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Aqueous Aldol Catalysis by a Nicotine Metabolite

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The aldol reaction, in addition to being an effective method for the formation of carbon−carbon bonds in organic synthesis, is also a critical biological reaction in the context of metabolism. A great deal of effort has been devoted to the discovery of stoichiometric and catalytic methods that predictably and efficiently generate aldol products with known stereochemistry.[1] Biological methodologies, such as aldolase enzymes[1,2] and catalytic antibodies,[3] have also been extensively developed for accomplishing aldol reactions with high efficiency and selectivity.

Recently, small organic molecules have been shown to serve as enantioselective catalysts for chemical reactions. Indeed, compounds such as proline will catalyze a variety of processes including the aldol reaction.[4,5] The proposed mechanism of this reaction is reminiscent of a type I aldolase, that is, the amine catalyst activates the aldol donor by forming an iminium ion which then is converted to the corresponding enamine nucleophile. Interestingly, proline, an essential building block of biomolecules, has not been shown to act as an "aldolase" under physiological conditions.

The use of water has been studied in the context of aqueous Mannich reactions and the total synthesis of compounds under physiological conditions can be found in the literature of the late 1930s.[6] Furthermore, although catalytic aqueous aldol reactions are documented, all involve metal-based Lewis acid activation of the acceptor and frequently use silyl enol ethers as aldol donors.[7] However, to our knowledge only enzymes have been shown to catalyze aldol reactions in water via an enamine-based mechanism.

Nornicotine **1** is a nicotine-related alkaloid that is found both endogenous in tobacco and as a minor metabolite of nicotine in vivo.[8] Nicotine has been identified as the addictive constituent of tobacco, while nornicotine has been found to be the only psychoactive nicotine metabolite.[9] Nornicotine has a longer half-life in vivo relative to its parent compound, nicotine,[10] and therefore heavy smokers can have relatively constant plasma levels of nornicotine. On the basis of our work in the treatment of nicotine addiction by immunopharmacotherapy,[11] we recognized the structural similarity between nornicotine and proline (Figure 1). Herein, we demonstrate that nornicotine, a biologically relevant constituent of tobacco, can serve as an aldol catalyst. This represents the first example of a small molecule organic catalyst for aldol reactions that operates exclusively in aqua.

Initially, we studied the reaction of acetone with 4-nitrobenzaldehyde. According to the precedent shown with proline,[4] we attempted to perform the reaction in anhydrous DMSO/acetone (4:1) with 30 mol % **1**. Interestingly, no aldol addition was observed, even after extended reaction times, varied amounts of **1**, and elevated temperatures. Furthermore, catalysis was not observed in any common organic solvents including chloroform, benzene, acetonitrile, THF, and DMF. However, by performing the reaction

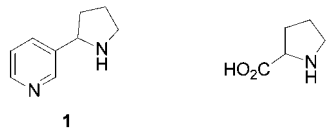

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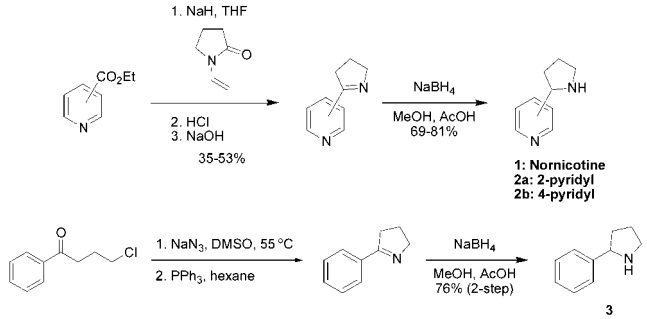
Figure 1. Structures of nornicotine **1** and proline.

Scheme 1. Synthesis of Nornicotine and Related Modified Alkaloids



in aqueous phosphate buffer (pH 7.4, 12 h), the aldol addition product, 4-hydroxy-4-(4-nitro-phenyl)-butan-2-one, was formed in 81% yield with no apparent dehydration or *retro*-aldolization of the product.[12,13] In the absence of catalyst, the aldol product was formed in less than 6% yield.

To determine if the observed catalysis was due to a unique property of nornicotine or if other substituted pyrrolidines could also catalyze the aldol reaction in water, compounds **2a**, **2b**, and **3** were synthesized according to known procedures (Scheme 1).[14] By using acetone as the donor and 4-nitrobenzaldehyde as the acceptor, the pseudo-first-order rate constant for each compound was determined (Table 1). Surprisingly, proline and pyrrolidine exhibited little difference as catalysts while the three pyridine-containing alkaloids displayed the highest rate enhancement over background. Furthermore, the nature of the aromatic ring clearly plays some role in the efficiency of the catalyst as 2-phenylpyrrolidine (**3**) showed acceleration over the uncatalyzed rate with a rate constant comparable to that of 2-pyridyl(2′-pyrrolidine) (**2a**). Finally, the combination of pyridine and pyrrolidine (1:1) did not serve as an effective catalyst (entry 6, Table 1).

There are two distinct possibilities for the catalytic mechanism of the observed aldol reaction, general base catalysis and covalent catalysis via an enamine nucleophile. A series of experiments was conducted to provide evidence for either of these mechanisms. Using 2D-ROESY ^{1}H NMR, the chemical exchange of acetone for the corresponding enamine was observed. Additionally, upon mixing nornicotine and acetone in buffer, we were able to trap the putative enamine species as the corresponding amine using $NaCNBH_3$, indicating the possibility of an aqueous enamine mechanism. Attempts at trapping the corresponding enamine intermediate between proline and acetone in water were not successful,

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Table 1. Pseudo-First-Order Rate Constants for the Reaction of Acetone with 4-Nitrobenzaldehyde in the Presence of Amine Catalysts (30 mol %) in Phosphate Buffer[a]

entry	catalyst	k_{obs} (min^{-1})
1	**1**	10.1×10^{-3}
2	**2a**	8.2×10^{-3}
3	**2b**	9.4×10^{-3}
4	**3**	6.4×10^{-3}
5	pyrrolidine	2.4×10^{-3}
6	pyrrolidine + pyridine (1:1)	1.3×10^{-3}
7	proline	1.8×10^{-3}
8	nicotine	0.7×10^{-3}
9	background	0.7×10^{-3}

[a] Kinetic assays were performed in aqueous buffer (200 mM sodium phosphate, pH 8.0) at 37 °C with 10% DMSO to enhance substrate solubility. The reaction was followed by monitoring generation of the aldol addition product by reversed-phase HPLC. The assay was started by addition of aldehyde (1−8 mM in DMSO) to a mixture of the catalyst (2.4 mM) and acetone (240 mM) in the aqueous buffer system. Rate constants were calculated by using linear regression analysis.

suggesting that the nornicotine-derived enamine is stabilized in some fashion to prevent hydrolysis relative to the proline-derived enamine. A general base mechanism could also be invoked; however, nicotine would be expected to catalyze the reaction at a comparable rate to nornicotine due to their similarities in pK_a. Yet, when nicotine was used as a catalyst, no rate acceleration was observed. The order of nornicotine was determined to be unity, implying the involvement of one nornicotine molecule in the transition state of the rate-determining step. An examination of the pH rate profile of this reaction showed that above pH 9.5, catalysis of the reaction by nornicotine becomes minimal as general base catalysis due to hydroxide dominates. However, between pH 7.5 and 8, optimal rate acceleration (k_{cat}/k_{bkgd}) was observed with over twenty turnovers at 2.5 mol % **1**.

Substrate specificity was briefly examined and it was found that activated aldehyde acceptors are critical for the reaction to proceed. Thus, aldehydes having suitable water solubility and electronic activation, as with 4-nitrobenzaldehyde and 2-chlorobenzaldehyde, are acceptors. This observation can be explained in the context of the proposed mechanism. If the hydrolysis of the enamine intermediate is significantly faster than carbon−carbon bond formation, no product will be formed. We have also looked at a small set of donors, simple ketones such as hydroxyacetone and cyclopentanone, that were found to be competent reaction partners with 4-nitrobenzaldehyde. Included in this list was also pyruvate, a biologically relevant compound in metabolism. The compilation of these findings suggests that with an appropriately activated aldehyde in vivo, nornicotine could catalyze aldol reactions as well as other enamine-based processes. This could have significant implications in not only glycolysis, but also the metabolism of ketone-containing drugs.

We have demonstrated that nornicotine can catalyze aldol reactions in water. Our findings suggest that nornicotine could catalyze in vivo aldol reactions as maximum rate acceleration is observed near physiological pH. The proposed mechanism will require further investigations, but based on the data presented, it appears that the catalysis occurs via an enamine nucleophile, contrary to the known rapid hydrolysis of enamines in water.[15] At this juncture, nornicotine would not be synthetically useful as a catalyst. However, the ability of nornicotine to catalyze the aldol reaction and other biologically significant enamine processes *in vivo* is highly relevant. Furthermore, to our knowledge, this demonstrates the only known example of a metabolite capable of serving as a catalyst.

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Supporting Information Available: Experimental procedures for the preparation of all compounds and conditions for kinetic experiments and measurement of order of nornicotine (PDF). This material is available free of charge via the Internet at http://pubs.acs.org.

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