

RESEARCH NOTE

STUDIES ON BILE PIGMENTS—3. OXIDATIVE PHOTODIMERIZATION OF A PHYTOCHROME P_r MODEL PIGMENT AND ITS THERMAL REVERSION

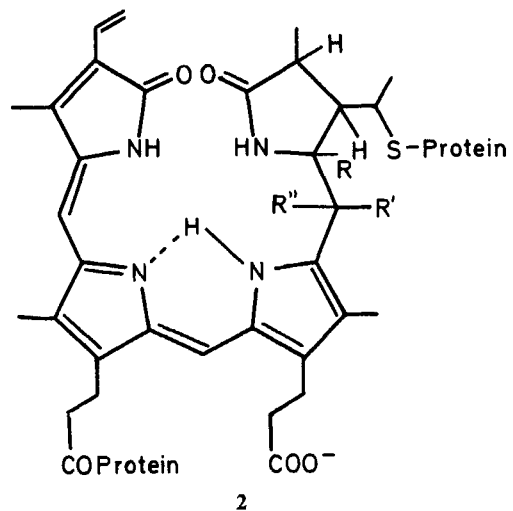
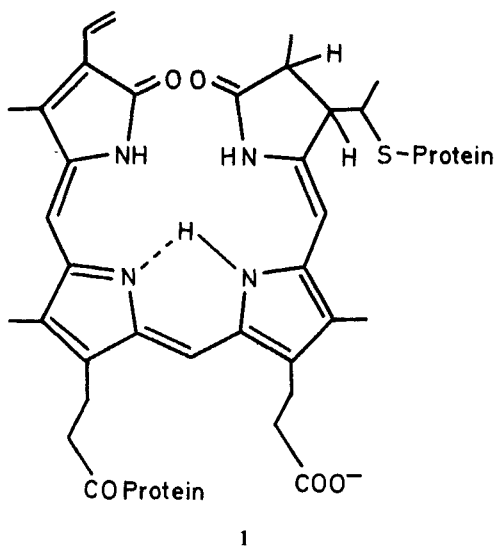
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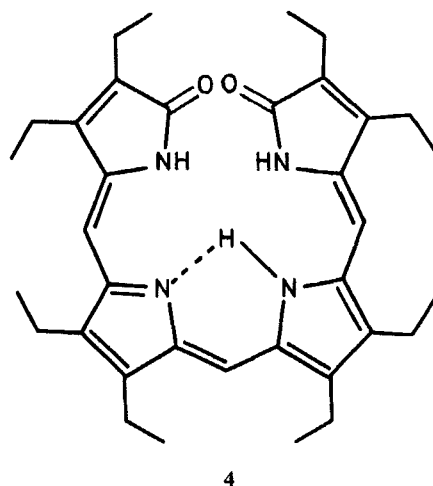
INTRODUCTION

Phytochrome, the photomorphogenetic reaction center pigment of green plants and some algae, has been proposed recently to contain two chemically different chromophores in its P_r and P_{fr} forms, respectively (Grombein *et al.*, 1975). P_r contains an A-dihydrobilin chromophore (1) closely related to that of phycocyanin. For P_{fr} , a violin (2) (or possibly purpurin) (Scheer *et al.*, 1976) type chromophore has been proposed, in which the conjugated system is shortened by one double bond. Conversion of P_r into P_{fr} is strictly photochemical, while the reversion of the less stable P_{fr} to P_r occurs thermally, too (cf. Briggs and Rice, 1972). We wish to report the oxidative photodimerization of 2,3-dihydrooctaethyl-1,19(21,24 H)-bilindione (3), a synthetic model for P_r (Scheer, 1976), into a dimeric violin, and its thermal cleavage back to the starting biline 3.

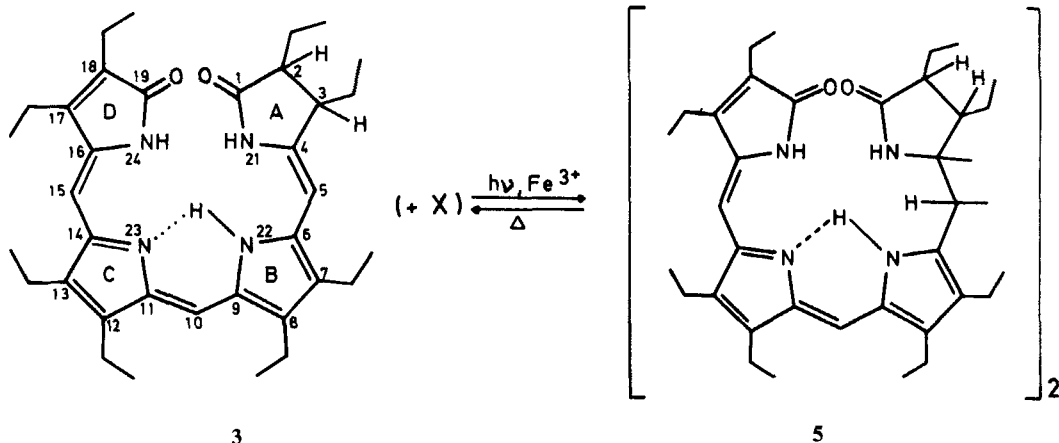


MATERIALS AND METHODS

The bilin 3 has been prepared by the method of Cavaleiro and Smith (1973) from octaethylporphyrin. $K_3[Fe(CN)_6]$ was reagent grade. All solvents were either purified according to standard procedures or were reagent grade. Preparative photochemical reactions were carried out under nitrogen in pyrex cylinders with the light of a 1000 W tungsten halogen source (13 W/m^2 light between



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550 and 620 nm). UV-visible absorption spectra were recorded with a DMR 22 (Zeiss, Germany), $^1\text{H-NMR}$ spectra with a HFX 90 (Bruker, Germany), mass spectra with a JDM-100 (Jeol, Japan), and IR spectra with an IR 5 instrument (Beckmann, Germany). Melting points were determined with a Kofler bank (Reichelt, Austria) and were uncorrected.

RESULTS AND DISCUSSION

Irradiation of the bilin **3** in methanol ($25\ \mu\text{M}$) under nitrogen in the presence of $\text{K}_3[\text{Fe}(\text{CN})_6]$ ($75\ \mu\text{M}$) for 15 min leads to a change in colour from blue to violet, corresponding to a shift of the long wavelength absorption maximum from 594 to 576 nm (Fig. 1). Workup and chromatography on silica H (Merck) yields, in the sequence of decreasing R_F values, the starting material **3**, the fully unsaturated octaethyl-1,19(21,24H)-bilindione **4**, and two products with a violin type UV-visible spectrum (Köst *et al.*, 1976), with a 4:1 excess of the slower one. Both have similar UV-visible spectra for their cations, free bases, zinc complexes and anions, as well as similar mass spectra, and they are therefore likely to be stereoisomers.

The dimeric structure **5** of the latter compound (40% yield, m.p. = $220\text{--}224^\circ$, prisms from methanol) is evidenced by the following data: (a) The mass spectrum* shows a peak of highest mass at 1112 m/e†. Main fragments in the high mass range are at 970

and 968 m/e. The 100% peak is at 556 m/e, and the fragmentation pattern below this peak is similar to that of the starting bilin **3**.

(b) The $^1\text{H-NMR}$ shows six signals in the methine proton range, four singlets (1 H each, $\delta = 6.76, 6.70, 6.10, 5.94$ ppm), and one AB double-doublet (2 H, $\delta = 4.63, 3.99$ ppm, $J = 13$ Hz); a multiplet at 2.3–2.7 ppm corresponding to ca. 28 H (Ring B, C, D— CH_2 , Ring A—CH), a multiplet at 1.6–2.0 ppm corresponding to 4 H (characteristic for the CH_2 protons at the saturated pyrrolidinone ring A), and a multiplet at 1.4–0.8 ppm corresponding to ca. 48 H (CH_3).

(c) Pronounced shoulders or splittings in the UV-visible spectra of the free base and the cation, but especially that of the zinc complex‡ are indicative of the presence of two rather than one chromophores.

(d) The dimer **5** can be partially cleaved back to the starting monomer **3** under strictly oxygen free conditions. When **5** is heated in a sealed tube under nitrogen for short periods of time above 150° , two main products were isolated from the reaction mixture by tlc (silica buffered with MgHCO_3 , eluted with methylene chloride containing 4% acetone at 4° in

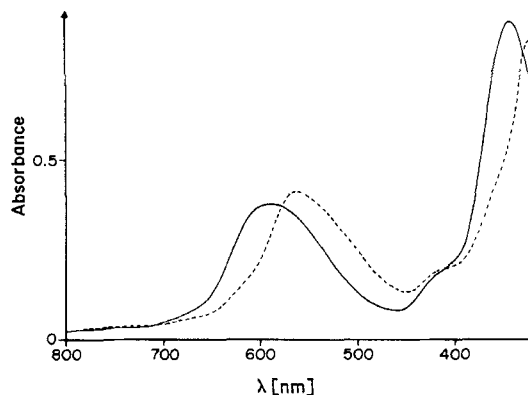


Figure 1. UV-visible spectra of a solution of the 2,3-dihydrobilin **3** ($25\ \mu\text{M}$) and $\text{K}_3[\text{Fe}(\text{CN})_6]$ ($75\ \mu\text{M}$) in methanol before irradiation (—) and after irradiation (-----) with white light (15 min, $\sim 1\ \text{kW/m}^2$ in the range between 550–620 nm). The shoulder around 430 nm arises from ferric cyanide.

* The mass spectra were confirmed recently by M. H. Studier in a modified time-of-flight spectrometer (Bendix).

† Dimerization of bilins in the mass spectrometer has been reported recently by Beuhler *et al.* (1976). Although we did observe with our instrument (75 eV ionization voltage, direct inlet from a glass capillary) peaks in the 700–900 m/e range for the monomer **3**, these did occur only at high sample temperatures, and their intensity was low and unrelated to that of the molecular ion of **3** at 556 m/e.

‡ The zinc complex of **5** is unstable under aerobic conditions, although less pronounced than that of the monomer **3** (Scheer *et al.*, 1976).

§ As both products are unstable under these conditions, extended reaction times lead to various by-products, among them **4**.

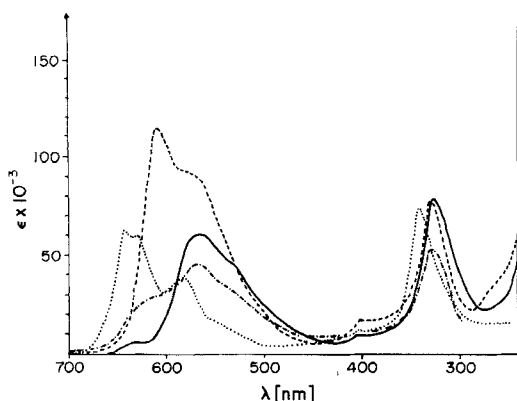


Figure 2. UV-visible spectra (λ , $\epsilon \times 10^{-3}$) of the dimer **5** in methanol (—), of its cation in 2M methanolic HCl (---), its zinc complex in a 0.01 M solution of Zn(OAc)₂ in methanol (.....), and of a solution of **5** in 5M methanolic KOH (-·-·-·-).

the dark) in about equal amounts, the monomer **3**, and a slightly less polar violin. **3** has been identified (by comparison with an authentic sample) by mass spectroscopy, UV-visible spectroscopy of the cation and free base, and by the characteristic oxidation reaction to a purpurin in the presence of zinc ions and strong base (Scheer *et al.*, 1976). The product ratio suggests the dimer to contain two bilins of different oxidation state, one formally in that of the bilin **3**, or alternatively, a disproportionation of **5**. However, the violin obtained by heating of the dimer **5** is unstable and yields, upon standing, additional **3** among other products. Thus, it rather seems to be a precursor of **3**, which would then indicate *both* "halves" of the dimer to be formally in the same oxidation state as is **3** (formula **5**). Judging from its reactivity, one might envisage **5** as being a Diels-Alder adduct. This would account (cf. Kwart and King, 1968) for the facile thermal reversion, and for the very similar melting point as compared to the monomer **3** (m.p. = 219–221°, Cavaleiro and Smith, 1973).

To our knowledge, this is the first report of an isolated and spectroscopically characterized dimeric bile pigment. Dimerization of bile pigments in mass spectrometer has been reported recently (Beuhler *et al.*, 1976) and discussed in terms of a gas phase reaction between one cation radical and one neutral molecule of **3**. Formation of a cation radical in the photodimerization reported here could be achieved by electron transfer from **3** in an excited state to ferricyanide. This sequence is supported by findings that (a) the bilin **3** is stable photochemically in the absence of ferricyanide, and (b) that it is stable in the dark in the presence of ferricyanide under the reaction conditions. However, the dimer **5** can be formed in the

dark in the presence of a large excess of ferricyanide or other oxidants. Thus, it has been identified as by-product in the preparation of **3** with thallium-trifluoroacetate.*

The A-dihydrobilin **3** and the dimer **5** exhibit the spectral characteristics of the chromophores of *P_r* and *P_{fr}*, respectively, if the influence of the protein is relieved by denaturation (Grombein *et al.*, 1975). Thus, the photodimerization of **3**, as well as the at least partial thermal reversion, might serve as a model reaction for the reversible transformations of the phytochrome chromophore. Correll *et al.* (1968) have suggested the interaction of two chromophors in the "large" phytochrome of about 120,000 molecular weight, but at present the photoreversible "small" phytochrome seems to contain only one chromophore per 60,000 molecular weight. However, this question is still not completely resolved (for a discussion, see Briggs and Rice, 1972). On the other hand, the reversible formation of **5** might serve as a different model, too. Attempts do induce a similar dimerization with the fully unsaturated bilin **4** have failed. Thus, the reaction seems to be a direct result of ring A-hydrogenation. One can then imagine that instead of reacting with a second bilin, the *P_r* chromophore adds reversibly to part of the protein, for example. To study this possibility, details of the reaction mechanism and of the structure of the reaction products are currently worked out.

CONCLUSION

The A-dihydrobilin **3**, a model for the phytochrome *P_r* chromophore, undergoes photochemical dimerization with visible light in the presence of K₃[Fe(CN)₆]. The dimer **5** has been characterized by UV-visible, ¹H-NMR, mass and IR spectroscopy. The chromophore of **5** is shortened by one double bond (Δ -4), as is that of *P_{fr}*. Partial reversion to the monomer is possible by heating **5** for short periods in the absence of oxygen.

* *Note in proof*: The latter observation has been confirmed recently by K. M. Smith (personal communication, 1976).

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