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Transimination and reduction of imines using NADH models

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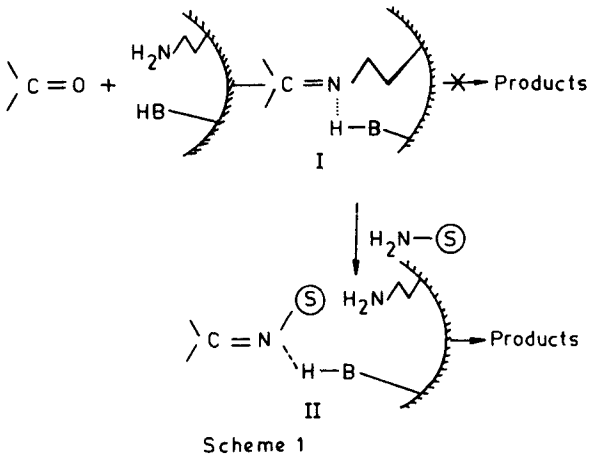
Abstract. Schiff bases prepared from aromatic aldehydes and alicyclic amines undergo transimination and reduction with 3,5-dicarboethoxy-2,6-dimethyl-1,4-dihydropyridine, as the NADH model in the presence of aromatic amines in acetic acid. This sequence of steps is analogous to a similar chemical mechanism proposed for glutamate dehydrogenase. In the absence of the NADH model, only the transimination process is observed.

Keywords. Transimination; NADH models; imines; 1,4-dihydropyridines; glutamate dehydrogenase; N-arylideneanilines; N-benzylaniline; N-benzylidenecyclohexylamine.

1. Introduction

There are a significant number of enzymes which catalyse transformations of substrates bearing a primary amino or a carbonyl functional group. Invariably, at the active site of these enzymes, (*e.g.* transaminases, aldolases, etc.), an amino group of a lysine residue is present (Snell and DiMari 1970). The role of this amino group is chiefly to assist the binding of a carbonyl carrying substrate or coenzyme through the formation of an imine bond as well as to activate the carbonyl carbon by further polarizing this imine bond by either protonation, hydrogen bonding or chelation of the more basic nitrogen. In some cases, this imine undergoes further amine exchange with an amino substrate and it is this newly formed imine which gives rise to the products (Lowe and Ingraham, 1974) and not the initial adduct (scheme 1). Why the initial enzyme-carbonyl adduct



Scheme 1

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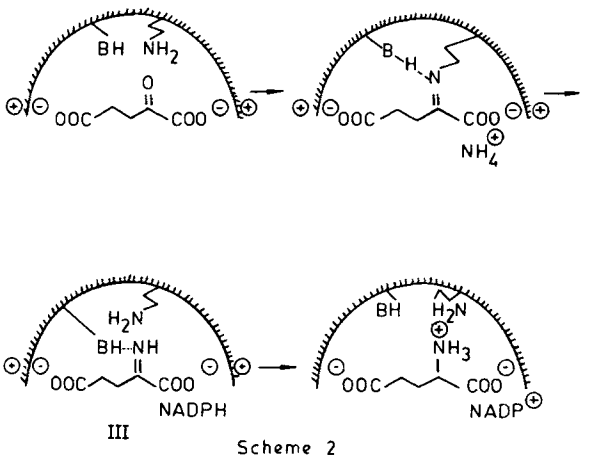


Scheme 2

does not result in products has not been investigated. In order to understand this selectivity we investigated a model system involving similar transimination and reduction of the imine bond by an NADH model compound. Such transimination and reduction is encountered in glutamate dehydrogenase where α-ketoglutaric acid is believed to be bound through Schiff base formation with a lysine residue (Smith *et al* 1975). General acid-catalysed transimination with ammonia gives α-iminoglutaric acid (III) to be reduced by NADPH to glutamic acid (scheme 2).

2. Experimental

2.1 *General method for preparation of imines*

Equimolar quantities of the amine and the aldehyde were taken in a minimum quantity of methanol and allowed to stand overnight at room temperature. Partial evaporation of methanol usually resulted in crystallization of the imines which were further purified by recrystallization from methanol. Imines derived from aliphatic amines are prepared by the azeotropic removal of water from a benzene solution of equimolar quantities of amine and aldehyde.

2.2 *Transimination with aromatic primary imines*

N-benzylidenecyclohexylamine (10 mmol) in glacial acetic acid (20 ml) was treated with aniline derivatives (10 mmol) for 15 min at room temperature. The acetic acid solution was poured over crushed ice and the solid that precipitated was filtered and recrystallized from methanol to give the transiminated product in more than 90% yields.

2.3 *Transimination and reduction using NADH model IV*

The experiment was repeated by adding 1,4-dihydropyridine (IV, 10 mmol). The reaction mixture was left overnight. Neutralization with sodium bicarbonate followed by extraction with ether and evaporation of dried extract gave the reduced trans-

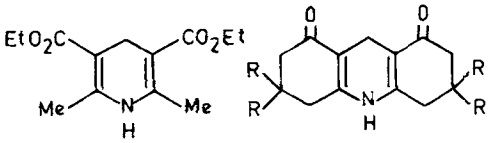
iminated products which were characterized by comparison of IR, TLC and ^1H NMR of authentic samples.

2.4 *NADH model reduction of N-arylidene aliphatic amines*

N-arylideneamines (ArCH : NR, R = C_6H_{11}, benzyl, phenethyl) (1 mmol) was taken in dry dichloromethane and the tricyclic NADH model compound (V, R=H) (1 mmol) added. Trifluoroacetic acid (3 drops) was added and the reaction was followed by TLC. After completion of the reaction, the solvent was evaporated and the residue triturated with dilute sodium hydroxide and extracted with ether. Products were separated by chromatography on silica gel and characterized by spectroscopic comparison with authentic samples.

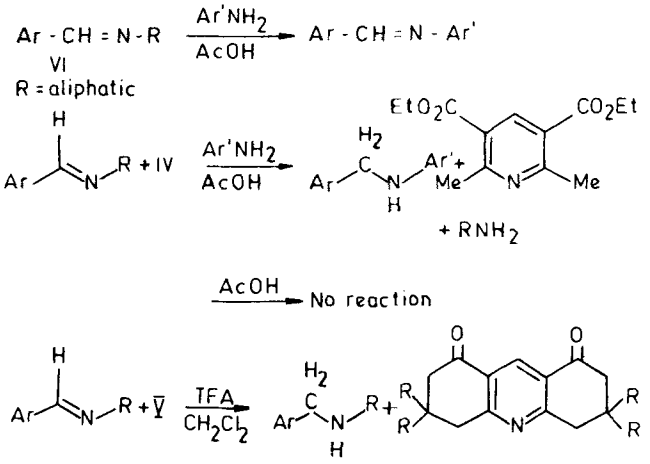
3. Results and discussion

We have shown earlier that aromatic imines which are normally not reduced by 1,4-dihydropyridines are reduced by either photoactivating the model compound IV (Singh *et al* 1978) or by using acetic acid (Singh and Sharma 1979) as the solvent and IV as the NADH model. Later on some tricyclic compounds such as V proved to be even better NADH models (Singh *et al* 1982). We see here that if one takes an imine (VI)



R = H or Me

IV V



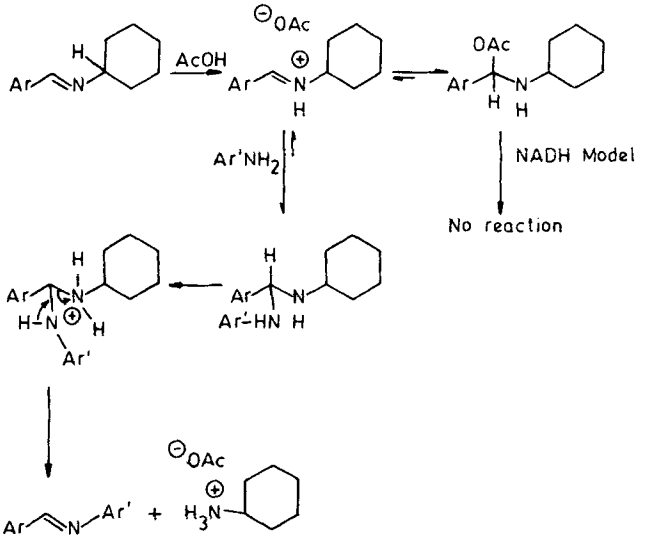
Scheme 3

formed from an aliphatic primary amine (RNH_2) and an aromatic aldehyde and treat it with an aromatic amine in acetic acid, the new imine resulting from transimination (scheme 3) is obtained. If the model compound IV is also added to the reaction mixture the reduction product results purely from the transiminated product and none from the preformed imine from the aliphatic amine (table 1). The conclusion that can be drawn is that if we have two primary amines in a weakly acidic environment in the presence of a carbonyl compound, only the less basic of these forms an imine bond. The more basic amine remains inactive due to easier protonation by the weak acid. Thus, in the present

Table 1. Amine exchange[a] and reduction[b] of N-arylidenecyclohexylamine (VI) with NADH model (IV) in glacial acetic acid at room temperature (25°C).

N-arylidene-cyclohexylamine	Amine($Ar'NH_2$) Ar'	Product yields Imine	Amine
VIa	C_6H_5	94·0	92·0
	m-$MeOC_6H_4$	85·0	80·5
	p-$NO_2C_6H_4$	82·0	82·0
VIb	C_6H_5	90·0	87·0
	p-$MeOC_6H_4$	76·0	81·3
VIc	C_6H_5	91·0	83·0
	p-$MeOC_6H_5$	82·0	78·0
VId	C_6H_5	82·0	76·0

[a] Exchange reaction was allowed to proceed at 25°C for 15 min and product isolated by aqueous work-up.
[b] Exchange and reduction using IV was carried out at 25°C for 15 hr. Imines are formed in the absence of NADH model IV, whereas amines are the products in the presence of IV.



Scheme 4

example, aromatic amines being very weak bases are not protonated by acetic acid unlike aliphatic amines such as phenethyl, benzyl or cyclohexylamine (scheme 4). This explains the selective formation of products from N-aryl imines rather than those from N-alkyl imines.

In glutamate dehydrogenase, although the α-amino group of a lysine also forms an imine with α-keto glutarate only the α-iminoglutarate formed with ammonia is reduced whereas the ε-amino group is released again. Ammonia being less basic than the lysine amino is selectively retained in the transimination process analogous to the above mentioned *in vitro* analogy. The weak acid which discriminates between ammonia and the lysine amino in glutamate dehydrogenase is probably a protonated histidine or some similar group.

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