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Salvinicins A and B, New Neoclerodane Diterpenes from *Salvia divinorum*

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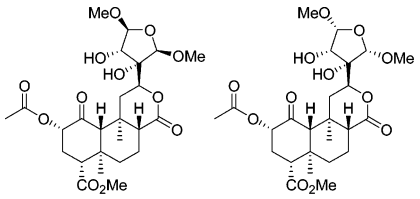
ABSTRACT



Two new neoclerodane diterpenes, salvinicins A (4) and B (5), were isolated from the dried leaves of *Salvia divinorum*. The structures of these compounds were elucidated by spectroscopic techniques, including ^{1}H and ^{13}C NMR, NOESY, HMQC, and HMBC. The absolute stereochemistry of these compounds was assigned on the basis of single-crystal X-ray crystallographic analysis of salvinicin A (4) and a 3,4-dichlorobenzoate derivative of salvinorin B.

The genus *Salvia* is one of the most widespread members of the Lamiaceae (formerly Labiatae) family and is featured prominently in the pharmacopeias of many countries throughout the world.[1] Among these is *Salvia divinorum* Epling & Játiva, a sage native to Oaxaca, Mexico. An infusion prepared from four or five pairs of fresh or dried leaves is used by the Mazatec Indians to stop diarrhea, relieve headache and rheumatism, and to treat a "semi-magical" disease known as *panzón de barrego* or swollen belly.[2] *S. divinorum* is also used in traditional spiritual practices of the Mazatecs to produce "mystical" or hallucinogenic experiences.[2]

The active ingredient in *S. divinorum* is salvinorin A (**1a**) (Figure 1).[3,4] Salvinorin A as well as salvinorin B (**1b**), were identified nearly simultaneously by Ortega and Valdés III et al.[5,6] A dose of 200−500 μg, when smoked, produces profound hallucinations lasting up to 1 h.[7,8] Interestingly, **1a** does not act at the presumed molecular target responsible for the actions of classical hallucinogens, the serotonin 5-HT$_{2A}$ receptor.[9−11] Rather, **1a** is a potent and selective κ opioid receptor agonist in vitro and in vivo.[12,13]

Presently, **1a** and *S. divinorum* are gaining popularity as recreational drugs.[14,15] Advertisements for dried *S. divinorum* leaves, as well as recipes for leaf extracts, elixirs, and

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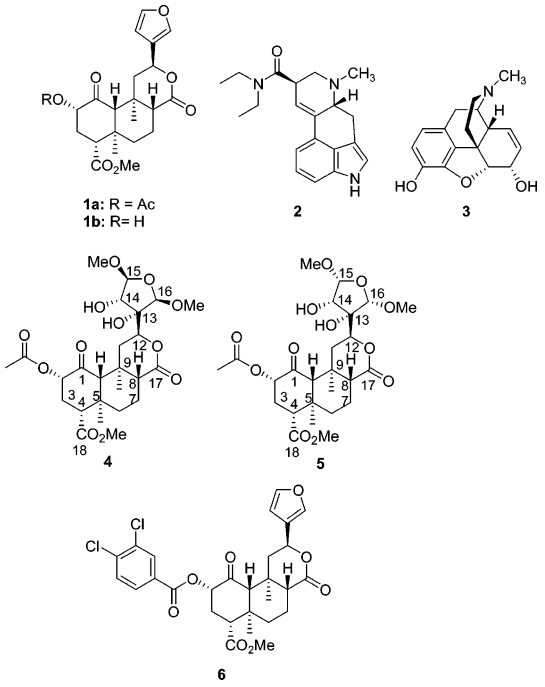

Figure 1. Structures of salvinorin A (**1a**), B (**1b**), LSD (**2**), morphine (**3**), salvinicin A (**4**), salvinicin B (**5**), and dichlorobenzoyl derivative **6**.

tinctures may be found posted on the Internet.[16] Young adults and adolescents have begun to smoke the leaves and leaf extracts of the plants to induce powerful hallucinations.[17]

Currently, *S. divinorum* is unregulated in most countries and available throughout the world for purchase over the Internet. However, it is listed as a controlled substance in Denmark, Australia, and Italy. Obtaining *S. divinorum* is easy in countries where it is unregulated, and it represents a cheap, easy solution for those who wish to experiment with drugs and perception altering substances. To date, U.S. laws for controlled substances do not ban the sale of *S. divinorum* or its active components. This has resulted in various on-line botanical companies advertising and selling *S. divinorum* as a legal alternative to other regulated plant hallucinogens. Therefore, it is predictable that its misuse will increase.

As a hallucinogen, **1a** is structurally unique. It bears no similarity to classical hallucinogens, such as LSD (**2**). Furthermore, it has no similarity to other opioid ligands, such as morphine (**3**). Given its potential for abuse, as well as its unique pharmacological properties, we[18−20] and others[21−23] have begun to study the chemistry and pharmacology associated with constituents from *S. divinorum*.

Previous phytochemical investigation of *S. divinorum* resulted in the identification of several neoclerodane diterpenes present in the leaves, salvinorins A−F and divinatorins A−C.[5,6,8,24,25] Here, we report the identification of two new neoclerodane diterpenes present in commercially available *S. divinorum* leaves.

Commercially available dried leaves of *S. divinorum* were extracted with acetone, and the acetone extract was subjected to repeated flash column chromatographies using a variety of solvent mixtures to afford salvinicins A (**4**) (65 mg) and B (**5**) (14 mg). Compound **4** gave a pseudomolecular ion peak at *m*/*z* 551.2080 ([M + Na]$^+$) in the HRESIMS suggesting a molecular formula of $C_{25}H_{36}O_{12}$ and an index of unsaturation of 8. Its IR spectrum showed absorption bands for hydroxyl (3426 cm^{-1}, broad), carbonyl (1718 cm^{-1}), and alkene (1646 cm^{-1}) functionalities.

Comparison of the ^{1}H and ^{13}C NMR spectra of **4** with those of **1a**[5,6] suggested that these compounds were structurally similar. Thus, most chemical shift assignments for the *trans*-decalin portion and ring A substituents of **4** could be determined readily by comparison with **1a**. The most striking features of the ^{1}H NMR spectrum of **4** were the absence of furanoid protons and the presence of two additional methoxyl signals (δ 3.47 and 3.36) and three additional oxymethine protons (δ 4.93, 4.70, and 4.43) as compared to **1a**.[5,6] The ^{13}C NMR spectrum showed resonances typical of dioxygenated carbons at δ 110.6 and 107.9. Two oxygen-linked carbons were observed at δ 80.4, of which one was quaternary and one was tertiary, as deduced from the DEPT-135 spectrum. HMQC analysis indicated that carbons at δ 110.6 and 107.9 were attached directly to protons at δ 4.93 and 4.70, respectively. Signals at δ 3.68 and 3.40 disappeared on addition of D_2O, suggesting the presence of two hydroxyl groups. The aforementioned spectral data suggested that **4** had lost the furan ring present in **1a** due to oxygenation. Presence of HMBC cross-peaks between the methoxyl signals at δ_H 3.47 and 3.36 and carbons at δ_C 110.6 and 107.9, respectively, allowed for placement of these groups at the α positions of the reduced furan ring. Further HMBC, HMQC, and COSY analysis enabled gross structural assignment of **4** deducing the structure shown. Assignment of

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Table 1. NMR Data for Compounds **4** and **5** in $CDCl_3$

entry	**4** δ_C	**4** δ_H (J in Hz)	HMBC[a]	**5** δ_C	**5** δ_H (J in Hz)
1	201.9		2, 3, 10	201.9	
2	74.9	5.17 dd (7.9, 12.3)	3, 4, 10	74.9	5.12 dd (8.1, 12.1)
3	30.9	2.27 m; 2.32 m	2	30.9	2.28 m; 2.31 m
4	53.4	2.74 dd (4.0, 12.8)	3, 10, 19	53.4	2.76 dd (4.3, 12.5)
5	42.0		3, 4, 6, 10, 19	42.1	
6	38.0	1.55 m; 1.77 ddd (2.9, 2.9, 10.1)	4, 8, 10, 19	38.1	1.57 m; 1.75 m
7	18.1	1.57 m; 2.08 m	8	18.2	1.57 m; 2.09 m
8	50.2	2.08 m	7, 11, 20	50.4	2.04 dd (2.9, 11.7)
9	34.6		7, 10, 11, 20	34.7	
10	64.1	2.18 s	4, 6, 11, 19, 20	64.0	2.22 s
11	35.2	1.67 dd (10.7, 12.8); 2.20 dd (6.2, 12.8)	12	35.6	1.57 m; 2.13 dd (6.1, 11.6)
12	80.4	4.82 dd (6.2, 10.7)	11, 14	77.2	4.90 dd (6.1, 11.6)
13	80.4			80.7	
14	80.0	4.43 dd (3.5, 4.5)	12, 15, 16	76.2	4.02 br s
15	110.6	4.93 d (3.5)	14, 16, 15-OCH_3	111.3	4.92 d (3.4)
16	107.9	4.70 s	12, 15, 16-OCH_3	108.4	4.94 s
17	172.2		7, 8	171.6	
18	171.6		3, 4	171.7	
19	16.1	1.08 s	4	16.2	1.07 s
20	15.3	1.33 s	8	15.1	1.35 s
2-$OCOCH_3$	169.8		2	170.7	
2-$OCOCH_3$	20.6	2.17 s		20.6	2.18 s
15-OCH_3	56.5	3.47 s	15	56.3	3.47 s
16-OCH_3	55.2	3.36 s	16	55.2	3.42 s
18-OCH_3	51.9	3.72 s		51.9	3.72 s
13-OH		3.68 s	16		4.15 br s
14-OH		3.40 d (4.5)			2.37 br s

[a] Protons correlating with carbon shift. These correlations were observed for both molecules.

relative stereochemistry of C-14 to C-16 was achieved with the aid of NOESY data. In the NOESY experiment, cross-peaks were observed between the α-tetrahydrofuran (THF) protons. A NOESY correlation was also observed between H-12 and H-14. No NOESY cross-peaks were seen between either of the α-THF protons and H-14, suggesting that the α-THF protons and H-14 were on opposite sides of the ring. Although such negative data are not conclusive, this assignment was confirmed by single-crystal X-ray diffraction analysis of **4** (Figure 2).

A molecular formula of $C_{25}H_{36}O_{12}$ was determined by HRESIMS (m/z 551.2089 $[M + Na]^+$) for compound **5** indicating an index of unsaturation of 8. Gross comparison of the NMR spectra of **4** and **5** suggested that they were structurally similar (Table 1). Like compound **4**, the ^1H NMR spectrum of **5** was devoid of aromatic signals and showed oxymethines (δ 4.94 and 4.92), which could be assigned to the α positions of the reduced furan ring. A D_2O exchange experiment indicated that **5** also possessed two hydroxyl groups; signals at δ 4.15 and 2.37 disappeared on addition of D_2O, and this was accompanied by resolution of the broad signal at δ 4.02 to a clean doublet. In an HMBC experiment, methoxyl protons at δ_H 3.47 and 3.42 correlated to dioxygenated carbons at δ_C 111.3 and 108.4, respectively, indicating a similar substitution pattern to that seen in **4**. Further HMBC analysis enabled elucidation of the structural connectivity of **5**. As in the NOESY spectrum of **4**, the NOESY spectrum of **5** showed cross-peaks between H-12 and H-14 (Figure 3). In addition, H-14 showed cross-peaks to H-15 and H-16 suggesting that H-14, H-15, and H-16 are on the same face of the tetrahydrofuran ring. Thus, the structure of **5** is proposed for salvinicin B.

The absolute stereochemistries of **1a** and **1b** were determined previously through use of a nonempirical exciton chirality circular dichroism (CD) method.[26] However, the absolute sterochemistry has not been confirmed unambiguously by X-ray crystallographic analysis. In an effort to

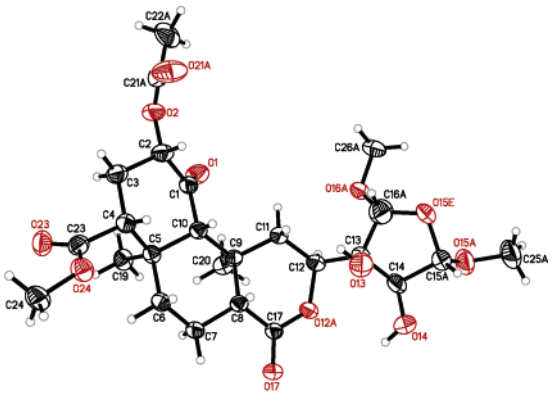

Figure 2. Results from the X-ray analysis on **4** drawn from the experimentally determined coordinates with the thermal parameters at the 20% probability. There was some disorder in the crystal structure that was successfully modeled. This figure represents the major component of that disorder.

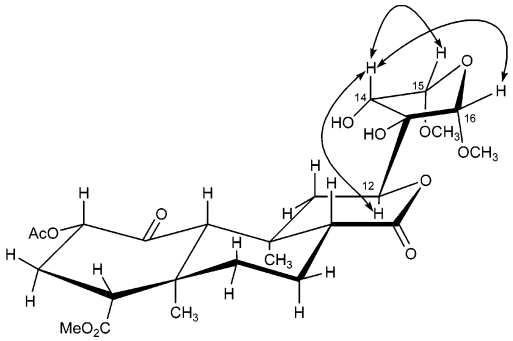

Figure 3. NOESY correlations in salvinicin B (**5**).

confirm the stereochemistry of **1a** and **1b** unequivocally, as well as that of salvinicins A and B, a 3,4-dichlorobenzoyl derivative (**6**) was prepared. This compound was obtained in 70% yield by reacting **1b** with 3,4-dichlorobenzoyl chloride under conditions described previously.[18] A single-crystal X-ray diffraction study of **6** was carried out, from which its absolute stereochemistry was determined (Flack parameter[27] = 0.00(3)) and is as shown (Figure 4). Thus,

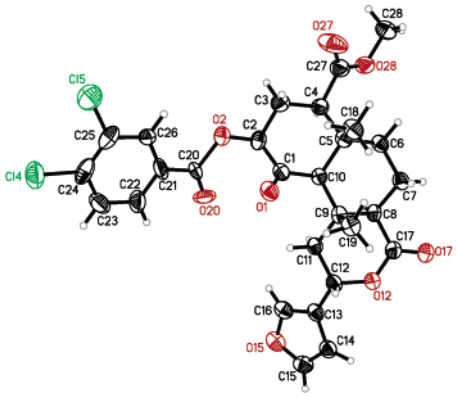

Figure 4. Results from the X-ray analysis on **6** drawn from the experimentally determined coordinates with the thermal parameters at the 20% probability. There was some disorder in the crystal structure that was successfully modeled. This figure represents the major component of that disorder.

the absolute stereochemistry of **1a** and **1b** was determined unambiguously, and by extension, the absolute stereostructure of **4** is proposed as depicted.

The 3,4-dihydroxy-2,5-dimethoxytetrahydro-3-furyl moiety seen in **4** and **5** is rare. This moiety has been reported previously in diterpenes of the clerodane and labdane classes, as well as in other classes of natural products.[28−33] However, this is the first report of this highly oxygenated tetrahydrofuran ring system in compounds isolated from the *Salvia* genus. In addition, this description is the first report of an X-ray crystallographic structure of this type of reduced furan ring system.

Pharmacologic evaluation of these compounds at μ, κ, and δ opioid receptors was then conducted using a [^{35}S]GTPγS assay.[34] Initial screening at 10 μM indicated that **4** and **5** showed activity at κ and μ receptors, respectively. Further work indicated that salvinicin A (**4**) exhibited partial κ agonist activity with an EC_{50} value of 4.1 ± 0.6 μM (E_{max} = 80% relative to (−)-U50,488H). Interestingly, salvinicin B (**5**) exhibited antagonist activity at μ receptors with a K_i of > 1.9 μM. This is the first report of a neoclerodane diterpene with opioid antagonist activity and represents a new lead in the development of opioid receptor antagonists.

At present, there are few methods for the synthesis of neoclerodane diterpenes, including **1a**. Recently, the first total synthesis of the neoclerodane (−)-methyl barbascoate from an (*R*)-(−)-Weiland−Meischer ketone analogue was reported.[35] This unique architecture, and the potential biological activity of these compounds, make them attractive targets for the synthetic chemist.

In conclusion, two novel neoclerodane diterpenes with opioid receptor activity have been isolated from commercially available *S. divinorum* leaves. Salvinicins A (**4**) and B (**5**) are unique neoclerodanes which possess a 3,4-dihydroxy-2,5-dimethoxytetrahydrofuran ring. The absolute stereochemistry of these molecules has been assigned through X-ray crystallographic analysis of **6**.

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Supporting Information Available: Experimental details on the isolation of **4** and **5**; physical and spectral data of **4**, **5**, and **6**; and X-ray data of **4** and **6**. This material is available free of charge via the Internet at http://pubs.acs.org.

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(36) Crystallographic data for salvinicin A (**4**) (deposition no. CDCC 265522) and the 3,4-dichlorobenzoyl derivative of salvinorin B (**6**) (deposition no. CDCC 265523) have been deposited at the Cambridge Crystallographic Data Centre. Copies of the data can be obtained free of charge, on application to the Director, CDCC, 12 Union Road, Cambridge CB2 1EZ, U.K. [Fax: 44-(0)1223-306033 or email: deposit@cdcc.cam.ac.uk].