Oxidation of α-Hydroxy Acids by an Oxidation-active Flavin Mimic bearing a Bipyridin-6-ylmethyl Moiety in the Presence of Zn^{2+} and a Base in *tert*-Butyl Alcohol

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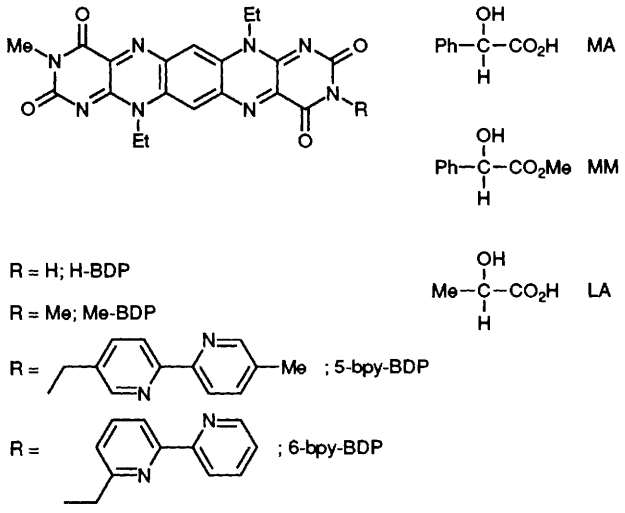
A benzo-dipteridine (BDP) having a bipyridin-6-ylmethyl moiety oxidizes α-hydroxy acids to give α-keto acids in the presence of Zn^{2+} and Et_3N in Bu^tOH, whereas a BDP bearing a bipyridin-5-ylmethyl moiety is unable to oxidize them under the same conditions; this is the first example of a D-lactate dehydrogenase model.

We have shown that oxidation-active flavin mimics are useful for the investigation of flavin-mediated oxidations in model systems.[1] For construction of more sophisticated systems, however, it would be necessary to assemble functional groups covalently and/or noncovalently[2] at the reaction site of the oxidation-active flavin mimics. D-Lactate dehydrogenases from bacterial and mammalian sources, which oxidize D-lactate to afford pyruvate, are the only flavoproteins to contain Zn^{2+}, although the roles of Zn^{2+} are not clearly understood.[3] We report that a benzo-dipteridine (BDP) bearing a bipyridin-6-ylmethyl moiety (6-bpy-BDP) oxidizes α-hydroxy acids to give α-keto acids in the presence of Zn^{2+} † and an amine base in Bu^tOH.

The 5- and 6-bpy-BDPs were synthesized from H-BDP‡ and 5-bromomethyl-5′-methyl-2,2′-bipyridine or 6-bromomethyl-2,2′-bipyridine in the presence of K_2CO_3 in dimethyl-

† The oxidation also occurred with Ni^{2+} or Co^{2+}.

‡ H-BDP was prepared as for Me-BDP except for the stepwise condensation of *N,N′*-diethyl-*p*-phenylenediamine with 6-chloro-3-methyluracil and 6-chlorouracil.[1c]



Scheme 1

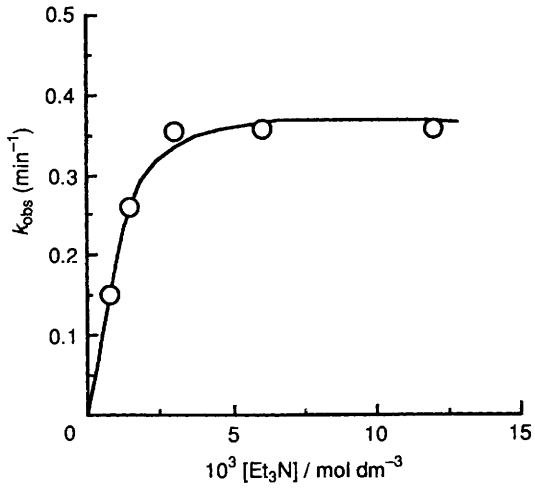
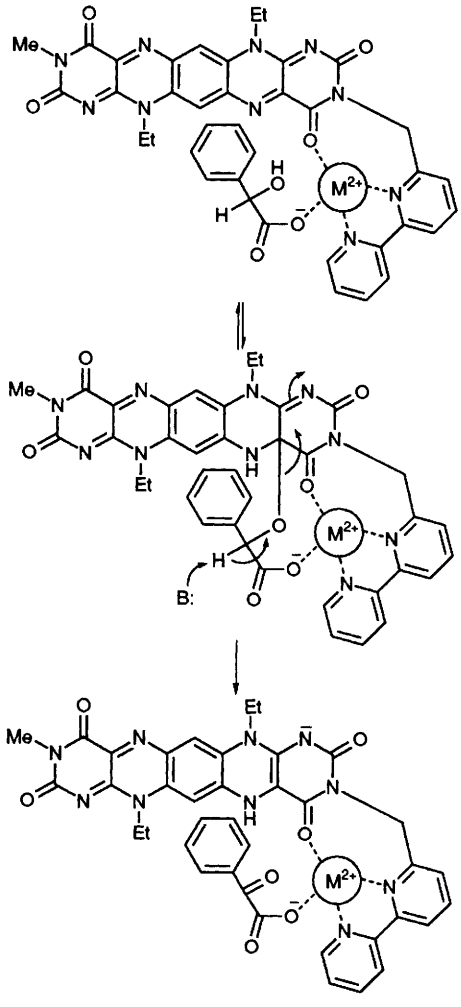


Fig. 1 k_{obs} *vs.* [Et_3N]; [6-bpy-BDP] = 1.0×10^{-5} mol dm^{-3}, [Zn^{2+}] = 1.0×10^{-4} mol dm^{-3}, [MA] = 5.0×10^{-4} mol dm^{-3}, Bu^tOH, N_2, 25 °C

formamide (DMF).§ The electronic absorption spectrum of 6-bpy-BDP was found to shift slightly by the addition of Zn^{2+} in Bu^tOH, which allowed us to calculate the binding constant for the 1:1 complex to be 2.38×10^5 mol^{-1} dm^3. Such a spectral shift was not observed for 5-bpy-BDP and Me-BDP under the same conditions. This suggests that the Zn^{2+} bound at the bipyridine moiety of 6-bpy-BDP is able to interact with BDP skeleton to cause electronic perturbation. In fact, inspection of Corey–Pauling–Koltun (CPK) molecular models reveals that the interaction of the metal ion bound at the bipyridine with the 2- or 4-carbonyl oxygen is geometrically possible only for 6-bpy-BDP.

Spectroscopic examination of the reaction of 6-bpy-BDP with mandelic acid (MA) in Bu^tOH showed that the oxidation (formation of reduced 6-bpy-BDP) occurs only when Zn^{2+} and Et_3N are present, and the reduced 6-bpy-BDP complexed with Zn^{2+} is stable towards O_2. After removal of the bound Zn^{2+} by acidification, the oxidized 6-bpy-BDP was regenerated by O_2 bubbling. Formation of benzoylformic acid (oxidation product of MA) was confirmed by HPLC.¶ On the other hand, MA was found not to be oxidized by 5-bpy-BDP or Me-BDP under the same conditions. This indicates that the positioning of the Zn^{2+} is crucial.

Pseudo-first-order rate constants were determined by following the absorption increase of reduced 6-bpy-BDP at 640 nm under anaerobic conditions. The relative reactivities of MA, methyl mandelate (MM) and lactic acid (LA) were found to be 20:8:1, indicating that the substrate having a carboxylate anion (MA *vs.* MM) and an acidic α-hydrogen (MA *vs.* LA) shows larger reactivity. More detailed kinetic studies on the oxidation of MA were conducted to get an insight into the oxidation mechanism. Plots of k_{obs} *vs.* [Zn^{2+}] or [MA] showed that k_{obs} reaches a maximum and decreases with the increase of [Zn^{2+}] or [MA] (data not shown), suggesting that the reaction proceeds *via* a complex of 6-bpy-BDP, Zn^{2+} and MA in which the pK_a values of the hydroxy and α-C-H hydrogens of MA are lowered.[4] The rate decrease at higher [Zn^{2+}] or [MA] may be explained by the decrease of the concentration of the reactive ternary complex due to the complex formation of Zn^{2+} and MA. Plots of k_{obs} *vs.* [Et_3N] showed a saturation curve (Fig. 1), suggesting that the base also functions through complex formation.



Scheme 2

The deuterium kinetic isotope effect (k_H/k_D) for the removal of hydrogen from the α-carbon of MA was found to be 7.2 ± 0.6,‖ indicating that α-C–H bond cleavage of MA is involved in the rate-determining step, and the hydrogen is

§ 5-bpy-BDP (60%), 6-bpy-BDP (63%), m.p. > 300 °C (from DMF). Satisfactory ^{1}H NMR data and elemental analyses were obtained.

¶ Column; Wakosil 5C18, solvent, H_2O–MeCN = 50:1 (*v*/*v*).

‖ PhCD(OH)CO_2H was prepared by the reduction of PhCOCO_2H with $NaBD_4$ (P. L. Polavarapu, L. P. Fontana and H. E. Smith, *J. Am. Chem. Soc.*, 1986, **108**, 911). The D-content of PhCD(OH)-CO_2H was 82.0%. The reaction conditions are the same as in Table 1.

Table 1 Rate constants under anaerobic and aerobic conditions[a]

Conditions	$k_{obs}/10^{-1}\,min^{-1}$
Anaerobic[b]	3.05 ± 0.15
Aerobic[c]	2.91 ± 0.09

[a] [6-bpy-BDP] = 1.0×10^{-5} mol dm^{-3}, [MA] = 5.00×10^{-4} mol dm^{-3}, [$Zn(NO_3)_2 \cdot 6H_2O$] = 1.00×10^{-4} mol dm^{-3}, [Et_3N] = 1.50×10^{-3} mol dm^{-3}. [b] N_2 bubbling for 15 min. [c] O_2 bubbling for 15 min.

considered to be half-transferred to the base.[5] Furthermore, it was found that the α-C–H hydrogen of MA does not exchange with solvent protons.** This suggests that the carbanion is not involved during the reaction. Furthermore the present oxidation was found to proceed at the same rate under aerobic and anaerobic conditions as shown in Table 1. This result suggests that the radical mechanism may be excluded.[6] It was also confirmed that Me-DBP readily oxidized $PhCH_2O^-$ in $PhCH_2OH$ to afford reduced Me-BDP and PhCHO.

Taking account of the fact that BDP is susceptible to a nucleophilic attack at the C(4a)-position, the present oxidation is considered to proceed *via* C(4a)-adduct formation followed by base-catalysed 1,2-elimination to give benzoylformic acid and the reduced 6-bpy-BDP as shown in Scheme 2.

** H-D exchange of MA was performed as follows: a solution of CD_3OD–MeCN (50:50 *v/v*) (1 ml) containing MA (3.3×10^{-2} mol dm^{-3}), $ZnCl_2$ (3.3×10^{-2} mol dm^{-3}), 1,8-diazabicyclo-[5.4.0]-undec-7-ene (DBU) (0.3 mol dm^{-3}) and the bipyridine (3.3×10^{-2} mol dm^{-3}) was stirred for 50 h at 25 °C.

This is the first example of a D-lactate dehydrogenase model, although the oxidation mechanism is different.[3]

The roles of Zn^{2+} can be summarized as follows; (*i*) Zn^{2+} bound with the bipyridine moiety improves the oxidation-activity of BDP owing to the coordination to the carbonyl oxygen; (*ii*) the bound Zn^{2+} acts as a binding site for an anionic substrate to form the ternary complex; and (*iii*) it activates the substrate by lowering the pK_a values of the hydroxy and α-C–H hydrogens. Similar roles of Zn^{2+} are conceivable in native D-lactate dehydrogenases, in which Zn^{2+} is known to be located in the vicinity of FAD.

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