The Complete Mechanism of an Aldol Condensation
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**Supporting Information**

**ABSTRACT:** Although aldol condensation is one of the most important organic reactions, capable of forming new C–C bonds, its mechanism has never been fully established. We now conclude that the rate-limiting step in the base-catalyzed aldol condensation of benzaldehydes with acetophenones, to produce chalcones, is the final loss of hydroxide and formation of the C=C bond. This conclusion is based on a study of the partitioning ratios of the intermediate ketols and on the solvent kinetic isotope effects, whereby the condensations are faster in D₂O than in H₂O, regardless of substitution.

**INTRODUCTION**

The aldol reaction and the aldol condensation are among the most versatile of organic reactions,¹ with >25000 entries in SciFinder. Each of these uses two carbonyl compounds, one as an electrophile and the other as a nucleophile. Each succeeds in forming a new carbon–carbon single bond, or else a carbon–carbon double bond, which distinguishes the aldol condensation. There are many variants, including the Claisen, Dieckmann, Henry, and Darzens condensations and the Knoevenagel and Perkin reactions. Because of their ability to construct larger molecules from smaller ones,²⁻⁹ or to effect cyclization,⁷⁻⁹ often with control of stereochemistry,¹⁰⁻¹² these reactions are a mainstay of organic synthesis. They are also common in metabolism, where aldolase, citrate synthase, and other enzymes catalyze aldol reactions and aldol condensations, or their reverse,¹³ leading to the suggestion that they reflect primordial metabolism.¹⁴,¹⁵

We are interested in the particular aldol reaction of a benzaldehyde 1 and an acetophenone 2 to form ketol (β-hydroxyketone) 3, which is then dehydrated to chalcone (benzylideneacetophenone) 4, as shown in Scheme 1. Chalcones have many medicinal and pharmacological properties, with antimicrobial, anticancer, anti-inflammatory, antimalarial, antibacterial, and antiproliferative activities.¹⁶ They are intermediates in the synthesis of various natural products,¹⁷,¹⁸ as well as unusual polycyclic aromatics.¹⁹ The aromatic rings stabilize 4 and increase the equilibrium constant for its formation, so that the reaction becomes more feasible for study.

The question we address is the mechanism of base-catalyzed chalcone formation, as a representative of the aldol condensation. It may be thought that this mechanism is well understood, but surprisingly, it has never been fully established. There are five steps, as shown in Scheme 2, although the last two are sometimes merged into a single dehydration step, perhaps merely for the sake of brevity.

According to an early kinetic study,²⁰ the rate, for Ar = Ph = Ar', is given by eq 1, where k is a third-order rate constant. Therefore, step 1 cannot be rate-limiting, because if it were, the rate would be independent of ArCHO concentration. For the aldol reaction, arrested at 3, step 2 must be rate-limiting, because the proton equilibration of step 3 is fast (although there are examples in which the enolization of step 1 is rate-limiting).²¹⁻²³

Received: April 27, 2016

DOI: 10.1021/acs.joc.6b00959
J. Org. Chem. XXXX, XXX, XXX--XXX
\[ \frac{d[\text{chalcone}]}{dt} = k'[\text{ArCHO}][\text{Ar'}\text{COCH}_3][\text{OH}^-] \]

Which step is the rate-limiting step of the aldol condensation, as distinguished from the aldol reaction? Noyce, Pryor, and Bottini studied the fate of the ketol intermediate, independently synthesized.\textsuperscript{24} They found that 3 \((\text{Ar} = \text{Ph} = \text{Ar'}\text{'})\) is converted in base to a mixture of 80% 1 and 2 and 20% 4. There has been disagreement about the mechanistic inference to be drawn from this 4:1 ratio. Noyce, Pryor, and Bottini inferred that “in dilute solutions the C–C bond forming step is rate-determining, with dehydration being rapid”. This inference is echoed in a recent advanced textbook: “Studies ... have shown that about 80% (sic) of [ketol] goes on to product. These reactions are faster than the overall reaction, so the second step must be rate-controlling.”\textsuperscript{25} An earlier monograph concluded, “observation that alkali transforms the intermediate \(\beta\)-hydroxy ketone to benzaldehyde and acetophenone more rapidly than it dehydrates it shows that the second step is not rate-controlling.”\textsuperscript{26} It should be noted that these two books draw exactly opposite conclusions about step 2, and that the recent one misquoted the experimental observation. We now resolve these contradictions.

According to one definition,\textsuperscript{27} the rate-limiting step of a multistep mechanism is the last one whose rate constant remains in the kinetic equation. Because ketol 3 reverts to precursors faster than it continues to chalcone, steps 1–3 of Scheme 2 are rapid and reversible and cannot be rate-limiting. This holds even in dilute solution, where step 2 is slower in the forward direction but not retarded in the reverse direction.

Therefore, dehydration must be rate-limiting. Although this can be represented as a single step, it is possible to distinguish enolization (step 4) from elimination of \(\text{OH}^-\) (step 5). Which one is rate-limiting, step 4, step 5, or their composite?

Kinetic isotope effects are often useful in elucidating reaction mechanisms and distinguishing the rate-limiting step.\textsuperscript{28,29} Indeed, this question can be answered by measuring the solvent deuterium kinetic isotope effect. Because step 1 is rapid and reversible, \(\text{Ar'}\text{COCH}_3\) in \(\text{D}_2\text{O}\) becomes \(\text{Ar'}\text{COCD}_3\) and 3 becomes \(\text{ArCHODCD}_2\text{COAr'}\). The deuterated 3 may be expected to form enolate 5 more slowly than undeuterated 3 does, as is generally seen in base-catalyzed enolizations, because of the lower zero-point energy of a C–D bond. A faster reaction in \(\text{D}_2\text{O}\) would then be strong evidence against step 4 as being rate-limiting. We also choose to ascertain whether the answer depends on substituents in the ary rings and even the extent to which the partition ratio of intermediate 3 might depend on substituents. We therefore have extended the earlier studies to some substituted benzaldehydes 1 and acetophenones 2.

Although earlier studies were often performed in ethanol, a solvent isotope effect is more readily interpreted in an aqueous medium. Then, to maintain the solubility of substrates and of the chalcone product, it was found to be necessary to add acetonitrile as a cosolvent to the \(\text{H}_2\text{O}\) or \(\text{D}_2\text{O}\). Fortunately, \(\text{CH}_3\text{CN}\) is sufficiently inert to base-catalyzed \(\text{H}/\text{D}\) exchange.\textsuperscript{30} We now report that the reaction is faster in \(\text{D}_2\text{O}\) than in \(\text{H}_2\text{O}\), and we conclude that elimination of \(\text{OH}^-\) is the rate-limiting step, regardless of substituents in the aromatic rings.

#### RESULTS AND DISCUSSION

**Partitioning of Ketol Intermediates.** Upon being treated with dilute base, ketols 3 partition between reversion to precursors 1 and 2 and progression to chalcone product 4. The partitioning ratio was evaluated from the absorbances of the product mixture at both the \(\lambda_{\text{max}}\) of chalcone 4, near 312 nm, and the isosbestic wavelength of benzaldehyde 1 and acetophenone 2, near 250 nm. Table 1 presents the ratio R

<table>
<thead>
<tr>
<th>Ar</th>
<th>Ar'</th>
<th>R</th>
</tr>
</thead>
<tbody>
<tr>
<td>Ph</td>
<td>Ph</td>
<td>6.4</td>
</tr>
<tr>
<td>pClPh</td>
<td>Ph</td>
<td>5.4</td>
</tr>
<tr>
<td>pO2NPh</td>
<td>Ph</td>
<td>5.8</td>
</tr>
<tr>
<td>pMePh</td>
<td>Ph</td>
<td>6.9</td>
</tr>
<tr>
<td>Ph</td>
<td>pClPh</td>
<td>6.9</td>
</tr>
<tr>
<td>Ph</td>
<td>pO2NPh</td>
<td>6.9</td>
</tr>
</tbody>
</table>

Thus, the rate is greater in \(\text{D}_2\text{O}\) than in \(\text{H}_2\text{O}\). For example, enolizations of simple ketones show a kinetic isotope effect \(k_{\text{D}/\text{H}}/k_{\text{H}/\text{H}} = 1/4\) to 1/7.\textsuperscript{33–35} A mechanism more closely analogous to steps 4 and 5 of Scheme 2 is often operative for elimination of \(\text{H}\) and a good leaving group, such as halide. Such a mechanism is designated as E1cb(irrev), but it is less likely here for the poorer leaving group hydroxide. Indeed, this mechanism would have shown a \(k_{\text{D}/\text{H}}/k_{\text{H}/\text{H}} = 1/7\).\textsuperscript{36}

The reaction is faster in \(\text{D}_2\text{O}\). This is consistent with the observations that base-catalyzed formation of epoxide from 2-haloethanols is faster in \(\text{D}_2\text{O}\) than in \(\text{H}_2\text{O}.\textsuperscript{30,37,38} Thus, the rate
Table 2. Rate Constants (M⁻¹ s⁻¹) for Base-Catalyzed Conversion of Benzaldehyde 1 and Acetophenone 2 to Chalcone 4 in H₂O or D₂O and kₓ/O/kₓ/H₂O Ratios

<table>
<thead>
<tr>
<th>% CH₃CN</th>
<th>Ar</th>
<th>Ar'</th>
<th>kₓ/H₂O</th>
<th>kₓ/D₂O</th>
<th>kₓ/D₂O/kₓ/H₂O</th>
</tr>
</thead>
<tbody>
<tr>
<td>26</td>
<td>Ph</td>
<td>Ph</td>
<td>0.0111 ± 0.0004</td>
<td>0.0127 ± 0.0005</td>
<td>1.14 ± 0.06</td>
</tr>
<tr>
<td>40</td>
<td>pClPh</td>
<td>Ph</td>
<td>0.0412 ± 0.0008</td>
<td>0.0506 ± 0.0007</td>
<td>1.23 ± 0.03</td>
</tr>
<tr>
<td>40</td>
<td>pO₂NPh</td>
<td>Ph</td>
<td>0.440 ± 0.019</td>
<td>0.512 ± 0.013</td>
<td>1.16 ± 0.06</td>
</tr>
<tr>
<td>40</td>
<td>Ph</td>
<td>pClPh</td>
<td>0.0298 ± 0.0009</td>
<td>0.0334 ± 0.0007</td>
<td>1.12 ± 0.04</td>
</tr>
<tr>
<td>40</td>
<td>Ph</td>
<td>pO₂NPh</td>
<td>0.158 ± 0.004</td>
<td>0.227 ± 0.014</td>
<td>1.43 ± 0.10</td>
</tr>
</tbody>
</table>

Scheme 3. E1cb Elimination of Methoxide

It is necessary to justify the simplification to pseudo-first-order kinetics. In principle, the stoichiometric OH⁻ concentration might partition itself among the anionic species of Scheme 2, leading to a catalytic cycle with a more complicated rate expression. Thus, Scheme 2 can alternatively be drawn as a set of catalytic cycles, as shown in Scheme 4. Such a drawing places onto the cycle not only the catalyst but also any species to which the catalyst is converted, while reactants and products are shown as entering or leaving the cycle. A catalytic cycle is advantageous for cases like Michaelis–Menten kinetics, in which a high concentration of substrate S can convert catalyst E to E-S. Such a complication does arise in proline-catalyzed aldol reactions, where the enamine intermediate is present at levels to which the catalyst is converted, while reactants and products. By using the partition ratios listed in Table 1, each of them can be separated into rate constants for conversion to 4 and reversion to 1 and 2, as also listed in Table 3. The value of 0.084 M⁻¹ s⁻¹ for Ar = Ph = Ar in 80% aqueous CH₃CN is in semiquantitative agreement with the values of 0.22 and 0.30 M⁻¹ s⁻¹ in the different solvents water and 95% aqueous ethanol, respectively.

Table 3. Rate Constants (M⁻¹ s⁻¹) for the Disappearance of Ketols 3, for Conversion to Chalcones 4, and for Reversion to Benzaldehydes 1 and Acetophenones 2

<table>
<thead>
<tr>
<th>Ar</th>
<th>Ar'</th>
<th>% CH₃CN</th>
<th>k₁</th>
<th>k₋₁</th>
<th>k₋₁/K₁</th>
</tr>
</thead>
<tbody>
<tr>
<td>Ph</td>
<td>Ph</td>
<td>80</td>
<td>0.084</td>
<td>0.011</td>
<td>0.073</td>
</tr>
<tr>
<td>pClPh</td>
<td>Ph</td>
<td>26</td>
<td>0.41</td>
<td>0.065</td>
<td>0.35</td>
</tr>
<tr>
<td>pO₂NPh</td>
<td>Ph</td>
<td>60</td>
<td>0.40</td>
<td>0.059</td>
<td>0.34</td>
</tr>
<tr>
<td>pMePh</td>
<td>Ph</td>
<td>70</td>
<td>0.20</td>
<td>0.025</td>
<td>0.17</td>
</tr>
<tr>
<td>Ph</td>
<td>pClPh</td>
<td>70</td>
<td>0.32</td>
<td>0.041</td>
<td>0.28</td>
</tr>
<tr>
<td>Ph</td>
<td>pO₂NPh</td>
<td>60</td>
<td>0.60</td>
<td>0.077</td>
<td>0.53</td>
</tr>
</tbody>
</table>

It should be noted that the transition state for conversion of 5 to 4 is still a rate-determining state even when this terminology is applied to the catalytic cycles of Scheme 4. Reaction Rates of Ketol Intermediates. For the sake of completeness, Table 3 lists rate constants k₅ for the base-catalyzed disappearance of ketols 3. By using the partition ratios listed in Table 1, each of them can be separated into rate constants for conversion to 4 and reversion to 1 and 2, as also listed in Table 3. The value of 0.084 M⁻¹ s⁻¹ for Ar = Ph = Ar in 80% aqueous CH₃CN is in semiquantitative agreement with the values of 0.22 and 0.30 M⁻¹ s⁻¹ in the different solvents water and 95% aqueous ethanol, respectively.

In terms of Scheme 2, it is readily seen that k₅ = (kₛK₄ + k₋₂/K₃)[OH⁻], where k₋₂ is the rate constant for the reverse reaction of step 2, which is rate-limiting for the reversion of 3 to 1 and 2. The individual terms of this rate constant correspond to the separate rate constants k₋₁ and k₋₁,K₃.

Above, we claimed that intermediate product 3 does not build up to any appreciable extent under our reaction conditions, because it is not sufficiently stable. As evidence of this claim, second-order rate constants k₅ for ketol disappearance in Table 3 are considerably larger than rate constants kₓ/H₂O for chalcone formation in Table 2, converted to pseudo-second-order rate constants kₓ/H₂O/[Ar’/COCH₃] at the typical [Ar’/COCH₃] of 0.02 M.

SUMMARY AND CONCLUSIONS

Our conclusion that step 5 of Scheme 2 is rate-limiting was also reached, although implicitly, by calculating rate and equilibrium constants by Marcus theory. In hindsight, we should not be surprised at this conclusion. If step 1 (enolization of CH₃COAr⁻) is not rate-limiting, then we might expect the similar step 4 [enolization of ArCH(OH)CH₂COAr⁻] not to be rate-limiting. This conclusion is not inescapable though, because enolization of CH₃COAr⁻ is followed by a bimolecular reaction whereas enolization of ArCH(OH)CH₂COAr⁻ is followed by a unimolecular step, and because enolization is calculated to be rate-limiting in the similar elimination of H⁺ and CH₃CO₂⁻ from CH₃YCOCH₂CH₂(OH)OCOCH₃CH₃ (Y = O or

C

DOI: 10.1021/acs.joc.6b00959
J. Org. Chem. XXXX, XXX, XXX--XXX
or S),\textsuperscript{45} where acetate is admittedly a much better leaving group. Certainly though, the results here are convincing experimental evidence of rate-limiting loss of OH\textsuperscript{−}.

Moreover, these results also provide evidence concerning the mechanism of the reverse reaction, the hydration of chalcone \textbf{4} followed by the retro-aldo condensation reverting to \textbf{1} and \textbf{2}. According to the principle of microscopic reversibility, the rate-limiting step for the reverse reaction must be the initial Michael addition of OH\textsuperscript{−} to the C=\textequiv=\textequiv bond.

Intermediate ketol \textbf{3} partitions predominantly (7:1) to precursors \textbf{1} and \textbf{2} regardless of substitution. Therefore, the first three steps in Scheme 2 are rapid and reversible. Because the rates of chalcone formation are faster in D\textsubscript{2}O than in H\textsubscript{2}O, regardless of substitution, all of the first four steps in Scheme 2 are rapid and reversible, and the rate-limiting step must be the loss of OH\textsuperscript{−} (step 5). This conclusion resolves the contradictions among refs \textsuperscript{24−26}.

All these results can be summarized in the energy diagram shown in Figure 1, constructed from these results (and others, as explained in the Supporting Information). The highest-energy transition state is for the final loss of OH\textsuperscript{−}, but it is not higher than the others by much. Another transition state might ask to the \textbf{C} bond.

\begin{figure}[h]
\centering
\includegraphics[width=0.5\textwidth]{energy_diagram.png}
\caption{(Free) energy diagram for aldol condensation of Scheme 2 (Ar = Ph = Ar').}
\end{figure}

\section*{Experimental Section}

\textbf{Materials.} Acetoneitrile was of a grade formulated for UHPLC-UV and purchased from Fisher Scientific. Commercial benzaldehyde and acetophenone and their substituted derivatives were purified by vacuum distillation or recrystallization and stored under N\textsubscript{2}. Each was as explained in the Supporting Information. The highest-energy transition state is for the final loss of OH\textsuperscript{−}, but it is not higher than the others by much. Another transition state might ask to the \textbf{C} bond.

\begin{align*}
\text{Rate Measurements.} & \text{ Rates of base-catalyzed condensation of benzoaldehyde 1 and acetophenone 2 to chalcone 4 were followed on a recording UV spectrophotometer by monitoring the absorbance of 4 at its } \lambda_{max} \text{ near 312 nm.} \\
\end{align*}

Because NaOH is a catalyst and because 2 is in excess, neither of their concentrations varies with time. Therefore, pseudo-first-order conditions apply, and the third-order kinetics of eq 1 simplifies to eq 2. Although the solution to eq 2 is \[ A_{\text{ArCHO}} = A_{\text{ArCHO}}^0 \exp(-k_{\text{obs}}t), \] the spectrophotometer measures absorbance \( A \) of product 4, as in eq 3, which was fit by nonlinear least squares.

\begin{align*}
v &= \text{d[chalcone]/dt} = -\text{d[ArCHO]/dt} = k_{\text{obs}}[\text{ArCHO}] \\
A &= A_{\text{ArCHO}} - (A_{\text{ArCHO}} - A_0) \exp(-k_{\text{obs}}t) \\
\end{align*}

\textbf{Extraction of Forward Rate Constant } \( k \). Because this reaction does not go to completion, it is necessary to extract the forward rate constant \( k \) of eq 1 from \( k_{\text{obs}} \) of eq 2. These are related by eq 4, in which an average equilibrium constant \( K_e \) can be evaluated from the final concentrations of benzoaldehyde 1, acetophenone 2, and chalcone 4. Rate constants were averaged over 4−17 experiments at various initial concentrations of 1, 2, and OH\textsuperscript{−} or OD\textsuperscript{−}. Further details of procedure are described in the Supporting Information.

\begin{align*}
k &= \frac{k_{\text{obs}}}{[\text{OH}^{-}] + K_e[2]} \\
\end{align*}

\section*{Associated Content}

\textbf{Supporting Information}

The Supporting Information is available free of charge on the ACS Publications website at DOI: 10.1021/acs.joc.6b00959.

Details of the procedure and construction of the energy diagram, representative time curves, reaction conditions, fitting parameters, and rate constants for formation of chalcones (PDF)
The Journal of Organic Chemistry

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Notes
The authors declare no competing financial interest.

ACKNOWLEDGMENTS

This research was supported by National Science Foundation Grant CHE11-48992. We are grateful to Prof. Robert Pomeroy for providing access to spectrophotometers and for helpful advice. We thank Ms. Janet B. Willis for some preliminary experiments.

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DOI: 10.1021/acs.joc.6b00959
J. Org. Chem. XXXX, XXX, XXX–XXX