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Reactivity of Pyrrole Pigments, Part 15 [1]: On the Oxidation of Bilirubins and Biliverdins

C. Acero, J. M. Ribó*, R. Solé, and **F. R. Trull**

Departament de Química Orgànica, Universitat de Barcelona, c/Martí i Franquès 1. E-08028 Barcelona, Catalunya, Spain

Summary. Characteristic oxidation reactions of bilin-1,19-diones (biliverdins) and biladien-*ac*-1,19-diones (bilirubins) were reinvestigated. These include bromine and iodine oxidation of biliverdin IXα, bilirubin IXα and their Zn(II) complexes, the reaction described by Siedel (1941) of mesobilirubin XIIIα dimethyl ester with nitric acid (Gmelin reaction) and Smith's oxidation (1977) of etiobiliverdin IVγ using Tl triacetate and Pb tetra-acetate. For some of these reaction products new structures were assigned on the basis of their spectrometric data. These structures agreed with the expected reactivity pattern of biliverdins and bilirubins.

Keywords. Bilipurpurins; Biliviolins; Electron transfer vs. Electrophilic attack.

Reaktivität von Pyrrolpigmenten, 15 Mitt.: Über die Oxidation von Bilirubinen und Biliverdinen

Zusammenfassung. Einige bekannte Oxydationen von Bilin-1,19-dionen (Biliverdinen) und Biladien-*ac*-dionen (Bilirubinen) wurden neuerlich untersucht, und zwar die Brom- und Jodoxydation von Biliverdin IXα und Bilirubin IXα sowie der entsprechenden Zn(II)-Komplexe, die Reaktion des Dimethylesters von Mesobilirubin XIIIα mit HNO_3 (Gmelinreaktion; Siedel 1941) und Smith's Oxydation von Etiobiliverdin IVγ mit Thaliumtriacetat und Bleitetraacetat. Für einige Reaktionsprodukte wurden aufgrund spektroskopischer Daten neue Strukturen-konsistent mit dem bekannten Reaktionsverhalten von Biliverdinen und Bilirubinen-formuliert.

Introduction

The redox interconversion between biladien-*ac*-1,19-diones (bilirubins) and bilin-1,19-diones (biliverdins) [2] is a well studied reaction [3], in part because of the biological significance of biliverdin reduction to bilirubin and the studies directed to the development of chemical analysis of bilirubin in biological media. The oxidation of bilin-1,19-diones involves the addition of nucleophiles from the medium, most often followed by fragmentation processes of the carbon skeleton [4–6]: in agreement with this, the irreversibility of the electrochemical oxidation of biliverdin has been demonstrated [1, 3g, 7]. As a result of the small differences between the oxidation potential values of bilirubins and biliverdins [1, 7], the majority of reagents which oxidize biladien-*ac*-1,19-diones oxidize the resulting bilin-1,19-diones as well. During the oxidation of bilirubins using such poorly

discriminating oxidizing agents a sequence of colour changes is observed. This is due to the initial oxidation of the biladiene-*ac* to bilindione (bathochromic shift) and to the subsequent bilindione π system shortening (hypsochromic shift), as a result of the addition of nucleophiles and fragmentation reactions. Among the most characteristic of these oxidation processes are:

(i) the so-called Gmelin reaction [4], i. e. oxidation of bilirubin by nitric acid (a reaction nearly 200 years old! [8]);
(ii) the oxidative nucleophile additions to the exocyclic double bond of the 5-ylidene-3-pyrrolin-2-one end fragment of biliverdins: for instance, using bromine as oxidant [5a] or, more recently, by anodic electrolysis [5d–e], or even by oxidation with Tl(III) and Pb(IV) [5b–c];
(iii) the oxidation of the Zn(II) complexes of bilirubins and biliverdins with iodine [6].

In the past, much experimental work has been published related to these oxydation processes, which helps in the interpretation of the reactivity of linear oligopyrroles. However, literature contains results concerning the bile pigment oxidation products, in which the proposed structures do not agree with their expected reactivity. These results arise criticism especially because of the absence of spectrometric data due to their early origin.

The Gmelin reaction [4, 8] consists of several reaction steps, the first one being the oxidation of bilirubins to biliverdins. These are subsequently converted (oxidative nitration) into 5-nitro bilindiones [4c, 9a] which, in spite of their π system extended through the four rings, show a biliviolin* or bilipurpurin UV/Vis absorption spectrum because of the Z configuration at C5 and the ca. 90° dihedral angle conformation at C4, C5, C6, C7 [9]. Nucleophilic substitution reactions to these reaction intermediates may also occur. Nucleophilic addition to these nitro derivatives in acidic media and subsequent fragmentation would give tripyrrin-14-carbaldehydes: this reaction pathway has been suggested on the basis of the fragmentation pathway experienced by analogous compounds [10]. However, during his investigation of the Gmelin reaction, Siedel [4b–d] never identified any tripyrrin type compounds among the reaction products from mesobiliverdin XIIIα dimethyl ester (see C in formula scheme); rather he reported bilipurpurins and bilicholetelins (four ring systems) as the only reaction products. Although some of these products agree with the expected reactivity of bile pigments [11], others needed to be further investigated on the basis of modern structural methods. We have already shown that the mesobilipurpurin XIIIα dimethyl ester (619 nm Zn^{++}) identified by Siedel [4b–c] as 4-nitroso-5-hydroxy biladiene-*cd*-1,19-dione is actually the corresponding 5-nitrobilin-1,19-dione [6c]. Here we present additional results on the identification of the products formed during the oxidation of C with concentrated nitric acid [4a–c].

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* In addition to the well known bilirubin and biliverdin nomenclature, in this paper the old bile pigment nomenclature of bilipurpurin, biliviolin and bilicholetelin will be used: biladien-*ab*-1,19-diones, tripyrrin-1-ones and bilen-*a*-1,19-diones, respectively.

Oxidation of bilindiones with bromine in the presence of alcohols results in colour changes similar to those of the Gmelin reaction [4b], and bilipurpurins (4,5-dialcoxy biladienes-*cd*) have been identified among the reaction products [4b, 5a, 9b]. These bilipurpurins are also obtained from bilindiones in the presence of methanol, both by anodic oxidation [5d–e] and with Tl(III) [5b–c]. Furthermore, it has been reported [5b–c] that oxidation of etiobiliverdin IVγ (**D**) with Tl(III) or *Pb*(IV) in acetic acid yields the 4-acetyl-bilin-1,5,19-trione **D1**, besides other reaction products and the characteristic tripyrrin-14-carbaldehyde **D4** (coming from a fragmentation reaction). However, the structure of bilipurpurin **D1** does not agree with the expected reactivity pattern for biliverdins and for the 4-methylene-3-pyrrolin-2-one fragment of bile pigments. Here we present results, which confirm the expected oxidative reactivity pattern of bile pigments and assign the right structure to the aforementioned 4-acetyl-bilin-1,5,19-trione.

Related to the reaction of biliverdins with bromine, a chemical test for biliverdins and bilirubins is known which is based on the change of electronic absorption spectra of their Zn(II) complexes after iodine addition [6]. The test is based upon the generation of a spectrum with red fluorescence typical of the biliviolin and bilipurpurin Zn complexes. Lemberg has studied the end products of this reaction [6b] and we have reported [6c] the true structure of chrysins – one of the end products – as 10,11-dihydro-14-formyltripyrrin-1-ones. Data concerning the formation of Zn(II) complexes of bilirubin and biliverdin, mainly in solution, have been published, as well as results about their reactivity towards oxidizing agents [12]. However, most of these data are difficult to correlate. Here we present results on the reactions of bilirubin IXα (**A**), biliverdin IXα (**B**) and their Zn complexes with iodine, comparing them with the analogous reactions with bromine.

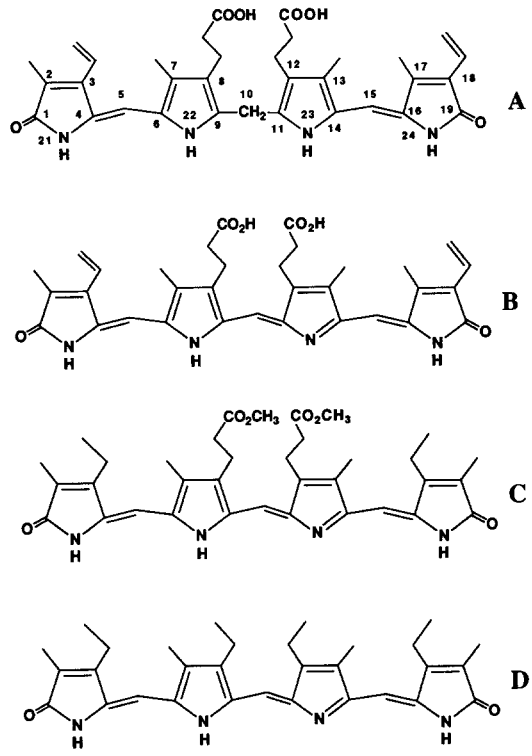
Results and Discussion

Oxidation of Bilirubin IXα (**A**), *Biliverdin IXα* (**B**) *and their* Zn(II) *Complexes with Bromine and Iodine*

The reaction of dimethylformamide (*DMF*) solutions of bilirubin IXα (**A**), biliverdin IXα (**B**) and their Zn(II) complexes with bromine or iodine were studied following the changes in their UV/Vis spectra after addition of a halogen solution in *DMF* (see Exp. Part). These spectral changes are fast and stable in the absence of oxygen.

Iodine addition changes the bilirubin (**A**) spectrum (λ_{max} 442 nm) only at a large halogen excess: reagent to substrate molar ratios above 7:1 are needed to achieve formation of biliverdin (λ_{max} 380, 650 nm) in excess with respect to the initial bilirubin. Biliverdin (**B**) itself is not oxidized by iodine and the changes in spectra observed (see below) must be attributed to the formation of some type of charge transfer complex. This is characterized by a 15 nm bathochromic shift and an hyperchromic effect on the low energy biliverdin absorption band. This hyperchromic effect is not very important and is probably accompanied by an hypsochromic effect on the high energy band. Unfortunately, absorption of iodine in this range does not allow the measurement of the corresponding absorption coefficient.

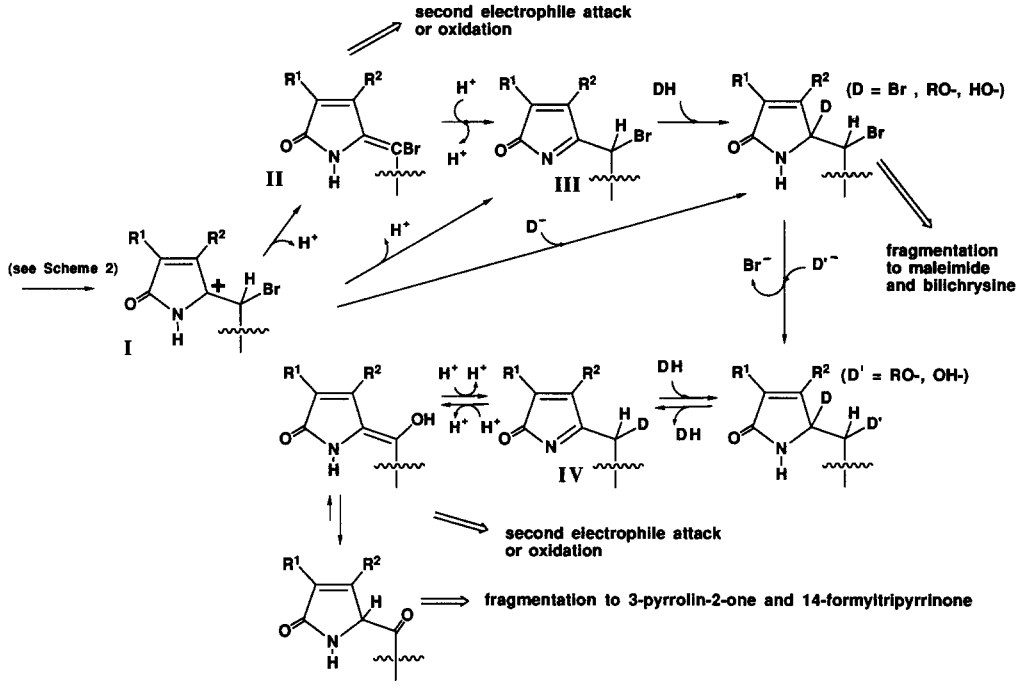
Bromine oxidizes very efficiently bilirubin (**A**) to biliverdin (**B**). The process is likely equimolar, and the formed biliverdin is then partially further oxidized to



bilipurpurins. However, bromine oxidizes **B** to bilipurpurins less efficiently than it oxidizes **A** to **B**: similar ratios of oxidized biliverdin are obtained when 1:1 or 7:1 bromine to bilirubin ratios are used.

These results indicate that iodine, with a lower reduction potential than bromine, can barely oxidize bilirubins to biliverdins: This lower reactivity of iodine vs. bromine is also observed with biliverdins. However, the small differences on anodic electrochemical potential values between bilirubins and the corresponding biliverdins (≈ 60 mV) [1, 3, 7] cannot account for the different reactivity of both substrates towards bromine. Rather, this difference in reactivity must be explained in terms of the different type of reaction of halogens with bilirubins and biliverdins. While oxidation of bilirubins to biliverdins (formal dehydrogenation) can be interpreted by means of radical mechanisms, electron transfer or electrophilic attack of a positively charged halogen species to the α pyrrole ring position (C9) followed by hydracid β elimination; in contrast, the irreversible steps of the oxidative transformation of biliverdins to bilipurpurins in the presence of halogens belong to the reaction type of the intermediates generated from the formal reaction of the unsaturated π system of biliverdins with a positively charged halogen species. The regiospecificity of the electrophile attack at the C5 position of biliverdins is experimentally proved [e.g. 4c, 4d, 13] and has been explained by semi-empirical calculations [11]: Scheme 1 shows the more likely reaction pathways from the intermediate **I** obtained following electrophilic attack to the biliverdin π system. The most important reaction steps, which can explain the reaction products isolated after the oxidation of biliverdins, are:

(i) recovery of the exocyclic double bond from **I** by deprotonation at carbon atom C5 giving **II** [13];
(ii) addition of a nucleophile to carbon atom C4, either directly to **I** or to a 2-oxo-(2*H*)-pyrrole intermediate (**III** or **IV**: such tautomeric structures of pyrrole-(2*H*)3-in-2-ones, in spite of not having been isolated, they have been proposed as intermediates in several bile pigment reactions [13, 14]);
(iii) facile nucleophilic substitution of the halogen atoms at sp^3 carbon atoms bonded to the α ring position of pyrrole rings and to the α position to a double bond C=N [13].



Scheme 1

The role of nucleophiles in this reaction is confirmed by the enhanced transformation of biliverdins to bilipurpurins in the presence of nucleophiles. In contrast with the results in *DMF* reported here, iodine in protic solvents oxidizes, although slowly, biliverdins to bilipurpurins [6c] and the reaction with bromine in methanol proceeds at an equimolar ratio [4c, 5a, 9b]. The reaction of biliverdins with halogens towards bilipurpurins shows the characteristics of both, an irreversible reaction, and of one which depends on the reagent excess. These observations are in good agreement with a first reaction step depending on a Nernst redox relationship. A first reaction step consisting of the oxidation of biliverdin to a radical cation would agree with the results reported here. This reaction pathway consisting of a primary one electron oxidation has already been proposed [5c, 9b] and is discussed below.

DMF solutions of the Zn complexes of **A** or **B** react with iodine more efficiently than the corresponding solutions without the addition of Zn(II) (see Exp. Part). Such a difference in reactivities agrees with the lower anodic potentials of the lactam NH deprotonated bilirubins and biliverdins, structures which are present in these Zn(II) complexes [1, 12c].

The structure of the transition metal complexes of bile pigments is not well known, because of their low stability constants and of the presence of more than one form in equilibrium, depending on the experimental conditions [12]. As a consequence of the necessary NH deprotonation and of the low stability constants, complex formation depends on the *pH* of the medium. This explains the differences reported in literature about the formation of some of these complexes: in *DMF* **A** does not form the Zn(II) complex using ZnI_2 [15], but according to the results reported here, the complex is formed if an excess of zinc acetate is used, i.e. the hydrolysis of the metal acetate salts seems to determine the formation of complexes of bile pigments with transition metals [16]. The structural analogy between the lactam NH deprotonated biliverdin anion and the biliverdin-Zn complex is shown by the corresponding UV/Vis spectra: The UV/Vis spectra in *DMF* of the Zn complex of **B** and of its dimethyl ester [12c] are very similar and both are significantly similar to those of lactam NH deprotonated biliverdins lacking free propionic acid substituents [17] (e.g. the diester **C** and the alkyl substituted biliverdin **D**).

The Zn complexes of **A** show a high reactivity towards iodine: addition of one equimolar amount of iodine results in a mixture containing a small amount of the initial bilirubin, a significant amount of the biliverdin-Zn complex (λ_{max} 715 nm), and as principal component the Zn complex of a bilipurpurin or biliviolin (λ_{max} 648 nm) showing a red fluorescence. This change in colour, together with a red fluorescence, is characteristic for the test of bilirubins using Zn(II) acetate plus iodine [6a]. Bromine in turn reacts with the Zn complex of **A** to give the biliverdin and bilipurpurin Zn complexes to a lesser extent. However, the decrease in intensity of the bilirubin-Zn complex UV/Vis absorption band is higher with bromine than with the same molar ratio of iodine and with the first, it is similar to that of uncomplexed **A** with bromine. Possibly, the enhanced reactivity of the Zn complex allows, in the case of bromine, other reactions of the π system in addition to those performed by iodine. Among those, one can speculate with direct oxidation towards propent-dyopent derivatives [18].

In the case of the Zn complex solutions of **B** reaction with iodine takes place at an equimolar ratio, but bromine needs a twofold ratio to give similar amounts of bilipurpurin and biliviolin complexes. In this latter case, the presence of absorption bands near 546 and 512 nm (which are not present when iodine is used) suggests further oxidation of the primary reaction products of the biliverdin Zn complex; towards urobilinoid- or dipyrrin-Zn complexes; i.e., the enhanced reactivity of the biliverdin-Zn complex allows either reaction of a second bromine on the initial substrate or bromine attack on the bilipurpurins and biliviolin Zn complexes, which result as primary reaction products.

The enhanced reactivity of the Zn complexes of bile pigments can be attributed to both, higher reactivity towards electrophiles, as a result of their higher electron density, and to the lower oxidation potential of the metal complexes compared to the free bile pigments [12c].

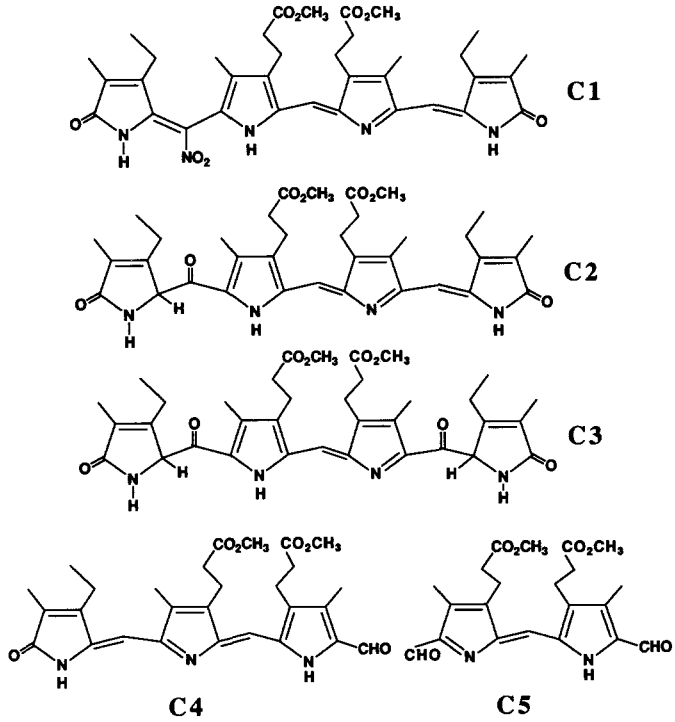
Bilipurpurins and Biliviolins from the Reaction of Mesobiliverdin XIIIα Dimethyl Ester with Nitric Acid

The study of the reaction products from the Gmelin reaction by Siedel [4b–d] had been performed upon the "symmetric" mesobilirubin XIIIα dimethyl ester in order to reduce the number of possible reaction products. We have followed the same strategy but started with the biliverdin **C**, the product corresponding to the first colour change observed during Gmelin test. The reaction was quenched [4b–c] at the biliviolin stage after addition of an aqueous NaOH solution. Preparative thin layer chromatography on silica gel using CH_2Cl_2/CH_3OH, 25:1 as eluent, yielded five fractions (*Rf*: 0.65 violet, 0.56 red, 0.50 blue, 0.45 red and 0.36 blue-violet). Siedel used column chromatography on talcum powder for this separation.

The blue fraction (*Rf* 0.50) was identified as unreacted biliverdin **C**.

We identified the fastest migrating fraction as the compound called by Siedel mesobilipurpurin XIIIα dimethyl ester (619 nm Zn^{++}), and later identified [6c] as 5-nitromesobiliverdin XIIIα dimethyl ester **C1**.

The red fraction of *Rf* 0.56 shows physical data very similar to those of the fraction called by Siedel [4b–c] mesobilipurpurin XIIIα dimethyl ester (622 nm Zn^{++}). For this bilipurpurin the structure of bilin-1,5,19-trione **C2** had been postulated [4b]. However, our spectral data permit to assign to this compound the structure of tripyrrin 14-carbaldehyde **C4** [^{1}H-NMR, MS (EI and FAB ionizating modes) and IR]. The physical and additional experimental data reported by Siedel for this fraction also agree with this tripyrrinone structure. The fluorescence spectrum of **C4** shows, by excitation at its absorption bands, emission at the red



region (λ_{em} 678 nm), especially intense for its Zn complex (λ_{em} 653). The physical properties of our *Rf* 0.56 fraction are slightly different to those described by Siedel for mesobilipurpurin XIIIα dimethyl ester (622 nm Zn^{++}). However, the small differences in melting point, elemental analysis and UV/Vis spectra observed must be due to the contamination of Siedel's sample by the bilipurpurin **C2** [in our chromatography *Rf* 0.45; see below]. Differences in the chromatographic behaviour of the reaction mixture under the conditions used by Siedel (talcum powder) and ours (silica gel) would make both results compatible.

The physical data of the red fraction of *Rf* 0.45 do not agree with any of the fractions isolated by Siedel. A structure of bilipurpurin of keto type (**C2**) can be assigned on the basis of our spectral data. However, this fraction was always contaminated with small percentages of additional compounds (with more than 85% corresponding to structure **C2**; ^{1}H-NMR, see Exp. Part). MS spectra (FAB): $(M+1)^+ = 631$ *m/z*, $M^+ = 630$ and the typical fragmentation of a ketone to a ketyl cation $(M-124)^+ = 506$ (loss of the final end ring) confirm the **C2** structure. In addition, this fraction shows a typical biliviolin or bilipurpurin UV/Vis absorption as well as its Zn complex. The fluorescence of **C2** (λ_{em} 585 nm), is different from that of a tripyrrin type of structure, such as **C4** (λ_{em} 653 nm), an observation that can be of interest to differentiate bilipurpurins of keto type and tripyrrin biliviolins. The main impurity of this fraction could be the choletelin of diketo type **C3**, on the basis of the MS (FAB) peaks at 647 and 669 *m/z*, $(M+1)^+$ and $(M+Na)^+$, respectively, as well as of the shoulder at about 470 nm in the UV/Vis spectrum. This absorption is characteristic of the bilen-*b*-1,19-dione chromophore.

The slowest migrating fraction could not be identified (see Exp. Part). However, its UV/Vis spectrum shows a first absorption in the bilipurpurin region, but lacking the characteristic double band, and a second absorption corresponding to biliverdins (see Exp. Part). The non fluorescent Zn complex excludes the possibility of a mixture containing bilipurpurins. The ^{1}H-NMR spectrum shows several olefinic signals, and the MS (FAB with *m/z* at 1000) contains a peak at 615 *m/z*, the same value corresponding to mesobiliverdin dimethyl ester, a compound which must be excluded however, on the basis of its very different *Rf* value. Additional peaks appear at higher mass (e.g. 748, 959 *m/z*). We suggest a dimeric or polymeric structure formed in a reaction of the radical cation or neutral radical which originated in the first reaction steps (see below).

Oxidation of Etiobiliverdin IVγ (3,8,12,17-Tetraethyl-2,7,13,18-tetramethylbilin-1,19(21H,24H)-dione) (**D**) *with Thallium(III) Triacetate and Lead(IV) Tetraacetate*

The experiments described by Smith [5b–c] on the oxidation of **D** with thallium triacetate and lead tetraacetate were repeated and the oxidation products isolated and identified.

The comparison of the reaction products obtained with both oxidizing agents at different reaction times (45', 24 h and 72 h) do not show any significant difference: the same reaction products in analogous relative ratios were obtained at each reaction time. Thin layer chromatographic (TLC) analysis (benzene:ether 1:1, or

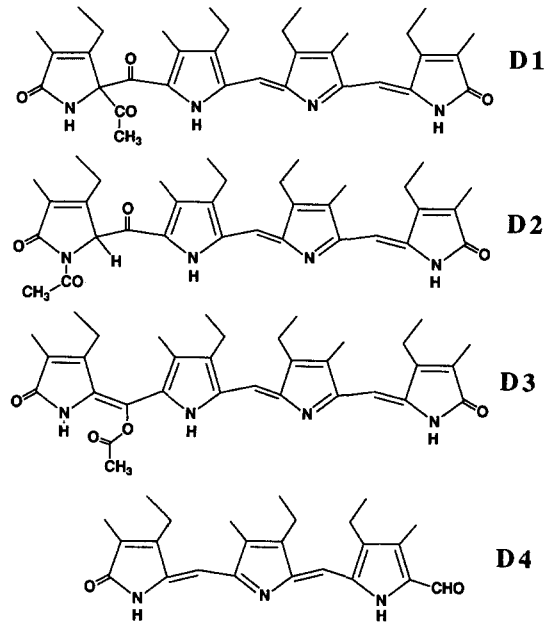
CH_2Cl_2:methanol 30:1) of the reaction mixture results in five fractions, although in the solvent system used by Smith [5c] only four fractions could be detected. We found the benzene:ether mixture especially suitable to separate the two fastest (F1 and F2, from the front to the origin respectively) and the slowest (F5) migrating fractions, while under these conditions the two remaining middle fractions (F3 and F4) were isolated as a single fraction, which was subsequently resolved by PTLC in CH_2Cl_2:methanol 30:1 (inversion of the elution order!; see Exp. Part).

F2 was identified by TLC, UV/Vis, IR and ^{1}H-NMR as unreacted starting biliverdin **D**.

F4 was identified as the tripyrrin-14-carbaldehyde **D4** (m.p., UV/Vis, IR, ^{1}H-NMR, ^{13}C-NMR), already described by Smith from an analogous reaction mixture [5c].

F1 shows the same physical and spectroscopic data as the product assigned by Smith [5c] with the structure of 4-acetyl-bilin-1,5,19-trione **D1**. However, a more detailed examination of its ^{1}H-NMR spectrum (a 200 MHz compared to 100 MHz instrument in Ref. [5c]), together with its – until now unreported – ^{13}C-NMR spectrum, points to structure **D2**. The ^{1}H-NMR singlet near 5.9 (ppm, δ) corresponds to two protons, not to one as assigned in Ref. [5c]: under high resolution conditions this signal is shown to contain a narrow singlet (5.88 ppm) and a wider one (5.89 ppm). Furthermore, the ^{13}C-NMR spectrum (DEPT experiment) shows only two olefinic =CH signals (113.6 and 95.9 ppm), and one CH signal corresponding to a tertiary sp^3 carbon atom (64.1 ppm): such a CH group, with low field resonating H and C nuclei, does not agree with structure **D1** and confirms structure **D2**. The remaining ^{13}C-NMR signals also agree with structure **D2**: e.g. only one carbonyl group of keto type (184.8 ppm) and three carboxyamide carbonyl groups (172.1, 171.8 and 171.1 ppm) are detected. MS fragmentation ($M^+ = 556$ m/z), with an important peak at 390 m/z $(M-194)^+$, shows the formation of a ketyl cation by loss of the 3-pyrrolin-2-one ring and of the acetyl group. In addition, its characteristic bilipurpurin absorption UV/Vis spectrum seems to exclude any additional *N* substitution: configurational and conformational hindrance would be expected to change the shape of the π conjugated chain and consequently its UV/Vis absorption spectrum.

F3 is the main reaction product ($\approx 30\%$ *w/w*) at short reaction time and is not described by Smith because of the coincidence of its *Rf* in the eluting system toluene:ethyl acetate 8:2 with the initial biliverdin **D**, however it can be separated by PTLC using successive chromatography in two eluting systems. Its UV/Vis spectrum shows a wide absorption near 550 nm, but its shape is not characteristic for bilipurpurin or biliviolin absorption (double band). A second, intense absorption at 364 nm together with a smaller absorption at 315 nm in place of a unique absorption about 320 nm confirm the differences in absorption between this compound and a bilipurpurin or biliviolin. The MS spectrum is very similar to that of the *N*-acetyl-bilintrione **D2** of fraction F1. This fact points to an isomer of **D2**. Actually, **D3** (F3) has been observed to decompose into **D2** (F1). ^{1}H-NMR and ^{13}C-NMR spectra indicate the presence of five non-ethylic methyl groups, two =CH groups of a tripyrrin residue and three carbonyl groups of lactam or ester type (172.3, 171.2, 170.4 ppm). The IR spectrum shows, in addition to the lactam groups, a distinct absorption at 1770 cm^{-1} plus a C=O stretching absorption at



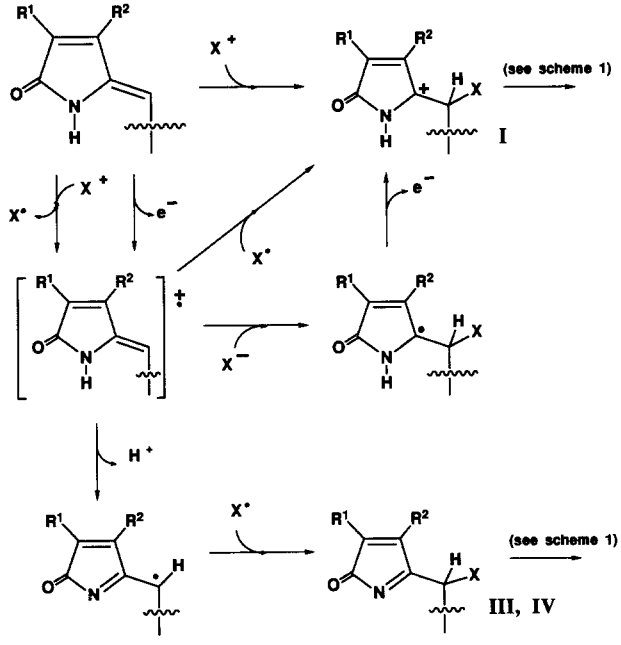
about 1200 cm^{-1}. In conclusion, these spectral data point to the presence of an enol acetate group at C5, i.e. to structure **D3**. Such a structure is confirmed by its chemical behaviour. **D3** decomposes in wet methanol giving **D2** besides **D4** and the compound of fraction F5. This observation is confirmed by the changes in each fraction yield with time (see below). In fact, **D3**, although not isolated, had already been proposed by Smith as the first reaction product or intermediate in the reaction of **D** with thallium triacetate and lead tetra-acetate [5c]. The absorption spectrum of **D3** (see above) should be attributed to a *Z* configuration at the exocyclic double bond at C4, as a result of the substitution at C5 [19] and, likely, to the presence of an internal hydrogen bond with the lactam NH. As a matter of fact, this is an analogous structure, (stabilized by similar effects) to the 5-nitro bilindiones [9b]; however, the UV/Vis absorptions near 364 nm and likely at about 600 nm (included on the wide band centered at 550 nm) might correspond to the presence of additional conformers with a π system of a biliverdin type.

The compound in F5 shows a typical biliviolin or bilipurpurin absorption spectrum. This compound could not been identified because it could not be isolated pure since it decomposes giving **D2** and other two unidentified derivatives, one shows a biliviolin-like spectrum and the other one a bilicholetelin-like one. This compound in F5 is also obtained from the decomposition of **D3** (without detection of ethyl methyl maleimide!). Its MS is similar to those of **D2** and **D3**. The ^{1}H-NMR spectrum indicates absence of C_{2v} symmetry, two olefinic signals corresponding to protons at C10 and C15 carbon atoms, one signal at 6.05 ppm, i.e. a similar chemical shift to the proton at C4 in **D2**, one sharp NH signal (NH of a 3-pyrrolin-2-one group?) and five singlet methyl groups. Its IR spectrum does not show a C=O absorption corresponding to an enol acetate, ester or to the N,N-diketyl group of **D2**. In conclusion, F5 is likely to be a **D2** isomer, with the acetyl group bonded to a nitrogen other than N21.

By increasing the oxidation time, the relative amounts of each fraction change: an expected decrease of the initial biliverdin (**D**), increase of the N-acetyl derivative **D2**, decrease of the enol acetate **D3** and increase of the compound in fraction F5. Furthermore, a relative increase of the tripyrrinone aldehyde **D4** is observed in lengthening the reaction time from 45′ to 24 h, but it could not be detected at all after 72 h. This result must be attributed to the easy oxidation of the carbaldehyde group. Hydrolysis in methanol of **D3** gives, in addition to **D2**, also **D4** and the compound of fraction F5, which agrees with the expected reactivity of an enol acetate.

Concluding Remarks

There is strong evidence that aromatic electrophilic substitution on systems with low oxidation potential, i.e. highly reactive systems, does not proceed via an intermediate carbocation, but rather through an initial electron transfer followed by coupling of the resulting radical species [21]. For instance, this reaction mechanism has been proposed in the case of porphyrin nitration with N_2O_4, because of the low oxidation potential of porphyrins [22]. Biliverdins have an even lower oxidation potential (about 0.2–0.3 V lower) and consequently an electron transfer mechanism in the attack of most of the electrophiles described here should be preferred. In this respect, 5-nitrobilin-1,19-dione fermation from bilin-1,19-dione nitration using nitrous acid is very significant. In this case, the first reaction step should be biliverdin oxidation to the radical cation, which can either couple to NO_2 and loose a proton – as proposed [9b] – or undergo a subsequent one electron oxidation and proton loss to add a nitrite nucleophile (Scheme 2).



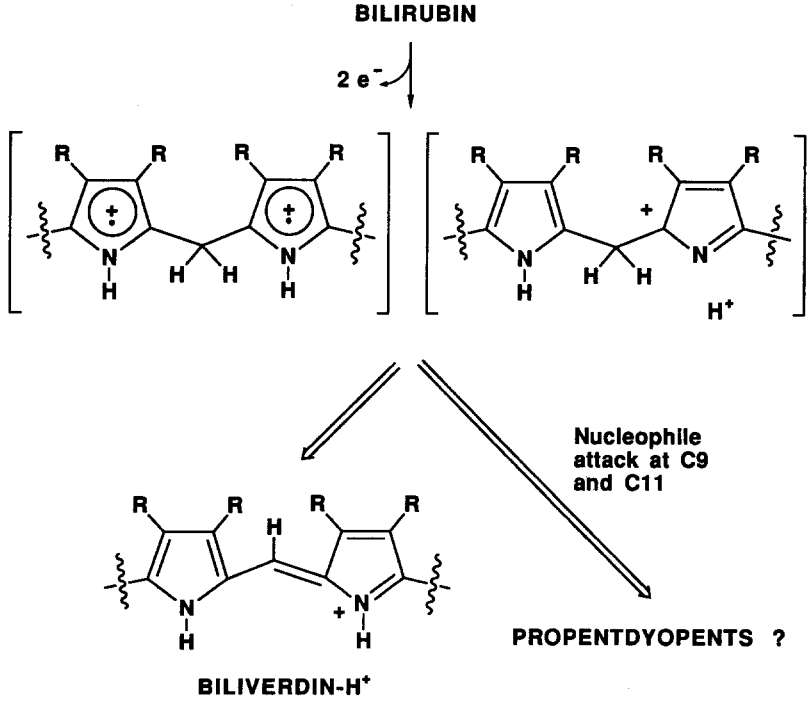
Scheme 2

The similar reaction products obtained by chemical and electrochemical [e.g. 5] oxidation of biliverdins and the same nature of these reaction products agree with reaction mechanisms involving nucleophile capture following loss of one electron. This is what probably occurs during the chemical oxidation of **D** in acetic acid to give **D3**.

Concerning the reactivity towards halogens, even though it is not possible to unequivocally distinguish between electrophilic attack or electron transfer, the results of the reaction of **A**, **B** and their Zn(II) complexes with iodine or bromine indicate a dependence on the substrate oxidation potential and on the halogen reduction potential for the first step i.e. towards a mechanism based on an initial electron transfer.

The expected orientation of electrophilic attack to biliverdins and bilirubins is basically the same as that of their respective radical cations towards nucleophiles. Simple semi-empirical reactivity calculations – based upon the HOMO coefficients – which predict the position for electrophilic attack in biliverdins and bilirubins (C5 in the case of biliverdins and C9 in the case of bilirubins [11]), cannot distinguish between reactivity towards electrophiles and the most reactive positions for nucleophilic attack to the corresponding radical cations.

Electrochemical evidence shows oxidation of bilirubins to occur through a two electron process [1, 3]: however, whether one electron is lost in each dipyrrinone half or two electrons are lost in the same dipyrrinone residue remains as an open question (see Scheme 3). Reactions of bilirubins towards electron deficient species



Scheme 3

show regiospecificity on positions C9 and C11 [11, 20]. The two most likely mechanisms for reaction of bilirubins with electron deficient species involve 1) bilirubin oxidation followed by nucleophile attack at C9 or 2) direct electrophilic attack to C9: The last mechanism seems to operate for non-oxidizing electrophiles, e.g. protons in the so-called "scrambling" reaction of bilirubins [12a, 20], and we assume oxidation to the radical cation as the most suitable mechanism for oxidizing electrophiles, e.g. halogens. Formation of the radical cation at C9 would also explain production of propentdyopents in alkaline media [18], this probably being the reaction pathway for the easily oxidizable bilirubin-Zn complexes. Similarly, the elusive, yet observed by some authors, scrambling reaction of bilirubins in base could involve radical cations obtained by oxidation of the easily oxidizable NH deprotonated bilirubin [1].

Experimental Part

Bilirubin IXα was of commercial origin (Janssen Chimica). The synthesis and properties of the following compounds are described in literature: biliverdin IXα [23], biliverdin XIIIα dimethyl ester [24, 25], etiobiliverdin IVγ (3,8,12,17-triethyl-2,7,13,18-trimethyl-bilin-1,19-dione) [26].

Thin layer chromatography (TLC) analysis was performed on silica gel with fluorescence indicator chromatofoils (DC-Alufolien F_{254}, Merck). Preparative thin layer chromatography (PTLC) was performed on 1 mm thick silica gel (Merck) plates.

Melting points were determined on a Kofler-Reichert microhot stage apparatus. UV/Vis spectra were recorded on a Perkin-Elmer Lambda 5 instrument. IR spectra were recorded on a Perkin-Elmer 681 spectrometer. MS on a Hewlett-Packard 5988A instrument equipped for FAB analysis with a Capillaritron Frasor (Xe). ^{1}H-NMR spectra were determined on a Geminis 200 Varian (200 MHz) instrument: chemical shifts are referred to TMS as internal reference. Fluorescence spectra were performed in an Aminco SPF500 ratio spectrofluorometer using solutions of the chromophore in CH_2Cl_2 of absorbance at λ_{max} 0.1.

Oxidation of Bilirubin IXα (**B**), *Biliverdin IXα* (**C**) *and their* Zn(II) *Complexes with Bromine and Iodine*

Exact volumes of a bromine or iodine $1.5 \cdot 10^{-3}$ mol l$^-$ solution in *DMF* (for residue analysis, Merck) were added to a $1.5 \cdot 10^{-5}$ mol l^{-1} solution of **A** or **B** in *DMF*. The UV/Vis spectral changes were recorded. These changes are fast and remain practically stable under water and oxygen exclusion. Oxidation experiments on the Zn(II) complexes of **A** and **B** were performed using similar solutions to which a zinc acetate excess was added to fully shift (by UV/Vis) the equilibrium towards the bile pigment Zn complex. For **A**, an eight fold molar excess was needed [isosbestic point; the complex showed λ_{max} 523 nm and 490 nm (sh)], while for **B** a twofold molar excess was sufficient [isosbestic point; the complex showed λ_{max} 713 nm, 389 nm (intensity ratio; 1:2) and 660 nm (sh)].

Experimental Procedure to Reproduce Siedel's Results of the Gmelin Reaction [4b–c]

The experimental procedure adapted from Ref. [4c] was the following. To a solution of 30 mg mesobiliverdin XIIIα dimethyl ester (**C**) (3,17-diethyl-8,12-di(2-methoxycarbonylethyl)-2,7,13,18-tetramethyl-bilin-1,19(21*H*,24*H*)-dione) in 20 ml CH_2Cl_2 contained in a separatory funnel a solution of 5 mg $NaNO_2$ in 4 ml 2 mol l^{-1} HNO_3 was added in several fractions under shaking. Addition was continued until the mixture showed a red-violet colour, at which point 4 ml of a 2 mol l^{-1} NaOH solution were added at once. The organic phase was separated, dried with anhydrous Na_2SO_4 and

evaporated under vacuum. The residue was analyzed by analytical TLC (see text) and separated by PTLC using CH_2Cl_2/CH_3OH, (25:1) as eluent. Five fractions were isolated (TLC *Rf*: 0.65, 0.56, 0.50, 0.45 and 0.36).

Fraction of Rf 0.65; 5-Nitromesobiliverdin XIIIα Dimethyl Ester [(3,17-Diethyl-8,12-di(2-methoxycarbonylethyl)-2,7,13,18-tetramethyl-5-nitrobilin-1,19(21H,24H)-dione] (**C1**)

Isolated in 30% yield and identified as **C1** [described by Siedel [4c] as the so-called mesobilipurpurin XIIIα dimethyl ester (619 nm Zn^{++}] according to its UV/Vis, IR, ^{1}H-NMR, MS(FAB, Xe) spectra, and melting point [6c].

Fraction of Rf 0.56; 3,17-Diethyl-14-formyl-8,12-di(2-methoxycarbonylethyl)-2,7,13-trimethyl-tripyrrin-1,19-dione (**C4**)

Isolated in 23% yield and identified as **C4** on the basis of its physical and spectroscopic data. These are very similar to those of the so-called mesobilipurpurin XIIIα dimethyl ester (622 nm, Zn^{++}) [4c]. According to our results, the last sample would consist of **C4** contaminated by the bilipurpurin identified as **C2** in this work (fraction of *Rf* 0.45 see below).

M.p. 155–157 °C. MS (FAB, Xe, *m/z*): 530 $(M+Na)^+$, 508 $(M+1)^+$, 507 $(M)^+$. UV/Vis [λ_{max} nm (ε)]: CH_2Cl_2: 535(10000), 504(10300), 470 sh, 320(24700); CH_3OH: 527, 499, 317; Zn(II) complex (Ethanol-CH_2Cl_2-Zn acetate): 617(12000), 573(7800), 331(20000).

Fluorescence spectrum: $\lambda_{exc} = 504$, 535 nm, $\lambda_{em} = 678$ nm. Fluorescence spectrum Zn complex: $\lambda_{exc} = 334$, 577 nm, $\lambda_{em} = 653$ nm. IR (film, cm^{-1}): 3300–3350, 1735, 1710, ^{1}H-NMR ($CDCl_3$, δ ppm): 9.82 (s, –CHO), 6.84 (s, C10 =CH), 5.90 (s, C5 =CH), 3.68 (s, $-COOCH_3$), 3.66 (s, $-COOCH_3$), 3.00 (t, $J = 7.1$ Hz, 2 H), 2.91 (t, $J = 7.1$ Hz, 2 H), 2.57 (t, $J = 7.1$ Hz, 4 H), 2.54 (q, $J = 7.9$ Hz, 2 H), 2.36 (s, C13 $-CH_3$), 2.09 (s, C7 $-CH_3$), 1.98 (s, C2 $-CH_3$), 1.20 (t, $J = 7.9$ Hz, 3 H).

Fraction of Rf 0.50; Mesobiliverdin XIIIα Dimethyl Ester (**C**)

Isolated in 6% yield and identified as unreacted **C**.

Fraction of Rf 0.45; 3,17-Diethyl-8,12-di(2-methoxycarbonylethyl)-2,7,13-trimethylbilin-1,5,19(4H,21H,24H)-trione (**C2**)

Isolated in 11% yield and identified as impure **C2** (>85%, from ^{1}H-NMR analysis). According to the UV/Vis sh. near 470 nm and the MS peaks, the accompanying impurity is probably 3,17-diethyl-8,12-di(2-methoxycarbonylethyl)2,7,13-trimethylbilin-1,5,15,19(4H, 16H, 21H, 24H)-tetraone (**C3**).

M.p. 209–212 °C. MS (FAB, Xe, *m/z*): **C2**; 631 (M+1)+, 630 (*M*)+, 506 (M−124)+. **C3**: 669 $(M+Na)^+$, 647 $(M+1)^+$. UV/Vis [λ_{max} nm (ε)]: CH_2Cl_2: 543 (12000), 506 (10500), 324 (26500), (470 sh from **C4**); CH_3OH: 537, 502, 322 Zn(II) Complex (CH_2Cl_2-Ethanol-Zn acetate): (stable bands after several minutes) 626 (14000), 583 (8000), 336 (18000).

Fluorescence spectrum: $\lambda_{exc} = 324$, 506 nm, $\lambda_{em} = 585$. Fluorescence spectrum Zn complex: $\lambda_{exc} = 336$, 630 nm, $\lambda_{em} = 655$ nm. IR (film, cm^{-1}): 3340, 1735, 1710, 1675, 1655. ^{1}H-NMR ($CDCl_3$, δ ppm): only main peaks, those assigned to impurities not given; 11.7 (s, NH), 10.23 (s, NH), 8.21 (s, NH), 6.97 (s, C10 =CH), 6.22 (s, C4 CH), 6.01 (s, C15 =CH), 3.68 (s, $-CO_2CH_3$), 3.64 (s, $-CO_2CH_3$), 3.00, 2.95 (2 t, $J = 7.1$ two $-CH_2-CH_2-COOCH_3$) 2.65–2.35 (m, two $-CH_2-CH_2-COOCH_3$) and two $-CH_2-CH_3$), 2.34 (s, C13 $-CH_3$), 2.11 (s, C7 $-CH_3$), 1.94 (s, C2 $-CH_3$), 1.88 (s, C18 $-CH_3$), 1.20 (t, $J = 7.9$ Hz, $-CH_2-CH_3$), 1.09 (t, $J = 7.9$ Hz, C17 $-CH_2-CH_3$).

Fraction of Rf 0.36

Isolated in 6% yield. The following data suggest a dimeric structure containing a biliverdin and bilipurpurin or biliviolin units.

M.p. 181–183 °C. MS (FAB, Xe, *m/z*): 927, 748, 615, 480, 467, 332. UV/Vis [λ_{max} nm]: CH_2Cl_2: 640 sh, 571 (wide), 370, 326 (intensity ratio 1:1.3:2.2); CH3OH: 558, 368, 323.

Oxidation of Etiobiliverdin IVγ (3,8,12,17-triethyl-2,7,13,18-trimethylbilin-1,19(21H,24H)-dione) (**D**), *with Thallium(III) Triacetate or Lead(IV) Tetraacetate*

The procedure described in Ref. [5c] was followed. To 30 mg **D** (0.060 mmol) in 3 ml HO*Ac*, was added under stirring, in several steps and during 30 minutes, an equimolar amount of the oxidizing agent (lead tetraacetate or thallium triacetate, crystallized in *Ac*OH prior to use). After 45 min, the mixture was poured onto 300 ml water and neutralized to $pH = 10$ (4% aq. Na_2CO_3 solution or concentrated aq. ammonia). The precipitated pigments were extracted with CH_2Cl_2, the organic phase washed with water, dried with Na_2SO_4 and evaporated to dryness. PTLC (eluent benzene:ether 1:1) permitted to separate the two fastest migrating fractions (F1 and F2), a mixture of the two middle ones (F3 plus F4) and the slowest migrating fraction (F5). F3 and F4 were separated by PTLC in CH_2Cl_2:CH_3OH 30:1. Table 1 summarizes the analytical TLC *Rf* values of each of these fractions in our two eluents, as well as in the eluting system (toluene:ethyl acetate 8:2) used in Ref. [5c] (the same *Rf* value of F2 and F3 in this last system, which explains why fraction F3, i.e. compound **D3**, was not detected by the authors of Ref. [5c]).

Additional oxidation experiments were performed following the same procedure but increasing the reaction time from 45 min to 24 and 72 h: the amounts of isolated fraction (mg per 100 mg of initial **D**) at these three reaction times were: F1, 7, 13 and 27; F2, 24, 23 and 11; F3, 34, 19, and 17; F4, 5, 8 and zero; F5, 6, 22 and 18.

Fraction F1: 21-Acetyl-3,8,12,17-tetraethyl-2,7,13,18-tetramethylbilin-1,5,19(5H,21H,24H)-trione (**D2**) *and Ethyl Methyl Maleimide*

This fraction contains small amounts of ethyl methyl maleimide, identified by its ^{1}H-NMR spectrum and by TLC (comparison with a pure sample). Removal of this imide can be achieved by fast extraction with a cold alkaline aqueous solution. The remaining compound shows the same physical properties and spectral data (m.p., IR, UV/Vis, MS, and ^{1}H-NMR) as the compound assigned to structure **D1** in Ref. [5c]. However, the ^{1}H-NMR 5.88 ppm singlet assigned in Ref. [5c] to one of the =CH groups was shown to consist of two singlets [5.88 and 5.89 ppm] corresponding to two protons. The ^{13}C-NMR spectrum (DEPT) confirms structure **D2** for this compound.

^{13}C-NMR ($CDCl_3$, δ ppm, DEPT): 184.8 (keto C=O), 172.1, 171.8 and 171.1 (three C=O), 169.6 (C=N), 156.2, 153.1, 147.6, 146.5, 144.8, 135.4, 133.5, 133.2, 132.5, 130.1, 130.0, 129.4 (12 quaternary sp^2 C), 113.6 (C10 =CH), 95.9 (C15), 64.1 (C4, CH), 23.9 (CH_3–CO), 20.5, 17.7, 17.4, 17.1 (four CH_3–CH_2), 15.9, 15.8, 14.1, 11.9 (four –CH_2–CH_3), 10.5, 9.5, 8.5, 8.3 (four CH_3–C=).

Table 1. TLC (SiO_2) *Rf* values of the five fractions isolated from the mixture obtained in the oxidation of etiobiliverdin IVγ (**D**) with lead(IV) tetra-acetate or thallium(III) triacetate

Fraction (Colour)	Benzene:Ether (1:1)	CH_2Cl_2:*Me*OH (30:1)	Toluene:Ethyl acetate (8:2)
F1 (red)	0.53	0.63	0.32
F2 (blue)	0.34	0.60	0.18
F3 (blue)	0.26	0.47	0.15
F4 (red)	0.24	0.22	0.15
F5 (violet)	0.1	0.18	0.1

Fraction F2: Etiobiliverdin IVγ [3,8,12,17-Tetraethyl-2,7,13,18-tetramethylbilin-1,19(21H,24H)-dione] (**D**)

Identified as the initial reaction product (**D**) by TLC, UV/Vis, IR and ^{1}H-NMR spectra.

Fraction F3: [5-Acetoxy-3,8,12,17-tetraethyl-2,7,13,18-tetramethylbilin-1,19(21H,24H)-dione] (**D3**)

Identified as **D3**, according to the following spectral and physical data.

M.p. 179–181 °C. MS(El, 70 eV: *m/z*): 556 $(M)^+$, 390, 282, 268, 178. UV/Vis [λ_{max} nm (ε)]: $CHCl_3$: 550 (9700), 364 (16500), 315 (13800). IR (film: cm^{-1}): 3300 (NH), 1770 (C=O vinyl acetate), 1700 (C=O), 1680, 1600, 1220 (C–O). ^{1}H-NMR ($CDCl_3$, δ ppm): 6.73 (s, C10 =CH), 5.94 (s, C15 =CH), 2.68–2.46 (m, four $\mathbf{CH_2}$–CH_3), 2.32 (s, –CH_3), 2.07 (two s, two –CH_3), 1.90 (s, –CH_3), 1.88 (s, –CH_3), 1.25–1.12 (m, four $\mathbf{CH_3}$–CH_2–). ^{13}C-NMR ($CDCl_3$, δ ppm, DEPT): 172.3, 171.2, 170.4 (three C=O), 160.5 (C=N), 146.9, 146.7, 145.4, 145.1, 141.6, 138.5, 135.9, 134.0, 131.0, 130.5, 130.2, 128.9, 125.8, 123.5 (fourteen sp^2 quaternary C atoms), 114.8 (C10 =CH), 96.6 (C15 =CH), 20.5 ($\mathbf{CH_3}$–COO–), 20.2, 17.8 (two –$\mathbf{CH_2}$–CH_3) 17.6 (two –$\mathbf{CH_2}$–CH_3), 15.9 (two $\mathbf{CH_3}$–CH_2–), 14.3, 13.3 (two $\mathbf{CH_3}$–CH_2–), 10.0, 9.4, 8.3, 8.0, (four –CH_3).

The enol acetate functional group was confirmed by heating 5 mg of F3 in 3 ml methanol to reflux during 40 minutes: partial decomposition under formation of F1, F4 and F5 occurs.

Fraction F4: 3,8,12-Triethyl-14-formyl-2,7,13-trimethyltripyrrin-1(15H, 16H)-one (**D4**)

Physical and spectral data were those described in the literature [5c].

^{13}C-NMR ($CDCl_3$, δ ppm, DEPT): 178.2 (–CHO), 172.1 (C=O), 169.6 (C=N), 153.6, 147.3, 146.3, 145.3, 134.1, 133.6, 133.3, 133.1, 130.4, 128.5 (ten sp^2 quaternary C atoms), 113.4 (C10 =CH), 95.6 (C5 =CH), 17.7, 17.6, 17.1 (three –$\mathbf{CH_2}$–CH_3), 15.9, 15.7, 14.2 (three $\mathbf{CH_3}$–CH_2), 9.5, 8.4, 8.3 (three –CH_3).

Fraction F5

A pure compound could not be obtained, basically as a result of its easy decomposition to **D2**.

UV/Vis [λ_{max} nm (ε)]: $CHCl_3$: 545, 510, 324 (intensity ratio; 0.9:1.0:2.0) and 364 sh. MS(El, 70 eV:*m/z*): Same spectrum as **D2** and **D3**. ^{1}H-NMR ($CDCl_3$, δ ppm): main signals assigned to the most abundant compound; 8.22 (NH), 6.74 (s, =CH), 6.05 (d, $J = 2$ Hz?), 5.90 (s, =CH), 2.70–2.40 (four –$\mathbf{CH_2}$–CH_3) 2.35 (s, CH_3), 2.11 (s, CH_3), 2.02 (s, CH_3), 1.83 (s, CH_3), 1.78 (s, CH_3), between 1.35–0.78 (four $\mathbf{CH_3}$–CH_2–).

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References

[1] Part 14. Ribó J. M., Farrera J.-A., Claret J., Grubmayr K. (1992) Bioelectrochem. Bioenerg. (J. Electroanal. Chem.) accepted for publication

[2] For bile pigment nomenclature see, IUPAC and IUB (1987) Pure and Appl. Chem. **59**: 779

[3] a) van Norman J. D. (1973) Anal. Chem. **45**: 173. b) Slifstein Ch., Ariel M. (1973) J. Electroanal. Chem. **48**: 447. c) van Norman J. D., Szentirmay R. (1974) Anal. Chem. **46**: 1456. d) Slifstein Ch., Ariel M. (1977) J. Electroanal. Chem. **75**: 551. e) Longhi P., Manitto P., Monti D., Mussini T., Rondinini S. (1981) Electrochim. Acta **26**: 541. f) Wang J., Luo D. B., Farias P. A. M. (1985) J. Electroanal. Chem. **185**: 61. g) Moussa F., Kanoute G., Herrenknecht C., Levillain P., Trivin F. (1988) Anal. Chem. **60**: 1179

[4] a) Fischer H., Orth H. (1937) In: Die Chemie des Pyrrols, II. Band, 1. Hälfte, pp. 631 and 712. b) Siedel W., Fröwis W. (1941) Hoppe Seyler's Z. Physiol. Chem. **56**: 37. c) Siedel W., Grams E. (1941) Hoppe Seyler's Z. physiol. Chem. **56**: 49. d) Siedel W. (1943) Chemie **56**: 185. e) Gossauer A., Plieninger H. (1979) In: Dolphin (ed.) The Porphyrins, vol. 6, part A. Academic Press, New York, p. 639

[5] a) von Dobeneck H., Sommer U., Brunner E., Lippacher E., Schnierle F. (1973) Liebig's Ann. Chem. **1973**: 1934. b) Eizavi F., Hudson M. F., Smith K. M. (1976) Tetrahedron Lett. **1976**: 3837. c) Eizavi F., Hudson M. F., Smith K. M. (1977) Tetrahedron **33**: 2959. d) Eizavi F., Lewis W. M., Smith K. M. (1977) Tetrahedron Lett. **1977**: 3083. e) Eizavi F., Lewis W. M., Smith K. M. (1979) J.C.S. Perkin I **1979**: 544

[6] a) Ref. 4a p. 632. b) Lemberg R., Lockwood W. H. (1939) J. Proc. R. Soc. New South Wales **72**: 69. c) Ribó J. M., Salgado A., Sesé M. L., Trull F. R., Vallès M. A. (1987) Tetrahedron **43**: 5321. d) Ref. 4e pp 612 and 640

[7] Pradko J. R., Pons S., Bandyopadhyay S., McAleer J. F., Scott Hinman A. (1984) Bioelectrochem. Bioenerg. **13**: 267. b) Cheng G., Yang Y., Dong S. (1991) Biolectrochem. Bioenerg. **26**: 345

[8] Falk H. (1989) In: The Chemistry of Linear Oligopyrroles and Bile Pigments. Springer Verlag, Berlin Heidelberg New York Tokyo, p. 45

[9] a) Bonfiglio J. V., Bonnett R., Hursthouse M. B., Malik K. M. A., Naithani S. C. (1977) J. Chem. Soc Chem. Comm. **1977**: 829. b) Bonfiglio J. V., Bonnett R., Buckley D., Hamzetash M. B., Hursthouse M. B., Malik K. M. A., Naithani S. C., Trotter J. (1982) J. Chem. Soc. Perkin I **1982**: 1291

[10] Ribó J. M., Trull F. (1983) Liebig's Ann. Chem. **1983**: 1

[11] a) Falk H., Gsaller H., Hubauer E., Müller N. (1985) Monatsh. Chem. **116**: 939. b) Caballol R., Ribó J. M., Trull F. R., Vallès A., (1987) Monatsh. Chem. **118**: 1011. c) For a general review on the reactivity of bile pigments see Ref. [8]

[12] a) McDonagh A. F. (1979) in Ref. [4e] p. 293. b) Ref. [8] p. 486. c) Fuhrhop J.-H., Salek A., Subramanian J., Mengersen C., Besecke S. (1975) Justus Liebig's Ann. Chem. **1975**: 1131

[13] Daroca A., Mercè R. Ribó J. M. Trull F. R., Vallès M. A. (1984) Monatsh. Chem. **115**: 357

[14] Ribó J. M., Vallès A. (1983) J. Chem. Res. (S) **1983**: 273 (M) 2501

[15] van Norman J., Szentimay R. (1974) Bioinorg. Chem. **4**: 37

[16] a) Falk H., privat communication. b) Farrera J.-A., Ribó J. M., unpublished results

[17] Anglada C., Claret, J., Crusats J., J.-A. Farrera, Ribó J. M., Trull F. R. (1990) Monatsh. Chem. **121**: 653

[18] von Dobeneck H., in Ref. [4e] p. 651

[19] Mercè R., Pujol M., Ribó J. M., Trull F. R., Valera G., Vallès M. A. (1986) Monatsh. Chem. **117**: 631

[20] Ref. [8] p. 502

[21] Perrin Ch. L. (1977) J. Am. Chem. Soc. **99**: 5516

[22] Liang-Chu Gong, Dolphin D. (1985) Can. J. Chem. **63**: 401

[23] McDonagh A. F., Palma L. A. (1980) Biochem. J. **189**: 193

[24] O'Carra P., Colleran E. (1941) J. Chromatog. **108**: 212

[25] Reisinger M., Trull F. R., Lightner D. (1985) J. Heterocycl. Chem. **22**: 1221

[26] Grunewald, J., Cullen R., Bredfeld J. (1975) Org. Prep. Proc. Int. **7**: 103

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