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# A new triterpenoid and eremophilanolide from *Ligularia przewalskii*

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ABSTRACT

A phytochemical study of the ethanolic extract of *Ligularia przewalskii* (Maxim.) Diels led to the isolation of two new terpenoids, (1βH,3βH)-epoxy-olean-13(18)-ene-3α,2-olide (**1**) and 8β-hydroxy-(10βH)-14β-methyl-6α-angeloyloxy eremophil-7(11)-en-8α,12-olide-15α-oic acid (**2**), along with 22 known compounds (**3**–**24**), of which compounds **3**–**13** were isolated from this plant for the first time. The structures of these compounds were established on the basis of spectroscopic methods. Compounds **1**–**2** were evaluated for their *in vitro* anti-proliferative activities against Hep-G2 and MCF-7 tumour cell lines. Compound **1** exhibited strong inhibitory activity against Hep-G2 cell growth, in contrast to moderate cytotoxic activity against MCF-7 cells. Although compound **2** showed strong inhibitory activity against MCF-7 cell growth, it appeared to have modest activity against Hep-G2 cells.



## 1. Introduction

*Ligularia przewalskii* (Maxim.) Diels is a perennial herb that is for the most part abundantly distributed in Sichuan, Qinghai, Gansu, Ningxia province. It grows on floodplains, foothills, forest edges and shrublands in altitudes of 1100–3700 m (Liu, 1989). It has been used traditionally in folk medicine for the treatment of asthma, hemoptysis, hepatitis and pulmonary tuberculosis (Guo, 1987).

Previous phytochemical studies on *L. przewalskii* have revealed that it is a rich source of eremophilane sesquiterpenes (Xu and Hu, 2008; Zhao et al., 1995) and furan derivatives (Jia and Zhao, 1994). To ascertain its chemical composition and medicinal value, the ethanol extract of *L. przewalskii* was investigated. Twenty-four compounds were isolated and identified as follows: (1βH,3βH)-epoxy-olean-13(18)-ene-3α,2-olide (**1**); 8β-hydroxy-(10βH)-14β-methyl-6α-angeloyloxy eremophil-7(11)-en-8α,12-olide-15α-oic acid (**2**); genkwanin (**3**) (Shi et al., 2010); luteolin (**4**) (Miyazawa and Hisama, 2003); apigenin (**5**) (Zhang et al., 2011); chrysoeriol (**6**) (Jia et al., 1986); sitost-4-en-3-one (**7**) (Joshi et al., 1974); β-daucosterol (**8**); scopoletin (**9**) (Zolek et al., 2003); furanoeremophilan-4β,6α-olide (**10**) (Moriyama and Takahashi, 1976); 5,6-dimethoxy-2-isopropenyl-benzofuran (**11**) (Murae et al., 1968); luteolin-7-O-β-D-glucoside (**12**) (Ren and Yang, 2001); and diosmin (**13**) (Nieto and Gutierrez, 1986) (Fig. 1); and additionally 10β-hydroxy-8β,9β-epoxyeremophil-7(11)-en-6α,15;8α,12-diolide (**14**) (Zhao et al., 1995); 8β-hydroxy-eremophil-7(11)-en-6α,15;8α,12-diolide (**15**) (Zhao et al., 1995); 2-acetyl-5,6-dimethoxybenzofuran (**16**) (Jia and Zhao, 1994); 10β-hydroxyeremophil-8(9),7(11)-dien-6α,15;8,12-diolide (**17**) (Zhao et al., 1995); 8β-hydroxy-6β-angeloyloxy eremophil-7(11)-en-8α,12-olide-15-oic acid (**18**) (Zhao et al., 1995); β-sitosterol (**19**); eremophil-7(11)-en-6α,15β;8α,12-diolide (**20**) (Zhao et al., 1995); eremophil-8(9),7(11)-dien-6α,15;8,12-diolide (**21**) (Zhao et al., 1995); 2-propenyl-5-acetyl-7-hydroxy-2,3-dihydrobenzofuran (7-hydroxtremetone) (**22**) (Jia and Zhao, 1994); euparin (**23**) (Bohlmann et al., 1977); 8β-methoxyeremophil-7(11)-en-6α,15; 8α,12-diolide (**24**) (Zhao et al., 1995). Among the isolates, compounds **1** and **2** were discovered to be new natural terpenoids. The structures were determined mainly on the basis of various spectroscopic evidence including 1D and 2D NMR, HRESIMS and IR data. The anti-tumour activities of compounds **1**–**2** were evaluated *in vitro*. Compound **1** exhibited cytotoxic activity against MCF-7 and Hep-G2 cell lines with $IC_{50}$ values of 42.9 and 15.0 μM, respectively. Compound **2** showed cytotoxic activity against MCF-7 and Hep-G2 cell lines with $IC_{50}$ values of 25.9 and 43.5 μM, respectively. Compound **1** showed strong inhibitory activity against Hep-G2 cell growth, and compound **2** showed strong inhibitory activity against

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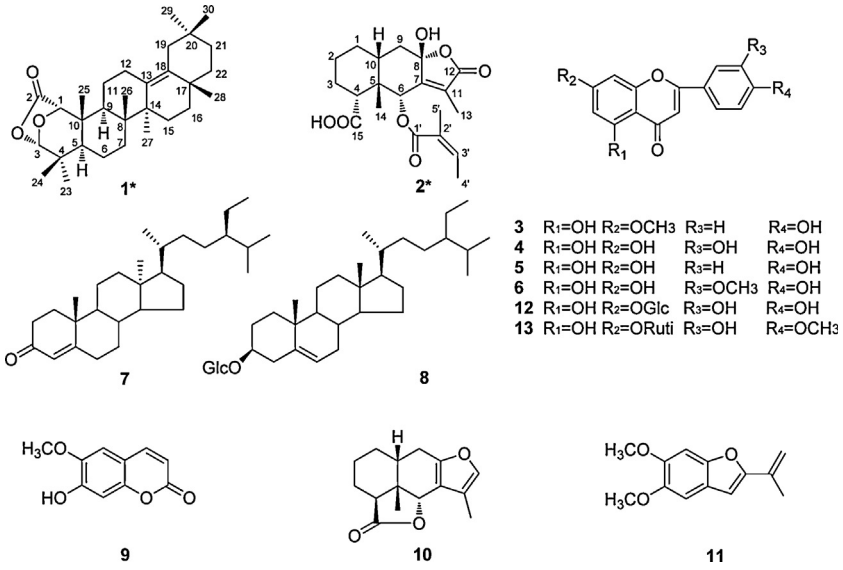


**Fig. 1.** Chemical structures of compounds **1**–**13**.

MCF-7 cell growth. Cisplatin was used as positive control. Herein, the isolation and structural elucidation of the two terpenoids will be described.

## 2. Results and discussion

The petroleum ether soluble fraction, ethyl acetate soluble fraction and n-BuOH soluble fraction of the EtOH-$H_2O$ (95:5) extract of the whole parts of *L. przewalskii* were submitted to multiple chromatographic steps to afford compounds **1**–**24**. The two new terpenoids were identified by means of IR, HRESIMS and NMR spectroscopy ($^1$H NMR, $^{13}$C NMR, HSQC, HMBC and ROESY).

Compound **1** was obtained as a white powder (mp: 256–257 °C, $[\alpha]_D^{20} = -18.4$ ($c = 1.00$, $CHCl_3$)). The molecular formula was deduced to be $C_{30}H_{46}O_3$ based on the $^1$H and $^{13}$C NMR data and the quasi-molecular ion peak at $m/z$ 477.3327 ($[M+Na]^+$, calc. 477.3345) in the HR-ESI-MS. The assignment was confirmed with the aid of 2D NMR (HSQC, HMBC and ROESY) spectra.

There were eight signals corresponding to methyl groups ($\delta$ 1.22, 1.18, 1.01, 0.99, 0.94, 0.92, 0.90 and 0.70) in the $^1$H NMR spectrum and 30 carbon signals in $^{13}$C NMR spectrum, which suggested that compound **1** was a triterpenoid. The IR spectrum exhibited an intense absorption band at 1795 cm$^{-1}$, which was ascribed to the carbonyl group of a γ-lactone, of which an ether linkage to the oxygen is implied, as no other carbonyl or hydroxyl was detected in the IR spectrum. In the $^1$H NMR spectrum two singlets were observed at $\delta$ 4.05 and $\delta$ 5.32, the only signals attributable to protons attached to the oxygen-bearing carbon atoms. The above substitution pattern could only correspond to the A ring of a triterpene skeleton (Aimi et al., 1981). Moreover, a tetrasubstituted double bond at $\delta$ 133.5 and 133.9 was indicated in the structure. The lack of a proton signal corresponding with the carbon signal at $\delta$ 172.2 further validated compound **1** as a lactone-containing structure. The proton signal at $\delta$ 5.32 (s, 1H) together with the carbon signal at $\delta$ 110.9 revealed that this carbon was connected to two oxygens (Basnet et al., 1994). The proton signal at $\delta$ 4.05 (s, 1H) together with the carbon signal at $\delta$ 78.7 revealed this carbon was connected to one oxygen and one carbonyl group (Basnet et al., 1994). The proposed arrangement was preferred on the basis of a comparison of physico-chemical data with the only reported structurally related oleanane triterpene, thysanolactone (Aimi et al., 1981). These data revealed that the compound was a triterpenoid with an olean-13(18)-ene skeleton, additionally featuring a lactone and an epoxy group. The proton and carbon signals of **1** are shown in Table 1.

In the HMBC spectrum (Fig. 2), the carbon resonance at $\delta$ 133.5 showed an HMBC correlation with H-27, and another HMBC

**Table 1**
$^1$H (500 MHz) and $^{13}$C (75 MHz) NMR data of compound **1** (in $CDCl_3$ at 30 °C; $\delta$ in ppm).

| Position | **1** | |
|---|---|---|
| | $\delta_H$ ($J$ in Hz) | $\delta_C$ |
| 1 | 4.05 s | 78.7 |
| 2 | | 172.2 |
| 3 | 5.32 s | 110.9 |
| 4 | | 36.4 |
| 5 | 1.37 m | 48.8 |
| 6a | 1.39 m | 17.6 |
| 6b | 1.44 m | |
| 7a | 1.47 m | 34.5 |
| 7b | 1.52 m | |
| 8 | | 41.1 |
| 9 | 2.12 m | 41.0 |
| 10 | | 40.1 |
| 11a | 1.48 m | 22.1 |
| 11b | 1.49 m | |
| 12a | 1.96 m | 24.2 |
| 12b | 2.64 m | |
| 13 | | 133.5 |
| 14 | | 45.2 |
| 15a | 1.04 m | 26.4 |
| 15b | 1.72 m | |
| 16a | 1.30 m | 39.3 |
| 16b | 1.34 m | |
| 17 | | 34.6 |
| 18 | | 133.9 |
| 19a | 1.64 m | 38.7 |
| 19b | 2.24 m | |
| 20 | | 33.3 |
| 21a | 1.12 m | 35.4 |
| 21b | 1.43 m | |
| 22a | 1.31 m | 36.4 |
| 22b | 1.34 m | |
| 23 | 0.99 s | 19.3 |
| 24 | 0.92 s | 24.3 |
| 25 | 1.22 s | 15.0 |
| 26 | 0.90 s | 18.1 |
| 27 | 1.18 s | 21.3 |
| 28 | 1.01 s | 23.8 |
| 29 | 0.94 s | 32.3 |
| 30 | 0.70 s | 24.0 |