Computational and Synthetic Investigation of Cationic Rearrangement in the Putative Biosynthesis of Justicane Triterpenoids

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Abstract: A biomimetic cationic structural rearrangement of the oleanolic acid framework is reported for the gram-scale synthesis and structural reassignment of justicioside E aglycone. The mechanism of the putative biosynthetic rearrangement is investigated with kinetic, computational, and synthetic approaches. The precursor to rearrangement was accessed through two strategic advancements: (1) synthesis of a 1,3-diketone via oxidation of a β-silyl enone, and (2) diastereoselective 1,3-diketone reduction to form a syn-1,3-diol using SmI₂ with PhSH as a key additive.

The pentacyclic triterpenoids are an ancient, populous and structurally diverse class of natural products with important and wide-ranging biological activities. Oleane triterpenoids are actively being developed for pharmaceutical purposes: oleanolic acid is used in China to treat liver disorders, and synthetic oleananes have advanced to clinical trials for chronic kidney disease. Decades of research have established that the biosyntheses of these pentacyclic triterpenoids occur via polyolefin cyclization of 2,3-oxidosqualene and subsequent cationic rearrangements of the D and E rings. In contrast, the pathways that reorganize the A and B rings of pentacyclic triterpenoids, as represented by the justicane (Figure 1), are not clearly understood. Herein we report a synthetic, kinetic, and computational evaluation of the interconversion of the oleane skeleton to that of the justicane class (i.e. 1–3). This work results in a structural reassignment and gram-scale laboratory chemical synthesis of 1.

A possible biosynthetic pathway of 1 involves reorganization of the A,B-ring system of justicioside A aglycone (3) from a 6,6-to the 5,7-ring system (Figure 1A). Similar decalin rearrangements by activation of the C1 position have been employed in small molecule synthesis, and the bicyclo[5.3.0]decane motif is widely observed in natural products. However, these decalin rearrangements have been reported to undergo competitive alkyl migrations, which prompted us to consider whether the formation of 2 might compete with the formation of 1. A detailed understanding of the reaction mechanism would aid in rationally employing this and other cationic rearrangement tactics in organic synthesis. The use of kinetic isotope effects and computational modelling in studying decalin rearrangements may contribute to rationalizing chemo- and stereoselective migrations, in addition to the analysis of product distribution that has been the main tool for mechanistic investigations on related systems.

An additional complication in the justicane case is that a stereospecific 1,2-alkyl migration of 3 would result in a cis-fused A,B-ring system (1), as opposed to the trans-A,B-ring fusion reported for justicioside E aglycone (C1-epi-1). To resolve this ambiguity, we conducted NMR prediction calculations (Figure 1B). Deviations from experimental data (in ppm) are shown for both assignments. The mean average deviation (MAD) and maximum difference (Max) from the reported ¹H- and ¹³C-NMR spectra are more consistent with the revised structure 1. Furthermore, statistical evaluation of the ¹H, ¹³C, and ¹H & ¹³C data sets using Goodman’s DP4 method provides a 100% probability that the reassignment is more accurate than the originally proposed structure. The structural reassignment may have implications toward other cationic rearrangements in organic synthesis.

![Figure 1. A. Potential structural reorganizations of justicioside A aglycone (3) towards 1 and 2. B. NMR predictions support structural reassignment of 1. MAD = mean average deviation (ppm), Max = maximum deviation (ppm). Statistical analysis was performed using the DP4 method.](image)
have implications for the biosynthetic mechanism of rearrangement, as a stereospecific concerted migration, among other possibilities, would be consistent with the formation of cis-fused 1.

We planned to elucidate the mechanism of this rearrangement by synthetic, computational,16 and kinetic investigations. It was thus necessary to access a justicioside A-type rearrangement precursor and examine the migration to justicioside E aglycone (1). The synthesis of justicioside E aglycone (1) began with the known enone 4, prepared from oleanolic acid in two steps (Scheme 1). It was envisioned that enone 4 could be converted to the 1,3-diketone via a two-step approach involving palladium-catalyzed β-silylation and subsequent oxidation. Our previously reported β-silylation protocol was modified and smoothly furnished 5 in 62% yield on 10.0-gram scale (see Supporting Information for details). β-Silyl enone 5 was then oxidized to the 1,3-diketone 6 with basic tert-butyl hydroperoxide.

At this stage, it was necessary to perform a diastereoselective reduction of the diketone moiety to the syn-1,3-diol. The use of hexamethylphosphoramide (HMPA) and thiophenol18 in the presence of SmI₂ uniquely effected the reduction to the desired product. Thiophenol may act as a hydrogen atom transfer agent to enhance the diastereoselectivity of thermodynamic ketone reductions. After initial proton-coupled electron transfer,19 it is possible that the orientability of the S–H bond facilitates H-atom abstraction by the samarium-complexed ketyl radical, as opposed to proton transfer to the samarium alkoxide from other proton donors. To our delight, we found that the conditions of our diketone formation and subsequent reduction were amendable to a one-pot transformation, resulting in the requisite syn-diol in 77% yield.

The syn-diol was selectively protected with benzoyl chloride in the presence of pyridine to yield migration precursor 7. Treatment of 7 with acid under mild conditions did not lead to the formation of 8. However, exposure to trifluoromethanesulfonic anhydride and pyridine spontaneously elicited the key rearrangement to the 5,7-ring system of justicioside E, forming 8 as a 10:1 mixture of olefin isomers in 86% yield. The structure of 8 was unambiguously assigned as the cis isomer by X-ray crystallography. This is consistent with the predicted structural reassignment and supports a stereospecific migration for ring rearrangement.

In exploring conditions for the alkyl migration, it was not observed that 1,2-methyl shift to form 9 was competitive under any conditions. This prompted us to prepare20 and investigate the reactivity of C1-epi-7. Treatment of C1-epi-7 with trifluoromethanesulfonic anhydride and pyridine induced a 1,2-methyl shift to form 9 as a mixture of olefin isomers in 93% yield with no detection of rearrangement product 8. Under all conditions explored, the activated derivatives of secondary alcohols 7 and C1-epi-7 migrated with complete stereochemical control to form 8 and 9, respectively. This selective migration of the bond antiperiplanar to the leaving group suggests a concerted or tight ion pairing mechanism with complete stereoelectronic control is operative, as the formation of an intermediate carbocation would result in common products from both starting materials.

Completion of the synthesis of 1 from 8 required enone formation with Collins reagent and global carbonyl reduction with lithium aluminium hydride. The spectral data for the material thus obtained are in excellent agreement with those reported for justicioside E aglycone (1).7

Scheme 1. Synthesis of justicioside E aglycone (1). Reagents and conditions: (3) PhMe₂SiLi (1.2 equiv), HMPA (2.9 equiv), THF (0.4 M), −78 °C, 5 min; [Pd(allyl)Cl]₂ (2.5 mol %), diethyl allyl phosphate (1.0 equiv), 70 °C, 40 min, 82%; (4) NaH (3.0 equiv), i-BuO₂H (6.0 equiv), NMP–THF (3:1, 0.08 M), 0 → 23 °C, 1 h; HMPA (36.0 equiv), PhSH (18.0 equiv), SmI₂ (13.1 equiv), 23 °C, 30 min, 77%; (5) BzCl (1.6 equiv), pyridine (0.25 M), 23 °C, 12 h, 76%; (6) Tf₂O (1.8 equiv), pyridine (3.7 equiv), CH₂Cl₂ (0.02 M), −78 → 23 °C, 3 h, 86%, 10:1 exo:endo olefin from 7; 93% from C1-epi-7; (7) CrO₃ (20.4 equiv), pyridine (40.6 equiv), CH₂Cl₂ (0.01 M), 23 °C, 12 h, 56%; (8) LIAH₄ (5.0 equiv), THF (0.02 M), 0 → 23 °C, 13 h, 71%, 10:1 dr.
With synthetic access to 1, we investigated the mechanism of the key bond rearrangement with kinetic and computational studies (Figure 2). A kinetic isotope effect (KIE) experiment was designed which required a C1-deuterated decalin precursor. Substrate 7-D was prepared, with deuterium incorporation at C1 and C3, and mixtures of 7 and 7-D were subjected to rearrangement reaction conditions in a one-pot competition experiment (Figure 2A). The deuterated substrate was used to simplify spectroscopic analysis (see Supporting Information), and it is not expected that deuteration at C3 would significantly impact the value of the KIE. The 2° KIE was calculated using the Singleton method to be 1.00 ± 0.01. This value reflects a lack of rehybridization at C1 and is indicative of a concerted 1,2-alkyl shift. This is consistent with the stereospecific migration of the group antiperiplanar to the C1 leaving group observed in 7 → 8 and C1-epi-7 → 9 (Scheme 1). A KIE of 1.00 does not support stepwise leaving group ionization and subsequent bond reorganization, for which a normal or inverse 2° KIE should be observed. The concerted nature of the synthetic transformation suggests the possibility that the biosynthetic transformation also proceeds via a concerted pathway.

While benzoate 7 undergoes concerted bond rearrangement under abiotic conditions, the enzymatic machinery responsible for the biosynthetic transformation might mediate the stepwise rearrangement mechanism. This possibility was investigated computationally. When the C1 carbocation of 7 was subjected to gas phase optimization, only energy-minimized structures corresponding to the 5,7-ring system or methyl-shift product were obtained, and all efforts to identify the initial C1 carbocation were unsuccessful. This suggests that the C1 carbocation of 7 is not a local minimum on the energetic landscape for the computational methods used. In contrast, the C1 carbocation 10 was observed as an energy minimized structure when subjected to gas-phase optimization (Figure 2B).

Unlike the C1 carbocation of 7, that of 10 benefits from increased stability: the pC1 orbital is situated in a pseudoequatorial orientation and is therefore stabilized by the nonbonding orbitals of the allylic hydroxyl group (n0 → pC1). This intermediate was modelled undergoing 1,2-methyl shift to 12 and 1,2-alkyl shift to 14. The 1,2-methyl shift pathway is kinetically accessible (ΔG° = +8.6 kcal/mol) and exergonic (ΔG = -13.5 kcal/mol). The 1,2-alkyl shift pathway leading to 14 is also thermodynamically favourable (ΔG = -9.5 kcal/mol), and the transformation is nearly barrierless (ΔG° = +0.2 kcal/mol). The kinetic favourability of the alkyl migration can be explained by the chair-like A-ring conformation of 10, which situates the pC1 orbital antiperiplanar to the migrating C5–C10 bond, facilitating ring rearrangement. The close energetic and geometrical homology between 10 and transition state 13 results in a low kinetic barrier to migration. In contrast, the transition state for the 1,2-methyl shift pathway (11) shows that a distortion to an A-ring half-chair conformer is necessary to allow migration via the axial pC1, hyperconjugation. Taken together, the results of the calculations and KIE measurement indicate that both the concerted and stepwise mechanisms would favour formation of the 5,7-ring system from a suitable decalin precursor without competitive methyl migration.

A. Kinetic isotope effect experiment

B. Gas phase biosynthetic study

Figure 2. A. Kinetic isotope effect experiment indicates a concerted ionization and rearrangement. B. Reaction coordinate for carbotactic rearrangement indicates kinetic preference for the 1,2-alkyl shift product 14 via transition state 13 and thermodynamic preference for the 1,2-methyl shift product 12 via transition state 11 (mPW1PW91/6-31+G(2d,p)/B3LYP/6-31+G(d,p) (gas phase)). Values listed are relative free energies (ΔG).
A gram-scale synthesis of justiciside E aglycone (1) is reported, featuring a substrate-controlled cationic rearrangement. This work has resulted in several synthetic advances and the structural reassignment of 1 by synthetic and computational methods. These results, along with a kinetic isotope effect study and a computational investigation into gas phase carbocation reactivity suggest two orthogonal means to control structural reorganization of neopentyl carbocations common to terpenoid frameworks, and offer insight into the 6,6- to 5,7-ring system rearrangement. This report further highlights the synergistic relationships between organic synthesis, mechanistic analysis, and computational chemistry, with similar investigations ongoing in our laboratory.26

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[15] C1-epi-7 was prepared from enone 4 via the following sequence: nucleophilic epoxidation, reductive opening, and diastereoselective reduction (see Supporting Information for details).


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