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ESR STUDIES OF COENZYME Q_1 CHROMANOXYL AND CHROMENOXYL RADICALS

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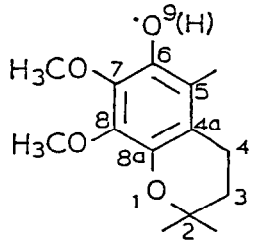
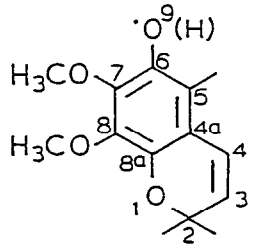
ESR measurements were performed for the coenzyme Q_1 chromanoxyl and chromenoxyl radicals obtained by oxidizing the phenol precursors with PbO_2 in toluene, and the proton hyperfine splittings were correctly determined.

The participation of coenzyme Q (ubiquinone) in a variety of biological processes such as electron transport and oxidative phosphorylation has been well established in recent years.[1,2] It was reported that coenzyme Q was converted to coenzyme Q chromenol in good yield with an aliphatic tertiary amine or by photochemical reaction.[3] The chromanol derivatives of coenzyme Q were prepared by refluxing the coenzyme Q in acetic acid with stannous chloride.[4] The biological activity of these chromanols and chromenols in vitamin E-deficient animals has been studied by several investigators.[5,6] For instance, the chromanol of hexahydrocoenzyme Q_4 was found to prevent encephalomalacia in the chick, the resorption-gestation syndrome in the vitamin E-deficient rat, and to cure nutritional muscular dystrophy in the rabbit. The coenzyme Q_{10} chromenol was reported active in the resorption-gestation syndrome in the vitamin E-deficient rat. It was reported that vitamin K_1 chromanol also show a high degree of vitamin E activity in the gestation-resorption syndrome in the rat and in the encephalomalacia in the chick.[7] In a previous paper, we studied the ESR spectra of the vitamin K_1 chromanoxyl and chromenoxyl radicals obtained by the PbO_2 oxidation of corresponding chromanol and chromenol in toluene at room temperature.[8]

In the present paper, ESR measurements were performed for the coenzyme Q_1 chromanoxyl and chromenoxyl radicals produced by the PbO_2 oxidation of the corresponding chromanol and chromenol in toluene under vacuum. The proton hyperfine splitting constants were correctly determined for each radical. From the results, the electronic structure of the Q_1 chromanoxyl and chromenoxyl radicals have



Coenzyme Q_1 chromanoxyl and chromanol



Coenzyme Q_1 chromenoxyl and chromenol

been discussed. When one compares the chemical structures of the vitamin E α-chromanoxyl and the chromanol derivative of hexahydrocoenzyme Q_4, it is seen that they are identical, except for the interchange of two methyl and two methoxy groups in the 7 and 8 positions. Therefore, these radicals may exist as reaction intermediates in the above biological processes, as described later.

The coenzyme Q_1 chromanol was prepared by refluxing the coenzyme Q_1 in acetic acid with excess stannous chloride for 30 minutes.[4] The reaction mixture was concentrated under reduced pressure to dryness. The residue was taken up in diethyl ether, washed with water, and dried over anhydrous sodium sulfate. After removal of the diethyl ether, the solid remained; this was recrystallized from petroleum ether (bp. 60-70°C) to give white crystals: mp. 92-94°C; UV (EtOH) λ_{max} 293 nm (ε=3480); NMR (CCl_4) δ 1.31 (6H, s, 2-C$(CH_3)_2$), 1.76 (2H, triplet, J=6.7 Hz, 3-CH_2), 2.01 (3H, s, 5-CH_3), 2.54 (2H, triplet, J=6.7 Hz, 4-CH_2), 3.72 (3H, s, 7-CH_3O), 3.86 (3H, s, 8-CH_3O), 5.12 (1H, s, OH) with Me_4Si as internal standard. Anal. Calcd for $C_{14}H_{20}O_4$: C, 66.65; H, 7.99. Found: C, 66.05; H, 8.09. The coenzyme Q_1 chromenol was prepared by refluxing the coenzyme Q_1 in triethylamine.[3]

The ESR spectrum of coenzyme Q_1 chromanoxyl radical measured at 20°C is given in Fig. 1(a). The radical is not stable, and the radical concentration decreases rapidly with time. Therefore, the intensity ratios of the ESR absorption deviate from those expected theoretically. The spectrum can be reconstructed with four groups of 3, 2, 3, and 4 equivalent protons, showing four different hyperfine splittings (5.70, 1.02, 0.41, and 0.10 G), respectively. These splittings are tentatively assigned to the methyl protons at C-5 ($a_5^{CH_3}$=5.70 G), two equivalent β-methylene protons at C-4 ($a_4^{CH_2}$= 1.02 G), methoxy protons at C-7 ($a_7^{CH_3O}$=0.41 G), methoxy protons at C-8

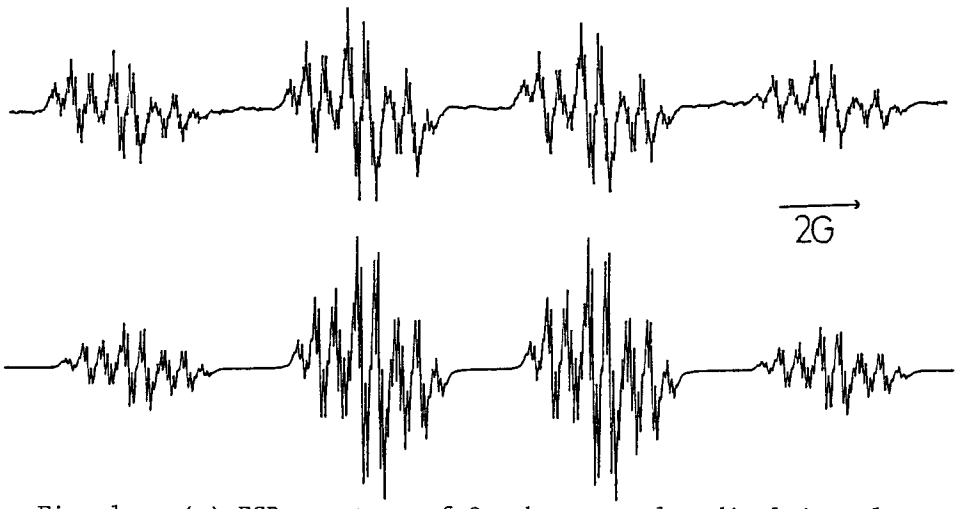


Fig. 1. (a) ESR spectrum of Q_1 chromanoxyl radical in toluene at 20°C. (b) Computer simulation of the spectrum in (a) using hyperfine splittings reported in Table I.

($a_8^{CH_3O}$=0.10 G), and one of two γ-methylene protons at C-3 ($a_3^{CH_2}$=0.10 G). A computer simulation of the ESR spectrum employing the above splitting constants of Q_1 chromanoxyl is shown in Fig. 1(b), and it is comparable with the experimental spectrum.

The ESR spectrum of the coenzyme Q_1 chromenoxyl radical in toluene at 20° C is shown in Fig. 2(a). The spectrum consists of three groups of hyperfine splittings (4.57 (quartet), 0.62 (quintet), and 0.16 (quartet) G). These couplings will be assigned to the methyl protons at C-5 ($a_5^{CH_3}$=4.57 G), methoxy protons at C-7 ($a_7^{CH_3O}$=0.62 G), methine proton at C-4 (a_4^{H}=0.62 G), and methoxy protons at C-8 ($a_8^{CH_3O}$=0.16 G). The spectrum is reproduced by a computer-generated spectrum employing the above coupling constants of Q_1 chromenoxyl (see Fig. 2(b)). The proton hyperfine splittings of these Q_1 chromanoxyl and chromenoxyl radicals are listed in Table I.

The experimental values of spin densities (ρ_i^{π}) were estimated using the relations, $a_i^{H}=27\rho_i^{\pi}$, $a_i^{CH_3}=27\rho_i^{\pi}$, $a_i^{CH_3O}=3.17\rho_i^{\pi}$,[9] and $a_{4a}^{CH_2}=54\cos^2 30°\times\rho_{4a}^{\pi}$, as performed for vitamin K_1 chromanoxyl and chromenoxyl radicals.[8] All the experimental spin densities ρ(exptl.) calculated from the ESR hyperfine splittings and g_{iso}-values are summarized in Table I. MO calculations of coenzyme Q 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 McLachlan MO calculations. The values of the MO parameters used are listed in Table I.[8-10] The 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.

It is well known that tocopherols (vitamin E) are localized in biomembranes and have functions as efficient inhibitors of lipid peroxidation. The potent antioxidant properties of the tocopherols have been ascribed to the oxidation reaction of the phenolic hydroxyl group with the production of the corresponding chromanoxyl radicals. The above chromanoxyl radicals are relatively stable, and are detectable at room

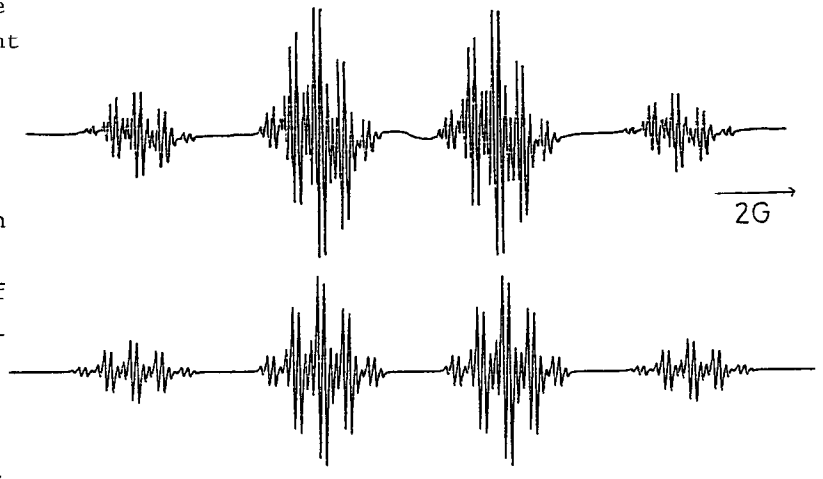


Fig. 2. (a) ESR spectrum of Q_1 chromenoxyl radical in toluene at 20°C. (b) Computer simulation of the spectrum in (a) using hyperfine splittings reported in Table I.

Table I. Hyperfine Splittings (a_i^H) (in Gauss), g_{iso}-Values, and Spin Densities (ρ_i^π) of the Coenzyme Q_1 Chromanoxyl and Chromenoxyl Radicals in Toluene.

Chromanoxyl	$a_5^{CH_3}$	$a_7^{CH_3O}$	$a_8^{CH_3O}$	$a_4^{CH_2}$	$a_3^{CH_2}$	g_{iso}
ESR	5.70[a]	0.41	0.10	1.02	0.10	2.00476[b]
ρ(Exptl.)	0.2111	0.1293	-0.0322	-0.0252[c]		
ρ(Calcd.)	0.2105	0.2105	-0.0336	-0.0336[c]		
Chromenoxyl	$a_5^{CH_3}$	$a_7^{CH_3O}$	$a_8^{CH_3O}$	a_4^H		g_{iso}
ESR	4.57[a]	0.62	0.16	0.62		2.00480[b]
ρ(Exptl.)	0.1693	0.1956	-0.0505	-0.0230		
ρ(Calcd.)	0.2024	0.2095	-0.0342	-0.0184		

[a]Experimental errors ±0.03 G. 1 G = 10^{-4}T. [b]Experimental errors ±0.00003. [c]Spin densities at C-4a. [d]MO parameters: $\alpha_{O_9}=\alpha+1.3\beta$, $\beta_{C_6\text{-}O_9}=1.5\beta$; $\alpha_{O_1}=\alpha+2.0\beta$, $\beta_{C_{8a}\text{-}O_1}=1.0\beta$; $\alpha_{C_5}=\alpha_{C_{4a}}=\alpha_{C_7}=\alpha_{C_8}=\alpha-0.1\beta$; and $\lambda=1.2$.

temperature by ESR.[10,11] The present coenzyme Q_1 chromanol and chromenol have a structure similar to that of vitamin E chromanol, producing corresponding chromanoxyl and chromenoxyl radicals, as described above. In fact, the coenzyme Q_1 chromanol and chromenol show biological activity like that of tocopherols in vitamin E deficient animals, as reported by several investigators.[5,6]

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