Intramolecular Transacylation in Salvorins D and E

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Received July 24, 2009

J. Nat. Prod. XXXX, xxx, 000

Extraction of fresh Salvia divinorum leaves afforded salvorins E and D as potential biosynthesis precursors of salvorin A, a major metabolite and a potent hallucinogen. Attempts at HPLC purification of salvorin E (2) with acetonitrile as a solvent revealed an equilibrium with its regioisomer, salvorin D (3), in a 3:5 ratio. The presence of both compounds was readily observed in the 1H NMR spectrum. This spontaneous formation of the mixture of isomers occurs via a dynamic intramolecular transacylation 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 salvorin 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 salvorin A (1), as potential biosynthesis intermediates. In the course of isolating the salvorin analogues a recurring problem was encountered with the purification of salvorin E (2). Salvorin 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 salvorins D (3) and E (2) in solution via transacytation, the first observation of such a spontaneous process in this pharmaco logically important class of neoclerodane diterpenoids.

The instability of salvorin E (2) was first noticed during analysis of its 1H 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 salvorin 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, δ, J = 5.1 Hz; H-2β, δ 4.46, brs] with those of salvorin D (3) strongly suggested a mixture of salvorin E (2) with its regioisomer, salvorin D (3). Similarly, when the 1H NMR spectrum of “pure” salvorin D (3) was recorded in CDCl3, the appearance of the H-1β and H-2β resonances of salvorin 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 salvorins 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 1H NMR experiments. Such a dynamic equilibrium between salvorins D (3) and E (2) clearly resulted from a process of intramolecular transacylation, which is stereochemically permitted by the 1α-axial and 2α-equatorial orientation of the O-acetyl and hydroxy group functionalties in these compounds. The 1,3-

Scheme 1. Mechanism of Intramolecular Transacylation of Salvorins D (3) and E (2)

α-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 transacylation 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 (salvorin C) or a 1,2-dihydroxy (salvorin 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 salvorins 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 salvorins 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 salvorins J containing the same functional groups at C-1 and C-2 as in salvorin E (2), a similar intramolecular O-acetyl group migration was not observed.3

A notable feature of the intramolecular transacylation reaction of salvorins D (3) and E (2) is the apparent preference for the formation of salvorin D (3) with an α-O-acetyl group as opposed to the α-equatorial arrangement in salvorin 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 salvorin D (3) thermodynamically less stable than salvorin E (2), where such sterically repulsive interactions are less pronounced. A possible explanation is that the free rotating 1-OH group in salvorin E (2) produces a 1,3-diaxial interaction with both the C-5 and C-9 methyl groups, while in salvorin 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

XXX American Chemical Society and American Society of Pharmacognosy
A 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.  

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/boat-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-1eq and H-11eq. 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-1eq/H-11eq interaction is enhanced by a conformational change of ring C that places the furanyl moiety in an 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.  

**Extraction and Isolation.** Extraction and isolation of salvinorin analogues were performed according to the procedure described in our previous report. 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. Salvinorins D and E were obtained in good yields.  

**Acknowledgment.** Financial support for L.M.K. from NIUST grant NA16RU149 is greatly acknowledged.

**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.

**References and Notes**


NP900447W