The Photoreduction of 8-Substituted 5-Deazaflavins and the 5-Deazaflavin Catalyzed Photoreduction of Methyl Viologen

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Dedicated to Professor Norman H. Cromwell

Prolonged illumination of 8-X-5-deazaflavins (X = Cl, N(CH₃)₂, NH₂, p-NH₂-C₆H₄) in the presence of an electron donor leads to the formation of a 5,5'-dimer and/or a 6,7-dihydro compound. The course and rate of these photoreductions were established and discussed in terms of electronic and steric effects, exerted by the substituent at position 8 and the electron donor. Pseudo first-order kinetics were found to apply to the photoreduction of 8-X-5-deazaflavins (X = Cl, NH₂, p-NH₂-C₆H₄) while the rate of the photoreduction of 8-X-5-deazaflavin (X = N(CH₃)₂) appeared to contain an autocatalytic element. The catalytic effect of 8-X-5-deazaflavins in the photoreduction of methyl viologen by EDTA was investigated. The substituent effect on the rate of the 8-X-5-deazaflavin mediated photoreduction of methyl viologen by EDTA was found to be comparable with that on the photoreduction rate of 8-X-5-deazaflavin in the presence of EDTA with the exception of 8-X-5-deazaflavin (X = N(CH₃)₂), which showed a remarkable relative enhancement of the reactivity towards methyl viologen photoreduction.

In context of the search for a 5-deazaflavin (dFlox)‡, which combines a low excitation energy with a high rate of catalysis of the photoreduction of flavoenzymes, we synthesized a number of water soluble 8-substituted 5-deazaflavins (Figure 1, **1a-g**) [1]. The effect of the substituent at
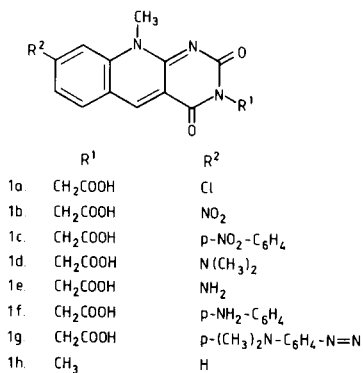

	R¹	R²
1a	CH₂COOH	Cl
1b	CH₂COOH	NO₂
1c	CH₂COOH	p-NO₂-C₆H₄
1d	CH₂COOH	N(CH₃)₂
1e	CH₂COOH	NH₂
1f	CH₂COOH	p-NH₂-C₆H₄
1g	CH₂COOH	p-(CH₃)₂N-C₆H₄-N=N
1h	CH₃	H

Figure 1. Structures of 5-deazaflavins.

position 8 on the reducing power of the 5-deazaflavosemiquinone radical (HdFl·), which is proposed to be the actual reducing species in the photoreduction system [2], was determined by measuring the half-wave potentials of **1a-g** by differential pulse polarography. It was found that the half-wave potentials become more negative with increasing electron donating character of the substituent [3].

Absorption, fluorescence and phosphorescence spectra as well as fluorescence and phosphorescence quantum yields of **1a-g** appeared to be highly influenced by the substituent at position 8 of the 5-deazaisoalloxazine skeleton [4]. The course of the photoreduction of **1a-g** in the presence of electron donors was studied. It was established that the photoreduction in case of **1a,1d,1e,1f** leads to the formation of the 5,5'-dimer (HdFl)₂ and/or the 6,7-dihydro-5-deazaflavin (6,7-H₂dFlred, **3**) (Scheme 1). Reduction of the C(6)-C(7) bond appeared to be promoted by strong electron donating substituents and electron donors with bulky substituents. The ratios between **2** and **3** were determined previously [4,5].

Scheme 1
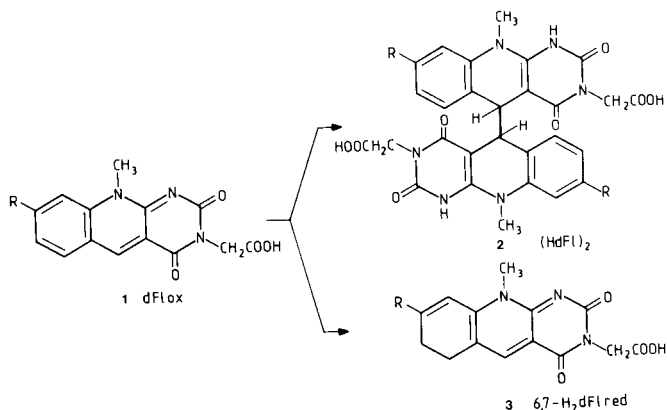
5-Deazaflavins containing a reducible substituent at position 8, *i.e.* **1b,1c,1g**, exhibited reduction of the substituent prior to reduction of the 5-deazaisoalloxazine skeleton [4].

The present report is concerned with a study of the rate of the photoreduction of **1a,1d,1e,1f** by various electron donors and a study of the substituent effect on the photocatalyzing ability of the dFlox's in the photoreduction of methyl viologen (MV^{2+}).

Materials and Methods.

Chemicals were obtained commercially *i.e.* triethanolamine (Baker, The Netherlands), oxalic acid and titrisol borate buffer of *p*H 8 (Merck, West Germany), ethylenediaminetetraacetate and methyl viologen (Janssen Chimica, Belgium). Compounds **1a-g** were prepared as described previously [1]. Analytical photochemical experiments were carried out in a Hellma 193 Suprasil 1 cuvette. Solutions contained in this cuvette were made anaerobic by flushing with oxygen-free argon for at least 30 minutes followed by repeated cycles of evacuation and flushing with oxygen-free argon. The light source was a 20 W blue fluorescent lamp (Sylvania F20T12-B, for the spectral output see [14] or a 250 W slide projector lamp at the low intensity setting. Spectroscopic monitoring was carried out on a Beckman DU-7 or a Varian DMS 90 UV-visible spectrophotometer.

Results and Discussion.

Mechanistic Considerations.

To measure the rate of the photoreduction we determined the change of the absorbance of the first absorption maximum (unless otherwise stated) in the presence of several electron donors as a function of time. From the logarithmic relation observed a pseudo first-order decay of dFlox was established (Table 1). This relation was found to

be rather accurate for more than 95% conversion of dFlox except for the photoreduction of **1d** by both oxalate and ethylenediaminetetraacetate (EDTA) (see below). Our observations are in contradiction with results reported [6] showing that the rate of disappearance of dFlox obeys pseudo first-order kinetics only up to 70% conversion in the presence of oxalate and that the rate thereafter decreases. Therefore, it is not unlikely that the formation of some $6,7\text{-}H_2$dFlred, which exhibits a distinct absorbance at the monitored wavelength [5] has been overlooked [6].

With triethanolamine (TEA) as electron donor the photoreduction of **1e** obeyed pseudo first-order kinetics only up to 65% conversion and that of **1d** went off too fitfully for a reproducible kinetic analysis, despite the distinct course of the reaction. This was probably due to the occurrence of a shift of the protonation equilibrium of TEA ($pK_a = 7.8$, [7] towards the non-oxidizable protonated species during the long irradiation period.
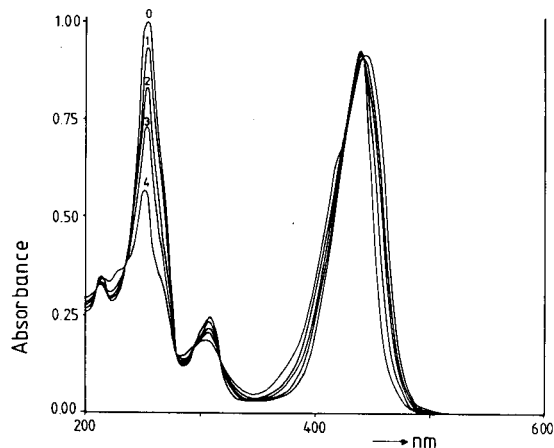

Figure 2a.   Photoreduction of **1d** (2.0×10^{-5} mol dm^{-3}) by oxalate (4×10^{-3} mol dm^{-3}) in borate buffer *p*H 8.0. Curves 0-4 correspond to irradiation times of 0, 89, 184, 278 and 444 hours, respectively.
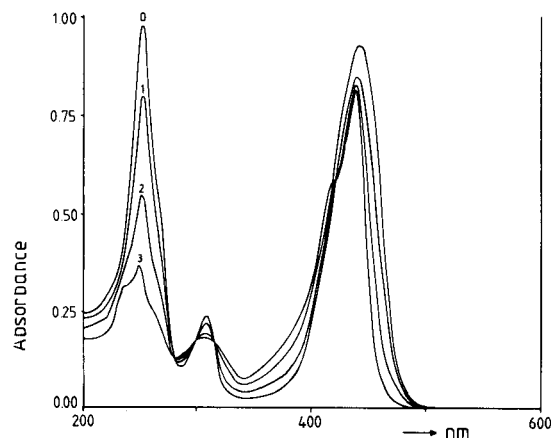
Table 1

Pseudo First-order Rate Constants (s^{-1}) of the
Photoreduction of 5-Deazaflavins with Various Electron Donors [a]
Using a 20 W Blue Fluorescent Lamp as the Light Source

Compound	EDTA	oxalate	TEA
1a	$(2.1 \pm 0.1) \, 10^{-2}$	$(1.6 \pm 0.1) \, 10^{-2}$	$(2.7 \pm 0.1) \, 10^{-3}$
1d [b]	1×10^{-6}	$(2.6 \pm 0.5) \, 10^{-7}$ [b]	
1e	$(9.1 \pm 0.2) \, 10^{-4}$	$(3.2 \pm 0.2) \, 10^{-6}$	$(1.4 \pm 0.1) \, 10^{-4}$
1f	$(1.2 \pm 0.1) \, 10^{-5}$		

[a] [dFlox] 2.0×10^{-5} mol dm^{-3}, [electron donor] 4×10^{-3} mol dm^{-3}, borate buffer *p*H 8.0. [b] The calculated constants for the photoreduction of **1d** to $k_1 = (2.6 \pm 0.1) \, 10^{-7}$ s^{-1}, $k_2 = (7.5 \pm 0.5) \, 10^{-2}$ dm^3 mol^{-1} s^{-1} in the presence of exalate and $k_1 = 1 \times 10^{-6}$ s^{-1} in the presence of EDTA are merely indications for the order of magnitude of the photoreduction rate (see text).

Figure 2b.   Photoreduction of **1d** (2.0×10^{-5} mol dm^{-3}) by EDTA (4×10^{-3} mol dm^{-3}) in borate buffer *p*H 8.0. Curves 0-3 correspond to irradiation times of 0, 30, 53 and 77 hours respectively.

The photoreduction of **1d** by oxalate (Figure 2) exhibited, when spectra were recorded at various irradiation times, the same spectral changes as were observed in the photoreduction of **1d** by TEA. Since the latter reaction leads to the formation of **3d** only [5], it is justified to conclude that the oxalate-mediated photoreduction of **1d** also yields only **3d**. This is supported by the fact that **3d** was not converted to **1d** on admitting air to the reaction mixture, which would have been expected when the reaction mixture contained dimer **2d** [5]. The results shown in Figure 2 can be expressed in quantitative terms by Eq 1 and 2, being valid during the full course of the photoreduction of **1d** by oxalate.

$$[dFlox] = [dFlox]_o - [6,7-H_2dFlred]$$

$$A_{252} = \varepsilon_{252}^{ox} [dFlox] + \varepsilon_{252}^{red} [6,7-H_2dFlred]$$

A_{252} is the absorbance at 252 nm and ε_{252}^{ox} and ε_{252}^{red} are the molar extinction coefficients of **1d** and **3d** respectively. Combination of Eq 1 and 2 leads to Eq 3, in which A_{252}^{∞} is the absorbance at 252 nm at infinite time. When A_{252}

$$A_{252} = (\varepsilon_{252}^{ox} - \varepsilon_{252}^{red}) [dFlox] + A_{252}^{\infty}$$

was plotted against time a flat S-shaped curve was obtained (Figure 3); this S-shaped form is typical for the involvement of autocatalysis in the dihydro formation [8].
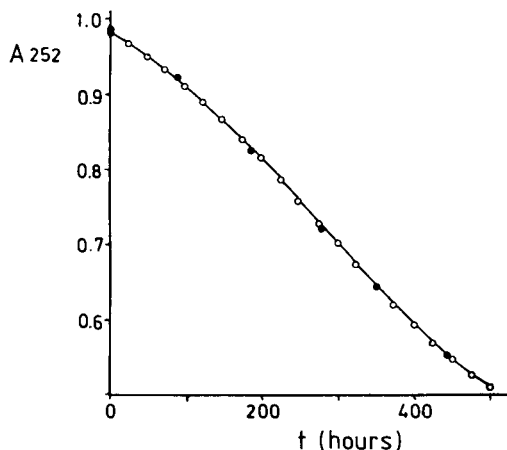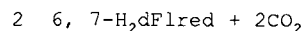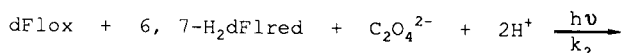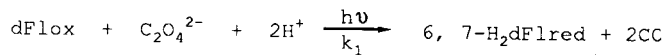

Figure 3. The partly autocatalytic decay of **1d** in the photoreduction by oxalate as found expression in the A_{252}-dependence on time; (O) values calculated according to Eq 3 and 8 with $k_1 = 2.6 \times 10^{-7} s^{-1}$ and $k_2 = 7.5 \times 10^{-2} dm^3 mol^{-1} s^{-1}$; (O) experimentally determined values.

In our case the pseudo first-order decay of **1d** yielding **3d** (Eq 4) is thought to be accompanied by a competing reaction, **3d** acting as a photocatalyst (Eq 5).

$$dFlox + C_2O_4^{2-} + 2H^+ \xrightarrow[k_1]{h\upsilon} 6,7-H_2dFlred + 2CC$$

$$dFlox + 6,7-H_2dFlred + C_2O_4^{2-} + 2H^+ \xrightarrow[k_2]{h\upsilon}$$

$$2 \quad 6,7-H_2dFlred + 2CO_2$$

The rate expression for the decay of **1d** is given by Eq 6, the concentration of oxalate being in excess.

$$\frac{d[dFlox]}{dt} = - (k_1 [dFlox] + k_2 [dFlox] [6,7-H_2dFlred])$$

Substitution of Eq 1 in Eq 6 leads to Eq 7, which on integration gives Eq 8.

$$\frac{d[dFlox]}{dt} = - \{ (k_1 + k_2 [dFlox]_o) [dFlox] - k_2 [dFlox]^2 \}$$

$$[dFlox] = \frac{(k_1 + k_2 [dFlox]_o) [dFlox]_o e^{-(k_1 + k_2 [dFlox]_o)t}}{k_1 + k_2 [dFlox]_o e^{-(k_1 + k_2 [dFlox]_o)t}}$$

By trial and error using Eq 3 and Eq 8 we were able to estimate values for k_1 and k_2 well in line with the calculated and experimentally determined absorbances at 252 nm (Figure 3, Table 1).

Also in the photoreduction of **1d** by EDTA an autocatalytic effect was found: when the time-dependent concentrations of **1d**, calculated from the recorded spectra (Figure 2b), were plotted against time a flat S-shaped curve was obtained again. The decrease of the absorbance at 442 nm, the loss of the isosbestic point in this region, and the observed small formation of **1d** after reoxidation with air are consistent with the formation of a small amount of **2d** next to **3d**. The formation of this mixture complicates the derivation of the rate law, since it is impossible to express the concentrations of **2d** and **3d** into [dFlox], required to derive a rate expression for the decay of **1d** (cf. Eq 7). Therefore, from our observations (Figure 2b) it is only justified to conclude that the photoreduction of **1d** by EDTA is several times faster compared to the photoreduction of **1d** by oxalate.

The data presented in Table 1 offer new elements for elucidating the mechanism of 5-deazaflavin photo-

chemistry both in relation to the substituent present at position 8 and the nature of the electron donor used. Particularly the element of steric interference appear to be important as will be shown below.

In dye photochemistry the common mechanism is the le^--transfer to yield the dye semiquinone anion and the substrate radical cation [9]. When the same electron donor is used, rate-differences in the le^--transfer to various dyes depend on the differences in the reduction potential, *i.e.* the electron affinity in the ground state, and the excitation energy, *i.e.* the energy by which the electron affinity is increased when the acceptor dye molecule is excited [9,10]. In order to investigate the applicability of this le^--transfer mechanism in our photosystems we determined the reduction potentials at pH 8.0 [3] and excitation energies of the various dFlox's (Table 2). The excitation energies were calculated from the phosphorescence spectra [4], since it has been established that the triplet state of dFlox is the reactive form in the photoreduction of dFlox [11,12]. Because the rate of photoreduction is influenced by the quantum yield of triplet formation, which may be approximated by the term $(1-\phi_f)$ [4,6,13] values for $(1-\phi_f)$ are included in Table 2.

Table 2

Reduction Potentials $(E'_{8.0})$, Triplet Energies $(E_{o.o})$ and Approximate Quantum Yields of Triplet Formation $(1-\phi_f)$ 5-Deazaflavins

| Compound	$E'_{8.0}$ in V	$E_{o.o}$ in eV	$1-\phi f$
la	-0.74	2.57	0.93
ld	-1.09	2.21	0.98
le	-1.04	2.40	0.55
lf	-0.95	2.48	1.00

From this Table it is clear that the mechanism of le^--transfer outlined above does not offer a completely satisfying explanation for the differences in photoreduction rates of dFlox's. This is exemplified by the photoreduction of **lf** by EDTA being about a hundred times slower than the photoreduction of **le** by EDTA, despite the fact that **lf** exhibits more favourable values for $E'_{8.0}$, $E_{o.o}$ and $(1-\phi_f)$ and a larger absorption of incident light with respect to the spectral output of the blue fluorescent lamp [14]. However, this feature can be explained by the initial formation of a sandwich-like triplet excimer T_E [6,15], which will be hindered by large substituents like the *p*-aminophenyl and dimethylamino group and, as a result, cause a decrease of the photoreduction rate of **ld** and **lf**.

An analogous explanation may be valid for the relatively low reactivity of **le** towards oxalate when the steric hindrance is caused by hydrogen bridged complex formation between the amino group and oxalate.

The composition of the reaction product of the various

photoreductions [4,5] points to the involvement of a steric as well as an electronic contribution in the reaction mechanism as outlined in Scheme 2. The present data are

Scheme 2
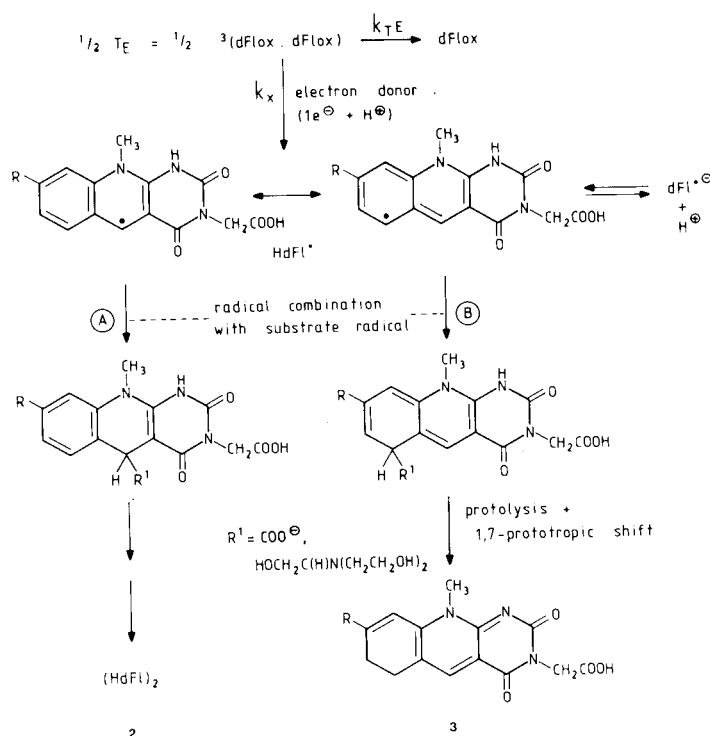
insufficient to specify the initial interaction between T_E and the electron donor. The le^--transfer from the electron donor to T_E, which in case of oxalate is accompanied by decarboxylation, yields the 5-deazaflavosemiquinone ($HdFl^\cdot$), which is stabilized by electron delocalization. Subsequent radical combination of $HdFl^\cdot$ and the radical of the electron donor may occur following two different pathways. Route A yields a C(5)-adduct, which is well established in 5-deazaflavin photochemistry and is thought to be the precursor in dimer formation [12,16]. Route B leads to the formation of the C(6)-adduct, which on protolysis and a 1,7-prototropic shift yields the 6,7-dihydro-5-deazaflavin (6,7-H_2dFlred).

This mechanism is consistent with the product composition resulting from the photoreduction of **1** *i.e.* (C(6)-adduct along with 6,7-H_2dFlred formation is favoured with a bulky electron donor like TEA encountering less steric hindrance with respect to C(5)-adduct formation. Photoreduction of dFlox by EDTA leads to the formation of $HdFl^\cdot$, which yields dFlox and H_2dFlred by dismutation, the latter is converted to dimer **2** as the ultimate product [12,17]. Hence, EDTA does not exhibit a steric effect, which otherwise would favor the formation of 6,7-H_2dFlred.

The preference of photoreduction of the C(6)-C(7) bond of dFlox's with a strong electron donating substituent at position 8 is explained by the following reasoning. It has been shown [18,19] that in roseoflavin (compound **1d**, in which C(5)H is replaced by N(5)) an intramolecular charge transfer from the 8-dimethylamino group to the pteridine moiety occurs, which is even more outspoken in the triplet state. It can be expected that in the triplet state of **1d,1e** a similar effect is operative, which enhances the electrophilicity of the benzenoid ring and hence favours its reduction following route B. The present data are not sufficient to give any insight into the transformation of the $2e^-$-reduced species into the dimer.

Photochemical Reduction of Methyl Viologen.

Common substrates for the dFlox catalyzed photoreactions are EDTA [2] and methyl viologen (MV^{2+}) in combination with a large variety of electron donors [14,20]. In order to investigate the efficiency of the various dFlox's as photocatalyst we illuminated a mixture of dFlox **1a,1d-1f** and EDTA in the presence of a 20 fold excess (relative to [dFlox]) of MV^{2+} and determined the rate of reduced methyl viologen ($MV^{\cdot+}$) formation by measuring the increase of the absorption at 600 nm ($\epsilon_{MV^{\cdot+}} = 8600$ mol^{-1} [14]) as a function of time. As a representative of the dFlox's with a reducible substituent at position 8 we also included **1g** ($R^2 = p$-$(CH_3)_2N$-C_6H_4-$N=N$, Figure 1) in the experiments requiring the 250 W slide projector lamp as light source. The rate of $MV^{\cdot+}$ formation appeared to be pseudo first-order in MV^{2+}. Rate constants of MV^{2+} reduction and those of dFlox reduction in the absence of MV^{2+}

Table 3

Pseudo First-order Rate Constants (s^{-1}) of the Photoreduction of dFlox by EDTA and of the Photoreduction of MV^{2+} by EDTA, Catalyzed by dFlox, using a 250 W Slide Projector Lamp as the Light Source

Compound	dFlox, EDTA [a]	MV^{2+}, dFlox, EDTA [b]
1a	$(6.5 \pm 0.1) 10^{-2}$	$(7.1 \pm 0.2) 10^{-3}$
1d	3×10^{-6} [c]	$(3.0 \pm 0.2) 10^{-5}$
1e	$(2.3 \pm 0.1) 10^{-3}$	$(3.8 \pm 0.2) 10^{-4}$
1f	$(1.7 \pm 0.1) 10^{-5}$	$(2.5 \pm 0.4) 10^{-6}$
1g	–	$(3.8 \pm 0.2) 10^{-4}$

[a] [dFlox] 2.0×10^{-5} mol dm^{-3}; [EDTA] 4×10^{-3} mol dm^{-3}; borate buffer, pH 8.0. [b] [MV^{2+}] 1.00×10^{-4} mol dm^{-3}; [dFlox] 5.0×10^{-6} mol dm^{-3}; [EDTA] 1×10^{-3} mol dm^{-3}; borate buffer, pH 8.0. [c] For **1d** a value of 3×10^{-6} s^{-1} was calculated and is merely indication for the order of magnitude of the photoreduction rate for **1d** (see text).

using EDTA are summarized in Table 3. With all dFlox's a quantitative formation of $MV^{\cdot+}$ was observed (2000% yield based on dFlox), which is consistent with the mechanism depicted in Scheme 3.

Scheme 3
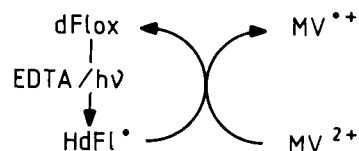
When **1g** was used as a photocatalyst we observed first a small decrease of the absorption at 600 nm accompanied by the disappearance of the purple colour of **1g**. After the purple colour had vanished completely, the absorption at 600 nm started to increase reaching a value of 0.86, which agrees with 100% formation of $MV^{\cdot+}$. Since the rate constant of MV^{2+} photoreduction by **1g** is the same as that found for **1e** and photoreduction of **1g** by EDTA leads to the formation of **1e** [4], these results indicate that prior to the reduction of MV^{2+} a quantative reduction of the azofunction of **1g** occurs yielding the photocatalyst **1e**. The easy conversion of **1g** into **1e** by reduction has previously been demonstrated [3].

The most striking result mentioned in Table 3 is the fact that the photoreduction of **1f** by EDTA is faster than that of **1d**, while the reversed reactivity is observed in the 5-deazaflavin mediated photoreduction of MV^{2+}. It can be suggested that the dismutation process of $HdFl^{\cdot}$ in the MV^{2+} photoreduction does not occur. It accounts for the higher rate of photoreduction of MV^{2+} by **1d** relative to **1f**, assuming that the dismutation process in case of **1d**, which leads to the formation of 6,7-H_2dFlred, is slow compared to the formation of 1,5-H_2dFlred in case of **1f**. Alternatively, the electron density in the 8-dimethylamino-5-deazaflavosemiquinone radical may be more centered in the benzenoid ring (especially at C(6)), making a less sterically hindered encounter between MV^{2+} and $HdFl^{\cdot}$ possible and hence a decreased activation energy of the $1e^-$-transfer process as a result. Still 20 hours of irradiation were required to reduce 90% of MV^{2+}.

REFERENCES AND NOTES

‡ Abbreviations: dFlox, oxidized 5-deazaflavin; $HdFl^{\cdot}$, 5-deazaflavosemiquinone radical; $(HdFl)_2$, 5-deazaflavin radical dimer; H_2dFlred, reduced 5-deazaflavin; MV^{2+}, methyl viologen; $MV^{\cdot+}$, reduced methyl viologen; EDTA, ethylenediaminetetraacetate; TEA, triethanolamine.

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