164. Dependence of the Regioselectivity of Photo-oxidative Ring Opening of the Chlorophyll Macrocycle on the Complexed Metal Ion

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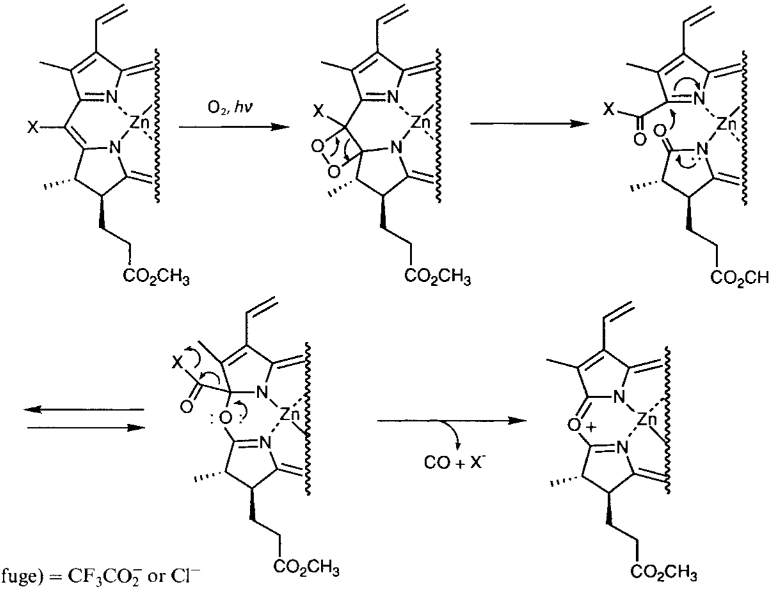
On irradiation with visible light in the presence of air, the zinc complex **1b** of pyropheophorbide-*a* methyl ester (**1a**) is photo-oxidized at the C(20) methine bridge yielding the corresponding 19-formyl-21*H*-bilin-1(23*H*)-one derivative **2**. Surprisingly, the cadmium complex **1c** is cleaved, under the same conditions, at the C(5) methine bridge affording the same type of bilinone which has been recently characterized as an early product of chlorophyll catabolism in plants.

In our recent report on the photo-oxidative ring opening of the zinc chelates of 20-trifluoroacetoxy and 20-chloro derivatives of pheophorbide-*a* methyl ester and pyropheophorbide-*a* methyl ester (**1a**) [1], we proposed a reaction mechanism in which one molecule of O_2 is added to the most nucleophilic methine bridge of the chlorophyll chromophore, before concerted loss of CO and the substituent at C(20) proceeds to yield

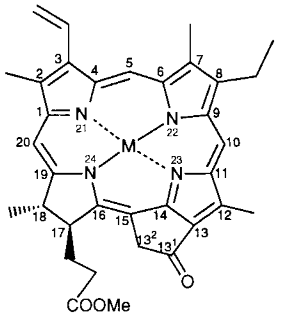
Scheme. *Possible Mechanism of Photo-oxidative Ring Cleavage of the Zinc Chelates of 20-Trifluoroacetoxy and 20-Chloro Derivatives of Pyropheophorbide-*a *Methyl Ester* (**1a**)[a]) (*cf.* [1])



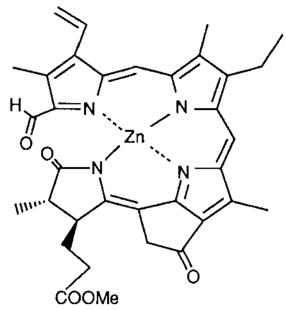
X (a nucleofuge) = $CF_3CO_2^-$ or Cl^-

[a]) For the sake of clarity, only the part of the molecule which is modified during the raction is represented in the partial structure, remainder as in **1**.

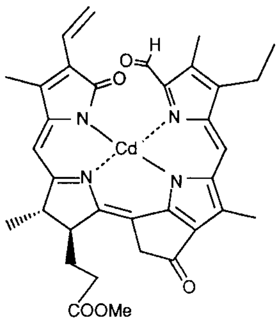
oxoniachlorin complexes as the reaction products (*cf. Scheme*). Owing to the improbable release of a hydride ion, as a nucleofuge, 19-formyl-21*H*-bilin-1(23*H*)-one complexes should be the final reaction products of the photo-oxygenation of C(20)-unsubstituted chlorophyll derivatives, in the same way as 20-methyl derivatives in the bacteriochlorophyll-*c* and -*e* series yield 19-acetyl-21*H*-bilin-1(23*H*)-ones (so-called photobilins) when irradiated with visible light in the presence of O_2 [2–4]. As a matter of fact, 19-formylbilinone complexes have been obtained on photo-oxidation of metalloporphyrins and metallochlorins with low half-wave oxidation potentials [5–7] but not yet, to the best of our knowledge, from chlorophyll derivatives (*cf.* [8]).



1a M = 2H
b M = Zn
c M = Cd



2



3

In our hands, photo-oxidation of the zinc chelate of pheophorbide-*a* methyl ester gave, after chromatographic separation, a mixture of two compounds, whose purification has failed until now. The corresponding pyropheophorbide-*a* derivative **1b**, on the contrary, afforded a rather sensitive brownish pigment **2** in *ca.* 50% yield, which could be purified with considerable loss by flash chromatography [9] and characterized by spectroscopic techniques. Particularly, the ^{1}H-NMR data with the presence of *s*'s at 6.89 and 7.41 ppm as well as the NOE's (*Table 1*) of **2** indicate that oxidative cleavage of the macrocycle takes place at the C(19)–C(20) bond. Until now, the isomeric formylbilinone which would result from the cleavage of the C(1)–C(20) bond of **1b** according to the mechanism depicted in the *Scheme*, when the appropriate resonance structure of the substrate is taken into consideration, could not be isolated.

Table 1. *^{1}H-NMR Signals* [ppm] *of* **2** *Assigned by Differential ^{1}H,^{1}H-NOE Experiments.* Percent enhancements of intensity are given in parentheses.

Irradiated signal	Enhanced signal	Interacting protons	Irradiated signal	Enhanced signal	Interacting protons
9.24	2.39 (2.9)	CHO,$CH_3(2^1)$	2.57	6.85 (1.6)	$CH_3(12^1)$,CH(10)
7.41	6.92 (10.9)	CH(5),$CH(3^1)$	2.39	9.24 (2.2)	$CH_3(2^1)$,CHO
	2.29 (6.8)	CH(5),$CH_3(7^1)$		6.92 (2.3)	$CH_3(2^1)$,$CH(3^1)$
6.85	2.59, 2.69 (1.4)	CH(10),$CH_2(8^1)$		5.54 (2.1)	$CH_3(2^1)$,$CH_2(3^2)$
	2.57 (4.2)	CH(10),$CH_3(12^1)$	2.29	7.41 (3.0)	$CH_3(7^1)$,CH(5)
	1.18 (1.4)	CH(10),$CH_3(8^2)$			

Bearing in mind that cadmium(II) porphyrinates have lower one-electron oxidation potentials than the Zn^{II} complexes [10], whose oxidation potentials, in their turn, are *ca.* 300 mV higher than those of the analogous metallochlorins [11], we anticipated a smoother completion of the photo-oxidation using the pyropheophorbide derivative **1c** instead of **1b**. In fact, however, the product mixture obtained from cadmium(II) pyropheophorbidato-*a* methyl ester (**1c**) proved to be more complex than in the case of the Zn^{II} chelate **1b**. Nevertheless, after a short reaction time, a greenish formylbilinone was isolated as the main product, along with a large amount of demetalled starting material and many unidentified by-products. After chromatographic purification, structure **3** was established for the main product by spectroscopic methods. Both the presence of *s*'s at 7.00 and 5.24 ppm and the characteristic *ABX* pattern in the ^{1}H-NMR, assigned to a vinyl group adjacent to a lactam carbonyl group [12–14], suggest that in this case, oxidative cleavage of the macrocycle has occurred at the C(5) methine bridge. This is corroborated by the NOE's of **3** given in *Table 2*. It is, moreover, noteworthy that according to the observed NOE's, the preferred conformation of the vinyl group in **2** is s-*cis* with respect to the endocyclic double bond, whereas in **3** it is s-*trans*.

Table 2. *^{1}H-NMR Signals* [ppm] *of* **3** *Assigned by Differential ^{1}H,^{1}H-NOE Experiments.* Percent enhancements of intensity are given in parentheses.

Irradiated signal	Enhanced signal	Interacting protons	Irradiated signal	Enhanced signal	Interacting protons
9.71	2.16 (3.7)	$CHO,CH_3(7^1)$	2.16	9.71 (2.8)	$CH_3(7^1),CHO$
7.00	2.51 (4.5)	$CH(10),CH_2(8^1)$		2.51 (0.7)	$CH_3(7^1),CH_2(8^1)$
	2.40 (6.8)	$CH(10),CH_3(12^1)$		1.11 (1.4)	$CH_3(7^1),CH_3(8^2)$
	1.11 (1.6)	$CH(10),CH_3(8^2)$	2.04	6.44 (2.9)	$CH_3(2^1),CH(3^1)$
5.24	2.59 (2.4)	$CH(20),CH(18)$		5.24 (2.7)	$CH_3(2^1),CH(20)$
	2.04 (7.4)	$CH(20),CH_3(2^1)$	1.20	5.24 (1.4)	$CH_3(18^1),CH(20)$
	1.20 (1.9)	$CH(20),CH_3(18^1)$		2.59 (3.0)	$CH_3(18^1),CH(18)$
2.40	7.00 (2.3)	$CH_3(12^1),CH(10)$		2.43 (1.9)	$CH_3(18^1),CH(17)$

As the first oxidation potentials of the ligand in Cd^{II} and Mg^{II} octaethylporphinate are closer to each other ($E_{1/2} = 0.55$ and 0.54 V, resp.) than to that of the Zn^{II} chelate ($E_{1/2} = 0.63$ V), the regioselectivity of the oxidative cleavage of the chlorin macrocycle of **1c** is not only interesting from the mechanistic point of view, but particularly also in connection with the structures of the primary products of chlorophyll breakdown in chloroplasts of senescent barley [15]¹) and in microalgae [16]. In both cases, cleavage of the chlorin macrocycle at the C(5) methine bridge has been observed, although on the basis of theoretical considerations [17] and the structure of some luciferins isolated from bioluminescent plancton [18] [19], initial electrophilic attack at the methine bridges adjacent to the reduced pyrrole ring has been predicted (*cf.* [20] [21]). The question, whether the different behaviour of **1b** and **1c** is conditioned by different mechanisms of the photo-oxidation reaction is at present under investigation.

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Experimental Part

General. See [1]. Pyropheophorbide-*a* methyl ester (**1a**) was prepared from chlorophyll *a*, obtained from *Spirulina geitlerii*, by standard procedures [22] [23]. MS and ^{1}H-NMR spectra (360.13 MHz) were measured by *F. Nydegger* and *F. Fehr*, resp. ^{13}C-NMR spectra (50.3 MHz): *Varian-Gemini-200* instrument; δ(H) and δ(C) in ppm rel. to $CHCl_3$ (δ(H) = 7.26 ppm) and $CDCl_3$ (δ(C) = 77.5 ppm), resp., as internal standards; ^{1}H-NMR assignments are based on multiplicity and/or by NOE correlations.

*(Pyropheophorbidato-*a *Methyl Ester)cadmium(II) (= [13-De(methoxycarbonyl)pheophorbidato-*a *Methyl Ester]cadmium(II);* **1c**). A soln. of $Cd(OAc)_2$ (300 mg) in MeOH (50 ml) was added to a soln. of **1a** (300 mg) in CH_2Cl_2 (50 ml) and refluxed for 2 h. Then the mixture was poured into H_2O and extracted with CH_2Cl_2 and the org. layer washed with H_2O and evaporated. The green residue was crystallized from CH_2Cl_2/hexane:322 mg (89%) of **1c**. M.p. 173–175°. UV/VIS (CH_2Cl_2): 658 (4.53), 620 (3.98), 428 (4.77), 412 (sh). ^{1}H-NMR ($CDCl_3$, $5.5 \cdot 10^{-3}$ M): 7.97 (*s*, CH(20)); 7.78 (*s*, CH(10)); 7.72 (*s*, CH(5)); 7.53 (*dd*, *J* = 17.9, 11.4, CH(3^1)); 6.02 (*dd*, *J* = 17.9, 1.5, CH(3^2), *trans*); 5.96 (*dd*, *J* = 11.4, 1.5, CH(3^2), *cis*); 5.04, 4.73 (*AB*, *J* = 19.6, CH_2(13^2)); 4.38 (*qd*, *J* = 7.0, 3.3, CH(18)); 4.03 (*m*, CH(17)); 3.73 (*s*, CH_3O); 3.19 (*s*, CH_3(12^1)); 3.15 (*s*, CH_3(2^1)); 2.9–2.4 (*m*, CH_2(17^1), CH_2(17^2)); 2.71 (*q*, *J* = 7.5, CH_2(8^1)); 2.31 (*s*, CH_3(7^1)); 1.85 (*d*, *J* = 7.0, CH_2(18^1)); 1.03 (*t*, *J* = 7.5, CH_3(8^2)). ^{13}C-NMR ($CDCl_3$): 196.4 (*s*, C(13^1)); 173.9 (*s*, C(17^3)); 168.1, 162.4, 156.3, 154.0, 150.1, 147.0, 146.9, 144.1, 142.3, 138.4, 135.4, 134.6, 132.4, 131.5 (14*s*, quat. C's); 130.1 (*d*, C(3^1)); 120.1 (*t*, C(3^2)); 105.8 (*s*, C(15)); 105.0, 97.4, 91.4 (3*d*, C(5), C(10), C(20)); 52.2 (*q*, CH_3O); 52.1, 49.9 (2*d*, C(17), C(18)); 49.2 (*t*, C(13^2)); 31.8, 30.3 (2*t*, C(17^1), C(17^2)); 23.4 (*q*, C(18^1)); 19.1 (*t*, C(8^1)); 16.9 (*q*, C(8^2)); 12.7, 12.5, 10.5 (3*q*, C(2^1), C(7^1), C(12^1)). FAB-MS (3-nitrobenzyl alcohol): 660 ($M^{+\cdot}$) and 661 (MH^+) for ^{114}Cd.

*(19,20-Dioxo-19,20-secopyropheophorbidato-*a *Methyl Ester)zinc(II)* (**2**). A soln. of zinc complex **1b** [24] (38 mg) in CH_2Cl_2 (350 ml) was irradiated with two tungsten lamps (each 250 W) at a distance of 30 cm in a reactor provided with H_2O refrigeration and O_2 bubbling. After 3 h, the soln. was quickly filtered through a short column (2 cm diam., 11 cm high) of silica gel *60* (*Merck;* 40–60 μ), and the product retained on the top was eluted with CH_2Cl_2/acetone 92:8. Thus, 3 mg (8%) of **1b** were recovered, and 15.9 mg (40%) of **2** were isolated from the immediately following brownish band (after crystallization from CH_2Cl_2/hexane). M.p. > 150° (dec.). UV/VIS (CH_2Cl_2): 858 (4.10), 610 (3.56), 568 (3.92), 456 (4.19), 334 (4.30). ^{1}H-NMR ($CDCl_3$, $1.1 \cdot 10^{-2}$ M): 9.24 (*s*, CHO); 7.41 (*s*, CH(5)); 6.92 (*dd*, *J* = 17.6, 11.7, CH(3^1)); 6.85 (*s*, CH(10)); 5.54 (*dd*, *J* = 17.6, 1.5, CH(3^2), *trans*); 5.53 (*dd*, *J* = 11.7, 1.5, CH(3^2), *cis*); 3.80, 3.56 (*AB*, *J* = 20.2, CH_2(13^2)); 3.73 (*s*, CH_3O); 2.81 (*dt*, *J* = 10.7, 3.3, CH(17)); 2.59, 2.69 (*AB* of ABX_3, J_{AB} = 14, *J* = 7.6, CH_2(8^1)); 2.62–2.45, 2.37–2.25, 1.90 (3*m*, 5 H, CH_2(17^1), CH_2(17^2), CH(18)); 2.57 (*s*, CH_3(12^1)); 2.39 (*s*, CH_3(2^1)); 2.29 (*s*, CH_3(7^1)); 1.18 (*t*, *J* = 7.5, CH_3(8^2)); 1.14 (*d*, *J* = 7.4, CH_3(18^1)). ^{13}C-NMR ($CDCl_3$): 195.8 (*s*, C(13^1)); 192.5 (*s*, C(1) ?); 186.8 (*d*, CHO); 173.5 (*s*, C(17^3)); 175.7, 175.6, 160.9, 158.9, 150.4, 150.2, 146.5, 145.0, 140.3, 139.9, 139.2, 136.8, 129.9 (13*s*, C(19), quat. C's); 128.9 (*d*, C(3^1)); 119.5 (*t*, C(3^2)); 123.6, 111.1 (2*d*, C(5), C(10)); 100.1 (*s*, C(15)); 52.2 (*q*, CH_3O); 49.6, 41.9 (2*d*, C(17), C(18)); 46.1 (*t*, C(13^2)); 31.3, 28.6 (2*t*, C(17^1), C(17^2)); 18.2 (*t*, C(8^1)); 17.1, 16.0 (2*q*, C(8^2), C(18^1)); 12.1, 11.1, 10.1 (3*q*, C(2^1), C(7^1), C(12^1)). FAB-MS (3-nitrobenzyl alcohol): 642 ($M^{+\cdot}$) and 643 (MH^+) for ^{64}Zn.

*(4,5-Dioxo-4,5-secopyropheophorbidato-*a *Methyl Ester)cadmium(II)* (**3**). A soln. of **1c** (30 mg) in CH_2Cl_2 (350 ml) was photo-oxygenated for 25 min under the same conditions as above. After evaporation of the solvent, 4 main components were isolated from the residue by TLC (silica gel, CH_2Cl_2/acetone 9:1): demetalled starting material (13.3 mg, 53%; R_f 0.8), a blueish and a brownish by-product (R_f 0.35 and 0.30, resp.) whose structures have not yet been elucidated, and greenish **3** (3.2 mg, 10%; R_f 0.45) which was extracted from silica gel with acetone and crystallized from CH_2Cl_2/hexane. M.p. > 150° (dec.). UV/VIS (CH_2Cl_2): 680 (3.93), 462 (4.40), 3.18 (4.38). ^{1}H-NMR ($CDCl_3$, $9.2 \cdot 10^{-3}$ M); 9.71 (*s*, CHO); 7.00 (*s*, CH(10)); 6.44 (*dd*, *J* = 17.6, 11.5, CH(3^1)); 6.10 (*dm*, *J* = 17.6, CH(3^2), *trans*); 5.39 (*dd*, *J* = 11.5, 2.2, CH(3^2), *cis*); 5.24 (*s*, CH(20)); 3.64 (*s*, CH_3O); 3.41, 3.25 (*AB*, *J* = 20.9, CH_2(13^2)); 2.59 (*qd*, *J* = 7.3, 1.8, CH(18)); 2.51 (*q*, *J* = 7.6, CH_2(8^1)); 2.40 (*s*, CH_3(12^1)); 2.43 (*m*, CH(17)); 2.32 (*m*, CH_2(17^2)); 2.16 (*s*, CH_3(7^1)); 2.04 (*s*, CH_3(2^1)); 1.95, 1.72 (2*m*, CH_2(17^1)); 1.20 (*d*, *J* = 7.3, CH_3(18^1)); 1.11 (*t*, *J* = 7.6, CH_3(8^2)). ^{13}C-NMR ($CDCl_3$): 194.8 (*s*, C(13^1)); 187.2 (*d*, CHO); 173.8, 173.2 (2*s*, C(4), C(17^3)); 183.1, 182.7, 163.6, 159.0, 153.5, 151.3, 147.0, 144.6, 143.8, 142.0, 132.5, 131.9, 129.3 (13*s*, quat. C's); 128.8, 126.7 (2*d*, C(3^1), C(10)); 122.0 (*t*, C(3^2)); 113.8 (*s*, C(15)); 95.1 (*d*, C(20)); 52.4 (*q*, CH_3O); 49.9, 47.6 (2*d*, C(17), C(18)); 45.8 (*t*, C(13^2)); 31.5, 28.4 (2*t*, C(17^1), C(17^2)); 20.1 (*q*, C(18^1)); 18.5 (*t*, C(8^1)); 16.8 (*q*, C(8^2)); 11.6, 10.4, 9.8 (3*q*, C(2^1), C(7^1), C(12^1)). FAB-MS (3-nitrobenzyl alcohol): 692 ($M^{+\cdot}$) and 693 (MH^+) for ^{114}Cd.

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