A Non-enzymatic Model of Δ^4-3-Ketosteroid Reductases[1]

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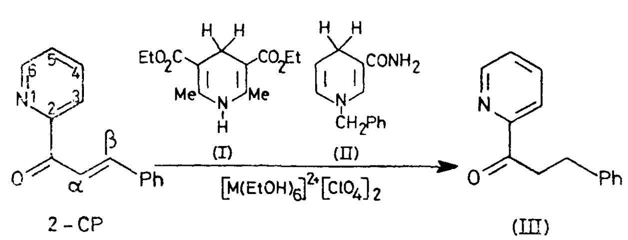
Summary Metal-ion electrophilic catalysis of the reduction of the double bond of an $\alpha\beta$-unsaturated ketone by a pyridine nucleotide coenzyme model has been demonstrated.

CONSIDERABLE interest has recently centred around the mechanism of action of pyridine nucleotide-linked Δ^4-3-ketosteroid reductases.[2,3] Enzymatic studies with mammalian 5α- and 5β-(Δ^4-3-keto) reductases,[3] using a steroidal substrate containing ^{18}O label at the carbonyl group, have shown that, with both enzymes, the label was retained in the reduced product. These results indicate that a covalent imine intermediate[2] is unlikely during the reduction process and that the $\alpha\beta$-unsaturated carbonyl group is activated, towards a hydride transfer from the (NADH) coenzyme, by co-ordination of the carbonyl oxygen with an electrophilic (catalytic) centre in the enzyme. The latter implications for the mechanism of the reductases prompt us to report the metal ion-catalysed reduction of an $\alpha\beta$-unsaturated ketone, by 1,4-dihydropyridines (NADH models).



Since carbonyl groups by themselves are weak ligands for co-ordination with metal cations (*e.g.* Mg^{2+} or Zn^{2+}), it was envisaged that a system containing a suitably located second binding-site should prove to be a more adequate substrate for the present study. A simple model which meets these requirements is the $\alpha\beta$-unsaturated ketone, 2-cinnamoylpyridine (2-CP), which contains a basic nitrogen function and a carbonyl group in a relative proximity that is ideally suited for bidentate chelation with a metal ion. In accordance with these expectations, 2-CP showed complexation with Mg^{2+} and Zn^{2+} {employed as $[M(EtOH)_6]^{2+}[ClO_4^-]_2$}[4] in acetonitrile, the solvent of choice for the reduction studies. Comparison of the ^{13}C n.m.r. spectra of mixtures of the magnesium ethanolate complex and the isomeric 2-, 3-, and 4-cinnamoylpyridines (2-CP, 3-CP, and 4-CP, respectively) attested to the binding of the metal to both the oxygen and the nitrogen atoms in the case of 2-CP. Particularly revealing were the large chemical shift displacements of the C_α-, C_β-, and carbonyl-carbons: $\Delta\delta$ [δ (complexed) $-$ δ (free)] $-2{\cdot}9$, $+9{\cdot}5$, and $+2{\cdot}6$ p.p.m., respectively, of 2-CP. Similar results were obtained for mixtures of 2-CP, 3-CP, 4-CP and $[Zn(EtOH)_6]^{2+}[ClO_4^-]_2$. Furthermore, a crystalline zinc complex of 2-CP, $[(2\text{-}CP)_2Zn][ClO_4]_2$, could be isolated, in which two Zn–O and two Zn–N stretching vibrations were visible in the far-i.r. spectrum.[5]

The $\alpha\beta$-unsaturated ketone 2-CP was inert towards typical NADH models such as the Hantzsch ester (I) or 1-benzyl-1,4-dihydronicotinamide (II). However, in the presence of 1·0 equiv. of $[M(EtOH)_6]^{2+}[ClO_4^-]_2$, in acetonitrile, 2-CP was quantitatively reduced to (III), by the aforementioned dihydropyridines, at room temperature, in a few minutes. To the best of our knowledge this represents the first example of metal ion catalysis of the reduction of a C=C bond of an $\alpha\beta$-unsaturated ketone by NADH-models. Reduction with the dideuterio-Hantzsch ester $[4,4\text{-}^2H_2]$-(I) yielded (III) which contained one deuterium atom at the β-carbon (n.m.r.). The rates of reduction of the cinnamoylpyridines (0·4M) by 1-benzyl-1,4-dihydronicotinamide (II) (0·4M) in the presence of 0·4M $[Mg(EtOH)_6]^{2+}[ClO^-]_2$, were followed by ^1H n.m.r. spectroscopy. The bimolecular rate constants in CD_3CN, at 23 °C, are as follows: k_2(2-CP) 10·3; k_2(3-CP) $6{\cdot}5 \times 10^{-3}$; k_2(4-CP) $3{\cdot}2 \times 10^{-2}$ l mol^{-1} min^{-1}. The latter results, coupled with the complexation studies, emphasize the importance of electrophilic catalysis in the NADH-mediated reduction of $\alpha\beta$-unsaturated ketones.

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