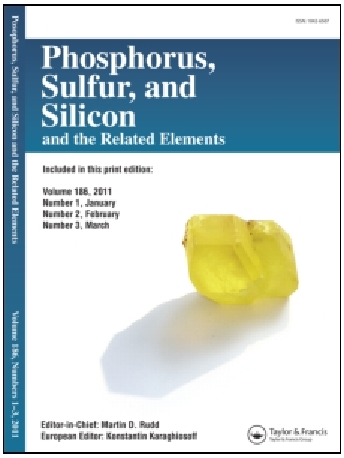

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SHORT COMMUNICATION

An Electron Spin Resonance Study of the Reactions of Dialkyl Phosphites and Pyrophosphite with Vitamin C: The Phosphonyl Ascorbate Radical Adduct

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The property of vitamin C in scavenging phosphonyl free radicals to yield persistent radical adducts is demonstrated and the structure of the phosphonyl–ascorbate radical is elucidated by electron spin resonance spectroscopy.

INTRODUCTION

The chemistry of vitamin C, L-ascorbic acid, has continued to receive much attention. The antioxidant property of vitamin C in some biological systems has been attributed to the ease of formation and the stability of the ascorbate radical anion which can be directly observed by electron spin resonance.[1–3] In enzymatic peroxidation systems, for example, dopamine β-mono-oxygenase releases ascorbate free radical as a product of the ascorbate oxidation.[4,5] While the primary role of vitamin C in lipid peroxidation is to scavenge the oxygen (alkoxy) radicals, which are the chain carrying intermediates in biological damage, little is known about its reactions with secondary lipid radicals. This report describes the competitive reactions of alkoxy radicals with vitamin C and dialkyl phosphites and pyrophosphites as well as the secondary free radical reactions between vitamin C and the oxidized phosphonyl radicals. The direct observation by electron spin resonance of the secondary radical adduct permits the analysis of the radical structure and the identification of the active reaction site in vitamin C towards phosphonyl radicals.

RESULTS AND DISCUSSION

When vitamin C was sparingly dissolved in liquid tert-butyl peroxide and the solution was subjected to UV irradiation or exposure to heat, the ascorbate radical anion with a typical proton splitting of 1.8 gauss was observed. The ascorbate radical anion was formed from the scavenging of the tert-butoxy radicals:

$$\mathrm{Me_3COOCMe_3} \xrightarrow{h\nu/\mathrm{heat}} 2\,\mathrm{Me_3C\dot{O}}, \qquad (1)$$

$$\mathrm{Me_3C\dot{O}} + \mathrm{AH^-}\ (\mathrm{ascorbate}) \rightarrow \mathrm{A^{\dot{-}}} + \mathrm{Me_3COH}. \qquad (2)$$

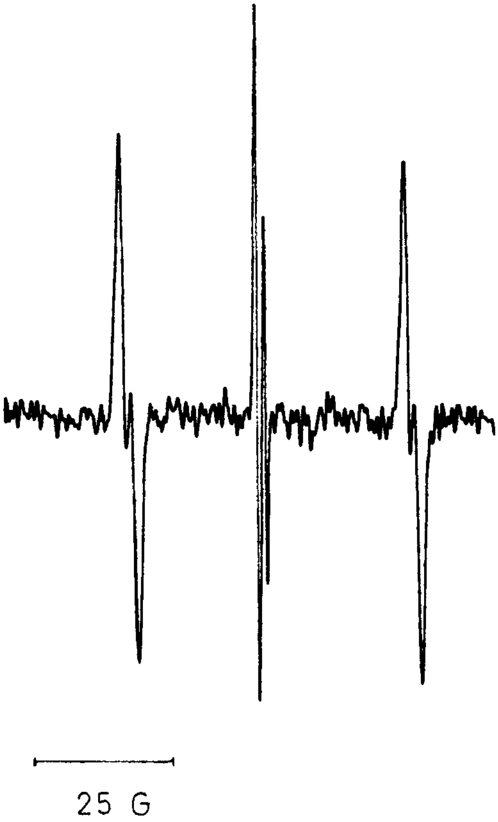


FIGURE 1 The e.s.r. spectrum at room temperature observed during the photolysis of diethyl phosphite solution containing vitamin C and *t*-butyl peroxide.

When vitamin C was dissolved in liquid diethyl phosphite, no radical formation was observed. However, upon the addition of a small amount of tert-butyl peroxide which initiated the photochemical or thermal oxidation processes, two major radical species were immediately observed within the e.s.r. cavity. A typical e.s.r. spectrum of the radicals formed is shown in Figure 1. The central doublet, having a splitting of 1.77 gauss is clearly the ascorbate radical anion. The other more intense spectrum which is a doublet of a smaller doublet can be interpreted according to a large ^{31}P splitting ($a_P = 53.2$ gauss) and a smaller proton splitting ($a_H = 1.6$ gauss). It was also apparent upon close examination that a very small doublet due to another proton with splitting less than 1 gauss was not completely resolved. In order to assign the proton couplings for this new radical species, vitamin C was repeatedly recrystallized in D_2O so that all the hydroxylic protons were deuterated. The reaction of the treated vitamin C with diethyl phosphite and tert-butyl peroxide gave an e.s.r. spectrum shown in Figure 2. A significant change is the disappearance of the splitting assigned to the C-3 proton of the radical adduct (see structure below). However, the unresolved proton splitting remained. These results led to the conclusion that the radical has the Structure I with the 1.6 gauss splitting due to the OH proton on C-3 and the partially resolved coupling presumably due to H-4.

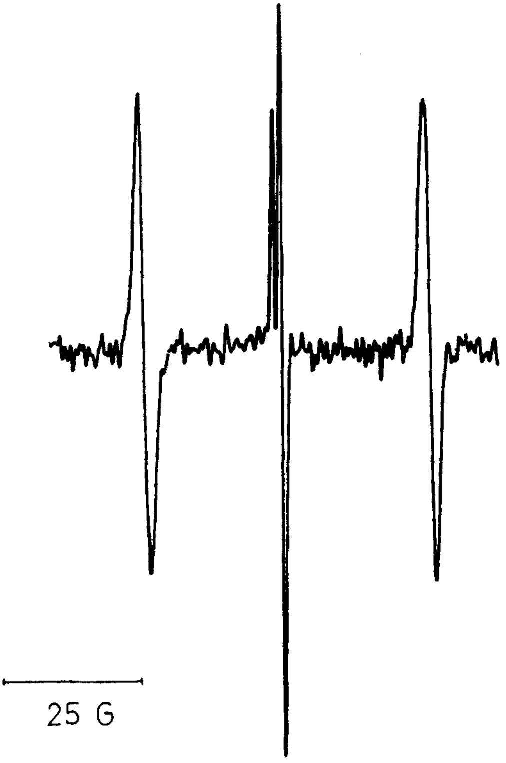
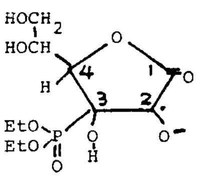
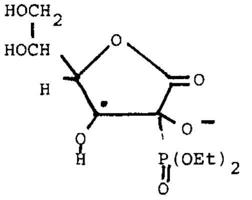


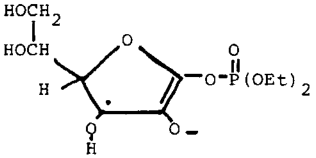
FIGURE 2 The e.s.r. spectrum at room temperature observed during the photolysis of diethyl phosphite solution containing partially deuterated (OD) vitamin C and *t*-butyl peroxide.



I



II



III

The alternative Structures **II** and **III** can be discarded because of the absence of a large (> 20 gauss) splitting due to the H-4 proton which would be expected for the radicals **II** and **III**.[6] Moreover, the hyperfine coupling constant of the ^{31}P nucleus is consistent with those observed when the diethyl phosphonyl radical was added to the C=C double bond of maleic anhydride.[7] The results also identify the active reaction site as the C=C double bond in vitamin C towards the scavenging of phosphonyl radicals. The formation of this radical **I** is readily understood by the following reactions:

$$Me_3\dot{O} + (RO)_2P(O)H \rightarrow Me_3COH + (RO)_2\dot{P}{=}O, \tag{3}$$

$$(RO)_2\dot{P}{=}O + AH^- \rightarrow \text{Radical } \dot{\mathbf{I}}. \tag{4}$$

Reaction (3) has been extensively studied by Ingold and coworkers.[8] In the present system, vitamin C was only slightly soluble in diethyl phosphite and therefore competitive reactions of the alkoxy radicals would have favored Reaction (3), as indicated by the higher concentration (large signal intensity) of radical adduct **I**. The property of vitamin C in scavenging phosphonyl radicals to yield persistent phosphonyl–ascorbate radical adducts is extremely interesting in biological systems involving peroxidation of phospholipids.

Similar results were observed in the reactions with either dimethyl phosphite or diethyl pyrophosphite. In the case of the pyrophosphite, the phosphonyl radical was generated by the following reaction with tert-butyl peroxide:

$$\mathrm{Me_3C\dot{O} + (EtO)_2P{-}O{-}P(EtO)_2 \rightarrow (EtO)_2\dot{P}{=}O + (EtO)_2POCMe_3}. \qquad (5)$$

In the dimethyl phosphonyl-ascorbate radical adduct, the phosphorus splitting is slightly larger, $a_P = 58.4$ gauss. This is consistent with the parent phosphonyl radical series in which the dimethyl phosphonyl radical has the largest splitting of 700 gauss, compared with the value of 687 gauss for the diethyl phosphonyl radical.[9] In biological systems containing mixed phosphite groups, the magnitude of the phosphorus splitting could be used to identify the reactive phosphite.

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