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ORIGINAL ARTICLE

Two new compounds from the leaves of *Forsythia suspensa* (Thunb.) Vahl

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Abstract: A new phenanthrene derivative and a new phenylethanoid glycoside were isolated from *Forsythia suspensa* (Thunb.) Vahl leaves, named as 5,6-dimethoxy-9,10-dihydrophenanthrene-1,2,7-triol (**1**), phenethyl alcohol 8-*O*-*α*-*L*-rhamnopyranosyl-(1→3)-*O*-*β*-*D*-glucopyranosyl-(1→4)-*O*-*β*-*D*-glucopyranoside (**2**). These structures were determined by analysing their physical and chemical properties and using high-resolution mass spectrometry, infrared spectroscopy, nuclear magnetic resonance and other spectroscopic techniques. In addition, the toxic activity of the isolated compounds on RAW 264.7 cells was evaluated, but the isolated compounds did not show significant activity.

Keywords: *Forsythia suspensa* (Thunb.) Vahl, phenylethanoid glycoside, phenanthrene derivative

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1 Introduction

Forsythia suspensa (Thunb.) Vahl (*F. suspensa*) is a traditional medicinal plant belonging to the Forsythia genus of the Oleaceae family. It is primarily produced in provinces such as Hebei, Shanxi, Shaanxi, and Henan (Zhang et al., 2015; Zhou et al., 2022), in addition to the traditional use of its fruits for medicinal purposes, the stems, leaves, flowers, and roots of *F. suspense* are also utilised in folk medicine (Zhou et al., 2011; Ge et al., 2016). According to historical herbal literature, the roots, stems, and leaves of *F. suspensa* have all been used as medicinal parts (Guo et al., 2016; McClements & Rao, 2011), with each part having distinct therapeutic effects. Since the 20th century, researchers have isolated various compounds from forsythia, primarily including phenethyl alcohol glycosides, lignans (Deng et al., 2021; Kuo et al., 2017; Dong et al., 2017), flavonoids, and essential oils (Wang et al., 2018; Yang et al., 2014). Modern pharmacological studies indicate that *F. suspensa* exhibits significant anti-inflammatory activity (Zappavigna et al., 2020; Cheng et al., 2015; Wang et al., 2016a; Yang et al., 2023; Fang et al., 2023). It has been demonstrated to effectively suppress lipopolysaccharide (LPS)-induced inflammatory responses, including downregulating the production of nitric oxide (NO), prostaglandin E2 (PGE2), and key inflammatory cytokines such as TNF-α, IL-1β, and IL-6. Additionally, it exhibits anticancer (Shirin & Mohamed, 2021; Adam & Evripidis, 2022; Sun et al., 2015; Zhao et al., 2015), antioxidant (Yu et al., 2021; Zhao et al., 2021; Li et al., 2021) and anti-fatigue activity, etc. (Wang et al., 2023). This study focused on the chemical composition of *F. suspensa* leaves, isolating a new phenanthrene derivative and a new phenylethanoid glycoside (Figure 1). Their chemical structures were clearly identified through physical and chemical property analysis and various spectroscopic techniques. This research further enriches the chemical composition database of *Forsythia suspensa*, providing a material basis for future exploration of the potential pharmacological activities of its components.

2 Materials and Methods

General experimental procedures, Plant material, Extraction and isolation, Anti-inflammatory activity, and Statistical analysis were listed in the Supplementary Information.

2.1 Spectroscopic Data of Compounds 1 and 2

5,6-dimethoxy-9,10-dihydrophenanthrene-1,2,7-triol (**1**): White amorphous powder, HRESIMS: *m/z* 289.1076 $[M+H]^+$, (Calculated for 289.1035 $[M+H]^+$). ^{1}H NMR (Pyridine-d_5, 400 MHz) and ^{13}C NMR (Pyridine-d_5, 100 MHz), see Table 1.

phenethyl alcohol 8-O-α-L-rhamnopyranosyl-(1→3)-O-β-D-glucopyranosyl-(1→4)-O-β-D-glucopyranoside (**2**): White amorphous powder, HRESIMS: *m/z* 593.2445 $[M+H]^+$, (Calculated for 593.2417 $[M+H]^+$). ^{1}H NMR (Pyridine-d_5, 400 MHz) and ^{13}C NMR (Pyridine-d_5, 100 MHz), see Table 2.

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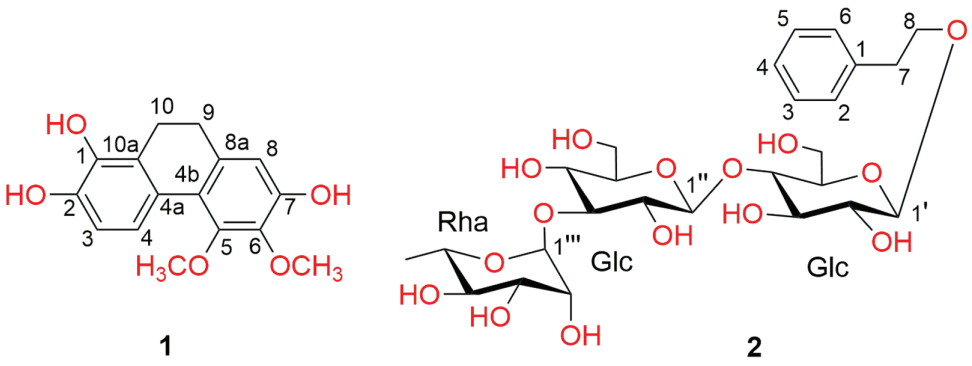

Figure 1. Structures of compounds **1** and **2**

Table 1. ^{1}H-NMR (400 MHz, in Pyridine-d_5) and ^{13}C-NMR (100 MHz, in Pyridine-d_5) spectral data of compound **1**

No.	δ_H (*J* in Hz)	δc
1	—	143.2
2	—	148.5
3	7.28, d, *J* = 7.8 Hz	115.1
4	6.95, d, *J* = 7.8 Hz	120.4
4a	—	122.0
4b	—	118.9
5	—	150.4
6	—	141.1
7	—	152.2
8	7.10, s	114.6
8a	—	138.0
9	2.65, m	32.1
10	2.71, m	31.5
10a	—	131.3
5-OCH_3	3.73, s	62.8
6-OCH_3	3.88, s	61.5

Table 2. ^{1}H-NMR (400 MHz, in Pyridine-d_5) and ^{13}C-NMR (100 MHz, in Pyridine-d_5) spectral data of compound **2**

No.	δ_H (*J* in Hz)	δc
1	—	139.3
2	7.18, d, *J* = 7.5 Hz	129.9
3	7.26, dd, *J* = 7.5, 7.5 Hz	129.3
4	7.21, t, *J* = 7.5 Hz	127.1
5	7.26, dd, *J* = 7.5, 7.5 Hz	129.3
6	7.18, d, *J* = 7.5 Hz	129.9
7	3.14, m	37.2
8	3.80, m 4.24, m	71.5
1′	4.78, d, *J* = 7.6 Hz	103.3
2′	4.19, m	78.1
3′	4.15, m	89.9
4′	4.03, m	75.4
5′	4.24, m	79.1
6′	4.58, dd, *J* = 2.5, 11.5 Hz 4.24, m	62.9
1″	5.08, d, *J* = 7.8 Hz	105.2
2″	4.03, d, *J* = 8.7 Hz	70.1
3″	4.13, dd, *J* = 9.0, 9.0 Hz	72.0
4″	4.21, m	78.9
5″	3.81, m	78.5
6″	4.45, dd, *J* = 2.5, 11.5 Hz 4.24, m	62.9
1‴	6.31, s	103.0
2‴	4.93, m	72.9
3‴	4.63, dd, *J* = 3.2, 9.3 Hz	73.3
4‴	4.34, s	74.6
5‴	4.83, m	70.3
6‴	1.72, d, *J* = 6.2 Hz	19.3

3 Results and Discussion

3.1 Structure Elucidation

Compound **1** was isolated as a white amorphous powder. The molecular formula $C_{16}H_{16}O_5$ was supported by the positive HR-ESI-MS molecular ion peak at *m/z* 289.1076 $[M+H]^+$ (calculated 289.1035 $[M+H]^+$). The ^{1}H NMR data of **1** (Table 1) exhibited four characteristic double bond signals at δ_H 7.28 (1H, d, *J* = 7.8 Hz, H-3), δ_H 7.10 (1H, s, H-8), δ_H 6.95 (1H, d, *J* = 7.8 Hz, H-4), two methoxy signals at δ_H 3.88 (3H, s, -OCH_3), 3.73 (3H, s, -OCH_3), two methylene signals δ_H 2.71 (2H, m, H-10), 2.65 (2H, m, H-9), and the rest is the parent nucleus proton signals. The ^{13}C NMR data of **1** (Table 1) displayed 16 carbon signals. In combination with the DEPT spectrum, two CH_2 signals (δ_C 31.5 and 32.1), two methoxy signals (δ_C 61.5 and 62.8), three CH signals (δ_C 114.6, 115.1 and 120.4), and nine C signals (δ_C 118.9, 122.0, 131.3, 138.0, 141.1, 143.2, 148.5, 150.4 and 152.2) could be identified. Its ^{1}H and ^{13}C NMR data were similar to those of 2,5,6-trihydroxy-3,4-dimethoxy-9,10-dihydrophenanthrene (Du et al., 2016). The main difference lies in the different positions of the two hydroxyl groups, with HMBC (Figure 2) correlations [H-4/C-2, C-4a, C-10; H-3/C-1, C-2, C-10a; H-8/C-4b, C-6, C-7, C-9] supported the identification of the OH groups at positions C-1, C-2, and C-7. A systematic literature search revealed compound **1** to be a new and unreported compound with the structure identified as 5,6-dimethoxy-9,10-dihydrophenanthrene-1,2,7-triol (Figure 1).

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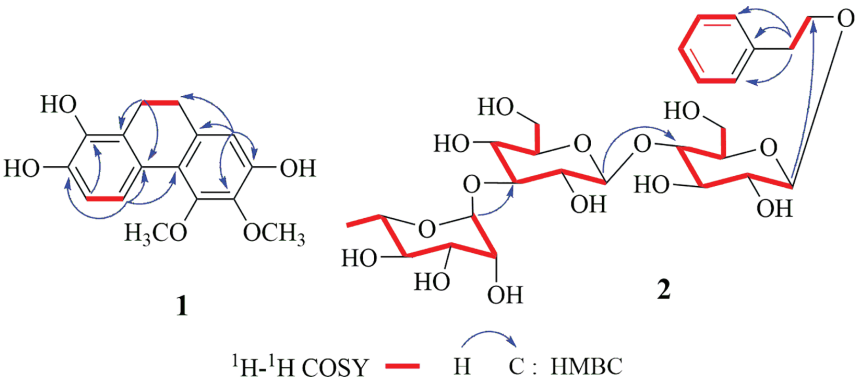

Figure 2. Key ^{1}H - ^{1}H COSY and HMBC correlations of compounds **1** and **2**

Compound **2** was obtained as a white amorphous powder. The molecular formula $C_{26}H_{40}O_{15}$ was supported by the positive HR-ESI-MS molecular ion peak at *m/z* 593.2445 $[M+H]^+$ (calculated 593.2417 $[M+H]^+$). The ^{1}H NMR data of **2** (Table 2) exhibited five characteristic double bond signals at δ_H 7.26 (2H, dd, *J* = 7.5, 7.5 Hz, H-3, 5), δ_H 7.21 (1H, t, *J* = 7.5 Hz, H-4), δ_H 7.18 (2H, d, *J* = 7.5 Hz, H-2, 6), four methylene signals δ_H 4.24 (1H, m, H-6′a), 4.58 (1H, dd, *J* = 2.5, 11.5 Hz, H-6′b), δ_H 4.24 (1H, m, H-6″a), 4.45 (1H, dd, *J* = 2.5, 11.5 Hz, H-6″b), δ_H 3.15 (2H, m, H-7), δ_H 3.80 (1H, m, H-8a), 4.24 (1H, m, H-8b), and one methyl signal δ_H 1.72 (3H, d, *J* = 6.2 Hz, H-6‴). The ^{13}C NMR data of **2** (Table 2) displayed 26 carbon signals, six aromatic carbons (δ_C 127.1, 129.3, 129.3, 129.2, 129.2, 139.3), three heteroatoms (δ_C 103.0, 103.3, 105.2) and one methyl carbon (δ_C 19.3). In combination with the DEPT spectrum, four CH_2 signals (δ_C 37.18, 62.90, 62.92 and 71.51), twenty CH signals (δ_C 70.1, 70.3, 72.0, 72.9, 73.3, 74.6, 75.4, 78.1, 78.5, 78.9, 79.1, 89.9, 103.0 103.3, 105.2, 127.1, 129.3, 129.3, 129.9 and 129.9), and one C signals (δ_C 139.3) could be identified. The HSQC spectrum allowed to attribute the carbon and hydrogen signals in the 2D NMR spectrum. The compound data is similar to phenethyl alcohol 8-*O*-*β*-*D*-glucopyranosyl-(1→2)-[*O*-α-*L*-arabinopyranosyl-(1→6)]-*O*-*β*-*D*-glucopyranoside (Ono et al., 2010). The HMBC spectrum of **2** showed the key correlations between H-1 of Glc″ and C-4 of Glc′; H-1 of Rha‴ and C-3 of Glc″; and H-1 of Glc′ and C-8 of aglycone (Figure 2). Moreover, acid hydrolysis deduced the D-glucose and L-rhamnose with a ratio of 2:1 in compound **2** (Figure S20). A systematic literature search revealed compound **2** to be a new and unreported compound with the structure of **2** was identified as phenethyl alcohol 8-*O*-*α*-*L*-rhamnopyranosyl-(1→3)-*O*-*β*-*D*-glucopyranosyl-(1→4)-*O*-*β*-*D*-glucopyranoside (Figure 1).

Anti-inflammatory activities were reported in lots of medicines (Shen et al., 2025; Song et al., 2024; Yang et al., 2024; Cao et al., 2023), which is closely related to the occurrence and development of various diseases (Zhou et al., 2023; Qiu et al., 2021; Shao et al., 2019; Wang et al., 2016b). In this study, *in vitro* activity studies were on compounds **1** and **2** were conducted (RAW 264.7 cells) (Zhang et al., 2025). However, no inhibitory activity was observed in the two compounds obtained from the separation (Table 3).

Table 3. Anti-inflammatory activities of isolated compounds **1** and **2**

Compounds	Cell lines IC_{50} (μM)[a]
1	>100
2	>100
Aminoguanidine Hydrochloride[b]	12.62 ± 1.52

Note: [a]IC_{50} values were expressed as mean ± SD (*n* = 3). [b]In the anti-inflammatory experiment, aminoguanidine hydrochloride was used as a positive control.

4 Conclusion

Two new compounds, 5,6-dimethoxy-9,10-dihydrophenanthrene-1,2,7-triol (**1**) and phenethyl alcohol 8-*O*-*α*-*L*-rhamnopyranosyl-(1→3)-*O*-*β*-*D*-glucopyranosyl-(1→4)-*O*-*β*-*D*-glucopyranoside (**2**) were isolated from the leaves of *Forsythia suspensa*, their structures were detected by multiple spectroscopic methods. The anti-inflammatory activities were conducted in RAW 264.7 cells, however, none of them showed significant activities. This study enriched the chemical diversity of *Forsythia suspensa*, in the further study, more bioactivities should be explored, such as antiviral, antioxidant, and immunosuppressive etc.

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Author Contributions

Na Wen: Writing—original draft, Investigation, Data curation, Project administration. Yuxuan Wang: Data curation.

Jingjing Su: Data curation. Dongdong Zhang: Writing—review & editing, Data curation, Supervision. Xuan Zhang: Writing—review & editing, Data curation, Supervision.

Availability of Data and Materials

The authors declare that the data supporting the findings of this study are available within the paper and its Supplementary Information files. Should any raw data files be needed in another format they are available from the corresponding author upon reasonable request. Source data are provided with this paper.

Conflicts of Interest

The authors declare that they do not have any conflict of interest.

Supporting Information

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

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