

Synthesis and spectral studies of natural chlorins having antioxidative activity

Lifu Ma and David Dolphin*

Department of Chemistry, The University of British Columbia, 2036 Main Mall,
Vancouver, B.C., Canada V6T 1Z1

Abstract: Starting from pheophorbide *a* methyl ester (**1**), a degradation product of chlorophyll *a*, novel natural antioxidants of chlorophyll *a* related chlorins **3**, **5(S)**, **5(R)**, **8(R)**, **9–13** have been synthesized in high yields. Key steps involve formation of **5(R)** and **5(S)** via a DBU-promoted asymmetric hydroxylation of **3**, and regioselective periodate oxidations of **5** to **8(R)** and **9**.

Since photosynthetic organisms began releasing oxygen into the primitive atmosphere 3.5 billion years ago, all living things faced the challenges of a new chemical process, oxidation! The appearance of oxygen, while affording the possibility of synthesizing highly energetic compounds, also generated molecules capable of damaging constituents of living structures, including proteins, nucleic acids and lipids (ref. 1).

It is therefore no surprise that all biological organisms have developed multiple and sophisticated ways to reduce the detrimental effects of oxygen. Thus, antioxidants were evolved in many organisms and microorganisms as a defense against these oxidations. Most antioxidants found in nature are hydroxylated and polyhydroxylated aromatic and heterocyclic compounds, such as vitamin A (retinol), vitamin C (ascorbic acid) and vitamin E (α -tocopherol) (ref. 2).

Recently, a new class of antioxidants, metal-free chlorins, have been isolated from marine organisms (ref. 3) and it is interesting and typical that nature should modify the very molecule that was producing oxygen (chlorophyll) in order to protect against over oxidation. Of these chlorins, $13^2,17^3$ -cyclopheophorbide *a* enol (**3**) was the first to be isolated from the marine sponge *Darwinella oxeata* (ref. 4). The structural difference between **3** and chlorophyll *a* is in the formation of an additional exocyclic ring VI. The rest of the antioxidative chlorins, which can be considered as the further oxidized products of **3**, 13^2 S-hydroxychlorophyllone *a* [**5(S)**], 13^2 R-hydroxychlorophyllone *a* [**5(R)**], 15^1 R-hydroxychlorophyllonelactone *a* [**8(R)**], 13^2 -oxypyropheophorbide *a* (**10**), purpurin 18 (**11**), purpurin 18 methyl ester (**12**), and chlorophyllonic acid *a* methyl ester (**13**), were recently found in marine animals such as the short-necked clam, *Ruditapes philippinarum*, the viscera of the scallop *Pactinopecten yessoensis* and attached diatoms and wafting diatoms (ref. 3).

These novel antioxidative chlorins share a similar structural framework and molecular substitution pattern to chlorophyll *a*. They are most likely to be biogenetically related compounds evolved from chlorophyll *a* (refs. 3, 5). The rich stereostructural diversity of these antioxidative chlorins and their important biological activity prompted us to develop synthetic routes to them and thus be able to produce sufficient amounts of material for subsequent antioxidative and biomedical investigations.

Antioxidants as therapeutic agents have shown various potentials in the treatment of inflammation, cancer, aging and neurodegenerative diseases (ref. 6). This contribution exemplifies our most recent progress on the syntheses of these antioxidative chlorins. A more comprehensive description of our earlier work in this area has been published previously (ref. 7).

Synthesis of Enol 3 and Asymmetric Hydroxylation

Our basic strategy for the synthesis of hydroxydiketones **5** (S) and **5** (R) is shown in Fig. 1. A degradation product of chlorophyll *a*, pheophorbide *a* methyl ester (**1**), extracted from *Spirulina maxima* alga was used as starting material (ref. 8). One key step was the Claisen-type intramolecular condensation of **2** to obtain **3** by modification of Eschenmoser's method (refs. 7, 9). Chlorin **3** is a common intermediate to all the antioxidative chlorins and exists only in the enolic form in solution and in the crystalline state (ref. 4).

A number of methods were investigated, which included aerial and iodine oxidation, to bring about the conversion of **3** to hydroxychlorophyllone *a* (**5**). None of them were successful since the two exocyclic rings of **3** are so labile that they can be readily cleaved, particularly by ionic bases. For example, treatment of **3** with $(\text{TMS})_2\text{NNa}$ or LDA followed by oxidation with 1-phenyl-*N*-(phenylsulfonyl)oxaziridine (ref. 10) at -78°C for 15 min, failed to give the desired product **5**, but gave a very polar yellowish-green material (24% yield), which was found, after treatment with diazomethane, to be chlorin p_6 trimethyl ester (**14**).

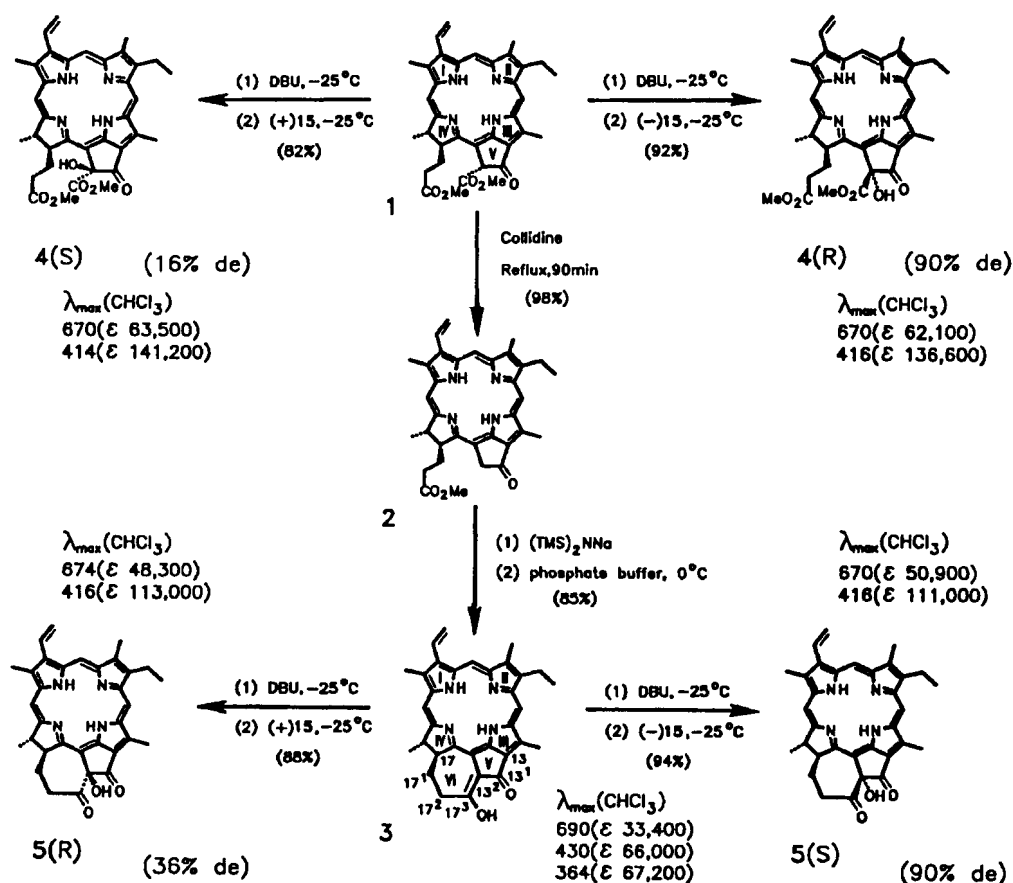


Fig. 1 Synthesis of **3**, **4**(R), **4**(S), **5**(R) and **5**(S)

Thus, for the asymmetric hydroxylation of **3** to give **5(S)**, **5(R)**, considerable attention was initially focussed on routes that avoided cleavage of the two exocyclic rings V and VI. 1,8-Diazabicyclo[5.4.0]undec-7-ene (DBU) was found to be efficient at promoting the reaction (ref. 7). Deprotonation of **3** with DBU followed by oxidation with (-)-(1R)-(10-camphorsulfonyl)oxaziridine [(-)**15**] gave a 94% yield of 13²-hydroxychlorophyllone *a* (**5**), obtained as an (S,R) mixture of 95% **5(S)** and 5% **5(R)** according to reversed-phase HPLC analysis. Reaction of **3** with (+)-(1S)-(10-camphorsulfonyl) oxaziridine [(+)**15**], the enantiomer of (-)**15**, under similar conditions, afforded an 88% yield of **5** as a mixture of 68% **5(R)** and 32% **5(S)**. The above mixtures were purified by preparative HPLC to furnish diastereomerically pure **5(S)** and **5(R)** (their UV-Vis spectral data are shown in Fig. 1).

The stereoselective difference between the reaction of **3** with (-)**15** and (+)**15** can be rationalized on the basis of the steric effects of the 17-propionic group, which hinders the bulky electrophile (+)**15** approach from the *re* face of the chlorin enolate. By the same methodology, optically-pure 13²R-hydroxy pheophorbide *a* methyl ester [**4(R)**] and its epimer [**4(S)**] were readily obtained (ref. 7).

Model Studies for the Oxidative Formation of 13²-Hydroxylactones

Because 13²-hydroxychlorophyllone *a* (**5**) possesses two similar carbonyl groups which are both reactive (though the carbonyl at the 17³-position appears to be less sterically encumbered and non-conjugated), the monoketone 13²-hydroxypheophorbide *a* methyl ester (**4**) was used as a model for hydroxylactonization.

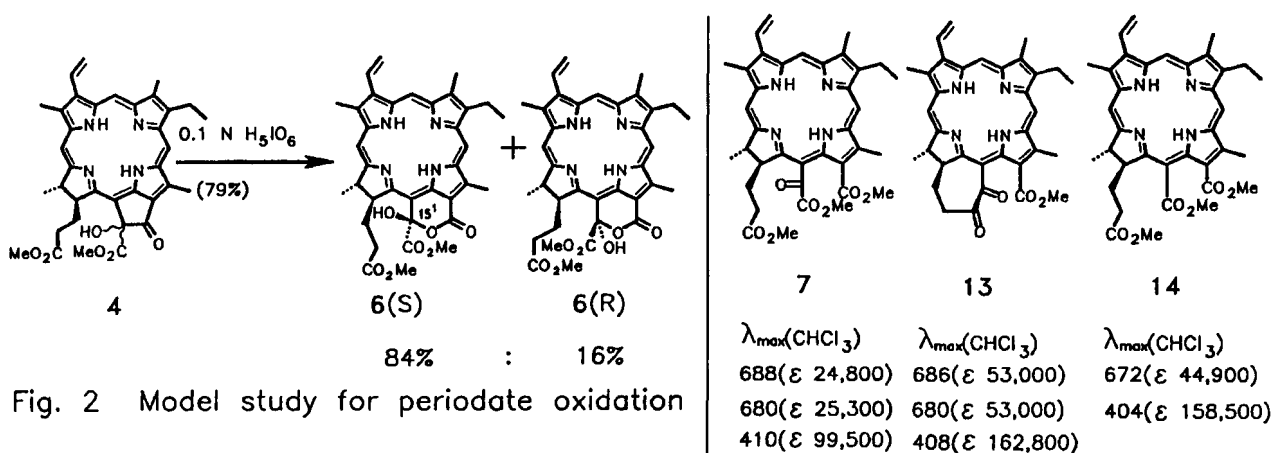


Fig. 2 Model study for periodate oxidation

Baeyer-Villiger type oxidation, MCPBA, and basic hydrolysis were unsuccessful for the oxidation conversion of **4** to **6**. The only method which worked in our hands was found to be periodate oxidation, which occurs only under acidic conditions. 0.1N Periodic acid in aqueous dioxane was found to be the best and 15¹-hydroxypurpurin 7-lactone dimethyl ester (**6**) was isolated in 79% yield. Under these conditions **6** was obtained as a diastereomeric mixture of 84% **6(S)** and 16% **6(R)** (see Fig. 2). Reaction of **6** with diazomethane gave a quantitatively conversion to purpurin 7 trimethyl ester (**7**).

Hydroxylactone **6** was found to be very sensitive to acid and base which give rise to epimerization via the reversible opening of the hydroxylactone ring. Either epimer of **4** has been shown to give the same diastereomeric mixture of **6** under the above oxidation conditions.

Preparation of Hydroxylactone 8(R), Diketones 9 and 13

When the initial experiment utilizing the above periodate oxidation were used to make hydroxychlorophyllone *a* 5, only a low yield (<10%) of the desired products were obtained, the major product (>50%) was the bis-oxidized product, purpurin 18 (11). This result indicated that "the mono-oxidized products" 8 and 10 were more reactive than the starting material 5. This was further confirmed by following the oxidations of 8 and 10, with periodic acid, which more rapidly both gave purpurin 18 (11).

Subsequent work has shown that regioselective oxidation of "one of the two carbonyl groups" can be achieved by using the appropriate choice of solvent. Addition of pyridine to the periodic acid resulted in the principle formation of 13²-oxopyropheophorbide *a* (10) (49%) and two minor products, purpurin 18 (11) (11% yield) and 15¹-hydroxychlorophyllonelactone *a* (8) (28% yield) (see Fig. 3). This procedure allowed for the preparation of 13²-oxopyropheophorbide *a* (10) from pheophorbide *a* methyl ester (3) in ~40% overall yield.

Chlorophyllonic acid *a* methyl ester (13) was readily obtained by direct methylation of 15¹-hydroxychlorophyllonelactone *a* (8) with diazomethane.

Confirmation of the structure of the synthetic compounds was achieved by ¹H, ¹³C NMR, ¹H-¹H COSY, UV-Vis, mass spectral and microanalytical studies. The spectral measurements were specially important for determining the absolute configuration of diastereomers at C-13² and C-15¹.

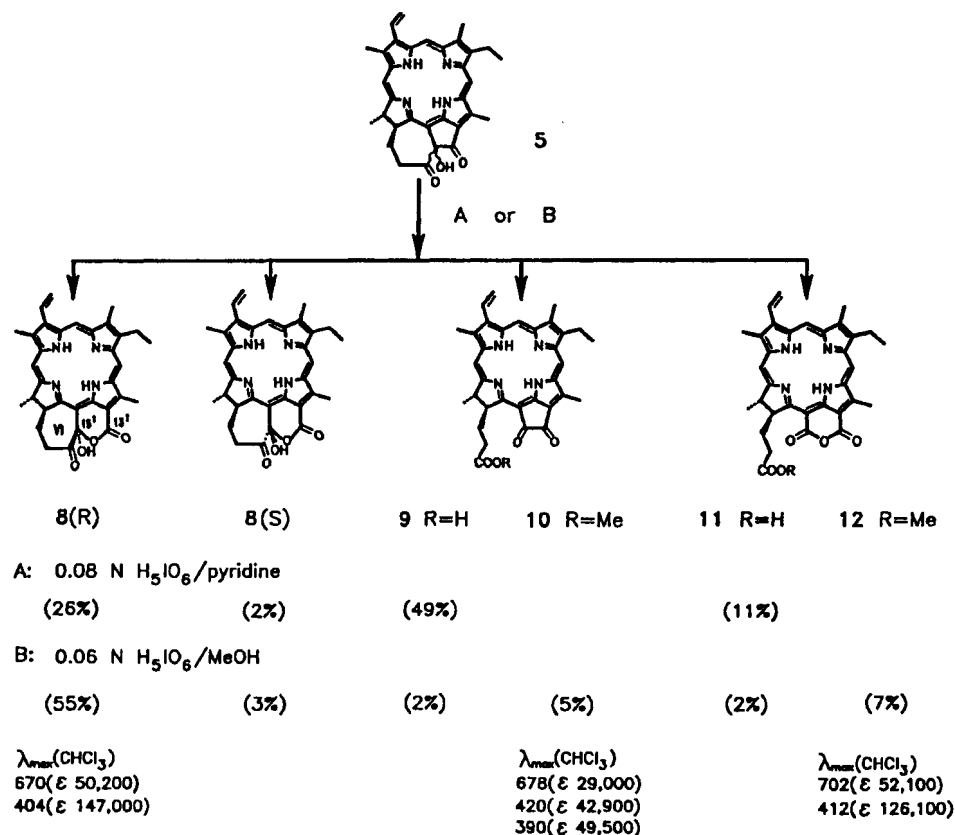


Fig. 3 Regioselective periodate oxidation of 5

ACKNOWLEDGMENT

This work was supported by the Canadian Natural Sciences and Engineering Research Council of Canada.

REFERENCES

1. Anthony W. D. Larkum. In *Chlorophylls*, Scheer, H., Eds., pp. 367-383, CRC Press: Boca Raton (1991).
Schopf, J. W. In *Earth's Earliest Biosphere: Its Origin and Evolution*, Schopf, J. W., Eds., pp.543, Princeton Univ. Press: Princeton, NJ (1983).
2. M-T. Huang, T. Ferraro and C-T. Ho. *ACS Symp. Ser.* **547**, 1 (1994).
3. K. Sakata, K. Yamamoto, H. Ishikawa, A. Yagi, H. Etoh and K. Ina. *Tetrahedron Lett.* **31**, 1165 (1990).
K. Sakata, K. Yamamoto and N. Watanabe. *ACS Symp. Ser.* **547**, 164 (1994).
N. Watanabe, K. Yamamoto, H. Ishikawa, A. Yagi, K. Sakata, L. S. Brinen and J. Clardy. *J. Nat. Prod.* **56**, 305 (1993).
K. Yamamoto, K. Sakata, N. Watanabe, A. Yagi, L. S. Brinen and J. Clardy. *Tetrahedron Lett.* **33**, 2587 (1992).
4. P. Karuso, P. R. Berquist, J. S. Buckleton, R. C. Cambie, G. R. Clark and C. E. F. Richard. *Tetrahedron Lett.*, **27**, 2177 (1986).
5. F. Montforts, B. Gerlach and F. Hoper. *Chem. Rev.* **94**, 327 (1994).
6. C. Auclair and C. Pasquier. In *Oxidative Stress, Cell Activation and Viral Infection*, C. Pasquier *et al* , Eds., pp.1, Birkhauser Verlag: Basel (1993).
7. L. Ma and D. Dolphin. *Tetrahedron: Asymmetry*, in press.
8. L. Ma and D. Dolphin, submitted for publication.
9. H. Falk, G. Hoornaert, H.P. Isenring and A. Eschenmoser. *Helv. Chim. Acta*, **58**, 2347 (1975).
H.P. Isenring, E. Zass, K. Smith, H. Falk, J.L. Luisier and A. Eschenmoser. *Helv. Chim. Acta*, **58**, 2357 (1975).
10. F.A. Davis and B. Chen. *Chem. Rev.*, **92**, 919 (1992).