Intramolecular Transacetylation in Salvinorins D and E

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Extraction of fresh *Salvia divinorum* leaves afforded salvinorins E and D as potential biosynthesis precursors of salvinorin A, a major metabolite and a potent hallucinogen. Attempts at HPLC purification of salvinorin E (**2**) with acetonitrile as a solvent revealed an equilibrium with its regioisomer, salvinorin D (**3**), in a 3:5 ratio. The presence of both compounds was readily observed in the ^{1}H NMR spectrum. This spontaneous formation of the mixture of isomers occurs via a dynamic intramolecular transacetylation process.

Salvia divinorum Epling & Jativa (Lamiaceae) has drawn much attention in recent years due to its profound psychopharmacological properties. The active component, the neoclerodane diterpenoid salvinorin A (**1**), is a potent κ-opioid agonist.[1] Several natural and synthetic analogues of **1** have been reported and their κ-opioid affinity profiles defined.[2] Directing our efforts on the biosynthesis of **1**, we focused on isolation of natural analogues of salvinorin A (**1**), as potential biosynthesis intermediates. In the course of isolating the salvinorin analogues a recurring problem was encountered with the purification of salvinorin E (**2**). Salvinorin E was obtained from the CHCl₃ extract of fresh *S. divinorum* leaves according to a method published previously.[3] Purification of compound **2**, using RP-HPLC, consistently led to the formation of regioisomeric salvinorin D (**3**). Herein, we report the dynamic equilibration of salvinorins D (**3**) and E (**2**) in solution via transacetylation, the first observation of such a spontaneous process in this pharmacologically important class of neoclerodane diterpenoids.

The instability of salvinorin E (**2**) was first noticed during analysis of its ^{1}H NMR spectrum in CDCl₃. This spectrum of a perceived pure sample unexpectedly showed evidence for an admixture with a closely related compound, in a 3:5 ratio. Analysis of the H-1 and H-2 resonances in this mixture revealed H-1β and H-2β of salvinorin E (**2**) as a broad singlet at δ 4.46 and a doublet of doublets at 5.40 (J = 2.3, 4.6 Hz), respectively.[4] Comparison of the chemical shifts of the same protons in the "contaminant" [H-1β, δ 5.70, d, J = 5.1 Hz; H-2β, δ 4.46, brs] with those of salvinorin D (**3**) strongly suggested a mixture of salvinorin E (**2**) with its regioisomer, salvinorin D (**3**). Similarly, when the ^{1}H NMR spectrum of "pure" salvinorin D (**3**) was recorded in CDCl₃, the appearance of the H-1β and H-2β resonances of salvinorin E (**2**) was observed, indicating a 5:3 ratio of isomers **3** and **2**. Notably, integration of the characteristic fingerprint H-12 resonances [δ 5.60 for **2** and 5.63 for **3**] confirmed the previously measured equilibrium ratios of the two regioisomers.

In attempts at the separation of salvinorins D (**3**) and E (**2**) using RP-HPLC in MeCN, retention times of 20.6 and 22.9 min were observed for **3** and **2**, respectively. However, when the samples of **2** and **3** from these well-separated peaks were reinjected, both showed the presence of an equilibrium mixture similar to that observed in the ^{1}H NMR experiments. Such a dynamic equilibrium between salvinorins D (**3**) and E (**2**) clearly resulted from a process of intramolecular transacetylation, which is stereochemically permitted by the 1α-axial and 2α-equatorial orientation of the *O*-acetyl and hydroxy group functionalities in these compounds. The 1,3-

Scheme 1. Mechanism of Intramolecular Transacetylation of Salvinorins D (**3**) and E (**2**)
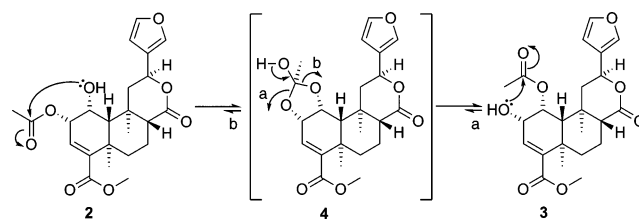
dioxolane intermediate (**4**) (Scheme 1) would facilitate the migration of the *O*-acetyl group from C-1 to C-2 and vice versa. Although the transacetylation may also occur intermolecularly, the intramolecular process may be favored, because no evidence could be found supporting the formation of a 1,2 di-*O*-acetyl (salvinorin C) or a 1,2-dihydroxy (salvinorin H) analogue.

Even though **2** was reported before, Munro and Rizzacasa did not mention problems with its purification, although they observed decomposition of **2** during extended storage or in attempts of its recrystallization from MeOH or hexanes/EtOAc.[4]

Similar to the observations of Munro and Rizzacasa, in our hands both salvinorins D (**3**) and E (**2**) also slowly decomposed into unidentified compounds during prolonged storage in MeCN solution. These authors, however, did not observe the facile interconversion of salvinorins D (**3**) and E (**2**) in solution. The intramolecular transesterification process was reported previously among natural products as one of the steps during the total synthesis of respirantin.[5] More relevant to the present situation is transesterification in the chondropsin class of compounds, in basic conditions,[6] or spontaneous rearrangement within longipinane derivatives.[7] It is interesting to note that in our recent report on salvinorins J containing the same functional groups at C-1 and C-2 as in salvinorin E (**2**), a similar intramolecular *O*-acetyl group migration was not observed.[3]

A notable feature of the intramolecular transacetylation interconversion of salvinorins D (**3**) and E (**2**) is the apparent preference for the formation of salvinorin D (**3**) with an α-axial *O*-acetyl group as opposed to the α-equatorial arrangement in salvinorin E (**2**). Superficially, the two 1,3-diaxial interactions of the 1α-axial OAc group and the C-5 and C-9 α-axial methyl groups should render salvinorin D (**3**) thermodynamically less stable than salvinorin E (**2**), where such sterically repulsive interactions are less pronounced. A possible explanation is that the free rotating 1-OH group in salvinorin E (**2**) produces a 1,3-diaxial interaction with both the C-5 and C-9 methyl groups, while in salvinorin D (**3**), the bulky 1-OAc group does not permit free rotation in the sterically congested space below the A ring and exerts predominant 1,3-diaxial interaction with the C-9 methyl group. This is supported by

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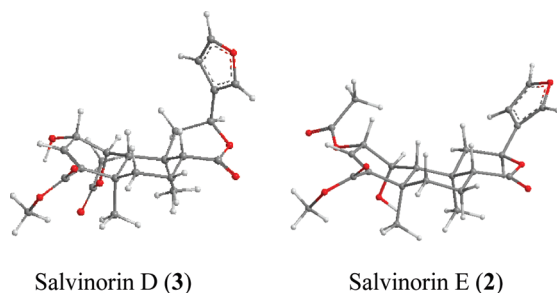　§ Research Institute of Pharmaceutical Sciences.

Salvinorin D (**3**)   Salvinorin E (**2**)

Figure 1. Conformations of salvinorins D (**3**) and E (**2**) optimized at the B3LYP/6-31G** level.

comparison of the ^{1}H NMR chemical shift data of the C-5 and C-9 methyl groups of **2** and **3**, indicating that the 1-OAc group affects the chemical shift of the C-9 methyl group significantly more ($\Delta\delta$ 0.25 vs 0.03) than that of the C-5 methyl group.[4]

Computational modeling was next employed to explain these phenomena. The starting conformations for salvinorins D (**3**) and E (**2**) were obtained by the MM2 method in ChemBio3D Ultra 11.0. Further geometrical optimization was carried out using density functional theory at the B3LYP/6-31G** level in the gas phase. Surprisingly, the total energy of the optimized conformation of **3** was indeed 4.78 kcal/mol lower than that of **2** (Supporting Information, Table S1). Salvinorin D (**3**) possesses chair-like/chair/boat-like conformations for the A, B, and C rings, in contrast to salvinorin E (**2**), which has chair-like/chair/chair-like conformations for these rings (Figure 1). The adoption of a boat-like conformation of the C ring in **3** may serve to reduce the severe van der Waals interaction of H-1*eq* and H-11*eq*. Such a bond rotation would secure a more axial orientation of 1-OAc and would also explain the enhanced shielding of the C-9 methyl group. In salvinorin E (**2**) the H-1*eq*/H-11*eq* interaction is enhanced by a conformational change of ring C that places the furanyl moiety in a equatorial orientation. More advanced computational methods, however, are required to probe the subtleties of these conformational changes.

Experimental Section

General Experimental Procedures. The NMR spectra of salvinorins D (**3**) and E (**2**) were recorded using a Bruker AV 400 MHz instrument with a tunable 3 mm carbon sensitive probe. Spectra were recorded in CDCl$_3$ and analyzed with Mnova Suite 5.3.2 software. The HPLC purification and analytical separations were performed using Delta Prep 4000 Waters equipment with dual-wavelength UV detector (Waters, Milford, MA).

Plant Material. *S. divinorum* plants were obtained from and identified by Daniel Siebert, *Salvia divinorum* Research and Information Center, Malibu, CA, in August 2008. Culturing procedures were used as described before.[3]

Extraction and Isolation. Extraction and isolation of salvinorin analogues were performed according to the procedure described in our previous report.[3] Briefly, salvinorins D (**3**) and E (**2**) were obtained from fresh biomass by extraction with CHCl$_3$. The crude extract was chromatographed using silica gel and preparative RP-HPLC, as previously described.[3] Salvinorins D and E were obtained in good yields.[3]

Supporting Information Available: Salvinorins D and E stability check graphs in different experimental conditions, NMR spectra, HPLC chromatograms, and conformational analysis data. This information is available free of charge via the Internet at http://pubs.acs.org.

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