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4-VINYL-4-DESETHYL CHLOROPHYLL A: CHARACTERISATION OF A NEW NATURALLY OCCURRING CHLOROPHYLL USING FAST ATOM BOMBARDMENT, FIELD DESORPTION AND "IN BEAM"ELECTRON IMPACT MASS SPECTROSCOPY

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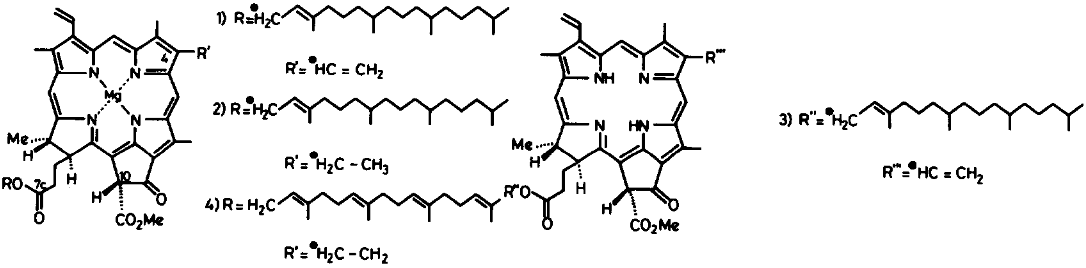
Summary: *A chlorophyll that has previously been isolated as the major chlorophyll in a mutant of maize, has been studied by 3 mass spectroscopic techniques, and has been shown to be 4-vinyl-4-desethyl chlorophyll a.*

It has been previously established that chloroplasts from a mutant of *Zea mays L.* exhibit structural and functional characteristics similar to those of immature chloroplasts and that these chloroplasts can photosynthesize *in vitro* efficiently[1]. It has been suggested that the chlorophyll in these plants differs structurally from chlorophyll a, and is probably derived from a biosynthetic precursor of chlorophyll a.[2,3] Recent n.m.r. investigations have demonstrated that this chlorophyll is the major chlorophyll in the mutant plants, and that chlorophyll a is absent or present only in small amounts.[4] Furthermore, these investigations are consistent with the proposed structure (1) differing from chlorophyll a (2) by the ethyl group at position 4



being replaced by a vinyl group. However, these studies did not enable us to fully characterise the structure; in particular we were uncertain about the nature of the hydrocarbon tail at position 7c, which is phytyl in chlorophyll a, but which is believed to be geranylgeranyl in biosynthetic precursors of chlorophyll a.[5,6] In this study we demonstrate that the hydrocarbon chain is phytyl, and also confirm the nature of the modification at position 4 in the macrocyclic ring by mass spectrometry.

Electron impact (EI) and chemical ionisation (CI) mass spectrometry do not generally provide structurally conclusive information for chlorophylls. Limited information has been obtained with Field Desorption (FD) and "In beam" EI mass spectroscopy,[7] although these techniques are difficult in practice since they both rely upon either chemical or electronic mass marking for molecular weight determination. Fast Atom Bombardment (FAB) mass spectroscopy,[8,9] a new technique for examining involatile or thermally labile compounds, has proved particularly useful in characterising this chlorophyll.

We attempted to take FAB spectra of both the chlorophyll from the mutant maize plants and chlorophyll a. The normal method of dissolving samples in a viscous matrix of glycerol did not yield a spectrum. Normally FAB is most effective for polar molecules, and the hydrocarbon chain of chlorophylls reduces their solubility in glycerol. However, we found that a mixture of chloroform and trigol as solvent for the chlorophylls, gave intense positive ion FAB spectra. Molecular ions corresponding to $M^{\cdot +}$ and $M+1^{+}$ occurred at m/z 892 and 893 (chlorophyll a) and m/z 890 and 891 (mutant chlorophyll) as illustrated in Figure 1. This 2 mass unit difference is consistent with one less reduced double bond in the mutant chlorophyll, compared to chlorophyll a, as expected from structure 1.

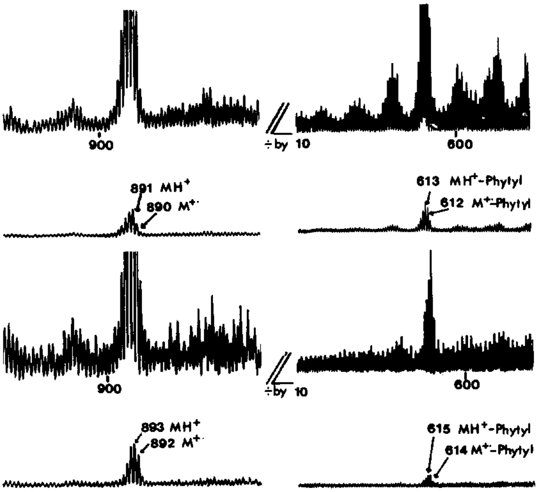


Figure 1

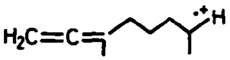
Portions of the FAB mass spectra of the chlorophyll from the mutant maize plant (above) and chlorophyll a (below)

Further peaks can be observed at m/z 614 and 615 (chlorophyll a) and m/z 612 and 613 (mutant chlorophyll). These peaks correspond to the loss of phytyl sidechain in chlorophyll a, and so are strong evidence that the difference between the two chlorophylls is on the macrocyclic ring.

We were unable to obtain other high mass fragments in the FAB mass spectrum. However chlorophylls pheophytinise in FD source (loss of Magnesium and protonation of the Nitrogens) and we obtained a molecular ion at m/z 868 corresponding to structure (3) from the mutant chlorophyll. A fragment ion at m/z 796 is observed arising from loss of -CHCO$_2$Me at position 10, which is expected for structure 1. Since the most reactive position in chlorophylls appears to be 10, yielding allomers[7] and pyrochlorophylls, this fragment ion is confirmation that this position has remained unchanged.

Neither of the above techniques provided us with direct evidence for the structure of the hydrocarbon tail. "In beam" EI spectra are the most difficult in practice and interpretation, and although they were generally irreproducible and short lived at high m/z values, strong reproducible fragment ions were observed at m/z 278 (structure (5)) and m/z 123 (structure (6)), for both chlorophylls. This is direct evidence for the degree of unsaturation of the sidechain, and confirms that it is phytol (structure (7)).

(5)



(6)

(7)

The combination of mass spectroscopic techniques, therefore, confirms that the chlorophyll occurring in the mutant of maize, previously called chlorophyll a$_1$ A436[3] is 4-vinyl-4-desethyl chlorophyll a.

The existence of this compound in large quantities is of considerable biosynthetic interest. Although the biosynthetic route to chlorophyll a has not been conclusively characterised, there is strong evidence that geranylgeranyl chlorophyllide a (structure (4)) is a precursor of chlorophyll a,[5] and this compound has been isolated in small quantities from developmentally immature horsechestnut leaves.[6] The vinyl group at position 4 is reduced prior to esterification of 7c with geranylgeraniol.

Acknowledgements

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