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Anti-hepatitis B virus activities of friedelolactones from *Viola diffusa* Ging

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ABSTRACT

Background: Hepatitis B virus (HBV) infection is the major factor of causing hepatitis B, cirrhosis and liver cancer. Interferon and nucleoside drugs, the main drugs to treat HBV infection, have disadvantages of scavenge difficulty and drug resistance respectively. *Viola diffusa* Ging is used as a traditional Chinese herbal medicine for the treatment of hepatitis.
Purpose: The aim of the study is to investigate the chemical constituents of *Viola diffusa* Ging and their anti-HBV activity.
Methods: Chemical constituents were extracted and purified by ethanol reflux extraction and chromatographic separation technology including D-101 Macroporous resin, silica gel, Sephadex LH-20 and preparative thin-layer chromatography. Their structures were elucidated on the basis of extensive NMR and MS data. Cytotoxicity and inhibiting effects on HBsAg and HBeAg secretion of HepG2.2.15 of all compounds except **10** were studied by MTT method and ELISA method.
Results: Three friedelolactones with naturally occurring *seco*-ring-*A* friedelane triterpenoids, 2β-hydroxy-3,4-seco-friedelolactone-27-oic acid (**1**), 2β, 28β-dihydroxy-3,4-seco-friedelolactone-27-oic acid (**2**) and 2β, 30β-dihydroxy-3,4-seco-friedelolactone-27-lactone (**3**), and a stigmastane, stigmast-25-ene-3β,5α,6β-triol (**11**) together with nine known compounds were isolated from the whole plant of *Viola diffusa* G. (Violaceae). Compounds **1–3, 9, 11, 12** exhibited significant activities of blocking both HBsAg and HBeAg secretion, and compound **4, 6, 7, 8** selectively inhibited HBeAg secretion while compound **13** selectively inhibited HBsAg secretion. IC_{50} values of compounds **1** and **2**, 26.2 μM and 33.7 μM for HBsAg, 8.0 μM and 15.2 μM for HBeAg, was significantly lower than that of positive control lamivudine.
Conclusion: Compounds **1–3, 11** are new compounds never reported before and the promising results demonstrate the potential of compound **1–3, 9, 11, 12** for the treatment of HBV infection.

Introduction

Highly oxidized metabolites are often found particularly among the tetracyclic triterpenes and steroids. In the oxidation process, ring-fission and subsequent modification of the skeleton is frequently accompanied. However, ring-fission is not restricted to these compounds; *seco*-derivatives have also been found among the pentacyclic triterpenes. However, naturally occurring *seco*-ring-*A* friedelane triterpenoids with the *A* ring expanded to seven-membered ring containing compounds, are relatively rare among secondary metabolites. Apetalactone was the first 3,4-*seco*-ring-*A* friedelane lactone, found in the leaf of two *Calophyllum* species (Govindachari et al. 1968). Two closely related compounds friedelolactone (Katai et al. 2006) and itoaic acid (Chai et al. 2009) were isolated respectively from *Euonymus japonicus* and *Itoa orientalis*. Beside 3,4-*seco* compounds, a natural 2,3-*seco*triterpenelithocarpic lactone (Hui et al. 1975) was described in the literature. Recently a new type of triterpene anhydride lobatanhydride (Rodríguez et al. 2009) was found from *Crossopetalum lobatum*. Although the known *seco*-ring-*A* friedelane lactones from nature are rare, they have been reported to have significant biological activity that includes, promotion rat mesenchymal stem cells (rMSCs) proliferation (Wang et al. 2010), cytotoxicty (Reyes et al. 2010), anti-inflammatory (Chai et al. 2009) and anti-dermatophytic activities (Kuiate et al. 2007).

Viola of *Violaceae*, containing about more than 500 species, is widely distributed in temperate zone, tropic and subtropics, and mainly in temperate zone of northern hemisphere. There are about

Abbreviations: CC_{50}, the concentration to inhibit Hep G2.2.15 cell proliferation by 50%; DEPT, distortionless enhancement by polarization transfer; DMEM, Dulbecco's modified Eagle's medium; DMSO, dimethyl sulfoxide; ELISA, enzyme-linked immunosorbent assay; HBeAg, hepatitis B e antigen; HBsAg, hepatitis B surface antigen; HBV, hepatitis B virus; HMBC, ^{1}H detected heternuclear multiple bond correlation; HMQC, ^{1}H detected heternuclear multiple quantum coherence; IC_{50}, 50% inhibition concentration against HBV synthesis; MTT, methyl thiazolyl tetrazolium; NOESY, nuclear Overhauser enhancement spectroscopy; SI, selectivity index; TLC, thin layer chromatography.

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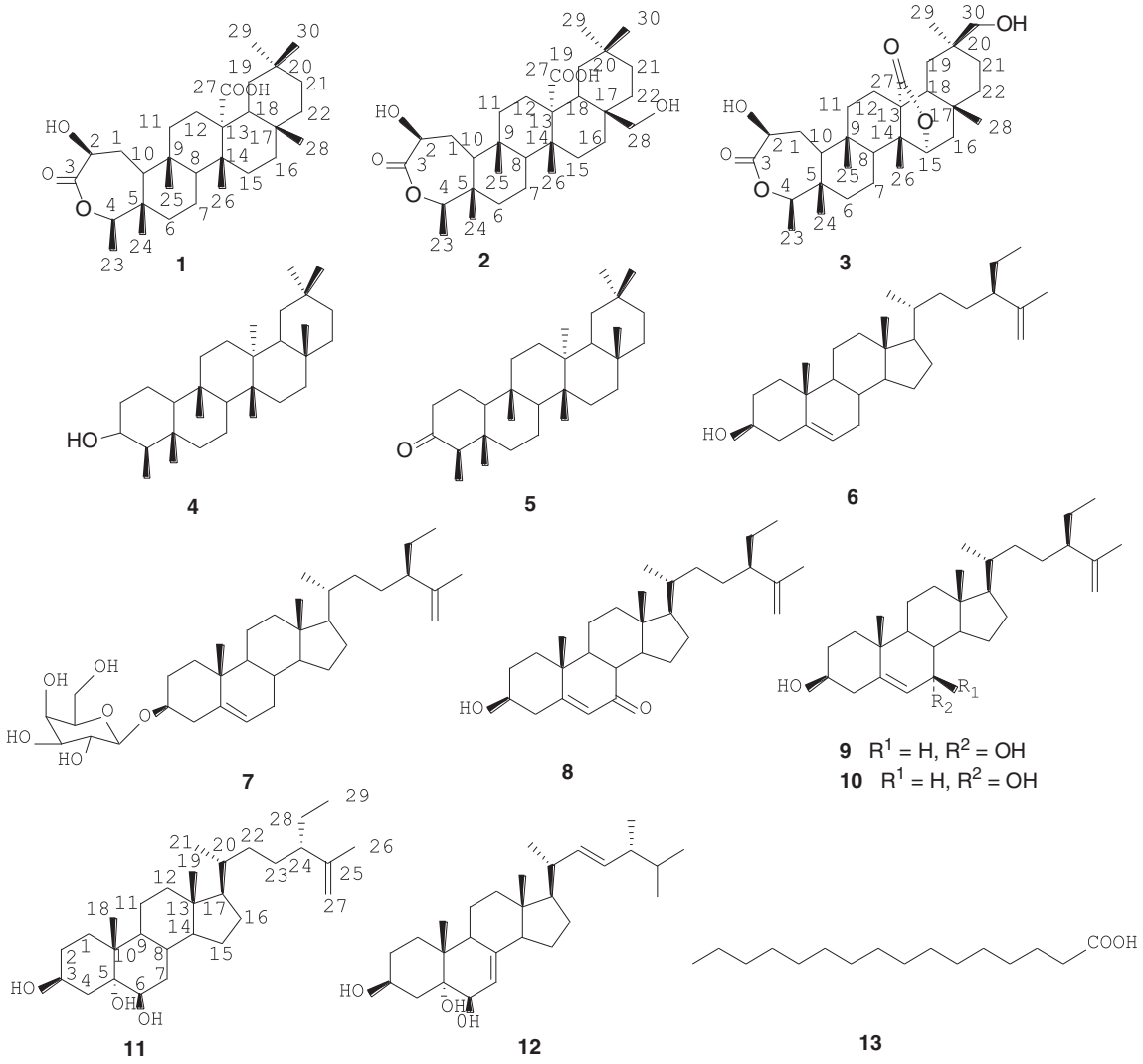

Fig. 1. Structures of compounds **1–13**.

111 species of *Viola* in China. They distribute in both southern and northern area and grow in the mountain forest, hillside meadow, roadside shrubs (Delectis Florae Republicae PoPularis Sinicae Agendae Academiae Sinicae Edita 1991). Since Péter et al. (Péter et al. 1980) identified two isomerides of violaxanthin from *Viola tricolor* by MS, IR and UV, the researches on the chemical constituents of *Viola* plants began to rise. At present, flavonoids (Lee et al. 1993), coumarins (Qin et al. 1994), terpenoids (Hu and Ding 1987), sterols (Asilbekova et al. 1999), polypeptides (Ulf et al. 2004), organic acids (Liu et al. 2011) and other components (Péter et al. 1980) have been isolated from *Viola* genus. Furthermore, the antibacterial (Zhu et al. 1986), anti-inflammatory (Li et al. 2010), anti-viral (Ngan et al. 1988), anti-oxidation (Shen and Xie 2009) and inhibiting K562 leukemia cell (Li et al. 2009) activities of these constituents have also been reported.

Viola diffusa G. is an annual or perennial herb of *Viola* genus mainly distributed in the southern part of P.R. China, and has been used as a traditional Chinese herbal medicine for the treatment of hepatitis, pleuritis, conjunctivitis, venomous snake bite and ulcerative carbuncle (Fujian Science and Technology Committee 1990). So far, its chemical constituents have not been systematically investigated. Here, we isolated of three new friedelolactones, violaic A (**1**), violaic B (**2**) and violalide (**3**), a new stigmastane, stigmast-25-ene-3β,5α, 6β-triol (**11**), and nine known compounds, epifriedelanol (**4**), friedelin (**5**), clerosterol (**6**), clerosterol galactoside (**7**), decorinone (**8**), decortinol (**9**), isodecortinol (**10**), cerevisterol (**12**) and palmitic acid (**13**) (Fig. 1), and evaluated their HBV surface antigen (HBsAg) and e antigen (HBeAg) secretion blocking effects on HBV-infected HepG2 2.2.15 cells of compounds **1–9, 11, 12, 13**. This is the first report of friedelolactones from the Violaceae.

Material and methods

Plant material

The whole plants of *Viola diffusa* G. were collected in Yongchun, Fujian, China, in August 2009 and authenticated by Professor Ji Ma, School of Chinese Medicine, Southern Medical University. A voucher specimen (No. 200908101) was deposited in the Chemistry Department of Traditional Chinese Medicine, Southern Medical University.

Extraction and isolation

The dried materials (10 kg) were successively extracted with 95% EtOH three times under reflux, 1.5 h each time. The extract was concentrated under vacuum to give a residue (0.9 kg), which was suspended in H_2O (5.5 L) and sequentially partitioned with petroleum ether (3 × 4 L), EtOAc (3 × 4 L), and n-BuOH (3 × 4 L). The n-BuOH