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Comprehensive NMR Reassignments of Lignans Derived from *Commiphora myrrha*

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Abstract

Eight stereoisomers of the lignan type were produced from the gum resin of *Commiphora myrrha* in this investigation and their characterisation is provided. These stereoisomers are (+)-*epi*-excelsin (**1**), (+)-5′-demethoxyepiexcelsin (2), (+)-*epi*-methoxypiperitol (**3**), (+)-*epi*-syringaresinol (**4**), (+)-methoxypiperitol (**5**), (+)-piperitol (**6**), (+)-syringaresinol (**7**) and (+)-*dia*-syringaresinol (**8**). The isolated and recognised secondary metabolites exhibited a wide range of structural features, including lignans. Correlations between the ^{1}H and ^{13}C NMR spectra of 7,7'-diaryl-7,9':7',9-dioxabicyclo[3.3.0]octane and those of other recognised lignans can be used to predict the arrangement in an unsymmetric (**1**-**4**) and symmetrical (**5**-**8**) substitution. We use techniques including ^{1}H-^{1}H COSY, HMQC, HMBC, and NOESY to characterise the ^{1}H and ^{13}C NMR spectra of three lignans: (+)-*epi*-excelsin (**1**), (+)-5′-demethoxyepiexcelsin (**2**) and (+)-*epi*-syringaresinol (**4**). We also propose updated structural NMR data for (+)-5′-demethoxyepiexcelsin (**2**) and (+)-*epi*-syringaresinol (**4**). To the best of our knowledge, (+)-*epi*-excelsin (**1**) its ^{13}C NMR assignment has never been published with its absolute configuration through CD spectrum. They were isolated from the genus *Commiphora* (**1**–**8**) for the first time. With IC_{50} values of 27.6, 29.5, and 31.8 µM, respectively, compounds **1**, **2**, and **4** showed tremendous inhibitory action against NO release, but compounds **3**, **5**, and **8** had weak activity > 50 µM.

Keywords: Gum, Resin, *Commiphora myrrha*, Lignans, NMR, *anti*-Inflammatory

1. Introduction

Through the application of herbal medicine, humans have historically interacted with respective surroundings. More than 80% of the world's population receives their primary healthcare from traditional medicine, according to the World Health Organization [1, 2]. Several illnesses have been treated and prevented with medicinal herbs since the dawn of time. They include a wide range of bioactive compounds. The most prominent plant bioactive compounds are sterols, flavonoids, terpenes, diterpenes, sesquiterpenes, and polyphenolic molecules. [2, 3]. Medicinal plants are a safer and cheaper source of drugs than chemically made pharmaceuticals, which have adverse or toxic side effects. [4].

More than 150 species belong to the genus *Commiphora* (Burseraceae), most of which are found in northeastern Africa, southern Arabia, and India [5]. The plant *Commiphora myrrha* (Nees) Engl. yields real myrrh, a resinous exudate (Figure **1**) that has been used for ages as an embalming ointment, a wound-healing treatment, and in other medical procedures [6]. Myrrh has been approved by the American Food and Drug Administration as a safe natural flavouring component in meals and beverages as well as a smell in cosmetics [7]. For a very long time [8], myrrh has been used to heal wound damage. Volatile oil, alcohol-soluble resins, and a gum that is water-soluble and comprises polysaccharides, proteins, and long-chain aliphatic derivatives make up myrrh. The myrrh's lipophilic component is made up of steroids, terpenes, and sterols [9]. Sesquiterpenes are the main constituents of the volatile component of myrrh, which has hundreds of metabolites thus far been found. In particular, furanosesquiterpenes like furanoelemanes, furanoeudesmanes, and furanogermacranes are what make myrrh oil unique [10]. Sesquiterpenoids, important volatile oil ingredients, have a number of

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biological properties, such as antibacterial, antifungal, and antiparasitic properties. It is also important to note that recent articles have revealed analgesic and anticancer properties *in-vitro* and *in-vivo* [11-14].

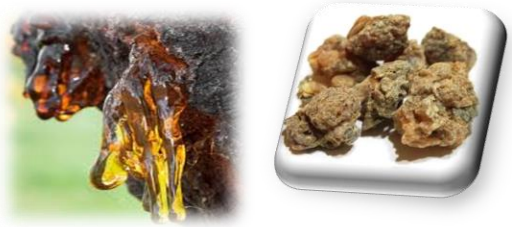

Figure 1. Resinous exudate from *C. myrrha*

A class of plant chemicals with the structural feature of two C_6-C_3 (*n*-propylbenzene) residues joined by a bond connecting the central (□) carbon atoms of each side-chain were first referred to as "lignans" by Haworth' [15] around 1940. McCredie et al. [16] proposed expanding the concept of lignans in 1969 to include any naturally occurring low-molecular-weight substances that are primarily created by the oxidative coupling of *p*-hydroxyphenylpropane units [17], (Figure **2**). In accordance with the majority of definitions, lignans are produced through the oxidative coupling of cinnamic acids and/or cinnamyl alcohols [18].

Lignans are prevalent in a broad variety of plants and microorganisms. Based on their diverse branching chains and different substitution patterns in the aromatic moiety, lignans can be divided into the following groups: dibenzocyclooctadiene, arylnaphthalene, aryltetralin, dibenzylbutane, dibenzylbutyrolactol, dibenzylbutyrolactone, furofuran, and furan type [19-21]. A sizable collection of naturally occurring *bis*-epoxylignans known as furofuran lignans are extensively spread across the plant world. 53 species of plants belonging to 41 genera and 27 plant families have been reported to contain furofuran lignans which have been discovered in all parts of the plants, including the roots, stems, leaves, bulbs, barks, and seeds [21].

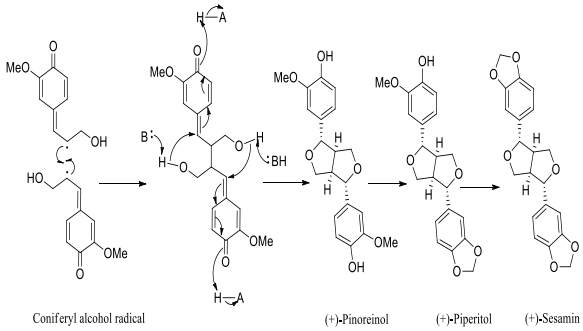

Figure 2. Shows how dirigent protein aids in the enantioselective production of pinoresinol and how sesamin synthase creates.

One of the main subclasses of the lignan family of natural products are the 7,7'-diaryl-7,9':7',9-dioxabicyclo[3.3.0]octane-containing furofuran lignans, also known as *bis*-epoxylignans linked through 8-8′, 7-O-9′, and 9-O-7′ (Figure **3**).

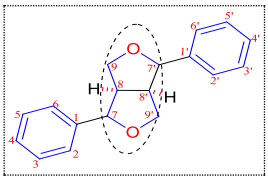

Figure 3. Numbering of furofuran lignan and carbon framework

Four asymmetric carbons are present in 7,7'-diaryl-7,9':7',9-dioxabicyclo[3.3.0]octane. The *cis* orientation of the merged tetrahydrofurofuran rings provides a limit on the number of potential enantiomeric couples [22]. The configurations of the bridge atoms 8 and 8' as a result of this limitation are either (*RR*) or (*SS*) [(*R*,*S*) or (*S*,*R*) would indicate a *trans* fusion of the tetrahydrofurofuran rings] [22]. In turn, this only permits three pairs of enantiomers when the aromatic moieties at C-7 and C-7' are same, and four when the remains of C-7 and C-7' are different. Configuration of (C-8,8',7,7') would be a-*RRRR* (**Type I**. Figure **4**), b-*RRRS* (**Type III.** Figure **4**), c-*RRSR*, d-*RRSS* (**Type II.** Figure **4**), with the corresponding enantiomers a-*SSSS*, b-*SSSR*, c-*SSRS*, d-*SSRR* : if both of arylic substitution are equivalent b = c in both cases. The lignans or 7,7'-diaryl-7,9':7',9-dioxabicyclo[3.3.0]octanes, of the fused *bis*-tetrahydrofuran series, were identified from their NMR spectra (Table **1**), and the pattern of substitution on the aromatic moieties was determined by the assignment of signals due to aromatic protons, methylenedioxy protons, and methoxyl protons. The isomer with both aryl groups *equatorial* (Figure **4**, Type **I**), the isomer with both aryl groups *axial* (Figure **4**, Type **II**), and the isomer with one aryl group *equatorial* and one *axial* (Figure **4**, Type **III**) are all possible in this family of lignans.

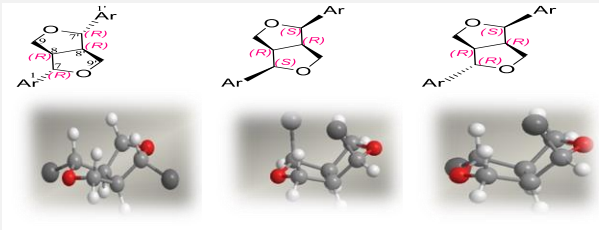

Figure 4. Lignans: three types of stereoisomers are possible

Type I. The isomer with both aryl groups equatorial
Type II. The isomer with both aryl groups axial
Type III. The isomer one aryl group equatorial and the other one axial

In a NMR study of the three types of lignans stereoisomers, it has been shown that the symmetrical di-*equatorial* (Figure **4**, Type **1**) and di-*axial* isomers (Figure **4**, Type **II**) have four aliphatic protons in different environments, rather than eight, while the *axial-equatorial* isomer (Figure **4**, Type **III**) has a methine C-8 proton different from that at C-8', and the benzylic proton at C-7 shows as a doublet at a different chemical shift from that due to H-7'. Each of the three isomers varies in terms of the locations of the C-9 and C-9' methylene protons. The anisotropic effect of the aromatic ring (shielding effect) causes the *axial* aryl groups in Type **III** (Figure **4**) to be kept relatively near to the *axial* protons in the opposing rings, causing these protons to resonate upfield (3.3-3.7 ppm) from the 'normal' location (3.8-4.0 ppm). Due to the deshielding effect of the *equatorial* aryl groups, the two equatorial methylene protons in the case of the di-*equatorial* isomer (Figure **4**, Type **I**) are withdrawn downfield to (4.2-4.4 ppm). One proton resonance shifts upfield to (3.25-3.45 ppm) and one shifts downfield to (4.2-4.4 ppm) in the remaining isomer (Figure **4**, Type **III**), which correspond to an *axial* and an *equatorial* aryl group, respectively.

A survey of the literature reveals that ^{13}C NMR spectroscopy has been significantly used in structural elucidation and stereochemistry assignment since the initial publication in 1974 [23]. Additionally, it has assisted in the revision of numerous structures that were created using only a small amount of information from ^{1}H NMR and MS data. However, material about ^{13}C shielding is still scattered throughout the literature and hasn't yet been carefully collated. This might be one of the explanations for why ^{13}C NMR spectrum analysis is typically disregarded in papers dealing with the isolation and chemical study of such molecules, despite their promise. Therefore, it was deemed appropriate to evaluate the ^{13}C NMR literature that is currently accessible for this significant class of natural compounds. In some instances, the assignments have been changed in light of more current information. Similar to this, based on various data, the stereochemistry of some of the compounds has been assigned and/or updated [24]. In this paper, we concentrate on discussing one of 7,7'-diaryl-7,9':7',9-dioxabicyclo[3.3.0]octane lignan derivatives. The structure and stereochemistry of these lignans (7,7'-diaryl-7,9':7',9-dioxabicyclo[3.3.0]octanes) have been successfully established using ^{13}C NMR spectroscopy. Particularly for C-1 and C-l', the frequencies values of the chemical shifts are indicative of the stereochemistry of the attachment to the fundamental skeleton [25]. Because of this, the chemical shift of C-1 for the *equatorial* 3,4-dimethoxyphenyl, 3,4-methylenedioxyphenyl, and 3,4,5-trimethoxyphenyl groups is in the range of δ_C 133.4-134.1, 134.9-135.6, and 136.6-137.6 ppm, respectively, whereas for an equivalent *axial* substituent, they appear at δ_C 130.8-131.4, 132.6-134.1, and 134.1 ppm. This suggests that switching array from an *equatorial* to an *axial* aryl substituent for C-1 will lead to an average increase in shielding of 2.3-3.0 ppm. The same is true for the *epi*- series, where every time an aryl group transfers from an *equatorial* to an *axial* orientation, the C-1 signal observations an average upfield shift of 2.5-2.7 ppm. Therefore, it would be possible to determine the stereochemistry of both aryl groups in the molecule using these criteria. Chemical shifts for C-7,7', C-8,8', and C-9,9' are also dependent on the stereochemistry of the molecule [24, 25]. These carbon atoms were present in compounds (Figure **4**, Type **1**), in the ranges δ_C 85.3-85.9, 53.7-54.4, and 71.3-72.0 ppm, respectively, with both aryl groups *equatorially* orientated. In contrast, these signals were found in dieudesmin [26], the only example with both aryl groups *axially* orientated, at δ_C 83.9, 49.5, and 68.7 ppm, respectively. On the acetylation of the phenolic hydroxyl groups, these changes are unaffected [24].

Several hydrogen signals from lignans have been characterised in certain articles simply as multiplets or even with incorrect multiplicity. Also, often discovered are inaccurate coupling constant values [27]. The emergence of natural product structure elucidation was an expected and heralded result of the already well-established area of natural product isolation acquiring scientific rationality in the early ^{19}th century [28-30]. The influence of NMR in determining the configuration of isolated or synthesised products has grown. Additionally, the development of new techniques and the ongoing advancement of NMR technology made it feasible to conduct considerably more extensive analyses, offering a variety of unique and significant data. Because they may be exploited as starting points for the structural elucidation of novel compounds or as a database for the quick identification of existing compounds, trustworthy NMR data can make structural elucidation faster and simpler.

The relative configurations of lignans having a 7,9′:7′,9-diepoxy moiety may now be detected with high accuracy using ^{1}H NMR spectroscopy, according to a recent survey [31]. The chemical shift discrepancies of H_2-9 and H_2-9′ ($\Delta\delta_{H\text{-}9}$ and $\Delta\delta_{H\text{-}9'}$) may be used to swiftly and easily identify the configurations of 8-H and 8-OH furofuran lignans. The rule applies to data gathered in methanol-d_4, DMSO-d_6, or $CDCl_3$. When the aromatic rings' C-7 or C-7' substituents may cause the furofuran molecule's dominant conformers to alter, the rule should be carefully applied [31]. Based on the ^{1}H NMR data of the furofuran lignans, the following is a synopsis of the connection between the chemical shift differences (H_2-9 and H_2-9′) and their related relative

configurations (See Figure **4**): for the furofuran lignans of the 8-H type:

Type **I** (Figure **4**): $\Delta\delta_{H-9}$ and $\Delta\delta_{H-9'}$ = 0.30-0.40 ppm, (also known as 7-H/8-H *trans*, 7′-H/8′-H *trans*).

Type **II** (Figure **4**): For 7-H/8-H *cis* and 7′-H/8-H *cis*, respectively, $\Delta\delta_{H-9}$ and $\Delta\delta_{H-9'}$ are equal to or less than 0.20 ppm.

Type **III** (Figure **4**): For $\Delta\delta_{H-9}$ = 0.25-0.36 ppm, $\Delta\delta_{H-9'}$ > 0.50 ppm, (7-H/8-H *trans*, 7′-H/8′-H *cis*).

Here, we provide a thorough assignment of the 1D and 2D NMR data obtained for those lignans, complete with measurements of all hydrogen coupling constants and an explanation of all signal multiplicities. Altered ^{1}H and ^{13}C NMR values, particularly for the furofuranic frame work for compounds **2** and **4**, as well as ^{13}C NMR data for compound **1**, in addition to the weak of **3**, **5-8** (> 50 □M) and moderate of **1**, **2** and **4** (IC_{50} values of 27.6, 29.5, and 31.8 □M respectively) inhibitory activity on NO release.

2. Experimental

2.1. Reagents and apparatus

On a Perkin-Elmer 241 MC polarimeter, optical rotations were recorded in $CHCl_3$. A UV-2450 spectrophotometer (Shimadzu, Kyoto, Japan) was used to find UV spectra in MeOH. Jasco J-710 spectropolarimeter updated to J-715 for CD Spectra. Thermo Fisher Scientific Inc., Waltham, Massachusetts, USA, used a Nicolet iN 10 Micro-FTIR spectrometer in transmission mode to generate infrared spectra (IR). On a Varian Unity 400BB spectrometer, NMR spectra were obtained using $CDCl_3$ as the solvent and a reference to residual solvent peaks ($CDCl_3$: □$_C$ 77.16, □$_H$ 7.26). HRESIMS was carried out using an LTQ Orbitrap XL mass spectrometer (Thermo Fisher Scientific Inc., Waltham, MA, USA). Using a Hitachi L-6200 HPLC instrument and either an Inertsil Prep-sil GL 10 x 250 mm stainless steel column or an Inertsil Prep-ODS GL 10 x 250 mm column, high performance liquid chromatography (HPLC) was carried out. A Hitachi L-7400 UV detector and a Shodex SE-61 RI detector were used to monitor the results. Each compound's elution solvent was indicated. C_{18} reverse-phased silica gel (YMC ODS-A gel, YMC Co., Ltd., Kyoto, Japan). For column chromatography (CC), silica gel (70-230, 230-400 mesh) (Merck) was utilised, and for thin layer chromatography (TLC) and preparative (TLC), silica gel 60 F-254 (Merck) was utilised. Column chromatography (CC) was carried out using Sephadex LH-20 (GE Health, Uppsala, Sweden). Thin layer chromatography (TLC) plates with GF254 silica gel precoated were used for thin layer chromatography (Qingdao Haiyang Chemical Co., Ltd., Qingdao, Shandong, China). Spots of TLC were visible in iodine vapour or after being sprayed with a solution of anisaldehyde-H_2SO_4-acetic acid-H_2O (4:6:80:100) and then heated.

2.2. Production of nitric oxide (NO) by RAW 264.7 macrophages.

Into 96-well plates (2 105 cells/well) containing RPMI 1640 media (HyClone) with 10% FBS in a humidified environment with 5% CO_2 at 37 °C, murine monocytic RAW264.7 macrophages were distributed. Following a 24-hour preincubation period, cells were exposed to successive dilutions of the test substance up to a maximum concentration of 25 □M for 18 hours while being exposed to 1 □g/mL LPS. To create various concentrations, the molecules were dissolved in DMSO and then further diluted in medium. NO generation in each well was measured by mixing 100 □L of Griess reagent (reagent A and reagent B, Sigma) with 100 □L of each supernatant from the cells that had been treated with LPS (Sigma) and compounds in triplicate. Using a 2104 Envision multilabel plate reader (Perkin-Elmer Life Sciences, Inc., Boston, MA, USA), samples' absorbance was measured at 570 nm, after 5 minutes of incubation. As a positive control, MG-132 (Sigma) was employed (IC_{50} = 2.6 □M).

2.3. Plant material

C. myrrh (Somali myrrh, Arabian myrrh), family Burseraceae, oleo-gum resin, was purchased from the Egyptian agricultural seeds, Herbs and Medicinal Plants Company. Figure **1** shows the brownish lumps that make up myrrh resin, which is present. It tastes harsh and has a fragrant smell. Until it was used for alcohol extraction, myrrh resin was ground in a mill into a fine powder.

2.4. Extraction and isolation

250 g of *C. myrrh* resin was boiled in 2.0 l of water for 30 minutes, while being constantly stirred. It was centrifuged after being cooled to room temperature. The supernatant was removed by decanting and lyophilized to produce a flaky powder (90.0 g). It wasn't stored since it wasn't soluble in water or organic solvents at room temperature. An aromatic extract was created by heating the residual material, extracting it with MeOH (3 x 1 l), and then evaporating it under decreased pressure (77.5 g). The EtOAc (3 x 150 ml) was agitated for 30 minutes with an aliquot of this extract (50 g). Under decreased pressure, the mixed ethyl acetate extracts were evaporated to produce EtOAc-soluble (6.5 g) and insoluble fractions (30.0 g). Hexane/acetone was used as the mobile phase in a gradient-driven silica gel MPLC (450 x 51 mm, 370 g of silica) to separate the EtOAc-soluble fraction. At a flow rate of 5 ml/min, the gradient was begun at 9.5 : 0.5 → 6 : 4 (hexane/acetone). Five fractions were created by combining the fractions based on comparable TLC (hexane/acetone) characteristics (I-V). We merged

Fr. II and III. Five fractions were produced when an aliquot (753.3 mg) of it was purified using silica-gel TLC with hexane/acetone 9.8: 0.2 (v/v) (A-E). Using Sephadex LH-20 and MeOH: CH_2Cl_2 (1: 1) as the mobile phase, fraction C (451.3 mg) was further purified to provide four fractions: (C-I, 80.1 mg), (C-II, 90.5 mg), (C-III, 60.6 mg), and (C-IV, 130.4 mg). Using preparatory HPLC (Jaigel ODS; MeOH/H_2O 9: 1, 3 ml/min; detection at 210 nm), Fr. (C-I) was purified to obtain compound (**1**, 40.0 mg) and (**2**, 23.3 mg). With the use of preparatory HPLC (Jaigel ODS; MeOH/H_2O 8:2; of 3 ml/min, detection, 254 nm), Fr. (C-II) was purified to yield the chemical (**3**, 51.3 mg) and (**4**, 21.5 mg). Two peaks appeared at 44.7 and 51.0 minutes following the HPLC purification of Fr. (C-II) (Jaigel ODS; MeOH/H_2O 6.5:3.5 (v/v); 3 ml/min; detection at 254 nm). Two pure compound (**5**, 22.4 mg) and compound after solvent removal (**6**, 20.6 mg). By using prep. HPLC (Jaigel ODS; MeOH/H_2O 55:45, 3 ml/min; detection at 210 nm), compounds (7, 55.8 mg) and (8, 33.6 mg) were extracted from the Fr. (C-IV), and the peaks eluted at 50.64 and 61.44 min were collected.

2.4.1 (+)-*Epi*-excelsin (1): (7*S*,7'*R*,8*R*,8'*R*)-7,7'-Bis(5-methoxy-3,4-methylenedioxyphenyl)-7,9':7',9-dioxabicyclo[3.3.0]octane: colorless gum; $[\alpha]_D^{25}$ = +113.5º (c 0.50 $CHCl_3$); UV ($CHCl_3$) λ_{max} (log ε) 244 (2.90), 276 (3.91) nm; IR ($CHCl_3$) υ_{max} 2948, 2892, 1638, 1510, 1456, 1432, 1362, 1324, 1224, 1216, 1136, 1094, 1044, 972, 932 cm^{-1}; ^1H NMR ($CDCl_3$, 400 Hz) and ^{13}C NMR ($CDCl_3$, 100 Hz) data, see Table **1**; CD data [210 (-3.00), 230 (+0.60), 272 (+0.15), 280 (0.00) and 288 (-0.16) nm; HRESIMS *m/z* 414.1291 ($C_{22}H_{22}O_8$ requires 414.1315).

2.4.2 (+)-5′-Demethoxyepiexcelsin (2): **(7*S*,7'*R*,8*R*,8'*R*)-7-(5-methoxy-3,4-methylenedioxyphenyl)-7'-(3,4-methylenedioxyphenyl)-7,9':7',9-dioxabicyclo[3.3.0]octane**: colorless gum; $[\alpha]_D^{25}$ = +110.0º (c 0.50 $CHCl_3$); UV ($CHCl_3$) λ_{max} (log ε) 256 (3.98), 276 (4.24) nm; IR ($CHCl_3$) υ_{max} 2928, 2872, 1634, 1604, 1506, 1450, 1426, 1262, 1236, 1134, 1090, 1030, 804, 760 cm^{-1}; ^1H NMR ($CDCl_3$, 400 Hz) and ^{13}C NMR ($CDCl_3$, 100 Hz) data, see Table **1**; HRESIMS *m/z* 385.1221 ($C_{21}H_{20}O_7$ requires 385.1224).

2.4.3 (+)-*Epi*-syringaresinol (4): **(7*S*,7'*R*,8*R*,8'*R*)-7,7'-Bis(4-hydroxy-3,5-dimethoxyphenyl)-7,9':7',9-dioxabicyclo[3.3.0]octane**: colorless gum; $[\alpha]_D^{25}$ = +110.0º (c 0.50 $CHCl_3$); UV ($CHCl_3$) λ_{max} (log ε) 256 (3.98), 276 (4.24) nm; IR ($CHCl_3$) υ_{max} 2928, 2872, 1634, 1604, 1506, 1450, 1426, 1262, 1236, 1134, 1090, 1030, 804, 760 cm^{-1}; ^1H NMR ($CDCl_3$, 400 Hz) and ^{13}C NMR ($CDCl_3$, 100 Hz) data, see Table **1**; HRESIMS *m/z* 385.1221 ($C_{21}H_{20}O_7$ requires 385.1224).

2.4.4 (+)-Syringaresinol (7): White powder; $[\alpha]_D^{25}$ = +55.5º (c 0.50 $CHCl_3$); UV ($CHCl_3$) λ_{max} (log ε) 210 (4.00), 233 (4.16), 271 (4.21) nm; IR ($CHCl_3$) υ_{max} 3340 (OH), 2923 (CH), 1567, 1462 (C=C), 1262 (CO) cm^{-1};EIMS m/z (rel. int %): 418 ([M]+, 24), 182 (34), 182 (100), 167 (34); ^1H NMR ($CDCl_3$, 400 Hz): δ_H 6.60 (4H, s, H-2/H-2'/H-6/H-6'), 4.75 (2H, d, J = 4.4 Hz, H-7/H-7'), 4.30 (2H, dd, *J* = 9.6, 3.6 Hz, H-9_{eq}/H-$9'_{eq}$), 3.93 (2H, dd, *J* = 9.6, 6.8 Hz, H-9_{axi}/H-$9'_{axi}$), 3.91 (12H, s, 3/3'/5/5'-OCH_3), 3.11 (2H, ddd, 6.8, 6.6, 4.4 Hz, H-8/H-8') and ^{13}C NMR ($CDCl_3$, 100 Hz): δ_C 147.1 (C-3/C-5/C-3'/C-5'), 134.3 (C-4/C-4'), 132.1 (C-1/C-1'), 102.7 (C-2/C-2'/C-6/C-6'), 86.0 (C-7/C-7'), 71.8 (C-9/C-9'), 56.3 (3/3'/5/5'-OCH_3), 54.3 (C-8/C-8').

2.4.5 (+)-*Dia*-syringaresinol (8): White amorphous powder; $[\alpha]_D^{25}$ = +100º (c 0.1 $CHCl_3$); UV ($CHCl_3$) λ_{max} (log ε) 212 (4.00), 240 (4.16), 278 (4.11) nm; IR ($CHCl_3$) υ_{max} 3400 (OH), 2923 (CH), 1600, 1470 (C=C), 1266 (CO) cm^{-1};EIMS m/z (rel. int %): 418 ($[M]^+$, 22), 182 (40), 182 (100), 167 (30); ^1H NMR ($CDCl_3$, 400 Hz): δ_H 6.61 (4H, s, H-2/H-2'/H-6/H-6'), 4.90 (2H, d, *J* = 4.4 Hz, H-7/H-7'), 3.91 (12H, s, 3/3'/5/5'-OCH_3), 3.73 (2H, dd, *J* = 9.6, 6.8 Hz, H-9_{axi}/H-$9'_{axi}$), 3.57 (2H, dd, *J* = 9.6, 3.6 Hz, H-9_{eq}/H-$9'_{eq}$), 3.18 (2H, m, H-8/H-8') and ^{13}C NMR ($CDCl_3$, 100 Hz): δ_C 147.0 (C-3/C-5/C-3'/C-5'), 133.9 (C-4/C-4'), 130 (C-1/C-1'), 103.1 (C-2/C-2'/C-6/C-6'), 84.2 (C-7/C-7'), 68.8 (C-9/C-9'), 56.3 (3/3'/5/5'-OCH_3), 49.5 (C-8/C-8').

3. Results and discussion

Following progressive extaction and partitioning, the EtOH portion of the gum resin of *C. myrrha* was yielded eight 7,9'-7',9-diepoxylignan recognised isolates (**1**–**8**), were produced after the EtOAc fractions were separated using a variety of column chromatographic methods (Figure. **5**). A variety of ^1H and ^{13}C NMR spectrum data, including COSY, NOESY, HSQC, and HMBC, were fully assigned based on a combination of 1D and 2D NMR investigations to make up for the dearth of fully assigned NMR spectral data for these kinds of compounds.

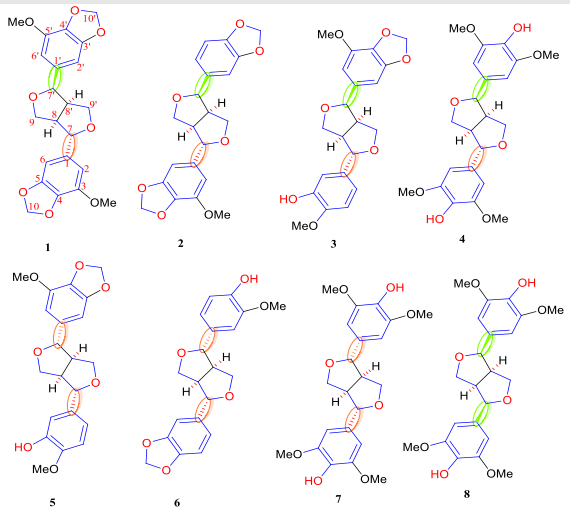

Figure 5. Chemical structure of the isolates lignans (The green bold means the A ring in *axial* orientation and the red dash indicates the B-ring in *equatorial* configuration)

A variety of aromatic and methoxy group signals caught our notice during a ^{1}H NMR analysis of diverse fractions. After further purification by HPLC, compounds having one to four methoxy groups, well-resolved aromatic protons, and numerous proton signals emanating from oxygenated and benzylic sites were found. The structures of the known natural products, **1**-**8** (Figure **5**), were verified by further investigation and structural elucidation utilising ^{13}C NMR, 2D-NMR, and HRESIMS data. By comparing experimental and published spectroscopic data, compounds (**1**–**8**) were isolated and termed as (+)-*epi*-excelsin (**1**) [32], (+)-5′-demethoxyepiexcelsin (**2**) [33], (+)-*epi*-methoxypiperitol (**3**) [34], (+)-*epi*-syringaresinol (**4**) [35-39] (in ref. [36] the same skeleton under the name of (+)-terpineol and in ref. [33] was resulted from hydrolysis of the glucosidated product), (+)-methoxypiperitol (**5**) [34, 40], (+)-piperitol (**6**) [34, 41-43], (+)-syringaresinol (**7**) [39, 44, 45] and (+)-*dia*-syringaresinol (**8**) [39, 46, 47].

Compound **1** (Figure **5**), also designated as *epi*-excelsin, was presented as a colorless gum with positive optical rotation equal +113.5º (*c* 0.50 $CHCl_3$) [48]. The chemical formula $C_{22}H_{22}O_8$ was confirmed using the positive HRESIMS at *m/z* 441.1097 $[M+Na]^+$ (calculated for $C_{22}H_{22}O_8Na$, 441.1100), ^{13}C-NMR, and DEPT spectra, which reflect twelve degrees of unsaturation. A furofuranic-type lignans may have been present in **1** based on the UV spectrum's absorption maxima at 212, 240, and 278 nm [47-49]. The prevalence of unsymmetrical C_6-C_3 moieties in the secondary metabolite **1** was noticed by ^{1}H NMR analysis. Two pairs of an un equivalence AB-type coupling pattern were visible in the ^{1}H NMR spectrum (Table **1**), indicating the presence of two 1,3,4,5-tetraubstituted phenyl groups [δ_H 6.59 (1H, brd, *J* = 1.2 Hz, H-2), 6.55 (1H, brd, *J* = 1.2 Hz, H-2'), 6.54 (1H, brd, *J* = 1.2 Hz, H-6') and 6.52 (1H, brd, *J* = 1.2 Hz, H-6)]. There still are two benzylic oxymethine protons at δ_H 4.82 (1H, d, *J* = 5.6 Hz, H-7') and 4.38 (1H, d, *J* = 7.2 Hz, H-7), two oxygen-containing methylenes [δ_H 4.11 (1H, dd, *J* = 9.6, 1.2 Hz, H-9β), 3.83 (1H, dd, *J* = 9.6, 6.4 Hz, H-9α), 3.83 (1H, dd, *J* = 9.6, 1.2 Hz, H-9'β) and 3.31 (1H, dd, *J* = 9.6, 6.2 Hz, H-9'α)], and two aliphatic methines [δ_H 3.30 (1H, m, H-8') and 2.88 (1H, q, *J* = 7.0 Hz, H-8)]. Two O-methyl singlets were found in the ^{1}H NMR spectrum at δ_H 3.85 (6H, s) and also showed signals indicative of the presence of a methylenedioxy group δ_H 5.96 (4H, brd , *J* = 1.2 Hz H_2-10,-10').

The 22 carbons in the ^{13}C-NMR (Table **1**) [including 12 carbons for two aromatic rings (four methines and eight quaternary] and DEPT spectrum, have been split into four sp^2 methine groups [δ_C 99.8 (C-2'), 104.8 (C-6'), 100.5 (C-2) and 105.2 (C-6)], four sp3 methine carbons [two of which were two oxymethine δ_C 87.6 (C-7), 82.0 (C-7') and the other two were aliphatics methane δ_C 54.6 (C-8') and 50.0 (C-8)], two ketal groups overlapping represented by one signal appeared at δ_C 101.4 , two oxymethylenes [δ_C 70.9 (C-9) and 69.6 (C-9')], two methoxy groups at δ_C 56.6 and eight non-protonated carbons for two aromatic rings [δ_C 149.0 (C-5), 148.7 (C-3'), 143.6 (C-3), 143.5 (C-5'), 135.7 (C-1), 134.3 (C-4), 134.1 (C-4') and 132.9 (C-1')]. All ^{1}H and ^{13}C NMR signal assignments were based on the results of the HMBC, HSQC, and ^{1}H-^{1}H COSY analyses. 2D NMR measurements were used to construct the structure of **1**.

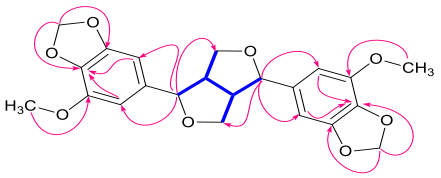

Figure 6. The Choose correlation of **1** between HMBC (arrow) and COSY (bold line)

A *bis*-tetrahydrofuran ring of the 7,7'-diaryl-7,9':7',9-dioxabicyclo[3.3.0]octane-type lignan was also observable, as evidenced by signals between δ_H 3.0 and 5.0 ppm [20, 50, 51]. Naturally present furofuranic-type lignans therefore have C-8, C-8' bond that frequently occurs in the *cis* configuration [34]. Dioxabicyclooctane existence was confirmed by the ^{1}H-^{1}H COSY correlation (Figure **6**), of H-7/H-8/H_2-9, H-8/H-8' and H-7'/H-8'/H_2-9'. Two 1,3,4,5-tetrasubstituted phenyl groups were validated by the ^{1}H-^{1}H COSY spectrum, which also revealed connectivities between the hydrogens H-6', H-2' of ring A and the hydrogens H-6, H-2 of ring B. HMBCs (Figure **6**) from H-7' to C-1', C-9', C-2', C-6';

from H-8' to C-9, C-7; from H_2-9 to C-7', C-8, C-8' as well as the diagnostic carbon chemical shifts at δ_C 54.6 (C-8'), 87.6 (C-7), 70.9 (C-9), 50.0 (C-8), 82.0 (C-7') and 69.6 (C-9'). Additional HMBCs of H-2, H-6/C-7 and H-2', H-6'/C-7' imply that both of the aryl rings were connected to dioxabicyclooctane *via* C-7 and C-7'. Additionally, HMBCs (Figure **6**) crosspeaks were seen between H-7 and C-1 (135.7), C-2 (105.5), and C-6 (100.2), proving that C-7 was connected to ring B. The benzyl group of the ring-A was located at the C-7′ position, as evidenced by the correlations between H-7′ and C-1′ (132.9), C-2′ (99.8), and C-6′ (104.8). The HMBC spectrum also enabled us to establish the location of the methoxyl groups, demonstrating a link between the 3H signals at δ_H 3.85 and C-3 (143.6) and C-3′ (148.7). According to the aromatic carbon shifts, compound **1** has aryl groups are 3-methoxy-4,5-methylenedioxyphenyl units. The HMBC findings gave clear confirmation of the structure of 7,7'-diaryl-7,9':7',9-dioxabicyclo[3.3.0]octane. A reliable piece of evidence regarding OCH_3's placement at benzene rings could come from the NOESY experiment (Figure **7**). As a consequence of comparing the ^{1}H NMR data of compound **1** with previously published data (previously identified from *Macropiper excelsum* by Russella & Fenemore [32]), it was possible to define all protons with the actual chemical shifts and coupling constants also while updating the chemical shifts of both benzylic protons.

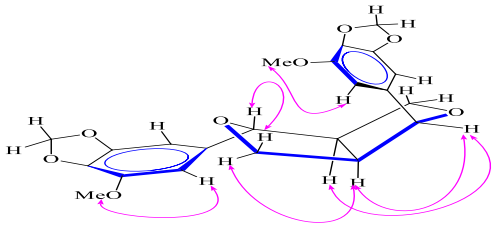

Figure 7. NOESY correlations of compound **1** with some selection

Analyses of the ^{1}H NMR and NOESY spectra of compound **1** were primarily used to identify the relative configurations. According to the 2D-NOESYexperiments, the molecule has an envelope conformation (Figure **7**) [52]. The envelope structure of the lignan moiety was supported by the chemical alterations of the H-atoms at C(2), C(6), C(2'), and C(6'), as well as the different spatial orientations of the aromatic rings in the molecule [52]. H_2-9 has chemical shift differential ($\Delta\delta_H$ = 0.28 ppm) and H_2-9' ($\Delta\delta_H$ = 0.52 ppm) has a indicating that H-7′/H-8' are *cis* whereas H-7/H-8 has a *trans* relative configuration [31]. The NOESY spectrum's critical corrections between H-7/H-8, $H\text{-}9_{axi}$ and H-7′/H-8', $H\text{-}9'_{eq}$ show that **1** has the identical stereochemistry at C-7, 8, and 7' and 8' as (+)-*epi*-methoxypiperitol (**3**) [34]. Furthermore, compound **1** has an CD data which identical to (+)-*epi*-syringaresinol (**7**) which was one of our proceuder isolates [210 (-3.00), 230 (+0.60), 272 (+0.15), 280 (0.00) and 288 (-0.16) nm][48]. Thus, it was unequivocally confirmed that the structure of *epi*-excelsin (**1**) is (+)-(7*S*,7'*R*,8*R*,8'*R*)-bis(5-methoxy-3,4-methylenedioxyphenyl)-7,9':7',9-diepoxylignane.

An alternative topic on NMR spectroscopy to have a thorough discussion of the first lignan skeleton **1**. There are three different types of stercoisorners since the aromatic substituents on C-7 and C-7' can either be *axial* or *equatorial* as mentioned in the introduction of the current document. Sets of requirements of compound **1** has ^{1}HNMR spectra led researchers to conclude it is an *axial-equatorial* isomer. This suggestion was based on three activities: (**A**) a discrepancy in the chemical shifts of the proton in the C-8' and C-8 methines (δ_H 3.30 and 2.88, respectively [shielding effect of an *equatorial* aryl ring on the vicinal proton]); (**B**) a distinction between the benzylic protons at C-7 and C-7' (δ_H 4.38 and 4.82, respectively), in terms of chemical shift [shielding effect of an *axial* aryl group on C-7']; and (**C**) The existence of an additional *equatorial* methylene proton (C-9) shifted to down field (δ_H 4.11) owing to deshielding by the equatorial aromatic ring at C-7, compared to an *axial* (C-9) methylene proton shifted to up field by $\Delta\delta_H$ = - 1.08 ppm. Otherwise, an *axial* (C-9') proton shifted up field by $\Delta\delta_H$ = - 0.52 ppm compared to an *equatorial* (C-9') proton by shielding effect of an *axial* aryl moiety at C-7'. While Greger and Hofer [48] assigned the stereochemistry of unsymmetrically substituted *bis*-tetrahydrofurans based on the Ianthanide-induced shift method. Chiba et al.[53] & Agrawal and Thakur [24], employed ^{13}C NMR spectroscopy to determine the locations of aromatic groups. The relative configurations assigned in the ongoing study were based on the ^{13}CNMR spectra. When identifying the stereochemistry of the attachment of the phenyl groups to the *bis*-tetrahydrofuran skeleton, the chemical shifts of C-l and C-1' are very helpful. The (+)-syringaresinol (**7**) molecule, which is di-*equatorially* substituted, has chemical shifts of δ_C 132.08 ppm on the 1 and 1' carbon atoms. With a chemical shift of δ_C 130.00 ppm, the di-*axial* 3,5-dimethoxy-2-hydroxyphenyl substituent of (+)-*dia*-syringaresinol(**8**) can be readily separated from the equatorial one. Owing to Pelter et al., [54] for unsymmetrical C_6-C_3 with two *axial* and *equatorial* aryl rings lignans the high field benzylic proton is *axial* in each case. The *axial* methine proton at C-7 shifted up field by the anisotropic shielding effect of an *axial* aryl group on C-7', when compare to *equatorial* methine at C-7' shifted to downfield $\Delta\delta_H$ = + 0.44 ppm. Strong evidence that for the orientation of both of aryl groups was provided by the downfield chemical shift of C-7 (δ_C 87.6) and C-8 (δ_C 54.5)

and the upfield chemical shift of C-7' (δ_C 82.0) and C-8' (δ_C 50.0) [54].

For $C_{21}H_{21}O_7$, (+)-5-demethoxyepiexcelsin (**2**) had a molecular ion at *m/z* 385.1221 (calcd. 385.1225) in the HRESIMS which was gave as a colorless gum, with $[\alpha]_D^{25}$ = + 116.1º. According to an analysis of its IR spectra, compound **2** has an aromatic moiety at 1615 and 1492 cm^{-1}. On the basis of the molecular formula, the ^{13}C NMR spectrum (Table **1**), and the DEPT experiment, tweleve indicators of hydrogen deficit (IHD) were identified. More spectrum information (Table **1**, Figures **1**) confirmed the structure of compound **2** should has a 7-(5-methoxy-3,4-methylenedioxyphenyl)-7'-(3,4-methylenedioxyphenyl)-7,9':7',9-diepoxylignane. A piperonyl group [δ_H 6.87 (1H, brs), 6.83 (1H, d), 6.78 (1H, d), and 5.95 (2H, brs] and a 3-methoxy-4,5-me thylenedioxyphenyl [δ_H 6.59 (1H, brs), 6.52 (1H, brs), 5.98 (2H, brs), and 3.86 (3H, s, OMe] were found in the ^1H and ^{13}C NMR spectral data (Table **1**). The analysis of the spectrum data and biogenetic considerations led to the conclusion that the remaining $C_6H_8O_2$ of the molecule is a 7,7'-dioxabicyclo-[3,3,0]-octane unit. The 3-methoxy-4,5-methylenedioxyphenyl unit was ascribed to C-7 of one of the dioxabicyclo-[3,3,0]-octane unit's tetrahydrofuran groups by examination of the HMBC spectrum of **2**, and the piperonyl group was assigned to C-7' of the second tetrahydrofuran unit. When the NMR spectra of compound **2** and **1** (Table **1**) were compared, the differences between the signals attributable to the ring A in **1** which was 5-methoxy-3,4-methylenedioxyphenyl transformed to be 3,4-methylenedioxyphenyl in **2**. The ^1H NMR spectra of **1** showed AB-type coupling features (*meta*-coupling) at δ_H 6.55 (1H, d, *J* = 1.2 Hz, H-2') and 6.54 (1H, d, *J* = 1.2 Hz, H-6') to ABX-type coupling patterns in **2** δ_H 6.87 (1H, d, *J* = 1.2 Hz, H-2'), 6.78 (1H, d, *J* = 8.1 Hz, H-5') and 6.83 (1H, dd, *J* = 8.1, 1.2 Hz, H-6') (Table **1**). The aryl moiety ring A in **1** [δ_C 100.2 (C-2'), 134.1 (C-4'), 143.5 (C-5') and 105.5 (C-6')]

Table 1. NMR Spectroscopic Data of Compounds **1**, **2** & **4**.

Position	(+)-*epi*-Excelsin (**1**)		(+)-5′-Demethoxyepiexcelsin (**2**)		(+)-*epi*-Syringaresinol (**4**)	
	^1H NMR δ_H (*J* in Hz)	^{13}C NMR δ_C	^1H NMR δ_H (*J* in Hz)	^{13}C NMR δ_C	^1H NMR δ_H (*J* in Hz)	^{13}C NMR $\delta\delta\delta\delta_C$
1	--------	135.7	--------	135.0	--------	132.3
2	6.59 brd (1.2)	104.8	6.59 brd (1.2)	104.8	6.60 brd (1.2)	103.0
3	--------	143.6	--------	143.5	--------	147.2
4	--------	134.3	--------	134.1	--------	134.3
5	--------	149.0	--------	148.8	--------	147.2
6	6.52 brd (1.2)	99.8	6.52 brd (1.2)	99.8	6.60 brd (1.2)	103.0
7	4.38 d (7.2)	87.6	4.40 d (7.2)	87.6	4.44 d (7.1)	87.1
8	2.88 q (7.0)	54.6	2.88 q (7.0)	54.5	2.91 q (7.0)	54.4
9eq = 9δ	4.11 dd (9.6, 1.2)	70.9	4.11 dd (9.6, 1.2)	70.9	4.16 dd (9.6, 1.2)	70.8
9axi = 9δ	3.83 dd (9.6, 6.4)		3.83 dd (9.6, 6.4)		3.87 dd (9.6, 6.4)	
10	5.96 brd (1.2)	101.4	5.96 brd (1.2)	101.4	-------	------
1'	----	132.9	--------	132.9	--------	130.0
2'	6.55 brd (1.2)	100.2	6.87 brd (1.2)	106.5	6.62 brd (1.2)	103.6
3'	------	148.7	------	147.2	--------	147.0
4'	------	134.1	------	147.9	--------	133.8
5'	-------	143.5	6.78 d (8.1)	108.1	--------	147.0
6'	6.54 brd (1.2)	105.5	6.83 dd (8.1, 1.2)	119.5	6.62 brd (1.2)	103.6
7'	4.82 d (5.6)	82.0	4.82 d (5.6)	82 .0	4.87 d (5.2)	81.5
8'	3.30 m	50.0	3.30 m	50.1	3.35 m	50.3
9'eq= 9'α	3.83 dd (9.6, 2.0)	69.6	3.83 dd (9.6, 2.0)	69.6	3.82 dd (9.5, 2.8)	69.6
9'axi = 9'β	3.31 dd (9.6, 6.2)		3.31 dd (9.6, 6.2)		3.35 dd (9.6, 6.2)	
10'	5.96 brd (1.2)	101.4	5.96 brd (1.2)	101.0	------	------
3-OMe	3.85 s	56.6	3.91 s	56.6	3.90 s	56.2
3'-OMe	------	------	------	------	3.90 s	56.2
5-OMe	------	------	------	------	3.90 s	56.2
5'-OMe	3.85 s	56.6	------	------	3.90 s	56.2

Table 2. Optical rotation and benzylic orientation for **1**-**8** according to NMR data.

Compd.	δ_H in ppm H_2-9	$\Delta\delta_{H\text{-}9}$ ppm	δ_H in ppm H_2-9'	$\Delta\delta_{H\text{-}9'}$ ppm	Type* (Figure 4)	7 δ_H in ppm (*J* in Hz)	7' δ_H in ppm (*J* in Hz)	7 δ_C in ppm (7H,8H-*cis* or *trans*)	7' δ_C in ppm (7'H,8'H-*cis* or *trans*)	$[\alpha]_D^{25}$
1	4.11 3.83	0.28	3.83 3.31	0.52	III	4.38 d (7.2)	4.82 d (5.6)	87.6 (*trans*)	82.0 (*cis*)	+113.5º
2	4.11 3.83	0.28	3.83 3.31	0.52	III	4.40 d (7.2)	4.82 d (5.6)	87.6 (*trans*)	82.0 (*cis*)	+110.0º
3	4.09 3.81	0.28	3.85 3.30	0.55	III	4.39 d (7.3)	4.80 d (5.5)	87.7 (*trans*)	82.0 (*cis*)	+ 97.1º
4	4.16 3.87	0.29	3.82 3.35	0.57	III	4.44 d (7.1)	4.87 d (5.2)	87.1 (*trans*)	81.5 (*cis*)	+110.0º
5	4.05 3.73	0.32	4.05 3.73	0.32	I	4.70 d (4.8)	4.69 d (5.1)	85.9 (*trans*)	85.8 (*trans*)	+85.5º
6	4.24 3.85	0.39	4.24 3.85	0.39	I	4.71 d (4.8)	4.70 d (5.1)	85.9 (*trans*)	85.8 (*trans*)	+ 53.5º
7	4.30 3.93	0.37	4.30 3.93	0.37	I	4.75 d (4.4)	4.75 d (4.4)	86.0 (*trans*)	86.0 (*trans*)	+55.5º
8	3.73 3.57	0.16	3.73 3.57	0.16	II	4.90 d (4.3)	4.90 d (4.3)	84.2 (*cis*)	82.0 (*cis*)	+110.0º

Type **I**: (also known as 7-H/8-H *trans*, 7′-H/8′-H *trans*): $\Delta\delta_{H\text{-}9}$ and $\Delta\delta_{H\text{-}9'}$ = 0.30-0.40 ppm. Type **II**: ($\Delta\delta_{H\text{-}9}$ and $\Delta\delta_{H\text{-}9'}$ < 0.20 for 7-H/8-H *cis* and 7′-H/8-H *cis*, respectively). Type **III**: (7-H/8-H *trans*, 7′-H/8′-H *cis*): $\Delta\delta_{H\text{-}9}$ = 0.25-0.36 ppm, $\Delta\delta_{H\text{-}9'}$ > 0.50 ppm.

undergoes some modifications in the ^{13}C NMR spectra that are present in **2** [δ_C 106.5 (C-2'), 147.9 (C-4'), 108.1 (C-5') and 119.5 (C-6')] (Table **1**). These alterations may be tried to justify by the acquisition of one more methoxy group at C-5' in **1** and its dismissal in **2** due to mesomeric effects [*ortho*: C-6', $\Delta\delta_C$ = 105.5 (**1**) – 119.5 (**2**) = - 14.0 ppm and C-4', $\Delta\delta_C$ = 134.1 (**1**) – 147.9 (**2**) = - 13.8 ppm]; [*para*: C-2', $\Delta\delta_C$ = 100.2 (**1**) – 106.5 (**2**) = - 6.3 ppm] and inductive effects [*ipso*: C-5', $\Delta\delta_C$ = 143.5 (**1**) - 108.1 (**2**) = + 35.4 ppm], respectively. We were capable of assessing and revise the NMR data, particularly C-7 and C-7' in addition to the detection of exact chemical shifts of H_2-9 and H_2-9' with their coupling constants, based on the aforementioned facts and comparison with prior literature of the goal compound **2** [33]. Thus, it was unequivocally confirmed that the structure of **2** (+)-(7*S*,7'*R*,8*R*,8'*R*)-7-(5-methoxy-3,4-methylenedioxyphenyl)-7'-(3,4-methylenedioxyphenyl)-7,9':7',9-diepoxylignane. As a direct consequence, the structure of **2** was constructed and given the designation (+)-5-demethoxyepiexcelsin (**2**) (Figure **5**).

(+)-*epi*-Syringaresinol (Figure **5**), is the designation for compound **4**, which was found as an amorphous powder. Using data from the ^1H and ^{13}C-NMR (Table **1**) as well as the sodiated molecular ion $[M+Na]^+$ at *m/z* 441.1054 (calcd. 441.1059) in the HRESIMS, the molecular formula was identified as $C_{22}H_{26}O_8$ (ten degrees of unsaturation). The IR spectra showed distinctive absorption due to the hydroxyl group (3432 cm^{-1}) and aromatic group (1646, 1549, and 1462 cm^{-1}). Two pairs of equivalent aromatic proton signals at δ_H 6.60 (2H, brd, *J* = 1.2 Hz), 6.62 (2H, brd, *J* = 1.2 Hz) and four magnetically equivalent methoxyls signal at δ_H 3.90 (12H, s) were already seen in the ^1H NMR spectrum of **4** in $CDCl_3$ suggested the presence of two phenyl rings with 1,3,4,5 tetra-substitutions. There are 12 aromatic carbons in total, according to an analysis of its ^{13}C NMR and DEPT spectrum of **4**, including six oxygenated quaternary carbons (δ_C 133.8, 134.3, 147.2 x 2 and 147.0 x 2), two quaternary carbons (δ_C 130.0 and 132.2) and four methines (δ_C 103.0 x 2 and 103.6 x 2). Additionally, there are four methines, comprising two oxygenated methine unit (δ_C 87.1 and 81.5) and two methines (δ_C 50.3 and 54.4) and four methoxy groups (δ_C 56.2), as well as two oxygenated methylenes (δ_C 69.6 and 70.8). Based upon the aforementioned characteristic data and the results of the ^1H-^1H COSY and HMBC studies, compound **4** could possess a furofuran lignan skeleton [20, 50, 51]. From δ_H 4.16, 3.87 (H_2-9) to δ_H 3.35 (H-8) which in turn couples to δ_H 2.91 (H-8') and δ_H 4.44 (H-7), and from H_2-9'/H-8'/H-7' according to the ^1H-^1H COSY data. Similar to it though, its HMBC data indicate that the proton at δ_H 2.91 (H-8′) correlates with the carbons at δ_C 130.0 (C-1′), 87.1 (C-7) and δ_H 4.87 (H-7′) correlates with δ_C 130.0 (C-1′), 103.6 (C-2′, -6'), and 69.6 (C-9′). The ^1H NMR data of **4** were very similar to those of

another furofuran lignan actually named (+)-syringaresinol (**7**) [35-39] and (+)-*dia*-syringaresinol (**8**) [39, 46, 47] (NMR data of both of them were reported in the experimental part) which were collected in schedule procedure of separation, with the exception that both of them express symmetrical furofuran lignans, which is demonstrated by the presence of only four proton signals in their ^{1}H NMR spectrum from δ_H 3.00 to 5.00 ppm as opposed to eight signals in **4**. Except for the replacement of the both aryl moiety in (+)-epiexcelsin (**1**), which was (5-methoxy-3,4-methylenedioxyphenyl) altered to a syringyl (4-hydroxy-3,5-dimothoxyphenyl), compound **4** has a great deal of aspects with (+)-epiexcelsin (**1**). The stereochemistry of **4** was recognized by the NOESY spectrum. The α-orientation (*equatorial*; H-7/H-8, *trans*) at C-7 was indicated by the down-field chemical shift of the δ_H 4.16 (H-9α=eq) as well as the up-field shift of H-7 at δ_H 4.44, and this assignment was further confirmed by the coupling constants of H-7 (J = 7.1 Hz) [55]. Another indicative in the NOESY spectrum of the opposite syringyl moiety β-orientation (*axial*; H-7'/H-8', *cis*) is the correlation between the down-field benzylic proton H-7' (δ_H 4.87) which shows a low coupling constant (J = 5.2 Hz) and the *axial* proton H-8' (δ_H 2.91) [34]. Through the use of a mixture of spectrum data from the COSY, HMQC, HMBC, and ROESY systems, the complete ^{1}H and ^{13}C NMR assignments were obtained (Table **1**). As a necessary consequence, the structure of **4** was confirmed to be (+)-*epi*-syringaresinol (Figure **5**)

In the existing literatures, conflicting account has been evolved. The isolation of (+)-*epi*-syringaresinol, from the stems of *Annona cherimola* for instance, is described by Chen et al. (1998) [39]. Despite the fact that their NMR results are nearly different from ours, including the signals the oxygenated methylenes and one of the methine protons of the furofurane skeleton were detected in a range without specification (^{1}H NMR), whereas both of the benzylic protons were detected in the reverse values in the ^{1}H NMR and have the same signal in ^{13}C NMR, and both of methine protons belonged to the *cis*-fused furan rings also, had the same chemical shift in the ^{13}C NMR. Thus, it was definitively demonstrated that *epi*-excelsin (1) had a (+) structure (7*S*,7'*R*,8*R*,8'*R*)-7,7'-bis(4-hydroxy-3,5-dimethoxyphenyl)-7,9':7',9-diepoxylignane.

A review of the furofuran lignan literature yielded significant stereochemical information that helped determine the relative and absolute configuration. Coniferyl alcohol, a derivative of cinnamyl alcohol, is enantioselectively dimerized to form the *cis*-fused bis-tetrahydrfuran (7,7'-dioxa-[3,3,0]-bicyclooctane) unit, which is utilised to make furofuran lignans [20]. This process proceeds through either enantiomeric series to create the (+)- or (-)-enantiomers series with the 8*R*,8′*R* and 8*S*,8′*S* configurations, respectively [56-58]. In the past, the aryl groups in these systems have been described as having *pseudo-axial* and *pseudo-equatorial* configurations [54], which are comparable to *exo*- and/or *endo*-relationships with regard to the oxygen atom in the surrounding ring (Figure **8**) [52].

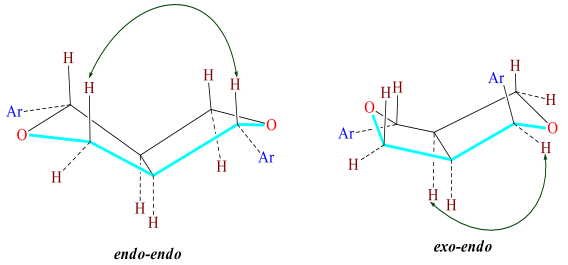

Figure 8. The stereochemistry of the diaryl-7,7'-terahydrofurofurans in the cis and trans forms.
Double arrows signify a NOESY connection.

A better match to describe these stereochemical interactions is the 7H, 8H, and 7′H, 8'H protons, which are *trans*-positioned instead of *axial* and *cis*-positioned instead of *equatorial*. This notation has been used in the past to avoid confusion when undertaking molecular modeling [59]. All of the isolated compounds suggested here are divided into three series: the first series {7,7′-*equatorial*, *axial* (7H,8H-*trans*/7′H,8′H-*cis*) [compounds **1**-**4**, (Figure **5**)}, beside with the second series {7,7'-di-equatorial (7H,8H-*trans*/7′H,8′H-*trans*) [compounds **5**-**7**, (Figure **5**)]}, in addition to the third series {7,7′-di-*axial* (7H,8H-*cis*/7′H,8′H-*cis*) [compounds **8**, (Figure **5**)]}. Based on the information acquired above as well as the variations in chemical shifts between the two oxygenated methylenes, the proton coupling constants for the benzylic sites for compounds the eight metabolites were also summarised (see Table 2).

After BV-2 cells were stimulated with 0.1 □g/mL of LPS in the presence or absence of various dosages of test substances for 20 h, the quantities of NO produced into culture medium were quantified using the Griess procedure [60]. The activity of compounds **1**, **2**, and **4** is moderate, whereas that of compounds **3**, 5-8 is weaker than 50 μM.

4. Conclusions

All hydrogens and carbons signals were assigned of the eight lignans. Complete ^{1}H and ^{13}C NMR signal assignments were utilised to determine their structures. Additionally, ^{1}H-^{1}H-COSY, HMQC, HMBC, and NOESY analyses were used to establish the stereochemistry of the lignans. Information from the HMQC and COSY spectra made it feasible to determine the majority of chemical shifts and coupling constants; information from the HMBC, *J*-resolved, and correlations in NOESY spectra made it possible to solve the more

challenging situations. Analysis of the tabular data reveals that the ^{13}C NMR chemical shift for C7 and C7′ in the *trans*/*cis*, *trans*/*trans*, and *cis*/*cis* cases is quite informative (Table **2**). Compounds **1**, **2** and **4** have moderate an anti-inflammatory activity and others (**3**,**5**-**8**) showed weak activity > 50 μM.

5. Conflicts of interest

"There are no conflicts to declare".

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