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# NADH Models

## II. Mechanism of Reduction of Electrophilic Olefins with 3,5-Diethoxycarbonyl-2,6-Dimethyl-1,4-Dihydropyridine (*1*)

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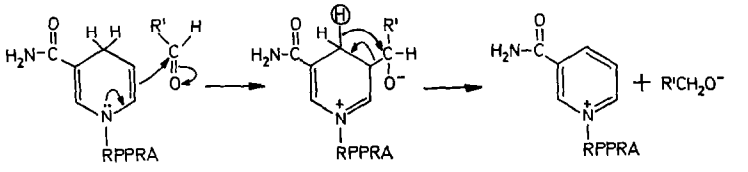
Reduction of unsaturated acyl chlorides (5a–c) and acyl anhydrides (9a, b) by Hantzsch ester 3a proceeds by direct transfer of a hydrogen species (presumably a hydride ion). Critically designed experiments rule out a mechanism involving an acyl intermediate of type 2. The implications of these results in the mechanism of NADH-mediated reductions are discussed.

Pyridine nucleotide-dependent redox enzymes play a vital role in life processes. The mechanism of action of the coenzyme has been investigated extensively, both in enzyme-catalyzed transformations (*2*) and suitably designed nonenzymatic model reactions (*3*). Despite these studies, details of the hydrogen transfer from the coenzyme to the substrate and, in particular, the role of the apoenzyme, are as yet not completely understood. Some attention has been, however, directed to this aspect in the last years. The significance of hydrogen bonding in the enzyme–NADH–substrate complex (*4a, b*) has been examined in this laboratory via reduction studies on a specially constructed carbonyl substrate, with an NADH model (*1*). A recent proposal (*5*) suggests that hydride transfer from NADH to a carbonyl system may occur intramolecularly in the covalent intermediate 1, formed by an enamine reaction of the dihydropyridine moiety with the electrophilic carbonyl substrate (Scheme 1). An intermediate of unspecified structure has been invoked on the basis of isotopic partitioning experiments in the model reduction of trifluoroacetophenone (*6*).

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SCHEME 1

The novel suggestion (*5*) for the detailed course of the hydride-transfer step, described in Scheme 1, prompted us to examine the mechanism of (double bond) reduction of

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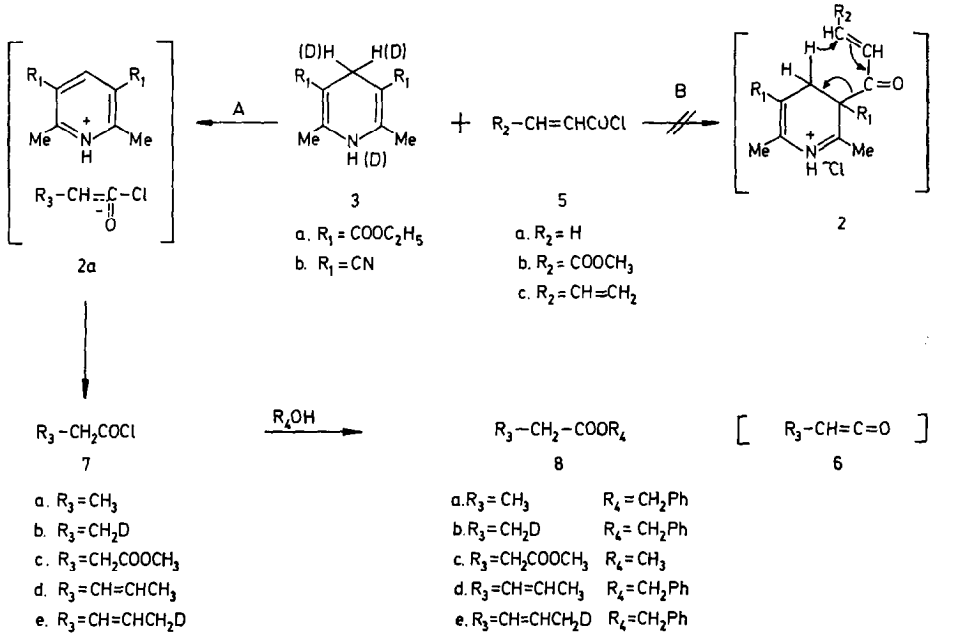
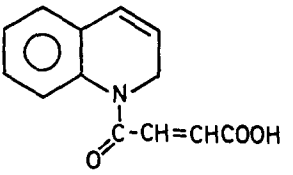
suitable electrophilic olefins with 1,4-dihydropyridine derivatives. If the latter reactions would proceed via a mechanism analogous to that presented in Scheme 1, covalent intermediates of general structure 2 (Scheme 2) would be formed, which would be expected to undergo an intramolecular hydride shift concerted with a heterolytic fragmentation. The overall process could also be interpreted in terms of the Woodward–Hoffmann rules (*7*). The recognition of an intermediate of type 2 would lend direct support to the involvement of C-acyl intermediates in NADH-mediated reductions. In the present and the first phase of our investigation, Hantzsch ester 3a was chosen as




SCHEME 2

a simple analog of the NADH system. While recognizing the deviation of 3a from an ideal model, it was felt that the shortcomings which it possessed were compensated for by several practical advantages. Prominent amongst these were stability under a range of reaction conditions and the opportunity of labeling at desired locations. Furthermore, several examples of reduction of electrophilic olefins with 3a are reported in the literature (*3*). A number of cases of homogeneous hydrogen transfers from hydroaromatic nitrogenous heterocycles to electrophilic olefins have been described by Braude and co-workers (*8*). Particularly noteworthy is their result: that while reduction of maleic anhydride by Hantzsch ester (3a) (101°C, 30 min) proceeds in over 90% yield; under identical conditions diethyl maleate is almost unaffected. This difference in behavior between the two electrophilic olefins can be interpreted in terms of the lower reactivity of the ester carbonyls in diethyl maleate, as compared to the anhydride carbonyls, toward the enamine function of the dihydropyridine system. Also, relevant to the present discussion is the observation (*9*) that hydrogen transfer from 1,2-dihydroquinoline to maleic anhydride proceeds via an isolable intermediate to which structure

4 has been assigned. This communication describes the results of the reaction of Hantzsch ester (3a) and its 4,4-dideutero derivative (3a–4,4-$d_2$) with a variety of unsaturated acid chlorides and anhydrides, and discusses the significance of these to the mechanism of reduction by dihydropyridine derivatives.

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## RESULTS

*Reaction of Hantzsch Esters 3a and 3a–4,4-$d_2$, (10) with Acid Chlorides*

The following procedure was employed for conducting the reaction between Hantzsch esters and the acid chlorides: A solution of equimolar quantities of the ester and the acid chloride in dioxane (~0.25 *M*) was heated with stirring at 85°C under $N_2$, until (almost) all of the ester had been consumed (tlc). An equivalent amount of benzyl alcohol (methanol in case of 5b) was added to the mixture, which was subsequently heated to reflux for 0.5–4 hr. After addition of water the neutral ester fraction was separated by extraction with ether (or in case of 5c, was further purified by chromatography over a silicagel column; eluent = chloroform) and the pure ester isolated by glc. The results are shown in Table 1. The structure of the esters, which are known

TABLE 1

REACTION OF HANTZSCH ESTER WITH ACID CHLORIDES

| Hantzsch ester | Acid chloride | Ester (Yield) |
|---|---|---|
| 3a | 5a | 8a (>70%) |
| 3a–4,4-$d_2$ | 5a | 8b (>70%) |
| 3a | 5b (*cis*) | 8c (>70%) |
| 3a | 5c (*trans*) | 8d[a] (10%) |
| 3a–4,4-$d_2$ | 5c (*trans*) | 8e[a] (10%) |

[a] Spectroscopic data suggest *cis* stereochemistry for the ester. Implications of this will be discussed elsewhere.

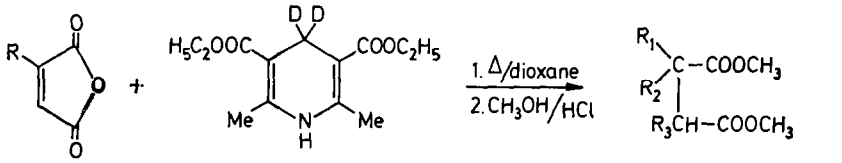
products, was established by spectral data. NMR spectra of 8b and 8e attested to the incorporation of one atom of deuterium per molecule at the indicated positions (Scheme 2).

*Reaction of 1,4,4-Trideuterated Hantzsch Ester with Acrylyl Chloride*[2]

The trideuterated ester 3a–1,4,4-$d_3$, mp 191–193°C, was prepared by exchanging the $N_1$–H of 3a–4,4-$d_2$ by refluxing a solution of the dideuterated compound in a mixture of dry tetrahydrofuran and $D_2O$ (2:1, v/v). NMR spectra in $d_6$-DMSO and $CDCl_3$ attested to the absence of the band corresponding to N–H. Furthermore, the ir spectrum (KBr) showed a strong absorption at 2498 $cm^{-1}$ (N-D). Reaction of the triply labeled Hantzsch ester with acrylyl chloride, followed by treatment with benzyl alcohol and isolation of the thereupon formed ester, according to the previously described procedure, yielded benzyl propionate-2,3-$d_2$; NMR($CDCl_3$) $\delta$ 1.12 d(2H, $CH_2D$), 2.37 t(1H, CHD), 5.11 s(2H, $CH_2Ph$), around 7.32, broad singlet (5H, aromatic protons.)

*Reaction of Deuterated Hantzsch Ester (3a–4,4-$d_2$) with Anhydrides*

A mixture of equimolar amounts of anhydride 9a or 9b and 3a–4,4-$d_2$ in dioxane (~0.25 *M*) was refluxed till no Hantzsch ester was detectable by tlc. The dioxane was removed under reduced pressure, a small quantity (5–10 ml) of methanol was added and the resulting solution was saturated with dry HCl and refluxed (20 min). The esters thus obtained were isolated by glc and identified by their ir and NMR spectra. The results are described in Scheme 3. The NMR spectra were utilized in recognizing the extent and location of deuterium incorporation. Reaction of 9b led to a mixture of 10b and 10c in the approximate ratio of 1:1 (*11*).




9 3a– 4,4-$d_2$ 10

a. R = H
b. R = $CH_3$

a. $R_1$ = D , $R_2$ = $R_3$ = H
b. $R_1$ = $CH_3$ , $R_2$ = D , $R_3$ = H
c. $R_1$ = $CH_3$ , $R_2$ = H , $R_3$ = D

SCHEME 3

*Rates of Reaction of Acrylyl Chloride with 3a and 3a–4,4-$d_2$*

The Hantzsch ester (3a), mp 189–190°C and deuterated Hantzsch ester (3a–4,4-$d_2$), mp 191–193°C employed, were chromatographically pure; tlc attested to exclusion of the corresponding pyridine (oxidation) derivatives. The purity of acrylyl chloride was checked via its refractive index [$n_D(20°) = 1.4339$] and its ir and NMR spectra. Rate measurements were carried out utilizing the following procedure: 0.417 mmoll of 3a or 3a–4,4-$d_2$ were placed in a $N_2$-flushed 25-ml flask covered with a rubber seal. To this, 20 ml of dry, degassed dioxane was added under nitrogen and the resulting solution warmed with magnetic stirring, to 40.6°C, in a Tamson thermostat. Acrylyl chloride

[2] We are indebted to Mr. R. A. Gase for the synthesis of the trideuterated Hantzsch ester and the study of its reaction with acrylyl chloride.

(0.418 mmoll) was injected into the solution of the Hantzsch ester (3a or 3a–4,4-$d_2$). Aliquots of 100 $\mu$l were withdrawn from the reaction mixture with a syringe and made up to a total volume of 25 ml with ethanol. The extinctions of the samples at $\lambda_{max}$ 372 nm (Hantzsch ester) and 282 and 273 nm (3,5-diethoxycarbonyl-2,6-dimethylpyridine) were measured in a PMQII Zeiss spectrophotometer. The measurements were carried out in triplicate and the bimolecular rate constants ($k_2$) were calculated from the rate of disappearance of the Hantzsch ester or the rate of appearance of 3,5-diethoxycarbonyl-lutidine, the two being identical ($k_2t = 1/C_0 - 1/C_t$ (*12*); see Fig. 1). A Hewlett-Packard table computer was employed for these calculations; $k_2$ values were determined by the method of least squares. The rate constants are presented in Table 2. Figure 1 is based on the average of three runs.

TABLE 2

RATE CONSTANTS OF THE REACTION OF HANTZSCH ESTERS WITH ACRYLYL CHLORIDE (DIOXANE, 40.6°C)

| Hantzsch ester | | 4,4-Dideutero-Hantzsch ester | |
|---|---|---|---|
| Run no. | $k_2$ ($M^{-1}$.min$^{-1}$) | Run no. | $k_2$ ($M^{-1}$.min$^{-1}$) |
| 1 | 0.419 ± 0.006 | 1 | 0.082 ± 0.002 |
| 2 | 0.394 ± 0.003 | 2 | 0.077 ± 0.002 |
| 3 | 0.417 ± 0.007 | 3 | 0.082 ± 0.002 |

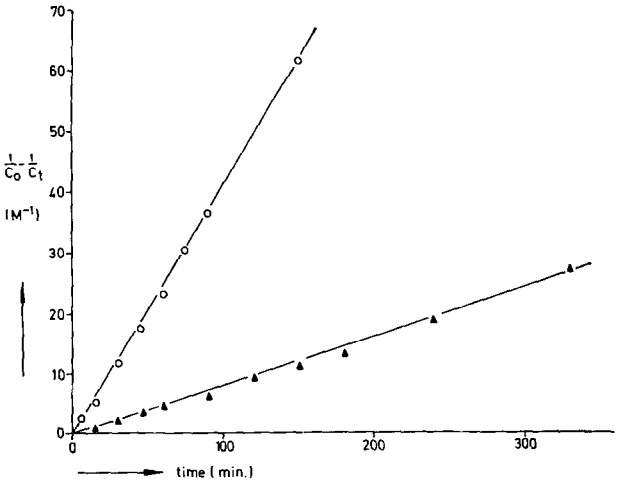



FIG. 1. Rates of reaction of Hantzsch ester (3a), —○—, and 4,4-dideutero-Hantzsch ester (3a–4,4-$d_2$), —▲—, with acrylylchloride (40.6°C).

## DISCUSSION

The hydride transfer step in the reductive alcoholysis of unsaturated acid chlorides (5a–c) and by analogy, the anhydrides 9a,b, by the Hantzsch ester, can proceed by two

possible mechanisms (see Scheme 2). According to mechanism A, hydride transfer to the terminal carbon of the double bond of the acid chloride proceeds via an intermolecular process, to result in an ion pair 2a (or an equivalent intermediate) which, after a proton donation by the pyridinium salt to the anionic moiety, gives the reduced acid chloride 7. The anion of 2a can, alternatively, release a chloride ion to form ketene 6. Both 6 and 7 would yield ester 8 upon alcoholysis. In mechanism B, on the other hand, the hydride transfer is visualized as an intramolecular process occurring in the acyl intermediate 2, which, in turn, is formed in an enamine-type reaction of the Hantzsch ester with the acid chloride.[3] Hydride transfer in 2, or an electrocyclic process (*7*), would cause fragmentation of 2 into a pyridinium salt and ketene 6. Reaction of the ketene (*6*) with an alcohol would subsequently result in the formation of the corresponding ester 8, which, in each case, is the isolated product of the reaction. The aforementioned results, as we shall see from the discussion in part I (*1*), provide evidence in support of mechanism A.

The kinetic data on the reduction of 5a by Hantzsch ester 3a and its 4,4-dideutero derivative identify it as a second-order reaction in which a C—H bond-fission is involved in the rate-determining step ($k_2H/k_2D \simeq 5$, Table 2). This result is compatible with either a rate-determining intermolecular hydride transfer (Mechanism A) or a fast acylation step (Mechanism B), followed by a slow hydride rearrangement-induced fragmentation (*9*) of intermediate 2 (Scheme 2). Careful spectral and kinetic monitoring of the reaction mixtures of 3a and 5a indicated that no spectroscopically identifiable intermediate was being formed, and furthermore, that disappearance of 3a and the appearance of 3,5-diethoxycarbonyl lutidine was proceeding concurrently at identical rates. While these observations tend to disfavour process B, they do not, however, exclude a special case of the same mechanism in which the intermediate (*2*) is present in a low steady-state concentration.

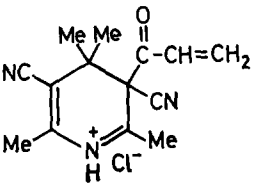
The reaction of 5a with 1,4,4-trideutero Hantzsch ester provides salient information concerning the mechanism of reduction. As noted, quenching of the reaction mixture with benzyl alcohol leads to the formation of benzyl propionate with a deuterium-labeling pattern represented by $CH_2D$–$CHD$–$COOCH_2C_6H_5$. Formation of the latter ester demands that both of the acquired hydrogens in the reduced product be derived exclusively from the Hantzsch ester. Such a mechanism rules out the involvement of a ketene intermediate (*6*) and, thereby, the operation of mechanism B in the overall reduction process. Elimination of the ketene intermediate from the mechanistic scene also throws light on the fate of intermediate 2a, postulated in mechanism A. Apparently, proton transfer—from the cation to the anion—in 2a is responsible for the conversion of 5a to the corresponding reduced acid chloride 7a, which reacts with benzyl alcohol to yield 8a. Further evidence in favor of mechanism A may be derived from experiments which directly argue against the acylation step in the reaction of 3a with unsaturated acyl derivatives. Reactions of 3b (*14*) and its 4,4-dimethyl derivative (*15*) with acrylyl chloride were examined with the objective of trapping intermediate 11. It was anticipated that the latter, if formed, would be spectrally identifiable, since lack of hydrogens at the 4-position should prevent the reduction step and thereby lead to accumulation of the intermediate. While normal reductive alcoholysis could be

[3] A theoretically possible mechanism starting with *N*-acylation of the ester is regarded as highly improbable owing to severe steric hindrance of the flanking methyl groups (13).

achieved with 3b as the hydrogen donor, its 4,4-dimethyl derivative was totally inert toward acid chloride 5a.

Reduction of anhydrides 9a and 9b also argue against an acylation–hydride transfer mechanism. Anhydride 9b reacted with 4,4-$d_2$ Hantzsch ester to give, after alcoholysis, equal amounts of esters 10b and 10c. An initial acylation reaction, according to mechanism B, would discriminate between the two carbonyl groups owing to the obvious steric factors. The reaction would have been expected to proceed by a preferential attack at the carbonyl remote from the methyl group, thus favoring the formation of diester 10b. Since the deuterium label distribution is almost statistical in the product ester (10b, c), it seems highly unlikely that an acylation step is involved.

Finally, results of reduction of the diene acyl chloride 5c with 3a and 3a–4,4-$d_2$ constitute a persuasive argument in favor of mechanism A. The products of the reaction, after alcoholysis, have been identified as 8d and 8e, respectively. The site of hydrogen (or deuterium) transfer is not the one anticipated in accordance with the six-membered process described in 2 ($R_2$=CH=$CH_2$) but is consistent with an intermolecular hydride transfer postulated in mechanism A.

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In conclusion it may be said that insofar as the reaction of unsaturated acid chlorides with Hantzsch ester serves as a model for the NADH-mediated reductions, there is, thus far, no experimental support for the recently suggested two-step mechanism.

## ACKNOWLEDGMENT

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