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ORIGINAL ARTICLE

Two new cucurbitane-type triterpenoids from the peel of *Trichosanthes kirilowii*

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Abstract: Two new cucurbitane-type triterpenoids (**1–2**) and one known compound (**3**) were obtained from the ethyl acetate extract of *Trichosanthes kirilowii* Maxim. The structures of these compounds were elucidated on the basis of spectroscopic data analysis and comparison with spectroscopic data in the literature. All compounds were evaluated for anti-inflammatory activity in LPS-induced RAW264.7 cells. Compounds **1** and **2** could inhibit the secretion of IL-6, TNF-α, and NO, with IC_{50} values ranging from 34.82 ± 1.54 to 86.14 ± 1.94 μM.

Keywords: *Trichosanthes kirilowii*, triterpenoids, anti-inflammatory

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1 Introduction

Trichosanthes kirilowii Maxim., a medicinal plant, is mainly distributed in the provinces of Hebei, Anhui, Henan, and Shandong in China. The peel of *Trichosanthes kirilowii* possesses the efficacy of clearing heat, resolving phlegm, promoting qi circulation, and relieving chest pain, which has been widely used in traditional Chinese medicine (Yu et al., 2018). The previous phytochemical investigations showed that the peel of *Trichosanthes kirilowii* contains diverse bioactive constituents, including terpenoids (Yang et al., 2023; Zou et al., 2025), flavonoids (Li et al., 2014), sterols (Yu et al., 2018), alkaloids (Lei et al., 2021; Fan et al., 2012), polysaccharides (Jing et al., 2024) and fructofuranoside (Lian et al., 2012). Among terpenoids, cucurbitane-type triterpenoids bearing a hydroxyl group at the C-16 position are commonly found in *Trichosanthes kirilowii* peel, however, substitution of the hydroxyl group at this position rarely occurs. Many of these compounds have been reported to exhibit anti-hyperlipidemia (Jing et al., 2024), anti-inflammatory (Lei et al., 2021; Zou et al., 2025), and anti-complementary activity (Yang et al., 2023; Zou et al., 2025). Furthermore, our preliminary experiment demonstrated that the water extract derived from the peel of *Trichosanthes kirilowii* exhibited anti-inflammatory activity, inhibiting NO production by 78.51% at a concentration of 50 mg/mL. To explore compounds with anti-inflammation activity, the water extract of the peels of *Trichosanthes kirilowii* was further investigated.

In our study, two undescribed cucurbitane-type triterpenoids (**1–2**) and one known compound (**3**) were isolated from the peel of *Trichosanthes kirilowii*. The known compound was identified as (3′*R*, 9′*S*)-3′-hydroxy-5′-megastigmen-7′-yn-9′-yl *β*-D-glucopyranoside (**3**) (Skouroumounis et al., 1995) based on the comparison with NMR data in the literature. Here, we report the details of the isolation and structural characterization of the isolates, as well as their anti-inflammatory activity.

2 Materials and Methods

2.1 Plant Material

The peels of *Trichosanthes kirilowii* Maxim. were obtained from Qianshan City, Anhui Province, China and identified by Professor Qingshan Yang, School of Pharmacy, Anhui University of Traditional Chinese Medicine. A voucher specimen (ID-P-202212) was stored in the School of Pharmacy, Anhui University of Traditional Chinese Medicine.

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2.2 General Experimental Procedures

The NMR spectra were performed on a Bruker AVANCE NEO 600 spectrometer. ESI-HRMS were performed on a Dionex UltiMate 3000 UPLC Thermo Scientific Q Exactive Focus Mass spectrometer. Semi-preparative HPLC was conducted using a SEP LC 52 instrument equipped with a CAPCELL PAK-MG II C18 column (250*10 mM, 5 μM). Optical rotation measurements were conducted using a JASCO P2000 polarimeter. ECD spectra were recorded on a JASCO J-1500 spectrometer.

2.3 Extraction and Isolation

The dried peel of *Trichosanthes kirilowii* (14 kg) were powdered and extracted thrice with boiling water (54 L, 1.5 h each). The filtrate was concentrated under reduced pressure. Anhydrous ethanol was then added to achieve a final concentration of 75% (v/v). The mixture solution was allowed to stand and then filtered. The supernatant was concentrated under reduced pressure to remove ethanol and then subjected to liquid-liquid extraction with ethyl acetate. The ethyl acetate layer was evaporated under reduced pressure to afford the ethyl acetate fraction (80 g).

The ethyl acetate extract (80 g) was subjected to silica gel column chromatography using a gradient elution with dichloromethane-methanol (50:1 to 9:1, v/v) to afford 14 fractions (A1–A14). Fraction A3 was further separated by silica gel column chromatography using an ethyl acetate-methanol gradient (40:1 to 1:1, v/v), yielding three subfractions (A3.1–A3.3). Subfraction A3.1 was then chromatographed on a Sephadex LH-20 column, eluting with methanol, to give five fractions (A3.1.1–A3.1.5). Among these, A3.1.5 was further purified by Sephadex LH-20 column chromatography (methanol) to afford four fractions (A3.1.5.1–A3.1.5.4). Fraction A3.1.5.2 was chromatographed on an ODS column with a water-methanol gradient (90:10 to 0:100, v/v), yielding three subfractions (A3.1.5.2.1–A3.1.5.2.3). Subfraction A3.1.5.2.2 was further purified by Sephadex LH-20 column chromatography (methanol), and semi-preparative HPLC (acetonitrile-water containing 0.1% formic acid, 33:67, v/v) to afford compound **1** (9.5 mg). Subfraction A3.1.5.2.3 was subjected to semi-preparative HPLC (acetonitrile-water containing 0.1% formic acid, 28:72, v/v) to yield compound **2** (5.5 mg). Fraction A3.1.5.4 was chromatographed on an ODS column with a water-methanol gradient (80:20 to 0:100, v/v), affording four subfractions (A3.1.5.4.1–A3.1.5.4.4). Subfraction A3.1.5.4.2 was purified by silica gel column (dichloromethane-methanol, 29:1, v/v), Sephadex LH-20 column (methanol), and semi-preparative HPLC (acetonitrile-water containing 0.1% formic acid, 17:83, v/v) to give compound **3** (4.5 mg).

2.3.1 Trikirilolide A (1)

White amorphous powder. $[\alpha]_D^{20.0}$: +52 (*c* 0.097, MeOH); CD (MeOH) λ_{max} ($\Delta\varepsilon$): 211 (+81.7), 301 (+15.5) and 332 (−3.9) nM; ^{1}H NMR (CD_3OD, 600 MHz) and ^{13}C NMR (CD_3OD, 150 MHz) spectral data were shown in Table 1; HR-ESI-MS: *m/z* 625.2975[M+ Na]$^+$ (calcd for $C_{33}H_{46}O_{10}Na^+$, 625.2989).

2.3.2 Trikirilolide B (2)

White amorphous powder. $[\alpha]_D^{20.0}$: +113 (*c* 0.082, MeOH); CD (MeOH) λ_{max} ($\Delta\varepsilon$): 208 (+71.6), 298 (+48.3) and 332 (−9.2); ^{1}H NMR (CD_3OD, 600 MHz) and ^{13}C NMR (CD_3OD, 150 MHz) spectral data were shown in Table 1. ESI-HRMS: *m/z* 625.2975[M+ Na]$^+$ (calcd for $C_{33}H_{46}O_{10}Na^+$, 625.2989).

2.4 Anti-Inflammatory Activity Assay

The anti-inflammatory activity was evaluated using RAW264.7 macrophages, which were stimulated with lipopolysaccharide (LPS). RAW264.7 cells were inoculated into 96-well plates at a density of 1×10^5 cells/well, and cultured for 24 h in DMEM at 37°C with 5% CO_2. The experiment was divided into three groups: the blank control, the model control, and the treatment group. The blank control group contained only cells and culture medium. The model control group was induced with LPS (1 μg·mL^{-1}). The treatment group was treated with 1 μg·mL^{-1} LPS along with different concentrations of isolates (12.5~100 μmol·L^{-1}). Dexamethasone (DEX) served as the positive control. The cells were incubated for 24 h. The concentration of nitric oxide (NO) was determined by the Griess method (Green et al., 1982), and the levels of TNF-α and IL-6 were measured using ELISA kits. Six replicates were set up for each experimental group.

3 Results and Discussion

3.1 Structure Elucidation

Compound **1** was obtained as a white amorphous powder. Its molecular formula was $C_{33}H_{46}O_{10}$ derived from a quasi-molecular ion peak at *m/z* 625.2975 [M+Na]$^+$ (calcd for $C_{33}H_{46}O_{10}Na^+$, 625.2989) in ESI-HRMS spectrum. The ^{1}H NMR spectrum (Table 1) exhibited eight singlet methyl signals at δ_H 0.99, 1.06, 1.28, 1.29, 1.34, 1.34, 1.35 and 1.42 (3H each, s), three olefin proton signals at δ_H 6.85 (1H, d, *J* = Hz Hz), 7.06 (1H, d, *J* = 15.6 Hz) and 5.80 (1H, m), indicating the presence of a double bond with an *E*-configuration, two oxygenated methine proton signals at δ_H 5.38 (1H, t, *J* = 7.8 Hz) and 4.57 (1H, dd, *J* = 13.2, 6.0 Hz). The ^{13}C NMR spectrum, combined with HSQC data, displayed 33 resonances, including 12 quaternary carbon signals (δC 49.1, 49.8, 51.6, 51.8, 71.5, 79.5, 142.1, 168.0, 170.0, 204.3, 213.9, 215.0), eight methyls (δ_C 19.2, 20.2, 20.6, 21.9, 24.8, 29.3, 29.3, 29.8), eight methines (δ_C 34.8, 43.7, 56.0, 76.4, 72.8, 120.6, 121.2, 156.6) and five methylenes (δ_C 24.8, 37.1, 43.9, 48.6, 49.7). An active methylene group (δ_H 3.16, δ_C 48.6) was observed in the NMR spectrum recorded in DMSO-d_6. The 1D NMR data of **1** were similar to those of cucurbitacin Y (Yang et al., 2023), except that a methoxyl group (δ_H 3.73, δ_C 52.8) was absent from **1**. The HMBC correlations (Figure 2) from H-16 to C-1′ confirmed that the malonate was attached at the C-16 position. Further analysis of ^{1}H-^{1}H COSY and HMBC data, the planar structure of **1** was established. The NOESY spectrum (Figure 3) showed the correlations of H-10 with H-2 and H_3-30, and of H-17 with H_3-30 and H_3-21, indicating that these protons were α-oriented. The NOESY correlations of H-16 with H_3-18, and of H-8 with H_3-18

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Table 1. ^{1}H NMR (600 MHz) and ^{13}C NMR(150 MHz) data of compounds **1** and **2** in CD_3OD

Position	1		2	
	δ_C	δ_H (*J* in Hz)	δ_C	δ_H (*J* in Hz)
1α	37.1	2.10 (1H, ddd, 12.2, 5.8, 3.5)	40.2	2.18 (1H, dd, 12.8, 4.8)
1*β*		1.20 (1H, ddd, 12.9, 12.2, 3.5)		2.33 (1H, t, 12.8)
2	72.8	4.57 (1H, dd, 13.2, 6.0)	211.9	–
3	213.9	–	81.4	3.95 (1H, s)
4	51.8	–	47.4	–
5	142.1	–	140.0	–
6	121.2	5.80 (1H, m)	122.6	5.96 (1H, br d, 5.2)
7α	24.8	2.40 (1H, m)	24.7	2.50 (1H, dd, 19.0, 7.2)
7*β*		1.96 (1H, m)		2.04 (1H, dt, 19.0, 5.8)
8	43.7	2.03 (1H, d, 7.9)	43.9	2.08 (1H, d, 8.0)
9	49.8	–	49.7	–
10	34.8	2.99 (1H, d, 12.9)	37.4	2.82 (1H, d, 12.8)
11	215.0	–	214.7	–
12α	49.7	3.50 (1H, d, 14.6)	49.4	3.35 (1H, d, 14.8)
12*β*		2.68 (1H, d, 14.6)		2.62 (1H, d, 14.8)
13	51.6	–	51.5	–
14	49.1	–	48.6	–
15α	43.9	1.99 (1H, dd, 13.8, 7.8)	44.0	1.95 (1H, dd, 14.0, 7.9)
15*β*		1.49 (1H, d, 13.8)		1.50 (1H, d, 14.0)
16	76.4	5.38 (1H, t, 7.8)	76.2	5.36 (1H, t, 7.8)
17	56.0	2.86 (1H, d, 7.4)	55.9	2.82 (1H, t, 7.2)
18	20.6	0.99 (3H, s)	20.7	0.97 (3H, s)
19	20.2	1.06 (3H, s)	20.3	1.17 (3H, s)
20	79.5	–	79.4	–
21	24.8	1.42 (3H, s)	24.8	1.40 (3H, s)
22	204.3	–	204.2	–
23	120.6	6.85 (1H, d, 15.6)	120.6	6.83 (1H, d, 15.6)
24	156.6	7.06 (1H, d, 15.6)	156.6	7.05 (1H, d, 15.6)
25	71.5	–	71.5	–
26	29.3	1.34 (3H, s)	29.3	1.33 (3H, s)
27	29.3	1.35 (3H, s)	29.3	1.35 (3H, s)
28	29.8	1.29 (3H, s)	24.6	1.29 (3H, s)
29	21.9	1.28 (3H, s)	21.6	0.85 (3H, s)
30	19.2	1.34 (3H, s)	19.2	1.24 (3H, s)
1′	168.0	–	168.3	–
[a]2′	48.6	3.16 (2H, s)	48.5	3.16 (2H, s)
3′	170.0	–	170.4	–

Note: [a]Recorded in DMSO-d_6.

and H_3-19 confirmed that protons of H-8, H-16, H_3-18, and H_3-19 were *β*-oriented. The absolute configuration of **1** was determined as 2*S*, 8*S*, 9*R*, 10*R*, 13*R*, 14*S*, 16*R*, 17*R*, 20*R* on the basis of the close similarity of its CD Cotton effects to those of cucurbitacin Y (Yang et al., 2023). Therefore, compound **1** was identified and named as trikirilolide A (Figure 1).

Compound **2**, a white amorphous powder, had the same molecular formula as compound **1**, $C_{33}H_{46}O_{10}$, based on the ESI-HRMS peak at *m/z* 625.2975 $[M+Na]^+$ (calcd for $C_{33}H_{46}O_{10}Na^+$, 625.2989). Detailed NMR analysis (Table 1) showed that the spectroscopic data of **2** were similar to those of **1**. The significant difference was observed in an oxygen-substituted methine group. A methine group (δ_H 3.95) was a singlet in **2**, while the corresponding methine signal (δ_H 4.57) in **1** exhibited a doublet of doublets (dd, *J* = 13.2, 6.0 Hz). The difference indicated that the methine group (δ_H 3.95) was located at the C-3 position in **2**. The HMBC correlations (Figure 2) from H-3 to C-2, C-4, C-28 and C-29, from H-28 to C-3, C-5 and C-29 confirmed the presence of a methine group at C-3 and a carbonyl group at C-2. In the NOESY spectrum, the NOESY correlations (Figure 3) of H-16 with H_3-18, and of H-8 with H_3-18 and H_3-19 confirmed that protons of H-8, H-16, H_3-18, and H_3-19 were *β*-oriented. The NOESY correlations of H-10 with H_3-29 and H_3-30, and of H-17 with H_3-30 and H_3-21, indicating that these protons were α-oriented. The NOESY correlations of H-3 with H-1*β* and

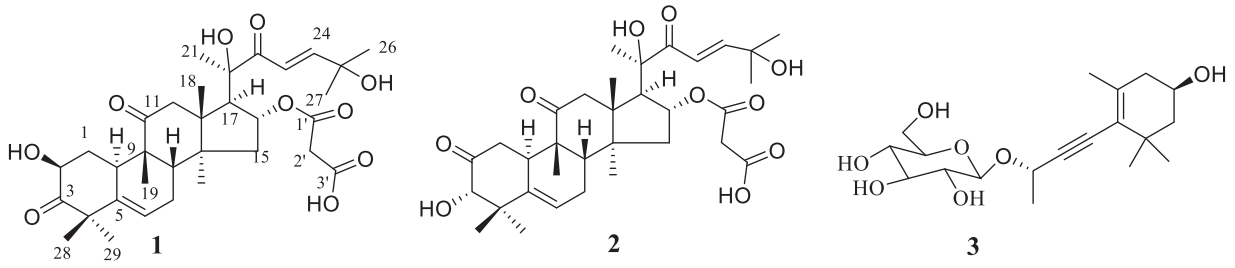

Figure 1. Structures of compounds **1–3**

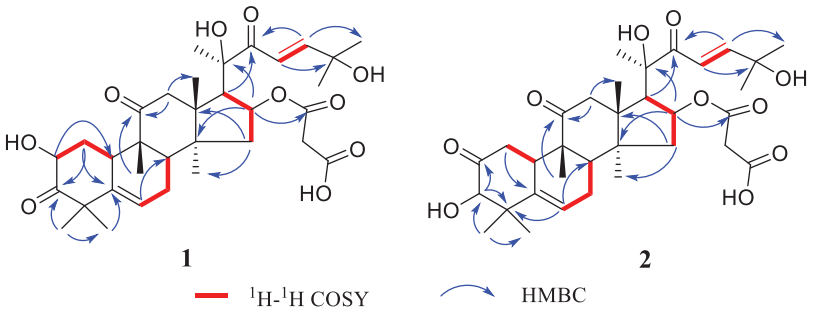

Figure 2. key ^{1}H–^{1}H COSY and HMBC correlations of compounds **1–2**

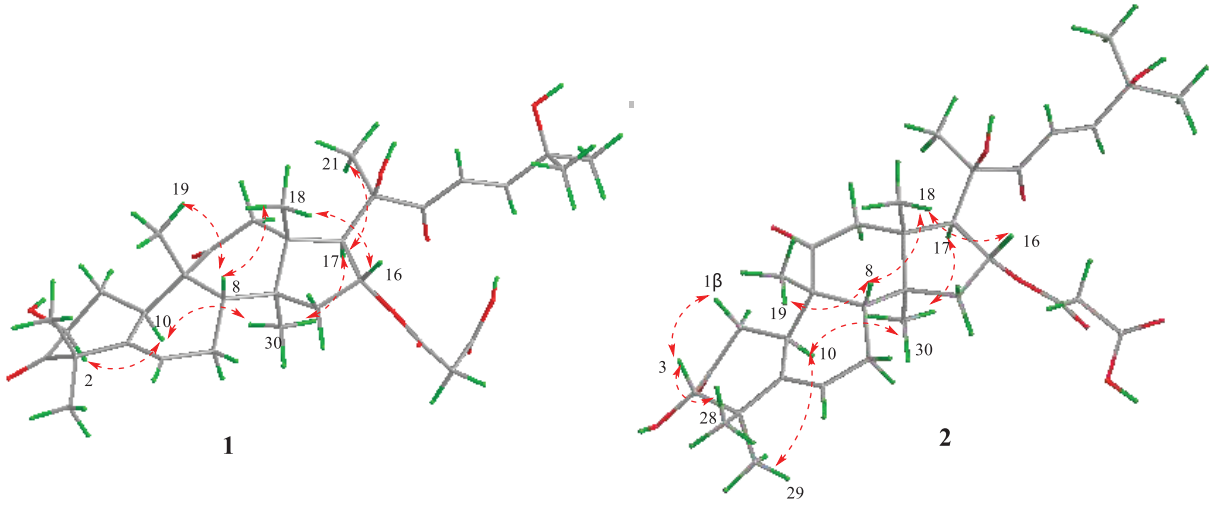

Figure 3. Key NOESY correlations of compounds **1–2**

H_3-28 indicated that the proton of H-3 is β-oriented (Sallam et al., 2010). The absolute configuration of **2** was assigned as 3*S*, 8*S*, 9*R*, 10*R*, 13*R*, 14*S*, 16*R*, 17*R*, 20*R* based on its similar CD spectrum to that of **1**. Thus, the structure of compound **2** was established, and named trikirilolide B.

3.2 Anti-Inflammatory Activity

The anti-inflammatory activities of compounds **1–3** were assessed in a LPS-induced RAW264.7 macrophage model by measuring the production of inflammatory mediators, including NO, TNF-α, and IL-6. The results (Table 2) showed that compounds **1** and **2** exhibited inhibitory effects on the production of NO, TNF-α, and IL-6 within the concentration range of 12.5–100 μM. The IC_{50} values of compound **1** for inhibiting the production of NO, TNF-α, and IL-6 were 34.82 ± 1.54, 55.54 ± 1.75, and 77.11 ± 1.85 μM, respectively, while those of compound **2** were 40.29 ± 1.61,

Table 2. Anti-inflammatory activity assays of compounds **1–3**

Compounds	IC_{50} Values(μM)		
	NO	TNF-α	IL-6
1	34.82 ± 1.54	55.54 ± 1.75	77.11 ± 1.85
2	40.29 ± 1.61	50.48 ± 1.70	86.14 ± 1.94
3	>100	64.57 ± 1.810	>100
DEX	13.93 ± 0.57	10.59 ± 0.93	13.12 ± 0.64

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50.48 ± 1.70, 86.14 ± 1.94 μM, respectively. Compound **3** exhibited inhibitory activity only against TNF-α production, with an IC_{50} of 64.57 ± 1.81 μM.

4 Conclusion

Three compounds were isolated from the peel of *Trichosanthes kirilowii*, including two new cucurbitane-type triterpenoids (**1–2**) and one known norsesquiterpenoid (**3**). Evaluation of anti-inflammatory activity revealed that two cucurbitane-type triterpenoids showed the potential anti-inflammatory effect.

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Author Contributions

Shulei Wan: Conceptualization, Methodology, Validation, Data curation, Formal analysis, Writing—original draft. Jiamin Yin: Conceptualization, Methodology, Validation, Data curation, Formal analysis, Writing—original draft. Fengqing Xu: Investigation, Methodology, Methodology, Technical assistance. Jinsong Liu: Design, Supervision, Funding acquisition. Deling Wu: Supervision, Funding acquisition. Manqin Yang: Conceptualization, Methodology, Writing-Reviewing. Renzhong Wang: Conceptualization, Methodology, Writing—Reviewing, Editing, Project administration.

Availability of Data and Materials

The authors declare that the data supporting the findings of this study are available within the paper and its Supplementary Information files. Should any raw data files be needed in another format they are available from the corresponding author upon reasonable request. Source data are provided with this paper.

Ethics Approval

No experimentation involving human participants or live animals was conducted in this study.

Conflicts of Interest

No potential conflict of interest was reported by the authors.

Supporting Information

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

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