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Three new 3,4-*seco*-cycloartane triterpenoids from the flower of *Gardenia jasminoides*

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ABSTRACT

Three new 3,4-*seco*-cycloartane triterpenoids (**1**-**3**), including an unusual C-1 oxygenated 3,4-*seco*-cycloartane triterpenoid lactone (**3**), along with six known triterpenoids, were isolated from 95% ethanol extract of the flower of *Gardenia jasminoides*. Their structures were determined by spectroscopic methods, especially 2D NMR and HRESIMS techniques. Compounds **3**, **4** (dikamaliartane D), and **7** (ursolic acid) showed promising cytotoxic activity against HepG2 human hepatocellular carcinoma cell line with IC_{50} values of 9.3, 27.7, and 39.3 μM, respectively.

1. Introduction

Gardenia jasminoides Ellis (Rubaceae) is an evergreen shrub or arbor widely distributed and cultivated in southern China for its ornamental and medicinal values (Flora of China, 1999). The leaf, root, fruit, and flower were separately applied in traditional Chinese medicine (TCM) for a variety of uses (Chinese Materia Medica, 1999). Besides the application in cosmetics and foods, the refreshing fragrance flower was usually decocted to treat cough and epistaxis in TCM. Previous phytochemical studies of the flower have resulted in the isolation of iridoids, flavonoids, terpenoids, phenolic acids, and organic acid esters (Song et al., 2013, 2014; Ragasa et al., 2007; Watanabe et al., 1994). As a part of our continuous research on cycloartane triterpenoids from medicinal plants (Zhou et al., 2013; Gan et al., 2015), the flower of *G. jasminoides* from Zhejiang province of China were studied phytochemically. Three new 3,4-*seco*-cycloartane triterpenoids (**1**-**3**, Fig. 1), along with six known triterpenoids (**4**-**9**), were isolated from the 95% ethanol extract. Their structures were determined by spectroscopic methods, especially 2D NMR and HRESIMS techniques. Cytotoxic activities of all the compounds were evaluated by a MTT assay on HepG2 human hepatocellular carcinoma cell line.

2. Results and discussion

Compound **1** was isolated as colorless needles. The molecular formula was determined as $C_{30}H_{46}O_5$ by a HRESIMS ion peak at 487.3425 $[M+H]^+$ (calcd for $C_{30}H_{47}O_5$, 487.3418). IR spectrum of **1** showed vibrational absorption peaks for carbonyl and double bond at 1726 and 1644 cm^{-1}, respectively. The ^1H NMR spectrum exhibited two shielded geminal hydrogen signals at δ_H 0.49 (1H, d, J = 4.2 Hz) and 0.81 (1H, d, J = 4.2 Hz) assignable to the CH_2-19 group of a cycloartane type triterpenoid. Three singlets at δ_H 1.81 (3H, s), 1.07 (3H, s), and 1.02 (3H, s) indicated three methyls linked to quaternary carbons. The ^{13}C NMR and DEPT (135) spectra showed one carbonyl carbon at δ_C 177.7, one trisubstituted double bond and one terminal double bond at δ_C 154.1, 139.0, 130.2, and 110.3, one oxygen-bearing methine at δ_C 75.1, and three oxygen-bearing methylenes at δ_C 73.0, 64.6, and 61.5. The above data showed a 3,4-*seco*-cycloartane type triterpenoid for **1**, similar to the known compound secaubryenol (Grougnet et al., 2006). Compared to secaubryenol, an additional unsaturated degree, the absence of CH_3-21, and the oxygen-bearing methine at δ_C 75.1 suggested the connection of CH_2(21)-O-CH(23) to form a five-membered ring in the side chain. The above deduction were further confirmed by 2D HMQC, ^1H-^1H COSY, and HMBC spectra (Fig. 2). Based on the HMQC data, ^1H-^1H COSY correlations revealed four spin systems drawn with bold bonds in Fig. 2. HMBC correlations from H_2-19 to C-1, C-10, C-5, C-11, C-9, and C-8 confirmed the cyclopropyl core. Correlations of H_2-1/C-3, H_2-2/C-3, and from H_2-28 to C-29, C-4, and C-5 showed the 3,4-*seco* structure. Key HMBC correlations of H-23/C-21 and H_2-21/C-23 confirmed the formation of the five-membered ring in the side chain. The relative configuration of **1** was determined by NOESY spectrum as shown in Fig. 3. Key NOESY correlations of H-19β/H-8, H-8/H_3-18, H_3-

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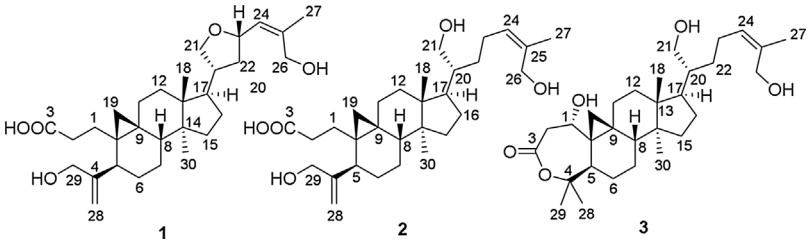

Fig. 1. Chemical structures of compounds **1–3.**

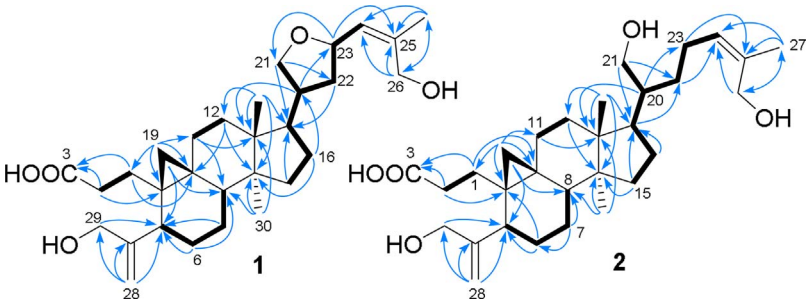

Fig. 2. Selected HMBC (H → C) correlations of compounds **1** and **2.**

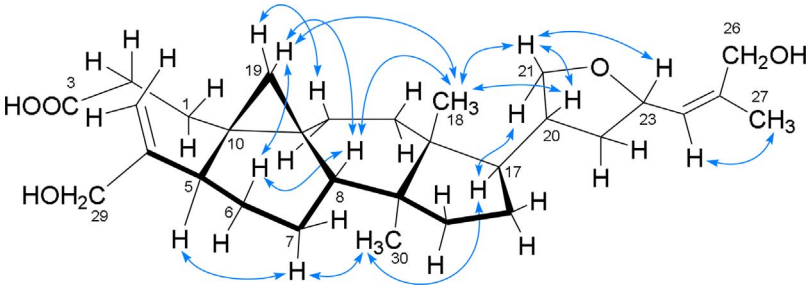

Fig. 3. Key NOESY (H ↔ H) correlations of compound **1**.

18/H-20, H_3-18/H-21β, H-21β/H-23 showed their β-orientation. Accordingly, the α-oriented hydrogens were identified by NOESY correlations of H-5/H-7α, H-7α/H_3-30, H_3-30/H-17, and H-17/H-21α. NOESY correlations between H-24 and H_3-27 revealed a *Z* configuration double bond in the side chain. Therefore, the structure of **1** was determined to be (17*R**,20*R**,23*S**,24*Z*)-26,29-dihydroxy-21,23-epoxy-3,4-secocycloart-4(28),24(25)-dien-3-oic acid (**1**).

The molecular formula of compound **2**, $C_{30}H_{48}O_5$, suggested one less unsaturated degree than that of **1**. The ^{1}H and ^{13}C NMR spectra of **2** showed similar peaks as that of **1**, except the absence of signals for the CH(23)-O moiety. The above data indicated the five-membered ring of **1** was cleaved in **2**. Further HMQC, ^{1}H–^{1}H COSY, HMBC, and NOESY experiments (Fig. 2) confirmed the above deduction and the structure of compound **2** was then determined as (17*R**,20*R**,24*Z*)-21,26,29-trihydroxy-3,4-secocycloart-4(28),24(25)-dien-3-oic acid (**2**).

Compound **3** was isolated as a white amorphous power and showed the same molecular formula ($C_{30}H_{48}O_5$) as compound **2** by HRESIMS data. IR spectrum of **3** showed the presence of carbonyl (1725 cm^{-1}) and double bond (1644 cm^{-1}). In the ^{1}H NMR spectrum, the cycloartane nature of **3** was firstly indicated by two strongly shielded hydrogen signals assignable to H_2-19 at δ_H 0.81 (1H, d, J = 4.9 Hz) and 0.75 (1H, d, J = 4.9 Hz). Instead of dehydrogenated C-28 and oxygenated C-29 signals in **2**, compound **3** showed five methyl singlets at δ_H 1.78, 1.50, 1.39, 1.05, and 1.02, while key signals of the side chain, H-24, H_2-26, and H_2-21 remain unchanged. Accordingly, the ^{13}C NMR and DEPT (135) spectra showed the presence of an additional oxygen-bearing quaternary carbon at δ_C 89.0 and an oxygen-bearing methine at δ_C 69.5, while the olefinic C-4 and CH_2-28, and CH_2OH-29 in **2** were absent from **3**. The above information implied the existence of a lactone bond between C-3 and C-4 of the *seco*-cycloartane ring A in compound **3** (Tuchinda et al., 2002). HMQC, ^{1}H-^{1}H COSY, and HMBC (Fig. 4) experiments were further carried out to confirm the skeleton and locate the oxygen-bearing carbons. Key HMBC signals from H_2-19 to the oxygen-bearing methine and from the methine hydrogen signal to C-3 showed its location at C-1. HMBC correlations of H_3-28 and H_3-29 to the oxygen-bearing quaternary carbon allowed assignment of it to C-4. Correlations of H_2-21/C-22, H_2-21/C-17, H_2-22/C-17, H-20/C-23, H_2-23/C-25, H_2-26/C-24, H_3-27/C-24, and H_3-27/C-26 confirmed a same side chain as **2**. Relative configuration of compound **3**, especially the newly occurred stereo center at C-1 was determined by NOESY spectrum. Lowest energy conformations of compound **3** were firstly identified via Monte Carlo searching using molecular mechanism with MMFF94 force field in the Spartan 08 program. Experimental NOESY correlations and the conformations were then analyzed in detail to determine the relative configuration. Key NOESY correlations of H-1/H-19α, H-19α/H-11β, H-1/H-11β, H-19β/H-8, H-19β/H_3-18, and H-8/H_3-18 indicated that these hydrogens are all β-oriented (Fig. 4). The structure of compound **3** was then determined as (1*S**,17*R**,20*R**,24*Z*)-1,21,26-trihydroxycycloart-24(25)-en-3-olide (**3**).

By comparing their NMR data with those reported in the literature, structures of the known compounds were identified as dikamaliartane D (**4**) (Kunert et al., 2009), gardenolic acid A (**5**) (Xu et al., 1987), gardenolic acid B (**6**) (Qin et al., 1989), ursolic acid (**7**) (Acebey-Castellon et al., 2011), rotundic acid (**8**) (Cao et al., 2014) and 3β-6β-23-trihydroxyolean-12-en-28-oic acid (**9**) (Khan and Sticher, 1993).

Compounds **1–9** were tested for their cytotoxic activity against HepG2 human hepatocellular carcinoma cell line. Compounds **3**, **4**, and **7** (Son et al., 2013) showed promising cytotoxic activity with IC_{50} values of 9.3, 27.7, and 39.3 μM, respectively.

In conclusion, three new 3,4-*seco*-cycloartane triterpenoids, (17*R**,20*R**,23*S**,24*Z*)-26,29-dihydroxy-21,23-epoxy-3,4-secocycloart-4(28),24(25)-dien-3-oic acid (**1**), (17*R**,20*R**,24*Z*)-21,26,29-