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RESEARCH NOTE

STUDIES ON PLANT BILE PIGMENTS. II. REGIOSELECTIVE PHOTOCHEMICAL AND ACID CATALYZED *Z,E* ISOMERIZATION OF DIHYDROBILINDIONE AS PHYTOCHROME MODEL

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Abstract—The (4 *Z*, 10 *Z*, 15*E*)-2,3-dihydrobilindione **4**, along with the fully unsaturated (*E*, *Z*, *Z*)-analogue **8**, has been prepared from the corresponding (*Z*, *Z*, *Z*)-isomer by a variation of Falk's method (Falk *et al.*, 1980). The photochemical and acid-catalyzed back-reactions have been studied by UV-vis and ^{1}H-NMR spectroscopy.

The photoconversion of **1**, the chromophore of the P_r form of phytochrome, is the primary photochemical reaction of this plant sensory pigment. The structure of the stable photoproduct, the P_{fr} chromophore, is still unknown. Photooxidation has been indicated earlier (Grombein *et al.*, 1975) and model reactions of this type have been investigated with the synthetic analogue **2** (Krauss and Scheer, 1981) bearing the characteristic A-dihydrobilin chromophore system (see Scheer, 1981). The characterization of *Z,E* isomeric bile pigments (Falk and Grubmayr, 1977; Falk *et al.*, 1978, 1980; Gossauer *et al.*, 1981), isotope exchange and spectroscopic studies (Song *et al.*, 1979; Sarkar and Song, 1981) and recent studies with P_{fr} chromopeptides (Thümmler *et al.*, 1981) again raised the possibility of a geometric (*Z,E*)-isomerization as the primary reaction of the phytochrome system. *Z,E*-Photoisomerizations have hitherto only been studied with the fully unsaturated biliverdin-type pigments (Falk and Grubmayr, 1977; Falk *et al.*, 1978, 1980; Gossauer *et al.*, 1981) and with bilirubins (McDonagh *et al.*, 1980; Holzwarth and Schaffner, 1981; de Groot *et al.*, 1982; Lightner *et al.*, 1979; Stoll *et al.*, 1979; Isobe and Onishi, 1981; Falk *et al.*, 1980). The $Z \rightarrow E$-isomerization occurs generally (Falk and Thirring, 1979, 1980) only with the rubinoid pigments, viz. the isomerization of biliverdin proceeds via reversibly formed rubinoid addition products (Holzwarth *et al.*, 1978; Manitto and Monti, 1979; Falk and Schlederer, 1978; Falk *et al.*, 1980; Kufer and Scheer, 1979, 1982) as the substrates proper of the photoreaction (Falk *et al.*, 1980), whereas the direct $E \rightarrow Z$(back-)photoisomerization is possible with the verdins as well as the rubins (Falk and Neufingerl, 1979).

We are currently investigating the *Z,E* interconversions with pigments like **2** bearing the characteristic A-dihydrobilindione chromophore of phytochrome. They also form reversibly rubinoid addition products (Kufer and Scheer, 1979, 1982), but with rather different chromophoric systems in the two 'halves' of the molecule. This raises the question of the regioselectivity of the isomerization in pigments like **2** which is relevant to the structure of the P_{fr}-chromophore.

The isomerization of the A-dihydrobilindione **2**–**4** was performed by a modification of Falk *et al.*'s (1980) general procedure. Compound **2** (8.3 mg) was treated with 2-mercaptoethanol (10 mℓ) in dimethylsulfoxide (30 mℓ) to yield the addition product **3** (λ_{max} = 421 nm, see Kufer and Scheer, 1982). It was irradiated for 10 min under N_2 with light from a 1000 W halogen lamp, filtered through 5 cm each of an aqueous solution of $NaNO_2$ (2.17 *M*) and a solution of $CuSO_4$ (0.035 *M*) in 2.7 *M* aqueous ammonia (transmission maximum: 426 nm, cut off; 0.1% transmission 390 nm; modified from Calvert and Pitts, 1966). After photolysis, the solution was partitioned between chloroform and water, thoroughly washed with water, and chromatographed on silica H (Merck, Darmstadt) plates with chloroform/methanol = 20:1 (Falk *et al.*, 1978). Due to the ready back-isomerisation of **4** to **2**, the work-up and further handling of **4** was performed either in complete darkness or, when necessary, with red safety lights. The (15 *E*)-isomer **4** (typical yield 4%) was isolated from the slower moving zone, in addition to the educt **2** (37%) as the major colored component, and a few oxidation products. It was rechromatographed on silica plates with carbon

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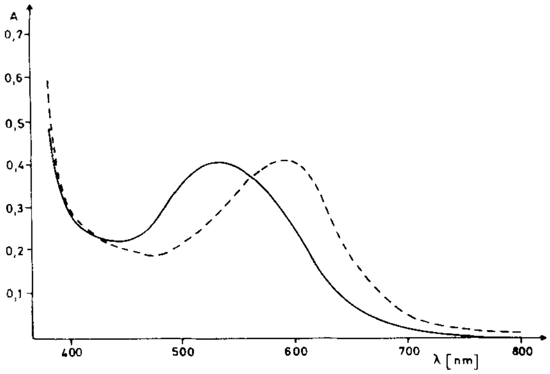
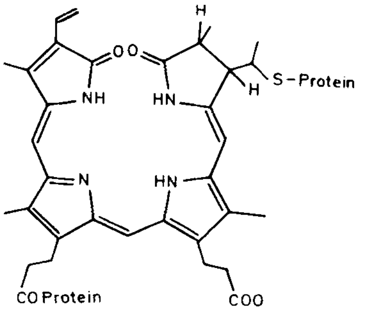


Figure 1. Photoisomerization of (4 *Z*, 10 *Z*, 15 *E*)-2,3-dihydrobilindione **4**. Visible absorption spectrum of **4** in acetone (——) and of the same solution after 3 min irradiation with white light (1400 W·m^{-2} in the range between 460 and 600 nm) (– – –). According to TLC analysis the product of the photoreaction was the corresponding (*Z*,*Z*,*Z*)-isomer **2**.

tetrachloride/acetone = 10:1. The structure of **4** was established as follows: (i) the UV-vis spectra of the free base (λ_{max} = 520 nm in acetone) and the cation (λ_{max} = 570 nm in acetone, 1% concentrated HCl) were red shifted by 76 and 85 nm with respect to the corresponding forms of the (15 *Z*)-isomer **2**. This matches with the data given by Blacha-Puller (1979) for the (15 *E*)-isomer of a dihydrobilindione prepared by total synthesis [λ_{max} = 533 (15 *E*) vs.: 605 nm (15 *Z*) in $CHCl_3$, 605 (15 *E*) and 676 nm (15 *Z*) in $CHCl_3$/HCl]. The shift is much larger than that expected for the 4 *E* isomer **5**, in which only the 4-double bond is uncoupled. (ii) In the NMR spectrum, the signals of the 5 H (5.38 ppm) and 10 H (6.93 ppm) remain unchanged, whereas the 15 H (6.16 ppm) signal is shifted by 0.23 ppm to lower field. A shift of this order is typical for methine-protons at the site of isomerization (Gossauer *et al.*, 1981; Falk *et al.*, 1978). In particular, the data are similar to the ones given by Blacha-Puller (1979) for a (15 *E*) (15 H at 6.21 ppm) and (15 *Z*)-isomer (15 H at 5.97 ppm) of a dihydrobilindione. (iii) Irradiation of **4** or its cation with white light yielded back the isomer **2** as the sole product (Fig. 1). The reaction was also studied by NMR spectroscopy. Under non-exchanging conditions, the 15 H signal was shifted back to its original position at 5.93 ppm in **2**, whereas the other signals remained unchanged.

To study the influence of the reduced ring A on the behavior of **2**, the isomerization was also studied with the bilindione **6** (octaethylbilindione) with ring A unsaturated. The conditions for the reaction sequence were less critical than with **2**. The addition product **7** was already formed quantitatively in the presence of 7.5% of the thiol, and the complete removal of 2-mercaptoethanol was also facilitated by the stability of **6** towards bases which allows washing with dilute KOH. The final yield of **8** (10%), however, was similar to the best yields obtained for the isomerization of **2**. Data of **8**: UV-vis: free base ($CHCl_3$): λ_{max} = 585,



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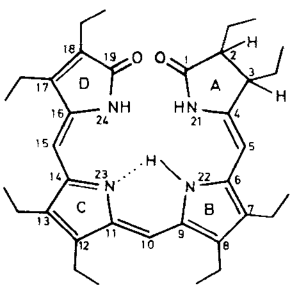

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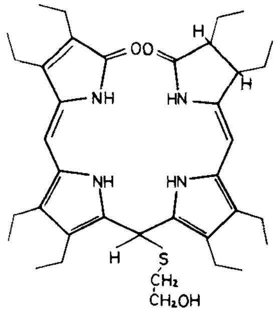

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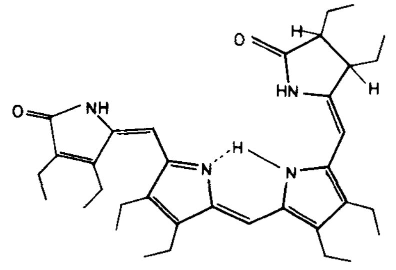

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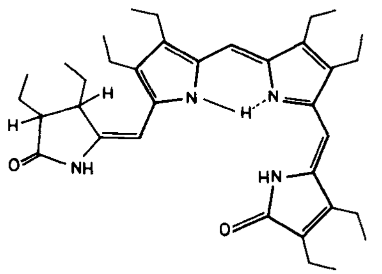

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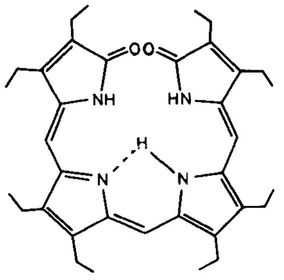

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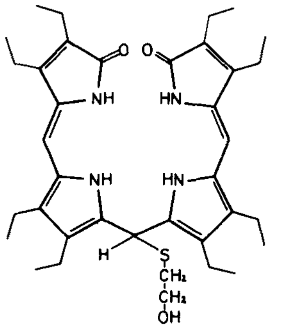

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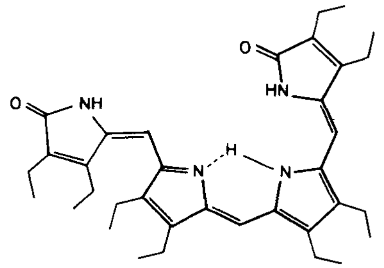

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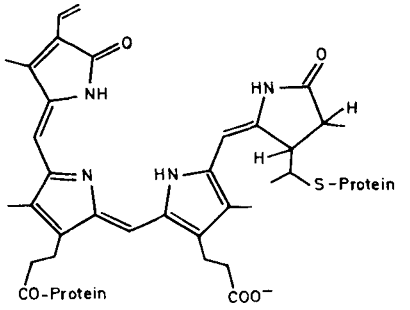

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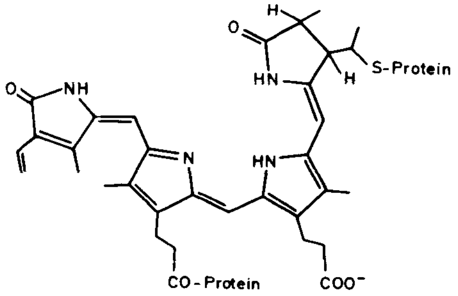

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368 nm; cation (CHCl$_3$/trifluoroacetic acid): λ_{max} = 598, 362 nm; ^{1}H-NMR in CDCl$_3$: 6.86 ppm (s, 10 H), 6.20 (s, 15 *E*H), 5.90 (s, 5 *Z*H), 2.2–2.8 (m, —CH$_2$—), 0.8–1.4 (m, —CH$_3$); in CDCl$_3$/d-trifluoroacetic acid: 7.28 (s, 10 H), 6.52 (s, 15 *E*H), 6.25 (s, 5 *Z*H), 2.3–3.0 (m, —CH$_2$—), 1.0–1.5 (m, —CH$_3$). These data are comparable to the ones reported by Falk *et al.* (1978) and Gossauer *et al.* (1981) for *E*,*Z*,*Z*-bilindiones of the verdin series, and in particular the assignment of the methine NMR signals has been made in analogy.

The (*E*)-isomer reverts photochemically to the (*Z*)-educt **6**. Like other (*E*)-isomers (Falk and Grubmayr, 1979), it also reverts in acidic solution in a dark reaction. Both the photochemical and the acid-catalyzed back reactions give isosbestic points (642, 460, 335, 311 nm in CHCl$_3$/trifluoroacetic acid) when followed by UV-vis spectroscopy, and yield the (*Z*,*Z*,*Z*)-isomer **6** as the sole product. The mechanism of the acid catalyzed reaction has been studied by ^{1}H-NMR in the presence of deuterated trifluoroacetic acid. There is apparently no simultaneous H/D exchange at C-15 with the isomerization (Fig. 2), indicating that no protonation–deprotonation mechanism at this position is operative for the isomerization. The isomerization is then likely to involve protonation at other sites and/or tautomerization similar to the recently suggested processes in the primary steps of phytochrome phototransformation (Sarkar and Song, 1981). The negligible proton exchange at C-15 also indicates that the electron density at the isomerized methine bridges is neither strongly increased in the (*E*,*Z*,*Z*)-isomer nor during the isomerization, as compared to the (*Z*,*Z*,*Z*)-isomer. The C-5 in **2** is selectively attacked by electrophilic reagents and the 5 H is readily exchanged with deuterated trifluoroacetic acid (Krauss, 1980). This is supported by MO calcu-

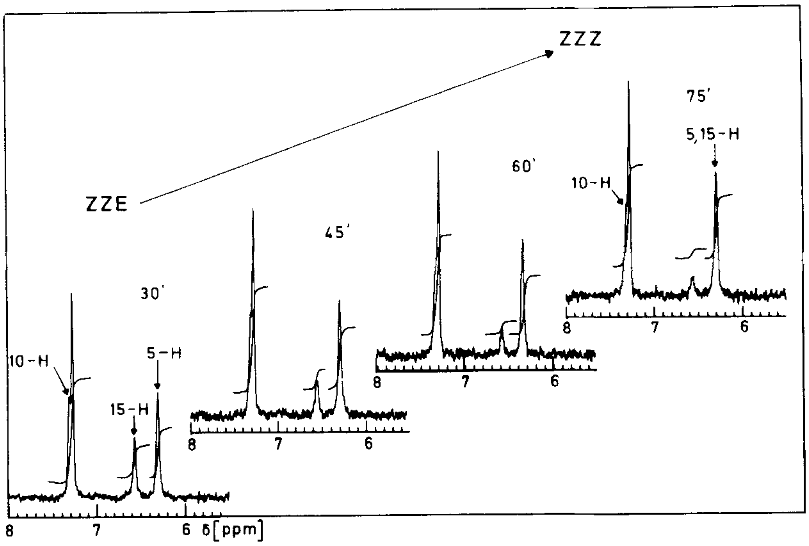

Figure 2. ^{1}H-NMR spectra of the acid catalyzed thermal isomerization of the (*E*, *Z*, *Z*)-bilindione **8** to the (*Z*, *Z*, *Z*)-isomer **6**. Only the range between δ = 6–8 ppm is shown, which contains the signals of the methine protons (indicated), as well as the signal of residual $CHCl_3$ at δ = 7.26 ppm. The first spectrum (lower left) was scanned 30 min after the addition of trifluoroacetic acid to a solution of **8** in $CDCl_3$. The subsequent spectra were scanned at intervals of 15 min.

lations indicating an increased electron density at the methine bridge next to the reduced ring (Scheer *et al.*, 1982).

The results obtained with **2** show that the *Z,E*-(photo)isomerizations of A-dihydrobilindiones occur along the lines previously established for biliverdins with an unsaturated ring A (see Falk *et al.*, 1980). A particular feature, however, of the A-dihydrobilindiones is their asymmetry. Two rather different products (**9**, **10**) can principally be formed by isomerization at either C-5 or C-15, respectively, a point which is obviously important to the phytochrome problem. The adduct **3** as the educt proper for photoisomerization has two absorption maxima (421 and 315 nm in the reaction mixture*) which are assigned to the dipyrromethenone chromophore comprising rings C and D, and to the vinylpyrrole chromophore comprising rings A and B, respectively (Kufer and Scheer, 1982). The irradiation of **3** has been carried out with filtered light absorbed preferentially by the dipyrromethenone chromophore, leading to an overall regioselective transformation of **2** to its (4 *Z*, 10 *Z*, 15 *E*) isomer **4**. The ready direct photoisomerization of **4** back to **2** compares well with that of denatured or peptic digested P_{fr} (Thümmler *et al.*, 1981), which would be in support of an *E,Z,Z* isomer like **4** as a P_{fr}-model, rather than pigments derived from photooxidative reactions (see Scheer, 1981). The absorption maximum of the *E,Z,Z*-**4** cation (570 nm) is, however, considerably shorter than that of denatured or peptic digested P_{fr} in acidic solvents (615 nm), even if the influence of the 18-vinyl-group is taken into account. It is, therefore, desirable to have at hand, too, the (4 *E*, 10 *Z*, 15 *Z*)-isomer **5** of **2**. Unsuccessful attempts for a total synthesis of a pigment bearing this chromophore (Blacha-Puller, 1981) indicate an even decreased stability as compared to the (4 *Z*, 10 *Z*, 15 *E*)-isomer. The effects of irradiation into the vinylpyrrole chromophore of **3** comprising rings A and B (Kufer and Scheer, 1982) are currently being studied.

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*The absorption maxima of bilirubins are strongly solvent dependent (see Kuenzle, 1970; Holzwarth *et al.*, 1978, 1981).

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