ENERGY TRANSFER REACTIONS IN AQUEOUS SOLUTION. THE RIBOFLAVIN-PHOTOOXIDATION OF AN ACRIDAN DERIVATIVE

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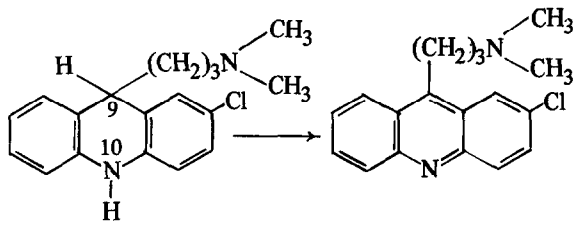
Evidence of triplet-triplet energy transfer in aqueous solution was first presented by Berends et al. [1–3] who studied the photodecomposition of the polyene fungicide Pimaricin and the photo-isomerization of stilbene derivatives sensitized by riboflavin and lumichrome. We wish to report our recent observation of an apparent triplet-triplet energy transfer in the FMN (mono-sodium salt of riboflavin 5-phosphate) sensitized anaerobic photooxidation of the antipsychotic drug Clomacran phosphate (I) (SK & F 14,336), a member of the acridan family [2-chloro-9-(3-dimethylamino-propyl)acridan phosphate (1:1)] to its acridine derivative (II).



(I) (II)

This reaction differs from the reaction between FMN and 9-aminomethylacridan in which the acridan derivative suffers a side-chain fragmentation to yield acridine and formaldehyde [4].

The acridan nucleus resembles that of phenothiazine, and the oxidation of both compounds appears to involve a semiquinone intermediate. There have been several recent indications that biological activity of phenothiazine anthelminthic compounds is related to semiquinone formation [5] and that activity of certain phenothiazine tranquilizers can be correlated with inhibition of electron transport [6]. The potential for energy transfer reactions in biological systems between excited state biomolecules and drugs such as acridans and phenothiazines merits consideration with respect to both the mechanism of action of such drugs and possible toxic manifestations. Studies of this nature are in progress in these laboratories.

Solutions were prepared daily in degassed water and reactions were run in 1 cm silica cells under an atmosphere of N_2 at 27 ± 0.1°C. The light source for Clomacran-FMN studies was the unfiltered light from a 500 W projection lamp, and for photolytic studies of Clomacran alone the light source was a Pen-Ray mercury lamp which emitted radiation of essentially 253.7 nm. Absorbance was measured in a Cary 15 recording spectrophotometer for the region 220–400 nm, and for kinetic studies absorbance was determined in a Gilford 240 spectrophotometer at 292 nm near the 282 nm maximum for Clomacran phosphate. Measurement at 292 nm, an isosbestic point for FMN and its photoproducts, facilitated correction for contribution of FMN to the total absorbance.

Irradiation of a solution of equimolar (5×10^{-5} M) concentrations of Clomacran phosphate and FMN with visible light under anaerobic conditions resulted in conversion of Clomacran (I) into its acridine derivative (II), as evidenced by the decrease in absorbance of the acridan peak at 282 nm and the appearance of acridine peaks at 252 and 350–360 nm. The product (II) was isolated by preparative thin-layer chromatography (TLC) and identified as 2-chloro-9-(3-dimethylaminopropyl)acridine on the basis of IR and UV spectra and TLC comparison with an authentic sample

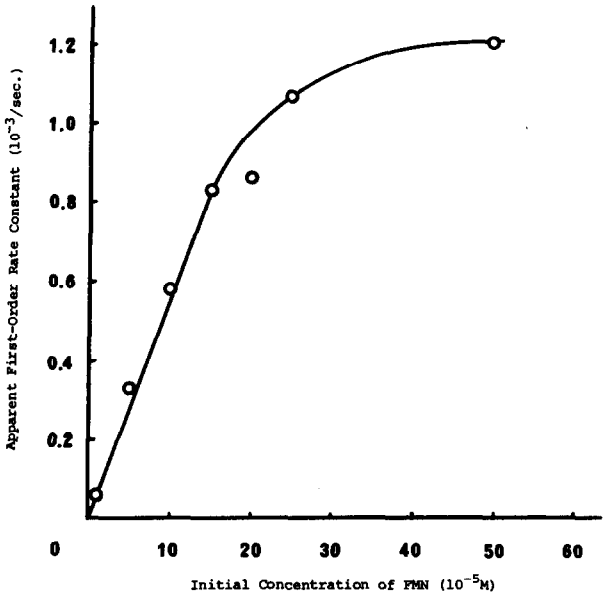


Fig. 1. The effect of riboflavin phosphate concentration on the rate of photo-oxidation of Clomacran phosphate (5×10^{-5} M).

provided by Smith, Kline & French Laboratories. Both light and FMN are essential to the reaction and oxygen retards the reaction. A kinetic study revealed that the reaction is first-order with respect to Clomacran phosphate (at a concentration of 5.0×10^{-5} M) and first-order with respect to FMN at concentrations of $0.5 \times 10^{-5} - 1.5 \times 10^{-4}$ M FMN, as indicated in fig. 1. At higher concentrations of FMN ($2.0 \times 10^{-4} - 5.0 \times 10^{-4}$ M) the dependency of reaction rate on FMN concentration is less than first-order, probably as a result of concentration quenching exhibited by FMN [7]. It should be noted that the pH values of the varying concentrations of riboflavin phosphate (from 5×10^{-5} to 5×10^{-4} M) were essentially the same. Loss of FMN during the anaerobic photolysis of Clomacran was insignificant relative to loss of Clomacran, indicating that the reaction is not a coupled oxidation-reduction with the FMN isoalloxazine nucleus serving as a hydrogen acceptor. The photolysis of Clamacran proceeded to completion with concentrations of Clomacran 100 times that of FMN.

It is proposed that the anaerobic photodecomposition of Clomacran by visible light in the presence of FMN proceeds via a triplet-triplet energy transfer from FMN to Clomacran. This conclusion is based on the observations that loss of FMN is insignificant relative to that of Clomacran, that reagents known to serve as quenchers of the triplet state of FMN, i.e. oxygen and 1×10^{-4} M KI, retarded the reaction, and fluorescence spectra of FMN in presence of Clomacran and 1×10^{-4} M KI indicated no quenching of the FMN singlet excited state.

When Clomacran was irradiated with ultraviolet light of 253.7 nm in absence of FMN, under anaerobic conditions, the resulting photo-product exhibited the same spectral characteristics as II, the photo-product of the FMN-sensitized reaction in visible light.

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