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Communication

Anti-inflammatory spirobisnaphthalene natural products from a plant-derived endophytic fungus *Edenia gomezpompae*

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ABSTRACT

Spirobisnaphthalenes comprise a relatively rare family of natural products that are normally isolated from fungi and occasionally from plants. Here we reported the discovery of seven new preussomerin-type spirobisnaphthalenes, preussomerins YT1-YT7 (**1**-**7**), and seven known ones (**8**-**14**), from the endophytic fungus *Edenia gomezpompae*, enriching the structural diversity of this family of natural products. Their structures were established by 1D and 2D NMR spectroscopy, HRESIMS analysis and comparison with previously reported compounds, with the absolute configurations of compounds **1** and **2** being further confirmed by single-crystal X-ray diffraction using Cu Kα radiation. The anti-inflammatory activities of all isolates were assessed by measuring the production of NO in LPS-induced RAW264.7 macrophage cells. Among them, compounds **8** and **13** exhibited potent inhibitory activities on the production of NO, with IC_{50} values of 2.61 and 1.32 μmol/L, respectively.

Spirobisnaphthalenes are a growing family of rare natural products containing two naphthalene-derived C10 units bridged through a spiroketal linkage [1]. Members of this family include preussomerins, decaspirones, palmarumycins and spiro-nonadienes which have been mainly isolated from fungi and although occasionally from plant sources [1–3]. The preussomerins belong to a relatively rare class of spirobisnaphthalene possessing a unique structural feature. They consist of two unsaturated bicyclo [4.4.0]decane units connected via three oxygen bridges through two spiroketal carbons [1]. This class of compounds have been reported to possess a variety of biological activities such as antifungal, antibacterial, cytotoxic, anti-inflammatory and anti-leishmanial activities [2,4–6]. Microbes derived from unexplored or underexplored ecological niches are becoming to be a potential source for discovering structurally interesting and biologically active natural products [7–10]. During our ongoing search for new bioactive metabolites from plant endophytic fungi [11–13], a fungus strain *E. gomezpompae* was isolated from an unidentified plant collected from Hainan Province of China. LC–MS analysis of its metabolites revealed a series of compounds with molecular weights ranging from 300 to 400 Dalton and a common maximum UV absorption at around 225 nm. Subsequent chemical investigation of this fungus afforded seven new preussomerin-type spirobisnaphthalenes, preussomerins YT1-YT7 (**1**-**7**), and seven known compounds, including preussomerin EG1 (**8**) [14], preussomerin EG2 (**9**) [14], preussomerin EG4 (**10**) [15], palmarumycin SA1 (**11**) [2], MB7056B (II) (**12**) [16], CJ-12,371 (**13**) [17], and palmarumycin CP17 (**14**) [18] (Fig. 1). In this paper, we report the details of isolation, structural elucidation, and anti-inflammatory activity of all isolates (**1**-**14**).

A 40-liter fermentation of the fungus *E. gomezpompae* was conducted and the broth was filtered and extracted with ethyl acetate. The afforded extract was subjected to silica gel column, Sephadex LH-20 column, reversed-phase ODS column chromatography and further purified by reversed-phase HPLC with a gradient CH_3CN-H_2O system to afford compounds **1**–**14**.

Preussomerin YT1 (**1**) was obtained as pale yellow crystals, and the molecular formula was determined to be $C_{22}H_{20}O_7$, corresponding to the $[M+Na]^+$ ion at *m*/*z* 419.1133 in its HRESIMS,

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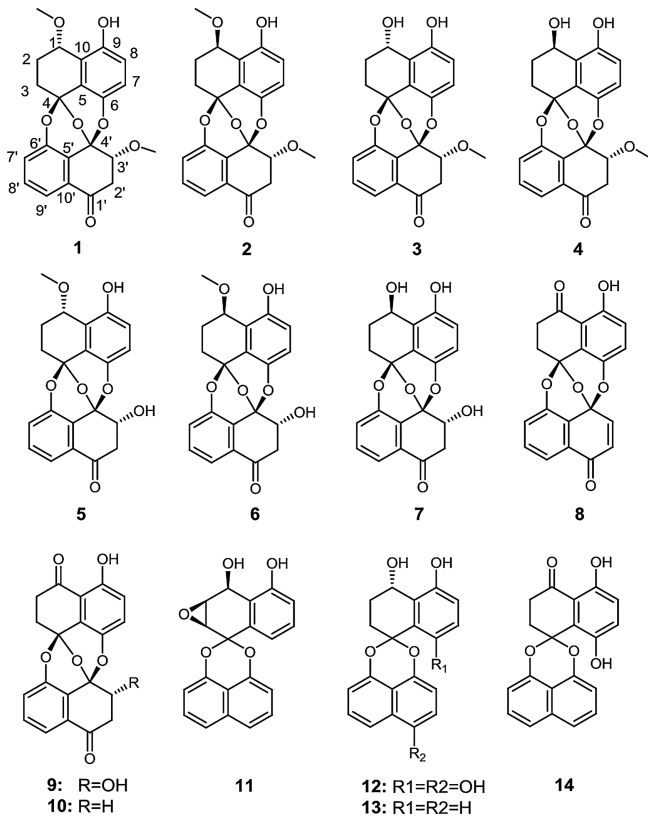

Fig. 1. Structures of compounds **1**–**14.**

requiring thirteen degrees of unsaturation. The NMR spectra contained characteristic signals for a trisubstituted aromatic ring [δ_H 7.09 (d, J =8.1 Hz, H-7′), 7.40 (t, J =8.0 Hz, H-8′) and 7.50 (d, J =7.6 Hz, H-9′); δ_C 121.0 (C-5′), 151.2 (C-6′), 121.0 (C-7′), 130.4 (C-8′), 118.8 (C-9′) and 130.8 (C-10′)], a tetrasubstituted aromatic ring [δ_H 6.68 (d, J =8.8 Hz, H-7) and 6.78 (d, J =8.8 Hz, H-8); δ_C 118.5 (C-5), 141.6 (C-6), 116.0 (C-7), 118.1 (C-8), 151.0 (C-9), and 122.2 (C-10)] and one phenolic group [br s, δ_H 8.19 (9-OH)] (Tables 1 and 2). Analysis of the ^{13}C NMR and HSQC spectra revealed two methoxyl groups at δ_C 56.3 and 58.5, one carbonyl carbon of a ketone at δ_C 193.3, two spiroketal carbons at δ_C 94.1 and 94.9, three methylenes at δ_C 40.6, 22.3 and 28.6 and two oxygenated methines at δ_C 71.6 and 79.6 (Table 2). The partial structures H (1)–H (2)–H (3), H (7)–H (8), H (2′)– H (3′) and H (7′)–H (8′)–H (9′) were established by their ^{1}H–^{1}H COSY correlations (Fig. 2). The key HMBC correlations from H-1 to C-2, C-3, C-5, C-9 and C-10; from H-2 to C-4; and from H-3 to C-4 and C-5 further confirmed the partial structure a (Fig. 3). Similarly, the partial structure b (Fig. 3) was verified by the HMBC correlations of H-9′ with C-1′ and of H-2′, H-3′, H-7′ and H-9′ with C-4′. Clearly, there were two unsaturations left. Thus, substructures a and b must be combined by three oxygens and the structure of **1** could be tentatively determined as shown in Fig. 1. The observation of the coupling constants of 2.4 Hz and 4.6 Hz between H-1 and H-2 and the coupling constants of 2.8 Hz between H-2′ and H-3′ proved that H-1 and H-3′ both occupied pseudoequatorial orientation. Ultimately, these deductions were strongly proven by single-crystal X-ray diffraction experiment using Cu Kα radiation with the absolute configurations being determined as 1*S*, 4*R*, 3′*R*, 4′*S* (Fig. 4).

Preussomerin YT2 (**2**) was isolated as pale yellow crystals and had the same molecular formula of $C_{22}H_{20}O_7$ as that of preussomerin YT1 (**1**) deduced from HRESIMS and NMR data (Tables 1 and 2). Analysis of the ^{1}H, ^{13}C NMR, ^{1}H-^{1}H COSY, HSQC and HMBC spectra of **2** revealed that all structural units appeared identical with those of **1**. Thus, compound **2** should have the same planar structure as **1** based on the above data. However, compounds **1** and **2** exhibited an epimeric relationship at a center, which is further supported by a comparison of their notable chemical shifts of the asymmetric center C-1. Meanwhile, the large $^3J_{1H,2H}$ of 8.0 Hz suggested the methoxy group at C-1 adopted the pseudoaxial position. Finally, the absolute configuration of **2** was confirmed as 1*R*, 4*R*, 3′*R*, 4′*S* by single-crystal X-ray diffraction using Cu Kα radiation (Fig. 4), which only differs from **1** at C-1.

Preussomerin YT3 (**3**) and preussomerin YT4 (**4**) were determined to have the same molecular formula of $C_{21}H_{18}O_7$ on the basis of HRESIMS (m/z 405.0953 $[M + Na]^+$). They had characteristic bis-spirobisnaphthalene NMR signals as found in preussomerin YT1 (**1**). Their NMR spectra closely resembled those of **1** except the absence of the methoxy group on C-1 in **1**. Furthermore, the presence of a hydroxyl group [δ_H 4.79 (d) and 5.34 (d)] instead of a methoxyl group at C-1 established a downfield shift of H-1 (δ_H 5.20, dd, J = 3.2, 6.4 Hz and 5.14, t, J = 7.3, 9.3 Hz) and a upfield shift of C-1 (δ_C 63.0 and 67.2). Their structures were confirmed by ^{1}H-^{1}H COSY correlations of H-1 with H-2 and of H-1 with OH-1 and the key HMBC correlations of OH-1 with C-1, C-2, C-10 (Fig. 2). For the stereochemistry of C-1, the hydroxyl group OH-1 in **3** was inferred as being placed in the equatorial position of the pseudochair ring owing to the small coupling constant observed between H-1 and H-2 ($^3J_{1H,2H}$ = 3.2 and 6.4 Hz), which were similar to those of **1**. Meanwhile, comparison of the ^{13}C NMR data (Table 2) of **3** at C-4, C-3′ and C-4′ with those of **1** indicated the same configurations as **1**. For the stereochemistry of **4**, the large coupling constants of H-1

Table 1
^{1}H NMR (400 MHz) spectroscopic data for compounds **1**–**7** in acetone-d_6.

No.	1	2	3	4	5	6	7
1	4.75 (dd, 4.6, 2.4)	4.98 (t, 8.0)	5.20 (dd, 6.4, 3.2)	5.14 (t, 7.3, 9.3)	4.73 (dd, 6.5, 3.4)	4.98 (t, 8.2)	5.12 (dd, 7.2, 9.9)
2	2.64, 2.31 (m)	2.57 (m)	2.77, 2.13 (m)	2.59, 2.50 (m)	2.63, 2.31 (m)	2.57 (m)	2.49, 2.58 (m)
3	2.31 (m)	2.56, 2.16 (m)	2.32 (m)	2.49, 2.19 (m)	2.29 (m)	2.49, 2.15 (m)	2.42, 2.15 (m)
7	6.68 (d, 8.8)	6.74 (d, 8.8)	6.68 (d, 8.8)	6.68 (d, 8.8)	6.67 (d, 8.8)	6.75 (d, 8.8)	6.68 (d, 8.8)
8	6.78 (d, 8.8)	6.77 (d, 8.8)	6.79 (t, 8.8)	6.76 (d, 8.8)	6.78 (d, 8.8)	6.76 (d, 8.8)	6.75 (d, 8.8)
1-OCH_3	3.49 (s)	3.47 (s)			3.49 (s)	3.47 (s)	
1-OH			4.79 (d, 4.3)	5.34 (d, 5.8)			
9-OH	8.19 (s)	7.85 (s)	8.71 (s)	8.59 (s)	8.21 (s)	7.82 (s)	
2′	3.36, 2.98 (dd, 18.0, 3.0)	3.36, 2.98 (dd, 18.0, 2.8)	3.35, 2.97 (dd, 18.1, 2.9)	3.35, 2.97 (dd, 18.0, 2.8)	3.35, 2.84 (dd, 18.2, 2.4)	3.36, 2.85 (dd, 18.1, 2.6)	3.36, 2.84 (dd, 18.0, 2.8)
3′	4.28 (t, 2.8)	4.27 (t, 2.4)	4.28 (t, 2.7)	4.27 (t, 2.8)	4.64 (dd, 6.1, 2.9)	4.63 (dd, 6.0, 2.9)	4.63 (t, 2.8)
7′	7.09 (d, 8.1)	7.16 (d, 8.1)	7.11 (d, 8.0)	7.13 (d, 8.1)	7.08 (d, 8.2)	7.14 (d, 8.1)	7.12 (dd, 8.1, 1.0)
8′	7.40 (t, 8.0)	7.43 (t, 7.9)	7.41 (t, 7.9)	7.44 (t, 7.9)	7.40 (t, 7.9)	7.43 (t, 7.9)	7.42 (t, 7.9)
9′	7.50 (d, 7.6)	7.52 (d, 7.6)	7.50 (d, 7.6)	7.51 (d, 7.7)	7.51 (d, 7.6)	7.52 (d, 7.7)	7.52 (dd, 7.7, 1.0)
3′-OCH_3	3.47 (s)	3.46 (s)	3.47(s)	3.46 (s)			
3′-OH					4.98 (brs)	4.95 (brs)	4.97 (brs)

Table 2
^{13}C NMR (100 MHz) spectroscopic data for compounds **1**–**7** in acetone-d_6.

No.	1	2	3	4	5	6	7
1	71.6	74.3	63.0	67.2	71.5	74.3	67.3
2	22.3	23.2	27.0	27.7	22.3	23.2	27.7
3	28.6	32.4	28.4	32.9	28.5	32.3	32.8
4	94.9	94.2	95.0	94.4	94.9	94.2	94.4
5	118.5	118.9	118.0	118.0	118.6	118.9	118.1
6	141.6	141.9	141.7	141.8	141.9	142.1	142.1
7	116.0	116.7	115.6	116.0	116.0	116.6	116.0
8	118.1	118.1	118.2	117.7	118.0	118.1	117.7
9	151.0	151.7	150.6	151.8	151.0	151.6	151.7
10	122.2	120.6	124.0	123.2	122.2	120.5	123.1
1-OCH_3	56.3	53.4			56.3	53.3	
1'	193.3	193.3	193.4	193.3	193.9	193.8	193.8
2'	40.6	40.5	40.6	40.6	42.4	42.4	42.4
3'	79.6	79.7	79.6	79.7	70.2	70.2	70.2
4'	94.1	94.0	94.1	93.9	93.8	93.8	93.7
5'	121.0	120.9	121.0	120.9	120.9	120.8	120.8
6'	151.2	151.1	151.3	151.0	151.4	151.4	151.2
7'	121.0	121.0	121.1	121.1	120.9	120.9	120.9
8'	130.4	130.6	130.5	130.5	130.4	130.5	130.4
9'	118.8	118.9	118.9	118.8	118.7	118.8	118.7
10'	130.8	130.9	130.9	130.8	130.9	130.9	130.4
3'-OCH_3	58.5	58.6	58.5	58.5			

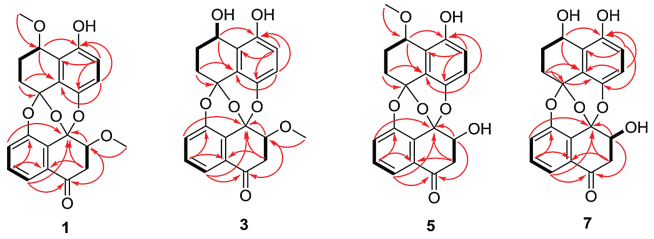

Fig. 2. Key ^{1}H-^{1}H COSY and HMBC correlations of compounds **1**, **3**, **5**, **7**.

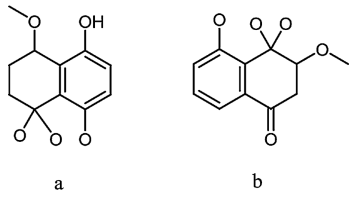

Fig. 3. Partial structures a and b.

suggested that the hydroxyl group OH-1 was located in the axial position of the pseudochair ring. The ^{13}C NMR data (Table 2) of **4** at C-4, C-3′ and C-4′ were same with those of **2**, implying these carbons have same configurations. Thus, combining with the biogenetic consideration, the absolute configurations of **3** and **4** were tentatively assigned as 1*S*, 4*R*, 3′*R*, 4′*S* and 1*R*, 4*R*, 3′R, 4′*S*, respectively (Fig. 1).

Preussomerin YT5 (**5**) and preussomerin YT6 (**6**) were shown to have the same molecular formula of $C_{21}H_{18}O_7$ based on their HRESIMS data. The ^{1}H and ^{13}C NMR spectra of **5** and **6** (Tables 1 and 2) showed similar chemical shifts to those of preussomerin YT1 (**1**). Comparison of the ^{1}H and ^{13}C NMR spectra of **5** and **6** with those of **1** indicated the absence of a methoxy group and the presence of an additional hydroxyl group on C-3′. This was confirmed by the correlations (Fig. 2) of the hydroxyl group with its vicinal protons (indicated by ^{1}H-^{1}H COSY and HMBC spectra). Through analysis of the ^{13}C NMR data of these chiral carbons and the protons coupling constants and from the biogenetic consideration, the absolute configurations of **5** and **6** were established to be the same as compounds **1** and **2**, respectively (Fig. 1).

Analysis of preussomerin YT7 (**7**) by HRESIMS and ^{13}C NMR suggested the molecular formula of $C_{20}H_{16}O_7$. Likewise, the ^{1}H and ^{13}C NMR data (Tables 1 and 2) of **7** displayed a similar pattern to those of **1** except for the absence of two oxygenated methyls on C-1 and C-3'. Comprehensive analysis of the ^{1}H, ^{13}C NMR, HSQC, ^{1}H-^{1}H COSY and HMBC spectra of **7** permitted the establishment of the structure of **7** as shown in Fig. 1. The absolute configuration could be deduced from the comparison of the ^{13}C NMR data and the protons coupling constants of **7** with those of **6**, established to be 1*R*, 4*R*, 3′*R*, 4′*S*.

Compounds **8**-**14** were identified as preussomerin EG1 (**8**) [14], preussomerin EG2 (**9**) [14], preussomerin EG4 (**10**) [15], palmarumycin SA1 (**11**) [2], MB7056B (II) (**12**) [16], CJ-12,371 (**13**) [17] and palmarumycin CP17 (**14**) [18] by comparing their NMR spectroscopic data with those reported in the literature.

Spirobisnaphthalenes have been reported to have anti-inflammatory activity [2,19]. Thus, all compounds (**1**-**14**) were firstly evaluated for their anti-inflammatory activity by monitoring their inhibitory effects on the production of NO in LPS-induced RAW264.7 macrophage cells. Among them, compounds **8** and **13** displayed significant anti-inflammatory activity at concentrations down to 3 μmol/L with IC_{50} values of 2.61 μmol/L and 1.32 μmol/L, with no obvious cytotoxicity, whereas the cell survival of RAW264.7 was very low in toxicity studies for other compounds as shown in Table 3.

In conclusion, we have identified seven new preussomerin-type spirobisnaphthalenes, preussomerins YT1-YT7 (**1**-**7**), and seven known compounds (**8**-**14**), from the endophytic fungus *E. gomezpompae*. Their structures were unambiguously determined by analysis of their NMR and HRESIMS data, with the absolute configurations of compounds **1** and **2** being confirmed by single-crystal X-ray diffraction. The new modifications of these isolated

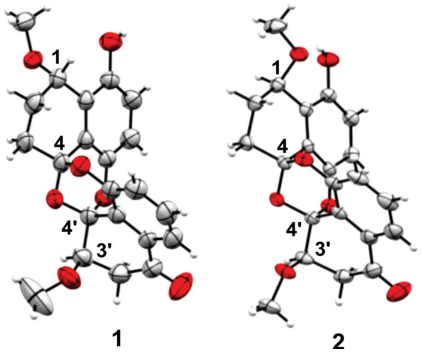

Fig. 4. X-ray crystallographic analysis of **1** and **2**.

Table 3
Inhibitory effects of compounds **1**–**14** on LPS-induced NO production in RAW264.7.

Compounds	IC_{50} (μmol/L)
Preussomerin YT1 (**1**)	>20
Preussomerin YT2 (**2**)	>20
Preussomerin YT3 (**3**)	>20
Preussomerin YT4 (**4**)	>20
Preussomerin YT5 (**5**)	>20
Preussomerin YT6 (**6**)	>20
Preussomerin YT7 (**7**)	>20
Preussomerin EG1 (**8**)	2.61
Preussomerin EG2 (**9**)	>20
Preussomerin EG4 (**10**)	>20
Palmarumycin SA1 (**11**)	>20
MB7056B (II) (**12**)	>20
CJ-12,371 (**13**)	1.32
Palmarumycin CP17 (**14**)	>20

new compounds expand the chemical diversity of the spirobisnaphthalene family of natural products. Furthermore, anti-inflammatory activity assays have showed that compounds **8** and **13** exhibited significant inhibitory activities on the production of NO in LPS-induced RAW264.7 macrophage cells with no obvious cytotoxicity, presenting us with a great opportunity to discover promising natural agents for new anti-inflammatory drugs.

Declaration of competing interest

The authors declare that they have no known competing financial interests or personal relationships that could have appeared to influence the work reported in this paper.

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Appendix A. Supplementary data

Supplementary material related to this article can be found, in the online version, at doi:https://doi.org/10.1016/j.cclet.2020.03.059.

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