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Neolignans and Sesquiterpenes from Leaves and Embryogenic Cultures of *Ocotea catharinensis* (Lauraceae)

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Foram isoladas de extratos de folhas de *Ocotea catharinensis* Mez (Lauraceae) quatorze neolignanas sendo nove benzofurânicas (incluindo três novas substâncias **1e**, **2f** e **4b**), uma *seco*-benzofurânica inédita (**3b**), duas biciclo[3.2.1]octânicas (incluindo a nova **5c**), dois novos dímeros biciclo[3.2.1]octânicos (**7a** e **7b**) e ainda dois sesquiterpenos (incluindo o novo humulanol **9**). Nos embriões somáticos de *O. catharinensis* foram identificadas sete neolignanas incluindo uma nova neolignana biciclo[3.2.1]octânica (**4a**).

The extracts from leaves of *Ocotea catharinensis* Mez (Lauraceae) were found to contain fourteen neolignans and two sesquiterpenes: nine benzofuran types (including three new compounds **1e**, **2f** and **4b**), one new *seco*-benzofuran type (**3b**), two bicyclo[3.2.1]octane types (including the new compound **5c**), two new dimers of bicyclo[3.2.1]octane type (**7a** and **7b**) and two sesquiterpenes (including a new humulanol **9**). In addition, seven neolignans were also showed to occur in somatic embryos of *O. catharinensis* including one new bicyclo[3.2.1]octane type (**4a**).

Keywords: *Ocotea catharinensis*, benzofuran neolignans, bicyclo[3.2.1]octane neolignans, humulane sesquiterpene, somatic embryos

Introduction

Ocotea catharinensis (Lauraceae) is a woody plant species found in southern Atlantic forest in Brazil, which produces excellent quality of timber. The extensive logging over the past thirty years associated with difficulties for propagation has led its natural population to be significant decrease. Since *O. catharinensis* has been included as endangered species, a somatic embryogenic system was developed aiming to a massive propagation.[1,2]

The *Ocotea* has been one of the most phytochemically investigated Lauraceous genus and their major secondary compounds were showed to be phenylpropanoid-derived including several sub-classes of neolignans.[3] Previous phytochemical studies carried out in leaves of *O. catharinensis* collected at Horto Florestal (Serra da Cantareira), São Paulo State, Brazil, reported the occurrence of benzofuran (**1b**, **1c**, **1d**, **2b**, **2c**, **2d**, **2e** and **2h**) and bicyclo[3.2.1]octane (**5a**, **5b** and **5d**) neolignans.[4] Representatives of both sub-classes of neolignans have also been previously isolated from barks and woods of a specimen collected in São Paulo State,[5,6] and also from wood and leaves of *O. porosa* ("imbuia") collected in Rio Grande do Sul State, southern Brazil.[7-9]

This work describes the isolation and characterization of major secondary compounds from leaves collected at Vale do Itajaí, Santa Catarina State, Brazil and from embryogenic cultures developed from the same plant source. The extracts from leaves afforded seven new neolignans **1e**, **2f**, **3b**, **4b**, **5c**, **7a**, **7b**, besides seven previously reported ones **1a**,[6,10] **1d**,[6] **2a**,[6,10,11] **2d**,[4] **2e**,[6] **2g**,[12] **5e**.[13] Additionally, a new sesquiterpene of humulane type (**9**), besides known

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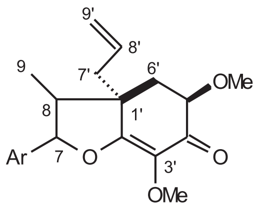
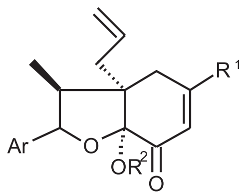
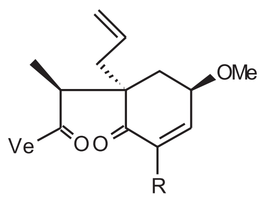


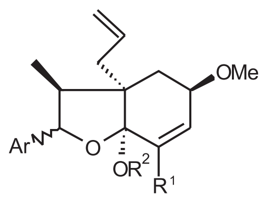
1a Ar = α-Pi, β-Me
1b Ar = β-Pi, α-Me
1c Ar = β-Pi, β-Me
1d Ar = β-Ve, β-Me
1e Ar = α-Tp, β-Me



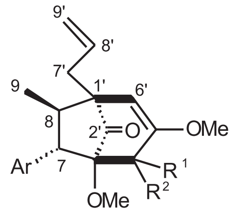
2a Ar = α-Pi, R¹ = H, R² = H
2b Ar = β-Pi, R¹ = H, R² = H
2c Ar = β-Pi, R¹ = H, R² = Me
2d Ar = α-Ve, R¹ = H, R² = H
2e Ar = β-Ve, R¹ = H, R² = H
2f Ar = β-Ve, R¹ = OMe, R² = H
2g Ar = α-Mp, R¹ = H, R² = H
2h Ar = β-Tp, R¹ = H, R² = H



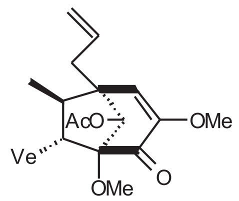
3a R = H
3b R = OMe



4a Ar = α-Pi, R¹ = OMe, R² = H
4b Ar = β-Mp, R¹ = OMe, R² = H
4c Ar = β-Ve, R¹ = H, R² = Me



5a Ar = Pi, R¹ = α-OH, R² = H
5b Ar = Pi, R¹ = β-OH, R² = H
5c Ar = Pi, R¹R² = O
5d Ar = Mp, R¹ = α-OH, R² = H
5e Ar = Mp, R¹R² = O



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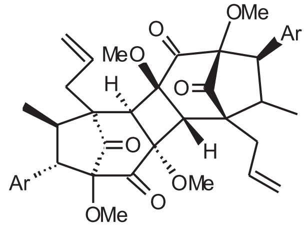
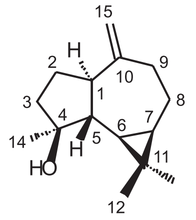
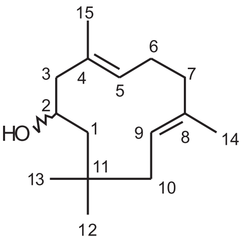


7a Ar = Pi
7b Ar= Mp



8 spathulenol



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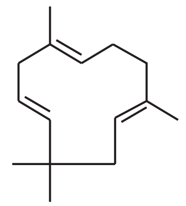

α-hummulane

Pi = piperonyl (3,4-methylenedioxyphenyl)
Ve = veratryl (3,4-dimethoxyphenyl)
Mp = 5-methoxy-3,4-methylenedioxyphenyl
Tp = 3,4,5-trimethoxyphenyl

spathulenol (**8**)[4,14] were isolated as well. The embryogenic cultures were shown to contain a new benzofuran neolignan **4a**, six already known benzofuran **1c**,[6,12] **1d**,[6] **2a**,[6,10,11] **2b**,[11,12] **2e**,[6] and a bicyclo[3.2.1]octane neolignan **6**.[15]

Results and Discussion

The defatted fraction of hexane extracts from *O. catharinensis* leaves and from *O. catharinensis* somatic embryos were submitted to flash chromatography followed by prep. TLC and/or circular chromatography (Chromatotron®). This procedure yielded nine new compounds (**1e**, **2f**, **3b**, **4a**, **4b**, **5c**, **7a**, **7b** and **9**), besides armenin-B (**1a**),[6,10] **1c**,[6,12] 5'-methoxyporosin (**1d**),[6] ferrearin-C (**2a**),[6,10,11] ferrearin-B (**2b**),[11,12] **2d**,[4] ferrearin-E (**2e**),[6] ferrearin-G (**2g**),[12] **5e**,[13] **6**[15] and spathulenol (**8**).[4,14]

The molecular formula of compound **1e** was determined by HRESI as $C_{23}H_{30}O_7$. Its ^{1}H NMR spectrum was quite similar to that of armenin-B (**1a**) ($C_{21}H_{24}O_6$), previously isolated from *Licaria armeniaca*[10] and from

Table 1. ^{1}H and ^{13}C NMR data of **1e**, **2f**, **3b**, **4a**, **4b** and **6** [δ, J (Hz), 200 MHz and 50 MHz, $CDCl_3$]

position	**1e**		**2f**		**3b**		**4a**	**4b**		**6**
	^{1}H	^{13}C	^{1}H	^{13}C	^{1}H	^{13}C	^{1}H	^{1}H	^{13}C	^{13}C
1		131.8		131.3		131.3			132.5	129.4
2	6.34 (brs)	102.5	6.63-6.76 (m)	110.8	7.38 (d, 2.0)	109.9	6.99 (d,1.5)	6.45 (brs)	106	113.9
3		153.4		148.2		148.7			148	148.2
4		136.1		148.6		149.9			134.2	147.3
5		153.3	6.63-6.76 (m)	109.7	6.80 (d, 8.4)	110.8	6.64 (d, 7.8)		143.1	110.6
6	6.45 (brs)	101.1	6.63-6.76 (m)	118.5	7.52 (dd, 8.4; 2.0)	122.8	6.73 (dd, 7.8; 1.5)	6.42 (brs)	106	122.8
7	5.20 (d, 1.9)	92.5	5.36 (d, 9.3)	82.4		202.3	4.43 (d, 9.9)	5.33 (d, 9.7)	82.3	57.1
8	2.37-2.66 (m)	44.3	2.75 (m)	43.2	4.0 (q, 7.5)	42.4	2.18-2.40 (m)	2.72 (d, 9.6)	43.3	49.1
9	1.15 (d, 7.4)	17.7	0.52 (d, 7.4)	11.4	1.10 (d, 7.5)	12.5	0.84 (d, 6.8)	0.57 (d, 7.5)	12.4	13.8
1’		47.3		51.6		50.4			49.9	50.8
2’		31.6		100.9		196.5			102	77.4
3’		77.3		196.9		152.8			152.5	89.9
4’		192.5	5.39 (s)	102.4	5.72 (brs)	132.9	4.78 (brs)	4.92 (brs)	97.5	193.7
5’	3.93 (dd, 12.1; 5.0)	132.3		169.3	4.3 (m)	72.8	4.03-4.12 (m)	4.08-4.13 (m)	74.3	151.8
6’	1.82 (dd, 12.0; 12.0)	169.7	2.45 (d, 16.5)	41.7	2.80 (dd, 12.9; 9.9)	34.6	2.00 (dd, 12.6; 5.0)	1.90 (dd, 12.3; 4.8)	31.7	119.3
	2.09-2.25 (m)				2.20 (dd, 12.9; 5.3)		1.40 (dd, 12.6; 10.0)	1.46 (dd, 10.0; 10.0)		
7’	2.09-2.25 (m)	41.3	2.31 (dd, 7.3; 5.5)	40.1	2.50 (dd, 14.4; 6.4)	39	2.18-2.40 (m)	2.43 (dd, 14.2; 7.1)	38.4	36.9
					2.30 (dd, 14.4; 8.8)			2.23 (dd, 14.2; 7.9)		
8’	5.54-5.65 (m)	132.3	5.64-5.73 (m)	133.4	5.58-5.68 (m)	132.9	5.86-5.95 (m)	5.90-5.95 (m)	135	133.8
9’	5.03 (dd, 14.6; 1.4)	120	5.00 (dd, 15.3; 1.9)	118.7	5.05 (dd, 12.0; 2.0)	118.6	5.03 (dd, 14.8; 1.8	5.12 (dd, 14.4; 1.8)	117.5	118.8
	4.72 (dd, 11.2; 1.4)		4.93 (dd, 10.0; 1.9)				5.03 (dd, 14.8; 1.8	5.08 (dd, 7.7; 1.8)		
CH_2O_2							5.86 (s)	5.92 (s)	101.3	
MeO-3	3.80 (s)	60.9	3.79 (s)	55.8	3.82 (s)	55.9				55.8
MeO-4	3.81 (s)	60.5	3.78 (s)	55.9	3.86 (s)	55.8				55.7
MeO-5	3.82 (s)	60.9						3.85 (s)	56.7	
MeO-3’	3.73 (s)	59.3			3.58 (s)	55.7	3.61 (s)	3.67 (s)	55.8	54.7
MeO-4’					3.34 (s)	55				
MeO-5’	3.56 (s)	55.9	3.75 (s)	55.6			3.34 (s)	3.37 (s)	55.1	55.5
OH-2’			3.66 (s)							
COMe-4’			3.66 (s)							21.0/169.0

O. catharinensis.[6] The only difference was assigned to the oxygenation pattern of the aromatic ring, which was determined as 3,4,5-trimethoxyphenyl in **1e** instead of 3,4-methylenedioxyphenyl in **1a**.

The ^{1}H NMR spectra of **2f** ($C_{21}H_{26}O_6$) and of ferrearin-E (**2e**) ($C_{20}H_{24}O_5$) isolated from *O. catharinensis*[6] were similar. The difference between both compounds consisted of an additional methoxyl group at β-position (C-5’, δ 169.3) of **2f**.

The molecular formula of compound **3b** was determined by HRESI as $C_{22}H_{28}O_6$. All the ^{1}H NMR data was similar to that of a neolignan **3a** ($C_{21}H_{26}O_5$) previously isolated from *O. porosa.*[16] Nevertheless, the ^{1}H and ^{13}C NMR spectra indicated that **3b** contained a methoxyl group at C-3’ (δ_H 3.58, δ_C 55.7) which was evidenced by the signal of C-3’ at δ_C 152.8 instead of δ_C 132.7 in **3a**. Its absolute configuration was determined as 8*S*,1’*R*,5’*R* based on the signal of optical rotation ($[\alpha]_D^{21}$ = −17° (MeOH, c = 0.92 g/100mL) and comparison with reported data for (–)-megaphone in which X-ray crystallographic studies was carried out.[16]

The compounds **4a** and **4b** were characterized as hexahydrobenzofuran neolignans by analysis of their ^{1}H and ^{13}C NMR spectra and comparison with **4c** which was previously isolated from *O. porosa.*[9] The aromatic rings were determined as 3,4-methylenedioxyphenyl and 5-methoxy-3,4-methylenedioxyphenyl for **4a** and **4b**, respectively. The relative stereochemistry between methyl and aryl groups was deduced from the anisotropic shielding effect caused by aromatic ring on *cis* methyl hydrogens as observed in the ^{1}H NMR spectra. The methyl (H-9) signals of **4a** (*trans*) and **4b** (*cis*) were observed at δ 0.84 (d, 6.8 Hz) and 0.57 (d, 7.5 Hz), respectively. The placement of hydroxyl group at C-2' and methoxyl group at C-3' were possible by comparison of ^{13}C NMR data and those described for **4c**.[9]

The molecular formula of compound **5c** was determined by HRESI as $C_{21}H_{22}O_6$. Its ^{1}H NMR were similar to that observed for previously reported **5e** ($C_{22}H_{24}O_7$) isolated from *Aniba simulans*,[13] but with a 3,4-methylenedioxy for **5c** instead of 5-methoxy-3,4-methylenedioxy observed for **5e**.

Compounds **7a** and **7b** had their structures determined by analysis of their IR, MS and NMR spectra. The IR spectra of **7a/7b** exhibited absorption bands at 1766 and 1714 cm^{-1}, assignable to two carbonyl groups for each compound. Their ^{1}H and ^{13}C NMR spectra resembled those of **5c/5e**, respectively. Nevertheless, the ^{13}C NMR signals associated to the olefinic double bond at C5´-C6´ were replaced by signals of quaternary carbinolic and methine signals at δ 88.4/52.6 (**7a**) and δ 88.4/52.7 (**7b**), respectively. The EIMS of **7a/7b** showed molecular ion at *m/z* 370 and 400, but the chemical ionization mass spectrometry (CIMS) provided a molecular ion peak $(M + H)^+$ at *m/z* 741 and *m/z* 801, respectively. These molecular ions combined with ^{1}H and ^{13}C NMR (PND and DEPT 135°), suggested a molecular formula $C_{42}H_{44}O_{12}$ and $C_{44}H_{48}O_{14}$ compatible with dimeric structures. Thus, the fragmentary ion peaks found at *m/z* 371 of **7a** and 401 of **7b** were assigned to the cleavage into monomers indicating that **7a** and **7b** were symmetric dimers of **5a** and **5e**. Based on similarities to **5a** and **5e** all ^{1}H and ^{13}C NMR data were assigned with the aid of ^{1}H-^{1}H COSY and HETCOR spectra (Table 2). In order to determine the relative stereochemistry at the cyclobutane ring, NOESY spectra showed cross-peaks between H-8 and H-7', H-7' and H-6', H-6' and OMe-5'. Thus, among the cyclobutane *syn*-adducts at C-5' and C-6' four dimers would be expected (Figure 1). Dimers III and IV having *cis* configuration at the cyclobutane would involve a considerable steric hindrance between the bulky groups of bicyclooctane neolignan, which would prevent such arrangement. For these reason, dimers I and II having *trans* configuration were considered as the mostly probable structures.

The molecular formula of **9** ($C_{15}H_{26}O$) was deduced from MS and ^{13}C NMR (PND and DEPT 135°) spectral data. The spectral characteristics of compound **9** were closely related to those of α-humulane[17] except for the existence of a hydroxyl group (IR ν_{max}/cm^{-1}: 3425) and two double bonds instead of three. All the ^{1}H and ^{13}C NMR signals were assigned by ^{1}H-^{1}H COSY and HETCOR techniques and were compatible with the structure depicted for humula-4,8-dien-2-ol (**9**).

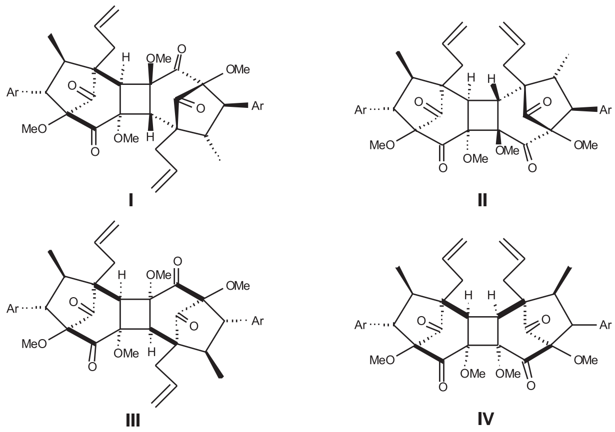

Figure 1. Possible structure of dimers for **7a/7b**.

The leaves of *O. catharinensis* collected in São Paulo and in Santa Catarina were showed to contain both benzofuran and bicyclo[3.2.1]octane type neolignans. The benzofuran neolignan **1d** was the major compound in the leaves collected in São Paulo while bicyclo[3.2.1]octane neolignans **5e/5c** were predominant in Santa Catarina specimen. The embryogenic cultures also produced both types of neolignans, but **1d** was the major compound.

Experimental

General

Prep. TLC was carried out on silica gel PF-254 (Merck) and CC on silica gel 60H (0.005-0.045 mm) (Merck). Optical rotations were measured on Jasco Mod. DIP-370. ^{1}H and ^{13}C NMR spectra were registered on a Bruker AC-200 spectrometers using $CDCl_3$ as solvent and TMS as internal standard. EIMS (70 eV) and CIMS (methane) were obtained on a HP 5988-A spectrometer. HRESIMS (positive mode) were recorded on a Bruker Daltonics microTOF. Elemental analysis were performed on a Perkin-Elmer CHN Elemental Analyser 2400. HPLC were performed on Gilson 321 using a reverse phase column (Supelco, C-18, 4.6 × 250 mm, 5 µm).

Plant material

The leaves of the *O. catharinensis* were collected in Brusque, Santa Catarina State, Brazil in June 1993. The species was collected and identified by Prof. Ademir Reis (Departamento de Botânica, UFSC). The voucher specimen (FLOR-17560) was deposited in the FLOR Herbarium (UFSC).

Table 2. ^{1}H and ^{13}C NMR of compounds **5c**, **5e**, **7a** and **7b** [δ, J (Hz), 200 MHz and 50 MHz, $CDCl_3$]

position	**5c**		**5e**		**7a**		**7b**	
	^{1}H	^{13}C	^{1}H	^{13}C	^{1}H	^{13}C	^{1}H	^{13}C
1		130.8		131.5		131.8		132.5
2	6.51 (brs)	109.4	6.14 (d, 1.4)	109.4	6.50 (s)	107.9	6.13 (s)	108.9
3		146.9		148.7		147.1		142.9
4		147.6		134.4		147.4		148.6
5	6.69 (d, 7.6)	107.9		142.9	6.70 (d, 8.4)	108.9		108.9
6	6.49 (d, 7.6)	122.9	6.17 (d, 1.4)	103.1	6.49 (dd, 8.4; 1.8)	122.2	6.13 (s)	122.2
7	2.54-2.59 (m)	53.9	2.46-2.51 (m)	54.1	2.89 (d, 4.5)	62.2	2.81 (d, 4.5)	62.4
8	2.19-2.24 (m)	47.1	2.15-2.22 (m)	47.0	2.53 (dd, 6.7; 4.5)	37.2	2.53 (dd, 7.0; 4.5)	37.2
9	0.96 (d, 6.6)	14.3	0.92 (d, 6.7)	14.4	1.18 (d, 6.7)	18.9	1.12 (d, 7.0)	18.9
1’		54.2		54.2		56.6		56.5
2		204.9		204.9		201.6*		201.5*
3’		93.7		93.6		85.2**		85.2**
4’		191.7		191.7		199.2*		198.1*
5’		152.7		152.8		88.4**		88.4**
6’	5.81 (s)	117.7	5.76 (s)	117.8	2.96 (s)	52.6	2.91 (s)	52.7
7’	2.52-2.58 (m)	35.8	2.46-2.51 (m)	35.7	2.23 (dd, 15.2; 5.7) 2.65 (dd, 15.2; 8.4)	34.4	2.20 (dt, 5.7; 2.9; 1.4) 2.45 (dd, 6.7; 4.6)	34.3
8’	5.87-5.96 (m)	132.9	5.82-5.91 m	132.9	5.80-5.92 (m)	132.6	5.80-5.87 (m)	132.6
9’	5.19 (d, 17.0) 5.17 (d, 10.3)	119.3	5.13 (d, 16.6) 5.11 (d, 10.6)	119.3	5.20 (d, 15.7) 5.23 (d, 11.2)	119.6	5.20 (d, 15.7) 5.25 (d, 11.6)	119.6
CH_2O_2	5.91 (d, 2.3)	101.1	5.88 (d, 1.3)	101.3	5.91 (s)	101.1	5.86 (d, 1.5)	101.4
MeO-5	-	-	3.78 (s)	55.8	-	-	3.78 (s)	56.1
MeO-3’	3.52 (s)	56.3	3.47 (s)	56.3	3.35 (s)	55.1	3.31 (s)	55.2
MeO-5’	3.73 (s)	53.9	3.67 (s)	56.5	3.61 (s)	58.4	3.55 (s)	58.4

*,**: Assignments may be reversed.

Table 3. ^{1}H and ^{13}C NMR data of compound **9** [δ, J (Hz), 200 MHz and 50 MHz, $CDCl_3$]

position	**9**	
	^{1}H	^{13}C
1	4.83-4.85	124.7
2	1.75-2.0 (m)	25.2
3	1.75-2.2 (m)	39.2
4		133.0
5	4.83-4.85 (m)	126.5
6	0.90-1.1 (m) 1.75-2.0 (m)	47.0
7	1.9-2.2 (m)	49.7
8	3.14 (ddd; 2.9; 2.7; 2.4)	70.0
9	1.75-2.2 (m)	39.3
10		132.2
11		33.6
12	0.95 (s)	31.2
13	1.05 (s)	27.7
14	1.44 (s)	15.9
15	1.59 (s)	18.3

Initiation and multiplication of embryogenic cultures

Embryogenic cultures were initiated from mature zygotic embryos of *O. catharinensis* according to described methodology.[1,2]

The somatic embryos produced at early cotyledonary stage (2-3 mm length) were inoculated in Woody Plant Medium (WPM, Sigma Co., USA) supplemented with 22.7 g L^{-1} sorbitol, 2 g L^{-1} Phytagel, 20 g L^{-1} sucrose and 400 mg L^{-1} glutamine (pH 5.8) and maintained at 25 °C and with photon flux of 22 µmol m^{-2} s^{-1} provided by fluorescent tubes under 16 h photoperiod.[2,18] After four weeks cultivation the somatic embryos at mature stage (≥ 5 mm) were transferred to Petri dishes (6 cm), spread over two filter papers, and maintained at 25 °C for 4 days for desiccation.

Extraction and isolation of the constituents from leaves

Dried and powdered leaves (395.0 g) were exhaustively extracted with hexane at room temp. Evaporation of the hexane under a reduced pressure gave a residue, which was partitioned between hexane and MeOH-H_2O (9:1). The hydroalcoholic phase was concentrated under reduced pressure yielding 2.4 g. This residue was submitted to flash chromatography column (silica gel, 150 g) and eluted with hexane-EtOAc mixtures at increasing polarities (7:3 to 0:1), yielding 120 fractions (30 mL each). Frs. 29-32 (33.0 mg) submitted to prep. TLC (silica gel, hexane-Et_2O, 4:1) gave spathulenol **8** (7.0 mg) and humula-4,8-dien-2-ol **9** (17.0 mg). Frs. 33-41 (76.0 mg) was also fractionated by prep. TLC (silica gel, cyclohexane-Me_2CO, 98:2) followed by prep. TLC (silica gel, hexane-$CHCl_3$-*iso*-PrOH, 94.5:5.0:0.5) and afforded **2d** (5.5 mg) and **2a** (4.9 mg). Frs. 44-59 (60.0 mg) submitted to prep. TLC (silica gel, cyclohexane-Me_2CO, 97:3) followed by prep. TLC (silica gel, $CHCl_3$-EtOAc-*iso*-PrOH, 94.5:5.0:0.5) gave **2g** (3.0 mg) and **6** (6.0 mg). Fr. 60 (24.0 mg) fractionated by prep. TLC (silica gel, cyclohexane-Me_2CO, 98:2) yielded **5c** (6.0 mg). Frs. 61-65 (440.0 mg) submitted to flash chromatography column (silica gel, $CHCl_3$-EtOAc-*iso*-PrOH, 89:10:1), affording three sub-fractions (S_1-S_3). Prep. TLC of sub-fraction S_1 (70.0 mg) (silica gel, $CHCl_3$-EtOAc-*iso*-PrOH, 94.5:5.0:0.5) afforded **7a** (17.0 mg) and prep. TLC of sub-fraction S_3 (80.0 mg) (silica gel, $CHCl_3$-EtOAc-*iso*-PrOH, 94.5:5.0:0.5) yielded **5c** (30.0 mg). Frs. 66-71 (272.0 mg) fractionated by prep. TLC [silica gel, cyclohexane-Me_2CO (97:3)] furnished **2e** (6.0 mg). Frs. 77-79 (150.0 mg) submitted to chromatotron (silica gel, hexane-EtOAc-*iso*-PrOH, 69:30:1), to give two sub-fractions (S_4 and S_5). Prep. TLC of sub-fraction S_4 (14.0 mg) (silica gel, cyclohexane-Me_2CO, 95:5) gave **2a** (5.0 mg) and **7b** (4.0 mg) and prep. TLC of sub-fraction S_5 (98.0 mg) (silica gel, cyclohexane-Me_2CO, 95:5) gave **5e** (70.0 mg). Frs. 80-81 (98.0 mg) submitted to prep. TLC (silica gel, CH_2Cl_2-Me_2CO, 95:5) yielded **4b** (12.0 mg). Fr. 82-86 (500.0 mg) submitted to chromatotron (silica gel, hexane-EtOAc-*iso*-PrOH, 89:10:1) afforded **2e** (7.2 mg) and two sub-fractions S_6 and S_7. Sub-fraction S_6 (400.0 mg) was further fractionated by flash chromatography column (silica gel, CH_2Cl_2-Me_2CO, 98:2) yielding **2f** (12.0 mg) and **3b** (9.0 mg). Sub-fraction S_7 (43.0 mg) was submitted to chromatotron (silica gel, CH_2Cl_2-Me_2CO, 95:5) to give **1a** (24.0 mg). Frs. 105-110 (87.0 mg) was submitted to chromatotron (silica gel, CH_2Cl_2-Me_2CO, 96:4) yielding **1d** and **1e** (5.5 mg) as a mixture.

Extraction and isolation of the constituents from somatic embryos

The desiccated somatic embryos (400.0 g) were frozen in Me_2CO with dry ice, ground and extracted with 500 mL MeOH-H_2O (4:1). The hydroalcoholic extracts was concentrated and successively partitioned with hexane and $CHCl_3$ (3 × 200 mL, each). The $CHCl_3$ residue (360.0 mg) was submitted to flash chromatography column (silica gel, 150 g) and eluted with CH_2Cl_2-Me_2O at increasing polarity (1:1 to 0:1), affording 160 fractions (20 mL each). Fr. 1 (18.0 mg) submitted to prep. TLC (cyclohexane-Me_2CO, 95:5) yielded **2a** (5.0 mg) and **6** (5.0 mg). Frs. 2-12 (7.0 mg) submitted to prep. TLC (cyclohexane-Me_2CO, 95:5) gave **2a** (1.0 mg) and **2e** (2.0 mg). Frs. 13-21 (20.5 mg) submitted to prep. TLC (cyclohexane-Me_2CO, 95:5) gave **2b** (3.0 mg), **2a** (4.0 mg) and **1c** (3.0 mg). Frs. 22-54 (43.0 mg) purified by prep. TLC (CH_2Cl_2-Me_2O, 9:1) yielded **4a** (3.0 mg) and **6** (4.0 mg). Frs. 145-158 (29.0 mg) fractionated by prep. TLC ($CHCl_3$-MeOH, 9:1) yielded **1d** (7.0 mg).

*(7S,8S,1’R,3’R)-3,4,5,3’,5’-Pentamethoxy-4’-oxo-$\Delta^{1,3,5,5',8'}$-8.1’,7.O.6’-neolignan (**1e**)*

Viscous oil; IR (film) ν_{max}/cm^{-1}: 3469, 2925, 1707, 1695, 1500, 1446, 1371, 1218, 1087, 1033, 925, 816, 772; ^1H and ^{13}C NMR, see Table 1; HRESIMS *m/z*: 419.2090 $[M+H]^+$ (calcd for $C_{23}H_{31}O_7$, 419.2071); EIMS (70 eV) *m/z* (rel. int.): 418(M^+, 34), 388(19), 377(100), 349(87), 317(29), 285(13), 208(44), 181(22), 91(13).

*rel(7R,8S,1’R,2’S)-2’-Hydroxy-3,4,5’-trimethoxy-3’-oxo-$\Delta^{1,3,5,4',8'}$-8.1’,7.O.2’-neolignan (**2f**)*

Viscous oil; $[\alpha]_D^{21} = -130^o$ (MeOH, $c = 0.20$ g/100mL); IR (film) ν_{max}/cm^{-1}: 3435, 2936, 2849, 1739, 1664, 1588, 1511, 1457, 1251, 1142, 1012, 762; ^1H and ^{13}C NMR, see Table 1; EIMS (70 eV) *m/z* (rel. int.): 374(M^+, 7), 194(17), 167(100), 166(66), 165(76), 139(56), 95(17), 77(21), 69(18).

*rel (8S, 1’R, 5’R)-3,4,3’,5’-Tetramethoxy-7,2’-dioxo-$\Delta^{1,3,5,3',8'}$-8.1’-neolignan (**3b**)*

Viscous oil; $[\alpha]_D^{21} = -17^o$ (MeOH, $c = 0.92$ g/100mL); IR (film) ν_{max}/cm^{-1}: 2957, 2914, 2860, 2348, 1739, 1620, 1511, 1457, 1371, 1263, 1229, 1144, 1023, 766; ^1H and ^{13}C NMR, see Table 1; HRESIMS *m/z*: 389.1983 $[M+H]^+$ (calcd for $C_{22}H_{29}O_6$, 389.1966); EIMS (70 eV) *m/z* (rel. int.): 388(M^+, 4), 352(30), 339(23), 324(10), 316(3), 165(100), 137(6).

*rel(7S,8S,1’R,2’S)-2’-Hydroxy-3,4-methylenedioxy-3’,5’-dimethoxy-$\Delta^{1,3,5,3',8'}$-8.1’,7.O.2’-neolignan (**4a**)*

Viscous oil; ^1H NMR see Table 1.

*rel(7R,8S,1’R,2’S)-2’-Hydroxy-3,4-methylenedioxy-5,3’,5’-trimethoxy-$\Delta^{1,3,5,3',8'}$-8.1’,7.O.2’-neolignan (**4b**)*

Viscous oil; ^1H and ^{13}C NMR, see Table 1.

*rel(7S,8R,1'R,3'R)-4'-Hydroxy-3,4-methylenedioxy-3',5'-dimethoxy-2',4'-dioxo-$\Delta^{1,3,5,5',8'}$-8.1',7.3'-neolignan (**5c**)*

Viscous oil; $[\alpha]_D^{21} = -18^o$ (MeOH, *c* = 3.75 g/100mL); IR (film) ν_{max}/cm^{-1}: 1765, 1698, 1504, 1491, 1247, 1094, 1039. ^1H and ^{13}C NMR, see Table 2; HRESIMS *m/z*: 371.1496 $[M+H]^+$ (calcd for $C_{21}H_{23}O_6$, 371.1496); EIMS (70 eV) *m/z* (rel. int.): 370 (M^+, 22), 329(80), 287(12), 269(9), 208(100), 149(82), 137(73), 77(31).

*(7S,8R,1'R,3'R)-4'-Hydroxy-3,4-methylenedioxy-3',5',5-trimethoxy-2',4'-dioxo-$\Delta^{1,3,5,5',8'}$-8.1',7.3'-neolignan (**5e**)*

Viscous oil; $[\alpha]_D^{21} = -29^o$ (MeOH, *c* = 4.48 g/100mL); ^1H and ^{13}C NMR, see Table 2; EIMS (70 eV) *m/z* (rel. int.): 400 (M^+, 52), 359(21), 331(10), 288(14), 219(100), 208(45), 207(43), 193(18), 192(66), 180(53), 165(34), 137(23), 91(14), 77(16).

***7a** (Dimer of **5c**)*

Viscous oil; $[\alpha]_D^{21} = +220^o$ (MeOH, *c* = 0.07 g/100mL); IR (film) ν_{max}/cm^{-1}: 2922, 1766, 1714, 1513, 1453, 1137, 1094,1044; ^1H and ^{13}C NMR, see Table 2; CIMS *m/z* (rel. int.): 741(M^+, 1), 579(1), 419(15), 391(91), 371(100), 341(18), 209(28), 163(37), 57(27).

***7b** (Dimer of **5e**)*

Viscous oil; $[\alpha]_D^{21} = +57^o$ (MeOH, *c* = 0.62 g/100mL); IR (film) ν_{max}/cm^{-1}: 1767, 1714, 1505, 1491, 1445, 1240, 1039; ^1H and ^{13}C NMR, see Table 2; CIMS *m/z* (rel. int.): 801(M^+, 1), 609(18), 429(76), 419(85), 401(100), 371(11), 209(17), 193(11).

*rel(8R)-Humulan-1,4-dien-8-ol (**9**)*

Solid amorphous. Found: C, 80.89 %, H, 11.65% ($C_{15}H_{26}O$ requires: C, 81.02%, H, 11.79%); IR (film) ν_{max}/cm^{-1}: 3425, 2946, 1707, 1446, 1371, 1022, 762; ^1H and ^{13}C NMR, see Table 3; EIMS (70 eV) *m/z* (rel. int.): 344(M^+, 27), 303(9), 271(12), 189(100), 179(26), 178(76), 166(25), 165(66), 151(59), 137(19), 115(15), 107(27), 91(35), 77(33).

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Supplementary Information

Supplementary information is available free of charge at http://jbcs.org.br, as PDF file.

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