Fitoterapia 118 (2017) 94–100

Contents lists available at ScienceDirect

Fitoterapia

journal homepage: www.elsevier.com/locate/fitote

Monomeric and dimeric ent-kauranoid-type diterpenoids from rabdosia japonica and their cytotoxicity and anti-HBV activities

Hong-Chuan Liu [a,b], Zhao-Bao Xiang [a,c], Qiang Wang [d], Bo-Yan Li [a], Yong-Sheng Jin [a,*], Hai-Sheng Chen [a,*]

[a] School of Pharmacy, Second Military Medical University, Shanghai 200433, China
[b] Department of Pharmacy, Beijing Chao-yang Hospital, Capital Medical University, Beijing 100020, China
[c] Key Laboratory of Natural Medicine Research of Chongqing Education Commission, Chongqing Technology and Business University, Chongqing 400067, China
[d] Department of Pharmacy, No. 202 Hospital of PLA, Shenyang 110000, China

ARTICLE INFO

Article history:
Received 19 December 2016
Received in revised form 8 March 2017
Accepted 10 March 2017
Available online 12 March 2017

Keywords:
Labiatae
Rabdosia japonica
ent-kaurane diterpenoid
Cytotoxicity
Anti-HBV activity

ABSTRACT

Two new *ent*-kauranoid-type diterpenoids (**1** and **2**) and one new rare dimer of *ent*-kauranoids (**3**) with a cyclobutane ring by a [2 + 2] cycloaddition, together with nine known diterpenoids (**4**–**12**) were obtained from the aerial parts of *Rabdosia japonica*. Their chemical structures were established by 1D and 2D NMR techniques and mass spectrometry and by comparison with spectroscopic data reported. All *ent*-kauranoids were test for their cytotoxic effects against A549, HCT116, CCRF-CEM and HL-60 tumor cell lines. Compounds **1**, **2**, **4**, **5**, **7**, **10** and **12** showed potent and selective cytotoxicity. In addition, some selected *ent*-kauranoids were test for their anti-HBV activities, and the results showed compound **8** had inhibitory effect on HBsAg with a 59% inhibition ratio at the concentration of 20 μg/mL.

1. Introduction

Rabdosia japonica (Burm. f.) Hara var. *glaucocalyx* (Maxim.) Hara, a plant of the genus *Rabdosia,* is mainly distributed over the northeast of China [1]. The *Rabdosia* species have been used in Chinese popular folk medicine for the treatments of hepatitis, gastricism, mastitis and cancer for a long history [2]. Previous phytochemical investigations on *R. japonica* have been reported the isolation of various *ent*-kauranoid-type diterpenoids with interesting cytotoxic activity [3–6]. As a continuation of our investigations on the chemical constituents and biological activities of *R. japonica* [5,7–9], three new (**1**–**3**) and nine known (**4**–**12**) *ent*-kauranoid-type diterpenoids were isolated and identified from the EtOAc extract of aerial parts of *R. japonica.* All diterpenoids (**1**—**12**) were evaluated for their cytotoxic activities against four human tumor cell lines (A549, CCRF-CEM, HCT116 and HL-60). The anti-HBV activities of some selected diterpenoids (**4**–**6, 8**–**10, 12**) were also described.

2. Results and discussion

The EtOH extract of the aerial parts of *R. japonica* was suspended in H_2O and then partitioned successively with petroleum ether, EtOAc, and *n*-BuOH. The entire EtOAc fraction was subjected repeatedly to column chromatography over silica gel to afford three new (**1**–**3**) and nine known (**4**–**12**) *ent*-kauranoid-type diterpenoids: glaucocalyxin A (**4**) [10], glaucocalyxin B (**5**) [11], glaucocalyxin C (**6**) [4], glaucocalyxin D (**7**) [10], glaucocalyxin E (**8**) [4], glaucocalyxin F (**9**) [10], oridonin (**10**) [12], minheryins I (**11**) [13], and glaucocalyxin X (**12**) [9] (Fig. 1).

Compound **1** (Fig. 1) was obtained as a white powder and its molecular formula was determined to be $C_{22}H_{32}O_5$ based on the HR-ESIMS (*m/z* 399.2132 $[M + Na]^+$, calcd for $C_{22}H_{32}O_5Na$, 399.2147) and ^{13}CNMR data. The UV spectrum showed a maximum band at 228 nm, which indicated the presence of α, β-unsaturated ketone. IR absorptions at 3502, 2960, 1754, and 1648 cm^{-1} implied the presence of hydroxy, carbonyl, and α, β-unsaturated ketone functionalities. The ^{13}C, DEPT and HMQC NMR spectra of **1** (Table 1) gave twenty-two carbon signals comprising four methyl, six methylene, six methine (three of them were oxygenated), and six quaternary carbons (including sp^2 carbon and one carbonyl), which was consistent with a skeleton of an *ent*-kaur-16-en-15-one [14]. The ^{1}HNMR spectrum (Table 1) showed the existence of three tertiary methyls (δ 1.20, 0.84, 1.02), three oxygenated methines (δ 3.21, 4.18, 6.00), an exocyclic double band (δ 6.18, 5.41) and one methyl group (δ 2.00). The ^{1}H and ^{13}CNMR data of **1** were highly similar to those of glaucocalyxin B (**5**), which had been isolated from *R. japonica* [6,9], except for the observation of signals for an oxymethine group at δ_H 3.21 (dd, J = 11.4, 4.8 Hz), and δ_C 78.4 in **1** instead of the C-3 carbonyl carbon at δ_C 219.7 in compound **5**, indicating the C-3 carbonyl group was replaced by a secondary hydroxy group. This was verified by the ^{1}H-^{1}H COSY correlations between H-2 and H-3. Meanwhile, the

* Corresponding authors.
E-mail addresses: ysjin@smmu.edu.cn, ysjinsmmu@163.com (Y.-S. Jin), hschen2012@163.com (H.-S. Chen).

http://dx.doi.org/10.1016/j.fitote.2017.03.006
0367-326X/

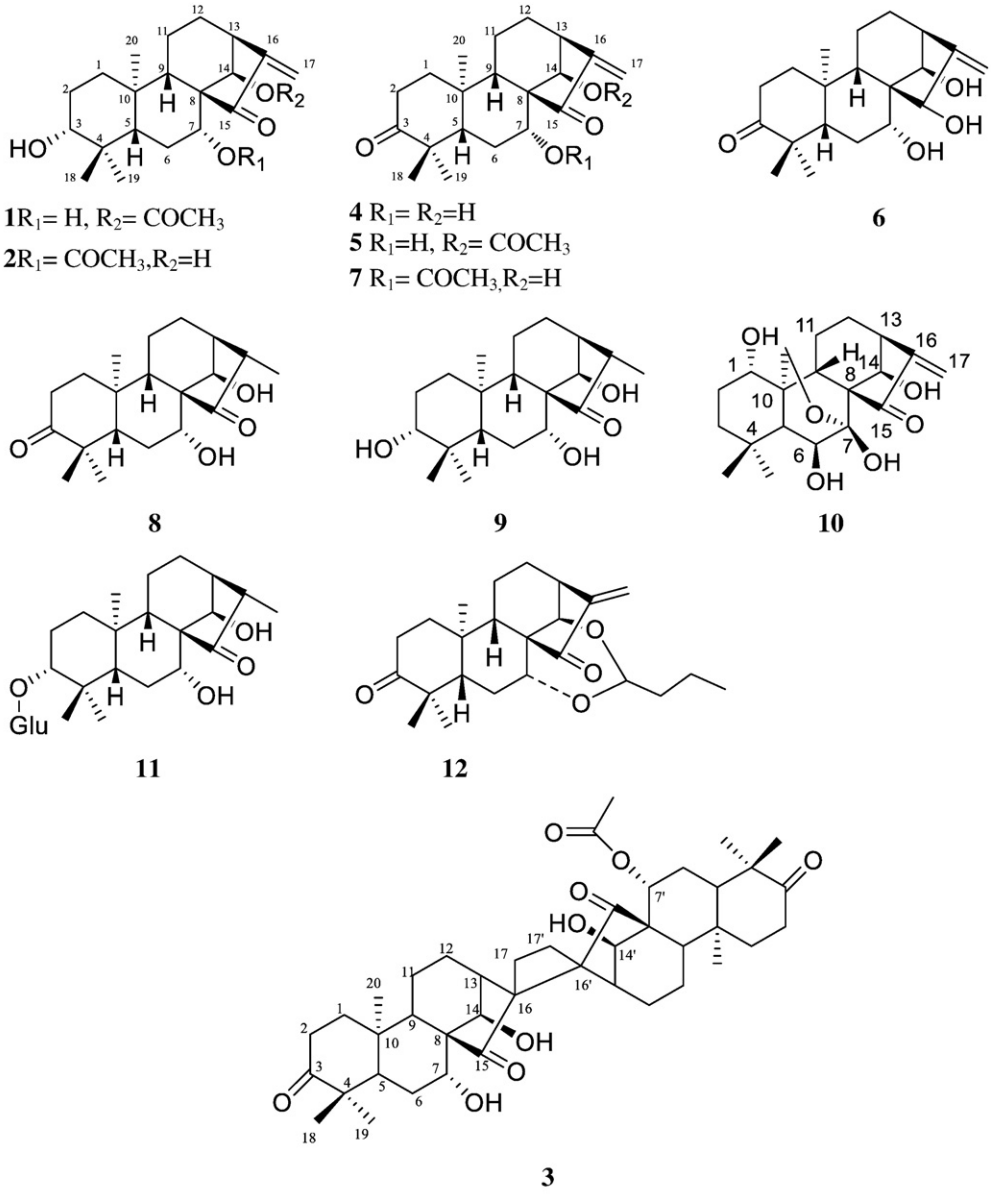

Fig. 1. Chemical structures of compounds **1**–**12.**

acetoxyl group at C-14 was confirmed by the HMBC correlations from H-14 to C-15, C-16 and carbonyl carbon of the acetyl group, and the ketone carbonyl group located at C-15 was confirmed by the HMBC correlations from H-17a to C-15 (Table 1).

The relative stereochemistry of the hydroxyl groups at C-7 and C-14 were determined by the analysis from NOESY experiments. The hydroxyl groups at C-7 and C-14 were shown to be in α and β orientations, respectively, as deduced from the NOESY correlations between H-7 (δ 4.18, dd, J = 12.0, 3.6 Hz) and H-9 (δ 1.35, m), and between H-14 (δ 6.00, brs) and H-12 (δ 2.19, m), H_3-20 (δ 1.20, s) demonstrated that the 7-OH and 14-OH adopted α and β orientations, respectively. The 3-OH was deduced to be α-oriented by NOESY correlations from H-3 (δ 3.21, dd, J = 11.4, 4.8 Hz) to H-5 (δ 1.05) as shown in Fig. 2. The coupling constants of H-3 (δ 3.21, dd, J = 11.4, 4.8 Hz) and H-7 (dd, J = 12.0, 3.6 Hz) further confirmed the 3-OH and 7-OH adopted α orientation, because the coupling constant between Ha-Ha is usually between 9 and 13 Hz. Finally, compound **1** was established as 14β-acetyloxy-3α,7α-dihydroxy-*ent*-kaur-16-en-15-one.

Compound **2** (Fig. 1) was obtained as a white powder. The molecular formula of **2** was determined to be $C_{22}H_{32}O_5$ by a *quasi*-molecular ion at *m/z* 377.2317 $[M + H]^+$ (calcd for $C_{22}H_{33}O_5$, 377.2328) in its HRESIMS. The NMR data of **2** (Table 1) were highly similar to those of **1**, except that distinct downfield shifts of H-7 in **2** (δ 5.41, dd, 12.0, 4.2 Hz) and of H-14 in **1** (δ 6.00, brs) in ^{1}HNMR. This demonstrated the acyl group is located at the C-7 position in **2** rather than at the C-14 position, which is further confirmed by HMBC correlations from H-7 to the carbonyl group of acetyl group (Table 1). The relative stereochemistry of the hydroxyl groups at C-7 and C-14 were deduced as α and β orientations from NOESY correlations between H-7 (δ 5.41, dd, J = 12.0, 4.2 Hz) and H-5 (δ 1.04), H-9 (δ 1.34, d, J = 8.4 Hz); H-14α (δ 4.88, s) and H_3-20 (δ 1.09, s). The 3-OH was deduced to be α-oriented by NOESY correlations from H-3 (δ 3.25, dd, J = 12.0, 4.8 Hz) to H-5 (δ 1.04) (Fig. 2). The coupling constants of H-3 (dd, J = 12.0, 4.8 Hz) and H-7 (dd, J = 12.0, 4.2 Hz) further confirmed the 3-OH and 7-OH adopted α orientation. Consequently, the structure of compound **2** was characterized as 7α-acetoxy-3α,14β-dihydroxy-*ent*-kaur-16-en-15-one.

Compound **3** (Fig. 1) was obtained as a white powder that analyzed for the molecular formula $C_{42}H_{58}O_9$ by HR-ESIMS data [*m/z* 705.4031[M-H]$^-$ (calcd for $C_{42}H_{57}O_9$, 705.4003, 741.3796 $[M + Cl]^-$ (calcd for $C_{42}H_{58}O_9Cl$, 741.3769)]. Its IR spectrum displayed the