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"IN BEAM" ELECTRON IMPACT MASS SPECTROMETRY. THE STRUCTURE OF A BACTERIOCHLOROPHYLL ALLOMER

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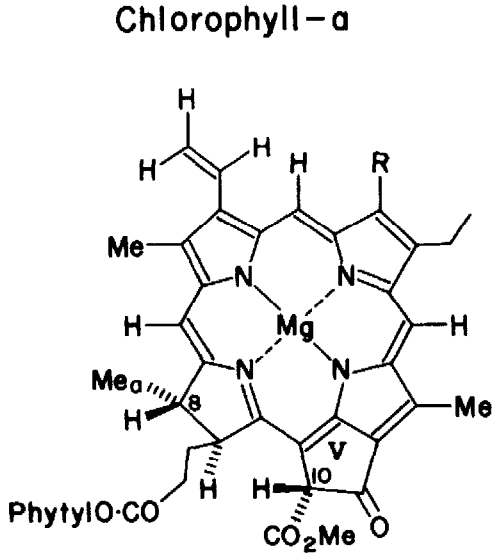
Summary. *By means of proton n.m.r. and "in beam" electron impact mass spectrometry, an allomer of bacteriochlorophyll is shown to be hydroxylated in ring V.*

Chlorophylls are notoriously unstable compounds, and during extensive isolation procedures, they are frequently modified chemically. The best known derivatives of chlorophyll-a (1, R=Me) are the allomers which have



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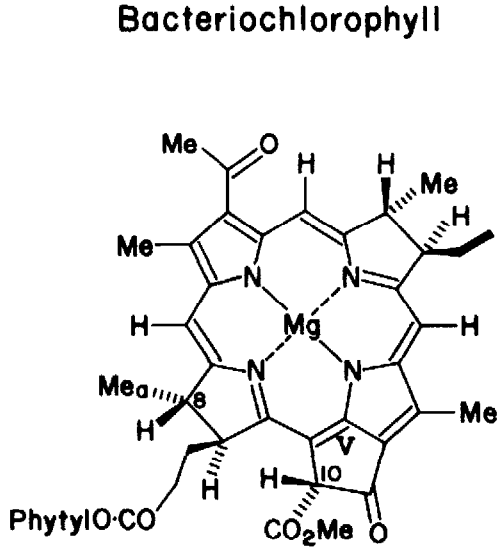

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modifications in ring V;[1,2] the corresponding chemistry of bacteriochlorophyll-a (2) is less well known as the compounds are even more reactive. We show here that "in beam" electron impact mass spectrometry[3-5] facilitates reliable molecular weight determinations, and thus aids rapid structure determination.

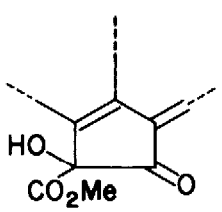
In the course of preparing (2) by our previously described method,[6] we have also isolated a new compound, (3), sometimes as the principle bacteriochlorin.[7] The UV/visible spectrum of (3) is virtually indistinguishable from that of (2), and the proton n.m.r. spectra are almost identical except for significant differences in both chemical shift and T_1 for a few protons (Table).[8]

Table

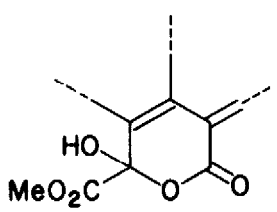
Differences in the ^1H NMR Spectra of Bacteriochlorophyll-a (2) and its Allomer (3)

	2		3	
Proton signal	δ (ppm)	T_1 (sec)	δ (ppm)	T_1 (sec)
H-10 (1H, s)	5.93	0.8	5.72	1.4
$-CO_2Me$ (3H, s)	3.76	0.8	3.55	0.6
-Me(8a) (3H, d)	1.67	0.4	1.50	0.4

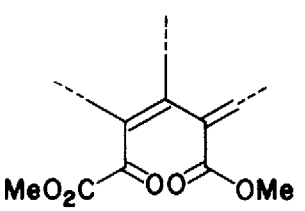
The δ5.72 signal in (3) is shown to be a hydroxyl proton by D_2O exchange and by its temperature dependent shift. The long T_1 of the hydroxyl proton is due to greater mobility and/or greater distance from other protons, but the short T_1 of the ester methyl protons is clearly due to steric effects.[6] The addition of base does not cause epimerisation. These data are consistent with (3) possessing a modified ring V of structure (3-i) or (3-ii), but they do not enable a distinction to be made.



(3-i)



(3-ii)



(3-iii)

Treatment of (3) with an excess of diazomethane gave no reaction; this is tentative evidence against (3-II) which would have been expected to afford (3-III).

Electron impact mass spectrometry of chlorophylls usually fails completely, and although field desorption sometimes affords good molecular ion peaks,[9] this technique is not routinely available to many workers. "In beam" electron impact mass spectrometry is little-used, but is a sensitive method for obtaining molecular weight information for many relatively polar molecules.[3-5] In this method, a solution of the sample is loaded onto the outside of the quartz tip of a direct insertion probe. The tip is then extended directly into the edge of the electron beam. The proximity of the sample to the electron beam aids volatilisation of intact molecules. With this technique, pheophytin-a (metal-free chlorophyll-a) gives ions at m/z 870.5 $(M)^{+\cdot}$, 839.5 $(M-OMe)^{+}$ and 811.5 $(M-CO_2Me)^{+}$. Chlorophyll-a gives ions at m/z 870.5 $(M-Mg^{++} + 2H^{+})^{+\cdot}$ and 871.5 $(M-Mg^{++} + 3H^{+})^{+}$, and chlorophyll-b (1, R=CHO), simply gives m/z 884.5 $(M-Mg^{++} + 2H^{+})^{+\cdot}$. Formally, these species correspond to pheophytins formed by loss of magnesium; however, the spectra are clearly and reproducibly different from those of the pheophytins themselves. The "in beam" mass spectrum of (3) gave only an intense peak at m/z 905.5, corresponding to protonated bacteriopheophytin + 16 Daltons. Thus (3) contains only one oxygen atom more than (2) and we conclude therefore that (3) has the structure (3-I). The stereochemistry of (3-I) is not certain, although the T_1 of the CO_2Me protons tentatively suggests that the ester group is β.[6]

In conclusion, it appears that "in beam" mass spectrometry is a powerful method for molecular weight determination of compounds which are too fragile for purely thermal volatilisation.

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7. An improved procedure eliminates this problem and greatly increases both the yield and stability of 2: R.G.B. and J.K.M.S. to be published.

8. Spectra were obtained at 100MHz and a temperature of 310K in d_6-acetone solution; concentrations were ca 10mM.

9. H.R. Morris, J.C. Waterton and J.K.M. Sanders, unpublished work.

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