SHORT REPORT

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A New Benzofuran from the Heartwood of *Dalbergia odorifera* T. Chen and Its Protective Effect on Hypoxia/Reoxygenation Injury in H9c2

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Abstract: A new benzofuran, named as (2*S*,3*S*)-5,6-dimethoxy-3-methyl-2-(3'-hydroxyphenyl)-2,3-dihydrobenzofuran **(1)**, along with the six known isoflavonoids, 2'-hydroxy-4',7-dimethoxyisoflavone **(2)**, 2′-methoxybiochanin A **(3)**, tectorigenin **(4)**, calycosin **(5)**, 7-hydroxy-2',4',5'-trimethoxyisoflavone **(6)** , orobol **(7)** were isolated from the heartwood of *Dalbergia odorifera* T.Chen. The structure of compounds was characterised by NMR spectroscopic data and comparisons with relevant literature data. The absolute structural configuration of compound 1 was determined through X-ray single crystal diffraction. Moreover, compounds **1-7** have no significant cytotoxic effects on H9c2 cells (IC_{50} > 200 μM). Compound **1-7** exhibit a significant protective effect against H/R (hypoxia/reoxygenation) induced H9c2 cell damage at 10~40, 5~40, 5~40, 5~40, 5~40, 5~40 and 5~10 μM ($P < 0.05$).

Keywords: *Dalbergia odorifera* T. Chen; benzofuran; H9c2; hypoxia/reoxygenation.

1. Plant Source

The heartwood of *Dalbergia odorifera* T.Chen was purchased from Danzhou, Hainan, China, in August 2019 and identified by Professor Rong-hua Liu from Jiangxi University of Chinese Medicine. A voucher specimen (No.201908) was deposited in the herbarium of Jiangxi University of Chinese Medicine, Nanchang, China.

2. Previous Studies

D. odorifera is also known as Hainan yellow rosewood, fragrant rosewood and so on [1]. Its wild resources are mainly distributed in western and southern parts of Hainan Province, China [2]. It is commonly

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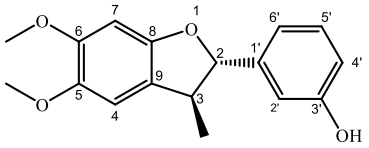
used to treat vomiting blood, traumatic bleeding, liver and rib pain, bruises, vomiting, abdominal pain, and chest paralysis and stabbing pain [3]. Modern pharmacological research has shown that it is significantly effective in treating cardiovascular disease by increasing vasodilation, coronary blood flow, and anti-thrombosis, as well as improving myocardial function [4-5]. Additionally, it has various pharmacological effects, including anti-inflammatory, antibacterial, antioxidant and anti-tumor properties [6-12]. Currently, over 200 compounds have been extracted from *D. odorifera*, comprising of flavonoids, volatile oils, sesquiterpenes, neoflavonoids, phenols, benzofurans, and other compounds [13].

3. Present Study

Dried heartwood (25.0 kg) of *D. odorifera* was pulverized to a coarse powder and extracted with 75% ethanol by heating and refluxing three times for 2 hours each time. 7.2 kg of ethanolic extract was obtained under reduced pressure, the extraction efficiency is 28.8%. Then, silica gel CC (column chromatography) was applied and PE (petroleum ether, 52.3 g), CH_2Cl_2 (dichloromethane, 877.2 g), EtOAc (ethyl acetate, 3700.0 g) and MeOH (methanol 679.0 g) fractions were obtained. The EtOAc fraction (3700.0 g) was chromatographed by silica gel CC with a gradient of PE- EtOAc (20:1 to 1:1, v/v) to give 9 fractions, A-I. Fr.F (448.0 g) was separated on silica gel CC with PE- EtOAc (40:1 to 1:1, v/v) as eluent to obtain 14 subfractions (F1-F14). Subfraction F7 (5.6 g) was separated to silica gel CC eluted with PE- EtOAc (12:1 to 5:1, v/v), 2 fractions (F7a-F7b) were obtained. Fr.F7b (0.84 g) was purified by Sephadex LH-20 CC (CH_2Cl_2-MeOH=1:1, v/v) and recrystallization (MeOH) to give compound **1** (12.0 mg). Subfraction F4 (3.8 g) was chromatographed on silica gel CC with a gradient elution of PE- EtOAc (20:1 to 5:1, v/v) to yield 3 fractions (F4a-F4c). Fr.F4b (1.4 g) was isolated by Sephadex LH-20 CC (CH_2Cl_2- MeOH=1:1, v/v) to obtain compound **2** (18.3 mg). Subfraction F8 (3.5 g) was subjected to silica gel CC using PE-acetone (40:1 to 2:1, v/v) elution to afford 7 fractions (F8a-F8g). Fr.F8e (0.8 g) was separated by Sephadex LH-20 CC (CH_2Cl_2-MeOH=1:1, v/v) yielding 4 fractions (F8e1-F8e4). Fr. F8e3 (0.3 g) was repeatedly enriched by Sephadex LH-20 CC (methanol) furnishing compound **3** (28.0 mg). Subfraction F12 (40.6 g) was applied silica gel CC using gradient elution with PE-EtOAc (25:1 to 1:1, v/v) resulting in the isolation of 7 fractions (F12a-F12g). Fr.F12e (8.9 g) was separated by chromatography to a large Sephadex LH-20 column (methanol) giving 6 fractions (F12e1-F12e6). Fr.F12e5 (1.4 g) was again isolated on a Sephadex LH-20 CC (CH_2Cl_2- MeOH= 1:1, v/v), 2 fractions (F12e5a-F12e5b), were obtained. Fr.F12e5a was dissolved in an appropriate amount of methanol, and after part of the methanol was naturally evaporate, crystals were precipitated, which was compound **4** (168.0 mg). Fr.G (440.0 g) was subjected to silica gel CC using CH_2Cl_2- MeOH (800:1 to 10:1, v/v) as eluant, obtaining 8 subfractions (G1-G8). Subfraction G6 (10.9 g) was separated applying silica gel CC with CH_2Cl_2- MeOH (400:1 to 10:1, v/v) gradient to obtian 9 fractions (G6a-G6i). Fr.G6h (6.5 g) was chromatographed on a large Sephadex LH-20 column (methanol) separation yielded 7 fractions (G6h1-G6h7). Fraction G6h3 (2.0 g) was purified multiple times through recrystallization to obtain compound **5** (531.0 mg). Subfraction G7 (20.0 g) was separated on silica gel CC using CH_2Cl_2- MeOH (350:1 to 10:1, v/v), giving 11 fractions (G7a-G7k). Frs.G7g (1.2 g) and G7i (1.3 g) were isolated by Sephadex LH-20 CC (CH_2Cl_2-MeOH=1:1, v/v) to yield 5 fractions (G7g1-G7g5) and 5 fractions (G7i1-G7i5), respectively. Frs.G7g1 (0.1 g) and G7i5 (0.3 g) were concentrated repeatedly by Sephadex LH-20 CC (CH_2Cl_2-MeOH=1:1, v/v) to afford compound **6** (16.8 mg) and compound **7** (73.8 mg), respectively.

(2*S*,3*S*)-5,6-dimethoxy-3-methyl-2-(3′-hydroxyphenyl)-2,3-dihydrobenzofuran (**1**): colorless crystal; UV (MeOH) λ_{max} 212.5 and 297.8 nm; IR (KBr) ν_{max} 3415 2930 1601 1491 1446cm^{-1}; ^{1}H-NMR (DMSO-d_6, 600 MHz) and ^{13}C-NMR (DMSO-d_6, 150 MHz) data, see Table 1; HRESIMS *m/z* measured 285.1128 [M -

H]$^-$, calculated 285.1132[M - H]$^-$.crystal data: $C_{17}H_{18}O_4$, M=286.33, a=10.1481(3) Å, b=7.0868(1) Å, c=10.7578(3) Å, α=90°, β=111.099(3)°, γ=90°, V=721.81(3) Å^3, T=293(2) K, space group P1211, Z=2, μ(Cu Kα)=0.764 mm^{-1}, 5907 reflections measured, 2054 independent reflections (R_{int}=0.0237, R_{sigma}=0.0269). The final R_1 values were 0.0309 ($I>2\sigma(I)$). The final $wR(F^2)$ values were 0.0804 ($I>2(I)$). The final R_1 values were 0.0317 (all data). The final $wR(F^2)$ values were 0.0814 (all data). The goodness of fit on F^2 was 1.081. Flack parameter = -0.2(16). Deposition number: CCDC2333621.



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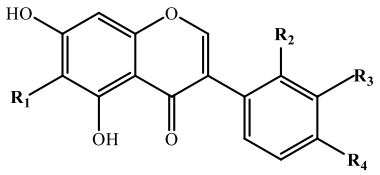
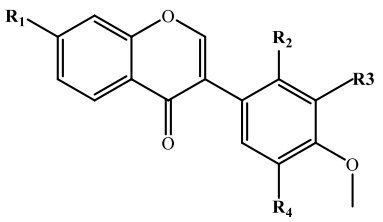


	R_1	R_2	R_3	R_4
3	H	OCH_3	H	OCH_3
4	OCH_3	H	H	OH
7	H	H	OH	OH



	R_1	R_2	R_3	R_4
2	OCH_3	OH	H	H
5	OH	H	OH	H
6	OH	OCH_3	H	OCH_3

Figure 1. structures of compounds **1**-**7**

Tabel 1. ^{1}H (600 MHz) and ^{13}C(151 MHz) NMR data of compound **1** (in DMSO-d_6) [δ (ppm)]

Position	δ_C	δ_H（***J* in Hz**）
1	-	-
2	91.6	5.08（1H，d，8.0）
3	45.4	3.26-3.20（1H，m）
4	109.1	6.85（1H，s）
5	143.4	-
6	149.4	-
7	95.3	6.59（1H，s）
8	152.7	-
9	121.6	-
1′	142.9	-
2′	112.4	6.78（1H，t，2.1）
3′	157.5	-
4′	114.9	6.71（1H，dd，7.6，2.1）
5′	129.6	7.17（1H，t，7.8）
6′	116.3	6.80（1H，d，7.5）
3-CH_3	18.6	1.33（3H，d，6.8）
5-OCH_3	56.6	3.69（3H，s）
6-OCH_3	55.8	3.73（3H，s）
3′-OH	-	9.44（1H，s）

In this study, we isolated and characterized the chemical constituents of the ethyl acetate portion of the heartwood of *D. odorifera*. We obtained a new benzofuran compound and six known compounds (Figure 1). The known compounds were identified as 2'-hydroxy-4',7-dimethoxyisoflavone **(2)** [14] , 2′-methoxybiochanin A **(3)** [15], tectorigenin **(4)** [16], calycosin **(5)** [17] , 7-hydroxy-2',4',5'-

trimethoxyisoflavone **(6)** [18] and orobol **(7)** [19] by comparing the NMR data with those reported in the literature.

Compound **1** is a colorless crystal. Its molecular formula of $C_{17}H_{18}O_4$ from the HRESIMS *m/z*: measured 285.1128 [M - H]-, calculated 285.1132[M - H]-. The ^{1}H-NMR spectrum (Table 1) showed a hydroxyl signal [δ_H 9.44 (1H, s)], two methoxy signals [δ_H 3.73 (3H, s, 6-OCH_3) and 3.69 (3H, s, 5-OCH_3)] and one methyl signal [δ_H 1.33 (3H, d, *J*=6.8, 3-CH_3)]. Analysis of the ^{13}C-NMR spectrum (Table 1) and HSQC spectrum of compound 1 revealed 17 carbons signals, including 12 aromatic carbons (δ_C 157.5, 152.8, 149.4, 143.4, 142.7, 129.6, 121.6, 116.3, 114.9, 112.4, 109.1, 95.3), two methoxy carbons (δ_C 56.6, 55.8), one tertiary carbon (δ_C 45.4), and one methyl carbon (δ_C 18.6). The carbon skeleton of the compound is similar to pterolinuses C reported in the literature (Table S1, see Supporting Information)[20]. After comprehensive analysis, it is preliminarily inferred that the compound is a dihydrobenzofuran compound with one hydroxyl, one methyl, and two methoxy substituents.

In the HMBC (Figure 2) profile of compound **1**, the correlations between H-4 (δ_H 6.85) and C-3 (δ_C 45.4)/C-6 (δ_C 149.4)/C-8 (δ_C 152.7) as well as between -OCH_3 (δ_H 3.73) and C-6 (δ_C 149.4) indicated the positions of the methoxyl (δ_H 3.73, δ_C 55.8) is at the C-6 position. Correlations between H-7 (δ_H 6.59) and C-5 (δ_C 143.4)/C-9 (δ_C 121.6), as well as between -OCH_3 (δ_H 3.69) and C-5 (δ_C 143. 4), revealed that methoxy (δ_H 3.69, δ_C 56.6) in the C-5 position; H-3 (δ_H 3.23) correlates with C-1′ (δ_C 142.7)/C-8 (δ_C 152.7), while 3-CH_3 (δ_H 1.33) correlates with C-2 (δ_C 91.6)/C-9 (δ_C 121.6), inferring that the methyl group (δ_H 1.33, δ_C 18.6) is at the C-3 position. Correlations from H-3' (δ_H 9.44) to C-2' (δ_C112.4)/C-4' (δ_C 114.9)/C-3' (δ_C 157.5) and H-2' (δ_H 6.78) to C-2 (δ_C 91.6)/C-6' (δ_C 116.3) were observed in the HMBC spectrum; Meanwhile the ^{1}H-^{1}H COSY spectrum (Figure 2) shows H-4' (δ_H 6.71)/H-5' (δ_H 7.17)/H-6' (δ_H 6.80) correlations; Based on these three pieces of evidence, we can determine that the hydroxyl group is located at C-3′ as well as at the position corresponding to these four protons. Finally, the single-crystal X-ray diffraction analysis of compound 1 (CCDC:2333621) determine that the absolute configuration of the compound is 2*S*, 3*S* (Figure 3).

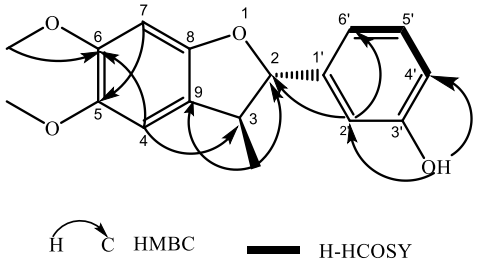


Figure 2. Key HMBC and COSY correlations of **1**

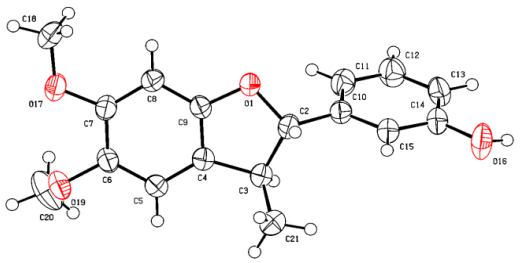


Figure 3. Ellipsoid diagram of **1** (ellipsoidal ratio of 50%)

The cytotoxic effects of compounds **1-7** on H9c2 cells were determined using the CCK-8 method. The IC_{50} of compounds **1-7** were all greater than 200 μM, indicating that none of the compounds had a significant cytotoxic effect on H9c2 cells. In addition, we investigated the protective effect of all identified compounds against H/R induced H9c2 cell damage[21]. The cell survival rates of compounds **1-7** in the range of 10~40, 5~40, 5~40, 5~40, 5~40, 5~40 and 5~10 μM were all statistically significantly different compared to the model group ($P < 0.05$), demonstrating that compounds **1-7** have a protective effect against H9c2 hypoxia-reperfusion injury (Figure 4 and Figure S31, see Supporting Information).

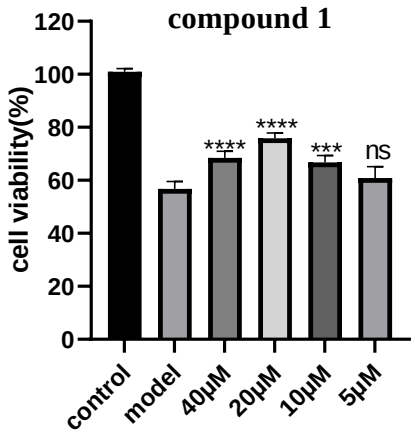


Figure 4. Protective effects of compounds 1 on H/R induced injury in H9c2. Values are expressed as the mean ± SD of 4 replicates; **P* < 0.05, ***P* < 0.01, ****P* <0.001 *****P* <0.0001 versus model group cell.

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Supporting Information

Supporting information accompanies this paper on http://www.acgpubs.org/journal/records-of-natural-products

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