

## The Photo-oxidation of *meso*-Tetraphenylporphyrins <sup>1</sup>

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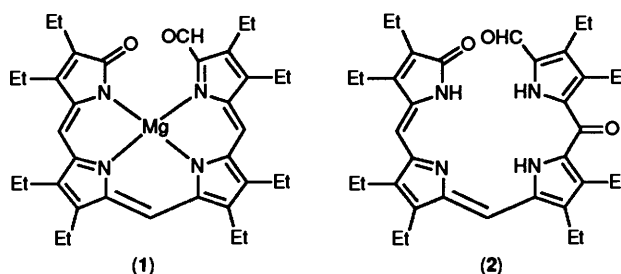
The photo-oxidation of *meso*-tetraphenylporphyrin (TPP) dianion in methanol affords a purple benzoyl bilinone (**5b**) by addition of singlet oxygen, followed by ring-opening and addition of methanol; similar products (**5a**) and (**5c**) can be formed by addition of water, or ethanol, respectively. The water adduct (**5a**) had previously been obtained by other workers from the photo-oxidation of metallo derivatives of TPP, by cerium(IV) or thallium(III) oxidation of TPP, or by treatment of the cation radical of TPP with nitrite, but a different structure had been assigned. Our new structure (**5**) has been deduced from detailed NMR and mass spectral studies including decoupling and NOE measurements, as well as field desorption mass spectra at low wire currents; the presence of quaternary *meso*-carbon atoms in structures (**5a**) and (**5b**) has been shown by the <sup>13</sup>C NMR spectra of the *meso* <sup>13</sup>C-labelled pigments. Confirmation of these assignments has been provided by photo-oxidation of the *meso*-tetra(4-methoxyphenyl)porphyrin and further spectral studies of the resulting bilinones (**7a**) and (**7b**), including NOE and <sup>13</sup>C-<sup>1</sup>H shift correlated NMR spectra.

The oxidative metabolism of haem to biliverdin and other bile pigments is a vital biochemical process both in mammalian and plant systems.<sup>2,3</sup> In contrast to the mammalian bile pigments, those in plants are not simply waste products. For example, the algal pigments phycocyanobilin and phycoerythrobilin which occur in the blue, green, and red algae, are accessory pigments in photosynthesis whilst phytochrome is an important growth regulator in a wide variety of plants.<sup>4</sup> On the other hand, it seems likely that the degradation of chlorophylls in senescent leaves involves photo-oxidation and cleavage of the macrocycle but the evidence for this is, so far, only circumstantial. Thus, whilst it has been shown that bacteriochlorophylls-*c* and -*e* slowly oxidise in air to form acetyl bilinones,<sup>5,6</sup> no firm evidence has yet been obtained that similar oxidative processes occur *in vivo*.

The intrinsic importance of all these natural processes has stimulated a great deal of interest in their chemistry during the last fifteen years.<sup>2,3,7,8</sup> It now seems certain that the plant bile pigments (like those in mammals) are formed from haem,<sup>9</sup> rather than from an intermediate in chlorophyll biosynthesis. The initial stage involves oxidation to a *meso*-oxygenated haem, followed by oxidative ring opening by molecular oxygen and loss of carbon monoxide and demetallation to biliverdin,<sup>10,11</sup> the latter then undergoes reduction and/or conjugation to form other bile pigments. These oxidative ring opening processes can be mimicked *in vitro* using purely chemical methods<sup>12</sup> although in the absence of enzymes all four biliverdin isomers are formed due to attack at each of the *meso*-positions of the haem.<sup>2,3</sup>

In contrast to the metabolism of haem, the photo-oxidation of metalloporphyrins (*i.e.* those formed from Group II metals) follows a different pathway. For example, Mauzerall and Fuhrhop<sup>13</sup> in their pioneering studies on the photo-oxidation of magnesium octaethylporphyrin showed that the primary oxidation product is the magnesium formyl bilinone (**1**).<sup>14</sup> Attempts to obtain the metal-free bilinone by treatment with dilute acid were thwarted by its apparent instability and further oxidation to a new bile pigment, formulated as the *meso*-oxo derivative (**2**).<sup>15</sup>

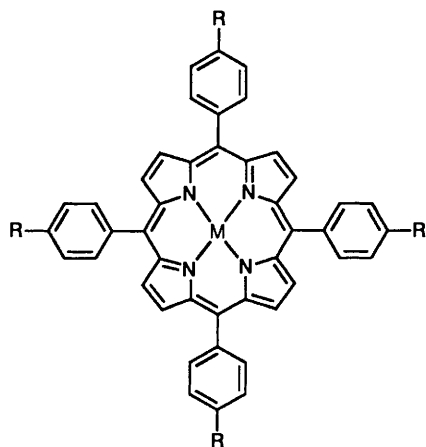
Zinc octaethylchlorin also undergoes photo-oxidation<sup>16,17</sup>



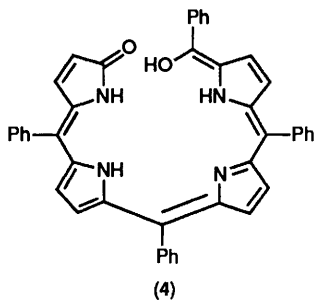
to form a mixture of dihydro zinc analogues of the magnesium bilinone (**1**); similar products are formed from the mono- and di-*meso*-methyl derivatives of zinc octaethylchlorin, ring opening occurring at the *meso*-methylated positions.<sup>17</sup> These reactions all provide simple models for the slow aerial oxidation of bacteriochlorophylls-*c* and -*e* to acetyl bilinones.<sup>5,6</sup> All these processes presumably involve cleavage by singlet oxygen (formed from triplet oxygen by sensitisation by the porphyrin or chlorin) as both oxygen atoms in the bilinones arise from the same molecule of dioxygen.<sup>18</sup> In contrast, in the degradation of haem to bile pigments both *in vivo* and *in vitro* the two terminal amide oxygen atoms of the bile pigment are derived from different molecules of dioxygen,<sup>19</sup> and the *meso*-carbon is extruded as carbon monoxide.

The degradation of chlorophylls in autumn leaves may also follow a similar type of photo-oxidative ring opening to that described in the previous paragraph. However, an alternative process, more closely akin to the biochemical degradation of haem, is also conceivable as evidenced by model studies with porphyrin and chlorin zinc complexes. Thus zinc octaethyl oxophlorin underwent photo-oxidation to form zinc octaethyl biliverdin<sup>20a</sup> and similarly zinc oxochlorins (formed from chlorophyll derivatives) afforded dihydrobiliverdins;<sup>20b</sup> in each case carbon monoxide was also formed from the *meso*-oxygenated carbon of the macrocycle.

*meso*-Tetraphenylporphyrin (TPP) metal complexes (3) also undergo photo-oxygenation in organic solvents;<sup>21,22</sup> magnesium, zinc, cadmium, and thallium complexes have been studied and the metal can be removed from the initial bile pigment complexes to form a stable purple pigment ( $\lambda_{\max}$  565 nm). This material, referred to as compound (A), was originally formulated as the bilinone (4) and the same product has been isolated from chemical oxidations of TPP with thallium(III) or cerium(IV) salts.<sup>23</sup> It has also been obtained from the cation radical of TPP metal complexes,<sup>24</sup> and from a zinc *meso*-methoxy isoporphyrin derivative from TPP<sup>25</sup> (see below); a similar purple complex has also been observed as a by-product in nitrations of TPP.<sup>26</sup>



(3) a R = H  
b R = OMe



(4)

Relatively little work has been carried out on the photo-oxidation of porphyrin free bases. Only in the case of protoporphyrin-IX have products of clearly defined structure been obtained and these were two isomeric chlorins derived from Diels-Alder addition of singlet oxygen to the vinyl groups and neighbouring peripheral double bonds.<sup>27</sup> The earliest reported examples of porphyrin photo-oxidation at the *meso*-position were described by Fischer,<sup>28</sup> who carried out oxidations of protoporphyrin in strongly basic media on the di-sodium salts, and obtained products of uncertain structure but which were probably analogues of those obtained more recently by Fuhrhop after demetallation of the metal formyl bilinones formed by photo-oxidation of metalloporphyrins.<sup>15</sup> We have now confirmed this conclusion by studying the photo-oxidation of the dianion of TPP using sodium methoxide as base in methanol-tetrahydrofuran (THF). The purple photo-oxidation product (B) had very similar visible spectroscopic properties to those of the oxidation product (A) obtained from TPP metal complexes. However, elemental analyses and NMR spectroscopy showed that it apparently contained methanol of solvation, whereas the original product (A) was thought to contain water of solvation. When product (A) was treated

with methanolic sulphuric acid and the product worked up by evaporation to dryness, followed by chromatography on alumina, product (B) was obtained; conversely treatment of product (B) with aqueous acid, followed by work up, afforded compound (A).

The original structure (4) assigned to the purple pigment (A) was based on interpretation of NMR and mass spectra.<sup>23</sup> The <sup>1</sup>H NMR spectrum showed three broad NH resonances at low field, a multiplet corresponding to the phenyl proton resonances, a series of doublets corresponding to the peripheral 'pyrrole' proton resonances, and a singlet at  $\delta$  6.37 attributed to a hydroxyl group.<sup>23</sup> We have confirmed these findings by determining the spectrum at 360 MHz and showing that the only resonances due to exchangeable protons (by shaking with D<sub>2</sub>O) are the three low field NH resonances, and the hydroxyl resonance (which corresponded to only *one* proton) (see Table 1). The NMR spectrum of the related pigment (B) prepared for the first time in the present studies (and shown in ref. 1), was very similar to that of pigment (A), the only major difference being the absence of the hydroxyl resonance and the presence of a methoxyl resonance at  $\delta$  3.19 in the spectrum of pigment (B) (Table 1); as with pigment (A) the three NH resonances were removed on shaking with D<sub>2</sub>O.

We have also prepared a third purple pigment (C) by carrying out photo-oxidation of TPP dianion using sodium ethoxide as base in ethanol-THF; the NMR spectrum of this compound was very similar to that of pigment (B) except that the methoxyl resonance was replaced by a multiplet at  $\delta$  3.38 and a triplet at  $\delta$  1.13 corresponding to an ethoxyl group (Table 1).

The electron impact mass spectrum<sup>22,23</sup> of pigment (A) showed an apparent molecular ion at  $m/z$  648 and this was confirmed in later studies<sup>21</sup> concerned with the mechanism of the oxidative ring opening process of TPP metal complexes using mixtures of <sup>16</sup>O<sub>2</sub> and <sup>18</sup>O<sub>2</sub> when the molecular ions were observed at  $m/z$  652 and 648 (but not 650). This showed that both terminal oxygen atoms in the bilinone were derived from the same molecule of the dioxygen. We have now redetermined the electron impact mass spectra of compound (A) and also those of the related pigments (B) and (C); all gave very similar spectra with base peaks at  $m/z$  648 essentially confirming the earlier results, although smaller peaks were also observed at  $m/z$  646 and 647.

Initially we considered the possibility that the mass spectral results with the three pigments could be accounted for by the assumption that they simply differed from each other by water, methanol or ethanol of 'solvation', which was lost in the course of volatilising the sample during the determination of the EI mass spectra. However, the NMR spectra did not show evidence for the additional hydroxyl group (of the water, methanol or ethanol) which would have been expected. Furthermore, it was not obvious why the TPP oxidation products should contain two additional hydrogen atoms compared with the corresponding photo-oxidation products from octaethylporphyrin metal complexes, or those from chlorophylls or chlorins. Moreover, the purple colour of the three pigments (A)-(C) ( $\lambda_{\max}$  565 nm) was more consistent with those of tripyrrenes (or violins) with three conjugated pyrrole rings, rather than with those of a fully conjugated bilatriene (or biliverdin) which are normally deep blue in colour ( $\lambda_{\max}$  620-630 nm).

Further evidence was obtained by determining the <sup>13</sup>C NMR spectra of both pigments (A) and (B) (Table 2). The spectrum of the hydroxy derivative (A) included carbonyl carbon signals at  $\delta$  184.5 and 173.3, and a quaternary carbon resonance at  $\delta$  75.0 as well as numerous signals between  $\delta$  120 and 160. A similar spectrum was reported by Matsuura *et al.*<sup>22</sup> The methoxy derivative (B) showed an additional methoxyl signal at  $\delta$  53.0, and the aliphatic quaternary carbon signal had moved to  $\delta$  80.7. This led us to consider the possibility that the quaternary

**Table 1.** <sup>1</sup>H NMR spectra of biladienones.<sup>a</sup>

Atom	(5a)	(5b)	(5c)	(7a)	(7b)
1-H	6.18 (overlapping)	6.29 (dd, <i>J</i> 6, 2 Hz)	6.25 (overlapping)	6.18 (overlapping)	6.26 (dd, <i>J</i> 6, 1 Hz)
2-H	6.88 (d, <i>J</i> 6 Hz)	6.93 (dd, <i>J</i> 6, 2 Hz)	6.91 (dd, <i>J</i> 5, 2 Hz)	6.90 (d, <i>J</i> 6 Hz)	6.93 (dd, <i>J</i> 6, 1 Hz)
3-H	6.31 (d, <i>J</i> 5 Hz)	6.28 (d, <i>J</i> 5 Hz)	6.28 (d, <i>J</i> 5 Hz)	6.38 (d, <i>J</i> 4.7 Hz)	6.33 (d, <i>J</i> 4.5 Hz)
4-H	6.78 (d, <i>J</i> 5 Hz)	6.71 (d, <i>J</i> 5 Hz)	6.69 (d, <i>J</i> 5 Hz)	6.82 (d, <i>J</i> 4.7 Hz)	6.76 (d, <i>J</i> 4.5 Hz)
5-H <sup>b</sup>	6.49 (d, <i>J</i> 4 Hz)	6.47 (d, <i>J</i> 5 Hz)	6.50 (d, <i>J</i> 5 Hz)	6.52 (d, <i>J</i> 4 Hz)	6.51 (d, <i>J</i> 4 Hz)
6-H <sup>b</sup>	6.18 (overlapping)	6.14 (d, <i>J</i> 5 Hz)	6.25 (overlapping)	6.18 (overlapping)	6.13 (d, <i>J</i> 4 Hz)
7-H	6.18 (overlapping)	6.26 (dd, <i>J</i> 5, 3 Hz)	6.25 (overlapping)	6.18 (overlapping)	6.23 (dd, <i>J</i> 4, 3 Hz)
8-H	6.82 (dd, <i>J</i> 5, 3 Hz)	6.82 (dd, <i>J</i> 5, 3 Hz)	6.77 (dd, <i>J</i> 5, 4 Hz)	6.82 (obscured)	6.81 (dd, <i>J</i> 4, 3 Hz)
R <sup>2</sup>	6.37 (OH)	3.19 (OMe)	3.38 (m, OCH <sub>2</sub> ) 1.13 (t, OCH <sub>2</sub> CH <sub>3</sub> )	6.32 (OH)	3.14 (OMe)
R <sup>1</sup>	—	—	—	3.80, 3.88 } OMe 3.88, 3.90 } OMe	3.80, 3.85 } OMe 3.85, 3.88 } OMe
α-3'-H				6.96 (d, <i>J</i> 8.6 Hz)	6.93 (d, <i>J</i> 8.6 Hz)
β-3'-H				6.98 (d, <i>J</i> 8.6 Hz)	6.97 (d, <i>J</i> 8.6 Hz)
γ-3'-H				6.89 (d, <i>J</i> 8.6 Hz)	6.88 (d, <i>J</i> 8.6 Hz)
δ-3'-H	7.35–7.55	7.35–7.55	7.3–7.6	6.95 (d, <i>J</i> 8.8 Hz)	6.91 (d, <i>J</i> 8.8 Hz)
α-2'-H	complex	complex	complex	7.29 (d, <i>J</i> 8.6 Hz)	7.28 (d, <i>J</i> 8.6 Hz)
β-2'-H				7.49 (d, <i>J</i> 8.6 Hz)	7.48 (d, <i>J</i> 8.6 Hz)
γ-2'-H				7.39 (d, <i>J</i> 8.6 Hz)	7.43 (d, 8.6)
δ-2'-H	7.91 (d, <i>J</i> 9 Hz)	7.87 (d, <i>J</i> 9 Hz)	7.83 (d, <i>J</i> 9 Hz)	7.91 (d, 8.8)	7.90 (d, 8.8)
NH <sub>A</sub>	10.8	10.2	9.8	11.0	10.2
NH <sub>C</sub>	12.4	12.5	12.3	12.3	12.5
NH <sub>D</sub>	9.95	11.3	10.8	9.9	11.3

<sup>a</sup> The numbering system adopted is that shown in structure (5). <sup>b</sup> For each compound, 5-H and 6-H did not show clear splitting due to coupling to NH, but showed some broadening which was removed on irradiation of NH<sub>C</sub>.

carbon resonances could be accounted for by assuming that water, methanol or ethanol [in the case of compounds (A)–(C) respectively] had added to the terminal carbonyl or enol group of structure (4); an alternative possibility was that the water or alcohol had added to one of the *meso*-positions (especially as this would account for the violet colour due to conjugation of only three pyrrole rings).

In order to provide an answer to these speculations, we decided to synthesise *meso*-<sup>13</sup>C-labelled TPP for further photo-oxidation experiments. Commercially available benzyl alcohol (<sup>13</sup>C labelled in the side-chain) was oxidised with manganese dioxide to <sup>13</sup>C-benzaldehyde and the latter condensed with pyrrole in boiling propionic acid in the usual way.<sup>29</sup> The *meso*-<sup>13</sup>C-labelled TPP was subjected to photo-oxidation in methanolic THF containing sodium methoxide and the <sup>13</sup>C NMR spectrum of the oxidation product was determined. This showed four intense signals at δ 184.6, 142.0, 120.1, and 80.7 (as shown in ref. 1); the lowest field resonance was assigned to the carbonyl of a terminal benzoyl group, and the highest (at δ 80.7) to the quaternary carbon atom, whilst the other two resonances were clearly due to *sp*<sup>2</sup> resonances.<sup>1</sup> Very similar results were observed with the <sup>13</sup>C-labelled pigment (A) (Table 2).

We therefore concluded that water, or methanol, had added to either the α- or γ-methine bridges of the primary oxidation product in the photo-oxidation reactions; addition to the β-methine bridge was ruled out by the visible spectrum which was consistent with conjugation of three rings (rather than two or four). The original structure (4)<sup>21–25</sup> assigned to pigment (A) was thus ruled out and indeed it was difficult to accommodate the two additional hydrogen atoms involved in structure (4) in any other reasonable formulation. This prompted us to re-examine the mass spectra, especially as a number of bile pigments had previously been shown to undergo disproportionation in the mass spectrometer, or to form (*M* + 1) and (*M* + 2) ions.<sup>30</sup> For these reasons we considered the two new structures (5) and (6) for the TPP oxidation products; these corresponded formally to addition of oxygen to the TPP,

**Table 2.** <sup>13</sup>C NMR spectra of the biladienones.<sup>a</sup>

(a) Carbons derived from <i>meso</i> -bridges of the porphyrins								
	γ	α	β	δ				
(5a)	75.0	121.3	142.7	184.5				
(5b)	80.7	120.1	142.0	184.6				
(7a)	74.6	121.1	142.9	183.3				
(7b)	80.4	120.1	142.2	183.4				
(b) Other quaternary carbons								
(7a)	128.5 (α-1'), 129.6 (β-1'), 130.3, 131.0 (δ-1'), 132.0, 135.8 (γ-1'), 139.0, 142.2, 149.4, 150.1, 159.6 (γ-4' and α-4'), 161.0 (β-4'), 162.5 (δ-4') 164.4, 173.2.							
(7b)	128.85, 129.7, 131.1, 131.5, 131.7, 133.2, 138.9, 139.2, 148.3, 151.0, 159.6, 159.7, 160.9, 162.4, 162.65, 172.1.							
(c) Peripheral pyrrole (CH) carbons								
	1	2	3	4	5	6	7	8
(7a)	123.9	137.6	125.6	134.3	125.2	111.6	109.5	118.9
(7b)	124.3	137.0	124.7	133.4	125.8	113.0	110.9	119.1
(d) Phenyl (CH) carbons								
	α-2',6'	β-2',6'	γ-2',6'	δ-2',6'	α, β, γ, and δ-3',5'			
(7a)	132.1	133.3	128.4	131.0	113.3, 113.5, 113.5, and 113.7			
(7b)	132.4	133.1	130.3	131.2	113.2, 113.3, 113.4, and 113.5			
(e) Methoxy carbons								
(5b)	53.0 γ- <i>meso</i> -OMe							
(7a)	55.2, 55.3, 55.3, 55.4 (α, β, γ, and δ-4'-OMe)							
(7b)	55.2, 55.3, 55.3, 55.3 (α, β, γ, and δ-4'-OMe), 52.8 γ- <i>meso</i> -OMe							

<sup>a</sup> Only the chemical shifts of the '*meso*'-carbon atoms of (5a) and (5b) and the *meso*-methoxy group of (5b) were assigned; the full spectrum of the *meso*-<sup>13</sup>C-labelled (5b) is shown in ref. 1.

The numbering scheme adopted is that shown in structure (5). The term *meso* is used to describe the carbons derived from the *meso*-bridge carbons of the parent porphyrin.

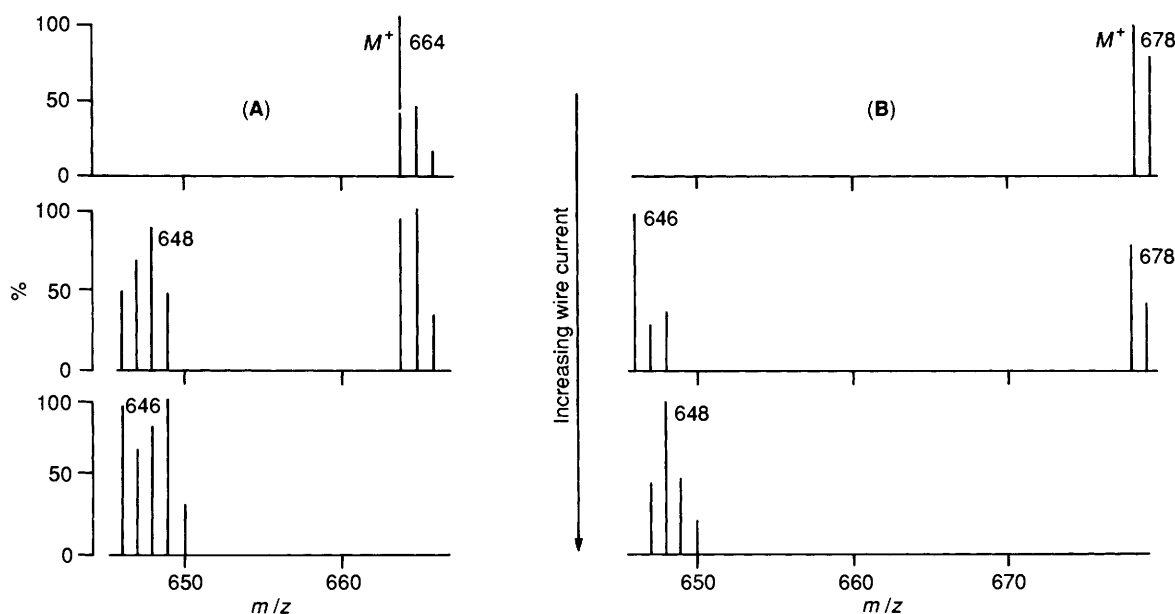
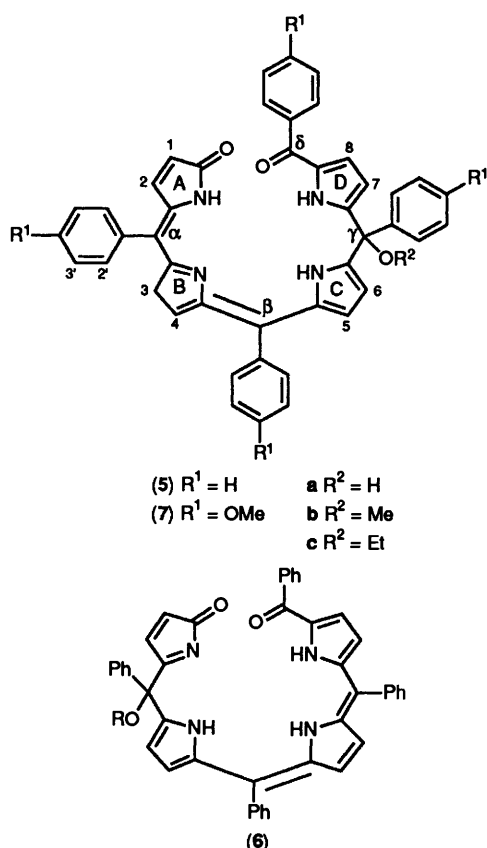


Figure 1. Field desorption mass spectra of purple pigments (A) and (B) [i.e. (5a) and (5b), respectively] showing the effects of increasing the wire current.

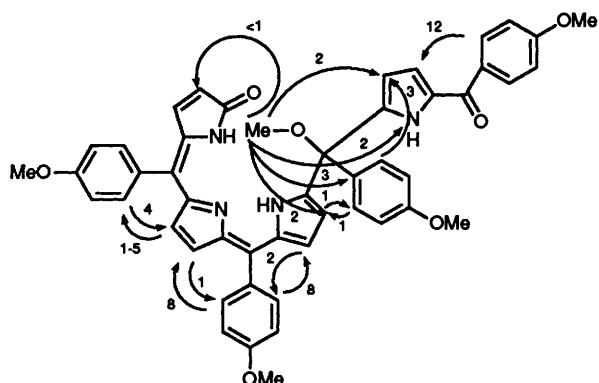


followed by attack of water, methanol or ethanol at either the  $\alpha$ - or  $\gamma$ -*meso*-positions of the primary oxidation product. Field desorption mass spectral experiments readily confirmed these conclusions; thus, when a series of spectra were run as the wire current was slowly increased, the initial spectrum of compound (B) showed only a molecular ion at  $m/z$  678 corresponding to structure (5b) or (6b); subsequently this peak diminished as a new peak at  $m/z$  646 (corresponding to loss of methanol) began

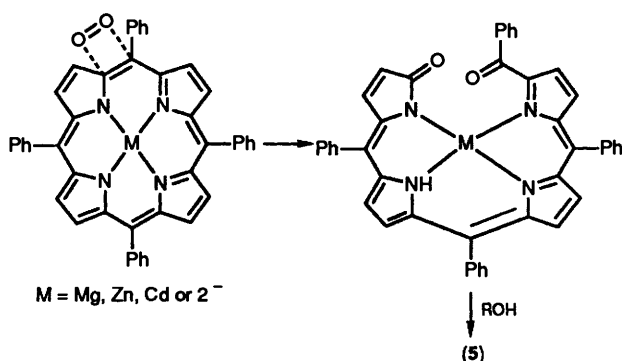
to appear, but as the wire current was further increased to 20  $\mu A$  the base peak changed to  $m/z$  648 and the compound finally decomposed (Figure 1). Similar results were obtained with pigment (A), the initial molecular ion at  $m/z$  664 being replaced by peaks at  $m/z$  646, 647, and 648.

Distinction between the two formulations (5) and (6) was achieved by further NMR studies involving decoupling and NOE difference measurements. For both compounds, decoupling and pseudo-INDOR experiments linked the pyrrole protons into three NH-pyrrole rings and one pyrrolenine ring. The four phenyl groups gave typical overlapping signals, but the spectra of both compounds manifested a low-field 2 H doublet [at  $\delta$  7.91 for compound (5a) and  $\delta$  7.87 for compound (5b)] which was clearly due to the two protons *ortho* to the carbonyl of the benzoyl group. Saturation of this signal caused an NOE of the pyrrole proton resonance at  $\delta$  6.82 in both compounds. For the methoxy compound (5b) saturation of the methoxy signal enhanced the pyrrole resonances at  $\delta$  6.14 and 6.26, both of which are coupled to NH protons. This established the structure and substitution of rings C and D in agreement with evidence from the  $^{13}C$  spectrum of the *meso*- $^{13}C$ -labelled compound described below. It was not possible to establish the tautomeric situation in rings A and B because of overlap of the phenyl signals. Our preliminary communication<sup>1</sup> suggested the formulation (5) to be most likely and this was confirmed unequivocally when the two analogous compounds (7a) and (7b) were prepared from *meso*-tetra-(4-methoxyphenyl)-porphyrin.

At 360 MHz the hydroxy (7a) and methoxy (7b) compounds showed  $^1H$  NMR spectra with the expected similarities to the spectra of the analogues (5a) and (5b) from TPP. The significant difference was that the aryl groups now gave pairs of doublets sufficiently separated from one another and from other resonances to allow complete assignment. Analysis of couplings again confirmed 3 NH-pyrrole rings and one pyrrolenine for both compounds (7a) and (7b). NOE measurements were made on both compounds at all positions not precluded by overlap or proximity of signals. Figure 2 shows the enhancements (%) observed for the methoxy compound (7b) and similar results were obtained for the hydroxy compound (7a). These measurements confirmed the structures completely and allowed all protons to be assigned with certainty. The chemical shifts and



**Figure 2.** Nuclear Overhauser difference effects observed with the purple biladienone (**7b**); the percentage enhancements are shown by the numbers on the arrows.



**Scheme.** Mechanism of photo-oxidation of metal complexes of tetra-arylporphyrins.

coupling constants are listed in Table 1. It was now possible to return to the TPP derivatives (**5a**) and (**5b**) and complete the assignments of pyrrole protons for rings A and B by analogy. Chemical shifts and coupling constants are also shown in Table 1.

The conformations of the products (**5**) and (**7**) are not known. Both methoxy compounds (**5b**) and (**7b**) showed a slight NOE at 1-H on irradiation of the *meso*-methoxy signal. It seems likely that these two compounds have a conformation in which the *meso*-methoxy group lies near to 1-H, at least for part of the time, *cf.* Figure 2. The NH chemical shifts of the two methoxy derivatives (**5b**) and (**7b**) are identical, and the NH chemical shifts of the hydroxy derivatives (**5**) and (**7a**) are close to one another but different in rings A and D from those of compounds (**5b**) and (**7b**). The NH assignments are based on their couplings to pyrrole protons assigned as described, and so they are not in doubt. It seems likely that the *meso*-hydroxy compounds have conformations different from those of the two *meso*-methoxy compounds, because of the possibility of hydrogen bonding to the NH protons or the carbonyl group of ring A.

More complete  $^{13}\text{C}$  NMR analysis was possible for the compounds (**7a**) and (**7b**) derived from *meso*-tetra-(4-methoxyphenyl)porphyrin. For both compounds,  $^{13}\text{C}$ - $^1\text{H}$  shift-correlated 2D spectra were obtained. This enabled all methine carbons to be assigned (Table 2) since all proton assignments had been completely established. For the hydroxy derivative (**7a**) a further 2D-NMR spectroscopic experiment was carried out by the COLOC procedure which correlates  $^{13}\text{C}$  and  $^1\text{H}$  resonances when coupled through two bonds by small coupling constants, typically 5 Hz. This makes it possible to assign quaternary carbon atoms from their coupling to assigned protons on adjacent carbons. In this way, twelve quaternary

carbon signals were assigned unambiguously, as recorded in Table 1; particularly interesting is the conclusive assignment of the four carbons derived from the *meso*-bridge carbons of the parent porphyrin. Unfortunately, with the limited material available, the signals from the eight pyrrole quaternary carbons were too weak for conclusive assignment. Presumably  $\delta$  173.1 represents the terminal carbonyl group of ring A but the others remain unknown. It was not considered necessary to carry out the COLOC procedure for the methoxy derivative (**7b**), but all signals (Table 2) were as expected by comparison with signals from derivative (**7a**).

Our results show unequivocally that the TPP oxidation products are represented by structure (**5**) and those from the corresponding tetra-(4-methoxyphenyl)porphyrin by structure (**7**), the only uncertainty being the conformations of the products. Attempts to obtain suitable crystals for X-ray studies have so far proved unsuccessful.

The photo-oxidation of metal complexes of *meso*-tetraphenylporphyrins occurs by essentially the same mechanism as the photo-oxidation of octaethylporphyrin and chlorin metal complexes and the photo-oxidation of bacteriochlorophyll derivatives (referred to earlier in this paper) *i.e.* singlet oxygen addition occurs followed by ring cleavage as shown in the Scheme. The reason for the secondary addition of water, or an alcohol, in the case of the TPP oxidation products is presumably that when the templating effect of the metal is removed the steric interactions (between the bulky terminal benzoyl group and the pyrrolenone ring at the other end of the molecule) are relieved by formation of a quaternary centre. Removal of the metal from the OEP photo-oxidation products affords the unstable *meso*-oxo bile pigment (**2**), perhaps by addition of water to a methine carbon, followed by oxidation to form an oxo-bridge; the *meso*-phenyl groups prevent the latter oxidation occurring in the TPP series. Nucleophilic additions do not appear to occur readily in the chlorin, or chlorophyll, series although prolonged oxidation of octaethylchlorin with thallium trifluoroacetate results in cleavage of one pyrrole ring and formation of tripyrrolic products.<sup>31</sup>

However, whilst the mechanism of the TPP photo-oxidations is reasonably clear, the mechanisms of the chemical oxidations, which lead to the same products are still uncertain. It was originally suggested that chemical oxidations with cerium(IV) or thallium(III) salts involved addition of two oxygen functions to a *meso*-position and a neighbouring  $\alpha$ -carbon atom followed by hydrolysis and loss of the metal ion to form the bilinone (**4**); in other reactions radical cations,<sup>24</sup> or dications, were invoked and in one case evidence was provided for formation of an intermediate isoporphyrin, by addition of a methoxyl group to a *meso*-bridge,<sup>25</sup> either the isoporphyrin or the radical cation was thought to undergo further oxidation followed by hydrolytic ring opening and loss of the metal ion, to form the bilinone (**4**). However, as we have now rigorously shown that the final products of the TPP oxidations have the general structure (**5**) rather than (**4**), the mechanisms previously suggested for the chemical oxidation must be modified. One possible explanation for the formation of the bilinones (**5**) in the chemical oxidation is that the bilinone (**4**) is the primary product from hydrolytic work-up after chemical oxidations, but that it is unstable and rapidly oxidises to structure (**5**) as soon as the metal is removed.

## Experimental

M.p.s were determined on a hot stage and are uncorrected. Reactions were monitored by TLC and HPLC and by UV and visible spectra as appropriate. Mass spectra were measured with a Varian CH5D spectrometer, either by field desorption (wire currents 5–20  $\mu\text{A}$ ) or by electron impact at 50  $\mu\text{A}$  and 70 eV with source temperatures in the range 200–250  $^\circ\text{C}$ .

$^1\text{H}$  and  $^{13}\text{C}$  NMR spectra were determined at 360 and 90 MHz, respectively, with a Bruker WM360 instrument for  $\text{CDCl}_3$  solutions, and chemical shifts are given as  $\delta$  values from internal TMS. Nuclear Overhauser effects were measured by the difference method using standard Bruker software. Presaturation pulses were applied before each acquisition (32 K points over 4 500 Hz), collecting 128 blocks of 8 with the decoupler alternating between the irradiation and 'blank' positions. A line-broadening of 1 Hz was applied to each subtracted FID before Fourier transformation. Quantitative values for enhancements were determined from integrals.  $^{13}\text{C}$ - $^1\text{H}$  shift-correlated spectra were measured using the Bruker XHCOORD routine optimised for  $J$  135 Hz, and longer range correlations were obtained using the Bruker COLOC routine optimised for  $J$  5 Hz.

*Photo-oxidation of the Dianion of meso-Tetraphenylporphyrin.*—(a) To a solution of sodium methoxide (105 mg) in dry methanol (0.5 ml) and dry THF (200 ml) was added solid meso-tetraphenylporphyrin (500 mg) and the mixture was stirred until the latter had dissolved to afford a deep green solution. This solution was irradiated with visible light (60 W tungsten lamp) and a stream of dry oxygen was bubbled slowly through it for 5 h. Solvent (*ca.*  $\frac{2}{3}$ ) was then removed on a rotary evaporator under reduced pressure at  $<40^\circ\text{C}$ , and the residual solution was diluted with water (150 ml). The product was extracted with dichloromethane ( $3 \times 50$  ml) and the combined extracts were dried ( $\text{MgSO}_4$ ) before being evaporated to dryness on the rotary evaporator. The residue was chromatographed on alumina (Grade III) eluting initially with toluene, which afforded unreacted meso-tetraphenylporphyrin (243 mg) after evaporation of the eluates. Elution with toluene-dichloromethane (1:1, v:v) afforded a minor (unidentified) red pigment, and finally elution with dichloromethane gave a purple eluate. The latter was shown to be homogeneous by TLC and HPLC and, after evaporation of solvent, the residue was recrystallised from dichloromethane-benzene to afford the purple pigment (A) (173 mg) (67% yield based on recovery TPP), m.p. 188–190  $^\circ\text{C}$ . This was subsequently identified by its spectroscopic characteristics (as described in the theoretical section) as the  $\gamma$ -methoxybiladienone (5b). (Found: C, 79.7; H, 5.4; N, 8.5.  $\text{C}_{45}\text{H}_{34}\text{N}_4\text{O}_3$  requires C, 79.6; H, 5.1; N, 8.3%;  $\lambda_{\text{max}}(\text{CHCl}_3)$  (log  $\epsilon_{\text{max}}$ ) 348 (4.43) and 565 (4.27) nm. The  $^1\text{H}$  NMR spectrum is shown in Table 1. NOEs were observed from  $\delta$  2'-H to 8-H (10%) and from  $\delta$  meso-OMe<sub>3</sub> to 6-H (1%), 7-H (1%), 1-H (1%), and  $\text{NH}_D$  (4%).

(b) Similar photo-oxidation reactions were carried out with sodium hydroxide in water and THF (instead of sodium methoxide in methanol and THF) and this afforded the purple  $\gamma$ -hydroxybiladienone (5a), m.p. 138–139  $^\circ\text{C}$  (65%) (Found: C, 79.2; H, 5.2; N, 8.5.  $\text{C}_{44}\text{H}_{32}\text{N}_4\text{O}_3$  requires C, 79.5; H, 4.9; N, 8.4%;  $\lambda_{\text{max}}(\text{CHCl}_3)$  345 and 565 nm. This was shown to be identical in all respects with the compounds previously prepared by photo-oxidation of TPP metal complexes,<sup>21,22</sup> or by cerium(IV) or thallium(III) salt oxidations of TPP metal complexes.<sup>23</sup> The  $^1\text{H}$  NMR spectrum is shown in Table 1. An NOE was observed from  $\delta$  2'-H to 8-H (10%).

(c) A similar photo-oxidation reaction using sodium ethoxide in ethanol afforded the purple  $\gamma$ -ethoxybiladienone (5c) as needles, m.p. 198–201  $^\circ\text{C}$  (70%). (Found: C, 79.4; H, 5.1; N, 7.9.  $\text{C}_{46}\text{H}_{36}\text{N}_4\text{O}_3$  requires C, 79.7; H, 5.2; N, 8.1%;  $\lambda_{\text{max}}(\text{CHCl}_3)$  345 and 565 nm. The  $^1\text{H}$  NMR spectrum is shown in Table 1.

*Interconversion of TPP Photo-oxidation Products.*—(a) The  $\gamma$ -hydroxybiladienone (5a) (20 mg) was dissolved in methanolic sulphuric acid (5%; 5 ml) and kept for 15 min at 20  $^\circ\text{C}$ . The solution was diluted with dichloromethane (100 ml) and the acid neutralised with dilute aqueous sodium carbonate (20 ml).

The organic phase was then washed with water ( $2 \times 25$  ml), dried ( $\text{MgSO}_4$ ) and evaporated to dryness. The residue was taken up in dichloromethane and chromatographed on alumina (Grade III). A minor unstable red fraction was first eluted, followed by the main purple fraction which was evaporated to dryness. The residue was recrystallised from dichloromethane-hexane to afford the purple  $\gamma$ -methoxybiladienone (5b) (10 mg), m.p. 188–190  $^\circ\text{C}$  which was shown to be identical (m.p., HPLC, NMR, and visible spectra) with the pigment formed by photo-oxidation of TPP dianion in methanol-THF.

(b) The  $\gamma$ -methoxybiladienone (5b) was converted into the  $\gamma$ -hydroxy derivative (5a) in a similar manner by shaking a dichloromethane solution with aqueous 2M hydrochloric acid.

The hydroxy and methoxy derivatives were readily separated from each other by HPLC on a column (250  $\times$  5 mm) Spherisorb 5 ODS with acetonitrile-water (80:20, v:v) using a UV detector set at 346 nm; at a flow rate of 1 ml min<sup>-1</sup> the hydroxy derivative (5a) eluted at 15.3 min and the methoxy derivative (5b) at 17.4 min.

*Photo-oxidation of the Dianion of meso-Tetra(4-methoxyphenyl)porphyrin.*—Photo-oxidation of the meso-tetra(4-methoxyphenyl)porphyrin was carried out using sodium methoxide in methanol-THF in the same manner as for meso-tetraphenylporphyrin. After work-up and chromatography, the purple  $\gamma$ -methoxybiladienone (7b), m.p. 196–198  $^\circ\text{C}$  was obtained in 53% yield. (Found: C, 73.5; H, 5.3; N, 6.8.  $\text{C}_{49}\text{H}_{42}\text{N}_4\text{O}_7$  requires C, 73.7; H, 5.3; N, 7.0%;  $\lambda_{\text{max}}(\text{CHCl}_3)$  (log  $\epsilon_{\text{max}}$ ): 375 (4.52) and 573 (4.31) nm. The  $^1\text{H}$  NMR spectrum is shown in Table 1, and NOEs are shown diagrammatically in Figure 2. The corresponding  $\gamma$ -hydroxybiladienone (7a) was also prepared in a similar manner in 70% yield, and formed purple needles, m.p. 187–189  $^\circ\text{C}$  (Found: C, 73.4; H, 5.1; N, 6.9.  $\text{C}_{48}\text{H}_{40}\text{N}_4\text{O}_7$  requires C, 73.45; H, 5.1; N, 7.1%;  $\lambda_{\text{max}}(\text{CHCl}_3)$  (log  $\epsilon$ ): 375 (4.51), 575 (4.38), and 591 sh (6.33) nm. The  $^1\text{H}$  NMR spectrum is shown in Table 1. NOEs were observed as follows:  $\alpha$ -2'-H to 2-H (6%) and 3-H (5%);  $\beta$ -2'-H to 4-H (9%), 5-H (7%) and  $\beta$ -3'-H (11%);  $\gamma$ -2'-H to OH (5%) and  $\gamma$ -3'-H (12%); and  $\delta$ -2'-H to 8-H (12%) and  $\delta$ -3'-H (12%).

*meso- $\alpha,\beta,\gamma,\delta$ - $^{13}\text{C}$ -Tetraphenylporphyrin.*—To a stirred suspension of active manganese dioxide<sup>32</sup> (6.5 g) in hexane (15 ml) was added benzyl alcohol (1.1 ml) and 1'- $^{13}\text{C}$ -benzyl alcohol (95%  $^{13}\text{C}$ ) (0.5 ml) in hexane (5 ml). The mixture was left to stir at 20  $^\circ\text{C}$  overnight and was then diluted with dichloromethane (5 ml) before centrifugation of the oxidant. The organic layer was decanted and the solid residue stirred with fresh dichloromethane ( $3 \times 10$  ml) before being centrifuged ( $\times 3$ ). The combined organic layers were evaporated to dryness to afford a mixture of unchanged benzyl alcohol, together with the desired labelled benzaldehyde (60% conversion by  $^1\text{H}$  NMR spectroscopy). This mixture was taken up in propionic acid (70 ml) and converted into meso  $^{13}\text{C}$ -labelled tetraphenylporphyrin by heating with freshly distilled pyrrole (1.5 ml) and work-up in the normal manner following the literature procedure.<sup>33</sup>

*$^{13}\text{C}$ -Labelled Biladienones (5a) and (5b).*—These were prepared from the meso- $^{13}\text{C}$ -labelled tetraphenylporphyrin by the same methods as described for the unlabelled compounds. The  $^1\text{H}$  NMR spectra were identical with those of the unlabelled compounds, whilst the  $^{13}\text{C}$  spectra showed enhanced signals for three meso-bridge carbons and the benzoyl carbonyl group (see spectrum in ref. 1).

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