Tetrahedron Letters,Vol.24,No.46,pp 5099-5102,1983 0040-4039/83 $3.00 + .00
Printed in Great Britain

ESR STUDIES OF VITAMIN K_1 CHROMANOXYL AND CHROMENOXYL RADICALS

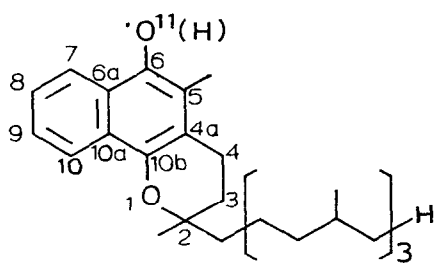
Kazuo MUKAI,* Chie MORIMOTO, and Kazuhiko ISHIZU
Department of Chemistry, Faculty of Science, Ehime University,
Matsuyama 790, Japan

The ESR spectra of vitamin K_1 chromanoxyl and chromenoxyl radicals were observed by oxidizing the phenol precursors with PbO_2 in toluene, and the proton hyperfine splittings and g_{iso}-values were corfectly determined.

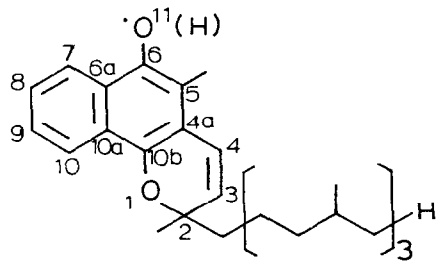
The importance of vitamin K in a variety of biological processes such as blood coagulation, oxidative phosphorylation, and electron transport has been well recognized in recent years.[1-3] The photolysis of vitamin K_1 in ethanol solution under anaerobic conditions has been investigated and the primary product was observed to be its chromenol.[4,5] The chromanol derivative of vitamin K_1 was identified as product of the enzymatic reduction of vitamin K_1.[3] Recently, Wilson et al. have studied the tetraphenylporphin-sensitized photooxidation of vitamin K chromanol and chromenol derivatives.[6]

In the present paper, ESR measurements were performed for the vitamin K_1 chromanoxyl and chromenoxyl radicals produced by the PbO_2 oxidation of corresponding chromanol and chromenol in toluene. The proton hyperfine splitting constants were correctly determined for each radical. From the results, the electronic structure of the vitamin K_1 chromanoxyl and chromenoxyl radicals have been discussed. These radicals may exist as reaction intermediates in the above biological processes and/or photooxidation reactions.

The vitamin K_1 chromanol and chromenol were prepared according to the method of Fujisawa et al.[4] and Wilson et al.,[6] respectively. The vitamin K_1 chromanoxyl and chromenoxyl radicals are not so stable, and, thus, the ESR



Vitamin K_1 chromanoxyl and chromanol



Vitamin K_1 chromenoxyl and chromenol

observations were performed on several samples under slightly different conditions of oxidation, varying the concentration of the chromanol and chromenol and the amount of PbO_2. All the ESR spectra have been measured in a sealed, degassed system.

The ESR spectrum of vitamin K_1 chromanoxyl radical measured at 20°C is given in Fig. 1(a). The spectrum can be reconstructed with three groups of 3, 4 and 2 equivalent protons, showing three different hyperfine splittings (8.06, 1.68 and 0.42 G), respectively. The largest quartet splitting (8.06 G) was easily assigned to methyl protons ($a_5^{CH_3}$). The assignment of the other two splittings has been performed by a comparison of experimentally obtained hyperfine splittings with calculated ones (see Table I), as described later. The tentative assignments are as follows: the larger quintet splitting (1.68 G) was attributed to two equivalent β-methylene protons ($a_{4a}^{CH_2}$) plus H-8 and H-10 ring protons ($a_8^H=a_{10}^H$), and the other triplet (0.42 G) to H-7 and H-9 ring protons ($a_7^H=a_9^H$).

The ESR spectrum of the vitamin K_1 chromenoxyl radical in toluene at 20°C is shown in Fig. 1(b). The spectrum consists of three groups of hyperfine splittings (7.21 (quartet), 1.88 (triplet), and 0.43 (quintet) G). The largest quartet splitting (7.21 G) was easily attributed to methyl protons ($a_5^{CH_3}$). The assignments of the remaining hyperfine splittings were made by comparison with the McLachlan MO calculations (see Table I). The results are as follows: $a_8^H=a_{10}^H=1.88$ G and $a_3^H=a_4^H=a_7^H=a_9^H=0.43$ G. The proton hyperfine splittings of these K_1 chromanoxyl and chromenoxyl radicals, together with the g_{iso}-values of these radicals, are listed in Table I.

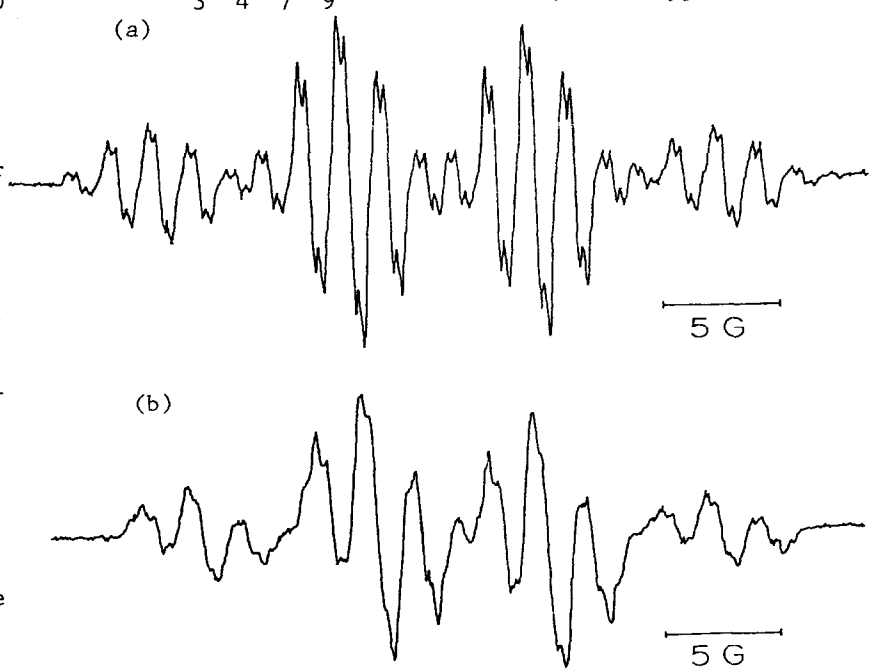


Fig. 1. (a) ESR spectrum of vitamin K_1 chromanoxyl radical in toluene at 20°C. (b) ESR spectrum of vitamin K_1 chromenoxyl radical in toluene at 20°C.

Assuming that the McConnell equations, $a_i^H=Q^H \cdot \rho_i^\pi$ and $a_i^{CH_3}=Q^{CH_3} \cdot \rho_i^\pi$, hold and that $Q^H=-27$ and $Q^{CH_3}=27$ G, the experimental value of the spin density (ρ_i^π) at the i th carbon atom was estimated.[7] In the case of the

Table I. Hyperfine Splittings (a_i^H) (in Gauss), g_{iso}-Values, and Spin Densities (ρ_i^π) of the Vitamin K_1 Chromanoxyl and Chromenoxyl Radicals in Toluene.

Chromanoxyl	a_5^{CH3}	a_8^H	a_{10}^H	a_7^H	a_9^H	a_{4a}^{CH2}	g_{iso}
ESR	8.06[a]	1.68	1.68	0.42	0.42	1.68	2.00427[b]
ρ(Exptl.)	0.2985	0.0622	0.0622	0.0156	-0.0156	-0.0415[c]	
ρ(Calcd.)	0.2655	0.0569	0.0663	0.0053	-0.0020	-0.0351[c]	

Chromenoxyl	a_5^{CH3}	a_8^H	a_{10}^H	a_7^H	a_9^H	a_4^H	a_3^H	g_{iso}
ESR	7.21[a]	1.88	1.88	0.43	0.43	0.43	0.43	2.00421[b]
ρ(Exptl.)	0.2670	0.0696	0.0696	0.0159	-0.0159	-0.0159	0.0159	
ρ(Calcd.)	0.2639	0.0535	0.0623	0.0070	-0.0000	-0.0182	0.0196	

[a]Experimental errors ±0.04 G. 1 G = 10^{-4}T. [b]Experimental errors ±0.00005. [c]Spin densities at C-4a.

K_1 chromanoxyl radical, one may expect that a rotation of the β-methylene residue would be largely restricted by its strong strain. As the result of the analysis of the ESR spectrum indicates, the two β-methylene protons of the K_1 chromanoxyl radical have an equivalent hyperfine splitting within the present resolution. Thus, the dihedral angle must be equivalent for the two β-protons, giving the value of $\theta=30^\circ$. Consequently, the experimental value of the spin density at C-4a carbon atom was estimated using the Heller-McConnell's equation,[8] $a_{4a}^{CH2}=54\cos^2 30^\circ \times \rho_{4a}^\pi$. All the experimental spin densities ρ(Exptl.) calculated from the ESR hyperfine splittings are listed in Table I. As is clear from the results listed in Table I, the hyperfine splittings obtained for the vitamin K_1 chromanoxyl and chromenoxyl radicals in toluene are similar to each other, except for those of heterocyclic ring. The g_{iso}-values of the K_1 chromanoxyl and chromenoxyl also show a good agreement with each other within the limits of experimental error, suggesting that these radicals have similar electronic structure each other.

MO calculations of vitamin K_1 chromanoxyl and chromenoxyl radicals have not been reported, as far as we are aware. Therefore, it is of interest to see how our results could be rationalized in terms of MO calculations. Numerous McLachlan MO calculations were made to obtain reasonable agreement with the experimental spin densities, and the final values of the MO parameters adopted are as follows: $\alpha_{O11}=\alpha+1.3\beta$, $\beta_{C_6\text{-}O11}=1.5\beta$; $\alpha_{O_1}=\alpha+2.0\beta$, $\beta_{C_{10b}\text{-}O_1}=1.0\beta$; and $\alpha_{C_5}=\alpha_{C_{4a}}=\alpha-0.1\beta$. The McLachlan MO parameter λ was given the standard value of 1.2. These parameters are the same as those used previously for the vitamin E α-chromanoxyl radical.[9] The McLachlan spin densities (ρ_i^π) calculated with these parameters are given in Table I. The

results of McLachlan MO calculations were found to be in satisfactory agreement with the 'experimental' spin densities evaluated from the hyperfine splitting constants, except for the C-7 and C-9 carbon atoms of small spin density.

The importance of tocopherols (vitamin E) as natural antioxidants, particularly in biomembranes with respect to lipid peroxidation, has been well recognized in recent years. The antioxidant properties of the tocopherols have been ascribed to the initial oxidation of the phenolic hydroxyl group, producing corresponding chromanoxyl radicals. The above chromanoxyl radicals formed in the first oxidation step of tocopherols are relatively stable, and are detectable at room temperature by ESR.[10] The present vitamin K_1 chromanol and chromenol have a structure similar to that of vitamin E chromanol, producing corresponding chromanoxyl and chromenoxyl radicals, as described above. Therefore, we can expect that the K_1 chromanol and chromenol function as an efficient inhibitor of lipid peroxidation, but there is no report regarding their absolute antioxidant effectiveness both in vivo and vitro.

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(Received in Japan 11 July 1983)