Acknowledgment.—We are indebted to the National Science Foundation for financial support (Grant No. G-17345).

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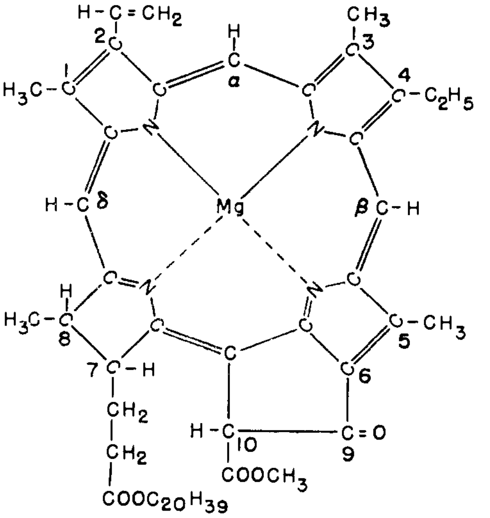
RECEIVED JUNE 22, 1962

SITE OF EXCHANGEABLE HYDROGEN IN CHLOROPHYLL *a* FROM PROTON MAGNETIC RESONANCE MEASUREMENTS ON DEUTERIO-CHLOROPHYLL *a*[1]

Sir:

As shown by an indirect infrared method, chlorophyll *a* possesses one slowly exchangeable hydrogen atom when the chlorophyll is treated with methanol in carbon tetrachloride solution at room temperature in the dark.[2] Although the position of the exchangeable hydrogen could not be established by the exchange method, considerations had indicated that the active hydrogen probably was located at the C-10 position (I).[3]

We have now carried out proton magnetic resonance measurements (Varian A-60 n.m.r. spectrometer equipped with an audio oscillator and counter) that confirm the presence of but one exchangeable hydrogen atom in chlorophyll *a* (under our exchange conditions). The exchangeable hydrogen is not located at the C-10 position, but is in fact on the δ-bridge carbon atom of the porphyrin ring (I). The n.m.r. observations were made with two materials; not only was the exchange followed in ordinary chlorophyll *a*, but also in the fully deuteriated chlorophyll *a* that we have available.[4] The use of the deuterio-chlorophyll made it possible to detect hydrogen resonances that might otherwise have been obscured in ordinary chlorophyll.



Chlorophyll *a*, isolated from spinach and carefully purified, exhibited three sharp, low field resonance bands ($\tau = 0.62, 0.85$, and 1.81, measured in $CDCl_3$, tetramethylsilane as internal indicator). These bands correspond to the three bands found in chlorin e_6 trimethyl ester and assigned to the α-, β- and δ-bridge hydrogen atoms by Woodward and Skaric.[5] All other hydrogen resonances in chlorophyll *a*, including the C-10 hydrogen, lie at higher fields. When chlorophyll *a* was treated with excess CH_3OD in CCl_4 for 48 hours,[2] the band at $\tau = 1.81$ disappeared, in a manner entirely analogous to that reported for chlorin by Woodward and Skaric.[5] When the exchanged chlorophyll *a* was treated with CH_3OH, the band reappeared. These facts indicate that the δ-bridge hydrogen atom undergoes the exchange.

To minimize uncertainties and ambiguities in the interpretation of the very complex chlorophyll n.m.r. spectrum, we followed the hydrogen exchange in deuterio-chlorophyll *a*.[4] This substance showed no proton resonances. On treatment with CH_3OH in CCl_4 for 48 hours, one, and only one, proton resonance appeared at $\tau = 1.81$. Again, treatment with CH_3OD caused the resonance peak to disappear. These results with deuterio-chlorophyll *a* demonstrate unequivocally that only one hydrogen atom undergoes exchange under the reaction conditions used here, and that the labile hydrogen is located on the porphyrin ring at the δ position.

The remarkable observations of Woodward and Skaric[5] on the chemical and exchange behavior of chlorins thus also appear to be valid for chlorophyll *a* itself. That the hydrogen at the δ position is more labile than the one at C-10 could only be established by measurements on chlorophyll itself rather than on derivatives lacking the central magnesium atom and the cyclopentanone ring. Although the conditions used for the hydrogen exchange are certainly not physiological, nevertheless the lability of the δ-hydrogen atom in chlorophyll *a* as established here may also be pertinent to the chemical behavior of chlorophyll *a* in photosynthesis.

We are deeply indebted to Dr. Gerhard L. Closs of the University of Chicago for guidance and instruction in the interpretation of the n.m.r. spectra.

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RECEIVED JULY 25, 1962

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[1] Based on work performed under the auspices of the U. S. Atomic Energy Commission.

[2] J. J. Katz, M. R. Thomas, H. L. Crespi and H. H. Strain, *J. Am. Chem. Soc.*, **83**, 4180 (1961).

[3] H. Fischer and S. Goebel, *Ann.*, **522**, 168 (1936); S. Aronoff, *Encyclopedia of Plant Physiology*, **5**, 234 (1960); W. Vishniac and I. A. Rose, *Nature*, **182**, 1089 (1958).

[4] H. H. Strain, M. R. Thomas, H. L. Crespi, M. I. Blake and J. J. Katz, *Ann. New York Acad. Sci.*, **84**, 617 (1960).

[5] R. B. Woodward and V. Skaric, *J. Am. Chem. Soc.*, **83**, 4676 (1961).

THE EFFECT OF H_2O ON THE γ-RADIOLYSIS OF AERATED CH_3OH[1]

Sir:

We wish to report a previously overlooked[2] effect of H_2O on the γ-radiolysis of aerated methanol which gives promise of serving as a useful tool in the study of radiolytic mechanism.

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[1] Research performed under the auspices of the United States Atomic Energy Commission.

[2] E. Hayon and J. J. Weiss, *J. Chem. Soc.*, 3970 (1961).