The First Non-enzymatic Reduction of Acetaldehyde and Analogues by an NADH Model Compound

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An acid-stable NADH model compound, 9,10-dihydro-10-methylacridine ($AcrH_2$) efficiently reduces acetaldehyde and analogues in the presence of $HClO_4$ in acetonitrile to yield 10-methylacridinium ion and the corresponding alcohols; rates of the acid-catalysed reduction of various aliphatic aldehydes and ketones by $AcrH_2$, which have small primary kinetic isotope effects ($k_H/k_D < 2.0$), are well correlated with those of the acid-catalysed electron transfer from the excited state of $[Ru(bpy)_3]^{2+}$ (bpy = 2,2′-bipyridine) to the same substrates.

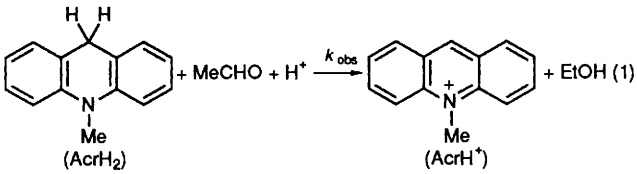
Alcohol dehydrogenases from various sources efficiently catalyse the reduction of acetaldehyde to ethanol, which is the most important substrate with regard to the physiological significance of ethanol metabolism as well as ethanol fermentation, by dihydronicotinamide adenine dinucleotide (NADH).[1—5] However, none has so far succeeded the efficient non-enzymatic reduction of acetaldehyde or analogues by NADH model compounds, although the reduction of activated aldehydes and ketones has been studied extensively.[6] Here we report the first successful reduction of acetaldehyde and analogues by an NADH model compound in the presence of perchloric acid in an aprotic solvent (acetonitrile), and this provides a chemical basis for the understanding of the origin of the catalytic activity of the NADH-dependent enzymatic reduction.

The active centres in alcohol dehydrogenases are believed to be acids (Zn^{2+} ion and an amino acid residue act as a Lewis acid and Brönsted acid, respectively) in a hydrophobic environment surrounded by proteins.[1—5] However, NADH or most NADH model compounds decompose readily in the presence of acid.[6] Here we use 9,10-dihydro-10-methylacridine ($AcrH_2$) as an NADH model compound, which is

Table 1. Acid-catalysed reduction of substrates (0.30 mol dm^{-3}) by $AcrH_2$ and $AcrD_2$ (4.0 × 10^{-2} mol dm^{-3}) in the presence of $HClO_4$ (0.10 mol dm^{-3}) in acetonitrile at 333 K. Reductant $AcrH_2$.

No.	Substrate	k_H/k_D[a]	Time /h	Product yield (%)
1	Acetaldehyde			Ethanol
	CH_3CHO	1.4	1	CH_3CH_2OH(100)
	CH_3CHO[c]		3	CH_3CHDOH(100)
2	Propionaldehyde			Propanol
	C_2H_5CHO	1.6	1	$C_2H_5CH_2OH$(100)
3	Butyraldehyde			Butanol
	$CH_3(CH_2)_2CHO$	1.9	1	$CH_3(CH_2)_2CH_2OH$(100)
4	Isovaleraldehyde			3-Methylbutanol
	$(CH_3)_2CHCH_2CHO$	1.1	2.5	$(CH_3)_2CHCH_2CH_2OH$ (100)
5	Isobutyraldehyde			2-Methylpropanol
	$(CH_3)_2CHCHO$	1.0	5	$(CH_3)_2CHCH_2OH$(100)
6	Pivalaldehyde			Neopentyl alcohol
	$(CH_3)_3CCHO$	1.0	19	$(CH_3)_3CCH_2OH$(69)
7	Acetone			Propan-2-ol
	$(CH_3)_2CO$	1.7	23	$(CH_3)_2CHOH$(25)
8	Fluoroacetone			Fluoropropan-2-ol[b]
	$(CFH_2)CH_3CO$	3.0	70	$(CFH_2)CH_3CHOH$(48)
9	Cyclohexanone			Cyclohexanol
	$C_6H_{10}(=O)$	2.1	5	$C_6H_{11}OH$(100)
10	Pyruvic acid			Lactic acid[b]
	CH_3COCO_2H	1.3	24	$CH_3CH(OH)CO_2H$(29)
	CH_3COCO_2H[c]		20	$CH_3CD(OH)CO_2H$(18)

[a] Determined from the ratio of the rate constants of $AcrH_2$ to $AcrD_2$ in the presence of $HClO_4$ (2.7 × 10^{-2} mol dm^{-3}). The experimental errors are ±10%; [b] Racemic mixtures. [c] Reductant $AcrD_2$.



stable in the presence of perchloric acid ($HClO_4$) and in acetonitrile (MeCN),[7] for the reduction of acetaldehyde and analogues.

An acid-stable NADH model compound ($AcrH_2$) shows no reactivity towards acetaldehyde in MeCN at 333 K in the dark. When $HClO_4$ (70%)† is added to the $AcrH_2$–acetaldehyde system, however, acetaldehyde is readily reduced by $AcrH_2$ to yield 10-methylacridinium ion ($AcrH^+$) and ethanol, quantitatively [equation (1)]. When $AcrH_2$ is replaced by the 9,9′-dideuteriated compound, 9,9′-[$^2H_2$]-9,10-dihydro-10-methylacridine ($AcrD_2$), deuterium is introduced to ethanol as shown in Table 1. The product yields were determined by ^1H NMR spectroscopic comparison with authentic materials independently obtained. Other aliphatic aldehydes and ketones can also be reduced by $AcrH_2$ in the presence of $HClO_4$ in MeCN at 333 K (Table 1).

In the case of enzymatic reactions, it is very difficult to determine the rates of catalytic step and the corresponding kinetic isotope effects directly, since the observed rates consist of complex functions of the binding of substrates and release of products as well as the catalytic interconversion of ternary complexes.[2,8] Our model system provides for the first time the

† For safety reasons $HClO_4$ containing water (30%) was used in this study.

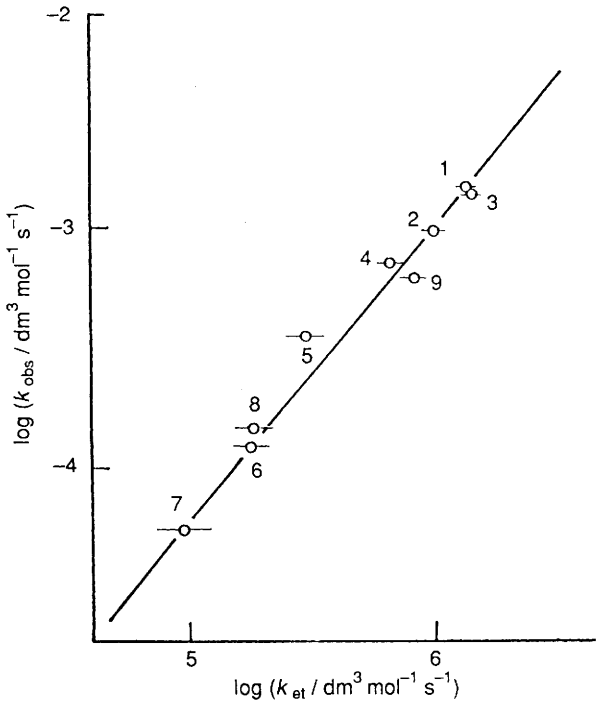


Figure 1. Plot of log k_{obs} for the acid-catalysed reduction of substrates by $AcrH_2$ in the presence of $HClO_4$ (2.7 × 10^{-2} mol dm^{-3}) in MeCN at 333 K *vs.* log k_{et} for the acid-catalysed electron-transfer from $[Ru(bpy)_3]^{2+*}$ to the same series of substrates in the presence of $HClO_4$ (2.0 mol dm^{-3}) at 298 K. Numbers refer to the substrates in Table 1.

opportunity to determine the k_H/k_D values for the acid-catalysed reduction of aliphatic aldehydes and ketones by an NADH model compound. Rates of the acid-catalysed reduction of various aliphatic aldehydes and ketones by $AcrH_2$ were followed by the increase in absorbance due to $AcrH^+$ (λ_{max} 358 nm), which obeyed second-order kinetics showing first-order dependence on the concentration of each reactant. The primary kinetic isotope effects were determined from the ratios of the observed second-order rate constants of $AcrH_2$ to $AcrD_2$ in the presence of $HClO_4$ (2.7 × 10^{-2} mol dm^{-3}) in MeCN at 333 K. The k_H/k_D values are uniformly small (k_H/k_D < 2 except for fluoroacetone) as shown in Table 1.

The electron-acceptor ability of carbonyl compounds is expected to increase in the presence of $HClO_4$ in MeCN.[9] Thus, in the presence of $HClO_4$ the luminescence of $[Ru(bpy)_3]^{2+*}$ (* denotes the excited state) can be quenched efficiently by electron transfer to acetaldehyde and other aliphatic aldehydes and ketones in MeCN [equation (2)], although no quenching has been observed in the absence of $HClO_4$. The k_{et} values of aliphatic aldehydes and ketones in the presence of $HClO_4$ (2.0 mol dm^{-3}) in MeCN were determined from the Stern–Volmer plots and the lifetime of $[Ru(bpy)_3]^{2+*}$ (τ 850 ns), which is the same as that in the absence of $HClO_4$.[9] When the k_{et} values are compared with the observed second-order rate constants (k_{obs}) of the acid-catalysed reduction of aliphatic aldehyde and ketones by $AcrH_2$ in the presence of a fixed concentration of $HClO_4$ (2.7 × 10^{-2} mol dm^{-3}), there exists a linear correlation between k_{et} and k_{obs} as shown in Figure 1 (pyruvic acid, which itself is acid, is not included in the plot). The linear correlation in Figure 1 together with the small k_H/k_D values in Table 1 demonstrates the importance of the acid-catalysed electron-

transfer step in decreasing the activation barrier for the reduction by an NADH model compound.

$$[Ru(bpy)_3]^{2+*} + MeCHO + H^+ \xrightarrow{k_{et}} [Ru(bpy)_3]^{3+} + EtOH \quad (2)$$

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