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Nucleophilic reactions of charge delocalised carotenoid mono- and dications†

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In the present study insight was gained on the larger complexity of cationic mixtures of diaryl (ϕ,ϕ-carotene, isorenieratene) and aliphatic (ψ,ψ-carotene, lycopene) carotenes, prepared by reaction with BF_3-etherate, compared with β,β-carotene. Chemical reactions of the mono- and dications prepared *in situ* from the allylic carotenols β,β-caroten-4-ol (isocryptoxanthin) and β,β-carotene-4,4′-diol (isozeaxanthin), and from isorenieratene and lycopene were investigated using selected O, N and S nucleophiles; water, methanol, azide and thioacetate. In total 22, including 18 new, neutral carotenoid products were isolated and identified by VIS, MS and NMR (in part) spectroscopy. Their structures were compatible with the structures of the cationic intermediates. The formal addition of hydride to the various dications, required to rationalise minor reaction products, is discussed in terms of more likely hydrogen radical or proton transfer in cationic reactions. Extensive *E*/*Z* isomerisation was observed for all quenching products. The potential use of carotenoid cations for the synthesis of 4,(4′)-substituted β,β-carotenes and 7-oxabicyclo[2,2,1]heptane derivatives is discussed.

Introduction

Previously we have prepared from β,β-carotene (**1**) the β,β-carotene dication (**2**) by treatment with BF_3-etherates in $CHCl_3$ solution, and elucidated the structure of this charge delocalised cation by NIR and detailed NMR spectroscopy at −15 °C.[1,2]

Subsequently we have obtained from β,β-caroten-4-ol (**3**, isocryptoxanthin) the monocation **4** (as four stereoisomers) by reaction with CF_3COOH in CH_2Cl_2 solution.[3,4] The corresponding bisallylic diol β,β-carotene-4,4′-diol (**5**, isozeaxanthin) was converted to the dication **6** by similar treatment, and to the symmetrical dicarbocation **7** (three stereoisomers) by reaction with the stronger Brønsted acid CF_3SO_3H.[4]

The structure elucidation of the monocation **4** and the dications **6** and **7** was conducted in the same manner by detailed NMR spectroscopy, including COSY, HSQC, HMBC and 2D ROESY techniques. The structures of the delocalised carotenoid cations prepared are given in Scheme 1. The distribution of the charge is illustrated by the diameters of the filled circles.

An alternative approach to gain structural information on the delocalised carotenoid cations is to study their reactions with suitable nucleophiles, including detailed product analysis.

The reactions of the β,β-carotene dication **2** (20 π electrons) with H_2O as nucleophile (in acetone) provided isocryptoxanthin (**3**), isocarotene (**8**), mutatochrome (**9**) and β,β-carotene (**1**).[2]

Treatment of the dication **2** with CH_3ONa/CH_3OH furnished the methyl ethers **10** and **11** and isocarotene (**8**). The reactions, summarised in Scheme 2, were rationalised and consistent with the dication structure determined by NMR spectroscopy.[2]

In the present work we report on the reactions of the charge delocalised monocation **4**, and dications **6** and **7** with selected O, N and S nucleophiles.

Also included are nucleophilic reactions performed with cations prepared *in situ* from carotenes with two aryl or two aliphatic end groups.

Results

Nucleophilic reaction of the monocation 4

The monocation **4**, resulting from β,β-caroten-4-ol (**3**) treated with CF_3COOH, was converted to *E*/*Z* isomerised **3** when reacted with H_2O as nucleophile in acetone.

Nucleophilic reactions of the dication 6

β,β-Carotene-4,4′-diol (**5**) or its diacetate **12** was used as substrate for generating the dication **6**, which was subsequently reacted with water as nucleophile. In both cases *E*/*Z* isomerised isozeaxanthin (**5**) was the major product. Methanolysis of the dication **6** provided mainly the *E*/*Z* isomerised dimethyl ether **13**, characterised by VIS, NMR and mass spectrometry.

When sodium azide was employed as the nucleophile, NMR and MS analyses identified the monoazide **14**, and the diazide **15**, as major products, Scheme 3. Attempts to convert the produced azides to amines by hydrogenation with Lindlar catalyst[5] or with $SnCl_2$[6] failed.

Reactions of the dication **6** with thioacetic acid as nucleophile provided three main products, which were tentatively identified as the monothioester **16**, the dithioester **17**,[7] and the hydroxythioester **18**, after partial characterisation by HPLC, VIS, MS and by ^{1}H NMR (**18** only).

Reduction of the dithioester **17** to the thiol with $LiAlH_4$ failed according to earlier experience.[7] Attempted conversion of **16**, **17** and **18** to the free thiols using KOH resulted in the formation of isocarotene (**8**), judged by HPLC, VIS and MS data.

Other carotenoid thioacetates[8] and azides[9] have been prepared previously by different approaches.

Nucleophilic reactions of the symmetrical dication 7 (22 π electrons)

The symmetrical dicarbocation **7**, obtained from β,β-carotene-4,4′-diol (**5**) with CF_3SO_3H, was reacted with H_2O in acetone. Product analysis including HPLC, TLC and spectroscopy (VIS, MS, and in part ^{1}H NMR) resulted in plausible identification of three major *E*/*Z* isomerised products as the carotenols **19**, **20** and 4′-hydroxy-β,β-caroten-4-one (**21**), Scheme 4 (for definition of P_1, P_2, P_3, and P_4, see Schemes 2 and 3). Product **19** with nonaene chromophore had molecular ion *m*/*z* 550, and MS showed M − 92, but no M − H_2O fragment ions. This product, characterised by NMR, was resistant to acetylation. Product **20** with decaene chromophore had the same molecular ion as **19** and also no fragment ion for loss of water upon MS. Product **21** had λ_{max} 462 nm, showed strong M − H_2O ion upon electron impact MS, and gave isozeaxanthin (**5**) upon $NaBH_4$ reduction.

Treatment of the dicarbocation **7** with methanol as a nucleophile provided several products analysed by HPLC, TLC, and VIS and mass spectroscopy. HPLC revealed strong *E*/*Z* isomerisation of each

† No. 6 in the series Charged Carotenoid Species. No. 5 = Ref. 4.

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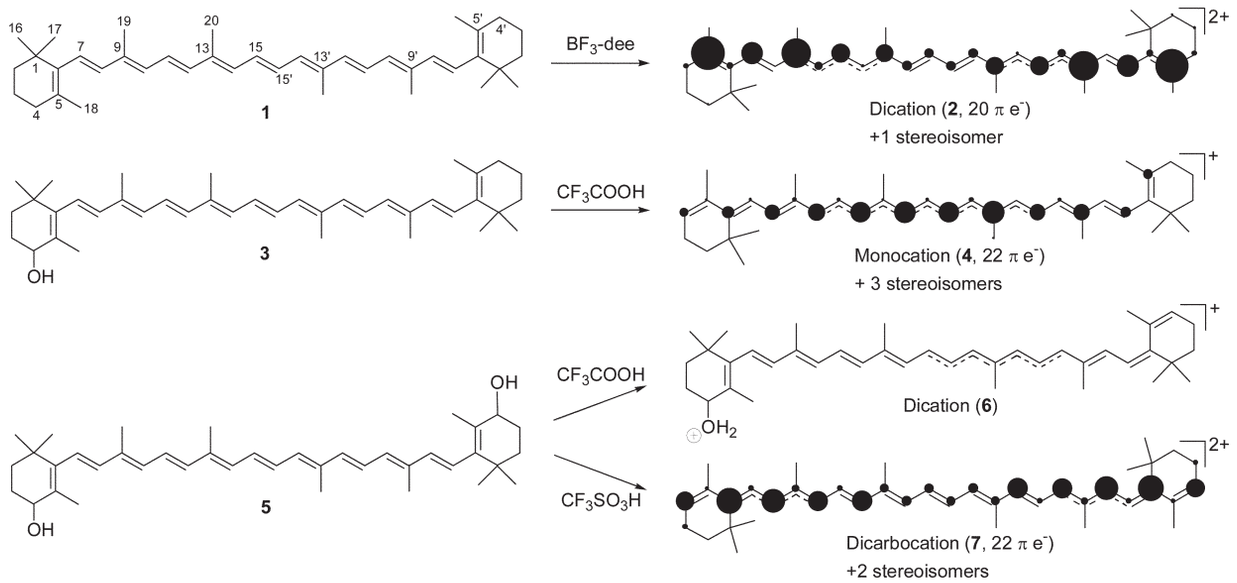
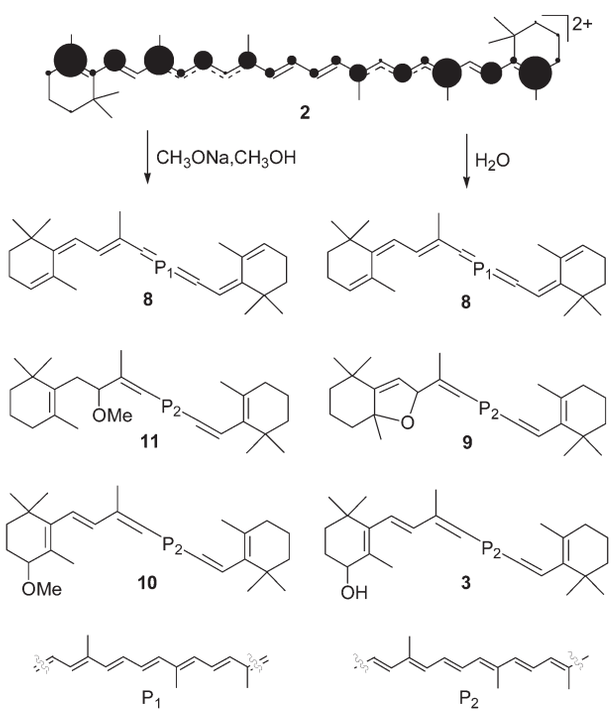
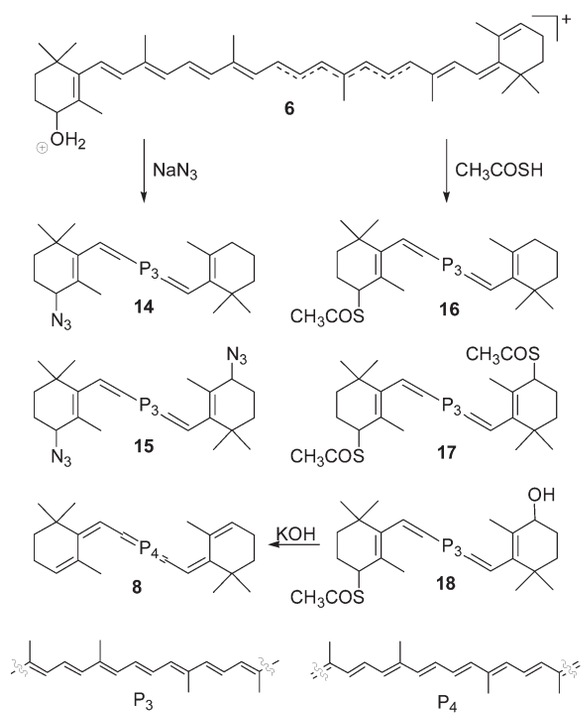


Scheme 1



Scheme 2



Scheme 3

product with up to eight peaks (isomers). Plausible structures were assigned based on relative polarity, VIS spectra (λ_{max} and spectral fine-structure, %III/II[10]) in comparison with known chromophores, including 3,4-didehydro-β,β-carotene, and MS data (molecular weight, number of double bond equivalents and fragmentation patterns). Products **8** and **22**–**25** are given in Scheme 4 according to polarity by TLC, assuming that **25a** had undergone dehydration to **25b** during work-up or MS analysis.

The formation of the products obtained by nucleophilic reactions from the dications **6** and **7** is rationalised in the Discussion.

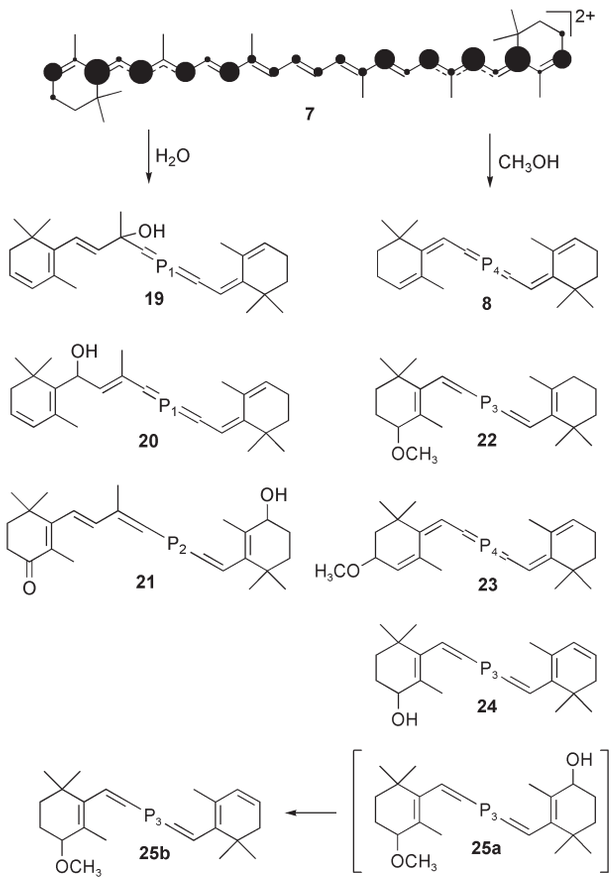
Preparation, characterisation and nucleophilic reactions of the isorenieratene (26) and lycopene (27) cations

As a supplement to the dicyclic cationic substrates prepared from β,β-carotene (**1**), its 4-ol (**3**) and 4,4′-diol (**5**) derivatives, it was considered of interest to examine nucleophilic reactions of cations prepared *in situ* from carotenes with two aryl end groups, isorenieratene (**26**), and with two aliphatic end groups, lycopene (**27**).

Isorenieratene (**26**) provided with BF_3-etherate in $CHCl_3$ or CH_2Cl_2 a product with λ_{max} 910 nm. EPR studies revealed the presence of free radicals in the reaction mixture, Fig. 1. A line width of *ca.* 13 G was compatible with a delocalised carotenoid cation radical.[11] The product mixture was less stable than for the β,β-carotene dication (**2**) and NMR analysis at −15 °C revealed a complex mixture where the expected dication **28** could not be identified. A minor monocation **29**, occasionally formed, was structurally identified, Scheme 5.[4]

Hydrolysis of the cationic intermediate with H_2O in acetone provided the mono-ol **30**, identified by VIS and MS data, and a diol **31**, identified by polarity, VIS and MS data. The by HPLC least polar products were tentatively identified as diastereomers of the furanoxide **32**, judged by VIS data, M = 544 ($C_{40}H_{48}O$) by MS, and reaction with CF_3COOH to a blue, tentative oxonium ion **33**. Reversion to λ_{max} 412 nm upon addition of KOH to **33** is compatible with the formation of the hemiketal (10-ol).[12]

Lycopene (**27**), treated with BF_3-etherate, provided a product with λ_{max} 940 nm in $CHCl_3$ and λ_{max} 920 nm in CH_2Cl_2, which according to NIR data appeared to be relatively stable.



Scheme 4

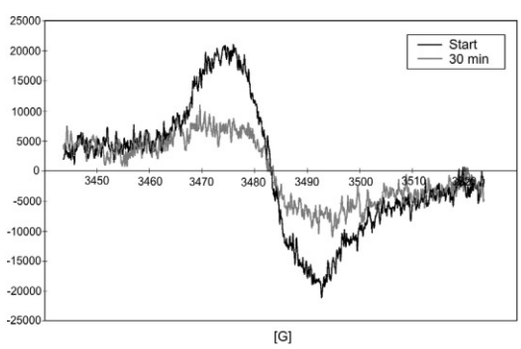


Fig. 1 EPR spectrum of isorenieratene (**26**) treated with BF_3-dee.

MS showed prominent ions at M + 4, M + 2, M, M − 2 and M − 4. EPR studies showed a line width of *ca.* 13 G, compatible with the presence of delocalised free radicals.[11] The amount of radicals was reduced over time, Fig 2.

NMR studies of the cation prepared from lycopene (**27**) were unsuccessful.[4] When the *in situ* prepared cationic mixture of lycopene (**27**, $M = 536$) was reacted with H_2O in acetone a large number of products (>70 peaks) were apparent by HPLC. Two major fractions isolated by TLC had MS compatible with $M = 536 + H_2O$ and $M = 536 + 2$ O, both fractions containing products with nonaene and decaene chromophores. Dication **34** and product **35**, Scheme 6, are referred to later.

Discussion

Nucleophilic reactions of monocation 4 and dications 6 and 7

When the monocation **4**, obtained from β,β-caroten-4-ol (**3**) treated with CF_3COOH,[3,4] was hydrolysed with H_2O in acetone, *E*/*Z* isomerized **3** was obtained. The result is consistent with the structure assigned to the delocalised monocation **4** and leading to carotenol formation with maximum length of the polyene chain. Also in the following, it was usually experienced that maximum length of the polyene system/product stability was a decisive factor in directing the nucleophilic attack. This was the case even when the highest partial positive charge was located in the middle of the polyene system.

The dication **6** with a protonated hydroxy group has a good leaving group for S_N2 reactions. Substitution at C-4′ is evident from all products obtained from **6** with various nucleophiles, Scheme 3. The hydroxy substituent in product **18** is considered as a remnant of the protonated hydroxy group in **6**. As to the fate of the second delocalised charge, addition of the nucleophile to the C-4 position was generally the case. For the minor products **14** and **16**, formally a hydride has been added to C-4, see below.

The products (**5**, **13**–**18**) obtained with water, methanol, azide or thioacetic acid as nucleophiles are compatible with the structure assigned to the dication **6**, and demonstrate the potential use of this dication in partial synthesis for obtaining C-4,4′ disubstituted derivatives of β,β-carotene (**1**). No in-chain substitution appeared to be favoured for this delocalised cation (**6**).

The symmetrical dication **7** was reacted with water (aqueous acetone) or methanol as nucleophiles, Scheme 4. With methanol, disubstitution at C-4,4′ (**25a**), monosubstitution at C-4 (**22**, **24**) and elimination of one proton (**23**, **24**, **25b**) were noted. The formation of products **19** and **23** is rationalised in Scheme 7. The hydroxy substituent of **24** and **25a** might be the result of incomplete dication formation, *cf.* **6**, of β,β-carotene-diol (**5**) with CF_3SO_3H. The didehydro product isocarotene (**8**) formally requires one hydride transfer.

The reactions of the dication **7** with water resulted partly in C-4,4′ disubstitution (**21**, considered obtained by ready oxidation of product **5**), and gave partly in-chain hydroxylation (**19**, **20**), as rationalised for product **19** in Scheme 7.

In conclusion, the products obtained by reaction of the symmetrical dication **7** with selected nucleophiles (water and methanol) were compatible with the structure assigned to this dication.

The large extent of *E*/*Z* isomerisation for the products isolated by reaction of **6** and **7** with nucleophiles was evident when employing an HPLC system suitable for the separation of geometrical isomers of carotenoids, and is consistent with product formation from delocalised carotenoid cations.[1–3] As an illustration is included the HPLC profile of the *E*/*Z* isomerised reaction mixture containing mainly isocarotene (**8**), obtained from β,β-caroten-4-ol (**3**) by treatment with dilute HCl *via* the monocation **4**, *cf.* ref. 3, Fig. 3.

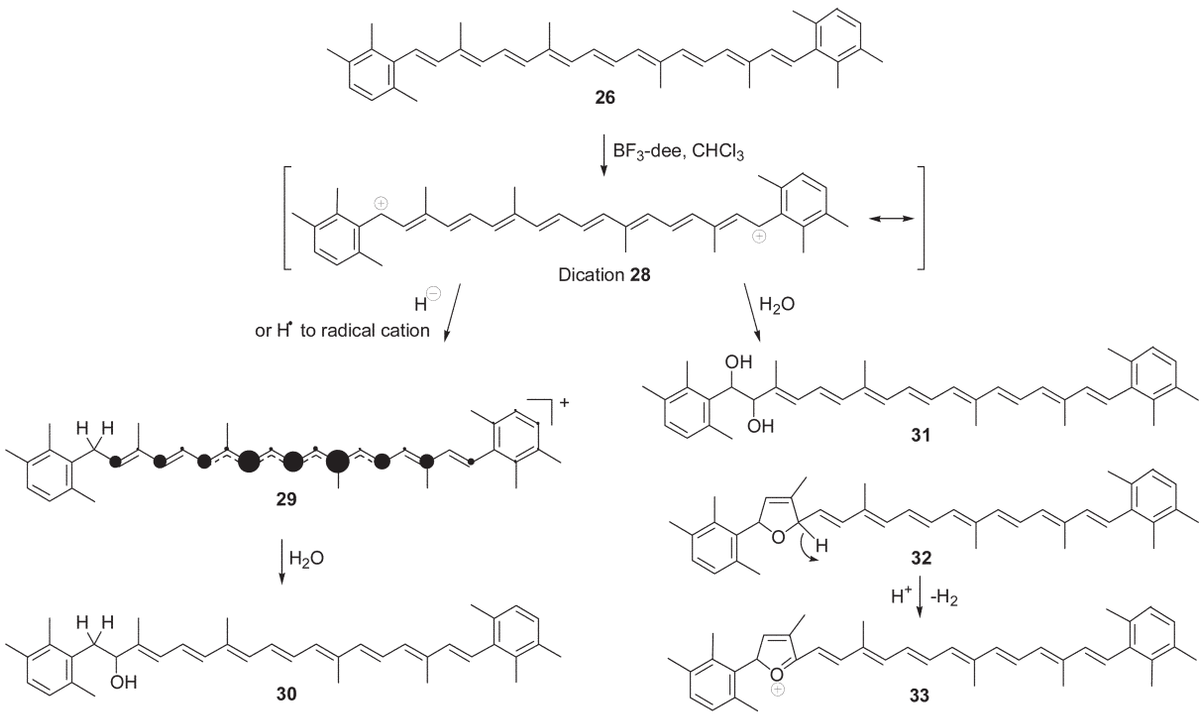
The strong *E*/*Z* isomerisation observed for all the carotenoid products is characteristic for the products formed in these reactions, as might be expected from the low rotational barrier of the cationic intermediates.[13] However, the structures of the carotenoid cations determined by NMR have shown the all-*E* configuration to be preferred also in the charged state.[1,2,4]

Except for the double bond connecting the end group to the polyene chain in *retro* carotenoids, only the *E*-configuration was observed in these cations. Similar observations have previously been made for α,ω-diphenyl polyenyl anions.[14,15] This preference for the all-*E* configuration has been explained by a higher charge delocalisation, which is due to a better overlap of the π-orbitals.

There seem to be two possible explanations for this apparent anomaly. Either the *E*/*Z* isomerisation occurs only upon the quenching of the cations, or, more likely, the *Z* isomers are present also in the charged state, but without being observed in the NMR experiments. If the latter explanation is correct, the lifetime of the *Z* isomers in the charged state must be too short to be detected within the NMR time frame.

Nucleophilic reactions of β,β-carotene dication (2) related dications

As to carotenoid cations prepared by reaction with BF_3-etherates from hydroxylated β,β-carotenes we have previously examined the reactions of (2*R*)-β,β-caroten-2-ol (**36**) and (2*R*,2′*R*)-β,β-carotene-2,2′-diol (**37**), resulting in the formation of the 7-oxabicyclo[2.2.1]heptane derivatives **38** and **39**, assumed to occur by internal attack by the hydroxy groups of a C-5(5′)-cation,[16]



Scheme 5

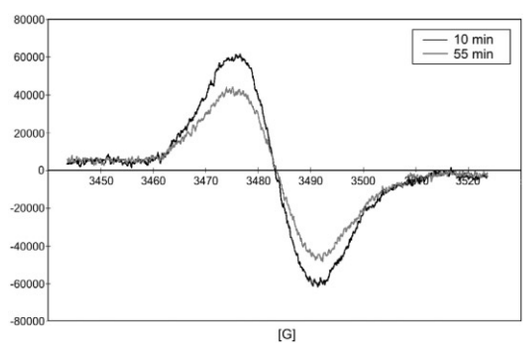
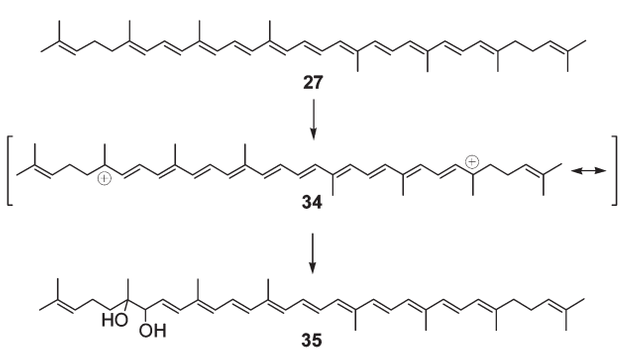


Fig. 2 EPR spectrum of lycopene (**27**) treated with BF_3-dee.



Scheme 6

Scheme 8. The relevant dications **40** and **41** obtained from **36** and **37** can now be formulated by analogy with the dication **2**, prepared from β,β-carotene (**1**), Scheme 8. The relatively high positive charges at C-5(5′) serve to explain the course and good yield of these nucleophilic reactions.

Nucleophilic reactions of cations obtained from isorenieratene (26) and lycopene (27)

The predicted dication **28** of isorenieratene (**26**), Scheme 5, could not be identified in the complex reaction mixture by NMR. The identified monocation **29** might be a product of the dication **28** formed by a formal hydride attack, *vide infra*. Reaction with H_2O in acetone provided the mono-ol **30**, which may be rationalised by reaction of the monocation **29** with H_2O, whereas the diol **31** is an expected product from the hypothetic dication **28**. The formation of the assumed furanoxide **32** can be depicted by analogy with the formation of the furanoxide mutatochrome (**9**, Scheme 2) from the β,β-carotene dication (**2**).[2]

The reaction products obtained from the isorenieratene (**26**) cation mixture are consequently compatible with the presence of the dication **28** and the monocation **29**. Moreover, the reaction products encountered, as well as the monocation **29**, support negligible delocalisation of the charge of **28** and **29** to the aromatic end groups, which would cause a reduction of the Hückel ($4n + 2$) aromaticity.

As for isorenieratene (**26**), the reaction of lycopene (**27**) with BF_3-etherate proceeds *via* free radicals. Athough absorption was observed in the NIR region no resolution in the NMR spectrum could be obtained. The opportunity for intermolecular cyclisations of cationic intermediates such as the hypothetical dication **34** is obvious. Quenching with H_2O in acetone revealed a very complex reaction mixture. Major coloured reaction products exhibited mainly decaene and nonaene chromophores. The apparent addition of H_2O to lycopene (**27**) might involve a formal addition of hydride to the dication **34**, and addition of H_2O to the resulting monocation, providing a mono-ol with decaene chromophore ($M = 554$)

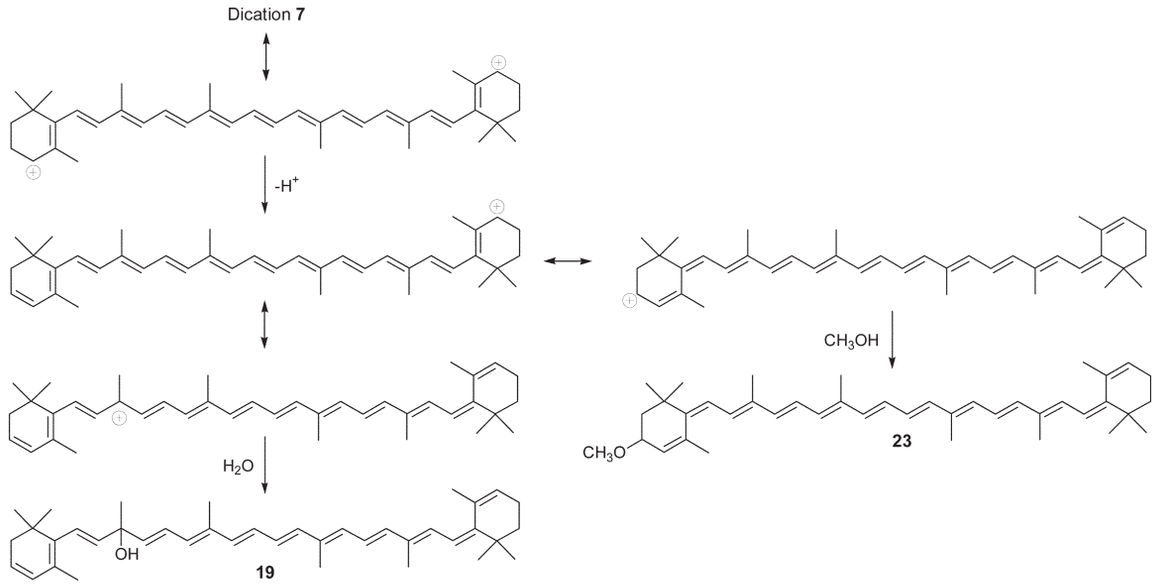
The alternative addition of 2 oxygens ($M = 568$) points to epoxide type products. Previously the formation of the 5,6-diol **35** requiring $M = 572$, Scheme 6, identified by chemical correlations, has been claimed from similar experiments.[17] This diol **35**, would be a predicted product of the dication **34**.

Electron impact mass spectra recorded of freshly prepared cationic mixtures of isorenieratene (**26**) and lycopene (**27**) showed no evidence of BF_3 addition products, *cf.* refs. 2,16,17. For lycopene (**27**), the MS might support hydrogen transfer in the ionised state, see below.

Hydrogen transfer reactions of carotenoids

Hydride as leaving group has been documented during the reaction of carotenoid 5,8-furanoxides with very strong acids, forming delocalised oxonium ions,[12] *cf.* **32** and **33**, Scheme 6. Also the formation of the monocation **42** from echinenone (**43**) reacted with CF_3COOH requires H^- as a leaving group,[4,18] Scheme 9. In all these cases, H_2 must be produced.

The rationalisation of products **8** (from **7**), **14**, **16**, **22**, and **29** encountered here formally requires the addition of a hydride to a dication, and has represented a puzzle. The combined evidence demonstrates that



Scheme 7

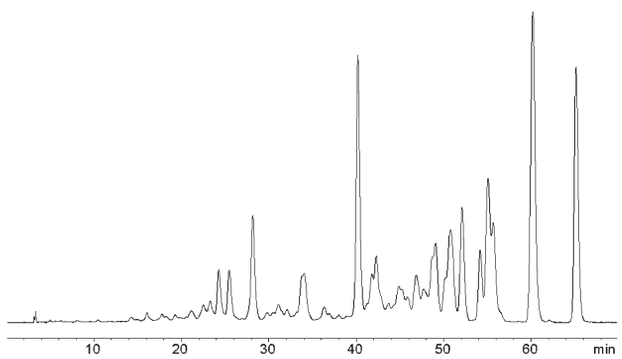
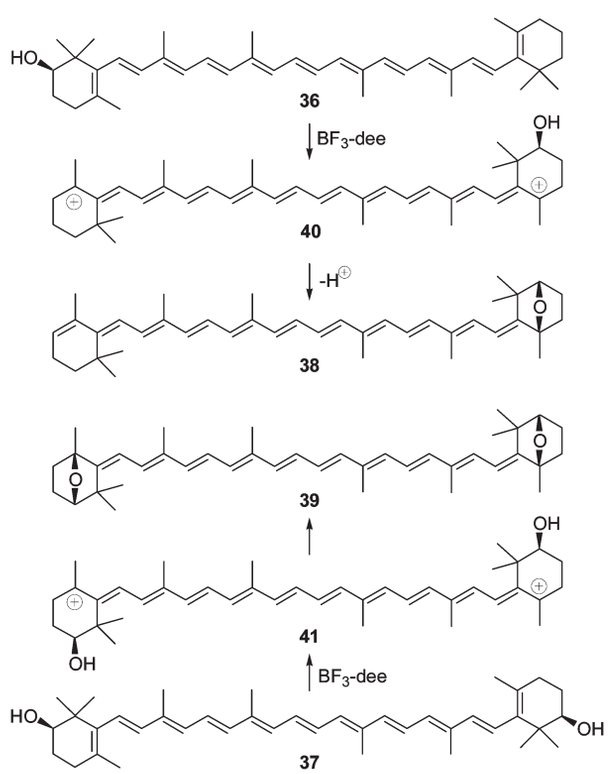
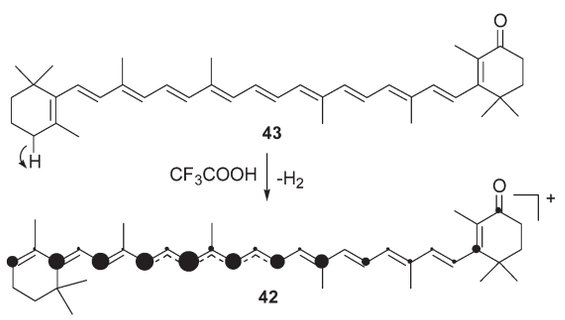


Fig. 3 HPLC (system 1) of the reaction mixture of isocryptoxanthin (**3**) treated with 0.03 N HCl in $CHCl_3$. For peak identification, see the experimental part.



Scheme 8



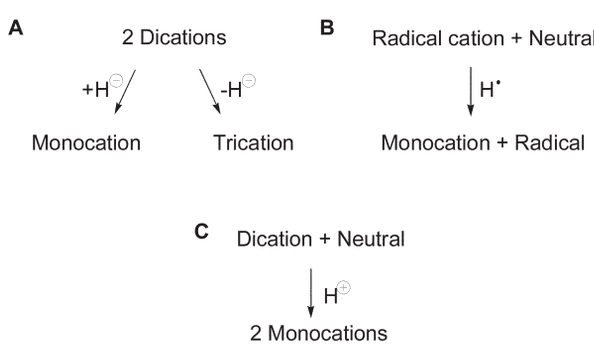
Scheme 9

i) Although higher proportions of products with apparent hydride-addition have been observed in particular solvent batches, no selective transfer of deuterium from either $CDCl_3$ or CD_3OD has been observed.

ii) Formal hydride transfer is observed using both BF_3-etherates and CF_3COOH as reagent for generating carotenoid cations. Free radicals are involved in the former, but not in the latter reaction. In strong CF_3COOH, H^- cannot survive.

iii) Hydrogen transfer occurs as a minor reaction (products usually in the order of 10% of the total) to a positively charged carbon atom at the end of the delocalised π-electron system, resulting in cations, which subsequently react with added nucleophiles. The monoazide **14** and monothioacetate **16** were obtained from the dication **6**, Scheme 3. Moreover, the methyl ether **22** was obtained from the symmetrical dication **7**.

The above evidence suggests that the formal hydride transfer may occur by reactions of the various dications, Scheme 10. However, referring to A, no experimental support has so far been obtained for the existence of a trication of this type, or derivatives thereof.



Scheme 10

The 7,8-dihydro-8-methoxy derivative **11** previously obtained from the β,β-carotene dication (**2**) was then rationalised as a hydrogen radical attack of an intermediary radical cation,[2] see B, Scheme 10. For carotenoid cations generated by treatment with BF_3-etherate, radical reactions involving transfer of hydrogen radicals remain an open alternative. For isorenieratene (**26**) treated with BF_3-etherate, the monocation **29** was isolated,[4] as well as product **30** when the reaction was quenched with water, Scheme 5. In this case the monocation with a hydrogen added was detected by NMR. The intensity of the signals from the monocation **29** was constant during the experiments (*ca.* 10 h), indicating that the H-transfer was taking place during the formation of the cations only, and that the neutral carotenoid was the H-donating species, compatible with routes B or C.

However, products **14**, **16** and **22** were formed in radical-free media (CF_3COOH). Here reaction C involving reaction between dication and neutral carotenoid with H^+ transfer is a plausible alternative to reaction A.

In summary, it appears more likely that hydrogen radical transfer reactions (reaction B), rather than hydride transfer reactions (reaction A) occur in free radical media, whereas hydride transfer (reaction A) or proton transfer (reaction C) are required in radical-free media. In strongly acidic media (CF_3COOH or CF_3SO_3H) proton transfer (C) is more likely.

Conclusions

The reactions of the cations **4**, **6** and **7**, prepared *in situ* from allylic β,β-carotenols with various O, N and S nucleophiles (water in acetone, methanol, sodium azide and thioacetate) were investigated. Product analysis included TLC, HPLC-DAD, VIS, MS and NMR spectroscopy, and chemical derivatisation reactions. In total, 22 neutral carotenoid products were identified, including 18 new carotenoids.

The potential use of the dications **6** and **7** for the synthesis of 4,(4′)-substituted β,β-carotenes, and of the 2-monohydroxy and 2,2′-dihydroxy analogues of the dication **2** for the preparation of 7-oxabicyclo[2.2.1]heptane derivatives is pointed out.

Insight was gained into the larger complexity of cationic mixtures of diaryl- and aliphatic carotenes, prepared by reaction with BF_3-etherate, compared with β,β-carotene (**1**).

The structures of all products with nucleophiles were rationalised from those of the delocalised cations. The formal hydride addition to carotenoid dications, required to rationalise the formation of some products (**8**, **14**, **16**, **22**, **30**), has been discussed in terms of cationic reations involving hydrogen radical or proton transfer reactions.

All carotenoid products obtained from the delocalised cations were strongly *E*/*Z* isomerised, according to HPLC analysis, compatible with cationic intermediates.

Experimental

Materials

Commercially available CF_3COOH from Acros and Merck and CF_3COOD from Acros were used. CF_3SO_3H and CF_3SO_3D were supplied from Aldrich. Ethanol stabiliser present in $CHCl_3$ was removed on an alumina column prior to use. Pyridine was distilled from BaO. BF_3-diethyl etherate (BF_3-dee) was obtained from Acros. Carotenoids used were synthetic samples obtained from Hoffmann-La Roche, except isorenieratene (**26**), which was prepared by total synthesis.[4]

General methods

Spectroscopy and chromatography. Reactions and manipulations were carried out as far as possible in darkness and under nitrogen atmosphere. Visible light (VIS) and near infrared (NIR) spectra were recorded on a Varian Cary 50 UV-VIS Spectrophotometer (190–1100 nm) or a Varian Cary 5 UV-VIS-NIR Spectrophotometer (170–3500 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 temperature 250 °C. NMR spectra were obtained in $CDCl_3$ on a Bruker Avance DPX 400 instrument using a 5 mm QNP probe, or a Bruker Avance DRX 500 instrument using a 5 mm TXI probe. Chemical shifts are cited relative to TMS with calibration against residual $CHCl_3$ at 7.27 ppm for ^1H.

General methods for the analysis of the cations by NIR and NMR are given elsewhere.[4]

EPR spectra were recorded on a Bruker ESP 300E instrument, rectangular cavity, flat cell.

HPLC was carried out on a Hewlett Packard Series 1050 instrument equipped with a diode array detector (DAD). Detection wavelengths were set at 420, 450 and 480 nm. VIS spectra of the carotenoid components were recorded on-line during chromatography. Spectral fine structure is reported as %III/II.[10] Three different HPLC systems were used:

System 1,[19] Waters YMC Carotenoid C30 column, 250 × 4.6 mm. 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}).

System 2, Interchrom Uptisphere 5 ODB column, 250 × 4.6 mm. Mobile phase 0 min: methanol : acetone (100 : 0 v/v 1.0 ml min^{-1}), 100 min: methanol : acetone (0 : 100 v/v, 1.0 ml min^{-1}).

System 3, Interchrom Uptisphere 5 ODB 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}).

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

Preparation of substrates (3, 5, 12, 26). β,β-Caroten-4-ol (isocryptoxanthin, **3**) and β,β-carotene-4,4′-diol (isozeaxanthin, **5**) were prepared by $NaBH_4$-reduction from synthetic β,β-caroten-4-one (echinenone, **43**) and β,β-carotene-4,4′-dione (canthaxanthin).[4] β,β-Carotene-4,4′-diol (**5**) was converted to its diacetate (**12**) by standard procedure. ϕ,ϕ-Carotene (isorenieratene, **26**) was prepared by total synthesis.[4]

Treatment of β,β-caroten-4-ol (3) with acids

VIS/NIR and NMR experiments.

0.03 M HCl at room temperature. β,β-Caroten-4-ol (**3**, *ca.* 20 μg) was dissolved in 0.03 M HCl in $CHCl_3$ (3 ml). The VIS/NIR spectrum was recorded at 5 min intervals. An immediate bathochromic shift to λ_{max} 479 nm was observed, as well as a weak absorption in NIR around 1010 nm. A slight decrease in both intensity and fine structure (%III/II) of the main peak occurred during 20 min.

0.03 M HCl at −20 °C. The experiment was performed as described above with the cuvette placed in a cuvette holder cooled by cold methanol (−20 °C) from a cryostat. The change of absorption maximum progressed much slower, giving approximately equal intensity of λ_{max} 464 nm and λ_{max} 487 nm after 20 min. Initially, the peak around 1010 nm was more pronounced than at room temperature, with 20% of the intensity of the main peak. However, the NIR absorption decayed rapidly to *ca.* 25% of the initial absorption during 20 min.

CF_3COOH at −20 °C. β,β-Caroten-4-ol (**3**, *ca.* 5 μg) was dissolved in $CHCl_3$ (3 ml) containing CF_3COOH (0.013 M) at −20 °C. The VIS/NIR spectrum was recorded with cooling as described above. An absorption maximum of λ_{max} 977 nm was measured, with 16% loss of intensity during 30 min. Exchange of $CHCl_3$ for CH_2Cl_2 gave λ_{max} 1028 nm, with <10% decay observed during 3 h.

For NMR analysis at −10 °C of the monocations **4**, see refs. 3,4.

Elimination of water with 0.03 M HCl.

At room temperature. β,β-Caroten-4-ol (**3**, *ca.* 20 μg) was dissolved in 0.03 M HCl in $CHCl_3$ (3 ml). After 20 min, the reaction mixture was washed with water and saturated NaCl solution

and analysed by HPLC, Fig. 3. HPLC (System 1) R_T = 28.1 (5%, λ_{max}/nm 475; %III/II 0; unidentified carotenoid), R_T = 40.2 (12%, λ_{max}/nm 465; %III/II 0; 3,4-didehydro-β,β-carotene), R_T = 50.7 (8%, λ_{max}/nm 439sh 461 491; %III/II 28; 4′,5′-didehydro-4,5′-*retro*-β,β-carotene, **8**), R_T = 52.1 (6%, λ_{max}/nm 440 465 495; %III/II 39; Neo D **8** (For neo-nomenclature see ref. 20)), R_T = 55.0 (7%, λ_{max}/nm 442 465 495; %III/II 36; **8**), R_T = 55.7 (5%, λ_{max}/nm 443sh 469 498; %III/II 48; Neo B **8**), R_T = 60.2 (16%, λ_{max}/nm 444sh 467 497; %III/II 38; Neo A **8**), R_T = 65.1 (12%, λ_{max}/nm 446 472 503; %III/II 51; all-*trans* **8**); HPLC (System 3) R_T = 15.9 (8%, λ_{max}/nm 425sh 451 479; %III/II 40; **3**), R_T = 16.8 (16%, λ_{max}/nm 423sh 449 479; %III/II 30; **3**), R_T = 29.4 (7%, λ_{max}/nm 439 459 491; %III/II 60; unidentified carotenoid), R_T = 30.7 (15%, λ_{max}/nm 461; %III/II 0; 3,4-didehydro-β,β-carotene), R_T = 33.6 (22%, λ_{max}/nm 441 465 495; %III/II 28; **8**), R_T = 34.2 (21%, λ_{max}/nm 444 471 499; %III/II 43; **8**).

At −20 °C. β,β-Caroten-4-ol (**3**, *ca.* 20 μg) was dissolved in 0.03 M HCl in $CHCl_3$ (2.5 ml) at −20 °C. After 5 min an aliquot was withdrawn and washed with water and saturated NaCl solution. The product mixture was analysed by HPLC. HPLC (System 1) R_T = 24.2 (6%, λ_{max}/nm 429sh 450 479; %III/II 50; all-*trans* **3**), R_T = 26.9 (7%, λ_{max}/nm 470; %III/II 0; unidentified carotenoid), R_T = 43.5 (6%, λ_{max}/nm 431sh 459 487; %III/II 40; **8**), R_T = 48.0 (5%, λ_{max}/nm 433sh 459 489; %III/II 50; Neo E **8**), R_T = 49.7 (8%, λ_{max}/nm 438 460 489; %III/II 40; **8**), R_T = 51.1 (6%, λ_{max}/nm 438 465 495; %III/II 45; **8**), R_T = 53.4 (6%, λ_{max}/nm 440 461 491; %III/II 50; **8**), R_T = 54.3 (7%, λ_{max}/nm 440sh 465 493; %III/II 40; Neo B **8**), R_T = 55.0 (5%, λ_{max}/nm 442 471 499; %III/II 50; **8**), R_T = 59.8 (15%, λ_{max}/nm 445sh 467 495; %III/II 45; Neo A **8**), R_T = 64.8 (11%, λ_{max}/nm 449 471 503; %III/II 60; all-*trans* **8**).

Isomerisation of β,β-caroten-4-ol (3). To β,β-caroten-4-ol (**3**, 0.35 mg), dissolved in CH_2Cl_2 (5 ml) at −10 °C, CF_3COOH (25 μl) was added, whereupon the reaction mixture turned blue. A cooled solution of 25% H_2O in acetone (10 ml) was added, giving a yellow reaction mixture. The pigments were transferred to hexane and the organic phase washed with water and saturated NaCl solution, pigment recovery 89% ($E_{1\%,\ 1\ cm}$ 2500), λ_{max}(hexane)/nm 425sh 448 475; %III/II 18. HPLC (System 1) R_T = 17.4 (9%, λ_{max}/nm 422sh 444 470; %III/II 11; 13-*cis* **3**), R_T = 18.3 (8%, λ_{max}/nm 420sh 444 470; %III/II 13; **3**), R_T = 21.0 (63%, λ_{max}/nm 428sh 451 478; %III/II 33; all-*trans* **3**), R_T = 23.7 (9%, λ_{max}/nm 422sh 447 474; %III/II 36; 9-*cis* **3**).

Treatment of β,β-carotene-4,4′-diol (5) with CF_3COOH

VIS/NIR and NMR experiments at −20 °C. β,β-Carotene-4,4′-diol (**5**, 16 μg) was dissolved in CH_2Cl_2 containing CF_3COOH (0.013 M) at −20 °C. The VIS/NIR spectrum was recorded with cooling as described above. λ_{max} 980 nm was measured, with <20% loss of intensity observed during 3 h.

NMR analysis at −20 °C of dication **6**, see ref. 4.

Reaction of dication 6 (from 5) with water. β,β-Carotene-4,4′-diol (**5**, 0.57 mg) was dissolved in CH_2Cl_2 (5 ml), and cooled to −10 °C. CF_3COOH (25 μl) was added, whereupon the reaction mixture turned blue. A cooled solution of 25% H_2O in acetone (8 ml) was added after 10 min stirring, giving an orange solution. The pigments were transferred to hexane and washed with water and saturated NaCl solution. Pigment recovery 100% ($E_{1\%,\ 1\ cm}$ 2500), λ_{max}(hexane)/nm 424sh 448 474, %III/II 22, $\%D_B/D_{II}$[10] 15; HPLC (System 1) R_T = 10.5 (5%, λ_{max}/nm 419sh 443 470; %III/II 33; **5**), R_T = 11.8 (55%, λ_{max}/nm 426sh 450 477; %III/II 27; all-*trans* **5**), R_T = 17.0 (6%, λ_{max}/nm 422sh 447 474; %III/II 61; 9-*cis* **5**), R_T = 21.7 (15%, λ_{max}/nm 426sh 452 480; %III/II 51; unidentified carotenoid).

Reaction of dication 6 (from 5) with methanol. β,β-Carotene-4,4′-diol (**5**, 1.0. mg) was dissolved in CH_2Cl_2 (6 ml), and cooled to −10 °C. CF_3COOH (40 μl) was added, whereupon the reaction mixture turned blue. Cooled methanol (10 ml) was added after 10 min stirring, giving an orange solution. The pigments were transferred to hexane and washed with water and saturated NaCl solution. Pigment recovery 56% ($E_{1\%,\ 1\ cm}$ 2500). The reaction mixture was characterised without further purification.

4,4-Dimethoxy-β,β-carotene (13). λ_{max}(hexane)/nm 424sh 449 475, %III/II 28, $\%A_B/A_{II}$ 13; HPLC (System 1) R_T = 18.7 (12%, λ_{max}/nm 420sh 445 471; %III/II 18; 13-*cis* **13**), R_T = 21.8 (55%, λ_{max}/nm 424sh 451 478 %III/II 37; all-*trans* **13**), R_T = 25.8 (4%, λ_{max}/nm 420sh 446 473; %III/II 44; 9-*cis* **13**), R_T = 28.4 (14%, λ_{max}/nm 426sh 452 479; %III/II 33; unidentified carotenoid); δ_H(400 MHz, $CDCl_3$) 1.02 (6H, 16/17/16′/17′-H), 1.04 (6H, 16/17/16′/17′-H), 1.26 (lipid), 1.37 (2/2′-H), 1.68 (2H, 2/2′-H), 1.77 (3/3′-H), 1.81 (6H, 18/18′-H), 1.82 (3/3′-H), 1.97 (19/19′-H), 1.98 (20/20′-H), 3.40 (6H, -OCH_3), 3.52 (2H, 4/4′-H), 6.10–6.23 (6H, 7/8/10/7′/8′/10′-H), 6.27 (2H, 14/14′-H), 6.38 (2H, 12/12′-H, $J_{11,12} = J_{11′,12′}$ = 14.8 Hz), 6.62–6.68 (4H, 11/15/11′/15′-H); *m/z* (EI) 597 (47%, M + 1), 596 (M^+, 100), 566 (32, M − HCOH), 564 (30, M − CH_3OH), 534 (14), 532 (16, M − 2 CH_3OH), 504 (13, M − 92), 474 (5, M − 92 − HCOH), 472 (5, M − 92 − CH_3OH), 442 (3, M − 92 − HCOH − CH_3OH), 440 (2, M − 92 − 2 CH_3OH).

Reaction of dication 6 (from 5) with NaN_3. β,β-Carotene-4,4′-diol (**5**, 1.3. mg) was dissolved in CH_2Cl_2 (5 ml) and cooled to −10 °C. CF_3COOH (40 μl) was added, whereupon the reaction mixture turned blue. After 10 min stirring, a cooled solution of NaN_3 (2.0 g) in water (5 ml) was added, giving a two-phase system. After vigorous stirring, the organic layer turned brown/orange. The pigments were transferred to hexane and washed with water and saturated NaCl solution to give an orange solution, λ_{max}(hexane)/nm 426sh 449 476, %III/II 26, $\%A_B/A_{II}$[10] 14; HPLC (System 1) R_T 16–33 min. Preparative TLC developed with 10% acetone in hexane and eluted with acetone gave two main products:

4-Azido-β,β-carotene (14, 38% yield). R_F 0.85–0.96; λ_{max}(acetone)/nm 426sh 451 476, %III/II 6; HPLC (System 1) R_T = 25.8 (9%, λ_{max}/nm 420sh 445 470; %III/II 17; 13-*cis* **14**), R_T = 26.5 (9%, λ_{max}/nm 420sh 444 472; **14**), R_T = 30.5 (54%, λ_{max}/nm 426sh 452 480; %III/II 34; all-*trans* **14**), R_T = 32.8 (11%, λ_{max}/nm 420sh 448 473; **14**), R_T = 34.4 (4%, λ_{max}/nm 420sh 448 472; **14**); δ_H(400 MHz, $CDCl_3$) 1.04–1.07 (12H, 16/17/16′/17′-H), 1.26 (lipid), 1.47 (2-H), 1.49 (2′-H), 1.63 (3′-H), 1.67 (2-H), 1.73 (3H, 18′-H), 1.84 (3H, 18-H), 1.89 (3-H), 1.95 (3-H), 1.98 (12H, 19/20/19′/20′-H), 2.03 (4′-H), 3.72 (1H, 4-H), 6.13–6.21 (6H, 7/8/10/7′/8′/10′-H), 6.27 (2H, 14/14′-H), 6.36 (1H, 12′-H, $J_{11′,12′}$ = 14.9 Hz), 6.39 (1H, 12-H, $J_{11,12}$ = 14.6 Hz), 6.63–6.70 (4H, 11/15/11′/15′-H); *m/z* (EI) 578 (10%, M + 1), 577 (M^+, 21), 549 (31, M − N_2), 536 (32), 535 (51, M − N_3), 534 (100, M − HN_3), 442 (21, M − 92 − HN_3).

Attempted reduction with $SnCl_2$[6] failed to give the desired 4-amino-β,β-carotene.

4,4′-Diazido-β,β-carotene (15, 53% yield). R_F 0.70–0.85; λ_{max}(acetone)/nm 426sh 451 478, %III/II 20; HPLC (System 1) R_T = 22.5 (16%, λ_{max}/nm 420sh 443 469; %III/II 17; 13-*cis* **15**), R_T = 25.4 (60%, λ_{max}/nm 424sh 451 478; %III/II 41; all-*trans* **15**), R_T = 29.4 (11%, λ_{max}/nm 422sh 446 473; %III/II 41; 9-*cis* **15**); δ_H(400 MHz, $CDCl_3$) 1.04 (6H, 16/17/16′/17′-H), 1.06 (6H, 16/17/16′/17′-H), 1.26 (lipid), 1.48 (2/2′-H), 1.67 (2H, 2/2′-H), 1.84 (6H, 18/18′-H), 1.88 (3/3′-H), 1.96 (3/3′-H), 1.98 (19/20/19′/20′-H), 3.72 (2H, 4/4′-H), 6.09–6.21 (6H, 7/8/10/7′/8′/10′-H), 6.28 (2H, 14/14′-H), 6.39 (2H, 12/12′-H, $J_{11,12} = J_{11′,12′}$ = 15.0 Hz), 6.62–6.69 (4H, 11/15/11′/15′-H); *m/z* (EI) 619 (8%, M + 1), 618 (M^+, 17), 590 (6, M − N_2), 576 (18, M − N_3), 575 (22, M − HN_3), 548 (31, M − N_2 − N_3), 547 (32, M − N_2 − HN_3), 535 (28), 534 (67), 533 (35), 532 (61, M − 2 HN_3), 517 (6), 484 (4, M − 92 − N_3), 456 (14, M − 92 − N_3 − N_2), 442 (14, M − 92 − 2 N_3), 440 (14, M − 92 − 2 HN_3), 43 (100).

Attempted hydrogenation with Lindlar catalyst[5] did not give the desired 4,4′-diamino-β,β-carotene.

Reaction of dication 6 (from 5) with CH_3COSH. β,β-Carotene-4,4′-diol (**5**, 1.7 mg) was dissolved in CH_2Cl_2 (6 ml), and cooled to −10 °C. CF_3COOH (40 μl) was added. After 10 min stirring, a cooled solution of 25% CH_3COSH in acetone (1.25 ml) was added. The pigments were transferred to hexane and washed with 5% $NaHCO_3$, 0.01 M NaOH and saturated NaCl solution. λ_{max}(hexane)/nm 410 441; HPLC (System 1) R_T 15–42 min. Preparative TLC developed with 10% acetone in hexane and eluted with acetone gave five main products, of which all had a distinct smell of the parent thioacid:

Unknown A (12% yield). R_F 0.88–0.96; λ_{max}(acetone)/nm 430sh 453 478sh.

Unknown B (6% yield). R_F 0.77–0.88; λ_{max}(acetone)/nm 429sh 453 479sh.

4-Acetylthio-β,β-carotene (16, 17% yield). R_F 0.69–0.77; λ_{max}(acetone)/nm 429sh 453 477, %III/II 2; HPLC (System 1) R_T = 27.2 (10%, λ_{max}/nm 420sh 446 472; 13-*cis* **16**), R_T = 28.8 (4%), R_T = 32.2 (54%, λ_{max}/nm 428sh 452 480; %III/II 24, all-*trans* **16**), R_T = 35.0 (14%, λ_{max}/nm 422sh 448 475; **16**); *m*/*z* (EI) 611 (12%, M + 1), 610 (M^+, 27), 536 (79), 535 (60), 534 (100, M − CH_3COSH), 533 (10), 532 (17), 456 (17), 442 (20, M − 92 − CH_3COSH).

4′-Acetylthio-β,β-caroten-4-ol (18, 19% yield). R_F 0.60–0.69; λ_{max}(acetone)/nm 429sh 455 478, %III/II 1; HPLC (System 1) R_T = 31.0 (5%, λ_{max}/nm 422sh 446 470sh; **18**), R_T = 31.3 (7%, λ_{max}/nm 424sh 447 474sh; **18**), R_T = 35.3 (68%, λ_{max}/nm 428sh 454 479, %III/II 21; all-*trans* **18**), R_T = 38.6 (5%, λ_{max}/nm 424sh 449 476; %III/II 24; **18**), R_T = 39.2 (5%, λ_{max}/nm 424sh 449 476; %III/II 22; **18**); *m*/*z* (EI) 627 (1.5%, M + 1), 626 (M^+, 3.1), 610 (12), 608 (11, M − H_2O), 536 (36), 535 (36, M − H_2O − CH_3COS), 534 (68, M − H_2O − CH_3COSH), 533 (19), 532 (30), 518 (8), 456 (6), 442 (13, M − 92 − H_2O − CH_3COSH), 91 (100).

4,4′-Diacetylthio-β,β-carotene (17, 17% yield). R_F 0.47–0.60; λ_{max}(acetone)/nm 428sh 454 479, %III/II 1; HPLC (System 1) R_T = 25.2 (13%, λ_{max}/nm 420sh 446 471; %III/II 8; 13-*cis* **17**), R_T = 28.9 (65%, λ_{max}/nm 426sh 453 480; %III/II 23; all-*trans* **17**), R_T = 33.0 (9%, λ_{max}/nm 424sh 448 472; %III/II 28; 9-*cis* **17**); δ_H(400 MHz, $CDCl_3$) 1.03 (16/17/16′/17′-H), 1.05 (16/17/16′/17′-H), 1.50 (2/2′-H), 1.65 (2/2′-H), 1.75 (3/3′-H), 1.96 (6H, 19/19′-H), 1.98 (6H, 20/20′-H), 2.13 (3/3′-H), 4.14 (2H, 4/4′-H), 6.11–6.19 (6H, 7/8/10/7′/8′/10′-H), 6.26 (2H, 14/14′-H), 6.37 (2H, 12/12′-H, $J_{11,12} = J_{11',12'}$ = 14.9 Hz), 6.61–6.68 (4H, 11/15/11′/15′-H), NMR spectrum shows low purity; *m*/*z* (EI) 685 (6%, M + 1), 684 (M^+, 12), 610 (16), 609 (18, M − CH_3COS), 608 (13, M − CH_3COSH), 536 (19), 535 (31), 534 (58), 533 (20), 532 (23, M − CH_3COSH), 442 (20), 43 (100). Attempted reductions of **17** with $LiAlH_4$ and $LiEt_3BH$ failed to give the desired β,β-carotene-4,4′-dithiol.

Hydrolysis with 10% KOH in methanol of a crude reaction mixture dissolved in diethyl ether gave, after aqueous work-up, mainly isomerised 4′,5′-didehydro-4,5′-*retro*-β,β-carotene (**8**), λ_{max}(diethyl ether)/nm 445sh 467 494, %III/II 4; HPLC (System 1) R_T = 44.3 (5%, λ_{max}/nm 440 462 492; %III/II 41; **8**), R_T = 46.4 (7%, λ_{max}/nm 440 462 492; %III/II 42; **8**), R_T = 48.0 (10%, λ_{max}/nm 440 466 495; %III/II 38; Neo D **8**), R_T = 51.2 (5%, λ_{max}/nm 441 466 495; %III/II 43; **8**), R_T = 51.9 (6%, λ_{max}/nm 445 470 500; %III/II 53; Neo B **8**), R_T = 56.6 (20%, λ_{max}/nm 446 470 499; %III/II 43; Neo A **8**), R_T = 61.8 (24%, λ_{max}/nm 447 473 504; %III/II 48; all-*trans* **8**); *m*/*z* (EI) 536 (17%, M + 2), 535 (45, M + 1), 534 (M^+, 100), 442 (16, M − 92), 428 (17, M − 106).

Treatment of 4,4′-diacetoxy-β,β-carotene (12) with CF_3COOH

For NMR analysis at −20 °C of dication **6**, see ref. 4.

Reaction of dication 6 (from 12) with water. 4,4′-Diacetoxy-β,β-carotene (**12**, 0.4. mg) was dissolved in CH_2Cl_2 (5 ml), and cooled to −10 °C. CF_3COOH (25 μl) was added, whereupon the reaction mixture turned blue. A cooled solution of 25% H_2O in acetone (10 ml) was added after 10 min stirring, giving an orange solution. The pigments were transferred to hexane and washed with water and saturated NaCl solution. Pigment recovery 95% ($E_{1\%,\ 1\ cm}$ 2500), λ_{max}(hexane)/nm 424sh 448 475, %III/II 23; HPLC (System 1) R_T = 10.5 (5%, λ_{max}/nm 424sh 445 474; %III/II 70; **5**), R_T = 11.8 (62%, λ_{max}/nm 426sh 450 477; %III/II 30; all-*trans* **5**), R_T = 16.1 (5%, λ_{max}/nm 426sh 452 480; %III/II 97; unidentified carotenoid), R_T = 17.0 (5%, λ_{max}/nm 424sh 447 476; %III/II 83; 9-*cis* **5**), R_T = 21.6 (6%, λ_{max}/nm 483; mixture).

Treatment of β,β-carotene-4,4′-diol (5) with CF_3SO_3H

VIS/NIR and NMR experiments at −15 °C. β,β-Carotene-4,4′-diol (**5**, *ca.* 10 μg) was dissolved in CH_2Cl_2 containing CF_3SO_3H (0.011 M) at −15 °C. The VIS/NIR spectrum was recorded as described above. λ_{max} 1020 nm was measured, with 18% loss of intensity observed during 1 h. However, the total intensity decay after 21 h storage at −20 °C was not greater than 22%.

For NMR analysis at −20 °C see ref. 4.

Reaction of dication 7 (from 5) with water. β,β-Carotene-4,4′-diol (**5**, 1.3 mg) was dissolved in CH_2Cl_2 (6 ml), and cooled to −15 °C. CF_3SO_3H (5 μl), dissolved in CH_2Cl_2 (2 ml), was added, whereupon the reaction mixture turned blue. An aliquot was withdrawn and examined by VIS/NIR, showing λ_{max} 1010–1020 nm. However, the colour changed to pale yellow upon dilution prior to VIS/NIR. The reaction mixture was stirred for 10 min, with no colour changes observed. A cooled solution of 25% H_2O in acetone (8 ml) was added, and the pigments were transferred to hexane and washed with water and saturated NaCl solution. A green colour appeared during evaporation. *N*-Ethyldiisopropylamine (4 drops) was added and the yellow colour reappeared. Pigment recovery 62% ($E_{1\%,\ 1\ cm}$ 2500), λ_{max}(hexane)/nm 447; HPLC (System 2) R_T 9–41 min. Products were isolated by preparative TLC developed with 20% acetone in hexane and eluted with acetone to give:

4′,5′-Didehydro-4,5′-retro-β,β-carotene (8, 32%). R_F 0.88–0.96; λ_{max}(acetone)/nm 447 470 497sh.

3,4-Didehydro-4′,9-retro-β,β-caroten-9-ol (19, 19%). R_F 0.78–0.84; λ_{max}(acetone)/nm 421 443 470, %III/II 30; HPLC (System 2) R_T = 25.1 (57%, λ_{max}/nm 419 441 467; %III/II 34; **19**), R_T = 25.6 (27%, λ_{max}/nm 418 442 471; %III/II 53; **19**), R_T = 41.9 (16%, λ_{max}/nm 453 479; %III/II 16; unidentified carotenoid); δ_H (500 MHz, $CDCl_3$) 1.32 (16′/17′-H), 1.54 (2′-H), 1.66 (19-H), 1.89 (18-H), 1.92 (18′-H), 1.98–2.02 (20/19′/20′-H), 2.14 (3′-H), 4.67 (8-H), 5.80 (4/4′-H), 5.96 (7-H), 6.40 (7′-H), 6.80 (8′-H) (Impure); *m*/*z* (EI) 551 (45, M + 1), 550 (M^+, 100), 458 (19, M − 92), 444 (17, M − 106).

An aliquot of product (**19**) was dissolved in pyridine (1.0 ml) and acetic anhydride was added (0.1 ml). Work-up after 18 h and subsequent analysis by TLC revealed no formation of new products.

3,4-Didehydro-4′,7-retro-β,β-caroten-7-ol (20, 11%). R_F 0.63–0.69; λ_{max}(acetone)/nm 434sh 456 484sh; HPLC (System 2) R_T = 23.5 (44%, λ_{max}/nm 430sh 452 483; %III/II 28; **20**), R_T = 24.2 (53%, λ_{max}/nm 435 458 485; %III/II 45; **20**); *m*/*z* (EI) 551 (1.8%, M + 1), 550 (M^+, 5.6), 548 (2.1), 534 (0.3), 458 (0.3, M − 92), 41 (100).

Mixture of unknowns (21%). R_F 0.34–0.49; λ_{max}(acetone)/nm 447.

4′-Hydroxy-β,β-caroten-4-one (21, 18%). R_F 0.26–0.31; λ_{max}(acetone)/nm 455, %III/II 0; HPLC (System 2) R_T = 9.7 (64%, λ_{max}/nm 462; %III/II 0; **21**), R_T = 11.2 (32%, λ_{max}/nm 449, %III/II 0; unidentified carotenoid); *m*/*z* (EI) 564 (1.9%, M − 2), 549 (8), 548 (20, M − 18), 456 (1.9), 41 (100).

An aliquot of product **21** was reacted with $NaBH_4$,[21] resulting in a hypsochromic shift and increased fine structure. HPLC (System 2)

R_T = 8.0 (47%, λ_{max}/nm 424sh 450 475; %III/II 27; β,β-carotene-4,4′-diol, **5**), R_T = 10.4 (24%, λ_{max}/nm 419sh 445 469; %III/II 32; unidentified carotenoid). **5** was identified by co-chromatography (HPLC) with an authentic sample.

Reaction of dication 7 (from 5) with methanol. β,β-Carotene-4,4′-diol (**5**, 1.1 mg) was dissolved in CH_2Cl_2 (10 ml), and cooled to −15 °C. CF_3SO_3H (5 µl), dissolved in CH_2Cl_2 (2 ml), was added, giving a blue reaction mixture. After 10 min stirring, methanol (1 ml) was added, whereupon the reaction mixture turned yellow. The pigments were transferred to hexane and washed with 5% $NaHCO_3$, water and saturated NaCl solution. A slightly brownish hue was removed upon addition of *N*-ethyldiisopropylamine (4 drops). Pigment recovery 71% ($E_{1\%,\ 1\ cm}$ 2500), λ_{max}(hexane)/nm 445; HPLC (System 2) R_T 17–42 min. Products were isolated by preparative TLC developed with 10% acetone in hexane and eluted with acetone to give:

4′,5′-Didehydro-4,5′-retro-β,β-carotene (8, 11%). R_F 0.85–0.91; λ_{max}(acetone)/nm 447 469 496sh; HPLC (System 2) R_T = 36.7 (9%, λ_{max}/nm 439 461 489; %III/II 36), R_T = 40.5 (7%, λ_{max}/nm 437sh 457 485; %III/II 19), R_T = 41.4 (32%, λ_{max}/nm 443 465 494; %III/II 24; **8**), R_T = 42.1 (29%, λ_{max}/nm 446 469 499; %III/II 37; **8**), R_T = 48.2 (7%, λ_{max}/nm 445sh 467 499; %III/II 21), R_T = 48.9 (7%, λ_{max}/nm 449sh 473 500; %III/II 20); *m*/*z* (EI) 535 (44%, M + 1), 534 (M^+, 97), 532 (23), 442 (19, M − 92), 428 (20, M − 106), 57 (100).

4-Methoxy-β,β-carotene (22, 8%). R_F 0.71–0.81; λ_{max}(acetone)/nm 450 473sh; HPLC (System 2) R_T = 26.8 (10%, λ_{max}/nm 445; %III/II 0; unidentified carotenoid), R_T = 27.4 (17%, λ_{max}/nm 461; %III/II 0; unidentified carotenoid), R_T = 28.1 (5%, λ_{max}/nm 457; %III/II 0; unidentified carotenoid), R_T = 31.0 (43%, λ_{max}/nm 427sh 450 476; %III/II 11; **22**), R_T = 31.6 (6%, λ_{max}/nm 423sh 446 471; %III/II 16; 9-*cis* **22**), R_T = 39.3 (11%, λ_{max}/nm 450 475sh; unidentified carotenoid); *m*/*z* (EI) 567 (44%, M + 1), 566 (M^+, 100), 565 (33), 564 (64), 535 (25), 534 (49, M − CH_3OH), 532 (25), 474 (17, M − 92), 442 (10, M − CH_3OH − 92).

Co-chromatography (TLC) with authentic **22**[2] gave no separation.

4′,5′-Didehydro-3-methoxy-4,5′-retro-β,β-carotene (23, 21%). R_F 0.56–0.65; λ_{max}(acetone)/nm 448sh 470 500, %II/II 17; HPLC (System 2) R_T = 31.4 (65%, λ_{max}/nm 443 467 496; %III/II 29; **23**), R_T = 32.1 (33%, λ_{max}/nm 446 471 502; %III/II 43; all-*trans* **23**); *m*/*z* (EI) 565 (41%, M + 1), 564 (M^+, 91), 534 (33), 533 (29), 532 (63, M − CH_3OH), 518 (18), 472 (13, M − 92), 458 (13, M − 106), 119 (100).

3′,4′-Didehydro-β,β-caroten-4-ol (24) and β,β-caroten-4-ol (3) (24%). R_F 0.12–0.21; λ_{max}(acetone)/nm 427sh 451 474sh; HPLC (System 2) R_T = 17.3 (5%, λ_{max}/nm 448, %III/II 0; unidentified carotenoid), R_T = 18.1 (36%, λ_{max}/nm 461; %III/II 0; all-*trans* **24**), R_T = 19.0 (11%, λ_{max}/nm 455; %III/II 0; **24**), R_T = 20.8 (38%, λ_{max}/nm 424sh 451 473; %III/II 10; **3**); *m*/*z* (EI) 553 (7%, M + 1, **3**), 552 (M^+, **3**, 18, M + 2, **24**), 551 (13, M + 1, **24**), 550 (M^+, **24**, 26), 536 (25), 535 (34), 534 (79, M − H_2O, **3**), 533 (30), 532 (65, M − H_2O, **24**), 442 (13, M − 92 − H_2O, **3**), 440 (10, M − 92 − H_2O, **24**), 91 (100).

Co-chromatography (HPLC, System 2) with authentic **3** gave no separation at R_T = 20.8.

4′,5′-Didehydro-4,5′-retro-β,β-caroten-3-ol (23b) and 4-methoxy-β,β-caroten-4′-ol (25a) (16%). R_F 0.06–0.12; λ_{max}(acetone)/nm 447sh 469 495sh; HPLC (System 2) R_T = 13.2 (13%, λ_{max}/nm 425sh 449 475; %III/II 22; **25a**), R_T = 20.7 (46%, λ_{max}/nm 446 469 498; %III/II 30; **23b**); MS of mixture containing **23b** and **25a**/**b**, *m*/*z* (EI) 565 (6%), 564 (11, M; **25b**), 551 (6), 550 (6, M; **23b**), 549 (6), 548 (7), 534 (9), 533 (13), 532 (24), 523 (6), 440 (4), 368 (7), 313 (8), 72 (100).

Treatment of ϕ,ϕ-carotene (26) with Lewis acid

UV/VIS, EIMS and ^1H (500 MHz) and ^{13}C (125 MHz) NMR data for ϕ,ϕ-carotene (**26**) were recently published elsewhere.[4]

VIS/NIR experiments. ϕ,ϕ-Carotene (**26**, 0.5 mg) was dissolved in the solvent (0.5 ml) and BF_3-dee (0.25 ml) was added at room temperature. The mixture was diluted and the UV/VIS/NIR spectrum recorded. λ_{max}/nm ($CHCl_3$) 910, 750 (weak) ($t_{½}$/min 10); λ_{max}/nm (CH_2Cl_2) 930, 750 (strong) ($t_{½}$/min 10); λ_{max}/nm (benzene) 730. For VIS/NIR experiments at −20 °C, see ref. 4.

EPR analysis. ϕ,ϕ-Carotene (**26**) was dissolved in $CHCl_3$. The same volume of BF_3-dee was added, and the solution analysed, Fig. 2. After 1 h, the signal was reduced by 35%; line width 14 G.

MS analysis. ϕ,ϕ-Carotene (**26**, 1 mg) was dissolved in $CHCl_3$ (0.4 ml) and BF_3-dee (0.4 ml) added. An aliquot was evaporated with N_2. Adding a drop of xylene facilitated the evaporation. The MS was recorded 15 min after the preparation of the reaction mixture. *m*/*z* (EI) 528 (M, 7%), 526 (M − 2, 9), 511 (2), 448 (2), 263 (8), 187 (11), 173 (70), 133 (31), 69 (23), 55 (27), 28 (100).

Reaction of ϕ,ϕ-carotene (26) cations with H_2O at room temperature. ϕ,ϕ-Carotene (**26**, 1.3 mg) was dissolved in $CHCl_3$ (2 ml) and BF_3-dee (0.54 ml) added. The mixture was flushed with N_2, shaken for 2 min and poured into 20% H_2O in acetone (30 ml). The carotenoids were transferred to hexane. HPLC (System 1) revealed the presence of *E*/*Z* isomerised ϕ,ϕ-carotene (**26**) and two more polar products, λ_{max}/nm (HPLC solvent) 385sh, 405, 430 (10% of total) and λ_{max}/nm (HPLC solvent) 408sh, 425, 455 (20% of total). MS was recorded of the reaction mixture, *m*/*z* (EI) 544 (9%), 528 (100), 513 (46), 395 (25), 355 (12), 315 (11), 275 (15), 211 (16), 173 (42), 133 (92). Test for elimination of allylic OH with 0.03 N HCl in $CHCl_3$[21] was negative.

Reaction of ϕ,ϕ-carotene (26) cations with H_2O at low temperature. In two parallel experiments, ϕ,ϕ-carotene (**26**, 1.0 mg) was dissolved in $CDCl_3$ (0.5 ml) and CH_2Cl_2 (0.5 ml) at −35 °C. To each solution was BF_3-dee (0.5 ml) added, giving a colour change to blue *via* green. After *ca.* 30 min, the reaction mixtures were poured into 20% H_2O in acetone (10 ml), providing yellow solutions. The pigments were transferred to hexane and washed with water. Pigment recovery 35% ($CDCl_3$) and 31% (CD_2Cl_2), $E_{1\%,\ 1\ cm}$ = 2200, λ_{max}/nm (hexane) 426. Both mixtures showed similar chromatographic behaviour by HPLC (System 3), and were combined prior to preparative TLC. Preparative TLC plates were developed with 10% acetone in hexane, and the products were eluted with acetone to give:

ϕ,ϕ-Carotene (26, 7%). R_F = 0.87–0.93; λ_{max}/nm (acetone) 428sh, 452, 478; %III/II 3.

Product 1 (32 isomers, 4%). R_F = 0.70–0.73, 0.77–0.83; λ_{max}/nm (acetone) 386sh, 406, 428; %III/II 17; R_T (System 2) = 19.4 (65%, λ_{max}/nm 383sh, 405, 427; %III/II 37), R_T (System 2) = 23.9 (26%, λ_{max}/nm 409, 429, 457; %III/II 51); *m*/*z* (EI) 545 (13%, M + 1), 544 (M^+, 30), 542 (17), 411 (9), 410 (23), 147 (83), 133 (100).

An aliquot of this TLC fraction was tested for allylic hydroxyl by treatment with 0.03 M HCl in $CHCl_3$,[21] giving no observable change of chromophore in UV/VIS. Likewise, an aliquot was dissolved in CF_3COOH, giving λ_{max}/nm (CF_3COOH) 687. Subsequent neutralisation with 10% KOH in methanol recovered the carotenoid spectrum, λ_{max}/nm 412.

Product 2 (32 isomer, 5%). R_F = 0.47–0.53; λ_{max}/nm (acetone) 384sh, 406, 425; %III/II 2; R_T (System 2) = 11.8 (23%, λ_{max}/nm 457), R_T (System 2) = 17.6 (43%, λ_{max}/nm 384sh, 405, 428; %III/II 30), R_T (System 2) = 19.3 (13%, λ_{max}/nm 405sh, 425, 445sh); *m*/*z* (EI) 545 (4%), 544 (12), 542 (24), 410 (9), 133 (100).

An aliquot of this TLC fraction was tested for allylic hydroxyl by treatment with 0.03 M HCl in $CHCl_3$,[21] giving no observable change of chromophore in UV/VIS. Likewise, an aliquot was dissolved in CF_3COOH, giving λ_{max}/nm (CF_3COOH) 605sh, 651, 722. Subsequent neutralisation with 10% KOH in methanol failed to give recovery of carotenoids.

Product 3 (unidentified, 4%). R_F = 0.35–0.43; λ_{max}/nm (acetone) 407; R_T (System 2) = 11.8 (64%, λ_{max}/nm 459), R_T (System 2) = 12.8 (9%, λ_{max}/nm 441); *m*/*z* (EI) 546 (1.5%), 544 (0.3), 530 (14), 413 (11), 412 (33), 410 (15), 133 (100).

7,8-Dihydro-φ,φ-caroten-8-ol (30, 67%). R_F = 0.25–0.32; λ_{max}/nm (acetone) 408sh, 429, 455; %III/II 37; R_T (System 2) = 16.0 (85%, λ_{max}/nm 405sh, 428, 453; %III/II 50), R_T (System 2) = 17.2 (12%, λ_{max}/nm 403sh, 423, 447; %III/II 59); δ_H(400 MHz, $CDCl_3$, Me_4Si) 1.96–2.00 (19/20/20′-H), 2.09 (19′-H), 2.24 (16′-H), 2.27–2.29 (16/17/17′/18″-H), 2.36 (18-H), 2.89 (dd, $^2J_{H,H}$ = 13.7 Hz, $^3J_{H,H}$ = 3.4 Hz, 7-H), 3.03 (dd, $^2J_{H,H}$ = 14.1 Hz, $^3J_{H,H}$ = 9.6 Hz, 7-H), 4.32 (8-H), 6.23 (10′-H), 6.25 (10-H), 6.29 (14/14′-H), 6.35 (12-H), 6.41 (12′-H), 6.54 (11-H), 6.61 (15/15′-H), 6.69 (11′-H), 6.97–7.00 (3/4/3′/4′-H); *m*/*z* (EI) 547 (1.2%, M + 1), 546 (M^+, 3.5), 530 (10), 528 (16, M − H_2O), 133 (100).

7,8-Dihydro-φ,φ-caroten-7,8-diol (31, 13%). R_F = 0.03–0.10; λ_{max}/nm (acetone) 405, 428, 455; HPLC (System 2) R_T = 10.9 (84%, λ_{max}/nm 406sh, 428, 454; %III/II 52), R_T = 11.9 (13%, λ_{max}/nm 403, 423, 448; %III/II 47); *m*/*z* (EI) 562 (M^+, 1.9%), 544 (10, M − H_2O), 412 (24), 410 (20), 133 (100).

Reproducibility of the low temperature experiments. Attempts to reproduce the results from the above experiments in larger scale failed, giving φ,φ-carotene (**26**) as the main product, with only minor amounts converted to other products. An experiment performed at room temperature with commercial $CHCl_3$ (with stabiliser present) as solvent, gave **30** and **26** as the main products in a 2 : 1 ratio. Performed with φ,φ-carotene (**26**) from another source, different reactions were observed in duplicate experiments. The reaction mixtures had λ_{max}/nm (hexane) 379, and evaporation of solvent caused a colour change to green. The orange colour was restored upon the addition of *N*-ethyldiisopropylamine (4 drops). Preparative TLC developed with 10% acetone in hexane and subsequent elution with acetone revealed the presence of numerous compounds with shorter chromophore (λ_{max}/nm (acetone) 360–385), covering a wide range of polarities (R_F = 0.07–0.80). Due to the low amounts of carotenoids present, characterisation was only attempted for two of these products.

Product A (31 isomer, 19%). R_F = 0.15–0.22; λ_{max}/nm (acetone) 380; R_T (System 2) = 9.4 (52%, λ_{max}/nm 381, 395sh); *m*/*z* (EI) 563 (1.0%, M + 1), 562 (M^+, 2.2), 560 (2), 544 (8, M − H_2O), 526 (6, M − 2 H_2O), 133 (100).

Product B (31 isomer, 24%). R_F = 0.07–0.14; λ_{max}/nm (acetone) 380; R_T (System 2) = 7.6 (53%, λ_{max}/nm 382, 396sh), R_T = 8.2 (42%, λ_{max}/nm 381, 396sh); *m*/*z* (EI) 563 (2.6%, M + 1), 562 (M^+, 5.6), 544 (10, M − H_2O), 526 (13, M − 2 H_2O), 347 (24, M − $C_{15}H_{19}O$), 133 (100).

A reaction was also performed in $CHCl_3$ with the ordinary stabiliser (ethanol) replaced with CD_3OD. The reaction mixture was worked up as described above. The compound of interest, 7,8-dihydro-φ,φ-caroten-8-ol (**30**), was isolated by preparative TLC.

7,8-Dihydro-φ,φ-caroten-8-ol (30, 11%). R_F = 0.29–0.39; λ_{max}/nm (acetone) 406sh, 428, 453; %III/II 11; *m*/*z* (EI) 547 (7.4%, M + 1), 546 (M^+, 14.6), 529 (13), 528 (26, M − H_2O), 436 (6), 413 (10), 133 (100). M + 1/M^+ = 0.51.

A similar study with CD_3OD added in 10-fold excess to the ethanol stabiliser already present, was undertaken with β,β-carotene (**1**). The dication formed by the addition of BF_3-dee, was quenched with CH_3ONa as described previously.[2] The compound of interest, 8-methoxy-7,8-dihydro-β,β-carotene was isolated with preparative HPLC. Full characterisation is presented elsewhere.[2]

8-Methoxy-7,8-dihydro-β,β-carotene (6%). R_T (System 3) = 30.9 min; *m*/*z* (EI) 569 (5.2%, M + 1), 568 (M^+, 7.7), 538 (10), 536 (17, M − CH_3OH), 434 (15), 431 (44), 119 (100). M + 1/M^+ = 68%.

Treatment of ψ,ψ-carotene (lycopene, 27) with Lewis acid

VIS/NIR experiments. ψ,ψ-Carotene (27, 1.0 mg) was dissolved in the solvent (1.0 ml) and BF_3-dee (0.5 ml) added at room temperature. The mixture was diluted and the UV/VIS/NIR spectrum recorded at room temperature. λ_{max}/nm ($CHCl_3$) 940 ($t_{½}$/h 1.5), λ_{min}/nm ($CHCl_3$) 1413; λ_{max}/nm (CH_2Cl_2) 920 ($t_{½}$/h 7.5); λ_{max}/nm (benzene) 850 ($t_{½}$/h 1).

EPR analysis. ψ,ψ-Carotene (**27**) was dissolved in $CHCl_3$, the same volume of BF_3-dee was added, and the solution analysed, Fig. 3, line width 13 G. After 45 min the signal intensity was reduced by 30%.

MS analysis. ψ,ψ-Carotene (**27**, 1.0 mg) was dissolved in $CHCl_3$ (1 ml), and BF_3-dee (1 ml) added. An aliquot was evaporated with N_2 and the MS was recorded 15 min after the preparation of the reaction mixture. *m*/*z* (EI) 540 (M + 4, 28%), 538 (M + 2, 72), 536 (M, 91), 534 (M − 2, 52), 532 (M − 4, 23), 521 (13), 493 (7), 347 (10), 267 (19), 233 (17), 145 (35), 105 (33), 91 (39), 28 (100).

NMR analysis. The cationic mixture was prepared in $CDCl_3$ as for the UV/VIS/NIR experiment in a glove box under argon. No meaningful NMR spectrum was achieved at −20 °C.[4]

Reaction of ψ,ψ-carotene (27) cations with H_2O. ψ,ψ-Carotene (27, 3.4 mg) was dissolved in $CHCl_3$ (3 ml), BF_3-dee (1 ml) was added, and the reaction mixture shaken for 2 min before addition of 20% H_2O in acetone (40 ml), whereupon the reaction mixture turned yellow. The products were transferred to hexane and analysed by UV/VIS, HPLC (System 1) and MS. λ_{max}/nm (HPLC solvent) 410, 434, 454 (pigment recovery 38%). MS of product mixture: *m*/*z* (EI) 568 (1%), 552 (5), 536 (100), 534 (43), 444 (12), 430 (6), 347 (6), 281 (6), 223 (14), 197 (23), 171 (24), 159 (3), 157 (34), 145 (47), 119 (42). HPLC (System 1) showed 7 major peaks with VIS spectra corresponding to heptaene chromophore (1 peak, 13%), octaene (2 peaks, 20%), nonaene (2 peaks, 13%), decaene (2 peaks, 6%) and undecaene (2 peaks, 2%). Preparative TLC on silica (5% acetone in heptane) showed 3 zones.

Fraction A. R_F = 0.88–0.94; λ_{max}/nm (acetone) 436, 456 (74% of total); HPLC (System 1) comprised 9 peaks with VIS data compatible with mainly decaene and undecaene chromophores.

Fraction B. R_F = 0.73–0.81; λ_{max}/nm (acetone) 406, 430, 456 (8%); HPLC (System 1) showed 6 peaks with VIS data compatible with nonaene and decaene chromophores; *m*/*z* (EI) 568 (M_1, 6%), 554 (M_2, 32%), 552 (M_1 − 16, M_2 − 2, 13), 536 (M_2 − 18, 19), 462 (M_1 − 106, M_2 − 92, 4), 410 (25), 368 (lipid, 11), 267 (11), 239 (15), 197 (23), 185 (26), 173 (28), 157 (37), 145 (50), 95 (51), 81 (53), 69 (68), 43 (100).

Fraction C. R_F = 0.47–0.56; λ_{max}/nm (acetone) 450, 464, 500 (17%); HPLC (System 1) exhibited 5 peaks with decaene and tentatively monocyclic undecaene chromophores; *m*/*z* (EI) 568 (M_1, 8%), 554 (M_2, 58), 552 (M_1 − 16, M_2 − 2, 20), 537 (33), 536 (M_2 − 18, 71), 534 (19), 462 (M_1 − 106, M_2 − 92, 12), 444 (M_2 − 18 − 106, 15), 410 (19), 333 (9), 281 (12), 239 (18), 223 (23), 209 (35), 197 (43), 171 (49), 159 (62), 157 (67), 145 (93), 119 (99), 69 (100).

Fraction C was tested for allylic hydroxyl by treatment with 0.03 N HCl.[21] HPLC analysis revealed no products with prolonged chromophore.

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