

STUDIES ON PLANT BILE PIGMENTS—6. PHOTOCHEMICALLY ASSISTED REACTION OF A-DIHYDROBILINDIONE WITH NUCLEOPHILES AS A MODEL FOR PHYTOCHROME INTERCONVERSION

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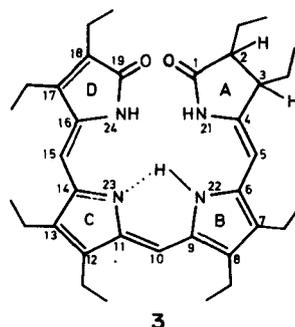
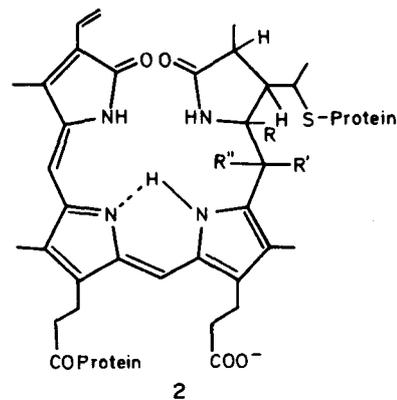
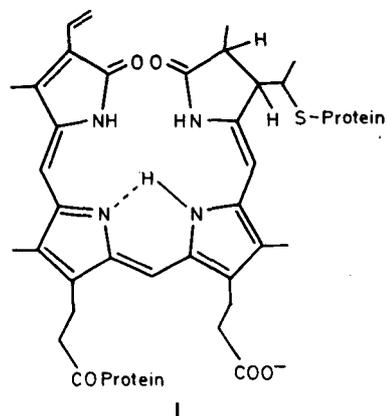
(Received 23 February 1979; accepted 1 May 1979)

Abstract—From the A-dihydrobilindione **3**, the pyridinium derivatives **6a,b** and analogues thereof have been obtained by a formal nucleophilic substitution. The reaction is rationalized as a photochemically assisted oxidation of **3** with a subsequent regioselective addition of pyridine at C-5. By thermolysis, **6a,b** yields back the parent bilin **3**, together with an oxidation product. The significance of the reaction with respect to the phytochrome interconversion is discussed.

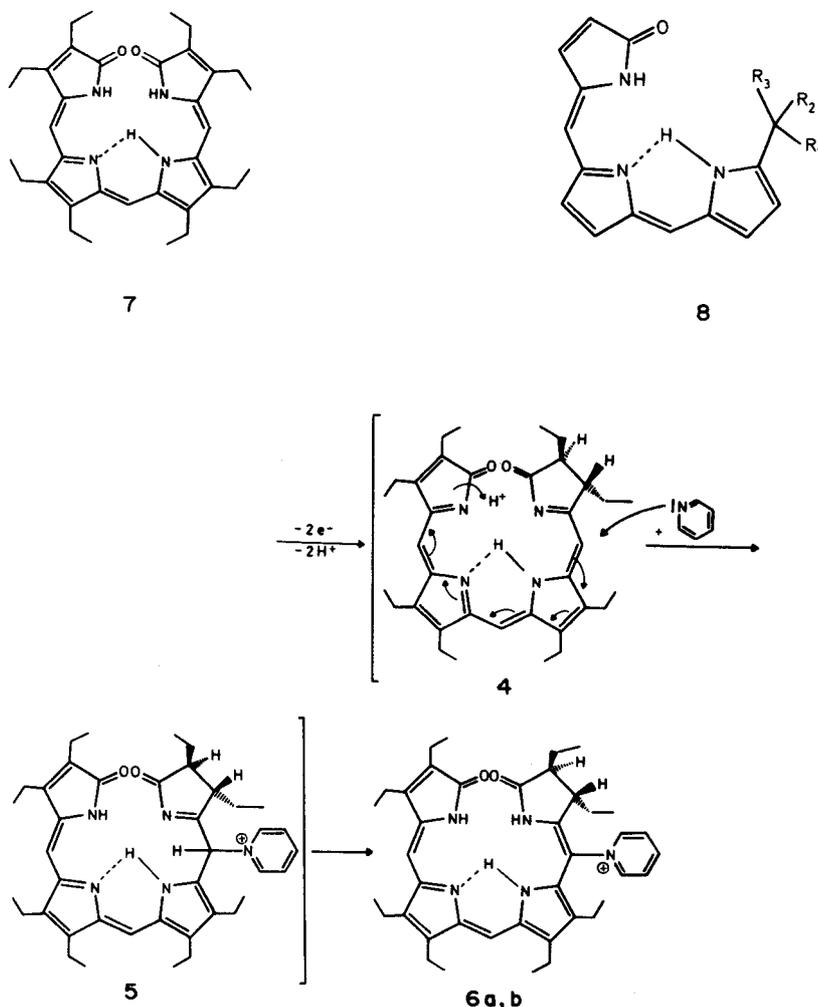
INTRODUCTION

Phytochrome, the photomorphogenetic reaction center pigment of higher green plants and some algae, contains a bile pigment chromophore covalently linked to the protein (Siegelman *et al.*, 1966; Rüdiger, 1975). The photochromic conversion between the two long-lived forms P_r and P_{fr} is the primary event of the photomorphogenetic reaction chain (Pratt, 1978; Briggs and Rice, 1972). P_r contains the A-dihydrobilindione chromophore **1** (Klein and Rüdiger, 1978; Schoch *et al.*, 1976; Klein *et al.*, 1977; Grombein *et al.*, 1975; Scheer, 1976). For the chromophore of P_{fr} , structure **2** with the β -pyrrolic side chains unaltered (Klein *et al.*, 1977) and a chromophore no longer containing the Δ 4,5-double bond (if judged from UV-vis spectra, Grombein *et al.*, 1975) has been proposed. This proposal implies a change in the molecular structure during the $P_r \rightleftharpoons P_{fr}$ interconversion and excludes conformational changes and/or protonation-deprotonation events as the *sole* structural difference between the two forms (see Scheer, 1978, for a discussion).

In exploiting the synthetic bilin **3** as a model for **1**, an increased reactivity at the Δ 4,5-double bond has been characterized for A-dihydrobilindiones, as compared to their fully unsaturated analogues, e.g. **7** (Scheer and Krauss, 1977; Scheer *et al.*, 1977). Specifically, the reversible photodimerization of **3** has been proposed as a model type reaction for the phytochrome interconversions (Scheer and Kraus, 1977). Since present evidence supports but one chromophore in the monomeric phytochrome (Pratt 1978; Briggs and Rice, 1972; and citations therein), the possibility of using partners other than a second bilin for addition reactions has been studied. Here, we report the



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Reaction scheme

reversible, photochemically assisted addition of nucleophiles of the pyridine type.

MATERIALS AND METHODS

The A-dihydrobilindione **3** was prepared by the method of Cavaleiro and Smith (1973) from octaethylporphyrin via octaethylchlorin (Whitlock *et al.*, 1969). I_2 and pyridine were reagent grade, the latter was dried over basic alumina (Woelm-ICN, Eschwege). All solvents were distilled prior to use or were reagent grade. Preparative photochemical reactions were carried out under nitrogen in pyrex cylinders at starting concentrations $c \approx 0.1$ mM of compound **3** with the light of a 1000 W tungsten halogen source (13 W/m^2 between 550 and 620 nm). UV-vis absorption spectra were recorded on a DMR 22 spectrophotometer (Zeiss, Oberkochen), and are reported in the format $\lambda_{\text{max}}[\text{nm}] (\epsilon \cdot 10^{-3})$. Mass spectra were obtained in the EI mode with a JMS-D 100 spectrometer (Jeol, Japan), and in the FD mode with a model 731 instrument (Varian-MAT, Bremen). ^1HMR spectra were recorded in the FT mode on a HFX 90 instrument (Bruker, Karlsruhe) with

$(\text{CH}_3)_4\text{Si}$ as internal standard. Chemical shifts are reported in $\delta[\text{ppm}]$. Column chromatography of the reaction mixtures was carried out by an improved HPLC method (Schoch *et al.*, 1978) on silica with a slightly alkaline ternary eluent system. For TLC, silica H (Merck, Darmstadt) was used.

RESULTS AND DISCUSSION

Irradiation of **3** in benzene/pyridine (4:1) in the presence of I_2 or other one-electron oxidants leads to a rapid colour change from blue to violet, similar to the change occurring during dimerization in the absence of pyridine (Scheer and Krauss, 1977) (Fig. 1). Chromatography of the product mixture yields only traces of the dimers, however. Two main products can be isolated, which migrate on silica only at high methanol concentrations, and which are irreversibly adsorbed upon prolonged exposure to silica. TLC: $R_f = 0.52$ and 0.62 , respectively, in dichloromethane/methanol = 9:1, containing 1% aqueous ammonia;

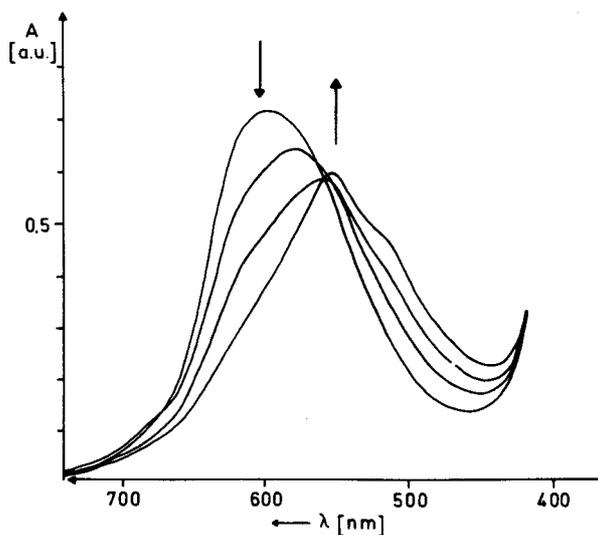


Figure 1. UV-vis spectra of the photochemical reaction of **3** ($40 \mu\text{M}$) in benzene containing pyridine (2.47 M) and I_2 ($19.0 \mu\text{M}$). Each trace corresponds to 1 min irradiation time, for the light conditions, see text. The arrows denote the change of absorption during the reaction.

HPLC: $V_R = 106$ and 182 m' , respectively, on $4 \times 300 \text{ mm}$ silica with dichloromethane/methanol = 1:1 containing 0.04% aqueous ammonia as eluent. The two products are interconvertible, e.g. by acid, and thus assigned as isomers. From the slower migrating zone, the isomer **6a** of 2,3-dihydro-5(1-pyridinium)-octaäthyl-1,19(21,24H)-bilindione has been isolated, the faster migration zone contains **6b**.

UV-vis (see also Fig. 2): *Cation* (corresponding to the chromophore free base): 545 (10.27), 326 (19.9); *dication* (corresponding to the chromophore monocation): 603 (25.5), 327 (21.4); *zwitterion* (corresponding

to the chromophore anion): 585 (9.7); *zinc complex*: 630 (17.5), 585 (9.7), 330 (25.5).

¹HMR: C^2HCl_3 : **6a**: 6.72 (H-10, s), 5.92 (H-15, s), 8.21 (Pyr-H-B,B', 'd'), 8.49 (Pyr-H-A, 't'), 9.04 (Pyr-H-C,C', 'd'). **6b**: 6.76 (H-10, s) 5.96 (H-15, s) 8.29 (Pyr-H-B,B', 'd'). 8.70 (Pyr-H-A, 't') 9.38 (Pyr-H-C, C', 'd'). $(\text{C}^2\text{H}_3)_2\text{CO}$: **6a**: 7.07 (H-10, s), 6.21 (H-15, s), 8.42 (Pyr-H-B,B', 'd'), 8.92 (Pyr-H-A, 't'), 9.59 (Pyr-H-C,C', 'd'). **6b**: 7.04 (H-10, s), 6.18 (H-15, s), 8.50 (Pyr-H-B,B', 'd'), 8.94 (Pyr-H-A, 't'), 9.57 (Pyr-H-C,C', 'd'). $\text{CF}_3\text{COO}^2\text{H}$: **6a**: 7.71 (H-10, s), 6.60 (H-15, s), 8.35 (Pyr-H-B,B', 'd'), 8.70 (Pyr-H-A, 't'), 9.11 (Pyr-H-C,C',

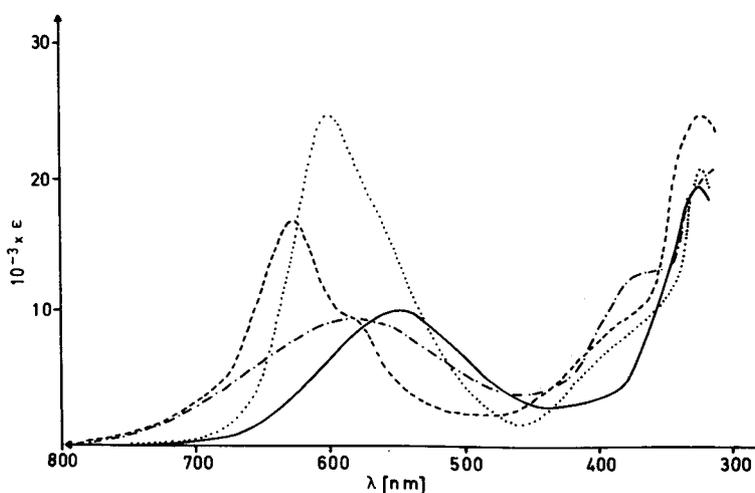


Figure 2. UV-vis spectra of the pyridinium salt **6a** in methanol (—), its protonated form obtained after addition of 0.25% of 35% HCl (·····), its deprotonated form obtained after addition of 0.25% methanolic KOH (— · — · —), and its Zn-complex obtained after addition of 0.25% saturated methanolic Zn-acetate (— · — · —). Extinction coefficients are based on the assumption of OH^- as counterion after silica chromatography.

'd'). **6b**: 7.68 (H-10, s), 6.48 (H-15, s), 8.43 (Pyr-H-B,B', 'd'), 8.80 (Pyr-H-A, 't'), 9.29 (Pyr-H-C,C', 'd').

MS:EI-Mode: 556 (100%), 554 (66%), 527 (46%), 432 (45%), 421 (24%), 418 (26%). *FD-Mode*: 634 (100%), 556 (6%).

Structure **6** of the pyridinium adducts has been established by the following arguments: (1) The ¹HMR spectrum shows the ABB'CC' pattern (two 'doublets' and one 'triplet' ($J_{\text{apparent}} \approx 7$ Hz) in the ratio 2:2:1) and the chemical shifts typical for *N*-substituted pyridinium ions (Abramovitch, 1974; Diner and Lown, 1971); (2) The FD mass spectrum has a peak at the highest mass of 634 *m/e* (base peak), the only major fragment occurs at 556/e. In the EI spectrum, there are no reproducible peaks above the basis peak at 556 *m/e*; (3) The chromatographic properties indicate an ionic compound; (4) The site of substitution is indicated from the ¹HMR spectrum. Of the three methine resonances in **3** assigned to H-5 (5.44 ppm), H-10 (6.56 ppm) and H-15 (5.97 ppm) (Cavaleiro and Smith, 1973), the ones of H-15 and H-10 are slightly shifted, but still present in **6a** and **6b** while the high-field signal characteristic for H-5 is lacking; (5) The UV-vis spectra (Fig. 2) of the monocation, dication, zwitterion and zinc complex are in the wavelength ranges typical of the free bases, cations, anions and zinc complexes, respectively, of 'bilivoliols' containing the tripyrrinone chromophore **8***. Thus, the Δ 4,5-double-bond, although still present, is spectroscopically no longer evident. This effect of large *meso*-substituents has been observed earlier by Bonfiglio *et al.* (1977) and related to the uncoupling of the adjacent rings by a pronounced twist between them; (6) The hydrogenated ring A is still present, as evidenced by the characteristic ¹HMR multiplet (Scheer *et al.*, 1977) at $\delta = 1.5$ –1.8 ppm (2',3'-CH₂), by the 556 *m/e* peak in the mass spectra, and by the reversion experiments; (7) The product is formulated as the tautomer **6** rather than **5** for the lack of a third *meso* proton signal in the ¹HMR spectrum, and for the generally higher basicity of N as compared to C.

Due to its ready interconversion, compound **6a** contained 19% **6b**. In addition to the thermal disproportionation (see below), this is another reason for us not being able to obtain a defined melting point. The isomer mixture **6a,b** can be analyzed by ¹HMR. The signals of the methine protons and of the pyridinium ring protons listed above are most suitable due to their large separation for the respective isomers, but doubling of some signals can even be

observed in the methyl- and methylene region. After equilibration in CF₃COO²H, the isomer ratio **6a**:**6b** = 2:1 has been obtained, as compared to 4.5:1 in the reaction mixture.

The stereochemical origin for **6** to occur as isomers can be related to steric hindrance of the *meso*-pyridinium and the β -pyrrolic substituents. By this means, rotation around the C-5, C-6 bond is hindered, leading to atropisomers. In conjunction with the asymmetric centers at ring A (transoid configuration of the ethyl groups), this gives rise to two diastereomeric pairs of enantiomers, even in case of the rings B, C and D being coplanar. Based on this model, the 5-nitrobilindione obtained from an achiral bilindione (Bonfiglio *et al.*, 1973) possibly is a racemate.

Reaction **3** \rightarrow **6** is formally a nucleophilic substitution at C-5. It can be rationalized by the sequence shown in Scheme 1. Electron abstraction by the oxidant and subsequent deprotonation by the base present leads to a radical, and by repetition to the hypothetical dehydrobilindione **4**, either one of which is attacked by pyridine at C-5 to yield **6** after tautomerization. Accordingly, the reaction is facilitated with the 2,4, 2,6 and 3,5-lutidines due to the inductive effect of the alkyl groups, and by increasing concentrations of the oxidant, I₂. Nucleophilic attack on cation radicals or dications of fully unsaturated bilindiones have been reported to occur at both the outer methine bridges (Eivazi *et al.*, 1977). Although we have not yet identified all by-products of the reaction of **3**, the predominant formation of **6a,b** supports again (Scheer, 1978), that hydrogenation of ring A activates selectively the adjacent methine bridge under oxidizing conditions.† Formation of **6a,b** is photochemically assisted, but occurs principally in the dark, too. An anaerobic solution of **3** (40 μ M) in benzene containing I₂ (7.75 μ M) and pyridine (2.47 M) has been quenched after 2 h standing in the dark by filtration over Na₂S₂O₃ into glycine buffer (pH 2.7). After workup, it contained 5% of the isomers **6a,b** (in addition to minor amounts of a dimeric violin), besides unaltered **3**. The dark reaction is accompanied by a slight blue-shift of the absorption spectrum, which indicates the formation of an intermediate. The educt **3** can be recovered in high yield from this intermediate, but the latter is an activated form of **3** which forms rapidly 5-oxobilindiones and formyltripyrinones (Scheer *et al.*, 1977) upon addition of Zn²⁺ and admission of air. A fresh solution of **3** with pyridine and I₂, in which the spectral changes are not yet visible, reacts at least an order of magnitude slower under otherwise identical conditions. At the present state, it is impossible to assign a specific structure (isomer, complex, or a more or less oxidized species) to this intermediate formed in the dark. As the photochemical reaction occurs rapidly even in freshly prepared solutions, the latter appears to be independent from the dark intermediate.

To account for the long-wavelength absorption of P_{1r}, Grombein *et al.* (1975) have invoked the chromo-

*The term 'violin' is used here as a phenomenological description designating linear oligopyrroles with a single visible absorption band of the free base around 560 nm. Formula **8** with R₁, R₂, R₃ \neq 0 is typical of these compounds. Historically, the term has been associated with 4,5-dihydro-1,19 (21, 24 H) bilindiones.

†The central methine bridge (C-10) is the principal point of nucleophilic attack in the (unoxidized) free bilins both for bilindiones (Falk and Schlederer, 1978; Holzwarth *et al.*, 1978) and dihydrobilindiones (Kufer and Scheer, 1979).

Table 1. Apparent pK -values for protonation (pK_1) and deprotonation (pK_2) of bile pigments. The values are determined by titration of the bilins ($c \approx 10^{-5} \mu M$) with HCl and KOH, respectively, in a 1:1 mixture of a saturated solution of guanidinium chloride in water and methanol (Scheer, 1976) monitored with a glass electrode

Compound	pK_1	pK_2
3	5.6*	10–11†
7	5.1*	10–11†
Dimer of 3	1.4	11
6a,b	4.2	9.2

*From Scheer (1976).

†Destruction at $pH > 10$ during titration.

phore to be present in a deprotonated form. Based on this suggestion, a model for the P_{fr} chromophore should not only have a violin-type absorption spectrum, but also a sufficiently low pK -value to be deprotonated under physiological conditions. Violins generally have too high pK -values in this respect (Falk and Leodolter, 1978), and this is true, too, for the dimeric violins. By contrast, the pK of 6a,b is considerably lower. Acid-base titrations of 6a,b in a mixed solvent system (Scheer, 1976) gave a poorly defined $pK = 9.1$ (Table 1) for the reversible deprotonation to the zwitterion (spectrum in Fig. 2). The pK decrease is rationalized by coulomb interactions, similar to, e.g. the pK decrease of the carboxyl group in amino acids.

Like the dimers described earlier (Scheer and

Krauss 1977), compound 6a,b reverts thermally to the educt 3. The reversion occurs already at lower temperatures, e.g. in the FD source of the mass spectrometer at 76° . If carried out on a micropreparative scale, 3 is accompanied by the bilindione 7 and another unidentified oxidation product of the violin type. In contrast to the cleavage of the dimers, the reversion reaction of 6a,b in the absence of an added reductant can only be accomplished by a disproportion reaction. This type of reaction has recently been indicated for the bilindione series, too (Eivazi *et al.*, 1977). It should be noted in this context that the *in vitro* dark reversion of P_{fr} to P_r is accelerated by reducing agents (Mumford and Jenner, 1971; Kufer and Scheer, 1979).

If the principle of a photochemically assisted nucleophilic addition is applied to the phytochrome interconversion, one would expect the protein side chains of cysteine, tyrosine, histidine or tryptophane as the most likely reaction partners. Preliminary results with the respective N- and C-protected amino acids have indicated that the latter three can add to compound 3, thus supporting this model.

Acknowledgements—This work was supported by a grant from the Deutsche Forschungsgemeinschaft Bonn-Bad Godesberg. We are indebted to the Münchner Universitätsgesellschaft for financial aid. We thank Prof. W. Rüdiger for helpful discussions and continuing support, Prof. H. Budzikiewicz (University, Köln) for providing the FD-mass spectra, and G. Schild for measurement of the 1HMR spectra.

Note added in proof. In an attempt to identify intermediates of the oxidative reaction, a long-lived π -cation radical produced from the zinc complexes of 3 and 7 has recently been characterized (Krauss, C. and H. Scheer (1979) *Tetrahedron Lett.*, in press).

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