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栗色鼠尾草中一个新的吉马烷型倍半萜内酯类化合物

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摘要 从栗色鼠尾草全草的乙醇提取物中分离得到一个新的吉马烷型倍半萜内酯类化合物 salviatanolide A (**1**). 化合物 **1** 的结构通过一维/二维 NMR 和 HRESIMS 波谱分析技术得到确认. 通过 X-ray 单晶衍射实验还确认了化合物 **1** 的绝对构型. 利用人源化秀丽隐杆线虫的阿尔兹海默症病理模型, 筛选了化合物 **1** 的抗阿尔兹海默症活性. 生物活性结果表明, 化合物 **1** 能显著地延缓秀丽隐杆线虫麻痹表型的阿尔兹海默症症状, 提示该化合物可作为抗阿尔兹海默症的候选化合物.

关键词 栗色鼠尾草; 吉马烷型倍半萜内酯; 抗阿尔兹海默症活性; 秀丽隐杆线虫

A New Germacrane-Type Sesquiterpene Lactone from *Salvia castanea*

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Abstract One new germacrane-type sesquiterpene lactone, named as salviatanolide A (**1**) was isolated from an ethanol extract of the whole plant of *Salvia castanea.* Its structure was elucidated by 1D and 2D-NMR and HRESIMS spectra, and its absolute configuration was determined by single crystal X-ray crystallography. The anti-Alzheimer's disease (AD) activity of **1** was evaluated using humanized *Caenorhabditis elegans* AD pathological model. Compound **1** significantly delayed AD-like symptoms of worm paralysis phenotype, which may be potential to be served as anti-AD agent candidate.

Keywords *Salvia castanea*; germacrane-type sesquiterpene lactone; anti-Alzheimer's disease activity; *Caenorhabditis elegans*

1 Introduction

The genus *Salvia* (family Labiatae) is composed of about 900 species throughout the temperate and tropical regions,[1] of which 78 species, 24 varieties, and 8 forms are found in China.[2] Many plants of this genus have long been used in folk medicine to treat various ailments all around the world.[3-4] Pharmacologically *Salvia* showed cardioactive,[5] antibacterial,[6] antioxidant,[7] antitumor,[8] antidiabetic,[9] and anti-inflammatory activities.[10] Previously phytochemical investigations of *Salvia* led to the isolation of diterpenoids, sesquiterpenoids, steroids, phenolic acids, and flavonoids.[11-13] Diterpenoids and sesquiterpenoids are the important phytochemical constituents of the genus *Salvia*.[14]

The *Salvia castanea* Diels is a perennial herb generally distributed in Southwestern China, especially in Yunnan and Sichuan.[15] It has been traditionally used as a traditional folk medicine for the treatment of blood stasis syndrome, menoxenia, dysmenorrhea, and upset insomnia in Southwestern China. In our previous phytochemical investigation,[16] a series of guaiane-type sesquiterpenoids were isolated from the dried whole plant of *S. castanea*. Among them, salcastanins A～C significantly delayed Alzheimer's disease (AD)-like symptoms of worm paralysis phenotype.

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In our continuing search for anti-Alzheimer's disease active sesquiterpenoids from *S. castanea*, further chemical investigation of this plant was conducted, which led to the isolation of a new germacrane-type sesquiterpene lactone, salviatanolide A (**1**) (Figure 1). The structure of **1** was a rare 1(10),3(4),7(11)-triene type of germacran-8,12-olide. In this paper, we reported the isolation, structural elucidation, and the biological activity of the new compound towards alleviating Alzheimer's disease (AD) like symptoms in AD *Caenorhabditis elegans*.

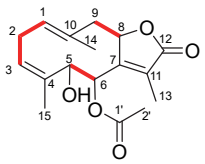

Figure 1 Chemical structure of compound **1**

2 Results and discussion

The ethanol extract of the dried whole plant of *S. castanea* was suspended in H_2O and successively partitioned with EtOAc. The EtOAc extract was purified by repeated column chromatography over silica gel, MCI, and semi-preparative high performance liquid chromatography (HPLC) to afford one new germacrane-type sesquiterpene lactone (**1**).

Table 1 ^{1}H NMR (600 MHz) and ^{13}C NMR(150 MHz) data of **1** in $CDCl_3$

Position	δ_H, mult. (*J* in Hz)	δ_C, type
1	5.77, t (8.4)	129.5, CH
2	2.89～2.95, m, *α*; 2.70～2.75, m, *β*	28.6, CH_2
3	5.30, brd (9.6)	123.8, CH
4	—	136.9, C
5	5.36, d (9.6)	71.3, CH
6	4.93, s	74.4, CH
7	—	160.1, C
8	5.27, dd (11.4, 5.4)	81.0, CH
9	3.03, dd (11.4, 5.4), *α*; 2.16, t (11.4), *β*	45.7, CH_2
10	—	130.5, C
11	—	127.8, C
12	—	173.9, C
13	2.02, brs	10.5, CH_3
14	1.79, s	16.1, CH_3
15	1.74, s	18.8, CH_3
1'	—	169.5, C
2'	2.12, s	20.5, CH_3
5-OH	2.49, d (10.8)	

Compound **1** was obtained as colorless needles. The molecular formula $C_{17}H_{22}O_5$, including seven degrees of unsaturation, was established by HRESIMS (*m/z* 307.1540 for $[M+H]^+$). The IR spectrum of **1** showed characteristic bands of hydroxyl (3494 cm^{-1}) and carbonyls (1749 and 1727 cm^{-1}). In its 1D NMR spectra (Table 1), the methyl at δ_H 2.12 (s, 3H), together with the corresponding carbon signals at δ_C 169.5 and 20.5, revealed the presence of an acetyl structural fragment. In addition to this acetyl group, the ^{1}H NMR spectrum (Table 1) of **1** exhibited signals for three methyls at δ_H 1.74 (s, 3H), 1.79 (s, 3H), and 2.02 (brs, 3H), two olefinic protons at δ_H 5.30 (brd, *J*=9.6 Hz, 1H) and 5.77 (t, *J*=8.4 Hz, 1H), and three oxygenated methines at δ_H 4.93 (s, 1H), 5.27 (dd, *J*=11.4, 5.4 Hz, 1H), and 5.36 (d, *J*=9.6 Hz, 1H). Excluding the two carbons forming the acetyl group, there were a further 15 resonances present in the ^{13}C NMR spectrum (Table 1). Utilizing DEPT (distortionless enhancement by polarization transfer) and HSQC (heteronuclear single quantum coherence) experiments, these 15 signals were sorted as three methyls, two methylenes, five methines (two olefinic and three oxygenated), and five quaternary carbons (four olefinic and one carbonyl), among which, carbon atoms at δ_C 160.1, 127.8 and 173.9 were attributed to an *α*,*β*-unsaturated *γ*-lactone. Two double bonds, an *α*,*β*-unsaturated *γ*-lactone, and an acetyl group accounted for six out of seven degrees of unsaturation, thus, the last one was concluded to be a ring.

The ^{1}H-^{1}H COSY correlations of H-1/H_2-2/H-3, H-5/H-6 and H-8/H_2-9 determined the structural fragments as shown in Figure 2. The HMBC correlations (Figure 2) from H_3-14 to C-1, C-9 and C-10, from H-1 to C-2 and C-3, from H_3-15 to C-3, C-4 and C-5, from H-3 to C-1 and C-2, from H-5 to C-3 and C-7, from H-6 to C-5, C-7 and C-8, from H_3-13 to C-7, C-11 and C-12, and from H-8 to C-7, C-9, C-11 and C-12, in combination with its degrees of unsaturation, confirmed that **1** had a skeleton of 7(11)-ene-germacran-8,12-olide.[17] One double bond was determined between C-1 and C-10 by the cross-peaks in the HMBC spectrum: H-1 with C-9, C-10 and C-14. The other double bond located at C-3 and C-4 was deduced from the HMBC correlations between H-3 and C-4, C-5 and C-15. The hydroxyl group was located at C-5 as a result of the observation of the HMBC correlations from H-5 to C-4 and C-15. The C-6 placement of the acetyl group was done via the key HMBC correlations from H-6 to C-1' (at δ_C 169.5, the carbonyl of the acetyl group).

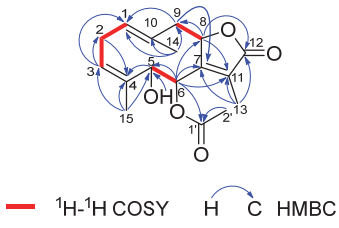

Figure 2 Key ^{1}H-^{1}H COSY and HMBC correlations of **1**

Moreover, the relative stereochemistry was confirmed by a NOESY (nuclear overhauser effect spectroscopy) experiment (Figure 3). The key NOESY correlations of H-8/H-6 and H-6/H-5 indicated that H-8, H-6, and H-5 were cofacial and assigned as *α*-oriented. The *E*-geometry of the double bond between C-1 and C-10, and *Z*-geometry of the double bond between C-3 and C-4 were assigned by NOESY cross-peaks of H_3-14 with H_2-2 and H_3-15 with H-3. To confirm the planar structure and its absolute configura-

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tion, a single-crystal X-ray diffraction experiment of **1** was performed by using Cu Kα radiation (Figure 4). The absolute configuration of **1** was determined as 5*R*,6*R*, and 8*R* with a Flack parameter of 0.01(8). Hence, compound **1** was elucidated and named salviatanolide A.

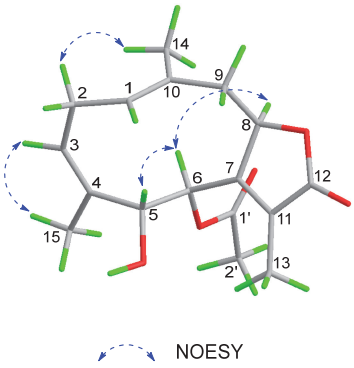

Figure 3 Key NOESY correlations of **1**

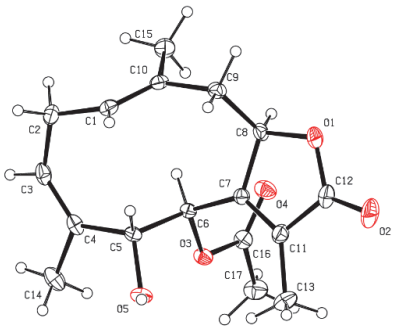

Figure 4 Single-crystal X-ray ORTEP drawing of **1**

Compound **1** was further evaluated the anti-AD activity by transgenic AD *Caenorhabditis elegans* pathological model in our present work. Memantine *et al.*[18] has been demonstrated that it is one of disease modifying drugs by decreasing Aβ levels and inhibiting Aβ toxicity, and it can significantly alleviate AD-like symptom of worm paralysis.[19] Therefore, memantine was used as a positive control. As expected, 100 μmol/L memantine significantly delayed AD worm paralysis, and 100 μmol/L compound **1** significantly alleviated AD-like symptoms ($p<0.05$) (Figure 5). The biological assay result indicated that compound **1** may be potential to be served as anti-AD agent candidate.

3 Conclusions

In conclusion, one new germacrene-type sesquiterpene lactone, salviatanolide A (**1**), was obtained from the whole plant of *S. castanea*. The structure of the new compound **1** was extensively elucidated and characterized by HRESIMS, IR, 1D and 2D NMR, and single-crystal X-ray diffraction techniques. Most of the germacrane-type sesquiterpene lactones have a double bond at 1(10) and 4(5) positons,[20] while compound **1** represents a rare 1(10),3(4),7(11)-triene-germacran-8,12-olide structure. Combined with the results of our previous phytochemical studies on *S. castanea* and

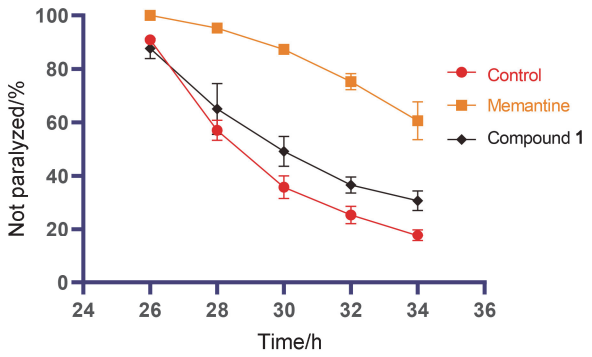

Figure 5 Anti-Alzheimer's disease (AD) activity of compound **1**, positive control and control

Worms were treated with 100 μmol/L compound **1**, 100 μmol/L memantine was regarded as positive control, and 0.1% dimethyl sulfoxide (DMSO) was regarded as control

the results of this study, it can be concluded that the characteristic constituents of *S. castanea* were guaiane-type and germacrane-type sesquiterpenoids. However, the content of germacrane-type sesquiterpenoids in *S. castanea* was less than that of guaiane-type sesquiterpenoids. Therefore, guaiane-type and germacrane-type sesquiterpenoids could be served as chemotaxonomic markers of *S. castanea*. Furthermore, the anti-AD activity of **1** was investigated through the transgenic AD worm pathological model. Compound **1** significantly delayed AD-like symptoms of worm paralysis phenotype. The present result exhibited that compound **1** could be a potential candidate compound against AD.

4 Experimental section

4.1 General experimental procedures

Melting points were determined on an X-4 digital display micromelting point apparatus (Beijing Tech Instrument Co., Ltd., Beijing, China) and are uncorrected. Optical rotations were measured on a Rudolph Autopol IV automatic polarimeter. IR spectra were recorded using a Nicolet NEXUS 670 FT-IR spectrometer. HRESIMS were determined on a Thermo LTQ Orbitrap Elite mass spectrometer. 1D and 2D NMR spectra were recorded on a 600 MHz Bruker AVANCE NEO-600 spectrometer with TMS as internal standard. Semi-preparative HPLC was performed on an H&E Quiksep LC-50 system, equipped with a UV-100D detector at 210 nm, and a Kromasil 100-5 C18 ODS column (250 mm×10 mm, 5 μm). Silica gel (200～300 mesh) used for column chromatography (CC) and silica gel GF254 (10～40 μm) used for thin layer chromatography (TLC) were purchased from Qingdao Marine Chemical Factory in China. MCI GEL CHP 20P (75～150 μm) was purchased from Mitsubishi Chemical Holdings in Japan.

4.2 Plant materials

The whole plant of *S. castanea* was collected from Lijiang City (Yunnan Province, China) in June 2018. The plant material was identified by Dr. Dong-Qing Fei (School of Pharmacy, Lanzhou University). A voucher specimen

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(No. 201806SC) has been deposited at the School of Pharmacy, Lanzhou University.

4.3 Extraction and isolation

The air-dried whole plant of *S. castanea* (7.5 kg) was crushed and exhaustively extracted with 95% EtOH for four times (7 days for each time) at room temperature. The combined extracts were concentrated in vacuum to give a residue (1400 g), which was suspended in H_2O and then subsequently partitioned with EtOAc and *n*-BuOH to afford EtOAc and *n*-BuOH soluble fraction. The EtOAc extract (528 g) was fractionated by silica gel (200～300 mesh) column chromatography eluted with petroleum ether (PE)-acetone (V∶V=40∶1, 20∶1, 10∶1, 5∶1, 2∶1, 1∶1) to give six fractions (Fr.A～Fr.F). Fr.E (160 g) was applied to an MCI gel column [eluted with EtOH/H_2O, V∶V=30∶70, 50∶50, 70∶30, 90∶10, 100∶0] to afford 4 subfractions (Fr.E1～Fr.E4). Fr.E3 (4 g) was chromatographed on a silica gel column eluted with a system of $CHCl_3$-CH_3OH (V∶V=25∶1) and then purified by semi-preparative HPLC (CH_3CN/H_2O, V∶V=65∶35, 2 mL/min) to give salviatanol A (**1**) (5 mg, t_R=18.2 min). Colorless needles, m.p. 206～208 ℃; $[\alpha]_D^{20}$+34.59 (*c* 0.10, MeOH); ^{1}H NMR (600 MHz, $CDCl_3$) see Table 1; ^{13}C NMR (150 MHz, $CDCl_3$) see Table 1; IR (KBr) ν_{max}: 3494, 2916, 1749, 1727, 1441, 1232, 1054, 736 cm^{-1}; HRESIMS calcd for $C_{17}H_{23}O_5$ $[M+H]^+$ 307.1540, found 307.1540.

Crystallographic data of **1**. $C_{17}H_{22}O_5$, M_r = 306.34, monoclinic, space group $P2_1$, a=0.970515(8) nm, b=0.827677(8) nm, c=0.997497(9) nm, α=90.00°, β=93.9464(8)°, γ=90.00°, V=0.79936(1) nm^3, T=150 K, Z=2, d=1.273 g/cm^3, μ(Cu Kα)=0.77 mm^{-1}, F(000)=328, crystal dimensions 0.07 mm×0.04 mm×0.02 mm were used for measurement on a Rigaku XtaLAB Synergy R, HyPix diffractometer with Cu Kα radiation (λ = 0.154184 nm). The total of 7802 reflections, and 3116 independent reflections (R_{int}=0.0311) were measured. The final R_1 value was 0.0319 [I>2σ(I)], and the final $wR(F^2)$ value was 0.0820 [(I>2σ(I)]. The final R_1 value was 0.0326 (all data), and the final $wR(F^2)$ value was 0.0812 (all data). The goodness of fit on F^2 was 1.051. The absolute structure parameter was 0.01(8). Crystallographic data for the structure of compound **1** have been deposited with the Cambridge Crystallographic Data Centre (deposition no. CCDC 2128434). Copies of these data can be obtained free of charge via www.ccdc.cam.ac.uk/conts/retrieving.html (or from the Cambridge Crystallographic Data Centre, 12 Union Road, Cambridge CB21EZ, U.K.; Fax (+44) 1223-336-033; or deposit@ccdc.cam.uk).

4.4 Bioactivity assay for Alzheimer's disease like phenotype of delaying paralysis in *Caenorhabditis elegans*

The transgenic *Caenorhabditis elegans* strain CL4176 with genotype of smg-1ts (myo-3/A$\beta_{1\text{-}42}$ long 3'-untranslated region (UTR)) was obtained from *Caenorhabditis* Genetics Center (CGC) (University of Minnesota, Minneapolis, MN). Worms were routinely cultured on nematode growth medium (NGM) plates with *Escherichia coli* OP50 as standard food resource at 16 ℃. 60～80 worm eggs were put on NGM, which contained 100 μmol/L tested compounds in 0.1% DMSO, and the 0.1% DMSO solvent was used as negative control in the whole bioactivity assay experiment. When the worms grew into L3 larvae, they were then transferred into another incubator which was set at 25 ℃ for another 26 h. Paralyzed worms were observed and counted under a dissecting microscope at 2 h interval till all worms got paralyzed. The anti-AD bioactivity of tested compound was indicated by the percentage of paralyzed worm individuals in the tested worm population throughout the whole experiment in contrast with the negative control group. For anti-AD activity assay, a log rank survival test was used to compare significance among treatments. p values at 0.05 or lower were considered statistically significant.

Supporting Information 1D and 2D NMR, HRESIMS, IR spectra, and X-ray crystallographic information file (CIF) of compound **1**. The Supporting Information is available free of charge via the Internet at http://sioc-journal.cn.

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