Fitoterapia 142 (2020) 104521

Contents lists available at ScienceDirect

Fitoterapia

journal homepage: www.elsevier.com/locate/fitote

Vasorelaxant 4,5-*seco*-abietane diterpenoids with diverse 6/6/6, 6/6/7, and 6/6/8 architectures from *Salvia prattii* Hemsl.

Fan Xia[a,b,c,1], Dan Luo[a,b,1], Tingting Wang[a], Xu Ji[a,*], Gang Xu[b,*]

[a] Key Laboratory of Medicinal Chemistry for Natural Resource, Ministry of Education and Yunnan Province, School of Chemical Science and Technology, Yunnan University, Kunming 650091, Yunnan, People's Republic of China

[b] State Key Laboratory of Phytochemistry and Plant Resources in West China, Kunming Institute of Botany, Chinese Academy of Sciences, Kunming 650201, Yunnan, People's Republic of China

[c] University of Chinese Academy of Sciences, Beijing 100049, People's Republic of China

ARTICLE INFO

Keywords:
Salvia prattii
4,5-*seco*-Abietane diterpenoid
Vasorelaxant activity

ABSTRACT

Salpratins A–D (**1**–**4**), four new 4,5-*seco*-abietane diterpenoids, along with twelve known analogues, featuring diverse 6/6/6, 6/6/7, and 6/6/8 rings system, were isolated from *Salvia prattii* Hemsl. Particularly, salpratin A is the first example of 4,5;12,13-bis-*seco*-abietane diterpenoid features with a 5/6/6/6 ring system. Their structures were determined by analyses of comprehensive NMR and MS spectroscopic data and single-crystal X-ray diffractions. In addition, compounds **1**, **3**, **4**, **6**, **7**, **8** and **14** showed potent vasorelaxant activity on endothelium-intact thoracic aorta rings precontracted with KCl.

1. Introduction

The name of the genus, *Salvia*, is derived from the Latin *salvare* (to heal or to be safe), making reference to the curative properties of the plants which were used as medicinal herbs throughout the world [1,2]. *S. miltiorrhiza* Bunge (Danshen or Tanshen in Chinese) is one of the most popular herbal traditional medicines in Asian countries and has been used extensively for the treatment of cardiovascular diseases (such as coronary artery diseases, angina pectoris), various type of hepatitis, chronic renal failure, and dysmenorrhea [1]. Investigated *Salvia* plants have produced an array of secondary metabolites, mainly abietane and clerodane types of diterpenoid, as well as polyphenol. Some of them, such as tanshinone IIA and salvinorin A, have attracted considerable attention from the chemical and biological communities for their broad spectrum of biological activities and novel structure [1]. Of the abietane diterpenoid, 4,5-*seco*-abietane diterpenoid constitutes a small but interesting group that originates from the ring cleavage of C-4/5 of the core abietane skeleton accompany with the immigration of Me-20 from C-10 to C-5 position, and they tend to form new 6/6/5, 6/6/6, 6/6/7, and 6/6/8 rings [1]. To date, about 55 naturally occurring 4,5-*seco*-abietane diterpenoids have been reported form nineteen plants of the genus *Salvia* [1,3–9]. In which, salvicine, the representative one, significantly inhibited the proliferation and growth of various solid tumours, including lung, gastric, liver, colonic, ovarian and cervical cancers, with better efficacy profiles than positive controls such as vincristine and etoposide [10].

We have focused on the diterpenoid constituents from the *Salvia* plants growing in southwest China for more than 10 years and discovered many diterpenoids with intriguing chemical structures and extensive biological activities [11–14]. In the course of our ongoing search for interesting secondary metabolite, *Salvia Prattii* Hemsl., an alternative of “Danshen” is widely utilized in traditional Tibetan medicines, was investigated in depth [15]. As a result, salpratins A–D (**1**–**4**), four new 4,5-*seco*-abietane diterpenoids, along with twelve known analogues, featuring diverse 6/6/6, 6/6/7, and 6/6/8 rings system (Fig. 1), were isolated. Their structures were determined by analyses of comprehensive NMR and MS spectroscopic data and single-crystal X-ray diffractions. It's noteworthy that salpratin A is the first example of 4,5;12,13-bis-*seco*-abietane diterpenoid features with a 5/6/6/6 ring system [16,17]. What's more, compounds **1**, **3**, **4**, **6**, **7**, **8**, and **14** showed potent vasorelaxant activity on endothelium-intact thoracic aorta rings precontracted with KCl, which can be seen as the first report of vasorelaxant activity of 4,5-*seco*-abietane diterpenoid. Herein, we describe the isolation, structural elucidation, and biological activity of these compounds.

* Corresponding authors.
E-mail addresses: jixu@ynu.edu.cn (X. Ji), xugang008@mail.kib.ac.cn (G. Xu).
[1] These authors contributed equally to this work.

https://doi.org/10.1016/j.fitote.2020.104521
Received 16 January 2020; Received in revised form 17 February 2020; Accepted 19 February 2020
Available online 21 February 2020
0367-326X/

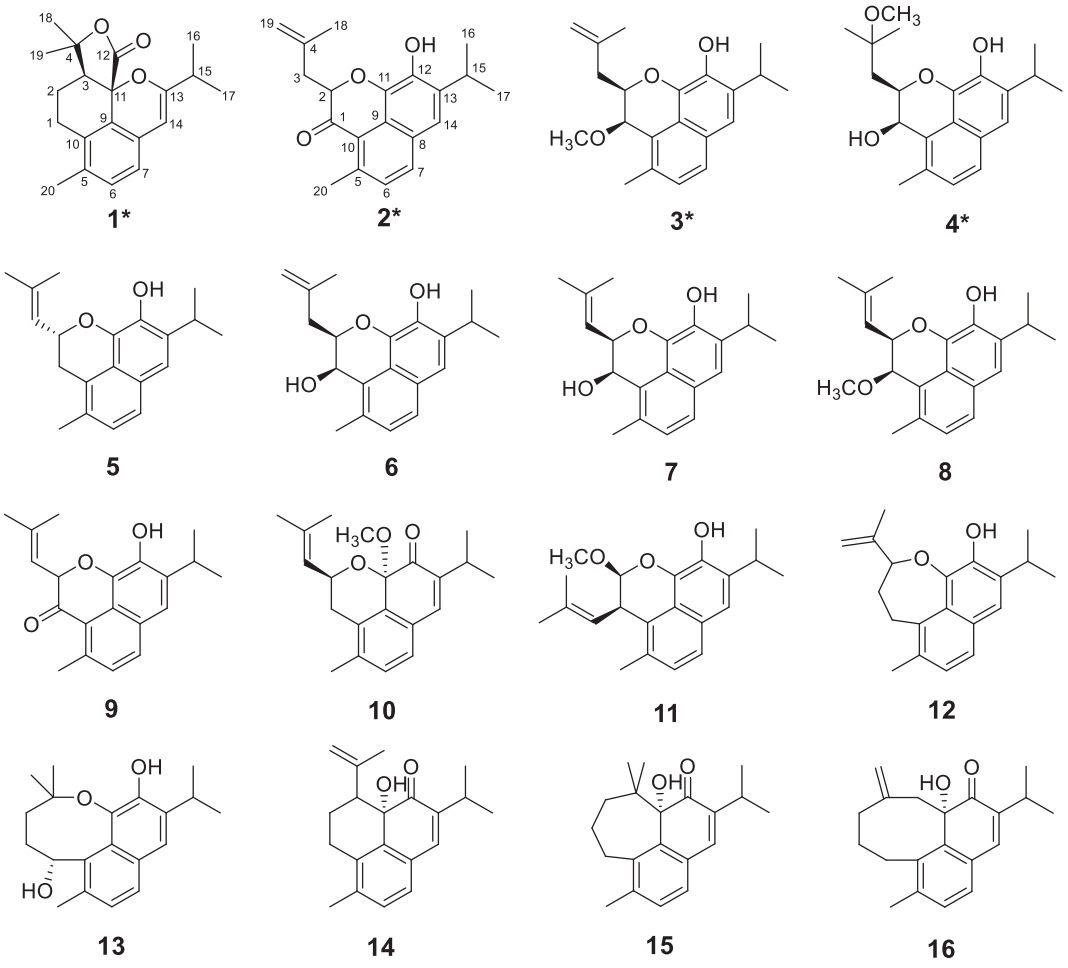

Fig. 1. Structures of **1–16** isolated from *Salvia prattii*.

2. Experimental

2.1. General experimental procedures

^{1}H, ^{13}C and 2D-NMR spectra were recorded on Bruker DRX-600 and DRX-800 spectrometer using TMS as an internal standard. Unless otherwise specified, chemical shifts (δ) are expressed in ppm with reference to the solvent signals. HRESIMS and HREIMS data were acquired on Agilent G6200 TOF mass spectrometer and Waters Autospec Premier P776 mass spectrometer, respectively. Optical rotations were measured on a Jasco P-1020 polarimeter. UV spectra were recorded on a Shimadzu UV-2401PC spectrometer. IR spectra were recorded on a Bruker FT-IR Tensor-27 infrared spectrophotometer with KBr disks. X-ray data were generated using a Bruker Apex Duo instrument. Silica gel (100–200 and 200–300 mesh, Qingdao Marine Chemical Co., Ltd., Qingdao, China), MCI gel (75–150 μm, Mitsubishi Chemical Corporation, Tokyo, Japan), and Amphichroic reversed-phase C-18 column (40–63 μm, Merck, Darmstadt, Germany) were used for column chromatography. Semi-preparative HPLC was performed on an Agilent 1100 HPLC with a Zorbarx SB-C_{18} (9.4 × 250 mm) column. Fractions were monitored by TLC (GF 254, Qingdao Marine Chemical Co., Ltd., Qingdao, China), and spots were visualized by heating silica gel plates immersed in 10% H_2SO_4 in ethanol.

2.2. Plant material

The root parts of *S. prattii* were collected in Zuogong prefecture Tibet, People's Republic of China, in July 2011. The plant was identified by Dr. Yu-Kun Wei, Shanghai Chenshan Plant Science Research Center, Chinese Academy of Sciences. A voucher specimen was deposited in Kunming Institute of Botany, Chinese Academy of Sciences with identification number 20110712.

2.3. Extraction and isolation

The root parts of the air-dried *S. prattii* (33.0 kg) were powdered and percolated with acetone at room temperature and filtered. The filtrate was evaporated in vacuo to be concentrated. The crude extract (1.6 kg) was subjected to a silica gel column chromatography eluted with $CHCl_3$ to afford a diterpenoid-rich fraction (730.0 g). This fraction was then chromatographed on a silica gel column, eluted with petroleum ether/acetone (from 500:1 to 0:1), to yield five fractions (Fr. A–E). Fraction B (223.0 g) was separated over a MCI gel column (MeOH-H_2O from 70:30 to 100:0) to obtain eight fractions (Fr. B1–B8). Fraction C (150.0 g) was separated over a MCI gel column (MeOH-H_2O from 60:40 to 100:0) to produce ten fractions (Fr. C1–C10).

Fraction B4 (16.7 g) was separated over a reversed-phase C-18 gel column (MeOH-H_2O from 80:15 to 100:0) to obtain nine fractions (Fr. B4a–B4i). Fr. B4f (3.2 g) was chromatographed on a silica gel column, eluted with petroleum ether-ethyl acetate (from 300:1 to 50:1), to yield six fractions (B4f-1–B4f-6). Fr. B4f-1 (560.0 mg) was purified by repeated silica gel columns and semi-preparative HPLC (90% MeCN-H_2O) to afford **5** (48.0 mg), and **12** (10.3 mg). Fr. B4h (0.8 g) was chromatographed on a silica gel column, eluted with petroleum ether-acetone (from 200:1 to 20:1), to yield six fractions (Fr. B4h-1–B4h-6). Fr. B4h-1 (14.0 mg) was purified by semi-preparative HPLC (85% MeOH-H_2O) to

afford **10** (2.8 mg). Fr. B4h-2 (85.0 mg) was purified by semi-preparative HPLC (80% MeCN-H_2O) to afford **3** (2.5 mg) and **15** (5.0 mg). Fr. B4h-6 (23.0 mg) was purified by semi-preparative HPLC (80% MeCN-H_2O) to afford **8** (6.7 mg). Fr. B4i (340.0 mg) was separated on a silica gel column, eluted with petroleum ethyl acetate (from 100:1 to 10:1), to yield four fractions (B4i-1–B4i-4). Fr. B4i-2 (56.0 mg) and Fr. B4i-3 (78.0 mg) were purified by silica gel column and semi-preparative HPLC (75% MeOH-H_2O) to afford **6** (2.8 mg) and **7** (5.0 mg), respectively.

Fraction B5 (79.0 g) was separated over a reversed-phase C-18 gel column (MeOH-H_2O from 75:25 to 100:0) to obtain five fractions (Fr. B5a–B5e). Fr. B5c (25.4 g) was chromatographed on a silica gel column, eluted with petroleum ether-ethyl acetate (from 200:1 to 20:1), to yield six fractions (B5c-1–B5c-6). Fr. B5c-2 (10.5 g) was separated over a reversed-phase C-18 column (MeOH-H_2O from 70:30 to 100:0) to obtain eight fractions (Fr. B5c-2a–B5c-2 h). Fr. B5c-2a (50.0 mg), Fr. B5c-2b (60.0 mg), and Fr. B5c-2c (90.0 mg) were purified by silica gel column and semi-preparative HPLC (80% MeCN-H_2O) to afford **14** (16.0 mg), **9** (16.0 mg), and **16** (4.0 mg), respectively. Fr. B5c-3 (3.5 g) was chromatographed on a silica gel column, eluted with petroleum ether-acetone (from 200:1 to 20:1), to yield eleven fractions (B5c-3a–B5c-3 k). Fr. B5c-3f (126.0 mg) was purified by repeated silica gel columns and semi-preparative HPLC (80% MeCN-H_2O) to afford **2** (3.0 mg).

Fraction B6 (22.0 g) was separated over a reversed-phase C-18 gel column (MeOH-H_2O from 70:30 to 100:0) to obtain ten fractions (Fr. B6a–B6j). Fr. B6h (520.0 mg) was chromatographed on a silica gel column, eluted with petroleum ether-ethyl acetate (from 100:1 to 10:1), to yield five fractions (B6h-1–B6h-5). B6h-2 (80.0 mg) and B6h-4 (100.0 mg) were purified by silica gel column and semi-preparative HPLC (70% MeCN-H_2O) to afford **1** (6.0 mg) and **11** (15.0 mg), respectively.

Fraction C5 (6.7 g) was chromatographed on a silica gel column, eluted with petroleum ether-acetone (from 100:1 to 20:1), to yield twelve fractions (C5a–C5l). Fr. C5h (800.0 mg) was purified by repeated silica gel columns and semi-preparative HPLC (75% MeCN-H_2O) to afford **4** (1.2 mg) and **13** (3.0 mg).

Salpratin A (**1**): colorless crystal; mp 102–103 °C; $[\alpha]$ $[22]_D$ + 80.8 (*c* 0.12, MeOH); UV (MeOH) λ_{max} (log ε) 202 (4.17), 216 (4.14), 238 (4.21), 291 (3.75) nm; IR (KBr) ν_{max} 2967, 2933, 1773, 1482, 1272, 1230, 1149, 986, 902, 833 cm^{-1}; ^1H and ^{13}C NMR data, see Tables 1 and 2; HREIMS *m/z* 312.1719 [M] + (calcd for $C_{20}H_{24}O_3$, 312.1725).

Crystal data for salpratin A (**1**): $C_{20}H_{24}O_3$, M = 312.39, orthorhombic, a = 6.57910(10) Å, b = 11.7349(2) Å, c = 21.6100(4) Å, α = 90.00°, β = 90.00°, γ = 90.00°, V = 1668.40(5) $Å^3$, T = 100(2) K, space group $P212121$, Z = 4, μ(CuKα) = 0.653 mm^{-1}, 8939 reflections measured, 2890 independent reflections (R_{int} = 0.0403). The final R_1 values were 0.0384 ($I > 2\sigma(I)$). The final w$R(F^2)$ values were 0.1030 ($I > 2\sigma(I)$). The final R_1 values were 0.0386 (all data). The final $wR(F^2)$ values were 0.1031 (all data). The goodness of fit on F^2 was 1.113. Flack parameter = 0.2(2). The Hooft parameter is 0.06(07) for 1165 Bijvoet pairs.

Salpratin B (**2**): yellow gum; $[\alpha]$ $[23]_D$ − 6.9 (*c* 0.14, MeOH); UV (MeOH) λ_{max} (log ε) 194 (4.33), 223 (4.56), 264 (4.18), 346 (3.48), 399 (3.40) nm; IR (KBr) ν_{max} 3429, 2962, 2927, 1685, 1635, 1433, 1369, 1178, 1029 cm^{-1}; ^1H and ^{13}C NMR data, see Tables 1 and 2; HRESIMS *m/z* 309.1490 $[M - H]^-$ (calcd for $C_{20}H_{21}O_3$, 309.1496).

Salpratin C (**3**): colorless gum; $[\alpha]_D^{25}$ − 12.0 (*c* 0.21, MeOH); UV (MeOH) λ_{max} (log ε) 194 (4.23), 218 (4.36), 247 (4.51), 311 (3.50), 339 (3.41) nm; IR (KBr) ν_{max} 3432, 2961, 2928, 1636, 1435, 1174, 1096, 584 cm^{-1}; ^1H and ^{13}C NMR data, see Tables 1 and 2; HRESIMS *m/z* 349.1775 $[M + Na]^+$ (calcd for $C_{21}H_{26}O_3Na$, 349.1774).

Salpratin D (**4**): colorless gum; $[\alpha]$ $[19]_D$ − 41.9 (*c* 0.07, MeOH); UV (MeOH) λ_{max} (log ε) 198 (3.88), 218 (4.11), 246 (4.31), 300 (3.26) nm; IR (KBr) ν_{max} 3382, 2964, 2924, 2852, 1735, 1635, 1455, 1368, 1177, 1080, 1016 cm^{-1}; ^1H and ^{13}C NMR data, see Tables 1 and 2; HRESIMS *m/z* 367.1883 $[M + Na]^+$ (calcd for $C_{21}H_{28}O_4Na$, 367.1880).

Table 1
The ^1H NMR data of compounds **1–4** in acetone-d_6 (δ in ppm and *J* in Hz).

No.	1[a]	2[a]	3[a]	4[b]
1	2.72, m 2.59, m		4.68, d (1.5)	4.95, dd (6.9, 1.4)
2	2.16, m 1.65, m	4.91, dd (6.3, 7.8)	4.99, m	4.87, m
3	2.62, m	2.51, m	2.04, overlap	1.46, m 1.40, m
6	7.06, d (7.6)	7.29, d (8.3)	7.15, d (8.4)	7.14, d (8.4)
7	6.84, d (7.6)	8.00, d (8.3)	7.64, d (8.4)	7.62, d (8.4)
14	5.75, s	7.48, s	7.26, s	7.26, s
15	2.40, sept (6.9)	3.46, sept (7.0)	3.40, sept (6.9)	3.43, sept (6.9)
16	1.12, d (6.9)	1.33, d (7.0)	1.30, d (6.9)	1.33, d (6.9)
17	1.10, d (6.9)	1.34, d (7.0)	1.29, d (6.9)	1.30, d (6.9)
18	1.81, s	4.84, s 4.67, s	4.77, s 4.48, s	1.26, s
19	1.46, s	1.83, s	1.81, s	1.12, s
20	2.20, s	2.76, s	2.47, s	2.52, s
1-OH				4.26, d (6.9)
1-OCH_3			3.36, s	
4-OCH_3				3.11, s
12-OH		7.94, s	7.40, s	7.38, s

[a] ^1H NMR was recorded at 600 MHz, [b] ^1H NMR was recorded at 800 MHz.

Table 2
The ^{13}C NMR data of compounds **1–4** in acetone-d_6 (δ in ppm and *J* in Hz).

No.	1[a]	2[a]	3[a]	4[b]
1	24.6, CH_2	194.6, C	74.5, CH	67.7, CH
2	24.0, CH_2	81.3, CH	75.2, CH	78.3, CH
3	47.4, CH	40.1, CH_2	39.7, CH_2	41.4, CH_2
4	85.0, C	141.5, C	142.5, C	74.3, C
5	122.0, C	139.8, C	133.8, C	132.8, C
6	130.8, CH	128.8, CH	127.1, CH	127.3, CH
7	122.2, CH	134.6, CH	128.2, CH	127.7, CH
8	131.2, C	127.2, C	127.0, C	127.2, C
9	134.3, C	123.6, C	120.7, C	120.7, C
10	135.5, C	120.4, C	123.1, C	126.3, C
11	82.0, C	133.5, C	133.4, C	133.8, C
12	170.6, C	143.1, C	140.9, C	141.0, C
13	158.4, C	138.7, C	137.5, C	137.5, C
14	101.8, CH	118.3, CH	116.4, CH	116.3, CH
15	32.6, CH	28.4, CH	28.4, CH	28.4, CH
16	20.2, CH_3	22.7, CH_3	23.0, CH_3	23.2, CH_3
17	20.3, CH_3	22.7, CH_3	22.7, CH_3	22.6, CH_3
18	29.8, CH_3	114.3, CH_2	113.6, CH_2	25.9, CH_3
19	24.9, CH_3	22.5, CH_3	22.5, CH_3	25.1, CH_3
20	19.3, CH_3	22.1, CH_3	18.2, CH_3	17.8, CH_3
1-OCH3			55.8	
4-OCH_3				49.3

[a] ^{13}C NMR was recorded at 125 MHz, [b] ^{13}C NMR was recorded at 200 MHz.

2.4. Vasorelaxant effect assay

Adult male SD rats (250–300 g) were kept in an animal room (Accreditation No. SYXK K2013–0004) with a constant temperature of 22 ± 2 °C, a humidity of 60 ± 5% and had free access to food and water. All animals were cared for compliance with the National Institutes of Health guide for the care and use of Laboratory animals (NIH Publications No. 8023, revised 1978). All experimental procedures were pre-approved by the Experimental Animal Ethic Committee of Kunming Institute of Botany, Chinese Academy of Sciences.

Vasorelaxant effects of 100 μmol/L of the compounds were evaluated on endothelium-intact thoracic aorta rings precontracted with KCl. Nifedipine, a calcium channel blocker, was used as a positive

control. Rat aortic rings were prepared according to that described [18,19]. Aortic rings were mounted on stainless steel hooks in organ baths containing 37 °C Krebs solution continuously bubbled with 95% O_2 and 5% CO_2, then equilibrated for 60 min under a resting tension of 1.5 g. After equilibration, the vessels were exposed to 1 μmol/L phenylephrine, followed by 10 μmol/L acetylcholine to check functional endothelial integrity. More than 80% relaxation of the ring was considered to be an endothelium-intact ring. Endothelium-intact rings precontracted with 60 mmol/L KCl were treated with different compounds for 30 min or 1 h, and the changes in the tension of aortic rings were recorded. The vasorelaxant effect of the compound was calculated as the percentage of KCl-induced vasoconstriction.

2.5. Statistical analysis

All data were presented as mean ± SD. Statistical comparisons were performed by one-way ANOVA followed by Bonferroni's test using SPSS 20.0. *P* value of less than or equal to 0.05 was regarded to be statistically significant.

3. Results and discussion

The acetone extract of *S. prattii* was subjected to purification using silica gel column chromatography and MCI gel column followed by reversed-phase C-18 silica gel and semi-preparative HPLC, and yielded four new 4,5-*seco*-abietane diterpenoids (salpratins A–D (**1**–**4**), together with twelve known ones sahandol (**5**) [20], ceratodiol (**6**) [4], de-O-ethylsalvonitin (**7**) [21], prionidipene A (**8**) [5], prionoid B (**9**) [22], sahandone B (**10**) [6], prionidipene B (**11**) [5], salviwardin C (**12**) [7], 10-isopropyl-2,2,6-trimethyl-2,3,4,5-tetrahydronaphtha-[1,8-bc]oxocine-5,11-diol (**13**) [8], saprirearine (**14**) [23], microstegiol (**15**) [9], candidissiol (**16**) [9].

Salpratin A (**1**) was isolated as colorless needle crystal, with molecular formula of $C_{20}H_{24}O_3$ was established by the HREIMS ($[M]^+$ peak at *m/z* 312.1719, calcd for 312.1725), indicating 9 degrees of unsaturation. The ^{13}C NMR and DEPT spectra revealed signals for 20 carbon resonances due to seven quaternary carbons and one carbonyl, five methines, two methylenes, and five methyls (Tables 1 and 2). The signals at δ_C 170.6 indicated the presence of one ester, which was further supported by the characteristic IR absorption band at 1773 cm^{-1}. Taking the 1D NMR spectra into accounts, the characteristic signals for 4,5-*seco*-abietane diterpenoid including the three singlet methyls [δ_H 1.81 (3H, s), δ_C 29.8, Me-18; δ_H 1.46 (3H, s), δ_C 24.9, Me-19; and δ_H 2.20 (3H, s), δ_C 19.3, Me-20], and an isopropyl group [δ_H 2.40 (1H, sept, J = 6.9 Hz), δ_C 32.6, CH-15; δ_H 1.12 (3H, d, J = 6.9 Hz), δ_C 20.2, Me-16; 1.10 (3H, d, J = 6.9 Hz), δ_C 20.3, Me-17] can be distinguished, suggesting that **1** should be derived from 4,5-*seco*-abietane diterpenoid [3–9].

The six-membered ring-A was deduced by the HMBC correlations of H-1 (δ_H 2.72, 2.59, m) with C-5 (δ_C 122.0), C-9 (δ_C 134.3), and C-10 (δ_C 135.5), of H-2 (δ_H 2.16, 1.65, m) and H-3 ((δ_H 2.62, m) with C-11 (δ_C 82.0), conjugated with the proton spin system of H-1/H-2/H-3 observed in the $^1H - {}^1H$ COSY spectrum (Fig. 2). Similarly, the HMBC correlations from H-20 to C-5, C-6 (δ_C 130.8), C-10, from H-6 (δ_H 7.06, d, J = 7.6 Hz) to C-8 (δ_C 131.2), and from H-7 (δ_H 6.84, d, J = 7.6 Hz) to C-9, as well as the proton spin system H-6/H-7 deduced from the $^1H - {}^1H$ COSY spectrum, established the six-membered ring-B.

In the HMBC spectrum, the correlations from H-18 (δ_H 1.81, s) and H-19 (δ_H 1.46, s) to C-4 (δ_C 85.0), and C-3 (δ_C 47.4), and from H-3 (δ_H 2.62, m) to C-11, and C-12 (δ_C 170.6) established the linkage of C-4/C-3/C-11/C-12 (Fig. 2). In addition, the characteristic chemical shift of C-4 and C-12 deduced the γ-lactone ring-D. Apart from the fifteen carbon signals occupied by rings A/B/D and the aforementioned isopropyl group, only two resonances (δ_C 158.4, C-13; δ_C 101.8, C-14) remained and were assignable to ring-C. Then, the isopropyl moiety was located at C-13 based on the HMBC correlations from H-16 (δ_H 1.12, d, J = 6.9 Hz) and H-17 (δ_H 1.10, d, J = 6.9 Hz) to C-13. In the HMBC spectrum, the correlations from H-14 (δ_H 5.75, s) to C-9 and C-7, from H-15 (δ_H 2.40, sept, J = 6.9 Hz) to C-13 and C-14, established the linkage of C-8/C-14/C-13 (Fig. 2). Finally, the oxygen bridge between C-11 and C-13 was determined by the obvious downfield chemical shift of C-11 (δ_C 82.0) and C-13. Subsequently, suitable crystals of **1** were prepared for single-crystal X-ray diffraction analysis. The final refinement on the Cu K*α* data of crystal [CCDC 1977136] further confirmed the structure of **1** and clarified the absolute configurations of **1** as 3*S*, 11*S* (Fig. 2).

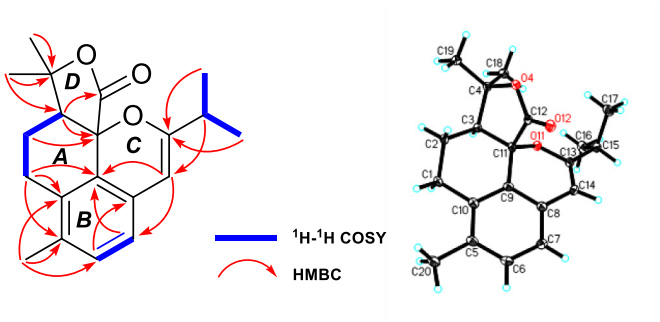

Fig. 2. Key HMBC (red arrow), $^1H-^1H$ COSY (blue bold) correlations of, and ORTEP drawing of **1**. (For interpretation of the references to colour in this figure legend, the reader is referred to the web version of this article.)

There are only 3 naturally occurring bis-*seco*-abietane diterpenoids that have been reported so far. Hyptisolide A, an 7,8;11,12-bis-*seco*-abietane diterpenoid, was isolated from *Hyptis crenata* [16]. (±)-Cryptomeriolide, a pair of racemic 4,5;11,12-bis-*seco*-abietane diterpenoids, were isolated from *Cryptomeria fortune* [17]. What's more, there is no 12,13-*seco*-abietane diterpenoid that have been reported so far. Hence, salpratin A (**1**) is the first naturally occurring 4,5;12,13-bis-*seco*-abietane diterpenoid, its C-4/C-5 bond and the C-12/C-13 bond both have been cleaved and formed two new six membered ring through an oxygen bridge between C-4/C-12, and C-11/C-13.

Salpratin B (**2**) was obtained as yellow gum. Its molecular formula $C_{20}H_{22}O_3$ was established by the ^{13}C NMR (Table 2) and negative HRESIMS (*m/z* 309.1490 $[M - H]^+$) data, indicating 10 degrees of unsaturation. The IR spectrum showed the absorption bands for hydroxyl (3429 cm^{-1}) and carbonyl (1685 cm^{-1}) groups. The ^{13}C NMR and DEPT spectra showed 20 carbon resonances due to eight quaternary carbons and one carbonyl, five methines, two methylenes, and four methyls. According to the characteristic signals [δ_H 4,84, 4.67 (2H, ds), δ_C 114.3, CH_2–18; δ_H 1.83 (3H, s), δ_C 22.5, Me-19; δ_H 2.76 (3H, s), δ_C 22.1, Me-20] and the isopropyl group [δ_H 3.46 (1H, sept, J = 7.0 Hz), δ_C 28.4, CH-15; δ_H 1.33 (3H, d, J = 7.0 Hz), δ_C 22.7, Me-16; δ_H 1.34 (3H, d, J = 7.0 Hz), δ_C 22.7, Me-17] (Tables 1 and 2), compound **2** should be derived from a 4,5-*seco*-abietane diterpenoid closely related to ceratodiol (**6**) [4]. Carefully comparison of the ^1H and ^{13}C NMR spectra of **2** with those of **6** indicated that the hydroxyl (δ_H 4.47, s) at C-1 (δ_C 66.2) in **6** should be oxidized to carbonyl (δ_C 194.6, C-1) in **2**. The HMBC correlation of H-2 (δ_H 4.91, dd, J = 6.3, 7.8 Hz) with C-1 was indicative of such a difference (Fig. 3).

The molecular formula of salpratin C (**3**) was determined as $C_{21}H_{26}O_3$ by analysis of its ^{13}C NMR and HRESIMS (*m/z* 349.1775, $[M + Na]^+$) data. Salpratin C (**3**) and ceratodiol (**6**) shared the same carbon skeletons by analysis of their NMR data. While the hydroxyl (δ_H 4.47, s) of C-1 (δ_C 66.2) in **6** was replaced by methoxyl group (δ_H 3.36, δ_C 55.8) of C-1 (δ_C 74.5) in **3**, which was confirmed by the HMBC correlation of 1-OCH_3 (δ_H 3.36, s) with C-1 (Fig. 3). The relative configuration of **3** was deduced *syn* for the substitutions at C-1 and C-2 from the small coupling between H-1 and H-2 ($J_{1,2}$ = 1.5 Hz), calculated for a dihedral angle of approximately 30° [4,5].

Compound **4** was obtained as a colorless oil. The HRESIMS of

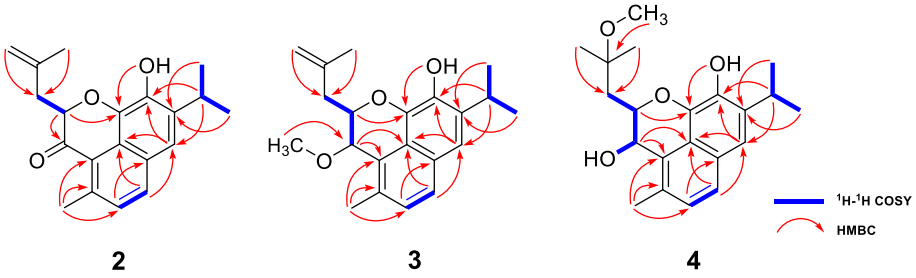

Fig. 3. Key HMBC (red arrow) and $^{1}H-^{1}H$ COSY (blue bold) correlations of **2–4**. (For interpretation of the references to colour in this figure legend, the reader is referred to the web version of this article.)

Table 3
Vasorelaxant effects of compounds on rat thoracic aortas precontracted with KCl.

Compounds (100 μM)	Maxiumum relaxant ratio (%) [a]	
	30 min	1 h
DMSO	3.43 ± 0.93	11.00 ± 3.72
1	44.94 ± 12.74 ##	66.49 ± 9.96 **
3	22.95 ± 1.85 ##	48.59 ± 6.30 **
4	52.80 ± 8.11 ##	68.62 ± 11.57 **
6	43.66 ± 6.69 ##	73.20 ± 3.86 **
7	65.14 ± 9.58 ##	95.21 ± 11.20 **
8	39.40 ± 4.50 ##	75.51 ± 8.19 **
14	38.30 ± 4.76 ##	63.81 ± 6.74 **
Nifedipine	91.67 ± 3.02 ##	92.80 ± 3.46 **

[a] Date expressed as means ± SD (n = 3).
P < .01 vs DMSO group at 30 min;
** P < .01 vs DMSO group at 1 h.

compound **4** showed m/z 367.1883 $[M + Na]^+$ (calcd for $C_{21}H_{28}O_4Na$, 367.1880). The ^{13}C NMR and DEPT spectra (Table 2) showed 21 carbon resonances due to eight quaternary carbons, six methines (including two oxygenated and three olefinic ones), one methylene, and six methyls (including an oxygenated). The ^{1}H and ^{13}C NMR data of **4** were similar to ceratodiol (**6**) except for the replacement of the olefinic methylene of in **6** instead of two methines (including an oxygenated) in **4**. Then, the methoxyl was located at C-4 was determined by the HMBC correlation from 4-OCH_3 (δ_H 3.11, s) to C-4 (δ_C 74.3) (Fig. 3). As in compound **3**, the substitution at C-1 and C-2 was deduced *syn* for **4** due to the small coupling between H-1 and H-2 ($J_{1,2}$ = 1.4 Hz). Therefore, the structure of compound **4** was confirmed and given the trivial name salpratin D.

Some *Salvia* species, such as *S. miltiorrhiza* Bunge, *S.yunnanensis* C. H. Wright, *S. przewalskii* Maxim., have been used as folk medicines for the treatment of coronary heart disease in China [1]. Therefore, the vasorelaxant effects of all the isolates at 100 μM on thoracic aorta rings precontracted with KCl were evaluated. As shown in Table 3, compared with DMSO group, compounds **1**, **3**, **4**, **6**, **7**, **8**, and **14** vasodilated the arteries. Among the compounds, compound **7** exhibited the highest vasodilative activity, and at 1 h it was comparable to nifedipine (95.21% vs 92.80%). Differing from nifedipine, the compounds showed much higher relaxant ratios at 1 h (about 50–95%) than those at 30 min (about 30–65%), indicating that they might require longer time to fully bind to the receptors to reach the maximal effects. More research is required to clarify the underlying mechanisms of the vasodilative effects of these compounds. Notably, this work can be seen as the first report of vasorelaxant activity of 4,5-*seco*-abietane diterpenoid. What's more, compounds **3**, **4**, **6**, **7** and **8** share the same 6/6/6 architecture, and have the hydroxyl or methoxyl group of C-1, which should be the important pharmacophores for vasorelaxant activity.

4. Conclusion

In summary, four new 4,5-*seco*-abietane diterpenoids, salpratins A–D (**1–4**) and twelve known analogues with diverse 6/6/6, 6/6/7, and 6/6/8 rings system were isolated from *Salvia prattii*. It's noteworthy that salpratin A (**1**) is a rare bis-*seco*-abietane with a 5/6/6/6 ring system formed by migration of an angular methyl group and bond-breaking of ring-A and ring-C. Bioassays displayed that compounds **1**, **3**, **4**, **6**, **7**, **8**, and **14** showed potent vasorelaxant activity on endothelium-intact thoracic aorta rings precontracted with KCl. The discoveries will enrich the structure type of 4,5-*seco*-abietane diterpenoid and its pharmacology, which would also draw more attention to its potential cardiovascular activity.

Declaration of Competing Interest

None.

Acknowledgments

The work was financially supported by the State Key Laboratory of Phytochemistry and Plant Resources in West China (P2019-ZZ05), the Second Tibetan Plateau Scientific Expedition and Research (STEP) program (2019QZKK0502). The Scientific Research Foundation for the Returned Overseas Chinese Scholars, State Education Ministry; the Research Programs of Youth Innovation Promotion Association in the Chinese Academy of Sciences (2014354).

Appendix A. Supplementary data

Supplementary data to this article can be found online at https://doi.org/10.1016/j.fitote.2020.104521.

References

[1] Y.B. Wu, Z.Y. Ni, Q.W. Shi, M. Dong, H. Kiyota, Y.C. Gu, B. Cong, Chem. Rev. 112 (2012) 5967–6026.
[2] Y.Z. Dong, S.L. Morris-Natschke, K.H. Lee, Nat. Prod. Rep. 28 (2011) 529–542.
[3] F. Xia, C.Y. Wu, X.W. Yang, X. Li, G. Xu, Nat. Pro. Bioprospect. 5 (2015) 307–312.
[4] H.H. Mirzaei, Q. Firuzi, J.N. Chandran, B. Schneider, A.R. Jassbi, Phytochem. Lett. 29 (2019) 129–133.
[5] L. Li, M. Zhou, G. Xue, W. Wang, X. Zhou, X. Wang, L. Kong, J. Luo, Bioorg. Chem. 81 (2018) 454–460.
[6] I. Salimilia, M.M. Farimani, H.R. Monsef-Esfahani, A.R. Gohari, Nat. Prod. Res. 30 (2015) 120–124.
[7] Q.L. Xiao, F. Xia, X.W. Yang, Y. Liao, L.X. Yang, Y.K. Wei, X. Li, G. Xu, Nat. Pro. Bioprospect. 5 (2015) 77–82.
[8] Y. Zhang, Y. Lu, L. Zhang, Q.T. Zheng, L.Z. Xu, S.L. Yang, J. Nat. Prod. 68 (2005) 1131–1133.
[9] G. Topcu, A. Ulubelen, J. Nat. Prod. 59 (1976) 734–737.
[10] Z.H. Miao, T. Tang, Y.X. Zhang, J.S. Zhang, J. Ding, Int. J. Cancer 106 (2003) 108–115.
[11] G. Xu, A.J. Hou, R.R. Wang, G.Y. Liang, Y.T. Zheng, Z.Y. Liu, X.L. Li, Y. Zhao, S.X. Huang, L.Y. Peng, Q.S. Zhao, Org. Lett. 8 (2006) 4453–4456.
[12] G. Xu, A.J. Hou, Y.T. Zheng, Y. Zhao, X.L. Li, L.Y. Peng, Q.S. Zhao, Org. Lett. 9 (2007) 291–293.
[13] G. Xu, X.W. Yang, C.Y. Wu, X.N. Li, J. Su, X. Deng, Y. Li, H.B. Qin, L.X. Yang,

Q.S. Zhao, Chem. Commun. 48 (2012) 4438–4440.
[14] F. Xia, W.Y. Li, X.W. Yang, J. Yang, X. Li, Y. Nian, G. Xu, Org. Lett. 21 (2019) 5670–5674.
[15] J. Dang, Y. Shao, J. Zhao, L. Mei, Y. Tao, Q. Wang, L. Zhang, J. Sep. Sci. 39 (2016) 3327–3338.
[16] Y.S. Yun, H. Fukaya, T. Nakane, A. Takano, S. Takahashi, Y. Takahashi, H. Inoue, Org. Lett. 16 (2014) 6188–6191.
[17] L. Feng, A. Mandi, C. Tang, T. Kurtan, S. Tang, C.Q. Ke, N. Shen, G. Lin, S. Yao, Y. Ye, J. Nat. Prod. 81 (2018) 2667–2672.
[18] H.D. Jiang, J. Cai, J.H. Xu, X.M. Zhou, Q. Xia, J. Ethnopharmacol. 101 (2005) 221–226.
[19] G.J. Chang, T.P. Lin, Y.S. Ko, M.S. Lin, Life Sci. 86 (2010) 869–879.
[20] S.N. Ebrahimi, S. Zimmermann, J. Zaugg, M. Smiesko, R. Brun, M. Hamburger, Planta Med. 79 (2013) 150–156.
[21] L.Z. Lin, G.A. Cordell, P. Lin, Phytochemistry 40 (1995) 1469–1471.
[22] J. Chang, J. Xu, M. Li, M. Zhao, J. Ding, J.S. Zhang, Planta Med. 71 (2005) 861–866.
[23] X. Chen, J. Ding, Y.M. Ye, J.S. Zhang, J. Nat. Prod. 65 (2002) 1016–1020.