THE COENZYME Q_{10} and VITAMIN K_1 SEMIQUINONE FREE RADICALS*

M. S. Blois, Jr. and J. E. Maling

Biophysics Laboratory
Stanford University
Stanford, California

Received July 11, 1960

The production of semiquinone free radicals by the univalent oxidation of quinols, and their subsequent study by means of electron spin resonance (e.s.r.) have been extensively reported over the past few years. (Ingram,1958).

Because of the roles of coenzyme Q (ubiquinone) and vitamin K_1 in biological electron transport and possibly in oxidative phosphorylation, the semiquinones of these compounds are of considerable interest. The e.s.r. spectrum of the vitamin K_1 semiquinone, the line splittings and g-value have been previously reported (Blois, 1957, Adams, 1958) but the line assignments had not at that time been made.

The e.s.r. spectrum of coenzyme Q_{10} semiquinone gives a convenient measure of the hyperfine spectral splitting due to the methyl and methylene protons. Its analysis, therefore, is not only of interest for its own sake but for the assistance it lends in interpreting the spectrum of vitamin K_1.

Pure coenzyme Q_{10}** was reduced to the quinol which was then dissolved in alkaline ethanol, and while undergoing air oxidation to form the semiquinone free radical, the e.s.r. spectrum at the top of Fig. 1 was observed. It will be noted there are nine evenly spaced components with a line separation of 1.1 gauss, and a g-value of 2.00467 ± .00002. The line assignment

*This research was supported by Grant A-2304 from the National Institutes of Health, U.S.P.H.S., and by Contract AF-1511, Office of Scientific Research, U. S. Air Force.

**We wish to express our appreciation to Professor A. L. Tappel of the University of California, Davis, for helpful discussions and for providing the Coenzyme Q_{10}.

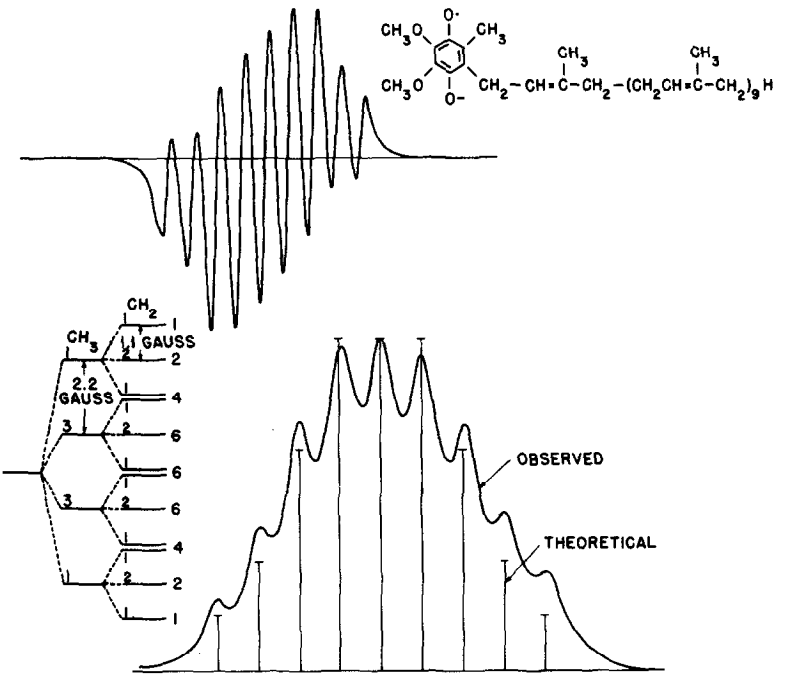

Fig. 1 The e.s.r. spectrum and line assignment for the semiquinone free radical of Coenzyme Q_{10}

---

is straightforward by assuming that the methylene protons produce the line splitting of 1.1 gauss. The quartet produced by the methyl protons will then have line separations of 2.2 gauss which is consistent with the value of 2+ gauss for the splitting of methyl protons attached to a single aromatic ring, as reported by Venkataráman and Fraenkel (1955). The nature of the agreement between the calculated and observed line intensities is shown in the lower part of Fig. 1, where the graphically integrated spectrometer output is shown together with the predicted intensities resulting from the assumed line assignment.

Using the preceding results, the line assignment for the semiquinone of vitamin K_1 can then be made by adding the effect of the protons of the second ring. To within the resolution available for this study, these protons may be considered equivalent and would be expected to produce a splitting of about 0.59 gauss as reported for the 2-methyl 1,4 naphthosemiquinone (Adams, 1958) or of 0.57 gauss for the 1,4 naphthosemiquinone

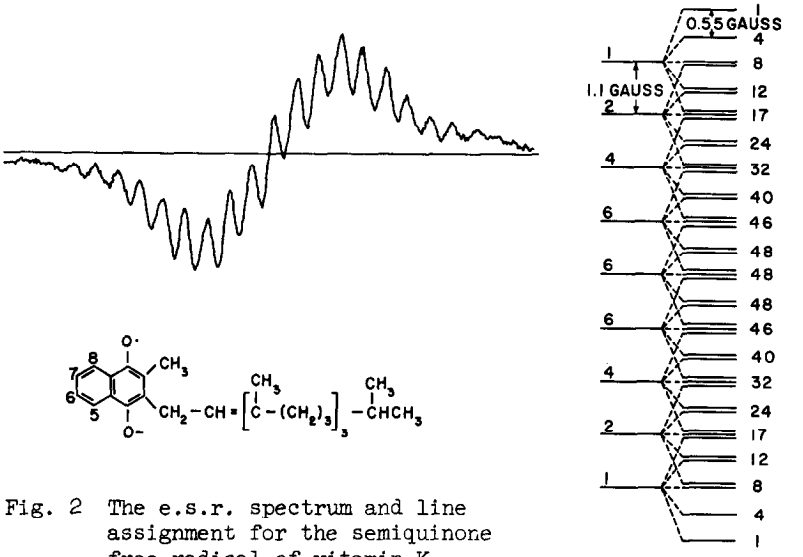

Fig. 2 The e.s.r. spectrum and line assignment for the semiquinone free radical of vitamin K_1 .

(Wertz, 1956). The e.s.r. spectrum of the vitamin K_1 radical is shown in the upper portion of Fig. 2, together with its line assignment shown to the right, which requires a splitting of 0.55 gauss for the effect of the added ring protons. The line assignment chosen thus yields the observed number of lines, a line separation close to that expected, and a ratio of line intensities which agrees with the integrated spectrum observed as follows:

Line No.	1	2	3	4	5	6	7	8	9	10	11 (center)
Observed:	5	10	15	21	30	46	65	91	107	119	123
Predicted:	3	9	17	29	37	53	71	91	102	106	106

It is of interest to note that these line assignments corroborate the view that the odd electron is fairly well localized to the aromatic nucleus of these radicals, and that it does not interact strongly with the aliphatic chain beyond the first methylene group. Improved instrumental resolution may show weaker interactions with protons further out the aliphatic chains and remove the equivalency of the ring protons in the vitamin K_1 semiquinone, but it is clear that the strong electron-proton interactions are those immediately associated with the aromatic nucleus. It would also seem probable that the

behavior of the odd electron is little affected whether the aliphatic portion is isoprenoid or a phytol, or whether this chain is lengthened or shortened.

In a sense then, and referring to the function of electron transport, one might think of the aromatic part as the "prosthetic" group of these molecules.

REFERENCES

Adams, M., Blois, M. S., Jr., and Sands, R. H., J. Chem. Phys., 28, 774 (1958).

Blois, M. S., Jr. and Sands, R. H., Proc. First National Biophysics Conference--1957, p. 97, Yale Univ. Press, 1959.

Ingram, D.J.E., Free Radicals as Studied by Electron Spin Resonance, Academic Press (1958).

Venkarataman, B. and Fraenkel, G. K., J. Chem. Phys., 23, 588 (1955).

Wertz, J. E. and Vivo, J. L., J. Chem. Phys., 24, 479 (1956).