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Preparation and structure elucidation by NMR of the charge delocalised β,β-carotene dication

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Abstract—The β,β-carotene dication (λ_{max} 985 nm at room temperature) prepared by treatment of β,β-carotene with BF_3–etherate had considerable stability. The reaction, monitored by EPR studies at −25°C, occurred by free radical intermediates. EIMS further revealed a $C_{40}H_{56}BF_3$ intermediate. The detailed structure of the charge delocalised β,β-carotene dication was established by COSY, HSQC, HMBC and 1D and 2D ROESY NMR techniques (600 MHz, $CDCl_3$, −25°C) leading to complete assignments of ^1H and ^{13}C chemical shifts and $^3J_{H,H}$ coupling constants. Considerable downfield shifts were observed relative to β,β-carotene. The results are consistent with charge delocalisation mainly in the C-5–C-9 and C-5′–C-9′ regions and with retro shifted double bonds in the central C-13–C-13′ region. The effect of the two delocalised charges on chemical shift (charge distribution) and bond distance ($^3J_{H,H}$) is discussed. © 2002 Elsevier Science Ltd. All rights reserved.

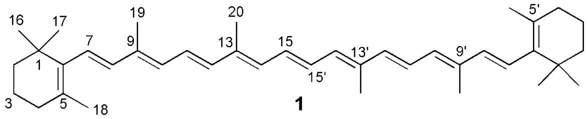
Carotenoid cations are likely intermediates in E1-type eliminations of allylic hydroxy, alkoxy and glycosyloxy groups[1] and have been hypothetic intermediates in reactions of BF_3 with carotenoids.[2]

Carotenoid cation radicals have been demonstrated in photosystem II, which is a membrane bound pigment–protein complex responsible for catalysing the oxidation of H_2O to O_2 in oxygenic photosynthesis.[3–6] Moreover, the presence of carotenoid dications have been suggested in photosynthetic reaction centres.[7]

The reactive charged carotenoid species relevant for biological systems have been studied by methods including NIR,[3,7–11] EPR,[4,12] ENDOR[5] and resonance Raman spectroscopy,[3] cyclic voltammetry,[7–9] and AM1 calculations.[13–16] The experimental data have not resulted in definitive structural assignments of such charged carotenoid species. No NMR data have been published.

The reaction between β,β-carotene **1** and BF_3–etherate, studied by Zechmeister's school in the 1950s,[17,18] has been reinvestigated by modern methods. Our studies have resulted in the isolation and structural elucidation of the dication of β,β-carotene **1** as a symmetrical charge delocalised dication.

The reaction between **1** and BF_3–etherate provided a complex with λ_{max} ($CHCl_3$) 985 nm at rt and λ_{max} 920 nm at −25°C. The stability was monitored by UV–vis–NIR studies. The absorption of the dication at −25°C showed a decrease in intensity of less than 20% during 2 h. This is remarkably stable for a cationic species, albeit consistent with the strong stabilisation from extensive delocalisation.



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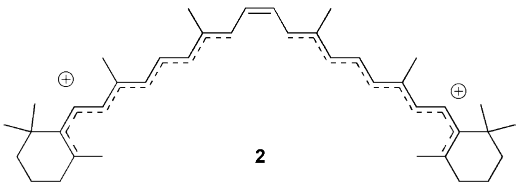

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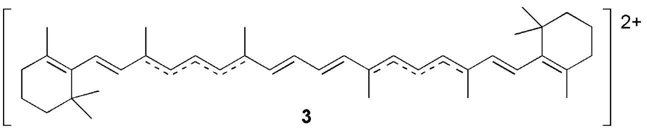

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The reaction, also monitored by EPR studies at −25°C, occurred via free radical intermediates. The EPR spectrum of the reaction mixture showed a weak signal with a linewidth of 14 G, which is in agreement with observed values for the β,β-carotene (**1**) cation radi-

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Keywords: carotenoid cation; β,β-carotene; dication; preparation; NMR; charge delocalisation; structure.

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cal.[19] The presence of a radical species is also supported by the observation that in freshly prepared samples the NMR resonances are broadened at the beginning of the recordings. Moreover, EIMS revealed an intermediate with molecular ion *m*/*z* 604 compatible with $C_{40}H_{56}BF_3$ and with fragmentations involving two consecutive losses of C_2H_2.

^{1}H NMR studies of the dication **3** were conducted in $CDCl_3$ at −25°C as an optimum temperature, and with suppression of the methyl/ethyl signals of the etherate reagent. However, no ^{1}H–^{13}C connectivities could be observed due to dominant signals from the etherate reagent. The dication was subsequently prepared using BF_3 complexed with $(CD_3)_2O$ as Lewis acid in order to minimise unwanted signals.

The ^{1}H NMR spectrum demonstrated a symmetrical dication. ^{1}H and ^{13}C chemical shifts were established using homonuclear ^{1}H–^{1}H COSY and heteronuclear ^{1}H–^{13}C HSQC and HMBC NMR techniques (Table 1). Initially the (15-*Z*)-configuration (**2**) was deduced[20] on the basis of a relatively small coupling constant for $J_{15,15'}$.[21] However, detailed 1D and 2D ROESY experiments led to a revised structure for the β,β-carotene dication **3**.

Whereas the assigned chemical shifts (^{1}H and ^{13}C) and $^3J_{H,H}$ coupling constants remained unchanged, the interactions in space shown in Fig. 1 clearly differentiated between structures **2** and **3** for the β,β-carotene dication. The reorientation of the six-membered rings relative to the polyene chain allows an increased delocalisation of the positive charges, evidenced by the large downfield ^{13}C shifts for C-5(5′). The effect of rotating the β-ring on spin density and bond lengths in the polyene chain has been studied in detail by the density functional method B3LYP[22] in the β,β-carotene cation radical.

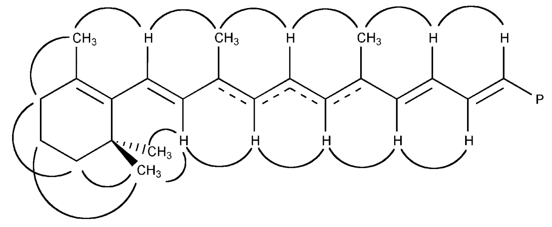

Figure 1. Through-space interactions in β,β-carotene dication **3** established by 1D and 2D ROESY.

Table 1. ^{13}C and ^{1}H NMR chemical shifts (ppm) relative to TMS for β,β-carotene **1** and β,β-carotene dication **3** in $CDCl_3$, and chemical shift differences for **3** relative to **1**

	^{13}C			^{1}H		
	1	**3**	Δ	**1**	**3**	Δ
1/1′	34.3	35.1	0.8			
2/2′	39.8	41.5	1.7	1.47	1.63	0.16
3/3′	19.4	22.7	3.3	1.62	1.72	0.10
4/4′	33.2	39.5	6.3	2.03	2.68	0.65
5/5′	129.3	181.2	51.9			
6/6′	138.0	146.3	8.3			
7/7′	126.7	159.7	33.0	6.16	8.56	2.40
8/8′	137.8	136.9	−0.9	6.15	7.39	1.24
9/9′	136.0	181.8	45.8			
10/10′	130.8	142.0	11.2	6.15	7.27	1.12
11/11′	125.1	153.6	28.5	6.65	8.40	1.75
12/12′	137.3	140.2	2.9	6.37	7.03	0.66
13/13′	136.4	163.4	27.0			
14/14′	132.4	145.7	13.3	6.27	7.10	0.83
15/15′	130.0	142.9	12.9	6.63	7.40	0.77
16/16′	29.0	28.8	−0.2	1.03	1.35	0.32
17/17′	29.0	28.8	−0.2	1.03	1.35	0.32
18/18′	21.7	24.6	2.9	1.72	2.39	0.67
19/19′	12.8	15.2	2.4	1.97	2.64	0.67
20/20′	12.8	14.2	1.4	1.97	2.43	0.46
			252.3			12.12

NMR shifts of the symmetrical structure **3** relative to the β,β-carotene **1** (Table 1) served to identify the charge distribution of the delocalised cation. The total ^{13}C chemical shift change of **3** relative to **1** was 504 ppm, consistent with the formation of a dication.[23] Also the total shift change for ^{1}H is consistent with the expected value per positive charge[24] for formation of a polyene dication. The downfield shift is most pronounced for the carbon atoms that can carry the positive charge by resonance (odd-numbered carbons) (Table 1).

The downfield shift increases towards the end of the polyene chain, and in an almost linear fashion for the odd numbered carbons (Fig. 2). Deviations for C-7 and C-9 are probably due to C-7 being a secondary carbon, whereas C-9 is a tertiary carbon, stabilising the positive charge better. The effect for the even-numbered carbon atoms not carrying the charge does not give a clear picture.

In the ^{1}H NMR, the size of the downfield shift in **3** versus **1** is largest at H-7(7′), even when the increased steric hindrance between 18(18′)-CH_3 and H-7(7′) from the rotation of the end groups is taken into consideration. A similar effect is observed for C-7(7′) in the ^{13}C NMR, which are also the positions with the largest change in chemical shift of all proton carrying carbons.

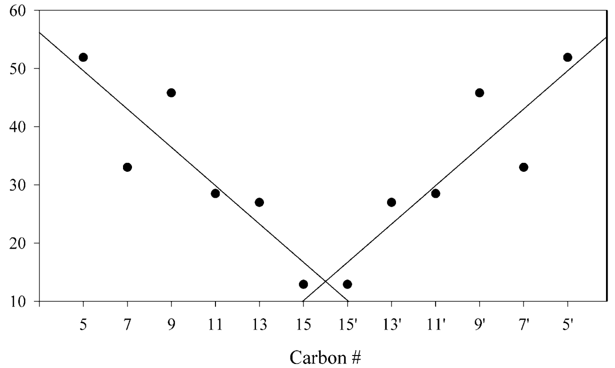

Figure 2. ^{13}C NMR downfield chemical shift differences between β,β-carotene **1** and β,β-carotene dication **3**.

Steric effects also explain the small upfield shift of C-8(8′), which will experience less steric hindrance in **3** versus **1**. Thus, the chemical shift data support a symmetrical delocalised dication with the charge preferentially in the C-5–C-9 and the C-5′–C-9′ regions.

The size of the coupling constants in the polyene chain of non-charged carotenoids has been generalised as 13.5–16.8 Hz for *trans* double bonds with a tendency to decrease towards the central part of the conjugated chain. The coupling constant across single bonds (s-*trans*) are normally 10.5–12.0 Hz with the larger values near the central part of the molecule.[21] These generalisations were not made for retro-carotenoids[25] in which the positions of double bonds are shifted. For *cis* double bonds, the range 11.5–12.8 Hz has been given.[21]

As depicted in Fig. 3, the coupling constants $J_{14,15}=$ 14.6 Hz and $J_{15,15'}=12.0$ Hz are of the same size as the coupling constants in **1** in this region ($J_{14,15}=14.4$ Hz and $J_{15,15'}=11.9$ Hz),[26] and are compatible with localised retro double/single bonds in the C-13,13′ region. The C-10,11 ($J_{10,11}=13.7$ Hz) and C-11,12 ($J_{11,12}=12.4$ Hz) bonds have similar bond character compatible with bond reversals in this region.

Structure **3** is also consistent with the value found for the coupling constant $J_{7,8}$ (15.1 Hz), which, though being somewhat smaller than in **1** (16.1 Hz), indicates a *trans* 7,8-double bond. Also, the C-5,6 bond is therefore expected to have a high degree of double bond character, forcing the C-4–C-5–C-6–C-1 structural element of the end group in a planar arrangement, consistent with the coupling constants in the ^{1}H NMR spectra. A planar polyene system is also a prerequisite for maximum electron delocalisation.

The total NMR evidence is best accommodated with structure **3**, with charge delocalisation in **3** primarily in the C-5–C-9 and C-5′–C-9′ regions. This structure is compatible with previously published semi-empirical Hartree-Fock AM1 calculations of the structure of the β,β-carotene dication, suggesting that the β,β-carotene dication would exist as a pair of charged solitons with bond reversals.[13,14]

Other AM1 calculations of bond length modifications in the β,β-carotene dication versus **1**, showing the largest changes in the central region compatible with bond reversals, and almost no change towards the end groups, do not contradict structure **3**.[15,16] These studies, however, suffer from the fact that they do not take into consideration the rotation of the end groups, thereby predicting a too small charge delocalisation here.[22]

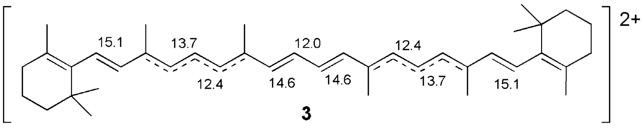

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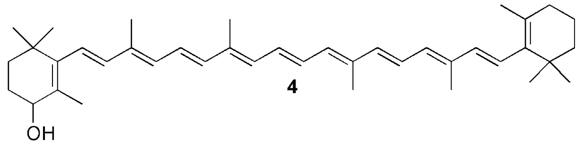
Figure 3. Coupling constants in Hz recorded for β,β-carotene dication **3**.

The β,β-carotene dication **3**, therefore, emerges as an example in which the effect of the cations on bond lengths and charge distribution is differently experienced in various regions of the polyene chain. The effect of the positive charge (as deduced from the chemical shifts) is largest towards the ends of the chain (C-5–C-9 and C-5′–C-9′), whereas the effect on the bond lengths (as deduced from the $^3J_{\mathrm{H,H}}$ coupling constants) is largest in the centre of the molecule (C-13–C-13′) leading to a reversal of double and single bonds in the centre. The regions C-9–C-13 and C-9′–C-13′ are characterised by delocalised bonds.

In relation to the previously proposed structure for the β,β-carotene dication with a central *cis*-bond **2**, it was considered of interest to examine the dication obtained from (15-*Z*)-β,β-carotene (15*Z*-**1**). The (15-*Z*)-isomer, which was completely stable in $CDCl_3$ in darkness at room temperature for 20 h, provided, according to ^{1}H NMR data, the same dication **3** with BF_3 complexed with $(CD_3)_2O$ after approximately 1 h at −25°C. It is concluded that the (15-*Z*)-bond isomerised as a consequence of the formation of the preferred delocalised dication structure.

The formation of the dication **3** and the $C_{40}H_{56}BF_3$ intermediate via a radical type process is rationalised in Scheme 1. The structure of the negative counter ion of the dication **3** is tentative, formulated by analogy,[27] and not established. Reaction of the dication **3** with a nucleophile (H_2O in acetone) provided strongly *E*/*Z* isomerised isocryptoxanthin **4** as the major product, (*E*/*Z*)-retro-dehydro-β,β-carotene **5** and mutatochrome **6** according to HPLC, UV, MS and ^{1}H NMR data. The resulting *E*/*Z* isomerisation is expected via cationic intermediates. The formation of the products **4**–**6** from the dication **3** may be rationalised on a mechanistic basis. Details will be presented elsewhere.



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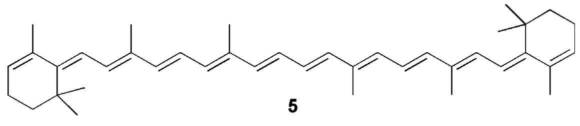

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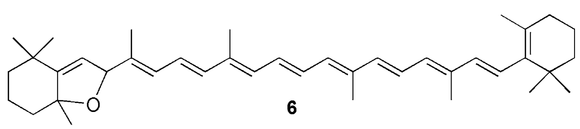

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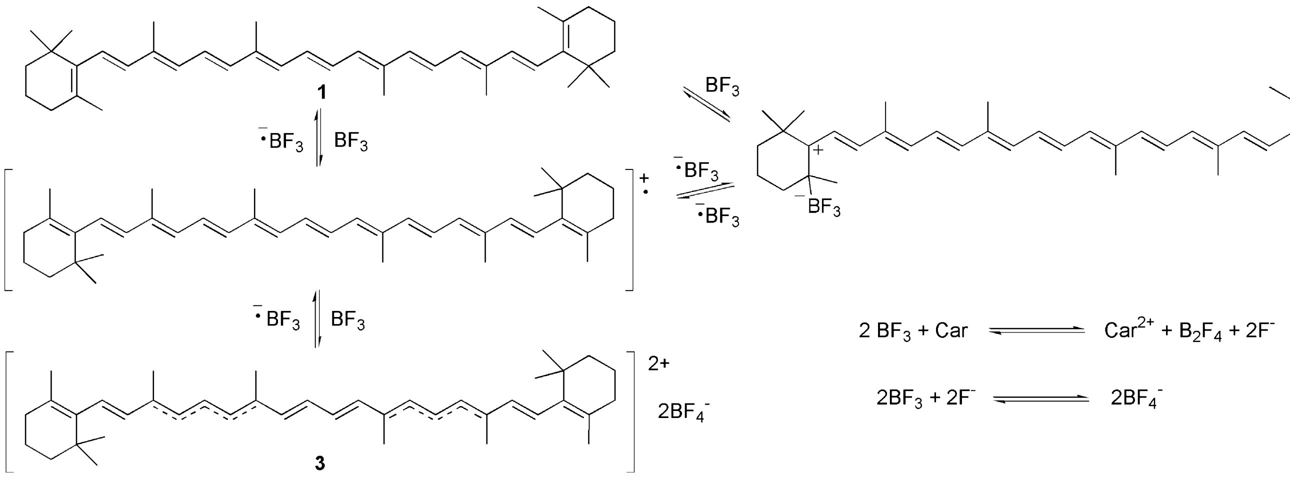

Scheme 1. Proposed formation of β,β-carotene dication **3** and its counterion from β,β-carotene **1** and BF_3–etherate.

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