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Hemisynthesis of 13^2,17^3-cyclomesopheophorbide-*a*-enol

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ABSTRACT

Cyclopheophorbides are often major chlorophyll-*a* degradation products in biogeochemical situations. Authentic standards are unavailable and facile generation of pure compounds is required. 13^2,17^3-Cyclomesopheophorbide-*a*-enol (mesoCYCLO) was prepared in moderate yields via a known Dieckmann-like condensation of mesopyropheophorbide-*a* methyl ester (mpPBID*a*ME). MesoCYCLO was purified by flash chromatography over a polymeric reversed phase (PRP-1™) material, negating the requirement for a crystallization step. Structural verification included ultraviolet-visible spectrometry, high resolution matrix (sulfur/CS_2) assisted laser desorption mass spectrometry, and nuclear magnetic resonance.

Cyclopheophorbides-*a*-enol (CYCLO, **2a**) is a well known biogeochemical degradation product of chlorophyll-a.[1,2] Several reports reveal the generation of CYCLO from chlorophyll-*a* (CHL*a*) in filter feeding marine organisms, such as sponges[3] and mollusks.[4–6] Geochemically, CYCLO is reported from a variety of sediments[1,7] and has been observed to form directly from pyropheophorbide-*a* (pPBID*a*, $\mathbf{1a}_1$) in a downhole sequence of sulfidic carbonates.[8] The exocyclic seven-membered ring forms via a dehydration–cyclization of pyropheophorbide-*a* free acid ($\mathbf{1a}_1$). Interest in the cyclopheophorbides also arises from their apparent direct relationship as diagenetic precursors to a variety of bicycloalkylporphyrins (BiCAP) in oil shales and petroleum crudes.[2,7,9,10]

CYCLO (**2a**) has been synthesized (Scheme 1) in vitro by Dieckmann-like (intramolecular Claisen)[11–13] cyclizations of pyropheophorbide-*a* methyl ester ($\mathbf{1a}_2$). Though a wide variety of 13^2,17^3-cyclopheophorbide-enols were synthesized by Falk et al.,[11] as far as we could find, mesoCYCLO has not been formed in vitro until the present Letter.

The title compound of the present study, 13^2,17^3-cyclomesopheophorbide-*a*-enol (mesoCYCLO, **2b**), has been identified by LC–PDA–MS as a minor constituent of sedimentary organic matter in Peru margin sediments.[8] In that Letter, the authors identified mesoCYCLO (**2b**) as cyclopheophorbide-518 (CPP518), indicating the nominal mass of the pigment. Previously, our group[2] has also termed CYCLO (**2a**) as phorbide686 and mesoCYCLO (**2b**) as chlorin678, indicating the wavelengths (nm) of their band I absorptions in ethyl ether.

The cyclopheophorbides have been found to be extremely unstable and often oxidatively rearrange to 13^2-(*S*/*R*)-hydroxychlorophyllones (**3**), 13^2-oxopheo-phorbide-*a* (**4**), and/or chlorophyllonic acid-*a* (**5**) and are therefore often referred to as antioxidants.[4,5,14–17] However, to date, there is no proof that these compounds are physiologically active as such in nature. The structures of these compounds are given in Figure 1.

In this Letter, we report the hemisynthesis of CYCLO (**2a**) and describe the analogous generation of mesoCYCLO (**2b**) from mesopyropheophorbide-*a* methyl ester (mpPBID*a*ME, **1b**). Each procedure followed the more recently modified methodology of Ocampo et al.[13;cf11,15] Additionally, we present proof (UV/Vis, ^{1}H NMR, HR-MALDI-TOF) of the structure of mesoCYCLO (**2b**) and add to the NMR data on CYCLO (**2a**). Significant analytical development was required and we describe the successful and facile purification of this and other cyclopheophorbides, as well as providing UV/Vis, mass and NMR spectra of the highly purified pigments.

All procedures were performed either in the dark or subdued yellow light and solutions were kept cold/frozen and under argon whenever possible.mpPBID*a*ME (**1b**) was prepared from hydrogenation of the vinyl group of pPBID*a*ME[18] ($\mathbf{1a}_2$) as follows; pPBID*a*ME(100 mg, 0.18 mmol) was dissolved in 99.9% anhydrous tetrahydrofuran (THF, 25 mL) and added to 4 mg (10%) palladium on charcoal catalyst.[19] This follows the procedure outlined by Jeandon et al.[20] Hydrogenation was performed under 1 atmosphere pressure (relative) for 200 min in a Parr hydrogenator using an ACE glass reaction vessel. The catalyst was removed by filtering the mixture through Celite and the solvent was removed in vacuo.

CYCLO (**2a**) or mesoCYCLO (**2b**) was prepared from pPBID*a*ME ($\mathbf{1a}_2$) or mpPBID*a*ME (**1b**), respectively, following a reported procedure[13] according to Scheme 1. For example, in the case of mesoCY-

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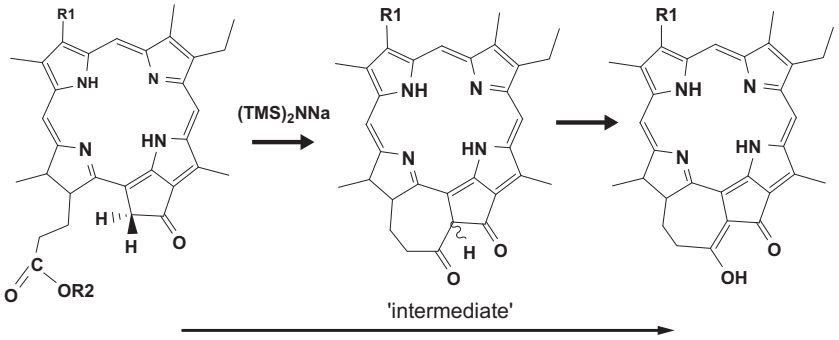

1a$_1$ R1 = -CH=CH$_2$ R2 = -H
1a$_2$ R1 = -CH=CH$_2$ R2 = -CH$_3$
1b R1= -CH$_2$-CH$_3$ R2 = -CH$_3$
2a R1 = -CH=CH$_2$ **2b** R1= -CH$_2$-CH$_3$

Scheme 1. Structural comparisons and cyclization reaction forming the cyclopheophorbide-*a*-enols.

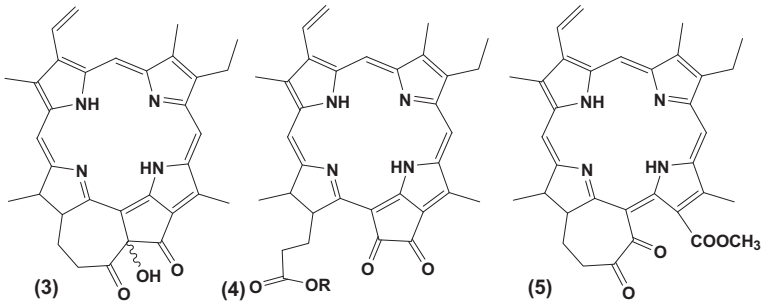

Figure 1. Structures[14–16] of oxidatively generated degradation products of (**2a**) CYCLO: (**3**) 13^2(*S*/*R*)-hydroxychlorophyllones, (**4**) 13^2-oxopheophorbide-*a*, (**5**) chlorophyllonic acid-*a*.

CLO (**2b**), mpPBID*a*ME (**1b**, 55.07 mg, 1.09 mmol) was added to 6 mL tetrahydrofuran (THF; 99.9% anhydrous inhibited with 250 ppm BHT) under a stream of ultra-high purity (UHP)-argon. Ten milliliters (1.0 mmol) of sodium bis(trimethylsilyl)-amide[21] in THF was added to that solution and stirred for about 8 min. The reaction mixture was quenched in an Ar-sparged deoxygenated mixture of CH_2Cl_2 (80 mL), saturated NaH_2PO_4 (20 mL) and degassed deionized ice (20 g). Following liquid:liquid extraction, the organic (CH_2Cl_2) layer was dried over anhydrous Na_2SO_4, filtered and evaporated in vacuo.

The crude product was then flash chromatographed over polymeric reversed phase (PRP-1™) using an isocratic solvent (90% acetonitrile) as we detailed previously.[22] This rapid and mild purification also negated the need for crystallization/recrystallization procedures. As we were interested in only the pure product, we did not trace yield, which would be difficult as we 'heart cut' the main fraction of the target compound (**2b**). A very gentle purification method was required since cyclopheophorbide-*a*-enols have been found to be very unstable compounds that can be easily oxidized to a variety of alternate cyclic pheophorbides. CYCLO (**2a**) is routinely altered to different oxidative artifacts, mainly 13^2(*S*/*R*)-hydroxychlorophyllones-*a* (**3**), during chromatographic and other isolation/purification procedures.[14–16,22] As with CYCLO (**2a**), when mesoCYCLO (**2b**) is chromatographed over normal phase silica or alumina in conventional column chromatography or analytical HPLC, it is mainly oxidized to artifacts and becomes barely detectable. The main artifacts compared to CYCLO (**2a**) analog have been found to be highly polar and are presumed to be the *meso*-chlorophyllonic acids (cf compd **22** in Ref. 15). To prevent undesirable oxidations, and due to successful purification using PRP-1™ as packing material,[22] a PRP-1™ analytical or semi-prep column was used for HPLC analysis. The HPLC (PRP-1™) chromatogram for the purification of mesoCYCLO (**2b**) is presented in Figure 2. The two peaks immediately following the solvent front are the artifacts generated over the column. To confirm this hypothesis, we collected a 'heart cut' of the mesoCYCLO (**2b**) peak between ~23–27 min and reinjected it. This was repeated several times and each time a new pair of these artifacts was generated from a previous run's heart cut. The reappearance of these two peaks on the chromatogram supported our hypothesis of artifact formation being directly related to injectate preparation and injection into the HPLC system. This occurred regardless of continual He sparging of solvents and/or the addition of anti-oxidants such as butylated-hydroxytoluene (BHT) or ascorbic acid to the injectate and developing solvent.

Like cyclopheophorbide-*a*-enol (CYCLO, **2a**), the Soret Band in the UV/Vis spectrum of pure mesoCYCLO (**2b**) is complex and consists of at least 4 overlapping individual bands. Additionally, the spectrum contains an intense band I appearing at 676 nm (Fig. 3a), which exhibits a bathochromic shift compared to the parent compound mesopyropheophorbide-*a* methyl ester (**1b**; λ_1 = 666 nm). Both band I and the Soret band exhibit hypsochromic shifts when compared to the main maxima of the CYCLO analog (Fig. 3b). This is expected and occurs as a consequence of reduction of the vinyl to ethyl at position 3 of the macrocycle.[8,23] The UV/Vis spectrum, an overall characteristic of the cyclopheophorbides, has broad split Soret(S) bands. Absorption maxima recorded in the HPLC eluant and relative intensities are given in brackets: λ_S = 358 [1.000], (407) [0.796], 423 [0.907], 449 [0.593], λ_{IV} = 528 [0.065], λ_{III} = 572 [0.093], λ_{II} = 619 [0.139], λ_I = 676 [0.426] nm.