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Novel neoclerodane diterpene derivatives from the smoke of salvinorin A

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Abstract

Salvinorin A is a naturally-occurring potent and selective kappa opioid receptor agonist, and smoking salvinorin A produces the most intense hallucinogenic effects in human. Eight neoclerodane diterpene derivatives were isolated from the smoke of salvinorin A, and their structures were identified by spectroscopic methods. The major structural changes include epimerizations, eliminations, and rearrangements.

Keywords

Salvinorin A; Neoclerodane diterpenoid; Smoke; Opioid receptor

Salvia divinorum is a rare member of the Lamiaceae (mint) family and has been traditionally used by the Mazatec Indians of northeastern Oaxaca in spiritual practices.¹ *S. divinorum* leaf and leaf preparations are widely available in Western Europe and the USA, notably on Internet sites.² Because of its hallucinogenic effects, *S. divinorum* (also known as magic mint) has been increasingly used as a marijuana substitute in non-ethnopharmacological settings.^{2–4} Salvinorin A (**1**), a non-nitrogenous neoclerodane diterpenoid isolated from *S. divinorum*,^{5,6} was characterized as a potent and selective kappa (κ) opioid receptor (KOR) agonist and responsible for the observed psychoactive effects.^{1,7–10} Inhalation of the vaporized smoke of **1** was proven to be the most efficient method for its hallucinogenic effects in human.¹ The effective dose as low as 200 to 500 μg produces profound hallucinations with similar potency as hallucinogen LSD (lysergic acid dirthyamide).¹ However, the active principles in the smoke of **1** are still unclear. In order to better understand the biological and pharmacological activities of **1**, we report the isolation and structural elucidation of eight neoclerodane diterpene derivatives resulted from the smoke extract of **1**.

Salvinorin A (**1**, 1.0 g) was heated in a glass flask immersed in an oil bath. When the temperature reached at 245 °C, the visible white vapor rose to the cold upper inner glass wall and condensed into solid. The excess vapor was collected by passing through a short tube into a chloroform solution. The heating process lasted for 10 min at 248 \pm 3 °C. The condensed solid was washed out with chloroform, and the combined chloroform solution was concentrated under reduced pressure and the temperature of the water bath was kept

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below 40 °C. The residue (150 mg) was chromatographed over silica gel and eluted with hexane-ethyl acetate to give eight compounds **1-8** (Figure 1). **1** and 8-epi-**1** (**2**) are the major components, while **3-8** are minor compounds. The known compounds **1-4** were identified by comparison with their published spectroscopic data.^{11,12}

Compound **5** is a colorless semi-solid. Its molecular formula was determined to be C₂₃H₂₈O₈ on the basis of HR ESI-MS at *m/z* 455.1679 [M+Na]⁺ (calcd 455.1682). The ¹H and ¹³C data of **5** were closely related to those of 8-episalvinorin A (**2**),¹¹ indicating that **5** is a derivative of **2**. The difference between **5** and **2** was observed in the ¹H and ¹³C NMR spectra, in which the ¹H and ¹³C chemical shifts of the ring A of **5** were different from those of **2**. Especially, the coupling pattern of H-2 (δ 4.88, t, *J* = 3.0 Hz) in **5** is indicative of equatorial orientation and the acetyl group at C-2 must have β -configuration. This was further confirmed by comparison of NMR data with those of 2-epi-salvinorin A.¹³ The ¹H and ¹³C signals of **5** were assigned by extensive 2D-NMR methods (Table 1). This conclusion was furthermore supported by the synthesis of **5** from **2** via intermediate 8-episalvinorin B (**9**)¹⁴ following the literature procedures (Scheme 1).^{13,15} Consequently, the structure of **5** was established as 2-epi-8-epi-salvinorin A.

Compound **6**, a colorless semi-solid, gave a pseudomolecular ion peak at *m/z* 315.1599 [(M + H)]⁺ in HR ESI-MS suggesting a molecular formula of C₁₉H₂₂O₄. The characteristic ¹H NMR spectrum revealed the presence of two tertiary methyl groups (δ 1.10 and 1.49), an oxygenated methine proton [δ 5.27 (dd, *J* = 0.9 Hz and 12.3 Hz)], and five olefinic protons [δ 5.94 (dd, *J* = 3.3 Hz and 10.2 Hz), 6.40 (t, *J* = 0.9 Hz), 6.78 (ddd, *J* = 1.8 Hz, 5.7 Hz and 9.9 Hz), 7.39 (t, *J* = 1.2 Hz) and 7.46 (d, *J* = 0.6 Hz)]. The ¹³C NMR spectrum of **6** revealed 19 carbon signals, which were classified by DEPT as two methyls (δ 19.8 and 23.7), four methylenes (δ 17.3, 35.6, 45.4 and 48.9), eight methines (δ 45.4, 63.3, 69.9, 108.4, 130.0, 139.4, 143.2 and 145.9), three quaternary carbons (δ 34.2, 38.5 and 123.6), and two carbonyl carbons (δ 174.2 and 198.5). Comparison of the ¹H and ¹³C NMR spectra of **6** with those of **5** suggested that the B-, C-, and furan-rings of these compounds were structurally similar. The most striking features of **6** were the absence of acetoxy and methyl ester groups and the presence of two additional olefinic signals. HMQC analysis indicated that the olefinic carbons at δ 130.0 and 145.9 were attached directly to the protons at δ 5.94 and 6.78, respectively. Interpretation of HMBC data (Figure 2) showed the following significant correlations: H-3 (δ 6.78) with C-5 (δ 38.5); H-4 (δ 2.10-2.20) with C-2 (δ 130.0), C-3 (δ 145.9) and C-10 (δ 63.3); and CH₃-19 (δ 1.10) with C-4 (δ 45.4), C-5 (δ 38.5), C-6 (δ 35.6) and C-10 (δ 63.3). This, together with the upfield shift of C-1 (δ 198.5), led to the establishment of the partial structure – a conjugated carbonyl group in A-ring. Table 1 showed the full assignments of the ¹H and ¹³C signals of **6** based on the COSY, HMQC and HMBC analysis. Furthermore, in the NOESY spectrum, H-20 showed cross peaks to H-8 and H-19, which fully supported that the relative stereochemistry of H-8 is α -orientation. On the basis of these data, the structure of **6** was proposed.

Compound **7** was obtained as colorless needles, and its molecular formula was deduced as C₂₀H₂₂O₆ by a pseudomolecular ion peak at *m/z* 359.1496 [(M + H)]⁺ in its HR ESI-MS spectrum. The ¹H NMR spectrum displayed signals of two tertiary methyls (δ 1.19 and 1.25), one oxygenated methine (δ 5.57) and three olefinic protons (δ 6.63, 7.66 and 7.75). The remaining protons were observed in aliphatic region from δ 1.2 to δ 3.0. The ¹³C NMR spectrum of **7** showed 20 carbon signals ascribed to two methyls, five methylenes, six methines, four quaternary carbons, and three carbonyl carbons. Assignments of the ¹H and ¹³C signals were performed by extended 2D NMR methods including ¹H-¹H COSY, HMQC and HMBC spectra (Table 2 and Figure 2), which indicated that the A-ring is a five member ring, and an additional anhydride is formed between C-1 and C-18. In the HMBC spectrum, the methylene group resonating at δ 2.01 and 2.71 (H-2) was correlated with C-1

(δ 170.0), C-5 (δ 43.1), C-9 (δ 36.3) and C-10 (δ 58.8), indicating that the methylene group was located at C-10 position (Figures 1 and 2). The coupling constants of H-8 (dd, $J = 3.0$ Hz and 10.8 Hz) and H-12 (dd, $J = 4.8$ Hz and 11.7 Hz) is in agreement with those of natural salvinorins, suggesting that H-8 was β -orientation. In the NOESY spectrum, H-8 (δ 2.94) showed significant cross peaks to H-2 (δ 2.71), while H-12 (δ 5.57) related to H-20 (δ 1.25). The stereochemistry of **7** was further confirmed by X-ray analysis (Figure 3). From these data, the structure of **7** was determined.

Compound **8** was confirmed to have the same molecular formula as **7** by measurement of its HR ESI-MS. The ^1H and ^{13}C NMR data of **8** were closely related to those of **7**, suggesting **8** to be an isomer of **7**. Using 2D NMR techniques, including COSY, HMQC and HMBC, permitted the assignments of all ^1H and ^{13}C NMR chemical shifts as shown in Table 2. In the ^1H NMR spectrum of **8**, the coupling constants of H-8 (br s) and H-12 (d, $J = 12.0$ Hz) suggested that H-8 was α -orientated. This was also evidenced by the correlations of H-20 with H-8 and H-19 in its NOESY spectrum. Accordingly, the structure of **8** was established as 8-epimer of **7**.

In conclusion, eight neoclerodane diterpene derivatives were isolated from the smoke of salvinorin A. The major structural changes of salvinorin A include epimerizations at C-2 and C-8, eliminations of acetoxy and methyl ester groups, and carbon-carbon rearrangements at C-1, C-2 and C-10. Compounds **7** and **8** are unique salvinorin derivatives with five member rings and anhydrides confirmed by x-ray analysis. Our findings provided information on the chemical components of smoke generated by heating salvinorin A. The biological data of these novel products will be reported in due course.

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17. X-ray crystallography of **7**: CCDC 782913 contains the supplementary crystallographic data. These can be obtained free of charge from the Cambridge Crystallographic Data Centre via; www.ccdc.cam.ac.uk/data_request/cif

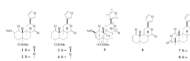


Figure 1.
Compounds **1-8** isolated from the smoke of salvinorin A

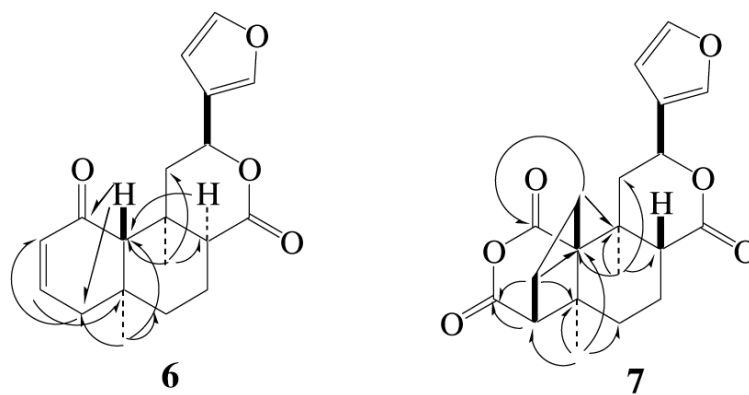


Figure 2.
Selected HMBC correlations of **6** and **7**

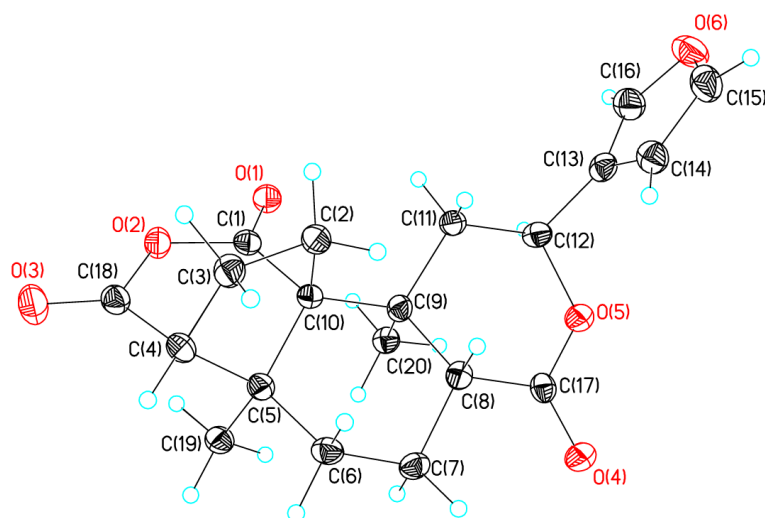
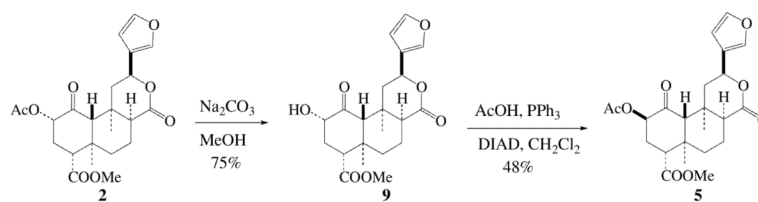


Figure 3.
Crystal structure of **7** showing 50% probability displacement ellipsoids



Scheme 1.
Conversion of **2** to **5**

Table 1

 ^1H (300 MHz) and ^{13}C NMR Data (75 MHz) for **5** and **6** in CDCl_3

No. C	5		6	
	δ_{H}	δ_{C}	δ_{H}	δ_{C}
1		204.4		198.5
2	4.88 (t, 3.0)	76.1	5.94 (dd, 3.3,10.2)	130.0
3	2.35 (m)	30.6	6.78 (ddd, 1.8, 5.7, 9.9)	145.9
	2.10-2.25 (m)			
4	2.90 (dd, 3.6, 13.2)	50.0	2.37 (dt, 3.0,18.9)	45.4
			2.10-2.20 (m)	
5		42.9		38.5
6	2.10-2.18 (m)	34.1	1.80-2.02 (m)	35.6
	1.55 (dt, 3.3, 10.5)		1.51-1.61 (m)	
7	2.15-2.25 (m)	17.5	2.10-2.20 (m)	17.3
	1.87 (dt, 4.5, 14.1)		1.80-2.02 (m)	
8	2.46 (d, 3.6)	45.4	2.51 (br s)	45.4
9		34.2		34.2
10	2.59 (s)	62.1	2.36 (s)	63.3
11	2.35 (m)	47.8	2.66 (dd, 1.8, 15.3)	48.9
	1.40 (dd, 12.3, 14.7)		1.72 (dd, 12.3, 15.3)	
12	5.26 (d, 12.0)	69.9	5.27 (dd, 0.9, 12.3)	69.9
13		123.4		123.6
14	6.36 (dd, 0.9, 1.8)	108.4	6.40 (t, 0.9)	108.4
15	7.40 (t, 1.8)	143.6	7.39 (t, 1.2)	143.2
16	7.44 (d, 0.9)	139.7	7.46 (d, 0.6)	139.4
17		173.7		174.2
18		172.5		
19	1.07 (s)	14.9	1.10 (s)	19.8
20	1.84 (s)	24.5	1.49 (s)	23.7
CO_2CH_3	3.69 (s)	51.7		
$-\text{COCH}_3$		169.6		
$-\text{COCH}_3$	2.14 (s)	21.1		

Table 2

 ^1H (300 MHz) and ^{13}C NMR Data (75 MHz) for **7** and **8**

No. C	7 ^a		8 ^b	
	δ_{H}	δ_{C}	δ_{H}	δ_{C}
1		170.0		170.0
2	2.71 (dt, 4.5, 11.4) 2.01 (m)	29.3	2.10-2.40 (m)	33.2
3	2.24 (m) 1.79 (dt, 4.2, 9.6)	21.9	2.10-2.40 (m) 1.80-1.90 (m)	22.0
4	2.84 (d, 7.2)	55.2	2.80 (d, 6.0)	55.9
5		43.1		43.1
6	1.44-1.54 (m) 1.37 (m)	28.8	1.90-2.00 (m) 1.22-1.36 (m)	25.9
7	1.88-1.95 (m) 1.55-1.65 (m)	17.4	2.20-2.40 (m) 1.90-2.00 (m)	16.6
8	2.94 (dd, 3.0, 10.8)	44.6	2.54 (br s)	43.4
9		36.3		36.8
10		58.8		60.5
11	2.55 (dd, 4.8, 13.2) 1.90-2.00 (m)	38.5	2.52 (d, 15.6) 2.10-2.30 (m)	43.9
12	5.57 (dd, 4.8, 11.7)	70.8	5.30 (d, 12.0)	69.8
13		125.5		123.3
14	6.63 (t, 1.8)	109.2	6.43 (br s)	108.4
15	7.66 (t, 1.5)	144.0	7.44 (br s)	143.7
16	7.75 (t, 0.9)	140.4	7.50 (br s)	139.7
17		171.7		174.9
18		169.7		168.8
19	1.19 (s)	20.8	1.32 (s)	20.3
20	1.25 (s)	15.8	1.58 (s)	26.2

^a**7** was measured in DMSO-d₆^b**8** were measured in CDCl₃.