Chemical Constituents of the Folk Medicinal Plant
**Argyreia acuta** Lour. and Their Anti-Inflammatory Activity

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**Abstract**

Phytochemical investigation of the folk medicinal plant *Argyreia acuta* Lour. has led to the isolation of a pair of new 3-alkylated coumarin enantiomers, (+)-acutamarin ([+]-1) and 3 known structurally related coumarins (2–4), along with 4 known flavonoids (5–8). The structures of these compounds were elucidated on the basis of extensive spectroscopic (including one-dimensional and two-dimensional nuclear magnetic resonance) analyses and by comparison of their spectral data with those reported in the literature. The absolute configurations of (−)-1 and (+)-1 were proposed by comparison of experimental and calculated electronic circular dichroism data. Compounds 5 and 7 exhibited in vitro anti-inflammatory activity by inhibiting the production of nitric oxide with IC_{50} values of 24.54 ± 0.36 μM and 10.60 ± 0.15 μM, respectively.

**Keywords**

*Argyreia acuta* Lour., 3-alkylated coumarin, flavonoids, anti-inflammatory activity, ECD calculation

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The genus *Argyreia* (Convulvulaceae) comprises approximately 90 species, which are mainly distributed in south and southeast Asia.1 Plants of this genus are known to have broad biological properties, such as cytotoxic,2 anti-inflammatory,3 hepatoprotective,4 and neuroprotective5 activities. Among them, *A. acuta* Lour., locally known as “Goudahao,” is a perennial liana or climbing vine growing in southwest mainland China and has been used as a folk herbal medicine for the treatment of inflammatory diseases, including acute or chronic bronchitis, cough, sore throat, and mouth ulcers.6 Previous phytochemical and biological investigations of this plant have resulted in the isolation of a series of resin glycosides with anti-inflammatory,3 hepatoprotective,6 and biological activity of these metabolites are reported herein.

**Results and Discussion**

Compound 1 was obtained as a yellow, amorphous solid. It was assigned the molecular formula C_{18}H_{16}O_{6} (11 degrees of unsaturation) on the basis of its HRESIMS data (m/z 351.0842 [M + Na]). The 1H and 13C NMR spectra of 1 displayed signals for 2 methyl groups including 1 O-methyl, 1 methine, 14 aromatic/olefinic carbons (6 of which were protonated), and 1 carboxyl carbon (δC 164.1). These data accounted for all the NMR resonances for 1 and agreed with the molecular formula C_{18}H_{16}O_{6}, except for 3 exchangeable protons. In the aromatic region of the 1H NMR spectrum, 3 singlets at δH 7.57 (H-4), 7.07 (H-5), and 6.74 (H-8) were consistent with a 36,7-trisubstituted coumarin core,11,12 which was supported by comparison of experimental and calculated electronic circular dichroism data. This discovery suggests the potential for future pharmaceutical development.
by heteronuclear multiple bond correlation (HMBC) cross-peaks from H-4 to C-2 (δ_C 164.1)/C-5 (δ_C 109.7)/C-8a (δ_C 150.0), from H-5 to C-4 (δ_C 140.1)/C-7 (δ_C 151.9)/C-8a (δ_C 150.0), and from H-8 to C-4a (δ_C 113.0)/C-6 (δ_C 147.0) (Figure 2). HMBC from the protons of the oxygenated methyl (δ_H 3.88, 6-OCH_3) to C-6 (δ_C 147.0) located the methoxy group at C-6, which was confirmed by the NOESY correlation of H-5 (δ_H 7.07) with 6-OCH_3. In turn, an ABX-type spin system comprised of the signals at δ_H 6.71 (H-2', d, J = 2.0 Hz), 6.70 (H-5', d, J = 8.1, Hz), and 6.61 (H-6', dd, J = 8.1, 2.1 Hz) revealed the presence of a 1-substituted 3,4-dihydroxyphenyl moiety, and relevant HMBC cross-peaks further supported this assignment (Figure 2). Finally, key HMBCs from Me-8' with C-7'/C-1'/C-3 and from H-7' to C-2/C-4/C-2'/C-6' indicated that C-3, C-1', and C-8' are all connected to the methine C-7'. On the basis of these data, the planar structure of 1 was established, as shown in Figure 1.

Compound 1 has 1 chiral center at C-7', but is optically inactive, suggesting that it was a racemic mixture. This is consistent with the transparent CD curve. Subsequent chiral resolution of 1 was performed using a Daicel Chiralpak IC type chiral HPLC column, leading to the separation of (–)-1 and (+)-1 in a ratio of approximately 1:1. As expected, both (–)-1 and (+)-1 showed opposite optical rotations and mirror-like Cotton effects in the electronic circular dichroic (ECD) curves (Figure 3). To determine absolute configurations of the enantiomers, ECD calculations were carried out at the CAM-B3LYP/def2-TZVP level in methanol using the PCM model. Results (Figure 3) indicated that the calculated ECD spectrum of (K)-1...
fitted well with the ECD measurement value of \((-\)-1, so that the absolute configurations of compounds \((-\)-1 and \((+\)-1 were established to be \(R\) and \(S\), respectively. Therefore, compounds \((-\)-1 and \((+\)-1 were assigned as \((R\)-3-(1-(3,4-dihydroxyphenyl)ethyl)-7-hydroxy-6-methoxycoumarin and \((S\)-3-(1-(3,4-dihydroxyphenyl)ethyl)-7-hydroxy-6-methoxycoumarin, respectively. They were named \((-\)-acutamarin \([(-\)-1] and \((+\)-acutamarin \([(+\)-1] after the plant origin.

Three known structurally related coumarins, scopoletin \((2)\), umbelliferone \((3)\), and scopolin \((4)\), together with 4 known flavonoids, \((2R,3R\)-7-O-methyl-dihydrokaempferol \((5)\), \(4’\)-7-di-O-methyl-kaempferol \((6)\), \(7\)-O-methyl-kaempferol \((7)\), and \(3’\)-7-di-O-methyl-quercetin \((8)\), were isolated and identified by spectral data (Supplemental Tables S2, S3 and S4) in comparison with those reported in literatures.

Compound 1 is a new member of the 3-alkylated coumarin family, which has valuable structural motifs with a wide range of applications in the chemical and pharmaceutical industries.\(^{18-20}\) To our knowledge, 3-(3,4-dihydroxybenzyl)-6,7-dihydroxy-6-methoxycoumarin isolated from *Onychium japonicum* is the closest naturally occurring precedent.\(^{21}\) In addition, it should be noted that 1 shares the same structural core with warfarin (Supplemental Figure S1), a famous synthetic 3-alkylated coumarin used as an anticoagulant drug. However, no inhibitory activity was observed for 1 in the evaluation of in vitro anti-platelet aggregation activity, suggesting that the substitution pattern of the core structure is critical for the potent anticoagulant effect.

All isolated compounds were tested for in vitro anti-inflammatory activities by measuring the NO production in LPS-stimulated RAW264.7 mouse macrophages and the results are shown in Table 1. Among them, only compounds 5 and 7 exhibited inhibition of NO production with IC\(_{50}\) values of 24.54 ± 0.36 μM and 10.60 ± 0.15 μM, respectively. L-NMMA was used as a positive control and presented an IC\(_{50}\) value of 39.82 ± 1.92 μM.

In summary, compound 1, a new racemic 3-alkylated coumarin, 3 known coumarin analogues \((2-4)\), and 4 known flavonoids \((5-8)\) were isolated from the folk medicinal plant *A. acuta*. Compound 1 was further resolved on a chiral column to yield \((-\)-1 and \((+\)-1 whose absolute configurations were elucidated by comparison of the observed and calculated ECDs. The new naturally occurring 3-alkylated coumarins \([(-\)-1 and \([(+\)-1] identified in this research expand the chemical space of coumarins. Among these isolates, compounds 5 and 7 showed inhibitory activities against NO production in activated macrophages. The findings may provide a clue for understanding the anti-inflammatory mechanism of *A. acuta*.

### Experimental

**General**

Optical rotations were obtained on a Jasco P-1020 Automatic Digital Polarimeter (JASCO), UV measurements on a Shimadzu UV-2700 (Shimadzu), and ECD data with a Jasco J-810 CD spectrometer (Agilent). \(^1\)H NMR (500 MHz), \(^13\)C NMR (125 MHz), and 2D NMR spectra were recorded on a Bruker AV-600 instrument (Bruker) (CD3OD, δ\(_{H}\) 3.30, δ\(_{C}\) 49.0). ESIMS data were obtained on an MDS SCIEX API 2000 LC/MS instrument (SCIEX), and HRESIMS data on an Agilent G6230 TOF mass spectrometer (Agilent). Semipreparative HPLC was performed with an HPLC system equipped with a Shimadzu LC-20AR pump (Shimadzu) using a YMC-pack ODS-A column (5 μm, 10 × 250 mm, YMC). Chiral resolution was performed using a Daicel Chiralpak IC type chiral HPLC column (3 μm, 4.6 × 250 mm, Daicel). For column chromatography, silica gel 60 (100 - 200 mesh, Qingdao Marine Chemical Ltd) and YMC ODS (75 μm, YMC) were used. TLC was performed using HSGF254 silica gel plates (Yantai Jiayou Silica Gel Development Co. Ltd).

### Plant Material

The whole herbs of *A. acuta* Lour were collected from Nanning City (22°45′N, 108°26′E), Guangxi Zhuang Autonomous

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**Table 1.** NO Inhibitory Activity of Isolated Compounds.

| Compound | IC\(_{50}\) (μM)\(^a\) |
|----------|-------------------------|
| 1a       | >50                     |
| 1b       | >50                     |
| 2        | >50                     |
| 3        | >50                     |
| 4        | >50                     |
| 5        | 24.54 ± 0.36            |
| 6        | >50                     |
| 7        | 10.60 ± 0.15            |
| 8        | >50                     |
| L-NMMA\(^b\) | 39.82 ± 1.92          |

\(^a\)The values represent mean ± SD from triplicate tests.

\(^b\)Positive control.
Region, China in June 2017, and identified by Prof. Songji Wei of College of Pharmacy, Guangxi University of Traditional Chinese Medicine, where a voucher specimen (No. YPC-001) is kept at the College of Pharmacy, Guangxi University of Traditional Chinese Medicine.

**Extraction and Isolation**

The air-dried, powdered aerial parts of *A. acuta* (15.0 kg) were extracted with 95% ethanol (35 L, 72 h), followed by 75% ethanol (2 × 35 L, each 72 h). A suspension of the concentrated ethanol extract (1.3 kg) in H2O (3 L) was partitioned in sequence with light petroleum, EtOAc and n-butanol (saturated with H2O), each 3 × 3 L, to give fractions soluble in light petroleum (60 g), EtOAc (200 g), n-butanol (500 g), and water (480 g), after evaporation under reduced pressure at 45°C. The EtOAc-soluble portion (200 g) was subjected to Si gel CC, eluted with a light petroleum/EtOAc gradient system, to afford fractions A to I. Fraction A (4.6 g) was chromatographed on a silica gel column, eluted with step gradient mixtures of light petroleum/EtOAc, followed by recrystallization with methanol to afford compound 6 (1.0 mg) as yellow needles. Fraction C (2.4 g) was subjected to CC on silica gel, eluted with a light petroleum/EtOAc gradient, and Sephadex LH-20, eluted with light petroleum − CHCl3 − CH3OH (1:1:1), followed by RP-18 semipreparative HPLC, to yield 1 (4.5 mg; CH3OH − H2O 11:9) to give 2 (4.0 mg; tR = 10.5 min), 7 (4.5 mg; tR = 27.0 min), and 8 (6.3 mg; tR = 29.9 min). Similarly, fractions E (4.5 g), G (1.0 g), and H (7.5 g) were fractionated on a Sephadex LH-20 column (light petroleum − CHCl3 − CH3OH 1:1:1), followed by separation by RP-18 semipreparative HPLC, to yield 5 (1.7 mg; CH3OH − H2O 7:3; tR = 12.5 min) and 3 (4.3 mg; CH3OH − H2O 2:8; tR = 22.5 min) from fraction E, and 1 (2.8 mg; CH3OH − H2O 4:6; tR = 19.3 min) from fraction G, and 4 (5.0 mg; CH3OH − H2O 2:8; tR = 11.5 min) from fraction H.

**Chiral Separation of 1**

Compound 1 (2.8 mg) was optically separated over a Daicel Chiralpak IC type chiral HPLC column using n-hexane/isopropanol (85:15) as eluent at a flow rate of 1 mL/min, yielding (−)-1 (1.1 mg; tR = 52.2 min) and (+)-1 (1.0 mg; tR = 64.6 min).

(±)-acutamarin (I): yellow, amorphous solid; UV (MeOH) λmax (log ε): 289 (3.97), 344 (4.14); 1H and 13C NMR data, see Table 2; HRESIMS: m/z 351.0842 [M+Na]+ (calcd for C18H16NaO6, 351.0845).

(−)-R-1: [α]D20 = −26.3 (c 0.12, MeOH); ECD (MeOH) λmax (Δε) 206 (−18.2), 237 (+ 2.0), 262 (−1.6), 347 (+ 0.7) nm.

(+)−S-1: [α]D20 + 22.4 (c 0.10, MeOH); ECD (MeOH) λmax (Δε) 206 (+ 20.2), 237 (−1.6), 262 (+ 1.6), 347 (−1.1) nm.

**Anti-Inflammatory Assay**

The anti-inflammatory activity of compounds 1 to 8 was evaluated by measuring inhibitory effects on NO production in LPS-induced RAW 264.7 macrophage cells. Briefly, RAW264.7 cells were seeded in 96-well plates and stimulated with 1 μg/mL of LPS. Test compounds (final concentration 50 μM) were added to 96-well plates at the same time. After overnight incubation, NO production was evaluated by measuring the absorbance at 570 nm. MTS was added to the remaining medium to detect cell viability to eliminate the compounds’ toxic effect on cells. L-NMMA was used as a positive control.

**Anti-Platelet Aggregation Activity Assay**

The anti-ADP-induced rabbit platelet aggregation assays were carried out as in the previous report.

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**Declaration of Conflicting Interests**

The authors declared no potential conflicts of interest with respect to the research, authorship, and/or publication of this article.

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### Table 2. 1H (500 MHz) and 13C (125 MHz) NMR Data for 1 in CD3OD.

| No. | δC, type | δH, mult. (J in Hz) |
|-----|----------|---------------------|
| 2   | 164.1, C |                     |
| 3   | 131.0, C |                     |
| 4   | 140.1, CH| 7.57, s             |
| 4a  | 113.0, C |                     |
| 5   | 109.7, CH| 7.07, s             |
| 6   | 147.0, C |                     |
| 7   | 151.9, C |                     |
| 8   | 103.5, CH| 6.74, s             |
| 8a  | 150.0, C |                     |
| 1'  | 136.9, C |                     |
| 2'  | 115.9, CH| 6.71, d (2.0)       |
| 3'  | 146.2, C |                     |
| 4'  | 144.9, C |                     |
| 5'  | 116.3, CH| 6.70, d (8.1)       |
| 6'  | 119.9, CH| 6.61, dd (8.1, 2.1) |
| 7'  | 39.8, CH | 4.08, q (7.2)       |
| 8'  | 20.6, CH3| 1.52, d (7.2)       |
| 6-OCH3 | 56.8, CH3| 3.88, s             |
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