# A New Cyclooxygenase Inhibitor from *Incarvillea arguta*

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A new anti-inflammatory spirovetivane-type sesquiterpenoid, designated as 1,10-didehydrolubimin (**1**) and thirteen known compounds (**2** – **14**) were isolated from the whole plants of the '*Yi*' ethnomedicinal plant *Incarvillea arguta*. Their structures were established based on spectral methods and by comparison of the spectral data with those reported previously. Compound **1** exhibited significant inhibitory effects on Cox-1 and Cox-2 and a negative effect against 5-Lox at the concentration of 100 $\mu$M.

*Key words: Incarvellea arguta*, Bignoniaceae, 1,10-Didehydrolubimin, Cyclooxygenase Inhibitor

## Introduction

*Incarvillea arguta* (Royle) Royle, family Bignoniaceae, called 'wabuyou', was mainly used to treat hepatitis, pharyngitis (by decoction drinking) and rheumatism (by pasting on the lesion) by the local 'Yi' people in the Yunnan and Sichuan Provinces, P. R. China [1]. Its specifications were promulgated in detail due to the favorable treatment [2]. It is generally agreed that proinflammatory leukotrienes (LTs) produced *via* the lipoxygenase (Lox) pathway, and prostaglandins (PGs) produced *via* the cyclooxygenases (Cox) pathway, are associated with adverse physiologic processes such as inflammation, fever and arthritis. For example, the Cox-2 pathway was a contributing factor to hepatic inflammation and fibrosis [3]. Particularly, Cox-2 and 5-Lox produce high amounts of prostaglandins and leukotrienes, respectively, in pathological lesions [4]. In order to discover the anti-inflammatory active secondary metabolites, the chemical investigation on this plant was carried out. Herein, we describe the isolation and structure elucidation of the new compound 1,10-didehydrolubimin (**1**) and thirteen known compounds (**2** – **14**) (Fig. 1) from the whole plants of *Incarvillea arguta*. The anti-inflammatory activity of 1,10-didehydrolubimin (**1**) against Cox-1, Cox-2 and 5-Lox was evaluated.

## Results and Discussion

Compound **1** was obtained as a colorless oil and has a molecular formula of $C_{15}H_{22}O_2$ based on the positive HRMS ((+)-ESI) (*m/z* = 235.1703, calcd. 235.1698 for $C_{15}H_{23}O_2$, $[M+H]^+$). The IR spectrum showed absorptions for hydroxyl (3423 $cm^{-1}$) and conjugated carbonyl groups (1694 $cm^{-1}$), respectively. The $^1$H NMR spectrum of **1** exhibited the presence of two methyl groups at $\delta$ = 1.03 (3H, d, $J$ = 6.9 Hz) and 1.75 (3H, s), a proton attached to a carbon atom bearing an oxygen atom at $\delta$ = 4.50 (1H, m), a terminal double bond at $\delta$ = 4.75 (1H, s) and 4.69 (1H, s), and an olefinic proton at $\delta$ = 6.49 (1H, s). The $^{13}$C NMR and DEPT spectra of **1** suggested two methyl carbons ($\delta$ = 16.8, 20.5), five methylene carbons ($\delta$ = 32.1, 33.2, 37.5, 41.1, 108.9), five methine carbons ($\delta$ = 38.9, 49.0, 67.7, 151.5, 194.8), and three quaternary carbons ($\delta$ = 46.5, 148.0, 150.6). Compared with the spectral data of lubimin [5], signals for one methylene carbon ($\delta$ = 25.9) and one methine carbon ($\delta$ = 58.4) disappeared; instead, an additional pair of olefinic carbons at $\delta$ = 151.6 and 150.6 were present, which suggested that dehydrogenation occurred in **1**. Besides, the chemical shift of the aldehyde carbon in lubimin ($\delta$ = 204.9) was shifted up-field to $\delta$ = 194.8. These facts indicated that the double bond is conjugated with the aldehyde group in **1**. This conjugation was confirmed by the correlations of the signals at $\delta$ = 46.5 (s, C-5) with $\delta$ = 9.43 (1H, s, H-14) and $\delta$ = 6.49 (1H, br. s, H-1), and $\delta$ = 194.8 (C-14, d) with $\delta$ = 6.49 (1H, br. s, H-1) in the HMBC spectrum of **1** (Fig. 2). The $\alpha$-orientations of the hydroxyl group at C-2 and the methyl at C-4 and H-8 were deduced from the X-ray diffraction analysis of its derivative 3-hydroxylubimin [6] and confirmed

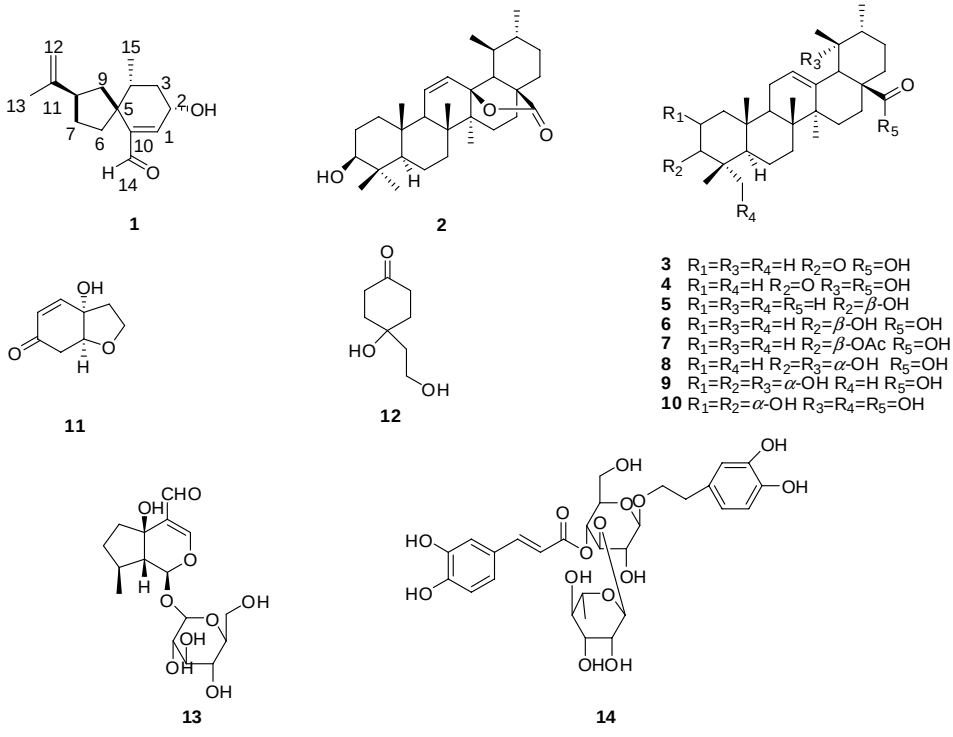


Fig. 1. Chemical structures of compounds **1** – **14**.

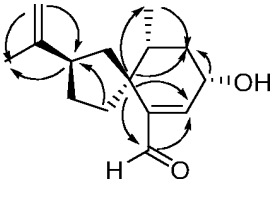

Fig. 2. Key HMBC correlations for compound **1**.

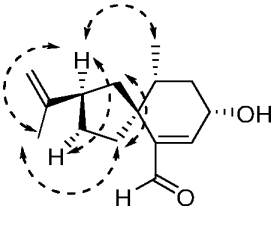

Fig. 3. Key ROESY correlations for compound **1**.

by the correlations of H-8 ($\delta$ = 2.61, m) with H-15 ($\delta$ = 1.03, d, $J$ = 6.9 Hz) and H-13 ($\delta$ = 1.75, s) in the ROESY spectrum of **1** (Fig. 3).

Based on the MS, $^1$H and $^{13}$C NMR data, the thirteen known compounds were determined to be ursolic acid lactone (**2**) [7], ursonic acid (**3**) [8], pomonic acid (**4**) [9], ursolic aldehyde (**5**) [10], ursolic acid (**6**) [11], acetylursolic acid (**7**) [12], 3-epipomolic acid (**8**) [13, 14], euscaphic acid (**9**) [15], myrianthic acid (**10**) [16], rengyolone (**11**) [17], cleroindicin B (**12**) [18], stansioside (**13**) [19], and acteoside (**14**) [20].

| Compound | Cox-1 | Cox-2 | 5-Lox |
|---|---|---|---|
| **1** | 90.7 | 89.9 | $< 0$ |
| SC-560 | 61.3 | – | – |
| NS-398 | – | 97.1 | – |
| Zileuton | – | – | 83.1 |

Table 1. *In vitro* anti-inflammatory activity of compound **1** (percentage inhibition).

The new compound was tested for anti-inflammatory activity against Cox-1, Cox-2 and 5-Lox by using the absorbance determination method (Table 1). After dissolving compound **1** in DMSO, the solvent was diluted with normal saline to a concentration of 100 $\mu$M. Using SC-560, NS-398 and zileuton as positive control, the percentage inhibition of compound **1** to Cox-1, Cox-2 and 5-Lox was 90.7 %, 89.9 % and negative, respectively.

## Experimental Section

*General*

Column chromatography was performed over silica gel (200 – 300 mesh, Qingdao Marine Chemical Inc., China), RP-18 silica gel (40 – 65 $\mu$m, Merck Company) and Sephadex LH-20 (40 – 70 $\mu$m, Pharmacia Fine Chemical Co., Ltd., Sweden). TLC was carried out on silica gel G plates, and spots were detected by 10 % sulfuric acid reagents

followed by heating. NMR spectra were recorded on Bruker AM-400 and DRX-500 spectrometers with TMS as internal standard. Optical rotation was measured with a Horiba SEPA-300 polarimeter. The IR spectrum was obtained on a Bruker Tensor-27 spectrometer with KBr pellets; MS spectra were recorded on an API Qstar Pulsar I spectrometer. The reagents used in the anti-inflammatory experiments such as Cox-1, Cox-2, 5-Lox, TMPD, assay buffer, arachidonic acid, NS-398, and the colorimetric substrate were all purchased from Cayman Chemical, U. S. A. The 96-well microplate and heme were purchased from Corning and Sigma, U. S. A, respectively. The absorbance values were determined on Safire 2 (Switzerland).

*Plant material*

The whole plants of *Incarvillea arguta* were collected in Yunnan Province, China in September 2006 and identified by Dr. Chun-Xia Zeng, Kunming Institute of Botany, CAS. A voucher specimen (KUN 20060912) has been deposited in the Herbarium of Kunming Institute of Botany, CAS.

*Extraction and isolation*

Air-dried powdered whole plants of *Incarvillea arguta* (2.4 kg) were extracted with 95 % aqueous MeOH (4 L) at r. t. (48 h × 3). After evaporating the solvents *in vacuo* at 50 °C, a residue (304 g) was obtained, which was dissolved in $H_2O$ (1 L) and then partitioned with EtOAc (1 L × 3). The EtOAc extract (100 g) was subjected to silica gel column chromatography (8.6 × 150 $cm^2$) and eluted with $CHCl_3$-$Me_2CO$ (from 1 : 0 to 1 : 1 v/v) to give seven fractions (I – VII). Fr. III (19.0 g) was subjected to silica gel CC (3.6 × 90 cm), eluting with $CHCl_3$-$Me_2CO$ (from 1 : 0 to 4 : 1 v/v) to afford five sub-fractions (A – E). Subfraction C (1.44 g) was then applied to the RP-18 chromatograph (1.4 × 30 cm) and eluted with MeOH-$H_2O$ (75 – 100 %, v/v) to give nine subfractions (C1-C9). Separation of C1 (170 mg) by silica gel CC (1 × 40 cm) eluted with petroleum ether-$Me_2CO$ (10 : 1) yielded compound **1** (11.0 mg).

*1,10-Didehydrolubimin (**1**)*

Colorless oil. – UV ($CHCl_3$): $\lambda_{max}$ ($\lg \varepsilon_{max}$) = 242 (4.40), 232 (4.04), 225 (4.05), 200 (3.98) nm. – $[\alpha]_D^{20}$ = +39.5 ($c$ = 0.75, $CHCl_3$). – IR (KBr): $\nu$ = 3423 (OH), 1694 (C=O) $cm^{-1}$. – $^1$H NMR ($CDCl_3$, 500 MHz): $\delta$ = 6.49 (br. s, 1H, H-1), 4.50 (m, 1H, H-2), 1.91, 1.36 (m, each 1H, H-3), 1.72 (t, $J$ = 4.9 Hz, 1H, H-4), 1.56 (t, $J$ = 3.7 Hz, 2H, H-6), 2.18, 1.86 (m, each 1H, H-7), 2.61 (m, 1H, H-8), 2.22, 1.48 (m, each 1H, H-9), 4.75, 4.69 (s, each 1H, H-12), 1.75 (s, 3H, H-13), 9.43 (s, 1H, H-14), 1.03 (d, $J$ = 6.9 Hz, 3H, H-15). – $^{13}$C NMR ($CDCl_3$, 125 MHz): $\delta$ = 151.6 (CH, C-1), 67.7 (CH, C-2), 37.5 ($CH_2$, C-3), 38.9 (CH, C-4), 46.5 (C, C-5), 33.2 ($CH_2$, C-6), 32.1 ($CH_2$, C-7), 49.1 (CH, C-8), 41.2 ($CH_2$, C-9), 150.6 (C, C-10), 148.0 (C, C-11), 108.9 ($CH_2$, C-12), 20.5 ($CH_3$, C-13), 194.8 (CH, C-14), 16.8 ($CH_3$, C-15). – HRMS ((+)-ESI): $m/z$ = 235.1703 (calcd. 235.1698 for $C_{15}H_{23}O_2$, $[M+H]^+$).

*In vitro anti-inflammatory assay*

The *in vitro* anti-inflammatory activity was performed according to the literature with minor modifications [21]. Briefly, the reaction system was incubated for 5 min at 25 °C after putting the collocated assay buffer, heme, compound **1** and Cox-1 or Cox-2 into the reaction system by sequence and agitated softly for several seconds after mixing with TMPD and arachidonic acid. The absorbance value was recorded at a wavelength of 590 nm after another 15 min of incubation at 25 °C. The performance of the assay was checked using SC-560 and NS-398 as positive controls. Compared with the inhibitory activity (percentage inhibition) of these positive controls to Cox-1 (61.3 %) and Cox-2 (97.1 %), the inhibitory effects of compound **1** on Cox-1 and Cox-2 were 90.7 % and 89.9 %, respectively. Different from the method mentioned above, the reaction system was added to the assay buffer with 5-Lox in the presence of the colorimetric substrate and compound **1** and then incubated for a period of 5 min at 25 °C. After the completion of the reaction, the chromogen was added, and the plate was shaken softly for a few seconds. Then another period of 5 min incubation was performed at 25 °C. The inhibitory effect against 5-Lox was determined by measuring the absorbance at a wavelength of 500 nm. Compared with the inhibitory activity of zileuton (positive control) to 5-Lox (83.1 %), the inhibition of compound **1** to 5-Lox was negative (Table 1).

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