STRUCTURAL MODIFICATION OF LIGNAN COMPOUNDS ISOLATED FROM *NECTANDRA* SPECIES (LAURACEAE)

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1-INTRODUCTION

In the genus *Nectandra*, the presence of certain types of secondary metabolites has been determined, including sesquiterpenes, phytosterols, polyalcohols, arylpropionic acid derivatives, flavonols, arylpropanoids, furofuran lignans, dihydrobenzofuran neolignans [1], and certain norlignans [2], alkaloids [3], tannins [4], diterpenes [5], and components of essential oils [6]. However, the chemotaxonomic characteristics are determined by the presence of lignan-type compounds [7]. The ultimate goal of structural modification of natural products is to obtain new drugs [8]. In that sense, there is a growing interest in lignans and their synthetic derivatives due to applications in cancer chemotherapy and various other pharmacological effects [9]. This work corresponds to the first report of this type of structural modification of lignan compounds (7,7'-epoxylignans and diaryldimethylbutane lignans) isolated from *Nectandra* species. Therefore, this work can be used as a starting point for structure-activity relationship studies.

EXPERIMENTAL

Materials and reagents

Benzyl bromide (Merck), acetone and toluene were freshly distilled before use. 2,3-Dichloro-5,6-dicyano-1,4-benzoquinone (DDQ) (Fluka purum ,97%), 1,4-dioxane (Aldrich, anhydrous 99.8%), pyridine (ACS reagent, ≥99.0%, Sigma-Aldrich) and acetic anhydride (ACS reagent, ≥98.0%, Sigma-Aldrich) were used. Purification of the products was carried out on a short silica gel column (100–200 mesh, Merck) using increasing percentage of ethyl acetate in hexane as elutant. NMR spectra: were recorded on a Bruker Avance 400 spectrometer (^{1}H 400 MHz, ^{13}C 100 MHz) using TMS as internal standard, in deuterated chloroform ($CDCl_3$) as solvent. The product ethers were characterized by comparing spectral data of known compounds described in the literature and analysis of the spectral data.

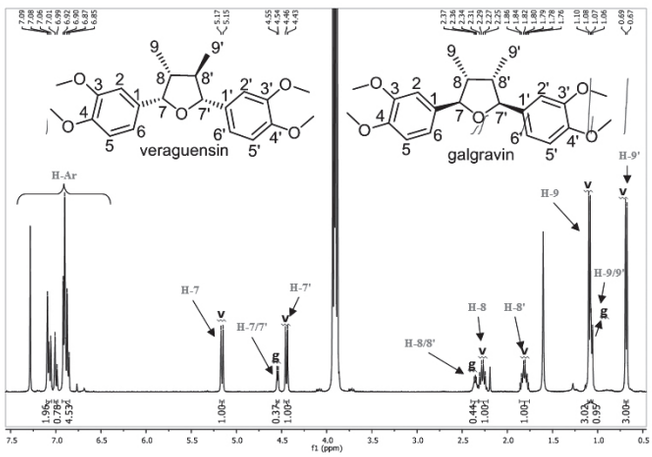

Figure 1. ^{1}H NMR (400 MHz, $CDCl_3$) Spectrum of Compound **1** (mixture of veraguensin/galgravin)

Veraguensin: ^{1}H NMR (400 MHz, $CDCl_3$), δ: 0.67 (3H, *d*, *J* = 7.0, H-9'), 1.07 (3H, *d*, *J* = 6.6, H-9), 1.79 (1H, *m*, H-8), 2.25 (1H, *m*, H-8'), 3.86 (*s*, OCH_3), 3.88 (*s*, OCH_3), 3.89 (*s*, OCH_3), 3.91 (*s*, OCH_3), 4.42 (1H, *d*, *J* = 9.3, H-7), 5.14 (1H, *d*, *J* = 8.6, H-7'), 6.86-7.08 (6H, *m*, H-2/5/6, H-2'/5'/6'). [**v**=*veraguensin*].

Galgravin: ^{1}H NMR (400 MHz, $CDCl_3$), δ: 1.05 (6H, *d*, *J* = 6.7, H-9/9'), 2.34 (2H, *m*, H-8/8'), 3.87 (*s*, OCH_3), 3.88 (*s*, OCH_3), 4.52 (2H, *d*, *J* = 6.4, H-7/7'), 6.85-6.99 (6H, *m*, H-2/5/6, H-2'/5'/6'). [**g**=*galgravin*].

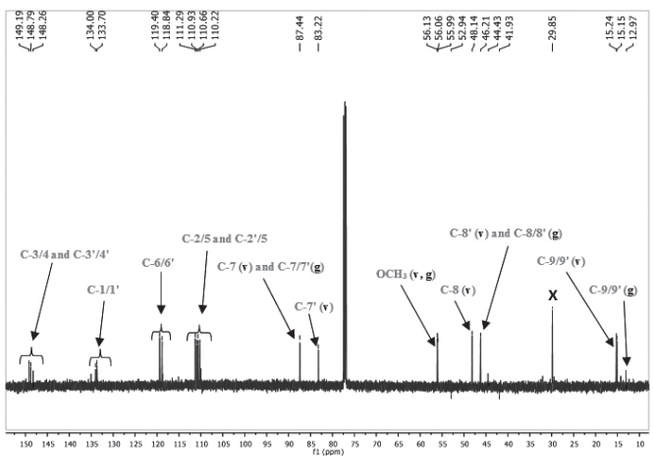

Figure 2. ^{13}C NMR (100 MHz, $CDCl_3$) Spectrum of Compound **1** (mixture of veraguensin/galgravin)

Veraguensin: ^{13}C NMR (100 MHz, $CDCl_3$) δ: 149.1 (C), 148.7 (C), 148.7 (C), 148.2 (C), 133.9 (C), 133.6 (C), 119.3 (CH), 118.8 (CH), 111.1 (CH), 110.8 (CH), 110.5 (CH), 110.1 (CH), 87.4 (CH), 83.1 (CH), 56.1 (CH_3), 56.0 (CH_3) (x2), 55.9 (CH_3), 48.0 (CH), 46.1 (CH), 15.2 (CH_3), 15.1 (CH_3).

Galgravin: ^{13}C NMR (100 MHz) δ: 12.9 (CH_3), 44.3 (*C*HCH_3), 55.8 (OCH_3), 55.9 (OCH_3), 87.2 (OCH(Ar)), 109.7 (Ar-C2), 110.9 (Ar-C5), 118.5 (Ar-C6), 134.8 (Ar-C1), 148.4 (Ar-C4), 148.9 (Ar-C3).

Extraction of Secondary Metabolites

Secondary metabolites were previously isolated from species of *Nectandra sp*. in the Natural Products Laboratory of the National University of Colombia and correspond to 7,7'-epoxilignan and diaryldimethylbutane lignans.

General Procedure

Aromatization of veraguensina and galgravin (1).

A solution of veraguensin and galgravin (90/10) **1**, (5,0 mmol) and DDQ (15,0 mmol) in toluene (20 mL) (the reaction mixture immediately turned deep green) was refluxed (100°C) for 6 h. The mixture was cooled, the precipitate collected, the solvent evaporated under reduced pressure, and the resulting residue purified by flash chromatography on silica gel (n-hexane/AcOEt= 7/3) to give 2,5-bis(3,4-dimethoxyphenyl)-3,4-dimethylfuran (45%) (**TL-1**) [10].

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^{1}H NMR (400 MHz, $CDCl_3$) spectral data of Veraguensin: δ: 0.67 (3H, *d*, *J* = 7.0, H-9'), 1.07 (3H, *d*, *J* = 6.6, H-9), 1.79 (1H, *m*, H-8), 2.25 (1H, *m*, H-8'), 3.86 (*s*, OCH_3), 3.88 (*s*, OCH_3), 3.89 (*s*, OCH_3), 3.91 (*s*, OCH_3), 4.42 (1H, *d*, *J* = 9.3, H-7), 5.14 (1H, *d*, *J* = 8.6, H-7'), 6.86-7.08 (6H, *m*, H-2/5/6, H-2'/5'/6').

^{1}H NMR (400 MHz, $CDCl_3$) spectral data of Galgravin: δ: 1.05 (6H, *d*, *J* = 6.7, H-9/9'), 2.34 (2H, *m*, H-8/8'), 3.87 (*s*, OCH_3), 3.88 (*s*, OCH_3), 4.52 (2H, *d*, *J* = 6.4, H-7/7'), 6.85-6.99 (6H, *m*, H-2/5/6, H-2'/5'/6').

^{1}H NMR (400 MHz, $CDCl_3$) spectral data of **TL-1**: δ 7.21 (4H, *dd*, *J* = 6.1, 1.9, H-2/2' and H-6/6'), 6.94 (2H, *d*, *J* = 8.9, H-5/5'), 3.95 (3H, *s*, O**C**$\mathbf{H_3}$-3/3'), 3.92 (3H, *s*, O**C**$\mathbf{H_3}$-4/4'), 2.22 (*s*, 6H, H-9/9') (*see supporting information*, Figure 3).

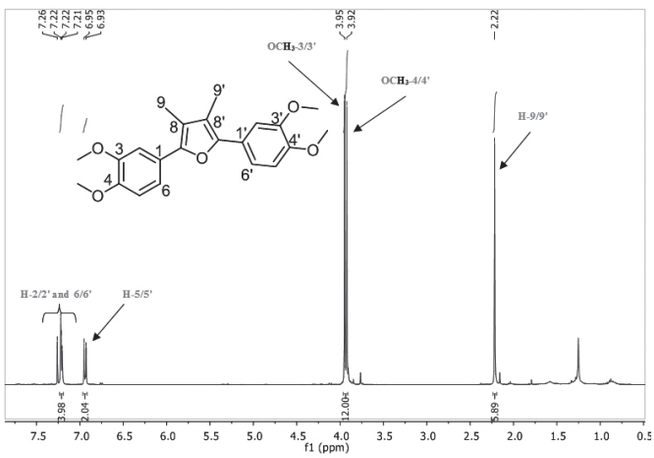

Figure 3. ^{1}H NMR (400 MHz, $CDCl_3$) Spectrum of Compound **1** modified (**TL-1**) (mixture of veraguensin/galgravin modified)

^{1}H NMR (400 MHz, $CDCl_3$) δ 7.21 (4H, *dd*, *J* = 6.1, 1.9, H-2/2' and H-6/6'), 6.94 (2H, *d*, *J* = 8.9, H-5/5'), 3.95 (6H, *s*, OCH_3-3/3'), 3.92 (6H, *s*, OCH_3-4/4'), 2.22 (6H, *s*, H-9/9').

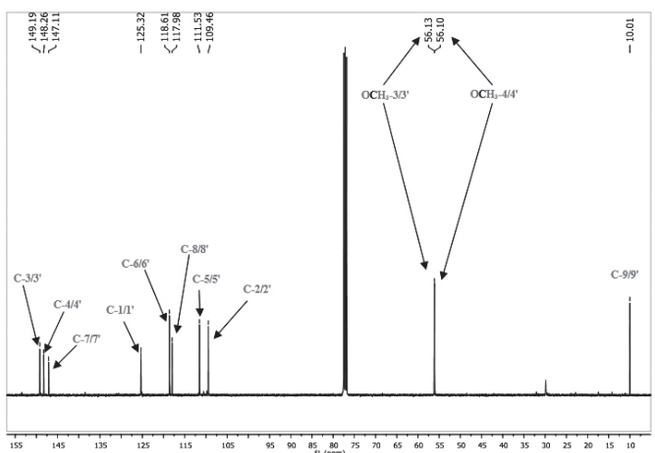

Figure 4. ^{13}C NMR (400 MHz, $CDCl_3$) Spectrum of Compound **1** modified (**TL-1**) (mixture of veraguensin/galgravin modified)

^{13}C NMR (100 MHz, $CDCl_3$) δ 149.2 (C) (x2), 148.3 (C) (x2), 147.1 (C) (x2), 125.3 (C) (x2), 118.6 (CH) (x2), 117.9 (C) (x2), 111.5 (CH) (x2), 109.5 (CH) (x2), 56.2 (CH_3), 56.1 (CH_3), 10.0 (CH_3) (x2).

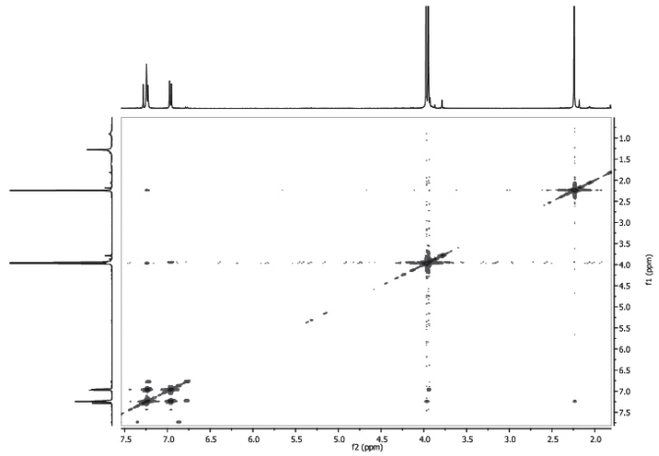

Figure 5. COSY Spectrum of Compound **1** modified (**TL-1**) (mixture of veraguensin/galgravin modified)

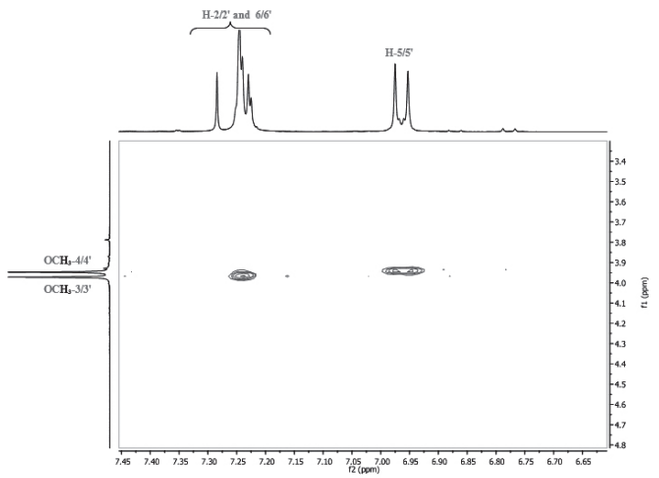

Figure 6. Expansion of the COSY Spectrum of Compound **1** modified (**TL-1**) (From 4.80 to 3.40 $_{f1}$ and From 7.45 to 6.65 $_{f2}$) (mixture of veraguensin/galgravin modified)

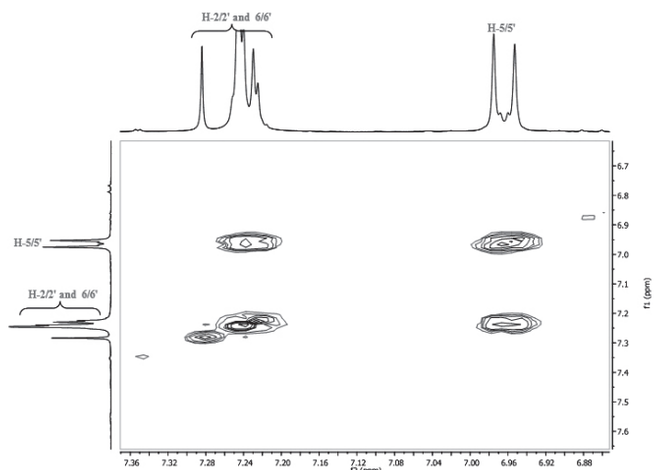

Figure 7. Expansion of the COSY Spectrum of Compound **1** modified (**TL-1**) (From 7.60 to 6.70 $_{f1}$ and From 7.36 to 6.88 $_{f2}$) (mixture of veraguensin/galgravin modified)

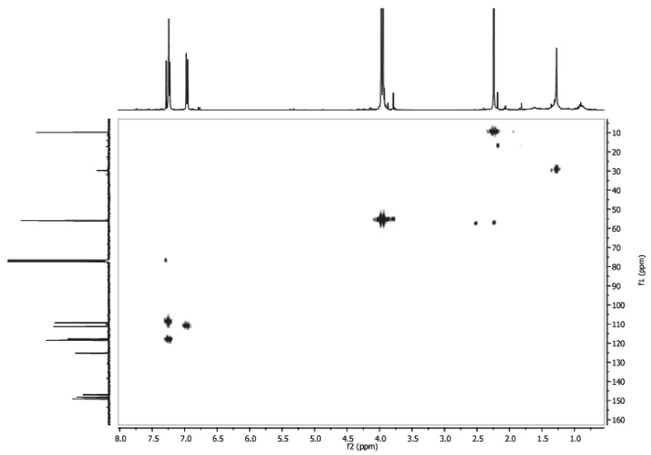

Figure 8. HMQC Spectrum of Compound **1** modified (**TL-1**) (mixture of veraguensin/galgravin modified)

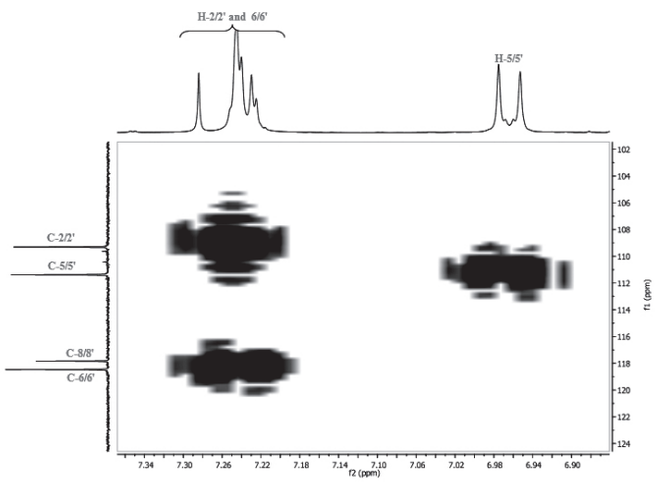

Figure 9. Expansion of the HMQC Spectrum of Compound **1** modified (**TL-1**) (From 124.0 to 102.0 $_{f1}$ and From 7.34 to 6.90 $_{f2}$) (mixture of veraguensin/galgravin modified)

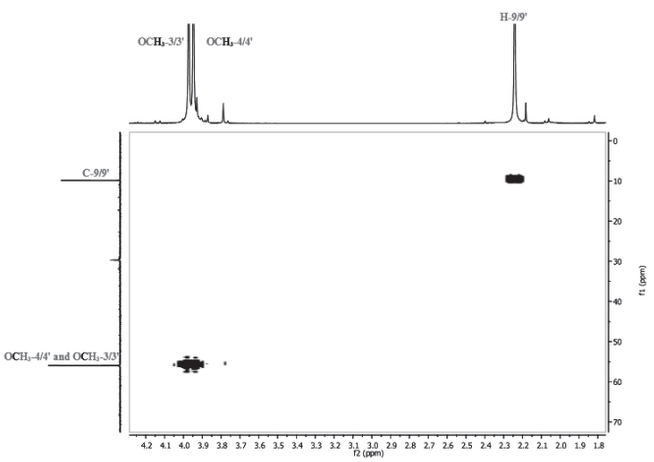

Figure 10. Expansion of the HMQC Spectrum of Compound **1** modified (**TL-1**) (From 70.0 to 0.0 $_{f1}$ and From 4.20 to 1.80 $_{f2}$) (mixture of veraguensin/galgravin modified)

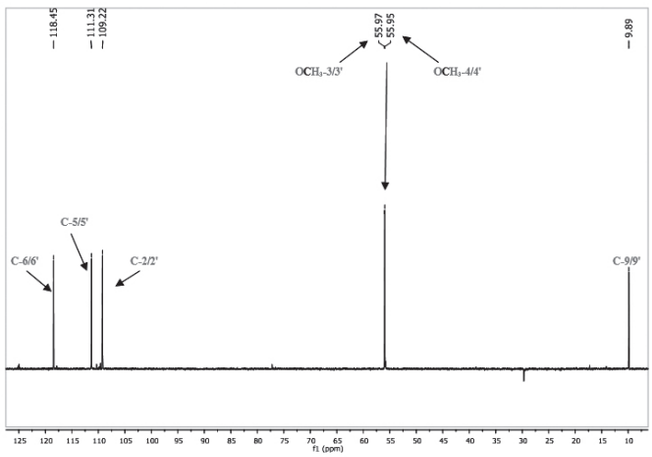

Figure 11. DEPT-135° Spectrum of Compound **1** modified (**TL-1**) (mixture of veraguensin/galgravin modified)

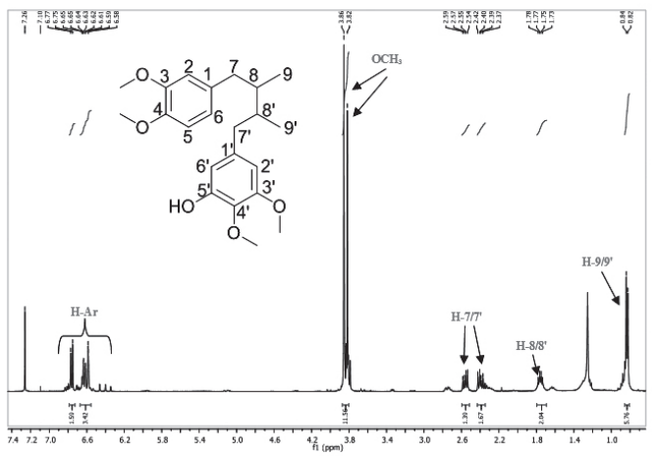

Figure 12. ^{1}H NMR (400 MHz, $CDCl_3$) Spectrum of Compound **2** (schineolignin B)

^{1}H NMR (400 MHz, $CDCl_3$) δ 6.76 (2H, *d*, *J* = 8.1, ArH), 6.65 (1H, *d*, *J* = 1.9, ArH), 6.63 (1H, *dd*, *J* = 8.1, 1.8, ArH), 6.58 (1H, *d*, *J* = 1.8, ArH), 2.56 (2H, *dd*, *J* = 13.5, 6.8, H-7/7'), 2.40 (2H, *dd*, *J* = 13.7, 7.8, 7/7'), 1.76 (2H, *dd*, *J* = 12.9, 6.5, H-8/8'), 0.83 (6H, *d*, *J* = 6.6, H-9/9').

Acetylation of schineolignin B (2).

Schineolignin B **2** (2,1 mmol), in a mixture of acetic anhydride and pyridine (5 mL/ 5 mL) was placed in a 50 mL pear-shaped flask. The mixture was stirred at 100 C for 15 h. Removal of the solvent under reduced pressure afforded a crude mixture, which was extracted with HCl solution followed by extracted with $NaHCO_3$ solution to give the products, which was purified by column chromatography on silica gel (n-hexane/AcOEt= 8/2) *Sephadex LH-20* in open column chromatography to give 5-(4-(3,4-dimethoxyphenyl)-2,3-dimethylbutyl)-2,3-dimethoxyphenyl acetate, 85% (**TL-2**) [11].

^{1}H NMR (400 MHz, $CDCl_3$) spectral data of Schineolignin B: ^{1}H NMR (400 MHz, $CDCl_3$) δ: 6.76 (1H, *d*, *J* = 8.1, Ar-**H**), 6.65 (1H, *d*, *J* = 1.9, Ar-**H**), 6.63 (1H, *dd*, *J* = 8.1, 1.8, Ar-**H**), 6.58 (1H, *d*, *J* = 1.8, Ar-**H**), 2.56 (2H, *dd*, *J* = 13.5, 6.8, H-7/7'), 2.40 (2H, *dd*, *J* = 13.7, 7.8, H-7/7'), 1.76 (2H, *dd*, *J* = 12.9, 6.5, H-8/8'), 0.83 (6H, *d*, *J* = 6.6, H-9/9').

^{1}H NMR (400 MHz, $CDCl_3$) spectral data of **TL-2**: δ: 6.76– 6.57 (5H, *m*, Ar-**H**), 3.86–3.81 (12H, *s*, 3 x O**CH$_3$**), 2.56 (2H, *dd*, *J* = 13.5, 6.7, H-7/7'), 2.40 (2H, *dd*, *J* = 13.5, 7.8, H-7/7'), 2.30 (3H, *s*, C**H$_3$**-CO_2-Ar), 1.79–1.73 (*m*, 2H, H-8/8'), 0.83 (*d*, *J* = 6.6, 6H, H-9/9') (*see supporting information*, Figure 13).

Benzylation of *meso*-dihydroguaiaretic acid and *threo*-dihydroguaiaretic acid (3).

A mixture of **3**(0.567 mmol) and sodium carbonate (11.4 mmol) in dry acetone (36 ml) was heated to reflux for 1 h under nitrogen. Then, benzyl bromide (0.63 ml, 5.67 mmol) was added and the mixture was heated under reflux for an additional 3 h. After cooling to room temperature, the reaction mixture was filtered. The filtrate was concentrated and distilled under reduced pressure in a rotary evaporator to remove the excess unreacted benzyl bromide. The residue was chromatographed on silica gel (hexane/AcOEt= 8/2) and *Sephadex LH-20* in open column chromatography to give 1-(benzyloxy)-4-(4-(3,4-dimethoxyphenyl)-2,3-dimethylbutyl)-2-methoxybenzene, 80% (**TL-3**) [12, 13].

^{1}H NMR (400 MHz, $CDCl_3$) spectral data of meso-dihydroguaiaretic acid and *threo*-dihydroguaiaretic acid: δ: 6.82 (*d*, *J* = 8.0, 2H), 6.78 (*dd*, *J* = 8.2, 2.3, 2H), 6.67 (*dd*, *J* = 8.1, 1.9, 1H), 6.63 (*d*, *J* = 1.8, 1H), 6.60 (*dd*, *J* = 8.0, 1.8, 2H), 6.59 (*d*, *J* = 1.9, 1H), 6.54 (*d*, *J* = 1.8, 2H), 2.75 (*dd*, *J* = 13.5, 5.0, 2H), 2.54 (*dd*, *J* = 13.5, 7.1, 2H), 2.40 (*dd*, *J* = 13.6, 7.6, 2H), 2.30 (*dd*, *J* = 13.5, 9.2, 1H), 1.76 (*dd*, *J* = 13.3, 6.7, 2H), 1.75 (*dd*, *J* = 13.0, 6.6, 2H), 0.85 (*dd*, *J* = 6.6, 2.5, 6H).

^{1}H NMR (400 MHz, $CDCl_3$) spectral data of **TL-3**: δ: 7.46 (2H, *d*, *J* = 7.3, H-2''/2''' and 6''/6'''), 7.38 (2H, *t*, *J* = 7.4, H-3''/3''' and H-5''/5'''), 7.32 (1H, *t*, *J* = 7.2, H-4''/4'''), 6.81 (2H, *d*, *J* = 9.3, Ar-H), 6.79 (2H, *d*, *J* = 8.3, Ar-H), 6.69 (1H, *d*, *J* = 1.6, Ar-H), 6.64 (1H, *dd*, *J* = 9.4, 1.5, Ar-H), 6.57 (1H, *dd*, *J* = 8.1, 1.6, Ar-H), 5.14 (4H, *s*, H-7''/7'''), 2.75 (2H, *dd*, *J* = 13.4, 4.9, H- 7/7'), 2.57 (2H, *dd*, *J* = 13.6, 6.7, H-7/7'), 2.40 (2H, *dd*, *J* = 13.6, 7.8, H-7/7'), 2.30 (2H, *dd*, *J* = 13.4, 9.3, H-7/7'), 0.86 (3H, *d*, *J* = 7.2, H-9/9'), 0.84 (3H, *d*, *J* = 6.8, H-9/9') (*see supporting information*, Figure 15).

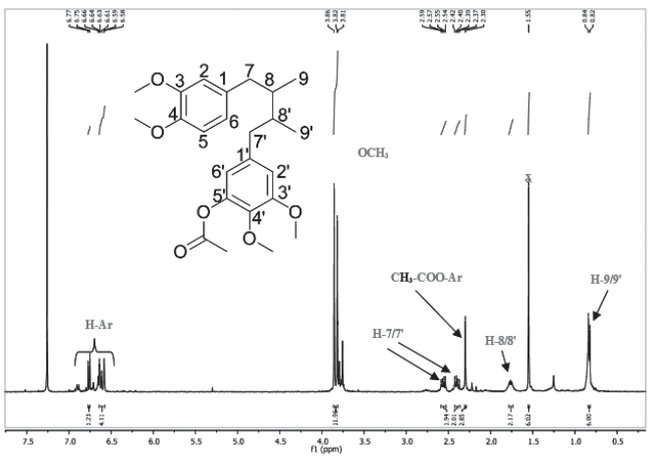

Figure 13. ^{1}H NMR (400 MHz, $CDCl_3$) Spectrum of Compound 2 modified (**TL-2**) (schineolignin B modified)

^{1}H NMR (400 MHz, $CDCl_3$) δ 6.76– 6.57 (5H, *m*, ArH), 3.86–3.81 (12H, *s*, 3 x OCH_3), 2.56 (2H, *dd*, *J* = 13.5, 6.7, H-7/7'), 2.40 (2H, *dd*, *J* = 13.5, 7.8, H-7/7'), 2.30 (3H, *s*, CH_3-CO), 1.79 – 1.73 (2H, *m*, H-8/8'), 0.83 (6H, *d*, *J* = 6.6, H-9/9').

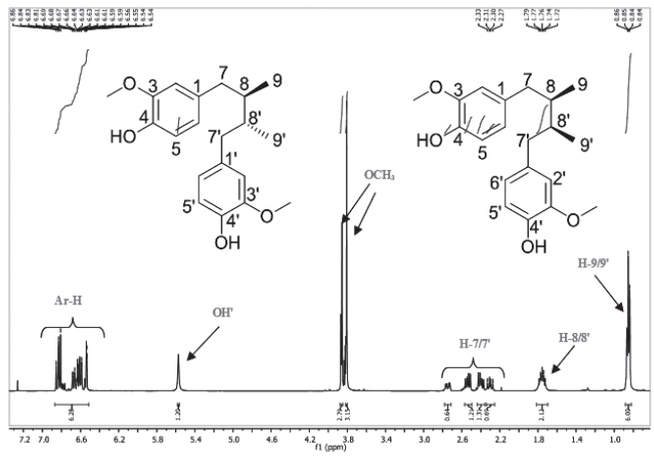

Figure 14. ^{1}H NMR (400 MHz, $CDCl_3$) Spectrum of Compound **3 (**mixture of *meso*-dihydroguaiaretic acid and *threo*-dihydroguaiaretic acid)

^{1}H NMR (400 MHz, $CDCl_3$) δ 6.82 (2H, *d*, *J* = 8.0), 6.78 (2H, *dd*, *J* = 8.2, 2.3), 6.67 (1H, *dd*, *J* = 8.1, 1.9), 6.63 (1H, *d*, *J* = 1.8), 6.60 (2H, *dd*, *J* = 8.0, 1.8), 6.59 (1H, *d*, *J* = 1.9), 6.54 (2H, *d*, *J* = 1.8), 5.44 (1H, OH), 2.75 (2H, *dd*, *J* = 13.5, 5.0), 2.54 (2H, *dd*, *J* = 13.5, 7.1), 2.40 (2H, *dd*, *J* = 13.6, 7.6), 2.30 (2H, *dd*, *J* = 13.5, 9.2), 1.76 (2H, *dd*, *J* = 13.3, 6.7), 1.75 (2H, *dd*, *J* = 13.0, 6.6), 0.85 (6H, *dd*, *J* = 6.6, 2.5).

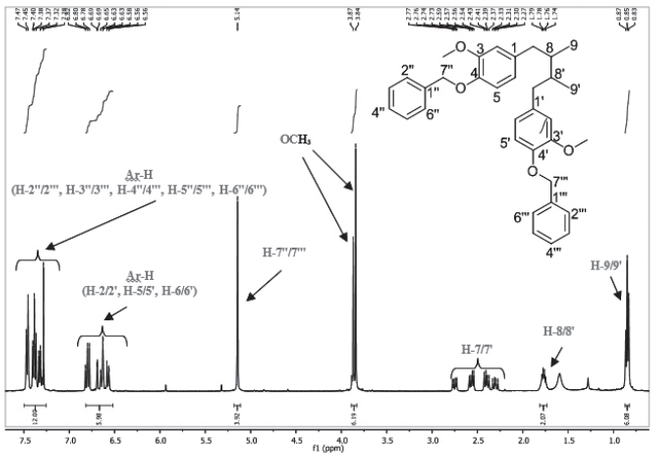

Figure 15. ^{1}H NMR (400 MHz, $CDCl_3$) Spectrum of Compound **3** modified (**TL-3)** (mixture of *meso*-dihydroguaiaretic acid and *threo*-dihydroguaiaretic acid modified)

^{1}H NMR (400 MHz, $CDCl_3$) ^{1}H NMR (400 MHz, $CDCl_3$) δ 7.46 (2H, *d*, *J* = 7.3, H-2''/2''' and 6''/6'''), 7.38 (2H, *t*, *J* = 7.4, H-3''/3''' and H-5''/5'''), 7.32 (1H, *t*, *J* = 7.2, H-4''/4'''), 6.81 (2H, *d*, *J* = 9.3, Ar-H), 6.79 (2H, *d*, *J* = 8.3, Ar-H), 6.69 (1H, *d*, *J* = 1.6, Ar-H), 6.64 (1H, *dd*, *J* = 9.4, 1.5, Ar-H), 6.57 (1H, *dd*, *J* = 8.1, 1.6, Ar-H), 5.14 (4H, *s*, H-7''/7'''), 2.75 (2H, *dd*, *J* = 13.4, 4.9, H-7/7'), 2.57 (2H, *dd*, *J* = 13.6, 6.7, H-7/7'), 2.40 (2H, *dd*, *J* = 13.6, 7.8, H-7/7'), 2.30 (2H, *dd*, *J* = 13.4, 9.3, H-7/7'), 0.86 (3H, *d*, *J* = 7.2, H-9/9'), 0.84 (3H, *d*, *J* = 6.8, H-9/9').

RESULTS AND DISCUSSION

Three structural transformation process are presented in this article; and corresponds to the first report of this type of structural modification of lignans isolated from *Nectandra* species. A direct method was developed for the conversion of compound **1** to furan-type lignan. Additionally, the structural transformation of compounds **2** [14] (benzylation); and compound **3** [15] (acetylation). The spectroscopic data comparison between the initial

and the transformed compound showed formation of derivatives compounds (*see supporting information* for details). Interestingly, few reports describe dehydrogenation, benzylation or acetylation of natural products isolates; to our knowledge the direct structural transformation of lignan compounds isolated from *Nectandra* species has yet to be documented.

Comparison of spectroscopic data between the starting material (veraguensin and galgravin) and the product (**TL- 1**) show the absence some characteristics signals [such as: 4.42 (1H, *d*, *J* = 9.3, H-7), 5.14 (1H, *d*, *J* = 8.6, H-7'), and 4.52 (2H, *d*, *J* = 6.4, H-7/7')], allow suggest the formation of **TL-1**.

The compound **TL-2** has a signal 2.30 (3H, *s*, CH_3-CO), among others; which it is characteristic of the formation of the product.

The compound **TL-3** has a signal 5.14 (4H, *s*, H-7''/7'''), among others; which it is characteristic of the formation of the product. Additionally, the compound formed is absent the signal generated by the hydroxyl group [5.44 (1H, *s*, OH)].

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