

## 115. Photochemistry of Biliverdin IX $\delta$ as a Model for the Study of the Photoproducts from Natural Biliverdin IX $\gamma$ (Pterobilin)

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### Summary

The configurations of biliverdin-IX  $\gamma$  and -IX  $\delta$  dimethyl esters **1** and **2** in solutions, have been studied using Nuclear-Overhauser-Effect (NOE) experiments. Irradiation (500-700 nm) of biliverdin IX  $\delta$  gave *syn-Z*  $\rightarrow$  *anti-E* isomerization of the central methine bridge and in aerated polar solutions, four new cyclized pigments were isolated for which structures **3**, **4**, **5** and **6** are proposed. Neobiliverdin IX  $\delta$  **3** is also formed in degassed solution ( $\Phi = 4 \cdot 10^{-5}$ ) but pigments **4**, **5** and **6** arise from photo-oxidation with O<sub>2</sub>. Biliverdin IX  $\delta$  appears to be a good model for the study of photo-reactions occurring on the vinyl groups of the natural biliverdin IX  $\gamma$  (pterobilin).

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**Introduction.** - Photo-excitation of bilirubin IX *a* in aerated solution leads to self-oxidation resulting in a variety of degradation products [1-6] such as maleimides, dipyrrole dialdehydes and propentdyopents. These reactions are self sensitized, involving triplet bilirubin (energy level 150 kJ mol<sup>-1</sup> [7]) and singlet oxygen [4] probably generated by energy transfer from triplet excited bilirubin. In oxygen-free medium, (*Z, Z*)-bilirubin IX *a* is isomerized to a mixture of (*Z, E*)-, (*E, Z*)- and (*E, E*)-isomers [8]. In some cases, photo-disproportion to III *a* and XIII *a* isomers [2], photo-oxidation to verdin [9] and addition of thiols, disulfides and alcohols to one vinyl group [10] [11] have been observed.

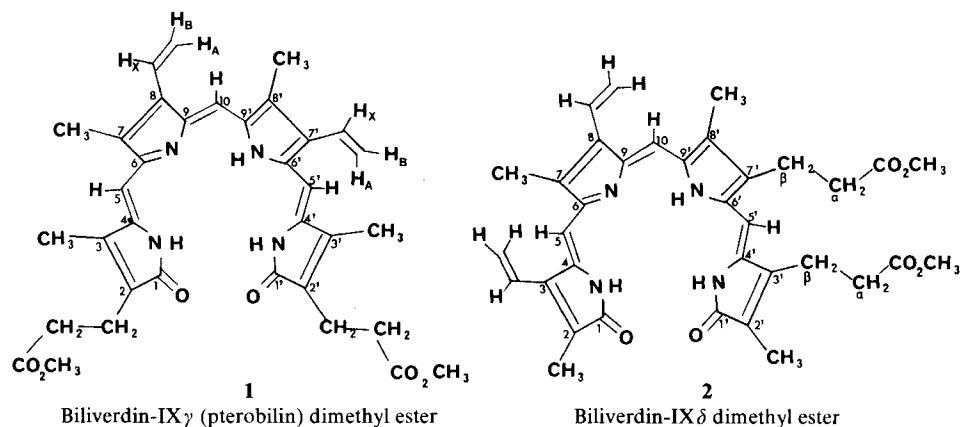
Under the action of light and oxygen, biliverdin IX *a* is more stable [5] [6] than bilirubin; this pigment quenches <sup>1</sup>O<sub>2</sub> with high efficiency and self sensitized photo-oxidation occurs very slowly [5] [6] [12]. Imides and propentdyopents are isolated after irradiation in the presence of rose bengal and a red derivative has also been obtained with laser-generated <sup>1</sup>O<sub>2</sub> in Freon 113 [12]. In the absence of oxygen, Falk *et al.* [13] reported *syn-Z, syn-Z, syn-Z*  $\rightarrow$  *syn-Z, syn-Z, syn-E + syn-E, syn-Z, syn-Z* photo-isomerizations with biliverdin IX *a* adsorbed on alumina or SiO<sub>2</sub>. Scheer & Krauss [14] observed an oxidative photo-dimerization occurring with a bilidione irradiated in a methanolic solution of K<sub>3</sub>Fe(CN)<sub>6</sub>.

In preceding papers [15] [16] we compared the photo-reactivity of biliverdin-IX isomers; whereas biliverdin IX *a* and IX  $\beta$  are relatively photo-stable in aerated

solution, biliverdins IX  $\gamma$  and IX  $\delta$  are much more reactive. The position of the vinyl groups in the tetrapyrrole molecule is determining for photo-reactivity. The irradiation of biliverdin IX  $\gamma$  (**1**, pterobilin), occurring in many *Lepidoptera* species [17], leads to the formation of a complex mixture of new pigments, proceeding from reactions of the C(7')-vinyl group, the C(8)-vinyl group or both, the only products identified being neobiliverdin IX  $\gamma$  (phorcabilin) and sarpedobilin [15] [18] [19]. These natural pigments have been isolated, as biliverdin IX  $\gamma$ , (pterobilin) [17] from *Lepidoptera* species.

We now report the photo-transformations of biliverdin-IX  $\delta$  dimethyl ester **2**, a good model for the study of the photo-reactions involving the C(8)-vinyl group, because of the relative photo-inertia of the C(3)-vinyl group.

**Results.** - *Configurations of biliverdin-IX  $\gamma$  and -IX  $\delta$  dimethyl esters 1 and 2 in solution.* Since the photochemistry of biliverdins involves the geometrical isomerization of the methine bridges, determination of the configuration of these molecules in solution at RT. in the dark was desirable. The problem may be resolved by high frequency  $^1\text{H-NMR}$ . (250 MHz) and the Nuclear Overhauser Effect (NOE), as employed by Lehner *et al.* [20] and Falk *et al.* [13b] in the study of the configuration of biliverdin IX  $\alpha$ .



*Biliverdin-IX  $\gamma$  dimethyl ester (1).* Irradiation of the methyl singlets at 2.06 and 2.08 ppm induced NOE (15%) on both singlets at 5.78 and 5.90. Furthermore, irradiation of methyl singlets at 2.14 and 2.25 ppm induces a NOE respectively on singlets at 5.78 (10%) and 6.65 (16%). Irradiation in the region 5.30–5.40 ppm ( $\text{CH}_2=$ ) produces a NOE on the singlets at 5.90 (17%) and 6.65 (15%). Finally, irradiation near 6.65 ppm  $\text{H-C}(10)$  and  $\text{H}_x$  from  $\text{H}_2\text{C}=\text{CH}-\text{C}(8)$  induces a NOE on the methyl singlets at 2.25 (5%) and 2.14 (3%) and decoupling of protons  $\text{H}_B$  (5.36) and  $\text{H}_A$  from  $\text{H}_2\text{C}=\text{CH}-\text{C}(8)$  (5.40 ppm).

These results permit unequivocal assignments of signals at 2.06 and 2.08 ppm to  $\text{H}_3\text{C}-\text{C}(3)$  and  $\text{H}_3\text{C}-\text{C}(3')$  and of the singlet at 2.14 to  $\text{H}_3\text{C}-\text{C}(7)$ , the remaining  $\text{H}_3\text{C}-\text{C}(8')$  resonating downfield at 2.25. The singlet at 5.78 ppm is attributed to

Table 1.  $^1H$ -NMR. spectra in  $CDCl_3$  (ppm from TMS) of biliverdin-IX $\gamma$  and -IX $\delta$  dimethyl esters **1** and **2**

	<b>1</b>	<b>2</b>
$CH_3-C=$ 	2.06 s { 6 H H <sub>3</sub> C-C(3) 2.08 s { H <sub>3</sub> C-C(3') 2.14 s 3 H H <sub>3</sub> C-C(7) 2.25 s 3 H H <sub>3</sub> C-C(8')	1.82 s 3 H { H <sub>3</sub> C-C(2) 1.90 s 3 H { H <sub>3</sub> C-C(2') 2.17 s 3 H { H <sub>3</sub> C-C(7) 2.18 s 3 H { H <sub>3</sub> C-C(8')
CH <sub>2</sub> -CH <sub>2</sub>	2.50 s 8 H	2.52 t 2 H 2.64 t 2 H 2.84 t 4 H: H <sub>2</sub> C-C(3'), H <sub>2</sub> C-C(7')
COOCH <sub>3</sub>	3.64 s 6 H	3.68 s 3 H 3.72 s 3 H
$H_x$ $H_B$ \      / C=C /      \ $H_A$	5.30 d x d H <sub>B</sub> from H <sub>2</sub> C=CH-C(7') 5.32 d x d H <sub>A</sub> from H <sub>2</sub> C=CH-C(7') 5.36 d x d H <sub>B</sub> from H <sub>2</sub> C=CH-C(8) 5.40 d x d H <sub>A</sub> from H <sub>2</sub> C=CH-C(8) 6.50 d x d H <sub>x</sub> from H <sub>2</sub> C=CH-C(7') 6.63 d x d H <sub>x</sub> from H <sub>2</sub> C=CH-C(8)	5.47 d x d H <sub>B</sub> from H <sub>2</sub> C=CH-C(8) 5.53 d x d H <sub>A</sub> from H <sub>2</sub> C=CH-C(8) 5.66 d x d H <sub>B</sub> from H <sub>2</sub> C=CH-C(3) 5.68 d x d H <sub>A</sub> from H <sub>2</sub> C=CH-C(3) 6.62 d x d H <sub>x</sub> from H <sub>2</sub> C=CH-C(3) 6.79 d x d H <sub>x</sub> from H <sub>2</sub> C=CH-C(8)
$J_{cis}$ = 12 Hz $J_{trans}$ = 18 Hz $J_{gem}$ = 2 Hz		
-CH=	5.78 s 1 H H-C(5) 5.90 s 1 H H-C(5') 6.65 s 1 H H-C(10)	6.00 s 1 H H-C(5') 6.05 s 1 H H-C(5) 6.86 s 1 H H-C(10)

H-C(5) and the one at 5.90 to H-C(5'); as expected, the signal at 6.65 ppm is due to the central proton H-C(10).

The observation of these NOE unambiguously demonstrates the *syn-Z* configuration of the three methine bridges and the geometry of the two vinyl groups of biliverdin-IX $\gamma$  dimethyl ester **1** in solution.

*Biliverdin-IX $\delta$  dimethyl ester (2)*. Irradiation of the two methyl singlets at 2.17 and 2.18 ppm induces NOE on the singlets at 6.86 (14%) and 6.05 (15%) and permits assignments of these latter to H-C(10) and to H-C(5). Irradiation of the triplet at 2.84 ppm (attributed to the  $\beta$ -CH<sub>2</sub> of the two propionyl chains) produces NOE (20%) on the singlet at 6.0 which consequently is attributed to H-C(5'). The high value of 20% is reasonably due to additive effects of the two  $\beta$ -H<sub>2</sub>C-C(7') and  $\beta$ -H<sub>2</sub>C-C(3') and proves that the 5' methine bridge has the *syn-Z* configuration. The results so far obtained concerning the NOE between the CH<sub>2</sub> of the vinyl groups and H-C(5) and H-C(10) are not clear. This is partly due to overlapping of signals and to the difficulty in resolving the decoupling effects from NOE. Consequently, it is not possible to establish the complete configuration of the methine bridges at positions 5 and 10 in the IX $\delta$  series, but as similar optical properties in visible light are observed for the IX $\gamma$  isomer, the same *syn-Z, syn-Z, syn-Z* configurations probably occur in the IX $\delta$  series.

*Photochemical transformation of biliverdin-IX $\delta$  dimethyl ester (2)*. Irradiation (500-700 nm) of an aerated solution of biliverdin-IX $\delta$  dimethyl ester (**2**) in dimethyl sulfoxide (DMSO), led to the formation of four new pigments, **3**, **4**, **5** and **6**, which cannot be interconverted photochemically.

When the solution was carefully degassed (ten freeze-pump-thaw cycles) the only transformation seen is **2**→**3**. The quantum yield calculated for the formation of **3** is  $\Phi = 4 \cdot 10^{-5}$  under these conditions.

The formation of the other pigments **4**, **5** and **6** was induced by a continuous passage of  $O_2$ . The quantum yields calculated after irradiation in the presence of  $O_2$  were  $1.5 \times 10^{-5}$  for **3**;  $3.7 \times 10^{-5}$  for **4**; based on these results, values of  $10^{-6}$  for **5** and  $8 \times 10^{-6}$  for **6** were evaluated. On the preparative scale, irradiation of a  $8 \cdot 10^{-5} M$  solution of **2** was continued for 1 h with a 800 W halogen lamp, the reaction leading to recovered **2** 20%, **3** 20%, **4** 33%, **5** 3%, **6** 10%. Compositions

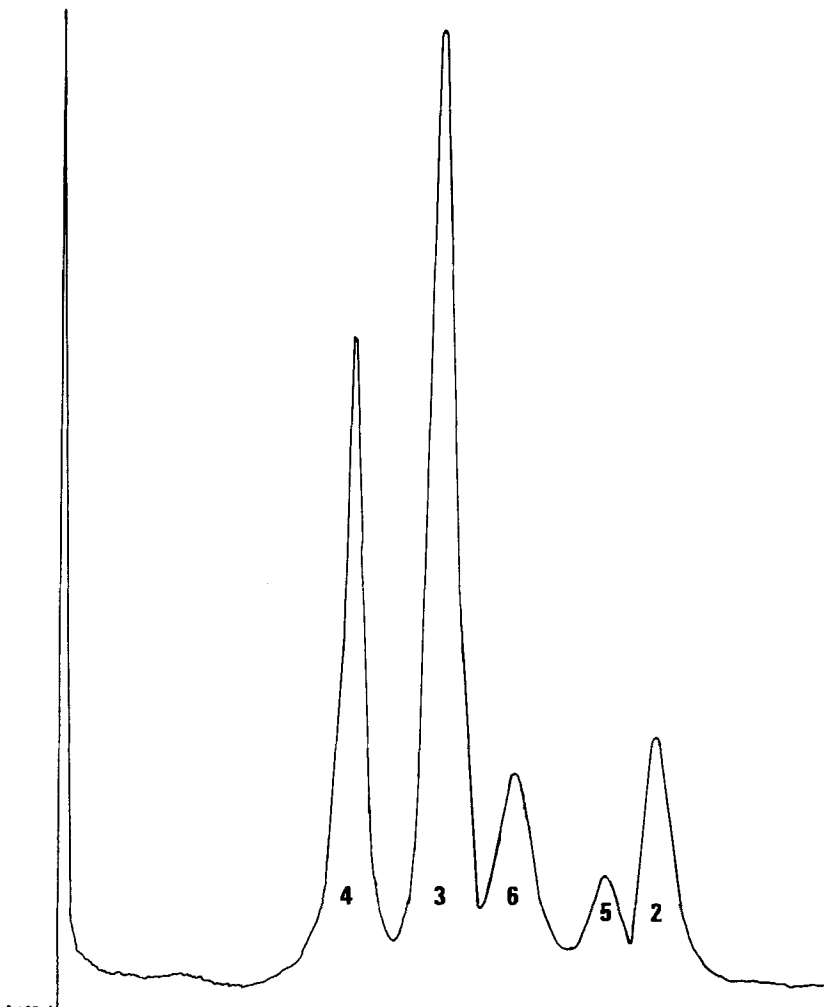


Fig. 1. High performance liquid chromatography of the mixture of pigments obtained after irradiation ( $O_2$ ) of biliverdin-IX $\delta$  dimethyl ester (**2**). Column:  $\mu$ Bondapak  $C_{18}$ , mobile phase 30%  $H_2O$  + 0.75% triethylamine in methanol,  $\lambda_{max}$  546 nm for detection.

after reactions were analyzed by high performance liquid chromatography (HPLC.) (Fig. 1) or thin layer chromatography (TLC.). All these photo-transformations need polar solvents, DMSO giving the best yields and benzene or dichloromethane no transformation at all. Irradiation of a stirred suspension in hexane of **2** adsorbed on neutral alumina also gave a good yield in the photo-transformation.

The addition of 1,4-diazabicyclo[2.2.2]octane (DABCO), a  $^1\text{O}_2$  quencher [21], to a DMSO solution of **2** saturated with  $\text{O}_2$  did not modify the reactivity. Nor did the addition of  $^1\text{O}_2$ -photosensitizers such as methylene blue or rose bengal accelerate the reaction; instead, maleimides were produced, owing to the addition of  $^1\text{O}_2$  to the methine bridges in **2**. The addition of galvinoxyl (free radical) modifies the yields, those from the transformation **2**  $\rightarrow$  **3** being lowered and those of **4**, **5** and **6**, being raised. However, other products were formed by radical additions and quantitative estimations were thus difficult.

*Structural analysis of photo-products from 2.* The pigment **3** [MS. (field desorption)  $610 = M^+$ ], is neobiliverdin-IX  $\delta$  dimethyl ester [15] [18]. Like **4**, **5** and **6**, neobiliverdin IX  $\delta$  has a new ring in the central part of the molecule and the methine bridge C(10) has the *anti-E* configuration. This results in modifications of the NMR. and the visible light spectra; the absorption at 375 nm nearly disappears (Fig. 2) and the 650 nm band shows hypsochromic shift and hyperchromic effect (for  $2\epsilon = 3.8 \cdot 10^4$  at 375 nm and  $1.3 \cdot 10^4$  at 650 nm). The *syn-Z*  $\rightarrow$  *anti-E* isomerization of the central part of the molecule results in a shift downfield ( $\Delta\delta$  0.44 ppm) of the H-C(10) signal in the NMR. spectrum. The signals for protons H-C(5) and H-C(5') are not shifted, in agreement with the possible conservation of the *syn-Z* configuration at C(5) and C(5') (the study of this by NOE experiments with Fourier transform has not been performed). Falk *et al.* [13b] observed smaller differences in the shifts ( $\Delta\delta$  0.16 and 0.22 ppm) for H-C(5) and H-C(5') owing to *syn-Z*  $\rightarrow$  *syn-E* isomerization of biliverdin IX  $\alpha$ .

Compound **4** [MS. (field desorption): 626,  $M^+$ , 608 ( $M - \text{H}_2\text{O}$ ) $^+$ ] is the hydroxy derivative of neobiliverdin IX  $\delta$ . The corresponding acetyl derivative (MS.: 668,  $M^+$ ) and the methyl ether **7** (640,  $M^+$ ) have been prepared. The chromic acid oxidation of **7** furnishes hematinic ester (**8**), methylvinylmaleimide (**9**), and the new imide **10** (364,  $M^+$ ) with a diimide structure retaining the methoxy group  $\text{C}_{17}\text{H}_{20}\text{N}_2\text{O}_7$  [MS.: 333,  $M^+ - \text{OCH}_3$ , 210,  $\text{C}_{10}\text{H}_{12}\text{NO}_4$  and 150, 210 -  $\text{HCOOCH}_3$ , Scheme 2]. Dehydration of **4** on refluxing 1 h in benzene with *p*-toluene sulfonic acid, yields **5** [MS. (field desorption): 608,  $M^+$ ]. Chromic oxidation leads only to hematinic ester (**8**) and to methylvinylmaleimide (**9**) the expected diimide being not observed owing to its further degradation.

The NMR. spectrum of **4** (Table 2) in  $\text{CDCl}_3$  shows signals consistent with methyl groups, only one vinyl group ( $J_{cis} = 12$  Hz,  $J_{trans} = 18$  Hz,  $J_{gem} = 3$  Hz) and an *AMX*-system:  $\text{H}_x$  5.14 ppm, allylic proton in equatorial position adjacent to the OH group and coupled with the vicinal equatorial proton  $\text{H}_M$  ( $J = 6$  Hz);  $\text{H}_A$  (3.84 ppm,  $J_{gem} = 14$  Hz) situated in the  $\alpha$ -position to the N-atom and in *trans*-diaxial position to the nitrogen doublet is shielded compared with the equatorial *gem* proton  $\text{H}_M$  (4.58 ppm). While neobiliverdin-IX  $\delta$  (**3**) (and-IX  $\gamma$ ) dimethyl esters exhibit badly resolved signals for the  $\text{CH}_2$ -protons of the 7 membered ring, the

rigidity of **4** imposed by the OH-group in the axial position leads to a nicely resolved system.

The NMR. spectrum of **5** (Table 2) displays signals for an *ABX*-system assigned to a vinyl group and two doublets (6.48 1H, 7.20 1H,  $J=10$  Hz) which may be attributed to the N-CH=CH-C=C-group in the central 7membered ring. The

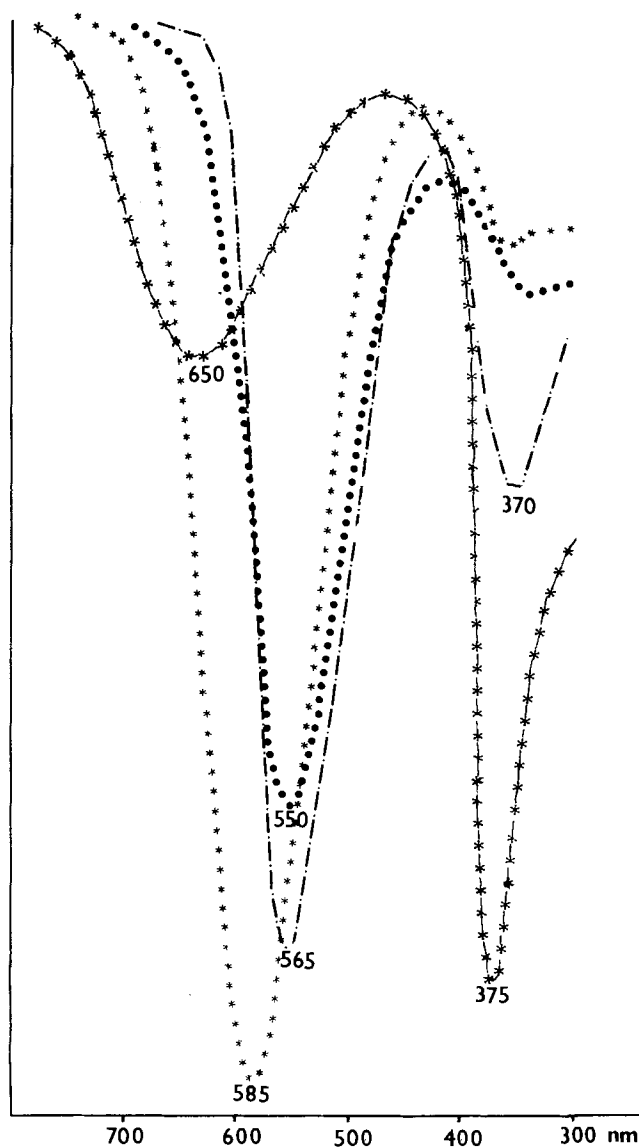
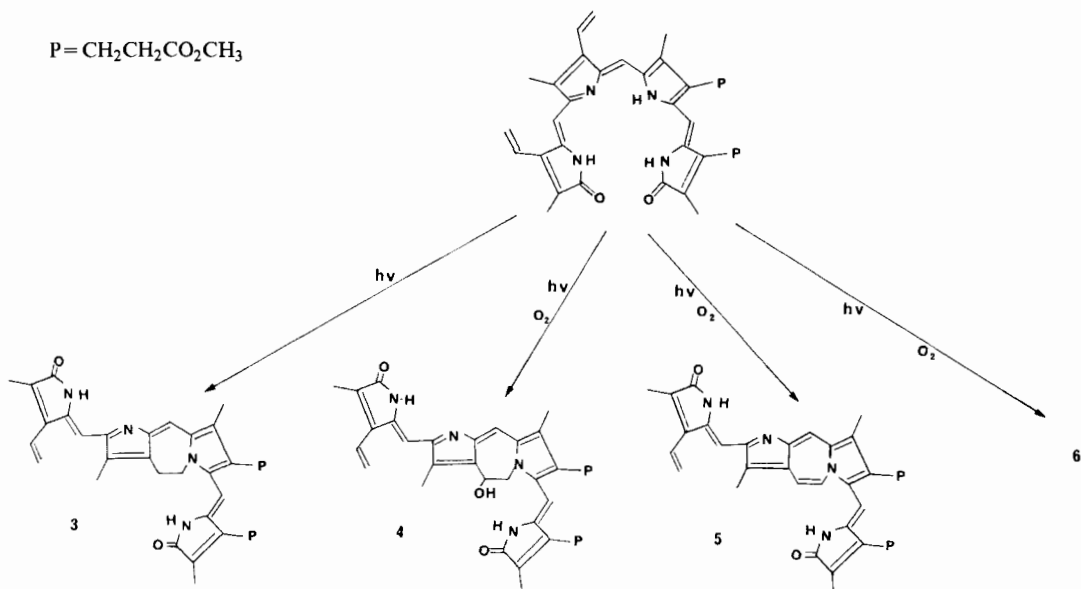
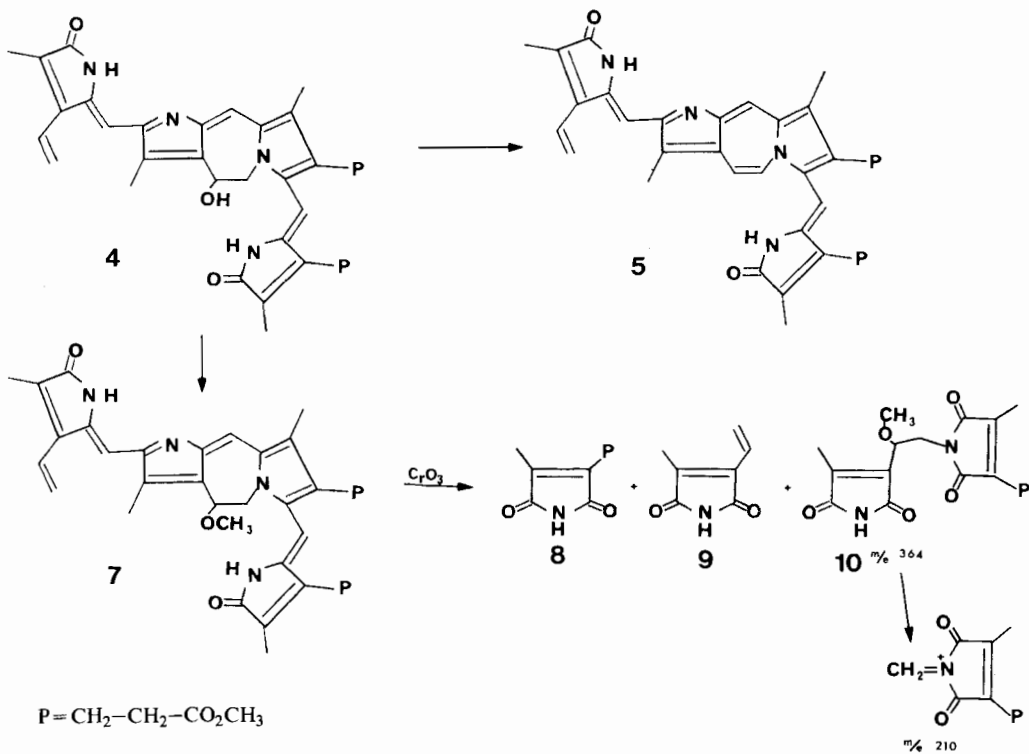


Fig. 2. Absorption spectra in MeOH of pigment 2-*x-x-x-x*-, pigment 3-*●●●●●●*-, pigment 4-*\*\*\**-, pigment 5-*-----*

Scheme 1. Irradiation (500-700 nm, DMSO) of biliverdin-IX  $\delta$  dimethyl ester (2)P = CH<sub>2</sub>CH<sub>2</sub>CO<sub>2</sub>CH<sub>3</sub>

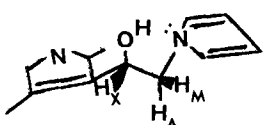
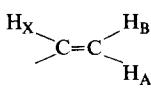
Scheme 2. Chemical reactions on pigment 4



downfield shifts of the methine bridge protons are consistent with the extension of the conjugated system in the structure.

Pigment **6** [MS. (field desorption): 624,  $M^+$ ] contains one more O-atom and two less H-atoms than **3**. Chromic acid oxidation gives hematinic ester (**8**), methylvinylmaleimide (**9**) and a new imide **11** (high resolution MS.). These results clearly suggest that pigment **6** is also formed through oxidation and cyclization, both reactions occurring on the two central pyrrole rings. In the NMR. spectrum (Table 2), the protons of four methyl groups resonate between 1.98 and 2.32 ppm. Signals for the *ABX*-system of one vinyl group are shifted as found in pigments **4** and **5** but two doublets (1 H) corresponding to two coupled vinylic protons are also observed at 6.10 and 6.44 ppm. The low coupling constant ( $J=3.8$  Hz) excludes the possibility of two *cis*-vicinal protons as in **5** and strongly suggests *gem*-protons of a methylenide group, situated either at C(7) or  $\beta$  to C(8). The identification of the new oxygen function in **6** remains unresolved. One possibility would be an epoxide function, for example on C(7)-C(8). A 2,3-epoxy-porphyrin has been previously reported to be readily converted into a keto-

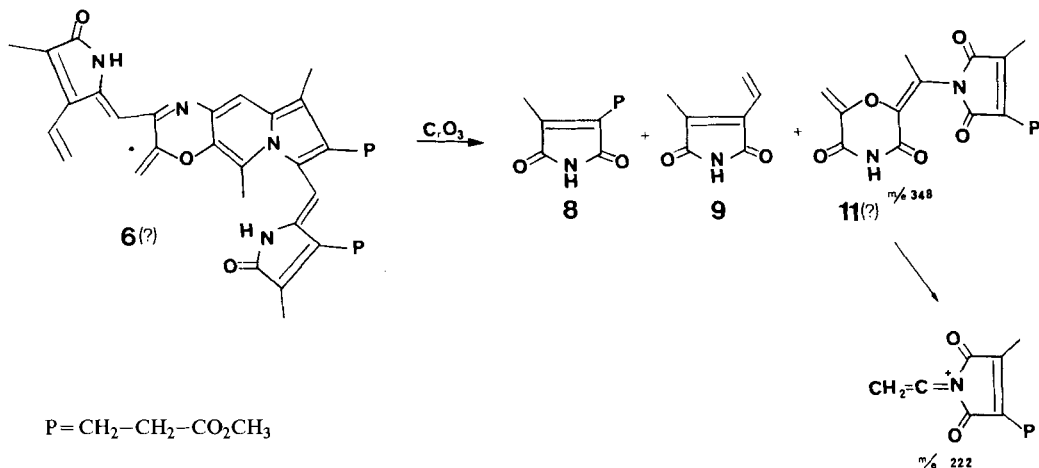
Table 2.  $^1\text{H-NMR}$ . spectra of pigments **4**, **5**, **6** (ppm from TMS, in  $\text{CDCl}_3$ ) and for *N-H* determinations in  $d_6$ -DMSO +  $\text{CF}_3\text{CO}_2\text{H}$  0.2%

In $\text{CDCl}_3$	<b>4</b>	<b>5</b>	<b>6</b>
C-CH <sub>3</sub>	1.94 <i>s</i> 3 H	1.98 <i>s</i> 3 H	1.98 <i>s</i> 3 H
	2.08 <i>s</i> 3 H	2.10 <i>s</i> 3 H	2.10 <i>s</i> 6 H
	2.18 <i>s</i> 3 H	2.30 <i>s</i> 3 H	2.32 <i>s</i> 3 H
	2.24 <i>s</i> 3 H	2.48 <i>s</i> 3 H	
CH <sub>2</sub> -CH <sub>2</sub>	2.48 <i>t</i> 2 H	2.58 <i>t</i> 2 H	2.54 <i>t</i> 2 H
	2.62 <i>t</i> 2 H	2.68 <i>t</i> 2 H	2.62 <i>t</i> 2 H
	2.88 <i>m</i> 4 H	2.96 <i>m</i> 4 H	2.86, 2.88 <i>m</i> 4 H
CO <sub>2</sub> CH <sub>3</sub>	3.64 <i>s</i> 3 H	3.64 <i>s</i> 3 H	3.65 <i>s</i> 3 H
	3.70 <i>s</i> 3 H	3.72 <i>s</i> 3 H	3.70 <i>s</i> 3 H
	3.84 <i>d</i> (14 Hz) H <sub>A</sub>		
	4.58 <i>d</i> × <i>d</i> (14 and 6 Hz) H <sub>M</sub>		
	5.14 <i>d</i> (6 Hz) H <sub>X</sub>		
	5.68 <i>d</i> (12 Hz) H <sub>B</sub>	5.68 <i>d</i> (12 Hz) H <sub>B</sub>	5.68 <i>d</i> (12 Hz) H <sub>B</sub>
	5.70 <i>d</i> (18 Hz) H <sub>A</sub>	5.72 <i>d</i> (18 Hz) H <sub>A</sub>	5.70 <i>d</i> (18 Hz) H <sub>A</sub>
	6.64 <i>d</i> × <i>d</i> (12 and 18 Hz) H <sub>X</sub>	6.66 <i>d</i> × <i>d</i> (12 and 18 Hz) H <sub>X</sub>	6.64 <i>d</i> × <i>d</i> (12 and 18 Hz) H <sub>X</sub>
		6.48 <i>d</i> (10 Hz) 1 H	6.10 <i>d</i> (3.8 Hz) 1 H
=C-H		7.20 <i>d</i> (10 Hz) 1 H	6.44 <i>d</i> (3.8 Hz) 1 H
	6.00 <i>s</i> 1 H	6.20 <i>s</i> 1 H	6.00 <i>s</i> 1 H
	6.08 <i>s</i> 1 H	6.22 <i>s</i> 1 H	6.14 <i>s</i> 1 H
	7.34 <i>s</i> 1 H	7.96 <i>s</i> 1 H	7.40 <i>s</i> 1 H
in $d_6$ -DMSO + $\text{CF}_3\text{CO}_2\text{H}$ 0.2%			
	N-H	10.25 1 H	9.80 1 H
	10.57 1 H	10.63 1 H	10.60 1 H



chlorin by acidic treatment [22]. Under the same conditions, **6** does not react either with MeOH/BF<sub>3</sub> or MeOH/SO<sub>4</sub>H<sub>2</sub>. Another possibility would be a 1,3-oxazine (*Scheme 3*) proceeding from a photo-oxidation at C(7)-C(8) followed by a rearrangement.

*Scheme 3. Hypothetical structures for pigment 6 and oxidation products 8, 9 and 11*

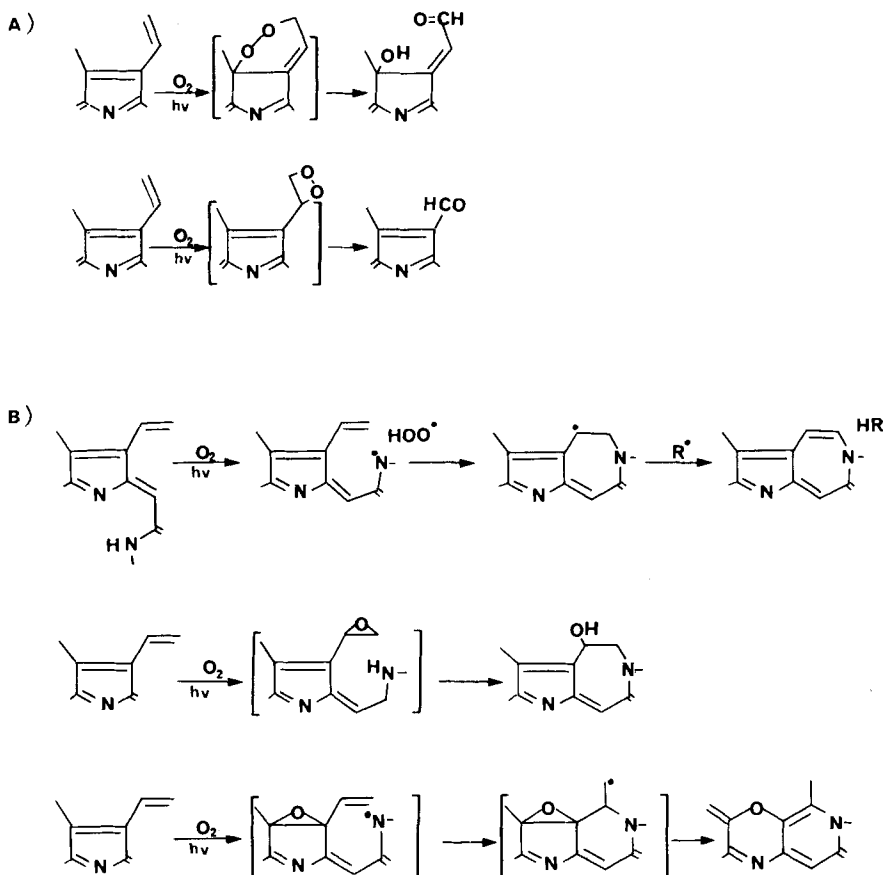


**Discussion.** - All the pigments isolated after irradiation of biliverdin-IX  $\delta$  ester at 500-700 nm, result from a *syn-Z*  $\rightarrow$  *anti-E* isomerization of the central methine bridge, and addition of the pyrrole amine function on the C(8)-group.

In absence of oxygen, the transformation **2**  $\rightarrow$  **3** corresponds to the addition of an amino function on a double bond activated by conjugation with electrophilic C=N or C=O groups [18]. In presence of oxygen, the reaction seems to have a radical character, in view of the observed modifications when R $\cdot$  is added. Such reactions could be induced through the elimination of H $\cdot$  from NH by the action of  $\cdot\bar{O}-\bar{O}\cdot$  (or R $\cdot$ ) as represented in *Scheme 4*. The insertion of oxygen is observed for the formation of **4** and **6** and the intermediate could be the 8 $\alpha$ ,8 $\beta$ -epoxide for **4** and the 7,8-epoxide for **6**. The 7,8-epoxide would rearrange with ring extension.

These photo-oxidations probably do not involve <sup>1</sup>O<sub>2</sub> but <sup>3</sup>O<sub>2</sub> as reported particularly for photo-epoxidation [26]. Experiments with <sup>1</sup>O<sub>2</sub>-sensitizers or quenchers support such a hypothesis, and biliverdin has been shown not to be a sensitizer for <sup>1</sup>O<sub>2</sub> [5]. The known reactions of bile pigments with <sup>1</sup>O<sub>2</sub> never occur on the vinyl groups; the red pigment obtained through reaction of biliverdin IX $\alpha$  with a laser generated <sup>1</sup>O<sub>2</sub> in Freon 113 is described as an endo-peroxide where the oxygen is attached at a position other than the vinyl bond [12] and *Lightner & Park* [23] have demonstrated the unreactivity of the vinyl group of bilirubin with <sup>1</sup>O<sub>2</sub>. Only the photo-oxidation of the vinyl groups of protoporphyrine IX [24] [25] has been reported. Two series of compounds are obtained and the most likely mechanisms involve in one case a 1,4-cycloaddition of <sup>1</sup>O<sub>2</sub> to the diene formed by one endocyclic double

Scheme 4. Proposed mechanisms for the photo-oxidation of pyrrole vinyl groups: A) in the photo-oxidation of protoporphyrin IX [24] [25]; B) hypothetical mechanisms for photo-oxidation of biliverdin IX $\delta$



bond and the vinyl group, and in the other case, a 1,2-addition to the vinyl group, giving a labile 1,2-dioxetane which further decomposes (Scheme 4). Results are not similar for biliverdin IX $\delta$ .

Thanks are due to Prof. *D.H.R. Barton* for discussions and for his interest, to Dr. *G. Roussi* for his help in the quantum yields determinations, also to Dr. *H.J. Veith (Darmstadt)* for the field desorption mass spectrometry, and to Dr. *D. Davoust (Paris)* for calculations by the LAOCOON method.

#### Experimental Part

*General remarks.* Field desorption mass spectrometry was performed on *Varian MAT-CH5/DF* and *MAT 731* apparatus and electron impact determination (high resolution) on an *AEI MS9* spectrometer.  $^1\text{H-NMR}$  were run at  $20^\circ$  on a *CAMECA 250* MHz spectrometer. Biliverdin esters **1** and **2** spectra and NOE experiments were performed in CW; the  $0.05\text{M}$   $\text{CDCl}_3$  (TMS as internal standard)

solutions were degassed *in vacuo* through 4 freeze-thaw cycles and tube sealed. The two vinylic systems in each biliverdin were resolved by double irradiation and analyzed by means of an algorithm LAOCOON III on a minicomputer Bruker BNC 28. Simulated and measured spectra were identical.  $^1\text{H-NMR}$ -spectra of pigments 3, 4, 5 and 6 were run in Fourier transform using 0.01M  $\text{CDCl}_3$  solutions and the systems resolved by double irradiation. Owing to the small amounts obtained, NOE experiments were not possible. Melting points were determined on a Kofler apparatus, and corrected.

Two different light sources were used for photochemical experiments: a *Bausch & Lomb* monochromator equipped with a 150 W Xenon lamp and an additional *Corning* glass filter CS 3.72 was used (selected wavelength 585 nm) for determination of quantum yields. For preparative experiments, a 800 W halogen superhot lamps *Osram* with *Corning* glass filter CS 3.69 was used.

*Biliverdin-IX isomers* were prepared [27] by coupled oxidation of hemin. The isolation of biliverdin esters was by TLC. on silica gel with  $\text{CH}_2\text{Cl}_2/\text{acetone}$  95:5. The last purification of biliverdin-IX $\delta$  dimethyl ester was carried out by HPLC. on a micro *Porasil* column in hexane/ $\text{CH}_2\text{Cl}_2/\text{ethanol}$  30:70:0.5, when the elution order is biliverdin-IX $\beta$ , -IX $\alpha$ , -IX $\gamma$  and -IX $\delta$  dimethyl esters. Biliverdin-IX $\gamma$  dimethyl ester is best purified by reversed phase HPLC. on a  $\mu\text{Bondapak C}_{18}$  column with acetone/water+0.75% triethylamine 11:9, the elution order being biliverdin dimethyl esters IX $\alpha$  [IX $\beta$ +IX $\delta$ ], and IX $\gamma$ .

After irradiation, the photo-pigments were extracted from DMSO with  $\text{CH}_2\text{Cl}_2/\text{H}_2\text{O}$  and purified by preparative TLC. on precoated silicagel plates *Merck*, with  $\text{CH}_2\text{Cl}_2/\text{acetone}$  9:1. The pigments crystallized from  $\text{CH}_2\text{Cl}_2/\text{hexane}$  (microcrystalline powder). For determination of quantum yields, the mixture was analyzed by reversed phase HPLC. on a  $\mu\text{Bondapak C}_{18}$  column with methanol/water+0.75% triethylamine, recording at 546 nm for detection.

*Quantitative yield determination.* Chemical actinometry was performed with aqueous 0.02M  $\text{K}(\text{Cr}(\text{NH}_3)_3(\text{NCS})_4$  (*Reinecke's salt*) [28]. The quantum yield calculated as the number of moles of thiocyanate released per einstein of light absorbed was 0.27 at 585 nm [28]. The concentration of released thiocyanate was determined with 0.1M  $\text{Fe}(\text{NO}_3)_3 \cdot 9\text{H}_2\text{O}$  in 0.5M  $\text{HClO}_4$  and measured by differential spectrophotometry (parallel dark run) at 450 nm,  $\epsilon = 4.3 \cdot 10^3$ ; the calculated intensity for the monochromator at 585 nm was  $2.2 \cdot 10^{-6}$  einstein/min.

Biliverdin-IX $\delta$  dimethyl ester  $4 \cdot 10^{-8}$  mol in 2 ml DMSO was irradiated for 30 min giving less than 10% of the photo-product. Experiments were carried out either in a cell with continuous bubbling of  $\text{O}_2$ , or in a cell connected with a degassing system. As all the incident light was not absorbed, transmission corrections were necessary. For the estimation of the quantum yields for pigments 5 and 6, the 800 W lamp was used and calculations made comparing with the formation of 3 and 4. The quantitative analyse were performed by HPLC.

*Pigments derivatives and chronic acid oxidation.* Neobiliverdin-IX $\delta$  dimethyl ester (3):  $\lambda_{\text{max}}$  methanol 550 nm,  $\epsilon = 4.5 \cdot 10^4$  (*Fig. 2*), m.p. 95–100°. Compound 4:  $\lambda_{\text{max}}$  methanol 585 nm,  $\epsilon = 3.5 \cdot 10^4$ ; m.p. 133–138°. The acetate of 4 was prepared by reaction in pyridine/acetic anhydride, 16 h at RT. Treatment of 4 with  $\text{MeOH}/\text{SO}_4\text{H}_2$  4:1 in sealed tube for 1.5 h at 90° gave 80% of the methyl ether 7, m.p. 78–83° and some dehydrated product 5. The pigment 5 was prepared by refluxing 1 mg of 4 and 10 mg dry *p*-toluenesulfonic acid in 15 ml toluene and 2 ml benzene for 1 h, the solution then being washed with water and evaporated:  $\lambda_{\text{max}}$  methanol 565 nm; m.p. 105–110°. Pigment 6:  $\lambda_{\text{max}}$  methanol 560 nm. Oxidation product 11:  $M^+$  at 348.0946 ( $\text{C}_{16}\text{H}_{16}\text{N}_2\text{O}_7$ , 100%), with 317.0766 ( $\text{C}_{15}\text{H}_{13}\text{N}_2\text{O}_6$  ( $M^+ - \text{OCH}_3$ )) 27%; 289.0819 ( $\text{C}_{14}\text{H}_{13}\text{N}_2\text{O}_5$  ( $M^+ - \text{CO}_2\text{CH}_3$ )) 6%; 222.0766 ( $\text{C}_{11}\text{H}_{12}\text{NO}_4$ , 12) 15%; 190.0500 ( $\text{C}_{10}\text{H}_8\text{NO}_3$  (222 -  $\text{CH}_3\text{OH}$ )) 26%; 162.0555 ( $\text{C}_9\text{H}_8\text{NO}_2$  (222 -  $\text{HCO}_2\text{CH}_3$ )) 24%.

Chromic acid oxidation [29] was performed at RT. directly on the thin layer plate. A solution of 0.34 g  $\text{CrO}_3$  in 20 ml  $\text{H}_2\text{O}/\text{SO}_4\text{H}_2$  1% was carefully added dropwise on the pigment adsorbed on  $\text{SiO}_2$  and after disappearance of the pigment colour and drying, developing was performed with  $\text{CCl}_4/\text{cyclohexane/ethyl acetate}$  5:1:3. Imides were visualized by treatment with  $\text{Cl}_2$  and spraying with a benzidine solution.

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