[1] a) G. A. Jeffrey in *Comprehensive Supramolecular Chemistry*, *Vol. 6* (Eds.: J. L. Atwood, J. E. D. Davies, D. D. MacNicol, F. Vögtle, K. S. Suslick), Pergamon, Oxford, **1996**, pp. 757 – 788; b) J. A. Ripmeester, *Ann. N.Y. Acad. Sci.* **2000**, *912*, 1 – 16.

[2] a) E. D. Sloan, Jr., *Hydrates of Natural Gas*, 2nd ed., Marcel Dekker, New York, **1998**; b) "Gas Hydrates: Challenges for the Future": *Ann. N.Y. Acad. Sci.* **2000**, *912*.

[3] a) J. Hernandez, N. Uras, J. P. Devlin, *J. Phys. Chem. B* **1998**, *102*, 4526 – 4535; b) A. K. Sum, R. C. Burruss, E. D. Sloan, Jr., *J. Phys. Chem. B* **1997**, *101*, 7371 – 7377; C. Tulk, J. A. Ripmeester, D. D. Klug, *Ann. N.Y. Acad. Sci.* **2000**, *912*, 859 – 872.

[4] a) M. J. Collins, C. I. Ratcliffe, J. A. Ripmeester, *J. Phys. Chem.* **1990**, *94*, 157 – 162; b) J. A. Ripmeester, C. I. Ratcliffe, *J. Phys. Chem.*, **1990**, *94*, 8773 – 8776; c) J. A. Ripmeester, C. I. Ratcliffe, *Energy Fuels* **1998**, *12*, 197 – 200.

[5] a) J. A. Ripmeester, *J. Am. Chem. Soc.* **1982**, *104*, 289 – 290; b) D. W. Davidson, Y. P. Handa, J. A. Ripmeester, *J. Phys. Chem.* **1986**, *90*, 6549 – 6552.

[6] a) M. A. Bouchiat, T. R. Carver, C. N. Varnum, *Phys. Rev. Lett.* **1960**, *5*, 373 – 375; b) B. C. Grover, *Phys. Rev. Lett.* **1978**, *40*, 391 – 392; c) W. Happer, E. Miron, S. Schaefer, D. Schreiber, W. A. van Wijngaarden, X. Zeng, *Phys. Rev. A* **1984**, *29*, 3092 – 3110; d) T. G. Walker, W. Happer, *Rev. Mod. Phys.* **1997**, *69*, 629 – 642; e) N. D. Blaskar, W. Happer, T. McCleland, *Phys. Rev. Lett.* **1982**, *49*, 25 – 28.

[7] T. Pietrass, H. C. Gaede, A. Bifone, A. Pines, J. A. Ripmeester, *J. Am. Chem. Soc.* **1995**, *117*, 7520 – 7525.

[8] I. L. Moudrakovski, A. Sanchez, C. I. Ratcliffe, J. A. Ripmeester, unpublished results.

[9] a) D. Raftery, H. Long, T. Meersmann, P. Grandinetti, L. Reven, A. Pines, *Phys. Rev. Lett.* **1991**, *66*, 584; b) I. L. Moudrakovski, A. Sanchez, C. I. Ratcliffe, J. A. Ripmeester, *J. Phys. Chem. B* **2000** *104*, 7306 – 7310.

[10] In previous work,[7] HP Xe was delivered to the ice sample in frozen form, and since it was necessary to wait for xenon to sublime, this contributed significantly to the uncertainty in the starting point for kinetic measurements. In this work, HP Xe was always delivered as a gas, allowing accurate sampling of the early stages of the interaction.

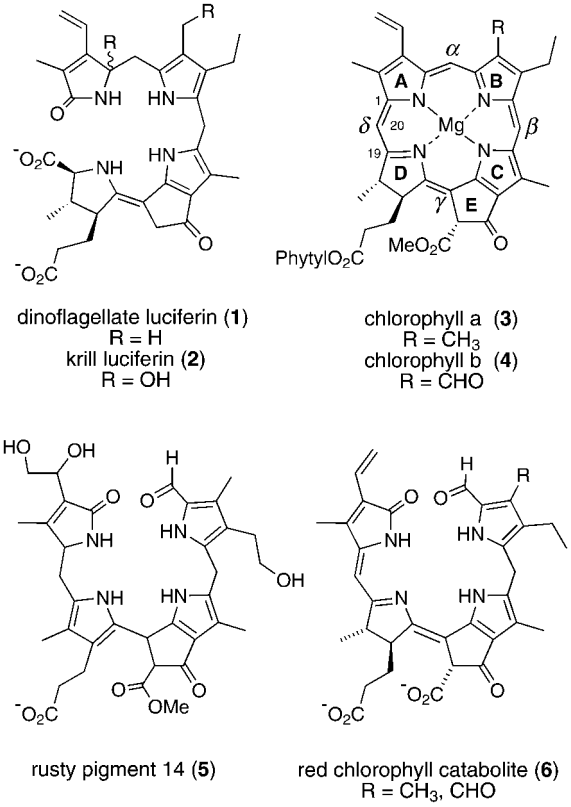
[11] We note here the FTIR observation of CO_2, normally a structure I hydrate guest, as occupying the large cage in Str. II hydrate in epitaxially grown hydrate thin films at low temperature: F. Fleyfel, J. P. Devlin, *J. Phys. Chem.* **1991**, *95*, 3811 – 3815.

[12] This will also explain the persistence of the signal of Xe in the small cages of str. II hydrate on application of a vacuum. If Str. I is formed strictly from the decomposition of the metastable str. II hydrate it will always be on the surface and decompose readily. However, Xe atoms can migrate deep into the str. II hydrate to fill the small cages as it is the stable phase and the dynamic properties of the THF hydrate water lattice are favorable for easy transport.

Chlorophyll Catabolism Leading to the Skeleton of Dinoflagellate and Krill Luciferins: Hypothesis and Model Studies**

George Topalov and Yoshito Kishi*

In the late 1980s, we reported the structures of dinoflagellate luciferin (**1**) and krill luciferin (**2**) (Scheme 1).[1] Recognizing their structural similarity with chlorophylls a and b (**3** and **4**, respectively), we speculate that dinoflagellate luciferin



Scheme 1. Structure of dinoflagellate and krill luciferins and representative chlorophyll catabolites.

(**1**) and krill luciferin (**2**) are derived through an oxidative ring cleavage at the C1–C20 bond of chlorophylls with retention of the C20 carbon atom as a carboxylate at ring D. Shortly after the structures of **1** and **2** were disclosed, chlorophyll catabolites from barley and *Chlorella protothecoides* were isolated and characterized (**5** and **6**, respectively; Scheme 1).[2, 3] It is evident that, unlike dinoflagellate and krill luciferins, these catabolites are formed through cleavage of the C4–C5 bond of chlorophylls with retention of the C5 carbon atom as a

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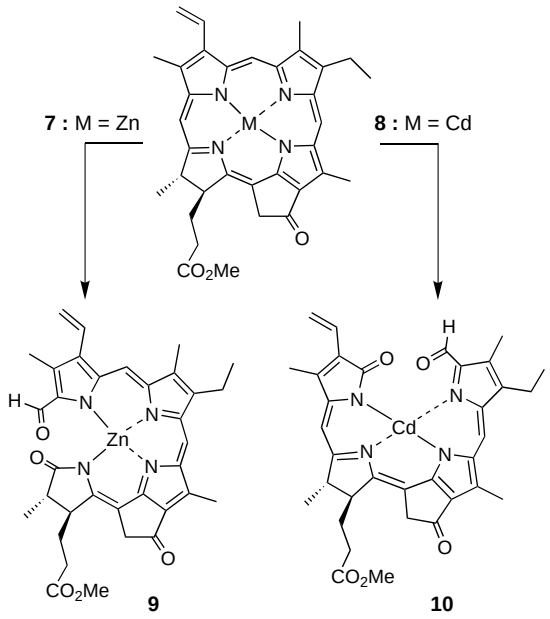
formyl group at ring B. Chemical bond cleavages are also known on Cd– and Zn–chelates of pyropheophorbide a (Scheme 2).[4] Interestingly, C4–C5 bond cleavage occurred for the Cd–chelate **8** to give **10**, whereas C19–C20 bond cleavage occurred for Zn–chelate **7** to give **9**.

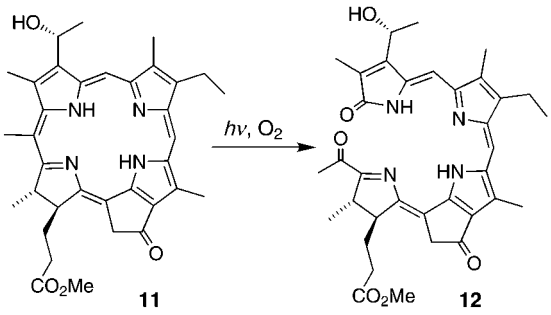


Scheme 2. Photooxidation of Cd– and Zn–chelates of pyropheophorbide a.

To the best of our knowledge, no example is known for the enzymatic or chemical cleavage of the C1–C20 bond, which would transform chlorophylls to the skeleton of dinoflagellate and krill luciferins (see Scheme 1). There are two intriguing aspects of this presumed mode of chlorophyll catabolism: 1) the selectivity in the bond cleavage at the α- versus δ-bridge, and 2) the selectivity in the bond cleavage at the C1–C20 versus C19–C20 bond.

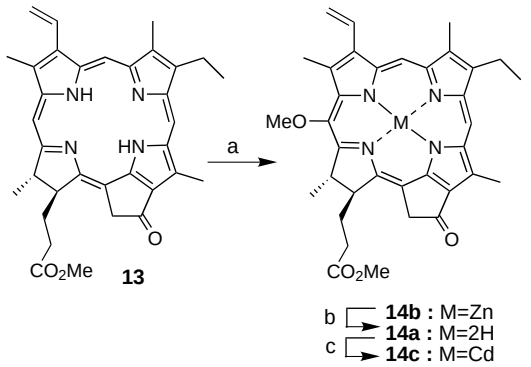
For several reasons, we have hypothesized that this presumed mode of chlorophyll catabolism may proceed through two distinct steps, that is, a hydroxylation or equivalent event at C20,[5] followed by cleavage of the C1–C20 bond. First, the δ-bridge of pheophorbides is known to be most prone to electrophilic attack.[6] Indeed, a C20-chlorinated chlorophyll was isolated from blue-green alga.[7] Second, the C20 carbon atom of a chlorophyll is retained as a carboxylate, instead of a formyl, in the luciferins **1** and **2**, which could be explained by the oxidation of C20 prior to bond cleavage. Third, the photooxidation of bacteriochlorophyll e or its derivatives (cf., **11**) is known to involve a selective cleavage of the C1–C20 bond (Scheme 3).[8] Although steric interactions between the methyl groups at C20 and C2 could be the source for the observed selectivity, electronic effects, exercised by the electron-donating methyl group, may also play an important role for an overall transformation of chlorophylls into the skeleton of dinoflagellate and krill luciferins.

To test this hypothesis, we planned to photooxidize a substrate bearing a methoxy group at C20 and its metal



Scheme 3. Photooxidation of bacteriochlorophyll e.

chelates. In practice, methyl 20-methoxychlorin (**14a**), along with its Zn– (**14b**) and Cd–chelates (**14c**), was synthesized from pyropheophorbide a methyl ester (**13**) (Scheme 4).[9]



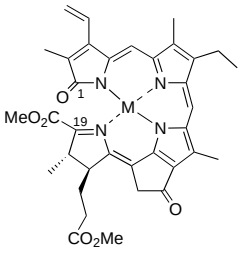
Scheme 4. Synthesis of methyl 20-methoxychlorin and its Zn– and Cd–chelates. Reagents and conditions: a) 1. $Zn(OAc)_2 \cdot 2H_2O$, 1:1 MeOH:CH_2Cl_2, 4 h (90%); 2. $Tl(O_2CCF_3)_3$ (1.1 equiv), THF, 15 min (80%);[16a] 3. 0.25 M NaOAc/MeOH, 1 h;[16b] 4. MeI/K_2CO_3, 1:1 THF:CH_3CN (30% over 2 steps). b) HCl aq./MeOH, 5 min (70%). c) $Cd(OAc)_2 \cdot 2H_2O$, 1:1 MeOH:CH_2Cl_2, 2 h (80%).

Photooxidation was carried out by irradiation of a dilute solution (ca 7.10^{-5} M) of the substrate by using three 300 W tungsten lamps at 30 cm from a reaction flask provided with cooling and oxygen bubbling. The solvent and the reaction time varied with the substrate; Cd–chelate **14c** was irradiated for 20–25 min in benzene, Zn–chelate **14b** for 20 min in benzene or dichloromethane, and the metal-free substrate **14a** for 3 h in benzene.

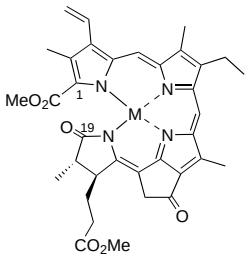
Table 1 summarizes the product distribution observed under these conditions. Most significantly, the photooxidation of chlorin **14a** smoothly proceeded to yield exclusively the C1–C20 bond cleaved product **15a** (Figure 1) in 45% yield, along with a substantial amount of the unconsumed starting material.[10]

The molecular weights of photooxidation products **15** and **16** were established by fast atom bombardment mass spectrometry (FAB-MS), and the presence of a carbomethoxy group but the absence of a formyl group in **15** and **16** were demonstrated by ^{1}H NMR spectroscopy. These experiments established that all the photooxidation products isolated were derived by cleavage of a δ-bridge by singlet oxygen, thereby leaving only two possible structures, that is, **15** and **16**. Differentiation of **15** from **16** could be made through

Table 1. Product distributions of the photooxidation of methyl 20-methoxychlorin and its Zn– and Cd–chelates.



15a–c



16a–c

14a (M = 2H)	→	**15a** (M = 2H) 45%	**16a** (M = 2H) none
14b (M = Zn)	→ none	**15b** (M = Zn)	**16b** (M = Zn) 45%
14c (M = Cd)	→	**15c** (M = Cd) ~5%	**16c** (M = Cd) ~20%

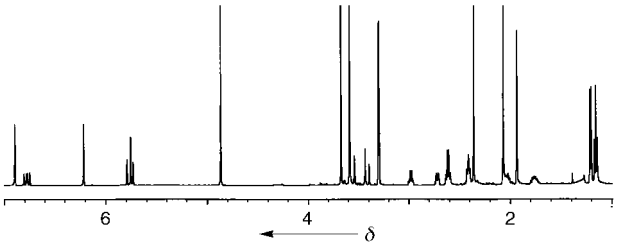


Figure 1. ^{1}H NMR spectrum of the photooxidation product **15a** (500 MHz, CD_3OD).

establishing the bond connectivity of the carbomethoxy group at C20 to the rest of the molecule. In spite of extensive efforts, however, we were unable to detect a cross-peak establishing the bond connectivity between the carbomethoxy group at C20 and the rest of the molecule through heteronuclear multiple bond correlation (HMBC) NMR experiments.[11] Thus, we relied on UV/Vis spectroscopy. Two possible photooxidation products were obtained only in the Cd–chelate series. The UV/Vis spectrum of **15c** (λ_{max} = 667, 437, 360 nm (MeOH)) was dramatically different from that of **16c** (λ_{max} = 886, 670, 571, 439, 380 nm (MeOH)). Next, **15c** was chemically correlated with **15a**; upon treatment with sodium diethyldithiocarbamate hydrate in methanol at room temerature, **15c** was cleanly converted to the metal-free product **15a**.[12] The UV spectrum of **15a** (λ_{max} = 570, 357, 317 nm (MeOH)) was almost superimposable on that of the methylketone **12** (λ_{max} = 576, 344 nm (MeOH)).[13] On the other hand, the UV spectra of **16b** (λ_{max} = 868, 612, 564 nm (CH_2Cl_2); 883, 666, 552, 432, 388 nm (MeOH)) and **16c** (λ_{max} = 886, 670, 571, 439, 380 nm (MeOH)) were very similar to that of **9** (λ_{max} = 858, 610, 568 nm (CH_2Cl_2); 857, 581, 455, 433, 376 nm (MeOH)). These results show that **15a** and **15c** belong to the C1–C20 bond-cleaved series, whereas **16b** and **16c** belong to the C19–C20 bond-cleaved series. The ^{13}C spectra of **15** and **16** provided further support to the assigned structures. The ^{13}C chemical shifts for C1 and C19 of **16b** were δ = 145.5 and 190.4 ($CDCl_3$), respectively, which compared well with the chemical shifts (δ = 150.4 and 192.5 ($CDCl_3$)) reported for **9**.[14] However, **15c** (δ_{C1} = 181.7 and δ_{C19} = 172.1 ([D_6]acetone)) and **15a** (δ_{C1} = 172.9 and δ_{C19} = 173.3 (CD_3OD)) gave ^{13}C chemical shifts significantly different from those reported for **9**.

The remarkable selectivity observed in the photooxidation of **14a** to **15a** lends strong support to the hypothesis that a hydroxy or equivalent group at C20 directs the C1–C20 bond cleavage that transforms chlorophylls into krill and dinoflagellate luciferins. The methoxy substituent at C20 is a powerful δ-bridge directing group even in the case of Cd–chelate **14c**.[15] This is in sharp contrast with the case where the Cd–chelate without the C20 methoxy substituent was photooxidized at the C4–C5 bond. The fact that δ-methoxy Zn–chelate **14b** is cleaved in the same way as the unsubstituted Zn–chelate **7** may imply that the methoxy group simply lowers the oxidation potential of the pyropheophorbide without influencing electron distribution within the aromatic system.

In conclusion, the photooxidation experiments suggest the existence of a new pathway for chlorophyll catabolism in dinoflagellates and krill. The selective and efficient bond cleavage at the C19–C20 bond reported immediately suggests a possible route for a synthesis of dinoflagellate and krill luciferins from chlorophylls a and b.

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[1] a) H. Nakamura, Y. Kishi, O. Shimomura, D. Morse, J. W. Hastings, *J. Am. Chem. Soc.* **1989**, *111*, 7607; b) H. Nakamura, B. Musicki, Y. Kishi, O. Shimomura, *J. Am. Chem. Soc.* **1988**, *110*, 2683.

[2] For a recent review, see: P. Matile, S. Hörtensteiner, H. Thomas, *Annu. Rev. Plant Physiol. Plant Mol. Biol.* **1999**, *50*, 67.

[3] a) B. Kräutler, B. Jaun, K. Bortlik, M. Schellenberg, P. Matile, *Angew. Chem.* **1991**, *103*, 1354; *Angew. Chem. Int. Ed. Engl.* **1991**, *30*, 1315; b) N. Engel, T. A. Jenny, V. Mooser, A. Gossauer, *FEBS Lett.* **1991**, *293*, 131; c) N. Engel, C. Curty, A. Gossauer, *Plant Physiol. Biochem.* **1996**, *34*, 77.

[4] J. Iturraspe, A. Gossauer, *Helv. Chim. Acta* **1991**, *74*, 1713.

[5] A C20 oxochlorin is known to exist in a keto form under neutral conditions but in an enolate form under basic conditions.

[6] R. B. Woodward, V. J. Skaric, *J. Am. Chem. Soc.* **1961**, *83*, 4647.

[7] H. Scheer, E. Gross, B. Nitsche, E. Cmiel, S. Schneider, W. Schäfer, H.-M. Schiebel, H.-R. Schulten, *Photochem. Photobiol.* **1986**, *43*, 559.

[8] a) G. W. Kenner, J. Rimmer, K. M. Smith, J. F. Unsworth, *Philos. Trans. R. Soc. London Ser. B* **1976**, *273*, 255; b) H. Brockmann, Jr., *Philos. Trans. R. Soc. London Ser. B* **1976**, *273*, 277; c) N. Risch, A. Schormann, H. Brockmann, *Tetrahedron Lett.* **1984**, *25*, 5993.

[9] We would like to thank Dr. Lynn Hawkins at the Eisai Research Institute of Boston for a generous gift of pyropheophorbide *a* methyl ester.

[10] Up to 35% of the starting material was recovered with reverse-phase (C-18) flash column chromatography.

[11] We are grateful to Dr. Kenichi Tezuka of this laboratory for performing the 2D NMR experiments.

[12] All attempts to demetalate **16b, c** to **16a** led to decomposition.

[13] The structure of a close analogue to **12** was established by X-ray analysis: W. S. Sheldrick, A. Borkenstein, U. Jürgens, H. Brockmann, Jr., *J. Chem. Res. (S)* **1981**, 266.

[14] The connectivity of the C20 formyl group to ring A was established through an NOE observed between the aldehyde proton and the methyl group at C2.[4]

[15] We have recently shown that the photooxidation of methyl 20-benzyloxychlorin (i.e., BnO instead of MeO in **14a**) yields the benzyl ester corresponding to **15a** in 45% yield.

[16] a) J. Iturraspe, A. Gossauer, *Photochem. Photobiol.* **1991**, *54*, 43; b) M. S. Huster, K. M. Smith, *Tetrahedron Lett.* **1988**, *45*, 5707.

  0044-8249/01/11320-4012 $ 17.50+.50/0