New Germacrane Sesquiterpenes from *Salvia chinensis*

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Two new germacrane sesquiterpenes, called salviadienol A (1) and salviadienol B (2), together with five known compounds, methyl-ent-4-epi-agath-18-oate (3), angelicoidenol (4), clovane-2β,9α-diol (5), dehydrovomifoliol (6) and blumenol A (7), were isolated from *Salvia chinensis*. Their structures were identified on the basis of spectral characteristics.

Key words *Salvia chinensis*; salviadienol A; salviadienol B; germacrane; sesquiterpene

Salvia chinensis BENTH. is an herbal medicinal plant distributed in the southern part of the Yangtze River in China. It has been used as a Chinese folk medicine for the treatment of hepatitis, nephritis, dysmenorrhea and several kinds of cancer.[1)] Several investigations showed that the chemical constituents of *Salvia* species were rich in triterpenoids, diterpenoids, monoterpenes and polyphenolics.[2—5)] However, further chemical studies on the aerial parts of *Salvia chinensis* were left uncharacterized. In the course of our chemical studies on this plant, two new germacrane sesquiterpenes, salviadienol A (**1**) and salviadienol B (**2**), together with five known compounds (**3**—**7**) were isolated from the 70% ethanol extract of the aerial parts of *Salvia chinensis*. This paper mainly deals with the isolation and structural elucidation of two new germacrane sesquiterpenes.

Results and Discussion

The 70% ethanol extract of the aerial parts of *Salvia chinensis* was suspended in water, and partitioned with petroleum ether (PE), $CHCl_3$, EtOAc, and *n*-butanol, successively. The $CHCl_3$ fraction was subjected to Sephadex LH-20 and repeated silica gel column chromatography, as well as ODS column chromatography, to afford two new compounds (**1**, **2**), called salviadienol A and salviadienol B, and five known compounds (**3**—**7**). Compounds **3**—**7** were identified as methyl-ent-4-epi-agath-18-oate (**3**),[6)] angelicoidenol (**4**),[7)] clovane-2β,9α-diol (**5**),[8)] dehydrovomifoliol (**6**),[9)] and blumenol A (**7**),[10)] respectively, by comparison of their spectroscopic data with those reported in the literature.

Compound **1** was obtained as colorless oil, and its molecular formula was determined as $C_{28}H_{42}O_{12}$ by HR-ESI-MS (*m*/*z* 593.2546 $[M+Na]^+$, Calcd 593.2574). The IR spectrum indicated absorption bands at 3470, 1744 cm^{-1} assignable to hydroxy and carbonyl groups. The ^{1}H-NMR spectrum (pyridine-d_5, Table 1) of **1** showed the presence of three methyls at δ 1.30 (3H, d, J=6.0 Hz) and 1.33 (6H, d, J=6.0 Hz), four acetoxy groups at δ 1.98 (3H, s), 1.99 (3H, s), 2.17 (3H, s) and 2.23 (3H, s), three olefinic protons at δ 5.44 (1H, s), 5.67 (1H, s) and 6.15 (1H, d, J=6.0 Hz). The ^{13}C-NMR and distortionless enhancement by polarization transfer (DEPT) spectra of **1** revealed 28 carbons (Table 1). One olefinic methylene (δ 119.6), one olefinic methine (δ 137.7) and two olefinic quaternary carbons (δ 132.3, 146.8) indicated the presence of two double bonds, including a terminal double bond. The remaining carbons were assigned to two oxymethylenes (δ 61.3, 67.9), five oxymethines (δ 66.3, 68.6, 68.7, 71.4, 81.9), one carbonyl group (δ 173.4), in addition to four acetoxy groups, three methyls, two sp^3 methylenes and three sp^3 methines. Therefore, with the eight degrees of unsaturation required by the molecular formula, **1** should be monocyclic. The heteronuclear multiple quantum coherence (HMQC) spectrum permitted the assignment of all protons to the corresponding carbon atoms as shown in Table 1. In the heteronuclear multiple bond coherence (HMBC) spectrum (Fig. 1), the long-range correlations between H-14 (δ 5.44, 5.67) and C-1 (δ 68.6), C-9 (δ 81.9) and C-10 (δ 146.8), between H-2 (δ 4.46) and H-8a (δ 2.52) and C-10, between H-9 (δ 5.76) and C-7 (δ 47.7), and between H-15a (δ 4.78) and C-3 (δ 33.4) and C-5 (δ 137.7) were observed. These results indicated a partial structure through C2–C1–C10–C9–C8–C7 as well as C3–C4–C5. In the ^{1}H–^{1}H COSY spectrum, H-11 (δ 2.43) was correlated with H-12b (δ 4.46), H-13 (δ 1.33), and H-7 (δ 1.93) which suggested the presence of an oxyisopropenyl group branching at C-7. Additionally, the correlations of H-2 (δ 4.46) with H-3a (δ 2.47), H-6 (δ 5.08) with H-5 (δ 6.15) and H-7 (δ 1.93), and H-7 (δ 1.93) with H-8a (δ 2.52) were observed. These suggested the linkages from C-2 to C-3 and C-5 to C-8. On the basis of all the evidence, the structure of **1** was determined to be a 10-membered carbocycle typical of the germacrane skeleton. As shown in Fig. 1, the ^{1}H–^{1}H COSY experiment indicated the presence of partial structures written in bold lines. The HMBC correlations between H-2′ (δ 2.95), H-3′ (δ 5.46) and H-5′ (δ 1.30) and carbonyl carbon C-1′ (δ 173.4) showed that the carbonyl carbon was linked at C-2′. The observation of the correlation between H-9 (δ 5.76) and C-1′ (δ 173.4) clarified that an oxy-isovaleryl group was located at C-9. The HMBC spectrum was used to determine the position of the acetoxy groups. The long-range correlations of H-1 (δ 6.33) with the signal at δ 170.4, H-15a (δ 4.78) with the signal at δ 170.6, H-12a (δ 4.60) with the signal at δ 171.2, and H-3′ (δ 5.46) with the signal at δ 170.2 implied that the four acetoxy groups were located at C-1, C-15, C-12 and C-3′, respectively. Significant long-range proton–carbon correlations are shown in Fig. 1.

With the help of the molecular model, the relative configuration of **1** was deduced from the NOESY spectrum (Fig. 2) and analysis of the couple constants (*J*). The endocyclic double bond at C-4 was determined to be *Z*, as H-5 showed a NOE correlation to H-3b, not to H-15. The NOE correlations

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Table 1. ^{1}H- (600 MHz) and ^{13}C-NMR (150 MHz) Data of Compounds **1** and **2**

Position	**1** (pyridine-d_5)		Position	**2** ($CDCl_3$)	
	δ_H (J Hz)	δ_C		δ_H (J Hz)	δ_C
1	6.33 (1H, dd, J=10.8, 1.8 Hz)	68.6 d	1	5.38 (1H, dd, J=10.8, 1.4 Hz)	132.5 d
2	4.46 (1H, m)	68.7 d	2a	2.34 (1H, ddd, J=12.6, 10.8, 4.8 Hz)	24.4 t
			2b	2.18 (1H, m)	
3a	2.47 (1H, m)	33.4 t	3a	2.55 (1H, dd, J=10.8, 3.0 Hz)	33.4 t
3b	2.08 (1H, m)		3b	2.10 (1H, overlapped)	
4		132.3 s	4		131.8 s
5	6.15 (1H, d, J=6.0 Hz)	137.7 d	5	5.52 (1H, d, J=7.8 Hz)	138.0 d
6	5.08 (1H, overlapped)	66.3 d	6	4.57 (1H, t-like, J=7.8 Hz)	66.2 d
7	1.93 (1H, m)	47.7 d	7	1.55 (1H, m)	49.4 d
8a	2.52 (1H, m)	33.5 t	8	5.29 (1H, dd, J=10.2, 1.2 Hz)	73.8 d
8b	2.06 (1H, m)				
9	5.76 (1H, d, J=10.8 Hz)	81.9 d	9	4.87 (1H, d, J=10.2 Hz)	77.8 d
10		146.8 s	10		132.9 s
11	2.43 (1H, m)	31.3 d	11	1.84 (1H, m)	30.8 d
12a	4.60 (1H, dd, J=10.8, 4.2 Hz)	67.9 t	12a	4.27 (1H, dd, J=11.0, 1.8 Hz)	67.0 t
12b	4.46 (1H, dd, J=10.8, 1.8 Hz)		12b	4.21 (1H, dd, J=11.0, 3.6 Hz)	
13	1.33 (3H, d, J=6.0 Hz)	18.4 q	13	1.14 (3H, d, J=6.0 Hz)	17.7 q
14a	5.67 (1H, s)	119.6 t	14	1.80 (3H, s)	19.0 q
14b	5.44 (1H, s)				
15a	4.78 (1H, d, J=12.6 Hz)	61.3 t	15a	4.46 (1H, d, J=12.6 Hz)	59.8 t
15b	4.36 (1H, d, J=12.6 Hz)		15b	4.28 (1H, d, J=12.6 Hz)	
1′		173.4 s	1′		172.5 s
2′	2.95 (1H, dq, J=12.0, 6.0 Hz)	45.6 d	2′	2.58 (1H, m)	44.7 d
3′	5.46 (1H, dq, J=12.0, 6.0 Hz)	71.4 d	3′	5.03 (1H, dt, J=12.6, 6.0 Hz)	70.9 d
4′	1.33 (3H, d, J=6.0 Hz)	18.3 q	4′	1.22 (3H, d, J=6.0 Hz)	18.0 q
5′	1.30 (3H, d, J=6.0 Hz)	12.4 q	5′	1.14 (3H, d, J=6.0 Hz)	12.4 q
1-OAc	2.23 (3H, s)	21.2 q	8-OAc	2.13 (3H, s)	21.0 q
		170.4 s			172.4 s
12-OAc	2.17 (3H, s)	20.8 q	12-OAc	2.11 (3H, s)	20.9 q
		171.2 s			171.0 s
15-OAc	1.98 (3H, s)	20.6 q	15-OAc	2.08 (3H, s)	20.6 q
		170.6 s			170.6 s
3′-OAc	1.99 (3H, s)	21.0 q	3′-OAc	2.04 (3H, s)	20.7 q
		170.2 s			170.1 s

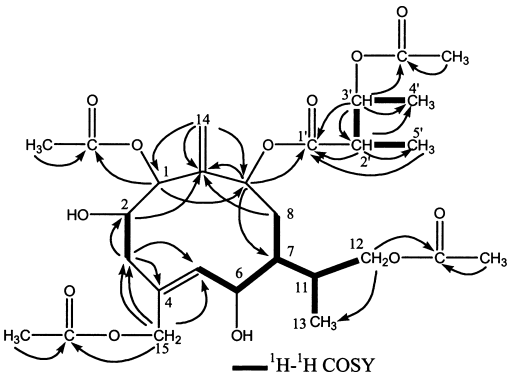

Fig. 1. Selected HMBC (H to C) Correlations for Compound **1**

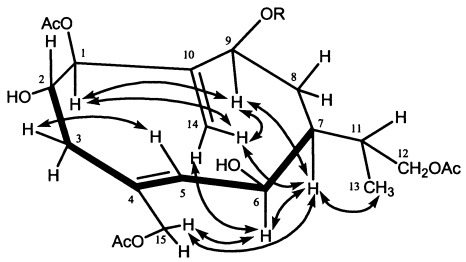

Fig. 2. Selected NOE Correlations for Compound **1**

(Fig. 2) of H-6/H_2-15, H-7/H_2-15and H-6/H-7 showed that H-6 and H-7 possess α-orientation.[11] Observation of NOEs of H-9/H-7 and H-1/H-9 indicated the α-orientation of H-1 and H-9. Analysis of the coupling constant for the H-1 revealed a $J_{1,2}$ value of 10.8 Hz, assignable to *trans*-orientation between the H-1 and H-2 protons. Inspection of a molecular model for the proposed structure revealed a dihedral angle between H-1 and H-2 was close to 180°, which fulfilled the NOESY correlations discussed above, and agreed with the magnitude of ^{1}H–^{1}H coupling between these two protons. Therefore, compound **1** was deduced to be (4*Z*)-9β-(3′-acetoxy-2′-methylbutyryloxy)-1β,12,15-triacetoxy-7αH-germacra-4,10(14)-diene-2α,6β-diol.

Compound **2** was also isolated as colorless oil, and high-resolution ESI-MS analysis revealed the molecular formula of **2** to be $C_{28}H_{42}O_{11}$ (*m/z* 553.2626 $[M-H]^-$, Calcd 553.2649). The IR spectrum showed the presence of hydroxy (3466 cm^{-1}) and carbonyl functions (1736 cm^{-1}). The ^{1}H-NMR spectrum (Table 1) of **2** displayed signals for four methyls at δ 1.14 (6H, d, J=6.0 Hz), 1.22 (3H, d, J=6.0 Hz) and 1.80 (3H, s), four acetoxy groups at δ 2.04 (3H, s), 2.08 (3H, s), 2.11 (3H, s) and 2.13 (3H, s), two olefinic protons at δ 5.38 (1H, dd, J=10.8, 1.4 Hz) and 5.52 (1H, d, J=7.8 Hz). The ^{13}C-NMR and DEPT spectra (Table 1) of **2** exhibited 28 carbons, two olefinic methines (δ 132.5, 138.0), two olefinic quaternary carbons (δ 131.8, 132.9), two oxymethylenes (δ 59.8, 67.0), four oxymethines (δ 66.2, 73.8, 77.8, 70.9), one

carbonyl group (δ 172.5), in addition to four acetoxy groups, four methyls, two sp^3 methylenes and three sp^3 methines. The proton and carbon signals were assigned from the HMQC spectrum (Table 1). Following the same NMR elucidation procedures used for **1**, the germacrane skeleton and the positions of substituents for **2** were determined by ^{1}H–^{1}H COSY and HMBC correlations (Fig. 3). The ^{1}H- and ^{13}C-NMR spectra (Table 1) of **2** were similar to those of **1**. Comparison of the NMR data indicated that signals of the exocylic double bond of **1** were replaced by signals of the endocyclic double bond and a methyl group attached to the double bond of **2**. In the HMBC spectrum of **2** (Fig. 3), correlations of the methyl proton H-14 (δ 1.80) and the olefinic proton H-1 (δ 5.38) with C-9 (δ 77.8) implied that the double bond of **2** was located between C-1 and C-10 instead of between C-10 and C-14 as in **1**. The location of the oxymethine group at C-8 was determined by the correlations between the proton at δ 5.29 (H-8) and signals of C-10 at δ 132.9 and C-11 at δ 30.8 in the HMBC spectrum. An acetoxy group was linked at C-8 by the long-range correlations of H-8 (δ 5.29) with the carbon signal at δ 172.4.

The relative configuration of compound **2** was determined from the NOESY spectrum (Fig. 4) and analysis of the coupling constants (J), along with inspection of the molecular model. The NOE correlation was observed between H-5 and H-3b, leading to the conclusion that the configuration of the C-4, C-5 double bond was *Z*-form. The C-1, C-10 double bond was determined to be *E*-form by NOE between H-1 and H-9. The NOE correlations of H-6/H_2-15, H-7/H_2-15 and H-6/H-7 showed that H-6 and H-7 were on the same face and possessed α-orientation.[11] The coupling patterns of the H-8 (dd, J=10.2, 1.2 Hz) and H-9 (d, J=10.2 Hz) led to confirmation of the *cis*-orientation of H-7/H-8 and the *trans*-orienta-

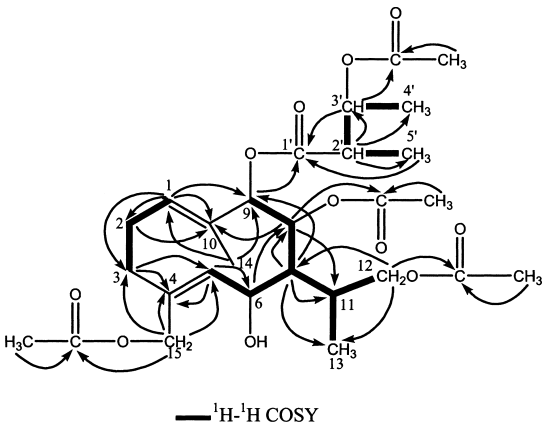

Fig. 3. Selected HMBC (H to C) Correlations for Compound **2**

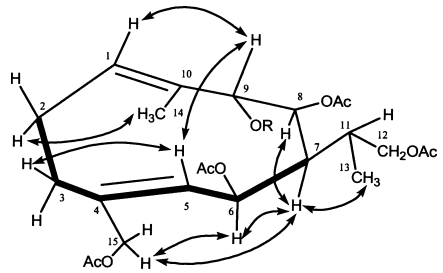

Fig. 4. Selected NOE Correlations for Compound **2**

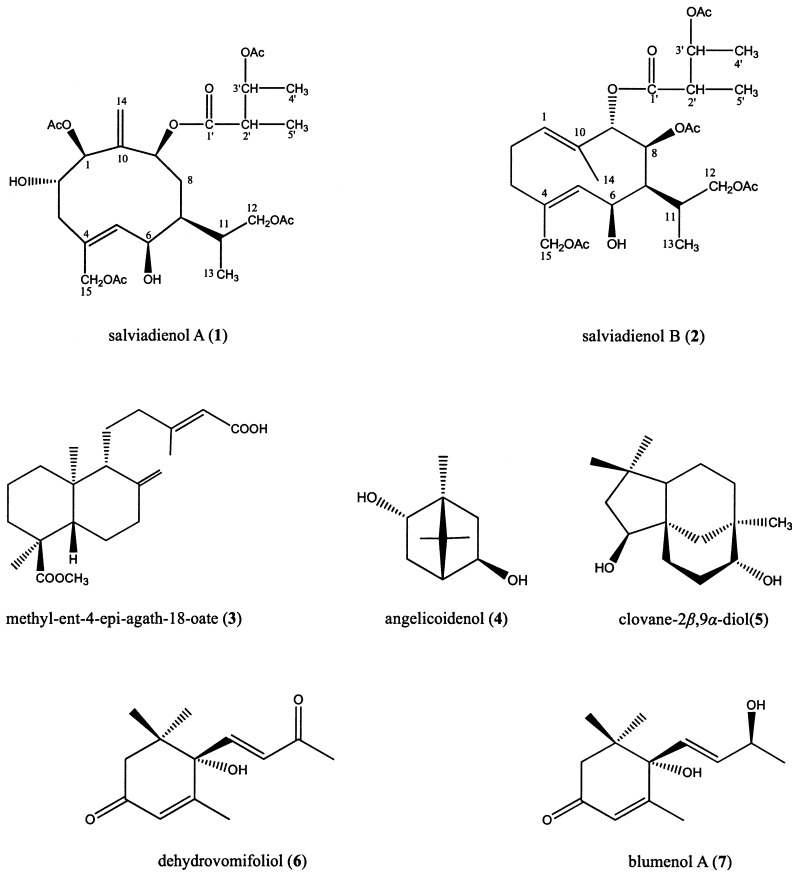

Fig. 5. The Structures of Compounds Isolated from *Salvia chinensis*

tion of H-8/H-9. Thus, H-8 and H-9 protons were deduced to be α-orientation and β-orientation.[12] The molecular model fits the observed NOE correlations and *J*-value. From these data compound **2** was confirmed to be (1*E*,4*Z*)-9α-(3′-acetoxy-2′-methylbutyryloxy)-8β,12,15-triacetoxy-7α*H*-germacra-1(10),4-diene-6β-ol.

Compounds **1** and **2** are the first germacrane sesquiterpenes obtained from this species. The closest known analogues have been observed from natural sources.[11—17] These precedents also show the 10-membered carbocycle of the germacrane skeleton, but with different arrangements in functionality. Substances **1** and **2**, on the other hand, are highly acylated members with oxy-isovaleryl groups being the most unusual ones. The related germacrane sesquiterpenes have been reported to show cytotoxic activities.[11,13,14] Quite disappointingly, compounds **1** and **2** were inactive against HL-60, the human leukemia cell line. The concentration which inhibits cell growth by 50% (IG_{50}) and the concentration which reduces cell viability by 50% (IC_{50}) both exceeded 100 μM.

Experimental

Optical rotations were measured with a Perkin-Elmer 241MC polarimeter. NMR spectra were recorded on a Bruker ARX-300 NMR spectrometer and a Bruker AV-600 NMR spectrometer. The chemical shifts were quoted relative to TMS and the coupling constants were in Hz. ESI-MS was conducted on an Agilent 1100 SL instrument. HR-ESI-MS were recorded on a NEC JMS−+100 cs instrument. FAB-MS was recorded on an Autospec Ulima-TOF instrument. IR spectra were conducted on a Perkin IFS-55 spectrometer. The chromatographic silica gel (200—300 mesh) was produced by Qingdao Ocean Chemical Factory and Sephadex LH-20 was purchased from GE Healthcare. ODS (50 μm) was produced by YMC Co., Ltd.

Plant Material The aerial parts of *Salvia chinensis* were purchased from Liaoning Medicinal Material Corporation, Shenyang, China and identified by Prof. Qishi Sun of Shenyang Pharmaceutical University.

Extraction and Isolation The aerial parts of *Salvia chinensis* (30 kg) were extracted three times with 70% EtOH under reflux for 3 h. Evaporation of the solvent under reduced pressure provided a 70% EtOH extract (1900 g). A suspension of the resulting extract in water was partitioned with petroleum ether (PE), $CHCl_3$, EtOAc, and *n*-BuOH, successively. The $CHCl_3$ extract (270 g) was fractionated by silica gel column chromatography (200—300 mesh) eluting with PE–acetone (100 : 1—100 : 100) to give five fractions. Fraction 3 was separated by silica gel column chromatography (200—300 mesh) eluting with PE–acetone (100 : 2—100 : 20) to give five sub-fractions. Sub-fraction 3 (PE–acetone, 10 : 1) was further separated by medium-pressure liquid chromatography (MPLC) with the mobile phase of MeOH–H_2O (90 : 10), to afford compound **3** (10 mg). Sub-fraction 4 (PE–acetone, 8 : 1) was further separated by repeated silica gel column chromatography ($CHCl_3$–acetone, 100 : 3), and purified by MPLC with MeOH–H_2O (70 : 30) to furnish new compounds **1** (15 mg) and **2** (40 mg). Sub-fraction 5 (PE–acetone, 5 : 1) was subjected to Chromatorex ODS chromatography (eluant MeOH–H_2O, 55 : 45), yielding compound **4** (20 mg). Fraction 4 was chromatographed by silica gel column with $CHCl_3$–CH_3OH (40 : 1) to obtain compound **5** (13 mg) and a fraction that was further purified by MPLC with MeOH–H_2O (50 : 50) to give compounds **6** (15 mg) and **7** (13 mg).

Compound **1**: Colorless oil, $[\alpha]_D^{25}$ +0.8° (*c*=0.36, pyridine). High-resolution ESI-MS: Calcd for $C_{28}H_{42}O_{12}Na$ $[M+Na]^+$: 593.2574; Found: 593.2546. IR (KBr): 3470, 2952, 1744, 1694, 1383, 1242, 1184, 1135, 1054, 1030 cm^{-1}. ^{1}H- and ^{13}C-NMR: given in Table 1. ESI-MS *m*/*z*: 593 $[M+Na]^+$.

Compound **2**: Colorless oil, $[\alpha]_D^{25}$ −5.3° (*c*=0.33, $CHCl_3$). High-resolution ESI-MS: Calcd for $C_{28}H_{41}O_{11}$ $[M-H]^-$: 553.2649; Found: 553.2626. IR (KBr): 3466, 2940, 1736, 1382, 1237, 1027 cm^{-1}. ^{1}H- and ^{13}C-NMR: given in Table 1. FAB-MS *m*/*z*: 577 $[M+Na]^+$.

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