Thermal Interconversions among 15-*cis*-, 13-*cis*-, and *all-trans*-β-Carotene: Kinetics, Arrhenius Parameters, Thermochemistry, and Potential Relevance to Anticarcinogenicity of *all-trans*-β-Carotene[1]

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Abstract: Rates of thermal, cis−trans rearrangement among *all-trans*-, 15-*cis*-, and 13-*cis*-β-carotene have been measured at temperatures in the range 37−69 °C. From the resulting specific rate constants, Arrhenius and Eyring parameters are derived. Positions of equilibrium are estimated experimentally and by force field calculations based on the Allinger MM2 program as improved by Roth (MM2-ERW), while enthalpies of activation for cis−trans isomerization to 11-, 9-, and 7-*cis*-β-carotenes are estimated by application of the Roth program augmented by the inclusion of the quantum mechanical program of Malrieu et al., EVBH (effective valence-bond Hamiltonian), expanded to encompass longer polyenes. Implications of the interaction of strain and delocalization in the rotation about the 7,8 double bond are presented. A procedure has been developed for the small scale preparation of 13-*cis*-β-carotene by heating *all-trans*-β-carotene at 80 °C for 8 h. Kinetically and thermodynamically accessible at 37 °C, 15-*cis*- or 13-*cis*-β-carotene or both become candidates for the role of true anticarcinogenic agent, whereupon *all-trans*-β-carotene would be relegated to the role of reservoir for the active species.

Since the first announcement by Dorogokuplya et al.[4] that β-carotene minimizes the appearance of skin cancer in mice painted with methylcholanthrene and the subsequent call to arms by Peto et al.[5] to join the fray, ever more attacks on the question of the possible role of β-carotene as a potent anticarcinogenic agent have been mounted.[6] By now strong evidence has been accumulated in support of a positive correlation between higher intake of β-carotene and lower incidence of lung cancer.[7] Its long-known function as a surrogate for vitamin A having been rejected as its sole role, β-carotene has become a chemopreventive or chemo-postponing agent of promise in aging,[8] immune deficiency,[9] senile cataracts,[10] and in several other types of cancer.[6,11] A timely, comprehensive overview of medical applications of carotenoids has come from Mathews-Roth.[12]

A not unexpected, concomitant increase in organic chemical activity has focused on β-carotene as an antioxidant, one of the components of a triad consisting additionally of vitamins E and C;[13,14] on its reaction with triplet oxygen;[15] and on its effectiveness as a quencher of singlet oxygen *vis-á-vis* lycopene, *inter alia*.[16] In general, this renewed interest in biological activity has stimulated a sympathetic interest in possibly novel chemistry of β-carotene, carotenoids, and polyenes in general. Our attention has focused on the possibility that purely thermally driven rotation about one or more of the double bonds in β-carotene may play a role in its putative anticarcinogenicity. This paper is directed specifically to kinetic and thermodynamic relations among the mono-cis isomers of *all-trans*-β-carotene (**2**) shown in Figure 1.

A widely regarded hypothesis has free radicals being among the most important DNA-damaging agents with which the body's defenses against mutagenesis and carcinogenesis have to contend.[17] In this action, *all-trans*-β-carotene, *inter alia*, should function as a lipid-soluble trap for free radicals. If a neutral, planar polyene can be considered to be a credible, free-

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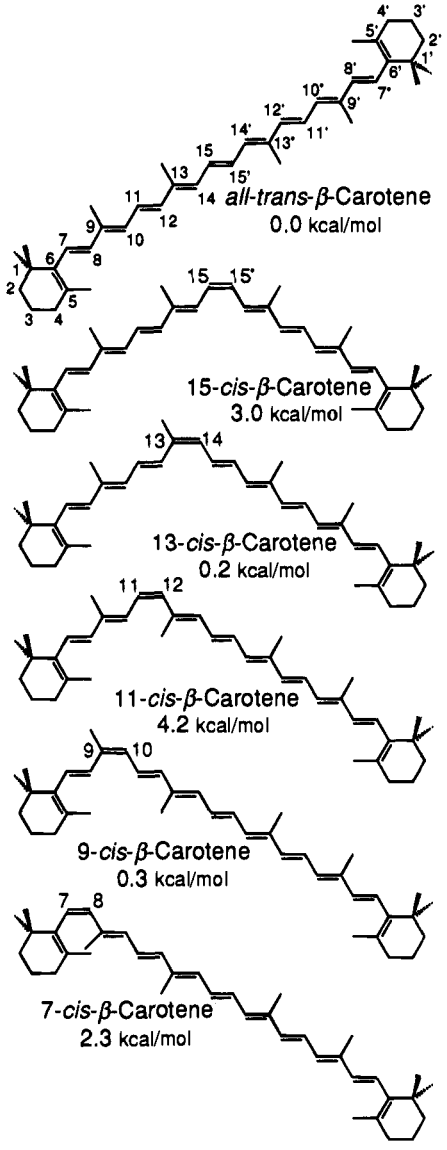

Figure 1. The mono-cis isomers of *all-trans*-β-carotene are shown along with their steric energies relative to *all-trans*-β-carotene, as calculated by the molecular mechanics program, MM-ERW.

radical trap, a diradical generated by twisting 90° about one of its double bonds should be even more effective, or so ran the initiating, conceptual stimulus for this work.

In the basic literature of polyenes, too little was known about the energetics of 90°-twisted double bonds in even short polyenes beyond ethylene and hexa-1,3,5-triene to conclude that a diradical-like, *all-trans*-β-carotene, twisted, for example, 90° about its 15,15′ double bond, should be considered a credible actor. A diradical formally generated by such a twist would consist of two nonatetraenyl radicals (or undecapentaenyl radicals if the conformationally nonplanar trimethylcyclohexenyl (TMCH) ring were to participate) as shown in Scheme 1.[18] The energy required to generate a 90°-diradical should be related quantitatively to the intrinsic enthalpy of stabilization of each of the component radicals. This quantity was neither known nor credibly to be estimated for the nonatetraenyl radical; nor was it known for the next shorter homologue, the heptatrienyl radical. Even for the pentadienyl radical, stabilization energies reported in the literature[19] covered such a wide range that extrapolation to nonatetraenyl would have been pointless. A diradical like that shown in Scheme 1 could just as plausibly have been of energy low enough to be in sensible equilibrium with *all-trans*-β-carotene or high enough to have served only as a model for the fleeting transition state in a thermally driven, cis−trans isomerization.

The energy of a 90°-twisted *all-trans*-β-carotene (**2**) is closely related to the height of the barrier that separates trans from cis isomers. References to thermal instability occur in the classical, pioneering work of Zechmeister[20] on the cis isomers of carotenoids and continue to the present. But for four,[21−24] these references are qualitative in nature. Striking examples are found in the thermal isomerization of various cis isomers of β-carotene to the least soluble of the isomers, *all-trans*-β-carotene, in the ultimate step of the current commercial syntheses.[25] In a much quoted paper, Tsukida, Saiki, and Sugiura heat *all-trans*-β-carotene neat at 190 °C for 15 min as a means of effecting thermal cis−trans rearrangement to at least 17 products, among which the mono-cis and several di-cis isomers have been identified.[26a] Longer heating for 5 h at 170 °C leads to extrusion of toluene and xylenes and smaller polyenes, structures of which accord with the elegantly elaborated mechanism of Byers.[27]

The goal of placing the 90°-twisted diradical in perspective might have been tackled directly by studying *all-trans*-β-carotene, but initially this approach was deemed unattractive by the absence in the literature of quantitatively reproducible analytical methods, a potentially unfavorable thermochemistry, and fear that the sheer number of cis isomers of β-carotene would overwhelm any analytical method.[28] The problem was tackled instead in two stages, of which the first was examination of a series of semirigid polyenes constrained to exist in only one trans and one cis configuration and constitutionally limited to the single reaction of thermal cis−trans configurational isomerization. The study was designed to bridge the gap between the three-carbon allyl radical and the nine-carbon nonatetraenyl radical and indicate whether a direct study of β-carotene might be justified. The missing stabilization energies were acquired[19,29] and, when applied to *all-trans*-β-carotene, led to a predicted enthalpy of activation for internal rotation about the central double bond (E_a = 24.5 kcal/mol, log A = 12.6, half-life of 9 h at 37 °C) that was much too high for a 90°-diradical to be in sensible equilibrium but low enough to

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(22) Pesek, C. A.; Warthesen, J. J.; Taoukis, P. S. *J. Agric. Food Chem.* **1990**, *38*, 41−45.

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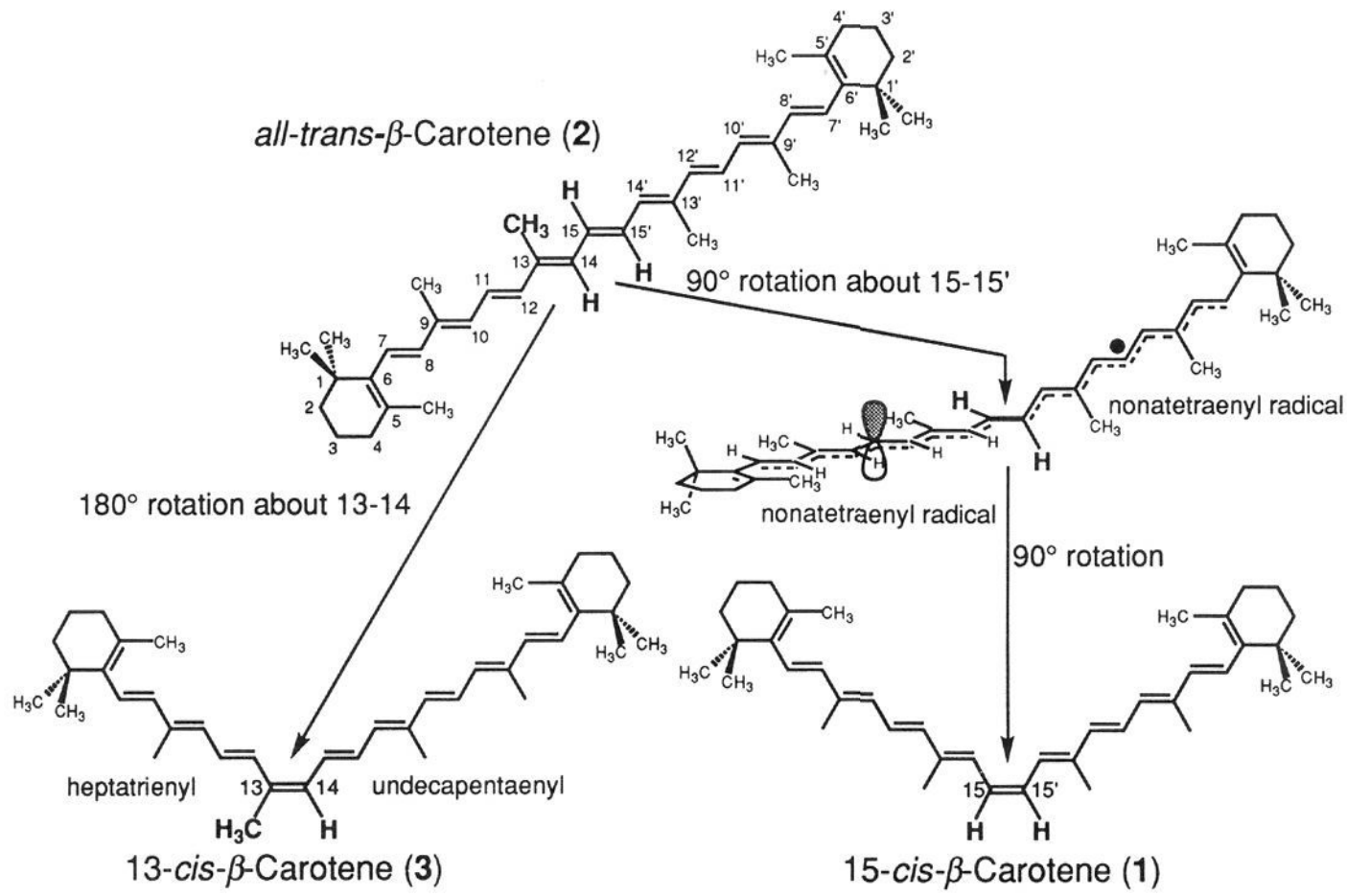
(27) Byers, J. *J. Org. Chem.* **1983**, *48*, 1515−1522.

(28) In addition to the all-trans and all-cis isomers, there are five mono-cis, 20 di-cis, 44 tri-cis, and 66 tetra-cis (and correspondingly five mono-trans, etc.) for a total of 272 configurational isomers!

(29) Doering, W. v. E.; Sarma, K. *J. Am. Chem. Soc.* **1992**, *114*, 6037−6043.

Scheme 1



suggest thermal instability at physiological temperature. They also implied the feasibility of a direct experimental examination of interconversions within a set limited to the three isomers, *all-trans*-, 15-*cis*-, and 13-*cis*-β-carotene, without undue interference from other isomers.[30] Despite the high number of stereoisomers of β-carotene with which one might have had to contend,[28] initial experiments confirmed qualitatively that only *all-trans*- (**2**), 15-*cis*- (**1**), and 13-*cis*-β-carotene (**3**) were kinetically competent at the lowest temperatures.

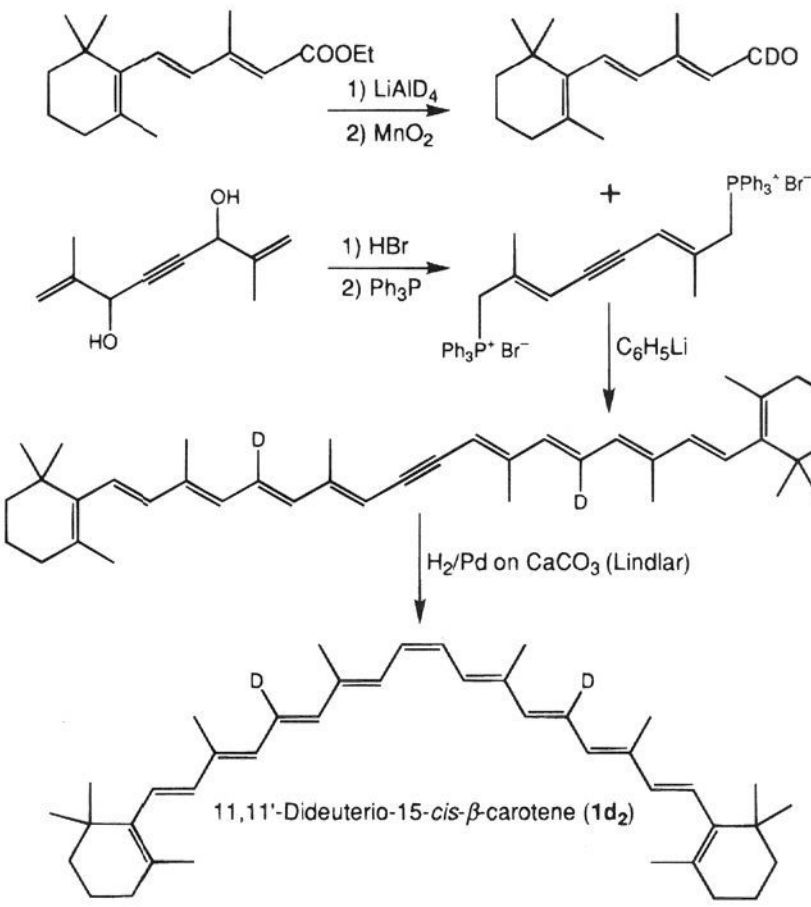
The main obstacle to mounting a quantitative kinetic study was identification of a routine, reliable analytical procedure of sufficient accuracy and precision. High-performance liquid chromatography based on Zechmeister's original employment of lime columns was exemplified by several variants,[26a,c] but failed in our hands to achieve the necessary level of reproducibility and precision. In the absence of explicit evaluation of its quantitative reliability, the technically elaborate and demanding method of Vecchi[26b] was not deemed appropriate to our limited resources. We chose ^{1}H NMR instead, although it was clear from the exhaustive NMR studies of Koyama and his colleagues that the signals from the 11 and 11′ hydrogen atoms of 15-*cis*- and *all-trans*-β-carotene would interfere with their simultaneous, quantitative estimation.[31] This drawback was removed by the synthesis of 11,11′-dideuterio-15-*cis*-β-carotene (**1d$_2$**) following the steps outlined in Scheme 2. A sample of 11,11′-dideuterio-*all-trans*-β-carotene (**2d$_2$**) was then easily obtained by conventional thermal isomerization of **1d$_2$**.[25]

Initial quantitative experiments were concentrated on the rearrangement of **1** to **2**, the fastest of the three rearrangements. The kinetics could be modeled in the *early stages* as a pseudo-first-order reaction because **2** was strongly favored at (pseudo)-equilibrium (93−95%). Owing to the instability of all β-carotenes to light, the reactions were conducted in the dark. Sensitivity to oxygen cautioned the use of previously evacuated, sealed NMR tubes. These were analyzed intermittently during the period of heating without being opened. Worries about

(30) For thermodynamic reasons, 11-cis was not anticipated, nor were 9-cis or 7-cis, but for kinetic reasons (*vide infra*).

(31) Koyama, Y.; Hosomi, M.; Hashimoto, H.; Shimamura, T. *J. Mol. Struct.* **1989**, *193*, 185−201.

Scheme 2



instability to acid were relieved by finding no change in rate constant in several experiments with added diazabicyclooctane (DABCO). Support for the pseudo-first-order hypothesis was provided by two experiments in which doubling of initial concentration of starting material occasioned no significant change in specific rate constant. The lowest temperature selected for the study of kinetics was 37.2 °C, not only for its physiological associations, but for its suitability for measurement of the slowest reaction rate. From a large body of data, given in full detail as supplementary material in Table S-I, specific rate constants, $k_{1,2}$, at various temperatures in the range 37.2−68.9 °C and Arrhenius parameters were derived, the results being recorded in Table 1.

In the next step, starting from **1d$_2$**, an attempt was made to elucidate specific rate constants, $k_{2,1}$, for the reverse rearrange-

Table 1. Pseudo-First-Order, Specific Rate Constants, $k_{1,2}$ and Arrhenius Parameters for the Thermal Isomerization of 11,11′-Dideuterio-15-*cis*-β-carotene (**1d$_2$**) to 11,11′-Dideuterio-*all-trans*-β-carotene (**2d$_2$**) in Benzene-d_6 Based on Analysis by NMR of the Disappearance of **1** and Appearance of **2**

T, °C	$k_{1,2}$[a]	T, °C	$k_{1,2}$[a]
37.2 ± 0.1	1.60 ± 0.03[b]	59.1 ± 0.1	28.7 ± 0.7
37.2 ± 0.1	1.61 ± 0.03[c]	64.1 ± 0.1	53.0 ± 1.1
37.2 ± 0.1	1.73 ± 0.04[d]	68.7 ± 0.1	86.5 ± 2.0[e]
37.2 ± 0.1	1.41 ± 0.06[b]	68.8 ± 0.2	93.1 ± 4.1[e]
43.3 ± 0.1	3.64 ± 0.04	69.0 ± 0.2	92.3 ± 2.3[f]
49.0 ± 0.1	9.28 ± 0.15	69.0 ± 0.2	98.6 ± 2.8[g]

Arrhenius plot [$1/T$ *vs* log k][h]

$E_1 = 26.8_6 \pm 0.18$ kcal/mol[i] log $A = 13.1_4 \pm 0.12$[i]

[a] In units of 10^{-6} s^{-1}. [b] [15-*cis*]$_{t=0}$ = 9.3 mM. [c] [15-*cis*]$_{t=0}$ = 3.7 mM, DABCO = 4 mM. [d] [15-*cis*]$_{t=0}$ = 7.4 mM, DABCO = 4 mM. [e] [15-*cis*]$_{t=0}$ = 11.1 mM. [f] [15-*cis*]$_{t=0}$ = 3.7 mM, DABCO = 0.5 mM. [g] [15-*cis*]$_{t=0}$ = 7.4 mM, DABCO = 0.5 mM. [h] Calculated from the data in Table S-I by linear regression using the usual first-order expression. [i] Double all standard errors for 90% confidence limits.

Table 2. Specific Rate Constants and Derived Arrhenius Parameters for the Thermal Rearrangement of 11,11′-Dideuterio-15-*cis*- (**1d$_2$**) to 11,11′-Dideuterio-*all-trans*- (**2d$_2$**) and 11,11′-Dideuterio-13-*cis*-β-carotene (**3d$_2$**) in Benzene-d_6

T, °C	$k_{1,2}$[a]	$k_{2,1}$[a]	$k_{2,3}$[a]	$k_{3,2}$[a]
37.2	1.63 ± 0.03	0.14 ± 0.01	0.64 ± 0.04	1.54 ± 0.13
43.1	3.84 ± 0.02	0.36 ± 0.02	1.31 ± 0.05	3.18 ± 0.15
64.0_7	55.9 ± 0.5	6.9 ± 0.5	19.5 ± 1.1	44.1 ± 4.2
68.6	94.8 ± 1.3	10.6 ± 1.0	39.6 ± 2.5	77.9 ± 7.0

Arrhenius plots [$1/T$ *vs* log k][b,c]

$k_{1,2}$: $E_a = 27.2_0 \pm 0.15$ kcal/mol; log $A = 13.3_8 \pm 0.10$
$k_{2,1}$: $E_a = 29.1_4 \pm 1.07$ kcal/mol; log $A = 13._{70} \pm 0.72$
$k_{2,3}$: $E_a = 27._{47} \pm 0.77$ kcal/mol; log $A = 13._{13} \pm 0.52$
$k_{3,2}$: $E_a = 26._{13} \pm 1.13$ kcal/mol; log $A = 12._{58} \pm 0.76$

[a] In units of 10^{-6} s^{-1}. [b] Calculated from the data in Table S-II for the model above by the four-rate-constant version of the Roth kinetic program, which incorporates the Marquardt procedure for determining experimental uncertainties: Marquardt, D. W. *J. Soc. Ind. Appl. Math.* **1963**, *11*, 431−441. [c] Double all standard errors for 90% confidence limits.

ment of **2d$_2$** to **1d$_2$**. This goal was not achieved with comparable accuracy or precision for two reasons: the faster reaction undergone by **2** by a significant factor was conversion to 13-*cis*-β-carotene (**3**); and only a few percent of **1** was present at equilibrium. These two factors conspired to make the contribution of the appearance of **1** to the disappearance of **2** too small for accurate dissection within the limits of the NMR analytical method. Results of markedly extending the time of reaction of **1** (from 40 h to 30 days, for example, at 37.2 °C) and treating the data (Table S-II, supplementary material) as a pair of reversible first-order reactions are given in Table 2. Although the uncertainty, (σ) in E_a and log A for the $k_{2,1}$ process are ~8 times greater than those for the $k_{1,2}$ process, the values obtained agree well with those in Table 1.

Starting with **2d$_2$**, $k_{2,1}$ is still determined with unacceptably high uncertainty, as is $k_{1,2}$. Only the rate constants, $k_{2,3}$, and derived Arrhenius parameters are of good, if not high, precision. The results, based on the data in Table S-III (supplementary material), are collected in Table 3.

When the starting compound is unlabeled **3**, only one product, **2**, can be analyzed by the NMR method. Whether unrecognized changes are responsible for the inexplicably large discrepancy in the values of $k_{2,3}$ and derived Arrhenius parameters (Tables 3 and 4) is unclear. Particularly surprising are the low values of 12.6 for log A in comparison to values for the other reactions in the 13.3 range. Recalculation of $E_a(k_{2,3})$ by substituting log A = 13.35 (Table 3) for log A = 12.57 (Table 4) leads to a value of 27.5 kcal/mol. No further comment is justified.

Table 3. Specific Rate Constants and Derived Arrhenius Parameters for the Thermal Rearrangement of 11,11′-Dideuterio-*all-trans*- (**2d$_2$**) to 13-*cis*- (**3d$_2$**) and 15-*cis*-β-Carotene (**1d$_2$**) in Benzene-d_6

T, °C	$k_{1,2}$[a]	$k_{2,1}$[a]	$k_{2,3}$[a]	$k_{3,2}$[a]
37.3	1.1 ± 0.2	0.085 ± 0.01	0.55 ± 0.01	1.43 ± 0.05
43.2	2.6 ± 0.9	0.19 ± 0.03	1.23 ± 0.03	3.27 ± 0.17
64.2	25.2 ± 25.7	2.66 ± 0.64	18.8 ± 0.7	38.8 ± 4.4
69.1	63.4 ± 10.5	6.7 ± 0.6	36.0 ± 0.7	79.7 ± 2.6

Arrhenius plots [$1/T$ *vs* log k][a,b]

$k_{1,2}$: $E_a = 26._0 \pm 2.9$ kcal/mol; log $A = 12._3 \pm 1.9$
$k_{2,1}$: $E_a = 28._6 \pm 1.2$ kcal/mol; log $A = 13._0 \pm 0.8$
$k_{2,3}$: $E_a = 27.86 \pm 0.24$ kcal/mol; log $A = 13.35 \pm 0.16$
$k_{3,2}$: $E_a = 26.71 \pm 0.45$ kcal/mol; log $A = 12.96 \pm 0.30$

[a] In units of 10^{-6} s^{-1}. [b] Calculated from the data in Table S-III for the model shown in Scheme 2 by the four-rate-constant version of the Roth kinetic program. [c] Double all standard errors for 90% confidence limits.

Table 4. Specific Rate Constants and Derived Arrhenius Parameters for the Thermal Rearrangement of 13-*cis*- (**3**) to *all-trans*-β-Carotene (**2**) in Benzene-d_6

T, °C	$k_{2,3}$[a]	$k_{3,2}$[a]
37.2	0.85 ± 0.03	1.66 ± 0.02
43.1	2.0_5 ± 0.11	3.8_4 ± 0.06
64.2	$26._1$ ± 0.7	$50._3$ ± 0.4
68.9	$47._8$ ± 1.4	$87._4$ ± 1.2

Arrhenius plots [$1/T$ *vs* log k][a,b]

$k_{2,3}$: $E_1 = 26.4 \pm 0.3$ kcal/mol; log $A = 12.57 \pm 0.20$
$k_{3,2}$: $E_a = 26.2 \pm 0.1$ kcal/mol; log $A = 12.69 \pm 0.08$

[a] In units of 10^{-6} s^{-1}. [b] Calculated by the first-order reversible version of the Roth kinetic program from the data in Table S-IV. [c] Double all standard errors for 90% confidence limits.

Table 5. Arrhenius Parameters Calculated from the Combined Data in Tables S-II, S-III, and S-IV for Thermal Interconversion among 15-*cis*- (**1**), 13-*cis*- (**3**), and *all-trans*-β-Carotene (**2**)

Arrhenius parameters [$1/T$ *vs* log k][a]

$k_{1,2}$: $E_a = 27.2 \pm 1.2$ kcal/mol; log $A = 13.3 \pm 0.7$
$k_{2,1}$: $K_a = 28.9 \pm 5.0$ kcal/mol; log $A = 13.4 \pm 1.1$
$k_{2,3}$: $E_a = 27.7 \pm 1.5$ kcal/mol; log $A = 13.3 \pm 0.8$
$k_{3,2}$: $E_a = 26.4 \pm 1.1$ kcal/mol; log $A = 12.8 \pm 0.3$

[a] Calculated for the model above by the special version of the Roth kinetics program designed to optimize Arrhenius parameters directly from data at varying temperatures and to provide experimental uncertainties at the 95% confidence level by the Marquardt procedure.

In an attempt to make full use of the kinetic data, a calculation was undertaken with the combined data from Tables S-II, S-III, and S-IV (supplementary material). To apply the special feature of the Roth program,[32] which optimizes Arrhenius parameters directly from individual points at different temperatures, data relating to **1** need to be constructed artificially to complete the set in Table 4. This was done by application of the activation parameters in Table 1. The three sets then became comparable and led to the activation parameters given in Table 5 (uncertainties from this program are reported at the 95% confidence level). Uncertainty in $k_{2,1}$ remains too high for significance. The level of confidence in the other three values is better, but certainly not high. The low value of log A and correspondingly low value of E_a for $k_{3,2}$ remain.

(32) Roth, W. R.; Adamczak, O.; Breuckmann, R.; Lennartz, H.-W.; Boese, R. *Chem. Ber.* **1991**, *124*, 2499−2521.

Table 6. Extrapolation of $\Delta H^{\ddagger}_{n.1.n}$ for Thermal *Cis–Trans* Isomerization of Symmetrical Semirigid Polyenes of Type *n*.1.*n* and Mono-*Cis* β-Carotenes by Means of a Geometric Function[a]

n	*n*.1.*n*	f_n[b]	estd $\Delta H^{\ddagger}$	exptl $\Delta H^{\ddagger}$		β-carotene	*n*.1.*n'*	estd $\Delta H^{\ddagger}$
1	1.1.1	0.0	(38.9)	38.9				
2	2.1.2	6.8	32.1	32.1				
3	3.1.3	11.3	27.6	27.5				
4	4.1.4	14.4	24.5	24.5	↔	15-cis	4.1.4	24.5
5	5.1.5	16.4	22.5			13-cis	3.1.5	25.0[c,d]
6	6.1.6	17.8	21.1			11-cis	2.1.6	26.6[c,d]
7	7.1.7	18.7	20.2			9-cis	1.1.7	29.6[c,d]
8	8.1.8	19.3	19.6			7-cis	0.1.8	38.8[c,d]
						[7-cis	1.1.8	29.3][c,d]
∞	∞.1.∞	20.5	18.4					

[a] $\Delta H^{\ddagger}_{n.1.n}$ (in kcal/mol) = $\Delta H^{\ddagger}_{1.1.1} - f_n$, where $f_n = a/(1 - r)\{(1 - r^{n-1})/(1 - r)\}$, n = number of double bonds in the polyenyl radical, $(2n + 1)$ = number of double bonds in the corresponding polyene (and number of carbon atoms in the polyenyl radical), $a/(1 - r) = 6.8$, and "best fit" values are $a = 2.25$, $r = 0.67$. [b] Stabilization enthalpies are given by the expression $SE_n = SE_1$ (13.5) + $f_n/2$. [c] Estimated values of $\Delta H^{\ddagger}$ apply to rearrangement starting from *all-trans*-β-carotene and are given by [(estd $\Delta H^{\ddagger})_{n.1.n}$ + (estd $\Delta H^{\ddagger})_{n'.1.n'}$)]/2. [d] A statistical entropy factgor of R ln 2 (0.50 kcal/mol at 37 °C) favors rearrangements to these isomers.

Inferences

These observations on β-carotene confirm a widely recognized warning that some purely thermal isomerization may be anticipated whenever carotenoids containing the typical sequence of 11 double bonds are handled for long at temperatures above ~35 °C. Coupled with the uniformly much lower solubility of all-trans isomers, such thermal isomerization can lead to the mistaken assignment of an all-trans configuration to compounds that originally might have had a 15-cis or 13-cis double bond.[20]

An earlier extrapolation of enthalpies of activation to longer radicals involved finding an algebraic expression relating stabilization energies to number of double bonds in the polyenyl radical. A geometric function, chosen arbitrarily[29,33] in the absence of a theoretically based expression to accommodate the three experimental values of $\Delta H^{\ddagger}$ for n = 2, 3, and 4, respectively, gave the values for n = 5, 6, 7, 8, and ∞ shown in the fourth column of Table 6 and in Figure 2.

On the assumption that rearrangements of β-carotene near its center proceed without appreciable assistance from the noncoplanar double bonds of the two TMCH rings, that is, behave like a 4.1.4 π-system, lycopene, as a polyene unequivocally of the 5.1.5 type, should undergo faster reversible thermal isomerization to 15-*cis*-lycopene with an enthalpy of activation predicted to be lower by ~2 kcal/mol. Support is found in the statement that "*cis*[15,15′-*cis*]-lycopene isomerizes much more easily, for example, than 15,15′-*cis*-β-carotene".[34]

To the question, why has biochemical nature not found good uses for prenoids beyond the level of eight (units of isoprene), an answer is suggested in the extrapolations given in Table 6. At the level of nonaprenoids (effectively polyenes of order 11) and almost certainly by decaprenoids (polyenes of order 13), centrally positioned cis isomers would be in fluxional equilibrium with their trans isomers at ambient temperatures. To be sure, crystal forces might generate sparingly soluble, easily isolable all-trans isomers, but these should no longer be configurationally stable in solution.

(33) A logarithmic, or even a parabolic, function fits the set of three data quite as well! Marginally to improve matters will require, at the least, a study of semirigid polyenes of order 11 and 13!

(34) Isler, O.; Gutmann, H.; Lindlar, H.; Montavon, M.; Rüegg, R.; Ryser, R.; Zeller, P. *Helv. Chem. Acta* **1956**, *39*, 463–473.

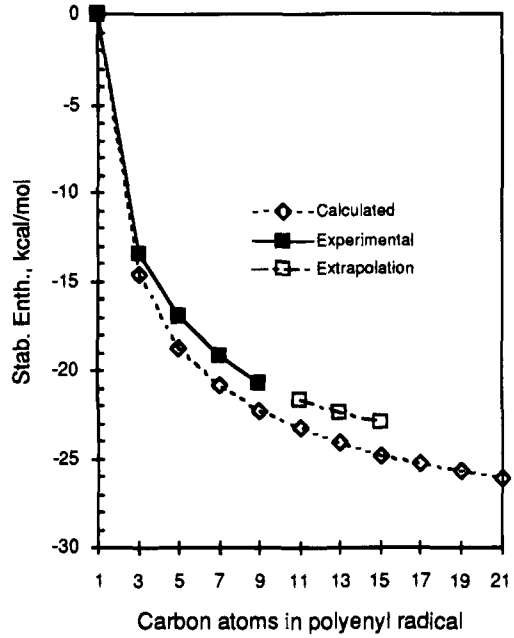


Figure 2. Shown are stabilization enthalpies of polyenyl radicals: experimental values[19,29] (black squares) and three values (open squares) extrapolated therefrom (see Table 6, column 4), and as calculated by the combined programs, MM2-ERW-EVBH (open diamonds) (see Table 7).

In this connection, Gebhard et al.[35a] have made the fascinating observation that provision of labeled *all-trans*-[14′-^{13}C]spheroidene of very high purity to the carotenoid-free, photosynthetic reaction center of *Rhodobacter sphaeroides* leads without apparent difficulty to the reconstituted 15-*cis*-spheroidene–protein complex. One explanation sees 15-*cis*-spheroidene, a polyene of the 6.1.3 type, being absorbed as fast as it is formed from *all-trans*-spheroidene by thermal rearrangement.

The recent, provocative finding of Sies and co-workers[36] that various human tissues contain 15-*cis*- and 13-*cis*-β-carotene (in a relatively constant ratio of 1:6) amounting to ~10% of *all-trans*-β-carotene is quite consistent with a slow thermal isomerization in cells unaccompanied by appreciable stereochemically selective rejection. By contrast, the ratio in serum (shorter residence time at 37 °C?) is markedly lower.

Preferred values for the activation parameters of the interconversions are shown in Scheme 3. That their accuracy and precision leave something to be desired can be appreciated from the range of calculated specific rate constants to which they lead (collected in Table 7). How these uncertainties in activation parameters might bear in a practical sense on an interest of nutritionists, for example, in the effect of cooking on the distribution among stereoisomers of β-carotene can be inferred from the specific rate constants calculated at 100 °C. These can also be expressed as times required to reach the halfway point[37] toward equilibrium concentrations.[38] For the transformation of **2** to **3**, for example, a time of about 11 min suffices. This value is qualitatively in agreement with literature, where it can be read that cooking and canning has been recognized for many years in all instances to lead to the transformation of

(35) (a) Gebhard, R.; van der Hoef, K.; Violette, C. A.; de Groot, H. J. M.; Frank, H. A.; Lugtenburg, J. *Pure Appl. Chem.* **1991**, *63*, 115–122. (b) Koyama, Y.; Kito, M.; Takii, T.; Saiki, K.; Tsukida, K.; Yamashita, J. *Biochem. Biophys. Acta* **1982**, *680*, 109–118.

(36) Stahl, W.; Schwarz, W.; Sundquist, A. R.; Sies, H. *Arch. Biochem. Biophys.* **1992**, *294*, 173–177.

(37) ln 2 = $k \times t_{1/2}$ in s^{-1} and s, respectively.

(38) $1/K_{2,1} + 1 + 1/K_{2,3} = 1/[\mathbf{2}]$; from mean values, $K_{2,1(100\ °C)} = 7.2$; $K_{2,3(100\ °C)} = 1.8$; [**1**] = 8.2%; [**2**] = 59.0%; [**3**] = 32.8% at (pseudo)-equilibrium at 100 °C.

Scheme 3

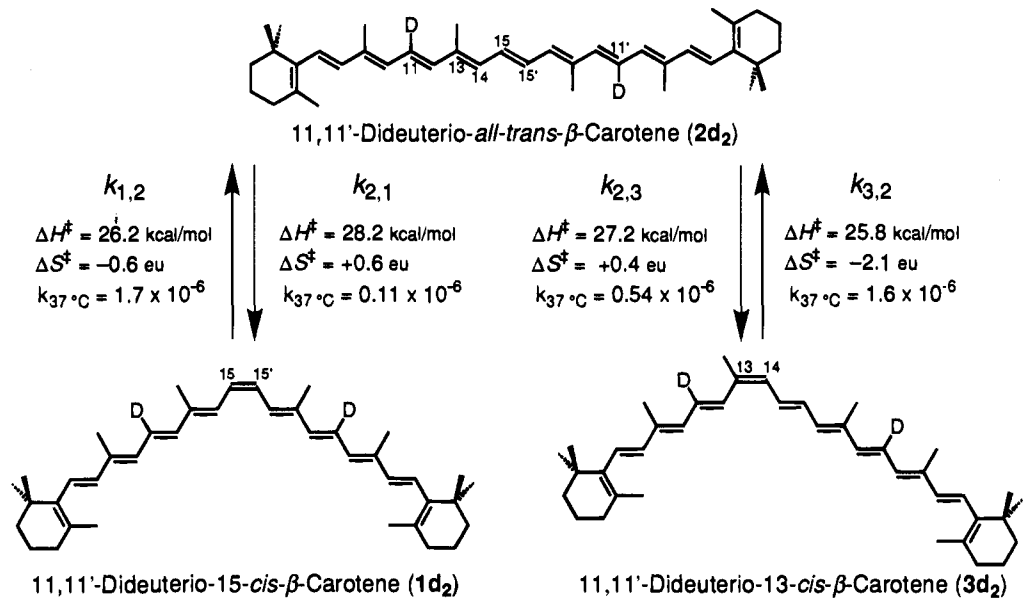


Table 7. First-Order, Specific Rate Constants[a] for Thermal Interconversions among 15-*cis*- (**1**), 13-*cis*- (**3**), and *all-trans*-β-Carotene (**2**) as Calculated from the Arrhenius Expressions Recorded in Table 1−5, Italicized Values Being Preferred

T, °C	$k_{1,2}$	$K_{2,1}$	$k_{2,1}$	$k2_{,3}$	$K_{2,3}$	$k_{3,2}$
			Table 1			
37.2	*1.68*					
68.9	*95.2*					
100.0	*2566*					
			Table 2			
37.2	1.69	11.3	0.15	0.61	2.5	1.51
68.9	100.4	8.3	12.1	37.9	2.0	76.8
100.0	2820	6.6	430	1100	1.7	1890
			Table 3			
37.2	0.99	13.0	0.076	*0.54*	2.6	1.42
68.9	49.4	8.9	5.56	*35.8*	2.2	78.5
100.0	1200	6.5	185	*1090*	1.9	2080
			Table 4			
37.2				0.91	1.9	1.75
68.9				48.3	1.9	89.6
100.0				1230	1.8	2230
			Table 5			
37.2	1.62	15.3	*0.106*	0.58	27	*1.59*
68.9	96.2	11.8	*8.14*	37.6	2.2	*83.6*
100.0	2690	9.6	*281*	1130	1.9	*2120*

[a] In units of 10^{-6} s^{-1}.

significant quantities of the all-trans **2** into **3** and 9-*cis*-β-carotene. As an example, a fresh sweet potato, the β-carotene fraction of which contains 5.1% of 13-*cis*-β-carotene and 0.0% of 9-*cis*-β-carotene, is converted, on being heated for 90 min at 116 °C as a *purée*, into a canned sweet potato, the β-carotene fraction of which now contains 14.5% of 13-*cis*-β-carotene, and 2.7% of 9-*cis*-β-carotene.[39]

To the biologist and epidemiologist concerned with possible thermal rearrangement of *all-trans*-β-carotene *in vivo* at 37.2 °C, it is relevant that the halfway point toward the equilibrium concentration of 13-*cis*-β-carotene will be attained in 5 days' time; slow to be sure, but perhaps comparable to the average residence time of β-carotene in cell membranes. Both 15-*cis*- and 13-*cis*-β-carotene are sufficiently competent kinetically and thermodynamically to qualify as the true anticarcinogenic agent in place of the conventionally accepted *all-trans*-β-carotene, which then would serve only as a reservoir for the thermally accessible active agent.

Extrapolation to 11-, 9- and 7-*cis*-β-Carotenes. Of the two elements, rates of cis−trans isomerization and positions of equilibria, that determine the practicality of thermal interconversion among the other mono-cis isomers, the latter, the thermodynamic, is the less complicated to handle in that the enthalpy term contributing to differences in free energy (entropy differences remain beyond our competence) can be usefully approximated by force field or molecular mechanical calculation. Were *accurate* absolute heats of formation required, this approach would not work: the β-carotenes are too big to ignore the cumulative effect of small inaccuracies in the various functions selected empirically best to fit collections of experimental heats of formation, themselves of significantly variable accuracy.

An especially severe problem is encountered in the interaction of the sterically bulky trimethylcyclohexenyl (TMCH) ring and the polyenyl system beginning with C7. It is already manifest in the ground state of the β-carotenoids and retinoids, for which several X-ray crystallographic determinations of structure[18] have revealed s-cis conformations with dihedral angles $\Delta^{5,6}-\Delta^{7,8}$ falling in the range −44° and −54° and 40° and 51°. The noncoplanarity originates in the large steric repulsions between the TMCH ring and the C9 methyl group in the 0° (s-cis, cisoid, or synperiplanar) and 180° (s-trans, transoid, or antiperiplanar) conformations. In unsubstituted butadiene, the latter conformation is more stable than the former by ~3 kcal/mol, while the 90° conformation representing the transition state between them is ~7 kcal/mol above the transoid (quantitatively, this function varies somewhat with degree of substitution). The extent of participation by $\Delta^{5,6}$ is determined by a balance between energy-lowering, π-electron delocalization, which is maximal at 0° or 180°, and the opposing energy-raising, steric repulsions, likewise maximal at 0° or 180°. Calculations on a truncated model of β-carotene consisting of the TMCH ring substituted at C6 by carbon atoms 7, 8, 9, and 10 (including the C9 methyl group) reveal the dependence of steric energy on dihedral angle $\Delta^{5,6}-\Delta^{7,8}$ shown in Figure 3. There are relatively flat minima between 45° and 75° and between 305° and 335° (not shown, but essentially identical in energy) in good accord with the

(39) Chandler, L. A.; Schwartz, S. J. *J. Agric. Food Chem.* **1988**, *36*, 129−133 and other references cited therein.

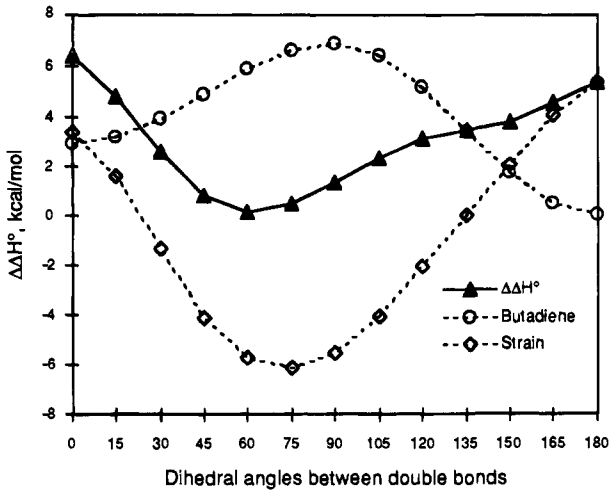


Figure 3. The interdependence of enthalpy of formation (arbitrary zero; kcal/mol) and dihedral angle (C1,2−C3,4 in butadiene; C5,6−C7,8 in β-carotene) as calculated by the combined MM2-ERW and EVBH programs. The upper curve (open circles) shows a typical torsional function linking *cis*- (0°) and *trans*- (180°) butadiene; the lower curve (open diamonds), the steric or strain energy of the interaction of the trimethylcyclohexene (TMCH) ring with the polyene substituent; and the central curve (black triangles), their sum, an approximation to the change in enthalpy of formation in β-carotene and dihedral angle. (The second hemisphere, 180°−360°, in which the polyene is cis to the pseudoaxial methyl group at C1, is not shown.)

crystallographic structures. Interconversion between the two minima passes over the transoid conformation (180°), a conjugative interaction which is greater than that in the alternative cisoid conformation by ~3 kcal/mol.[40]

Calculations of *differences* in heats of formation are sufficient to the purpose, and suitably truncated fragments of the β-carotenes suffice.[41] Results are included in Figure 1 as differences in steric energy in kcalories/mole relative to *all-trans*-β-carotene as naught.[42] The enthalpy differences for 15-*cis*- and 13-*cis*-β-carotene are qualitatively in accord with the free energy differences derived from the equilibrium constants listed in Table 7: at 37.2 °C, $\Delta\Delta G°$ values for 15-*cis*- and 13-*cis*-β-carotene are 1.7 and 0.6 kcal/mol, respectively. Also in accord is the failure to observe detectable amounts of 11-*cis*-β-carotene. Like 13-*cis*-β-carotene, substantial amounts of 9-*cis*-β-carotene are expected and indeed become available when thermal (pseudo)-equilibrium is reached at a higher temperature (110 °C). The same is true of 7-*cis*-β-carotene at even higher temperature,[26a] a perhaps surprising observation, but in accord with the prediction that 7-*cis*-β-carotene is thermodynamically somewhat more favored than 15-*cis*-β-carotene.

In order to estimate specific rate constants for the interconversions of *all-trans*-β-carotene and 11-*cis*-, 9-*cis*-, and 7-*cis*-β-carotene in even crudely quantitative terms, stabilization enthalpies for the two component radicals at the 90° stage need

(40) For further elaboration of the conformational problem, see the discussion of 13-*cis*-retinal by the following: Simmons, C. J.; Liu, R. S. H.; Denny, M.; Seff, K. *Acta Crystallogr.* **1981**, *B37*, 2197−2205. See also: Chen, R.; Colmenares, L. U.; Thiel, J. R.; Liu, R. S. H. *Tetrahedron Lett.* **1994**, *35*, 7177−7180.

(41) Differences in steric energy are calculated for isomeric cis−trans pairs by the MM2 program of Allinger as made available in the CSC Chem3D Plus program by Cambridge Scientific Computing, Inc. Truncated models for 7- and 9-*cis*-β-carotene include fragments 1,1,5,9-tetramethyl-(C1−C12) and 1,1,5,9,13-pentamethyl-(C1−C14); for 11-*cis*-β-carotene, 9,13-dimethyl-(C7−C15′) and 1,1,5,9,13-pentamethyl-(C1−C15′) (also for 13-*cis*-β-carotene); for 13-*cis*-β-carotene, 9,13,13′,9′-tetramethyl-(C7−C7′) (also for 15-*cis*-β-carotene); and for 15,15′-*cis*-β-carotene, 13,13′-dimethyl-(C11−C11′).

(42) The cis isomers, apart from 15-*cis*-β-carotene, are favored by the symmetry factor $R \ln 2$ (0.4 kcal/mol at 37.2 °C).

Table 8. Calculations by MM2-ERW-EVBH Heats of Formation (kcal/mol) of All-Trans Polyenes of *n* Double Bonds, $C_{2n}H_{2n+2}$, and Delocalized and Localized All-Trans Polyenyl Radicals, $CH_2{=}CH(CH{=})_{n-1}CH_2{\cdot}$, and Enthalpies of Stabilization, $\Delta\Delta H°_{stab}$, and Activation, $\Delta H^{\ddagger}_{rot}$,[a] for Trans−Cis Isomerization about the Central Double Bond in Polyenes of Odd Order[b]

polyene/radical·	$\Delta_fH°$ [d,e]	$\Delta H^{\ddagger}_{rot}$[a]	$\Delta_fH°_{deloc}$[d]	$\Delta_fH°_{loc}$[f]	$\Delta\Delta H°_{stab}$[h]
C_{22}	145.5	30.2			
C_{12}·			146.9	173.0	−26.1
C_{20}	132.2				
C_{19}·			134.0	169.7	−25.7
C_{18}	118.9	32.2			
C_{17}·			121.1	146.4	−25.3
C_{16}	105.6				
C_{15}·			108.3	133.1	−24.8
C_{14}	92.32	35.2			
C_{13}·			95.7	118.8	−24.1
C_{12}	79.03				
C_{11}·			83.2	106.5	−23.3
C_{10}	65.73	39.6			
C_9·			70.9	93.2	−22.3
C_8	52.44				
C_7·			59.1	79.9	−20.8
C_6	39.14	47.8			
C_5·			48.0	66.7	−18.7
C_4	25.85				
C_3·			38.8	53.4	−14.6
C_2	12.55				
C_1·			35.8	35.8	0.0

[a] $\Delta H^{\ddagger}_{rot} = 2\Delta_fH°$[delocalized $CH_2{=}CH(CH{=}CH)_{(n-3)/2}CH_2{\cdot}$] + 9.3 kcal/mol[c] − $\Delta_fH°$[polyenes, $C_{2n}H_{2n+2}$, *n* odd]. [b] (2*n* + 1) double bonds, $C_{2(2n+1)}$. [c] Increment for replacing two CH bonds by C−C + H_2. [d] Calculated by the MM2-ERW-EVBH program of Roth et al.[46] in which SE_1, $\Delta_fH°(CH_3{\cdot})$, and $\Delta_fH°$(90°−twisted ethene) are taken to be 14.6, 35.8, and 65.9 kcal/mol, respectively. [e] $\Delta_fH° = 12.55$(ethene) + (*n* − 1)(13.295). [f] $\Delta_fH°$[$(CH_2{=}CH(CH{=}CH)_{n-1}CH_3)$] + 48.6[g] ($\Delta\Delta_fH°$ [R−CH_3 → R−$CH_2{\cdot}$]. [g] According to the following: Seetula, J. A.; Russell, J. J.; Gutman, D. *J. Am. Chem. Soc.* **1990**, *112*, 1347−1353. [h] $\Delta_fH°_{deloc} - \Delta_fH°_{loc}$.

to be estimated. From the extrapolations in Table 6 based on the model compounds,[19,29] incremental increases in stabilization enthalpies become smaller as the order of the radical increases from 5 through 8. This conclusion also emerges from the theoretical calculations of enthalpies of polyenic radicals by the MM2-ERW-EVBH program, shown numerically in columns 4, 5, and 6 of Table 8 and graphically in Figure 2. In qualitative terms, rates are expected to decrease progressively as the rearranging double bond departs further from the central position because the gain in stabilization on the longer side of the diradical fails ever more severely to compensate for the loss of stabilization on the shorter side.

Although the predicted increase of 2.1 kcal/mol in the enthalpy of activation for isomerization to 11-*cis*-β-carotene (Table 6) might still have allowed its appearance to be detected, the severe thermodynamic bias in its enthalpy of formation, noted above, conspires against detection at equilibrium. A qualitative observation bearing on the lability of Δ,[11,12] is the report that a crude sample of synthetic 11-*cis*-β-carotene melts at 70 °C and has resolidified by 80 °C to an impure sample of *all-trans*-β-carotene.[43]

Rearrangement of **2** to 9-*cis*-β-carotene is thermodynamically feasible, but the enthalpy of activation for rotation about $\Delta^{9,10}$ is predicted to be higher by 5.1 kcal/mol if the shorter radical is formulated without participation of $\Delta^{5,6}$ as allyl (*n* = 1; SE_1 = 13.5 kcal/mol), or higher by 3.4 kcal/mol if formulated with participation of $\Delta^{5,6}$ as pentadienyl (*n* = 2; SE_2 = 16.9 kcal/mol). Qualitatively in agreement, traces of 9-*cis*-β-carotene may just be detectable in our longest runs at 69 °C. Consistently,

(43) Surmatis, J. D. U.S. Patent 3367985, Feb 6, 1968.

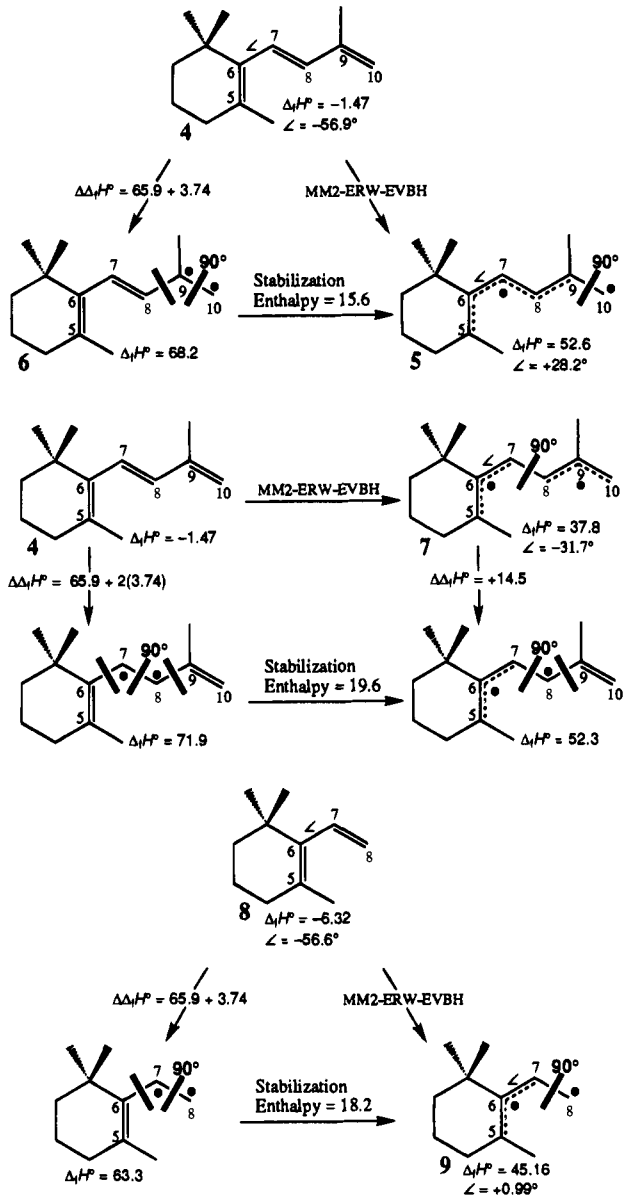
9-*cis*-β-carotene rearranges only very slowly at that temperature (see NMR spectra in supplementary material). In this respect, the report of Pesek, Warthensen, and Taoukis[22] that **2** rearranges at 25 °C to **3** and 9-*cis*-β-carotene, no mention being made of 15-*cis*-β-carotene, is in conflict with our results[44] and those of Kuki, Koyama, and Nagae,[24] who report the formation of 15-*cis*-β-carotene (~8%), 13-*cis*-β-carotene (24%), and a smaller amount of 9-*cis*-β-carotene (~6%) after 1 h at 80 °C.

Attempts to follow the transformation of an equilibrium mixture of **2**, 15-, and 13-mono-*cis*-β-carotene (and most likely 13,13′-di-*cis*-β-carotene, as well) were thwarted by analytical complexities not resolvable by the NMR analysis. Starting with pure 9-*cis*-β-carotene led to no improvement, as might have been expected from the semiquantitative findings of Kuki, Koyama, and Nagae in their brief kinetic examination of thermal rearrangements of mono-cis isomers.[24] The spectrum rapidly became very complicated apparently owing to rearrangement to 9,13′-di-*cis*-, 9,15-di-*cis*-, and 9,13-di-*cis*-β-carotene being faster than rotation about $\Delta^{9,10}$ to produce 9-trans isomers and thence **2**.

Rotation about $\Delta^{7,8}$ has a value of $\Delta H^{\ddagger} = 38.8$ kcal/mol when estimated without participation by $\Delta^{5,6}$ and is doubtless too high. A more reasonable value closer to 29.3 kcal/mol is estimated with participation by $\Delta^{5,6}$ (Table 6, column eight). That rotation about $\Delta^{7,8}$ plays a role in the thermal behavior of **2** at higher temperature (195 ± 5 °C/5 min) is clear from the work of Tsukida and Saiki,[45] but an assessment of relative rates is apparently precluded by the ease with which 7-*cis*-β-carotene undergoes triene cyclization. Finding less than 1.5% of **2** when 7-*cis*-β-carotene is heated for 1 h at 80 °C corresponds to a minimum value of E_a of 29 kcal/mol.[24]

Estimation of relative rates of conversion of *all-trans*-β-carotene to 7- and 9-*cis*-β-carotene by theory, as already noted above, is complicated by the non-coplanarity of the TMCH ring and its nontrivial contribution to the stabilization energies of the shorter radicals. The problem is particularly acute with 7-*cis*-β-carotene because the stabilization enthalpy of the allyl radical is high enough (13.5 kcal/mol) to buy large amounts of strain energy and the twisting of trigonal C8 to 90° drastically alters steric interactions operating in the ground state (*vide supra*). In respect to rotation about $\Delta^{9,10}$, should the shorter radical be viewed as pentadienyl with full participation of the 5,6 double bond or as allyl with no participation, or as somewhere in between? This problem has been addressed by application of the program, MM2-ERW-EVBH, developed by Roth, Staemmler, Neumann, and Schmuck.[46] This program is preferred for evaluation of heats of formation not only of polyenes but of polyenyl radicals and the diradicals corresponding to 90°-twisted polyenes. It conjoins the MM2, molecular force field program of Allinger,[47] optimized to a more current potential function for rotation about the 2,3 bond of butadienes (MM2-ERW),[32] and an extended version of the effective valence

Scheme 4



bond Hamiltonian (EVBH) of Malrieu and Maynau,[48] recalibrated to an intrinsic barrier for rotation in ethylene of 65.9 kcal/mol.[49]

When this program is applied to a simplified model, **4**, of β-carotene rotating about its 7,8 and 9,10 double bonds, the results shown in Scheme 4 are obtained. In the $\Delta^{9,10}$, 90°-twisted state, **5**, dihedral angle $\Delta^{5,6}-\Delta^{7,8}$ has contracted from −56.9° to 28.2°, consistent with a stabilization energy of 15.6 kcal/mol.[50] This is somewhat greater than expected of a simple allyl radical but smaller than the 16.9 kcal/mol expected of a fully planar (*E,E*) pentadienyl radical. In other words it appears that a more favorable compromise has been reached by

(44) We have not attempted to identify the origin of the discrepancy, but note in respect to identification of materials the only relevant comments: "The source of β-carotene... was selected because it was determined to contain appreciable amounts of *all-trans*-, 9-*cis*-, and 13-*cis*-β-carotenes" and "Peak identification was accomplished against a β-carotene standard and comparison of absorption spectra and retention properties of the isomers with published data."

(45) Tsukida, K.; Saiki, K. *J. Nutr. Sci. Vitaminol.* **1983**, *29*, 111−122.

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(47) Burkert, U.; Allinger, N. L. *Molecular Mechanics*; American Chemical Society: Washington, 1982.

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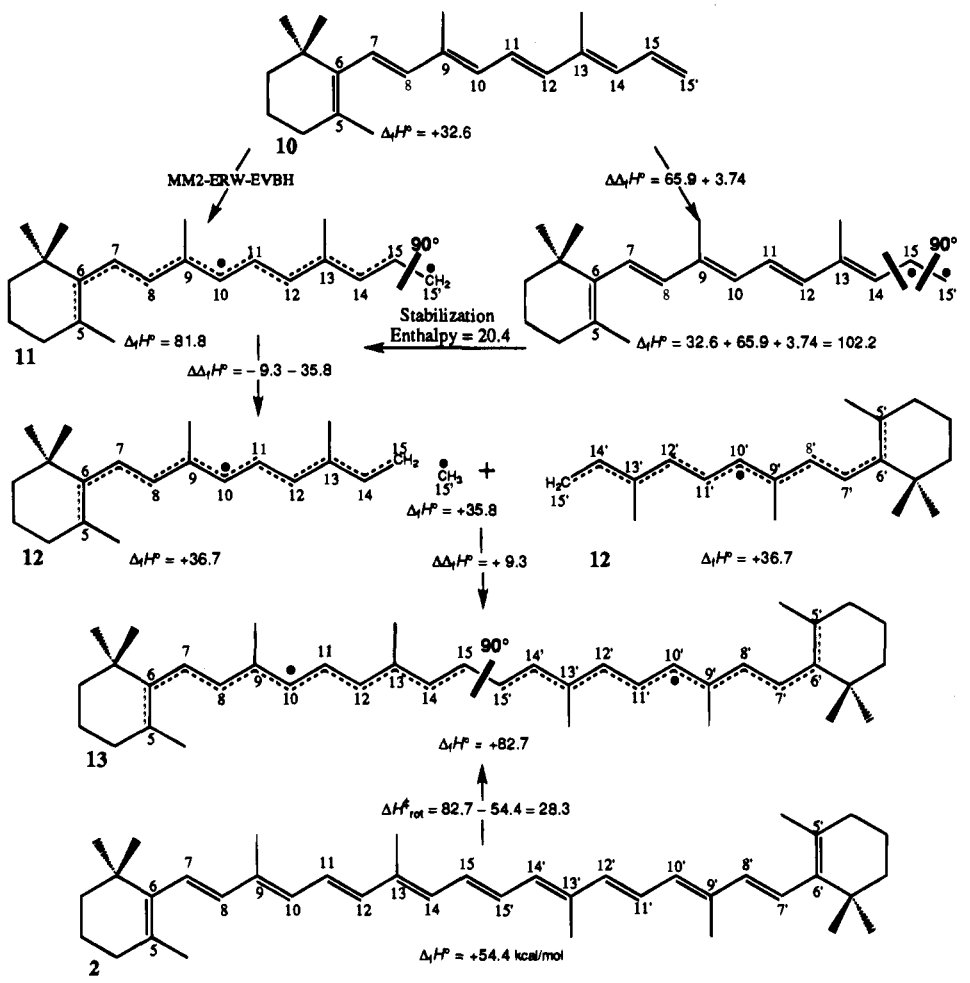
(50) The heat of formation of a fully localized diradical, **6**, is estimated according to the definition of stabilization energy of polyenyl radicals used in this work[19,29] by deconjugating the $\Delta^{7,8}-\Delta^{9,10}$ system, that is, by adding the Kistiakowsky energy of 3.74 kcal/mol, and twisting the now isolated 9,10 double bond by 90° at the cost of the intrinsic rotational barrier of 65.9 kcal/mol.[49]

Scheme 5



increasing both energy-raising steric energy and energy-lowering delocalization by approaching more closely to (cisoid) coplanarity.

A major consequence of rotation about the 7,8 double bond is a marked decrease in steric interaction between C8 and its substituents in the 90°-twisted state, **7**. The dihedral angle decreases from −56.9° to −31.7° and thereby allows much of the allylic stabilization to be realized. The resulting enthalpy of stabilization generates an activation enthalpy for rotation about $\Delta^{7,8}$ comparable to, and perhaps lower than, that for rotation about $\Delta^{9,10}$. In 6-vinyl-1,1,5-trimethylcyclohex-5-ene, **8**, as the model, complete coplanarity is achieved in the allylic part of diradical **9** owing to the smaller bulk of the C8 methylene group. Essentially all of the adverse steric interaction of the initially planar vinyl group is relieved when it becomes a 90°-vinyl group at the transition state. Although pursuit of other implications of this insight for the chemistry of β-carotene is inappropriate here, note that attack of a reagent at C8 involves losing only one Kistiakowsky unit of conjugative interaction (3.74 kcal/mol) in contrast to the loss of two in attack further along the chain.

A theoretical estimate of the enthalpy of activation of rotation about $\Delta^{15,15'}$ in β-carotene, too large a molecule for direct calculation of its allied diradicals, is made accessible by calculation of the heats of formation of β-carotene and the truncated diradical models in Scheme 5. From the heat of formation of model **10** twisted to 90° about $\Delta^{15,15'}$, **11**, a heat of formation for the semi-β-carotene radical, **12**, is calculated to be 36.7 kcal/mol by addition of the heat of hydrogenation of a C−C bond (taken to be −9.3 kcal/mol) and subtraction of the heat of formation of methyl radical (taken to be 35.8 kcal/mol for the sake of internal consistency). By reversal of the process, two semi-β-carotene radicals **12** may be combined by dehydrogenation to generate a model of 90°-twisted β-carotene, **13**. From the resulting heat of formation of 82.7 kcal/mol, an enthalpy of activation, $\Delta H^\ddagger$, of isomerization of *all-trans*-β-carotene to 15-*cis*-β-carotene is thereby estimated to be 28.3 kcal/mol, in good agreement with the experimental value of 27.9 ± 1.2 kcal/mol (Table 3).

In conclusion, we express our conviction that interventionist studies of the possible effect of supplementary carotenoids on the incidence of various cancers should not be limited to experimentation with the admittedly very readily available *all-trans*-β-carotene, but should be extended to include at the least 15-*cis*-, 13-*cis*-, and 9-*cis*-β-carotenes. Although some fresh vegetables, such as carrots, contain only *all-trans*-β-carotene, others, including squash, spinach, and collards, and fruits, such as peaches, apricots, and plums, contain substantial quantities of 13-*cis*- and 9-*cis*-β-carotenes and di-cis isomers as well. Our present finding that 13-*cis*- and 15-*cis*-β-carotenes are generated unavoidably, if slowly, at 37 °C provides further stimulus to probe the role of other stereoisomers, particularly since the demonstration by Sies and his co-workers that these two isomers and 9-*cis*-β-carotene are regular components of not long dead tissues![36] Additionally, we concur with Sies that further study should include lycopene, a thermally, substantially more reactive carotene. No matter how seductive the ready availability of *all-trans*-β-carotene may have been, the ever deepening rut into which the epidemiological studies have fallen thereby is not

lightly to be overlooked.[51] The other isomers are clearly just as "natural" as the all-trans, quite as appropriate for a rating of "Generally Regarded As Safe" by the United States Food and Drug Administration, and possibly more effective.

Experimental Section

General Methods. ^{1}H-NMR and ^{13}C-NMR (125.8 MHz) spectra are recorded on a Bruker AM-500 instrument in C_6D_6 and in $CDCl_3$, respectively, unless otherwise noted, and are reported as parts per million (ppm) from TMS (δ). Spin−lattice relaxation times (T_1) are determined by the inversion recovery method in degassed C_6D_6. Infrared spectra are recorded on a Perkin-Elmer Model 337 grating spectrophotometer and are reported in wavenumbers (cm^{-1}). Liquid samples are observed as thin films on NaCl plates; solid samples, as KBr pellets. UV−vis absorption spectra are measured with a Cary 219 spectrophotometer in CH_2Cl_2 and are reported as λ_{max} (ϵ_{max}). High-resolution, electron impact, mass spectra (HRMS) are obtained on a JEOL AX 505 spectrometer and data system and are recorded at a mass resolution of 1 part in 10 000.

2,7-Dimethylocta-2,6-dien-4-ynylenebis(triphenylphosphonium bromide). This intermediate was prepared following the sequence developed by Surmatis and Ofner.[52] The diol, 2,7-dimethylocta-1,7-dien-4-yne-3,6-diol, was crystallized from toluene (charcoal treatment): 33.4 g (40%) from 70 g of methacrolein; mp 89−91 °C (lit.[52] mp 88−91 °C); IR 3240 (br), 2940, 1655, 1450, 1380, 1285, 1125, 1015, 905, 695, 650; ^{1}H-NMR 1.78 (s, 6 H, Me-2,7), 2.51 (d, 2H, J = 5.2 Hz, H-3,6), 4.70 (d, 2 H, J = 5.2 Hz, HO-3,6), 4.79 (s, 2 H, vinyl), 5.17 (s, 2 H, vinyl); ^{13}C-NMR 18.1, 66.0, 84.8, 112.5, 143.6. Conversion to 1,8-dibromo-2,7-dimethylocta-2,6-dien-4-yne afforded 22.8 g (65%) from 20 g of diol: mp 38−40 °C (lit.[52] mp 40 °C); ^{1}H-NMR 1.82 (s, 6 H, Me-2,7), 3.43 (s, 4 H, 1,8-CH_2Br), 5.44 (s, 2 H, H-3,6); ^{13}C-NMR 17.8, 38.0, 92.2, 110.3, 146.2. As this compound was not reliably stable, it was converted to the triphenylphosphonium bromide without further purification to afford 63 g (97%) from 22.8 g of dibromide: after recrystallization from MeOH/EtOAc, mp 254−256 °C (dec) (lit.[52] mp 256−258 °C); IR 3400 (br), 2820, 2785, 1430, 1100, 740, 720, 690; ^{1}H-NMR (CD_3OD) 1.60 (s, 6 H, Me-2,7), 4.39 (d, 4 H, J_{H-P} = 16.3 Hz, 1,8-CH_2PPh_3Br), 5.48 (s, 2 H, H-3,6), 7.66−7.84 (m, 30 H, C_6H_6); ^{13}C-NMR (CD_3OD) 21.6, 33.4 (d), 119.0, 119.7, 131.5, 135.2, 136.6.

Ethyl β-Ionylideneacetate. This compound was prepared by the method of Shriver et al.[53] Triethyl phosphonoacetate (70 g, 0.312 mol) was added slowly to a stirred suspension of NaH (9.6 g of 80% oil dispersion) in THF (150 mL) at 0 °C during 1 h. To this mixture was added β-ionone (30 g, 0.156 mol) in THF (150 mL) over a 30-min period. After being stirred at 0 °C for 3 h and at room temperature for an additional 15 h, the reaction mixture was quenched with icewater, extracted with ether (3 × 200 mL), dried with anhydrous Na_2SO_4, concentrated *in vacuo*, and distilled under reduced pressure to give a colorless liquid (31.5 g, 77%) as a mixture of ethyl 9-*trans*- (91%) and 9-*cis*- (9%) β-ionylideneacetate: bp 145−150 °C/2 mmHg; IR (liquid) 2940, 2880, 2840, 1715, 1610, 1450, 1370, 1235, 1155, 1050, 975; ^{1}H-NMR 0.99 (s, 6 H, Me-1,1′, trans), 1.02 (t, 3H, J = 7.0 hz, Et), 1.12 (s, 6 H, Me-1,1′, cis), 1.36−1.42 (m, 2 H, CH_2-2), 1.48−1.54 (m, 2 H, CH_2-3), 1.60 (s, 3 H, Me-5), 1.75 (s, 3 H, Me-9, cis), 1.86 (t, 3 H, $J_{3,4}$ = 5.8 Hz, CH_2-4), 2.43 (s, 3 H, Me-9, trans), 4.01 (q, 2 H, J = 7.0 Hz, Et, cis), 4.06 (q, 2 H, J = 7.0 Hz, Et, trans), 5.75 (s, 1 H, H-10, cis), 5.92 (s, 1 H, H-10, trans), 6.08 (d, 1 H, $J_{7,8}$ = 16.1 Hz, H-7), 6.48 (d, 1 H, $J_{7,8}$ = 16.1 Hz, H-8, trans), 6.58 (d, 1 H, $J_{7,8}$ = 16.1 Hz, H-8, cis); ^{13}C-NMR 13.5, 14.3, 19.1, 21.5, 28.8, 33.0, 34.1, 39.4, 59.5, 118.0, 130.9, 133.5, 136.2, 137.1, 152.7, 167.2 (−CO_2−).

β-Ionylideneethyl-*11,11-d*$_2$ Alcohol. Ethyl β-ionylideneacetate (26 g, 0.099 mol) was reduced with $LiAlD_4$ (5 g, 0.119 mol) in dry THF (200 mL) at 0 °C during 30 min following procedures of Robeson et al.[54] and Wendler et al.[55] to give the alcohol as a colorless oil: 19.95 g, 91%; bp 130−137 °C/0.2 mmHg; 9-trans (81%) and 9-cis (19%) isomers; IR 3340 (br), 2920, 2870, 2840, 2200, 2100, 1620, 1450, 1385, 1360, 1220, 1135, 1085, 1030, 970, 890; ^{1}H-NMR 1.07 (s, 6 H, Me-1,1′, cis), 1.09 (s, 6 H, Me-1,1′, trans), 1.42−1.48 (m, 2 H, CH_2-2), 1.53−1.57 (m, 2 H, CH_2-3), 1.64 (s, 3 H, Me-5, trans), 1.72 (s, 3 H, Me-5, cis), 1.74 (s, 3 H, Me-9, trans), 1.82 (s, 3 H, Me-9, cis), 1.93 (t, 2 H, CH_2-4), 5.43 (s, 1 H, H-10, cis), 5.55 (s, 1 H, H-10, trans), 6.16 (s, 2 H, H-7,8, trans), 6.24 (d, 1 H, $J_{7,8}$ = 16.1 Hz, H-7, cis), 6.53 (d, 1 H, $J_{7,8}$ = 16.1 Hz, H-8, cis); ^{13}C-NMR 12.3, 19.1, 21.5, 28.7, 32.8, 34.0, 39.4, 58.5 (quint, CD_2OH), 126.7, 128.3, 128.7, 136.5, 136.9, 137.4.

β-Ionylideneacetaldehyde-*formyl-d*. Following the procedure of Wendler et al.,[55] β-ionylideneethyl-*11,11-d*$_2$ alcohol (15 g) in light petroleum ether (750 mL) was mechanically stirred for 24 h at room temperature, under argon with active MnO_2 (150 g).[56] The usual workup afforded the product as a yellow viscous liquid (12 g, 81%) consisting of 9-trans (84%) and 9-cis (16%) aldehyde: bp 130−135 °C/0.1 mmHg; IR (liquid) 2970, 2930, 2860, 2830, 2100, 1660, 1610, 1450, 1390, 1360, 1350, 1260, 1205, 1160, 1000, 970, 900; ^{1}H-NMR 0.97 (s, 6 H, Me-1,1′, cis), 0.98 (s, 6 H, Me-1,1′, trans), 1.37−1.40 (m, 2 H, CH_2-2), 1.47−1.53 (m, 2 H, CH_2-3), 1.55 (s, 3 H, Me-5, cis), 1.57 (3 H, s, Me-5, trans), 1.59 (s, 3 H, Me-9, trans), 1.70 (s, 3 H, Me-9, cis) 1.85 (t, 2 H, J = 6.3 Hz, CH_2-4), 5.78 (s, 1 H, H-10, trans), 5.98 (d, 1 H, $J_{7,8}$ = 16.1 Hz, H-7, cis), 6.44 (d, 1 H, $J_{7,8}$ = 16.1 Hz, H-8, cis); ^{13}C-NMR 12.8, 19.0, 21.6, 28.8, 33.1, 34.2, 39.4, 127.7, 128.6, 132.6, 135.5, 137.0, 154.9, 191.0 (t, −CDO); HRMS calcd for $C_{15}H_{21}D$ 219.1734, found 219.1721.

15,15′-Dehydro-11,11′-dideuterio-β-carotene. Prepared by a Wittig reaction,[52] 2,7-dimethylocta-2,6-dien-4-ynylenebis(triphenylphosphonium bromide) (3.75 g, 4.6 mmol) was added slowly with vigorous stirring under argon (deep violet red) to phenyllithium (9.2 mmol, 1.8 M solution in cyclohexane/ether, 7:3) in dry ethyl ether (50 mL). After 30 min, a solution of β-ionylideneacetaldehyde-*formyl-d* (2 g, 9.1 mmol) in ethyl ether (20 mL) was added dropwise. The mixture was then heated at reflux for 8 h, cooled to 0 °C, and diluted with MeOH (5 mL). Removal of solvents by evaporation *in vacuo* left a residue, which was dissolved in CH_2Cl_2/hexane (1:1) and loaded on a basic alumina column. Elution with CH_2Cl_2/hexane (1:1) afforded an orange product, which was further purified by two recrystallizations from hexane: 850 mg (35%); mp 153−154 °C (lit.[52] mp 154 °C, undeuterated); UV−vis 464 (77 200), 441 (91 000); ^{1}H-NMR 1.14 (s, 12 H, s, di-Me-1,1′), 1.47−1.49 (m, 4 H, CH_2-2,2′), 1.57−1.62 (m, 4 H, CH_2-3,3′), 1.80 (s, 6 H, Me), 1.85 (s, 6 H, Me), 2.15 (s, 6 H, Me), 1.97 (t, 4 H, $J_{3,4}$ = $J_{3',4'}$ = 6.2 Hz, CH_2-4,4′), 5.84 (s, 2 H, H-14,14′), 6.16 (s, 2 H), 6.27 (s, 2 H), 6.33 (s, 4 H); ^{13}C-NMR 12.8, 15.3, 19.2, 21.7, 29.0, 33.1, 34.3, 39.6, 98.0 (acetylenic C), 110.4, 127.4, 129.6, 130.1, 134.8, 137.5, 137.6, 138.0, 146.6; HRMS calcd for $C_{40}H_{52}D_2$ 536.4354, found 536.4335.

15,15′-*cis*-11,11′-Dideuterio-β-carotene (1d$_2$). Lindlar catalyst (100 mg) (amount used depending on the activity of catalyst) was added to a suspension of 15,15′-dehydro-β-carotene (200 mg) in hexane (50 mL) containing 1 drop of quinoline. A gentle stream of hydrogen was passed into the well-stirred mixture for 10 min (reaction monitored by ^{1}H-NMR). The catalyst was then filtered and washed with CH_2Cl_2, filtrate being then evaporated to dryness. The crude product was recrystallized twice from benzene/MeOH: 160 mg (80%); mp 143−144 °C (lit.[34] mp 150−151 °C, undeuterated); UV−vis 486 (69 400), 461 (84 400), 346 (38 900); ^{1}H-NMR[57] 1.14 (s, 12 H, di-Me-1,1′), 1.47−1.49 (m, 4 H, CH_2-2,2′), 1.57−1.62 (m, 4 H, CH_2-3,3′), 1.79 (s, 6 H, Me-5,5′), 1.87 (s, 6 H, Me-13,13′), 1.91 (s, 6 H, Me-9,9′), 1.96 (t, 4 H, $J_{3,4}$ = $J_{3',4'}$ = 6.1 Hz, CH_2-4,4′), 6.31 (d, 2 H, $J_{7,8}$ = $J_{7',8'}$ = 16.1 Hz, H-7,7′), 6.34 (s, 2 H, H-10,10′), 6.37 (d, 2 H, $J_{7,8}$ = $J_{7',8'}$ = 16.1 Hz, H-8,8′), 6.43 (sext, 2 H, $J_{15,15'}$ = 10.5 Hz, $J_{14,15}$ = $J_{14',15'}$ = 12.9 Hz, $J_{14,15'}$ =

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$J_{14',15}$ = 1.7 Hz, H-15,15'), 6.48 (s, 2 H, H-12,12'), 6.85 (q, 2 H, $J_{14,15}$ = $J_{14',15'}$ = 12.9 Hz, $J_{14,15'}$ = $J_{14',15}$ = 1.7 Hz, H-14,14'); ^{13}C-NMR 12.5, 12.8, 19.7, 22.0, 29.2, 33.3, 34.6, 39.9, 126.1, 126.9, 129.3, 131.7, 136.1, 137.4, 138.1, 138.4, 138.7; HRMS calcd for $C_{40}H_{54}D_2$ 538.4510, found 538.4515.

***all-trans*-11,11'-Dideuterio-β-carotene (2d$_2$).** Thermal rearrangement of **1d$_2$** (70 mg) was effected in hexane (20 mL) under reflux for 10 h (argon) while the reaction mixture was protected from light. Concentration *in vacuo* and standing in the refrigerator overnight afforded *all-trans*-11,11'-dideuterio-β-carotene: 60 mg (86%); mp 171−173 °C; UV−vis 488 (99 700), 462 (116 500); ^{1}H-NMR[57] 1.15 (s, 12 H, diMe-1,1'), 1.48−1.50 (m, 4 H, CH_2-2,2'), 1.57−1.62 (m, 4 H, CH_2-3,3'), 1.81 (s, 6 H, Me-5,5'), 1.86 (s, 6 H, Me-13,13'), 1.93 (s, 6 H, Me-9,9'), 1.98 (t, 4 H, $J_{3,4}$ = $J_{3',4'}$ = 6.1 Hz, CH_2-4,4'), 6.31 (q, 2 H, $J_{14,15}$ = $J_{14',15'}$ = 11.7 Hz, $J_{14,15'}$ = $J_{14',15}$ = 1.0 Hz, H-14,14'), 6.32 (d, 2 H, $J_{7,8}$ = $J_{7',8'}$ = 16.0 Hz, H-7,7'), 6.34 (s, 2 H, H-10,10'), 6.39 (d, 2 H, $J_{7,8}$ = $J_{7',8'}$ = 16.0 Hz, H-8,8'), 6.47 (s, 2 H, H-12,12'), 6.67 (sext, 2 H, $J_{15,15'}$ = 14.2 Hz, $J_{14,15}$ = $J_{14',15'}$ = 11.7 Hz, $J_{14,15'}$ = $J_{14',15}$ = 1.0 Hz, H-15,15'); ^{13}C-NMR 12.8, 12.9, 19.7, 22.0, 29.2, 33.3, 34.6, 40.0, 126.8, 129.4, 130.6, 131.8, 133.2, 135.9, 136.7, 137.9, 138.4, 138.7; HRMS calcd for $C_{40}H_{54}D_2$ 538.4510, found 538.4490.

13-*cis*-β-Carotene (3). A solution of *all-trans*-β-carotene (100 mg; Aldrich; recrystallized twice from benzene/MeOH) in benzene (50 mL) was boiled under reflux for 8 h under argon. Dilution of the cooled reaction mixture with MeOH (20 mL) followed by partial concentration *in vacuo* afforded 53 mg of crystalline *all-trans*-β-carotene. The filtrate, found by ^{1}H NMR to consist of **1** (15%), **3** (63%), and **2** (22%), was concentrated *in vacuo*, redissolved in heptane, and chromatographed ($Ca(OH)_2$) using heptane as eluant, at ~4 °C to minimize isomerization and degradation. Success in this separation mainly depends on the quality of $Ca(OH)_2$ (material from Aldrich Chemical Co. gave the best results). The order of elution was 15,15'-*cis*-, 13-*cis*-, and *all-trans*-β-carotene. 13-*cis*-β-Carotene, obtained in 20% yield, was recrystallized from CH_2Cl_2/MeOH: mp 138−139 °C (lit.[43] mp 139 °C); UV−vis 484 (56 400), 459 (72 500), 436 (59 100), 356 (36 000); ^{1}H-NMR essentially identical to that reported;[31] ^{13}C-NMR (C_6D_6, 125.8 MHz) 12.8, 19.7, 20.8, 22.0, 29.2, 30.2, 33.3, 34.6, 40.0, 125.3, 126.7, 126.8, 127.2, 129.4, 129.5, 129.8, 129.9, 131.6, 131.8, 131.9, 133.0, 134.9, 135.8, 136.5, 136.6, 138.1, 138.3, 138.4, 138.5, 138.7.

Kinetics of Thermal Isomerization of 15,15'-*cis*-11,11'-Dideuterio- (1d$_2$), *all-trans*-11,11'-Dideuterio- (2d$_2$), and 13-*cis*-β-Carotene (3). Solutions of each of the above three β-carotene isomers (3.7−11.1 mM) in C_6D_6 containing 18-crown-6 ether (1.6 mM) as internal standard are prepared and placed in Pyrex NMR tubes (528 × 5 mm), previously cleaned by soaking in 30% NH_4OH, washing with analytical grade acetone, and drying in an oven at 100 °C. After three freeze−pump−thaw cycles, the NMR tubes are sealed under vacuum (10^{-3} mmHg) and then heated in a thermostated oil bath, equipped with a proportional temperature controller (YSI Model 72), at 37, 43, or 64 °C for the specified period of time; heating at 69 °C is in a vapor bath above boiling *n*-hexane. Temperatures are recorded with a digital thermometer (Digisense Model 8528-30, Cole Parmer Instrument Company) employing a J-type thermocouple as temperature sensor or with an iron−constantan thermocouple connected to a millivolt potentiometer (Leeds and Northrup Model 8686) using an icewater reference junction. The resulting mixture of isomers is analyzed on a Bruker AM-500 NMR spectrometer by using proton signals at 6.85 (H-14,14', T_1 = 0.72 s) of **1d$_2$**, 6.67 (H-15,15', T_1 = 1.01 s) of **2d$_2$**, and 6.12 (H-14, T_1 = 1.30 s), 6.92 (H-15, T_1 = 1.01 s), and 7.03 (H-12, T_1 = 0.87 s) of **3**. The methylene signal of 18-crown-6 ether (3.51, T_1 = 1.80 s) is used for calculation of recoveries. Approximately 128 scans are collected with 90° pulses and a pulse interval of 9.0 s, which is 5 tirnes the longest T_1 (internal standard). In order to eliminate the possibility of proton catalysis, some kinetics were run both in the presence and in the absence of DABCO (0.0005 M): the specific rate constants were unchanged. In order to ensure that only first-order reactions were taking place, some measurements were made at different initial concentrations of β-carotene isomers without effecting any change in the resulting specific rate constants. In order to measure the rates and equilibrium constants for the interconversion among 15,15'-*cis*-, *all-trans*-, and 13-*cis*-β-carotene, four different sets of data were generated.

Set 1 represents kinetics starting from **1d$_2$** and observing both the disappearance of **1d$_2$** and the appearance of **2d$_2$**. Measurements were taken as long as no new isomer appeared besides **2**. The concentrations of **1** and **2** were calculated from the ^{1}H-NMR spectra by measuring the areas at 6.85 (H-14,14') and 6.67 (H-15,15'), respectively, as described above. The data are given as supplementary material in Table S-I and the results in Table 1.

Set 2 represents kinetic runs starting from **1d$_2$** and following both the disappearance of **1d$_2$** and the appearance of **2d$_2$** and **3d$_2$**. Observations are continued as long as **1, 2,** and **3** are the only components. The concentration of **1d$_2$** is deduced from the area of the quartet at 6.85 (H-14,14'). Because this quartet partially overlaps the signal at 6.92 corresponding to H-15 of **3d$_2$**, the area, $A_{15,15'\text{-}cis}$, is estimated by multiplying A_1, the area of the two middle peaks of the quartet, by a correction factor of 1.14, itself obtained from the ^{1}H-NMR spectrum of an authentic sample of **1d$_2$**, by dividing the area of the entire quartet at 6.85 by the area of the two middle peaks of the same quartet. The concentration of 13-*cis*-β-carotene is obtained by measuring only the area at 6.12 (H-14), since the proton signal at 6.92 (H-15) partially overlaps the signal at 6.85 from **1d$_2$**, and the signal at 7.03 (H-12 of 13-*cis*-β-carotene) overlaps the spinning side bands of C_6D_6. The concentration of **2d$_2$** is obtained from the signal at 6.67 (H-15,15' of *all-trans*-β-carotene). Since this sextet partially overlaps the signal at 6.61 corresponding to H-15' of 13-*cis*-β-carotene, the area of the four middle peaks of the sextet is measured and multiplied by a correction factor, 1.08, which is determined from the ^{1}H-NMR spectrum of an authentic sample of **2d$_2$** by dividing the total area of the sextet at 6.67 (H-15,15') by the area of the four middle peaks of the same sextet. The data are given as supplementary material in Table S-II and the results in Table 2.

Set 3 represents kinetics starting from **2d$_2$** and following both the disappearance of **2d$_2$** and the appearance of **1d$_2$** and **3d$_2$**. Measurements again are made to a point where signals only from the three isomers could be observed. Concentrations of these isomers are estimated as in set 2. The data are given as supplementary material in Table S-III and the results in Table 3.

Data in set 4 represent kinetics starting from 13-*cis*-β-carotene (**3**) and observing both its disappearance and the appearance of *all-trans*-β-carotene. Measurements are continued as long as signals from only the two isomers, 13-cis and all-trans, are observed. Relative concentrations of 13-*cis*-β-carotene are recorded as the mean of the areas of the three proton signals at 6.12 (H-14), 6.92 (H-15), and 7.03 (H-12); those of *all-trans*-β-carotene as in data set 2. The data are given as supplementary material in Table S-IV and the results in Table 4.

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Supplementary Material Available: Tables S-I−S-IV containing unprocessed data from the study of the kinetics of thermal rearrangement of 15,15'-*cis*-11,11'-d_2-β-carotene (**1d$_2$**), *all-trans*-11,11'-d_2-β-carotene (**2d$_2$**), and 13-*cis*-β-carotene (**3**) and nine ^{1}H-NMR spectra including those of pure samples of *all-trans*-, 9-, 13-, and 15-*cis*-β-carotene, **1d$_2$**, and **2d$_2$**, and three of 9-*cis*-β-carotene heated at 69 °C (27 pages). This material is contained in many libraries on microfiche, immediately follows this article in the microfilm version of the journal, can be ordered from the ACS, and can be downloaded from the Internet; see any current masthead page for ordering information and Internet access instructions.

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