Splendidins A – C, Three New Clerodane Diterpenoids from *Salvia splendens*

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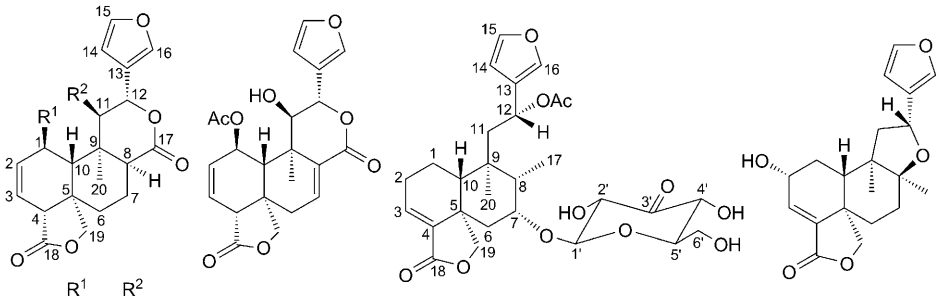
Three new clerodane diterpenoids, splendidins A – C (**1** – **3**, resp.), were isolated from *Salvia splendens*, together with six known ones. Their structures were elucidated by extensive spectroscopic analysis. Splendidin C (**3**) was the first diterpenoid glucoside reported from this plant. These compounds were evaluated for their cytotoxic activity; however, none of them were cytotoxic.

Introduction. – *Salvia* is an important genus consisting of *ca.* 900 species in the family Labiatae, growing in the temperate and warmer zones of the world [1]. Various plants of this genus are widely used in folk medicine in China [2]. *Salvia splendens* is a Brazilian species which is now widely used for ornamental purposes. There are several reports on the chemical investigation of *S. splendens* [3 – 7]. In our continuous search for bioactive metabolites from *Salvia* [8], we re-investigated the constituents of *S. splendens* collected in Kunming Institute of Botany. Three new clerodane diterpenoids, splendidins A – C (**1** – **3**, resp.), together with six known clerodane diterpenoids, salviarin (**4**) [3], splenolide B (**5**) [6], splendidin (**6**) [4], splenolide A (**7**) [6], salvisplendin A (**8**) [7], and salvisplendin D (**9**) [7], were isolated from *S. splendens*. Here, we describe the isolation and structure elucidation of the new compounds **1** – **3**.

Results and Discussion. – Splendidin A (**1**) was obtained as a white amorphous solid. Its molecular formula was established as $C_{20}H_{22}O_7$ by the HR-ESI-MS (*m*/*z* 397.1254 ($[M+Na]^+$; calc. 397.1263), indicating ten degrees of unsaturation. The IR spectrum implied the presence of OH (3429 cm^{-1}) and C=O (1768 and 1731 cm^{-1}) groups. The NMR spectra (*Tables 1* and *2*) exhibited 20 C-atom signals including those for one Me group, three CH_2 (one oxygenated), eleven CH (three oxygenated and five olefinic) groups, and three quaternary (one olefinic) and two ester C=O C-atoms. Considering the types of the compounds previously isolated from this plant [3 – 7], along with the characteristic signals of the furan ring at δ(H) 6.78 (*d*, $J = 1.6$, H–C(14)), 7.61 (*d*, $J = 1.6$, H–C(15)), and 7.86 (*s*, H–C(16)); δ(C) 125.2 (*s*, C(13)), 109.9 (*d*, C(14)), 144.1 (*d*, C(15)), and 142.0 (*d*, C(16)) and signals of a noticeable CH_2O moiety at δ(H) 4.55, 4.36 (each *d*, $J = 9.2$, CH_2(19)); δ(C) 71.7 (*t*, C(19)), compound **1** could be determined as a clerodane diterpenoid.



	R^1	R^2
1	OH	OH
4	H	H
5	H	AcO
6	AcO	AcO
7	OH	H
8	H	OH

2 **3** **9**

Table 1. *^{1}H-NMR Data for Compounds* **1**–**3**. δ in ppm, J in Hz.

	1[a])	**2**[b])	**3**[c])
H–C(1) or CH_2(1)	4.63 (*d*, *J* = 9.6)	5.85 (*d*, *J* = 10.8)	1.05 (br. *d*, *J* = 9.8, H_α), 1.46 (br. *d*, *J* = 9.8, H_β)
H–C(2) or CH_2(2)	6.24 (*dd*, *J* = 8.0, 2.4)	5.87–5.91 (*m*)	2.18–2.26 (*m*, H_α), 1.95–2.02 (*m*, H_β)
H–C(3)	5.81 (*dd*, *J* = 8.0, 2.4)	5.77–5.81 (*m*)	6.61 (br. *d*, *J* = 4.8)
H–C(4)	3.06 (*d*, *J* = 1.4)	3.18 (*d*, *J* = 2.5)	–
CH_2(6)	1.77 (br. *d*, *J* = 14.0, H_α), 1.57 (*dd*, *J* = 14.0, 12.0, H_β)	2.52 (*dd*, *J* = 18.5, 6.0, H_α), 2.38 (*d*, *J* = 18.5, H_β)	2.28 (H_α, br. *d*, *J* = 7.2), 1.17 (H_β, br. *d*, *J* = 7.2)
CH_2(7) or H–C(7)	1.99–2.07 (*m*, H_α), 2.49 (*dd*, *J* = 14.0, 2.4, H_β)	7.22 (*d*, *J* = 6.0)	4.12 (br. *s*)
H–C(8)	2.85 (br. *s*)	–	1.78–1.83 (*m*)
H–C(10)	2.42 (*d*, *J* = 9.6)	3.59 (*d*, *J* = 11.0)	1.89 (*d*, 9.6)
H–C(11) or CH_2(11)	3.99 (*d*, *J* = 10.4)	4.65 (br. *s*)	2.22 (*dd*, *J* = 12.8, 6.8, H_α), 1.77 (*dd*, *J* = 12.8, 2.0, H_β)
H–C(12)	5.99 (*d*, *J* = 10.4)	5.91 (br. *s*)	5.82 (*dd*, *J* = 6.8, 2.0)
H–C(14)	6.78 (*d*, *J* = 1.6)	6.55 (*d*, *J* = 1.6)	6.40 (*d*, *J* = 1.2)
H–C(15)	7.61 (*d*, *J* = 1.6)	7.62 (br. *d*, *J* = 1.6)	7.38 (*d*, *J* = 1.2)
H–C(16)	7.86 (*s*)	7.76 (*s*)	7.42 (*s*)
Me(17)	–	–	1.17 (*d*, *J* = 5.6)
CH_2(19)	4.55 (*d*, *J* = 9.2, H_a), 4.36 (*d*, *J* = 9.2, H_b)	4.40 (*d*, *J* = 9.0, H_a), 4.06 (*d*, *J* = 9.0, H_b)	5.34 (*d*, *J* = 5.6, H_a), 3.84 (*d*, *J* = 5.6, H_b)
Me(20)	1.35 (*s*)	1.06 (*s*)	0.82 (*s*)
AcO–C(1)	–	2.05 (*s*)	–
AcO–C(12)	–	–	2.01 (*s*)
H–C(1′)	–	–	4.45 (*d*, *J* = 6.4)
H–C(2′)	–	–	4.22 (*d*, *J* = 6.4)
H–C(4′)	–	–	4.37 (*d*, *J* = 7.6)
H–C(5′)	–	–	3.36 (*d*, *J* = 6.8)
CH_2(6′)	–	–	4.03 (*d*, *J* = 9.6, H_a), 3.91 (*dd*, *J* = 9.6, 6.8, H_b)

[a]) Recorded in C_5D_5N; 400 MHz. [b]) Recorded in C_5D_5N; 500 MHz. [c]) Recorded in $CDCl_3$; 400 MHz.

Table 2. *^{13}C-NMR Data of* **1**–**3**. δ in ppm.

	1[a])	**2**[b])	**3**[c])	C-Atom	**1**[a])	**2**[b])	**3**[c])
C(1)	64.2 (*d*)	69.5 (*d*)	19.3 (*t*)	C(15)	144.1 (*d*)	144.6 (*d*)	143.3 (*d*)
C(2)	135.2 (*d*)	131.0 (*d*)	26.9 (*t*)	C(16)	142.0 (*d*)	139.4 (*d*)	139.7 (*d*)
C(3)	120.7 (*d*)	122.4 (*d*)	135.5 (*d*)	C(17)	171.1 (*s*)	165.5 (*s*)	12.1 (*q*)
C(4)	52.7 (*d*)	50.9 (*d*)	138.5 (s)	C(18)	175.9 (*s*)	175.1 (*s*)	170.1 (*s*)
C(5)	42.9 (*s*)	40.5 (*s*)	44.5 (*s*)	C(19)	71.3 (*t*)	71.7 (*t*)	72.2 (*t*)
C(6)	32.5 (*t*)	36.1 (*t*)	35.2 (*t*)	C(20)	20.2 (*q*)	20.9 (*q*)	18.9 (*q*)
C(7)	20.0 (*t*)	138.0 (*d*)	77.8 (*d*)	*Ac*O–C(1)	–	170.1 (*s*), 21.2 (*q*)	–
C(8)	50.4 (*d*)	133.2 (*s*)	40.9 (*d*)	*Ac*O–C(12)	–	–	170.2 (*s*), 21.1 (*q*)
C(9)	40.7 (*s*)	41.9 (*s*)	38.9 (*s*)	C(1′)	–	–	101.4 (*d*)
C(10)	45.5 (*d*)	38.3 (*d*)	48.2 (*d*)	C(2′)	–	–	76.7 (*d*)
C(11)	77.7 (*d*)	74.4 (*d*)	72.3 (*t*)	C(3′)	–	–	206.6 (*s*)
C(12)	74.5 (*d*)	82.0 (*d*)	64.3 (*d*)	C(4′)	–	–	72.5 (*d*)
C(13)	125.2 (*s*)	126.4 (*s*)	125.8 (*s*)	C(5′)	–	–	76.0 (*d*)
C(14)	109.9 (*d*)	108.6 (*d*)	108.4 (*d*)	C(6′)	–	–	61.9 (*t*)

[a]) Recorded in C_5D_5N; 100 MHz. [b]) Recorded in C_5D_5N; 125 MHz. [c]) Recorded in $CDCl_3$; 100 MHz.

The spectral data of **1** were almost identical to those reported [4] for splendidin (**6**), and the key difference was the absence of the AcO–C(1) and AcO–C(11) groups in the former. The upfield shifts of the signals of H–C(1) (δ(H) 4.63) and H–C(11) (δ(H) 3.99) as well as the formula $C_{20}H_{22}O_7$ supported the above conclusion.

The relative configuration of **1** was established *via* the ROESY spectrum (*Fig. 1*). The presence of ROESY correlations H–C(1)/Me(20) and H–C(11)/Me(20) verified that both HO–C(1) and HO–C(11) were β-oriented.

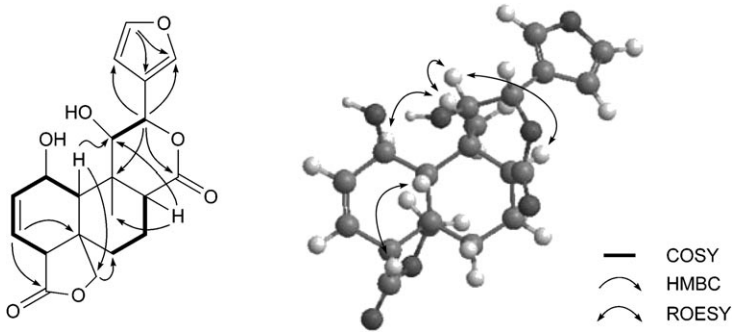


Fig. 1. *Key 2D-NMR correlations of* **1**

Splendidin B (**2**), a white amorphous solid, showed a *quasi*-molecular-ion peak at *m*/*z* 437.1207 ($[M+Na]^+$; calc. 437.1212), corresponding to the molecular formula $C_{22}H_{22}O_8$. The ^{13}C-NMR spectrum (*Table 2*) indicated the presence of a furan ring (δ(C) 108.6 (*d*), 126.4 (*s*), 139.4 (*d*), 144.6 (*d*)), three C=O C-atoms (δ(C) 175.1, 170.1, 165.5), a disubstituted C=C bond (δ(C) 122.4 (*d*), 131.0 (*d*)), and a trisubstituted C=C bond (δ(C) 133.2 (*s*), 138.0 (*d*)). The 1D-NMR data (*Tables 1* and *2*) of **2** were similar to those of **1**, except for the presence of a trisubstituted C=C bond in **2**. The HMBC

correlations Me(20)/C(8), H–C(11)/C(8), H–C(7)/C(5), H–C(7)/C(6), H–C(7)/C(8), H–C(7)/C(9), and H–C(7)/C(17) further confirmed the presence of the trisubstituted C(7)=C(8) bond. Another difference was that the HO–C(1) of **1** was replaced by an AcO group in **2**, which was confirmed by the HMBC cross-peak H–C(1)/C=O of the AcO group. The ROESY correlations H–C(1)/Me(20) and H–C(11)/Me(20) indicated the same β-orientation of AcO–C(1) and HO–C(11). Accordingly, the structure of splendidin B was established.

Splendidin C (**3**), obtained as yellow gum, had a molecular formula of $C_{28}H_{36}O_{11}$, determined by the HR-ESI-MS (*m/z* 571.2152 ($[M+Na]^+$; calc. 571.2155)). The IR absorptions indicated the presence of OH (3446 cm^{-1}) and C=O (1767 and 1735 cm^{-1}) groups. The 1D-NMR spectra (*Tables 1* and *2*) exhibited signals of three Me groups at δ(H) 0.82 (*s*, Me(20)), 1.17 (*d*, $J=5.6$, Me(17)), and 2.01 (*s*, AcO–C(12)), of a furan ring at δ(H) 6.40 (*d*, $J=1.2$, H–C(14)), 7.38 (*d*, $J=1.2$, H–C(15)), and 7.42 (*s*, H–C(16)); δ(C) 125.8 (*s*, C(13)), 108.4 (*d*, C(14)), 143.3 (*d*, C(15)), and 139.7 (*d*, C(16)), of a trisubstituted C=C bond at δ(C) 135.5 (*d*, C(3)) and 138.5 (*s*, C(4)), of a D-ribohexo-3-ulopyranoside moiety [9][10] at δ(H) 4.45 (*d*, $J=6.4$, H–C(1′)), 4.22 (*d*, $J=6.4$, H–C(2′)), 4.37 (*d*, $J=7.6$, H–C(4′)), 3.36 (*d*, $J=6.8$, H–C(5′)), 4.03 (*d*, $J=9.6$, H_a–C(6′)), and 3.91 (*dd*, $J=9.6$, 6.8, H_b–C(6′)); δ(C) 101.4 (*d*, C(1′)), 76.7 (*d*, C(2′)), 206.6 (*s*, C(3′)), 72.5 (*d*, C(4′)), 76.0 (*d*, C(5′)), and 61.9 (*t*, C(6′)), and of a pair of CH_2O H-atoms at δ(H) 5.34 (*d*, $J=5.6$, H_a–C(19)) and 3.84 (*d*, $J=5.6$, H_b–C(19)). The above mentioned data showed similarities to those of salvisplendin C [7], a clerodane diterpenoid isolated from the same plant, except that an OH group was replaced by a sugar moiety. The HMBC H–C(1′)/C(7) confirmed that the D-ribohexo-3-ulopyranoside moiety was located at C(7).

The relative configuration of **3** was determined by analysis of the ROESY spectrum (*Fig. 2*) and the coupling constant. The β-configuration of the sugar was deduced from the large coupling constant ($J=6.4$ Hz) of the anomeric H-atom H–C(1′) in the ^{1}H-NMR spectrum. The ROESY correlations H–C(10)/H–C(12), H–C(8)/H–C(12), and H–C(7)/H–C(10) showed that these H-atoms were β-oriented. The relative orientations of other positions in **3** were the same as those of salvisplendin C [7]. Therefore, the structure of **3** was elucidated as shown in *Fig. 2*. This was the first diterpenoid glucoside isolated from this plant.

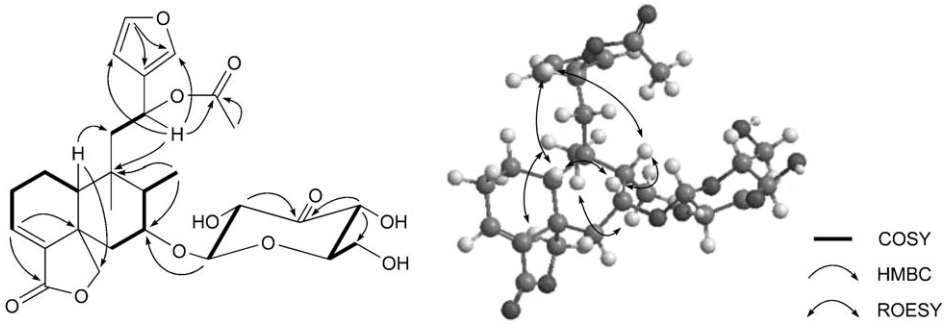


Fig. 2. *Key 2D-NMR correlations of* **3**

The structures of the known compounds were identified by comparison of their spectroscopic data with those reported in the literature.

Compounds **1**–**9** were tested for cytotoxicity against HL-60, A-549, SMMC-7721, PANC-1, and SK-BR-3 cell lines *in vitro*. However, all of them were inactive.

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Experimental Part

General. Solvents were distilled before use. Column chromatography (CC): silica gel (SiO_2; 200–300 mesh; *Qingdao Marine Chemical Inc.*, Qingdao, P. R. China), silica gel *H* (10–40 μm; *Qingdao Marine Chemical Inc.*), *Lichroprep RP-18* gel (40–63 μm; *Merck*, D-Darmstadt), or *MCI* gel (75–150 μm, *Mitsubishi Chemical Corporation*, Japan); fractions were monitored by TLC, and spots were visualized by heating SiO_2 plates sprayed with 10% H_2SO_4 in EtOH. Optical rotations: *Horiba SEPA-300* polarimeter. UV Spectra: *Shimadzu UV-2401A* spectrometer. IR Specta: *Bio-Rad FTS-135* spectrometer; KBr pellets. 1D- and 2D-NMR spectra: *Bruker AM-400* and *DRX-500* spectrometers with TMS as internal standard; unless specified, chemical shifts (δ) in ppm with reference to the solvent signals. MS: *VG Autospec-3000* spectrometer.

Plant Material. The aerial parts of *S. splendens* were collected in Kunming Institute of Botany, Yunman Province, P. R. China, in April 2009. The sample was identified by Prof. *Xi-Wen Li*, and a voucher specimen (KIB 090401) has been deposited with the State Key Laboratory of Phytochemistry and Plant Resources in West China, Kunming Institute of Botany, Chinese Academy of Sciences.

Extraction and Isolation. The air-dried and powdered aerial parts of *S. splendens* (5.5 kg) were extracted with Me_2CO (3 × 15 l, each 24 h) at r.t. and filtered. The filtrate was evaporated to give a residue, which was suspended in H_2O and then extracted with AcOEt. The AcOEt extract (185 g) was decolorized on *MCI* gel (90% MeOH) and then was subjected to CC (SiO_2; petroleum ether (PE)/Me_2CO, gradient system) to afford *Frs. 1–4*. *Fr. 2* was subjected to CC (SiO_2; PE/Me_2CO, increasing polarity) and then further purified by *Sephadex LH-20* ($CHCl_3$/MeOH 1:1) to yield **4** (500 mg), **5** (50 mg), and **6** (1200 mg). *Fr. 3* was further purified by CC (SiO_2; PE/Me_2CO 8:2) to provide *Frs. 3.1–3.3*. *Fr. 3.1* was subjected to *Sephadex LH-20* ($CHCl_3$/MeOH, 1:1) and then applied to repeated CC (SiO_2) to yield compounds **7** (15 mg), **8** (8 mg), and **9** (190 mg). *Fr. 3.2* was subjected to *Sephadex LH-20* ($CHCl_3$/MeOH, 1:1) and then further purified by CC (SiO_2; PE/Me_2CO 8:1) to yield **1** (90 mg) and **2** (120 mg). *Fr. 3.3* was subjected to CC (*RP-18*; 40 → 80% MeOH/H_2O), and further purifitied by *Sephadex LH-20* (MeOH) to give compound **3** (68 mg).

Splendidin A (=*15,16-Epoxy-1β,11β-dihydroxy-8α*H,*12β*H-*cleroda-2,13(16),14-triene-17,12:18,19-diolide* = (*3*S,*4*R,*4a*R,*4b*S,*5*R,*7a*R,*10a*S,*12a*S)-*3-(Furan-3-yl)-3,4,4a,5,7a,11,12,12a-octahydro-4,5-dihydroxy-4a-methyl-1*H-*[2]benzofuro[4,3a-*f*]isochromene-1,8(4b*H)-*dione*; **1**). White amorphous solid. $[\alpha]_D^{12.4} = -30.3$ ($c = 0.24$, $CHCl_3$). UV ($CHCl_3$): 208 (0.92). IR (KBr): 3429, 1768, 1731, 1287, 1176, 1151, 1017. ^{1}H- and ^{13}C-NMR: see *Tables 1* and *2*, resp. ESI-MS (pos.): 397 ($[M+Na]^+$). HR-ESI-MS (pos.): 397.1254 ($[M+Na]^+$, $C_{20}H_{22}NaO_7^+$; calc. 397.1263).

Splendidin B (=*15,16-Epoxy-1β*-O-*acetyl-11β-hydroxy-12β*H-*cleroda-2,7,13(16),14-tetraene-17,12:18,19-diolide* = (*3*S,*4*R,*4a*R,*4b*S,*5*R,*7a*R,*10a*S)-*3-(Furan-3-yl)-3,4,4a,4b,5,7a,8,11-octahydro-4-hydroxy-4a-methyl-1,8-dioxo-1*H-*[2]benzofuro[4,3a-*f*]isochromen-5-yl Acetate*; **2**). White amorphous solid. $[\alpha]_D^{12.9} = -168.7$ ($c = 0.13$, $CHCl_3$). UV ($CHCl_3$): 213 (1.10). IR (KBr): 3447, 2907, 1785, 1720, 1656, 1263, 1029. ^{1}H- and ^{13}C-NMR: see *Tables 1* and *2*, resp. ESI-MS (pos.): 437 ($[M+Na]^+$). HR-ESI-MS (pos.): 437.1207 ($[M+Na]^+$, $C_{22}H_{22}NaO_8^+$; calc. 437.1212).

Splendidin C (=*15,16-Epoxy-12α*-O-*acetylcleroda-3,13(16),14-triene-18,19-olide-7α*-O-*β*-D-*ribo-hexo-3-ulopyranoside* = (*1*R*)-*1-(Furan-3-yl)-2-[(6a*R,*7*R,*8*S,*9*R,*10a*S)-*9-(β*-D-ribo-*hexopyranosyl-3-uloseoxy)-3,5,6,6a,7,8,9,10-octahydro-7,8-dimethyl-3-oxonaphtho[1,8a-c]furan-7-yl]ethyl Acetate*; **3**). Yellow gum. $[\alpha]_D^{12.8} = -84.9$ ($c = 0.13$, $CHCl_3$). UV ($CHCl_3$): 208 (1.21). IR (KBr): 3446, 2932, 1767, 1735,

1372, 1240, 1032. ^{1}H- and ^{13}C-NMR: see *Tables 1* and *2*, resp. ESI-MS (pos.): 571 ($[M+Na]^+$). HR-ESI-MS (pos.): 571.2152 ($[M+Na]^+$, $C_{28}H_{36}NaO_{11}^+$; calc. 571.2155).

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