Flavonoids from *Lonchocarpus araripensis* (Leguminosae): Identification and Total ^{1}H and ^{13}C Resonance Assignment

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Abstract

*The NMR study of the flavonoids 6a,11a-dihydro-9-methoxy-6H-benzofuran[3,2-C] benzopiran-3-ol (**1**) and (2,3-cis-3,4-cis-3,4,5,8-tetramethoxy-[1˝,2˝ : 6,7]-furanoflavan (**2**) is described. In addition to convencional 1D NMR methods, 2D shift-correlated NMR techniques (COSY, HMQC, HMBC and NOESY) were used for the complete ^{1}H and ^{13}C resonance assignments. The relative stereochemistry at the asymmetric centers was established by nOe difference experiments. The compounds **1** and **2** are novel to Lonchocarpus araripensis.*

Keywords: *Lonchocarpus araripensis*, Leguminosae, flavonoids, ^{1}H and ^{13}C NMR, 2D NMR, nOe difference spectra

1.0 Introduction

The genus *Lonchocarpus* (Leguminosae) is found to be rich in phenol compounds, including flavones, chalcones, flavonols, flavans, flavanones, and aurones.(Alvarez-Solano *et al.*, 2000; Borges-Argaez *et al.*, 2002; Lawson *et al.*, 2006; Magalhães *et al.*, 1996; Magalhães *et al.*, 1999; Nascimento *et al.*, 1976). Furan and pyran moieties located at ring A in a linear or angular position, linked to either C-6/C-7 or C-7/C-8, respectively, are a common characteristic of the flavonoids produced by plants of this genus (Alvarez-Solano *et al.*, 2000; Borges-Argaez *et al.*, 2002; Lima *et al.*, 2009; Magalhães *et al.*, 1996; Magalhães *et al.*, 1999; Nascimento *et al.*, 1976; Nascimento and Mors, 1981). The flavonoids have aroused considerable interest recently because of their potential beneficial effects on human health and have been reported to have antiviral, anti-allergic, antiplatelet, anti-inflammatory, antitumor and antioxidant activities. The antioxidant activity depends on their molecular structure, and structural characteristics of certain flavonoids found in hops and beer confer surprisingly potent antioxidant activity exceeding that of red wine, tea, or soy (Pouységu *et al*., 2011; Sakihma *et al*., 2002). In this paper we assess the potential utilization of two-dimensional NMR spectroscopy to establish the complete ^{1}H and ^{13}C resonance assignment of a methoxylated pterocarpane (**1**) and of a methoxylated furanoflavonoid (**2**), first isolated from roots of *L. araripensis*, a perennial plant found in the northeast Brazil. These data can be used in further research into other flavonoids.

2.0 Results and Discussion

2.1 Chemistry: Structure elucidation and assignment of compounds

The signals corresponding to quaternary, methine, methilene and methyl carbon atoms of **1** and **2** were identified by comparative analysis of BB and DEPT ^{13}C NMR spectra. A ^{1}H NMR signal doublet (J = 8.0 Hz) of **1** at δ_H 7.37 (1H) was coupled to the signals due oxygenated and non-hydrogenated sp^2 carbons at δ_C 157.57 and 156.81, as well as to oxygenated methine sp^3 carbon at δ_C 78.82, according to HMBC spectrum.

In this same spectrum, the signal at δ_C 156.81 showed connectivities with the signals at δ_H 4.23 (1H) and 3.63 (1H) which in turn, were correlated to diastereotopics methylene hydrogens (H-6' and H-6") attached at an oxygenated carbon (H_2C-6, δ_C 66.72) according to HMQC spectrum. Thus, the signal at δ_H 7.37 was assigned to H-1 while those at δ_C 157.57, 156.81 and 78.82, were to C-3, C-4a and C-11a, respectively. On the other hand, the H-1 allowed the obvious localization of H-2 at δ_H 6.65 (1H, *dd*, *J* = 8.0 and 2.4 Hz) in the ^{1}H x ^{1}H COSY technique which in turn determined the chemical shift (δ_C 110.10) of the C-2 using the HMQC experiment. The signal doublet (*J* = 2.3 Hz) in the ^{1}H NMR spectrum (500 MHz) at δ_H 6.42 was assigned to hydrogen H-4, confirmed by its correlation with the signals at δ_C 110.10, 157.57, 156.81 and 112.57 due to carbon atoms C-2, C-3, C-4a and C-11b, respectively, in the HMBC spectrum. In addition, the HMQC experiment correlated the carbon at δ_C 103.88 (C-4) with the doublet of the hydrogen at δ_H 6.42 (H-4).

The third doublet (*J* = 8.8 Hz) in aromatic region of ^{1}H NMR spectrum localized at δ_H 7.13 (1H) was attributed to H-7. In turn, this signal showed connectivities with the signals at δ_C 161.30 (C-9) and 160.85 (C-11) in the HMBC spectrum as well as with the signal at δ_H 6.46 (m) in the COSY spectrum which was easily assigned to H-8. Finally, the hydrogen H-10 (almost overlapped to H-8) was observed in δ_H 6.44 as a broad signal integrating as 2H (H-8 and H-10) on the basis in the correlations with the carbon atoms C-9 (δ_C 161.30), C-11 (160.85), C-6b (119.38) and C-8 (106.63) in the HMBC spectrum. In addition, the HMQC experiment exhibited the correlation of the carbon at δ_C 97.13 (C-10) with the hydrogen at δ_H at 6.44 (H-10). Confirmation of various others ^{1}H assignments based on HMBC was undertaken through the use of a COSY experiment. Starting with the readily assigned H-11a resonance at δ_H 5.49 [*d*, *J* = 6.9 Hz (δ_C 78.82)] the single cross-peak reveals its connectivity to the resonance at δ_H 3.53 (*m*, 1H, H-6a) which, in turn, shows coupling to the resonances at 3.63 (*m*, 1H, H-6') and 4.23 (*m*, 1H, H-6").

All these assignments were confirmed through HMQC spectrum (Table 1). Based on this analysis and by comparison with literature data (Chalmers, 1977; Demuner *et al*., 2002), ^{1}H and ^{13}C NMR assignments of **1** (Figure 1; $C_{16}H_{14}O_4$, $[M]^+$ 270), identified as 6a,11a-dihydro-9-methoxy-6H-benzofuran[3,2-C] benzopiran-3-ol, were made and are listed in Table 1. The value (6.9 Hz) of the coupling constant between the hydrogens H-11a and H-6a is in agreement with the ring fusion B/C *cis*, as naturally observed in this type of metabolite. A differential nOe experiment was performed with irradiation of the H-11a (δ_H 5.49) hydrogen and the experiment showed significant nOe on H-6a (δ_H 3.53), confirming that these two-hydrogen atoms are on the same side of the molecule. Other nOe interactions were also detected (Table 1) and confirmed chemical shifts of the hydrogens H-8 and H-10 as well as the location of methoxy group at C-9.

The ^{1}H NMR spectrum of **2** showed a downfield double doublet for one hydrogen at δ_H 3.92 and two downfield doublets, each corresponding to one hydrogen, at δ_H 5.03 and 4.80, all associated with hydrogens attached to saturated carbons. In addition, the spectrum also showed signals to four methoxy groups (δ 3.30, 3.36, 4.05 and 4.07); an α,β-disubstituted-furan moiety [δ 7.51 (*d*, *J* = 2.3, H-1") and 6.87 (*d*, *J* = 2.3, H-2")]; a monosubstituted benzene ring [δ 7.48 (*dd*, *J* = 7.7, 1.5, 2H), 7.37 (*t*, *J* = 7.7, 2H) and 7.33 (*m*, 1H)]. All these data are consistent with the structure of a 3,4-dimethoxi flavan. The presence of the furan ring is in agreement with the substitution pattern of other previously isolated flavonoids from *Lonchocarpus* (Magalhães et al., 1996; Magalhães et al., 1999). The ^{1}H x ^{1}H-COSY spectrum indicated that the signal at δ 3.92 (*dd*, *J* = 6.6 and 4.3 Hz, 1H) was coupled those at δ 5.03 (*d*, *J* = 6.6 Hz, 1H) and at δ 4.80 (*d*, *J* = 4.3 Hz, 1H) and they assigned to H-3, H-2 and H-4, respectively, showing one -O-C_2H-C_3H(OMe)-C_4H(OMe)- oxygenated system flavan ring. The HMBC spectrum was successfully used to attribute the chemical shifts of the hydrogenated as well as almost all non-hydrogenated carbons ^{13}C signals, which showed correlations (except for C-8) with characteristics ^{1}H signals (Table 2).

On the other hand, in the 2D NOESY experiment the furan was found to be linearly fused with the A ring because the signal at δ 6.87 (H-2") revealed cross peak with resonance at δ_H 4.07 (CH_3O-5). Consequently, as the fourth methoxyl group was attached to carbon C-8, the connectivity of the signal at δ_H 4.05 (CH_3O-8) with the signal at δ_C 129.1 identified the carbon C-8. Based on ^{1}H-^{1}H COSY, ^{1}H-^{13}C HMQC, ^{1}H-^{13}C HMBC and NOESY data and by comparison with literature data (Magalhães *et al*., 1996; Nascimento and Mors, 1981) ^{1}H and ^{13}C NMR assignments of **2** (Figure 1; $C_{21}H_{22}O_6$, $[M]^+$ 370), identified as (2,3-*cis*-3,4-*cis*-3,4,5,8-tetramethoxy-[1",2" : 6,7]-furanoflavan, were made and are listed in Table 2.

In order to gain more structural information and establish the relative stereochemistry at the asymmetric centers, nOe difference experiments were carried out (Table 3).

Irradiation at δ_H 5.03 (H-2α, *d*, 6.6 Hz) resulted in 2.9% nOe at δ_H 3.92 (H-3α, *dd*, 6.6 and 4.3 Hz), 0.5% nOe at δ_H 4.80 (H-4α, *d*, 4.3 Hz) and 6.3% nOe at δ_H 7.48 (H-2'/H-6', *dd*, 7.5 and 1.5 Hz). Irradiation at δ_H 3.92 (H-3α) resulted in 1.6% nOe at δ_H 5.03 (H-2α), 2.5% nOe at δ_H 4.80 (H-4α), 2.6% nOe at δ_H 7.48 (H-2'/H-6'), 3.7% nOe at δ_H 3.30 (MeO-3) and 0.9% nOe at δ_H 3.36 (MeO-4). Irradiation at δ_H 4.80 (H-4α) resulted in 3.7% nOe at δ_H 3.92 (H-3α), 0,7% nOe at 5.03 (H-2α), 4.5% nOe at δ_H 3.36 (Me-O-4). In addition, irradiation at δ_H 4.80 (H-4α) produced about a 0.7% nOe at δ_H 4.07 (MeO-5). Considering that a half-chair conformation is preferentially assumed by the C ring, was possible to establish the relative configuration shown in Figure 2.

Additional data obtained in the 2D shift-correlated NMR (^{1}H, COSY, HMQC, HMBC and NOESY) experiments were used to confirm the structures **1** and **2** and allowed the ^{1}H and ^{13}C chemical shift assignments as summarized in Tables 1 and 2.

3.0 Experimental

3.1 General experimental procedures

NMR spectra: All the experiments were performed on Bruker spectrometers DPX 300 [^{1}H (300 MHz; ^{13}C (75 MHz)] and DRX 500 [^{1}H (500.1 MHz; ^{13}C 125.77 MHz)] with NMR spectra measured at 27 ^{o} C using $CDCl_3$. Chemical shifts are given on the δ scale and were relatively referenced to the residual $CHCl_3$ (δ ^{1}H 7.27) ^{1}H NMR and to the central peak of the $CDCl_3$ for ^{13}C NMR. One- and two-dimensional ^{1}H and ^{13}C spectra were performed by standard Bruker's pulse programs [zg30 (^{1}H), zgpg30 (^{13}C-BBHD), dept135 (^{13}C-DEPT 135), cosygpqf (^{1}H, ^{1}H-COSY), etgp (HSQC), gplpndqf (HMBC), noesygpph (^{1}H, ^{1}H-NOESY) and selnogp (difference nOe spectra) using either a 5-mm dual ^{13}C/^{1}H probe for normal (^{13}C) detection or a 5-mm multinuclear inverse z-gradient probe for inverse (^{1}H) detection. As many as 32 and 64 k data point sets, with a spectral width of 12 and 31 kHz, were collected for ^{1}H and ^{13}C unidimensional spectra, respectively. Inversely detected 2D heteronuclear correlated spectra were collected over 1 k data points with a spectral width of 5 kHz in F2,and 27 kHz x 256 points in F1. Data processing was performed with 1 k x 256 blocks, using backward linear prediction in F1 to generate the final data matrix.

3.2 Plant material: *Lonchocarpus araripensis* was collected at Acarape County, State of Ceará, Brazil. A voucher of the plant which was identified by Prof. Edson P. Nunes is deposited at the herbarium Prisco Bezerra do Departamento de Biologia, Universidade Federal do Ceará, Brazil, under number 11074.

3.3 Extraction and isolation: Dried and pulverized log stem (4.0 kg) were successively percolated in n-hexane (1 x 25 l) and in EtOH (1 x 25 l) at room temperature. The EtOH extracts were concentrated under reduced pressure to yield 41.1 g of a crude extract, which was fractioned over silica gel by elution with hexane, hexane-EtOAc, EtOAc and EtOH. The hexane-EtOAc fraction (4.8 g) was chromatographed on a silica gel column using hexane and increasing amounts of EtOAc to yield 62 fractions of which 14-42 (hexane-EtOAc 9 : 1, 2.8 g) were pooled on the basis of tlc profiles. This was subjected to a second cc on a silica gel using hexane-EtOAc 8 : 2 to yield 42 fractions of which 43-60 (1.23 g) were pooled on the basis of tlc profiles. This was subjected to a third cc on a silica gel using hexane-EtOAc 7 : 3 to yield fractions from which 14-42 (264 mg) were similarly selected for a final flash chromatography on silica gel. Elution with hexane-EtOAc 6 : 4 afforded 35 fractions of which 11-17 were pooled on the basis of tlc profiles to give compound **1** (96.0 mg; $[\alpha]^{25}_D$ -101,47 (CCl_4, *c*=0,0075). Dried and pulverized peel stem (3.0 kg) were successively percolated in n-hexane (1 x 25 l) and in EtOH (1 x 25 l) at room temperature. The hexane extracts were concentrated under reduced pressure to yield 32.0 g of a crude extract which was fractionated with hezane, EtOAc and EtOH. The solvent was removed from hexane fraction under vacuum, and the solid residue (24.8 g) was chromatographed over Si gel column and eluted with mixtures of hexane-EtOAc in increasing polarity. A precipitate was obtained from the hexane-EtOAc 9 : 1 fraction (18.2 g) after partial evaporation of the solvent system which was recrystallized to give **2** (2.5 g; $[\alpha]^{25}_D$ +17,24 (CCl_4, *c*=0,075). Column chromatography was rung using silica gel 60 (60-230 mesh, and TLC was performed on precoated silica gel polyester sheets (kiesegel 60 F_{254}, 0.20 mm, Merck). The compounds were detected by spraying with vanillin/perchloric acid/EtOH solution followed by heating at 100 ^{o}C.

Conclusions

This work demonstrated a practical application of spectroscopic techniques in the identification of complex natural products. Particularly, they demonstrated the use of two-dimensional NMR spectroscopy and nOe difference measurements to establish the total ^{1}H and ^{13}C resonance assignment and the relative stereochemistry in these types of compounds.

These data can be utilized in fully understanding the correlations between their molecular conformation and the biological activity and further investigation of the other natural products of the same nature.

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References

Alvarez-Solano, D.; Reyes-Chilpa, R.; Jimenez-Estrada,M.; Gomez-Garibay, F.; Chavez-Uribe, I.; Sousa-Sanchez, M. (2000) Flavanones and 3-hydroxyflavanones from *Lonchocarpus oaxacensis*. *Phytochemistry* **55**: 953-957.

Borges-Argaez, R.; Pena-Rodriguez, L.M.; Waterman, P.G. (2002) Flavonoids from two *Lonchocarpus* species of the Yucatan Penisula. *Phytochemistry* **60**: 533-540.

Chalmers, A. A. (1977) 13C NMR of pterocarpans, *Tetrahedron* **33**: 1735-1737. Demuner, A.J.; Barbosa, L.C.A.; Nascimento, J.C.; Vieira, J.J. (2002) Isolamento e avaliação da atividade nematicida de constituintes químicos de *Mucuna cinera* contra *Meloidogyne incognita e Heterodera glycines. Quimica Nova* 335-339.

Lawson, M.A.; Kaouadji, M.; Allais, D.P.; Champavier, Y.; Chulia, A.J. (2006) Substituted tubaic acids, new oxidative rotenoid metabolites from *Lonchocarpus nicou*. *Tetrahedron* **47**: 451-454.

Lima, A.F.; Mileo, P.G.M.; Andrade-Neto, M.; Braz-Filho, R.; Silveira, E.R.; Pessoa, O.D.L. (2009) ^{1}H and ^{13}C assignments of new methoxylated furanoflavonoids from *Lonchocarpus araripensis*. *Magn. Reson. Chem* **47**: 165-168.

Magalhães, A.F.; Tozzi, A.M.G.A.; Sales, B.H.L.N.; Magalhães, E.G. (1996) Twenty-three flavonoids fom *Lonchocarpus subglaucescens*. *Phytochemistry* **42**: 1459-1471.

Magalhães, A.F.; Ruiz, A.L.T.G.; Tozzi, A.M.G.A. (1999) Dihydroflavanols and flavanones from *Lonchocarpus atropurpureos* roots. *Phytochemistry* **52**: 1681-1685.

Nascimento, M.C.; Diaz, R.L.V.; Mors, W.B. (1976) Flavonoids of *Derris obtusa*: Aurones and auronols. *Phytochemistry* **15**: 1553-1558.)

Nascimento, M.C.; Mors, W.B. (1981) Flavonoids from *Derris araripensis*. *Phytochemistry* **20**: 147-152.

Pouységu, L.; Deffieux, D.; Malik, G.; Natangelo, A.; Quideau, S. (2011) Polyphenol researche's cover gallery. *Angew. Chem.* **50**: 586-621.

Sakihama, Y.; Cohen, M.F.; Grace, S.C.; Yamasaki, H. (2002) Plant phenolic antioxidant and prooxidant activities: phenolics-induced oxidative damage mediated by metal in plants. *Toxicology* **177**: 67-80.

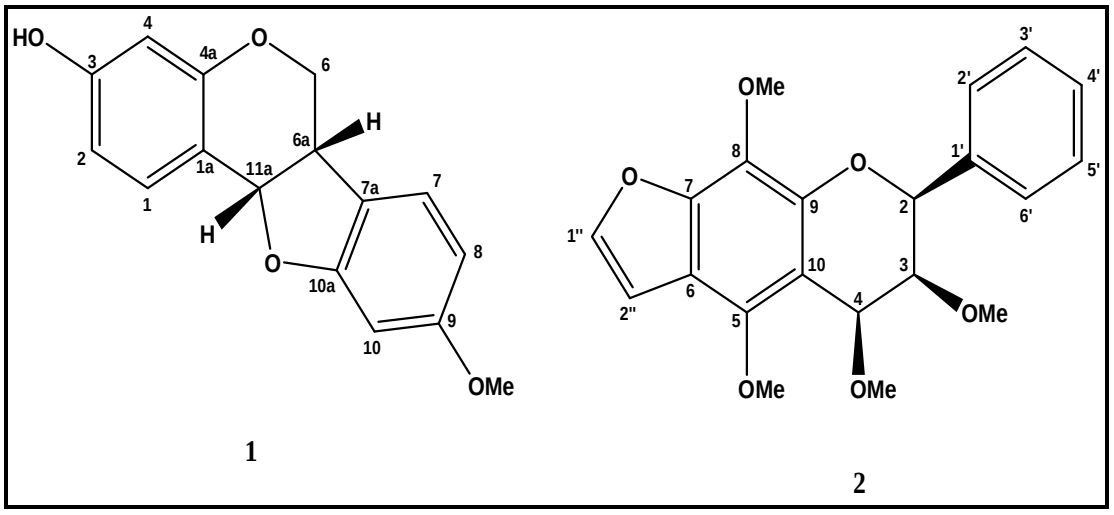

Figure 1: Structures of compounds **1** and **2**

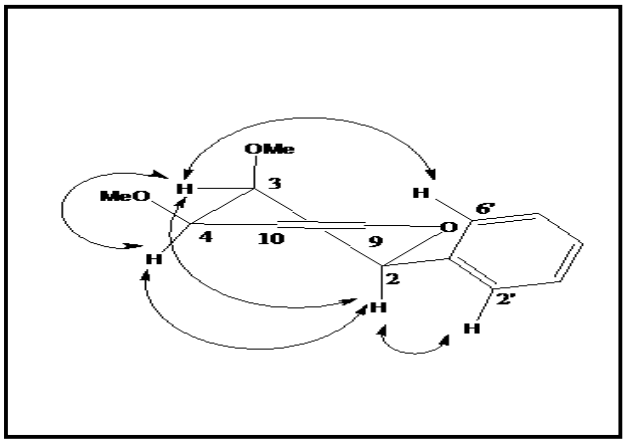

Figure 2: Relative configuration for compound **2**.

Table 1: NMR data for methoxylated pterocarpane 1*

	^{1}H x ^{13}C-HMQC		^{1}H x ^{1}H-COSY	^{1}H x ^{13}C-HMBC		
Atom	δ_C	δ_H		$^2J_{CH}$	$^3J_{CH}$	dif. nOe
C						
1[a]	-	-		H-11a	H-2	-
3	-	-			H-1	
4[a]	-	-			H-1, H-11[a]	-
7[a]	-	-		H-6'	H-8/H-10	-
9	-	-		H-8/10	H-7, OCH_3	-
10a	-	-		H-10		-
CH						
1	132.3	7.37 (*d*, 8.0 Hz)	H-2		H-11[a]	-
2	110.1	6.56 (*dd*, 8.0 and 2.4 Hz)	H-1		H-4	-
4	103.8	6.42 (*d*, 2.3)			H-2	-
7		7.13 (*d*, 8.8)	H-8			-
8		6.46 (*m*)	H-7		H-10	-
10		6.46 (*m*)			H-8	-
6[a]	39.7	3.53 (*m*)	H-6', H-6, H-11a	H-6'		
11a	78.8	5.49 (*d*, 6.9 Hz)	H-6[a]		H-1, H-6, H-6"	H-6a, H-1
CH_2						
6	66.7	3.63 (*t*, 9.7 Hz)	H-6'		H-11[a]	-
		4.23 (*dd*, 9.7 and 6.3 Hz)	H-6"			
CH_3O-9	55.7	3.71 (*s*)				H-8, H-10

*Multiplicity of signals of carbon atoms deduced by comparative analysis of BB and DEPT ^{13}C NMR spectra. Chemical shifts of hydrogens atoms obtained from 1D ^{1}H NMR. The 2D ^{1}H x ^{1}H COSY and 2D ^{1}H x ^{13}C HMQC spectra were also used in these assignments.

Table 2: NMR data for methoxylated furanoflavonoid 2*

	^{1}H x ^{13}C-HMQC		^{1}H x ^{1}H-COSY	^{1}H x ^{13}C-HMBC		^{1}H x ^{1}H-NOESY
Atom	δ_C	δ_H		$^2J_{CH}$	$^3J_{CH}$	
C						
2	-	-				
3	-	-				
4	-	-				
5	147.5	-			H-4, MeO-5	
6	114.0	-		H-2"	H-1"	
7	148.8	-			H-1, H-2"	
8	129.9	-			MeO-8	
9	145.3	-			H-2, H-4	
10	111.3	-		H-4	H-3	
1'	139.1	-		H-2	H-3, H-3', H-5'	
CH		-				
2	80.8	5.03 (*d*, 6.6 Hz)	H-3		H-2'/H-6'	H-2'/H-6'
3	83.2	3.92 (*dd*, 6.6 and 4.3 Hz)	H-2, H-4	H-2, H-4	MeO-3	H-4, H-2'/H-6', MeO-3
4	74.7	4.80 (*d*, 4.3 Hz)	H-3	H-3	H-2, MeO-4	H-3, MeO-4
2'/6'	126.8	7.48 (*dd*, 7.5 and 1.5 Hz)			H-2, H-4'	
3'/5'	128.4	7.37 (*t*, 7.5 Hz)				
4'	127.9	7.33		H-3'/H-5'	H-2'/H-6'	
1"	143.7	7.51 (*d*, 2.3 Hz)	H-2"	H-2"		
2"	105.0	6.87 (*d*, 2.3 Hz)	H-1"	H-1"		MeO-5
MeO-3	58.5	3.30 (*s*)			H-3	
MeO-4	56.9	3.36 (*s*)			H-4	
MeO-5	60.8	4.07 (*s*)				
MeO-8	61.5	4.05 (*s*)				

*Multiplicity of signals of carbon atoms deduced by comparative analysis of BB and DEPT ^{13}C NMR spectra. Chemical shifts of hydrogens atoms obtained from 1D ^{1}H NMR. The 2D ^{1}H x ^{1}H COSY and 2D ^{1}H x ^{13}C HMQC spectra were also used in these assignments.

Table 3: **NOe difference spectral data of 2: irradiations at H-2, H-3 and H-4 hydrogens atoms.**

Irradiation		**Observed nOe**		
H	δ_H	H	δ_H	Percentagem (%)
H-2	5.03 (*d*, 6.6 Hz)	H-2'/H-6'	7.48	6.29
		H-4	4.80	0.51
		H-3	3.92	2.86
H-4	4.80 (*d*, 4.3 Hz)	H-2	5.03	0.74
		H-3	3.92	3.76
		MeO-5	4.07	0.71
		MeO-4	3.36	4.55
		MeO-3	3.30	1.85
H-3	3.92 (*dd*, 6.6 and 4.3 Hz)	H-2'/H-6'	6.87	2.60
		H-2	5.03	1.59
		H-4	4.80	2.51
		MeO-4	3.36	0.88
		MeO-3	3.30	3.69