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Characterization of covalent Ene adduct intermediates in "hydride equivalent" transfers in a dihydropyridine model for NADH reduction reactions

R. Daniel Libby *, Ryan A. Mehl [1]

Moravian College, Chemistry Department, Bethlehem, PA 18018, United States

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ABSTRACT

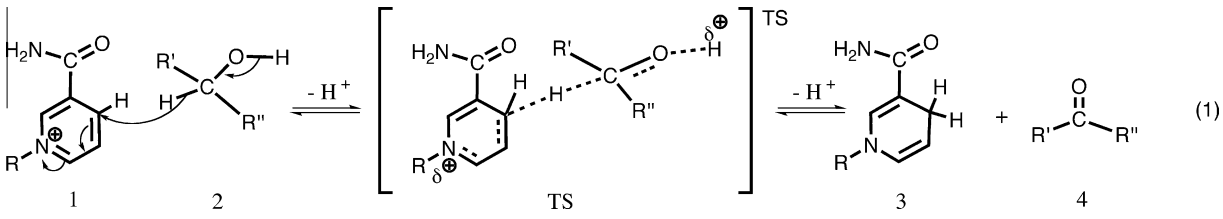
A study of the reactions of an NADH model, 1,4-di(trimethylsilyl)-1,4-dihydropyridine, **7**, with a series of α,β-unsaturated cyano and carbonyl compounds has produced the first direct evidence for an obligatory covalent adduct between a dihydropyridine and substrate in a reduction reaction. The reactions were monitored by NMR spectroscopy. In all reactions studied, the covalent adduct was the first new species detected and its decomposition to form products could be observed. Concentrations of adducts were sufficiently high at steady-state that their structures could be determined directly from NMR spectra of the reaction mixtures; adduct structures are those expected from an Ene reaction between **7** and the substrate. This first reaction step results in transfer of the C_4 hydrogen nucleus of **7** to the substrate and formation of a covalent bond between C_2 of the dihydropyridine ring and the substrate α-atom. Discovery of these Ene-adduct intermediates completes the spectrum of mechanisms observed in NADH model reactions to span those with free radical intermediates, no detectable intermediates and now covalent intermediates. The geometry of the transition state for formation of the Ene adduct is compared with those of theoretical transition state models and crystal structures of enzyme–substrate/inhibitor complexes to suggest a relative orientation for the dihydropyridine ring and the substrate in an initial cyclic transition state that is flexible enough to accommodate all observed mechanistic outcomes.

1. Introduction

Since Westheimer et al. [1] demonstrated that there is a direct hydrogen transfer from ethanol to NAD^+ in the alcohol dehydrogenase catalyzed reaction, much work has been focused on the chemical mechanism of this transfer [2]. Both radical and ionic mechanisms have been proposed but to date no direct conclusive evidence for any covalent intermediate species has been presented for any enzymatic or model nicotinamide hydride transfer reaction. Thus, the currently prevailing enzymatic mechanistic model is a one-step hydride transfer process, often referred to as a "hydride equivalent transfer", which is generally written as in Eq. (1) with a transition state (**TS**) that has C4 of the nicotinamide ring (**1**), the H nucleus being transferred, and the carbon and oxygen atoms of the substrate (**2**) collinear.

In 1971 Hamilton proposed an ionic mechanism in which the hydrogen transfer from substrate to NAD^+ is accomplished through an electrocyclic reaction [3]. Hamilton's mechanism (Eq. (2)) requires an intermediate, **5** or **6**, with a covalent bond between the oxygen atom of the substrate (**2**) and either C2 or C6 of NAD^+ (**1**).

The first step of Hamilton's mechanism is a nucleophilic addition of **2** to the iminium function of the NAD^+ (**1**). The second step



* Corresponding author. Fax: +1 610 625 7918.
E-mail address: rdlibby@chem.moravian.edu (R.D. Libby).
[1] Present address: Department of Biochemistry & Biophysics, 2011 Agricultural Life Sciences Building, Oregon State University, Corvallis, OR 97331-7305, United States.

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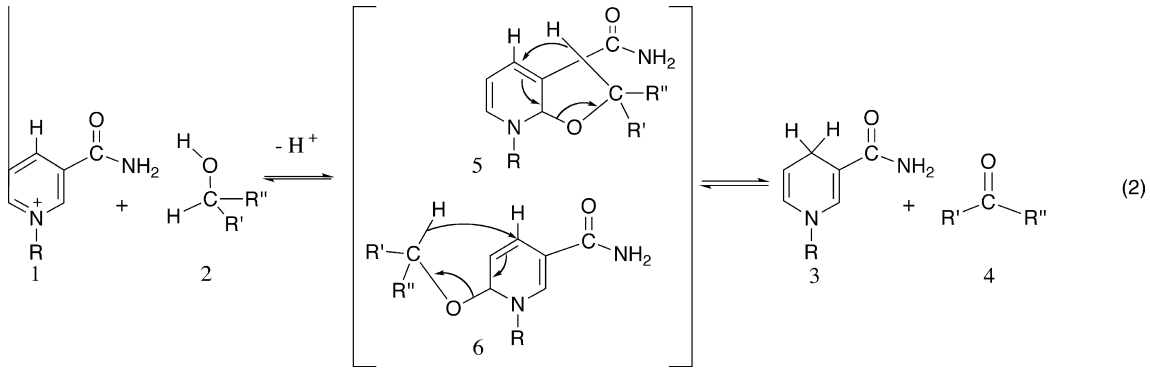
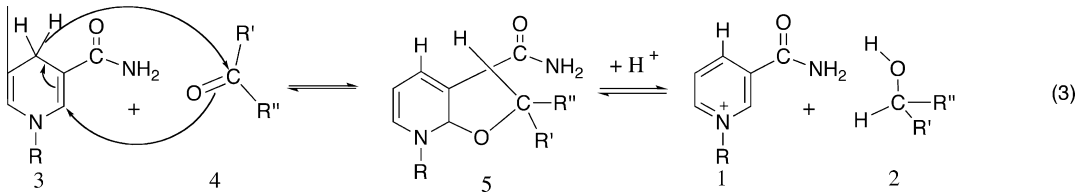
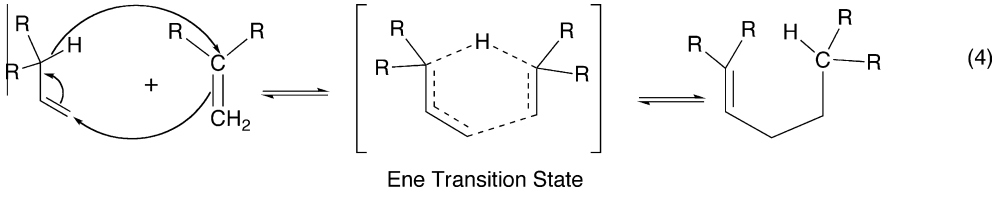




is a cyclic intramolecular hydrogen transfer known as a Retro-Ene reaction, a well-documented pathway for decomposition of allyl ethers and thioethers [4].

When the reaction of Eq. (2) is viewed in the reverse direction (Eq. (3)), the first step is seen to be an electrocyclic Ene reaction, a type of reaction first characterized by Alder et al. in 1943 [5]. We will refer to the mechanism proposed by Hamilton as the Ene mechanism for hydride transfer in nicotinamide reactions. Ene reactions involve transition states with aromatic character, which contributes to lowering their energies (Eq. (4)) [4].



Because of a lack of evidence for covalent intermediates in NADH reactions, the Ene mechanism for hydride transfer has not received much consideration in the literature. We now report the direct NMR observation of covalent intermediates in the reduction of a series of α,β-unsaturated cyano and carbonyl compounds (**8a–e**) by 1,4-di(trimethylsilyl)-1,4-dihydropyridine, **7** (Fig. 1). Although our model is quite different from NADH, our observations support the fundamental possibility of involvement of the Ene mechanism for hydride transfer in dihydropyridine reactions completing the spectrum of mechanisms observed in NADH model reactions from those with single electron transfers, to apparently concerted hydride transfers (no detectable intermediates) [2] and now to Ene reactions (covalent intermediate Ene-adducts characterized). The Ene mechanism also suggests a relative orientation for the dihydropyridine ring and the substrate in an initial cyclic transition state that is flexible enough to accommodate all of the observed mechanisms.

2. Experimental

2.1. General

Acrylonitrile (2-propenitrile, **8a**) (Sigma–Aldrich) was distilled while protected from moisture with $CaCl_2$ drying tubes and was stored under argon until used, tetrahydrofuran (Sigma–Aldrich) was purified by drying with LiAlH4 and distillation under a positive argon pressure with protection by $CaCl_2$ drying tubes [6], pyridine (Sigma–Aldrich) was distilled and stored under argon until used,

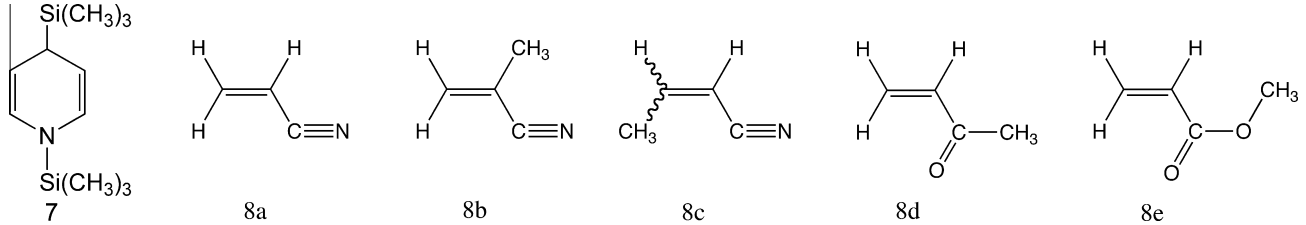


Fig. 1. 1,4-ditrimethylsilyl-1,4-dihydropyridine, **7** and α,β-unsaturated cyano and carbonyl electron accepting reagents: acrylonitrile, **8a**; methacrylonitrile, **8b**; (*E,Z*) crotonitrile, **8c**; methyl vinyl ketone, **8d**; methyl acrylate, **8e**.