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# ELECTROPHILIC SUBSTITUTION AT THE δ-METHINE BRIDGE OF PHEOPHORBIDE a and a'

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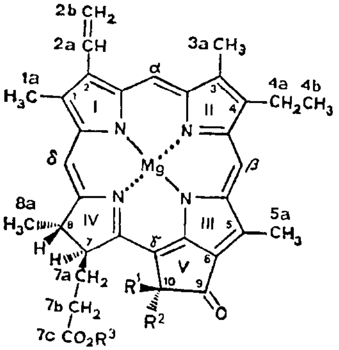
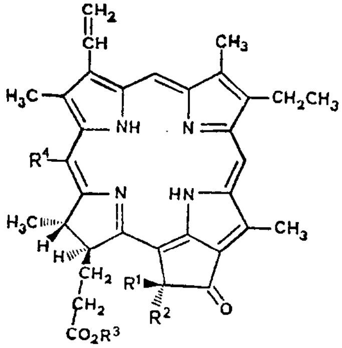
Abstract: A chloro-substituted derivative was prepared from chlorophyll a'. The derivative was shown to be δ-chloro-methyl pheophorbide a by UV/VIS, MS and 'H NMR.

The susceptibility of simple chlorins to selective electrophilic attack at the γ and δ bridge positions is a well-known fact.[1-4] However, there seems to be very little information on electrophilic substitutions in phorbins (1-6) possessing an enolizable β-keto ester system in ring V. The C10-hydrogen has been shown to be more easily exhangeable than the δ-hydrogen by deuterium-exhange experiments.[5,6] In this publication, we show that under certain conditions, electrophilic substitution occurs selectively at the δ-position of pheophorbide a (3) and a' (4) while the 10- and other positions remain intact.





$R^3 = C_{20}H_{39}$ = phytyl

1 : $R^1 = H$, $R^2 = CO_2CH_3$
2 : $R^1 = CO_2CH_3$, $R^2 = H$

3 : $R^1 = H$, $R^2 = CO_2CH_3$, $R^3 = R^4 = H$
4 : $R^1 = CO_2CH_3$, $R^2 = H$, $R^3 = R^4 = H$
5 : $R^1 = H$, $R^2 = CO_2CH_3$, $R^3 = CH_3$, $R^4 = H$
6 : $R^1 = CO_2CH_3$, $R^2 = H$, $R^3 = CH_3$, $R^4 = H$
7 : $R^1 = H$, $R^2 = CO_2CH_3$, $R^3 = CH_3$, $R^4 = Cl$
8 : $R^1 = CO_2CH_3$, $R^2 = H$, $R^3 = CH_3$, $R^4 = Cl$

On trying to prepare pheophorbide a'(4) from chlorophyll a'(2) using 30 % (w/w) hydrochloric acid-diethyl ether,[9] we observed that a new red derivative was formed when the acid phase was diluted with water from the city waterworks. At an [HCl] of 16 %, this red derivative distributed principally into the ether phase while pheophorbide a'/a remained in the acid phase. Since the water used contained chlorine 0.2-0.5 mg/l and $Fe^{3+}$ < 0.05 mg/l, it was plausible that a substitution reaction had occurred. Treatment of chlorophyll a (1) under similar conditions produced a smaller amount of the red derivative. The washed ether solution of the derivative was evaporated to dryness and the pigments in the residue were esterified with methanol in pyridine-THF employing ethyl chloroformate for activation.[10] The methylated derivative was further purified by chromatography on a sucrose column using pyridine-petroleum ether, 1:15, v/v, as the eluent.

The electronic spectrum of the new derivative exhibited $\lambda_{max}$ ($\varepsilon \cdot 10^{-3}$) in THF at: 675.0 (32.5), 615 (3.99), 546 (9.26), 514 (6.54), 483 (3.11), 412.0 (70.2), 329 (13.8) and 273 (8.75) nm. The MS of the derivative showed the principal molecular ion at m/e 640 (100) and main fragments at m/e 582 (78) and 495 (39). Comparison of the MS with that obtained from methyl pheophorbide *a* (5), showing the principal molecular ion at m/e 606 (100) and main fragments at m/e 548 (27) and 459 (27),[11,12] proved that the new derivative contained a chlorine atom. The 'H NMR spectrum of the chloro-derivative lacked the signal corresponding to the δ-H. In other regards the spectrum was quite similar to that of 5; Δδ obtained significant values only in the instance of the 12-$CH_3$, 2a-$H_x$, 8-H and 8-$CH_3$ protons which are adjacent to the δ-methine bridge.

These results conclusively establish the structure of the new derivative as δ-chloromethyl pheophorbide a (7). Noteworthy is that the C-10 proton did not undergo substitution under the experimental conditions used. The NMR spectrum of the chloro-derivative also showed that the configuration at C-10 was predominantly *R*. Although the configuration originally was 10 *S*, the conversion to the thermodynamically more stable 10 *R* form took place, in part, during the HCl-ether treatment and, in part, during the methylation and chromatographic procedures where pyridine was used. In these solvents, the enolization and epimerization in ring V are facile reactions.[4,8] Two small signals were observed in the NMR spectrum of 7, which were located 0.03 and 0.05 ppm to higher field from the signals corresponding to β-H and α-H. This implies the presence of 10-15 % of the prime derivative (8) in the preparation.

The fact that Chl *a*' (2) was found to be more susceptible to electrophilic substitution than Chl *a* (1) under the experimental conditions used, is in accordance with the results obtained by 'H NMR.[7,8] The NMR results show that changes occur in the π-electron system of the chlorin macrocycle when the epimerization at C-10 takes place. These changes were interpreted as arising from the conformational alterations caused by steric strain.[8] Further investigations, attempting to reveal in detail the true differences between C-10 epimeric chlorophyll derivatives, are underway in our laboratory.

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