Divinatorins A−C, New Neoclerodane Diterpenoids from the Controlled Sage *Salvia divinorum*

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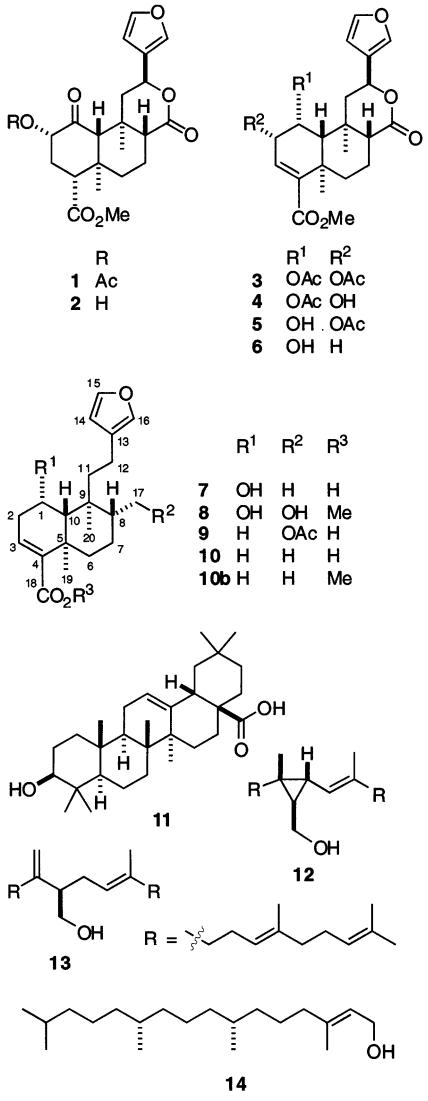
Three new neoclerodane diterpenoids, divinatorins A−C (**7**−**9**), have been isolated from the leaves of *Salvia divinorum*. The compounds were identified by spectroscopic methods as derivatives of the antibiotic (−)-hardwickiic acid (**10**), which was also isolated, along with four other known terpenoids. Neither the crude extract nor **7**−**9** displayed antimicrobial activity.

Salvia divinorum Epling & Játiva (Lamiaceae) is a sage used medicinally by the Mazatec Indians of Oaxaca, Mexico. The leaves contain salvinorin A (**1**),[1] a potent hallucinogen acting at the kappa opioid receptor.[2] Recently, the plant has gained notoriety as a legal hallucinogen, sold openly on the Internet. As a result, the plant and compound **1** have been prohibited in Australia[3] (a recent bill to prohibit the plant in the United States was not enacted).[4] The enforcement of such controls is likely to be hampered by the plant's nondescript appearance and the very limited chemical data available.[5] Until recently, only four compounds had been isolated from this species: salvinorins A (**1**), B (**2**),[6] and C (**3**)[7] and loliolide.[8] GC/MS analysis also indicated the possible presence of neophytadiene and stigmasterol.[5]

Recently we reported the isolation of salvinorins D−F (**4**−**6**) from the acetone extract of commercial *S. divinorum*,[9] employing chromatography on activated carbon to separate the terpenoids from complicating pigments. This work generated several mixed fractions, which appeared initially to be inseparable. Exhaustive chromatography on silica gel, employing high silica ratios and diverse solvent systems, has now yielded divinatorins A−C (**7**−**9**), along with the known terpenoids (−)-hardwickiic acid (**10**)[10] and oleanolic acid (**11**).[11]

In addition, an extraction of locally grown leaves was undertaken. The acetone extract was again chromatographed on activated carbon; elution with EtOAc/petrol gave partial separation of two terpenoid fractions. The first, after chromatography on silica gel, yielded the known terpenoids presqualene alcohol (**12**),[12] peplusol (**13**),[13] and (*E*)-phytol (**14**).[14] The second mixture was recrystallized from MeOH to give **1**, albeit in much lower yield than from the commercial material (0.56 g/kg).

The structures of **7**−**9** were elucidated chiefly by NMR (^{1}H,^{13}C, DEPT, HMQC, HMBC, COSY, and NOESY in each case). The ^{1}H NMR spectra suggested that they were derivatives of **10**. The molecular formula of **7**, $C_{20}H_{28}O_4$ (HRESIMS), implied the presence of a single hydroxyl substituent, which was confirmed by an IR absorption at 3392 cm^{-1}. This was located at C-1 on the basis of 2D NMR: the oxymethine at δ 4.49 showed couplings to H-2 and -10 (COSY) and C-3, -9, and -10 (HMBC). The configuration was confirmed by an H-1 to H-11 cross-peak in the NOESY spectrum.



The ^{1}H NMR spectrum of **8** suggested a methyl ester (δ 3.71) with two hydroxyl groups [δ 1.49 (2H, D_2O-exchangeable)]. A strong IR absorption band occurred at 3434 cm^{-1}. The molecular formula, $C_{21}H_{30}O_5$ (HRESIMS), was consistent with this proposal. One of the hydroxyl groups was again located at C-1, showing the same couplings as in **7**. The second was located at C-17, on the basis of the couplings of the oxymethylene signals (δ 3.38 and 3.84) to H-8 (COSY) and to C-7, -8, and -9 (HMBC). The NOESY

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Table 1. ^{1}H NMR Data (400 MHz) for Compounds **7**−**9**[a]

	δ, m, J (Hz)		
H	**7**	**8**	**9**
1	4.49 br d (4.8)	4.46 br d (4.9)	1.70 m
			1.46 m
2	2.56 ddd (20.1, 5.1, 2.8)	2.53 ddd (19.9, 5.1, 2.8)	2.35 dt (20.5, 5.1)
	2.40 m	2.34 m	2.19 m
3	6.90 dd (4.8, 2.7)	6.65 dd (4.8, 2.7)	6.89 dd (4.4, 2.9)
6	2.40 m	2.36 m	2.53 dt (13.2, 3.2)
	1.20 m	1.16 m	1.15 td (13.2, 3.6)
7	1.57 m	1.87 m	1.74 m
	1.43 m	1.56 m	1.48 m
8	1.55 m	1.58 m	1.79 m
10	1.45 br s	1.45 br s	1.42 br d (12.1)
11	1.85 ddd (14.7, 12.8, 4.7)	1.91 m	1.75 m
	1.68 m	1.77 m	1.63 m
12	2.33 m	2.42 td (13.6, 4.6)	2.40 td (13.8, 4.2)
	2.05 ddd (14.3, 12.9, 4.7)	2.08 ddd (14.1, 12.8, 4.7)	2.20 m
14	6.25 br s	6.25 br s	6.28 br s
15	7.36 t (1.6)	7.35 t (1.7)	7.35 t (1.6)
16	7.20 br s	7.20 br s	7.22 br s
17	0.84 d (6.0)	3.84 dd (10.5, 3.7)	4.26 dd (11.0, 4.1)
		3.38 dd (10.5, 8.0)	3.79 dd (11.0, 8.4)
19	1.64 s	1.66 s	1.27 s
20	1.15 s	1.18 s	0.83 s
CO_2CH_3		3.71 s	
$OCOCH_3$			2.03 s
OH		1.49 br s	

[a] In $CDCl_3$ as solvent and internal standard (7.26 ppm).

spectrum showed H-1 to H-11 and H-17 to H-20 cross-peaks, confirming the configuration at these centers.

The ^{1}H NMR spectrum of **9** showed an acetyl methyl signal (δ 2.03). The oxymethylene signals, shifted downfield to δ 3.79 and 4.26, showed the same couplings as in **8**, establishing the 17-acetoxy structure shown. This was consistent with the molecular formula, $C_{22}H_{30}O_5$ (HRESIMS). The expected relative stereochemistry of compounds **7**−**9** was confirmed in each case by NOESY cross-peaks from H-20 to H-17 and -19 (setting C-5, -8, and -9), and H-10 to H-12 (setting C-10). The absolute stereochemistry shown is common to all clerodanes isolated from the Lamiaceae,[15] including **1**[16] and **10**.[17]

Since compound **10** has previously been shown to display potent, broad-spectrum antimicrobial activity,[10] **7**−**9** were screened against standard antibiotic-susceptible strains of *Escherichia coli*, *Staphylococcus aureus*, *Bacillus subtilis*, and *Candida albicans*, using standard microdilution[18,19] and disk diffusion[20] assays. Compounds **7**−**9** showed no activity against any of the test organisms at 100 μg/mL or 100 μg/disk. These data extend the remarkably stringent structure−activity requirements of **10**.[10] To probe this further, we decided to screen (+)-hardwickiic acid (*ent*-**10**). This was isolated from copaiba balsam as the methyl ester.[21] Hydrolysis proved challenging: reflux in KOH/MeOH gave only slow decomposition, but microwave irradiation on KF/Al_2O_3[22] provided *ent*-**10** in low yield. *ent*-**10** proved active against *S. aureus* (MIC 25 μg/mL) and *B. subtilis* (MIC 12.5 μg/mL, 10 mm zone of inhibition), but much less potent than its enantiomer (MIC 0.78 μg/mL against *B. subtilis*).[10]

A previous non-peer-reviewed investigation[23] reported the acetone extract of *S. divinorum* to be active against a wide range of bacteria. We were unable to confirm these results. The acetone extract of the commercial material, as well as **1**, showed no activity at 100 μg/mL or 100 μg/disk. Probably insufficient **10** and **11** were present to elicit an effect (**11**, like **10**, is active against *B. subtilis* and *S. aureus*).[24]

Experimental Section

General Experimental Procedures. Instruments and materials were as described previously.[9] Flash column chromatography was performed on Merck silica gel 60. Silica:solute mass ratios up to 400:1 were used for difficult separations (ΔR_f < 0.05). Vacuum chromatography was performed on Merck activated carbon 2183. 'Petrol' refers to the petroleum ether fraction boiling at 40−60 °C.

Plant Materials. *S. divinorum* plants, cultivated in Melbourne, were harvested in February 2003. A voucher specimen was deposited at the National Herbarium of Victoria (accession number MEL 2145478). Copaiba balsam was donated by Australian Botanical Products (Hallam, Victoria).

Antimicrobial Tests. The crude extract and pure compounds were tested against *Escherichia coli* (ATCC 25922), *Staphylococcus aureus* (ATCC 29213), *Bacillus subtilis* (ATCC 6633), and *Candida albicans* (ATCC 90028) using standard broth microdilution[18,19] (100−0.19 μg/mL using 2-fold serial dilutions) and disc-diffusion[20] assays (100 μg/disk). All measurements were performed in duplicate. Streptomycin sulfate and amphotericin B were used as positive controls.

Extraction and Isolation. Dried commercial *S. divinorum* leaves (860 g) were extracted as described previously.[9] The mother liquor from recrystallization of **1** was subjected to flash column chromatography (FCC) on silica gel in a 5−50% acetone/CH_2Cl_2 gradient. This was divided based on TLC (10% acetone/CH_2Cl_2) into four series: A (656 mg), B (150 mg), C (359 mg), and D (77 mg).

Series A: 90 mg was subjected to FCC, eluting with a gradient from 50 to 80% Et_2O/petrol, to give **10** (6 mg).

Series C: trituration in Et_2O gave **4** (75 mg). FCC of the mother liquor (60−100% Et_2O/petrol) gave four fractions based on TLC (70% Et_2O/petrol): C1 (55 mg), C2 (119 mg), C3 (57 mg), and C4 (26 mg)

Fraction C1: repeated FCC (20% acetone/petrol and 40−60% Et_2O/petrol) gave **9** (23 mg) and **11** (3 mg).

Fraction C2: repeated FCC (25% acetone/petrol and 60−100% Et_2O/petrol) gave **8** (32 mg).

Fraction C3: extensive FCC (Et_2O/petrol, acetone/petrol, and EtOAc/petrol) gave additional **8** (total yield 41 mg) and a mixture of **5** and **6**, which were separated as described previously.[9]

Fraction C4 gave pure **4** (total yield 114 mg).

Table 2. ^{13}C NMR Data (100 MHz) for Compounds **7**−**9**[a]

	δ		
C	**7**	**8**	**9**
1	64.7	64.3	17.0
2	38.1	38.0	27.4
3	136.2	133.2	140.3
4	140.8	141.4	141.2
5	37.4	37.1	37.4
6	38.6	38.0	35.2
7	27.4	21.9	22.3
8	37.1	44.8	40.9
9	39.7	39.1	38.4
10	49.0	48.7	46.8
11	39.1	38.8	38.9
12	18.2	18.2	18.3
13	125.2	124.9	125.2
14	110.9	110.8	110.9
15	142.8	142.8	142.8
16	138.4	138.4	138.5
17	15.7	63.9	66.1
18	171.8	167.3	171.9
19	21.4	21.4	20.5
20	19.8	20.9	19.0
CO_2CH_3		51.3	
$OCOCH_3$			21.0
$OCOCH_3$			171.2

[a] In $CDCl_3$ as solvent and internal standard (77 ppm).

Series D: repeated FCC (60% Et_2O/petrol and 4% MeOH/CH_2Cl_2) gave **7** (36 mg).

Extraction of Australian Material. Dried, powdered Australian-grown *S. divinorum* leaves (224 g) were steeped in acetone for 30 min (3 × 250 mL). Filtration and evaporation under reduced pressure gave a dark green tar (7 g). This was purified by vacuum column chromatography on a mixture of activated carbon (75 g) and diatomite filter aid (~1:1), eluting with a gradient from 50 to 20% EtOAc/petrol, to give series E (97 mg) and F (279 mg) based on TLC (70% Et_2O/petrol).

Series E: repeated FCC (1% acetone/CH_2Cl_2 and 20% Et_2O/petrol) gave **13** (6 mg). Further FCC (0.75% MeOH/CH_2Cl_2 and 1% EtOH/$CHCl_3$) gave **12** (23 mg) and **14** (12 mg).

Series F: two recrystallizations from MeOH gave **1** (126 mg).

Divinatorin A (7): amber resin; $[\alpha]^{19}_D$ −53° (*c* 1.8, CH_2Cl_2); FTIR (film) ν_{max} 3392, 2927, 2874, 2648, 1684, 1456, 1411, 1386, 1245 cm^{-1}; ^1H and ^{13}C NMR, see Tables 1 and 2; HRESIMS [M + Na^+] *m*/*z* 355.1864 (calcd for $C_{20}H_{28}O_4Na^+$, 355.1880); TLC, see Table S1.

Divinatorin B (8): amber resin; $[\alpha]^{20}_D$ −54° (*c* 2.1, $CHCl_3$); FTIR (film) ν_{max} 3434, 2930, 2881, 1714, 1437, 1236, 1067 cm^{-1}; ^1H and ^{13}C NMR, see Tables 1 and 2; HRESIMS [M + Na^+] *m*/*z* 385.1988 (calcd for $C_{21}H_{30}O_5Na^+$, 385.1985); TLC, see Table S1.

Divinatorin C (9): amber resin; $[\alpha]^{25}_D$ −110° (*c* 1.1, $CHCl_3$); FTIR (film) ν_{max} 2960, 2873, 1738, 1681, 1236, 1025 cm^{-1}; ^1H and ^{13}C NMR, see Tables 1 and 2; HRESIMS [M + Na^+] *m*/*z* 397.1989 (calcd for $C_{22}H_{30}O_5Na^+$, 397.1985); TLC, see Table S1.

Methyl (−)-hardwickiate (10b): amber syrup; $[\alpha]^{25}_D$ −115° (*c* 0.03, $CHCl_3$) [lit.[10] −104°]; ^1H and ^{13}C NMR, FTIR, and EIMS (70 eV) matched literature values.[10]

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Supporting Information Available: ^1H and ^{13}C NMR spectra for **7**−**9**; TLC data for **1**−**14**; isolation procedure for *ent*-**10**. This material is available free of charge via the Internet at http://pubs.acs.org.

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