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Trypanocidal tetrahydrofuran lignans from inflorescences of *Piper solmsianum*

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Dedicated to the memory of Professor Jeffrey B. Harborne

Abstract

In addition to sitosterol, syringaldehyde, 3,4,5-trimethoxybenzoic acid, isoelemicin and grandisin, two new tetrahydrofuran lignans were isolated from *Piper solmsianun* and characterized as *rel*-(7*R*,8*R*,7′*R*,8′*R*)-3′,4′-methylenedioxy-3,4,5,5′-tetramethoxy-7,7′-epoxylignan and *rel*-(7*R*,8*R*,7′*R*,8′*R*)-3,4,3′,4′-dimethylenedioxy-5,5′-dimethoxy-7,7′-epoxylignan on the basis of spectroscopic data, including 2D NMR spectrometric techniques. Their *in vitro* activity were determined against the trypomastigote form of *Trypanossoma cruzi*.

Keywords: *Piper solmsianum*; Piperaceae; Tetrahydrofuran lignans; *Trypanosoma cruzi*

1. Introduction

Piperaceae species are abundant in the tropics and are important components of secondary vegetation. Due to their economic, medicinal and ecological importance, a number of species have been phytochemically investigated yielding several classes of secondary compounds (Sengupta and Ray, 1987; Parmar et al., 1997, 1998). The phytochemical studies carried out thus far on Brazilian species yielded amides (Giesbrech et al., 1981; Alécio et al., 1998; Santos and Chaves, 1999a; Navickiene et al., 2000, Silva et al., 2002), phenylpropanoids (Santos and Chaves, 1999b; Benevides et al., 1999), prenylated benzoic acids and chromenes (Baldoqui et al., 1999; Moreira et al., 1998a, 1998b), lignan/neolignans (Moreira et al., 1995; Benevides et al., 1999; Martins et al., 2000), and aristolactams (Araujo Jr. et al., 1999).

The leaves and stem barks from two different specimens of *P. solmsianum* have previously been investigated and yielded one benzofuran lignan and one glycosylated flavonoid (Moreira et al., 1995) as well as five phenylpropanoids and two tetrahydrofuran lignans (Martins et al., 2000). In continuation of this investigation on *P. solmsianum,* the isolation of isoelemicin (**1**), syringaldehyde (**2**), 3,4,5-trimethoxybenzoic acid (**3**), sitosterol (**4**), grandisin (**5**) besides two new tetrahydrofuran lignans *rel*-(7*R*,8*R*,7′*R*,8′*R*)-3′,4′-methylenedioxy-3,4,5,5′-tetramethoxy-7,7′-epoxylignan (**6**) and *rel*-(7*R*,8*R*,7′*R*,8′*R*)-3,4,3′,4′-dimethylenedioxy-5,5′-dimethoxy-7,7′-epoxylignan (**7**) from inflorescences is reported. Additionally, since the tetrahydrofuran lignans veraguensin and grandisin (**5**), previously isolated from *Virola surinamensis* (Lopes et al., 1998), were proved to be the most potent *in vitro* natural products against trypomastigote form of *Trypanossoma cruzi,* both lignans **6** and **7** had their potential activities evaluated as well.

2. Results and discussion

The EtOAc extract from inflorescence of *P. solmsianum* was subjected to a series of chromatographic separations over Si-gel, resulting in the isolation of isoelemicin (**1**), syringaldehyde (**2**), 3,4,5-trimethoxy-

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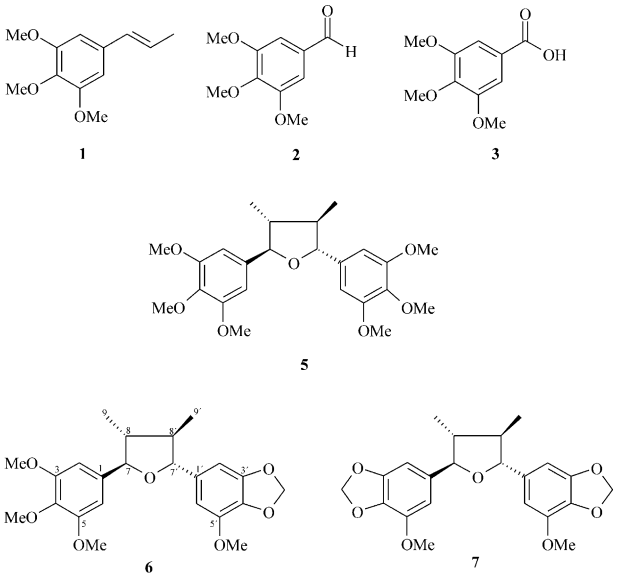

benzoic acid (**3**), sitosterol (**4**), grandisin (**5**) and two new tetrahydrofuran lignans (**6** and **7**). The known compounds **1**–**5** were identified by comparison of ^{1}H and ^{13}C–NMR spectroscopic and EI-MS data with those in the literature (Barbosa-Filho et al., 1989; Almad et al., 1992; Holloway and Scheinmann, 1974). Their identities were confirmed further by co-injection in GC analysis with authentic compounds.

The ^{13}C NMR spectra (BBD and DEPT 135°) of **6** showed signals assignable to six methyl, one methylene, eight methine and eight quaternary carbons. The EI-mass spectral data showing a molecular peak at m/z 416 Da were compatible with a molecular formula of $C_{23}H_{28}O_7$. The IR spectrum showed an intense absorption band at 1133 cm^{-1}, suggesting an ether functionality. Since no absorptions near 3500 and 1700 cm^{-1} were observed, the presence of hydroxyl or carbonyl groups were not considered further.

The ^{1}H NMR spectrum of **6** was very similar to that of grandisin **5** (Sarkanen and Wallis, 1973; Martins et al., 2000). The set of signals at δ 0.96 (2 CH_3, *d*, 4.5 Hz), 1.67 (2 CH, *m*), and 4.52 (2 CH, *d*, 7.2 Hz), whose connectivities were established by HMBC spectrum, confirmed the symmetric tetrahydrofuran ring moiety. The remaining signals at δ 5.87 (*s*, 2H), 3.84 (3H), 3.80 (6H) and 3.76 (3H) were assigned to a methylenedioxyphenyl and four aromatic methoxyl groups. This information, associated to aromatic hydrogens signals at δ 6.54 (*s*, 2H) and 6.52 (*s*, 2H), determined the two aromatic rings as 3,4,5-trimethoxyphenyl and 5′-methoxy-3′,4′-methylenedioxyphenyl for this lignan. The relative stereochemistry in the tetrahydrofuran ring was defined as all *trans* based on the coupling constants values observed in ^{1}H NMR spectrum (Table 1).

All these assignments were confirmed by HMBC correlations, in which mutual cross peaks between C-7/H-2, H-6, H-8′ and H-9 and between C-7′/H-2′, H-6′, H-

Table 1
NMR spectral data for lignans **6** and **7** [δ, 300 (^{1}H), 75 MHz (^{13}C) and 125/500 MHz (HMBC), $CDCl_3$, *J* given in Hz]

	6			**7**		
C	δ_C	δ_H	HMBC (C→H)	δ_C	δ_H	HMBC (C→H)
1	137.8	–	H-2, H-6, H-8	134.6	–	H-2, H-6, H-8
2	103.1	6.54 (*s*, 1H)	H-6, H-7	105.8	6.51 (*s*, 1H)	H-6, H-7
3	153.2	–	–	143.5	–	–
4	137.4	–	H-2, H-6	137.1	–	H-2, H-6
5	153.2	–	-	148.9	–	–
6	103.1	6.54 (*s*, 1H)	H-2, H-7	101.4	6.51 (*s*, 1H)	H-2, H-7
7	88.3	4.52 (*d*, 7.2, 1H)	H-2, H-6, H-8′, H-9	88.4	4.50 (*d*, 9.0, 1H)	H-2, H-6, H-8′, H-9
8	50.8	1.67 (*m*, 1H)	H-7′, H-8′, H-9′	51.0	1.68 (*m*, 1H)	H-7′,H-8″, H-9′
9	13.8	0.96 (*d*, 4.5, 3H)	H-7, H-8′	13.9	0.96 (*d*, 6.3, 3H)	H-7, H-8′
1′	134.5	–	H-2′, H-6′, H-8′	134.6	–	H-2′, H-6′, H-8′
2′	105.7	6.52 (*s*, 1H)	H-6′, H-7′	105.8	6.51 (*s*, 1H)	H-6′, H-7′
3′	143.4	–	–	143.5	–	-
4′	137.1	–	H-2′, H-6′	137.1	–	H-2′, H-6′
5′	148.8	–	–	148.9	–	–
6′	100.2	6.52 (*s*, 1H)	H-2′, H-7′	100.3	6.51 (*s*, 1H)	H-2′, H-7′
7′	88.4	4.52 (*d*, 7.2, 1H)	H-2′, H-6′, H-8, H-9′	88.4	4.50 (*d*, 9.0, 1H)	H-2′, H-6′, H-8, H-9′
8′	51.1	1.67 (*m*, 1H)	H-7, H-8, H-9	51.0	1.68 (*m*, 1H)	H-7, H-8, H-9
9′	13.9	0.96 (*d*, 4.5, 3H)	H-7′, H-8	13.9	0.96 (*d*, 6.3, 3H)	H-7′, H-8
O_2CH_2	101.3	5.87 (*s*, 2H)	–	101.4	5.88 (*s*, 2H)	–
O_2CH_2	–	–	–	101.4	5.88 (*s*, 2H)	–
OCH_3/3	56.1	3.80 (*s*, 3H)	–	–	–	–
OCH_3/4	60.7	3.76 (*s*, 3H)	–	–	–	–
OCH_3/5	56.1	3.80 (*s*, 3H)	–	56.7	3.85 (*s*, 3H)	–
OCH_3/5′	56.6	3.84 (*s*, 3H)	–	56.7	3.85 (*s*, 3H)	–

8 and H-9′ were observed. Therefore, the structure for the new lignan **6** was determined as *rel*-(7*R*,8*R*,7′*R*,8′*R*)-3′,4′-methylenedioxy-3,4,5,5′-tetramethoxy-7,7′-epoxylignan.

The structure of compound **7** was determined based on the similarities of spectrometric data with **6**. Its ^{1}H NMR spectrum showed signals at δ 6.51 (*s*, 2 Ar–H), 5.88 (*s*, O_2CH_2), 4.52 (*d*, 9.0 Hz, 2H), 3.84 (*s*, 2 OCH_3), 0.96 (*d*, 6.3 Hz, 2 CH_3). These data were compatible with a tetrahydrofuran lignan having the 5,5′-dimethoxy-3,4,3′,4′-dimethylenedioxyphenyl groups. The same all *trans* configuration as that of lignan **6** could be determined based on $J = 9.0$ Hz at δ 4.50 (2H, H-7/H-7′) and also by ^{13}C NMR data (Table 1). The molecular ion peak at *m*/*z* 400 Da in the EI-MS was in agreement with the molecular formula $C_{22}H_{24}O_7$. EI-MS spectrum also showed fragment ions at *m*/*z* 208 and 192 Da, assignable to the methoxymethylenedioxyoxopropanephenyl and methoxymethylenedioxypropenylphenyl ions, respectively (Holloway and Scheinmann, 1974). The ^{13}C NMR spectroscopic data (BBD and DEPT 135°) of **6** were again in full accordance with a symmetric tetrahydrofuran lignan (Table 1). In an HMBC experiment, cross-peaks between C-7/ H-2, H-6, H-8′ and H-9 and between C-7′/H-2′, H-6′, H-8 and H-9′ as well as between C-1/H-2, H-6 and H-8 and between C-1′/H-2′, H-6′ and H-8′ were observed. Thus, **7** was determined as *rel*-(7*R*,8*R*,7′*R*,8′*R*)-3,4,3′,4′-dimethylenedioxy-5,5′-dimethoxy-7,7′-epoxylignan.

In a previous study carried out with leaves and stem barks of *P. solmsianum*, five phenylpropanoids and two tetrahydrofuran lignans, including grandisin **5** (Martins et al., 2000) were described. In this work, grandisin **5** and isoelemicin **1** were also detected in its inflorescence as major metabolites, but two new additional tetrahydrofuran lignans (**6** and **7**), one phenylpropanoid and two possibly degradation derivatives from isoelemicin **1** (syringaldehyde **2** and 3,4,5-trimethoxybenzoic acid **3**) were described.

Since the lignan grandisin **5** has previously been shown to be a potent in vitro antichagasic compound against the trypomastigote form of *Trypanosoma cruzi* (Lopes et al., 1998), both lignans **6** and **7** were similarly evaluated (Table 2). The lignan **7** bearing two 5-methoxy-3,4-methylenedioxyphenyl groups showed higher activity than 6 and grandisin (**5**), but there was no direct correlation between the substitution pattern and the trypanocide activity since grandisin **5** was more active than **6**. This aspect should be the subject of future investigations including synthetic lignans.

Table 2
In vitro evaluation of trypanocidal activity of tetrahydrofuran lignans on trypomastigote form of *Trypanosoma cruzi* (Y strain)

Compounds	Concentration (μg/ml) l/% of lysis (±S.D.)			IC_{50} (μg/ml)
	5.0	25.0	50.0	
5	52.4±5.6	89.1±8.1	92.8±3.9	8.74
6	23.7±5.3	61.1±2.4	68.3±6.9	17.6
7	55.2±6.2	68.7±0.5	81.0±2.2	3.47

Positive control — gentian violet (250μg/ml).

3. Experimental

3.1. General

EI-MS were measured at 70 eV on a HP 5990/5988 A spectrometer. ^{1}H and ^{13}C NMR spectra were measured in a Bruker DPX-300 (300 and 75 MHz) while 2D experiments (HMQC and HMBC) were recorded in a Bruker DRX-500 (500 and 125 MHz) using $CDCl_3$ (Aldrich) as solvent and TMS as int. standard. Chemical shifts were reported in δ units (ppm) and coupling constants (*J*) in Hz. Optical rotations were measured at $\lambda = 589$ nm in a digital polarimeter JASCO model DIP-370. UV spectra were recorded in MeOH using a HP 8452 A spectrophotometer. IR spectra were measured in KBr pellets in a Perkin-Elmer Infrared Spectrometer model 1750. Elemental analyses were performed on a Perkin-Elmer CHN Elemental Analyser 2400. Silica gel (Merck, 70–230 mesh) was used for CC and silica-gel 60 PF_{254} Merck (0.50 and 1 mm) for anal. and prep. TLC. Spots on chromatograms were detected under UV light (254 and 365 nm) and by spraying with H_2SO_4 60% and ceric sulphate solutions followed by heating.

3.2. Plant material

Inflorescence of *P. solmsianum* were collected in Núcleo de Picinguaba, City of Ubatuba, São Paulo State, Brazil in October of 2000. Identification of the botanical material has been reported previously (Martins et al., 2000).

3.3. Chromatographic isolation procedures

Dried inflorescence (325 g) of *P. solmsianum* were milled and extracted with EtOAc which after conc. in vacuum yielded 21 g of crude extract. This extract was submitted to CC and eluted with a gradient of hex–EtOAc, followed by prep. TLC (hex–EtOAc 9:1 and 8:2) to afford **1** (1.10 g), **2** (11 mg), **3** (13 mg), **4** (31 mg), **5** (215 mg), **6** (93 mg), and **7** (9 mg).

3.4. rel-(7*R*,8*R*,7′*R*,8′*R*)-3′,4′-Methylenedioxy-3,4,5,5′-tetramethoxy-7,7′-epoxylignan (6)

Pale yellow oil. $[\alpha]_D^{21}$ −4.5 (MeOH; *c* 0.01). IR (KBr) ν_{max} cm^{-1}: 2957, 2924, 1634, 1506, 1457, 1435, 1091, 1031. UV λ_{max} (MeOH) nm (ϵ): 254 (12650), 273 (5440). ^{1}H and ^{13}C NMR: see Table 1. EI-MS *m*/*z* (rel. int.):

416 $[M]^+$ (18), 236 (14), 224 (32), 220 (29), 208 (100), 205 (50), 192 (19), 175 (24), 165 (12), 147 (13), 135 (6), 91 (12), 77 (9). Found C, 66.27%; H, 6.84%, requires C, 66.33%; H, 6.78%.

3.5. *rel-(7R,8R,7′R,8′R)-3,4,3′,4′-Dimethylenedioxy-5,5′-dimethoxy-7,7′-epoxylignan (7)*

Pale yellow oil. $[\alpha]_D^{21}$ −10.1 (MeOH; *c* 0.01). IR (KBr) ν_{max} cm^{-1}: 2957, 2894, 1634, 1593, 1505, 1459, 1426, 1130, 1096, 1033. UV λ_{max} (MeOH) nm (ϵ): 254 (15745), 277 (4270). ^1H and ^{13}C NMR: see Table 1. EI-MS *m*/*z* (rel. int.): 400 $[M]^+$ (14), 220 (55), 208 (74), 205 (53), 192 (100), 175 (44), 165 (22), 147 (23), 91 (14), 77 (10). Found C, 66.05%; H, 5.99%, requires C, 65.99%; H, 6.04%.

3.6. *In vitro bioassay*

Bioassays were carried out using blood collected by cardiac puncture of Swiss albino mice in the parasitemy peak (7th day) after infection with the Y strain of *T. cruzi*. The infected blood was diluted with blood of healthy mice to achieve a concentration of 10^6 trypomastigote forms/ml. The standard solutions (in DMSO) were added into the infected blood to provide concentrations of 5.0, 25.0 and 50.0 μg/ml, respectively. The plates were incubated at 4 °C during 24 h and the number of parasites determined according to method described (Brener, 1962). The bioassays were performed in triplicate on microtiter plates (96 wells), which contained 200 μl of mixture/well. Negative and positive controls containing either DMSO or gentian violet were ran in parallel.

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