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Curcumolide, a unique sesquiterpenoid with anti-inflammatory properties from *Curcuma wenyujin*

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ABSTRACT

Curcumolide, a novel sesquiterpenoid with a unique 5/6/5 tricyclic skeleton, was isolated from *Curcuma wenyujin*. The structure and absolute configuration were elucidated by extensive NMR, ECD data analysis, and a single-crystal X-ray study. This molecule exhibited significant anti-inflammatory effects in LPS-induced RAW 264.7 macrophages. It suppressed LPS-induced NF-κB activation, including the nuclear translocation and DNA binding activity of NF-κB, and decreased tumor necrosis factor-α (TNF-α), interleukin-6 (IL-6), interleukin-1β (IL-1β), nitric oxide (NO) and reactive oxygen species (ROS) production. Therefore, Curcumolide may have therapeutic potential for treating inflammatory diseases by inhibiting NF-κB activation and pro-inflammatory mediator production.

Chronic inflammation is a hallmark of many human diseases, such as rheumatic arthritis, arteriosclerosis, obesity, diabetes, neurodegenerative diseases and even cancer.[1–6] Steroids and cyclooxygenase inhibitors have remarkable effects on anti-inflammatory, which have been applied in clinic for more than one century. However, they often suffer from several side effects in patients, such as atrial fibrillation, gastrointestinal erosions, and renal and hepatic insufficiency.[7,8] Thus, there is an urgent need to find new and safe anti-inflammatory compounds. The nuclear factor-kappa B (NF-κB) pathway has been implicated in the pathogenesis of chronic inflammatory diseases.[9] NF-κB, mainly the p50–p65 heterodimer, normally exists as an inactive state tightly bound to the inhibitory protein of IκB in the cytoplasm. In response to a variety of stimuli, such as inflammatory cytokines, bacterial lipopolysaccharides (LPS), and reactive oxygen species (ROS), the proteasome dependent degradation of IκB allows the translocation of NF-κB to the nucleus, interacting with κB elements in the promoter region of a variety of inflammatory response genes, and induces the transcription of pro-inflammatory mediators, including tumor necrosis factor (TNF)-α, interleukin (IL) 1β, IL-6, inducible nitric-oxide synthase (iNOS), and cyclooxygenase (COX). These mediators play important roles in mediating inflammatory responses.[10] Inhibition of these mediators is beneficial for the treatment of inflammatory diseases and has become an important strategy.[11]

Curcuma wenyujin Y.H. Chen et C. Ling (Zingiberaceae) is cultivated in the southeast of China, its rhizomes have been used as a Traditional Chinese Medicine for the treatment of arthralgia, jaundice, thoracic-abdominal pain, and dysmenorrhea.[12] Phytochemical studies on this plant have led to the isolation of various sesquiterpenoids including guaiane, cadinane, germacrane and eudesmane with different oxygenations and cleavage patterns,[13–20] which shows anti-inflammatory,[21] antitumor,[22–24] antioxidant,[17] and inhibition of platelet aggregation.[25] In order to determine the anti-inflammatory constituents, a novel sesquiterpenoid with a rare 5/6/5 tricyclic skeleton, namely curcumolide (**1**), was discovered from this plant. In this study, the isolation, structural elucidation, and anti-inflammatory activities of the compound are reported.

Dried rhizomes of *C. wenyujin* (20 kg) were powdered and extracted with 95% EtOH (10 L × 3) at room temperature. The Ethanol extract was evaporated to dryness under reduced pressure and the residue (1700 g) was suspended in water and then partitioned successively with petroleum ether (bp 60–90 °C), EtOAc, and *n*-BuOH to give three corresponding portions. The EtOAc extract (600 g) was subjected to silica gel column chromatography and eluted with a petroleum ether–Me_2CO (50:1 to 1:1) gradient solvent system to afford eight major fractions (1–8). Fraction 4 (35 g) was subjected to silica gel column chromatography by elution with petroleum ether–EtOAc (20:1 to 1:1) to afford four

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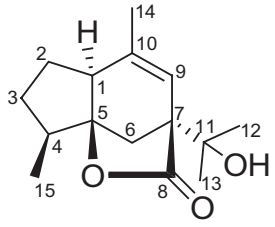
subfractions (4a–4d). Subfraction 4d (8 g) was separated on silica gel column chromatography eluting with petroleum ether–Me_2CO (20:1), and further purified by an ODS column (C-18, eluted with MeOH–H_2O 70:30) to obtain compound **1** (46 mg, >99% purity, as determined by HPLC).



Curcumolide (1)

Curcumolide (**1**),[26] obtained as colorless crystals, had a molecular formula of $C_{15}H_{22}O_3$ as determined by HRESIMS (*m*/*z* 273.1469 $[M+Na]^+$, calcd 273.1461) and NMR data, indicating five degrees of unsaturation. The ^1H NMR spectrum of **1** showed four methyl group signals, including an olefinic methyl singlet at δ_H 1.71 (s, H_3-14), a methyl doublet at δ_H 1.06 (d, J = 6.2 Hz, H_3-15)], and two methyl singlets at δ_H1.21 (s, H_3-12) and δ_H 1.33 (s, H_3-13), in addition to an olefinic proton signal at δ_H 5.94 (s, H-9). The ^{13}C NMR spectrum presented a total of fifteen carbon resonances, including a carbonyl carbon (δ_C 179.4), two olefinic carbons [δ_C 139.4 (qC) and 121.7 (CH)], two oxygenated carbons [δ_C 92.1 (qC) and 70.7 (qC)] (Table 1).

The HMQC spectrum assigned all protons and corresponding carbons in the molecule. Two degrees of unsaturation, accounted for by the functional groups (a carbonyl and an olefinic double bond) from five in the molecule, suggested a tricyclic structure in **1**. Detailed analysis of 2D NMR spectroscopic data established a rare structure of the 5/6/5-membered tricyclic backbone. The ^1H–^1H COSY correlations allowed to establish the partial structure of consecutive proton system extending from H-1 [δ_H 2.49 (1H, t, J = 11.4 Hz)] to H_3-15 via H_2-2 [δ_H 1.91 (1H, m), 1.59 (1H, m)], H_2-3 [δ_H 2.07 (1H, m), 1.54 (1H, m)], and H-4 (δ_H 2.08 (1H, m)). The HMBC correlations observed from H_3-15 to C-3 (δ_C 29.9), C-4 (δ_C 38.4), and C-5 (δ_C 92.1), from H_3-14 to C-1 (δ_C 51.2), C-10 (δ_C 139.4), and C-9 (δ_C 121.7), from H-1 to C-4, C-5, and C-6 (δ_C 37.1), and from H_2-6 [δ_H 2.34 (1H, d, J = 10.8 Hz), 1.77 (1H, d, J = 10.8 Hz)] to C-7 (δ_C 56.8) and C-9, led to the assignment of a cyclopentane moiety (ring A) fused to a six-membered ring B at C-1 and C-5, and ring B contained a methyl group and double bond that were located at C-10 and C-9/C-10, respectively. Additional HMBC correlations from both H_3-12 and H_3-13 to a hydroxylated quaternary carbon C-11 at δ_C 70.7 (qC), two methyl carbon at δ_C 24.9 (CH_3) and 26.1 (CH_3), and a quaternary carbon C-7 indicated the attachment of a 2-hydroxyisopropane group to C-7. Moreover, the presence of a γ-lactone moiety (ring C) fused to ring B at C-5 and C-7 was revealed by the HMBC correlations from H_2-6 to an oxygenated quaternary carbon C-5, C-7, and carbonyl carbon C-8 (δ_C 179.4), and from olefinic proton H-9 to C-6, C-7, and C-8, in association with the IR absorption at 1739 cm^{-1}. Consequently, the gross structure of **1** was elucidated as shown in Figure 1.

The relative stereochemistry of **1** was established by NOESY spectroscopic analysis (Fig. 1). The key NOE cross peaks were observed between H-1/H-4, H-1/H-6a, H-6a/H_3-12, and H-6a/H_3-13, suggesting that H-1, H-4, H-6a, and 2-hydroxyisopropane group were oriented toward the same side, and requiring rings A/B and B/C were *trans*- and *cis*-fused, respectively. An X-ray crystallography analysis was performed. A perspective of single molecule of **1** was given in Figure 2,[27] which confirmed the structure deduced by NMR studies.

The absolute configuration of **1** was established by measurement of the ECD spectrum and comparison with calculated ECD data.[28] According to the established relative configuration, curcumolide (**1**) should be one of the two enantiomers (1R,4R,5R,7R)- or (1S,4S,5S,7S)-. As shown in Figure 3, the experimental ECD spectra agreed well with the calculated ECD curves of (1S,4S,5S,7S)-enantiomer. Thus, the absolute configuration of **1** was established as 1S,4S,5S,7S.

In order to identify the anti-inflammatory effect of **1**, the pro-inflammatory cytokines (TNF-α, IL-1β, IL-6), nitric oxide (NO), ROS, and NF-κB were measured in LPS-induced RAW 264.7 macrophages treated with **1**, and the viability of cultured RAW

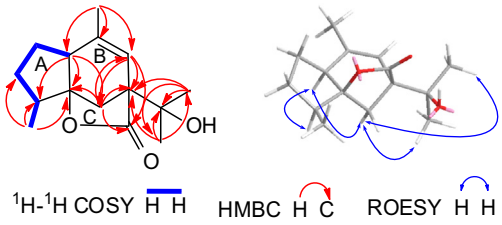


Figure 1. Key 2D NMR correlations of **1**.

Table 1
^1H (600 MHz) and ^{13}C (150 MHz) NMR Data of **1**[a] in $CDCl_3$ (δ in ppm, J in Hz)

No.	δ_H	δ_C
1	2.49 (1H, t, 11.4)	51.2 CH
2a	1.91 (1H, m)	23.3 CH_2
2b	1.59 (1H, m)	
3a	2.07 (1H, m)	29.9 CH_2
3b	1.54 (1H, m)	
4	2.08 (1H, m)	38.4 CH
5		92.1 qC
6a	2.34 (1H, d, 10.8)	37.1 CH_2
6b	1.77 (1H, d, 10.8)	
7		56.8 qC
8		179.4 qC
9	5.94 (1H, s)	121.6 CH
10		139.4 qC
11		70.7 qC
12	1.21 (3H, s)	24.9 CH_3
13	1.33 (3H, s)	26.1 CH_3
14	1.71 (3H, s)	20.5 CH_3
15	1.06 (3H, d, 6.2)	12.4 CH_3

[a] Data were assigned by HSQC, HMBC, ^1H–^1H COSY, and ROESY spectra.

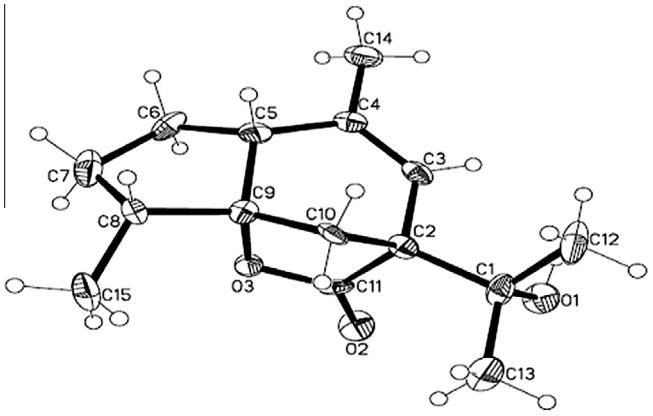


Figure 2. X-ray structure of **1**.