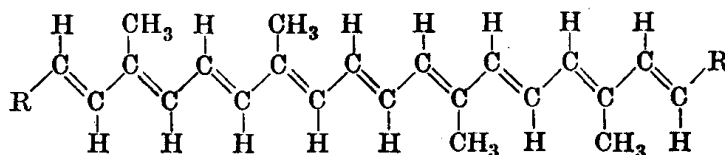


CHAPTER IX
CAROTENOIDS

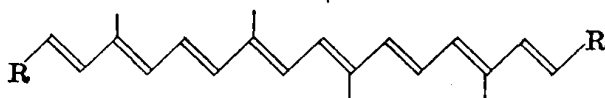
§1. Introduction. The carotenoids are yellow or orange pigments which are widely distributed in plants and animals. Chlorophyll is always associated with the carotenoids carotene and lutein; the carotenoids act as photosensitisers in conjunction with chlorophyll. When chlorophyll is absent, *e.g.*, in fungi, then the carotenoids are mainly responsible for colour. Carotenoids are also known as lipochromes or chromolipids because they are fat-soluble pigments. They give a deep blue colour with concentrated sulphuric acid and with a chloroform solution of antimony trichloride (the Carr-Price reaction); this Carr-Price reaction is the basis of one method of the quantitative estimation of carotenoids. Some carotenoids are hydrocarbons; these are known as the *carotenes*. Other carotenoids are oxygenated derivatives of the carotenes; these are the *xanthophylls*. There are also acids, the *carotenoid acids*, and esters, the *xanthophyll esters*.

Chemically, the carotenoids are polyenes, and almost all the carotenoid hydrocarbons have the molecular formula $C_{40}H_{56}$. Also, since the carbon skeleton of these compounds has a polyisoprene structure, they may be regarded as tetraterpenes (*cf.* §1. VIII).

In most of the carotenoids, the central portion of the molecule is composed of a long conjugated chain comprised of four isoprene units, the centre two of which are joined tail to tail. The ends of the chain may be two open-chain structures, or one open-chain structure and one ring, or two rings. The colour of the carotenoids is attributed to the extended conjugation of the central chain (see Vol. I). X-ray analysis has shown that in the majority of natural carotenoids, the double bonds are in the *trans*-position; a few natural carotenoids are *cis*-. Thus, if we represent the ends of the chain by R (where R may be an open-chain structure or a ring system), *trans*-carotenes may be written:



If we use the conventional formulæ of terpenes (§4. VIII), the above formula will be the following (the reader should write out in this way the various formulæ given in the text; see §6 for an example):



§2. Carotenes. Carotene was first isolated by Wackenroder (1831) from carrots (this was the origin of the name *carotin*, which was later changed to *carotene*). The molecular formula of carotene, however, was not determined until 1907, when Willstätter showed it was $C_{40}H_{56}$. Carotene was shown to be unsaturated, and when treated with a *small* amount of iodine, it forms a crystalline di-iodide, $C_{40}H_{56}I_2$. Kuhn (1929) separated this di-iodide into two fractions by means of fractional crystallisation. Treatment of each fraction with thiosulphate regenerated the corresponding carotenes, which were designated α - and β -carotene. Kuhn *et al.* (1933) then found that

chromatography gives a much better separation of the carotenes themselves, and in this way isolated a third isomer, which he designated γ -carotene.

α -Carotene, m.p. 187–187.5°; optically active (dextrorotatory).

β -Carotene, m.p. 184.5°; optically inactive.

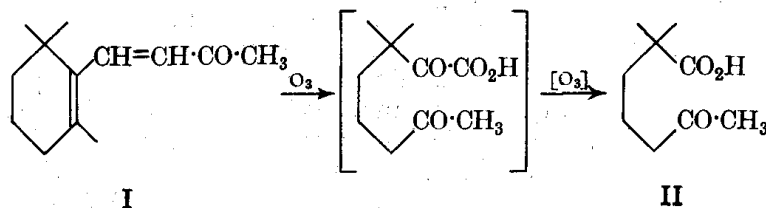
γ -Carotene, m.p. 176.5°; optically inactive.

It appears that all three carotenes occur together in nature, but their relative proportions vary with the source, *e.g.*, carrots contain 15 per cent. α , 85 per cent. β and 0.1 per cent. γ . Carotenes are obtained commercially by chromatography, two of the best sources being carrots and alfalfa.

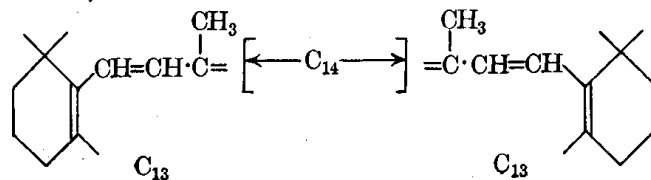
Biosynthetic studies of the carotenes have been carried out, and the pathways are those for the terpenes (§32a. VIII). Thus Braithwaite *et al.* (1957) and Grob (1957) have shown that labelled mevalonic acid is incorporated into β -carotene. Scheuer *et al.* (1959) have also shown that this acid is incorporated into lycopene. Furthermore, Modi *et al.* (1961) have isolated mevalonic acid from carrots.

§3. β -Carotene, $C_{40}H_{56}$. When catalytically hydrogenated (platinum), β -carotene forms perhydro- β -carotene, $C_{40}H_{78}$. Thus β -carotene contains eleven double bonds, and since the formula of perhydro- β -carotene corresponds to the general formula C_nH_{2n-2} , it follows that the compound contains two rings.

When exposed to air, β -carotene develops the odour of violets. Since this odour is characteristic of β -ionone, it was thought that this residue is present in β -carotene (see §6. VIII). This was confirmed by the fact that the oxidation of a benzene solution of β -carotene with cold aqueous potassium permanganate gives β -ionone. Now β -ionone, I, on ozonolysis, gives, among other things, gericonic acid, II (Karrer *et al.*, 1929).

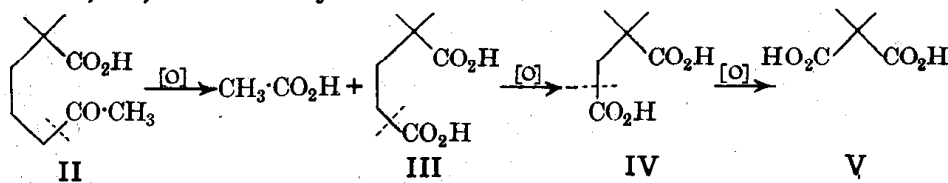


β -Carotene, on ozonolysis, gives gericonic acid in an amount that corresponds to the presence of two β -ionone residues (Karrer *et al.*, 1930). Thus a tentative structure for β -carotene is:

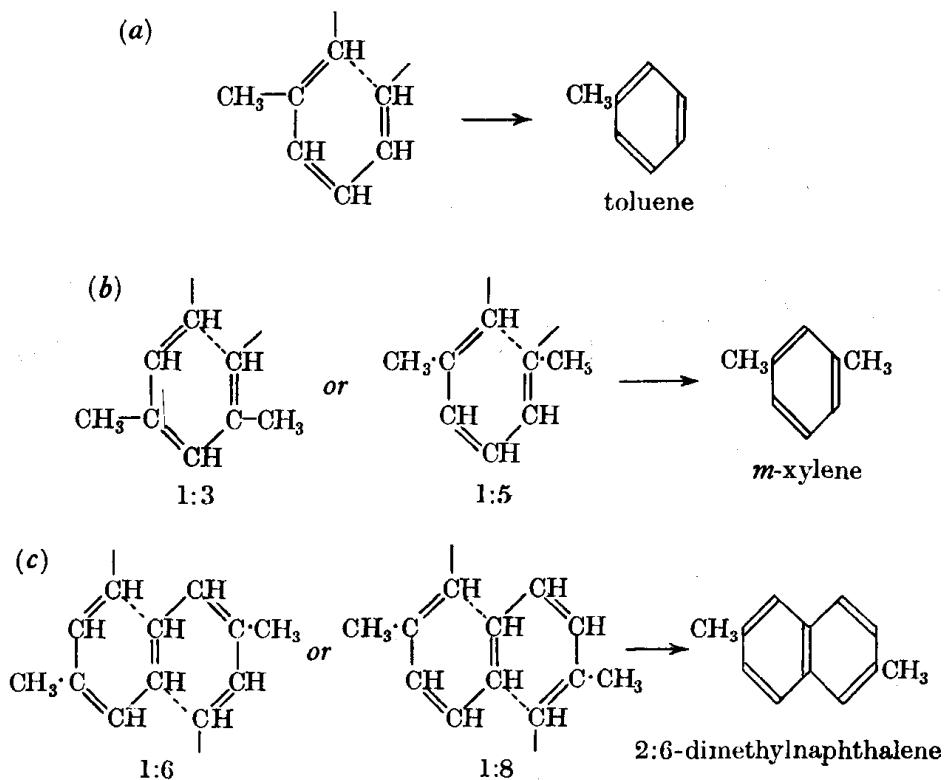


Since the colour of β -carotene is due to extended conjugation (§1), the C_{14} portion of the molecule will be conjugated. The presence of conjugation in this central portion is confirmed by the fact that β -carotene forms an adduct with five molecules of maleic anhydride (Nakamiya, 1936).

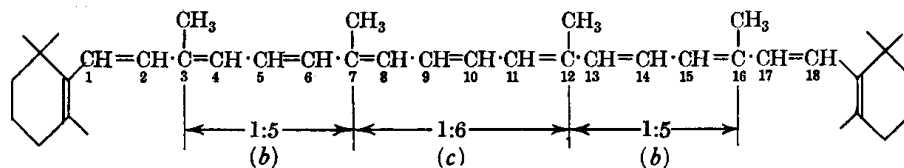
Gericonic acid, on oxidation with cold aqueous potassium permanganate, forms a mixture of acetic acid, α : α -dimethylglutaric, III, α : α -dimethylsuccinic, IV, and dimethylmalonic acids, V.



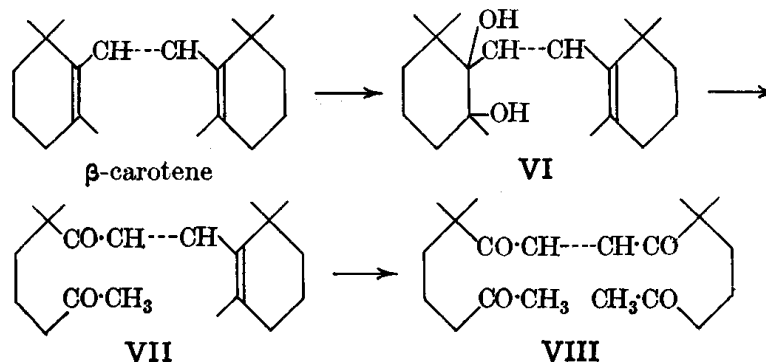
Oxidation of β -carotene in benzene solution with cold aqueous permanganate gives a mixture of β -ionone, III, IV, V, and acetic acid, the amount of acetic acid being more than can be accounted for by the presence of two β -ionone residues. Thus there must be some methyl side-chains in the central C_{14} portion of the molecule. Since it is essential to know the exact number of these methyl side-chains, this led to the development of the **Kuhn-Roth methyl side-chain determination** (1931). The first method used was to oxidise the carotenoid with alkaline permanganate, but later chromic acid (chromium trioxide in sulphuric acid) was found to be more reliable, the methyl group in the fragment $-C(CH_3)=$ being always oxidised to acetic acid. It was found that alkaline permanganate only oxidises the fragment $=C(CH_3)-CH=$ to acetic acid, and fragments such as $=C(CH_3)-CH_2-$ are incompletely oxidised to acetic acid, or not attacked at all (Karrer *et al.*, 1930). Since a molecule ending in an *isopropylidene* group also gives acetic acid on oxidation with chromic acid, this end group is determined by ozonolysis, the acetone so formed being estimated volumetrically. Application of the Kuhn-Roth methyl side-chain determination to β -carotene gave four molecules of acetic acid, thus indicating that there are four $-C(CH_3)=$ groups in the *chain*. The positions of two of these have already been tentatively placed in the two end β -ionone residues (see tentative structure above), and so the problem is now to find the positions of the remaining two. This was done as follows. Distillation of carotenoids under normal conditions brings about decomposition with the formation of aromatic compounds. Thus the distillation of β -carotene produces toluene, *m*-xylene and 2:6-dimethylnaphthalene (Kuhn *et al.*, 1933). The formation of these compounds may be explained by the cyclisation of fragments of the polyene chain, without the β -ionone rings being involved. The following types of chain fragments would give the desired aromatic products:



The following *symmetrical* structure for β -carotene would satisfy the requirements of (a), (b) and (c); the tail to tail union of the two isoprene units at the centre should be noted.



This symmetrical formula for β -carotene has been confirmed by the following oxidation experiments (Kuhn *et al.*, 1932-1935). When β -carotene is oxidised *rapidly* with potassium dichromate, dihydroxy- β -carotene, VI, is obtained and this, on oxidation with lead tetra-acetate, gives semi- β -carotenone, VII, a diketone. Since both VI and VII contain the *same* number of carbon atoms as β -carotene, it follows that the *double bond in one of the β -ionone rings* has been oxidised; otherwise there would have been chain scission had the chain been oxidised. Oxidation of semi- β -carotenone with chromium trioxide produces β -carotenone, VIII, a tetraketone which also has the same number of carbon atoms as β -carotene. Thus, in this compound, the *other β -ionone ring* is opened. Now only *one* dihydroxy- β -carotene and *one* semi- β -carotenone are obtained, and this can be explained only by assuming a symmetrical structure for β -carotene. Thus the oxidations may be formulated:



This structure for β -carotene has been confirmed by synthesis, *e.g.*, that of Karrer *et al.* (1950). The acetylenic carbinol IX is treated with ethylmagnesium bromide and the product is treated as shown on opposite page.