Geometrical Isomerization of Carotenoids in Dichloromethane

Antony S. Jeevarajan, Chih-Chang Wei and Lowell D. Kispert*
Department of Chemistry, University of Alabama, Tuscaloosa, AL 35487, USA

In a 3 mmol dm^{-3} solution of all-*trans*-canthaxanthin (**I**) in HPLC grade dichloromethane *cis*-isomers are formed. Optical absorptions due to *cis*-isomers in addition to those due to an intermediate are observed in 3 mmol dm^{-3} solutions of all-*trans*-β-carotene(**II**) and 8′-apo-β-caroten-8′-al (**III**), as a result of 0.1–0.2 mmol dm^{-3} acid normally found in HPLC grade dichloromethane. The *cis*-isomers were separated by HPLC and characterized by optical and NMR spectroscopic techniques. AM1 calculations performed on the ground state and protonated **I** showed that 9-*cis* and 13-*cis*-isomers will be formed by acid induced isomerization in greater yield than other *cis* and *cis,cis*-isomers. This was observed experimentally. If an excess amount of hydrochloric acid (~1 mol dm^{-3}) is added to the solution of dichloromethane containing the carotenoids, radical cations of the carotenoids are formed.

Carotenoids are necessary for the survival of photosynthetic organisms. They serve as light-harvesting pigments as well as provide photoprotection against singlet oxygen.[1,2] Carotenoids, a class of unsaturated hydrocarbons and their oxygenated derivatives are hydrophobic molecules, usually associated with photosynthetic membranes, bound noncovalently to specific pigment protein complexes.

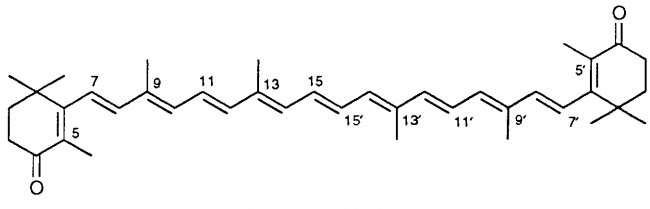
Extensive reviews exist on the carotenoid function in photosynthetic bacteria.[1,2] However, numerous properties of carotenoids are still not understood. For instance, carotenoid radical cations have been observed optically to form at the photosystem-II reaction centre;[3] the reason for this is not clear. Furthermore, there is evidence that β-carotene can function as an effective radical-trapping antioxidant.[4] It has been proposed that the radical trapping potential of β-carotene might originate near body temperature (37 °C) from a configuration twisted to 90° about the 15,15′-double bond during *trans* to *cis* isomerization.[5] In this configuration an orthogonal diradical can be formed[5] which would have exceptional reactivity toward free radicals. Carotenoid cation radicals can also be formed electrochemically in a dichloromethane solution in equilibrium with carotenoid dications and carotenoids. The comproportionation constant (K_{com} ~ 10^3) lies toward the formation of cation radicals for carotenoids which possess keto groups or strong electron acceptor groups[6,7] (Scheme 1). On the other hand, carotenoids which have electron donor groups or are nonpolar like β-carotene exhibit comproportionation constants (10^{-2}–10^{-4}) that lie toward the formation of dications; the reason for the formation of β-carotene dication preferentially over the cation radical is not understood. Possibly, a twisted geometry occurs for the cation radical before the ejection of the second electron as proposed for some sterically hindered substituted ethylene molecules.[8]

$$Car^{++} + Car \overset{K_{com}}{\rightleftharpoons} 2\,Car^{\bullet+}$$

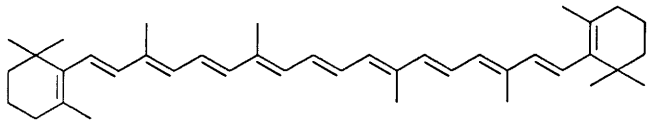
Scheme 1

Other properties of carotenoid cation radicals have also been studied. Carotenoid cation radicals can be formed[9] as part of a donor–acceptor pair, $C^{\bullet+}$–P–P–$Q^{\bullet-}$ with a lifetime of 250 μs upon light irradiation of a tetrad molecule consisting of two porphyrins (P) positioned between a carotenoid (C) and a quinone (Q). Spin-echo measurements[10] have detected the carotenoid cation radical and show that the EPR linewidth is consistent with that observed for the species formed electrochemically.[11] The carotenoid cation radical has been prepared in solid silica gel or nafion supports and their electron nuclear double resonance spectra (ENDOR) have also been reported.[12]

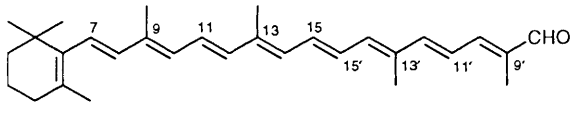
The current study on *cis–trans* isomerization of carotenoids in dichloromethane solution was motivated by the observation that the lifetime of the carotenoid cation radical formed electrochemically in dichloromethane was typically minutes in dilute solution[13] and about an hour in concentrated solution. In contrast, photochemically generated carotenoid cation radicals in carbon tetrachloride solution using time-resolved electron paramagnetic resonance (TREPR) techniques have a lifetime of a few microseconds. When solvents with different dielectric constants like dichloromethane or acetonitrile were used, very different results were obtained.[14] Examination of the solvent dependence on the TREPR results led to the following observations described here regarding acid induced *cis* isomerization.



Canthaxanthin (**I**)



β-Carotene (**II**)



8′-Apo-β-caroten-8′-al (**III**)

Optical spectra were recorded for all-*trans*-canthaxanthin (**I**), β-carotene (**II**) and 8′-apo-β-caroten-8′-al (**III**) dissolved in a number of solvents. It is observed that **I**, **II** and **III** in HPLC

grade dichloromethane form *cis*-isomers and **II** and **III** also form intermediates. If an excess of hydrochloric acid (~ 1 mol dm^{-3}) is added to the dichloromethane–carotenoid solution, radical cations of the corresponding carotenoids are formed. The extent of isomerization is quantified by separation of the *cis*-isomers using high performance liquid chromatography (HPLC) and their identification by NMR measurements, and predicted by AM1 calculations of the protonated carotenoids.

Experimental

Canthaxanthin (**I**), and 8′-apo-β-caroten-8′-al (**III**) were obtained from Fluka. β-Carotene (**II**) was obtained from Sigma. 3-Methylpentane, benzene, cyclohexane and propan-2-ol were obtained from Aldrich. Dichloromethane, carbon tetrachloride and acetonitrile of HPLC grade were obtained from Fisher.

A Varian DMS-400 UV–VIS spectrophotometer was used to obtain the optical absorption spectrum. A 1 cm cell was used for all spectra.

NMR spectra were determined using Bruker AM500 (^{1}H, 500.13 MHz) and AM360 (^{1}H, 360.13 MHz; ^{13}C, 90.56 MHz; 5 mm ^{1}H/^{13}C dual probe) instruments at 22 °C.

For the HPLC measurements, a Shimadzu LC-600 pump with a SPD-6AV optical detector was used. The analytical column of 25 cm length and 4.6 mm ID was used with 5 μm 201 TP (C_{18}) as the stationary reverse phase and 100% acetonitrile as mobile phase. The optical detector was set at 465 nm for canthaxanthin (**I**) and at 457 nm for 8′-apo-β-caroten-8′-al (**III**).

The amount of acid present in HPLC grade dichloromethane was measured as follows: water (5 cm^3) was added to the dichloromethane and shaken thoroughly. The aqueous layer was separated using a separating funnel. The extraction procedure was repeated ten times and the hydrogen ion concentration present in the aqueous layer was measured using a Beckman Instruments Model 4500 digital pH meter with a Fisher combination electrode. The dichloromethane used was found to contain 0.1 to 0.2 mmol dm^{-3} acid.

The optical spectra and HPLC spectra were digitized using UNPLOTIT software and reproduced in the figures. The AM1 (Austin Model 1) calculations were done using the HYPER-CHEM software running on a Gateway 2000 486-DX2-50MHz personal computer.

Results and Discussion

Canthaxanthin (**I**)—The UV–VIS absorption spectra of carotenoids have been extensively studied.[15] A typical spectrum contains an absorption maximum (λ_1) in the visible region, one (λ_2) in the near UV and another one (λ_3) in the UV region [Fig. 1(*a*)]. The origin of the visible absorption is due to $S_0 \longrightarrow S_2$ transition.[16] For an all-*trans*-isomer, a strong λ_1 band and weak λ_3 are present and the λ_2 band is absent. In the case of *cis*-isomers, a strong λ_1 band, a weak λ_2 band and a very weak λ_3 band are commonly present.

The absorption spectra of canthaxanthin in a number of solvents with varying dielectric constants were measured. The optical spectra of all-*trans*-canthaxanthin in all the solvents (Table 1) except that in dichloromethane were similar to the spectrum of the all-*trans*-isomer in Fig. 1(*a*). In Fig. 1(*b*), the spectrum of all-*trans*-isomer in dichloromethane is given. It is very striking to note that the λ_2 band or the '*cis*-peak' is present only when the solvent is dichloromethane suggesting that some geometrical isomerism has occurred. To determine this, the same HPLC column and procedure as normally used in this laboratory to separate different geometrical isomers from thermally isomerized canthaxanthin was employed. All-*trans*-canthaxanthin was dissolved in carbon tetrachloride and was injected into the 201 TP(C_{18}) column; eluted with 100% acetonitrile. The chromatogram is shown in Fig. 2(*a*). The different peaks, retention times and peak areas are given in Table 2. All-*trans*-canthaxanthin was also dissolved in dichloromethane and was injected into the column and eluted with 100% acetonitrile. The chromatogram is shown in Fig. 2(*b*) and it is very clear that there are additional peaks along with the all-*trans* peak. The different peaks are assigned to various *cis*-isomers based on published data[17,18] and our HPLC and NMR results.[19] The predominant isomers appear to be 13-*cis* and 9-*cis* and their NMR spectral data are given in Table 3. The fractions of the various peaks were collected and the absorption spectra taken. The absorption spectrum of the fraction collected from peak C in Fig. 2(*b*) is shown in Fig 1(*a*) (13-*cis*). To obtain the absorption spectrum, the eluent, acetonitrile was evaporated and the compound was dissolved in carbon tetrachloride. The absorption spectrum [Fig. 1(*a*)] of the *cis*-isomer clearly shows λ_1 and λ_2 peaks and the λ_2 peak is similar to the λ_2 peak in Fig. 1(*b*), where all-*trans*-canthaxanthin was dissolved in dichloromethane.

It is known that dichloromethane contains a small amount of acid and it is experimentally found to contain 0.1 to 0.2 mmol dm^{-3} acid. The acid is removed by adding anhydrous potassium

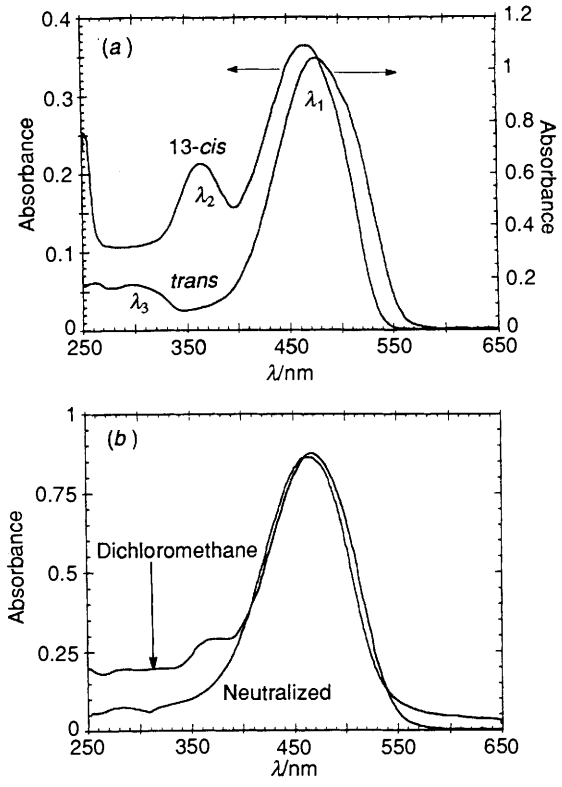


Fig. 1 Absorption spectra of all-*trans*- and 13-*cis*-canthaxanthin (**I**) in (*a*) carbon tetrachloride and (*b*) dichloromethane and neutralized dichloromethane

Table 1 Absorption spectral data of canthaxanthin (**I**) in different solvents

Solvent	Dielectric constant	λ_1/nm	λ_2/nm	λ_3/nm
Cyclohexane	2.02	463.8	—	290.0
Benzene	2.28	479.4	—	298.6
Carbon tetrachloride	2.24	474.5	—	292.7
Chloroform	4.80	481.2	—	298.6
Dichloromethane	9.08	470.0	372	—
Propan-2-ol	18.3	472.1	—	293.1
Acetonitrile	36	469.4	—	292.2

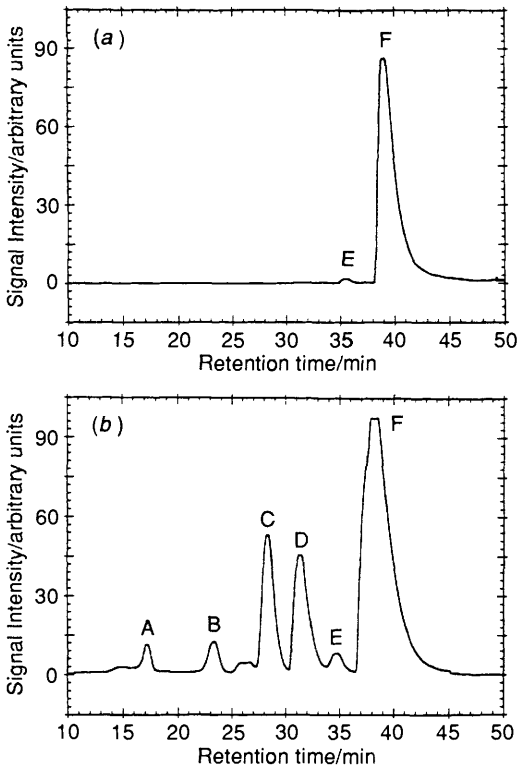


Fig. 2 HPLC of canthaxanthin (**I**) dissolved in (*a*) carbon tetrachloride and (*b*) dichloromethane

carbonate, especially for experiments involving porphyrin.[20] In our experiments dichloromethane treated with anhydrous potassium carbonate (hereafter referred as neutralized dichloromethane) was used for the following experiments. The optical spectrum of canthaxanthin in neutralized dichloromethane was taken [Fig. 1(*b*)] and was very similar to the spectrum obtained in a number of other solvents. It is very important to note that the '*cis*-peak' is not present in the optical spectrum of canthaxanthin in neutralized dichloromethane. The HPLC separation indicates the above solution contains 98% *trans*- and 2% *cis*-isomer.

The experiments with neutralized dichloromethane suggest that small amount of acid present in HPLC grade dichloromethane is responsible for the formation of *cis*-isomers from the all-*trans*-canthaxanthin. Another set of experiments suggested a method of increasing the yield of *cis*-isomers. In our laboratory, preparative HPLC technique was used regularly to separate sufficient amounts (mg quantities) for spectroscopic investigation. The necessary amount of canthaxanthin was dissolved in dichloromethane and the solvent was evaporated under low pressure. The process is repeated two to three times to increase the concentration of the *cis*-isomers. Finally, the necessary amount of dichloromethane was added to dissolve the all-*trans*-canthaxanthin and the solution was used for separating *cis*-isomers.

In a number of experiments, an excess of hydrochloric acid (~1–4 mol dm^{-3}) was added to a solution of all-*trans*-canthaxanthin in dichloromethane. The optical spectrum (Fig. 3) contained an absorption in the region of 800–900 nm with a

Table 2 HPLC Data of canthaxanthin (**I**) in different solvents monitored at 465 nm

Solvent	Peak	Retention time/min	Peak area (%)	Probable isomer	Ref.
Carbon tetrachloride	E	35.4	1.2	*a*	
	F	39.3	98.8	all-*trans*	17
Dichloromethane	A	17.0	1.9	*a*	
	B	23.3	2.7	13-*cis*-13′-*cis*	18, 19
	C	28.4	14.0	13-*cis*	18, 19
	D	31.4	14.8	9-*cis*	18, 19
	E	34.6	1.2	*a*	
	F	38.4	62.1	all-*trans*	18, 19
Acetonitrile	F	37.7	100.0	all-*trans*	18, 19

[a] Not characterized.

Table 3 ^{1}H NMR chemical shifts (in ppm) for canthaxanthin (**I**) and 8′-apo-β-caroten-8′-al (**III**) isomers (solvent $CDCl_3$)

Compound	Olefinic protons							Methyl protons				Methylene protons		
	7 7′	8 8′	10 10′	11 11′	12 12′	14 14′	15 15′	1 1′	5 5′	9 9′	13 13′	2 2′	3 3′	4 4′
Canthaxanthin														
all-*trans*	6.24	6.37	6.30	6.65	6.43	6.30	6.68	1.20	1.87	2.00	2.00	1.88	2.51	—
13-*cis*	6.25 6.23	6.37	6.33 6.27	6.65 6.64	6.97 6.43	6.16 6.27	6.82 6.57	1.19	1.87	1.99	1.99	1.85	2.51	—
9-*cis*	6.23	6.90 6.35	6.21 6.27	6.71 6.64	6.37 6.43	6.27	6.66	1.19	1.90	1.97	2.00	1.86	2.52	—
8′-Apo-β-caroten-8′-al														
all-*trans*	6.21 —	6.14 —	6.16 6.94	6.72 6.63	6.36 6.73	6.27 6.55	6.77 6.69	1.03 —	1.72 —	1.98 1.90	2.00	1.47 —	1.61 —	2.03 —
13-*cis*[a]	6.23 —	6.15 —	6.21 6.95	6.72 6.69	6.89 6.74	6.13 6.43	6.93 6.57	1.04 —	1.73 —	1.99 1.90	1.99 2.01	1.48 —	1.63 —	2.03 —

[a] 360 MHz. All other spectra at 500 MHz.

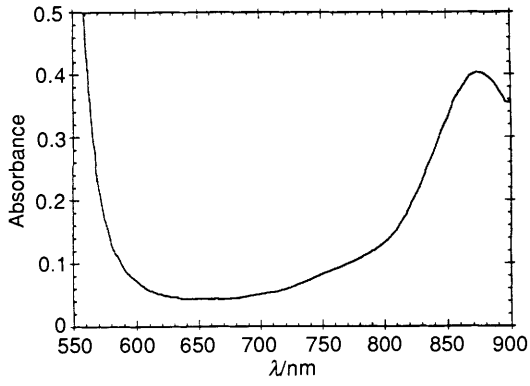


Fig. 3 Absorption spectrum (550–900 nm) of canthaxanthin (**I**) in highly acidic (~ 1.0 mol dm^{-3}) dichloromethane solution

peak at 871 nm. This spectrum is very similar to the optical spectrum of the electrochemically generated radical cation of canthaxanthin.[11] The optical spectrum in the 800–900 nm region lasted for nearly an hour. An estimate of the concentration of the radical cation in the above experiment is 4 μmol dm^{-3} with an estimated[21] extinction coefficient of ~ 10^5 dm^3 mol^{-1} cm^{-1}. Experiments were done with highly acidic acetonitrile (dielectric constant = 36) and the absorption due to radical cation was not seen. It is known[7] that the radical cation of **I** decays rapidly in the presence of even a small amount of water. A small amount of water present in acetonitrile may react with the radical cation and the absorption could not be seen in our experiments. When HPLC separation was done on this highly acidic canthaxanthin in dichloromethane solution, we observed a number of components with retention times less than 10 minutes. The low retention time components may be the degradation products of the radical cation of **I** and are currently under investigation.

β-*Carotene* (**II**).—The optical spectrum of β-carotene was measured in benzene, cyclohexane, carbon tetrachloride, acetonitrile and dichloromethane. The characteristics of the optical spectrum are quite different in dichloromethane, compared to those with other solvents. The optical spectrum of β-carotene in dichloromethane taken after two hours of the dissolution is shown in Fig. 4(*a*). The concentrated (~ 1 mmol dm^{-3}) solution is diluted before taking the optical spectrum. Although the fine vibrational structure in the 400–500 nm range was not seen, there was a significant contribution in the '*cis*-peak' region and a new absorption was observed in the 600–800 nm range (λ_{max} 735 nm). Recently, Pesek *et al.*[22] reported the formation of *cis*-isomers of β-carotene in dichloromethane and chloroform and they did not offer an explanation for the isomerization.

HPLC techniques were employed to separate any *cis*-isomers and it was found that 30% of the all-*trans*-β-carotene in dichloromethane solution had been converted to *cis*-isomers. The NMR studies of the *cis*-isomers are not yet completed. Since acidity was previously responsible for isomerization of canthaxanthin, β-carotene was dissolved in neutralized dichloromethane and the optical spectrum obtained is shown in Fig. 4(*b*). The spectrum contains the vibrational progression, does not contain absorption due to *cis*-isomers, and there is no absorption in the 600–800 nm range (λ_{max} 735 nm). The origin of the optical absorption in the 600–800 nm range (λ_{max} 735 nm) is discussed below.

It is known[23] in optical spectroscopy of astaxanthin and other carotenoids that the presence of hydrochloric acid gives an absorption in the 600–800 nm range (λ_{max} 648 nm) and is attributed to the formation of a degradation product. In the

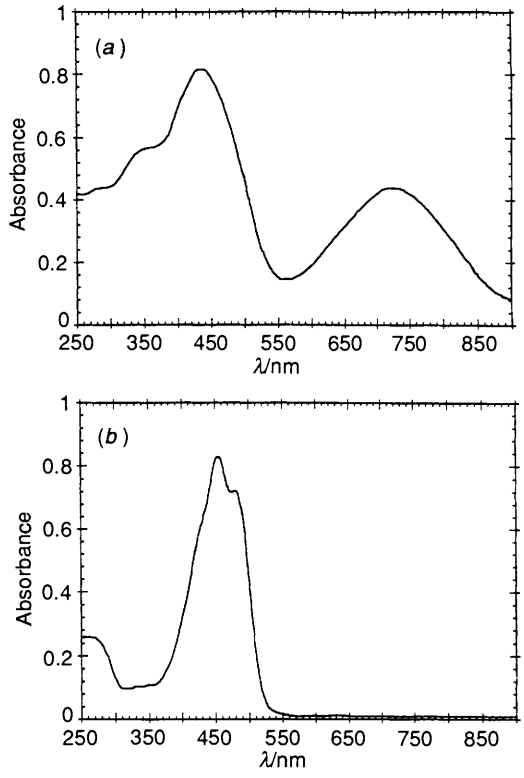


Fig. 4 Absorption spectrum of β-carotene (**II**) in (*a*) dichloromethane and (*b*) neutralized dichloromethane

presence of trifluoroacetic acid the absorption moves to even higher wavelength (λ_{max} 840 nm). In the latter experiment, the absorption is attributed to a charge-transfer (CT) complex. From our spectroelectrochemical work,[24] we can suggest that the absorption in the latter experiment is indeed due to radical cation of astaxanthin. Mallik *et al.*[25] performed optical spectroscopy of β-carotene solid in the presence of the vapours of nitric acid, iodine, bromine and iodine chloride. The optical spectrum in the presence of nitric acid vapour also showed absorption in the 600–800 nm range (λ_{max} 720 nm) and in the presence of all other vapours there was an absorption in the 700–900 nm range (λ_{max} 865 nm for iodine, 885 nm for bromine and 901 nm for iodine chloride). They suggest that the absorption in all the cases is due to a CT complex. From the work of Ding *et al.*,[26] it is known that the latter absorption is due to cation radical of β-carotene. The former result (λ_{max} 720 nm) corroborates our result (λ_{max} 735 nm). The above results suggest that the optical absorption in the range of 600–800 nm (λ_{max} 735 nm) of the β-carotene in dichloromethane solution may be due to a CT complex of acidic dichloromethane with β-carotene. In this paper the absorption due to a species in the 600–800 nm region (λ_{max} 735 nm) is addressed as an intermediate involving hydrogen ion, dichloromethane and β-carotene.

Craft and Soares[27] studied the stability of β-carotene in different solvents and they found out that β-carotene degraded quickly in dichloromethane and cyclohexanone when compared to other solvents. The following experiments were carried out to monitor the formation and decay of the intermediate in dichloromethane solution, which may explain Craft and Soares' results. The optical spectrum of β-carotene in neutralized dichloromethane does not contain the absorption due to the intermediate, but exhibits resolved vibrational structure due to the neutral carotenoid. When β-carotene was dissolved in dichloromethane containing a trace of HCl, the absorption spectrum contained the higher wavelength (485 nm) vibrational structure. After 4 minutes, the vibrational structure broadened and a structure appeared at the lower wavelength. When the

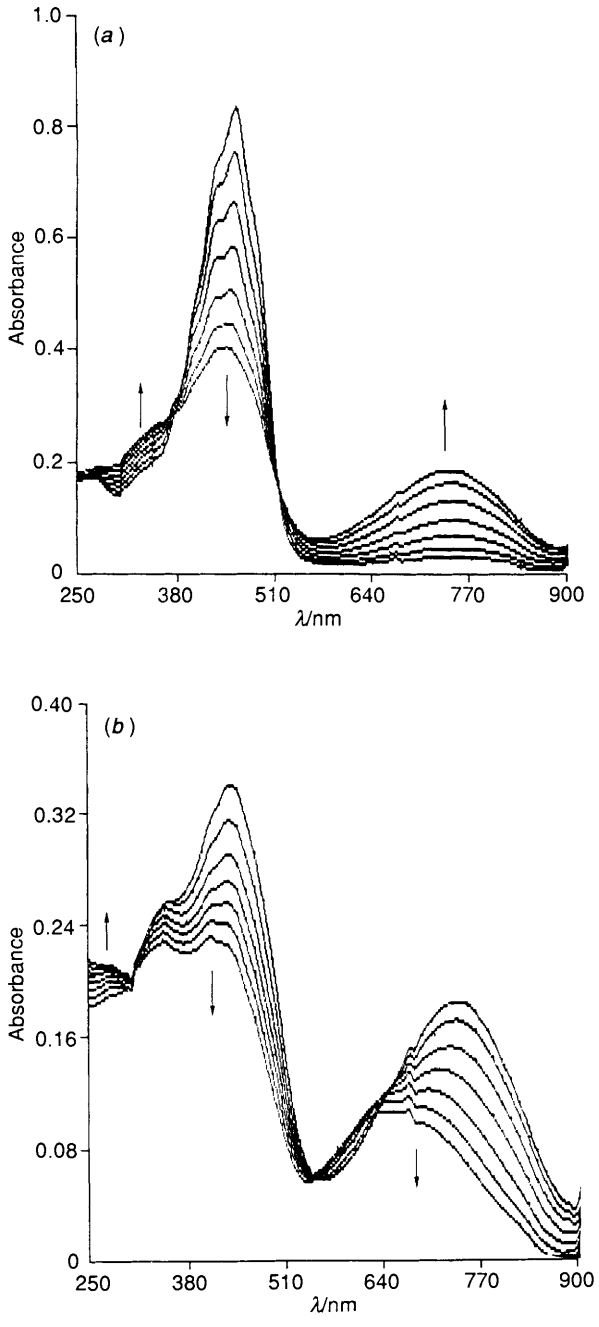


Fig. 5 (*a*) Formation of an intermediate of β-carotene (**II**) in dichloromethane at three minutes intervals and (*b*) decay of the intermediate of β-carotene (**II**) in dichloromethane in three minutes intervals

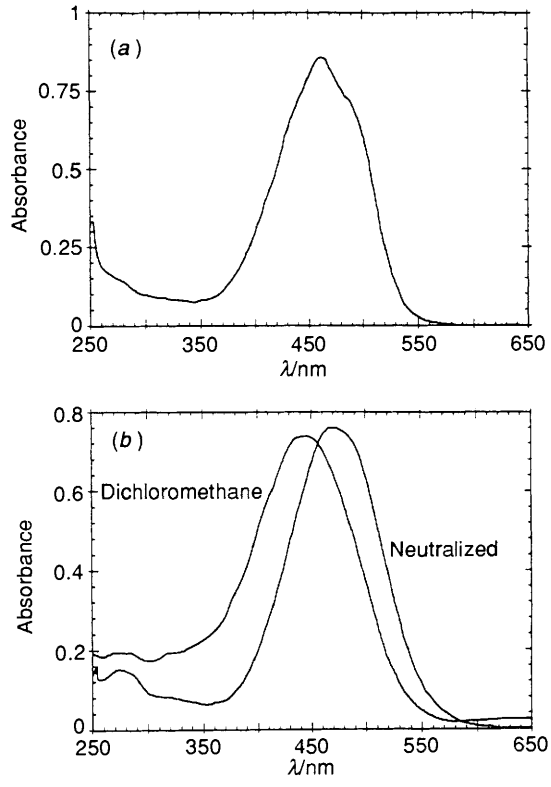


Fig. 6 Absorption spectrum of 8′-apo-β-caroten-8′-al (**III**) in (*a*) carbon tetrachloride and (*b*) dichloromethane and neutralized dichloromethane

spectrum was taken from 6 minutes onwards with an interval of 3 minutes, the formation of the intermediate was clearly seen. The spectra are shown in Fig. 5(*a*). When the intensity of the peak in the 470 nm range decreased, the spectral intensity in the wavelength region 250–300 nm and 600–800 nm increased.

After 30 minutes, the intensity of the absorption at 470 nm and 600–800 nm range began to decrease [Fig. 5(*b*)]. Apparently, the rate of decay of the intermediate supersedes the rate of formation of the intermediate. The optical spectra are shown in Fig. 6(*b*). Within two hours, the primary peak at 470 nm and the absorption due to the intermediate in the 600–800 nm range completely vanished giving rise to a spectrum with an absorption in the 250–400 nm range. The above set of experiments were done in complete darkness. These experiments suggest that when β-carotene solution was prepared in HPLC grade dichloromethane containing 0.1–0.2 mmol dm $^{-3}$ of acid, the sample may degrade through the formation of the intermediate. When β-carotene was dissolved in neutralized dichloromethane, there was no formation of the intermediate as well as no decay of the primary peak at 470 nm of β-carotene [Fig. 4(*b*)] for more than four hours.

Recently, Truscott *et al.*[28] reported the optical absorption spectrum of β-carotene in the ZSM-5 zeolite. They observed an absorption in the range of 600–900 nm and attributed the absorption to the forbidden $S_0 \longrightarrow S_1$ transition. Previously, Wolf *et al.*[29] reported the $S_0 \longrightarrow S_1$ transition (λ_{max} 680 nm) of β-carotene single crystal using the Raman excitation profile. From our present study and previous ENDOR results,[30] it is necessary to make a few comments on the paper by Truscott *et al.*[28] ZSM-5 Zeolite contains Bronsted and Lewis sites and the concentration of these sites depends on the Si/Al ratio.[31] The shift in the position of the $S_0 \longrightarrow S_2$ transition and the observation of the transition in the range 600–900 nm are probably due to the interaction of Bronsted acidic sites with β-carotene. The spectrum reported in ref. 28 is very similar to Fig. 5(*a*). Truscott *et al.*[28] also reported that they could not generate the cation radicals in ZSM-5 zeolite. However, it is possible to generate the cation radicals by using the method described in ref. 30. It may be concluded from the above discussion that it is very unlikely that the absorption reported in ref. 28 is due to the $S_0 \longrightarrow S_1$ transition. The single photon absorption spectroscopic technique[32] used to observe the $S_0 \longrightarrow S_1$ transition of neurosporene in solution would be the appropriate tool to observe the $S_0 \longrightarrow S_1$ transition of β-carotene.

8′-*Apo*-β-*caroten*-8′-*al* (**III**).—The optical and HPLC separation methods used for compound **III** are similar to those of β-carotene. The optical spectra of **III** in carbon tetrachloride and in dichloromethane are shown in Fig. 6(*a*) and (*b*), respectively. The spectrum of **III** in dichloromethane appeared to have the

Table 4 HPLC Data of 8′-apo-β-caroten-8′-al (**III**) in different solvents monitored at 457 nm

Solvent	Peak	Retention time/min	Peak area (%)	Probable isomer	Ref.
Carbon tetrachloride	B	20.5	2.2	13-*cis*	20
	E	29.1	96.8	all-*trans*	17
Dichloromethane	A	17.3	12.0	13′-*cis*-11′-*cis*	33
	B	19.8	15.6	13-*cis*	20
	C	23.9	1.7	*a*	
	D	25.1	1.5	*a*	
	E	28.3	68.8	all-*trans*	17
Acetonitrile	B	20.2	5.8	13-*cis*	20
	E	28.5	91.5	all-*trans*	17

[a] Not characterized.

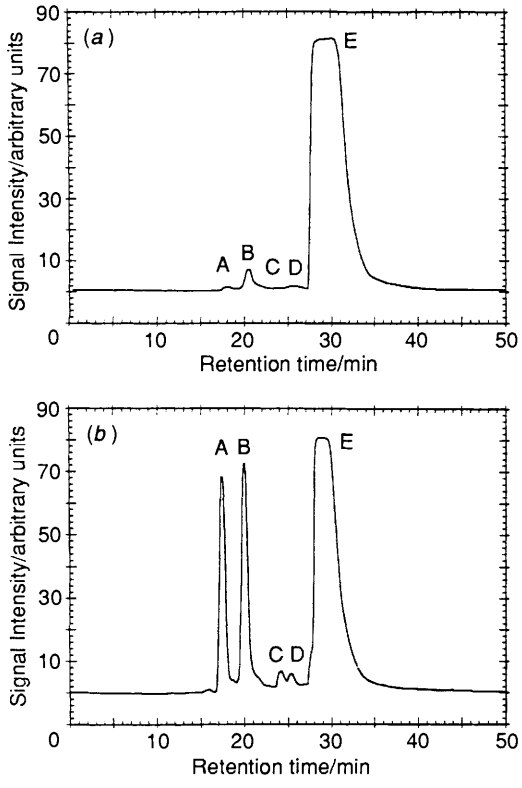


Fig. 7 HPLC of 8′-apo-β-caroten-8′-al (**III**) dissolved in (*a*) carbon tetrachloride and (*b*) dichloromethane

'*cis*-peak'. The HPLC separations of a solution of **III** prepared in carbon tetrachloride and dichloromethane are shown in Fig. 7(*a*) and (*b*), respectively. The spectrum in Fig. 7(*b*) clearly shows that 30% of the all-*trans*-isomer is converted into *cis*-isomers in dichloromethane. The results are given in Table 4. The isomers were separated and characterized by optical and NMR spectroscopic techniques. The 13-*cis*- and 13′-*cis*-11′-*cis*-isomers are the predominant isomers. The NMR data of all-*trans*- and 13-*cis*-isomers are given in Table 3.

The optical spectrum of **III** in dichloromethane in the range of 550–900 nm was also measured. The spectrum (Fig. 8) contained a weak absorption ($A \sim 0.25$) in the 600–750 nm range (λ_{max} 632 nm). The nature of the absorbing species may be an intermediate similar to that of β-carotene.

The above experiments involving carotenoids in a number of solvents highlight the special nature of the dichloromethane solvent. Also, in the electrochemical literature, the solvent of choice for cation radical formation is dichloromethane. The stability of cation radicals in dichloromethane may be attributed to two primary characteristics. (1) Dichloromethane contains 0.1–0.2 mmol dm^{-3} acid so the pH of the solution is lower than the p*K*a

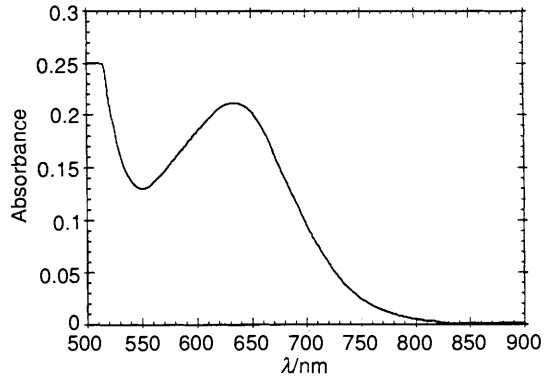


Fig. 8 Absorption spectrum (intermediate) of 8′-apo-β-caroten-8′-al (**III**) in dichloromethane

of the cation radical (Scheme 2). (2) The solubility of water in dichloromethane is very low, preventing one of the important decay paths of the cation radicals (Scheme 3).

$$Car^{\bullet+} \overset{K_a}{\rightleftharpoons} \underset{\text{(less H atom)}}{Car^{\bullet}} + H^+$$

Scheme 2

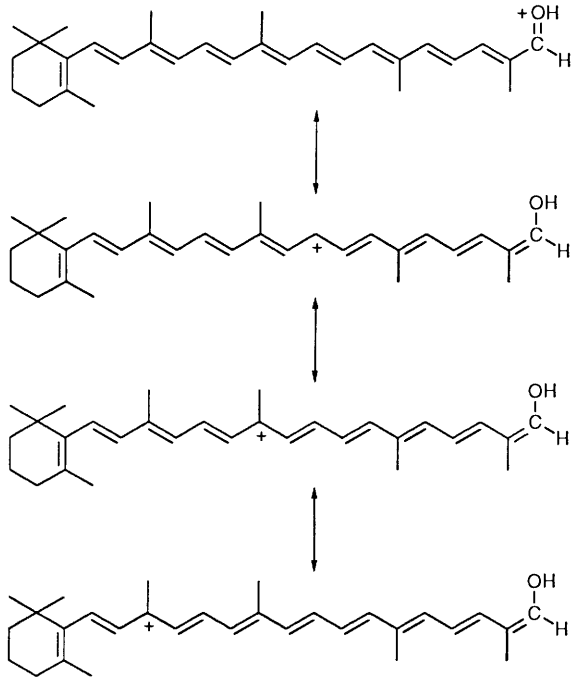
$$Car^{\bullet+} + H_2O \longrightarrow \text{Products}$$

Scheme 3

NMR Spectral Data Analysis.—There are several different types of protons in the molecular structure of carotenoids. The diagnostic NMR spectral data (Table 3) come from the olefinic protons on the central polyene structure. Fourteen protons in the conjugated backbone in canthaxanthin are separated by the intervening methyl groups into five blocks which have two, three, four, three and two protons. Twelve protons in the conjugated backbone in 8′-apo-β-caroten-8′-al have four blocks except the last two protons. Vicinal and/or long range couplings are expected to take place in each block. In the case of the all-*trans*-canthaxanthin, the molecule is symmetric, so the protons in the two halves of the molecule are equivalent. The NMR signals of the seven vinyl protons in one half of the central polyene chain are classified into three groups. They are (i) two doublets (7-H and 8-H), (ii) two doublets (10-H and 12-H), one doublet-doublet (11-H) and (iii) a pair of sextets (14-H and 15-H). The vicinal coupling constants of *J* (CH=CH) and *J* (CH–CH) are in the ranges 15–16 and 11–12 Hz.[34] In the above discussion, the olefinic protons are referred to as *n*-H, where *n* is the number of the carbon atom to which the particular proton is attached.

From the known chemical shift values for the all-*trans*-canthaxanthin (**I**) and 8′-apo-β-caroten-8′-al (**III**) and the isomerization shift data of different *cis*-isomer structures,[35] the chemical shifts of the protons of the *cis*-isomers of **I** and **III** listed in Table 3 are assigned.

Possible Protonation Sites Leading to Isomerization.—It is experimentally shown that **I**, **II** and **III** form geometrical isomers in dichloromethane containing 0.1–0.2 mmol dm^{-3} of acid. AM1 calculations are carried out for the protonated carotenoids and the possible sites for the isomerization reactions were explored. Qualitatively, one can draw equivalent structures of a protonated carotenoid (Scheme 4). 8′-Apo-β-caroten-8′-al (**III**) is taken as an example for the protonated carotenoid.



Scheme 4

An increase in positive charge at the site of isomerization (C-9, 13, 15 and not shown C-13′ and 9′) is observed. Thus structures of **I**, **II** and **III** and their protonated analogues are geometry optimized by AM1-RHF using the HYPERCHEM software. The bond lengths and bond angles of **I**, **II** and **III** are similar to that of the data found using X-ray crystallography.[36] The AM1 charge densities are given for the respective ground state structures in Fig. 9. In the case of **I**, a proton is added at one of the carbonyl oxygen atoms and the structure was geometry optimized by AM1-RHF. The AM1 charge densities are given for the protonated compound **I** in Fig. 9(*a*). From symmetry, the charge densities can be deduced if the other oxygen is protonated. If the charges are compared between the ground state and the protonated compound **I**, it is seen that at positions 9, 11, 13, 15, 14′, 12′, 10′, 8′ and 6′ there is more positive charge density in the protonated species ($\Delta\rho$ = 0.04, 0.06, 0.09, 0.11, 0.14, 0.17, 0.21, 0.20 and 0.18 increase, respectively) than the ground state. The above observation suggests that free rotation around the carbon atoms with more positive charge density is possible, giving rise to different geometrical isomers. For instance, more positive charge at positions 9, 13, 15, 14′, 10′ and 8′ yields the 9-*cis*, 13-*cis*, 15-*cis*, 13′-*cis*, 9′-*cis* and 7′-*cis*-isomers, respectively. However, the total energies of the 7′-*cis* and 7-*cis*-13′-*cis*-isomers calculated by AM1 occur at ~46 kcal mol^{-1} above those for the all-*trans*-isomer. The energies of 15-*cis*, 9-*cis*-15-*cis* and 13-*cis*-15-*cis*-isomers are calculated to occur at 1.6 kcal mol^{-1} above the all-*trans*-isomer, while the 9-*cis*, 13-*cis*, 9-*cis*-13-*cis*, 9-*cis*-13′-*cis*-isomers are all within 0.1 kcal mol^{-1} of the lowest all-*trans* structure, the 13-*cis* being the lowest in energy. Experimentally, we observed and identified by NMR techniques the formation

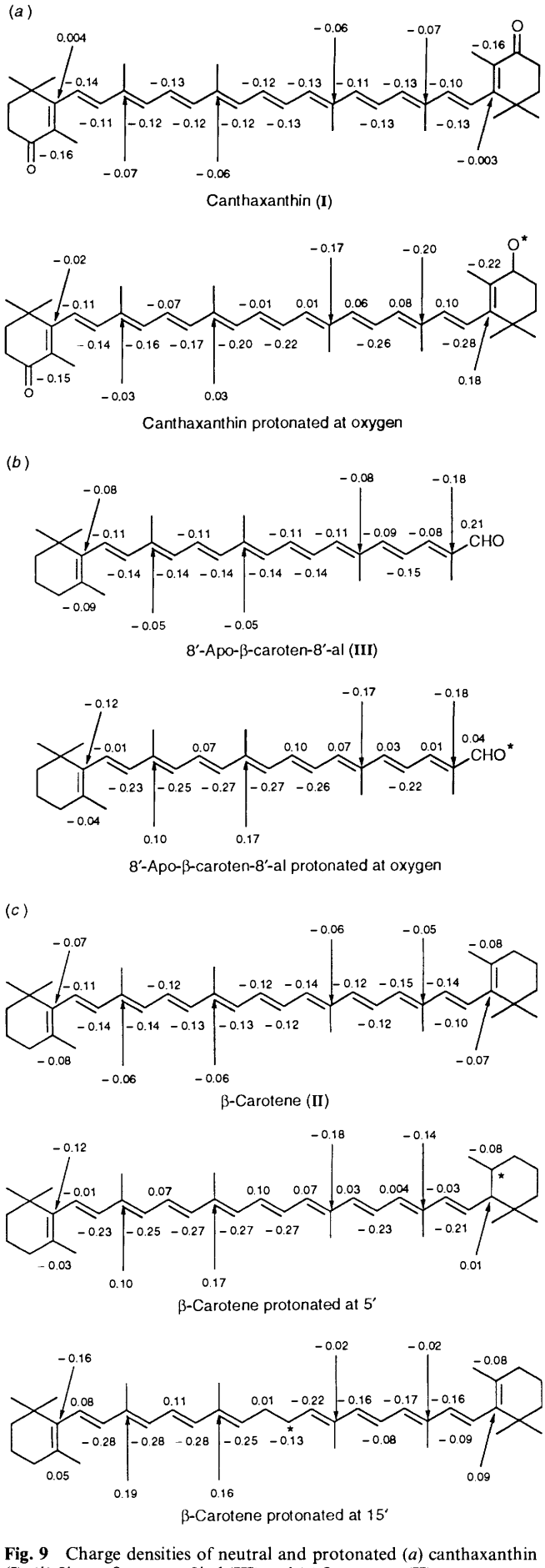


Fig. 9 Charge densities of neutral and protonated (*a*) canthaxanthin (**I**), (*b*) 8′-apo-β-caroten-8′-al (**III**) and (*c*) β-carotene (**II**)

of the 9- and 13-*cis*-isomers in greater yield than the other *cis*-isomers. Large quantities of *cis*-isomers were not detected by NMR.

Similar calculations were also performed for compounds **III** and **II** and the results are given in Figs. 9(*b*) and (*c*), respectively. In the case of **III**, the proton is added at the carbonyl oxygen atom [Fig. 9(*b*)]. If the AM1 charge densities are compared between the ground state and the protonated compound **III**, it is seen that at positions 9 and 13, there is more positive charge density (an increase of 0.15 and 0.22, respectively) in the protonated species than the ground state, the largest increase occurring at carbon 13. The difference between the AM1 calculated total energy of the 13-*cis*, 9-*cis*-13-*cis*, 9-*cis*, 9-*cis*-13′-*cis* and 13′-*cis*-11′-*cis*-isomers and the all-*trans*-isomer of **III** is less than 0.2 kcal mol^{-1} with the 13-*cis* the lowest in energy. In our experiments we identified by NMR the formation of 13′-*cis*-11′-*cis*-isomer[33] [peak A in Fig. 7(*b*)] and 13-*cis*-isomer [peak B in Fig. 7(*b*)] as major peaks. The 9-*cis*, 9-*cis*-13-*cis* and 9-*cis*-13′-*cis*-isomers are also predicted to occur. However, peak A showed an NMR signal due to 9-*cis* and *cis*,*cis*-isomers which have been very difficult to separate and assign at this time. The 15-*cis*, 9-*cis*-15-*cis* and 13-*cis*-15-*cis*-isomers are predicted to occur at 1.6 kcal mol^{-1} above the all-*trans*-isomer, while the 7-*cis* and 7-*cis*-13′-*cis* are predicted by AM1 calculation to occur at 48.4 kcal mol^{-1} above the all-*trans*-isomer. These isomers are not observed in our experiments in agreement with the AM1 calculations.

In the case of compound **II**, a proton is added at the 15′ position and also at the 5′ position. If the AM1 charge densities are compared between the ground state and 15′ protonated compound **II**, it is seen that at positions 9 and 13 there is more positive charge density (an increase of 0.25 and 0.22, respectively) in the protonated species than the ground state. In the case of 5′ protonated compound **II**, it is seen that at positions 9, 13, 15, 14′ and 10′ there is more positive charge density (an increase of 0.16, 0.23, 0.22, 0.21 and 0.15, respectively) in the protonated species than the ground state. The difference between the AM1 calculated total energy of 9-*cis*, 9-*cis*-13-*cis* and 9-*cis*-13′-*cis*-isomers and the all-*trans*-isomer of **II** is less than 0.2 kcal mol^{-1} and would favour *cis*-isomer formation. The 13-*cis*-15-*cis*, 15-*cis* and 9-*cis*-15-*cis* were calculated to occur at 1.6 kcal above the all-*trans*, while the 7-*cis* and 7-*cis*-13′-*cis* were predicted to occur at 31.0 and 31.4 kcal above the all-*trans*, respectively. Experimentally, the 9-*cis*, 13-*cis*, 9-*cis*-13-*cis*, 9-*cis*-13′-*cis* and possibly other *cis*-isomers were observed by HPLC in more or less similar concentrations possibly due to the presence of several sites for protonation.

Conclusion

The carotenoids **I**, **II** and **III** form *cis*-isomers in solution due to 0.1–0.2 mmol dm^{-3} acid present in HPLC grade dichloromethane. In addition, the carotenoids **II** and **III** form intermediates in dichloromethane and the mode of decay of **II** and **III** may be through the formation of these intermediates. In addition, carotenoids **I**, **II** and **III** form radical cations in highly acidic (~1 mol dm^{-3} HCl) dichloromethane solution. The formation of *cis*-isomers, the intermediate and the radical cation formation are detected by their characteristic optical spectra. The *cis*-isomers were separated by HPLC and identified by optical and NMR spectroscopic techniques. AM1 calculations were carried out on the ground state and the protonated carotenoids. Noting the position where the increase in positive charge occurred upon protonation of the carotenoids and relative energies of the *cis*-isomers, AM1 calculations predict the formation of 9-*cis* and 13-*cis* isomers in greater yield for **I** than the other *cis*-isomers. The acid induced *cis* isomerization may be a very clean way to form selected carotenoid *cis*-isomers.

Acknowledgements

This work was supported by the Division of Chemical Sciences, Office of Basic Energy Sciences, Office of Energy Research of the U.S. Department of Energy under Grant No. DE-FG05-86ER13465. Drs. C. Devadoss of University of Illinois at Urbana-Champaigne, M. R. Wasielewski of Argonne National Laboratory, W. P. Jencks of Brandeis University, E. S. Hand and R. M. Metzger are thanked for useful discussions. Dr. M. M. Khaled is thanked for carrying out the AM1 calculations of the *cis*-isomers.

References

1 Y. Koyama, *J. Photochem. Photobiology*, 1991, **B9**, 265 and refs. therein.
2 H. A. Frank, C. A. Violette, J. K. Trautman, A. P. Shreve, T. G. Owens and A. C. Albrecht, *Pure Appl. Chem.*, 1991, **63**, 109.
3 P. Mathis and A. W. Rutherford, *Biochim. Biophys. Acta*, 1984, **767**, 217.
4 G. W. Burton and K. U. Ingold, *Science*, 1984, **224**, 569.
5 W. von E. Doering and T. Kitagawa, *J. Am. Chem. Soc.*, 1991, **113**, 4288.
6 M. Khaled, A. Hadjipetrou, L. D. Kispert and R. D. Allendoerfer, *J. Phys. Chem.*, 1991, **95**, 2438.
7 M. Khaled, Ph. D. Dissertation, University of Alabama, Tuscaloosa, 1992.
8 J. Phelps and A. J. Bard, *J. Electroanal. Chem.*, 1976, **68**, 313.
9 D. Gust, T. A. Moore, A. L. Moore, S. J. Lee, E. Bittersmann, D. K. Luttrull, A. A. Rehms, J. M. DeGraziano, X. C. Ma, F. Gao, R. E. Belford and T. T. Trier, *Science*, 1990, **248**, 199 and refs. therein.
10 K. Hasharoni, H. Levanon, J. Tang, M. K. Bowman, J. R. Norris, D. Gust, T. A. Moore and A. L. Moore, *J. Am. Chem. Soc.*, 1990, **112**, 6477.
11 J. L. Grant, V. J. Kramer, R. Ding and L. D. Kispert, *J. Am. Chem. Soc.*, 1988, **110**, 2155.
12 Y. Wu, L. Piekara-Sady and L. D. Kispert, *Chem. Phys. Lett.*, 1991, **180**, 573.
13 M. Khaled, A. Hadjipetrou and L. D. Kispert, *J. Phys. Chem.*, 1990, **94**, 5164.
14 A. S. Jeevarajan, M. Khaled, M. D. E. Forbes and L. D. Kispert, *Z. Phys. Chem., Neue Folge (Frankfurt)*, 1993, in the press.
15 L. Zechmeister, A. L. Le Rosen, W. A. Schroeder, A. Polgar and L. Pauling, *J. Am. Chem. Soc.*, 1943, **65**, 1940.
16 B. DeCoster, R. L. Christensen, R. Gebhard, J. Lugtenburg, R. Farhoosh and H. A. Frank, *Biochem. Biophys. Acta*, 1992, **1102**, 107.
17 W. Vetter, G. Englert, N. Rigassi and U. Schwieter, *Carotenoids*, ed. O. Isler, Birkhauser Verlag, Basel, Switzerland, 1971, p. 228.
18 H. Hashimoto, Y. Koyama and T. Shimamura, *J. Chromatogr.*, 1988, **448**, 182 and refs. therein.
19 K. Ouderkirk, K. Chen, E. Hand and L. D. Kispert, unpublished work.
20 D. Gust, T. A. Moore, A. L. Moore, F. Gao, D. Luttrull, J. M. De Graziano, X. C. Ma, L. R. Makings, S. J. Lee, T. T. Trier, E. Bittersmann, G. R. Seely, S. Woodward, R. V. Bensasson, M. Rougee, F. C. De Schryver and M. V. Auweraer, *J. Am. Chem. Soc.*, 1991, **113**, 3638.
21 J. Lafferty, A. C. Roach, R. S. Sinclair, T. G. Truscott and E. J. Land, *J. Chem. Soc., Faraday Trans. 1*, 1977, **73**, 416.
22 C. A. Pesek, J. J. Warthesen and P. S. Taoukis, *J. Agric. Food Chem.*, 1990, **38**, 41.
23 M. Buchwald and W. P. Jencks, *Biochemistry*, 1968, **7**, 834.
24 A. S. Jeevarajan, L. D. Kispert and Xiangli Wu, *Chem. Phys. Lett.*, 1994, in the press.
25 B. Mallik, K. M. Jain and T. N. Misra, *Biochem. J.*, 1980, **189**, 547.
26 R. Ding, J. L. Grant, R. M. Metzger and L. D. Kispert, *J. Phys. Chem.*, 1988, **92**, 4600.
27 N. E. Craft and J. H. Soares, Jr., *J. Agric. Food Chem.*, 1992, **40**, 431.
28 J. L. Haley, A. N. Fitch, R. Goyal, C. Lambert, T. G. Truscott,

J. N. Chacon, D. Stirling and W. Schalch, *J. Chem. Soc., Chem. Commun.*, 1992, 1175.
29 K. Gaier, A. Angerhofer and H. C. Wolf, *Chem. Phys. Lett.*, 1991, **187**, 103.
30 A. S. Jeevarajan, L. D. Kispert and L. Piekara-Sady, *Chem. Phys. Lett.*, 1993, **209**, 269.
31 M. Crocker, R. H. M. Herold, M. H. W. Sonnemans, C. A. Emeis, A. E. Wilson and J. N. van der Moolen, *J. Phys. Chem.*, 1993, **97**, 432.
32 M. Mimuro, U. Nagashima, S. Nagaoka, S. Takaichi, I. Yamazaki, Y. Nishimura and T. Katoh, *Chem. Phys. Lett.*, 1993, **204**, 101.
33 Y. Miki, T. Kameyama, Y. Koyama and Y. Watanabe, *J. Phys. Chem.*, 1993, **97**, 6142.
34 Y. Koyama, M. Hosomi, H. Hashimoto and T. Shimamura, *J. Mol. Struct.*, 1989, **93**, 185.
35 H. Kläui, *Carotenoid Chemistry and Biochemistry*, eds. G. Britton and T. W. Goodwin, Pergamon Press, New York, 1981, p. 309.
36 M. O. Senge, H. Hope and K. M. Smith, *Z. Naturforsch., Teil C*, 1992, **47**, 474.

Paper 3/06046B
Received 11*th October* 1993
Accepted 18*th November* 1993