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A facile method for the preparation of deuterium labeled salvinorin A: synthesis of [2,2,2-$^2H_3$]-salvinorin A

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Abstract—Salvinorin A is a novel hallucinogen isolated from the widely available leaves of *Salvia divinorum*. Based on its mechanism of action, salvinorin A has shown potential as a stimulant abuse therapeutic. However, there are no methods for the detection of salvinorin A or its metabolites in biological fluids. In order to begin developing salvinorin A as a potential therapeutic, an understanding of its metabolism is needed. Here, a straightforward synthesis of a deuterium labeled analog of salvinorin A and its utility as an internal standard for the detection of salvinorin A and its metabolites in biological fluids by LC–MS is described.

Salvinorin A (**1a**) (Fig. 1) is a neoclerodane diterpene originally isolated from the leaves of *Salvia divinorum* by Ortega et al. in 1982[1] and soon thereafter, Valdés III et al.[2] *S. divinorum* is a plant from the Sage family that has been used in traditional spiritual practices by the Mazatec Indians of Oaxaca, Mexico to produce 'mystical' or hallucinogenic experiences.[3] A smoked dose of 200–500 μg of salvinorin A, the presumed active ingredient, produces profound hallucinations lasting up to 1 h.[4,5] Thus, it has a potency that is similar to the highly active synthetic hallucinogen LSD (**2**).

Recreational use of the Mexican mint, *S. divinorum*, has recently increased rapidly.[6] Young adults and adolescents have begun to smoke the leaves and leaf extracts of the plants to induce powerful hallucinations. Recipes for leaf extracts, elixirs, and tinctures may be found posted on the Internet.[7] Currently, Mexican youths have begun to use the plant as a substitute for marijuana.[8] In addition, a large number of *S. divinorum* plants were seized at a large-scale plantation in Switzerland, indicating this plant's increasing use as a recreational drug in Europe.[8] Due to the recent increase in the popularity of the plant among both European and American teens, the DEA has recently placed it on the list of drugs of concern.[6]

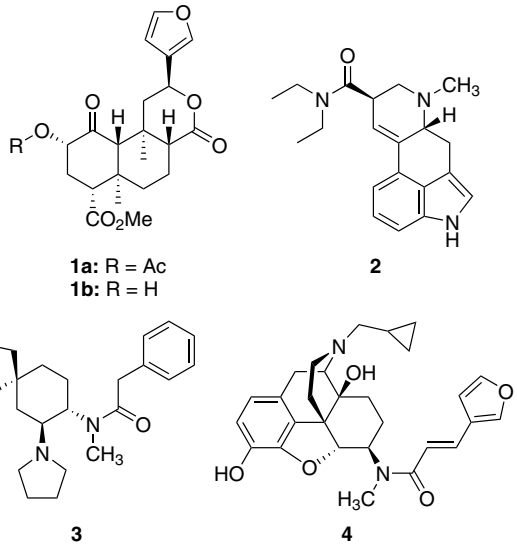

Figure 1. Structures of salvinorin A (**1a**), salvinorin B (**1b**), LSD (**2**), U69,593 (**3**), and TRK-820 (**4**).

Currently, *S. divinorum* is unregulated in most countries and available throughout the world by purchasing it

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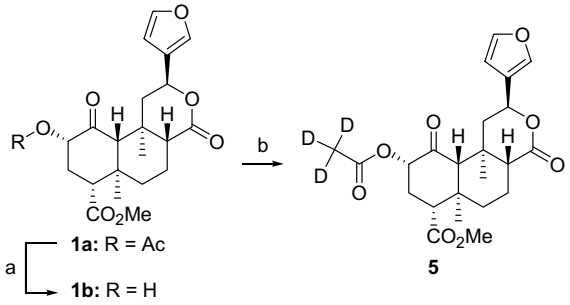
over the Internet but is listed as a controlled substance in Denmark and Australia. Obtaining *S. divinorum* is easy in countries where it is unregulated and it is a cheap, easy solution for many youths who wish to experiment with drugs and perception altering substances. At present, US laws for controlled substances do not ban the use of *S. divinorum* or its active components. This has resulted in various on-line botanical companies advertising and selling *S. divinorum* as a legal alternative to other regulated plant hallucinogens. As mentioned earlier, these botanical companies also sell extracts, salvinorin A enhanced extracts, and *S. divinorum* tinctures and elixirs.[7] It is predictable that its misuse will increase rapidly.

Curiously, salvinorin A does not act at the presumed molecular target responsible for the actions of classical hallucinogens, the serotonin 5-HT_{2A} receptor.[9–12] Rather, studies have shown that salvinorin A is a potent and selective κ opioid receptor agonist. Interestingly, salvinorin A (**1a**) shares little structural similarity with other κ agonists such as U69,593 (**3**) and TRK-820 (**4**).

Selective κ opioid receptor agonists have been explored as analgesics with potential for reduced tolerance and dependence. However, psychotomimesis, dysphoria, and diuresis are associated with their therapeutic use. Interestingly, a large body of evidence indicates that κ receptors may be involved in the modulation of some abuse related effects of CNS stimulants.[13–16] These findings indicate that κ opioid receptors may be involved in the antagonism of some abuse related effects of cocaine, offering a novel pharmacological approach to treat cocaine abuse.

As part of our program to develop novel stimulant abuse therapeutics, we sought to investigate the psychopharmacology of salvinorin A. Presently, the identity of the metabolites of salvinorin A are unknown. It has been suggested that salvinorin B (**1b**) (Fig. 1) is a metabolite of salvinorin A.[5] However, this has not been shown definitively either in vitro or in vivo. In order to better study the metabolism of salvinorin A in vivo, the development of a validated liquid chromatography–mass spectrum (LC–MS) method for the determination of salvinorin A and its potential metabolites, such as salvinorin B, in biological fluids was needed. As is generally agreed, stable isotopically labeled analogs with the same molecular structure of a compound are the best internal standards for the LC–MS assay. To this end, we describe the synthesis of an analog that is specifically labeled with deuterium starting from salvinorin A (Scheme 1).

Rather than begin a lengthy total synthesis of salvinorin A, we initially focused on extracting salvinorin A from the dried leaves of *S. divinorum*. Commercially available leaves were extracted as previously described[1,2,17] and afforded salvinorin A. However, we then set out to further improve the bioyield of salvinorin A. Modification of extraction procedure[18] resulted in the isolation of 7.5 g of salvinorin A from 1.5 kg of dried leaves. This process has resulted in an improved bioyield of salvinorin A compared to previously described methodology. Efforts were then shifted to the preparation of salvinorin B. Basic hydrolysis of the C-2 acetate using sodium carbonate in MeOH afforded salvinorin B in 77% yield.[19] Curiously, these conditions do not result in the cleavage of the C-18 methyl ester as noted by the presence of a methyl singlet at 3.7 ppm in the ^{1}H NMR spectrum. This is likely due to the C-18 position being more sterically hindered than the C-2 position. More vigorous conditions, such as heat and NaOH, are required for the cleavage of this group. However, this leads to the opening of the lactone ring, as well as epimerization of the 8-position. Reacetylation of salvinorin B using d_6-acetic anhydride in the presence of a catalytic amount of DMAP afforded [2,2,2-$^2H_3$]-salvinorin A (**5**) in 80% yield.[20]



Scheme 1. Reagents and conditions: (a) Na_2CO_3, MeOH, 77%; (b) $(Cd_3CO_2)_2O$, DMAP, CH_2Cl_2, 80%.

Analysis of isotopic purity of **5** was performed by tabulating mass intensities from the peak top and subtracting mass intensities from a nearby blank region of the chromatogram.[21] Approximately 86% of the total ion intensity is due to the d_3 species, most of the remainder corresponds to the d_2 species (Fig. 2).

A calibration curve for salvinorin A was constructed by spiking eight 0.25 mL aliquots of human plasma with 100 ng of **5** and 1, 5, 10, 25, 50, 100, 250, or 500 ng salvinorin A, respectively. Controls at 40, 200, and 400 ng, as well as a blank and a blank 0 were prepared in the same manner. All samples were extracted using Waters Oasis SPE cartridges.[21] Salvinorins were eluted with 75% CH_2Cl_2/CH_3CN. After evaporation, samples were reconstituted in 0.1 mL of 75% CH_3CN/H_2O. Analysis was done with a Shimadzu LCMS-2010A with an APCI

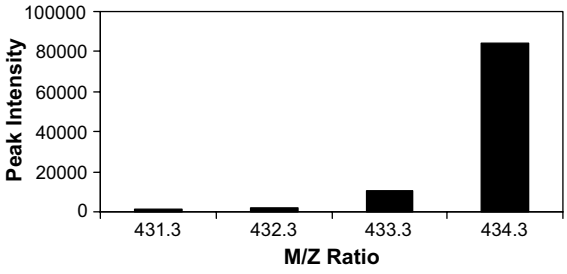


Figure 2. Analysis of isotopic purity of **5**.

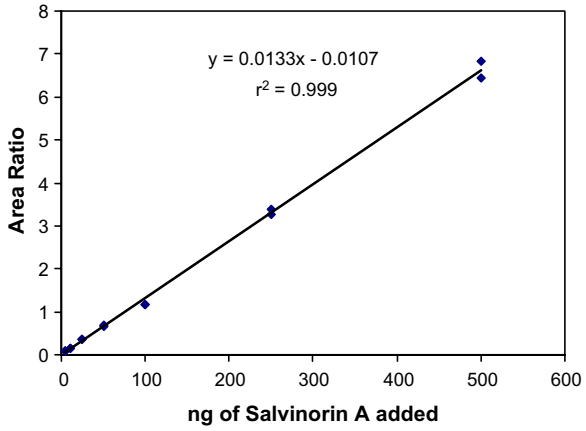


Figure 3. Calibration curve for **1a** in human plasma.

probe operating in negative ion mode. [M−1] ions were obtained and used for quantitation. Separation was done using a Phenomenex Synergi Polar-RP column (150 mm × 2 mm, 4 μm), eluted isocratically with 50% CH_3CN/4 mM NH_4OAc adjusted to pH 7.2. The calibration curve was linear with a coefficient (r^2) value of 0.999 (Fig. 3).

In conclusion, an improved extraction method for the isolation of salvinorin A from the leaves of *S. divinorum* is described. Synthesis of [2,2,2-$^2H_3$]-salvinorin A (**5**) was achieved in two steps from salvinorin A. An LC–MS method employing deuterium-labeled **5** was also developed and found to be suitable for the detection of salvinorin A and its metabolites in biological fluids. This method allows us and others to better monitor the concentration of salvinorin A in biological fluids. Additional studies characterizing the structure–activity relationships and metabolism of salvinorin A are currently under investigation and will be presented in due course.

Acknowledgements

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18. Dried *Salvia divinorum* leaves (1.5 kg), obtained commercially from Ethnogens.com, were ground to a fine powder and percolated with acetone (5 × 4 L). The acetone extract was concentrated under reduced pressure to afford a crude green gum (93 g), which was subjected to column chromatography on silica gel with elution in *n*-hexanes containing increasing amounts EtOAc. Fractions eluting in 20% *n*-hexanes/EtOAc contained salvinorin A (TLC) and other minor diterpenes and some pigmented material. These fractions were pooled and concentrated in vacuo to give a green gum (24 g). A mixture of the crude green gum, acetic anhydride (50 mL, 530 mmol) and DMAP (0.2 g) in CH_2Cl_2 (250 mL) was stirred at rt overnight. The CH_2Cl_2 solution was washed sequentially with 1 N HCl (2 × 500 mL), 2 N NaOH (100 mL), and H_2O (2 × 100 mL). The CH_2Cl_2 solution was dried (Na_2SO_4) and the solvent was removed under reduced pressure to afford a yellow-green gum (23 g). The resulting gum was subjected to column chromatography on silica gel. Elution was performed in 1000 mL aliquots of a mixture of *n*-hexanes/EtOAc in increments of 10% EtOAc with the final elution in neat EtOAc. Fractions eluting in 30% *n*-hexanes/EtOAc and subsequent fractions were pooled and the solvent was removed under reduced pressure affording salvinorin A (7.5 g, 0.5%) as a green powder, mp 235–238 °C (lit.[1,2] 240–242 °C).
19. A mixture of **1a** (3.5 g, 8.0 mmol) and Na_2CO_3 (3.4 g, 32.2 mmol) in absolute MeOH (150 mL) was stirred at room temperature for 4 h. The solvent was removed under reduced pressure and CH_2Cl_2 (500 mL) was added to the crude residue. The organic extract was washed successively with 2 N HCl (50 mL) and saturated NaCl (50 mL) and dried (Na_2SO_4). The solvent was removed under reduced pressure and MeOH (100 mL) was added to the residue. The resulting solid was collected by filtration and dried to afford 2.4 g (77%) of **1b** as a white solid, mp 211–214 °C (lit.[2] 213–216 °C).
20. A solution of **1b** (0.1 g, 0.3 mmol), d_6-acetic anhydride (0.1 g, 1.3 mmol) and a catalytic amount of DMAP in CH_2Cl_2 (20 mL) was stirred at room temperature for 2 h. Absolute MeOH (15 mL) was added and the solvent was removed under reduced pressure. CH_2Cl_2 (25 mL) was added to the residue and the solution was washed with 10% HCl (3 × 20 mL) and saturated NaCl (3 × 20 mL) and dried (Na_2SO_4). Removal of the solvent under reduced pressure afforded 0.09 g (80%) of **5** as a white solid, mp

237–240 °C: ^{1}H NMR (300 MHz, $CDCl_3$) δ 1.12 (s, 3H), 1.46 (s, 3H), 1.80 (m, 1H), 2.07 (dd, J = 3.0, 11.6 Hz, 1H), 2.31 (m, 2H), 2.51 (dd, J = 5.4, 13.2 Hz, 1H), 2.75 (dd, J = 6.3, 10.2 Hz, 1H), 3.73 (s, 3H), 5.15 (dd, J = 9.9, 9.9 Hz, 1H), 5.53 (dd, J = 4.8, 12.0 Hz, 1H), 6.37 (dd, J = 0.9, 1.5 Hz, 1H), 7.39 (dd, J = 1.5, 1.8 Hz, 1H), 7.41 (dd, J = 0.9, 1.5 Hz, 1H).

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