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Article

Detailed ^{1}H and ^{13}C NMR Spectral Data Assignment for Two Dihydrobenzofuran Neolignans

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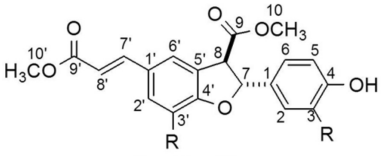
In this work we present a complete proton (^{1}H) and carbon 13 (^{13}C) nuclear magnetic resonance (NMR) spectral analysis of two synthetic dihydrofuran neolignans (±)-*trans*-dehydrodicoumarate dimethyl ester and (±)-*trans*-dehydrodiferulate dimethyl ester. Unequivocal assignments were achieved by ^{1}H NMR, proton decoupled ^{13}C ($^{13}C\{^{1}H\}$) NMR spectra, gradient-selected correlation spectroscopy (gCOSY), *J*-resolved, gradient-selected heteronuclear multiple quantum coherence (gHMQC), gradient-selected heteronuclear multiple bond coherence (gHMBC) and nuclear Overhauser effect spectroscopy (NOESY) experiments. All hydrogen coupling constants were measured, clarifying all the hydrogen signals multiplicities. Computational methods were also used to simulate the ^{1}H and ^{13}C chemical shifts and showed good agreement with the *trans* configuration of the substituents at C_7 and C_8.

Keywords: neolignans, oxidative coupling, *J*-resolved, benzofurans

Introduction

Neolignans (NL) are a class of plant-derived natural products which are produced from shikimic acid pathway.[1] They differ from related lignans by the way the two C_6C_3 units are joined by other bonds. According to the International Union of Pure and Applied Chemistry (IUPAC) recommendations, the term lignan refers to structures where the two C_6C_3 units are β,β' (8-8') linked, whereas the term neolignan must be used for compounds that originate from coupling other than 8-8' coupling.[2]

Among NL, compounds exhibiting a dihydrobenzofuran moiety as structure feature have attracted special attention because their wide range of biological activities, such as antioxidant,[3] antitumor,[4] anti-inflammatory,[5] antileishmanial,[6] trypanocidal,[7,8] insecticidal[9] and cytotoxic.[3] Because of these biological activities, several synthetic methodologies have been proposed to build the basic skeleton of dihydrobenzofuran neolignans (DBNL).[10-12] However, the oxidative coupling of phenylpropanoids is so far the most commonly reported synthetic route to obtain DBNL, such as compounds (±)-*trans*-dehydrodicoumarate dimethyl ester (**2a**) and (±)-*trans*-dehydrodiferulate dimethyl ester (**2b**; Figure 1). Compound **2b** is reported to have antileishmanial,[13] antiplasmodial,[13] cytotoxic,[13] antiangiogenic,[14] antitumor,[15] and antioxidant[16] activities. Despite of these biological activities, nuclear magnetic resonance (NMR) data found in literature for both



2a: R = H
2b: R = OCH_3

Figure 1. Structures of dihydrobenzofuran neolignans **2a** and **2b**.

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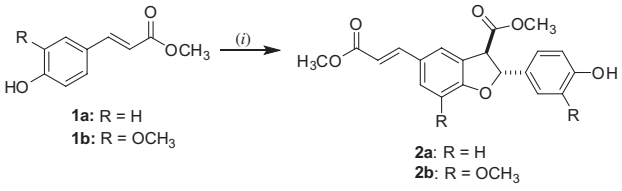
compounds are generally incomplete and, in some cases, inaccurate.[14,15,17,18]

Owing to our interest in the detailed NMR study of natural[19-21] and synthetic[22-25] compounds, in this study we have performed a thorough assignment of all proton (^{1}H) and carbon 13 (^{13}C) NMR data for the synthetic dihydrobenzofuran neolignans **2a** and **2b** using one- (1D) and two-dimensional (2D) NMR techniques.

Results and Discussion

The (±)-*trans*-dehydrodicoumaroate dimethyl ester (**2a**) and (±)-*trans*-dehydrodiferulate dimethyl ester (**2b**) were synthesized according to previous reported procedure,[15,17,26] which was outlined in Scheme 1. The ^{1}H and ^{13}C NMR data for these compounds were previously published[14,15,17,18] but presented some imprecisions that should be corrected.



Scheme 1. (*i*) Ag_2O, $(CH_3)_2CO$:C_6H_6 3:5, r.t., 20 h (**2a**: 36% yield; **2b**: 43% yield).

The main ^{1}H and ^{13}C NMR data for (±)-*trans*-dehydrodicoumaroate dimethyl ester (**2a**) and (±)-*trans*-dehydrodiferulate dimethyl ester (**2b**) are presented in Tables 1 and 3. Two-dimensional NMR data (gradient-selected correlation spectroscopy, gCOSY; gradient-selected heteronuclear multiple quantum coherence, gHMQC; gradient-selected heteronuclear multiple bond coherence, gHMBC; and nuclear Overhauser effect spectroscopy, NOESY) for the same compounds are given in Tables 2 and 4, respectively. Firstly, the ^{1}H NMR spectra were analyzed in detail, which made it possible to verify all chemical shifts. Further analysis of ^{1}H NMR spectra led to the measurement of most homonuclear hydrogen coupling constants. Some *J* values were measured only in *J*-resolved spectrum and all couplings were confirmed by gCOSY experiments. Then, most signals of the proton decoupled ^{13}C (^{13}C{^{1}H}) NMR spectra were assigned through gHMQC and distortionless enhancement by polarization transfer (DEPT) 135 experiments. The assignment of non-hydrogenated carbons was carried out by the use of gHMBC information and by comparison with calculated spectra.

^{1}H and ^{13}C NMR data previously reported for compound **2a** and **2b** were obtained in $CDCl_3$ or acetone-d_6. Most of the signals in the ^{1}H NMR spectrum were between δ_H 6.0 and δ_H 8.0, but the hydrogen signal multiplicities are ambiguous. In this work, we found that for compound **2a** in acetone-d_6, the signals at δ_H 7.6-7.7 are referred to four hydrogen atoms and their overlapping precluded their correct assignment (Figure 2). Therefore, $CDCl_3$ provided much clearer spectra for **2a**, but not for **2b**, due to the solvent influence on chemical shifts. For compound **2b**, three hydrogen atoms resonate at δ_H 6.91 in the ^{1}H HMR spectrum in $CDCl_3$**.** On the other hand, the ^{1}H NMR signals of **2b** were resolved by using acetone-d_6 as solvent, which allowed verification of the multiplicities, observation of the chemical shifts and measurement of the coupling constants.

The ^{1}H NMR (400 MHz, $CDCl_3$) of compound **2a** (Table 1) showed resonances for one *trans* disubstituted

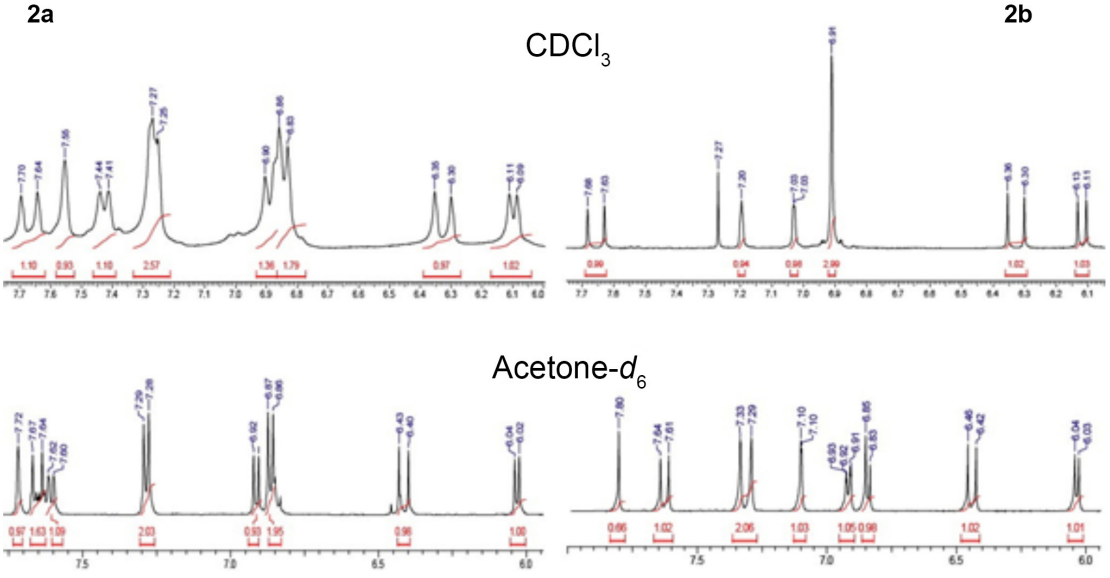


Figure 2. Expansions of the ^{1}H NMR spectrum of compounds **2a** and **2b** obtained in $CDCl_3$ and acetone-d_6.

double bond at δ_H 6.32 (d, 1H, *J* 15.9 Hz) and δ_H 7.66 (ddd, 1H, *J* 0.6, 1.1, 15.9 Hz). The smaller coupling constant values of the signal at δ_H 7.66 could be measured only in the *J*-resolved spectrum. Analysis of the ^{1}H-^{1}H COSY spectrum data (Table 2) revealed some long-range couplings (^{4}J): H_7/H_2, H_7/H_6, $H_{7'}/H_{2'}$, $H_{7'}/H_{6'}$. From ^{1}H NMR and ^{1}H-^{1}H COSY spectra it was possible to establish the spin systems corresponding to the $C_{3'}/C_{2'}/C_{6'}/C_8$ and $C_2/C_3/C_5/C_6/C_7$ portions of **2a**.

Table 1. ^{1}H and ^{13}C NMR data assignments for compound **2a** (400 MHz, $CDCl_3$)

	δ_C[a]	δ_H (integral, multiplicity[b]), *J* / Hz
1	132.0 (C)	–
2=6	127.5 (CH)	7.27 (2H, ddd, $J_{2,5} = J_{6,3}$ 0.3, $J_{2,7} = J_{6,7}$ 0.6, $J_{2,3} = J_{6,5}$ 8.3)
3=5	115.7 (CH)	6.84 (2H, dd, $J_{3,6} = J_{5,2}$ 0.3, $J_{3,2} = J_{5,6}$ 8.3)
4	156.1 (C)	–
7	87.7 (CH)	6.09 (1H, dt, $J_{7,2} = J_{7,6}$ 0.6, $J_{7,8}$ 7.2)
8	55.1 (CH)	4.27 (1H, dd, $J_{8,6'}$ 1.4, $J_{8,7}$ 7.2)
9	170.9 (C)	–
10	52.9 (CH_3)	3.83 (3H, s)
1'	127.8 (C)	–
2'	130.8 (CH)	7.43 (1H, ddd, $J_{2',7'}$ 1.1, $J_{2',6'}$ 2.0, $J_{2',3'}$ 8.3)
3'	110.3 (CH)	6.89 (1H, dd, $J_{3',6'}$ 0.4, $J_{3',2'}$ 8.3)
4'	161.2 (C)	–
5'	125.1 (C)	–
6'	124.9 (CH)	7.55 (1H, dddd, $J_{6',3'}$ 0.4, $J_{6',7'}$ 0.7, $J_{6',8}$ 1.4, $J_{6',2'}$ 2.0)
7'	144.7 (CH)	7.66 (1H, ddd, $J_{7',6'}$ 0.7, $J_{7',2'}$ 1.1, $J_{7',8'}$ 15.9)
8'	115.2 (CH)	6.32 (1H, d, $J_{8',7'}$ 15.9)
9'	167.9 (C)	–
10'	51.7 (CH_3)	3.81 (3H, s)

[a]Multiplicities assigned on the basis of distortionless enhancement by polarization transfer (DEPT) 135 experiments; [b]multiplicities and coupling constant values measured within ^{1}H NMR and *J*-resolved spectra with the help from ^{1}H-^{1}H correlation spectroscopy (COSY) results.

Assignments of the carbonyl C_9 and $C_{9'}$ and methoxy groups C_{10} and $C_{10'}$ are directly performed and those groups can clearly be differentiated on the basis of the gHMBC spectrum (Table 2). The gHMBC correlations are observed between δ_C at 170.9 and the signals at δ_H 4.27 (H_8), 6.09 (H_7), and 3.83 (s, 3H); therefore, the δ_C at 170.9 is attributed to C_9, and δ_H at 3.83 is assigned to H_{10}. On the other hand, gHMBC correlations between δ_C at 167.9 and the signals at δ_H 7.66 ($H_{7'}$), 7.55 ($H_{7'}$) and 3.81 (s, 3H) allowed to assign the δ_C 167.9 to $C_{9'}$, and the δ_H 3.81 to $H_{10'}$. In addition, the δ_c 51.7 and δ_c 52.9 were assigned to $C_{10'}$ and C_{10}, respectively, on the basis of the correlations observed in the gHMQC spectrum with δ_H 3.81 ($H_{10'}$) and δ_H 3.83 (H_{10}). Finally, the non-hydrogenated sp^2-hybridized carbons $C_{1'}$ (127.8), $C_{4'}$ (161.2) and $C_{5'}$ (125.1) were unambiguously assigned to $C_{1'}$, $C_{4'}$ and $C_{5'}$ on the basis of their long-range C–H correlations in the gHMBC spectrum with δ_H 6.32 ($H_{8'}$), 7.43 ($H_{2'}$) and 6.09 (H_7), respectively. Similarly, the assignment of C_1 and C_4 to δ_C 132.0 and 156.1 was established on the basis of the correlations with δ_H 7.27 (H_2=H_6) and 6.84 (H_3=H_5), respectively. Considering that C_4 is expected to be unshielded when compared to C_1 due to the inductive effect of the oxygen hydroxyl, this corroborates the assignment.

Table 2. 2D NMR data for compound **2a** (400 MHz, $CDCl_3$)

C	H	gCOSY[a]	gHMBC[b]	gHMQC[c]	NOESY[d]
1	–	–	H_3=H_5, H_7, H_8	–	–
2=6	2=6	H_3, H_5, H_7	H_3=H_5, H_7	H_2=H_6	H_7, H_8
3=5	3=5	H_2, H_6	H_5	H_3=H_5	–
4	–	–	H_3=H_5, H_2=H_6	–	–
7	7	H_2=H_6, H_8	H_6, H_8	H_7	H_2=H_6*
8	8	H_7, $H_{6'}$	H_7, $H_{6'}$	H_8	H_2=H_6, $H_{6'}$*
9	–	–	H_7, H_8, H_{10}	–	–
10	10	–	-	H_{10}	–
1'	–	–	$H_{3'}$, $H_{8'}$	–	–
2'	2'	$H_{3'}$, $H_{6'}$, $H_{7'}$	$H_{6'}$, $H_{7'}$	$H_{2'}$	$H_{7'}$, $H_{8'}$*
3'	3'	$H_{2'}$, $H_{6'}$	-	$H_{3'}$	–
4'	–	–	$H_{2'}$, $H_{3'}$, $H_{6'}$, H_7, H_8	–	–
5'	–	–	H_8, $H_{3'}$	–	–
6'	6'	$H_{2'}$, $H_{3'}$, $H_{7'}$, H_8	$H_{2'}$, $H_{7'}$, H_8	$H_{6'}$	$H_{8'}$, $H_{7'}$, H_8*
7'	7'	$H_{2'}$, $H_{8'}$, $H_{6'}$	$H_{2'}$, $H_{6'}$, $H_{8'}$	$H_{7'}$	$H_{6'}$*, $H_{2'}$
8'	8'	$H_{7'}$	$H_{7'}$	$H_{8'}$	$H_{6'}$*, $H_{2'}$
9'	–	–	$H_{7'}$, $H_{8'}$, $H_{10'}$	–	–
10'	10'	–	–	$H_{10'}$	–

[a]Gradient-selected correlation spectroscopy; [b]gradient-selected heteronuclear multiple bond coherence; [c]gradient-selected heteronuclear multiple quantum coherence; [d]nuclear Overhauser effect spectroscopy. *mean weak correlation.

The ^{1}H NMR (400 MHz, acetone-d_6) data of compound **2b** are shown in Table 3 and their 2D NMR data are compilated in Table 4.

The structure of compound **2b** is related to the natural dimer 3',4-di-*O*-methylcedrusin, which is one of the active compounds in dragon's blood. This blood-red latex, produced by some *Croton* species growing in the South America, is employed in traditional medicine for wound-healing and anticancer properties.[27] Lemière *et al.*[17] have previously reported the synthesis of 3',4-di-*O*-methylcedrusin and other related neolignans, including compound **2b**, and

Table 3. ^{1}H and ^{13}C NMR data assignments for compound **2b** (400 MHz, acetone-d_6)

	δ_C[a]	δ_H (integral, multiplicity[b]); J / Hz
1	132.5 (C)	–
2	111.2 (CH)	7.10 (1H, ddd, $J_{2,5}$ 0.3, $J_{2,7}$ 0.8, $J_{2,6}$ 2.1)
3	149.1 (C)	–
4	148.5 (C)	–
5	116.3 (CH)	6.84 (1H, dd, $J_{5,2}$ 0.3, $J_{5,6}$ 8.3)
6	120.7 (CH)	6.92 (1H, ddd, $J_{6,7}$ 0.6, $J_{6,2}$ 2.1, $J_{6,5}$ 8.3)
7	88.8 (CH)	6.04 (1H, ddd, $J_{7,6}$ 0.6, $J_{7,2}$ 0.8, $J_{7,8}$ 7.3)
8	57.0 (CH)	4.47 (1H, dd, $J_{8,6'}$ 1.4, $J_{8,7}$ 7.3)
9	172.1 (C=O)	–
10	53.5 (CH_3)	3.81 (3H, s)
11	56.4 (CH_3)	3.84 (3H, s)
1'	129.9 (C)	–
2'	113.9 (CH)	7.33 (1H, dd, $J_{2',7'}$ 0.4, $J_{2'6'}$ 2.6)
3'	146.3 (C)	–
4'	151.5 (C)	–
5'	127.8 (C)	–
6'	119.5 (CH)	7.29 (1H, ddd, $J_{6',7'}$ 0.8, $J_{6',8}$ 1.4, $J_{6',2'}$ 2.6)
7'	145.9 (CH)	7.63 (1H, ddd, $J_{7',2'}$ 0.4, $J_{7',6'}$ 0.8, $J_{7',8'}$ 15.8)
8'	116.8 (CH)	6.44 (1H, d, $J_{8',7'}$ 15.8)
9'	168.2 (C)	–
10'	52.1 (CH_3)	3.73 (3H, s)
11'	56.8 (CH_3)	3.92 (3H, s)

[a]Multiplicities assigned on the basis of distortionless enhancement by polarization transfer (DEPT) 135 experiments; [b]multiplicities and coupling constant values measured within ^{1}H-NMR and J-resolved spectra with the help from ^{1}H-^{1}H correlation spectroscopy (COSY) results.

Table 4. 2D NMR data for compound **2b** (400 MHz, acetone-d_6)

C	H	gCOSY[a]	gHMBC[b]	gHMQC[c]	NOESY[d]
1	–	–	H_2, H_6, H_7, H_8	–	–
2	2	H_5, H_6, H_7	H_5, H_6, H_7	H_2	H_7, H_8, H_{11}
3	–	–	H_2, H_5, H_{11}	H_3	–
4	–	–	H_2, H_5, H_6	–	–
5	5	H_2, H_6	H_6	H_5	–
6	6	H_2, H_5, H_7	H_2, H_5	H_6	H_7, H_8
7	7	H_2, H_6, H_8	H_2, H_6, H_8	H_7	H_6*, H_2
8	8	$H_{6'}$, H_7	H_2, $H_{6'}$	H_8	$H_{6'}$*, H_2, H_6
9	–	–	H_7, H_8, H_{10}	H_9	–
10	10	–	-	H_{10}	–
11	11	–	-	H_{11}	H_2
1'	–	–	$H_{7'}$, $H_{8'}$	$H_{1'}$	–
2'	2'	$H_{6'}$, $H_{7'}$	$H_{6'}$, $H_{7'}$	$H_{2'}$	$H_{7'}$, $H_{8'}$*, $H_{11'}$
3'	–	–	$H_{11'}$	–	–
4'	–	–	$H_{2'}$, $H_{6'}$, H_7, H_8	–	–
5'	–	–	H_7, $H_{8'}$	–	–
6'	6'	$H_{7'}$, $H_{2'}$, H_8	$H_{2'}$, $H_{7'}$, H_8	$H_{6'}$	$H_{8'}$, $H_{7'}$, H_8*
7'	7'	$H_{2'}$, $H_{6'}$, $H_{8'}$	$H_{2'}$, $H_{6'}$, $H_{8'}$	$H_{7'}$	$H_{6'}$, $H_{2'}$,
8'	8'	$H_{7'}$	$H_{7'}$	$H_{8'}$	$H_{6'}$, $H_{2'}$
9'	–	–	$H_{8'}$, $H_{10'}$	–	–
10'	10'	–	–	$H_{10'}$	–
11'	11'	–	–	$H_{11'}$	$H_{2'}$

[a]Gradient-selected correlation spectroscopy; [b]gradient-selected heteronuclear multiple bond coherence; [c]gradient-selected heteronuclear multiple quantum coherence; [d]nuclear Overhauser effect spectroscopy. *mean weak correlation.

assigned the ^{13}C NMR data of these compounds on the basis of DEPT experiments and long-range of heteronuclear correlation (HETCOR) correlations. In this work, we found that the ^{13}C NMR data assignment based on DEPT, gHMQC and gHMBC were similar to that reported by Lemière *et al.*[17] and therefore, will not be discussed in details here. On the other hand, the ^{1}H NMR data of compound **2b** available in the literature seems inaccurate. Multiplicities of the signals of the ^{1}H NMR spectrum of **2b** are often reported as singlet (H_{10}, $H_{10'}$, H_{11} and $H_{11'}$), doublet (H_2, H_5, H_7, H_8, $H_{7'}$ and $H_{8'}$), doublet of doublets (H_6) or broad singlet ($H_{2'}$ and $H_{6'}$) and have not been previously explored. In this work, ^{1}H-^{1}H COSY and 2D J-resolved spectra were used to understand the multiplicity and to measure the coupling constants.

As reported for compound **2a**, analysis of the ^{1}H-^{1}H COSY spectrum of **2b** (Table 4) revealed a long-range coupling (^{4}J) of $H_{7'}$ (ddd, 1H, δ_H 7.63) with $H_{2'}$ (δ_H 7.33) and $H_{6'}$ (δ_H 7.29). The coupling constant values $J_{7',6'}$ and $J_{7',2'}$ were measured in the J-resolved spectrum to be 0.8 Hz and 0.4 Hz, respectively. Similarly, the signal at $H_{2'}$ (dd, δ_H 7.33) correlates with δ_H 7.29 ($H_{6'}$, $J_{2',6'}$ 2.6 Hz) and δ_H 7.63 ($H_{7'}$). A long-range coupling (^{4}J) between $H_{6'}$ and H_8 was also deduced from the correlations between δ_H 7.29 (ddd, $H_{6'}$) and 4.47 (dd, $H_{8'}$, $J_{7',8}$ 1.4 Hz) in the ^{1}H-^{1}H COSY spectrum. It was possible to establish the spin systems corresponding to the $C_{2'}$/$C_{6'}$/$C_{7'}$/C_8 and C_2/C_3/C_5/C_6/C_7 portions of **2b**. The long-range coupling (^{4}J) of both H_2 and H_6 with H_7 has not been previously reported in the literature. In this work, we could measure the scalar coupling constants $J_{2,7}$ and $J_{6,7}$ in the J-resolved spectrum as being 0.8 and 0.6 Hz, respectively.

The relative stereochemistry of the substituents at C_7 and C_8 in (±)-**2a** and (±)-**2b**, only the *trans*-(7*R*,8*R*) stereoisomers, are reported in Figure 1 and Scheme 1 was determined on the basis of the $J_{7,8}$ value and some theoretical calculations, all corroborated by nuclear Overhauser effect (NOE) data (Figure 3).

Firstly, a comparison of $J_{7,8}$ values for **2a** and **2b** with J values reported for other dihydrobenzofuran neolignans,[28] showed a clear agreement with the *trans* configuration. It is

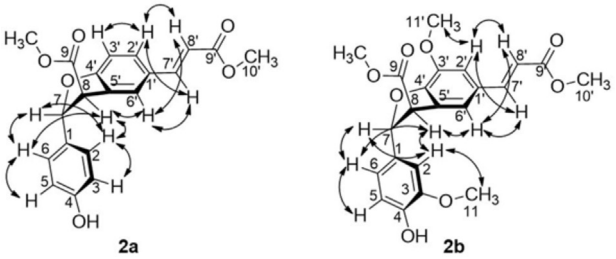


Figure 3. Main nuclear Overhauser effect (NOE) correlations observed in the nuclear Overhauser effect spectroscopy (NOESY) spectra of compounds **2a** and **2b**.

well-established in the literature that the coupling constant $J_{7,8}$ in the skeleton of neolignans is higher for *cis* isomers (8.2-8.4 Hz) than for the *trans* isomer (6.5-7.3 Hz).[28] Nevertheless, it has also been reported that conclusions on the relative stereochemistry in five membered rings based on *J* values for vicinal hydrogens cannot be so reliable for some compounds, as these hydrogens are susceptible to a great variety of dihedral angles and that *cis* or *trans* H–H coupling constants can be exactly the same.[29] On the other hand, Muñoz and Joseph-Nathan[30] suggested that different stereoisomers might show rather large differences in their ^{13}C chemical shifts, and that these differences can be used for the structural identification, reassignment and confirmation. Thus, we decided to use theoretical calculations of ^{1}H and ^{13}C chemical shifts as an extra effort to elucidate the relative stereochemistry of **2a** and **2b**. We hence calculated the ^{1}H and ^{13}C chemical shifts for *trans*-(7*R*,8*R* and 7*S*,8*S*) and *cis*-(7*S*,8*R* and 7*R*,8*S*) stereoisomers of compounds **2a** and **2b** and plotted these results in a cross-comparison to experimental values obtained for these compounds**.** In our case, each group of experimental data was compared to the group of *cis* and *trans* calculated data. The database that shows better agreement with experimental data should indicate which isomer we are dealing with. As an evaluation of this comparison, two main values were considered: the root mean square (rms) error and the coefficient of determination (R^2), as recently used to clarify conformation and configuration of several structures.[31] This first one, the rms error, was obtained by the comparison of chemical shift values atom by atom, both hydrogen and carbon. In this case, rms value was always lower for *trans* compounds, regardless the comparison made: only ^{1}H chemical shifts (δ_H), only ^{13}C chemical shifts (δ_C) or δ_H plus δ_C. Table 5 shows the rms values obtained, which clearly indicates *trans* configuration for both compounds **2a** and **2b**.

The coefficients of determination were obtained from graphics where ^{1}H and ^{13}C experimental chemical shift values were plotted in one axis and the corresponding calculated values in the other one. Three different graphics were drawn for each structure: one with δ_H, one with δ_C and one with both δ_H and δ_C. Invariably, the values obtained for *trans* structures were closer to the experimental rather than the *cis* values. Figure 4 shows the example of R^2 obtained for compounds **2a** and **2b** *versus* ^{1}H and ^{13}C chemical shifts

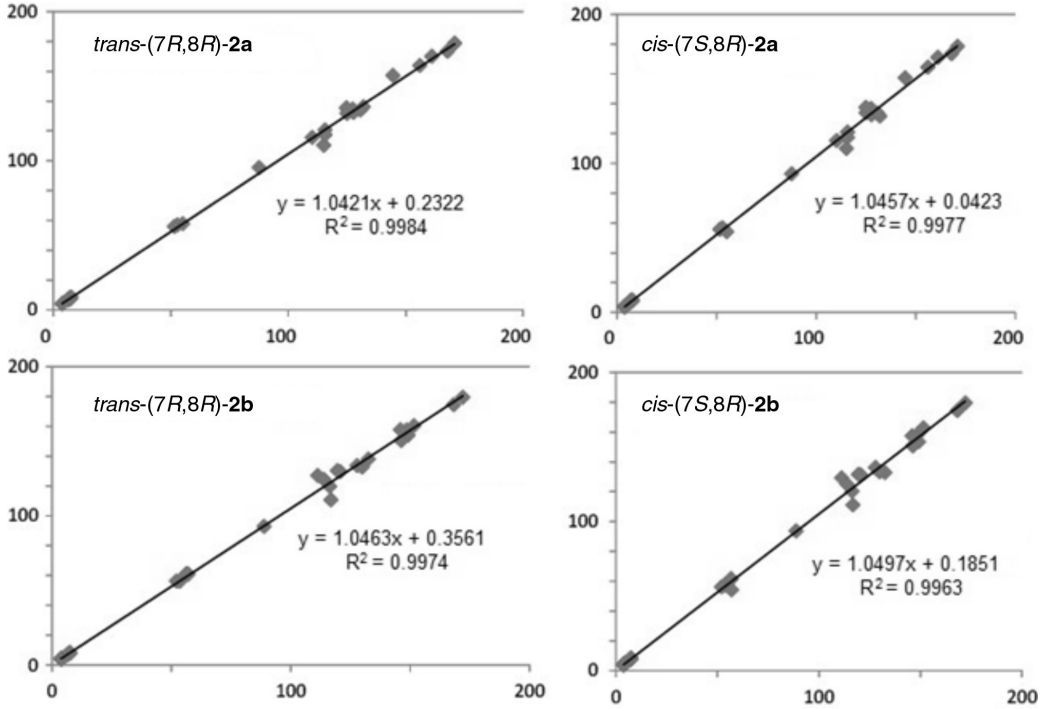


Figure 4. Graphics with ^{1}H plus ^{13}C chemical shift ($\delta_H + \delta_C$, ppm) experimental values *vs.* calculated values for *trans*-(7*R*,8*R*) and *cis*-(7*S*,8*R*) diasteroisomers of neolignans **2a** and **2b**.

for the *trans*-(7*R*,8*R*) structure. The R^2 values for **2a** and **2b** *trans*-(7*R*,8*R*) were 0.9984 and 0.9974, respectively; while the R^2 value for the diasteroisomers *cis*-(7*S*,8*R*) were 0.9977 and 0.9963, respectively. These data also indicate that the obtained compounds have a *trans* configuration.

Moreover, H_7 exhibited significant NOE correlation with H_2 and H_6, in the NOESY spectrum (Table 4). However, NOE correlation of H_7 with H_8 is weak, indicating the relative *trans* configuration for compounds **2a** and **2b**. In addition, the *trans* stereochemistry is also consistent with the diastereoselectivity observed in previously reported syntheses of dihydrobenzofuran neolignans by oxidative coupling, in which the main product is normally a *trans* racemic mixture.[32] In this work, the formation of a *trans* racemic mixture for both **2a** and **2b** was confirmed on the basis of their specific optical rotation values ($[\alpha]_D^{25} = 0°$).

Table 5. Root mean square (rms) values from the comparison of experimental ^{1}H and ^{13}C chemical shifts of compounds **2a** and **2b** with those calculated for their *cis*-(7*S*,8*R*) and *trans*-(7*R*,8*R*) diastereoisomers

	Compound **2a**[a]		Compound **2b**[b]	
	trans	*cis*	*trans*	*cis*
Only δ_H	0.46	0.49	0.39	0.42
Only δ_C	6.52	6.91	7.32	7.92
$\delta_H + \delta_C$	5.09	5.39	5.81	6.29

[a]Calculated in $CDCl_3$; [b]calculated in acetone-d_6.

Conclusions

The complete and unequivocal assignments of ^{1}H and ^{13}C NMR data for two dihydrobenzofuran neolignans are achieved, leaving no ambiguities. This work included the measurement of all hydrogen homonuclear coupling constants values and all hydrogen signal multiplicities were clarified. Confirmation of the relative stereochemistry was also achieved by density functional theory (DFT) calculations and NOE experiments. This study provides an important ^{1}H and ^{13}C NMR database for these two substances and eliminates all previous ambiguities. The stereochemistry was also confirmed by means of *J* values comparison. This is the first complete assignment reported for each one of these two compounds.

Experimental

Synthesis of compounds **2a** and **2b**

Dihydrobenzofuran neolignans **2a** and **2b** were synthesized as previously reported.[15,17,26] Briefly, compounds **2a** and **2b** were obtained by oxidative coupling of methyl coumarate (**1a**) and methyl ferulate (**1b**) using Ag_2O as oxidant. The reactions were carried out employing a mixture of acetone and benzene (5:8, v/v) in a two-necked flask with aluminum foil, equipped with a magnetic stirrer and a gas tube of N_2 for 20 h at room temperature. The product was purified by column chromatography (2.2 × 100 cm, silica gel 60, 0.040-0.063 mm) with hexane and ethyl acetate (2:1, v/v) as eluent affording compounds **1** (36% yield) and **2** (43% yield) as mixture of *trans*-enantiomers. All structures were confirmed by NMR analysis.

NMR analyses

All ^{1}H and ^{13}C NMR experiments were performed on a Bruker Avance DRX400 spectrometer (Karlsruhe, Germany, 400.13 MHz for ^{1}H and 100.61 MHz for ^{13}C). A direct 5-mm probe head (BBO) was used for ^{13}C{^{1}H} NMR experiments and an inverse 5-mm probe head (BBI) was used for other experiments. The ^{1}H NMR spectra were acquired with a solar water heating (SWH) of 8.28 kHz, a time domain (TD) of 64 K, and a number of scans (NS) of 16, which provided a digital resolution of ca. 0.126 Hz (^{1}H 30° pulse width = 8.5 μs). As for the ^{13}C NMR spectra, an SWH of 23.98 kHz was employed, with TD of 32K and NS of 1024, giving a digital resolution of ca. 0.732 Hz (^{13}C 30° pulse width = 14.25 μs). DEPT (512 scans), ^{1}H/^{1}H and ^{13}C/^{1}H 2D chemical shift correlation experiments were carried out using standard pulse sequences supplied by the spectrometer manufacturer. Long-range ^{13}C/^{1}H chemical shift correlations were obtained in experiments with delay values optimized for ^2J(C,H) = 8 Hz. Experiments were performed at 300 K and the concentrations for all samples were in the range 10-15 mg mL^{-1}, in $CDCl_3$ or acetone-d_6, using tetramethylsilane (TMS) as internal reference.

Computational methods

Full geometry optimization and vibrational frequency calculations were carried out using the Gaussian09 program package,[33] employing the B3LYP hybrid functional[34] and 6-311+G(2d,p) basis set.[35] The nature of the stationary point was determined by performing Hessian matrix analysis. ^{1}H and ^{13}C NMR chemical shifts values are calculated within Gauge-Independent Atomic Orbital (GIAO) method,[36-38] using the TMS as the reference molecule. The mixed option was included to consider the Fermi contact contribution and improve the accuracy of spin-spin coupling constants.[39] All NMR calculations were performed at the mPW1PW91/6-311+G(2d,p) level of theory, following the recommendations from Tantillo and co-workers[40-42] for ^{1}H and ^{13}C computed chemical shifts.

In addition, the solvent effect in the NMR calculations was taken into account via the self-consistent reaction field (SCRF) approach.[43]

Supplementary Information

^{1}H NMR, ^{13}C NMR and 2D NMR, IR and mass spectra of compounds are available free of charge at http://jbcs.sbq.org.br as a PDF file.

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