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Autoxidation of Salvinorin A under Basic Conditions

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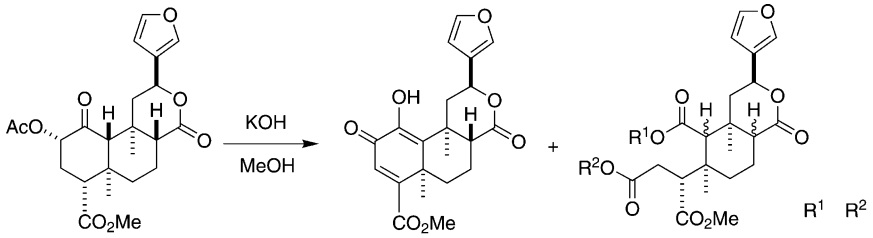
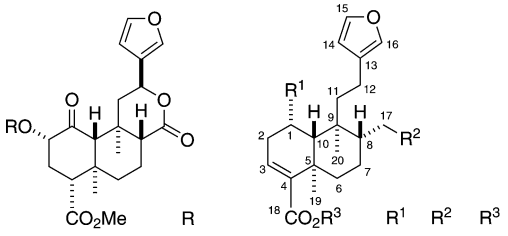


Treatment of salvinorin A (**1a**) with KOH in MeOH gave the enedione **3**, for which the dienone structure **7** was recently proposed. Also isolated, after methylation, were the secotriesters **4a**−**c**. A mechanism for this unusual series of autoxidations is proposed. Surprisingly, **4a** showed weak affinity at the κ-opioid receptor. Divinatorins A−C (**2a**−**c**) showed no affinity at opioid receptors. Attempted reduction of **3** to a novel salvinorin diol **(9d)** was unsuccessful, but careful deacetylation of salvinorin C (**9a**) provided a viable route to this compound. A general method for identifying salvinorin 8-epimers by TLC is also presented.

Introduction

Salvinorin A (**1a**)[1] is a neoclerodane diterpenoid isolated from the Mexican medicinal plant *Salvia divinorum*. Compound **1a** is an agonist at the κ-opioid receptor (KOR)[2] and is the most potent naturally occurring hallucinogen known.[3] As a result, both the compound and the plant itself have been prohibited in some countries and several US states. Compound **1a** has also attracted growing scientific interest, being the only nonnitrogenous KOR agonist known, with no apparent structural similarity to other ligands. This anomaly has inspired several groups to study the structure−activity relationships of **1a**.[4−11]

We recently showed that the ketone and lactone are not involved in activation of the KOR by **1a**, while the methyl ester and furan ring are.[10] Consistent with this, recent work has confirmed that steric hindrance around



	R		R^1	R^2	R^3
1a	Ac	**2a**	OH	H	H
1b	H	**2b**	OH	OH	Me
1c	Et	**2c**	H	OAc	H
1d	MOM	**2d**	OH	OAc	Me
1e	Bz	**2e**	OH	CHO	Me

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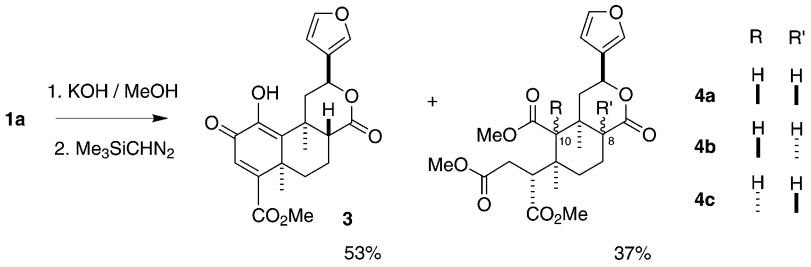
SCHEME 1



the furan ring[12] or the C-18 ester[9] greatly reduce binding affinity. Most work, however, has been devoted to modification of the acetoxy function. More hindered esters show greatly reduced affinity at the KOR.[4−6,8] The deacetyl compound salvinorin B (**1b**)[13] also shows little[8] or no[5] affinity. Nonetheless, the acyloxy group is not essential for activity. Some notable examples are the ethyl ether **1c**,[4] which displays comparable affinity at the KOR to **1a**, and even more remarkably the methoxymethyl ether **1d**,[8] which exhibits substantially increased (sub-nanomolar) affinity and potency. Another surprise is the benzoate **1e**,[6] which is a potent μ-opioid receptor agonist. Some of these compounds also exhibit antagonist activity.[10,12] Complementing this work, site-directed mutagenesis suggests that **1a** and analogues activate the KOR via different residues than conventional ligands.[11] That such a small body of work should generate so many noteworthy results establishes **1a** as a valuable lead for the study of opioid receptors and for the development of new ligands.

Results and Discussion

For the purposes of our derivative work, we needed to cleave the methyl ester. While searching for a suitable method, we treated **1a** with 1 M KOH in MeOH. To our surprise, the major product, found in the neutral fraction, was enedione **3** (Scheme 1). The ^{1}H spectrum showed two new singlets at δ 6.91 and 6.99 ppm. The peak at 6.91 showed no COSY or HMQC cross-peak and exchanged with D_2O. Such strongly deshielded exchangeable peaks are typical of cyclic α-diones, whose enol tautomers are stabilized by internal H-bonds.[14] Consistent with this, the compound exhibited strong IR absorptions at 3373 and 1651 cm^{-1} (OH and enol C=C). The structure was further elucidated by analysis of the HMBC spectrum. The quaternary C-10 peak, located unambiguously by its correlations to the H-19 and 20 methyls, showed a correlation to the enolic proton, placing the enol at C-1 and the ketone at C-2. The vinylic H-3 peak showed the expected correlations to C-1, -4, -5, and -18 (see Figure 1). UV absorptions at 215, 249, and 324 nm confirmed

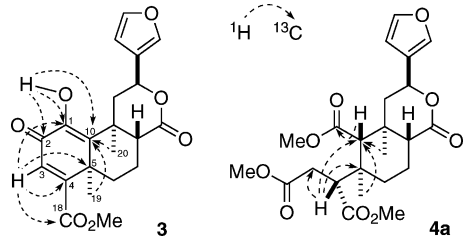


FIGURE 1. Key HMBC correlations of **3** and **4a**.

an extended π system (compare **9a**:[15] 208 nm). HRESIMS confirmed the molecular formula. The remainder of the structure, unchanged from the salvinorins, was fully elucidated and assigned by NMR experiments (DEPT, COSY, HMQC, HMBC, and NOE). The H-12 coupling constants were closer to those of 8-*epi*-**1a**[10] than of **1a**, suggesting that epimerization at C-8 may have occurred (as is typical for salvinorins and analogues under basic conditions).[10] However, the β configuration of H-8 was evidenced by a diaxial coupling constant (9.7 Hz); in addition, irradiation of H-12 gave an NOE enhancement of H-20. The structure of **3** thus established is remarkably similar to the recently isolated salvinorin G (**6**).[7]

The base-soluble fraction was difficult to analyze, smearing on TLC and giving a poorly resolved ^{1}H NMR spectrum. Surprisingly, at least eight peaks were apparent in the methoxy region. After methylation with Me_3SiCHN_2,[16] TLC showed only a single spot. ^{1}H NMR analysis, however, revealed three major compounds, which were separated with difficulty by HPLC. Baseline resolution was not achieved, necessitating repeated repurification and poor recoveries. The major product was identified as 1,2-secotriester **4a** based on extensive NMR experiments. The H-10 singlet showed an HMBC correlation to the new C-1 ester carbonyl (Figure 1). H-4 showed correlations to C-3, -5, and -10 and formed an isolated spin system with the two deshielded H-3 peaks. This confirmed the location of the new methyl esters at C-1 and -2, although the three esters were not sufficiently resolved in the 2D spectra to allow individual assignment. The remaining NMR data was very similar to that of **1a**. The chemical shift and coupling constants of H-12 were nearly identical to those of **1a**, confirming the configuration at C-8. HRESIMS confirmed the molecular formula. The second major product was identified as the

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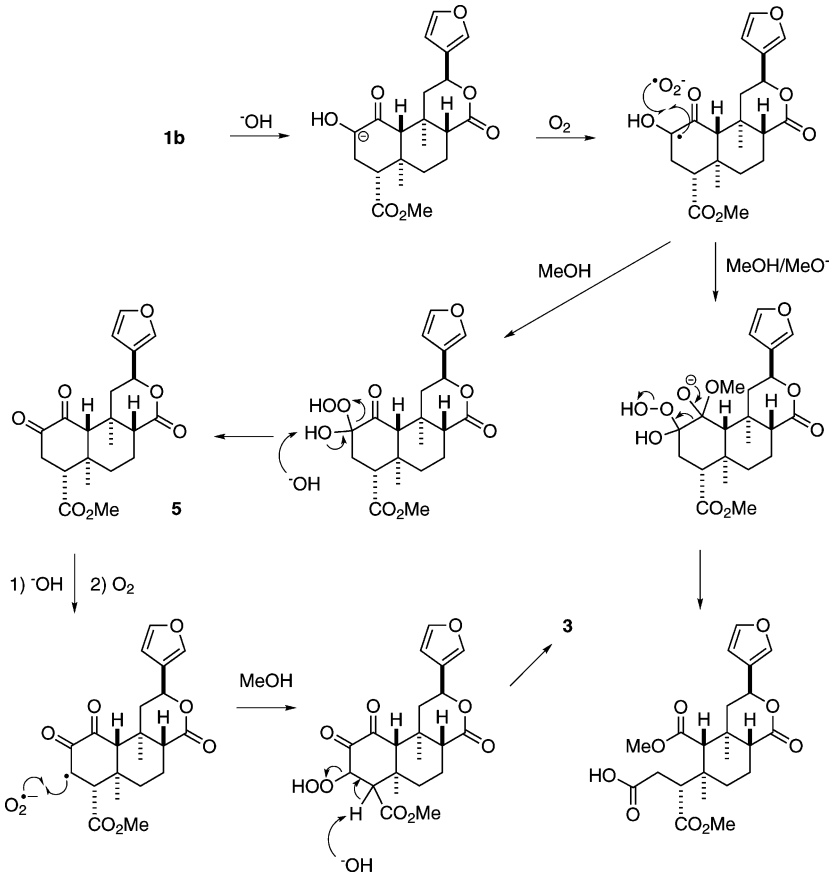
SCHEME 2. Proposed Mechanism of the Autoxidation



8-epimer **4b** on the basis of the shifts and coupling constants of H-8 and -12 (nearly identical to those of 8-*epi*-**1a**).[10] Assignment of the remaining data was straightforward. 2D NMR showed the same correlations as **4a**; HRESIMS again confirmed the molecular formula. Interestingly, although **4a** and **4b** cospotted by TLC, they gave different colors with vanillin/H_2SO_4 in EtOH. After development with a heat gun and several minutes' cooling, **4a** gave a pink/purple color, while **4b** appeared blue. Similar colors were observed for salvinorins A−E and several derivatives. In all pairs other than **4a/4b**, the 8-epimer gave a higher R_f in Et_2O or EtOAc/petroleum ether. Given the tendency for salvinorins to epimerize at C-8 under basic conditions, this information should prove useful for future derivative work.

The third (minor) compound decomposed in $CDCl_3$ before characterization was completed, but was tentatively assigned as **4c**. HRESIMS established that the compound was also an isomer of **4a**, and the appearance of the same couplings in the COSY spectrum suggested another stereoisomer. The coupling constants of H-8 and -12 established that the C-ring configurations matched those of **4a**. Indeed, all of the coupling constants determined were close to those in **4a**, whereas many chemical shifts showed large changes. This implied a change in the electronic environment of the coupling protons, without a change in their configuration. The most plausible candidate structure was therefore the 10-epimer **4c**, since H-10 is not coupled. Placing a large substituent in an axial configuration would be expected to affect the conformation of both remaining rings and, hence, the chemical shifts around those rings. By contrast, inversion at C-4 would not be expected to dramatically alter the conformations of the rings but would be expected to alter the coupling constants with the H-3 protons. These couplings were scarcely changed, while the chemical shifts of H-4, -7, -8, -10, -11, and -12 (but not H-3) were dramatically altered. Thus, **4c** is the more plausible structure. The particularly large change at H-4, shifted downfield by 0.83 ppm, might be due to falling within the deshielding region of the C-1 carbonyl. Interestingly, Brown found that when **1a** was refluxed with KCN in CD_3OD, deuterium exchange occurred at H-2, -8, and -10 but not H-4.[17] In the absence of NOE data, however, the proposed structure **4c** must remain tentative.

Our results conflict with recently published reports. Tidgewell et al.[18] reported that heating **1a** with NaOH in MeOH caused cleavage of the methyl ester and opening of the lactone, without giving further detail. Since lactone hydrolysis would be reversed upon neutralization, we interpret this as methanolysis. Presumably, ester cleavage was inferred from the formation of a base-soluble fraction, and lactone methanolysis from the methoxy peaks in the ^1H NMR spectrum. As shown above, however, the acidic fraction and its methoxy peaks result from cleavage of the α-hydroxy ketone. Refluxing

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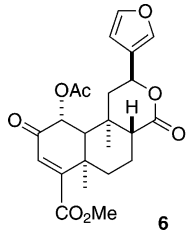
the 1-hydroxy derivative **8a**[13] in KOH/MeOH for 30 min resulted only in epimerization at C-8, confirming that neither ester cleavage nor methanolysis occur under these conditions. More recently, Lee et al. treated **1a** with $Ba(OH)_2$ in MeOH. The ^1H and ^{13}C NMR data of the product are nearly identical to **3**, apart from the omission of the multiplet at δ 1.77−1.67.[8] On the basis of NMR analysis, they propose the dienone structure **7**, which is not consistent with the additional data presented here. Specifically, HRMS shows no molecular ion corresponding to $C_{21}H_{22}O_6$ (**7**), but a prominent peak corresponding to $C_{21}H_{22}O_7$ (**3**). Further, the singlet at 6.91 cannot be attached to C-1, since it exchanges with D_2O, has no HMQC cross-peak, and lacks the expected HMBC correlations to C-3, -5, and -9. A corresponding methine peak is also absent from the DEPT spectra. Moreover, deoxygenation would not be expected under these conditions.

By contrast, autoxidation of α-hydroxy ketones (acyloins or α-ketols) to α-diones (diosphenols) under basic conditions is well-established.[19−21] The reaction consumes 1 equiv of O_2, generating H_2O_2.[21] While the autoxidation of unsubstituted ketones requires stronger bases such as *t*-BuOK, α-hydroxy ketones are more readily enolized, and the reaction proceeds with KOH.[20] A proposed mechanism, via saturated dione **5**, is shown in Scheme 2 (the initial deacetylation to **1b** is not shown). Dehydrogenation of **5** to form **3** is more unusual. While there have been several reports of dehydrogenation of 1,4-diones in alcoholic KOH,[22] we have not located such a reaction involving a 4-ketoester. However, α-diones are much more readily enolized than unsubstituted ketones; in the case of **3**, no trace of the 1-keto tautomer was detectable by NMR. It is therefore plausible that **5** should be extremely reactive, and it is not surprising that this compound was not isolated. Consistent with this, Brown's attempts to prepare **5** via PCC oxidation of **1b** gave no isolable product.[17] The alternate pathway, bond cleavage to give the secodiesters, has numerous precedents.[20,23] We have based our proposed mechanism on the generally accepted formation of hydroperoxide intermediates,[20] although this mechanism has been disputed.[23] Tautomerization of the enolate or radical will give the regioisomeric diester, which along with epimerization at C-8 and -10 explains the numerous methoxy peaks in the ^1H NMR spectrum of the crude product.



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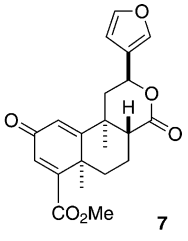

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The yield and selectivity of autoxidations of this type are subject to strong solvent effects.[20] We therefore performed the reaction in EtOH, *i*-PrOH, and *t*-BuOH. The resulting neutral and acidic fractions were noticeably more complex. In each case, **3** was contaminated by inseparable impurities (presumably including the 8-epimer); the original selection of MeOH thus proved fortuitous. The reaction proceeded with only traces of oxygen, even when performed under N_2 in MeOH presaturated with N_2. This is again typical.[20,23] Nonetheless, we found the reaction faster and more consistent when the solution was saturated with O_2. Anther useful refinement was the use of dilute KOH rather than $NaHCO_3$ to extract the extremely hydrophobic diesters during workup.

The new compounds **3** and **4a** were screened for binding affinity at cloned opioid receptors in vitro, as previously described.[10] Compound **3** was inactive at the KOR (K_i > 10 μM), as Lee et al. reported for **7**.[8] Surprisingly, **4a** showed weak affinity at the KOR (K_i = 2.9 ± 0.4 μM). Both compounds were inactive at δ and μ subtypes. The result for **4a** provides further evidence that the 2-acyloxy function in **1a** is not essential for binding, but that modifying this function usually reduces affinity. In light of these findings, we also screened divinatorins A−C (**2a**−**c**),[24] which we had previously isolated from *S. divinorum*. These compounds showed no affinity at κ, δ, or μ subtypes (K_i > 10 μM). Lee et al. also found **2c** to be inactive at the KOR.[7] Intriguingly, however, the recently isolated divinatorin D (**2d**)[7] showed weak affinity (K_i = 230 nM vs 1.0 nM for **1a**). Thus, either of the A or C rings can be cleaved without total loss of affinity. Apart from this greater conformational freedom, **2d** not only lacks the 2-acetoxy function but also possesses a 1α-hydroxy group, which drastically reduces activity in analogues of **1a**.[10] The structure−activity relationships of this compound are thus markedly different from those of **1a**. Given that its deacetyl analogues **2b** and **2e**[7] are inactive, perhaps the 17-acetoxy function substitutes for the furan ring in binding. However, **2c**, which also possesses this function, is inactive. Further exploration of this productive region of chemical space is clearly warranted.

Given the unexpected installation of the 3,4 double bond in **3**, we thought the compound might provide a route to diol **9d**. This would then give access to salvinorins C (**9a**),[15] D (**9b**), and E (**9c**),[25] which occur in *S. divinorum* in much lower levels than **1a**. However, $NaBH_4$ in EtOH/CH_2Cl_2 resulted in conjugate addition and epimerization, giving the known 8-*epi*-diol **8b**[15] in low yield (Scheme 3). Attempted reduction with $NaBH_4$−$CeCl_3$ in MeOH[26] (with or without sonication)[27] was also unsuccessful, giving a complex mixture whose unstable major components retained the characteristic enedione peaks at δ 6.8 and 7.0 ppm (these products cospotted with the starting material, making the reaction difficult to follow, but gave a darker purple with vanillin). Enolized α-diones form complexes with a variety of metal salts such as $FeCl_3$;[28] it appears likely that such an enolic

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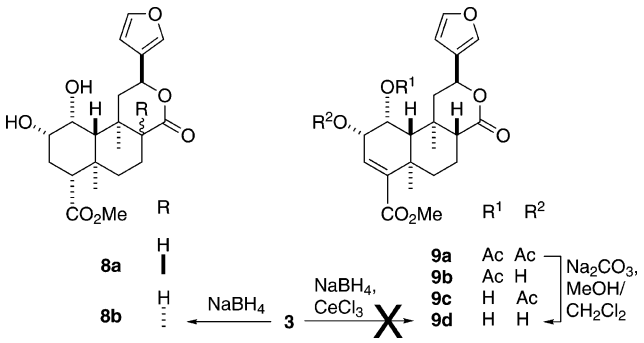
SCHEME 3. Attempted Reductions of 3



complex forms in preference to the desired ketone−solvent−Ce^{3+} complex,[26] and the reaction therefore does not follow the desired course. The only successful precedent we located for this reduction involved nonenolizable enediones.[29] It might be possible to prevent this problem by protection of the enol, but attempted TES protection was unsuccessful.

As an alternative route, the target diol **9d** was prepared by deacetylation of **9a**. Tidgewell et al.'s now-standard conditions for **1a** (Na_2CO_3 in minimal MeOH)[18] gave almost exlusively the 8-epimer, but we found this could be prevented by addition of CH_2Cl_2, affording **9d** from **9a** or **9b** in up to 86% yield. Reacetylation under standard conditions progressed slowly through intermediate **9b** and **9c** to give **9a**. While no one has yet isolated **9d** from *S. divinorum*, it may be that the compound occurs in the plant in very low levels. This would be expected if its formation were the rate-limiting step on the path to **9a**−**c**. Alternatively, current isolation procedures may give poor recoveries. Indeed, we found that like **1b**, the compound precipitates when loaded on silica gel in most solvent systems. Stripping the column with $MeOH/CH_2Cl_2$ was required to achieve satisfactory recovery.

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Conclusions

In summary, we have identified the actual products of the treatment of **1a** with hydroxide in MeOH, resolving recent conflicting reports. One of these products, **4a**, shows mild affinity at the KOR, further elucidating the structure−activity relationships of **1a**. Finally, interconversion of salvinorins C−E (**9a**−**c**) with the novel diol **9d** provides further confirmation of the structure of these compounds.

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Supporting Information Available: Experimental details, characterization data, ^1H NMR spectra, and IUPAC International Chemical Identifiers (InChIs) of compounds **3**, **4a**−**c**, **8b**, and **9d**. This material is available free of charge via the Internet at http://pubs.acs.org.

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