J. Chem. Sci., Vol. 119, No. 3, May 2007, pp. 253–258.

Isomerization of β-carotene by titanium tetrachloride catalyst

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MS received 22 February 2007; revised 4 May 2007

Abstract. Isomerization of all-*trans*-β-carotene occurs during shaking with 0·5% of titanium tetrachloride catalyst in methylene chloride at room temperature. In the present study we compared two types of columns C18 and C30 and various solvent systems for the separation of β-carotene and its *cis* isomers by high performance liquid chromatography (HPLC). Results showed that β-carotene isomers were resolved by employing a C30 column with a mobile phase of methanol (100%) (A) and methylene chloride (100%) (B) under a gradient elution condition. A total of eleven *cis* isomers and one all-*trans*-β-carotene isomer were resolved within 50 min at a flow rate of 1 ml/min and detection wave-length of 470 nm.

Keywords. β-Carotene; liquid chromatography; isomerization; titanium tetrachloride catalyst.

1. Introduction

β-carotene is an important carotenoid compound that is widely distributed in fruits and vegetables. It has received considerable attention in the past decade because of their beneficial effects on human health. Epidemiological studies have shown that the consumption of fruits and vegetables high in carotenoid content can elevate all-*trans*-β-carotene levels in the blood, which in turn can protective against some fatal diseases such as skin and stomach cancer.[1,2] In addition to being a vitamin A precursor, all-*trans*-β-carotene (β-carotene, C_{2h} symmetry) is also effective antioxidant because of the presence of a long chain of conjugated carbon-carbon double bonds.[3,4]

Most β-carotene is naturally present in the *trans* form; however, there are still significant amounts of *cis* forms of β-carotene in foods. The *cis* isomers of β-carotene in foods may be identified by the methods such as extraction, chromatography etc. It has been reported that the chlorinated solvents can promote isomerization of *trans* conjugated polyenes such as β-carotene during extraction.[5] Also, the isomerization of β-carotene was found to be relatively higher in non-polar solvents than that of polar solvents.[6] Because of the increased awarness of the involvement of *trans*/*cis* isomerization of carotenoids in many biochemical and biological processes, numerous methods of isolation and detection of *trans*/*cis* isomers,[7,9] as well as their preparation, have been developed. The methods leading to formation of mixtures of *cis* and *trans* isomers include refluxing in organic solvents, melting of crystals, contact for prolonged period with certain active surfaces, treatment with acids, and irradiation of solution with or without iodine catalyst.[10,11]

During food processing, β-carotene may undergo degradation and isomerization simultaneously and the formation of *cis* isomers of β-carotene may reduce its color intensity and biological activity. Some recent studies suggested that several *cis* isomers of β-carotene such as 9-*cis* and 13-*cis* β-carotene are present in human serum.[12]

Traditionally, the separation of carotenoids in food samples is often carried out by HPLC with a C18 column. However, most HPLC methods employing a C18 column failed to resolve all-*trans* carotenoids and its *cis* isomers. To remedy this problem the application of a C30 column for separation of all-*trans* carotenoids and its *cis* isomers has been developed.[13,14] Now for the first time we report that *trans*/*cis* isomerization can also be achieved by using 0·5% (v/v) titanium tetrachloride catalyst in methylene chloride with mild shaking at room temperature. The peaks due to isomerized β-carotene are separated by HPLC. Figure 1 shows structures of the predominant geometrical isomers of β-carotene.

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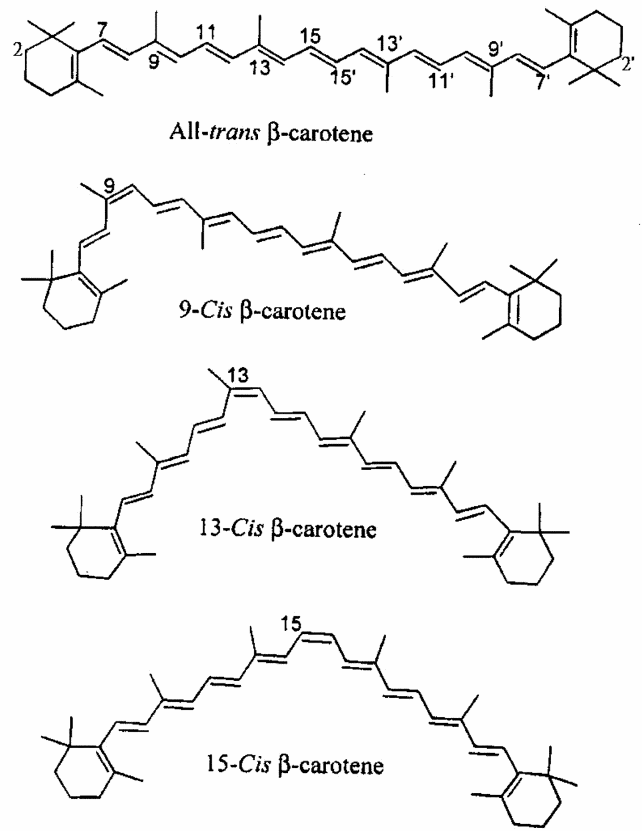

Figure 1. Structures of major isomers of β-carotene.

2. Experimental

2.1 *Materials*

All-*trans*-β-carotene standard was obtained from Sigma Co. (St. Louis, MO, USA). The HPLC-grade solvents, including methanol, methylene chloride, acetone, acetonitrile, titanium tetrachloride and hexane were from Mallinckrodt Co. (Paris, KY, USA). Deionized water was prepared using Milli-Q water purification system (Millipore Co., Bedford, MA, USA). A C18 column (Hypersil 5-19626: 150 × 4·6 mm i.d., 5 μm particle size) was purchased from Thermo Electron Co. (Bellefonte, PA, USA) and a C30 column (YMCRP 30: 250 × 4·6 mm i.d., 5 μm particle size) was from Waters Corp. (Milford, MA, USA).

2.2 *Instrumentation*

The HPLC system is composed of a Phenomenex DG-440 degasser (Phenomenex Co., Torrance, CA, USA), a Rheodyne model 7161 injector (Rheodyne Co., CA, USA), an Agilent model 1100 UV-Vis detector, a Jasco MD-915 photodiode-array detector (Tokyo, Japan), and a Borwin computer software system. The sonicator (model 2210R-DTH) was from Branson Co., (Danbury, CT, USA). The freeze-dryer (model FD-24) was from Chin-Ming Co., (Taipei, Taiwan).

2.3 *Titanium tetrachloride catalysed isomerization of all-trans-β-carotene*

Five numbers of 10 ml vials were taken, each vial containing 2 ml of 100 μg/ml (100 ppm) of all-*trans*-β-carotene standard in methylene chloride. The methylene chloride was removed by passing nitrogen gas. After drying, the standard residue was redissolved in 2 ml of 0·5% (v/v) titanium tetrachloride containing methylene chloride solution. All the vials were subjected to mild shaking at 200 rpm for 1, 3, 5, 7 and 9 h at room temperature. After shaking the standards were then dried under a stream of nitrogen. The residue were finally dissolved in 100 μl sample solvent of methanol : methylene chloride (55 : 45, v/v) and filtered through a 0·2 μm membrane filter for the HPLC analysis. The sample which underwent five hours shaking yielded high number of *cis* isomers of β-carotene than the others. From the HPLC chromatogram the *cis-trans* isomers were tentatively identified based on spectral characteristics and Q-ratios [i.e., ratio of absorbances at the near-UV maxima and main absorbance maxima], reported in the literature[15–20] (tables 1 and 2).

2.4 *HPLC analysis of β-carotene isomers*

Various binary and ternary solvent systems were tried for the separation efficiency of β-carotene isomers. Two different binary solvent systems in different proportions, one system containing methanol–methylene chloride (99 : 1, 97 : 3, and 95 : 5, v/v) were used in isocratic condition and another system containing methanol-isopropanol (95 : 5, v/v) (A) and methylene chloride (100%) (B) was used in the gradient condition. Likewise, two ternary solvent systems in different proportions, one system containing *n*-butanol/acetonitrile/methylene chloride (30 : 70 : 10, v/v/v) and another system containing isopropanol/acetonitrile/methylene chloride (25 : 70 : 5, 25 : 70 : 10 and 25 : 70 : 20, v/v/v) were used. The solvent strength of each mobile phase was carefully controlled by calculating the polarity index. In addition, the separation efficiency in different

Table 1. Retention time, retention factor (k'), separation factor (α), and Q-ratio of isomerized β-carotene by titanium tetrachloride catalyst.

Peak no.	Compound	Retention time (min)	k'[a]	α[b]	Q-ratio found	Q-ratio reported
1	*Cis*-β-carotene	19·17	4·36	1·19	–	–
2	*Cis*-β-carotene	22·20	5·20	1·11	–	–
3	*Cis*-β-carotene	24·24	5·77	1·07	–	–
4	15 or 15′-*cis*-β-carotene	25·64	6·16	1·04	0·44	0·43[d]
5	9 or 9′-*cis*-β-carotene	26·61	6·43	1·08	0·13	0·09[e]
6	13 or 13′-*cis*-β-carotene	28·56	6·97	1·12	0·36	0·35[f]
7	All-*trans*-β-carotene	31·51	7·81	1·04	0·08	0·08[g]
8	13 or 13′-*cis*-β-carotene	32·72	8·14	1·08	0·35	0·35[f]
9	*Cis*-β-carotene	35·12	8·81	1·04	–	–
10	15 or 15′-*cis*-β-carotene	36·28	9·13	1·03	0·43	0·43[d]
11	9 or 9′-*cis*-β-carotene	37·12	9·37	1·04	0·12	0·09[e]
12	*Cis*-β-carotene	38·48	9·75	1·03	–	–

[a]k' (Retention factor or capacity factor) = $(t_{R1} - t_0)/t_0$, where t_R denotes retention time of sample components and t_0 denotes retention time of sample solvent
[b]α (Selectivity factor or separation factor) = $(t_{R2} - t_0)/(t_{R1} - t_0)$
[c]–, Data not available
[d]A gradient mobile phase of 1-butanol-acetonitrile (30 : 70, v/v) and methylene chloride (from 99 : 1, v/v to 90 : 10, v/v) was used by Lin and Chen[15]
[e]A mobile phase of methanol-methyl-*tert*-butyl ether (MTBE) (75 : 25, v/v) was used by Bohm *et al*[16]
[f]A mobile phase of acetone-hexane (3 : 97, v/v) was used by Tsukida *et al*[17]
[g]A mobile phase of methanol–methylene chloride–isopropanol (89 : 1 : 10, v/v/v) was used by Tai and Chen[18]

Table 2. Identification data for all-*trans* and *cis* forms of β-carotene after catalysed by titanium tetrachloride.

Peak no.	Compound	Retention time (min)	λ (nm) (in-line)[a]			λ (nm) (reported)		
1	*Cis*-β-carotene	19·17	413	433	458	413	437	458[b]
2	*Cis*-β-carotene	22·20	417	441	464	417	441	464[c]
3	*Cis*-β-carotene	24·24	417	435	465	417	447	471[c]
4	15 or 15′-*cis*-β-carotene	25·64	419	447	471	421	443	470[d]
5	9 or 9′-*cis*-β-carotene	26·61	418	447	471	422	447	473[e]
6	13 or 13′-*cis*-β-carotene	28·56	419	447	471	419	442	465[f]
7	All-*trans*-β-carotene	31·51	429	453	477	426	454	478[g]
8	13 or 13′-*cis*-β-carotene	32·72	419	447	471	419	442	465[f]
9	*Cis*-β-carotene	35·12	417	441	465	417	441	459[c]
10	15 or 15′-*cis*-β-carotene	36·28	419	447	471	421	443	470[d]
11	9 or 9′-*cis*-β-carotene	37·12	418	447	471	422	447	473[e]
12	*Cis*-β-carotene	38·48	417	441	471	417	441	471[c]

[a]A gradient mobile phase of methanol (100%) (A) and methylene chloride (100%) (B) from (90 : 10, v/v to 52 : 48, v/v) was used
[b]A mobile phase of methanol–methylene chloride (99 : 1, v/v) was used by Chen *et al*[19]
[c]A mobile phase of methanol–isopropanol (99 : 1, v/v) and methylene chloride (from 100 : 0, v/v to 70 : 30, v/v) was used by Chen *et al*[20]
[d]A gradient mobile phase of 1-butanol-acetonitrile (30 : 70, v/v) and methylene chloride (from 99 : 1, v/v to 90 : 10, v/v) was used by Lin and Chen[15]
[e]A mobile phase of methanol–methyl-*tert*-buty lether (MTBE) (75 : 25, v/v) was used by Bohm *et al*[16]
[f]A mobile phase of acetone-hexane (3 : 97, v/v) was used by Tsukida *et al*[17]
[g]A mobile phase of methanol–methylene chloride–isopropanol (89 : 1 : 10, v/v/v) was used by Tai and Chen[18]

sample solvents were also studied. The separation efficiency was evaluated by retention factor (k') and separation factor (α). After various studies, the most appropriate mobile phase was found to be methanol (100%) (A) and methylene chloride (100%) (B) with the gradient elution which was explained in the results and discussion section. The most suitable sample solvent was found to be methanol–methylene

chloride (55 : 45, v/v). The analytical C30 column was used for comparison. The flow rate was 1·0 ml/min and column temperature was 25°C with detection at 470 nm. The injection volume was 20 μl.

3. Results and Discussion

3.1 *HPLC separation of β-carotene isomers*

Various HPLC conditions have been developed to separate the isomers of β-carotene using C18 and C30 columns. It was observed that the C30 column could resolve high numbers of more carotenoid isomers than C18 column, which may be due to a greater hydrophobic interaction between C30 stationary phase with the isomers of β-carotene.[13,14] Thus, a C30 column was selected instead of a C18 column for separation of the isomers of β-carotene.

After various studies, a gradient mobile phase of methanol (100%) (A) and methylene chloride (100%) (B) was developed : 90% A and 10% B in the beginning, maintained for 5 min, decreased to 78% A in 15 min, 62% A in 30 min, 52% A in 40 min, maintained for 10 min and returned to 100% A in 55 min. A total of 12 β-carotene isomers including one all-*trans* isomer were resolved within 50 min.

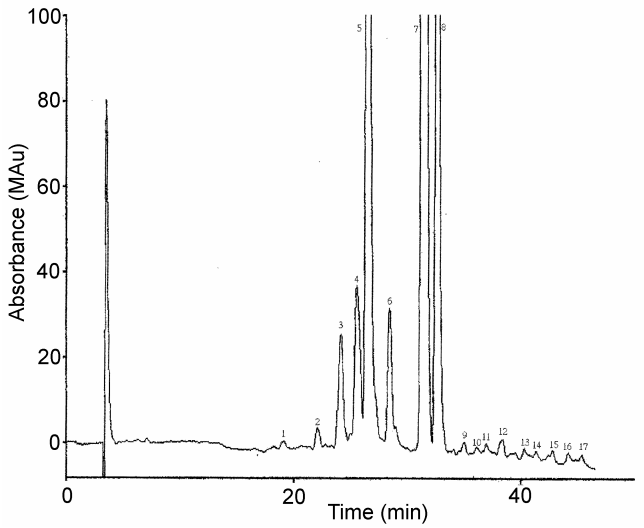

Figure 2. Chromatographic separation of geometrical β-carotene isomers catalysed by 0·5% titanium tetrachloride in methylene chloride. Chromatographic conditions described in text. Peaks: 1. *cis*-β-carotene; 2. *cis*-β-carotene; 3. *cis*-β-carotene; 4. 15 or 15′-*cis*-β-carotene; 5. 9 or 9′-*cis*-β-carotene; 6. 13 or 13′-*cis*-β-carotene; 7. all-*trans*-β-carotene; 8. 13 or 13′-*cis*-β-carotene; 9. *cis*-β-carotene; 10. 15 or 15′-*cis*-β-carotene; 11. 9 or 9′-*cis*-β-carotene; 12. *cis*-β-carotene.

Figure 2 shows the various geometrical isomers of the β-carotene isomers separated using the YMC C30 column. The separation efficiency was assessed from k' and α values, where k' denotes retention factor or capacity factor ($k' = (t_R - t_0)/t_0$) and α denotes selectivity factor or separation factor ($\alpha = (t_{R2} - t_0)/(t_{R1} - t_0)$). It has been well established that the k' values should be controlled between 0·5 to 20 and the α values should be more than one for good separation to occur[21] (table 1). In a similar way high degree of shape selectivity by a 5 μm polymeric C30 column has been demonstrated for the geometrical isomers of α-carotene, lutein, β-cryptoxanthin, lycopene and zeaxanthin, in addition to β-carotene.[13] For the separations of geometrical carotenoid isomers, the stationary phase is reported to be superior to existing reverse phase liquid chromatography (RPLC) columns that are commonly employed for this application.[13] Appreciable separations of geometrical carotenoid isomers were also been achieved in normal-phase liquid chromatography using calcium hydroxide, alumina, silica, and nitrile bonded stationary phases systems.[22–29]

3.2 *Electronic absorption spectra of β-carotene isomers*

The electronic absorption spectral characteristics obtained for each of the β-carotene isomers are shown in tables 1 and 2. All of the spectra were characteristic of β,β-carotenoids with respect to its fine structures (~400 to 500 nm).[30,31] A total of 12 peaks were resolved. Peak 7 was positively identified as all-*trans*-β-carotene with the standard. The other peaks were tentatively identified as *cis*-isomers of β-carotene based on spectral characteristics and Q–ratios as reported in the literature[15–20] (tables 1 and 2). Peaks 4 and 10 were identified as 15 or 15′-*cis*-β-carotene, while peaks 6 and 8 were identified as 13 or 13′-*cis*-β-carotene. Peaks 5 and 11 were identified as 9 or 9′-*cis*-β-carotene. For these all *cis*-β-carotenes the hypsochromic shifts of 6 nm were detected from their main absorption maxima, relative to that of all-*trans*-β-carotene (table 2). In general, a hypsochromic shift of about 5 nm is observed with the introduction of a *cis* bond in a carotenoid structure, but the specific magnitude of these shifts depends on the position of the *cis* bond. Because absorbance in the near UV region generally increases as the position of the *cis* bond approaches the centre of the conjugated systems,[30–32] rather strong

absorbance in this region by the 15 or 15′-*cis*-isomers was expected. This absorbance is reflected in the Q values for these isomers, which are greater than those of the all-*trans*-, 13 or 13′-*cis*-, and 9 or 9′-*cis*-isomers (table 1). The Q values of 15 or 15′-*cis*- and 13 or 13′-*cis*-*β*-carotene are typical as in reference.[20] The 9 or 9′-*cis*-isomers Q values are similar as reported in.[6,33] No *cis* position was assigned to the other peaks (1, 2, 3, 9 and 12) because of inconsistent wavelength shift and no Q ratio is available.

3.3 *Mechanism*

It is known that Lewis acids such as titanium tetrachloride and ferric chloride, can catalyse *cis-trans* isomerization of compounds containing double bonds through the formation of an intermediate radical carbocation.[34] The radical carbocation is sp^3 hybridized and also an sp^2 double bond of polyenes converted into a single bond. Subsequently, free rotation about the new single bond would then form *cis*-isomers according to the following possible mechanism for the isomerization of *β*-carotene.

$$(\beta\text{-carotene})_{trans} - e \leftrightarrow (\beta\text{-carotene}^{\bullet+})_{trans}$$

$$(\beta\text{-carotene}^{\bullet+})_{trans} \leftrightarrow (\beta\text{-carotene}^{\bullet+})_{cis}$$

$$(\beta\text{-carotene}^{\bullet+})_{cis} + e \leftrightarrow (\beta\text{-carotene})_{cis}$$

This mechanism is supported by recent studies.[34–36]

4. Conclusion

The present research: (1) demonstrates that the catalytic activity of titanium tetrachloride towards the formation of geometrical isomers of *β*-carotene with high degree of shape selectivity by the polymeric C30 stationary phase; (2) unambiguously confirms the double bond configuration of *β*-carotene isomers in reverse phase liquid chromatography; and (3) supplements the existing spectroscopic data on *β*-carotene isomers.

Acknowledgements

The authors gratefully acknowledge financial supports from the National Science Council of Republic of China (R.O.C), Taiwan.

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