



Biological Activities of Lignoids from Amazon Myristicaceae Species: *Virola michelii*, *V. mollissima*, *V. pavonis* and *Iryanthera juruensis*[#]

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O trabalho consiste na re-investigação dos frutos de *Iryanthera juruensis* e *Virola pavonis* e das folhas de *V. michelii*, bem como no estudo dos frutos de *V. mollissima*. A partir de *I. juruensis* foram isoladas uma neolignana ariltetralínica (**1**) e uma neolignana tetraidrofurânica (**2**), enquanto que de *Virola pavonis* foram isoladas neolignanas benzofurânicas (**6-9**), tetraidrofurânicas (**2**, **11-13**, **17**), bifênica (**10**), diastereoisômeros de uma hidróxi-neolignana 8.O.4' (**14-15**) e outras. *V. mollissima* acumula a neolignana ariltetralônica **4** ou o seu derivado *seco* (**5**). As folhas de *V. michelii* apresentaram a ocorrência de lignanas furofurânicas (**18-19**). Os lignóides **1** e **2** obtidos dos arilos de *I. juruensis* apresentaram atividade leishmanicida contra a forma promastigota de *Leishmania amazonensis*.

This work revisits the fruits of *Iryanthera juruensis* and *Virola pavonis* and the leaves from *V. michelii*, as well as describing a study of the fruits of *V. mollissima*. In *I. juruensis* aryltetraline neolignan (**1**) and tetrahydrofuran neolignan (**2**), were found while from *V. pavonis* neolignans of benzofuran type (**6-9**), the tetrahydrofuran type (**2**, **11-13**, **17**) and the biphenyl type (**10**), in addition to diastereoisomers of the 8.O.4'-oxyneolignan type (**14** and **15**) and others were isolated. The *V. mollissima* accumulates the aryltetralone neolignan **4** and its *seco* derivative (**5**). The lignoids **1** and **2** obtained from *I. juruensis* arils possess antileishmanial activity against the promastigote form of *Leishmania amazonensis*.

Keywords: lignoids, *Iryanthera juruensis*, *Virola michelii*, *Virola mollissima*, *Virola pavonis*, Myristicaceae

Introduction

Myristicaceae is a botanical family composed of 18 genera and about 500 species with pantropical

distribution. The species are normally trees, exceptionally shrubs, frequently found in lowland forests. In Brazil, this family is represented by the genera *Compsonoura*, *Iryanthera*, *Osteophloeum*, *Otoba* (syn. *Dialyanthera*) and *Virola*, which are concentrated in the Amazon region, where the myristicaceous species are popularly known as “*ucuúba*”.^{1,2}

The species belonging to this family became the target of intense study when Schultes reported that some indigenous tribes of the Amazon used the barks and resins

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[#] This paper is dedicated to Prof. Otto R. Gottlieb, who made an important phytochemical contribution to plant species from the Amazon rain forest, describing the occurrence of neolignans in Lauraceae and Myristicaceae species (Gottlieb, O. R.; Yoshida, M.; Lignans, in Natural Products of Woody Plants: Chemicals Extraneous to the Lignocellulosic Cell Wall, J.W. Rowe, ed., Springer-Verlag, Berlin, 1989, ch. 7.3, pp. 439-511).

of *Virola* spp. for the preparation of hallucinogenic snuffs and arrow poisons for hunting.¹

The phytochemical investigations on myristicaceous species have shown the accumulation of a variety of lignoids in their tissues, which have relevant pharmacological activities that are well documented in the literature.^{3,4} While there are several studies about the chemical composition of Amazon Myristicaceae species, the biological activities of these species could be better studied. This paper aims to contribute to the chemosystematics of Myristicaceae species and to assay some biological activities *in vitro*, by reporting a phytochemical re-investigation of the fruits from *Iryanthera juruensis* and *Virola pavonis* and of the leaves from *Virola michelii*, as well as a new study of the fruits of *Virola mollissima* as well as the results of biological assays of isolated lignoids.

Experimental

General

¹H and ¹³C NMR spectra were recorded on spectrometers from Bruker AC-200 and Varian INOVA 200, 4.7 T, operating at 200 MHz and 50 MHz, respectively, Varian INOVA 300, 7.4 T, operating at 300 MHz and 75 MHz, respectively, and Varian INOVA 500, 11.7 T, operating at 500 MHz and 125 MHz, respectively. The samples were dissolved in CDCl₃ or CD₃CN, purchased from Aldrich or CIL.

Plant material

About 1.0 kg of ripe fruits from *I. juruensis* Warburg were collected on the campus of the Universidade Federal do Amazonas (March 2003), in Manaus-AM, while the fruits of *V. mollissima* (Poepp. ex A. DC.) Warburg and *V. pavonis* (A. DC.) A.C. Smith were obtained from the Adolpho Ducke Reserve, Manaus-AM, in November 2003, and March 2004, respectively. The botanical materials collected were identified by José Ferreira Ramos. *Virola michelii* Heckel leaves were collected on Campus I of the Instituto Nacional de Pesquisas da Amazônia (INPA), Manaus-AM, in December 2004. Vouchers were deposited at the INPA herbarium under the numbers: 213356 (I.j.), 179760 (V.mo.), 214917 (V.p.) and 215688 (V.mi.).

Extraction and isolation

The ripe fruits (*I. juruensis*, *V. mollissima* and *V. pavonis*) were manually separated into pericarps and seeds and the latter into arils, seed coats and almonds, which were dried,

milled and macerated individually with hexane followed by EtOH, during a total period of fourteen days. After the extractions, the solutions were concentrated under reduced pressure yielding their hexane and EtOH extracts. The crude hexane extracts were dissolved in 9:1 MeOH:water, and submitted to partition with hexane, yielding the hexane and hydromethanolic fractions. Similarly, the crude EtOH extracts were partitioned with hexane, CHCl₃ and EtOAc, generating four additional fractions.

The CHCl₃ residue of the EtOH extract from *I. juruensis* arils (960.0 mg) was submitted to column chromatography on silica gel eluting with hexane:EtOAc in mixtures of increasing polarity, affording 20 fractions that were analyzed by TLC and pooled into 4 subfractions (IJ-A1 to IJ-A4). The subfraction coded IJ-A2 (422.0 mg) gave a white precipitate which was identified as guaiacin (**1**, 136.0 mg),⁵ while IJ-A3 (52.0 mg) was fractionated by preparative TLC (silica gel and hexane:EtOAc 7:3) providing verrucosin (**2**, 18.0 mg).⁶

The hydroalcoholic residue of the hexane extract of *V. mollissima* pericarps (2.5 g) was fractionated by silica gel column chromatography and eluted with hexane:EtOAc of increasing polarity, resulting in the isolation of grandisin (**3**, 13.8 mg).^{7,8} The hexane residue of the EtOH extract from the arils (3.84 g) showed formation of crystals, which recrystallized in CH₂Cl₂ afforded hydroxy-oxo-otobain (**4**, 11.0 mg).^{9,10} The precipitate (555.0 mg) of the hexane extract *V. mollissima* arils with hot MeOH was submitted to column chromatography on silica gel and eluted with hexane:EtOAc of increasing polarity, yielding 67 fractions which were pooled into four groups (VM-A1 to VM-A4) after TLC analysis. The purification of VM-A4 by preparative TLC yielded *seco*-otobain (**5**, 12.0 mg).⁹

The CHCl₃ residue of the EtOH extract from *V. pavonis* arils (2.4 g) was first fractionated by column chromatography with silica gel and eluted with hexane:EtOAc of increasing polarity. This procedure yielded 17 fractions that were reduced to 5 fractions according their TLC profiles (VP-A1 to VP-A5). Fraction VP-A1 (479.0 mg) was submitted to preparative TLC, and eluted with 98:2, CHCl₃:EtOAc to afford carinatin (**6**, 133.0 mg), carinatidin (**7**, 48.0 mg), dihydrocarinatin (**8**, 103.0 mg) and dihydrocarinatidin (**9**, 60.0 mg).¹¹ Fraction VP-A2 (159.0 mg), through preparative TLC (silica gel and 4:1 hexane:EtOAc), led to the isolation of dehydrodieugenol (**10**, 24.0 mg).¹² After several steps to column chromatography and preparative TLC, fraction VP-A3 (197.0 mg) gave three tetrahydrofuran neolignans: galgravin (**11**, 16.0 mg),¹³ nectandrin A (**12**, 7.5 mg)¹⁴ and galbelgin (**13**, 12.0 mg).¹⁵ Fraction VP-A4 (43.0 mg) was submitted to preparative TLC using 85:15 hexane:EtOAc provided the *erythro* (**14**, 5.4 mg) and the *threo* (**15**,

3.0 mg) isomers of an oxyneolignan.¹⁶ VP-A5 (54.0 mg) provided verrucosin (**2**, 3.3 mg) after fractionation by column chromatography over silica gel and elution with hexane:EtOAc of increasing polarity. The CHCl_3 residue of the EtOH extract from *V. pavonis* pericarps fractionated by column chromatography and eluted with hexane:EtOAc at increasing polarity provided the fractions VP-P1 to VP-P4. The purification of VP-P2 and VP-P3 by preparative TLC with hexane:Me₂CO 4:1 as eluent provided carinatone (**16**, 4.5 mg)¹⁷ and nectandrin B (**17**, 3.0 mg),⁶ respectively.

The ground leaves of *V. michelii* (730.0 g) were extracted for six hours in a Soxhlet apparatus, first with hexane and then with EtOH. After that, the solutions were concentrated under reduced pressure yielding 28.4 g of hexane extract and 89.5 g of EtOH extract. Then, the crude EtOH extract was dissolved in MeOH:water 9:1, and partitioned with hexane and EtOAc yielding three residues. After TLC analysis, the EtOAc residue (29.2 g) was submitted to column chromatography using silica gel and CHCl_3 :MeOH in mixtures of increasing polarity, collecting 13 fractions. Fractions 1-5, after fractionation on preparative TLC using for elution the systems: hexane:EtOAc (98:2), hexane:Et₂O (8:2) and CHCl_3 : Me₂CO (9:1), allowed the isolation of eudesmin (**18**, 27.3 mg)¹⁸ and phylligenol (**19**, 4.8 mg).¹⁹

Biological assay

Antioxidant assay

The assay was performed with DPPH (Sigma-Aldrich) in MeOH solution and the 50% effective dose (ED_{50}) was determined following the procedure described by Chang *et al.*²⁰

In vitro antileishmanial assay

Promastigote forms of *Leishmania amazonensis* (WHO/BR/00/LT0016), *L. braziliensis* (MHOM/BR/75/M2903) and *L. chagasi* (MHOM/BR/72/strain 46) were maintained in M199 liquid media supplemented with 10% fetal calf serum²¹ and 2% human urine²² at 26 °C. About 1×10^7 promastigote cells were separately incubated in 24-well plates at 26 °C for 72 h in the presence of increasing concentrations of samples. The number of cells in each culture was estimated by counting in a Neubauer chamber.

Results and Discussion

The use of adsorption chromatographic methods allowed the isolation and identification of two lignoids from the fruits of *Iryanthera juruensis*, three from *Virola mollissima*, twelve from *V. pavonis* and two from *V. michelii* leaves. The identification of isolated compounds was based

especially on interpretations of their ¹H and ¹³C NMR spectra and compared with reported data, since these are known compounds.

As described in the literature,²³ species belonging to the genus *Virola* accumulate lignoids with a great variety of carbon skeleta. In *I. juruensis* Warburg aryltetralone neolignan (**1**) and a tetrahydrofuran lignan (**2**) were found. *V. mollissima* fruits presented the occurrence of the aryltetralone neolignan **4** and its *seco* derivative (**5**). From *V. pavonis* (A. DC.) A. C. Smith neolignans of benzofuran type (**6** and **7**), dihydrobenzofuran type (**8** and **9**), tetrahydrofuran type (**2**, **11-13**, **17**) and biphenyl neolignan (**10**) were isolated, besides two diastereomeric forms of 8.O.4'-oxyneolignan (**14** and **15**). The *V. michelii* leaves afforded two furofuran lignans, **18** and **19**.

The NMR data of **5** and **19** allowed the unambiguous assignment of chemical shifts. The structure of *seco*-lignoid **5** was suggested⁹ to be produced by a retro Friedel-Crafts reaction from aryltetralone neolignan. In this work, the extensive studies using NMR techniques such as gCOSY, gHSQC and gHMBC allowed the correct assignment agreeing with those previously described (see Table 1). Through a 1D nOe (Figure 2) experiment with **5** it is possible to observe the effect of the oxy-methylene hydrogens H-1a and H-1b with the methyl hydrogens CH₃-3 and of H-4 with CH₃-2. Figure 2 represents a unique possible conformation of **5**, according to the observed nOe, confirming the stereochemistry previously proposed.

The literature does not register the chemical shifts of the aromatic hydrogens phylligenol **19**, because aromatic hydrogens in the ¹H NMR spectrum of guaiacyl and veratryl rings present continuous absorption. An experiment was performed at 40 °C, using Gaussian transformation on the FID in order to result in better resolution of aromatic hydrogens, where it was possible to observe the aromatic hydrogens (Figure 3). Between δ 6.83-6.94 can be observed hydrogens at δ 6.94 (*d*, *J* 1.5 Hz, H-2), at δ 6.91 (*d*, *J* 2.0 Hz, H-2'), at δ 6.89 (*d*, *J* 8.0 Hz, H-5'), centered at δ 6.86 (H-6 and H-5, close to an A₂ system), δ 6.85 (*d*, *J* 8.0 Hz, H-5), δ 6.85 (*dd*, *J* 8.0 and 2.0 Hz, H-6').

The allocation of the hydrogens from ring furofuran of **16** was confirmed by analysis of its gCOSY spectrum, in which observed correlations are consistent with a spin system of H-7:H-8:H-9a:H-9b, H-7':H-8':H-9'a:H-9'b, besides the correlation between the hydrogens H-8:H-8'. The analysis of this system was based by comparison of chemical shifts of hydrogens H-7 and H-7' with models available in the literature.

In our previous work, we communicated that compound **4** showed promising results in the bioautographic test and in a solid medium quantitative activities against xylophagus

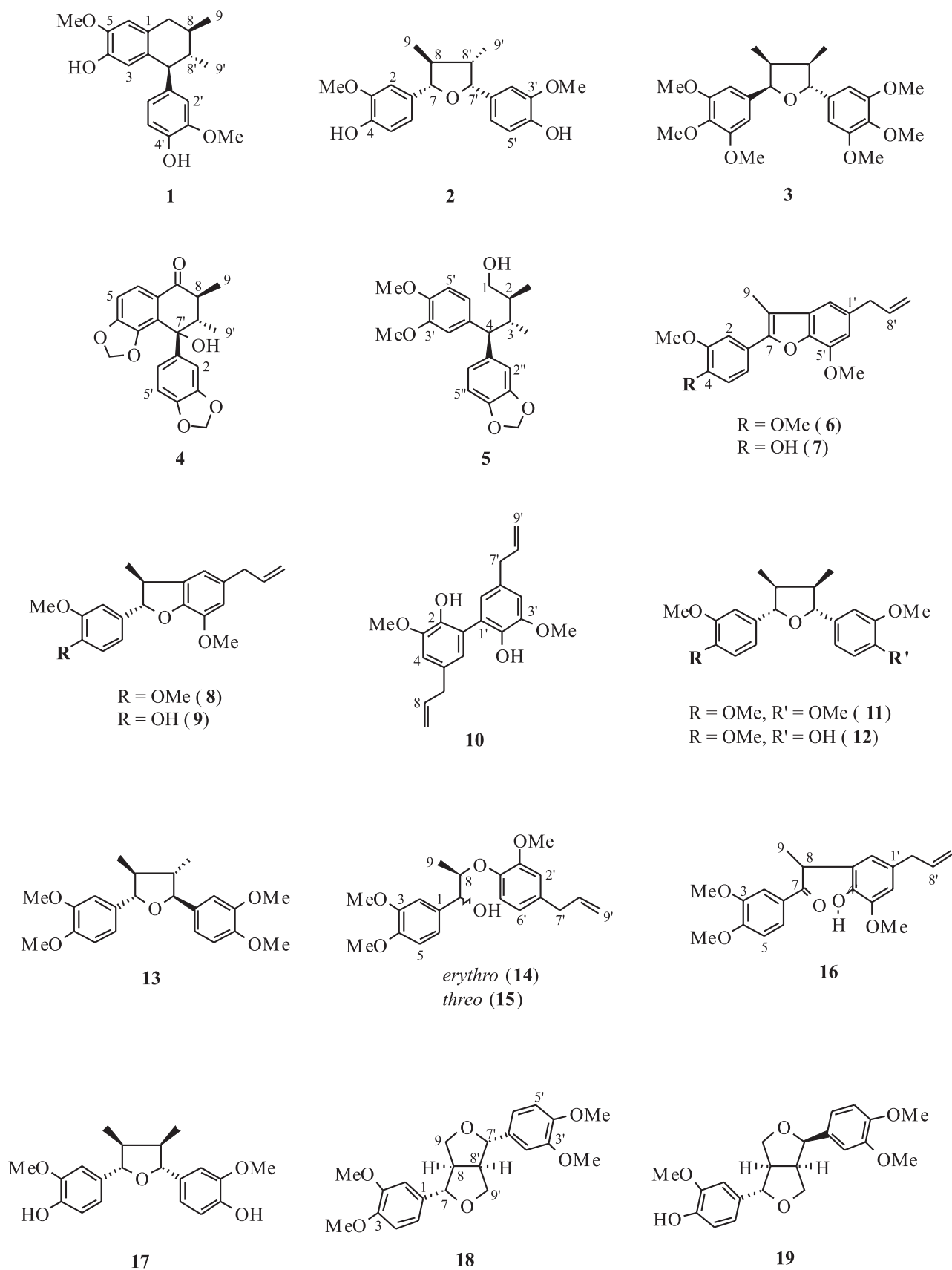
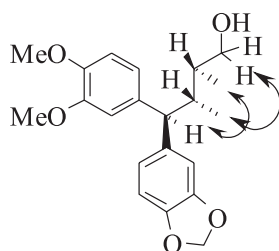


Figure 1. Structures of lignoids.

Table 1. ¹H NMR data (11.7 T, δ , J in Hz) and correlations in 2D diagrams of **5**

Hydrogens	δ / ppm, multiplicity	gCOSY ¹ H (δ)	gHSBC ¹³ C (δ)	gHMBC ¹³ C (δ)
1	3.47 (<i>dd</i> , J 10.4; 6.5 Hz)			
3.51 (<i>dd</i> , J 10.4; 8.2 Hz)		67.2	9.8	
2	1.77 (<i>dddq</i> , J 8.2; 6.5; unresolved; 6.9 Hz)	3.47/3.51	36.3	
3	2.60 (<i>ddq</i> , J 11.9; unresolved; 6.9 Hz)	3.53	36.1	
4	3.53 (<i>d</i> , J 11.9 Hz)		56.2	12.0/108.2/121.1
/111.5/119.7				
2'	6.81 (<i>d</i> , J 2.1 Hz)		111.5	149.2/137.2
5'	6.79 (<i>d</i> , J 8.2 Hz)		111.7	
6'	6.86 (<i>dd</i> , J 8.2; 2.1 Hz)	6.79	119.8	67.2
2''	6.81 (<i>d</i> , J 1.5 Hz)		108.2	148.0
5''	6.70 (<i>d</i> , J 7.9 Hz)	6.76	108.4	139.3
6''	6.76 (<i>dd</i> , J 7.9; 1.5 Hz)		121.1	
2-Me	0.76 (<i>d</i> , J 6.9 Hz)	1.77	12.0	
3-Me	0.70 (<i>d</i> , J 6.9 Hz)	2.60	9.8	
MeO	3.83(<i>s</i>)		56.1	
MeO	3.87 (<i>s</i>)		56.2	
3'',4''-CH ₂ O ₂	5.88 (<i>d</i> , J 1.4 Hz)			
5.90 (<i>d</i> , J 1.4 Hz)		101.1		

**Figure 2.** Observed nOe experiment of **5**.

fungi *Pycnoporus sanguineus*, *Trametes villosa* and *Lenzites work* were determined. This experiment revealed the potential of *V. mollissima* in the development of antifungal products against wood decaying fungi.²⁴

An antioxidant assay by the radical scavenger DPPH was performed on *V. michelii* extract, their fractions and phylligenol. The 50% effective dose (ED₅₀) of 16.0 $\mu\text{g mL}^{-1}$ was found for the crude EtOH extract and for its aqueous EtOH fraction. The EtOAc fraction obtained after partition of crude extract showed a ED₅₀ of 20.4 $\mu\text{g mL}^{-1}$. After purification of the EtOAc fraction by preparative TLC, the major fraction lost the antioxidant activity, with ED₅₀ = 278.0 $\mu\text{g mL}^{-1}$. The phylligenol isolated from this fraction presented ED₅₀ = 108.0 $\mu\text{g mL}^{-1}$. The ED₅₀ determined for quercetin was 5.3 $\mu\text{g mL}^{-1}$.

The lignoids **1** and **2** obtained from *I. juruensis* arils presented higher antileishmanial activity among the assayed lignoids against the promastigote forms of *Leishmania amazonensis* when compared with promastigote forms of *L.*

Table 2. Sensitivity of *Leishmania* sp. promastigote forms to lignoids

Lignoids	<i>L. amazonensis</i> <i>L. braziliensis</i> <i>L. chagasi</i>		
	IC ₅₀ / ($\mu\text{g mL}^{-1}$)		
1	45.0	98.0	184.0
2	27.0	100.0	170.0
6	> 250	> 250	> 250
7	76.0	> 250	> 250
8	100.0	> 250	> 250
9	> 250	> 250	> 250
10	148.0	150.0	> 250
11	> 250	> 250	> 250
12	> 250	> 250	> 250
13	> 250	> 250	> 250
14	239.0	230.0	> 250
15	> 250	> 250	> 250
17	> 250	> 250	> 250

braziliensis and *L. chagasi*, as shown in the Table 2. The 50% inhibition concentration, IC₅₀, was 27.0 and 45.0 $\mu\text{g mL}^{-1}$ of **1** and **2**, observed for *L. amazonensis*, suggests further studies for potential use of these compounds.

Spectrometric data

Rel-(8*R*,7'*S*,8'*S*)-4,4'-dihydroxy-3,3'-dimethoxy- $\Delta^{1,3,5,1',3',5'}$ -8,8',6,7'-neolignan (**1**), *guaiacina*

¹H NMR (200 MHz, CDCl₃), δ : 6.54 (1H, *s*, H-6); 6.26 (1H, *s*, H-3); 2.59 (1H, *dd*, J 15.8; 10.1 Hz, H-7ax); 2.75

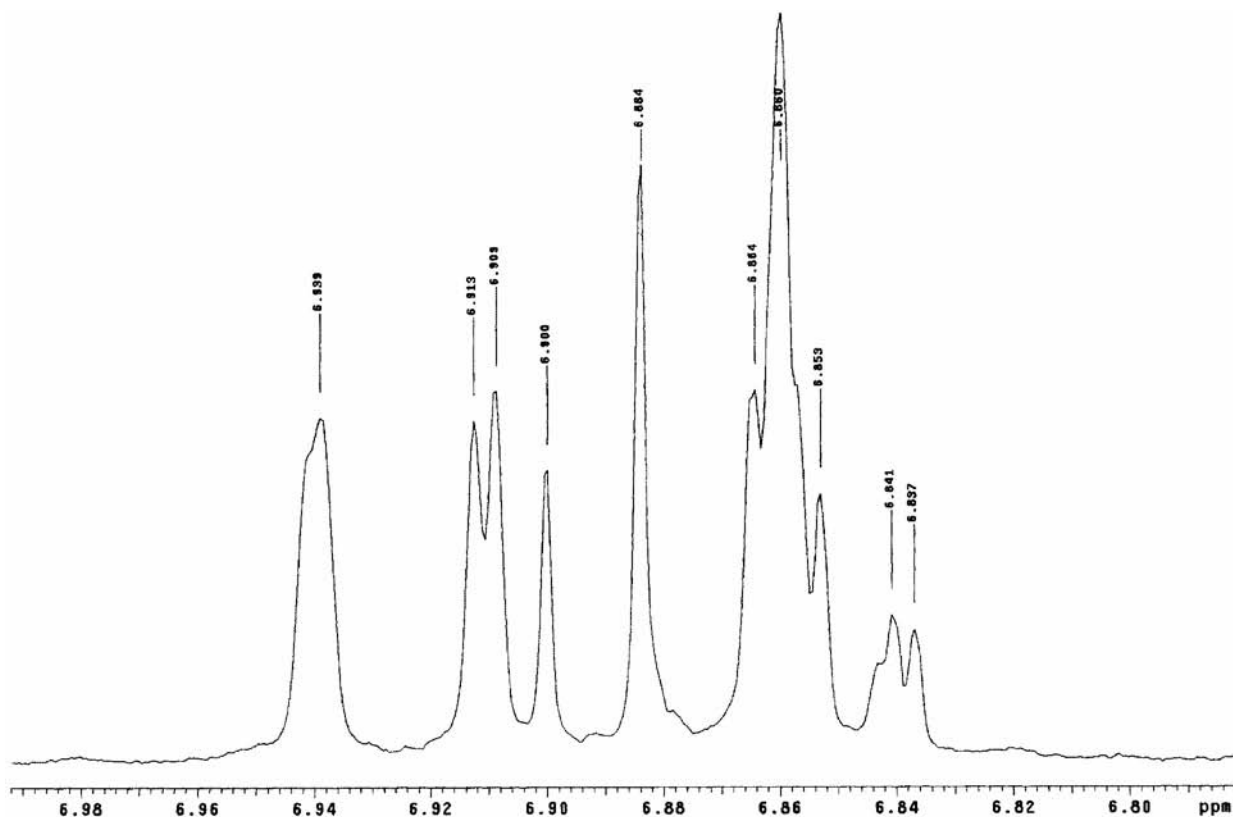


Figure 3. Aromatic region in ^1H NMR spectrum of **19** (500 MHz, CD_3CN).

(1H, *dd*, J 15.8; 4.4 Hz, H-7_{eq}); 1.52 (1H/1H, *m*, H-8/8'); 1.07 (3H, *d*, J 6.2 Hz, H-9); 6.55 (1H, *d*, J 2.2 Hz, H-2'); 6.83 (1H, *d*, J 8.4 Hz, H-5'); 6.63 (1H, *dd*, J 8.4; 2.2 Hz, H-6'); 3.37 (1H, *d*, J 10.3 Hz, H-7'); 0.85 (3H, *d*, J 6.2 Hz, H-9'); 3.82 (3H, *s*, MeO-3); 3.84 (3H, *s*, MeO-3'); 5.28 (1H, *s*, OH-C4); 5.48 (1H, *s*, OH-C4').

^{13}C NMR (50 MHz, CDCl_3), δ : 128.4 (C-1); 133.5 (C-2); 115.5 (C-3); 143.3 (C-4); 143.9 (C-5); 110.0 (C-6); 39.1 (C-7); 43.7 (C-8); 20.0 (C-9); 138.3 (C-1'); 111.5 (C-2'); 146.5 (C-3'); 144.6 (C-4'); 113.9 (C-5'); 122.5 (C-6'); 54.2 (C-7'); 35.6 (C-8'); 17.2 (C-9'); 55.8 (MeO-3); 55.9 (MeO-3').

MS (70 eV), m/z (Rel. Int. %) M^+ 328 (100), 241 (94), 271 (43), 189 (32), 136 (143).

Rel-(7*S*,8*S*,7'*R*,8'*S*)-4,4'-dihydroxy-3,3'-dimethoxy- $\Delta^{1,3,5,1',3',5'}$ -8,8',7*O*.7'-neolignan (**2**), verrucosin

^1H NMR (200 MHz, CDCl_3), δ : 5.11 (1H, *d*, J 8.5 Hz, H-7); 4.40 (1H, *d*, J 9.0 Hz, H-7'); 2.26 (1H, *m*, H-8); 1.76 (1H, *m*, H-8'); 1.06 (3H, *d*, J 6.5 Hz, H-9); 0.66 (3H, *d*, J 7.0 Hz, H-9'); 7.05-6.80 (6H, *m*, Ar-H); 3.91 (3H, *s*, MeO-3); 3.85 (3H, *s*, MeO-3'); 5.63 (1H, *sl*, OH-4/4').

^{13}C NMR (50 MHz, CDCl_3), δ : 132.8 (C-1); 109.5 (C-2); 146.5 (C-3); 145.2 (C-4); 114.1 (C-5); 119.3 (C-6); 87.3 (C-7); 47.8 (C-8); 14.9 (C-9); 132.2 (C-1'); 109.8 (C-2');

146.2 (C-3'); 144.6 (C-4'); 113.8 (C-5'); 119.9 (C-6'); 83.1 (C-7'); 46.0 (C-8'); 15.0 (C-9'); 55.8 (MeO-3/3').

MS (70 eV), m/z (Rel. Int. %): M^+ 192 (100), 151 (64), 177 (57), 152 (21), 164 (18).

Rel-(7*R*,8*S*,7'*R*,8'*R*)-3,4,5,3',4',5'-hexamethoxy- $\Delta^{1,3,5,1',3',5'}$ -8,8',7*O*.7'-neolignan (**3**), grandisin

^1H NMR (300 MHz, CDCl_3), δ : 6.45 (2H, *ls*, H-2/6); 4.27 (1H, *d*, J 9.0 Hz, H-7); 1.80 (1H, *m*, H-8); 1.12 (3H, *d*, J 6.5 Hz, H-9); 6.58 (2H, *s*, H-2'/6'); 5.12 (1H, *d*, J 8.5 Hz, H-7'); 2.25 (1H, *m*, H-8'); 0.69 (3H, *d*, J 7.0 Hz, H-9'); 3.81 (3H, *s*, MeO-4); 3.83 (3H, *s*, MeO-4'); 3.86 (3H, *s*, MeO-3); 3.87 (3H, *s*, MeO-3'); 3.88 (3H, *s*, MeO-5); 3.89 (3H, *s*, MeO-5').

^{13}C NMR (75 MHz, CDCl_3), δ : 134.5 (C-1); 134.1 (C-1'); 103.8 (C-2/2'/6); 153.5 (C-3); 153.3 (C-3'); 136.9 (C-4); 136.7 (C-4'); 153.5 (C-5); 153.1 (C-5'); 104.1 (C-6'); 87.6 (C-7); 87.4 (C-7'); 46.3 (C-8); 48.1 (C-8'); 15.1 (C-9); 14.3 (C-9'); 56.3 (MeO-3/5/3'/5'); 61.1 (MeO-4/4').

Rel-(8*S*,7'*S*,8'*R*)-7'-hydroxy-3,4,3',4'-dimethylenedioxy-7-oxo- $\Delta^{1,3,5,1',3',5'}$ -8,8',6,7'-neolignan (**4**), hydroxy-oxotobain

^1H NMR (300 MHz, CDCl_3), δ : 1.20 (3H, *d*, J 7.0 Hz, H-9); 0.93 (3H, *d*, J 7.0 Hz, H-9'); 2.90 (1H, *dq*, J 7.0;

unresolved Hz, H-8); 2.14 (1H, *dq*, *J* 7.0; unresolved Hz, H-8'); 7.71 (1H, *d*, *J* 8.0 Hz, H-6); 6.79 (1H, *dd*, *J* 8.5; 1.2 Hz, H-6'); 6.89 (1H, *d*, *J* 8.0 Hz, H-5); 6.74 (1H, *d*, *J* 8.5 Hz, H-5'); 6.81 (1H, *d*, *J* 1.2 Hz, H-2'); 5.72 and 5.86 (2H, *d*, *J* 1.2 Hz, OCH₂O); 5.97 (2H, *s*, OCH₂O).

¹³C NMR (75 MHz, CDCl₃), δ: 126.6 (C-1); 140.4 (C-1'); 128.4 (C-2); 106.6 (C-2'); 144.5 (C-3); 146.2 (C-3'); 152.5 (C-4); 147.3 (C-4'); 108.8 (C-5); 107.4 (C-5'); 122.8 (C-6); 118.9 (C-6'); 198.5 (C-7); 74.8 (C-7'); 43.3 (C-8); 46.7 (C-8'); 121.0 (C-9); 12.0 (C-9'); 100.9 and 101.0 (OCH₂O).

Rel-(2*S*,3*R*,4*R*)-2,3-dimethyl-4-(3',4'-dimethoxyphenyl)-4-(3'',4''-methylenedioxyphenyl)-butan-1-ol (5), *secotobain*

¹H NMR (300 MHz, CDCl₃), see the Table 1. ¹³C NMR (75 MHz, CDCl₃), δ: 67.2 (C-1); 36.3 (C-2); 36.1 (C-3); 56.2 (C-4); 12.0 (H₃C-2); 9.8 (H₃C-3); 137.2 (C-1'); 108.2 (C-2'); 149.2 (C-3'); 147.6 (C-4'); 111.5 (C-5'); 119.8 (C-6'); 139.3 (C-1''); 108.4 (C-2''); 148.0 (C-3''); 145.9 (C-4''); 111.7 (C-5''); 121.1 (C-6''); 101.1 (OCH₂O); 56.2 (MeO-3); 56.1 (MeO-4).

3,4,3'-Trimethoxy-Δ^{1,3,5,7,1',3',5',8'}-8.5',7.0.4'-neolignan (6), *carinatin*

¹H NMR (200 MHz, CDCl₃), δ: 7.37-7.33 (2H, *m*, H-2/6); 6.96 (1H, *d*, *J* 9.0 Hz, H-5); 2.43 (3H, *s*, H-9); 6.65 (1H, *ls*, H-2'); 6.94 (1H, *d*, *J* 1.2 Hz, H-6'); 3.49 (2H, *d*, *J* 7.0 Hz, H-7'); 6.06 (1H, *ddt*, *J* 16.5; 10.0; 6.5 Hz, H-8'); 5.19-5.09 (2H, *m*, H-9'); 3.99 (3H, *s*, MeO-3); 3.94 (3H, *s*, MeO-4); 4.03 (3H, *s*, MeO-3').

¹³C NMR (50 MHz, CDCl₃), δ: 133.0 (C-1); 107.5 (C-2); 148.9 (C-3 and C-4); 110.9 (C-5); 119.9 (C-6); 151.2 (C-7); 110.3 (C-8); 9.6 (C-9); 135.1 (C-1'); 110.0 (C-2'); 144.7 (C-3'); 141.5 (C-4'); 124.3 (C-5'); 111.1 (C-6'); 40.6 (C-7'); 138.0 (C-8'); 115.6 (C-9'); 56.1 (MeO-3); 56.0 (MeO-4); 55.9 (MeO-3').

4-Hydroxy-3,3'-dimethoxy-Δ^{1,3,5,7,1',3',5',8'}-8.5',7.0.4'-neolignan (7), *carinatidin*

¹H NMR (200 MHz, CDCl₃), δ: 7.33-7.25 (2H, *m*, H-2/6); 6.99 (1H, *d*, *J* 7.9 Hz, H-5); 2.41 (3H, *s*, H-9); 6.64 (1H, *ls*, H-2'); 6.93 (1H, *ls*, H-6'); 3.48 (2H, *d*, *J* 6.5 Hz, H-7'); 6.02 (1H, *ddt*, *J* 16.5; 10.0; 6.5 Hz, H-8'); 5.18-5.07 (2H, *m*, H-9'); 3.98 (3H, *s*, MeO-3); 5.77 (1H, *ls*, OH-4); 4.02 (3H, *s*, MeO-3').

¹³C NMR (50 MHz, CDCl₃), δ: 133.0 (C-1); 107.5 (C-2); 146.6 (C-3); 145.7 (C-4); 114.4 (C-5); 120.6 (C-6); 151.4 (C-7); 110.0 (C-8); 9.6 (C-9); 135.1 (C-1'); 109.5 (C-2'); 144.7 (C-3'); 141.4 (C-4'); 123.8 (C-5'); 111.0 (C-6'); 40.6 (C-7'); 138.0 (C-8'); 115.6 (C-9'); 56.1 (MeO-3/3').

Rel-(7*S*,8*S*)-3,4,3'-trimethoxy-Δ^{1,3,5,1',3',5',8'}-8.5',7.0.4'-neolignan (8), *dihydrocarinatin*

¹H NMR (200 MHz, CDCl₃), δ: 7.00-6.95 (2H, *m*, H-2/6); 6.85 (1H, *d*, *J* 8.0 Hz, H-5); 5.11 (1H, *d*, *J* 9.5 Hz, H-7); 3.51-3.43 (1H, *m*, H-8); 1.38 (3H, *d*, *J* 6.5 Hz, H-9); 6.62 (1H, *s*, H-2'); 6.64 (1H, *s*, H-6'); 3.37 (2H, *d*, *J* 6.5 Hz, H-7'); 5.99 (1H, *ddt*, *J* 17.0; 10.0; 6.5 Hz, H-8'); 5.16-5.06 (2H, *m*, H-9'); 3.89 (9H, *s*, MeO-3/4/3').

¹³C NMR (CDCl₃, 50 MHz), δ: 137.9 (C-1); 109.5 (C-2); 149.1 (C-3 and C-4); 110.7 (C-5); 119.3 (C-6); 93.6 (C-7); 45.7 (C-8); 17.5 (C-9); 133.1 (C-1'); 111.8 (C-2'); 145.1 (C-3'); 144.1 (C-4'); 133.5 (C-5'); 115.6 (C-6'); 40.2 (C-7'); 137.9 (C-8'); 115.6 (C-9'); 55.9 (MeO-3/4/3').

Rel-(7*S*,8*S*)-4-hydroxy-3,3'-dimethoxy-Δ^{1,3,5,1',3',5',8'}-8.5',7.0.4'-neolignan (9), *dihydrocarinatidin*

¹H NMR (200 MHz, CDCl₃), δ: 6.99-6.91 (3H, *m*, H-2/5/6); 5.09 (1H, *d*, *J* 10.0 Hz, H-7); 3.50-3.45 (1H, *m*, H-8); 1.38 (3H, *d*, *J* 7.0 Hz, H-9); 6.62 (1H, *s*, H-2'); 6.64 (1H, *s*, H-6'); 3.37 (2H, *d*, *J* 6.5 Hz, H-7'); 5.98 (1H, *ddt*, *J* 17.0; 10.0; 6.5 Hz, H-8'); 5.17-5.06 (2H, *m*, H-9'); 3.89 (6H, *s*, MeO-3/3'); 5.31 (1H, *s*, OH-4).

¹³C NMR (50 MHz, CDCl₃), δ: 132.2 (C-1); 108.9 (C-2); 146.7 (C-3); 145.7 (C-4); 114.0 (C-5); 119.9 (C-6); 93.7 (C-7); 45.8 (C-8); 17.4 (C-9); 133.5 (C-1'); 111.8 (C-2'); 144.0 (C-3'); 108.9 (C-4'); 133.2 (C-5'); 115.6 (C-6'); 40.2 (C-7'); 137.9 (C-8'); 55.9 (MeO-3/3').

4,4'-Dihydroxy-3,3'-dimethoxy-Δ^{1,3,5,8,1',3',5',8'}-5.5'-neolignan (10), *dehydrodieugenol*

¹H NMR (200 MHz, CDCl₃), δ: 6.74 (2H, *d*, *J* 2.0 Hz, H-2/2'); 6.76 (2H, *d*, *J* 2.0 Hz, H-6/6'); 3.37 (4H, *d*, *J* 6.5 Hz, H-7/7'); 5.97 (2H, *ddt*, *J* 17.0; 10.0; 6.5 Hz, H-8/8'); 5.17-5.05 (4H, *m*, H-9/9'); 3.92 (6H, *s*, MeO-5/5').

¹³C NMR (50 MHz, CDCl₃), δ: 131.9 (C-1/1'); 123.1 (C-2/2'); 124.4 (C-3/3'); 140.8 (C-4/4'); 147.2 (C-5/5'); 110.6 (C-6/6'); 39.9 (C-7/7'); 137.6 (C-8/8'); 115.7 (C-9/9'); 56.0 (MeO-5/5').

Rel-(7*S*,8*S*,7'*R*,8'*R*)-3,4,3',4'-tetramethoxy-Δ^{1,3,5,1',3',5',8'}-8.8',7.0.7'-neolignan (11), *galgravin*

¹H NMR (200 MHz, CDCl₃), δ: 4.53 (2H, *d*, *J* 6.5 Hz, H-7/7'); 2.34 (2H, *m*, H-8/8'); 1.05 (6H, *d*, *J* 6.5 Hz, H-9/9'); 6.99-6.85 (6H, *m*, Ar-H); 3.89 (6H, *s*, MeO-3/3'); 3.88 (6H, *s*, MeO-4/4').

¹³C NMR (50 MHz, CDCl₃), δ: 134.7 (C-1/1'); 109.6 (C-2/2'); 148.9 (C-3/3'); 148.4 (C-4/4'); 110.9 (C-5/5'); 118.5 (C-6/6'); 87.2 (C-7/7'); 55.8 (MeO-3/4/3'/4'); 44.3 (C-8/8'); 12.9 (C-9/9').

MS (70 eV), *m/z* (Rel. Int. %): M⁺ 206 (100), 191 (61), 372 (24), 165 (20); 178 (13); 194 (4), 166 (4).

Rel-(7S,8S,7'R,8'R)-4'-hydroxy-3,4,3'-trimethoxy- $\Delta^{1,3,5,1',3',5'}$ -8,8',7.O.7'-neolignan (12), nectandrin A

$^1\text{H NMR}$ (200 MHz, CDCl_3), δ : 4.67 (2H, *d*, *J* 9.0 Hz, H-7/7'); 1.79 (2H, *m*, H-8/8'); 1.05 (6H, *d*, *J* 6.0 Hz, H-9/9'); 7.0-6.83 (6H, *m*, Ar-H); 3.92 (6H, *s*, MeO-3/3'); 3.86 (6H, *s*, MeO-4/4').

$^{13}\text{C NMR}$ (50 MHz, CDCl_3), δ : 134.8 (C-1); 109.7 (C-2); 148.9 (C-3); 148.4 (C-4); 110.9 (C-5); 118.5 (C-6); 87.2 (C-7); 44.2 (C-8); 12.8 (C-9); 134.1 (C-1'); 109.1 (C-2'); 146.4 (C-3'); 145.0 (C-4'); 114.1 (C-5'); 119.2 (C-6'); 87.3 (C-7'); 44.3 (C-8'); 12.9 (C-9'); 55.9 (MeO-4); 55.8 (MeO-3/3').

MS (70 eV), *m/z* (Rel. Int. %): M^+ 206 (100), 191 (74), 178 (44), 372 (20), 194 (20), 165 (7).

Rel-(7S,8S,7'S,8'S)-3,4,3',4'-tetramethoxy- $\Delta^{1,3,5,1',3',5'}$ -8,8',7.O.7'-neolignan (13), galbelgin

$^1\text{H NMR}$ (200 MHz, CDCl_3), δ : 4.49 (2H, *d*, *J* 6.0 Hz, H-7/7'); 2.33 (2H, *m*, H-8/8'); 1.03 (6H, *d*, *J* 6.5 Hz, H-9/9'); 7.04-6.85 (6H, *m*, Ar-H); 3.88 (6H, *s*, MeO-3/3'); 5.59 (2H, *s*, OH-C4/4').

$^{13}\text{C NMR}$ (50 MHz, CDCl_3), δ : 134.9 (C-1/1'); 109.1 (C-2/2'); 149.0 (C-3/3'); 148.5 (C-4/4'); 110.8 (C-5/5'); 118.6 (C-6/6'); 88.3 (C-7/7'); 55.8 (MeO-3/4/3'/4'); 50.9 (C-8/8'); 13.8 (C-9/9').

MS (70 eV), *m/z* (Rel. Int. %): M^+ 192 (100), 177 (54), 151 (44), 164 (17), 152 (14); 180 (10), 344 (7).

Rel-(7S,8R)-7-hydroxy-3,4,3'-trimethoxy- $\Delta^{1,3,5,1',3',5'}$ -8.O.4'-neolignan (14), erythro

$^1\text{H NMR}$ (200 MHz, CDCl_3), δ : 7.00-6.75 (6H, *m*, Ar-H); 4.84 (1H, *d*, *J* 2.6 Hz, H-7); 4.33 (1H, *m*, H-8); 1.18 (3H, *d*, *J* 6.6 Hz, H-9); 3.37 (2H, *d*, *J* 7.0 Hz, H-7'); 5.98 (1H, *ddt*, *J* 16.6; 10.3; 6.6 Hz, H-8'); 5.16-5.07 (2H, *m*, H-9'); 3.90 (3H, *s*, MeO-3); 3.89 (3H, *s*, MeO-3'); 3.88 (3H, *s*, MeO-4).

$^{13}\text{C NMR}$ (50 MHz, CDCl_3), δ : 132.5 (C-1); 110.7 (C-2); 148.1 (C-3); 151.4 (C-4); 109.4 (C-5); 121.1 (C-6); 73.4 (C-7); 82.5 (C-8); 13.5 (C-9); 135.5 (C-1'); 112.4 (C-2'); 148.8 (C-3'); 144.7 (C-4'); 118.4 (C-5'); 119.9 (C-6'); 39.9 (C-7'); 137.2 (C-8'); 115.9 (C-9'); 55.8 (MeO-3/4/3').

Rel-(7R,8R)-7-hydroxy-3,4,3'-trimethoxy- $\Delta^{1,3,5,1',3',5'}$ -8.O.4'-neolignan (15), threo

$^1\text{H NMR}$ (200 MHz, CDCl_3), δ : 6.97-6.72 (6H, *m*, Ar-H); 4.64 (1H, *d*, *J* 8.3 Hz, H-7); 4.06 (1H, *m*, H-8); 1.17 (3H, *d*, *J* 6.1 Hz, H-9); 3.37 (2H, *d*, *J* 6.6 Hz, H-7'); 5.95 (1H, *ddt*, *J* 16.6; 10.5; 6.6 Hz, H-8'); 5.15-5.07 (2H, *m*, H-9'); 3.91 (3H, *s*, MeO-3); 3.89 (3H, *s*, MeO-3'); 3.88 (3H, *s*, MeO-4).

$^{13}\text{C NMR}$ (50 MHz, CDCl_3), δ : 132.3 (C-1); 110.3 (C-2); 148.2 (C-3); 150.0 (C-4); 109.6 (C-5); 120.3 (C-6);

77.5 (C-7); 82.8 (C-8); 16.2 (C-9); 134.3 (C-1'); 111.9 (C-2'); 148.4 (C-3'); 145.3 (C-4'); 118.2 (C-5'); 119.3 (C-6'); 39.3 (C-7'); 136.8 (C-8'); 115.2 (C-9'); 55.2 (MeO-3/4/3').

Rel-(8S)-4'-hydroxy-3,4,3'-trimethoxy-7-oxo- $\Delta^{1,3,5,1',3',5'}$ -8.5'-neolignan (16), carinatone

$^1\text{H NMR}$ (200 MHz, CDCl_3), δ : 7.62 (1H, *d*, *J* 2 Hz, H-2); 6.82 (1H, *d*, *J* 8.5 Hz, H-5); 7.73 (1H, *dd*, *J* 8.5; 2 Hz, H-6); 5.06 (1H, *q*, *J* 7 Hz, H-8); 1.47 (3H, *d*, *J* 6.5 Hz, H-9); 6.53 (1H, *d*, *J* 2 Hz, H-2'); 6.56 (1H, *d*, *J* 2 Hz, H-6'); 3.23 (2H, *d*, *J* 6.5 Hz, H-7'); 5.87 (1H, *m*, H-8'); 5.03-4.97 (2H, *m*, H-9'); 3.89 (6H, *s*, MeO-3/5'); 3.87 (3H, *s*, MeO-4); 5.31 (1H, *s*, OH-4').

Rel-(7S,8S,7'R,8'R)-4,4'-dihydroxy-3,3'-dimethoxy- $\Delta^{1,3,5,1',3',5'}$ -8,8',7.O.7'-neolignan (17), nectandrin B

$^1\text{H NMR}$ (200 MHz, CDCl_3), δ : 4.52/4.51 (1H, *d*, *J* 6.5 Hz, H-7/7'); 2.34 (1H, *m*, H-8/8'); 1.05/1.04 (3H, *d*, *J* 6.5 Hz, H-9/9'); 6.99-6.85 (6H, *m*, Ar-H); 3.89 (6H, *s*, MeO-3/3'); 3.88 (3H, *s*, MeO-4'); 5.59 (1H, *s*, OH-4').

$^{13}\text{C NMR}$ (50 MHz, CDCl_3), δ : 134.2 (C-1/1'); 109.1 (C-2/2'); 146.4 (C-3/3'); 145.0 (C-4/4'); 114.1 (C-5/5'); 119.3 (C-6/6'); 87.3 (C-7/7'); 55.8 (MeO-3/3'); 44.3 (C-8/8'); 12.9 (C-9/9').

MS (70 eV), *m/z* (Rel. Int. %): M^+ 192 (100), 206 (81), 191 (66), 177 (56), 151 (47), 178 (16), 152 (5), 164 (9).

Rel-(7S,8R,7'S,8'R)-3,4,3',4'-tetramethoxy- $\Delta^{1,3,5,1',3',5'}$ -8,8',9.O.7',7.O.9'-lignan (18), eudesmin

$^1\text{H NMR}$ (500 MHz, CDCl_3), δ : 6.91 (2H, *d*, *J* 2.0 Hz, H-2/2'); 6.84 (2H, *d*, *J* 8.0 Hz, H-5/5'); 6.88 (2H, *dd*, *J* 8.5; 2.0 Hz, H-6/6'); 4.76 (2H, *d*, *J* 4.5 Hz, H-7/7'); 3.11 (2H, *ddt*, *J* 9.5; 4.5; 2.0 Hz, H-8/8'); 4.26 (2H, *dd*, *J* 9.0; 6.5 Hz, H-9a/9'a); 3.84-3.86 (2 H, *m*, H-9b/9'b); 3.88 (6H, *s*, MeO-4/4'); 3.90 (6H, *s*, MeO-3,3').

$^{13}\text{C NMR}$ (125 MHz, CDCl_3), δ : 133.6 (C-1/1'); 109.3 (C-2/2'); 148.7 (C-3/3'); 149.3 (C-4/4'); 111.1 (C-5/5'); 118.3 (C-6/6'); 85.8 (C-7/7'); 54.2 (C-8/8'); 71.8 (C-9/9'); 55.9 (MeO-3/3'); 56.0 (MeO-4/4').

MS (70 eV), *m/z* (Rel. Int. %): M^+ 386 (100), 151 (31), 165 (22), 356 (13).

Rel-(7S,8R,7'R,8'R)-4-hydroxy-3,3',4'-trimethoxy- $\Delta^{1,3,5,1',3',5'}$ -8,8',9.O.7',7.O.9'-lignan (19), phylligenol

$^1\text{H NMR}$ (500 MHz, CD_3CN), δ : 6.99/6.97 (2H, *d*, *J* 1.5/2.0 Hz, H-2/2'); 6.81/6.94 (2H, *d*, *J* 8.0/8.0 Hz, H-5/5'); 6.87/6.94 (1H/1H, *dd*, *J* 8.0; 1.5/8.0; 2.0 Hz, H-6/6'); 4.41/4.86 (1H/1H, *d*, *J* 7.0/5.5 Hz, H-7/7'); 2.92/3.44 (1H/2H, *m*, H-8/8'); 4.12/3.20 (1H/2H, *d/m*, *J* 9.5 Hz, H-9a/9'a); 3.84/3.80 (2H/2H, *m/m*, H-9b/9'b);

3.89/3.84 (6H, s, MeO-3/3'); 3.82 (3H, s, MeO-4'); 5.58 (1H, ls, OH-C4).

^{13}C NMR (125 MHz, CD_3CN), δ : 133.9/131.9 (C-1/1'); 109.9/109.8 (C-2/2'); 147.4/148.2 (C-3/3'); 145.8/149.2 (C-4/4'); 114.6/111.7 (C-5/5'); 119.1/118.0 (C-6/6'); 87.8/81.9 (C-7/7'); 54.5/50.1 (C-8/8'); 70.9/69.4 (C-9/9'); 55.9/55.5 (MeO-3/3'); 55.6 (MeO-4').

MS (70 eV), m/z (Rel. Int. %): M^+ 372 (100), 156 (25), 137 (13).

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