

Studies on Allylic Oxidation of Carotenoids

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Catalysis by iodine and light of the aerobic oxidation of carotenoids containing allylic hydroxyl groups to the corresponding ketones is reported. Molecular oxygen (air) can with advantage be replaced by *p*-chloranil as oxidant, and the catalytic effect of iodine and light on the allylic oxidation with *p*-chloranil is demonstrated. Illumination by means of standardized artificial light is the method of choice. On exposure to artificial Na-light the production of undesirable side-products was suppressed, and a nearly quantitative yield of keto-products was obtained.

The method has been employed for the partial synthesis of 4-hydroxy-4'-keto- β -carotene (IV) from isozeaxanthin (III), and compound (IV) has been characterized.

Efficient methods for the selective oxidation of allylic hydroxyl groups to the corresponding ketones are of importance for the structural elucidation of naturally occurring carotenoids and for the partial synthesis of conjugated keto-carotenoids from hydroxylated compounds. Owing to the general instability of carotenoid pigments only a few methods are usable, notably allylic oxidation by *p*-chloranil as introduced to the carotenoid field by Warren and Weedon¹ and oxidation by silver oxide described by Bodea, Nicoara and Salontai.² Oxidation of allylic hydroxyl groups with active manganous dioxide according to Attenburrow *et al.*³ which has proved so useful in the vitamin A field, has also found a certain application in the carotenoid series.⁴ However, the method is not always reliable,¹ and in our experience not sufficiently gentle on semi-micro scale.

In the present paper a further contribution to this field is given.

RESULTS AND DISCUSSION

During the iodine catalyzed stereoisomerization routinely carried out in this laboratory for the characterization of the stereoisomeric sets of new carotenoids (*cf.* Ref. 5), we observed oxidation of hydride-reduced okenone (okenol)⁶ and hydride-reduced warmingone (warmingol),⁷ to the corresponding ketones. These experiments were carried out in daylight in the presence of catalytic amounts of iodine (*ca.* 1 % of the carotenoid) in a presumably

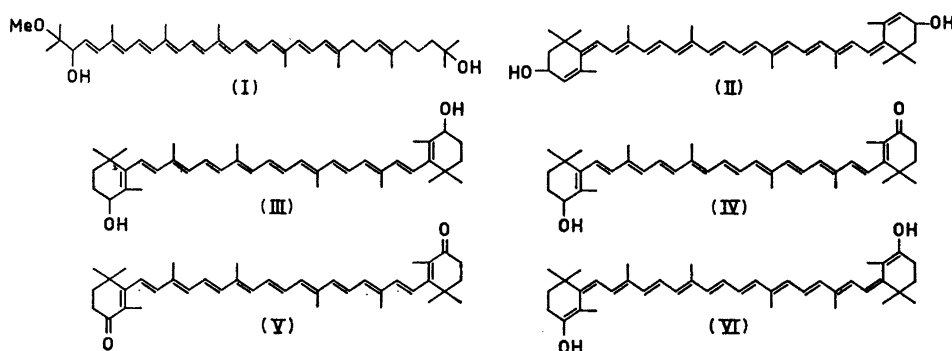
inert atmosphere. However, when under otherwise similar experimental conditions pure nitrogen was continuously passed through the system, no oxidation took place. Thus the oxidant involved evidently was molecular oxygen.

Similarly, iodine catalysis in the light, of various carotenoids like hydride-reduced OH-spheroidenone (I), eschscholtzanthin (II), and isozeaxanthin

Table 1. Oxidation by air of carotenoids containing allylic hydroxyl groups to the corresponding ketones under the catalytic influence of iodine and daylight.

Carotenoid	Structure	Reaction time in h	Reaction mixture	
			Carotenoid	In % of recovered carotenoid
Reduced OH-spheroidenone	I	3½	Red. OH-spheroidenone	80
			OH-spheroidenone	20
Eschscholtzanthin	II	24	Eschscholtzanthin	70
			Rhodoxanthin	30
Isozeaxanthin	III	4	Isozeaxanthin	10
			4-Hydroxy-4'-keto-β-carotene	40
			Canthaxanthin	50
			Warmingol	90
Warmingol	?	4	Warmingone	10
			Okenol	45
Okenol	?	3	Okenone	55

(III) gave rise to the corresponding conjugated keto-carotenoids in variable yield, when oxygen was present in the reaction vessel (Table 1).



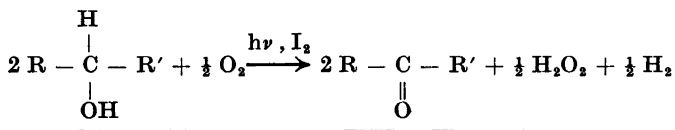
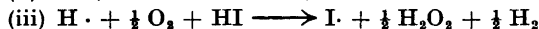
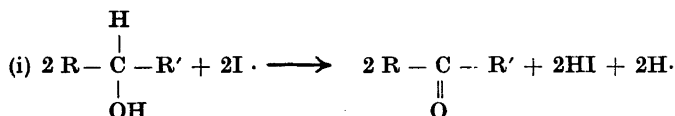
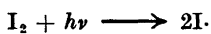
Isozeaxanthin (III) was used for the establishment of the experimental conditions required for the allylic oxidation; see Table 2. In the absence of iodine, light and oxygen caused no significant allylic oxidation during the 4 h test period, under conditions when the presence of iodine caused a 40 % conversion to (IV) and 50 % to canthaxanthin (V). Similarly the light require-

Table 2. The catalytic influence of iodine and daylight on the oxidation of isozeaxanthin by air. Reaction time 4 h.

Carotenoid	Carotenoid in % of recovered pigment			
	I ₂ /N ₂ /light	I ₂ /air/darkness	air/light	I ₂ /air/light
Isozeaxanthin	100	100	90	10
4-Hydroxy-4'-keto- -β-carotene	0	0	0	40
Canthaxanthin	0	0	10	50

ment of the reaction was demonstrated. No oxidation took place in the presence of oxygen and iodine during 4 h in darkness.

It can therefore be inferred that oxidation of carotenoids containing allylic hydroxyl to the corresponding ketones can be performed by molecular oxygen under the catalytic influence of iodine and light, possibly according to the following scheme:



The photooxidation of hydriodic acid (eqns. ii, iii) has been studied by Cook and Bates.⁸

The presence of oxygen in the system was not a desirable feature, since undesired photooxidations occurred as side-reactions. With longer reaction intervals low pigment recovery was observed. An attempt was therefore made to replace oxygen with *p*-chloranil. Tetrachlorohydroquinone formed according to the latter reaction was further expected to serve as an antioxidant.

Allylic oxidation by means of *p*-chloranil in the carotenoid series was introduced by Warren and Weedon,¹ and has since been successfully employed also in this laboratory. However, the yield of the keto-carotenoid produced has never exceeded 27 %.^{1,9}

The catalytic influence of iodine and light on the allylic oxidation of isozeaxanthin (III) with *p*-chloranil was subsequently demonstrated in a number of experiments. Two typical results are presented in Table 3. In this case the pigment recovery was high (ca. 90 %) and reproducible. In the pre-

Table 3. Catalytic influence of iodine in daylight on the allylic oxidation of isozeaxanthin by *p*-chloranil.

Expt. No.	Initial sample in mg	Total carotenoid recovery in %	Carotenoid	Carotenoid in % of recovered carotenoid											
				With iodine						No iodine					
				0 h	3 h	6 h	23 h	47 h	0 h	3 h	6 h	23 h	47 h		
1	1	91	Isozeaxanthin (III)	100	80	10	5		100	90	80	80	80		
			4-Hydroxy-4'-keto- β -carotene (IV)	trace	20	40	15	trace	10	20	20	20	20		
			Canthaxanthin (V)	—	—	50	80	—	—	—	—	—	—	—	
2	5	88	Isozeaxanthin (III)	100	86		47	—	100	96	78	29	29		
			4-Hydroxy-4'-keto- β -carotene (IV)	—	14		18	5	—	4	11	19	19		
			Canthaxanthin (V)	—	—		8	54	—	—	—	—	10	10	
			Side-product 1	—	—		7	30	—	—	11	12			
			Side-product 2 (polar)	—	—		20	5	—	—	—	—	30		

sence of iodine considerably better transformation to the mono- and diketo compounds was observed, and it can be inferred that the yields in the allylic oxidation with *p*-chloranil can be further improved by employing iodine as catalyst in the light.

Illumination by daylight has the obvious drawback of unreproducible light intensities. Furthermore, during prolonged illumination of isozeaxanthin (III) the formation of two sideproducts with isozeaxanthin spectrum and lower polarity was often observed (see Expt. 2, Table 3). These side-products were also encountered when the reaction was carried out in daylight in the absence of iodine, and they did not belong to the stereoisomeric sets of (III), (IV), or (V). The more polar one had adsorptive properties indicative of a mono-hydroxy-carotenoid, whereas apparently no free hydroxyl groups were present in the other side-product. The side-products were regarded as light-induced reaction products whose formation was caused by *p*-chloranil, and were not further investigated.

It was subsequently demonstrated that daylight could be efficiently replaced by white electric light; see Table 4. However, the method of choice

Table 4. Allylic oxidation of isozeaxanthin by *p*-chloranil under the catalytic influence of iodine and light of different wavelengths.

Specifications	Expt. 1	Expt. 2	Expt. 3
	Daylight (UV + visible)	Electric white light (400 – 700 m μ)	Artificial Na-light (580 – 590 m μ)
Reaction period in h	23	22	21
Initial sample in mg	5	10	12
Recovery in %	95	95	88
<i>Products in % of total</i>			
Isozeaxanthin (III)	47	21	—
4-Hydroxy-4'-keto- β -carotene (IV)	18	33	13
Canthaxanthin (V)	8	33	84
Side-product 1	7	2	3
Side-product 2 (polar)	20	12	—
	} 27	} 14	} 3

appeared to be illumination with artificial Na-light. The formation of the side-products was reduced when standardized white electric light was employed, and nearly completely eliminated with artificial Na-light; see Table 4. The synthesis of canthaxanthin (V) *via* (IV) from isozeaxanthin (III) was very efficiently carried out under standardized conditions involving illumination with artificial Na-light; see Tables 4 and 5. Carotenoids exhibit negligible adsorption in this spectral region, and the light requirement of the iodine

Table 5. Partial synthesis of 4-hydroxy-4'-keto- β -carotene from isozeaxanthin by oxidation with *p*-chloranil under the catalytic influence of iodine and artificial Na-light.

Specifications	Expt. 1	Expt. 2
Reaction period in h	15	10
Initial sample in mg	9	12
Recovery in %	85	86
Products in % of total:		
Isozeaxanthin	4	42
4-Hydroxy-4'-keto- β -carotene	25	37
Canthaxanthin	70	18

catalyzed oxidation reaction seems to be determined by light activation of the iodine involved. In benzene solution iodine has a broad absorption maximum at 500 $m\mu$ with significant extinction also at 580–590 $m\mu$.

An obvious disadvantage of the present method is of course the fact that illumination in the presence of iodine causes *cis-trans* isomerization of the carotenoids involved.⁵

The present reaction was used for the preparation of 4-hydroxy-4'-keto- β -carotene (IV) from isozeaxanthin (III). The partition ratio of (IV) has been published by Petrcek and Zechmeister,¹⁰ but evidently no further description has been published. A characterization of (IV) is therefore included.

4-Hydroxy-4'-keto- β -carotene (IV) crystallized as needles from ether/petroleum ether, m.p. 175°C. The melting point was intermediate between those of isozeaxanthin (III) (149–151°C)¹¹ and canthaxanthin (V) (216–217°C).¹² The extinction curve in visible light is presented in Fig. 1 together with those of isozeaxanthin and canthaxanthin, and the IR-spectra of these three carote-

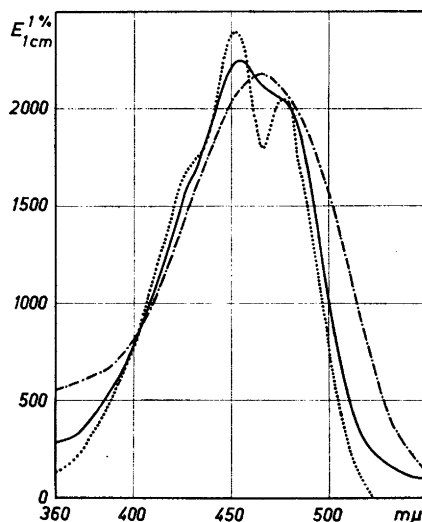


Fig. 1. Visible light absorption spectra in pet. ether of — 4-Hydroxy-4'-keto- β -carotene (IV), Isozeaxanthin (III), - . - Canthaxanthin (V).

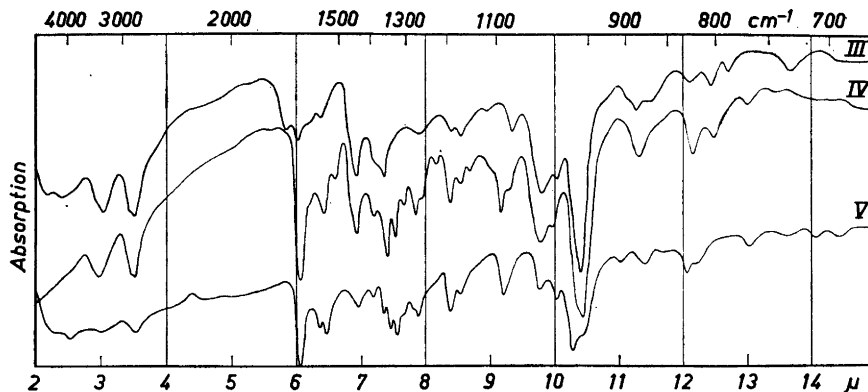


Fig. 2. Infrared spectra in KBr of isozeaxanthin (III), 4-hydroxy-4'-keto- β -carotene (IV), and canthaxanthin (V).

noids are given in Fig. 2. Further characteristic data of (IV) are to be found in the Experimental section. The mono-keto compound (IV) showed no tendency towards enolization on a weakly alkaline alumina column, and was not autoxidized to canthaxanthin (V) in the presence of alkali. If enolization to (VI) occurred, autoxidation to (V) would be expected as judged from the results of Kuhn and coworkers with related carotenoids (*e.g.* Refs. 13, 14). The production of the blue-coloured enol-salt of (VI) *in vacuo* was not observed.

EXPERIMENTAL

Materials. Hydride-reduced OH-spheroidenone (I), warmingol and okenol were produced by LiAlH_4 -reduction as described elsewhere.¹⁵⁻¹⁷ Eschscholtzanthin (II) was similarly synthesized from rhodoxanthin. The isozeaxanthin (III), canthaxanthin (V) and echinenone were synthetic samples. Except for the petroleum ether (b.p. 40–70°C) and acetone, solvents and reagents used were of analytical grade.

Methods. All reactions were carried out at room temperature in glass-stoppered Pyrex flasks. Reactions tested in darkness were carried out in a dark room. The following light sources were used:

- Indirect daylight, approximately 80 lux.
- White electric light from a 200 W incandescent lamp mounted at a distance of 70 cm from the reaction vessel; light intensity 20 lux measured at the reaction vessel.
- Artificial Na-light was prepared by passing the light from a 200 W incandescent lamp through a lens and a filter transparent to light of 5791–5893 Å; light intensity 20 lux measured at the reaction vessel.

The light intensities were measured with a Ljuskultur Luxmeter with colour-correcting filter.

For reactions carried out in inert atmosphere, pure nitrogen was bubbled through the reaction mixture during the test period. Aerobic reactions were carried out in unshaken flasks under a gas phase of air.

In most experiments samples of 0.1–10 mg carotenoids were used. Pet. ether or pet. ether-benzene mixtures (5–50 ml) were used as solvent. Iodine was added in pet. ether solution in amounts of 1–5 % (by weight) of the carotenoid.

Experiments involving oxidation with *p*-chloranil were carried out with isozeaxanthin (III) (1–10 mg) in benzene (5–50 ml) and ethanol (0.5–2 ml). *p*-Chloranil (3 times the

weight of the carotenoid) was added, and pure nitrogen was bubbled through the solution for 10 min. Iodine (1–5 % of the weight of the carotenoid) was added in pet. ether solution (0.5–5 ml). The mixture was stirred mechanically during the experiment.

Aliquots withdrawn at intervals were analysed by paper chromatography according to the method of Jensen and Liaaen Jensen,¹⁸ followed by spectrophotometric examination of the eluted pigments. The products were identified from absorption spectra in visible light and by co-chromatography tests with authentic specimens.

In larger-scale experiments the reaction products were transferred to ether in a separating funnel in the usual manner. The ether extract was washed with water, dried over anhydrous sodium sulphate, and the carotenoid mixture submitted to column chromatography on Woelm neutral, deactivated alumina, activity grade 2.¹⁹

In calculation of pigment recovery the drop in extinction coefficient due to inevitable *cis*-isomerization in the presence of iodine was allowed for. The extinction coefficient ($E_{1\text{ cm}}^{1\%}$ at the main maximum in pet. ether) used for canthaxanthin was 1900²⁰ and for (III) and (IV) 2000.

Visible light absorption spectra were recorded on a Beckman DB recording spectrophotometer and IR-spectra on a Perkin Elmer Model 21 Instrument.

4-Hydroxy-4'-keto- β -carotene (IV)

Preparation. The procedure outlined above was used, and the result is presented in Table 5.

Crystallization. (IV) was obtained as dark red, long, thin needles forming rosettes on recrystallization from ether-pet. ether (m.p. 175°C uncorr., measured in an evacuated tube in an Electrothermal melting point apparatus); yield about 1 mg.

Adsorptive properties. (IV) was adsorbed between canthaxanthin (V) and isozeaxanthin (III). The three compounds required 5–10 %, 15–25 %, and 25–35 % acetone in pet. ether for elution from deactivated alumina. *Trans* (IV) had $R_F = 0.31$ on kieselguhr paper¹⁶ when 5 % acetone-pet. ether was used as developer. In the same system *trans* canthaxanthin had $R_F = 0.56$ and *trans* isozeaxanthin $R_F = 0.18$.

Absorption spectra in visible light. Measured in pet. ether the absorption spectrum was rather round-shaped with abs. max. at 454 and (474) m μ , $E_{1\text{ cm}}^{1\%} = 2250$ at 454 m μ ; see Fig. 1. Isozeaxanthin (III) exhibited abs. max. at (428), 451 and 479 m μ and canthaxanthin (V) abs. max. at 463 m μ in the same solvent. The spectrum of echinenone (4-keto- β -carotene) in pet. ether appeared slightly broader than that of (IV).

IR-spectrum of 0.34 mg (IV) in 0.2 g KBr was measured according to the technique described elsewhere;²¹ see Fig. 2. The IR-spectra of isozeaxanthin (III) (1.2 mg in 0.6 g KBr) and canthaxanthin (V) (1.0 mg in 0.6 g KBr) were measured for comparison. Bands for allylic hydroxyl were located at 3300 and 1020 cm⁻¹ and for conjugated carbonyl at 1655 cm⁻¹. Only in canthaxanthin (III) was the band for *trans* disubstituted double bonds around 960 cm⁻¹ split into a doublet.

Partition ratio was measured according to the method of Petracek and Zechmeister¹⁰; found for pet.ether/ 95 % methanol, 31:69.

Iodine catalyzed cis-trans equilibrium mixture. To 1 mg (IV) in 30 ml benzene was added 30 μ g I₂ in 0.3 ml pet. ether. Nitrogen was passed continuously through the mixture during 3 h illumination with artificial Na-light. The reaction mixture contained *trans* 4-hydroxy-4'-keto- β -carotene, $R_F = 0.46$, max 460 and (480) m μ in acetone (68 % of total) and neo-4-hydroxy-4'-keto- β -carotene A, $R_F = 0.55$, max 454 and (475) m μ in acetone (32 % of total). 5 % Acetone-pet.ether was used for development of the chromatogram. The neo A isomer had a weak *cis*-peak at 350 m μ , and was reversibly isomerized to *trans* in daylight.

Behaviour towards alkali treatment. (IV) (0.12 mg) in a 5 % KOH-methanol solution (10 ml) was shaken with air for 1 h at room temperature. The carotenoids were transferred to ether in a separating funnel in the usual manner; spectrophotometrically determined pigment recovery 84 %. The reaction mixture contained (IV) only, as judged from paper-chromatographic examination.

In another experiment (IV) (0.12 mg) in dry pyridine (2 ml) was treated with 10 % KOH-methanol (4 ml) in a two-finger device under vacuum (0.1 mm Hg). No blue-coloured enol salt was formed.

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