Terpenoids from the Roots of *Stellera chamaejasme* (L.) and Their Bioactivities

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Posted Date: 17 October 2023

doi: 10.20944/preprints202310.1083.v1

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Article

Terpenoids from the Roots of *Stellera chamaejasme* (L.) and Their Bioactivities

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Abstract: An undescribed diterpene, stellerterpenoid A (**1**) and two undescribed sesquiterpenoids, stellerterpenoids B and C (**2**-**3**), together with six known compounds, were isolated from the roots of *Stellera chamaejasme* L. Their structures were elucidated by extensive spectroscopic data (1D, 2D NMR, IR, UV and HR-ESI-MS). The absolute configuration of **1**-**3** were elucidated based on ECD calculation. Among of them, stellerterpenoid A was a rare 13, 14 - *seco* nortigliane diterpenoid and stellerterpenoid B was a guaiacane type sesquiterpenoid with an unusual 1, 2-diketone moiety. The known stelleraguaianone B (**5**) exhibited moderate activity to suppress NO production in LPS - simulated macrophages with an IC_{50} value of 24.76 ± 0.4 μM. None of the compounds showed inhibitory influenza virus and tumor activity.

Keywords: *Stellera chamaejasme* L; stellerterpenoids A–C; ECD calculation; NO inhibition

Introduction

Stellera chamaejasme L (Thymelaeaceae) is a toxic plant that is widely distributed in north and southwest China and used to treat skin diseases, psoriasis, chronic tracheitis, and tuberculosis [1–4]. The spread of *S. chamaejasme* in degraded grassland is increasingly serious, which affects the sustainable development and ecological security of grassland animal husbandry in China [3]. *S. chamaejasme* originally recorded in the Shen Nong Ben Cao Jing and has the effects of clearing heat and detoxifying, detumescence, reducing inflammation, stopping ulcers, and removing saprophytic muscle [5–9]. Many studies have been conducted on its chemical composition and pharmacological activities in recent decades. A series of compounds have been reported, including highly oxidized daphnane-type diterpenes [10,11], guaiane-type sesquiterpenoids [12–16], and unusual C-3/C-3''-biflavanones [17–20]. Some compounds from *S. chamaejasme* have been shown to demonstrate many different and interesting biological activities. For example, gnidimacrin, a 1-alkyldaphnane-type diterpene was found to have strong anti-cancer activity and broad anticancer spectrum [21]. Daphnane diterpenes, stelleralides C-E, exhibited more potent anti-HIV activity (EC_{50} = 0.73~0.98 nM) than zidovudine (positive controls, EC_{50} = 32 nM) [22]. Chamechromone can significantly inhibit the expression of proinflammatory cytokines in RAW 264.7 cells [23].

In our continuous research work, methanol extract of *S. chamaejasme* were phytochmically investigated. As a result, nine terpenoids, including one new diterpene and two new sesquiterpenoids named stellerterpenoids A–C (**1**-**3**) and six known terpenoids (**4**-**9**) were identified from the ethyl acetate extract (Figure 1). In addition, the anti-inflammatory, anti-influenza virus and anti-tumor activity of the nine compounds was tested. Herein, the isolation, identification, structural characterization, and biological assessment of these compounds are reported.

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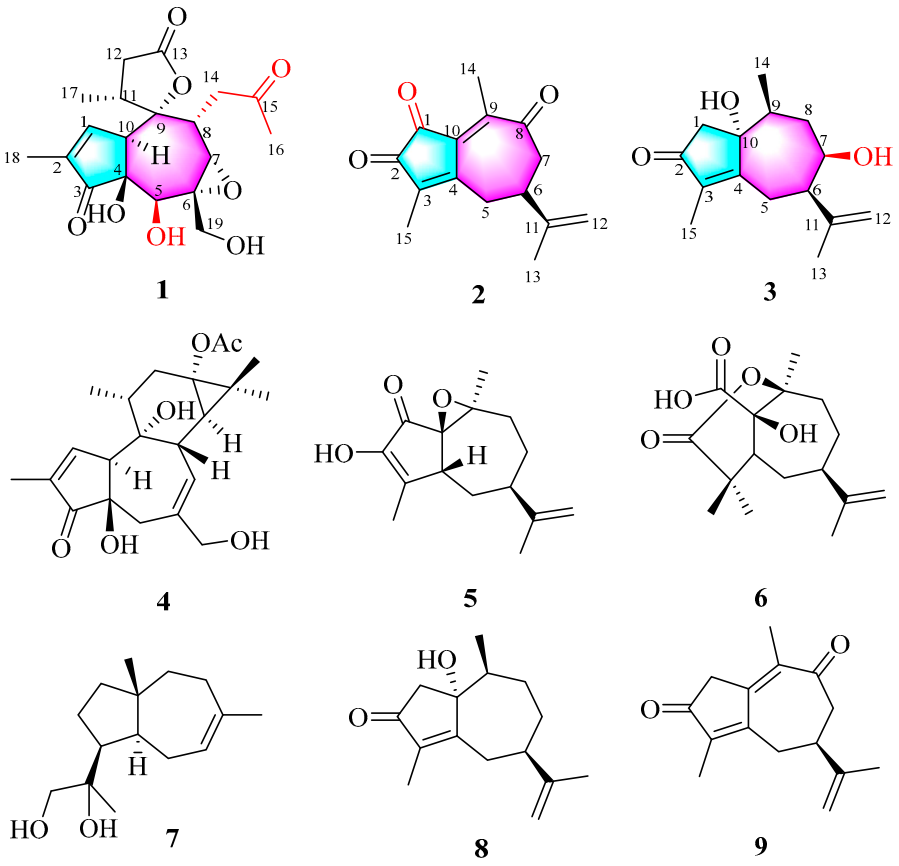

Figure 1. Structures of compounds 1–9 isolated from *Stellera chamaejasme* L.

Results and Discussion

2.1. Structural Elucidation of Three New Compounds (1, 2, and 3)

Stellerterpenoid A (**1**), yellow oil, had a molecular formula of $C_{19}H_{24}O_8$ deduced from the quasi-molecular ion peak at *m/z* 403.1344 $[M + Na]^+$ (calcd 403.1363) in the HRESIMS, with eight degrees of unsaturation. The ^{1}H NMR spectrum (Table 1) of **1** displayed the presence of three methyls at δ_H 2.19 (s, H-16), 1.78 (s, H-18) and 1.18 (d, *J* = 7.0 Hz, H-17), an oxymethines at δ_H 3.85 (s, H-19), a suite of methines including an olefinic proton at δ_H 7.48 (d, *J* = 1.8 Hz, H-1), and four methines at δ_H 4.31 (s, H-5), 3.57 (ddd, *J* = 10.6, 5.4, 2.4 Hz, H-8), 3.24 (d, *J* = 6.0 Hz, H-7), and 2.74 (m, H-11). Its ^{13}C NMR and DEPT spectra (Table 2) revealed 19 carbon signals, including three methyls [δ_C 30.2 (C-16), 18.0 (C-17), and 10.0 (C-18)], three methylenes [δ_C 63.6 (C-19), 42.6 (C-14), and 38.9 (C-12)], six methines [δ_C 158.3 (C-1), 75.9 (C-5), 58.8 (C-7), 51.0 (C-10) and 40.3 (C-8)], and seven quaternary carbons consisting of two carbonyl [δ_C 209.8 (C-3), 209.2 (C-15)], one ester carbonyl group at δ_C 177.9 (C-13), one olefinic at δ_C 137.7 (C-2) and three oxygenated ones at δ_C 91.8 (C-9), 76.2 (C-4) and 66.0 (C-6).

The ^{1}H and ^{13}C-NMR spectral data of **1** were similar to those of crotonianoid A [24] except for the occurrence of signals due to a methylene group (δ_C 42.6, C-14) and a ketone carbonyl (δ_C 209.2, C-15) in **1** instead of the $\Delta^{14,15}$ double bond (δ_C 123.0, 136.5) in crotonianoid A. The phenomena suggested that the $\Delta^{14,15}$ double bond in crotonianoid A was oxygenated, thus interpreting the presence of the methylene and ketone carbonyl in **1**. In addition, the methylene of C-5 [δ_C 38.8, δ_H 2.41 (d, *J* = 18.6 Hz), 2.66 (d, *J* =18.6 Hz)] in crotonianoid A was replaced by one oxy methine [δ_C 75.9, δ_H 4.31 (s)] and the double bond was replaced with one epoxy structure at C-6 and C-7. The C-9 and C-13 via an oxygen atom to generate a five-membered lactone ring was demonstrated by signal offset are similar to crotonianoid A [24]. The HMBC cross-peaks from H-8 (δ_H 3.57) to C-14 (δ_C 42.6) and C-15 (δ_C 209.2) and from H-16 (δ_H 2.19) to C-14 (δ_C 42.6) and C-15 (δ_C 209.2) supported the location of the acetone unit at C-8. In addition, the HMBC correlations from H-11 (δ_H 2.74) to C-8 (δ_C 40.3), C-9 (δ_C 91.8), C-10 (δ_C 51.0), C-12 (δ_C 38.9) and C-13 (δ_C 177.9).

The relative configuration of **1** was established by the analysis of NOESY spectrum. The correlations of H-7/H-11, H-8/H-11and H-12β (Figure 3) allowed H-7, H-8, H-11, and C-9–C-11 bond

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were assigned β-directed, while the NOESY cross-peaks H-10/H-5 and H-17, and H-12α/H-17 indicated that H-5, H-10, and Me-17 were α-oriented, and H-10 and 4-OH were *trans*-oriented. The comparison of the calculated and experimental ECD spectra of **1** assigned its absolute configuration to be 4*S*, 5*S*, 6*R*, 7*S*, 8*R*, 10*R*, 11*R* (Figure 4a). Therefore, the structure of **1** was fully elucidated to be a rare 13, 14-*seco* nortigliane and named stellerterpenoid A. To be the best our knowledge, it is the second report of 13, 14-*seco* nortigliane diterpenoid besides crotonianoid A.

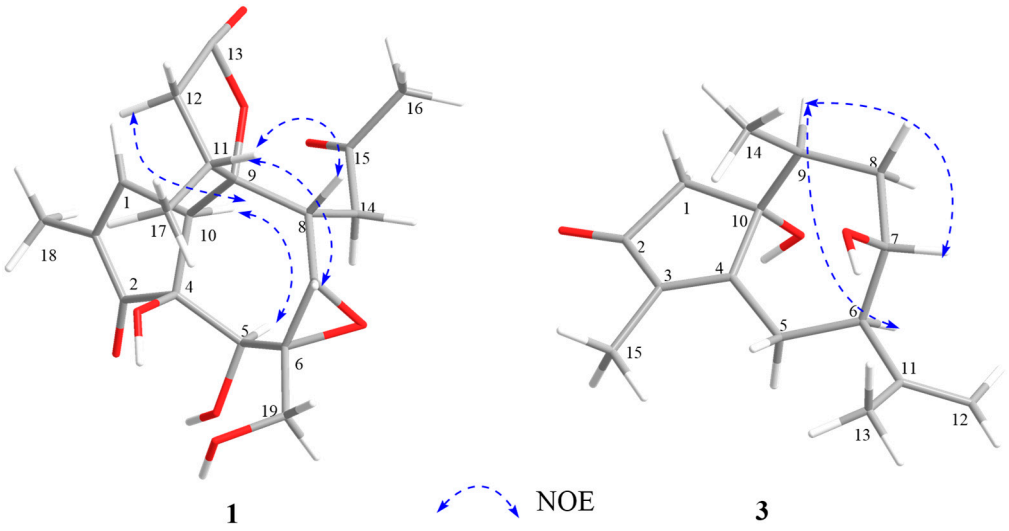

Figure 3. Key NOESY correlations of compounds 1 and 3.

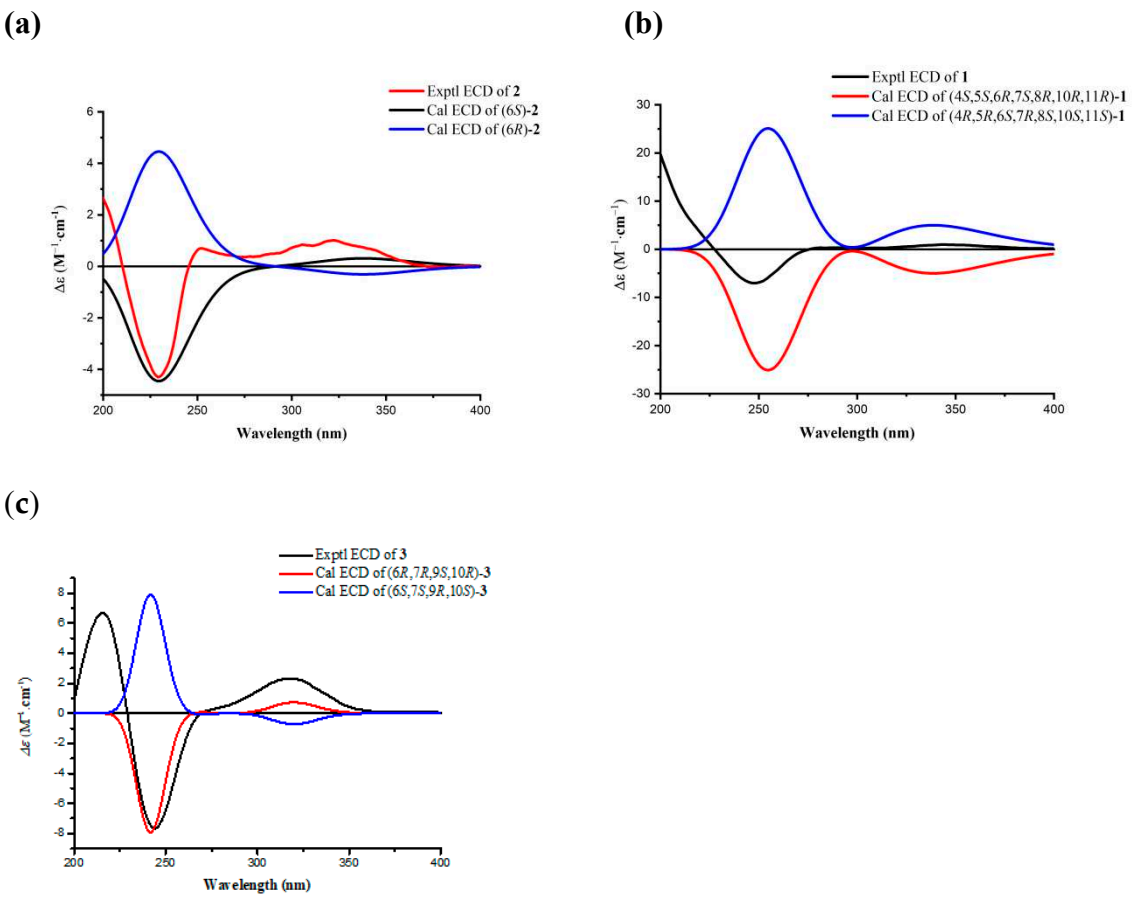

Figure 4. Calculated and experimental ECD spectra of compounds 1 - 3.

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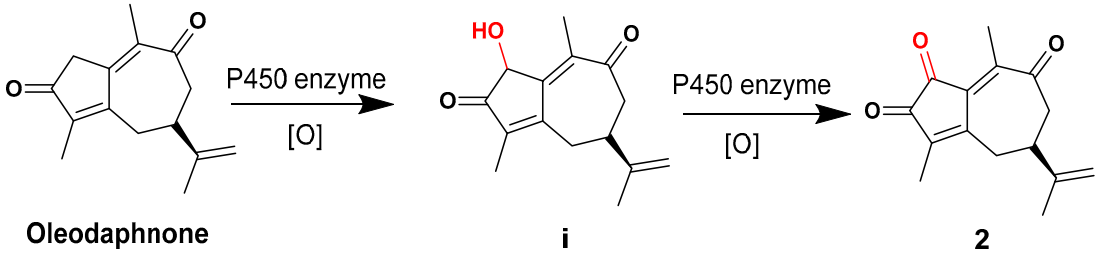
Stellerterpenoid B (**2**) was obtained as yellow oil with a molecular formula of $C_{15}H_{16}O_3$ according to its HRESIMS at *m*/*z* 267.0981 [M + Na]$^+$ (calcd 267.0992), suggesting eight degrees of unsaturation. The ^{1}H NMR spectrum (Table 1) of **2** exhibited the presence of a one double bond at δ_H 4.81 (s, H-12b) and 4.80 (s, H-12a) and three methyl peaks at δ_H 1.95 (s, H-14), 1.85 (s, H-15), and 1.80 (s, H-13). The ^{13}C NMR spectrum (Table 2) in combination with the DEPT spectrum showed 15 carbon signals, including three methyls in the high magnetic field at δ_c 20.4 (C-13), 17.3(C-14), and 8.8 (C-15), an olefinic carbons at δ_c 111.3 (C-12), two methylene at δ_c 50.6 (C-7) and 36.3 (C-5), one methine at δ_c 40.3 (C-6) and eight quaternary carbons δ_c 204.3 (C-2), 202.6 (C-1,8), 165.1 (C-4), 148.9 (C-11), 146.1 (C-3), 145.9 (C-10) and 132.6 (C-9). The NMR feature indicated that **2** was a guaiacane type sesquiterpene.

The NMR spectral data of **2** were very similar to those of oleodaphnone [25] except for the replacement of one ketone carbonyl in **2** by one methylene in oleodaphnone. This observation suggested that one methylene in oleodaphnone was oxygeanted into one ketone carbonyl in **2**. The HMBC correlation from H-14 (δ_H 1.95) to C-1 (δ_C 202.6) verified that the ketone was located at C-1. The absolute configuration of **2** was determined by the ECD data. The calculated ECD spectrum agreed well with the experimental ECD spectrum of (6*S*)-**2** (Figure 4b). Therefore, the structure of **2** was elucidated to be a guaiacane sesquiterpene with a unusual 1,2-diketone moiety and named as stellerterpenoid B.

Since 1,2-diketone fragment is rare in natural compounds, the biosynthetic pathway of **2** were proposed based on a biosynthetic precursor oleodaphnone (Scheme 1). The C-1 of oleodaphnone is oxidized by P450 enzyme to form hydroxyl intermediate **i**, which is further oxidized to form **2** [26].



Scheme 1. Proposed Biosynthetic Pathway for 2.

Stellerterpenoid C (**3**) was obtained as white oil. Its HRESIMS exhibited a quasi-molecular ion peak at *m*/*z* 273.1454 [M + Na]$^+$ (calcd 273.1461), suggesting a molecular formula of $C_{15}H_{22}O_3$ with five degrees of unsaturation. The ^{1}H NMR spectroscopic data (Table 1) show the presence of three methyls at δ_H 1.80 (s, H-13), 1.64 (s, H-15) and 0.85 (d, *J* = 7.2 Hz, H-14), four methylenes at δ_H 4.79 (s, H-12a), δ_H 4.76 (s, H-12b), 2.57 (d, *J* =18.2 Hz, H-1a), 2.34 (d, *J* =18.2 Hz, H-1b) and 2.20 (ddd, *J* =13.8, 10.0, 3.5 Hz, H-8), 1.75 (ddd, *J* =13.8, 5.4, 3.5 Hz, H-8), and threes methines at δ_H 3.69 (m, H-7), 3.00 (m, H-6) and 2.29 (m, H-9).The ^{13}C NMR spectrum showed 15 signals, which were ascribed to three methyls at δ_C 19.9 (C-13), 15.6 (C-14), and 7.9 (C-15), four methylenes at δ_C 112.8 (C-12), 50.3 (C-1), 32.2 (C-5), and 40.8 (C-8), three methylates at δ_C 70.5 (C-7), 51.3 (C-6), and 37.9 (C-9), and five quaternary carbons at δ_C 208.3 (C-2), 138.9 (C-3), 173.8 (C-4), 82.2 (C-10), and 149.0 (C-11) (Table 2).

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Table 1. The ^{1}H-NMR data (600 MHz, δ_H, mult, *J* in Hz) of 1–3 in CD_3OD.

No.	1	2	3
1	7.48 d (1.8)		2.57 d (18.2) 2.34 d (18.2)
2			
3			
4			
5	4.31 s	3.03 d (17.2) 2.89 d (6.5)	2.69 d (6.7)
6		2.76 m	3.00 m
7	3.24 d (6.0)	2.94 m 2.85 m	3.69 m
8	3.57 ddd (10.6, 5.4, 2.4)		2.20 ddd (13.8, 10.0, 3.5) 1.75 ddd (13.8, 5.4, 3.5)
9			2.29 m
10	3.47 d (2.6)		
11	2.74 m		
12	3.09 dd (18.1, 8.1) 2.27 dd (18.1, 4.4)	4.81 s 4.80 s	4.79 s 4.76 s
13		1.80 s	1.80 s
14	2.83 dd (18.1, 10.6) 2.63 dd (18.1, 2.6)	1.95 s	0.85 d (7.2)
15		1.85 s	1.64 s
16	2.19 s		
17	1.18 d (7.0)		
18	1.78 s		
19	3.85 s		

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Table 2. The ^{13}C-NMR data (150 MHz, δ_C) of 1–3 in CD_3OD.

No.	1	2	3
1	158.3	202.6	50.3
2	137.7	204.3	208.3
3	209.8	146.1	138.9
4	76.2	165.1	173.8
5	75.9	36.3	32.2
6	66.0	40.3	51.3
7	58.8	50.6	70.5
8	40.3	202.6	40.8
9	91.8	132.6	37.9
10	51.0	145.9	82.2
11	39.2	148.9	149.0
12	38.9	111.3	112.8
13	177.9	20.4	19.9
14	42.6	17.3	15.6
15	209.2	8.8	7.9
16	30.2		
17	18.0		
18	10.0		
19	63.6		

The NMR spectra of **3** were extremely similar to those of known wikstronone C [27], except that the signals of one methylene in the referenced compound were replaced by one oxy methine in **3**. The HMBC correlations from H-7 (δ_H 3.69) to C-8 (δ_C 40.8) and C-6 (δ_C 51.3), together with the ^{1}H-^{1}H COSY spin system of H-6/H-7/H-8/H-9, indicated that a hydroxy was connected with C-7 in **3** to allow for the presence of oxymethine (Figure 2). In the NOESY spectrum, δ_H 0.85 (H_3-14) correlated with δ_H 2.57 (H-1β) suggested that CH_3-14 is β-oriented. The NOESY correlations of H-7/H-9 and H-6/H-9 suggested the α-orientation of H-6 and H-7 (Figure 3). The 10-OH is defined as β configuration because signal of C-10 (δ_C 82.2) is similar to C-10 (δ_C 83.2) in wikstronone C. The comparison of the calculated and experimental ECD spectra of **3** (Figure 4c) assigned its stereochemistry to be 6*R*, 7*R*, 9*S*, 10*R*. Hence, the structure of **3** was characterized and named stellerterpenoid C.

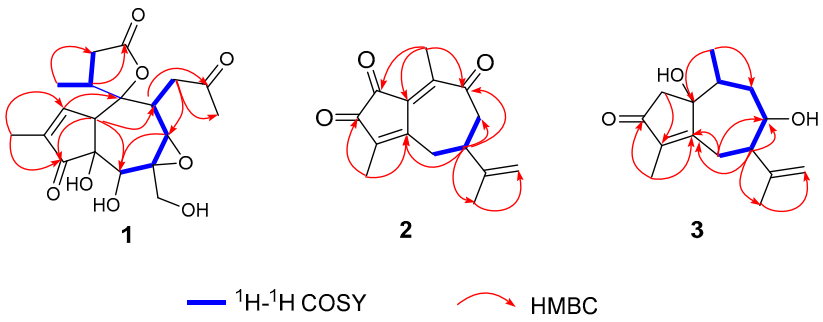

Figure 2. ^{1} H–^{1} H COSY and key HMBC correlations of compounds 1–3.

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The known compounds were identified as prostratin (**4**) [28], Stelleraguaianone B (**5**) [12], Chamaejasnoid A (**6**) [29], auranticanol L (**7**) [30], wikstronone C (**8**) [27], and oleodaphnone (**9**) [25].

2.2. Biological Studies

Compounds **1**-**9** were evaluated for their inhibition on nitric oxide (NO) production induced by lipopolysaccharide (LPS) in RAW 264.7 macrophages. The research results shows that compound **5** displayed the mild activity to inhibit NO production (IC_{50} = 24.76 ± 0.4 μM) (Table 3).

Table 3. Inhibitory effects of compounds 1-9 on LPS-induced NO production in RAW 264.7 macrophages.

Compds	[a] IC_{50} (μM)	[b] CC_{50} (μM)	Compds	IC_{50} (μM)	CC_{50} (μM)
1	>50	[c] NT	**6**	>50	NT
2	>50	NT	**7**	>50	NT
3	>50	NT	**8**	>50	NT
4	>50	NT	**9**	>50	NT
5	24.76 ± 0.4	>50	[d] **L-NMMA**	12.30 ± 3.4	>50

[a] IC_{50}: 50% inhibitory concentration. (Mean ± SD of 3 tests.) [b] CC_{50}: 50% cytotoxic concentration. (Mean ± SD of 3 tests.) [c] NT represents compounds were not tested due to insufficient amounts. [d] L-NMMA was used as positive control.

The inhibitory rate of HepG2, A549 and HeLa cells growth of compounds **1–9** was measured by the MTT assay. Unfortunately, none of these nine compounds is active.

Furthermore, compounds **1–9** were also tested for anti-influenza virus activity against A/Puerto Rico/8/1934 (H1N1) virus, and nucleozin. However, none of the compounds showed inhibitory activity.

Materials and Methods

1.1. General Experimental Procedures

Optical rotations were recorded on a Jasco P-1020 polarimeter (JASCO Corporation, Tokyo, Japan). CD spectra were obtained on a Jasco J715 spectropo- larimeter. UV spectra were measured using a UV-8000 spectrophotometer (Shanghai Metash instruments Co.,Ltd, Shanghai, China). IR spectra were recorded on Bio-Rad FTS-135 spectrophotometer (Bio-rad Company, California, USA). NMR data were determined by Bruker Avance III HD-600 (Bruker BioSpin Group, Rheinstetten, Germany). ESI-MS and HR-ESI-MS were recorded on an APIQ star-Pulsar spectrometer (Applied Biosystems Corporation, Canada). The rotary evaporator is n-1300V-W type (Tokyo Physicochemical Co., LTD, Kyoto, Japan). Semipreparative HPLC was used Agilent 1260 (ZORABAX SB-C18, 4.6 mm × 250 mm, 1 ml/min; 9.4 mm × 250 mm, 3 ml/min.) (Agilent Technology Co., Ltd, California, USA) and NP7005-10C (CC: Nucifera C8M, 4.6 mm × 250 mm, 1 ml/min; 20× 250 mm,10 ml/min) (Hanbon Sci.& Tech, Jiangsu, China). Preparative HPLC used methanol and acetonitrile (pure reagents) were purchased from Merk Company (Merck, Darmstadt, Germany). Sephadex LH-20 (25-100 μm; Amersham Biosciences AB, Uppsala, Sweden), Thin layer silica gel plate (GF254) was purchased from Qingdao Marine Chemical Plant (Qingdao, China) and spots were visualized by heating silica gel plates sprayed with 5% H_2SO_4-EtOH.

1.1. Plant Material

The dried roots of *Stellera chamaejasme* L were collected in Dali city, Yunnan Province. People's Republic of China, and identified by Dr Zhijun Zhang, Kunming University of Science and Technology. A voucher specimen (KUMST20211007) has been deposited at the Key Laboratory of Phytochemistry, Kunming University of Science and Technology.

3.3. Extraction and Isolation

Dried roots of *Stellera chamaejasme* (11.0 kg) was crushed and extracted with 70% acetone/H_2O three times (3 × 50 L) at room temperature to give a crude extract (10.1 kg) under reduced pressure distillation. The extract was mixed with water (15.0 L), and followed by successive partition with petroleum ether (3 × 15 L) and EtOAc (3 × 15 L). The EtOAc extract (3.35 kg) was separated by silica

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gel (2.5 × 160 cm) using a gradient of petroleum ether/EtOAc (5:1-1:1, v/v) and $CHCl_3$/MeOH (3:1–1:1, v/v) to give eight fractions (Fr. A~H).

Fr. C (45.75 g) was separated by silica gel (8 × 160 cm) using a gradient solvent petroleum ether/EtOAc (5:1–1:1, v/v) to afford six fractions (Fr. C1~C6). Fr. C3 (3.3 g) was repeatedly separated by silica gel (6 × 70 cm, CH_2Cl_2/MeOH, 10:1-1:1, v/v) to afford four fractions (Fr. C3-1~C3-4). Fr. C3-1 (198.4 mg) was purified by Sephadex LH-20 (MeOH) to yield **2** (8.3 mg), **5** (13.3 mg), **6** (3.7 mg), **7** (6.2 mg), and **8** (12.4 mg). Fr. C3-2 (1.2 g) was applied to a silica gel column (2 × 60 cm) washed with petroleum ether-acetone (15:1–1:1, v/v) to provide fractions C3-2-2 (63.7 mg), which was further separated by semi-preparative HPLC (MeOH/H_2O, 57:43, v/v) to afford **3** (3.7 mg) and **9** (3.8 mg). Fr. C4 (7.5 g) was subjected to silica gel (10 × 160 cm) using $CHCl_3$/MeOH (20:1–1:1, v/v) to give six fractions (Fr. C4-1~ C4-6). Fr. C4-2 (840 mg) was separated by silica gel column chromatography (1 × 60 cm, petroleum ether/CH_2Cl_2, 1:10-1:1, v/v) to afford fractions C4-2-2 (355.8 mg). Fr.C4-2-2 (355.8 mg) was purified by Sephadex LH-20 eluted with $CHCl_3$-MeOH (1:1, v/v) and then was used to silica gel ($CHCl_3$ / MeOH, 15:1, v/v, 2 × 70 cm) to yield **1** (39.3 mg) and **4** (105.3 mg).

3.4. Details of New Compounds

3.4.1. Stellerterpenoid A (1)

Yellow oil; $[\alpha]_D^{24}$ −37.1 (c 0.24, MeOH); UV (MeOH) λ_{max} (log ε) 244 (1.88) nm; IR (KBr) ν_{max} 3449, 2918, 2920, 1769, 1703, 1631, 1424, 1361, 1292, 1220, 1164, 1089, 1040, 966, 930, 834, 800, 724, 676, 585, 530, 418 cm^{-1}; ^1H NMR (CD_3OD, 600 MHz) and ^{13}C NMR (CD_3OD, 150 MHz) data see Table 1 and Table 2; HRESIMS *m/z* 403.1344 $[M + Na]^+$ (calcd 403.1363).

3.4.2. Stellerterpenoid B (2)

Yellow oil; $[\alpha]_D^{24}$ −11.0 (c 0.05, MeOH); UV (MeOH) λ_{max} (log ε) 207 (1.09) nm, 230 (1.01) nm and 301 (1.59) nm; IR (KBr) ν_{max} 3444, 2919, 2851, 1702, 1647, 1444, 1383, 1309, 1262, 1179, 1092, 896, 802 cm^{-1}; ^1H NMR (CD_3OD, 600 MHz) and ^{13}C NMR (CD_3OD, 150 MHz) data see Table 1 and Table 2; HRESIMS *m/z* 267.0981 $[M + Na]^+$ (calcd 267.0992).

3.4.3. Stellerterpenoid C (3)

Yellow oil; $[\alpha]_D^{24}$ +91.8 (c 0.10, MeOH); UV (MeOH) λ_{max} (log ε) 239 (1.56) nm; IR (KBr) ν_{max} 3458, 3070, 2972, 2928, 2852, 1688, 1629, 1454, 1382, 1319, 1230, 1188, 1164, 1110, 1050, 978, 878, 807, 729, 655, 552, 463 cm^{-1}; ^1H NMR (CD_3OD, 600 MHz) and ^{13}C NMR (CD_3OD, 150 MHz) data see Table 1 and Table 2; HRESIMS *m/z* 273.1454 $[M + Na]^+$ (calcd 273.1461).

3.5. ECD Calculations for 1, 2 and 3

Based on the conformation determined from ROESY spectra and Chem 3D modeling, the low-energy conformers of model compound (4*S*,5*S*,6*R*,7*S*,8*R*,10*R*,11*R*) -**1**, (6*R*)-**2**, (6*R*,7*R*,9*R*,10*R*)-**3** were generated within 10 kcal/mol energy window under MMFF94S via the software CONFLEX [31]. The selected conformers of each model compound with highest distribution (fuef63, fuef4 and fuef72-1 conformers for compounds **1-3**, respectively were further optimized by the density functional theory method at the B3LYP/6-31G (d) level. The ECD calculations were using TD-DFT-B3LYP/6-311G (+, 2d, p) of theory on optimized geometries through the CPCM model (in MeOH) [32]. The calculated ECD curves were generated using SpecDis 1.71 [33] and all the above calculations were carried out with the Gaussian 16 package of programs. All ECD curves were weighted by Boltzmann distribution after UV correction.

3.6. Determination of NO Production

Compounds **1-9** were evaluated for their anti-inflammatory activity using a LPS-induced NO production model. The NO production was calculated by measuring the content of nitrite in the supernatant of the culture by Griess reaction method. Cell viability was measured via MTT assay. The bioassays for NO production and cell viability of **1-9** were determined by a procedure described previously [34].

3.7. Anti-Influenza Virus Assay

Influenza strain A/Puerto Rico/8/1934 (H1N1) was used in this study. For the inhibitory activity assays, compounds **1-9** were dissolved in DMSO and Oseltamivir was used as positive control. MDCK cells (104/well) were inoculated in 96-well plates and cultured for 24 h, compounds of different concentrations (from 1.56 μM to 50 μM) were mixed with influenza virus (100 $TCID_{50}$) and added into the cells. IC_{50} (50% virus inhibitory concentration) was calculated using the software CalcuSyn (Biosoft, Cambridge, UK) [35].

3.8. Anti-Tumor Assay

Cytotoxic activity assays against HepG2, A549 and HeLa cell lines were seeded on 96 well microplates and cultured in DMEM medium with 10% fetal bovine serum (FBS) at 37 ℃ and 5% CO_2 for 24 h.. Spetra-Max M2 (Molecular Devices, USA) was employed to record the optical density (λ = 490 nm). SPSS 21.0 software was used for data evaluation. IC_{50} values were calculated based on the mean OD measured three times versus concentration curves of drugs. Adriamycin (10 mM, purity 99%, Solar bio Science&Technology Co. Ltd., Beijing, China) was used as the positive control [36].

Conclusions

In summary, three previously unreported terpenoids including an undescribed diterpene (**1**), two new guaiacane type sesquiterpenoids (**2**-**3**) and six known ones (**4**-**9**) were isolated from root of *Stellera chamaejasme*. The absolute configuration of compounds **1–3** were established by the experimental and calculated ECD. Compound **5** exhibited the moderate activity to inhibit NO production in LPS-induced RAW 264.7 macrophages, presenting the potential of anti-inflammation.

Credit author statement: Design the study: Xuanqin Chen; drafting the manuscript: Juan Wu; bioactive assay: Caicen Liao; critical revision of the manuscript: Rongtao Li; assisting the chemical experiment: Zhujun Ye.

Declaration of competing interest: The authors declare that they have no competing financial interests or personal relationships that could have appeared to influence the work reported in this paper.

Acknowledgments: This work was supported by the National Natural Science Foundation of China(22067012, 82073737), the key R&D program of Yunnan Province (202103AC10005), the Open Fund of State Key Laboratory of Tea Plant Biology and Utilization (SKLTOF20190110), and the Innovative Team of Yunan Province (No.2019HCO18).

Appendix A. Supplementary: Supplementary material related to this article can be found, in the online version.

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