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Aryltetralols from *Holostylis reniformis* and syntheses of lignan analogous

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ABSTRACT

Two new lignans, an aryltetralol and its methyl ether analogous, were isolated from *Holostylis reniformis* (Aristolochiaceae) together with futokadsurin C and (−)-8′-*epi*-aristoligone. The latter was also obtained as an enantiomeric mixture by synthesis and was transformed into aryltetralols and aryltetralenes that were subjected to chiral-HPLC separations. The compound structures were determined by spectroscopic methods. Several of these lignans had their antiplasmodial activity (against *Plasmodium falciparum*, W2 clone, anti-HRPII) and toxicity to mammalian kidney cells (MDL_{50}) evaluated. (−)-Cyclogalgravin and (−)-aristoligol exhibited activity ($IC_{50} \sim 10.8$ and 8.4 μM, respectively), the latter exhibited lower toxicity.

1. Introduction

Holostylis reniformis Duch. (Aristolochiaceae) synthesizes a variety of seco compounds, including lignans and sesquiterpenes (da Silva and Lopes, 2004, 2006; Lopes et al., 2012; Martins et al., 2014; Pereira et al., 2012). More than 25 aryltetralone and furan lignans without oxygenation at C-9,9′ have been isolated from extracts of this species (da Silva and Lopes, 2004, 2006; Lopes et al., 2012), and isoeugenol is shown to be their biosynthetic intermediate (da Silva and Lopes, 2004, 2006; Messiano et al., 2008, 2009). The anti-*Plasmodium falciparum* activities and toxicities of lignans and extracts obtained from *H. reniformis* have been demonstrated (da Silva et al., 2004; da Silva and Lopes, 2004; de Andrade-Neto et al., 2007). As part of our continuing studies on the chemical constituents of *H. reniformis*, in this paper we report the results of the *in vitro* antiplasmodial and toxicity evaluation of two new natural lignans (**1** and **2**) and of aryltetralone, aryltetralol, and aryltetralene lignans obtained by regioselective syntheses. These lignans belong to 2,7′-cyclolignan subclass of lignans or arylnaphthalene type of lignans. Lignans of this subclass, without oxygenation at C-9,9′, have already been obtained by syntheses using commercially available compounds (Kuo and Lin, 1993; Takeya et al., 1983; Yvon et al., 2001), *via* transformations of 7,7′-epoxylignans (furan lignans) (Blears and Haworth, 1958; Crossley and Djerassi, 1962; Purushothaman et al., 1984), and by biotransformations from fungi (Messiano et al., 2010). However, stereoselective syntheses of 2,7′-cyclolignans normally involve more than 10 steps (Peng et al., 2013; Rye and Barker, 2011), and those regioselective carried out in fewer steps showed poor yields (Barba et al., 1990; Iguchi et al., 1978). Aiming to obtain lignan analogous to advances in pharmacological studies of these lignans, this report describes a sequence for the syntheses of aryltetralone, aryltetralol, and aryltetralene lignans in four, five, and six steps, successively by improving a route for obtaining aryltetralone lignans proposed by Müller and Vajda (1952).

2. Results and discussion

The acetone extract of *H. reniformis* roots was subjected to column chromatography followed by HPLC to yield two minor new lignans (**1** and **2**, Fig. 1) together with the known compounds (−)-futokadsurin C (**3**) and (−)-8′-*epi*-aristoligone (**4a**). They were analyzed by spectroscopic methods, and the known compounds

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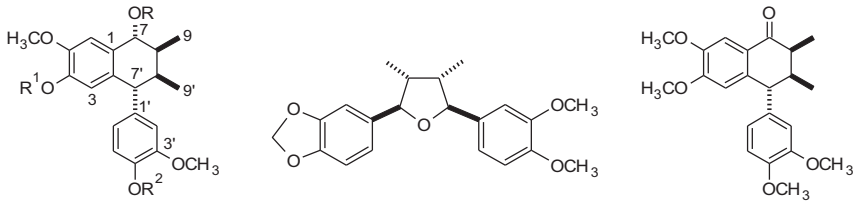

	R	R^1	R^2
1	H	H	CH_3
2	CH_3	H	CH_3
5	H	CH_3	CH_3
6	CH_3	CH_3	CH_3
7	CH_3	CH_3	H

3 **4a**

Fig. 1. Chemical structures of compounds **1**-**3**, **4a**, and **5**–**7**.

were identified by comparison with data reported in the literature (da Silva and Lopes, 2004, 2006; Konishi et al., 2005; Kuo et al., 1989) (Fig. 1).

The ^1H and ^{13}C NMR, IR, and UV spectroscopic data for compounds **1** and **2** were very similar to those reported for the aryltetralol derivatives, previously isolated from *H. reniformis* and obtained by microbial transformation of the aryltetralin lignans **5**-**7** (da Silva and Lopes, 2006; Messiano et al., 2008, 2010). Compounds **1** and **2** were also suggested to be aryltetralol lignans based on the HRMS spectra. They displayed quasi-molecular ions at *m*/*z* 381.1663 $[M+Na]^+$ and at *m*/*z* 371.1445 $[M-H]^-$, respectively, which were consistent with the molecular formula $C_{21}H_{26}O_5$ for **1** and $C_{22}H_{28}O_5$ for **2** (14 μ higher than **1**). The IR spectra of both compounds showed characteristic absorptions of hydroxyl groups at ~3440 cm^{-1}. The ^1H and ^{13}C NMR spectra of both compounds (Tables 1 and 2, Figs. S1–S4) suggested the presence of a veratryl group (C ring), and one methoxyl group linked to 1,2,4,5-tetrasubstituted aromatic ring (A). These spectra also showed signals for two methyl groups and four methine carbons, of which one is oxygenated. In addition, signals for hydroxyl groups at $\delta_H \sim 5.4$ were observed. Compound **2** showed additional resonances reminiscent of an aliphatic methoxyl group (δ_C 56.0; δ_H 3.40). The differences between the chemical shifts of carbons and hydrogens on the B ring of **1** and **2**, particularly of CH-7 (**1**: δ_C 74.1; δ_H 4.38; **2**: δ_C 83.4; δ_H 3.92) and CH-8 (**1**: δ_C 39.4; δ_H 1.99; **2**: δ_C 34.6; δ_H 2.19), suggested that these compounds differ by the presence of a methoxyl group at C-7 in **2** instead of a hydroxyl group in **1**. Furthermore, gHMBC experiments supported correlations between C-7 and OCH_3-7, H-6, and 3H-9. The magnitude of the coupling constants of the methine hydrogens in the B ring evidenced *trans* diaxial, *cis* axial–equatorial, and *trans* diequatorial positions for H-7′,8′ ($J = 10.0 \pm 0.5$ Hz), H-8′,8 ($J = 3.5$ Hz), and H-7,8 ($J = 3.7 \pm 0.3$ Hz), respectively. Moreover, gNOESY experiments showed spatial interactions between H-7 and 3H-9, as well as between H-7′ and H-3, H-2′, H-6′, 3H-9, and 3H-9′ for both compounds. Furthermore, spatial interactions of H-6 (δ 6.68) with H-7, OCH_3-7 (δ 3.40), and OCH_3-5 (δ 3.84) were observed in gNOESY experiments of **2**. Both compounds showed very similar CD curves with a positive Cotton effect at $\lambda \sim 290$ nm, consistent with a 7′*R* configuration (da Silva et al., 2004). Thus, the absolute configuration of **1** and **2** was determined as (7*R*,7′*R*,8*S*,8′*S*), which was identical to that previously determined for **5**–**7** (da Silva and Lopes, 2006; Messiano et al., 2010).

The synthesis of aryltetralone (**4** and **11**), aryltetralol (**12**), and aryltetralene (**13**) lignans was achieved from four to six steps, successively, starting from stable and easily available materials. Improving the scheme proposed by Müller and Vajda (1952), via Reformatsky, by using veratrylacetone and α-bromopropianate,

Table 1
^1H NMR spectroscopic data for compounds **1** and **2** ($CDCl_3$, 11.7 T).

Position	**1**		**2**	
H	δ_H (J in Hz)[a]	gNOESY	δ_H (J in Hz)[a]	gNOESY
3	6.28, d (1.0)	7′	6.29, d (0.5)	7′
6	6.78, s	7, OCH_3-5	6.68, s	7, OCH_3-5, OCH_3-7
7	4.38, d (4.0)	6, 8, 9	3.92, d (3.5)	6, 8, 9, OCH_3-7
8	1.99, ddq (4.0, 3.5, 7.5)	7, 9, 8′, 9′	2.19, ddq (3.5, 3.5, 7.0)	7, 9, 8′, OCH_3-7
9	0.83, d (7.5)	7, 8, 7′, 9′	0.79, d (7.0)	7, 8, 7′, 9′
2′	6.55, d (2.0)	7′, 8′, OCH_3-3′	6.55, d (2.0)	7′, 8′, OCH_3-3′
5′	6.70, d (8.0)	OCH_3-4′	6.71, d (8.0)	OCH_3-4′
6′	6.56, dd (8.0, 2.0)	5′, 7′, 8′	6.63, dd (8.0, 2.0)	7′
7′	3.36, br d (9.5)	3, 9, 2′, 6′, 9′	3.30, br d (10.5)	3, 9, 2′, 6′, 9′
8′	2.32, ddq (9.5, 3.5, 7.0)	8, 2′, 6′, 9′	2.35, ddq, (10.5, 3.5, 7.0)	8, 2′, 9′
9′	0.81, d (7.0)	8, 9, 8′	0.81, d (7.0)	8′
OCH_3-3′	3.73, s	2′	3.73, s	
OCH_3-4′	3.78, s	5′	3.80, s	
OCH_3-5	3.81, s	6	3.84, s	
OCH_3-7			3.40, s	7, 8
OH-4	5.49, br s		5.37, br s	

[a] Multiplicities were determined with the assistance of ^1H–^1H COSY spectra and simulation using ACD/C + ^1H NMR predictors (ACD, 2010).

Table 2
^{13}C NMR spectroscopic data for compounds **1** and **2** ($CDCl_3$, 11.7 T).

Position	**1**		**2**	
H	δ_C, type[a]	gHMBC[b]	δ_C, type[a]	gHMBC[b]
1	128.6, C	H-3, 7, 7′	126.0, C	H-3
2	133.0, C	H-6, 7, 7′	134.0, C	H-6
3	115.6, CH	H-7′	115.5, CH	
4	145.3, C	H-3 and/or H-6	145.3, C	H-6
5	145.4, C	H-3 and/or H-6, OCH_3-5	144.9, C	H-3, OCH_3-5
6	111.4, CH	H-7	112.2, CH	H-7
7	74.1, CH	H-9	83.4, CH	H-6, 9, OCH_3-7
8	39.4, CH	H-9	34.6, CH	H-7′, 9′
9	11.7, CH_3	H-7	11.1, CH_3	
1′	138.1, C	H-5′, 7′	138.4, C	H-5′
2′	112.4, CH	H-6′, 7′	112.2, CH	H-6′, H-7′′
3′	148.9, C	H-5′, OCH_3-3′	148.9, C	H-5′, OCH_3-3′
4′	147.5, C	H-2′, 6′, OCH_3-4′	147.4, C	H-2′, 6′, OCH_3-4′
5′	111.0, CH		110.8, CH	
6′	121.7, CH	H-2′, 5′, 7′	121.8, CH	H-2′
7′	48.7, CH	H-3, 2′, 6′, 9′	48.8, CH	H-3, 2′, 9′
8′	35.1, CH	H-7, 9, 7′	35.3, CH	
9′	16.8, CH_3	H-7′	17.2, CH_3	H-7′
OCH_3-3′	55.9, CH_3		56.0, CH_3	
OCH_3-4′	55.9, CH_3		55.9, CH_3	
OCH_3-5	55.9, CH_3		55.9, CH_3	
OCH_3-7			56.0, CH_3	

[a] Chemical shifts and multiplicities were determined with the assistance of DEPT and gHMQC experiments.
[b] gHMBC correlations, optimized for 8 Hz, are from carbon stated to the indicated hydrogen(s).

two pairs of aryltetralone enantiomers (**4** + **11**) were obtained, after HPLC separation, in four steps with good yield (87.5%). The major bottleneck in **4** + **11** synthesis was the lactonization after Reformatsky reaction. By extracting the intermediate lactone (**9**) with $CHCl_3$ instead of distilling it (Müller and Vajda, 1952), the yield of this step increased from 28% to 99.8%.

After separation by HPLC, each pair of enantiomers (**4** and **11**) was evaluated *in vitro* for their antiplasmodial activity. The immunoenzymatic test with monoclonal antibodies to the parasite protein histidine- and alanine-rich (HRPII), as shown in SI, was used to assess the inhibition of growth of *P. falciparum* in the presence of these compounds (Krettli et al., 2009; Noedl et al., 2002). Enantiomers **11** and enantiomers **4** showed to be inactive ($IC_{50} \geq 82$ μM, Table S1), suggesting that the stereochemistry is very important for the antiplasmodial activity of this type of lignans. Aiming to obtain pure enantiomers to advance in the biological tests and chemical transformations, to evaluate the importance of C-7 carbonyl group for the activity, samples of **4** were analyzed by chiral-HPLC by using several conditions in analytical scales (columns, solvents and flows). Unfortunately, no suitable condition to separate the enantiomers **4** in a semi-preparative scale was achieved. Thus, samples of **4** + **11** and the natural aryltetralone **4a** [(−)-8′-*epi*-aristoligone] were individually subjected to reduction with $NaBH_4$ in methanol to give **12** and **12a**, respectively. The structure of **12a** was confirmed by comparison of its 1D and 2D NMR spectra and optical activity ($[\alpha]_D = -27$ ($CHCl_3$, *c* 0.10)) with those of an authentic sample of (−)-aristoligol (**12a**) $[\alpha]_D = -20$ ($CHCl_3$, *c* 0.10) (da Silva and Lopes, 2006). Interestingly, the stereochemistry of C-7 of **12a** is different from that of the natural aryltetralol (**1**). Finally, subjecting **12** and **12a** to dehydration with *p*-toluenesulfonic acid, the products **13** and **13a** were obtained in 83.3% and 85% yield, respectively (Fig. 2).

A better chromatographic condition, with lower t_R, was achieved for the separation of the enantiomers **13** than for the aryltetralones **4** using chiral columns (Fig. S5). Thus, **13** was subjected to semi-preparative chiral-HPLC to give (−)-cyclogalgravin **13a** ($t_R = 14.3$ min, 50.9%) and (+)-cyclogalgravin **13b** ($t_R = 13.4$ min, 49.1%).

The similarity of the ^1H and ^{13}C NMR data and optical activities of **13a** ($[\alpha]_D = -96$ ($CHCl_3$; *c* 0.21), $[\theta]_{290} - 10330$) with those from the natural product ($[\alpha]_D = -106$ ($CHCl_3$; *c* 1.07), $[\theta]_{290} - 1254$) (da Silva and Lopes, 2006) confirmed the assigned relative and absolute configurations for **13a**.

The compound **12a** and **13a** exhibited the highest activity (IC_{50} 8.4 and 10.8 μM, respectively), whereas **13b** showed moderate activity (IC_{50} 30 μM) (Table S1). The natural aryltetralols **1** and **5** also showed moderate activity (IC_{50} 33.6 and 33.1 μM, respectively), while the C-7 OCH_3 analogous **2** and **6** were inactive (Table S1). These results suggest that steric effects also have influence on the activity. Among the tested lignans, **12a** and **13a** exhibited good selectivity index (SI > 28).

3. Experimental

3.1. General experimental procedures

One-dimensional (^1H, ^{13}C, DEPT, HOMODEC, TOCSY, and gNOESY) and two-dimensional (^1H–^1H gCOSY, gNOESY, gHMQC, gHSQC, and gHMBC) NMR experiments were performed on a Varian INOVA 500 spectrometer (11.7 T) at 500 MHz (^1H), and 126 MHz (^{13}C), using deuterated solvents ($CDCl_3$ and DMSO-d_6) (P 99.9% D) as an internal standards for ^{13}C NMR chemical shifts and residual solvent as an internal standard for ^1H NMR. δ values are reported relative to TMS. Mass spectra (ESI-MS) were obtained on a LCQ *Fleet*—Thermo Scientific, and flow injection into the electrospray source was used for LC-ESI-MS. High-resolution mass spectra (HRMS) were obtained on a Bruker Daltonics ultrOTOFq (ESI-TOFMS). IR spectra were obtained on a PerkinElmer 1600 FT-IR spectrometer using KBr discs. Optical rotations were measured on a PerkinElmer 341-LC polarimeter. Ultraviolet (UV) absorptions were measured on a PerkinElmer UV–vis Lambda 14P diode array spectrophotometer. Circular dichroism (CD) spectra were recorded on a JASCO J-815 spectrometer, using 0.2 mm cell. HPLC analyses were performed using a Shimadzu liquid chromatograph (SPD-10 Avp), equipped with UV–vis and 341-LC polarimeter detectors, and using a Jasco LC-NetII/ADC, equipped with photodiode array (MD-2018 Plus) and CD (2095 Plus) detectors, and chromatograms were acquired at 270 nm and 254 nm. The columns RP-18 (C18, Varian), Chiralpak® IC (DAICEL), Lux 5 μ Cellulose-1 (Phenomenex®), and β-CD BR Chiralpak® (YMC) having a particle size of 5 μm, were used for analytical analysis (250 × 4.6 mm) and for semi-preparative analysis (250 × 10 mm). All reactions were monitored by TLC using 0.25 mm E. Merk silica gel plates (60 PF_{254}) with UV, I_2 vapor, or 10% H_2SO_4—heat as developing agent. All reactions were carried out under N_2 atmosphere with freshly distilled solvents under anhydrous conditions unless otherwise noted. Reagents were purchased from Sigma–Aldrich Co., and used without purification, except where noted. Solvents employed were HPLC grade from Mallinckrodt. All yields refer to chromatographically and spectroscopically (^1H and ^{13}C NMR, α_D) homogenous material unless otherwise stated. Ultrapure water was obtained from Milli-Q Gradient A10 from Millipore.

3.2. Plant materials

The plant materials were collected in Ituiutaba, MG, Brazil, in February 2010, and identified as *Holostylis reniformis* Duch. by Dr. Vinícius C. Souza and Dr. Lindolpho Cappellari Jr. A voucher specimen (ESA 110,744/2010) was deposited at the herbarium of the Escola Superior de Agricultura, Luiz de Queiroz (ESALQ), Piracicaba, SP, Brazil. Authorization CGEN/MMA number 10,586/2012-1. The material was separated according to the plant parts and dried (~45 °C).

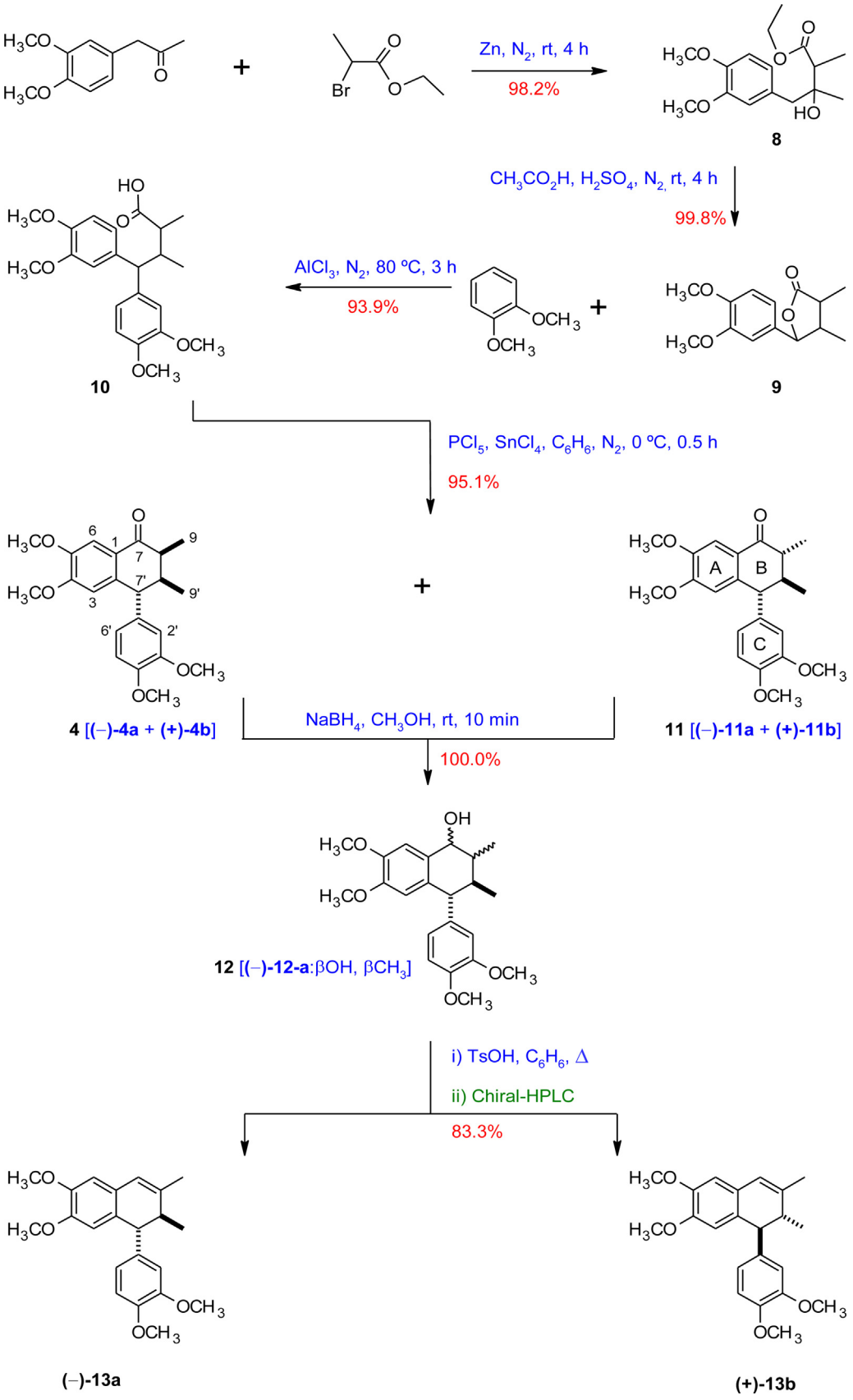

Fig. 2. Total syntheses of aryltetralenes **13a** and **13b.**

3.3. Extraction and isolation of the chemical constituents

The roots (3.7 kg) were ground and exhaustively extracted successively at room temperature with hexanes, acetone, and ethanol [4 × (~200 mL, 2 days and shaken manually every 12 h for 2 min) each solvent] (da Silva and Lopes, 2006; Messiano et al., 2009). The residues were extracted with ethanol in a Soxhlet apparatus and the extracts were individually concentrated.

The crude acetone extract (6.6 g) was subjected to CC (40.0 × 5.0 cm, silica gel 60H, 203.5 g, *n*-hexanes/EtOAc gradient, 19:1 to 100% EtOAc) to give 31 fractions (*ca.* 120 mL each), as previously described (da Silva et al., 2004). After semi-preparative HPLC (C18, MeOH/H_2O, 7:3, flow rate: 8 mL/min) fractions 16 and 19 gave **3** (8.2 mg) and **4a** (1140.0 mg), respectively. Fraction 23 (280.0 mg) gave **1** (43.5 mg) and **2** (8.9 mg) after semi-preparative HPLC (C18, MeOH/H_2O, 3:2, flow rate: 8 mL/min).

3.3.1. (−)-(7R,7′R,8S,8′S)-4,7-Dihydroxy-3′,4′,5-trimethoxy-2,7′-cyclolignan [(−)-4-O-demethyl-7-hydroxyisogalbulin, **1**]

Yellow oil ($CHCl_3$); $[\alpha]_D^{25}$ −38 (*c* 0.7, $CHCl_3$); UV λ_{max}^{MeOH} see Fig. S6; IR (KBr) ν_{max} 3434, 1590, 1513, 1463 cm^{-1}; ^1H and ^{13}C NMR ($CDCl_3$) see Tables 1 and 2; ESIMS 20 eV, positive mode, *m*/*z* (rel. int.): 359 $[M+H]^+$ (100); HRESIMS (probe) 4.5 eV, positive mode, *m*/*z* (rel. int.): 381.1663 $[M+Na]^+$ (100) (calcd for $C_{21}H_{26}O_5Na$, 381.1672); CD ($CHCl_3$, *c* 0.50): $[\theta]_{307}$ −5626, $[\theta]_{296}$ +22895, $[\theta]_{279}$ −44311, $[\theta]_{260}$ −14739, $[\theta]_{241}$ −141569.

3.3.2. (7R,7′R,8S,8′S)-4-Hydroxy-3′,4′,5,7-tetramethoxy-2,7′-cyclolignan [(−)-4-O-demethyl-7-methoxyisogalbulin, **2**]

Yellow oil ($CHCl_3$); $[\alpha]_D^{25}$ −26 (*c* 0.6, $CHCl_3$); UV λ_{max}^{MeOH} see Fig. S6; IR (KBr) ν_{max} 3446, 1590, 1520, 1430 cm^{-1}; ^1H and ^{13}C NMR ($CDCl_3$) see Tables 1 and 2; HRESIMS (probe) 4.5 eV, negative mode, *m*/*z* (rel. int.): 371.1445 $[M-H]^-$ (100) (calcd for $C_{22}H_{27}O_5$, 371.1853); CD ($CHCl_3$, *c* 0.57): $[\theta]_{297}$ +5494, $[\theta]_{278}$ −18353, $[\theta]_{245}$ −113950.

3.3.3. (7R,7′R,8S,8′S)-7-Hydroxy-3′,4′,4,5-tetramethoxy-2,7′-cyclolignan [(−)-holostylol, **12a**]

Yellow oil ($CHCl_3$); $[\alpha]_D^{25}$ −28 (*c* 0.10, $CHCl_3$) lit. (da Silva et al., 2006) $[\alpha]_D^{25}$ −20 (*c* 1.6, $CHCl_3$). ^1H NMR ($CDCl_3$, 500 MHz): δ 6.16 (1H, s, H-3), 7.08 (1H, s, H-6), 4.93 (1H, d, *J* = 4.5 Hz, H-7), 2.16 (1H, ddq, *J* = 3.0, 4.5, 7.0 Hz, H-8), 0.86 (3H, d, *J* = 7.0 Hz, H-9), 6.48 (1H, d, *J* = 2.0 Hz, H-2′), 6.72 (1H, d, *J* = 8.0 Hz, H-5′), 6.58 (1H, dd, *J* = 8.0, 2.0 Hz, H-6′), 3.48 (1H, d, *J* = 10.0 Hz, H-7′), 2.01 (1H, ddq, *J* = 3.0, 10.0, 7.0 Hz, H-8′), 0.84 (3H, d, *J* = 7.0 Hz, H-9′), 3.54 (s, OCH_3-4), 3.84 (s, OCH_3-5), 3.74 (s, OCH_3-3′), 3.81 (s, OCH_3-4′).

3.3.4. (7′R,8′S)-3′,4′,4,5-Tetramethoxy-2,7′-cyclolignan-7-eno [(−)-cyclogalgravin, **13a**]

Yellow oil ($CHCl_3$); $[\alpha]_D^{25}$ − 103 (*c* 0.10, $CHCl_3$), lit. (da Silva et al., 2006) $[\alpha]_D^{25}$ −106 (*c* 1.07, $CHCl_3$). ^1H NMR ($CDCl_3$, 300 MHz): δ 6.49 (1H, s, H-3), 6.56 (1H, s, H-6), 6.08 (1H, br s, H-7), 1.73 (3H, d, *J* = 1,2 Hz, H-9), 6.60 (1H, d, *J* = 2.1 Hz, H-2′), 6.65 (1H, d, *J* = 8.4 Hz, H-5′), 6.49 (1H, dd, *J* = 8.4, 2.1 Hz, H-6′), 3.62 (1H, d, *J* = 3.3 Hz, H-7′), 2.33 (1H, dq, *J* = 7.2, 3.3 Hz, H-8′), 1.02 (3H, d, *J* = 7.2 Hz, H-9′), 3.76 (s, OCH_3-4), 3.82 (s, OCH_3-5); 3.72 (s, OCH_3-3′), 3.72 (s, OCH_3-4′).

3.4. Syntheses and chemical transformations

3.4.1. Benzenebutanoic acid, β-hydroxy-3,4-dimethoxy-α,β-dimethyl-, ethyl ester (**8**):

α-Bromopropionate (2.89 g, 17.4 mmol) was dropwise added to a stirred solution of veratrylacetone (3.08 g, 17.2 mmol) in 9 mL of dry benzene (under N_2) and zinc (previously washed with acetone and activated at 100 °C for 8 h (Chavan, 2004)). The stirring solution was heated until the reflux began, this being maintained for 4 h. Then, the mixture was cooled and the ester (4.24 g, 16.89 mmol, 98.2%) extracted with $CHCl_3$ (3 × 50 mL).

3.4.2. α,β-Dimethyl-γ-(3,4-dimethoxyphenyl) butyrolactone (**9**)

The ester (3.92 g, 15.6 mmol) was dissolved in CH_3CO_2H (13 mL) and then ice-cooling. To this cooling and stirring solution sulfuric acid (3 mL) was added by drops over 1 h. After 4 h on steam-bath, the organic portion was successively extracted with $CHCl_3$ (3 × 50 mL), neutralized with $NaHCO_3$, washed with water and dried ($CaCl_2$), and concentrated at reduced pressure to give the lactone **9** (3.43 g, 15.6 mmol, 99.8%).

3.4.3. 4,4-Bis(3,4-dimethoxyphenyl)-2,3-dimethyl-butyric acid (**10**)

A solution of $AlCl_3$ (2.23 g, 16.8 mmol) in veratrol (1 mL, 7.97 mmol) was slowly dropwise to veratrol (1 mL, 7.97 mmol, 15 min) and the lactone **9** (2.2 g, 8.78 mmol). The resulting mixture was then stirred at 80 °C for 3 h, and then the complex was decomposed by dropwise addition of EtOH (5 mL) at room temperature, followed by addition of 10% HCl (10 mL). After disappearance of solid particles, the mixture was extracted with $CHCl_3$ (3 × 50 mL), washed with 8% $NaHCO_3$ (3 × 50 mL) and then with 10% HCl until a white solution was obtained. The removal of solvents under reduced pressure yielded the intermediary acid **10** (3.20 g, 8.24 mmol, 93.8 %).

3.4.4. 8′-epi-Aristoligone and aristoligone (**4** + **11**)

The acid **10** (3.02 g, 8.1 mmol) was dissolved in dry benzene (6 mL), then PCl_5 (1.98 g, 9.5 mmol) was added to this solution, which was kept stirring in ice-bath for 30 min. Then, the stirring mixture was warmed up to 40 °C and occasionally shacked until the reactants dissolved. After that, the mixture was chilled and a solution of $SnCl_4$ (2.84 g, 10.9 mmol) in dry benzene (4 mL) was added to it. In a few minutes, a red precipitate was observed, which was dissolved with concentrate HCl. A mixture comprising the enantiomer pairs **4** + **11** (2.85 g, 7.7 mmol, 95.1%) was obtained by extraction with $CHCl_3$, followed by removal of the solvents.

3.4.5. Aryltetralol **12**

To a stirring solution of **4** + **11** (2.00 g, 5.40 mmol) in MeOH (20 mL) a methanol solution of $NaBH_4$ (204.2 mg, 5.40 mmol, 20 mL) was added. The mixture was stirred in an ice-bath for 4 h. Following reduction, the excess of $NaBH_4$ was quenched by dropwise addition of MeOH and water. The organic phase resulting from EtOAc (3 × 30 mL) extraction was washed with water, dried ($MgSO_4$), and concentrated to yield **12** (2.01 g, 5.40 mmol, 100.0%).

3.4.6. (−)-(7′R,8′S)- and (+)-(7′S,8′R)-3′,4′,4,5-Tetramethoxy-2,7′-cyclolignan-7-ene [(−)-cyclogalgravin, **13a** and (+)-cyclogalgravin, **13b**]

To a stirring solution of the alcohol 1**2** (1.93 g, 5.20 mmol) in dry benzene (15 mL) TsOH (0.72 g, 0.40 mmol) was slowly added. After stirring under reflux, this solution was cooled, extracted with $CHCl_3$ and the solvent removed under reduced pressure to give **13** (1.53 g, 4.33 mmol, 83.3%). This product was subjected to chiral-HPLC analysis [cellulose tris-(3,5-dichlorophenylcarbamate column) to give (−)-**13a** (0.78 g, 2.20 mmol, ee > 99.9%), and (+)-**13b** (0.75 g, 2.13 mmol, ee > 99.9%).

(−)-**13a**: yellow oil ($CHCl_3$); $[\alpha]_D^{25}$ −96 (c 0.21, MeOH); (+)-**13b**: Yellow oil ($CHCl_3$); $[\alpha]_D^{25}$ +106 (c 0.20, MeOH), lit. (Messiano et al., 2010)] $[\alpha]_D^{25}$ −106 (c 1.07, $CHCl_3$); CD ($CHCl_3$, c 0.3): $[\theta]_{296}$ +24783, $[\theta]_{290}$ +10406, $[\theta]_{274}$ +71184, $[\theta]_{249}$ −15366, $[\theta]_{240}$ +543, $[\theta]_{226}$ +71706, $[\theta]_{220}$−52760, $[\theta]_{210}$ −153856, $[\theta]_{201}$ +113946.

*3.4.7. HPLC separation of **4** + **11***

Aliquots (30 mg) of **4** + **11** were subjected to semi-preparative HPLC (20 times) using RP-C18 ODS Chrompack column, UV (λ 254 nm) detector, flow rate of 8 mL/min, and MeOH/H_2O (7:3, v/v) as mobile phases.

*3.4.8. Chiral-HPLC analysis of **4** (**4a** + **4b**) and **13** (**13a** + **13b**)*

Aryltetralone (**4**, 4 mg) and aryltetralene (**13**, 4 mg) lignans were subjected to HPLC (44 times each sample) using Chiralpak® IC column, UV (λ 270 nm) and polarimeter (λ 365 nm) detectors, flow rate of 0.5 mL/min and 0.7 mL/min for **4** and **13**, respectively, and *n*-hexanes/EtOAc (17:3, v/v for **4** and 87:13, v/v for **13**) as mobile phases (Fig. S5).

4. Conclusions

Two new lignans were isolated from *H. reniformis*. Aryltetralone, aryltetralol, and aryltetralene lignans were synthesized by a regioselective route in four, five, and six steps, successively, with good overall yields (81.0%, 80.9%, and 67.6%). (−)-Cyclogalgravin (**13a**) and (−)-aristoligol (**12a**) exhibited activity ($IC_{50} \sim 10.8$ and 8.4 μM, respectively), the latter exhibited lower toxicity.

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Appendix A. Supplementary data

Supplementary data associated with this article can be found, in the online version, at http://dx.doi.org/10.1016/j.phytol.2015.06.001.

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