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Photooxidation of Chlorophyll b by Quinones. Studied by Chemically Induced
Dynamic Nuclear Polarization[†]
(NMR/excited states/radical pairs)

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Running Title: Photooxidation of Chlorophyll b by Quinones

[†]Work performed under the auspices of the U. S. Atomic Energy Commission.

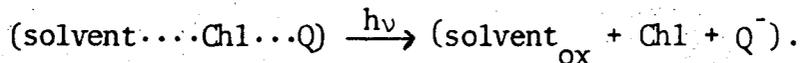
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ABSTRACT The photooxidation of chlorophyll b by 1,4-benzoquinone and 2,6-dimethyl-1,4-benzoquinone was investigated using the technique of chemically induced dynamic nuclear polarization. Polarization of the proton magnetic resonance lines of the quinone was detected. A mechanism for the photooxidation was postulated which invokes the reaction of the quinone with the excited singlet state of the chlorophyll to form a radical pair. This mechanism, together with a theoretical model and parameters taken from the literature, yields a theoretical PMR spectrum for the polarized quinone which agrees well with that observed experimentally.

INTRODUCTION

The photosensitized oxidation of chlorophyll by different oxidizing agents is being investigated in several laboratories (1-17), in order to provide guidelines for understanding the primary steps in photosynthesis. Among the various oxidants, the reaction of chlorophyll with various quinones is of special interest. It is now well established that this reaction proceeds through free radical intermediates, but the mechanistic details are still controversial (16). There are conflicting data on the nature of the excited states of chlorophyll with which the quinones react. The main sources of information about the nature of the precursors involved were obtained from flash photolysis, ESR, and fluorescence studies with β -carotene as a competing quencher of the excited states. It was assumed that β -carotene reacts only with the triplet state of the excited chlorophyll. It was found (13,14) that addition of quinone reduced the concentration of the excited triplet of the chlorophyll but not its lifetime. This suggests that the quinone reacts with a precursor of the chlorophyll triplet. However, ESR studies showed that β -carotene quenches the semi-quinone radical formation, which was explained in terms of competition between the quinones and the β -carotene for the chlorophyll triplet (9,11).

As a way out of this dilemma, Tollin (13,14,17) suggested that a reaction occurs involving a ternary complex of the form:



The suggestion was that the chlorophyll photosensitizes the oxidation of solvent molecules and the solvent radical which is formed reacts with the quinone. Recently Harbour and Tollin (17) reported observations of the solvent radical in low temperature ESR experiments; for ethanol as the

solvent, the ethoxy radical was identified.

Chemical reactions involving radical pairs can give rise to nuclear spin polarization, which is manifest by enhanced NMR absorption and/or emission. This phenomenon is commonly denoted chemically induced dynamic nuclear polarization (CIDNP) (18,19). This technique appeared eminently suitable for attacking this problem, and we report here direct evidence of the nature of the precursors in the photooxidation of chlorophyll by quinones.

A necessary condition for producing polarized NMR spectra of this sort is a competition between spin dependent annihilation of the radical pair to form a singlet and spin independent scattering to form the two separate radicals (20-26). This condition seems to be satisfied in this case, since the reaction is reversible and the radicals of both Chl^+ and semiquinone have been observed by EPR (16). Furthermore, some data obtained by fluorescence, flash photolysis and ESR were interpreted in terms of the existence of radical pairs (1,3,16). Since the CIDNP technique is sensitive to the multiplicity of the precursor of the radical pair as well as the chemical nature of the participants, it seemed to be a promising approach for clarification of some of the mechanistic aspects of this reaction.

MATERIALS AND TECHNIQUE

The light induced PMR spectra were recorded, using a Varian A-60 spectrometer equipped with a light guide, as described elsewhere (27). 1,4-Benzoquinone was purified by vacuum sublimation. All other chemicals were the most highly purified among those commercially available, and were used without further purification. Corning glass filters were used

for selecting the desired spectral range. Calibrated mesh filters were used to change the light intensity. An Hanovia 1000 W high pressure mercury xenon lamp in a Schoeffel housing was used as the light source.

We found no effect upon deoxygenating the sample, except for the observation that the deoxygenated samples remained stable for longer periods of irradiation. Fresh solutions were used for each experiment.

RESULTS

Fig. 1 shows spectra for a solution of 5×10^{-5} M chlorophyll b with 10^{-2} M 1,4-benzoquinone in perdeuterated methanol. The lower trace shows the spectrum before irradiation and the upper trace shows the spectrum during irradiation with visible light using an O-51 Corning filter, which removes all light with wavelengths shorter than 400 nm. The magnetic field increases from left to right. The line which appears in the low field part of the spectrum is due to the ring protons of 1,4-benzoquinone. The remaining features are due to the solvent. It can be seen that upon irradiation the ring protons of the 1,4-benzoquinone are enhanced in intensity while the shape and intensity of the methyl protons of the solvent remained unchanged. The absorption peak of the OH protons changes shape and shifts to higher field due to local heating, but integration of the signal revealed that there is no change in intensity.

Fig. 2 shows spectra of 2,6-dimethyl-1,4-benzoquinone taken under the same conditions as in Fig. 1. A and B are scans recorded before irradiation under low and high resolution conditions, respectively, while C and D were recorded during irradiation.

The PMR spectrum of 2,6-dimethyl-1,4-benzoquinone is of the type $AX_3A'X'_3$ (28). While we were unable to resolve the ring proton lines, the methyl lines were fully resolved with a coupling constant of

$J_{ax} = 0.7 \pm 0.2$ Hz. Upon irradiation the ring protons of 2,6-dimethyl-1,4-benzoquinone are enhanced in intensity while the signals due to the methyl protons appear as emission lines with a different distribution of intensities within the triplet compared to the unirradiated sample. In addition, an unidentified peak appears on the low field side of (a) in trace C. This peak arises from some unidentified polarized irradiation product. Another feature of the spectra, not shown, is that after prolonged irradiation the enhanced absorption lines of the ring protons become emission lines. We will not deal with this problem here, as we are not sure as to the mechanisms involved. Our working hypothesis is that this occurs from new product(s) whose chemical shift does not differ from that of the original quinones' aromatic protons. Work is in progress to clarify this point.

The polarization of an NMR line which results from transitions between nuclear states n and m is defined by:

$$P_{mn} = \frac{V_{mn} - V_{mn}^0}{V_{mn}^0} \quad (1)$$

where V_{mn} is the observed intensity of the NMR signal and V_{mn}^0 is the intensity of the signal at Boltzman equilibrium.

No polarization of the quinone peaks occurred in the absence of chlorophyll. No polarization was observed when the chlorophyll was replaced with chlorophyllin. Also, no polarization was observed when the deuterated methanol solvent was replaced by CCl_4 .

When the system chlorophyll + quinone was excited with red light, by using Corning filter 2-61 which removed all wavelengths shorter than 600 nm, the same pattern of polarization was observed but the magnitude

was reduced by more than 50%; this indicates that both visible absorption bands of the chlorophyll participate in the reaction. No further enhancement of the polarization was observed by removing all filters and extending the spectral range of the excitation into the ultraviolet. It was found that the polarization was proportional to the light intensity.

Theory: Details of the theory used to calculate the polarized spectra can be found elsewhere (23). Here we will give only the final formulas involved in those calculations, and we mention the physical meaning of the parameters involved.

Based on the density matrix treatment of the radical pair, the theoretical expression for P_{mn} in Eq. 1 is:

$$P_{mn} = \frac{LkT}{g_N \beta_N H_0 \sum_n [D \cdot P]} \gamma T_1^{nm} (\rho_{SS}^n - \rho_{SS}^m) \quad (2)$$

In this equation, D·P describes the diamagnetic product formed by annihilation of the radical pair, $L = \prod_i (2I_i + 1)$, the total number of nuclear levels of D·P, g_N is the nuclear g factor, β_N is the nuclear magneton, T is the absolute temperature, H_0 is the intensity of the magnetic field, k is the Boltzman factor, $\gamma = k_1/k_{-2}$ where k_1 is the rate constant for the radical pair annihilation and k_{-2} is the rate constant for scattering of the radical pair to give two separate radicals (see scheme I), T_1^{nm} is the spin lattice relaxation time from state m to state n, and ρ_{SS} is the diagonal density matrix element for the singlet state of the radical pair, which is given by:

$$\rho_{SS} = \frac{\alpha_{SS} + \mu(2 + \gamma) \alpha_{TT}}{1 + \gamma + \mu(2 + \gamma)} \quad (3)$$

In Eq. 3, α is a matrix which describes the rate of formation of the radical pair; its exact form will depend on the nature of the precursor

of the radical pair. If the total rate of formation of the radical pair is given by k^* , then for a singlet precursor

$$\alpha_{TT} = 0 \quad \text{and} \quad \alpha_{SS} = k^*,$$

for a triplet precursor

$$\alpha_{SS} = 0 \quad \text{and} \quad \alpha_{TT} = k^*/3$$

and if the radical pair is formed from two separate radicals, then

$$\alpha_{SS} = \alpha_{TT} = k^*/4.$$

μ is given by

$$\mu = \frac{H_{ST}^2/k_{-2}^2}{(1+\gamma/2)^2 + 4J_e^2/k_{-2}^2 + (2+\gamma) H_{ST}^2/k_{-2}^2} \quad (4)$$

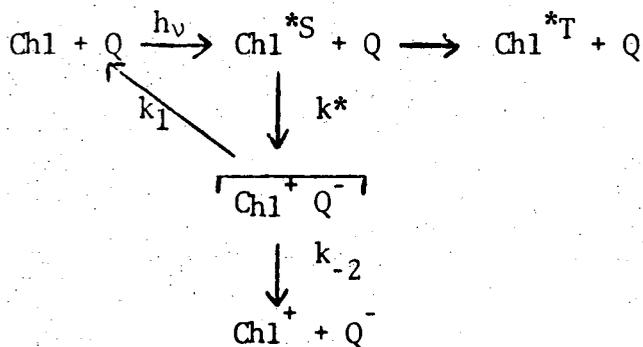
In Eq. 4, J_e is the electron exchange integral in energy units, which is related to the energy separation of the singlet and the T_0 triplet state of the radical pair, and H_{ST} is the off-diagonal matrix element of the spin-Hamiltonian which mixes the singlet with the T_0 state of the triplet manifold and is given by:

$$H_{ST} = 1/2 [\beta H_0 \Delta g + \sum_j^a A_j N_j - \sum_k^b A_k N_k] \quad (5)$$

In Eq. 5, a and b are the two radicals which form the radical pair, Δg is the difference in their electronic g factors, A_j and A_k are their scalar nuclear hyperfine interactions, N_j and N_k are their nuclear spin quantum numbers which describe the state of the j^{th} and k^{th} spin (for protons 1/2 and -1/2), and β is the electron Bohr magneton.

DISCUSSION

The stick spectrum given in Fig. 2(E) was constructed by using Eq. 2 with the appropriate parameters, based on the following mechanism:



Scheme I

The chlorophyll is excited to its singlet excited state from which it can go via intersystem crossing to the excited triplet state. The quinone quenches the excited singlet. As a result, the radical pair $\boxed{\text{Chl}^+ \text{Q}^-}$ is formed. The radical pair can dissociate to give the semiquinone radical and Chl^+ or it can annihilate to give back the starting materials. This mechanism can account for the reversibility of the reaction as well as for the fact that both the semiquinone radical and Chl^+ have been detected by EPR (16).

According to this scheme, the constituents of the radical pair are Chl^+ and Q^- . The g value of Chl^+ is 2.0025 (29) and that of 2,6-dimethyl-1,4-semiquinone radical is 2.00445 (30). The magnitudes of the hyperfine interaction of the CH_3 protons is $A_{\text{CH}_3} = +1.87$ g, and that of the aromatic protons $A_{\text{H}} = -2.22$ g (30). The signs were deduced from the well known difference in coupling mechanism between these two kinds of protons and the unpaired electron spin density in semiquinone radicals. While the coupling of the CH_3 protons is via hyperconjugation and hence A_{CH_3} will be positive, the coupling of the aromatic protons is via polarization of the

C-H σ electrons by the unpaired π electron, and hence A_{CH_3} will be negative (31).

The asymmetry of the methyl triplet is determined by the sign of the indirect coupling constant, J_N . For the calculated stick spectrum in Fig. 2(E), which agrees reasonably well with the experimentally observed spectrum, Fig. 2(D), a negative sign was required, in agreement with other allylic coupling constants, e.g., toluene (32). The electron-electron exchange interaction was taken as $J_e = 10^8$ Hz; the justifications for using this value are given elsewhere (23). The parameters given thus far are sufficient for calculating H_{ST} from Eq. 5.

Much less is known about the kinetic parameters which appear in Scheme I. The existence of biradicals of the nature postulated here was suggested previously to explain various optical and EPR observations (1,3,16). Hales and Bolton (16) postulate that they observe the EPR spectrum of the $\text{Chl}^+ \text{Q}^-$ biradical at room temperature and that the biradical decays with a first order rate constant of $1.2 \times 10^3 \text{ sec}^{-1}$. We have some reservations as to the correctness of the assignment of their rapidly decaying ESR signal as the $\text{Chl}^+ \text{Q}^-$ biradical, and thus do not choose to use their value for the rate of disappearance in our calculations. In view of lack of other sources of information, we took the literature value for the rate constant for the reaction $\text{Chl}^+ + \text{Q}^-$ which is $(3.0 \pm 1.0) \times 10^9 \text{ l mole}^{-1} \text{ sec}^{-1}$ (10,7,12). We normalized this value to an apparent quinone concentration in which the distance between the quinone and the chlorophyll would be 6 \AA and multiplied the result by a steric factor which accounts for only one semiquinone radical per Chl^+ . Assuming that the radical pair dissociates with equal probabilities through its singlet and the three triplet channels, we arrived at the following values for the rate constants:

$$k_1 = 5 \times 10^8 \text{ sec}^{-1} \quad \text{and} \quad k_{-2} = 1.5 \times 10^9 \text{ sec}^{-1}$$

In view of the fact that we did not measure quantum yields for the polarization, the only information that we have about k^* is its linear dependence upon the light intensity which derives from the predicted linear dependence of the polarization. It should be mentioned that the accuracy of our estimation of the rate constants will have only a marginal effect on the relative shape of the simulated spectrum, but will have a pronounced effect on the absolute magnitude of the observed polarization. The shape of the polarized spectrum is very sensitive to the relative values of Δg and the hyperfine constants of the various protons.

Finally, as the precursor is a singlet

$$\alpha_{SS} = k^* \quad \text{and} \quad \alpha_{TT} = 0$$

Attempts to fit the spectra for a triplet precursor or two separate radicals gave spectra inverted relative to the experimental. It was impossible to fit the spectra to the radical pair proposed by Tollin et al. (13,14,17), namely, $\text{CH}_3\text{O} \cdot \text{Q}^-$. Using the g value assigned by Harbour and Tollin (17) for the ethoxy radical, $g = 2.0049$, and assuming that the methoxy radical will have a similar g value, the Δg between the methoxy and the semiquinone radicals is too small compared to the hyperfine interactions to account for the observed polarization. Simulated spectra which are based on those values look totally different from those observed experimentally.

Although the experimental results presented here are in reasonable agreement with the postulate of a singlet precursor of the radical pair, we cannot exclude some participation of the chlorophyll triplet. From eqs. 2 to 5 one can see that if the same products are formed from singlet

and triplet precursors, the condition for the absence of any polarization is that $\alpha_{SS} = \alpha_{TT} (1 + \gamma)$. The fact that we observe polarization characteristic of a singlet precursor is indicative that at least 30% of the radical pairs form through the singlet channel. We can not assign a more accurate fraction as the absolute quantum yield of the polarization was not measured.

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FIGURE CAPTIONS

Fig. 1. 60 MHz PMR spectra of a solution of 10^{-2} M 1,4-benzoquinone and 5×10^{-5} M chlorophyll b in CD_3OD . A: before irradiation. B: during irradiation with visible light. (a) 1,4-benzoquinone; (b) CD_3OD .

Fig. 2. 60 MHz PMR spectra of a solution of 10^{-2} M 2,6-dimethyl-1,4-benzoquinone and 5×10^{-5} M chlorophyll b in CD_3OD . (A) and (B): before irradiation. (C) and (D): during irradiation with visible light. (E) Theoretically constructed stick spectrum. (a) Methyl protons of the quinone; (b) aromatic protons of the quinones; (c) solvent lines.

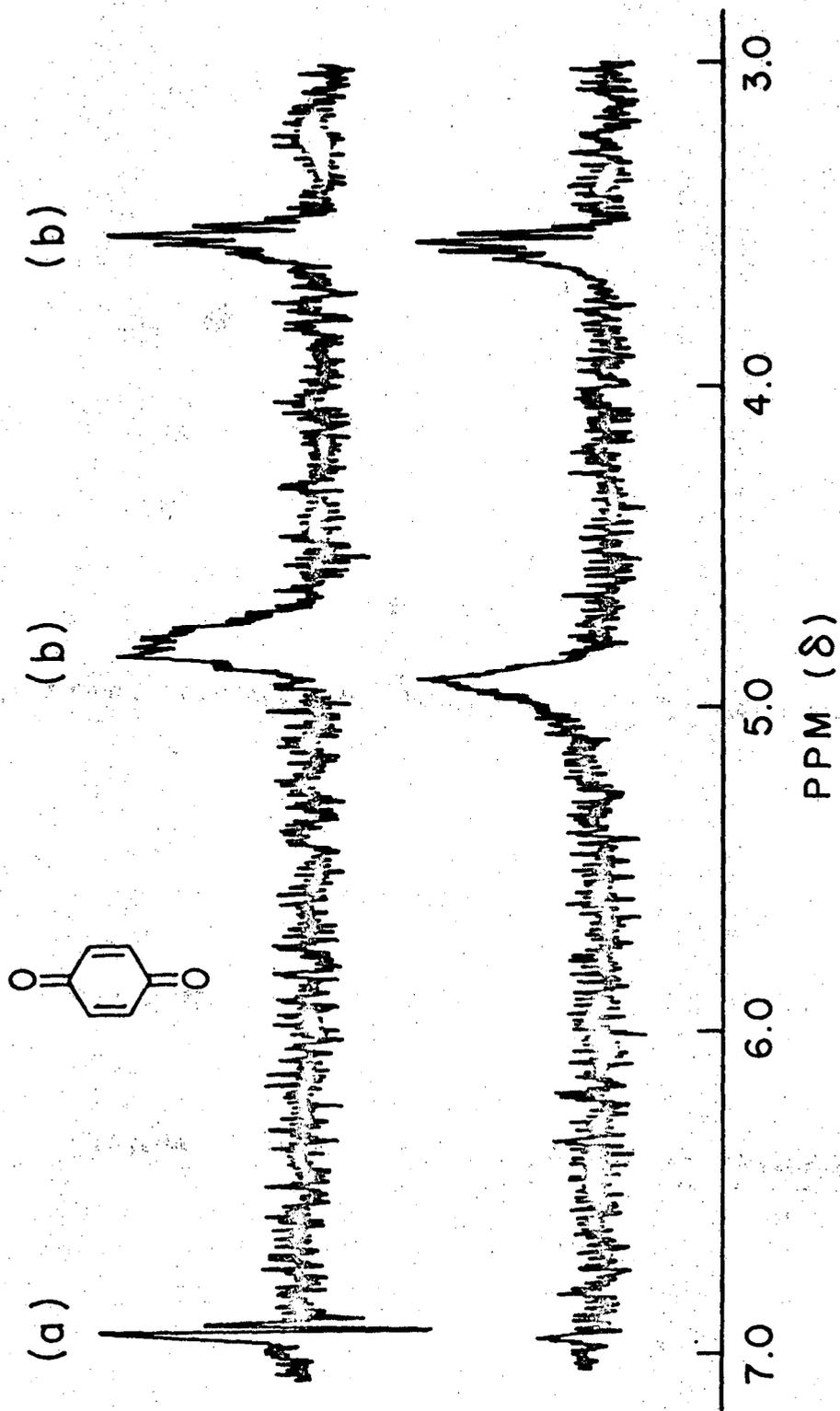
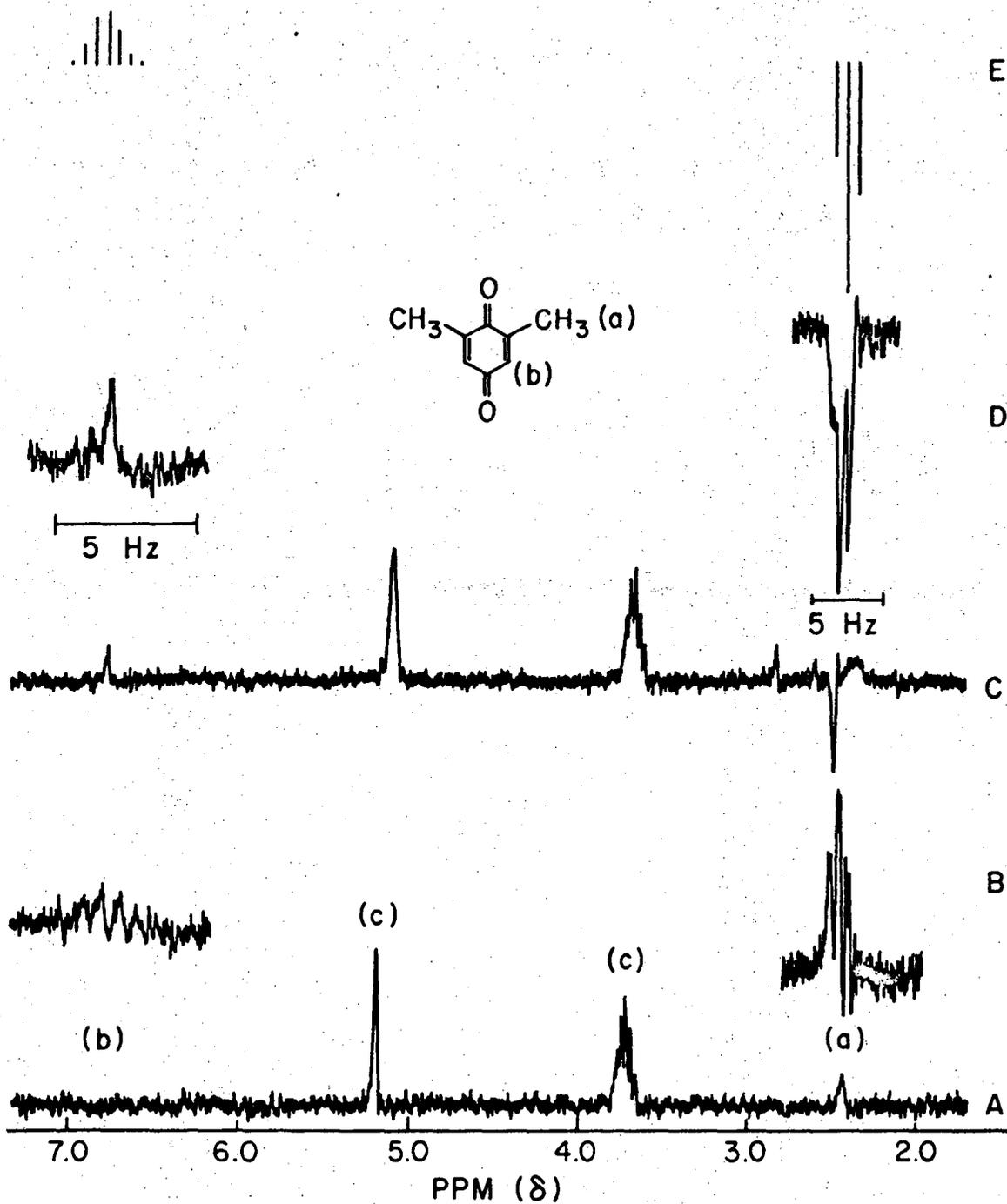


Fig. 1

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Fig. 2

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