Isolation and Structural Elucidation of Different Geometrical Isomers of Lycopene

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Abstract: Five geometrical isomers of lycopene were isolated from a photoisomerized mixture on a semi-preparative C_{30} column and characterized by UV-vis spectroscopy, mass spectroscopy, and nuclear magnetic resonance (NMR) spectroscopy. ^{1}H NMR and 2D NMR measurements were used to unambiguously assign the double bond configuration of five isomers: *(5Z,9'Z)-, (9Z)-, (5Z,9Z)-*, *(all-E)-*, and *(5Z)*-lycopene. This will allow other laboratories to use the results for their C_{30} HPLC. In addition, it is possible to investigate structure-activity relationships for these lycopene isomers which will improve the understanding of their physiological role in biological tissues.

Key words: Lycopene, geometrical isomers, UV-vis spectroscopy, mass spectrometry, NMR spectroscopy

Introduction

Lycopene is an acyclic carotenoid with 11 linearly arranged conjugated double bonds, found only in a few food items. Tomatoes and tomato-based products are the main dietary lycopene sources for humans. Further sources of lycopene include apricot, guava, watermelon, papaya, pink grapefruit, sea buckthorn, rosehip, etc. Lycopene lacks the β-ionone ring and therefore has no provitamin A activity. Numerous investigations focus currently on carotenoids as biological antioxidants. The carotenoids with nine or more conjugated double bonds are able to quench singlet oxygen with increasing activity depending on the number of conjugated double bonds [1]. Within the carotenoids, lycopene is the most effective quencher of singlet oxygen [2]. Recent investigations by using the TEAC (Trolox equivalent antioxidant capacity) assay showed significantly different antioxidant activity for lycopene isomers depending on the geometrical structure [3].

Isomerization of carotenoids, often encountered in food processing, is affected by both temperature and light exposure. However, in tomatoes and tomato-based foods *(all-E)*-lycopene is predominant, accounting for 90–98% of total lycopene [4]. In contrast, in benign or malignant human prostate tissues, *(all-E)*-lycopene accounts for on-

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ly 12–21% of total lycopene, and *(Z)*-isomers for 79–88%. In serum, lycopene consists of 58–73% *(Z)*-isomers [5]. A recent human intervention study showed that there was no significant *(Z)-(E)* isomerization of lycopene in the human stomach. The fact that lycopene *(Z)*-isomers are poorly transported by the chylomicrons and thus poorly absorbed, strongly suggests that an *(E)-(Z)* isomerization of lycopene occurs in the human body at a post-enterocyte level [6].

In most investigations on lycopene, the different *(Z)*-isomers are often only tentatively identified by using the UV-vis data as well as their high-performance liquid chromatography (HPLC) retention behavior compared to published separations. Thus, the aim of this study was to unambiguously identify prominent geometrical lycopene *(Z)*-isomers in a photoisomerized mixture by using different spectroscopic methods. These investigations were done by using the nowadays most common C_{30} HPLC.

Material and Methods

Chemicals

(all-E)-Lycopene was a gift from BASF (Ludwigshafen, Germany). Iodine was purchased from Merck (Darmstadt, Germany). Cyclohexane, toluene, petroleum ether, ethanol, dichloromethane, and acetone were of analytical grade, and methanol, methyl tert-butyl ether (MTBE), and hexane were of HPLC quality. Deuterated chloroform ($CDCl_3$) was obtained from Euriso-top (Saarbrücken, Germany).

Iodine isomerization

(all-E)-Lycopene standard solution (150–200 µg/mL) in cyclohexane/toluene (8+2, v/v) was used for isomerization. Iodine crystals were added to the standard at approximately 5% of the carotenoid weight and photoisomerization was performed according to the method of Zechmeister [7]. The resulting mixture of lycopene metabolites was used for fractionation as described below. Four prominent *(Z)*-isomers (Figure 1 showing a separation on the preparative C_{30} column) were isolated for further investigations.

Fractionation

The isomers were fractionated at room temperature using a HPLC pump model L-7100 (Merck, Darmstadt, Germany), detector model Lambda 1000 (Bischoff, Leonberg, Germany) and integrator model Chromatopac C-R6A (Shimadzu, Duisburg, Germany). For separation, a preparative C_{30} (300 × 10.0 mm, 5 µm) column (YMC Europe, Schermbeck, Germany), preceded by a C_{18} ProntoSil 120-5-C18 H (10 × 4.0 mm, 5 µm) column (Bischoff, Leonberg, Germany) was used. Mixtures (see details below) of methanol and MTBE constituted a mobile phase at a flow rate of 4.0 mL/minute; the detection wavelength was 450 nm. Due to the low stability of the isolated isomers of lycopene, all steps had to be completed rapidly and under subdued light.

The pre-fractionation was necessary to provide the isomers for the NMR measurements at adequate concentrations, for isomers **1–3**. The mixture of isomers was dissolved in cyclohexane/toluene (8+2, v/v). The separation was carried out with a mobile phase consisting of a mixture of methanol and MTBE (1+1, v/v). The isolated extract contained the three isomers *(Z)*-lycopene isomer **1**, *(Z)*-lycopene isomer **2**, and *(Z)*-lycopene isomer **3**. A concentrated solution of this mixture was stable for two weeks at –30°C as shown by analytical HPLC (data not shown).

Prior to the separation of several isomers, the mixtures were dried under a flow of nitrogen at room temperature. After dilution with the mobile phase, several isomers were fractionated using a mixture of methanol and methyl tert-butyl ether (6+4, v/v) as mobile phase. The eluates with the separated isomers were concentrated under vacuum at room temperature in a rotary evaporator. The residue of the solvent was dried under a nitrogen flow at room temperature. The purity of the separated isomers was checked by means of an analytical C_{30} HPLC-diode array detector (DAD) as described below, and found to range between 95 and 100%. In addition, for isomers **1–3** purity was checked by using the preparative HPLC system as described above due to its better separation of these isomers.

HPLC analysis

The mixture of isomers as well as the isolated single compounds were analyzed using a HPLC pump model L-6200 (Merck, Darmstadt, Germany), autosampler model AS-2000 (Merck), column oven model CTO-10AC (Shimadzu, Duisburg, Germany) and diode array detector model L-4500 (Merck). For separation, an analytical scale polymeric C_{30} (250 × 4.6 mm, 5 µm) column (YMC Europe, Schermbeck, Germany), preceded by a C_{18} ProntoSil 120-5-C18 H (10 × 4.0 mm, 5 µm) column (Bischoff, Leonberg, Germany) was used. As mobile phase (1.3 mL/minute) the following gradient procedure consisting of methanol (solvent A) and methyl tert-butyl ether (solvent B) was used: 1) Initial conditions 90% solvent A and 10% solvent B, 2) a 35-minute linear gradient to 45% solvent B, 3) a 10-minute linear gradient to 60% solvent B, 4) 40% solvent A and 60% solvent B for 11 minutes, 5) a

4-minute linear gradient to 10% solvent B. The column temperature was 23 ± 1°C, and injection volume was 50 µL [8].

UV-vis spectroscopy

The absorbance spectra of isolated isomers were measured in hexane, petroleum ether, MTBE, ethanol, dichloromethane, and acetone directly after fractionation using an UV-vis spectrophotometer (model V-530, Jasco, Groß-Umstadt, Germany).

HPLC-MS analysis

Dried isomers were transported cooled under argon ambience and stored at –18°C until analysis. Before analysis, residues were redissolved in MTBE/methanol (1+1, v/v). The HPLC-mass spectrometry (MS) was performed on an HP1100 modular HPLC system (Hewlett Packard, Waldbronn, Germany), coupled to a Micromass (Manchester, UK) VG platform II quadrupole mass spectrometer, using an APCI interface, operated in the positive mode to generate quasimolecular ions ($[M+H]^+$). Definite interface parameters as well as information about instrument calibration were described in detail previously [9]. Mass spectra of lycopene isomers were recorded in a m/z 200–1000 scan range and the UV-vis absorbance measurement was monitored at 450 nm (DAD). Data were acquired and processed using MassLynx 3.2 software. For HPLC separation, a YMC analytical column (250 × 4.6 mm, 5 µm; YMC Europe, Schermbeck, Germany) with C_{30} material including a precolumn (10 × 4.6 mm, 5 µm) maintained at 35 ± 1°C was used. The mobile phase consisted of two mixtures of methanol, MTBE, and water (A = 81+15+4, v/v/v and B = 6+90+4, v/v/v), using a gradient program (minutes/%A): 0/99, 39/44, 45/0, 48/0, 52/99, 55/99 at a flow rate of 1 mL/minute. The injection volume was 20 µL.

NMR analysis

To eliminate residual solvent, the separated isomers were dried again under vacuum for two hours in a desiccator. Dried isomers were transported cooled under argon ambience and stored at –18°C until actual analysis. For NMR analysis, 160–290 µg of the isolated lycopene isomers dissolved in 175 µL $CDCl_3$ were used. The NMR solvent and the sample tube were flushed with nitrogen in order to remove oxygen prior to data acquisition. NMR spectra were recorded on a Varian Unity Inova 500 MHz NMR equipped with a 3 mm ID-PFG probe. ^{1}H chemical shifts were referenced to the residual solvent signal at $\delta = 7.27$ ppm ($CDCl_3$) relative to TMS. ^{1}H NMR and 2D NMR (gCOSY, TOCSY, TROESY) measurements were performed using standard Varian pulse sequences.

Results and Discussion

Iodine-mediated isomerization of *(all-E)*-lycopene resulted in a mixture of *(all-E)*-lycopene, *(Z)*-isomers, and oxidation products. Five isomers (Figure 1), being the same as recently investigated on their antioxidant activity [3] as well as being partly present in plasma samples [10], with retention times (preparative column) between 30 and 60 minutes, were fractionated for characterization. These five peaks represented 25–72% (mean value of 15 isomerization days) of the peaks in the mixture obtained by iodine mediated isomerization. The large variation depended on different amounts of oxidation products while the relative contents of the *(Z)*-isomers were nearly constant (ratio: peaks (1+2+3):4:5 = 1.5 ± 0.4:1.0:1.5 ± 0.3.; *(all-E)*-lycopene (peak 4) was the basis for the calculation of the ratio).

UV-vis: The UV-vis data of the five isomers investigated are shown in Table I. Differences in the wavelength corresponding to maximal absorbance were observed depending on the solvent used. For *(all-E)*-lycopene **(4)**, the wavelength of the maximum varied between 469 nm in petroleum ether and 482 nm in dichloromethane. Isomer **5** exhibited the same spectral characteristics in all solvents as *(all-E)*-lycopene **(4)**. Thus, this isomer was suspected to be a mono-*(Z)*-isomer with *(Z)*-double bond far from the center of the molecule. In contrast, the spectra of the isomers **1–3** showed a hypsochromic shift of 5–7 nm, being normally a hint for mono-*(Z)*-isomers. In our experiment, one *(Z)*-double bond of the di-*(Z)*-isomers was far from the center of the molecule. The ratio $\varepsilon_2/\varepsilon_1$ (absorption intensity at the near-UV maxima to absorption intensity at the main absorption maximum) is another useful

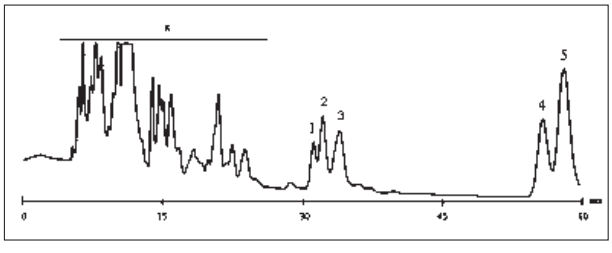

Figure 1: Chromatographic separation of *(all-E)*-lycopene and four lycopene *(Z)*-isomers (25–50 ng per isomer) using a preparative C_{30} column at room temperature, DAD 450 nm (see text for further chromatographic conditions). **1, 2, 3, 5** = lycopene *(Z)*-isomers, **4** = *(all-E)*-lycopene, **x** = other lycopene metabolites.

Table I: Electronic absorption of geometrical lycopene isomers investigated in pure organic solvents

Isomer	Absorption maxima[a] [$\varepsilon_2/\varepsilon_1$][b]	
	Hexane	Petroleum ether
4 *(all-E)-*	295, 363, 444, (471), 502 [0.09]	295, 361, 443, (469), 501 [0.12]
1 *(5Z, 9'Z)*	296, 361, 438, (464), 496 [0.21]	296, 361, 438, (464), 495 [0.19]
2 *(9Z)*	296, 361, 438, (465), 496 [0.18]	296, 361, 438, (464), 495 [0.18]
3 *(5Z, 9Z)*	296, 361, 438, (465), 496 [0.16]	296, 361, 438, (464), 495 [0.17]
5 *(5Z)*	295, 362, 443, (470), 502 [0.09]	295, 361, 443, (469), 500 [0.09]
	MTBE	Ethanol
4 *(all-E)-*	294, 361, 445, (472), 503 [0.18]	295, 363, 445, (472), 503 [0.17]
1 *(5Z, 9'Z)*	296, 361, 440, (466), 497 [0.23]	297, 361, 441, (466), 497 [0.21]
2 *(9Z)*	296, 361, 440, (466), 497 [0.19]	296, 361, 441, (466), 497 [0.21]
3 *(5Z, 9Z)*	296, 361, 440, (466), 497 [0.18]	296, 361, 441, (467), 497 [0.21]
5 *(5Z)*	295, 362, 445, (472), 503 [0.10]	295, 362, 446, (472), 503 [0.16]
	Dichloromethane	Acetone
4 *(all-E)-*	nd, 369, 455, (482), 515 [0.16]	nd, 363, 447, (473), 505 [0.13]
1 *(5Z, 9'Z)*	nd, 368, 450, (476), 509 [0.30]	nd, 362, 442, (468), 499 [0.28]
2 *(9Z)*	nd, 368, 450, (476), 509 [0.30]	nd, 362, 442, (468), 499 [0.14]
3 *(5Z, 9Z)*	nd, 368, 450, (476), 509 [0.14]	nd, 362, 442, (468), 499 [0.15]
5 *(5Z)*	nd, 368, 455, (482), 515 [0.10]	nd, 363, 447, (473), 505 [0.08]

[a] Units are nm. Values in parentheses represent the main absorption maxima.
[b] Ratio of absorption intensity (ε_2) at the near-UV maxima (361–369 nm) to absorption intensity (ε_1) at the main absorption maximum (464–482 nm).
nd: not detected.

parameter to describe carotenoid isomers. This value is nearly zero for *(all-E)*-isomers, and increases with position of the *(Z)*-double bond getting closer to the center of the molecule. Hence this ratio is highest for *(15Z)*-isomers of carotenoids. This study resulted in ratios of 0.09–0.18 for the *(all-E)*-lycopene **(4)**, 0.08–0.16 for isomer **5**, and 0.14–0.30 for the isomers **1–3**. These results also characterize isomer **5** as one with a *(Z)*-double bond far from the center of the molecule. The higher ratios of the three isomers **1–3** describe a position of the *(Z)*-double bond up to the 9- or 9'-position.

HPLC-MS: All isolated isomers showed spectra with strong mass signals at m/z 537.4, corresponding to the quasimolecular ion of lycopene. Fragmentation did not occur, and adduct ions were not present. The isotopic ratio of ions at m/z 537.4/538.4/539.4 calculated for $C_{40}H_{57}$ is in a good agreement with that measured for all lycopene isomers; the intensity of the $[M+H]^+$-signal indicated the presence of a C_{40} skeleton.

NMR: In order to unambiguously identify the structures of the major lycopene isomers obtained by iodine isomerization of *(all-E)*-lycopene (Figure 2), the individual isomers **1–5** were semi-preparatively isolated and investigated by ^{1}H NMR. Spectral assignment of the proton signals was performed by 2D NMR including gCOSY, TROESY, and TOCSY. Compared to *(all-E)*-lycopene **(4)**, a downfield shift of $\Delta\delta = 0.04$ ppm of H-2 and $\Delta\delta = 0.11$ ppm of H-4 as well as ROEs between Me-18 and H-6, H-4 and H-7 established the *(5Z)*-configuration in compound **5** (Table II). As in the case of the *(5Z)*-configuration, *(9Z)*-isomer **2** showed also some typical chemical shift differences compared to *(all-E)*-lycopene **(4)**: downfield shift of $\Delta\delta = 0.08$ ppm of H-6, $\Delta\delta = 0.02$ ppm of H-7, $\Delta\delta = 0.54$ ppm of H-8, and $\Delta\delta = 0.17$ ppm of H-11 as well as upfield shift of $\Delta\delta = 0.14$ ppm of H-10, and $\Delta\delta = 0.07$ ppm of H-12. A crosspeak between H-10 und Me-19 in the ROESY spectrum clearly established the *(9Z)*-configuration. Compounds **1** and **3** (Figure 2) showed almost identical ^{1}H NMR spectra in the olefinic region (Figure 3). The major difference is a chemical shift difference ($\Delta\delta = 0.03$ ppm)

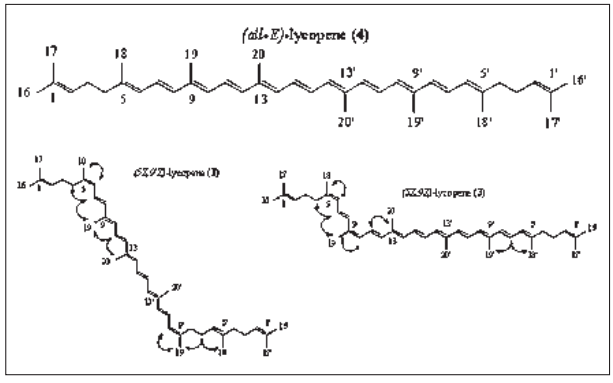

Figure 2: The structures of *(all-E)*-lycopene and of compounds **1** *(5Z, 9'Z)* and **3** *(5Z, 9Z)*. The arrows indicate important ROESY correlations.

Table II: [1]H NMR of lycopene isomers. $CDCl_3$, ref= 7.27 ppm, ϑ = 25°C, 500 MHz

	[1]H NMR δ [ppm], *mult*, *J* [Hz] [a], ov = overlapped, *s* = singlet, *d* = doublet, *t* = triplet				
	1 *(5Z, 9'Z)*	**2** *(9Z)*	**3** *(5Z, 9Z)*	**4** *(all-E)*	**5** *(5Z)*
2	5.16, *bt,* 7.2	5.13, ov	5.16, *bt,* 7.2		5.16, *bt,* 7.2
				5.12, *bt,* 6.8	
2'	5.13, *bt,* 6.8	5.12, ov	5.12, *bt,* 6.8		5.12, *bt,* 7.0
3 (2H)	2.12, ov	2.14, ov	2.14, ov		2.14, ov
				2.13, ov	
3' (2H)	2.14, ov	2.13, ov	2.13, ov		2.13, ov
4 (2H)	2.23, *t,* 7.2	2.13, ov	2.24, *bt,* 7.4		2.24, *bt,* 7.4
				2.13, ov	
4' (2H)	2.14, ov	2.13, ov	2.13, ov		2.13, ov
6	5.95, *d,* 11.4	6.04, *d,* 10.9	6.03, *d,* 10.7		5.95, *d,* 10.9
				5.96, *d,* 10.9	
6'	6.04, *d,* 11.1	5.96, *d,* 10.9	5.96, *d,* 10.8		5.96, *d,* 10.9
7	6.50, *dd,* 11.2, 15.1	6.52, *dd,* 10.9, 15.0	6.52, *dd,* 11.1, 14.9		6.50, *dd,* 11.0, 15.1
				6.50, *dd,* 10.9, 15.1	
7'	6.52, *dd,* 10.9, 14.9	6.50, *dd,* 10.9, 15.1	6.50, *dd,* 10.8, 15.1		6.50, *dd,* 11.0, 15.1
8	6.23, *d,* 15.1	6.80, *d,* 15.1	6.77, *d,* 14.9		6.26, *d,* 15.0
				6.26, *d,* 15.0	
8'	6.80, *d,* 15.0	6.26, *d,* 15.1	6.26, *d,* 15.0		6.26, *d,* 15.0
10	6.19, *d,* 11.5	6.05, *d,* 11.6	6.05, *d,* 11.6		6.19, *d,* 11.3
				6.19, *d,* 11.4	
10'	6.05, *d,* 11.5	6.19, *d,* 11.5	6.19, *d,* 11.5		6.19, *d,* 11.3
11	6.64, ov	6.81, *dd,* 11.6, 14.9	6.80, *dd,* 11.5, 14.9		6.64, ov
				6.64, ov	
11'	6.81, *dd,* 11.4, 14.9	6.64, ov	6.64, ov		6.64, ov
12	6.36, *d,* 14.9	6.29, *d,* 15.0	6.29, *d,* 15.1		6.36, *d,* 14.9
				6.36, *d,* 14.9	
12'	6.29, *d,* 14.9	6.36, *d,* 14.9	6.36, *d,* 15.0		6.36, *d,* 14.9
14	6.26, ov	6.26, ov	6.26, ov		6.26, ov
				6.26, ov	
14'	6.26, ov	6.26, ov	6.26, ov		6.26, ov
15	6.63, ov	6.64, ov	6.64, ov		6.64, ov
				6.64, ov	
15'	6.63, ov	6.64, ov	6.64, ov		6.64, ov
16 (3H)	1.696, *s*	1.702, *s*	1.696, ov		1.697, ov
				1.698, *s*	
16' (3H)	1.703, *s*	1.696, *s*	1.696, ov		1.697, ov
17 (3H)	1.632, *s*	1.632, *s*	1.633, *s*		1.635, *s*
				1.624, *s*	
17' (3H)	1.632, *s*	1.623, *s*	1.624, *s*		1.624, *s*
18 (3H)	1.836, *s*	1.836, *s*	1.853, *s*		1.836, *s*
				1.829, *s*	
18' (3H)	1.836, *s*	1.828, *s*	1.829, *s*		1.829, *s*
19 (3H)	1.962, *s*	1.977, ov	1.965, *s*		1.963[b]
				1.979, ov	
19' (3H)	1.977, *s*	1.977, ov	1.977, ov		1.979[b],ov
20 (3H)	1.977, *s*	1.989, *s*	1.984, *s*		1.979, ov
				1.979, ov	
20' (3H)	1.989, *s*	1.977, ov	1.977, ov		1.979, ov

[a] Observed coupling constants were not averaged. Assignments based on gCOSY, TOCSY, and TROESY spectra.
[b] Assignments maybe interchanged.

between the doublet at δ = 6.80 ppm of **1** and δ = 6.77 ppm of **3**. Significant differences were observed in the chemical shift region of the methyl groups as shown in Figure 4. Inspection of the 2D NMR spectra revealed both the peak pattern of *(5Z)*- and *(9Z)*-configurations in both compounds **1** and **3**. The ROESY-correlations between H-4 and H-7, Me-18 and H-6, H-7 and Me-19, Me-19 and H-10 unambiguously identified **3** as *(5Z, 9Z)*-lycopene whereas ROEs between H-4 and H-7, Me-18 and H-6, H-7 and Me-19, Me-19 and H-11, H-10' and Me-19', Me-19' and H-7', H-7' and Me-18' established **1** as *(5Z, 9'Z)*-lycopene.

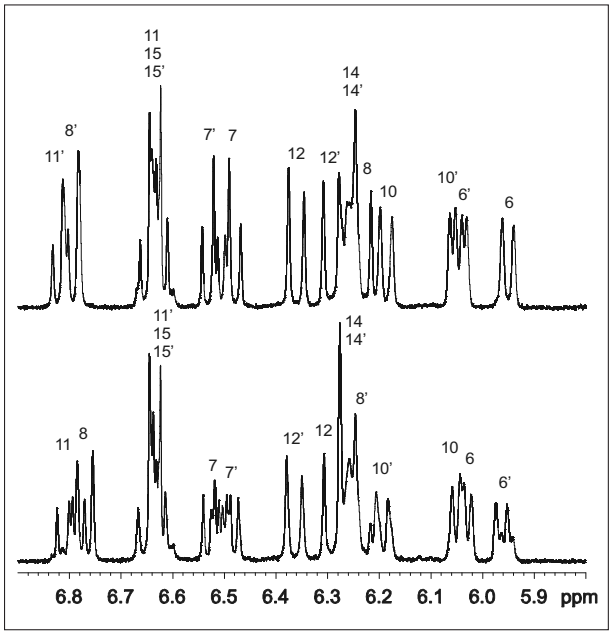

Figure 3: The ^{1}H NMR spectra of the olefinic region of **1** *(5Z, 9'Z)* (upper trace) and **3** *(5Z, 9Z)* (lower trace).

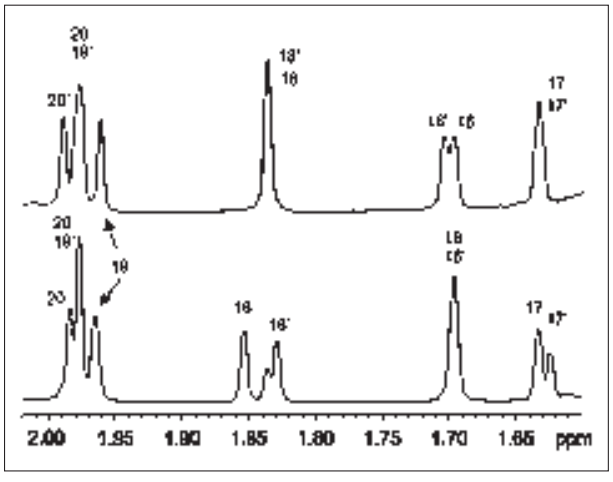

Figure 4: The ^{1}H NMR spectra of the region of the methyl groups of **1** *(5Z, 9'Z)* (upper trace) and **3** *(5Z, 9Z)* (lower trace).

Comparison of the ^{1}H NMR data for compounds **1, 2, 4,** and **5** with literature values [11] were in a good agreement except for the chemical shift of H-8 in compound 2 *(9Z)*. In the study presented here $\delta = 6.80$ was found whereas Hengartner *et al* reported $\delta = 6.70$ for that proton. These authors isolated compounds **1** and **2** by using C_{18} HLPC and synthesized isomers **4** and **5**. In another study [12] where liquid chromatography (LC)-NMR was used for the characterization of different *(Z)*-isomers of lycopene, Strohschein identified compounds **2**, and **5** by this method

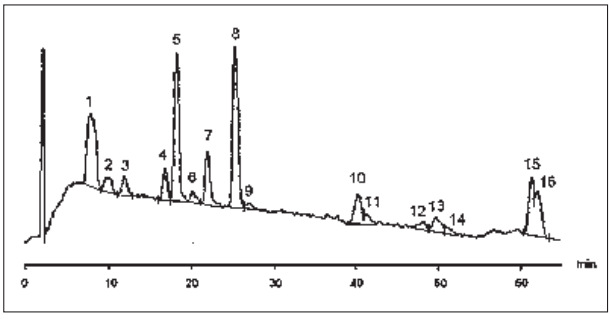

Figure 5: HPLC chromatogram of a plasma extract using an analytical C_{30} column at 23 ± 1°C (further chromatographic conditions are the same as described in the text for the mixture of isomers and the isolated single compounds). 1 = *(all-E)*-lutein, 2 = *(all-E)*-zeaxanthin, 3 = *(all-E)*-canthaxanthin, 4 = *(all-E)*-β-cryptoxanthin, 5 = echinenone (IS), 6 = *(13Z)*-β-carotene, 7 = *(all-E)*-β-carotene, 8 = *(all-E)*-β-carotene, 9 = *(9Z)*-β-carotene, 10 = *(13Z)*-lycopene, 11 = *(15Z)*-lycopene, 12 = *(5Z, 9'Z)*-lycopene **(isomer 1)**, 13 = *(9Z)*-lycopene **(isomer 2)**, 14 = *(5Z, 9Z)*-lycopene **(isomer 3)**, 15 = *(all-E)*-lycopene **(isomer 4)**, 16 = *(5Z)*-lycopene **(isomer 5)**.

and compound **3** by conventional NMR after isolation. The author used a silica gel column and acetone as a mobile phase. A recent paper [13] describing the analysis of lycopene isomers in tomato extracts and human serum, identified only compounds **2** and **5** by using LC-NMR (C_{30} column, acetone/water gradient or methanol/MTBE/water gradient as mobile phase). In another paper [14], Breitenbach *et al* showed the compounds 2–5 in a C_{30} chromatogram (methanol/MTBE/water gradient as mobile phase) of a lycopene isomer standard without giving any information about identification of carotenoids. Even a current paper [15] investigating lycopene isomers in tomato pulp only tentatively identified two of the here presented *(Z)*-isomers (**2** and **5**) by using spectroscopic data as well absorbance ratios from the literature. To our knowledge, the identification of the *(5Z)*-isomer seems to be erroneous. In addition, the authors used a very unusual mobile phase (1-butanol/acetonitrile/methylene chloride gradient) for their separation. Tiziani *et al* (2006) used off-line NMR experiments to characterize lycopene *(Z)*-isomers in extracts from tomato juice. They did not isolate single *(Z)*-isomers. However, they identified the compounds **2** and **5** and allocated them to a C_{30} separation using a methanol/MTBE gradient as mobile phase [16].

To the best of our knowledge, this paper presents for the first time ever the unambiguous identification of two lycopene (di-*Z*)-isomers related to the known, most common HPLC separation on a C_{30} column. This will allow other laboratories to use the results for their C_{30} HPLC. Thus, six *(Z)*-isomers of lycopene in human plasma (Figure 5) are now characterized. In addition, it is possible to

investigate structure-activity relationships for these lycopene isomers which will improve the understanding of their physiological role in biological tissues.

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