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Aryltetralol and aryltetralone lignans from *Holostylis reniformis*

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Abstract

Aryltetralol and aryltetralone lignans were isolated from the hexane extracts of the roots of *Holostylis reniformis*. Their structures were determined by spectroscopic methods and chemical transformations.

Keywords: *Holostylis reniformis*; Aristolochiaceae; Structures elucidation; Aryltetralone lignans; Aryltetralol lignans; Lignans

1. Introduction

Holostylis reniformis Duch. (Aristolochiaceae) is a rich source of aryltetralone lignans. Previous studies on this species have led to the isolation and structural determination of 10 lignans, including two 7,8-seco-lignans from extracts of the roots (da Silva and Lopes, 2004). An evaluation of the anti-plasmodial activity of the extracts and their chemical constituents showed that the apolar extracts and aryltetralone lignans were the most active against *Plasmodium berghei* and *P. falciparum* in vivo and in vitro bioassays, respectively (da Silva et al., 2004).

In a continuation of our examination of *H. reniformis*, in this study we more closely investigated the hexane extract of the roots. This analysis lead to the isolation and structural determination of five new lignans (**1**–**5**), including four aryltetralone lignans, of which two (**3** and **4**) were epimers of the cagayanone, and an aryltetralol (**5**), which was an epimer of the aristoligol (**5b**). In addition, an aryltetralene (**6**), which was an enantiomer of the cyclogalgravin, together with the known lignans galbacin (**7**) (Watanabe and Lopes, 1995), wulignan A_2 (**8**), and epishisandrone (**9**) (Jia-Sen et al., 1988), were isolated. Furthermore, lignans (−)-aristotetralone (**10**), (−)-aristoligone (**11**), (+)-8,8′-*epi*-aristoligone (**12**), (−)-8′-*epi*-aristoligone (**13**), (−)-8′-*epi*-holostylone (**14**), (−)-8-*epi*-holostylone (**15**), (−)-4′-*O*-methylenshicine (**16**), and (−)-7,8-seco-holostylone A (**17**), which have been previously isolated from this plant, were obtained (da Silva and Lopes, 2004). The structures of the new compounds were determined by spectroscopic methods and chemical transformations.

2. Results and discussion

The hexane extract of the roots of *H. reniformis* gave 17 lignans (**1**–**17**) by chromatographic separation followed by semi-preparative HPLC. Lignans **7**–**17** were identified by comparing their spectroscopic (IR, MS, ^{1}H and ^{13}C NMR) data and α_D values to those reported in the literature (Jia-Sen et al., 1988; Watanabe and Lopes, 1995; da Silva and Lopes, 2004).

The ESI-MS of compound **1** displayed a *quasi*-molecular ion $[M+H]^+$ at *m*/*z* 357, and its ^{13}C and ^{1}H NMR spectra showed a total of 21 carbons and 24 hydrogens, respectively, which were consistent with the molecular formula $C_{21}H_{24}O_5$. The IR spectrum of **1** showed characteristic absorption bands of a hydroxyl group at 3433 cm^{-1} and an aromatic ketone at 1663 cm^{-1}. The ^{1}H and ^{13}C NMR, ^{1}H–^{1}H COSY, *g*HMQC, and *g*HMBC experiments suggested the presence of three aromatic methoxyl groups,

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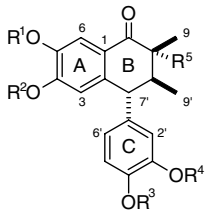
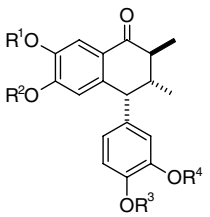
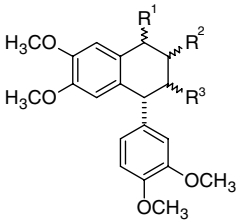


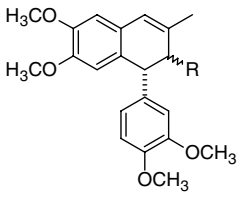
	R^1	R^2	R^3	R^4	R^5
1	CH_3	CH_3	H	CH_3	H
2	CH_3	CH_3	CH_3	CH_3	OH
4	CH_2		CH_2		H
13	CH_3	CH_3	CH_3	CH_3	H



	R^1	R^2	R^3	R^4
3	CH_2		CH_2	
11	CH_3	CH_3	CH_3	CH_3



	R^1	R^2	R^3
5	αOH	βCH_3	βCH_3
5a	βOH	αCH_3	βCH_3
5b	αOH	βCH_3	αCH_3
5c	βOH	βCH_3	βCH_3
12	=O	αCH_3	βCH_3



	R
6	βCH_3
6a	αCH_3

one hydroxyl group, 1,2,4,5-tetrasubstituted and 1,3,4-trisubstituted aromatic rings, two methyl groups, one carbonyl carbon, and three methine carbons in the molecule (Tables 1 and 2). 1D-*g*NOESY and *g*HMBC experiments allowed us to establish the position of the aromatic substituents, with the hydroxyl group linked to C-4′ on the C ring. Moreover, 2D experiments enabled the precise assignment of all hydrogens and carbons in the basic structure of an aryltetralone lignan. The *J* values determined for the hydrogens were confirmed by selective proton irradiations (Table 2). Lignan **1** did not show any characteristic *trans* di-axial oriented methine hydrogens ($J \cong 11.5$ Hz) on the *B* ring (Lopes et al., 1982). 1D-*g*NOESY experiments showed spatial interactions between 3H-9′ and H-7′, and between H-8′ and H-8, H-7′, and H-2′. 3H-9′ did not show any spatial interaction with H-8. These data suggested a *syn* orientation for H-8 and H-8′, as well as a *trans* orientation for H-8′ and H-7′. Thus, its relative configuration was established by analyzing coupling constants and the spatial interaction between the hydrogens on the B ring, as well as between these hydrogens and the substituents on this ring (Table 2, Fig. 1).

The IR spectrum of compound **2** also showed characteristic absorption bands of a hydroxyl group at 3432 cm^{-1} and an aromatic ketone at 1668 cm^{-1}. The EI-MS of **2** showed a molecular ion M^+ at *m*/*z* 386, and its ^{13}C and ^1H NMR spectra revealed a total of 22 carbons and 26 hydrogens, respectively, which were consistent with the molecular formula $C_{22}H_{26}O_6$. Moreover, these spectra supported the presence of four aromatic methoxyl groups and two aromatic rings, one of which was veratryl and the other was 1,2,4,5-tetrasubstituted, which suggested that **2** was also an aryltetralone lignan. The main differences between **1** and **2** were signals for a methoxyl group at C-4′ instead of a hydroxyl group, and signals for an additional hydroxyl group at C-8 (δ_C 75.6). The latter significantly affected the chemical shifts of C-8′ ($\Delta\delta = +4.1$) and C-9 ($\Delta\delta = +7.4$) due to a β effect, and of C-9′ ($\Delta\delta = -3.5$) due to a γ effect. In addition, the multiplicities and chemical shifts of 3H-9 (δ_H 1.25, *s*) and H-8′ (δ_H 2.31, *dq*, 11.0, 6.5 Hz) were different. The magnitude of the coupling constant between H-7′ and H-8′ ($J = 11.0$ Hz) suggested a *trans* di-axial orientation for these hydrogens on the B ring, which was further supported by 1D-*g*NOESY experiments. The experiments showed spatial correlations between H-7′ and 3H-9′, as well as between H-8′ and H-6′ and H-2′. Furthermore, spatial correlation between H-7′ and 3H-9 was observed and aided in the determination of the conformations and relative configuration for this lignan (Fig. 1).

An analysis similar to that described for aryltetralones (**10**–**16**), previously isolated from this species (da Silva and Lopes, 2004), was carried out. This included an evaluation of the Cotton effect at $\lambda = 323 \pm 10$ nm to determine the absolute configuration of the stereocenter C-8 and allowed us to determine that **1** had an 8*S* configuration and **2** an 8*R* configuration, since they showed a negative Cotton effect at $\lambda = 314$ nm and a positive Cotton effect at $\lambda = 332$ nm, respectively. Therefore, the absolute configurations should be 7′*R*,8*S*,8′*S* for **1** [named (−)-holostyligone] and 7′*R*,8*R*,8′*S* for **2** [named (−)-8′-*epi*-8-hydroxy-aristoligone].

The ^1H and ^{13}C NMR, IR, and UV spectroscopic data for compounds **3** and **4** were very similar.

Their ESI-MS showed a *quasi*-molecular ion $[M+H]^+$ at *m*/*z* 339, and their ^1H and ^{13}C NMR spectra (Tables 1 and 3) showed signals for 18 hydrogens and 20 carbons, which were consistent with the molecular formula $C_{20}H_{18}O_5$ and results of an elemental analysis. The IR spectra of these compounds showed an absorption band characteristic of an aromatic ketone at 1665 ± 3 cm^{-1}. A detailed analysis of ^1H and ^{13}C NMR, ^1H–^1H COSY, *g*HMQC, and *g*HMBC experiments enabled the precise assignment of all hydrogens and carbons in the basic structure of the lignan cagayanone (Kuo et al., 1989). Except for the chemical shifts determined for H-6, 3H-9′, and methylenedioxyl hydrogens, all of the other chemical shifts differed significantly for the three lignans (Table 3), which suggested that they were epimers. ^1H–^1H COSY and ^1H selective irradiation NMR experiments for lignan **3** showed coupling con-

Table 1
^{13}C NMR spectroscopic data for compounds **1**–**4** ($CDCl_3$, 126 MHz)[a]

C	**1**		**2**		**3**		**4**	
	$^{13}C(\delta)$	*g*HMBC	$^{13}C(\delta)$	*g*HMBC	$^{13}C(\delta)$	*g*HMBC	$^{13}C(\delta)$	*g*HMBC
1	125.6	H-3	122.7	H-3	127.1	H-7′, H-3	127.0	H-7′, H-3
2	138.8	H-7′, H-6	141.7	H-7′, H-6	142.7	H-7′, H-6	140.8	H-8′, H-7′, H-6
3	111.9		111.9		108.5		109.6	
4	153.7	OCH_3-4, H-6	154.2	OCH_3-4, H-6, H-3	152.2	OCH_2O, H-6, H-3	151.8	OCH_2O, H-6, H-3
5	148.2	OCH_3-5, H-3	148.2	OCH_3-5, H-6, H-3	147.5	OCH_2O, H-6, H-3	147.2	OCH_2O, H-6, H-3
6	108.2		108.4		106.2		105.6	
7	199.9	H-9, H-6	201.3	H-9, H-6, OH	199.0	H-9, H-8, H-6	199.4	H-9, H-8, H-8′
8	42.7	H-9, H-9′	75.6	H-9, H-9′	42.7	H-9, H-9′; H-7′	43.7	H-9, H-9′, H-7′
9	11.9		19.3		12.7	H-8′	12.0	
1′	135.7	H-7′	135.6	H-7′, H-5′	133.1	H-7′, H-5′	137.2	H-7′, H-5′
2′	111.0		[b]		110.3	H-7′, H-6′	108.6	H-7′, H-6′
3′	146.7	OCH_3-3′	148.5	OCH_3-3′, H-5′	147.4	H-5′	147.4	OCH_2O, H-5′, H-2′
4′	144.4		149.4	OCH_3-4′	146.4	OCH_2O, H-2′	146.8	OCH_2O, H-6′
5′	114.2	OH	111.4		107.9		108.2	
6′	121.9	H-7′, H-2′	122.0 (*br*)		123.2	H-7′, H-2′	122.0	H-7′, H-2′
7′	50.4	H-9′	51.1	H-9′, H-3	50.6	H-9′, H-6′, H-3, H-2′	50.6	H-9′, H-6′, H-3, H-2′
8′	42.6	H-9, H-9′	46.7	OH, H-9, H-9′, H-7′	39.5	H-9, H-9′, H-7′	41.0	H-9, H-9′, H-7′
9′	15.9		12.4		18.0	H-7′	15.9	H-7′
OCH_3-3′	56.0		55.8					
OCH_3-4	56.0		55.9					
OCH_3-4′			56.0					
OCH_3-5	56.0		56.1					
OCH_2O-3′,4′					101.0		100.8	
OCH_2O-4,5					101.6		101.8	

[a] ^{13}C NMR data were assigned with assistance of *g*HMQC and *g*HMBC experiments.
[b] Signal not observed.

Table 2
^{1}H NMR spectroscopic data for compounds **1** and **2** ($CDCl_3$, 500 MHz, *J* in Hz)[a]

H	**1**		**2**	
	δ	1D-*g*NOESY	δ	2D-*g*NOESY
3	6.37 *s*	OCH_3-4	6.17 *s*	OCH_3-4
6	7.50 *s*	OCH_3-5	7.43 *s*	OCH_3-5
8	2.72 *dq* (4.0, 7.0)	H-9, H-8′		
9	1.07 *d* (7.0)	H-9′, H-8, H-8′	1.25 *s*	H-9′, H-7′
2′	6.48 *d* (2.0)		6.48 *br s*	
5′	6.78 *d* (8.0)		6.79 *d* (8.0)	OCH_3-4′
6′	6.50 *dd* (8.0, 2.0)		6.69 *br d* (8.0)	
7′	3.90 *d* (5.5)		3.62 *br d* (11.0)	H-9, H-9′
8′	2.35 *ddq* (5.5, 4.0, 7.0)	H-9′, H-8, H-7′, H-2′	2.31 *dq* (11.0, 6.5)	H-9′, H-6′, H-2′
9′	0.93 *d* (7.0)	H-9, H-8′, H-7′	0.94 *d* (6.5)	H-9, H-8′, H-7′
OCH_3-3′	3.74 *s*	H-2′	3.73 *br s*	
OCH_3-4′			3.84 *s*	H-5′
OCH_3-4	3.71 *s*	H-3	3.58 *s*	H-3
OCH_3-5	3.89 *s*	H-6	3.88 *s*	H-6
OH	5.47 *br s*[b]		4.00 *br s*[b]	

[a] Multiplicities were determined with the assistance of ^{1}H–^{1}H COSY.
[b] Signal disappears with addition of D_2O.

stant for *trans* di-axial H-8, H-8′ ($J = 11.0$ Hz), whereas **4** did not show a *J* value with a *trans* di-axial characteristic magnitude. 1D-*g*NOESY experiments for **3** and **4** showed spatial correlations between hydrogens on the B rings and the substituents of these rings, which enabled us to establish the most stable conformations and the relative configurations for **3** and **4** (Table 3, Fig. 1). Hence, **3** and **4** were named (−)-cagayanone A and (−)-cagayanone B, respectively. Both lignans showed a negative Cotton effect at $\lambda \cong 318$ nm, which was consistent with an 8*S* configuration. Thus, the absolute configurations 7′*R*,8*S*,8′*R* and 7′*R*,8*S*,8′*S* were determined for **3** and **4**, respectively.

The IR spectrum of lignan **5** did not show an absorption band characteristic of an aromatic ketone, as observed for lignans **1**–**4**. The ^{1}H and ^{13}C NMR spectra showed signals for two aromatic rings (veratryl and 1,2,4,5-tetrasubstituted), four methine carbons, and two methyl groups (Table 4, for ^{13}C NMR spectroscopic data see Section 4).

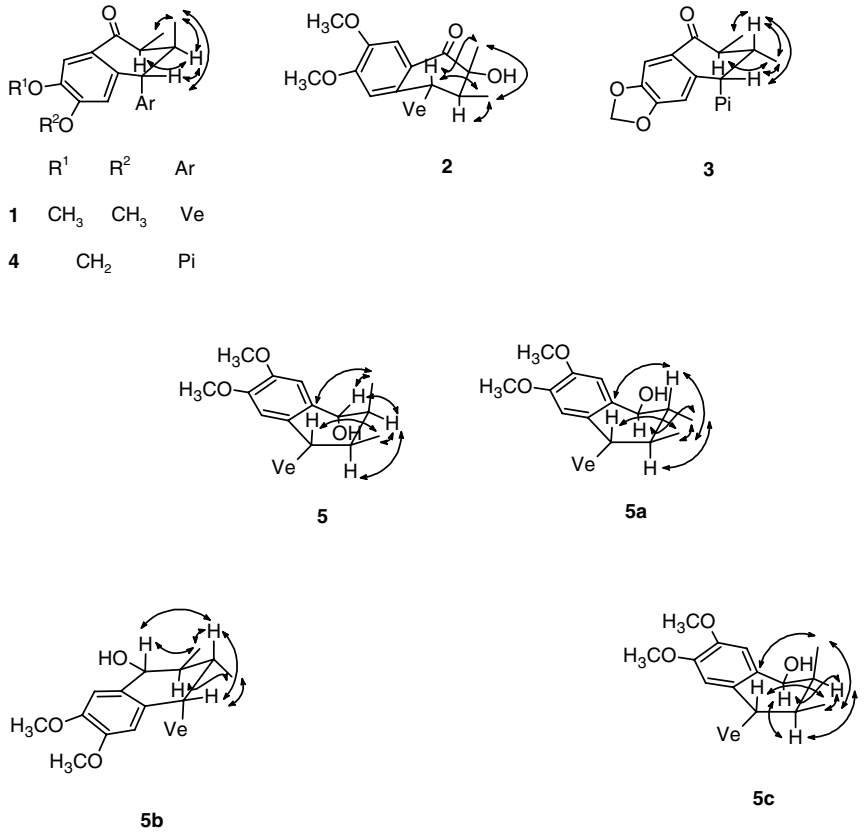


Fig. 1. Selected nOe interactions and possible conformations for lignans **1**–**5** and **5a**–**5c**.

Table 3
^{1}H NMR spectroscopic data for compounds **3**, **4**, and cagayanone ($CDCl_3$, 500 MHz, *J* in Hz)[a]

H	**3**		**4**		Cagayanone (Kuo et al., 1989)
	δ	1D-*g*NOESY	*δ*	1D-*g*NOESY	
3	6.44 *s*		6.35 *s*		6.17 *s*
6	7.46 *s*		7.44 *s*		7.44 *s*
8	2.35 *dq* (11.0, 6.5)	H-9, H-9′, H-6, H-2′	2.69 *dq* (3.5, 7.0)	H-9	2.32 *m*
9	1.13 *d* (6.5)	H-9′, H-8, H-8′	1.06 *d* (7.0)	H-9′, H-8	1.26 *d* (6.6)
2′	6.40 *d* (2.0)		6.47 *d* (1.5)	H-7′	6.51 *d* (1.5)
5′	6.65 *d* (8.0)		6.68 *d* (8.0)		6.76 *d* (6.3)
6′	6.43 *dd* (8.0, 2.0)		6.46 *dd* (8.0, 1.5)	H-7′	6.63 *dd* (6.3, 1.5)
7′	3.99 *d* (4.5)	H-9′, H-8′, H-6′, H-2′	3.85 *d* (5.5)	H-9′, H-8′, H-6′, H-3, H-2′	3.61 *d* (10.8)
8′	2.25 *ddq* (11.0, 4.5, 7.0)	H-9, H-9′, H-7′	2.30 *ddq* (5.5, 3.5, 6.5)	H-9′, H-8, H-7′, H-6′ H-2′	1.99 *m*
9′	0.90 *d* (7.0)	H-9; H-8, H-8′, H-7′, H-6′, H-2′	0.91 *d* (6.5)	H-9, H-8′, H-7′	0.90 *d* (6.6)
OCH_2O-4,5	5.93, 5.90 2*d* ($w_{1/2}$ 1.5)		5.92 *s*		5.93 *s*
OCH_2O-3′,4′	5.86, 5.85 2*d* ($w_{1/2}$ 1.5)		5.88 *s*		5.91 *s*

[a] Multiplicities were determined with the assistance of ^{1}H–^{1}H COSY.

Instead of a carbonyl group, signals for a carbinolic benzyl group ($\delta_{C\text{-}7}$ 74.0, $\delta_{H\text{-}7}$ 4.40) were noted. The carbinolic hydrogen showed a nOe correlation with H-6 (*δ* 6.83) at a frequency lower than those of aryltetralone lignans. These data, together with the ion at *m/z* 355 $[M+H-H_2O]^+$ in the APCI-MS spectra, suggested that compound **5** was an aryltetralol lignan. ^{1}H selective irradiation NMR experiments aided in determining the *J* values and chemical shifts based on first-order coupling hydrogens, which were further confirmed by spectral simulations using the program FOMSC3 (FOMSC3, 2004).

The magnitudes of the coupling constants between the methine hydrogens ($J_{H\text{-}7',8'} = 9.0$ Hz, $J_{H\text{-}8',8} = 3.1$ Hz, $J_{H\text{-}7,8} = 4.5$ Hz) suggested a *trans* di-axial orientation of H-7′, H-8′, a *cis* axial-equatorial orientation of H-8, H-8′, as well as a *trans* di-equatorial orientation of H-7, H-8. These suggestions were supported by 2D-*g*NOESY experiments, which showed spatial correlations between H-7 and H-8, 3H-9, and between H-8′ and 3H-9′, H-8, H-6′, and H-2′. Moreover, H-7′ was spatially correlated with 3H-9 (Fig. 1). To establish the absolute configuration of **5**, three aryltetralols were prepared by the reduction of

Table 4
^{1}H NMR spectroscopic data for compounds **5** and **5a**–**5c** ($CDCl_3$, 500 MHz, *J* in Hz)[a,b]

H	**5**		**5a**		**5b**		**5c**	
	δ	2D-*g*NOESY	δ	1D-*g*NOESY	δ	1D-*g*NOESY	δ	1D-*g*NOESY
3	6.22 *s*	OCH_3-4, H-7′	6.08 *s*		6.31 *s*		6.15 *s*	
6	6.83 *s*	OCH_3-5, H-7	7.08 *s*		7.06 *s*		7.07 *s*	
7	4.40 *d* (4.5)	H-9, H-8, H-6	4.36 *dd* (9.7, 9.0)	H-9, H-8′, H-6	4.24 *d* (9.0)	H-9, H-8′, H-6	4.93 *d* (4.5)	H-8, H-8′, H-6, OH
8	2.00 *ddq* (4.5, 3.1, 7.0)	H-9, H-8′, H-7	1.46 *ddq* (11.0, 9.7, 6.5)		1.65 *ddq* (11.5, 9.0, 6.5)		2.15 *ddq* (4.5, 3.0, 7.0)	H-9, H-9′, H-7
9	0.87 *d* (7.0)	H-8, H-7, H-7′	1.17 *d* (6.5)	H-9′, H-8, H-8′	1.01 *d* (6.5)	H-9′, H-8, H-8′, H-7	0.83 *d* (7.0)	H-8, H-7′, OH
2′	6.56 *d* (2.0)	OCH_3-3′, H-8′, H-7′	6.48 *d* (2.0)		6.52 *d* (2.0)		6.47 *d* (2.0)	
5′	6.73 *d* (8.0)	OCH_3-4′	6.75 *d* (8.0)		6.67 *d* (8.0)		6.71 *d* (8.0)	
6′	6.58 *dd* (8.0, 2.0)	H-8′, H-7′	6.62 *dd* (8.0, 2.0)		6.43 *dd* (8.0, 2.0)		6.56 *dd* (8.0, 2.0)	
7′	3.45 *d* (9.0)	H-9, H-9′, H-6′, H-3, H-2′	3.42 *d* (10.5)	H-9′, H-8, H-6′, H-3, H-2′	3.84 *d* (5.0)	H-9′, H-8′, H-6′, H-3, H-2′	3.46 *d* (10.5)	H-9, H-9′, H-6′, H-3, H-2′
8′	2.30 *ddq* (9.0, 3.1, 7.0)	H-9′, H-8, H-6′, H-2′	1.61 *ddq* (11.0, 10.5, 6.5)		1.88 *ddq* (11.5, 5.0, 7.0)		2.00 *ddq* (10.5, 3.0, 7.0)	H-9′, H-7, H-6′, H-2′
9′	0.86 *d* (7.0)	H-8′	0.82 *d* (6.5)	H-9, H-8, H-8′, H-7′, H-6′, H-2′	0.78 *d* (7.0)	H-9, H-8, H-8′, H-7′, H-6′, H-2′	0.85 *d* (7.0)	H-8, H-8′, H-7′, H-6′, H-2′
OCH_3-3′	3.75 *s*	H-2′	3.74 *s*		3.74 *s*		3.73 *s*	
OCH_3-4′	3.82 *s*	H-5′	3.82 *s*		3.78 *s*		3.80 *s*	
OCH_3-4	3.57 *s*	H-3	3.51 *s*		3.66 *s*		3.53 *s*	
OCH_3-5	3.84 *s*	H-6	3.83 *s*		3.85 *s*		3.83 *s*	
OH	[c]		1.54 *d* (9.0)		1.83		1.44	

[a] Multiplicities were determined with the assistance of ^{1}H–^{1}H COSY.
[b] Simulated ^{1}H NMR spectra were in total agreement with the experimental spectra.
[c] Signal not observed.

Table 5
^{1}H NMR spectroscopic data for compounds **6** and **6a** ($CDCl_3$, 500 MHz, *J* in Hz)[a]

	6		**6a**	
	δ	1D-*g*NOESY	δ	1D-*g*NOESY
3	6.48 *s*		6.57 *br s*	
6	6.55 *s*		6.56 *s*	
7	6.11 *br s*		6.13 *br s*	
9	1.73 *d* (1.0)		1.83 *br s*	H-9′, H-8′, H-7
2′	6.59 *d* (2.0)		6.77 *m*	
5′	6.64 *d* (8.5)		6.77 *m*	
6′	6.49 *dd* (8.5, 2.0)		6.77 *m*	
7′	3.60 *d* (3.5)	H-9′, H-8′, H-6′, H-3, H-2′	4.02 *d* (6.5)	H-9′, H-8′, H-6′, H-3, H-2′
8′	2.32 *dq* (3.5, 7.0)	H-9, H-9′, H-7′, H-6′, H-2′	2.39 *dq* (6.5, 7.5)	H-9, H-9′, H-7′
9′	1.01 *d* (7.0)	H-9, H-8′, H-7′	0.80 *d* (7.5)	H-9, H-8′, H-7′, H-6′, H-2′
$4\times OCH_3$	3.75 *s*, 3.81 *s*, 3.71 *s*, 3.71 *s*		3.81 *s*, 3.82 *s*, 3.66 *s*, 3.77 *s*	

[a] Multiplicities were determined with the assistance of ^{1}H–^{1}H COSY.

aryltetralones (**11**–**13**) with $LiAlH_4$, the absolute configurations of which have been well established (da Silva and Lopes, 2004). A known aryltetralol was obtained from **11**, and was identified as (−)-aristoligol (**5b**), which was previously obtained by the reduction of (−)-aristoligone (Urzúa and Shamma, 1988). Two new aryltetralols (**5a** and **5c**) were obtained from **12** and **13**, respectively. A comparative analysis of the ^{1}H NMR spectra of **5** with those of derivatives showed that H-7′ and H-8′ of **5** ($J = 9.0$ Hz), **5a** ($J = 10.5$ Hz), and **5c** ($J = 10.5$ Hz) were *trans* dial-axial oriented, like H-8 and H-8′ of **5a** ($J = 11.0$ Hz) and **5b** ($J = 11.5$ Hz), and H-7 and H-8 of **5a** ($J = 9.7$ Hz) and **5b** ($J = 9.0$ Hz). Thus, **5** should have a relative configuration different from the other derivatives. This deduction was further supported by 1D-*g*NOESY experiments, which aided in establishing the relative configurations of all methine hydrogens on the B rings (Table 4, Fig. 1). Furthermore, *g*HMQC and *g*HMBC experiments aided in ascribing the chemical shifts for carbons and hydrogens of **5** and **5a**–**5c**. Derivative **5c** showed chemical shift for C-9 (δ 6.6) at lowest frequency due to γ effects (CH-7′, CH_3-9′, OH-7), which were in agreement with the relative configurations established. Since the configurations of the stereocenters C-7′, C-8, and C-8′ of **5a**–**5c** are known, their absolute configurations could be determined to be 7*S*,7′*R*,8*R*,8′*S*, 7*R*,7′*R*,8*S*,8′*R*, and 7*S*,7′*R*,8*S*,8′*S*, respectively. Furthermore, the CD curves of these derivatives were very similar, with positive Cotton effects at $\lambda \cong 289$ nm characteristic of an α-aryl at C-7′ (7′*R*) (Lopes et al., 1982). The CD curve of **5** also showed a positive effect at $\lambda = 288$ nm, which is consistent with a 7′*R* configuration. Hence, the absolute configuration of the natural aryltetralol **5** (named holostylol A) was determined to be 7*R*,7′*R*,8*S*,8′*S*.

Dehydrations of **5b** and **5c** gave **6a** and **6**, respectively. A comparative analysis of their spectroscopic data (^{1}H, ^{13}C NMR, IR, and ESI-MS) and optical activity (Table 5, Section 4) allowed us to identify **6** as the natural compound isolated from *H. reniformis*. Therefore, the absolute configurations of **6** and **6a** were determined to be 7′*R*,8′*S* and 7′*R*,8′*R*, respectively. Furthermore, comparison of the optical activity of **6** ($[\alpha]_D = -105.9°$) with those reported in the literature for aryltetralene lignans led us to establish that **6** was an enantiomer of (+)-cyclogalbelgin, also known as a methyl derivative of (+)-myrisfragransin (Miyazawa et al., 1996), and as (+)-cyclogalgravin, which was previously obtained by acid transformation of tetrahydrofuran lignans, such as galbelgin, saucernetin and veraguensin (Rao, 1978; Fonseca et al., 1979; Rao and Alvarez, 1982).

3. Conclusions

Although tetrahydrofuran lignans have been transformed into aryltetralone lignans, and both types of lignans have been isolated from *H. reniformis*, it seems more likely that compound **6** is a biosynthetic derivative of aryltetralones, such as **13**. Indeed, these metabolites are plentiful in this species and the presumed aryltetralol intermediate (**5**) has been isolated from the same plant part.

In addition to previously isolated lignans from the antiplasmodial extract of *H. reniformis*, this paper describes the structure determination of nine lignans, which can lead to further investigations in search for anti-malarial drugs.

4. Experimental

4.1. General and experimental procedures

1D- (^{1}H, ^{13}C, and *g*NOESY) and 2D- (^{1}H–^{1}H *g*COSY, *g*HMQC, *g*HMBC, and *g*NOESY) NMR experiments were recorded on a Varian INOVA 500 spectrometer (11.7 T) at 500 MHz (^{1}H) and 126 MHz (^{13}C), with the solvents used as an internal standard. Mass spectra (EI-MS, APCI-MS, and ESI-MS) were obtained on a Fisons Platform II, and flow injection into the electrospray source was used for ESI-MS. IR spectra were obtained on a Nicolet-730 FT-IR spectrometer using KBr discs. UV absorptions were measured on a Hewlett–Packard 8452A diode array spectrophotometer. Optical rotations were measured on a Polamat A Carl Zeiss Jena. 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 RP18 (Shimadzu, C18, 3.9 × 150 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

The plant material was collected in Ituiutaba, MG, Brazil, in February, 2003, and identified as *Holostylis reniformis* Duch. by Dr. Condorcet Aranha and by Dr. Lindolpho Cappellari Júnior. A voucher specimen (ESA 88282) was deposited at the herbarium of the Escola Superior de Agricultura, Luiz de Queiroz (ESALQ), Piracicaba, SP, Brazil. The material was separated into plant parts, dried (~45 °C) and ground.

4.3. Extraction and isolation

Dry material from the roots (800 g) of *H. reniformis* was extracted exhaustively at room temp. with hexane, Me_2CO, and EtOH successively, and the extracts were individually concentrated.

The hexane extract (6.17 g) was fractionated by CC (silica gel, 151.0 g, hexane–EtOAc gradient) to give 28 fractions. Fractions 7, 11–18, and 20 were individually subjected to semi-prep. HPLC (MeOH–H_2O 3:2). Fraction 7 gave **3** (5.9 mg), **4** (2.0 mg), **7** (22.8 mg), and **10** (2.6 mg). Fraction 11 gave **10** (25.7 mg) and **16** (22.1 mg). Fraction 12 and 13 were joined to give **11** (255.7 mg), **12** (275.8 mg), and **13** (28.1 mg). Fraction 14 gave **1** (6.3 mg), **2** (4.7 mg), **15** (5.8 mg), and **14** (17.4 mg). Fraction 15 gave **8** (5.0 mg) and **9** (14.5 mg). Fraction 16 gave **14** (23.5 mg) and **15** (6.3 mg). Fraction 17 gave **5** (3.3 mg). Fraction 20 gave **17** (2.5 mg). Fraction 18 was washed with hexane to give **6** (4.6 mg).

4.3.1. (7′R,8S,8′S)-4′-Hydroxy-3′,4,5-trimethoxy-2,7′-cyclolignan-7-one [(−)-holostyligone, 1]

Amorphous yellow solid; $[\alpha]_D^{25}$ − 27.0° ($CHCl_3$; *c* 1.18); UV λ_{max} (MeOH) nm (log ε): 234 (4.0), 234 (3.9), 277 (3.8), 310 (3.6); IR (KBr) ν_{max} 3433, 2972, 2923, 1663 cm^{-1}; for ^1H and ^{13}C NMR spectra, see Tables 1 and 2; CD (MeOH; *c* 0.1): $[\theta]_{212}$ +9240, $[\theta]_{223}$ +1650, $[\theta]_{238}$ +12,540; $[\theta]_{248}$ 0, $[\theta]_{256}$ −1320, $[\theta]_{264}$ 0, $[\theta]_{275}$ +990, $[\theta]_{277}$ 0, $[\theta]_{291}$ −5610, $[\theta]_{300}$ −2640, $[\theta]_{314}$ −4950; positive ESI-MS (probe) 20 eV, *m/z* (rel. int.): 357 $[M+H]^+$ (100), 237 (15), 219 (3), 165 (2). Found: C, 70.8; H, 6.8. $C_{21}H_{24}O_5$ requires: C, 70.9; H, 6.8%.

4.3.2. (7′R,8R,8′S)-8-Hydroxy-3′,4,4′,5-tetramethoxy-2,7′-cyclolignan-7-one [(−)-8′-epi-8-hydroxy-aristoligone, 2]

Amorphous yellow solid; $[\alpha]_D^{25}$ − 14.0° ($CHCl_3$; *c* 1.3); UV λ_{max} (MeOH) nm (log ε) 235 (4.0), 212 (3.9) 277 (3.7), 310 (3.4); IR (KBr) ν_{max} 3432, 2927, 2847, 1668, 1589, 1510 cm^{-1}; for ^1H and ^{13}C NMR spectra, see Tables 1 and 2; CD (MeOH; *c* 0.1): $[\theta]_{212}$ +14,850, $[\theta]_{220}$ 0; $[\theta]_{225}$ −4950, $[\theta]_{230}$ 0, $[\theta]_{240}$ +15,840, $[\theta]_{255}$ 0, $[\theta]_{270}$ +6930, $[\theta]_{279}$ 0, $[\theta]_{288}$ −11,550, $[\theta]_{293}$ −10,230, $[\theta]_{303}$ −11,550, $[\theta]_{324}$ 0, $[\theta]_{332}$ +3960; positive EI-MS (probe) 20 eV, *m/z* (rel. int.): 386 $[M]^{+\cdot}$ (67), 221 (100), 204 (81), 182 (74), 165 (98) 149 (95). Found: C, 68.4; H, 6.8. $C_{22}H_{26}O_6$ requires: C, 68.5; H, 6.8%.

4.3.3. (7′R,8S,8′R)-3′,4′:4,5-Bis(methylenedioxy)-2,7′-cyclolignan-7-one [(−)-cagayanone A, 3]

Amorphous yellow solid; $[\alpha]_D^{25}$ − 27.4° ($CHCl_3$; *c* 0.78); UV λ_{max} (MeOH) nm (log ε): 235 (3.9), 278 (3.5), 320 (3.4); IR (KBr) ν_{max} 2931, 2847, 1663 cm^{-1}; for ^1H and ^{13}C NMR spectra, see Tables 1 and 3; CD (MeOH; *c* 0.1): $[\theta]_{221}$ 0; $[\theta]_{240}$ +4620, $[\theta]_{256}$ +330, $[\theta]_{262}$ 0, $[\theta]_{267}$ −330, $[\theta]_{272}$ 0, $[\theta]_{274}$ +330, $[\theta]_{292}$ −1320, $[\theta]_{306}$ −1650, $[\theta]_{319}$ −2970; positive ESI-MS (probe) 35 eV, *m/z* (rel. int.): 339 $[M+H]^+$ (100), 217 (36), 189 (4). Found: C, 71.0; H, 5.4. $C_{20}H_{18}O_5$ requires: C, 71.1; H, 5.4%.

4.3.4. (7′R,8S,8′S)-3′,4′:4,5-Bis(methylenedioxy)-2,7′-cyclolignan-7-one [(−)-cagayanone B, 4]

Amorphous yellow solid; $[\alpha]_D^{25}$ − 33.0° ($CHCl_3$; *c* 0.45); UV λ_{max} (MeOH) nm (log ε): 240 (3.2), 277 (3.2), 324 (3.2); IR (KBr) ν_{max} 3022, 2926, 2864, 1668 cm^{-1}; for ^1H and ^{13}C NMR spectra, see Tables 1 and 3; CD (MeOH; *c* 0.1): $[\theta]_{223}$ +11,220; $[\theta]_{238}$ +36,300, $[\theta]_{255}$ +1320, $[\theta]_{262}$ 0, $[\theta]_{273}$ +7920, $[\theta]_{279}$ 0, $[\theta]_{292}$ −22,440, $[\theta]_{308}$ −6930, $[\theta]_{318}$ −6270; positive ESI-MS (probe) 30 eV, *m/z* (rel. int.): 339 $[M+H]^+$ (15), 138 (100), 121 (58), 217 (34). Found: C, 71.1; H, 5.3. $C_{20}H_{18}O_5$ requires: C, 71.1; H, 5.4%.

4.3.5. (7R,7′R,8S,8′S)-3′,4′,4,5-Tetramethoxy-2,7′-cyclolignan-7-ol [(−)-8′-epi-aristoligol, holostylol A, 5]

Amorphous yellow solid; $[\alpha]_D^{25}$ − 36.0° ($CHCl_3$; *c* 0.83); UV λ_{max} (MeOH) nm (log ε) 213 (3.9), 231 (3.7) 280 (3.3); IR (KBr) ν_{max} 3440, 2947, 2847, 1596 cm^{-1}; ^{13}C NMR (126 MHz, $CDCl_3$): δ 129.4 (*s*, C-1), 131.9 (*s*, C-2), 112.6 (*d*, C-3), 148.9 (*s*, C-4), 147.9 (*s*, C-5), 111.8 (*d*, C-6), 74.0 (*d*, C-7), 39.2 (*d*, C-8), 12.0 (*q*, C-9), 138.2 (*s*, C-1′), 112.3 (*d*, C-2′), 148.8 (*s*, C-3′), 147.5 (*s*, C-4′), 110.9 (*d*, C-5′), 121.7 (*d*, C-6′), 49.3 (*d*, C-7′), 35.9 (*d*, C-8′), 16.7 (*q*, C-9′), 55.8, 2 × 55.9, 56.0 (4 × *q*, OCH_3-3′,4′,4,5); for ^1H NMR spectra, see Table 4; CD (MeOH; *c* 0.1): $[\theta]_{220}$ +4290, $[\theta]_{226}$ 0; $[\theta]_{238}$ −11,550, $[\theta]_{254}$ −990, $[\theta]_{272}$ −3950, $[\theta]_{280}$ 0, $[\theta]_{288}$ +5610, $[\theta]_{299}$ 0; positive APCI-MS (probe) 40 eV, *m/z* (rel. int.): 355 $[M+H-H_2O]^+$ (13), 217 (100), 202 (22), 165 (82) 151 (54). Found: C, 71.0; H, 7.6. $C_{22}H_{28}O_5$ requires: C, 71.0; H, 7.6%.

4.3.6. (7′R,8′S)-3′,4′,4,5-Tetramethoxy-2,7′-cyclolignan-7-ene [(−)-cyclogalgravin, 6]

Amorphous yellow solid; $[\alpha]_D^{25}$ − 105.9° ($CHCl_3$; *c* 1.07); UV λ_{max} (MeOH) nm (log ε): 241 (3.7), 280 (3.4); IR (KBr) ν_{max} 3444, 2918, 2844, 1708, 1663 cm^{-1}; ^{13}C NMR (126 MHz, $CDCl_3$): δ 127.3 (*s*, C-1), 127.4 (*s*, C-2), 113.0

(*d*, C-3), 147.6 (*s*, C-4), 147.5 (*s*, C-5), 109.1 (*d*, C-6), 121.1 (*d*, C-7), 138.8 (*s*, C-8), 22.1 (*q*, C-9), 138.2 (*s*, C-1′), 111.1 (*d*, C-2′), 148.7 (*s*, C-3′), 147.8 (*s*, C-4′), 111.0 (*d*, C-5′), 119.6 (*d*, C-6′), 50.9 (*d*, C-7′), 42.0 (*d*, C-8′), 18.7 (*q*, C-9′), 55.8 ($2\times q$, OCH_3-3′,4), 2×55.9 ($2\times q$, OCH_3-4′,5) (^{13}C NMR data agree with those reported in the literature (Fonseca et al., 1979) except for assignments of several carbons that should be interchanged); for ^1H NMR spectra, see Table 5; CD (MeOH; *c* 0.1): $[\theta]_{225}$ +990, $[\theta]_{227}$ +1320; $[\theta]_{231}$ 0, $[\theta]_{237}$ −1650, $[\theta]_{259}$ −330, $[\theta]_{271}$ −3960, $[\theta]_{290}$ −1254, $[\theta]_{300}$ −1303; positive APCI-MS (probe) 20 eV *m/z* (rel. int.): 355 $[M+H]^+$ (5), 219 (100), 149 (20), 121 (18). Found: C, 74.6; H, 7.4. $C_{22}H_{26}O_4$ requires: C, 74.6; H, 7.4%.

4.3.7. Wulignan A_2 (**8**)

Amorphous yellow solid; $[\alpha]_D^{25}$ − 55.0° ($CHCl_3$; *c* 0.90), [lit. $[\alpha]_D^{14}$ − 61.2° ($CHCl_3$; *c* 0.17) (Jia-Sen et al., 1988)]; ^1H NMR, UV, IR, and CD data agree with those reported in the literature (Jia-Sen et al., 1988); ^{13}C NMR (126 MHz, $CDCl_3$): δ 125.2 (*s*, C-1), 140.0 (*s*, C-2), 115.4 (*d*, C-3), 150.7 (*s*, C-4), 145.8 (*s*, C-5), 108.9 (*d*, C-6), 200.2 (*s*, C-7), 41.0 (*d*, C-8), 11.7 (*q*, C-9), 135.5 (*s*, C-1′), 111.9 (*d*, C-2′), 146.7 (*s*, C-3′), 144.4 (*s*, C-4′), 114.2 (*d*, C-5′), 121.9 (*d*, C-6′), 49.8 (*d*, C-7′), 42.0 (*d*, C-8′), 16.0 (*q*, C-9′), 55.9 (*q*, OCH_3-3′), 56.1 (*q*, OCH_3-5).

4.3.8. Epischisandrone (**9**)

Amorphous yellow solid; $[\alpha]_D^{25}$ − 24.0° ($CHCl_3$; *c* 1.25), [lit. $[\alpha]_D^{14}$ + 5.50° ($CHCl_3$; *c* 1.05) (Jia-Sen et al., 1988)]; ^1H NMR, UV, IR, and CD data agree with those reported in the literature (Jia-Sen et al., 1988); ^{13}C NMR (126 MHz, $CDCl_3$): δ 126.4 (*s*, C-1), 137.8 (*s*, C-2), 111.3 (*d*, C-3), 151.3 (*s*, C-4), 144.7 (*s*, C-5), 111.9 (*d*, C-6), 200.0 (*s*, C-7), 42.6 (*d*, C-8), 11.9 (*q*, C-9), 136.5 (*s*, C-1′), 112.0 (*d*, C-2′), 149.1 (*s*, C-3′), 147.3 (*s*, C-4′), 111.1 (*d*, C-5′), 121.4 (*d*, C-6′), 50.5 (*d*, C-7′), 42.5 (*d*, C-8′), 15.9 (*q*, C-9′), 55.9 (*q*, OCH_3-3′), 2×56.0 ($2\times q$, OCH_3-4′,5).

4.4. Chemical transformations

A solution of **12** (5.2 mg) in dry THF was added dropwise to a suspension of $LiAlH_4$ (48.5 mg) in THF (0.5 ml). The mixture was then stirred (30 min), treated with THF saturated with H_2O until H_2 ceased to evolve, and then with aqueous saturated NH_4Cl solution. The solution was extracted with $CHCl_3$ (4×2 ml), and the organic solutions were combined, dried ($MgSO_4$), and evaporated. The residue (4.6 mg) was characterized as **5a**.

4.4.1. (7*S*,7′*R*,8*R*,8′*S*)-3′,4′,4,5-Tetramethoxy-2,7′-cyclolignan-7-ol [(−)-holostylol B, **5a**]

Amorphous yellow solid; $[\alpha]_D^{25}$ − 35.0° ($CHCl_3$; *c* 0.75); IR (KBr) ν_{max} 3409, 2972, 1618, 1512 cm^{-1}; ^{13}C NMR (126 MHz, MeOH-d_4): δ 132.8 (*s*, C-1), (C-2, not observed), 112.4 (*d*, C-3), 147.5, 147.4, (2 *s*, C-4,5), 109.7 (*d*, C-6), 74.1 (*d*, C-7), 44.0 (*d*, C-8), 14.6 (*q*, C-9), 139.0 (*s*, C-1′), 112.8 (*d*, C-2′), 149.5, (*s*, C-3′), 148.0 (*s*, C-4′), 111.6 (*d*, C-5′), 122.5 (*d*, C-6′), 53.5 (*d*, C-7′), 41.5 (*d*, C-8′), 15.6 (*q*, C-9′), 3×55.8 ($3\times q$, OCH_3-3′,4′,5), 55.3 (*q*, OCH_3-4); for ^1H NMR spectra, see Table 4; CD (MeOH; *c* 0.1): $[\theta]_{220}$ 0, $[\theta]_{221}$ −792; $[\theta]_{225}$ −627, $[\theta]_{236}$ −6699, $[\theta]_{254}$ −1122, $[\theta]_{276}$ −4422, $[\theta]_{282}$ 0, $[\theta]_{288}$ +3630, $[\theta]_{296}$ 0, $[\theta]_{300}$ −1452.

Treatment of **11** (38.0 mg) and **13** (28.0 mg) with $LiAlH_4$ (97.0 mg) in THF (1 ml), according to the reduction procedure described previously, gave **5b** (34.7 mg) and **5c** (23.9 mg), respectively.

4.4.2. (7*R*,7′*R*,8*S*,8′*R*)-3′,4′,4,5-Tetramethoxy-2,7′-cyclolignan-7-ol [(−)-aristoligol, **5b**]

Amorphous yellow solid; $[\alpha]_D^{25}$ − 106.5° ($CHCl_3$; *c* 1.8), lit. 167.0° ($CHCl_3$; *c* 2.2); IR (KBr) ν_{max} 3458, 2937, 1604, 1510 cm^{-1}; for ^1H NMR spectra, see Table 4; ^{13}C NMR (126 MHz, $CDCl_3$): δ 130.1 (*s*, C-1), 132.1 (*s*, C-2), 111.7 (*d*, C-3), 147.9 (*s*, C-4), 147.8 (*s*, C-5), 109.2 (*d*, C-6), 75.9 (*d*, C-7), 37.6 (*d*, C-8), 15.8 (*q*, C-9), 134.9 (*s*, C-1′), 114.1 (*d*, C-2′), 147.5 (*s*, C-3′), 147.3 (*s*, C-4′), 110.3 (*d*, C-5′), 122.7 (*d*, C-6′), 50.5 (*d*, C-7′), 37.4 (*d*, C-8′), 17.5 (*q*, C-9′), 4×55.9 ($4\times q$, OCH_3-3′,4′,4,5); CD (MeOH; *c* 0.1) $[\theta]_{214}$ 0, $[\theta]_{222}$ +7260, $[\theta]_{227}$ 0, $[\theta]_{239}$ −35,640, $[\theta]_{253}$ −990, $[\theta]_{273}$ −15,840, $[\theta]_{286}$ 0, $[\theta]_{289}$ +1320, $[\theta]_{293}$ 0, $[\theta]_{295}$ −990, comparable to the literature (Urzúa and Shamma, 1988).

4.4.3. (7*S*,7′*R*,8*S*,8′*S*)-3′,4′,4,5-Tetramethoxy-2,7′-cyclolignan-7-ol [(−)-holostylol C, **5c**]

Amorphous yellow solid; $[\alpha]_D^{25}$ − 20.0° ($CHCl_3$; *c* 1.6); IR (KBr) ν_{max} 3402, 2959, 1602, 1512 cm^{-1}; ^{13}C NMR (126 MHz, $CDCl_3$): δ 130.6 (*s*, C-1), 131.3 (*s*, C-2), 112.0 (*d*, C-3), 147.7 (*s*, C-4), 147.3, 147.9, ($2\times s$, C-4′,5), 108.5 (*d*, C-6), 72.5 (*d*, C-7), 39.9 (*d*, C-8), 6.6 (*q*, C-9), 138.2 (*s*, C-1′), 112.2 (*d*, C-2′), 148.8 (*s*, C-3′), 110.8 (*d*, C-5′), 121.6 (*d*, C-6′), 49.3 (*d*, C-7′), 39.4 (*d*, C-8′), 17.7 (*q*, C-9′), 4×55.7 ($4\times q$, OCH_3-3′,4′,4,5); for ^1H NMR spectra, see Table 4; CD (MeOH; *c* 0.1): $[\theta]_{222}$ +7260, $[\theta]_{229}$ 0, $[\theta]_{239}$ −28,710, $[\theta]_{251}$ −990, $[\theta]_{283}$ −1584, $[\theta]_{289}$ +1320, $[\theta]_{293}$ 0, $[\theta]_{299}$ −990.

Solutions of **5b** (18.6 mg) and **5c** (17.7 mg) in dry C_6H_6 (5 ml) and PTSA (8.8 mg) were individually stirred at room temp. for 1 h under N_2 atmosphere. The solutions were then washed with aqueous saturated $NaHCO_3$ solution, extracted with C_6H_6, and then dried ($MgSO_4$), filtered and evaporated. The resulting organic residues were characterized as **6** (14.5 mg) and **6a** (13.9 mg), respectively.

4.4.4. (7′*R*,8′*R*)-3′,4′,4,5-Tetramethoxy-2,7′-cyclolignan-7-ene [(+)-7′-epi-cyclogalgravin, **6a**]

Amorphous yellow solid; $[\alpha]_D^{25}$ + 57.0° ($CHCl_3$; *c* 1.3); IR (KBr) ν_{max} 2923, 1600, 1510 cm^{-1}; ^{13}C NMR (126 MHz, $CDCl_3$): δ 147.1, 147.4, 147.6, 148.3 ($4\times s$, C-3′,4′,4,5), 140.2 (*s*, C-8), 133.6 (*s*, C-1′), 127.8, 128.4 ($2\times s$, C-1,2), 122.1 (*d*, C-7), 121.8 (*d*, C-6′), 113.2, 111.6, 110.8, 109.2 ($4\times d$, C-3,6,2′,5′), 4×55.9 ($4\times q$, OCH_3-3′,4′,4,5), 50.0 (*d*, C-7′), 39.5 (*d*, C-8′), 21.4 (*q*, C-9), 13.1 (*q*, C-9′); for ^1H NMR spectra, see Table 5; CD (MeOH; *c* 0.1): $[\theta]_{230}$

+56,298, $[\theta]_{254}$ 0; $[\theta]_{275}$ −40,590, $[\theta]_{293}$ −23,463, $[\theta]_{309}$ −8184.

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