A computational investigation of the uncatalysed and water-catalysed acyl rearrangements in ingenol esters

Asja A. Kroeger and Amir Karton*

School of Molecular Sciences, The University of Western Australia, Perth, WA 6009, Australia

Ingenol esters have been identified as potent anticancer and HIV latency reversing agents. Ingenol-3-angelate was recently approved as a topical treatment for precancerous actinic keratosis skin lesions. It was found, however, that ingenol esters can undergo a series of acyl rearrangements, which may affect their biological potency and the shelf life of drug formulations. We use double-hybrid density functional theory to explore the mechanisms for the uncatalysed and water-catalysed acyl migrations in a model ingenol ester. The uncatalysed reaction may proceed either via a concerted mechanism or via a stepwise mechanism that involves a chiral orthoester intermediate. We find that the stepwise pathway is kinetically preferred by a significant amount of $\Delta\Delta H^\ddagger_{298} = 35.3 \text{ kJ mol}^{-1}$. The uncatalysed 3-O-acyl to 5-O-acyl and 5-O-acyl to 20-O-acyl stepwise rearrangements involve cyclisation and ring-opening steps, both concomitant with a proton transfer. We find that the ring-opening step is the rate-determining step (RDS) for both rearrangements, with reaction barrier heights of $\Delta H^\ddagger_{298} = 251.6$ and $177.1 \text{ kJ mol}^{-1}$, respectively. The proton transfers in the cyclisation and ring-opening steps may be catalysed by a water molecule. The water catalyst reduces the reaction barrier heights of these steps by over 90 kJ mol^{-1} .

Keywords: Double-hybrid DFT, acyl migration, ingenol-3-angelate, water catalysis.

*Corresponding author's E-mail: amir.karton@uwa.edu.au.

1. Introduction

Ingenol esters, members of the family of polycyclic diterpenoids, were first discovered in 1986 by Opferkuch and Hecker.¹ Known for their toxicity, the compounds received little attention in medical research² until usage of *euphorbia peplus* sap as a home remedy against skin cancer in Queensland, Australia³ led to the re-discovery of ingenol-3-angelate as a potent anticancer compound (Figure 1).⁴ In 2012 the compound was approved as a topical treatment for precancerous actinic keratosis skin lesions.⁴

Fig. 1. Schematic representation of the structure of an ingenol-3-ester.

Although ingenol-3-angelate is the most studied ingenol ester,⁵ bioassays on other natural and synthetic derivatives indicate a great diversity in their biological activities ranging from antiviral,⁶ to anti-tumour⁷ and tumour promoting⁸ properties. Recently, ingenol esters have been the focus of renewed interest in biomedical research as it emerged from in vitro studies that the compounds can reverse latency in HIV opening up a new avenue in research towards a potential cure.^{9,10,11,12,13}

While both the polyhydroxylic rim and ester substitution of ingenol derivatives have been identified as essential for high biological potency, these structural features cause the compounds to be prone to slow but problematic degradation via acyl migrations, even at physiological pH, especially in the presence of moisture.^{14,15,16} The rearrangements are

known to shorten drug shelf life^{16,17} as well as to hamper structure determination and bioassay research.^{2,17} It has further been suggested by Bertelsen et al. that degradation via acyl migrations may decrease drug effectiveness.¹⁷ Experimental structure activity research aimed at identifying both stable and biologically potent ingenol esters is ongoing^{17,18} and knowledge of the mechanism of the neutral acyl migration could make it possible to predict stability at physiological pH prior to synthesis. However, despite numerous accounts of neutral acyl migrations in experimental studies,^{19,20,21,22,23} the mechanism of the reaction is not well understood.

Previous theoretical explorations of the mechanism of the neutral acyl migration are scarce and stem from the fields of lipid and carbohydrate chemistry where the acyl migration is well known as a side reaction reducing regioselectivity of syntheses^{24,25,26,27} as well as a potential mechanism of inducing toxicity in the acyl glucuronide metabolites of numerous non-steroidal anti-inflammatory drugs.^{28,29,30} Two studies by Compton et al. on glycerols examined acyl migrations in ester-protected hexopyranoses using semiempirical and density functional theory (DFT) calculations.^{31,32} These studies invoked energy barriers from proposed orthoester intermediates. However, this approach has been found to give vastly underestimated reaction barriers.^{31,32} Activation energies from transition structure calculations were reported by Rangelov et al.³³ for the neutral rearrangement of monoformulated tetrahydrofuranol at the PCM-B3LYP/6-31+G level of theory. They reported high barriers of 230 and 200 kJ mol⁻¹ for the concerted and stepwise pathways, respectively.

In the present study we use double-hybrid DFT (DHDFT) calculations to elucidate the mechanism of the 3-O-acyl to 5-O-acyl and 5-O-acyl to 20-O-acyl migrations in a model of ingenol ester. The considered acyl migration reactions are illustrated in Figure 2. Based on the results of a radiocarbon labelling study by Doerschuk³⁴ precluding intermolecular

mechanisms involving hydrolysis and re-esterification for the acyl migration, intramolecular concerted and stepwise pathways are compared. We also consider the water-catalysed stepwise mechanism and show that a water catalyst can significantly reduce the reaction barriers for the acyl migration.

Fig. 2. Reaction scheme for the acyl migration reactions in a reduced model ingenol ester considered in the present work.

2. Computational Details

In order to study the mechanism of the 3-O-acyl to 5-O-acyl and 5-O-acyl to 20-O-acyl migrations in ingenol esters a model system including the rigid polycyclic backbone but excluding free alkyl substituents further from the reaction centre was chosen (Figure 2). The geometries of all structures were optimised using the B3LYP-D3 exchange-correlation DFT functional^{35, 36, 37, 38} in conjunction with the 6-31G(2df,p) Pople-style basis set.^{39, 40, 41} Empirical D3 dispersion corrections^{42,43} were included using the Becke–Johnson⁴⁴ damping potential as recommended in Ref. 38 (denoted by the suffix -D3). Bulk solvent effects in aqueous solution were included using the charge-density-based SMD continuum solvation model.⁴⁵ The resulting level of theory is denoted by SMD(water)-B3LYP-D3/6-31G(2df,p). Harmonic vibrational analyses were performed at the same level of theory to confirm each stationary point as either an equilibrium structure (i.e., all real frequencies) or a transition

structure (i.e., with one imaginary frequency). The connectivity of the local minima and first-order saddle points was confirmed by performing intrinsic reaction coordinate calculations.^{46,47} In order to ensure that the equilibrium structures are in their minimum-energy conformations we carried out systematic conformational searches using the MMFF94 molecular mechanics force-field with the Avogadro program.⁴⁸ The geometries of the low-lying conformations were then re-optimised at the SMD(water)-B3LYP-D3/6-31G(2df,p) level of theory and the lowest-energy conformation was selected.

Double-hybrid DFT calculations⁴⁹ were performed in order to obtain accurate electronic energies for the equilibrium and transition structures located along the uncatalysed and catalysed reaction pathways considered in this work. These DHDFT procedures involve both Hartree-Fock-like exchange and MP2-like correlation from second-order Møller–Plesset perturbation theory. Double-hybrid DFT has been found to produce thermochemical properties (such as reaction energies and barrier heights) with mean absolute deviations (MADs) approaching the threshold of “chemical accuracy” (arbitrarily defined as 1 kcal mol⁻¹ $\approx$ 4.2 kJ mol⁻¹) from a wide range of accurate thermochemical determinations.^{49,50,51,52,53,54,55,56,57} In the present work we used the spin-component-scaled DSD-PBEP86-D3 DHDFT functional of Kozuch and Martin^{50,51} in conjunction with the Def2-TZVPP basis set of Weigend and Ahlrichs.⁵⁸ These single-point energy calculations were carried out on top of the SMD(water)-B3LYP-D3/6-31G(2df,p) optimised geometries.

We note in passing that we have confirmed for one of the reaction pathways that re-optimising the geometries using the larger Def2-TZVPP basis set has practically no effect on the final reaction profile (see Table S1 of the Supporting Information). The DSD-PBEP86-D3 electronic energies were converted to enthalpies at 298 K (ΔH_{298}) using zero-point vibrational energies and thermal corrections obtained at the SMD(water)-B3LYP-D3/6-31G(2df,p) level of theory. Corrections for bulk solvent effects in aqueous solution were added to the gas-

phase ΔH_{298} values using the SMD model at the M05-2X/6-31G(d) level of theory⁵⁹ as recommended by Marenich, Cramer, and Truhlar.⁴⁵ All the DFT and ab initio calculations were carried out with the Gaussian 09 and Gaussian 16 program suites.^{60,61}

To assist in the analysis of reaction path stereoselectivity natural bond orbital (NBO) calculations^{62,63,64} at the B3LYP/6-31G(d,p) level of theory were performed via the NBO6⁶⁵ and Gaussian 09 program suites. NBO analysis has been established as a capable method for estimating hyperconjugative stabilisation at both the ground and transition state.^{66,67,68,69,70} It transforms the canonical delocalised Hartree-Fock MOs into complete and orthonormal localised occupied Lewis-type and unoccupied non-Lewis type natural bond orbitals where deviation from the ideal Lewis structure indicates delocalisation. The magnitude of delocalisation between molecular fragments can either be estimated by deletion of off-diagonal Fock matrix elements between the interacting orbitals or by second-order perturbation analysis.^{63,64} Alabugin and Zeidan have reported excellent linear correlation between the results of deletion and perturbation analysis.⁶⁹ We report stabilisation energies resulting from second-order perturbation theory analysis, referred to as $E^{(2)}$ energies.

3. Results and Discussion

Three pathways, a concerted, a stepwise and a water-catalysed stepwise mechanism were explored for the 3-O-acyl to 5-O-acyl and following 5-O-acyl to 20-O-acyl rearrangements in the model ingenol ester. An additional eight-step pathway involving zwitterionic intermediates resulting from proton transfer preceding nucleophilic attack at the carbonyl and subsequent departure of the hydroxyl leaving group can be envisaged. However, the related transition structures could not be located on the potential energy surface.

3.1 Concerted mechanism for the acyl rearrangements

The concerted mechanism of the acyl migration reaction involves simultaneous attack of the hydroxyl group at the ester carbonyl carbon and proton transfer of the hydroxyl proton concomitant with cleavage of the initial ester C–O bond. The reaction profiles for the concerted 3-O-acyl to 5-O-acyl and 5-O-acyl to 20-O-acyl rearrangements are shown in Figure 3. These reactions proceed via a strained four-membered cyclic transition structure and with a reaction centre in close proximity to the steric bulk of the ingenol backbone. The concerted pathway yields prohibitively high barriers of 296.1 and 190.6 kJ mol⁻¹ for the two migration reactions, respectively. Both transition states are late in the reaction at the carbonyl as well as the proton transfer. The breaking bonds in the proton transfer (**TS1**: O...H 1.49, **TS2**: O...H 1.43 Å) as well as in the reaction at the carbonyl (**TS1**: O...C 2.05, **TS2**: O...C 1.94 Å) are strongly elongated while the forming bonds are close to their equilibrium values (**TS1**: O...H 1.09 and O...C 1.61, **TS2**: O...H 1.08 and O...C 1.60 Å).

Fig. 3. Reaction profile (DSD-PBEP86-D3/Def2-TZVPP, ΔH_{298} , kJ mol⁻¹) for the uncatalysed concerted acyl rearrangements in the model ingenol ester.

The strained four-membered transition structures result in unfavourable trajectories for the incoming and leaving nucleophiles (**TS1**: $\angle \text{O}-\text{C}-\text{O}$ 78.3°, **TS2**: $\angle \text{O}-\text{C}-\text{O}$ 78.7°). Experimental and computational work by Bürgi et al. on the reaction pathways at the carbonyl suggest that the lowest energy trajectory, which minimises repulsive interactions while allowing for high orbital overlap between the HOMO of the incoming nucleophile and the unoccupied carbonyl π^* orbital, is achieved at an angle of about 107° between the nucleophile and carbonyl. Deviation from this trajectory has been found to lead to a very rapid increase in the reaction barrier.^{71, 72}

A notable difference of 105.5 kJ mol⁻¹ is observed between the activation energies for the 3-O-acyl to 5-O-acyl and the 5-O-acyl to 20-O-acyl rearrangements. This difference can

be partly explained by the fact that the first rearrangement involves a 2° hydroxyl group introducing conformational rigidity, while the 1° hydroxyl group of the second rearrangement allows for a higher rotational freedom. In addition, steric hindrance by the 3° 4-hydroxyl group in the transition structure further encumbers the 3-O-acyl to 5-O-acyl migration.

3.2 Stepwise mechanism for the acyl rearrangements

The stepwise mechanisms of the acyl migration here explored include a cyclisation step achieved by nucleophilic attack of the hydroxyl group at the carbonyl and concomitant proton transfer from the hydroxyl group to the carbonyl oxygen of the ester leading to a cyclic orthoester intermediate. This intermediate involves a new stereocentre at the carbonyl carbon. As such the reaction may proceed via two different pathways with the **stereochemistry** outcomes depending on the preferred relative orientations of the prochiral carbonyl and nucleophile in the transition structure. Subsequent ring opening and another proton transfer afford the migration product. Figure 4 shows the TSs and intermediates involved in the uncatalysed stepwise acyl migrations. Section 3.2.1 explores the uncatalysed stepwise mechanism in detail, whilst section 3.2.2 considers the water-catalysed stepwise mechanism.

Fig. 4. Mechanisms of the uncatalysed and water-catalysed stepwise 3-O-acyl to 5-O-acyl migration in the model ingenol ester via two diastereomeric intermediates.

3.2.1 Uncatalysed stepwise mechanism for the acyl rearrangements

Figure 5 gives the reaction profile for the uncatalysed stepwise mechanism for the 3-O-acyl to 5-O-acyl and 5-O-acyl to 20-O-acyl rearrangements. Pathway **a** in Figure 4, which involves the R enantiomer of the intermediate, affords the lowest activation energies for both the 3-O-acyl to 5-O-acyl and 5-O-acyl to 20-O-acyl rearrangements. In particular, we obtain reaction barriers of 234.4 (3-O-acyl to 5-O-acyl cyclisation step), 251.6 (3-O-acyl to 5-O-acyl ring-opening step), 176.9 (5-O-acyl to 20-O-acyl cyclisation step), and 177.1 (5-O-acyl to 20-O-acyl ring-opening step) kJ mol^{-1} (Figure 5). Thus, for each of the acyl rearrangements, the ring-opening step is the rate-determining step (RDS). Comparing these reaction barriers with those obtained for the concerted rearrangements, the stepwise mechanism leads to reductions in the energy barriers of 44.5 and 13.5 kJ mol^{-1} for the first and second rearrangements, respectively (Figures 3 and 5). While in both the concerted and stepwise mechanisms the proton transfer proceeds via a strained 4-membered cyclic transition structure, in the stepwise mechanism the reaction centre has shifted away from the ingenol backbone reducing steric

crowding, especially in the transition structure of the 3-O-acyl to 5-O-acyl rearrangement.

Fig. 5. Reaction profile (DSD-PBEP86-D3/Def2-TZVPP, ΔH_{298} , kJ mol⁻¹) for the uncatalysed stepwise (top) and the water-catalysed stepwise (bottom) acyl rearrangements in the model ingenol ester. Relative enthalpies are given for the mechanisms proceeding via both possible diastereomeric intermediates (shown in Figure 4), where the enthalpies pertaining to the less favoured pathway are given in parentheses.

The stepwise mechanism leading to an S-configuration in the newly formed stereocentre of the intermediate (Figure 4b) is kinetically unfavourable compared to the pathway involving the R enantiomer. In particular, the differences in the barriers heights between the two pathways ($\Delta\Delta H_{298}$) are $\Delta\Delta H_{298} = 9.4$ (**TS1**) and 25.7 (**TS3**) kJ mol⁻¹ in the cyclisation steps and $\Delta\Delta H_{298} = 9.2$ (**TS2**) and 35.5 (**TS4**) kJ mol⁻¹ in the ring-opening steps.

In both rearrangements as well as for both pathways **a** and **b** the cyclisation steps have lower activation enthalpies relative to the ring-opening steps. In particular, they are lower by

17.2 and 17.0 kJ mol⁻¹ for pathways **a** and **b** of the 3-O-acyl to 5-O-acyl migration, and by 25.9 and 38.7 kJ mol⁻¹ for pathways **a** and **b** of the 5-O-acyl to 20-O-acyl migration.

Figure 6 shows a schematic representation of the transition structures involved in pathways **a** and **b**. Compared to the concerted rearrangements the stepwise pathways lead to improvements in the trajectories of the nucleophiles represented by the $\angle \text{O-C-O}$ angles between the nucleophilic oxygen and carbonyl bond in the formation and decomposition of the tetrahedral intermediate. These trajectories are listed in Table 1. In the concerted mechanism we obtain $\angle \text{O-C-O}$ angles of 78.3° (**TS1**) and 78.7° (**TS2**), while in the stepwise mechanism we obtain $\angle \text{O-C-O}$ angles ranging between 79.9° (**TS2a**) and 93.9° (**TS3b**) (Table 1). The larger $\angle \text{O-C-O}$ angles in the stepwise mechanism indicate an increase in the stabilising Bürgi–Dunitz interactions.^{71,72} Interestingly, despite favourable trajectories in pathway **b** of the stepwise mechanism compared to pathway **a** (Table 1), pathway **b** leads to higher activation energies. A rationalisation for this is proposed in Section 3.3.

Fig. 6. Schematic representation of the transition structures of the stepwise ingenol rearrangements for pathways **a** (upper pane) and **b** (lower pane) with selected bond lengths in Å.

Table 1. Trajectories of nucleophiles in the cyclisation and ring-opening transition structures in the stepwise mechanisms shown in Figures 4 and 5. The tabulated values are the $\angle \text{O-C-O}$ angle between the nucleophilic oxygen and carbonyl bond for the transition structures of the uncatalysed pathways (**a** and **b**, shown in Fig. 6) and water-catalysed pathways (**c** and **d**, shown in Fig. 7).

Pathway	TS1	TS2	TS3	TS4
a	81.5°	79.9°	92.9°	82.9°
b	90.5°	93.5°	93.9°	83.5°
c	98.2°	99.8°	109.8°	103.6°
d	105.4°	107.9°	108.5°	105.6°

3.2.2 Water-catalysed stepwise mechanism for the acyl rearrangements

It is well known that a water molecule can significantly enhance the rates of hydrogen-transfer reactions relative to the rates of the uncatalysed reactions.^{54,73,74,75,76,77,78} Thus, **TS1–4** in the stepwise mechanism (Figures 4a and 4b) could potentially be catalysed by a water catalyst, in which the water acts as a proton shuttle between the exo- and endocyclic oxygens in both steps. The TSs and intermediates involved in the water-catalysed rearrangements are shown in Figures 4c and 4d.

Figure 5 gives the reaction profile for the water-catalysed stepwise mechanism for the 3-O-acyl to 5-O-acyl and 5-O-acyl to 20-O-acyl rearrangements. Inclusion of a catalytic water molecule decreases the activation energies of the stepwise rearrangements by as much as 98.9 and 90.1 kJ mol⁻¹ for the 3-O-acyl to 5-O-acyl and 5-O-acyl to 20-O-acyl rearrangement, respectively when comparing the lowest energy pathways **a** and **d** (Figures 4 and 5). Participation of a water molecule in the reactions expands the cyclic transition structures of the proton transfers from unfavourable 4-membered to more favourable 6-membered rings reducing strain and thus stabilising the structures. Importantly, this more relaxed ring system allows for significantly improved trajectories of the nucleophilic hydroxyl in the formation and decomposition of the tetrahedral intermediate, now very close to the ideal Bürgi–Dunitz trajectory (Table 1).^{71,72} It is instructive to compare the trajectories for the energetically favoured pathways for the uncatalysed and water-catalysed pathways (pathways **a** and **d**, respectively). The $\angle\text{O-C-O}$ angle for the uncatalysed pathway (**a**) ranges between 79.9–92.9°, whilst for the water-catalysed pathway (**d**) this angle ranges between 105.4–108.5° (Table 1).

We note that while in the uncatalysed reaction, the transition structures leading to stereocentres of R-configuration are favoured (pathway **a**), with a water catalyst, reaction pathway **d** leading to stereocentres of S-configuration is favoured. The differences in

activation energies between the two pathways are, however, notably smaller than in the uncatalysed reaction ($\Delta\Delta H_{298} = 7.8$ (TS1'), 7.0 (TS2'), 3.2 (TS3') and 14.5 (TS4') kJ mol⁻¹, Figure 5). The lower barriers for pathway **d** are consistent with the fact that the forming and breaking C...O bonds are shorter in the transition structures of pathway **d** (1.79 (TS1'), 1.69 (TS2'), 1.59 (TS3'), and 1.89 (TS4')) compared to those of pathway **c** (2.21 (TS1'), 2.12 (TS2'), 1.62 (TS3'), and 1.94 (TS4')) Å (see Figure 7).

Fig. 7. Schematic representation of the transition structures of the water-catalysed stepwise ingenol rearrangements for pathways **c** (upper pane) and **d** (lower pane) with selected bond lengths in Å.

With highly unfavourable trajectories in the uncatalysed pathways, **TS2** and **TS4** corresponding to the ring-opening steps, are clearly rate limiting for each of the acyl migration steps. Participation of a catalytic water molecule improves the trajectories of the nucleophiles such that they are equally favourable to those observed for the cyclisation steps (Table 1). Accordingly, the transition states of the cyclisation and ring-opening steps are

associated with similar activation energies such that there is no longer a clear rate-determining step. In particular, there are negligible differences in activation energies between the cyclisation and ring-opening steps, namely, $\Delta\Delta H_{298} = 0.5$ (3-O-acyl to 5-O-acyl migration **d**) and 2.2 (5-O-acyl to 20-O-acyl migration **d**) kJ mol^{-1} .

From experimental studies it is well known that acyl migration reactions can be both base and acid catalysed.^{22,23,34, 79} Acid catalysis is assumed to involve activation of the electrophilic carbonyl by protonation of the carbonyl oxygen. The base catalysed pathway is assumed to be initiated by deprotonation of the attacking OH group improving its nucleophilicity.^{34,79} As both a weak acid and base the role of water can be understood as that of a bifunctional catalyst in this reaction, activating both the nucleophile and electrophile through hydrogen bonding while facilitating proton transfer. The very significant reductions in the activation energies upon participation of water in the reaction observed here establish the possibility of bifunctional catalysis for the neutral acyl migration reaction and should be generalisable to other bifunctional catalysts. So far the observation of slow acyl migration reactions in the absence of acid or base could not be rationalised mechanistically.³³ The possibility for bifunctional activation explored here offers a plausible explanation.

3.3 Stereochemistry of the reaction pathways

Both stepwise mechanisms explored here (pathways **a–d**) show reaction path stereoselectivity that is consistent throughout both possible migrations. Rationalisation of this requires consideration of the conformations of the 6-membered cyclic transition structures in the formation of the diastereomeric orthoesters. Figure 8 illustrates the two conformations involved in the transition structures leading to intermediates with R- and S-conformations in the uncatalysed stepwise 3-O-acyl to 5-O-acyl migration. Due to the rigidity of the polycyclic ingenol backbone the 6-membered transition structures are locked into a single chair

conformation with no possibility for a conformational flip. Alternative boat conformations could not be located on the potential energy surfaces of neither the catalysed nor uncatalysed pathways (except in the case of **TS3a**).⁸⁰ This is likely due to high steric interference from the ingenol backbone and its out-of-plane substituents. As such, the preference of exocyclic substituents for the axial and equatorial orientation determines reaction path stereoselectivity.

Fig. 8. Schematic representation of the transition structure conformations for pathways **a** and **b** leading to the R- and S-conformations of the intermediate in the uncatalysed stepwise 3-O-acyl to 5-O-acyl rearrangement.

From conformational analyses of cyclohexane derivatives it is well known that the thermodynamically favoured conformations put substituents with highest steric bulk in equatorial orientation reducing interference with other substituents.⁷⁹ When catalysed the reaction path, as expected, favours transition structures locating the carbonyl C=O substituent in equatorial position. Given the path stereoselectivity of the uncatalysed reaction for pathway **a**, however, the transition structures of this reaction show a contrasting preference for structures with the larger forming or breaking carbonyl C=O substituent in axial orientation. The following will show that this behaviour may be rationalised by considering the general anomeric effect.

A contrasting preference of electronegative substituents in the anomeric position of pyranoses for an axial orientation over an equatorial one was first observed by Edward⁸¹ and Lemieux and Chu.⁸² Known, based on its origin in carbohydrate chemistry, as the “anomeric” effect this phenomenon has been widely studied and extended to other 2-substituted heterocycles as well as to acyclic molecules.⁸³ It has been generalised in its definition to describe a preference of electronegative substituents for a gauche over an antiperiplanar conformation in a general R-X-Z-Y system, where Z (generally carbon) is less electronegative than Y and X possesses lone pairs of electrons.^{84,85,86} This general definition can be applied to the transition structures of the acyl rearrangements under consideration when envisaging the carbonyl carbon as Z, the carbonyl oxygen as Y and the endocyclic oxygen as X. In the case of the uncatalysed reaction this model now correctly predicts the transition structures of pathway **a** as favourable (Figure 9).

Fig. 9. Newman projections of schematic representations of the transition structures of the uncatalysed stepwise acyl rearrangements.

To rationalise this it is necessary to consider the roles of destabilising electrostatic interactions in the anti-staggered conformation and stabilising stereoelectronic interactions in the gauche-staggered conformation in the transition structures.^{84,85,86,87,88,89,90,91,92,93} These can be invoked to explain the notable stereoselectivity of the reaction path of the uncatalysed acyl rearrangements in the ingenol ester. As illustrated by figure 10a electrostatic repulsion of

aligned dipoles in the transition structures with anti-staggered conformation has a destabilising effect. Negative $n_o \rightarrow \sigma^*_{C-O}$ hyperconjugation between a lone pair of the endocyclic oxygen (X) and an antibonding orbital of the carbonyl stabilises transition structures of gauche-staggered conformation. Such an interaction is less accessible in the anti-staggered conformation due to unfavourable orbital orientations (Figure 10b).^{84,85,86,87,88,89,90,91,92,93}

Fig. 10. Schematic representation of the electrostatic and hyperconjugative interactions in the transition structures of the ingenol acyl rearrangement contributing to the observed anomeric effect.

While these interactions in systems subject to an anomeric effect have been the topic of numerous studies, the largest focus has so far been on the study of ground state structures.^{68,86,89,91,94} As hyperconjugation is known to stabilise transition structures^{66,67,70,95,96} and since anomeric hyperconjugation has been found to be amplified in transition structures,^{67,97} the relative magnitudes of the hyperconjugative interactions between the X-

oxygen lone pairs and carbonyl antibonds in the anti- and gauche-staggered transition structures of the acyl migrations were studied via second-order perturbation analysis. Indeed the hyperconjugative stabilisation experienced by all transition structures of pathway **a** and **c** is more than double that calculated for pathway **b** and **d** (see Table 2 and Figure 11).

Table 2. Stabilising ($E^{(2)}$) energies from second order perturbation analysis in kJ mol⁻¹.

Pathway	TS1	TS2	TS3	TS4	TS4
Gauche-staggered (pathway a)	41.0	44.9	42.3	56.4	56.4
Anti-staggered (pathway b)	10.0	20.9	15.6	11.5	11.5
Gauche-staggered (pathway c)	48.6	47.9	38.7	50.3	50.3
Anti-staggered (pathway d)	10.4	12.3	9.9	11.3	11.3

Fig. 11. Three-dimensional orbital rendering of the hyperconjugation interaction in **TS1a** (created using NBOview 2.0).

4. Conclusions

We use DHDFT calculations to investigate the uncatalysed and water-catalysed reaction mechanisms for the 3-O-acyl to 5-O-acyl and 5-O-acyl to 20-O-acyl rearrangements in ingenol-3-ester under neutral conditions. We draw the following conclusions:

1. In both the uncatalysed concerted and stepwise mechanisms the 3-O-acyl to 5-O-acyl rearrangement involves significantly higher reaction barrier heights than the 5-O-acyl to 20-O-acyl rearrangement. In particular, we obtain the following differences in barrier heights between the two rearrangements 105.5 (concerted mechanism) and 74.5 (stepwise mechanism) kJ mol^{-1} . These differences are rationalised based on the greater conformational flexibility in the transition structures for the latter rearrangement and steric hindrance by the 3° 4-hydroxyl group in the 3-O-acyl to 5-O-acyl migration.
2. The uncatalysed stepwise mechanism involves reaction barriers that are lower by 44.5 (3-O-acyl to 5-O-acyl rearrangement) and 13.5 (5-O-acyl to 20-O-acyl rearrangement) kJ mol^{-1} than those for the concerted mechanism.
3. Participation of a catalytic water molecule in the stepwise mechanism significantly reduces the activation energies by allowing for nearly ideal Bürgi–Dunitz trajectories of the nucleophiles relative to the carbonyl and through bifunctional activation of the carbonyl electrophile and hydroxyl nucleophile. In particular, a water catalyst reduces the activation energies of the RDS by 98.4 (3-O-acyl to 5-O-acyl rearrangement) and 90.1 (5-O-acyl to 20-O-acyl rearrangement) kJ mol^{-1} .
4. The stepwise mechanisms of the acyl migrations show reaction path stereoselectivity, which can be rationalised based on stereoelectronic, electrostatic, and steric interactions.

Our results provide important insights into the molecular mechanisms of acyl rearrangements in ingenol esters. Most notably, that (i) under neutral conditions this rearrangement is water-catalysed and (ii) the catalysed 3-O-acyl to 5-O-acyl migration has a reaction barrier which is higher by 66.2 kJ mol^{-1} than the 5-O-acyl to 20-O-acyl

rearrangement. We hope that the results of this study will aid the design of ingenol ester-based drugs less prone to acyl rearrangements.

Supporting Information

Reaction profile (DSD-PBEP86-D3/Def2-TZVPP, ΔH_{298} , kJ mol⁻¹) for the uncatalysed concerted acyl rearrangement in the model ingenol ester calculated using SMD(water)-B3LYP-D3/Def2-TZVPP optimised geometries (Table S1). SMD(water)-B3LYP-D3/6-31G(2df,p) optimised geometries for all the local minima and transition structures considered in this work (Table S2). Full references for the Gaussian 09 and Gaussian 16 program suites (Table S3).

Acknowledgements

Dedicated to our colleague and friend Prof. Graham Chandler on the occasion of his 80th birthday. This research was undertaken with the assistance of resources from the National Computational Infrastructure (NCI), which is supported by the Australian Government. We also acknowledge system administration support provided by the Faculty of Science at UWA to the Linux cluster of the Karton group. We gratefully acknowledge an Australian Research Council (ARC) Future Fellowship (to A.K., project number: FT170100373).

Conflicts of Interest

The authors declare no conflicts of interest

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