

# LiOH promoted allomerization of pyropheophorbide *a*. A convenient synthesis of 13<sup>2</sup>-oxopyropheophorbide *a* and its unusual enolization

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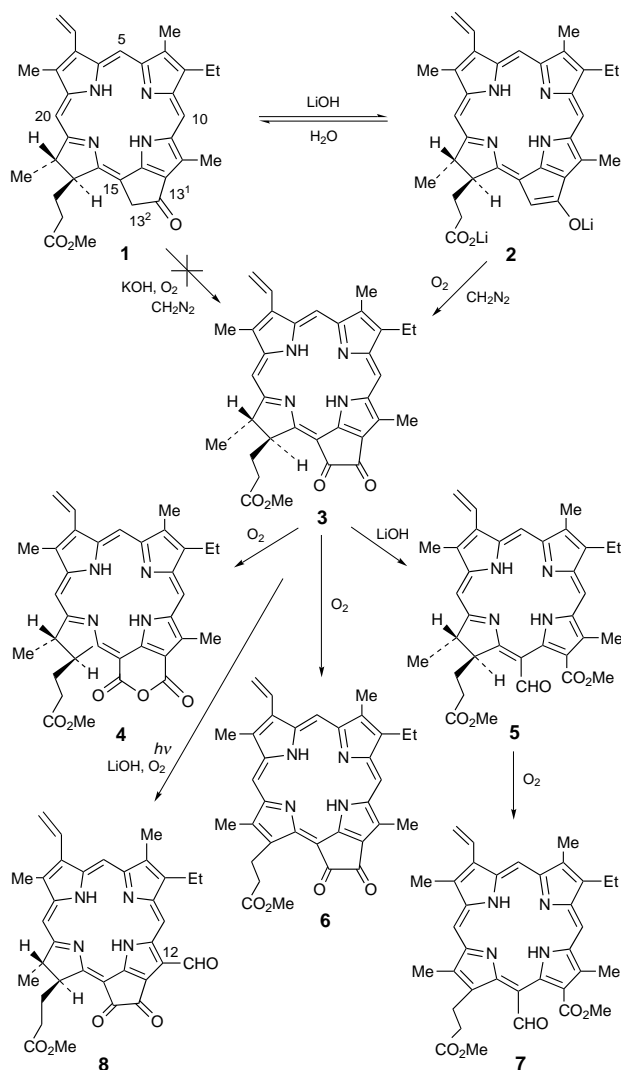
Treatment of pyropheophorbide *a* with aqueous LiOH–THF gave the corresponding enolate, which on *in situ* oxidation produced the corresponding 13<sup>2</sup>-oxo derivative in 79% yield; prolonged autoxidation gave purpurin-18 and purpurin-5 as major products and a mechanism for the formation of 12-formyl derivative from 13<sup>2</sup>-oxopyropheophorbide *a* under unusual enolization is discussed; the electronic absorption spectroscopy data of the enolic intermediates showed a considerable perturbation in the  $\pi$ -systems of the macrocycle.

The term ‘allomerization’ was first introduced to chlorophyll chemistry by Willstatter,<sup>1</sup> who used it to describe then unknown modification reactions of chlorophylls occurring on standing in alcohol solution in contact with air. Thus, allomerization is identical with the term ‘autoxidation’, which implies oxidation by triplex oxygen (<sup>3</sup>O<sub>2</sub>). In chlorophyll chemistry, besides allomerization, the enolization of the isocyclic ring has been studied extensively and it is now well established that allomerization takes place after the formation of chlorophyll enolates.<sup>2</sup> The isocyclic ring of all chlorophylls (except the chlorobium Chls) is a substituted  $\beta$ -keto ester and therefore has a tendency to enolize. Due to the activation by the two carbonyl functions, the 13<sup>2</sup>-hydrogen atom is abnormally acidic. On the basis of oxidation potentials, Wasielewski *et al.*<sup>3</sup> have proposed an enolate ion of chlorophyll *a* as the possible primary electron donor in Photosystem I of plant photosynthesis.

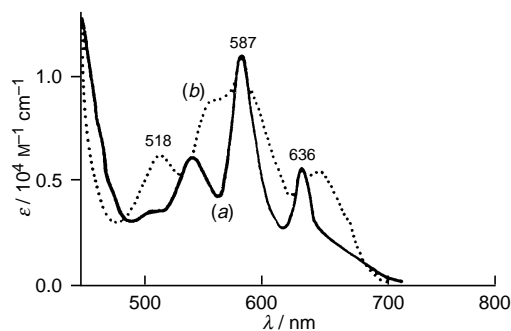
We recently reported the formation of a formyl analog as a minor product in the synthesis of purpurin-18 imides.<sup>4</sup> In our investigation on the mechanism for the formation of such formyl derivatives, we used a variety of chlorins including 13<sup>2</sup>-oxopyropheophorbide *a* as substrates. Here we report a simple and efficient method for the preparation of diketone **3**, its ‘alomerization’ behavior, spectroscopic studies and a possible mechanism for the formation of the corresponding 12-formyl analog.

In contrast to natural chlorophylls and their free bases, pyropheophorbide *a* **1** which lacks an acidic hydrogen at 13<sup>2</sup>-position was believed not to undergo enolization. This was further confirmed by Conant and Hyde<sup>5</sup> on the basis of a negative Molisch ‘phase test’. We reinvestigated this reaction under milder basic conditions, and observed that pyropheophorbide *a* on treatment with LiOH indeed produced stable enolate derivative **2** (Scheme 1), which was detected spectroscopically. It exhibited a characteristic UV–VIS spectrum with the split and red shifted Soret band at  $\lambda_{\max} = 449$  nm. Stronger bases, such as KOH and NaOH, did not produce any spectroscopic changes. This phenomenon could be explained due to the well-known property of lithium salts to form partially covalent bonds.<sup>2</sup> As a result of enolization, methyl pyropheophorbide *a* **1** was completely autoxidized on stirring in THF at room temperature for extended time (12 h) to produce 13<sup>2</sup>-oxo derivative **3** in 79% yield. Prolonged oxidation (72 h) gave the products of further degradation, which after standard work-up and CH<sub>2</sub>N<sub>2</sub> treatment were separated by preparative chromatography. The faster moving band was identified as purpurin-18 methyl ester **4** (32%).<sup>6</sup> The slower moving band was charac-

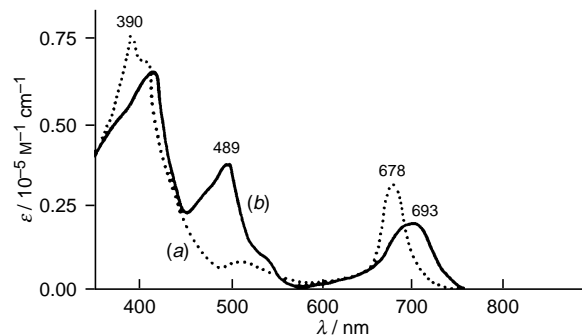
terized as purpurin-5 dimethyl ester **5** (a key product in Woodward’s ‘purpurin reaction’) and was isolated in 38% yield.<sup>7</sup> The formation of chlorin **5** is obviously due to the basic hydrolysis of the dicarbonyl C–C bond of the diketocyclopentane ring. The other two gray–green porphyrins were isolated in small amounts (*ca.* 2%) and were identified as 13<sup>2</sup>-oxophylloerythrin methyl ester **6** and 15-*meso*-formylrhododoporphyrin XV dimethyl ester **7**. Both porphyrins showed unique ‘reverse-rhodo’ visible absorption spectra (Fig. 1) which were found to be different from those generally reported in porphyrin chemistry.<sup>8</sup> These newly discovered green porphyrins can be compared to those related to verdins, or recently reported emeraldins containing fused anhydride or imide ring systems.<sup>9</sup>



Scheme 1



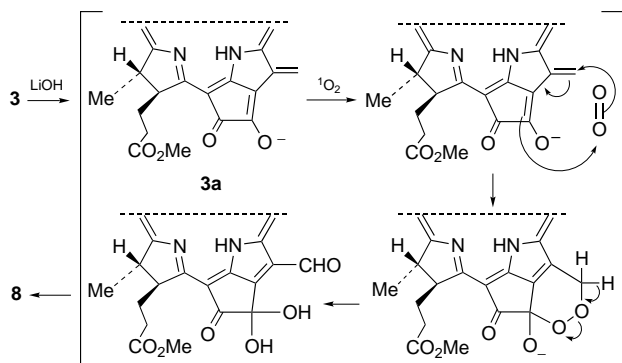
**Fig. 1** Electronic absorption spectra (in  $\text{CH}_2\text{Cl}_2$ ) of (a) dioxoporphyrin **6** and (b) porphyrin **7**



**Fig. 2** Electronic absorption spectra (in  $\text{CH}_2\text{Cl}_2$ ) of (a) chlorin **3** and (b) its enolate **3a**

Further purification of this complex mixture also gave a novel chlorin (5% yield) with a significantly red shifted absorption  $Q_y$ -band at 710 nm and a Soret band at 440 nm. Compared to 13<sup>2</sup>-oxopyropheophorbide **a**, the <sup>1</sup>H NMR spectrum of this chlorin showed the presence of a distinctive singlet for a formyl group at  $\delta$  11.08 and disappearance of the resonances for the 12-methyl protons. The mass spectrum of this product gave a molecular ion peak at  $m/z$  576, confirming the replacement one of the methyl group by a formyl substituent. The <sup>1</sup>H NMR and 2D ROESY data supported the structure as 12-demethyl-12-formyl-13<sup>2</sup>-oxopyropheophorbide **a** methyl ester **8**. The formation of chlorin **8** from 13<sup>2</sup>-oxopyropheophorbide **a** **3**, which lacks protons at 13<sup>2</sup>-position, possibly proceeds *via* a vinylogous enolization involving the methyl protons attached to the adjacent pyrrolic ring (ring C). This process would certainly be facilitated by the presence of strong electron-withdrawing carbonyl functions at the adjacent cyclopentane ring. A proposed mechanism for the formation of chlorin **8** is shown in Scheme 2. The key step in this reaction is the formation of a reactive intermediate species **3a**. Reaction of enolate **3a** with singlet oxygen, *via* [4 + 2] cycloaddition and subsequent rearrangement, would generate the 12-formyl analog **8**. Chlorins in general are known to convert molecular oxygen to singlet oxygen (<sup>1</sup>O<sub>2</sub>) on irradiating with light. In order to further confirm the [4 + 2] cycloaddition mechanism with singlet oxygen, we used purpurin-18 imide,<sup>4</sup> as a more stable substrate, which could also undergo vinylogous enolization similar to **3a**. Photooxidation of purpurin-18 imide in aqueous LiOH-THF on exposing to sunlight produced the corresponding 12-formyl derivative in high yield (up to 60%). When the reaction was performed in the dark, the 12-formyl analog was not detected.<sup>10</sup>

Despite intensive studies in chlorophyll chemistry, this is the first example in which this type of enolization is observed. The



**Scheme 2**

evidence of enolization in 13<sup>2</sup>-oxopyropheophorbide **a** **3** was also confirmed by its specific UV-VIS spectrum (splitting of the Soret band), a characteristic property of enolates in pheophorbide system (Fig. 2). The enolic form **3a** produced under other basic conditions (KOH, NaOH) was found to be stable in non-polar solvents. However, traces of alcohol or water completely destroyed the potassium and sodium enolates, while the lithium salt appeared to be quite stable.

The chemistry discussed here might explain the origin of deoxyphyloerythrietioporphylin (12-demethyl-DPEP), a minor petroporphyrin structure isolated by Callot.<sup>11</sup>

The newly discovered LiOH promoted enolization of pyropheophorbide **a** has opened a simple and effective way for the preparation of 13<sup>2</sup>-oxopyropheophorbide **a** **3** which had previously been isolated as a by-product in minor quantities.<sup>6</sup> Further studies are underway to explore the reactivity of the cyclopentanedione ring system for the preparation of novel chlorins with linear conjugation as photosensitizers for photodynamic therapy and models for photosynthetic reaction centers.

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## Notes and References

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- 1 R. Willstätter and A. Stoll, *Untersuchungen über Chlorophyll*, Springer, Berlin, 1913.
- 2 P. H. Hynninen, in *Chlorophylls*, ed. H. Scheer, CRC Press, Ann Arbor, 1991, p. 145.
- 3 M. R. Wasielewski, J. R. Norris, L. L. Shipman, C. P. Lin and W. A. Svec, *Proc. Natl. Acad. Sci. USA*, 1981, **78**, 2957.
- 4 A. N. Kozyrev, G. Zheng, C. Zhu, T. J. Dougherty, K. M. Smith and R. K. Pandey, *Tetrahedron Lett.*, 1996, **37**, 6431.
- 5 J. K. Conant and J. F. Hyde, *J. Am. Chem. Soc.*, 1929, **51**, 3668.
- 6 L. Ma and D. Dolphin, *J. Org. Chem.*, 1996, **61**, 2501.
- 7 R. B. Woodward, W. A. Ayer, J. M. Beaton, F. Bickelhaupt, R. Bonnett, P. Buchschacher, G. L. Closs, H. Dutler, J. Hannan, F. P. Hauck, S. Ito, A. Langemann, E. Le Goff, W. Leimgruber, W. Lwowski, J. Sauer, Z. Valenta and H. Volz, *Tetrahedron*, 1990, **46**, 7599.
- 8 K. M. Smith, *General features of the structure and chemistry of porphyrin compounds*, in *Porphyrins and Metalloporphyrins*, ed. K. M. Smith, Elsevier, Amsterdam, 1975.
- 9 A. N. Kozyrev, G. Zheng, E. Lazarou, T. J. Dougherty, K. M. Smith and R. K. Pandey, *Tetrahedron Lett.*, 1997, **38**, 3335.
- 10 A. N. Kozyrev and R. K. Pandey, unpublished results.
- 11 J. Verne-Mismer, R. Ocampo, C. Bauder, H. L. Callot and P. Albrecht, *Energy Fuels*, 1990, **4**, 639.

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