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高卢蜜环菌中一个新的甾体类化合物

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摘要 对蜜环菌 *Armillaria gallica* Marxm. & Romagn.发酵提取物进行化学成分研究, 从中分离得到 1 个新的甾体类化合物 armillanal D (**1**), 1 个新天然产物 VD2-1 (**8**)以及 6 个已知化合物. 综合运用 1D、2D 的核磁共振波谱、高分辨质谱和电子圆二色性(ECD)谱对化合物 **1** 的结构进行了鉴定. 在小鼠巨噬细胞(J774A.1)中, 评价了除化合物 **8** 以外所有化合物对尼日利亚菌素(Nigericin)诱导的 NLRP3 炎症小体激活的抑制作用, chaxine C (**6**)表现出较好的抗炎活性, 其 IC_{50} 值为(4.97±0.81) μmol/L.

关键词 高卢蜜环菌; 真菌代谢产物; 甾体; 抗炎活性

A New Steroid from *Armillaria gallica*

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Abstract A chemical investigation on the cultures extract of the basidiomycete *Armillaria gallica* Marxm. & Romagn. led to the isolation of eight compounds, including a new steroid armillanal D (**1**), a new natural product VD2-1 (**8**) and six known compounds. The structure of **1** was elucidated on the basis of extensive 1D and 2D-NMR spectra analysis, HR-ESI-MS, and electronic circular dichroism (ECD) spectra. The inhibitory activity of these compounds except **8** on NLRP3 inflammasome activation in Nigericin-induced mouse macrophage (J774A.1) cells was evaluated. Chaxine C (**6**) exhibited potential anti-inflammatory activity *in vitro* with IC_{50} value of (4.97±0.81) μmol/L.

Keywords *Armillaria gallica*; fungal metabolite; steroid; anti-inflammatory activity

1 Introduction

Armillaria is a member of the genera of the Basidiomycota family, which comprises about 70 known species.[1-2] *Armillaria gallica* Marxm. & Romagn. is a fungus symbiotic with *Gastrodia elata* (Chinese medicinal herb "Tian-

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ma"), which is a rootless and lesfless orchid.[3-4] Structurally diverse secondary metabolites have been found in the fungi of *Armillaria*, including protoilludane sesquiterpene aryl esters,[5-8] protoilludane norsesquiterpenoid esters,[9-10] steroids, triterpenes,[11] isoflavones[12] and alkaloids.[13] These compounds have attracted considerable interest because of a diverse array of activities such as anti-tumor,[7] acetylcholinesterase (AChE) inhibitory activity,[8] antibacterial[14] and so on. The chemical investigation of the secondary metabolites from *Armillaria gallica* Marxm. & Romagn. was carried out, which led to the identification of a new steroid (**1**), and a new natural product (**8**), together with six known compounds (**2**～**7**) (Figure 1). Herein, the isolation and structure elucidation of compound **1** and the inhibitory activities of compounds **1**～**7** on the NLRP3 inflammasome were reported.

2 Results and discussion

2.1 Structure elucidation of compound 1

Armillanal D (**1**) was isolated as a white amorphous powder. The molecular formula of compound **1** was established as $C_{31}H_{48}O_5$ via HR-ESI-MS (*m/z* 518.3837) $[M+NH_4]^+$ ion peak (calcd for $C_{31}H_{52}NO_5$ 518.3840), indicating eight degrees of unsaturation. The ^{1}H NMR spectrum (Table 1) showed five methyl doublets, two methyl singlets, two doubles of doublets and two doublets attributed to four olefinic protons, and one quartet and one multiplet characteristic of two oxygenated methines. The ^{13}C NMR data (Table 1) along with the heteronuclear single quantum coherence (HSQC) experiment displayed twelve

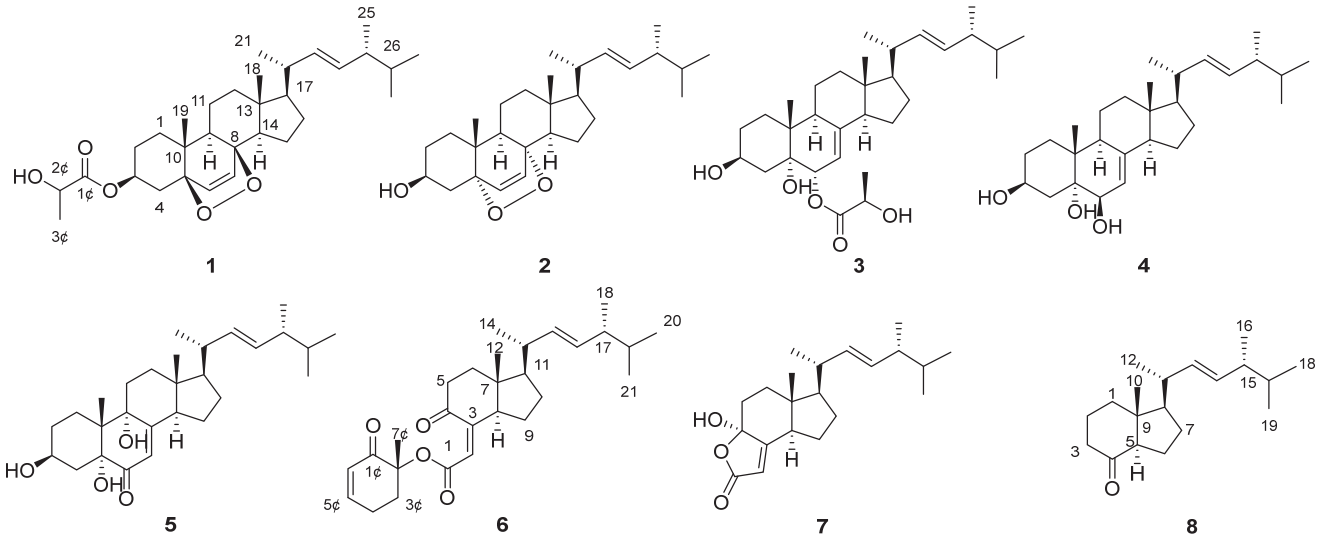

Figure 1 Chemical structures of compounds **1**～**8**

Table 1 ^{1}H (400 MHz) and ^{13}C (100 MHz) NMR data of compound **1** in CD_3OD

Position	δ_C (type)	δ_H (*J* in Hz)	Position	δ_C (type)	δ_H (*J* in Hz)
1	35.5 (CH_2)	1.93～1.97 (m, 1H)			1.26 (overlap)
		1.76 (overlap)	16	29.8 (CH_2)	1.76 (overlap)
2	27.2 (CH_2)	1.84～1.89 (m, 1H)			1.37～1.42 (m, 1H)
		1.65～1.69 (m, 1H)	17	57.5 (CH)	1.26 (overlap)
3	71.3 (CH)	4.92～4.98 (m, 1H)	18	13.3 (CH_3)	0.86 (s, 3H)
4	34.0 (CH_2)	2.04～2.13 (m, 2H)	19	18.5 (CH_3)	0.93 (s, 3H)
5	83.2 (C)		20	41.2 (CH)	2.02～2.07 (m, 1H)
6	136.3 (CH)	6.28 (d, 8.5, 1H)	21	21.4 (CH_3)	1.02 (d, 6.6, 3H)
7	131.9 (CH)	6.55 (d, 8.5, 1H)	22	136.8 (CH)	5.18 (dd, 15.3, 8.3, 1H)
8	80.8 (C)		23	133.5 (CH)	5.25 (dd, 15.3, 7.7, 1H)
9	52.7 (CH)	1.47 (overlap)	24	44.3 (CH)	1.82～1.87 (m, 1H)
10	38.2 (C)		25	20.1 (CH_3)	0.84 (d, 6.8, 3H)
11	21.5 (CH_2)	1.53 (overlap)	26	34.4 (CH)	1.47 (overlap)
		1.49 (overlap)	27	20.5 (CH_3)	0.86 (d, 6.7, 3H)
12	40.6 (CH_2)	1.97～2.00 (m, 1H)	28	18.2 (CH_3)	0.93 (d, 6.7, 3H)
		1.26 (overlap)	1'	175.7 (C)	
13	45.8 (C)		2'	67.9 (CH)	4.20 (q, 6.9, 1H)
14	53.0 (CH)	1.53 (overlap)	3'	20.6 (CH_3)	1.35 (d, 6.9, 3H)
15	24.4 (CH_2)	1.54～1.58 (m, 1H)			

methines containing four olefinic methines (C-6, C-7, C-22 and C-23) and two oxygenated methines (C-3 and C-2'), seven methylenes, seven methyls, and five quaternary carbon atoms containing two oxygenated quaternary carbons (C-5 and C-8) and one ester carbonyl carbon (C-1'). The ^{1}H NMR and ^{13}C NMR spectra of compound **1** were closely similar to those of (22*E*,24*R*)-5α,8α-epidioxyergosta-6,22-dien-3-ol (**2**), suggesting that it was presumably a derivative of **2**.[15] The major difference was the presence of a 2-hydroxy-propionyloxy group in **1** (Figure 1) instead of a hydroxyl group in **2**, which was supported by the heteronuclear multiple-bond correlation (HMBC) correlations (Figure 2) of H-3' (δ_H 1.35) with C-1' (δ_C 175.7) and C-2' (δ_C 67.9), and H-2' (δ_H 4.20) with C-1' (δ_C 175.7) and C-3' (δ_C 20.6). The HMBC correlation between H-3 (δ_H 4.92～4.98) and C-1' (δ_C 175.7) suggested that the 2-hydroxypropionyloxy group located at C-3. The planar structure of **1** was determined by analysis of the other ^{1}H-^{1}H correlation spectroscopy (COSY) and HMBC correlations.

The relative configuration of compound **1** was determined by analysis of nuclear overhauser effect spectroscopy (NOESY) correlations. The NOESY correlations of H-3/H-1α (δ_H 1.93～1.97), H-9/H-1α (δ_H 1.93～1.97), H-9/H-17 and H-14/H-17 indicated that H-1α, H-3, H-9, H-14 and H-17 were α-oriented. Likewise, the NOESY correlations of H_3-19/H-1β (δ_H 1.76), H_3-19/H_3-18 and H_3-18/H-20 indicated that H-1β, H_3-18, H_3-19 and H-20 were β-oriented (Figure 3). Additionally, the double bond $\Delta^{22(23)}$ was assigned with an *E*-configuration from coupling constants. However, the configurations of C-5 and C-8 could not be assigned from the NOESY data. Therefore, the theoretical ECD spectra of compound **1** possible diastereoisomers (3*S*,5*S*,8*S*,9*R*,10*R*,13*R*,14*R*,17*R*,20*R*,24*R*)-**1a**, (3*R*,5*R*,8*R*,9*S*,10*S*,13*S*,14*S*,17*S*,20*S*,24*S*)-**1b**, (3*S*,5*R*,8*R*,9*R*,10*R*,13*R*,14*R*,17*R*,20*R*,24*R*)-**1c** and (3*R*,5*S*,8*S*,9*S*,10*S*,13*S*,14*S*,17*S*,20*S*,24*S*)-**1d** were calculated at the B3LYP/6-31+G(d,p) level in methanol with the time-dependent density functional theory (TDDFT) (Figure 3). The calculated spectrum of (3*S*,5*R*,8*R*,9*R*,10*R*,13*R*,14*R*, 17*R*,20*R*,24*R*)-**1c** was in good agreement with the experimental spectrum, and its absolute configuration can be determined, as shown in Figure 1. It is worth noting that the configuration of the chiral carbon in 2-hydroxypropionyloxy unit can not be determined by ECD calculations because of its flexible nature.

2.2 Biological activity

In this study, compounds **1**～**7** were tested for their inhibitory effect on NLRP3 inflammasome (Figure 4, Table 2). The initial screening results showed that compound **6** had a lactate dehydrogenase (LDH) release inhibition rate greater than 50% at a concentration of 10 μmol/L, which showed anti-inflammatory activity.

Table 2 LDH release inhibition of compound **6** in nigericin-induced mouse macrophage (J774A.1) cells[a]

Compound	IC_{50} (means±SD)/(μmol•L^{-1})
6	4.97±0.81
Andrographolide	8.11±0.71

[a] Compared with the LPS+Nig group, *P<0.05, **P<0.01, and ***P<0.001.

3 Conclusions

In summary, a new steroid, a new natural product and six known compounds were obtained from cultures of *Armillaria gallica* Marxm. & Romagn. The structures of **1**～**8** were elucidated by extensive NMR spectroscopic data analysis, and the experimental ECD spectra of compound **1**

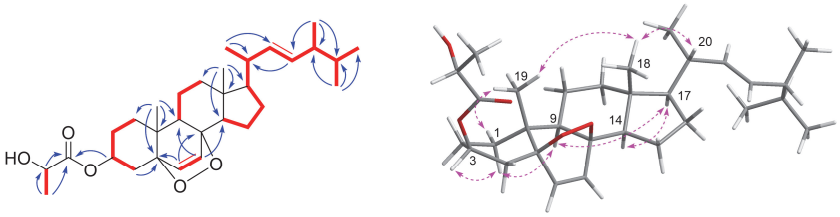

Figure 2 Key ^{1}H-^{1}H COSY (▬), HMBC (⌒) and NOESY (⌒) correlations of compound **1**

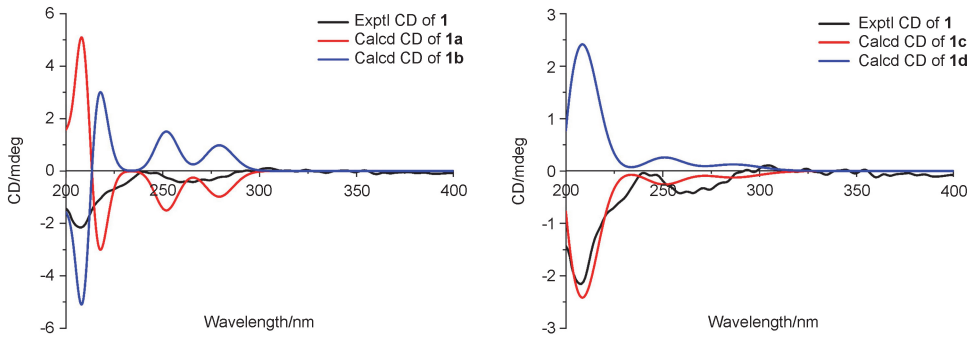

Figure 3 Calculated and experimental ECD spectra for **1**

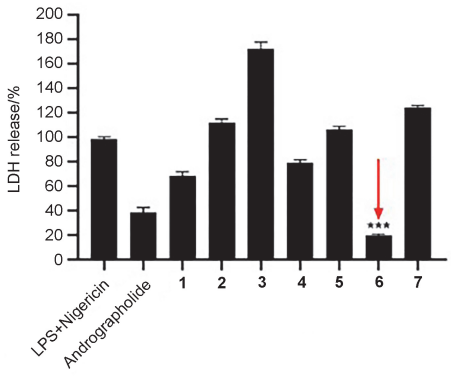

Figure 4 Inhibition of NLRP3 inflammasome by compounds **1**～**7**

Aandrographolide (10 μmol/L) was used as the positive control, and the concentration of compounds was 10 μmol/L.

were compared with the ECD spectra calculated by the time-dependent density functional method to determine the absolute configuration of compound **1**. Compounds **1**～**7** were screened for their anti-inflammatory activities and compound **6** showed the best inhibition of inflammatory vesicle activation with an IC_{50} value of (4.97±0.81) μmol/L. Compounds **1**～**8** have not been isolated from *Gastrodia elata*, suggesting that a symbiotic relationship between *Armillaria gallica* and *Gastrodia elata* may lead to changes in chemical composition that may be related to their growing environment and interactions.

4 Experimental section

4.1 General experiment

NMR spectra were recorded on a Bruker AV-400 instrument or a Bruker AV-III-600 instrument using TMS as an internal standard, and HR-ESI-MS data were measured on an Agilent1100 HPLC-TOF mass spectrometer. IR spectra were measured on a Nicolet iS10 FT IR spectrometer. UV and ECD spectra were measured on an Applied Photophysics V100 Chirascan instrument. Optical rotations were recorded on an Autopol VI polarimeter at 25 ℃. Semi-preparative high performance liquid chromatography (HPLC) was performed on an Agilent 1260 liquid chromatograph with an Agilent Zorbax SB-C18 column (250 mm×9.4 mm, i.d., 5 μm). Column chromatography (CC) was performed using MCI gel CHP 20P (75～150 μm, Mitsubishi Chemical Industries, Japan), Sephadex LH-20 (Amersham Pharmacia, Sweden), and Silica gel (80～100 mesh and 200～300 mesh, Qingdao Marine Chemical, Ltd.). Fractions were monitored by thin-layer chromatography (TLC), and spots were visualized under UV light (254 nm) and heated after spraying with 8% aqueous H_2SO_4.

4.2 Fungal material

The fungal strain was isolated from the epidermis in the underground portion of *Gastrodia elata* collected from a plantation field at Xiaocaoba, Yiliang, Yunnan Province, China in December 2022. By analyzing the internal transcribed spacer (ITS) region of the rRNA sequences, it was determined that the strain has the closest genetic relationship with Armillaria gallica (GenBank accession number: PP526995). The voucher specimen (CGMCC No. 6758) was deposited at College of Agronomy and Life Sciences, Zhaotong University. The fungal strain was cultured on potato dextrose agar slants at 28 ℃ for 3 d. The agar plugs were cut into small pieces (approximately 0.5 cm×0.5 cm×0.5 cm) under aseptic conditions, and 15 pieces of agar plugs were used to inoculate into three Erlenmeyer flasks (250 mL), each containing 50 mL of medium (1% malt extract, 0.4% glucose, and 0.4% yeast extract), and sterilized by autoclave. The three flasks inoculated with medium were incubated on a shaker at 28 ℃, 170 r/min for 5 d to prepare seed cultures. Potato dextrose broth (30 L) was then placed in a fermenter and fermented for 5 d at 28 ℃ and 170 r/min.

4.3 Extraction and isolation

The mycelium of *A. gallica* (8.0 kg) was extracted with 95% EtOH (25 L×3). The EtOH extract was partitioned with H_2O and ethyl acetate (EA) to afford the EA solution (174 g). The EA fraction was fragmented by MCI column chromatography (CH_3OH/H_2O gradient elute, 50%, 70% and 90%) to obtain three fractions. Fr.1 was divided into five fractions (Fr.1a～Fr.1e) on a silica gel CC with CH_2Cl_2/MeOH (V∶V=100∶1 to 1∶1). Fr.1c was subjected to silica gel CC eluting with $CHCl_3$/acetone (V∶V=100∶1) to yield four sub-fractions (Fr.1c1～Fr.1c4). Fr.1c2 was fractionated by silica gel column eluting petroleum ether (PE)/EA (V∶V=2∶1) to afford compound **4** (50.0 mg). Fr.1c3 was further separated by preparative TLC (P-TLC) (PE/EA, V∶V=1∶1) to obtained compounds **3** (8.6 mg) and **5** (12.0 mg). Fr.1d was fractionated into Fr.1d1～Fr.1d5 using silica gel CC (PE/EA, V∶V=10∶1 to 1∶1). Compound **6** (5.0 mg) was afforded from Fr.1d4 via preparative TLC (P-TLC) (PE/EA, V∶V=2∶1). Fr.2 was divided into five fractions (Fr.2a～Fr.2e) on a silica gel CC (PE/EA, V∶V=15∶1 to 1∶1). Fr.2b was separated by semi-preparative HPLC (CH_3CN/H_2O, V∶V=73∶27, 3.0 mL/min) to yield compound **8** (1.2 mg, t_R=10 min). Silica gel CC purification of Fr.2d was accomplished by elution with PE/EA (V∶V=2∶1) to afford compound **7** (36.9 mg). Compound **2** (200.3 mg) was crystallized from Fr.3. Fr.3 was further fractionated by Sephadex LH-20 chromatography (CH_3OH) to produce 4 sub-fractions (Fr.3a～Fr.3d). Compound **1** (5.4 mg) was obtained from Fr.3c using preparative TLC (P-TLC) (PE/EA, V∶V=1∶1).

Armillanal D (**1**): White amorphous powder; $[\alpha]_D^{25}$ + 11.41 (*c* 0.056, MeOH); UV (MeOH) λ_{max} [log ε/(L•mol^{-1}•cm^{-1})]: 200 (3.00) nm; ECD (MeOH) λ_{max} [$\Delta\varepsilon$/(L•mol^{-1}•cm^{-1})]: 266 (−0.42), 244 (−0.09), 207 (−2.16) nm; ^{1}H NMR and ^{13}C NMR data see Table 1; IR (KBr) ν_{max}: 3430, 2957, 2930, 2873, 1734, 1459, 1376, 1222, 1130, 1042 and 971 cm^{-1}; HR-ESI-MS calcd for $C_{31}H_{52}NO_5$ $[M+NH_4]^+$ 518.3840, found 518.3837.

(22*E*,24*R*)-5*α*,8*α*-Epidioxyergosta-6,22-dien-3-ol (**2**):

White amorphous powder. ^{1}H NMR (400 MHz, $CDCl_3$) δ: 6.50 (d, J = 8.5 Hz, H-7, 1H), 6.24 (d, J = 8.5 Hz, H-6, 1H), 5.22 (dd, J = 15.2, 7.5 Hz, H-23, 1H), 5.13 (dd, J = 15.2, 8.1 Hz, H-22, 1H), 3.91～4.04 (m, 1H, H-3), 0.99 (d, J = 6.6 Hz, H-21, 3H), 0.90 (d, J = 6.9 Hz, H-28, 3H), 0.83 (s, 3H, H-19), 0.93 (d, J = 6.6 Hz, H-27, 3H), 0.81 (s, 3H, H-18), 0.81 (d, J = 6.6 Hz, H-26, 3H); ^{13}C NMR (100 MHz, $CDCl_3$) δ: 135.5 (C-6), 135.3 (C-22), 132.4 (C-23), 130.9 (C-7), 82.3 (C-5), 79.6 (C-8), 66.6 (C-3), 56.3 (C-17), 51.8 (C-9), 51.2 (C-14), 44.7 (C-13), 42.9 (C-24), 39.9 (C-20), 39.5 (C-12), 37.1 (C-4), 37.0 (C-10), 34.8 (C-1), 33.2 (C-25), 30.2 (C-2), 28.8 (C-16), 23.5 (C-15), 21.0 (C-11), 20.8 (C-27), 20.1 (C-26), 19.8 (C-21), 18.3 (C-19), 17.7 (C-28), 13.0 (C-18); HR-ESI-MS calcd for $C_{28}H_{45}O_3$ $[M+H]^+$ 429.3363, found 429.3359. Compound **2** was identified as (22*E*,24*R*)-5α,8α-epidioxyergosta-6,22-dien-3-ol by comparing spectroscopic data with those of previously reported compounds.[15]

Quadristerol B (**3**): White amorphous powder. ^{1}H NMR (600 MHz, $CDCl_3$) δ: 5.22 (dd, J=15.2, 7.6 Hz, H-23, 1H), 5.16 (dd, J=15.2, 8.3 Hz, H-22, 1H), 4.92 (d, J=5.3 Hz, H-7, 1H), 4.26 (d, J=6.9 Hz, H-2', 1H), 1.44 (d, J=6.8 Hz, H-3', 3H), 1.05 (s, 3H, H-19), 1.02 (d, J=6.5 Hz, H-21, 3H), 0.91 (d, J=6.8 Hz, H-25, 3H), 0.83 (d, J=6.8 Hz, H-27, 3H), 0.82 (d, J=6.7 Hz, H-28, 3H), 0.58 (s, 3H, H-18); ^{13}C NMR (150 MHz, $CDCl_3$), δ: 175.4 (C-1'), 146.9 (C-8), 135.4 (C-22), 132.4 (C-23), 113.6 (C-7), 75.3 (C-6), 74.8 (C-5), 67.4 (C-2'), 67.1 (C-3), 56.1 (C-17), 55.1 (C-14), 44.0 (C-13), 43.5 (C-9), 43.0 (C-24), 40.5 (C-20), 39.3 (C-4), 39.3 (C-12), 37.4 (C-10), 33.2 (C-26), 32.5 (C-1), 30.7 (C-2), 28.0 (C-16), 22.9 (C-15), 22.2 (C-11), 21.3 (C-21), 20.4 (C-3'), 20.1 (C-28), 19.8 (C-27), 18.4 (C-19), 17.8 (C-25), 12.5 (C-18); HR-ESI-MS calcd for $C_{32}H_{51}O_7$ $[M+COOH]^-$ 547.3640, found 547.3642. Compound **3** was identified as quadristerol B by comparing spectroscopic data with those of previously reported compounds.[16]

(22E,24R)-Ergosta-7,22-diene-3β,5α,6β-triol (**4**): White amorphous powder. ^{1}H NMR (400 MHz, Pyridine-d_5) δ: 5.26 (dd, J=15.2, 7.2 Hz, H-23, 1H), 5.19 (dd, J=15.2, 8.0 Hz, H-22, 1H), 4.81～4.91 (m, 1H, H-3), 4.35 (d, J=4.5 Hz, H-6, 1H), 3.05 (dd, J=13.2, 11.2 Hz, H-4, 1H), 1.53 (s, 3H, H-19), 1.08 (d, J=6.6 Hz, H-21, 3H), 0.97 (d, J=6.8 Hz, H-28, 3H), 0.88 (d, J=6.7 Hz, H-26, 3H), 0.87 (d, J=6.7 Hz, H-27, 3H), 0.68 (s, 3H, H-18); ^{13}C NMR (100 MHz, Pyridine-d_5) δ: 142.0 (C-8), 136.7 (C-22), 132.5 (C-23), 121.0 (C-7), 76.6 (C-5), 74.7 (C-6), 68.0 (C-3), 56.5 (C-17), 55.7 (C-14), 44.2 (C-9, 13), 43.5 (C-24), 42.4 (C-4), 41.4 (C-20), 40.4 (C-12), 38.5 (C-10), 34.3 (C-1), 33.8 (C-26), 33.1 (C-2), 29.0 (C-16), 23.9 (C-15), 22.9 (C-11), 21.9 (C-21), 20.6 (C-27), 20.3 (C-28), 19.3 (C-19), 18.3 (C-25), 13.0 (C-18); HR-ESI-MS calcd for $C_{28}H_{43}O$ (M^+-OH-H_2O) 395.3309, found 395.3306; for $C_{28}H_{41}$ ($M^+-OH-2H_2O$) 377.3202, found 377.3197. Compound **4** was identified as (22*E*,24*R*)-ergosta-7,22-diene-3β,5α,6β-triol by comparing spectroscopic data with those of previously reported compounds.[17]

3β,5α,9α-Trihydroxy-ergosta-7,22-dien-6-one (**5**): White amorphous powder. ^{1}H NMR (400 MHz, Pyridine-d_5) δ: 5.28 (dd, J=15.2, 7.5 Hz, H-23, 1H), 5.19 (dd, J=15.2, 8.0 Hz, H-22, 1H), 4.62～4.74 (m, 1H, H-3), 3.01 (t, J=9.4 Hz, H-4, 1H), 1.17 (s, 3H, H-19), 1.06 (d, J=6.6 Hz, H-21, 3H), 0.97 (d, J=6.8 Hz, H-28, 3H), 0.88 (d, J=6.7 Hz, H-26, 3H), 0.87 (d, J=6.7 Hz, H-27, 3H), 0.65 (3H, s, H-18); ^{13}C NMR (100 MHz, Pyridine-d_5) δ: 199.4 (C-6), 164.5 (C-8), 136.1 (C-22), 132.7 (C-23), 120.6 (C-7), 80.1 (C-5), 75.4 (C-9), 67.1 (C-3), 56.4 (C-17), 52.3 (C-14), 45.7 (C-13), 43.4 (C-24), 42.5 (C-10), 41.0 (C-20), 38.5 (C-4), 35.7 (C-12), 33.6 (C-25), 31.8 (C-2), 29.3 (C-11), 28.7 (C-16), 26.8 (C-1), 23.0 (C-15), 21.6 (C-21), 20.7 (C-19), 20.5 (C-27), 20.2 (C-26), 18.2 (C-28), 12.7 (C-18); HR-ESI-MS calcd for $C_{28}H_{44}NaO_4$ $[M+Na]^+$467.3132, found 467.3133. Compound **5** was identified as 3β,5α,9α-trihydroxyergosta-7,22-dien-6-one by comparing spectroscopic data with those of previously reported compounds.[18]

Chaxine C (**6**): White amorphous powder. ^{1}H NMR (400 MHz, $CDCl_3$) δ: 6.86～4.92 (m, 1H, H-5'), 6.04 (d, J=10.5 Hz, H-6', 1H), 5.64 (d, J=2.3 Hz, H-2, 1H), 5.26 (dd, J=15.2, 7.7 Hz, H-16, 1H), 5.14 (dd, J=15.2, 8.5 Hz, H-15, 1H), 2.88～2.98 (m, 1H, H-3'), 2.67～2.78 (m, 1H, H-5), 2.43～2.52 (m, 3H, H-8, 4'), 2.17～2.24 (m, 1H, H-6), 1.47 (s, 3H, H-7'), 1.02 (d, J=6.6 Hz, H-14, 3H), 0.90 (d, J=6.8 Hz, H-18, 3H), 0.86 (s, 3H, H-12), 0.83 (d, J=6.7 Hz, H-20, 3H), 0.81 (d, J=6.7 Hz, H-21, 3H); ^{13}C NMR (100 MHz, $CDCl_3$) δ: 204.9 (C-4), 196.0 (C-1'), 164.4 (C-1), 156.5 (C-3), 149.0 (C-5'), 134.7 (C-15), 133.0 (C-16), 128.2 (C-6'), 117.5 (C-2), 81.3 (C-2'), 58.0 (C-8), 55.4 (C-11), 46.6 (C-7), 43.0 (C-17), 40.3 (C-13), 39.2 (C-5), 38.1 (C-6), 33.2 (C-19), 32.2 (C-3'), 29.2 (C-10), 24.9 (C-4'), 22.0 (C-7'), 21.8 (C-9), 21.1 (C-14), 20.1 (C-21), 19.8 (C-20), 17.7 (C-18), 12.2 (C-12); HR-ESI-MS calcd for $C_{28}H_{40}NaO_4$ $[M+Na]^+$ 463.2819, found 463.2816. Compound **6** was identified as chaxine C by comparing spectroscopic data with those of previously reported compounds.[19]

Demethylincisterol A_3 (**7**): White amorphous powder. ^{1}H NMR (400 MHz, $CDCl_3$) δ: 5.58 (d, J=1.8 Hz, H-2, 1H), 5.24 (dd, J=15.3, 7.5 Hz, H-16, 1H), 5.15 (dd, J=15.3, 8.2 Hz, H-15, 1H), 2.61～2.68 (m, 1H, H-8), 2.26～2.34 (m, 1H, H-5), 1.02 (d, J=6.6 Hz, H-14, 3H), 0.91 (d, J=6.8 Hz, H-18, 3H), 0.83 (d, J=6.8 Hz, H-21, 3H), 0.82 (d, J=6.8 Hz, H-20, 3H), 0.58 (s, 3H, H-12); ^{13}C NMR (100 MHz, $CDCl_3$), δ: 172.2 (C-1), 171.4 (C-3), 134.8 (C-15), 132.9 (C-16), 112.1 (C-2), 105.6 (C-4), 55.4 (C-11), 50.5 (C-8), 49.0 (C-7), 42.9 (C-17), 40.2 (C-13), 35.3 (C-5), 35.2 (C-6), 33.2 (C-19), 29.0 (C-10), 21.5 (C-9), 21.1 (C-14), 20.1 (C-21), 19.8 (C-20), 17.7 (C-18), 11.9 (C-12); HR-ESI-MS calcd for $C_{21}H_{33}O_3$ $[M+H]^+$ 333.2424, found 333.2428. Compound **7** was identified as demethylincisterol A_3 by comparing spectroscopic data with those of previously reported compounds.[20]

(1*R*,3a*R*,7a*R*)-1-((2*R*,*E*)-5,6-Dimethylhept-3-en-2-yl)-7a-methyloctahydro-4*H*-inden-4-one (**8**): White amorphous powder. ^{1}H NMR (600 MHz, $CDCl_3$) δ: 5.24 (dd, J=15.3, 7.6 Hz, H-14, 1H), 5.17 (dd, J=15.3, 8.4 Hz, H-13, 1H),

2.38～2.44 (m, 1H, H-2), 2.31～2.37 (m, 1H, H-2), 2.11～2.17 (overlap, 3H, H-1, 4, 11), 1.89～1.94 (m, 1H, H-6), 1.82～1.87 (m, 1H, H-15), 1.76～1.79 (m, 1H, H-7), 1.71～1.74 (m, 1H, H-1), 1.62～1.66 (m, 1H, H-6), 1.45～1.49 (m, 1H, H-17), 1.13 (s, 3H, H-10), 1.06 (d, J=6.7 Hz, H-12, 3H), 0.91 (d, J=6.8 Hz, H-16, 3H), 0.83 (d, J=6.8 Hz, H-18, 3H), 0.81 (d, J=6.8 Hz, H-19, 3H); ^{13}C NMR (150 MHz, $CDCl_3$), δ: 212.3 (C-4), 134.9 (C-13), 132.9 (C-14), 62.5 (C-5), 51.8 (C-8), 49.7 (C-9), 43.0 (C-15), 39.3 (C-11), 36.6 (C-2), 33.2 (C-17), 32.9 (C-1), 31.4 (C-7), 29.9 (C-3), 25.1 (C-6), 22.1 (C-10), 21.4 (C-12), 20.1 (C-19), 19.8 (C-18), 17.7 (C-16). Compound **8** was identified as (1*R*, 3a*R*,7a*R*)-1-((2*R*,*E*)-5,6-dimethylhept-3-en-2-yl)-7a-methyloctahydro-4*H*-inden-4-one by comparing spectroscopic data with those of previously reported compounds.[21]

4.4 TDDFT-ECD calculations

The ECD calculations and simulations of the calculated ECD spectra were performed using the same methodology that was previously documented in the literature.[22]

4.5 Anti-inflammatory activity assay

The J774A.1 cells were purchased from the Kunming Cell Bank (Kunming, China). J774A.1 cells were cultured with DMEM medium containing 10% fetal bovine serum, and inoculate logarithmic growth stage cells with a cell density of 1.5×10^5/well into a 24 well plate, and cultured overnight. Then the next day, cell supernatant was discarded and addition of lipopolysaccharide (LPS) formulated with Ultra-Fectin at a concentration of 200 ng/mL was stimulated for 3.5 h to allow the first signal to be generated. Then the supernatant was removed, and 10 μmol/L of the compound was added and incubated for 30 min, using andrographolide as the positive drug. Finally, Nigericin (10 μmol/L) was added for 1 h of stimulation to activate NLRP3 inflammasome. Inhibition of lactate dehydrogenase (LDH) release from collected cell supernatant was assayed by an LDH assay kit.

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Supporting Information 1D and 2D NMR, HR-ESI-MS, UV, IR and optical rotation spectra for compound **1**, NMR spectra of compounds **2**～**8**, and HR-ESI-MS spectra of compounds **2**～**7**. The Supporting Information is available free of charge via the Internet at http://sioc-journal.cn.

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