Artemisia argyi

Keywords

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Abstract

Chemical analysis of the aerial parts of Artemisia argyi H. Lév. & Vaniot led to the isolation of 6 lignans, including a new lignan glycoside, artemisiaside A, using various chromatographic techniques. Detailed spectroscopic (including 1D, 2D- nuclear magnetic resonance) and high resolution mass spectroscopy procedures, and electronic circular dichroism were used to ascertain the structural orientations of these compounds. The anti-inflammatory activities of compounds 1 to 6 were evaluated by measuring their inhibitory effects on lipopolysaccharide (LPS) -induced nitric oxide (NO) production in RAW264.7 LPS-activated macrophages. At 50 µM, compound 1 showed moderate anti-inflammatory activity with an inhibition rate of 61.2%.

Keywords

Artemisia argyi, compositae, lignan glycosides, anti-inflammatory activity

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Introduction

Compositae, the largest family of flowering plants, encompasses more than 1600 genera and 22,000 species, with a worldwide distribution in all continents except Antarctica. This family has enormous economic benefits by providing food, cosmetics, and pharmaceuticals. Artemisia is a large genus in the Compositae family, with more than 350 species distributed across parts of the northern hemisphere temperate regions. Artemisia species have been used to treat a variety of diseases, from simple fevers to malaria. Secondary metabolites of Artemisia species reported in the literature include phenolic compounds, alkaloids, terpenoids, sterols, and coumarins. Especially, the discovery of artemisinin from Artemisia annua has brought more attention to this genus. Artemisia argyi, a perennial plant, known as wormwood in traditional Chinese medicines, is mainly found across parts of eastern Asia and Europe. The stems and leaves of A argyi have a particularly strong fragrance. The whole plant was generally used as a Chinese traditional herb to treat warming meridians to dissipate cold, to stop bleeding, as an anti-inflammatory, to relieve cough-melt sputum, and so on. In addition, it can also be used as a dietary source for treating gastric mucosal injury or unhealthy eating habits. Previous study has shown that the leaves and stems of A argyi mainly contain volatile oil, sesquiterpenoids, flavonoids, phenols, alkaloids, and sugars. In our phytochemical investigation of the methanol extract of the aerial parts of A argyi, we report the isolation and structure determination of 3 lignans and 3 lignan glycosides, including 1 previously undescribed compound (I), and 5 known compounds (2-6). Their anti-inflammatory activities were assessed by measuring the inhibitory effects on lipopolysaccharide (LPS)-induced NO production in macrophages activated by RAW264.7 LPS.

Result and Discussion

Compound 1 was obtained as a colorless amorphous powder. Based on the high resolution electrospray ionization mass spectroscopy (HRESIMS) ion peak at m/z 623.2341 [M-H]⁻ (calcd 623.2340), its molecular formula was determined as C₃₀H₄₀O₁₄, suggesting eleven degrees of unsaturation. Analysis of the ¹³C nuclear magnetic resonance (NMR) data (Table 1) with the aid of distortionless enhancement by polarization transfer experiment uncovered the presence of 1 methyl, 4 methylenes (including 3 oxygenated), 11 methines (including 3 aromatics, 5 oxygenated), 10 quaternary carbons (including 9 aromatics, 1 acetyl), and 4 methoxyl groups. The ¹H and ¹³C NMR

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data (Table 1) were reminiscent of 2-acetoxy-methyl-1,2,3,4-tetrahydro-7-hydroxy-1-(4-hydroxy-3,5-dimethoxyphenyl)-3-hydroxymethyl-8,6-dimethoxynaphthalene (compound 1b). However, it is noteworthy that there is a slight difference in the carbon chemical shift at C-7 and C-7′, which probably resulted from the introduction of a sugar moiety at C-9. The key 1H detected heteronuclear multiple bond correlation (HMBC) correlations (Figure 2) of H-9 with C-1′ confirmed that the glycosyl moiety was attached to C-9. The planar structure of 1 was further determined by a comprehensive interpretation of the 1H-1H COSY, HSQC, and HMBC spectra. To determine the absolute configuration of 1, it was acid hydrolyzed to obtain an aglycon and sugar. For the aglycone (1c), due to the low amount, the 1H NMR and 13C NMR data could not be completed, but CD (Figure 3) and specific rotation data (Supplementary Figure S22) were provided; its electronic circular dichroism (ECD) spectrum was almost identical to that of urinitetralin, phyltetralin, and strychnovanosides B-C, and the absolute configuration of the sugar was confirmed as D-glucose by comparison with standard glucose using a chiral HPLC column analysis (Supplementary Figure S20). Thus, the absolute configuration of 1 was established as 7′R, 8′L, 8′S. Accordingly, compound 1 was established as shown in Figure 1 and was named artemisiaside A.

By comparing their spectroscopic data with those reported in the literature, the other 5 known compounds 2-6 were identified as (−)-lyoniresinol 3α-O-β-D-glucopyranoside, (6R,7S,8S)-7α-[β-D-glucopyranosyl(1→2)]-lyoniresinol, (+)-lyoniresinol, 5-methoxy-(+)-isolericiresinol, and burselignan, respectively.

As a traditional (folk) medicine, A. argyi exhibits multifarious bioactivities and is used to control dysmenorrhea, abdominal pain, and inflammation. For instance, the essential oil from A. argyi (AAEO) dose-dependently suppressed the release of pro-inflammatory mediators (nitric oxide [NO], PGE2, and ROS) and cytokines (TNF-α, IL-6, IFN-β, and MCP-1) in LPS-induced RAW264.7 macrophages. As well as the crude extract, dehydromarticactin A was also identified as an active component of this plant against inflammation of the lung. Moreover, several sesquiterpenoids obtained from A. argyi have shown an enormous potential for inhibitory activity of NO production in vitro. Therefore, in order to explore and enrich the variety of compounds with anti-inflammatory activity from A. argyi, the anti-inflammatory activities of compounds 1 to 6 were evaluated by measuring their inhibitory effects on LPS-induced NO production in RAW264.7 cells (Table 2). At the same concentration, the inhibition rate of the positive control (L-NMMA) was 55.5%.

**Table 1.** 1H and 13C NMR Data of Compounds 1 and 1b.

| No. | δH (J in Hz) | δC, type | No. | δH (J in Hz) | δC, type |
|-----|--------------|----------|-----|--------------|----------|
| 1   | 129.9 s      |          | 1a  | 129.5 s      |          |
| 2   | 125.9 s      |          | 2   | 125.6 s      |          |
| 3   | 147.4 s      |          | 3   | 146.8 s      |          |
| 4   | 138.9 s      |          | 4   | 138.3 s      |          |
| 5   | 148.8 s      |          | 5   | 147.8 s      |          |
| 6   | 6.58 (s) 107.6, d |          | 6   | 6.57 (s) 107.0, d |          |
| 7   | 2.84 (dd, 15.3, 4.3) 34.1, t |          | 7   | 2.70 33.4, t |          |
| 8   | 1.86 (m) 38.6, d |          | 8   | 1.67 40.2, d |          |
| 9   | 3.98 (dd, 9.6, 4.5) 73.8, t |          | 9   | 3.64 65.4, t |          |
| 10  | 3.48 (dd, 9.6, 6.6) 35.4 |          | 10  | 3.54 |          |
| 3-OMe | 3.28 (s) 59.9, q |          | 3-OMe | 3.33 (s) 59.3, q |          |
| 5-OMe | 3.85 (s) 56.5, q |          | 5-OMe | 3.82 (s) 56.2, q |          |
| 1′  | 138.9 s |          | 1′  | 138.4 s |          |
| 2′  | 6.32 (s) 106.7, d |          | 2′  | 6.40 (s) 106.7, d |          |
| 3′  | 149.0 s |          | 3′  | 148.3 s |          |
| 4′  | 134.7 s |          | 4′  | 134.9 s |          |
| 5′  | 149.0 s |          | 5′  | 148.3 s |          |
| 6′  | 6.32 (s) 106.7, d |          | 6′  | 6.40 (s) 106.7, d |          |
| 7′  | 4.14 (d,6.6) 43.4, d |          | 7′  | 4.20 (d,6.2) 42.6, d |          |
| 8′  | 2.15 (m) 46.2, d |          | 8′  | 2.21 44.9, d |          |
| 9′  | 4.21 (overlap) 65.8, t |          | 9′  | 4.13 65.3, t |          |
| 9′-carbonyl | 173.3 s |          | 9′-carbonyl | 171.2 s |          |
| 9′-acetyl | 2.08 (s) 20.9, q |          | 9′-acetyl | 20.8, q |          |
| 3′-OMe | 3.73 (s) 56.7, q |          | 3′-OMe | 3.71 (s) 56.6, q |          |
| 5′-OMe | 3.73 (s) 56.7, q |          | 5′-OMe | 3.71 (s) 56.6, q |          |
| 1′″  | 4.20 (d, 7.8) 104.7, d |          | 1′″  | 3.16 (dd, 8.0, 7.9) 75.1, d |          |
| 2′″  | 3.32 (overlap) 78.1, d |          | 2′″  | 3.26 (overlap) 71.6, d |          |
| 3′″  | 2.32 (overlap) 77.9, d |          | 3′″  | 2.32 (overlap) 77.9, d |          |
| 4′″  | 3.83 (overlap) 62.7, t |          | 4′″  | 3.83 (overlap) 62.7, t |          |
| 6′″  | 3.65 (dd, 11.9, 5.4) |          | 6′″  | 3.65 (dd, 11.9, 5.4) |          |

The 1H and 13C NMR data of compound 1 were recorded at 600 MHz and 150 MHz, with CD3OD as solvent, respectively.

The 1H and 13C NMR data of the compound reported in the literature were recorded with a Bruker DRX-360, with (CD3)2CO as solvent.

**Table 2.** Inhibitory Rate of Lipopolysaccharide (LPS)-Induced Nitric Oxide (NO) Production in RAW264.7 Cells Treated With Compounds 1 to 6.

| Compound | Concentration (µM) | NO Inhibitory Rate (%) |
|----------|-------------------|------------------------|
| 1        | 50                | 61.2 ± 0.5             |
| 2        | 50                | 25.5 ± 2.5             |
| 3        | 50                | 21.6 ± 2.0             |
| 4        | 50                | 18.7 ± 2.4             |
| 5        | 50                | 22.6 ± 1.8             |
| 6        | 50                | 19.5 ± 2.5             |
| L-NMMA   | 50                | 55.5 ± 2.3             |

Conclusion

Six lignan analogues, including 1 new lignan glycoside, were obtained from the aerial parts of A. argyi. Their structures were determined from extensive spectroscopic and mass
spectrometric data and ECD. Compounds 1 to 6 were assessed by measuring the inhibitory effects on LPS-induced NO production in RAW264.7 LPS-activated macrophages. The results indicated that compound 1 has moderate anti-inflammatory activity.

**Experimental**

**General Experimental Procedures**

Column chromatography (CC) was performed on silica gel (200-300 mesh, Qingdao Marine Chemical Factory), Sephadex LH-20 (20-100 μm, Amersham Pharmacia Biotech), and MCI gel CHP-20P (70-150 μm, Mitsubishi Chemical Corp.). Standard thin-layer chromatography (TLC) was carried out on silica gel (GF254, 10-40 μm, Qingdao Marine Chemical Factory). Compounds were visualized on the TLC plates under UV light and by spraying with 5% H₂SO₄ in EtOH (v/v) followed by heating. HPLC analyses were performed on an Agilent 1260 HPLC system using an ODS column (C18, 250 × 4.6 mm, YMC Pak, 5 μm; detector: UV) and DAICEL chiral column (AGP 100.4 100 × 4.0) with a flow rate of 1.0 mL/min. Preparative HPLC was performed on an Agilent 1100 series with a Zorbax SB-C18 column (5 μm, 9.4 × 150 mm, Agilent) with a flow rate of 8.0 mL/min. NMR experiments were carried out using a cryogenic Bruker AV-600 spectrometer with TMS as internal standard. Mass spectra were measured on an Agilent G6230 spectrometer. UV spectra were obtained on either a Shimadzu 2700 or 2401 PC double-beam spectrophotometer. Optical rotations were measured with a Horiba SEPA-300 polarimeter. ECD experiment was conducted with an Agilent Applied Photophysics circular dichroism spectrometer.

**Plant Material**

The aerial parts of *A. argyi* (Compositae) were collected from Xichang (GPS, 30°52′N/104°44′E) of Sichuan province, China, in July 2021 (wet season). The plant was identified by Prof. Qing-Shan Yang (Anhui University of Chinese Medicine), and an authentic sample (A20210708) is kept in the Laboratory of Research and Development of Medicinal Plants in the Panxi Region.

**Extraction and Isolation**

Air-dried and powdered aerial parts of *A. argyi* (2 kg) were milled and soaked in methanol (3 L) at room temperature for 48 h, and the extraction was conducted 3 times under the same conditions. The combined extract was evaporated to dryness under reduced pressure to yield an oily residue (106 g), which was further subjected to silica gel CC eluting with CH₂Cl₂ and then with CH₂Cl₂/MeOH stepwise-gradient (50:0, 25:1, 20:1, 15:1, 10:1, 5:1, 2:1, 0:1, v/v) to give 3 fractions (Frs. I-III).

Frac. I (15 g) was fractionated by silica gel CC with CH₂Cl₂/MeOH (from 50:1 to 20:1, v/v) as the eluent to give 3 subfractions (Frs. I-A-IC). Frac. IC (500 mg) was applied to a silica gel
column and eluted with CH₂Cl₂/MeOH (30:1, v/v) to afford 4 additional subfractions (Fr. IC-1-Fr. IC-4). Fr. IC-2 (108 mg) was passed through a Sephadex LH-20 column eluting with CH₂Cl₂/MeOH (1:1, v/v) to obtain 3 additional subfractions (Fr. IC-2a-Fr. IC-2C). Fr. IC-2b (30 mg) was separated on a Sephadex LH-20 column eluting with CH₂Cl₂/MeOH (1:1, v/v) and then subjected to semi-preparative RP-HPLC using MeCN-H₂O (15:85-25:75, v/v) as the mobile phase to yield 4 (tᵣ 30 min, 3.2 mg), 5 (tᵣ 34 min, 4.8 mg), and 6 (tᵣ 40 min, 5.2 mg).

Fr. III (12 g) was separated on a silica gel column eluting with CH₂Cl₂/MeOH (from 15:1 to 1:1, v/v) to afford 3 subfractions (Frs. IIIA-IIIC). Fr. IIIA (3 g) was subjected to silica gel CC with CH₂Cl₂/MeOH (5:1, v/v) as the eluent and then was chromatographed over a Sephadex LH-20 column eluting with CH₂Cl₂/MeOH (1:1, v/v) to obtain 3 additional subfractions (Fr. IIIA-1-Fr. IIIA-3). Fr. IIIA-2 (76 mg) was separated on a Sephadex LH-20 column eluting with CH₂Cl₂/MeOH (1:1, v/v) and then was subjected to semi-preparative RP-HPLC using MeCN-H₂O (5:95-20:80, v/v) as the mobile phase to yield 1 (tᵣ 23 min, 2.1 mg), 2 (tᵣ 25 min, 4.8 mg), and 3 (tᵣ 30 min, 5.6 mg).

**Artemisiaside A (1).** Colorless amorphous powder, [α]D²⁵ −4 (c 0.02, MeOH); UV (MeOH) λ_max (log ε) 275 (3.54) nm; ECD (MeOH) λ_max (Δε) 312 (+0.33), 299 (+0.33), 274 (−0.91), 265 (−0.36), 244 (−3.68), 225 (+2.92), 213 (+4.96).

**Determination of Sugar Components**

Compound 1 (2 mg) was refluxed in 10% HCl/dioxane 1:1 (2 mL) for 2 h. The reaction mixture was extracted with ethyl acetate (5 mL × 3) and further purified by silica gel CC using light petroleum–acetone (5:1) to give compound 1c (0.4 mg). The sugar component in the aqueous layer was evaporated under reduced pressure, dissolved in water (1 mL) and subjected to HPLC analysis (Column: ChromCore Sugar-10Ca, 6 μm; Mobile Phase: 0.5 mL/min). Standard D-glucose (Sigma) was subjected to the same reaction and HPLC analysis under the same conditions.

**Measurement of Nitric Oxide Production**

NO production was assayed in supernatants of cultured RAW264.7 cells using an NO assay kit (Beyotime Institute of Biotechnology). Cells were seeded in 96 well culture plates, and pretreated with the tested compounds (50 µM) and stimulated with LPS (1 µg/mL) for 24 h. The supernatant was mixed with an equal volume of Griess reagent (1% sulfanilamide, 0.1% naphthylethylenediamine dihydrochloride).
and 2.5% phosphoric acid) and incubated at room temperature for 24 h. The concentration of nitrite was measured by reading the absorbance at 570 nm. 21-22 L-NMMA was used as a positive control.

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Declaration of Conflicting Interests

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Supplemental Material

Supplemental material for this article is available online.

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Appendix

Appendix A. Supplementary Data

1D NMR (¹H and ¹³C), 2D NMR (¹H-¹H COSY, HSQC, HMBC, and ROESY), high resolution mass spectroscopy, UV, and experimental ECD spectra of compound 1, and HPLC analysis of hydrolyzed sugar of compound 1 and the standard glucose. Supplementary data for this article can be found online at https://doi.org/10.1177/1934578X221118552