Studies on the mechanism of the Carr–Price blue colour reaction†

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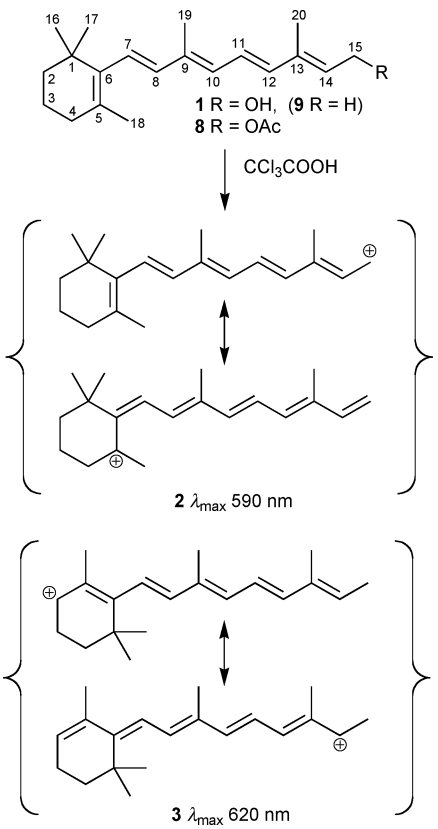
The reaction of retinoids (retinol, retinyl acetate and anhydroretinol) with Brønsted acids was studied as a model system for the Carr–Price reaction. The anhydroretinylic cation was characterised by VIS and 2D NMR spectroscopy, including an estimate of the charge distribution and region of bond inversion, observed in a mixture of identified *E*/*Z* isomers. Products obtained by quenching with NaOMe–MeOH were identified by HPLC and MS. The classical Carr–Price reaction between retinol (vitamin A) and the Lewis acid $SbCl_3$ in saturated chloroform solution was reinvestigated by VIS, NMR, EPR, dynamic light scattering and chemical quenching. Whereas product instability and failure to provide informative NMR spectra indicated a radical cation, EPR results excluded free-radical species. Dynamic light scattering experiments, in comparison with model systems, revealed strong aggregation for the Carr–Price complex, rationalising the low stability, NMR problems and dimerisation observed by chemical quenching. The VIS data support structural similarity of the blue Carr–Price product with the delocalised anhydroretinylic cation, and a detailed structure of the antimony complex is evaluated.

Introduction

Early efforts to quantify vitamin A activity by chemical analysis, in particular in cod-liver oils, concentrated on the blue colour reaction taking place when retinol (**1**, vitamin A) is treated with acid. Both Brønsted and Lewis acids, such as sulfuric acid and arsenic trichloride, could be used, although many of the tested reagents had several drawbacks; interaction with impurities or solvents, poor reproducibility and unstable absorptions were common problems. The development of the Carr–Price reaction overcame most of these problems, although the blue absorption fades rather quickly.[1] In this reaction the retinol-containing mixture is treated with a saturated solution of $SbCl_3$ in chloroform, and the blue product is subjected to photometric analysis. The intensity of the blue absorption was successfully correlated with vitamin A activity. This became the standard method for quantitative vitamin A analysis,[2] and the method was later extended for analysis of polyenes in general.[3] Based on experience, the observed colour could be correlated with the number of conjugated double bonds, and was thus an important tool as a first analysis of chromatographic fractions in polyene synthesis. A more formal correlation between absorption maxima (λ_{max}) and number of double bonds in the substrate was later published for polyenes treated with sulfuric acid.[4,5]

Polyenes treated with Brønsted acids were shown to give cationic products with delocalised charges, either through elimination of leaving groups, or by direct protonation of the polyene chain.[4–7] For Lewis acid reactions, in particular with BF_3–etherates, addition of the Lewis acid to the polyene chain to form a zwitterion with a positive charge in the polyene chain was suggested.[8] On the basis of VIS absorptions measured for the retinylic cation (**2**) and the anhydroretinylic cation (**3**), reported for treatment of various retinoids with Brønsted acids, Scheme 1,[9,10] the structure



Scheme 1

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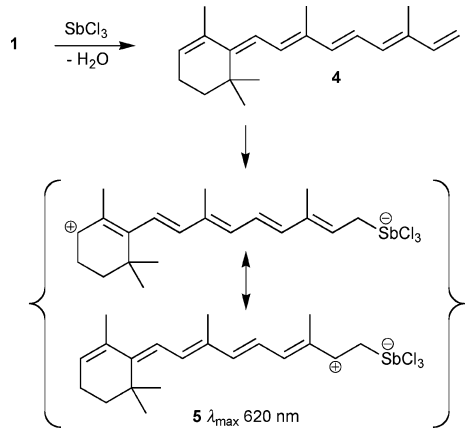
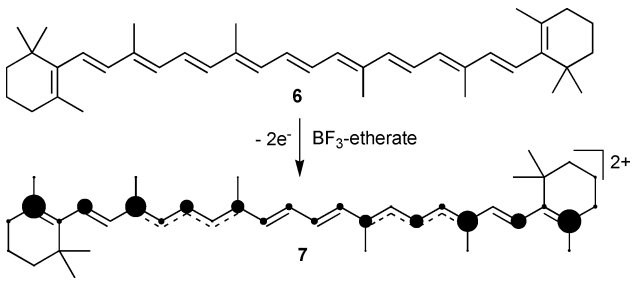
of the Carr–Price product was suggested to have $SbCl_3$ covalently added to carbon 15 on an anhydroretinol (**4**) intermediate. This would provide a delocalised cation (**5**) of a similar type as the anhydroretinylic cation (**3**), Scheme 2.[11]



Scheme 2

Our reinvestigation of the reaction between β,β-carotene (**6**) with BF_3–etherates revealed the formation of the β,β-carotene dication (**7**) as the product, and thus no BF_3 covalently added to the polyene chain, Scheme 3.[12] By the detection of an EPR signal, the reaction is suggested to take place through two successive one-electron transfers.[12]



Scheme 3

$SbCl_3$ is a Lewis acid containing lone-pair electrons. Sb^{III} can undergo extensive complex formation with neutral donors where the lone pair may be stereochemically active.[13] As a reagent, $SbCl_3$ can perform one-electron abstractions.[14] VIS absorption spectra of several radical retinoid species, obtained by pulse radiolysis or chemical oxidation, have been recorded, with λ_{max} ranging from 575 to 635 nm.[15,16] Recent EPR analysis of the treatment of selected carotenoids and retinoids with the Carr–Price reagent did, however, reveal that whereas carotenoids readily form cation radicals when treated with $SbCl_3$, no radicals could be detected for the retinoids.[17] The product between retinol (**1**) and $SbCl_3$ should therefore be of diamagnetic nature.

In this work we have re-examined the mechanism of the Carr–Price reaction, aiming at characterising the product by UV/VIS spectroscopy, NMR analysis, dynamic light scattering and chemical quenching reactions.

Results and discussion

Treatment of retinoids with Brønsted acids

We have investigated the UV/VIS absorption of retinol (**1**), anhydroretinol (**4**) and retinyl acetate (**8**, Scheme 1) upon treatment with Brønsted acids. Results are given in Table 1.

The treatment of retinyl acetate (**8**) with 50 mol% H_2SO_4 in methanol reproduced the VIS-absorption reported for the retinylic cation (**2**).[9] Retinol (**1**) treated with trifluoroacetic acid gave rapid elimination to anhydroretinol (**4**), and only at lowered temperatures was a weak absorption with λ_{max} 623 nm observed. When the stronger acid trifluoromethanesulfonic acid was used, a much more stable absorption was observed for all the three retinoids tested, providing a cation of sufficient stability for characterisation by low temperature NMR. The values obtained are in reasonable agreement with those previously reported for the anhydroretinylic cation (**3**).[10] The observed absorption maxima of the blue products relative to those measured for the Carr–Price product,[17] points towards structural similarities, as was the basis for the hypothesis presented for the Carr–Price reaction in the seventies.[11]

NMR analysis at −15 °C of the retinoids **1**, **4**, and **8** treated with deuterated trifluoromethanesulfonic acid in $CDCl_3$ gave spectra compatible with formation of the anhydroretinylic cation (**3**), confirming the structural assignment made earlier from UV/VIS data.[11] The full ^1H chemical-shift assignment and partial ^{13}C chemical-shift assignment of the dominant stereoisomer of the cation **3** as prepared from **1**, is given in Table 2. Also included is a comparison between the chemical shifts of the anhydroretinylic cation (**3**) and a neutral model compound, using the chemical shifts of anhydroretinol (**2**, for ^1H) or the corresponding carotenoid end group (for ^{13}C) up to C-9,[3,18] and axerophthene (**9**, Scheme 1) for the rest of the molecule.[3,19] The total downfield ^1H chemical shift is in fair agreement with the value of 10.6 ppm per charge found in the Spiesecke–Schneider relationship,[20] and the corresponding ^{13}C value of 239 ppm per charge is close to values obtained for other polyenyl cations of isoprenoid origin.[21] The two lacking

Table 1 UV/VIS absorptions of cations formed by treatment of retinoids with Brønsted acids

	Acid	Solvent	T/°C	λ_{max}/nm	λ_{max}/nm[b]	$t_{1/2}$/min
Retinol (**1**)	CF_3CO_2H	$CHCl_3$	−10	623[a]	—	—
Retinol (**1**)	CF_3SO_3H	CH_2Cl_2	−15	618	620	360[c]
Retinyl acetate (**8**)	CF_3SO_3H	CH_2Cl_2	−15	618	—	360[c]
Retinyl acetate (**8**)	H_2SO_4	CH_3OH	−30	595	—	4
Anhydroretinol (**4**)	CF_3SO_3H	CH_2Cl_2	−15	618	623	360[c]

[a] Formation of anhydroretinol (**4**) dominating the spectra. No cation absorptions observed at room temperature. [b] Reported for the Carr–Price product obtained with $SbCl_3$ in $CHCl_3$, ref. 17. [c] Extrapolated assuming first-order decay.

Table 2 ^{1}H and ^{13}C chemical shifts for anhydroretinylic cation **3a**, and shift changes relative to model compounds in $CDCl_3$ solution

	δ_H/ppm			δ_C/ppm		
Carbon	**3a**	Literature value[a]	Δ	**3a**	Literature value[a]	Δ
1	—	—	—	37.7	35.1	2.6
2	1.72	1.49	0.23	39.0	40.8	−1.8
3	2.49	2.09	0.40	24.6	23.0	1.6
4	6.92	5.78	1.14	153.7	128.6	25.1
5	—	—	—	137.9	134.1	3.8
6	—	—	—	184.1	146.1	38.0
7	6.95	6.38	0.57	n/a	120.3	n/a
8	8.65	6.78	1.87	168.9	130.4	38.5
9	—	—	—	142.7	135.5	7.2
10	8.21	6.08	2.13	175.6	130.7	44.9
11	7.03	6.47	0.56	n/a	125.9	n/a
12	7.81	6.27	1.54	171.1	138.0	33.1
13	—	—	—	140.0	134.6	5.4
14	6.96	5.59	1.37	159.6	122.6	37.0
15	2.15	1.76	0.39	17.5	14.0	3.5
16/17	1.50	1.29	0.21	29.7	29.0	0.7
18	2.10	1.91	0.19	21.1	21.7	−0.6
19	2.21	1.94	0.27	11.8	12.1	−0.3
20	2.02	1.81	0.21	11.7	12.1	−0.4
Σ	—	—	14.86	—	—	239.0

[a] Anhydroretinol (**4**) for δ_H or the corresponding carotenoid end group for δ_C used as reference for positions 1–9 and 16–19, respectively. Axerophthene (**9**) used for the rest of the molecule. Data from ref. 3, 18 and 19.

^{13}C shifts at C-7 and C-11 occur at positions in the chain not expected to be downfield shifted based on resonance structures.

From retinol (**1**), the ^{1}H chemical shifts of the cation **3** drifted during aquisition, up to 0.16 ppm, and in Table 2 the initial values are reported. NMR of acid treated anhydroretinol (**4**) and retinyl acetate (**8**) did not show this behaviour, instead constant chemical shifts were observed. This anomaly is not rationalised.

The anhydroretinylic cation (**3**) appears as *E*/*Z* isomers around the C-6 to C-7 bond, with the *E*-isomer (**3a**) dominating over the *Z*-isomer (**3b**) in a 1.2 : 1 ratio, Fig. 1. Similar isomerisation has been observed in related cations, *e.g.* the C_{40} 4-dehydro-β,β-carotenyl monocation **10**, Fig. 2.[22] Moreover, the hydrogens on Me-15 appears as a triplet with $^{3}J_{H,H} = 6.7$ Hz. This is likely

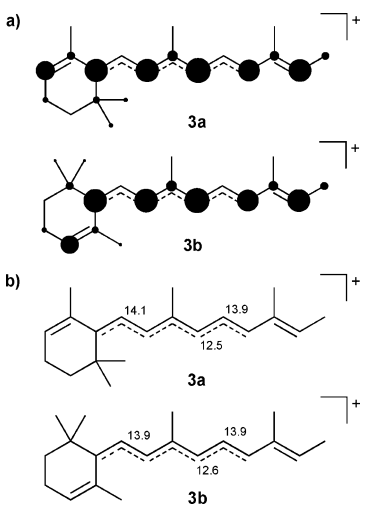


Fig. 1 a) Estimated charge distribution from ^{13}C chemical shifts of anhydroretinylic cations (**3a**/**3b**), with partial positive charge illustrated by the area of the filled circles. b) $^{3}J_{H,H}$ coupling constants, given in Hz.

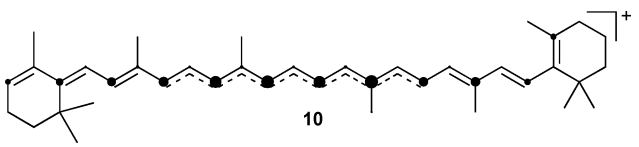
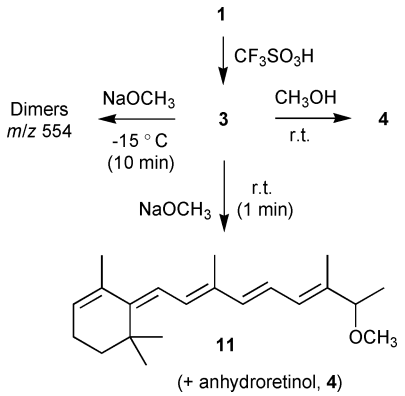


Fig. 2 Charge distribution for the 4-dehydro-β,β-carotenyl monocation (**10**); from ref. 22.

to arise from overlap of the two expected doublets from *E*/*Z* isomerisation of the terminal double bond in the polyene. The charge distributions of the cations **3a**/**3b**, estimated from the ^{13}C chemical-shift difference, are visualised in Fig. 1a. A rather even distribution of the positive charge on C-4, C-6, C-8, C-10, C-12 and C-14 is seen, in contrast to the charge distribution of the C_{40} 4-dehydro-β,β-carotenyl monocation **10**, Fig. 2, where the charge density drops off markedly towards the ends.[22] This phenomenon emphasizes the soliton-like nature of the monocation **10**.[21] The $^{3}J_{H,H}$ coupling constants for the olefinic protons of the anhydroretinylic cation (**3a**/**3b**), shown in Fig. 1b, were all in the intermediate region between the expected values for single and double bonds in similar systems. For comparison, typical values for $^{3}J_{H\text{-}7,H\text{-}8}$ in double bonds are 15.2–16.8 Hz,[18] and 12 Hz for single bonds.[3] In the H-10 to H-12 region, values for $^{3}J_{H,H}$ of 11.3 Hz and 14.8 Hz for single and double bonds, respectively, are typical,[18] leading to the double bond inversion region C-6 to C-12.

Quenching of the anhydroretinylic cation (**3**) prepared from retinol (**1**), with methanol or sodium methoxide as nucleophiles, was attempted at different temperatures and with varying reaction times. Some results are shown in Scheme 4. Judged by MS, dimerisation was the main reaction pathway when the cation **3** was quenched with sodium methoxide after 10 min at −15 °C. The exact structure of the dimers was not established. The same reaction at room temperature, with quenching after 1 min, gave mainly anhydroretinol (**4**), but also ethers. The



Scheme 4

UV/VIS absorption data for the main ether product was similar to early reports for the axerophthene (**9**, Scheme 1) chromophore.[2] However, this assignment was later shown to be in error.[23] Instead, the data is consistent with formation of the ether bond at C-14, giving the structure **11**, as shown in Scheme 4. Due to the use of chloroform with an ethanol stabiliser in this experiment, both methyl and ethyl ethers were formed. Quenching with methanol at room temperature gave anhydroretinol (**4**) as the dominating product.

In conclusion, treatment of retinol (**1**), retinyl acetate (**8**) or anhydroretinol (**4**) with trifluoromethanesulfonic acid led to the anhydroretinylic cations **3a**/**3b**, the structures of which, including the distribution of the positive charge, were unequivocally determined by NMR spectroscopy. The quenching products, including C_{40} dimers ($M = 554$), the C-14 methyl ether **11** and anhydroretinol (**4**), are consistent with the anhydroretinyl cation (**3**) intermediate.

Treatment of retinoids with $SbCl_3$–$CHCl_3$: the Carr–Price reaction

As seen from Table 1, the VIS spectra recorded for the cation **3** produced from the retinoids treated with Brønsted acid were similar to those reported by treatment with $SbCl_3$ in chloroform,[11] and verified in our experiments.[17] However, no NMR spectra could be obtained for this blue product. Since cations and radical cations of polyenes are known to have similar λ_{max} values for their VIS/NIR absorption,[24] a paramagnetic radical cation was suspected by the reaction with $SbCl_3$. This could also serve to explain the low stability observed for the Carr–Price product relative to the anhydroretinylic cation (**3**) obtained by Brønsted acid treatment. However, it was clearly demonstrated that, in contrast to the C_{40} carotenoids, the tested retinoids (**1**, **4**, and **8**) did not provide any radicals with $SbCl_3$ in chloroform.[17] The failure to obtain NMR spectra and low stability of the blue Carr–Price product was finally explained by facile aggregation, demonstrated by the dynamic light scattering data presented in Fig. 3. BF_3–diethyl etherate was included in this investigation as an additional Lewis acid.

For light scattering experiments in general, aggregates scatter significantly more light than monomers. This explains the experimental results given in Fig. 3. Solutions of retinol (**1**), β,β-carotene (**6**) and the reagents alone showed only modest scattering of light. The situation was strikingly different for the mixture of $SbCl_3$ and retinol (**1**), where aggregates gave rise to a large increase in the scattered intensity.‡ The other reagent–polyene combinations also showed increased scattering relative to the pure solutions, however, not to the extent observed for the original Carr–Price reaction. The equivalent hydrodynamic radii of the aggregates were determined to be 90–100 nm for retinol (**1**) and β,β-carotene (**6**) in mixture with $SbCl_3$, Fig. 4. For the BF_3–diethyl etherate mixtures, with lesser aggregate abundance, somewhat larger aggregate sizes were measured, Fig. 3 and Fig. 4.

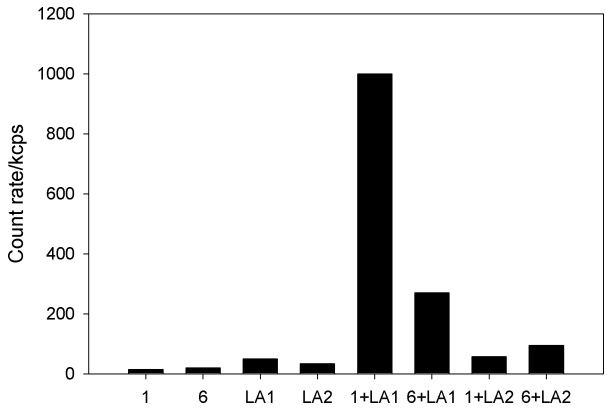


Fig. 3 Scattered intensity of chloroform solutions of retinol (**1**, 1.1 mg mL^{-1}), β,β-carotene (**6**, 1.8 mg mL^{-1}), $SbCl_3$ (LA1, 100 mg mL^{-1}) and BF_3–diethyl etherate (LA2, 10 vol.%), and corresponding mixtures.

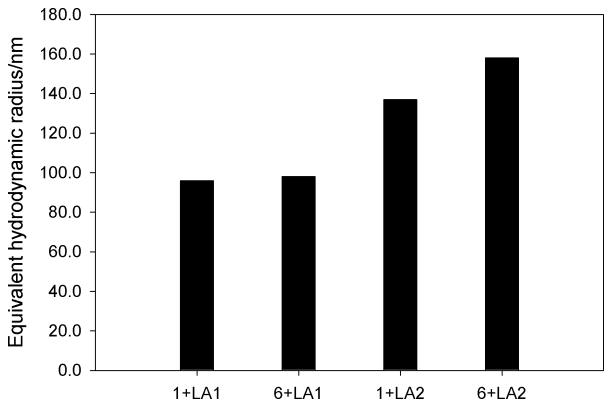
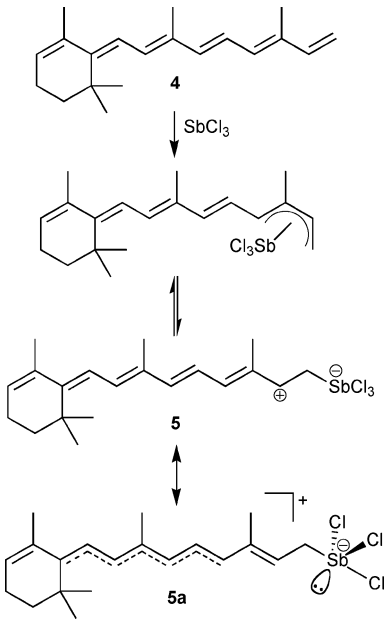


Fig. 4 Equivalent hydrodynamic radii for 3 mL chloroform solutions of $SbCl_3$ (LA1, 100 mg mL^{-1}) and BF_3–diethyl etherate (LA2, 10 vol.%), mixed with 2 drops of retinol (**1**, 1.1 mg mL^{-1}) or β,β-carotene (**6**, 1.8 mg mL^{-1}).

In contrast to β,β-carotene dication (**7**), prepared from β,β-carotene (**6**) and BF_3–etherates, that gave interpretable NMR spectra,[12] the broadening of NMR signals in the case of the original Carr–Price conditions relative to BF_3–etherates must be ascribed to higher aggregation tendency in the former.

X-Ray analysis of aryl adducts of $SbCl_3$ demonstrated the presence of tetrameric chlorine-bridged Sb_4Cl_{12} units, cross-linked by the arenes.[25,26] The binding showed slight deviation from a centric (η^6) coordination. Similar larger clusters might be envisaged in our aggregate solutions, for instance through η^4 coordination of the anhydroretinol intermediate (**4**), as illustrated in Scheme 5. However, the UV/VIS absorption strongly suggest

‡ Due to the light absorption of the Carr–Price product in the vicinity of the laser wavelength (632.8 nm), the scattered intensity is also underestimated.



Scheme 5

an important contribution from the charge-separated zwitterion **5**, considered earlier, *cf.* **5**, Scheme 2.[11] Very similar UV/VIS absorption for the anhydroretinylic cation **3a**/**3b** and the Carr–Price product favours the analogous, more detailed structure **5a** for the major isomer. By analogy with the estimated charge distribution in **3a**/**3b**, Fig. 1, a similar delocalisation of the charge in **5a**, Scheme 5, seems reasonable. The energy gained by charge delocalisation in the $SbCl_3$ adduct **5a** has a counterpart in the aromaticity maintained in the aryl adducts mentioned above.[25,26] Antimony might be expected to exist in a trigonal bipyramidal geometry in **5a**, with regard to the stereochemically active lone pair present in $SbCl_3$.[13] However, the observed aggregation points towards extensive cross-bridging and formation of more complex structural units. Other examples of compounds with covalent Sb–C bonds are known.[27]

The lack of stability for the Carr–Price product may also be explained by aggregate formation, bringing reactive species in relatively close proximity. Decomposition of retinoid cations to form larger units are reported,[9] as well as dimer formation in retinoid radical cations.[15,28]

Addition of sodium methoxide as a nucleophile afforded formation of two classes of dimers with short chromophores as the main products, formally corresponding to addition of either retinol (**1**) + anhydroretinol (**4**), with $M = 554$, or to even less polar products. Dimerisation may thus be considered to be a major pathway for decomposition of the blue product formed in the Carr–Price reaction. The formation of even higher molecular weight addition products was not investigated.

Conclusions

The reaction between the retinoids retinol (**1**), retinyl acetate (**8**) and anhydroretinol (**4**) with Brønsted acids was studied as a model system, leading to a detailed structure of the blue, delocalised anhydroretinylic cation (**3**).

Our reinvestigation of the classical Carr–Price blue colour reaction between vitamin A (retinol, **1**) and the Lewis acid $SbCl_3$ in chloroform solution solved two major problems: i) the relative instability of the product *versus* the anhydroretinylic cation (**3**), and ii) the failure to obtain NMR spectra. A radical cation structure of the Carr–Price product was excluded in a separate study.[17] These results could instead be rationalised by facile aggregation, demonstrated by dynamic light scattering experiments.

Very similar VIS data for the anhydroretinylic cation (**3**) and the Carr–Price product supported structural analogy. The detailed structure proposed for the Carr–Price product based on the delocalised anhydroretinylic cation (**3**) and with the antimony in a trigonal bipyramidal configuration is based on literature data for antimony complexes. Further cross-bridging to more complex structural units in the aggregates is considered to be likely.

Chemical quenching of both the anhydroretinylic cation (**3**) and the Carr–Price product with nucleophiles were consistent with the cationic structures. The formation of dimers appears to be a prominent pathway for decomposition of these cations.

Experimental

Materials

Synthetic retinol (**1**) was purchased from Sigma, whereas β,β-carotene (**6**) was supplied by F. Hoffmann-La Roche. Retinol (**1**) was converted to retinyl acetate (**8**) by a standard procedure with acetic anhydride in pyridine. Anhydroretinol (**4**) was prepared by treatment of retinol (**1**) with an acetic acid–potassium acetate mixture.[29],§ Purification of **4** and **8** was performed by preparative TLC. The acids were supplied by Merck (trifluoroacetic acid, sulfuric acid), Aldrich (trifluoromethanesulfonic acid, and deuterated trifluoromethanesulfonic acid) and Acros ($SbCl_3$, BF_3–diethyl etherate). Chloroform was purified on an alumina column (basic, activity I) prior to use.[30] All other solvents were used without further purification.

Methods

All pigments were stored under nitrogen atmosphere in a freezer (−20 °C). Ultraviolet (UV) and visible light (VIS) spectra were recorded on a Varian Cary 50 UV/VIS spectrophotometer (190–1100 nm). Spectral fine structure is reported as %III/II.[31] During low temperature experiments the spectrophotometer was equipped with a cuvette holder with internal circulating cooling fluid (methanol) delivered from a cryostat. Condensation of water was prevented with a continuous flow of nitrogen through the sample compartment.

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 20–300 °C, at a transient of 10 °C min^{-1}. NMR spectra were obtained on a Bruker Avance DRX 500 instrument, using a 5 mm inverse probe (TXI). ^{13}C chemical-shift data was acquired using inverse ^{1}H-detected experiments. Chemical shifts are cited relative to TMS with calibration against chloroform at 7.27 ppm and 77.0 ppm for ^{1}H and ^{13}C, respectively.

§ The method reported by Shantz *et al.*[36], and Petracek and Zechmeister[37] is recommended.

HPLC was carried out on a Hewlett Packard Series 1050 instrument equipped with a diode array detector (DAD). Detection wavelengths were set at 280, 330 and 380 nm, and the selected wavelength for integration is given in parenthesis. UV/VIS spectra of the retinoids were recorded on-line during chromatography. System: Interchrom Uptisphere 5 ODB column, 250 × 4.6 mm. Mobile phase 0 min, methanol (1.0 mL min^{-1}); 25 min, methanol–*tert*-butyl methyl ether (87.5 : 12.5 v/v, 1.0 mL min^{-1}); 45 min, methanol–*tert*-butyl methyl ether (55 : 45 v/v, 1.0 mL min^{-1}). A similar column with 10 mm inner diameter was used for preparative separations.

Dynamic light scattering measurements were performed using an ALV DLS/SLS-5022F compact goniometer and an ALV-5000/E multiple τ-digital correlator (ALV, Langen, Germany). The light source was a 22 mW He–Ne laser (Uniphase, Witney Oxon, UK). The temperature of the sample was 24 °C, and the scattering angle was set to 90°. Data analysis was performed with the CONTIN method available in the ALV software package.[32] In the absence of information on the aggregate shape, the equivalent hydrodynamic radius was calculated.[33]

Characterisation of starting materials

Retinol (1). λ_{max}(chloroform)/nm 333; HPLC R_T = 5.8 min. ^{1}H NMR, ^{13}C NMR and mass spectra were in agreement with literature data.[3,19,34]

Anhydroretinol (4). λ_{max}(hexane)/nm 351 369 391, %III/II 66. HPLC R_T = 10.9 min (λ_{max}/nm 349sh, 365, 383), R_T = 11.1 min (λ_{max}/nm 351, 369, 389).

Retinyl acetate (8). λ_{max}(dichloromethane)/nm 332; HPLC R_T = 7.7 min.

^{1}H NMR and mass spectra for **4** and **8** were in agreement with literature data.[3,34]

UV/VIS experiments

a) With trifluoroacetic acid. UV/VIS experiments with trifluoroacetic acid were carried out both at room temperature and at −10 °C. The acid concentration was 1.5 mg mL^{-1} in chloroform. Retinol (**1**, 50 μL of a 0.21 mg mL^{-1} solution) was added to 3 mL of the acid solution in the cuvette. Formation of anhydroretinol (λ_{max}/nm 358, 377, 398, %III/II 43) dominated the spectra at both temperatures, but at the lowest temperature a weak absorption at 623 nm could be observed, Table 1.

b) With sulfuric acid. The experiment was performed at −30 °C with an acid concentration of 50 mol% H_2SO_4 in methanol. To a 3 mL acid solution, an aliquot from the methanol stock solution of retinyl acetate (**8**) was added. UV/VIS spectra were recorded every min for 5 min. High viscosity prevented proper mixing.

c) With trifluoromethanesulfonic acid. UV/VIS experiments were performed at −15 °C with an acid concentration of 1.7 mg mL^{-1} in dichloromethane. To 3 mL acid solution, aliquots from dichloromethane stock solutions of retinol (**1**), anhydroretinol (**4**) and retinyl acetate (**8**) were added in separate experiments. UV/VIS spectra were recorded every 5 min for 1 h, Table 1.

NMR experiments

a) With trifluoromethanesulfonic acid. Characterisation by NMR was done at −15 °C. General procedure: To a cooled solution of retinol (**1**, 2.0–2.5 mg) in 0.5 mL $CDCl_3$, 5 μL deuterated trifluoromethanesulfonic acid was added, causing an immediate colour change to blue. ^{1}H, ^{1}H–^{1}H COSY, 2D ROESY, ^{1}H–^{13}C HMSC,[35] ^{1}H–^{13}C HMQC and ^{1}H–^{13}C HSQC spectra were recorded. Results are given in Table 2.

The same protocol was employed for NMR characterisation (^{1}H, ^{1}H–^{1}H COSY) of cations formed from anhydroretinol (**4**, 0.1 mg). For NMR characterisation (^{1}H, ^{1}H–^{1}H COSY) of cations formed from retinyl acetate, 1.5 mg retinyl acetate (**8**) and 10 μL deuterated trifluoromethanesulfonic acid was employed.

b) With antimony trichloride. Blue solution was achieved at −15 °C with 0.6 mg retinol (**1**) added to 0.5 mL 7% $SbCl_3$ in $CDCl_3$. However, only severely broadened peaks could be observed in the ^{1}H NMR spectra. The experiments were complicated by difficult shimming, poor stability and precipitation of reagent at temperatures below −15 °C.

Dynamic light scattering experiments

Solutions of retinol (**1**, 1.1 mg mL^{-1}), β,β-carotene (**6**, 1.8 mg mL^{-1}), $SbCl_3$ (100 mg mL^{-1}) and BF_3–diethyl etherate (10 vol.%) were prepared. The samples were carefully filtered through a 5 μm filter. Measurements were performed on pure solutions and on low concentration mixtures, prepared with two drops of polyene solution (**1** and **6**) added to 3 mL Lewis acid solutions. For the mixtures, care was taken to minimise time between mixing and measurement.

Reactions of anhydroretinylic cation (3) with nucleophiles

a) Methanol at room temperature. Retinol (**1**, 5.0 mg, 0.017 mmol) was dissolved in chloroform (5.0 mL). Trifluoromethanesulfonic acid (10 μL), dissolved in chloroform (2.0 mL), was added, causing an immediate colour change to dark blue. The reaction mixture was quenched with methanol (1.0 mL) after 1 min, and regained its pale yellow colour. The pigments were transferred to hexane and washed with water and aqueous, saturated NaCl solution. After evaporation of solvents, the crude product mixture was redissolved in *tert*-butyl methyl ether and analysed by HPLC, showing anhydroretinol (**4**) as the main product (HPLC R_T = 11.0 min, 67% (380 nm), λ_{max}/nm 349, 366, 387, %III/II 67).

b) Sodium methoxide at room temperature. Retinol (**1**, 5.0 mg, 0.017 mmol) was dissolved in chloroform (5.0 mL). Trifluoromethanesulfonic acid (10 μL), dissolved in chloroform (2.0 mL, containing 1% ethanol), was added, causing an immediate colour change to dark blue. The reaction mixture was quenched with sodium methoxide in methanol (1.0 mL, 30%) after 1 min, and regained its yellow colour. The pigments were transferred to hexane and washed with water and aqueous, saturated NaCl solution. After evaporation of solvents, the crude product mixture was redissolved in *tert*-butyl methyl ether and analysed by HPLC, and subsequently separated in four fractions by preparative HPLC.

14-Methoxy-4,15-*retro*-deoxyretinol (11). HPLC R_T = 9.7 min, 16% (330 nm), λ_{max}/nm 331, 347, 365, %III/II 51; *m/z* (EI) 300 (M^+, 100%, base peak), 268 (15, M − CH_3OH).

14-Ethoxy-4,15-*retro*-deoxyretinol. HPLC R_T = 10.5 min, 7% (330 nm), λ_{max}/nm 333, 349, 367, %III/II 48; *m/z* (EI) 314 (M^+, 100%, base peak), 284 (10), 268 (12, M − EtOH), 260 (16).

Anhydroretinol (4). HPLC R_T = 11.7 min, 21% (330 nm), λ_{max}/nm 349, 367, 385, %III/II 63; *m/z* (EI) 268 (M^+, 100%, base peak).

Dimer mixture. HPLC R_T = 36–39 min, 14% (330 nm), λ_{max}/nm 305–323; *m/z* (EI) 536 (M^+, 16%), 268 (100, base peak).

c) Sodium methoxide at −15 °C. Retinol (**1**, 5.0 mg, 0.017 mmol) was dissolved in chloroform (5.0 mL) immersed in a dry ice–ethylene glycol bath to keep the solution at the desired temperature. Trifluoromethanesulfonic acid (10 μL), dissolved in chloroform (2.0 mL) and cooled to −15 °C, was added, causing an immediate colour change to dark blue. The reaction mixture was quenched with sodium methoxide in methanol (1.0 mL, 30%) solution kept at the same temperature after 10 min, and regained its yellow colour after a few seconds. The pigments were transferred to hexane and washed with water and aqueous, saturated NaCl solution. After evaporation of solvents, the crude product mixture was redissolved in *tert*-butyl methyl ether and analysed by HPLC, and subsequently separated in two fractions by preparative HPLC.

Dimer I. HPLC R_T = 17.6 min, 50% (280 nm), λ_{max}/nm 281sh, 291, 303 sh; *m/z* (EI) 555 (20%, M + 1), 554 (M^+, 48), 431 (15), 365 (22), 351 (11), 285 (20), 269 (45), 268 (100, base peak).

Dimer II. HPLC R_T = 18.8 min, 21% (280 nm), λ_{max}/nm 289; *m/z* (EI) 555 (34%, M + 1), 554 (M^+, 81), 431 (44), 364 (20), 351 (39), 285 (76), 269 (100, base peak), 268 (40).

Reaction of Carr–Price product with sodium methoxide

Retinol (**1**, 10.0 mg, 0.035 mmol) was dissolved in chloroform (5.0 mL) at room temperature. $SbCl_3$ (200 mg, 0.88 mmol) in chloroform (2.0 mL) was added, causing an immediate colour change to dark blue. The reaction mixture was quenched with sodium methoxide in methanol (1.0 mL, 30%) after 30 s, and regained its yellow colour. A few drops of *N*-ethyl diisopropylamine was added, and the pigments transferred to hexane and washed with water and aqueous, saturated NaCl solution. After evaporation of solvents, the crude product mixture was redissolved in *tert*-butyl methyl ether and analysed by HPLC, showing mainly formation of dimers, *vide supra* (HPLC R_T = 17.3 min, 21% (280 nm), λ_{max}/nm 281sh, 291, 303 sh; R_T = 18.5 min, 11%, λ_{max}/nm 287; R_T = 36.6 min, 11%, λ_{max}/nm 291; R_T = 37.7 min, 11%, λ_{max}/nm 291). Reduction of the reaction temperature to −15 °C gave similar results.

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Notes and references

1 F. H. Carr and E. A. Price, *Biochem. J.*, 1926, **20**, 497–501.
2 T. Moore, *Vitamin A*, Elsevier, Amsterdam, 1957.
3 U. Schwieter, G. Englert, N. Rigassi and W. Vetter, *Pure Appl. Chem.*, 1969, **20**, 365–420.
4 T. S. Sorensen, *J. Am. Chem. Soc.*, 1965, **87**, 5075–5084.
5 T. S. Sorensen, in *Carbonium Ions Vol 2: Methods of Formation and Major Types*, ed. G. A. Olah and P. von R. Schleyer, Wiley-Interscience, New York, 1970, pp. 807-835.
6 A. Wassermann, *J. Chem. Soc.*, 1959, 979–982.
7 N. C. Deno, C. U. Pittman, Jr. and J. O. Turner, *J. Am. Chem. Soc.*, 1965, **87**, 2153–2157.
8 W. V. Bush and L. Zechmeister, *J. Am. Chem. Soc.*, 1958, **80**, 2991–2999.
9 P. E. Blatz and D. L. Pippert, *J. Am. Chem. Soc.*, 1968, **90**, 1296–1300.
10 P. E. Blatz, N. Baumgartner, V. Balasubramaniyan, P. Balasubramaniyan and E. Stedman, *Photochem. Photobiol.*, 1971, **14**, 531–549.
11 P. E. Blatz and A. Estrada, *Anal. Chem.*, 1972, **44**, 570–573.
12 B. F. Lutnaes, L. Bruås, G. Kildahl-Andersen, J. Krane and S. Liaaen-Jensen, *Org. Biomol. Chem.*, 2003, **1**, 4064–4072.
13 F. A. Cotton and G. Wilkinson, *Advanced Inorganic Chemistry*, John Wiley & Sons, New York, 5th edn, 1988, pp. 396–397.
14 A. C. Buchanan III, R. Livingston, A. S. Dworkin and G. P. Smith, *J. Phys. Chem.*, 1980, **84**, 423–427.
15 K. Bobrowski and P. K. Das, *J. Phys. Chem.*, 1985, **89**, 5079–5085.
16 Q.-X. Guo, T. Ren, B. Li, J. Wang, Y.-C. Liu, T.-C. Tu and J.-R. Dong, *Chin. J. Chem.*, 1994, **12**, 66–72.
17 G. Kildahl-Andersen, T. A. Konovalova, A. L. Focsan, L. D. Kispert, T. Anthonsen and S. Liaaen-Jensen, unpublished work.
18 G. Englert, in *Carotenoids*, ed. G. Britton, H. Pfander and S. Liaaen-Jensen, Birkhäuser, Basel, 1995, vol. 1B: Spectroscopy, pp. 147–260.
19 G. Englert, *Helv. Chim. Acta*, 1975, **58**, 2367–2390.
20 H. Spiesecke and W. G. Schneider, *Tetrahedron Lett.*, 1961, **3**, 468–472.
21 B. F. Lutnaes, G. Kildahl-Andersen, J. Krane and S. Liaaen-Jensen, *J. Am. Chem. Soc.*, 2004, **126**, 8981–8990.
22 G. Kildahl-Andersen, B. F. Lutnaes, J. Krane and S. Liaaen-Jensen, *Org. Lett.*, 2003, **5**, 2675–2678.
23 R. L. Christensen and B. E. Kohler, *Photochem. Photobiol.*, 1973, **18**, 293–301.
24 V. V. Konovalov and L. D. Kispert, *J. Chem. Soc., Perkin Trans. 2*, 1999, 901–909.
25 H. Schmidbaur, R. Nowak, O. Steigelmann and G. Müller, *Chem. Ber.*, 1990, **123**, 1221–1226.
26 T. Probst, O. Steigelmann, J. Riede and H. Schmidbaur, *Chem. Ber.*, 1991, **124**, 1089–1093.
27 P. J. Durrant and B. Durrant, *Introduction to Advanced Inorganic Chemistry*, Longman, London, 2nd edn, 1970, pp. 778–779.
28 K. Bobrowski and P. K. Das, *J. Phys. Chem.*, 1986, **90**, 927–931.
29 W. Oroshnik, G. Karmas and A. D. Mebane, *J. Am. Chem. Soc.*, 1952, **74**, 3807–3813.
30 M. Casey, J. Leonard, B. Lygo and G. Procter, *Advanced Practical Organic Chemistry*, Blackie, Glasgow, 1990, pp. 35–36.
31 G. Britton, in *Carotenoids*, ed. G. Britton, H. Pfander and S. Liaaen-Jensen, Birkhäuser, Basel, 1995, vol. 1B: Spectroscopy, pp. 13–62.
32 S. W. Provencher, *CONTIN (Version 2) Users manual, technical report EMBL-DA07*, Max-Planck-Institut Biophysikalische Chemie, Göttingen, 1984.
33 N. C. Santos and M. A. R. B. Castanho, *Biophys. J.*, 1996, **71**, 1641–1650.
34 R. L. Lin, G. R. Waller, E. D. Mitchell, K. S. Yang and E. C. Nelson, *Anal. Biochem.*, 1970, **35**, 435–441.
35 R. Burger, C. Schorn and P. Bigler, *J. Magn. Reson.*, 2001, **148**, 88–94.
36 E. M. Shantz, J. D. Cawley and N. D. Emtree, *J. Am. Chem. Soc.*, 1943, **65**, 901–906.
37 F. J. Petracek and L. Zechmeister, *J. Am. Chem. Soc.*, 1956, **78**, 3188–3191.