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Bioactive Neolignans and Other Compounds from *Magnolia grandiflora* L.: Isolation and Antiplasmodial Activity

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Abstract

Bioassay-guided fractionation of a methanol extract of *Magnolia grandiflora* against *Plasmodium falciparum* yielded two new (**1** and **2**) and six known (**3 – 8**) bioactive compounds. The structures of the new compounds were assigned by mass spectrometric and 1D and 2D NMR data. Known compounds were identified by comparison of ^{1}H NMR and MS data with literature data. The two known neolignans **3** and **4** showed moderate antiplasmodial activity with IC_{50} values of 2.8 ± 0.1 and 3.4 ± 0.1 μM, respectively. Weak antiplasmodial activity was recorded for compounds **1**, **2**, **5**, **6**, **7** and **8**, with IC_{50} values of 38 ± 2, 23 ± 2, 16.5 ± 0.2, 86 ± 1, 44 ± 4 and 114 ± 9 μM, respectively.

Keywords

Antiplasmodial activity; Neolignans; *Magnolia grandiflora* (Magnoliaceae); 1D-and 2D-NMR; Mass spectrometry

Introduction

Malaria is one of the world's most devastating diseases, with over 200 million people being infected every year and over 400,000 deaths in 2015 alone, most of them among children in

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Conflict of Interest

The authors declare no conflict of interest.

Author Contributions Statement

David Kingston conceived and designed the experiments, Abdul Latif isolated the compounds, and Abdul Latif and Yongle Du determined their structures. Seema Dalal, Maria Fernández-Murga, and Emilio Merino carried out the antiplasmodial bioassays under the direction of Maria Cassera, and Michael Goetz provided the crude extract.

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sub-Saharan Africa.[1] Unfortunately the lethal malaria parasite, *Plasmodium falciparum*, has become resistant to most of the antimalarial drugs that were effective in the past, such as quinine, chloroquine, sulphadoxine-pyrimethamine, and mefloquine.[2] Drug resistance to artemisinin, which is used in combination therapy with other antimalarials as the first-line treatment for uncomplicated *P. falciparum* malaria, has also been observed.[3] Therefore, there is a continuing need to develop novel and more effective drugs against malaria. Various reviews have describe the current state of antimalarial drug research,[4–7] but in the words of one review "although there has been a dramatic improvement in the pipeline of new antimalarial molecules over the past decade, the glass is still rather empty".[8]

As part of a systematic search for antimalarial agents from plant extracts from the Natural Product Discovery Institute Repository, a detanninized methanol partition fraction of the twigs and fruit of *Magnolia grandiflora* was found to show moderate activity against the Dd2 strain of *P. falciparum. M. grandiflora* is a well-investigated plant species, and is known to contain alkaloids,[9] flavonoids, phenolic compounds,[10] glycosides,[11, 12] sesquiterpene lactones,[13–15] and volatile compounds.[16] It has been reported in Traditional Chinese Medicine as a reliever of colds, headaches, and stomach ache.[9] It has not however been reported to contain any antimalarial compounds, and it was thus selected for investigation. Herein, we report the isolation and structure elucidation of two new (**1** and **2**) and six known (**3** – **8**) compounds with various degrees of antimalarial activity.

Results and Discussion

A methanol extract of *M. grandiflora* was subjected to liquid-liquid partitioning to eventually afford an ethyl acetate soluble fraction with an IC_{50} value of 10 μg/mL against the pyrimethamine, chloroquine, and mefloquine-resistant Dd2 strain of *P. falciparum*. Bioassay-guided isolation using solid phase extraction over C_{18} silica followed by C_{18} HPLC gave two new compounds (**1** and **2**) along with six known compounds (**3** – **8**) as shown in Figure-1. The known compounds were identified as 4′-*O*-methyl honokiol (**3**),[17] magnolol (**4**),[18] honokiol (**5**),[19] 3-methoxymagnolol (**6**),[18] isomagnolol (**7**),[20] and ketone (**8**)[21, 22] after comparison of their mass spectrometric and ^{1}H NMR data (Figure S13–S18) with experimental values.

Compound **1** was isolated as a colorless oil and was assigned the molecular formula $C_{18}H_{20}O_2$ based on its sodiated molecular ion peak at *m/z* 291.1352 $[M + Na]^+$ in its HRESIMS. Its ^{1}H NMR data (Table 1) displayed three aromatic protons of an ABX system at δ_H 6.73 (1H, J = 8.0 Hz, H-2), 6.96 (1H, J = 8.0, 1.8 Hz, H-3), and 6.98 (1H, J = 1.8 Hz, H-5). Signals for two allyl groups were present at δ_H 3.34 (2H, d, J = 6.6 Hz, H-7), 2.47 – 2.51 (1H, m, H-7′a), 2.53 – 2.60 (1H, m, H-7′b), H-5.90 – 5.95 (1H, m, H-8), 5.6 – 5.76 (1H, m, H- H-8′), 5.03 – 5.12 (2H, m, H-9), and 5.04 – 5.12 (2H, m, H-9′). Proton shifts at δ_H 5.62 (1H, d, J = 10.0 Hz) and 5.95 – 6.00 (1H, m) were assigned to H-5′ and H-6′, respectively. Moreover, two oxygenated methine and two methylene protons were also observed at chemical shift values of δ_H 4.14 (1H, dq, J = 8.4, 4.5 Hz, H-1′), δ_H 4.71 (1H, broad t, J = 3.9 Hz, H-3′), δ_H 2.00 – 2.08 (1H, m, H-2′a) and δ_H 2.41 – 2.48 (1H, m, H-2′b). Its ^{13}C NMR spectrum (Table 1) showed the presence of 18 carbon signals. An HSQC experiment in combination with ^{13}C NMR data identified five methylenes at δ_C 31.6,

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39.8, 41.4, 115.6 and 118.6, two sp^3-methines at δ_C 62.2 and 85.3, and seven sp^2-methines at δ_C 110.1, 123.2, 128.0, 128.6, 131.4, 133.2, and 137.8. The remaining 4 carbon signals were classified as quaternary carbons with resonances at δ_C 47.8, 133.2 (2C), and 156.0. The NMR data (Table 1) of **1** is similar to that of ketone (**8**)[21, 22] except for the presence of a hydroxymethine at δ_C 62.2 (C-1′) instead of the ketone carbonyl at δ_C 195.2 (C-1′). An HMBC experiment on **1** (Figure 2) indicated correlations of H-7 (δ_H 3.34) with C-4 (δ_C 133.2), C-3 (δ_C 128.6) and C-5 (δ_C 123.2) confirming the presence of an allyl group on the aromatic ring. The presence of a second allyl group at C-4′ was validated from correlations between H-7′ (δ_H 2.56) to C-4′ (δ_C 47.8), and C-3′ (δ_C 85.3). The position of the double bond was fixed between C-5′ and C-6′ by the correlations of H-5′ (δ_H 5.62) with C-4′, C-1′, and C-3′. The position of C-3′ (δ_C 85.3) was confirmed on the basis of its correlations to H-5′ (δ_H 5.62) and H-7′ (δ_H 2.56). A COSY spectrum (Figure 2) was also obtained for compound **1**, and cross peaks were located for H-1′ to H-2′a/b and H-6′ and H-2′a/b to H-3′, indicating the presence of a cyclohexene ring (C-1′ through C-6′) in **1**. Compound **1** was thus identified as 4,4′-diallyl-1,2,6,4′-tetrahydrodibenzo[*b*,*d*]furan-3′-ol.

Compound **2**, also a colorless oil, was assigned the molecular formula $C_{26}H_{27}NO_2$ (*m/z* 386.2107 $[M + H]^+$, calcd. 386.2115) on the basis of its HRESIMS. ^{1}H NMR data (Table 1) exhibited the presence of two ABX substituted benzene rings and a 1,4-disubstituted benzene ring. Protons of the two ABX aromatic rings were assigned chemical shift values of δ_H 7.11 (1H, m, overlapped, H-2′), 7.02 (1H, m, overlapped, H-2), 6.89 (1H, m, H-5), 6.77 (1H, d, J = 8.2 Hz, H-5′), 7.03 (1H,dd, J = 8.8, 2.3 Hz, H-6), and 7.24 (1H, dd, J = 8.2, 2.2 Hz, H-6′). The 1,4-disubstituted third aromatic ring had proton signals at δ_H 7.11 (2H, d, J = 8.4 Hz, H-2″ and H-6″) and 6.80 (2H, d, J = 8.4 Hz, H-3″ and H-5″). Chemical shifts of 3.34 (2H, dt, J = 6.7, 1.7 Hz, H-7), 3.21 (2H, dt, J = 6.3, 1.7 Hz, H-7′), 5.97 (1H, ddt, J = 16.8, 10.0, 6.7 Hz, H-8), 5.82 (1H, ddt, J = 16.6, 10.2, 6.3 Hz, H-8′), 5.04 (1H, dq, J = 10.0, 1.7, H-9a), 5.08 (1H, dq, J = 17.0, 1.7, H-9b), 4.99 (1H, dq, J = 17.0, 1.7, H-9a′) and 5.01 (1H, dq, J = 10.2, 1.7, H-9b′) were assigned to two allyl groups on the separate aromatic rings. Additional signals characteristic of an ethylamino group[23] were observed at δ_H 2.90 (2H, t, J = 6.9 Hz, H-7″) and 3.41 (2H, dt J = 6.9, 6.9 Hz, H-8″). ^{13}C NMR data (Table 1) for compound **2** displayed a total of 26 carbon atoms. An HSQC NMR experiment in combination with ^{13}C NMR data classified these carbon atoms into 6 methylenes (δ_C 34.6, 36.6, 39.6, 45.1, 115.6, and 116.8), 12 methines (δ_C 111.2, 115.4, 115.6 (2C), 128.4, 128.5 (2C), 130.1 (3C), 135.5, and 138.0) and 7 quaternary carbons (δ_C 124.8, 128.0, 130.2, 130.6, 131.4, and 154.3 (2C). Assignment of the ^{13}C NMR spectrum of **2** was made on the basis of its HMQC spectrum and by comparison with the ^{13}C NMR spectra of honokiol analogs.[24] An HMBC NMR spectrum (Figure 3) showed key correlations for **2**, including cross-peaks for the three-bond correlation H-2″/H-6″ to C-7″ (δ_C 34.6), for the two-bond correlation H-7″ to C-8″ (δ_C 45.1), and for the three bond correlations H-2 to C-1′ (δ_C 125.3) and to C-4 (δ_C 151.1), H-7 to C-2 (δ_C 130.3), and H-7′ to C-2′ (δ_C 130.6) and C-4′ (δ_C 146.1). These correlations established the structure of **2** as 3,3′-diallyl-4′-((4-hydroxyphenethyl)amino)-[1,1′-biphenyl]-4-ol.

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Compound **2** is most probably formed by oxidative coupling of 2-allyphenol followed by condensation of the intermediate with *p*-hydroxyphenethylamine. Figure 4 shows an example of one plausible pathway.

The isolated compounds **1–8** were evaluated for their antiplasmodial activity against the Dd2 strain of *P. falciparum*. Compounds **3** and **4** showed moderate antimalarial activity with IC_{50} values of 2.8 ± 0.06 and 3.4 ± 0.08 μM, respectively. Compounds **1**, **2**, **5**, **6**, **7**, and **8** had weaker activities with IC_{50} values of 37.5 ± 2.00, 22.7 ± 1.81, 16.6 ± 0.2, 86.1 ± 0.6, 44.4 ± 4.1 and 114 ± 9 μM, respectively.

The fact that simple lignans show moderate antiplasmodial activity is not unprecedented. Thus several lignans with antiplasmodial activity from African medicinal plants are reported in the review by Ntie-Kang et al.,[6] and lignans with antiplasmodial activity have been reported from *Asparagus africanus*[25] and *Carrisa edulis*.[26] Neolignans have also shown good antiplasmodial activity.[27] One point of interest is that antiplasmodial activity appears to be strongly dependent on the substitution pattern of simple lignans. Thus compounds **3** and **4** were active at the single digit micromolar level, but compound **5**, isomeric with **4**, was fivefold less potent, and compound **6**, with an extra hydroxyl group, was thirtyfold less potent than **3**.

Experimental Part

General Experimental Procedures

UV spectra were measured on Shimadzu a UV-1201 spectrophotometer. ^{1}H- and ^{13}C NMR spectra were recorded on a Bruker Avance 500 spectrometer in $CDCl_3$ with TMS as internal standard. An Agilent 6220 mass spectrometer was used to obtain high resolution mass spectra. Solid phase extraction was performed using RP-18 silica gel (40–63μm, EM Science, Germany). Semi-preparative HPLC was performed on a semipreparative Phenomenex C18 column (5μm, 250 × 10 mm), using Shimadzu LC-10AT pumps coupled with a Shimadzu SPD M10A diode array detector, and a SCL-10A system controller.

Antiplasmodial Bioassay

The effect of each fraction or compound on parasite growth of the Dd2 strain of *P. falciparum* was measured in a 72 h growth assay in the presence of compound as descibed previously [28, 29]. Briefly, ring stage parasite cultures (100 μL per well, with 1% hematocrit and 1% parasitemia) were grown for 72 h in the presence of increasing concentration of the drug in a 5% CO_2, 5% O_2, and 90% N_2 gas mixture at 37 °C. After 72 h in culture, parasite viability was determined by DNA quantitation using SYBR Green I assay [29]. The half-maximum inhibitory concentration (IC_{50}) calculation was performed with KaleidaGraph software using a nonlinear regression curve fitting. IC_{50} values are the average of three independent determinations with each determination in duplicate and are expressed ± SEM. Artemisinin was used as the positive control with an IC_{50} of 6 ± 2 nM.

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Plant Material

The plant material was collected in Santa Barbara, CA on September 11, 1995 by Cori Morenberg and Jan Wienpahl (NYBG) in the Santa Barbara Alice Keck Memorial Park, along Arrelaga St. 34°25′ N, 119°42′ W. http://sweetgum.nybg.org/science/vh/specimen_details.php?irn=284841. Voucher specimen CM00144c.

Extraction and Initial Fractionation

The dried and powdered twigs and fruit of *M. grandiflora* (175 g) were exhaustively extracted with MeOH in two 24-hour percolation steps followed by partition into hexane, methylene chloride and an aqueous methanolic fractions. The latter was detanninized by passage through a column of polyvinyl pyrrolidone to give the active methanolic fraction 0038279-03C, X-4568 (about 10 g). For purposes of fractionation and purification, 1.5 g of extract was shipped to Virginia Tech for bioassay-guided isolation.

Isolation of Bioactive Constituents

The crude extract (1.3 g) was first detanninized again by dissolving in MeOH and passing it through a polyamide column. A total of 932 mg detanninized extract was collected after elution and evaporation. It was dissolved in 200 mL of 90% aquous methanol and extracted with hexanes (200 × 5 mL). A total of 69 mg hexanes fraction was obtained. The 90% aqeous methanol extact was evaporated, suspended in 200 mL water, and then extracted with EtOAc (200 × 5 mL); evaporation of this EtOAc-soluble fraction gave 317 mg residue. The water layer was concentrated to a brown reside (530 mg). The EtOAc fraction had the highest antiplasmodial activity, with an IC_{50} value between 5 and 2.5 μg/mL. It was separated into seven sub-fractions (F1–F7) by open column chromatography over RP-18 silica gel with a MeOH/H_2O gradient. Sub-fraction F5 (eluted with 90% MeOH/H_2O) was the most active fraction with an IC_{50} value between 2.5 and 1.5 μg/mL. Semi-preparative C_{18} HPLC (MeOH/H_2O sovent system) on F5 yielded nine subfractions (F5-1 to F5-9). Fraction F5-8 was further purified on HPLC using a C_{18} column with a MeOH/H_2O solvent system to give compound **3** (3.2 mg, t_R 19.2 min). Fraction F5-6 was subjected to HPLC purification on a C_{18} column using MeCN/H_2O as solvent system to afford compounds **4** (2.0 mg, t_R 12.76 min) and **6** (0.3 mg, t_R 14.5 min). F5-5 was purified by HPLC on a C_{18} column using the MeCN/H_2O solvent system to obtain compound **1** (0.5 mg, t_R 12.1 min). The seventh fraction F5-7 yieded compounds **2** (0.6 mg, t_R 14.2 min) and **7** (0.3 mg, t_R 18.8 min) on HPLC separation on a C_{18} column using the MeCN/H_2O solvent system. HPLC of fraction F5-3 on a C_{18} column (MeCN/H_2O solvent system) resulted in the isolation of compounds **5** (2.7 mg, t_R 10.1 min) and **8** (0.3 mg, t_R 14.4 min).

Structural Identification

Characterization data of compounds **1** and **2** are shown below and their ^{1}H NMR, ^{13}C NMR, HSQC, and HMBC spectra are reproduced in the Supporting Information. The ^{1}H NMR spectra of compounds **3** – **8** are also reproduced in the Supporting Information.

4,4′-Diallyl-1,2,6,4′-tetrahydrodibenzo[*b,d*]furan-3′-ol; 1—Colorless oil. $[\alpha]_D^{21}$-33.8 (c = 0.020, MeOH), LC-UV [(acetonitrile in H_2O)] λ_{max} 225, 285 nm. ^{1}H

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NMR ($CDCl_3$, 500 MHz) and ^{13}C NMR ($CDCl_3$, 125 MHz), see Table 1. ESI-HRMS *m/z* 291.1352 $[M + Na]^+$; $C_{18}H_{20}O_2Na^+$ (calc. 291.1361).

3,3′-Diallyl-4′-((4-hydroxyphenethyl)amino)-[1,1′-biphenyl]-4-ol; 2—LC-UV [(acetonitrile in H_2O)] λ_{max} 219, 280 nm. ^1H NMR ($CDCl_3$, 500 MHz) and ^{13}C NMR ($CDCl_3$, 125 MHz), see Table 1. ESI-HRMS *m/z* 386.2107 $[M + H]^+$; $C_{26}H_{28}NO_2^+$ (calc. 386.2120).

Supplementary Material

Refer to Web version on PubMed Central for supplementary material.

Acknowledgments

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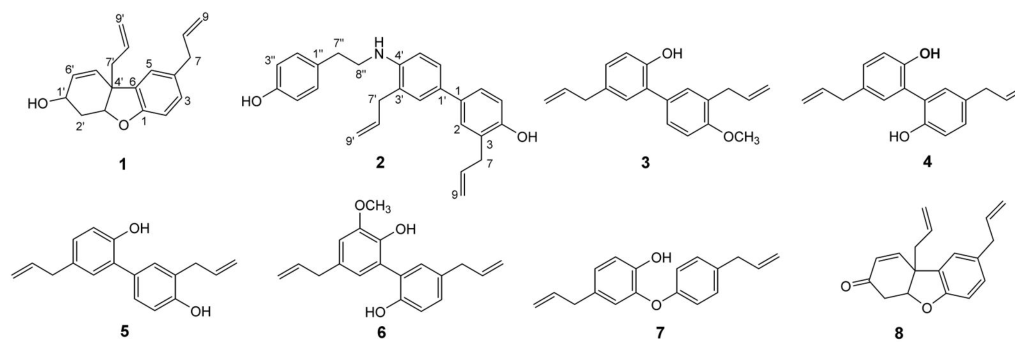

Figure 1.

Structures of compounds **1–8**.

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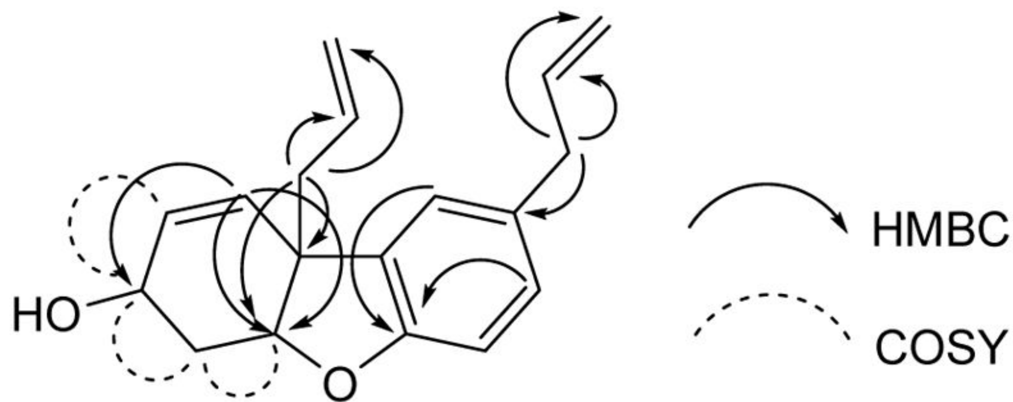

Figure 2.
Key HMBC and COSY correlations of compound **1**

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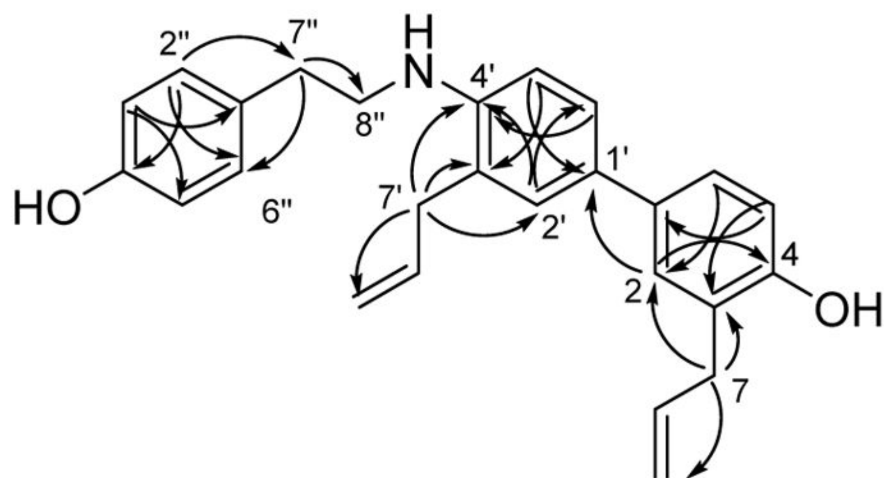

Figure 3.
Key HMBC correlations of compound **2**

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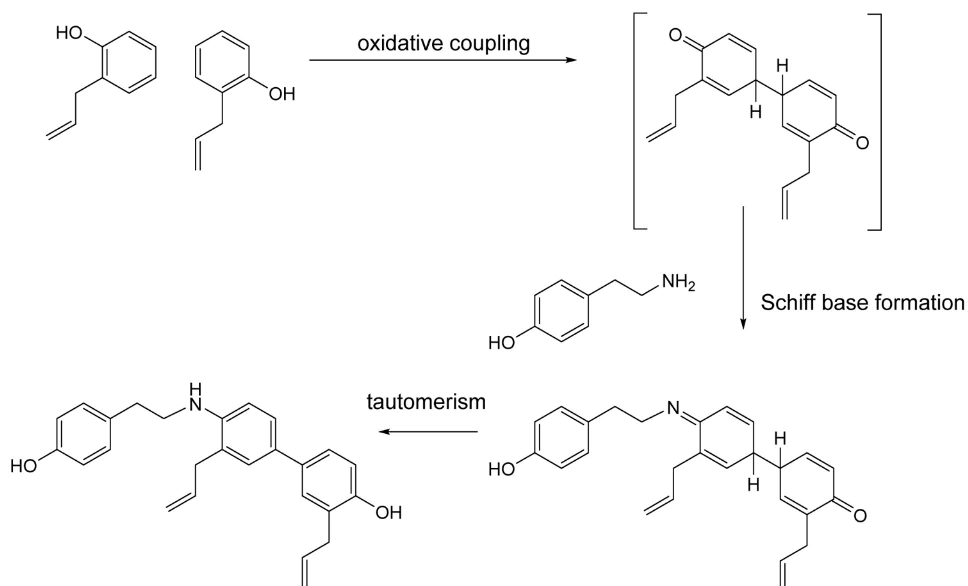

Scheme 1.
Plausible biosynthetic pathway for compound **2**

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Table 1

^{1}H- and ^{13}C NMR data of compounds **1** and **2**.

Position	1		2	
	^{1}H NMR (*J* in Hz)	^{13}C NMR	^{1}H NMR (*J* in Hz)	^{13}C NMR
1	-	156.2	-	128.0
2	6.73, d, *J* = 8.0	110.3	7.02, d, *J* = 2.3	130.3
3	6.96, dd, *J* = 8.0, 1.8	128.6	-	132.2
4	-	133.4	-	151.0
5	6.98, d, *J* = 1.8	123.4	6.89, d, *J* = 8.8	115.4
6	-	133.2	7.03, dd, *J* = 8.8, 2.3	128.5
7	3.34, d, *J* = 6.6	39.9	3.34, dt, *J* = 6.7, 1.7	39.6
8	5.90 – 5.95, m	138.0	5.97, ddt, *J* = 16.8, 10.0, 6.7	138.0
9	5.03 – 5.12, m	115.8	5.04, dq, *J* = 10.0, 1.7 5.08, dq, *J* = 17.0, 1.7	115.6
1′	4.14, dq, *J* = 8.4, 4.5	62.4	-	125.3
2′/a or b	2.00 – 2.08, m, 2.41 – 2.48, m	31.8	7.11, d, *J* = 2.2	130.6
3′	4.71, br t, *J* = 3.9	85.6	-	124.8
4′	-	47.9	-	146.1
5′	5.62, d, *J* = 10.0	131.6	6.77, d, *J* = 8.2	111.2
6′	5.95–6.00, m	128.2	7.24, dd, *J* = 8.2, 2.2	128.5
7′/a or b	2.47 – 2.51, m, 2.53 – 2.60, m	41.6	3.21, dt, *J* = 6.3, 1.7	36.6
8′	5.66 – 5.76, m	133.4	5.82, ddt, *J* = 16.6, 10.2, 6.3	135.5
9′	5.04 – 5.12, m	118.7	4.99, dq, *J* = 17.0, 1.7 5.01, dq, *J* = 10.2, 1.7	116.8
1″	-	-	-	131.3
2″	-	-	7.11, d, *J* = 8.4	130.1
3″	-	-	6.80, d, *J* = 8.4	115.6
4″	-	-	-	154.3
5″	-	-	6.80, d, *J* = 8.4	115.6
6″	-	-	7.11, d, *J* = 8.4	130.1
7″			2.90, t, *J* = 6.9	34.6
8″			3.41, dt, *J* = 6.9, 6.9	45.1
NH			3.85, br s	
OH			5.21, s	
OH			4.70, s	