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Clerodane diterpenoids from the Chinese liverwort *Jamesoniella autumnalis* and their anti-inflammatory activity

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ABSTRACT

Nine previously undescribed clerodane-type diterpenoids, jamesoniellides M−T and one *ent*-labdane-type diterpenoid, as well as one known analogue, were isolated from the Chinese liverwort *Jamesoniella autumnalis* (DC.) Stephani. Their structures were determined using MS, NMR spectroscopy, and electronic circular dichroism (ECD) calculations. Inhibition on LPS-induced NO production in RAW 264.7 murine macrophages was investigated, and the results showed that jamesoniellides Q−S exhibited moderate anti-inflammatory activity, with 50–80% maximum inhibition rate of NO production under the nontoxic tested concentration.

1. Introduction

Clerodane diterpenoids are a widespread class of natural products (Li et al., 2016) that have attracted considerable interest because of their noteworthy biological activities, including insect antifeedant (Klein Gebbinck et al., 2002), antitumour (Hayashi et al., 2002), and antimicrobial (Marthanda et al., 2005) activities. Liverworts, as a rich source of bioactive substances (Asakawa, 1982, 2007; Asakawa and Ludwiczuk, 2017; Asakawa et al., 2013a, 2013b; Zinsmeister et al., 1991), have produced many exceptional clerodane-type diterpenoids such as novel caged clerodanes (e.g., scaparvins A–E and jamesoniellide C) from *Scapania parva* (Guo et al., 2011) and *Jamesoniella autumnalis* (Tazaki et al., 1994), degraded clerodanes from *Jamesoniella colorata* (Toyota et al., 2010), and 8,9-*seco*-clerodanes (e.g., jamesoniellides A and J and cephaloziellins E-G) and 9,10-*seco*-clerodanes (e.g., jamesoniellides D, E, H, and I and cephaloziellins H–K) from *Jamesoniella autumnalis* (Blechschmidt and Becker, 2004; Tazaki et al., 1995, 1999) and *Cephaloziella kiaeri* (Li et al., 2014), respectively.

To increase our knowledge of the chemical and biological diversity of clerodane-type diterpenoids, the chemical investigation of the Chinese liverwort *Jamesoniella autumnalis* (DC.) Stephani, collected from the Changbai Mountain in Jilin province, China, led to the discovery of 9 previously undescribed clerodane-type diterpenoids, jamesoniellides M−T, of which compounds **1**–**8** were determined to be *seco*-clerodanes. In addition, one previously undescribed *bisnor-ent*-labdane (**10**) as well as one known *nor-ent*-labdane diterpenoid, 15-*nor*-14-oxolabda-8 (17),12E-dien-19-ol (**11**), were obtained. Structural elucidation of the undescribed compounds was achieved by spectroscopic methods and by comparison of experimental and calculated ECDs. Herein, we report the isolation, structural elucidation and anti-inflammatory activity of these previously undescribed compounds.

2. Results and discussion

2.1. Isolation and identification of undescribed compounds

The EtOH–H_2O (95:5, v/v) extract of *J. autumnalis* was fractionated by chromatography over MCI gel, silica gel and Sephadex LH-20 and was then further purified by semipreparative HPLC to afford 11 compounds (Fig. 1). Preliminary NMR spectroscopic analysis of the previously undescribed compounds established the presence of a clerodane skeleton in compounds **1**–**9** and an *ent*-labdane skeleton in compound **10**. The structure of the known compound **11** was determined by comparison of its 1D NMR and ESI-MS data with those previously reported (Gan et al., 2009).

Compound **1** was obtained as an amorphous powder. Its molecular formula, $C_{22}H_{30}O_8$ (*m/z* 440.2278 $[M + NH_4]^+$, calcd 440.2279), with 8 degrees of unsaturation, was established based on HRESIMS data. The 1D NMR spectra (Tables 1 and 4) showed signals consistent with the presence of two methyl groups [δ_H 1.15 (d, J = 5.0 Hz, H_3-17) and 1.14 (s, H_3-19)], two methoxy groups [δ_H 3.61 (s, H_3-21) and 3.65 (s, H_3-22)], three oxygenated methines [δ_H 3.56 (m, H-8), 5.09 (ddt, J = 10.9,

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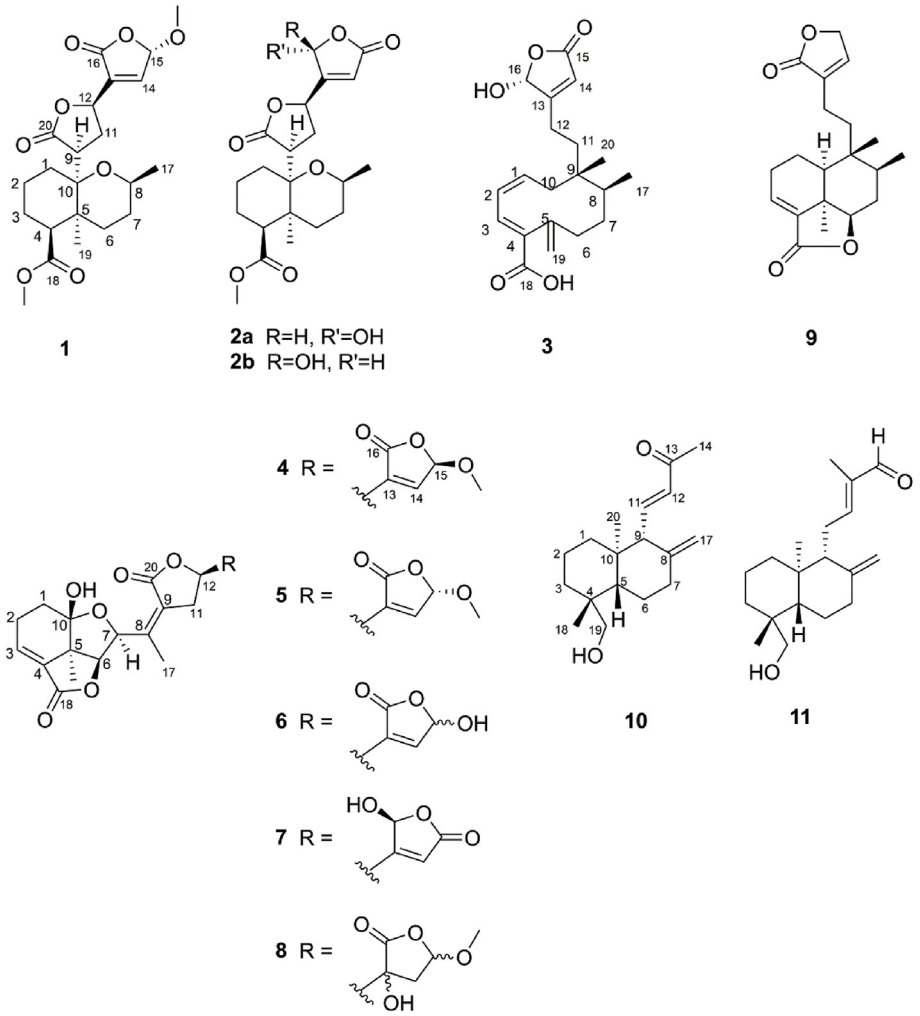

Fig. 1. Structures of compounds **1–11**.

6.4, 1.5 Hz, H-12) and 5.84 (s, H-15); δ_C 68.1 (C-8), 70.8 (C-12) and 103.3 (C-15)], one ester group [δ_C 175.1 (C-18)] and two lactone carbonyl groups [δ_C 174.6 (C-20) and δ_C 168.6 (C-16)]. Further analysis of its 1D NMR data suggested that compound **1** was similar to jamesoniellide A (Blechschmidt, 2004) except for the side chain linked to C-12. The existence of the 15-methoxy-13-buten-16,15-olide moiety linked to C-12 was proved by HMBC correlations from H-14 to C-13, C-15, C-16 and C-12 and from the methoxy protons to C-15. The presence of a 20,12-lactone ring was supported by HMBC correlations from H-11 to C-9, C-12 and C-20 as well as the degrees of unsaturation. HMBC correlations between H-8 and C-10 confirmed the presence of the 8,10-six membered epoxy ring. These data suggest that compound **1** is a *seco*-clerodane diterpene cleaved between C-8 and C-9. The relative configuration of **1** was established by performing NOE experiments. Correlations from H_3-19 to H-4, H-8 and H-9 and from H-9 to H-12 determined their cofacial orientation. H-1α and H-1β were determined by NOE correlations with H_3-19 and H-4, respectively. The configuration of C-15 and the absolute configuration of **1** were determined by comparing experimental and calculated ECD spectra (Fig. 4). Thus, compound **1** was identified as (4*R*,5*S*,8*S*,9*S*,10*S*,12*R*,15*S*)-8,10-epoxy-15,18-dimethoxy-8,9-*seco*-13-clerodene-20,12:16,15-diolide and was named jamesoniellide M due to similarities between it and other jamesoniellide family members isolated from the same species (Tazaki et al., 1995, 1999).

Following purification of the compounds from *J. autumnalis*, one compound mixture (**2**: **2a** and **2b**) was also obtained. The 1D NMR spectrum displayed signals consistent with a pair of isomers with nearly identical spectral data. The ^{1}H and ^{13}C NMR data (Table 2) and the molecular formula $C_{21}H_{28}O_8$ (*m/z* 409.1857 $[M + H]^+$, calcd 409.1857) of the mixture **2** were similar to those of compound **1** with the exception of the obvious distinctions related to the C-12 side chain. The typical downfield shift of C-13 (δ_C 163.7) as well as the upfield shift of C-14 (δ_C 119.2 and 118.9) proved the existence of a 15,16-olide instead of the 16,15-olide moiety (Bläs et al., 2004). Accordingly, **2** was identified as an approximately 1:1 mixture of *seco*-clerodane diterpenoids that share the same planar structure. The distinguishing characteristics between **2a** and **2b** include subtle differences in the chemical shifts of C-12, C-14 and C-16. NOESY experiments (Fig. S16) were performed to confirm the relative configurations of **2a** and **2b**. For example, H-4 showed correlations with H_3-19, H-2α and H-7α, and H_3-19 showed correlations with H-8 and H-9. A correlation between H-12 and H-9 indicated that these protons have the same orientation. This information proved that **2a** and **2b** have the same relative configuration except for C-16, which led us to conclude that **2a** and **2b** are C-16 epimers. To determine the absolute configuration of **2**, we calculated the ECDs for both **2a** and **2b**, and then mixed the two spectra using a 1:1 ratio. As a result, the calculated ECD spectra of the mixture coincided with the experimental one (Fig. 5). Therefore, the absolute configuration of **2** was determined as being epimeric at C-16 as shown.

Compound **3** was assigned the molecular formula $C_{20}H_{26}O_5$ based on HRESIMS data (*m/z* 345.1708 $[M - H]^-$, calcd 345.1707) and a ^{13}C NMR spectrum that indicated eight indices of hydrogen deficiency. The IR spectrum showed absorptions at 3356, 1735 and 1687 cm^{-1} due to one hydroxy group and two carbonyl groups, respectively. The ^{1}H NMR