A new 11,10-guaiane-type sesquiterpenoid from the roots of Stellera chamaejasme Linn

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Abstract
Chamaejasmone F, a new 11,10-guaiane-type sesquiterpenoid, is isolated from the roots of Stellera chamaejasme Linn., along with four known compounds including two guaiane sesquiterpenoids and two lignans. The structure of the new compound is elucidated by extensive spectroscopic analysis, especially two-dimensional nuclear magnetic resonance spectroscopy and high-resolution electrospray ionization mass spectrometry. The known compounds 1 (β-hydroxy-10β-H-guaia-4,11-dien-3-one) and (+)-hinokinin are isolated from the genus Stellera for the first time. In an in vitro assay, chamaejasmone F shows moderate cytotoxic activity against the human triple negative breast cancer cell line MB-MDA-231.

Keywords
cytotoxic, guaiane, lignan, sesquiterpenoid, Stellera chamaejasme

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Introduction
The genus Stellera (Thymelaeaceae) is composed of perennial herbs or shrubs that are distributed in temperate regions of Asia.1 Only two species in this genus have been found in China: Stellera chamaejasme Linn. and Stellera formosana Hayata ex Li. S. chamaejasme is the representative species of the genus, the dried roots of which are also known as “Rui-Xiang-Lang-Du” in China. Although regarded as highly toxic, S. chamaejasme Linn. has been widely used in traditional Chinese Medicine for the treatment of several disorders such as scabies, tinea, chronic tracheitis, and tuberculosis.2–4 Modern pharmacological studies have also revealed that S. chamaejasme has antitumor, antibacterial, anti-HIV, anti-epileptic, anticonvulsant, anti-inflammatory, immunity regulation, and insecticidal activity.5 Previous chemical investigations on the plant led to the discovery of flavonoids, coumarins, terpenoids, and lignans.2,5 Herein, we report the isolation, structure elucidation, and biological evaluation of a new 11,10-guaine-type sesquiterpenoid from the roots of S. chamaejasme Linn.

Results and discussion
Extensive column chromatography (CC) of the ethanol extract of the roots of S. chamaejasme Linn. afforded one new compound and four known compounds (Figure 1). The known compounds were identified as stelleraguanianone A (2),6 1β-hydroxy-10β-H-guaia-4,11-dien-3-one (3),7 (+)-hinokinin (4),8 and pinoresinol (5)9 by comparing their spectroscopic data with those reported in literature. To the best of our knowledge, compounds 3 and 4 are isolated from the genus Stellera for the first time.

Chamaejasmone F (1) was obtained as colorless oil. A molecular formula of C16H22O4Na was deduced from the HR-ESI-MS peak at m/z 301.1413 ([M + Na]+, C16H22O4Na; calcd 301.1410), corresponding to six degrees of unsaturation. The IR spectrum of 1 showed absorption bands for hydroxy (3396 cm⁻¹) and carbonyl (1754 and 1714 cm⁻¹) groups. In the 1H NMR spectrum of 1, displayed 16 carbon resonances including one methoxy, three methyls, three methylenes, three methine (one olefinic methine at δC 145.9, 175.1, and 216.7). These data suggested the existence of two carbonyls and one trisubstituted...
double bond in 1, accounting for three degrees of unsaturation. Hence, the remaining unsaturation required 1 to be tricyclic. In addition, the NMR spectrum of 1 exhibited characteristic signals of a guaiane-type sesquiterpenoid, the only sesquiterpenoid type found in the genus Stellera to date. All the aforementioned information indicated that 1 was probably a tricyclic guaiane-type sesquiterpenoid.

Analysis of the 1H NMR, 13C NMR, and HSQC spectra of 1 enabled us to assign all the protons to their bound carbons. After detailed comparison, the NMR data of 1 was found to share similarities with those of chamaejasmone D, a tricyclic 11,10-guaiane-type sesquiterpenoid previously isolated from the same plant, implying that they were structurally related. The distinctive NMR resonances in 1 were due to the carbonyl (δc 175.1) and the methoxy groups (δh 3.71 and δc 52.2) which replaced a hydroxymethyl in chamaejasmone D. By comprehensive analysis of the 2D NMR spectra of 1 (Figure 2), an intact tricyclic 11,10-guaiane moiety can be established. Specifically, 1H−1H COSY plots between H-2/H-3, H-3/H-4, H-4/H-5, H-4/H-15, and H-5/H-6 revealed the presence of a partial structure =CH(2)−CH2(3)−CH(4)/CH3(15)−CH(5)−CH2(6). The HMBC correlations from H-5 to C-1 and C-2 indicated the linkage between C-2 and C-5 via C-1 to form the five-membered ring of the guaiane moiety. The HMBC spectrum of 1 also showed correlations from H-2 to C-10, from H2-14 to C-1, C-9, and C-10, from H2-6 to C-1 and C-7, and from H2-6 and H2-9 to the ketone carbon C-8, which constructed a seven-membered ring that was fused with the five-membered ring via C-1 and C-5. Additional HMBC correlations from H3-13 and H3-14 to the quaternary carbons C-10 and C-11 demonstrated the connection of C-10 and C-11 to form a tricyclic 11,10-guaiane moiety. The additional carbonyl (δc 175.1) was deduced to be located at C-12 based on the HMBC correlation from H3-13 to C-12. Furthermore, the HMBC correlation from H2-16 to C-12 anchored the methoxy to the carbonyl (δc 175.1) to form the methyl ester. Thus, compound 1 was elucidated to be an analogue of chamaejasmone D possessing a methyl carboxylate at C-12. The relative stereochemistry of 1 was deduced to be the same as that of chamaejasmone D by a NOESY experiment (Figure 2).

In an in vitro assay, chamaejasmone F (1) exhibited moderate cytotoxic activity against the human triple negative breast cancer cell line MDA-MB-231 with an IC50 value of 23.78 ± 0.89 µM as compared with the positive control taxol (IC50 = 0.31 ± 0.03 µM).

Guaiane-type sesquiterpenoids belong to a special group of natural products with a basic skeletal structure containing a five-membered ring, a seven-membered ring, two methyl (anchoring at C-4 and C-10) and one isopropyl group (anchoring at C-7). The basic skeleton is prone to rearrange to form a 12,6-guaiane lactone, a 12,8-guaiane lactone, pseudoguaianolides, an 11,1-guaiane, an 11,6-guaiane, and an 11,10-guaiane, significantly increasing the structural diversity of guaiane-type sesquiterpenoids. This type of natural products has been reported to exhibit various biological activities, such as antitumor, anti-inflammatory, and antibacterial. Many of these compounds have been widely used in pharmaceutical and perfume industries. Thymeleaeaceae
plants are rich in guaiane-type sesquiterpenoids.12 As compared with other genus in the Thymelaeaceae family, for example, Aquilaria,13–18 the genus Stellera Linn. has not been well studied for the presence of guaiane-type sesquiterpenoids, with only 12 compounds reported to date.6,10,19,20 A recent study reported the identification of four guaiane synthases from the transcriptome of S. chamaejasme Linn., implying the great biosynthetic potential of guaiane-type sesquiterpenoids in this plant.12 Hence, S. chamaejasme Linn. represents an untapped source of guaiane-type sesquiterpenoids and warrants systematic investigation.

Experimental

All solvents used were of analytical grade. Optical rotations were determined on a Perkin-Elmer 341 spectrophotometer. IR spectra were recorded on a Thermo-Nicolet-6700 spectrometer. NMR spectra were recorded on an Agilent-6210-Lc/Tof mass spectrometer. Silica gel (300–400 mesh, Qingdao Haiyang Chemical Plant, Qingdao, P.R. China), MCI CHP 20P gel (75−150 µm, YMC Co. Ltd., Kyoto, Japan) were used for CC, and a precoated silica gel GF254 plate (Qingdao Haiyang Chemical Plant, Qingdao, P.R. China) was used for TLC.

Plant material was collected from the Tibetan Autonomous Prefecture of Garzê of Sichuan Province, P.R. China, in July 2016 and identified as S. chamaejasme Linn. by Prof. Wen-Hong Liu of Zhejiang Chinese Medical University. A voucher specimen (No. SC20160706) has been deposited at Hangzhou Zhongmeihuadong Pharmaceutical Co Ltd.

The roots of S. chamaejasme Linn. (1 kg) were pulverized and extracted with 95% ethanol (3 × 5 L, 3 d each) at room temperature to give a crude extract after evaporation. The crude extract (95 g) was suspended in water and partitioned with EtOAc (3 × 1 L). The organic phase was concentrated under reduced pressure to yield an EtOAc-soluble residue (23 g), which was subjected to silica gel CC eluting with a gradient of petroleum ether/EtOAc (40:1→1:1, v/v) to furnish four fractions Frs. A−D. Fraction B (5.3 g) was separated by CC on MCI CHP 20P gel eluting with a gradient of MeOH/H2O (80:20→95:5, v/v) to give five subfractions Frs. B1−B5. Compound 1 (5.4 mg) was obtained from fraction B3 by silica gel CC eluting with petroleum ether/acetone (5:1, v/v) followed by CC on ODS C18 gel eluting with MeOH/H2O (70:30, v/v). Fraction B4 was purified by silica gel CC eluted with petroleum ether/acetone (8:1, v/v) to afford compound 4 (4.8 mg). Compound 1 (1.7 mg) was also separated by CC on MCI CHP 20P gel eluting with a gradient of MeOH/H2O (40:60→90:10, v/v) to give three subfractions Frs. C1−C3. Fraction C1 was subjected to ODS C18 CC eluting with MeOH/H2O (60:40, v/v) followed by CC on HW-40C gel eluting with methanol to yield compound 2 (3.1 mg). Compounds 3 (6.6 mg) and 5 (8.3 mg) was obtained successively from fraction C2 by CC on ODS C18 gel eluting with a gradient of MeOH/H2O (50:50→55:45, v/v). Chamaejasmone F (1): colorless oil. [α]D20 36.7 (c 0.06, MeOH). IR (KBr): vmax 3396, 2959, 2929, 1754, 1714, 1454, 1408, 1380, 1291, 1261, 1135, 1065, 1033 cm−1. 1H and 13C NMR: see Table 1. HR-ESI-MS: m/z [M + Na]+ calcd for C16H22O4Na: 301.1410; found: 301.1413.

Cytotoxicity Assay: compound 1 was evaluated for cytotoxicity against the human triple negative breast cancer cell line MDA-MB-231 by means of the MTT ([3-(4,5-dimethylthiazol-2-yl)-2,5-diphenyltetrazolium bromide]) assay following a protocol reported in the literature.21 The cell line was purchased from the China Center for Type Culture Collection, Wuhan University.

Declaration of conflicting interests

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Supplemental material

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