JOURNAL
OF
THE CHEMICAL SOCIETY

1. *The Oxidation of β-Carotene in Solution by Oxygen.*

By R. F. HUNTER and R. M. KRAKENBERGER.

Examination of the products formed by oxidation of solutions of β-carotene in arachis oil and benzene by oxygen indicates that this proceeds by way of attack of the first and then the second terminal double bond of the conjugated system.

ALTHOUGH it is well known that the carotenes undergo rapid oxidation in contact with air, little is known of the reactions involved other than that they lead to ultimate loss of colour with gain in weight and increased solubility. It has been shown by Baur (*Helv. Chim. Acta*, 1936, **19**, 210; 1937, **20**, 402) that ultra-violet irradiation of α-carotene in chloroform causes initial rapid autocatalysed absorption of oxygen with deepening of colour followed by further absorption, independent of light, leading to loss of colour. The first stage is reversible and is probably connected with the formation of dipole association products (cf. von Halban, Briegleb, *et al.*, *Z. physikal. Chem.*, 1925, **117**, 461; 1932, *B*, **19**, 255; 1934, **27**, 161; Hunter, Qureishy, and Samuel, *J.*, 1936, 1576). It is evident that the early stages of actual oxidation of carotene by air will involve attack of the extended conjugated system which might be expected to commence at one of the terminal double bonds of the β-ionone ring, since resonance should cause progressive loss of " double-bonded character " towards the centre of the system (Zechmeister, Le Rosen, Schroeder, Polgar, and Pauling, *J. Amer. Chem. Soc.*, 1943, **65**, 1940). This has been borne out by an examination of the oxidation of β-carotene by oxygen both in arachis oil and in benzene.

The experiment on the oxidation of β-carotene in arachis oil by oxygen was carried out at 50°. The progress of oxidation was followed by determination of E (1%, 1 cm.) values at the highest carotene absorption band and by a limited number of chromatographic analyses. As will be seen from Table I, oxidation proceeded at a much slower rate than might have been anticipated on *a priori* grounds.

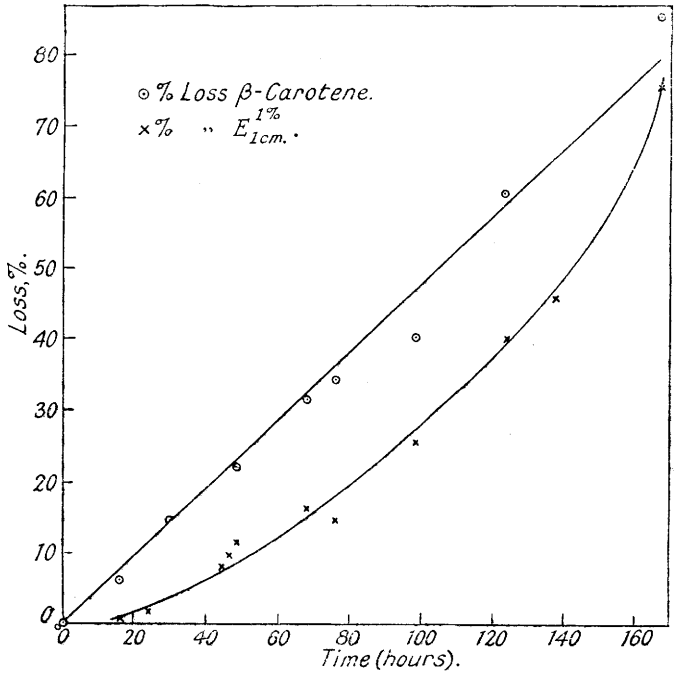
TABLE I.

Oxidation of β-carotene in arachis oil at 50°.

	Carotene content (50 g. of oil).			E (1%, 1cm.) at 464 mμ.		
Time (hrs.).	Initial.	After oxidation,	Loss, %.	Initial.	After oxidation.	Loss, %.
16	0·0568	0·0529	6·0	2·61	2·59	0·8
24	—	—	—	2·61	2·57	1·5
30	0·0489	0·0418	14·5	—	—	—
45	—	—	—	2·61	2·40	8·1
46½	—	—	—	3·86	3·48	9·8
49	0·0839	0·0654	22·0	3·86	3·42	11·4
69	0·0722	0·0493	31·7	3·32	2·79	16·1
77	0·0568	0·0374	34·2	2·61	2·23	14·6
99½	0·0359	0·0214	40·3	1·65	1·12	25·5
125	0·0359	0·0140	60·9	1·65	0·988	40·1
138½	—	—	—	2·46	1·34	46·0
168	0·0489	0·0698	85·8	2·46	0·69	75·9

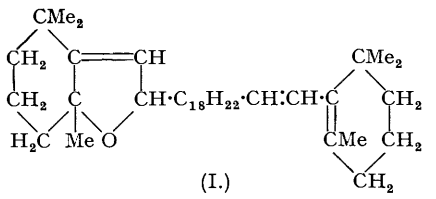
After 212 hours, complete bleaching had taken place. The comparison of the % loss of carotene and % loss in E(1%, 1 cm.) at 464 mμ with time is shown in the diagram. The former appears to be in simple proportion, but the loss of E(1%, 1 cm.) value increases with time owing, evidently, to the disappearance of oxidised carotenes having absorption spectra similar to that of β-carotene itself.

Chromatographic separation of the unsaponifiable matter obtained from " oxidised " solutions of β-carotene in arachis oil furnished carotenoids having the spectroscopic characteristics of mutatochrome (I) (Karrer and Jucker, *Helv. Chim. Acta*, 1945, **28**, 427; formerly regarded as " β-carotene oxide," Euler, Karrer, and Walker, *ibid.*, 1932, **15**, 1507), aurochrome

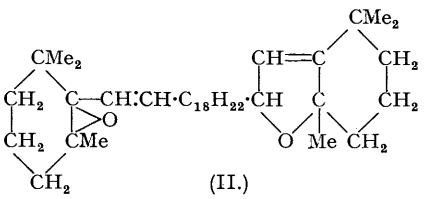


Effect of oxygen on β-carotene in arachis oil at 50°.

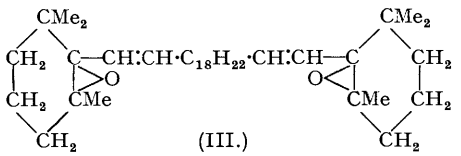
($C_{40}H_{56}O_2$ with both β-ionone rings oxidised as in I), luteochrome (II), β-carotene diepoxide (III), semi-β-carotenone (IV), and β-carotenone (as IV but with both β-ionone rings oxidised).



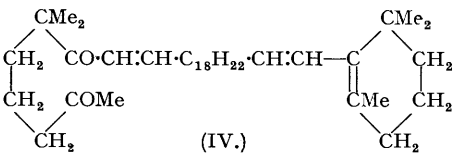
(I.)



(II.)



(III.)



(IV.)

[$C_{18}H_{22}$ = ·CMe:CH·CH:CH·CMe:CH·CH:CH·CH:CMe·CH:CH·CH:CMe·.]

Evidence was also obtained of the presence of β-carotene epoxide (as III, but with only one β-ionone ring oxidised).

A similar experiment, in which a solution of β-carotene in benzene was treated with oxygen at 50°, provided a striking contrast in the rate of oxidation, as indicated in Table II. The difference is evidently attributable to the presence of the natural anti-oxidant and unsaturated glycerides in arachis oil. Chromatographic separation of the products of oxidation furnished carotenoids having the spectroscopic characteristics of β-carotene epoxide, mutatochrome (I), luteochrome (II), and semi-β-carotenone (IV). The course of oxidation of β-carotene by oxygen followed in arachis oil and in benzene is therefore similar, and evidently consists in

TABLE II.

Time (hrs.).	E(1%, 1 cm.) at 464 mμ.	β-Carotene (g./100 c.c.).	Loss, %: in E (1%, 1 cm.).	in carotene.
0	3·19	0·134	0	0
2	3·12	0·134	2·2	0
4	3·03	0·132	5·0	1·9
6	2·73	0·105	14·4	22·0
8	1·50	0·062	53·0	54·2
24	0	0	100	100

attack on a terminal double bond to give β-carotene epoxide which is converted in the presence of acid into mutatochrome, which then undergoes fission to yield semi-β-carotenone; and a similar attack of the second β-ionone ring leading to the production of luteochrome, aurochrome, and β-carotenone.

EXPERIMENTAL.

Oxidation in Arachis Oil.—(i) A solution of β-carotene [0·53 g., E(1%, 1 cm.) = 2120 at 464 mμ in benzene] in warm benzene (8 c.c.) was gradually diluted with fresh deodorised refined arachis oil (*ca.* 500 g.), and the resulting solution was heated at 50° under reduced carbon dioxide pressure for 10 minutes in order to remove as much benzene as possible without causing very substantial *cis*-isomerisation. The resulting solution, which had E(1%, 1 cm.) 2·61 at 463 mμ in benzene on the Hilger–Nutting spectrophotometer, was stored in carbon dioxide.

A carotene estimation was made by saponification of the oil (50 g.) with potassium hydroxide (15 g.), water (25 c.c.), alcohol (75 c.c.), and light petroleum (50 c.c.) for 2—2½ hours and chromatographic separation of the petrol-soluble unsaponifiable matter on Savory and Moore's " Mayfair " alumina under slight carbon dioxide pressure, with the usual precautions (Hunter and Scott, *Biochem. J.*, 1941, **35**, 31; 1944, **38**, 212). The carotene zone was eluted with ether–benzene–absolute alcohol. The residue obtained by evaporation under reduced carbon dioxide pressure furnished a residue whose solution in benzene had a log I_0/I value corresponding to 0·0568 g. of β-carotene [the E(1%, 1 cm.) value of 2·61 for the oil corresponds to a β-carotene content of 0·1135%].

Portions of about 55 g. of this solution (or other similarly prepared solutions) were treated with a stream of oxygen at 50° for periods ranging from 16 to 212 hours. The E(1%, 1 cm.) value in benzene at 463 mμ of the oxidised sample was determined on the Hilger–Nutting spectrophotometer, and 50 g. of the remainder were assayed for carotene by chromatographic separation in which the *cis*-isomers were included with the all-*trans*-β-carotene.

(ii) The products formed during oxidation were examined in the cases of solutions in arachis oil which had been treated with oxygen for 99½ and 10 hours (corresponding to carotene losses of 40 and 3%, respectively). Two experiments were made with regard to the more prolonged oxidation. In each case, 500 g. of a solution of β-carotene (*ca.* 0·5 g.) in arachis oil were kept at 50° in a thermostat during the passage of a stream of oxygen. The resulting oil was saponified in the usual way and extracted with light petrol and thereafter with ether, and the combined extracts were washed, dried (Na_2SO_4), and evaporated with the usual precautions, and the residue chromatographed on " Mayfair " alumina. The chromatogram was divided into 3 or 4 groups of zones which were separately rechromatographed.

The first experiment (99½ hours) furnished a chromatogram of some 14 zones, the lowest of which contained β-carotene, and the three upper zones oxidised carotenoids with the following spectroscopic characteristics: Zone 14, abs. max. at 447 and 427 mμ; zone 13, abs. max. at 447 and 427 mμ; zone 12, abs. max. at 451 and 431 mμ in carbon disulphide (maxima at 620, 600, 590, and 632 mμ respectively in the antimony trichloride reaction). Zone 8 furnished a carotenoid having the spectroscopic characteristics of aurochrome (abs. max. at 457 and 428 mμ in carbon disulphide and at 587 mμ in the antimony trichloride reaction), and zone 4 yielded a carotenoid corresponding to mutatochrome (abs. max. at 486, 458, and 428 mμ in carbon disulphide and at 595 mμ in the antimony trichloride reaction). Zone 2 furnished material having spectroscopic characteristics close to those of β-carotene epoxide.

In a second and similar experiment, zones containing carotenoids having the spectroscopic characteristics of luteochrome (abs. max. at 480, 451, and 419 mμ in carbon disulphide) and β-carotenone (abs. max. at 536, 497, and 466 mμ) were also obtained. In this case, a small quantity of crystalline mutatochrome was isolated from the appropriate zone.

In the third experiment (10 hours), the chromatogram furnished zones containing carotenoids having the spectroscopic characteristics of mutatochrome (abs. max. at 485, 457, and 428 mμ), β-carotenone (539, 499, 474, and 448 mμ), β-carotene epoxide (512, 482, and 452 mμ), and semi-β-carotenone (538, 499, and 465 mμ in carbon disulphide). This chromatogram differed from those of the previous experiments in that it was more weakly coloured and the absorption spectra of the oxidation products were more sharply defined.

Oxidation in Benzene.—(i) A solution of β-carotene (200 c.c.), prepared from β-carotene [E(1%, 1 cm.) = 2100; *ca.* 0·5 g.] and benzene (" AnalaR," Towers, 500 c.c.) in a vessel fitted with a reflux, was treated at 50° (thermostat) with a stream of oxygen (previously saturated with benzene by passage through a bubbler containing the solvent) at a fixed rate. At intervals, 2-c.c. and 25-c.c. samples were withdrawn and examined with respect to E(1%, 1 cm.) value and carotene content, the latter being separated by chromatography on alumina after dilution with light petrol and spectroscopically determined in the usual way.

(ii) A solution of β-carotene [E(1%, 1 cm.) = 2350; *ca.* 1·5 g.] in purified benzene (1000 c.c.) was treated with a slow stream of oxygen at 50° for 5 hours, during which the E(1%, 1 cm.) value decreased from 3·65 to 3·1. The solution was evaporated under reduced carbon dioxide pressure with the usual

precautions and the residue was chromatographed on "Mayfair" alumina. The chromatogram consisted of 17 zones which furnished substances having the following spectroscopic characteristics:

Zone.	Colour.	Abs. max. (mμ) in CS_2.	$SbCl_3$ reaction.
17	Orange	Vague	Vague, 599 mμ. Greenish
16	Pink	,,	Green
15	Yellow	512, *481*, 457	588 and 494 mμ. Blue-grey
14	Pink	483, 458	Blue ⟶ green
13	Red	484, *469*	Mauve ⟶ green
12	Orange-yellow	Vague	*728*, *667*, 595, and 479 mμ. Green
11	Orange	,,	481 mμ. Green
10	Pink	,,	637, 585, *493* mμ. Green
9	Fawn	,,	588 mμ. Deep blue
8	Canary-yellow	482, 456	—
7	Lemon-yellow	486, *453*	640 and 597 mμ. Blue
6	Yellow	474, *451*	*662* and 592 mμ. Blue
5	Orange	510, 480, 458	585 mμ. Blue
4	Pink	536, 502, 468	585 mμ. Violet
3	Pink	538, 503, 470	588 mμ. Violet-blue
2	Pink	532, 499, 468	586 mμ. Violet
1	Orange	527, 494, 465	*663* mμ. Blue-green

The spectroscopic characteristics of zones 1—4 correspond to semi-β-carotenone and oxy-semi-β-carotenone, those of zone 5 to β-carotene epoxide, of zone 7 to mutatochrome, and of zone 14 to luteochrome.

We thank the Directors of Lever Brothers and Unilever Limited for permission to publish these results.

RESEARCH DEPARTMENT, LEVER BROTHERS AND UNILEVER LTD.,
PORT SUNLIGHT.

[*Received, April 15th*, 1946.]