Flavin-Catalyzed Photooxidation of an Acridan Drug: A Reinvestigation

W.-R. KNAPPE

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Abstract □ Anaerobic irradiation of flavin derivatives (IIa and IIb) in the presence of 2-chloro-9-(3-dimethylaminopropyl)acridan phosphate (Ia) resulted in the reduction of the flavins to IVa and IVb and oxidation of an equimolar amount of Ia to IIIa. This photoreduction occurred from the first excited triplet state of IIa and IIb and proceeded *via* a covalent intermediate (V) between flavin and the acridan derivative. If the N-10 of the acridan was blocked by a methyl group, *e.g.*, Ic, V could be observed spectrophotometrically at −40°, decomposing homolytically on warming. With N-10 unsubstituted acridan derivatives, *e.g.*, Ia and Ib, V could not be observed because of fast heterolytic decomposition, yielding IVa, IVb, and the acridine compounds IIIa and IIIb. All photoreactions showed a kinetic isotope effect between 1.50 and 2.20 when position 9 of the acridan compounds was substituted by deuterium instead of hydrogen.

Keyphrases □ Acridan, substituted—mechanism of anaerobic flavin-catalyzed photooxidation □ Flavin-catalyzed photooxidation–substituted acridan, mechanism studied □ Photooxidation, flavin catalyzed—substituted acridan, mechanism studied

Recently, the anaerobic photodecomposition of an acridan drug, 2-chloro-9-(3-dimethylaminopropyl)acridan phosphate (Ia)[1], was studied in the presence of riboflavin 5′-phosphate (IIa) (1, 2). It was postulated that the photooxidation of the acridan derivative Ia to its acridine derivative IIIa does not involve a photoreduction of the flavin IIa but proceeds as follows (2).

The flavin is photoexcited to the first excited singlet state, which, on intersystem crossing, yields the triplet state (Scheme I).

$$\text{IIa} \xrightarrow{h\nu} {}^1\text{IIa}^* \xrightarrow{\text{intersystem crossing}} {}^3\text{IIa}^*$$

Scheme I

The flavin transfers its energy to the acridan, with generation of the acridan triplet and flavin ground state (Scheme II).

$${}^3\text{IIa}^* + \text{Ia} \rightarrow {}^1\text{IIa}^\circ + {}^3\text{Ia}^*$$

Scheme II

The acridan triplet decomposes to IIIa and hydrogen (Scheme III).

$${}^3\text{Ia}^* \rightarrow \text{IIIa} + \text{H}_2$$

Scheme III

Since the triplet state of acridan lies 292 kJ/mole above the ground state (3) (and the energy of the triplet of Ia should be almost the same) and the flavin triplet has only an energy of 209 kJ/mole (4), such an energy transfer is not possible. Energy transfer reactions from the flavin triplet to low lying ground states of substituted olefins (*e.g.*, stilbene or retinol), resulting in *cis–trans*-isomerization (5), or to triplet oxygen, yielding singlet oxygen (6), are known, but the energy transfer must be thermodynamically possible in all cases.

The flavin triplet is known to oxidize substrates, however, yielding reduced flavin (7); in this respect, it resembles the flavin in flavoenzymes (7). In these redox reactions, 4a-substituted 4a,5-dihydroflavin derivatives presumably are the first intermediates, as was shown recently (8).

EXPERIMENTAL

Reagents—Compounds Ia[1] and IIa[2] were used as supplied. 3-Methyllumiflavin (IIb) (9), acridan (Ib) (10), *N*-methylacridan (Ic) (11), 9,9-dimethylacridan (Id) (12), 9,9,10-trimethylacridan (Ie) (13), and *N,N′*-dimethyl-9,9′-diacridanyl (Ii) (14) were synthesized according to literature procedures.

9,9-Dideuterioacridan (If) (≈98% 9,9-D_2 from ^{1}H-NMR) was prepared from 9-deuterioacridine (15) by reduction with lithium aluminum deuteride; 9,9-dideuterio-*N*-methylacridan (Ig) (≈98%, 9,9-D_2 from ^{1}H-NMR) was obtained by reduction of *N*-methylacridone with sodium in *O*-D-butanol (16). Compound 9-D-Ia (Ih) (≈96% 9-D) was synthesized from IIIa (2). The acetonitrile used was freshly distilled from phosphorus(V) oxide.

Spectroscopy[3]—The anaerobic irradiations were performed in 1-cm quartz cells of the Thunberg type after argon (<1 ppm O_2) was bubbled through the solutions for 30 min. The light source was a 250-w medium pressure mercury arc with a 300-nm cutoff filter, a 250-w/24-v tungsten halogen lamp with a 442-nm narrow band interference filter[4] (8 nm half-width, B-40), or a broad band interference filter[4] (K 2), transparent from 420 to 480 nm. The quantum yields were calculated from the light intensities measured bolometrically (17).

RESULTS AND DISCUSSION

When an aqueous solution of Ia (6.67×10^{-4} *M*) and 3-methyllumiflavin (IIb, 1×10^{-4} *M*) at pH 9 was irradiated under strictly anaerobic conditions with light of 442 nm, a slow reduction of the flavin occurred, giving 3-methyl-1,5-dihydrolumiflavin (IVb) with a quantum yield of 0.012. This photoreduction was accompanied by the oxidation of an equimolar amount of Ia to its acridine analog IIIa as shown by the increased absorbance at 360 nm and by TLC comparison with an authentic sample. The same reaction was observed using light of a medium pressure mercury arc with a cutoff filter at 300 nm. Spectra are shown in Fig. 1.

Upon admission of oxygen, IVb was reoxidized instantaneously nearly quantitatively. The loss of 10% was due to some side reactions, presumably with the dimethylaminopropyl side chain of Ia and, more probably, due to the prolonged short wavelength irradiation. With filtered light (420–480 or 442 nm), IIb was recovered quantitatively upon admission of oxygen.

The same results were obtained using riboflavin 5′-phosphate (IIa) instead of IIb; the photodestruction of IIa (intramolecular reaction with its side chain) proceeded at pH 9 with a very small quantum yield and was quenched by the Ia present, presumably *via* formation of a hydrophobic complex (18).

The well-known dihydroflavin derivatives IVa and IVb (8) were identified by their characteristic, very fast reoxidation with air, yielding the oxidized flavins IIa and IIb. Further proof of structure was obtained by subtraction of the spectra of IIa and IIb from the spectra of the reaction mixture, resulting in the spectra of IVa and IVb.

The observation (1, 2) that IIa was not reduced under these conditions is probably due to nonanaerobic conditions. Therefore, the main reactions can be formulated in the following way.

The flavin is photoexcited by the long wavelength light and yields, *via* intersystem crossing its triplet state, ^{3}IIb*, according to Scheme I. The

[1] Smith Kline and French, Philadelphia, PA 19101.
[2] Hoffmann-La Roche, Basel, Switzerland.
[3] UV spectra were recorded with a Varian 635 M spectrophotometer.
[4] Balzers.

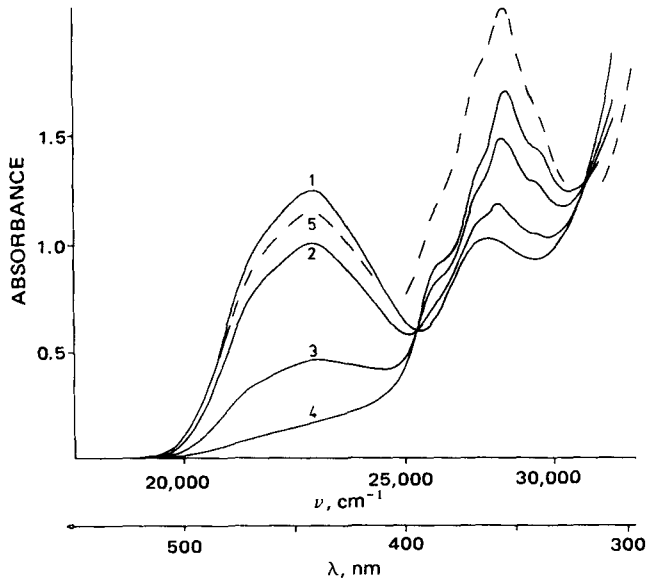


Figure 1—*Spectral course of the photoreduction of IIb (1×10^{-4} M) with Ia (6.67×10^{-4} M) in pH 9 aqueous phosphate buffer under strictly anaerobic conditions. The light source was a 250-w medium pressure mercury arc with a 300-nm cutoff filter. Key: 1, starting material; 2, 3, and 4, after illumination for 3, 13, and 43 min, respectively; and 5, after admission of oxygen.*

reported quenching experiments with iodide (1, 2) could be verified. All intermolecular photoreactions of flavin derivatives, except with solvent molecules, start from the triplet state (7).

The electron-deficient flavin triplet then attacks the acridan substrate, yielding in an overall reaction the reduced flavin IV*b* and the acridine III*a* (Scheme IV).

$$^3\text{II}b^* + \text{I}a \rightarrow \text{IV}b + \text{III}a$$

Scheme IV

On admission of oxygen, IV*b* is reoxidized to II*b*, accompanied by formation of water and hydrogen peroxide (Scheme V).

$$\text{IV}b + O_2 \rightarrow \text{II}b + H_2O_{(2)}$$

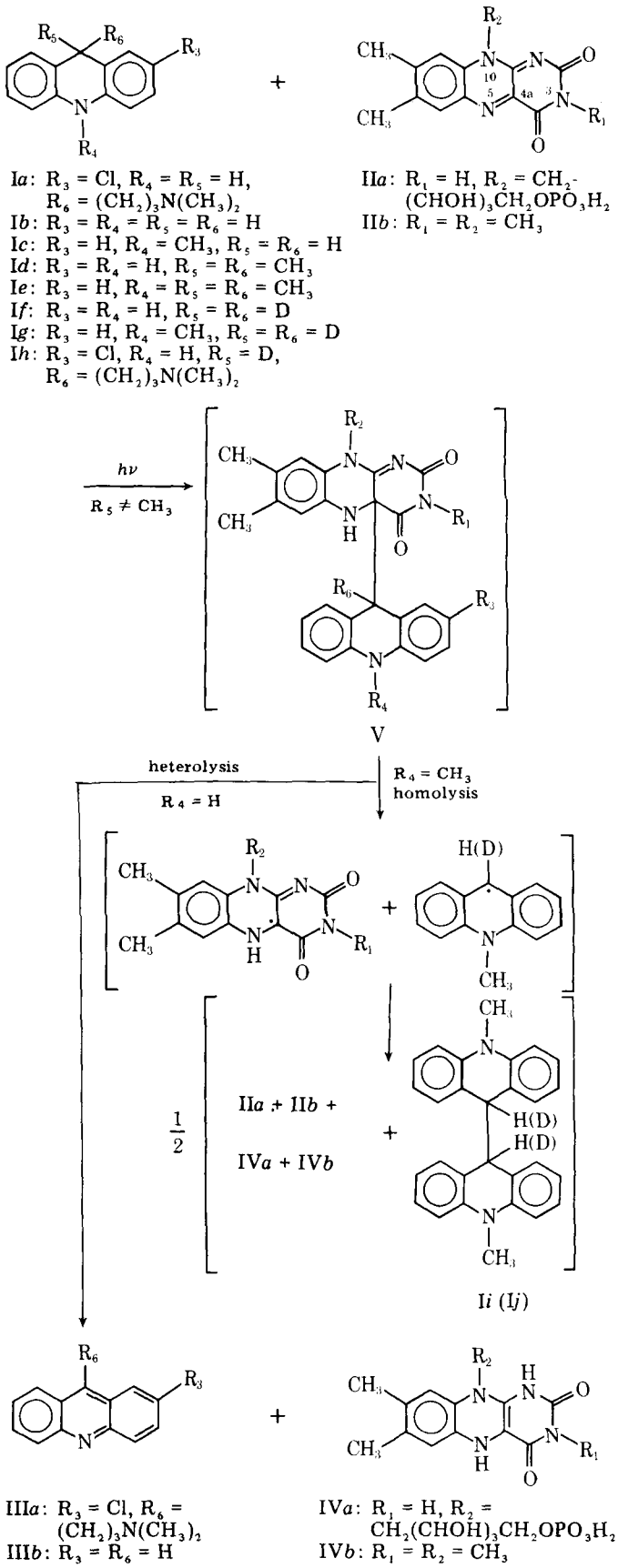
Scheme V

Under aerobic conditions, no IV*b* could be detected, since it is reoxidized instantaneously [half time of 6.1 sec (19, 20)] according to Scheme IV. Only the oxidation of I*a* to III*a* was observed, proceeding with a quantum yield of 0.10. This higher quantum yield, compared to the value of 0.012 in the anaerobic case, might be explained by the fact that singlet oxygen, which can be produced by energy transfer from the flavin triplet to the oxygen triplet (6), oxidizes I*a* with a higher efficiency than does the flavin triplet itself.

To gain further insight into the mechanism of this flavin-catalyzed photooxidation of I*a*, simpler acridan derivatives were investigated; 9,9-dimethylacridan (I*d*) and 9,9,10-trimethylacridan (I*e*) were not attacked by the flavin triplet. Therefore, the attack of the flavin triplet on I*a* does not occur at the N-10 position but at position 9 of the acridan nucleus. This finding was proved by the fact that acridan (I*b*) and *N*-methylacridan (I*c*) showed an even higher reactivity toward the flavin triplet than I*a*. The photoreaction of II*b* proceeds with quantum yields of 0.039 and 0.18 in acetonitrile at +25° (Table I).

When the photoreaction of II*b* with I*c* was performed at −40°, a new maximum at 368 nm (log ε 3.87) was observed in the UV spectrum (8), which is characteristic for a 4a-substituted 4a,5-dihydroflavin of type V (Scheme VI). Upon warming the solution to +25°, this intermediate decomposed homolytically, yielding the flavin radical, which disproportionated to 50% II*b* and 50% IV*b*, and the acridanyl radical, which dimerized to I*i*. Compound I*i* could be identified by TLC as the only oxidation product of I*c* (8).

When performing the same reaction with the N-10 unsubstituted I*b*, no intermediate adduct could be detected spectroscopically at −40° (8). Only a reduction of II*b* to IV*b* and an oxidation of an equimolar amount of I*b* to III*b*, identified by TLC comparison with acridine, was observed. In this case, the adduct of type V split heterolytically in a thermodynamically favored manner, yielding the oxidized aromatic substrate (III*b*) and the 1,5-dihydroflavin (IV*b*).



Scheme VI

In the first reaction step yielding the 4a-adduct (V), a CH bond is split, and a primary kinetic isotope effect should be observable when I*b* is

Table I—Quantum Yields (Φ) of the Anaerobic Photoreduction of IIb (1×10^{-4} M) in the Presence of Acridan Derivatives (6.67×10^{-4} M)

Acridan Derivative	Φ	Solvent
Ia	0.012	pH 9 phosphate buffer, anaerobic
Ia	0.10[a]	pH 9 phosphate buffer, aerobic
Ib	0.039	Acetonitrile, anaerobic
If	0.018	Acetonitrile, anaerobic, $\alpha_{H/D} = 2.17$
Ic	0.18	Acetonitrile, anaerobic
Ig	0.12	Acetonitrile, anaerobic, $\alpha_{H/D} = 1.50$
Id	0.000	Acetonitrile, anaerobic
Ie	0.000	Acetonitrile, anaerobic

[a] Quantum yield for the IIb-sensitized oxidation of Ia.

compared to its deuterated analog (If). A primary isotope effect of 2.20 ± 0.10 was found, and the N-10 methylated derivatives (Ic and Ig) showed a smaller isotope effect of 1.50 (Table I).

Therefore, the oxidation of Ia by flavin, which proceeds not only on illumination but even very slowly (0.21 M^{-1} sec^{-1} at pH 4) in the dark, is likely to proceed *via* intermediate formation of an adduct of Structure V, which decomposes heterolytically as pointed out for acridan. This proposal is confirmed on repeating the isotope effect measurements of Digenis *et al.* (2). Compound Ih, prepared according to their method (2), showed a primary isotope effect of 2.1 ± 0.20 when compared to Ia. For inexplicable reasons, Digenis *et al.* (2) found no isotope effects.

Therefore, it can be concluded that Ia as well as other acridan derivatives is photooxidized by the flavin triplet *via* covalent intermediates, which split heterolytically when the N-10 position is not blocked by an alkyl group. If the N-10 position is blocked, *e.g.*, in Ic, the covalent intermediate of type V can be observed spectroscopically at low temperatures, splitting homolytically upon heating.

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Effect of Ionization on Absorption of Cephalosporins

JOYCE L. DeYOUNG ˣ, HETTY G. H. TAN, H. E. HUBER, and M. A. ZOGLIO

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Abstract □ To explore the relative absorbabilities of different ionic forms of cephalosporins, the absorption rates of four compounds were measured in the pH 5–9 region using an *in situ* rat gut technique. Cephalexin, cephradine, and cephaloglycin have some oral activity, while 3-[(acetyloxy)methyl]-8-oxo-7-[[(4-oxo-1(4*H*)-pyridinyl)acetyl]-amino]-5-thia-1-azabicyclo[4.2.0]oct-2-ene-2-carboxylic acid (I) has insignificant oral activity. The pH–species profiles calculated from their ionization constants showed that cephalexin, cephradine, and cephaloglycin have a large proportion of uncharged molecules plus zwitterions in the pH range of the small intestine, while I exists as the anion throughout this range. When the species profiles are compared with the pH–absorption rate profiles for cephalexin, cephradine, and I, the results are consistent with a model in which the zwitterionic and/or uncharged forms of the molecules are well absorbed, whereas the anions show little or no absorption. Although it has a pH profile for zwitterions plus uncharged molecules similar to cephalexin, cephaloglycin shows poor absorption, suggesting that the ratio of uncharged molecules to zwitterions may be important in absorption.

Keyphrases □ Absorption, GI—various cephalosporins, effect of ionization, rats □ Cephalosporins, various—GI absorption, effect of ionization, rats □ Ionization—effect on GI absorption of various cephalosporins, rats □ Antibacterials—various cephalosporins, GI absorption, effect of ionization, rats

There has been very little published concerning the mechanism of cephalosporin absorption. Some cephalosporins are known to be actively secreted into the renal tubules (1), suggesting that an active process could also be important in absorption. Penzotti and Poole (2) investigated this possibility using everted rat intestinal sacs; for some penicillins and cephalosporins, including cephalexin and cephaloglycin, they found no evidence for a specialized transport mechanism.

If these compounds are passively absorbed, the extent of their ionization in the pH region of the GI tract could determine partly the ease with which they are absorbed. Therefore, the absorption rates of cephalexin, cephradine, cephaloglycin, and 3-[(acetyloxy)methyl]-8-oxo-7-[[(4-