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The charge delocalised β,β-carotene dication—preparation, structure elucidation by NMR and reactions with nucleophiles† ‡

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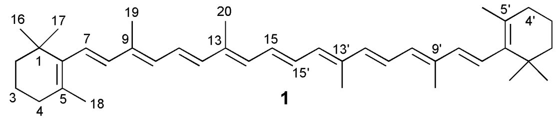
The reaction between β,β-carotene and BF_3-etherates has been investigated, leading to structural elucidation of the blue product, formed in appropriate organic solvents, as a symmetrical charge delocalised dication (λ_{max} 985 nm at room temperature in $CHCl_3$) with considerable stability. The reaction, monitored by EPR studies at −25 °C, occurred *via* free radical intermediates. A $C_{40}H_{56}BF_3$ intermediate was captured by EIMS. The detailed structure of the dication was established by COSY, HSQC, HMBC and 1D and 2D ROESY NMR techniques (600 MHz, $CDCl_3$, −20 °C) leading to complete assignments of ^1H and ^{13}C chemical shifts and $^3J_{H,H}$ coupling constants. The effects of the two delocalised charges on chemical shift (charge distribution) and bond distance ($^3J_{H,H}$) were considered. The results are consistent with charge delocalisation mainly in the C-5–C-9 and C-5′–C-9′ regions and with bond inversion to *retro* shifted double bonds in the central C-13–C-13′ region. A convention for denoting the charge delocalisation and bond types is presented. The experimental results are discussed relative to previous theoretical calculations of the β,β-carotene dication structure. (All-*E*) and (15-*Z*)-β,β-carotene provided the same dication. The NIR spectra and stability of dications prepared in the same manner from the related carotenes 20,20′-dinor-β,β-carotene, heptapreno-β,β-carotene and nonapreno-β,β-carotene were examined for comparison. Reactions of the β,β-carotene dication with selected nucleophiles provided products including isocryptoxanthin, isocarotene and mutatochrome with H_2O as nucleophile, and isocryptoxanthin methyl ether, 8-methoxy-7,8-dihydro-β,β-carotene and isocarotene with CH_3ONa as nucleophile. The formation of these products is rationalised from the structure assigned to the dication.

Introduction

Early work on reactions of carotenoids with strong acids or with Lewis acids, BF_3-etherates in particular, has been summarised by Zechmeister.[1] It was recognised early on that carotenoids form strong blue complexes with BF_3-etherates in appropriate organic solvents and that these complexes could be rapidly cleaved with water or alcohol. Treatment of the blue BF_3 complex of β,β-carotene (**1**) with water provided isocryptoxanthin (**2**, β,β-caroten-4-ol) and with base *retro*-dehydrocarotene (**3**, isocarotene; 4′,5′-didehydro-4,5′-*retro*-β,β-carotene). The reactions were formulated *via* resonance stabilised dication intermediates with covalently bound BF_3 groups of type **4**.[1]

We have carried out a modern reinvestigation of this reaction aiming at characterisation and structure elucidation of the blue BF_3 complex, and detailed analyses of the quenching reactions. The blue complex has been identified as a charge delocalised dication **5** of β,β-carotene (**1**). A priority note has been published.[2] Recently, we have also studied mono- and dications obtained from β,β-carotene (**1**) containing allylic hydroxy groups in the C-4 and C-4,4′ positions.[3] An up-to-date survey on charged carotenoid species, including their established and potential functions in biological systems, is available.[4]

Doping of carotenoids in the solid phase with iodine vapour is reported to give charge transfer complexes with good conductivity properties.[5–8] Iodine complexes of several carotenoids have been prepared.[9,10] For iodine complexes made in organic solutions, cationic, radical cationic and dicationic structures have been considered.[9,11–14]



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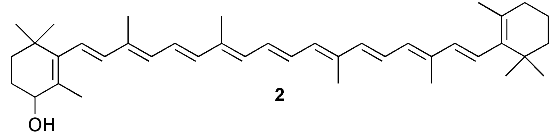

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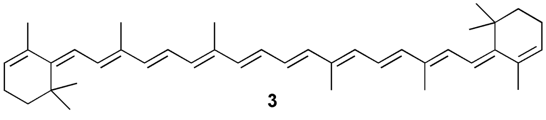

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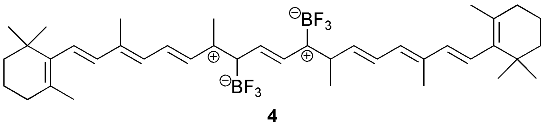

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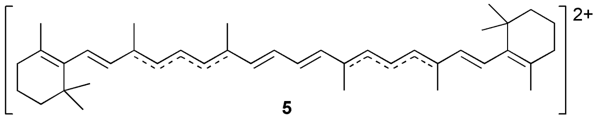

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The main methods employed for studies of charged carotenoid species include: NIR,[15–20] EPR,[21,22] ENDOR[23] and resonance Raman[15] spectroscopy, cyclic voltammetry[16–18] and AM1 calculations.[12,13,24,25]

This study is the first successful application of NMR spectroscopy for the detailed structural assignment of a delocalised carotenoid carbocation.

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† No. 3 in the series 'Charged carotenoid species'. No. 1 = ref. 2, No. 2 = ref. 3.

‡ Electronic supplementary information (ESI) available: 2D ROESY NMR spectrum of β,β-carotene dication (**5**) in $CDCl_3$ at −20 °C. See http://www.rsc.org/suppdata/ob/b3/b307531a/

DOI: 10.1039/b307531a

Table 1 NIR absorptions and estimated half lives for β,β-carotene dication (**5**) in various solvents at room temperature and at −20 °C.

	Room temperature		−20 °C	
Solvent	λ_{max}/nm	$t_{½}$/h	λ_{max}/nm	$t_{½}$/h
$CHCl_3$	960[a]	3.5	925	35[d]
CH_2Cl_2	920[b]	1[b]		
Benzene	840	4		
CCl_4	710	4		
Acetone	950[c]	1		

[a] λ_{min} at 1413 nm. [b] New λ_{max} 755 nm. [c] Much unreacted **1**. [d] Extrapolated.

Results and discussion

Preparation, characterisation and structure elucidation of β,β-carotene dication (5)

In the following section, evidence will be presented for the identification and structure elucidation of the blue product obtained from β,β-carotene (**1**) by treatment with BF_3-etherates[1] as the charge delocalised dication **5**.

The dication **5** was prepared by treatment of β,β-carotene (**1**) in organic solvents with BF_3 diethyl etherate (BF_3-dee), BF_3 dimethyl etherate (BF_3-dme) or BF_3 tetrahydrofuran etherate (BF_3-THF) added in different proportions at room temperature or −20 °C. The resulting NIR absorptions and estimated half-lives of **5** are presented in Table 1.

A hypsochromic shift of 35 nm was observed when lowering the temperature from room temperature to −20 °C in $CHCl_3$ solution. The highest specific absorption coefficients were observed for λ_{max} in $CHCl_3$ and CH_2Cl_2, estimated $E_{1\%,1\ cm}$ 2920 at 925 nm in $CHCl_3$ at −20 °C. This is a higher specific absorption coefficient than for β,β-carotene (**1**). The reported value for **1** in $CHCl_3$ at room temperature is $E_{1\%,1\ cm}$ 2396.[26] Chloroform was considered the most suitable solvent. A typical experiment demonstrating the formation of the dication **5** in $CHCl_3$ solution at room temperature using a 2 : 1 ratio between $CHCl_3$ and BF_3-dee is shown in Fig. 1.

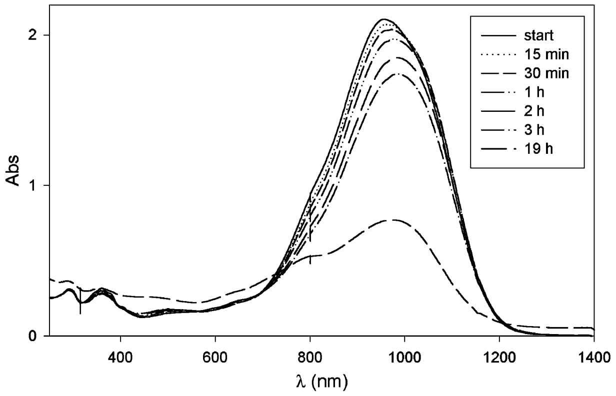


Fig. 1 UV-VIS-NIR spectrum of β,β-carotene dication (**5**), freshly prepared from β,β-carotene (**1**) and BF_3-dee in a 1 : 2 ratio between BF_3-dee and $CHCl_3$.

The remarkable stability of the dication **5** is demonstrated in Table 1 and Fig. 2.

The course of formation of the dication **5** by BF_3-dee treatment of β,β-carotene (**1**) was monitored by EPR, demonstrating free radical intermediates. The EPR spectrum at room temperature showed a weak signal with a linewidth of 14 G. At −25 °C the linewidth was 15–16 G and it increased further to 18 G when the temperature was lowered to 180 K, see Fig. 3.

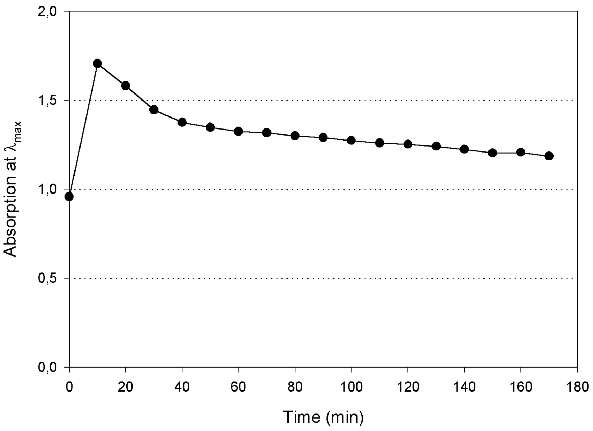


Fig. 2 Stability of β,β-carotene dication (**5**) in $CHCl_3$ at −20 °C monitored by absorption at λ_{max} ~920 nm.

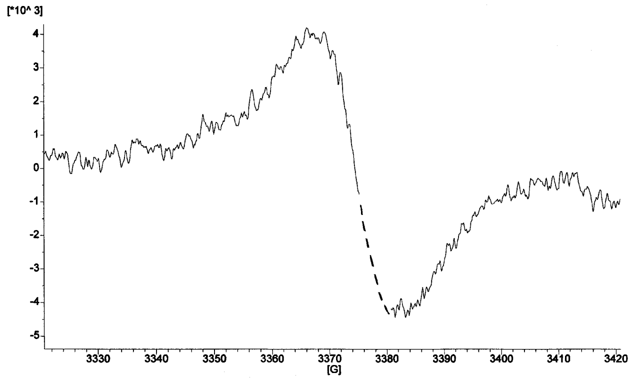
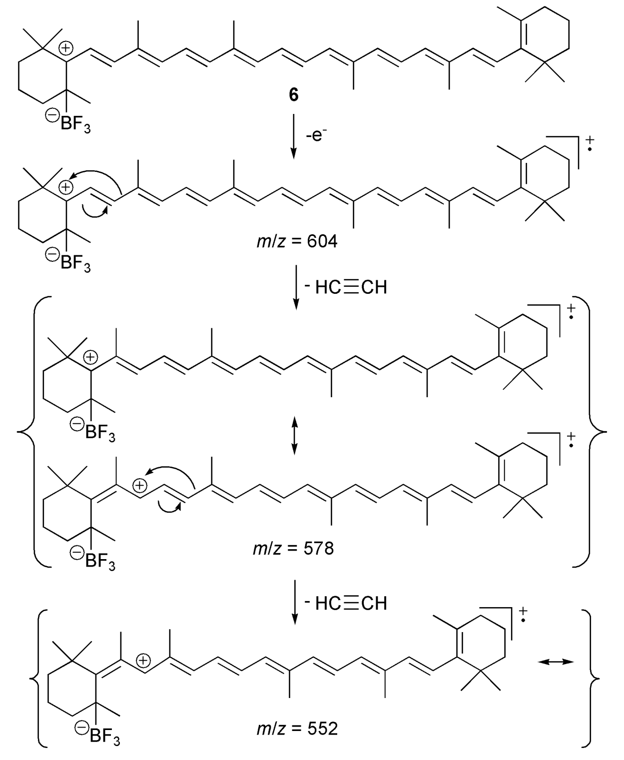


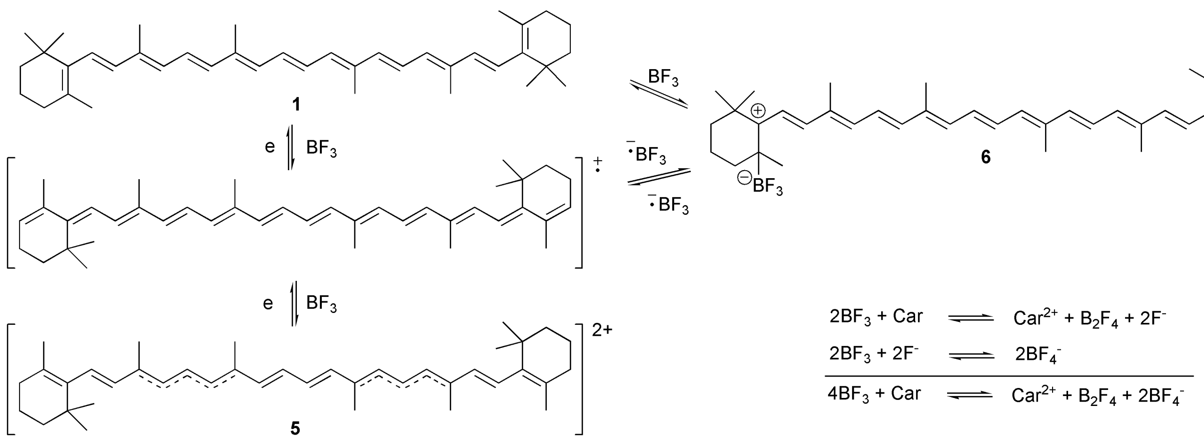
Fig. 3 EPR spectrum of β,β-carotene (**1**) in $CHCl_3$ recorded after addition of BF_3-etherate at −25 °C.

The observed linewidth is in agreement with published values for the delocalised β,β-carotene (**1**) radical cation.[14] Moreover, EIMS analyses of a freshly prepared reaction mixture of β,β-carotene (**1**) and BF_3-dee in $CHCl_3$ caught an ion consistent with the BF_3-adduct **6**, with fragment ions compatible with two consecutive losses of acetylene (26 mass units), rationalised in Scheme 1.

From the evidence obtained, it is concluded that the formation of the dication **5** proceeds by radical mechanism by two



Scheme 1



$$2BF_3 + Car \rightleftharpoons Car^{2+} + B_2F_4 + 2F^-$$
$$2BF_3 + 2F^- \rightleftharpoons 2BF_4^-$$
$$4BF_3 + Car \rightleftharpoons Car^{2+} + B_2F_4 + 2BF_4^-$$

Scheme 2

one-electron transfers from β,β-carotene (**1**) to BF_3-dee, as rationalised in Scheme 2. The structure of the negative counter-ion of the dication **5** as BF_4^- is tentatively formulated by analogy[27] and is not established.

The presence of radical species in this reaction was also supported by the observation that the NMR resonances were initially broadened when mixing β,β-carotene (**1**) and the BF_3-etherate before the spectrum of the dication **5** could be obtained.

^1H NMR studies of the dication **5** were performed in $CDCl_3$ at −25 °C as an optimum temperature, and with suppression of the methyl/ethyl signals of the etherate reagent. However, due to dominant signals from the etherate reagent, no ^1H–^{13}C connectivities could be observed. Therefore, the dication **5** was subsequently prepared using BF_3 complexed with $(CD_3)_2O$ as Lewis acid.

The ^1H NMR spectrum clearly demonstrated a symmetrical dication. The 2D ^1H–^1H COSY NMR spectrum of **5** is shown in Fig. 4.

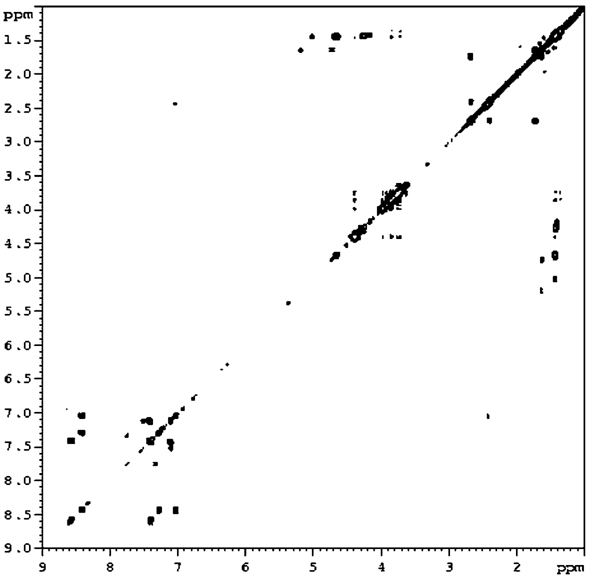


Fig. 4 ^1H–^1H COSY NMR (600 MHz) spectrum of β,β-carotene dication (**5**) at −20 °C in $CDCl_3$.

The ^1H and ^{13}C chemical shifts were established using homonuclear ^1H–^1H COSY and heteronuclear ^1H–^{13}C HSQC and HMBC techniques, see assignments for the dication **5** in Scheme 3. The through-space interactions in the dication **5** shown in Scheme 3, demonstrate the reorientation of the rings relative to the polyene chain in **5** *versus* β,β-carotene (**1**).

The differences in ^1H chemical shifts and particularly in ^{13}C chemical shifts for β,β-carotene dication (**5**) relative to β,β-carotene (**1**) were used for identifying the charge distribution in the dication **5**. ^1H NMR chemical shifts and ^{13}C chemical shifts for β,β-carotene (**1**) are given on the half-structures in Scheme 3, as well as the differences in ^1H chemical shifts and in ^{13}C chemical shifts. The bond order is not considered at this stage of the structure elucidation. The total ^{13}C chemical shift difference of the dication **5** relative to **1** was 504 ppm. This is consistent with the formation of a dication.[28] Moreover, the total ^1H chemical shift difference of 35.82 ppm, counting methylene protons twice and methyl protons three times, was compatible with the expected value per positive charge[29] for the formation of a polyene dication. The reported[2] value of 24.24 ppm is hereby corrected.

As shown in Scheme 3, the downfield shift for the dication **5** is most pronounced for the carbon atoms C-5, C-7 and C-9. The downfield ^{13}C shifts increased towards the end of the polyene chain with a linear trend for the odd-numbered carbon atoms, as shown in Fig. 5. Deviations for C-7/7′ and C-9/9′ may be due to secondary (C-7/7′) *versus* tertiary (C-9/9′) carbon, where the tertiary carbon is expected to stabilise the positive charge better. The even-numbered carbons are carrying less positive charge, as seen from the smaller downfield shift differences, Fig. 5. The shift differences of these carbons show a general increase towards the centre of the molecule.

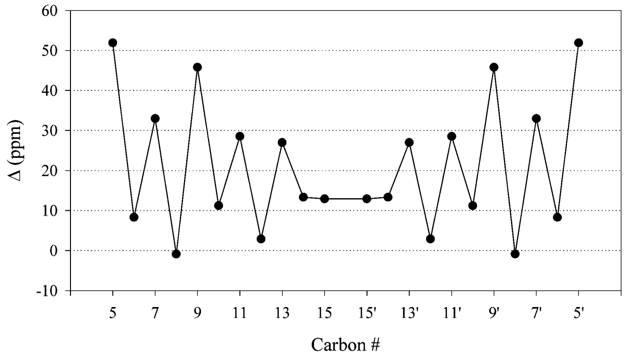
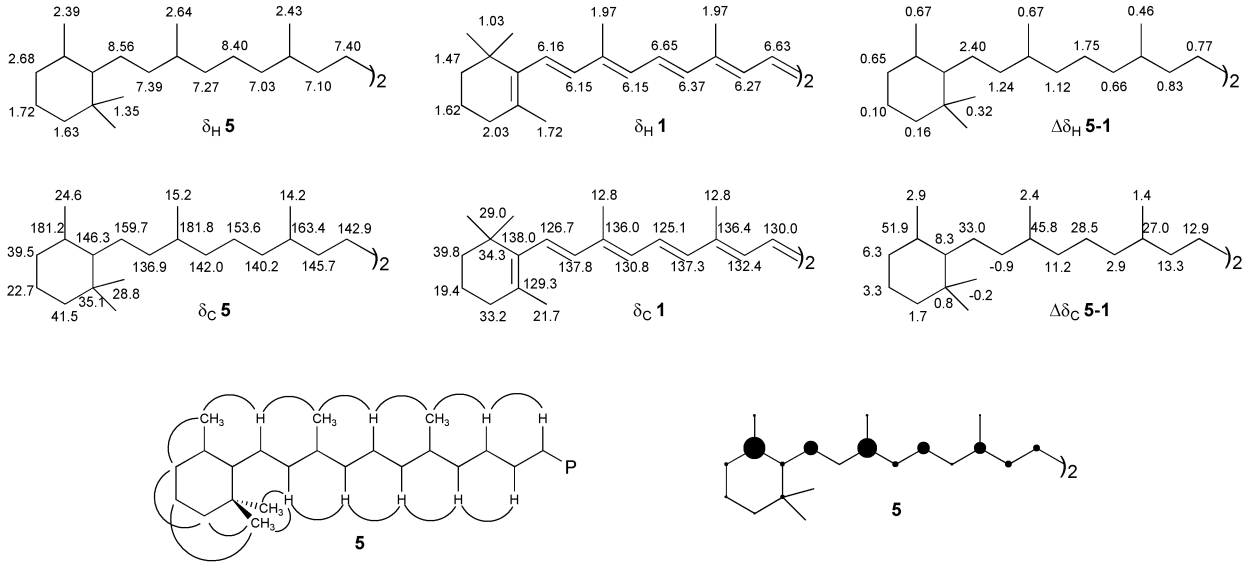


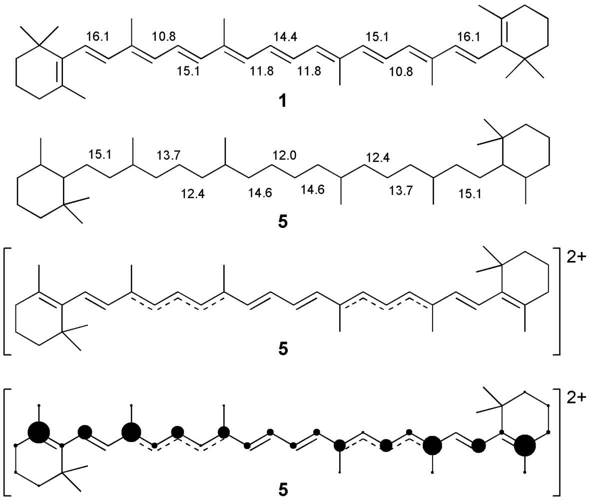
Fig. 5 ^{13}C NMR downfield shift difference for carbons in the polyene chain for β,β-carotene dication (**5**) relative to β,β-carotene (**1**).

As shown in Scheme 3, the size of the downfield ^1H NMR shift of the dication **5** relative to **1** is largest at H-7/7′, even when larger steric hindrance between H-7/7′ and Me-18/18′ in **5** than between H-7/7′ and the geminal dimethyl groups in **1** is taken into consideration. Indeed, a similar effect is noted for C-7/7′ in the ^{13}C NMR. These are the carbons showing the largest downfield chemical shifts of all proton carrying carbon atoms. Steric effects may also explain the small upfield shifts of C-8/8′, see Scheme 3, because C-8/8′ will experience less steric hindrance in the dication **5** than in neutral β,β-carotene (**1**).



Scheme 3

In conclusion, the chemical shift data support a symmetrical delocalised dication with the charge preferentially in the C-5–C-9 and C-5′–C-9′ regions, as illustrated at the bottom of Scheme 4. Larger filled circles indicate higher charge density.



Scheme 4

The size of the coupling constants established for β,β-carotene dication (**5**) was used to define the bond character. Previous generalisations for neutral carotenoids have defined the region for the coupling constants of *trans* double bonds as 13.5–16.8 Hz with a tendency to decrease towards the central part of the polyene chain. The coupling constants across single bonds (s-*trans*) are lower, usually 10.5–12.0 Hz, with the larger values near the central part of the conjugated chain.[30] However, these generalisations do not include *retro*-carotenoids,[30,31] where the positions of the double bonds are shifted, as in isocarotene (**3**). For *cis* double bonds the range 11.5–12.8 Hz has been concluded.[30]

All $^3J_{H,H}$ coupling constants of the polyene chain were determined from the ^{1}H NMR spectrum for the dication **5**. However, while the two-spin system (H-7–H-8) and the three-spin system (H-10–H-11–H-12) gave first order spectra, so that the coupling constants could be determined directly from the ^{1}H NMR spectrum, the coupling constants of the central four-spin system were detemined by spectrum simulation using WinDaisy software. The experimental and simulated spectra of the H-14/14′ multiplet are shown in Fig. 6.

In Scheme 4 the coupling constants of β,β-carotene (**1**) and its dication **5** are compared. It follows that the single bond coupling constants ($J_{14,15}$ = 11.8 Hz[32] for **1** and $J_{15,15'}$ = 12.0 Hz for **5**) and the double bond coupling constants ($J_{15,15'}$ = 14.4 Hz[32] for **1** and $J_{14,15}$ = 14.6 Hz for **5**) in the central region are of the same size, albeit with bond reversal. This is compatible with localised *retro* double/single bonds in the C-13–C-13′ region in **5**, Scheme 4. The C-10,11 ($J_{10,11}$= 13.7 Hz) and C-11,12 ($J_{11,12}$ = 12.4 Hz) bonds have similar bond character, compatible with bond reversal in this region (dotted bonds). Expected coupling constants of β,β-carotene (**1**) are $J_{10,11}$= 10.8 Hz and $J_{11,12}$ = 15.1 Hz.[32]

Considering the ends of the polyene chain, the coupling constant $J_{7,8}$ = 15.1 Hz, although smaller than in β,β-carotene (**1**) ($J_{7,8}$ = 16.1 Hz),[32] is compatible with a *trans* 7,8-double bond. It is inferred that the C-5,6 bond must also have a high degree of double bond character, thereby forcing the C-4–C-5–C-6–C-1 structural element of the end groups into a planar arrangement, compatible with ^{1}H NMR spectra of the end groups. A planar polyene is also a prerequisite for maximum electron delocalisation.

In the ^{1}H–^{1}H COSY spectrum, long-range couplings can be seen from in-chain methyl groups to protons *trans* to the methyl group (H-19 to H-10 and from H-20 to H-14), but not to protons in an s-*trans* position (H-19 to H-8 and H-20 to H-12) for β,β-carotene (**1**), as shown in Fig. 7. The long-range couplings are indicated by solid bonds. For the dication **5**, these long-range couplings can be seen from H-20 to H-12 and from H-19 to H-8 (strongest) and H-10, supporting the rearrangements of the double bonds.

The total NMR evidence is consequently best accomodated with structure **5** for the β,β-carotene dication, Scheme 4, where dotted lines indicate intermediate bond type. Including the symbols used in Scheme 3, indicating the charge distribution, the bottom structure **5** in Scheme 4, is more informative.

Using more conventional structures, the β,β-carotene dication (**5**) may alternatively be represented as a resonance hybrid with main contributing structures carrying the positive charge alternatively at the C-5, C-7, C-9, C-9′, C-7′ and C-5′ positions, Scheme 5, compatible with charge repulsion.

Austin Model 1 (AM1) theoretical calculations have previously been used for obtaining information on the structure of the dication of β,β-carotene.[12,13,25] In structure **7**, the dication was depicted as a pair of charged solitons.[12,13] Information gained on bond lengths, charge distribution and orbital energies resulted in structure **8** for the dication.[25] The doubly charged cation posessed two spinless charged symmetry equivalent

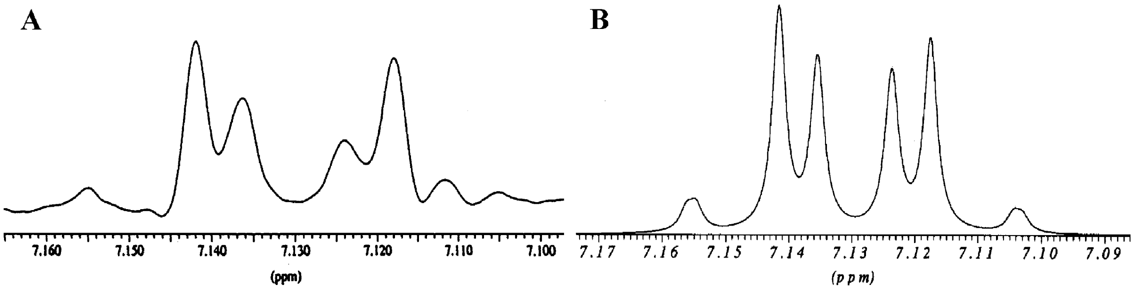


Fig. 6 Experimental (A) and simulated (B) multiplet for H-14/14′.

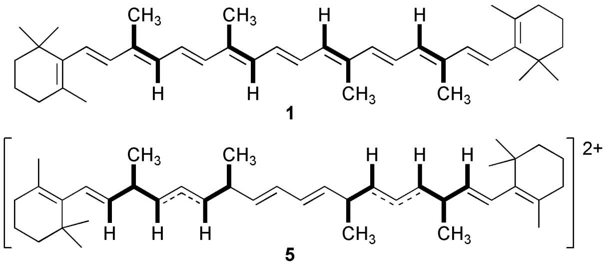
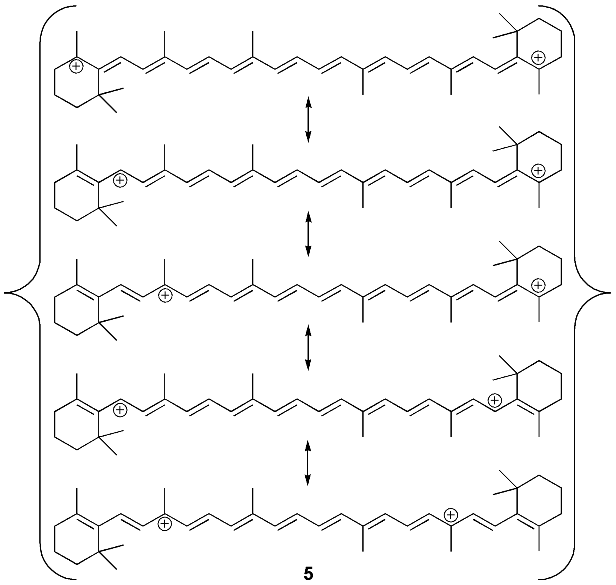


Fig. 7 Long-range couplings from in-chain methyl groups in the ^1H–^1H COSY spectrum.



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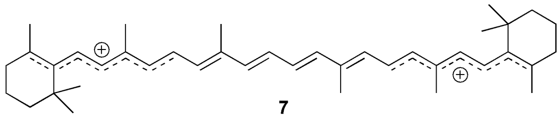
Scheme 5

areas, which developed more or less ideal bond equalisations. However, in the central part of the molecule, strong bond alternations were assigned, and a reversal of double and single bonds relative to β,β-carotene (**1**).[25] It should be pointed out that both β,β-carotene (**1**) and **7** were depicted with rotated end groups,[12,13] but rotation of the C-6,7 and C-6′,7′ bonds was not treated in these calculations.

It is interesting to note that the dication structure **5**, here elucidated by NMR, incorporates elements predicted by theoretical calculations including a pair of charged solitons (**7**, **8**) and reversal of double and single bonds in the central part of the molecule (**7**, **8**).



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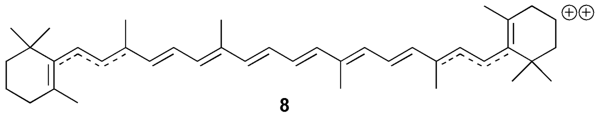

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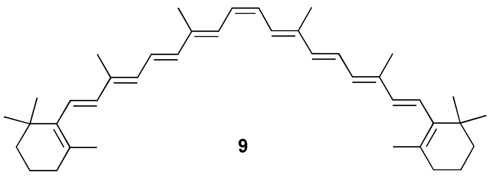
The effect of ring rotation on spin density and bond lengths in the polyene chain has been studied by application of the density functional method B3LYP for the β,β-carotene cation radical.[33] Rotated C-6,7 and C-6′,7′ single bonds, as calculated for β,β-carotene cation radical,[33] was established here for β,β-carotene dication (**5**) by NMR data.

Application of multireference Møller–Plesset theory confirmed the general tendency observed for carotenoids, that the first transition of the dications of polyenes are at a higher energy (shorter λ_{max}) than the intense transition of the radical cation and at a lower energy (longer λ_{max}) than the first allowed transition of the neutral species.[34] However, concerning λ_{max} of β,β-carotene dication (**5**), given in Table 1, it should be pointed out that the λ_{max} recorded here are at considerably longer wavelengths than the previously published λ_{max}, 817 nm in CH_2Cl_2 at room temperature.[35] Since the cation radical and dication of β,β-carotene (**1**) in the latter investigation were produced by the action of $AlCl_3$ in CH_2Cl_2, it appears that the effect of the negatively charged counter ion on the NIR spectrum needs to be considered.

Comparative study with dications prepared from other model carotenes

Four synthetic model carotenes, (15*Z*)-β,β-carotene (**9**),[36] 20,20′-dinor-β,β-carotene (**10**),[37] heptapreno-β,β-carotene (**11**)[38] and nonapreno-β,β-carotene (**12**)[38] were available.



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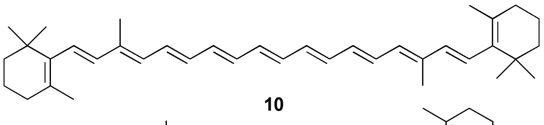

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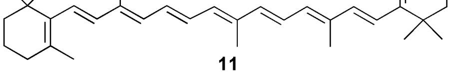

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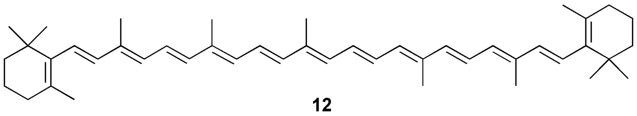

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(15*Z*)-β,β-carotene (**9**) provided the same β,β-carotene dication (**5**) as β,β-carotene (**1**), consistent with expected isomerisation of cationic intermediates to the most stable all-*trans* dication configuration (**5**).

It was expected that the lateral C-19, 20, 19′, 20′ methyl groups with positive inductive effect serve to stabilise the dication **5** containing positive charge at adjacent carbons (C-9,13,9′,13′). However, in a comparative study in CH_2Cl_2 at

-15 °C, the stability of the dication of the derivative **10** was the same as for the dication **5**.

The dications of the C_{35} model (**11**), with 9 conjugated double bonds, and the C_{45} model (**12**), with 13 conjugated double bonds, were of interest concerning λ_{max} and stability. As expected, the dications of the nonaene **11** absorbed at shorter wavelengths (793, 880 nm) and that of the tridecaene **12** at longer wavelengths (956, 1050 nm) than that of the undecaene **1** (**5**), which absorbed at 928 nm in CH_2Cl_2 at -15 °C. Thus, the dication of the tridecaene **12**, not investigated by NMR, is the carotenoid dication studied so far absorbing at the longest wavelength. The stability of the unsymmetrical dications of **11** and **12**, monitored by NIR spectroscopy in CH_2Cl_2 at -15 °C, appeared to be higher than for **5**.

Reactions of β,β-carotene dication (5) with nucleophiles

In principle, structural information may be obtained from reactions of a carotenoid cation with suitable nucleophiles. β,β-Carotene dication (**5**) has been reacted with i) water as nucleophile in acetone at room temperature and ii) CH_3ONa in methanol at -20 °C.

The dication **5** in $CHCl_3$ was treated with water in acetone, and the reaction mixture analysed by TLC and HPLC. The product mixture contained β,β-carotene (**1**, *ca.* 5% of total recovered), isocryptoxanthin (**2**, 51%) and isocarotene (**3**, 20%). Isocryptoxanthin (**2**) was identified by HPLC/VIS data, including co-chromatography with an authentic sample prepared by $LiAlH_4$ reduction of echinenone (β,β-caroten-4-one), MS and ^1H NMR data, as well as allylic dehydration to isocarotene (**3**) with acidified chloroform.[39,40] Isocarotene (**3**) was identified by HPLC/VIS data and mutatochrome (**13**) by VIS and characteristic MS fragmentation including $[M-80]^+$, homopyrylium (m/z = 205) and pyrylium (m/z = 165) ions.[41] A suitable HPLC system[42] revealed the high degree of *cis*-isomerisation of products **2** (di-*cis* : mono-*cis* : all-*trans* : mono-*cis ca.* 2 : 2 : 6 : 1) and **3**, compatible with cationic intermediates. The formation of products **2**, **3** and **13** by nucleophilic attack on the dication **5** by H_2O (**2**, **13**) or by elimination (**3**) is rationalised in Scheme 6.

Treatment of β,β-carotene dication (**5**) with 5% CH_3ONa in methanol at -20 °C provided isocryptoxanthin methyl ether (**14**, 4-methoxy-β,β-carotene, 16–23%), isocarotene (**3**, 16–18%) and 8-methoxy-7,8-dihydro-β,β-carotene (**15**, 2–23%). Isocryptoxanthin methyl ether (**14**) was identified by HLPC/VIS. EIMS data and an HPLC profile of *cis*-isomerised **3** will be presented elsewhere. The structure elucidation of the 8-methoxy-derivative **15** with monocyclic octaene chromophore rested on HPLC/VIS, EIMS and ^1H NMR including ^1H–^1H COSY. Location of the methoxy group at C-8 rather than C-9 was compatible with ^1H NMR data revealing an ABX spin system with a methine proton at 3.60 ppm, and with the MS fragmentation providing a strong fragment ion at m/z 431 compatible with cleavage of the C-7,8 single bond. Allylic elimination with HCl in $CHCl_3$ of **15** provided β,β-carotene (**1**) and confirmed the assigned structure.

As for the reaction of **5** above quenched with water, considerable *cis*/*trans* isomerisation was also noted for the products in the present reaction. Thus, product isocarotene (**3**) was resolved by HPLC into several stereoisomers in a 39 : (20+2+6+2 = 20) all-*trans* : *cis* ratio unsuitable for ^1H NMR analysis, and isocryptoxanthin methyl ether (**14**) was resolved into three stereoisomers in a 1.0 : 0.63 : 0.30 ratio. The results are consistent with cationic intermediates.

Whereas the formation of products **14** and **3** are readily rationalised on the basis of structure **5** for the dication (Scheme 6), the formation of the monocyclic octaene methoxy derivative **15** requires the addition of a hydrogen, formulated in Scheme 6 as addition of a hydrogen radical to the radical cation from an unidentified donor. A minor product (< 2% of total), encountered upon treatment of the dication **5** with H_2O in acetone, corresponded, according to MS fragmentation reactions, to the allylic 8-ol **16**, analogous to the 8-methoxy derivative **15** above.

Concerning the product distribution for i) water as a nucleophile at room temperature *versus* ii) methoxide as a nucleophile at -20 °C: in the latter reaction much more β,β-carotene (**1**) was recovered (*ca.* 45%), oxygenated substitution products dominated under the former conditions (*ca.* 70% *versus ca.* 20% of total recovered) and the elimination product isocarotene (**3**) constituted around 20% of recovered carotenoids in both cases. Since β,β-carotene (**1**) was quantitatively converted to the dication **5** according to VIS/NIR and NMR data, the recovered, strongly isomerised β,β-carotene (**1**) in these reactions must have been formed by electron transfer to **5** from the counter ion.

In conclusion, the products obtained from β,β-carotene dication (**5**) by reaction with selected nucleophiles were in good agreement with the structure determined for **5** by NMR spectroscopy.

Experimental

General methods

Chemical manipulations were carried out in darkness, as far as possible, and under nitrogen or argon atmosphere. Visible light (VIS) and near infrared (NIR) spectra were recorded on a Varian Cary 5 UV-VIS-NIR spectrophotometer (220–1500 nm) or a Varian Cary 50 UV-VIS spectrophotometer (190–1100 nm). EI mass spectra were recorded on a Finnigan MAT 95XL ThermoQuest spectrometer with a direct inlet to the ion source, 70 eV, ion source 250 °C. Diagnostically useful ions only are cited. EPR spectra were obtained at room temperature on a Bruker ESP 300E instrument, rectangular cavity, flat cell sample holder at 248 K and 180 K on a Bruker EMX instrument using an HS resonator/probe and an ER4131VT variable temperature unit with liquid nitrogen for cooling.

^1H NMR spectra of neutral carotenoids were recorded on a Bruker Avance DPX 400 instrument, using a 5 mm QNP probe. NMR spectra of charged carotenoids were obtained 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 (7.37 ppm and 78.8 ppm in solutions with BF_3) for ^1H and ^{13}C respectively.

HPLC was carried out on a Hewlett Packard instrument series 1050 equipped with a diode array detector. Detection wavelengths were set at 335, 420, 450 and 480 nm. VIS spectra of the carotenoid components were recorded on-line during chromatography using two different HPLC systems:

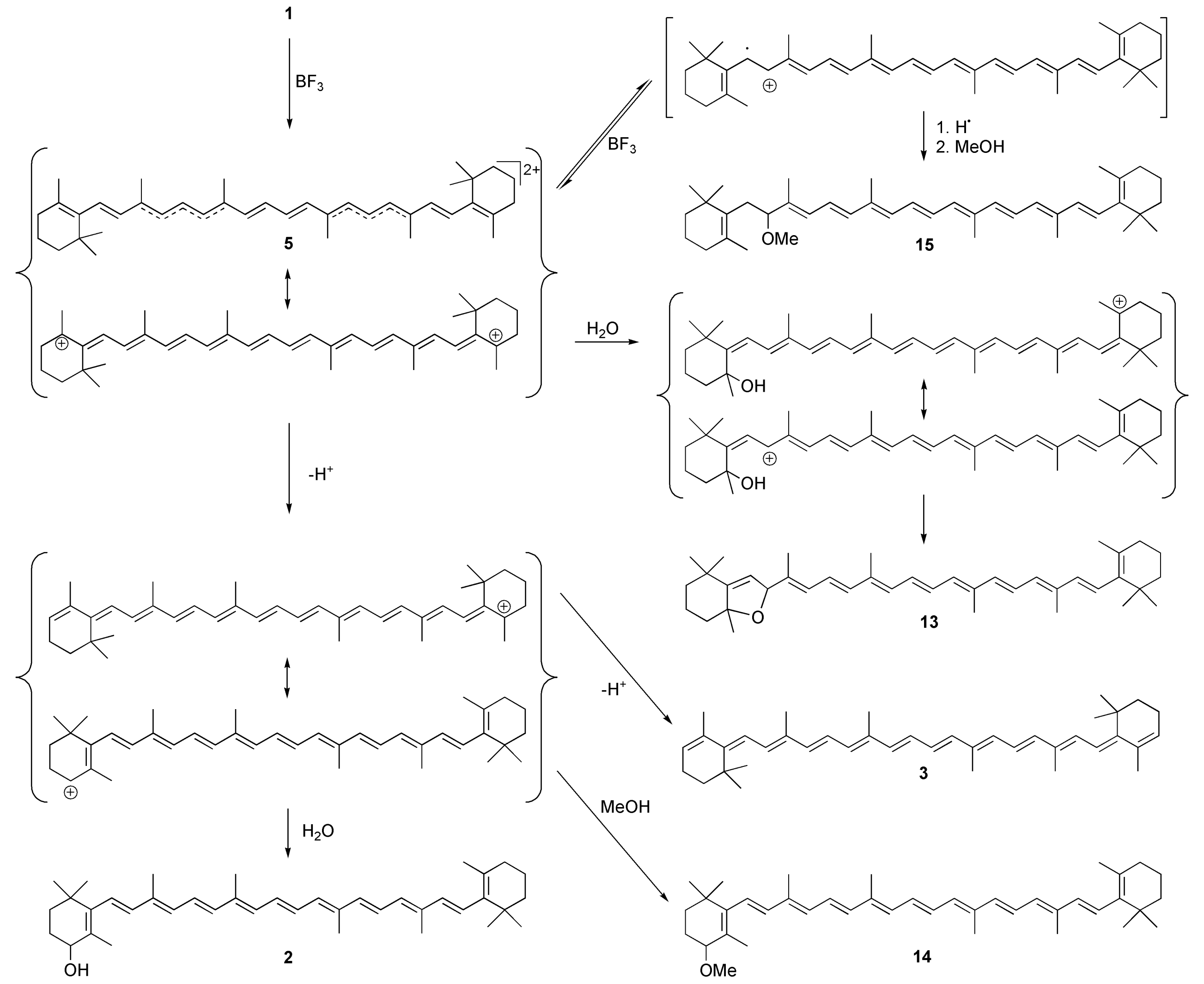
System 1,[42] Waters YMC Carotenoid C30 column, 250 × 4.6 mm. Mobile phase 0 min: methanol : *t*-butyl methyl ether : water (81 : 15 : 4 v/v/v, 1.0 ml min^{-1}), 60 min: methanol : *t*-butyl methyl ether : water (31 : 65 : 4 v/v/v, 1.0 ml min^{-1}), 70 min: methanol : *t*-butyl methyl ether : water (16 : 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**) and isocarotene (**3**).

System 2, Interchrom Uptisphere 50DB column, 250 × 4.6 mm. Mobile phase 0 min: methanol : acetone (90 : 10 v/v, 1.0 ml min^{-1}), 90 min: methanol : acetone (0 : 100 v/v, 1.0 ml min^{-1}). This reversed phase system gives less resolution of *cis*/*trans* isomers of carotenes.

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

Reactions of β,β-carotene (1) with BF_3-etherates

β,β-carotene (1). Synthetic **1** from Hoffmann-La Roche was used. HPLC (System 1) 91% all-*trans* (R_T = 37.8 min), 3% 13-*cis* (32.6), 0.2% 9-*cis* (40.0), other *cis* isomers *ca.* 6%; λ_{max}(heptane)/nm 424sh, 446 ($E_{1\%,1cm}$ 2390), 476; λ_{max}($CHCl_3$)/nm 430sh, 460, 486; δ_H(600 MHz, $CDCl_3$; Me_4Si) see Scheme 3;



Scheme 6

δ_C(150 MHz, CDCl$_3$, Me$_4$Si) see Scheme 3; *m*/*z* (EI) 538 (16%, M + 2), 537 (43, M + 1), 536 (M$^+$, 100), 444 (26, M − 92), 430 (2, M − 106).

VIS/NIR experiments at room temperature. β,β-carotene (**1**, 1 mg) was dissolved in the solvent (1 ml) and BF$_3$-dee (2 ml) added. An aliquot was diluted to a concentration suitable for VIS/NIR analysis. The reaction was monitored by VIS/NIR spectroscopy; Fig. 1, Table 1.

VIS/NIR experiments at −20 °C. β,β-carotene (**1**, 2.3 mg) was dissolved in CDCl$_3$ (1 ml) and BF$_3$-dee (1 ml) added at −35 °C. An aliquot (15 µl) was transferred to a cuvette containing CDCl$_3$ (3 ml) at −20 °C. The VIS/NIR spectrum was recorded at 10 min intervals (−20 °C), Table 1, with the cuvette placed in a cuvette holder cooled by cold methanol (−20 °C) from a cryostat. Mist developing on the cuvette caused some baseline problems.

EPR analysis at room temperature

To β,β-carotene (**1**, a few mg) dissolved in CHCl$_3$ was added the same volume of BF$_3$-dee. The reaction was monitored by EPR spectroscopy. The observed linewidth was 14 G (1.4 mT). After 1 h the original signal was reduced to 65%.

EPR analysis at −25 °C and −93 °C

To a 9.3 mM solution of β,β-carotene (**1**) in CHCl$_3$ was added the same volume of BF$_3$-dee. The EPR line width was 15–16 G at −25 °C and 18 G at −93 °C, Fig. 3.

EIMS analysis

β,β-carotene (**1**, 1 mg) was dissolved in CHCl$_3$ (1 ml) and BF$_3$-dee (1 ml) added. An aliquot was withdrawn and the solvent blown off with N$_2$ upon addition of xylene. EIMS was recorded 25 min after the reactants were mixed.

m/*z* (EI) 604 (M$^+$, 27%), 578 (29, M − C$_2$H$_2$), 552 (9, M − C$_2$H$_2$–C$_2$H$_2$), 450 (6), 394 (11), 339 (19), 313 (7), 262 (35), 243 (12), 223 (11), 165 (15), 119 (17), 109 (21), 95 (36), 83 (41), 55 (59), 28 (100).

NMR analysis at −20 °C

BF$_3$-dme was distilled at 126–128 °C and CDCl$_3$ dried over a MgSO$_4$ column prior to the experiment.

The dication was generated under argon atmosphere. β,β-carotene (**1**, *ca.* 3 mg) or (15*Z*)-β,β-carotene (**13**, *ca.* 3 mg) was dissolved in CDCl$_3$ (0.3 ml) and cooled (dry ice–isopropanol). Chilled BF$_3$-etherate (0.3 ml) was added and the mixture transferred to a chilled NMR tube and analysed by 600 MHz NMR experiments at −20 °C. In freshly prepared reaction mixtures, the NMR resonances were broadened, and the best NMR spectra was therefore obtained after some minutes, but before decomposition. In a separate experiment the results from NMR and VIS/NIR were correlated by checking the VIS/NIR spectra (λ_{max} 925 nm) prior to, and after, an NMR experiment.

1D ^{1}H NMR spectra were obtained with a relaxation delay of 1 s. The gs-COSY[43] spectra were recorded in magnitude mode using a 90° read pulse. Phase-sensitive 2D ROESY spectra were obtained by the States-TPPI method.[44] All

ROESY experients were performed with a mixing time of 450 ms. Presaturation was used to reduce the intensity of the methyl signal from the BF_3-dme reagent in the ^1H, COSY and ROESY spectra.

gs-HSQC spectra[45] were recorded in the phase-sensitive mode with echo/anti-echo acquisition. Different windows were used for the olefinic and aliphatic regions, optimised for a $^1J_{C,H}$ coupling of 160 and 140 Hz, respectively. gs-HMBC spectra[46] were recorded in the phase-sensitive mode using States-TPPI. The experiment was optimised for a $^nJ_{C,H}$ coupling constant of 11 Hz.

β,β-Carotene dication (5)

Prepared from **1** or **13** with BF_3–OMe_2, BF_3–OEt_2 or BF_3–THF as Lewis acids; λ_{max} see Table 1, Fig. 1; δ_H (600 MHz, $CDCl_3$, Me_4Si, −20 °C) see Scheme 3 and Scheme 4. Long-range couplings from the in-chain methyl groups are shown in Fig. 7; δ_C (150 MHz, $CDCl_3$, Me_4Si, −20 °C) see Scheme 3.

Preparation of deuterated BF_3-etherates

Deuterated BF_3-etherates necessary for determination of ^{13}C chemical shifts were prepared as follows:

BF_3–OMe_2-d_6. Deuterated BF_3-dme was prepared by reacting BF_3 (g) with $(CD_3)_2O$ (g), giving BF_3–$O(CD_3)_2$ as a condensate. Purification by distillation gave the product as a colourless liquid, bp 126–128 °C.

BF_3–THF-d_8. THF-d_8 and BF_3-dee were mixed in equimolar quantities. Diethyl ether was distilled from the mixture at 36–38 °C. After pressure reduction, deuterated BF_3–THF etherate was collected at 80–82 °C as a colourless liquid.

Preparation and properties of other dications

Dications of β,β-carotene (**1**), 20,20′-dinor-β,β-carotene (**14**), heptapreno-β,β-carotene (**15**) and nonapreno-β,β-carotene (**16**) were prepared at the 1–2 mg scale at −15 °C in CH_2Cl_2 with BF_3-dee as described for β,β-carotene (**1**) at −20 °C. VIS/NIR spectra were recorded every 5 min. at −15 °C for 2 h. Complete conversion of the substrate occurred immediately to products with λ_{max} of **1**: 928 nm, **14**: 931 nm, **15**: 793 (880) nm and **16**: 956 (1050) nm. After 2 h, the absorption at λ_{max} for the corresponding dications had dropped 10% for nonapreno-β,β-carotene (**16**), 16% for 20,20′-dinor-β,β-carotene (**14**), 22% for heptapreno-β,β-carotene (**15**) and 23% for β,β-carotene (**1**).

Reactions of β,β-carotene dication (5) with nucleophiles

Water in acetone as nucleophile. Procedure adapted from ref. 47. β,β-Carotene (**1**, 3.2 mg) was dissolved in $CHCl_3$ (3 ml) and BF_3-dee (1 ml) was added at room temperature. The reaction mixture, which immediately turned black, was flushed with N_2, shaken for 2 min and poured into 20% H_2O in acetone (40 ml). A colour change to yellow-orange occurred. Hexane (6 ml) was added. The organic phase was washed with water and analysed. Pigment recovery 40% ($E_{1\%,1cm}$ = 2500), λ_{max}(hexane)/nm 443; HPLC (System 1): R_T 19–49 min. Products were isolated by preparative TLC developed with 5% acetone in hexane and eluted with acetone to give:

β,β-carotene (1) (5%). R_F = 0.97; R_T = 37.8 min (λ_{max}/nm 430, 452, 480).

Isocryptoxanthin (2, β,β-caroten-4-ol) (51%). R_F = 0.12–0.23; λ_{max}(acetone)/nm 428, 451, 484; R_T = 19.7 (18%, λ_{max}/nm 420sh, 444, 473; $\%D_B/D_{II}$[26,48] = 40; di-*cis*), R_T = 21.5 (18% λ_{max}/nm 420sh, 447, 477; $\%D_B/D_{II}$ = 30; mono-*cis* A), R_T = 23.4 (55%, λ_{max}/nm 430sh, 452, 480; $\%D_B/D_{II}$ = 0; all-*trans*), R_T = 26.1 (9%, λ_{max}/nm 428sh, 452, 480; $\%D_B/D_{II}$ = 10; mono-*cis* U); δ_H(400 MHz, $CDCl_3$, Me_4Si) 1.03 (12H, 16/17/16′/17′-H), 1.72 (3H, 18′-H), 1.83 (3H, 18-H), 2.00 (12H, 19/20/19′/10′-H), 4.02 (1H, 4-H), 6.16 (4H, 7,8,7′,8′-H). *m*/*z* (EI) 552 (M^+, 13%), 550 (16, M − 2), 534 (100, M − H_2O), 442 (23, M − H_2O − 92), 428 (4, M − H_2O − 106).

An aliquot of product **2** was tested for allylic hydroxyl by treatment with 0.03 M HCl–$CHCl_3$[39] providing *cis*/*trans* isomerised isocarotene (**3**), which by HPLC (System 1) showed: R_T = 46.4 (22%, λ_{max}/nm 435sh, 465, 495); R_T = 49.5 (12%, λ_{max}/nm 440sh, 465, 495); R_T = 50.2 (13%, λ_{max}/nm 445sh, 470, 500); R_T = 54.8 (35%, λ_{max}/nm 440sh, 470, 500); R_T = 59.8 (17%, λ_{max}/nm 445sh, 470, 505).

For direct comparison of product **2** with an authentic sample synthetic echinenone (β,β-caroten-4-one) was reduced with $LiAlH_4$ in dry diethyl ether by standard procedure,[39,40] providing all-*trans* isocryptoxanthin (**2**), R_T = 23.4 (System 1).

Isocarotene (3, 4′,5′-didehydro-4,5′-retro-β,β-carotene) (20%). R_F = 0.9; For R_T see under **2** and below.

Mutatochrome (13, 5,8-epoxy-5,8-dihydro-β,β-carotene) (20%). R_F = 0.74–0.81; R_T = 20.2 (50%, λ_{max}/nm 405, 430, 455); R_T = 28.5 (50%, λ_{max}/nm 410sh, 430, 455), tentatively identified as two C-8 epimers; *m*/*z* (EI) 552 (M^+, 71%), 550 (16, M − 2), 536 (14, M − 16), 472 (73, M − 80), 205 (100, homopyrylium), 165 (54, pyrylium) with characteristic fragmentation pattern.[41]

7,8-Dihydro-β,β-caroten-8-ol (16) (2%). R_F = 0.33; R_T = 20.2 (λ_{max}/nm 405sh, 430, 455); *m*/*z* (EI) 554 (M^+, 95%), 536 (98, M − H_2O), 462 (15, M − 92), 444 (M − H_2O − 92), 417 (22, M − 137), 119 (100).

CH_3ONa in CH_3OH as nucleophile

To β,β-carotene (**1**, 5.5 mg) in $CDCl_3$ (2 ml) was added BF_3-dee (2 ml) at −20 °C. The solution immediately turned black. A 5% solution of CH_3ONa in CH_3OH (7 ml) was added after 5 min. The colour of the reaction mixture changed to yellow. The pigments were transferred to $CHCl_3$ and the organic phase washed with water to give a pigment recovery of 49–62%. The composition of the reaction mixture was analysed by HPLC (System 2) and TLC (2% acetone in hexane). The results from two experiments are given:

β,β-carotene (1) (53–39%). R_F = 0.56–0.60, R_T = 35.8–36.6, *cis*-isomerised.

Isocarotene (3) (16–18%). R_F = 0.51–0.56; R_T = 33.7 (7%, λ_{max}/nm 441, 460, 485; *cis* B), R_T = 34.5 (50%, λ_{max}/nm 442, 465, 495; *cis* A), R_T = 35.1 (43%, λ_{max}/nm 447, 473, 501; all-*trans*).

Isocryptoxanthin methyl ether (14, 4-methoxy-β,β-carotene) (16–23%). R_F = 0.35–0.41; R_T = 27.9 (33%; λ_{max}/nm 425sh, 449, 473; *cis* A), R_T = 28.2 (52%; λ_{max}/nm 429sh, 451, 478; all-*trans*), R_T = 28.5 (16%; λ_{max}/nm 423sh, 447, 473; *cis* U); δ_H(400 MHz, $CDCl_3$, Me_4Si) 1.03–1.07 (12H, 16/17/16′/17′-H), 1.38 (1H, 2_a-H), 1.48 (2H, 2′-H), 1.63 (2H, 3′-H), 1.67 (1H, 2_b-H), 1.73 (3H, 18′-H), 1.74 (1H, 3_a-H), 1.81 (3H, 18-H), 1.82 (1H, 3_b-H), 1.98–2.00 (12H, 19/20/19′/20′-H) 2.03 (2H, 4′-H), 3.40 (3H, 4-OMe), 3.52 (1H, 4-H), 6.12–6.18 (6H, 7/8/10/7′/8′/10′-H), 6.27 (2H, 14/14′-H), 6.38 (2H, 12/12′-H), 6.64 (2H, 15/15′-H, 6.66 (2H, 11/11′-H); *m*/*z* (EI) 566 (M^+, 50%), 534 (100, M − CH_3OH). Test for allylic methoxyl was carried out with 0.03 M HCl in $CHCl_3$,[39] resulting in a bathochromic shift of 16 nm.

8-Methoxy-7,8-dihydro-β,β-carotene (15) (2–23%). R_F = 0.15–0.18; λ_{max} (acetone)/nm 410sh, 428, 453; δ_H(400 MHz, $CDCl_3$, Me_4Si) 1.02–1.07 (12H, 16/17/16′/17′-H), 1.45 (2H, 2-H), 1.49 (2H, 2′-H′), 1.61 (2H, 3-H), 1.64 (2H, 3′-H), 1.74 (3H, 18′-H), 1.80 (3H, 18-H), 1.95 (2H, 4-H), 1.96–2.02 (12H, 19/20/19′/20′-H) 2.03 (2H, 4′-H), 2.22 (1H, 7-H), 2.39 (1H, 7-H), 3.15 (3H, 8-OMe), 3.61 (1H, 8-H), 6.06 (1H, 10-H), 6.13–6.19 (3H, 7′/8′/10′-H), 6.25 (2H, 14/14′-H), 6.31 (1H, 12-H), 6.36 (1H, 12′-H), 6.50 (1H, 11-H), 6.63 (2H, 15/15′-H), 6.66 (1H, 11′-H); *m*/*z* (EI) 568 (M^+, 49%), 536 (43, M − CH_3OH), 431 (100), 137 (27). Test for allylic methoxyl with 0.03 M HCl in $CHCl_3$[39] resulted in a bathochromic shift of 16 nm. Subsequent HPLC/VIS (System 1) revealed the formation of β,β-carotene (**1**), > 90% of total pigment recovered.

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