Photochemistry of (Iso)Alloxazines, V
Mechanism of the Photoreaction of Flavin with 1,4-Cyclohexadiene
and Analogous Compounds

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Non-Radical Photoreduction, Covalent Adducts, Homolytic/Heterolytic Decomposition

On illumination flavin reacts from its triplet state with dihydroaromatic systems at room temperature yielding 1,5-dihydroflavin. Substrates which are substituted with methyl groups to hinder aromatisation (3,3-dimethyl-1-phenyl-1,4-cyclohexadiene, ergosterol, N-methylacridan, 1,3,10-trimethyl-1,5-dihydro-5-deazaisoalloxazine) yield at — 40 °C 4a-substituted 4a,5-dihydroflavins (adducts), which on warming split homolytically, yielding a 1:1:1 mixture of 1,5-dihydroflavin/starting flavin/dimerized substrate after disproportionation and dimerisation, resp.

In the case of unblocked substrates these adducts are not UV-detectable even at — 80 °C but split heterolytically, yielding 1,5-dihydroflavin and oxidized substrate in a 1:1-ratio.

Isoalloxazine derivatives, e.g. lumiflavin, riboflavin, FMN or FAD, as well as flavoenzymes can be reduced chemically or photochemically by a number of compounds (“substrates”), yielding their 1,5-dihydro-form. Although a large number of investigations have been published, the mechanism of these reductions is not known: all three possibilities are still discussed: a two step radical-mechanism, hydride, or carbanion transfer. In this paper some experiments, mainly UV-spectroscopic observations of covalent intermediates are reported. The results favor a carbanion or group transfer mechanism.

Materials and Methods

3-Methylumiflavin, 3,7,8,10-tetramethylalloxazine (I), Fl(ox) reacts with 1,4-cyclohexadiene from its first excited triplet state yielding unsubstituted 3-methyl-1,5-dihydroflavin (2, 1,5-H2Flred; λmax 400 sh, 340 sh, 295 nm). The reaction with cyclopentadiene under identical conditions yield in contrast the 4a-(1-cyclopentadienyl)-3-methyl-4a,5-dihydroflavin (3a, 4a-RFlredH; λmaxfH=0] 362 nm, log ε 3.78).

These different results might be explained by the hypothesis that in the case of 1,4-cyclopentadiene as a substrate a 4a-adduct is also formed. This adduct, however, undergoes rapid and irreversible decomposition by breaking of the flavin(4a)-cyclopentadiene bond and a C–H bond, yielding benzene and 1,5-dihydroflavin, cf. Scheme 3. Substitution of one methylene group in the substrate by a dimethylmethylene group [C(CH3)2] should render this fragmentation more difficult. Therefore 3,3-dimethyl-1-phenyl-1,4-cyclohexadiene was subjected to the photoreaction of 1 in acetonitrile in the absence of oxygen.

Source was a slightly modified slide projector with a 250 W/24 V tungsten halogen lamp and a metal interference filter (K2, Balzers) transparent from 420–480 nm. The UV-spectra were recorded with a Cary 118 C spectrophotometer.

Results and Discussion

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At +25 °C the characteristic UV spectrum of the unsubstituted 1,5-dihydro derivative (2) was observed, cf. curve 5 in Fig. 1. However, when the irradiation was performed and the UV spectrum recorded at —40 °C, the spectrum 2 in Fig. 1 resulted (λ_max 360 nm, log ε 3.78), which is characteristic of a 4a-adduct (5-adducts in most cases absorb between 315 and 330 nm, 4a-adducts between 340 and 360 nm)².⁹

On warming the solution to room temperature exactly 50% of 1 was reformed, cf. curve 3; this decomposition started at about —20 °C. The other 50% of 1 was formed upon admission of oxygen, see curve 4.

From these data the following reaction scheme can be postulated: At —40 °C 1 reacts upon light excitation with 3,3-dimethyl-1-phenyl-1,4-cyclohexadiene yielding the adduct 3b.¹⁰ As the temperature increase, the bond between the flavin and the substituent is cleaved homolytically. The resulting flavin radical disproportionates, yielding 50% of Fl_ox 1 and 50% of 1,5-H_2Fl_red 2, the latter being oxidized to 1 upon admission of oxygen. The products of the cyclohexadienyl substituent were not further investigated. From the literature it is known¹¹,¹² that cyclohexadienyl radicals dispropor-

Analogous adducts were observed spectrophotometrically at low temperatures with ergosterol, N-methylacridan and 1,3,10-trimethyl-1,5-dihydro-5-deazaalloxazine, cf. Scheme 2. At room temperature these adducts decompose homolytically, as can be seen from the formation of exactly 50% of 1. On the other hand in all these cases the dimerisation products of the 4a-substituent (bis-ergostatrienol, N,N'-dimethyl-9,9'-bisacridan and

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Fig. 1. Spectral course of the photoreaction of 3-methylumiflavin (6 × 10⁻⁵ M) and 3,3-dimethyl-1-phenyl-1,4-cyclohexadiene (6 × 10⁻⁴ M) in acetonitrile under anaerobic conditions.

Curve 1: Spectrum of starting material; 2: Photoprodut at —40 °C (= 3b); 3: Products after warming to +25 °C (= 50% 3-methylumiflavin + 50% 3-methyl-1,5-dihydrolumiflavin); 4: Curve 2, 3 or 5 after admission of oxygen (= 100% 3-methylumiflavin); 5: Spectrum of the photoprodut at +25 °C (= 100% 3-methyl-1,5-dihydrolumiflavin).
in 1962 to yield 1,5-dihydroflavin and bisergosta-trienol \(^{13}\). These authors proposed an attack of the flavin triplet on the substrate, yielding flavin radical and ergosterol radical directly. The spectral observation (\(\lambda_{\text{max}}\) 360 nm) in the case of ergosterol shows that upon attack of the flavin triplet on position 7 of the steroid the adduct 3e is formed, which decomposes on warming, consistent with scheme 1.

With unsubstituted 1,4-cyclohexadiene even at \(-80 \, ^{\circ}\text{C}\) (butyronitrile or 2-methyltetrahydrofuran as solvent) no adduct could be observed spectroscopically; the only product was the dihydroflavin 2. Similarly, with acridan and 3,10-dimethyl-1,5-dihydro-5-deazaisoalloxazine only 2 and exactly equimolar amounts of the oxidized substrates acridine and 3,10-dimethyl-5-deazaisoalloxazine, resp., were obtained. No dimers of these substrates could be detected by tlc.

Therefore it is concluded that in these cases 4a-adducts are formed initially, and these adducts decompose heterolytically, yielding the oxidized (aromatic) substrate and 1,5-dihydroflavin 2, cf. Scheme 3.

A mechanism involving radicals cannot explain the exact equimolar formation of oxidized substrate and reduced flavin 2, since acridanyl-\(^{14}\) and 5-deazaisoalloxazinyl-radicals\(^{15}\) should dimerize at least to some extent.

The mechanism of the photoreaction of flavin with 1,4-cyclohexadiene and analogous compounds can be interpreted as an electrophilic substitution of the electron-deficient flavin triplet on the substrate. In a non-radical reaction a \(\sigma\)-bond between a methylene group and the 4a-position of the flavin is formed.

The thermal decomposition of this adduct occurs heterolytically even at \(T < -80 \, ^{\circ}\text{C}\) if the substituent can aromatize. In this reaction 100\% 1,5-dihydroflavin 2 and equimolar amounts of oxidized substrate are formed. If an aromatization is not possible because the methylene or NH-group is blocked by methyl groups, the thermal decomposition of the adduct begins at about \(-20 \, ^{\circ}\text{C}\) and is strictly homolytical. The flavin radicals disproportionate, yielding 50\% 1,5-dihydroflavin, whereas the substrate radicals dimerize.

Non-photochemical reductions of the flavin system are presently under investigation to investigate the possibility that the group-transfer mechanism is of general validity.

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The attack of the flavin triplet may also occur at the allylic positions; the $^1$H NMR spectra are under investigation.


G. Vermeersch, N. Febvay-Gabot, S. Caplain, and A. Lablache-Combie, Tetrahedron 31, 867 [1975], and literature quoted therein.