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On the structure of carotenoid iodine complexes†

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Previous work on carotenoid–iodine complexes is briefly reviewed. The formation of iodine complexes of β,β-carotene and of (3*R*,3′*R*)-β,β-carotene-3,3′-diol (zeaxanthin) has been studied by modern methods including UV/VIS/NIR, IR MS, EPR, ENDOR and NMR (^{1}H, ^{1}H–^{1}H COSY, TOCSY, 2D ROESY, ^{1}H–^{13}C HSQC and ^{1}H–^{13}C HMBC) spectroscopy, and chemical reactions monitored by HPLC, TLC and spectral analysis (VIS, MS, ^{1}H NMR). β,β-Carotene formed a solid complex $C_{40}H_{56} \times 4I$ with iodine in hexane and a solvent complex with λ_{max} 1010 nm in chlorinated solvents. Iodine was not covalently bound to the carotene. Spectroscopic and chemical evidence is consistent with the representation of the β,β-carotene–iodine complex containing iodine in a π complex with cationic/radical cationic properties. Extensive *E*/*Z* isomerisation was noted for all quenching products obtained in acetone, with thiosulfate, by dilution, or by reaction with nucleophile (MeOH). Key products obtained from the β,β-carotene–iodine complex were 4′,5′-didehydro-4,5′-*retro*-β,β-carotene (isocarotene) and 4-methoxy-β,β-carotene. The zeaxanthin–iodine complex was not suitable for a practical synthesis of (3*S*,3′*S*)-4′,5′-didehydro-4,5′-*retro*-β,β-carotene-3,3′-diol (eschscholtzxanthin).

Introduction

Even though carotenoid–iodine complexes have been studied since 1886,[1] structural details remain unknown. The properties of such complexes depend on the method of preparation.

Solid iodine added to carotenoids in benzene solution provided a complex with elemental composition compatible with $C_{40}H_{56}I_3$.[1–3] The iodine was present as I_3^- according to Mössbauer spectrometric data.[3]

Mixing solutions of iodine and excess carotene in diethyl ether provided violet crystals, $C_{40}H_{56}I_2$.[4,5]

A mixture of solutions of iodine and β,β-carotene (**1**, Scheme 1) in hexane provided di- or tetraiodine complexes, which upon treatment with acetone or thiosulfate furnished isocarotene (4′,5′-didehydro-4,5′-*retro*-β,β-carotene, **2**, Scheme 4 later).[4,6–8]

If chloroform, dichloromethane or 1,2-dichloroethane was used as solvent, a complex was formed with NIR absorption around 1000 nm, ascribed to a charge transfer complex[9,10] $(C_{40}H_{56}I^+)I_3^-$ or alternatively a cationic,[11,12] dicationic[10] or radical cation[13,14] structure. Several carotenoids have been used as substrates.[12,13]

Doping of various carotenoids with iodine vapour, providing charge transfer complexes,[15–18] has been used in conductivity studies.

Our recent interest in *E*/*Z* and *R*/*S* (of allenes) isomerisation of carotenoids,[19–24] using iodine as catalyst, initiated by Karrer in 1929,[25] and extensively used by Zechmeister,[26] prompted us to examine carotenoid–iodine complexes by modern methods. Mainly β,β-carotene (**1**) has been used here as the carotenoid substrate, whereas zeaxanthin (β,β-carotene-3,3′-diol, **3**) has been partly employed.

We have characterised a solid complex of iodine and β,β-carotene (**1**) obtained from heptane and a solution complex formed in chloroform, dichloromethane or benzene solutions by spectroscopic methods and chemical reactions.[27]

In the meantime we have gained experience on structural elucidation of carotenoid carbocations by low-temperature NMR spectroscopy and their reactions with nucleophiles.[28–33] These results are relevant for the interpretation of the structure of carotenoid–iodine complexes.

Results and discussion

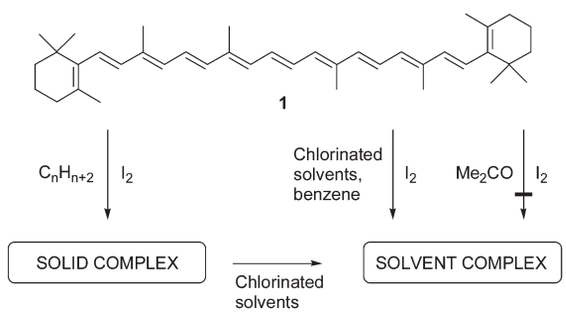
Complex formation and stability

A black solid complex was formed by mixing solutions of β,β-carotene (**1**) and iodine (4–15 times molar excess) in heptane at −20 °C,[4] or preferably at 6 °C which results in larger particles.

A solvent complex (λ_{max} around 1000 nm) was formed spontaneously at room temperature in chloroform, dichloromethane and 1,2-dichloroethane, and more slowly in benzene. The slower reaction in benzene than in the chlorinated solvents may be due to the formation of a benzene–iodine charge-transfer complex.[34] The solvent complex was also formed by dissolving the solid complex in benzene and any of the above mentioned chlorinated solvents.

The rationalisation was made that solvents in which iodine was dissolved molecularly, providing a pink-red colour (λ_{max} *ca.* 500 nm), were applicable, whereas solvents in which iodine was dissolved in the ionic form, as the triiodide (yellow-brown, λ_{max} 295 and 364 nm) did not yield solvent complexes. The results are summarised in Scheme 1.

The stability of the solvent complex was monitored by UV/VIS/NIR spectroscopy at room temperature. The half-life time in dichloromethane was higher (*ca.* 5 h) than in chloroform (*ca.* 2 h). The rate of formation and decomposition of the solvent complex in dichloromethane at room temperature is illustrated in Fig. 1.



Scheme 1 Solvents used for the formation of iodine complexes.

† No. 7 in the series Charged Carotenoid Species. No. 6 = Ref. 33.

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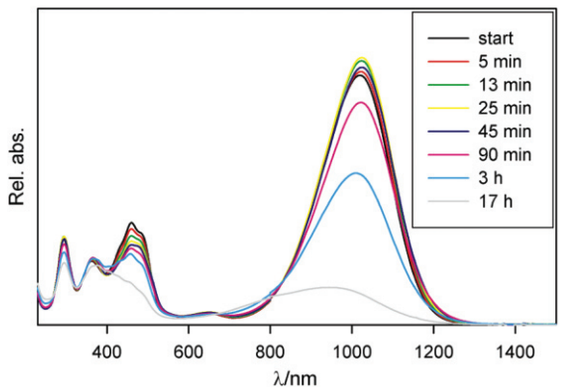


Fig. 1 UV/VIS/NIR spectra of the β,β-carotene (**1**)–iodine complex in CH_2Cl_2.

The solid complex

Elemental analysis was consistent with the composition $C_{40}H_{56}I_4$, compatible with the calculated amount of iodine consumed in the reaction. X-Ray analysis failed due to the amorphous consistency of the solid precipitate.

The IR (nujol) spectrum of the solid complex differed from that of β,β-carotene (**1**) with three strong broad absorption bands at 1440, 1125 and 960 cm^{-1}. No absorption around 500 cm^{-1} for the C–I stretch was detected. These data are in line with previous data for polythiophene[35] and β,β-carotene (**1**)[10,36,37] doped by treatment with iodine vapour, for **1** doped with SO_3,[37] and for doped polyacetylene.[38] These doped molecules also showed 3–4 strong absorption bands in this region, which have been rationalised as being due to charged self-localised excitations.[35] The nature of these excitations (soliton, polaron or other) was not determined.[35]

Both EI and FAB MS supported the absence of covalently bound iodine. The EIMS showed ions at *m/z* 540 (**1** + 4, 3%), 538 (**1** + 2, 6%), 536 (**1**, 7%), 534 (**1** − 2, 3%), 254 (I_2, 100%), 128 (HI, 24%), 127 (I, 30%).

Conductivity measurements showed no conductivity for the solid complex. This was interpreted as a lack of a π-stacked sandwich-type structure in the amorphous solid, which is a prerequisite for this type of intermolecular conductivity.

In contrast to the solid-state NMR data for iodine doped polyacetylene,[39] no iodine-bound carbons appeared to be present in the ^{13}C CP-MAS NMR spectrum of the solid β,β-carotene (**1**)–iodine complex. However, the broadening and downfield shift of the signals from the solid complex compared to neutral β,β-carotene (**1**) indicates that there is charge on the polyene chain as in the doped polyacetylene, Fig. 2. The shift range of approximately 125–160 ppm (majority between 125–145 ppm) is compatible with the shift range and distribution previously found for carotenoid monocations.[30–32] A carotenoid dication would be expected to have carbon resonances down to 180 ppm, and also a higher proportion of chemical shift in the region downfield of 145 ppm.[28,29,32]

The origin of the sharp resonances overlaid on the broad main-part of the signals, which is not consistent with the chemical shifts of non-complexed β,β-carotene (**1**), was not rationalised. The 2D ^{13}C-T_1 correlation spectrum shows that the broad and sharp resonances have a similar T_1 of about 0.75 s, indicating that the broadening might be due to differences in the T_2 relaxation, which is compatible with a radical contribution.

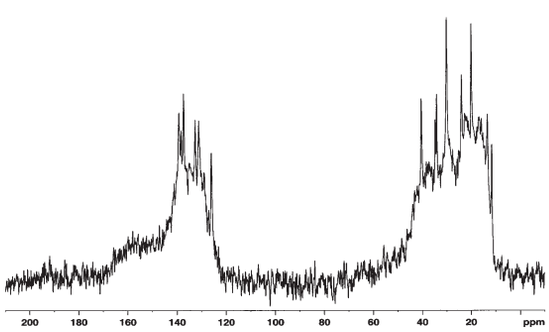


Fig. 2 ^{13}C CP-MAS NMR spectrum (150 MHz) of the solid β,β-carotene (**1**)–iodine complex.

The solvent complex

Spectroscopic data. The reaction of β,β-carotene (**1**) with iodine in $CDCl_3$ solution was monitored by EPR, demonstrating that radical species were present in the complex, Fig. 3. The solvents were mixed at −20 °C under nitrogen. The *g*-factor was indicative of a carbon-centred radical, and showed the same temperature dependence as reported previously.[13] The S/N ratio increased as the temperature was lowered, and when the sample went from the liquid to the solid state, as expected.

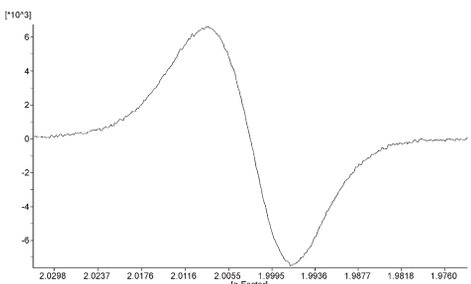


Fig. 3 EPR spectrum of the β,β-carotene (**1**)–iodine complex in $CDCl_3$ at 77 K. Linewidth: 20 G.

The EPR line-width increased as the temperature was lowered. At −20 °C, the line-width was 14 G, in agreement with previous calculations for the radical cation of β,β-carotene (**1**).[40] When the temperature was lowered, the line-width increased. At 70 K, the line-width was 20 G, Fig. 3. The large line-width for the observed radical indicated a strongly delocalised radical.

The ENDOR data showed no evidence for coupling to iodine nuclei. The ENDOR spectrum exhibited three doublets with splittings of 8.1 MHz, 4.7 MHz and 2.7 MHz. These splittings are consistent with what has been previously observed under similar conditions for the β,β-carotene (**1**) radical cation.[41] For the β,β-carotene (**1**) radical cation, however, the intensities of all the doublets were similar, while for the β,β-carotene (**1**)–iodine complex, the doublet with a splitting of 2.7 MHz was twice the intensity of the doublet with a splitting of 8.1 MHz.

EIMS of the solvent complex showed peaks at *m/z* 538 (**1** + 2, 13%), 536 (**1**, 4), 254 (I_2), 128 (HI) and 127 (I). Only in one of several attempts (EIMS, FAB), when solid iodine was added to a chloroform solution, could a weak ion demonstrating covalent binding of iodine to **1** be detected (*m/z* 663, $C_{40}H_{55}I$, relative intensity 0.017%).

The UV/VIS/NIR spectrum with λ_{max} 1050 nm in CH_2Cl_2, Fig. 1, exhibited weaker absorption at 294 and 363 nm, compatible with the presence of I_3^-.[3] A weak absorption in the 450 nm region was ascribed to non-complexed β,β-carotene (**1**).

The IR spectrum of the solvent complex in $CHCl_3$ differed from that of β,β-carotene (**1**) with absorptions at 1415, 1070 and 985, resembling the spectrum of the solid complex.

NMR studies are treated below.

$C_{40}H_{57}$ monocarbocation 4. Attempted determination of the structure of the solvent complex by NMR failed because the signals obtained from the complex were very broadened, and the signal-to-noise ratio was low, especially in the olefinic region. Despite several attempts, including lowering the temperature in steps down to −50 °C, the only signals that could be interpreted resembled those arising from the β-end group of β,β-carotene (**1**).

However, in some experiments performed with a particular batch of deuterated chloroform, after the initial broadening, the signals became sharper, and the S/N ratio increased,

Table 1 NMR data for 5-hydro-β,β-carotenyl cation (**4**)

	δ_H (ppm)	δ_C (ppm)	$^3J_{H,H}$/Hz
1		38.5	
2	1.39–1.54	41.2	
3	1.47–1.82	16.9	
4	1.57–1.79	—[a]	
5	3.37	32.2	
6		172.2	
7	6.54	120.2	12.3
8	7.38	145.3	12.3
9		137.4	
10	7.63	161.2	13.3
11	6.91	126.3	12.0–13.3
12	7.71	161.5	12.0
13		140.9	
14	7.91	164.4	13.3
15	7.07	132.3	12.8–13.3
16	1.19	30.4	
17	1.20	30.8	
18	1.24	22.8	
19	2.04	12.0	H-8[b]
20	2.16	12.7	H-12[b]
1′		34.3	
2′	1.48	39.7	
3′	1.61	19.2	
4′	2.11	34.5	
5′		—[a]	
6′		138.1	
7′	6.77	136.7	15.9
8′	6.43	137.1	15.9
9′		152.2	
10′	6.54	133.1	12.2
11′	7.60	141.8	14.2–12.2
12′	6.76	137.9	14.2
13′		163.7	
14′	6.87	137.0	12.3
15′	8.13	155.7	12.3–12.8
16′	1.08	28.8	
17′	1.08	28.8	
18′	1.81	22.1	
19′	2.18	13.2	H-10[b]
20′	2.41	15.1	H-14[b]

[a] ^{13}C shifts could not be unequivocally assigned. [b] Long-range couplings detected in the ^{1}H–^{1}H COSY spectrum.

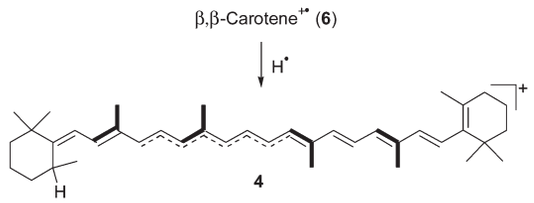
showing a single, downfield shifted, unsymmetrical carotenoid. This carotenoid was analysed by a series of NMR techniques (^{1}H, ^{1}H–^{1}H COSY, TOSCY, 2D ROESY, ^{1}H–^{13}C HSQC and ^{1}H–^{13}C HMBC) at 600 MHz and −20 °C.

The spectra were analysed using the same approach as described previously.[32] In this case, a signal at 3.37 ppm presented a puzzle, and was first ascribed to an iodine-carrying carbon, but as the MS data showed that no iodine was covalently bound to the carotenoid, this possibility was disregarded. The NMR data also indicated that one of the β-end groups was unsymmetrically substituted, which made the analysis of the chemical shifts difficult due to the large increase in transitions, reducing the S/N ratio significantly.

However, the proton at 3.37 ppm could be assigned to the C-5 position of this end group, explaining the lack of symmetry. This methine proton had a similar chemical shift as the α-proton of the 2-vinyl-2-adamantyl cation (at 3.42 ppm),[42] which is also in a similar position due to the locking of the 5-proton in an axial position by the methyl groups in relative 1,3-positions.

From the obtained data, all ^{1}H and ^{13}C (except C-4 and C-5′) chemical shifts for 5-hydro-β,β-carotenyl monocation (**4**) were assigned, Table 1. The positions of the double, single, and intermediate bonds were determined from the $^3J_{H,H}$ coupling constants of the olefinic protons, Table 1, combined with the long-range couplings from the methyl groups, as indicated in Scheme 2. However, determination of the charge distribution of the monocarbocation **4** was not possible, since ^{13}C NMR data for a neutral model of the left half of **4** is not available.

The origin of hydrogen transfer (here to C-5 in **4**), as H^+, $H^\bullet$ or H^-, to carotenoid dications (*e.g.* **5**, Scheme 4) and cation radicals (*e.g.* **6**, Scheme 2) from neutral carotenoids has recently been discussed.[33] In radical containing media, hydrogen radical transfer to cation radicals was favoured,[33] as shown in Scheme 2.



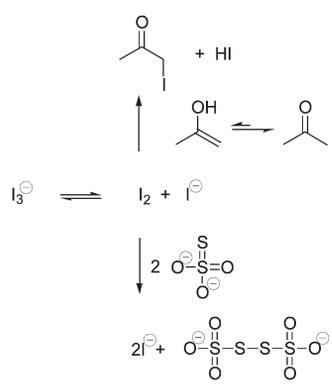
Scheme 2 Structure of the 5-hydro-β,β-carotenyl monocation **4**, and its possible origin. Long-range HMBC couplings from the methyl groups are indicated in bold.

Cleavage of complexes in acetone, with thiosulfate, or by dilution

The solid complex when dissolved in acetone or treated with thiosulfate is reported to give isocarotene (**2**).[8] Thus, in the present work, the solid complex dissolved in acetone or reacted with $Na_2S_2O_3$ in dichloromethane provided, according to HPLC analysis, strongly isomerised isocarotene (**2**, 15 geometrical isomers) besides strongly isomerised β,β-carotene (**1**) and minor products.

Similar treatment of the solvent complex with $Na_2S_2O_3$ in chloroform, dichloromethane or benzene gave higher yields of *E*/*Z* isomerised isocarotene (**2**). The highest yield of **2** (51% of total) was obtained in chloroform. *E*/*Z* isomerised **2** exhibited the predicted MS fragmentations.

Isocarotene (**2**) was also formed by dilution of the $CDCl_3$ solution in the absence of other reagents. Reaction between thiosulfate and iodine[43] rationalises the cleavage of the solid and solvent complexes, as does the reaction between iodine and acetone,[44] Scheme 3.



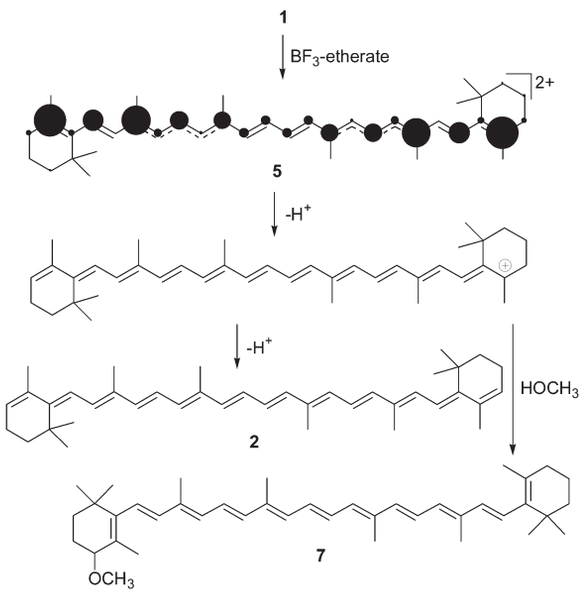
Scheme 3 Scavenging of iodine from I_3^- by thiosulfate and acetone.

The formation of isocarotene (**2**) upon dilution may be ascribed to complex cleavage by destabilisation due to a larger distance between the counter ions of the complex.

Reactions of complexes with nucleophile (CH_3OH)

The solid complex dissolved very slowly in methanol to provide a complicated product mixture (86 peaks by HPLC), of which all-*E*-β,β-carotene (**1**) was the major product in 20% yield. No *Z*-isomers of β,β-carotene (**1**) were found in the product mixture. When treated with methanol, the solvent complex in chloroform provided, according to HPLC data, *E*/*Z* isomerised isocarotene (**2**, 2–14%) and partly characterised methoxylated products (28–48%), besides several minor products. The major methoxylated products exhibited β,β-carotene (**1**) type chromophores and represented, according to MS data, mono- and dimethoxylated derivatives. 4-Methoxy-β,β-carotene (**7**) was identified by VIS, MS and ^{1}H NMR data and conversion to isocarotene (**2**) with acidified chloroform.[45]

4-Methoxy-β,β-carotene (**7**) and isocarotene (**2**) have recently been prepared from β,β-carotene dicarbocation (**5**, 20 πe^-), obtained from β,β-carotene (**1**) with BF_3–diethyl etherate, upon quenching with methanol,[29] Scheme 4.



Scheme 4 Formation of isocarotene (**2**) and 4-methoxy-β,β-carotene (**7**) from the β,β-carotene dication (**5**).

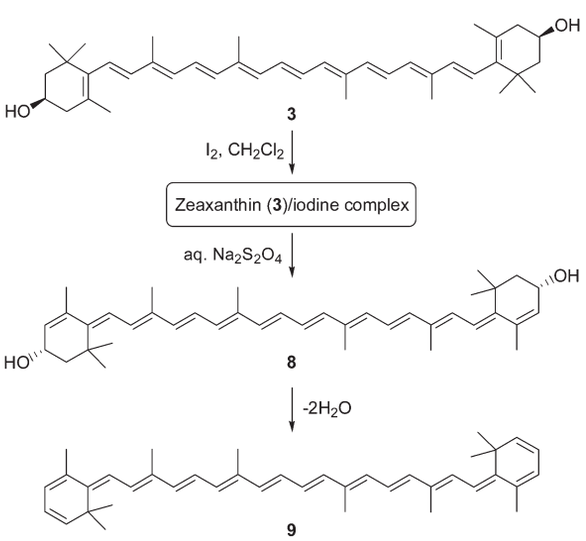
Zeaxanthin (3) solvent complex

Dichloromethane was the preferable solvent for complex formation of zeaxanthin (**3**) with iodine. A solvent complex was also formed in $CHCl_3$, albeit of low solubility. Zeaxanthin (**3**) was not sufficiently soluble in hexane for forming a solid complex.

The solvent complex in CH_2Cl_2 had λ_{max} 1015 nm, decomposed rapidly at room temperature, and was more stable at −20 °C.

The NMR spectra obtained of the zeaxanthin (**3**)–iodine complex were similar to the spectra obtained for the β,β-carotene (**1**) complex, with strong signal broadening. The only information provided was the downfield shifted methyl groups, which indicated a positive charge on the carotenoid polyene system.

It was of interest to investigate a conversion of (3*R*,3′*R*)-zeaxanthin (**3**) *via* the iodine complex to (3*S*,3′*S*)-4′,5′-didehydro-4,5′-*retro*-β,β-carotene-3,3′-diol (eschscholtzxanthin, **8**), Scheme 5, by analogy with the reaction of β,β-carotene (**1**) to isocarotene (**2**), Scheme 4.



Scheme 5 Conversion of (3*R*,3′*R*)-zeaxanthin (**7**) to (3*S*,3′*S*)-eschscholtzxanthin (**8**), and dehydration to bisanhydroeschscholtzxanthin (**9**).

Low pigment recovery and reproducibility besides a strongly *E*/*Z* isomerised product hampered the identification of eschscholtzxanthin **8** by HPLC and VIS spectroscopy, in comparison with authentic **7** prepared by $NaBH_4$ reduction of the corresponding dione rhodoxanthin (4′,5′-didehydro-4,5′-*retro*-β,β-carotene-3,3′-dione). Product **8** was readily transformed to bisanhydroeschscholzxanthin (**9**) with a characteristic MS fragmentation pattern, resulting in the formation of the aromatic tropylium ion (*m*/*z* 133). Monoanhydroeschscholtzxanthin (**10**, $C_{40}H_{51}OH$) was also tentatively identified by EIMS, and a $C_{40}H_{51}I$ product ascribed to reaction of the polyene with HI.

It is concluded that the zeaxanthin (**3**) iodine complex is not suitable for a practical synthesis of eschscholtzxanthin (**8**).

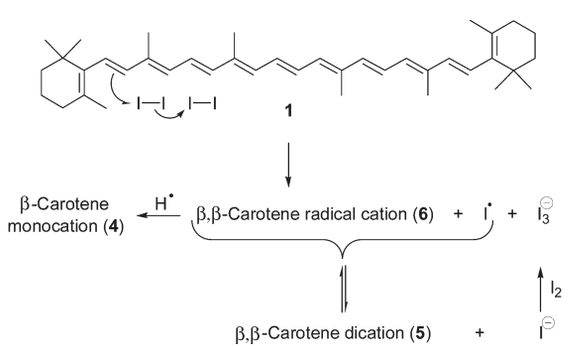
Structure of the β,β-carotene (1)–iodine complex

A rationalisation of the present results for the β,β-carotene (**1**) iodine complex follows here. In none of the carotenoid–iodine complexes prepared according to previous methods was iodine covalently bound to the carotenoid. This is consistent with previous *ab initio* calculations revealing that addition of iodine to a polyene is an endothermic process.[10]

In the solvent complex formed from the solid complex ($C_{40}H_{56} \times 4I$) in polar, non-nucleophilic, preferably chlorinated solvents, or directly from β,β-carotene (**1**) and iodine in the latter solvents, Scheme 1, β,β-carotene (**1**) is present in an ionised form, carrying positive charges caused by interaction with iodine. The counter ion, determined by VIS data to be I_3^-, is known to be stabilised in chlorinated solvents.[46] However, in solutions with high iodine concentration, also the formation of higher order iodides is expected (I_5^-, I_7^-, *etc.*).[46]

The NIR spectra of the solvent complexes are compatible with a carotenoid radical cation[14] or carotenoid mono- and dications.[29,30] Quenching products of the solvent complex with thiosulfate or acetone, Scheme 3, and the reaction with nucleophile (CH_3OH) are compatible with reactions of β,β-carotene dication (**6**) as substrate in its solvent complex, Scheme 4. This implies the transfer of two electrons from β,β-carotene (**1**) to iodine, resembling the situation for treatment of β,β-carotene (**1**) with BF_3–diethyl etherate.[29]

According to the EPR results, radical species are involved in the solvent complex. A plausible reaction scheme for the formation of the radical cation **6** and dication **5** of β,β-carotene (**1**) upon treatment with iodine is given in Scheme 6. This route implies that I^+ serves as an oxidising agent, removing stepwise, *via* $I^{\bullet}$, two electrons from the carotenoid polyene chain.

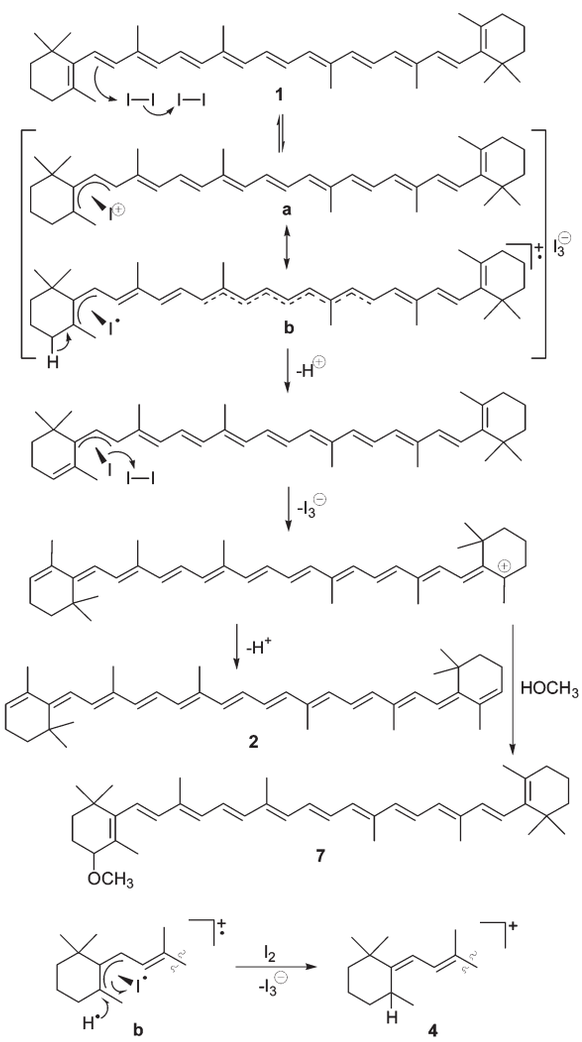


Scheme 6 Representation of the β,β-carotene (**1**)–iodine complex as an equilibrium between radical cation **6** + $I^{\bullet}$ and dication **5** + I^+.

The high degree of *E*/*Z* isomerised products (**1**, **3**, **5**, **7**) obtained by quenching of the iodine complexes by i) reacting the complexing iodine or ii) reacting the complexed carotenoid with a nucleophile, is consistent with cationic intermediates.[33]

However, iodine radicals were not detected by EPR spectroscopy. Moreover, the presence of the dication **5** could not be documented by NMR, in contrast to earlier experience.[29]

In Scheme 7, the β,β-carotene (**1**)–iodine complex is suggested as a tetrahapto (η_4) π complex which overcomes these problems. However, no experimental evidence as to the number of binding sites for the iodine has been obtained. The complex could therefore equally well be represented as a η_2 or a η_1 π complex. In principle, iodine may attack any π bond in the polyene system if the η_2 or the η_1 π complex is preferred for the β,β-carotene (**1**)–iodine system. Recent results obtained by computational methods for the complex between benzene and the iodine radical[47] indicate that a η_1 π complex is energetically favoured over the η_2 and η_6 π complexes, and the η_1 σ complex for that system. The η_4 π complex was not considered relevant for the benzene–iodine complex.



Scheme 7 Alternative representation of the β,β-carotene (**1**)–iodine complex, with iodine bound in a η_4 π complex, and rationalisation of the formation of isocarotene (**2**), 4-methoxy-β,β-carotene (**7**), and monocation **4**.

The computational study also showed that there was a very low degree of spin and charge transfer between iodine and benzene in that complex.[47] This is in agreement with the previous findings by EPR, that not more than 2% of the β,β-carotene (**1**) treated with I_2 was in the radical form.[2,13,48] Our model is compatible with the observed carotenoid radical in the EPR and ENDOR experiments.

The products observed in the quenching of the solvent complex may also be formed from this complex, without the formation of the free β,β-carotene dication (**5**), which was not observed in the NMR experiments, as shown in Scheme 7. Also the absorption at 450 nm in the VIS/NIR spectrum, Fig. 1, of the iodine complex may be explained by our preferred model as the free carotenoid in equilibrium with the carotenoid–iodine complex, Scheme 7.

Also the occasional formation of the monocation **4** may be formulated by reaction of the radical resonance structure **b** with a hydrogen radical, Scheme 7.

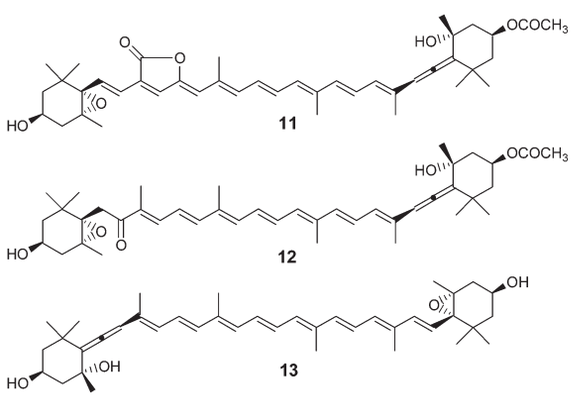
In chlorinated solvents, the favoured solvation of triiodide results in a complex in solution. However, in alkane solvents, where the solubility of the triiodide is low, the immediate precipitation of the carotenoid complex with the triiodide counter ion would result in the formation of the solid complex. Thus the solid complex is considered as the alkane insoluble salt with the same structure as the solvent complex given in Scheme 7.

The current picture, based on further experimental data, thus incorporates features of previous suggestions,[3–14] as mentioned in the introduction.

On iodine-catalysed photochemical stereoisomerisation

The relevance of the present results on carotenoid iodine complexes for the photochemical *E*/*Z* isomerisation of carotenoids in the presence of catalytic amounts of iodine[26] needs consideration.

Whereas the mechanism of photochemical *E*/*Z* and allenic *R*/*S* isomerisation using diphenyl diselenide as catalyst of carotenoids such as peridinin (**11**), fucoxanthin (**12**) and neoxanthin (**13**), Scheme 8, has been suggested,[23,49] no generally accepted mechanism has so far been published for the photo-induced *E*/*Z* or *R*/*S* (allene) iodine catalysed stereoisomerisation of carotenoids.



Scheme 8 Structures of the allenic carotenoids peridinin (**11**), fucoxanthin (**12**) and neoxanthin (**13**).

Relevant data for iodine catalysed stereoisomerisation was recently reviewed.[23] AM1 calculations have shown that the energy barrier of *cis*/*trans* isomerisation is much lower for the radical cation (*ca.* 24 kcal mol^{-1}) and dication (*ca.* 3 kcal mol^{-1}) than in the neutral carotenoid (*ca.* 55 kcal mol^{-1}).[50]

Kinetic studies on the iodine catalysed stereoisomerisation of a series of carotenoids indicated that the isomerisation occurs through a series of first-order reversible reaction steps.[51] Mechanisms of geometrical *cis*/*trans* (*E*/*Z*) isomerisation *via* energy transfer and electron transfer have recently been discussed.[52]

Decomposition of the solvent complex, depicted in Scheme 7, resulted in extensively *E*/*Z* isomerised products, as might be predicted on structural grounds. The resonance forms are either of cationic type (**a**), or of free radical cationic type (**b**). However, the photochemical events, during the *E*/*Z* isomerisation of carotenoids in the presence of catalytic amounts of iodine, remains unknown.

Experimental

General methods

Synthetic β,β-carotene (**1**) and (3*R*,3′*R*)-zeaxanthin (**3**) were used. General precautions for work with carotenoids were taken.[53] Visible light (VIS) spectra were recorded on a

Perkin Elmer 552 Spectrophotometer and near infrared (NIR) spectra on a Varian Cary 5 UV-VIS-NIR spectrophotometer. EI mass spectra were recorded on a VG 7070S spectrometer with a direct inlet to the ion source (70 eV). Diagnostically useful ions only are cited. X-Band EPR spectra were obtained at room temperature on a Bruker ESP 300E spectrometer using a rectangular cavity and a flat cell; and at 248 K and 180 K on a Bruker EMX spectrometer using an HS resonator and a ER 4131VT variable temperature unit with liquid nitrogen for cooling. X-Band pulse EPR/ENDOR spectra were recorded at 12 K on a Bruker E580 spectrometer with an E560-P DICE ENDOR unit using the Flexline ENDOR resonator (ER 4118X-MD5-EN).

^{1}H NMR spectra were recorded on a Bruker Avance DPX 400 instrument, using a 5 mm QNP probe, or on a Bruker Avance DRX 600 instrument, using a 5 mm inverse probe (QXI). $CDCl_3$ was used as solvent and as internal standard. Chemical shifts are cited relative to TMS with calibration against $CHCl_3$ at 7.27 ppm and $CDCl_3$ at 77.0 ppm for ^{1}H and ^{13}C, respectively.

^{13}C Solid-state CP-MAS NMR spectra were recorded on a Bruker Avance 600 instrument operating at 150.86 MHz using a 2.5 mm rotor, 1 ms contact time, with spinning rates ensuring that all side-bands fall outside the spectrum.

HPLC was carried out on a Hewlett Packard instrument series 1050 equipped with a diode array detector, and using a Waters YMC Carotenoid C30, 250 × 4.6 mm column, mobile phase 0 min: methanol : *tert*-butyl methyl ether : water (81 : 15 : 4 v/v/v, 1.0 ml min^{-1}), 60 min: methanol : *tert*-butyl methyl ether : water (31 : 65 : 4 v/v/v, 1.0 ml min^{-1}), 70 min: methanol : *tert*-butyl methyl ether : water (16 : 80 : 4 v/v/v, 1.0 ml min^{-1}). This reversed phase system offers excellent separation of *cis*/*trans* isomers of β,β-carotene (**1**)[54] and isocarotene (**2**). VIS spectra of the carotenoid components were recorded on-line during chromatography.

Preparative TLC was carried out on self-made TLC plates (silica : calcium carbonate 2 : 1).

The solid complex

Preparation and characterisation. The solid complex was formed according to a procedure modified from Kuhn and Lederer,[4] in order to obtain larger solid precipitates. To a solution of β,β-carotene (**1**, 107.8 mg, 0.20 mmol) in heptane (180 ml) was added a solution of iodine (205.6 mg, 1.62 mmol) in heptane (70 ml) at room temperature. The solution, which immediately turned black, was left overnight at 6 °C for the formed precipitates to aggregate. The solid complex was filtered from the solution, and washed on the filter to remove free (excess) iodine with cold (−20 °C) heptane. The solid complex formed was dried under reduced pressure to give a black powder, in an evacuated tube mp 155–160 °C (decomp.); Elemental analysis (found C, 45.9; H, 4.8; I, 49.0%. $C_{40}H_{56}I_4$ requires C, 46.0; H, 5.4; I, 48.6%); a molar excess of 6 was used in a parallel experiment (found C, 45.9; H, 5.0; I, 48.7%); ν_{max}(nujol)/cm^{-1} 1440, 1125 and 960; ν_{max}(KBr)/cm^{-1} 1460, 1115 and 980; δ_c (150 MHz, solid state) see Fig. 2; *m*/*z* (EI) 540 (**1** + 4, 3%), 538 (**1** + 2, 6), 536 (**1**, 7), 534 (**1** − 2, 3), 254 (I_2, 100), 128 (HI, 24), 127 (I, 30).

According to X-ray analysis, the solid complex was amorphous. Dissolving the solid complex in $CHCl_3$ or CH_2Cl_2 gave a black solution with absorption maximum as for the solvent complex, *vide infra.*

The conductivity was measured on a four-point probe instrument under atmospheric conditions at room temperature. A tablet was prepared of the solid complex under vacuum at 10 ton pressure. The thickness of the tablet was measured, and the conductivity, σ/S cm^{-1}, calculated from Equation 1:

$$\sigma = \ln 2\, I\, (\pi d U)^{-1} \quad (1)$$

Table 2 Conductivity of doped and undoped β,β-carotene (**1**), and of the solid β,β-carotene (**1**)–iodine complex

Sample	*d*/cm	*I*/mA	*U*/V	σ/mS cm^{-1}
β,β-Carotene (**1**)	0.042	0	0	0
Doped β,β-carotene (**1**)	0.042	2.22	7.82	1.49
Solid complex, sample 1	0.049	0	0.33	0
Solid complex, sample 2	0.041	0	0.19	0

where *I* is the current (in A) through the tablet, *U* is the voltage (in V) imposed and *d* is the thickness of the tablet (in cm). As a control the conductivity of a tablet of β,β-carotene (**1**) was measured before and after doping with iodine vapour for 2 days. The results (average of three measurements) are given in Table 2.

Reactions

Conversion to isocarotene (2). The solid complex was dissolved in CH_2Cl_2. The resulting solution was treated with 10% aq. $Na_2S_2O_3$ twice, washed with water and extracted into heptane. HPLC analysis showed β,β-carotene (**1**, 12%, 3 isomers), isocarotene (**2**, 29%, 15 isomers) and other carotenoids (59%).

Isocarotene (**2**) was isolated from the reaction mixture by preparative TLC (2% acetone in heptane). Elution with acetone of the zone with R_F 0.60–0.73 gave isocarotene, λ_{max}/nm (Me_2CO) 464; *m*/*z* (EI) 536 (M + 2, 49%), 534 (M, 100), 442 (M − 92, 11), 428 (M − 106, 10), 267 (M^{2+}, 12), 209 (32), 157 (44), 145 (56), 119 (69), 95 (90).

As an alternative method of preparation, the solid complex was dissolved in acetone.[4] According to HPLC analysis β,β-carotene (**1**, 19%, 3 isomers), isocarotene (**2**, 34%, 17 isomers) and other carotenoids (47%) were formed after treatment of the resulting solution with 10% aq. $Na_2S_2O_3$ as described above.

Reactions with nucleophile (MeOH). The solid β,β-carotene (**1**)–iodine complex was dissolved in MeOH. The complex only dissolved slowly to provide a greenish black solution, which was treated with 10% aq. $Na_2S_2O_3$ as described above. HPLC analysis showed a total of 86 peaks. The major carotenoid present was identified as (all-*E*)-β,β-carotene (**1**, 21%) from R_T and the VIS spectrum. No isocarotene (**2**) or *Z*-isomers of **1** were observed.

The solvent complex

Preparation and characterisation. The solvent complex was formed by treatment of β,β-carotene (**1**) in $CHCl_3$ or CH_2Cl_2 solution with iodine dissolved in the same solvent. A range of molar excesses of iodine was tested. Increasing the molar excess from 6 to 10 resulted in a reduction in the absorption ratio of β,β-carotene (**1**) (459 nm) relative to the absorption from the solvent complex (1014 nm) from 0.2 to 0.1. Further increase in the iodine excess did not reduce this ratio further. The solvent complex, when monitored by VIS/NIR spectroscopy, showed a stability of several hours. The stability was higher at lower temperatures. λ_{max}/nm (CH_2Cl_2) 294 (I_3^-), 363 (I_3^-), 459 (**1**), 1014 (solvent complex); ν_{max}($CHCl_3$)/cm^{-1} 1457s, 1420m, 1119s, 1081m, 991m, 964m; *m*/*z* (EI) 538 (**1** + 2, 13%), 536 (**1**, 14), 534 (**1** − 2, 6), 254 (I_2, 8), 128 (HI, 100), 127 (I, 57).

NMR data. For characterisation by NMR, β,β-carotene (**1**, 3.0 mg, 5.6 mmol) was dissolved in $CDCl_3$ (0.3 ml), and cooled to −20 °C. A solution of iodine (5.0 mg, 39 mmol) in $CDCl_3$ (0.3 ml) was added, and the solution inserted in a cold (−20 °C) NMR tube flushed with Ar. 600 MHz NMR spectra (^{1}H, ^{1}H–^{1}H COSY, TOCSY, 2D ROESY, ^{1}H–^{13}C HSQC and ^{1}H–^{13}C HMBC) were recorded at −20 °C. The NMR spectra showed very broad signals downfield shifted relative to

β,β-carotene (**1**). Due to the broadening of the signals, the spectrum appeared as two unsymmetrical humps in the olefinic and the aliphatic regions. The shape of these humps initially changed rapidly, but a steady state was reached after some time.

When using one particular batch of $CDCl_3$, however, one set of sharp signals appeared. From the obtained NMR spectra, these signals could be ascribed to the 5-hydro-β,β-carotenyl monocation (**4**). For δ_H/δ_C (600/150 MHz, $CDCl_3$, −20 °C, Me_4Si), see Table 1.

Pulse EPR and ENDOR data. The pulse ENDOR spectrum was collected at 12 K using the Davies ENDOR sequence (π_{PREP}–t_{RF}–$\pi/2$–τ–π, with an RF pulse during time t_{RF}) with pulse lengths π_{PREP} = 400 ns, $\pi/2$ = 200 ns, π = 400 ns and an RF pulse of 15 μs. Samples were prepared as for the NMR experiments. The solution was inserted into the cooled resonator for measurement. The ENDOR spectrum was measured at the center of the EPR line and exhibited three doublets centered around the ^1H Larmor frequency (ν_L = 14.8 at B_0 = 3475 G). The doublets showed splittings of 8.1 MHz, 4.7 MHz and 2.7 MHz.

Reactions. ***Conversion to isocarotene (2).*** The solvent complex was prepared in CH_2Cl_2 from β,β-carotene (**1**) as described above, with 10 equivalents of iodine. The resulting black solution was treated with 10% aq. $Na_2S_2O_3$, followed by work-up as described above for the solid complex dissolved in CH_2Cl_2. Analysis by HPLC showed β,β-carotene (**1**, 20%, 5 isomers), isocarotene (**2**, 37%, 16 isomers) and other carotenoids (37%). A sample prepared in $CDCl_3$ was worked up after NMR analysis (8 h, −20 °C), and showed β,β-carotene (**1**, 29%, 3 isomers), isocarotene (**2**, 51%, 15 isomers), and other carotenoids 20%. In a blind-test, β,β-carotene in CH_2Cl_2 was treated with 10% aq $Na_2S_2O_3$, and worked up. This sample showed β,β-carotene (**1**, 85%), no isocarotene (**2**), and 15% other carotenoids.

Reactions with nucleophile. The solvent complex was prepared from β,β-carotene (**1**, 1.0 mg) and iodine (2.5 mg) in CH_2Cl_2 (1.0 ml) as described above. Shortly after preparation, MeOH (0.5 ml) was added as nucleophile. The colour immediately changed to greenish black, and then slowly turned orange. The solution was washed with 10% aq $Na_2S_2O_3$ (2×) and water, taken to dryness under reduced pressure, and redissolved in acetone. HPLC analysis showed a complex reaction mixture, where methoxylated β,β-carotenes (**1**) (30%), β,β-carotene (**1**, 18%) and isocarotene (**2**, 15%) were tentatively identified from VIS spectra and polarity data.

The reaction mixture was separated into 4 zones by preparative TLC (3% acetone in heptane). Zone A, isocarotene (**2**); R_F = 0.73; λ_{max}/nm (HPLC System 1, R_T = 40–60 min) *ca.* 470; *m*/*z* (EI) 534 (M, 88%), 442 (M − 92, 10), 428 (M − 106, 8), 267 (M^{2+}, 11), 209 (30), 157 (41), 119 (66), 95 (82), 69 (100). Zone B, dimethoxy-β,β-carotene; R_F = 0.47; λ_{max}/nm (HPLC, R_T = 25–35 min) *ca.* 450; *m*/*z* (EI) 598 (M, 6%), 566 (M − MeOH, 50), 534 (M − MeOH − MeOH, 33), 474 (M − MeOH − 92, 4), 442 (M − MeOH − MeOH − 92, 3), 267 ([M − MeOH − MeOH]$^{2+}$, 7), 209 (13), 157 (24), 145 (29), 119 (43), 75 ($C_3H_7O_2$, 100); δ_H (400 MHz, $CDCl_3$) singlets at 3.40 ppm and 3.68 ppm ascribed to methoxy group, signals at 4.1 and 4.2 ppm with coupling into the aliphatic region ascribed to methine proton with oxygen substituent, in-chain protons as for **1**. Zone C, methoxylated β,β-carotene; R_F = 0.33; λ_{max}/nm (HPLC System 1, R_T = 14–28 min) 400–450). Zone D, several polar compounds; R_F = 0; λ_{max}/nm (HPLC System 1, R_T = 5–25 min) 370–400; *m*/*z* (EI) tentatively three molecular ions, 628, 598 and 582, from which the loss of 3, 2 and 1 moles of water was observed, respectively; base peak at *m*/*z* 75 ($C_3H_7O_2$).

The reaction mixture was treated with 0.03% HCl in CH_2Cl_2 to provide β,β-carotene (**1**, 33%), isocarotene (**2**, 24%) other carotenoids (43%). The amount of **2** corresponded to the amount of carotenoid in zones A and B above, while the amount of **1** corresponded to the amount of carotenoid in zone B.

Treatment of the solvent complex with 5% KOH in MeOH provided the same reaction mixture as for treatment with MeOH.

Iodine solvent complex with zeaxanthin (3)

Preparation and characterisation. The solvent complex between iodine and zeaxanthin (**3**) was prepared in the same manner as the solvent complex with β,β-carotene (**1**). The zeaxanthin (**3**) complex precipitated from $CHCl_3$. λ_{max}/nm (CH_2Cl_2) 295 (I_3^-), 366 (I_3^-), 456 (**3**), 1015 (solvent complex); δ_H (600 MHz, CD_2Cl_2, −20 °C) spectrum similar to that of the β,β-carotene (**1**) solvent complex described above, but poor solubility of **3** at −20 °C.

Conversion to eschscholtzxanthin (8). The zeaxanthin (**3**) solvent complex was treated with 10% aq $Na_2S_2O_3$, and worked up as described above for the conversion of the solid β,β-carotene (**1**) complex to isocarotene (**3**). Analysis of the resulting product mixture by HPLC showed zeaxanthin (**3**, 51%, 6 isomers), monoanhydroeschscholtzxanthin (13%, 3 isomers), eschscholtzxanthin (**8**, 13%, 15 isomers), bisanhydroeschscholtzxanthin (**9**, 1%), other carotenoid 20%. **8** was isolated from the product mixture by preparative TLC (3% acetone in heptane). R_F = 0.92; λ_{max}/nm (Me_2CO) 464 nm).
The product was rechromatographed by preparative TLC (hexane). HPLC analysis revealed the conversion of **8** to **9**. λ_{max}/nm (MeOH : *t*-BuOMe : H_2O 31 : 65 : 4) 434, 500, 532; *m*/*z* (EI) 530 (M, 61%), 516 (M − 14, 35), 438 (M − 92, 8), 424 (M − 106, 6), 265 (M^{2+}, 14), 159 (59), 133 (76), 119 (100).

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References

1 M. A. Arnauld, *C. R. Hebd. Seances Acad. Sci.*, 1886, **102**, 1119–1122.
2 C. M. Huggins and O. H. J. LeBlanc, *Nature*, 1960, **186**, 552–553.
3 T. Matsuyama, H. Sakai, H. Yamaoka and Y. Maeda, *J. Chem. Soc., Dalton Trans.*, 1982, 229–231.
4 R. Kuhn and E. Lederer, *Chem. Ber.*, 1932, **65B**, 637–640.
5 R. Willstätter and W. Mieg, *Justus Liebigs Ann. Chem.*, 1907, **355**, 1–28.
6 R. Kuhn and E. Lederer, *Chem. Ber.*, 1931, **64B**, 1349–1357.
7 R. Kuhn and E. Lederer, *Naturwissenschaften*, 1931, **19**, 309.
8 P. Karrer and G. Schwab, *Helv. Chim. Acta*, 1940, **23**, 578–581.
9 J. H. Lupinski, *J. Phys. Chem.*, 1963, **67**, 2725–2728.
10 E. Ehrenfreund, D. Moses, A. J. Heeger, J. Cornil and J. L. Bredas, *Chem. Phys. Lett.*, 1992, **196**, 84–90.
11 T. G. Ebrey, *J. Phys. Chem.*, 1967, **71**, 1963–1964.
12 N. T. Ioffe, A. A. Engovatov and V. G. Mairanovskii, *Zh. Obshch. Khim.*, 1976, **46**, 1638–1644.
13 R. Ding, J. L. Grant, R. M. Metzger and L. D. Kispert, *J. Phys. Chem.*, 1988, **92**, 4600–4606.
14 J. A. Jeevarajan, C. C. Wei, A. S. Jeevarajan and L. D. Kispert, *J. Phys. Chem.*, 1996, **100**, 5637–5641.
15 B. Mallik, K. M. Jain and T. N. Misra, *Biochem. J.*, 1980, **189**, 547–552.
16 P. Pal and T. N. Misra, *J. Phys. D. Appl. Phys.*, 1990, **23**, 218–222.
17 S. Sen, P. Pal and T. N. Misra, *J. Mater. Sci.*, 1993, **28**, 1367–1371.
18 D. Ghosh, S. Hazra, P. Pal and T. N. Misra, *Bull. Mater. Sci.*, 1993, **16**, 127–135.
19 J. A. Haugan, G. Englert, E. Glinz and S. Liaaen-Jensen, *Acta Chem. Scand.*, 1992, **46**, 389–395.

20 J. A. Haugan and S. Liaaen-Jensen, *Tetrahedron Lett.*, 1994, **35**, 2245–2248.
21 J. A. Haugan, G. Englert, T. Aakermann, E. Glinz and S. Liaaen-Jensen, *Acta Chem. Scand.*, 1994, **48**, 769–779.
22 T.-R. Lindal and S. Liaaen-Jensen, *Acta Chem. Scand.*, 1997, **51**, 1128–1131.
23 T. Refvem, A. Strand, B. Kjeldstad, J. A. Haugan and S. Liaaen-Jensen, *Acta Chem. Scand.*, 1999, **53**, 114–123.
24 A. Strand, K. Kvernberg, A. M. Karlsen and S. Liaaen-Jensen, *Biochem. Syst. Ecol.*, 2000, **28**, 443–455.
25 P. Karrer, A. Helfenstein, R. Widmer and T. B. van Itallie, *Helv. Chim. Acta*, 1929, **12**, 741–756.
26 L. Zechmeister, *Cis–trans Isomeric Carotenoids Vitamins A and Arylpolyenes*, Springer Verlag, Wien, 1962.
27 B. F. Lutnaes, J. Krane and S. Liaaen-Jensen, in *Book of Abstracts, 13th Int. Carotenoid Symp.*, Honolulu, 2002, p. 108.
28 B. F. Lutnaes, L. Bruas, J. Krane and S. Liaaen-Jensen, *Tetrahedron Lett.*, 2002, **43**, 5149–5152.
29 B. F. Lutnaes, L. Bruas, G. Kildahl-Andersen, J. Krane and S. Liaaen-Jensen, *Org. Biomol. Chem.*, 2003, **1**, 4064–4072.
30 G. Kildahl-Andersen, B. F. Lutnaes, J. Krane and S. Liaaen-Jensen, *Org. Lett.*, 2003, **5**, 2675–2678.
31 G. Kildahl-Andersen, B. F. Lutnaes and S. Liaaen-Jensen, *Org. Biomol. Chem.*, 2004, **2**, 489–498.
32 B. F. Lutnaes, G. Kildahl-Andersen, J. Krane and S. Liaaen-Jensen, *J. Am. Chem. Soc.*, 2004, **126**, 8981–8990.
33 G. Kildahl-Andersen, L. Bruås, B. F. Lutnaes and S. Liaaen-Jensen, *Org. Biomol. Chem.*, 2004, **2**, 2496–2506.
34 A. Streitwieser, C. H. Heathcock and E. M. Kosower, *Introduction to Organic Chemistry*, MacMillan Publishing Company, New York, 1992.
35 Y. Furukawa, *J. Phys. Chem.*, 1996, **100**, 15644–15653.
36 E. Ehrenfreund, D. Moses, K. Lee, A. J. Heeger, J. Cornil and J. L. Bredas, *Synth. Met.*, 1993, **57**, 4707–4713.
37 I. Harada, Y. Furukawa, M. Tasumi, H. Shirakawa and S. Ikeda, *Chem. Lett.*, 1980, **3**, 267–270.
38 H. Shirakawa, *Rev. Mod. Phys.*, 2001, **73**, 713–718.
39 I. Heinmaa, M. Alla, A. Vainrub, E. Lippmaa, M. L. Khidekel, A. I. Kotov and G. I. Kozub, *J. Phys. Colloq.*, 1983, **44**, 357–360.
40 J. L. Grant, V. J. Kramer, R. Ding and L. D. Kispert, *J. Am. Chem. Soc.*, 1988, **110**, 2151–2157.
41 P. Faller, T. Maly, A. W. Rutherford and F. MacMillan, *Biochemistry*, 2001, **40**, 320–326.
42 G. K. S. Prakash, V. P. Reddy, G. Rasul, J. Casanova and G. A. Olah, *J. Am. Chem. Soc.*, 1992, **114**, 3076–3078.
43 P. G. Desideri, L. Lepri and D. Heimler, in *Encyclopaedia of Electrochemistry of the Elements*, ed. A. J. Bard, Marcel Dekker, New York, 1973, vol. I, pp 91–153.
44 M. Hulce and M. J. Chapdelaine, in *Comprehensive Organic Synthesis*, ed. B. M. Trost and I. Fleming, Pergamon Press, Oxford, 1991, vol. 4, pp. 237–268.
45 R. Entschel and P. Karrer, *Helv. Chim. Acta*, 1958, **41**, 402–413.
46 A. I. Popov, in *Halogen Chemistry*, ed. V. Gutmann, Academic Press, London, 1967, vol. 1, pp. 225–264.
47 M.-L. Tsao, C. M. Hadad and M. S. Platz, *J. Am. Chem. Soc.*, 2003, **125**, 8390–8399.
48 J. Cornil, E. Ehrenfreund, D. Moses, A. Heeger and J. Bredas, *Mater. Sci. Forum*, 1993, **122**, 41–49.
49 A. Strand and S. Liaaen-Jensen, *J. Chem. Soc., Perkin Trans. 1*, 2000, 595–598.
50 G. Gao, C. C. Wei, A. S. Jeevarajan and L. D. Kispert, *J. Phys. Chem.*, 1996, **100**, 5362–5366.
51 P. Molnar, T. Kortvelyesi, Z. Matus and J. Szabolcs, *J. Chem. Res., Synop.*, 1997, **4**, 120–121.
52 N. Polyakov, T. Leshina and L. Kispert, *RIKEN Rev.*, 2002, **44**, 140–143.
53 K. Schiedt and S. Liaaen-Jensen, in *Carotenoids Vol. 1A. Isolation and Analysis*, ed. G. Britton, S. Liaaen-Jensen and H. Pfander, Birkhäuser, Basel, 1995, pp. 81–108.
54 L. C. Sander, K. E. Sharpless, N. E. Craft and S. A. Wise, *Anal. Chem.*, 1994, **66**, 1667–1674.