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SHORT REPORT

Two undescribed phenylpropanoid derivatives from *Notopterygium incisum* roots with anti-inflammatory potential

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Abstract: Two previously undescribed phenylpropanoid derivatives, including a terpenylated coumarin (**1**) and a ferulic acid analogue (**2**), were isolated from the roots of *Notopterygium incisum* (Umbelliferae). Their structures were successfully established using spectroscopic techniques, including mass spectrometry (MS), nuclear magnetic resonance (NMR), and electronic circular dichroism (ECD) spectroscopy. In vitro anti-inflammatory assay using a lipopolysaccharide-stimulated RAW264.7 macrophages indicated compounds **1** and **2** showed anti-inflammatory potential by inhibiting the secretion of NO at the concentra-tion of 7.5 μM.

Keywords: *Notopterygium incisum*, phenylpropanoid derivatives, nitric oxide, anti-inflammatory

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1 Plant Source

The roots of *N. incisum* were purchased from Sichuan Neautus Pharmaceutical Co., Ltd., and were identified by Prof. Guangzhi Wang who worked at School of Pharmacy, Chengdu University of TCM. The specimen of *N. incisum* with the collection number CDCM 20221225. has been preserved in the Herbarium of Chengdu University of TCM.

2 Previous Studies

Notopterygium incisum Ting ex H. T. Chang, a well-known herb in traditional Chinese medicine, has been utilized for centuries to treat various inflammation-related conditions, including musculoskeletal disorders and arthritis (Azietaku et al., 2017). Previous phytochemical investigations have indicated that *N. incisum* is a rich source of various natural products, including volatile oils, prenylidenol, and coumarins, which exhibit notable bioactivities (Jiang et al., 2025).

3 Present Study

The roots of *N. incisum* (30 kg) were powdered and subjected to percolation extraction with 95% ethanol. After recovery of ethanol, 1.5 kg dried extract was obtained. The extract was separated by silica gel column chromatography, and eluted with petroleum ether and acetone (94:6 to 0:100, V/V), yielding five fractions (Fr.1–Fr.5). Fr. 2 (722 g) was further fractionated by a D101 macroporous resin column chromatography (180 × 1,200 mM) with a MeOH/H_2O as mobile phase with a gradient of 0:100, 30:70, 60:40, and 95:5. Each gradient was eluted with five column volumes. The 95% methanol fraction (448 g) was subjected to silica gel column chromatography and eluted with petroleum ether and ethyl acetate (100:0 to 0:100, V/V), resulting in thirteen subfractions (Fr.2.1–Fr.2.13). Fraction 2.5 (23.8 g) was further separated on a Sephadex LH-20 column using dichloromethane and methanol (50:50, V/V) as eluents, yielding three fractions (Fr.2.5.1–Fr.2.5.3). Fraction 2.5.2 (8.5 g) was subjected to semi-preparative HPLC under the conditions of methanol: water (75:25, V/V, 10 mL/min) for 60 min, resulting in ten subfractions (Fr.2.5.2.1–Fr.2.5.2.10). Fraction 2.5.2.6 (3.2 g) was further separated by preparative TLC using a dichloromethane-methanol (94:6, V/V) mobile phase, resulting in four bands (Fr.2.5.2.6.1–Fr.2.5.2.6.4). Fraction 2.5.2.6.3 (16.2 mg) was purified by semi-preparative HPLC using methanol-water (78:22, V/V,

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Table 1. The ^{1}H-NMR (600 MHz) and ^{13}C-NMR (150 MHz) data of compound **1**[a]

No.	δ_H (*J* in Hz)	δ_C, type	No.	δ_H (*J* in Hz)	δ_C, type
2	–	161.8	2′	1.83–1.90, m; 1.91–2.00, m	41.2
3	6.19, d (9.4)	112.7	3′	–	84.5
4	7.57, d (9.5)	143.5	4′	1.82–1.91, m	37.5
4a	–	112.0	5′	1.85–1.95, m; 2.10–2.16, m	31.2
5	7.12, s	128.5	6′	4.49, m	83.7
6	–	126.8	7′	–	144.7
7	–	159.1	8′	4.87, s; 5.07, s	111.2
8	6.82, s	104.6	9′	1.76, s	18.2
8a	–	154.9	10′	1.30, s	26.8
1′	2.76, m; 2.69, m	24.8			

Note: a: measured in $CDCl_3$.

Table 2. The ^{1}H-NMR (600 MHz) and ^{13}C-NMR (150 MHz) data of compound **2**[a]

No.	δ_H (*J* in Hz)	δ_C, type	No.	δ_H (*J* in Hz)	δ_C, type
1	–	127.1	2′	1.61–1.68, m; 2.13–2.18, m	33.8
2	7.03, d (1.9)	109.5	3′	2.00–2.06	38.5
3	–	148.2	4′	1.46–1.52, m; 2.22–2.27, m	27.3
4	–	146.9	5′	2.08–2.13, m; 2.34–2.41, m	38.1
5	6.92, d (8.2)	114.9	6′	–	219.9
6	7.08, dd (8.2, 1.9)	123.2	7′	1.84–1.90, m	55.0
7	7.61, d (15.9)	145.2	8′	2.34–2.40, m	25.6
8	6.28, d (16.0)	115.4	9′	5.26, dtt (10.5, 8.0, 1.8)	125.3
9	–	167.4	10′	5.44, dtt (10.5, 7.2, 1.6)	134.0
10	3.93, s	56.1	11′	2.01–2.12, m	20.8
1′	4.25–4.33, m	62.6	12′	0.96, t (7.6)	14.3

Note: a: measured in $CDCl_3$.

3 mL/min), obtaining **3** (3.33 mg). Fraction 2.5.3 (6.6 g) was subjected to semi-preparative HPLC under the conditions of methanol: water (75:25, V/V, 10 mL/min) for 60 min, resulting in seven subfractions (Fr.2.5.3.1–Fr.2.5.3.7). Fraction 2.5.3.1 (3.1 g) was further separated by preparative TLC using a dichloromethane-methanol (94:6, V/V) mobile phase, resulting in four bands (Fr.2.5.3.1.1–Fr.2.5.3.1.4). Fraction 2.5.3.1.2 (3.9 mg) was purified by semi-preparative HPLC using methanol-water (75:25, V/V, 3 mL/min), obtaining **2** (1.27 mg). Fraction 2.5.3.4 (2.6 g) was subjected to preparative TLC with dichloromethane-methanol (94:6, V/V), yielding five bands (Fr.2.5.3.4.1–Fr.2.5.3.4.5). Fraction 2.5.3.3.4 (16.4 mg) was recrystallization, resulting in **4** (2.6 mg). Fraction 2.5.3.5 (1.0 g) was further separated by preparative TLC using a dichloromethane-methanol (94:6, V/V) mobile phase, resulting in three bands (Fr.2.5.3.5.1–Fr.2.5.3.5.3). Fraction 2.5.3.5.2 (3.1 mg) was purified by semi-preparative HPLC using methanol-water (V/V, 3 mL/min), obtaining **1** (1.06 mg). Fraction 2.5.3.5.3 (1.8 mg) was purified by semi-preparative HPLC using methanol-water (78:22, V/V, 3 mL/min), obtaining **5** (0.86 mg).

Compound 1: Yellow gum; $[\alpha]_D^{20}$ = −5.0 (*c* 0.020, MeOH); UV (MeOH) λ_{max}: 204 (3.00), 221 (0.80), 332(0.75) nM; IR (KBr) ν_{max}: 3413.9, 2915.7, 2850.3, 1732.2, 1698.4, 1633.1, 1387.4, 1105.6, 1042.5 cm^{-1}, HRESIMS *m/z* 337.1406 [M+Na]$^+$ (calcd. for $C_{19}H_{22}NaO_4^+$, 337.1411), ΔE = −0.0005 Da, ppm = −1.48 ppm. ^{1}H-NMR, ^{13}C-NMR data are shown in Table 1.

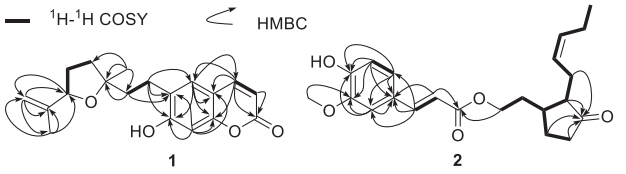

Figure 1. The key ^{1}H-^{1}H COSY, HMBC correlations of **1**–**2**

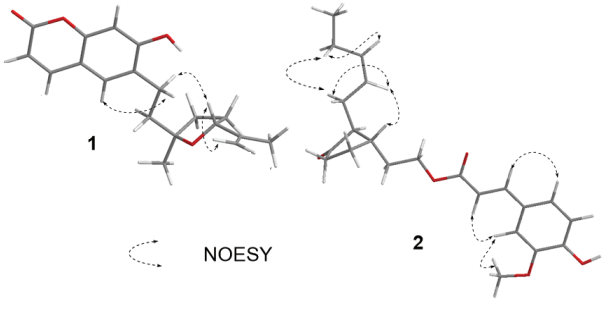

Figure 2. The key NOESY correlations of **1**–**2**

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Compound 2: Yellow gum; $[\alpha]_D^{20}$ = −35.1 (*c* 0.037, MeOH); UV (MeOH) λ_{max}: 261 (1.07), 237 (0.87), 297 (0.98), 326 (1.48) nM; IR (KBr) ν_{max}: 3402.6, 2922.5, 2848.1, 1734.5, 1691.7, 1642.1, 1513.6, 1452.7, 1376.1, 1261.1, 1164.2, 1107.8, 1056.0 cm^{-1}, HRESIMS *m/z* 371.1867 $[M-H]^-$ (calcd. for $C_{22}H_{27}O_5^-$, 371.1864), ΔE = 0.0003 Da, ppm = 0.81 ppm. ^{1}H-NMR, ^{13}C-NMR data are shown in Table 2.

Compound **1** was obtained as yellow gum. Its molecular formula was established as $C_{19}H_{22}O_4$ on the basis of HRESIMS data (*m/z* 337.1406 $[M+Na]^+$, calcd. for $C_{19}H_{22}O_4Na^+$: 337.1411). The IR spectra data showed that **1** contains hydroxyl (3413.9 cm^{-1}), methyl (2915.7 and 2850.3cm^{-1}), ester carbonyl (1732.2 cm^{-1}), phenyl (1698.4 and 1633.1 cm^{-1}). The ^{1}H-NMR spectrum of **1** exhibited signal corresponding to six olefinic protons and two methyl groups. Additionally, the ^{13}C-NMR spectrum revealed that **1** contains a total of 19 carbon atoms, which include one ester carbonyl, ten olefinic carbons, and six sp^3 carbons. The ^{1}H-^{1}H COSY correlations of H-3/H-4, as well as the HMBC correlations of H-4/C-2, C-5, C-4a, C-8a, H-5/C-4, C-4a, C-7, C-8a, and H-8/c-4a, C-6, C-7, C-8a indicated the presence of a coumarin moiety; The ^{1}H-^{1}H COSY correlations of H-1′/H-2′, and H-4′/H-5′/H-6′ combined with the HMBC correlations of H-1′/C-5, C-6, C-7, H-2′/C-3′, H-10′/C-2′, C-3′, C-4′ and H-8′/C-6′, C-7′, C-9′ generated the planar structure of compound **1** (Figure 1). The NOESY correlations of H-1′/H-6′ indicated the relative configuration of **1** should be 3′*S**,6′*R** (Figure 2). Subsequent calculations of the ECD (Zou et al., 2024), as illustrated in Figure 3, indicate that the calculated curve for 3′*S*,6′*R*-**1** aligns closely with the experimental spectra. Consequently, the absolute configuration of **1** has been determined to be 3′*S*,6′*R*.

Compound **2** was obtained as yellow gum. The molecular formula of **2** was deduced to be $C_{22}H_{28}O_5$ based on the HRESIMS peak at *m/z*: 371.1867 $[M-H]^-$ (calcd. for $C_{22}H_{27}O_5^-$). The IR spectrum of **2** present the signals of hydroxyl (3402.6 cm^{-1}), methyl (2922.5 and 2848.1 cm^{-1}), carbonyl (1734.5 cm^{-1}), phenyl (1691.7 and 1642.1 cm^{-1}). The ^{1}H NMR spectrum of **2** reveals the presence of signals corresponding to an ABX coupling system indicative of a trisubstituted phenyl. Additionally, it displays four olefinic protons, as well as signals from an oxy-methyl group and a methyl group. The ^{13}C NMR spectrum indicated **2** contains 22 carbons including a ketone carbonyl, an ester carbonyl, ten olefinic carbons, and ten sp^3 carbons. The ^{1}H-^{1}H COSY of H-7/H-8, H-5/H-6 and the HMBC correlations of H-2/C-1, C-3, C-4, C-6; H-5/C-1, C-3, C-4, C-6; H-6/C-1, C-2, C-4, C-5; 3-OMe/C-3; H-7/C-1, C-2, C-6, C-9; H-8/C-1, C-9 combined with the large coupling constants (*J* = 15.9 Hz) between H-7 and H-8 indicated the presence of a ferulic moiety, The ^{1}H-^{1}H COSY of H-1′/H-2′/H-3′/H-4′/H-5′; H-3′/H-7′/H-8′/H-9′/H-10′/H-11′/H-12′ and the HMBC correlations of H-4′/C-6′; H-5′/C-6′, C-7; H-7′/C-6′; H-8′/C-6′ established the planar structure of **2** (Figure 1). Due to the signal overlap between H-3′ (δ 2.00–2.06) and H-11′ (δ 2.01–2.12), it was difficult to assign the NOESY signal between δ 5.26 and δ 2.01–2.10. The coupling constant of 10.5 Hz indicates the *cis* configuration of $\Delta^{9'}$. Therefore, the aforementioned NOESY signal was assigned to the correlations of H-9′/H-3′, suggesting that the relative configuration of **2** should be 3′*S**, 7′*S** (Figure 2). The absolute configuration of 2 was finally confirmed to be 3′S, 7′S-2 following further ECD calculations (Figure 3).

According to the SciFinder database, compounds **1** and **2** were isolated as new natural products (Figure S and Figure S). Additionally, three known coumarins were isolated alongside them, identified as 6-isopentenyloxy-7-methoxycoumarin (**3**) (Azelmat et al., 2015), pandanusin A (**4**) (Nguyen et al., 2016), and ninhvanin B (**5**) (Huong et al., 2019) through a comparison of their spectral data with previously reported literature (Figure 4). These coumarins and ferulic acid derivatives serve as supportive markers for distinguishing *N. incisum*.

Notopterygium incisum Ting ex H. T. Chang, a well-known herb in traditional Chinese medicine, has been utilized for centuries to treat various inflammation-related conditions,

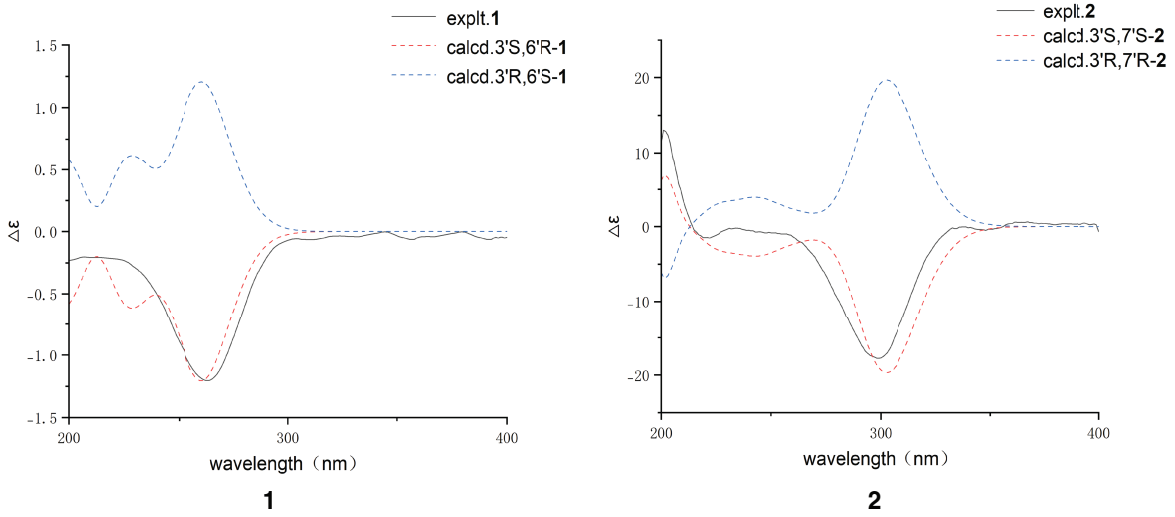

Figure 3. The experimental ECD spectra and calculated curves of **1**–**2**

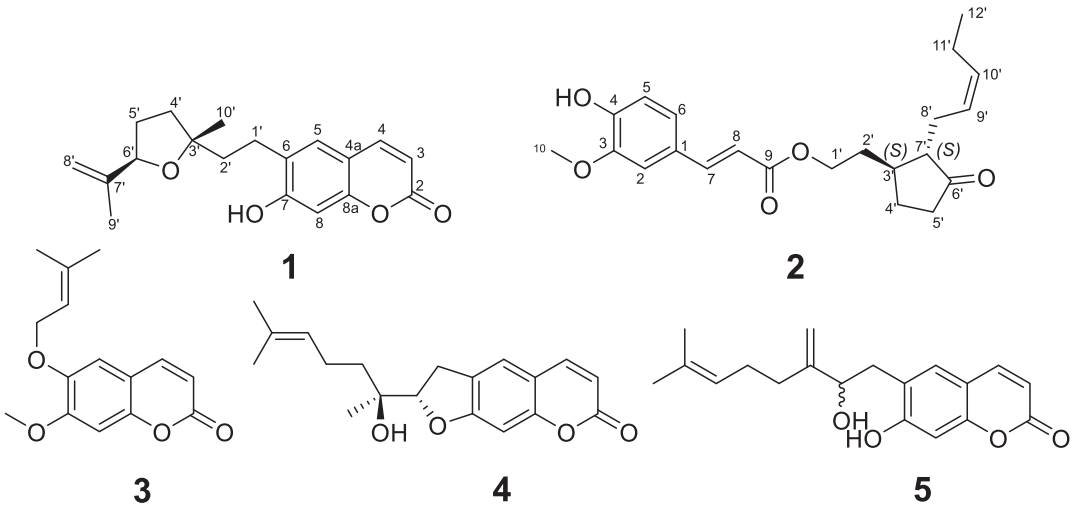

Figure 4. The chemical structures of **1**–**2**

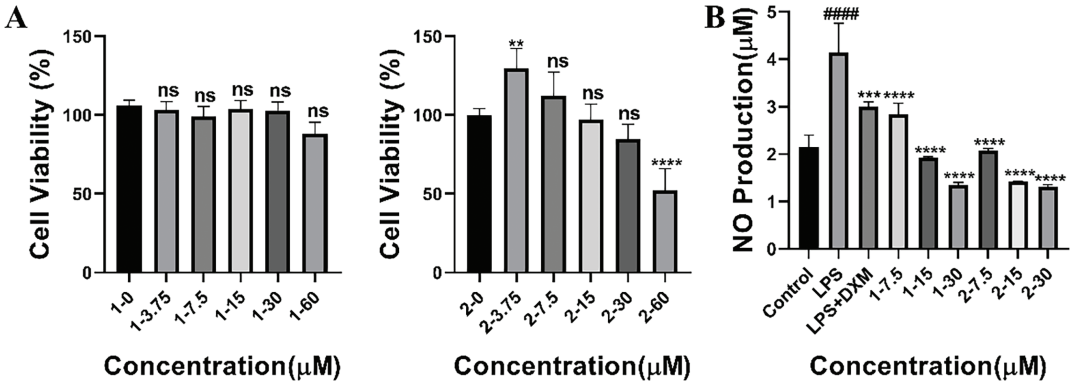

Figure 5. The cytotoxicity against RAW 264.7 macrophages and the NO inhibition of **1**–**2**

including musculoskeletal disorders and arthritis (Azietaku et al., 2017). Anti-inflammatory assays using dexamethasone (DXM) as a positive control demonstrated that compounds **1** and **2** exhibit significant efficacy in reducing nitric oxide (NO) levels at a concentration of 7.5 μM in LPS-stimulated RAW 264.7 macrophages (Figure 5).

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Author Contributions

Fei-Yuan Deng: Writing—original draft, Investigation, Data curation, Li Huang: Investigation, Data curation. Li-Lian Zhao: Data curation. Peng Tan: Validation. Yun Deng: Supervision, Funding acquisition. Li-Jun Huang: Validation, Writing—review & editing, Funding acquisition.

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 did not report any potential conflicts of interest.

Supporting Information

Supplementary data to this article can be found online at http://www.acgpubs.org/journal/records-of-natural-product.

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