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Five new triterpenoidal saponins from the roots of *Ilex cornuta* and their protective effects against H_2O_2-induced cardiomyocytes injury

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ABSTRACT

Five new ursane-type triterpenoidal saponins (**1**–**5**), together with five known ones (**6**–**10**), were isolated from the EtOH extract of the roots of *Ilex cornuta*. The structures of saponins **1**–**5** were elucidated as 19α-hydroxyurs-12-en-28-oic acid 3β-O-β-D-glucuronopyranoside (**1**), 19α-hydroxyurs-12-en-28-oic acid 3β-O-β-D-glucuronopyranoside-6-O-ethyl ester (**2**), 19α-hydroxyurs-12-en-28-oic acid 3β-O-α-L-arabinopyranosyl-(1 → 2)-β-D-glucuronopyranoside (**3**), 3β-O-[α-L-arabinopyranosyl-(1 → 2)-β-D-glucuronopyranosyl]-19α-hydroxyurs-12-en-28-oic acid 28-O-β-D-glucopyranosyl ester (**4**) and 3β-O-[α-L-arabinopyranosyl-(1 → 2)-β-D-glucuronopyranoside-6-O-methyl ester]-19α-hydroxyurs-12-en-28-oic acid 28-O-β-D-glucopyranosyl ester (**5**), on the basis of spectroscopic analyses (IR, ESI-MS, HR-ESI-MS, 1D and 2D NMR) and chemical reactions. Protective effects of compounds **1**–**10** against H_2O_2-induced H9c2 cardiomyocyte injury were tested. Compounds **1**–**5**, **7**, and **10** showed cell-protective effects. Among them compound **5** exhibited the highest activity. No significant DPPH radical scavenging activity was observed for compounds **1**–**10**.

1. Introduction

Ilex (Aquifoliaceae) species are distributed widely in the People's Republic of China, and some are used as folk medicines. For example, *Ilex cornuta* and *I. latifolia* are traditionally used for the treatment of headache, toothache, bloodshot eyes, and tinnitus [1]. Previous studies on the *Ilex* genus have led to the isolation of triterpenes [2], triterpenoidal saponins [3,4], hemiterpene glycosides [5], and phenolic compounds [6]. Our previous studies have resulted in the isolation of a series of triterpenoidal saponins from *I. cornuta* [7–9], which showed potential protective effect against cardiomyocyte injury induced by H_2O_2.

Ischemic heart disease (IHD) is a frequently occurring disease with high fatality rate worldwide. Reactive oxygen species (ROS) may be the major cause and lead to the intracellular oxidative damage of biomolecules. H_2O_2, superoxide and hydroxyl radicals may readily cross cellular membranes and induce severe damages to the adjacent biomolecules [10]. Growing evidence has showed that triterpenoidal saponins inhibit the production of nitric oxide and ROS in murine macrophages and show excellent antioxidant activity in diabetes mellitus [11,12]. The extract of *I. cornuta* was reported to have protective effects on myocardial ischemia [13] and some isolates from this plant possessed moderate cell-protective effects [7]. To continue our effort for searching active compounds from this plant, we investigated the EtOH extract of the roots of *I. cornuta*, which

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led to the isolation of five new ursane-type triterpenoidal saponins (**1**–**5**) (Fig. 1), along with five known ones. The roots of *I. cornuta* have been used in traditional Chinese medicine for treatment of various disorders [2]. In this paper, we report the isolation and structural elucidation of these saponins, as well as their antioxidant activities and protective effects against H_2O_2-induced injury in cultured H9c2 cardiomyocytes.

2. Result and discussion

Five new ursane-type triterpenoidal saponins, compounds **1**–**5**, together with five known ones (**6**–**10**) were obtained from the roots of *I. cornuta* after multi-step chromatographic separations. Compounds **6**–**10** were determined as 19α-hydroxyurs-12-en-28-oic acid 28-O-β-D-glucopyranosyl ester (**6**) [14], 19α-hydroxyurs-12-en-28-oic acid 3β-O-β-D-glucuronopyranoside-6-O-methyl ester (**7**) [15], 3β-O-β-D-glucopyranosyl-19α-hydroxyurs-12-en-28-oic acid 28-O-β-D-glucopyranosyl ester (**8**) [16], 19α,23-dihydroxyurs-12-en-28-oic acid 3β-O-α-L-arabinopyranoside (**9**) [17], and 19α-hydroxyurs-12-en-28-oic acid 3β-O-α-L-arabinopyranosyl-(1 → 2)-β-D-glucuronopyranoside-6-O-methyl ester (**10**) [9], in comparison with data in literature. The 1D- and 2D-NMR spectra and MS analyses were performed for chemical structural elucidation of the newly isolated compounds **1**–**5** (Fig. 1). The structures of monosaccharides in the glycoside moieties of the compounds were determined by gas chromatography (GC) analyses after acid hydrolysis.

Compound **1** was obtained as white amorphous powder. The molecular formula of $C_{36}H_{56}O_{10}$ was determined on the basis of its HR-ESI-MS $[M-H]^-$ ion peak at *m/z* 647.3830 (calcd. 647.3795). The ^{1}H-NMR spectrum showed seven methyl proton singlets at δ_H 1.84 (3H, s, Me-27), 1.39 (3H, s, Me-23), 1.53(3H, s, Me-29), 1.14 (3H, s, Me-26), 1.21 (3H, s, Me-24), 0.92(3H, s, Me-25), and 1.07 (3H, s, Me-30), an olefinic proton signal at δ 5.67 (1H, br s, H-12), and an oxygenated methine proton signal at δ 3.49 (1H, dd, $J = 4.0$, 12.0 Hz, H-3). The ^{13}C-NMR spectrum of **1** showed the presence of two olefinic carbons at δ_C 128.2 (C-12) and 140.0 (C-13), an oxymethine carbon at δ_C 89.2 (C-3), a quaternary carbon at δ_C 72.8 (C-19), and a carboxylic carbon at δ_C 180.8 (C-28) (Table 1). In addition, the ^{13}C-NMR spectrum also exhibited the signals of one glucuronopyranosyl (GlcA) group [δ_C 107.2 (C-1 of GlcA), 75.7 (C-2 of GlcA), 78.4 (C-3 of GlcA), 73.8 (C-4 of GlcA), 77.3 (C-5 of GlcA) and 173.6 (C-6 of GlcA)] (Table 2). The NMR spectra indicated that **1** was a 19α-hydroxy-ursolic acid derivative by comparing its spectroscopic data with previously published data of Ilexasoside A [18]. In the ^{1}H-NMR spectrum, an anomeric proton was observed at δ_H 5.08 (1H, s), which showed the HSQC correlation with the anomeric carbon at δ_C 107.2 (C-1 of GlcA). The sugar residue yielded from acid hydrolysis of **1** was identified as D-glucuronic acid by GC analysis. The linkage position of the GlcA group on the aglycone

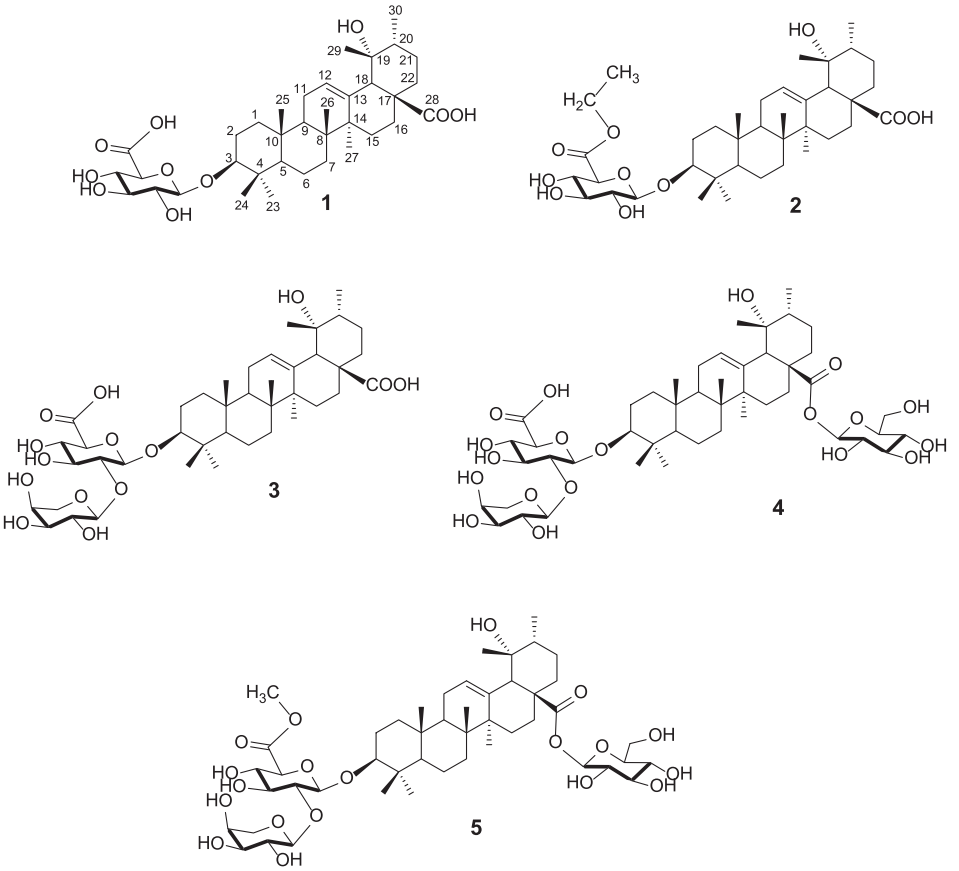

Fig. 1. Structures of compounds **1**–**5**.