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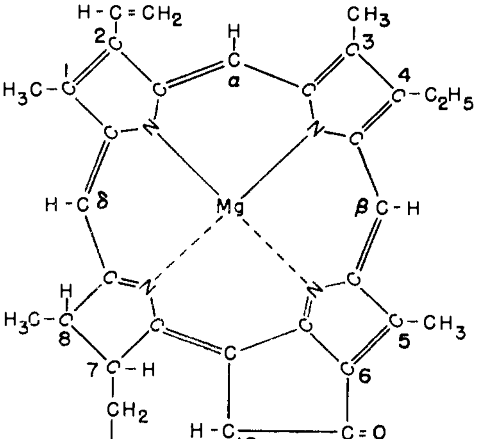
SITE OF EXCHANGEABLE HYDROGEN IN CHLOROPHYLL *a* FROM PROTON MAGNETIC RESONANCE MEASUREMENTS ON DEUTERIO-CHLOROPHYLL *a*[1]

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 (τ = 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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(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).

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RECEIVED JULY 25, 1962

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).

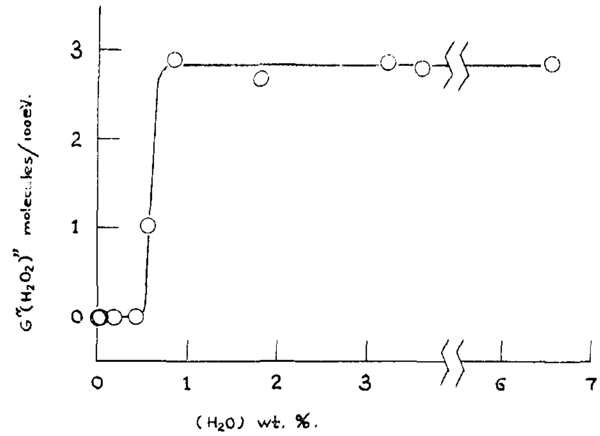

Fig. 1.

Eastman "Spectro Grade" methanol, purified by refluxing with $NaBH_4$ for two hours and rectifying on a Todd column, taking only the middle third, and dried by refluxing with fresh magnesium turnings for eight hours, was distilled through a column directly from the $Mg(OCH_3)_2$ solution into carefully dried radiation cells, with exclusion of atmospheric moisture and CO_2. Aeration was effected by sweeping with dry, CO_2-free, filtered air for twenty minutes. The concentration of oxygen in air-saturated methanol, according to the I.C.T., is approximately $1.5 \times 10^{-3} M$ at 19°. Cells, cleaned by soaking in hot nitric-sulfuric acid mixture, rinsing with water, soaking in aq. ammonia and rinsing with distilled water, were filled so as to leave less than 1 cc. of air space when ground glass stoppers were introduced. Irradiations in Schwarz-Allen type sources[3] were principally at a dose rate to methanol in the vicinity of 2.02×10^{17} eV. ml.$^{-1}$ min.$^{-1}$ (Fricke dosimetry, corrected for the difference in electron density). Formaldehyde,[4] ethylene glycol[5] and peroxide[6] were determined by established procedures and formic acid by a method differing from that of Grant[7] largely by prior addition of $NaHCO_3$ and removal of methanol and volatile products by codistillation with benzene.

In disagreement with Hayon and Weiss,[2] who report $G(H_2O_2) = 2.89$, doses in the range $1\text{-}2 \times 10^{18}$ eV. ml.$^{-1}$ produce *no* detectable peroxide, either at the standard dose rate or at a rate $^1/_{20}$ as large. Peroxide (presumably $H_2O_2 + CH_3OOH$) is formed when small amounts of water are present. The dependence of peroxide yield on water concentration is remarkable and is represented in Fig. 1. Although more rigorous work is required to determine accurately the concentration of H_2O at which $G''(H_2O_2)''$ increases abruptly from zero, data now available are consistent with a value of 0.5% by wt. or somewhat higher. An increase in water concentration of no more than 0.3% by wt. increases $G''(H_2O_2)''$ from a value indistinguishable from zero to its plateau value of 2.8.

The abrupt increase of $G(H_2O_2)$ at concentrations of H_2O in the vicinity of 0.3 M suggests that water may be titrating an adventitious impurity which destroys peroxide or inhibits its formation. That an impurity could be present at concentrations of the order of 0.3 M in the methanol described above is very unlikely. The possibility remains, however, that water reacts *reversibly* with some substance which destroys or inhibits formation of peroxide and that magnitudes of equilibrium and rate constants and the concentration of the impurity are such that effective inhibition of suppression of peroxide occurs at rather high concentrations of water. The exclusion of this class of explanation is very difficult although specific solutes can be tested directly.[8]

An alternative possibility is that water is, indeed, essential to the formation of peroxide in otherwise pure aerated methanol. Because of the limited available data, interpretations founded on this viewpoint serve at present largely to suggest further experiments. One such line of speculation assumes that O_2 does not scavenge the forms of "hydrogen atoms" formed in this medium, *e.g.*, the solvated electrons and hydrogen atoms which have been suggested.[9] It has been shown[10] that the corresponding aqueous species[11] scavenge O_2, however. The present data suggest that, at a mole fraction of water in the vicinity of 0.01, species with reactivity similar to the aqueous species become significant. It is not apparent, however, why the aqueous and methanolic species should possess quite different reactivity.

We find no ethylene glycol production in aerated dry or wet methanol, in agreement with Hayon and Weiss,[2] a result which suggests that this product arises entirely from coupling of radicals in bulk solution. $G(HCO_2H)$ is 1.8 ± 0.2, differences among results in dry and wet methanol at the standard dose rate and dry methanol at the lower dose rate falling within the standard deviation. Hayon and Weiss assumed but did not demonstrate the formation of this product.

Our values of $G(CH_2O)$ fall in the range 10 ± 1, independent of the presence of water or dose rate, whereas Hayon and Weiss[2] report 4.28. That this discrepancy may be due to the presence of benzene in the methanol employed by Hayon and Weiss

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(3) H. A. Schwarz and A. O. Allen, *Nucleonics*, **12**, 58 (1954).

(4) C. E. Bricker and H. R. Johnson, *Ind. Eng. Chem., Anal. Ed.*, **17**, 400 (1945).

(5) N. N. Lichtin, *J. Phys. Chem.*, **63**, 1449 (1959).

(6) C. J. Hochanadel, *ibid.*, **56**, 587 (1952).

(7) W. M. Grant, *Anal. Chem.*, **20**, 267 (1948).

(8) It has been suggested by a referee that reaction with methyl borate might be responsible for the observed effect of water. This follows from the observation of R. P. Porter, *J. Phys. Chem.*, **61**, 1260 (1957), that methanol vapor reacts extensively with Pyrex to give methyl borate which has a major influence on the photolysis of methanol. We have found that liquid methanol does indeed extract boron from Pyrex, *e.g.*, the boron concentration which in untreated Eastman "Spectrograde" methanol taken directly from the supplier's bottles is less than $5 \times 10^{-5} M$ increases to about $4 \times 10^{-3} M$ during rectification, according to an analytical procedure which involves prior hydrolysis of methyl borate followed by spectrophotometric determination by the method of W. T. Dieble, E. Truog and K. C. Berger, *Anal. Chem.*, **26**, 418 (1954). The drying procedure described above reduced the boron concentration to below $5 \times 10^{-5} M$ measured before and after irradiation, however. Since plateau peroxide yields were not affected by 0.3 M $B(OCH_3)_3$ added prior to irradiation, it seems extremely improbable that borate is responsible for the observed effect.

(9) J. H. Baxendale and F. W. Mellows, *J. Am. Chem. Soc.*, **83**, 4720 (1961).

(10) G. Czapski and A. O. Allen, *J. Phys. Chem.*, **66**, 262 (1962).

(11) G. Czapski and H. A. Schwarz, *ibid.*, **66**, 472 (1962).

is suggested by our findings that one supplier's methanol (Fisher, Certified Reagent) contains spectroscopically detectable benzene which is removable with low efficiency by rectification and that 0.2 wt. per cent. of benzene added to purified Eastman "Spectro Grade" methanol reduced $G(CH_2O)$ to 5.5. No benzene was detectable in the latter before or after purification but it is possible that the relatively poor precision of our $G(CH_2O)$ values may reflect variable trace amounts of benzene. The present high value of $G(CH_2O)$ suggests that CH_2O may be formed in a short chain reaction.

A detailed study of oxygenated methanolic solutions is now in progress. A survey of the effect of water on peroxide production from other substrates has been initiated.

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THE INFRARED SPECTRUM AND EVIDENCE FOR THE STRUCTURE OF A NEW METAL CARBONYL[1]

Sir:

The infrared spectra, structures, and hybridizations of metal pentacarbonyls derived from the hexacarbonyls of Cr, Mo, and W are of considerable interest. Several workers[2,3,4] have postulated their existence as intermediates in the production of hexacarbonyl derivatives of the type $M(CO)_{6-n}(L)_n$ by ultraviolet irradiation (L = ether, nitrile, amine, etc.). Such reactions are thought to be initiated by the mechanism

$$M(CO)_6 + h\nu \longrightarrow M(CO)_6^* \longrightarrow M(CO)_5 + CO$$

We have obtained evidence to support this view.

0.02 mmole of $W(CO)_6$ was dissolved in 2 ml. of a specially purified 1:4 methylcyclohexane–isopentane mixture, and the resulting solution was placed under nitrogen in a thin quartz tube and cooled to 77° K. The resulting glass was irradiated until a yellow color appeared. At the instant the glass was melted the solution was withdrawn from the tube, and the infrared spectrum was taken immediately. The spectrum showed peaks at 2075 (w), 1936 (s), and 1912 (ms) cm.$^{-1}$ in the carbonyl stretching region; such a spectrum is characteristic of a square pyramid of five carbonyl groups about the metal atom.[5] The species was found to have a half-life of about two minutes at room temperature. The experiment was repeated using *n*-hexane as the solvent, and bands were found at 1935 and 1911 cm.$^{-1}$.

Of the four species which one might reasonably expect to be the product of the photolysis, one, $W_2(CO)_{10}^{=}$, may be rejected immediately because its spectrum shows carbonyl stretching bands[6] at 1944, 1906, and 1882 cm.$^{-1}$. The monomer $W(CO)_5^-$ may also be discounted because extensive electron spin resonance studies show no evidence for the existence of unpaired electrons in our species. The structure $W(CO)_5^{=}$ is also open to serious question because it is highly unlikely that the other product of such a photodissociation, CO^{++}, could exist long enough to permit the observed reversibility[3] of the reaction and because one would expect the carbonyl stretching bands for such a complex to be at much lower frequencies than those observed. The carbonyl stretching frequencies for $Fe(CO)_5$ are at 2034 and 2014 cm.$^{-1}$ and for $Mn(CO)_5^-$ are at 1898 and 1863[7] cm.$^{-1}$, illustrating the pronounced lowering of frequency when even one electron is introduced into a five-coördinated structure. Finally, the solubility of the complex in non-polar solvents is a strong argument against any of the three charged structures discussed.

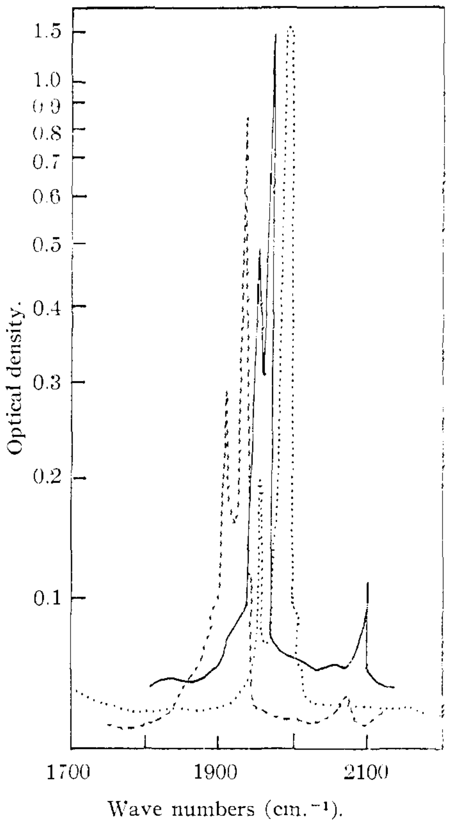

Fig. 1.—Carbonyl stretching region of infrared for $W(CO)_6$, $W(CO)_5$ and $W(CO)_5(CHCH)$: ......, $W(CO)_6$; ———, $W(CO)_5(CHCH)$; - - - -, $W(CO)_5$.

In support of the proposed structure we have analytically and spectrophotometrically proven that $W(CO)_5(CH_3CN)$ is immediately formed when acetonitrile is introduced into a solution containing the species. Furthermore, the spectrum of $W(CO)_5(CHCH)$ shows carbonyl stretching bands at 2097, 1967, and 1954 cm.$^{-1}$, in line with the expectation that the poor charge donating ability of acetylene coupled with its outstanding ability to accept any excess charge accumulated on the metal atom should slightly increase the CO bond order from that found in the postulated species. We believe that $W(CO)_5$ is a d^2sp^2 hybrid and that the vacant p orbital is responsible for its extreme reactivity.

---

[1] Supported by A.E.C. contract AT-(40-1)-2434.

[2] L. E. Orgel, *Nature*, **191**, 1387 (1961).

[3] G. R. Dobson, M. A. El Sayed, I. W. Stolz and R. K. Sheline, *Inorg. Chem.*, **1**, 526 (1962).

[4] W. Strohmeier, *Chem. Ber.*, **94**, 3337 (1961).

[5] L. E. Orgel, *Inorg. Chem.*, **1**, 25 (1962).

[6] W. Hieber, W. Beck and G. Braun, *Angew. Chem.*, **72**, 795 (1960).

[7] W. F. Edgell, *J. Am. Chem. Soc.*, **82**, 1254 (1960).