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Stelleraguaianone B and C, two new sesquiterpenoids from *Stellera chamaejasme* L.

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ABSTRACT

Two new sesquiterpenoids were isolated from *Stellera chamaejasme* L., known as the traditional Chinese herb 'Rui Xiang Lang Du'. The compounds were elucidated as stelleraguaianone B (**1**) and C (**2**) by comprehensive spectroscopic analysis, including 1D and 2D NMR as well as HRESIMS, and by comparing their NMR data with known compounds. In addition, the structure of **1** was further confirmed by single-crystal X-ray diffraction analysis. Both the compounds were evaluated for their cytotoxicity on common tumour cell lines *in vitro*, which revealed that compound **1** exhibits cytotoxic activity on A549 cells, while **2** has no activity.

1. Introduction

Stellera chamaejasme L. (Thymelaeaceae), also known as 'Lang Du', is a traditional Chinese medicinal perennial herb that is widespread in the northern and southwestern regions of China. The root of the plant contains medicinal components and has shown to treat oedema, phlegm, inflamed lymph nodes, and parasite diseases. Previous phytochemistry studies revealed that the chemical composition of *S. chamaejasme* L. includes diterpenoid **[1,8]**, sesquiterpenoid **[1]** and lignans **[1]** compounds. Furthermore, the current several research have reported that the plant was focused on cytotoxic **[1–5]**, antibacterial **[6]**, antiviral **[7,8]**, and anti-tumour **[9–12]** properties. Herein, the chemical constitution and activity of the isolated *S. chamaejasme* L. sesquiterpenes were evaluated.

To investigate the chemical components and biological activity, two new sesquiterpenoids, denoted stelleraguaianone B (**1**) and C (**2**), were isolated from a *S. chamaejasme* L. plant obtained from Yunnan province (Fig. 1). The cytotoxicity of the two compounds on tumour cell lines was also examined in this paper.

2. Results and discussion

Stelleraguaianone B (**1**) was obtained as colourless orthorhombic crystals (MeOH) with $[\alpha]_D^{21}$–93.9 (*c* 0.02 MeOH). Its molecular formula, $C_{15}H_{20}O_3$, with 6 degrees of unsaturation was established by HRESIMS (*m/z* 271.1308 $[M + Na]^+$, calcd for $C_{15}H_{20}O_3Na$, 271.1305) and ^{13}C NMR spectroscopic data (Table 1). The IR absorption peaks at 3359, 1700, and 1623 cm^{-1} indicate the presence of hydroxyl, carbonyl, and double-bond groups, respectively. All 15 carbons were well resolved in the ^{13}C NMR and DEPT spectra and were classified as three methyl groups (δ_C 22.4, 18.1, 12.5), four methylene groups (exocyclic double bond at low field δ_C 109.9), two methine groups and six quaternary carbons (cyclic carbonyl δ_C 196.6, oxygenated quaternary carbons δ_C 148.1, 67.8, 66.8). The ^1H NMR spectroscopic data show three methyl peaks (δ_H 2.00, 1.76, 1.59) and one double bond (δ_H 4.81, 4.76). Three additional rings were determined based on the six indices of hydrogen deficiency deduced by the molecular formula. The linkage of the structural fragments with quaternary carbons was determined by 2D NMR ($^1H-^1H$ COSY, HSQC, HMBC and ROESY) data (Fig. 2 and Fig. S4-S7 in Supplementary Material). $^1H-^1H$ COSY and HSQC correlations reveal the existence of one structural fragment, indicated by the bold bonds in Fig. 2a. The exocyclic CH_2=$CHCH_3$ structure segment was confirmed by the HMBC (Fig. 2a) correlation of H-12/C-11, C-7, and C-13. The location of CH_3 (δ_C 12.5) on C-4 was determined by the obvious correlation of H-15 and quaternary carbons C-4, C-5, C-3, which also indicates the existence of the enol form segment in compound **1**. A 7-membered ring structure is revealed by the correlation of H-7/C-5 with C-10; H-6/C-1 with C-8, and H-14/C-1 with C-9, verifying the location

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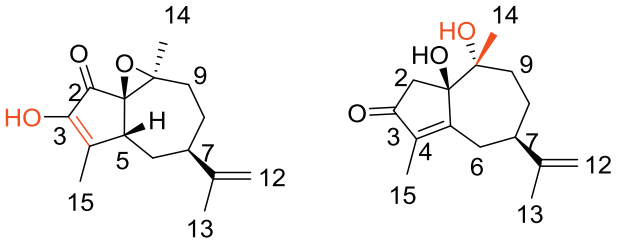

1 2

Fig. 1. Structures of stelleraguaianone B (**1**) and C (**2**).

Table 1
^{1}H and ^{13}C NMR Spectroscopic Data of **1** and **2** (500 and 125 MHz in $CDCl_3$ and DMSO-d_6; δ in ppm, J in Hz).

	1[a]		2[b]	
Position	δ_H	δ_C, type	δ_H	δ_C, type
1		67.8, C		80.8, C
2		196.6, C	2.53, d (17.8) 2.13, d (17.8)	46.6, CH_2
3		145.7, C		206.0, C
4		149.1, C		173.5, C
5	2.71, d (10.2)	40.1, CH		135.5, C
6	1.55, m; 1.95, m	30.7, CH_2	2.67, dd (11.6, 2.2) 2.34, dd (11.6, 2.2)	29.9, CH_2
7	2.18, m	41.8, CH	2.00, m	46.0, CH
8	1.61, m; 1.49, m	22.5, CH_2	1.80, m; 1.45, m	27.1, CH_2
9	2.13, m; 1.95, m	33.5, CH_2	1.92, m; 1.33, m	34.7, CH_2
10		66.8, C		72.8, C
11		148.1, C		150.0, C
12	4.81, s; 4.76, s	109.9, CH_2	4.74, m; 4.67, m	109.2, CH_2
13	1.76, s	22.4, CH_3	1.71, s	19.8, CH_3
14	1.59, s	18.1, CH_3	1.03, s	25.2, CH_3
15	2.00, d (1.3)	12.5, CH_3	1.56, s	7.2, CH_3
1-OH			5.12, brs	
10-OH			4.55, brs	

[a] Recorded in $CDCl_3$. [b] Recorded in DMSO-d_6.

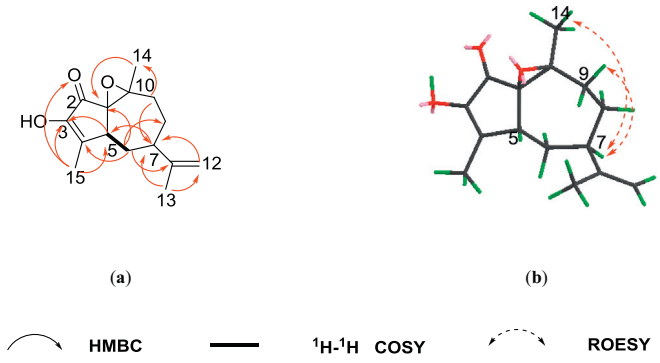

Fig. 2. Structural analysis of **1**: (**a**) ^{1}H–^{1}H COSY (bold) and key HMBC of **1**; (**b**) ROESY correlations of **1**.

of CH_2=$CHCH_3$ segment on C-7. An epoxy substructure was identified by the correlation of H-14 and quaternary carbons C-10 and C-1. Therefore, compound **1** was deduced to be a sesquiterpenoid composed of a 5-membered ring and 7-membered ring. The relative configuration of **1** was elucidated from the ROESY spectrum (Fig. 2b), where the ROESY correlation of H-9/H-7 and Me-10/H-7 reveals these protons to be cofacial. Thus, the protons were assigned arbitrarily as α-oriented, while the H-5 configuration was β-oriented.

The absolute configuration of **1** was elucidated by Cu-target single-crystal X-ray diffraction for further identification (Fig. 3). A colourless

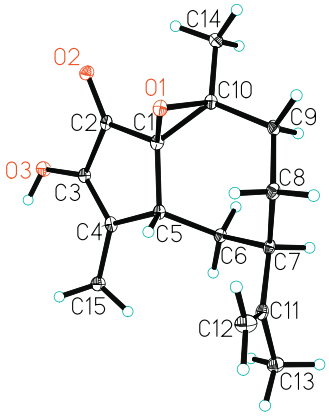

Fig. 3. X-ray ORTEP drawing of stelleraguaianone B (**1**). (CCDC. 1,880,822).

monoclinic crystal of **1** was obtained from MeOH. Intensity crystal data were collected at 100(2) K on a Bruker APEX DUO diffractometer equipped with an APEX II CCD using Cu Kα radiation. Bruker SAINT software was used for cell refinement and data reduction. The H atoms were placed in calculated positions and refined using a riding model. Molecular graphics were computed with PLATON. Crystallographic data (excluding the structure factor tables) for **1** have been deposited with the Cambridge Crystallographic Data Center as supplementary publication (deposit number CCDC 1880822, crystal data: see Fig. S14 and Table S1–7 in Supplementary Material). Fig. 3 displays the construction of the gross structure of stelleraguaianone B (**1**). In addition, there is a interesting phenomenon that compound **1** is a tautomeric form in the solid state, this could be due to C-2 carbonyl and C3-C4 double bond form conjugated system which average the electron density of C-3 that make enol form be relatively stable.

Compound **2** was isolated as a colourless amorphous powder, and its molecular formula was determined to be $C_{15}H_{22}O_3$ by high-resolution electrospray ionization mass spectrometry (HRESIMS), which give an m/z of 273.1479 for $[M + Na]^+$ (calcd for $C_{15}H_{22}O_3Na$, 273.1461) with 5 indices of hydrogen deficiency. The wide absorption peak at 3419 cm^{-1} in the IR spectrum indicates the presence of a hydroxyl group, while IR absorption peaks at 1705 and 1643 cm^{-1} are respective of carbonyl and double bond groups. ^{1}H NMR spectroscopic data (Table 1) show three methyl peaks (δ_H 1.71, 1.56, 1.03), one double bond (δ_H 4.74, 4.67), and two broad singlets (δ_H 5.12, 4.55). ^{13}C NMR and DEPT spectra data analysis reveal 15 carbon signals, including three methyl groups (δ_C 25.2, 19.8, 7.2), five methylene groups (exocyclic double bond at low field δ_C 109.2), one methine and six quaternary carbons (cyclic carbonyl δ_C 206.0). Despite the three unsaturated bonds, **2** was determined to have a bicyclic structure.

Based on ^{1}H and ^{13}C NMR data, the difference between compound **2** and 1β-hydroxy-10βH-guaia-4,11-dien-3-one **[13,14]** is the presence of one more quaternary carbon in **2** and the absence of one methine in the former. The 2D NMR (^{1}H–^{1}H COSY, HSQC, HMBC, and ROESY) (Fig. 4 and Fig. S10-S13 in Supplementary Material) spectrum further confirms the carbon skeletal structure of **2**, where an exocyclic CH_2=$CHCH_3$ structure segment is indicated by the correlation of H-12/C-11, C-7, and C-13 in the HMBC spectrum. A 5-membered ring structure is suggested by the correlation of H-2/C-1 with C-10, C-4, C-5, and C-3 and H-6/C-8 with C-7, C-1, C-4, and C-5. A linkage between CH_3 (δ_C 7.2) and C-4 is due to the correlation of H-15 and quaternary carbons C-4, C-5, and C-3. A 7-membered ring structure is supported by the correlation of H-8/C-10, C-11 and H-9/C-7, C-10, C-1 with the location of the CH_2=$CHCH_3$ segment on C-7. The respective location of two oxhydryl groups on C-1 and C-10 (Fig. 4a) is indicated by the correlation of the hydroxyl signal (δ_H 5.12) with C-2, C-10, C-1 and C-5 and hydroxyl signal (δ_H 4.55) with C-14, C-9, C-10 and C-1.

The relative configuration of **2** was inferred from ROESY spectrum

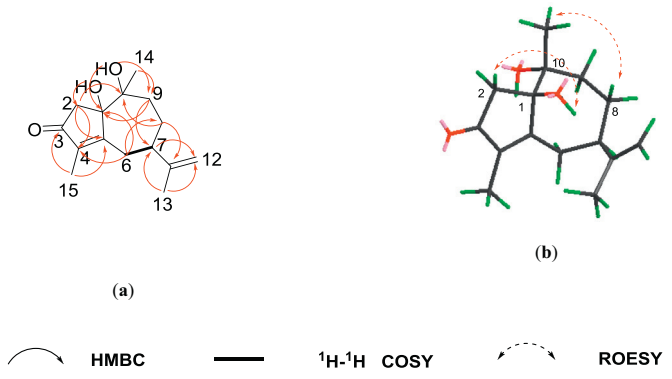

Fig. 4. Structural analysis of **2**: (**a**) ^{1}H–^{1}H COSY (bold) and key HMBC of **2**; (**b**) ROESY correlations of **2**.

data (Fig. 4b). The ROESY spectrum correlations of 10-OH/8α-H, 10-OH/6α-H, 10-OH/2α-H, and 10-CH_3/8β-H, along with correlative signal of 1-OH/2β-H, indicates that the two oxhydryl groups, 1β-OH and 10α-OH, are bifacial. From all data, the structure and relative configuration of **2**, named stelleraguaianone C, were determined as shown in Fig. 4.

There were few reports on sesquiterpenoids in *S. chamaejasme* L. but in plants of the same family (Thymelaeaceae) such as *Daphne aurantiaca* Diels, had been revealed to contain sesquiterpenoids **[15]** although characterized by the main production of diterpenoids. This might be related to the evolution of plant biosynthesis pathways under different growth conditions in different regions. Therefore, the possible biosynthetic pathways of two new sesquiterpenoids isolated from *S. chamaejasme* L. were speculated.

The structures of the two compounds which belonging to guaiane-type sesquiterpenoid were structurally close. A plausible biosynthetic pathway for them was proposed as shown in Fig. 5. A series of oxidation of guainane might lead to the compounds **1** and **2**. 3-OH is on the same plane as the C=O, which not only formed intramolecular hydrogen bond, but also composed to be a conjugated system, resulting in **1** becoming a more stable enolized α-dicarbonyl structure.

3. Experimental section

3.1. General experimental procedures

Optical rotations were measured with a JASCOP-1020 polarimeter. UV spectra were recorded on a Shimadzu UV-2401A. IR spectra were determined on a Bruker Tensor-27 infrared spectrophotometer with KBr disks. Melting points were measured using an X-4 apparatus (Yingyu Yuhua Instrument Factory, Gongyi, Henan Province, China). ESI and HRESIMS data were recorded using Agilent G6500 Series Q-TOF. NMR experiments were conducted on Bruker AM-400, DRX-500, or Avance III 600 spectrometers using residual $CDCl_3$ and DMSO-d_6 or TMS as the internal standard. Column chromatography (CC) was performed on silica gel (60–80 mesh, 200–300 mesh, 300–400 mesh, Qingdao Haiyang Chemical Co. Ltd., Qingdao, China), Sephadex LH-20 (40–70 μm, Amersham Pharmacia Biotech AB, Uppsala, Sweden), and Lichroprep RP-C18 gel (40–63 μm, Merck, Darmstadt, Germany). Precoated silica gel 60 GF_{254} (Merck, Darmstadt, Germany) was used for TLC analyses. Semipreparative HPLC analyses were performed on an Agilent 1100 liquid chromatograph with a Waters XBridge C_{18} column (i.d. 4.6 × 250 mm, 5 μm, 1.0 mL/min) and developed with MeOH-H_2O at room temperature (rt). All regular solvents and reagents were of reagent grade and purchased from Sigma-Aldrich Chemical Co. (St. Louis, MO, USA), Acros Organics (Geel, Belgium), and J&K Scientific (Beijing, China).

3.2. Plant materials

An entire *S. chamaejasme* L. plant was collected from Xiaozhongdian in Shangri-la, Yunnan Province, China in September 2015 and was identified by Prof. Yang Niu of Kunming Institute of Botany. A voucher specimen (20150929) was deposited at the State Key Laboratory of Phytochemistry and Plant Resources in West China.

3.3. Extraction and isolation

The air-dried, powdered twigs of *S. chamaejasme* L. (7.0 kg) were soaked with MeOH (3 × 50 L) and were extracted three times (4, 3, then 3 h) under reflux. The combined MeOH extracts were concentrated under vacuum to obtain a crude residue (1.0 kg), which was suspended in water and then partitioned with EtOAc. The EtOAc portion (758 g) was subjected to passage over a silica gel column eluted with a gradient of Pe-EtOAc (from 1:0 to 1:1, *v*/v) to yield three major fractions (1–3). Fraction 1 (18 g) was separated over an MCI gel column (MeOH-H_2O from 3:7 to 10:0, v/v), then purified by Sephadex LH-20 (MeOH) and repeated silica gel column chromatography eluted with a gradient of Pe-EtOAc (from 8:1 to 5:1, v/v). Subfractions were further purified by semipreparative HPLC (MeOH-H_2O, 4:6, v/v) to obtain compounds **1** (87.0 mg) and **2** (13.7 mg).

3.4. Characterization of compounds *1* and *2*

3-Hydroxy-1,10-epoxy-guaia-3,11-dien-2-one (**1**): A colourless orthorhombic crystal (MeOH); $[\alpha]_D^{21}$–93.9 (*c* 0.02 MeOH); UV (MeOH) λ_{max} (log ε) 274 (4.01) nm, 202 (3.73); IR (KBr) ν_{max} 3359, 2966, 2947, 2886, 1700, 1623, 1452, 1398, 1294, 1266, 636, 605 cm^{-1}; HRESIMS (positive) *m/z* 271.1308 $[M + Na]^+$ (calcd for $C_{15}H_{20}O_3Na$, 271.1305); ^{1}H NMR (500 MHz, $CDCl_3$) and ^{13}C NMR (125 MHz, $CDCl_3$) data see Table 1.

1β,10α-Dihydroxy-guaia-4,11-dien-3-one (**2**): A colourless amorphous powder; $[\alpha]_D^{21}$–84.5 (*c* 0.28 MeOH); UV (MeOH) λ_{max} (log ε) 241 (4.03) nm, 197 (3.72); IR (KBr) ν_{max} 3419, 2966, 2969, 2924, 2858, 1705, 1643, 1440, 1383, 1338, 885 cm^{-1}; HRESIMS (positive) *m/z* 273.1439 $[M + Na]^+$ (calcd for $C_{15}H_{22}O_3Na$, 273.1461); ^{1}H NMR (500 MHz, DMSO-d_6) and ^{13}C NMR (125 MHz, DMSO-d_6) data see Table 1.

3.5. Cytotoxic activity bioassay

The tested human cancer cell lines were seeded in 96-well plates (1 × 10^4 cells/well), and treated with the compounds at various concentrations of 1.5625, 3.125, 6.25, 12.5, 25, and 50 μM. After the treatment for 48 h, MTT (0.5 mg/mL) solution was added to each well and kept at 37 °C for 4 h. After removing the supernatant, DMSO (150 μL) was added into dissolve formazan crystals for the

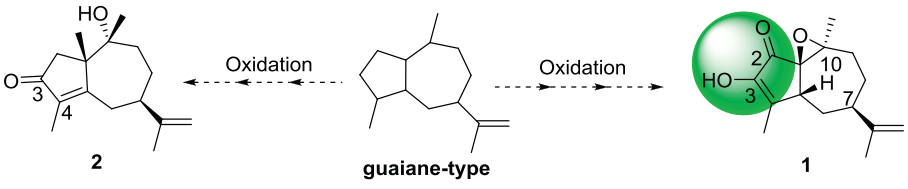

Fig. 5. Hypothetical biosynthetic pathway to **1** and **2**.

measurement of the absorbance at 570 nm using a microplate reader (TECAN A-5082, Magellan, Austria). Half-maximal inhibitory concentration (IC_{50}) values were calculated as drug concentrations for inhibiting 50% growth compared to control untreated cells.

Both the compounds were tested for cytotoxicity on HL60, A549, SMMC-7721, MCF-7, and SW480 tumour cell lines. Results show that compound **1** exhibits cytotoxicity on A549 cells with an IC_{50} of 8.52 μM, while compound **2** has no inhibitory activity on any of the tumour cell lines.

4. Conclusions

In this research, two new sesquiterpenoids were isolated from *S. chamaejasme* L. although their isolation from a plant family characterized by the production of diterpenoids and not sesquiterpenoids. A possible biosynthetic pathway of two new sesquiterpenoids were speculated. After evaluating the cytotoxicity of the sesquiterpenoids on A549, SMMC-7721, MCF-7, and SW480 tumour cell lines, compound **1** exhibited cytotoxicity on A549 cells with an IC_{50} of 8.52 μM, while compound **2** showed no activity. Further mechanistic studies are still underway, and the results will be reported in due course.

Conflict of interest

We declare that we do not have any commercial or associative interest that represent a conflict of interest in connection with the work submitted.

Acknowledgments

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Appendix A. Supplementary data

Supplementary data to this article can be found online at https://doi.org/10.1016/j.fitote.2019.03.024.

References

[1] L.P. Liu, K. Han, W. Chen, Y.Y. Zhang, L.J. Tong, T. Peng, H. Xie, J. Ding, H.B. Wang, Topoisomerase II inhibitors from the roots of *Stellera chamaejasme* L, Bioorgan. Med. Chem. 22 (2014) 4198–4203.
[2] Z.Q. Yan, L.M. Zeng, H. Jin, B. Qin, Potential ecological roles of flavonoids from *Stellera chamaejasme*, Plant Signal. Behav. 10 (2015) (e1001225/1-e1001225/3).
[3] H.Y. Cui, H. Jin, Q. Liu, Z. Q, L. Yan, B. Qin Ding, Nematicidal metabolites from roots of *Stellera chamaejasme* against Bursaphelenchus xylophilus and Bursaphelenchus mucronatus, Pest Manag. Sci. 70 (2014) 827–835.
[4] L. Pan, X.Z. Li, Z.Q. Yan, H.R. Guo, B. Qin, Phytotoxicity of umbelliferone and its analogs: structure-activity relationships and action mechanisms, Plant. Physiol. Bioch. 97 (2015) 272–277.
[5] H.R. Guo, H.Y. Cui, H. Jin, Z.Q. Yan, L. Ding, B. Qin, Potential allelochemicals in root zone soils of *Stellera chamaejasme* L. and variations at different geographical growing sites, Plant Growth Regul. 77 (2015) 335–342.
[6] B. Jo, N. Park, J. Jegal, S. Choi, S.W. Lee, H. Jin, S. Kim, M.H. Yang, A new flavonoid from *Stellera chamaejasme* L., stechamone, alleviated 2,4-dinitrochlorobenzene-induced atopic dermatitis-like skin lesions in a murine mode, Int. Immunopharmacol. 59 (2018) 113–119.
[7] T. Ikegawa, A. Ikegawa, Chamaejasmin and euchamaejasmin extraction from *Stellera chamaejasme* and their antiviral activities, Seimei Kagaku Kenkyusho Jugen (1996) 6.
[8] M. Yan, Y. Lu, C.H. Chen, Y. Zhao, K.H. Lee, D.F. Chen, Stelleralides D-J and anti-HIV Daphnane Diterpenes from *Stellera chamaejasme*, J. Nat. Prod. 78 (2015) 2712–2718.
[9] Q. Li, X.X. Kan, J. Yin, L.D. Sun, Y.J. Wang, Y.J. Li, Q. Yang, H.B. Xiao, Y. Chen, X.G. Weng, Chamaejasmine B induces the anergy of vascular endothelial cells to VEGFA pro-angiogenic signal by autophagic regulation of VEGFR2 in breast cancer, Front. Pharmacol. 8 (2017) (963/1-963/12).
[10] X.X. Kan, Q. Li, X. Chen, Y.J. Wang, Y.J. Li, Q. Yang, H.B. Xiao, Z.X. Wang, Y. Chen, X.G. Weng, A novel cell cycle blocker extracted from *Stellera chamaejasme* L. inhibits the proliferation of hepatocarcinoma cells, Oncol. Rep. 35 (2016) 3480–3488.
[11] X.N. Liu, Q. Yang, G.L. Zhang, Y.J. Li, Y. Chen, X.G. Weng, Y.J. Wang, Y.W. Wang, X.X. Zhu, Anti-tumor pharmacological evaluation of extracts from *Stellera chamaejasme* L. based on hollow fiber assay, BMC. Complem. Altern. M. 14 (2014) (116/1-116/8).
[12] C. Zhang, S.S. Zhou, L.Y. Feng, D.Y. Zhang, N.M. Lin, L.H. Zhang, J.P. Pan, J.B. Wang, J. Li, In vitro anti-cancer activity of chamaejasmenin B and neochamaejasmin C isolated from the root of *Stellera chamaejasme* L, Acta Pharmacol. Sin. 34 (2013) 262–270.
[13] F. Bohlmann, C. Zdero, R.M. King, H. Robinson, A tetracyclic sesquiterpene, further isocedrene and guaiene derivatives from *Jungia stuebelii*, Phytochemistry 22 (1983) 1201–1206.
[14] F.M. Xu, T. Morikawa, H. Matsuda, K. Ninomiya, M. Yoshikawa, Structures of new sesquiterpenes and hepatoprotective constituents from the Egyptian herbal medicine *Cyperus longus*, J. Nat. Prod. 67 (2004) 569–576.
[15] S. Liang, Y.H. Shen, Y. Feng, J.M. Tian, X.H. Liu, Z. Xiong, W.D. Zhang, Terpenoids from *Daphne aurantiaca* and their potential Anti-inflammatory activity, J. Nat. Prod. 73 (2010) 532–535.