

Salvinorins D–F, New Neoclerodane Diterpenoids from *Salvia divinorum*, and an Improved Method for the Isolation of Salvinorin A

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Received December 11, 2002

Three new neoclerodane diterpenoids, salvinorins D–F (**4**–**6**), have been isolated from the leaves of *Salvia divinorum*. The structures were elucidated by chemical and spectroscopic methods, particularly 1D and 2D NMR. A simplified isolation method using chromatography on activated carbon also gave improved yields of the controlled substance salvinorin A (**1**) and of salvinorin C (**3**).

Salvia divinorum Epling & Játiva (Lamiaceae) is a sage native to Oaxaca, Mexico. An infusion prepared from the leaves is used by the Mazatec Indians for the treatment of various illnesses including headache and diarrhea. At high doses, the infusion induces hallucinations.¹ Salvinorins A (**1**),² B (**2**),³ and C (**3**)⁴ have been isolated from the leaves; in mice, **1** produces sedation, while **2** is inactive.³ The status of **3** remains unclear; a mixture of **1** and **3** was significantly more potent than **1** alone, but the pure compound was not tested, and the results of this investigation appear to have been confounded, possibly by toxicity.⁴ Salvinorin A (**1**) binds potently and selectively to the kappa opioid receptor⁵ and produces effects typical of κ -opioids,⁶ including confusion, depersonalization, and hallucinations.^{7,8} A recent case report suggests *S. divinorum* may have antidepressant activity.⁹ This is in accord with another recent study which found the κ -opioid U-50,488H effective against an animal model of depression.¹⁰ Similarly, the relief of abdominal pain (particularly gut distension) by κ -opioids¹¹ suggests a plausible basis for Mazatec use of *S. divinorum* against abdominal swelling.¹

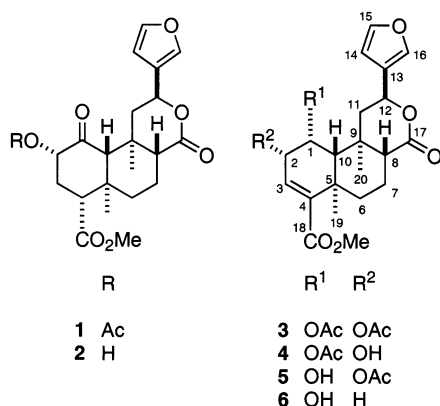
During the development of an improved procedure for the isolation of **1** and **3** from *S. divinorum*, three new compounds were isolated: salvinorins D, E, and F (**4**–**6**). Using several solvents, Gruber demonstrated¹² that extrac-

in the crude extract proved troublesome; the green-black color made it almost impossible to distinguish the phases during the solvent partitioning procedure employed by Valdes and co-workers.³ Some coloration persisted even after chromatography and recrystallization, as noted previously,¹² and the numerous pigment spots greatly complicated TLC analysis. We therefore decided to decolorize the crude extract by chromatography on activated carbon.¹³ This gave an amber terpenoid mixture from which pure **1** was isolated by recrystallization from methanol (giving a total yield of 3.4 g/kg). Repeated flash chromatography of the mother liquor on silica gel gave **2**–**4** and a mixture of **5** and **6**, which were separated by normal-phase HPLC. Compounds **3**, **5**, and **6** were isolated as clear resins. Attempted recrystallizations from methanol and hexane/EtOAc were unsuccessful and caused substantial decomposition. Compound **4** was triturated in diethyl ether, and it was subsequently found that this is also effective for **1**, which could greatly reduce the decomposition accompanying recrystallization from alcohols.

The structures of salvinorins D, E, and F (**4**–**6**) were assigned by comparison of NMR data with the published values for **3** and analogues,⁴ along with decoupling, HMQC, HMBC, and DEPT experiments. HRESIMS established the molecular formula of **4** and **5** as C₂₃H₂₈O₈. The ¹H NMR spectra were also extremely similar. Compared to **3**, the spectra of **4** and **5** showed only one acetyl peak and gained one new peak (δ 2.01 in **4**; δ 1.94 in **5**) which exchanged with D₂O. The presence of a hydroxyl group was confirmed by IR spectroscopy (3475 cm⁻¹ in **4**; 3510 cm⁻¹ in **5**). These data suggested that the compounds were the two possible monoacetates of **3**.

The two compounds were readily differentiated by ¹H NMR. Relative to **3**, the H-2 signal of **4** was shifted upfield from δ 5.55 to δ 4.44 and showed a strong coupling to the hydroxylic proton ($J = 6.7$ Hz) as well as the expected couplings to H-1 (5.6 Hz) and H-3 (2.4 Hz). The HMBC spectrum showed correlations between H-2 and C-4, as well as H-1 and C-5. The proposed structure of **4** was verified by acetylation, which proceeded smoothly to give **3**, identical in all respects with the isolated material (work with *cis*-diol derivatives of **1** showed that while the 2-position is readily esterified, the 1-position is unreactive).^{4,14} The implied structure of **5** was confirmed by ¹H NMR. Relative to **3**, the H-1 signal of **5** was shifted from δ 5.76 to δ 4.46. Irradiation sharpened the H-10 singlet. The HMBC spectrum again showed a correlation between H-2 and C-4.

The molecular formula of compound **6**, C₂₁H₂₆O₆, was established by HRESIMS. In contrast to **4** and **5**, ¹H NMR



tion at room temperature gives a higher recovery than the refluxing solvent used in the original procedures.^{2,3} His results suggest that **1** decomposes rapidly in hot solution. We found that extraction at room temperature in acetone gave an excellent recovery. However, the pigments present

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Table 1. ^1H NMR Data (400 MHz, CDCl_3) of Compounds **4–6** [δ (ppm), m, J (Hz)]

proton	4	5	6
1	5.70 br d (5.6)	4.46 ddd (4.7,1.6,1.3)	4.52 ddd (5.5,4.6,2.4)
2 α			2.35 br dd (20.1,4.7)
2 β	4.44 ddd (6.7,5.6,2.4)	5.40 dd (4.7,2.4)	2.60 ddd (20.1,5.5,3.0)
3	6.54 dd(2.4,1.2)	6.43 dd (2.4,1.3)	6.67 ddd (4.7,3.0,2.4)
6 α	2.56 dt (13.3,3.4)	2.52 ddd (12.5,3.0,2.6)	2.53 dt (13.3,3.2)
6 β	1.19 m	1.16–1.25 m	1.18 td (13.3,3.4)
7 α	1.78 m	1.84 m	1.82 m
7 β	2.09–2.17 m	2.07–2.18 m	2.08–2.17 m
8	2.13 dd (13.5,3.5)	2.07–2.18 m	2.08–2.17 m
10	1.42 br s	1.30 br s	1.25 br s
11 α	2.54 dd (13.1,5.6)	2.46 dd (13.1,6.0)	2.50 dd (13.2,5.8)
11 β	1.64 dd (13.1,11.2)	1.62 dd (13.1,11.2)	1.62 dd (13.2,11.2)
12	5.53 dd (11.2,5.6)	5.60 dd (11.2,6.0)	5.60 dd (11.2,5.8)
14	6.40 br s	6.41 br s	6.41 d (1.6)
15	7.42 t (1.6)	7.42 m	7.42 t (1.6)
16	7.44 br s	7.44 br s	7.44 br s
19	1.69 s	1.72 s	1.71 s
20	1.22 s	1.47 s	1.48 s
CO_2CH_3	3.74 s	3.73 s	3.72 s
OCOCH_3	2.15 s	2.17 s	
OH	2.01 d (6.7)	1.94 br s	1.29 d (4.6)

showed no acetyl peak and only one oxygenated methine signal, with two new diastereotopic protons at δ 2.35 and 2.60 coupling to H-1 and H-3. This implied the 2-deoxy structure shown. Protons H-2 α and H-2 β were distinguished by their coupling constants: molecular modeling¹⁵ predicted $J_{1\beta,2\beta} = 5.4$ Hz (observed: 5.5 Hz) and $J_{1\beta,2\alpha} = 1.5$ Hz (irradiation of H-1 sharpened the H-2 α peaks by 0.4 Hz).

A number of neoclerodane diterpenoids, very similar in structure to the salvinorins, display a broad range of activities against insects.¹⁶ Salvinorins C–F (**3–6**) fit an experimentally determined pharmacophore for antifeedant activity against *Tenebrio molitor*,¹⁷ while **1** and **2** contain the key features for antifeedant activity against *Spodoptera littoralis*.¹⁸ Research into *S. divinorum* has thus yielded promising leads in a remarkably wide range of areas of therapeutic and agrochemical interest. It should be noted, however, that *S. divinorum* and salvinorin A (**1**) are now controlled substances in Australia and research involving them requires a State Health Department permit.¹⁹

Experimental Section

General Experimental Procedures. Uncorrected melting points were determined using a Reichert hot-stage apparatus. Optical rotations were measured on a JASCO DIP-1000 digital polarimeter. IR spectra were recorded using a Bio-Rad FTS 165 FT-IR spectrophotometer. ^1H NMR (400 MHz) and ^{13}C NMR (100 MHz) spectra were recorded on Varian Inova 400 and Unity Plus 400 instruments. HRESIMS were run on a Bruker 4.7T BioAPEX FTMS. TLC was conducted on Merck silica gel 60 F₂₅₄ plates visualized with phosphomolybdic acid in ethanol. HPLC was performed on a Spherex 5 μm silica column (250 \times 10 mm) at a flow rate of 2 mL min⁻¹. Column chromatography was performed on Merck silica gel 60 or Merck activated carbon 2183, at an adsorbent:solute mass ratio of 30:1 unless otherwise indicated. "Petrol" refers to the fraction boiling at 40–60 °C.

Plant Material. Dried *S. divinorum* leaves, cultivated in Oaxaca, Mexico, were purchased in April 2002 from Salvia Space Ethnobotanicals (Berkeley, CA). Voucher specimens were deposited at the National Herbarium of Victoria (accession number MEL 2101361) and the University of Melbourne Herbarium (MELU sn).

Extraction and Isolation. Dried *S. divinorum* leaves (860 g) were powdered and steeped for 1 h in acetone (3 \times 1 L). Filtration and evaporation under reduced pressure gave a dark green tar (30.5 g). This was purified by flash column chroma-

Table 2. ^{13}C NMR (100 MHz) of Compounds **4–6** [δ (ppm)]

carbon	4	5	6
1	66.5	64.3	63.9
2	68.7	72.3	38.0
3	135.7	131.5	133.4
4	141.2	143.4	140.6
5	37.6	37.8	36.6
6	37.0	37.0	37.3
7	18.3	18.4	18.6
8	51.8	51.7	52.2
9	37.0	37.5	37.7
10	52.5	54.0	54.8
11	43.9	44.4	44.4
12	71.6	71.7	71.7
13	125.4	125.8	125.9
14	108.4	108.4	108.4
15	143.8	143.9	143.8
16	139.4	139.3	139.3
17	171.5	169.8	172.1
18	166.2	166.0	166.9
19	21.6	21.9	21.6
20	15.6	16.2	16.4
CO_2CH_3	51.7	51.8	51.5
OCOCH_3	21.2	21.0	
OCOCH_3	171.5	171.8	

tography (FCC) on an equal mixture of activated carbon and filter aid, eluting with a gradient from acetone to petrol, to give an amber semicrystalline mass (5.73 g). Several recrystallizations from methanol and ethanol gave **1** (2.64 g). The mother liquor was purified by FCC on silica gel (5–50% acetone/ CH_2Cl_2 gradient). This was divided by TLC into three series: A (656 mg), B (150 mg), and C (359 mg).

Series A. In a typical case, 71 mg was subjected to FCC on silica gel (14 g), eluting with a gradient from 50 to 80% Et_2O /petrol, to give **3** (total yield 219 mg, 0.25 g/kg) and additional **1**, which was recrystallized from ethanol (total yield 2.9 g, 3.4 g/kg).

Series B. FCC on silica gel (35 g) in 70–90% Et_2O /petrol and recrystallization from methanol gave **2** (13 mg). **1** and **2** were identified by ^1H NMR and mp.³

Series C. Trituration in Et_2O gave **4** (75 mg). The mother liquor was subjected to repeated FCC on silica gel (Et_2O /petrol). Final purification by HPLC (60% EtOAc /petrol; t_R 9.8 min for **5** and 10.7 min for **6**) and drying under high vacuum (96 h at 45 °C) gave **5** (2.8 mg) and **6** (1.1 mg).

Salvinorin D (4): fine colorless crystals, mp 185–187 °C; $[\alpha]_D^{17} +66.6^\circ$ (c 1.0, CH_2Cl_2); IR (thin film) ν_{max} 3475, 3146, 2952, 2861, 1723, 1505, 1435, 1371, 1228, 1142, 1027, 875, 788 cm^{-1} ; ^1H and ^{13}C NMR, see Tables 1 and 2; HRESIMS m/z 455.1672 (calcd for $\text{C}_{23}\text{H}_{28}\text{O}_8\text{Na}$, 455.1676); TLC, see Table S1.

Salvinorin E (5): clear resin; $[\alpha]_{\text{D}}^{17} +46.4^{\circ}$ (*c* 0.14, CHCl_3); IR (thin film) ν_{max} 3510, 3144, 2952, 2858, 1722, 1505, 1436, 1374, 1228, 1142, 1070, 1029, 875, 805 cm^{-1} ; ^1H and ^{13}C NMR, see Tables 1 and 2; HRESIMS *m/z* 455.1687 (calcd for $\text{C}_{23}\text{H}_{28}\text{O}_8\text{Na}$, 455.1676); TLC, see Table S1.

Salvinorin F (6): clear resin; $[\alpha]_{\text{D}}^{16} -20^{\circ}$ (*c* 0.05, CHCl_3); IR (thin film) ν_{max} 3514, 3147, 2951, 2857, 1712, 1505, 1436, 1372, 1318, 1232, 1144, 1070, 1028, 875, 797 cm^{-1} ; ^1H and ^{13}C NMR, see Tables 1 and 2; HRESIMS *m/z* 397.1610 (calcd for $\text{C}_{21}\text{H}_{26}\text{O}_6\text{Na}$, 397.1622); TLC, see Table S1.

Acetylation of 4. Ac_2O (250 μL , 2.6 mmol) was added to a solution of **4** (11 mg, 25.4 μmol) in dry pyridine (2.5 mL) and stirred under argon. After 3.5 h, TLC (10% acetone/ CH_2Cl_2) indicated completion. The reaction mixture was diluted with ice water and extracted with Et_2O ($\times 3$). The organic phase was washed with saturated NaHCO_3 , saturated CuSO_4 , water, and brine and dried over MgSO_4 . Filtration and evaporation in vacuo gave a cloudy resin (12 mg). FCC on silica gel (55% Et_2O /petrol) gave **3** as a clear resin (8.4 mg, 70%), identical with the natural material by TLC, ^1H and ^{13}C NMR, IR, and optical rotation: $[\alpha]_{\text{D}}^{16} +69.4^{\circ}$ (*c* 0.40, CHCl_3); natural material: $[\alpha]_{\text{D}}^{16} +70.5^{\circ}$ (*c* 0.55, CHCl_3); lit.:^{4,20} $[\alpha]_{\text{D}}^{22} +49.3^{\circ}$ (*c* 0.61, CHCl_3).

Acknowledgment. We thank the Melbourne University Research Grants Scheme for financial support, and the Commonwealth Department of Education, Science and Training for an Australian Postgraduate Award (T.M.)

Supporting Information Available: Table of TLC data for compounds **1–6** and NMR spectra (^1H , ^{13}C , and DEPT) for compounds **3–6**. This material is available free of charge via the Internet at <http://pubs.acs.org>.

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- (19) *Standard for the Uniform Scheduling of Drugs and Poisons*; Commonwealth Department of Health and Ageing: Canberra, 2002; Vol. 17; pp 230, 232. See under the (incorrect) systematic name: "8-METHOXYCARBONYL-4A,8A-DIMETHYL-6-ACETOXY-5-KETO-3,4,4B,7,9,10,10A-SEPTAHYDRO-3-(4-FURANYL)-2,1-NAPHTHO-[4,3-E]PYRONE". Among many other errors, note the use of "septa" for hepta and the incorrect numbering, apparently based on phenanthrene.
- (20) Our spectroscopic data were otherwise in accord, except for the ^1H NMR assignments of H-19 and H-20, which should be reversed (HMQC and HMBC data).

NP0205699