their useful comments in the course of this work. T.W.E. is grateful both for the Foreign Lectureship at the University of Tsukuba and to the National Science Foundation (Grant NSF INT 8407544), within the U.S.–Japan Program of Cooperation in Photoconversion and Photosynthesis, which made this collaboration possible. This work was in part supported by a Grant-In-Aid for Scientific Research to K.T. by the Ministry of Education, Science and Culture (Japan) and by the Office of Basic Energy Sciences of the U.S. Department of Energy. This is document No. NDRL-2948 from the Notre Dame Radiation Laboratory.

Registry No. *cis*-BPE^{2+}·2I$^-$, 101342-61-4; *trans*-BPE^{2+}·2I$^-$, 24274-78-0; BPE$^{\bullet+}$, 46740-62-9; O$_2$, 7782-44-7.

Carotenoid Cation Radicals: Electrochemical, Optical, and EPR Study

Janice L. Grant, Vincent J. Kramer,[†] Ruisong Ding, and Lowell D. Kispert*

Contribution from the Department of Chemistry, University of Alabama, Tuscaloosa, Alabama 35487-9671. *Received August 17, 1987*

Abstract: Cyclic voltammetry, absorption spectroscopy, and electron paramagnetic resonance spectroscopy (EPR) have been employed to study the production of carotenoid cation radicals via electrochemical means in various solvents [tetrahydrofuran (THF), dichloromethane (CH_2Cl_2), and dichloroethane ($C_2H_4Cl_2$)]. The electrooxidation of purified β-carotene, β-apo-8′-carotenal, and canthaxanthin in THF was found to proceed via a two-electron process. In the chlorinated solvents, the oxidation of β-carotene again occurred by the transfer of two electrons; in contrast, however, β-apo-8′-carotenal and canthaxanthin were oxidized via the transfer of only one electron. In all cases the apparent number of electrons transferred was found to be strongly dependent on the purity of the carotenoids. Electronic absorption measurements showed that the products generated at the first anodic peak potential have intense absorptions in the near-infrared (IR). Literature reports have shown that the only carotenoid oxidation products that absorb in this spectral region are carotenoid cations and cation radicals. EPR measurements of the electrooxidized products showed single-line spectra with a *g* value (2.0026) typical of hydrocarbon cation radicals. Furthermore, the oxidized β-carotene solutions exhibited an EPR line width of ~14 G, which is consistent with the value determined by second moment calculations of the β-carotene cation radical monomer. This is conclusive evidence that the electrochemical oxidation of carotenoids produces cation radicals. The one-electron oxidation of both β-apo-8′-carotenal and canthaxanthin directly results in the production of these radicals; however, the two-electron oxidation of β-carotene produces dications, which are believed to react with the neutral species to produce the cation radical, according to $C + C^{2+} \rightarrow 2C^{\bullet+}$ (where C = β-carotene). The stability of the carotenoid cation radicals was shown to be highly solvent dependent, with the β-carotene cation radical exhibiting the longest lifetime in CH_2Cl_2.

Carotenoid compounds are present in the chloroplasts of photosynthetic green plants and are essential for the plants' survival. The role of the carotenoids is believed to be twofold:[1-4] (1) they serve as photoproduct devices by preventing the formation of damaging singlet oxygen; (2) they act as antenna pigments for the absorption of light energy in the spectral region where chlorophyll is not an efficient absorber; they then transfer this energy to chlorophylls for the performance of photosynthesis.

Carotenoids are also thought to play another role in the photosynthetic process. Although the exact location of the carotenoids within the photosynthetic membrane has not yet been determined, it is known that they are in close association with the chlorophyll-containing PSII reaction center.[5,6] Because of this close association and also because of their extended conjugated systems (β-carotene has 11 double bonds), evidence for carotenoid-mediated redox reactions has been sought. Mathis and his coworkers[7,8] recently reported that light excitation of chloroplasts produces absorption changes due to formation of a carotenoid cation radical at the PSII reaction center.

Light-driven electron-transport processes across membranes are essential to the development of artificial photosynthetic systems that would convert solar energy into chemical energy. Recent work by Moore et al.[9-11] has shown that molecular triad molecules consisting of porphyrins covalently linked to both carotenoids and quinones (C–P–Q) can achieve photodriven electron transfers in good yield. Excitation of the C–P–Q moiety yields the porphyrin first excited singlet state C–^{1}P–Q, which donates an electron to the quinone to form C–P$^{\bullet+}$–Q$^{\bullet-}$. A second electron-transfer reaction yields a final charge-separated state C$^{\bullet+}$–P–Q$^{\bullet-}$, which in solution has a lifetime on the order of microseconds. This evidence further suggests that carotenoids can play an active role in the photosynthetic electron-transport chain. Moreover, the carotenoid cation radical $C^{\bullet+}$ appears to be an integral part of the electron-transfer process, and it follows that detailed knowledge of the carotenoids and their oxidation products is important to the overall understanding of these processes.

The oxidation of carotenoids has been previously studied by photochemical,[7,8,12] radiolytic,[13-15] and chemical means.[16-20]

[†] 1987 Summer participant in the NSF-sponsored REU Program.

(1) Goedheer, J. C. *Ann. Rev. Plant Physiol.* **1972**, *23*, 87.
(2) Sauer, K. *Bioenergetics of Photosynthesis*; Academic: New York, 1975; pp 115–181.
(3) Renger, G.; Wolff, Ch. *Biochim. Biophys. Acta* **1977**, *460*, 47.
(4) Chessin, M.; Livingston, R.; Truscott, T. G. *Trans. Faraday Soc.* **1966**, *62*, 1519.
(5) Lawlor, D. W. *Photosynthesis: Metabolism, Control, and Physiology*; Wiley: New York, 1987; p 43.
(6) Goodwin, T. W. *The Biochemistry of the Carotenoids: Plants*; Chapman and Hale: London, 1980; Vol. 1. p 79.
(7) Mathis, P.; Rutherford, A. W. *Biochim. Biophys. Acta* **1984**, *767*, 217.
(8) Schenck, C. C.; Diner, B.; Mathis, P.; Satoh, K. *Biochim. Biophys. Acta* **1982**, *680*, 216.
(9) Seta, P.; Bienvenue, E.; Moore, A. L.; Mathis, P.; Bensasson, R. V.; Liddell, P.; Pessiki, P. J.; Joy, A.; Moore, T. A.; Gust, D. *Nature (London)* **1985**, *316*, 653.
(10) Moore, T. A.; Gust, D.; Mathis, P.; Mialocq, J. C.; Chachaty, C.; Bensasson, R. V.; Land, E. J.; Doizi, D.; Liddell, P. A.; Lehman, W. R.; Nemeth, G. A.; Moore, A. L. *Nature (London)* **1984**, *307*, 630.
(11) Gust, D.; Moore, T. A.; Liddell, P. A.; Nemeth, G. A.; Makings, L. R.; Moore, A. L.; Barrett, D.; Pessiki, P. J.; Bensasson, R. V.; Raigee, M.; Chachaty, C.; De Schryver, F. C.; Van de Anweraer, M.; Holzwarth, A. R.; Connolly, J. S. *J. Am. Chem. Soc.* **1987**, *109*, 846.

0002-7863/88/1510-2151$01.50/0

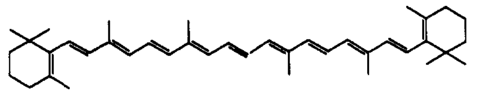
Results of photochemistry and radiation chemistry experiments in hexane solvent indicate that carotenoid cation radicals are formed on oxidation, but that the lifetimes of such species are extremely short (on the order of microseconds).[21] On the other hand chemical oxidation, using electron-acceptor molecules such as I_2, TCNQ, and DDQ, yields long-lived species with lifetimes of several minutes to hours. However, the products are not simple carotenoid cation radicals but, rather, ion pairs of a complex nature.[16-20] Hence, any studies of the physical characteristics of carotenoid cation radicals must necessarily be preceded by an investigation of methods whereby the radicals can be stabilized for longer periods of time.

Few electrochemical studies of carotenoids have been reported. Takahashi et al.[22,23] were the first to report the reduction properties of β-carotene in an aprotic solvent mixture. However, a later study by Mairanovsky et al.[24] showed some of the early results to be incorrect and that the first cathodic wave of β-carotene corresponds to a reversible, one-electron transfer. These authors later confirmed the formation of the β-carotene anion radical by EPR detection of the anionic species following controlled potential electrolysis in the EPR cavity.[25] The electrochemical oxidation, however, is not as straightforward. Mairanovsky et al.[26] and Park[27] studied the electrochemical oxidation of β-carotene in nonaqueous systems. Park's[27] cyclic voltammetry (CV) data showed the oxidation of β-carotene in tetrahydrofuran to be an irreversible two-electron process. However, controlled potential coulometry showed that four or five electrons may be involved in the overall oxidation reaction. Mairanovsky et al.[26] reported cyclic voltammetry of β-carotene, at a hanging drop mercury electrode, in the presence of a mixture of benzene and acetonitrile (1:2, v/v). At scan rates ≥ 10 V/s the oxidation process was determined to be a reversible, one-stage transfer of two electrons. Neither Park nor Mairanovsky reported the presence of a cation radical intermediate.

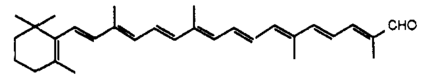
The general aim of this investigation is to determine whether carotenoid cation radicals can be produced, and stabilized, electrochemically. Hence, we have undertaken a detailed study of the electrooxidation of various carotenoids (β-carotene (I), β-apo-8′-carotenal (II), and canthaxanthin (III)) using the techniques of cyclic voltammetry, controlled-potential electrolysis (cpe) in conjunction with optical spectroscopy, and EPR spectroscopy coupled with in situ electrolysis. We report the successful generation of carotenoid cation radicals via electrochemical oxidation and, furthermore, the stabilization of these radicals for several minutes in CH_2Cl_2 and $C_2H_4Cl_2$ solvents.

Experimental Section

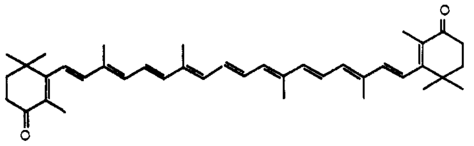
Materials. Acetonitrile (CH_3CN) and benzene (C_6H_6) were distilled over CaH_2. Methylene chloride (CH_2Cl_2), 1,2-dichloroethane ($C_2H_4Cl_2$), and tetrahydrofuran (THF), all Aldrich HPLC grade, were bubbled with



(I)



(II)



(III)

nitrogen for 30 min to remove oxygen and used without further purification. In initial experiments methylene chloride and dichloroethane were distilled over CaH_2, tetrahydrofuran was distilled over a mixture of potassium and benzophenone, and the solvents were transferred to the CV cell under a blanket of nitrogen. However, cyclic voltammograms of these solvents proved to be the same as those for the HPLC-grade solvents, and in all subsequent experiments the HPLC-grade materials were used. All solvents were transferred to the flask containing the carotenoid and electrolyte in a glovebag filled with argon. *N*,*N*-Dimethylformamide (DMF, Fisher Spectroanalyzed), for the reference filling solution, was used as supplied. Tetra-*n*-butylammonium hexafluorophosphate (TBAHFP), polarographic grade, was used as supplied from Fluka and stored under vacuum in a desiccator. Tetra-*n*-butylammonium iodide (TBAI), purchased from Aldrich, was used without further purification. Argon and nitrogen gases (Atlas Welding, 99.9% pure) were passed through 8-in. drying tubes filled with Drierite (anhydrous $CaSO_4$) to remove remaining water.

All carotenoids were purified on a column packed with silica gel (Davison Chemicals, Davisil 62). β-Carotene (Sigma) was eluted with CCl_4, β-apo-8′-carotenal (Fluka) with a mixture of petroleum ether and acetone (20:1, v/v), and canthaxanthin (Fluka) with a CCl_4–acetone mixture (20:1, v/v). The purity of each compound was verified by TLC and UV–vis spectroscopy. β-Carotene purity was also checked via NMR. All carotenoids were stored under argon in the freezer. It is extremely difficult to stress that even with the care taken to purify and store the carotenoid samples, it was still necessary to repurify them immediately (i.e., not more than a day) before use. For instance, immediately following column purification of the β-carotene sample, only one TLC spot was observed under UV light. This spot corresponded to the pure β-carotene. A cyclic voltammogram of this sample in CH_2Cl solvent indicated two electrons transferred in the oxidation process (i.e., $n_{app} = 2$). After care was taken to store this pure sample under argon and in the freezer, the sample was examined again several days layer for purity. This time TLC indicated the presence of a second, less intense spot and cyclic voltammetry showed an n_{app} value closer to one. Following repurification of this same sample on the column, the CV data again showed $n_{app} = 2$. Aging of the β-carotene samples did not, however, appear to affect the absorption maximum and EPR g values.

In order to investigate the effect of small amounts of water on the electrochemistry, all carotenoid solutions were run in both the presence and absence of activated alumina. In all cases the alumina had no detectable effect on the anodic CVs. Bubbling with O_2 for 1–2 min also had no immediate detectable effect. We therefore conclude that as long as an initial effort is made to exclude water and oxygen from all materials and glassware, the major factor in the accurate determination of the oxidation characteristics via electrochemical techniques is the purity of the carotenoid samples themselves.

Apparatus. All glassware was soaked in a KOH–EtOH bath, rinsed with distilled water and acetone, and finally dried in an oven at 110 °C. The glassware was allowed to cool either under a stream of nitrogen or in the argon-filled glovebag.

A three-electrode cell, where a platinum disk (area ~ 0.02 cm^2) and a platinum wire were used as working and auxiliary electrodes, respectively, was used for cyclic voltammetry measurements. An SCE electrode was used as the reference electrode in THF solutions. In all other solvents the quasi reference electrode was a half-cell Ag/0.04M TBAI in DMF, connected with an intermediate bridge containing 0.08M TBAHFP in DMF. The formal potential of the ferrocene/ferrocenium couple in THF is +0.65 V vs the SCE compared with +1.12 V vs the quasi reference electrode in CH_2Cl_2 and in $C_2H_4Cl_2$. All measurements were

---

(12) Mathis, P.; Vermeglio, A. *Photochem. Photobiol.* **1972**, *15*, 157.

(13) Dawe, E. A.; Land, E. J. *J. Chem. Soc., Faraday Trans. 1* **1975**, *71*, 2162.

(14) Almgren, M.; Thomas, J. K. *Photochem. Photobiol.* **1979**, *31*, 329.

(15) Lafferty, J.; Roach, A.; Sinclair, R. S.; Truscott, T. G.; Land, E. J. *J. Chem. Soc., Faraday Trans. 1* **1977**, *73*, 416.

(16) Ioffee, N. T.; Engovatov, A. A.; Mairanovskii, V. G. *Zh. Obshch. Khim.* **1976**, *46* (7), 1638–1644.

(17) Mallik, B.; Jain, K. M.; Misra, T. N. *Indian J. Biochem. Biophys.* **1978**, *15*, 233.

(18) Ebrey, T. G. *J. Phys. Chem.* **1967**, *71* (6), 1963.

(19) Lupinski, J. H. *J. Phys. Chem.* **1963**, *67*, 2725.

(20) Ding, R.; Grant, J. L.; Metzger, R. M.; Kispert, L. D., unpublished results.

(21) Bensasson, R. V.; Land, E. J.; Truscott, T. G. *Flash Photolysis and Pulse Radiolysis*; Pergamon: Oxford, 1983; p 77.

(22) Takahashi, R. *Rev. Polarogr.* **1961**, *9*, 247.

(23) Takahashi, R.; Tachi, I. *Agric. Biol. Chem.* **1962**, *26*, 771, 777.

(24) Mairanovsky, V. G.; Engovatov, A. A.; Samokhvalov, G. I. *Zh. Org. Khim.* **1970**, *6*, 632.

(25) Ioffee, N. T.; Engovatov, A. A.; Mairanovsky, V. G. *J. Gen. Chem. USSR (Engl. Transl.)* **1975**, 939.

(26) Mairanovsky, V. G.; Engovatov, A. A.; Ioffee, N. T.; Samokhvalov, G. I. *J. Electroanal. Chem.* **1975**, *66*, 123.

(27) Park, S. M. *J. Electrochem. Soc.* **1978**, *125* (2), 216.

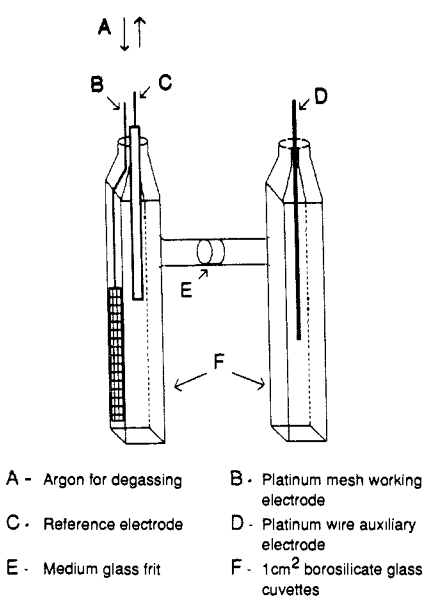


Figure 1. Three-electrode, two-compartment borosilicate glass cell used for recording absorption spectra during in situ electrolysis.

Table I. Cyclic Voltammetry of 10^{-3} M Solutions of β-Carotene, β-Apo-8′-carotenal, and Canthaxanthin in THF, CH_2Cl_2, and $C_2H_4Cl_2$

	peak potential vs SCE, V	n_{app}[a]	ΔE,[b] mV (±10)
	β-Carotene		
THF	0.76	2.0	i[c]
CH_2Cl_2	0.78	1.9	90
$C_2H_4Cl_2$	0.76	1.8	70
	β-Apo-8′-carotenal		
THF	0.90	2.6	i[c]
CH_2Cl_2	0.91	1.1	100
$C_2H_4Cl_2$	0.90	1.1	80
	Canthaxanthin		
THF	0.93	1.7	i[c]
CH_2Cl_2	0.90	0.89	80
$C_2H_4Cl_2$	0.89	0.86	90

[a] n_{app}: number of electrons passed, determined by comparison of peak height with the one-electron oxidation wave of 9,10-diphenylanthracene, assuming approximately equal diffusion coefficients. [b] ΔE: $E_{pa} - E_{pc}$. [c] i: irreversible wave.

performed in a stationary solution over neutral activated alumina, under a blanket of nitrogen, in the presence of red light and at room temperature. The *iR* drop was compensated by the positive feedback built in the potentiostat used. In all cases a solvent-supporting electrolyte solution was scanned over the solvent window to ensure the absence of electroactive impurities. A Bioanalytical Systems (BAS) CV-27 cyclic voltammograph was used for CV measurements. CVs were recorded on either a Houston Instruments Omnigraphic X-Y recorder (scan rates ≤500 mV/s) or a Tektronix 7844 dual-beam oscilloscope in conjunction with the Tektronix C-53 oscilloscope camera (scan rates ≥500 mV/s).

Optical spectra were recorded during in situ electrolysis in a custom-designed, three-electrode, two-compartment glass cuvette arrangement (Figure 1). The working electrode (platinum gauze) was separated from the auxiliary electrode (platinum wire) via a medium glass frit. Reference electrodes were identical with those used to record the cyclic voltammograms for the same solvent–electrolyte systems. A needle attached to either an argon- or N_2-filled balloon was used to degass the working half-cell solution and also provide constant stirring during the electrolysis. A Cary 14 recording spectrophotometer was used for recording electronic absorption spectra of the electrolysis products.

EPR measurements were recorded during in situ electrolysis, using a two-electrode, custom-designed Pyrex electrolytic flat-cell, with a large platinum gauze electrode as the anode and a platinum wire as the cathode. The potential was applied with a PAR voltage–current reference source, Model TC 100.2, and the current monitored with a Fluke 8024B multimeter. EPR spectra of the electrolysis products were recorded with a Varian E-12 spectrometer. The magnetic field was measured with a Bruker NMR gaussmeter, Model ER 035M, and the microwave frequency measured with a frequency counter, Model H/P 5246 L.

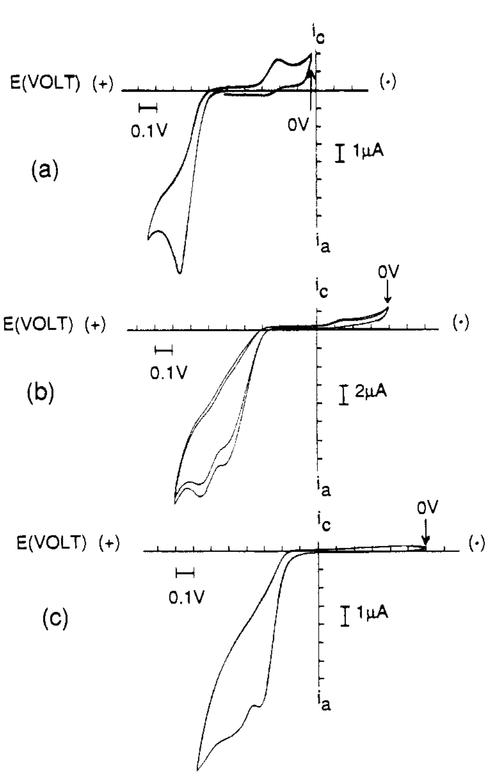


Figure 2. Cyclic voltammograms of the oxidation of 10^{-3} M solutions of I–III at a Pt disk electrode in THF with 0.08M TBAHFP as supporting electrolyte; scan rate, 100 mV/s. Key: (a) β-carotene, (b) β-apo-8′-carotenal, (c) canthaxanthin.

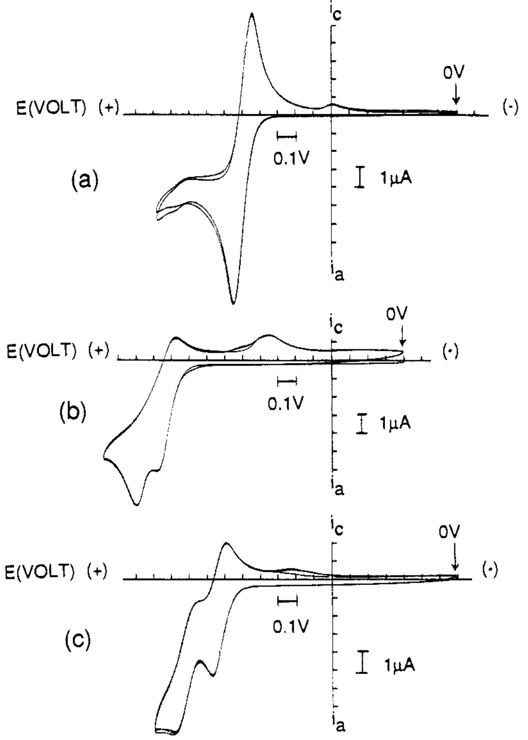


Figure 3. Cyclic voltammograms of the oxidation of 10^{-3} M solutions of I–III at a Pt disk electrode in CH_2Cl_2 with 0.08M TBAHFP as supporting electrolyte; scan rate, 100 mV/s. Key: (a) β-carotene, (b) β-apo-8′-carotenal, (c) canthaxanthin.

Results and Discussion

Cyclic Voltammetry. Cyclic voltammograms (CVs) that show the anodic behavior of β-carotene (I), β-apo-8′-carotenal (II), and canthaxanthin (III) in the solvents THF, CH_2Cl_2, and $C_2H_4Cl_2$ are shown in Figures 2–4, respectively. The electrode potentials

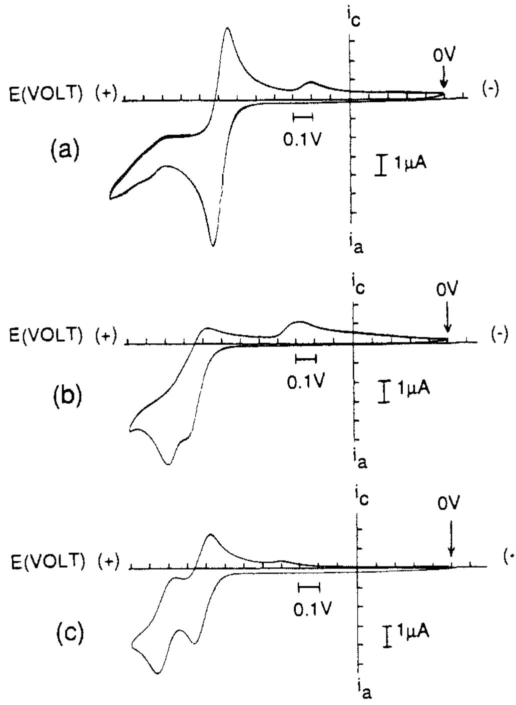


Figure 4. Cyclic voltammograms of the oxidation of 10^{-3} M solutions of I–III at a Pt disk electrode in $C_2H_4Cl_2$ with 0.08M TBAHFP as supporting electrolyte; scan rate, 100 mV/s. Key: (a) β-carotene, (b) β-apo-8′-carotenal, (c) canthaxanthin.

for the first oxidation waves of I–III are listed in Table I.

The CV results of I–III in the various solvent systems investigated are outlined below.

(i) THF. It is evident from Figure 2 that all oxidations in THF are irreversible; this was also found to be true even at relatively high scan rates i.e., ≥1 V/s (results not shown). The first anodic peak potential occurs at +0.76 V vs SCE for β-carotene, which is in good agreement with the CV result of Park.[27] CVs for II and III in THF have not been previously reported, and it is worthy of note that the first anodic waves of both β-apo-8′-carotenal and canthaxanthin are more positive than that of β-carotene by approximately 0.15 V. A close-following second oxidation wave was observed for II and III; however, our interest here lies with the first wave of each compound, and other oxidation waves will not be discussed.

The numbers of electrons transferred (n_{app}) was calculated by a comparison of wave heights with the height of the one-electron anodic wave of 9,10-diphenylanthracene under the same experimental conditions, assuming approximately equal diffusion coefficients. n_{app} values were determined for each compound in each solvent system studied and are listed in Table I. It is apparent from the CVs of I (Figure 2a) and III (Figure 2c) and from the values of n_{app} (Table I) that the oxidations of β-carotene and canthaxanthin in THF are characteristic of a two-electron-transfer process (EE) followed by rapid chemical (C) reaction, (i.e., the electrode processes have EEC character). The rapidity of the following chemical reaction is evidenced by the fact that the two-electron oxidation wave remains irreversible even at much higher scan rates (results not shown). The result for β-carotene is in good agreement with Park,[27] who also found the first oxidation potential of β-carotene in THF to involve the transfer of two electrons in an irreversible process. The CV data of Park[27] were measured at the relatively slow scan rate of 100 mV/s, and the resulting anodic wave did not yield evidence of a cation radical intermediate. In the present study, we obtained CVs at scan rates up to 10 V/s (results not shown), and these also showed no evidence for an intermediate radical species. The oxidation behavior of β-apo-8′-carotenal is more difficult to interpret and appears to involve either two or three electrons (i.e., n_{app} = 2.6; Table I). However, comparison of the first oxidation wave height (i_a) with the height of the first reduction wave (i_c) yielded a value of 2 for the ratio i_a/i_c. This indicates that the oxidation of II also involves two electrons.

It should be noted that the first oxidation potentials of all three carotenoids lie in the region at which the THF solvent window approaches its positive limit. Hence, the background current due to THF contributes to the measured oxidation current for the carotenoids, and for the 9,10-diphenylanthracene standard. The value of n_{app} is therefore difficult to determine accurately. However, it is not important that the number of electrons transferred be *exactly* known in this case, since it will later be shown that none of the three carotenoids studied produce cation radicals upon electrochemical oxidation in THF. *The important factor is that the THF solvent reacts chemically with the oxidized carotenoids, preventing the formation of radical cations.*

(ii) CH_2Cl_2. CVs showing the oxidation behavior of I–III in CH_2Cl_2 are shown in Figure 3. The peak potentials are listed in Table I. The most obvious feature of all three scans is that a cathodic wave is evident on the reverse scan. The reverse wave is apparent even at relatively low scan rates (100 mV/s), indicating that, unlike the behavior in THF, any chemical reaction following the oxidation must be slow.

The values of n_{app} for β-carotene, β-apo-8′-carotenal, and canthaxanthin are 1.9, 1.1, and 0.89, respectively, indicating that the oxidation of β-carotene is a reversible two-electron process, while the oxidations of β-apo-8′-carotenal and canthaxanthin involve only one electron. The reversible nature of the carotenoid oxidation waves allowed the values of $\Delta E = E_{pa} - E_{pc} = 0.059$ V/n (n = number of electrons transferred) to be determined, and these results are listed in Table I. It is apparent that all three values of ΔE are larger than would be expected for either a simple one-electron- (ΔE = 59 mV) or two-electron- (ΔE = 29.5 mV) transfer process. The simplest explanation for this would be the large solution resistance due to the use of nonaqueous solvents. The BAS CV-27 voltammograph has an internal positive feedback system that compensates for some of the *iR* drop across the working and auxiliary electrodes but has no external facility for adding further *iR* compensation. Hence, there may still be some *iR* loss due to solvent resistance. In order to determine whether this is the case, similar experiments were conducted with a voltammograph (IBM Model 225A), which has a capacity for manually "dialing-in" various amounts of *iR* compensation. The data obtained with this apparatus gave smaller ΔE values, but they were still in excess of those expected for one- and two-electron processes, e.g.: ΔE(β-carotene/CH_2Cl_2) = 65 ± 10 mV (IBM) vs 90 ± 10 mV (BAS) vs 29.5 mV (two-electrons, theory); ΔE-(canthaxanthin/CH_2Cl_2) = 70 ± 10 mV (IBM) vs 80 ± 10 mV (BAS) vs 59 mV (one-electron, theory).

In the cases of β-apo-8′-carotenal and canthaxanthin, the ΔE value for the first wave was difficult to obtain accurately because of the close-following second oxidation wave. Hence, it is difficult to distinguish between the two one-electron mechanisms, EC or E, for the first oxidations of these two carotenoids. In any case, *it is important to note that the oxidations of II and III in THF involved the transfer of two electrons, while in CH_2Cl_2 the oxidations only involve the transfer of one electron.*

For β-carotene, the ΔE value *appears* to be far in excess of that for a simple two-electron mechanism EE, even if the solution resistance is compensated. Often a large ΔE value reflects the presence of a coupled chemical reaction C,[28,29] which may indicate an overall reaction of the type ECE, where E represents an electron transfer at the electrode surface. Indeed, literature reports do indicate that hydrocarbons typically exhibit ECE character during oxidation.[30] However, Mairanovsky et al.[26] reported a simple two-electron transfer of the type EE for the electrochemical oxidation of β-carotene in a mixture of benzene and acetonitrile. Furthermore, Richardson and Taube[31] developed a theory for

---

(28) Kissinger, P. T.; Heineman, W. R. *Laboratory Techniques in Electroanalytical Chemistry*; M. Dekker: New York, 1984; pp 86–93.

(29) Bard, A. J.; Faulkner, L. R. *Electrochemical Methods*; Wiley: New York, 1980; pp 429–465.

(30) Adams, R. N. *Acc. Chem. Res.* **1969**, *2*, 175.

(31) Richardson, D. E.; Taube, H. *Inorg. Chem.* **1981**, *20* (4), 1278.

multistep charge-transfer reactions of the type shown in eq 1

$$A \xrightarrow{n_1e,\ E_1°} B \xrightarrow{n_2e,\ E_2°} C \qquad (1)$$

(where n_i is the number of electrons in step i, and $E_i°$ is the standard potential of step i), which shows that for a simple two-electron-transfer process (i.e., $n_1 = n_2 = 1$) the peak separation ΔE varies between 29.8 and 252 mV as $E_2° - E_1°$ (i.e., $\Delta E_{1/2}$) varies between 200 mV and -200 mV. *Thus a peak separation of 29.8 mV would only be expected if $E_2° - E_1°$ equals 200 mV.* Their results show that a peak separation of ~65 mV, as determined for the oxidation of β-carotene in the present study, can readily be accounted for if the $\Delta E_{1/2}$ value is on the order of 30–50 mV. Furthermore, if $\Delta E_{1/2}$ *is* ~30–50 mV, then a half-width $E_p - E_{p/2}$ equal to 57.0–68.3 should be observed; a value of ~60 mV is obtained from the CV shown in Figure 3a, which compares favorably with that predicted by theory. Moreover, Richardson and Taube have generated, from the theory, the current–potential curve for $\Delta E_{1/2}$ = 35.61 mV, which clearly shows that the two oxidation waves overlap and appear as only one wave, as seen in our experimental results (Figure 3a). Thus, there is considerable evidence that the two-electron oxidation of β-carotene occurs via a simple EE process.

(iii) $C_2H_4Cl_2$. The CVs of I–III in $C_2H_4Cl_2$ are shown in Figure 4. The anodic behavior of all three compounds is similar to that observed in CH_2Cl_2; i.e., the oxidation of β-carotene appears to involve a reversible two-electron process, while the oxidations of β-apo-8′-carotenal and canthaxanthin involve a one-electron process (see Table I).

The anodic behavior of β-carotene in a mixture of benzene and acetonitrile has been studied by Mairanovsky et al.[26] using both dc polarography and cyclic voltammetry, and was shown to involve a reversible two-electron process. Although no details of the $C_2H_4Cl_2$ solvent system were given, Mairanovsky et al.[26] indicated that it yielded similar results to those in benzene–acetonitrile. Our results (i.e., n_{app} = 1.8) indicate a two-electron transfer also and are therefore in general agreement with Mairanovsky et al.[26] The ΔE value of 70 mV is larger than that of 31 mV, which Mairanovsky et al. found for the oxidation of β-carotene in benzene–acetonitrile and, as outlined above, might indicate an EE mechanism in which the $\Delta E_{1/2}$ is approximately 30–50 mV.

As of the time of writing we are not aware of any other studies of the electrochemical oxidation of canthaxanthin or β-apo-8′-carotenal in this solvent.

In addition to the first oxidation wave and reverse cathodic wave, a second cathodic peak at more negative potentials was also observed for each carotenoid in each solvent system (see Figures 2–4). This peak (vs SCE) occurs at ~0.25 V for I, ~0.35 V for II, and ~0.45 V for III. Addition of small amounts (i.e., a few drops) of water did not affect the height of this wave, which indicates that it is not due to an interaction with trace amounts of water in the solution. Mairanovsky et al.[26] also observed this wave for β-carotene in both $C_2H_4Cl_2$ and a mixture of benzene and acetonitrile. This suggests that the peak is not the result of solvent interaction, since $C_2H_4Cl_2$ is particularly stable toward electrophilic interaction. Mairanovsky et al.[26] suggested that the peak is due to a reduction of RH^+ according to eq 2, where RH_2^{2+}

$$RH_2^{2+} \xrightarrow{-H^+} RH^+ \xrightarrow{e^-} RH^\cdot \xrightarrow{\text{rapidly}} \text{products} \qquad (2)$$

is the β-carotene dication. This conclusion has been challenged by Park,[27] who observed *two* additional cathodic peaks, at ~0.2 and ~0.1 V vs SCE, for β-carotene in THF. Park[27] concluded from controlled potential oxidation measurements that the peak at 0.2 V corresponds to reduction of an intermediate, while the -0.1 V peak is the reduction of H^+. However, the two oxidation waves of β-carotene overlap, and it is difficult to obtain further mechanistic information from the cyclic voltammogram. We have therefore used canthaxanthin, which has two clearly resolved oxidation waves, to learn more about the second cathodic wave. In this study, it was found that if the potential is reversed after the first oxidation wave of canthaxanthin, the peak at ~0.45 V vs SCE disappears completely. This indicates that the second oxidation wave, and, hence, dication formation, is necessary in order for the second cathodic peak to be observed, suggesting that the mechanism proposed by Mairanovsky et al.[26] is not unreasonable.

Table II. Absorption Spectroscopy during Controlled-Potential Electrolysis of 10^{-3} M Solutions of I–III in THF, CH_2Cl_2, and $C_2H_4Cl_2$

	potential vs SCE, V	λ_{max}, nm	$\tau_{1/2}$,[a] min
	β-Carotene		
THF	0.76	955	0.75
CH_2Cl_2	0.78	1000	14
$C_2H_4Cl_2$	0.76	1025	0.75
	β-Apo-8′-carotenal		
THF	0.90		
CH_2Cl_2	0.91	840	12
$C_2H_4Cl_2$	0.90	842	5
	Canthaxanthin		
THF	0.93		
CH_2Cl_2	0.90	890	6
$C_2H_4Cl_2$	0.89	890	6

[a] $\tau_{1/2}$: half-life of the electrolysis product at λ_{max}.

Optical Absorption. It has been well recorded in the literature that carotenoid cations and, in particular, cation radicals exhibit absorption bands in the wavelength region 800–1500 nm.[16,30,32,33] Hence, in order to determine whether carotenoid cation radicals are formed during electrooxidation, the absorption spectra of I–III in all three solvent systems were recorded over the wavelength range 750–1200 nm during controlled–potential electrolysis (cpe) at the first anodic peak potential. The measurements were carried out in the cell shown in Figure 1. The spectra were accumulated 2 min after application of the potential, and the data are summarized in Table II. Results for each solvent studied are outlined below.

(i) THF. Controlled-potential electrolysis of β-carotene gave rise to a new band with λ_{max} = 955 nm and a half-life $\tau_{1/2}$ of 0.75 min. No new absorption bands in the near-infrared were observed for compounds II and III.

According to the CV measurements, all three carotenoids exhibit EEC type oxidation behavior in THF. Therefore, we would not expect to see the carotenoid cation radicals following controlled potential oxidation, since the initial product would be a dication species RH_2^{2+} (where RH_2 is the neutral carotenoid). In each case, however RH_2^{2+} reacts chemically to produce a secondary product. Obviously, in the case of β-apo-8′-carotenal and canthaxanthin this product is not a cation (or radical cation) since no new bands were observed in the near-IR. On the other hand, the β-carotene dication *does* give rise to a short-lived species, with λ_{max} = 955 nm, following chemical reaction. A search of the literature has shown that the neutral carotenoids and their isomeric forms, as well as other possible oxidation products such as the alcohols, carbonyls, or acids, all absorb in the UV–vis region of the spectrum.[34] In addition, any shortening of the conjugated chain length would result in a blue shift of the absorption maximum, and not a red shift, as observed.[35] The only carotenoid species that absorb in the near-IR are the cations,[16,32,33] radical cations,[35] and radical anions;[35] the dications are readily obtainable from reaction with H_2SO_4 or $TiCl_4$[19] and do not show absorption in this region. Hence, the band at 955 nm can only be derived from singly charged "cationic" (i.e., cation or radical cation) species.

(ii) CH_2Cl_2. Electrooxidation in CH_2Cl_2 produces new bands in the absorption spectra of β-carotene, β-apo-8′-carotenal, and canthaxanthin, with peak maxima at 1000, 840, and 890 nm, respectively. The half-life $\tau_{1/2}$ of each new absorbing species is on the order of several minutes (see Table II).

---

(32) Sorenson, T. S. *J. Am. Chem. Soc.* **1965**, *87*, 5075.

(33) Wassermann, A. *J. Chem. Soc.* **1954**, 4329.

(34) Isler, O. *Carotenoids*; Berkhauser Verlag: Basel, 1971.

(35) Bensasson, R. V.; Land, E. J.; Truscott, T. G. *Flash Photolysis and Pulse Radiolysis*; Pergamon: Oxford, 1983; p 74.

The absorption maxima for various polyenes and their radical ions (derived from flash photolysis and pulse radiolysis) have been summarized by Bensasson et al.[35] The general trend is that the absorption maximum decreases with a concomitant decrease in the number of double bonds. For example, the dodecapreno-β-carotene cation radical (19 double bonds) absorbs at 1480 nm in hexane, the β-carotene cation radical (11 double bonds) has a peak maximum at 1040 nm, the canthaxanthin cation radical (11 double bonds + 2 C=O) absorbs at 960 nm, and the septatreno-β-carotene cation radical (with 9 double bonds) absorbs at 915 nm. β-apo-8′-carotenal (9 double bonds + C=O) is not listed in the table compiled by Bensasson et al.;[35] however, it is reasonable to assume that it will absorb in the same region as septatreno-β-carotene. Hence, the possibility exists that the products that absorb at 1000, 840, and 890 nm are the carotenoid cation radicals.

However, carotenoid cations also absorb in the near-IR region. For example, Ioffee et al.[16] have shown that the interaction of β-carotene with trifluoroacetic acid in $C_2H_4Cl_2$ is accompanied by the formation of an intense band with λ_{max} 990 nm (960 nm in benzene). The authors suggested that this absorption is due to a carbonium ion RH_3^+ formed by protonation of β-carotene by the acid. One should note that this species has only one double bond less than the RH^+ species proposed by Mairanovsky et al.[26] in reaction 2 above, and according to the data compiled by Bensasson et al.[35] both species should absorb in a similar region of the spectrum. Following the lead of Ioffee et al.,[16] we prepared 4.2×10^{-5} M solutions of I–III in $C_2H_4Cl_2$, with each solution containing 4×10^{-1} M trifluoroacetic acid. The β-carotene mixture produced an intense band, with λ_{max} 1,020 nm; the β-apo-8′-carotenal mixture gave a new band approximately one-tenth as intense as the β-carotene band, with λ_{max} 830 nm; the canthaxanthin mixture also gave a less intense band, with λ_{max} 880 nm. These λ_{max} values are almost identical with those observed in the near-IR following controlled potential oxidation. Thus, the electrochemically generated products might also be carotenoid cations.

CV measurements of II and III in CH_2Cl_2 showed that both oxidations involve one electron, and although the oxidation of β-carotene is a two-electron process, the products absorb in the near-IR. These results suggest that the oxidation of compounds I–III in CH_2Cl_2 results in the production of cation radicals. Of course, the radical species are paramagnetic, whereas the cations are diamagnetic; hence, a definitive answer can be arrived at by the use of EPR spectroscopy. We have undertaken electrolysis of I–III in conjunction with EPR spectroscopy, and these results are discussed in the following EPR section.

(iii) $C_2H_4Cl_2$. Controlled-potential electrolysis in $C_2H_4Cl_2$ results in the production of new absorption bands for each of the carotenoids I–III. The peak maxima are similar to those obtained in CH_2Cl_2, i.e., β-carotene, λ_{max} 1025 nm; β-apo-8′-carotenal, λ_{max} 840 nm, and canthaxanthin, λ_{max} 890 nm.

Again, the results suggest that either cations or cation radicals are produced in each carotenoid solution following controlled potential oxidation. Further discussion of this point is given in the EPR section.

An interesting and important point is that the half-life of each product is considerably shorter in $C_2H_4Cl_2$ than in CH_2Cl_2 (Table III), indicating that the products are more stable in CH_2Cl_2 solvent.

Electron Paramagnetic Resonance. EPR spectra of 10^{-3} M solutions of I–III in THF, CH_2Cl_2, and $C_2H_4Cl_2$ were recorded following in situ electrochemical oxidation. The spectra were recorded at 2 V_{Pt}, using the two-electrode system described above. A summary of the g values and line widths is presented in Table III. A discussion of the results obtained in each solvent system is given below.

(i) THF. No radical species were produced following electrochemical oxidation of I–III.

For carotenoids II and III this is in accordance with both the CV and absorption results. β-carotene, however, showed an absorption band at 955 nm following oxidation at the first anodic peak potential (see Table II). The EPR result indicates that the band at 955 nm is *not* a radical. Since the only other species that absorbs in the near-IR is the cation, *the products of β-carotene oxidation in THF must be cations*, (probably derived from the EEC reaction, as determined by CV).

Table III. EPR of 10^{-3} M Solutions of I–III in THF, CH_2Cl_2, and $C_2H_4Cl_2$ during in Situ Electrolysis at 2 V (Pt Electrode)

	g (±0.0002)	ΔH_{pp}, G (±0.5)
	β-Carotene	
THF		
CH_2Cl_2	2.0026	13.6
$C_2H_4Cl_2$	2.0024	13.4
	β-Apo-8′-carotenal	
THF		
CH_2Cl_2	2.0026	19.8
$C_2H_4Cl_2$	2.0027	18.1
	Canthaxanthin	
THF		
CH_2Cl_2	2.0026	14.8
$C_2H_4Cl_2$	2.0026	14.7

(ii) CH_2Cl_2 and $C_2H_4Cl_2$. Cation radicals were produced in each of solutions I–III following electrochemical oxidation in the cavity.

Each spectrum consisted of a single line with $g \sim 2.0026$. In each case, the expected hyperfine structure was not observed, and each line was relatively broad, i.e., $\Delta H_{pp} \sim$ 13–14 G for I; 18–20 G for II; 14–15 G for III (Table III). The g values are characteristic of hydrocarbon cation radicals,[36] which indicates that *the oxidation products are not simply carotenoid cations that have similar electronic absorption spectra but are indeed carotenoid cation radicals.* Furthermore, the line width of 13–14 G for the oxidized β-carotene product is in good agreement with the results of second moment calculations of the β-carotene cation radical[37] in solution, assuming a Gaussian line shape and the spin densities deduced from an INDO molecular orbital calculation of the β-carotene cation radical, which predicted a line width $\Delta H_{pp} \sim 14$ G.

A significant feature of the EPR spectra is that the intensities of all lines were greater in CH_2Cl_2 than in $C_2H_4Cl_2$ solvent. This probably reflects the greater stability of the radical species in CH_2Cl_2. Evidence for this was previously established in the absorption measurements, where we observed longer half-lives of the absorbing species in CH_2Cl_2 than in $C_2H_4Cl_2$ (see Table II).

For II and III the CV data showed that the oxidation process involves transfer of only one electron (Table I). Moreover, the absorption spectra showed new bands at 840 and 890 nm, respectively (Table II). The appearance of the EPR spectra following electrochemical oxidation in situ indicates that both compounds give rise to paramagnetic species. Hence, we conclude that the products of the single-electron oxidation of II and III in the chlorinated solvents are carotenoid cation radicals.

Anodic CV measurements of β-carotene, however, showed a two-electron-transfer process of the type EE, which results in formation of the β-carotene dication. Absorption measurements showed new bands at ~1,000 nm, following controlled potential oxidation at the first anodic peak potential (Table II), and EPR results showed conclusively that the electrochemically generated oxidation products are β-carotene cation radicals. (Of course, we cannot rule out the possibility that cations are also produced in the oxidation of the carotenoids and that these species absorb light in the same spectral region but cannot be detected in EPR measurements.) We suggest that the β-carotene cation radical might be derived from an electron-transfer reaction between the neutral species and the electrogenerated dication according to eq 3, where C = β-carotene. As discussed earlier, the EE mechanism

$$C + C^{2+} \rightarrow 2C^{\cdot+} \qquad (3)$$

---

[36] Wertz, J. E.; Bolton, J. R. *Electron Spin Resonance: Theory and Practical Applications*; McGraw-Hill: New York, 1972; p 384.

[37] Bradford, E.; Kispert, L. D., unpublished results.

can be rationalized if $E_2° - E_1° = 30–50$ mV (i.e., $E_2°$, $C^{•+} \rightarrow C^{2+} + e^- > E_1°$, $C \rightarrow C^{•+} + e^-$). This indicates that for reaction 3 $\Delta E° < 0$, which implies that $\Delta G° > 0$, and the forward reaction of 3 is not spontaneous. However, these values are based on theory and do not take into consideration such important experimental effects as solvation, which may cause a small, but sufficient, change in the $\Delta E°$ to produce a spontaneous reaction.

We therefore conclude that compounds II and III directly generate radical cations upon electrochemical oxidation in CH_2Cl_2 and $C_2H_4Cl_2$ solvents through a one-electron process. On the other hand, the two-electron oxidation of β-carotene results in the primary production of dications, which undergo further reaction with the neutral β-carotene molecule to form the radical cation species. This result might seem surprising, since II and III are carbonyl compounds and would be expected to stabilize anion radical, but perhaps not cation radical, formation. Similarly, β-carotene does not contain carbonyl groups and might be expected to generate the radical cation, rather than the dication species. However, this argument does not consider the fact that compounds II and III have long conjugated chains (canthaxanthin has 11 double bonds and β-apo-8′-carotenal has 9 double bonds) attached to the carbonyl group(s). Thus, in the case of β-apo-8′-carotenal, the cation radical may be stabilized at the end of the chain away from the C=O group, while for canthaxanthin, the radical could be stabilized along the chain, somewhere between the two carbonyl end groups. This suggests that β-carotene, which has no C=O substituents and 11 double bonds, might readily accommodate the dication species.

Conclusions

The results of CV measurements of β-carotene in THF, CH_2Cl_2, and $C_2H_4Cl_2$ indicate that the oxidation process occurs via a two-electron-transfer mechanism. In contrast, CV measurements of II and III showed that the oxidation in THF involves two-electrons, while in CH_2Cl_2 and $C_2H_4Cl_2$ the process occurs via the transfer of only one electron. Absorption measurements of β-carotene in all three solvents showed that new bands are formed in the near-IR following controlled potential oxidation at the first anodic peak potential. Absorption measurements of II and III showed new bands were formed only in the chlorinated solvents. Previous literature reports indicated that these bands could be due to either cations or radical cations. EPR measurements showed definitely that the electrochemically generated oxidation products in CH_2Cl_2 and $C_2H_4Cl_2$ are carotenoid cation radicals. In THF solution, however, β-carotene oxidation gives rise to a cation that is not a radical. The half-lives of all radical species are on the order of several minutes in CH_2Cl_2 but are considerably shorter in $C_2H_4Cl_2$. For the carotenoid radicals the order of solvent stability is therefore $CH_2Cl_2 > C_2H_4Cl_2 >$ THF.

We have thus shown that carotenoid cation radicals can be formed electrochemically in nonaqueous solvent systems, and furthermore, these radicals can be stabilized for several minutes in the solvent CH_2Cl_2.

Acknowledgment. This work was supported by the Division of Chemical Sciences, Office of Basic Energy Science, Dept. of Energy under Grant DE-FG05-86ER13465. We thank M. Wasielewski for many useful discussions, and A. J. Bard, B. P. Sullivan, and L. O. Spreer for helpful hints on electrochemical techniques. We also thank Professor L. Bottomley for use of the IBM instrument at Georgia Institute of Technology, Atlanta, and Grant Holder and John Helfrick, Jr., for assistance with those experiments, Professor M. Cava for loan of the BAS equipment, and M. Kendrick for use of the Cary 14. V.J.K. thanks NSF for a Summer Undergraduate stipend as part of the 1987 REU Site at The University of Alabama.

Photochemical Generation of [4]Paracyclophanes from 1,4-Tetramethylene Dewar Benzenes: Their Electronic Absorption Spectra and Reactions with Alcohols

Takashi Tsuji* and Shinya Nishida

Contribution from the Department of Chemistry, Faculty of Science, Hokkaido University, Sapporo 060, Japan. *Received August 31, 1987*

Abstract: Irradiation of 1,4-tetramethylene Dewar benzene (**1a**) in alcohols (methanol, ethanol, 2-propanol) affords 1-alkoxybicyclo[4.2.2]deca-7,9-dienes **3a**–**3c**, formal adducts of the alcohols to the central σ bond of **1a**. Photolyses of the 7-methoxymethyl and the 7-methoxycarbonyl derivatives **1b** and **1c** in ethanol produce mixtures of regioisomeric adducts, viz. 1- and 4-ethoxy derivatives **3** and **3′**. 2,5-Ethano bridging of **1a** leads to a complete loss of photochemical reactivity toward methanol. Thus, the compound **9**, the 2,5-ethano derivative of **1a**, gives merely benzobicyclo[2.2.2]oct-2-ene upon prolonged irradiation in methanol. Irradiation of **1a**–**1c** in rigid glass with a low-pressure Hg lamp at 77 K leads to development of absorptions in the UV–visible region that show systematic substituent-induced red shifts. The generated species, which are thermally extremely unstable, appear to revert to **1a**–**1c**, respectively, with 366-nm light irradiation. In contrast, no corresponding new absorption appears upon irradiation of **9** at 77 K. Evidence is presented to show that the irradiation of **1a**–**1c** leads to the formation of [4]paracyclophanes **2a**–**2c** from which the alcohol adducts **3** and **3′** are derived and that the electronic absorption spectra observed at 77 K are due to **2a**–**2c**. The contrasting behavior of **9** is accounted for in terms of still higher strain associated with the corresponding benzenoid derivative **10** than that in **2**.

Geometrically distorted unsaturated compounds have attracted considerable interest in recent years.[1] Paracyclophanes having a short bridge have presented a particular challenge in this field, and efforts to prepare paracyclophanes having very short bridges have led to a recent achievement of generating [5]paracyclophanes.[2] Despite severe bending of the benzene ring, it has been suggested that [5]paracyclophanes still retain aromatic character,[2,3] though they appear to lack thermal stability enough

---

(1) (a) Greenberg, A.; Liebman, J. F. *Strained Organic Molecules*; Academic: New York, 1978. (b) Shea, K. J. *Tetrahedron* **1980**, *36*, 1683. (c) Szeimies, G. *Reactive Intermediates*; Plenum: New York, 1983; Vol. 3, Chapter 5. (d) Keehn, P. M.; Rosenfeld, S. M. *Cyclophanes*; Academic: New York, 1983. (e) Wiberg, K. B. *Angew. Chem., Int. Ed. Engl.* **1986**, *25*, 312.

(2) (a) Jenneskens, L. W.; De Kanter, F. J. J.; Kraakman, P. A.; Turkenburg, L. A. M.; Koolhaas, W. E.; De Wolf, W. H.; Bickelhaupt, F.; Tobe, Y.; Kaneda, T.; Kakiuchi, K.; Odaira, Y. *J. Am. Chem. Soc.* **1985**, *107*, 3716. (b) Tobe, Y.; Kaneda, T.; Kakiuchi, K.; Odaira, Y. *Chem. Lett.* **1985**, 1301. (c) Kostermans, G. B. M.; DeWolf, W. H.; Bickelhaupt, F. *Tetrahedron Lett.* **1986**, *27*, 1095. (d) Kostermans, G. B. M.; DeWolf, W. H.; Bickelhaupt, F. *Tetrahedron*, **1987**, *43*, 2955.

0002-7863/88/1510-2157$01.50/0 © 1988 American Chemical Society