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Bis((5-allyl-2-(benzo[*d*][1,3]dioxol-5-yl)benzofuran-7-yl)oxy)methane: An Unusual *Nor*-Neolignan Dimer from *Magnolia grandiflora* L.

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Abstract: An unreported and unusual *nor*-neolignan dimer, namely bis((5-allyl-2-(benzo[*d*][1,3]dioxol-5-yl)benzofuran-7-yl)oxy)methane (**1**), was isolated from the bark-derived chloroform extract of *Magnolia grandiflora* L. Compound **1** was structurally elucidated through the detailed analysis of the spectroscopic data (one- and two-dimensional nuclear magnetic resonance, infrared, and high-resolution mass spectrometry). The effect of compound **1** on the mycelial growth of *Fusarium oxysporum* was also determined, affording moderate antifungal activity.

Keywords: Magnoliacea; *Magnolia grandiflora*; *nor*-neolignan; dimer; antifungal

1. Introduction

Magnolia grandiflora L. (Magnoliaceae), commonly known as "Southern Magnolia", is a pyramidal leafy tree that can grow to 30 meters high, forming blooms in spring or summer and even advanced autumn [1]. It is a highly attractive tree since it can be exploited as an ornamental plant and for the products provided (e.g., essential oils, resins, phenylpropanoids, and alkaloids) [2]. This plant comes originally from the USA but is highly distributed worldwide, including Colombia. *M. grandiflora* was introduced into Colombia for ornamental purposes and is part of the urban forestry along Bogotá city due to its glossy leaves, voluminous structure, and scented flowers, contributing several ecosystem services [3,4]. Additionally, *M. grandiflora* has various uses in traditional medicine to treat ailments related to inflammatory complications (e.g., rheumatism and asthma), nasal congestion, sinusitis, and fever, and has exhibited activity as a sedative, anticonvulsant, antioxidant, antitumor, antispasmodic, and antimicrobial [5,6].

M. grandiflora, as other *Magnolia* and Magnoliid plants, is well known to produce lignans and neolignans [7–9], which correspond to specialized metabolites naturally produced by the coupling of two phenylpropanoids or propenyl(allyl)phenols [10]. Lignans are biosynthetically differenced from the neolignans by the coupling type, particularly by the 8-8′ (lignans) or other than 8-8′ (neolignans) connectivity [11]. Among neolignans, the benzofuran-containing compounds have an 8-5′,7-O-4′ coupling, comprising a particular type of allylphenol dimers (also recognized as 2-arylbenzofurans), which have attracted high interest owing to their roles in plant defense against phytopathogens. Indeed, hydroxylated benzofurans, e.g., cicerfuran, have been implicated in chickpeas (*Cicer bijugum*) defense against Fusarium wilt [12]. A fascinating subclass is related to the benzofuran-type *nor*-neolignans. These metabolites are characterized when carbon C9(9′) is missing, whose structural variant provides remarkable biological properties [13].

Although this tree provides relevant urban and medicinal benefits, the search for specialized metabolites from *M. grandiflora* specimens established in this Andean city has not been conducted so far. Thus, as part of our research on the chemistry and antifungal compounds from exotic plants growing in Colombia, an unreported *nor*-neolignan dimer

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(**1**), a derivative of the reported compound **2**, was isolated from the chloroform extract of the bark of *M. grandiflora*. The isolated compound (**1**) was structurally elucidated (Figure 1) by employing the diagnostic analysis of the spectroscopic data (Supplementary Materials, Figures S1–S8). In addition, the antifungal activity was also evaluated against the phytopathogen *Fusarium oxysporum*.

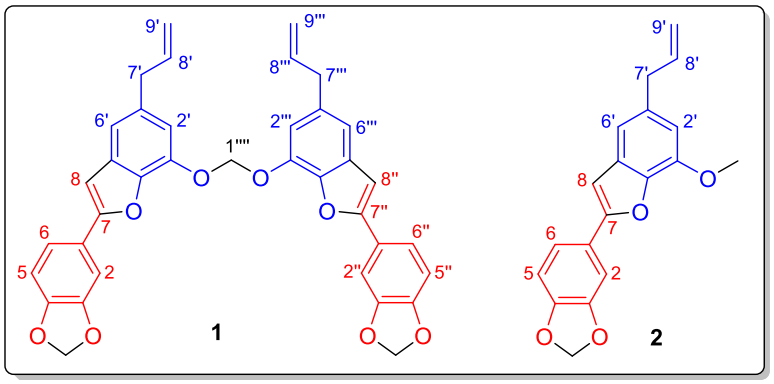

Figure 1. Structure of *nor*-neolignans **1** and **2**.

2. Results and Discussion

The dried bark of *M. grandiflora* was consecutively partitioned by conventional maceration with *n*-hexane, chloroform, and methanol to afford the respective crude extracts. The effect of the extracts on the *F. oxysporum* mycelial growth inhibition was then evaluated, and the chloroform extract caught our attention due to the observed activity (96% growth inhibition at 500 μg/mL). Hence, this extract was fractionated by vacuum column chromatography (VCC) on silica, gradient-eluted with *n*-hexane/ethyl acetate (EtOAc) mixtures. The resulting thin-layer-chromatography (TLC)-monitored fractions were depurated by CC on silica gel using gradients of *n*-hexane/chloroform, affording compound **1** as an abundant phytocomponent.

Compound (**1**) was isolated as an amorphous and colorless solid from *n*-hexane/chloroform. The molecular formula was determined to be $C_{37}H_{28}O_8$ by HRMS data (*m/z* 601.1873, $[M + H]^+$) with twenty-two unsaturation degrees. The infrared (ATR) spectrum (Figure S1) revealed bands for an oxygenated unsaturated compound, i.e., double bonds (1710 and 1530 cm^{-1}) and C-O stretching (1270 and 1050 cm^{-1}). Eleven signals were observed in the ^1H NMR spectrum (Figure S2), involving three singlets (δ_H 6.78, 5.99, and 5.59), five doublets (δ_H 7.36, 6.89, 6.98, 6.68, and 3.50), a double-doublet (δ_H 7.43), and two double–double-triplets (δ_H 6.11 and 5.21). The ^{13}C NMR spectrum (Figure S3) showed nineteen signals. A deep analysis of the DEPT and HMQC experiments indicated that such signals comprised eight quaternary carbons, seven methine carbons, and four methylene carbons.

The combined examination of the ^1H and ^{13}C NMR data (Table 1), indicated a profile highly similar to that of a previously reported *nor*-neolignan, i.e., 3′-methoxy-3,4-methylenedioxy-4′,7-epoxy-9-*nor*-8,5′-neolignan-7,8′-diene (**2**), isolated from various magnoliids plants, such as *Anaxagorea clavata* (Annonaceae) [14], *Nectandra purpurascens* (Lauraceae) [15], *N. lineata* (Lauraceae) [13], and *Pleurothyrium cinereum* (Lauraceae) [16], and synthesized employing an intramolecular Wittig cyclization [17]. However, the ^1H NMR data of compound **1** solely differed from those of **2** by a singlet signal at δ_H 5.59 in **2**, integrating for two hydrogens, instead of a singlet signal at δ_H 4.03, integrating for three hydrogens, assigned to a methoxyl group in **2** [14,15], which is absent in **1**. In addition, the ^{13}C NMR data also showed a difference between **1** and **2** by a signal at δ_c 89.7 instead of a signal at δ_c 56.0. These signals corresponded to a CH_2 and CH_3, respectively, defined by the DEPT experiments (Figure S3). The HMQC (Figure S4) experiment revealed a correlation between δ_H 5.59 and δ_c 89.7. This information indicated that compound **1** seemed to be a dimeric form of a derivative of **2**, which was confirmed by the analysis of the MS data (601.1873 $[M + H]^+$ (Figure S3) versus. 308 M^+ [14]) and the integration of the ^1H NMR

spectrum, as when the signal at δ_H 5.59 was adopted as the reference for integration (assuming $\int = 2$), the other signals showed a twofold integration increase. The dimer linking was established by the HMBC experiment (Figure S5), through the three-bond correlation between methylene at δ_H 5.59 and the aromatic quaternary carbon at δ_C 144.9. In this regard, the dimerization of **2** to produce **1** was rationalized through a methylenedioxy bridge (–OC1′′′′O–) to connect the aromatic ring of the benzofuran moieties of both monomers. A dimeric neolignan containing a peroxide bridge, named bishonokiol A, was previously isolated from *M. grandiflora* seeds [18], which exposed the capacity of this plant to produce dimeric forms of neolignans. The other ^{1}H-^{1}H COSY, HMQC, and HMBC correlations of **1** (Figure 2) were found to be identical to the monomer **2** [14,15]. Thus, compound **1** was determined to be bis((5-allyl-2-(benzo[*d*][1,3]dioxol-5-yl)benzofuran-7-yl)oxy)methane as an unreported and unusual *nor*-neolignan dimer from *M. grandiflora* bark.

Table 1. ^{13}C NMR and ^{1}H NMR ($CDCl_3$) data of compound **1** ($CDCl_3$).

Position	Type	δ_C	δ_H (multpl., *J* (Hz), Integral)
1, 1′′	C × 2	124.8	
2, 2′′	CH × 2	105.5	7.36, (d, *J* = 1.8, 1H)
3, 3′′	C × 2	148.1	
4, 4′′	C × 2	148.2	
5, 5′′	CH × 2	108.6	6.89, (d, *J* = 8.1, 1H)
6, 6′′	CH × 2	119.2	7.43, (dd, *J* = 8.1, 1.8, 1H)
7, 7′′	C × 2	156.1	
8, 8′′	CH × 2	100.5	6.78, (s, 1H)
1′, 1′′′	C × 2	135.8	
2′, 2′′′	CH × 2	107.6	6.98, (d, *J* = 1.5, 1H)
3′, 3′′′	C × 2	144.9	
4′, 4′′′	C × 2	142.7	
5′, 5′′′	C × 2	131.2	
6′, 6′′′	CH × 2	112.7	6.68, (d, *J* = 1.5, 1H)
7′, 7′′′	CH_2 × 1	40.6	3.50, (d, *J* = 6.7, 2H)
8′, 8′′′	CH × 1	138.1	6.11, (ddt, *J* = 16.8, 10.1, 6.7, 1H)
9′, 9′′′	CH_2 × 2	115.7	5.26–5.14, (ddt, *J* = 10.3, 1.9, 1.2, 2H)
1′′′′	CH_2	89.7	5.59, (s, 2H)
OCH_2O	CH_2 × 2	101.4	5.99, (s, 2H)

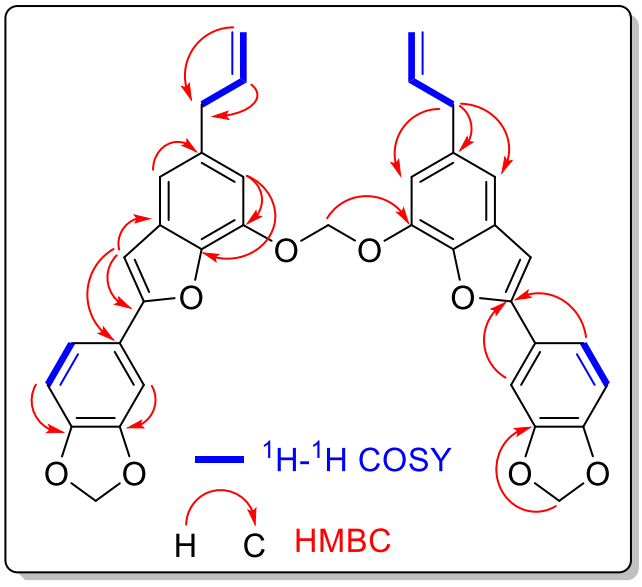

Figure 2. ^{1}H-^{1}H COSY and HMBC correlations of *nor*-neolignan **1**.

Compound **1** was evaluated against *F. oxysporum* employing an amended-medium assay to assess its mycelial growth inhibition [19]. The test *nor*-neolignan exhibited a dose-dependent response, affording a half-maximal inhibitory concentration (IC_{50}) of 53.5 μM and involving a fungistatic effect. Compound **1** exhibited 18.6-fold less activity than the positive control (i.e., iprodione, IC_{50} = 2.88 μM). Hence, the outcome indicated that compound **1** has moderate antifungal activity. Some benzofurans are related to the plant defense against phytopathogens, e.g., *F. oxysporum* f. sp. *ciceri*, having a relevant effect on spore germination (100% at 250 μg/mL) [12], although hydroxylation appeared to be a critical structural factor for a better antifungal activity [20].

3. Materials and Methods

3.1. General Experimental Procedures

The infrared (IR) spectrum was recorded on a Jasco FTIR-4600 (Jasco Inc., Tokyo, Japan) over the solid directly by the attenuated total reflection (ATR). The NMR spectra, as well as one-dimensional (DEPT 135) and two-dimensional (^{1}H-^{1}H COSY, HMQC, and HMBC) experiments, were recorded on a Bruker Avance 400 spectrometer (400 MHz for ^{1}H and 100 MHz for ^{13}C) using tetramethylsilane (TMS) as an internal standard. All shifts are given in δ (ppm) using the TMS signal as the reference. All coupling constants (*J*) are given in Hz. The high-resolution mass spectrum was recorded on an Agilent Technologies 1260 Liquid Chromatography system coupled to a quadrupole-time-of-flight (Q-ToF) mass analyzer with dual Agilent jet stream electrospray ionization (AJS ESI) (Agilent, Santa Clara, CA, USA). Thin-layer chromatography (TLC) using silica gel 60 F_{254} TLC plates (Merck KGaA, Darmstadt, Germany) and mobile phases comprising solvent mixtures of *n*-hexane, EtOAc, and chloroform were used. TLC-developed plates were observed under UV light (254 and 365 nm) and derivatized using I_2 vapor and Hannessian's reagent (aqueous solution of ammonium molybdate, cerium sulfate, and H_2SO_4). Silica gel 60 (0.063–0.200 mm), silica gel 60 (0.04–0.063 mm), and silica gel 60 HF254 (Merck KGaA, Darmstadt, Germany) were used for column chromatography (CC), flash chromatography (flash CC), and vacuum liquid chromatography (VLC), respectively.

3.2. Plant Material

The bark of *M. grandiflora* was collected from the urban forestry of Bogotá city in June 2017 (coordinates: N 4°39′26.7″ W 74°6′49.4″). The plant was identified, and a voucher specimen was deposited in the Herbario Nacional Colombiano (COL591171).

3.3. Extraction and Isolation

The bark (568 g) of *M. grandiflora* was dried and ground and subsequently partitioned by conventional maceration using consecutively different solvents increasing polarity. This partition started with *n*-hexane (2 L), involving stirring (100 rpm) and a daily solvent removal replaced by a fresh one for three days (i.e., three extractions × 2 L). The plant residue was dried, subsequently employed chloroform through the same procedure (i.e., three extractions × 2 L), and finally, methanol was used (i.e., three extractions × 2 L). Each resulting mixture was filtrated and concentrated under reduced pressure to afford the respective crude extracts, i.e., *n*-hexane (23 g), chloroform (16 g), and methanol (33 g). A portion (10 g) of chloroform extract was fractionated by VLC using *n*-hexane/EtOAc mixtures in gradient elution, obtaining fourteen fractions (f1 to f14). Fraction f6 (1186 mg) was depurated by flash CC using *n*-hexane/chloroform (8:2) as the mobile phase, affording five further fractions (f6.1 to f6.5). Fraction f6.2 (726 mg) was purified using flash CC on silica gel *n*-hexane/chloroform (9:1), yielding compound **1** (439 mg).

Bis((5-allyl-2-(benzo[d][1,3]*dioxol-5-yl)benzofuran-7-yl)oxy)methane* (**1**): colorless amorphous powder (mp 126–127 °C); IR ν_{max} (ATR) 2944, 1710, 1530, 1420, 1270, 1230, 1100, and 1050 cm^{-1}; ^{1}H (400 MHz) and ^{13}C (100 MHz) NMR, see Table 1; HRESIMS (positive mode) *m/z* 601.1873 $[M + H]^+$, (calcd. for $C_{37}H_{29}O_8$, 601.1862).

3.4. Antifungal Activity

The antifungal activity of compound **1** was explored against *Fusarium oxysporum* through the 12-well plate amended-medium protocol previously reported to evaluate the in vitro mycelial growth inhibition [19]. The IC_{50} value was calculated from a dose–response plot (i.e., log(doses) versus mycelial growth inhibition percentage) through nonlinear regression using GraphPad Prism 9.0 for Windows. Iprodione was used as the positive control. Once the assay ended at the highest test dose (1 µg/mL), a 2-mm fungal plug was transferred to a fresh medium-containing well to determine whether it could grow or not to classify the effect as fungistatic or fungicidal, respectively.

4. Conclusions

An unusual and unreported *nor*-neolignan dimer (**1**), namely bis((5-allyl-2-(benzo[*d*][1,3]dioxol-5-yl)benzofuran-7-yl)oxy)methane, was isolated from *M. grandiflora* bark-derived chloroform extract. Compound **1** exhibited a moderate antifungal activity against *F. oxysporum* (IC_{50} = 53.5 µM), implying a fungistatic effect.

Supplementary Materials: The following supporting information can be downloaded online, Figure S1. Infrared spectrum of **1** (ATR), Figure S2. ^{1}H NMR spectrum of **1** (400 MHz, $CDCl_3$), Figure S3. ^{13}C NMR spectrum of **1** (100 MHz, $CDCl_3$), Figure S4. DEPT 135 spectrum of **1** (100 MHz, $CDCl_3$), Figure S5. HMQC spectrum of **1**, Figure S6. ^{1}H-^{1}H COSY spectrum of **1**, Figure S7. HMBC spectrum of **1**, Figure S8. HRMS spectrum of **1**.

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