Selected *cis/trans* isomers of carotenoids formed by bulk electrolysis and iron(III) chloride oxidation

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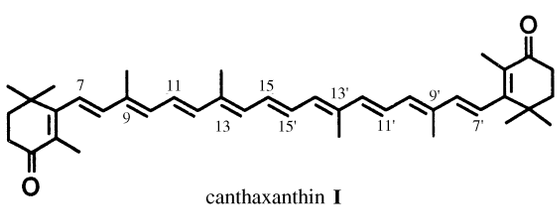
Bulk electrolysis and chemical oxidation with $FeCl_3$ of all-*trans* canthaxanthin (I) and 8′-apo-β-caroten-8′-al (II) gave primarily the 9- and 13-*cis*-isomers, which were separated by HPLC and identified by ^{1}H NMR spectroscopy. Optical absorption measurements showed that the 15-*cis*, 9,13-di-*cis* isomers of I are also formed by these methods. In the case of the unsymmetrical compound II, additional isomers were formed. The *cis* isomers account for about 40–60% of products formed. Formation of the isomers is believed to occur by rotation about certain bonds in the cation radicals or dications, which are formed in the oxidation processes. The neutral *cis* species are then formed by an electron exchange reaction of the *cis*-cation radicals with neutral all-*trans* carotenoids in solution. The electrochemical and iron(III) chloride oxidation induced isomerization are shown to be efficient and improved methods for forming selected carotenoid isomers.

Introduction

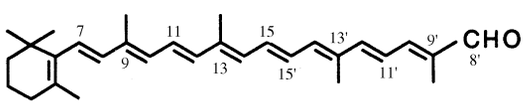
Carotenoids (Car) are naturally occurring, intensely coloured, pigments formed in numerous photosynthetic plants and algae where they serve the two important functions of light harvesting and photoprotection.[1,2] Approximately 600 naturally occurring carotenoids have been isolated and identified.[3] Although higher organisms cannot synthesize these compounds, the carotenoids are essential for their survival. It has been suggested that carotenoids may also protect against cancers[4] probably due to their antioxidant and free radical quenching properties.[5] Further, geometrical configuration is known to affect the biochemistry of carotenoids. For example, in purple bacteria, the natural selection of the carotenoid configurations has been found. The 15-*cis* carotenoids are selected in the reaction centres but the all-*trans* isomers are present in the light-harvesting complexes of photosynthetic organisms.[1] Geometrical isomers of carotenoids may have physiological or metabolic roles that are still being investigated. Facile and/or improved methods of preparation and isolation of isomers are therefore of considerable importance.

Carotenoids are generally present in the most stable form, in which all double bonds have the *trans* configuration. Numerous methods are available for partial geometrical isomerization of an all-*trans* carotenoid or any of its *cis*-isomers, such as heating of solid samples,[6] irradiating solutions of carotenoids in the presence of iodine (2%),[6] or treating the compounds with dichloromethane containing *ca*. 0.1 mM acid.[7] The resulting mixtures generally consist of the all-*trans*-compound and two or three predominant *cis*-isomers, referred to as the main or preferred isomers, in which steric interactions are similar to those in the all-*trans* geometrical isomers.[6,8] This is the case for configurational changes of the double bonds at positions 9, 13 and/or 15. The isomers can usually be separated by appropriate chromatographic techniques, and the location of the *cis*-double bonds can be established by ^{1}H NMR or optical spectroscopy. The presence of *cis* double bonds results in a hypsochromic shift of the main absorption band in the visible spectrum as well as a decrease in its intensity.[9] The typical optical absorption spectrum of a *trans*-carotenoid consists of strong absorption in the visible region (λ_1) and a weak absorption (λ_3) in the UV region. In the case of *cis*-isomers, a strong λ_1 band, a weak λ_2 band, the so-called '*cis* peak', and a very weak λ_3 band are usually present.

Here, we report that *cis/trans* isomerization can also be achieved by bulk electrolysis or by chemical oxidation with iron(III) chloride in dichloromethane, and these methods are an improvement over existing technologies for producing *cis* isomers of carotenoids. Optical absorption and/or ^{1}H NMR spectra of the materials formed from all-*trans* canthaxanthin (**I**) and 8′-apo-β-caroten-8′-al (**II**) and separated by HPLC showed



canthaxanthin **I**



8'-apo-β-caroten-8'-al **II**

the formation of *cis* isomers. The electrochemical and chemical oxidation induced isomerizations are shown to be efficient methods for forming selected carotenoid isomers.

Experimental

Chemicals

Canthaxanthin (**I**), 8′-apo-β-caroten-8′-al (**II**) and tetrabutylammonium hexafluorophosphate (TBAHFP) were purchased from Fluka (Buchs, Switzerland). According to HPLC, the sample of **I** consisted of 95% of the *trans*-isomer, that of **II** contained 99% of the *trans*-isomer. Both were used without further purification. Anhydrous dichloromethane and iron(III) chloride were obtained from Aldrich. Acetonitrile, diethyl ether and hexane of HPLC grade were obtained from Fisher.

Electrochemical set-up

Electrochemical oxidations were monitored using the Bio Analytical Systems BAS-100W electrochemical analyser. For cyclic voltammetry measurements, the working electrode was a platinum disk electrode (diam. = 1.6 mm), the auxiliary elec-

trode was a platinum wire and the pseudo-reference electrode was a Ag/AgCl electrode.

The electrochemical cell[15] divided into two sections was equipped with requisite quartz windows and used for electrochemical and simultaneous optical measurements. A 10 ml cylindrical undivided cell was used for bulk electrolysis, the working electrode was a platinum coil, the auxiliary electrode was a platinum wire and the pseudo-reference electrode was a silver wire. Dichloromethane solutions, prepared in a dry box under a nitrogen atmosphere just before use, were 0.1 M in TBAHFP and 0.3 to 0.5 mM in carotenoids. Stirred carotenoid solutions with TBAHFP were bulk electrolysed at 4 °C at the chosen potentials and then subjected to HPLC separation at 22 °C. Saturated stock solution of iron(III) chloride (180 μM) in dichloromethane was prepared at room temp. The chemical oxidation reactions were carried out by treating a 3 ml carotenoid solution with 130 μl iron(III) chloride solution.

Chromatographic separation

For the HPLC separations, a Shimadzu LC-600 pump equipped with a Rheodyne Model 7125 injector and a SPD-6AV UV–VIS detector (Columbia, MD, USA) was used. A Vydac 201 TP54 polymeric C18 column (250 × 4.6 mm id) packed with 5 μm particles (Separations Group, Hesperia, CA, USA) and 100% acetonitrile as the mobile phase were used for **I**. A LiChrosorb Si 60 column (250 × 4.0 mm id) packed with 10 μm particles (Merck, Darmstadt, Germany) and 5% diethyl ether in hexane as the mobile phase were used for **II**. The flow rate was 1 ml min^{-1}, and the detector was set at 465 nm for **I** and at 450 nm for **II**.

^{1}H NMR and optical absorption measurements

^{1}H NMR spectra of the major HPLC peaks isolated from the reaction mixtures were determined with a Bruker AM360 (^{1}H, 360.13 MHz) instrument at 25 °C and the optical absorption spectra were recorded with a double beam Shimadzu UV-1601 UV–VIS spectrophotometer (190–1100 nm) using 1 cm quartz cells.

Identification of geometrical isomers is based on the following criteria: (*i*) compared to the all-*trans*-species, *cis*-isomers show a hypsochromic shift of the main absorption band (λ_1).[8,9] (*ii*) Spectra of *cis*-isomers show a reduction in fine structure and in the absorption coefficient of the most intense band. (*iii*) *cis*-Isomers have an additional absorption band (λ_2), which is most intense for the central *cis* (C15) isomer. (*iv*) The position of the *cis*-double bonds affects the value of *Q*, which is defined as the absorbance ratio of the *cis* peak (λ_2) to the main absorption peak (λ_1).[10–14]

Results and discussion

It has been established[15–20] that electrochemical oxidation of carotenoids involves at least three heterogeneous (electrode) reactions [eqns. (1)–(3)] and three homogeneous (solution) reactions [eqns. (4)–(6)] which are shown in Scheme 1. Note that

Electrode reactions

$$\mathrm{Car} \overset{E^\circ_1}{\rightleftharpoons} \mathrm{Car}^{\bullet+} + \mathrm{e}^- \quad (1)$$

$$\mathrm{Car}^{\bullet+} \overset{E^\circ_2}{\rightleftharpoons} \mathrm{Car}^{2+} + \mathrm{e}^- \quad (2)$$

$$^*\mathrm{Car}^{+} + \mathrm{e}^- \overset{E^\circ_3}{\rightleftharpoons} {}^*\mathrm{Car}^{\bullet} \quad (3)$$

Homogeneous reactions

$$\mathrm{Car}^{2+} + \mathrm{Car} \overset{K_{com}}{\rightleftharpoons} 2\,\mathrm{Car}^{\bullet+} \quad (4)$$

$$\mathrm{Car}^{2+} \overset{K_{dp}}{\rightleftharpoons} {}^*\mathrm{Car}^{+} + \mathrm{H}^+ \quad (5)$$

$$\mathrm{Car}^{\bullet+} \overset{K'_{dp}}{\rightleftharpoons} {}^*\mathrm{Car}^{\bullet} + \mathrm{H}^+ \quad (6)$$

where *Car represents the carotenoid with one less proton.

Scheme 1

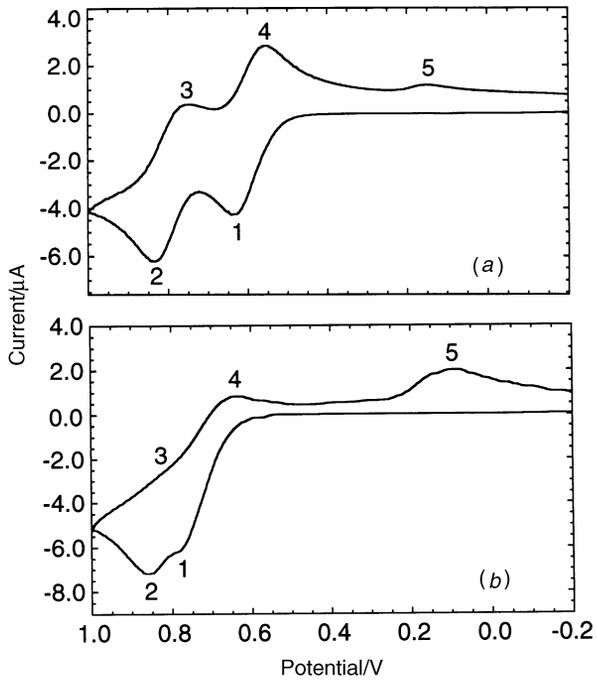


Fig. 1 Cyclic voltammograms of 1.0 mM of (*a*) all-*trans* canthaxanthin (**I**) and (*b*) 8′-apo-β-caroten-8′-al (**II**) in CH_2Cl_2 with 0.1 M TBAHFP as the supporting electrolyte. Scan rate: 100 mV s^{-1}.

carotenoid cation radicals are formed not only by a one-electron oxidation [eqn. (1)] but also in an equilibrium reaction of neutral carotenoid and its dication [eqn. (4)].

For canthaxanthin (**I**), the oxidation potentials of reactions 1 and 2 differ considerably so that exclusive formation of radical cations is possible by application of sufficiently low potentials. This is illustrated by the cyclic voltammogram (CV) shown in Fig. 1(*a*), where peaks 1 and 2 are due to reactions 1 and 2, peaks 3 and 4 are due to the reverse of reactions 2 and 1, respectively, and peak 5 results from reaction 3. The relative amplitudes of peaks 3 and 4 indicate that both the dication and the radical cation are fairly stable. In contrast, the analogous species formed from 8′-apo-β-caroten-8′-al (**II**) are much less stable [Fig. 1(*b*)], and the oxidation potentials for peaks 1 and 2 are more similar.

Simultaneous bulk electrolysis and optical absorption spectroscopy

Canthaxanthin (I). The optical absorption spectrum of all-*trans* canthaxanthin in dichloromethane with TBAHFP supporting electrolyte is shown in spectrum 1, Fig. 2(*a*). Like the spectra of other all-*trans* carotenoids, the spectrum contains a strong absorption maximum (λ_1) in the visible region and a weak one (λ_3) in the UV region. In the case of *cis*-isomers, a strong λ_1 band and a weak λ_2 band, the so-called '*cis* peak', are present.

In Fig. 2(*a*), scans 2–5, the optical spectra after different periods of bulk electrolysis of all-*trans* canthaxanthin at 900 mV are shown. The absorption in the 800–1000 nm region is characteristic of the electrochemically generated cation radical of canthaxanthin.[17] As the intensity of the peak in the 480 nm range decreases, the spectral intensity in the wavelength region 800–1000 nm increases. The solutions of the electrochemically oxidized carotenoid were used for HPLC analysis. The chromatograms of species formed from all-*trans* canthaxanthin after different periods of bulk electrolysis are shown in Fig. 2(*b*). By using a polymeric ODS (Vydac 201 TP54 column) it was possible to separate all-*trans* canthaxanthin and four of its *cis*-isomers [assignment in Fig. 2(*c*)] within 16 min. Peaks with retention times less than 6 min are attributed to degradation

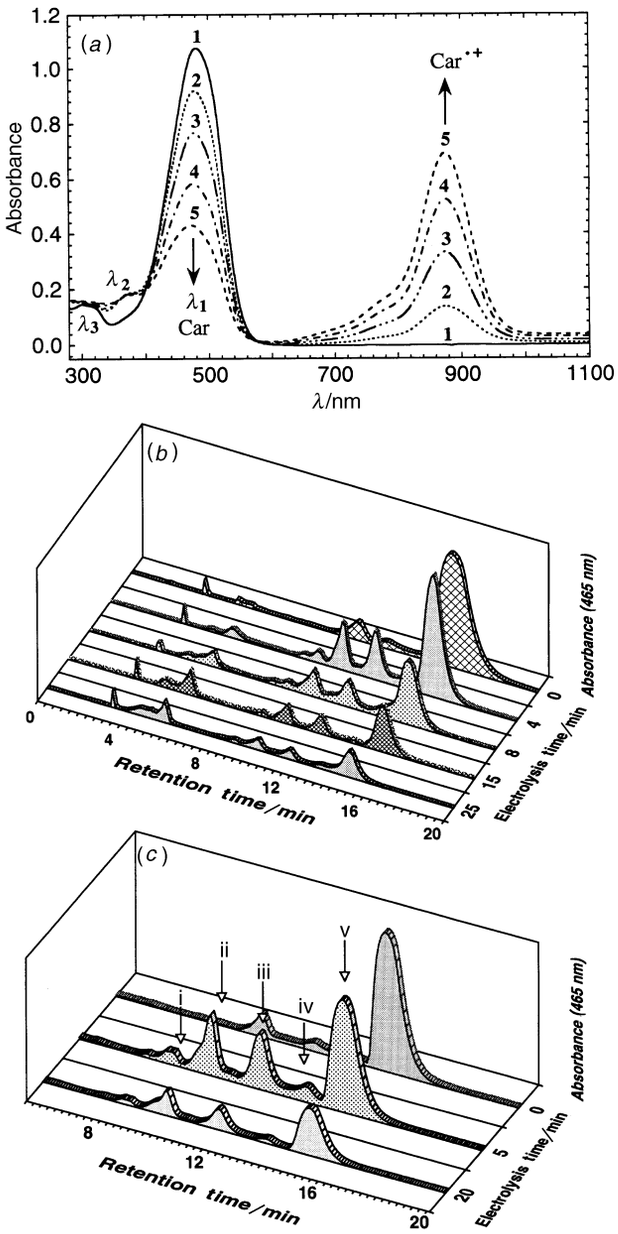


Fig. 2 (*a*) Optical absorption spectra of species formed after (1) 0, (2) 4, (3) 8, (4) 15 and (5) 25 min of bulk electrolysis of all-*trans* canthaxanthin (**I**) at 900 mV and 4 °C. (*b*) Chromatograms of species formed from all-*trans* canthaxanthin (**I**) (0.5 mM in CH_2Cl_2 with 0.1 M TBAHFP) after (1) 0, (2) 4, (3) 8, (4) 15 and (5) 25 min of bulk electrolysis at 900 mV and 4 °C. (*c*) Chromatograms of species formed from all-*trans* canthaxanthin (**I**) (0.3 mM in CH_2Cl_2 with 0.1 M TBAHFP) after (1) 0, (2) 5 and (3) 20 min of bulk electrolysis at 500 mV and 4 °C. Assignment of peaks is as follows: i, 15-*cis*; ii, 13-*cis*; iii, 9-*cis*; iv, 9,13-di-*cis*; v, all-*trans*.

products. The order of elution was 15-*cis*, 13-*cis*, 9-*cis*, 9,13-di-*cis* and all-*trans* canthaxanthin. The identification of *cis* isomers is based on spectral characteristics, *Q* ratios (Table 1) and, in the case of the 9- and 13-*cis* compounds isolated from the reaction mixtures, ^{1}H NMR analysis.[7,10–14] The ^{1}H NMR spectra were the same as those previously reported. The predominant isomers are all-*trans*, 13-*cis* and 9-*cis*.

While Fig. 2(*b*) shows the results after different periods of bulk electrolysis of **I** at 900 mV ($>E^\circ_2$), where both cation radicals and dications are formed; Fig. 2(*c*) shows the results after 5 and 20 min of bulk electrolysis of **I** at 500 mV ($<E^\circ_1$), where only cation radicals are formed. The HPLC chromatograms indicate that the same *cis* isomers are generated at both oxidation potentials. All the peaks are smaller after prolonged electrolysis due to the low stability of *cis* isomers. A possible

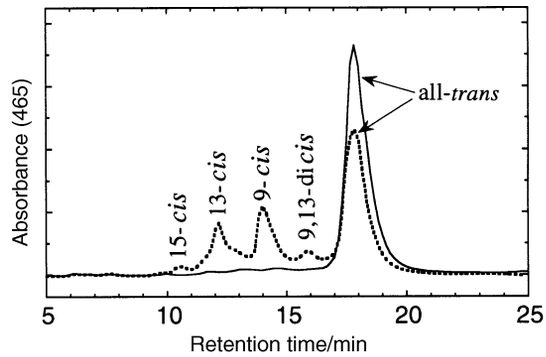


Fig. 3 Chromatograms of species formed from 0.3 mM all-*trans* canthaxanthin (**I**) in CH_2Cl_2 and the presence of 0 μM (———) and 8 μM (------) iron(III) chloride

mechanism which accounts for the isomerizations is shown in Scheme 2. The oxidized species *trans*-Car$^{\bullet+}$ and *trans*-Car^{2+}

$$trans\text{-Car}^{\bullet+} \rightleftharpoons cis\text{-Car}^{\bullet+} \quad (7)$$

$$trans\text{-Car}^{2+} \rightleftharpoons cis\text{-Car}^{2+} \quad (8)$$

$$cis\text{-Car}^{2+} + trans\text{-Car} \overset{K_{com}}{\rightleftharpoons} cis\text{-Car}^{\bullet+} + trans\text{-Car}^{\bullet+} \quad (9)$$

$$cis\text{-Car}^{\bullet+} + trans\text{-Car} \overset{\text{Electron exchange}}{\rightleftharpoons} cis\text{-Car} + trans\text{-Car}^{\bullet+} \quad (10)$$

Scheme 2

could be converted to the corresponding *cis*-species [eqns. (7) and (8)]. Our AM1 molecular orbital calculations[21] show that the energy barriers of configurational transformation from *trans* to *cis* are much lower in the cation radical and dication species than in the neutral molecule. The *cis*-dication reacts with the neutral *trans*-carotenoid in the known comproportionation equilibrium to give both the *cis*- and *trans*-Car$^{\bullet+}$ species [eqn. (9)]. Subsequent electron exchange with the excess neutral carotenoid can then generate the neutral *cis*-isomer [eqn. (10)].

It is not known whether the isomers are formed only from the cation radical, or also from the dication, since cation radicals are also formed in the equilibrium reaction [Scheme 1, eqn. (4)]. However, in Fig. 2(*c*), it was clear that it was only necessary to form carotenoid cation radicals to generate the isomers. Approximately 50% of the all-*trans* canthaxanthin was converted to *cis* isomers during 4–6 min of bulk electrolysis at 4 °C. After prolonged bulk electrolysis the proportion of isomers remained about the same, but the total concentration was greatly decreased and the amount of degradation compounds increased.

Oxidation of carotenoids with $FeCl_3$ leading to isomerization

Oxidation of conjugated polymeric compounds with iron(III) chloride is known to produce cation radicals.[22,23] In our experiments, treatment of 0.3 mM of compound **I** with 8 μM $FeCl_3$ also resulted in the formation of the radical cation as shown by the appearance of the absorption maximum in the 800–1000 nm spectral region. Further, HPLC analysis (Fig. 3) shows that not only are the same *cis*-isomers (as were obtained by bulk electrolysis) formed, but they are also formed in the same relative amounts. The above experiments suggest that carotenoid radical cation, formed either electrochemically in dichloromethane or by chemical oxidation with iron(III) chloride, may induce *cis* isomerization.

8′-Apo-β-caroten-8′-al (II). The experiments which were discussed for canthaxanthin were also carried out with compound **II**. The chromatogram of the species formed from all-*trans* **II** after 5 min of bulk electrolysis is shown in Fig. 4(*a*). By using a LiChrosorb Si 60 column[14] it was possible to separate the all-*trans* compound and as much as 60% of its *cis* isomers. The

Table 1 *Q*-ratios for canthaxanthin (**I**), 8′-apo-β-caroten-8′-al (**II**) and their *cis*-isomers

	I		**II**	
Isomer	This work (CH_3CN)	Literature[a] (hexane)	This work (solution)[b]	Literature[a] (hexane)
All-*trans*	0.10	0.08	0.01	0.01
9-*cis*	0.25	0.21	0.02	0.02
13-*cis*	0.47	0.48	0.34	0.41
15-*cis*	0.60	0.55	0.45	0.48
13′-*cis*	—	—	0.30	0.37
9,13-di-*cis*	0.16	0.10	0.11	0.09
9,13′-di-*cis*	—	—	0.07	0.08

[a] Reported values of *Q*-ratio are from refs. 12–14. [b] 5% Diethyl ether–hexane.

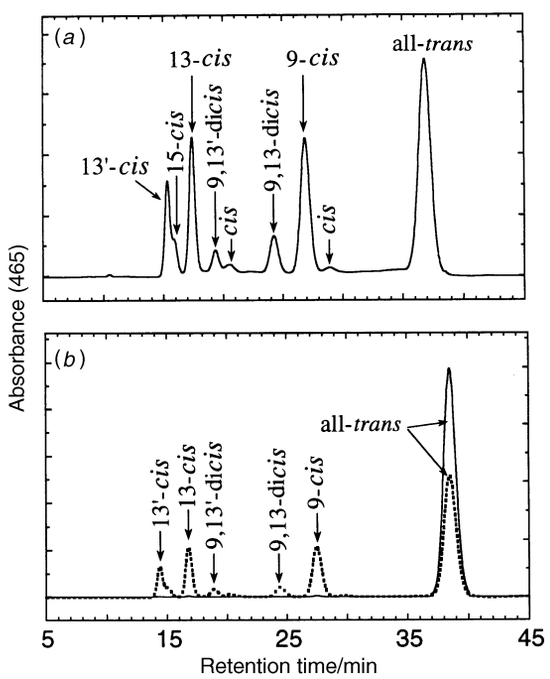


Fig. 4 (*a*) Chromatogram of species formed from all-*trans* 8′-apo-β-caroten-8′-al (**II**) (0.3 mM in CH_2Cl_2 with 0.1 M TBAHFP) after 5 min of bulk electrolysis at 800 mV and 4 °C. (*b*) Chromatograms of species formed from 0.3 mM all-*trans* 8′-apo-β-caroten-8′-al (**II**) in CH_2Cl_2 and the presence of 0 μM (———) and 8 μM (------) iron(III) chloride.

identification of *cis* isomers was also based on spectral characteristics, *Q* ratios (Table 1) and ^{1}H NMR spectroscopy.[7,14] The optical absorption spectrum of the species formed from 0.3 mM of **II** by treatment with 8 μM iron(III) chloride in dichloromethane at 22 °C displayed a maximum in the 800–900 nm region which is attributed to the cation radical. HPLC [Fig. 4(*b*)] of the mixture obtained from **II** and $FeCl_3$ showed that the isomers and their relative amounts were the same as those generated electrochemically. Approximately 40% of all-*trans* isomer is converted into *cis* isomers.

The percentage conversion to *cis*-isomers using thermal and iodine-catalysed isomerization is 25 and 35%, respectively, which is lower than in the electrochemical method described above. Degradation in the thermal method is more extensive than that in the electrochemical and $FeCl_3$ oxidation methods. The fact that higher yields of *cis*-isomers are obtained by the electrochemical method is attributable to better control of the reaction conditions (electrolysis time and potential) and use of much lower temperatures.

Conclusions

All-*trans* canthaxanthin (**I**) and 8′-apo-β-caroten-8′-al (**II**) are converted to *cis*-isomers during bulk electrolysis and treatment with $FeCl_3$ by intermediate oxidation to cation radicals and dications in dichloromethane. The formation of radical cations is detected by their characteristic optical spectra. The *cis*-isomers were separated by HPLC and identified by optical absorption (*Q*-ratio) and ^{1}H NMR spectroscopic techniques. As much as 60% conversion to *cis*-isomers was obtained. Although this conversion was achieved by use of an analytical column, we expect that a similar yield would be achieved on a preparative scale. The electrochemical and iron(III) chloride oxidations are efficient methods to form selected carotenoid geometrical isomers.

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