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Quinoid glycosides from *Forsythia suspensa*

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ABSTRACT

Phytochemical investigation on *Forsythia suspensa* (Thunb.) Vahl afforded 10 compounds, including quinoid glycosides, lignan glycosides, phenylethanoid glycoside and allylbenzene glycoside together with 13 known ones. Their structures were established based on extensive spectroscopic data analyses, including IR, UV, HRESIMS, 1D NMR and 2D NMR. Absolute configurations were determined by ECD calculation method and chemical degradation. In addition, all compounds were evaluated for their antiviral activity against influenza A (H1N1) virus and several were further evaluated against respiratory syncytial virus (RSV) *in vitro*. Among them, two previously known compounds showed significant activities against RSV with EC_{50} values of 3.43 and 6.72 μM.

1. Introduction

Forsythia suspensa (Thunb.) Vahl is widely distributed in China, Japan, Korea and many European countries. The dried fruit of this plant, locally named 'Lianqiao', is a well known herbal medicine in China for the treatment of pyrexia and infections (Cui et al., 2010). A number of phenylethanoid glycosides (Wang et al., 2009), lignan glycosides (Chang et al., 2008; Piao et al., 2008), quinoid glycosides (Ming et al., 1998), triterpenes (Rouf et al., 2001) and alkaloids (Zhang et al., 2002) have been isolated from the fruit of this plant. Some of them possess various bioactivities, including antiviral (Li et al., 2011), antibacterial (Qu et al., 2012) and anti-inflammatory activities (Dai et al., 2009). Our investigations on the fruit of *F. suspensa* in searching for antiviral components led to the isolation of 10 new compounds, along with 13 known ones. Herein, we mainly described the isolation and structural elucidation of the new compounds, as well as the antiviral activities against influenza A (H1N1) virus and respiratory syncytial virus (RSV).

2. Results and discussion

The dried fruit of *F. suspensa* was extracted with EtOH–H_2O (60:40), and then the extract was subjected to various column chromatographies to yield 10 new compounds, forsythensides G–L (**1**–**6**) and forsythialansides A–D (**7**–**10**) (Fig. 1). Moreover, 13 known compounds were also isolated, and their structures were identified as cornoside (**11**) (Lu et al., 2009), forsythenside A (**12**) (Ming et al., 1998), icariside E4 (**13**) (Miyase et al., 1989), dihydrodehydrodiconiferyl alcohol-4-*O*-*β*-D-glucoside (**14**) (Ou yang et al., 2011), calceolarioside B (**15**) (Nicoletti et al., 1986), forsythoside A (**16**) (Wang et al., 2009), sasanquin (**17**) (Zhao et al., 1999), epipinoresinol-4-*O*-*β*-D-glucoside (**18**) (Nishibe et al., 1984), phillyrin (**19**) (Nishibe et al., 1984), epipinoresinol-4-*O*-*β*-D-glucoside (**20**) (Rahman et al., 1990), pinoresinol-4-*O*-*β*-D-glucoside (**21**) (Kim et al., 2005), pinoresinol (**22**) (Li et al., 2012) and syringaresinol-4-*O*-*β*-D-glucoside (**23**) (Li et al., 2001), respectively.

Compound **1** was obtained as light yellow gum. Its molecular formula was deduced to be $C_{24}H_{34}O_{10}$ by HRESIMS. The ^{13}C NMR spectrum showed 24 carbon signals. The characteristic chemical shift of one carbonyl group (δ 187.8), along with four olefinic carbons (δ 154.4 × 2, 128.0, 127.9), two methylene carbons (δ 65.8, 41.0) and a quaternary carbon (δ 69.2) indicated that compound **1** had a 1-hydroxy-1-hydroxyethyl-2,5-cyclohexadienone moiety (Wang et al., 2008). This was further confirmed by HMBC correlations (Fig. 2).

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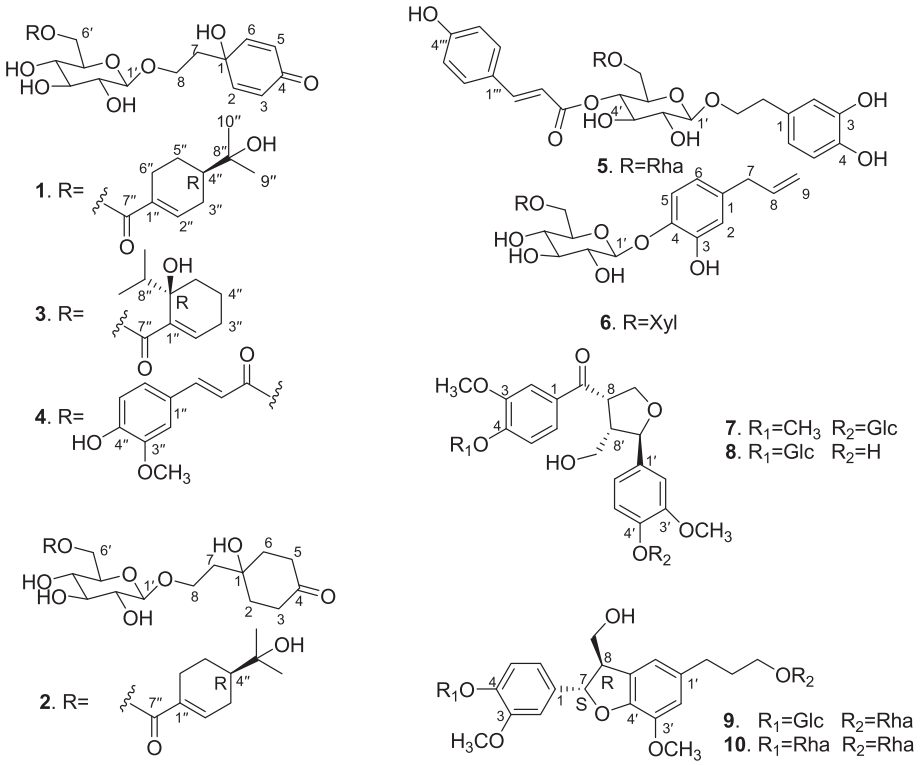

Fig. 1. Structural formulae of compounds **1**–**10**.

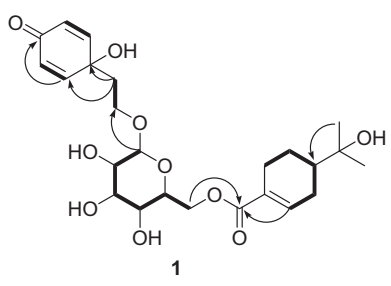

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Fig. 2. Key HMBC (→) and ^{1}H, ^{1}H-COSY (▬) correlations of **1**.

The anomeric carbon resonance at δ 104.4 showed the existence of a monosaccharide moiety. Acid hydrolysis and HPLC analysis were performed according to the method of Tanaka et al. (2007), which suggested that the monosaccharide was D-glucose. Moreover, the β configuration was prompted by the large coupling constant of the anomeric proton (δ 4.24, d, J = 7.8 Hz).

In addition, typical carbon signals at δ 131.2, 141.5 and 168.7 showed the existence of an α,β-unsaturated carbonyl group. In the ^{1}H, ^{1}H-COSY spectrum, the ^{1}H, ^{1}H spin system (H2″/H3″, H3″/H4″, H4″/H5″, and H5″/H6″) enabled deduction of the fragment –CH–CH_2–CH–CH_2–CH_2–. In the HMBC spectrum, correlations of H-2″/C-6″, C-7″ and C-4″, and those of H-9″/C-8″ and C-4″ suggested the presence of an oleuropeic acid unit (Tian et al., 2009). Moreover, the linkages of three moieties were established on the basis of HMBC correlations of H-1′/C-8 and H-6′/C-7″ (Fig. 2).

The absolute configuration of **1** was determined by chemical degradation and optical rotation analysis. Methanolysis of **1** was performed with NaOMe in MeOH. After neutralization with HCOOH, the EtOAc extract was obtained and evaporated to dryness, then the residue was dissolved in MeOH and further purified by preparative HPLC to yield (+) oleuropeic acid (**1a**) {$[\alpha]_D^{20}$ +45.3 ($CHCl_3$)}, which indicated that the absolute configuration of C-4″ was *R* (Nakanishi et al., 2005). Thus, the structure of **1** was deduced as 6-*O*-[(*R*)-oleuropeoyl]-1-*O*-[2-(1-hydroxy-4-oxo-2,5-cyclohexadien-1-yl)ethyl]-β-D-glucopyranose, trivially named forsythenside G.

Compounds **2**–**5** were also isolated as light yellow gums. The molecular formula of **2**, $C_{24}H_{38}O_{10}$, was established by analysis of HRESIMS data. The ^{13}C NMR and DEPT-135 spectroscopic data of **2** were similar to those of **1** except that the two olefinic groups (δ 154.4 × 2, 128.0, 127.9) in **1**, were replaced by four methylenes (δ 37.9 × 2, 37.8 × 2) in **2**, which suggested that the 2,5-cyclohexadienone moiety in **1** was replaced by a cyclohexanone moiety in **2**. Methanolysis of **2** with NaOMe in MeOH also gave (+)-oleuropeic acid (**1a**) {$[\alpha]_D^{20}$ +38.0 ($CHCl_3$)}; therefore, the absolute configuration of C-4″ in **2** was assigned to be *R*. Thus, compound **2** was assigned as 6-*O*-[(*R*)-oleuropeoyl]-1-*O*-[2-(1-hydroxy-4-oxocyclohexyl)ethyl]-β-D-glucopyranose, it was given the trivial name forsythenside H.

Compound **3** had the same molecular formula with **1**, which was determined by HRESIMS data. The ^{13}C NMR and DEPT-135 data of **3** also showed the presence of a monosaccharide moiety and a 1-hydroxy-1-hydroxyethyl-2,5-cyclohexadienone unit, as same as compound **1**. The remaining 10 carbon signals consisted of one α,β-unsaturated carbonyl group (δ 168.6, 139.3, 130.6), two methyls (δ 17.3, 17.2), three methylenes (δ 35.6, 31.4, 22.4), one methine (δ 38.6) and one quaternary carbon signal (δ 72.4). Those resonances demonstrated the presence of a 6-hydroxy-6-isopropylcyclohex-1-enecarboxylic acid moiety (Dai et al., 2005). Furthermore, the linkages of the three moieties were based on the HMBC correlations of H-1′/C-8, and H-6′/C-7″.

Methanolysis of **3** was also performed with NaOMe in MeOH to yield 6-hydroxy-6-isopropylcyclohex-1-enecarboxylic acid (**3a**). The electronic circular dichroism (ECD) spectrum of **3a** was calculated by time dependent density functional theory (TDDFT). The calculated ECD data matched well with the experimental ECD data of **3a** (Fig. 3), allowing the assignment of the absolute configuration

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