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Facile Electron Uptake by Carotenoids Under Mild, Non-Radiative Conditions: Formation of Carotenoid Anions

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Carotenoids – prominent antioxidants (= electron donors) – are commonly not recognized as electron acceptors. Nonetheless, carotenoids can take up electrons under mild chemical conditions: dioxocarotenoids in alkaline DMSO and DMF form stable deeply colored carotenoid dianions, which react with different additives to new carotenoid derivatives.

Introduction

The highly unsaturated polyenic carotenoids (Car) are widely known as antioxidants (reductants) for their electron-releasing capacity towards radicals.[1] The reverse reaction, uptake of electrons by Car (oxidant reaction) has not yet been observed in nature and is therefore not considered relevant for the antiradical properties of carotenoids.[2] Literally understood, Car are antioxidants for reactive oxygen species, inter alia hydroxy radical $HO^{\bullet}$, peroxyl radical $HO_2^{\bullet}$ and alkoxyl radical $RO^{\bullet}$, but not for superoxide anion radical $O_2^{\bullet-}$.[3] Nonetheless, it has recently been demonstrated that carotenoids scavenge $O_2^{\bullet-}$ as well.[4–6] The direction of electron transfer was not addressed in these investigations; however, electron release from $O_2^{\bullet-}$ to Car instead from Car to $O_2^{\bullet-}$ appears an alternative route. In fact, it has been demonstrated that Car could favorably act as electron acceptor.[7,8] Especially dioxocarotenoids, particularly astaxanthin and canthaxanthin, are expected to scavenge $O_2^{\bullet-}$ by electron uptake.[9] So far, electron uptake by Car has been investigated by quite elaborated methods: chemical reactions with metals (Al-Hg, Na, K, Cs),[10–13] and metal organic compounds (e.g. *s*BuLi),[14] electrochemical methods,[15] ionizing rays (pulse radiolysis)[2,16,17] and flash photolysis.[18] Notwithstanding, electron transfer to Car should be possible without relying on particular scrupulous procedures. The methylsulfinylmethide (dimsyl) anion $H_3CS(=O)CH_2^-$ ($DMSO^-$), prepared from DMSO with alkali (*s*BuOK, KOH),[19] is not only a powerful base and nucleophile but also a reducing agent.[20,21] Similarly, but far less mentioned, the DMF anion $HC(=O)NCH_3CH_2^-$ (DMF^-) reduces appropriate compounds.[20] Electron transfer to unsaturated dicarbonyl compounds is a well-established reaction.[22–24] Specifically, the various quinones in animal and plant cells exert their effects by consecutive single electron transfer.[25,26] It is, therefore, quite astonishing that the many ketocarotenoids and carotenoid aldehydes remained unnoticed in analogous electron transfer studies.

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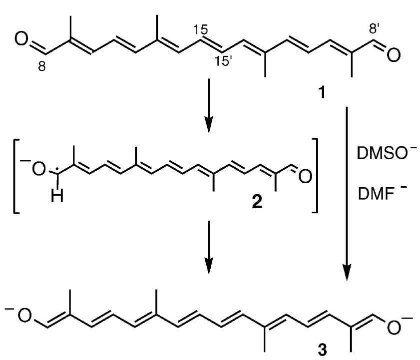
We describe here a straightforward reaction in which Car, hereafter defined as crocetindial (C20-dialdehyde **1**), a naturally occurring oxidation product of β,β-carotene,[27,28] easily accepts electrons at ambient conditions. The presented method of generating dienolate anions by electron transfer to α,ω-polyene aldeydes refers to related prior research on generating anion radicals by single electron transfer to (un)-saturated mono or vicinal diketones.[29–32]

Several oxocarotenoids have been exploratorily tested in alkaline DMSO and DMF. In this paper we concentrate to exemplify the reaction with **1** (Scheme 1).



Scheme 1. Charge delocalization in **3** not indicated.

Results and Discussion

Blue anion. When a solution of MeOK in MeOH was injected under nitrogen to DMSO or DMF in which **1** was dissolved, the color changed within 10 s resulting in a complete conversion (isosbestic point at 500 nm) from orange **1** (λ_{max} = 450 nm) to a new blue product (λ_{max} = 570 nm, Figure 1). Comparison of the ^{13}C NMR signals from the blue solutions in alkaline [D_6]DMSO with those of Car **1** (7 C=C) revealed a distinct up-shift of the polyene chain signals, which is indicative of a more electron rich molecule. It was identified as charged delocalized Car^{2-} **3** (8 C=C) with the charge partially directed to oxygen (Figure 2, Scheme 1).

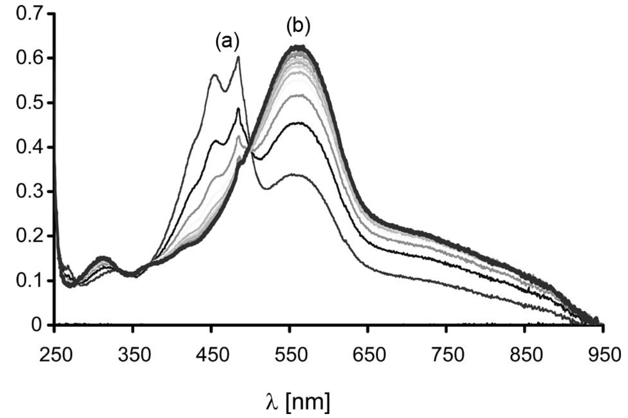


Figure 1. UV/Vis spectra of reaction Car **1** (a) → Car^{2-} **3** (b) in alkaline DMSO. Scans recorded at intervals of 2 s.

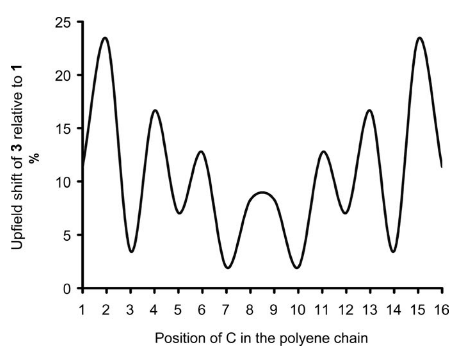
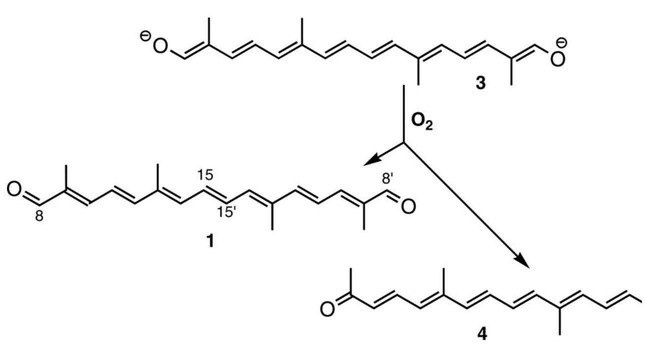


Figure 2. ^{13}C NMR signals of Car^{2-} **3** compared to ^{13}C NMR signals of Car **1**, upfield shift change expressed in % relative to Car **1**.

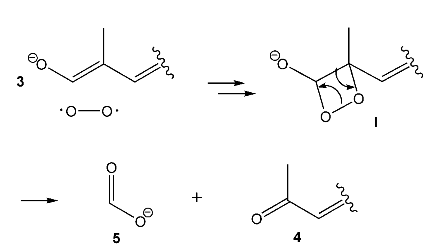
The unspectacular reaction of Scheme 1 finally confirms that carotenoids can accept electrons at ambient conditions. If assuming that $DMSO^-$ or DMF^- initiates a stepwise single electron transfer,[21] a delocalized ketyl radical $Car^{\cdot-}$ **2** might occur as intermediate (Scheme 1).[32] A single electron uptake would then reflect the course of reaction under radiolytical and electrochemical conditions.[11,15,17] We have not been able to detect short-lived $Car^{\cdot-}$ **2** by its NIR absorption (Figure 1).[18] Therefore, the prompt appearance of Car^{2-} **3** suggests either a direct two electron uptake, a rapid, successive one-electron attachment or a single electron uptake followed by an immediate disproportionation reaction 2 $Car^{\cdot-}$ → Car^{2-} + Car.[14] A probable dimerisation 2 $Car^{\cdot-}$ → Car-Car^{2-} has been excluded.[33]

Despite exhaustive investigations the mechanistic evidence for electron transfer reactions in alkaline DMSO remains uncertain: a cryptically formulated multistep addition reaction of $DMSO^-$ with dicarbonyl compounds was explicitly presented as "belief".[32] A direct one-electron transfer from $DMSO^-$ to unsaturated compounds with consequential formation of $DMSO^{\cdot}$ has been referred to as "unbelievable".[32] Accordingly, the detection of $DMSO^{\cdot}$ and its decay has not been addressed in previous studies.[29–32] The reaction mechanism for electron transfer from DMF^- to unsaturated compounds is probably not less ambiguous.[20] Whatever reservations can be forwarded on the electron-transfer mechanism from $DMSO^-$ and DMF^-, Car (**1**) undeniably takes up electrons and is reduced to give the blue dianion Car^{2-} (**3**) (Scheme 1).

Secondary products. C18 diketone: The solutions of Car^{2-} (**3**) in DMSO or DMF were stable in closed vials at room temperature for several months. However, when Car^{2-} **3** solutions were exposed to air, the blue color vanished and Car (**1**) was regenerated together with ketone **4** (Scheme 2). Concurrent NMR detection of formate **5** allowed the formulation of a tentative mechanism for the loss of two carbon atoms in **4** (Scheme 3). Enolate **3** may react with ground state oxygen $^3O_2$ to dioxethane intermediate **I**,[34,35] which, by electrocyclic arrangements, forms the shortened diketone **4** with double bond transposition, together with **5** (Scheme 3). Anions have been found to react with $^3O_2$ in alkaline DMF.[36] The reaction from C20 dialdehyde **1** to C18 diketone **4** represents the first synthesis of this paprika carotenoid.[37]

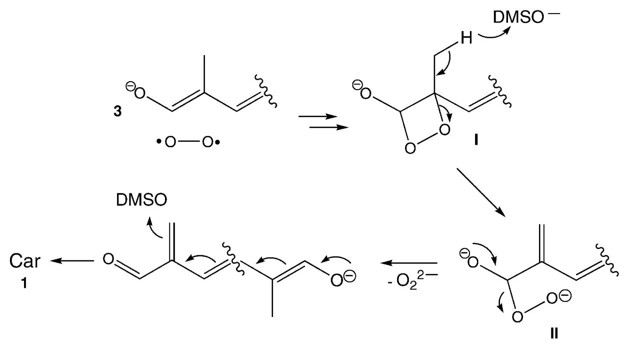


Scheme 2.



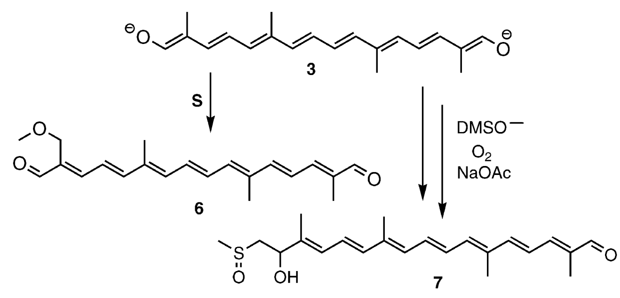
Scheme 3.

Regeneration of Car 1: **1** was always formed in substantial amounts after access of air to the blue anion solution. The regeneration of **1** could be explained by abstraction of a proton from dioxetane **I** by $DMSO^-$ resulting in intermediate peroxy anion **II** from which peroxide O_2^{2-} is eliminated by carbonyl formation followed by tautomerisation to **1** (Scheme 4). The reaction of anions with $^3O_2$ gives peroxidate O_2^{2-}.[38]

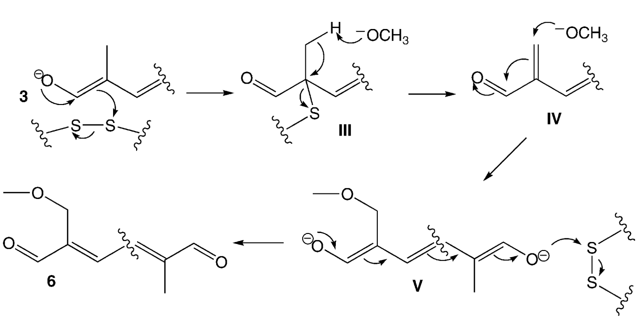


Scheme 4.

Methyl ether 6: When sulfur powder was added to the blue dianion **3** solution, methyl ether **6** was formed (Scheme 5). We reasoned that S could react with anion **3** to intermediate **III**. A base-catalyzed elimination of sulfide gives **IV** to which methylate (present in the solution to alkalify the DMSO) is added in a Michael-type addition. The enolate intermediate **V** then tautomerizes to methyl ether **6** (Scheme 6).



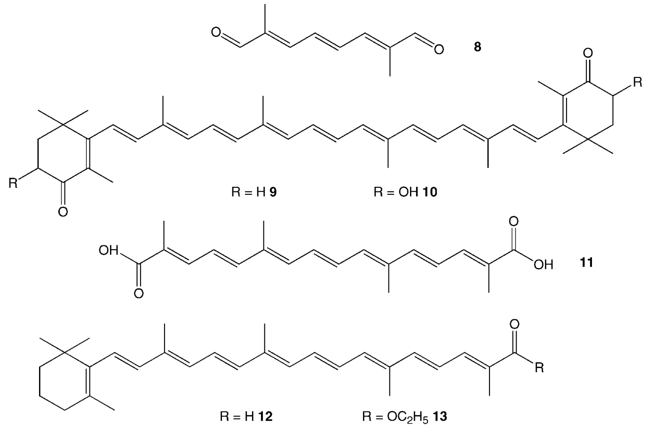
Scheme 5.



Scheme 6.

DMSO adduct 7: When NaOAc was added to the blue DMSO solution of **3** methylsulfinyl alcohol **7** was formed (Scheme 5). The same reaction in DMF did not provide the analogous DMF adduct. The role of NaOAc is not clear. It may accelerate the nucleophilic reaction of DMSO$^-$ with one of the carbonyl groups in regenerated **1** after air exposure or may stabilize product **7** by preventing the expected dehydration to a conjugated methylsulfinyl-polyene. Similar DMSO adducts with aromatic aldehydes are known.[39]

The fact that DMSO$^-$ and DMF$^-$ can act as a base, nucleophile and reducing agent indicates the possibility of several competing reactions; intermediates and reaction conditions may determine the outcome. A precise mechanistic discussion on product formation (see Schemes 3, 4, 6) seems provisional at this juncture since indicative byproducts have not yet been identified. Secondary products from carotenoid anions appear structurally more varied than from carotenoid cations.[40] The specified electron transfer reaction to **1** may be corroborated with compounds listed in Scheme 7. The yellow color of a C10-dialdehyde **8** solution in alkaline DMSO or DMF changed to red after addition of base illustrating the formation of the anticipated dianion. The red color disappeared immediately in air; formation of products analogous to those given in Scheme 1, though with shorter chain, is expected. A slower color change occurred with canthaxanthin (**9**), astaxanthin (**10**) and crocetin (**11**) in alkaline DMSO or DMF. The sparingly soluble monocarbonyl compounds C30-aldehyde **12** and C30-ester **13** gave the distinctive color change only after several days; a reaction pathway to anions different from dioxocompounds **1**, **8**–**11** is probable.



Scheme 7.

Although the described method cannot mimic relevant biological electron uptake conditions, alkaline DMF and DMSO easily allows characterization, and determination of the spectroscopic properties, of (di)oxocarotenoid anions. Especially the potential uptake of electrons by canthaxanthin (**9**) and astaxanthin (**10**) warrants further examinations.

Concluding Remark

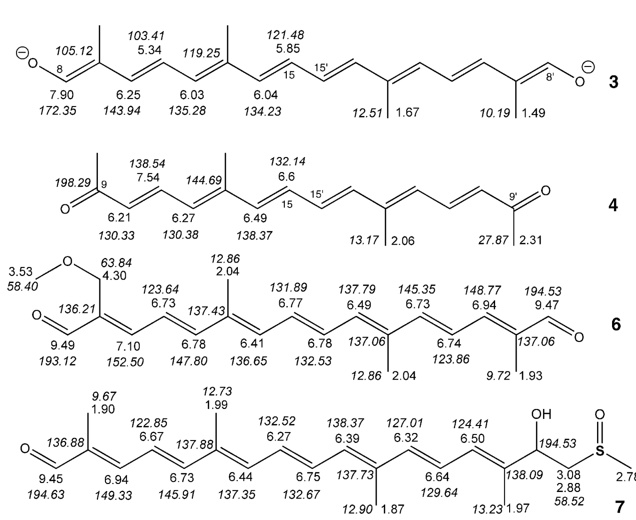
The role of carotenoids as electron acceptors has so far been underestimated apparently because of methodological constraints. Alkaline DMSO and DMF offer now a convenient bench-top method for studying the in vitro electron uptake reactions of oxocarotenoids without relying on difficult chemical reactions, electrochemical arrangements or specialized facilities requiring radiation sources. Even though carotenoids are exclusively defined as antioxidants (reductants),[1] the actual experimental results confirm the predictions[7–9] that carotenoids can occur as "antireductants", or synonymously expressed, as "oxidants". While the scientific antagonisms "oxidant" and "reductant" are unequivocally defined, the vernacular has only assimilated "antioxidant", particularly for carotenoids. It may therefore be justified to advocate the use of the redundant term "antireductant"[7,8,41,42] as the consequently entailed antonym for "antioxidant", in contrast to the likewise redundant term "prooxidant".[43]

Experimental Section

CAUTION: Carotenoids, when exposed to air, can unexpectedly oxidize with explosive effects. Explosions have been reported when working with $DMSO^-$.[44,45]

General: NMR spectra were recorded on Bruker Avance 400 MHz and 600 MHz instruments in $CDCl_3$ with 0.05% TMS as internal standard. The precise assignment of protons and carbons in the products was achieved by iterative interpretation of spectra from different NMR techniques (^{1}H, ^{13}C, DEPT, homo and hetero nuclear COSY, HMBC). UV/Vis spectra were obtained on a single beam Thermo Spectronic Helios γ and a HP 8453 diode array spectrometer. MS spectra were run on a MAT 95XL (TermoQuest Finigan). Solvents of spectroscopic quality were used for spectra recording; for reaction and elution solvents were of p.a. quality. Carotenoids **1**, **8**, **9**, **10** and **13** were provided by BASF AG, Ludwigshafen, Germany. Acids **11** and **12** were obtained after purification and hydrolysis of crocin (Fluka) and C30 ethyl ester **13**.[46,47]

Preparation and Work-Up Procedure: Car (**1**) (20 mg, 0.067 mmol) was dissolved under a strong stream of N_2 in dry deoxygenated DMSO or DMF (4 mL). MeOK (25% in MeOH, 100 µL) was added and the reaction stirred at room temperature under a continuous stream of N_2 for 2 h. The reaction became evident by a color change from orange **1** to blue **3**. Anion **3** was quenched by air, addition of sulfur or NaOAc (1 g, respectively) and stirred for 15 min. Extraction of the reaction mixture with CH_2Cl_2/H_2O, drying the organic phase with Na_2SO_4 and solvent removal under reduced pressure gave a residue from which the products were separated by flash chromatography and TLC silica plates. UV/Vis showed quantitative formation of **3**: Figure 1. The broad shoulder at 700 nm could either indicate the presence of small amounts of long-wavelengths absorbing species or may be inherent to the electronic properties of **3**. For NMR characterization **1** (10 mg, 0.034 mmol) was dissolved in deoxygenated [D6]DMSO (1 mL) in an NMR tube and kept under a steady N_2 stream. CD_3OK (25% in CD_3OD, 5 µL) was added with a syringe and the NMR tube was sealed. ^{1}H and ^{13}C NMR: see Scheme 8.



Scheme 8. ^{1}H and ^{13}C NMR in $CDCl_3$.

Regeneration of 1: The presence of **1** after reaction of **3** with O_2, S and NaOAc was verified by co-chromatography on analytical TLC.

C18-Diketone 4: Ketone **4** (9 mg, 50% yield in DMSO) was isolated from the blue solution after exposing to air. MS (EI, 70 eV): m/z = 269.9, UV/Vis: (CH_2Cl_2): λ = 409 nm. ^{1}H and ^{13}C NMR: Scheme 8. Formate $HC(=O)O^-$ was detected in a separate NMR sample after bubbling with air; ^{1}H NMR: δ = 8.29 ppm. ^{13}C NMR: δ = 165.86 ppm. The formation of **4** and formate is similar to the carbon dehomologation reaction of enamines.[48]

Methylether 6: Addition of sulfur powder (S_8) to the blue solution gave **6** (10.5 mg, 48% yield in DMSO). MS (EI, 70 eV): m/z = 326.5. UV/Vis (CH_2Cl_2): λ = 449 and 475 nm. ^{1}H and ^{13}C NMR: see Scheme 8. TLC shows the presence of other minor products.

The products from the reaction in DMF were identical with **4** and **6** obtained in DMSO (TLC evidence).

DMSO Adduct 7: After addition of NaOAc to the blue solution and work-up in air compound **7** was obtained (5 mg, 20% yield). UV/Vis (CH_2Cl_2): λ_{max} = 435 nm. MS (EI, 70 eV): m/z = 374.6. ^{1}H and ^{13}C NMR: see Scheme 8.

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