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THE RIBOFLAVIN CATALYZED PHOTODECOMPOSITION OF 9-AMINOMETHYLACRIDAN

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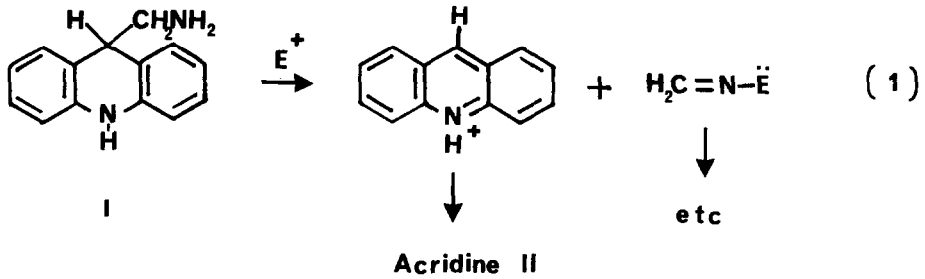
Previously we have reported an unusual oxidative amine fragmentation of 9-aminomethylacridan (9-AMA) (I) to acridine (II) (Eq. 1) by pyridoxal[1], sodium 1,2-napthoquinone-4-sulfonate, and nitrous acid[2]. Drawing upon our previous data[1,2], we proposed a mechanism for the formation of acridine through a covalent bond formation between the primary amine nitrogen of I and the electrophile (E^+ = pyridoxal, quinone, or NO^+).



I

Acridine II

(1)

The reaction depicted in Eq. 1 can be considered a model for biological transformations involving fragmentation of amines in the following general manner:

$$RCH_2NH_2 \xrightarrow{\text{electrophile}} R^+ \xrightarrow{NH_3} RNH_2 \longrightarrow \text{etc.} \qquad (2)$$

This is examplified by the biosynthesis of gramine[3] from tryptophan[4] and the presumed biosynthesis of p-hydroxybenzylamine from tyrosine[5].

Now, we wish to report our recent observation in the FMN (mono-sodium salt of riboflavin-5-phosphate) sensitized anaerobic photodecomposition of I to II.

Fresh solutions of I were prepared daily in degassed methanol-water (double distilled) (1:1). Reactions were run in a 1 cm quartz thunberg tube under N_2 at 27±0.1°C. The light source was an unfiltered 500W projector lamp placed 62 inches from the cell. A Cary 15 recording spectrophotometer was used to measure absorbance in the 220-400 nm region. Absorbance at 292 nm region (near the 278 nm maximum for I) was read on a Gilford 240 spectrophotometer for the kinetic studies. Measurements at 292 nm, an isosbestic point for FMN[6] and its photoproducts, facilitated correction for the contribution of FMN to the total absorbance.

In the typical experiment, irradiation of a solution of equimolar (5×10^{-5}M) amounts of I and FMN in methanol-water (1:1) with visible light under N_2 atmosphere resulted in a complete conversion of I to II in 120 seconds as evidenced by the decrease in absorbance of the acridan peak at 278 nm and the appearance of the acridine peaks at 252 and 350-360 nm. Subsequently the methanol was evaporated from the reaction mixture (3 ml) by blowing with N_2. The reaction was then extracted with chloroform. The chloroform extracts were evaporated to dryness under N_2 and the residue subjected to two-dimensional thin-layer chromatography (Silica Gel, 0.25 mm). The R_f values of the authentic acridine and the reaction product (visualized with UV irradiation) in the first developing solvent (butanol-5N acetic acid, 7:3) were 0.62 and 0.63. The R_f values in the second solvent system (3% NH_4Cl in H_2O) were 0.08 and 0.08. The photoproduct was eluted from the chromatogram with 10 ml of methanol. Determination of the absorbance of this solution at 255 nm ($\epsilon = 2.0 \times 10^5$) showed that I was quantitatively converted to acridine (II). The solution also exhibited maxima at 255 and 350-360 nm which are characteristic of II. The conversion of I to II is severely self-quenched at concentrations of I higher than 0.5×10^{-4}M. Thus it was necessary to use two-dimensional TLC and subsequent UV spectroscopy for identification of the acridine (II). No acridine (II) was produced when 9-aminomethylacridine[7] (III) was subjected to the above TLC treatment. Thus, the possibility that the reaction might lead to products II and III[8] with the latter being converted to the former during the isolation was eliminated.

Kinetic studies revealed that the reaction is first-order with respect to I (at 5.0×10^{-5}M and 5.0×10^{-5}M FMN) (Fig. 1). The dependency of the reaction rate on FMN concentration

is less than first order at concentrations higher than 1.0×10^{-4}M. Under these conditions the rate appears to be seriously slowed, possibly as a result of concentration quenching[10]

Loss of FMN during the reaction is insignificant relative to loss of I, indicating that the reaction is not a coupled oxidation-reduction with the isoalloxazine moiety participating as an electron acceptor. Furthermore, the photolysis of I proceeded to completion with catalytic amounts of FMN (as low as 100 times less than that of I). Known quenchers of the triplet state of FMN, such as oxygen and KI, retarded the reaction (Fig. 2). The reaction was found to be severely retarded by 1×10^{-4}M KI (Fig. 3). In addition, fluorescence spectra of FMN in the presence of I and 1×10^{-4}M KI indicated no quenching of the singlet excited state. I remained unchanged when exposed to light in the absence of FMN or in the presence of FMN in the absence of light excitation.

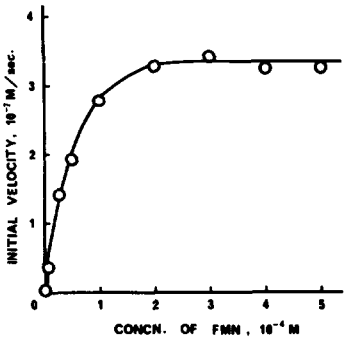


Fig. 1

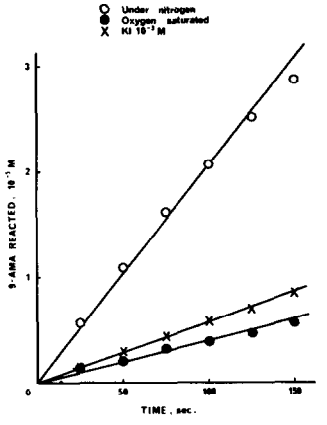


Fig. 2

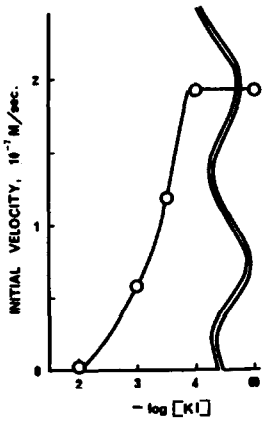


Fig. 3

Fig. 1: Effect of FMN concentration on the Anaerobic Photodecomposition of 9-Aminomethylacridan (9-AMA; 5.0×10^{-5}M).

Fig. 2: Effect of Quenchers on the Photodecomposition of 9-Aminomethylacridan (9-AMA; 5.0×10^{-5}M) in the presence of FMN (5.0×10^{-5}M).

Fig. 3: Effect of KI on the Anaerobic Photodecomposition of 9-AMA (5.0×10^{-5}M) in the presence of FMN (5.0×10^{-5}M).

The possibility that traces of oxygen are required for the anaerobic photodecomposition of I by FMN cannot be completely excluded. However, since the reaction appears to proceed at a slower rate in oxygen (Fig. 2), this species probably does not play an important role in the loss of the two electrons (Eq. 1). In this connection, we are currently investigating the fate of the 1-carbon fragment arising from C^{14}-9-AMA labeled at its methylenic carbon.

Although we are not as yet prepared to advance a detailed mechanistic rationalization for the anaerobic photodecomposition of I to II by visible light in the presence of FMN, we suggest that biological fragmentations of the type represented in Eq. 2 could be catalyzed by other than pyridoxal dependent enzymes[3,5]. Perhaps an attractive possibility is that of energy transfer reaction from an excited FMN species (possibly a triplet state) to I. The great potential of intermolecular energy transfer phenomena in biological systems has been recently demonstrated by the work of White[11,12].

References and Notes

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4. Both tryptophan and I have the common structural unit -NH-C=C-C-C-NH_2 hence reactions of the latter with electrophiles can be considered as models for the biological transformations.
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7. 9-Aminomethylacridine was produced by treating I with $NaNO_2$ in 10% H_2So_4 according to our previously developed method[2].
8. The production of III was considered a good possibility in light of our recent findings that 2-chloro-9-(3-dimethylaminopropyl) acridan was converted under similar conditions to its acridine derivative[9].
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