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

Masha Elkin[+], Anthony C. Scruse[+], Aneta Turlik, and Timothy R. Newhouse*

Dedicated to Larry E. Overman on the occasion of his 75th birthday

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 SmI$_2$ 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.[1] Oleanane triterpenoids are actively being developed for pharmaceutical purposes:[2] oleanolic acid is used in China to treat liver disorders,[3] and synthetic oleananes have advanced to clinical trials for chronic kidney disease.[2b] 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.[4–6] In contrast, the pathways that reorganize the A and B rings of pentacyclic triterpenoids, as represented by the justicanes (Figure 1),[7] 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).[7] Similar decalin rearrangements by activation of the C1 position have been employed in small molecule synthesis,[8] and the bicyclo-[5.3.0]decane motif is widely observed in natural products.[9] However, these decalin rearrangements have been reported to undergo competitive alkyl migrations,[10,11] which prompted us to consider whether the formation of **2** might compete with the formation of **1**. A detailed understanding of the reaction
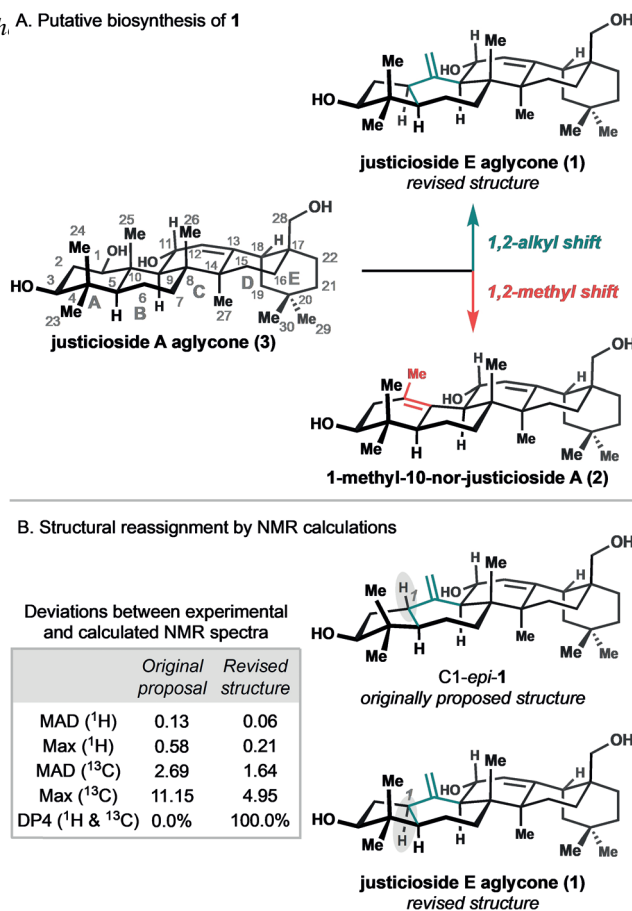

Figure 1. A) Potential structural reorganizations of justicioside A aglycone (**3**) towards **1** and **2**. B) NMR predictions support structural reassignment of **1**. MAD = mean average deviation (ppm). Max = maximum deviation (ppm). Statistical analysis was performed using the DP4 method.

	Original proposal	Revised structure
MAD (^{1}H)	0.13	0.06
Max (^{1}H)	0.58	0.21
MAD (^{13}C)	2.69	1.64
Max (^{13}C)	11.15	4.95
DP4 (^{1}H & ^{13}C)	0.0%	100.0%

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mechanism would aid in rationally employing this and other cationic rearrangement tactics in organic synthesis. The use of kinetic isotope effects and computational modelling[12] 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.[10,11]

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**).[7] To resolve this ambiguity, we conducted NMR prediction calculations (Figure 1B).[13,14] Deviations from experimental data (in ppm) are shown for both assignments. The mean

average deviation (MAD) and maximum difference (Max) from the reported ^{1}H- and ^{13}C- NMR spectra are more consistent with the revised structure **1**. Furthermore, statistical evaluation of the ^{1}H, ^{13}C, and ^{1}H & ^{13}C data sets using Goodman's DP4 method provides a 100% probability that the reassignment is more accurate than the originally proposed structure.[15] The structural reassignment may 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 82% yield on 10.0-gram scale (see Supporting Information for details).[17] 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 thiophenol[18] in the presence of SmI$_2$ 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 polarizability 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.
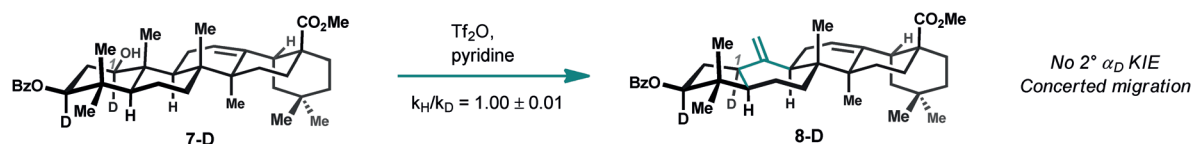
The *syn*-diol was selectively protected with benzoyl chloride in the presence of pyridine to yield migration precursor **7**. Treatment of **7** with acid under mild conditions did not lead to the formation of **8**. However, exposure to trifluoromethanesulfonic anhydride and pyridine spontaneously 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 prepare[20] 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 antiper-
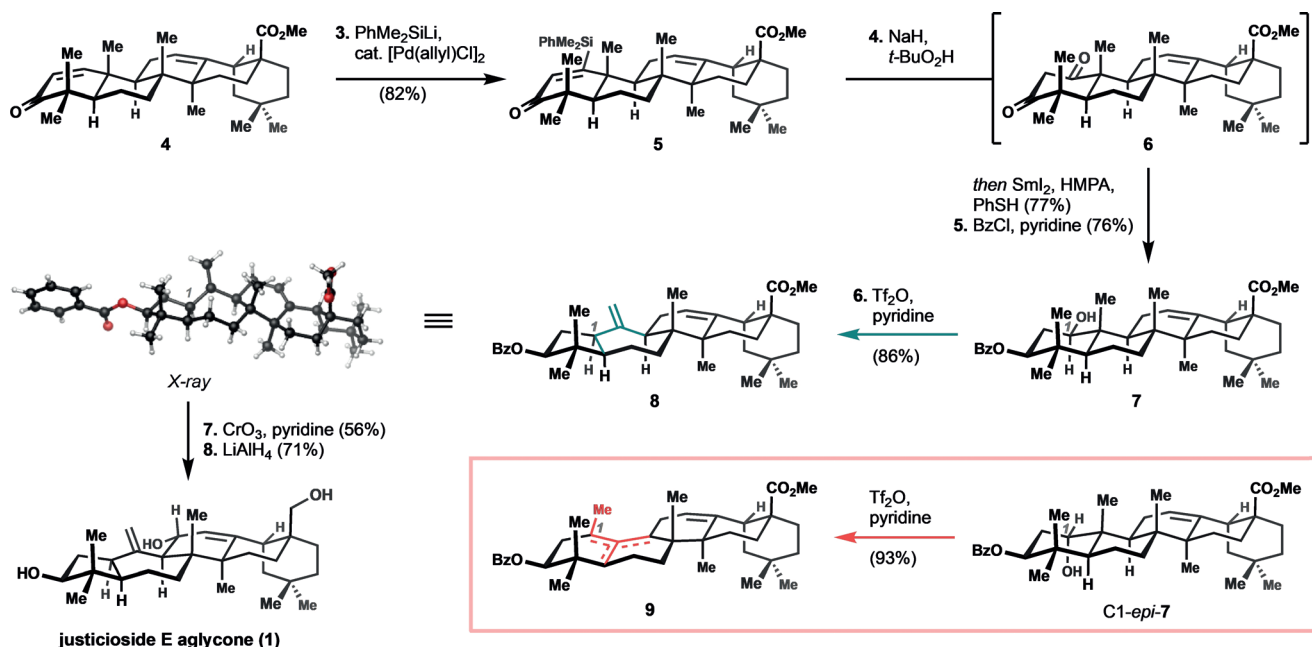
Scheme 1. Synthesis of justicioside E aglycone (**1**). Reagents and conditions: (3) PhMe$_2$SiLi (1.2 equiv), HMPA (2.9 equiv), THF (0.4 M), −78 °C, 5 m; [Pd(allyl)Cl]$_2$ (2.5 mol%), diethyl allyl phosphate (1.0 equiv), 70 °C, 40 m, 82%; (4) NaH (3.0 equiv), t-BuO$_2$H (6.0 equiv), NMP-THF (3:1, 0.08 M), 0 → 23 °C, 1 h; HMPA (36.0 equiv), PhSH (18.0 equiv), SmI$_2$ (13.1 equiv), 23 °C, 30 m, 77%; (5) BzCl (1.6 equiv), pyridine (0.25 M), 23 °C, 12 h, 76%; (6) Tf$_2$O (1.8 equiv), pyridine (3.7 equiv), CH$_2$Cl$_2$ (0.02 M), −78 → 23 °C, 3 h, 86%, 10:1 *exo:endo* olefin from **7**; 93% from C1-*epi*-**7**; (7) CrO$_3$ (20.4 equiv), pyridine (40.6 equiv), CH$_2$Cl$_2$ (0.01 M), 23 °C, 12 h, 56%; (8) LiAlH$_4$ (5.0 equiv), THF (0.02 M), 0 → 23 °C, 13 h, 71%, 10:1 dr.
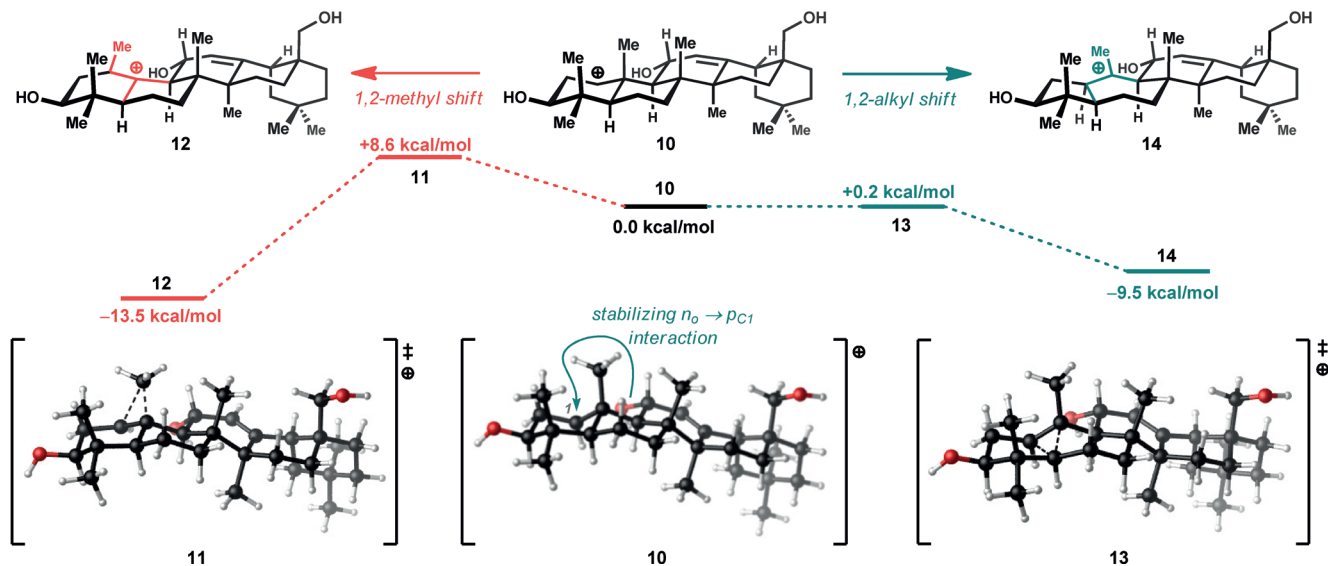
Figure 2. A) Kinetic isotope effect experiment indicates a concerted ionization and rearrangement. B) Reaction coordinate for carbocationic rearrangement indicates kinetic preference for the 1,2-alkyl shift product **14** via transition state **13** and thermodynamic preference for the 1,2-methyl shift product **12** via transition state **11** (mPW1PW91/6–311+G(2d,p)//B3LYP/6–31+G(d,p) (gas phase)).[24] Values listed are relative free energies (ΔG).

iplanar 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 aluminum hydride. The spectral data for the material thus obtained are in excellent agreement with those reported for justicioside E aglycone (**1**).[7]

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.[21] 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 dideuterated 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.[22] The 2° α_D KIE was calculated using the Singleton method to be 1.00 ± 0.01.[23] 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 mechanistic possibility was investigated computationally. When the C1 carbocation of **7** was subjected to gas phase optimization,[24] 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 p_{C1} orbital is situated in a pseudoequatorial orientation and is therefore stabilized by the nonbonding orbitals of the allylic hydroxyl group ($n_O \to p_{C1}$).[25] 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 ($\Delta G^\ddagger = +8.6$ kcal mol^{-1}) and exergonic ($\Delta G = -13.5$ kcal mol^{-1}). The 1,2-alkyl

shift pathway leading to **14** is also thermodynamically favorable ($\Delta G = -9.5$ kcal mol^{-1}), and the transformation is nearly barrierless ($\Delta G^{\ddagger} = +0.2$ kcal mol^{-1}). The kinetic favorability of the alkyl migration can be explained by the chair-like A-ring conformation of **10**, which situates the p_{C1} orbital antiperiplanar to the migrating C5-C10 bond, facilitating ring rearrangement.[25] 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 p_{C1} hyperconjomer.[25] Taken together, the results of the calculations and KIE measurement indicate that both the concerted and stepwise mechanisms would favor formation of the 5,7-ring system from a suitable decalin precursor without competitive methyl migration.

A gram-scale synthesis of justicioside 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]

Conflict of interest

The authors declare no conflict of interest.

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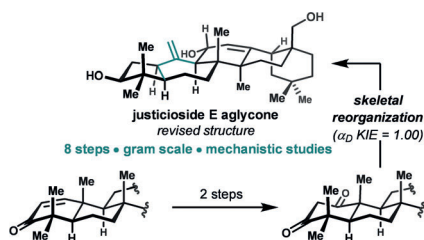
Communications

Biomimetic Synthesis

M. Elkin, A. C. Scruse, A. Turlik,
T. R. Newhouse* ——— ▉▉▉▉–▉▉▉▉

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

justicioside E aglycone
revised structure
8 steps • gram scale • mechanistic studies

skeletal reorganization
(α_D KIE = 1.00)

2 steps

Rearrangement process: A biomimetic cationic structural rearrangement of the oleanolic acid framework is reported for the gram-scale synthesis of justicioside E aglycone. The mechanism of the putative biosynthetic rearrangement is investigated with kinetic, computational, and synthetic approaches.

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