Configuration and stability of naturally occurring all-cis-tetrahydrofuran lignans from *Piper solmsianum*

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The study of variability of the tetrahydrofuran lignan (−)-grandisin in leaves of *Piper solmsianum* (Piperaceae) revealed two unknown compounds, that were isolated and determined to be the all-cis tetrahydrofuran lignans 1a [rel-(7R,8S,7'S,8'R)-3,4,5,7,8-pentamethoxy-7,7'-epoxy lignan] and 1b [rel-(7R,8S,7'S,8'R)-3',4'-methylenedioxy-3,4,7,7'-tetramethoxy-7,7'-epoxy lignan]. Their structures were determined by spectroscopic analysis including 1D and 2D-NMR while their configurations were determined by ECD associated to the density functional theory (DFT) at the B3LYP/6-31G(d,p) level. The hydrogen bonds between methoxy groups in trimethoxylphenyl rings stabilizes the all-trans tetrahydrofuran lignan grandisin by 6.5 kcal mol⁻¹ as compared to the corresponding all-cis isomer of grandisin. The occurrence of all-cis tetrahydrofuran lignans as natural products is a very rare event.

Introduction

Lignans are dimers of phenylpropanoid units widely found in the plant kingdom and with a variety of biological properties such as cytotoxic, antioxidant, antimicrobial, anti-inflammatory, antiviral and immunosuppressive activities. The dimerization process in their formation requires an oxidative coupling mechanism forming a β-β bonding, which can be followed by further reactions yielding eight different sub-classes including tetrahydrofuran, dihydrofuran, dibenzylbutane, dibenzylbutyrolactone, aryltetralin, arynaphthalene, dibenzocylooctadiene and dibenzylbutyrolactol lignans. Tetrahydrofuran (THFs) lignans can be further subdivided in 7,7'-monooepoxy, 7,9'-monooepoxy- and 9,9'-monooepoxy-lignans. The THFs lignans have been described so far from several plant species and their structural diversity results from the type of substituent in the aromatic ring that can be hydroxy, methoxy, methylenedioxy and a combination of them. The relative configuration among aryls and methyl groups can give rise to further stereochemistry diversity. In case of having the same aromatic rings, ten isomers are possible consisting of four diastereomeric pairs besides two meso forms. The (−)-grandisin is an example of the all-trans tetrahydrofuran lignan, which was formerly isolated from the Lauraceae plants, *Litsea grandis* and then from *Licaria aurea* and *Cryptocarya crassinervia*. Grandisin has also been isolated from monocotyledoneous Araceae, from other Angiosperms families such as Piperaceae, Myristicaceae, Magnoliaceae, Annonaceae but also from Rosids including Meliaceae, Violaceae species, and other plant species. The THFs lignans have displayed several biological activities such as anti-PAF, antiprotozoal, larvicidal, cytotoxic, estrogenic, anti-inflammatory, and NF-κB inhibitory activity. Thus, some studies have also been addressed to generate analogues. To date, the natural occurrence of all-cis tetrahydrofuran lignan has only been described from *Anogeissus acuminata* (Combretaceae) and from *Illicium floridanum* (Illiciacae). Besides, a synthetic version of all-cis had been obtained by catalytic hydrogenation of a furan derivative. Additionally, a series of synthetic tetrahydrofuran lignans with various stereochemistry were prepared, including the all-cis tetrahydrofurugiaacin B, in order to evaluate their plant growth inhibitory activity. Herein we report on the isolation and structural determination of two novel all-cis tetrahydrofuran lignans 1a and 1b from the leaves of two specimens of *Piper solmsianum*. Besides, two known lignans 2b and 3a were also isolated (Fig. 1). The structures of the last two tetrahydrofuran lignans had been only reported without the assignment of their configurations or optical rotations. The determination of configuration of THFs lignans (1a, 1b, 2b and 3a) were carried out using electronic circular dichroism (ECD) having (−)-grandisin as reference data since its configuration was previously reported based on crystallographic studies. Additionally, since natural all-cis tetrahydrofuran lignans, such as 1a and 1b, are of rare occurrence a study of structural stability were undertaken using density functional theory (DFT) at the B3LYP/6-31G(d,p) level. The relative energies of lignans 1a and 3a were compared to that of all-trans lignan (−)-grandisin (2a). Solvent effects were analysed by the self-
consistency reaction field (SCRF) method, and the energy difference in water and cyclohexane environments were determined with the polarizable continuum model PCM(B3LYP/6-31G(d,p)) method.

**Results and discussions**

**Analysis of crude extracts from *P. solmsianum***

The tetrahydrofuran lignan (−)-grandisin has displayed several bioactivities and thus, the initial approach was to determine its variability in specimens of *P. solmsianum* occurring in several sites in São Paulo State. The initial survey in samples from leaves of different sites revealed common profiles based on the occurrence of phenylpropanoids apioline–dillapiole and grandisin (2a). Then, the analysis of chemical variability of a population of *P. solmsianum* occurring in the Campus of University of São Paulo was undertaken and several samples collected presented an unusual chromatographic profile with one major peak with shorter retention time than that of grandisin. These extracts were submitted to 1H NMR analysis, which suggested the occurrence of a major tetrahydrofuran lignan (1a) with similar oxygenation pattern in the aromatic rings with six methoxy groups. Crude extracts from different *P. solmsianum* individuals collected in Alberto Lögren State Park analysed by HPLC indicated a second tetrahydrofuran lignan, having methoxy and 3,4-methylenedioxy groups (1b). Thus, both compounds were isolated and had their structures determined by spectroscopic analysis.

**Structural determination of lignans 1a, 1b, 2b and 3a**

The lignan 1a was isolated as a pale white powder with purity of 99.6% by HPLC. Its HR-ESIMS spectrum displayed a quasi-molecular ion [M + H]+ peak at 99.6% by HPLC. Its HR-ESIMS spectrum displayed a quasi-molecular ion [M + H]+ peak at m/z 433.2232 corresponding to

**Table 1 NMR spectral data**

| Lignan | δ 1H | δ 13C | HMBC |
|--------|------|-------|------|
| 1a     | —    | 137.0 | C6, C2 |
| 2      | 6.67 (s, 1H) | 103.6 | C6, C7, C8, C4, C3 |
| 3      | —    | 153.1 | C2, OCH3 |
| 4      | —    | 136.0 | C-6, OCH3-4 |
| 5      | —    | 153.1 | C-6, OCH3-5 |
| 6      | 6.67 (s, 1H) | 103.6 | C2, C7, C8, C4, C5 |
| 7      | 5.11 | 82.9  | C9, C8, C2, C3, C1 |
| 8      | 2.70 (m, 1H) | 41.6  | C7, C7', C8, C9, C9', C1 |
| 9      | 0.64 | 11.6  | C9, C8, C8', C7, C1 |
| 1a'    | —    | 137.0 | C6', C2' |
| 2'     | 6.67 (s, 1H) | 103.6 | C6', C7', C8', C4, C3' |
| 3'     | —    | 153.1 | O2CH3, C2' |
| 4'     | —    | 136.0 | O2CH3, C6' |
| 5'     | —    | 153.1 | C6', OCH3-5' |
| 6'     | 6.67 (s, 1H) | 103.6 | C2', C7', C8', C4', C5' |
| 7'     | 5.11 | 82.9  | C9, C8, C2, C3, C1' |
| 8'     | 2.70 (m, 1H) | 41.6  | C7, C7', C8, C9, C9', C1' |
| 9'     | 0.64 | 11.6  | C9, C8, C8', C7, C1' |
| 1b     | —    | 135.3 | C6, C2 |
| 2      | 6.65 (s, 1H) | 103.5 | C6, C7 |
| 3      | —    | 153.0 | C2, OCH3-3 |
| 4      | —    | 137.5 | C6, OCH3-4 |
| 5      | —    | 153.0 | C6, OCH3-5 |
| 6      | 6.65 (s, 1H) | 103.5 | C2, C7 |
| 7      | 5.09 | 82.8  | C9, C2, C3 |
| 8      | 2.67 (m, 1H) | 41.5  | C7, C7', C8, C9, C9' |
| 9      | 0.64 | 11.6  | C8, C8', C7 |

Note: 500 MHz for 1H and 125 MHz for 13C; compounds dissolved in CDCl3; δ in ppm relative to TMS, J in Hz.
the molecular formula C_{23}H_{33}O_{7}. The IR of 1a showed no absorptions near 3400 cm\(^{-1}\), but an intense band at 1128 cm\(^{-1}\) suggesting C-O-C functions. The simplicity of the \(^1\)H and \(^13\)C NMR spectra (Fig. S1 and S2; ES\(^{†}\)) indicated that 1a is a symmetrical compound (Table 1). The dimethyl-tetrahydrofuran moiety could be readily recognized in the \(^1\)H NMR spectrum by the doublets at \(\delta_{H} 6.06 (6H, J = 6.7 \text{ Hz, } H-9/H-9')\) and 5.11 (2H, \(J = 6.7 \text{ Hz, } H-7/H-7')\), and a multiplet at \(\delta_{H} 2.70 (2H, H-8/H-8')\). The singlet at \(\delta_{H} 6.74 (2H, H-2', H-6/H-6')\) was assigned to the two equivalent ortho-methoxy aromatic hydrogens, while the intense singlets at \(\delta_{H} 3.84 (4 \times \text{OCH}_3; 3,3',5',5')\) and 3.86 (2 \times \text{OCH}_3; 4,4') corresponded to the six methoxy groups. The structure of 1a bearing two 3,4,5-trimethoxyphenyl groups was corresponded to the six methoxy groups. The structure of all-determined as a (Tables 1 and S1). The dimethyl-tetrahydrofuran group appeared as two broad singlets at \(\delta_{H} 0.64 (6H, H-9/H-9')\) and 5.11, respectively and the coupling constants of \(J_{H-9/H-9'}\) Hz for the H-9/H-9' hydrogens is compatible with a dihedral angle of approximately 30°, indicating all-cis relationship between the methyl and aryl substituents. The up-field shifted methyl carbons at \(\delta_{C} 11.6\) in the \(^13\)C NMR is contrasting to the all-trans isomer grandisin (2a), which appeared at \(\delta_{C} 13.9\). This shielding effect has been assigned to the anisotropic effect of the pseudo-axially oriented aromatic ring on the methyl groups of 1a. The chemical shifts of C-7/C-7' observed at \(\delta_{C} 82.9\), and of C-8/C-8' at \(\delta_{C} 41.6\), provided further evidences for the cis configurations between the methyl and aryl groups of 1a (Tables 1 and S1). All the \(^1\)H and \(^13\)C NMR data indicated that the lignan 1a is all-cis isomer of 2a (grandisin)\(^{23}\) thus, the structure was determined as rel-(7R,8S,7'S,8'R)-3,4,5,5'-tetramethoxy-7,7'-epoxylignan.

Compound 1b was isolated as pale-yellow oil by preparative TLC of a methanolic extract of the leaves of P. solmsianum with 99.2% purity indicated by HPLC analysis. The molecular formula of 1b was determined as C_{23}H_{32}O_{7} according to the quasi-molecular ion [M + H]\(^{+}\) observed in the HR-ESIMS at m/z 417.1915.

Analysis of the \(^1\)H and \(^13\)C NMR spectra of 1b indicated the structure of an all-cis tetrahydrofuran lignan similarly to 1a (Table 1; Fig. S5 and S6\(^{†}\)). The major differences were associated to the replacement of two methoxy by one methylenedioxy group, which account for the difference of 16 Da between the molecular ions of 1a and 1b. The chemical shift of the equivalent aromatic hydrogens of the 3,4,5-trimethoxyphenyl ring (\(\delta_{H} 6.65, s, 2H\)) was similar to that of 1a, while the hydrogens of the second aromatic ring appeared as two broad singlets at \(\delta_{H} 6.63\) and 6.61 and were assigned to the two aromatic hydrogens of the 3-methoxy-4,5-methylenedioxyphenyl ring. The HMBC contour plot (Fig. S8\(^{†}\)) displayed correlations between the oxymethine protons (H-7/H-7') to C-6/C-6' and C-2/C-2', and between the H-7/H-7' hydrogens to signals of C-9/C-9' (Fig. 2). All NMR data, including HSQC (Fig. S7\(^{†}\)), confirmed the assignments made and thus 1b was determined as rel-(7R,8S,7'S,8'R)-3',4'-methylenedioxy-3,4,5,5'-tetramethoxy-7,7'-epoxylignan.

Compound 2b has been reported previously as rel-(7R,8S,7'S,8'-R)-3',4'-methylenedioxy-3,4,5,5'-tetramethoxy-7,7'-epoxylignan.\(^{35}\)

The compound 3a, determined as (-)-epigandisin based on the spectrometric data analysis, is a lignan formerly isolated from the stem bark of Aglaia leptantha but characterized in a mixture with (-)-grandisin (2a) with an estimated [\(\delta_{310}^{21}\)] value of \(-15 \pm 10\).\(^{39}\) (-)-Epigandisin, without configuration assigned, was also reported from leaves of Rhaphidophora decursiva.\(^{40}\)

### Determination of relative configurations of lignans 1a, 1b, 2b and 3a

The relative configurations of the THF lignans 1a, 1b, 2b and 3a were determined by comparison of ECD spectra with that of (-)-grandisin (2a) (Fig. 3), in which its configuration had been previously determined by X-ray analysis.\(^{7}\) Lignan 1a is optically inactive and no Cotton effect was observed in its ECD curve. The ECD curve of lignan 2b was very similar to that of (-)-grandisin (2a) with a negative Cotton effect at 233 nm, indicating the trans configuration between the aromatic rings. In contrast, the lignans 1b and 3a showed a positive Cotton effect at 220 nm in the ECD curve, i.e. opposite to that of 2a, hence the cis configuration between the aromatic rings were assigned (Fig. 3).
point energy calculation was performed, with the PCM (B3LYP) SCRF models using the 6-31G(d,p) basis set. The tight SCF convergence criteria were specified for all jobs and the predicted energy and solvent effects in cyclohexane and water media were calculated (ESI, Table S3†).

The calculations at the B3LYP/6-31G(d,p) level indicated lignan 2a as the most stable among the lignans 1a and 3a in gas phase, cyclohexane and water. An exception was observed for lignans 2a and 3a, which have similar electronic energy in water medium (Tables S2 and S3†). The calculations showed that inclusion of solvent polarity, and only the change in the energy produced in the high polar medium (i.e., water) was more effective in stabilizing the conformers as compared to the gas phase or apolar solvent (i.e., cyclohexane).

Gas phase optimization geometry of the THF lignans
A remarkable feature in the analysis of the geometrical parameters of 1a, 1b, 2a, 2b and 3a lignans is the stabilization resulting from intramolecular C–H…O hydrogen bonding. Energy calculations predict that the all-trans THF lignan 2a would be 0.42 kcal mol⁻¹ more stable than epigrandisin 3a, and 6.5 kcal mol⁻¹ more stable than the all-cis THF lignan 1a. Such sequence of stability is somewhat compatible with the rare natural occurrence of all-cis THF lignan isomer. In fact, there are only two previous report for it. It is also noteworthy that the methyl group (C9) attached to the THF ring lies close to the aromatic rings in the all-cis lignan 1a, revealing one further possible hydrogen bonding contributing to an unfavourable gauche conformation around the C8 bond (Fig. S9†). Accordingly, the calculated angle at C8-C8’-C7’ is 3° smaller than the corresponding C7-C8-C8’ angle at the THF ring, a feature also observed in 1b. Additionally, the phenyl rings in 1a lie closer to the THF ring compared with the all-trans isomer, resulting in smaller dihedral angles, which is less favourable.

The results concerning structural changes associated to the phenyl rings suggested that the geometrical equilibrium structures of 1b and 2b are dependent on intramolecular hydrogen bonding. On the other hand, the geometrical energy minimization of 2b taking into account both hydrogens but not hydrogen bonding (a difference of ~1 kcal mol⁻¹), showed that these additional hydrogen bonds are not energetically significant. It is also worth to note that in these geometry optimizations, the methoxy groups attached to each of the phenyl ring do not necessary lie close to the ring plane, but assumed the most favourable hydrogen bonding with conformers differing from those of crystallographic data.†

The calculated equilibrium geometries for lignans 1a–2a are shown in Fig. S9 and S10 (ESI†), and the main geometrical parameters, obtained after minimized energies using the B3LYP method, are presented in Tables S4–S7 (ESI†).

Relative energies of the THF isomers without methoxy groups in the phenyl rings
In order to compare the behavior of the THF lignan isomers, a singular qualitative calculation was made for the ten possible tetrahydrofuran isomers (four diastereomeric pairs and two meso forms) (Table S8†) with a lower level of theory as B3LYP/3-21G*. The methoxy groups were not included in the calculation in order to minimize the effect of the hydrogen bondings in the stabilities and thus to determine only the effect of configuration in the stabilities of THF rings. The electronic energy of the optimized structures indicates that the all trans and the all cis structures are the most and the least stable among the ten stereoisomers, respectively (Table S8†). With this finding, we can predict that the configuration and the degree of methoxylation contribute to the stabilization of the THFs lignans.

Conclusions
The structures and absolute configurations of two novel THF lignans (1a, 1b), isolated from leaves of the species P. solmsianum, and of two known lignans (2b, 3a), were determined. In addition, the theoretical electronic calculations provided a new data set for the structures of the THF lignans in gas phase showing that the configuration of the tetrahydrofuran
ring and intramolecular hydrogen bonding are related to the geometrical equilibrium energy profile of the conformers. The calculated energies in the gas phase and in solvents (water and cyclohexane) indicated that lignan 2a is the most stable among the isomeric lignans 1a–3a. Moreover, the calculated data for 1b and 2b could explain the theoretical equilibrium geometries of these lignans since the configuration of the THF ring and intramolecular hydrogen bonding originated from the methoxyl groups in the phenyl ring, compared to the methylenedioxy moiety, contributed to the stabilization of the THF lignan structures.

**Experimental**

**Plant material**

Leaves of *Piper solmsianum* C. DC were collected at the Instituto de Biociências (IB), Universidade de São Paulo (SP, Brazil) and at the Horto Florestal (HF), Instituto Florestal do Estado de São Paulo (SP, Brazil) in January 2003 (Permit ICMBio-15780-1). Plants were identified by Dr Elsie Franklin Guimarães (Instituto de Pesquisas Jardim Botânico do Rio de Janeiro, RJ, Brazil), and voucher specimens were deposited at the herbarium of the Instituto de Pesquisas Jardim Botânico do Rio de Janeiro (IB specimen – Kato-0367; HF specimen – Kato-0175).

**Extraction and isolation of lignan 1a**

Dried leaves (3.2 g) of *P. solmsianum* (IB specimens) were milled and the powder extracted with CH2Cl2 for 48 h. The extract was concentrated under vacuum to yield 300 mg of a residue that was submitted to CC over C18 column (7 g, 15 × 2.5 cm i.d.). Elution with mixtures of H2O : MeOH containing increasing amounts of MeOH (from 50 to 100%) gave 10 fractions. Fractions 3 and 4 (eluted with 70% MeOH) were pooled, extracted with CH2Cl2 (3 × 100 mL), the organic fractions dried over anhydrous Na2SO4, and concentrated under vacuum to yield 1a (6 mg). Data for compound 1a: pale yellow powder; [α]21D 0 (c 1 × 10−4 mol L−1, CH3Cl2); UV λmax 225 nm (c 3.0 × 10−2 mol L−1 MeOH); CD θ220 nm + 6.6 (c 2.5 × 10−3 mol L−1 MeOH); IR νmax cm−1: 2965, 2935, 1634, 1507, 1233, 1228, 1095, 843; 1H and 13C NMR: see Table 1; EI/MS m/z (rel. int): 416 [M]+ (14), 220 (100), 205 (85), 236 (14), 208 (21); HR-ESIMS [M + H]+ calcd for C24H33O7 433.2221, found 433.2234. Data for compound 3a: pale yellow oil; [α]21D 0 − 71.4 (c 1 × 10−4 mol L−1, CH3Cl2); 1H and 13C NMR.15

**Conflicts of interest**

There are no conflicts to declare.

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