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Configurational analysis of cubebins and bicubebin from *Aristolochia lagesiana* and *Aristolochia pubescens*

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Abstract

(8*S*,8′*R*,9*S*)-, (8*R*,8′*R*,9*R*)-, and (8*R*,8′*R*,9*S*)-cubebins, together with (8*R*,8′*R*,8″*R*,8‴*R*,9*R*,9″*S*)-bicubebin, were isolated from *Aristolochia lagesiana* and *Aristolochia pubescens*. Their structures were determined by spectroscopic methods, including ^{1}H and ^{13}C NMR spectroscopy at low temperatures, and by chemical transformations.

Keywords: *Aristolochia pubescens*; *Aristolochia lagesiana*; Aristolochiaceae; Lignan; Cubebin; Butyrolactol; Bicubebin

1. Introduction

Previous studies on *Aristolochia pubescens* led to the isolation of 7 lignans, 5 neolignans, 2 sesquiterpenes, 2 diterpenes, 8 aristolochic acids and derivatives, and 3 aristolactams (Nascimento and Lopes, 1999, 2000, 2003; Nascimento et al., 2000). As part of our continuing studies on the Aristolochiaceae family, in this paper we report the isolation and structural elucidation of 8,8′-*cis*-cubebin (**1**), 8,8′-*trans*-cubebins (**2a** and **2b**) and 8,8′-*trans*-bicubebin A (**3**) from *A. pubescens* and *Aristolochia lagesiana*.

Cubebin is a dibenzylbutyrolactone lignan type, which is known to reduce larval viability in *Anticarsia gemmatalis* to give rise to malformed adult insects (Nascimento et al., 2004), to exhibit antifeedant activity for insect pests (Harmatha and Dinan, 2003), and to show moderate anti-inflammatory and analgesic activities (Silva et al., 2005). Cubebin has been isolated from several species in various families, such as Aristolochiaceae, Myristicaceae, Rutaceae, and Piperaceae (Lopes et al., 2001; Bastos et al., 2001; Koul et al., 1983; Blumenthal et al., 1997). Generally, cubebin, whether obtained from a natural source or synthesized, has been identified as a mixture of two 8,8′-*trans* diastereomers at C-9 (Koul et al., 1983; Blumenthal et al., 1997; Wei-Ming et al., 1987; Badheka et al., 1987; Chatterjee et al., 1968; Rehnberg and Magnusson, 1988, 1990; Ward, 1990; Koul et al., 1988). However, the configuration of this anomeric center is still unclear, as with other butyrolactol lignans (Brown and Daugan, 1989; Li et al., 2003; Gözler et al., 1996; Bhandari et al., 1998; Rücker and Langmann, 1978; Barrero et al., 1994; Cambie et al., 1985).

2. Results and discussion

Complex mixtures of cubebins were obtained from an ethanol extract of the tubercula of *A. pubescens* and an acetone extract from the roots of *A. lagesiana*. These mixtures were subjected to preparative TLC followed by semi-preparative HPLC to give **1**, **2** (**2a** + **2b**), and **3**.

The ^{1}H and ^{13}C NMR, UV, and IR data for compound **1** were very similar to those reported for cubebin (Koul et al., 1983; Blumenthal et al., 1997; Wei-Ming et al., 1987; Badheka et al., 1987). Compound **1** was suggested

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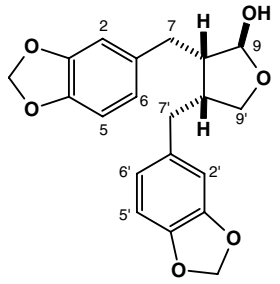

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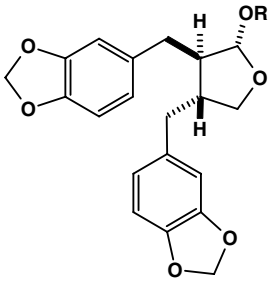
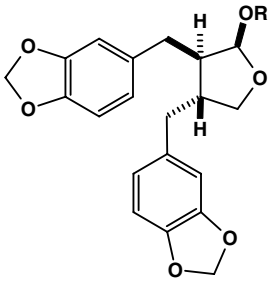
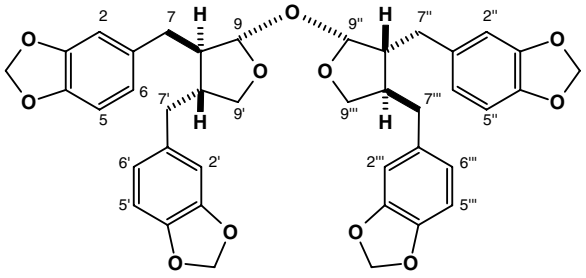


	R
2a	H
2c	CH_3



	R
2b	H
2d	CH_3



3

to be cubebin since it displayed *quasi*-molecular ions at *m*/*z* 379 $[M + Na]^+$ and *m*/*z* 357 $[M + H]^+$, which were consistent with the molecular formula $C_{20}H_{20}O_6$. The ^1H and ^{13}C NMR spectra showed signals for two methylenedioxybenzyl, two methynic (δ_C: 52.3, 45.7; δ_{2H}: 2.02), anomeric (δ_C: 104.3, δ_H: 5.04), and carbinolic (δ_C: 72.1, δ_H: 3.83, 3.32) groups (Tables 1 and 2). These latter comprise the butyrolactol ring. Analysis of ^1H–^1H COSY, *g*HMQC, and *g*HMBC data enabled the precise assignments of all hydrogens and carbons in the basic structure.

Compound **2** was also suggested to be a cubebin based on the similarity of its ESI-MS, UV, IR, ^1H and ^{13}C NMR spectra to those of **1**. The main differences between the ^1H and ^{13}C NMR spectra of **2** and **1** were signals for

Table 1
^{13}C NMR spectroscopic data for compounds **1**, **2a** and **2b** (30 °C, $CDCl_3$, 126 MHz)

C	1	2a	2b
1	133.5	133.3	133.8
2	109.2	108.9	108.9
3	147.6	147.6	147.7
4	145.8	145.7	145.7
5	108.1[a]	108.0	108.2[a]
6	121.8	121.4	121.3
7	38.4	38.4	33.6
8	52.3	53.0	51.9
9	104.3	103.3	98.8
1′	134.3	134.1	134.5
2′	108.9	109.1	109.3
3′	147.6	147.5	147.5
4′	145.8	145.9	145.9
5′	108.0[a]	108.0	108.1[a]
6′	121.4	121.7	121.6
7′	39.0	39.2	38.8
8′	45.7	45.8	42.9
9′	72.1	72.1	72.5
$2 \times OCH_2O$	100.8, 100.7	2×100.8	2×100.8

[a] Assignments may be interchangeable within the same column.

Table 2
^1H NMR spectroscopic data for compounds **1**, **2a** and **2b** (30 °C, $CDCl_3$, 500 MHz, *J* in Hz)

H	1	2a	2b
2	6.49 *d* (1.5)	6.45 *d* (1.5)	6.56 *d* (1.5)
5	6.61 *d* (7.5)	6.61 *d* (8.5)	6.66 *d* (8.0)
6	6.47 *dd* (7.5, 1.5)	6.44 *dd* (8.5, 1.5)	6.52 *dd* (8.0, 1.5)
7	2.33 *dd* (14.0, 8,0), 2.56 *dd* (14.0, 7.5)	2.37 *m*, 2.60 *dd* (13.5, 7.5)	2.37 *m*, 2.52 *m*
8	2.02 *m*	2.07 *m*	1.93 *m*
9	5.04 *d* (1.5)	5.15 *d* (1.5)	5.15 *d* (4.5)
2′	6.42 *d* (1.5)	6.51 *d* (1.5)	6.67 *d* (2.0)
5′	6.61 *d* (7.5)	6.63 *d* (7.5)	6.66 *d* (8.0)
6′	6.41 *dd* (7.5, 1.5)	6.49 *dd* (7.5, 1.5)	6.62 *dd* (8.0, 2.0)
7′	2.44 *m*	2.50 *m*, 2.52 *m*	2.50 *m*, 2.70 *dd* (14.0, 10.0)
8′	2.02 *m*	2.07 *m*	2.37 *m*
9′	3.32 *t* (8.5), 3.83 *dd* (8.5, 7.0)	3.73 *dd* (8.5, 8.0), 3.93 *dd* (8.5, 7.0)	3.50 *dd* (8.5, 7.5), 4.03 *t* (8.5)
OCH_2O	5.85 *d* ($W_{1/2}$ 1.5), 5.84 *d* ($W_{1/2}$ 1.5), 5.82 *d* ($W_{1/2}$ 1.5), 5.81 *d* ($W_{1/2}$ 1.5)	4×5.85 *d* ($W_{1/2}$ 1.5)	4×5.85 *d* ($W_{1/2}$ 1.5)
OH	2.02 *br s*	1.72 *br s*	2.86 *br s*

two sets of: methylenedioxybenzyl, two methynic, anomeric, and carbinolic groups (Tables 1 and 2). Based on integration of the signals observed in the ^{1}H NMR spectrum and on the intensity of the signals in the ^{13}C NMR spectrum (obtained at 30 °C), as well as on the observed correlations between hydrogens by ^{1}H–^{1}H COSY, and between hydrogens and carbons by *g*HMQC and *g*HMBC, it was determined that compound **2** consisted of two cubebins in a 3:2 proportion (**2a**:**2b**). Interestingly, the anomeric hydrogens of **2a** and **2b** showed the same chemical shift at δ 5.15, but different coupling constants (*d*, $J = 1.5$ and 4.5 Hz) at 30 °C, whereas the anomeric carbons showed signals at δ 103.3 and 98.8 for **2a** and **2b**, respectively. ^{1}H–^{1}H COSY experiments showed strong correlations between all four carbinolic hydrogens ($\delta_{H\text{-}9'}$: 4.03, 3.93, 3.73, and 3.50). These data suggested two hypotheses: there are two epimers (at C-9 and/or C-8) in equilibrium, or the cubebin species exist in conformational equilibrium.

Lactols can undergo ring-opening and -closing in solution. This phenomenon has been observed, for example, in carbohydrates (Pihlaja and Kleinpeter, 1994), trityloxymethyl butyrolactols (Li et al., 2004), and butyrolactol lignans (Wei-Ming et al., 1987). Therefore, dynamic molecular processes were investigated using NMR techniques. Under appropriate NMR conditions (temperatures above 26 °C, and long periods of storage in $CDCl_3$ solution), the resulting interconversion, **2a** $\rightleftharpoons$ **2b**, was manifested through the presence of additional signals at δ_H: 8.74 and δ_C: 203.0, which are characteristic of an aldehyde group, among others signals. The effects of temperature on the chemical shifts and the correlations between hydrogens of compounds **1** and **2** were also investigated. The ^{1}H NMR experiments, including 1D-*g*NOESY and ^{1}H–^{1}H COSY, were performed from 30, 26, 20 to 10, 0, −10 −20, −30, −40, and −50 °C, while keeping other conditions (solvent, concentration, pulse sequence, and recording conditions) constant.

While the change in temperature did not significantly affect the hydrogen or carbon chemical shifts of both compounds, this did aid in the unequivocal assignment of the structures, since some regions of the spectra such as the aromatic and anomeric regions were less crowded at lower temperature. Moreover, these $\Delta\delta$ allowed to determine the multiplicities and chemical shifts for the anomeric hydrogens of each species of compound **2** (Tables 1–3). Furthermore, the simulated ^{1}H NMR spectra by using the FOMSC3 program (FOMSC3, 2004) for first order coupling hydrogens of **2a** and **2b** compounds were in total agreement with the experimental spectra.

1D-*g*NOESY experiments with compound **1** showed interactions between the carbinolic hydrogen H-9′b1 (at lower frequency, δ 3.32) and H-9′b2 (at higher frequency, δ 3.83), as well as between H-9′b1 and H-2′ (δ 6.42), H-6′ (δ 6.41), 2H-7′ (δ 2.44), H-9 (δ 5.04), and H-8 and/or H-8′ (δ 2.02), whereas H-9′b2 was spatially correlated with H-9′b1 and with the methynic(s) hydrogen(s) at δ 2.02. H-9 did not show any correlation with the methynic hydrogens. These data suggested a *syn* orientation of H-9′b1 and H-9, as well as a *syn* orientation of H-9′b2, H-8′, and H-8 (Fig. 1).

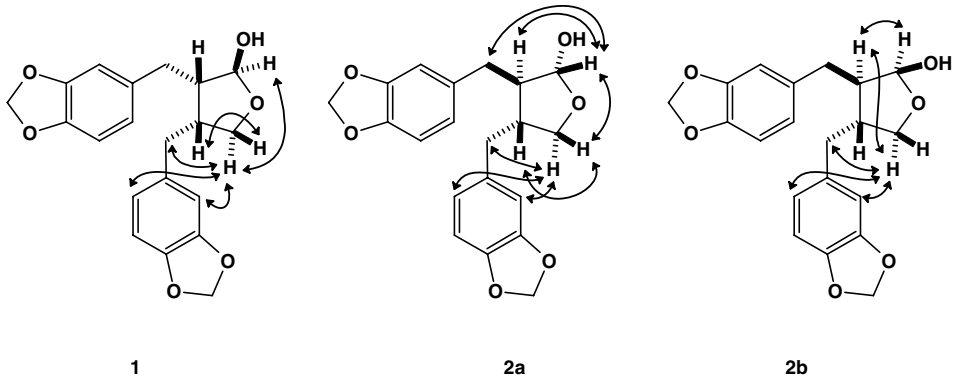
1D-*g*NOESY experiments with compound **2** showed spatial interactions between the carbinolic hydrogens H-9′b1 (**2a**: δ 3.73, **2b**: δ 3.50) and H-9′b2 (**2a**: δ 3.93, **2b**: δ 4.03), and between H-9′b1 and H-2′ (**2a**: δ 6.51, **2b**: δ 6.67), H-6′ (**2a**: δ 6.49, **2b**: δ 6.62), and 2H-7′ (**2a**: δ 2.50, 2.52, **2b**: δ 2.50, 2.70). Furthermore, the anomeric hydrogen of **2b** showed a strong correlation with H-8, and the latter gave a strong correlation with H-9′b1, which suggested that they were in a *syn* orientation, with the hydroxyl group *cis* to the methylenedioxybenzyl group at C-8 (Fig. 1). This suggestion is consistent with the upfield chemical shifts of C-7 (δ 33.6) due to the γ effect of the hydroxyl group. Correlations between H-9 and H-8, 2H-7, and H-9′b2 were observed for **2a**. These correlations suggested that H-9 was in an "equatorial" position, and in a *syn* orientation with H-9′b2 (Fig. 1).

The substituents on the lactol rings could favor the possibility that these molecules exist as a single conformation in solution (Lightner and Gurst, 2000; Lambert et al., 1974). To determine the most stable conformation, the relative configuration, and the absolute configuration for these compounds, they were transformed into hinokinins, the absolute configurations of which have been well established (Lopes et al., 1983; Batterbee et al., 1969; Burden et al., 1969). While no oxidation product was obtained from compound **1**, only one oxidation product was obtained from compound **2** under the same experimental conditions. The CD curve and α_D (−28.9°) for the oxidized

Table 3
Selected ^{1}H and ^{13}C NMR spectroscopic data for compounds **1**–**3** at −10 °C

H and C	**1**		**2a**		**2b**		**3**	
	δ_H[a]	δ_C[b]	δ_H[a]	δ_C[b]	δ_H[a]	δ_C[b]	δ_H[a]	δ_C[b]
9	5.06 *d* (1.0)	103.9	5.18 *d* (1.0)	103.1	5.17 *d* (4.0)	98.6	4.76 *d* (1.5)	108.0
9′	3.28 *t* (8.5), 3.85 *dd* (8.5, 7.0)	72.1	3.74 *dd* (8.5, 8.0), 3.95 *dd* (8.5, 7.0)	72.0	3.52 *dd* (8.5, 7.5), 4.01 *t* (8.5)	72.6	3.63 *t* (8.0), 3.92 *dd* (8.0, 7.0)	72.5
9″							4.80 *d* (4.5)	104.4
9‴							3.56 *dd* (8.5, 7.0), 4.03 *dd* (8.5, 8.0)	72.5

[a] Recorded in $CDCl_3$, 500 MHz, *J* in Hz.
[b] Recorded in $CDCl_3$, 126 MHz.



1 2a 2b

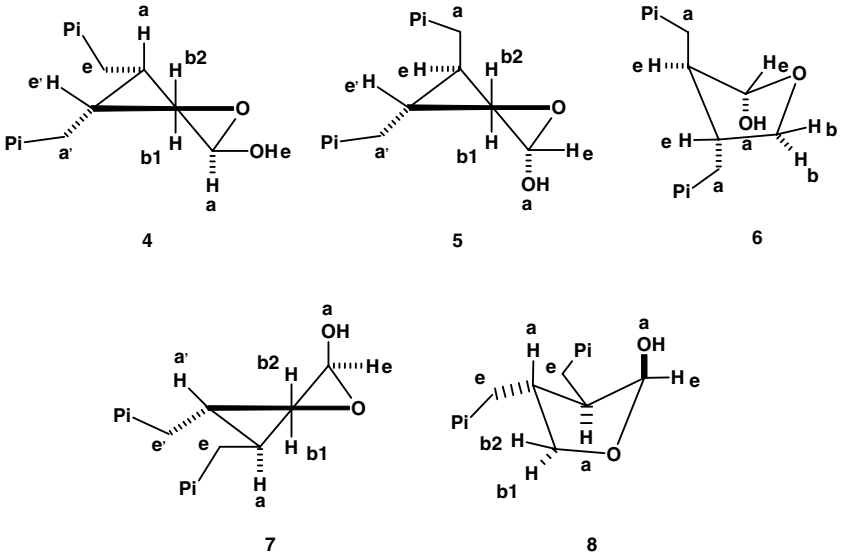
Fig. 1. Selected nOe interactions observed for compounds **1**, **2a** and **2b**.

product allowed it to be identified as (−)-hinokinin, which has an 8*R*,8′*R* configuration. Therefore, the anomers **2a** and **2b** should have the same configuration at the corresponding stereogenic centers (8*R*,8′*R*).

Even though the problems posed to establish the relative and absolute configurations of saturated hetero five-membered rings are more complex than those for six-membered rings (Pihlaja and Kleinpeter, 1994; Lightner and Gurst, 2000; Lambert et al., 1974), the coupling constants between the carbinolic hydrogens (H-9′) and between these and H-8′ were the same magnitude ($J \cong 8$ Hz) for **1**, **2a**, and **2b**, which suggested that they have the same spatial arrangements, and the torsion angles that involve C-8′ and C-9′ are very similar in all three molecules. Considering the two possible conformations for the lactol ring in the three cubebins, half-chair and envelope, and all data previously discussed, the conformations that better represented these molecules should be **4** for **1**, **5** and **6** for **2a**, and **7** and **8** for **2b**, in which the carbinolic hydrogens are bisected (Fig. 2). The CD curves of **1** and **2** were very similar, which suggested that the Cotton effects observed at 290 and 230 nm depended on the stereogenic center C-8′ (Fig. 3).

Based on the above spectroscopic findings, the cubebins were characterized as (8*S*,8′*R*,9*S*)-cubebin (**1**), (8*R*,8′*R*,9*R*)-cubebin (**2a**), and (8*R*,8′*R*,9*S*)-cubebin (**2b**), which is consistent with most of the configurations previously established for cubebin **2a** (α-cubebin or *epi*-cubebin) and **2b** (β-cubebin or cubebin) (Wei-Ming et al., 1987; Badheka et al., 1987; Rücker et al., 1981). Furthermore, two acetals were obtained from the anomers **2a** and **2b**, and they were, respectively, identified as α-methylcubebin (**2c**) and β-methylcubebin (**2d**) (Blumenthal et al., 1997; Rücker et al., 1981).

The ^{1}H and ^{13}C NMR spectra and *g*HMQC experiments showed a total of 40 carbons and 38 hydrogens for lignan **3**. The ESI-MS spectra displayed *quasi*-molecular ions at *m*/*z* 717 $[M + Na]^+$ and 695 $[M + H]^+$, which were consistent with the molecular formula $C_{40}H_{38}O_{11}$ and the results of an elemental analysis. This formula corresponded to dehydration of two cubebin units. The NMR spectra were also very similar to those of cubebins discussed previously



4 5 6 7 8

Fig. 2. Conformations for **1** (**4**), **2a** (**5** and **6**) and **2b** (**7** and **8**), showing axial (a), pseudoaxial (a′), equatorial (e), pseudoequatorial (e′), and bisected (b) exocyclic positions.

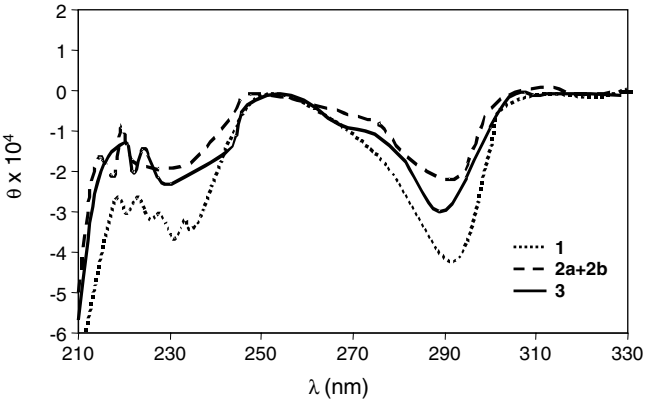


Fig. 3. CD curves for lignans **1–3**.

(Table 4). Indeed, they showed signals for two sets of: two methylenedioxybenzyl groups, one anomeric group, one carbinolic group, and two methynic groups, as previously observed for **2**. Furthermore, a detailed analysis of *g*HMBC, 1D-*g*NOESY, and ^{1}H–^{1}H COSY experiments allowed us to establish two cubebin units (Table 4 and Fig. 3). ^{1}H selective irradiation NMR experiments also aided in determining the *J* values and chemical shifts ascribed for first order coupling hydrogens, which were further confirmed by spectral simulations using the FOMSC3 program (FOMSC3, 2004). The main differences between these spectra from those of **2** were that the proportion of the cubebins units was 1:1 instead of 3:2, and the absence of signals for hydroxyl hydrogens. Furthermore, ^{1}H–^{1}H COSY experiments did not show any correlations between the carbinolic hydrogens of one unit with those of the other unit (2H-9′ with 2H-9‴), even at low temperatures. However, nOes were observed between the anomeric hydrogen from each unit. Moreover, *g*HMBC experiments showed correlations between the anomeric carbon from one unit with anomeric hydrogen from the other unit (δ_C: 108.6 with δ_H: 4.81, and δ_C: 104.2 with δ_H: 4.76), which suggested that the units were linked through C-9 → O → C-9″ (Fig. 4). This suggestion was further supported by analysis of the chemical shifts of C-9 and C-9″. Considering that the replacement of a hydroxyl group in lactols by an ether group caused $\Delta\delta \cong +6$ ppm for the anomeric carbon (Blumenthal et al., 1997), as observed for **2a** and **2c**, as well as for **2b** and **2d**, it could be deduced that the unit containing C-9 should be similar to **2a** and that containing C-9″ should be similar to **2b**. This deduction was corroborated by a comparison of all NMR spectroscopic data obtained from **3** with those obtained from **2**, including those obtained by 1D-*g*NOESY experiments (Figs. 1 and 4). Based on an analysis of all these data, including the coupling constants observed for anomeric hydrogens, as well as the spatial interactions between the hydrogens and the substituents on the lactol ring, as previously described for **1**, **2a**, and **2b**, the most stable conformations and the relative configurations could be established as being **5** and/or **6** for unity I and **7** and/or **8** for unity II for **3**. Taking into account that compound **3** was isolated together with **2** and that they showed very similar CD curves, the structure of **3** was determined to be (8*R*,8′*R*,8″*R*,8‴*R*,9*R*,9″*S*)-cubebin (named bicubebin A). Moreover, bicubebin A did not give acetals or hinokinins when subjected, respectively, to etherification and oxidation under the same experimental conditions as compound **2**, which is consistent with the proposed structure.

Table 4
^{13}C and ^{1}H NMR spectroscopic data for compound **3**

C and H	δ ^{13}C[a,b,c]	δ ^{1}H[c,d,e]	1D-gNOESY
1	133.4		
2	109.0	6.46 *d* (1.5)	
3	147.7		
4	145.8		
5	108.1	6.60 *d* (8.0)	
6	121.7	6.40 *dd* (8.0, 1.5)	
7	38.5	2.32 *dd* (13.5, 8.0), 2.47 *dd* (13.5, 7.5)	
8	52.7	2.11 *dddd* (8.0, 7.5, 6.0, 2.0)	
9	108.6	4.76 *d* (2.0)	H-7, H-8, H-9″
1′	134.6		
2′	109.0	6.43 *d* (1.5)	
3′	147.6		
4′	146.0		
5′	108.2	6.58 *d* (8.0)	
6′	121.4[b]	6.42 *dd* (8.0, 1.5)	
7′	39.4	2.51 *d* (8.0, 2H)	
8′	45.4	2.04 *dddt* (8.5, 7.5, 6.0, 8.0)	
9′	72.4	3.92 *t* (8.5), 3.62 *dd* (8.5, 7.5)	H-8′, H-9′, H-2′, H-7′, H-8, H-9′
1″	134.1		
2″	109.1	6.51 *d* (2.0)	
3″	147.7		
4″	145.7		
5″	108.1	6.60 *d* (8.5)	
6″	121.3[b]	6.44 *dd* (8.5, 2.0)	
7″	33.9	2.49 *dd* (13.5, 8.5), 2.43 *dd* (13.5, 6.5)	
8″	51.7	1.95 *tdd* (8.5, 6.5, 5.0)	
9″	104.2	4.81 *d* (5.0)	H-8″, H-9
1‴	134.2	6.54 *d* (2.0)	
2‴	109.0		
3‴	147.6		
4‴	145.8		
5‴	108.2	6.64 *d* (8.0)	
6‴	121.5	6.50 *dd* (8.0, 2.0)	
7‴	39.4	2.36 *dd* (13.5, 9.5), 2.60 *dd* (13.5, 5.0)	
8‴	43.6	2.27 *dddt* (9.5, 7.0, 5.0, 8.5)	
9‴	72.4	3.51 *dd* (8.5, 7.0), 4.00 *t* (8.5)	H-2‴, H-6‴, H-7‴, H-8″, H-9‴, H-8‴, H-9‴
OCH_2O	4 × 100.8	5.77 *d* ($W_{1/2}$ 1.5), 5.82 *d* ($W_{1/2}$ 1.5), 6 × 5.85 *br s*	

[a] Recorded in $CDCl_3$, 126 MHz (30 °C).
[b] Assignments may be interchangeable within the same column.
[c] The ^{1}H and ^{13}C NMR data were assigned with the assistance of *g*HMQC and *g*HMBC experiments.
[d] Recorded in $CDCl_3$, 500 MHz, *J* in Hz (30 °C).
[e] Multiplicities were determined with the assistance of ^{1}H–^{1}H COSY and TOCSY, 500 MHz.

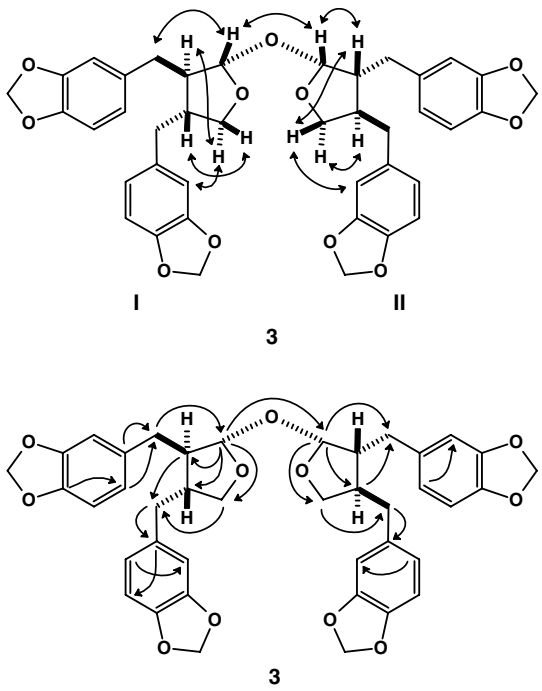


Fig. 4. (a) Selected nOe interactions (↔), (b) selected *g*HMBC correlations (→) for compound **3**.

3. Conclusion

The ring-opening of cubebins **2** in $CDCl_3$ solution, leading to inversion at the stereogenic center where the hydroxyl group is located, was confirmed by detection of the intermediary aldehyde by NMR. The equilibrium between the anomers **2a** and **2b** from 30 to −50 °C was on the same time-scale as the observation of signals by NMR spectroscopy. Even though it was not possible to establish whether both anomers (**2a** or **2b**) are natural compounds, this paper reports their absolute configurations. Furthermore, an 8,8′-*cis*-cubebin and a bicubebin A were described for the first time.

4. Experimental

4.1. General

The 1D (^1H, ^{13}C, DEPT, and *g*NOESY) and 2D (^1H–^1H *g*COSY, *g*HMQC, *g*HMBC, and *g*TOCSY) NMR experiments were recorded on a Varian INOVA 500 spectrometer (11.7 T) at 500 MHz (^1H) and 126 MHz (^{13}C), using the solvents as an internal standard. Mass spectra (ESI-MS) were obtained on a Fisons Platform II, and flow injection into the electrospray source was used. IR spectra were obtained on a Perkin–Elmer 1600 FT-IR spectrometer using KBr discs. UV absorptions were measured on a Perkin–Elmer UV–Vis Lambda 14P spectrophotometer. Optical rotations were measured on a Polamat A Carl Zeiss Jena polarimeter. Circular dichroism spectra were recorded on a JASCO J-720 spectrometer. HPLC analyses were carried out using a Shimadzu liquid chromatograph 10Avp equipped with a UV–Vis detector. Columns were RP-18 (Shimadzu, C_{18}, 250 × 4.6 mm for analytical analysis and 250 × 20 mm for semi-preparative analysis), and chromatograms were acquired at 254 nm. TLC: Silica gel 60 PF_{254}.

4.2. Plant material

Plant materials were collected in Ituiutaba, SP, Brazil, and identified as *A. pubescens* Will. ex Duch., and *A. lagesiana* Ule. var. intermedia Hoehne by Dr. Condorcet Aranha and Dr. Lindolpho Cappellari Júnior. Vouchers specimens of *A. lagesiana* (ESA 88885 02/13/2003) and *A. pubescens* (ESA 88882 01/20/1998) were deposited at the herbarium of the Escola Superior de Agricultura, Luiz de Queiroz (ESALQ), Piracicaba, SP, Brazil. The materials were separated by plant parts, dried (∼45 °C) and ground.

4.3. Extraction and isolation

Ground roots (245.6 g) of *A. lagesiana* and tubercula (728.1 g) of *A. pubescens* were extracted exhaustively at room temperature with hexane, Me_2CO and EtOH, successively, and the extracts were then individually concentrated. Some of the crude acetone extract of the roots of *A. lagesiana* (2.3 g) was washed with hexane, Me_2CO and MeOH, successively. The Me_2CO fraction (0.5 g) was subjected to preparative TLC (hexane/EtOAc, 7:3) to give a mixture of cubebins (**1** + **2a** + **2b** + **3**, 38.2 mg). This mixture was subjected to preparative TLC (hexane–EtOAc 7:3) followed by semi-preparative HPLC (C-18, MeOH/H_2O 4:1) to give **1** (18.4 mg), **2a** + **2b** (4.8 mg), and **3** (7.7 mg). The crude ethanol extract of the tubercula of *A. pubescens* (13.4 g) was washed with hot EtOH as described previously (Nascimento and Lopes, 2003). The solution was concentrated and subjected to partitioning between MeOH/H_2O/$CHCl_3$ (1:1:1). After concentration, the organic phase was subjected to CC (silica gel, $CHCl_3$/MeOH gradient) to give 33 fractions (Nascimento and Lopes, 2003). Fraction 6 (23 mg) after preparative TLC (hexane–EtOAc 7:3) gave three sub-fractions that by semi-preparative HPLC (C-18, MeOH/H_2O 4:1) gave **1** (8.4 mg), **2a** + **2b** (2.8 mg), and **3** (3.9 mg).

4.4. (8*S*,8′*R*,9*S*)-cubebin (*1*)

White crystal; $[\alpha]_D^{26}$ −102.3 (*c* 0.64, $CHCl_3$); CD (*c* 0.1, CH_3OH): $[\theta]_{223}$ −9569, $[\theta]_{230}$ −12,769, $[\theta]_{254}$ −455, $[\theta]_{270}$ −4124, $[\theta]_{292}$ −15,145, $[\theta]_{306}$ −908. UV and IR data agree with those reported in the literature (Koul et al., 1983; Blumenthal et al., 1997; Wei-Ming et al., 1987; Badheka et al., 1987). For ^1H and ^{13}C NMR spectra, see Tables 1 and 2; positive ESI-MS (probe) 70 eV, *m*/*z* (rel. int.): 379 $[M + Na]^+$ (100), 357 $[M + H]^+$ (72). Found: C, 67.3; H, 5.9. $C_{20}H_{20}O_6$ requires: C, 67.4; H, 5.7%.

4.5. (8R,8′R,9R)- and (8R,8′R,9S)-cubebin (2a + 2b)

White crystal; $[\alpha]_D^{26}$ −53.8 (*c* 2.58, $CHCl_3$); CD (*c* 0.1, CH_3OH): $[\theta]_{219}$ −3476, $[\theta]_{229}$ −7267, $[\theta]_{254}$ +178, $[\theta]_{269}$ −2939, $[\theta]_{272}$ −2498, $[\theta]_{290}$ −8157, $[\theta]_{304}$ −526. UV and IR data agree with those reported in the literature (Koul et al., 1983; Blumenthal et al., 1997; Wei-Ming et al., 1987; Badheka et al., 1987). For ^1H and ^{13}C NMR spectra, see Tables 1 and 2; positive ESI-MS (probe) 70 eV, *m/z* (rel. int.): 379 $[M + Na]^+$ (100), 357 $[M + H]^+$ (70). Found: C, 67.4; H, 5.8. $C_{20}H_{20}O_6$ requires: C, 67.4; H, 5.7%.

4.6. (8R,8′R,9R)- and (8R,8′R,9S)-methylcubebin (2c + 2d)

To dry DMSO (400 μL) was added KOH (11.3 mg). After stirring for 20 min, a solution of **2a** + **2b** (4.3 mg, 0.012 mmol) in DMSO (600 μL) was added, followed immediately by 10 μL of CH_3I (0.032 mmol) at room temperature. Stirring was continued for 1 h, and the mixture was then poured into H_2O (10 mL) and extracted with CH_2Cl_2 (3 × 10 mL). The combined organic phases were washed with H_2O (10 mL), dried (Na_2SO_4) and concentrated (Johnstone and Rose, 1979). Purification of the product by preparative TLC (hexane/EtOAc, 7:3) yielded **2c** + **2d** (1.8 mg, 0.005 mmol). ^1H and ^{13}C NMR, UV and IR data agree with those reported in the literature (Blumenthal et al., 1997; Rücker et al., 1981).

4.7. (8R,8′R,8″R,8‴R,9R,9″S)-bicubebin A (3)

White amorphous solid; $[\alpha]_D^{26}$ −54.8 (*c* 0.36, $CHCl_3$); CD (*c* 0.1, CH_3OH): $[\theta]_{220}$ −9508, $[\theta]_{231}$ −16,260, $[\theta]_{253}$ + 91, $[\theta]_{270}$ −6742, $[\theta]_{273}$ −6104, $[\theta]_{290}$ −20,587, $[\theta]_{306}$ −451. For ^1H and ^{13}C NMR spectra, see Table 4; positive ESI-MS (probe) 70 eV, *m/z* (rel. int.): 717 $[M + Na]^+$ (78), 695 $[M + H]^+$ (100). Found: C, 68.9; H, 5.8. $C_{40}H_{38}O_{11}$ requires: C, 69.2; H, 5.5%.

4.8. (−)-Hinokinin

Chromium trioxide (40.0 mg, 4.0 mmol) was added to a magnetically stirred solution of dry pyridine (54.2 μL) in CH_2Cl_2 (800 μL). The mixture was stirred for 15 min at room temperature. At the end of this period, a solution of **2a** + **2b** (20.0 mg, 0.056 mmol) in 900 μL of CH_2Cl_2 was added in one portion. After stirring for 4 h, the solution was decanted from the residue, which was washed with Et_2O (Ratcliffe and Rodehorst, 1970). The combined organic solutions were dried and the crude product was purified by preparative TLC (hexane/EtOAc, 7:3) to give hinokinin (13.6 mg, 0.038 mmol): $[\alpha]_D^{26}$ −28.9° (*c* 0.31, $CHCl_3$); ^1H and ^{13}C NMR, UV, and IR data agree with those reported in the literature (Lopes et al., 1983).

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References

Badheka, L.P., Prabhu, B.R., Mulchandani, N.B., 1987. Lignans of *Piper cubeba*. Phytochemistry 26, 2033–2036.

Barrero, A.F., Haïdour, A., Dorado, M.M., Grávalos, D.G., Quesada, T.G., 1994. Lignans from the wood of *Abies pinsapo*. J. Nat. Prod. 57, 713–719.

Bastos, J.K., Carvalho, J.C.T., Souza, G.H.B. de, Pedrazzi, A.H.P., Sarti, S.J., 2001. Anti-inflammatory activity of cubebin, a lignan from the leaves of *Zanthoxyllum naranjillo* Griseb. J. Ethnopharm. 75, 279–282.

Batterbee, J.E., Burden, R.S., Crombie, L., Whiting, D.A., 1969. Chemistry and synthesis of the lignan (−)-cubebin. J. Chem. Soc. (C), 2470–2477.

Bhandari, S.P.S., Babu, U.V., Garg, H.S., 1998. A lignan from *Piper chaba* stems. Phytochemistry 47, 1435–1436.

Blumenthal, E.E. de A., Silva, M.S. da, Yoshida, M., 1997. Lignoids, flavonoids and polyketides of *Virola surinamensis*. Phytochemistry 46, 745–749.

Brown, E., Daugan, A., 1989. Lignanes. 10. Preparation des (*R*) et (*S*)-(−)-β-piperonyl et β-veratryl-γ-butyrolactones et leur utilization dans la synthese totale de lignanes optiquement actifs. Tetrahedron 45, 141–154.

Burden, R.S., Crombie, L., Whiting, D.A., 1969. The extractives of *Heliopsis scabra*: constitution of two new lignans. J. Chem. Soc. (C), 693–701.

Cambie, R.C., Clark, G.R., Craw, P.A., Jones, T.C., Rutledge, P.S., Woodgate, P.D., 1985. Chemistry of the Podocarpaceae. LXIX. Further lignans from the wood of *Dacrydium intermedium*. Aust. J. Chem. 38, 1631–1645.

Chatterjee, A., Basa, S.C., Ray, A.B., 1968. Spectral properties of cubebin. J. Indian Chem. Soc. 45, 723–725.

FOMSC3 (First Order Multiplet Simulator/Checker), 2004. Available from: <http://artemis.ffclrp.usp.br/Software_P>.

Gözler, B., Rentsch, D., Gözler, T., Ünver, N., Hesse, M., 1996. Lignans, alkaloids and coumarins from *Haplophyllum vulcanicum*. Phytochemistry 42, 695–699.

Harmatha, J., Dinan, L., 2003. Biological activities of lignans and stilbenoids associated with plant-insect chemical interactions. Phytochem. Rev. 2, 321–330.

Johnstone, R.A.W., Rose, M.E., 1979. A rapid, simple, and mild procedure for alkylation of phenols, alcohols, amides and acids. Tetrahedron 35, 2169–2173.

Koul, S.K., Taneja, S.C., Dhar, K.L., Atal, C.K., 1983. Lignans of *Piper clusii*. Phytochemistry 22, 999–1000.

Koul, S.K., Taneja, S.C., Pushpangadan, P., Dhar, K.L., 1988. Lignans of *Piper trichostachyon*. Phytochemistry 27, 1479–1482.

Lambert, J.B., Papay, J.J., Khan, S.A., Kappauf, K.A., Magyar, E.S., 1974. Conformational analysis of five-membered rings. J. Am. Chem. Soc. 96, 6112–6118.

Li, L., Thompson, R., Sowa Jr., J.R., Clausen, A., Dowling, T., 2004. Kinetic study on the epimerization of trityloxymethyl butyrolactol by liquid chromatography. J. Chromatogr. A 1043, 171–175.

Li, N., Wu, J.-L., Sakai, J.-I., Ando, M., 2003. Dibenzylbutyrolactone and dibenzylbutanediol lignans from *Peperomia duclouxii*. J. Nat. Prod. 66, 1421–1426.

Lightner, D.A., Gurst, J.E., 2000. Organic Conformational Analysis and Stereochemistry from Circular Dichroism Spectroscopy. Wiley-VCH, New York.

Lopes, L.M.X., Nascimento, I.R., Silva, T. da, 2001. Phytochemistry of the Aristolochiaceae family. In: Mohan, R.M.M. (Ed.), Research Advances in Phytochemistry, vol. 2. Global Research Network, Kerala, pp. 19–108.

Lopes, L.M.X., Yoshida, M., Gottlieb, O.R., 1983. Dibenzylbutyrolactone lignans from *Virola sebifera*. Phytochemistry 22, 1516–1518.

Nascimento, I.R., Lopes, L.M.X., 1999. 2,3-Dihydrobenzofuran neolignans from *Aristolochia pubescens*. Phytochemistry 52, 345–350.

Nascimento, I.R., Lopes, L.M.X., 2000. 2,3-Dihydrobenzofuran neolignans from *Aristolochia pubescens*. Phytochemistry 53, 621.

Nascimento, I.R., Lopes, L.M.X., 2003. Diterpene esters of aristolochic acids from *Aristolochia pubescens*. Phytochemistry 63, 953–957.

Nascimento, I.R., Lopes, L.M.X., Davin, L.B., Lewis, N.G., 2000. Stereoselective synthesis of 8,9-lidarinediols. Tetrahedron 56, 9181–9193.

Nascimento, I.R., Murata, A.T., Bortoli, S.A., Lopes, L.M.X., 2004. Insecticidal activity of chemical constituents from *Aristolochia pubescens* against *Anticarsia gemmatalis* larvae. Pest Manag. Sci. 60, 413–416.

Pihlaja, K., Kleinpeter, E., 1994. Carbon-13 NMR Chemical Shifts in Structural and Stereochemical Analysis. Wiley-VCH, New York.

Ratcliffe, R., Rodehorst, R., 1970. Improved procedure for oxidations with the chromium trioxide-pyridine complex. J. Org. Chem. 35, 4000–4002.

Rehnberg, N., Magnusson, G., 1988. Total synthesis of the lignans (−)- and (+)-burseran, (−)-cubebin, and (−)-hinokinin by diastereoselective conjugate addition of benzyl anions to 2-(*R*)- and (*S*)-benzyloxy-2,5-dihydro-4-(3,4-methylenedioxybenzoyl)furan. Tetrahedron Lett. 29, 3599–3602.

Rehnberg, N., Magnusson, G., 1990. General conjugate-addition method for the synthesis of enantiomerically pure lignans. Total synthesis of (−)- and (+)-burseran, (−)-dehydroxycubebin, (−)-trichostin, (−)-cubebin, (−)-5″-methoxyhinokinin, and (−)-hinokinin. J. Org. Chem. 55, 4340–4349.

Rücker, G., Langmann, B., 1978. 3,4-Dimethoxy-3,4-desmethylendioxycubebin, ein neues lignan aus *Aristolochia triangularis*. Tetrahedron Lett. 5, 457–458.

Rücker, G., Langmann, B., Siqueira, N.S. de, 1981. Inhaltsstoffe von *Aristolochia triangularis*. Planta Med. 41, 143–149.

Silva, R. da, Souza, G.H.B. de, Silva, A.A. da, Souza, V.A. de, Pereira, A.C., Royo, V. de A., Silva, M.L.A. de, Donate, P.M., Araújo, A.L.S. de M., Carvalho, J.C.T., Bastos, J.K., 2005. Synthesis and biological activity evaluation of lignan lactones derived from (−)-cubebin. Bioorg. Med. Chem. Lett. 15, 1033–1037.

Ward, R.S., 1990. Asymmetric synthesis of lignans. Tetrahedron 46, 5029–5041.

Wei-Ming, C., Mayer, R., Rücker, G., 1987. Orientierung der hydroxylgruppe des (−)-cubebins und seines epimeren. Arch. Pharm. 320, 374–376.