SHORT REPORT

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A New Diarylbenzophenone from *Selaginella tamariscina*

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Abstract: A new diarylbenzophenone, namely selagibenzophenone C (**1**), along with 1,3-di-*p*-hydroxyphenyl-4-penten-1-one, (**2**) and unciflavone E (**3**) were isolated from *Selaginella tamariscina*. Their structures were elucidated on the basis of spectroscopic and mass spectrometric analyses, including 1D-, 2D-NMR and HRESIMS. Compound **1** represents the first example of naturally occurring diarylbenzophenone. Compound **2** was firstly isolated from the genus of *Selaginella*.

Keywords: *Selaginella*; *Selaginella tamariscina*; diarylbenzophenone; selagibenzophenone C; norlignan.

1. Plant Source

The whole herbs of *Selaginella tamariscina* was collected from ShaoYang City, Hunan Province, R. P. China, on September, 2012 and identified by Prof. Jin-Ping Li (XiangYa School of Pharmaceutical Sciences, Central South University, China). The voucher specimen (JB-005) was deposited in Laboratory of Phytochemistry, School of Medicine, Hunan Normal University.

2. Previous Studies

Selaginella tamariscina, a species of *selaginella*, was included in the 2015 edition of Chinese pharmacopoeia [1], which has been used a traditional herb for the treatment of thrombocytopenic purpura, chronic hepatitis, hyperglycemia, amenorrhea, metrorrhagia and inflammation [2].

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Pharmacological studies showed it exhibited good bioactivity such as anti-cancer [3-5], anti-inflammatory effect [6,7], inhibitory effects on PTP1B enzyme [8,9] and acts as a potent inhibitor to phosphodiesterase-4 (PDE4) [10]. The phytochemical researches revealed flavonoids [7,9], lignans [11], and selaginellins [12,13] as the main chemical constituents of *S. tamariscina*.

3. Present Study

The air-dried *S. tamariscina* (30 kg) was extracted with 70% EtOH (10 L × 2 h × 2 times). The extract was concentrated to 5 L and suspended in H_2O (5 L) and partitioned successively with petroleum ether, EtOAc and n-BuOH. The n-BuOH extract was subjected to a macroporous resin (HPD-100) column with EtOH-H_2O gradient elution (30%, 50%, 70%, 95%) to obtain 4 fractions (A-D). The 50% portion (B) was subjected to a silica gel column (200-300 mesh) eluted with CH_2Cl_2/MeOH (from 100:0 to 0:100, V/V) to obtain sixty fractions (*Fr*. B1-B60). *Fr*.B12-B15 was further separated via ODS silica gel column eluted with MeOH-H_2O (60%, 70%, 80%, 90%, 100%) to provide 92 subfractions (S-*Fr*. 1-95). The S-*Fr*. 80 was purified with semi-Preparative HPLC to give **2**. The fractions of B26-B32 was separated by ODS silica gel column with gradient elution of MeOH-H_2O (60%, 70%, 80%, 90%, 100%) to provide 90 subfractions (S-*Fr*. 1-140). The S-*Fr*. 31 was further purified with semi-preparative HPLC to obatain **3**. The S-*Fr*. 72-73 was further purified with semi-Preparative HPLC to obatain **1** (Figure 1).

Selagibenzophenone C ***(1)****:* Red powder. ^{1}H-NMR and ^{13}C-NMR (CD_3OD) see Table 1; HR-ESI-MS calcd for $C_{25}H_{19}O_4$ $[M + H]^+$ *m/z*: 383.1286; found *m/z*:383.1283.

1,3-Di-p-hydroxyphenyl-4-penten-1-one ***(2)****:* White powder. ^{1}H-NMR (CD_3OD, 400 MHz) δ_H 3.30 (2H, m, H-2), 3.93 (1H, dd, *J* = 7.2, 15.2 Hz, H-3), 6.02 (1H, m, H-4), 4.98 (2H, m, H-5), 7.86 (2H, d, *J* = 8.4 Hz, H-2′,6′), 6.83 (2H, d, *J* = 8.4 Hz, H-3′,5′), 7.06 (2H, d, *J* = 8.5 Hz, H-2″,6″), 6.71 (2H, d, *J* = 8.5 Hz, H-3″,5″); ^{13}C-NMR (CD_3OD, 100 MHz) δ_C 199.9 (C-1), 44.7 (C-2), 46.1 (C-3), 142.9 (C-4), 114.2 (C-5), 130.3 (C-1′), 130.5 (C-2′,6′), 116.2 (C-3′,5′), 163.8 (C-4′), 135.2 (C-1″), 129.7 (C-2″,6″), 116.2 (C-3″,C-5″), 156.8 (C-4″).

Unciflavone E ***(3)****:* Yellow powder. ^{1}H-NMR (DMSO-d_6, 400 MHz) δ_H 6.76 (1H, s, H-3), 6.24 (1H, s, H-6), 7.60 (2H, d, *J* = 8.4 Hz, H-2′,6′), 6.75 (2H, d, *J* = 8.4 Hz, H-3′,5′), 6.98 (1H, d, *J* = 8.4 Hz, H-3″), 7.87 (1H, dd, *J* = 2.4, 8.4 Hz, H-4″), 7.96 (1H, d, *J* = 2.4 Hz), 2.47 (3H, s, 8″-CH_3); ^{13}C-NMR (DMSO-d_6, 100 MHz) δ_C 163.5 (C-2), 102.8 (C-3), 182.2 (C-4), 160.8 (C-5), 100.2 (C-6), 165.9 (C-7), 105.6 (C-8), 154.9 (C-9), 103.2 (C-10), 120.8 (C-1′), 128.6 (C-2′,6′), 116.1 (C-3′,5′), 161.3 (C-4′), 120.7 (C-1″), 162.6 (C-2″), 116.1 (C-3″), 130.1 (C-4″), 129.8 (C-5″), 134.2 (C-6″), 196.4 (C-7″), 26.7 (C-8″).

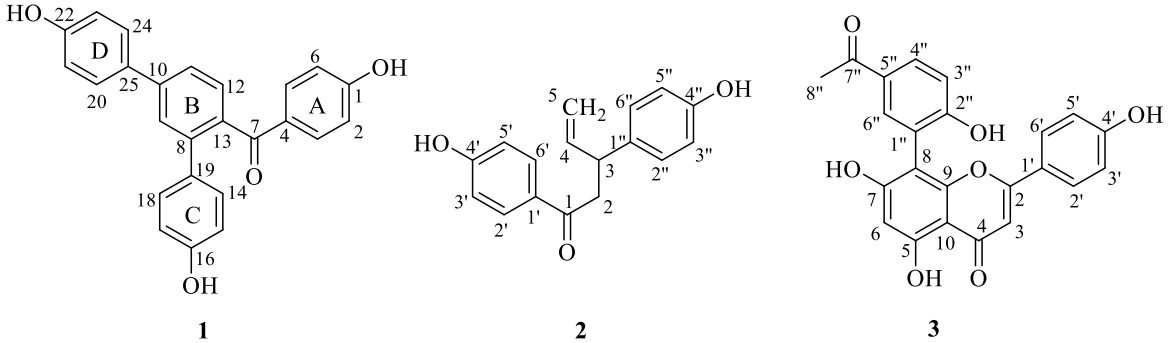

Figure 1. Structures of compounds **1**-**3**

Compound **1** was obtained as red powder, which showed an ion peak at *m/z* 383.1286 $[M + H]^+$ in HR-ESI-MS indicating its molecular formula of $C_{25}H_{18}O_4$ (calcd. for $C_{25}H_{19}O_4$: 383.1283),

indicating 17 degrees of unsaturation. The ^{1}H NMR spectrum of **1** displayed the NMR features for a 1,2,4-tri-substituted benzene ring [δ_H 7.62 (1H, d, J = 1.8 Hz), 7.64 (1H, dd, J = 7.8, 1.8 Hz), 7.45 (1H, d, J = 7.8 Hz)] which was confirmed by the correlations between H-11 and H-12 in the COSY spectrum, three *para*-substituted benzene rings as AA′BB′ coupling system corresponding to twelve methines which was also confirmed by HSQC spectrum. Except for 16 degrees of unsaturation for the four benzene rings, the structure of **1** still remains one degree of unsaturation. The ^{13}C NMR spectrum displayed the presence of twenty-four aromatic carbons consisting of six carbon signals for the 1,2,4-tri-substituted benzene ring and eighteen carbon signals for three *para*-substituted benzene rings, and one ketone carbon signal corresponding to one degree of unsaturation.

The HMBC spectrum of **1** showed (Figure 2) correlations from δ_H 7.55 [H-C(3,5)] to δ_C 163.9 (C-1), from δ_H 7.14 [H-C(14,18)] to δ_C 158.0 (C-16), and from δ_H 6.59 [H-C(20,24)] to δ_C 158.8 (C-22) suggested that the three *para*-substituted benzene rings (rings A, C and D) are *p*-hydroxyphenyl. The HMBC correlations from δ_H 7.55 [H-C(3,5)], 7.45 [H-C(12)] to δ_C 200.2 (C-7), from δ_H 6.70 [H-C(2,6)] to δ_C 130.3 (C-4), and from δ_H 7.62 [H-C(9)], 7.64 [H-C(11)] to δ_C 138.3 (C-13) indicated rings A and B established a mother nucleus structure of benzophenone. The HMBC corelations from δ_H 7.62 [H-C(9)], 6.67 [H-C(15,17)] to δ_C 133.1 (C-19), and from δ_H 7.14 [H-C(14,18)], 7.45 [H-C(12)] to δ_C 142.6 (C-8), suggested that ring C was linked to the C-8 of ring B. Furthermore, the HMBC correlations from δ_H 7.62 [H-C(9)], 7.64 [H-C(11)] and 6.92 [H-C(21, 23)] to δ_C 132.6 (C-25), and from δ_H 6.59 [H-C(20,24)], 7.45 [H-C(12)] to δ_C 144.3 (C-10), suggested ring D was linked to the C-10 of ring B. Thus, compound **1** was elucidated as (4,4"-dihydroxy-[1,1':3',1"-terphenyl]-4'-yl)(4-hydroxyphenyl)methanone, a new diarylbenzophenone named as selagibenzophenone C. To the best of our knowledge, compound **1** is the first example of diarylbenzophenone as natural sources.

Table 1. ^{1}H NMR (400 MHz) and ^{13}C NMR (100 MHz) data for **1** in CD_3OD (δ in ppm)

No.	δ_C[a]	δ_H (mult., J in Hz)
1	163.9, C	
2,6	116.0, CH	6.70 (1H, d, J = 8.4 Hz)
3,5	133.8, CH	7.55 (1H, d, J = 8.4 Hz)
4	130.3, C	
7	200.2, C	
8	142.6, C	
9	128.7, CH	7.62 (1H, d, J = 1.8 Hz)
10	144.3, C	
11	125.4, CH	7.64 (1H, dd, J = 7.8, 1.8 Hz)
12	130.1, CH	7.45 (1H, d, J = 7.8 Hz)
13	138.3, C	
14,18	131.2, CH	7.14 (2H, d, J = 8.4 Hz)
15,17	116.1, CH	6.67 (2H, d, J = 8.4 Hz)
16	158.0, C	
19	133.1, C	
20,24	129.3, CH	6.59 (2H, d, J = 8.4 Hz)
21,23	116.8, CH	6.92 (2H, d, J = 8.4 Hz)
22	158.8, C	
25	132.6, C	

[a]Multiplicities inferred from HSQC experiments.

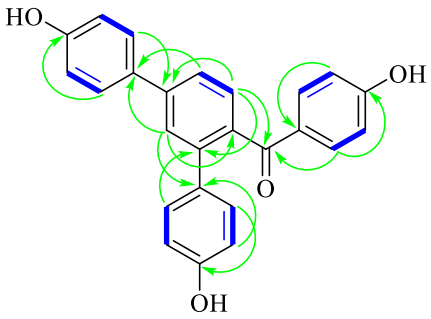

Figure 2. Key ^{1}H-^{1}H COSY (▬) and HMBC (⟶) correlations for compound **1**

The two known compounds were identified as 1,3-di-p-hydroxyphenyl-4-penten-1-one (**2**) [14] and unciflavone E (**3**) [15] by comparing their NMR data with those reported in the literature. Compound **2** is a norlignan which was firstly isolated from this genus.

In the genus of *Selaginella*, two species (*S. tamariscina* and *S. pulvinata*) attracted more attention for its pharmacologic action and structural diversity of secondary metabolites. In Chinese, these two species are often replaced by each other. Many studies revealed that they have a lot of the same structure types of their chemical constituents. In previous studies, the first natural source of triarylbenzophenone was isolated from *S. pulvinata* [16], following that we reported the second one from *S. tamariscina* [17]. We carry out a continued study on the bioactive secondary metabolite of *S. tamariscina*, and the first naturally occurring diarylbenzophenone was found in our present study. Our study added new structure type to this plant. Maybe more of such compounds will be isolated from *Selaginella*.

The natural products with basic structure of benzophenone were isolated from many plants and exhibited cytotoxic and antibacterial activity [18-20]. In terms of biosynthetic pathway, we suspected that diarylbenzophenones and triarylbenzophenones are synthesis via the cross-coupling reactions base on the benzophenone. Nawaz MA., et al [21] reported a synthetic method of diarylbenzophenones based on site-selective suzuki cross-coupling reaction. Khera R.A., et al. [22] reported the suzuki and sonogashira cross-coupling reactions to synthesize diarylbenzophenones.

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Supporting Information

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