

Figure 2. Cross sections through the  $^{13}\text{C}$  signals of MeLeu<sup>10</sup> in the plot of Figure 1. The proton assignments are indicated at the signals.

amino acid can be assigned by their coupling to identical protons. For example, the seven *N*-methyl carbons exhibit strong cross peaks to the methyl protons ( $^1J_{\text{CH}}$ ) as in a H,C-COSY spectrum. In addition, cross peaks to the  $\alpha$ -protons of the corresponding amino acids are observed ( $^3J_{\text{CH}}$ ) in the H,C-COLOG spectrum. Similarly, at the  $\alpha$ -carbons cross peaks to the  $\alpha$ -protons ( $^1J_{\text{CH}}$ ) and to the *N*-methyl protons ( $^3J_{\text{CH}}$ ) occur. Hence connectivity information of protons and carbons is obtained as indicated in Figure 1 for MeVal<sup>11</sup>.

Due to the complexity of the spectrum it is convenient for further interpretation to analyze cross sections through the carbon resonances. As a representative example we show those of the MeLeu<sup>10</sup> residue (Figure 2). As mentioned above, the  $\alpha$ -carbon exhibits cross peaks to the *N*-methyl protons and the  $\alpha$ -proton. Further signals are found at the position of both  $\beta$ -protons and the  $\gamma$ -proton. This is almost the complete spin system, projected on the  $\alpha$ -carbon. The missing  $\delta$ -protons are already found at the resonance of the  $\beta$ -carbon together with a cross peak to the  $\alpha$ -proton and to both  $\beta$ -protons. The  $\gamma$ -carbon shows a weak cross peak to the  $\alpha$ -proton in addition to peaks to the  $\gamma$ -proton as well as to the  $\delta$ -methyl protons. The  $\delta$ -carbons exhibit cross peaks only to directly attached protons. The coupling pattern of all other amino acids can be traced out in the same way.

Although not all two- and three-bond couplings are detected in the spectrum, it is evident that it contains so much redundant information that in general it appears to be the most informative technique for the determination of proton and carbon connectivities.

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## An Unusual CIDEP Observation in the Photochemical Reactions of Benzophenone and Ascorbyl Palmitate: The Elusive Neutral Ascorbate Radical

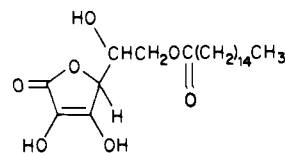
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The biochemical and biophysical roles of vitamin C (ascorbic acid) *in vivo* have received much attention in recent years.<sup>1</sup> In addition to its recognized antioxidant activity<sup>2</sup> this compound has been implicated as a potential therapeutic agent for treatment of ailments ranging from the common cold<sup>3</sup> to cancer.<sup>4</sup> With the establishment of the CIDEP technique as a powerful tool for mechanistic and kinetic studies of photochemical and thermal reactions,<sup>5</sup> the nature of the free radical processes involved in radiolysis<sup>6</sup> and photolysis<sup>7</sup> of vitamin C has been examined and explained by the conventional phototriplet and radical pair mechanisms. However, the relative insolubility of vitamin C has restricted its study to polar solvents.

There is currently considerable interest in the thermal and photochemical reactions of vitamin C derivatives.<sup>8</sup> Ascorbyl palmitate which has utility as an antioxidant in foodstuffs, par-

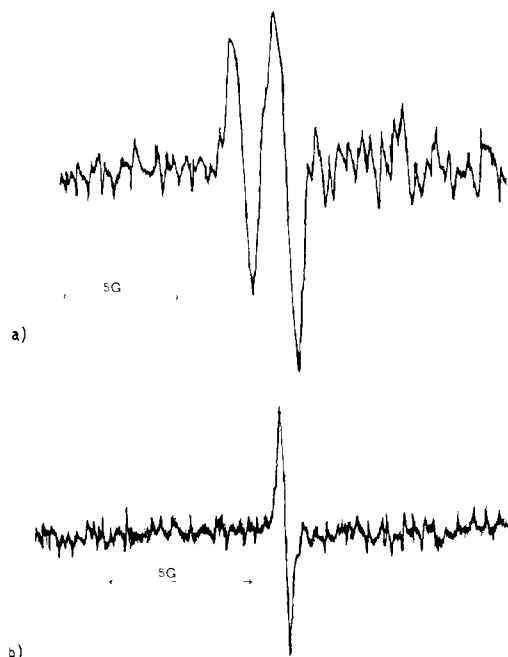


ticularly fats and oils,<sup>9</sup> is of interest as a model for ESR and CIDEP studies; its lipophilic hydrocarbon chain facilitates the study of reactions in nonpolar organic solvents. In this work we report a rather unusual CIDEP observation for the photochemical interaction of ascorbyl palmitate (H<sub>2</sub>AP) with several benzophenone derivatives in organic solvents. To our surprise one hyperfine component of the ascorbyl radical spectrum exhibited strong emission while the other component did not show any polarization. This phenomenon can be rationalized by phototriplet CIDEP theory<sup>5</sup> combined with secondary ionization of the elusive neutral HAP radical to the observed radical anion.

CW photolysis of an isopropyl alcohol/toluene (3:7 v/v) solution containing typically  $5 \times 10^{-2}$  M H<sub>2</sub>AP and benzophenone, respectively, produced an ESR spectrum as shown in Figure 1a. The central doublet is due to the ascorbate radical anion (AP<sup>-</sup>) with a *g* factor of 2.0051 and  $a_{\text{H}} = 1.69$  G.<sup>10,14</sup> The peripheral weaker lines in Figure 1a can be attributed to the benzophenone ketyl radical (Ph<sub>2</sub>ĊOH); this same radical could be produced by photolysis of benzophenone in the absence of H<sub>2</sub>AP.

As shown in Figure 1a the two hyperfine components of AP<sup>-</sup> differ dramatically in intensity. Similar results were obtained using 4,4'-dichloro-, 4,4'-dimethyl-, 4,4'-dimethoxybenzophenone with

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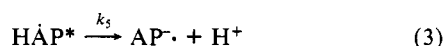
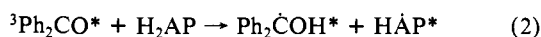


**Figure 1.** CW ESR spectra observed during the photolysis of benzophenone and ascorbyl palmitate (a) in IPA/toluene at room temperature and (b) in IPA/toluene solution containing 5% v/v H<sub>2</sub>SO<sub>4</sub>.

the largest intensity variation occurring in the latter case. Time-resolved CIDEP experiments (using technique described previously<sup>12</sup>) showed strong emissive polarization with a  $T_1$  of 4  $\mu$ s for the high-field component of AP<sup>-</sup> only and no polarization for the low-field component. Emissive polarization with a considerably longer  $T_1$  of 14  $\mu$ s was noted for Ph<sub>2</sub>COH. Lowering the temperature of substituting ethanol as the solvent did not alter the pattern of the polarization.

Addition of base (10% v/v NaOH) to the solution of H<sub>2</sub>AP and benzophenone resulted in a "normal" CW ESR spectrum having hyperfine lines of equal intensity for AP<sup>-</sup>. For this system time-resolved CIDEP experiments showed weak emissive polarization for both hyperfine lines. However, photolysis of a solution of H<sub>2</sub>AP and benzophenone containing 5% v/v H<sub>2</sub>SO<sub>4</sub> resulted in a single-line ESR spectrum (Figure 1b) having  $g$  factor 2.0048. In time-resolved experiments strong emissive polarization with  $T_1$  of 6  $\mu$ s was noted for this single line. These unusual CIDEP observations can be accounted for by considering some of the chemical reactions involved in such a system.

The relative proportions and pH dependence of the dissociated forms of H<sub>2</sub>A in solution, i.e., HA<sup>-</sup> and A<sup>2-</sup>, can be estimated spectrophotometrically.<sup>13</sup> Both H<sub>2</sub>A and H<sub>2</sub>AP are predominantly unionized in ethanol solution. ESR studies on radicals derived from ascorbic acid have demonstrated that A<sup>-</sup> is the most stable species formed over the pH range 0-9. However, Laroff, Fessenden, and Schuler<sup>10</sup> have observed the neutral radical H $\dot{A}$  with an estimated  $g$  factor of 2.00482 is very acidic solution (4 M HClO<sub>4</sub>). The  $pK_a$  of H $\dot{A}$  is thought to be  $\sim 0.4$ ,<sup>10</sup> thus rapid deprotonation to A<sup>-</sup> is expected in nonacidic media. However, in organic solvents H $\dot{A}P^*$  may dissociate to a lesser extent. The following reactions may explain the CW and CIDEP observations of the H<sub>2</sub>AP/benzophenone system in neutral, basic, and acidic solution:



The well-established phototriplet mechanism of CIDEP explains the formation of the emissively polarized benzophenone ketyl radical and H $\dot{A}P^*$  by hydrogen abstraction from H<sub>2</sub>AP by the polarized triplet benzophenone (eq 2). In neutral solution the

radical anion AP<sup>-</sup>, resulting from dissociation of H $\dot{A}P^*$  is unpolarized indicating that the first-order rate constant for deprotonation ( $k_5$ ) is less than  $1/T_1$  for H $\dot{A}P^*$  (i.e.,  $k_5 < 0.2 \times 10^6 \text{ s}^{-1}$ ). The anomalous emissive polarization on the high-field line of AP<sup>-</sup> is in fact a result of the presence of H $\dot{A}P^*$  which only partially overlaps the AP<sup>-</sup> spectrum due to the difference in their  $g$  values. This spectral overlap also accounts for the distortion of line intensities in the CW ESR spectrum (Figure 1a) since the H $\dot{A}P^*$  radical underlies only the high-field component of AP<sup>-</sup>. Upon addition of NaOH the deprotonation rate was greatly enhanced and the equilibrium was shifted entirely to the right, giving a "normal" CW ESR spectrum of AP<sup>-</sup>. If  $k_5$  is comparable to or greater than  $1/T_1$  of H $\dot{A}P^*$ , the resulting radical anion AP<sup>-</sup> will exhibit emissive polarization which will be observed on both hyperfine components; the magnitude of polarization retained will be dependent upon the relative magnitude of  $k_5$  to  $1/T_1$ . Conversely, addition of a strong acid may well shift the deprotonation equilibrium to the left, resulting in observation of the neutral radical H $\dot{A}P^*$  only in the CW spectrum. This radical exhibited strong emissive polarization in the time-resolved experiment as predicted by theory.

The results demonstrate the usefulness of the CIDEP technique in mechanistic studies. In the present case, the combination of the physical technique and chemical information led to a significant finding of the "elusive" neutral ascorbate radical as well as the nature of the primary photoreaction of benzophenone with ascorbates.

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#### Aggregation of a Bacteriochlorophyllide $d$ : Direct Evidence for a Nonsymmetric Aggregate in Solution

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Despite intense interest and activity, the molecular organization of the chlorophylls (Chls) and bacteriochlorophylls (BChls) in vivo remains open to question. A considerable body of spectroscopic data has provided evidence for dimerization, aggregation, and chlorophyll-protein interactions to account for the various BChl forms observed in vivo.<sup>3</sup> One may arbitrarily distinguish between the symmetric<sup>4</sup> and nonsymmetric models;<sup>5</sup> the latter include the Fong,<sup>6</sup> Shipman,<sup>7</sup> and synthetic<sup>8</sup> models for Chl and BChl reaction centers. We describe here the first direct experimental evidence for the nonsymmetric nature of a methyl bacteriochlorophyllide (BChlide; generically, a pyrochlorophyllide) dimer in solution and derive the detailed geometry of the dimer.

When BChlide  $d$  [Et,Et]<sup>9,10</sup> (1) is dissolved in CDCl<sub>3</sub>, an ex-

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