Thermal Degradation of 7, 8-Dimethyl-10-Formylmethylisoalloxazine in Acid Solution: A Kinetic Study

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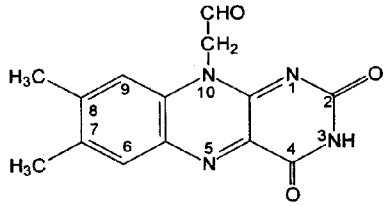
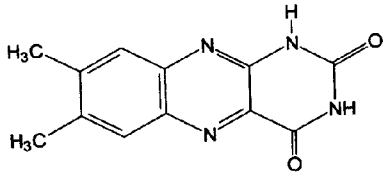
Summary: The thermal degradation of 7, 8-dimethyl-10-formylmethylisoalloxazine (FMF) in acid solution under anaerobic conditions has been studied. A specific spectrophotometric method has been used to determine FMF in the presence of degradation products. FMF has been found to undergo second-order reaction in the dark to form lumichrome and side chain products. The second-order rate constants for the reactions at 40-60 °C are in the range of 1.05 – 4.40 L mole^{-1} min^{-1}. The activation energy and the frequency factor for the reaction have been calculated as 15.0 kcal/mole (62.8 kJ/mole) and 2.43 x 10^{10} L mole^{-1} min^{-1}, respectively. A reaction scheme for the bimolecular degradation of FMF has been suggested.

Introduction

7,8-Dimethyl-10-formylmethylisoalloxazine (formylmethylflavin, FMF) was isolated and identified as an intermediate in the anaerobic photolysis of riboflavin (RF) [1, 2]. In neutral and alkaline solution FMF is hydrolysed to lumichrome (LC) and lumiflavin (LF) as the major photo-degradation products of RF [3-8]. It is relatively stable in acid solution and gives rise to LC on photodegradation of RF [9, 10]. Thus FMF plays an important role in the photolysis and product distribution of RF in acid and alkaline solutions. The present study has been conducted on the thermal degradation of FMF in acid solution leading to the formation of LC under anaerobic conditions in the dark to evaluate the kinetics of the reaction using a specific analytical method. The chemical structure of FMF and LC are shown below:



(FMF)



(LC)

Results and Discussion

Thin-layer Chromatography

Thin-layer chromatography (TLC) of the thermally degraded solutions of FMF (20-25% loss) using solvent systems A and B showed the presence of FMF and LC on comparison of their R_f values and characteristic fluorescence (*i.e.*, yellow green and sky blue, respectively) with those of the authentic compounds. This indicates the formation of LC as the only degradation product of FMF in acid solution (pH 4.5). The intensity of the LC spot increased with temperature suggesting an increase in the rate of reaction.

Assay Method

The assay method used in this work (see Experimental) is a specific spectrophotometric method developed and applied by Ahmad *et al.*, [5, 6, 9, 10] to the study of the hydrolysis and photolysis of FMF in aqueous solution. The application of the assay method to the determination of the concentrations of FMF and LC in the thermal degradation of FMF at 60 °C is shown in Table-1. It gives an almost constant molar balance (FMF + LC), with respect to the initial concentration of FMF (*i.e.*, 10^{-4} M), on the basis of mole to mole conversion (*i.e.*, one mole of FMF giving rise to one mole of LC) at 60 minutes interval, indicating the accuracy and reproducibility of the method.

Kinetics of Degradation

The molar concentrations of FMF determined during the degradation reactions at 40-60 °C

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Table-1: Thermal degradation of 10^{-4} FMF solution at pH 4.5 (60 °C); concentrations of FMF and LC.

Time (min)	FMF (M × 10^5)	LC (M × 10^5)	Total (M × 10^5)
0	10.00	—	10.00
60	9.73	0.29	10.02
120	9.44	0.55	9.99
180	9.24	0.76	10.00
240	8.98	0.98	9.96
300	8.88	1.20	10.08
360	8.60	1.39	9.99
420	8.39	1.63	10.02
480	8.25	1.79	10.04

were subjected to the kinetic treatment and the reactions were found to follow second-order kinetics. The second-order rate constants calculated from the slopes of the straight lines (1/C, *vs.* t) [11] lie in the range of 1.05-4.40 L mole^{-1} min^{-1} and are reported in Table-2.

Table-2: Second-order rate constants for the degradation of FMF at pH 4.5.

Temperature (°C)	k, L mole^{-1} min^{-1}	Correlation coefficient
40	1.05	0.998
50	2.22	0.998
60	4.40	0.999

The formation of LC as the only product of thermal degradation of FMF may be proved kinetically. The second-order integral rate equation can be written as

$$1/C_t = 1/C_i + kt \quad (1)$$

where C_i is the initial concentration and C_t is the concentration at time 't' of FMF. The values of C_t at 60 °C can be directly obtained from Table-1 or can be calculated from the values of [LC] in Table-1 as C_t^* = C_i – [LC]. The values of second-order rate constants obtained from the plots of 1/C *vs.* t and 1/C* *vs.* t are 4.4 and 4.5 L mole^{-1} min^{-1}, respectively. The good agreement between the two rate constants is a proof that LC is the only product of thermal degradation of FMF.

Effect of Temperature

The rate of a chemical reaction is generally proportional to the number of collisions per unit time. Since the number of collisions increase with an increase in temperature, the rate of reaction also increases with increasing temperature. The effect of temperature on the rate of reaction is given by the Arrhenius equation [12],

$$k = Ae^{-E_a/RT} \quad (2)$$

$$\text{or} \quad \log k = \log A - \frac{E_a}{2.303} \cdot \frac{1}{RT} \quad (3)$$

where k is the specific rate constant, A is known as the frequency factor, E_a is the activation energy, R is the gas constant, 1.987 cal/deg mole, and T is the absolute temperature. A plot of log k against $1/T$ for the reaction at 40 – 60 °C is shown in Fig 1. The slope of the straight line is $-E_a/2.303$ R and the intercept on the vertical axis is log A. The apparent value of the activation energy for the reaction has been determined as 15.0 kcal/mole (62.8kJ/ mole) and the frequency factor is 2.43 × 10^{10} L mole^{-1} min^{-1}. The magnitude of activation energy indicates that the molecule is fairly stable towards heat in acid solution. A value of 20 kcal/mole has been reported for the hydrolysis of riboflavin in alkaline solution [13]. This pertains to the isoalloxazine ring cleavage reaction.

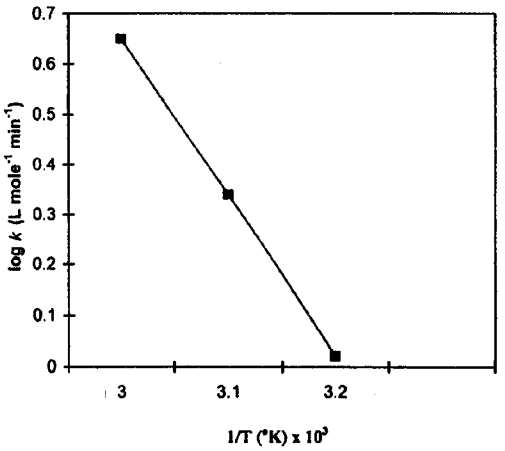

Fig. 1: Arrhenius plot for the thermal degradation of FMF.

Mode of Reaction

On the basis of the kinetic data it has been found that FMF undergoes thermal degradation by a second-order reaction suggesting a bimolecular mechanism to produce LC according to the following reactions.

$$2\text{FMF} \xrightarrow[\text{acid}]{\text{heat}} \text{FMF (reduced)} + \text{FMF (oxidized)} \quad (4)$$

FMF (oxidized) ⟶ LC + side chain products (5)

The thermally activated FMF molecule is probably reduced at the expense of the oxidation of the second molecule to form LC and the side chain products. The reduced form of FMF ($FMFH_2$) may involve the reduction of the isoalloxazine nucleus at N-1 and N-5 in the conjugated system ($-N_1=C-C=N_5$) or may be a cyclic intermediate [7]. The nature of the oxidized form of FMF is not known.

Experimental

7,8-Dimethyl-10-formylmethylisoalloxazine was prepared by the method of Fall and Petering [14]. Lumichrome was obtained from Sigma Chemical Co. All reagents and solvents were of the purest form available from BDH/Merck.

Thermal Degradation

A 10^{-4} M aqueous solution of FMF was prepared at pH 4.5 (adjusted with 0.001 M HCl) in a 100 ml volumetric flask and deoxygenated for about one hour by bubbling oxygen free nitrogen. The nitrogen was previously passed through an alkaline solution of pyrogallol (15% in 50% NaOH) solution [15] to remove traces of oxygen and subsequently through distilled water. The flask was then immersed in a thermostat bath maintained at 40-60 ± 1 °C using a Shanden Circotherm II unit. The reaction was carried out in the dark under a steady stream of nitrogen and the pH of the solution was maintained by the addition of 0.001 M HCl or 0.001 M NaOH solution using a Pye autotitrator in conjunction with a Pye pH meter. Samples were withdrawn at appropriate intervals for thin-layer chromatography and spectrophotometric assay.

Thin-layer Chromatography

Thin-layer chromatography of the degraded solutions of FMF was performed on 250-μm cellulose (Whatman CC41) plates and the following solvent systems were used: (A) 1-butanol-acetic acid-water (40: 10:50, v/v, organic phase); and (B) 1-butanol-1-propanol-acetic acid-water (50:30:2:18, v/v) [5]. FMF and degradation products were detected by their characteristic fluorescence emission under UV light (366 nm).

Assay Method

An appropriate aliquot of the degraded solution of FMF was pipetted out in a 25 ml beaker and immediately cooled in an ice bath to stop the reaction. A 5 ml portion of the cooled solution was placed in a 10 ml volumetric flask and made upto volume with pH 2.0 buffer (KCl-HCl). At this pH, FMF is stable in its protonated form (pK_a 3.5) [16]. The solution was extracted with chloroform to remove LC. The absorbance of the aqueous layer was measured at 385 nm to determine the concentration of FMF. The chloroform extract was evaporated to dryness under reduced pressure, the residue redissolved in acetate buffer (pH 4.5) and the absorbance of the solution measured at 356 nm to determine the concentration of LC. The details of the assay method are reported elsewhere [9].

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