Global Journal of Chemistry, Biology and Physics
Volume.8, Number 1;January-March, 2023;
ISSN: 2836-9572| Impact Factor: 7.49
https://zapjournals.com/Journals/index.php/gjcbp
Published By: Zendo Academic Publishing

KINETIC ANALYSIS OF IMINE AND HYDRAZONE HYDROLYSIS IN A PHYSIOLOGICALLY RELEVANT CONTEXT

Dr. Ahmed Ali Al-Mansoori[1], Prof. Layla Amira Al-Hussein[2], Dr. Karim Saad Al-Abdullah[3]

Article Info

Keywords: Nucleophilic addition
Primary amino groups
Aldehydes
Kinetics
Hydrolysis

Abstract

The addition of nucleophilic species to carbonyl groups, leading to the formation of tetrahedral intermediates that subsequently undergo an addition-elimination mechanism, is a fundamental chemical process. Such reactions are particularly significant in the context of primary amino groups and aldehydes, as they serve as model reactions for various biological processes. This study delves into the kinetic aspects of the hydrolysis of imines, which are intermediates formed during these reactions, with a focus on their behavior in aqueous acidic environments.

The hydrolysis of imines, especially those derived from primary amino groups and aldehydes, is a complex process with several potential reaction pathways. To gain insights into the kinetics and mechanisms governing these reactions, we examined the work of Hine et al. [1] and Cords and Jencks [2], who have contributed valuable insights in this area. Their research laid the foundation for our investigation.

In this study, we present a comprehensive analysis of the kinetics of imine hydrolysis in aqueous acid. We aim to elucidate the factors influencing the rate of this reaction and its mechanistic details. By employing kinetic methods, we have gathered data that sheds light on the reaction intermediates and the overall reaction mechanism. Our findings offer a deeper understanding of the behavior of primary amino group-containing compounds when reacting with aldehydes, particularly in acidic conditions, which have relevance in both synthetic and biological contexts.

1. Introduction

Certain nucleophilic species can add to carbonyl groups to give a tetrahedral intermediate which are unstable, and which break down to form new double bonds by an addition-elimination mechanism. An important class of reactions is that between compounds containing primary amino groups and aldehydes. These reactions are models

[1] Department of Chemical Sciences, Faculty of Science and Arts, Jordan University of Science and Technology.

of processes that are of interest in biological reactions. The hydrolysis of an imine occurs readily in aqueous acid and has been studied in detail by kinetic methods. Hine et al. [1] Cords and Jencks [2]

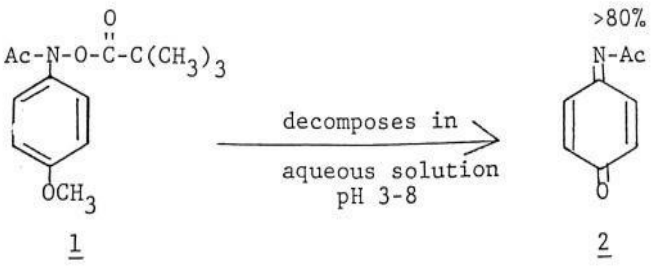
Imines are known to be unstable toward dissociation to their components and for few biomedical applications in vivo they are reduced to amines to prevent their breakdown to the starting materials, this is of course a limitation to their use in biological systems. Examples in literature support the conclusion that imines once formed are reduced to be useful. Lutz described a method for overcoming the problem in which, by covalently attaching free alkylamino groups to the carbohydrate moieties of the surface glycoproteins, they increased the number of amino groups available for cross-linking. Schweizer et al. [3] Modification of glycoproteins involves Schiff's base formation (imine) between the aryl alkyl diamine and aldehyde groups produced by oxidation of the glycoprotein terminal carbohydrate group. The formed Schiff's bases are reduced by sodium cyanoborohydride to stabilize them.

Klemm devised a method for the preparation of a radio iodinated derivative of ganglioside GM_1. Klemm et al. [4]

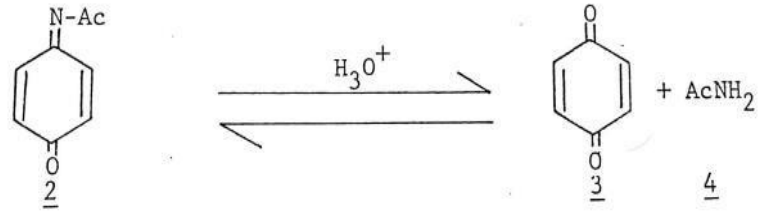
Carbon 6 of the terminal galactosyl residue of GM_1 was converted to an aldehyde by galactose oxidase. Once the imine was formed by this oxidation product of GM_1 with tyramine it was reduced with sodium cyanoborohydride. But hydrazone derivatives of hydrazides may be more stable to hydrolysis so that upon being formed there is no need to reduce them, and this could offer a way for drug loading via dextran conjugates to monoclonal antibodies. Heindel et al. [5] Molecules containing carbon–nitrogen double bonds are prevalent in both chemical and biological contexts. Kalia and Raines [6] The foundations for our current understanding of carbon–nitrogen double bond formation and hydrolysis were laid by seminal early work on hydrazone hydrolysis and formation, Conant, and Bartlett [7] and by contributions from mechanistic studies on enzymes that utilize pyridoxal phosphate. Metzler et al. [8] In particular, the meticulous kinetic analyses of Jencks resulted in the delineation of a carbinolamine intermediate in carbon–nitrogen double-bond formation and hydrolysis, and elucidation of the general mechanism of carbonyl-group addition reactions. Cords and Jencks [2]

Although the larger stability of hydrazones than imines are well-appreciated, a consensus on the comparative stability of hydrazones is lacking. More recently, other workers have discussed the stability of the hydrazones and used applications, Dirksen et al. [9] but without direct comparisons.

HPLC studies done by Novak showed that 80-100% of N-(pivaloyl)-4-methoxy-acetanilide, a model for suspected carcinogenic metabolites of phenacetin, decomposes into N-acetyl-p-benzoquinone imine (2 in Scheme 1). In aqueous solutions containing potassium chloride at pH< 6.0, compound 2 decomposes in a first-order manner by an acid-catalyzed process into p-benzoquinone, and acetamide (3, and 4 in Scheme 1). Novak et al. [10]



Scheme 1



2. Experimental

All melting points were determined on a Fisher-Johns melting point apparatus and were uncorrected. NMR spectra were taken in DMSO-d_6 as a solvent on a JEOL FX90-Q spectrometer using tetramethyl silane (TMS) as an internal standard. Infrared spectra were determined in KBr tablet on a Perkin-Elmer model 283 infrared spectrophotometer.

N-(p-nitro benzylidene)-4-hydroxyphenylethylamine (The Imine Scheme 2)

In a round-bottomed flask containing 20 ml of a 1:1 mixture of THF: ethanol was placed 0.112 g (0.74 mmol) of tyramine, 0.102 g (0.74 mmol) of 4-nitrobezaldehyde and two drops of acetic acid. The reaction mixture was refluxed with stirring for 2 hr. and evacuated to dryness in vacuo. The imine was recrystallized from THF. The yield was 1.90 g (95%); MP. 145-148 ^{o}C; IR (KBr) 3100 cm^{-1} (OH); 1530 and 1350 cm^{-1} (NO_2); ^{1}H NMR(DMSO-d_6); **δ**

8.40 (s,1, -CH=N-); **δ** 8.30 (d,2, AR-H); **δ** 7.95(d,2, Ar-H); **δ** 7.05(d,2, -CH_2-); **δ** 6.65(d,2,Ar-H); **δ** 3.75(s,2,-CH_2-); **δ**

2.85(s,2,-CH_2-). Anal. Calcd for $C_{15}H_{14}N_2O_3$. ½H_2O: C, 64.52; H, 5.38; N, 10.03

Found: C, 64.09; H, 5.58; N, 9.61

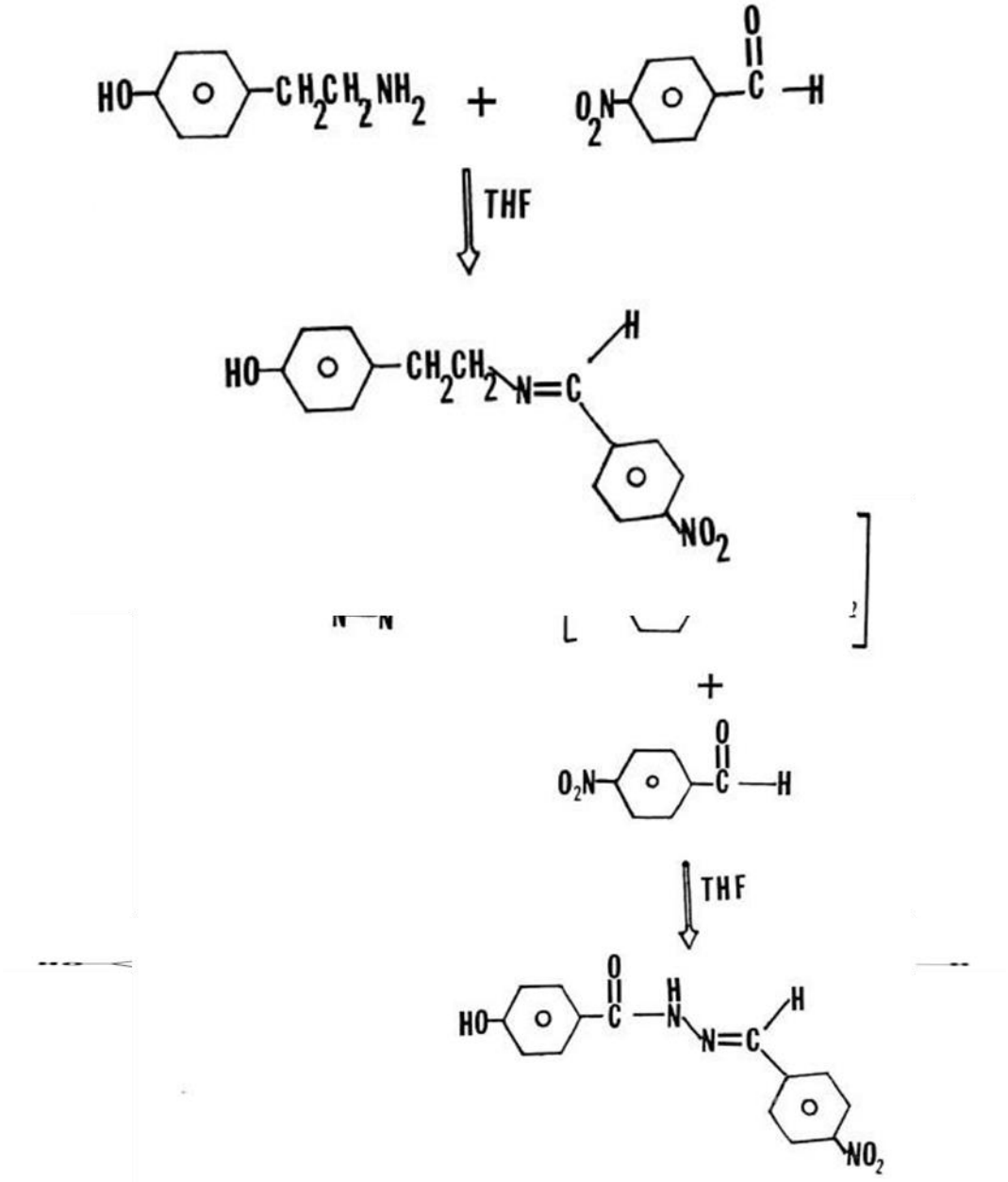
N_1-(p-nitro benzylidene)-N_2-(4-hydroxybenzoyl) hydrazine (The Hydrazone Scheme 3)

A solution of 0.30 g (1.85 mmol) of 2-(4-hydroxyphenyl)-1,3,4-oxadiazole, 0.20 ml (2.56 mmol) of concentrated hydrochloric acid, and 15 ml of THF was refluxed with stirring for 3 hr. The reaction mixture was then evaporated to dryness in vacuo to remove excess acid. THF (15 ml) containing 0.28 g (1.85 mmol) of 4nitrobenzaldehyde was added to the reaction flask and refluxed with stirring for 2 hr.

The hydrazone which was obtained following evaporation to dryness was recrystallized from ethanol and then dried. The yield was 0.48 g (90%); MP. Above 300 ^{o}C; IR (KBr); 3220 cm^{-1}(OH); 3150 cm^{-1}(NH); 1650 cm^{1}(C=O); 1550 and 1340 cm^{-1}(NO_2). ^{1}H NMR (DMSO-d_6); **δ** 11.95 (s,1, -CONH); **δ** 10.20 (s,1, Ar-OH); **δ** 8.55 (s,1, CH=N); **δ** 8.45 (d,2, Ar-H); **δ** 7.75 (d,2, Ar-H); **δ** 6.85 (d,2, Ar-H). Anal. Calcd for $C_{14}H_{11}N_3O_5$ C, 58.95; H, 3.85;

N, 14.74 Found: C, 58.65; H, 4.06; N, 14.70

Scheme 2 (The Imine)



Scheme 3 (The Hydrazone)

To evaluate potential internal standard references to be used as qualitative and quantitative markers in highpressure liquid chromatography (HPLC) studies, potassium acid phthalate (KHP) was compared to phenol. KHP was the optimum choice since it eluted early in the HPLC trace at about two minutes of post-injection compared to ten minutes for phenol. KHP was also deemed likely to experience minimum interferences with other components. Preparation of 1.0 x 10^{-3} M Solutions of N-(p-nitro benzylidene)-4-hydroxyphenylethylamine and N_1- (p-nitro benzylidene)-N_2-(4-hydroxybenzoyl) hydrazine.

A solution of 100 ml of 1.0 x 10^{-3} M of N-(p-nitro benzylidene)-4-hydroxyphenylethylamine was prepared by dissolving 0.027 g of this imine in 40 ml of acetonitrile. Then 1 ml of a 6.25 x 10^{-3} M solution of KHP was added and the volume was made up to the 100 ml mark of a volumetric flask with PBS, pH 7.40. Another solution of N_1- (pnitro benzylidene)-N_2-(4-hydroxybenzoyl) hydrazine was prepared by dissolving 0.0285 g of this

hydrazone in 40 ml of acetonitrile. To it also, 1 ml of a 6.25 x 10^{-3} M solution of KHP was added and the volume was made up to the 100 ml mark of a volumetric flask with PBS, pH 7.40.

3. Results and discussions

This study compared the hydrolysis of N-(p-nitro benzylidene)-4-hydroxyphenylethylamine (the imine) and N_1-(p-nitro benzylidene)-N_2-(4-hydroxybenzoyl) hydrazine (the hydrazone), under simulated physiological conditions. The hydrolytic reactions were studied by an HPLC method using a reversed phase C_{18} column (Solvent:60 % water, 40 % CH_3OH).

Table 1 showed that all elution times were corrected relative to that of the standard reference (KHP) taken as zero minute. The evidence for hydrolysis, that is, the loss of starting material and appearance of one of the products (tyramine), was evident after incubation at 37 ^oC for 72 hr., in phosphate buffered saline (PBS) pH 7.40, for the imine. However, no evidence for hydrolysis was observed for the hydrazone, even after 750 hr. of incubation. This behavior was expected since the C=N bond of the hydrazone is much more stable to hydrolysis than that of the imine.

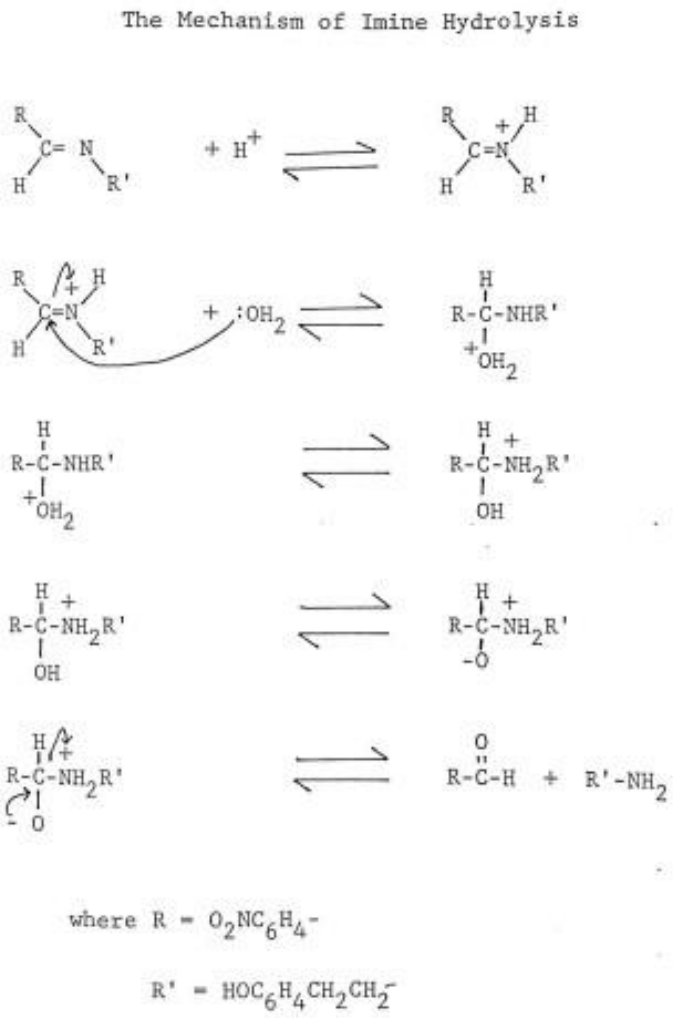
Table 1 All elution times are corrected relative to that of the standard potassium acid phthalate takes 0.00 minutes. C_{18} column: Solvent 60 % water, 40 % CH_3OH

Flow Rate: 1 ml/min.
Chart Speed: 0.25 cm/min.
Detector: Fixed UV at 254 nm.
Sample: 10 ml or 20 ml.
Solvent: CH_3CN and CH_3OH.

Compound	t_R (min)	t_R (corrected)
Potassium acid phthalate	2.4	0.0
4-Hydroxybenzoic acid hydrazide	13.2	10.8
p-Nitro benzaldehyde	16.2	13.8
Tyramine	4.5	2.1
The Imine (3+4)	16.2	13.6
The Hydrazone (2+3)	4.4	2.0

Scheme 4 showed the mechanism of imine hydrolysis. The relative rates of the various steps are a function of the pH of the solution and the basicity of the imine. In the alkaline range, the rate-determining step is usually the nucleophilic attack on the protonated C=N bond.

At intermediate pH values, water replaces hydroxide ion as the dominant nucleophile. In acidic solution, the rate-determining step becomes the breakdown of the tetrahedral intermediate. In this study, a pH of 7.40 was used and therefore water is expected to be the dominant nucleophile. It is expected that N-(p-nitro benzylidene)-4hydroxyphenylethylamine hydrolyzes by this general imine-hydrolysis mechanism.



Scheme 4

Table 2 showed the results of the HPLC study of the imine hydrolysis expressed as height of tyramine peak and height of the standard reference (KHP), and since the height of the peak represents concentration, then what really, we measured was the concentration of both tyramine and KHP.

Table 2 HPLC peak heights of imine hydrolysis in PBS pH 7.40 ± 0.01 and 37°C

No	H_1 (mm)	H_2 (mm)	H1/ H2 (mm)	Time (days)
1	8	63	0.127	3
2	24	116	0.207	4
3	11	89	0.186	4.2
4	10	35	0.286	6.3
5	12	30	0.400	11
6	12	41	0.293	11
7	10	25	0.400	13
8	7	27	0.260	18
9	9	26	0.340	20
10	7	32	0.234	21

11	5	30	0.167	21
12	7	44	0.159	25

H_1 = Height of tyramine peak. @ H_2 = Height of potassium acid phthalate

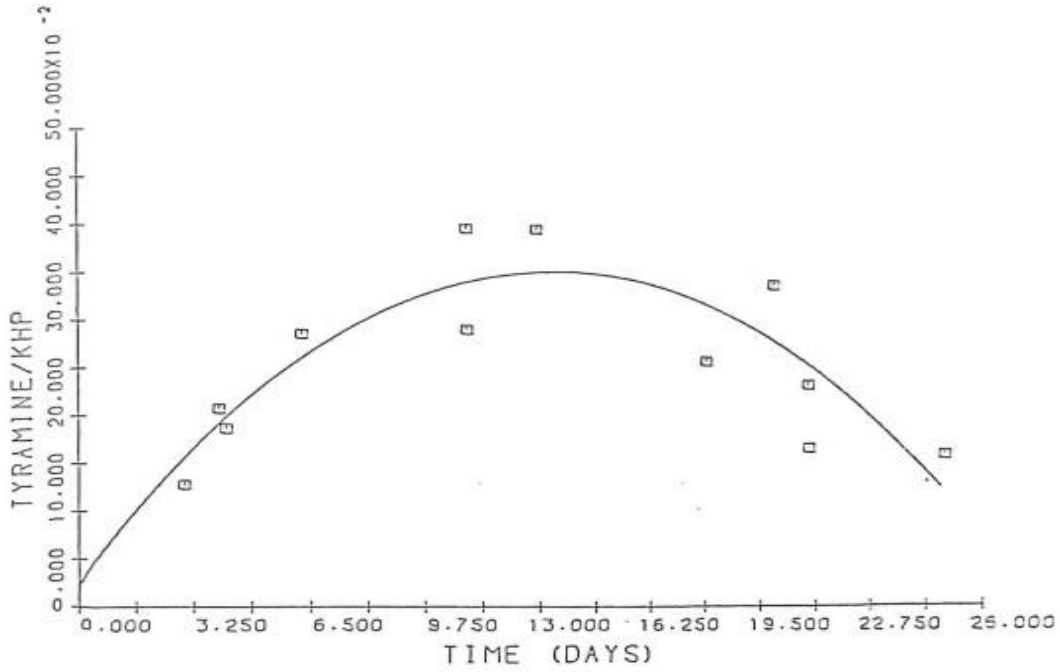


Figure 1 Plot of HPLC peak height (mm) vs. time for tyramine during the hydrolysis of N-(p-nitro benzylidene)-4-hydroxyphenylethylamine in 0.05 M phosphate buffer at pH 7.40

Fig.1 showed a plot of [tyramine]/ [KHP] vs time, typically it should resemble a plot done by Novak [6] which it did until it reached a maximum concentration of the tyramine, then it decreased. This behavior could be explained by the fact that tyramine is known to be oxidized to an aldehyde in the presence of oxygen, and since this study did not exclude oxygen then, indeed that was what happened. We got different peaks in the HPLC study which were not accounted for except for that of tyramine.

4. Conclusions

In the present study it was established that derivatives of the hydrazone would indeed be more stable in vivo than that of the imine derivatives. The hydrolytic stability of aryl carboxylic acid hydrazone was compared to that of similar tyramine imine under simulated physiological conditions (i.e., pH 7.40 and 37 °C), the latter being employed in indirect radioiodinations. The aryl carboxylic acid hydrazone was virtually inert to hydrolysis under the above conditions which caused the imine bond to rupture. This property made it possible to avoid the traditional borohydride reductions normally required in prosthetic derivatization of amines to carbonyl-containing biomolecules. A look at the chemical literature showed that there was an interest in studying imines and hydrazones since these derivatives have important applications in biological systems.

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