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New 4,5-*seco*-20(10→5)-*abeo*-Abietane Diterpenoids with Anti-Inflammatory Activity from *Isodon lophanthoides* var. *graciliflorus* (Benth.) H.Hara

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Three new 4,5-*seco*-20(10→5)-*abeo*-abietane diterpenoids, 16-hydroxysalvilenone (**1**), 15-hydroxysalprionin (**2**), and 11β,15-dihydroxysalprionin-12-one (**3**), and nine known abietane diterpenoids, **4**–**12**, along with one known sempervirane diterpenoid, hispidanol A (**13**), were isolated from the aerial parts of *Isodon lophanthoides* var. *graciliflorus*. The structures of compounds **1**–**3** were determined on the basis of spectroscopic methods including extensive analysis of NMR and mass spectroscopic data. All diterpenoids were tested for their TNF-α inhibitory effects on LPS-induced RAW264.7 cells. Compound **9** (16-acetoxyhorminone) was the most potent with an IC_{50} value of 3.97 ± 0.70 μM.

Keywords: *Isodon lophanthoides*, 4,5-*seco*-20(10→5)-*abeo*-abietane diterpenoid, structure elucidation, anti-inflammatory activity, biological activity..

Introduction

The aerial parts of *Isodon lophanthoides* are empirically employed as antimalarial and anti-inflammatory agents and also for the treatment of enteritis and jaundice.[1] Diterpenoids are the predominant constituents of *I. lophanthoides* and its varieties. Among its varieties, *Isodon lophanthoides* var. *graciliflorus* (Benth.) H.Hara is perennial herb distributed in Fujian, Guangdong, and Jiangxi provinces of China.[2] It is known in China by the name *'Tiancao'* and has been commercially farmed as a source of '*Xihuangcao*', a folk Chinese medicine for the treatment of acute icterohepatitis, cholecystitis, and enteritis as well as a herb for health promoting beverages such as tea and instant granules.[3] Previous study on this plant resulted in the isolation of ten abietane diterpenoids, which six abietane ones demonstrated potent cytotoxicity.[4] In our ongoing search for bioactive diterpenoids from this plant, an investigation of the chemical constituents of *I. lophanthoides* var. *graciliflorus*, which was collected in Guangzhou, led to isolation of three new 4,5-*seco*-20(10→5)-*abeo*-abietane diterpenoids, 16-hydroxysalvilenone (**1**), 15-hydroxysalprionin (**2**), and 11β,15-dihydroxysalprionin-12-one (**3**), along with ten known compounds, **4**–**13**. Herein, the structural elucidation of these new abietanoids and their anti-inflammatory evaluation are presented.

Results and Discussion

Structure Elucidation

Compound **1** (*Figure 1*), light amorphous powder, was assigned a molecular formula $C_{20}H_{20}O_3$ with 11

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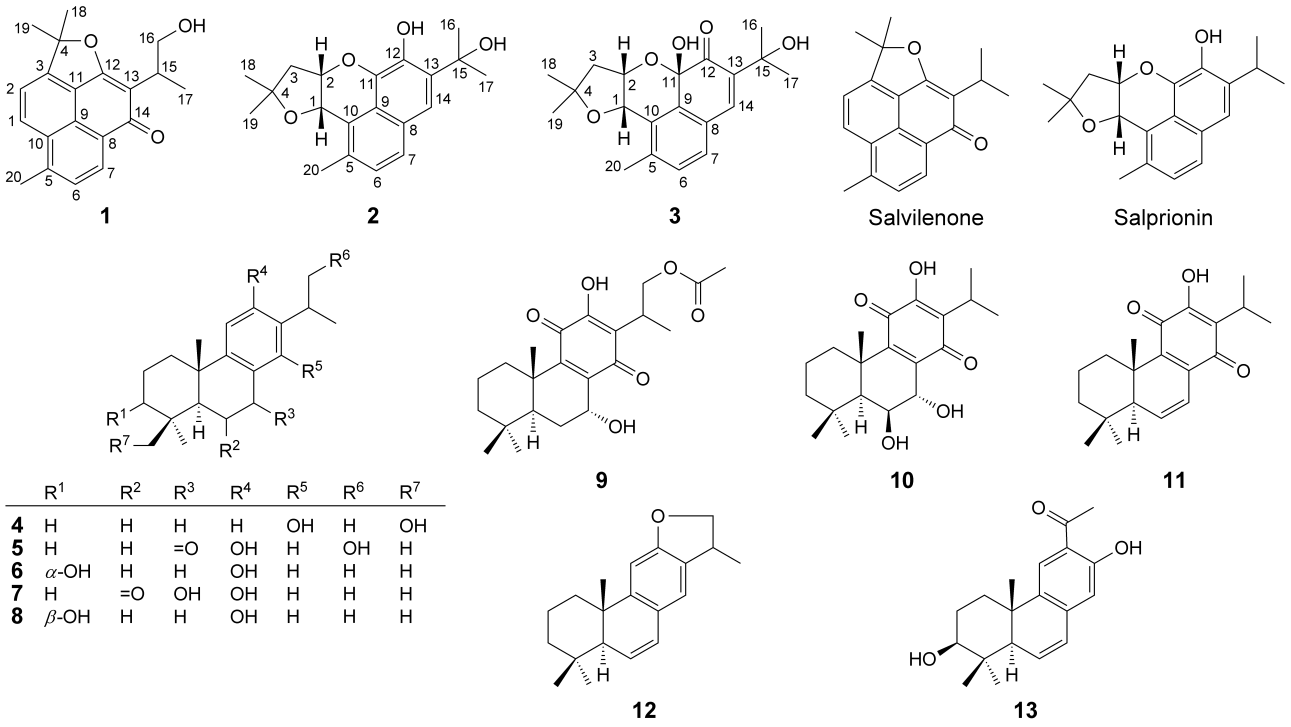


	R^1	R^2	R^3	R^4	R^5	R^6	R^7
4	H	H	H	H	OH	H	OH
5	H	H	=O	OH	H	OH	H
6	α-OH	H	H	OH	H	H	H
7	H	=O	OH	OH	H	H	H
8	β-OH	H	H	OH	H	H	H

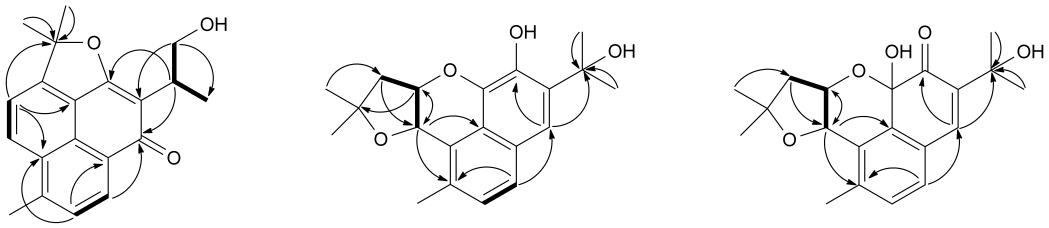
Figure 1. Structures of compounds **1**–**13**, salvilenone and salprionin.

degrees of unsaturation as deduced by positive ion mode HR-ESI-MS (*m/z* 309.1488 $[M+H]^+$). The IR spectrum of **1** showed absorption bands at 3421, 1670, 1606, 1563 and 1527 cm^{-1}, which were indicative of hydroxy group, α,β-unsaturated ketone carbonyl group and aromatic ring moiety. The ^{1}H-NMR spectrum of **1** (*Table 1*) displayed three singlet methyl groups at δ(H) 1.81 (6H, *s*, 2 Me), 2.81 (3H, *s*), one doublet methyl group at δ(H) 1.34 (3H, *d*, $J=7.2$), two hydroxymethyl protons at δ(H) 3.86 (1H, dd, $J=7.2$, 10.8) and 3.79 (1H, dd, $J=7.2$, 10.8), along with two groups of AB coupling aromatic protons at δ(H) 8.31 (1H, d, $J=8.4$), 7.68 (1H, d, $J=8.4$), 8.24 (1H, d, $J=7.2$), and 7.60 (1H, d, $J=7.2$). Twenty carbon resonances occurred in the ^{13}C-NMR spectrum (*Table 1*), including 10 aromatic carbons of a naphthalenyl (δ(C) 130.5, 120.3, 149.3, 143.6, 130.1, 130.8, 128.5, 124.2, 131.5, 125.8), two olefinic carbons and one carbonyl carbon for an α,β-unsaturated ketone functionality (δ(C) 169.1, 116.3, 188.0), seven aliphatic carbon resonances for four methyl groups (δ(C) 26.9, 26.8, 19.3, 16.0), one methine group (δ(C) 34.1), and one hydroxymethyl group (δ(C) 66.4), and one quaternary carbon at δ(C) 99.2. The above evidences suggested that compound **1** should be a phenalen-8-one derivative similar to a known compound salvilenone,[5] which could be biogenetically derived from 4,5-*seco*-20(10→5)-*abeo*-abietane diterpenoid. All protons and carbons were assigned by analysis of 1D and 2D NMR spectroscopic data. The NMR spectroscopic data of **1** was quite close to those of salvilenone, except that the Me-16 of salvilenone was replaced by a hydroxymethyl group in compound **1**. The key ^{1}H,^{1}H-COSY correlations (*Figure 2*) of the methine proton at δ(H) 3.43 with hydroxymethyl protons (δ(H) 3.86 and 3.79) and methyl protons (δ(H) 1.34), in combined with key HMBCs (*Figure 2*) from H_2-16 to C-13 (δ(C) 116.3) and C-17 (δ(C) 16.0), and from H-15 (δ(H) 3.41–3.45) to C-12 (δ(C) 169.1) and C-14 (δ(C) 188.0), confirmed that one 16-hydroxymethylated isopropyl was linked to C-13 position. The methyl group at C-5 position was determined by key HMBCs (*Figure 2*) from the methyl proton at δ(H) 2.81 to C-6 (δ(C) 130.1) and C-10 (δ(C) 131.5). Deducting ten degrees of unsaturation of phenalen-8-one skeleton, there is still one degree of unsaturation in structure of **1**, indicating the presence of an addition ring. The downfield chemical shifts of C-4 (δ(C) 99.2) and C-12 (δ(C) 169.1) revealed that C-4 and C-12 were connected by one oxygen atom and formed one five-membered epoxy ring. Therefore, the structure of **1** is proposed to be 16-hydroxysalvilenone.

Table 1. ^{1}H- and ^{13}C-NMR spectral data for compounds **1**–**3** (in CD_3OD).[a]

Position	**1**		**2**		**3**	
	δ(C)	δ(H)	δ(C)	δ(H)	δ(C)	δ(H)
1	130.5	8.31 (*d*, *J* = 8.4)	74.8	5.21 (br. *d*, *J* = 1.9)	72.3	4.80 (*d*, *J* = 2.9)
2	120.3	7.68 (*d*, *J* = 8.4)	80.5	4.55 (br. *s*)	74.7	4.98–4.99 (*m*)
3	149.3		47.0	2.47 (*d*, *J* = 14.0)	46.3	2.35 (*dd*, *J* = 6.2, 14.0)
				2.37 (*d*, *J* = 14.0)		2.23 (br. *d*, *J* = 14.0)
4	99.2		82.5		82.9	
5	143.6		134.9		141.0	
6	130.1	8.24 (*d*, *J* = 7.2)	127.6	7.16 (*d*, *J* = 8.3)	132.2	7.23 (*d*, *J* = 7.7)
7	130.8	7.60 (*d*, *J* = 7.2)	129.3	7.64 (*d*, *J* = 8.3)	130.0	7.21 (*d*, *J* = 7.7)
8	128.5		127.2		128.3	
9	124.2		122.0		135.2	
10	131.5		123.2		132.7	
11	125.8		137.0		91.2	
12	169.1		140.7		197.3	
13	116.3		137.6		141.7	
14	188.0		116.9	7.41 (*s*)	138.0	7.45 (*s*)
15	34.1	3.41–3.45 (*m*)	74.7		72.1	
16	66.4	3.86 (*dd*, *J* = 7.2, 10.8)	29.4	1.69 (*s*, overlapped)	28.1	1.47 (*s*)
		3.79 (*dd*, *J* = 7.2, 10.8)				
17	16.0	1.34 (*d*, *J* = 7.2)	30.2	1.69 (*s*, overlapped)	30.7	1.51 (*s*)
18	26.9	1.81 (*s*, overlapped)	30.0	1.45 (*s*)	29.1	1.36 (*s*)
19	26.8	1.81 (*s*, overlapped)	30.0	1.39 (*s*)	29.9	1.30 (*s*)
20	19.3	2.81 (*s*)	17.9	2.49 (*s*)	18.6	2.40 (*s*)

[a] δ in ppm, *J* in Hz. ^{1}H-NMR: 600 MHz, ^{13}C-NMR: 150 MHz.



1 **2** **3**

Figure 2. Key ^{1}H,^{1}H-COSY (–), HMBC (H→C) and ROESY (H↔H) data of compounds **1**–**3**.

Compound **2** was obtained as yellow amorphous powder, and had a molecular formula $C_{20}H_{24}O_4$ with nine degrees of unsaturation due to analysis of its positive ion mode HR-ESI-MS (*m/z* 351.1565 $[M+Na]^+$). The ^{1}H-NMR spectrum (*Table 1*) of **2** indicated presence of an AB coupling system of two aromatic protons at δ(H) 7.16 (*d*, *J* = 8.3, H-6, 1H) and 7.64 (*d*, *J* = 8.3, H-7, 1H), five singlet methyl signals at δ(H) 1.69 (6H, *s*, 2 Me), 1.45 (3H, *s*), 1.39 (3H, *s*) and 2.49 (3H, *s*), and two oxygenated proton signals at δ(H) 5.21 (1H, br. *d*, *J* = 1.9) and 4.55 (br. *s*), two proton signals for one methylene group at δ(H) 2.47 (1H, *d*, *J* = 14.0) and 2.37 (1H, *d*, *J* = 14.0). The ^{13}C-NMR spectra (*Table 1*) displayed 20 carbon resonances due to 9 quaternary carbons (δ(C) 82.5, 134.9, 127.2, 122.0, 123.2, 137.0, 140.7, 137.6, 74.7), five methine resonances (δ(C) 74.8, 80.5, 127.6, 129.3, 116.9), one methylene (δ(C) 47.0) and five methyl groups (δ(C) 29.4, 30.2, 30.0, 30.0, 17.9). A careful analysis of the HSQC, ^{1}H,^{1}H-COSY, and HMBC spectroscopic data suggested **2** also to be a 4,5-*seco*-20(10→5)-*abeo*-abietane derivative, and its ^{1}H- and ^{13}C-NMR data exhibited great similarity to those of known compound salprionin.[6] Compared with those of salprionin, ^{13}C-NMR of **2** demonstrated an additional O-bearing quaternary carbon atom at δ(C) 74.7, instead of the methine carbon at C-15 position in salprionin. Moreover, the ^{13}C chemical shifts of C-16 and C-17 of **2** were shifted downfield to δ(C) 29.4 and

30.2. These evidences disclosed that compound **2** is a 15-hydroxylated derivative of salprionin. Key HMBCs of Me-16 (δ(H) 1.69), Me-17 (δ(H) 1.69), and H-14 (δ(H) 7.41) with C-15 (δ(C) 74.7) confirmed the above deduction. The ^{13}C-NMR chemical shifts of C-1 (δ(C) 74.8 (*d*)), C-4 (δ(C) 82.5 (*s*)), C-2 (δ(C) 80.5), and C-11 (δ(C) 137.0), along with the ^{1}H,^{1}H-COSY correlations of H-1 (δ(H) 5.21 (br. *d*, $J=1.9$))/H-2 (δ(H) 4.55 (br. *s*)) and of H-2/H-3 (δ(H) 2.37), revealed that two epoxy rings were formed between C-1 and C-4, and between C-2 and C-11, respectively. Observation of the HMBCs from H-14 (δ(H) 7.41 (*s*)) to C-12 (δ(C) 140.7 (*s*)) permitted the assignment of a hydroxy group at C-12. The HMBCs between the methyl at δ(H) 2.49 and C-5, C-6 and C-10 attached the methyl at C-5 position. The relative configurations of H-1 and H-2 of **2** were suggested to be *cis* due to the small coupling constant between H-1 and H-2 ($J=1.9$) and key ROESY correlations of H-1 (δ(H) 5.21) and H-2 (δ(H) 4.55) (*Figure 2*). Considering the structural resemblances of compound **2** and salprionin[5] and their quite close NMR data except for those of C-13, C-15, C-16, and C-17, from biogenetic view, the relative configurations of H-1 and H-2 were proposed to be β-configuration. Consequently, the structure of **2** was established as 15-hydroxysalprionin.

Compound **3** was obtained as amorphous powder. Its molecular formula was established to be $C_{20}H_{24}O_5$ by positive ion mode HR-ESI-MS (*m/z* 367.1519 $[M+Na]^+$). Comparison of the spectroscopic data of **3** with those of **2** revealed that they were quite similar, except for the moiety at C-11 and C-12. Observation of the presence of a hemiketal carbon at C-11 (δ(C) 91.2 (*s*)) and at ketone C=O at C-12 (δ(C) 197.3 (*s*)), instead of two olefinic carbon atoms (δ(C) 140.7 (*s*) and 137.6 (*s*)) in the ^{13}C-NMR spectrum of **2**, implied that compound **3** should be an oxidized product of **2** with an 11-hydroxy-12-one moiety. The HMBCs observed from H-14 (δ(H) 7.45 (*s*)) to C-12, together with the IR spectrum of which showed hydroxy absorption (3427 cm^{-1}) and an α,β-unsaturated ketone carbonyl group (1691 and 1605 cm^{-1}), confirmed the presence of 11-hydroxy-12-one. The other structure moiety of **3** was characterized to be identical with that of **2** on the basis of HMBCs of Me-16 (δ(H) 1.47), Me-17 (δ(H) 1.51), and H-14 with C-15 (δ(C) 72.1), of H-1 (δ(H) 4.80) with C-9 (δ(C) 135.2) and C-10 (δ(C) 132.7), of H-3 with C-1 (δ(C) 72.3), and of Me-18 with C-1. The small coupling constant between H-1 (δ(H) 4.80 (*d*)) and H-2 ($J=2.9$), in combined with key ROESY correlation between H-1 and H-2 (*Figure 2*), indicated the presence of *cis* configuration of H-1 and H-2. Considering that compound **3** was biogenetically derived from **2**, the relative configurations of H-1 and H-2 were proposed to be the same β-oriented as **2**. The relative configuration of 11-hydroxy could not be determined because of lacking direct ROESY correlation. Compared with compound **2**, the chemical shift of H-2 was shifted downfield from δ(H) 4.55 in **2** to δ(H) 4.98–4.99 in **3**, while the chemical shift of C-2 was shifted highfield from δ(C) 80.5 to δ(C) 74.7. These differences could be explained by inducting and γ-gauche effects of 11-hydroxy on H-2 and C-2, respectively, if the 11-hydroxy took the same β-orientation as H-2. Therefore, compound **3** was determined as 11β,15-dihydroxysalprionin-12-one.

Also, ten known compounds were isolated from the same plant, and their structures were identified as abieta-8,11,13-triene-14,19-diol (**4**),[4] crossogumerin C (**5**),[7] 3α-hinokiol (**6**),[8] trilobinone (**7**),[9] hinokiol (**8**),[10] 16-acetoxyhorminone (**9**),[11,12] 6β,7α-dihydroxyroyleanon (**10**),[13] royleanone (**11**),[14,15] 12,16-epoxy-8,11,13-abietatriene (**12**),[16] and hispidanol A (**13**),[17] respectively, by comparing their spectroscopic data with those in the literatures.

Anti-Inflammatory Activity

Compounds **1**–**13** isolated from *I. lophanthoides* var. *graciliflorus* were estimated for TNF-α inhibitory effects on LPS-induced RAW264.7 cells with Dexamethasone as the positive control (*Table 2*). All compounds exhibited better TNF-α inhibitory effects on LPS-induced RAW264.7 cells than that of the positive control. Compound **9** was the most potent with an IC_{50} value of 3.97 ± 0.70 μM. Among them, compounds **1** and **5**–**12** exhibited significant anti-inflammatory activities with $IC_{50} < 10.0$ μM. Compounds **9**, **10**, and

Table 2. Anti-inflammatory activities of compounds **1**–**13**.

Sample	IC_{50} [μM][a]	Sample	IC_{50} [μM][a]
1	7.07 ± 1.20	8	8.54 ± 1.16
2	28.92 ± 3.32	9	3.97 ± 0.70
3	26.12 ± 1.39	10	4.22 ± 0.31
4	31.33 ± 2.58	11	6.65 ± 0.25
5	8.06 ± 1.39	12	9.19 ± 0.56
6	9.10 ± 1.09	13	53.99 ± 4.23
7	6.63 ± 0.47		
Dexamethasone[b]	509.60 ± 26.53		

[a] The data represent the means ± SD ($n=3$) from three independent experiments. [b] Positive control.

11 exhibited remarkable TNF-α inhibitory effects on LPS-induced RAW264.7 cells may be due to their *para*-quinone structure, while *para*-quinone structure showed weak cytotoxic activities against RAW264.7 cell lines with $CC_{50} > 200$ μM. Compared the anti-inflammatory activities of abietane diterpenoids, sempervirane diterpenoid, hispidanol A (**13**), exhibited weak activity with the IC_{50} value of 53.99 ± 4.23 μM.

Conclusions

In summary, 12 abietane diterpenoids including three new 4,5-*seco*-20(10→5)-*abeo*-abietane diterpenoids and one sempervirane diterpenoid were isolated from *I. lophanthoides* var. *graciliflorus*. Twelve abietane diterpenoids were classified into two groups: abietane and 4,5-*seco*-abietane. Abietane group is widely distributed in the genus *Isodon*, and nine abietane compounds isolated are divided into two subgroups, depending on the oxidation of the C ring. One subgroup is the oxidation and dehydrogenation of C ring to give the new aromatic C ring, the other group is the oxidation of C ring to get the *para*-quinone C ring. The abietane analogs with *para*-quinone C ring showed remarkable TNF-α inhibitory effects on LPS-induced RAW264.7 cells. However, *para*-quinone C ring also exhibited weak cytotoxic activities against RAW264.7 cell lines.

Experimental Section

General

NMR spectra were recorded on a Bruker AM-400 spectrometer, a Bruker DRX-500 spectrometer, and a Bruker Avance III-600 spectrometer in CD_3COCD_3 or CD_3OD with TMS as internal standard. Mass spectra were taken on a VG Auto spec-3000 spectrometer or on a Finnigan MAT 90 instrument. Optical rotations were measured with a PerkinElmer model 241 polarimeter. Ultraviolet absorption spectra were recorded on a Shimadzu UV-2401PC spectrophotometer. IR spectra were scanned using a Bio-Rad Tensor27 spectrometer as KBr pellets. Materials for column chromatography were silica gel (200–300 mesh; Qingdao Marine Chemical Inc.), Sephadex LH-20 (40–70 μm; Merck Co., Ltd.), and RP-18 (40–60 μm; YMC, Milford, MA). The positive control was Dexamethasone (water-soluble; Sigma Chemical Co., St. Louis, Missouri). Spots were visualized under UV light (254 nm) or by spraying with 10% H_2SO_4 in 95% EtOH (v/v) followed by heating.

Plant Material

The aerial parts of *I. lophanthoides* var. *graciliflorus* (Benth.) H.Hara were collected from Guangzhou city, Guangdong Province, P. R. China, in May 2015. Identification of the plant was performed by Dr. Li–Li Huang of Hong Kong Baptwast University. A voucher specimen (LLW 0028) was deposited with Hong Kong Baptwast University.

Extraction and Isolation

Powder (12 kg) of the dried aerial parts was extracted with acetone (30 L×4) at room temperature. Filtration and condensation of the solution to dryness under vacuum yielded an extract (400 g). The extract was dissolved in distilled water (3 L) and then fractionated with ethyl acetate and BuOH, respectively. The ethyl acetate part (354 g) was subjected to silica gel column chromatograph (PE/CP, 100:0 to 0:100 (v/v)) to give ten fractions (A_1–A_{10}).

Fraction A_3 (40 g) was initially subjected to MCI gel CC (eluted by MeOH/H_2O, 90:10 v/v) to remove the pigment, then separated by silica gel CC (PE/CP, 90:10 to 50:50 (v/v)) to get five subfractions (B_1–B_5). Subfraction B_1 was chromatographed on silica gel (200–300 mesh) eluted with PE/CP (20:1) to afford two subfractions ($B_{1\text{-}1}$, $B_{1\text{-}2}$). Subfraction $B_{1\text{-}1}$ was purified by Sephadex LH-20 (PE/CP, 1:1 (v/v)) to yield **10** (21.9 mg). Subfraction $B_{1\text{-}2}$ was purified on semi-preparative HPLC eluted with MeOH/H_2O (65:35 (v/v)) to get **9** (7.6 mg). Subfraction B_2 was purified by Sephadex LH-20 (PE/CP, 1:1, (v/v)) and semipreparative HPLC (MeOH/H_2O, 75:25 (v/v)) to obtain **11** (4.2 mg) and **12** (2.6 mg).

The pigment of fraction A_4 (25 g) was removed by MCI gel, then separated by silica gel CC (PE/CP, 100:0 to 0:100 (v/v)) to give four subfractions (A_4–$A_{4\text{-}4}$). Subfraction $A_{4\text{-}1}$ was subjected to silica gel CC (PE/EAC, 30:1 to 1:1 (v/v)) to give three subfractions ($A_{4\text{-}1\text{-}1}$–$A_{4\text{-}1\text{-}3}$). Subfraction $A_{4\text{-}1\text{-}1}$ afforded **2** (2.8 mg) and **3** (7.3 mg) by semipreparative HPLC (MeOH/H_2O, 75:25 (v/v)). Subfraction $A_{4\text{-}1\text{-}2}$ was separated by semipreparative HPLC (MeOH/H_2O, 75:25 (v/v)), then purified by Sephadex LH-20 (PE/CP, 1:1 (v/v)) to obtain **1** (4.2 mg). Subfraction $A_{4\text{-}1\text{-}3}$ was subjected to silica gel CC (PE/AcOEt, 5:1 (v/v)), then purified by Sephadex LH-20 (MeOH/$CHCl_3$, 1:1, (v/

v)) and semipreparative HPLC (MeOH/H_2O, 75:25 (v/v)) to give **13** (16 mg), **6** (6.5 mg), respectively. Subfraction $A_{4\text{-}2}$ was isolated over silica gel CC (PE/AcOEt, 15:1 to 1:1 (v/v)) and further in a state of separation through Sephadex LH-20 (MeOH/$CHCl_3$, 1:1 (v/v)) to furnish **4** (60.5 mg), **5** (20.1 mg), **8** (86.4 mg). Subfraction $A_{4\text{-}3}$ afforded **7** (2.2 mg) by semipreparative HPLC (MeOH/H_2O, 75:25 (v/v)).

16-Hydroxysalvilenone (**1**). Light amorphous powder. $[\alpha]_D^{25} = +2.22$ ($c = 0.21$, MeOH). UV (MeOH, λ_{max} (log ε)): 213 (4.21), 237 (4.27) nm. IR (KBr): 3421, 2971, 2929, 1726, 1670, 1605, 1563, 1526, 1441, 1373, 1335, 1229, 1086, 813, 759 cm^{-1}. ^{1}H- and ^{13}C-NMR: see *Table 1*. HR-ESI-MS: 309.1488 ($[M+H]^+$, $C_{20}H_{21}O_3^+$; calc. 309.1485).

15-Hydroxysalprionin (**2**). Yellow amorphous powder. $[\alpha]_D^{25} = -8.2$ ($c = 0.13$, MeOH). UV (MeOH, λ_{max} (log ε)): 215 (4.37), 242 (4.49) nm. IR (KBr): 3430, 2972, 2933, 1709, 1376, 1263, 1171, 1137, 1058, 1031 cm^{-1}. ^{1}H- and ^{13}C-NMR: see *Table 1*. HR-ESI-MS: 351.1565 ($[M+Na]^+$, $C_{20}H_{24}O_4Na^+$, calc. 351.1567).

11β,15-Dihydroxysalprionin-12-one (**3**). Yellow amorphous powder. $[\alpha]_D^{25} = -5.7$ ($c = 0.28$, MeOH). UV (MeOH, λ_{max} (log ε)): 203 (4.11), 243 (4.08) nm. IR (KBr): 3427, 2971, 1691, 1605, 1442, 1379, 1367, 1173, 1055, 1011, 914, 813 cm^{-1}. ^{1}H- and ^{13}C-NMR: see *Table 1*. HR-ESI-MS: 367.1519 ($[M+Na]^+$, $C_{20}H_{24}O_5Na^+$, calc. 367.1516).

Anti-Inflammatory Assay

The anti-inflammatory activities of compounds were evaluated on cell model of inflammation induced by lipopolysaccharide. RAW264.7 cells in logarithmic growth stage at a concentration of 1×10^5 cells/mL were inoculated into 96-well plate with 100 μL per well. After cell adherence, 1 μg/mL of lipopolysaccharide (LPS) and various gradient concentrations (200, 40, 8, 1.6, 0.32 μg/mL) of compounds were added respectively in triplicate. Cell culture medium was used as the blank control and Dexamethasone was used as the positive control. After 24 h of culture, the cell culture supernatants were collected, and the levels of TNF-alpha (TNF-α) were detected by ELISA kit. The 50% inhibitory concentration (IC_{50}) on the production of TNF-α was calculated.

Cytotoxicity Assay

MTT was used to assess the cytotoxicity of compounds on RAW264.7 cells. Briefly, cells at a concentration of 1×10^5 cells/mL were inoculated into 96-well plate with 100 μL per well. After cell adherence, various gradient concentrations (200, 40, 8, 1.6, 0.32 μg/mL) of compounds were added respectively in triplicate. Cells were incubated for 24 h at 37 °C, in a 5% CO_2-humidified incubator. Then, MTT (5 mg/mL in PBS) was added to each well. After incubating for 4 h, 100 μl of DMSO was added, and the plate was incubated at 37 °C overnight. The plate was read on a Bio-Tek Elx 800 ELISA reader at 570 nm. The 50% cytotoxic concentration (CC_{50}) was calculated.

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Author Contribution Statement

Dr. L.-L. Wong contributed in the collection and identification of plant material. Dr. X. Zhang contributed to the biological study and to the writing of the biological part. W.-F. Chen did the structural isolation of compounds. F. Xia participated in the structural elucidation of compounds. F. -X. Zhou performed the auxiliary experiments. Dr. L.-P. Tang and Dr. X. Li were the supervisor of the present work, checked the structure determination of the isolated compounds and the biological study, and completed the redaction of the article. All authors approved the final version for publication.

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