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Comparative studies on radical cation formation from carotenoids and retinoids

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Abstract—A comparative study of radical cation formation from selected polyenes, namely carotenoids (C_{40}) and retinoids (C_{20}), has been carried out by treatment with the Lewis acids BF_3 as its etherate or $SbCl_3$. The reaction in chloroform was monitored by vis/NIR and EPR spectroscopy at variable temperature. β,β-Carotene, β,β-caroten-4-ol, retinol (vitamin A), retinyl acetate and anhydroretinol were used as substrates. It is concluded that whereas BF_3–diethyl etherate or $SbCl_3$ is capable of effecting one-electron transfers to produce radical cations from the longer polyenes (carotenoids), no radical cations were obtained from the retinoids. The results of $SbCl_3$ treatment of the retinoids have a bearing on the current studies on the mechanism of the Carr–Price blue colour reaction previously used for quantitative analysis of vitamin A.

1. Introduction

For understanding free radical reactions of carotenoids and related polyenes the initial formation of radical species is essential. The carotenoids are asymmetric in their electron donor or acceptor properties, that is, they are more susceptible to oxidations to cations than reduction to anions.[1] Several studies on the formation of carotenoid and retinoid radical cations have been reported, using pulse radiolysis, laser flash photolysis and electrochemical or chemical methods.[1–10] The radicals are detected using vis–NIR absorption spectroscopy,[2,3,8] EPR and ENDOR methods,[9,10] or indirectly by EPR with spin-trapping techniques.[11,12] Carotenoid radical cations are also formed in photosystem II, as a part of the electron transfer sequence from cytochrome b_{559} to $P^{\cdot +}_{680}$.[13]

The previous interpretation of Lewis acid reactions with polyenes led to a reinvestigation of the reaction between β,β-carotene (**1**) and BF_3-etherates.[14] The resulting NIR-absorbing product was proven by NMR to be a delocalised dication, in which two electrons were removed from the starting polyene. An EPR signal was detected, supporting a reaction pathway through two successive one-electron transfers. $FeCl_3$ has also been employed as a one-electron transfer reagent for the generation of the radical cation and the dication,[8] whereas iodine as Lewis acid has given a more complicated picture than the other two, albeit with the presence of radical species shown by EPR.[15,16]

Lewis acid reactions of retinoids are also well known, in particular the Carr–Price reaction, in which the retinoid is treated with a saturated chloroform solution of $SbCl_3$. The resulting blue, unstable product, absorbing at 620 nm, was used for spectrophotometric analysis of retinol (**2**, vitamin A) content.[17] In the polyene field, the Carr–Price reaction was also used as an initial test on chromatographic fractions, roughly correlating the observed colour with the number of conjugated double bonds.[18] Based on vis-absorption spectra and kinetic considerations, cationic addition products between the dehydration product anhydroretinol (**3**) and $SbCl_3$ have been suggested.[19] However, the formation of aryl radical cations in molten $SbCl_3$ has been shown by EPR.[20] It was suggested that the reactive species in $SbCl_3$ is in fact $SbCl_5$,[21] which is a common impurity in commercial $SbCl_3$, but the ability to perform one-electron abstractions does not rely on the presence of this impurity.[20]

Keywords: BF_3-etherate; $SbCl_3$; NIR; EPR; Line width.

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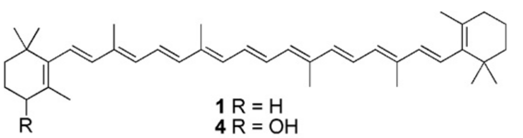
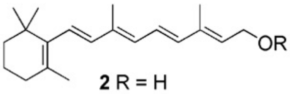
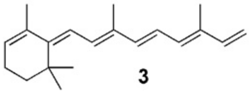
Chemical preparation of radical cations from retinol (**2**) are reported, using the single electron oxidant tris(*p*-bromophenyl)aminium hexachloroantimonate.[9] The radical was characterised by EPR and UV–vis spectroscopy, and supported by irradiation experiments. There are, to our knowledge, no other reports of the chemical preparations of retinyl radical cations. In this work, we have examined the treatment of carotenoids (C_{40}) and retinoids (C_{20}) in chloroform with the Lewis acids (i) BF_3 as its diethyl etherate and, (ii) $SbCl_3$, in order to obtain information on the formation of radical species by EPR studies. β,β-Carotene (**1**), β,β-caroten-4-ol (**4**, isocryptoxanthin), retinol (**2**), retinyl acetate (**5**) and anhydroretinol (**3**, anhydrovitamin A) were used as substrates.



1 R = H
4 R = OH



2 R = H
5 R = $COCH_3$



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2. Experimental methods

β,β-Carotene (**1**, Hoffmann-La Roche) and retinol (**2**, Fluka) were available. Carotenol **4** was prepared by $NaBH_4$ reduction of the corresponding ketone,[22] and anhydroretinol (**3**) by the treatment of **2** with acetic acid/potassium acetate,[23] or preferably, with acidic chloroform.[24] vis–NIR spectra of the polyenes were recorded, with temperature control, for 1 h in the previously reported manner,[14] in chloroform solutions containing 10% w/v of $SbCl_3$ or 1:10 v/v of BF_3–diethyl etherate. EPR measurements were performed with an X-band (9.1 GHz) Varian E-12 spectrometer (Palo Alto, California) equipped with a rectangular cavity. The magnetic field was measured with a Bruker (Billerica, MA) ESR 035 M gaussmeter, and the microwave frequency was measured with a HP 5245 L frequency counter. EPR spectra were recorded by the use of Scientific Software Services' setup consisting of a PIDM, data acquisition computer, connected to a Varian E-12 and EWWIN-data acquisition software. DPPH standard ($g = 2.00351$) was used for g value calibration.

Samples for measurements of EPR spectra were prepared using 0.15 mL of a 3–6 mM polyene solution in chloroform, degassed with freeze-thaw cycles and stored under argon, mixed with 0.20 mL of the Lewis acid solution, either 5–6% w/v $SbCl_3$ in chloroform, or 48% BF_3–diethyl etherate, used without dilution. The Lewis acid solutions were degassed by argon-flushing prior to mixing with the polyene. The Lewis acid was added to partially frozen polyene solutions, and the EPR tube cooled to 77 K and sealed, giving a layer of unreacted polyene at the bottom of the tube. The top layer turned coloured, ranging from purple to turquoise, green and blue, indicative of the formation of polyenyl radical cations or cations. Further mixing was promoted by thaw-freeze cycles, using a variable temperature assembly. Capillary room temperature experiments were performed with the mixtures of the equal volumes of pigment and $SbCl_3$ solutions.

3. Results and discussion

Results and conditions for vis/NIR experiments are given in Table 1, with stabilities given as half-life, $t_{1/2}$. The data obtained for the carotenoids (**1**, **4**) treated with $SbCl_3$ are compatible with published values for carotenoid radical cations obtained with $FeCl_3$.[8] Values obtained for the retinoids (**2**, **3**) are consistent with the literature data.[17,19] The low stability of the retinoid (**2**, **3**) products should be noted, thus requiring EPR experiments at low temperature.

The results of EPR measurements at 77 K, and after further mixing by thaw-freeze cycles, are given in Table 2, also including the capillary room temperature experiments. Measured line widths, ΔH_{pp}, for the carotenoids examined are given in Table 3. The initial line width values obtained at 77 K had low reproducibility, which may be rationalised by sample inhomogeneity in the frozen state.

BF_3 may be used as a Lewis acid as an alternative to the treatment of carotenoids with Brønsted acids. Only β,β-carotene (**1**) gave facile radical formation in the presence of BF_3–diethyl etherate, consistent with the previous observation of radical species by EPR during the formation of the β,β-carotene dication.[14] The corresponding allylic carotenol (**4**) gave a weaker EPR signal only after heating.

The reaction of polyenes with $SbCl_3$ is of particular interest for elucidating the mechanism of the Carr–Price

Table 1. Absorption of retinoids and carotenoids treated with $SbCl_3$ or BF_3-etherate

Polyene	Lewis acid	Temperature (°C)	λ_{max} (nm)	$t_{1/2}$ (min)
Retinol (**2**)	$SbCl_3$	20	620	7
Retinol (**2**)	$SbCl_3$	0	620	65
Retinol (**2**)	BF_3-etherate	0	611	60
Anhydroretinol (**3**)	$SbCl_3$	0	623	70
β,β-Carotene (**1**)	$SbCl_3$	0	1020[a]	[b]
β,β-Carotene (**1**)	BF_3-etherate	20[c]	985[c]	[b]
β,β-Carotene (**1**)	BF_3-etherate	−25[c]	920[c]	[b]
β,β-Caroten-4-ol (**4**)	$SbCl_3$	0	1025	[b]

[a] Initial absorption 950 nm. Additional shoulder at 935 nm present in all following scans.

[b] Much more stable than the retinoids, typically 20–30% intensity loss after 1 h.

[c] From Ref. 14.

Table 2. Detected free radical signals by EPR measurements, X = detected signal, 0 = no signal, W = weak signal, — = not attempted. Multiple entries correspond to multiple samples

Polyene	Lewis acid	77 K	Low temperature[a]	Capillary rt
β,β-Carotene (**1**)	$SbCl_3$	XX	XX	X
β,β-Caroten-4-ol (**4**)	$SbCl_3$	0X	XX	—
Retinol (**2**)	$SbCl_3$	00	0	0
Retinyl acetate (**5**)	$SbCl_3$	0	0	0
Anhydroretinol (**3**)	$SbCl_3$	0	0	0
β,β-Carotene (**1**)	BF_3-etherate	X	X	X[b]
β,β-Caroten-4-ol (**4**)	BF_3-etherate	0	W	—
Retinol (**2**)	BF_3-etherate	0	0	—
Retinyl acetate (**5**)	BF_3-etherate	0	0	—
Anhydroretinol (**3**)	BF_3-etherate	0	0	—

[a] Measured at 195–207 K after thaw-freeze cycle.

[b] From Ref. 14.

Table 3. Measured line widths, ΔH_{pp}, of carotenoids treated with Lewis acids

Polyene	Lewis acid	Low temperature[a]	Capillary rt
β,β-Carotene (**1**)	$SbCl_3$	4.6 G; 6.0 G	12.4 G
β,β-Carotene (**1**)	BF_3-etherate	2.4 G	14 G[b]
β,β-Caroten-4-ol (**4**)	$SbCl_3$	10.2 G; 9.6 G	—
β,β-Caroten-4-ol (**4**)	BF_3-etherate	9.5 G	—

[a] Measured at 195–207 K after thaw-freeze cycle.

[b] From Ref. 14.

reaction of retinol (**2**), providing a blue (λ_{max} 620 nm in $CHCl_3$) product, previously used for the quantitative analysis of vitamin A. Both carotenoids (**1** and **4**) gave the formation of radical cations, proving the ability of the $SbCl_3$ reagent to perform one-electron abstractions, exemplified for β,β-carotene (**1**) in Figure 1. By analogy with the reaction suggested for arenes,[20] the following redox equation may be formulated:

$$\mathrm{Car} + \frac{1}{3}\mathrm{SbCl_3} \rightleftharpoons \mathrm{Car}^{\bullet+} + \frac{1}{3}\mathrm{Sb}^0 + \mathrm{Cl}^-$$

However, no evidence for the formation of radical species was obtained in the presence of $SbCl_3$ for either retinol (**2**) or its acetate (**5**), nor for anhydroretinol (**3**), which has been suggested as an intermediate in the Carr–Price reaction,[19] cf. Table 2.

The EPR signal of β,β-carotene (**1**) treated with $SbCl_3$, shown in Figure 1, has a *g* value of 2.0028, which is characteristic of the carotenoid radical cation. However, the line width of 4.6–6.0 G, Table 3, is narrower than that usually found for the isolated radical cation (12–14 G). This narrowing is presumably due to a reaction product of Cl^- anions, formed by the reduction of $SbCl_3$,[20] and $Car^{\bullet+}$. It has previously been demonstrated that $Car^{\bullet+}$ prepared by $FeCl_3$ or I_2 chemical oxidation can be stabilised by I_3^- or Cl^- ions.[12,15,16] When $[I_2] > [Car]$, these radical cations form complexes of $[Car^{\bullet+}I_3^-]$ or dimer $[C_{40}H_{56}]_2^{\bullet+}I_3^-$ or trimer $[C_{40}H_{56}]_3^{\bullet+}I_3^-$, where the line widths equaled 14 G, or 7–8 G, or 4–5 G, respectively.[15] Also the observed line width for β,β-carotene (**1**) treated with BF_3–etherate was small at low temperature, indicating complex formation with the BF_4^- counterion. However, room temperature values for the capillary experiments are in the normal range, as are the values for β,β-caroten-4-ol (**4**).

It is concluded that whereas BF_3-etherates or $SbCl_3$ are capable of effecting one-electron transfers from the longer polyenes (**1**, **4**), the mode of reaction with the shorter polyenes (**2**, **3** and **5**) involves no radical species. This result may reflect the ease of oxidation of the longer polyenes (carotenoids) relative to the retinoids, providing more stabilised delocalised radical cation products. Electrochemical investigations have revealed a lower oxidation potential for β,β-carotene (**1**) relative to retinol (**2**) and anhydroretinol (**3**), although the data also suggest the direct formation of β,β-carotene dication.[1,7] Our NIR data for β,β-carotene (**1**) treated with $SbCl_3$ supports radical cation formation, whereas the presence of a shoulder at lower wavelength might indicate partial conversion to the dication.

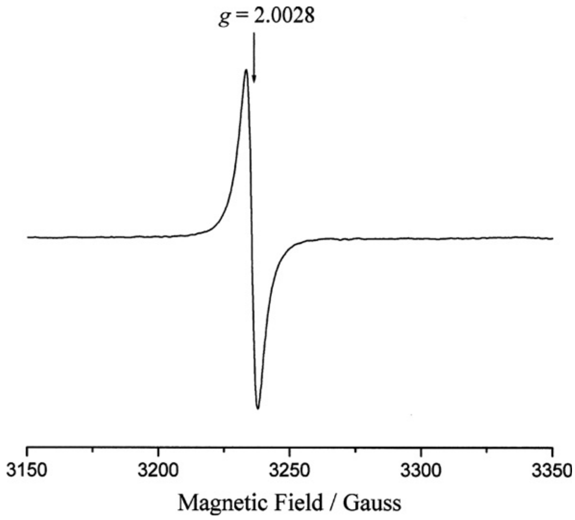

Figure 1. EPR spectrum of β,β-carotene (**1**) treated with $SbCl_3$ and measured at 207 K. Parameters: microwave power = 5 mW, microwave frequency = 9.066 GHz, frequency amplitude = 100 kHz, modulation amplitude = 1.6 G, gain = 1.25×10^4.

Further detailed studies on the Carr–Price reaction of retinoids are published separately.[25]

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