Geir Kildahl-Andersen

Polyenyl cations and radical cations – synthesis, spectroscopic properties and reactions

Thesis for the degree philosophiae doctor

Trondheim, November 2007

Norwegian University of Science and Technology
Faculty of Natural Sciences and Technology
Department of Chemistry

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Preface

The work presented in this thesis was carried out at the Department of Chemistry at the Norwegian University of Science and Technology (NTNU), and was funded by The Research Council of Norway. I am thankful for the continuous support and advice from my supervisors Prof. Thorleif Anthonsen and Prof. em. Synnøve Liaaen-Jensen, and feel honoured to be part of a long tradition of natural products research at NTH/AVH and its present incarnation as NTNU.

I would like to acknowledge the contributions from all my co-authors in the articles given as appendix to this thesis. In addition to my supervisors, some others deserve particular mention.

Prof. Jostein Krane turned my attention towards the carotenoid field in the first place, when I approached him in 2002 and asked about the possibility of doing a Master thesis in NMR and organic chemistry. His valuable advice and involvement in early parts of this project is greatly appreciated, and his premature departure from the Faculty is regrettable.

Dr. ing. Bjart Frode Lutnæs, former graduate student of the natural products chemistry group, has been an excellent tutor and co-worker, as well as a social resource. I like to think of us as an efficient team.

During my one-month stay at Prof. Lowell D. Kispert's group at University of Alabama, graduate student Mrs. Ligia Focsan had to put up with me living in her living room for the entire period. Such sacrifices in the name of science should not go by unnoticed. The side-effect of being included in the Romanian student community in Tuscaloosa made it an even more interesting experience.

The majority of the mass spectral data presented in the articles has been acquired by Associate prof. Helge Kjøsen and Mrs. Julie Jackson, for which I am grateful. In addition, I have had many valuable discussions with other members of the academic staff at the Department of Chemistry over the years.

Lastly, I would like to thank my present and former colleagues among the Ph.D. students at our Department. You have managed to create a supportive environment and a social network which have been of great importance to me. Thank you!

Geir Kildahl-Andersen
Trondheim, November 2007

Contents

Abstract

In the present work, polyene precursors for the formation of charged polyenyl species were synthesised. Their conversion to charge delocalised radical cations and diamagnetic mono- and dications was carried out by treatment with Brønsted and Lewis acids. Reaction conditions were optimised to achieve sufficient stability of the charged polyenes for characterisation by modern spectroscopic methods; in particular near-infrared absorption spectroscopy (NIR), two-dimensional nuclear magnetic resonance (2D NMR), electron paramagnetic resonance (EPR) and dynamic light scattering.

The full assignment of ^{13}C NMR chemical shifts of mono- and dications was used as basis for charge distribution estimates in charge delocalised polyenes. Coulombic repulsions are dominant features in dicationic systems, whereas monocations show a more even distribution of charge throughout the polyene system. The size limit of charge delocalisation regions, suggested by current theories, has been investigated using an empirical approach.

Treatment of the cationic polyenes with nucleophiles gave conversion to neutral compounds, which were characterised by mass spectrometry (MS) and ultra-violet/visible absorption spectroscopy (UV/Vis), in addition to NMR. In most cases, the identified neutral compounds supported the structural characterisation of their charged precursors.

The work is of relevance to the classical blue colour reactions of polyenes developed in the early 20^{th} century. The chemistry behind these reactions has previously not been elucidated. Only during the last 15-20 years has the instrumental methods necessary for the study of this chemistry been available.

In current studies on the colouration mechanism of the carotenoproteins present in external tissues of some marine organisms, a polarisation of the polyene chain appear to be of importance. Moreover, radical cations of polyenes are unstable intermediates in Nature, where they are shown to be of importance in photosynthetic processes in green plants and bacteria.

The delocalised polyenylic cations studied here, representing a new direction of structural studies in the carotenoid/retinoid field, are of interest in a wider context for carbocation structure and stability in basic organic chemistry and the various applied aspects outlined above.

List of appended papers

Paper I
Bjart Frode Lutnaes, Liv Bruås, Geir Kildahl-Andersen, Jostein Krane and Synnøve Liaaen-Jensen
The charge delocalised β,β-carotene dication – preparation, structure elucidation by NMR and reactions with nucleophiles
Org. Biomol. Chem., 2003, **1**, 4064-4072.

Paper II
Geir Kildahl-Andersen, Bjart Frode Lutnaes, Jostein Krane and Synnøve Liaaen-Jensen
Structure Elucidation of Polyene Systems with Extensive Charge Delocalization – Carbocations from Allylic Carotenols
Org. Lett., 2003, **5**, 2675-2678.

Paper III
Bjart Frode Lutnaes, Geir Kildahl-Andersen, Jostein Krane and Synnøve Liaaen-Jensen
Delocalized Carotenoid Cations in Relation to the Soliton Model
J. Am. Chem. Soc., 2004, **126**, 8981-8990.

Paper IV
Geir Kildahl-Andersen, Thorleif Anthonsen and Synnøve Liaaen-Jensen
Longer polyenyl cations in relation to soliton theory
Org. Biomol. Chem., 2007, **5**, 2803-2811.

Paper V
Geir Kildahl-Andersen, Liv Bruås, Bjart Frode Lutnaes and Synnøve Liaaen-Jensen
Nucleophilic reactions of charge delocalised carotenoid mono- and dications
Org. Biomol. Chem., 2004, **2**, 2496-2506.

Paper VI
Geir Kildahl-Andersen, Bjart Frode Lutnaes and Synnøve Liaaen-Jensen
Protonated canthaxanthins as models for blue carotenoproteins
Org. Biomol. Chem., 2004, **2**, 489-498.

Paper VII
Geir Kildahl-Andersen, Thorleif Anthonsen and Synnøve Liaaen-Jensen
Novel diapocarotenoid dications with VIS/NIR absorption
Tetrahedron Lett., 2006, **47**, 4693-4696.

Paper VIII
Geir Kildahl-Andersen, Tatyana A. Konovalova, A. Ligia Focsan, Lowell D. Kispert, Thorleif Anthonsen and Synnøve Liaaen-Jensen
Comparative studies on radical cation formation from carotenoids and retinoids
Tetrahedron Lett., 2007, **48**, 8196-8199.

Paper IX
Geir Kildahl-Andersen, Stine Nalum Naess, Petter B. Aslaksen, Thorleif Anthonsen and Synnøve Liaaen-Jensen
Studies on the mechanism of the Carr-Price blue colour reaction
Org. Biomol. Chem., 2007, **5**, 3027-3033.

Abbreviations and symbols

DAD	Diode-Array Detector
EPR	Electron Paramagnetic Resonance
HPLC	High-Performance Liquid Chromatography
IUPAC	International Union of Pure and Applied Chemistry
$^{n}J_{X,Y}$	Coupling constant through n bonds between nuclei X and Y
l	Soliton half-width
λ_{max}	Absorption maximum
m	Number of sp^2 hybridised carbons
NIR	Near Infrared
NMR	Nuclear Magnetic Resonance
TLC	Thin Layer Chromatography
$t_{½}$	Half-life
THF	Tetrahydrofuran
UV	Ultra Violet
Vis	Visible light

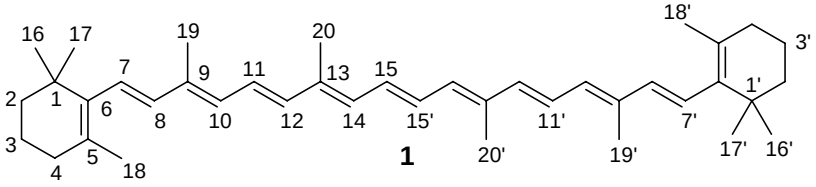
1. Introduction

1.1 Polyenes

Although by definition, any organic compound containing more than one carbon-carbon double bond may be described as a polyene, in normal use the term describes a class of compounds containing fragments of alternating single and double bonds.[1] In Nature, such systems are widespread, the carotenoids being the most prominent group, exemplified by β,β-carotene (**1**). A semirational nomenclature for carotenoids is defined by IUPAC, and the numbering of the carotenoid skeleton given on structure **1**.[2] The first isolation of carotene was carried out in 1831. The development of chromatographic techninques around 1900 by Tswett made it possible to seperate leaf pigments into different classes, such as chlorophylls, carotenes (hydrocarbons) and xanthophylls (oxygen containing carotenoids).[3] Together with chlorophylls, the carotenoids are important for light harvesting and photoprotection in photosynthesis. The presence of carotenoid radical cations in Photosystem II in green plants has been demonstrated.[4] Formation of carotenoid radical cation intermediates in phototropic bacteria has also been observed.[5]

The recognition of the carotenoids as polyenes came in 1928, by catalytic hydrogenation experiments. At the same time a clear link between the intense colour of the polyenes and the extended conjugated double bond system (the chromophore) was established by chemical synthesis of a homologous series of ω,ω'-diphenylpolyenes.[6] Finally, a few years later, Karrer and co-workers established the structure of β,β-carotene (**1**).[7] Today, more than 750 naturally occurring carotenoids are known.[8]

In animals, some carotenes are cleaved to form Vitamin A, or retinol (**2**). The structure determination was again performed by Karrer's group.[9] Retinol (**2**) is essential for vision, where its corresponding aldehyde, retinal, forms a Schiff base with the rhodopsin photoreceptor of the retina.[10] The shorter polyene system, compared to the carotenoids, gives retinol (**2**) a pale yellow colour. In natural products chemistry, both the retinoids and carotenoids belong to the larger class of terpenoids, formally built up from isoprene units. In this work, the polyenes studied have terpenoid-like structure.



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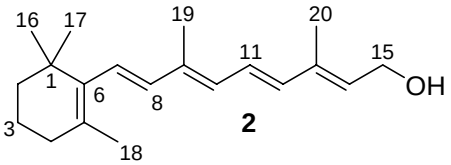
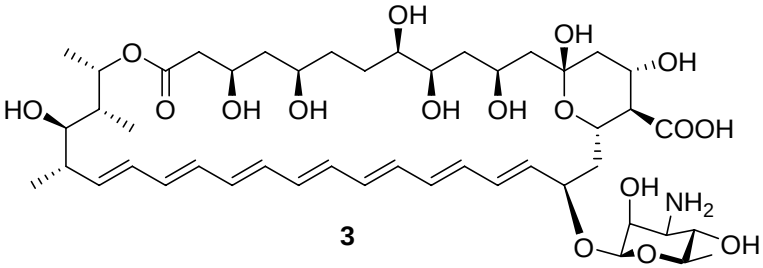


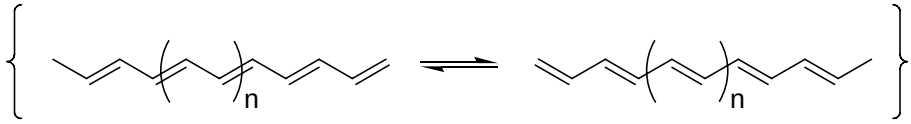
2

An unrelated class of naturally occurring polyenes are found among the antifungal macrolide antibiotics. Amphotericin B (**3**), isolated from *Streptomyces nodosus*, is an example, and has been used in treatment of fungal infections for over 40 years.[11]



3

Of the many synthetic polyenes reported in the literature, the polymer *trans*-polyacetylene (**4**) is of particular relevance to the present work. The interconversion between the two degenerate states of lowest energy leads to bond-inversion regions, called solitions.[12]



4

1.2 Early colour reactions of carotenoids and retinoids

The first identification of retinol (**2**) as a specific substance different from the provitamin A carotenoids, was established by a colour reaction. It had long been known that when carotenoids was treated with the strong acid H_2SO_4, a colour change to blue occurred.[13] Cod-liver oils, high in Vitamin A content, gave bright purple colours when treated with the same reagent. It was correctly inferred that the different colour corresponded to another pigment class.[14]

The rapid fading of the colour prevented the use of the colour reaction with the sulfuric acid reagent for measuring Vitamin A activity by photometric methods. Arsenic trichloride was suggested as an alternative reagent,[15] but handling of liquid arsenic trichloride in routine analysis was problematic. As an alternative, Carr and Price suggested the use of antimony trichloride in a saturated chloroform solution.[16] This was known as the Carr-Price reaction and became the standard method for quantitative analysis of Vitamin A. Also this method suffered from low stability of the blue product formed in the reaction.

Later, Zechmeister and co-workers investigated the formation of blue complexes between carotenoids and boron trifluoride etherates, and cleavage products upon addition of nucleophiles.[17] Furthermore, strongly coloured complexes of carotenoids treated with iodine has been known since the 19th century.[18] A specific blue colour reaction for identification of epoxide functions in carotenoids using protic acids has also been developed.[19]

1.3 Structure and stability of carbocations, free radicals and radical cations

The classical colour reactions mentioned above are based on the presence of a delocalised positive charge in the polyene system, see Section 1.4. The existence of stable carbocations was shown already at the start of the 20th century, by the preparation of triarylmethyl cations. However, alkyl cations was for a long time considered to be unstable and short-lived. Not until the developement of very strong acids in non-nucleophilic media, superacids, were stable alkyl carbocations achieved.[20] Investigation of the *tert*-butyl cation by NMR revealed the central carbon atom to be deshielded by more than 300 ppm compared to the corresponding alkane, whereas the isopropyl cation featured a proton resonance at 13 ppm for the central hydrogen. The large change in resonance frequency was also indicative of a rehybridisation of the charge-bearing carbon atom from sp^3 to sp^2,[20] meaning that the carbocation has a trigonal planar geometry, with perpendicular vacant *p*-orbitals, Figure 1 a). This is generally the case.

Carbocations are stabilised by electron-donating substituents, such as alkyl groups and heteroatoms with lone pairs.[21] For alkyl cations, the stability therefore follows the trend *tert* > *sec* > *prim* > methyl. Delocalisation of charge (resonance) further stabilises the cation. This is the case for benzyl and allyl cations, Figure 1 b), as well as for the triarylmethyl cations mentioned. Formation of aromatic systems, according to Hückel's rule, offer further stabilisation.[21]

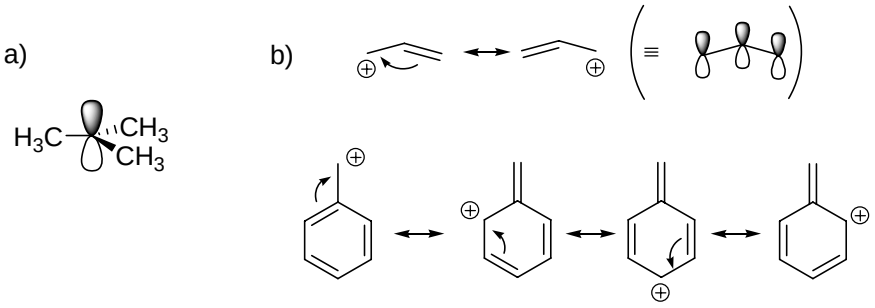


Figure 1. a) Structure of *tert*-butyl cation. b) Charge delocalisation by resonance in the allyl and benzyl cations.

Simple alkyl radicals, *i.e*. species with an unpaired electron, are generally of pyramidal structure, although with small inversion barriers.[21] As for carbocations, alkyl substitution provides stabilisation of the free radical. Allyl and benzyl radicals are planar in order to maximise stabilisation by electron delocalisation. This may be illustrated by the resonance forms depicted in Figure 2. Both electron-attracting and donating substituents can stabilise free radicals.[21]

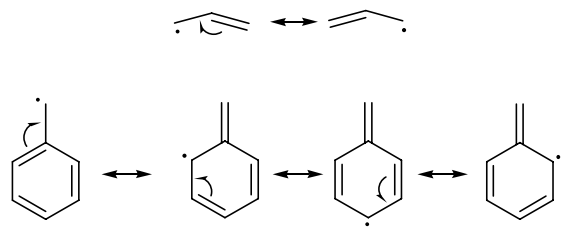


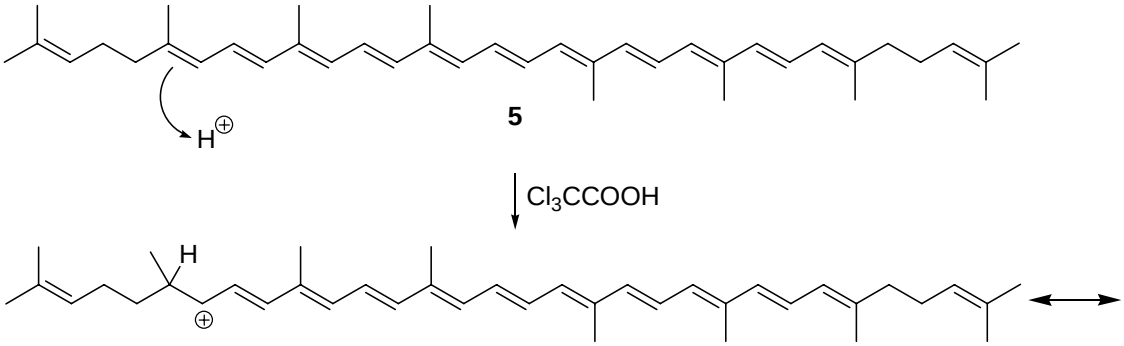
Figure 2. Resonance stabilisation of allyl and benzyl radicals.

Delocalisation of charge and unpaired electrons may take place within the same system of conjugated sp^2-hybridised carbon atoms, and makes formation of delocalised radical cations and radical anions possible. A radical cation exhibits properties of both a free radical and a cation.

1.4 Reaction modes of polyenes treated with acid

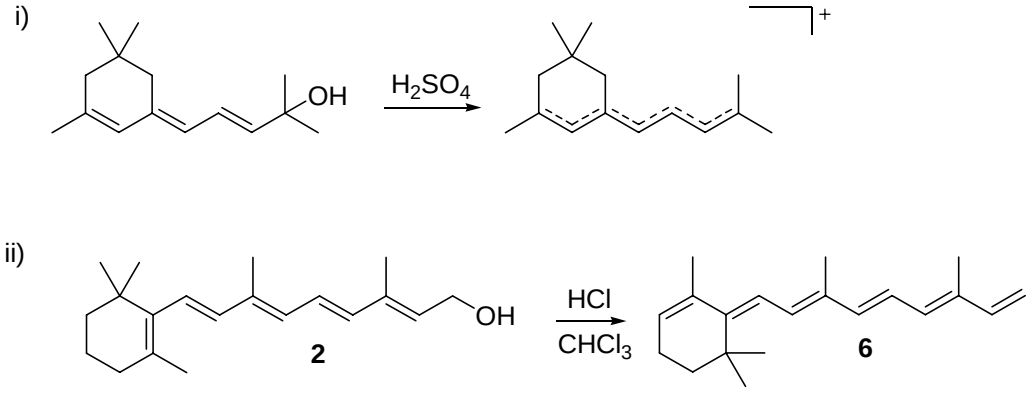
A formal division should be made between the different reagents used for the classical colour reactions, Section 1.2. They are all electron-deficient, which makes them Lewis acids. Boron, from group III in the periodic table, has three valence electrons, and by covalent binding to three fluorine atoms are still two electrons short of forming a full octet in the outer electron shell. The complex between the gas boron trifluoride and ethers formed by electron pair donation of the ether gives relatively stable solutions of the electron deficient BF_3 molecule. Arsenic and antimony trichlorides are weak Lewis acids, despite the full octet of electrons in their outer shell.[22] Iodine forms charge-transfer complexes with a wide variety of electron donors.[22] Only sulfuric acid of the reagents treated in Section 1.2 has Brønsted acidity, *i. e.* ability to donate protons. The reaction modes of protic acids versus Lewis acids should be looked upon separately.

For the reaction of protic acids with simple hydrocarbon polyenes, such as β,β-carotene (**1**), protonation of the polyene chain to form cationic polyenylic products with the charge delocalised in the polyene system was suggested at an early stage.[23] For the acyclic carotene lycopene (**5**, ψ,ψ-carotene), the experimental data was compatible with protonation at the terminal position,[24] thus giving the positive charge the longest possible polyene region for delocalisation, as depicted in Scheme 1. This reaction mode of hydrocarbon polyenes when treated with protic acids are found throughout the literature. However, there are also alternative pathways.



Scheme 1

If the polyene in question possesses a leaving group in allylic position, loss of the leaving group may take place instead of, or in addition to, direct protonation. Depending on the conditions, the reaction may stop at the cation stage, exemplified by reaction i) in Scheme 2,[25] or proceed to form the elimination product, as shown in the preparation of anhydroretinol (**6**) from retinol (**2**), reaction ii).[26,27]



Scheme 2

For Lewis acids, analogous reaction pathways with polyenes may occur. In additon, removal of single electrons by red-ox reactions are observed.[28] This results in the formation of radical cations, in which the remaining unpaired electron and the positive charge are delocalised in the polyene system. In contrast to the other pathways, no rehybridisation of any carbon atoms take place during such oxidations.

1.5 Present work

The polyenes studied in the present work fall into four main categories; i) C_{40} carotenoids, ii) carotene-like polyenes with elongated chromophores, iii) symmetrical diapocarotenoids with shorter polyene systems, and iv) retinoids (15-apocarotenoids or Vitamin A derivatives). Polyenyl cations and radical cations have been prepared from both hydrocarbon polyenes and more functionalised polyenes. Systems with allylic leaving groups or allylic carbonyl groups are included.

Preparation of charged polyenes by the action of protic acids was achieved by treatment with trifluoroacetic acid or trifluoromethanesulfonic acid. The non-protic acids employed were boron trifluoride etherates, antimony trichloride and triphenylcarbenium tetrafluoroborate. NMR-compatible deuterated analogs have been used where applicable.

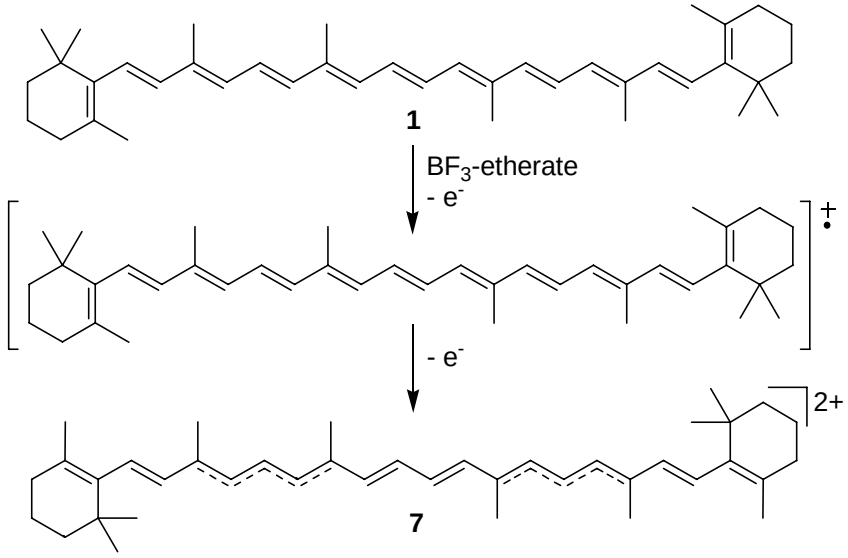
Formation and stability of polyenyl cations were monitored by Vis/NIR absorption spectroscopy at variable temperature. Structure elucidations of the diamagnetic cations, including charge distribution estimates, were obtained from low-temperature NMR spectra of the charged species. Radical cation formation was demonstrated by EPR spectroscopy. The reactivity of polyenyl cations with selected nucleophiles was investigated, and mechanisms for the formation of reaction products were rationalised based on the cation structure.

Insight was gained on the mechanism of action on polyenes for the Lewis acids antimony trichloride and boron trifluoride etherates. Details of delocalisation pattern in polyenyl mono- and dications are reported. The results are also considered in relation to the structure of solitions in infinitely long polyene systems, *i.e*. polyacetylene. The relevance of charge-transfer in the binding mechanism of carotenoids in blue carotenoproteins is discussed.

2. Cations from C_{40}-carotenoids and longer polyene analogs

2.1 Preparation of β,β-carotene dication with BF_3-etherates (Paper I)

Pioneering work on the reaction of carotenoids with BF_3-diethyl etherate was performed by Zechmeister's group in the fifties,[29-31] mainly by studies on the resulting blue complex. A reinvestigation of the reaction between β,β-carotene (**1**) and boron trifluoride dimethyl etherate, using modern spectroscopic techniques including Vis/NIR, NMR and EPR spectroscopy, showed the blue product to be a delocalised dication (**7**), Scheme 3.[32] The absorption maxima was in the NIR region of the electronic spectrum, with λ_{max} 985 nm at room temperature, and considerable stability was observed. Radical species were shown to be present by EPR spectroscopy, supporting a reaction mechanism with two successive one-electron oxidations of the carotenoid. NMR spectra showed considerable downfield shift compared to neutral β,β-carotene (**1**).



Scheme 3

This reinvestigation was further pursued in Paper I in this thesis, which highlights many important spectroscopic properties of cationic polyenes. Total downfield chemical shifts of the dication **7** relative to the starting material **1** was 35.82 ppm for ^{1}H, and 504 ppm for ^{13}C. These values are above the expected values for aryl dications,[33] but a wide range of ^{13}C downfield shifts are exhibited in other cationic systems.[34] An estimate of the charge distribution was made by calculating individual ^{13}C chemical shift differences along the carbon chain relative to β,β-carotene (**1**), based on the fact that local π-electron density is the dominant effect influencing the ^{13}C chemical shift.[35] This is shown in Figure 3, with the charge density correlated with the size of the filled circles. Higher charge density towards the ends of the polyene system are compatible with charge repulsions, whereas the alternating pattern are expected from resonance structures. From $^3J_{H,H}$ coupling constants and $^3J_{H,C}$ long-range couplings, two regions with intermediate bond lengths and the *retro*-configuration[36] in the central part were established.

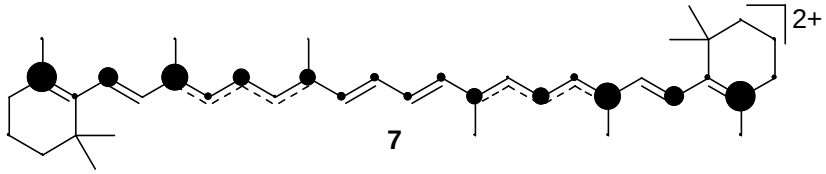
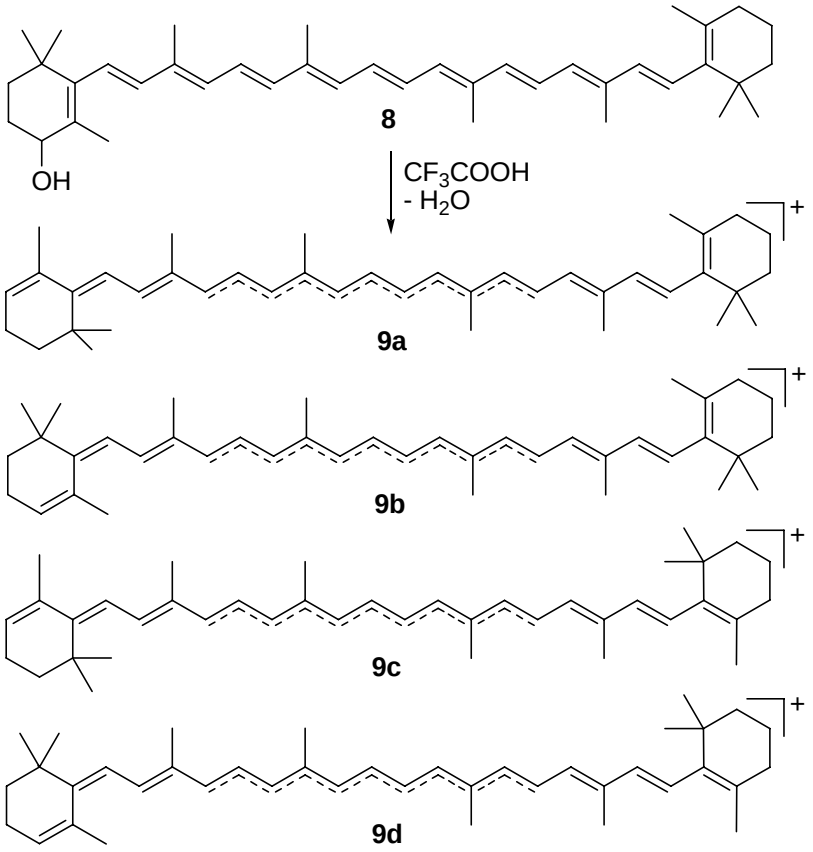


Figure 3. Estimated charge distribution for β,β-carotene dication (**7**).

2.2 Monocations prepared by allylic elimination of isocryptoxanthin (Papers II,III)

Allylic elimination of hydroxy and ether groups using 0.03 M HCl in $CHCl_3$ is a standard analytic reaction for structure elucidation of carotenoids.[19] The extension of the polyene chain by one double bond is readily identified by Vis spectroscopy, *cf.* Scheme 2 ii), Section 1.4. The proposed mechanism for the reaction suggested an intermediate delocalised carbocation.[19,37] By monitoring the reaction of isocryptoxanthin (**8**, (4*RS*)-β,β-caroten-4-ol) with HCl in the Vis/NIR region, a transient species with λ_{max} above 1000 nm was observed. A stable absorption at 1028 nm was observed at -20 °C when trifluoroacetic acid in CH_2Cl_2 was used as acid. NMR at low temperature resulted in structure elucidation of the four isomeric 4-dehydro-β,β-carotenyl monocations **9a**-**9d**, Scheme 4. They differ in stereochemistry at the C-6,7 double bond, and both s-*cis* and s-*trans* forms are observed for the C-6',7' single bond. Coupling constants and heteronuclear long-range couplings were used for determination of the bond-inversion region.



Scheme 4

The carbocations **9** have 22 π-electrons delocalised over 23 sp^2 hybridised carbon atoms. Chemical shifts for a neutral model compound was constructed from a combination of β,β-carotene (**1**) for the main part of the cation, and the appropriate stereoisomer of isocarotene (**10**, 4',5'-didehydro-4,5'-*retro*-β,β-carotene, see Section 2.6, Scheme 13) for positions C-1 – C-11 and C-16 – C-19. The charge distributions for the monocations **9a** and **9b** were estimated based on the ^{13}C chemical shift difference relative to the neutral model, and are visualised in Figure 4. The charge is delocalised mainly in the central parts of the cation, and the charge density drops off towards the ends. Also, as for dication **7**, the alternating pattern with charge on every other carbon atom, as expected from the different resonance contributions, is apparent. Total downfield ^{13}C chemical shift adds to 254 ppm for the **9a** stereoisomer relative to the neutral model, compatible with a carotenoid monocation.

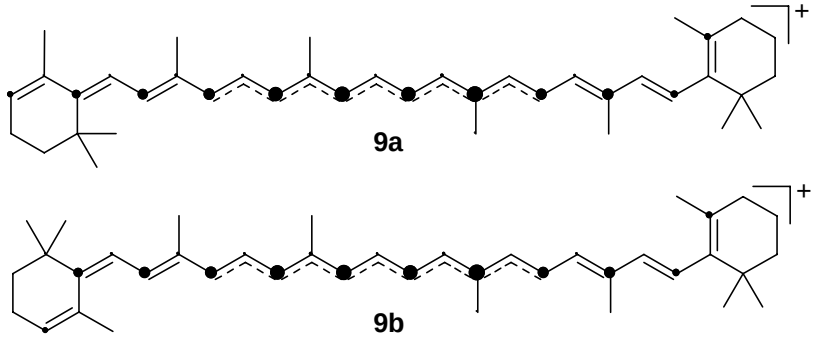
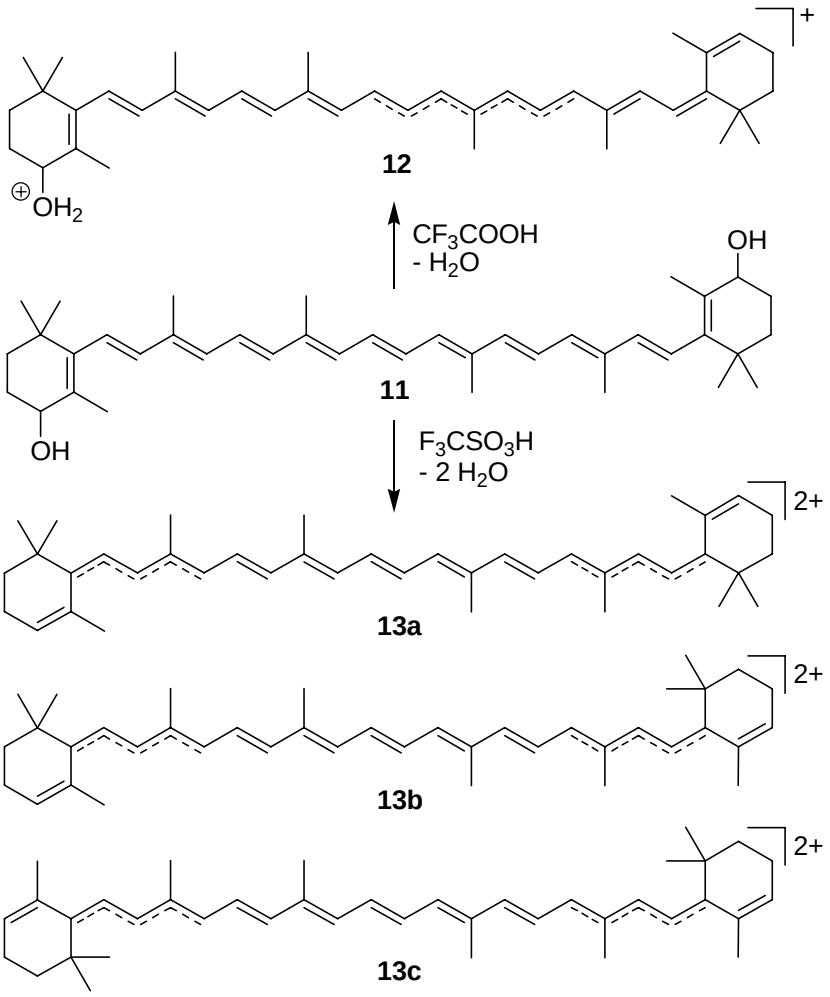


Figure 4. Estimated charge distribution for 4-dehydro-β,β-carotenyl monocations **9a** and **9b**.

2.3 Dications prepared by allylic elimination of isozeaxanthin (Paper III)

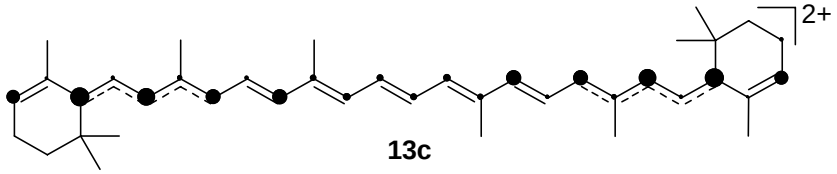
Double elimination of water from racemic isozeaxanthin (**11**, β,β-caroten-4,4'-diol) using trifluoroacetic acid in CH_2Cl_2 was attempted. Vis/NIR spectroscopy showed λ_{max} 1028 nm for the resulting product. Structure elucidation by low temperature NMR led to the identification of the dication **12**, Scheme 5. The partial chemical shift assignment of the polyene chain for **12** resembled that of the monocations **9**, leading to the conclusion of only one positive charge delocalised over 23 sp^2 hybridised carbon atoms. The proton in 4-position was shifted downfield to 5.43 ppm from 4.01 ppm.[38] This result was rationalised by protonation of the oxygen without eliminiation, causing the second positive charge. Similar downfield shifts of proton neighbours to positively charged oxygen are reported.[39]



Scheme 5

By the same method acetylated isozeaxanthin (4,4'-diacetoxy-β,β-carotene) provided the same dication **12** as reaction product. Further attempts to improve the leaving group ability of the hydroxy groups in isozeaxanthin (**11**) by preparation of trifluoroacetates caused decomposition during the synthesis. Conversion of **11** to the dication with two delocalised charges, structures **13a**-**13c**, was achieved by using the stronger acid trifluoromethanesulfonic acid. Vis/NIR spectra at -15 °C exhibited λ_{max} 1022 nm. Structure elucidation by NMR confirmed the structure of the dication to consist of a mixture of *E*/*Z*-isomers involving the C-6,7 and C-6',7' double bonds, Scheme 5. Since the central part of the dications are shared, the ratio 11:45:44 for **13a** : **13b** : **13c** was determined from the 3:2 predominance of the 6-*E* configuration over 6-*Z*.

Formally, dications **13** correspond to removal of two electrons from isocarotene (**10**, see Section 2.6) and has 22 π-electrons delocalised over 24 sp^2 hybridised carbon atoms. Using all-*E* isocarotene (**10**, Scheme 13) and 6,6'-di-*Z* **10** as neutral models, the charge distribution of **13a** and **13c** was determined, illustrated for **13c** in Figure 5. As for the β,β-carotene dication **7**, the effect of Coulombic repulsion is apparent, with the charge density highest towards the ends of the polyene. A total ^{13}C downfield shift for **13c** of 480 ppm compared to isocarotene (**10**) was calculated, consistent with the double charge.



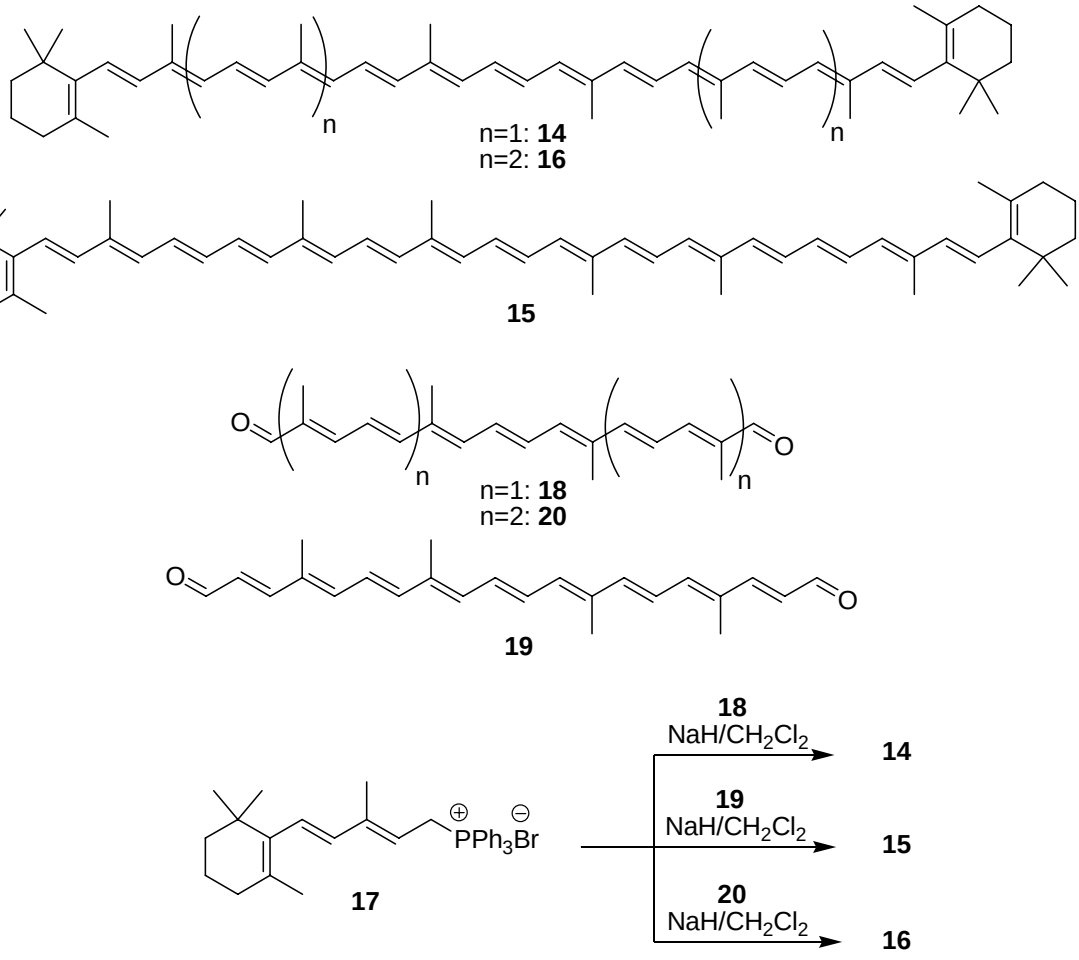
13c

Figure 5. Estimated charge distribution for all-*E* isocarotene dication (**13c**).

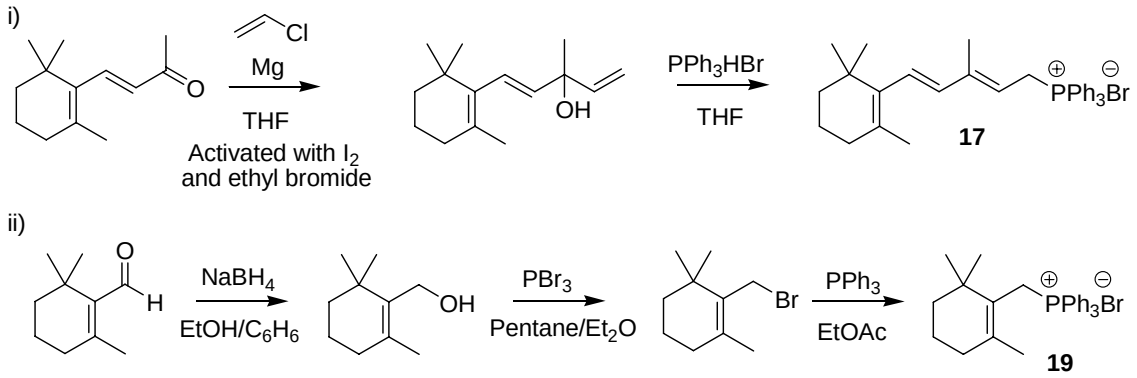
2.4 Cations from carotene analogs with extended polyene system

2.4.1 Synthesis of C_{50}-, C_{54}- and C_{60}-carotene analogs (Paper IV)

Decapreno-β-carotene (**14**, C_{50}-β-carotene), C_{54}-β-carotene (**15**) and dodecapreno-β-carotene (**16,** C_{60}-β-carotene) were synthesised from a common C_{15} Wittig salt **17** and the diapocarotenals **18**-**20**, Scheme 6. The phosphonium salt **17** was prepared according to Scheme 7 i).[40,41] NaH was used as base for generating the ylide in the Wittig coupling reaction.[42] Attempted synthesis of C_{44}-β-carotene from diapocarotenal **18** and the C_{10} phosphonium salt **19**, prepared according to Scheme 7 ii),[43] failed, using NaH or BuLi as base.



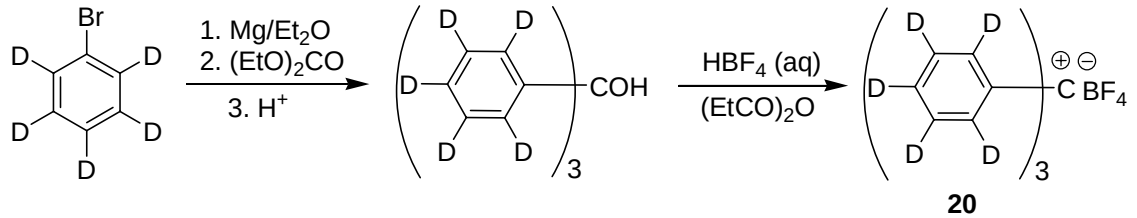
Scheme 6



Scheme 7

2.4.2 Properties of cations prepared from C_{50}-, C_{54}- and C_{60}-carotene analogs (Paper IV)

The original idea of introducing good leaving groups in allylic posistions in the carotene analogs **14-16** was abandoned because of poor yields in the hydroxylation step. Instead, elimination of the unusual leaving group of hydride ions was pursued. Hydride elimination from carotenoid 5,8-epoxides upon acid treatment are reported,[44] and hydride donor reactivities of unsaturated hydrocarbons have been investigated.[45] The hydride acceptor triphenylcarbenium tetrafluoroborate-d_{15} (**20**) was synthesised according to scheme 8.[46,47]



Scheme 8

NIR investigations using trifluoroacetic acid and triphenylcarbenium tetrafluoroborate gave contradictory results, with λ_{max} varying with temperature, acid reagent and concentration. It was concluded that λ_{max} values obtained with 0.013 M trifluoroacetic acid most likely corresponded to monocation absorption spectra, Figure 6, and lower λ_{max} values obtained under other experimental conditions might arise from dication formation (see Chapter 4).

NMR spectra of β,β-carotene (**1**) treated with triphenylcarbenium tetrafluoroborate-d_{15} (**20**) or dilute trifluoroacetic acid-*d* showed formation of the 4-dehydro-β,β-carotenyl monocation (**9**). Hydride is thus an acceptable leaving group in carotenes. For decapreno-β-carotene (**14**), however, the deuterated reagent **20** gave non-interpretable spectra. When trifluoroacetic acid-*d* was used for the elongated carotene analogs **14-16**, severe broadening of the signals was observed. Formation of radicals might be a reasonable explanation, based on observations in similar systems.[48,49] A qualitative treatment of the broadened signals with numerical calculation of the average olefinic ^1H

chemical shifts suggested monocation formation for decapreno-β-carotene (**14**), mono- and dication for C_{54}-β-carotene (**15**), and dication formation from dodecapreno-β-carotene (**16**).

2.4.3 Relevance for charge distribution in solitons (Papers III,IV)

Early investigations of shorter polyenyl cations showed a linear correlation between the polyene length and absorption maxima in optical spectra.[50,51] This is in agreement with the free-electron model,[52] which assumes electrons to be mobile and free to move within the entire π-electron system without restriction. An opposing view to this model was forwarded by the Su, Schrieffer and Heeger (SSH) soliton theory.[12,53] According to this theory, the charge is carried in defects in the polyene chain of limited width, restricting the charge delocalisation. Light absorption in doped polyacetylene is measured to 1650-1900 nm,[54,55] and the linear correlation from the free-electron model is thus not valid to infinity.

The absorption maxima plotted against the number of sp^2 hybridised carbons of the reported shorter polyenyl cations,[50] together with the maxima recorded for monocations from β,β-carotene (**1**) and the C_{50}-, C_{54}- and C_{60}-carotene analogs **14**-**16** are given in Figure 6. Deviations from the linearity of the free-electron model is seen already for the monocation **9** from β,β-carotene (**1**) with 23 sp^2 carbon atoms. This may be taken as evidence for the manifestation of free solitons in these longer polyenes.

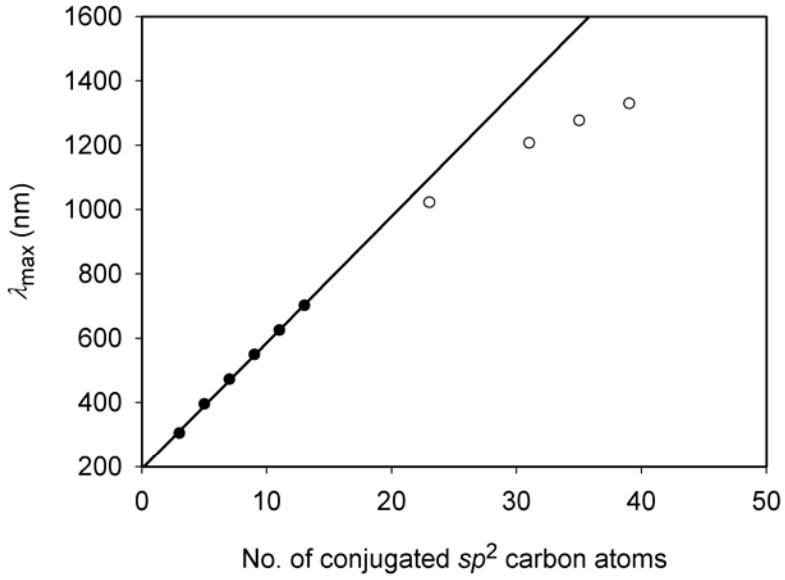


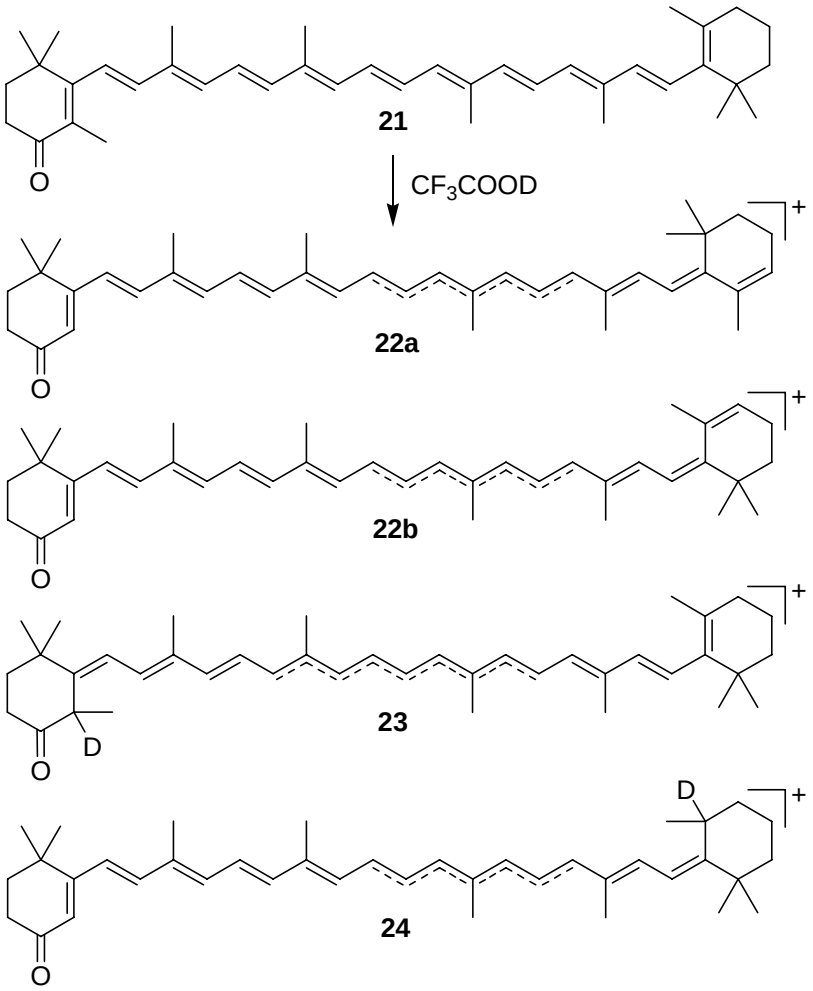
Figure 6. Absorption maxima at room temperature as function of the number of conjugated sp^2 hybridised carbon atoms in polyenyl monocations. ● From ref.[50], in 80-96% sulfuric acid. ○ Polyenes **1**, **14**-**16**, dissolved in 0.013 M trifluoroacetic acid in CH_2Cl_2.

In the SSH theory, the soliton is described by a charge density wave, with a width defined by the soliton half-width *l*. From the half-width parameter, a mathematical

description of bond lengths and charge distribution is available.[56] The 4-dehydro-β,β-carotenyl monocation (**9**) is the longest delocalised cation fully characterised by NMR spectroscopy, including charge distribution. By regression analysis, a solition half width of l = 7.8 was calculated for **9**. Because of the mobility of a free soliton,[12,57,58] NMR of longer, charged polyenes is not expected to give reliable charge distribution data, due to signal averaging in dynamic systems.[59]

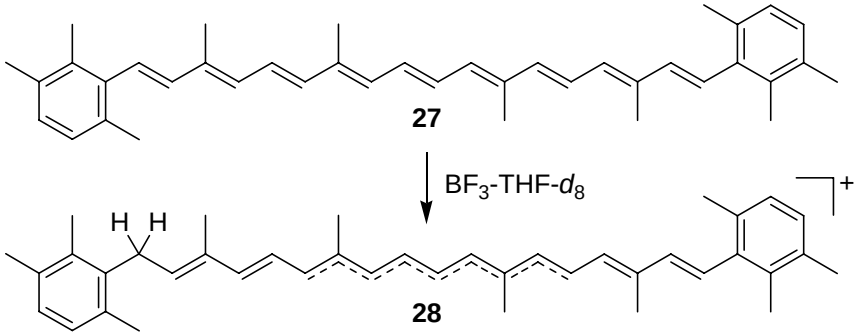
2.5 Miscellaneous C_{40}-cations (Paper III)

Treatment of echinenone (**21**, β,β-caroten-4-one) with trifluoroacetic acid resulted in formation of the monocation **22** as the main product, as observed by low temperature NMR, Scheme 9. The formation of this cation must be explained by hydride elimination. The monocation was present as 6',7'-*E* (**22a**) and 6'7'-*Z* (**22b**) stereoisomers. A full chemical shift assignment was made. In addition, two minor products corresponding to protonation of the polyene chain was partially assigned by NMR. Protonation was observed at 5-position (**23**) or 5'-position (**24**).



Scheme 9

Treatment of several carotenoids with boron trifluoride etherates, including lycopene (**5**), astaxanthin (**25**, see Section 3.1), canthaxanthin (**26**, see Section 3.1) and isorenieratene (**27**, ϕ,ϕ-carotene), was attempted. The monocation **28** was formed as the main product upon treatment of isorenieratene (**27**) with the boron trifluoride THF-d_8 complex, Scheme 10. The mechanism of formation for the monocation **28** is discussed in Section 2.7.

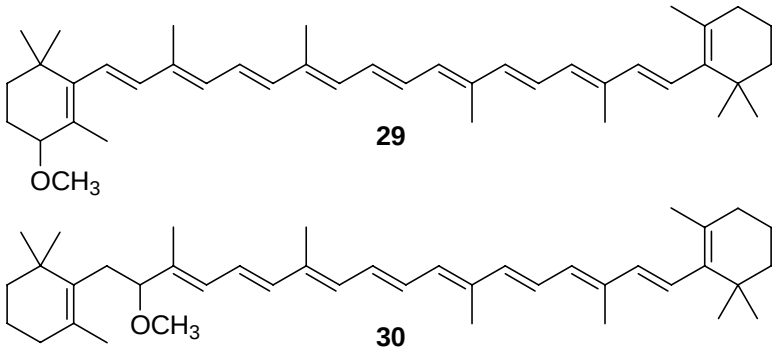


Scheme 10

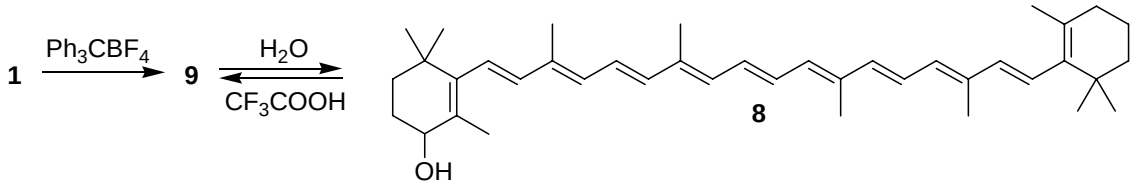
2.6 Nucleophilic reactions of C_{40}-cations (Papers I,IV,V)

The reaction of the polyenyl cations with various nucleophiles has been investigated, with product analysis by TLC, HPLC-DAD, UV/Vis, MS and ^{1}H NMR methods. Generally, the identified compounds can be rationalised from their parent cation structure, either by loss of H^+ or by addition of a nucleophile (either anionic, or with loss of H^+ after the addition). In addition, some structures require formal addition of hydrides or hydrogen radicals. A preference to form products providing the longest possible polyene chain was observed. Products isolated in these reactions were strongly *E*/*Z*-isomerised, as evidenced by HPLC analysis using a C_{30} stationary phase column.[60] This is compatible with cationic intermediates and can be rationalised by the low rotational barriers calculated for carotenyl mono- and dications.[61]

Addition of H_2O to the β,β-carotene dication (**7**) gave isocryptoxanthin (**8**) as main product, whereas sodium methoxide mainly gave recovered β,β-carotene (**1**). In addition, two methyl ethers, **29** (4-methoxy-β,β-carotene) and **30** (8-methoxy-7,8-dihydro-β,β-carotene), were identified.

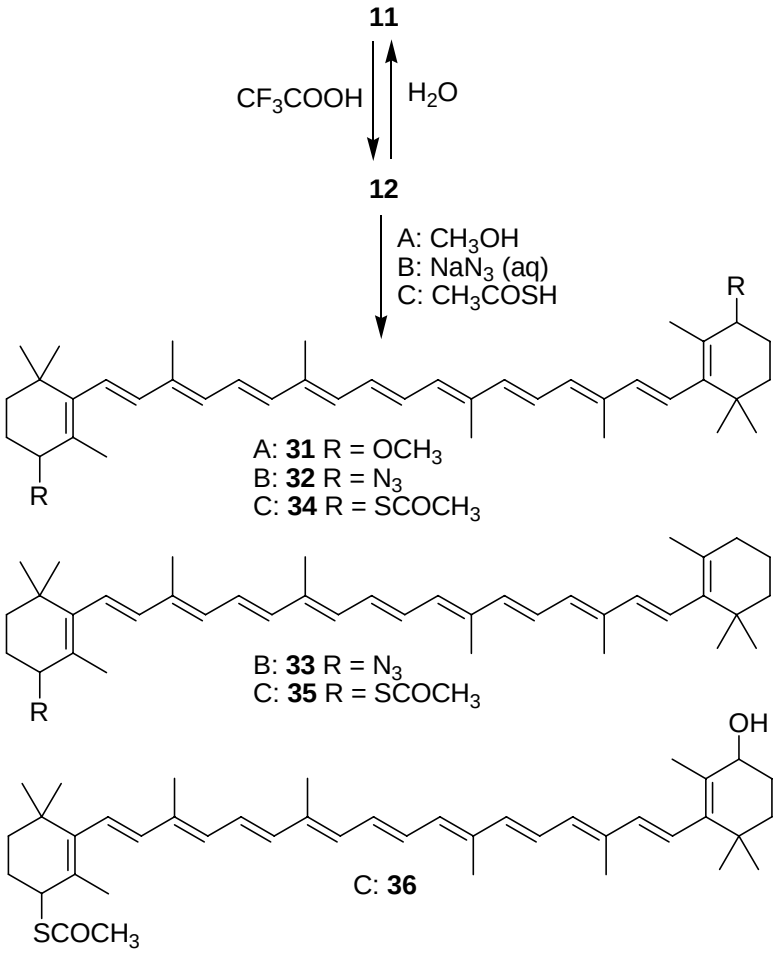


Treatment of the 4-dehydro-β,β-carotenyl monocation **9** with H_2O, prepared from either allylic elimination of isocryptoxanthin (**8**) or by hydride abstraction from β,β-carotene (**1**) using the trityl cation **20**, gave **8** as the main product, Scheme 11.



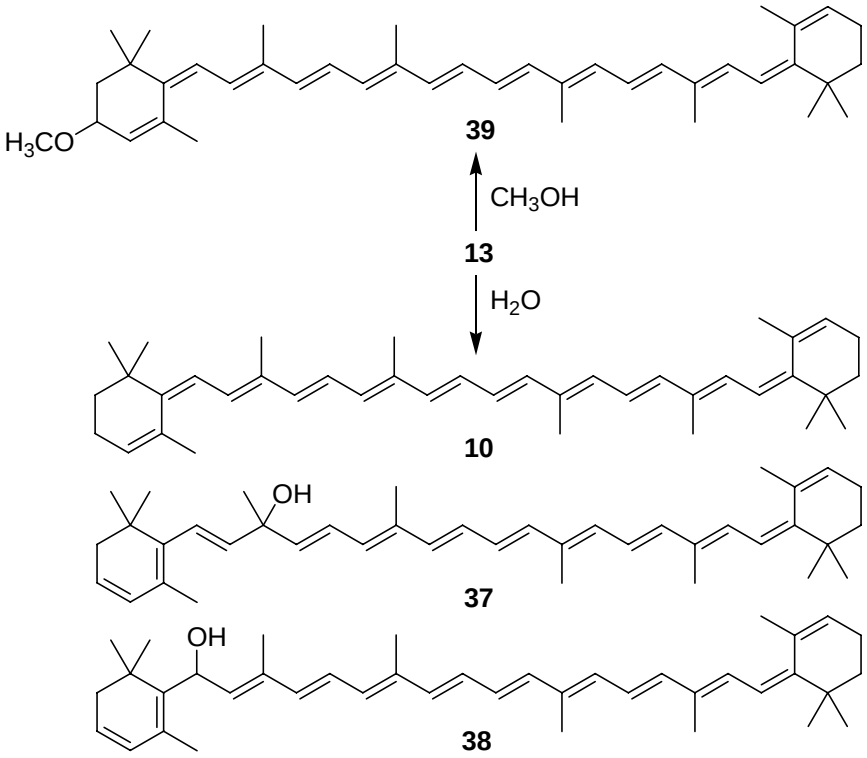
Scheme 11

The dication **12**, with a protonated hydroxy moiety, gave as main products 4,4'-disubstituted β,β-carotenes with O-, N- and S-nucleophiles. Thus, addition of H_2O to **12** gave recovery of isozeaxanthin (**11**). Addition of CH_3OH gave the 4,4'-dimethoxy ether **31**, Scheme 12. Treatment of the dication **12** with aqueous NaN_3 resulted in the formation of the 4,4'-diazido-β,β-carotene (**32**), as well as 4-azido-β,β-carotene (**33**) as a minor product. When thioacetic acid was used as nucleophile, three different compounds in equal amounts were identified, 4,4'-diacetylthio-β,β-carotene (**34**), 4-acetylthio-β,β-carotene (**35**) and 4'-acetylthio-β,β-caroten-4-ol (**36**).



Scheme 12

Reaction of the isocarotene dication **13** with H_2O and CH_3OH as nucleophiles gave more complex product mixtures, Scheme 13. Isocarotene (**10**) was the most prominent product in the reaction with H_2O, in addition to the atypic in-chain hydroxylated species **37** (3,4-didehydro-4',9-*retro*-β,β-caroten-9-ol) and **38** (3,4-didehydro-4',7-*retro*-β,β-caroten-7-ol). The methyl ether **39** (4',5'-didehydro-3-methoxy-4,5'-*retro*-β,β-carotene) was tentatively identified from the reaction with CH_3OH as the most prominent product, among several others.



Scheme 13

2.7 Hydride transfer in carotenoid reactions (Paper IV,V)

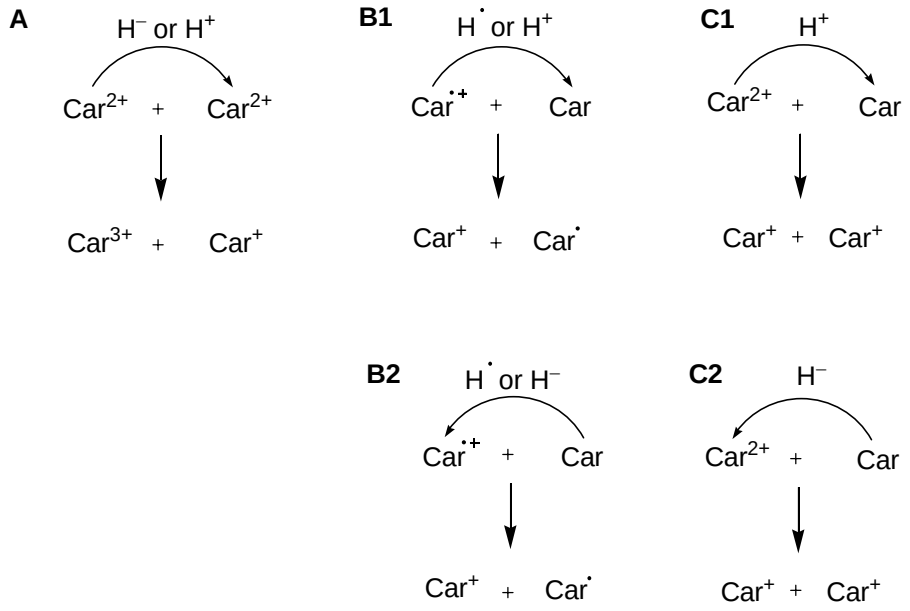
As was demonstrated in Section 2.4.2 for β,β-carotene (**1**), carotenes may serve as hydride donors, providing delocalised carotenoid cations. Also, the monocation **28** in Section 2.5, obtained with BF_3 etherates, has an additional hydrogen at C-7, which may arise from a hydride (or hydrogen radical) transfer from the diaryl carotene substrate. Since it has been demonstrated that carotenoid radicals may be formed by treatment with BF_3 etherates, Section 5, and also in reaction media containing Brønsted acids as the only reagent,[48] mechanisms involving radical reactions should not be excluded.

In the following examples involving nucleophilic reactions of C_{40} cations (Section 2.6) i) the formation of isocarotene (**10**) from isocarotene dication (**13,** Scheme 13), ii) the methyl ether **30** from β,β-carotene dication (**7**), and iii) the two different 4-monosubstituted products (**33** and **35**) from the dication **12** (Scheme 12), a mechanism including addition of either hydride ions or hydrogen radicals is required in order to rationalise the products. Referring to Scheme 14, the mechanisms for A (from dications), B1 (from radical cation + neutral carotenoid) and C1 (from dication + neutral carotenoid), cited from Paper V, have been expanded by alternatives B2 and C2.

Although the mechanisms may serve to explain products arising from hydride transfer, it should be noted that only products where carotenoids serve as hydride acceptors have been observed in nucleophilic reactions.

Formation of the two monosubstituted products **33** and **35** from dication **12** (Scheme 12) and isocarotene (**10)** from isocarotene dication (**13**, Scheme 13) is best explained by mechanism C2. Formation of the monocation **28** (Scheme 10), and the dihydro methyl ether **30** (below Scheme 10) might be envisaged by mechanisms B1, B2, C1 and C2, taking the use of boron trifluoride etherates into consideration. Mechanism A, with its trication formation, is regarded as unlikely.

In conclusion it is confirmed that carotenoids may serve as hydride donors, forming stabilised carotenoid cations. The evidence also suggests that carotenoids serve as hydride acceptors in nucleophilic reactions *via* carotenoid cations.



Scheme 14

3. Colouration mechanisms for carotenoproteins

3.1 Protonated canthaxanthin as model for blue carotenoproteins (Paper VI)

Protein-bound carotenoids are responsible for the mauve-blue-black colour of several tissues in invertebrate animals. Such carotenoproteins are present in the carapace of members of the *Crustacea* and *Echinodermata* families.[62] The most studied example is the carotenoprotein of the lobster, *Homarus gammarus*. In the lobster, two molecules of astaxanthin (**25**, 3,3'-dihydroxy-β,β-caroten-4,4'-dione) are bound non-covalently to two apoproteins in β-crustacyanin, absorbing at λ_{max} 587 nm in phosphate buffer, which is significantly shifted compared to free astaxanthin, with λ_{max} 480 nm in acetone (**25**).[62] Aggregation of eigth β-crustacyanin units provides α-crustacyanin, which is predominant in the lobster carapace. In α-crustacyanin the absorption is futher bathochromically shifted to λ_{max} 632 nm.[63]

X-ray studies of β-crustacyanin have established the structure of the apoproteins and the amino acid residues binding the astaxanthin (**25**) molecules.[64,65] A simplifeid view of the binding is presented in Figure 7. Prior to the results of the X-ray analysis, the colouration mechanism was believed to occur by protonation of the keto-groups in astaxanthin (**25**),[63,66] or a polarisation. In view of the amino acid environment and pH considerations, the polarisation mechanism was not confirmed.[65]

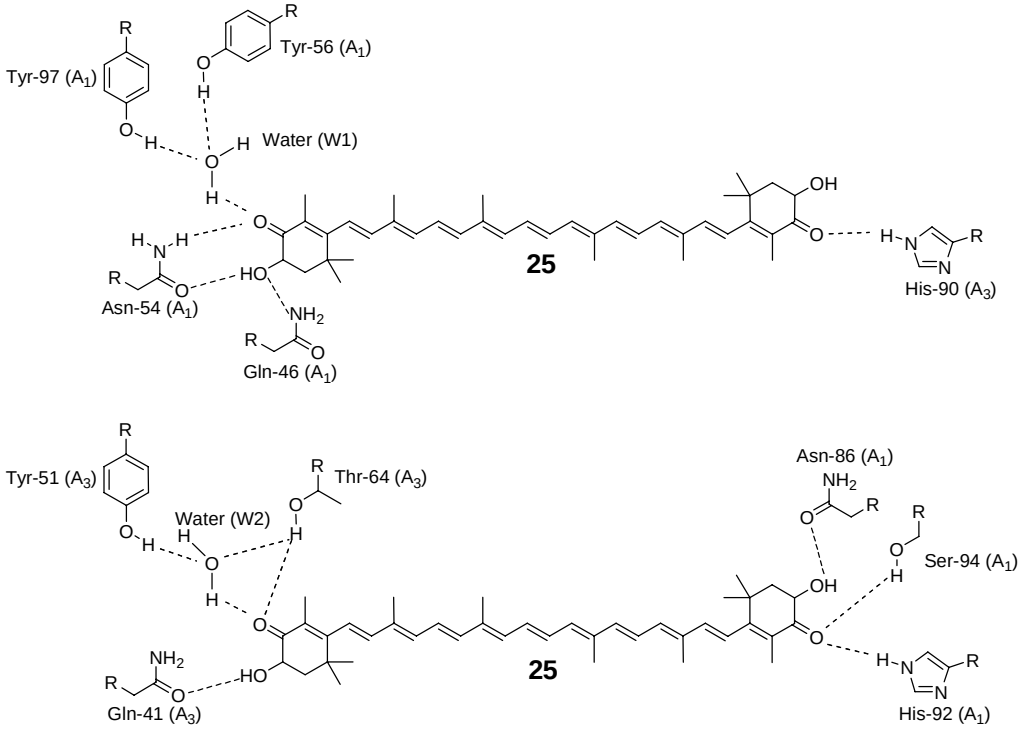
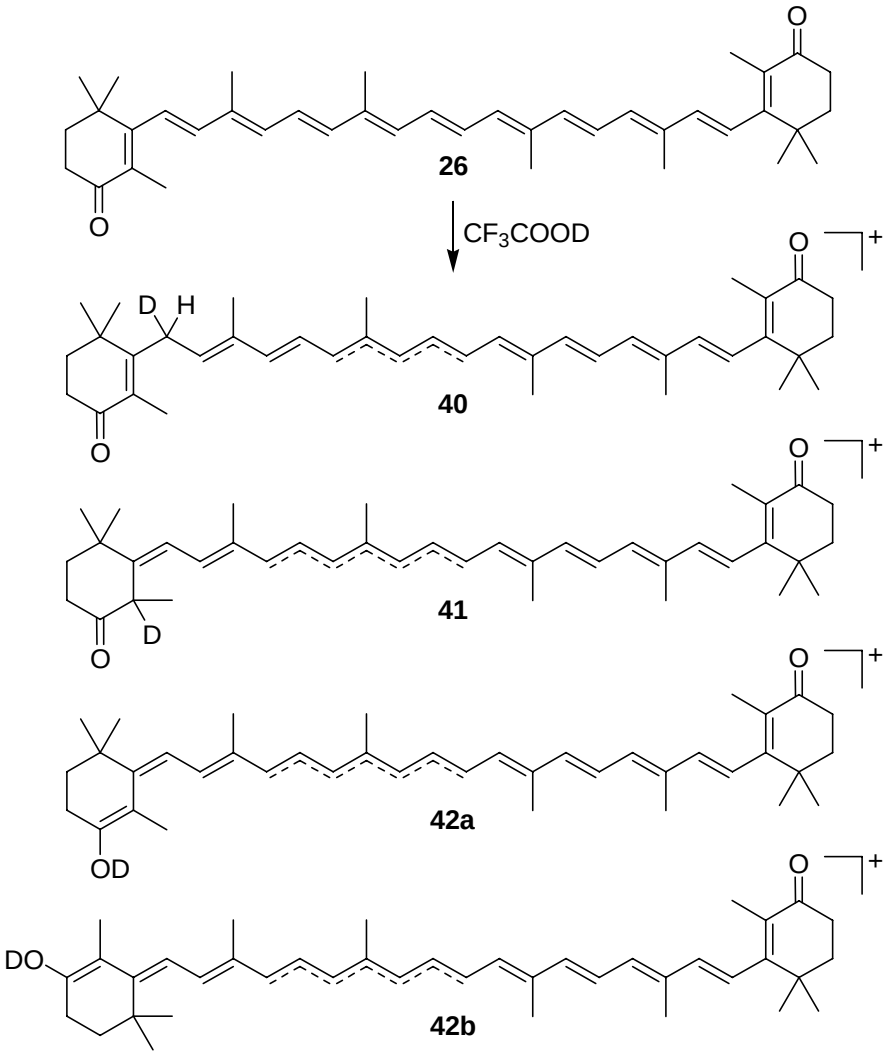


Figure 7. Astaxanthin (**25**) binding in β-crustacyanin. Adapted from X-ray data.[64]

As a model for the binding of astaxanthin (**25**) in β-crustacyanin, the treatment of canthaxanthin (**26**, β,β-caroten-4,4'-dione) with various Brønsted acids was

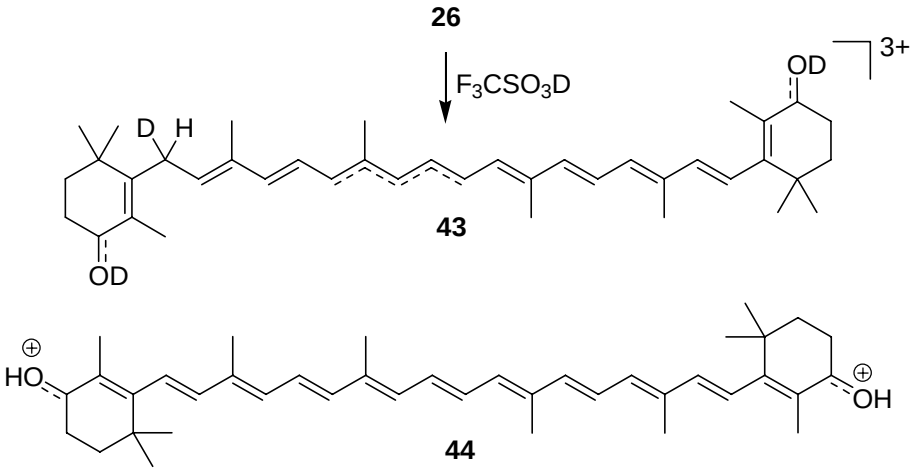
investigated, Paper VI. By employing trifluoroacetic acid from two different manufacturers, the monocations **40**, **41** and **42** were identified by low temperature NMR spectroscopy, Scheme 15, and a complete chemical shift assignment for ^{1}H and ^{13}C was made. The monocations **40** and **41**, corresponding to C-7 and C-5 protonation, respectively, were present in samples prepared with acid from both manufacturers. Product **42** from protonation of one carbonyl group, with subsequent enolisation, was observed as the major product in addition to **40** and **41** in trifluoroacetic acid from one manufacturer. This product consisted of two *E*/*Z* stereoisomers **42a** and **42b** around the C-6,7 double bond.



Scheme 15

When the stronger acid trifluoromethanesulfonic acid was employed, the canthaxanthin trication **43** was identified as the product, judged by NMR spectroscopy, Scheme 16. From the ^{13}C chemical shift data of the trication **43** and the C-7 monocation **40**, a model based on the ^{13}C chemical shift data difference **43** – **40** was devised for the charge distribution of the hypothetical O-4,4' diprotonated canthaxanthin (**44**), Scheme 16. Most of the charge was found to be localised on the carbonyl groups, but due to the possible rehybridisation of the atoms in the C=O double bond, only small ^{13}C chemical

shift changes on C-4,4' in **44** could be expected from the model. However, delocalisation of the charge to even-numbered atoms in the polyene chain was predicted, giving downfield shifts of up to 30 ppm for the C-6,6' position in **44** compared to the neutral canthaxanthin (**26**), Figure 8.



Scheme 16

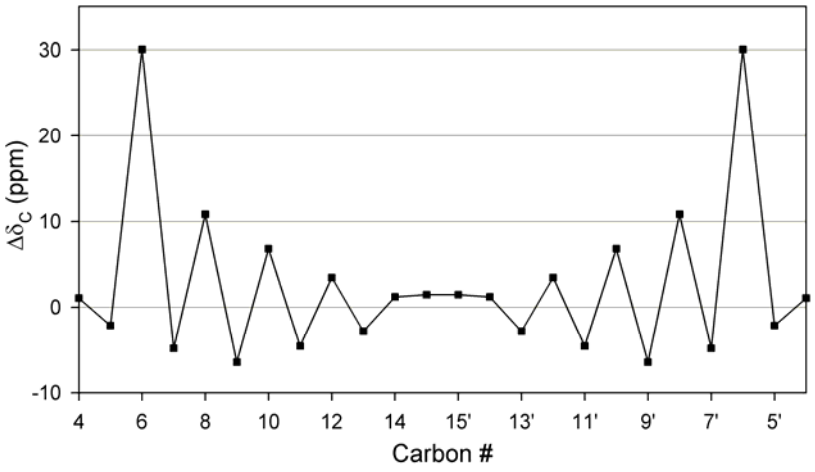


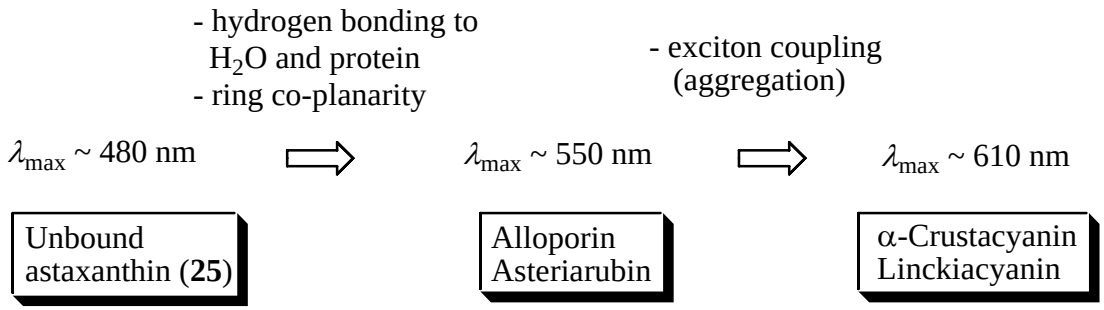
Figure 8. Expected downfield ^{13}C chemical shifts of hypothetical O-4,4' diprotonated canthaxanthin (**44**) relative to canthaxanthin (**26**).

3.2 Recent progress on the colouration mechanism in crustacyanins

Solid state NMR studies on selectively ^{13}C-enriched astaxanthin recombined to α-crustacyanin showed only small differences in chemical shifts for even-numbered carbons in α-crustacyanin compared to astaxanthin (**25**) in solution.[67] For C-6,6' and C-8,8', which from the model presented in Figure 8 should exhibit a difference in ^{13}C chemical shift of 30 and 10 ppm for a fully diprotonated carotenoid, respectively, no difference was found. A large contribution to the bathochromic shift arising from

protonation or polarisation was therefore ruled out, consistent with the X-ray data for β-crustacyanin.[67]

Based on calculations, the co-planarity of the rings with the aliphatic polyene chain should account for about a third of the bathochromic colour shift.[67] However, the major contribution to the bathochromic shift of astaxanthin (**25**) in the absorption spectra of crustacyanins was explained by an exciton coupling effect. Bathochromic shifts are observed in exciton coupling systems if the angle between two electronic transition moments is larger than 90°.[68,69] In β-crustacyanin, the two astaxanthin molecules are at a close distance, 7 Å, and form an exciton coupling system with an angle of 120°. Combined with the elongation of the conjugation of the π-system caused by co-planarity of the cyclic end group, illustrated in Figure 7, and the effect of hydrogen bonding of the keto-groups, a calculated value for λ_{max} in β-crustacyanin of 650 nm was found.[67] Further proof of the importance of aggregation effects for the colour in lobster may be found in the additional bathochromic shift of 45 nm going from β-crustacyanin subunits to α-crustacyanin. Experimental data from femtosecond time-resolved spectroscopic studies on α-crustacyanin were found to be in agreement with dimerisation of astaxanthin (**25**) as the main cause of the bathochromic shift.[70] X-ray data for another carotenoprotein, present in cyanobacteria *Arthrospira maxima*, has revealed the incorporation of monomeric 3'-hydroxyechinenone (3'-hydroxy-β,β-caroten-4-one).[71] The smaller bathochromic shift (55 nm) of λ_{max} observed in this carotenoprotein (λ_{max} up to 505 nm) is compatible with hydrogen bonding and extended π-conjugation by ring co-planarity, and with exciton effects absent. The blue (λ_{max} 554 nm) carotenoprotein asteriarubin from starfish (*Asterias rubens*) has a single mono- or diacetylenic astaxanthin (7,8-didehydro- or 7,8,7',8'-tetradehydro-) molecule per protein oligomer,[72] where exciton interaction also may be disregarded. The blue alloporin (λ_{max} 545 nm) of coral origin also contains only one astaxanthin (**25**) molecule per protein monomer.[73] On the other hand, in linckiacyanin (λ_{max} 612 nm) from the blue starfish *Linckia laevigata*, (3*S*,3'*S*)-astaxanthin is the dominant carotenoid and exciton interaction between carotenoids has been suggested.[74] A simple hypothesis is advanced in Scheme 17.



Scheme 17

As pointed out in a recent computational study, the protonation state of the histidine residues shown in Figure 7 is not known from the X-ray analysis, and protonation of astaxanthin (**25**) from these residues may give rise to bathochromic shifts of the same magnitude as the ones observed.[75] Furthermore, only small bathochromic shifts (5 nm

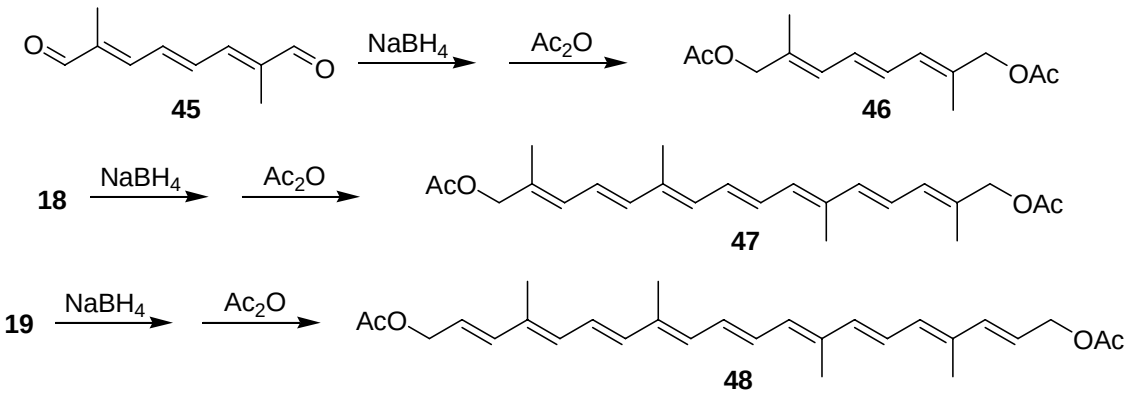
or less in organic solvents) was observed in a study of chiral carotenoid dimers.[76] Both because of the commercial need for a stable blue colorant,[66] and the remaining uncertainty in establishing the colouration mechanism in blue carotenoproteins, efforts should be made to prepare synthetic carotenoid dimers mimicking the stereochemical relationships between the two carotenoid molecules found in β-crustacyanin.

4. Shorter polyenylic dications

4.1 Preparation of C_{10}-, C_{20}- and C_{24}-dications (Paper VII)

Light absorption of neutral hydrocarbon polyenes reach a theoretical maximum value at 608 nm.[77] As was treated in Chapter 3, the complexing of astaxanthin (**25**) with proteins to provide α-crustacyanin shifted the absorption of a neutral carotenoid bathochromically around 150 nm to λ_{max} 632 nm. The blue fucoxanthin oxonium ion, prepared by hydrochloric acid treatment of the carotenoid fucoxanthin absorbs at λ_{max} 686 nm.[78] Moreover, the polyenyl cations presented in Chapters 2 and 3 all have absorptions above λ_{max} 870 nm, except the canthaxanthin trication (**43**, λ_{max} 692 nm).

In order to fill the gap in the Vis/NIR absorptions of carotenoid-related compounds in the 700-900 nm region, a series of possible dication precursors with shorter chromophores was synthesised. The available dialdehydes 2,7-dimethyl-2,4,6-octatriene-1,8-dial (**45**, C_{10}), crocetindial (**18**, C_{20}, Scheme 6, Section 2.4.1) and bixindial (**19**, C_{24}, Scheme 6, Section 2.4.1) were reduced to the corresponding diols by $NaBH_4$ and subsequently converted to their diacetates **46**-**48**, Scheme 18, thus introducing leaving groups for dication formation.

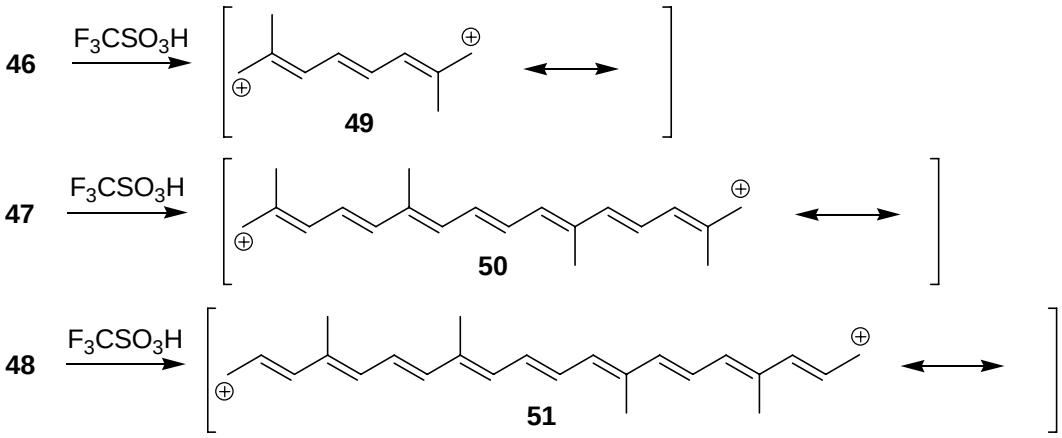


Scheme 18

Treatment of 1,8-diacetoxy-2,7-dimethyl-2,4,6-octatriene (**46**) with trifluoromethanesulfonic acid in dichloromethane yielded an initial product absorbing at λ_{max} 469 nm, which is in agreement with monocation formation, compared to related structures.[50] The reaction progressed partially to a second product absorbing at λ_{max} 438 nm. Treatment of 8,8'-diacetoxy-8,8'-diapocarotene (**47**) and 6,6'-diacetoxy-8,8'-diapocarotene (**48**) with trifluoromethanesulfonic acid gave clean conversion to products with stable NIR-absorptions at λ_{max} 735 and λ_{max} 850, respectively. Low-temperature NMR of the resulting products after acid treatment was attempted without success, partly due to solubility problems.

The observed absorption maxima were lower than values expected for monocations of comparable chain length,[51] as expected for dication formation,[79] and formation of the delocalised dications **49**-**51**, Scheme 19, was therefore inferred. A shoulder on the short-wavelength side, characteristic of delocalised cations,[50] was observed in the Vis/NIR spectra for **49**-**51**. It should be noted at this point that the stabilities of **49**-**51**, in particular the C_{24}-dication **51**, with a $t_{½}$ of only 10 min, are much lower than for the

cations presented in the preceeding chapters. Decay mechanisms involving cyclisation reactions may be expected.[51] In retrospect, successful NMR spectra of dications could have been obtained by introducing alkyl substituents in the terminal positions.[80] This could stabilise the delocalised charge and slow down decay reactions. Solubility in apolar solvents would also increase.



Scheme 19

4.2 Correlation between λ_{max} and chain length for polyenyl dications (Paper VII)

Analogous to the correlation between λ_{max} and number of delocalised bonds published for polyene monocations,[51] shown in Figure 6, Section 2.4.3, the data gathered for dications **49**-**51**, together with the β,β-carotene dication **7** and isocarotene dication **13**, was used as basis for a similar, empirical correlation for polyene dications. In order to provide data in a comparable form, both the correlations for monocations and dications was written using the number m of sp^2 hybridised carbon atoms as variable. Equation (1) gives the correlation formula for monocations, whereas the correlation for the dications, obtained by least-square error linear regression from the dication data points, is presented in equation (2). As may be seen from Figure 9, the agreement is satisfactory.

Monocations: $\lambda_{max} = (204.8 + 38.3m)$ nm (1)

Dications: $\lambda_{max} = (156.0 + 35.4m)$ nm (2)

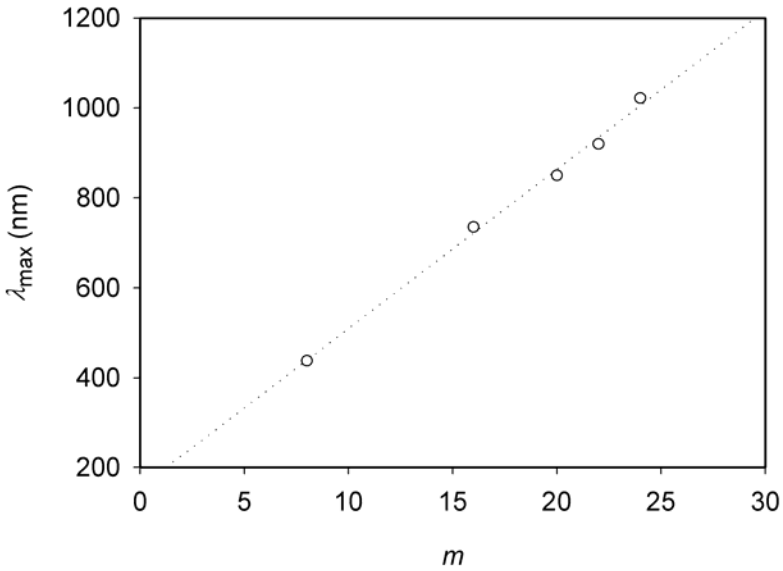
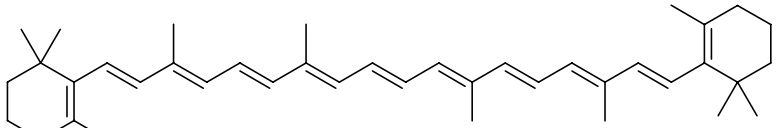


Figure 9. Absorption maxima as function of the number *m* of conjugated sp^2 hybridised carbon atoms in polyenyl dications. Data points for dications from left: **49**-**51**, **7** and **13**.

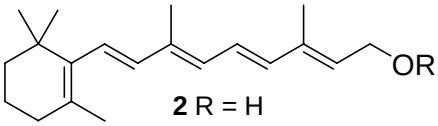
5. Preparation of polyenylic radical cations with Lewis acids (Paper VIII)

Polyenes are in general anti-oxidants, which is related to their properties as electron donors. It has been established by electrochemical methods that both carotenoids and retinoids are more susceptible to oxidation to cationic products than reduction to anionic products.[81] Several methods for preparation of radical cations are reported.[28] Formation of polyenylic radical cations by chemical oxidation of polyenes are reported with several reagents, such as the treatment of β,β-carotene (**1**) with boron trifloride etherates (Section 2.1),[32] carotenoids treated with iron(III)chloride and iodine,[18,79,82] and treatment of retinol (**2**) with the single electron oxidant tris(*p*-bromophenyl)aminium hexachloroantimonate.[83] Formation of arene radical cations in molten antimony trichloride solution has been demonstrated by EPR studies.[84] EPR has also been used for the detection of carotenoid radicals.[85]

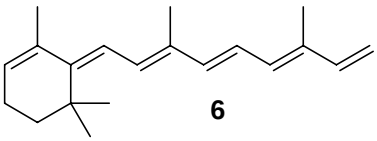
The question of radical cation formation in relation to the Carr-Price reaction, Chapter 6, justified an investigation of single electron oxidant properties of the reagent used in the reaction, antimony trichloride in chloroform solution. The reaction of polyenes with the two Lewis acids boron trifluoride diethyl etherate and antimony trichloride was studied by Vis/NIR and EPR. Employed as substrates were the carotenoids β,β-carotene (**1**) and isocryptoxanthin (**8**), and three retinoids, retinol (**2**), anhydroretinol (**6**) and retinyl acetate (**52**).



1 R = H
8 R = OH



2 R = H
52 R = $COCH_3$



6

Vis spectra of retinol (**2**) and anhydroretinol (**6**) treated with antimony trichloride in chloroform solution showed λ_{max} at 620 nm and 623 nm, respectively, for the blue products formed. This is in agreement with published values.[86] For β,β-carotene (**1**) and isocryptoxanthin (**8**), λ_{max} at 1020 nm and above was measured for the products, which is similar to λ_{max} of the diamagnetic monocation **9** (Section 2.2), but also in agreement with published values for radical cations from β,β-carotene (**1**).[79] This coincidence of closely similar NIR absorption (λ_{max}) of carotenoid radical cations and cations necessitates the application of EPR for the identification of radical cations.

EPR studies performed on frozen samples of the carotenoid substrates mixed with antimony trichloride in chloroform solution showed the presence of radicals for the products of carotenoids **1** and **8**. A represenatative spectrum is shown in Figure 10 for the radical cation from β,β-carotene (**1**). The single signal spectrum is characteristic for carotenoid radicals.[82] The linewidths observed for frozen samples of the radical cation obtained from β,β-carotene (**1**) treated with antimony trichloride were in agreement with formation of dimeric or trimeric complexes.[82] The results demonstrate the ability of antimony trichloride to perform one-electron oxidations of carotenoids. Weaker signals were observed for isocryptoxanthin (**8**) relative to β,β-carotene (**1**) in rapidly frozen samples, which may indicate an intermediate arising from initial elimination of the hydroxy group prior to radical formation. Thaw-freeze cycles gave increasing signal amplitude, ascribed to further progress of the reaction caused by mixing in the liquid state.

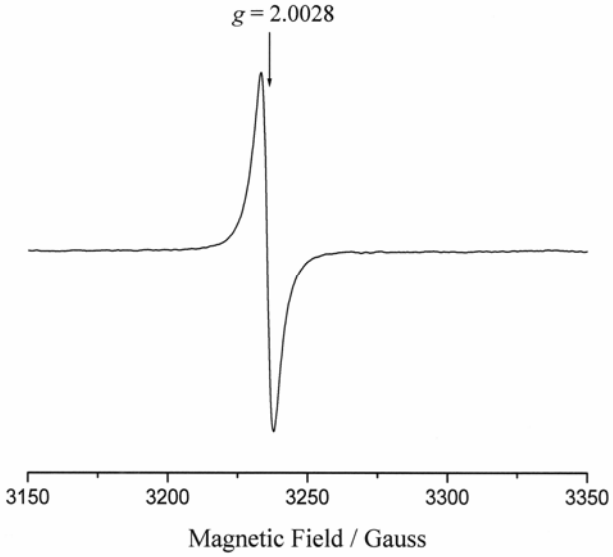


Figure 10. EPR spectrum of β,β-carotene (**1**) treated with $SbCl_3$, measured at 207 K.

Similar experiments performed on the retinoids (**2**, **6**, **52**) treated with antimony trichloride gave no EPR signals at any of the conditions attempted (frozen, frozen after thaw-freeze cycles, or at room temperature in capillary tubes). This led to the conclusion that formation of radical cations did not take place in these reactions. When the Lewis acid boron trifluoride diethyl etherate was employed, only β,β-carotene (**1**) provided a strong signal, whereas a weak signal was observed for isocryptoxanthin (**8**). Again, none of the retinoids gave a signal in the EPR spectra. From the blue colour visually observed during these reactions, it is inferred that carotenoid radical cations were formed from **1** and **8** and non-radical cationic products from the retinoids **2**, **6** and **52** (Chapter 6.)

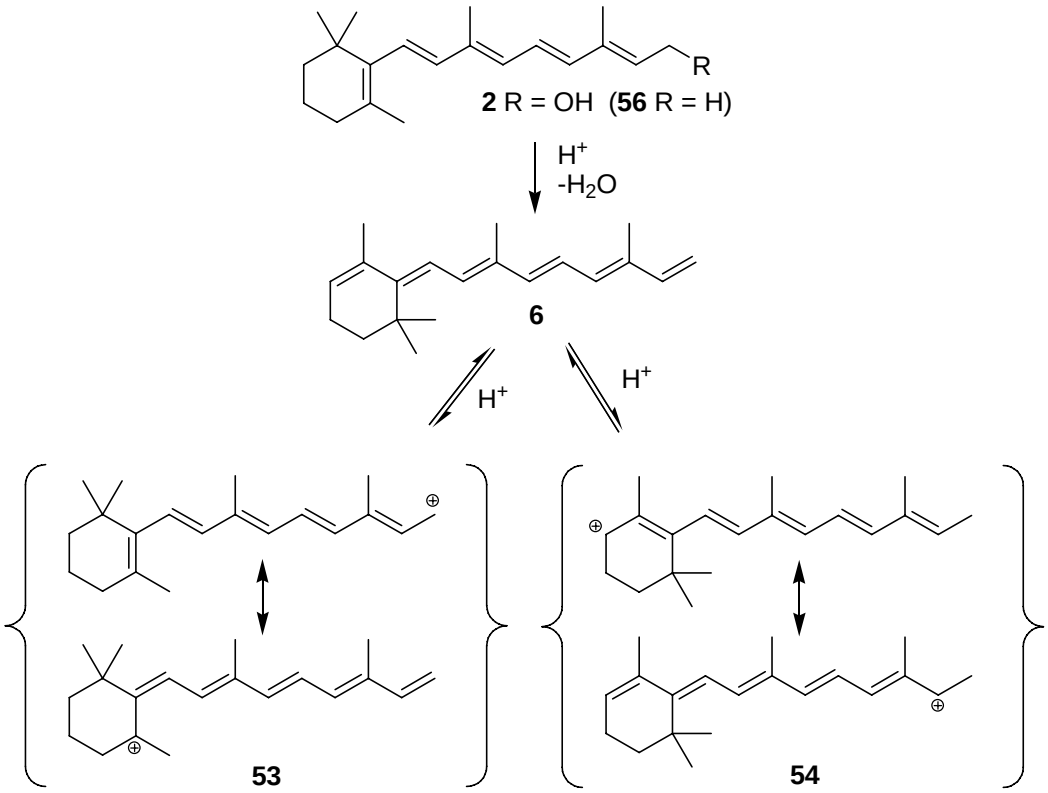
These results were rationalised on the basis of the electrochemical properties of carotenes compared to the retinoids. Lower oxidation potentials have been measured for β,β-carotene (**1**) relative to retinol (**2**) or anhydroretinol (**6**).[81,87] It was concluded that antimony trichloride or boron trifluoride etherates do not act on the retinoids as one-electron oxidation agents. For antimony trichloride, other reaction pathways was concluded (Chapter 6).

6. Studies on the Carr-Price reaction of retinoids

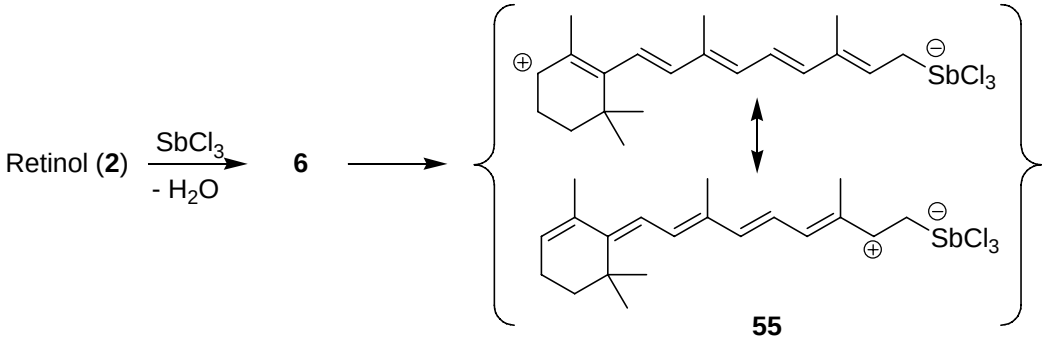
A brief historical introduction to the classical Carr-Price blue colour reaction of Vitamin A (**2**) and related retinoids was given in Section 1.2, dealing with early colour reactions of carotenoids and retinoids.

6.1 Structure determination of the model anhydroretinylic cation (Paper IX)

Treatment of several retinoids with iodine or Brønsted acids showed the formation of mainly two different delocalised cationic species, based on their Vis absorptions.[88,89] The retinyl cation (**53**) was found to have λ_{max} around 590 nm, while the anhydroretinyl cation (**54**) had λ_{max} at 620 nm, Scheme 20. Based on these values for the absorption maxima, it was hypothesised that the main absorbing species in the Carr-Price blue colour reaction, involving treatment of retinoids with a saturated chloroform solution of antimony trichloride, Section 1.2, was the anhydroretinyl cation analog **55**, Scheme 21.[86]



Scheme 20



Scheme 21

As a parallel to similar studies on carotenoids, the treatment of retinoids with Brønsted acids was investigated. Stable absorptions for the reaction products of retinol (**2**), anhydroretinol (**6**) and retinyl acetate (**52**) with λ_{max} 618 nm in dichloromethane was observed upon treatment with trifluoromethanesulfonic acid at -15 °C. Low temperature NMR experiments of this blue product resulted in our studies in full ^{1}H and partial ^{13}C chemical shift assignments of the anhydroretinylic cation (**54**), thereby confirming the identification made in the earlier Vis-studies, *cf.* [86]. The NMR data revealed the formation of both the 6,7-*E* stereoisomer **54a** and the 6,7-*Z* stereoisomer **54b**, with the former present in a 1.2/1 excess. Also, the terminal 15-Me group appeared as a skewed triplet in the ^{1}H spectrum, which is likely to arise from overlapping doublets from *E*/*Z*-isomers of the C-13,14 double bond. The charge distribution of the anhydroretinyl cation **54**, visualised in Figure 11, was estimated by comparing its ^{13}C chemical shifts with those of a hypothetical neutral model constructed from a combination of ^{13}C chemical shifts from anhydroretinol (**6**) and axerophthene (**56**, deoxyretinol, Scheme 20). The even distribution of charge along the polyene chain in such a short polyene system is pointed out. This is in contrast to the charge distributions presented in the longer delocalised systems presented in Chapter 2, where limitations from the maximum soliton width or electrostatic repulsion forces are readily observable in the charge distribution pattern.

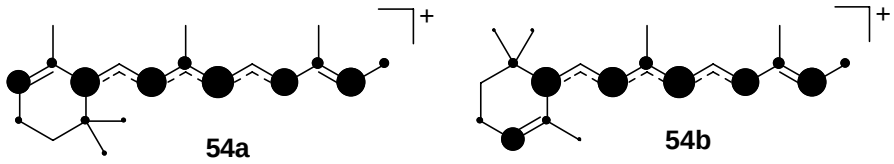


Figure 11. Estimated charge distribution for anhydroretinyl cation (**54a,b**).

6.2 Dynamic light scattering experiments on the Carr-Price product (Paper IX)

NMR of retinol (**2**) treated with the Carr-Price reagent, antimony trichloride in chloroform, gave no interpretable results due to severe line broadening of the signals. The hypothesis that the blue product of the Carr-Price reaction was a radical cation was abandoned in Chapter 5. Radical cations could have explained i) the instability of the Carr-Price product relative to that of the anhydroretinylic cation **54a**,**b** and ii) the lack

of interpretable NMR spectra. Signal broadening in NMR can otherwise be caused by the formation of aggregates with slow rates of tumbling.[90] Formation of complexes with Sb^{III} has been reported.[22,91,92] A reinvestigation of the Carr-Price reaction, aiming at the detection of aggregates was therefore undertaken, using dynamic light scattering as the experimental method.[93]

Chloroform solutions of β,β-carotene (**1**), of retinol (**2**), of antimony trichloride and of boron trifluoride diethyl etherate were prepared and analysed by dynamic light scattering. Only modest scattering of light was observed for all four samples. Light scattering measurements of mixtures were performed on Lewis acid solutions with two drops of polyene solution added. The result for the mixture of retinol (**2**) and antimony trichloride was strikingly different from the pure solutions, as shown in Figure 12. The mixture of β,β-carotene (**1**) and antimony trichloride also exhibited some scattering, whereas the scattered intensity of both boron trifluoride mixtures was consistently lower.

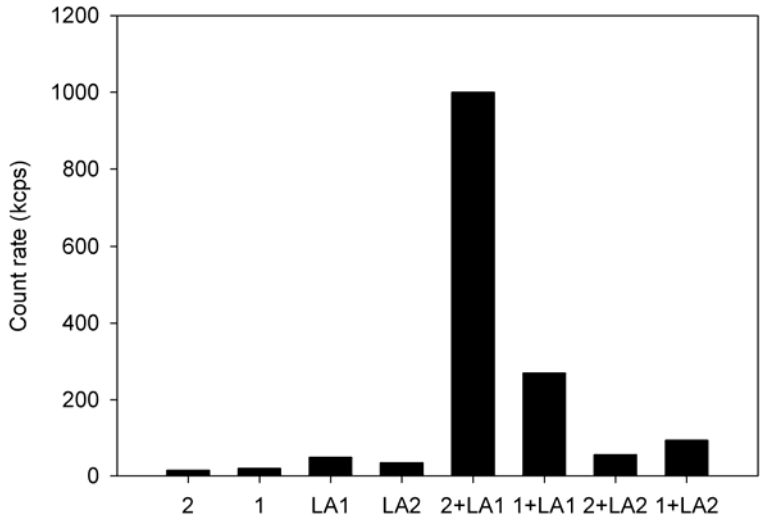
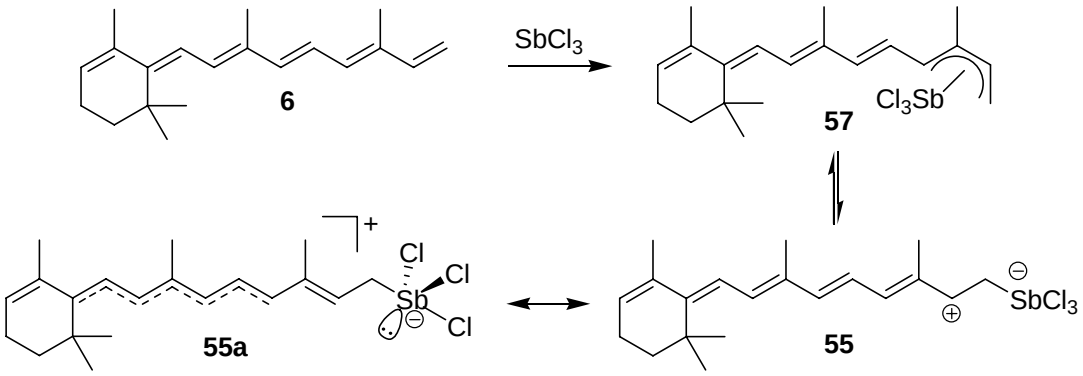


Figure 12. Light scattering of chloroform solutions of retinol (**2**), β,β-carotene (**1**), $SbCl_3$ (**LA1**) and BF_3-diethyl etherate (**LA2**), and corresponding mixtures.

6.3 Reaction mechanism of the Carr-Price reaction (Paper IX)

The light scattering data presented in Section 6.2 was in agreement with the formation of aggregates in the reaction between retinol (**1**) and antimony trichloride. This result also explains the signal broadening observed in the NMR spectrum. Based on η^6-complexes reported for arenes,[91,92] analogous η^4-complexes might be envisaged for the Carr-Price product, structure **57**, Scheme 22. However, from the similarities in the Vis spectra of the Carr-Price product compared to the anhydroretinyl cation (**54**), both with λ_{max} around 620 nm, contribution from the zwitterion structure **55** was expected, as originally suggested.[86] Structure **55a** in Scheme 22 suggested here for the monomeric form of the Carr-Price blue coloured product shows a trigonal bipyramidal geometry around the antimony atom, including the stereochemically active lone pair.[22] However, extensive cross-bonding and formation of more complex structures in the aggregates are likely.

The low stability of the blue Carr-Price product can also be understood from the aggregation. Reactive species are present in close proximity in the aggregates. Addition of sodium methoxide as a nucleophile to the Carr-Price product provided dimeric products, supporting intermolecular retinoid reactions as a major decay pathway for the blue coloured product.



Scheme 22

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