SEMIQUINONE PHOSPHATE: AN OXIDATION INTERMEDIATE OF QUINOL PHOSPHATES*

A. Bond** and H. S. Mason

Department of Biochemistry, University of Oregon Medical School, Portland

Received November 19, 1962

High transfer potential phosphate intermediates are formed during the oxidation of quinol monophosphates (1,2) and it has been suggested that the intermediates are semiquinone phosphates (3). We have been studying phosphate activation by one-electron transfer; in the present paper we report the detection by electron spin resonance spectroscopy of a semiquinone phosphate.

A Varian V-4500 spectrometer system with 100 kc field modulation and appropriate field strength and klystron frequency monitors were used. Two sets of conditions were employed: (a) the sample was prepared by rapid mixing and rapid freezing, using a modification of the apparatus of Bray (4); and (b) the sample was prepared by mixing reagents with a syringe drive, then examined at room temperature during constant flow.

The peroxidatic oxidation of 2,3-dimethyl-1,4-naphthoquinol-1-phosphate*** produced 2,3-dimethylnaphthoquinone rapidly and almost quantitatively. Pyrophosphate was detected chromatographically as a reaction product. Since peroxidatic oxidations occur by a one-electron transfer mechanism (5), it appears likely that semiquinone phosphate was involved in the reaction. Under

---

* This study was supported by grants from the American Cancer Society and the United States Public Health Service (AM-971).

** Predoctoral Fellow of the United States Public Health Service.

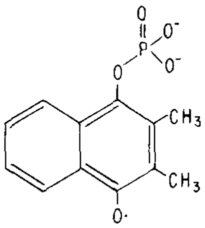
*** We are greatly indebted to the Hoffman-LaRoche Company for the quinol phosphates used in this study.

optimum conditions (peroxidase, 1×10^{-6} M; naphthoquinol phosphate, 1×10^{-2} M; H_2O_2, 5.5×10^{-4} M; pH 4.5 in 0.1 M succinate buffer) a small free radical signal was obtained from samples frozen to -165° within 20 msec after mixing, but no hyperfine structure was observed and the radical could not be identified. Similar results were obtained when Ce^{+4} in dilute H_2SO_4 was employed as an oxidant. This reagent also acts by one-electron transfer.

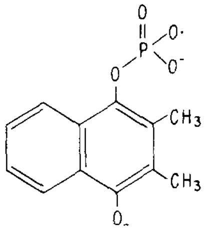
On the assumption that the decay rate of a semiquinone phosphate would decrease with increasing pH, 2,3-dimethyl-1,4-naphthoquinol-1-phosphate was oxidized by alkaline permanganate (substrate, 0.017 M; $KMnO_4$, 0.006 M; Na_2CO_3, 0.55M; pH, 10.95). A sample quenched to -165°, 150 msec after mixing, gave a strong ESR signal but at the temperature of observation only the major splittings were observed.

The room temperature experiment was carried out under the same conditions except that the sample was delivered at a constant rate to a flow cell in the spectrometer cavity, 140 msec after mixing. A highly resolved signal was obtained, 51.9 gauss wide, $g = 2.004$. This signal was different from the spectrum of 2,3-dimethylnaphtho-1,4-semiquinone previously observed by Wertz and Vivo (6) and confirmed by us. The radical had a half-life of about 3 seconds under the reaction conditions we employed.

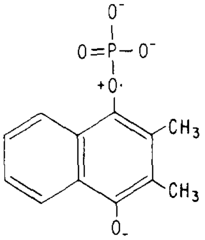
Several canonical forms may contribute to the structure of 2,3-dimethylnaphtho-1,4-semiquinone-1-phosphate, for example, structures I, II, and III. Our analysis of the spectrum suggests that the unpaired electron interacts strongly with the phosphorus atom, and that structure III is a principal contributor. The P^{31} nucleus, with spin = 1/2, causes a predominant doublet splitting (Figure 1, energy diagram) of 18.5 gauss. The three methyl protons in the 2-position cause a four-line secondary splitting of 7.8 gauss with relative intensities of 1-3-3-1, the two quartets overlapping and producing the overall shape of the integrated signal (Figure I). Each of these four lines is split into four tertiary lines with a separation of 1.3 gauss, by the three methyl protons in the 3-position. Finally, the four protons on the adjacent ring interact to produce a five-line splitting. In all, a 160-line spectrum should occur, 46 of which are apparent at the resolution used in these experiments.



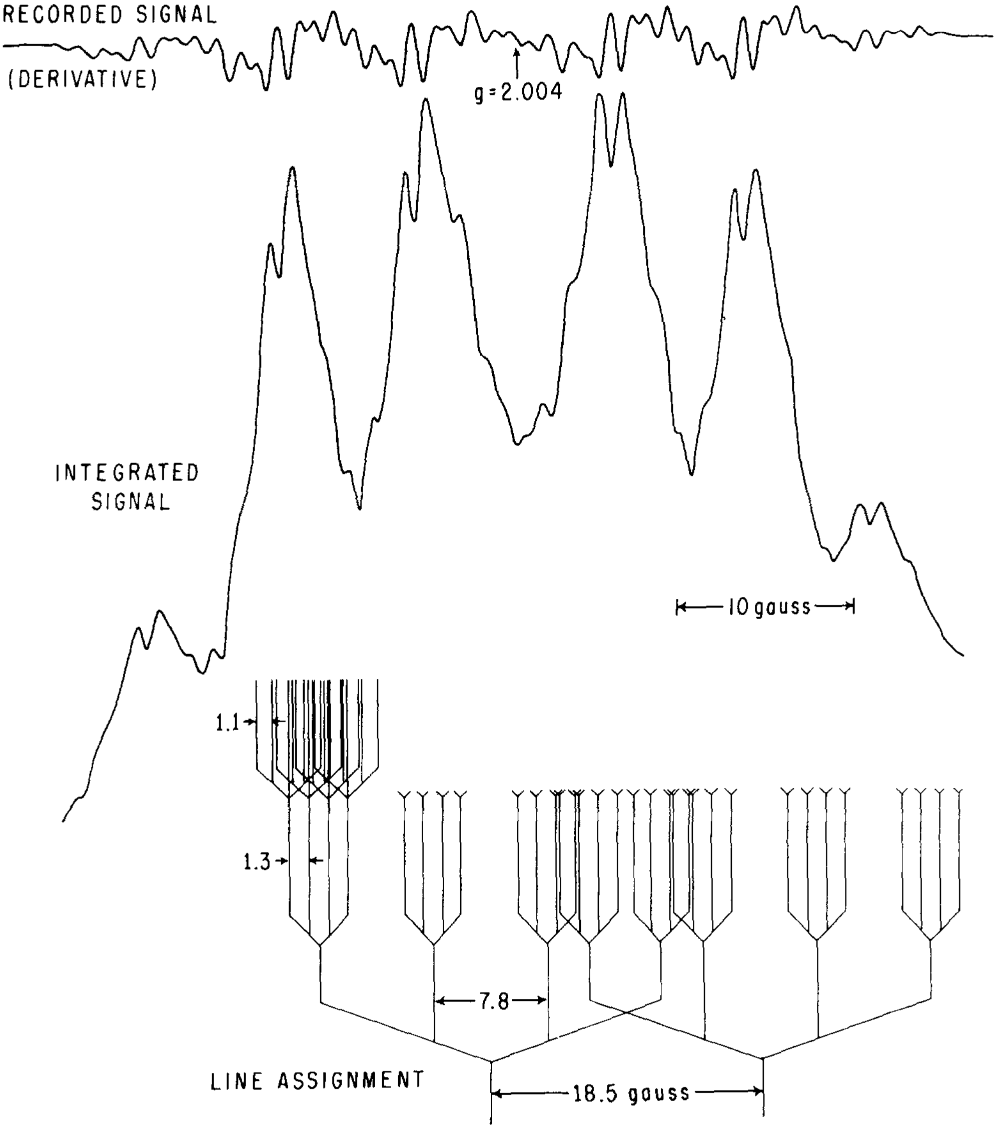
I



II



III



Only the 2,3-dimethylnaphtho-1,4-semiquinone-1-phosphate signal appeared during the course of the reaction; its decay was not accompanied by the appearance of any other free radical. We are now investigating the mechanism of decay of this and other oxidatively-formed semiquinone phosphates.

References

1. V. M. Clark, G. W. Kirby, and A. R. Todd, Nature 181, 1650 (1958).
2. V. M. Clark, D. W. Hutchinson, and A. R. Todd, Nature, 187, 59 (1960).
3. K. Harrison, Nature 181, 1131 (1958).
4. R. C. Bray, Biochem. J., 81, 189 (1961).
5. I. Yamazaki, H. S. Mason, and L. Piette, J. Biol. Chem., 235, 2444 (1960).
6. J. E. Wertz and J. L. Vivo, J. Chem. Phys., 24, 479 (1956).