (Aristolochiaceae), known by its common name Saragosa (or Saragez), is widely distributed in Ecuador and South America. The aerial parts of this plant have been empirically used in folk medicine as antispasmodic, anticancer, antimalarial, and anti-inflammatory agents, as an emmenagogue, and as a treatment for snake bites.[1] Few reports on the chemical constituents of *A. constricta* have appeared thus far. Four aristolactams, six protopine alkaloids, and a berberine alkaloid have been isolated from the EtOH extract, together with known sinapic acid, β-hydroxy-3′,4′-dimethoxyphenylethyl glucoside, and common aristolochic acids.[2] The protopine alkaloids markedly inhibited electrically induced (ECI), acetylcholine-induced (AChI), and histamine-induced contractions in isolated guinea-pig ileum.[2a,e] However, the results of our preliminary study concerning the relaxant effects of *n*-hexane, $CHCl_3$, and EtOH extracts of *A. constricta* on guinea-pig ileal contraction differed from the reported data. Specifically, we found that the *n*-hexane and $CHCl_3$ extracts inhibited ECI and AChI contractions in isolated guinea-pig ileum, whereas the EtOH extract had only a weak inhibitory effect. Therefore, we sought to isolate the antispasmodic constituents in the *n*-hexane and $CHCl_3$ extracts. As a result, 19 known components (six lignans, five diterpenes, three aristolochic acid derivatives, two tetralones, one sesquiterpene, one sitosterol, and one amide) and one new diterpeno−lignan hybrid (**1**) were isolated. Of these, some lignans and diterpenes, but not the new hybrid, showed inhibitory effects on ECI and AChI contractions in isolated guinea-pig ileum. Here we report the pharmacological effects and a structural determination of the diterpeno−lignan hybrid composed of (−)-cubebin (**2**) and (−)-kaur-15-en-17-ol (**3**).

Results and Discussion

The stems of *A. constricta*, collected at Mocache, LosRios Province, in the coastal region of Ecuador, were successively extracted with *n*-hexane, $CHCl_3$, and EtOH. Each extract was used to perform antispasmodic tests on an isolated guinea-pig ileum preparation. None of the extracts alone induced either contraction or relaxation under resting conditions (data not shown). The *n*-hexane and $CHCl_3$ extracts (0.03−0.1 mg/mL) potently inhibited ECI and AChI contractions in a concentration-dependent manner, as shown in Table 1. In contrast, the EtOH extract had only a weak inhibitory effect on ECI and AChI contractions. On the basis of these results, we speculate that antispasmodic constituents are mainly present in the *n*-hexane and $CHCl_3$ extracts. Rastrelli et al. have reported that the MeOH extract exhibits an inhibitory effect on ECI contraction, whereas petroleum and $CHCl_3$ extracts do not. These discrepancies might be caused by the differences in both experimental setups in the antispasmodic tests; however, it is reasonable to deduce that the neurogenic contraction should be equally evaluated in these setups because the technique is well-established and commonly used by research groups worldwide. Thus, the differences between our results and the findings of Rastrelli et al. may be attributable to plant sources used, but not experimental conditions.

Table 1. Effects of *n*-Hexane, $CHCl_3$, and EtOH Extracts on ECI and AChI Contractions in Isolated Guinea-Pig Ileum[a]

extract	concentration (mg/mL)	ECI contraction (%)	AChI contraction (%)
n-hexane	0.03	47.1 ± 5.4 [b]	45.4 ± 13.0 [c]
	0.1	5.7 ± 1.5 [b]	10.9 ± 4.6 [b]
$CHCl_3$	0.03	52.1 ± 10.4 [b]	37.8 ± 13.5 [c]
	0.1	9.5 ± 3.6 [b]	2.9 ± 0.6 [b]
EtOH	0.03	77.8 ± 8.14	94.4 ± 5.5
	0.1	49.7 ± 7.0 [b]	64.6 ± 8.7 [c]

[a] Contraction (%) is expressed as a percentage against control contraction induced by electrical stimulation or ACh in the absence of samples. Each value is the mean ± SEM of four or five animals. [b] $P < 0.01$, significantly different from control contraction (%) (paired *t* test). [c] $P < 0.05$.

Separation of the chemical constituents of the *n*-hexane extract by chromatographic techniques led to the isolation of (−)-cubebin (**2**),[3] a 3,4-dibenzyltetrahydrofuran-type lignan with a hemiacetal function. On the other hand, 20 components were isolated from the $CHCl_3$ extract, of which 19 were identified as six lignans [(−)-cubebin (**2**), (−)-hinokinin (**4**),[4] (−)-pluviatolide (**5**),[5] (−)-haplomyrfolol (**6**),[6] (−)-dihydrocubebin (**7**),[7] and 9-*O*-methylcubebin[8]], five kaurene-type diterpenes [(−)-kaur-15-en-17-ol (**3**),[9] (−)-kaur-16-en-19-oic acid (**8**),[10] (−)-kauran-16α,17-diol (**9**),[11] (−)-kaur-

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Table 2. ^{1}H NMR (600 MHz, $CDCl_3$) Data of **1**, **2**, and **3**

position	**1a** δ_H (*J* in Hz)	**1b** δ_H (*J* in Hz)	**2a** δ_H (*J* in Hz)	**2b** δ_H (*J* in Hz)	**3** δ_H (*J* in Hz)
1	0.70−0.80, m	0.70−0.80, m			0.71−0.80, m
	1.79, dd (12.6,3.6)	1.79, dd (12.6,3.6)			1.79, d (12.4)
2	1.23−1.30, m	1.23−1.30, m			1.26−1.42, m
3	1.11−1.15, m	1.11−1.15, m			1.13, td (14.4, 5.2)
	1.36−1.40, m	1.36−1.40, m			1.26−1.42, m
5	0.70−0.80, m	0.70−0.80, m			0.71−0.80, m
6	1.46−1.62, m	1.46−1.62, m			1.49−1.67, m
7	1.46−1.62, m	1.46−1.62, m			1.49−1.67, m
9	0.82−0.90, m	0.98−1.01, m			0.96−1.04, m
11	1.46−1.62, m	1.46−1.62, m			1.49−1.67, m
12	1.46−1.62, m	1.46−1.62, m			1.49−1.67, m
13	2.37−2.42, m	2.51−2.58, m			2.54−2.56, m
14	1.23−1.30, m	1.36−1.40, m			1.26−1.42, m
	2.04, d (10.2)	2.09, d (10.4)			2.12, d (10.4)
15	5.16, s	5.32, s			5.36, s
17	3.97, d (13.7)	3.85, d (12.9)			4.190, s
	4.13, d (13.7)	4.19, d (12.9)			4.194, s
18	0.85, s	0.85, s			0.85, s
19	0.80, s	0.80, s			0.80, s
20	1.02, s	1.04, s			1.02, s
2′	6.52−6.72, m	6.52−6.72, m	6.50−6.74, m	6.50−6.74, m	
5′	6.52−6.72, m	6.52−6.72, m	6.50−6.74, m	6.50−6.74, m	
6′	6.52−6.72, m	6.52−6.72, m	6.50−6.74, m	6.50−6.74, m	
7′	2.41−2.45, m	2.51−2.58, m	2.40−2.45, m	2.57−2.68, m	
	2.51−2.58, m	2.77, dd (13.2, 9.6)	2.57−2.68, m	2.78, d (10.0)	
8′	2.17−2.20, m	1.99−2.03, m	2.11−2.15, m	1.90−2.01, m	
9′	4.83, s	4.81, d (4.7)	5.21, br *s*	5.21, br *s*	
2″	6.52−6.72, m	6.52−6.72, m	6.50−6.74, m	6.50−6.74, m	
5″	6.52−6.72, m	6.52−6.72, m	6.50−6.74, m	6.50−6.74, m	
6″	6.52−6.72, m	6.52−6.72, m	6.50−6.74, m	6.50−6.74, m	
7″	2.41−2.45, m	2.41−2.45, m	2.57−2.68, m	2.40−2.45, m	
	2.60−2.62, m	2.71, dd (13.2, 4.9)	2.57−2.68, m	2.74, d (9.2)	
8″	2.11−2.12, m	2.37−2.42, m	2.11−2.15, m	2.40−2.45, m	
9″	3.64, dd (8.4,8.4)	3.57, dd (7.3, 7.3)	3.78, t (8.4)	3.56, t (8.0)	
	3.96−4.00, m	3.96−4.00, m	3.93, dd (8.4, 6.8)	4.09, t (8.1)	
OCH_2O	5.92−5.93, m	5.92−5.93, m	5.91−5.92, m	5.91−5.92, m	
OH			2.95, br s	2.97, br s	

ene,[12] and (−)-kauran-16α,17,18-triol[13]], three aristolochic acid derivatives [aristolochic acid-1 (**10**),[14] aristololactam A II,[15] and cepharanone B[16]], two tetralones (aristelegone-A[17] and (+)-4,7-dimethyl-6-methoxy-1-tetralone (**11**)[18]), one sesquiterpene (cadalene[19]), one sterol (β-sitosterol glycoside[20]), and one amide (*N-trans*-feruloyltyramine[21]). Aristolochic acid-1 (**10**), a naturally occurring nephrotoxin[22] and carcinotoxin,[23] was obtained as the most abundant component of the $CHCl_3$ extract, with (−)-cubebin (**2**) and (−)-kaur-16-en-19-oic acid (**8**) as the other major components.

Cubebin (**2**) has three sterogenic centers on a lactol ring, including an epimerizable hemiacetal carbon. Lopes et al.[3] recently isolated (−)-*cis*- and (−)-*trans*-cubebin as a single isomer and part of inseparable diastereomeric isomers at the C-9′ hemiacetal carbon, respectively, and precisely analyzed their stereochemistry using NMR data. On the basis of their discussion, we conclude that our (−)-cubebin (**2**) is a 3:2 diastereomeric mixture of the *trans* derivatives (see Tables 2 and 3).

A new compound, **1**, was isolated as an optically active, colorless gum. The ^{1}H (Table 2) and ^{13}C (Table 3) NMR spectra suggested that **1** was a hybrid compound of (−)-cubebin (**2**) and (−)-kaur-15-en-17-ol (**3**) because of the presence of proton and carbon signals assignable to methyl, methylene, methine, and quaternary carbons in each component. The signals appeared complex due to the presence of an inseparable diastereomeric mixture including the acetal functionality of the (−)-cubebin unit. Correlations of the carbons and protons could be deduced by DEPT, HMQC, and HMBC experiments, strongly indicating that **1** was produced through dehydration between the hydroxy functions of the cubebin and kaurene units. The ether bridge was established by HMBC correlations between the 17-hydrogens [δ 3.97 and 4.13 (for **1a**) and δ 3.85 and 4.19 (for **1b**)] and the 9′-carbon [δ 107.0 (for **1a**) and δ 104.1 (for **1b**)] and between the 9′-hydrogen [δ 4.83 (for **1a**) and δ 4.81 (for **1b**)] and the 17-carbon [δ 64.4 (for **1a**) and δ 65.2 (for **1b**)]. In addition, the EIMS spectrum showed a molecular ion peak (M^+) at *m/z* 626, which supported an expected hybrid composition ($C_{40}H_{50}O_6$). In fact, characteristic fragment peaks due to cubebin (**2**) ($C_{20}H_{20}O_6$) and kaur-15-en-17-ol (**3**) ($C_{20}H_{32}O$) units were observed as shown in Table 4. From these data, the new diterpeno−lignan hybrid **1** was reasonably deduced to be an inseparable diastereomeric mixture of 9-*O*-[(−)-kaur-15-en-17-oxyl]cubebin (Figure 1).

To establish the above conclusion, treatment of a mixture of (−)-cubebin (**2**) and (−)-kaur-15-en-17-ol (**3**) in the presence of 0.1 M camphorsulfonic acid (CSA) at room temperature for 3 days followed by heating at 40 °C for 2 days yielded an acetal identical to the new hybrid **1**, although in low yield (2.4%). Aristolochic acid esters connected to (−)-kauran-16α,17-diol are known to be hybrid components of the *Aristolochia* species.[24] However, this is the first case of the isolation of a unique diterpeno−lignan hybrid from the same species.

Next, the antispasmodic effects of the isolated constituents [five lignans: (−)-cubebin (**2**), (−)-hinokinin (**4**), (−)-pluviatolide (**5**), (−)-haplomyrfolol (**6**), and (−)-dihydrocubebin (**7**); three diterpenes: (−)-kaur-15-en-17-ol (**3**), (−)-kaur-16-en-19-oic acid (**8**), and (−)-kauran-16α,17-diol (**9**); and three others: the new diterpeno−lignan hybrid (**1**), aristolochic acid-1 (**10**), and (+)-4,7-dimethyl-6-methoxy-1-tetralone (**11**)] on ECI and AChI contractions were studied in guinea-pig ileum (Figures 2 and 3). The Ca^{2+} channel blocker verapamil was also examined as a positive control. The IC_{50} values for the antispasmodic effects of the tested samples are listed in Table 5. As shown in Figures 2 and 3, the activities of

Table 3. ^{13}C NMR (150 MHz, $CDCl_3$) Data of **1**, **2**, and **3**

position	**1a** δ_C mult.	**1b** δ_C mult.	**2a** δ_C mult.	**2b** δ_C mult.	**3** δ_C mult.
1	40.4,[a] CH_2	40.5,[a]CH_2			40.5, CH_2
2	18.6, CH_2	18.6, CH_2			18.6, CH_2
3	42.1, CH_2	42.1, CH_2			42.1, CH_2
4	33.3, qC	33.3, qC			33.3, qC
5	55.9, CH	56.0, CH			56.0, CH
6	19.3, CH_2	19.3, CH_2			19.2, CH_2
7	39.2, CH_2	39.2, CH_2			39.3, CH_2
8	49.0, qC	49.0, qC			49.0, qC
9	48.2, CH	48.5, CH			48.4, CH
10	39.6, qC	39.6, qC			39.5, qC
11	18.6, CH_2	18.6, CH_2			18.6, CH_2
12	25.3, CH_2	25.6, CH_2			25.7, CH_2
13	41.1, CH	41.7, CH			41.2, CH
14	43.6, CH_2	43.8, CH_2			43.9, CH_2
15	137.8, CH	137.4, CH			136.1, CH
16	142.7, qC	143.2, qC			145.9, qC
17	64.4, CH_2	65.2, CH_2			61.3, CH_2
18	33.6, CH_3	33.6, CH_3			33.6, CH_3
19	21.6, CH_3	21.6, CH_3			21.6, CH_3
20	17.7, CH_3	17.7, CH_3			17.7, CH_3
1′	134.1,[a] qC	134.9,[a] qC	133.3, qC	134.5, qC	
2′	109.2, CH	109.3, CH	108.1, CH	108.2, CH	
3′	147.7,[a] qC	147.6,[a] qC	147.6, qC	147.7, qC	
4′	145.8,[a] qC	145.9,[a] qC	145.9, qC	146.0,[a]qC	
5′	108.1, CH	108.1, CH	109.2, CH	108.9, CH	
6′	121.7, CH	121.6, CH	121.8, CH	121.6, CH	
7′	38.8, CH_2	33.7, CH_2	39.2, CH_2	33.6, CH_2	
8′	52.4, CH	52.2, CH	53.1, CH	52.0, CH	
9′	107.0, CH	104.1, CH	103.4, CH	98.8, CH	
1″	133.6,[a] qC	134.3,[a] qC	134.1, qC	133.9, qC	
2″	109.0, CH	109.0, CH	108.9, CH	108.2, CH	
3″	147.5,[a] qC	147.5,[a] qC	147.6, qC	147.5, qC	
4″	145.7,[a] qC	145.6,[a] qC	145.8, qC	145.7, qC	
5″	108.1, CH	108.1, CH	108.1, CH	109.3, CH	
6″	121.4, CH	121.4, CH	121.4, CH	121.4, CH	
7″	39.2, CH_2	39.5, CH_2	38.4, CH_2	38.9, CH_2	
8″	45.9, CH	43.4, CH	45.9, CH	42.9, CH	
9″	71.9, CH_2	72.2, CH_2	72.2, CH_2	72.6, CH_2	
2 × OCH_2O	100.8, CH_2	100.8, CH_2	100.8, CH_2	100.8, CH_2	

[a] Assignments in the same column are interchangeable.

several constituents were more potent than those of the *n*-hexane and $CHCl_3$ extracts.

The lignans [(−)-cubebin (**2**), (−)-hinokinin (**4**), (−)-pluviatolide (**5**), and (−)-haplomyrfolol (**6**)] and (−)-kaur-16-en-19-oic acid (**9**) markedly and significantly inhibited ECI and AChI contractions to the same extent. In addition, verapamil also inhibited these contractions with similar potency. In our antispasmodic test, acetylcholine (Ach) acts on the muscarinic M_3 receptors in smooth muscle cells, leading to the induction of ileal contraction, whereas electrical stimulation excites the parasympathetic postsynaptic neurons to elicit Ach release from the nerve endings. Because (−)-cubebin (**2**), (−)-hinokinin (**4**), (−)-pluviatolide (**5**), (−)-haplomyrfolol (**6**), and (−)-kaur-16-en-19-oic acid (**8**) have an inhibitory effect on both ECI and AChI contractions, these constituents are thought to inhibit the mechanism of smooth muscle contraction.

We found that aristolochic acid-1 (**10**) potently inhibited ECI contraction, but hardly inhibited AChI contraction in the guinea-pig ileum preparation (Figures 2 and 3). The site of action for aristolochic acid-1 (**10**) may differ from those for the above five isolates. Aristolochic acid-1 (**10**) preferentially inhibits neurogenic contraction rather than the contraction elicited by activation of the muscarinic M3 receptors in smooth muscle cells. Therefore, aristolochic acid-1 (**10**) is thought to have an inhibitory effect on neurotransmitter release in the gut.

It has been reported that (−)-kaur-16-en-19-oic acid (**8**), which inhibited both ECI and AChI contractions in the present study, shows antispasmodic activity[25] and that the presence of a carboxylic acid function is important for the inhibitory activity.[25c] In fact, kaurenoic acid **8** is an interesting compound, exhibiting a variety of pharmacological actions including cytotoxicity,[26] inhibition of protein tyrosine phosphatase 1B,[27] genotoxicity,[28] antimicrobial activity,[29] antiproliferative activity,[30] and reduction of sperm motility.[31] In contrast, dibenzyldihydrofuran-type lignans show many pharmacological actions such as trypanocidal activity,[32] analgesic activity,[33] anti-inflammatory activity,[33b,c,34] antigenotoxicity,[35] oral pathogenicity,[36] cytotoxicity,[37] antiproliferative activity,[38] antiviral activity,[39] insecticidal activity,[40] cytochrome P-450 inhibition,[41] and melanogenesis stimulation activity.[42] Thus, the antispasmodic activity of (−)-cubebin (**2**), (−)-hinokinin (**4**), and (−)-pluviatolide (**5**) observed here presents an additional pharmacological action for dibenzyldihydrofuran-type lignans. The importance of the carboxylic acid function in the kaurenoic acid **8**[25c] suggests that the lactol and lactone functions in the lignan structures may act as key functions for the activity. In the present study, although (−)-cubebin (**2**), which carries a lactol function, has potent antispasmodic activity, 9-*O*-[(−)-kaur-15-en-17-oxyl]cubebin (**1**), in which a lactol in the cubebin moiety was protected as an ether tether, did not show any antispasmodic activity. Therefore, the lactol or lactone function in the lignan structures may act as a pharmacophore for the antispasmodic activity.

In conclusion, the *n*-hexane and $CHCl_3$ extracts of *A. constricta* showed relaxant activities against ECI and AChI contractions in isolated guinea-pig ileum. (−)-Cubebin (**2**), (−)-hinokinin (**4**), (−)-pluviatolide (**5**), (−)-haplomyrfolol (**6**), and (−)-kaur-16-en-19-oic acid (**8**) have antispasmodic effects, probably due to the inhibition of the mechanism of smooth muscle contraction. Aristolochic acid-1

Table 4. EIMS Data (%) of **1**, **2**, and **3**

m/z	**1**	**2**[a]	**3**[b]
626	13	−	−
356	8	36	−
355	24	−	−
354	15	−	−
340	15	−	−
339	19	12	−
338	64	55	−
337	11	−	−
288	9	−	73
273	8	−	61
271	7	−	53
255	7	−	57
204	10	−	−
203	69	49	−
202	26	30	−
190	7	−	−
173	14	19	−
163	13	−	66
162	9	−	−
161	45	+	−
160	−	20	−
148	6	+	+
147	8	−	33
145	9	+	33
137	9	+	35
136	57	66	21
135	100	100	40
133	6	−	+
131	10	+	22
123	11	+	75
122	6	+	37
121	7	+	39
119	7	−	32
117	12	+	33
115	8	15	+
110	10	−	60
109	8	−	52
107	8	−	35
105	17	+	49
95	12	−	66
93	10	−	49
92	9	−	42
91	19	+	100
83	6	−	37
82	6	+	26
81	58	100	85
79	14	+	62
77	17	26	47
69	20	−	90
67	10	−	55

[a] "+" means the presence of peak but lower than 10% intensity. [b] "+" means the presence of peak but lower than 20% intensity.

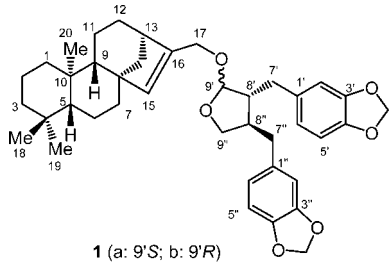

Figure 1. Structure of a new diterpeno−lignan hydrid, 9-*O*-[(−)-kaur-15-en-17-oxyl]cubebin (**1**).

(**10**) exhibits an antispasmodic effect, probably through an inhibitory effect on neurotransmitter release in the gut. These pharmacological effects may validate the use of "Saragosa" as an antispasmodic in folk medicine. The present results are at variance with previous reports,[2a,c–e] in which protopine-type alkaloids isolated from the

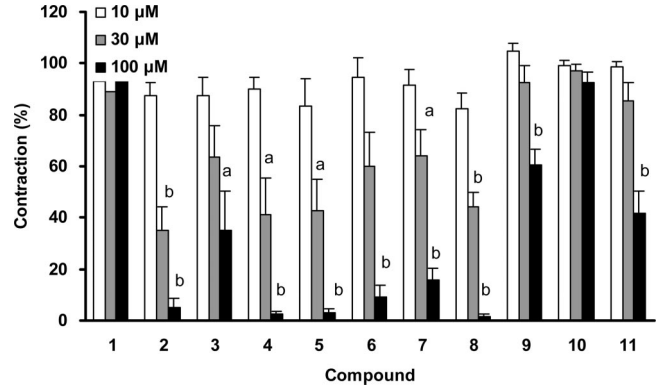

Figure 2. Effects of the selected isolates of *A. constricta* on ECI contraction in isolated guinea-pig ileum. Contraction (%) is expressed as a percentage against control contraction induced by electrical stimulation in the absence of samples. Each value shows the mean ± SEM of four or five animals. [a]$P < 0.05$, [b]$P < 0.01$, significantly different from control contraction (%) (paired *t* test).

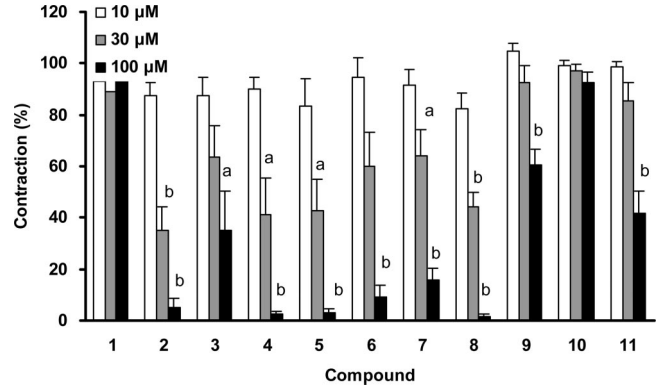

Figure 3. Effects of the selected isolates of *A. constricta* on AChI contraction in isolated guinea-pig ileum. Contraction (%) is expressed as a percentage against control contraction induced by Ach in the absence of samples. Each value shows the mean ± SEM of four or five animals. [a]$P < 0.05$, [b]$P < 0.01$, significantly different from control contraction (%) (paired *t* test).

Table 5. IC_{50} Values of Constituents of *A. constricta* Inhibiting ECI and AChI Contractions in Isolated Guinea-Pig Ileum[a]

compound	ECI contraction (%)	AChI contraction (%)
1	>100.0	>100.0
2	51.6 ± 4.5	23.6 ± 4.5
3	34.1 ± 4.2	>100.0
4	69.8 ± 5.9	27.3 ± 5.9
5	58.3 ± 10.1	26.8 ± 10.1
6	52.8 ± 6.4	39.5 ± 6.4
7	>100.0	40.4 ± 8.5
8	28.2 ± 8.5	25.4 ± 4.2
9	>100.0	>100.0
10	64.0 ± 14.1	>100.0
11	>100.0	89.8 ± 18.7
verapamil	0.25 ± 0.05	0.28 ± 0.03

[a] Each value shows the mean ± SEM of four or five animals.

EtOH extract had been identified as the active principals. Although the reason for this discrepancy is not clear, the discrepancy may be caused by the differences of the parts and/or the growing places of plant sources used for each experiment. In addition, 9-*O*-[(−)-kaur-15-en-17-oxyl]cubebin (**1**) was isolated as a new diterpeno−lignan hybrid, although it did not exhibit any antispasmodic activity.

Experimental Section

General Experimental Procedures. Melting points were determined on a micromelting point hot-stage apparatus (Yanagimoto) and are uncorrected. IR spectra were recorded on a JASCO IR-300 E spectrophotometer. Optical rotations were recorded on a JASCO DIP-360 polarimeter. EIMS were measured on a JEOL GC-Mate spectrometer. ^{1}H and ^{13}C NMR spectra were recorded with JEOL JNM ECP 400 and 600 spectrometers with TMS as an internal reference. For column chromatography (CC) and flash chromatography (FC), Si gel 60 (70−230 mesh ASTM; Merck) and Si gel 60 (230−400 mesh ASTM; Merck) were used, respectively, while for TLC and preparative TLC (p-TLC), Si gel 60 F254 (Merck) was used. A Biotage C18HS 12+M column was used for reversed-phase flash chromatography (RFC).

Plant Material. Stems of *A. constricta* were collected at Mocache, LosRios Province, in the coastal region of Ecuador in 2004. The plant material was verified by Prof. Enrique Torres Bolanos (Facultad de Ciencias Químicas, Universidad de Guayasquil, Ecuador). A voucher specimen (BETB-1) has been deposited at the herbarium of the Facultad de Ciencias Químicas, Universidad de Guayasquil, Ecuador.

Extraction and Isolation. Dried and powered plant material (413 g) was successively extracted using a Soxhlet apparatus with *n*-hexane, $CHCl_3$, and EtOH as solvents and, after evaporation of the solvent, yielded each extract. A part (295 mg) of the *n*-hexane extract (8.43 g) was subjected to CC followed by washing with *n*-hexane−Et_2O to afford (−)-cubebin (**2**) (21 mg). The $CHCl_3$ extract (7.83 g) was washed with *n*-hexane followed by washing of the insoluble fraction with Et_2O to give three fractions [*n*-hexane-soluble (Fr. A, 2.71 g), Et_2O-soluble (Fr. B, 2.44 g), and Et_2O-insoluble (1.56 g) fractions]. The Et_2O-insoluble fraction was further divided into two fractions by washing with MeOH [MeOH-soluble (Fr. C, 0.837 g) and MeOH-insoluble (Fr. D, 0.602 g) fractions]. These four fractions (Fr. A−D) were subjected to repeated separations using CC, FC, and p-TLC using various solvent systems, and the isolated components are listed in order of polarity as follows. Six components were isolated from Fr. A [cadalene (2 mg), (−)-kaurene (7 mg), (−)-9-[(−)-kaur-15-en-17-oxyl]cubebin (**1**) (5 mg), 9-*O*-methylcubebin (3 mg), (−)-kaur-16-en-19-oic acid (**8**) (170 mg), and (−)-kaur-15-en-17-ol (**3**) (68 mg)], 14 components from Fr. B [(−)-kaur-16-en-19-oic acid (**8**) (15 mg), (−)-hinokinin (**4**) (26 mg), (+)-4,7-dimethyl-6-methoxy-1-tetralone (**11**) (30 mg), (−)-kaur-15-en-17-ol (**3**) (22 mg), (−)-pluviatolide (**5**) (10 mg), aristelegone-A (2 mg), (−)-cubebin (**2**) (168 mg), cepharanone B (5 mg), (−)-dihydrocubebin (**7**) (8 mg), (−)-haplomyrfolol (**6**) (11 mg), (−)-kauran-16α,17-diol (**9**) (58 mg), aristololactam A II (6 mg), (−)-kauran-16α,17,18-triol (3 mg), and *N-trans*-feruloyltyramine (7 mg)], four components from Fr. C [aristolochic acid-I (**10**) (15 mg), aristololactam A II (4 mg), *N-trans*-feruloyltyramine (16 mg), and β-sitosterol glycoside (5 mg)], and one component [aristolochic acid-I (**10**) (433 mg)] from Fr. D.

(−)-9-[(−)-Kaur-15-en-17-oxyl]cubebin (1): colorless, amorphous gum; $[\alpha]^{24}_D$ −10.9 (*c* 0.11, $CHCl_3$); IR (ATR) ν_{max} 3440, 2921, 1737 cm^{-1}; ^{1}H and ^{13}C NMR, see Tables 2 and 3, respectively; EIMS, see Table 4.

(−)-Cubebin (2): yellow prisms by standing after the separation; mp 123−124 °C (lit.[3b] mp 125−128 °C); $[\alpha]^{21}_D$ −51.8 (*c* 0.86, $CHCl_3$) (lit.[3b] $[\alpha]^{25}_D$ −43.9 (*c* 0.5, $CHCl_3$)); IR (ATR) ν_{max} 3346 cm^{-1}; ^{1}H and ^{13}C NMR, see Tables 2 and 3, respectively; EIMS, see Table 4.

(−)-Kaur-15-en-17-ol (3): pale brown prisms by standing after the separation; mp 128−131 °C (lit.[9b] mp 134−136 °C); $[\alpha]^{23}_D$ −24.8 (*c* 0.40, $CHCl_3$) (lit.[9b] $[\alpha]^{17}_D$ −25.5 (*c* 0.5, $CHCl_3$)); IR (ATR) ν_{max} 3324 cm^{-1}; ^{1}H and ^{13}C NMR, see Tables 2 and 3, respectively; EIMS, see Table 4.

(−)-Hinokinin (4): yellow gum; $[\alpha]^{22}_D$ −31.1 (*c* 0.65, $CHCl_3$) (lit.[4b] $[\alpha]^{26}_D$ −30 (*c* 0.99, $CHCl_3$)); IR (ATR) ν_{max} 1767 cm^{-1}; ^{1}H NMR ($CDCl_3$, 400 MHz) δ 2.44−2.49 (2H, m, H-8′, H-7′a), 2.51−2.59 (2H, m, H-8, H-7′b), 2.84 (1H, dd, *J* = 14.0, 7.2 Hz, H-7a), 2.98 (1H, dd, *J* = 14.0, 5.2 Hz, H-7b), 3.86 (1H, dd, *J* = 9.4, 7.6 Hz, H-9′a), 4.13 (1H, dd, *J* = 9.4, 6.8 Hz, H-9′b), 5.93−5.94 (4H, m, OCH_2O), 6.45−6.49 (2H, m, H-6 or H-6′, H-2), 6.60 (1H, dd, *J* = 8.0, 1.6 Hz, H-6 or H-6′), 6.63 (1H, d, *J* = 1.6 Hz, H-2′), 6.70 (1H, d, *J* = 8.0 Hz, H-5), 6.73 (1H, d, *J* = 7.6 Hz, H-5′); ^{13}C NMR ($CDCl_3$, 100 MHz) δ 34.8 (C-7), 38.3 (C-7′), 41.3 (C-8′), 46.4 (C-8), 71.1 (C-9′), 100.97 (OCH_2O), 100.98 (OCH_2O), 108.2 (C-5), 108.3 (C-5′), 108.8 (C-2), 109.4 (C-5′), 121.5 (C-6), 122.2 (C-6′), 131.3 (C-1′), 131.6 (C-1), 146.3 (C-4 or C-4′), 146.4 (C-4 or C-4′), 147.8 (C-3 or C-3′), 147.9 (C-3 or C-3′), 178.4 (C-9); EIMS *m/z* 355 $[M + H]^+$ (9).

(−)-Pluviatolide (5): pale brown crystals by standing after the separation; mp 162 °C (lit.[5b] mp 160 °C); $[\alpha]^{24}_D$ −43.2 (*c* 0.06, $CHCl_3$) (lit.[5b] $[\alpha]_D$ −35.5 (*c* 1.00, $CHCl_3$)); IR (ATR) ν_{max} 3411, 1760 cm^{-1}; ^{1}H NMR ($CDCl_3$, 400 MHz) δ 2.45−2.50 (2H, m, H-7′a, H-8′), 2.53−2.57 (1H, m, H-8), 2.59−2.61 (1H, m, H-7′b), 2.90 (1H, dd, *J* = 14.0, 6.9 Hz, H-7a), 2.96 (1H, dd, *J* = 14.0, 5.2 Hz, H-7b), 3.84−3.87 (1H, m, H-9′a), 3.85 (3H, s, OCH_3), 4.11 (1H, dd, *J* = 9.0, 7.1 Hz, H-9′b), 5.53 (1H, s, OH), 5.93−5.94 (2H, m, OCH_2O), 6.45 (1H, s, H-2′), 6.46 (1H, d, *J* = 1.9 Hz, H-6′), 6.62 (1H, dd, *J* = 8.3, 1.9 Hz, H-6), 6.66 (1H, d, *J* = 1.9 Hz, H-2), 6.69 (1H, d, *J* = 7.7 Hz, H-5′), 6.83 (1H, d, *J* = 8.0 Hz, H-5); ^{13}C NMR ($CDCl_3$, 150 MHz) δ 34.6 (C-7), 38.3 (C-7′), 41.0 (C-8′), 46.6 (C-8), 55.9 (OCH_3), 71.2 (C-9′), 101.0 (OCH_2O), 108.1 (C-5′), 108.8 (C-2′), 111.5 (C-2), 114.2 (C-5), 122.1 (C-6), 121.5 (C-6′), 129.4 (C-1), 131.6 (C-1′), 144.5 (C-4), 146.3 (C-3), 146.7 (C-4′), 147.9 (C-3′), 178.7 (C-9); EIMS *m/z* 356 $[M]^+$ (24).

(−)-Kaur-16-en-19-oic acid (8): colorless prisms by standing after the separation; mp 164 °C (lit.[10a] mp 162−163 °C); $[\alpha]^{23}_D$ −99.6 (*c* 0.65, $CHCl_3$) (lit.[10b] $[\alpha]_D$ −110 (*c* 1.00, $CHCl_3$)); IR (ATR) ν_{max} 1689 cm^{-1}; ^{1}H NMR ($CDCl_3$, 400 MHz) δ 0.81 (1H, dt, *J* = 13.6, 4.0 Hz, H-1a), 0.95 (3H, s, H-20), 1.01 (1H, dt, *J* = 13.2, 4.4 Hz, H-3a), 1.03−1.09 (2H, m, H-5, H-9), 1.14 (1H, dd, *J* = 11.2, 4.8 Hz, H-14a), 1.24 (3H, s, H-18), 1.41−1.62 (7H, m, H-2, H-7, H-11a, H-12), 1.80−1.90 (4H, m, H-1b, H-6, H-11b), 1.99 (1H, d, *J* = 10.0 Hz, H-14b), 2.04−2.06 (2H, m, H-15), 2.16 (1H, d, *J* = 14.4 Hz, H-3b), 2.64 (1H, br s, H-13), 4.74 (1H, br s, H-17a), 4.80 (1H, br s, H-17b); ^{13}C NMR ($CDCl_3$, 100 MHz) δ 15.6 (C-20), 18.4 (C-11), 19.1 (C-2), 21.8 (C-6), 29.0 (C-18), 33.1 (C-12), 37.8 (C-3), 39.7 (C-10), 39.7 (C-14), 40.7 (C-1), 41.3 (C-7), 43.7 (C-4), 43.8 (C-13), 44.2 (C-8), 49.0 (C-15), 55.1 (C-9), 57.1 (C-5), 103.0 (C-17), 155.9 (C-16), 184.5 (C-19); EIMS *m/z* 303 $[M + H]^+$ (24).

Smooth Muscle Contraction Test Using Guinea-Pig Ileum. Male albino guinea pigs weighing 300−450 g were used for all experiments. Contraction of smooth muscle was measured in the ileal preparation as described previously.[43] The isolated ileum was placed in Krebs−Henseleit solution (mM): NaCl, 112.8; $NaHCO_3$, 25.00; glucose, 11.49; KCl, 5.90; $CaCl_2$, 1.97; NaH_2PO_4, 1.22; and $MgCl_2$, 1.18. The ileum was set up under 1 g tension in a 10 mL organ bath containing the nutrient solution. The bath was maintained at 38.5 °C and continuously bubbled with a gas mixture of 95% O_2 and 5% CO_2. After a 0.5 h equilibration period, the ileum was stimulated with a maximum response to ACh (3 μM, three times) to check its suitability. Contractions were isotonically recorded using an isotonic transducer (TD-112S, Nihon Koden, Tokyo, Japan), a balancing box (JD-112S, Nihon Koden, Tokyo, Japan), and a Powerlab system (AD Instruments, Castle Hill, Australia). After stable control contraction by ACh (3 μM), the isolated ileum was transmurally stimulated by platinum needle-ring electrodes using square-wave pulses of supramaximal voltage (38.6 V, monophasic pulses 0.2 Hz for 0.3 ms pulse duration) using a stimulator (SEN-3301, Nihon Koden, Tokyo, Japan) and a DC strain amplifier (SEG 3104, Nihon Koden, Tokyo, Japan). The experiments on ECI were performed after a stable twitch contraction was obtained by electrical stimulation in the absence of samples. The ileal responses were observed by cumulatively increased concentration of the samples (0.03 and 0.1 mg/mL for extracts, 10, 30, and 100 μM for compounds). The experiments on AChI were conducted in the presence of each concentration (a noncumulative manner) of samples (0.03 and 0.1 mg/mL for extracts, 10, 30, and 100 μM for compounds) after at least three stable contractions induced by ACh (3 μM). The samples were added 20 min before the addition of ACh. The height of the contraction induced by ACh was measured in the absence and in the presence of the samples. Contraction (%) was determined by dividing the contractile height under the sample-treated condition by that under the nontreated condition. The extract and the isolated pure compounds were dissolved in DMSO. Stock solutions were stored at −4 °C, and a fresh dilution was made daily in the nutrient solution. The final concentration of DMSO was less than 1%. All data are shown as the mean ± SEM of the results obtained from four or five animals. Statistical analyses were performed with a two-tailed paired *t* test for paired observations of two groups. A P value < 0.05 was considered statistically significant.

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