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# Hebecarposides A–K, antiproliferative lanostane-type triterpene glycosides from the leaves of *Lyonia ovalifolia* var. *hebecarpa*

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ABSTRACT

Eleven previously undescribed lanostane-type triterpene glycosides, hebecarposides A–K, were isolated from the leaves of *Lyonia ovalifolia* var. *hebecarpa* (Ericaceae), along with two known analogues, lyonifolosides L and O. The structures of hebecarposides A–K were established by extensive spectroscopic analysis and chemical methods, and the absolute configuration of C-24 in hebecarposides A and E was determined to be *S* and *R*, respectively, by a $Mo_2(OAc)_4$–induced electronic circular dichroism method. This is the first report of the presence of lanostane-type triterpene glycosides in *L. ovalifolia* var. *hebecarpa*. All compounds were evaluated for their antiproliferative activities against five cancer cell lines, SMMC-7721, HL-60, SW480, MCF-7, and A-549, and a normal epithelial cell line BEAS-2B, and none of them showed general cytotoxity to the normal cell line BEAS-2B. Interestingly, hebecarposides C, D, G, and K selectively inhibited the proliferation of HL-60 and SMMC-7721 cell lines, and hebecarposides C and D showed significant anti-proliferative activities against A-549 cell lines than the positive control, *cis*-platin. In addition, hebecarposides C and H exhibited more potent anti-proliferative activities against MCF-7 than *cis*-platin.



## 1. Introduction

Lanostane (also known as 4,4,14-trimethylcholestane)-type triterpenoids possessing a 6/6/6/5 tetracyclic carbon ring system and eight methyls are widely distributed in mushrooms (mainly *Ganoderma*) and plants (Duru and Tel, 2015; Hamid et al., 2015; Isaka et al., 2017; Jin et al., 2014; Tohtahon et al., 2017). Although lanostane triterpene glycosides are the main components of sea cucumbers (Holothurioidea and Echinodermata) and mushrooms (Aminin et al., 2014; Kalinin et al., 2015; Lee et al., 2012), they are relatively rare in plants, and only reported from Liliaceae (Adinolfi et al., 1993; Ono et al., 2011; Ori et al., 2003a), Hyacinthaceae (Ori et al., 2003b), Verbenaceae (Okwu and Offiong, 2009), Leguminosae (Mamedova et al., 2003), and Ericaceae (Lv et al., 2016). Ericaceae plants are famous for their beautiful flowers and their structurally intriguing and bioactive diterpenoids components (Zhang et al., 2013, 2015; Zhou et al., 2017a, 2017b; 2018a, 2018b). However, studies of Ericaceae triterpenoids are not thorough, and the main triterpene skeletons are ursane, oleanane, lupane (Bukreyeva et al., 2013; Dai and Yu, 2005; Huang et al., 2007; Way et al., 2014; Yao et al., 2006), and dammarane (Dai and Yu, 2005). Recently, Lv et al. (2016) reported the isolation of lanostane and cycloartane triterpene glycosides with potent antiviral activity from the twigs and leaves of *Lyonia ovalifolia*.

*Lyonia* is a small genus of Ericaceae family, and is mainly distributed in Eastern Asia and North America. There are about 35 species of *Lyonia* in the world, and only six species and five varieties in China. *Lyonia ovalifolia* var. *hebecarpa* (Franch. ex F.B. Forbes & Hemsl.) Chun (Ericaceae) is endemic to China, and is mainly distributed in Jiangsu, Anhui, Zhejiang, Guangdong, Guangxi, and Yunnan provinces (Editorial Committee of the Flora of China, 1991). The branches and leaves are used as a folk medicine for an astringent agent. Phytochemical studies on *L. ovalifolia* var. *hebecarpa* are rare, only a megastigmane sesquiterpene glycoside and two steroids were reported (Wang et al., 2001). In a course of search for bioactive compounds from Ericaceae plants (Zhang et al., 2013, 2015; Zhou et al., 2017a, 2017b; 2018a, 2018b), the leaves of *L. ovalifolia* var. *hebecarpa* were investigated, leading to the isolation

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of eleven previously undescribed lanostane triterpene glycosides (**1**–**11**) and two known analogues (**12** and **13**) (Fig. 1). This is the first report of lanostane triterpene glycosides from *L. ovalifolia* var. *hebecarpa*. In this paper, the isolation, structure elucidation, and antiproliferative activities of thirteen lanostane triterpene glucosides (**1**–**13**) are described.

## 2. Results and discussion

The air-dried leaves of *L. ovalifolia* var. *hebecarpa* were extracted with 95% aqueous EtOH. The crude extract was suspended in $H_2O$ and then partitioned excessively with petroleum ether and chloroform. The chloroform fraction was repeatedly subjected to silica gel, reversed phase (RP) $C_{18}$ silica gel, and Sephadex LH−20 column chromatography, as well as HPLC on a semipreparative XB−$C_{18}$ column to yield eleven previously undescribed lanostane-type triterpene glycosides (**1**–**11**) and two known analogues (**12** and **13**). Known lanostane-type triterpene glycosides were identified to be lyonifolosides L (**12**) and O (**13**) (Lv et al., 2016), respectively, by comparison of their spectroscopic data with those reported in the literature.

Hebecarposide A (**1**) was obtained as a white amorphous power. Its molecular formula was established as $C_{35}H_{58}O_9$ by the HRESIMS at *m/z* 645.3978 $[M + Na]^+$ (calcd for $C_{35}H_{58}O_9Na$, 645.3979) and $^{13}C$ NMR data, indicating seven indices of hydrogen deficiency. The $^1H$ NMR data of **1** (Table 1) showed resonances for seven methyl groups at $\delta_H$ 0.82 (3H, s, $CH_3$-18), 1.07 (3H, s, $CH_3$-19), 0.96 (3H, d, $J = 6.2$ Hz, $CH_3$-21), 1.16 (3H, s, $CH_3$-26), 1.13 (3H, s, $CH_3$-27), 0.98 (3H, s, $CH_3$-28), and 0.90 (3H, s, $CH_3$-29), an oxymethine at $\delta_H$ 3.39 (1H, br s, H-3$\beta$), and an arabinopyranosyl unit at $\delta_H$ 4.20 (1H, d, $J = 6.2$ Hz, H-1′), 3.53 (1H, m, H-2′), 3.52 (1H, m, H-3′), 3.81 (1H, dd, $J = 4.0$, 2.9 Hz, H-4′), 3.48 (1H, dd, $J = 12.4$, 1.2 Hz, H-5′a), and 3.85 (1H, dd, $J = 12.4$, 2.9 Hz, H-5′b) (Lv et al., 2016). The $^{13}C$ NMR and DEPT data of **1** (Table 2) exhibited a total of 36 carbon resonances corresponding to seven methyls, ten methylenes, three methines including two oxymethines at $\delta_C$ 82.2 (C-3) and 80.6 (C-24), four quaternary carbons, one oxygenated tertiary carbon at $\delta_C$ 74.1 (C-25), two tertiary $sp^2$ carbons at $\delta_C$ 128.9 (C-8) and 141.9 (C-9), a carboxyl group at $\delta_C$ 180.5 (C-30), and an arabinopyranosyl unit at $\delta_C$ 102.1 (C-1′), 72.6 (C-2′), 74.5 (C-3′), 69.9 (C-4′), and 67.0 (C-5′) (Lv et al., 2016). Apart from three indices of hydrogen deficiency occupied by a double bond, a carboxyl group, and an arabinopyranosyl unit, the remaining four indices of hydrogen deficiency suggested that compound **1** is a tetracyclic triterpene glycoside. The NMR data of **1** resembled those of **12** (lyonifoloside L) (Lv et al., 2016), except for an arabinopyranosyl unit in **1**, instead of a glucopyranosyl unit in **12**. The glycosidation at C-3 was proved by the cross-peaks from the anomeric proton H-1′ to C-3 ($\delta_C$ 82.2) and from H-3 to the anomeric carbon C-1' ($\delta_C$ 102.1) in the HMBC spectrum of **1**. The planar structure of **1** was confirmed by $^1H-^1H$ COSY, HSQC, and HMBC analyses (Fig. 2). The broad single peak of H-3 ($\delta_H$ 3.39, br s) in the $^1H$ NMR established its equatorial position in the chair conformation of the cylcohexane in ring A (Fig. 2), and H-3 was in a $\beta$-orientation (Lv et al., 2016). NOESY analysis (Fig. 3) and comparison of NMR data with **12** suggested that the relative configuration of **1** is same to **12**, ignoring the sugar moieties.

To determine the absolute configuration of the arabinopyranose, **1** was hydrolyzed by 2 mM HCl to obtain the sugar, and then, the trimethylsilylthiazolidine derivatives of the sugar and standards, D and L-arabinose, were prepared. By comparing the retention times of these three trimethylsilylthiazolidine derivatives obtained from gas chromatography (GC), the absolute configuration of the arabinopyranose in **1** was determined to be L. The coupling constant of the anomeric hydrogen $J = 6.2$ Hz ($\delta_H$ 4.20, d, H-1′) established the $\alpha$-arabinopyranosyl linkage in **1**. Due to the existence of a *vic*-diols unit in the side chain, a dimolybdenum tetraaccetate $[Mo_2(OAc)_4]$ −induced electronic circular dichroism (IECD) experiment was implemented to establish the absolute configuration of C-24 in **1** (Frelek et al., 2003; Gorecki et al., 2006, 2007; Snatzke et al., 1981). To eliminate the effects of the arabinose and carboxylic acid on the IECD, **1** was hydrolyzed by 0.6 mM TsOH·$H_2O$, instead of HCl, to afford the genuine aglycone **1a**, and then, **1a** was methylated with $CH_3I$ to yield an ester **1b**. As shown in Fig. 4, the $Mo_2(OAc)_4$-induced ECD spectrum of the ester **1b** exhibited a positive Cotton effect at 316 nm, suggesting the *S* configuration of C-24 (Frelek et al., 2003; Gorecki et al., 2006, 2007; Snatzke et al.,

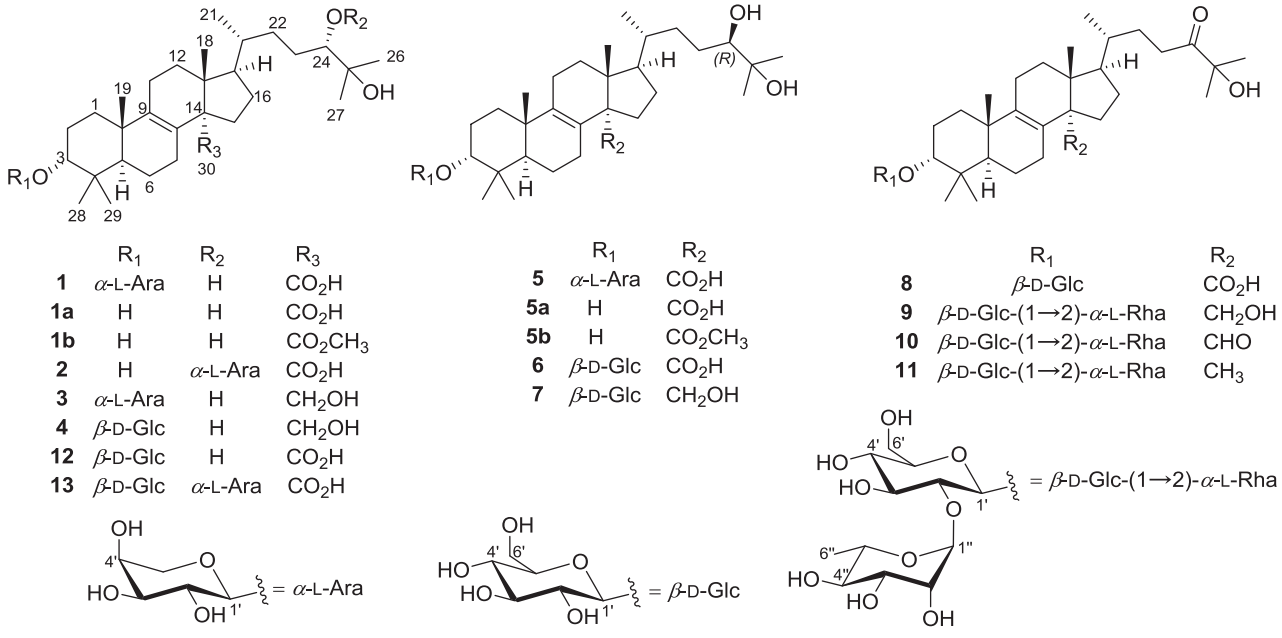


**Fig. 1.** Chemical structures of lanostane-type triterpene glycosides **1**–**13**.