# HYDROGEN TRANSFER IN NICOTINAMIDE ADENINE DINUCLEOTIDE MODELS

A thesis presented by

JEREMY DAVID SAMMES

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Chemistry Department
Imperial College
London SW7

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## Abstract

This thesis is concerned with the synthesis of model systems which simulate the alcohol-dehydrogenase catalysed nicotinamide adenine dinucleotide-substrate oxido-reduction. Of the factors which may be important in the enzymic process, the proximity effect of coenzyme and substrate in the ternary complex has been neglected in studies of model compounds to date. The synthesis is described of several molecular systems which incorporate a potential substrate grouping sterically adjacent to the pyridine 4-position of the coenzyme analogue. The properties of one alcohol-$NAD^+$ counterpart, N-methyl-3-(2'-hydroxymethylbenzoyl)-pyridinium iodide, are considered in detail. This molecule was completely stable under ordinary conditions and showed no tendency to undergo an intramolecular redox reaction. N-2,6-dichlorobenzyl-1,4-dihydronicotinyl-2'-benzaldehyde, a synthetic aldehyde-NADH analogue was also stable at room temperature in the absence of air and light. This compound did not transfer a hydride ion internally from the dihydropyridine to the aldehyde under the conditions studied, although it seems likely that more activated molecules would.

A hydrogen shift was observed, on photolysis of the latter molecule, and the corresponding alcohol pyridinium species N-2,6-dichlorobenzyl-3-(2'-hydroxymethyl-benzoyl)-pyridinium salt was produced. The reaction was shown to be an intramolecular process, to involve transfer of a hydrogen radical or anion and to follow approximately first order kinetics. Studies using deuterium

suggested that the hydrogen atom migrated to the aldehyde carbon atom. The implications of these results vis-à-vis the enzymic reaction are discussed.

## Acknowledgements

I would like to express my grateful thanks to Dr D.A. Widdowson for his continued help and encouragement during the course of this work, and to my fellow colleagues, past and present, for their many useful discussions.

The assistance of Mr A.P. Coleman and all the technical staff of the Whiffen and New Hofmann Laboratories is also acknowledged with thanks. Micro-analyses were kindly performed by Mr K.I. Jones and his staff, and proton magnetic resonance spectra were run by Mrs I. Boston and Mr P.N. Jenkins. Mr J.N. Bilton and Mrs J. Lee are also thanked for numerous mass spectra.

Finally, I sincerely thank the Salters Institute of Industrial Chemistry for providing a generous maintenance grant, and for taking such an interest in this project.

Awake my St. John! Leave all meaner things
To low ambition and the pride of kings.
Let us, since life can little more supply
Than just to look about us and to die,
Epatiate free o'er all this scene of man,
A mighty maze! but not without a plan.

Alexander Pope

To Sheila

# INDEX

# REVIEW

# INTRODUCTION

The pyridine nucleotides are of fundamental importance as cofactors in biological oxidoreduction reactions. The ubiquitous nature of these coenzymes which occur with a variety of oxidoreductases and substrates in nature has prompted much research into the structure and mechanism of the enzymic redox reactions. Liver and yeast alcohol dehydrogenases have been studied in most detail, owing to the relatively uncomplicated intermediates involved, and this review will be related mainly to these enzymes.

Once the structure of the pyridine nucleotides was established, it became possible to gain some insight into the nature of the "active site" of the protein using the standard techniques of enzyme chemistry. This work is by no means complete, but a considerable amount is now known about the factors which may influence the nature of this redox process.

The first half of this review is concerned with the evidence that has been assembled about the nature of the enzyme-coupled coenzyme-substrate redox reaction, and in particular whether ionic or radical species are involved. It is necessary first, however, to understand something of the electronic, steric and chemical influences present at the active site, and the treatment here will be to present the evidence about these first. The manner in which the environment of the coenzyme changes, first as it binds to the enzyme, and subsequently on addition of substrate is then considered. Finally the stereochemistry of the redox process will be briefly reviewed and the evidence for hydride transfer presented in detail.

The second section deals with model redox systems involving simple analogues of the coenzyme and substrate which have been

studied in an attempt to gather more information about the in vivo process. The theoretical requirements for useful model coenzymes and substrates are first discussed, and the remaining 3 sections deal with the ionic, homolytic and photolytic reactions respectively of these model systems. An attempt has been made continually to refer the conclusions derived from these model systems back to the enzymic reaction.

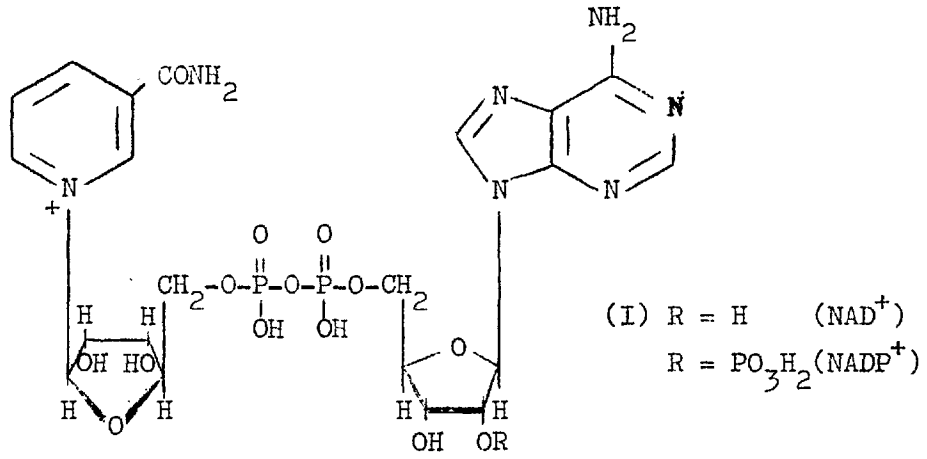
## 1. History and Nomenclature of Nicotinamide Adenine Dinucleotide

In 1935, Warburg, Christian and Griese[1] were able to isolate and elucidate the structure of the "hydrogen transporting coenzyme" which occurred in red blood cells. They established that this coenzyme consisted of 3 phosphate molecules bound in an unspecified way to a dinucleotide of nicotinamide and adenine. The following year it was shown independently by Warburg[2] and von Euler[3] that the dialysable cofactor "cozymase" isolable from fermented yeast[4] contained the same dinucleotide, but coupled to only 2 phosphate groups. The (German) names "triphospho-pyridinnucleotid" and "diphospho-pyridinnucleotid" were used[2] respectively to distinguish these compounds from other pyridine nucleotides already known. Subsequent workers have referred to them by the inaccurate transliteration eg. "diphosphopyridine-nucleotide" (DPN), which whilst still in current usage fails to describe the structure (Fig 1) of the diphosphate molecule. The terms DPN (and TPN) are gradually being superseded by "nicotinamide adenine dinucleotide (phosphate)", as recommended by the Commission of Enzymes of the International Union of Biochemistry.[5,6] Various abbreviated forms of these names have appeared, but in this thesis the reduced and oxidised forms of the diphosphate coenzyme will be referred to as "NADH" and "$NAD^+$" respectively in line with the half equation:

$$NAD^+ + H^+ + 2e \rightleftharpoons NADH.$$

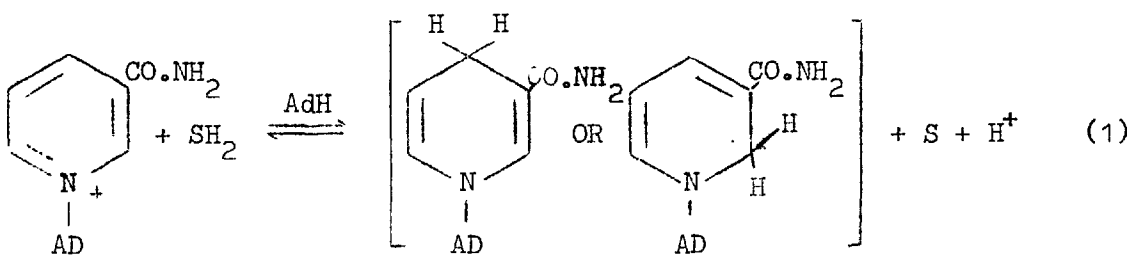
Where the state of ionisation of the coenzyme-substrate system is unclear, the terms "NAD" and "reduced NAD" have also been used[5,7]. Further considerations will be restricted to $NAD^+$ and NADH, although much of the following also applies to the triphosphate coenzyme ($NADP^+$ and NADPH).

## 2. Structure of Nicotinamide Adenine Dinucleotide




(Fig 1)

The structure of $NAD^+$ (I) has been thoroughly established[8-11] by degradation, detailed studies of various linkages, and synthesis[12]. More recently, the conformation of the molecule bound to lactate dehydrogenase has been demonstrated[13]. Alcohol-dehydrogenase (AdH) catalysed reduction of the molecule with ethanol converts the pyridinium moiety reversibly to a dihydropyridine NADH, whose exact structure was resolved only fairly recently (vide infra).



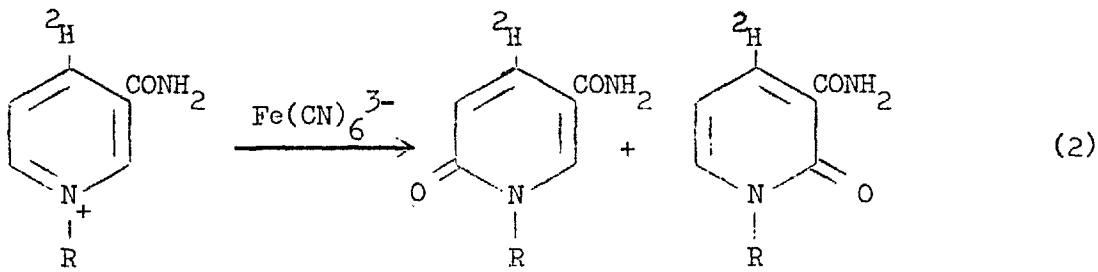

(II) (III)

(AD = Adenine Dinucleotide)

(S = Oxidised substrate = $CH_3CHO$)

The reduced coenzyme was originally believed to be the 2-isomer (III) on the basis of its absorption spectrum (340 nm)[2], and from studies of model compounds[2,14]. Thus, the ability of a model dihydropyridine to react with maleic anhydride was taken as evidence for the 1,2 structure[15]. In fact, a Diels-Alder adduct was not obtained, but the pyridinium species[16].

In the early 1950's Westheimer's group published a method for deuterium labelling the coenzyme specifically at the site of reduction[17,18] by an <u>in vivo</u> reduction of $NAD^+$ by $[1\text{-}^2H]$ ethanol. By subsequent oxidation of the 2 and 6 positions to carbonyl groups by ferricyanide with no deuterium loss, Pullman and his collaborators demonstrated that this deuterium resided in the 4-position[19]. Model compounds (with a similar chromophore) were similarly demonstrated to be 1,4-dihydropyridines (2). Reduction of $NAD^+$ itself (and many other pyridinium compounds) by sodium dithionite (hydrosulphite) in aqueous medium at pH 7-9 has long been known[2] to give exclusively NADH which is enzymically active (and dihydropyridines with similar ultra-violet spectra). Thus the structure (II) for NADH was proposed. A series of papers have since firmly established this as correct by synthesis[20], ultra-violet[21,22], infra-red[23] and proton magnetic resonance[24] studies, and further experiments with model compounds[25].




(2)

<u>No deuterium loss</u>

3. The Binding of the Coenzyme to the Enzyme and Substrate

In the absence of catalytic amounts of alcohol dehydrogenase, the redox reaction (1) between coenzyme and substrate is virtually absent. The enzyme may thus be considered to activate one or both reacting species in a number of ways:

(A) $AdH + NADH \rightleftharpoons$ AdH-NADH complex (3)

followed by either (i) $AdH\text{-}NADH + S \rightleftharpoons$ AdH-NADH-S complex (4)

$AdH\text{-}NADH\text{-}S + H^+ \rightleftharpoons AdH + NAD^+ + SH_2$ (5)

or (ii) $AdH\text{-}NADH + H^+ \rightleftharpoons$ reduced $AdH + NAD^+$ (6)

reduced $AdH + S \rightleftharpoons AdH + SH_2$ (7)

(B) $AdH + S \rightleftharpoons$ AdH-5 complex

followed by a reaction scheme analagous to (4) - (7)

There is no evidence in the literature to suggest the presence of discrete enzyme-substrate complexes (reaction sequence B). Also, if such complexes existed only fleetingly, it might nevertheless be possible to observe redox reactions between different substrates. Such reactions have only ever been observed in the presence of coenzymes[26-28]. The failure to demonstrate an independent enzyme-substrate reaction is evidence against this mechanism. (However, see ref 57, p50).

There is by constrast, abundant evidence for the existence of enzyme-coenzyme complexes[29-32] (3) which are stable reversibly formed systems. Redox reactions between coenzymes in the absence of substrate (6) have not been observed in the case of alcohol dehydrogenase[33,34], although certain transhydrogenases can catalyse such transformations[45-36]. These factors make the overall sequence (3)-(6)-(7) appear unlikely since equation (6) is by necessity reversible, and a "reduced AdH"

species has not been reported. Finally, detailed kinetic studies have shown that a separate 2-stage bimolecular process is not involved (for a review see refs. 37, 38).

The alternative mechanism (3)-(4)-(5) involves a ternary complex, with the implication that substrate and coenzyme are brought into close proximity and together are activated by the enzyme. Westheimer's classic work[17-18] using deuterium-labelled substrate established beyond doubt that hydrogen was transferred quantitatively between this species and the coenzyme. No labelled hydrogen was found in the enzyme or solvents. Earlier ideas about the redox process involving exchangeable protons and electron transfer[39-40] were thus refuted. This work lent support to the idea that if a ternary complex was involved then coenzyme and substrate were sterically adjacent, but it did not prove it. Nevertheless, any proposed "reduced enzyme" or other hydrogen carrier group in the system would clearly not be exchangeable, and the obvious candidates were sulphydryl and histidine groups[41]. Recently, Schellenberg's group[42-43] and others[44] have provided evidence for the transfer of tritium from $[1\text{-}^{3}H]$ ethanol to a tryptophan residue present at the active site[45], and it seems likely that this grouping lies close to the nicotinamide ring 2 position of the coenzyme[46], and may be involved in the redox process in several enzyme systems.

The idea of a ternary complex, whereby the coenzyme binds first and is followed by the substrate, was first propounded by Theorell and Chance in 1951[47]. Since then inhibitor-competition studies on substrates and substrate-analogues with the enzyme-coenzyme system have lent support to their "compulsory binding order" mechanism. Thus hydroxylamine[48] and certain mercaptans[49] compete with ethanol for a

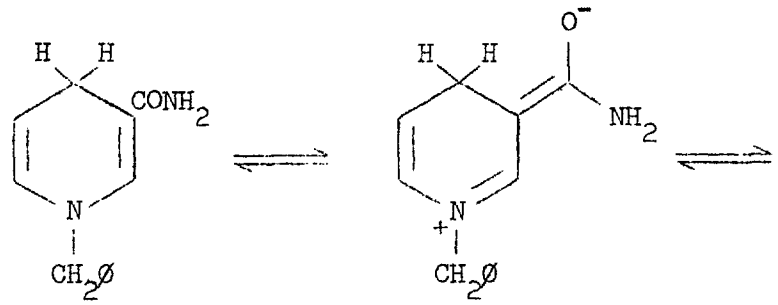
binding site on the $NAD^+$-liver AdH complex, and amides similarly compete with acetaldehyde for an NADH-enzyme complex[50-52]. The discovery that NADH possessed a fluorescent maximum at ~460nm which was profoundly changed on formation of binary and ternary complexes has since allowed the detailed analysis of the mechanism of formation and dissociation of these complexes, and established them beyond reasonable doubt (for a review see ref 53).

These considerations suggest that at a molecular level, the enzyme first binds the coenzyme, and this system is able in an unspecified way to bind the substrate. The ternary complex then reversibly undergoes the observed transfer of hydrogen, the substrate and coenzyme respectively finally diffusing away again.

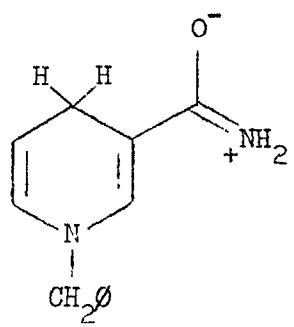
## 4. Modifications to the Coenzyme on binding to Alcohol Dehydrogenase

Several effects have been observed when the coenzyme-enzyme complex is formed. The environment of the coenzyme is modified electronically, chemically and sterically, and these effects are considered here.

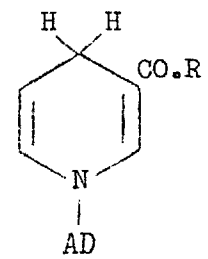
The absorption maxima of 1,4-dihydropyridines (~350nm) although untypical of an "isolated" enamine chromophore have been theoretically substantiated by molecular orbital calculations[54] and clearly involve the 5,6 double bond. Cilento *et al*[21] obtained the moderate value of 3.89D for the dipole moment of N-benzyl-1,4-dihydronicotinamide (IV) which they attributed to contribution by resonance structure (IVa). However, the position of the absorption maximum was only moderately solvent dependent[21], and they concluded that the cross-conjugated structure (IVb) was a more important resonance form. 3-Acetyl-1,4-dihydropyridines (e.g. VI) cannot show this latter contribution, and indeed, absorb at longer wavelengths (~ 365nm).



(IV) (IVa)



(IVb)



(V) R = NHOH
(VI) R = $CH_3$
(VII) R = Ø

Attempts to discover the importance of the electron donating amino group are complicated by steric and electronic considerations. Thus NADH (II) and its analogue (V) have almost the same chromophore (340nm) and redox potential (320mV), yet on complexing to alcohol dehydrogenase, react with substrate at very different rates and the benzoyl derivative (VII) does not act at all as a coenzyme for yeast AdH.

The observation that the absorption maximum of NADH was shifted from 340nm to 325nm on binding to horse liver AdH[29] (Table 1), indicated a pronounced electronic modification to the coenzyme chromophore in opposition to the resonance form analagous to (IVa). This shift has been allied to the increased ability of bound NADH to donate hydrogen from the 4-position. Thus Sund considered that "the specific binding of the coenzyme to the enzyme not only creates favourable steric conditions for the enzyme [to add substrate] but also leads to a change in the reactivity of the coenzyme"[57].

| Coenzyme | $\lambda_{max}$ free | $\lambda_{max}$ bound |
|---|---|---|
| NADH | 340 | 325 |
| 3-Acetylpyridine ADH | 365 | 350 |
| 3-Formylpyridine ADH | 355 | 340 |

Binding of coenzyme to liver alcohol dehydrogenase[57]

Table 1

The observation that a shift is observed with coenzyme analogues (Table 1) indicates that enzymically enhanced donation from the amide nitrogen (IVb) is not responsible. Sulphydryl groups have been implicated by some workers[29,59] to explain the effect. Kaplan and Ciotti[58], and Cilento et al[21] have suggested that a direct bond may be formed between the coenzyme ring nitrogen and an enzymic group. If this group were able to withdraw electrons from the ring it would account for the change. Kosower[60] has suggested a plausible ternary structure (Fig 2) which puts a protonated ε-amino group of lysine 3Å from the ring nitrogen group and would account for the observed shift. Hydrogen bonds to ribose and acetaldehyde help to hold the structure rigid. It also seems possible that a protonated tryptophan residue at this site could be responsible for the absorption change[45,46].

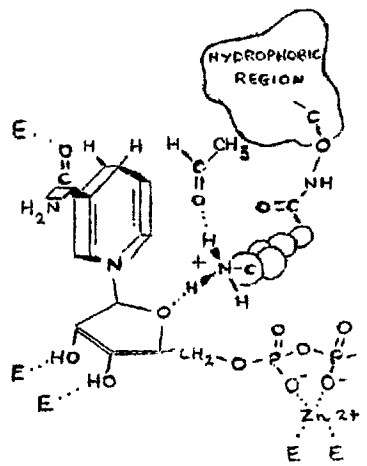



Fig 2[60]

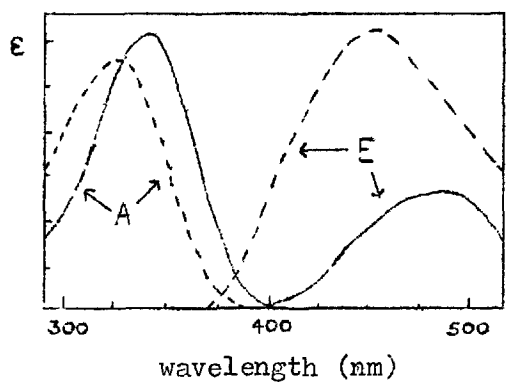



Fig 3 Absorption (A) and Emission (E) curves for NADH free (——) and bound to horse liver AdH (- - -).[61]

NADH also has an emission spectrum which is modified on binding (Fig 3) as was first discovered by Boyer and Theorell in 1955[62]. Generally, there is a manyfold enhancement in intensity, and a

shift to shorter wavelength. The intensity increase has been considered to be due either to rigid orientation of the coenzyme moiety on the enzyme surface which caused inhibition of an internal conversion operating in the unbound molecule, or to "sensitized fluorescence"[61], (transfer of excitation energy from enzymic aromatic amino residues to the bound NADH). The excitation maxima of free NADH were found to be at 260 and 340 nm,[63] corresponding to the absorption maxima of the adenine and dihydronicotinamide rings respectively. Fission of the pyrophosphate bond of NADH,[63] or dissociation by polar solvents or increased temperature,[64] lead to loss of the 260nm excitation maximum. This indicates that the two rings are in juxtaposition in the free state, to allow adenine excitation to cause nicotinamide fluorescence[63-64]. On binding to liver AdH, the 260nm excitation is also lost, suggesting a more open conformation in vivo. The apparently anomolous proton magnetic resonance studies of Kaplan and Ramaswamy[65a] in which the bound 2-proton is strongly deshielded, and which these workers ascribed to an eclipsing adenine ring, may also be due to the protonated amine previously implicated. The open conformation is supported by the recent X-ray crystallographic studies of Adams et al[13] with lactate dehydrogenase. The shift in fluorescence maximum from 462 to 440 nm[65b], and enhanced polarisation both suggest an environment of relatively low dielectric constant.

There is some evidence to suggest the presence of sulphydryl groups near the active site of liver AdH. Thus the binary complex absorption maximum at 325nm was extinguished by the addition of p-chloromercuribenzoate, and free NADH (340nm) was formed[29,59]. Heavy metals had a similar effect[66a]. Also the rate of reaction of p-chloromercuribenzoate with enzymic sulphydryl groups is considerably

slower in the ternary complex than with free enzyme[66b]. Iodoacetic acid inhibits this enzyme similarly, although it does not affect the binary NADH-enzyme complex[67]. It can also be introduced into certain NADH analogue-enzyme complexes[68], so it is not clear whether the responsible thiol group is involved in binding the complex (perhaps via a disulphide linkage[68]) or is merely near the active site. Sund and Theorell linked the sudden increase in dissociation constant of the NADH-AdH complex above pH 9 with the known pK ($\sim$10) of sulphydryl groups[57].

Many workers have implicated zinc in binary and ternary structure postulates. Thus 1,10-phenanthrolene competes with NADH[69], and optical rotatory dispersion studies of the two binary complexes show characteristically similar Cotton effects (for a review see ref 70). It is likely that zinc is involved in both binding processes and hence that this metal is at least near the redox active site. (For a summary of other chelating agents see ref 57 p47).

The redox potential of unbound $NAD^+$-NADH at -320mV[39] is considerably altered (by +60-80mV) on binding to liver AdH[57], thus favouring the formation of the reduced coenzyme, and hence oxidised substrate. In addition, the reduced coenzyme forms the stronger complex, and hence competes with $NAD^+$ and alters the position of equilibrium.

Much less is known about the changes in properties of $NAD^+$ on binding to substrate, because the usual techniques of absorption and emission spectroscopy cannot be applied. However, it is known that the anion affinity of $NAD^+$ (for attack in the pyridine 4-position) is considerably increased on binding[8,71-74] favouring the formation of reduced coenzyme. Several of the previously mentioned

factors (adjacent sulphydryl, zinc or quaternary nitrogen groupings) could contribute to this observation.

Throughout the preceding treatment it has been assumed that the "active sites" on a given enzyme molecule are identical and independent, and that $NAD^+$ and NADH occupy the same site and are hence mutually exclusive. These assumptions are well founded (for a synopsis see ref. 37 p54) as is the 1:1 stoichiometry of coenzyme-zinc couples[75].

## 5. The Effect of Substrate Addition

The substrate can be considered to attack the binary coenzyme-AdH complex (a) by binding to the nicotinamide moiety, (b) by direct combination with the enzyme surface or (c) some combination of these. It is necessary to see how this binding so activates both coenzyme and substrate as to allow the redox reaction to take place.

The fluorescence spectra of NADH coupled to yeast or liver AdH shows an intensity increase on addition of a variety of substrates including amides and imidazole[50-52]. An absorption maximum shift is also observed when pyrazole is added to the $NAD^+$-AdH system[76]. In these and other cases[77-78] the implication is that the coenzyme is modified and hence to some extent responsible for binding the substrate. However, it may be that the mode of addition here is unnatural, since many of these substrates couple to the coenzyme non-enzymatically as well[49,77,79]. Ethanol and acetaldehyde do not cause fluorescence enhancement of NADH coupled to AdH, nor do they form strong complexes with it non-enzymatically.

The second possibility, that the substrate binds exclusively to the enzyme seems unlikely since enzyme-substrate binary complexes ought then to be observable, unless the binding of the coenzyme is considered to generate a neighbouring substrate site[80] (perhaps by conformational changes).

The most likely mechanism, as considered by many workers, has involved both enzyme and coenzyme in the ternary complex formation, zinc often playing a prominent role. Hydrogen bonds are also probably important in the activation process[81]. The structure of the ternary complex has yet to be proved and the most likely postulates will be outlined in the next section. Very little about the precise structure

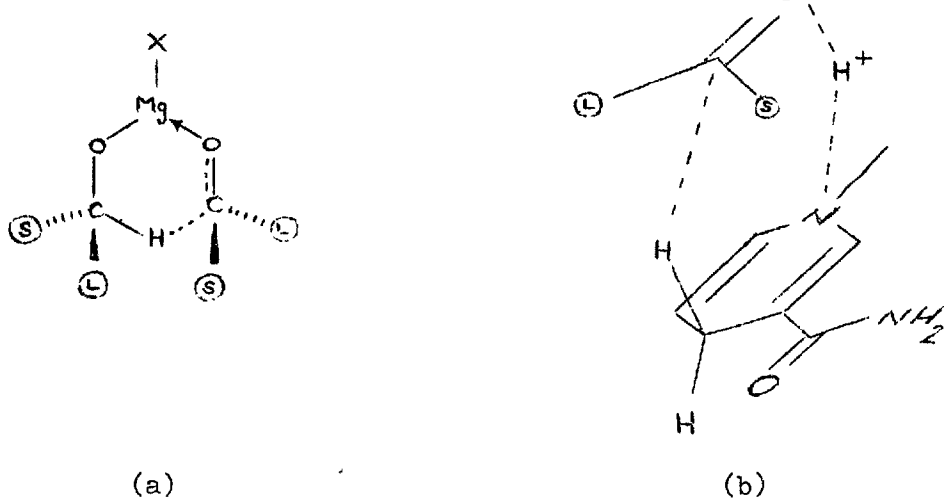
can be gleaned from the chemical or spectrophotometric properties of these complexes, and the proposed intermediates represent a balance of the available data.

Although the alcohol dehydrogenases can tolerate only relatively minor alterations in the coenzyme structure (specifically to the pyridine 3-position and adenine ring) and still remain active, the binary coenzyme-enzyme complex is less specific. Thus horse liver AdH-$NAD^+$ will oxidise a wide range of alcohols including aryl alcohols, many of them appreciably faster than ethanol itself.[57] Conversely, the corresponding AdH-NADH system reduces many aldehydes and ketones,[57] and several workers have attempted to deduce something of the steric requirements of the active site by correlation of structure with reaction rate[83-84]. Thus it was observed that an increase in homology from ethanol up to n-hexanol corresponded to an increased reaction rate,[57] and Westheimer's group suggested the presence of an enzymic hydrocarbon chain surrounding a cleft into which the alkyl portion could fit, other positions being sterically unfavourable[41]. (c.f. Fig 2)[60].

## 6. The Nature of the Hydrogen Transfer in the Ternary Complex

That the transfer of hydrogen was quantitative between coenzyme and substrate had been demonstrated by Westheimer's group[17-18]. They also observed that this transfer appeared to be stereospecific. Thus $\left[1,1\text{-di-}^{2}H\right]$-ethanol transferred exactly one deuterium atom to $NAD^{+}$ at equilibrium. The resulting $NAD^{2}H$ was enzymatically reoxidised to deuterium-free $NAD^{+}$ with acetaldehyde, clearly showing that only one side (side A)[85-86] of the pyridine ring underwent transfer. This specificity is a general enzymic property apparently, and a wide range of enzyme systems tested show absolute specificity for side A or B of the pyridine ring (for a summary see ref 37 p24). Thus in vivo, the nicotinamide ribose is probably held rigidly on the enzyme surface, as must be one other ring position (presumably the carbamido group) so that rotation of the ring is not possible. This is in accord with the previously noted fluorescence studies. Further, the reactive site occurs only on one side of the ring, the nature of the binary complex effectively preventing substrate attack on the other side. Similarly, a series of workers have shown that the substrate also reacts stereospecifically. Thus $\left[1\text{-}^{2}H\right]$- acetaldehyde is reduced exclusively to (-)S- $\left[1\text{-}^{2}H\right]$- ethanol[90] by NADH-AdH, and this binary complex reduces other aldehydes with the same absolute configuration[90-91]. The same is true of oxidations with $NAD^{+}$.[90] When secondary alcohols are converted to ketones or vice-versa, the same absolute stereochemistry applies, the less polar substituent in each case appearing in the same relative position[87,83,92]. These studies show that the substrate too is bound fairly rigidly in the ternary complex, and to account for the specificity observed, must be constrained at at least two points[41], by the enzyme, coenzyme or both.

Prelog[93] has suggested that a Meerwein-Ponndorf-Oppenauer type of mechanism may account for the stereoselectivity (Fig 4). Thus in the redox reaction between an assymmetric alcohol and ketone, the configuration of the 6-membered transition state is determined by the trans disposition of the large (L) groups, and hence the small (S) groups (Fig 4a)[87].




(a) (b)

(Fig 4)

The analogy with (Fig 4b) transition state is clear, although this effect alone does not explain the ability of certain enzymes (D and L-lactic dehydrogenase) to produce different enantiomorphs by NADH reduction of the same carbonyl compound on the same side of the pyridine ring[87]. Several workers have invoked a "hydrophobic region" which could attract the large (L) substituent preferentially (Fig 2), and Westheimer[41] considered that the magnitude of this aligning force would be sufficiently large. However, the situation is not yet resolved[94]. The implications of the more recent findings that hydrogen is transferred to a tryptophan residue,[42-43] also present at the active site have not been fully explored. Most workers have assumed that coenzyme and substrate are closely aligned on the enzyme

surface, and several such stereoselective intermediates have been proposed. A tryptophan (or other) intermediate hydrogen carrier considerably complicates the stereochemistry of the active site, and since nothing is yet known about the binding of any such intermediate, it has not been possible to propose meaningful ternary structures which incorporate this feature.

## 7. The Evidence for Hydride Ion Transfer *in vivo*

Several different mechanisms have been proposed for the reversible transfer of hydrogen between coenzyme and substrate. These involve polar or radical species:

$$NAD^+ \overset{2e}{\rightleftharpoons} NAD^- \overset{H^+}{\rightleftharpoons} NADH \qquad (8)$$

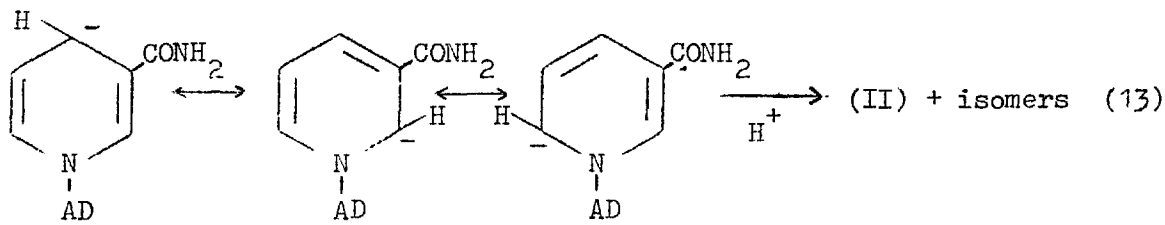
$$NAD^+ \overset{e}{\rightleftharpoons} NAD^\circ \overset{e}{\rightleftharpoons} NAD^- \overset{H^+}{\rightleftharpoons} NADH \qquad (9)$$

$$NAD^+ \overset{e}{\rightleftharpoons} NAD^\circ \overset{H^\circ}{\rightleftharpoons} NADH \qquad (10)$$

$$NAD^+ \overset{H^-}{\rightleftharpoons} NADH \qquad (11)$$

$$NAD^+ + HSH \rightleftharpoons NAD^{\delta+}\text{--}H\text{--}S\overset{\delta+}{H} \rightleftharpoons NADH + S\overset{+}{H} \rightleftharpoons NADH + S + H^+ \qquad (12)$$

Mechanisms involving transfer of a proton (8) and (9) can be immediately eliminated since there is abundant evidence[17-18] that the transfer of hydrogen is quantitative, and that no exchange occurs with solvent. The absence of 2- and 6-dihydropyridine isomers in NADH further negates discrete proton transfer (13). The remaining




(13)

mechanisms allow for direct transfer, and Westheimer emphasised that either radical (10) or hydride ion (11)(12) transfer might occur *in vivo*[41].

At one time it was considered that all oxidation-reduction

reactions necessarily proceed by one-electron steps[95-96]. This idea has since been thoroughly disproved[97a,98], and many organic and inorganic reactions have been shown to proceed by one or other of these mechanisms. However, it is not possible to distinguish theoretically between them since electron transfer can occur many times faster than chemical bond fracture, and it is likely that many borderline cases occur *in vivo* and in the laboratory. For practical purposes, the existence of radical intermediates is demonstrated by their sensitivity to light, oxygen, radical traps, other free radicals *etc.*[97b]

The great majority of studies undertaken to attempt to answer this central question about the nature of the *in vivo* transfer, have been concerned with model analogues of $NAD^+$ and NADH with a variety of hydrogen donors and acceptors (see next section). Such conclusions as can be drawn from these reactions have left the nature of the enzymic system unsolved. Very little work has been done with systems convincingly similar to the natural ternary complex, or indeed with this complex itself. Thus it has not been possible to date to replace the substrate with a known radical generator/acceptor *in vivo* and obtain a hydrogen transfer reaction. The use of radical traps with the ternary complex has not been reported, although steric considerations would make the results of such an investigation necessarily vague. Photolysis of the ternary complex is also unhelpful owing to the complexity of the products obtained. The only direct study of the system has been the work of Commoner *et al.*[99] They observed "an exceedingly low steady-state concentration of unpaired electrons" by electron spin resonance measurements on yeast alcohol dehydrogenase between 1-10 minutes after mixing the

components. The signal, near the limit of detectibility of their instrument, disappeared after ~ 20 minutes. Westheimer has suggested, however, that an impurity or an insignificant side-reaction could be responsible for these observations[100-101].

More positive results have been obtained in recent years by the production of the semiquinone $NAD^{\circ}$ non enzymically both by oxidation and reduction, but these will properly be considered later. Land and Swallow[102] have generated $NAD^{\circ}$ in vitro and successfully reacted it with oxygen in simulation of the NAD-peroxidase system. This enzymic system in particular has been considered possibly to proceed through radical intermediates[103].

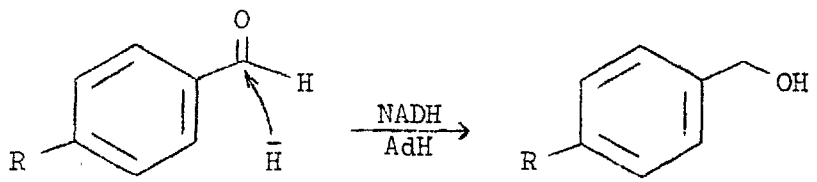
Although the evidence for radical-atom transfer is slight, it seems possible that it might occur in some enzymic systems. There is, however, good evidence that in the AdH ternary complex, the transfer is ionic (11)(12).

A great deal of circumstantial evidence for simultaneous transfer of hydrogen plus 2 electrons has been assembled. Thus throughout the preceeding discussion, the electronic and chemical modifications to the coenzyme on binding to enzyme and to substrate have been noted. These effects (ultraviolet and fluorescent spectra, redox potentials, binding constants) appear to change considerably, and have been considered to reflect an "activation-process" of the coenzyme. Thus direct evidence is available for the increased anion (hydride) affinity of bound $NAD^{+}$ over the free form, and the absorption spectrum of bound NADH is consistent with a polar molecule in which the positive end of the dipole is near the ring nitrogen (see preceding discussion). If a radical transfer is assumed to occur, then these effects must be largely irrelevant, which appears

unlikely. Several model systems which have been demonstrated to transfer hydride ion also have physical properties not unlike those of the "activated" coenzyme.

McGuire and Tompkins[104] successfully transferred hydrogen from the triphosphate coenzyme NADPH to the β carbon atom of the αβ -unsaturated ketone androstendione. This Michael-type addition, proved by $\left[{}^{3}H\right]$ tracer experiments, was performed in the presence of an impure enzyme from rat-liver, and so results are not conclusive, but are certainly in line with polar addition.

More recently, Blomquist has provided more direct evidence for a hydride transfer in the horse liver AdH system[105]. He compared the rates of reduction of a series of p-substituted benzaldehydes by NADH in the ternary complex, and found a direct relationship between the rates and the $\sigma$ constants of the ring-substituent bonds (14).




(14)

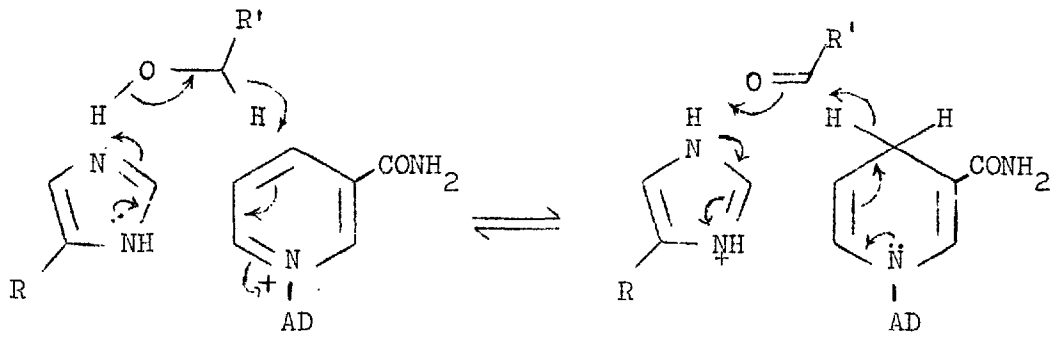
(R=H, $CH_3$, $CH_3O$, Cl, $NO_2$)

Winer[106] had already obtained relative rates for n-propanol (146) and allyl alcohol (192) oxidation by $NAD^+$ (ethanol = 100), in the reverse process, and these findings are in accord with the previous results.

The difference between equations (11) and (12) is that in (11) a discrete hydride ion is involved whereas in (12) the shift takes place via a binary intermediate. The latter reaction is

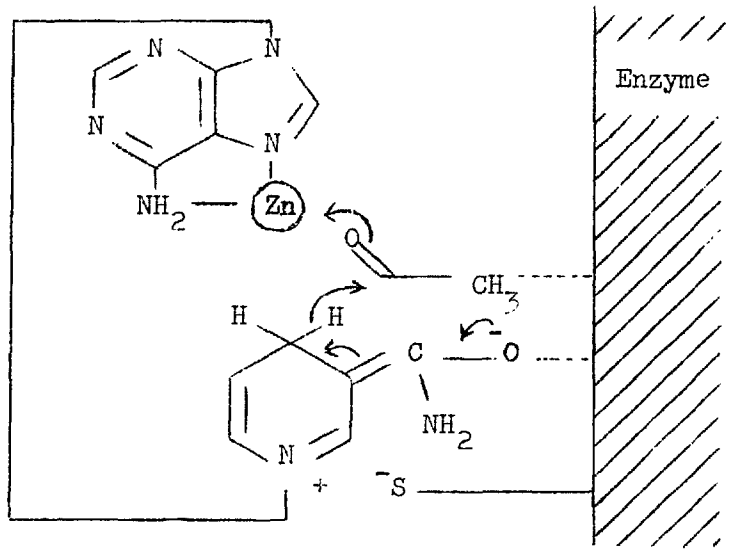
probably the more accurate, although clearly there is no absolute distinction between these possibilities.

A number of structures have been proposed for the ternary complex which simulate equation (12) by involving polar intermediates. One such structure has already been presented (Fig 2)[60], in which a protonated ε-amino group of lysine activates the acetaldehyde carbonyl function by development of a partial negative charge on the oxygen. Winer and Schwert[80] considered that an imidazole grouping might have the same effect, and more recently, Ringold[107] proposed a similar complex involving this grouping (Fig 5) as the major polarising factor. Harris has presented some evidence for the proximity of a histidine residue at the active site[108].



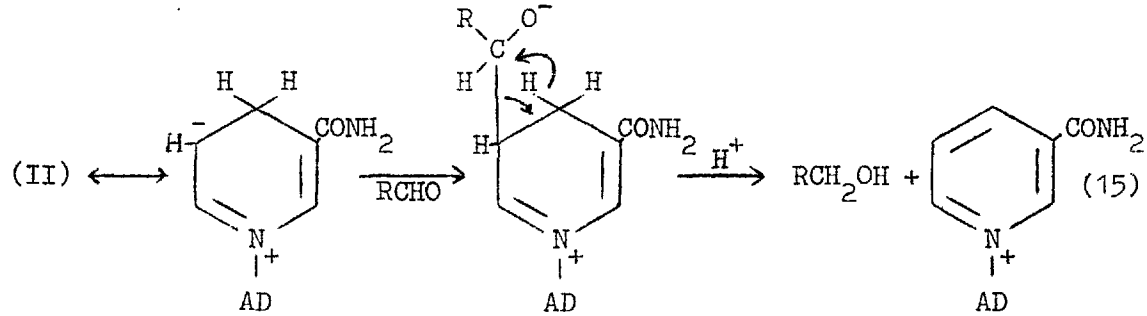

(Fig 5)

Zinc has been implicated directly in the activation process by a number of workers[66a, 109-111], who have also considered it important as the binding site both for the substrate and the adenine[111] end of the coenzyme. A typical ternary structure is given in (Fig 6), which has the Meerwein-Ponndorf-Oppenauer characteristics previously noted.




Although liver and yeast alcohol dehydrogenases contain zinc, this is not a universal property of all enzymes which require $NAD^+$. Thus Colowick et al[112] considered it probable that whilst zinc may play a part in binding the substrate it is not likely to be intimately connected with the transfer per se, if a universal mechanism is to be postulated. The binding role of zinc has been demonstrated by crystallisation of model compounds such as adenosine-zinc-thiophenol[113].

A rather different type of ternary complex has been recently suggested by Dunn[114] which involves enamine addition of the NADH to substrate aldehyde by a direct bond. The addition complex then decomposes via a 1,3-hydride shift to give the observed products (15).



The idea of the substrate binding solely to coenzyme is not new,[77,115] and is in line with the compulsory binding order mechanism. However, the driving force for the rearrangement (15) is not clear, even considering the polarising influences of neighbouring enzymic groups. A radical mechanism is clearly excluded.

In summary, there is considerable evidence to suggest that the in vivo hydrogen transfer between coenzyme and enzyme takes place within a polar environment, and between chemically activated groups which are held sterically adjacent on the enzyme surface. The migrating species may be a hydride ion but is more likely to contain a partial negative charge, so that the redox process includes an intramolecular Meerwein-Ponndorf-Oppenauer steady-state intermediate. A radical process has not been excluded, but appears to be unlikely in the AdH system.

## Non-Enzymic redox reactions of the Coenzyme and Analogues

### 1. Introduction

In the absence of enzyme, the redox process between coenzyme and substrate is very slow, and the coenzyme (NADH) may be replaced by a large number of alkyl and aralkyl 1,4-dihydronicotinamides, nicotinonitriles or Hantzsch[116] compounds with variable effect. Since there is no "activation" of the coenzyme and/or substrate here, it follows that to achieve reaction, one or both reagents must be activated in some way. In almost all the work reported so far in the literature, the practice has been to employ highly reactive substrates with the coenzyme or models of it in order to achieve hydrogen transfer. No direct work has been attempted with an "activated coenzyme" model system[117a] and few workers[117b] have attempted to bring the reagents into close proximity in a manner analogous to the in vivo reaction. The results that have emerged are confused, but it is clear that the coenzyme reacts at a rate comparable with many other dihydropyridines in vitro and will be treated as such in this review.

### 2. Requirements for an NADH Model

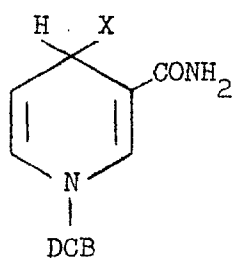
If a polar hydrogen transfer is assumed to take place on the enzyme surface, a model system should incorporate physical features which are characteristic of the bound reagents in the natural state. It has been previously noted that the observed fluorescence and polarisation enhancement of the coenzyme on binding are probably due to the rigid manner in which it is held in the complex, but

since this aspect of the binding process is not easy to simulate and has thus not been studied it will not be considered further.

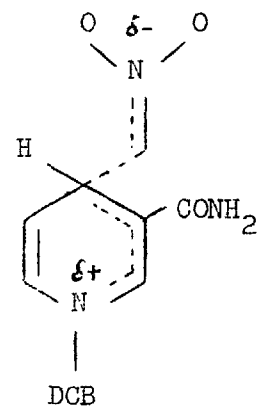
The ultraviolet shift on binding has been simulated in model compounds by 3 types of ring substitution.

A) 1,4-Addition Complexes: - Pyridinium salts react with a wide variety of nucleophiles to yield 1,4-addition complexes which have properties in common with 1,4-dihydropyridines[71,118]. The position of the observed absorption maximum depends upon the nature of X (Table 2). Substituents which are able to withdraw electrons from the ring by either an inductive or more particularly a mesomeric effect increase the contribution of a pyridinium resonance structure (Fig 7), thus causing an absorption shift to shorter wavelengths. A relationship has been found[71] between the stability of the corresponding complexes (and thus the strength of the C-X bond) and the position of the absorption maximum. From this evidence, it appears possible that the shift observed *in vivo* may be due partly to the presence of a strongly electron attracting group adjacent to the 4-hydrogen, such as an (activated) substrate molecule.

Table 2

| Addition Complex | X | (DMF) $\lambda_{max}$ (nm) |
|---|---|---|
|  | -SH | 348 |
| | -H | 343 |
| | $-OSO_2R^*$ | 340 |
| | $-SCH_2CH_3$ | 330 |
| | -CN | 329 |
| | $-CH_2NO_2$ | 326 |
| *4,4' dimer | | |





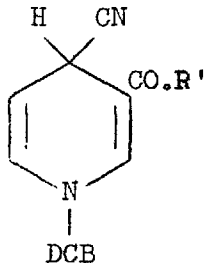

(Fig 7)

B) Replacement of Amide function in NADH Models: - Wallenfels et al[71] prepared a series of 4-cyano nicotinyl analogues of NADH (Table 3), and obtained a direct correlation between their absorption maxima and the stability of the cyano complex ($K_s$ in equation 16).

$$K_s = [\text{Complex}] \,/\, [\text{Pyridinium Salt}^+].[CN^-] \qquad (16)$$

Substitution in this position as in (A) obviously favours or hinders the enamine resonance structure (cf IVa), and thus the available partial negative charge at C-4.

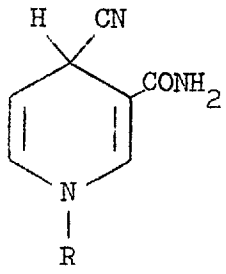
Table 3

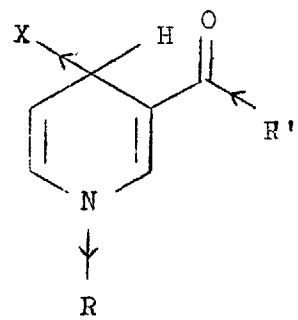
| Addition Complex | R' | conc$^{d}$ aq. KCN $\lambda$ max | log.$K_s$ | Redox Potential $E_o'$ (mV) |
|---|---|---|---|---|
|  | $N(CH_3)_2$ | 329 | -0.2 | -391 |
| | $OCH_3$ | 342 | +0.9 | -358 |
| | $OCH_2CH_3$ | 344 | +1.1 | -354 |
| | $NH_2$ | 339 | +1.2 | -349 |
| | $CH_3$ | 355 | +3.4 | -287 |



C) Replacement of Adenine Dinucleotide group - If the pyridine N-ribose linkage is replaced by alkyl or aralkyl groups, a further correlation may be seen between the position of the absorption maximum of the cyano complex, its stability constant and the electron-withdrawing power of the group[71]. A relative partial negative charge on C-4 is facilitated by electron withdrawing groups at $N^1$, (Table 4).

Table 4

| Addition Complex | R | Conc[d] Aq. KCN λ max | log $K_s$ | Redox Pot[1] $E_o'$ (mV) |
|---|---|---|---|---|
|  | $CH_2CH_2CH_3$ | 345 | -0.1 | -387 |
| | $CH_2\emptyset$ | 341 | +0.8 | -361 |
| | DCB | 339 | +1.2 | -349 |
| | AD | 327 | +2.4 | -320 |
| | $CH_2OCH_2\emptyset$ | 326 | +2.9 | -300 |
| | Tetra-acetylglucose | 319 | +4.1 | -267 |



(Fig 8)

In summary, the in vivo absorption binding shift has been simulated by models systems with substituents R,R' and X (Fig 8) with electron affinities as indicated by the arrows. It seems likely that some or all of these factors may be present in the ternary complex. R and R' clearly have an indirect influence on the strength of the C-X (C-H) bond, and X has a direct effect.

The redox potentials of NADH models are a more direct measure of their chemical similarity to bound NADH, but relatively little

work has been done on these. Wallenfels et al have calculated them[71] from a consideration of the $K_s$ values of the cyano compounds mentioned above, and some values are tabulated for comparison (Tables 3 and 4). Enzymic binding of NADH to liver AdH causes the potential to become more positive by ~80 mV, and thus favours the formation of reduced coenzyme and oxidised substrate.

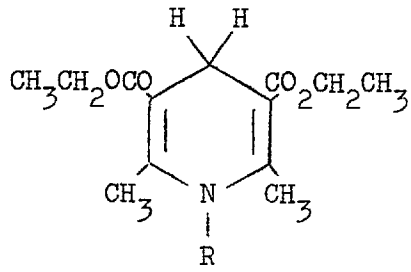
## 3. Requirements for a Substrate Model

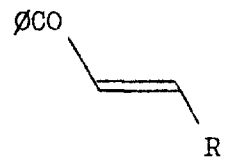
Much less is known about this aspect of the ternary complex, but assuming that a polar reaction has to be simulated, aldehydes and ketones with some polar character will have to be employed. This has generally been achieved by attaching electron withdrawing groups to the carbonyl function, although recently Pandit and Mas Cabré[81] have achieved some success with intra-molecularly hydrogen-bonded aldehydes.

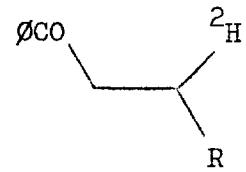
## 4. Two Electron Transfer in Model Systems

### A) Oxidation of NADH Analogues

There have been a number of reactions reported in the literature in which a dihydropyridine transfers hydrogen in Michael fashion to an $\alpha\beta$-unsaturated ketone. Braude, Hannah and Linstead[16] amongst many other related reactions, achieved the reduction of maleic anhydride to succinic anhydride in excellent yield using the "Hantzsch ester" 2,6-dimethyl-3,5-dicarbethoxy-1,4-dihydropyridine (VIII) as an NADH model. The oxidation product was the corresponding pyridine. Graves[119] identified the position of attack as $\beta$ to the carbonyl function in the reduction of benzoylacrylic acid (X) with the [4,4-di-$^2$H]







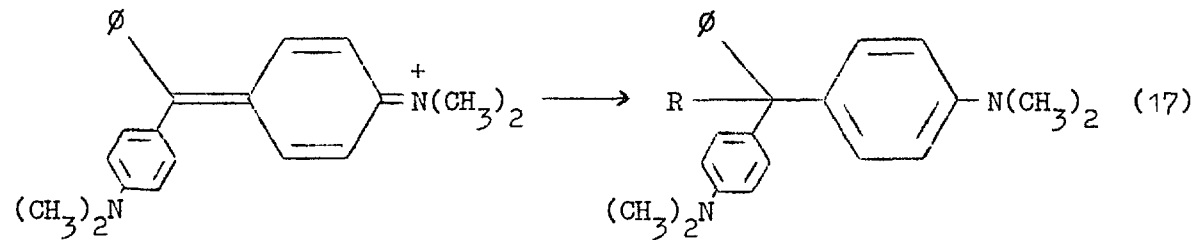
(VIII, R = H) (X, R = $CO_2H$) (XI, R = $CO_2H$)

(IX, R = $CH_3$) (XII, R = $CF_3$) (XIII, R = $CF_3$)

analogue of (VIII). The reduced product was (XI). Westheimer's group[120] investigated the reaction further and obtained equally good results with the corresponding trifluoromethyl derivative (XII) and deduced that the effect of the carboxyl/trifluoromethyl group was electrostatic. Deuterium studies again showed Michael attack to give (XIII) with both compounds (VIII) and (IX), the latter simulating NADH more closely. However, attempted extension of the reaction to more recognisable NADH models (eg. N-benzyl-1,4-dihydronicotinamide) failed. This may have been due to the fact that the successful reactions were carried out at pH 6.5-7.0 owing to the extreme alkaline susceptibility of the oxidant, and that 1,4-dihydropyridines are very acid labile[121a] (Hantzsch compounds less so)[121b]. This handicap is overcome *in vivo* where the localised pH is controlled by the enzyme.

Ionic transfer from N-benzyl-1,4-dihydronicotinamide to other more highly polarised substrate analogues has been observed. Mauzerall and Westheimer[25] first used this compound to reduce malachite green in high yield to the *leuco* compound (XIV) and it

now seems probable that this reaction (17) proceeds by polar addition[21,122] although there has been some doubt about this[25,123,96].

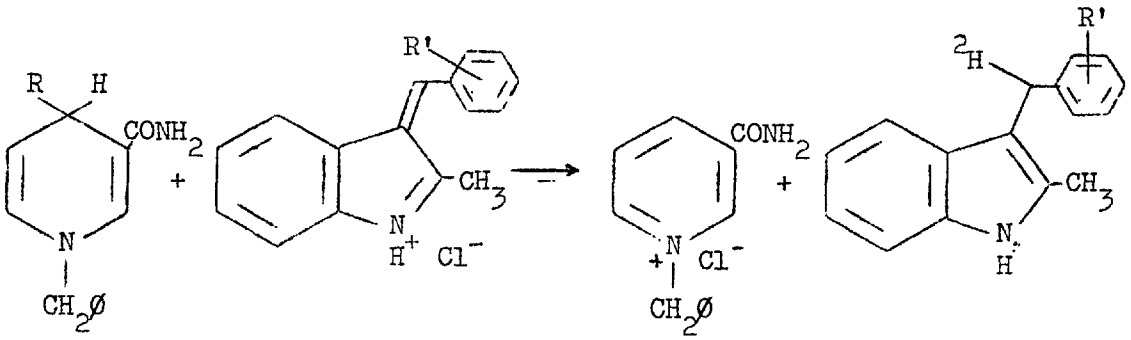


(XIV, R = H)
(XV, R = CN)

Recently, Roesler[122] reduced this and four other related dyes with the same dihydropyridine and concluded that a hydride transfer reaction took place from a kinetic consideration of the processes. He also noted a radical-like side-reaction when solvents were not purified, and air and light were not excluded. Wallenfels[21] obtained further support for an ionic mechanism in the reduction of malachite green by the analogous transfer of cyanide from the corresponding cyano addition complex (XVI) to form leucocyano malachite green (XV). The more facile fracture of the C-CN bond in the reducing agent (XVI) is known to proceed by by an ionic mechanism[117].

Huffman and Bruice[44] reduced the interesting tryptophan model indolenine salt with the Hantzsch ester (VIII) in acetonitrile, and showed that radical inhibitors had no effect in this reaction. They favoured an ionic mechanism, and suggested that a charge transfer intermediate (vide infra) might be involved. Schellenberg and his collaborators[124] also recently studied the incorporation of

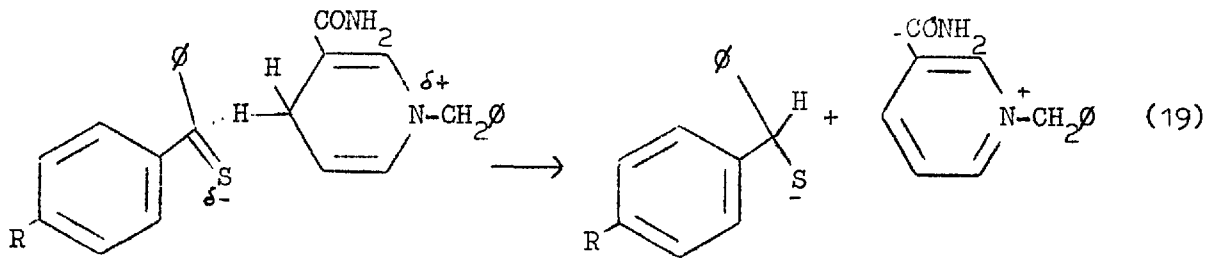
deuterium into a series of indolenine salts, from the N-benzyl-[4-$^2$H]-1,4-dihydropyridine (XVII). The redox reaction was complete at 25° in ethanol in 5-10 minutes, and deuterium was quantitatively incorporated into the benzylidene position (18).




(XVI, R = CN)
(XVII, R = $^2$H)

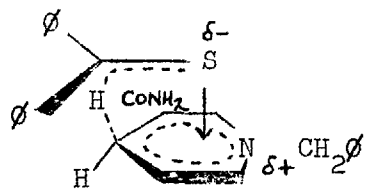
The effect of substitution (R') in the aromatic ring on the corresponding reaction rate was compared for R'=p-methoxy, o-chloro and p-nitro groups. The rate of reaction was shown to increase markedly in this order, which is again in accord with a polar mechanism.

Direct reduction of a ketone by a dihydropyridine has been achieved in only a relatively small number of cases. Abeles, Hutton and Westheimer[125] reduced thiobenzophenone in a classic paper which convincingly showed that polar addition to the thioketone carbon atom took place (19). Electron withdrawing substituents in one aromatic ring caused an increase in reaction rate, as did the use of more polar reaction media. These workers suggested that the greater polarisability of the thioketone carbonyl group over the carbonyl group was responsible for the ease of its reduction.



(XVIII) (19)

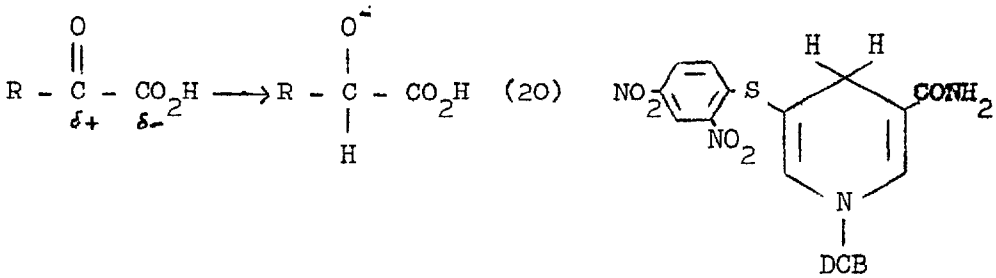
Radical inhibitors, light and oxygen had negligible effect on the course of the reaction, and once again, deuterium studies proved the direct attack of hydrogen with a marked isotope effect. It was noticed, however, that the rate of reduction did not seem to be dependant on pH and it was concluded that a proton was not involved in the activated intermediate as might have been expected. Kosower[116a,126] has suggested a charge-transfer intermediate complex to explain this (Fig 9) and his proposed structure is reminiscent of the enzymic complex.



(Fig 9)

Direct reduction of the "true" ketones pyruvic acid[25,128a] and benzoylformic acid[127a] was first achieved by Westheimer's group, but only with the apparently more reactive Hantzsch ester (VIII). Yields of the products (lactic acid and mandelic acid respectively) were very poor, but it was nevertheless possible to

show that direct hydrogen transfer had taken place, which was probably anionic (20). Again, the carboxyl group must be assumed to be responsible for creating a ketonic partial positive charge. Wallenfels and Hofman[127b] obtained 5-7% yields of lactic acid by reducing pyruvic acid (20) in the presence of the modified NADH analogue (XIX), which is presumably midway between the reactivities of the two major models (IV and VIII).



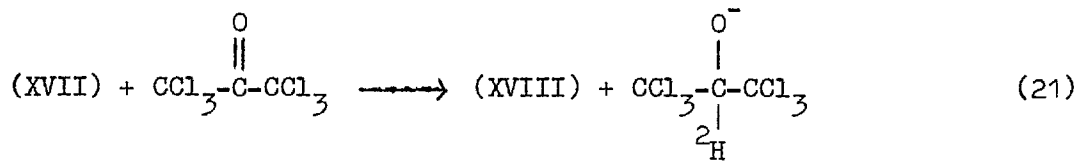
(20)



(XIX)

Hydrogen bonding between the carboxyl and carbonyl groupings could also be partly responsible for activation of the latter here.

Dittmer and others more recently have reduced hexachloroacetone with the N-benzyl compound (IV) in variable yields under a variety of conditions.[128-129] It appears from their work that two separate reaction paths were followed, either of which could be favoured by suitable reaction conditions. In polar solvents (nitromethane, formamide) with free radical inhibitors present, they obtained reasonable quantities of hexachloroisopropanol and the corresponding benzylochloride salt. Deuterium tracer studies showed incorporation in the 2-position of the alcohol as expected (21).

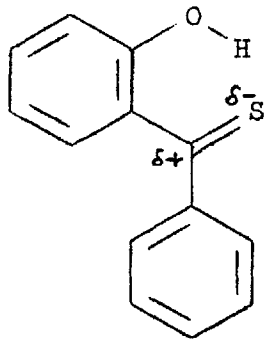


(21)

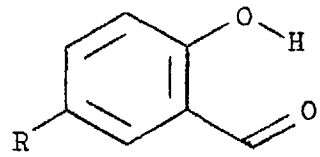
A polar intermediate was indicated here, and a charge-transfer complex was proposed, completely analogous to the one suggested by Kosower[127] for thiobenzophenone (Fig 9). Lombardo[130] recently extended this reaction to a number of 3-substituted 1,4-dihydropyridines and found that an increase in the electron withdrawing power of the 3-substituent caused a decrease in reaction rate as expected. An increase in the solvent polarity had the same effect. He also showed that the hydrogen transfer occurred prior to or during the rate-determining step and concluded that this step was probably the conversion of an outer charge-transfer complex to an inner complex.

This reaction can also be considered to be a radical transfer under suitable conditions (vide infra). Some work has also been done on related halo-ketones and aldehydes[128-130], but yields were generally lower, and much less is known about the mode of reaction.

Hydrogen-bonding to the carbonyl group has been exploited as a polarising influence by very few workers. Westheimer's group[125] had observed that in the reduction of thiobenzophenone, p-hydroxy and o-methoxy groups in the aromatic nucleus hindered the reaction as expected. However, an o-hydroxy group accelerated the reaction which they considered indicated the greater polarising ability of a hydrogen bond over the competing resonance effect (Fig 10).



(Fig 10)



(XX, R = H)
(XXI, R = $NO_2$)

Pandit and Mas Cabré[81] have very recently reduced salicylaldehyde (XX) in "small amount" with the Hantzsch ester (VIII) and its dideutero derivative, in refluxing ethylene glycol. Introduction of a 5-nitro group (XXI) greatly facilitated the reaction which proceeded in ethanol at 40° in unstated yield. Deuterium was incorporated in the benzylic position as predicted. In contrast, no reaction was observed with benzaldehyde, or m-nitrobenzaldehyde. The relative inefficiency of this reaction compared with the enzymic one has been suggested[81] as partly due to the greater degree of orbital steering[131] which the enzymic system may achieve over such models, and at present it is not possible to obtain a meaningful estimate of the hydrogen-bond effect in vivo. It seems possible that it may be larger than earlier supposed, however.

Substrate analogues have been "activated" in two further ways. Dittmer and Fouty[132] obtained a ring opening reaction of cyclobutanone with dihydropyridine (IV), the activation being derived from the relief of ring strain. More significant is the

recent discovery by Khidekel's group[133] that ketones may be suitably activated by reaction with alkali metals. Thus a solution of sodium and benzophenone in dioxan treated with the dihydropyridine (VIII) or (IV) gave benzhydrol in 80% and 27% yields respectively. Experiments with other metals (lithium, magnesium and zinc Grignards) gave successively lower yields, but the reaction worked with certain other aromatic aldehydes. In each case, the major by-product was the expected pinacol, presumably from a competing radical reaction.

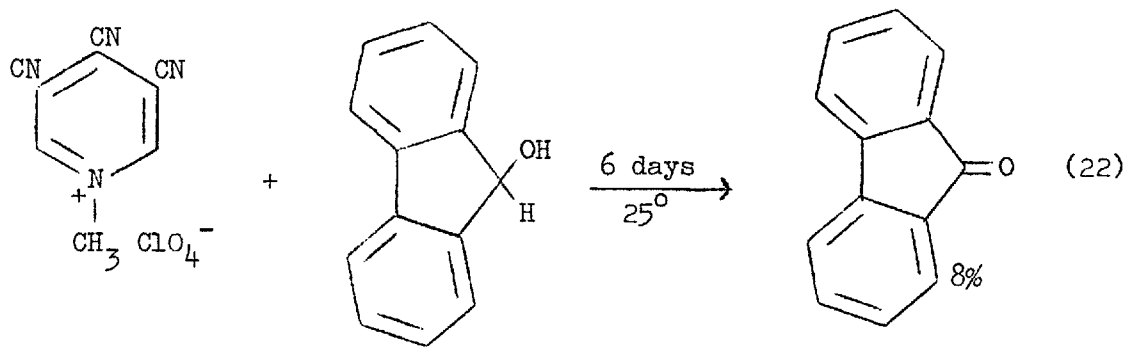
All these studies have shown that by suitably polarising the substrate analogue, a direct, polar hydrogen transfer can be made to occur in vitro from a suitable 1,4-dihydropyridine. A number of other non-ketonic systems have also been reduced with NADH models from time to time[134-139], by an apparently two-electron process, but these will not be further considered because they do not really parallel the system under examination here.

B) Reduction of $NAD^+$ Analogues

Much less is known about reduction of pyridinium salts to dihydropyridines by organic reducing agents. The reduction with sodium dithionite in aqueous medium at pH 6-9 is well documented and generates almost exclusively the 1,4-isomer. The mechanism of the reaction has been much disputed[140-141,126] but it seems probable that initial attack in the 4-position by sulphoxylate ion is followed by a cyclic rearrangement and evolution of sulphur dioxide[142-144]. In any case, this reaction has been considered to be ionic for a long time[140,145-146]. Reduction with sodium borohydride yields all three dihydropyridine isomers, depending on conditions[147,148],

and this reaction clearly also involves an incipient hydride ion[149].

So far, the only reduction which parallels the *in vivo* system is the reaction of N-methyl-3,4,5-tricyanopyridinium perchlorate with fluorenol (22)[150]. The mechanism of the reaction has not been examined, but the authors appear to favour radical intermediates, so it will be considered further in the next section. The primary reduced species was not identified, although it may have been the




(22)

2-hydroxy-1,2-dihydropyridine[150a] or 1,2-dihydropyridine[150b].

## 5. One Electron Transfer in Model Systems

Although the preceding section has presented a considerable amount of evidence for hydrogen anion donation by NADH and its models to a variety of acceptors, it is well-known that free-radical intermediates are also subject to polar influences[151]. Whilst it is clear that in many or most of the reactions so far dealt with, an incipient hydride ion is probably involved, certain other types of reaction have been found to proceed by a radical pathway, and some of these reactions are considered here. The accumulating evidence for hydride transfer in the AdH system has led workers to look for radical intermediates in other NAD-

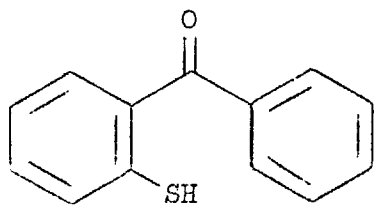
enzyme systems, notably the peroxidase system.

A) Oxidation of NADH and Analogues

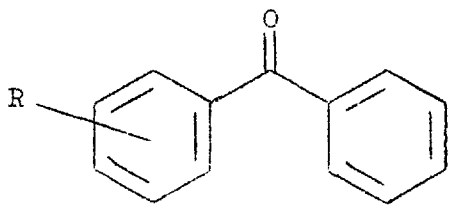
In the early work on these reactions, radical mechanisms seem to have been favoured. Methylene blue was first reduced to its colourless leuco base by Karrer et al[152] in the mid 1930's by a variety of 1,4-dihydropyridines, although the mechanism was not investigated. Mauzerall and Westheimer[25] actually favoured a radical process to explain the related reduction of malachite green on the known[96,123] ability of this dye and quinones in general to form free radicals. This prompted some research into the ability of quinones and related substances to act as acceptors for dihydropyridines. Wosilait and Nason[153] compared the rates of variously substituted quinones with NADH itself, but obtained an order of reactivity which they considered indicated an ionic mechanism rather than a free radical one. Braude et al[154] assumed that chloranil was similarly reduced by the Hantzsch ester (VIII) via a hydride transfer, and Wallenfels and Gellrich[155] studied the rates of oxidation of various models with a range of quinones. These rates were critically dependent upon the redox potential of the quinone oxidant, and rather less so upon the nature (Fig 8) of R and R', (X = H) of the dihydropyridine employed, or on the pH of the redox medium. Sund[53] in a recent review considered this unequivocal evidence in favour of a hydride transfer, although a free-radical reaction cannot be ruled out on this evidence alone. A Russian group[156] have since observed an E.S.R. quintet in the oxidation of (IV) with p-benzoquinone and 1,4-naphthoquinone. This was not observed with chloranil or 2,6-di-t-butyl benzoquinone

although as these workers point out, the steady-state free radical concentration may simply have been too small to be detected. Related compounds like phenazines [157] and the biologically important riboflavin[158-159] have also been studied and the mechanisms are not resolved for certain. Schellenberg and Hellerman[159] compared the rates of oxidation of NADH itself with "conventional" one-electron and two-electron oxidants, as well as with riboflavin, methylene blue and 2,6-dichlorophenol-indophenol[155]. Interestingly, the "two-electron" systems (o-iodosobensoate, triphenyltetrazolium ion etc) were totally inert, the "one-electron" systems (spirocyclohexylporphyrexide, porphyrindene)[160] were reduced almost instantly in high yield, and the quinonoid systems reacted at various (much slower) rates. In addition, they obtained comparable (slow) rates with ferricyanide ion[161] and Fenton's reagent[162]. From these studies, no clear picture has emerged although the balance seems in favour[121b] of a radical-initiated process, which perhaps involves charge-transfer[127] stabilised intermediates.

Schellenberg and Westheimer[163] have obtained a fairly convincing radical-initiated reduction of 2-mercaptobenzophenone (XXII) with the Hantzsch ester (VIII). The corresponding 2-mercaptobenzhydrol (XXVI) and pyridine ester (XXV) were isolated in 79% and 90% yields respectively, after 4 hours in hot ethanol. The reaction proceeded rapidly at room temperature in the presence of air or Fenton's reagent, but failed with the benzophenone series (XXIII).

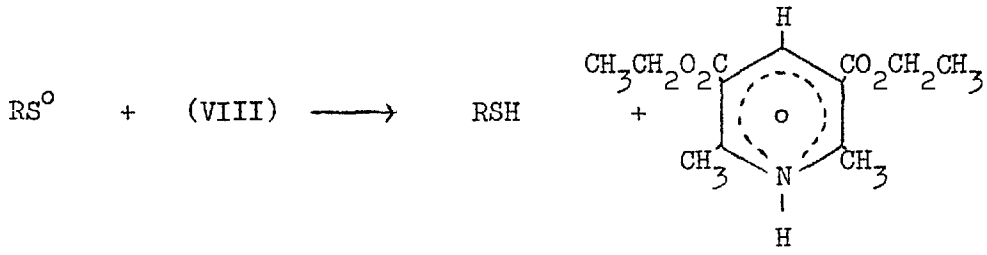



(XXII)

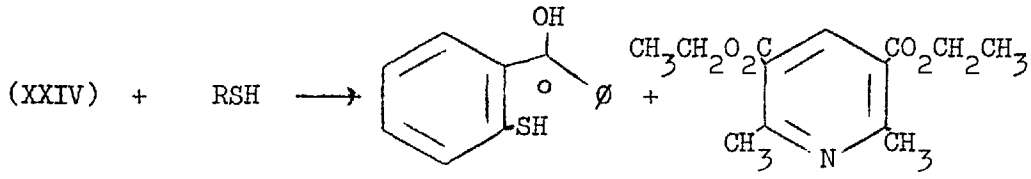



(XXIII, R=H, 2-$NH_2$,2-OH,4-SH)

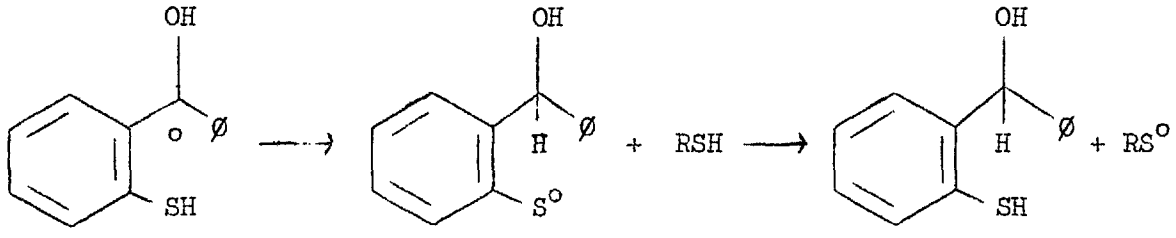
Experiments with deuterium indicated that hydrogen was incorporated from the medium, and hence that direct hydrogen transfer did not occur. Thus as the authors pointed out the system is not really analogous to the enzymic one. The mechanism proposed was that of initiation by ferrous ion in hydrogen peroxide followed by propogation via the thiyl radical ($RS^{o}$) of (XXII).



(XXIV)



(XXV)

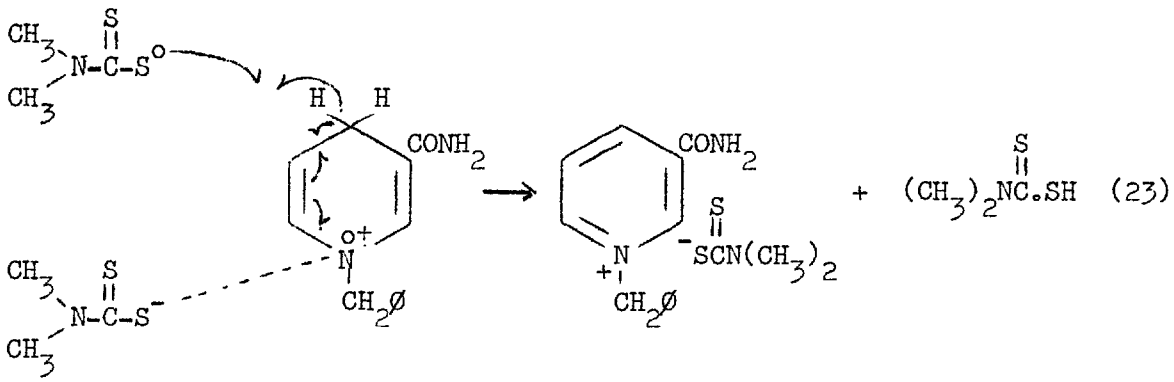


(XXVI)

The necessity for the neighbouring mercapto group is clear from the proposed reaction sequence, and the participation of these groups in radical reactions is well-documented[164].

Wang, Linnell and Wang[165] have recently oxidised the dihydropyridine (IV) with tetramethylthiuram disulphide (TMTD) and mono-

sulphide (TMTM) by an apparently homolytic process[166]. The oxidation product was identified as the pyridinium N,N-dimethyldithiocarbamate salt (in 65% yield), and this may be another example of a charge-transfer[126] complex. The reaction is presumed to begin with homolytic cleavage of the oxidant followed by attack of the thiyl radical at the dihydropyridine 4-position and formation of the observed products (23).



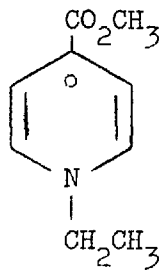

(23)

The importance of these two reactions is that a thiol group is supposed to be present near or at the active site in AdH. It has so far not been possible to demonstrate that it is within the reactive sphere of the NADH pyridine 4-position but if this is demonstrated, it will be necessary to reconsider whether free radicals are involved in the enzymic system.

This latter model system more closely resembles the peroxidase enzymic reaction; a disulphide linkage replacing the peroxide one. A considerable quantity of work has been done recently on aerobic and peroxide oxidation of NADH and its models, catalysed by various metals and organic radical generators[157b,167-70]. Once again these systems will not be considered in detail as they do not

parallel the AdH reaction. However, from these studies has emerged sufficient data to suggest that NADH and dihydropyridines in general can participate in purely homolytic reactions under suitable conditions, and it would seem likely that enzyme-catalysed radical reactions involving NADH may well occur *in vivo*.

A further contribution was made by Kosower and Poziomek[171] in the successful isolation and characterisation of N-ethyl-4-carbomethoxypyridinyl (XXVII). This stable free-radical could be rapidly oxidised to the pyridinium chloride by a series of chlorinated hydrocarbons[171-172].



(XXVII)

$$\left.\begin{array}{lcl} RCl & \longrightarrow & R^{\circ} + Cl^{\circ} \\ R^{\circ} + NADH & \longrightarrow & RH + NAD^{\circ} \\ NAD^{\circ} + Cl^{\circ} & \longrightarrow & NADCl \\ NADCl & \longrightarrow & NAD^{+}Cl^{-} \end{array}\right\} \qquad (24)$$

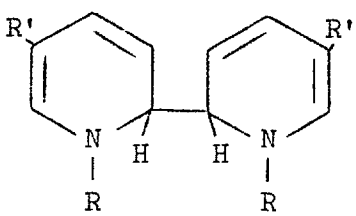
This reaction was compared to the oxidation of 1,4-dihydropyridines with hexachloroacetone[128], since pentachloroacetone and tetrachloroacetone were observed amongst the products in non-polar solvents. In Dittmer's reaction[128] radical inhibitors had little effect on the yield of pyridinium salt formed, but drastically affected the yield of pentachloroacetone. Conversely, the presence of t-butylhydroperoxide or ultraviolet light increased the yields of these chlorinated ketones at the expense of hexachloroisopropanol. Thus two competing mechanisms appeared to be in progress, the first as

previously described and the second involving homolytic cleavage of carbon-chlorine bonds followed by radical oxidation of the dihydropyridine (24, R = $CCl_3COCCl_2$-)

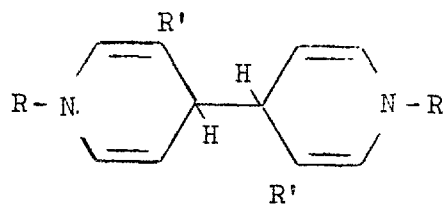
In conclusion, Blaedel and Haas[173] have recently oxidised a series of 1,4-dihydronicotinamides in acetonitrile at platinum and carbon electrodes. In basic madium they obtained quantitative yields of the corresponding salts by two one-electron oxidation processes.

B) Reduction of $NAD^+$ and Analogues

Little is known about one-electron reduction of the oxidised system. The majority of the work has centred on the electrochemical and polarographic reduction. The most useful information from these sources is that in such reductions, dimeric products are often formed, especially 6,6'-dimers (XXVIII).



(XXVIII)



(XXIX)

Wallenfels and Gelrich[174] reduced various pyridinium salts with chromous chloride, a zinc/copper couple or magnesium to such dimers, and Burnett and Underwood[175] isolated them by reduction at a dropping mercury electrode. However, the 4,4'-dimer (XXIX) has been suggested to be the product of polarographic reduction of $NAD^+$, and this isomer

may also result from other one-electron reductions in some cases[171] (for a review see ref. 176). At higher polarographic potentials, $NAD^+$ is reduced in variable amount to NADH[176], although other reduction products are always present. The significance of this work is that pyridine radicals are intermediates (since the first half-wave potentials are concentration and pH-independent), and these intermediates could conceivably be generated by suitable chemical oxidising and reducing agents.

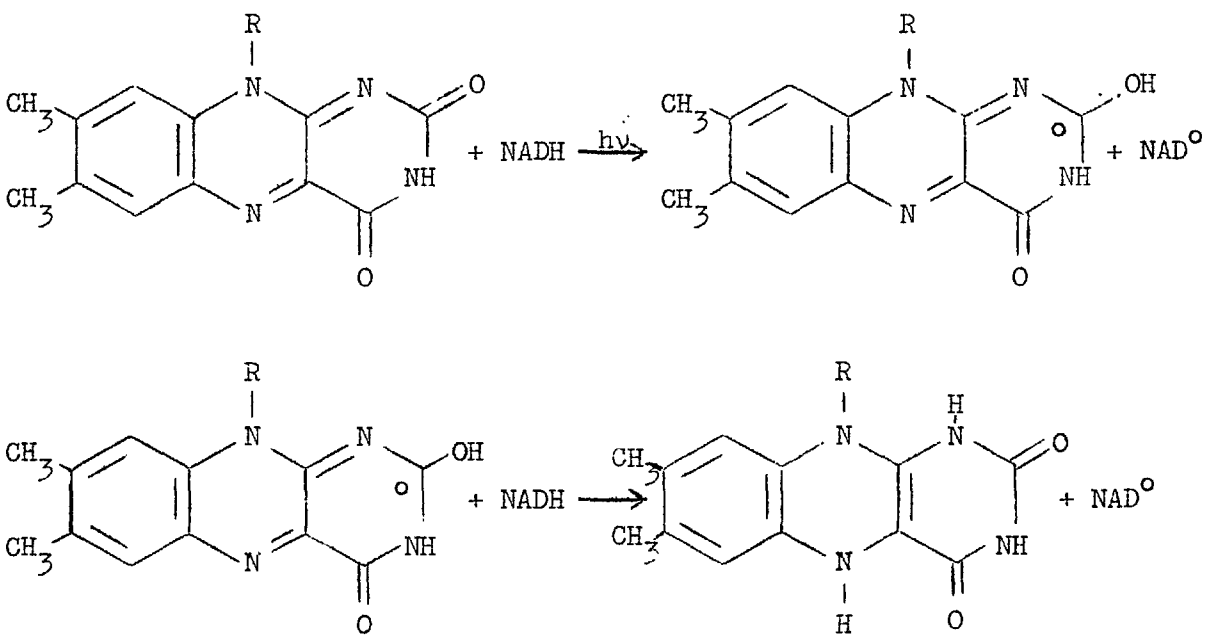
The reduction of N-methyl-3,4,5-tricyanopyridinium perchlorate by fluorenol[150] (22) has been mentioned. The yield of fluorenone is very poor despite the extremely high anion affinity of the salt, and it seems possible that the reaction may be free-radical in character. The primary reduced species may have been the 2-hydroxy-1,2-dihydropyridine, since this was rapidly oxidised in air to the corresponding 2-pyridone *via* an intermediate which exhibited an E.S.R. spectrum[150]. A similar observation was made by Wang[177] in a photochemical study of N-methylnicotinamide salts (*vide infra*).

In summary, it appears that there is some evidence for the occurrence of free-radical intermediates in redox model reactions of the coenzyme and its analogues, particularly in the presence of peroxides or participating sulphydryl groups. There is less evidence to suggest the existence of such intermediates in systems closely approximating the AdH enzyme-coenzyme-substrate reaction.

## 6. The Photochemistry of the Coenzyme and Analogues

### A) Irradiation of NADH

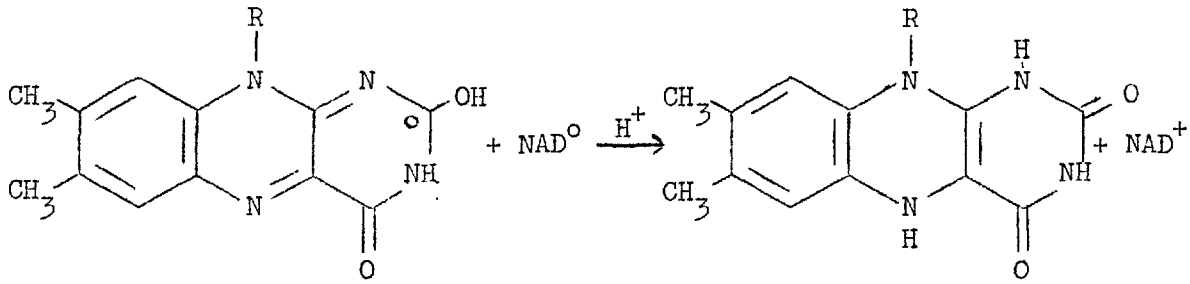
In an extension of their previous work on the aerobic, light-catalysed oxidation of nitrogen compounds by riboflavin phosphate, Frisnell and Mackenzie[178] found that NADH could be rapidly and efficiently oxidised to a product, which in turn could be chemically reduced back to NADH with dithionite. This product was assumed to be $NAD^+$ but the 4,4'-dimer of NADH has since been suggested[171]. The flavin was reduced to the colourless leuco form, and under these conditions was immediately reoxidised by the oxygen present. The role of oxygen was demonstrated by an anaerobic run. The reaction was shown to be dependent on light and the presence of riboflavin phosphate and it was concluded that a transfer of two hydrogen atoms was the major process taking place. A reaction mechanism, involving the formation of the 4,4'-dimer may be the more important, however:




$$2NAD^{\circ} \longrightarrow 4,4'\text{-dimer (XXIX)}$$

(R = Ribose phosphate)

It was suggested that both the reagents might be excited by the radiation in the first step, although the quinonoid riboflavin would seem to offer the most resonance stabilisation for initial excitation. The formation of the 4,4'-dimeric oxidation product has very recently received support from the work of Hanschmann and Berg[179]. They irradiated NADH in anaerobic solvent at 254 nm and claimed that the product was enzymically inactive and was the 4,4'-dimer. However, the "black light" fluorescent lamp used by Frisnell and Mackenzie allowed the excitation of both the riboflavin and the NADH chromophores, and if the riboflavin radical is taken to be the more stable, it will compete with $NAD^{o}$ dimer formation, to form the fully oxidised $NAD^{+}$:




This competing reaction would be less important with irradiation at 254 nm, since only the NADH chromophore is excited at this wavelength. (Fluorescence studies have indicated that the conformational overlap of adenine and nicotinamide rings in solution allows adenine excitation at 250 nm to be transmitted to the nicotinamide ring and cause characteristic fluorescence. Such coupling is not available to the riboflavin moiety, and thus the dimeric product predominates.) Further support for these ideas comes from Wagner et al[180] who obtained enzymically fully activated $NAD^{+}$ on photolytic oxidation of NADH with methylene blue or 2,6-dichlorophenol-indophenol. They

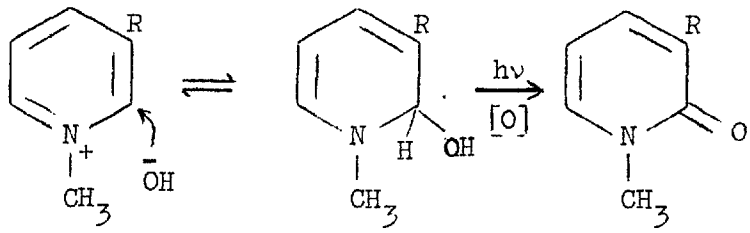
conducted their photolysis at 600-700 nm, thus precluding all direct coenzyme excitation. However, Abelson and his coworkers[181] oxidised NADH alone in phosphate and in tris buffer by photolysis at 254 nm, and in contrast to later work[179] obtained 25-60% yields of enzymically active $NAD^+$. Major by-products were the mononucleotide and riboside moieties, so the conditions here were probably more vigorous. They also found that the oxidation was accelerated or inhibited by trace quantities of acetaldehyde or ethanol respectively. Land and Swallow[102] very recently studied the inorganic oxidation of NADH by pulse X-ray radiolysis. They provide evidence that the NAD-dimer may be further attacked on prolonged photolysis and eventually oxidised to $NAD^+$. This may explain Abelson's findings. In a detailed paper they also present a method for quantitatively oxidising NADH *via* $NAD^o$ to $NAD^+$ using a halogen radical which is apparently more selective than the hydroxyl radical generated by Fenton's reagent *etc*. This work is primarily to be compared with the peroxidase system.

B) Irradiation of $NAD^+$ and Model Compounds

Runnstrom *et al*[182] first noticed in 1934 that exposure of $NAD^+$ or $NADP^+$ to light caused loss of enzymic activity. A series of workers[183-184] found that the modification to the coenzyme was specifically in the nicotinamide ring on ultraviolet irradiation, and that a product was formed which absorbed at 340 nm but was not NADH. This finding was corroborated by irradiation with X-rays[185] and $\gamma$-rays[186] in deoxygenated solutions containing ethanol, although these latter workers rather assumed that the product was NADH.

Since the photochemical changes were confined to the pyridine

ring, the most recent studies have been with simple N-alkyl pyridinium salts. The most important study has come from Wang[177] who has identified the photolysis product from both nicotinamide and nicotinonitrile methiodides as the corresponding 2-pyridones, in neutral aqueous solution. He formulated the reaction as follows (R = $CONH_2$, CN):

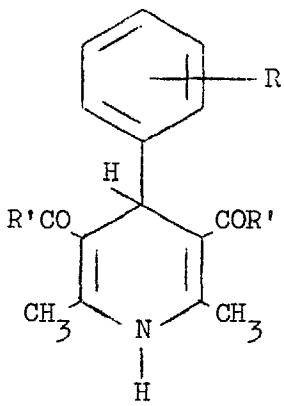



These oxidation products have absorption maxima around 325 nm, although some 4-and 6-isomer may also be present, the former absorbing at longer wave-length. The formulation of the intermediate oxygen-labile 2-hydroxy compound is reminiscent of the work of Wallenfels and Hanstein[150] who detected a free radical in this final oxidation step. No detailed mechanisms have been postulated for this stage.

C) Irradiation of NADH Models

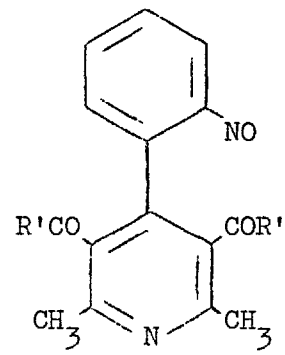
Whilst preparing a series of Hantzsch compounds, Hinkel et al[190] noticed the remarkable lability of the 2'-nitrophenyl compound (XXX) to light. Berson and Brown[188] investigated this photolysis and compared it with that of the 4'-isomer (XXXI). The latter was completely stable in sunlight or irradiation from a mercury arc

lamp, whereas the former underwent a reaction even in diffuse daylight rapidly and quantitatively. The product of reaction was shown to be the intra-molecular redox 2'-nitrosopyridine compound (XXXII).



(XXX, R = 2-$NO_2$)

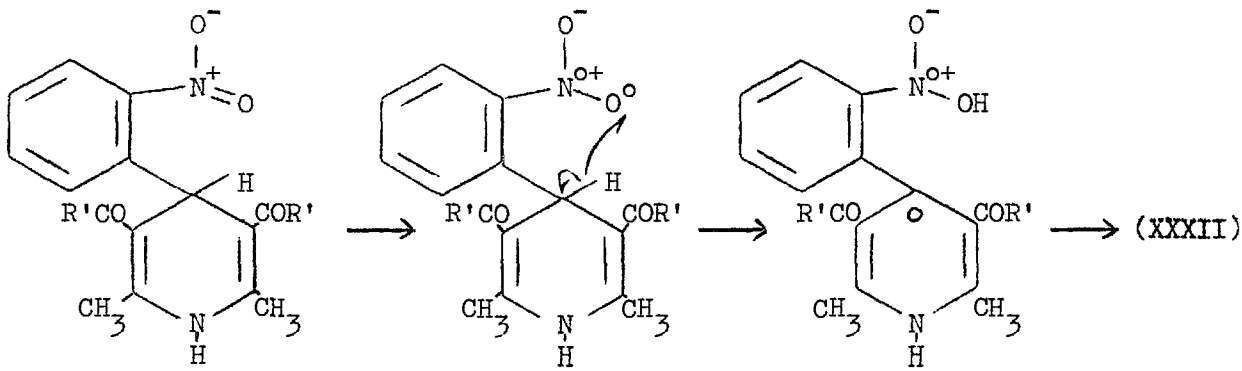
(XXXI, R = 4-$NO_2$)



R' = $CH_3$, $OCH_2CH_3$

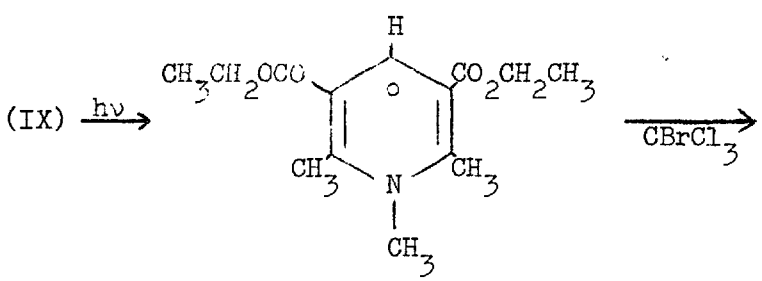
(XXXII)

The effective wavelength was around 366 nm, closely corresponding to the dihydropyridine chromophore absorption, but also to the nitrophenyl chromophore. These authors were not committed to any particular mechanism, but did not exclude the following type which involves an intermediate similar to the highly stable (XXVII).

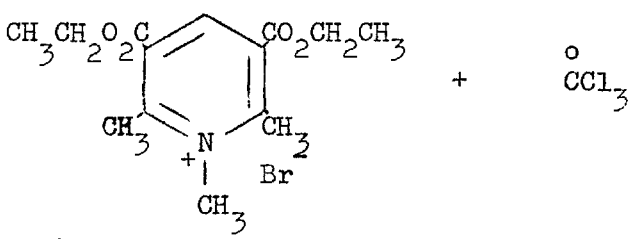


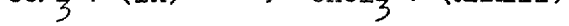
(R' = $CH_3$, $OCH_2CH_3$)

Further evidence for the existence of 4-pyridinyl radicals from Hantzsch compounds was furnished by Westheimer and his collaborators[189]. They oxidised the Hantzsch esters (VIII and IX) with bromotrichloromethane in a photolysis that was almost independent of the irradiated wavelength. They concluded that the chain initiation step could not be homolytic cleavage of the halomethane for reasons which they detailed. The reaction was certainly free-radical in nature (from a consideration of quantum yields), and thus it was necessary to invoke a pyridinyl radical as the chain initiation step. Once begun, the chain could be propagated by a number of dark mechanisms involving the trichloromethyl radical, finally giving chloroform and the pyridinium bromide salt in good yield. Deuterium tracer studies confirmed that hydrogen was transferred directly to the halocarbon, thus excluding solvent participation from the reaction.

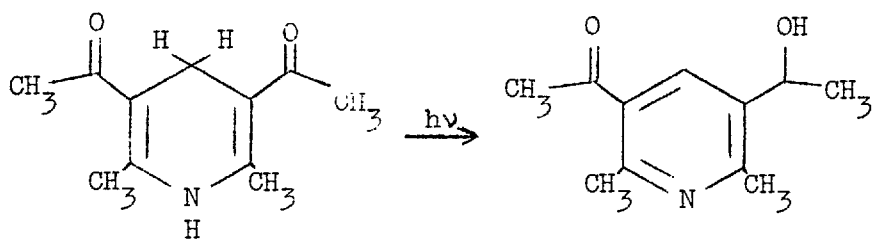


(XXXIII)





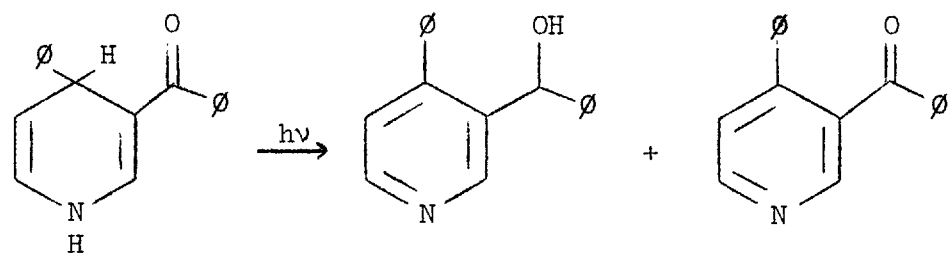
The preceeding oxidations of model compounds have all been concerned with Hantzsch compounds. Eisner and her coworkers[190] have recently investigated certain other less heavily substituted 1,4-dihydropyridines. They have found that in particular, the 2,6-dimethyl grouping inhibits dimerisation reactions, several of which they have explored. Their work highlights the idea that simple 1,4-dihydronicotinamides (including NADH) are open to several competing photolytic reactions including bond cleavage, dimer formation and disproportionation reactions. Amongst other reactions, they have reported the interesting (intramolecular) redox photolysis (25) which is not unlike Berson and Brown's reaction.



(25)

Deuterium tracer experiments have not been performed on this system, so the site of initial attack on the carbonyl group is not known, and little can be said about the possible mechanism.

A related reaction was reported earlier by Nelson and McKay[191] in which the more stabilised (XXXIV) was apparently both oxidised and autoxidised:



(XXXIV)

Evidence for the existence of 4-pyridinyl radicals of NADH and dihydropyridines has come recently both from oxidation of NADH by halogen radicals[102], and reduction of $NAD^+$[192] by solvated electrons.

In conclusion, it is evident that under certain conditions, NADH and its models can be oxidised photolytically. The extent to which these processes are relevant to the enzymic ones have yet to be assessed.

# RESULTS AND DISCUSSION

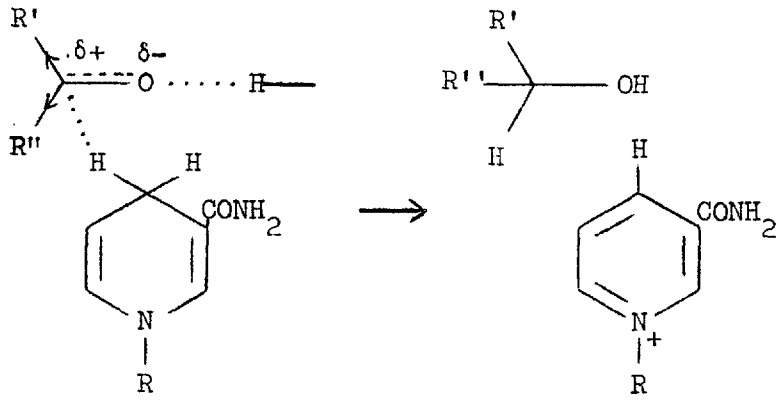
## INTRODUCTION

The mechanism of NADH-mediated enzymic reductions has received a great deal of attention through model studies involving potential substrates and 1,4-dihydropyridines. In the absence of alcohol dehydrogenase (AdH), the hydride transfer between NADH and acetaldehyde (or $NAD^+$ and ethanol) is inefficient, and the practice has been to replace normal substrates with highly activated ones in order to achieve an *in vitro* redox reaction. The majority of the work to date has centred on the oxidation of NADH or 1,4-dihydropyridines with highly activated carbonyl compounds, for two reasons. Firstly the *in vivo* reaction suggests an extremely favourable equilibrium constant ($K_s$) for this reaction[97b]:

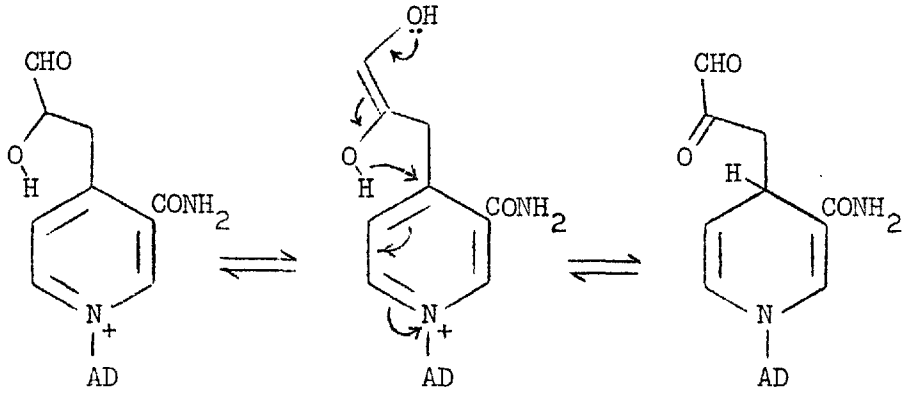
$$K_s = \frac{[NADH][CH_3.CHO][H^+]}{[NAD^+][CH_3CH_2OH]} \sim 10^{-11}$$

Thus, only one case is known[150] of the reduction of a pyridinium salt by an alcohol non-enzymatically. Secondly, the activation of the carbonyl group of the substrate analogue - ie: the generation of a partial positive charge on the carbon atom - appears to parallel the effect of the ternary complex on the naturally bound substrate. Consequently, redox reactions have been demonstrated using carbonyl groups polarised by neighbouring electron-attracting groups[128], and by hydrogen bonds[81] to the carbonyl oxygen.

Few workers have attempted to couple a substrate analogue directly to a dihydropyridine such that the reacting groups were held sterically adjacent. Such alignment of the reactants should



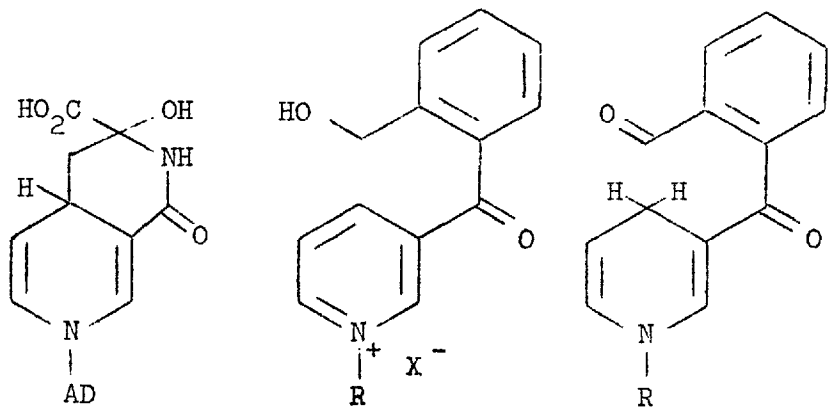
also give rise to an apparent enhancement of reactivity, since diffusion desolvation processes would be minimised (i.e. $\Delta S$ in the reaction would be small). Burton et al[193] have made a series of $NAD^+$ analogues in which the pyridine 4-position was additionally substituted. These include the potential model (I) which could conceivably undergo the rearrangement (I→II) under suitable conditions.



(I) (II)

However, there is little driving force for the reaction in this case, and the equilibrium would be predicted to lie on the side of pyridinium-alcohol. These workers did not report any unusual properties of this and related compounds. More recently, Di Sabato[117b] prepared an adduct (III) of $NAD^+$ with pyruvate, and demonstrated the reducing

properties of the complex with suitable hydride acceptors. In no case has an intra-molecular hydride transfer been shown to occur in such a complex.

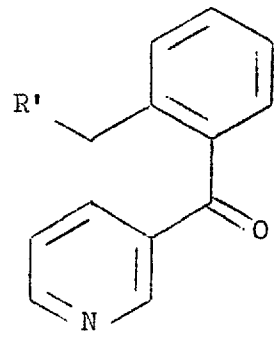



(III) (IV) (V)

It was decided to synthesise compounds (IV) and (V) which incorporate this coupled coenzyme-substrate feature in order to see whether an intramolecular redox reaction could be induced to take place in such a system. It is evident from the proposed structures that conformational alignment of the reacting sites is not constrained, but it was felt that the degree of coupling achieved here ought to increase the apparent reactivity of the sites by several orders of magnitude.

# PART I

## The Preparation and Characterisation of $NAD^+$ - Alcohol Model Compounds (IV)



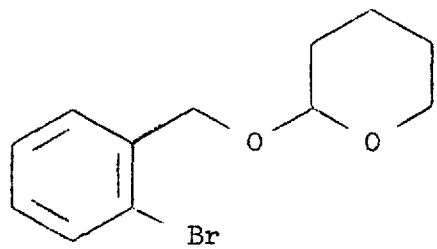

(VI, R' = OH)

(VII, R' = O.THP)

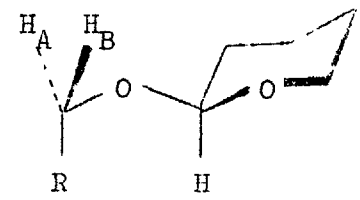
The model precursor 2-nicotinyl benzyl alcohol (VI) was prepared from nicotinaldehyde and the Grignard reagent of 2-bromobenzyl alcohol tetrahydropyranyl ether *via* a six stage synthetic route and in approximately 28% overall yield. Because of the simplicity of this reaction sequence, no other routes were investigated (eg. the condensation of pyridine-3-magnesium bromide with phthalic anhydride, followed by reduction). A series of pyridinium salts (IV) were prepared by the condensation of (VI) with the appropriate alkyl or aralkyl halide. Of these salts, the most readily characterised was the methiodide (IV, R=$CH_3$, X=I) and the stability of this compound *vis-à-vis* an intramolecular redox reaction was investigated.

(A) Preparation

2-Bromotoluene was oxidised with chromium trioxide in acetic anhydride[194] to 2-bromobenzaldehyde in 67% overall yield via the α,α -diacetate. The intermediate compound was hydrolysed anaerobically to avoid further oxidation. The aldehyde was subsequently reduced in high yield to the corresponding benzyl alcohol with lithium aluminium hydride (LAH) in refluxing ether and the product isolated as colourless needles. Protection of the hydroxyl function as the tetrahydropyranyl (THP) ether proceeded smoothly in anhydrous ether containing a trace of concd. hydrochloric acid and 2 equivalents of 2,3-dihydropyran, affording the ether (VIII) almost quantitatively.




(VIII)

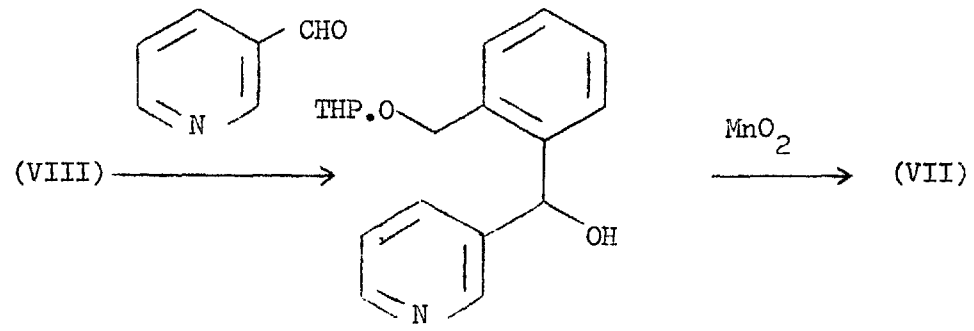



(IX)

This compound exhibited a proton magnetic resonance AB quartet centred on 5.20 τ and 5.48 τ consistent with benzylic geminal splitting (J, 13.0 Hz), and caused presumably by hindered rotation about the O-benzyl $CH_2$-bond (IX, R = 2-bromophenyl). Subsequent compounds (VII, X) containing this grouping showed similar characteristic splitting.

Initial attempts to form the magnesium Grignard with the bromo-

ether were unsuccessful, and it was decided to prepare the lithio-compound. n-Butyl lithium was prepared in good yield by the standard procedure[195] with the modifications noted. Attempts to filter the solution from lithium bromide were hazardous since positive pressure was required on the sintered glass funnel and filtration was inefficient. The precipitate settled after 24 hours, however, and the Grignard solution could be decanted off by syringe as required. The lithio THP ether formed easily and condensed with nicotinaldehyde to give 2-(α-hydroxy-3-picolyl)-benzyl alcohol THP ether (X) in 66% yield:




(X)

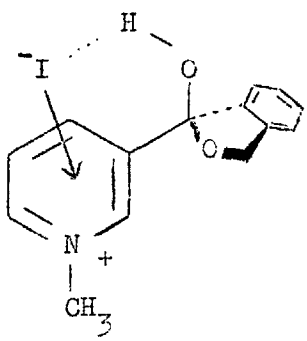
After further experimentation, the magnesium Grignard of (VIII) was made in scrupulously dry refluxing tetrahydrofuran (THF), containing iodine catalyst. With this compound, a considerably improved yield of (X) was obtained in a cleaner reaction. Manganese dioxide oxidation of this diaryl carbinol afforded (VII) in 80% amount as colourless platelets from petrol. The mass spectrum of this compound showed a weak mass ion at 297m.u.with base peak at 213 m.u. corresponding to loss of THP. An intense peak at 85 m.u. corroborated this.

Hydrolysis of the protecting group proved remarkably difficult. Stirring the ether with an equivalent of p-toluene sulphonic acid in aqueous ethanol at room temperature for 3 days had almost no effect. Refluxing the solution with p-toluene-sulphonic or mineral acid catalysts[197] resulted in modest yields of (VI) with some decomposition. A slight excess of perchloric acid in THF was more satisfactory and 50-55% yields of the required alcohol could be isolated after stirring for 3 days at room temperature. The best method was to use p-toluene-sulphonic acid (1.5 equivalents) in refluxing 60% aqueous acetone. By this technique the ether was hydrolysed in better than 70% yield after 40 minutes. Basic workup afforded the product (VI) as a colourless viscous oil which would not solidify. The p.m.r. of the benzyl $CH_2$ had collapsed to a singlet at 5.46τ , and at 6.3τ was a single exchangeable proton. an accurate mass measurement of 213.0794 and $\nu_{max}$ 3250 $cm^{-1}$ were also in accord with the expected 2-nicotinyl benzyl alcohol (VI).

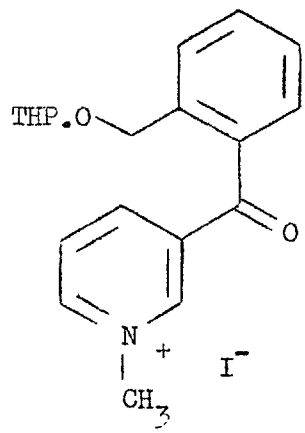
The N-benzyl bromide model salt (IV, R=$CH_2Ø$, X=Br) was readily prepared in high yield by refluxing the free base (VI) with a slight excess of benzyl bromide[148] in dry acetone for 4 hours. This solvent had the advantage that this and subsequent products often crystallised directly from the reaction mixture, although several side reactions of pyridinium salts with acetone have been reported[198]. Surprisingly, the benzyl bromide salt was a hygroscopic powder, and attempts to crystallise it from a variety of solvents resulted in intractible gums. Concentrated aqueous solutions were accordingly treated with potassium iodide, picric acid and picrolonic acid respectively in an attempt to render the com-

pound crystalline by anion exchange. These attempts failed.

The N-methyl-p-toluene sulphonate and N-methiodide salts were consequently prepared, by stirring the respective reagents in dry benzene at room temperature. The former compound was semi-solid and could be recrystallised from ethanol-chloroform, but it reverted to a semi-solid mass on standing. The N-methiodide (IV, R=$CH_3$, X=I) was by contrast highly crystalline m.p. 281-2° and pale yellow, as is characteristic of these compounds, which have been shown to be charge-transfer salts[121b]. The infra-red spectrum showed an intense sharp band at $\nu_{max}$ 3200 $cm^{-1}$ and a medium intensity sharp band at $\nu_{max}$ 1640 $cm^{-1}$. The absence of an absorption around 1680 $cm^{-1}$ implied that a lactol had formed, and the sharpness and position of the hydroxyl stretching frequency suggested hydrogen bonding - perhaps to the bound iodide ions (XI).




(XI)




(XII)

The ultraviolet spectrum showed $\lambda_{max}$ (water) 225, 264 nm, which is typical for N-alkyl nicotinyl salts indicating an open structure in aqueous or ethanolic solution. The p.m.r. spectrum was taken in $D_2O$ on a 100 MHz spectrometer, and the benzyl-$CH_2$ group was a

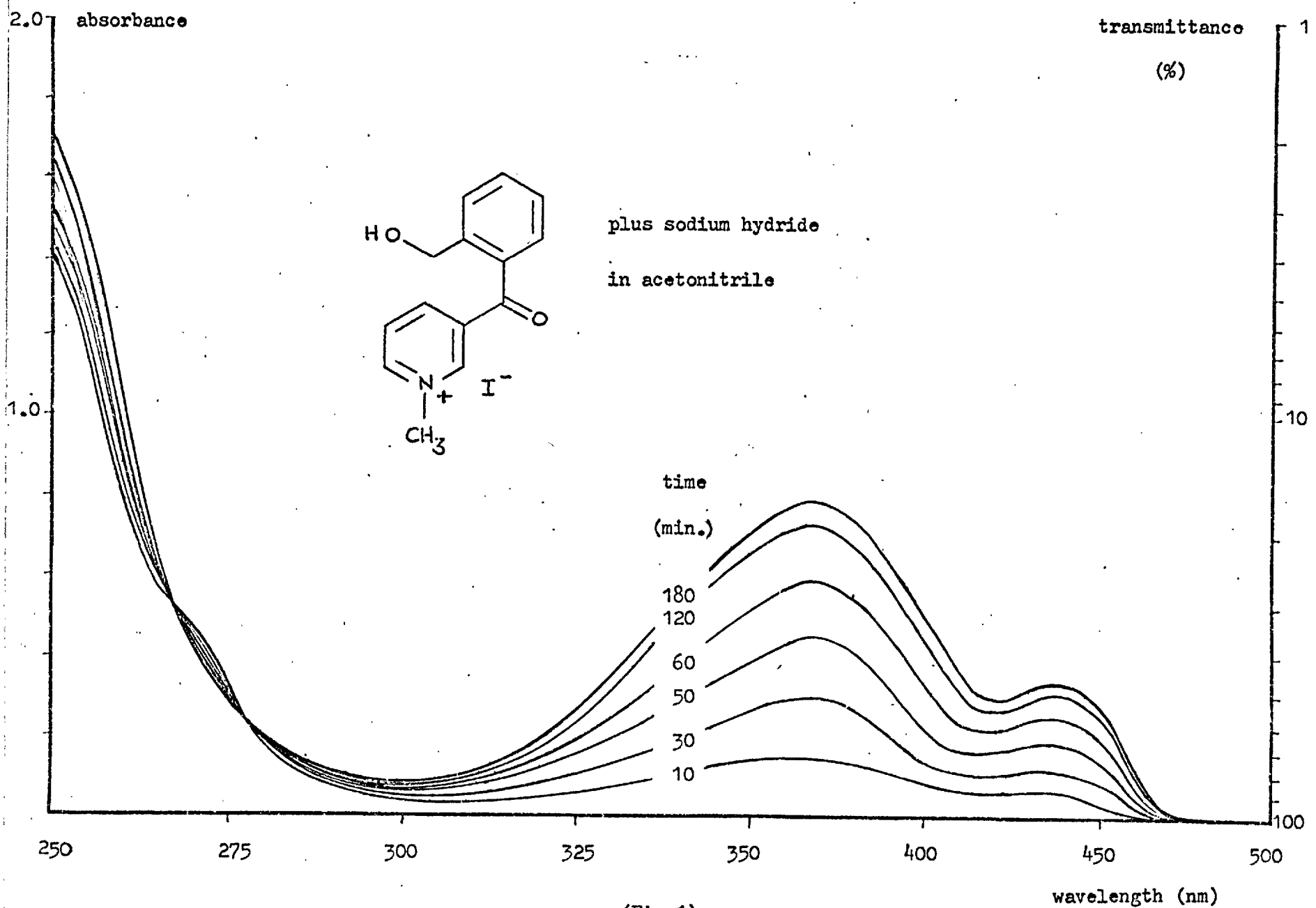
sharp singlet, also indicating an open structure. The analysis was in accord with the required compound.

For comparison, the methiodide (XII) of the pyridine THP ether (VII) was prepared and crystallised from ethanol mp. 171°. The infra-red spectrum showed two bands at $\nu_{max}$ 1681, 1637 $cm^{-1}$ as predicted. The p.m.r. and ultra-violet spectra were otherwise very similar to those of the alcohol-methiodide.

(B) Properties of (IV, R=$CH_3$, X=I)

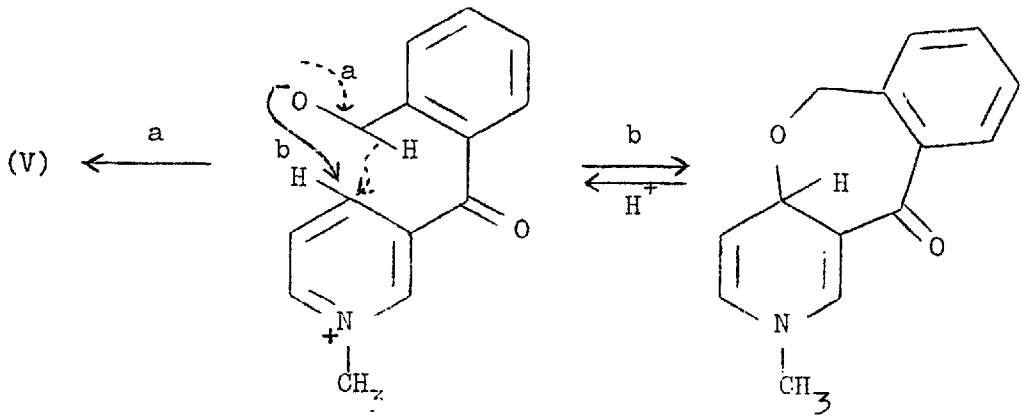
The model $NAD^+$-alcohol salt was completely stable under ordinary conditions, and showed no tendency to form a dihydropyridine whatsoever. Thus solutions of the salt in a variety of polar solvents were indefinitely stable at room temperature, as measured by increase in absorption around 350 nm. Similarly solutions refluxed aerobically in ethanol for 1 week, or heated in DMF at 140° for 48 hours showed no change. The melting point (281-2°) of the compound was sharp, and the melt was colourless at 300° under nitrogen Finally dilute mineral acid and base had no effect on the ultra-violet chromophore.

To a solution of the compound in dry acetonitrile in a quartz cuvette was added a slight excess of sodium hydride in the minimum amount of the same solvent, and the reaction followed spectrophotometrically over 3 hours, by which time the new bands at 368 nm and 435 nm had reached a maximum (Fig 1). Removal of the alcohol proton could conceivably result in one or other of the mechanistic paths (XIIIa or b) both of which would result in the appearance of dihydropyridine-like chromophores. Attack at the ring 2-position


absorbance
2.0
1.0
transmittance
(%)
1
10
100
HO
O
N+
I-
CH3
plus sodium hydride
in acetonitrile
time
(min.)
180
120
60
50
30
10
250
275
300
325
350
400
450
500
wavelength (nm)

(Fig 1)

could occur analagously.




(XIII) (XIV)

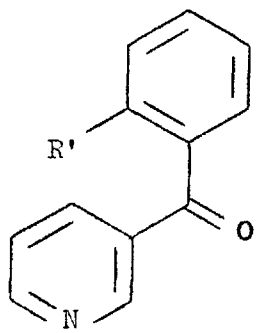
Addition of a proton source to the reaction product immediately decolourised it, and on workup, the original pyridinium salt was isolated ($m_p$. , mixed mp.). This suggested that reaction sequence b was the preferred path, since the aldehyde dihydropyridine (V) would not be expected to undergo the reverse process so rapidly. This was confirmed by later synthesis of compounds (V) as stable, isolable crystalline solids.

The appearance of the 435 nm band indicated the competing reaction of attack of the alcoholate anion on the ring 2 position. The position of this band is in agreement with previously reported 1,2-dihydropyridines[21,199]. Since the extinction coefficients of 1,2- and 1,4-dihydropyridines are generally approximately the same[199], it follows from (Fig 1 ) that attack at the 4-position is preferred by a factor of 2 over attack at the 2-position. This is to be expected from steric considerations. More bulky groups on the nitrogen should therefore increase the preponderance of the conformer required for the redox reaction at the 4-position.

## PART II

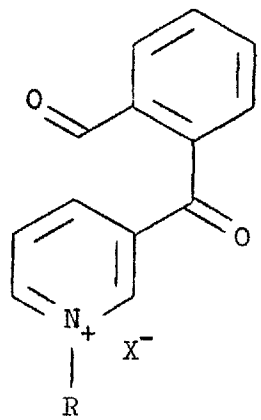
## The Preparation and Characterisation of NADH-Aldehyde Model Compounds (V)

Several routes to the model precursor (XV) were investigated including oxidation of (VI). A more direct route, and the one finally chosen involved the Grignard coupling of 2-bromotoluene with nicotinaldehyde to give (XVI) after allylic oxidation. Chromic anhydride oxidation of the methyl group followed by N-quaternisation yielded (XVII) which could be reduced to (V) with sodium dithionite.




(XV, R'= CHO)

(XVI, R'= $CH_3$)




(XVII)

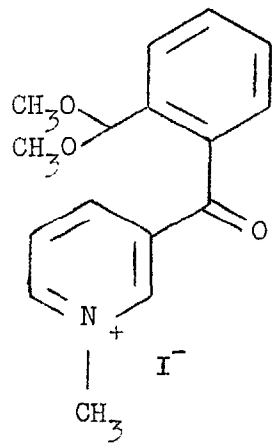
The preparation of important related compounds is presented hereunder.

### (A) Preparation

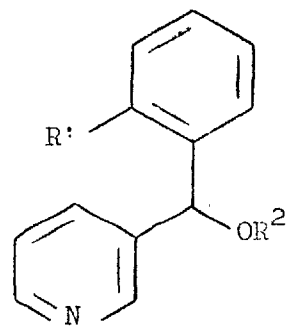
A small portion of 2-nicotinyl benzaldehyde (XV) was prepared by direct manganese dioxide oxidation of the corresponding benzyl alcohol (VI). The product crystallised from benzene-petrol in 65%

yield as colourless plates. The spectral data ( $\nu_{max}$ 1690, 1675 $cm^{-1}$, $\tau$ 0.07) and analysis were in accord with the required aldehyde.

A portion of this compound was stirred with methyl iodide in an attempt to make the aldehyde salt (XVII, R=$CH_3$, X=I).



(XVIII)



(XIX, R' = $CH_3$, $R^2$= H)
(XX, R' = $CH(OAc)_2$, $R^2$= Ac)
(XXI, R' = CHO, $R^2$= Ac)

The resulting pale yellow crystalline solid was recrystallised from ethyl acetate, but the p.m.r. spectrum, although similar to that of the alcohol salt (IV), contained 2 additional 3-proton singlets at 6.44 and 6.77 $\tau$ respectively, which were assigned to the methyl groups of a dimethyl acetal (XVIII). The analytical data showed the carbon content to be 0.37% low, but was otherwise in agreement with this structure. The reaction was consequently repeated with dry reagents, diluted in benzene, and with only a small excess of methyl iodide, to prevent acetal formation, but a series of runs produced starting material with varying amounts of (XVIII). The difficulty in making (XVII, R=$CH_3$, X=I) coupled with the fact that

N-methyl dihydropyridines tend not to be solids, prompted the preparation of alternative pyridinium salts.

In order to prepare sufficient pyridine-aldehyde (XV) for these experiments, a more direct route, via the pyridine-toluene (XVI) was investigated. 2-Bromotoluene magnesium Grignard was prepared as before and condensed smoothly with nicotinaldehyde in warm THF to give the carbinol (XIX) in 80% yield as a highly crystalline product, mp. 129-30°. Infra-red absorption at $\nu_{max}$ 3200 $cm^{-1}$ and p.m.r. singlets at 7.66 τ (3H), 5.23 τ (1H, exchangeable with $D_2O$) and 4.01 τ (1H), corresponding to methyl, hydroxyl and benzylic protons respectively, confirmed the structure, which was further corroborated by analysis.

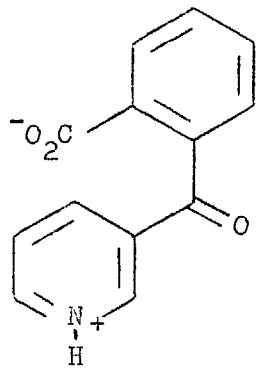
Various oxidising conditions were applied to this compound in an attempt to prepare the keto-aldehyde (XV) in one step. Chromium trioxide in acetic anhydride at 5° gave the triacetylated product (XX) in moderate yield as a crude colourless liquid. This compound partly hydrolysed on an alumina column to give 2-(α-acetoxy-3-picolyl) benzaldehyde (XXI) in 28% yield based on starting material $\nu_{max}$ (liquid film) 2760, 1740 and 1695 $cm^{-1}$, τ 0.20. The picrate derivative formed easily, and its combustion analysis was correct for the assigned acetoxy benzaldehyde structure. More vigorous hydrolysis of the intermediate triacetate gave 3 products as shown by t.l.c., none of which corresponded (infra-red)to the benzaldehyde-alcohol or acetate. The major component at Rf 0.25 (acetone-benzene 5:95) was tentatively considered to be an anthrone derivative since it contained no hydroxyl or aldehyde groups (p.m.r.) but exhibited 2 strong carbonyl absorptions $\nu_{max}$ (liquid film) 1715, 1675 $cm^{-1}$.

Ceric ammonium nitrate[203] oxidation of the alcohol-toluene (XIX) afforded small quantities of the keto-toluene (XVI), and selenium dioxide in anaerobic refluxing chlorobenzene[204] produced similar amounts of the keto-aldehyde (XV) although competing thermal decomposition was an important factor in the latter case.

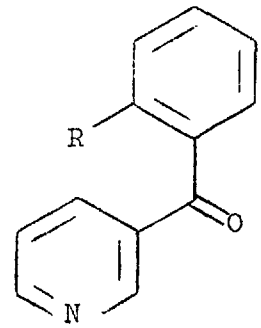
From these results, it was decided to perform the oxidation in two stages, by oxidation of the hydroxyl group, followed by the methyl group. Manganese dioxide oxidation of the hydroxyl group afforded the expected ketone (XVI) in virtually quantitative yield after stirring for 8 hours in chloroform. Unlike the alcohol, this compound would not crystallise, and combustion analysis was conducted on the picrate derivative. The infra-red spectrum contained a single carbonyl absorption at 1670 $cm^{-1}$, and no hydroxyl stretch. The p.m.r. spectrum showed that only aromatic protons and 1 methyl group (which had shifted to $\tau$7.59) was present.

Nishimura's standard oxidation of toluenes to $\underline{\alpha},\underline{\alpha}$-diacetates with chromic anhydride[194] was again employed to oxidize the keto-toluene (XV). If the reaction temperature was maintained at 5-10°[194] there was considerable further oxidation of the diacetate, and on hydrolysis of the crude, intermediate reaction product, the major component was the corresponding 2-nicotinylbenzoic acid betaine (XXII)(31%). The infra-red spectrum of this internal salt contained bands at 2500 and 1910 $cm^{-1}$ (broad), besides the carbonyl stretch at 1680 $cm^{-1}$. The p.m.r. of the carefully neutralised compound in $D_2O$ was featureless, the broad aromatic absorptions could be assigned to typical pyridine or pyridinium positions. The ultra-violet spectrum showed only very weak end absorption $\lambda_{max}$ 233 nm ($\epsilon$ ~520). The salt was crystallised from ethanol and analysed correctly for

(XXII).



(XXII)



(XXIIIa, R = $CO_2CH_3$)
(XXIIIb, R = $CO_2CH_2CH_3$)
(XXIV, R = $CH(OAc)_2$)

Repeating the reaction at $-5^{\circ}$ during addition of oxidant, coupled with efficient stirring to avoid local excess of oxidant resulted in greatly improved yields of the required α,α -diacetate (XXIV), with only small amounts of contaminating mixed anhydride. Purification of this intermediate was not attempted, and it was hydrolysed directly. Acid hydrolysis with p-toluene sulphonic acid in refluxing aqueous methanol, or with mineral acid in methanol at room temperature resulted in moderate yields of the keto-aldehyde (XV) after column chromatography. One equivalent of sodium in absolute ethanol or methanol reacted rapidly with the diacetate at room temperature, however, and a 65% yield of aldehyde was recorded after purification on a column. Rapid chromatography was necessary, since the compound was found to decompose slowly on alumina, a negligible amount remaining after 24 hours.

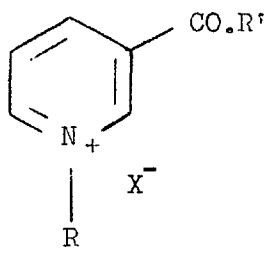
The major by-product from the base-catalysed hydrolysis in ethanol or methanol was the corresponding methyl (XXIIIa) or ethyl (XXIIIb) ester respectively. Both these compounds were stable,

highly crystalline compounds and their structures were confirmed by spectral and analytical data. A sample of the methyl ester was also prepared from the betaine (XXII) for comparison.

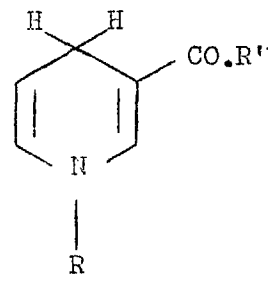
Smaller quantities of the model precursor (XV) were prepared from by-products of the reaction route. Thus the combined esters were easily reduced to the corresponding diol with excess LAH in THF. Oxidation of the reaction product with manganese dioxide gave (XV) in 70% overall yield.

A series of N-alkyl and N-aralkyl salts (XXV) were prepared from nicotinamide ($R'=NH_2$) and ethyl nicotinate ($R'=OCH_2CH_3$) as simple analogues of the required salts (XVII). Benzyl chloride, benzyl bromide, methyl iodide and 2,6-dichlorobenzyl bromide all reacted rapidly and quantitatively with nicotinamide in refluxing acetone or methanol, to form the corresponding known [148,200] quaternary salts which could be recrystallised from ethanol or methanol. The 2,6-dichlorobenzyl bromide salts were of particular interest for 2 reasons. Firstly it was hoped that the bulky nature of the group would contribute to the conformational alignment of the pyridine 4-position with the aldehyde group in (XVII, R=DCB). Secondly, the electronic influence of this grouping on the final dihydropyridine model (V) should be similar to the effect[71] of the ribose grouping in the *in vivo* coenzyme system. This aryl halide was consequently made from 2,6-dichlorotoluene by bromination with N-bromosuccinimide[201] in the usual way. Several of the corresponding pyridine salts were subjected to anion exchange with potassium iodide or sodium perchlorate. The products were also highly crystalline easily characterised compounds.

By contrast, the N-benzyl and N-2,6-dichlorobenzyl salts of 2-nicotinyl benzaldehyde (XVII, R=$CH_2Ø$, R=DCB respectively) formed as pale yellow amorphous solids which could not be induced to crystallize. They were hygroscopic and difficult to handle, and attempted recrystallisation resulted in gums. Stirring with cold ether regenerated the amorphous solid. After initial difficulty, the dichlorobenzyl salt was obtained analytically pure, and infra-red and p.m.r. spectra were in agreement with the predicted structure. These salts would not form mulls with nujol so the infra-red spectra were recorded as KBr discs. Anion exchange of aqueous solutions of the salts with iodide, perchlorate, picrate and picrolonate produced further gums or amorphous solids, and thus complete characterisation of the compounds was not attempted. For comparison the DCB salt (XXV, R'=o-Ø.$CO_2CH_3$) of the methyl ester (XXIIIa) was prepared. This was a crystalline solid which easily recrystallised from ethanol as colourless prisms and was fully characterised.




(XXV)

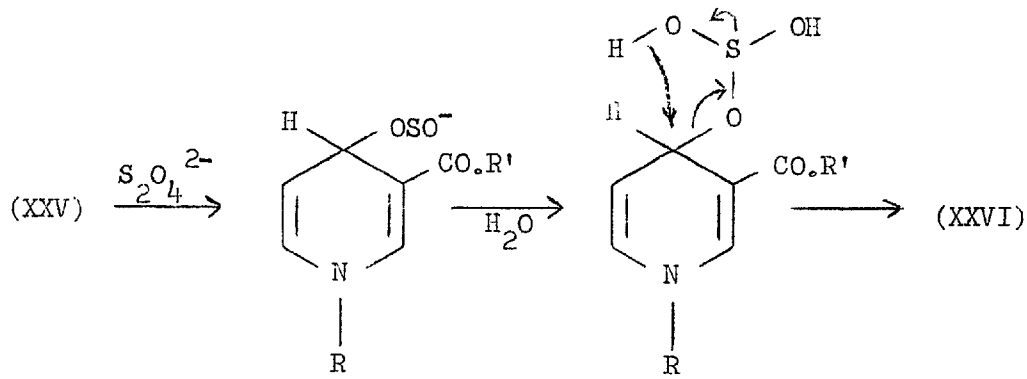



(XXVI)

The analysis and p.m.r. confirmed the presence of a bound molecule of ethanol of crystallisation. In $CDCl_3$, the p.m.r. spectrum exhibited absorptions at τ 8.80 (3H triplet), 6.34 (2H quartet) and

7.97 (1H singlet) all of which disappeared on shaking with 2 aliquots of $D_2O$. The integration and combustion analysis were in agreement with a 1:1 ratio of ethanol:salt.

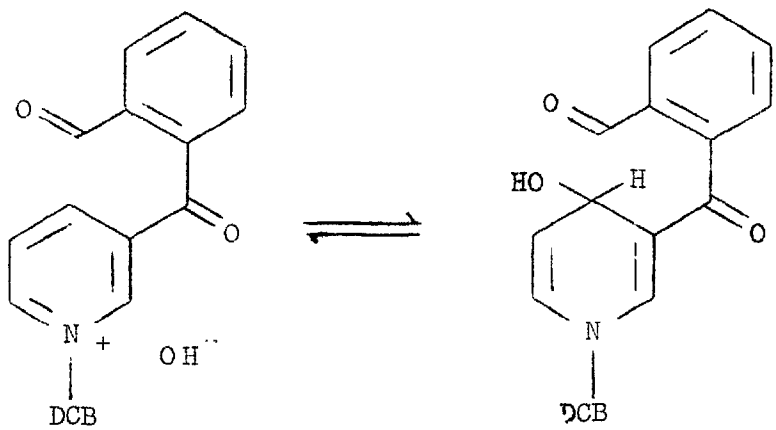
Sodium dithionite in aqueous medium has long been known to reduce quaternary nicotinyl salts almost exclusively to the corresponding 1,4-dihydropyridine isomers (XXVI). It is unique in this respect, and surprisingly very little work has been done on the best conditions for reduction. The mechanism of reduction has long been disputed[126,140-4], but from recent corroborated work[142-4] probably involves the intermediate (XXVII).




(XXVII)

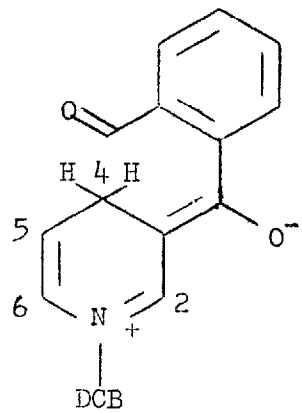
If the breakdown of the 4-sulphinate proceeds as shown, this will be acid catalysed. However, sodium carbonate or hydroxide have generally been used to buffer the reducing medium[152,25]. The pH must be kept above 7, since acid-catalysed 5,6-addition to 1,4-dihydropyridines is well-documented[201]. The intermediate sulphinate has been shown to be quite stable in strongly basic solution, and has been isolated and characterised[144]. It was found in the current work that the use of sodium bicarbonate buffer[205] resulted in rapid breakdown of this intermediate, and in general better yields of the

dihydropyridines were obtained than reported elsewhere. In the preparation of the more complex dihydropyridines required in this study, it was found necessary to buffer only the reducing medium. Addition of aqueous bicarbonate or carbonate to solutions of the salts (XVII) caused the formation of a yellow colouration and eventually a yellow gummy solid. This was attributed to reversible attack of hydroxyl anion in the 4-position of the ring.




The formation of this addition compound interfered with the dithionite reduction, and was avoided by adding the well-buffered reducing agent solution in one lot to a well-stirred solution of the salt in faintly acidic medium. Reduction was considerably faster than addition under these conditions. By this technique, a 64% yield of the model dihydropyridine aldehyde (V, R=DCB) was prepared. The yellow powdery solid was recrystallised with some difficulty from oxygen-free benzene, in the absence of light, and stored at $0^{\circ}$ under dry nitrogen. When freshly made, it was lemon-yellow and fluorescent, but rapidly went off in the air and light to a buff-coloured solid. The ultra-violet spectrum showed $\lambda_{max}$ 378 nm ($\varepsilon$ 10,100 ) - considerably higher than the N-dichlorobenzyl-1,4-

dihydropyridines of nicotinamide (XXVI, R'=$NH_2$; $\lambda_{max}$ 350 nm)[155] or of ethyl nicotinate (XXVI, R'=$OCH_2CH_3$; $\lambda_{max}$=352 nm)[155]. This is to be expected from the increased stabilisation afforded the resonance structure (XXVIII).




(XXVIII)

Table 1

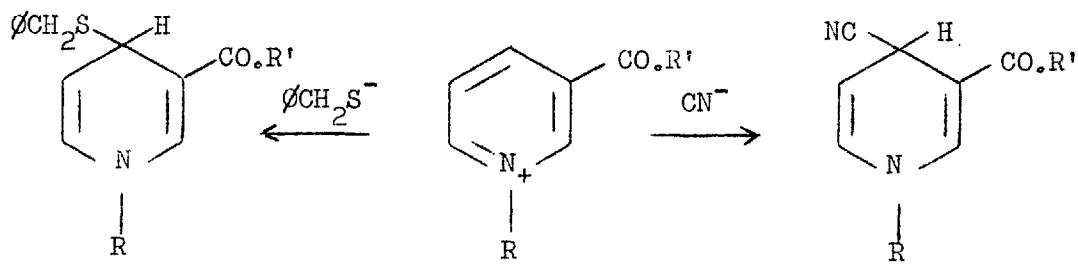
| Dihydropyridine Proton (τ) | 2 | 4 | 5 | 6 |
|---|---|---|---|---|
| (XXVI, R=DCB, R'=$NH_2$) | 2.75 | 6.88 | 5.23 | 4.12 |
| (V, R=DCB) | 3.43 | 6.76 | 5.01 | 4.13 |

The infra-red spectrum exhibited carbonyl absorptions at $\nu_{max}$ 1705 and 1675 $cm^{-1}$. The p.m.r. spectrum contained the typical 1,4-dihydropyridine splitting pattern, with the exception of a marked shift in the 2-proton position (Table 1) and an aldehyde proton singlet at τ 0.01. The compound analysed correctly and accurate mass ion measurement was 371.0464 m.u. (calc$^{d}$ 371.0480) for the $^{35}Cl$ isotope. The DCB group complicated the mass spectrum of this and related dihydropyridines, the peak height ratio for

$^{35}Cl_2$:$^{35}Cl^{37}Cl$:$^{37}Cl_2$ isotopes being 57:37:6.

The properties of this model NADH-aldehyde compound are discussed in section (B), and its remarkable light sensitivity in Part III. Initial experiments indicated that a light-catalysed internal rearrangement occurred which resulted in the formation of the corresponding $NAD^+$-alcohol system (IV). In order to verify this, it was necessary to characterise the salts produced after photolysis, and these were already known to be difficult to handle. The remainder of this section deals with attempts made to convert pyridinium salts to easily characterisable derivatives in high yield.

The pyridinium-alcohol corresponding to the redox product of (V, R=DCB) was (IV, R=DCB, X=Br). This latter compound was prepared in the usual way, and was isolated in 86% yield as a crude hygroscopic powder which could not be induced to crystallise under a variety of conditions. The iodide and perchlorate salts were similarly non-crystalline. 1,4-addition compounds of pyridinium salts are well-known[118], and amongst these, the mercaptide and cyanide ions form strong complexes:




(XXV) (XXIX)

A solution of benzyl mercaptan in benzene consecutively treated with sodium hydride and the salt (IV, R=DCB, X=Br) gave a red oily product which would not crystallise, although it was almost certainly the required compound (ultra-violet and p.m.r. spectra). Since the aim of the work was to find a highly crystalline derivative, this method was abandoned. The cyanide complex (XXIX, R=DCB, R'=o-Ø.$CH_2OH$) also formed as an impure yellow gum in 20% yield and was positively identified by its ultra-violet spectrum $\lambda_{max}$ 327 nm and accurate mass measurement. By contrast, the simple cyanide addition compound (XXIX, R=DCB, R'=$OCH_2CH_3$) formed in 70% yield as a highly cyrstalline compound which was fully characterised with ease. These results suggested that the o-substituted phenyl group effectively crowds the pyridine 4-position, and thus that the DCB grouping is an important factor in forming the required conformational orientation of these molecules. In view of this, it was decided simply to reduce the salt with sodium dithionite to the dihydropyridine (XXX) as the required derivative.

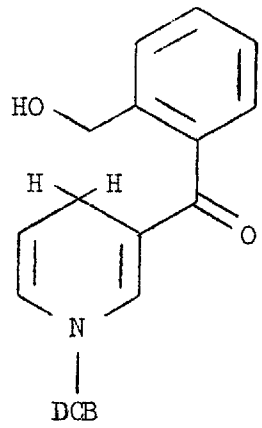
The alcohol-dihydropyridine (XXX) was consequently prepared in 67% yield by dithionite reduction of the corresponding pyridinium salt (XXV, R=DCB, R'=o-Ø.$CH_2OH$). It could also be made by selective reduction of the model aldehyde (V, R=DCB) with sodium borohydride. The infra-red spectrum exhibited a carbonyl stretch at 1670 $cm^{-1}$ and hydroxyl stretch at 3300 $cm^{-1}$. The ultra-violet spectrum ($\lambda_{max}$ 374 nm) and p.m.r. were similar to those of the model system (V, R=DCB). The 2-pyridine proton $\tau$ 3.18 was intermediate in chemical shift between those of the model and simple dihydronicotinamide (Table 1) as expected for the reduced resonance

contribution (e.g. XXVIII) in this compound. The mass spectrum is considered in detail in the next section. Although the yield from this reduction was not high, it was sufficiently good to enable the salt photolysis-product to be identified and characterised by mass spectral analysis. The preparation of 2 deuterium isotopes of this compound are also discussed in the following section.

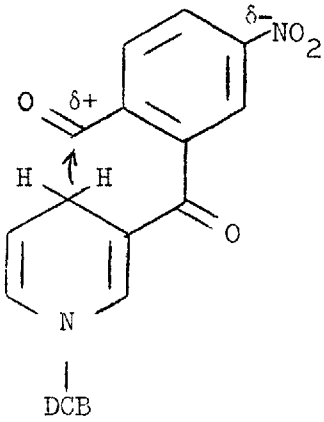
B) Properties of the Model (V, R=DCB)

The compound was found to be completely stable in the solid state and in neutral solvents in the absence of light or air. Aerobic oxidation of the aldehyde grouping appeared to be facile, the resulting acid adding on to the 6-position of another pyridine ring intermolecularly to form polymeric products. The compound could be purified by rapid chromatography on alumina and was stored at $0^{\circ}$ under nitrogen. It was not greatly affected after refluxing in benzene or ethanol for 72 hours in the dark and under nitrogen, the 378 nm absorption having decreased ~25% during this period. An attempted thermal rearrangement in refluxing pyridine containing a trace of concentrated hydrochloric acid resulted in a complex mixture of products along with unchanged starting material. The aqueous extract of the reaction was worked up by sodium dithionite reduction and the products examined for the rearranged alcohol-dihydropyridine, but no band corresponding to this compound could be observed by t.l.c.

It was clear by this stage that a direct hydride transfer between the two groupings in the model system did not occur under ordinary conditions. It is possible that such a transfer might occur with the system (XXXI).




(XXX)




(XXXI)

It was thought that sufficient polarisation of the carbonyl group might also be effected by simple protonation, and various acidic media were investigated as potential protonating agents which would not add to the 5,6-double bond. The results are summarised in Table 2. The "half-lives" refer to the time required to halve the intensity of the ultraviolet absorption maximum at ~ 350 nm. In each case, loss of this chromophore was concomitant with the appearance of the characteristic 1,4,5,6-tetrahydropyridine chromophore at ~ 290 nm.

These results showed the instability of 1,4-dihydronicotinamides below pH 6.8, and also that the model system was equally susceptible to 5,6 addition. Attempts to activate the model by protonation were consequently abandoned.

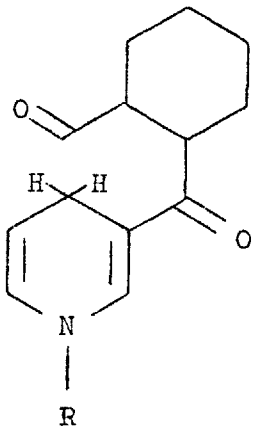
It is likely that the resonance stability afforded the mesomeric structure (XXVIII) is a major factor in impeding the transfer of hydride ion in this system (see Review). Replacement of the benzene ring by the cyclohexane or better, piperidine analogues

Table 2

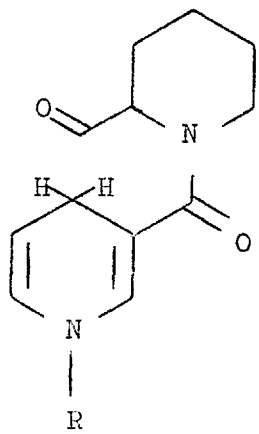
Stability of 1,4-dihydropyridines (XXVI) to 5,6 acid catalysed attack

| R | R' | Solvent | Acid (trace) or buffer | Half Life (mins) |
|---|---|---|---|---|
| DCB | $NH_2$ | Abs. methanol | HCl | 3.5 |
| DCB | $NH_2$ | Abs. methanol | $HClO_4$ | < 0.5 |
| Benzyl | $NH_2$ | Abs. methanol | HCl | 1.5 |
| Benzyl | $NH_2$ | Abs. methanol | glac AcOH | < 0.5 |
| Benzyl | $NH_2$ | dry benzene | $HClO_4$ | 40 |
| Benzyl | $NH_2$ | dry chloroform | $BF_3.Et_2O$ | very fast |
| Benzyl | $NH_2$ | 50% methanol | Buffer pH 4.8 | 30 |
| Benzyl | $NH_2$ | 50% ethanol | Buffer pH 4.8 | 41 |
| Benzyl | $NH_2$ | 50% methanol | Buffer pH 6.8 | 6 hours |
| Benzyl | $NH_2$ | 50% methanol | Buffer pH 8.5 | stable |
| DCB | $\underline{o}$-$C_6H_4$.CHO | 50% methanol | Buffer pH 4.8 | 55 |

would result in models which are more closely related energetically to bound NADH-substrate. Thus for the current system $\lambda_{max}$ is 378 nm which represents a considerably more reactive species. In contrast, systems (XXXII) and (XXXIII) would be predicted to have absorption maxima at 360 nm and 335 nm respectively, the latter being an almost idealised model compound.



(XXXII)



(XXXIII)

Although a hydride transfer was not observed with either class of redox isomers investigated, an interesting photolytic rearrangement occurs with (V, R=DCB) which will be discussed below.

## PART III

### Photolysis of NADH - Aldehyde Model System (V, R = DCB)

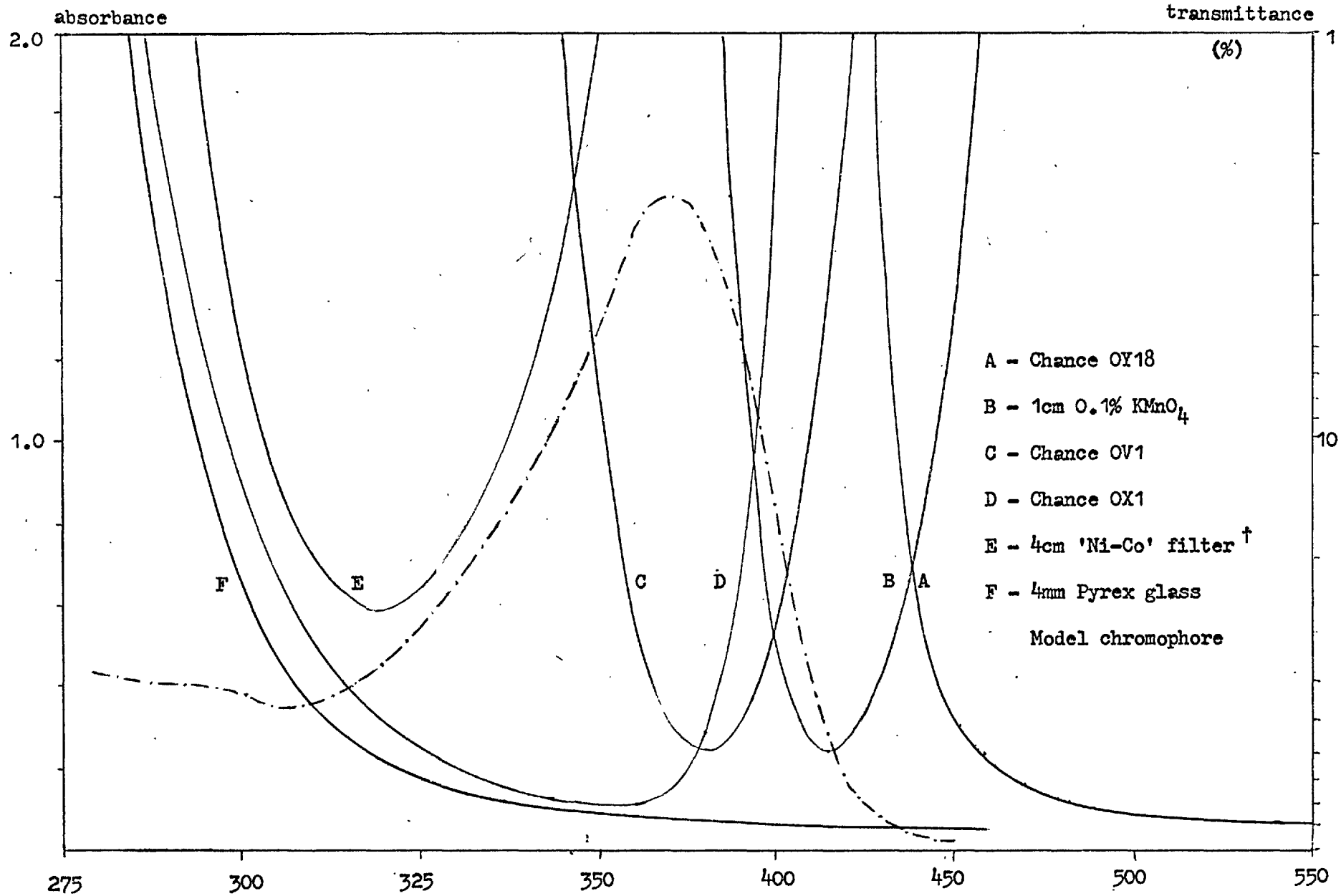
It has been noted that fresh samples of the model compound deteriorated rapidly on exposure to air. It was found that an anaerobic specimen also deteriorated in the prescence of daylight to give a buff-coloured non-fluorescent solid. More particularly, a solution in dry chloroform under nitrogen had lost the characteristic 378nm chromophore after 2 days on the bench in diffused daylight. Workup of this solution gave a very small amount of water-soluble material with a chromophore very similar to thos of pyridinium salts.

The absence of reaction on heating a solution in the dark, coupled with the rapid effect of a medium-pressure mercury lamp at 15° confirmed that the reaction was photolytic. It was consequently repeated on a larger scale and in benzene solution, the compound being photolysed at 14° under nitrogen for about 6 hours by which time the loss of the 378nm maximum was virtually complete. The chromophore change was complex, and no isosbestic points were observed. The solution was worked up for water-soluble products which were treated with aqueous dithionite to reduce any pyridinium salts to the corresponding 1,4-dihydropyridines. The crude mixture (which contained a large number of products) was purified by preparative scale chromatography and a major component was isolated (Rf 0.3, methanol-benzene, 5:95). This compound was fluorescent, exhibited an absorption maximum at 374nm and had a mass spectrum almost identical to that of the hydroxymethylene compound (XXX).

In order to demonstrate that this compound could not arise by simple dithionite reduction of the aromatic aldehyde group in (V), a solution of benzaldehyde in aqueous ethanol was treated with a large excess of aqueous dithionite at 40° for 4 hours. No benzyl alcohol could be detected by t.l.c. on workup of the reaction mixture.

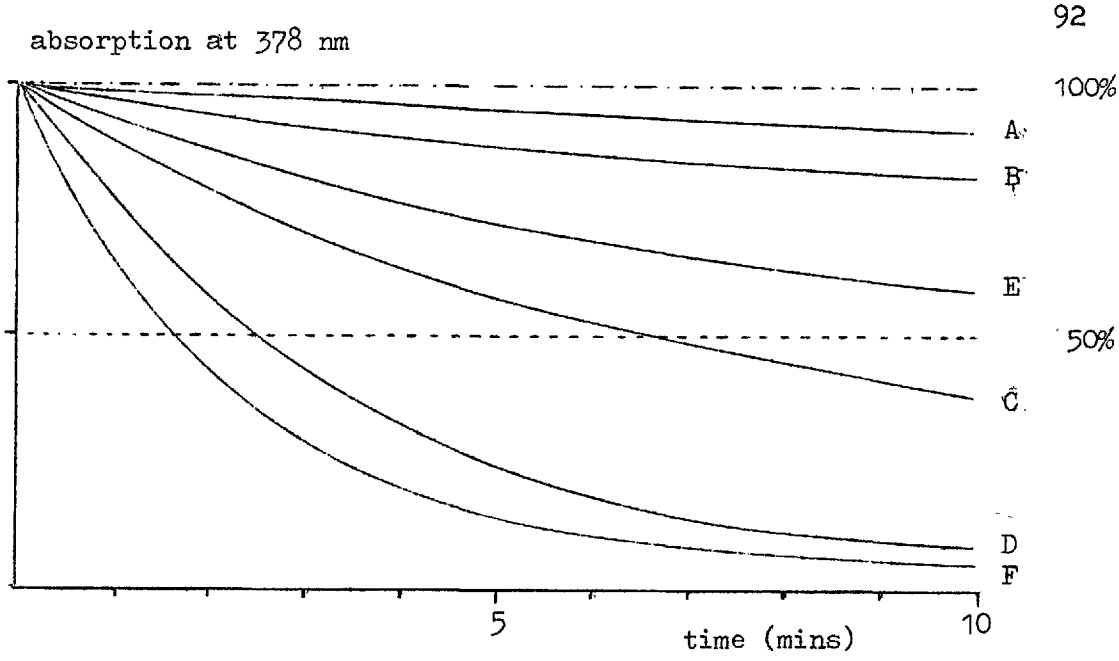
The remainder of the photolysis products were not investigated in detail. The major byproduct of the reaction was probably polymeric material since it was insoluble in common solvents. Recently, Eisner and her collaborators[190] have fully characterised a number of photolytic products of 3,5-disubstituted 1,4-dihydropyridines as dimers, which they obtained in good yields. It is possible that similar dimer-formation could occur in the current system.

The responsible absorption wavelength was ascertained from a series of runs with the filter systems (fig. 2, graph 1). The apparatus used was constructed such that the area of irradiated solution, the temperature of the quartz cuvette and its distance from lamp source remained constant. The course of the reactions were followed spectrophotometrically and from the results it was clear that filter D (Chance OX1) with a broad transmittance band between 320-380nm was about as effective as no filter at all. Since the photolyses were all performed in pyrex cooling vessels, irradiation below ~ 300nm was clearly not responsible for the activation process. Filter A, which transmitted only visible light likewise prevented any reaction.




† $NiSO_4.6H_2O$ (4.6g) + $CoSO_4.7H_2O$ (1.4g) in 10ml soln.

(Fig. 2)




(Graph 1)

The molecule could have been activated by excitation of either the aldehyde carbonyl double bond, of the dihydropyridine ring, or conceivably of both. The model chromophore can be considered to comprise a typical dihydronicotinyl moiety cross-conjugated with a phthaloyl chromophore. The former have absorption maxima in the 330-380nm region ($\epsilon \sim 10000$), and the latter absorb around 280-300nm ($\epsilon \sim 1000$). These observations suggest immediately that the dihydropyridine ring was the primary excited species here, and the implication is that the aldehyde carbonyl was not activated during the process, since the carbonyl triplet state would be at a higher energy level, and the excited dihydropyridine could not decay <u>via</u> such an energy transfer. Further evidence against carbonyl excitation is that the normal $n \rightarrow \pi^*$ excited state would not facilitate subsequent hydrogen-abstraction by the carbon atom, but rather by the oxygen atom.

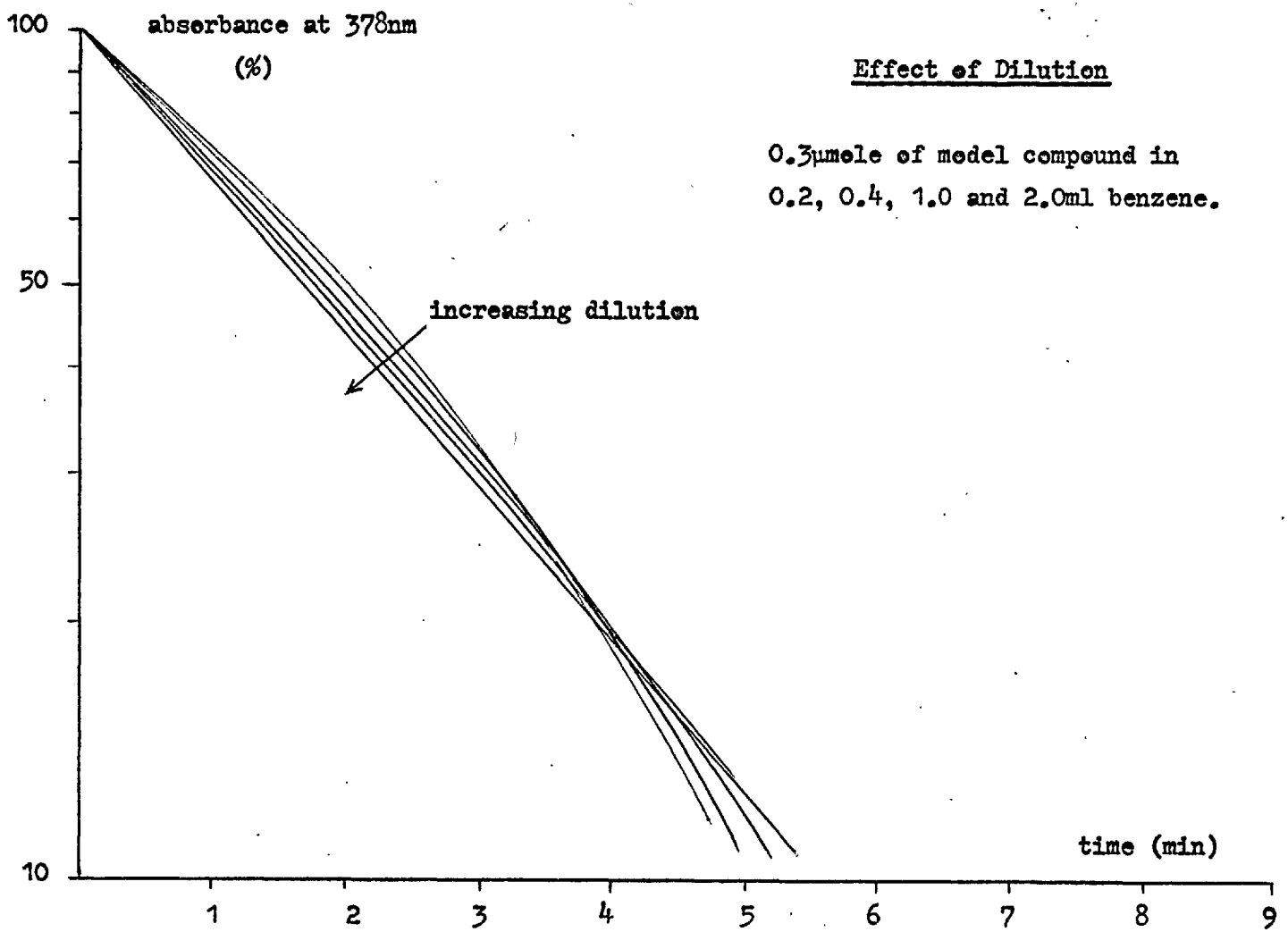
A series of runs were performed in benzene at different

concentrations and the results are plotted (graph 2). An increase in dilution had very little apparent effect on the pseudo first-order nature of the reaction, and thus the quantum yield was independent of the concentration. Analysis was not carried out on the reaction products at different concentrations however, so it was not possible to decide from this information alone whether the reaction was intermolecular or intramolecular.

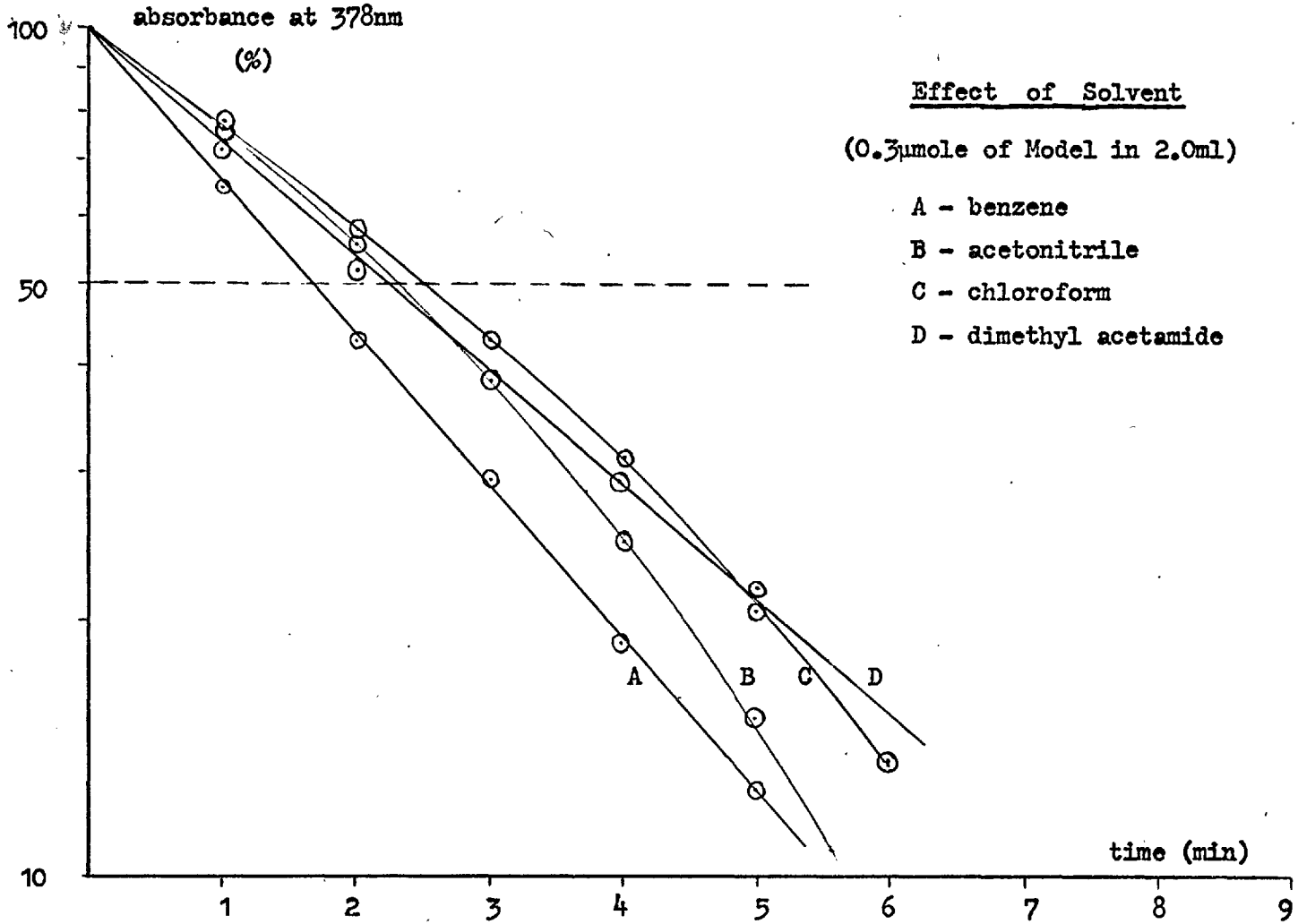
It was important to discover whether the polarity of the environment of the molecule influenced the rate of loss of the 378nm chromophore, since this could have an effect on a migrating species. The photolysis was consequently conducted in 4 different solvents: benzene, acetonitrile, dimethyl acetamide and chloroform respectively. Because of the complexity of competing reactions these observations were necessarily of a qualitative nature, but it was nevertheless possible to show that the reaction rates did not vary significantly between these solvents (graph 3). Thus to a first approximation, the quantum yield was independent of the solvent type, suggesting that the migrating species was not charged. Reactions involving transfer of ionic species or electrons are usually solvent-dependent[125], although it is possible that in an internally constrained intramolecular process, the effect of solvent might be small.

At this stage it was not possible to be sure whether the observed reaction was intramolecular, whether an ionic or neutral hydrogen species was transferred, or whether the transfer was direct or involved the participation of solvent.

More direct information was expected from the reaction of


100
absorbance at 378nm
(%)
50
10
Effect of Dilution
0.3μmole of model compound in
0.2, 0.4, 1.0 and 2.0ml benzene.
increasing dilution
time (min)
1
2
3
4
5
6
7
8
9

(Graph 2)


absorbance at 378nm
(%)
100
50
10
1
2
3
4
5
6
7
8
9
time (min)
A
B
C
D
Effect of Solvent
(0.3µmole of Model in 2.0ml)
A - benzene
B - acetonitrile
C - chloroform
D - dimethyl acetamide

(Graph 3)

the model compound in the prescence of radical initiators. Samples of the compound were set aside in benzene in the dark and in the prescence of dibenzoyl peroxide and of t-butyl hydroperoxide respectively. There was almost no change in the chromophores of the 2 samples after 2 weeks.

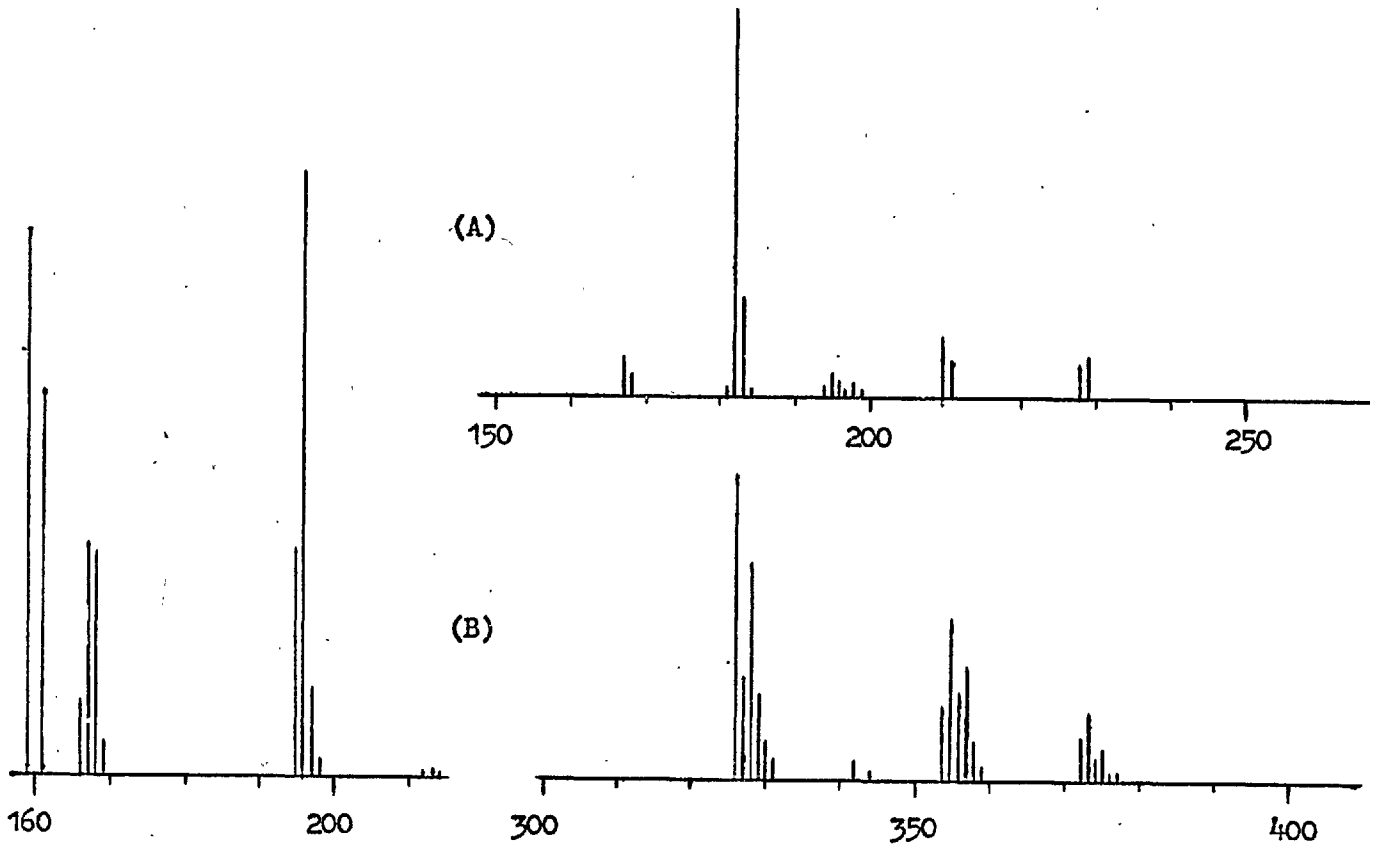
Runs were also performed in the prescence of pure oxygen and pure nitrogen respectively, both in acetonitrile and benzene. There was very little significant difference in the rates here, although oxygen appeared to facilitate the loss of $\lambda_{max}$ 378nm marginally. Land and Swallow[192] have observed the aerobic oxidation of NADH to NAD$^{\circ}$ under similar conditions.

If a radical intermediate is proposed for the reaction, these observations are difficult to explain. Oxygen should markedly have affected the reaction rate, and peroxides would be expected to react with the dihydropyridine moiety[192]. It is conceivable that the bulky nature of the peroxide groups would hinder the formation of a dihydropyridine radical species because of the proximity of the interfering aldehyde group, but this effect would not be expected to be large. The alternative possibility is that the migrating species was a hydride ion from a photolytically activated dihydropyridine. In order to determine the molecularity of the reaction, whether solvent was involved, and the site of attack, the mass spectra of the reduced photolysis product, of the authentic alcohol-dihydropyridine (XXX) and of two deutero-isomers were prepared. It has been noted that the mass spectra of the first two systems mentioned were identical. Because of the complexity of these

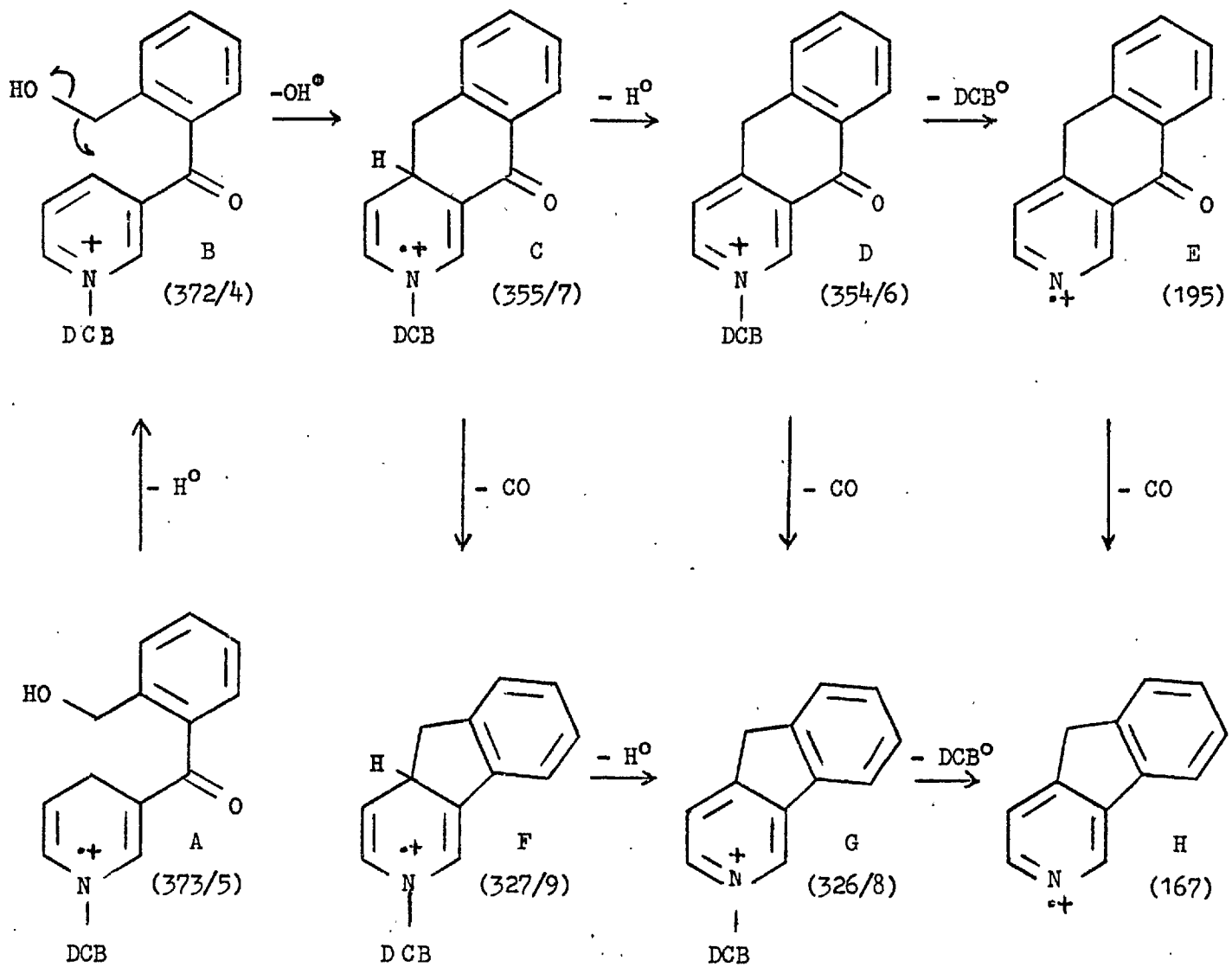
spectra, the closely related N-methyl compound was prepared by reduction of (IV, R = $CH_3$) with sodium dithionite in the usual way. The mass spectrum of this compound was very much simpler and is considered first (fig. 3A). The mass ion at 229 m.u. was preceded by an almost equally intense peak at 228 m.u. which corresponded to loss of hydrogen probably from the pyridine ring. Consecutive loss of a hydroxyl radical followed by a hydrogen atom gave rise to peaks at 211 and 210 m.u. respectively. Loss of the diaryl carbonyl group gave the base peak at 182 m.u. which could lose the N-methyl group to give the peaks at 167 and 168 m.u.

The more complex N-(2,6-dichlorobenzyl)-alcohol (V), had a very similar splitting pattern (fig. 3B) with the exception of the more facile loss of the DCB grouping over the methyl grouping as expected. This resulted in the appearance of a similar pattern corresponding to the debenzylated model with ($M^+$ - Ar) at 214 m.u. Similarly, the peak intensities at 196, 195, 168 and 167 m.u. were correspondingly increased. The intense peaks at 159 and 161 m.u. corresponded to $DCB^+$. A possible fragmentation pattern which was substantiated later by deuterium studies is given in (fig. 4). The peaks at 196 and 168 m.u., not included here, presumably arise by the addition of $H^o$ to the respective pyridine species. Ring fusion at the pyridine 2-position was considered unlikely from steric considerations, but a small amount may have occurred in the alkylated system. Early loss of the N-alkyl group in some fragments would facilitate ring 2-fusion.

All these major peaks could be accounted for in the spectra




(Fig. 3)


HO
-OH°
- H°
- DCB°
B
(372/4)
C
(355/7)
D
(354/6)
E
(195)
DCB
- H°
- CO
- CO
- CO
A
(373/5)
F
(327/9)
G
(326/8)
H
(167)

(Fig. 4)

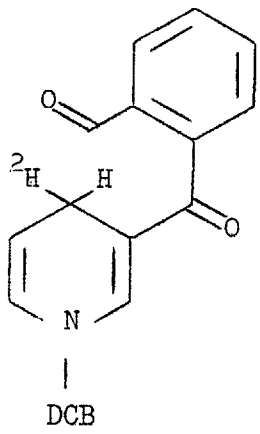
of both the authentic alcohol-dihydropyridine and the photolysis product. Thus, in summary:

$$(V,\ R = DCB) \xrightarrow[2.\ S_2O_4^{2-}]{1.\ h\nu} (XXX)$$

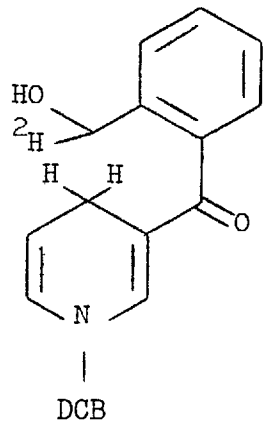
In order to discover whether an intramolecular hydrogen shift was responsible for this result, the $[4\text{-}^2H]$- model aldehyde (XXXIV) was prepared. Ideally the $[4,4\text{-di-}^2H]$ - compound was required, but an efficient oxidising agent could not be found which allowed multi-stage deuterium incorporation. Thus the corresponding pyridinium salt was reduced with dry dithionite in $^2H_2O$ containing $Na^2HCO_3$ buffer, and the required monodeutero model system isolated in moderate yield. Attempts to oxidise this to the pyridinium species with a number of oxidising agents including hexachloroacetone[128] gave very poor yields of the oxidised salt. Reduction in $^2H_2O$ as before resulted in unworkably low amounts of model containing $\sim 1.5$ atoms of deuterium per molecule. In contrast, simple 1,4-dihydronicotinamides afforded 15% yields of reduced material containing $\sim 1.94$ atoms of deuterium following 2 oxidation and 3 reduction stages, which represents an oxidation isotope effect $k_H/k_D \sim 4$. Since this was achieved with hexachloroacetone it is further evidence (see Review) for the polar as opposed to radical nature of this oxidant under these conditions[128](acetone, room temperature).

Because of these difficulties, it was decided to use the mono-deuterated model system (XXXIV) in the photolysis, and to identify the product by its mass spectrum. It was necessary

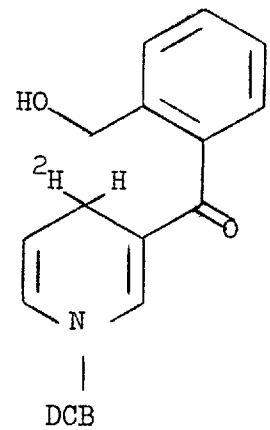
also to make the corresponding $\left[{}^{2}H\right]$ - isomers of (XXX) i.e. (XXXV) and (XXXVI).




(XXXIV)



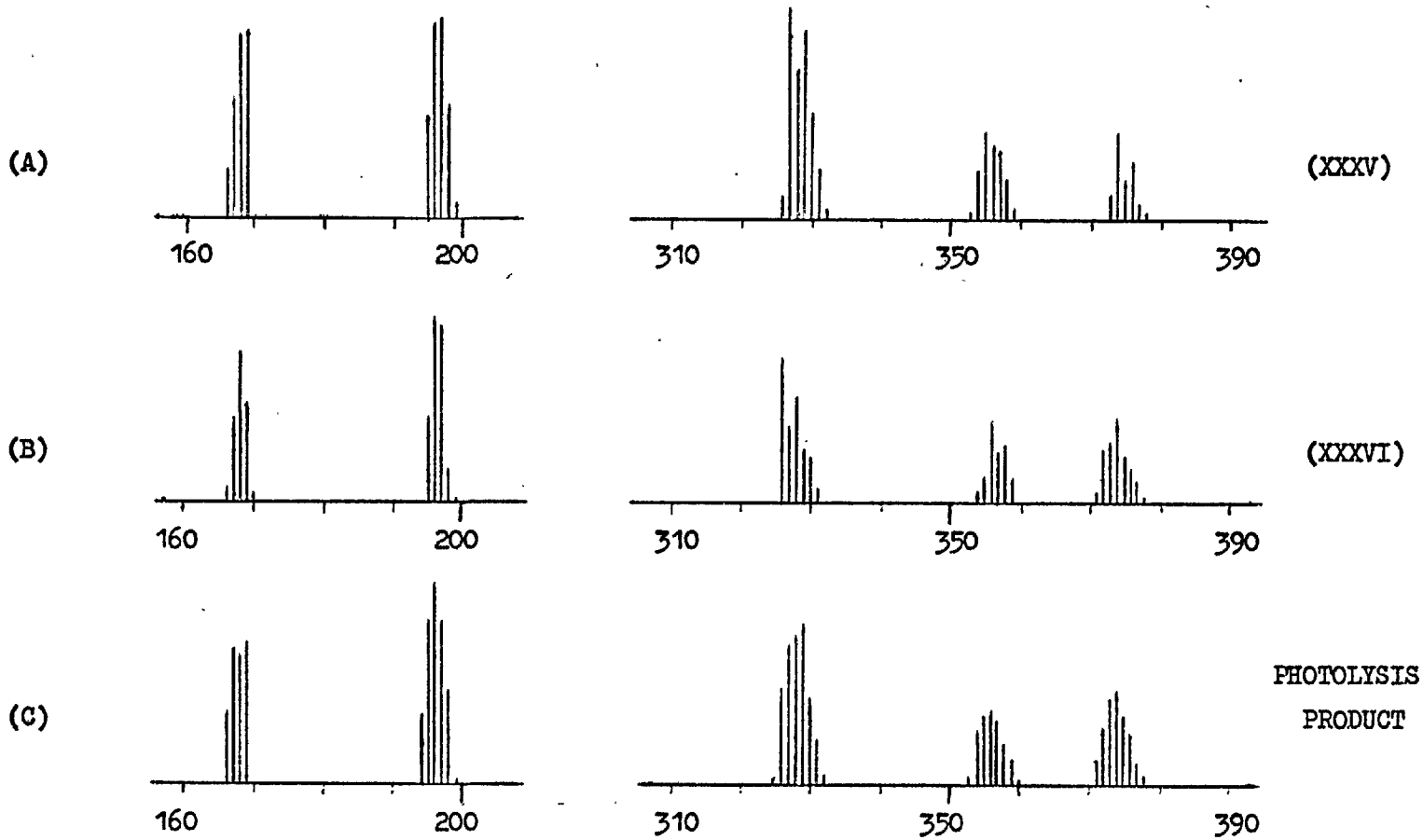

(XXXV)




(XXXVI)

Compound (XXXV)was prepared in high yield by the reduction of the model compound (V, R = DCB) with sodium borohydride-$d_4$ in methanol. The $\left[4\text{-}{}^{2}H\right]$ - isomer (XXXVI) was similarly prepared by borohydride reduction of (XXXIV). Obviously if the photolysis involved migration of hydrogen from the 4-position to the aldehyde carbon atom, the reduced product would be expected to contain both (XXXV) and (XXXVI). If solvent were involved, or hydrogen transfer was to the aldehyde oxygen, then less than one deuterium atom per molecule would be observed in the reduced product. The mass spectra of (XXXV) and (XXXVI) will be discussed first.

As expected, the spectrum of (XXXV) was identical to that of the undeuterated molecule (XXX) except that each peak was 1 m.u. higher (fig. 5A). The shape and position of the complex at 373-378 m.u. indicated that it contained greater than 95% monodeuterated material. The complex at 354 - 359 m.u.,contained spurious peaks which complicated its appearance, but a metastable at ~ 340 m.u.


(A)
160
200
310
350
390
(XXXV)
(B)
160
200
310
350
390
(XXXVI)
(C)
160
200
310
350
390
PHOTOLYSIS
PRODUCT

(Fig. 5 )

was in accord with loss of $OH^{\circ}$ from fragment B (fig. 4) at 373 m.u. to fragment C at 355 m.u. (calculated position, 339.8 m.u.). A small amount of scrambling of the deuterium (or possibly early loss of N-alkyl group) caused the lower molecular weight fragments to contain some undeuterated material.

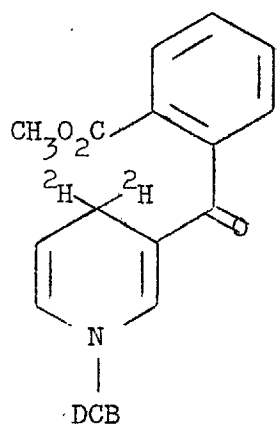
The spectrum of (XXXVI) was more complex (fig. 5B). The mass ion and $M^{+}-1$ pattern could be resolved into the two splitting patterns characteristic of the undeuterated and monodeuterated models. Thus the first hydrogen atom lost (A → B, fig. 4) was definitely from the 4-position, and not from the hydroxyl or benzyl groups. Species (B, C and F) gave correspondingly more complex patterns, because they also contained only some of the original deuterium, but E and H contained a majority of undeuterated material as predicted. Ideally, these latter species would be the most suitable for observing deuterium in the photolysis product, since theoretically, only benzylic deuterium would remain in these fragments. However, some scrambling and some attack at the ring 2-position after debenzylation caused the appearance of a certain amount of both these isotopes in (XXXV) and (XXXVI).

As expected, the mass spectrum of the mono-deuterated, reduced photolysis product was difficult to analyse in detail (fig.5C) owing to the complexity of the splitting pattern. It did contain some deuterium though, as evidenced by the appearance of a pair of peaks at 374 and 376 m.u. These were weaker than in the isotopes (XXXV) and (XXXVI) where the peak at 374 m.u. was the most intense of the band group. Almost as important was the peak pair at 373 and 375 m.u. which could have corresponded to

undeuterated material. If so, this could have arisen by the photolytic transfer of deuterium either directly to the aldehyde oxygen, or to solvent, with subsequent loss on workup. However, the remainder of the spectrum could be accounted for by the prescence of a combination of the two deutero-isomers, so it seems most likely that deuterium was retained in the molecule on photolysis. This important finding precluded the participation of solvent in the transfer process, and also suggested that the reaction was intramolecular, since otherwise both di- deuterated and undeuterated material would have been observed in the products. It was clear that the product contained some of the isotope (XXXV) since the 327 and 329 m.u. peaks were otherwise unaccountably large. It was not possible to obtain an accurate estimate of the amount of this isotopic isomer, but it was probably present in 20-40%. The remainder of the pattern could be approximately accounted for by the other isotope (XXXVI) which contained deuterium in the original 4-position. A reconstruction of the mass spectrum from these two isotopic isomers gave a spectrum which was very similar to that of the photolysis product.

In order to further demonstrate that the reaction was intra- and not inter-molecular, 2 experiments were performed. Simple Hantzsch[116] compounds have been found[190] to be quite stable in the prescence of ultra-violet light, presumably because dimerisation reactions are hindered here unlike the molecules under current investigation. Equimolar amounts of 1,2,6-trimethyl-3,5-dicarbethoxy-1,4-dihydropyridine and benzaldehyde were consequently photolysed for 24 hours and the mixture worked up for pyridinium

salt and benzyl alcohol. Neither compound could be detected although the concentrations used in this reaction were considerably greater than those of the model system. In addition, an equimolar mixture of the model aldehyde and the [4,4-di-$^2$H] - 1,4-dihydropyridine ester (XXXVII) were together photolysed in benzene, and the product worked up after dithionite reduction for alcohol-dihydropyridine. The mass spectrum of this compound contained no deuterium, and was identical to the spectrum given (fig. 3B). The methyl ester was prepared by the same technique used to make the deuterated aldehyde, and in this case oxidation with hexachloroacetone was efficient enough to allow for 3 reduction stages, and the incorporation of $\sim$ 1.7 atoms of [$^2$H]. An independent photolysis of this ester alone gave no alcohol-dihydropyridine under the same conditions according to t.l.c., although the chromophore was lost at almost the same rate as was that of the model, suggesting that the rate of competing dimerisation reactions was of the same order of magnitude as the hydrogen transfer.




(XXXVII)

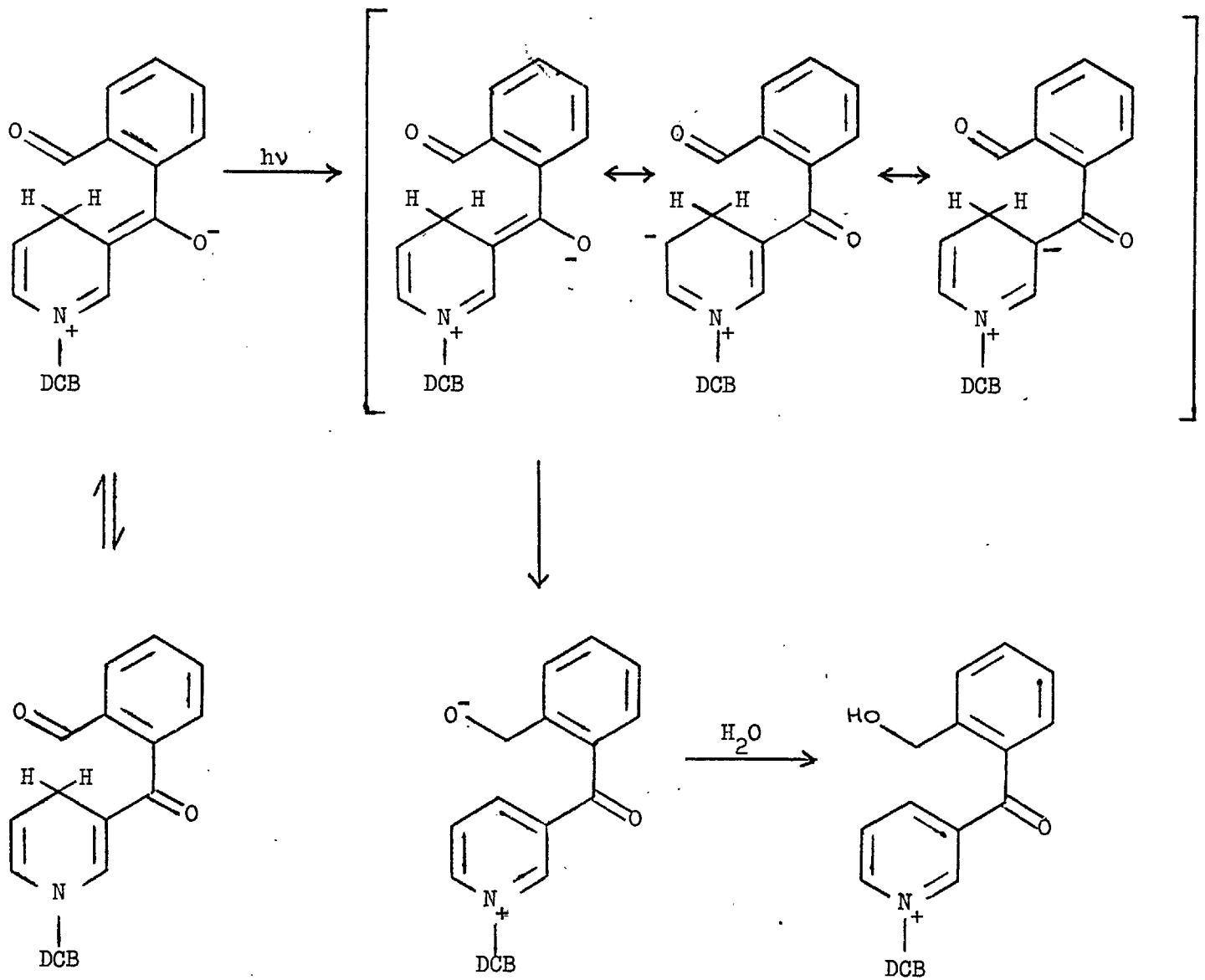
From these results it would appear that hydrogen was transferred photochemically from the dihydropyridine 4-position

directly to the aldehyde carbon atom by an intramolecular process, presumably via a 7-membered ring transition state. The process probably originated from dihydropyridine ring activation, and the balance of information suggests that a hydride ion rather than a radical atom may have been the migrating species. The rate of loss of the dihydropyridine chromophore was not dependent on the prescence of oxygen, and radical generators were also ineffective.

One possible mechanism involves the rapid reaction of a photo-excited singlet state[221] originating from the enolate ion (fig. 6). The enolate mesomer has been shown to make an important contribution to the model chromophore[21] and it is possible that photolytic activation of the ring is sufficient to enable it to undergo the observed transformation. The lack of solvent dependence of the reaction could reflect the constrained nature of the molecule and the fast transfer.

An alternative possibility is that a sigmatropic shift could account for the transformation. If the molecule is considered to undergo the transfer in its mesomeric state (XXVIII) the ring 4-position is coupled to the aldehyde carbonyl group by a conjugated system, either 1,6- or 1,10-. The transfer to carbon rather than oxygen could simply reflect the steric proximity of the former over the latter, a 7-membered transition state being favoured over an 8-membered state.

In summary, the complexity of the photolytic reactions, and the added complexity of the mass spectra of the photo-rearranged product precluded a definite conclusion about the exact nature of the transfer process. In order to clarify

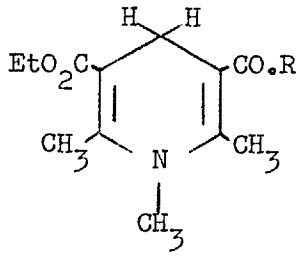



(Fig. 6)

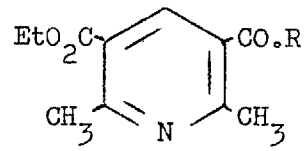
these questions, it would be necessary to consider the reactions of a model system closely akin to the one described but inert to dimer and polymer formation. It should also be crystalline yet contain a simple N-alkyl grouping. The preparation of a series of precursors to such a model system is considered in the next section. The question of sigmatropic rearrangement could be clarified by the preparation of unconjugated models eg. (XXXII) and (XXXIII)

## PART IV

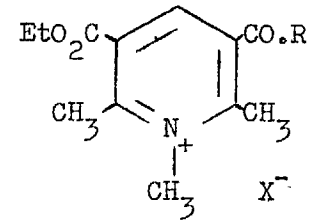
### Pathways to Potential Models related to Hantzsch Compounds[116]



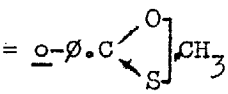
(XXXVIII)



(XXXIX)



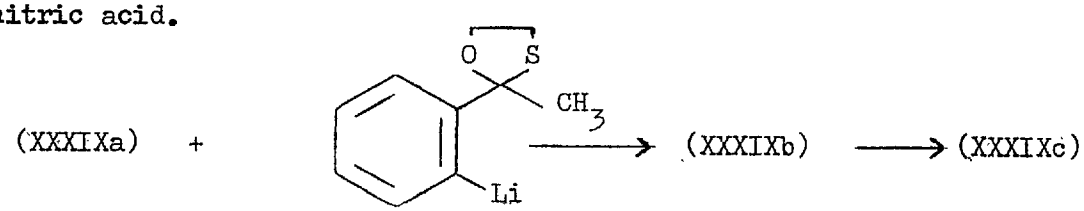
(XL)

(a) R = OEt, (b) R =  , (c) R = o-Ø.CO.$CH_3$

Because of the complexity of competing reactions which could occur photolytically at the nucleus of simple 1,4-dihydropyridines, the model system (XXXVIIIc) was considered. These N-methyl compounds, unlike those of the simpler NADH analogues are highly crystalline, and should be inert to light-catalysed bimolecular attack at the ring nucleus. In addition, the 2-methyl group would be expected to perform the same function as the DCB group in the previous model, in favouring the required conformation for intramolecular transfer. A complete synthesis of this model system has not been achieved, although the corresponding pyridine (XXXIXc) has been made in good yield and fully characterised. Attempts to alkylate the pyridine nitrogen with a number of alkylating agents, including triethoxonium fluoborate failed. If this could be achieved, the final reduction to the model system with dithionite would be a simple matter.

(A) Preparation of 2,6-Dimethyl-3 -(2'-acetyl)benzoyl-5-carbethoxypyridine (XXXIXc)

The diethyl ester (XXXIXa) was prepared by the standard method[206] from ethyl acetoacetate, formaldehyde and ammonia, followed by oxidation of the resulting dihydropyridine with nitric acid.



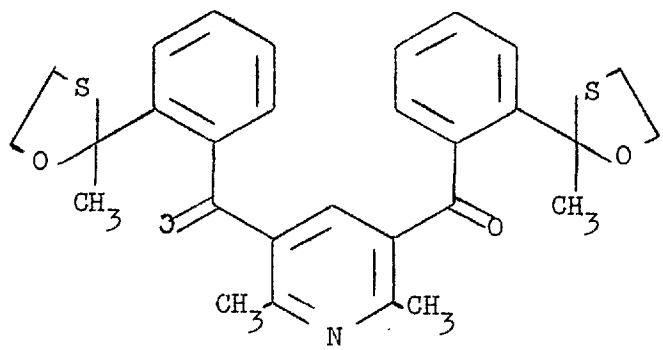

(XLI)

2-Bromoacetophenone was prepared via a 3-stage synthesis from acetophenone. The 2-nitro. compound was reduced, diazotised and coupled with cuprous bromide by standard chemical techniques[207] in 19% overall yield from acetophenone.

The hemithioketal of this compound was prepared in high yield by condensation with β-mercatoethanol in ether containing boron trifluoride[208]. The highly crystalline product was fully characterised. The infra-red spectrum showed no carbonyl absorption, and the p.m.r. spectrum contained complex methylene multiplets at τ 6.7 - 7.3 and 5.3 - 6.3 corresponding to the $S-CH_3$ and $O-CH_3$ protons respectively.

Reaction of this bromoketal compound with n-butyl lithium proceeded smoothely in ether-petrol and was complete within 2 hours at room temperature. This Grignard compound (XLI) was subsequently added dropwise to a vigorously stirred solution of

the pyridine-ester (XXXIXa) at room temperature and in an inert atmosphere, in an attempt to force attack at only one of the two carbethoxyl groups in the molecule. Various conditions were tried, the best yields of the required mono-ester (XXXIXb) resulting when low temperature ($-5^{\circ}$) and concentrated solutions of the diester were used. The crude reaction product contained a number of components, including the mono-ester, starting material diester, de-bromo hemithioketal and the bis-hemithioketal compound (XLII) arising from secondary attack of the lithium compound on the mono-ester.



(XLII)

An attempt to remove non-pyridine byproducts by washing a strongly acidic solution of the crude products with benzene failed, because the monoester pyridine nitrogen was apparently insufficiently basic to be protonated effectively in this medium. Consequently the product was isolated by column chromatography in 35% yield as a colourless, crystalline solid mp. $91.5-92.5^{\circ}$. The infra-red spectrum showed 2 types of carbonyl absorption at 1725 and 1678$cm^{-1}$, and the p.m.r. spectrum confirmed a 1:1 ratio of ethyl to aromatic protons. The 2 and 6 methyl groups at $\tau$ 7.17 were also split by $\sim$ 0.5Hz, and there was an additional

methyl group at τ 8.05. The major by-product also a colourless crystalline solid in 18% yield was fully characterised as the bis-hemithioketal compound (XLII). The infra-red and p.m.r. spectra reflected the symmetry of this molecule unlike the structure of (XXXIXb), and its composition was confirmed by analysis.

Removal of the hemithioketal protecting group to give (XXXIXc) was easily effected with mercuric chloride in acetic acid/acetate buffer[209]. The crude product crystallised in vacuo and was recrystallised from benzene-petrol. The infra-red spectrum showed 3 carbonyl absorptions $\nu_{max}$ 1720, 1680 $cm^{-1}$, the latter being a doublet. The p.m.r. spectrum confirmed the loss of hemithioketal methylene protons, and the 2 and 6 methyl proton singlets were further separated (τ 7.09 and 7.13). More especially, the acetyl methyl had moved downfield to τ 7.48.

(B) Attempted quaternisation of (XXXIXc)

In order to achieve a model system comparable to the NADH-aldehyde analogue (V) it was necessary to quaternise this compound and finally reduce with dithionite in the usual way. Quaternisation has not been achieved under the following conditions:

Excess methyl iodide failed to react with the base at room temperature in 48 hours. Heating the reagents in a small Carius tube at 80° or 120° for 24 hours under nitrogen also failed to give any methiodide salt, although some decomposition occurred. Methyl p-toluene sulphonate similarly had no effect after refluxing in ethanol for 48 hours under nitrogen.

The more electrophilic dimethyl sulphate has been used to quaternise many such Hantzsch compounds[189]. Various sets of con-

ditions were tried including heating equal quantities of the purified reagents at 60° under nitrogen for 2 hours. The reaction mixtures were worked up with water, washed with ether and the aqueous fraction reduced immediately with dithionite. Careful work-up of the reduced aqueous phase gave mixtures of products none of which showed a dihydropyridine absorption in the 350 nm region.

Finally, the pyridine compound was treated with an excess of triethoxonium fluoborate in dry methylene dichloride. The solution was set aside for 24 hours and then refluxed 24 hours. T.l.c. indicated that no reaction had occurred, but it was possible that the salt dissociated again on alumina. The solution was poured into excess buffered dithionite and vigorously shaken. No dihydropyridine compound was isolated.

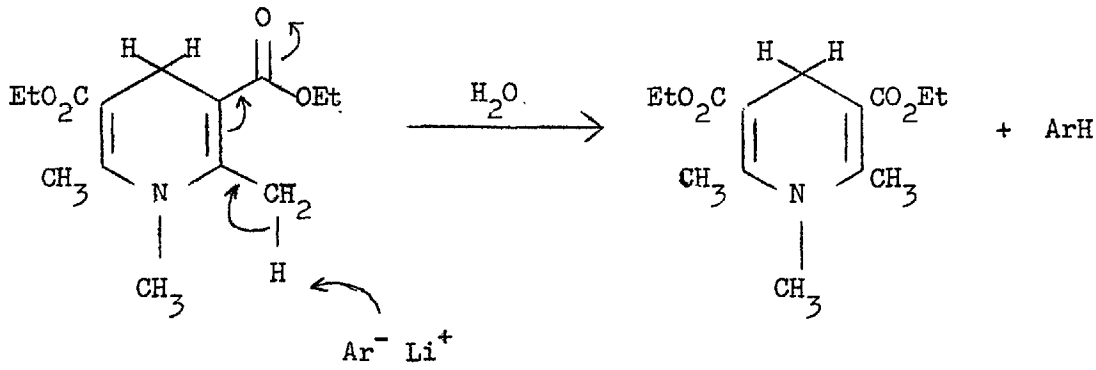
It appears that the nitrogen atom has virtually no basicity in this molecule because of the powerful electron-withdrawing groups in the 3 and 5 positions. It must be assumed that the phthaloyl moiety has considerably more electron attracting power than the ethoxycarbonyl group. It was noted earlier that the base could be removed from strongly acidic medium by simply washing with benzene.

Since the simple Hantzsch salt (XLa) is well-known[189] it was decided to make this compound, reduce it with dithionite to the corresponding dihydropyridine diester[15] (XXXVIIIa) and attempt to couple this molecule with the hemithioketal Grignard compound:

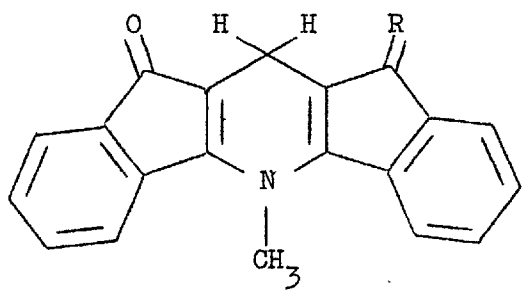
$$\text{(XXXVIIIa)} + \text{(XLI)} \longrightarrow \text{(XXXVIIIb)}$$

The lithium compound apparently did not attack the dihydropyridine carbethoxy group, however, but probably the less-hindered,

acidic 2-methyl hydrogens. The reaction product contained unchanged dihydropyridine diester along with acetophenone hemithioketal.




It is possible that the required condensation could be achieved with the dihydropyridine (XLIII) prepared from indan-1,3-dione, ammonia and formaldehyde in the usual way[206]. This molecule contains no centre for nucleophilic attack except the carbonyl group, and has the advantage that it is sterically constrained.




(XLIII, R = O)

(XLIV, R = CH.CH.CHO)
X

Condensation of this molecule with a suitable aliphatic Grignard compound would give a range of potential models (XLIV) which are not conjugated to the aldehyde, and should be stable to competing

photolytic reactions. The effect of X would be two fold. Its bulk determines the preferred conformation of the redox site and its electronic properties could affect the lability of the aldehyde group towards reduction.

In summary, it appears from the work of Eisner and others[190] that Hantzsch compounds must be employed in investigations involving the light-catalysed transfer of hydrogen between coenzyme and substrate models. That such transfers can occur in model systems has been shown here using a simple 3-substituted 1,4-dihydropyridine although the complex nature of competing photolytic reactions precluded detailed study of the actual transfer process.

# EXPERIMENTAL SECTION

All melting points were determined on a Kofler block and are uncorrected. Infra-red spectra were taken as nujol mulls (exceptions being indicated) on a Unicam SP200 spectrophotometer. Proton magnetic resonance (p.m.r.) spectra were recorded in deuteriochloroform unless otherwise stated, with either a Varian A60,T60 or HA100 spectrometer. Ultra-violet spectra were recorded on a Unicam SP800 spectrophotometer in absolute methanol unless stated otherwise. Mass spectra were determined on either a an A.E.I. MS9 or Perkin-Elmer 270 mass spectrometer.

Thin layer chromatography (t.l.c.) plates were prepared from Merck $GF_{254}$ Type E alumina activated at 110° for 1 hour.

Benzene, ether, cyclohexane and petroleum ether fraction bp. 60-80° (petrol) were dried over sodium. Tetrahydrofuran (T.H.F.) was dried over phosphorous pentoxide and distilled from lithium aluminium hydride (L.A.H.). Acetonitrile was dried over and distilled from calcium hydride. Dimethyl acetamide (D.M.A.) was distilled from calcium chloride. Acetone was dried over 4Å molecular sieves, and pyridine was distilled from potassium hydroxide pellets.

Solvent mixtures are described as ratios of volumes.

PART I

The Preparation and Characterisation of $NAD^+$- Alcohol Model Compounds (IV)

2-Bromo-α,α-diacetoxytoluene - 2-Bromotoluene (51.4g 0.3 mole) was oxidised with chromium trioxide in acetic anhydride at 5-10° according to the method of Nishimura[194]. The diacetate was isolated in 80% yield (68.8g) as a white crystalline solid, mp. 84-5° (lit.[210] mp. 84-6°); $\nu_{max}$ 1760, 1240, 770cm$^{-1}$; τ ~2.6 (4H, m, arom H), 7.88 (6H, s, acetate), 2.09 (1H, s, aldehyde H).

2-Bromobenzaldehyde - Hydrolysis of the diacetate (68.8g 0.24mole) under nitrogen with warm mineral acid[194], followed by distillation of the crude product, yielded 2-bromobenzaldehyde in 67% overall yield (36.9g, bp. 80-2°/5mm. Hg) as a low-melting solid, mp. 20-1° (lit.[210] mp. 20-1°); $\nu_{max}$ 1695, 1590, 770cm$^{-1}$.

2-Bromobenzyl Alcohol - L.A.H. reduction of the aldehyde (36.8g 0.20mole) in refluxing anhydrous ether (100ml) afforded 2-bromobenzyl alcohol in 95% yield (35.4g)as a white solid which recrystallised from petrol as colourless needles, mp. 80.0-.5° (lit.[211] mp. 80-1°); $\nu_{max}$ 3300, 1472, 760cm$^{-1}$.

2-Bromobenzyl Alcohol Tetrahydropyranyl Ether (VIII) - The alcohol (33.5g 0.18mole) was stirred for 3 hours with redistilled 2,3-dihydropyran[212] (30.2g 0.36mole) in anhydrous ether (500ml) containing a trace of concentrated hydrochloric acid at room temperature. Distillation via an efficient column yielded 2-bromo-

benzyl alcohol tetrahydropyranyl ether (46.2g 95%), bp. 138-40°/ 4mm. Hg, $\nu_{max}$(liquid film) 2940, 1130, 1038 $cm^{-1}$, τ 2.53 (4H, m, arom H), 5.20 and 5.48 (1H, d (J, 13Hz), benzyl H), 5.27 (1H, s, 2-pyranyl H), ~8.4 (6H, m, 3-5pyranyl H), ~6.3 (2H, m, 6pyranyl H); [found: C, 53.33%; H, 5.73%; Br, 29.52%. $C_{12}H_{15}BrO_2$ (271.16) requires: C, 53.16%; H, 5.58%; Br, 29.47%].

**n-Butyl Lithium** - Initial difficulty with the standard procedure[195] resulted in the following modifications. Lithium wire was used in place of pieces, the solvent employed was hexane-ether (1:1), and argon replaced nitrogen. The hazard in filtering the final solution was overcome by allowing the sediment to settle, and decanting off the n-butyl lithium solution which was then standardised in the usual way[195]. It could be stored for a month at 0° under argon.

2-(α-Hydroxy-3-picolyl)-benzyl Alcohol Tetrahydropyranyl Ether (X)

Method A - 2-Bromobenzyl alcohol tetrahydropyranyl ether (40.6g 0.15mole)in dry ether (500ml) was dropwise added to a solution of n-butyl lithium (0.15mole) in the same solvent at 0°. The reaction was allowed to warm up to room temperature over 1 hour and stirred for a further 2 hours by which time it was a pale cloudy yellow. Redistilled 3-pyridine carboxaldehyde (16.1g 0.15mole) in ether (400ml) was added dropwise over 1 hour, causing a yellow suspension to separate towards the end of the addition. After a further hour at room temperature, the product was worked up with saturated ammonium chloride, run onto a short alumina column, and the products eluted with benzene. Benzene-ether (50:1) removed the prod-

uct 2-(α-hydroxy-3-picolyl)-benzyl alcohol tetrahydropyranyl ether as a colourless high-boiling liquid (29.7g 66% based on the bromo-ether). Attempts to crystallize the material were unsuccessful, $\nu_{max}$ (liquid film) 3400, 3210, 1595cm$^{-1}$; τ2.63 (4H, ~s, arom H), 5.08 and 5.57 (1H, d (J, 12Hz), benzyl H), 5.30 (1H, s, 2pyranyl H), 8.36 (6H, m, 3-5pyranyl H), 6.32 (2H, m, 6pyranyl H), 3.81 (1H, s, α-picolyl H), 5.22 (1H, s, OH), 1.33,2.16,~2.6 and 1.46 (1H, m, respect. 2,4,5 and 6 pyridine H) $J_{45}$, 9Hz, $J_{56}$, 4.5Hz; [found: C, 72.06%; H, 6.87%; N, 4.46%. $C_{18}H_{21}NO_3$ (299.42) requires: C, 72.22%; H, 7.07%; N, 4.60%].

Method B - Dry magnesium turnings (2.4g 0.1g atom) in refluxing dry THF (150ml) containing iodine (0.05g) as catalyst, were heated with the bromo-ether (20ml of a solution containing 27.0g 0.1mole in 250ml THF). The solution was vigorously stirred until the reaction began, and the remainder of the bromo-ether added over 1 hour. The cooled Grignard reagent was treated with 3-pyridine carboxaldehyde (10.7g 0.1mole) in THF (200ml) as in the preceeding method, and the reaction worked up in the same way giving the product in 70-75% yield.

2-Nicotinylbenzyl Alcohol Tetrahydropyranyl Ether (VII) - Oxidation of the diaryl carbinol (X) (15.0g 0.05mole) with activated manganese dioxide[196] (150g) in purified chloroform (500 ml) was complete by t.l.c.(ether) in 40 minutes at room temperature. The oxidant was filtered off and well-washed with chloroform (300ml). Evaporation of the filtrate in vacuo gave crystals of 2-nicotinylbenzyl alcohol tetrahydropyranyl ether

(11.8g 80%), recrystallized from petrol as colourless plates, mp. 77-8°, $\nu_{max}$ 1672, 1581, 1120, 1020cm$^{-1}$; $\lambda_{max}$ (ethanol) 234 (ε 10550), 262nm (ε 6570); τ2.70 (4H, m, arom H), 5.20 and 5.46 (1H, d (J, 14Hz), benzyl H), 5.55 (1H, m, 2pyranyl H), 8.60 (6H, m, 3-5pyranyl H), 6.55 (2H, m, 6pyranyl H), ~1.2,1.94,2.68 and ~1.2 (1H, m, respect. 2,4,5 and 6 pyridine H), $J_{56}$, ~6Hz, $J_{45}$, ~8Hz, [found: C, 72.57%; H, 6.37%; N, 4.66%. $C_{18}H_{19}NO_3$ (297.34) requires: C, 72.70%; H, 6.44%; N, 4.71%].

2-Nicotinylbenzyl Alcohol (VI) - Method A - Hydrolysis of the ether (14.8g 0.05mole) in THF (500ml) with a slight excess of perchloric acid (6.0g 0.06mole) was near completion (t.l.c. acetone-benzene 5:95) after 3 days at room temperature. The solution was poured into saturated sodium bicarbonate (100ml), the aqueous layer separated and washed with ether (50ml), and the combined organic fraction evaporated in vacuo. The crude oily residue was partitioned between ether and N hydrochloric acid (60ml), and the aqueous phase washed with 2 portions of ether. The ice-cooled aqueous fraction was carefully basified with aqueous caustic soda and the product extracted into ether (3 portions of 100ml). The dried ($Na_2SO_4$) organic fraction was evaporated in vacuo to yield 2-nicotinyl benzyl alcohol in 50-55% yield as a colourless, viscous liquid which failed to crystallize under a variety of conditions, $\nu_{max}$ (liquid film) 3250, 1670, 1595cm$^{-1}$; τ~2.7 (4H, m, arom H), 5.46 (2H, s, benzyl $CH_2$), ~6.3 (1H, s, OH), 1.23,~2.1,~2.5 and 1.28 (1H, m, respect. 2,4,5 and 6 pyridine H); accurate mass measurement: [found: 213.0794.

$C_{13}H_{11}NO_2$ requires 213.0790].

Method B - The pyranyl ether (14.8g 0.05mole) was refluxed in 60% aqueous acetone (1.5l) with p-toluene sulphonic acid (13.8g 0.08mole) for 40 minutes, when the hydrolysis was essentially complete (t.l.c. acetone-benzene, 5:95). The bulk of the solvent was carefully distilled off in vacuo, and the concentrated residue worked up as before. The yield was 70-75% (~7.4g).

Attempts to hydrolyse the ether in refluxing aqueous ethanol with p-toluene sulphonic acid, hydrochloric or sulphuric acids[197], resulted in some decomposition and modest yields (~30%) after prolonged heating.

2-Nicotinylbenzyl Alcohol N-Benzyl Bromide Salt (IV, R = $CH_2\emptyset$, X = Br) - To a solution of the pyridine-alcohol (VI) (1.07g 5mmole) in dry acetone (25ml) was added redistilled benzyl bromide (1.20g 7mmole), the solution refluxed for 4 hours, cooled and filtered. The yellow amorphous solid was treated with dry ether and finally afforded an amorphous yellow hygroscopic powder (1.72g 90%)which could not be crystallized. Further purification was not attempted, $\nu_{max}$ (KBr disc) 3400, 1635, 1460, 770cm$^{-1}$; $\lambda_{max}$ 262nm (ε ~5700). Attempts to render this salt crystalline by adding concentrated solution of potassium iodide, picric or picrolonic acid to concentrated solutions of the salt yielded further oils or amorphous solids after workup.

2-Nicotinylbenzyl Alcohol N-Methyl p-Toluene Sulphonate Salt (IV, R = $CH_3$, X = OTs) - To a solution of the pyridine-alcohol

(1.07g 5mmole)in dry benzene (25ml) was added recrystallized methyl p-toluene sulphonate[200] (1.3g 7mmole) and the solution set aside for 48 hours. The solid product was filtered off and recrystallized from ethanol-chloroform (1.12g 56%), mp. 97-9°, $\nu_{max}$ 3210, 1645cm$^{-1}$; $\lambda_{max}$ 268nm (ε 12800); τ (DMSO-$d_6$) 2.70 (4H, m, arom H), 4.77 (2H, s, benzyl $CH_2$), 5.69 (3H, s, $CH_3$), 0.92,1.46,2.00 and 1.14 (1H, m, respect. 2,4,5 and 6 pyridine H), $J_{45}$,8.5Hz, $J_{56}$, 6.0Hz, 2.59 and 3.00 (2H, d,(J, 8Hz), anion arom H), 7.79 (3H, s, anion $CH_3$).

2-Nicotinylbenzyl Alcohol N-Methiodide Salt (IV, R = $CH_3$, X = I) - A solution of the pyridine-alcohol (1.07g 5mmole) in dry benzene (25ml) was treated with dry redistilled methyl iodide (3.6g 25mmole) in one lot. Pale yellow crystals began to separate from solution after a few minutes, and the mixture was set aside for 12 hours at room temperature. 2-Nicotinylbenzyl alcohol N-methiodide salt was collected by filtration in 70% yield (1.24g). A portion was recrystallised from absolute methanol as pale yellow platelets, mp. 281-2°, $\nu_{max}$ 3200, 1640, 1472cm$^{-1}$; $\lambda_{max}$ 266 (ε 4500), (ethanol) 264nm (ε 4745); τ ($^2H_2O$/acetone) 2.50 (4H, m, arom H), 4.55 (2H, s, benzyl $CH_2$), 5.56 (3H, s, $CH_3$), 0.96,1.32,1.91 and 1.20 (1H, m, respect. 2,4,5 and 6 pyridine H), $J_{45}$, 7.0Hz, $J_{56}$, 6.5Hz; [found: C, 47.18%; H, 4.12%; N, 3.69%. $C_{14}H_{14}INO_2$ (355.18) requires: C, 47.33%; H, 3.96%; N, 3.93%].

2-Nicotinylbenzyl Alcohol Tetrahydropyranyl Ether N-Methiodide

Salt (XII) - The corresponding pyridine ether (VII) (150mg 0.5mmole) was treated with methyl iodide (750mg 5mmole) in the absence of other solvent at room temperature for 3 days. On removal of solvent, the crude product 2-nicotinylbenzyl alcohol tetrahydropyranyl ether N-methiodide salt readily crystallized from absolute ethanol as pale yellow platelets (750mg 70%), mp. 171-2°; $\nu_{max}$ 1681, 1637, 1471cm$^{-1}$; $\lambda_{max}$ ($CH_3CN$) 247 (ε 15280), 268 (ε 7020), 276nm (ε 5465); τ ($^2H_2O$/acetone) 2.50 (4H, m, arom H), 4.33 (2H, s, benzyl $CH_2$), 5.67 (3H, s, $CH_3$), 5.60 (1H, s, 2pyranyl H), 8.67 (6H, m, 3-5pyranyl H), 6.68 (2H, m, 6pyranyl H), 0.97,1.97,1.23 and 1.23 (1H, m, respect. 2,4,5 and 6 pyridine H); [found: C, 51.77%; H, 4.92%; N, 3.22%. $C_{19}H_{22}INO_3$(439.29) requires: C, 51.90%; H, 5.04%; N, 3.20%].

Properties of $NAD^+$ - Alcohol Model (IV, R = $CH_3$, X = I)

1. Solvent Stability - The salt readily dissolved in water ethanol, acetonitrile, DMF, DMSO and was sparingly soluble in ethyl acetate and chloroform. Solutions were stable indefinitely in these solvents at room temperature, and the salt was easily recovered from them.

2. Thermal Stability - A sample (10mg) of the methiodide salt was refluxed for 1 week in pure absolute ethanol (5ml), both in the prescence and absence of air. No spectrophotometric changes were observed. A similar sample in pure DMF, externally heated in refluxing xylene for 48 hours similarly showed no absorption changes in the 350nm region.

3. Action of Aqueous Acid and Base - To portions of the salt (0.5mg) dissolved in water and in aqueous ethanol were added 1 drop 10% caustic soda, and 1 drop 10% hydrochloric acid respectively. No spectrophotometric changes were recorded.

4. Action of Sodium Hydride - To a solution of the alcohol-salt (350µg 1µmole) in dry acetonitrile (10ml) in a quartz cuvette (1.0cm), was added sodium hydride (1.5 equivalents) in the same solvent (1.0ml), and the reaction followed spectrophotometrically. The absorption bands at 358 and 435nm had essentially reached a maximum after 3 hours, isosbestic points appearing at 267 and 278nm (Fig 1). The resulting orange-yellow solution was stable for several days in the absence of air or moisture, but decolourised in the air. Addition of water, saturated ammonium chloride or dry crystalline ammonium chloride also resulted in decolourisation of the sample. The reaction was repeated on a 300-fold scale, in proportionately less solvent (300ml), the reaction being followed as before and finally quenched with water. The organic solvent was carefully evaporated at room temperature, and the aqueous fraction exhaustively extracted into ether. The residue from combining the ether fractions and evaporating in vacuo appeared to contain only polar material (t.l.c. Rf 0.1 acetone-benzene, 1:9), which did not fluoresce under an ulta-violet lamp (medium pressure). Saturated aqueous potassium iodide was added to the aqueous fraction (1 vol) followed by continuous chloroform extraction. The organic fraction was dried ($Na_2SO_4$) and evaporated in vacuo

to yield a white crystalline salt identified as the original starting material (IV, R = $CH_3$, X = I) by mp., mixed mp. and infra-red spectrum.

# PART II

## The Preparation and Characterisation of NADH-Aldehyde Model Compounds (V)

2-Nicotinylbenzaldehyde (XV) - A solution of the corresponding benzyl alcohol (VI) (10.7g 0.05 mole) in benzene or chloroform (11) was oxidised with excess activated manganese dioxide[196] (100g) at room temperature in 40 minutes, (t.l.c. acetone-benzene, 1:99). The mixture was filtered, the filter cake washed well with chloroform (400 ml) and the filtrate evaporated to dryness at the pump. The semi-solid 2-nicotinyl-benzaldehyde (6.8g 65%) was recrystallised from benzene-petrol as colourless plates, mp. 95-6°; $\nu_{max}$ 1690, 1675, 1585, 940 $cm^{-1}$; $\lambda_{max}$ (ethanol) 228 nm ($\epsilon$ 1060); $\tau$ 2.46 (4H, m, arom H), 0.07 (1H, s, aldehyde H), 1.23, 1.90, 2.05 and 1.30 (1H, m, respect. 2,4,5 and 6 pyridine H), $J_{56}$, 5.0Hz, $J_{45}$, 7.7Hz, $J_{46}$, 1.8Hz, $J_{26}$, 1.8Hz, $J_{24}$, ~0Hz; [found: C, 73.78%; H, 4.38%; N, 6.55%. $C_{13}H_9NO_2$ (211.22) requires: C, 73.92%; H, 4.29%; N, 6.63%].

2-Nicotinylbenzaldehyde Dimethylacetal N-Methiodide Salt (XVIII) - The pyridine-aldehyde (XV) (0.21g 1mmole) was stirred for 24 hours in excess dry methyl iodide (1.42g 10mmole), evaporated to dryness and the yellow gummy residue triturated with benzene-ether. The resulting pale yellow powder was recrystallised from ethyl acetate as pale yellow microcrystals of 2-nicotinyl-benzaldehyde dimethylacetal-N-methiodide salt (0.27g 65%), mp. 147-8°; $\nu_{max}$ 3260, 1640, 1080, 790 $cm^{-1}$; $\lambda_{max}$ (ethanol) 267nm ($\epsilon$ 4120); (DMSO-$d_6$)

2.60 (4H, m, arom H), 3.70 (1H, s, acetyl H), 6.44 and 6.77 (3H, s, $OCH_3$), 5.61 (3H, s, $NCH_3$), 0.86,1.56,1.98 and 1.05 (1H, m, respect. 2,4,5 and 6 pyridine H), $J_{56}$, 6.0Hz, $J_{45}$ 8.0Hz; [found: C, 46.86%; H, 4.37%; N, 3.44%. $C_{16}H_{18}INO_4$ (415.15) (monohydrate) requires: C, 46.49%; H, 4.34%; N, 3.39%].

The reaction was repeated using the pyridine-aldehyde (0.12g 1mmole) with methyl iodide (0.14g 1mmole) alone, and in benzene (0.5ml) under rigorously dry conditions. (The methyl iodide was dried over calcium chloride and redistilled). A third reaction was conducted with the same quantities of reagents in benzene at 100° under nitrogen in a Carius tube for 4 hours. In each case a majority of starting material was isolated on workup (mp., $\nu_{max}$) along with small quantities of the dimethyl acetal (XVIII) (mixed mp.).

2-(α-Hydroxy-3-picolyl)-toluene (XIX) - To a well-stirred suspension of dry magnesium turnings (2.9g 0.12g atom) in warm dry THF (10ml) was added a portion of 2-bromotoluene (0.01mole of a solution of 17.1g 0.1mole in 50ml same solvent), and a crystal of iodine. The reaction mixture was maintained at 40° until the reaction commenced, and the remainder of the bromotoluene added in the usual way (as a solution in an additional 200ml THF). The mixture was refluxed a further 30 minutes and cooled to room temperature. The Grignard reagent was treated in the normal way with 3-pyridine carboxaldehyde (10.7g 0.1mole) in THF (100ml) over 20 minutes, and the pale yellow mixture finally refluxed for a further 30 minutes and cooled.

The reaction was quenched with saturated ammonium chloride and worked up. The crude product (16.1g 80%) was readily recrystallized from benzene as colourless cubes of 2-(α-hydroxy-3-picolyl)-toluene, mp. 129-30°; $\nu_{max}$ 3200, 1599, 1460, 770cm$^{-1}$; $\lambda_{max}$ 257 (ε 2980), 262 (ε 3300), 267nm (ε 2440); τ2.78 (4H, m, arom H), 4.01 (1H, s, benzyl CH), 5.23 (1H, s (broad), OH), 7.66 (3H, s, $H_3$), 1.42,2.34,2.36 and 1.56 (1H, m, respect. 2, 4,5 and 6 pyridine H), $J_{56}$, 4.0Hz, $J_{45}$, 7.5Hz; [found: C, 78.30%; H, 6.54%; N, 6.98%. $C_{13}H_{13}NO$ (199.31) requires: C, 78.36%; H, 6.58%; N, 7.03%]. Picrate (recrystallized from ethanol) mp. 149-50°; picrolonate (ethanol) mp. 205-6°.

2-(α-Acetoxy-3-picolyl)-benzaldehyde (XXI) - Chromium trioxide in acetic anhydride under the standard conditions[194] oxidised the alcohol-toluene compound (XIX) (1.0g 5mmole) to the expected tri-acetylated product (XX) in 50-55% yield. The crude reaction mixture was poured into ice-water (200ml), extracted into chloroform (3 portions of 50ml) and the combined organic ice-cooled. Vigorous shaking with 1N caustic soda solution (2 portions of 20ml), was followed by washing with water to colourless aqueous washings, drying ($Na_2SO_4$), and removal of solvent, $\nu_{max}$ (crude oil) 1760, 1740cm$^{-1}$. Attempted purification of this compound on an alumina column (100g Grade V) eluted with acetone-petrol (1:100) afforded a colourless mobile liquid 2-(α-acetoxy-3-picolyl)-benzaldehyde (0.38g 28% overall yield from alcohol-toluene), $\nu_{max}$ (liquid film) 2760, 1740, 1695cm$^{-1}$; τ~2.2 (4H, m, arom H), 0.20 (1H, s, aldehyde H),

7.85 (3H, s, $CH_3$), 2.68 (1H, s, benzyl H), 1.30, ~2.2, ~2.4 and 1.41 (1H, m, respect. 2,4,5 and 6 pyridine H). The picrate derivative formed easily and was recrystallized from aqueous ethanol, mp. 172-3°; [found: C, 52.01%; H, 3.50%; N, 11.54%. $C_{21}H_{16}N_4O_{10}$ (484.40) requires: C, 52.07%; H, 3.33%; N, 11.57%]. Attempted hydrolysis of the crude intermediate triacetylated oxidation product under the usual conditions[194] gave rise to 3 major components (t.l.c. acetone-benzene 5:95) at Rf 0.10, 0.25 and 0.55 none of which corresponded to the alcohol-aldehyde expected, or to the corresponding keto-aldehyde (p.m.r., infra-red).

Attempted Oxidation of (XIX) to (XV) Directly

1. Ceric Ammonium Nitrate[203] - Ceric ion (3.3g 6mole-equiv) in 50% aqueous acetic acid (15ml) was added to a solution of the alcohol-toluene (0.2g 1mmole) in the same solvent (5ml), and the solution heated briefly to 90° (5 min). After ice-cooling, the solution was just neutralised with 1N sodium hydroxide and extracted into chloroform (3 portions of 10ml). T.l.c. indicated that no keto-aldehyde was present in the mixtures of products. The major component under a variety of conditions of temperature (30-100°) was the ketone (XVI) along with unchanged starting material (infra-red). Reactions were also performed in perchloric acid - water (3:2) and sulphuric acid - water (1:1); in each case the reaction mixture appeared to remain unchanged until the temperature reached ~80° when charring occurred.

2. Selenium Dioxide[204] - Excess selenium dioxide (0.45g 4 mmole) oxidised the alcohol (0.2g 1mmole) to the ketone (XVI) after 20hours in refluxing chlorobenzene (5ml) under nitrogen. T.l.c. showed that the reaction mixture contained a small quantity ( 10%) of the required keto-aldehyde (XV) but further refluxing resulted in gradual thermal decomposition.

2-Nicotinyltoluene (XVI) - The alcohol-toluene (XIX) (10g 0.05 mole) was quantitatively oxidised to the corresponding ketone with manganese dioxide[196] (120g) in pure chloroform (500ml) in 8 hours at room temperature. Workup as before[196] yielded 2-nicotinyltoluene (9.5g 96%) as a colourless viscous liquid which could not be crystallized, $\nu_{max}$ (liquid film) 1670, 1588, 940cm$^{-1}$; $\tau$2.60 (4H, m, arom H), 7.59 (3H, s, $CH_3$), 1.00,1.81,2.54 and 1.14 (1H, m, respect. 2,4,5 and 6 pyridine H), $J_{56}$, 4.5Hz, $J_{45}$, 8.0Hz, $J_{26}$, 1.8Hz, $J_{46}$, 1.8Hz, $J_{24}$, ~0Hz. The picrate derivative formed easily and was recrystallized from ethanol, mp. 174-5°; [found: C, 53.39%; H, 3.48%; N, 13.32%. $C_{19}H_{14}N_4O_8$ (426.31) requires: C, 53.53%; H, 3.31%; N, 13.14%]. The semicarbazone was also prepared and recrystallized from petrol, mp. 171-2°.

2-Nicotinyltoluene-α,α-diacetate (XXIV) - The keto-toluene (XVI) (6g 0.03mole) was oxidised to the corresponding diacetate with chromium trioxide in acetic anhydride under the standard conditions[194] with the following modifications: The reaction temperature was maintained at -5° during the addition of oxidant instead of 5-10°, and an inert atmosphere was employed. Under

these conditions, and with efficient stirring the reaction was complete in about 4 hours. The mixture was worked up in the usual way[194] and afforded 2-nicotinyltoluene-α,α-diacetate (~5g 55%) as a rather unstable viscous pale yellow liquid which was not fully characterised, τ 2.6 (4H, m, arom H), 7.94 and 8.08 (3H, s, acetyl $CH_3$), 1.24,~2.0,~2.0 and 1.42 (1H, m, respect. 2, 4,5 and 6 pyridine H).

2-Nicotinylbenzoic Acid Betaine (XXII) - Oxidation of the keto-toluene (XVI) (6g 0.03mole) with chromium trioxide in acetic anhydride according to the literature procedure[194] at 5-10°, followed by workup of the reaction product as described before gave an oily product which was hydrolysed directly, without characterisation. The hydrolysis product was filtered from the carefully neutralised aqueous solution as a pale yellow solid which was soluble in both dilute acid and base, but insoluble in benzene and chloroform. It was recrystallized from absolute ethanol and characterised as 2-nicotinylbenzoic acid betaine (2.1g 31%), mp. 176-7°; $\nu_{max}$ 2500, 1910 (broad), 1680, 960cm$^{-1}$; $\lambda_{max}$ 233 nm (ε 520); τ ($^2H_2O$/t-BuOH) ~2.4 (4H, m, arom H), 1.22,1.84,~2.2 and 1.29 (1H, m, respect. 2,4,5 and 6 pyridine H), $J_{56}$, 4.5Hz, $J_{45}$, ~8Hz; [found: C, 68.61%; H, 4.14%; N, 6.12%. $C_{13}H_9NO_3$ (227.22) requires: C, 68.72%; H, 3.99%; N, 6.16%].

2-Nicotinyl Methyl Benzoate (XXIIIa) - The pure betaine salt (XXII) (2.0g 8.8mmole) was dissolved in absolute methanol (50ml) which was previously saturated with dry hydrogen chloride gas,

and cooled to 0° [213]. The solution was allowed to warm up to room temperature overnight, the solvent removed in vacuo and the residue treated with another volume of methanolic hydrogen chloride (50ml) at 0°, and again allowed to warm up overnight. Removal of the solvent a second time afforded 2-nicotinyl methyl benzoate (1.6g 75%) as a crystalline mass which was easily recrystallized from benzene-petrol, mp. 74.0-.5°; $\nu_{max}$ 1705, 1675, 1590, 940cm$^{-1}$; $\lambda_{max}$ 228 (ε 14440), 266nm (ε 5100); τ 2.5 (4H, m, arom H), 6.24 (3H, s, $CH_3$), 1.18, 1.90, 1.95 and 1.32 (1H, m, respect. 2,4,5 and 6 pyridine H), $J_{56}$, 4.8Hz, $J_{45}$, 7.0Hz, $J_{26}$, 1.9Hz, $J_{46}$, 1.8Hz, $J_{24}$, 0Hz; [found: C, 69.67%; H, 4.62%; N, 5.78%. $C_{14}H_{11}NO_3$ (241.25) requires: C, 69.70%; H, 4.60%; N, 5.81%].

2-Nicotinylbenzaldehyde (XV) (large scale preparation) - The crude diacetate compound (XXIV) was hydrolysed under both acidic and basic conditions:

1. Acid hydrolysis - p-Toluene sulphonic acid (1.72g 10mmole) in methanol-water (3:7) (30ml) effected hydrolysis of the α,α-diacetate (1.56g 5mmole) after prolonged periods of refluxing (48 hours). Basic workup followed by chromatography afforded a modest yield of the required aldehyde (45%). 10% Mineral acid (20ml) in methanol (20ml) hydrolysed the compound (3.5g 10mmole) in 3 days at room temperature; refluxing the solution caused decomposition. A similar yield was obtained after chromatography.

2. Base hydrolysis - A solution of the diacetate (7.0g 20 mmole) in absolute ethanol (20ml) was treated with sodium (0.46g

20mg atom) in the same solvent (10ml). A white precipitate formed immediately, and the reaction mixture was shaken vigorously for 30 minutes. Saturated aqueous ammonium chloride solution (10ml) was run in, and the product extracted into ether. The resulting crude aldehyde was contaminated with an ester, and required purification on an alumina column (200g grade V, eluted with 200ml benzene to remove the ester, 400ml acetone-benzene (2:98) to recover the aldehyde), (2.74g 65%), mp. 94-5°.

2-Nicotinyl Ethyl Benzoate (XXIIIb) - The major byproduct from the preceeding reaction hydrolysis was 2-nicotinyl ethyl benzoate (0.44g 9%) which was recrystallized from ether-petrol as colourless cubes, mp. 61.0- .5°; $\nu_{max}$ 1715, 1675, 1280, 930 $cm^{-1}$; $\lambda_{max}$ 230 (ε 14600), 267nm (ε 5280); $\tau$ ~2.5 (4H, m, arom H), 8.87 (3H, t (J, 7.0Hz), $CH_3$), 5.87 (2H, q, $CH_2$), 1.17,1.92, 1.97 and 1.28 (1H, m, respect. 2,4,5 and 6 pyridine H), $J_{56}$, 4.8Hz, $J_{45}$, 7.0Hz, $J_{26}$, 2.0Hz, $J_{46}$, 1.8Hz, $J_{24}$, ~0Hz; [found: C, 70.83%; H, 5.17%; N, 5.43%. $C_{15}H_{13}NO_3$ (255.10) requires: C, 70.68%; H, 5.13%; N, 5.49%].

2-Nicotinylbenzaldehyde (XV) (other routes)

1. Hydrolysis of the acetate (XXI) (0.37g 1.5mmole) with dilute mineral acid (10ml) in ethanol (5ml) followed by basic work-up yielded a colourless oil. This was oxidised with activated manganese dioxide (5g)[196] in pure chloroform (200ml) to give the expected keto-aldehyde (0.16g 50% overall yield) after 8 hours.

2. Reduction of the combined methyl and ethyl esters (XXIII) (15mmole) in dry THF (30ml) was effected with L.A.H. (0.68g 18mmole) in the same solvent. The reaction was conducted at 0°, and allowed to warm up to room temperature overnight. It became bright red at first, the colour gradually fading to a pale straw, and was worked up after 8 hours with saturated ammonium chloride (10ml) in the usual way, to yield a pale yellow mobile liquid, τ2.82 (4H, m, arom H), 5.60 (2H, s, benzyl $CH_2$), 4.05 (1H, s, benzyl CH), ~4.9 (2H, s(broad), 2 OH), 1.84,2.40, ~2.8 and 1.92 (1H, m, respect. 2,4,5 and 6 pyridine H), $J_{45}$, ~8Hz, $J_{56}$~4Hz. This diol was not further characterised, but directly oxidised with activated manganese dioxide[196] (40g) in chloroform (150ml) to the keto-aldehyde in ten hours at room temperature and in 70% overall yield (2.2g). The product was recrystallized from benzene-petrol, mp. 94-5°.

2,6-Dichlorobenzyl Bromide - To a solution of 2,6-dichlorotoluene (32g 0.2mole) in dry carbon tetrachloride (500ml) was added N-bromosuccinimide (34g 0.2mole) and the mixture refluxed for 72 hours[201]. The cooled mixture was filtered, the filtrate evaporated to dryness taken up in petrol and refiltered through a short alumina column. The product was recrystallized from 40-60 petrol (41g 86%), mp. 55-6° (lit.[214] mp. 55°).

Salts of Nicotinamide (XXV, R = $NH_2$) - The following N-alkyl and N-aralkyl nicotinamide salts were made in virtually quantitative yields by standard methods[148]:

N-benzyl chloride salt, mp. 235-6° (lit.[200] mp. 236.5-.7°);
N-benzyl bromide salt, mp. 203-4° (lit.[215] mp. 205°);
N-(2,6-dichlorobenzyl) bromide salt, mp. 246-8° (lit.[214] mp. 246-8°);
N-methiodide salt, mp. 203-4° (lit.[14] mp. 204°). The following salts were prepared by addition of saturated aqueous potassium iodide to concentrated aqueous solutions of the corresponding bromide salts
N-benzyl iodide salt, mp. 168-70° (lit.[200] mp. 170.3-.5°);
N-(2,6-dichlorobenzyl) iodide salt, mp. 196-7° (lit.[148] mp. 196°).

Salts of Ethyl Nicotinate (XXV, R = $OCH_2CH_3$) - Refluxing was required for longer periods of time to prepare these carboxylate salts, but yields were still high (80-85%):
N-benzyl bromide salt, mp. 141-3° [found: C, 55.69%; H, 5.28%; Br, 24.62%; N, 4.09%. $C_{15}H_{16}BrNO_2$ (322.21) requires: C, 55.93%; H, 5.00%; Br, 24.79%; N, 4.34%];
N-methiodide salt, mp. 98-9° (lit.[216] mp. 100°);
N-(2,6-dichlorobenzyl) perchlorate salt,,mp. 157-8°,[found: C, 43.80%; H, 3.61%; Cl, 25.59%; N, 3.36%. $C_{15}H_{14}Cl_3NO_6$ (410.64) requires: C, 43.89%; H, 3.44%; Cl, 25.91%; N, 3.41%];

N-(2,6-Dichlorobenzyl)-2-nicotinyl Methyl Benzoate Bromide (XXV, R = DCB, R' = $CO_2CH_3$, X = Br) - Equimolar quantities of 2-nicotinyl methyl benzoate (1.2g 5mmole) and 2,6-dichlorobenzyl bromide (1.2g) were refluxed in analar acetone (30ml) for 10 hours[148]. The solvent was distilled off, the gummy residue triturated with anhydrous ether, and the resulting solid recrystallized

from ethanol as colourless prisms of N-(2,6-dichlorobenzyl)-2-nicotinyl methyl benzoate bromide (1.94g 74%), mp. 101-3°; $\nu_{max}$ 1720, 1680, 1475, 1280cm$^{-1}$; $\lambda_{max}$ (ethanol) 272 (ε 6190), 324nm (ε 310), τ ~2.4 (7H, m, arom H), 6.29 (3H, s, $CH_3$), 3.50 (2H, s, benzyl $CH_2$), 1.08,1.40,1.94 and 1.35 (1H, m, respect. 2,4,5 and 6 pyridine H), 8.80 (3H, t ($J_{Et}$, 6.8Hz), $CH_3$), 6.34, (2H, q, $CH_2$), 7.97 (1H, s, OH), [found: C, 52.42%; H, 4.12%; N, 2.58%. $C_{23}H_{22}BrCl_2NO_4$ (527.25) requires: C, 52.41%; H,4.21%; N, 2.66%].

N-Benzyl-2-nicotinylbenzaldehyde Bromide (XVII), R = $CH_2\emptyset$, X = Br) - The pyridine-aldehyde (XV) (1.05g 5mmole)and benzyl bromide (1.03g 6mmole) were refluxed together in analar acetone (30ml) overnight[148], evaporated to dryness and stirred with anhydrous ether. N-Benzyl-2-nicotinylbenzaldehyde bromide precipitated as a yellow, amorphous hygroscopic powder (1.6g 82%) which was not fully characterised. Attempts to crystallize it from ethanol, methanol,ethyl acetate,ether,benzene or combinations of these solvents failed, causing it to revert to a gum, $\nu_{max}$ (KBr disc) 1690, 1635, 1465cm$^{-1}$; $\lambda_{max}$ 264nm (ε 5960); τ ($^2H_2O$/acetone) 2.87 (9H, m, arom H), 4.48 (2H, s, benzyl $CH_2$), 0.08 (1H, s, aldehyde H), 1.17,1.66,2.22 and 1.40 (1H, m, respect. 2,4,5 and 6 pyridine H).

N-(2,6-Dichlorobenzyl)-2-nicotinylbenzaldehyde Bromide (XVII, R = DCB, X = Br) - This salt was prepared like the previous one from 2,6-dichlorobenzyl bromide and the pyridine-aldehyde

(XV) (1.05g 5mmole), and was isolated in 67% yield as a pale yellow amorphous solid (1.5g) which could not be induced to crystallize, $\nu_{max}$ (KBr disc) 1695, 1635, 1450cm$^{-1}$; $\lambda_{max}$ 270nm (ε 4640); τ ($^2H_2O$/acetone) 2.57 (7H, m, arom H), 3.87 (2H, s, benzyl $CH_2$), 0.08 (1H, s, aldehyde H), 0.90,1.25,1.83 and 1.03 (1H, m, respect. 2,4,5 and 6 pyridine H), $J_{45}$, 8.4Hz, $J_{56}$, 6.2Hz; [found: C, 50.76%; H, 3.45%; N, 2.81%. $C_{20}H_{16}BrCl_2NO_3$ (469.17) requires: C, 51.19%; H, 3.44%; N, 2.98% ]. Attempts to render this compound and the previous one crystalline by exchanging the anion (X = I, picrate or picrolonate) yielded further gums or hygroscopic powders. Further characterisation of these compounds was not attempted.

N-Aralkyl-1,4-dihydronicotinamides (XXVI, R' = $NH_2$)

1. N-Benzyl-1,4-dihydronicotinamide - The standard procedure using sodium dithionite (hydrosulphite) (1.5 equivs) in aqueous sodium carbonate[25] as the medium for the reduction of N-benzylnicotinamide bromide (0.05mole) gave a gummy product which was moderately stable at this pH. Warming at 50° for 15 minutes caused the slow evolution of sulphur dioxide, and solidification of the compound. Replacement of the sodium carbonate with bicarbonate[205] effected the reduction more reproducibly and rapidly. To a solution of the salt (14.7g 0.05mole) in water (200ml) was added a solution of the reducing agent (14.8g 0.07mole) in 50% saturated aqueous sodium bicarbonate (100ml) in a 1l flask plus air condenser. The solution fleetingly became bright orange and immediately deposited a yellow gummy solid. This intermediate rapidly solid-

ified on vigorous shaking at room temperature or after briefly warming, evolved sulphur dioxide and left an almost colourless supernatamt liquid phase. The product readily recrystallized from ethanol as long yellow needles (8.6g 88%), mp. 119-21° (lit.[25] mp. 120-2°); $\lambda_{max}$ 354 (ε 7720) (lit.[25] $\lambda_{max}$ 355nm (ε 7700). This buffer modification was used in all subsequent reductions.

2. N-(2,6-Dichlorobenzyl)-1,4-dihydronicotinamide - The product was recrystallized from ethanol-water (9:1). (85%), mp. 147-9° (lit.[214] mp. dec >150°); $\lambda_{max}$ 350 (ε 7480) (lit.[214] $\lambda_{max}$ 350nm (ε 7510).

N-(2,6-Dichlorobenzyl)-3-carbethoxy-1,4-dihydropyridine (XXVI, R = DCB, R' = $OCH_2CH_3$) - Prepared in the usual way from ethyl nicotinate N-(2,6-dichlorobenzyl)-bromide salt and recrystallized from ethanol water (9:1), mp. 79-80° (lit.[148] mp. 76-8°); $\lambda_{max}$ 210 (ε 22600), 350 (ε 7780) (lit.[148] $\lambda_{max}$ 352nm (ε 7990).

: N-(2,6-Dichlorobenzyl)-1,4-dihydronicotinyl-2'-methyl benzoate (XXVI, R = DCB, R' = o-Ø.$CO_2CH_3$) - The corresponding bromide salt (XXV, R = DCB, R' = $CO_2CH_3$, X = Br) (1.45g 3mmole) was reduced to N-(2,6-dichlorobenzyl)-1,4-dihydronicotinyl -2'-methyl benzoate as yellow prisms (0.9g 75%) which were recrystallized from absolute ethanol, mp. 142-4°; $\nu_{max}$ 1720, 1675, 1625, 725cm$^{-1}$; $\lambda_{max}$ 370nm (ε 9600); τ ~2.7 (7H, m, arom H), 6.19 (3H, s, $CH_3$), 5.56 (2H, s, benzyl $CH_2$), 3.49,5.03 and 4.12 (1H, m, respect. 2, 5 and 6 pyridine H), 6.74 (2H, m, 4 pyridine H), $J_{45}$, 3.5Hz, $J_{56}$, 8.5Hz, $J_{26}$,1.5Hz, $J_{46}$, ~1Hz; mass spectrum: 401/3, 386/8,369/71

340/2, 242,241, 238/40, 210, 182, 159/61m.u., [found: C, 62.60%; H, 4.22%; N, 3.30%. $C_{21}H_{17}Cl_2NO_3$ (402.28) requires: C, 62.69%; H, 4.26%; N, 3.48% ].

Reaction of Benzaldehyde with Sodium Dithionite - To a solution of rdistilled benzaldehyde (1.1g 10mmole) in absolute ethanol (50ml) was added sodium dithionite (30mmole) in 20% saturated aqueous sodium bicarbonate (50ml) and the solution warmed to 40° for 4 hours under nitrogen. The bulk of the solvent was removed at room temperature in vacuo, the aqueous residue extracted with ether (3 portions of 20ml), the organic fraction dried ($Na_2SO_4$) and the solvent removed, $\nu_{max}$ no band at 3500cm$^{-1}$,; $\tau$ 0.01 (1H, s, aldehyde H), ~2.7 (5H, m, arom H). No benzyl alcohol was detected by t.l.c.

N-(2,6-Dichlorobenzyl)-1,4-dihydronicotinyl-2'-benzaldehyde (V, R = DCB) - The crude bromide salt (XVII, R = DCB, X = Br) (1.8g 4mmole) was dissolved in water (30ml) with vigorous shaking, and decanted from any residual tar. The solution was filtered if necessary and washed with ether. After warming the solution to 30°and protecting the flask from light and air, the buffered reducing agent was added in the usual way (5.5mmole) with stirring. The mixture was allowed to cool slowly overnight and the powdery yellow solid filtered. N-(2,6-Dichlorobenzyl)-1,4-dihydronicotinyl-2'-benzaldehyde (0.95g 64%) was recrystallized with some difficulty from benzene, mp. 140-1°; $\nu_{max}$ 1705, 1675, 1620, 780cm$^{-1}$; $\lambda_{max}$ 243 ($\epsilon$ 10800), 378 ($\epsilon$ 10100); (chlorodorm) 245 ($\epsilon$ 15050), 380nm ($\epsilon$ 11900); $\tau$ 2.75 (7H, m, arom H), 0.01

(1H, s, aldehyde H), 5.55 (2H, s, benzyl $CH_2$), 3.43,5.01 and 4.13 (1H, m, respect. 2,5 and 6 pyridine H), 6.76 (2H, m, 4 pyridine H), $J_{56}$, 8.2Hz, $J_{45}$, 3.5Hz, $J_{26}$, 1.8Hz, $J_{46}$, ~1Hz; mass spectrum: 371/3, 342/4, 326/8, 212, 159/61 m.u., [accurate mass measurement:- found: 371.0464, 212.0711. $C_{20}H_{15}{}^{35}Cl_2NO_2$ and $C_{13}H_{10}NO_2$ require: 371.0480, 212.0711 respectively], [found: C, 64.81%; H, 4.12%; Cl, 18.76%; N, 3.56%. $C_{20}H_{15}Cl_2NO_2$ (372.26) requires: C, 64.54%; H, 4.06%; Cl, 19.05%; N, 3.76%]. The semicarbazone derivative was also prepared and crystallized from benzene mp. 204-5°.

N-(2,6-Dichlorobenzyl)-2-nicotinylbenzyl Alcohol Bromide (IV, R = DCB, X = Br) - This salt was prepared in the usual way from 2,6-dichlorobenzyl bromide (1.5g 6mmole) and the pyridine-alcohol (VI) (2.6g 5mmole) and isolated as a crude, unstable, hygroscopic powder (2.6g 86%) which could not be crystallized and which was consequently not fully characterised, $\nu_{max}$ 3420, 1635, 1580, 775cm$^{-1}$; $\lambda_{max}$ 264nm ($\epsilon$ ~6300). Picrate derivative, $\nu_{max}$ 1640, 1620, 1575cm$^{-1}$. The iodide and perchlorate salts were not crystalline.

Addition Compounds of the Pyridinium Salt (IV, R = DCB, X = Br)

1. Benzyl Mercaptan[118] - To a solution of redistilled benzyl mercaptan (0.12g 1mmole) in dry benzene (5ml) was added purified sodium hydride (0.027g 1.1mmole) under nitrogen, and the mixture stirred for 1 hour at room temperature. The salt (IV, R = DCB, X = Br) (0.37g 1mmole) was added in 1 lot whereupon the

solution became bright red, and the solid gradually dissolved. Workup under neutral conditions followed by t.l.c. yielded a major component at Rf 0.2 (acetone-benzene, 1:99) as a red oil in small amount which was not further purified, $\lambda_{max}$ 342nm.

2. Cyanide[71,118] - A purified, concentrated aqueous solution of the salt (IV, R = DCB, X = Br) (0.12g 1mmole) was treated with saturated potassium cyanide (0.32g 5mmole), the mixture vigorously shaken for 10 minutes, and then extracted into ether. Chromatography on alumina (acetone-benzene, 1:9) afforded 3 components, one of which at Rf 0.80 (20%) was isolated and identified ($\lambda_{max}$ 327nm; mass spectrum) as N-(2,6-dichlorobenzyl)-1,4-dihydronicotinyl-4-cyano-2'-benzyl alcohol (XXIX, R = DCB, R' = o-Ø.$CH_2OH$), mass spectrum: 398/400, 265/7, 252/4, 239, 200 m.u., [found: 398.0592. $C_{21}H_{16}{}^{35}Cl_2N_2O$ requires: 398.0589].

N-(2,6-Dichlorobenzyl)-3-carbethoxy-4-cyano-1,4-dihydropyridine (XXIX, R = DCB, R' = $OCH_2CH_3$) - The reaction was performed on the same molar scale as before, using N-(2,6-dichlorobenzyl)-ethyl nicotinate bromide (0.4g). The resulting orange gum crystallized on brief stirring with ether-petrol, and was recrystallized from absolute ethanol as long yellow needles (0.24g 70%), mp. 134-6°; $\nu_{max}$ 2250, 1682, 1600$cm^{-1}$; $\lambda_{max}$ 340 (ε 14000) (lit.[71] $\lambda_{max}$ 344nm); τ 2.64 (3H, m, arom H), 5.29 (2H, s, benzyl $CH_2$), 5.80 (2H, q ($J_{Et}$, 7.0Hz), ethyl $CH_2$), 8.74 (3H, t, ethyl $CH_3$), 2.76,5.21 and 3.91 (1H, m, respect. 2,5 and 6 pyridine H), 5.51 (2H, m, 4 pyridine H), $J_{45}$, 4.8Hz, $J_{56}$, 8.0Hz, $J_{26}$, 1.2Hz; [found: C, 56.91%; H, 4.19%; Cl, 20.90%; N, 8.29%. $C_{16}H_{14}Cl_2N_2O_2$ (337.21) requires: C, 56.99%; H, 4.18%; Cl, 21.03%; N, 8.31%].

N-(2,6-Dichlorobenzyl)-1,4-dihydronicotinyl-2'-benzyl Alcohol (XXX)

Method 1. - The corresponding pyridinium bromide (IV,R = DCB, X = Br) (0.45g 1mmole) was reduced with buffered dithionite in the usual way. The yield of crude material was 0.25g (67%).

Method 2. - The corresponding dihydronicotinylbenzaldehyde (V, R = DCB) (0.074g 0.2mmole) as a solution in absolute ethanol (40ml) under nitrogen was treated dropwise with ethanolic sodium borohydride[23] (0.0024g 0.06mmole) at room temperature. The part-suspension dissolved within a few minutes to give an orange-yellow solution, and t.l.c. (acetone-petrol, 1:9) indicated complete absence of starting material (Rf 0.45) and formation of a more polar component (Rf 0.20). Workup with water followed by recrystallization from ethanol yielded yellow needles (0.068g 90%) of N-(2,6-dichlorobenzyl)-1,4-dihydronicotinyl-2'-benzyl alcohol mp.114-18°; $\nu_{max}$ 3300, 1670, 1620, 1590cm$^{-1}$; $\lambda_{max}$ 374 (ε 8670); τ 2.75 (7H, m, arom H), 5.50 (2H, s, DCB $CH_2$), 5.57 (2H, s, carbinol $CH_2$), ~6.4 (1H, s (exchangeable), OH), 3.18,5.02 and 4.12 (1H, m, respect. 2,5 and 6 pyridine H), 6.77 (2H, m, 4 pyridine H), $J_{56}$, 8.1Hz, $J_{45}$, 4.0Hz, $J_{26}$, ~1.8Hz, $J_{46}$, 1.6Hz, $J_{24}$, ~0Hz; mass spectrum: 373/5, 372/4, 355/7, 354/6, 327/9, 326/8, 214, 213, 196, 195, 168, 167, 159/61 m.u. (fig. ); [accurate mass measurement:- found: 373.0636, 326.0507, 196.0767. $C_{20}H_{17}{}^{35}Cl_2NO_2$ requires: 373.0636, $C_{19}H_{14}{}^{35}Cl_2N$ requires: 326.0503, $C_{13}H_{10}NO$ requires: 196.0762].

N-Methyl-1,4-dihydronicotinyl-2'-benzyl Alcohol (XXVI, R = $CH_3$, R' = o-Ø.$CH_2OH$) - The methiodide salt (IV, R = $CH_3$, X = I) (0.35g

1mmole) was reduced with dithionite ion in bicarbonate buffer in the usual way and was isolated as a bright yellow mobile liquid (0.17g 74%), $\nu_{max}$ (liquid film) 3250, 1670cm$^{-1}$; $\lambda_{max}$ 375 ($\epsilon$ ~7800); $\tau$ 2.75 (4H, m, arom H), 7.13 (3H, s, $CH_3$), 5.58 (2H, s, $CH_2$), 6.67 (1H, s (broad, exchangeable), alcohol H), 3.44,5.00 and 4.37 (1H, m, respect. 2,5 and 6 pyridine H), 6.49 (2H, m, 4 pyridine H), $J_{56}$, 8.0Hz, $J_{45}$, 3.9Hz, $J_{26}$, 1.2Hz, $J_{46}$, 1.5Hz, $J_{24}$, ~0Hz; mass spectrum: 229, 228, 211, 210, 195, 182, 168, 167 m.u.; accurate mass measurement:- [found: 229.1097, 182.0965. $C_{14}H_{15}NO_2$ requires: 229.1103, $C_{13}H_{12}N$ requires: 182.0970].

Oxidation Reactions of 1,4-Dihydropyridines

1. 2,3-Dichloro-5,6-dicyanobenzoquinone (DDQ)[16] - To a solution of N-benzyl-1,4-dihydronicotinamide (0.21g 1mmole) in anhydrous pure benzene (1ml) was added DDQ (0.23g 1mmole) in the same solvent (1ml) by dropwise addition at room temperature and under nitrogen. A deep cherry-red colour fleetingly appeared with each drop, until near the end of the end of the addition, when the solution became intense greenish-red. Filtration after 20 minutes gave a small amount of a pale green precipitate which was insoluble in protic solvents and had an indeterminate infra-red spectrum. The filtrate yielded a gum yielded a gum which dissolved in non-protic solvents and was largely unchanged starting material (infra-red spectrum).

2. Manganic Tris-(acetylacetonate) (MTA)[217] - This oxidant was prepared accoding to the standard procedure[217] from acetyl acetone and manganous chloride in 70% yield. To a solution of

N-benzyl-1,4-dihydronicotinamide (0.21g 1mmole) in dry acetonitrile[218] (2ml) was added MTA (0.42g 1.2mmole) in the same solvent (2ml). There was no colour change after 24 hours at room temperature, or after a further 24 hours refluxing. The solvent was carefully removed, 10% aqueous sodium chloride added, and the solution washed with ether. Addition of saturated potassium iodide (1ml) to the aqueous solution caused no precipitation after 1 week at 0°. Continuous extraction with chloroform yielded no product. Replacement of the reaction solvent with chloroform likewise yielded no product.

3. Lead Tetraacetate - This oxidant was recrystallized from acetic acid/acetic anhydride and stored over $P_2O_5$ in the dark. N-(2,6-Dichlorobenzyl)-ethyl 1,4-dihydronicotinate (3g 10mmole) in dry redistilled pyridine (10ml) was treated dropwise with a solution of the oxidant (5g 11mmole in 10ml) at room temperature. The solution became dark red and was set aside for 8 hours, poured into water and washed with ether. To the aqueous fraction was added sodium sulphate, and the lead sulphate filtered off. To the filtrate was added buffered dithionite (1.5 equivs) and a modest yield of the expected dihydropyridine (0.75g 25%) was recorded (mp.,mixed mp.).

When the method was applied to the dihydronicotinylbenzaldehyde (V, R = DCB) only trace quantities of the required redox starting material were isolated under the conditions cited.

4. Ferric Chloride - Dropwise addition of the anhydrous oxidant (1.2 equivs) in dry ether (5ml) to N-(2,6-dichlorobenzyl) ethyl-1,4-dihydronicotinate (3g 10mmole) in ether (10ml) caused

the solution to turn greenish and finally deposit a small amount of a white precipitate. Workup after 3 hours at room temperature by pouring into sodium carbonate solution, filtering and washing the filtrate with ether, gave a colourless solution which on addition of dithionite yielded ~5% of the dihydropyridine. Similar oxidations in dry acetone and aqueous systems gave respectively ~8% and ~1% of the product on workup.

5. Potassium Ferricyanide[159,161b] - To an aqueous suspension of N-benzyl-1,4-dihydronicotinamide (2.1g 10mmole in 10ml) was added the oxidant (7.8g 20mmole) in 50% saturated aqueous sodium bicarbonate (30ml). The suspension gradually dissolved to give an orange solution which faded to pale yellow after 24 hours at 35°. The warm solution, when treated with excess buffered dithionite (30mmole), deposited an orange gum which rapidly solidified to give the original dihydropyridine (1.7g 80%) by p.m.r., mp. and mixed mp.

Application of the method to ethyl nicotinate derivatives afforded much lower yields (15-25%) after 1 oxidation-reduction step, in water, sat$^{d}$. KBr solution or sat$^{d}$. $NaHCO_3$ solution. The model system (V, R = DCB) furnished unworkably low yields by this technique (2-5%).

6. Hexachloroacetone[128] - The reported[128b] oxidation of N-benzyl-1,4-dihydronicotinamide to the pyridinium chloride salt in dry acetone or cyclohexene was repeated and the product was isolated in 60-70% yield (mp. 233-4°, mixed mp. 233-4°). The ethyl nicotinate derivatives were oxidised in comparable yield (65-75%). Direct reduction of the internediate salts with

dithionite afforded the starting materials in 55-60% yields. Oxidation of the model compound (V, R = DCB) followed by similar reduction gave the starting material in workable (15-20%) quantities.

[4-$^2$H]-1,4-Dihydropyridines

[$^2$H]-Sodium Bicarbonate - Deuterated sodium bicarbonate ($Na^2HCO_3$) was prepared by passing thoroughly dry carbon dioxide gas into a solution of freshly-ignited sodium carbonate (2.1g 20mmole) in deuterium oxide ($^2H_2O$ 99%) (20ml) with stirring for 12 hours or until the pH was ~8.5. The solution was cooled to 0° and the product filtered and stored in a desiccator over calcium chloride.

1. N-(2,6-Dichlorobenzyl)-[4,4-di-$^2$H]-3-carbethoxy-1,4-dihydropyridine - The pyridinium salt (2g 6mmole) was reduced in the usual way, with the exclusion of moisture, and using $Na^2HCO_3$, dry dithionite and $^2H_2O$. Mass spectrum and p.m.r. analyses indicated a total of 48-50% deuterium incorporation in the ring 4-position. The dihydropyridine was reoxidised with hexachloroacetone[128] to the pyridinium salt by the method described, and this redox process repeated twice more. The second $^2$H-reduction incorporated ~90% total deuterium in the 4-position (isotope effect $k_H/k_D \simeq 4$). A third redox stage effected ~97% total deuterium incorporation (0.3g 15% overall yield).

2. N-(2,6-Dichlorobenzyl)-[4,4-di-$^2$H]-1,4-dihydronicotinyl-2'-methyl Benzoate (XXXVII) - The corresponding bromide salt (XXV) (0.96g 2mmole) was 3 times reduced and reoxidised with [4-$^2$H] inc-

orporation as described above. The isotope effect was $k_H/k_D \simeq 3.5$, but the overall yield was low (0.085g 8%). Mass spectrum indicated the prescence of ~90% deuterium in the 4-position.

3. N-(2,6-Dichlorobenzyl)-[4-$^2$H]-1,4-dihydronicotinyl-2'-benzaldehyde (XXXIV) - The monodeuterated model aldehyde was prepared in low yield (0.044g 12%) from the undeuterated model (0.37g 1mmole). Subsequent redox stages gave unworkably small amounts of material. Deuterium incorporation was ~48% on the first redox stage.

4. N-(2,6-Dichlorobenzyl)-[4-$^2$H]-1,4-dihydronicotinyl-2'-benzyl Alcohol (XXXVI) - Reduction of the preceding reaction product (0.037g 0.1mmole) with sodium borohydride as previously described afforded the title compound in 95% yield (p.m.r. and mass spectral comparison with (XXX).

N-(2,6-Dichlorobenzyl)-1,4-dihydronicotinyl-2'-[$\alpha$-$^2$H]-benzyl Alcohol (XXXV) - Reduction of the undeuterated model compound (V, R = DCB) (0.037g 0.1mmole) with sodium borodeuteride ($NaB^2H_4$) in an exactly analagous manner yielded the [$\alpha$-$^2$H] isomer of the preceding compound by p.m.r. and mass spectra.

## PART III

### Photolysis of NADH - Aldehyde Model Compound (V, R = DCB)

(A) General Stability of the Model - The ensuing 3 sections refer to the behaviour of the model compound in the absence of air or light. Oxidation proceeded slowly in the prescence of air, leading to complex mixtures of products, mainly polymeric in nature. It could be purified by dissolving in warm benzene filtering through bentonite, and removal of the solvent under strictly anaerobic conditions. Rapid chromatography on alumina with acetone-benzene (3:97) afforded analytically pure material.

1. Solvent Stability - The compound was stable in the solid state but was stored at $0^{\circ}$. It was soluble in common neutral organic solvents (chloroform, acetonitrile, THF, ether, acetone) less so in benzene and absolute ethanol, and sparingly soluble in petrol and water. Solutions were stable at room temperature ($\lambda_{max}$ 378nm decreased ~10% in 2 months in pure benzene).

2. Thermal Stability - A solution of the model compound (0.56mg 1.5μmole) in carefully purified ethanol or benzene (10ml) was refluxed gently for 72 hours and the reaction followed spectrophotometrically. The absorption band at 378nm had decreased ~25% after this time, and t.l.c. indicated the formation of complex decomposition products which were not investigated. The thermolysis was also conducted in pure dry refluxing pyridine (10ml) containing concd. hydrochloric acid (trace). This solution became dark during 1½ hours and appeared greenish. Refluxing was continued for 24 hours and the bulk of the solvent

distilled off under nitrogen at room temperature. Ammonium chloride solution (10% 1ml) was added and the aqueous phase washed with ether (3 portions of 2ml),until almost colourless. Excess buffered dithionite ion was added (1mmole) and the solution vigorously shaken and set aside for 1 hour. Extraction into chloroform (3 portions of 5ml) followed by t.l.c. and ultra-violet analysis showed that no dihydropyridine corresponding to (XXX) was present. The major component (Rf 0.05), was not further investigated.

3. Effect of pH - Dilute solutions of the dihydronicotinamides (XXVI, R' = $NH_2$) (~1.5μmole) in 4 different solvents were treated with several acids or buffers, and the rate of disappearance of the band at around 350nm was recorded as the half life in minutes. One solvent-buffer system was applied to the model for comparison (Table 2).

(B) Preliminary Photolytic Reactions - A solution of the dihydropyridine (0.37g 1mmole) in dry redistilled benzene (1.5l) was flushed with dry nitrogen and photolysed with a medium pressure mercury lamp at constant temperature (14-5°) and volume. The reaction which was followed spectrophotometrically, was complete in ~6 hours. No isosbestic points were observed, and the final chromophore was complex. The pale yellow cloudy solution was concentrated at room temperature in vacuo to 10ml and ammonium chloride solution (10% 10ml) added with vigorous shaking. The filtered aqueous fraction was isolated and the organic fraction again washed with dilute ammonium chloride. The combined aqueous portions were treated with buffered dithionite (2 equivs) in the usual way, and after standing for 2 hours, extracted into chloroform. The solution was dried ($Na_2SO_4$), evaporated to dryness under nitrogen and the residue purified by t.l.c. (methanol-benzene, 5:95). A major component

(Rf 0.3) was shown to be identical to the alcohol-pyridine (XXX) by t.l.c. and by mass spectrum analysis. The major photolysis product filtered from solution was insoluble in benzene, ether and water, but soluble in chloroform and ethanol. It had indeterminate infra-red and p.m.r. spectra, and was very polar (Rf 0.1, ethanol-benzene, 1:9). It was not further investigated. The organic filtrate, after extraction with dilute ammonium chloride, yielded a small quantity of a yellow gum (~ 25%) soluble in common organic solvents.

1. Effect of Filters - A 5l beaker was fitted with cooling coil, thermometer and mechanical stirrer, and filled with distilled water. A medium-pressure mercury lamp was placed as close as possible (~ 2cm) to the beaker, and the apparatus surrounded by metal foil. A pyrex test tube was covered with aluminium foil and a 1cm x 2cm window cut in the foil. The tube was immersed to the level of the lamp, and as close to it as possible (4cm). The lamp was struck and the apparatus allowed to equilibrate for 1-2 hours before each run at 15°. The model compound (100µg) was dissolved in benzene (2.0ml) in a 1.0cm quartz cuvette, and flushed briefly with nitrogen and stoppered. The cell was placed in the tube and the solution photolysed using the filter combinations A through F in successive runs to shield the window (fig.2). The rate of loss of absorption at 378nm was followed spectrophotometrically, and the results plotted as % absorption *vs.* time (graph 1). Reproducibility of the half reaction times for a given run was ~ 10% under these conditions.

2. Effect of Dilution - The photolysis was conducted in benzene flushed with nitrogen, with 4 concentrations of the model aldehyde: 0.3μmole in 0.2, 0.4, 1.0 and 2.0ml solvent, and the results plotted graphically (graph 2).

3. Effect of Solvent - The photolysis was conducted under the same conditions with no additional filter and in the following pure solvents: benzene, chloroform, acetonitrile and dimethyl acetamide. The results were plotted graphically (graph 3).

4. Effect of Oxygen - Two similar solutions of the model in benzene were flushed with oxygen and nitrogen respectively, and photolysed with no additional filter. The photolysis was also conducted in acetonitrile.

5. Effect of Radical Generators - A solution of the compound (0.3μmole) in benzene (2ml) was kept in the dark at room temperature in the prescence of dibenzoyl peroxide ( ~ 10μg). The absorption maximum at 378nm had dereased 15% after 3 weeks. t-Butyl hydroperoxide had a similar effect.

(C) Degree of Intermolecularity

1. Photolysis of a Hantzsch Compound in the prescence of an Aldehyde - 1,2,6-Trimethyl-3,5-dicarbethoxy-1,4-dihydropyridine (0.27g 1mmole) and redistilled benzaldehyde (0.1g 1mmole) were photolysed in benzene (400ml) at 15° with a medium pressure mercury lamp for 24 hours. Decrease in $\lambda_{max}$ 352nm was ~ 25% after this time. Water was added (50ml), the aqueous phase separated and washed with benzene. $\lambda_{max}$ < 220nm for the aqueous

phase. The organic fraction was a complex mixture of products (t.l.c., acetone- benzene, 5:95), but no band which corresponded to authentic benzyl alcohol could be detected.

2. Photolysis of [4-$^2$H]-Model Compound (XXXIV) - The deuterated model system (0.1mmole) was photolysed in benzene (100ml) and then reduced with dithionite in the usual way. The product was worked up (t.l.c., acetone-benzene, 1:4), and the band corresponding to the hydroxymethylene compound (XXX) isolated, and the mass spectrum recorded.

3. Photolysis of Model in the prescence of [4,4-di-$^2$H]-2'-Methyl Benzoate (XXXVII) - The model compound (0.074g 0.2mmole) and the ester (0.085g 0.2mmole) were together photolysed in benzene (300ml) in the usual way, and the products reduced with dithionite in bicarbonate. The complex reaction products (t.l.c., acetone-benzene, 1:4) were not investigated, except the low-intensity band corresponding to the hydroxymethylene compound (XXX) which was isolated and its mass spectrum recorded.

(D) Competing Reactions

1. Photolysis of N-(2,6-Dichlorobenzyl)-3-carbethoxy-1,4-dihydropyridine (XXVI) - The compound (0.3μmole) was photolysed in benzene (2ml) under the usual conditions for 2 hours. The half-reaction time was ~3.5 minutes. The crude product was suspended in buffered dithionite (1mmole) for 1 hour and the reaction products separated by t.l.c. (acetone-benzene, 1:4). No component corresponding to the hydroxymethylene compound (XXX) could be detected.

## PART IV

### Pathways to Potential Models related to Hantzsch[116] Compounds

2,6-Dimethyl-3,5-dicarbethoxy-1,4-dihydropyridine[206] - Ethyl acetoacetate (100g 0.77mole) was cooled to 0° and treated with 40% aqueous formaldehyde (30.4g 0.4mole) and diethylamine (8 drops) according to the standard procedure. The mixtu e was vigorously stirred and allowed to warm up to room temperature over a period of 3 hours, and stirred for a further 3 days. Workup by the recommended method (after reaction with dry ammonia)[206] afforded the yellow dihydropyridine compound in 88% yield (89g), mp. 176-8° (lit.[30] mp. 175-80°); τ 8.77 (6H, t($J_{Et}$, 6.8Hz), ethyl $CH_3$), 5.89 (4H, q, ethyl $CH_2$), 7.85 (6H, s, 2,6-$CH_3$), 6.77 (2H, s, 4 pyridine H), 4.38 (1H, s, NH).

2,6-Dimethyl-3,5-dicarbethoxypyridine (XXXIXa)[206a] - The crude dihydropyridine was cautiously treated with water (100ml), concd. nitric acid (25ml) and concd. sulphuric acid (18ml) with cooling and swirling. The mixture was slowly heated to 80° and maintained at this temperature for 25 minutes. The cooled, cherry-red solution was treated with ice,(200g) and made strongly alkaline with ammonium hydroxide (S.G. 0.88). The off-white solid was filtered off (65g 76%) and recrystallized from ethanol, mp. 72-73° (lit.[206b] mp. 72°); τ 8.60 (6H, t($J_{Et}$, 7.6Hz), ethyl $CH_3$), 5.59 (4H, q, ethyl $CH_2$), 7.16 (6H, s, 2,6-$CH_3$), 1.34 (1H, s, 4 pyridine H).

2-Bromobenzaldehyde Ethylenedithioacetal - 2-Bromobenzaldehyde

(5.5g 30mmole in dry ether (50ml) was treated with ethane dithiol (3.1g 33mmole), followed by boron trifluoride etherate (2.5ml)[208] and the stoppered reaction vessel set aside for 48 hours. The solution was poured into 4% sodium hydroxide solution (30ml), vigorously shaken and the aqueous fraction discarded. The ether layer was washed twice with water, dried ($Na_2SO_4$) and the solvent removed *in vacuo*. The colouless oil (7.0g 92%) showed no infra-red absorption around 1690cm$^{-1}$; $\tau \sim 2.6$ (4H, m, arom H), 6.69 (4H, s, acetal $CH_2$), 4.01 (1H, s, benzyl CH); accurate mass measurement:- [found: 259.9347. $C_9H_9BrS_2$ requires: 259.9330].

Attempted Preparation of 2-Lithiobenzaldehyde Ethylenedithioacetal - A solution of the bromo-acetal (2.6g 10mmole) in dry ether (50ml) was flushed with dry argon and cooled to 0°. *n*-Butyl lithium (11mmole) as a solution in hexane (10ml) was added dropwise with stirring from a syringe. The solution became brown-yellow on initial addition of the Grignard reagent and finally a deep orange red. A portion was quenched with water, extracted into ether and chromatographed. T.l.c. (benzene) showed the prescence of a trace amount of starting material, along with a major component at the origin. The remainder of the reaction mixture was quenched with water and worked up. The crude oily product showed no p.m.r. absorptions other than aromatic ones.

2-Nitroacetophenone[207] - Acetophenone (120g 1mole) was run slowly into vigorously stirred fuming nitric acid (S.G. 1.5, 280ml) maintained between -10° and -5°. The mixture was stirred 40 minutes at 0°, poured into crushed ice and made up to 2l with cold

water. The precipitate (3-isomer) was removed by filtration and the filter cake well washed with ice-water. The filtrate was cautiously basified with sodium hydroxide and the product extracted into ether. The organic extract was washed with water, dried($MgSO_4$) and the ether distilled off. Ice-cooling the oily residue caused more 3-isomer to separate, and this was removed. Yield of crude 2-nitroacetophenone 49g (30%).

2-Aminoacetophenone[207] - The total crude nitro compound was dissolved in ethanol (400ml), 5% palladium/charcoal (1.5g) added, and hydrogenated at atmospheric pressure. Hydrogen uptake was 22.5l (theoretical uptake 22.3l) over 3 hours, the temperature rising to $\sim 35^{\circ}$ for the first hour. The crude product was distilled, bp. 100-104° at 1.8mm. Hg (lit.[207] bp. 124° at 10mm. Hg) (74% yield).

2-Bromoacetophenone[207] - 2-Aminoacetophenone (30g 0.22mole) was dissolved in 36% hydrobromic acid (92ml) and water (90ml), cooled to -5° and diazotised eith sodium nitrite (16g 0.23mole) in ice-cold water (27ml). Cupric sulphate hydrate (40.6g 0.16 mole) and potassium bromide (27.2g 0.22mole) were dissolved in water (180ml) and reduced with sulphur dioxide until colourless. The solid cuprous bromide was collected and dissolved in hydrobromic acid (S.G. 1.49) 75ml) and this cooled solution added to the diazotised solution. The mixture was heated for 2 hours on the steam bath and finally steam-distilled (2l distillate). The crude product was extracted into ether, washed with dilute base, acid and water. The dried ($CaCl_2$) solution was distilled,

bp. 95-99° at 2mm. Hg (lit.[207] bp. 112° at 10mm. Hg) 38g (85%) (lit. yield 80%).

2-Bromoacetophenone Ethylenehemithioketal[208] - 2-Bromoacetophenone (36g 0.18mole) was dissolved in dry ether (100ml) and treated with β-mercaptoethanol (18g 0.24mole) in ether (100ml) followed by boron trifluoride (30ml). The solution was set aside 48 hours and poured into saturated sodium bicarbonate solution (100ml). The organic layer was isolated, successively washed with 50% saturated bicarbonate and with water, dried ($Na_2SO_4$) and the solvent evaporated *in vacuo*. Trituration of the residue with petrol-ether yielded colourless crystals of 2-bromoacetophenone ethylenehemithioketal (40g 86%) which was recrystallized from petrol, mp. 61-61.5°; $\nu_{max}$ 1565, 1470, 770cm$^{-1}$: τ 7.96 (3H, s, $CH_3$), 2.3-3.2 (4H, m, arom H), 6.7-7.3 (2H, m, S-$CH_2$), 5.4-6.3 (2H, m, O-$CH_2$); [found: C, 46.40%; H, 4.20%; Br, 30.87%; S, 12.44%. $C_{10}H_{11}BrOS$ (259.17) requires: C, 46.50%; H, 4.26; Br, 30.70%; S, 12.40%].

2,6-Dimethyl-3-(2'-acetyl ethylenehemithioketal)benzoyl-5-carbethoxypyridine (XXXIXb) - 2-Bromoacetophenone hemithioketal (5.2g 20mmole) was dissolved in anhydrous ether (50ml) in a pressure-equalised dropping funnel fitted with serum cap and under argon. To the swirled solution was dropwise added n-butyl lithium (22mmole) in hexane (20ml) *via* syringe, and the pale yellow cloudy solution set aside for 2 hours. 2,6-Dimethyl-3,5-dicarbethoxypyridine (5.0g 20mmole) was dissolved in dry refluxing ether (20ml) and the Grignard reagent very slowly added with vigorous stirring in

the normal manner. The solution became cherry-red initially and deposited a pale yellow precipitate. Refluxing was continued for 30 minutes, and the solution was cooled and quenched with saturated ammonium chloride solution. The ethereal fraction was isolated rapidly, dried ($Na_2CO_3$) and the solvent evaporated *in vacuo* to give a pale yellow oil containing 5 major components A to E respectively by t.l.c. (petrol-benzene, 1:1) at Rf 0.80, 0.75, 0.40, 0.25, 0.15. The crude oil was chromatographed on grade III alumina with petrol-benzene (7:3), and the 5 components identified by their p.m.r. spectra. A: Acetophenone hemithioketal plus hydrocarbons (n-octane) (comparison with authentic samples), B: Acetophenone hemithioketal. C: Crystallized on standing (1.75g 35%) pyridine diester starting material. D: Crystallized on standing, recrystallized from benzene-petrol (5:95) as colourless cubes of 2,6-dimethyl-3(2'-acetyl ethylenehemithioketal)benzoyl-5-carbethoxypyridine (2.7g 35%), mp. 91.5-92.5°; $\nu_{max}$ 1725, 1678, 1132, 680cm$^{-1}$; $\lambda_{max}$ 242 ($\varepsilon \sim 8300$), 276nm ($\varepsilon$ 4860); $\tau$ ~2.6 (4H, m, arom H), 8.74 (3H, t($J_{Et}$, 6.9Hz), ethyl $CH_3$), 5.74 (2H, q, ethyl $CH_2$), 5.8-6.4 (2H, m, O-$CH_2$), 6.7-7.3 (2H, m, S-$CH_2$), 8.05 (3H, s, ketal $CH_3$), 7.17 and 7.18 (3H, s, 2,6-$CH_3$), 1.94 (1H, s, 4 pyridine H); [found: C, 65.20%; H, 6.07%; N, 3.79%; S, 8.27%. $C_{21}H_{23}NO_4S$ requires: C, 65.44%; H, 6.02%; N, 3.63%; S, 8.30%].

2,6-Dimethyl-3,5-bis(2'-acetyl ethylene hemithioketal)benzoyl pyridine (XLII) - E: (previous reaction) crystallized on standing and was recrystallized from a small amount of benzene as colourless cubes of 2,6-dimethyl-3,5-bis(2'-acetyl ethylenehemithio-

ketal)benzoyl pyridine (1.85g 18%), mp. 190-2°; $\nu_{max}$ 1668, 1130, 925cm$^{-1}$; $\lambda_{max}$ 249 (ε 15300), ~ 277nm (ε ~6100); τ ~2.7 (8H, m, arom H), 5.7-6.7 (2h, m, O-$CH_2$), 6.8-7.6 (2H, m, S-$CH_2$), 8.19 (6H, s, ketal $CH_3$), 7.16 ( 6H, s, 2,6-$CH_3$), 2.58 (1H, s, 4 pyridine H); [found: C, 67.00%; H, 5.61%; N, 2.40%; S, 12.44%. $C_{29}H_{29}NO_4S$ (519.53) requires: C, 67.04%; H, 5.63%; N, 2.70%; S, 12.32%].

2,6-Dimethyl-3(2'-acetyl)benzoyl-5-carbethoxypyridine (XXXIXc) - A solution of the hemithioketal compound (XXXIXb) (0.385g 1 mmole) in glacial acetic acid (20ml) was treated successively with a warm solution of mercuric chloride (0.28g 1.1mmole) in acetic acid (5ml) and potassium acetate (0.25g) in acetic acid (8ml)[209]. A heavy solid precipitated immediately; the mixture was vigorously shaken for 10 minutes, filtered through celite, and the filter pad washed with ether and water. The filtrate was cooled, and cautiously made just basic with dilute sodium hydroxide, and the product extracted into ether. The organic fraction was washed with water, dried ($Na_2SO_4$) and the solvent removed in vacuo. 2,6-Dimethyl-3(2'acetyl)benzoyl-5-carbethoxypyridine crystallized at once and was recrystallized from benzene-petrol (3:7) as colourless prisms (0.27g 82%), mp. 92.5-3.5°; $\nu_{max}$ 1720, 1680, 1144, 660cm$^{-1}$; $\lambda_{max}$ 247 (ε 13900), 274nm (ε 6050); τ ~ 2.4 (4H, m, arom H), 8.72 (3H, t($J_{Et}$, 6.9Hz), ethyl $CH_3$), 5.73 (2H, q, ethyl $CH_2$), 7.48 (3H, s, acetyl $CH_3$), 7.09 and 7.13 (3H, s, 2 and 6 $CH_3$ respect.), 2.01 (1H, s, 4 pyridine H); [found: C, 70.28%; H, 5.84%; N, 4.20%. $C_{19}H_{19}NO_4$ (325.36) requires: C, 70.13%; H, 5.89%; N, 4.31%].

Attempted Preparation of N-Alkyl-2,6-dimethyl-3-(2'-acetyl)-benzoyl-5-carbethoxypyridine Salts (XLc)

1. Methyl Iodide - The pyridine compound (XXXIXc) (0.033g 0.1mmole) was stirred for 48 hours in pure methyl iodide (2ml) at room temperature. Evaporation of solvent yielded unchanged starting material (t.l.c., p.m.r. spectrum). The reaction was repeated at 80° and 120° in 2 small Carius tubes under nitrogen for 24 hours. No methylated product was observed.

2. Methyl p-Toluenesulphonate - The pyridine compound (0.033g 0.1mmole) was refluxed with methyl p-toluenesulphonate (0.068g 0.4mmole) in absolute ethanol (2ml) under nitrogen for 48 hours, with no apparent reaction (t.l.c., acetone-benzene, 1:49)

3. Dimethyl Sulphate[189] - The pyridine compound (0.03g 0.1 mmole) was heated to 60° with acid-free dimethyl sulphate (0.015g 0.12mmole) under nitrogen for 2 hours. The oily amber liquid was cooled, wateradded (2ml) and the solution washed with ether until the washings were colourless. The dark red aqueous solution was immediately treated with buffered dithionite (1mmole), and filtered from a small amount of tar. The mixture was set aside for 2 hours and extracted with chloroform. The solution showed $\lambda_{max}$ below 330nm. The mixture resolved into 6 components (t.l.c. acetone-benzene, 5:95), but mass spectrum confirmed that none was the required product.

4. Triethoxonium Fluoborate[219] - The reagent was prepared by the literature method, weighed (0.19g 1mmole) under dry ether, the ether evaporated under nitrogen and dry methylene dichloride added (1ml). The solution was transferred to a solution of the pyridine

compound (0.033g 0.1mmole) in the same solvent (2ml) under nitrogen. T.l.c. (acetone-benzene, 1:49)showed no reaction after 48 hours, so the reaction mixture was refluxed a further 48 hours. The solution was poured directly into buffered dithionite (1mmole), but a chloroform extract contained no component with a suitable absorption spectrum.

1,2,6-Trimethyl-3,5-dicarbethoxypyridinium Perchlorate[189] (XLa, X = $ClO_4$) - The corresponding pyridine ester (XXXIXa) (0.5g 2m mole) was suspended in pure dimethyl sulphate (0.29g 2.3mmole) and set aside at 75° for 10 hours. The resulting brown gum was dissolved in water (2.5ml), washed with ether and treated with 1F sodium perchlorate (5ml). The mixture was warmed to dissolve the oily precipitate and allowed to cool slowly. The solid was collected, but was not further puified owing to difficulty with recrystallization (0.58g 80%), τ 8.59 (6H, t($J_{Et}$, 7.8Hz) ethyl $CH_3$), 5.58 (4H, q, ethyl $CH_2$), 5.84 (3H, s, N-$CH_3$), 6.96 (6H, s, 2,6-$CH_3$), 1.00 (1H, s, 4 pyridine H).

1,2,6-Trimethyl-3,5-dicarbethoxy-1,4-dihydropyridine[15] (XXXVIIIa) - The crude perchlorate salt (0.55g 1.5mmole) was reduced with buffered dithionite (3mmole) by the usual procedure, and the product recrystallized from petrol (0.3g 75%), mp. 85-6° (lit. mp. 85.8-6.9°[120], 86-7°[220]); τ 8.72 (6H, t($J_{Et}$, 7.0Hz), ethyl $CH_3$), 5.83 (4H, q, ethyl $CH_2$), 6.85 (3H, s, N-$CH_3$), 6.90 (2H, s, 4 pyridine H), 7.61 (6H, s, 2,6-$CH_3$).

Attempted Preparation of 1,2,6-Trimethyl-3-(2'-acetyl ethylene hemi-

thioketal)benzoyl-5-carbethoxy-1,4-dihydropyridine (XXXVIIIb) - A solution of the diethyl ester (XXXVIIIa) (2.67g 10mmole in ether (50ml) at 0° under argon was treated dropwise with 2-lithioacetophenone hemithioketal (10mmole) prepared as previously described. The solution was stirred for 1 hour at 10° and worked up with saturated ammonium chloride solution. The crude semi-solid product was an equimolar mixture of the diester starting material and acetophenone ethylenehemithioketal (t.l.c., p.m.r. spectrum comparison with authentic samples).

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