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Studies on Plant Bile Pigments, II[1]

Chemical and Photochemical Oxygenation of a Phytochrome P_r Chromophore Model Pigment to Purpurins

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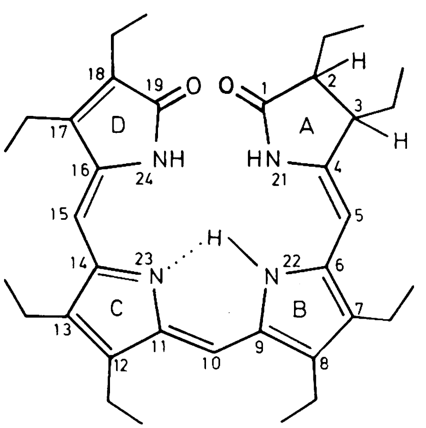
Summary: The chemical and photochemical oxygenation of 2,3-dihydrooctaethyl-1,19(21,24*H*)-bilindione (**1**), as a model for the chromophores of both phytochrome P_r and phycocyanin, has been studied in neutral and alkaline solution, and in the presence of zinc ions. By comparison with octaethyl-1,19(21,24*H*)-bilindione (**5**), the influence of ring A hydrogenation on the reactivity of bilins has been assessed. In the dark, **1** is attacked selectively and rapidly at C-5 yielding "purpurins", while **5** reacts slowly, and is attacked predominantly at C-10. Photooxidation of **5** yields the *tri*pyrrinic "purpurin" **7** only. Photoreactivity of **1** is considerably enhanced, yielding "purpurins" and "violins" rapidly. In spite of UV-vis and mass spectroscopic similarities, the "purpurin" **7** differs from the "purpurins" **6a, b** by the loss of ring A. The facile cleavage at the C-5 methine bridge and the spectroscopic properties of "purpurins" are discussed.

Untersuchungen an pflanzlichen Gallenfarbstoffen, II. Chemische und photochemische Oxygenierung eines Modellpigments für den Phytochrom-P_r-Chromophor zu Purpurinen

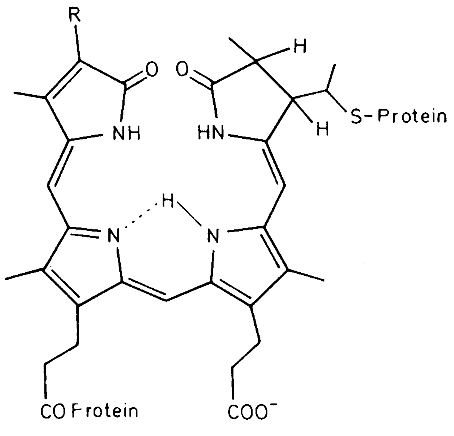
Zusammenfassung: Die chemische und photochemische Oxygenierung von 2,3-Dihydro-octaäthyl-1,19(21,24*H*)-bilindion (**1**), einem Modellpigment für die Chromophore von Phytochrom P_r und von Phycocyanin, wurde in neutraler und alkalischer Lösung, und in Gegenwart von Zink-Ionen untersucht. Durch Vergleich mit dem vollständig ungesättigten Octaäthyl-1,19(21,24*H*)-bilindion (**5**) wurde der Einfluß des hydrierten Ringes A auf die Reaktivität der Biline studiert. Verbindung **1** reagiert im Dunkeln schnell und selektiv zu „Purpurinen" durch oxidativen Angriff an C-5, während **5** nur langsam reagiert und vorwiegend an C-10 angegriffen wird. Die Photooxidation von **5** führt selektiv zu dem *Tri*pyrrin „Purpurin" **7**. Das Dihydrobilin **1** reagiert wesentlich schneller, wobei neben „Purpurinen" auch „Violine" entstehen. Trotz weitgehend übereinstimmender elektronenanregungs- und massenspektroskopischer Daten unterscheidet sich das „Purpurin" **7** von den „Purpurinen" **6a, b** durch den Verlust des Ringes A. Die leichte Spaltbarkeit der C-5-Methinbrücke und die spektroskopischen Befunde werden diskutiert.

Key words: Bile pigments, tripyrrins, phytochrome model, purpurins, photooxidation

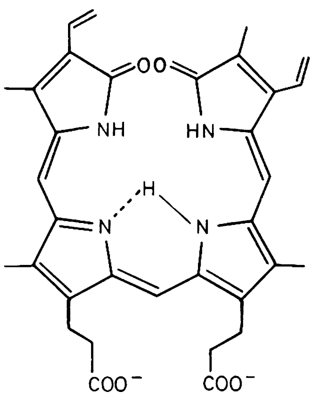
2,3-Dihydrooctaethyl-1,19(21,24*H*)-bilindione (**1**) has been proposed recently[1] as a model for the chromophores of both phycocyanin (2), the blue antenna pigment of bluegreen algae, and phytochrome, the "red" photomorphogenetic reaction centre pigment of higher plants and some algae, in its P_r form (**3**). The two biliproteins contain a closely related 2,3-dihydrobilin chromophore which is covalently linked to the protein. (For recent reviews, see ref.[2-4]). The photochromic reaction of this chromophore has been suggested as the primary reaction of the series of events leading to the phytochrome response[5,6]. In this reaction, the length of conjugation is shortened by one double bond[7], while the β-pyrrolic substituents remain the same (if judged from chromic acid degradation*).



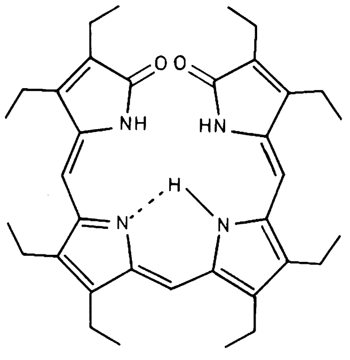
1: 2,3-Dihydrooctaethyl-1,19(21,24*H*)-bilindione



2: R = Ethyl; Phycocyanin
3: R = Vinyl; Phytochrome P_r



4: Biliverdin



5: Octaethyl-1,19(21,24*H*)-bilindione

The latter results suggest that the basis for the phytochrome response might be an intrinsic property of the A-dihydrobilin chromophore which is then modified by the substituents, and mainly the protein. In phytochrome it is enhanced, but suppressed in phycocyanin.

To further evaluate this possibility, we are currently investigating the reactivity of this chromophore by means of the synthetic[8] 2,3-dihydrobilin **1**. The chromophores of **2** and **3** can be cleaved off the biliprotein only in low yield, and not without chemical modification[9-14]. Therefore, the fully unsaturated biliverdin (**4**) has been commonly used as a model[15-17]. As hydrogena-

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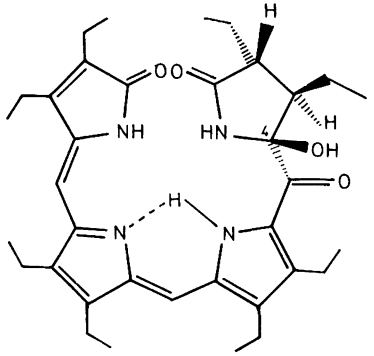
* Klein, G., Grombein, S. & Rüdiger, W., to be published.

tion of one ring has a profound influence in cyclic tetrapyrroles[18], we are currently studying the 2,3-dihydrobilin **1** as a model for **2** and **3** to assess the influence of ring A hydrogenation in open chain tetrapyrroles. In this presentation, photochemical and chemical oxidations of **1** leading to "purpurins"* are reported, together with parallel investigations on the fully unsaturated bilin **5**.

Results and Discussion

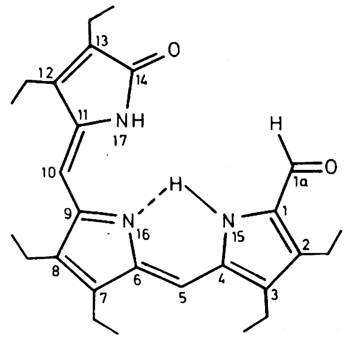
Dark oxidation

If a solution of **1** in methanol is treated with strong base, or zinc ions, or both, the resulting anions and zinc complexes, respectively, are very unstable under aerobic conditions. The zinc complex reacts rapidly to an intermediate absorbing at 755 and 372 mn, which is further transformed to stable products absorbing in the 550 - 650 nm range (Fig. 1 a). After workup, two octaethyl-2,3-dihydro-4-hydroxy-1,5,19(21,24*H*)-bilintrione zinc complexes and the free base **6a** were identified as main products by thin-layer chromatography. Demetalation yields as the main product the octaethyl-1,5,19-bilintrione **6a** accompanied by its diastereomer **6b**, as well as traces of octaethylbilindione **5**. If the anion of **1** absorbing at 766 nm is allowed to react with oxygen, a slower reaction leading to a series of absorptions between 630 and 450 nm is observed (Fig. 1 b). The complexity of the reaction, suggested by the lack of isosbestic points and the number of low intensity absorption bands, is confirmed by the product analysis. The main product is the 1-formyl-14(17*H*)tripyrrinone **7**, while **6a** and **6b** (inverted relative yields, as compared to the reaction of the zinc complex), the bilindione **5**, and a non-polar "violin" have been identified as byproducts. In addition, a number of more highly oxizdized polar products are visible on thin-layer chromatograms.



6a: Octaethyl-2,3-dihydro-4-hydroxy-1,5,19(21,24*H*)-bilintrione

6b: C-4 epimer



7: 2,3,7,8,12,13-Hexaethyl-1-formyl-17(15*H*)-tripyrrinone

If zinc ions and strong bases are applied simultaneously, the resulting "anion" of **1** absorbing at 720 nm (P 720*) (Fig. 1 c) is oxidized rapidly in spectroscopically nearly quantitative yield to the zinc complex of the 1-formyl-14(17*H*)-tripyrrinone (**Zn-7**) (λ_{max} = 625,580 nm).

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* Nomenclature follows the IUPAC-IUB suggestions[19] in the case of bilins of known structure (for numbering, see formulas 1 and 7). The terms "violins" and "purpurins" are strictly operative in terms of the free base absorption spectrum and colour (see discussion). It should be noted, that the term "purpurin" has been used, too, with a different meaning in hydroporphyrin chemistry[19,20] as well as for 1,2,4-trihydroxyanthraquinone.

* The educt "P 720" has been suggested from titration experiments to be the monoanion of **1**[1]. However, as it is accessible both by treatment of **1** first with Zn $(OAc)_2$, and then with sodium methoxide, and vice versa, an involvement of the metal in formation of this product is suggested. Bile pigments bind increasingly strongly to zinc and other metals at increasing pH[21], and metallobilin-rearrangements can be caused by acidity changes of the medium[21,22]. Thus, the role of metals in "P 720" formation and reactivity is currently being studied in more detail.

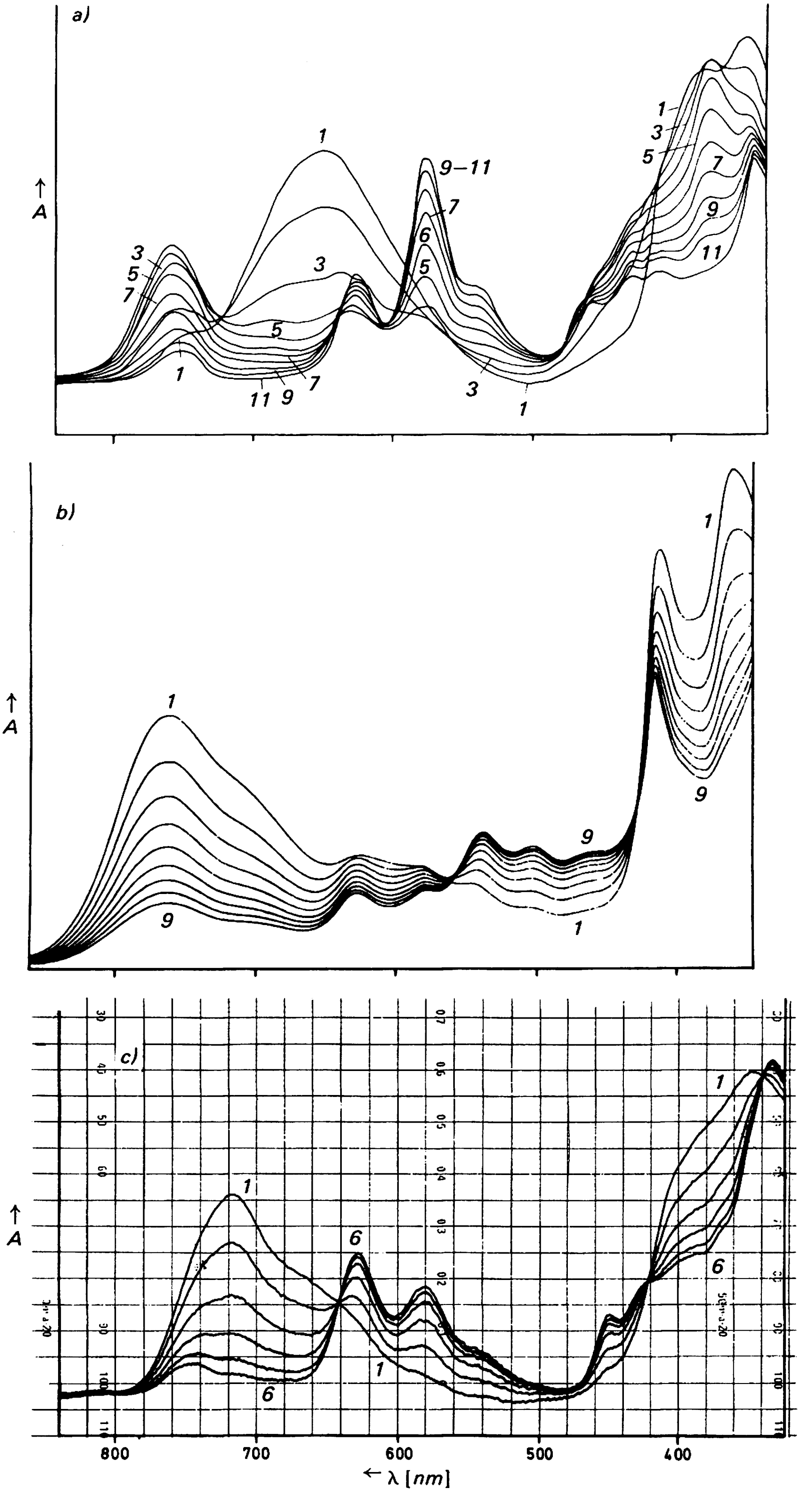

Fig. 1. UV-vis spectra obtained during dark oxygenation of the bilin **1** in methanol (10^{-5}M).

a) In the presence of zinc acetate (10^{-2}M). Each trace corresponds to 15 min reaction time at 23 °C;

b) In the presence of sodium methoxide (1M). Each trace corresponds to 30 min reaction time at 23 °C;

c) In the presence of sodium methoxide (1M) and zinc acetate (10^{-2}M). Each trace corresponds to 15 min reaction time at 23 °C.

Compared to the high reactivity of the A-dihydrobilin **1**, the fully unsaturated bilin **5** reacts under the same conditions only slowly. At the time when the reaction of the anion or the zinc complex of **1** is complete to 90%, only 7% of the anion and 10% of the zinc complex of **5**, respectively, have been oxidized, corresponding to a decrease of the quasi-first order rate constants by a factor of 25 and 22. In the presence of both zinc and base, the relative reactivity of **5** is even more decreased. In additon to the much slower reaction rates, there is also a very pronounced change in the product distribution. The products of **1** are mainly 1,5,19(21,24*H*)-bilintriones or 1-formyltripyrrinones, which arise by attack at the C-5 methine bridge. By contrast, the latter are only minor by-products of the dark oxidation of **5**. The main reaction products do not absorb noticeably above $\lambda = 450$ nm. A series of yellow coloured compounds can be separated on silica. If judged from their absorption spectra, they contain dipyrrinic chromophores. Since the 1,5,19-(21,24*H*)-bilintriones **6a, b** and the 1-formyl-14(17*H*)-tripyrrinone **7** are comparably stable under the reaction conditions, these products probably arise by direct attack at the C-10 position, and possibly further oxidation.

Photooxidation

The main product of the photooxygenation of **1** in neutral organic solvents with visible light ($\lambda \geqslant 320$ nm) is the 1-formyl-14(17*H*)-tripyrrinone **7**. A variety of by-products is indicated by the UV-vis spectrum of the reaction mixture (Fig. 2) and has been separated by thin-layer chromatography. Although the yield of **7** and the reaction time can be markedly affected by the type of solvent used, the general pattern of the product distribution is the same in most solvents. For the reactions in benzene, methylene chloride and methanol, by-product analysis has been carried out by chromatographic separation on silica, and subsequent characterization of the chromophore type by UV-vis spectroscopy of the free bases, and usually of their cations, anions and zinc complexes as well. Two general types of products absorbing at $\lambda \geqslant 500$ nm have been observed: "Purpurins" (and their metal complexes) with a double peaked maximum around 540 and 505 nm (625 and 580 nm for the metal complexes), and "violins" with maximum absorption of the free bases around 560 nm. While only two other "purpurins" (the bilins (**6a, b**)) are formed in minor amounts besides the main product **7**, at least seven different violins have been characterized. In view of the recent isolation of a *dimeric* "violin"[23], and of the possible number of asymmetric C-atoms, it is likely that this variety is at least partly due to the formation of isomers. The yield of the main product **7** was highest in methylene chloride, and decreased in methanol and benzene.

Compared to the ease and the complexity of the photooxygenation of **1**, the fully unsaturated **5** reacts very slowly, but also much more selectively. **7** is practically the only coloured product, formed upon irradiation of **5** under the same conditions.

It is accompanied only by traces of a non-polar "violin", and by metal complexes of **7**. Both the

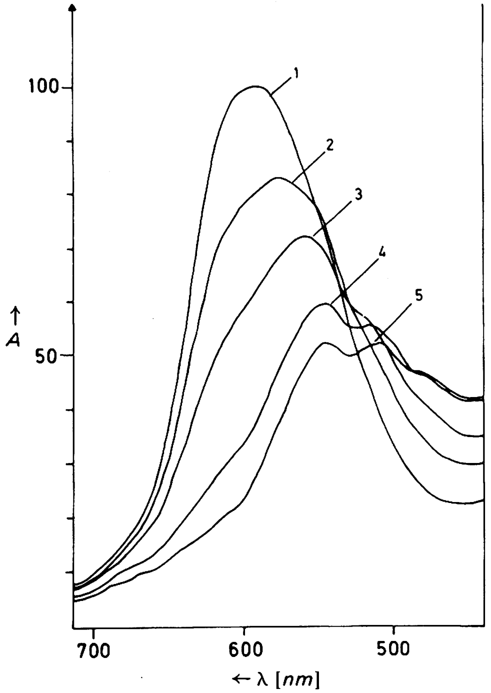

Fig. 2. UV-vis spectra obtained during photooxygenation of the bilin **1** in air saturated methanol (10^{-5}M) with white light ($\lambda \geqslant 320$ nm, ~ 10^6 lx). Each trace corresponds to 4 min irradiation time.

latter products are derivatives of, and convertible back to, the "purpurin" 7. Thus, the principal point of attack during photooxygenation in neutral solvents is at the C-5 methine bridge in both 1 and 5. However, although the directing influence of ring A hydrogenation (attack at C-5 or C-10) is less pronounced in the *photo*oxygenation under the reaction conditions, it still affects strongly the rate constants and the product distribution*.

Two types of photochemical reactions of 1,19(21,24*H*)-bilindiones ("biliverdins") have been observed before. They involve either vinyl groups, suitably positioned for intramolecular cyclizations[24-26], or they are initiated by attack of singlet oxygen[27-29] to α,α'-pyrrol C-atoms to yield cyclic imides and dipyrrins, but no tri- or tetrapyrrins (for the photooxidative bleaching of phycocyanin, c.f. ref.[30]; and for the photooxidation of a 4,5-dioxybilin-Tl(III) complex, c.f. ref.[31]). Our results suggest a third mechanism** to be operative, in which the principal point of attack is the C-5 methine bridge. This reaction is very slow, but very selective in the fully unsaturated bilin 5, leading to the 1-formyltripyrrinone 7 only. The reactivity is enhanced considerably, however, if ring A is hydrogenated at C-2, C-3, but at the same time a variety of byproducts is formed which currently are being analyzed.

Structure of products

The products **6a** and **6b** (which are accessible, too, from octaethylchlorin; see experimental part) have identical UV-vis spectra of their free bases, cations, "anions", and zinc complexes, and very similar mass, ^{1}H NMR and IR spectra, which suggested their being stereoisomers. This is supported by their reversible interconversion under alkaline conditions (see below). The ^{1}H NMR spectra of both **6a** and **6b** show at $\delta = 1.5 - 1.9$ ppm the multiplets (2- and 3-CH_2) characteristic for bilins with one reduced ring (A), and the two stereoisomers arise then from the presence of a third asymmetric C-atom at C-4. The 4-OH substituent has been proved by mass spectroscopy. The molecular ion at 588 *m/e* is of low intensity, but shows a prominent $(M - 18)^\oplus$ peak that corresponds to the loss of water. The main fragmentation occurs by cleavage at the 5-carbonyl bridge to yield ions at 433 and 432 *m/e* (loss of ring A only) and at 404 and 405 *m/e* (loss of ring A plus 5-CO). All the latter ions lack $(X - 18)^\oplus$ daughter ions. The only major difference between the mass-spectra of the diastereomers is a prominent $(M - 16)^\oplus$ ion in **6a**, which is absent in **6b**, indicating a less suitably positioned hydrogen for water abstraction in **6a**. The stereochemistry of **6a** with the 4-OH group *cisoid* to the 3-ethyl group is deduced from the ^{1}H NMR spectrum. The isolated triplets at highest field assigned to the 2a- and 3a-CH_3 groups absorb at $\delta = 0.89$ and 1.04 ppm, respectively, in **6a**, as compared $\delta = 0.92$ and 1.02 in **6b**. In both cases, the CH_3 group *cisoid* to the 4-OH group is shifted to lower field.

The diastereomeric purpurins **6a** and **6b** have also been obtained by oxidation of octaethylchlorin with thallium trifluoroacetate by a modification of the method of Cavaleiro and Smith[8], and subsequent separation on silica. The occurrence of two diastereomers gives further support to the proposed structure containing an asymmetric C-4. When treated with methanolic potassium hydroxide and worked up under acidic conditions to demetalate any metal complex, the two diastereomers are interconvertible. From both **6a** and **6b** an equilibrium mixture containing about 70% of **6a** and 30% of **6b** was obtained. The excess of **6a** is probably due to steric hindrance.

The UV-vis spectra of the free base, cation, "anion" and zinc complex of the 1-formyl*tri*pyrrinone 7 are similar to the spectra of the corresponding forms of the *tetra*pyrrintriones **6a** and **6b**. The highest-mass peak in the mass spectrum of 7 is at 586 *m/e*, and at lower *m/e* values the

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[*] The influence of ring A hydrogenation is even more pronounced in the photooxidation of 1 and 5, respectively, with ferric ions. Both in neutral[23] and in acidic solutions, 1 reacts selectively to "violins", while 5 is essentially inert. Details of this reaction are currently being investigated.

[**] Preliminary results indicate that the rate constant and the product distribution are not affected by the presence of either methylene blue or β-carotene if the reaction is carried out in methanol, but is strongly affected in methylene chloride. Thus, involvement of singlet oxygen in a rate-determining step is suggested in the latter solvent, but less likely in methanol.

mass spectra of **6a, b** and **7** are similar. On this basis, the *tetra*pyrrinic structure (2,3-dehydro-**6a,b**) was originally proposed* for **7**. This was supported by the consistent observation** of a small, metastable ion at 321 *m/e*, which suggested loss of the 153 *m/e* to form a fragment ion at 433 *m/e* (100%).*** However, in spite of this evidence, the assigned tripyrrinic structure **7** was proved by combustion analysis (found: 74.55% C, vs. calculated 74.79% C and 71.64% C for the 1-formyltripyrrinone **7** and 2,3-dehydro-**6a, b**, respectively), and by the ^{1}H NMR spectrum (singlet for 1 H at $\delta = 9.8$ ppm). The ion at 433 *m/e* is therefore the molecular ion and the peaks at masses above this are artifacts. Analogous unexpected and misleading peaks at mass units above $M^{\oplus}$ also have been observed in our mass spectrometer, in the spectrum of the 1-formyltripyrrinone **9a**[31]+, the structure of which has been confirmed recently by X-ray analysis++.

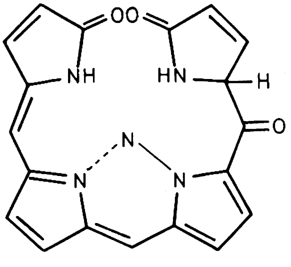
The smooth and selective cleavage of ring A, especially in the dark, is a new aspect of bile pigment chemistry. The selective degradation of a dihydroxybilin-Tl(III) complex has been described recently by Eivazi et al.[31], but the results presented here suggest this type of reaction to be more common than previously expected. In view of the difficulties in distinguishing the *tri-* and *tetra*pyrrinic purpurins by either UV-vis or mass spectroscopy (see above), some of the products encountered in the Gmelin reaction may turn out to be tripyrrins too.

The reaction mechanism of ring A cleavage is unknown. We have been unable to convert either **6a** or **6b** into **7** in reasonable yields under the conditions which lead to formation of **7** starting from **1** or **5**, which renders **6** an unlikely intermediate.

While the photochemical reaction could proceed via a known mechanism[32,33] [viz. addition of singlet (?, see footnote** p. 190) oxygen to the $\Delta^{4,5}$ bond, and splitting of the dioxetane formed] the dark reaction is more difficult to rationalize. It may include formation of a 4,5-dihydroxybilin-anion ("violin" anion), and subsequent cleavage of the remaining C-4, C-5 single bond by retro aldol condensation, benzilic acid rearrangement, or the like.

The "purpurin" chromophore

"Bilipurpurins" have been reported several times as oxidation products of bilins, especially as intermediates of the Gmelin reaction[2,34-37]. The term originally defined the violet and purple ("rotviolett") pigments of the Gmelin reaction[35]. However, this definition includes products with a variety of chromophoric systems. A more precise, although still phenomenological, definition which has evolved more recently includes only orange-red compounds exhibiting a typical double-peaked absorption of the free base around 540 and 505 nm (c.f. ref.[2,36], which have been formulated tentatively as 1,5,19-bilintriones (**8**). The structures of three compounds exhibiting such purpurin UV-vis spectra have been elucidated[8,21,31]. They all possess the proposed tripyrrindione chromophore **9**, but none of them corresponds to **8**, and only one is a true bile pigment (*tetra*pyrrin). Synthetic 1-carboxytripyrrinones like **9b**, and their metal complexes,



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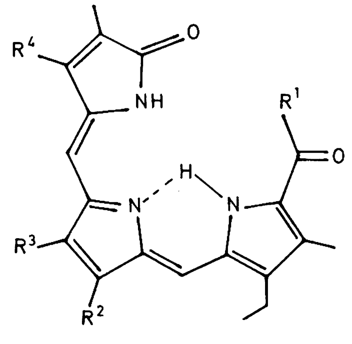
* Change in proof after consideration of referees comments and additional experiments.

** We have to thank Dr. *W. Schäfer* (Max Planck Institut für Biochemie, Martinsried) and *O. Seligmann* (Institut für Pharmazeutische Chemie der Universität, München) for the confirmation of the results on different spectrometers.

*** Ions at mass units above 433 *m/e* have been studied by defocusing and DADI experiments. Preliminary results indicate both parent ions contributing to the 433 *m/e* ion, and fragmentations of the 586 *m/e* ion, although the 586 → 433 fragmentation has not been observed. We are obliged to Dr. *E. Jacob* (Institut für Klinische Chemie, München-Großhadern) for these measurements.

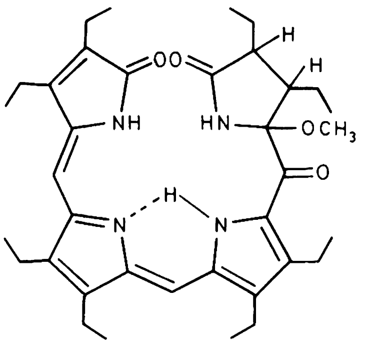
+ We thank Prof. *K. M. Smith* for providing to us a sample of **9a**.

++ Smith, K.M., & Cullen, D. (1976) private communication.



9a: $R^1 = H$, $R^2 = R^4 = C_2H_5$, $R^3 = CH_3$
9b: $R^1 = OH$, $R^2 = R^4 = CH_3$, $R^3 = C_2H_5$

have been investigated by Plieninger and Stumpf[21]. The 1-formyltripyrrinone **9a** has been obtained by sequential dark and photochemical oxidation of a synthetic 1,19(21,24*H*)-bilindione[31]. The 4-methoxylated 1,5,19-bilintrione **10** (possibly as a mixture of two diasteromers) has been prepared from octaethylchlorin by oxidation with thallium trifluoroacetate[8]. According to our results, both types of purpurins (i.e. tripyrrolic and tetrapyrrolic) can be obtained simultaneously, or selectively, by chemical and photochemical oxidations of 1,19(21,24*H*)bilindiones.



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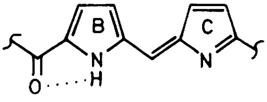
It is noteworthy that all tetrapyrrolic purpurins of established structure (viz. **6a**, **6b** and **10**) contain a 4-oxy substituent which, therefore, seems to be characteristic for this class of bilins. The oxygenation at C-4 may be compared to the formation of similar dipyrrinic products obtained by v. Dobeneck et al.[38,39]. In the latter case, the formation has been related to the instability of the azacylopentadienone system. The "bilipurpurins" **6a,b** and **10** may then be derived by addition to an intermediate containing an azacyclopentene ring A.

The formyltripyrrinone **7** has, at least formally, the same chromophore as the bilintriones **6a,b** and **10**. The similar UV-vis spectra indicate a similar conformation, too, for the free bases, cations and zinc complexes of the tripyrrinic "purpurins", and the tetrapyrrinic "(bili)purpurins". The only significant difference in the UV-vis spectra between the two types is the considerably increased extinction coefficient of the formyltripyrrinone cation as compared to the bilintriones **6a, b**, which may indicate a more rigid, planar structure in the former. The recent X-ray analysis of **9a** has shown a "porphyrin-like" planar crystal structure with the three N-atoms facing each other.

"Purpurins" are an exception to the general rule that bile pigments and other linear oligopyrroles have broad, unstructed absorption bands. The zinc complexes of **6a** and **6b** exhibit a similar characteristic two peaked absorption as the free bases, although shifted by about 80 nm to the red, and with unequal intensities (Fig. 3). In both cases, the band width ($W_{1/2}$, half width at half height) of each peak is around 16 nm (~ 500 cm^{-1}), as compared to 34 nm (~ 1000 cm^{-1}) for the red band of **1**. By contrast, the cation of **6a, b** exhibits again a broad "bile pigment spectrum" ($W_{1/2}$ = 28 nm). It has been argued[40,41] that bile pigments are flexible in solution, and that the broad absorption constitutes but an envelope of the absorptions of various conformers with slightly different excitation energies. On this basis, the sharp bands of purpurins, and their zinc complexes, indicate that they are rather rigid. The double peak possibly arises from two conformers fixed by strong H-bonding of NH-22 in ring B to either the 5-CO group (**11**) or N-23 (**12**). In zinc complexes these conformations would persist,



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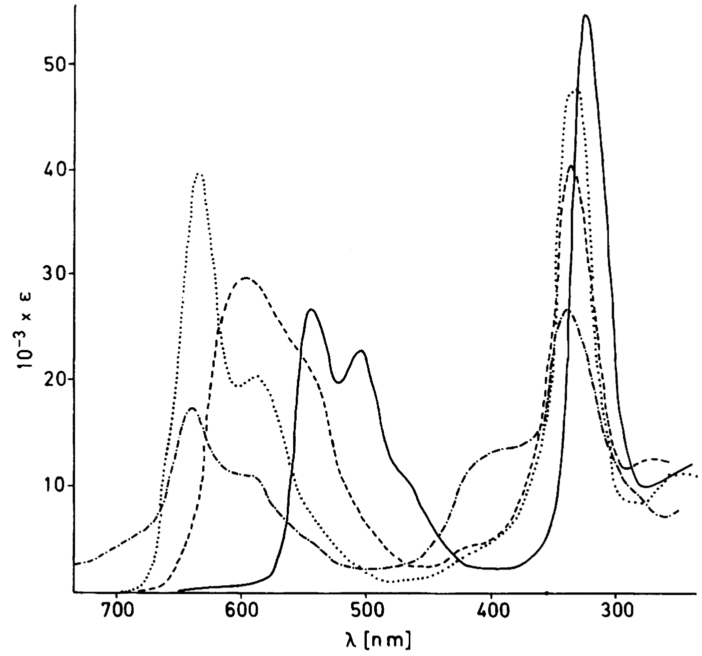


Fig. 3. UV-vis spectra of the bilintrione **6a** in methanol (——), of its cation in 1M methanolic HCl (– – –), of its zinc complex in 10^{-2}M methanolic zinc acetate solution (•••••), and of **6a** in 5M methanolic KOH (–•–•–).

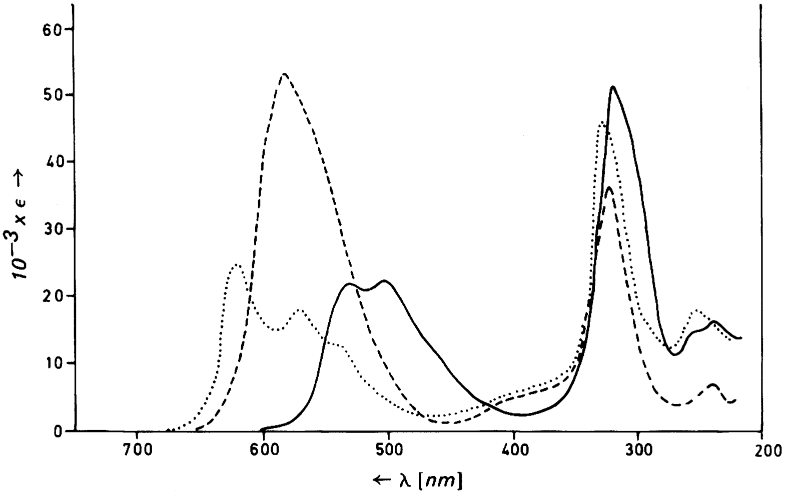
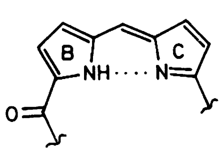


Fig. 4. UV-vis spectra of the 1-formyltripyrrinone **7** in methanol (——), of its cation in 1M methanolic HCl (– – –), and of its zinc complex in 10^{-2}M methanolic zinc acetate solution (••••••). The spectrum of **7** dissolved in 5M methanolic KOH resembles that of the zinc complex, but the product decomposes rapidly to form colourless oxidation products.

though in different proportions. However, the H-bonds are disrupted in the cation, leading again to a higher degree of flexibility. The UV-vis spectra of the 1-formylbilin **7** show similar features under these conditions (Fig. 4), indicating only minor contributions of ring A.



12

A potentially important aspect of "purpurin" chemistry, is their strong binding to metal ions, especially with regard to the binding of phytochrome P_{fr} to membranes[42-46]. The 1-carboxytripyrrinone **9b** binds not only transition metals, but also alkali and alkaline earth metal ions (e.g. $K^{\oplus}$, $Ca^{2\oplus}$) at higher pH to form complexes soluble in organic solvents[21]. Preliminary results suggest strong binding of the 1-formyltripyrrinone **7** and the bilintriones **6a, b** to metal ions, too. Treatment with strong bases leads to spectral changes similar to metal complex formation (Fig. 3 and 4) with an apparent p*K* of 9. Reversion to the free bases is possible only below pH 7. Thus, the "anions" observed may in fact be metal complexes. The picture is made even more complicated by the observation that the complexes of **9b** with alkali metal ions have UV-vis spectra very similar to those of the free base **9b**[21].

The facile metal complex formation also renders the "purpurins" difficult to purify. If judged from the instantaneous formation of the metal complexes upon additon of $Zn(OAc)_2$ to a methanolic solution of "purpurins" (and other bile pigments), the activation energy for metal insertion is negligible as compared, e.g., to porphyrins[47]. Thus, "purpurins" are effective scavengers for metal ions, and chromatography with commercial reagent grade solvents and adsorbents always leads to more or less pronounced metalation, which results in the formation of a blue, strongly fluorescing tail (see experimental part).

Experimental part

2,3,7,8,12,13,17,18-Octaethyl-2,3-dihydro-1,19(21,24*H*)-bilindione (**1**) and 2,3,7,8,12,13,17,18-octaethyl-1,19(21,24*H*)-bilindione (**5**) were prepared by modified methods of Cavaleïro and Smith[8] from octaethylporphyrin via trans-octaethylchlorin[48]. Solvents for preparative reactions were purified by standard procedures and distilled prior to use, analytical grade solvents were used without further purification in all spectroscopic studies. Preparative thin-layer chromatography was performed on silica H (Merck, Darmstadt), analytical thin-layer chromatography on silica HPDC precoated plates (Merck, Darmstadt).

Photochemical studies were carried out using a xenon source (Osram XBO 500) and suitable filters, or at 350 nm with a photochemical reactor with a mini go-round (The New England UV company, USA). Preparative irradiations were done with a 1000 W tungsten halogen source in pyrex tubes at concentrations with $A \leqslant 2$ absorption units. Intensities (lux) were determined with a luxmeter (Gossen).

UV-vis spectra were recorded in methanol, if not stated otherwise (Figs. 3 and 4) on a DMR 22 (Zeiss) spectrophotometer. Mass spectra (75 eV, direct inlet) were obtained on a JMS-D100 (Jeol, Japan) mass spectrometer, IR spectra on an IR 5 (Beckman, Germany) instrument. ^1H NMR spectra (δ [ppm]) were recorded on a HFX 90 (Bruker, Germany) spectrometer in FT mode in C^2HCl_3 with Me_4Si as internal standard. p*K* values were measured as previously reported[1] in a mixed buffer system. Combustion analyses were carried out in a model 1102 elemental analyzer (Carlo Erba, Italy).

Diastereomeric trans-2,3,7,8,12,13,17,18-octaethyl-2,3-dihydro-4-hydroxy-1,5,19(21,24H)-ethylbilintriones (6a, b)

trans-Octaethylchlorin (320 mg) in absolute methylene chloride (55 m*l*) was stirred for 45 s with thallium (III) trifluoroacetate (4.8 g) in absolute tetrahydrofuran (40 m*l*) and worked up by washing with 20% saturated $NaHCO_3$ and water, and drying with NaCl.

The reaction mixture, dissolved in carbon tetrachloride (10 m*l*), was adsorbed on a silica column (3 x 20 cm), and after 4 h standing on the column eluted with carbon tetrachloride containing 8% acetone. After evaporation of the solvent, the crude product was chromatographed on alumina, activity III, with methylene chloride containing 2% acetone. Crystallisation from methylene chloride/methanol yielded 35 mg (10%) of **6a**, and 42 mg (13%) of the less polar **6b**.

6a $C_{35}H_{48}N_4O_4$ (588.8) mp. 209 - 211 °C.
Calc. C 71.39 H 8.22 N 9.51
Found C 70.93 H 8.24 N 9.05
UV-vis [$\lambda(\epsilon \times 10^{-3})$]: *free base:* 545 (25.5), 509 (22.2), 470 (10.8, sh.), 324 (51.3), 268 (11.0), 248 (12.0, sh.); *cation:* 605 (29.9), 337 (39.0), 270 (24.4, sh.), 260 (11.9); *zinc complex:* 637 (37.4), 592 (19.4), 540 (5.9, sh.), 342 (44.4, sh.), 338 (44.8), 268 (12.2), 250 (12.5); *"anion":* 690 (4.6, sh.), 641 (16.8), 595 (10.4, sh.), 422 (9.7, sh.), 400 (13.0, sh.), 333 nm (25.1).
Mass spectrum: $M^{\oplus}$= 588 (4%), 572 (9%), 570 (20%), 554 (3%), 541 (2%), 433 (50%), 432 (100%), 273 (33%). *^1H NMR:* 11.70, 10.27, 8.07, 6.15 (s, NH, OH), 6.03, 6.79 (s. methine-H), 2.83, 2.61, 2.60, 2.58, 2.44, 2.42 (q, Ring B, C, D, $-CH_2$), 2.2 - 2.8 (m, Ring A-CH), 1.4 - 1.9 (m, Ring A-CH_2), 1.24, 2 x 1.20, 3 x 1.16, 1.04, 0.89 (t, CH_3).
IR: 1693, 1667, 1628 (C=O), 1600 (C=N) cm^{-1}.

6b $C_{35}H_{48}N_4O_4$ (588.8) mp. 197 - 199 °C.
Calc. C 71.39 H 8.22 N 9.51
Found C 71.25 H 8.31 N 9.37

UV-vis [λ($\epsilon \times 10^{-3}$), see Fig. 3]: *free base:* 545 (27.6) 509 (23.5), 470 (11.7, sh.), 324 (57.0), 277 (12.7, sh.), 267 (13.8), 247 (14.3, sh.); *cation:* 605 (32.8), 570 (27.3, sh.), 390 (5.8, sh.), 338 (39.7), 262 (13.2); *zinc complex:* 632 (39.1), 586 (20.7), 540 (7.5, sh.), 342 (44.5, sh.), 337 (45.9), 271 (10.8); *"anion":* 683 (7.2, sh.), 639 (14.2), 600 (11.0, sh.), 423 (10.8, sh.), 402 (13.0), 334 nm (28.1).
Mass spectrum: $M^{\oplus}$ = 588 (7%), 570 (30%), 555 (6%), 541 (7%), 433 (96%), 432 (100%).
^1H NMR: 11.6, 10.23, 8.06, 6.26 (s, 3 NH, 1 OH), 6.77, 6.01 (s, methine-H), 2.84, 2 × 2.60, 2.56, 2.50, 2.41 (q, Ring B, C, D-CH_2), 2.2. - 3.0 (m, Ring A-CH), 1.58 - 1.90 (m, Ring A-CH_2), 1.23, 2 × 1.21, 3 × 1.15, 1.02, 0.92 (t, CH_3).
IR: 1701, 1664, 1627, 1635 (C=O), 1593 (C=N) cm^{-1}.

Isomerization of 6a and 6b

1 mg of each isomer **6a** and **6b**, was dissolved in 5M methanolic KOH (5 m*l*) and stirred at ambient temperature. Isomerization was followed by acidic workup of portions, and thin-layer chromatography. After 5 h, equilibrium was reached in both samples with a ratio **6a**/**6b** 2:1.

2,3,7,8,12,13-Hexaethyl-1-formyl-14(17H)-tripyrrinone (7)

a) Photochemical preparation: A solution of 1 (29 mg) in methylene chloride (90 m*l*) was flushed with air while being irradiated for 1 h with a tungsten lamp (distance 1 m, ≈ 12000 lx). The solvent was evaporated in vacuo, and the reaction mixture separated on thin-layer chromatography with carbon tetrachloride containing 10% acetone. In order of decreasing R_F values, the educt 1 (10 mg), the fully unsaturated bilin 5, the bilintrione 7 (4 mg, 20%) and metal complexes of 7 (1 mg = 5%) were isolated. The latter can be demetalated with dilute HCl to yield additional 7 after a second chromatography.

$C_{27}H_{35}N_3O_2$ (433.6); mp. 149 °C (needles, from methanol)

Calc.	C 74.79	H 8.14	N 9.69
Found	C 74.55	H 8.12	N 9.58

UV-vis [λ($\epsilon \times 10^{-3}$), see Fig.4]: *free base:* 535 (22.0), 504 (22.9), 325 (41.8), 265 (14.5, sh.), 245 (16.5); *cation:* 584 (53.8), 380 (6.8, sh.), 328 (37.2), 260 (6, sh.); *zinc complex:* 624 (24.7), 576 (17.2), 535 (8.3, sh.), 332 (45.6), 265 nm (17.5).
Mass spectrum: 586 (2%), 556 (2%), 455 (23%), 433 (100%), 418 (55%). *^1H NMR:* 9.8 (s, CHO), 6.67, 588 (s, CH), 2.4 - 2.8 (m, CH_2), 0.8 - 1.4 (m, CH_3).
IR: 3400 (broad, NH, OH), 1710, 1660 (sh.), 1640 (C=O), 1595 (C=N) cm^{-1}.

b) By chemical oxidation: A solution of 1 (37 mg) in methanol (300 m*l*) was treated with a saturated solution of zinc acetate in methanol (15 m*l*), and 5M methanolic KOH (30 m*l*), stirred at ambient temperature for 15 min, and partititioned between water and methylene chloride. The organic phase was acidified, to yield after work up, thin-layer chromatography and crystallization from methanol the 1-formyltripyrrinone 7 (16 mg, 56%) besides the educt 1 (5 mg) and the bilin 5 (2 mg).

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