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Thermal degradation products derived from the smoke of *Salvia divinorum* leaves

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ABSTRACT

Smoking of *Salvia divinorum* leaves is the most common method for its psychotropic effects. Eleven thermal degradation products, including a new neoclerodane diterpene derivative, were isolated from the smoke of *S. divinorum* leaves, and their structures were identified by spectroscopic methods. The isolated compounds were evaluated for their binding affinities at the opioid receptors, and salvinorin A is still the most potent kappa opioid receptor agonist.

Salvia divinorum, a Mexican psychoactive plant, has been used in traditional spiritual ritual for centuries. Its commercial products, leaves and concentrated extracts, are widely available on the internet.[1,2] Smoking the leaves represents one of the common practices for experiencing the psychotropic effects, including hallucination and mood changes.[3–5] The concerns of its safety and pharmacological side effects, especially among the adolescence population, have led to a ban on the possession and selling of *S. divinorum* in several U.S. states.[6] Recently, González et al. reported that smoking the concentrated extract of *Salvia* leaves was the major route of administration among recreational users, and the effects were more potent and intense.[7] Chemical studies of *S. divinorum* have yielded the structurally related neoclerodane diterpenoids salvinorins A–I, divinatorins A–F, salvinicins A and B, and salvidivins A–D.[8] Among those compounds, salvinorin A (**1**) was identified as a potent and selective kappa opioid receptor (KOPR) agonist and the key ingredient responsible for psychoactive effects.[9,10] However, the chemical components of the smoke of *S. divinorum* leaves are still unclear. Therefore, in order to understand the biological and pharmacological principles of the smoke of *S. divinorum*, we report herein the isolation and structural elucidation of eleven thermal degradation products including one new compound (**4**) from the smoke of leaves.

The dried leaves (750 g) or concentrated extract (10×, 90 g) of *S. divinorum* was burned and the smoke was collected in a series of cold traps filled with $CHCl_3$ and H_2O (see Supplementary data). The solutions were evaporated under reduced pressure to obtain $CHCl_3$ and H_2O extracts, respectively. Based on the binding results at the opioid receptors, only $CHCl_3$ extract showed potent binding activities. Subsequently, the $CHCl_3$ extracts (40 g from leaves and 10 g from concentrated extract) were chromatographed, respectively, on silica gel columns and eluted with *n*-hexane–AcOEt to give sub-fractions, which were subjected to repeated silica gel or C-18 column chromatography to give eleven thermo-degradation products. Salvinorin A (**1**, 0.5 mg, see Fig. 1),[11] pyrocoll (10 mg),[12] 3-methyl-indole (8 mg),[13] indole (5 mg),[14,15] 2-hydroxypyridine (20 mg),[16,17] 3-hydroxypyridine (15 mg),[18] 3-hydroxy-2-methylpyridine (10 mg),[19] and β-sitosterol (50 mg)[20] were isolated from the $CHCl_3$ fraction of burned *Salvia* leaves, while salvinorin A (**1**, 53 mg),[11] 8-epi-salvinorin A (**2**, 35 mg),[21] 2-deacetoxy-8-epi-salvinorin A (**3**, 15 mg),[22] 1-deacetoxy-8-epi-salvinorin G (**4**, 2 mg), and β-sitosterol (250 mg)[20] were obtained from the $CHCl_3$ fraction of burned *Salvia* extract. The known compounds were identified by comparison with their published data or authentic samples. It should be noted that the isolated nitrogenous compounds have been reported as thermal decomposition products isolated from the smoke of tobacco cigarette.[23]

Compound **4** was obtained as a white powder with $[\alpha]_D^{23}$ −32 (*c* 0.16, $CHCl_3$). Its molecular formula was determined to be $C_{21}H_{24}O_6$ on the basis of HR ESI-MS at *m/z* 395.1467 $[M+Na]^+$ (calcd 395.1471). The IR spectrum disclosed the absorption bands due to carbonyl groups (1731 and 1693 cm^{-1}). The characteristic ^1H NMR spectrum revealed the presence of two tertiary methyl groups (δ 1.33 and 1.48), an oxygenated methine proton (δ 5.28), and four olefinic protons (δ 6.26, 6.40, 7.42, and 7.47). The ^{13}C NMR spectrum of **4** revealed 21 carbon signals, which were ascribed to three methyls (δ 17.9, 23.6, and 52.3), four methylenes

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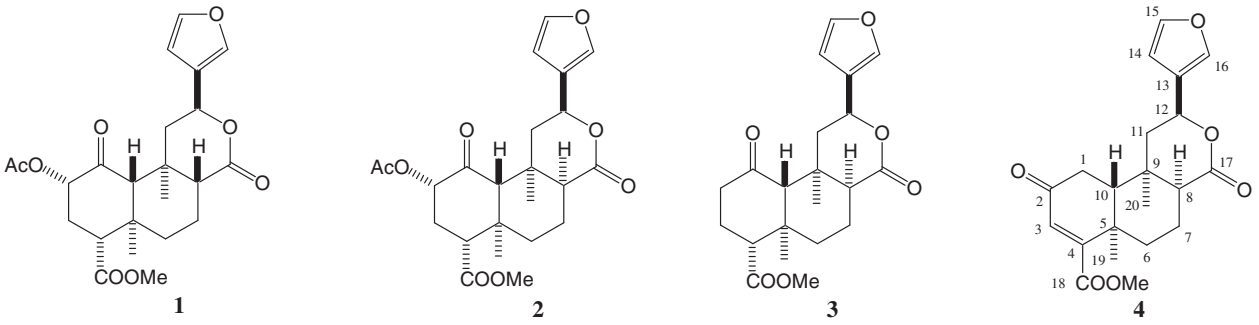

Figure 1. Neoclerodane diterpene derivatives isolated from the smoke of *S. divinorum* leaves.

(δ 17.7, 29.6, 35.3, and 48.2), seven methines (δ 44.2, 50.7, 69.6, 108.3, 129.4, 139.8, and 143.8), four quaternary carbons (δ 35.4, 38.4, 123.4, and 159.6), and three carbonyl carbons (δ 166.6, 173.5, and 198.9). Comparison of the ^{1}H and ^{13}C NMR spectra of **4** with those of salvinorin G[11] and deacetylsalvinorin G[24] suggested that these compounds were structurally similar. According to the ^{1}H–^{1}H COSY, HMQC, and HMBC spectra (Table 1 and Fig. 2), it was confirmed that the oxygen-containing group at C-1 was eliminated. The relative stereochemistry of H-8 in **4** was assigned as α-orientation, based on NOESY cross-peak between H-8 and H-12, and comparison of the ^{13}C NMR chemical shifts at C-8, C-11, C-12, C-13, C-17, and C-20 with those of natural and unnatural salvinorin derivatives.[22,24] Accordingly, the structure of **4** was determined as 1-deacetoxy-8-epi-salvinorin G.

Among the isolated compounds, we have previously reported that salvinorin A (**1**) has high affinity (K_i = 1.0 ± 0.1 nM), and 8-epi-salvinorin A (**2**) has modest affinity (K_i = 77 ± 4 nM) at the KOPR.[21] All other compounds were screened for binding affinity at opioid receptors in membranes of CHO cells stably transfected with an opioid receptor (see Supplementary data). At 3 μM, none of the compounds inhibited [^{3}H]diprenorphine binding to mu, delta, and kappa opioid receptors by more than 50%, indicating that they did not have significant binding affinity. Our findings provide scientific explanation as to why smoking the concentrated extracts is more effective than smoking the leaves. Salvinorins **1**–**4** were found in much higher quantity in the smoke of concentrated extract than those in the smoke of leaves. Total 105 mg of salvinorins **1**–**4** were isolated from the smoke of 90 g of concentrated extracts, while only 0.5 mg of **1** was isolated from that of 750 g of *Salvia* leaves.

Table 1
^{1}H (300 MHz) and ^{13}C NMR data (75 MHz) for **4** in $CDCl_3$

No. C	δ_H	δ_C
1	2.53 (dd, 14.1, 17.4) 2.38 (dd, 3.3, 17.4)	35.3
2		198.9
3	6.26 (s)	129.4
4		159.6
5		38.4
6	2.14 (dt, 12.9, 3.6) 1.64–1.78 (m)	29.6
7	2.28 (dq, 14.4, 2.4) 1.88–2.00 (m)	17.7
8	2.55 (d, 3.3)	44.2
9		35.4
10	1.83 (dd, 3.3, 14.1)	50.7
11	1.90–2.02 (m) 1.64–1.78 (m)	48.2
12	5.28 (d, 12.6)	69.6
13		123.4
14	6.40 (d, 0.9)	108.3
15	7.42 (t, 1.8)	143.8
16	7.47 (d, 0.6)	139.8
17		173.5
18		166.6
19	1.48 (s)	17.9
20	1.33 (s)	23.6
CO_2CH_3	3.79 (s)	52.3

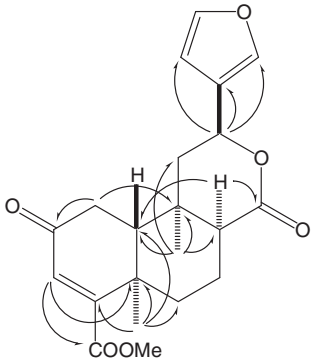

Figure 2. Significant HMBC correlations of **4**.

Burning the *Salvia* leaves produced a significant amount of 8-epi-salvinorin derivatives (**2**–**4**). Previous chemical studies have demonstrated that **1** and its derivatives readily underwent epimerization at C-8 under basic conditions.[25] The mechanism of epimerization proposed by Munro et al. involves enolization of the lactone.[26,27] Further epimerization studies demonstrated that the equilibrium favors the H-8α epimer over the H-8β stereochemistry.[28,29] Because the energy barrier between the chair and boat conformation of δ-lactones is small, the difference between C8-axial and equatorial substituents is minimized.[28] Therefore, the thermodynamically favored products of natural salvinorins in the smoke of *S. divinorum* leaves could be 8-epi-salvinorin derivatives.

In summary, eleven thermal degradation products were isolated from the smoke of *S. divinorum* leaves. The major structural changes of neoclerodane diterpenoids include the C-8 epimerization and acetoxy elimination. Biological binding data suggest that salvinorin A (**1**) is still the key ingredient responsible for psychoactive effects.

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Supplementary data

Supplementary data associated with this article can be found, in the online version, at doi:10.1016/j.tetlet.2010.08.033.

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