EXPERIENTIA

Vol. 33 – Fasc. 5 Pag. 555–696 15. 5. 1977

GENERALIA

Progress in the field of fat-soluble vitamins and carotenoids*

by O. Isler[1]

F. Hoffmann-La Roche & Co., Limited, CH–4002 Basle (Switzerland); present address: Am Landungssteg, CH–8592 Uttwil

Summary. A review is given of the development in the field of fat-soluble vitamins and carotenoids during the last 10 years. Special emphasis is placed on the chemistry of these compounds, but relevant biological results are included, too.

Although research in the field of fat-soluble vitamins started approximately 70 years ago, the interest in carotenoids has a much longer history. Publications on the abundant natural carotenoidal pigments appeared already around 1820. The close connection between these 2 fields was dramatically demonstrated by Karrer's elucidation of the structures of β-carotene (**1**) and of vitamin A (**2**) in 1931[2].

From structural considerations we distinguish today between 4 different types of fat-soluble vitamins[3].

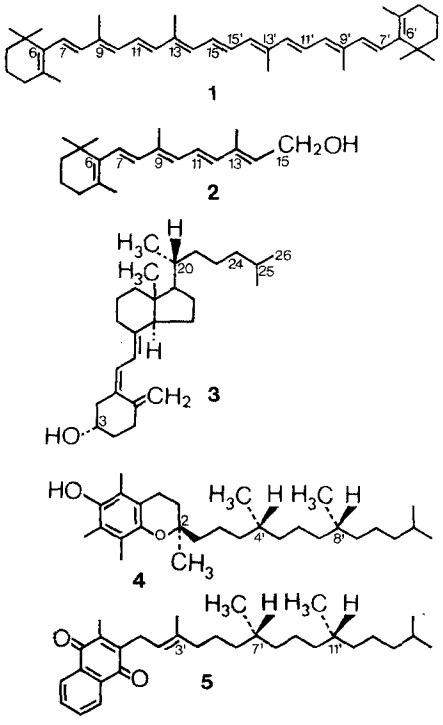


Fig. 1. The structural formula of fat soluble vitamins.

These are vitamin A (**2**) and the structurally closely related carotenoids of which some exhibit vitamin A activity, then D-vitamins [e.g. vitamin D_3 (**3**)], vitamin E (**4**), and the K-vitamins [e.g. vitamin K_1 (**5**)]. Their major natural sources and their deficiency symptoms are indicated in figure 2.

Vitamin A. Vitamin A deficiency today is still a very serious problem since it is estimated that every year about 100,000 children turn blind because of lack of this vitamin. Especially affected are the countries around the Mediterranean, in the middle east, southeast Asia and in central America.

1 Acknowledgment. My contribution to the field of fat-soluble vitamins and carotenoids would have been impossible without the counsel and skilful collaboration of many colleages. Special thanks I owe to Dr H. J. Mayer for supplying stereochemical drawings of vitamin A used in this text and to Dr F. Kienzle who translated the manuscript into English. To them and to Drs Rüegg, Marbet, Schudel, Schwieter und Mr Ryser and to many other collaborators at Hoffmann-La Roche, I express my deep gratitude for their dedication and help.

2 P. Karrer, A. Helfenstein, H. Wehrli and A. Wettstein, Helv. chim. Acta *13*, 1084 (1930). – P. Karrer, R. Morf and K. Schoepp, Helv. chim. Acta *14*, 1036 (1931).

3 For a review see: O. Isler, Experientia *26*, 225 (1970). – Vitams Horm., New York *32*, 129 (1974). – R. Ammon and W. Dirscherl (ed.), in: Fermente, Hormone, Vitamine, vol. III/1, p. 162. Georg-Thieme-Verlag, Stuttgart 1974. – W. H. Sebrell, jr, and R. S. Harris (ed.), in: The Vitamins, 2nd ed., vol. 1, p. 1–303 (1967); vol. 3, p. 155–301, 416–522 (1971); vol. 5, p. 165–317 (1972). Academic Press, New York and London. – D. B. McCormick and L. D. Wright, in: Methods in Enzymology, vol. 18, part C, p. 241. Ed. S. P. Colowick and N. O. Kaplan. Academic Press, New York and London 1971. – Kirk-Othmer, in: Encyclopedia of Chemical Technology, 2nd ed., vol. 21, p. 490–509, 549–600. Interscience Publishers, New York 1970. – R. A. Morton, in: International Encyclopaedia of Food and Nutrition, vol. 9. Ed. H. M. Sinclair. Pergamon Press, Oxford 1970.

* 17th Paul Karrer lecture presented 23 June 1976, in the aula of the University of Zürich.

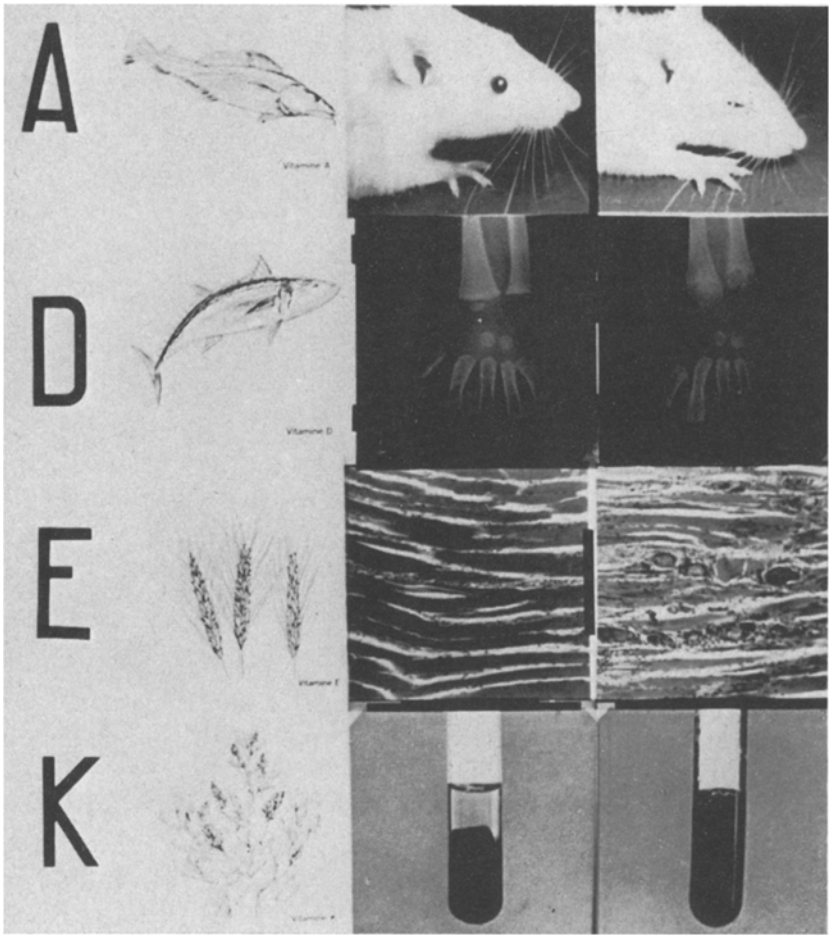


Fig. 2. The natural sources and deficiency symptoms of fat soluble vitamins.

Carotenoids are the source of all vitamin A; β-carotene is thereby the most important provitamin A. The biogenesis of carotenoids follows the usual path of terpenoid synthesis up to the C_{20}-unit geranylgeranyl pyrophosphate. Dimerisation of 2 C_{20}-units leads via prephytoene, a C_{40}-pyrophosphate compound, to the hydrocarbon phytoene. Through introduction of additional double-bonds, neurosporene and finally lycopene are formed. From these all other carotenoids and xanthophylls, the oxygenated carotenoids are derived (see figure 3). Degradation of one or both ends of the carotenoid molecule leads to apocarotenoids. Further

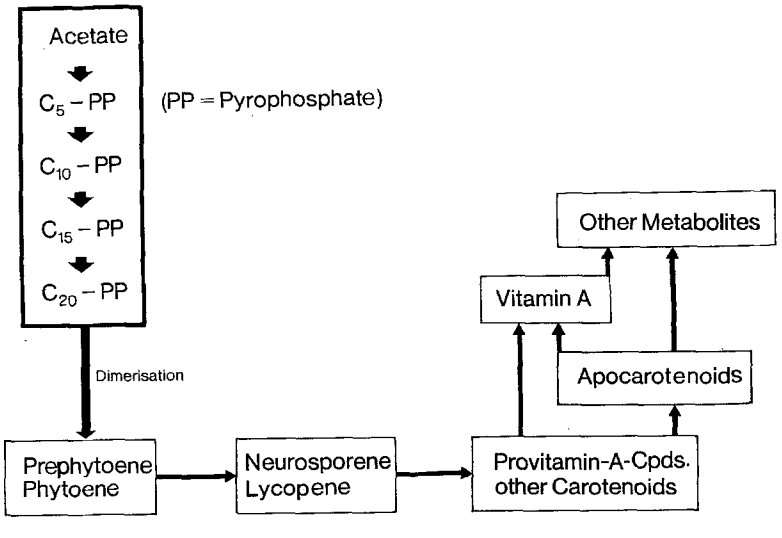


Fig. 3. The biogenesis of carotenoids and vitamin A.

degradation yields shorter metabolites, some of which play a role as natural plant-growth regulators or insecticides[4]. However, the most important metabolite from a human point of view is vitamin A.

Man and animals possess in the intestinal mucosa enzymes capable of degrading the provitamin A to vitamin A-aldehyde (**6**). A reductase reduces **6** to vitamin A (**2**) which, through interaction with the important 'retinol-binding protein', is transported either to the target tissues or to the liver, where it is stored in esterified form mainly as palmitate. When necessary these esters can be reconverted to **2** or **6**. In contrast, the in vivo oxidation of **6** to vitamin A-acid (**7**) is irreversible. So far little is known of the further fate of **7**. Finally a specific isomerase occuring in the retina isomerizes **6** to 11-cis-vitamin A-aldehyde (**8**), a constituent of the protein rhodopsin in the photoreceptor system of the eye (figure 4).

Most of these transformations are also possible in vitro if light and air are carefully excluded. For instance **2** may be obtained from **6** by oxidation with activated manganese dioxide, the reverse reaction may be effected with sodium borohydride. More forceful oxidation of **6** with silver oxide yields the acid **7** which with lithium aluminium hydride can be reduced to the alcohol **2** (figure 5).

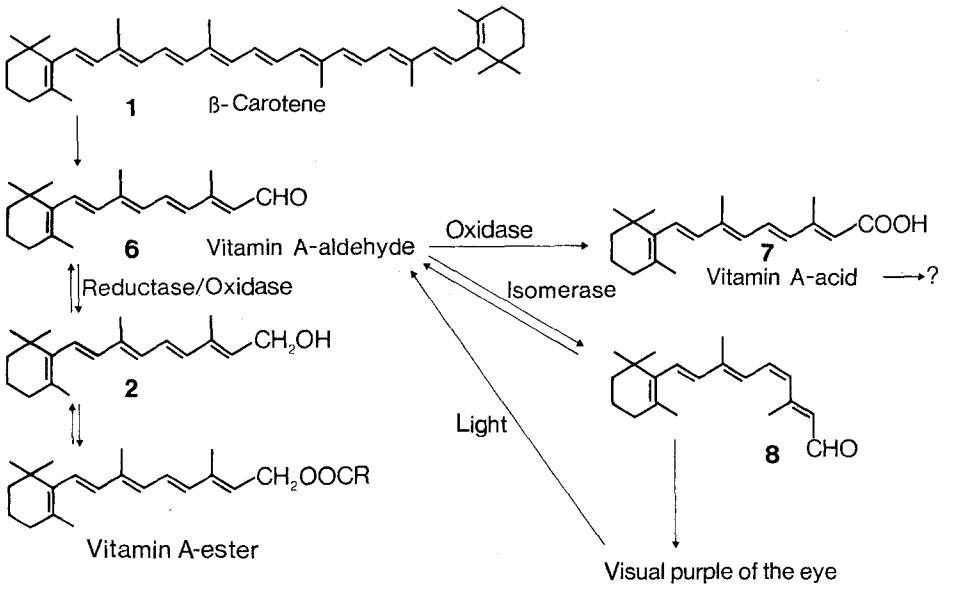


Fig. 4. Vitamin A-active substances from β-carotene.

Vitamin A is indispensable for growth, vision, and reproduction. It may play a role in the biogenesis of glycoproteins. Vitamin A-aldehyde and its 11-cis-isomer is the key to the chemistry of vision. Vitamin A-acid is responsible for the growth and differentiation of epitheleal tissues. Vitamin A-acid (**7**) is commercially available for the treatment of skin diseases like acne and psoriasis[5]. Unfortunately some side-effects

4 T. W. Goodwin, Biochem. J. *123*, 293 (1971). – G. Britton, in: Chemistry and Biochemistry of Plant Pigments, 2nd ed., vol. 1, p. 262. Ed. T. W. Goodwin. Academic Press, London and New York 1976. – E. D. Beytia and J. W. Porter, Ann. Rev. Biochem. *45*, 113 (1976).

5 W. Bollag and F. Ott, Acta derm.-vener., suppl. *74*, 163 (1975). – W. Bollag, Cancer Chemother. Rep. *55* (1), 53 (1971). – W. Bollag, Eur. J. Cancer *8*, 689 (1972).

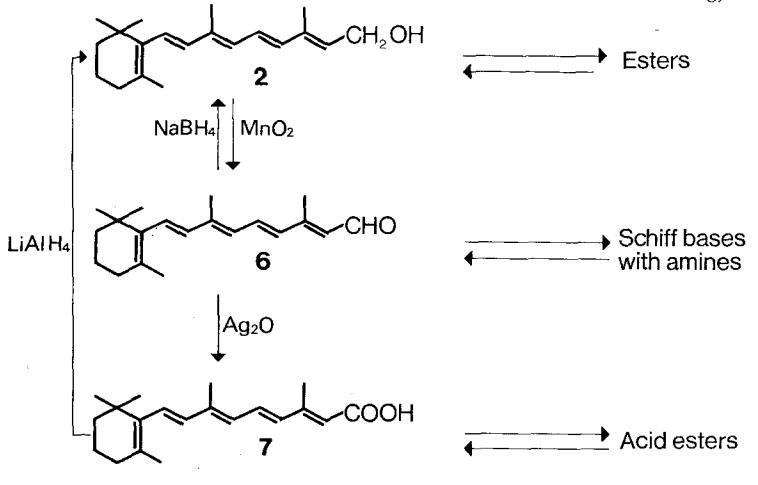


Fig. 5. In vitro conversions of vitamin A-aldehyde.

may be observed on its application. Recently a group at Roche succeeded in synthesizing a number of structurally related compounds with remarkably improved properties. Amongst these are the aromatic retinoids Ro 10-9359 and Ro 11-1430 which are as active as **7** but 10 times more compatible. Besides acne and psoriasis precanceroses respond to these new compounds. They might even be effective in cancer prophylaxis[6].

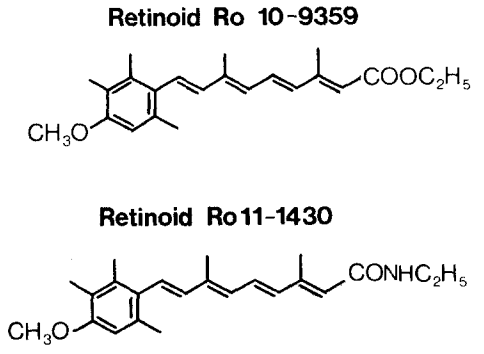


Fig. 6. Vitamin A-acid analogs.

All industrial syntheses of vitamin A and the carotenoids start today with β-ionone (**9**). This monocyclic C_{13}-ketone is produced from acetone via methyl heptenone (**10**) and pseudo-ionone (**11**). In this scheme 2 carbons are added in the form of acetylene and 3 carbons through condensation with isopropenyl ether, a method discovered and developed by Saucy and Marbet[7]. Pseudo-ionone is then cyclized with strong acid to **9** (figure 7).

Condensation of **9** with chloroacetic acid ester, the so-called Darzens reaction, leads to the C_{14}-aldehyde (**12**) of the Roche synthesis with a methylene group between the ring and the side chain. On the other hand acetylene addition to **9**, followed by partial hydrogenation over Lindlar catalyst yields C_{15}-vinylionol (**13**) a key compound in the technical synthesis of the BASF. Of more theoretical interest is the preparation of cis- and trans-C_{15}-aldehyde. Horner reaction of **9** furnishes a mixture of the cis- and trans-acid esters (**14**). Upon saponification the resulting mixture of acids is separated by fractional crystallization from benzene. The pure trans- or cis-acids are then reduced with lithium aluminium hydride to the corresponding alcohols which are oxidized with manganese dioxide to the desired aldehydes (figure 8).

The 4 double bonds of the vitamin A side chain may give rise to 16 stereo isomers. In 1939 Linus Pauling

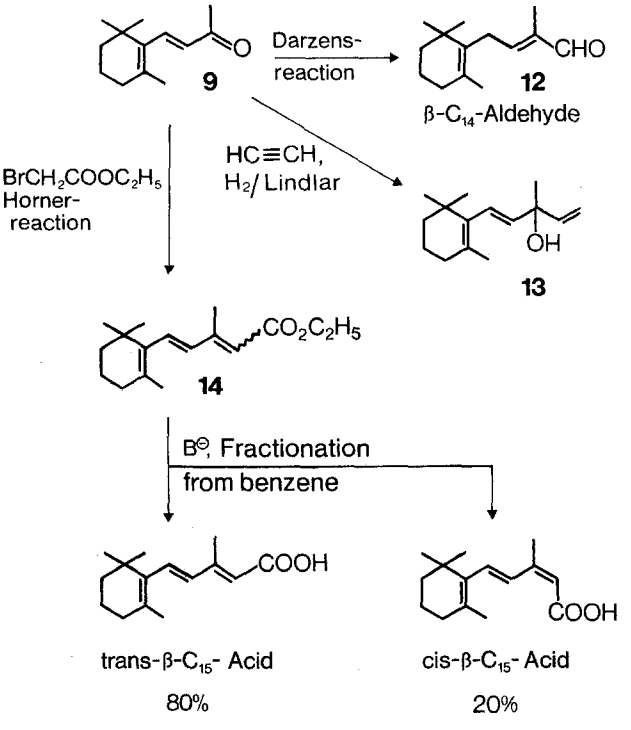


Fig. 8. β-Ionone as an intermediate in the synthesis of various vitamin A isomers.

believed from theoretical considerations that this number might be smaller[8]. Pauling differentiated between hindered and unhindered cis-isomers (figure 9). The unhindered isomers possess cis-double bonds with little or no steric hindrance of the hydrogen atoms in 1,4-position (case a). Cis-isomers with one methyl

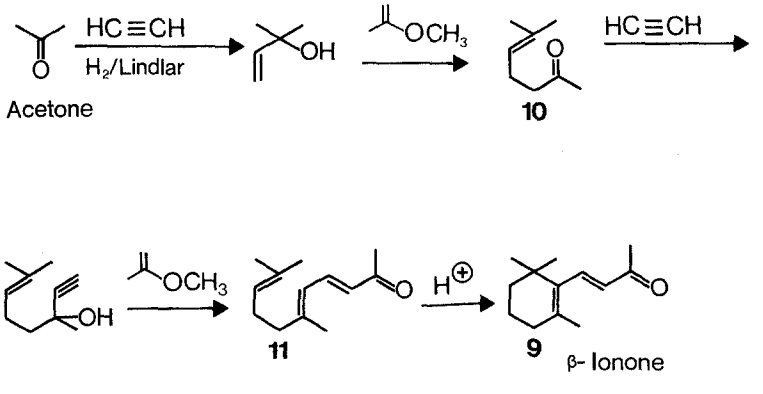


Fig. 7. The technical synthesis of β-ionone.

substituent (case b) and these with 2 methyl groups in 1,4-position (case c) show strong steric interference and are, according to Pauling, extremely fast isomerized to the trans-isomer or even not existent. However, it became soon apparent, that it was possible to produce hindered cis-isomers and that one, namely the 11-cis retinal was even used by nature for the visual process.

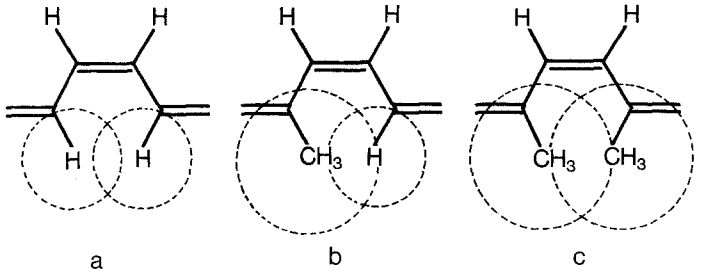


Fig. 9. Steric interference in polyenes.

In figure 10 the 4 possible unhindered isomers of vitamin A are shown. The 13-cis-isomer is easily isomerized to the all-trans form by the action of catalytic amounts of iodine and heat. The 9-cis double bond, however, is stable under these conditions.

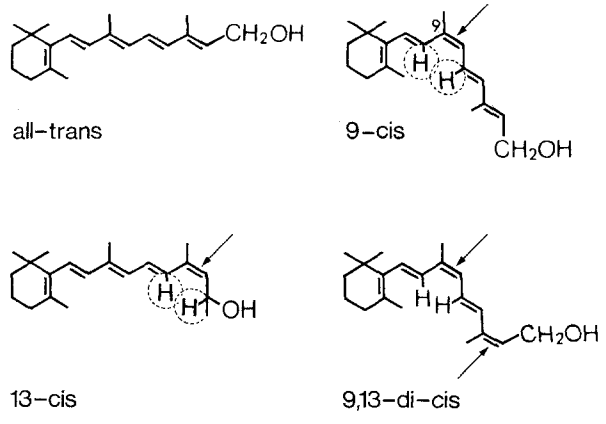


Fig. 10. Unhindered isomers of vitamin A.

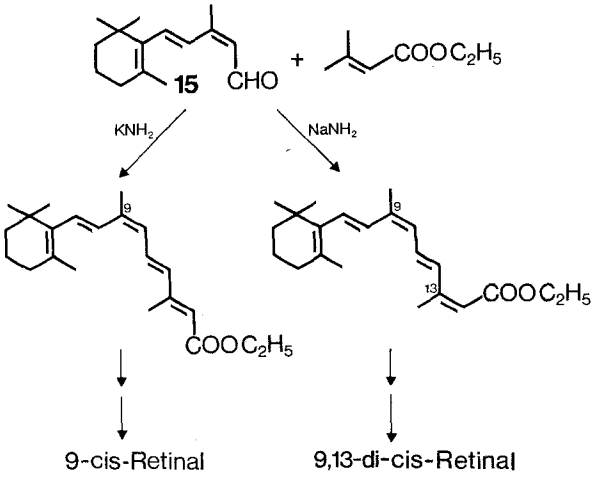


Fig. 11. The synthesis of 9-cis- and 9,13-di-cis-vitamin A.

The 9-cis- and 9,13-di-cis-vitamin A compounds have been synthesized[9] from the C_{15}-aldehyde **15** as shown in figure 11. Analogously starting with the corresponding trans-C_{15}-aldehyde 13-cis-retinal and all-trans-retinal could be prepared.

Of the 12 possible hindered isomers so far only three are known (figure 12), and these are easily isomerized to the all-trans compound. The 11-cis compound, as already mentioned, participates in the visual process. The 11,13-di-cis compound has been synthesized but recognized as physiologically not effective. The third isomer, the 7-cis vitamin A has been obtained by Liu[10] but in impure form accompanied by its 7,9-di-cis isomer. Liu's synthesis uses benzanthrone as a sensitizer in a photo-isomerization of β-ionone or β-ionylidene derivatives to obtain a starting material with the 7-cis configuration. With these in hand his synthesis follows established routes.

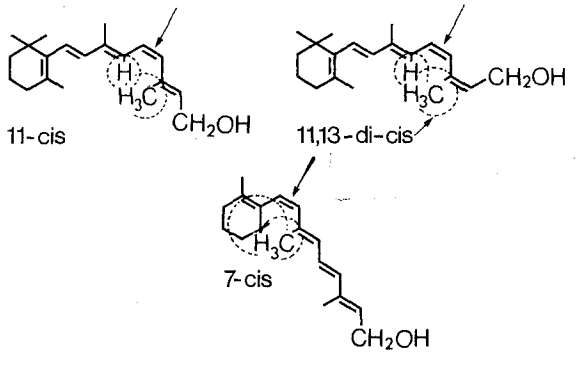


Fig. 12. The known isomers of vitamin A.

6 W. Bollag, Eur. J. Cancer *10*, 731 (1974); *11*, 721 (1975). – W. Bollag, Chemotherapy *21*, 236 (1975). – H. J. Mayer, W. Bollag, R. Haenni and R. Rüegg, Experientia (in press). – W. Bollag and A. Hanck, Acta vitam. enzymol. (in press).

7 G. Saucy and R. Marbet, Helv. chim. Acta *50*, 1158, 2091 (1967).

8 L. Pauling, Fortschr. Chem. org. NatStoffe *3*, 203 (1939). – L. Zechmeister, in: cis-trans Isomeric Carotenoids, Vitamins A and Arylpolyenes. Springer-Verlag, Vienna 1962.

9 U. Schwieter, C. v. Planta, R. Rüegg and O. Isler, Helv. chim. Acta *45*, 528 (1962). – S. Futterman and M. H. Rollins, J. biol. Chem. *248*, 7773 (1973).

10 V. Ramamurthy and R. S. H. Liu, Tetrahedron *31*, 193, 201 (1975). – A. E. Asato and R. S. H. Liu, J. Am. chem. Soc. *97*, 4128 (1975).

Today the preferred method for detection and separation of these various isomers is high pressure chromatography. This method can be employed with all fat-soluble vitamins and allows analyses with very small amounts. It is interesting to note that in the technical synthesis of vitamin A developed by Hoffmann-La Roche 30 years ago[11] various intermediates possess cis-configuration (figure 13), although this was not realized at that time. The criteria for choosing this particular route were economic considerations. Only much later, using NMR-spectroscopy was the fact detected that the product of the Grignard reaction was a mono-cis compound which upon partial hydrogenation was converted to a di-cis compound. The acidic dehydration leads directly and in good yield to all-trans-vitamin A[12].

The important 11-cis-vitamin A-aldehyde was prepared analogously by Oroshnik[13] (figure 14) using the higher boiling trans-pentol (**17**) instead of the cis-pentol (**16**). In this case acidic dehydration was carried out before the partial hydrogenation. In the same way 11,13-di-cis-retinal can be prepared with the pentol **16** instead of **17**.

The 11-cis-vitamin A-acetate may also be obtained using the synthesis of the BASF (figure 15). A Wittig-condensation of the C_{15}-phosphonium salt **18** with γ-acetoxy-tiglic aldehyde (**19**) yields all-trans-vitamin A-acetate and the corresponding 11-cis-isomer. The latter may be isolated but it is usually directly isomerized to the all-trans-compound[14].

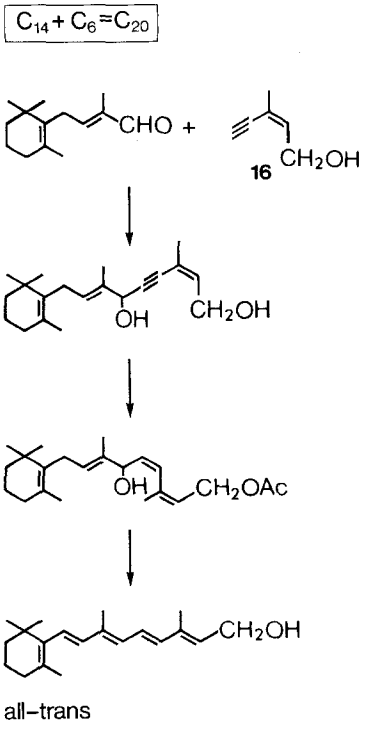


Fig. 13. The technical synthesis of vitamin A by Roche.

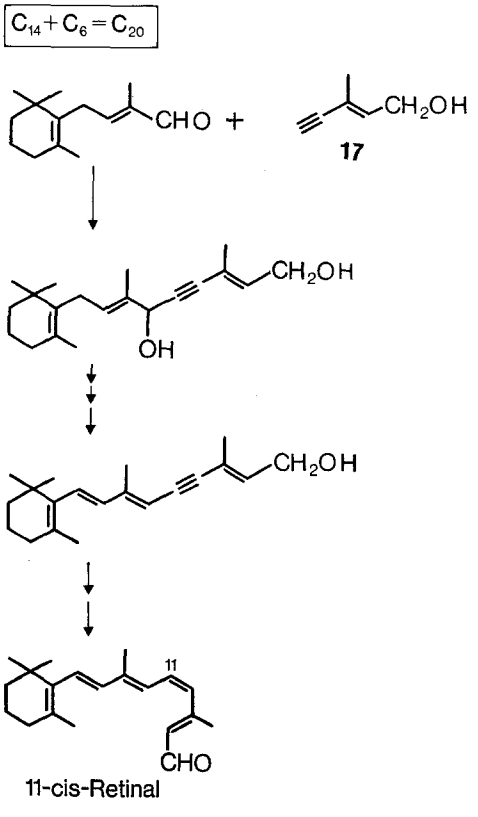


Fig. 14. The synthesis of 11-cis-retinal.

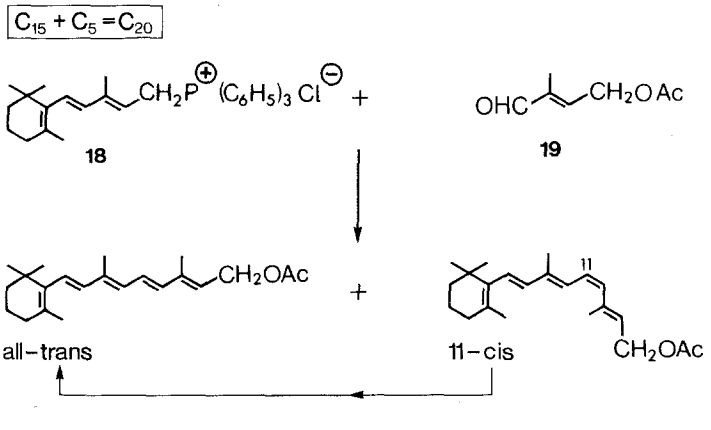


Fig. 15. The technical synthesis of the BASF.

In human medicine vitamin A is used for the treatment of night blindness, xerophthalmia, and in changes of the mucous membrane. In the veterinary field vitamin A is applied in the raising and feeding of domestic animals, in fertility disturbances, and in stress situation. X-ray structure determination of all-trans-vitamin A-acetate[15], vitamin A-aldehyde[16], as well as of the corresponding monoclinic and triclinic modifications of vitamin A-acid[17] showed that the polyene chain was bent like a sword in all 4 cases. This fact may be explained by the steric interaction of the methyl groups with the hydrogen atom at the adjacent double bond. Both vitamin A-acetate and the aldehyde possess a 6,7-s-cis-conformation. With vitamin A-acid the labile monoclinic modification was found to have the 6,7-s-trans-conformation, the stable triclinic form again showed 6,7-s-cis-conformation. This influence of ATP. The third important protein is the so-called 'retinal-binding protein' which regulates the vitamin A

11 O. Isler, W. Huber, A. Ronco and M. Kofler, Helv. chim. Acta *30*, 1911 (1947).
12 C. v. Planta, U. Schwieter, L. Chopard-dit-Jean, R. Rüegg, M. Kofler and O. Isler, Helv. chim. Acta *45*, 548 (1962).
13 W. Oroshnik, J. Am. chem. Soc. *78*, 2651 (1956).
14 U. Schwieter, C. v. Planta, R. Rüegg and O. Isler, Helv. chim. Acta *45*, 541 (1962). – H. Pommer, Angew. Chem. *72*, 811 (1960).
15 W. E. Oberhänsli, H. P. Wagner and O. Isler, Acta Crystallogr. *B 30*, 161 (1974).
16 T. Hamanaka, T. Mitsui, T. Ashida and M. Kakudo, Acta Crystallogr. *B 28*, 214 (1972).
17 C. H. Stam, Acta Crystallogr. *B 28*, 2936 (1972).
18 R. Gilardi, I. L. Karle, J. Karle and W. Sperling, Nature, Lond. *232*, 187 (1971).
19 E. L. Menger, Accts chem. Res. *8*, No. 3 (1975). – H. Langer (Ed.), in: Biochemistry and Physiology of Visual Pigments. Springer-Verlag, Berlin, Heidelberg and New York 1973.
20 D. Oesterhelt, Angew. Chem. *88*, 16 (1976). – R. Henderson and P. N. T. Unwin, Nature, Lond. *257*, 28 (1975).

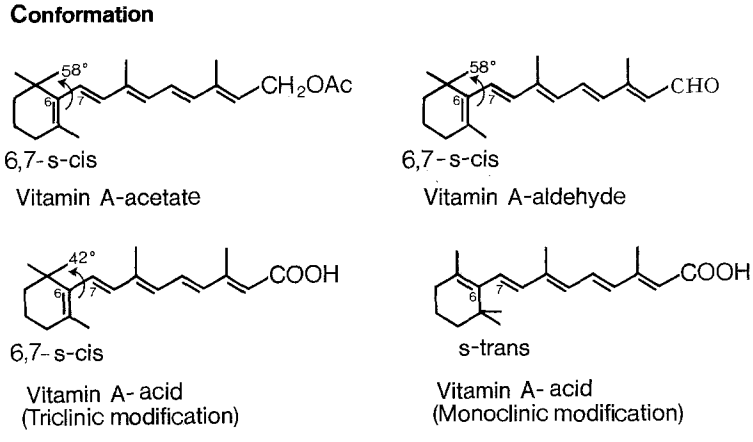


Fig. 16. The conformation of vitamin A and analogs.

the crystal structure on the conformation is astonishing (figure 16). The angles between the plane of the cyclohexene ring and that of the polyene chain is 58° in vitamin A-acetate and vitamin A-aldehyde. The same angle in the triclinic vitamin A-acid is only 42°. An x-ray structure projection of 11-cis-retinal and of its trans-isomer is shown in figure 17. 11-cis-Retinal which condenses with opsin to the visual purple occurs in the crystalline form in s-cis-conformation[18]. The angle between the ring and the polyene chain is here 40°. The triene system from C-7 to C-12 is flat. The chain end, however, is turned out of this plane from carbon-12 onward by 39°.

Presently, 3 vitamin A-protein complexes are the subject of intensive investigations: The already mentioned visual purple with a molecular weight of approximately 36,000 and the prosthetic group 11-cis-retinal[19]. Then the bacterial rhodopsin of the purple membranes of Halobacterium halobium. Its prosthetic groups are all-trans- and 13-cis-retinal[20]. The protein component has a molecular weight of 26,000. Halobacterium halobium can grow in a sodium chloride-containing medium and needs light in its synthesis of

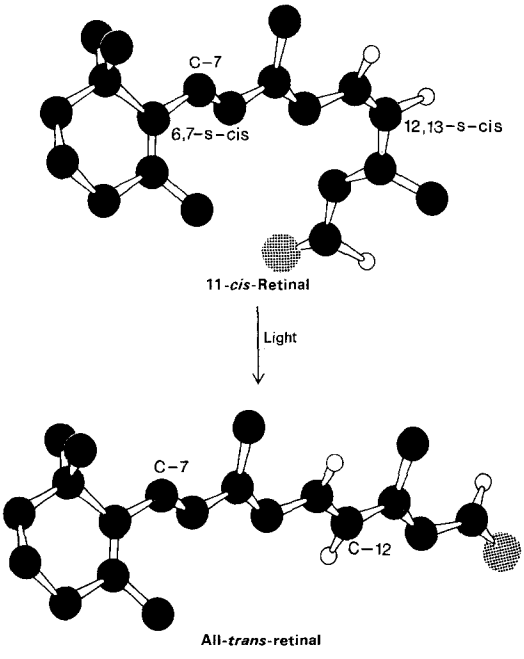


Fig. 17. X-ray structures of 11-cis- and all-trans-retinal. (From : 'Biochemistry' by L. Stryer, W. H. Freeman and Company, San Francisco, Copyright © 1975).

transport in the body[21]. The questions so far not answered are: How does this protein bind vitamin A, how does it deposite vitamin A at its target tissues and how many children turn blind despite of the presence of vitamin A because this protein is lacking?

Carotenoids. Carotenoids[22] are responsible for many colors of fruits, vegetables, roots, flowers, and autumn leaves. They are the cause of the colors of butter, egg yolk, many algae, mushrooms and crustaceae. The flesh of fish and the skin and feathers of many birds owe their pigmentation to carotenoids. It is estimated that nature produces 100 million tons of carotenoids a year. The application of modern physical methods has led to a rapid development in this relatively old field of chemistry. In 1950, the structures of approximately 50 carotenoids were known; in 1971 there were 300, and in 1976 we know the structures of 400 naturally occurring carotenoids.

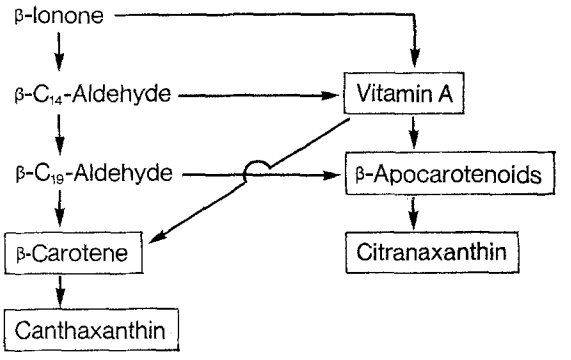


Fig. 19. The industrially synthesized carotenoids.

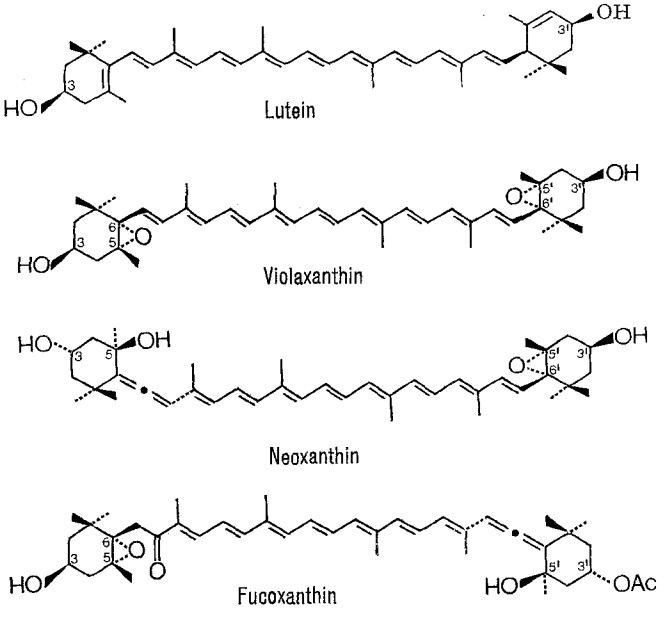


Fig. 18. The most abundant natural carotenoids.

The most common carotenoids are lutein, violaxanthin, neoxanthin and fucoxanthin (figure 18). The structurally complicated fucoxanthin is by far the most abundant of these. The absolute configurations of the chiral centres of these 4 carotenoids are known. 5 years ago, Weedon wrote a review article on chirality and conformation of carotenoids[23]. Then the chiral centres of 25 carotenoids were known. Today, we know the absolute configuration of more than 50.

Of the carotenoidal hydrocarbons and provitamin A compounds only a dozen possess chiral centres. In contrast to that, most of the xanthophylls are optically active. Today, only 5 carotenoids are being produced on an industrial scale (figure 19). These are β-carotene,

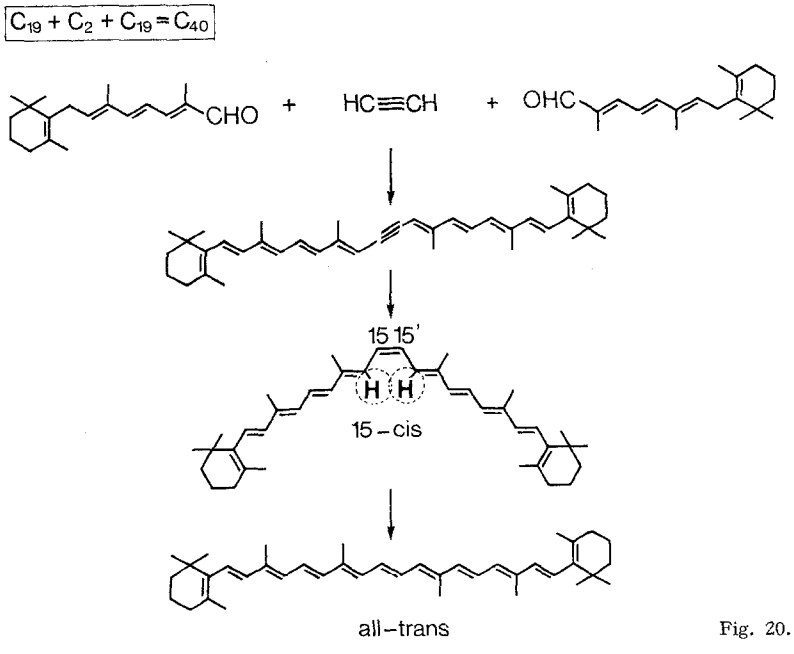


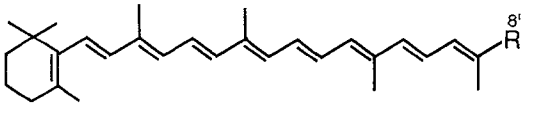
Fig. 20. Key steps in the Roche synthesis of β-carotene.

canthaxanthin, 2 C_{30}-apo-carotenoids, and the C_{33}-apo-carotenoid citranaxanthin. With the exception of canthaxanthin, all these exhibit provitamin A-activity. All the industrial syntheses start with β-ionone. This has already been described[24].

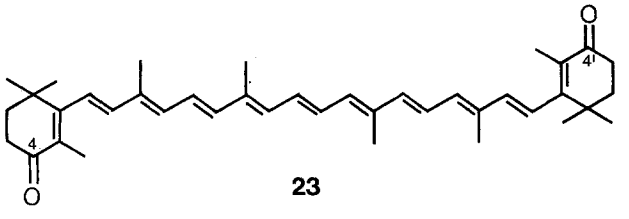
β-Carotene is the most important human source of vitamin A. It was not only the carotenoid that was synthesized first on a laboratory scale[25] but also the first to be produced industrially[26]. In figure 20, an outline is given of the key steps in the manufacturing procedure of Hoffmann-La Roche which is based on Inhoffen's first synthesis. 2 C_{19}-aldehydes are being condensed with acetylene to give 15,15'-didehydro-β-carotene. Catalytic partial hydrogenation of this carotenoid yields first 15-cis-β-carotene which is easily isomerized in boiling petroleum ether to the all-trans-compound.

β-Apo-8'-carotenoic acid ethyl ester (**20**) has approximately the same color as β-carotene. The corresponding apo-carotenal (**21**) and citranaxanthin (**22**) which arise from **21** through condensation with acetone, are slightly more reddish in color. Canthaxanthin (**23**), a compound obtained from β-carotene, is light red, and, in contrast to compounds **20**–**22**, more stable. The dark red torularhodin ester (**24**) occurs naturally in the red yeast Torula rubra. Dihydroxyisorenieratene (**25**), a carotenoid with aromatic end-groups, was isolated from sponges and mycobacteria. Its structure resembles the aromatic vitamin A-acid analogs mentioned above. All these oxygen-containing carotenoids (figure 21) are being absorbed by mammals and birds in their flesh in contrast to the normal carotenoidal hydrocarbons which are deposited.

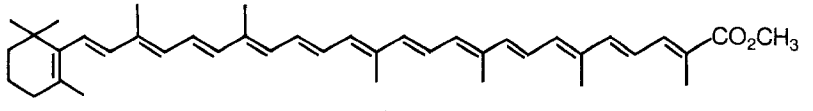
X-ray investigations have so far been carried out with β-carotene, canthaxanthin, and their corresponding 15,15'-didehydro-compounds[27]. In all cases it was found that the practically linear polyene chain was not a straight zig-zag chain, but showed an s-shaped



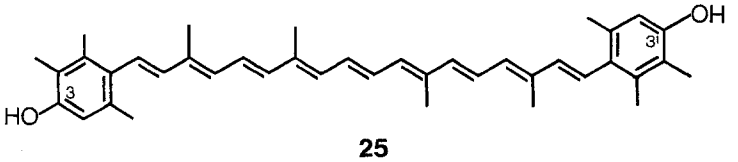
20, R = $COOC_2H_5$, **21**, R = CHO, **22**, R_1 = CH=CH-CO-CH_3



23



24



25

Fig. 21. A few representative examples of carotenoids.

21 Vitams Horm. *32*, 167 (1974).

22 For a review see: O. Isler (Ed.), in: Carotenoids. Birkhäuser-Verlag, Basel and Stuttgart 1971. – O. Straub, in: Key to Carotenoids. Birkhäuser-Verlag, Basel and Stuttgart 1976. – T. W. Goodwin (Ed.), in: Chemistry and Biochemistry of Plant Pigments, 2nd ed., vol. 1, p. 225; vol. 2, p. 38. Academic Press, London and New York 1976. – 3rd International Symposium on Carotenoids, Pure appl. Chem. *35*, 1 (1973); 4th International Symposium on Carotenoids, Pure appl. Chem. (in press).

23 B. C. L. Weedon, in: Carotenoids, p. 267. Ed. O. Isler. Birkhäuser-Verlag, Basel and Stuttgart 1971.

24 O. Isler and F. Kienzle, in: The Chemistry of Synthetic Dyes, vol. 9. Ed. K. Venkataraman. Academic Press, New York and London (in press).

25 P. Karrer and C. H. Eugster, Helv. chim. Acta *33*, 1172 (1950). – H. H. Inhoffen, F. Bohlmann, K. Bertram, G. Rummert and H. Pommer, Justus Liebigs Annln Chem. *570*, 54 (1950).

26 O. Isler, H. Lindlar, M. Montavon, R. Rüegg and P. Zeller, Helv. chim. Acta *39*, 249 (1956).

27 J. C. J. Bart and C. H. MacGillavry, Acta Crystallogr. *B 24*, 1569, 1587 (1968). – C. Sterling, Acta Crystallogr. *17*, 1224 (1964). – W. G. Sly, Acta Crystallogr. *17*, 511 (1964).

bending. This effect appears to be due to the non-bonded interaction between the side-chain methyl groups and the hydrogen atoms at C-11, C-15, C-11′ and C-15′. There is little doubt that the same situation is not only found in the crystal but also in solutions. The situation at the junction of the ring with the polyene chain is, however, different. As we have already seen for the vitamin A derivatives, the cyclohexene ring double bond is not co-planar with the polyene chain. The deviation again is influenced by the crystal lattice. For triclinic crystals of canthaxanthin the angle between the cyclohexene double bond and that of the neighbouring double bond in the polyene chain is slightly different for the two end groups, namely 52° and 56°, respectively (figure 22). For 15,15′-dihydrocanthaxanthin these angles are 43° and for β-carotene a 40° deviation is observed.

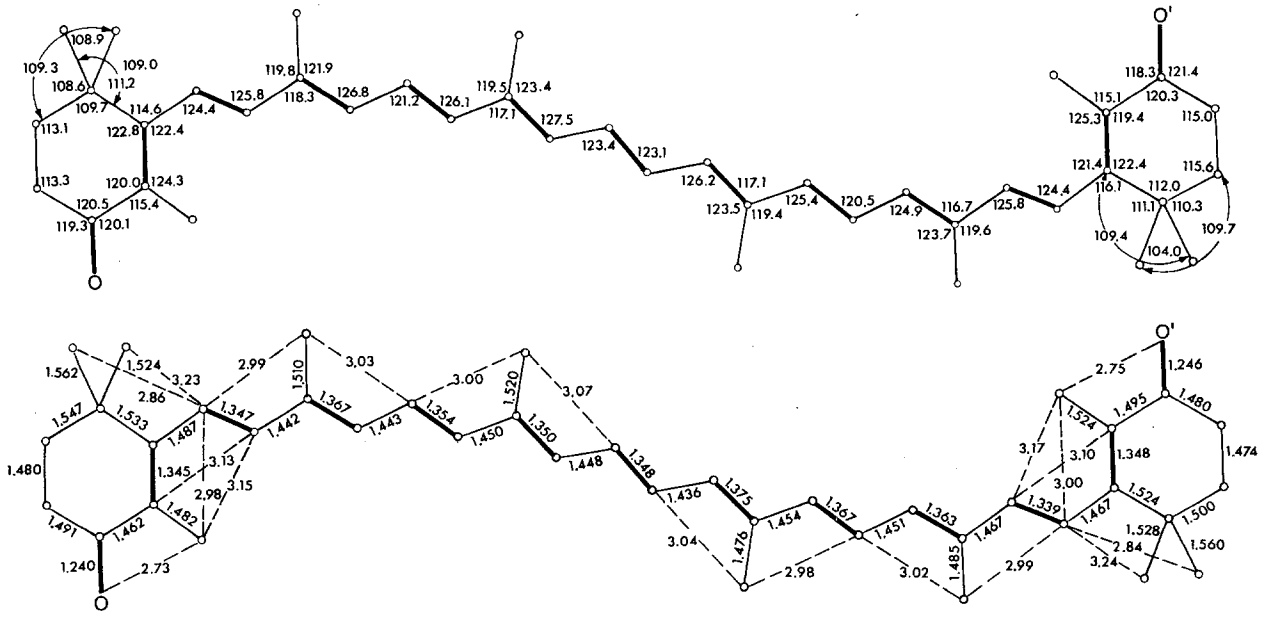


Fig. 22. Molecular structure of canthaxanthin, bond lengths and angles (Bart and MacGillavry[27]).

Recently, (3R,3′R)-zeaxanthin (**26**)[28], (3S,3′S)-astaxanthin (**27**), actinioerythrol (**28**) and violerythrin (**29**)[29] have been obtained by total synthesis. Zeaxanthin is the major pigment of corn; it is however, also found in many other plants, algae and bacteria. Astaxanthin occurs in many marine organisms like fish and crustaceae; it is the prosthetic group in many carotenoproteins (figure 23). Remarkable is the recent discovery of (3R,3′R)-astaxanthin, i.e. a carotenoid with the opposite absolute configuration, in Phaffia rhodozyma[30]. This finding points to a difference in the biosynthetic routes. Racemic naturally occurring carotenoids are not known.

The total synthesis of optically active zeaxanthin starts with keto-isophorone (**30**). The chirality is introduced enzymatically[28]. From there, the synthesis follows established procedures (figure 24). The resulting (3R,3′R)-zeaxanthin is identical in all respects with the natural product. This actually represents the first successful synthesis of an optically active xanthophyll. The carotenoids **28** and **29** belong to the class of 2,2′-dinor-carotenoids of which **28** is the only naturally occurring example so far known. These 5-ring carotenoids are deeper in color than their 6-ring counterpart, a fact that might be explained assuming coplanarity of the cyclopentene double bond with the polyene chain. Actinioerythrol occurs as a di-ester (actinioerythrin) in the sea anemone Actinia equina. Violerythrin, the oxidation product of **28**, is, surpris-

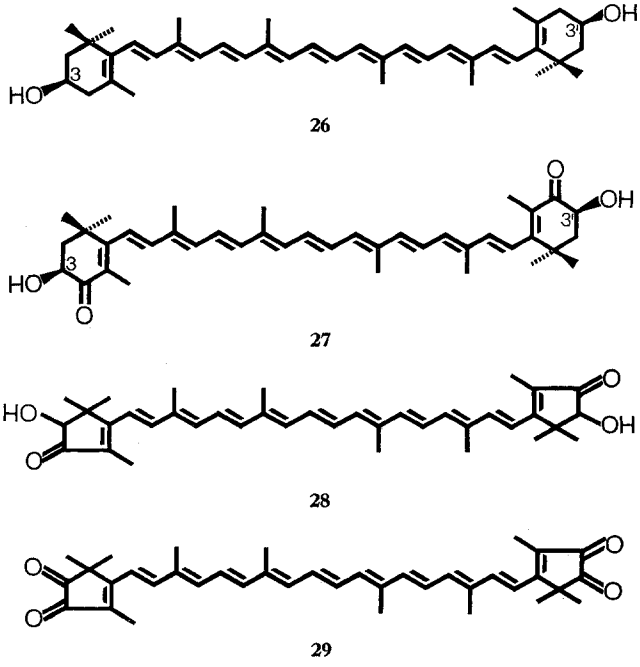


Fig. 23. Recently synthesized carotenoids.

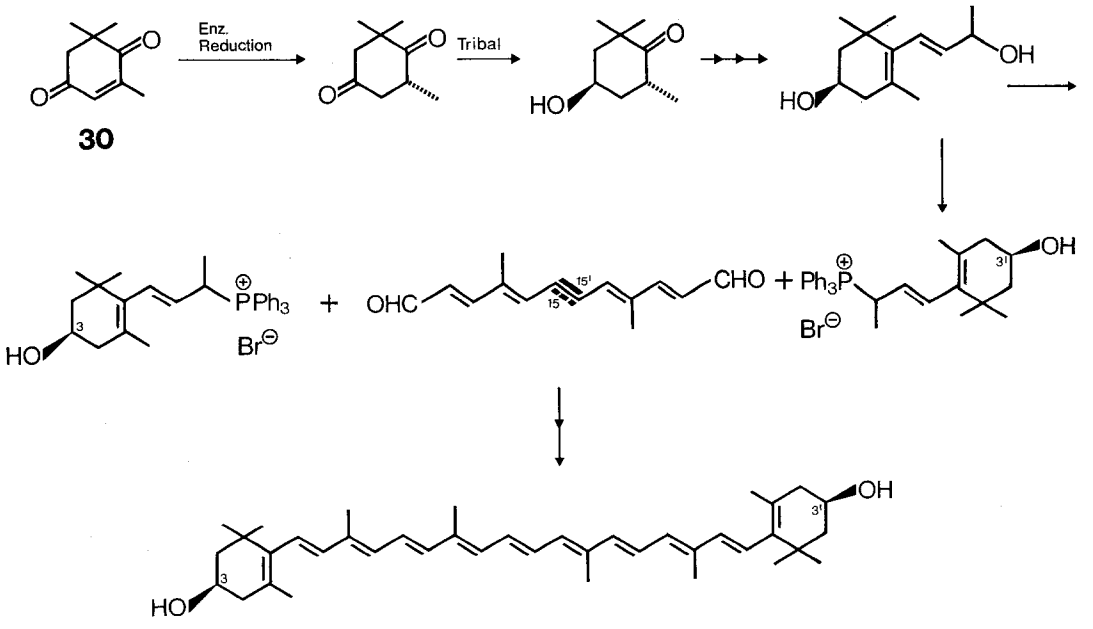


Fig. 24. (3R,3'R)-Zeaxanthin from ketoisophorone.

ingly, blue in color. These 2,2'-dinor-carotenoids have been obtained from astacene through a ring-contraction and by total synthesis from acetone[29].

The synthesis of violerythrin[29] starts with a trimethyl cyclopentenone easily accessible from acetone and acetylene. Interestingly, the polyene chain could not be linked to this ring component with either a Wittig or a Horner reaction. However, the olefinic side chain could successfully be introduced with the help of the recently discovered sulfone olefination reaction. Once parts of the polyene chain were linked with the endgroup, Wittig olefinations could be used for the completion of the synthesis. 2,2'-Dinor-canthaxanthin, a key intermediate, was then oxidized with selenium dioxide to violerythrin (figure 25). Although already more than 400 naturally occurring carotenoids are known, new ones are still being discovered. Some interesting examples are shown in figure 26. Alloxanthin possesses 2 acetylenic bonds, and rhodopinal is one of the few naturally occurring

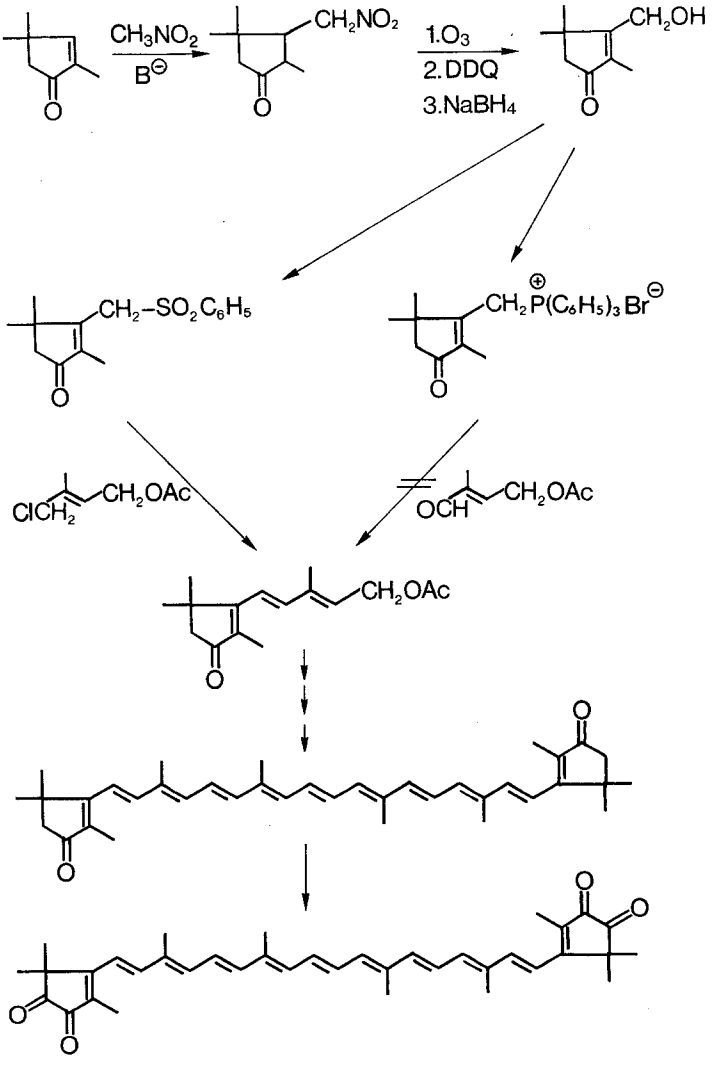


Fig. 25. The synthesis of violerythrin.

28 H. G. W. Leuenberger, W. Boguth, E. Widmer and R. Zell, Helv. chim. Acta *59*, 1832 (1976). – H. J. Mayer et al., Helv. chim. Acta (in preparation).

29 F. Kienzle and R. E. Minder, Helv. chim. Acta *59*, 439 (1976).

30 A. G. Andrewes and M. P. Starr, Phytochemistry *15*, 1009 (1976).

carotenoids with a cis double bond. Sarcinaxanthin, a C_{50}-carotenoid, has 2 additional isopentyl residues attached at positions 2 and 2', and finally 4,4'-diapophytoene which arises from presqualene is the mother substance of several C_{30}-apo-carotenoids.

Of the biological functions of carotenoids relatively little is known. One knows that they are accessory pigments in the photosynthesis. One finds them, therefore, in company of chlorophyll in all green plants. They also may protect tissues against the harmful effects of light. So far their pharmaceutical value is limited. β-Carotene or a mixture thereof and canthaxanthin given orally helps in cases of erythropoietic protoporphyria[31] and perhaps thalassemia, 2 rare genetic diseases. Industrially, carotenoids are important as natural and harmless colorants for food and feed. Of biological significance are some possible metabolites of carotenoids (figure 27), for instance the plant-growth regulators abscisic acid[32] and xanthoxin[33] which seem to be ubiquitous in nature; trisporic acid[34], a sexual attractant of fungi, and grasshopper ketone[35], an insecticide secreted by certain grasshoppers. The origin of trisporic acid from β-carotene has been proved through radioactive labelling. For the other compounds, definite proofs are still missing, although xanthoxin and abscisic acid may be obtained

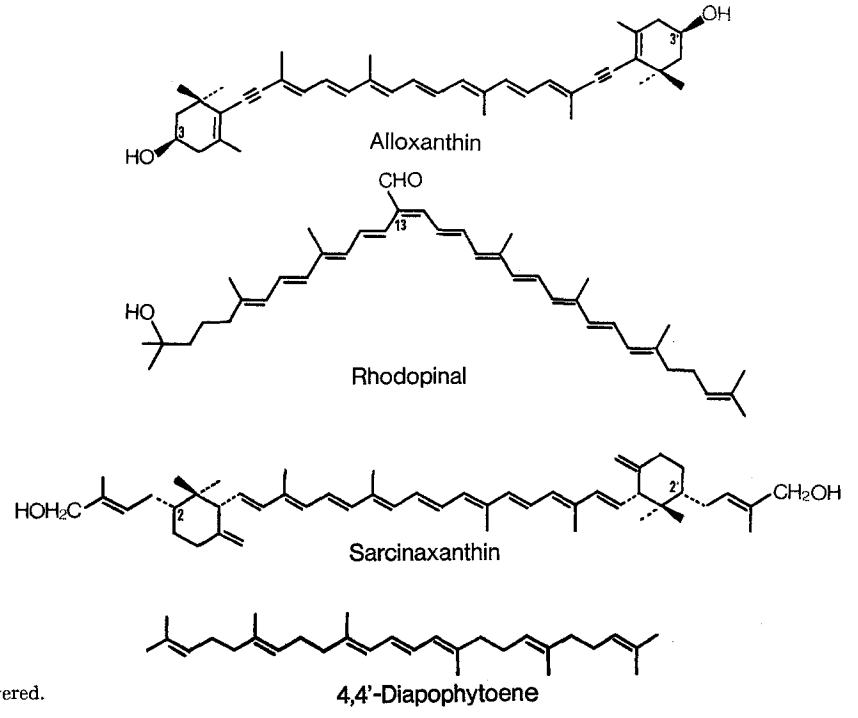


Fig. 26. Some new carotenoids recently discovered.

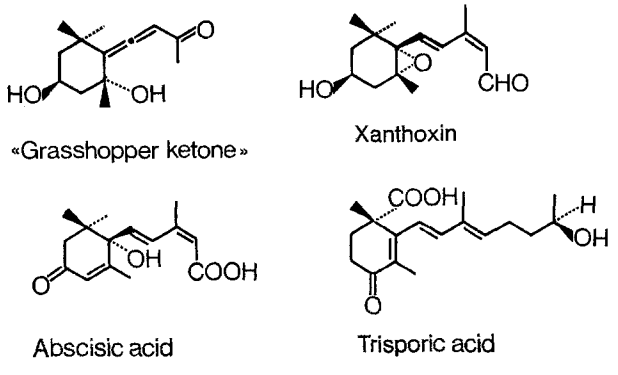


Fig. 27. Possible metabolites of carotenoids.

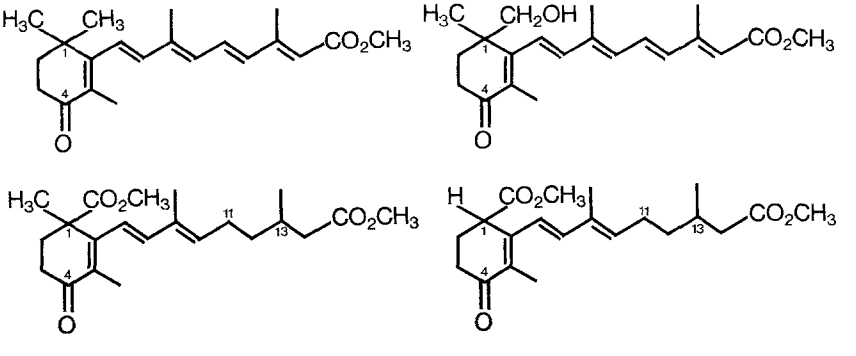


Fig. 28. Metabolites of vitamin A and vitamin A-acid.

from violaxanthin in vitro. In the rat, vitamin A and vitamin A-acid are metabolized to the compounds shown in figure 28[36]. First a keto function is being introduced in position 4, then the geminal methyl groups are being oxidized. Partial hydrogenation of the polyene chain and removal of 1 methyl group seem to be the next steps in the degradation. These metabolites remind us of trisporic acid.

Vitamin D[37]. The biosynthesis of vitamin D[38], too, follows the usual path of terpenoid synthesis up to the C_{15}-compound farnesyl pyrophosphate. Dimerisation of the latter leads via presqualene to squalene. From there, lanosterol and then cholesterol and provitamin D are obtained. Most cholesterol is being transformed to steroidal hormones, the preferred regulators for many physiological functions in mammals. However, a small percentage of cholesterol is converted to provitamin D (7-dehydro-cholesterol) which on exposure to light rearranges to vitamin D_3 (cholecalciferol). The active principle is 1,25-dihydroxy-vitamin D which

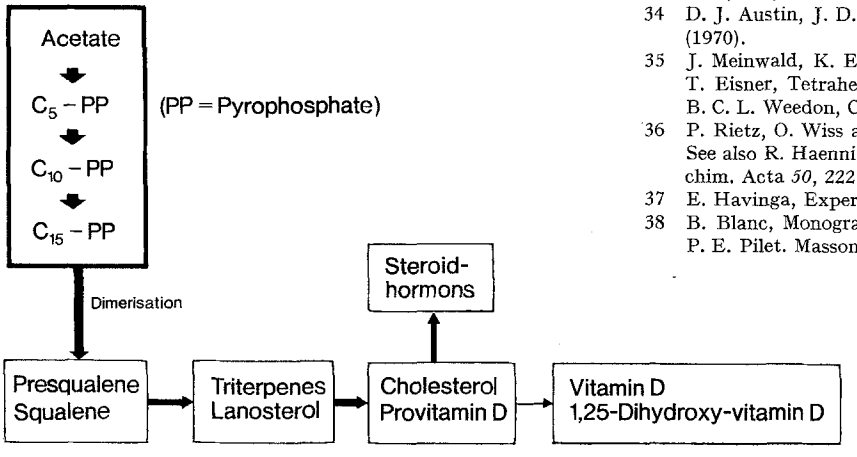


Fig. 29. The biosynthesis of vitamin D.

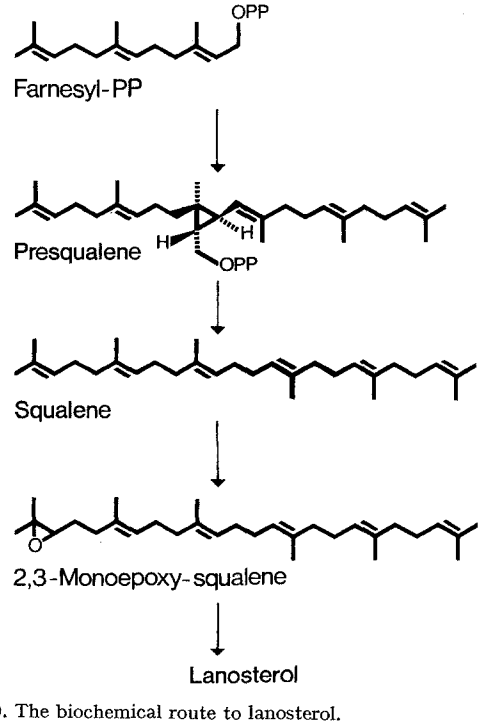


Fig. 30. The biochemical route to lanosterol.

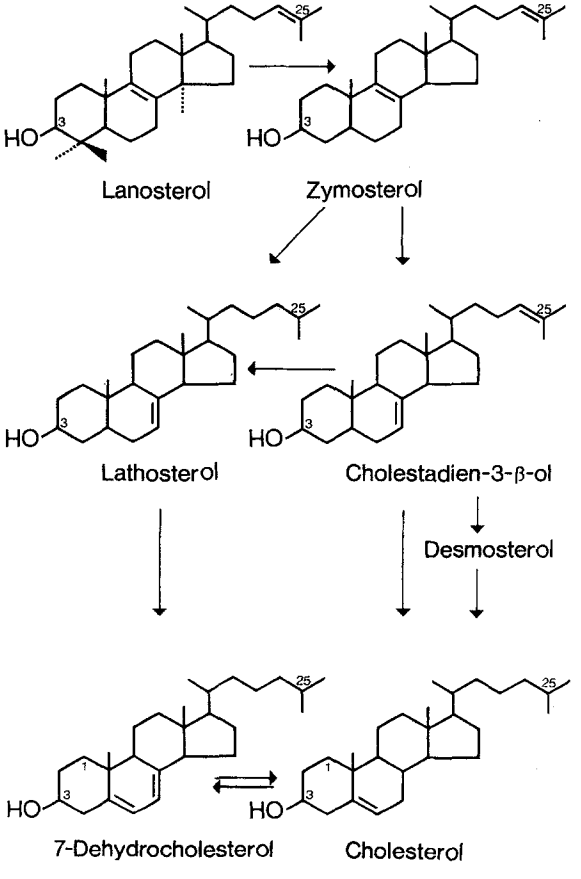


Fig. 31. The conversion of lanosterol to cholesterol.

31 V. A. DeLeo, M. Poh-Fitzpatrick, M. Mathews-Roth and L. C. Harber, Am. J. Med. *60*, 8 (1976). – P. Fritsch, F. Gschnait, H. Hoenigsmann and K. Wolff, Br. J. Derm. *94*, 263 (1976). – N. Pollitt, Br. J. Derm. *93*, 721 (1975).
32 H. J. Mayer, N. Rigassi, U. Schwieter and B. C. L. Weedon, Helv. chim. Acta *59*, 1424 (1976). – B. V. Milborrow, Ann. Rev. Plant Physiol. *25*, 259 (1974).
33 H. F. Taylor and R. S. Burden, Proc. Roy. Soc. Lond. *B 180*, 317 (1972).
34 D. J. Austin, J. D. Bu'lock and D. Drake, Experientia *26*, 348 (1970).
35 J. Meinwald, K. Erickson, M. Hartshorn, Y. C. Meinwald and T. Eisner, Tetrahedron Lett. *1968*, 2959. – S. W. Rossel and B. C. L. Weedon, Chem. Commun. *1969*, 85.
36 P. Rietz, O. Wiss and F. Weber, Vitams Horm. *32*, 237 (1974). See also R. Haenni, F. Bigler, W. Meister and G. Englert, Helv. chim. Acta *50*, 2221 (1976).
37 E. Havinga, Experientia *29*, 1181 (1973).
38 B. Blanc, Monographies de Physiologie Végétale, vol. 10. Ed. P. E. Pilet. Masson & Cie, Paris 1973.

arises through 2 enzymatic hydroxylations. This compound has been recognized as the actual active vitamin D.

Interesting details of this biochemical route have become known during recent years. 2 molecules of farnesyl pyrophosphate condense to give presqualene with a cyclopropane ring bearing an additional CH_2O-pyrophosphate group. From there, the symmetrical squalene is formed. The cyclization of squalene to lanosterol is initiated by an epoxidation of the double bond at position 2 (figure 30). In the conversion of lanosterol to zymosterol 3 CH_3-groups are lost. 2 pathways may then yield cholesterol, namely via cholestadienol and desmosterol or via lathosterol and the provitamin D 7-dehydrocholesterol. It seems that an equilibrium between 7-dehydrocholesterol and cholesterol exists in biological systems (figure 31).

The whole biochemical route is now firmly established through experiments with radioactive labelled compounds. In 1956 we had synthesized squalene from acetone and nerolidol and shown its identity with the natural product[39]. We succeeded in 1957 in incorporating radioactive labelled mevalonic acid in cholesterol. Through degradation we proved the position of the label[40]. Through x-ray analysis the absolute configuration of vitamin D_3 has been determined[41]. A complete new chapter in vitamin D research started with the recognition of the highly active metabolite 1,25-dihydroxy-vitamin D[42]. It was shown that the D-vitamins are first hydroxylated in position 25 in the liver and then, after migration to the kidney, another hydroxy group is introduced in position 1. The 1,25-dihydroxy-vitamin D is the compound actively engaged in the calciumphosphate exchange in the walls of intestines and in the bones.

Clinically, these new metabolites are being tested for their effect in the homeostasis of calcium, in renal osteodystrophia and in osteoporosis. In the veterinary field, their importance in the calcification of eggs and in the treatment of milk feaver is being investigated. One of the many recent syntheses of 1,25-vitamin D is shown in figure 32. The key intermediate 1,25-dihydroxy-cholesterol was obtained from O-acetyl-pregnenolone in a multistep synthesis[43]. At present, the whole field of vitamin D is being actively investigated from biologists as well as chemists. 24,25- and 25,26-dihydroxy-, 1,24,25-trihydroxy- and 3-desoxy-1,25-dihydroxy-cholecalciferol and the corresponding ergocalciferol compounds are being synthesized and tested. It is hoped in analogy to the steroid hormones that molecular modifications may result in an increase in efficiency and specificity.

Vitamin E. The absolute configuration of natural α-tocopherol (vitamin E) was determined by us in 1963[44,45]. It was found that all three chiral centres possess R-configuration. Of these, the centre at the chromane ring seems to have the most influence on the activity of the compound. The β-, γ- and δ-tocopherols, too, possess R-configuration, so do the tocotrienols whose double bonds exhibit trans-geometry. Racemic synthetic α-tocopherol shows only about 70% of the biological activity. Its technical synthesis (figure 33) uses as a key step a condensation of isophytol and trimethyl hydroquinone. The latter is obtained from acetone by a stepwise synthesis whereby each time 2 or 3 carbon atoms are added.

The biosynthesis of vitamin E follows the usual path of terpenoid synthesis up to geranylgeranyl pyrophos-

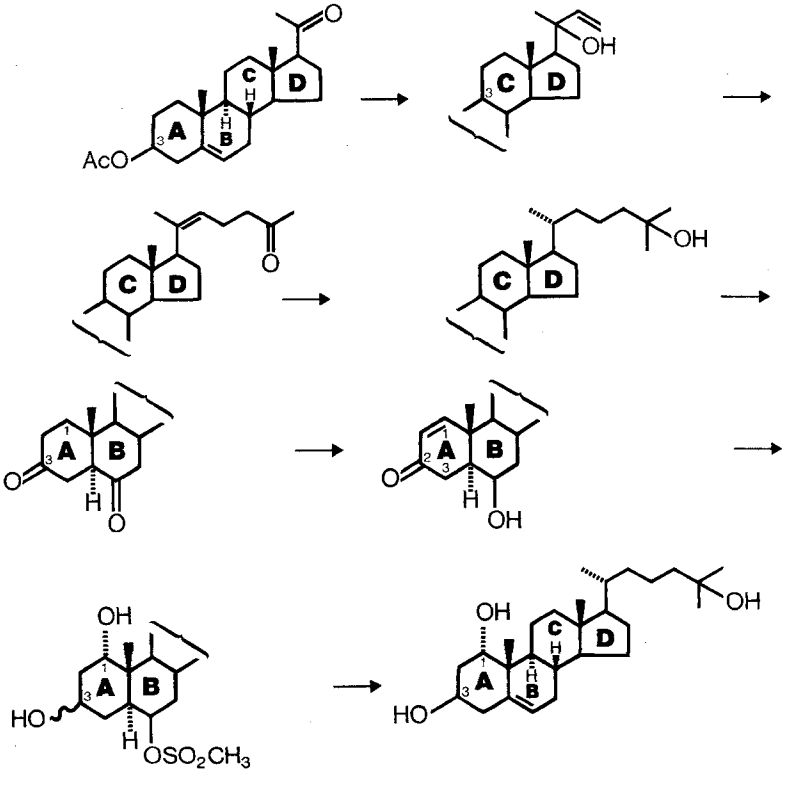


Fig. 32. The synthesis of 1,25-dihydroxy-cholesterol.

phate. 2 different pathways are, according to Pennock, possible from there[46]. In the main route, which is found in leaves and in most tissues of plants and algae, phytyl pyrophosphate condenses with homogentisic acid (or a derivative thereof) to give δ-tocopherol. Methylation, which is effected by S-adenosyl methionine, leads via β- and γ-tocopherol to α-tocopherol. On the other hand, in the 'tocotrienol route' homogentisic acid (or a derivative thereof) condenses with geranylgeranyl pyrophosphate to give δ-tocotrienol. After methylation with S-adenosyl methionine, β- and γ-tocotrienol and finally α-tocotrienol, are formed. The last step in this route, which occurs in tissues where tocotrienols are found such as latex, α-tocopherol arises through hydrogenation (figure 34).

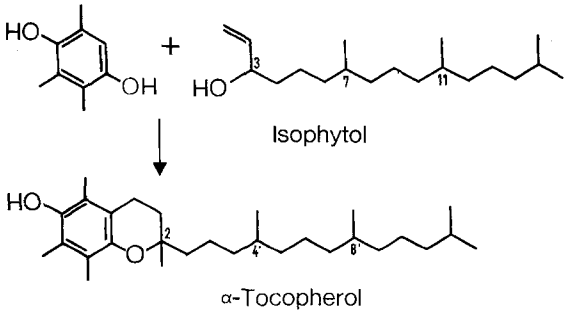


Fig. 33. The synthesis of α-tocopherol.

The biological precursors of homogentisic acid are shikimic acid and likely chorismic acid, prephenic acid, and p-hydroxyphenylpyruvic acid (figure 34). Vitamin E, a natural anti-oxidans, stabilizes unsaturated fatty acids. It is used in the veterinary field, in the prophylaxis of muscle dystrophy, and encephalomalacy. Natural α-tocopherol exhibits only a low optical rotation of $[\alpha]_D + 0.75°$. However, upon oxidation with potassium ferricyanide, a dimer with a spiro-dienone structure is obtained with an optical

39 O. Isler, R. Rüegg, L. Chopard-dit-Jean, H. Wagner and K. Bernhard, Helv. chim. Acta *49*, 897 (1956).
40 O. Isler, R. Rüegg, J. Würsch, K. F. Gey and A. Pletscher, Helv. chim. Acta *40*, 2369 (1957).
41 D. Crowfoot-Hodgkin, B. M. Rimmer, J. D. Dunitz and K. N. Trueblood, J. chem. Soc. *1963*, 4945.
42 M. F. Holick, H. K. Schnoes, H. F. De Luca, T. Suda and R. J. Cousins, Biochemistry *10*, 2799 (1971). – D. E. M. Lawson, D. R. Fraser, E. Kodicek, H. R. Morris and D. H. Williams, Nature, Lond. *230*, 228 (1971). – A. W. Norman, J. F. Myrtle, R. J. Midgett, H. G. Nowicki, V. Williams and G. Popják, Science *173*, 51 (1971).
43 T. A. Narwid, J. F. Blount, J. A. Iacobelli and M. R. Uskoković, Helv. chim. Acta *57*, 781 (1974).
44 H. Mayer, P. Schudel, R. Rüegg and O. Isler, Helv. chim. Acta *46*, 963 (1963).
45 H. Mayer, P. Schudel, R. Rüegg and O. Isler, Helv. chim. Acta *46*, 650 (1963).
46 D. R. Threlfall, Vitams and Horm. *29*, 153 (1971). – W. Janiszowska and J. F. Pennock, Vitams Horm. *34*, 77 (1976).

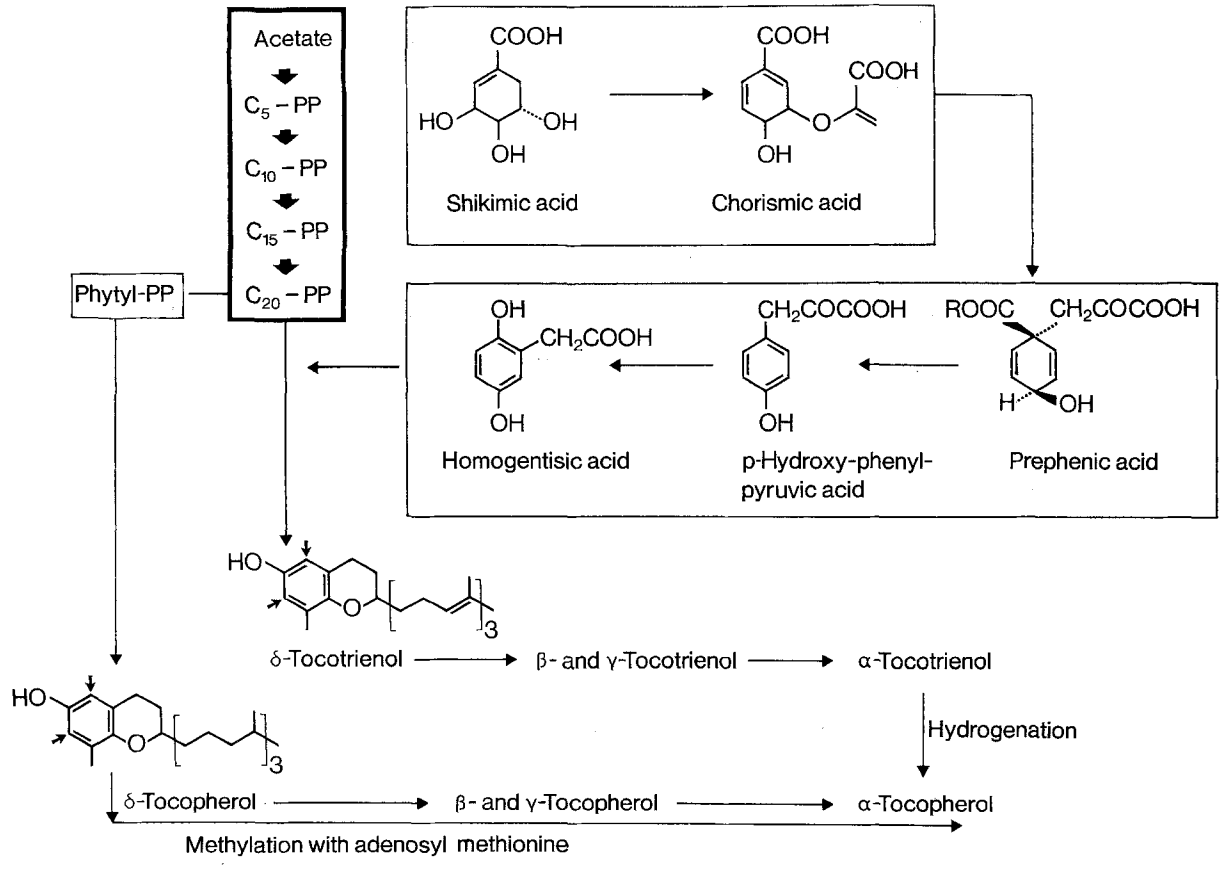


Fig. 34. The biosynthesis of α-tocopherol.

rotation $[\alpha]_D + 26°$. This compound has been used for the determination of the configuration at C-2 (figure 35)[47].

Another interesting reaction of α-tocopherol is its oxidation with ferric chloride which yields α-tocopherol quinone. Treatment of that compound with acetyl chloride closes the chromane ring again with retention of the side-chain oxygen atom[48]. This fact was ascertained through radioactive labelling. Treatment with zinc/hydrochloric acid and then with lithium aluminum hydride regenerates α-tocopherol with an 87% retention of the R-configuration. On the other hand, reduction of the α-tocopherol quinone with hydrogen to the corresponding hydroquinone and subsequent cyclization yields, with an 84% inversion, the α-tocopherol with S-configuration (figure 36).

Syntheses of natural α-tocopherol have been accomplished in several laboratories. For instance, using trimethyl hydroquinone and natural phytol as starting materials, we synthesized the corresponding optically active C_{14}-chromane aldehyde and a C_{15}-phosphonium salt, respectively (figure 37)[45]. Wittig condensation, hydrogenation, and finally removal of the protecting acetate furnished α-tocopherol identical in all respects with the natural product.

In another, more efficient synthesis, Saucy and coworkers succeeded in preparing the homologous C_{15}-chromane aldehyde which was similarly condensed with a C_{14}-phosphonium salt obtained through degradation of phytol. The same workers could also prepare in a similar manner natural α-tocotrienol[49].

Vitamin K. In the technical synthesis of vitamin K_1 (or phylloquinone), the 1-monobenzoate of 2-methylnaphthohydroquinone is condensed with phytol or isophytol. Subsequent saponification and oxidation fur-

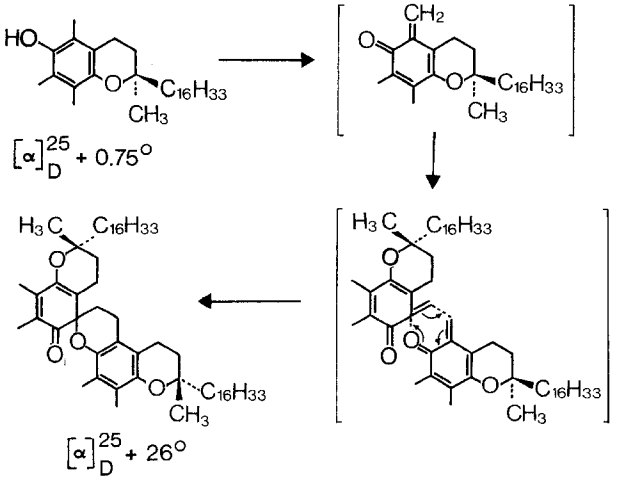


Fig. 35. Reaction of α-tocopherol with $K_3[Fe(CN)_6]$.

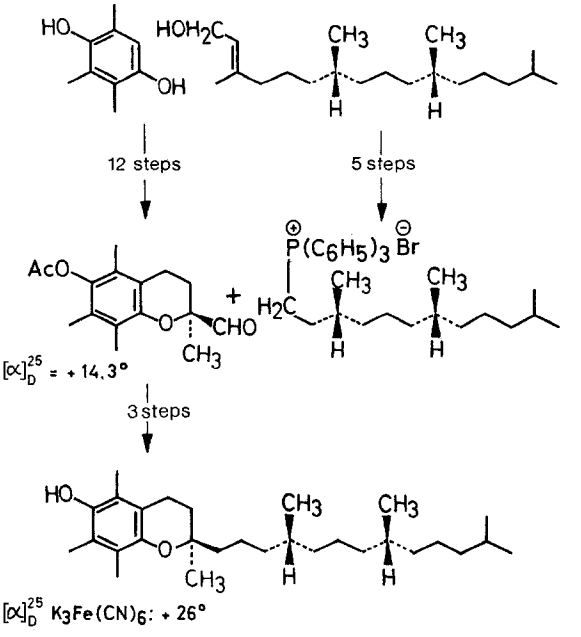


Fig. 37. Total synthesis of natural α-tocopherol.

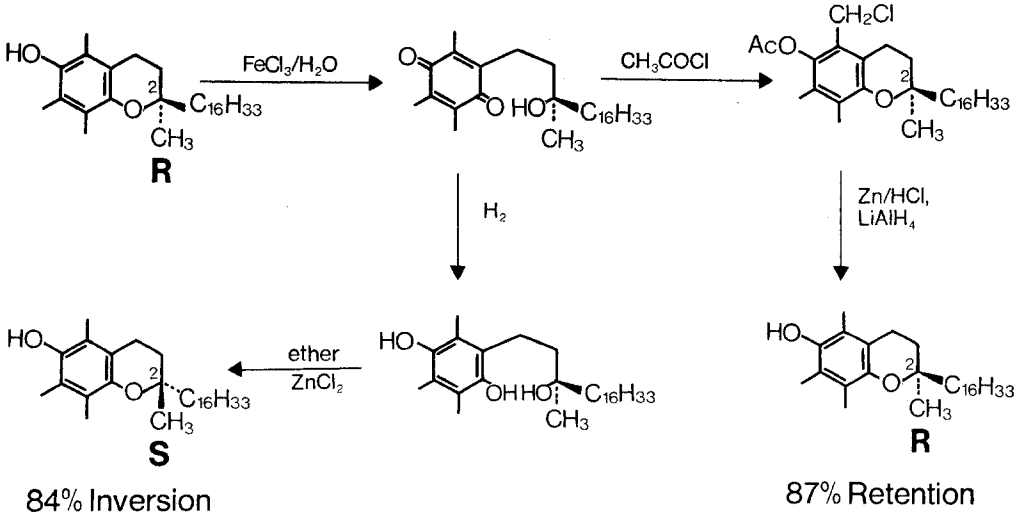


Fig. 36. Reaction of α-tocopherol.

nishes phylloquinone (figure 39). The absolute configuration of the chiral centres of natural vitamin K_1 was determinded by us to be R. The substituents at the double bond are as in phytol trans oriented[50]. The biological efficiency of racemic vitamin K_1 (from iso-

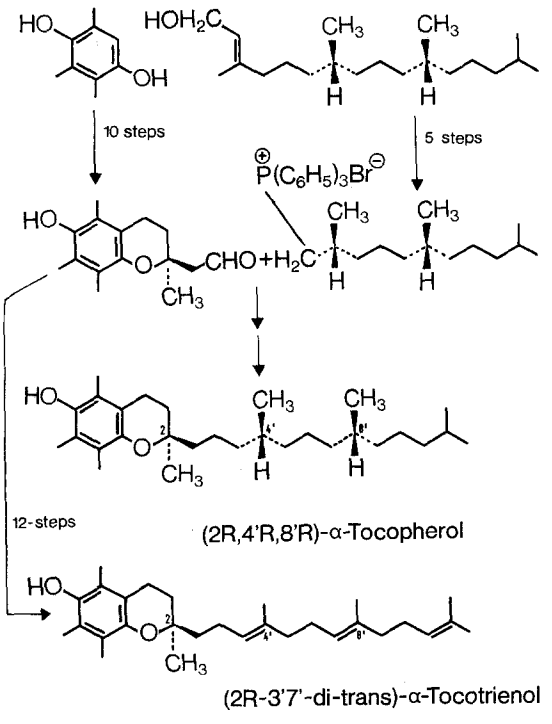


Fig. 38. The synthesis of α-tocopherol and α-tocotrienol.

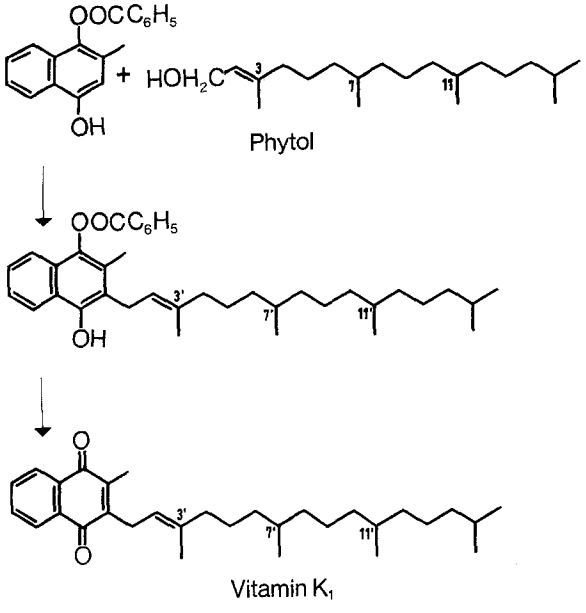


Fig. 39. The technical synthesis of vitamin K_1.

47 P. Schudel, H. Mayer, J. Metzger, R. Rüegg and O. Isler, Helv. chim. Acta *46*, 636 (1963).
48 H. Mayer, W. Vetter, J. Metzger, R. Rüegg and O. Isler, Helv. chim. Acta *50*, 1168 (1967). – P. Schudel, H. Mayer, J. Metzger, R. Rüegg and O. Isler, Helv. chim. Acta *46*, 333 (1963).
49 J. W. Scott, F. T. Bizarro, D. R. Parrish and G. Saucy, Helv. chim. Acta *59*, 290 (1976).
50 H. Mayer, U. Gloor, O. Isler, R. Rüegg and O. Wiss, Helv. chim. Acta *47*, 221 (1964).

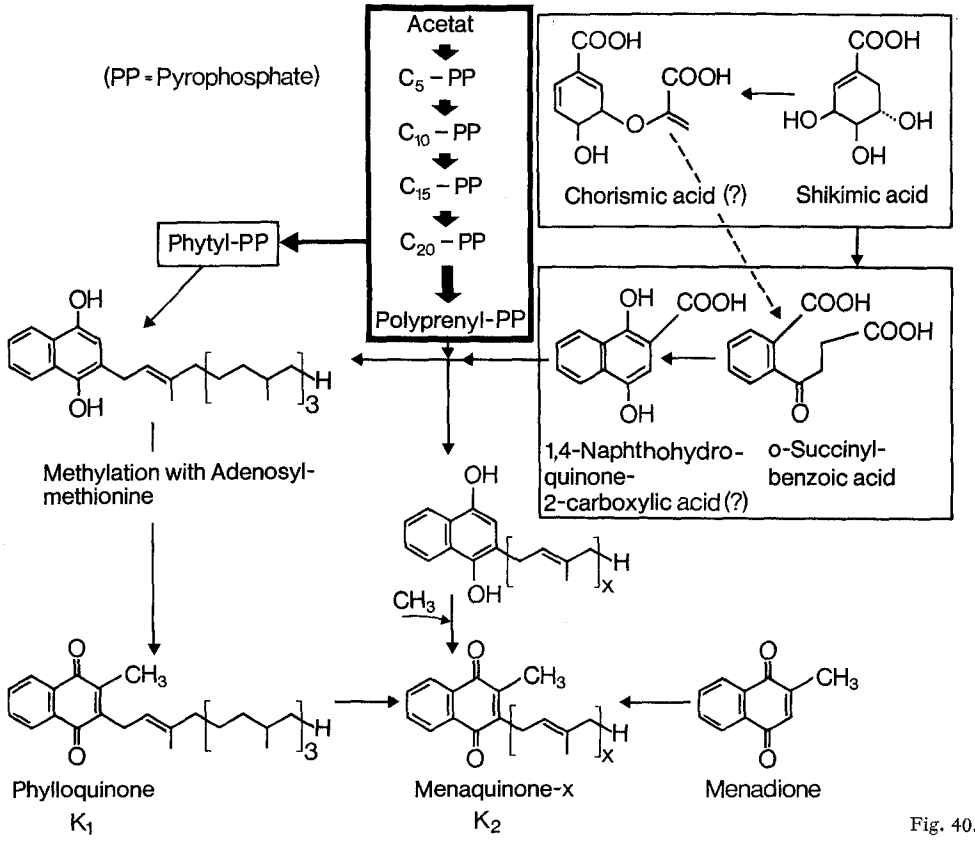


Fig. 40. The biosynthesis of vitamin K.

phytol) and that of the natural optically active compound (from phytol) is approximately the same[51] (table). According to Martius[52], an exchange of the original side chain with a geranylgeranyl side chain takes place in the organism of all mammals for all vitamin K compounds. That would mean that menaquinone-4 (or vitamin $K_{2(20)}$) would be the actual vitamin K of man and animals.

Like vitamin E the biosynthesis of vitamin K uses shikimic acid as the starting material for the aromatic part. Possibly chorismic acid, o-succinylbenzoic acid and 1,4-naphthohydroquinone-2-carbonic acid are further intermediates. There are strong indications that in most plants and microorganisms in the subsequent steps phytylation and polyprenylation precedes methylation. Methylation again is performed with S-adenosyl methionine. In mammals menadione is converted to menaquinone[53] (figure 40). The K-vitamins take part in the synthesis of prothrombin and other blood constituents necessary for blood coagulation. Closely related to vitamin E and K are the ubiquinones, which serve as electron-transfer agents, and the plastoquinones, which take part in the photosynthesis in plants (figure 41)[54].

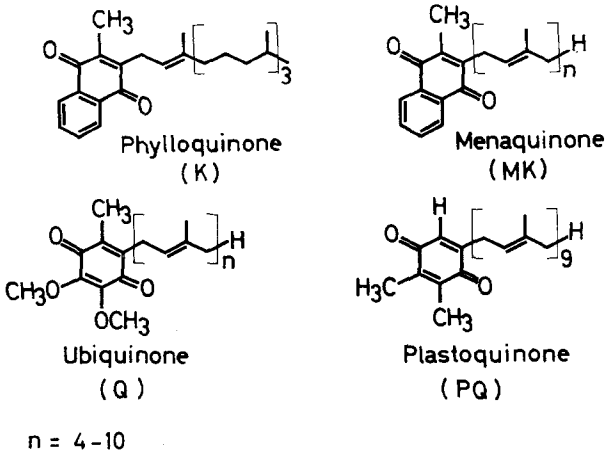


Fig. 41. Terpenoid quinones.

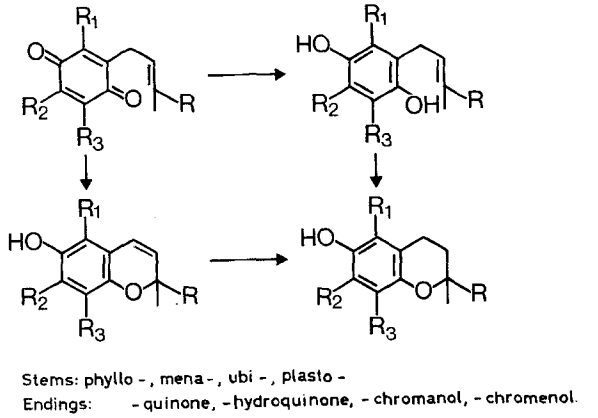


Fig. 42. General conversions of terpenoid quinones.

These compounds, generally called terpenoid quinones, may form a hydroquinone, a quinone, a chromanol or a chromenol (figure 42).

It is noteworthy that the vitamins E and K, as well as the ubiquinones and plastoquinones are metabolized in a similar way. Always the side chain is first degraded till only 7 carbon atoms are left. Excretion in the urine occurs then in form of the lactone or its corresponding hydroxy acid (figure 43)[55].

Conclusion. The development of physical methods had an enormous impact on the field of fat-soluble vitamins and carotenoids. With their help the absolute stereochemistry of many of these compounds could be elucidated, and that, in turn, lead to the total synthesis of the natural compounds and many of their unnatural analogs.

The biosynthesis revealed common precursors for all these vitamins and carotenoids. The vitamins A and D as well as the carotenoids are true terpenoids. The vitamins E and K contain additional aromatic rings derived in both cases from shikimic acid and the methyl groups from S-adenosyl methionine (figure 44).

Similarly, the technical syntheses of these compounds are also based on 1 common starting material, namely acetone (figure 45).

Dam's coagulation test with vitamin K-deficient chicken

	Quick time
Vitamin K_1 from phytol	85 ± 7.4
Vitamin K_1 from isophytol	87 ± 7.1
Controls: Vitamin K deficient chicken	180
Normal chicken	50 ± 2.2

Results from O. Wiss. Dosage 56 µg/kg body weight.

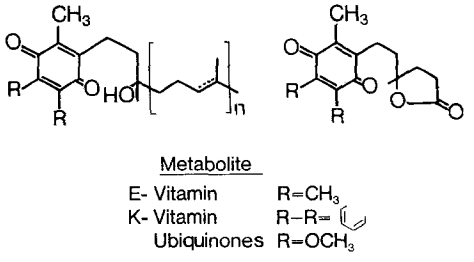


Fig. 43. Metabolites of terpenoid quinones.

51 O. Isler and O. Wiss, Vitams Horm. *17*, 53 (1959).
52 C. Martius, Angew. Chem. *73*, 597 (1961).
53 R. M. Baldwin, C. D. Snyder and H. Rapoport, Biochemistry *13*, 1523 (1974).
54 R. A. Morton, Biol. Rev. *46*, 47 (1971). – R. A. Morton (Ed.), in: Biochemistry of Quinones. Academic Press, London and New York 1965.
55 U. Gloor, J. Würsch, H. Mayer, O. Isler and O. Wiss, Helv. chim. Acta *49*, 2582 (1966).

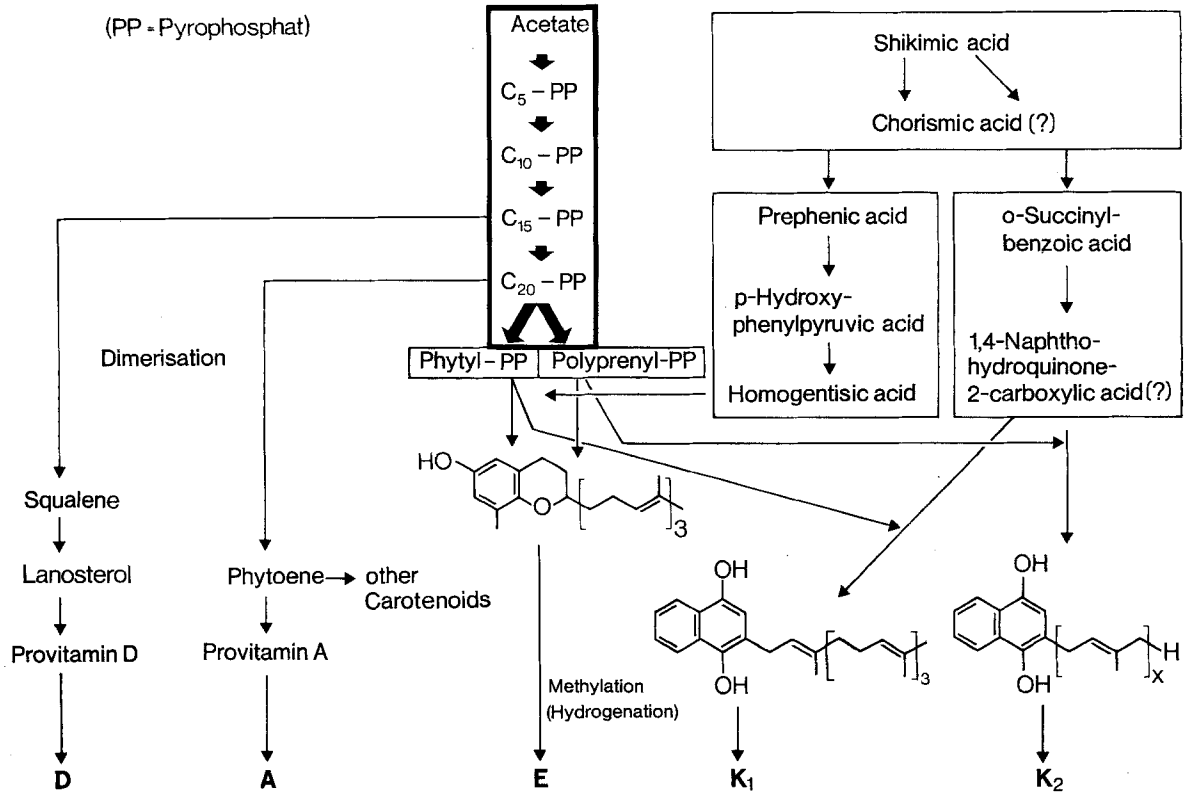


Fig. 44. The biochemical syntheses of fat-soluble vitamins and carotenoids.

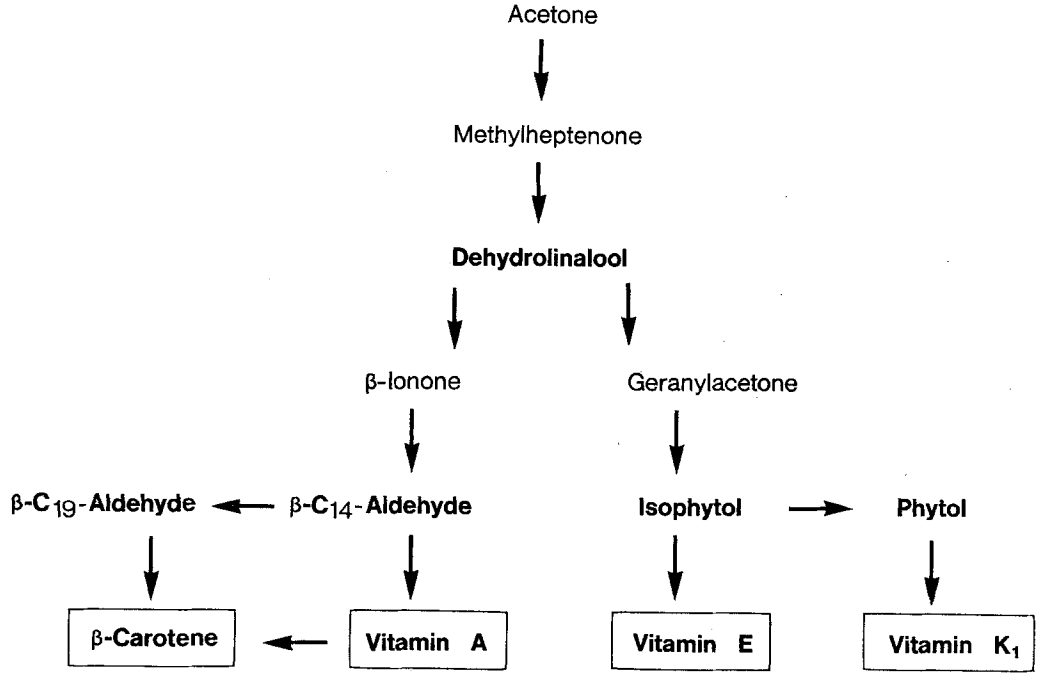


Fig. 45. The technical syntheses of fat-soluble vitamins and carotenoids.

The common intermediate dehydrolinalool can then be converted via geranylacetone, isophytol and phytol to the vitamins E and K_1 or via the intermediate β-ionone to vitamin A and to β-carotene.

It is certain that the interesting biological properties discovered for the vitamin D metabolites and the aromatic vitamin A-acids (retinoids) will also stimulate the research in the field of the other fat-soluble vitamins and carotenoids. Especially the biological functions of the latter are largely unknown.