Structural Elucidation of the Main *cis*- β -Carotenes

Kiyoshi TSUKIDA, Kayoko SAIKI, and Makiko SUGIURA¹

Kobe Women's College of Pharmacy, Motoyama-kitamachi,
Kobe 658, Japan

(Received July 20, 1981)

Summary Different isomerization experiments on all-*trans*- β -carotene (I) yielded multi-component mixtures of *cis* isomers. Separation and isolation of these isomers were achieved by classic adsorption chromatography on a lime column. Investigation of their 200 MHz ¹H- and 50.3 MHz ¹³C-NMR spectra led to the structural elucidation of the main *cis*- β -carotenes, namely the 9-monocis structure (II) for neo β -carotene U, and the 13-monocis structure (III) for neo β -carotene B. Formation of other hindered monocis (7-*cis* and 11-*cis*) and 15-monocis isomers should be carefully examined by a suitable method such as high-performance liquid chromatography.

Key Words *cis-trans* isomerization, *cis*- β -carotene, neo β -carotene U, neo β -carotene B, ¹H-NMR, ¹³C-NMR, refocussed INEPT

Artificial stereoisomerization of all-*trans*- β -carotene (I) has been known to produce twelve *cis* forms in total (theoretically 272 *trans/cis* isomers can exist), among which neo U (adsorbed above the all-*trans* pigment on a lime column) and neo B (adsorbed below it) are the two main stereoisomers(1), though the latter is also a genuine constituent of *Osmanthus fragrans* flowers(2).

By careful inspection of ultraviolet (UV) and infrared (IR) spectral data, Zechmeister *et al.* interpreted neo β -carotene U as a peripheral 9-monocis- β -carotene and also predicted the 9,15-dicis(3), later the revised 9,13'-dicis structure to neo β -carotene B(4). Though structural elucidation of these isomers was considerably hampered by the fact that only UV and IR spectroscopic data were available at that time, it is also true that no criteria has been presented on these assignments during the past two decades, mainly because of their lesser bio-significance as provitamin A. However, recent growing interest in the chromophoric behavior of carotenoid of carotene-containing bio-molecules and microorganisms prompted us to report our results obtained by modern spectral analysis. In the present paper, we report on the isomerization of all-*trans*- β -carotene and identification by ¹H- and ¹³C-nuclear magnetic resonance (NMR) spectroscopy of the

¹ 月田 潔, 斎木加代子, 杉浦眞喜子

main *cis*- β -carotenes. It is hoped that our data and conclusion will be informative and useful for future application to the structural elucidation of other geometrical isomers of retinoidal and carotenoidal compounds hitherto unknown.

MATERIALS AND METHODS

Chemicals. Lime (calcium hydroxide, guaranteed reagent) was purchased from Nakarai Chemicals, Ltd., Kyoto. All-*trans*- β -carotene was kindly provided by Dr. T. Takahashi, Sumitomo Chemical Co., Ltd., Osaka. Neo β -carotenes U and B were prepared by iodine-catalyzed photoisomerization of the all-*trans* compound.

Stereoisomerization. The following experiments a)–g) were conducted by K.T. in 1956 in collaboration with Dr. L. Zechmeister at the California Institute of Technology, Pasadena. The hexane solution (150–200 ml) containing all-*trans*- β -carotene (20 mg) was used throughout experiments a)–e). After being isomerized, the solution was evaporated *in vacuo*, dissolved in hexane, and poured onto a lime column (45 $\times$ 4.5 cm), then developed with 2% acetone in hexane. Each chromatogram was cut out separately, eluted with acetone, transferred into hexane, and estimated photometrically.

- a) Standing: the hexane solution was left to stand under diffuse light at room temperature for 5 days.
- b) Insolation: the hexane solution was exposed to sunshine for 5 hr.
- c) Iodine-catalyzed photoisomerization: the hexane solution containing I₂ (0.5 mg/20 mg carotene) was exposed to sunshine for 1 hr.
- d) HCl-treatment: the hexane solution was mechanically shaken with c-HCl (100 ml) for 30 min, washed with water and dried over Na₂SO₄.
- e) Refluxing: the hexane solution was refluxed in darkness, under an atmosphere of N₂ for 1 hr.
- f) Melting crystals: crystals (20 mg) were melted in a sealed glass tube and kept in a bath at 190–200°C for 15 min. The melt was rapidly solidified in ice-water

Table 1. Isomerization of all-*trans*- β -carotene (I) and spectral (UV and MS) characteristics of isomeric β -carotenes.

Procedure	Percentage composition				
	neo U	:	all- <i>trans</i>	:	neo B ^a
a) Standing	0	:	75	:	25
b) Insolation	0	:	80	:	20
c) I ₂ -Catalysis, in light	19	:	49	:	32
d) HCl treatment	18	:	48	:	34
e) Refluxing	0	:	79	:	21
f) Melting crystals	26	:	25	:	49
g) Naphthalene melt	22	:	30	:	48

Isomers	UV (nm, in hexane)	MS (M ⁺ peak)		
	$\lambda_{\max}$ [ϵ] ^c	Calc.	Found	Formula
All- <i>trans</i>	478, 450 [139 000], 425 (infl) <i>cis</i> -peak 337 [8 000]	536.438	536.438	C ₄₀ H ₅₆
Quasi-equilibrium mixture ^b	472, 446 [118 000], 423 (infl) <i>cis</i> -peak 336 [20 800]	—	—	—
Neo U	473, 445 [131 000], 421 (infl) <i>cis</i> -peak 338 [12 800]	536.438	536.439	C ₄₀ H ₅₆
Neo B	467, 442 [105 000], 418 (infl) <i>cis</i> -peak 336 [37 500]	536.438	536.438	C ₄₀ H ₅₆

^a Contains small amounts of neo A, C, D, and E. ^b The hexane solution containing all-*trans*- β -carotene (32 μ g/ml) and I₂ (0.1 μ g/ml) was irradiated by a fluorescent lamp (20W) from a 15-cm distance for 10 min. ^c Calculated from the known values (ϵ) for all-*trans*- β -carotene.

and dissolved in hexane.

g) Naphthalene melt: crystals (20 mg) were mixed with naphthalene (100 mg), placed in a sealed test tube, and kept in a bath at 190–195°C for 15 min. The melt was rapidly solidified in ice-water and dissolved in hexane.

Spectra. Spectral measurements were recorded with the following instruments: UV with a Shimadzu UV 200S ($\lambda_{\max}$ in nm, hexane), and 200 MHz ^1H -NMR or 50.3 MHz ^{13}C -NMR with a Varian XL-200 NMR spectrometer (δ in ppm, CDCl_3).

Table 2. ^1H -NMR data of stereoisomeric β -carotenes (200 MHz, δ in ppm, CDCl_3).

Protons	All- <i>trans</i> (I)	Neo U (II)	Neo B (III)
1,1,1',1'-Me	1.03	1.03+1.05	1.03+1.04
2,2'-H ₂	≈ 1.50	≈ 1.48	≈ 1.49
3,3'-H ₂	≈ 1.62	≈ 1.62	≈ 1.62
5'-Me	} 1.72	1.71	} 1.72
5-Me		1.75	
9,9',13,13'-Me	1.98	1.97	1.97+1.98
4,4'-H ₂	≈ 2.03	≈ 2.02	≈ 2.02
10-H	} 6.17 d (J12)	6.07 d (J11) ^b	} ≈ 6.16
10'-H		6.16 d (J11)	
7,7'-H	} ≈ 6.17	} ≈ 6.16	6.12 ^a ABq (J16)
8'-H			} $\approx 6.21^a$ ABq-like
8-H		6.69 d (J16)	
14-H	} ≈ 6.27 d-like ^{a,b}	} ≈ 6.26 d-like ^a	≈ 6.21 d-like
14'-H			6.29 d (J11)
12-H	} 6.36 d (J15) ^b	6.30 d (J15) ^b	6.90 d (J15)
12'-H		6.36 d (J15)	6.36 d (J15)
15-H	} 6.62 dd (J11, 15) ^b	} 6.62 dd (J11, 15)	6.81 dd (J11, 16)
15'-H			6.57 dd (J11, 16)
11-H	} 6.67 dd (J12, 15) ^b	6.78 dd (J11, 15) ^b	} 6.66 dd (J11, 15)
11'-H		6.66 dd (J11, 15)	

^a Center position. ^b Confirmed by decoupling experiments.

Table 3. Chemical shift differences ($\delta_{\text{cis}} - \delta_{\text{trans}}$) of the ^1H -NMR signals of *cis*- β -carotenes and related compounds (200 MHz, δ in ppm, CDCl_3 ; all the changes larger than ± 0.03 ppm are compiled; positive shifts mean downfield in the *cis* compound).

Isomers	H-8	H-10	H-11	H-12	H-14	H-15	H-15'	5-Me
Neo β -carotene U (II)	+0.52	-0.10	+0.11	-0.06	—	—	—	+0.03
9- <i>cis</i> -Astaxanthin ^a (7)	+0.54	-0.06	+0.05	-0.10	—	—	—	+0.03
Neo β -carotene B (III)	?	—	—	+0.54	-0.06	+0.19	-0.05	—
13- <i>cis</i> -Astaxanthin ^a (7)	—	—	—	+0.53	-0.12	+0.16	-0.08	—

^a All-*trans*-astaxanthin, see the formula (IV).

tetramethylsilane being used as an internal standard). For CH carbon selection in the ^{13}C -NMR spectrum, the refocused INEPT (insensitive nuclei enhanced by polarization transfer) technique (5) was employed. Electron-impact ionization mass spectra (MS) were determined with a JEOL JMS-01SG double focus high resolution spectrometer equipped with a direct-inlet system.

RESULTS AND DISCUSSION

1. Stereoisomerization of all-trans- β -carotene

Isomerization yields multi-component mixtures of *cis* isomers (Table 1). It is evident that neo U and neo B are the main *cis* isomers, though some other *cis* forms are also included in a mixture.

2. Spectra and structures

Mass spectral measurements clearly indicate that both neo U and neo B are β -carotene isomers (from M^+ (Table 1) and fragmentation behavior), though no

Fig. 1. Olefinic part of the ^1H -NMR of neo β -carotenes U and B (200 MHz, in CDCl_3).

further information can be obtained on their configurations. It is known, however, that the ^1H -NMR spectra of carotenoids, even their highly crowded olefinic parts, can be largely interpreted if they are recorded at sufficiently high magnetic field (7). For present purposes, highly resolved ^1H - and ^{13}C -NMR data including the isomerization shifts ($\delta_{\text{cis}} - \delta_{\text{trans}}$) are considered, the structural assignments being straightforward.

a) *Neo* β -carotene U. A peripheral monocis structure is deduced from its UV characteristics (λ_{max} shift and *cis*-peak intensity) (Table 1). ^1H -NMR strongly suggests the existence of a *cis* environment around the C-9 position (Tables 2 and 3, Fig. 1). Though recently developed binuclear lanthanide NMR shift reagent $[\text{Yb}(\text{fod})_3 + \text{Ag}(\text{fod})_3]^*$ (8) is not effective for present analysis, ^{13}C -NMR measurements (complete decoupling and the INEPT technique) (Figs. 2 and 3) finally give

Fig. 2. ^{13}C -NMR spectra (sp^3 region) of all-*trans*- β -carotene (50.3 MHz, in CDCl_3 ; complete decoupling and off-resonance decoupling).

* fod = 1,1,1,2,2,3,3-heptafluoro-7,7-dimethyl-4,6-octanedionate.

Fig. 3. ^{13}C -NMR spectra (sp^2 region) of neo β -carotene U (50.3 MHz, in CDCl_3 ; complete decoupling, off-resonance decoupling, and CH selection by the refocused INEPT technique).

decisive evidence for the structure of this isomer as 9-monocis- β -carotene (**II**) (Tables 4 and 5).

b) Neo β -carotene B. A monocis or a dicis structure containing at least one near-central *cis* or a central *cis* configuration is suggested from its UV characteristics (λ_{max} shift and *cis*-peak intensity) (Table 1). ^1H -NMR strongly indicates the existence of a *cis* atmosphere not around the C-9 or C-9', but around the C-13 position (Tables 2 and 3, Fig. 1). In addition to the above evidence, ^{13}C -NMR is again able to give congruous information for the structure of this isomer as 13-monocis- β -carotene (**III**) (Tables 4 and 5, Fig. 4).

Separation and isolation of other minor monocis isomers (central 15-*cis* as well as hindered 7- and 11-*cis*) by high-performance liquid chromatography are being pursued.

Table 4. ^{13}C -NMR data of stereoisomeric β -carotenes (50.3 MHz, δ in ppm, CDCl_3).

Carbon	All-trans (I)		Neo U (II)	Neo B (III)
	Ref. 10)	Found		
1,1'	34.4	34.33s ^a	≈ 34.27 br	34.30
2,2'	39.8	39.70t	39.59 + 39.68	39.67
3,3'	19.4	19.32t	19.29	19.29
4,4'	33.2	33.15t	33.11	33.13
5	129.3	129.34s	129.51 ^a	129.48
5'			129.36 ^a	129.34
6	138.0	137.97s	138.24	137.89 ^c
6'			137.95	137.93 ^c
7	126.7	126.66d	128.44	126.97
7'			126.64	126.59
8	137.8	137.86d	129.87	137.73 ^f
8'			137.80	137.81 ^f
9	136.0	135.99s	134.60	136.71
9'			135.99	135.91
10	130.8	130.91d	129.36	130.84 ^g
10'			130.86	
11	125.1	125.06d	123.86	126.36
11'			125.00	124.90
12	137.3	137.29d	136.52	129.07 ^h
12'			137.26	137.27
13	136.4	136.47s	≈ 136.38 br	134.85
13'				136.29
14	132.4	132.46d	132.30 ^b	130.75 ^g
14'			132.42 ^b	132.28
15	130.0	130.02d	130.01	128.72
15'				129.14 ^h
1,1,1',1'-Me	29.0	29.01q	29.00	28.99
5-Me	21.7	21.79q	21.89 ^c	21.78
5'-Me			21.77 ^c	
9-Me	12.8	12.77q	20.79	12.80
9'-Me			12.77 ^d	
13-Me	12.8	12.82q	12.86 ^d	20.76
13'-Me			12.83 ^d	12.80

Splitting in off-resonance. s, singlet; d, doublet; t, triplet; q, quartet; br, broad. ^{a-h} May be interchanged.

Table 5. Chemical shift differences ($\delta_{cis}-\delta_{trans}$) of the ^{13}C -NMR signals of *cis*- β -carotenes and related compounds (50.3 MHz, δ in ppm, CDCl_3 ; all the changes larger than ± 0.7 ppm are compiled; positive shifts mean downfield in the *cis* compound).

Isomers	C-5	C-7	C-8	C-9	C-10	C-11	C-12	C-13	C-14	C-15	C-15'	9-Me	13-Me
Neo β -carotene U (II)	—	+1.8	-8.0	-1.4	-1.6	-1.2	-0.8	—	—	—	—	+8.0	—
Neo zeaxanthin B ^a	—	+2.1	-8.0	-1.6	-1.6	-1.0	-0.9	—	—	—	—	+8.3	—
(9- <i>cis</i> -zeaxanthin; violeoxanthin) (9)	—	—	—	—	—	—	—	—	—	—	—	—	—
9- <i>cis</i> -Retinol ^b (10)	—	+2.1 (+1.8)	-7.7	-0.8 (-1.3)	-1.5	-0.8 (-1.0)	-0.8	—	—	—	—	+8.2 (+8.3)	—
Neo β -carotene B (III)	—	—	—	+0.7	—	+1.3	-8.2	-1.6	-1.7	-1.3	-0.9	—	+7.9
Neo zeaxanthin A ^a	—	—	—	—	—	+1.1	-8.4	-1.5	-1.5	-1.3	—	—	+7.9
(13- <i>cis</i> -zeaxanthin) (11)	—	—	—	—	—	—	—	—	—	—	—	—	—
13- <i>cis</i> -Retinol ^b (10)	—	—	—	+1.2 (+0.8)	—	+2.0 (+2.3)	-7.9	-1.0 (-)	-1.7 (-1.9)	-0.8 (-0.7)	—	—	+7.8

^a All-*trans* zeaxanthin, see the formula (V). ^b All-*trans* retinol, see the formula (VI).

Fig. 4. ^{13}C -NMR spectra (sp^2 region) of neo β -carotene B (50.3 MHz, in CDCl_3 ; complete decoupling and CH selection by the refocussed INEPT technique).

REFERENCES

- 1) Zechmeister, L. (1962): β -Carotene set, in *Cis-Trans Isomeric Carotenoids, Vitamins A, and Arylpolyenes*, Springer-Verlag, Wien, pp. 80–84.
- 2) Suzuki, N., and Tsukida, K. (1959): Carotenoids of the flowers of *Osmanthus fragrans*. *Chem. Pharm. Bull.*, **7**, 133–134.
- 3) Polgár, A., and Zechmeister, L. (1942): Isomerization of β -carotene. Isolation of a stereoisomer with increased adsorption affinity. *J. Am. Chem. Soc.*, **64**, 1856–1861.
- 4) Lunde, K., and Zechmeister, L. (1955): Infrared spectra and *cis-trans* configurations of some carotenoid pigments. *J. Am. Chem. Soc.*, **77**, 1647–1653.
- 5) Gray, G. A. (1980): Use of INEPT-based pulse sequences for sensitivity enhancement and carbon discrimination on the XL-200. *Varian Instruments at Work*, No. NMR-14, 1–8.
- 6) Zechmeister, L., and Polgár, A. (1943): *Cis-trans* isomerization and spectral characteristics of carotenoids and some related compounds. *J. Am. Chem. Soc.*, **65**, 1522–1528.
- 7) Englert, G., and Vecchi, M. (1980): *Trans/cis* isomerization of astaxanthin diacetate/Isolation by HPLC and identification by ^1H -NMR spectroscopy of three

- mono-*cis*- and six di-*cis*-isomers. *Helv. Chim. Acta*, **63**, 1711-1718.
- 8) Wenzel, T. J., and Sievers, R. E. (1981): Binuclear complexes of lanthanide (III) and silver (I) and their function as shift reagents for olefins, aromatics, and halogenated compounds. *Anal. Chem.*, **53**, 393-399.
 - 9) Moss, G. P. (1976): Carbon-13 NMR spectra of carotenoids. *Pure Appl. Chem.*, **47**, 97-102.
 - 10) Englert, G. (1975): A ^{13}C -NMR study of *cis-trans* isomeric vitamins A, carotenoids and related compounds. *Helv. Chim. Acta*, **58**, 2367-2390.
 - 11) Szabolcs, J. (1976): Some studies on the stereochemistry of carotenoids. *Pure Appl. Chem.*, **47**, 147-159.