

Synthesis and Properties of 3-Vinyl-3-Desmethyl Chlorophyll a

T.J. Michalski, J.E. Hunt, J.C. Hindman, and J.J. Katz*

Chemistry Division

Argonne National Laboratory

Argonne, IL 60439

Abstract: The title compound 1 has been prepared from chlorophyll b by a highly chemoselective Wittig reaction, and its spectroscopic properties characterized.

Derivatives of Chl a containing two vinyl side-chains have attracted considerable interest recently as postulated intermediates in the biosynthesis of the chlorophylls and as normal constituents of green plant chloroplasts (1). The inference that divinyl chlorophyll derivatives are present in green plants was based largely on visible absorption and fluorescence measurements. Bazzaz et al. (2), and Brereton et al. (3) have reported the isolation and identification of 4-vinyl-4-desethyl chlorophylls a and b by fast atom bombardment mass spectroscopy, but the optical properties of these compounds have not been characterized. Chlorophyll c, a prominent photosynthetic pigment in brown marine algae and diatoms, has long been known to contain two vinyl side-chains (4), but this compound, as well as the recently isolated divinyl protochlorophyll a (5), is a pheoporphyria, and these differ significantly in optical properties from the true chlorophylls. The spectroscopic properties of divinyl derivatives of the chlorophylls have thus remained a matter of conjecture. We have therefore developed a convenient one-step procedure for the preparation of 3-vinyl-3-desmethyl Chl a 1 (Fig. 1), and have characterized its optical and other spectroscopic properties.

The synthetic scheme for the preparation of 1 is based on a Wittig reaction between chlorophyll b (Chl b) 2 and triphenylphosphonium methylide. Chl b (2 mg, 2.2×10^{-6} M) was dissolved in 1 ml of a 1:1 (v/v) mixture of diethylether/tetrahydrofuran, and treated with 0.5 ml (8.6×10^{-6} M) of triphenylphosphonium methylide, which was generated from the bromide salt dissolved in the same solvent mixture by addition of butyl lithium in hexane. Addition of the first molar equivalent of Wittig reagent resulted in the formation of a red-brown intermediate whose visible absorption spectrum is very similar to that reported for the Chl a Molisch phase test intermediate (6). The reaction mixture was kept at room temperature under nitrogen for 30 min, after which it was partitioned against diethylether and washed 3 times with water. The organic layer was concentrated by evaporation. The product 1 was isolated in 60% yield by HPLC on a Zorbax 5 μ C-18 column; the chromatogram was developed with a

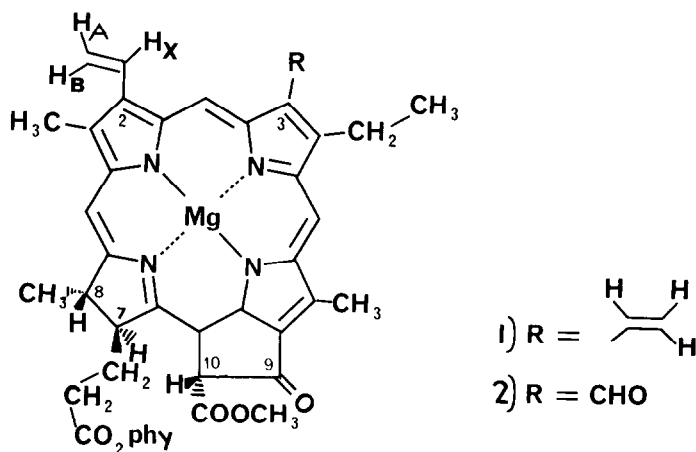


Figure 1. Structure of 3-vinyl-3-desmethyl chlorophyll a.

methanol/tetrahydrofuran/acetonitrile (5:2.1:2.9) mixture. Small amounts of what were shown to be unreacted Chl b 2 and the 10-hydroxy derivative of 1 were also collected.

The structure of the product 1 is fully supported by NMR, mass spectra, and FT-IR. ^1H -NMR in CD_3COCD_3 showed resonances at 9.77, 9.42, and 8.53 ppm that can be assigned to the α , β , and δ methine protons respectively; the resonance indicative of a formyl proton is not present in the low field region. The chemical shifts and coupling constants of the protons of the two vinyl groups at the 2 and 3 positions can also be fully assigned: 3- H_X at 8.05 ppm, with $J_{XB} = 17.7$ Hz and $J_{XZ} = 11.6$ Hz; 2- H_X at 8.04 ppm and $J_{XB} = 17.8$ Hz and $J_{XA} = 11.6$ Hz; 3- H_B and 2- H_B and 6.19 and 6.18 ppm, with $J_{BX} = 18.1$ Hz; 3- H_A at 5.93 ppm and $J_{AB} = 1.3$ Hz, $J_{AX} = 11.3$ Hz; 2- H_A at 6.02 ppm and $J_{AB} = 1.3$ Hz and $J_{AX} = 11.5$ Hz. All other resonances follow the expected pattern for Chl a.

The ^{252}Cf -PDMS mass spectrum of 1 (Figure 2) showed a molecular ion at 905.64 ± 0.08 amu; the calculated value for 2 is 905.52 amu. The fragment ion at m/z 627 establishes the presence of an intact phytol chain in 1. The fragmentation pattern also confirms the presence of an intact carbomethoxy group at position 10 in ring V. Reaction of 1 with molecular oxygen forms a small amount of the 10-hydroxy derivative of 1; the identity of this by-product is established by HPLC and ^{252}Cf PDMS: found 920.9, calculated 921.5 for 10-hydroxy-3-vinyl-3-desmethyl Chl a.

The FT-IR spectrum of 1 in THF showed a strong keto $\text{C}=\text{O}$ absorption at 1694 cm^{-1} and ester $\text{C}=\text{O}$ absorption at 1737 cm^{-1} , confirming the presence of an intact ring V. The FT-IR spectra of Chl a and 1 in the $1800\text{--}1600\text{ cm}^{-1}$ region are identical in intensity and frequency. The absence of an absorption peak at 1663 cm^{-1} characteristic of the formyl $\text{C}=\text{O}$ group in Chl b is further confirmation of the transformation of the formyl group in the starting material. The highly selective nature of the Wittig reaction was shown by the transformation of 2-formyl methylpyropheophorbide a to 2-vinyl-2-desmethyl methylpyropheophorbide a. This reaction offers an attractive and general method for the chemoselective derivatization of Chl b without

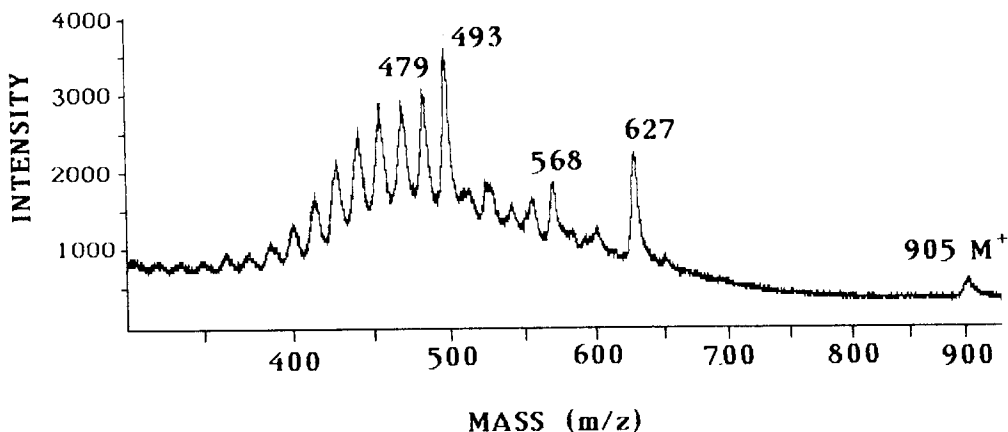


Figure 2. ^{252}Cf -PDMS mass spectrum of 3-vinyl-3-desmethyl chlorophyll a. Note molecular ion at m/z 905. Note fragment ion at m/z 627 corresponding to loss of esterifying phytyl alcohol.

the loss of the central magnesium atom, the disruption of ring V, on the use of protective groups at any stage of the procedure (see also ref. 7).

The visible absorption and fluorescence maxima of 3-vinyl-3-desmethyl Chl a and Chl a in three solvents are compared in Table I. In all three solvents, there is a slight shift of the $Q_y(0,0)$ (red band) transition to higher energy of the order of 10–20 Å. A more significant shift in 1 to higher energy is observed in the transition energies in the Soret (blue) region of the spectrum. The changes in the ratios of the amplitude of the blue and red absorption bands is a reflection of the greater effect of an additional vinyl group on the Soret band transitions than on the red $Q_y(0,0)$ transition. The transition dipole strength in the red for Chl a and 1 appear to be very similar. The fluorescence quantum yield in pyridine solution for 1 is about 10% smaller than that of Chl a. Within experimental error the fluorescence lifetimes (at 680 nm) for the two compounds is the same. The close resemblance in the optical properties of 1 and Chl a suggests that more than ordinary caution must be exercised in the identification of divinyl chlorophyll a derivatives in vivo entirely on the basis of optical criteria.

References

1. C.A. Rebeiz, S.M. Wu, M. Kuhadja, H. Daniell and E.J. Perkins, *Mol. Cell Biochem* (1983) 57, 97–125.
2. M.B. Bazzaz, C.V. Bradley, and R.G. Brereton, *Tetrahedron Lett.* (1982) 23, 1211–1214.
3. R.G. Brereton, M.B. Bazzaz, S. Santikarn and D.H. Williams, *Tetrahedron Lett.* (1983) 24, 5775–5778.
4. H.H. Strain, B.T. Cope, G.N. McDonald, W.A. Svec and J.J. Katz, *Phytochemistry* (1971) 10, 1109–1114.

5. S.M. Wu and C.A. Rebeiz, *Tetrahedron* (1984) 40, 659-664.
6. A. Weller, *J. Am. Chem. Soc.* (1954) 76, 5819.
7. K.M. Smith, M.J. Bushell, J. Rimmer, and J.F. Unsworth, *J. Am. Chem. Soc.* (1980) 102, 2437-2448.
8. M. Wasielewski, J. Hunt, J.J. Katz and T.J. Michalski, to be published.

TABLE 1
Comparison of Visible Absorption and Fluorescence Maxima of Chlorophyll a
and 3-Vinyl-3-Desmethyl Chlorophyll a (1)

System		λ_{\max} (blue) (nm)	λ_{\max} (red) (nm)	λ_{\max} (blue)/ λ_{\max} (red) (nm)	λ_{fl}^d (nm)
Acetone	Chl <u>a</u> ^a	432	662	1.31	672, 772
	<u>1</u> ^b	437	660	1.53	670, 717
Diethylether	Chl <u>a</u> ^a	432	660	1.34	668, 719
	<u>1</u> ^b	435	657	1.45	665, 715
Pyridine	Chl <u>a</u> ^c	442	670	1.4	680, 730
	<u>1</u> ^c	452	669	1.7	680, 730

(a) 1.7×10^{-3} M; (b) 1.4×10^{-3} M; (c) 5×10^{-4} M; (d) 337 nm excitation.

(Received in USA 1 July 1985)

*Work performed under the auspices of the Office of Basic Energy Sciences, Division of Chemical Sciences, U.S. Department of Energy under contract W-31-109-ENG-38.