

Neo-clerodane Diterpenes from the Hallucinogenic Sage *Salvia divinorum*

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Seven new neo-clerodane diterpenes, salvidivins A (**2**), B (**3**), C (**4**), and D (**5**), salvinorins H (**6**) and I (**7**), and divinorin F (**8**), along with eight known neo-clerodane diterpenes, salvinorins A (**1**)–F, divinorins A and B, and seven other constituents, were isolated from the hallucinogenic sage *Salvia divinorum*. The structures of **1**–**7** were elucidated on the basis of 2D NMR spectroscopic studies.

The Mexican hallucinogenic sage *Salvia divinorum* Epling & Játiva (Lamiaceae), which is called “diviner’s sage” or “magic mint”, contains the neo-clerodane diterpene salvinorin A (**1**) as a hallucinogenic active constituent.^{1,2} Recently, due to an increase in the popularity of this hallucinogenic plant as a recreational drug, a number of countries have begun to regulate either or both *S. divinorum* and salvinorin A (**1**) as controlled substances.³ Salvinorin A (**1**) is a potent naturally occurring non-nitrogenous κ -opioid selective agonist, and hence it is considered to be of interest for the development of novel therapeutic agents for Alzheimer’s disease.⁴ After the hallucinogenic actions of **1** were revealed, several research groups have studied *S. divinorum* and the salvinorins, and this has resulted in an increasing number of reports on the isolation and synthesis of new neo-clerodane diterpenes during the past few years.^{5–18} Recently, two new neo-clerodane diterpenes, salvinicins A and B, were isolated from this same plant material, and it was demonstrated that salvinicin A is a partial κ -opioid agonist, whereas salvinicin B is the first μ -opioid antagonist having a neo-clerodane skeleton.^{19,20} Such research reports have shown further possibilities for the use of *S. divinorum* as a resource for new bioactive compounds, and this encouraged us to attempt the isolation of new salvinorin-like diterpenoids from this hallucinogenic sage. Herein, we report a study on the isolation and structure determination of the new compounds **1**–**7** from this plant.

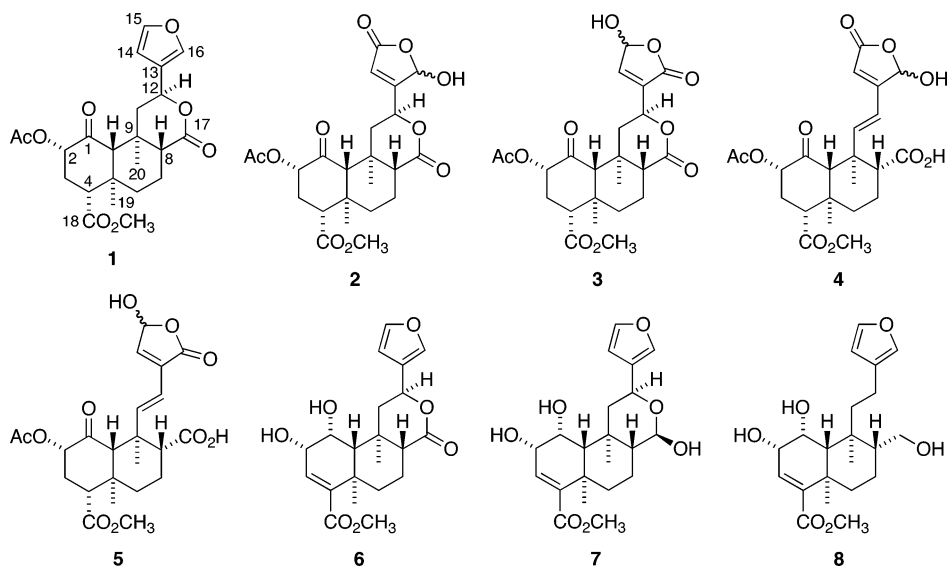
Results and Discussion

A dichloromethane-soluble portion (72 g) of the methanol extract (123 g) of the commercially available dried leaves (970 g) of *S. divinorum* was subjected to silica gel open-column chromatography using an *n*-hexane–ethyl acetate solvent mixture to afford 12 fractions. The fractions, which showed pink to purple spots on silica gel TLC using a vanillin–phosphoric acid reagent, were further separated by ODS-medium-pressure liquid chromatography (MPLC) using aqueous methanol as elution solvent. These additional fractions were then applied repeatedly to ODS-HPLC using aqueous acetonitrile as elution solvent to yield seven new neo-clerodane diterpenes, named salvidivins A (**2**), B (**3**), C (**4**), and D (**5**), salvinorins H (**6**) and I (**7**), and divinorin F (**8**). In addition to these substances, eight known neo-clerodane diterpenes, salvinorins A (**1**)–F^{1,2,5,7} and divinorins A and B,⁶ as well as six other constituents that have not been reported from this plant, nepetoidin B,^{21,22} dehydrovomifoliol,²³ isololiolide,²⁴ methyl caffate, methyl 3,4-dihydroxybenzoate, and 3,4-dihydroxybenzaldehyde, along with a previously reported compound, loliolide,²⁵ were also isolated. The structures of these known compounds were identified by comparison with their published data or with commercially available compounds.

Compounds **2** and **3** gave pseudomolecular ion peaks at m/z 465.1789 and 465.1765 $[M + H]^+$, respectively, in the HRESIMS, suggesting a molecular formula of $C_{23}H_{29}O_{10}$ in each case. The solubility of both compounds in several deuterated solvents such as $CDCl_3$, CD_3OD , and pyridine- d_5 was low, and the 1H NMR spectra measured in $DMSO-d_6$ showed broadened peaks. Therefore, the NMR spectra for their structure elucidation were measured in $CDCl_3$ – CD_3OD (ca. 1:1) mixtures. Even in this solvent mixture, some of the peaks were broadened in both the 1H and ^{13}C NMR spectra, especially in the olefinic regions. Comparison of the 1H and ^{13}C NMR spectra of **2** and **3** with those of salvinorin A (**1**) suggested that these compounds are structurally similar. In fact, the chemical shift assignments for the A and B rings, and their respective substituents of **2** and **3**, could be assigned readily by comparison to **1**; this was confirmed by analysis of the HMBC spectrum, as shown in Figure 1. The biggest problem with both the 1H and ^{13}C NMR spectra of **2** and **3** was that the signals that should have been assignable to the furan ring of **1** were extremely broad. Only one of the four carbon signals assignable to a furan ring unit of **2** appeared as a peak at δ_C 171.4 in the ^{13}C NMR spectrum. In the HSQC spectrum of **2**, a broad methine proton signal at δ_H 6.10 showed a cross-peak with a broad carbon signal that appeared at δ_C 117.8, and another broad signal at δ_H 6.18 exhibited a cross-peak with an extremely broad signal at around δ_C 98. Also in the HMBC spectrum of **2** a cross-peak was observed between one methylene proton of C-11 at δ_H 1.68 and an extremely broad carbon signal at around δ_C 167.5. In the case of **3**, all four carbon signals corresponding to the furan ring unit could not be observed as discrete peaks. In the HSQC spectrum of **3**, a broad methine proton at δ_H 7.20 showed a cross-peak with a broad carbon signal that appeared around δ_C 147.5. On the other hand, in the HMBC spectrum of **3**, the H-12 methine proton at δ_H 5.40 showed cross-peaks with broad carbon signals around δ_C 136, 147.5, and 170. However, the remaining extremely broad carbon signal at around δ_C 98 did not exhibit any cross-peaks. Although only limited information could be obtained for the furan derivative moieties of **2** and **3**, the signals around δ_C 170 were assigned to carbonyl carbons. Further, a quaternary carbon at C-13 (δ_C 167.5 for **2**, δ_C 136 for **3**) suggested one double bond in the moiety, and the remaining carbon signal around δ_C 98 could be assigned to a hemiacetal. From the HMBC correlations depicted in Figure 1, the structures of **2** and **3** were assigned as shown. Therefore, it is concluded that the furan derivative moieties of both **2** and **3** represent a pair of geometrical isomers of the γ -hydroxy- α,β -unsaturated γ -lactone, which differ from each other at the linkage position to C-12; **2** is linked at the β -position, whereas **3** is linked at the α -position from the carbonyl carbon of the lactone. The relative configuration of **2** and **3** was determined by ROESY NMR correlations, as shown in Figure 1, except for the hemiacetal proton,

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Chart 1



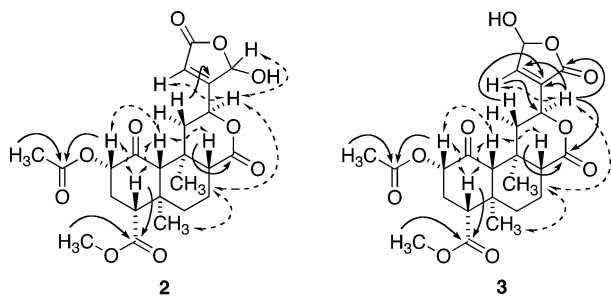
which did not result in any informative cross-peaks. Thus, structures **2** and **3**, respectively, were proposed for salvidivins A and B.

Compounds **4** and **5** were confirmed to have the same molecular formula as **2** and **3**, respectively, by measurement of their HRESIMS data. Their NMR spectra obtained in CD₃OD showed essentially the same pattern; further, **4** and **5** showed differences in the chemical shifts in the low-field region in both their ¹H and ¹³C NMR spectra. In the ¹H NMR spectra, one set of *trans* olefinic protons (δ_{H} 6.33, 6.50, $J = 16.3$ Hz for **4**; δ_{H} 6.13, 6.72, $J = 16.2$ Hz for **5**) was observed, and these were assigned as protons of a double bond between C-11 and C-12 since HMBC correlations were observed with C-8, C-9, C-10, and C-20. These olefinic protons also had HMBC correlations with carbons at δ_{C} 99.9 and 164.3 for **4** and at δ_{C} 133, 144.5, and 172.1 for **5**, as shown in Figure 2; these carbons were assigned as furan ring resonances connected to C-12. Further, HMBC NMR spectroscopic analysis revealed the presence of the same γ -hydroxy- α,β -unsaturated γ -lactone moiety in **2** and **3**. The HMBC spectrum of **5** also confirmed a C-17 carboxylic acid group instead of the lactone ring in the case of **1–3**, whereas the HMBC spectrum of **4** failed to give long-range correlations from any proton to a carbon at δ_{C} 172.6 assigned to C-17. Similar to the ROESY NMR spectra of **2** and **3**, the ROESY spectra of **4** and **5** revealed the same relative configuration for the A and B rings as **1**, as shown in Figure 2. From these data, the structures of **4** and **5** were determined for salvidivins C and D, respectively. In the ¹H NMR spectrum of **4**, H-10 had a split peak, and broad signals of H-8 and H-12 were observed. H-12 showed ROESY correlations with H-14, H-16, and H-20, whereas H-11 exhibited correlations with H-8, H-10, H-14, H-16, and H-20 in the ROESY spectrum of **4**. These observations suggested that rotational conformers with respect to bonds between C-9 and C-11 and between C-12 and C-13 are evident. A similar consideration

also applied to **5**, in which both H-11 and H-12 showed ROESY correlations with H-8, H-10, H-14, and H-20. The same applied to **2** and **3**, which showed broad signals around the γ -lactone moiety.

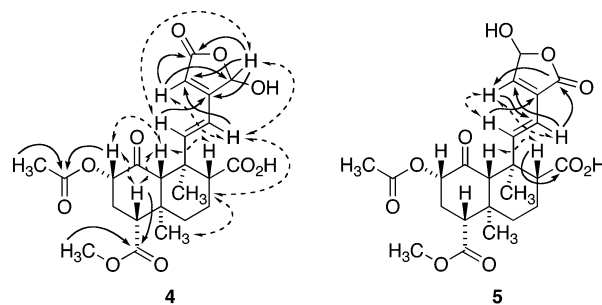
Compound **6** gave a pseudomolecular ion peak at m/z 391.1779 [M + H]⁺ in the HRESIMS, suggesting a molecular formula of C₂₁H₂₇O₇. In the ¹H NMR spectrum, four olefinic protons, of which three were a pair of normal furan rings as in **1**, an absence of acetyl methyl protons, and one additional oxymethine proton were assigned by a general comparison with **1**. Moreover, in the ¹³C NMR spectrum, the absence of a carbonyl carbon, which was assignable to C-1, and the appearance of one additional double bond were evident by comparison with **1**. Further, observations from the HMBC and ROESY NMR spectra, shown in Figure 3, supported the structure of **6** as salvinorin H, which is the deacetylated derivative of salvinorins C–E.^{5,7}

Compound **7** showed pseudomolecular ion peaks at m/z 807.3 [2M + Na]⁺ and 415.2 [M + Na]⁺ in the ESIMS and a peak at m/z 415.1783 [M + Na]⁺ in the HRESIMS, suggesting a molecular formula of C₂₁H₂₈O₇. In the ¹H NMR spectrum, the appearance of two oxymethine protons assignable to H-1 and H-2 and four olefinic protons assignable to a furan ring and the H-3 methine resembled those of **6** fairly closely. One oxymethine proton assignable to H-12 was shifted upfield and one additional oxymethine appeared at δ_{H} 4.72, in contrast to **6**. The additional oxymethine signal was finally assigned as H-17, since HMBC correlations were observed between the oxymethine proton and C-8, and C-9 and between the oxymethine carbon at δ_{C} 95.5 and H-8, as shown in Figure 3. Therefore, it was concluded that the lactone ring in **6** is partially reduced to a cyclic acetal in the case of **7**. The relative stereochemistry of the acetal hydroxyl group was assigned with β -orientation since ROESY NMR correlations were observed between H-12,



—: HMBC correlations - - - : ROESY correlations

Figure 1. Principal HMBC and ROESY correlations of **2** and **3**.



—: HMBC correlations - - - : ROESY correlations

Figure 2. Principal HMBC and ROESY correlations of **4** and **5**.

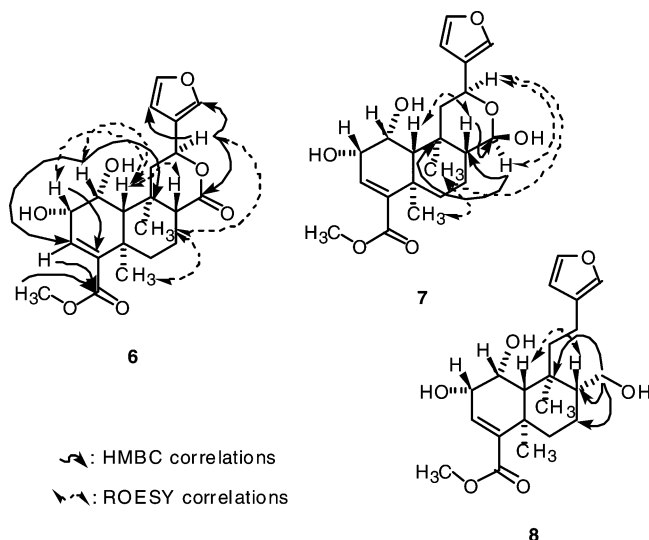


Figure 3. Principal HMBC and ROESY correlations of **6**–**8**.

H-17, and H-20. On the basis of these data, the structure **7** was proposed for salvinorin I.

Compound **8** was assigned a molecular formula of $C_{21}H_{30}O_6$, as suggested from the pseudomolecular ion peaks at m/z of 779.4 [$2M + Na$] $^+$ and 401.2 [$M + Na$] $^+$ in the ESIMS and a peak at m/z of 401.1952 [$M + Na$] $^+$ in the HRESIMS. In the 1H NMR spectrum, the appearance of two oxymethine protons assignable to H-1 and H-2 and four olefinic protons assignable to a furan ring and H-3 methines closely resembled those of **6** and **7**, although one set of oxymethylene protons was observed instead of the disappearance of the H-12 oxymethine proton. A detailed HMBC spectroscopic analysis led to the conclusion that **8** is a divinatorin-type neo-clerodane diterpene that possesses a decalin ring and a furan ring without a lactone ring.^{6,12} ROESY NMR spectroscopic analyses confirmed the relative stereochemistry of this isolate. Consequently, the structure **8** was proposed for divinatorin F.

In conclusion, seven new neo-clerodane diterpenes have been isolated from commercially available *S. divinorum*. Salvidivins A (**2**), B (**3**), C (**4**), and D (**5**) are unique neo-clerodane diterpenes that possess a γ -hydroxy- α,β -unsaturated γ -lactone moiety, and the pairs **2** and **3**, and **4** and **5**, respectively, are geometrical isomers at the γ -lactone moiety. It appears that **2** and **3** are important precursors of salvinicins A and B; salvinicin A is described as being a partial agonist of the κ -opioid receptor, whereas salvinicin B is reported to be the first μ -opioid antagonist having a neo-clerodane skeleton.^{19,20} Salvinorin H (**6**) has a 1,2-dihydroxy substitution on the A ring. The occurrence of salvinorin H (**6**) was predicted previously;¹³ this is because similar salvinorins acetylated at C-1 (salvinorin D), at C-2 (salvinorin E), or at both sites (salvinorin C) have already been isolated from *S. divinorum*.^{5,7} Salvinorin I (**7**) is a derivative of **6**, which is partially reduced at C-17, while divinatorin F (**8**) seems to be a precursor of **6**; these three substances have the same A, B ring substitution pattern in their molecules. It would be interesting to ascertain whether or not any of these new compounds also exhibit agonist/antagonist activities against various types of opioid receptors.

Experimental Section

General Experimental Procedures. Melting points were determined on a Yanaco MP-J3 micro melting point apparatus and are uncorrected. Optical rotations were measured with a JASCO P-1030 polarimeter. UV, CD, and IR spectra were obtained with a JASCO V-560 UV/vis spectrophotometer, a JASCO J-820 spectropolarimeter, and a JASCO FT/IR-6300 spectrometer with ATR option, respectively. 1D and 2D 1H and ^{13}C NMR spectra were recorded on a Varian Unity INOVA 500 spectrometer at 300 K using Varian standard pulse sequences. Phase-sensitive ROESY experiments were conducted with a mixing time of 300 ms. A 3.57 ms (140 Hz) delay was used to optimize one-bond coupling in the HSQC spectra and suppress it in the HMBC spectra, and the evolution delay for long-range couplings in the HMBC spectra was set to 62.5 ms (8 Hz). ESITOFMS and HRESITOFMS were obtained on a Q-TOF micro-mass spectrometer (Micromass/Waters). Silica gel open-column chromatography was performed on silica gel 60 (Merck). Medium-pressure liquid chromatography (MPLC)

Table 1. 1H NMR (500 MHz) and ^{13}C NMR (125 MHz) Spectroscopic Data for **2** and **3**

position	salvidivin A (2) in $CDCl_3$ – CD_3OD , 1:1		salvidivin B (3) in $CDCl_3$ – CD_3OD , 1:1	
	δ_C , mult.	δ_H , mult. (J in Hz)	δ_C , mult.	δ_H , mult. (J in Hz)
1	203.4, qC		203.4, qC	
2	76.0, CH	5.20, dd (7.3, 12.2)	76.0, CH	5.20, dd (7.6, 12.5)
3	31.3, CH_2	2.27, q-like (13.2)	31.4, CH_2	2.25, q-like (12.9)
		2.34, ddd (3.9, 7.6, 13.2)		2.31, ^d dt (3.7, 7.6)
4	53.6, CH	2.90, dd (3.7, 13.2)	53.6, CH	2.92, dd (3.7, 13.2)
5	42.6		42.6, qC	
6	38.3, CH_2	1.68, ^c br-t (12.5)	35.9, CH_2	1.68, ^c br-t (12.2)
		1.79, br-dd (2.9, 10.0)		1.79, br-dt (3.1, 13.1)
7	18.7, CH_2	1.62, br-dt (2.9, 13.2)	18.7, CH_2	1.61, br-dt (3.5, 13.3)
		2.12, br-d (10.5)		2.12, br-dd (3.2, 13.7)
8	51.2, ^a CH	2.36, br-dd (2.7, 11.5)	51.1, CH	2.35, ^d br-dd (3.4, 10.5)
9	35.9, ^a qC		38.4, qC	
10	63.4, CH	2.45, ^d s	63.5, CH	2.47, s
11	41.0, ^a CH_2	1.68, ^c br-t (12.5)	40.6, CH_2	1.68, ^c br-t (12.2)
		2.45, ^d br-s		2.46, dd (5.7, 13.4)
12	73.9, ^a CH	5.49, br-s	72.6, ^a CH	5.40, dd (5.7, 11.8)
13	167.5, ^b qC		136, ^b qC	
14	117.8, ^a CH	6.10, br-s	147.5, ^b CH	7.20, br-s
15	171.4, qC		98, ^b qC	6.18, br-s
16	98, ^b CH	6.18, br-s	170, ^b CH	
17	172.1, qC		172.6, qC	
18	172.8, qC		172.9, qC	
19	16.7, CH_3	1.11, s	16.6, CH_3	1.10, s
20	15.3, ^a CH_3	1.43, s	15.2, CH_3	1.43, s
$OCOCH_3$ (2)	171.2, qC		171.0, qC	
$OCOCH_3$ (2)	20.7, CH_3	2.18, s	20.6, CH_3	2.15, s
$COOCH_3$ (4)	52.3, CH_3	3.74, s	52.2, CH_3	3.73, s

^a Carbon signal appeared as a broad signal. ^b Carbon signal extremely broad or did not appear clearly; chemical shift value determined from HSQC and/or HMBC spectra. ^c Signals superimposed on each other. ^d Assignments may be exchanged with each other.

Table 2. ¹H NMR (500 MHz) and ¹³C NMR (125 MHz) Spectroscopic Data for **4** and **5**

position	salvidivin C (4) in CD ₃ OD		salvidivin D (5) in CD ₃ OD	
	δ _C , mult.	δ _H , mult. (<i>J</i> in Hz)	δ _C , mult.	δ _H , mult. (<i>J</i> in Hz)
1	203.9, qC		203.9, qC	
2	77.0, CH	5.19, dd (7.1, 12.2)	76.9, CH	5.20, dd (7.2, 12.6)
3	32.2, CH ₂	2.15, q-like (13.0)	32.2, CH ₂	2.15, q-like (12.9)
4	54.1, ^a CH	2.29, ddd (3.5, 7.2, 13.1)		2.28, ddd (3.5, 7.2, 12.9)
5	43.6, qC	3.01, dd (3.4, 13.4)	54.0, CH	3.02, dd (3.5, 13.3)
6	38.8, CH ₂	1.73, ^a m	43.4, qC	
7	22.0, CH ₂	1.73, ^a m	38.8, CH ₂	1.72, br-d (7.3)
8	54.1, ^a CH	1.78, br-ddd (3.2, 3.4, 11.1)	22.1, CH ₂	1.75, br-dd (3.4, 13.4)
9	42.5, qC	1.95, br-ddt (5.9, 12.5, 12.7)		1.94, m
10	61.6, CH 61.3, CH	2.42, br-s	54.2, CH	2.41, dd (2.9, 12.5)
11	152.6, CH		42.1, qC	
12	120.4, CH	2.81, 2.87, br-s	61.7, CH	2.81, s
13	164.3, qC	6.50, d (16.3)	147.5, CH	6.72, d (16.2)
14	116.5, CH	6.33, br-d (16.3)	118.2, CH	6.13, d (16.2)
15	173.7, ^b qC		133.0, qC	
16	99.9, CH	5.92, s	144.5, CH	7.03, s
17	176.2, qC		98.4, CH	6.04, br-s
18	173.6, ^b qC	6.13, s	172.1, qC	
19	16.4, CH ₃	1.05, s	176.3, qC	
20	15.9, CH ₃	1.56, s	173.7, qC	
OCOCH ₃ (2)	171.6, qC		16.5, CH ₃	1.06, s
OCOCH ₃ (2)	20.5, CH ₃	2.09, s	15.8, CH ₃	1.55, s
COOCH ₃ (4)	52.2, CH ₃	3.70, s	171.5, qC	
			20.5, CH ₃	2.08, s
			52.2, CH ₃	3.70, s

^a Signals superimposed on each other. ^b Assignments may be exchanged with each other.

Table 3. ¹H NMR (500 MHz) and ¹³C NMR (125 MHz) Spectroscopic Data for **6–8**

position	salvinorin H (6) in CDCl ₃ –CD ₃ OD = 1:1		salvinorin I (7) in CD ₃ OD		divinatorin F (8) in CDCl ₃	
	δ _C , mult.	δ _H , mult. (<i>J</i> in Hz)	δ _C	δ _H , mult. (<i>J</i> in Hz)	δ _C	δ _H , mult. (<i>J</i> in Hz)
1	66.1, CH	4.25, d (4.9)	66.5, CH	4.22, d (4.8)	66.3, CH	4.30, d (4.6)
2	70.3, CH	4.17, dd (2.2, 4.9)	71.1, CH	4.14, dd (2.2, 4.8)	70.0, CH	4.24, dd (2.3, 4.8)
3	138.1, CH	6.49, d (2.2)	138.6, CH	6.45, ^b d (2.2)	135.3, CH	6.45, d (2.3)
4	141.9, qC		142.7, qC		143.0, qC	
5	38.3, ^a qC		39.2, qC		38.0, qC	
6	37.7, CH ₂	1.18, dt (3.4, 13.2)	39.3, CH ₂	1.13, br-dt (1.1, 8.6)	37.7, CH ₂	1.15, dt (3.5, 12.9)
7	19.3, CH ₂	2.44, dt (3.4, 13.2)		2.39, dt (3.4, 12.9)		2.32, dt (3.3, 12.9)
8	52.2, CH	1.77, br-dq (2.8, 13.5)	19.4, CH ₂	1.53, dq (3.4, 13.4)	21.8, CH ₂	1.54, dq (3.4, 12.9)
9	38.2, ^a qC	2.00, br-dq (3.4, 14.4)		1.78, dq (3.4, 13.5)		1.85, m
10	54.5, CH	2.44, br-dd (2.7, 12.2)	54.7, CH	1.19, br-dt (2.2, 5.8)	44.5, CH	1.60, m
11	44.6, CH ₂		38.3, qC		38.8, qC	
12	73.3, CH	1.28, br-s	56.2, CH	1.16, br-s	48.1, CH	1.42, br-s
13	126.8, qC	1.69, br-d (10.3)	46.9, CH ₂	1.26, br-t (12.1)	38.9, CH ₂	1.78, br-dt (4.4, 12.7)
14	109.3, CH	2.51, dd (6.1, 13.2)		1.95, dd (3.2, 12.1)		1.88, br-dt (5.1, 15.1)
15	144.7, CH	5.63, dd (5.9, 11.0)	67.6, CH	4.98, dd (2.3, 11.6)	18.3, CH ₂	2.07, br-dt (4.7, 13.5)
16	140.6, CH					4.84, br-dt (4.2, 13.5)
17	174.5, qC		128.7, qC		124.8, qC	
18	168.1, qC	6.47, d (1.5)	110.1, CH	6.46, ^b t (0.9)	110.9, CH	6.25, d (0.7)
19	22.2, CH ₃	7.45, d (1.7)	144.1, CH	7.42, t (1.6)	142.9, CH	7.34, d (1.6)
20	16.4, CH ₃	7.52, t (0.7)	140.4, CH	7.48, d (0.7)	138.5, CH	7.20, s
COOCH ₃ (4)	51.9, CH ₃		95.5, CH	4.72, d (9.0)	63.9, CH ₂	3.38, dd (8.1, 10.5)
						3.83, dd (3.7, 10.5)
			168.5, qC		167.1, qC	
			22.8, CH ₃	1.68, s	21.9, CH ₃	1.65, s
			16.5, CH ₃	1.45, s	21.2, CH ₃	1.18, s
			51.9, CH ₃	3.69, s	51.6, CH ₃	3.72, s

^a Assignments may be exchanged with each other. ^b Signals superimposed on each other.

was performed with a prepacked glass column (Ultra Pack: 26 mm i.d. × 300 mm for medium-scale separation, 50 mm i.d. × 300 mm for large-scale separation; Yamazen Corporation, Kyoto, Japan) packed with 50 μm octadecyl silica gel (ODS). HPLC was performed with an Inertsil PREP-ODS column (6 mm i.d. × 250 mm for analysis, 20 mm i.d. × 250 mm for preparative; GL Science Inc., Tokyo, Japan) packed with 10 μm ODS. TLC was conducted on precoated silica gel 60 F₂₅₄ (Merck) and/or RP-18 F_{254s} (Merck), and the spots were detected by heating after spraying with vanillin–phosphoric acid reagent.

Plant Material. Dried *S. divinorum* leaves were purchased in June 2005 from Ethnogens.com (Lawrence, KS). Voucher specimens were deposited at the Medicinal Herbarium, Faculty of Pharmaceutical

Sciences at Kagawa campus, Tokushima Bunri University, specimen # 050601-001.

Extraction and Isolation. Commercial dried *S. divinorum* leaves (970 g) were powdered and extracted with MeOH three times around 40 to 50 °C, and the MeOH solution was evaporated in vacuo under 40 °C to yield a MeOH extract (123 g). The extract was partitioned between CH₂Cl₂ and water three times and evaporated to give a CH₂Cl₂-soluble portion (72 g). The CH₂Cl₂-soluble portion was then chromatographed over a silica gel open column (0.6 kg; 50 × 540 mm) eluted with an *n*-hexane–EtOAc gradient solvent system (10:0, 8:2, 1:1, 0:10, then MeOH) to yield 12 fractions. Fraction (Fr.) numbers 4 to 10 showed pink to purple spots by TLC (*n*-hexane–

EtOAc, 1:1) by spraying with vanillin–phosphoric acid spray reagent. The positive fractions were separated by ODS MPLC with aqueous MeOH as elution solvent. The MPLC-derived fractions were further purified by ODS HPLC with aqueous acetonitrile as elution solvent to yield seven new compounds: salvidivins A (**2**, 85 mg) and B (**3**, 80 mg) from Fr. 5–8 eluted with 35% acetonitrile; salvidivins C (**4**, 41 mg) and D (**5**, 42 mg) from Fr. 5 eluted with 33% acetonitrile; salvinorin H (**6**, 120 mg) from Fr. 5–7 eluted with 35% acetonitrile; and salvinorin I (**7**, 6.5 mg) and divinatorin F (**8**, 15 mg) from Fr. 5 eluted with 33% and 35% acetonitrile, respectively. The known salvinorins A (**1**, 2.0 g), B (37 mg), C (176 mg), D (180 mg), E (28 mg), and F (100 mg) and divinatorins A (219 mg) and B (29 mg) were also isolated from Fr. 4–10. Along with them, seven other known constituents, nepetoidin B (15 mg), dehydromifoliol (1.5 mg), isololiolide (5.4 mg), methyl caffeate (11 mg), methyl 3,4-dihydroxybenzoate (2.9 mg), 3,4-dihydroxybenzaldehyde (2.7 mg), and loliolide (26 mg), were isolated from Fr. 4–10.

Salvidivin A (2): amorphous solid; mp 217–222 °C; $[\alpha]_D^{24}$ –69.6 (c 0.28, CHCl₃); UV (MeOH) λ_{\max} (log ϵ) 206 (4.07), 253 (sh, 2.88) nm; CD (MeOH) λ_{\max} ($\Delta\epsilon$) 292 (–1.6), 225 (–3.9) nm; IR (ATR) 3324, 2932, 1725, 1456, 1376, 1277, 1230, 1198, 1165, 1134, 1083, 1048, 951, 893, 861, 783, 688, 605 cm^{–1}; ¹H NMR (CDCl₃–CD₃OD, 1:1, 500 MHz) and ¹³C NMR (CDCl₃–CD₃OD, 1:1, 125 MHz), see Table 1; ESITOFMS m/z 929.4 (15, [2M + H]⁺), 465.2 (7, [M + H]⁺), 447.2 (100, [M – H₂O]⁺); HRESITOFMS m/z 465.1789 (calcd for C₂₃H₂₉O₁₀, 465.1761).

Salvidivin B (3): amorphous solid; mp 216–221 °C; $[\alpha]_D^{24}$ –54.2 (c 0.28, MeOH); UV (MeOH) λ_{\max} (log ϵ) 203.5 (3.92), 248 (sh, 3.15) nm; CD (MeOH) λ_{\max} ($\Delta\epsilon$) 291.5 (–1.9), 222 (–4.4) nm; IR (ATR) 3462, 2955, 1772, 1729, 1702, 1456, 1378, 1276, 1210, 1164, 1147, 1092, 1048, 1005, 935, 886, 770 cm^{–1}; ¹H NMR (CDCl₃–CD₃OD, 1:1, 500 MHz) and ¹³C NMR (CDCl₃–CD₃OD, 1:1, 125 MHz), see Table 1; ESITOFMS m/z 929.4 (55, [2M + H]⁺), 465.2 (17, [M + H]⁺), 387.1 (100, [M – H₂O – CO₂Me]⁺); HRESITOFMS m/z 465.1765 (calcd for C₂₃H₂₉O₁₀, 465.1761).

Salvidivin C (4): amorphous solid; mp 123–127 °C; $[\alpha]_D^{22}$ –133.3 (c 0.22, CHCl₃); UV (MeOH) λ_{\max} (log ϵ) 269 (3.93) nm; CD (MeOH) λ_{\max} ($\Delta\epsilon$) 303 (0.2), 266.5 (–3.3), 226 (1.4); IR (ATR) 2953, 1717, 1645, 1437, 1375, 1234, 1169, 1126, 1048, 948, 889, 772 cm^{–1}; ¹H NMR (CD₃OD, 500 MHz) and ¹³C NMR (CD₃OD, 125 MHz), see Table 1; ESITOFMS m/z 929.4 (15, [2M + H]⁺), 465.2 (14, [M + H]⁺), 447.2 (100, [M – H₂O]⁺); HRESITOFMS m/z 465.1765 (calcd for C₂₃H₂₉O₁₀, 465.1761).

Salvidivin D (5): amorphous solid; mp 185–193 °C; $[\alpha]_D^{24}$ –107.2 (c 0.27, MeOH); UV (MeOH) λ_{\max} (log ϵ) 261 (3.95) nm; CD (MeOH) λ_{\max} ($\Delta\epsilon$) 261.5 (–3.2), 225 (–3.6) nm; IR (ATR) 3381, 2953, 1770, 1729, 1705, 1684, 1438, 1384, 1340, 1281, 1225, 1082, 1009, 928, 774, 662 cm^{–1}; ¹H NMR (CD₃OD, 500 MHz) and ¹³C NMR (CD₃OD, 125 MHz), see Table 1; ESITOFMS m/z 929.4 (2, [2M + H]⁺), 465.2 (12, [M + H]⁺), 387.1 (100, [M – H₂O – CO₂Me]⁺); HRESITOFMS m/z 465.1772 (calcd for C₂₃H₂₉O₁₀, 465.1761).

Salvinorin H (6): amorphous solid; mp 95–103 °C; $[\alpha]_D^{22}$ 29.1 (c 0.22, CHCl₃); UV (MeOH) λ_{\max} (log ϵ) 209.5 (4.03) nm; CD (MeOH) λ_{\max} ($\Delta\epsilon$) 257.5 (–1.5), 228 (7.0), 203.5 (–8.6) nm; IR (ATR) 3444, 2952, 1715, 1507, 1435, 1375, 1314, 1225, 1142, 1071, 1026, 949, 875, 787, 686, 601 cm^{–1}; ¹H NMR (CDCl₃–CD₃OD, 1:1, 500 MHz) and ¹³C NMR (CDCl₃–CD₃OD, 1:1, 125 MHz), see Table 2; ESITOFMS m/z 391.2 (42, [M + H]⁺), 373.2 (100, [M – H₂O]⁺); HRESITOFMS m/z 391.1779 (calcd for C₂₁H₂₇O₇, 391.1757).

Salvinorin I (7): amorphous solid; mp 217–220 °C; $[\alpha]_D^{22}$ –4.2 (c 0.11, MeOH); UV (MeOH) λ_{\max} (log ϵ) 210.5 (3.99) nm; CD (MeOH) λ_{\max} ($\Delta\epsilon$) 258.5 (–1.1), 228 (9.2), 203 (–6.9) nm; IR (ATR) 3400, 2945, 1692, 1541, 1507, 1438, 1240, 1174, 1130, 1085, 1039, 1022, 1000, 968, 875, 808, 684, 602 cm^{–1}; ¹H NMR (CD₃OD, 500 MHz) and ¹³C NMR (CD₃OD, 125 MHz), see Table 2; ESITOFMS m/z 807.3 (30, [2M + Na]⁺), 415.2 (32, [M + Na]⁺), 375.2 (22, [M – H₂O]⁺), 255.1 (100); HRESITOFMS m/z 415.1783 (calcd for C₂₁H₂₈O₇Na, 415.1733).

Divinatorin F (8): amorphous solid; mp 97–99 °C; $[\alpha]_D^{23}$ 8.4 (c 0.12, MeOH); UV (MeOH) λ_{\max} (log ϵ) 213 (3.99) nm; CD (MeOH)

λ_{\max} ($\Delta\epsilon$) 260.5 (–0.9), 227.5 (10.2), 202 (–8.9) nm; IR (ATR) 3395, 2928, 2878, 1704, 1434, 1226, 1164, 1055, 1026, 1007, 873, 777, 600 cm^{–1}; ¹H NMR (CDCl₃, 500 MHz) and ¹³C NMR (CDCl₃, 125 MHz), see Table 2; ESITOFMS m/z 779.4 (18, [2M + Na]⁺), 401.2 (45, [M + Na]⁺), 343.2 (61), 311.2 (100); HRESITOFMS m/z 401.1952 (calcd for C₂₁H₃₀O₆Na, 401.1940).

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References and Notes

- Ortega, A.; Blount, J. F.; Manchand, P. S. *J. Chem. Soc., Perkin Trans. 1* **1982**, 2505–2508.
- Valdes, L. J., III; Butler, W. M.; Hatfield, G. M.; Paul, A. G.; Koreeda, M. *J. Org. Chem.* **1984**, *49*, 4716–4720.
- Giroud, C.; Felber, F.; Augsburg, M.; Horisberger, B.; Rivier, L.; Mangin, P. *Forensic Sci. Int.* **2000**, *112*, 143–150.
- Sheffler, D. J.; Roth, B. L. *Trends Pharmacol. Sci.* **2003**, *24*, 107–109.
- Valdes, L. J., III; Chang, H.-M.; Visger, D. C.; Koreeda, M. *Org. Lett.* **2001**, *3*, 3935–3937.
- Bigham, A. K.; Munro, T. A.; Rizzacasa, M. A.; Robins-Browne, R. M. *J. Nat. Prod.* **2003**, *66*, 1242–1244.
- Munro, T. A.; Rizzacasa, M. A. *J. Nat. Prod.* **2003**, *66*, 703–705.
- Beguín, C.; Richards, M. R.; Wang, Y.; Chen, Y.; Liu-Chen, L.-Y.; Ma, Z.; Lee, D. Y. W.; Carlezon, W. A.; Cohen, B. M. *Bioorg. Med. Chem. Lett.* **2005**, *15*, 2761–2765.
- Harding, W. W.; Tidgewell, K.; Byrd, N.; Cobb, H.; Dersch, C. M.; Butelman, E. R.; Rothman, R. B.; Prisinzano, T. E. *J. Med. Chem.* **2005**, *48*, 4765–4771.
- Lee, D. Y. W.; He, M.; Kondaveti, L.; Liu-Chen, L.-Y.; Ma, Z.; Wang, Y.; Chen, Y.; Li, J.-G.; Beguin, C.; Carlezon, W. A.; Cohen, B. *Bioorg. Med. Chem. Lett.* **2005**, *15*, 4169–4173.
- Lee, D. Y. W.; Karnati, V. V. R.; He, M.; Liu-Chen, L.-Y.; Kondaveti, L.; Ma, Z.; Wang, Y.; Chen, Y.; Beguin, C.; Carlezon, W. A.; Cohen, B. *Bioorg. Med. Chem. Lett.* **2005**, *15*, 3744–3747.
- Lee, D. Y. W.; Ma, Z.; Liu-Chen, L.-Y.; Wang, Y.; Chen, Y.; Carlezon, W. A.; Cohen, B. *Bioorg. Med. Chem.* **2005**, *13*, 5635–5639.
- Munro, T. A.; Goetchius, G. W.; Roth, B. L.; Vortherms, T. A.; Rizzacasa, M. A. *J. Org. Chem.* **2005**, *70*, 10057–10061.
- Munro, T. A.; Rizzacasa, M. A.; Roth, B. L.; Toth, B. A.; Yan, F. *J. Med. Chem.* **2005**, *48*, 345–348.
- Beguín, C.; Richards, M. R.; Li, J.-G.; Wang, Y.; Xu, W.; Liu-Chen, L.-Y.; Carlezon, W. A.; Cohen, B. M. *Bioorg. Med. Chem. Lett.* **2006**, *16*, 4679–4685.
- Harding, W. W.; Schmidt, M.; Tidgewell, K.; Kannan, P.; Holden, K. G.; Dersch, C. M.; Rothman, R. B.; Prisinzano, T. E. *Bioorg. Med. Chem. Lett.* **2006**, *16*, 3170–3174.
- Lee, D. Y. W.; He, M.; Liu-Chen, L.-Y.; Wang, Y.; Li, J.-G.; Xu, W.; Ma, Z.; Carlezon, W. A.; Cohen, B. *Bioorg. Med. Chem. Lett.* **2006**, *16*, 5498–5502.
- Tidgewell, K.; Harding, W. W.; Lozama, A.; Cobb, H.; Shah, K.; Kannan, P.; Dersch, C. M.; Parrish, D.; Deschamps, J. R.; Rothman, R. B.; Prisinzano, T. E. *J. Nat. Prod.* **2006**, *69*, 914–918.
- Harding, W. W.; Tidgewell, K.; Schmidt, M.; Shah, K.; Dersch, C. M.; Snyder, J.; Parrish, D.; Deschamps, J. R.; Rothman, R. B.; Prisinzano, T. E. *Org. Lett.* **2005**, *7*, 3017–3020.
- Harding, W. W.; Schmidt, M.; Tidgewell, K.; Kannan, P.; Holden, K. G.; Gilmour, B.; Navarro, H.; Rothman, R. B.; Prisinzano, T. E. *J. Nat. Prod.* **2006**, *69*, 107–112.
- Nakanishi, T.; Nishi, M.; Inada, A.; Obata, H.; Tanabe, N.; Abe, S.; Wakashiro, M. *Chem. Pharm. Bull.* **1990**, *38*, 1772–1774.
- Grayer, R. J.; Eckert, M. R.; Veitch, N. C.; Kite, G. C.; Marin, P. D.; Kokubun, T.; Simmonds, M. S. J.; Paton, A. J. *Phytochemistry* **2003**, *64*, 519–528.
- Kato, T.; Tsunakawa, M.; Sasaki, N.; Aizawa, H.; Fujita, K.; Kitahara, Y.; Takahashi, N. *Phytochemistry* **1977**, *16*, 45–48.
- Mori, K.; Khlebnikov, V. *Liebigs Ann. Chem.* **1993**, *77*–82.
- Valdes, L. J., III. *J. Nat. Prod.* **1986**, *49*, 171.

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