SHORT REPORT

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A New Lignan with Potential Anti-inflammatory Activity from *Notopterygium incisum*

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Abstract: *Notopterygium incisum* is widely used in clinical practice because of its complex chemical composition and remarkable pharmacological effects. In this study, a previously undescribed lignan (**1**) was isolated from *N. incisum*. Its structure was evaluated through examinations of the NMR, HREIMS data and ECD calculation. Compound **1** demonstrated significant anti-inflammatory potential by inhibiting the secretion of nitric oxide (NO) in lipopolysaccharide (LPS)-induced macrophages, with an IC_{50} value of 3.57 μM.

Keywords: *Notopterygium incisum*; lignan; anti-inflammatory activity.

1. Plant Source

The roots and rhizomes of *Notopterygium incisum* utilized in this study were sourced from Sichuan Neautus Pharmaceutical Co., Ltd. The authenticity of the plant material was validated by Prof. Guang-Zhi Wang, who is affiliated with Chengdu University of Traditional Chinese Medicine. Moreover, the specimen of *N. incium* currently preserved in the plant specimen chamber with a collection number of 20221225/CDCM/SCA at the Herbarium of Chengdu University of Traditional Chinese Medicine.

2. Previous Studies

In our continuous pursuit to investigate natural products featuring novel structures and anti-inflammatory properties sourced from traditional Chinese medicines in southwestern China, a chemical analysis of *N. incisum* resulted in the extraction of several previously uncharacterized coumarins [1-3] exhibiting anti-inflammatory effects.

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3. Present Study

A combined weight of 30 kg of roots and rhizomes from *N. incisum* was transformed into a powder, then extracted with 95% ethanol, resulting in the production of dried extract. Following this, this extract was then dissolved by distilled water and fractionated by a column D101 macroporous resin measuring (80 × 1200 mm) employing a methanol/water gradient. The gradient consisted of varying ratios, specifically starting with a composition of 0% methanol to 100% water, transitioning to 30% methanol and 70% water, followed by 60% methanol and 40% water, and finally reaching a concentration of 95% methanol and 5% water. The fraction eluted with 60% methanol, weighing 101.3 g, underwent silica gel column chromatography. A gradient of petroleum ether and acetone was employed, gradually changing from a 100:0 to a 50:50 volume-to-volume ratio. This process resulted in the collection of eight distinct fractions, labeled Fr.1 to Fr.8. Fr.3, which weighed 18.51 g, underwent further separation using a silica gel column. In this step, petroleum ether and ethyl acetate were used as mobile phases, also adjusted from a 100:0 to a 50:50 volume-to-volume ratio. This further purification resulted in the formation of sixteen sub-fractions (labeled Fr.3.1 to Fr.3.16). Subsequently, Fr.3.6 fractionated using a Sephadex LH-20 column chromatography. The elution process involved a mixture of dichloromethane and methanol in a 50:50 ratio. Five sub-fractions, (labeled Fr.3.6.1 to Fr.3.6.5) were successfully yielded. Fraction 3.6.4 was further purified by semi-preparative HPLC utilizing a C18 column (5μm, 10×250mm), and yielded compound **1** (methanol-water: 68:32; 3 mL/min; t_R: 51.0 min; 4.7 mg). Fr 3.6.1 was further fractionated through semi-preparative HPLC with a C18 column (5μm, 10×250mm) and resulted in nine sub-fractions, labeled Fr.3.6.1.1 to Fr.3.6.1.9. Fr.3.6.1.3 was subjected to semi-preparative HPLC with the same C18 column, using a methanol-water mixture at a ratio of 68:32 and a flow rate of 3 mL/min and yield 2.3 mg of compound **2**, which eluted at a retention time of 13.0 min. Similarly, Fr.3.6.1.4 was further purified to isolate compound **3** (0.8 mg) at the same methanol-water mixture and flow rate were used, with the compound eluting at a retention time of 18.0 min. Besides, Fr 3.6.2 was refined using semi-preparative HPLC on the same C18 column. This process employed a methanol-water mixture at a ratio of 65:35 and a flow rate of 3 mL/min. This refinement led to the isolation of compound **4** (4.0 mg), which eluted at a retention time of 57.0 min, and compound **5** (2.1 mg), which eluted at a retention time of 59.0 min.

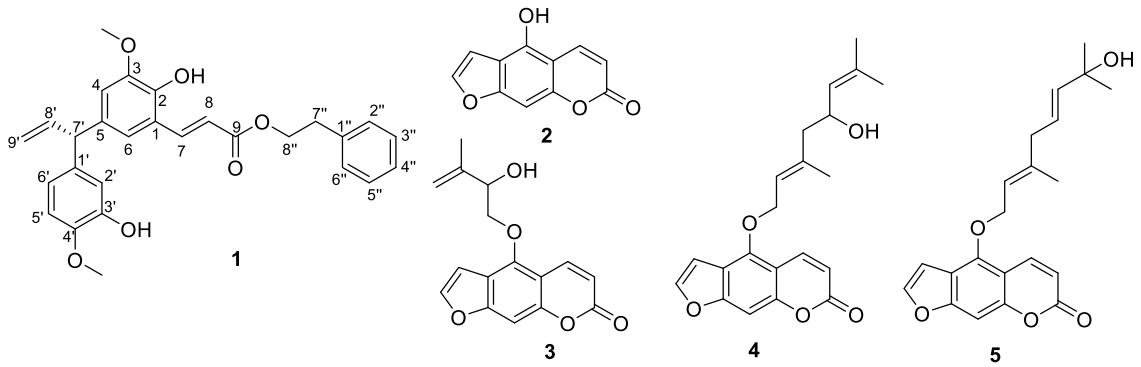

Figure 1. The structures of compounds **1-5**

*Compound **1**:* yellow gum; $[\alpha]_D^{20}$ = + 9.1 (c = 0.22, MeOH), IR (KBr): 3417, 2920, 2848, 1705, 1629, 1595, 1511, 1427, 1384, 1272, 1152, 1032, 700 cm^{-1}; UV (MeOH) λ_{max} 203 (5.08), 237 (2.03), 288 (1.16), 327 (1.62) nm; CD (c 1.74, MeOH) λ_{max} (Δε) 204 (20.31), 220 (-3.05), 240 (0.26) nm; HRESIMS *m/z* 478.2201 $[M+NH_4]^+$ (calcd. 478.2225). For ^1H and ^{13}C NMR data, see Table 1.

Compound **1** was obtained as a yellow gum. Its molecular formula was confirmed to be $C_{28}H_{28}O_6$, by HRESIMS adduct ion peak at *m/z* 478.2201 $[H+HN_4]^+$. The infrared (IR) spectrum revealed prominent absorption bands at 3417 and 1705 cm^{-1}, indicative of hydroxyl and carbonyl

functional groups, respectively. These findings suggest the presence of these functional groups within the compound's structure. The ^{1}H NMR spectrum provided further insights into the compound's architecture, displaying characteristic signals that are consistent with a polyaromatic framework. Notably, it revealed ten aromatic protons distributed across three distinct phenyl groups observed at δ_H 7.32 (H-2", H-6"), 7.26-7.20 (H-3", H-5", H-4"), 6.94 (H-4), 6.91 (H-6), 6.85 (H-5'), 6.72 (H-2'), and 6.70 (H-6'), along with a disubstituted double bond [δ_H 6.23 (H-8), 7.56 (H-7)] and a terminal vinyl group displaying resonances at δ_H 6.27 (H-8'), 4.96 (H-9'a), and 5.23 (H-9'b). Oxygenated functionalities were identified as two methoxy groups at δ_H 3.84 (3-OMe) and 3.91 (4'-OMe), while two methylene groups resonated at δ_H 3.01 (H-7") and 4.41 (H-8"). The ^{13}C NMR analysis of compound **1** displayed a signal at δ167.3 (C-9) indicative of a carbonyl group. Signals corresponding to twenty sp^2-hybridized carbon atoms, consistent with aromatic and olefinic carbons. Five sp^3 hybridized carbon atoms, supporting the presence of methoxy and methylene groups.

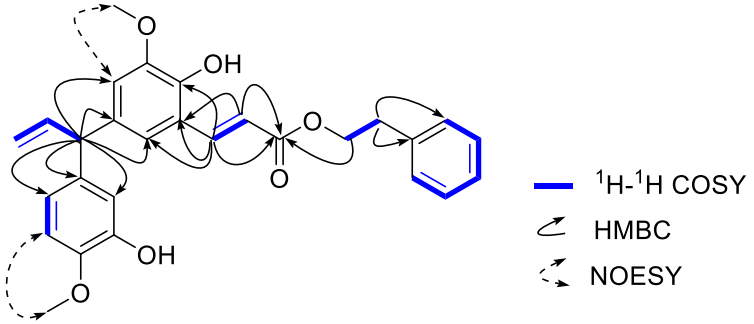

Figure 2. The key ^{1}H-^{1}H COSY, HMBC and NOESY correlations of **1**

The analysis results from HSQC and HMBC for compound **1** illustrated the existence of three phenyl groups. Among these, one is identified as a tetrasubstituted phenyl, characterized by aromatic protons at δ_H 6.94 (1H, d, J = 2.1 Hz, H-4), 6.91 (1H, d, J = 1.9 Hz, H-6) correlated with carbons at δ_C 146.5 (C-3), 144.3 (C-4), 134.1 (C-1), 121.3 (C-5), 114.3 (C-6), and 111.3 (C-2). The significant coupling constants observed between H-7" and H-8" indicate the *E*-configuration of the disubstituted double bond. The HMBC correlations (Figure 2) show the relationships between H-7 and C-1, C-2, C-6, C-9, along with connections between H-8 and C-1 and C-9, developing the initial C6-C3 unit within the lignan framework. A trisubstituted phenyl group exhibiting an ABX coupling system [δ_H 6.85 (d, J = 8.1 Hz, H-5'), 6.72 (d, J = 2.1 Hz, H-2'), 6.70 (dd, J = 8.1, 2.1 Hz, H-6'); δ_C 146.8 (C-2'), 145.4 (C-3'), 129.6 (C-5'), 126.3 (C-1'), 123.5 (C-6'), 107.4 (C-4')] was linked to a second C6-C3 moiety via ^{1}H-^{1}H COSY correlations (H-7'/H-8'/H-9') and HMBC correlations (H-7'/C-1', C-2', C-6'). The third substituent, a 2-hydroxyethylphenyl group, displayed aromatic signals at δ_H 7.32 (2H, t, J = 7.3 Hz, H-2", H-6" and 7.26–7.20 (3H, m, H-3", H-5", H-4"), with aliphatic protons at 3.01 (2H, t, J = 7.1 Hz, H-7") and 4.41 (2H, t, J = 7.1 Hz, H-8") correlated to δ_C 138.1 (C-1"), 129.1 (C-3", C-5"), 128.7 (C-2", C-6"), 126.7 (C-4"), 65.0 (C-8"), 35.9 (C-7"). The methoxy group positions were confirmed through HMBC correlations that connected 4'-OMe to C-4' and 3-OMe to C-3. Additionally, NOESY correlations involving 4'-OMe/H-5' and 3-OMe/H-4 were also considered. Key correlations were observed between the proton at position H-8" and the carbon at position C-9. Additionally, interactions between the proton at H-7' and the carbons at positions C-4, C-5, and C-6 as well as the proton at H-7" and the carbon at position C-1", C-2", C-6" provided further structural insights. Hence, the planar structure of compound **1** was established. The absolute configuration was determined to be 7'S. This conclusion was supported by subsequent ECD analyses [4-5], which is illustrated in Figure 3. As reported in the SciFinder database, compound **1** was identified as a new lignan, exhibiting a similarity of 92% with the most comparable compounds.

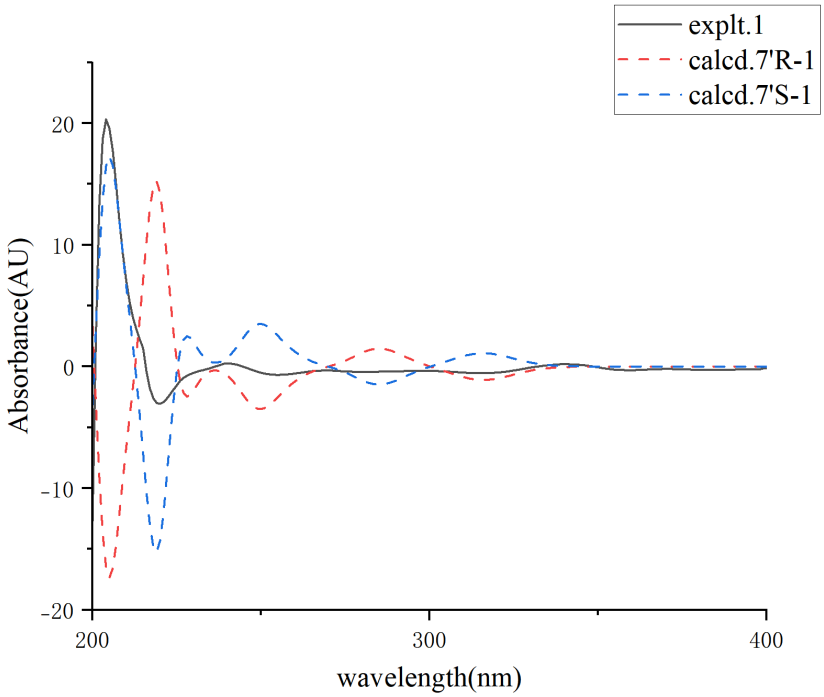

Figure 3 The experimental ECD spectra and calculated curves of **1**

Compounds **2-5** were elucidated to be name Bergaptol[6], name Pabulenol[7], name Notopterol[8], name Notoptol[9] by comparing spectral data in literatures.

In a series of anti-inflammatory assays with dexamethasone (DXM) as positive control, compound **1** demonstrated significant efficacy in reducing inflammatory markers. Specifically, at a concentration of 7.5 μM, compound **1** also significantly downregulated key pro-inflammatory gene expression (IL-1β and IL-6) in LPS-stimulated RAW 264.7 macrophages. Besides, it markedly decreased nitric oxide (NO) production with a IC_{50} of 3.57 μM. These effects are illustrated in Figure 4, highlighting the potential of compound **1** as a promising anti-inflammatory hit-compound.

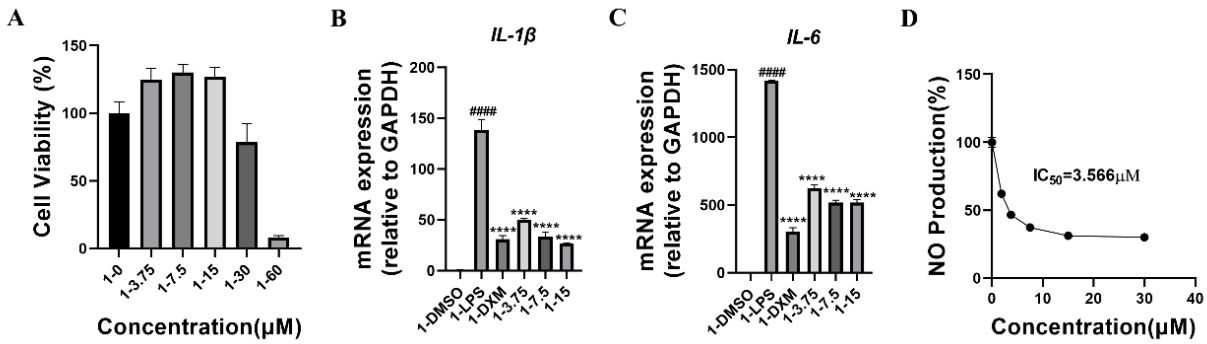

Figure 4. Cell viability, RT-PCR test results and IC_{50} determination for compound **1**. A: Cell viability results for compound **1**. B: Expression levels of inflammatory cytokines IL-1β for compound **1**. C: Expression levels of inflammatory cytokines IL-6 for compound **1**. D: IC_{50} of Compound **1** for NO production in RAW 264.7 macrophages. Data are expressed as the mean ± SD. ####p ＜0.0001, compared with the normal control group. ********p＜0.0001, compared with the LPS group.

Table 1. The NMR data of compound **1** in $CDCl_3$(δ in ppm, J in Hz)

Compound 1	δ_C	δ_H J **(Hz)**
1	126.3	-
2	146.8	-
3	145.4	-
4	107.4	6.94 (d, 2.0)
5	129.6	-
6	123.5	6.91 (d, 2.0)
7	145.6	7.56 (d, 15.8)
8	115.4	6.23 (d, 15.8)
9	167.3	-
1'	134.1	-
2'	111.3	6.72 (d, 2.0)
3'	146.5	-
4'	144.3	-
5'	121.3	6.70 (dd, 8.0, 2.0)
6'	114.3	6.85 (d, 8.0)
7'	47.3	5.05 (d, 6.6)
8'	139.7	6.27 (ddd, 17.0, 10.1, 6.6)
9'	116.6	5.23 (dt, 10.1, 1.6), 4.96 (dt, 17.0,
1"	138.1	1.6)
2", 6"	128.7	-
3", 5"	129.1	7.32 (t, 7.3)
4"	126.7	7.26-7.20 (m)
7"	35.9	7.26-7.20 (m)
8"	64.9	3.01 (t, 7.2)
3-OMe	56.1	4.41 (t, 7.2)
4'-OMe	56.3	3.84 (s)
2-OH	-	3.91 (s)
3'-OH	-	5.99 (s)
		5.50 (s)

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Supporting Information

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

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