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Characterization and Thermal Isomerization of (*all-E*)-Lycopene

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ABSTRACT: A large amount of (*all-E*)-lycopene was successfully purified from tomato paste using an improved method that included a procedure to wash crystalline powder with acetone. The total yield of the pure (*all-E*) form was at least 30%. The melting point of (*all-E*)-lycopene was determined to be 176.35 °C by differential scanning calorimetry (DSC) measurements. Bathochromic shifts were observed in the absorption maxima of all solvents tested (at most a 36 nm shift for λ_2 in carbon disulfide, as was observed in hexane) and were accompanied by absorbance decreases, namely, a hypochromic effect, showing a higher correlation between the position and the intensity of the main absorption bands. This bathochromic shift was dependent upon the polarizability of the solvent rather than its polarity. The structure of (*all-E*)-lycopene in $CDCl_3$ and C_6D_6 was identified on the basis of one- and two-dimensional nuclear magnetic resonance (NMR) spectra, including ^1H and ^{13}C NMR, homonuclear correlation spectroscopy ($^1H-^1H$ COSY), heteronuclear multiple-quantum coherence (HMQC), and heteronuclear multiple-bond connectivity (HMBC). The rate constants of the decrease in (*all-E*)-lycopene with hexane and benzene were calculated to be 3.19×10^{-5} and 3.55×10^{-5} s^{-1}, respectively. The equilibrium constants between (*all-E*) and (13*Z*) isomers were estimated to be 0.29 in hexane and 0.31 in benzene, respectively, from the point at which the amount of (13*Z*)-lycopene reached its maximum.

KEYWORDS: *lycopene, purification, tomatoes, spectrum analysis, thermal isomerization, density functional theory*

INTRODUCTION

Lycopene is a well-known carotenoid found abundantly in vegetables and fruits with a red color, such as tomatoes, red carrots,[1] watermelons, and gac (*Momordica cochinchinensis*),[2] as well as in microorganisms, such as *Dunaliella salina*,[3] *Chlorella* spp.,[4,5] and *Blakeslea trispora*.[6,7] Lycopene, like other carotenoids, is responsible for the characteristic bright color of these organisms and plays a protective role against oxidative stress.[8−10] The natural benefits of lycopene have been applied not only to food and dietary supplements as edible colorants and antioxidants but also to medical approaches to cancer and arteriosclerosis prevention,[11−13] taking advantage of its physiological properties and biocompatibility. These useful functions of lycopene, the molecular formula of which is $C_{40}H_{56}$, have been attributed to its chemical structure containing many unsaturated bonds, in which 11 double bonds are conjugated (Figure 1), and more effectively allow for the absorption of relatively long-wavelength light and quench singlet oxygen. Therefore, many researchers have studied this useful pigment and published excellent reports from the middle of the 20th century.[14−19] However, these studies were performed with lycopenes prepared from different origins and with different purification degrees, which may lead to a misunderstanding because of different values for basic physicochemical properties.

Although most of the lycopenes are known to be present in the (*all-E*) configuration in tomatoes and other vegetables, 71 kinds of (*Z*) isomers are theoretically possible.[20] However, bioavailability of the (*Z*) isomers of lycopene by lymph-cannulated ferrets[21] and a human intestinal cell model[22] was shown to be significantly greater than that of the (*all-E*) isomer. Therefore, an efficient method to improve the isomerization of (*all-E*) to the (*Z*) configuration is desired, with isomerization kinetics only being investigated in unpurified mixtures.[23,24]

Under these circumstances, we performed an extraction of (*all-E*)-lycopene with higher purity from a tomato paste and determined its physical and chemical properties, including some spectrophotometric measurements. The isomerization of lycopene by a heating apparatus was also investigated by chromatography. The results of our study give new criteria for the identification of lycopene and contribute to the fundamental chemistry of this carotenoid in the food science and technology field.

MATERIALS AND METHODS

Reagents. All reagents and solvents used were analytical-grade, except for methanol, which was high-performance liquid chromatography (HPLC)-grade (Sigma-Aldrich, St. Louis, MO), and were used without any further purification.

Extraction and Purification of (*all-E*)-Lycopene. All procedures were performed at room temperature, unless otherwise indicated. A total of 500 mL of dichloromethane was added to 50 g of tomato paste (Kagome Co., Ltd., Tokyo, Japan; lycopene content, 8−12 g/kg) in an Erlenmeyer flask, and the mixture was stirred for 60 min in darkness. The organic layer was separated with a separatory funnel, and repetitive extraction was performed on the resulting suspension by the same volume of dichloromethane. The solvent was evaporated on a rotary evaporator under a vacuum (170 mmHg) at 25 °C for 30 min. The crude extract (345 mg) containing lycopene was dissolved in 15

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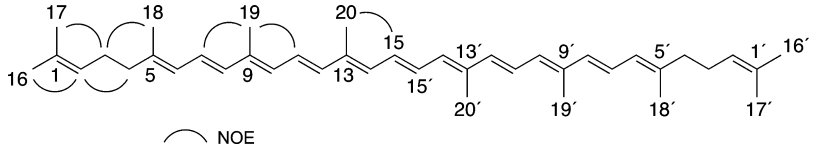

Figure 1. Chemical structure of (*all-E*)-lycopene. The nuclear Overhauser effect (NOE) correlations observed in the two-dimensional NMR measurements are shown as curved lines in one half side of the symmetrical structure of the lycopene.

Table 1. Absorption Maxima and Molar Extinction Coefficients of (*all-E*)-Lycopene from Tomato[a]

solvent	λ_1		λ_2		λ_3		λ_{max} calcd[b]
methanol	444.0	*c*	469.5	*c*	501.5	*c*	486.5
hexane	444.0	(118)	471.0	(182)	502.5	(168)	470.5
hexane/chloroform (98:2)[d]	443	(123)	470	(187)	502	(172)	*c*
MTBE[e]	445.5	(120)	472.0	(180)	503.5	(164)	*c*
ethanol	446.0	*c*	472.5	*c*	504.0	*c*	473.0
acetonitrile	446.0	*c*	472.5	*c*	504.0	*c*	*c*
acetone	447.0	(118)	474.0	(178)	505.5	(162)	*c*
ethyl acetate	447.5	(118)	474.0	(179)	506.0	(162)	*c*
cyclohexane	448.5	(116)	476.0	(177)	508.5	(159)	478.0
tetrahydrofuran	452.0	(114)	479.0	(173)	512.0	(153)	*c*
dichloromethane	455.0	(113)	482.5	(170)	515.5	(150)	*c*
chloroform	456.5	(110)	484.5	(164)	518.0	(143)	483.5
toluene	456.5	(110)	485.0	(165)	519.5	(144)	*c*
benzene	457.5	(110)	485.5	(164)	520.0	(144)	487.5
anisole	460.5	(104)	489.0	(157)	524.5	(137)	*c*
dimethyl phthalate	461.5	*c*	489.5	*c*	525.0	*c*	*c*
pyridine	462.5	(104)	490.5	(155)	526.0	(137)	490.0
benzonitrile	463.5	(101)	491.5	(150)	527.0	(132)	*c*
N,*N*-dimethylaniline	464.0	(102)	492.5	(148)	529.0	(130)	*c*
carbon disulfide	478.0	(89)	507.0	(133)	544.5	(123)	505.5

[a] λ_{1-3} in nm; values in parentheses, $\varepsilon \times 10^{-3}$. [b] Calculated according to the literature.[32−34] [c] Not determined. [d] Synthetic (*all-E*)-lycopene.[38] [e] Methyl *tert*-butyl ether, containing 0.1% dichloromethane.

mL of benzene at 60 °C within 10 min and recrystallized at 4 °C for 4 h under shading. The resulting crystals were collected by suction filtration on a Kiriyama funnel (number 5B filter paper), rinsed with 100 mL of acetone, and dried *in vacuo*: 188 mg of fine red crystalline powder, with a melting point (mp) of 176.35 °C [differential scanning calorimetry (DSC)]; HPLC, ≥99.3%; ultraviolet/visible (UV/vis), Table 1; infrared (IR) (KBr), Table 2; nuclear magnetic resonance (NMR), Table 3; high-resolution mass spectrometry−fast atom bombardment (HRMS−FAB) (*m*/*z*) [M + H] $^+$ calcd for $C_{40}H_{57}$, 537.4460; found, 537.4418.

Thermal Isomerization of Lycopene. Purified (*all-E*)-lycopene dissolved in 100 mL of organic solvent (hexane, 9.76 μM; benzene, 198 μM) was transferred into a three-neck flask equipped with a mechanical stirrer, a reflux condenser, and a gas inlet tube. The isomerization of lycopene was conducted at 50 °C with an oil bath in darkness under an argon stream. The reaction mixtures were sampled at intervals, and the contents of the lycopene isomers were analyzed by reversed-phase HPLC. The recoveries of the total lycopene isomers with hexane and benzene during the sampling periods were estimated to be 87.5 and 61.9%, respectively, by the HPLC method.

Table 2. IR Absorption Bands of (*all-E*)-Lycopene Extracted and Purified from Tomato Paste and Their Calculated Values

	frequency (cm^{-1})	
origin	found	calcd
C−H stretch, alkene	3038, 3020 m	3072−3055, 3033−3007 m
C−H stretch, methylene/methyl	2968, 2912, 2854 s	2981−2962, 2926−2894, 2894 m to s
C═C stretch	1627, 1552 w	1636, 1558 m
C−H deformation, methylene/methyl	1441 m	1460−1444 m
C−H deformation, methyl	1391, 1364 m	1399−1375, 1364−1350 m
C−H out-of-plane, (*E*) disubstituted double bond	960 s	976−946 s

UV−Vis, Fourier Transform Infrared (FTIR), Mass, and NMR Spectroscopic Analyses. UV−vis spectra of the purified lycopene were measured in organic solvents over a scanning range of 200−600 nm, and the λ maxima of the compounds were determined. Spectra were recorded with a Hitachi U-2910 spectrophotometer (Tokyo, Japan).

IR spectra were obtained by JASCO FT/IR 4100 (Tokyo) using the KBr disc in the range of 4000−400 cm^{-1}.

The HRMS of (*all-E*)-lycopene was recorded in the positive-ion mode by FAB+ on a JEOL JMS-700T instrument (Tokyo), using 3-nitrobenzyl alcohol as the matrix.

NMR spectra of (*all-E*)-lycopene were recorded using a JEOL JMN-LA400 FT 400 NMR spectrometer at 400 MHz (^{1}H) and 100 MHz (^{13}C). Chemical shifts were recorded as the δ value (ppm) using tetramethylsilane (TMS) as an internal standard. Spectra were observed on $CDCl_3$ and benzene-d_6 (C_6D_6).

DSC. The melting point of purified (*all-E*)-lycopene was determined by DSC using a DSC-60A system (Shimadzu, Kyoto, Japan). DSC measurements were performed with aluminum sample pans and empty reference pans. Both the sample and reference were scanned at a heating rate of 5 K/min from 303 to 473 K under a nitrogen atmosphere with a flow rate of 50 mL/min. The mass of the sample was 7 mg. All measurements were performed in triplicate.

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Table 3. Refinement of ^{1}H and ^{13}C NMR Assignments of (*all-E*)-Lycopene from Tomato[a]

proton	*d* in ppm (multiplicity, coupling constant in Hz)		carbon	*d* in ppm	
	in $CDCl_3$ [synthetic lycopene][38]	in C_6D_6		in $CDCl_3$ [synthetic]	in C_6D_6
			C(1), C(1′)	131.72 [131.64]	131.58
H−C(2), H−C(2′)	5.11 (m) [5.11]	5.23 (m)	C(2), C(2′)	123.96 [124.12]	124.50
2H−C(3), 2H−C(3′)	2.11 (m) [ca. 2.11]	2.18 (m)	C(3), C(3′)	26.70 [26.83]	27.10
2H−C(4), 2H−C(4′)	2.11 (m) [ca. 2.11]	2.18 (m)	C(4), C(4′)	40.23 [40.30]	40.62
			C(5), C(5′)	139.47 [139.30]	138.95
H−C(6), H−C(6′)	5.95 (d, *J* = 11.0) [5.95]	6.16 (d, *J* = 11.0)	C(6), C(6′)	125.74 [125.94]	126.77
H−C(7), H−C(7′)	6.49 (dd, *J* = 11.0, 15.0) [6.49]	6.67 (dd, *J* = 11.0, 15.1)	C(7), C(7′)	124.79 [124.87]	125.31
H−C(8), H−C(8′)	6.25 (d, *J* = 15.0) [6.25]	6.44 (d, *J* = 15.1)	C(8), C(8′)	135.40 [135.54]	136.17
			C(9), C(9′)	136.15 [136.15]	136.37
H−C(10), H−C(10′)	6.18 (d, *J* = 11.4) [6.18]	6.36 (d, *J* = 11.6)	C(10), C(10′)	131.55 [131.64]	132.39
H−C(11), H−C(11′)	6.64 (dd, *J* = 11.4, 14.9) [6.64]	6.77 (dd, *J* = 11.6, 15.0)	C(11), C(11′)	125.15 [125.21]	125.66
H−C(12), H−C(12′)	6.35 (d, *J* = 14.9) [6.35]	6.49 (d, *J* = 15.0)	C(12), C(12′)	137.35 [137.46]	138.00
			C(13), C(13′)	136.56 [136.54]	136.83
H−C(14), H−C(14′)	6.24 (m) [6.25]	6.34 (AA′ of AA′BB′ system)	C(14), C(14′)	132.64 [132.71]	133.39
H−C(15), H−C(15′)	6.62 (m) [6.62]	6.70 (BB′ of AA′BB′ system)	C(15), C(15′)	130.07 [130.17]	130.70
3H−C(16), 3H−C(16′)	1.687 (s) [1.688]	1.674 (s)	C(16), C(16′)	25.68 [25.66]	25.84
3H−C(17), 3H−C(17′)	1.614 (s) [1.612]	1.568 (s)	C(17), C(17′)	17.70 [17.70]	17.73
3H−C(18), 3H−C(18′)	1.818 (s) [1.818]	1.749 (s)	C(18), C(18′)	16.99 [16.97]	16.90
3H−C(19), 3H−C(19′)	1.968 (s) [1.968]	1.925 (s)	C(19), C(19′)	12.90 [12.90]	12.98
3H−C(20), 3H−C(20′)	1.968 (s) [1.968]	1.876 (s)	C(20), C(20′)	12.79 [12.81]	12.86

[a] ^{1}H and ^{13}C NMR were recorded at 400 and 100 MHz, respectively. s, singlet; d, doublet; dd, doublet of doublets; and m, multiplet.

HPLC Analysis. Reversed-phase HPLC analysis with a photodiode array detector (SPD-M10AVP, Shimadzu, Kyoto, Japan) was performed under the following conditions: column, YMC Carotenoid (250 × 4.6 mm inner diameter, 5 μm particles, YMC, Kyoto, Japan); solvent A, methanol/methyl *tert*-butyl ether (MTBE)/ H_2O (75:15:10, v/v/v); solvent B, methanol/MTBE/H_2O (7:90:3, v/v/v); gradient, started with 100% eluent A and ended with 100% eluent B over a period of 35 min; flow rate, 3.0 mL/min; and column temperature, 22 °C. A typical chromatogram of the lycopene isomers was obtained with a retention time and absorption maxima at (13*Z*)-lycopene [24.6 min; 440.0, 465.0, and 496.5 nm; and (*Z*) peak[25] at 361 nm with a relative intensity of 59.2% D_B/D_{II}], (9*Z*)-lycopene [27.6 min; 441.0, 467.0, and 497.5 nm; and (*Z*) peak at 361 nm with 13.7% D_B/D_{II}], (*all-E*)-lycopene (31.9 min; 445.0, 472.5, and 503.5 nm); and (5*Z*)-lycopene (32.6 min; 445.0, 472.0, and 503.5 nm). The quantification of all lycopenes was performed by peak area integration at 470 nm, showing a reliable approximation for the analysis of isomers.[26,27]

Computational Analysis. *Ab initio* and density functional theory (DFT) calculations on the infrared spectrum of (*all-E*)-lycopene were performed with Gaussian 03 software using the B3LYP functional and 6-31G(*d*) basis set.

RESULTS AND DISCUSSION

Physical Properties of (*all-E*)-Lycopene. In this study, a large amount of (*all-E*)-lycopene was successfully purified from tomato samples without laborious chromatographic procedures.[28,29] This improved method included a procedure to wash crystalline powder with acetone, in which the solubility of (*all-E*)-lycopene was low (ca. 0.75 mg/mL).[30] The total yield of the pure (*all-E*) form (purity ≥ 99.3% by HPLC) was at least 30% when the lycopene content of the tomato paste was considered. The DSC curve for the purified lycopene showed two possible melting points of 170.66 and 176.35 °C. The lower value may be attributed to the (*Z*)-lycopenes, which arose from the (*all-E*) form because of the heating process. The content of the (*all-E*) form was reduced to 61.6% by reversed-phase HPLC for

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lycopene samples after the DSC measurement. The melting point of (*all-E*)-lycopene was then determined to be 176.35 °C, which was consistent with the value obtained by Manchand et al.[18]

Lycopene has an electron spectrum characterized by 11 conjugated double bonds, which geometrically impose a linear and highly planar structure. In hexane, (*all-E*)-lycopene showed strong absorption maxima at 502.5, 471.0, and 444.0 nm with molar extinction coefficients estimated as 168×10^3, 182×10^3, and 118×10^3 (Table 1), corresponding to vibrational transition energies of 0−0, 0−1, and 0−2, respectively. The peak at approximately 360 nm, the so-called (*Z*) peak,[25,31] was not observed in this sample.

Furthermore, absorption maxima and molar extinction coefficients with (*all-E*)-lycopene were measured in various organic solvents to investigate the solvent effect on the electronic spectrum of the molecule (Table 1). All values for the maxima (λ_2) of the fine structure in this study were also consistent with the calculated values according to an empirical rule.[32−34] The values (λ_1, λ_2, and λ_3) listed in this table were plotted as a function of wavelength in Figure 2A. From these

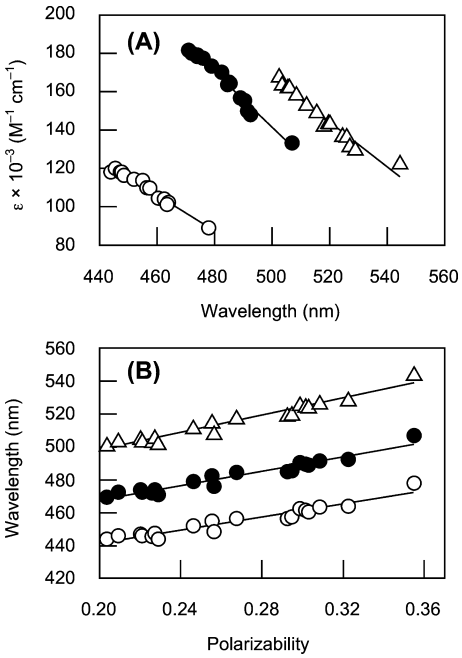

Figure 2. Relationships (A) between the absorption maxima and molar extinction coefficients of (*all-E*)-lycopene in various solvents and (B) between the polarizabilities of the solvents and the absorption maxima. The values of (○) λ_1, (●) λ_2, and (△) λ_3 are from Table 1. Polarizability of the solvent is calculated as follows: $(n^2 - 1)/(n^2 + 2)$, where *n* is the refractive index of the solvent.[39]

results, bathochromic shifts in the absorption maxima were observed in all solvents tested (at most a 36 nm shift for λ_2 in carbon disulfide, as was observed in hexane) and were accompanied by absorbance decreases, namely, a hypochromic effect, showing a higher correlation between the position and the intensity of the main absorption bands. Although many studies suggested that the bathochromic shift had been independently reported previously,[35,36] the highly purified (*all-E*)-lycopene had first enabled a discussion of the solvent effect on this carotenoid. This bathochromic shift depends upon the polarizability of the solvent because of high correlation between them (Figure 2B)[37] rather than its polarity (data not shown). This is the first report showing the solvent effect on the electron spectra of (*all-E*)-lycopene, because this was difficult to evaluate before as a result of samples having different purification grades with different origins. This finding contributes to the fundamental chemistry of carotenoids and will be a new criterion for the identification and evaluation of lycopene in agriculture, food, and medical fields.

The IR spectrum of (*all-E*)-lycopene was measured, and characteristic absorptions were shown in Table 2, along with those calculated by the Gaussian program. Observed and calculated values from C−H and C═C stretches and C−H out-of-plane attributed to the alkene as well as other origins were consistent with each other. This computational estimation with high fidelity may depend upon the restriction of the molecular motion of lycopene caused by the 11 conjugated double bonds. Therefore, these observations will facilitate an evaluation of the relative free energy of (*Z*) isomer lycopenes, which may be contained in foods or originate from heat-induced isomerization.

NMR Assignment of (*all-E*)-Lycopene. The structure of (*all-E*)-lycopene has been identified on the basis of one- and two-dimensional NMR spectra, including ^{1}H and ^{13}C NMR, homonuclear correlation spectroscopy (^{1}H−^{1}H COSY), heteronuclear multiple-quantum coherence (HMQC), and heteronuclear multiple-bond connectivity (HMBC). Chemical shifts for proton and carbon signals of the (*all-E*)-lycopene in this work were good accordance with those of the synthetic (*all-E*)-lycopene[38] (Table 3). Spectral data on (*all-E*)-lycopene in $CDCl_3$ were also independently reported by different literature;[40−42] however, some values reported were not consistent among the previous studies, e.g., for the coupling constants of protons H−C(11), H−C(11′) and the chemical-shift value of carbon atoms C(14), C(14′). In our study with thoroughly purified lycopene, the coupling constant values between H−C(11), H−C(11′) and H−C(10), H−C(10′) and between H−C(11), H−C(11′) and H−C(12), H−C(12′) were measured as 11.4 and 14.9 Hz, respectively. The chemical shift for C(14), C(14′) could be assigned to the signal observed at 132.64 ppm by the HMQC experiment, and refinement of the NMR signal assignment of (*all-E*)-lycopene was then achieved in $CDCl_3$.

Measurements were subsequently performed in another solvent, C_6D_6, in expectation of NMR signal charts distinct from those obtained in $CDCl_3$ because of differences in their physical properties, such as polarity, resonance, and viscosity. Proton and ^{13}C signals in C_6D_6 were preliminarily assigned by the results obtained in $CDCl_3$ and ascertained by ^{1}H homonuclear decoupling and the nuclear Overhauser effect difference (NOE-dif) experiments in addition to the above two-dimensional measurements. As shown in Table 3, chemical shifts in methyl protons between H−C(19), H−C(19′) and H−C(20), H−C(20′) were discriminated in C_6D_6 at 1.925 and 1.876 ppm, respectively (Table 3), whereas these signals appeared as a singlet at 1.968 ppm in $CDCl_3$ (this study and refs 40 and 41). Furthermore, the coupling system between H−C(14), H−C(14′) and H−C(15), H−C(15′) could be analyzed in C_6D_6, whereas their corresponding signals in $CDCl_3$ overlapped with H−C(8), H−C(8′) and H−C(11), H−C(11′), respectively, and were assigned to a multiplet. The observed spin signal occurred in the AA′BB′-type system, to which similar coupling was assigned in some carotenoids, such as prolycopene and (9*Z*,9′*Z*)-7,8,7′,8′-tetrahydrolycopene,[38,43]

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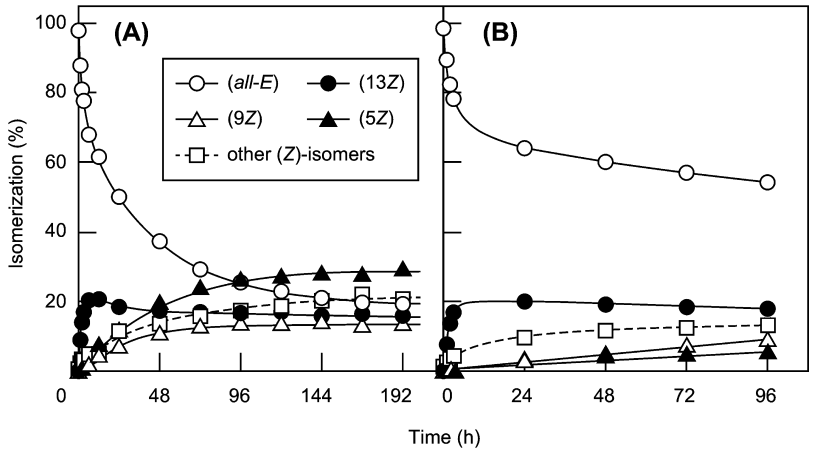

Figure 3. Isomerization of (*all-E*)-lycopene to (*Z*) isomers in (A) hexane and (B) benzene at 50 °C. Changes in the content percentage of (○) (*all-E*)-, (●) (13*Z*)-, (△) (9*Z*)-, and (▲) (5*Z*)-lycopene, as well as the other (*Z*) isomers (□), are presented with regard to the total amount of lycopenes resulting at each point of sampling.

and the full assignment of ^{1}H and ^{13}C signals was then given in Table 3. The unambiguous determination attained in this study will also help to analyze the (*Z*) isomers occurring in natural sources and those generated from the isomerization of (*all-E*)-lycopene by a heating process.

Thermal Isomerization of (*all-E*)-Lycopene. The isomerization of (*all-E*)-lycopene to (*Z*) isomers was investigated in hexane and benzene at 50 °C. The contents of (*all-E*)-lycopene rapidly decreased within a few hours in both solvents at this temperature and reached less than 20% for hexane and 55% for benzene during the test periods (Figure 3). Corresponding to the decrease in (*all-E*)-lycopene, (13*Z*)-lycopene emerged as the first (*Z*) isomer observed, and the content of the isomer gradually decreased after reaching its maximum of ca. 20% at 10 h. These decreases reflect the regeneration of (*all-E*)-lycopene and subsequent isomerization to other (*Z*) forms. The generation rates for (9*Z*) and (5*Z*) isomers were different in both solvents, and this may have led to the selective enrichment of a desirable isomer by taking advantage of the solvent effect.

The rate constants for the isomerization of lycopenes were then investigated on approximation to the one-order reaction.[44,45] The rate constants of the decrease in (*all-E*)-lycopene with hexane and benzene were calculated as 3.19×10^{-5} and 3.55×10^{-5} s^{-1}, respectively. It is necessary to evaluate the main kinetic parameters of lycopene isomerization in such a complex system that several equilibrium states can coexist. At the very early stage of these reactions, the reaction rate for the formation of (13*Z*)-lycopene overcome that of other isomers, which indicated that the reduction rate of (*all-E*)-lycopene could be approximate to the production rate of (13*Z*) isomer. The values obtained with lycopene were shown to be greater than those of the corresponding (13*Z*)-*β*-carotene and its derivative at 64 °C, apart from (15*Z*)-lycopene, which could not be observed in our or other studies.[44] According to the above approximation, the equilibrium constants between (*all-E*) and (13*Z*) isomers were estimated to be 0.29 in hexane and 0.31 in benzene, respectively, from the point at which the amount of (13*Z*)-lycopene reached its maximum.

Fundamental data for (*all-E*)-lycopene were obtained in the present study using an extremely purified extract from tomato paste, which provided a new insight into the spectroscopic and geometrical properties of lycopene. Moreover, the obtained thermal information of (*E*−*Z*) isomerization will contribute not only to the efficient production of (*Z*)-lycopenes that may give many benefits to human wellness but also to the identification of chemical and physical properties of the (*Z*) isomers.

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Notes

The authors declare no competing financial interest.

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