Thermal Stereoisomerization of All-(*E*)- β -Carotene: (*Z*)- β -Carotenes and Electrocyclized β -Carotenes

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Summary Thermal isomerization of synthetic all-(*E*)- β -carotene yielded a multi-component mixture composed of more than sixteen (*Z*)- β -carotenes and two thermo-cyclized isomers. They were separated and purified by high-performance liquid chromatography. Ten of these were characterized by spectroscopic methods as four mono-(*Z*)-, five di-(*Z*)-, and one tri-(*Z*)- β -carotenes; the 7-(*Z*)-, 9-(*Z*)-, 13-(*Z*)-, 15-(*Z*)-, 9.13-(*Z*)-, 9.15-(*Z*)-, 9.13'-(*Z*)-, 13.15-(*Z*)-, 7.13'-(*Z*)-, and 9.9'.13-(*Z*)-isomers were included. Stereochemistry of six additional (*Z*)-isomers obtained is, as yet, unknown, though one of them might have the 9.9'-(*Z*)-geometry in the molecule. This is the first report on the formation and identification of the 'sterically hindered' 7-(*Z*)-isomers as well as the (5→10)-thermo-cyclized products in the field of alicyclic carotenoids.

Key Words all-(*E*)- β -carotene, (*Z*)- β -carotenes, 'hindered (*Z*)'- β -carotenes, electrocyclized β -carotenes, thermal stereoisomerization, HPLC

Artificial stereoisomerization of all-(*E*)- β -carotene (**1**) has been reported to produce twelve (*Z*)-forms in total (**1**), though 272 (*E*)/(*Z*) geometrical isomers can exist theoretically. Recent growing interest in the roles of (*Z*)-carotenoids in biomolecules has prompted us to report at first on the structural determination of the two main stereoisomers of β -carotene (**2**), and then on the isolation of the novel 'sterically hindered' 7-(*Z*)-isomer (**3**). At the same time, a thermal isomerization mixture of all-(*E*)- β -carotene produced by melting the crystals was found to contain more than eighteen different ingredients which were well resolved by high-performance liquid chromatography (HPLC) on a lime column using a mixture of *n*-hexane and acetone at an ambient temperature (**4**). We now give here a full account of our results on the spectral characteristics (ultraviolet/visible spectra [UV/VIS] and nuclear magnetic resonance spectra [NMR]) of these (*Z*)-isomers and thermo-cyclized products newly recognized.

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MATERIALS AND METHODS

Chemicals. All-(*E*)- β -carotene was a gift from Dr. T. Takahashi, Sumitomo Chemical Co., Ltd., Osaka. All solvents and reagents were of analytical grade and used without further purification.

Spectra. Spectral measurements were recorded with the following instruments: UV/VIS with a Shimadzu UV 200S ($\lambda_{\max}$, nm in *n*-hexane), and 200 MHz ^1H -NMR or 50.3 MHz ^{13}C -NMR with a Varian XL-200 NMR spectrometer (δ ppm in CDCl_3 ; tetramethylsilane being used as an internal reference). For the purpose of carbon discrimination in the ^{13}C -NMR data, the refocussed INEPT (insensitive nuclei enhanced by polarization transfer) technique was employed (2). Electron-impact ionization mass spectra (MS) were determined with a Hitachi M-80 mass spectrometer; fragmentation being analyzed by the linked scan technique.

Chromatography

1) **HPLC.** HPLC was performed on a Shimadzu LC2 Liquid Chromatograph. Absorbance at 450 or 335 nm was monitored with an UV/VIS detector. Preparation of a lime-packed column (stainless-steel tubing, 4×250 mm) was as previously described (4). An injected sample was eluted by 0.1–2% acetone in *n*-hexane at a flow rate of 1 ml/min.

2) **Column chromatography.** A sample was eluted with 1% acetone in *n*-hexane on a lime column (4.5×40 cm).

3) **Preparative thin-layer chromatography (pTLC).** A mixture was developed and eluted with *n*-hexane on an aluminium oxide 60F₂₅₄ (type E) plate (0.25 mm-thick).

Thermal isomerization. All-(*E*)- β -carotene was isomerized by immersing a thin-walled evacuated tube containing the crystals into a bath of 190–200°C for 15 min. The melt was rapidly solidified in iced water and dissolved in *n*-hexane. Analysis was performed by HPLC (4) and spectroscopic methods.

RESULTS AND DISCUSSION

HPLC separation

A complicated mixture containing thermally isomerized products was analyzed by HPLC. At least eighteen different ingredients were well resolved as described

Table 1. UV/VIS characteristics of β -carotene isomers in *n*-hexane.

β -Carotene isomer	HPLC peak	UV/VIS $\lambda_{\max}$ nm ($\epsilon \times 10^{-4}$ a) in <i>n</i> -hexane					Ratio of ϵ at $\lambda_{\max}$ II to ϵ at (Z)-peak
		Fine structure			(Z)-peak	Overtone	
		I	II	III			
All-(E)	13	478	450 (13.90)	425 (sh)	337 (0.80)	270 (2.00)	17.4
7-(Z)	17	474	446 (13.87)	422 (sh)	337 (0.86)	270 (1.71)	16.1
9-(Z)	15	473	445 (12.97) ^b	421 (sh)	338 (1.13) ^b	255 (1.36)	11.5
13-(Z)	7	467	442 (10.73) ^b	418 (sh)	336 (3.88) ^b	274 (1.65)	2.8
15-(Z)	3	475	448 (10.32)	425 (sh)	335 (5.46)	274 (1.59)	1.9
9,9'-(Z)	16	464	438 (12.15)	415 (sh)	333 (1.42)	261 (1.19)	8.6
13,15-(Z)	2	463	436 (10.43)	414 (sh)	339 (1.49)	282 (2.80)	7.0
9,13-(Z)	9	465	439 (11.98)	415 (sh)	335 (2.02)	275 (1.53)	5.9
9,15-(Z)	8	468	441 (10.95)	417 (sh)	334 (2.20)	277 (1.80)	5.0
9,13'-(Z)	6	464	438 (11.18)	415 (sh)	334 (2.33)	279 (2.39)	4.8
7,13'-(Z)	12	467	440 (11.17)	416 (sh)	334 (2.81)	270 (1.51)	4.0
9,9',13-(Z)	11	456	432 (11.40)	409 (sh)	339 (1.16)	269 (1.78)	9.8
?	1	457 (sh)	431 (9.21)	410 (sh)	337 (3.32)	280 (1.80)	2.8
?	4	467 (sh)	441 (10.33)	415 (sh)	336 (3.52)	278 (2.43)	2.9
?	5	465	439 (11.70)	415 (sh)	335 (2.24)	274 (1.74)	5.2
Electrocyclized isomer	0 ^c	426	404 (4.85)	385 (sh)	297 (0.62)		7.8

^a Calculated from the values for all-(E)- β -carotene and the quasi-equilibrium mixture therefrom at a definite temperature. ^b Previous values (2) were revised. ^c Iodine-catalyzed isomerization gave no evidence that this substance was also a member of the β -carotene set.

previously, *viz.*, electrocyclized thermo-products and seventeen β -carotenes including the unchanged all-(*E*)-, four mono-(*Z*)-, five di-(*Z*)-, one tri-(*Z*)-, and six unknown isomers (4). Contrary to the statement of Zechmeister (5), occurrence of the 'hindered (*Z*)' isomers (7- and 7.13'-*Z*) as well as the central-*Z* isomer (15-*Z*) is particularly noteworthy. Furthermore, thermo-cyclized products were noticed for the first time in the field of alicyclic carotenoids.

Isomer identification

It was quite a laborious task in accumulating sufficient (*Z*)-specimen for measurement of spectra. For this purpose, HPLC running had to be repeated in a dark room. MS (M^+ and fragmentation behavior) and UV clearly demonstrated that these ingredients were (*E*)/(*Z*) members of β -carotene except for peak 0 (and peak 0'), though MS gave no further information on their stereochemistry. Identification of (*Z*)- β -carotenes was accomplished mainly by UV/VIS (Table 1) and ^1H -NMR (200 MHz) (Tables 2 and 3), and in part by ^{13}C -NMR (50.3 MHz) (2, 3). When the assignment of most of the ^1H -NMR signals was nearly completed, diagnostic features of each mono-(*Z*) structure were apparent. For this purpose, special attention was paid to differences in chemical shifts in the NMR spectrum of each (*Z*)-isomer compared with those of the all-(*E*)-form. Then these $\Delta\delta$ values (isomerization shift; Tables 4 and 5) in each mono-(*Z*)-isomer were able to serve as basis for assignment of signals of the particular di-(*Z*) form. For example, the resonances of the 9 (or 7).13-di-(*Z*)-isomer and those of the 9 (or 7).13'-di-(*Z*)-isomer were surprisingly well predicted as described below, when the additivity of

Table 2. Methyl proton chemical shifts in ^1H -NMR (200 MHz) of β -carotene isomers in CDCl_3 (δ ppm).

β -Carotene isomer	HPLC peak ^a	Methyl proton chemical shifts				
		1.1	1'.1'	5	5'	9.9'.13.13'
All-(<i>E</i>)	13	1.03	1.03	1.72	1.72	1.98
7-(<i>Z</i>)	17	1.04	+	1.02	1.52	1.71
9-(<i>Z</i>)	15	1.05		1.03	1.75	1.71
13-(<i>Z</i>)	7	1.04		1.03	1.72	1.72
15-(<i>Z</i>)	3	1.03		1.03	1.72	1.72
13.15-(<i>Z</i>)	2	1.03		1.03	1.72	1.72
9.13-(<i>Z</i>)	9	1.04		1.03	1.75	1.72
9.15-(<i>Z</i>)	8	1.04		1.03	1.76	1.72
9.13'-(<i>Z</i>)	6	1.04		1.04	1.75	1.72
7.13'-(<i>Z</i>)	12	1.04	+	1.03	1.52	1.72
9.9'.13-(<i>Z</i>)	11	1.04		1.04	1.75	1.75

^a Peak numbers refer to HPLC peak elution order (4).

Table 3. Vinyl proton chemical shifts in $^1\text{H-NMR}$ (200 MHz) of β -carotene isomers in CDCl_3 (δ ppm).

β -Carotene isomer	HPLC peak ^a	Vinyl proton chemical shifts														
		7	7'	8	8'	10	10'	11	11'	12	12'	14	14'	15	15'	
All-(E)	13	≈ 6.17	≈ 6.17	≈ 6.17	≈ 6.17	6.17	6.17	6.67	6.67	6.36	6.36	≈ 6.27	≈ 6.27	≈ 6.62	≈ 6.62	
7-(Z)	17	5.83	6.15	6.05	6.15	≈ 6.21	6.15	≈ 6.60	≈ 6.6	≈ 6.32	6.35	≈ 6.26	≈ 6.26	≈ 6.60	≈ 6.60	
9-(Z)	15	≈ 6.16	≈ 6.16	6.69	≈ 6.16	6.07	6.16	6.78	≈ 6.66	6.30	6.36	≈ 6.26	≈ 6.26	≈ 6.62	≈ 6.62	
13-(Z)	7	≈ 6.17	≈ 6.17	≈ 6.17	≈ 6.17	≈ 6.16	≈ 6.16	6.66	6.66	6.90	6.36	≈ 6.21	6.29	≈ 6.81	≈ 6.57	
15-(Z)	3	≈ 6.15	≈ 6.15	≈ 6.15	≈ 6.15	≈ 6.15	≈ 6.15	6.68	6.68	6.41	6.41	6.66	6.66	6.39	6.39	
13,15-(Z)	2	≈ 6.15	≈ 6.15	≈ 6.15	≈ 6.15	≈ 6.19	≈ 6.15	6.6?	6.6?	6.89	6.5?	6.6?	≈ 6.69	6.6?	≈ 6.38	
9,13-(Z)	9	6.14	6.14	6.74	6.14	6.05	6.14	≈ 6.79	≈ 6.66	6.82	6.35	≈ 6.25	≈ 6.25	≈ 6.80	≈ 6.57	
9,15-(Z)	8	6.15	6.15	6.67	6.15	6.06	6.15	6.78	6.67	6.35	6.40	≈ 6.65	≈ 6.65	≈ 6.37	≈ 6.37	
9,13'-(Z)	6	6.16	6.16	6.75	6.16	6.05	6.15	≈ 6.79	≈ 6.66	6.28	6.88	≈ 6.27	≈ 6.19	≈ 6.57	≈ 6.80	
7,13'-(Z)	12	5.84	6.16	6.05	6.16	≈ 6.21	≈ 6.18	≈ 6.6	≈ 6.7	6.32	6.89	≈ 6.3	≈ 6.2	≈ 6.6	6.81	
9,9',13-(Z)	11	≈ 6.14	≈ 6.14	6.75	6.75	6.05	6.05	≈ 6.67	≈ 6.67	6.81	6.35	≈ 6.24	≈ 6.24	≈ 6.8	≈ 6.6	

^a Peak numbers refer to HPLC peak elution order (4).

Table 4. Isomerization shifts ($\Delta\delta = \delta_{(Z)} - \delta_{(E)}$) of the ^1H -NMR signals in mono-(*Z*)- β -carotenes and related compounds (200 MHz, δ ppm in CDCl_3).

(Z)-Structure	Isomer	Protons						
		a	b	c	d	e	5-Me	9-Me
7-(Z)	-0.34 [-0.35]	-0.12 -0.15	+0.04 +0.04	-0.07 -0.08	-0.04 -0.04	-0.20 -0.29	-0.11 -0.08] ^a
9-(Z)		+0.52 [+0.52]	-0.10 -0.09	+0.11 +0.11	-0.06 -0.06	+0.03 +0.04] ^b
13-(Z)		+0.54 [+0.54]	-0.06 -0.14	+0.19 +0.17	-0.05 -0.07] ^b
11-(Z)	[+0.34	-0.46	-0.45	+0.09] ^c
15-(Z)	+0.05 [+0.07]	+0.39 +0.41	-0.23 -0.24] ^b				

All the changes larger than ± 0.04 ppm are compiled and reference values are given in parentheses. Positive shifts mean downfield in the (*Z*) compounds. ^a Reported values for 7-(*Z*)-3-dehydroretinal (6). ^b Reported values for (*Z*)- β -carotenes (7). ^c Reported values for 11-(*Z*)-retinal (8).

each $\Delta\delta$ value of the individual mono-(*Z*) structural pattern was considered.

Example 1. Differentiation of 9.13- and 9.13'-(*Z*)- β -carotenes.

9.13-(*Z*) Found 12-H δ 6.82, 12'-H δ 6.35
Calcd. 6.84, 6.36

9.13'-(*Z*) Found 12-H δ 6.28, 12'-H δ 6.88
Calcd. 6.30, 6.90

Example 2. Assignment of the peak 12 to 7.13'-(*Z*)- β -carotene.

Found 12-H δ 6.32 and 12'-H δ 6.89

Calcd. for 7.13-(*Z*) 12-H δ 6.86 and 12'-H δ 6.36

Calcd. for 7.13'-(*Z*) 12-H δ 6.32 and 12'-H δ 6.90

Individual *J* values were not given in Table 3, since they were found in their usual ranges as known from related compounds. In several cases the assignment was confirmed by decoupling experiments. Thus, ten of sixteen (*Z*)- β -carotenes were identified as shown in Table 1. Though there was some uncertainty about the structures of six additional (*Z*)-components because of their minute amounts for positive identification, one of these (peak 16) might have the 9.9'-(*Z*) configuration in the molecule.

Table 5. Isomerization shifts ($\Delta\delta = \delta_{(Z)} - \delta_{(E)}$) of the ^{13}C -NMR signals in mono-(Z)- β -carotenes and related compounds (50.3 MHz, δ ppm in CDCl_3).

(Z)-Structure	Isomer	Protons						Ref.
		a	b	1.1-Me	5-Me	9-Me	13-Me
7-(Z)	?	?	-0.23	-1.01 ^a	+1.62 ^a		(3)
9-(Z)	-7.99				+8.02		(2)
	13-(Z)	-8.22					+7.94	(2)
11-(Z)	[-3.82	-4.25			-0.60	+4.90]	(9) ^b
15-(Z)	-5.70	-4.46			-0.28		(10)
		[-5.6	-4.5			-0.2]		

All the changes larger than ± 4 ppm (in the sp^2 -C region) and ± 0.2 ppm (in the methyl carbons) are compiled and reference values are given in parentheses. Negative shifts mean upfield in the (Z)-compounds. ^a Tentative assignment. ^b Reported values for 11-(Z)-retinal.

Fig. 1. UV/VIS spectra of novel β -carotene isomers in n -hexane. I, 7-(Z)- β -carotene; II, all-(E)- β -carotene; III, electrocyclized β -carotene (II).

Fig. 2. ¹H-NMR spectra of 7-(Z)-β-carotene and electrocyclized β-carotene (II) (200 MHz, in CDCl₃). A, 1.1-Me; B, 1'.1'-Me; C, 5-Me; D, 5'-Me; E, 9-Me; F, 13-Me; G, 9'-Me + 13'-Me.

'Hindered (Z)' β-carotenes

'Sterically hindered' 7-(Z)-β-carotene was obtained in a stable state and somewhat surprisingly had very similar UV/VIS characteristics to those of the all-(E) pigment (Fig. 1). Existence of this novel geometry was established by the formation and separation of the higher 7-(Z)-isomer, 7.13'-di-(Z)-β-carotene, and by spectral analysis. The ¹H-NMR gave the most useful evidence; in the case of 7-(Z)-β-carotene, chemical shifts of the 5-Me protons (δ 1.52 ppm) and of the 7-H (δ 5.83 ppm, *J* 12 Hz) as well as the similarity in the rest of the NMR parameters to the all-(E) values clearly indicated that the C 7-8 double bond has the (Z)-configuration (Fig. 2). The other 'hindered' and the last mono-(Z)-isomer, 11-(Z)-β-carotene, has not been recognized as yet in our experiments, possibly because of its thermal instability as described earlier (11).

Fig. 3. ^{13}C -NMR spectra (sp^3 -region) of electrocyclized β -carotene (II) (50.3 MHz, in CDCl_3). A, 9'-Me + 13'-Me; B, 13-Me; C, 3-C; D, 3'-C; E, 5'-Me; F, 9-Me; G, 5-Me; H, 1'.1'-Me; I, 1.1-Me; J, 4'-C; K, 1'-C; L, 1-C; M, 4-C; N, 5-C; O, 2'-C; P, 2-C; Q, 10-C. COM, complete decoupling; INEPT, insensitive nuclei enhanced by polarization transfer.

Adsorptivity and stability

It is interesting to note that not only the ratio of ϵ at $\lambda_{\max}$ to ϵ at (Z)-peak but also the relative adsorption affinity of each mono-(Z)-isomer seems to be a function of the molecular shape and hence of the spatial configuration (Table 1), viz. the 15-(Z)-isomer has the most bent shape and the least adsorptivity while the 7-(Z)-isomer is *vice versa*. The stability of the isomers was checked by re-injection in an HPLC apparatus on a part of the sample. In spite of the longer accumulation periods, they were again found to be fully stable using the determining spectra.

(5→10)-Electrocyclized 5.10-trans-all-(E)- β -carotene (II) (peak 0 substance)

Thermal isomerization of all-(E)- β -carotene crystals produced sufficient peak 0 substance for isolation and identification. The major fraction containing this product other than (E)/(Z)- β -carotenes was obtained employing column chromatography, and the subsequent pTLC purification gave a yellowish oil, the peak 0 substance; R_f (TLC) 0.77; R_t (HPLC) 2.2 min; MS (m/z), 536.439 (M^+ 536.438, calcd. for $C_{40}H_{56}$), 444 ($M - 92$ [toluene]), 430 ($M - 106$ [xylene]), and 347 ($M - 189$ [cyclized part]); UV (Fig. 1 and Table 1); $^1\text{H-NMR}$ (Fig. 2 and Table 6); $^{13}\text{C-NMR}$

Table 6. $^1\text{H-}$ and $^{13}\text{C-NMR}$ spectral data of the electrocyclized β -carotene (II).

$^1\text{H-NMR}$ (200 MHz)		$^{13}\text{C-NMR}$ (50.3 MHz)		
δ ppm and splitting	H	δ ppm	INEPT ^b	$sp^3\text{-C}$
1.03 s	1',1'-Me	12.77	+	9'-Me + 13'-Me
1.04 s + 1.09 s	1.1-Me	13.06	+	13-Me ^c
1.12 s	5-Me	18.34	—	3
1.71 s	9-Me + 5'-Me	19.29	—	3'
1.85 s	13-Me	21.77	+	5'-Me
1.96 s	9'-Me + 13'-Me	21.87	+	9-Me
1.99 (<i>d</i> , <i>J</i> 10) ^a	10-H	25.05	+	5-Me ^c
5.46 <i>dd</i> , <i>J</i> 10, 15.5 ^a	11-H	28.99	+	1',1'-Me
5.57 <i>dq</i> , <i>J</i> 5.5, 1.5 ^a	8-H	31.42	+	1-Me
5.76 <i>d</i> , <i>J</i> 5.5 ^a	7-H	32.02	+	1-Me
5.85 <i>d</i> , <i>J</i> 15.5 ^a	12-H	33.12	—	4'
≈ 6.14 s	7',8'-H	34.29	×	1'
6.14 <i>d</i> , <i>J</i> 10	10'-H	34.77	×	1
≈ 6.19 <i>d</i> , <i>J</i> ≈ 15	14,14'-H	35.79	—	4
6.34 <i>d</i> , <i>J</i> 14.5	12'-H	38.02	×	5
≈ 6.56	15,11',15'-H	39.67	—	2'
		40.04	—	2
		58.48	+	10

^a Decoupling experiment. ^b +, peak appears upwards of the base line (Me or CH carbon); —, peak appears downward (CH_2 carbon); ×, peak disappears (quart. carbon).

^c Assignment was given by SEL technique.

(Fig. 3 and Table 6). The peak 0 substance was shown to have the same molecular weight ($M=536$) as other (*Z*)- β -carotenes, but its $\lambda_{\max}$ occurred at 404 nm, 46 nm shorter than all-(*E*)- β -carotene. Such a significant blue shift indicated a lesser degree of conjugation (8 conj. C=C) relative to the parent (11 conj. C=C) (see also *3 in Table 1). Thus, the starting molecule was considered to suffer a thermal reaction over the conjugated triene system (C-5 to C-10), keeping the octaene residue (C-11 to C-5') unaffected. Employing decoupling experiments (^1H -NMR) and INEPT as well as selective decoupling (SEL) techniques (^{13}C -NMR), it was verified that 13 vinyl protons and 20 sp^3 -carbons (10 Me, 6 CH_2 , 1 CH, and 3 quart. C) exist in the molecule (Cf. 14 vinyl protons and 18 sp^3 -carbons [10 Me, 6 CH_2 , and 2 quart. C] in β -carotene). Unequivocal assignments of ^1H -NMR (Fig. 2 and Table 6) and of ^{13}C -NMR data (Fig. 3 and Table 6) were made by comparison with the results on β -carotene (2) and electrocyclic retinoids (12-14). The chemical shifts of 7-H, 8-H, 10-H, 11-H, 12-H, and 5-Me as well as $J_{7,8}=5.5\text{ Hz}$ were diagnostic features of this compound.

Now all spectral data for this novel thermo-product are congruent with the (5 $\rightarrow$ 10)-6e-electrocyclized structure **II** and the stereochemistry of the new bond is believed to be 5.10-*trans* from its *disrotatory* thermo-cyclization mode, instead of *conrotatory* in photo-cyclization (12). It is evident that the hitherto unnoticed 6e-electrocyclized all-(*E*)- β -carotene can be produced from all-(*E*)- β -carotene via the 7-(*Z*)-isomer intermediate involving the 5-, 7-, and 9- C=C bonds.

(5 $\rightarrow$ 10)-Electrocyclized 5.10-trans-9'-(Z)- β -carotene (9'-Z-II; peak 0' substance)

Peak 0' was not recognized initially as a new peak on HPLC tracing as it was eluted together with peak 0 substance under the operated condition. They were resolvable on a TLC plate (R_f 0.84 for the peak 0' and 0.77 for the peak 0). Though sufficient spectral data were not available on this compound (M^+ 536; UV 397 nm in *n*-hexane), the chemical shifts of the 5'-Me protons at δ 1.76 ppm and of the 8'-H at δ ca. 6.68 ppm as well as the similarities of the rest of the NMR parameters to the values from all-(*E*)-**II** strongly suggested that the 9'-10' C=C bond would have the (*Z*)-configuration.

Our findings are the first examples of the formation of the electrocyclic highly-conjugated alicyclic carotenoid and are expected to offer an important insight into the general thermochemistry of polyenes.

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