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Revised structure of deacetyl-1,10-didehydrosalvinorin G

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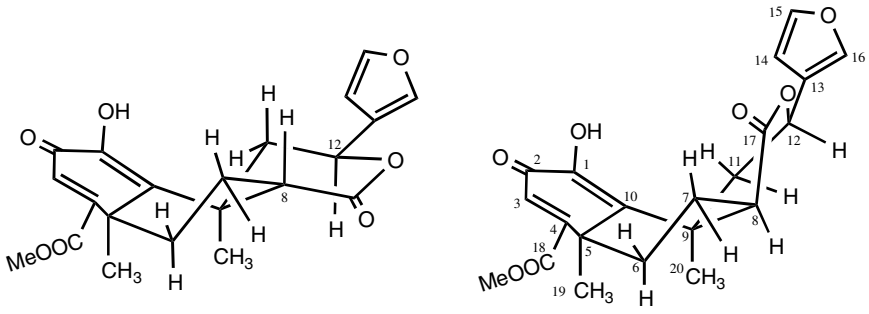
Abstract—In comparison with the NMR data of salvinorin A and its 8-epimer, the published structure of deacetyl-1,10-didehydrosalvinorin G was revised to its 8-epimer. The stereochemistry of 8-*epi*-deacetyl-1,10-didehydrosalvinorin G was further confirmed by NOESY and chemical synthesis.

Salvinorin A (**1a**), a non-nitrogenous neoclerodane diterpenoid, was isolated from the Mexican medicinal plant *Salvia divinorum*.[1,2] Compound **1a** was identified as a potent and selective kappa (κ) opioid receptor (KOR) agonist and as the key ingredient for psychoactive effects.[3–5] During the course of structure–activity relationship (SAR) studies,[6–18] it was found that **1a** and its derivatives readily underwent epimerization at C-8 under basic conditions. Using various inorganic bases, such as $NaBH_4$,[2,12,19] $NaHCO_3$,[20] Na_2CO_3,[7,12] K_2CO_3,[8] LiSEt[10] and LiI,[11] **1a** produced corresponding natural (8-Hβ) and unnatural (8-Hα, also called 8-*epi*-) mixtures. Surprisingly, treatment of **1a** with excess strong base KOH or $Ba(OH)_2$ gave a natural salvinorin derivative deacetyl-1,10-didehydrosalvinorin G that was claimed by different research groups to have structures **2a** and **3**.[13,8] In general, the affinity and potency of natural salvinorin derivatives show much higher KOR binding activities than those of unnatural 8-epimers.[8,12,15,21] Since the configuration of a molecule can significantly affect KOR binding, it is essential to establish a reliable method which would unambiguously determine the stereochemistry at C-8 of salvinorins. This prompted us to conduct a comprehensive analysis of the ^1H and ^{13}C NMR data of natural and unnatural salvinorin derivatives. The differences of **1a** and its 8-epimer (**1b**) in their ^1H and ^{13}C NMR spectra are discussed in this Letter. Based on the summarized NMR data and chemical synthesis, the stereochemistry of **2a** at C-8 is revised to its 8-epimer (**2b**) as shown in Figure 1.



2a **2b**

Figure 1. Conformational structures of **2a** and **2b**.

Keywords: Salvinorin A; Epimer; NMR; Revised structure.
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In connection with our previous study related to determining the stereochemistry of betulinic acids,[22] we recognized that the ^{1}H and ^{13}C NMR data of salvinorins and their corresponding 8-epimers have significant differences. A pair of 8-epimers (**1a** and **1b**), isolated[9] and synthesized[8] in our group, was selected as standard compounds for NMR analyses. Using 2D NMR techniques, including COSY, NOESY, HMQC and HMBC, permitted the full assignments of all ^{1}H and ^{13}C NMR chemical shifts. The relative stereochemistry at the C-8 position of these compounds was unambiguously determined, based on their ^{1}H and ^{13}C NMR data. For instance, in the ^{1}H NMR spectra, the C-8-H of **1a** resonated at δ 2.07 (dd, $J = 3.0$ and 12.0 Hz), confirming axial orientation, while that of **1b** resonated at δ 2.45 (d, $J = 2.7$ Hz) for equatorial orientation. The chemical shift change ($\Delta\delta$) between **1a** and **1b** is about 0.38 ppm (Table 1). In addition, the C-12-H of **1b** shifted upfield ($\Delta\delta$ 0.27 ppm) to δ 5.26 (dd, $J = 1.8$ and 12.0 Hz) in comparison with that of **1a** at δ 5.53 (dd, $J = 4.8$ and 11.7 Hz). The coupling constants of H-12 revealed the axial orientation of H-12 in both **1a** and **1b**. The X-ray analyses showed that the lactone ring in **1a** adopts a chair confirmation,[1,2,17] while the one in **1b** is a boat conformation.[21] The different conformation may explain the J values differences of H-12 between **1a** and **1b**. On the other hand, the H-8 configuration also strongly affects the ^{13}C NMR data in B- and C-ring carbons. For instance, the methylene carbon (C-11), carbonyl carbon (C-17) and axial methyl carbon (C-20) of **1b** showed lower-field chemical shifts in comparison with those of **1a** (Table 2), while the ^{13}C resonances of C-6, C-8, C-12, C-13 and C-19 of **1b** shifted to upper field. In summary, the characteristic carbon peaks of C-12, C-17 and C-20 can be employed readily for identification of natural and unnatural 8-epimers. In the previous published papers,[10,13,21] 8-epimers were mainly determined based on the coupling constants of H-8 and H-12, irradiation of H-12 for a NOE enhancement of H-8, and a general TLC Rf value. Our generalized NMR data should provide reliable information for identification of future salvinorin analogs, including C-8 epimers.

Table 1. ^{1}H NMR data (300 MHz) at H-8 and -12 for **1a**, **1b** and **2b** in $CDCl_3$

Compound	**1a**[a]	**1b**[a]	$\Delta\delta_{\mathbf{1b-1a}}$	**2b**
H-8 (δ)	2.07	2.45	+0.38	2.99
J value	dd, 3.0, 12.0	d, 2.7		dd, 5.1, 9.6
H-12 (δ)	5.53	5.26	−0.27	5.45
J value	dd, 4.8, 11.7	dd, 1.8, 12.0		dd, 2.4, 12.3

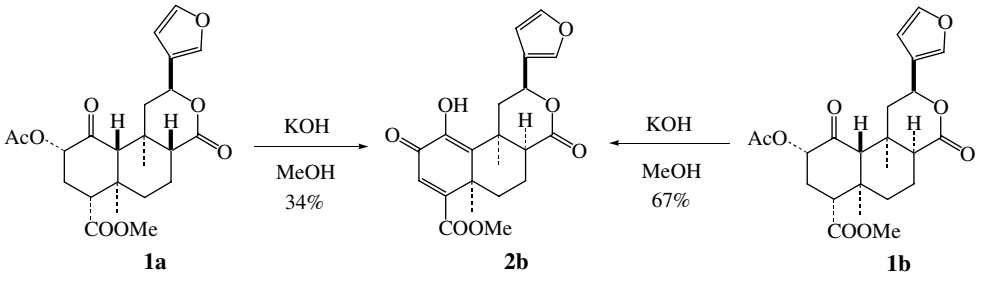
[a] See Refs. 10, 20 and 23 for full ^{1}H NMR assignments of **1a** and **1b**.

We reported previously that treatment of **1a** with $Ba(OH)_2$ in MeOH gave an unexpected oxidative-elimination product that was assigned as structure **3**.[8] In a very short time span, the structure of **3** was revised to deacetyl-1,10-didehydrosalvinorin G (**2a**), which was synthesized in 1 M KOH methanol solution.[13] The structural revision was based on extensive NMR experiments, including ^{1}H NMR, ^{13}C NMR, DEPT, COSY, HMQC, HMBC and NOE, and HR-ESI-MS. Following the published procedure (Scheme 1),[13] indeed, we isolated the same product.[24] The NMR data were in full

Table 2. ^{13}C NMR data (75 MHz) for **1a**, **1b** and **2b** in $CDCl_3$

Carbon #	**1a**	**1b**	$\Delta\delta_{\mathbf{1b-1a}}$[a]	**2b**	Carbon #	**1a**	**1b**	$\Delta\delta_{\mathbf{1b-1a}}$[a]	**2b**
1	202.0	202.3		145.1	13	125.2	123.4	−1.8	124.4
2	75.0	75.2		180.7	14	108.4	108.5		108.4
3	30.7	30.7		128.2	15	143.7	143.5		143.7
4	53.6	52.9		157.5	16	139.4	139.7		139.6
5	42.1	42.2		42.3	17	171.1	173.4	+2.3	173.2
6	38.1	34.0	−4.1	28.3	18	171.5	171.8		165.4
7	18.1	17.6		21.9	19	16.4	15.2	−1.2	30.3
8	51.4	45.2	−6.2	44.8	20	15.2	24.6	+9.4	24.4
9	35.4	34.7		37.6	–COO*Me*	52.0	51.7		52.6
10	64.1	64.0		140.0	–*C*OCH$_3$	170.0	169.7		—
11	43.4	48.0	+4.6	36.8	–CO*C*H$_3$	20.6	20.5		—
12	72.0	70.0	−2.0	70.8					

[a] Data is given when $\Delta\delta_{\mathbf{1b-1a}}$ is more than 1.0 ppm.



Scheme 1.

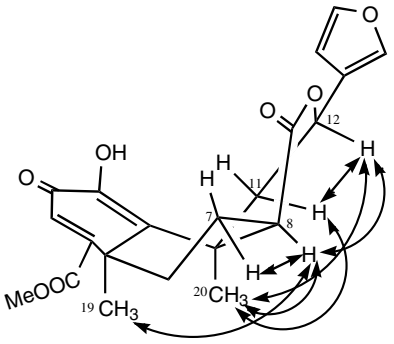
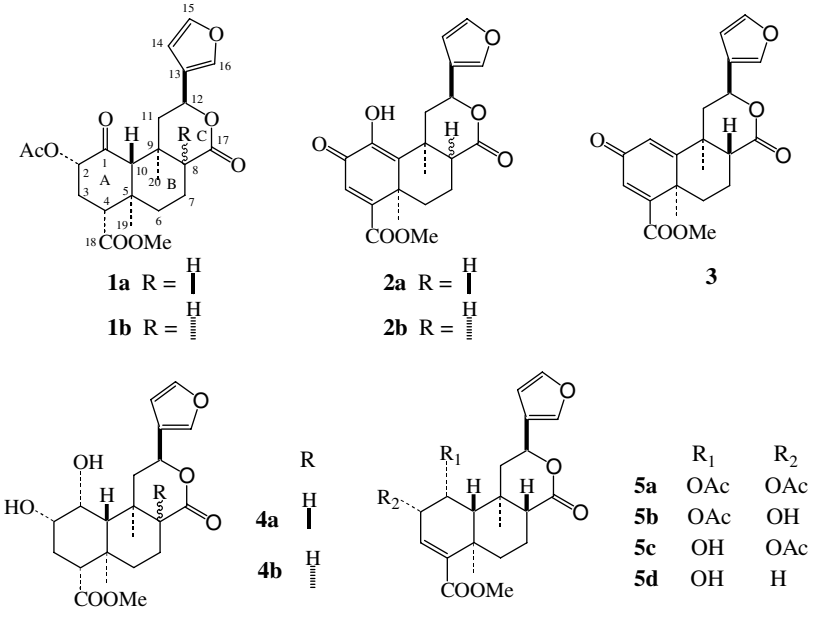


Figure 2. Key NOE interactions of **2b**.

agreement with previous report[13] except that the assignments of H-7α (δ 1.98) and H-7β (δ 2.24) should be reversed as H-7α (δ 2.24) and H-7β (δ 1.98). After careful comparison of our ^{1}H and ^{13}C NMR data with those of **1a** and **1b** (Tables 1 and 2), we found that structure **2a** assigned to the product was incorrect,[13] and the correct structure should be 8-*epi*-deacetyl-1,10-didehydrosalvinorin G (**2b**, Fig. 1). In the ^{1}H NMR spectrum, the C-8-H of **2b** shifted much lower field to δ 2.99 (dd, $J = 5.1$ and 9.6 Hz), while the C-12-H shifted upfield slightly compared with that of **1a** (Table 1). The H-12 coupling constants (dd, $J = 2.4$ and 12.3 Hz) of **2b** were closer to those of **1b** (dd, $J = 1.8$ and 12.0 Hz) than of **1a** (dd, $J = 4.8$ and 11.7 Hz), but the H-8 of **2b** showed the J values (5.1 and 9.6 Hz) for axial orientation.[13] However, this perplexing configuration at C-8-H was soon resolved by comparing the C-ring ^{13}C NMR data of **2b** with those of **1a** and **1b** (Table 2). The ^{13}C NMR chemical shifts at C-8, C-12, C-17 and C-20 are very similar to those of **1b**, indicating that the orientation of H-8 is α. The revised structure **2b** was further confirmed by the NOE interactions shown in Figure 2. In the NOESY spectrum (see Supplementary data), H-8 (δ 2.99) showed cross peaks to H-7α (δ 2.24, strong), H-7β (δ 1.98, weak), H-19 (δ 1.72) and H-20 (δ 1.67), while H-12 (δ 5.45) related to H-11α (δ 3.11, strong), H-11β (δ 2.02, weak) and H-20 (δ 1.67). It should be noted that the crossed peak between H-8 and H-12 was very weak. Furthermore, when **1b**, the 8-epimer of **1a**, was treated with 1 M KOH in MeOH, it afforded endione **2b** in a 67% isolated yield (Scheme 1).[24] Valdes et al.[2] reduced **1a** with $NaBH_4$ in *i*-PrOH and afforded equal amounts of 8-epimeric mixtures (diol **4a** and 8-*epi*-diol **4b**), while Munro et al.[13] reduced **2a** with $NaBH_4$ in $EtOH/CH_2Cl_2$ and obtained sole product—8-*epi*-diol **4b**. All of this evidence supports structure **2b**.

The coupling constants of H-8 in **2b** were misleading in comparison with those of other 8-*epi*-salvinorins. This is the main reason for Munro et al.[13] to determine H-8 as β configuration. Because of the double bond between C-1 and C-10, the conformation of **2b** is distorted. It is known that A/B/C rings in **1a** are in chair/chair/chair conformation[1,2] and A/B/C rings in **1b** are in chair/chair/boat conformation.[21] Based on NOESY and molecular modeling analyses, A/B/C rings in **2b** should be face-down boat/twist-chair/twist-chair conformation (Fig. 1). Under this circumstance, both H-7β and H-8 resemble axial orientations and showed a closer diaxial coupling constant (9.6 Hz). Without sufficient NOE data,[13] the incorrect assignments of H-7α and H-7β in **2b** might depend on those of the known salvinorins C (**5a**), D (**5b**), E (**5c**) and F (**5d**), isolated from *S. divinorum* by Munro and Rizzacasa.[25] Because the chemical shifts of H-7 and H-8 in **5a**, **5b**, **5c** and **5d** overlapped,[19,25] the unambiguous assignments of H-7α and H-7β with NOESY spectra became very difficult. Unexpectedly, one year later, Munro gave the revised assignments of H-7α and H-7β of **2b** without any scientific explanation.[20] Based on our NOE data, all protons in **2b** were fully assigned.[24]

In conclusion, a concise and informative NMR method for the determination of the C-8-H configuration of salvinorins was established. Based on this method, the correct product obtained by the treatment of **1a** with hydroxide in MeOH has been identified. The C-8



epimeric structure (**2b**) was confirmed by NOESY spectrum and chemical conversion.

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Supplementary data

Copies of ^{1}H and ^{13}C NMR spectra of **1a**, **1b** and **2b**, and 2D NOESY spectrum of **2b**. Supplementary data associated with this article can be found, in the online version, at doi:10.1016/j.tetlet.2007.05.179.

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24. *Synthesis of* **2b**. Compound **1a** (40 mg, 92.6 μmol) was added to a solution of KOH in MeOH (1 M, 3 mL) and stirred at room temperature for 1 h. Following the previous procedure,[13] crude **2b** was obtained. Further purification by silica gel column [hexane/AcOEt (2:1)] gave pure **2b** (12 mg, yield 34%). In the same manner, **2b** was also obtained from **1b** in 67% isolated yield. The ^{1}H and ^{13}C NMR chemical shifts of **2b** were in full agreement with those of published data.[8,13] The H-6α and H-6β were assigned as δ 2.53 and δ 1.67–1.77, respectively. The H-7α and H-7β were revised to δ 2.24 and δ 1.98, respectively. EI-MS *m*/*z*: 386 (M^{+}).
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