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Chemical Constituents from *Typhonium giganteum* Rhizome and Their Antioxidant, Tyrosinase Inhibitory Activities

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Abstract: Fourteen constituents were isolated from the rhizome of *Typhonium giganteum*, which included one new compound, bis(2-ethylbutyl) terephthalate (**1**), along with thirteen known compounds (**2**–**14**). The structures of these compounds were elucidated by physical data analyses such as 1D NMR, 2D NMR and HR-ESI-MS. All compounds were tested for their antioxidant and tyrosinase inhibitory activities. Compounds **2**, **5** exhibited obvious DPPH radical scavenging activities, while compounds **2**, **5**, **8**, and **14** showed significant tyrosinase inhibitory activities.

Keywords: *Typhonium giganteum*; terephthalate; DPPH; tyrosinase.

1. Introduction

Typhonium giganteum, a plant belonging to the genus Typhonium of the family Araceae, is widespread in China, Malaysia and India. Its rhizome, known as *Baifuzi*, has been recorded in the Chinese Pharmacopoeia for the treatment of epilepsia, tetanus, and stroke [1]. Previous phytochemical studies on the rhizome of *T. giganteum* have revealed the presence of essential oils, organic acids, amino acids, nucleosides, and cerebrosides [2–6]. It has been reported that the extract from *T. giganteum* showed obvious tyrosinase inhibitory activity, and thus commonly used as skin-whitening agents since ancient times in China [7–8]. However, the exact material basis behind remains uncertain. In the course of screening for biologically novel antityrosinase agents from *T. giganteum*, one new compound (**1**) together with thirteen known compounds (**2**–**14**) were isolated (Figure 1). In the subsequent antioxidant and anti-tyrosinase assays, compounds **2** and **5** exhibited obvious DPPH radical scavenging activities, while compounds **2**, **5**, **8**, and **14** showed strong tyrosinase inhibitory activities. Herein, the purification, structure characterization, antioxidant and tyrosinase inhibitory evaluation of these compounds are reported.

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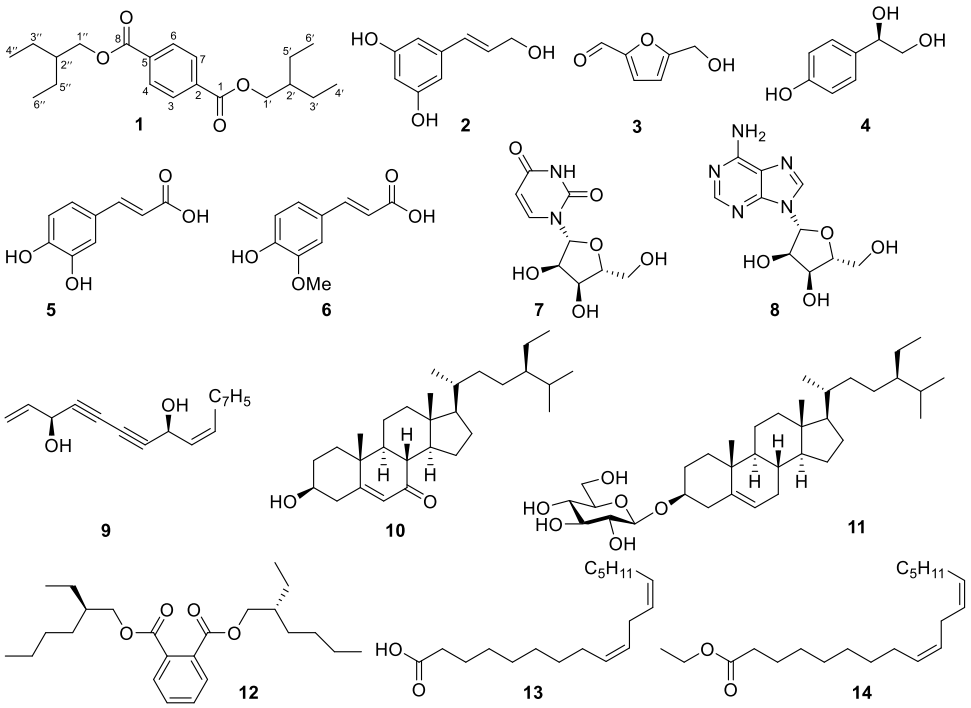

Figure 1. Structures of compounds **1**–**14**

2. Materials and Methods

2.1. General Experimental Procedures

IR and UV spectra were determined using FTIR-650 and Puxi TU-1950 instruments, respectively. NMR spectra were recorded on a Bruker AM-400 spectrometer. High-resolution electrospray ionization mass spectrum (HR-ESI-MS) was carried out on a Waters Xevo G2-XS QTof spectrometer. Column chromatography (CC) was performed using silica gel (Qingdao Marine Chemical Inc., China), ODS (50 μm, Fuji Silysia Chemical Ltd., Japan), and Sephadex LH-20 (GE Healthcare Bio-Sciences AB, Sweden). TLC was performed with silica gel 60 F254 (Yantai Chemical Industry Research Institute).

2.2. Plant Material

The fresh rhizome of *Typhonium giganteum* was collected in Yuzhou, China, in October 2019, and identified by Prof. Lin Yang at Lanzhou University of Technology. A voucher specimen (SPH2019B) was deposited in the herbarium of Food and Pharmacy College, Xuchang University.

2.3. Extraction and Isolation

The air-dried rhizome of *Typhonium giganteum* (9.8 kg) was extracted with 95% EtOH at room temperature to afford a crude extract of 503 g after removal of the solvent. The extract was then suspended in H_2O and extracted with EtOAc and *n*-BuOH, respectively. The EtOAc soluble portion (125 g) was subjected to silica gel CC using petroleum ether–EtOAc (80:1 to 1:2) as eluent to give six fractions F1–F6. Fraction F1 (13.4 g, eluted by petroleum ether–EtOAc 80:1) was subsequently purified using RP-C_{18} CC (MeOH–H_2O, 80:20 to 100:0) to afford five subfractions (F1-1 to F1-5). After purification with Sephadex LH-20 column (CH_2Cl_2), subfraction F1-1 gave compounds **3** (21.3

mg) and **10** (5.7 mg), subfraction F1-3 gave compounds **1** (22.8 mg) and **11** (16.8 mg). Subfraction F1-5 was subjected to a RP-C_{18} CC eluted with MeOH-H_2O (90:10), giving compound **14** (16.8 mg). Fraction F2 (8.3 g, eluted by petroleum ether–EtOAc 50:1) was further separated using Sephadex LH-20 column (CH_2Cl_2-MeOH 1:1) to give six subfractions (F2-1 to F2-6). Compounds **9** (11.3 mg), **2** (19.4 mg) were obtained from F2-1, F2-5 by silica gel CC, respectively. Fraction F3 (5.6 g, eluted by petroleum ether–EtOAc 20:1) was passed through a RP-C_{18} CC eluted with MeOH-H_2O (70:30) to give four subfractions (F3-1 to F3-4). Subfraction F3-1 was chromatographed on a silica gel CC to give compounds **4** (14.2 mg) and **13** (10.6 mg). Subfraction F5 (34.6 g, eluted by petroleum ether–EtOAc 10:1 to 1:2) was purified by Sephadex LH-20 column (CH_2Cl_2–MeOH 1:1) to give eight subfractions (F5-1 to F5-8). Compound **12** (15.7 mg) was separated from F5-1 by RP-C_{18} CC eluted with MeOH-H_2O (60:40). After further purification with silica gel CC, subfraction F5-5 gave compounds **6** (15.1 mg) and **7** (6.7 mg), subfraction F5-7 gave compounds **5** (8.4 mg) and **8** (5.2 mg).

*Bis(2-ethylbutyl) terephthalate (**1**):* Colorless oil. IR (KBr) ν_{max} 2965, 2933, 1720, 1270, 1116, 1103, 1020, 757, 732 cm^{-1}. UV λ_{max} (MeOH) nm (log ε): 244 (3.8). HRESIMS *m/z* 333.2062 $[M - H]^-$ (calcd for $C_{20}H_{29}O_4$, 333.2066). ^1H NMR (400 MHz, $CDCl_3$): δ_H 8.07 (4H, br s, H-3, H-4, H-6, H-7), 4.23 (4H, m, H_2-1', H_2-1"), 1.65-1.62 (2H, m, H-2', H-2"), 1.46-1.39 (8H, m, H_2-3', H_2-5', H_2-3", H_2-5"), 0.92 (12H, t, J = 7.6 Hz, H_3-4', H_3-6', H_3-4", H_3-6"); ^{13}C NMR (100 MHz, $CDCl_3$): δ_C 166.1 (C-1, C-8), 134.4 (C-2, C-5), 129.6 (C-3, C-4, C-6, C-7), 67.5 (C-1', C-1"), 40.6 (C-2', C-2"), 23.7 (C-3', C-5', C-3", C-5"), 11.3 (C-4', C-6', C-4", C-6").

2.4. DPPH Radical Scavenging Assay

DPPH radical scavenging assay was conducted according to literature procedure with slight modification [9]. Briefly, 20 μL of a sample solution (in DMSO, 1 mM) and 180 μL of 0.2 mM DPPH methanol solution were added to 96-well microplate. L-ascorbic acid was used as the positive control. Mixtures were then incubated for 30 min in the dark, and antioxidant activities were determined by measuring absorbances at 517 nm using a Multiskan FC microplate reader. Experiments were performed in triplicate. The DPPH radical scavenging activity was calculated by: Radical scavenging activity (%) =$[1 - (As/Ac)] \times 100$, where Ac is the absorbance of the non-treated control and As is the absorbance of tested compound.

2.5. Mushroom Tyrosinase Inhibition Assay

The mushroom tyrosinase inhibition activities of compounds **1**–**14** were tested according to the literature procedure [10]. Compounds (**1**–**14**, 10 μL, 25 μM) and mushroom tyrosinase (20 μL, 1000 U/mL) (Psaitong, China) in a potassium phosphate buffer (50 mM, pH 6.5) were added to 170 μL of an assay mixture containing a 10 : 10 : 9 ratio of L-tyrosine solution (1 mM), potassium phosphate buffer (1 mM), and distilled water in a 96-well microplate. The reaction mixture was incubated at 37 °C for half an hour. Percentage tyrosinase inhibition was determined by measuring optical densities at 450 nm using a Multiskan FC microplate reader (Thermo Fisher Scientific, Inc.). Kojic acid (25 μM) was chosen as the positive control. Three independent experiments were repeated. The % inhibition was determined by $[1 - (As/Ac)] \times 100$, where As is the absorbance of tested compound and Ac the non-treated control. Statistical analysis was determined using GraphPad Prism 5 software, and the results were expressed as means ± SEMs. The inhibitory rate > 5% is considered active.

3. Results and Discussion

3.1. Structure Elucidation

Compound **1** was isolated as a colorless oil. Its molecular formula $C_{20}H_{30}O_4$, with six degrees of unsaturation, was established based on its quasi-molecular ion peak at *m/z* 333.2062 $[M - H]^-$ (calcd for $C_{20}H_{29}O_4$, 333.2066) in the HR-ESI-MS spectrum. The ^1H NMR spectrum of **1** revealed the existence of four aromatic protons at δ_H 8.07 (4H, br s, H-3, H-4, H-6, H-7), two methine protons at δ_H

1.65-1.62 (2H, m, H-2', H-2"), twelve methylene protons at δ_H 4.23 (4H, m, H_2-1', H_2-1"), 1.46-1.39 (8H, m, H_2-3', H_2-5', H_2-3", H_2-5"), and four methyls at δ_H 0.92 (12H, t, J = 7.6 Hz, H_3-4', H_3-6', H_3-4", H_3-6"). Detailed NMR data analysis indicated the compound to be bis(2-ethylbutyl) terephthalate, which was confirmed by the ^{1}H-^{1}H COSY correlations of H-4'/H-3'/H-2'/(H-1')/H-5'/H-6', H-4"/H-3"/H-2"/(H-1")/H-5"/H-6", and the key HMBC correlations from H-1' to C-1, and H-1" to C-8 (Figure 2). On the basis of detailed 2D NMR analysis, the structure of **1** was determined as bis(2-ethylbutyl) terephthalate.

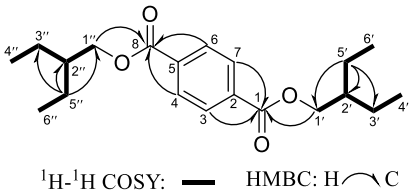

Figure 2. Key ^{1}H-^{1}H COSY and HMBC correlations for compound **1**

On the basis of detailed NMR spectroscopic analysis and comparison with those reported data in the literatures, the known compounds **2**−**14** were identified as caffeoyl alcohol (**2**) [11], 5-hydroxymethylfurfural (**3**) [12], phomoaspardiol (**4**) [13], caffeic acid (**5**) [14], ferulic acid (**6**) [15], uridine (**7**) [16], adenosine (**8**) [17], falcarindiol (**9**) [18], 3*β*-hydroxystigmast-5-en-7-one (**10**) [19], daucosterol (**11**) [20], di-(2-ethylhexyl) phthalate (**12**) [21], linoleic acid (**13**) [22], and ethyl linoleate (**14**) [23], respectively. Compounds **1**−**2**, **4**, **9** and **12** were reported from *T. giganteum* for the first time, which would enrich our knowledge about the chemical diversity of *T. giganteum.*

3.2. Antioxidant and Tyrosinase Inhibitory Activities

All the isolated compounds (**1**–**14**) were tested for their DPPH radical scavenging activities, with L-ascorbic acid as the positive control. As shown in Table 1, compounds **2** and **5** exhibited obvious DPPH radical scavenging activities: 46.86±1.11% for **2**; 57.69±0.88% for **5**; 71.01±0.66% for L-ascorbic acid. According to the previous reports [24–25], the DPPH radical scavenging activities of **2** and **5** might be ascribed to the hydroxyl substituents at their aromatic structures.

Table 1. DPPH radical scavenging activity of compounds **1**–**14** and L-ascorbic acid[a]

Compound	DPPH radical scavenging activity (%)	Compound	DPPH radical scavenging activity (%)
1	14.99±1.58	**9**	16.31±1.78
2	46.86±1.11	**10**	11.70±1.61
3	18.33±2.26	**11**	13.55±1.69
4	20.00±1.86	**12**	12.51±1.63
5	57.69±0.88	**13**	14.24±1.92
6	17.75±1.53	**14**	15.85±1.60
7	17.46±1.52	L-ascorbic acid	71.01±0.66
8	17.00±1.63		

[a] The radical scavenging effects of compounds **1**–**14** and L-ascorbic acid were measured at a concentration of 1 mM. Three independent experiments were performed and results were expressed as means ± SEMs.

At a concentration of 25 μM, the mushroom tyrosinase inhibitory activities of compounds **1**–**14** and kojic acid (positive control) were evaluated (Table 2). However, only compounds **2**, **5**, **8** and **14** showed obivious tyrosinase inhibition activities. Just as Pillaiyar's group has reported [26], entities with strong tyrosinase inhibitory activity usually contained several aromatic hydroxyl (**2**, **5**) or amino groups (**8**). These compounds may be responsible for the skin-whitening function of the extract of *Typhonium giganteum* rhizome.

Table 2. Inhibitory effects of compounds **2**, **5**, **8**, **14** and of kojic acid on mushroom tyrosinase[a]

Compound	Tyrosinase inhibition (%)
2	14.64±0.92
5	21.31±1.01
8	11.12±1.22
14	15.69±0.67
kojic acid (positive control)	25.29±0.86

[a] Tyrosinase inhibitions were measured at a derivative concentration of 25 μM, with L-tyrosine as the substrate. Results were expressed as means ± SEMs.

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Supporting Information

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