

C₄₅- and C₅₀-carotenoids

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Abstract A review on the occurrence, structures, biosynthesis, functions and especially on the synthesis of optically active C₄₅- and C₅₀-carotenoids is given.

1. INTRODUCTION

Carotenoids are an important class of natural pigments which occur ubiquitously in Nature. Up to now more than 600 different carotenoids have been isolated from natural sources ^{1, 2} and it is estimated that 10⁸ tons of carotenoids are produced annually by Nature. Most of these compounds have been isolated from higher plants, animals and eubacteria. In addition, the occurrence of carotenoids in archaeobacteria, i.e. in *Halococcus* and *Halobacteria* has been reported. Furthermore, the presence of carotenoids in the serum of men is of utmost importance for health and this field has recently been subject to many investigations.

The majority of the carotenoids are C₄₀-compounds containing eight isoprenoid units (tetraterpenes) and all carotenoids may be formally derived from the acyclic C₄₀H₅₆ structure **1** (see Fig. 1) having a long central chain of conjugated double bonds.

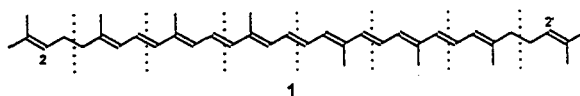


Fig. 1. Acyclic C₄₀H₅₆ structure 1

The importance of the carotenoids is mainly due to their different functions in biological systems. This includes the participation in photosynthesis, quenching of singlet oxygen and other reactive oxygenating species, antimutagenic activity, immunoenhancement, stimulation of inter-cellular communication and the provitamin A activity. Carotenoids e.g. β , β -carotene and canthaxanthin are produced on an industrial scale and as they are considered to be safe, they are currently used primarily as colorants in food products such as margarine.

2. C₄₅- AND C₅₀-CAROTENOIDS

2.1. Occurrence and Structures

In 1966, decaprenoxanthin (**16**), the first carotenoid containing more than 40 carbon atoms was isolated from *Flavobacterium dehydrogenans*³. Since then ca. 30 naturally occurring C₄₅- and C₅₀-carotenoids have been reported^{1, 2, 4}. These compounds have been mainly isolated from extremely halophilic bacteria (*Halobacteria*, *Halococcus*), which belong to the archaeobacteria, and from moderately halophilic bacteria and *Corynebacterium poinsettiae*, which belong to the eubacteria. As some of these compounds have not been characterized by modern spectroscopic methods, especially high resolution NMR-spectroscopy, the constitution of these natural products remained uncertain.

In the C₄₅- and C₅₀-carotenoids the additional C₅-unit is always attached to the C(2) and C(2') of the C₄₀-compound. These carbon atoms become asymmetric, and the stereochemistry remained unknown for a long time. According to their end group the C₄₅- and C₅₀-carotenoids can be divided into two groups, *acyclic* and *cyclic* compounds. The constitution of the acyclic end groups are shown in *Figure 2*.

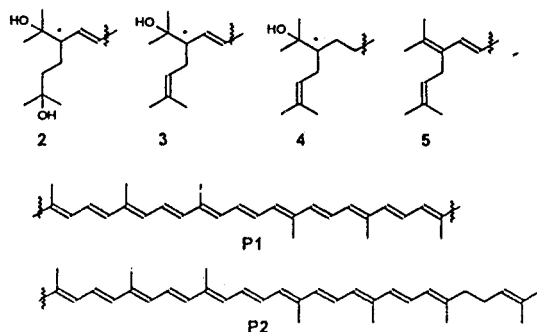


Fig. 2. Constitution of main *acyclic* end groups and polyene systems of *acyclic* C₄₅- and C₅₀-carotenoids

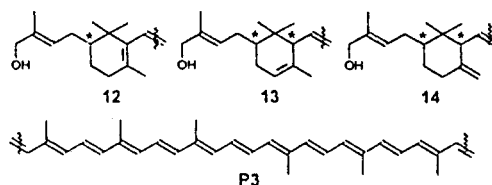


Fig. 3. Constitution of main *cyclic* end groups and polyene systems of *cyclic* C₄₅- and C₅₀-carotenoids

The most important representatives of this group are bacterioruberin **6** containing twice the end group **2** with the polyene chain **P1** (**6** = **2-P1-2**), monoanhydrobacterioruberin **7** (**2-P1-3**), bisanhydrobacterioruberin **8** (**3-P1-3**) and trisanhydrobacterioruberin **9** (**3-P1-5**). Furthermore, 3,4,3',4'-tetrahydrobis-anhydrobacterioruberin **10** (**4-P1-4**), with single bonds at the 3,4- and 3',4'-position respectively has been isolated. The most prominent C₄₅-carotenoid is 2-isopentenyl-3,4-dehydrorhodopin **11** (**3-P2**). In addition, the isolation of the mono- and diglucoside of bacterioruberin has been reported.

The main end groups of the *cyclic* C₄₅- and C₅₀-carotenoids are shown in *Figure 3*.

They differ in the position of the double bond in the ring system. Whereas in the β -end group **12** only one asymmetric C-atom is present, the ϵ -(**13**) and the γ -

end group (14) contain two asymmetric centers. The main natural cyclic C₅₀-carotenoids are C.p. 450 15 (12-P3-12) decaprenoxanthin 16 (13-P3-13) and sarcinaxanthin 17 (14-P3-14), together with the mono- and diglucosides of 16 and 17.

2.2. Biosynthesis

Based on labelling experiments, inhibition of the biosynthesis with nicotine, diphenylamines etc. and investigations of mutants ⁵, the biosynthetic pathway shown in Figure 4 for *acyclic* C₄₅- and C₅₀-carotenoids has been postulated.

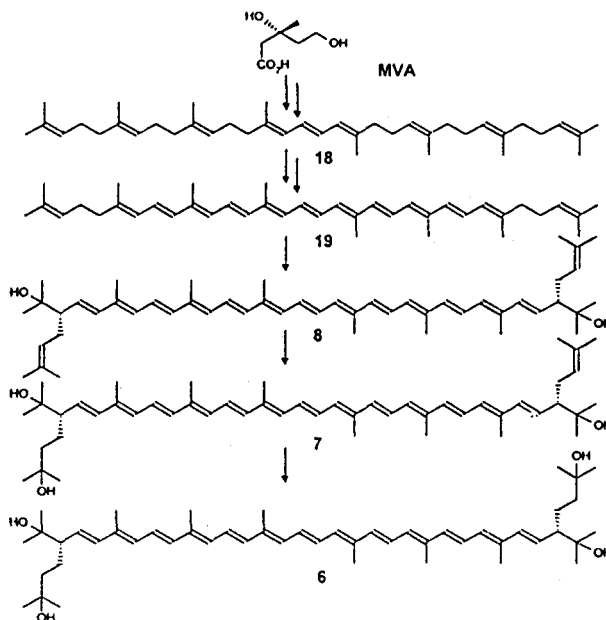


Fig. 4. Proposed biosynthesis of *acyclic* C₅₀-carotenoids

As in organisms which produce C₄₀-carotenoids as main pigments the biosynthesis of the C₄₅- and C₅₀-carotenoids starts with (3*R*)-mevalonic acid, which is converted via (all-*E*)-phytoene (18) and other intermediates to (all-*E*)-lycopene (19). Remarkable is the fact that all these intermediates possess the (all-*E*)-configuration, which is in contrast to higher plants and other bacteria where (15*Z*)-isomers have been reported. Afterwards lycopene (19) is prenylated at C(2) and C(2') to give the C₄₅- and C₅₀-skeleton respectively.

The prenylation is followed by the hydroxylation at the side chain to give finally (all-*E*)-bacterioruberin 6. Recently the influence of light, growth medium, salinity and the time of harvest on the carotenoid composition of *Halobacterium* sp. SB 183 was investigated ⁶. The total content of carotenoids is not significantly changed by increased light, but some new, yet unidentified, compounds are observed. Enhanced salinity (up to 35%) increases the total content of carotenoid, especially of bisanhydrobacterioruberin 8. Furthermore, it was shown that at an early stage of growth the portion of (*Z*)-isomers is enhanced. It must be pointed out that many questions remain open. Up to now, no experiments have been reported on the biosynthesis of *cyclic* C₅₀-carotenoids.

2.3. Function

In eucaryotic cells cholesterol and other sterols act as reinforcers of the cell membrane. It has been shown that in procaryonts and especially in archaeobacteria, hopanoids and polar carotenoids fulfill this function ^{7, 8}. For this function the length, the rigidity and the amphiphilic character of the molecule are essential. Whereas sterols and hopanoids act as "pegs", the carotenoids which are longer act as "rivets". This hypothesis is supported by experiments in which C₅₀-carotenoids have been incorporated into vesicles built up by phospholipids.

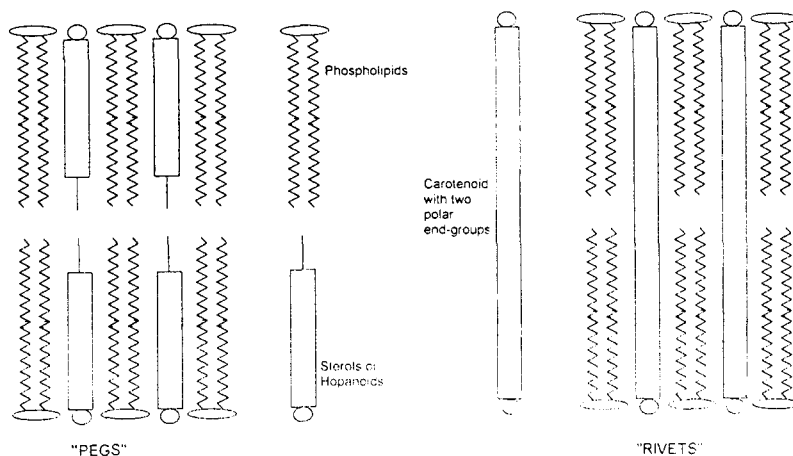


Fig. 5. Reinforcement of cell membranes

2.4. Synthesis

In view of the elucidation of the structure of the C₄₅- and C₅₀-carotenoids, especially their stereochemistry at C(2) and C(2') the synthesis of these compounds in optically active form was investigated. In the synthesis of C₄₀-carotenoids the strategies C₁₀ + C₂₀ + C'₁₀ = C₄₀ and C₁₅ + C₁₀ + C'₁₅ = C₄₀ are often used. In analogy the strategy C₁₅ + C₂₀ + C'₁₅ = C₅₀ and C₁₅ + C₃₀ = C₄₅ have been chosen for the acyclic compounds. For the synthesis of the cyclic C₅₀-carotenoids the strategy C₂₀ + C₁₀ + C₂₀ = C₅₀ was selected. For the olefination the *Wittig* reaction which is very often used in carotenoid synthesis was chosen.

2.4.1. Acyclic C₄₅- and C₅₀-carotenoids

The synthesis of the three optically active C₁₅- building blocks **27** - **29** starts with the methyl ester of (*R*)-3-hydroxybutyric acid (**20**) (Figure 6).

The key reaction is the α -alkylation of the dilithium derivative of the ester which gave **21** in good chemical (76%) and optical yield (> 90%) ⁹. This compound was transformed in 3 steps (reduction, benzoylation, oxidation; yield 52%) to **22** which was converted to the key intermediate **23** in four steps (acetalization, hydrolysis, oxidation, *Wittig* reaction; yield 52%). From this compound the end group (*S*)-**24** of (2*S*,2'*S*)-bacterioruberin **6** was synthesized in 7 steps in 21% yield. The C₁₅-building block (*S*)-**25**, the end group for the preparation of (2*S*,2'*S*)-monoanhydrobacterioruberin **7**, (2*S*,2'*S*)-bis-anhydrobacterioruberin **8**,

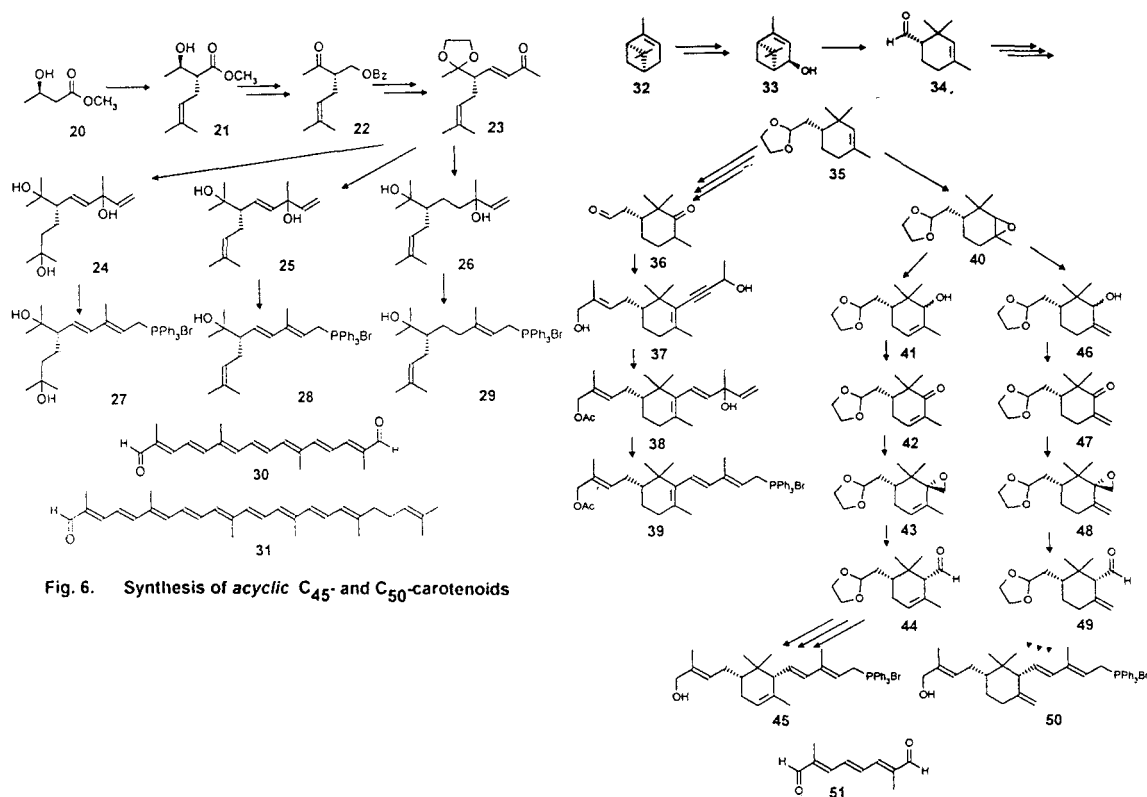


Fig. 6. Synthesis of acyclic C₄₅- and C₅₀-carotenoids

Fig. 7. Synthesis of cyclic C₅₀-carotenoids

(*S*)-trisanhydrobacterioruberin **9** and (*S*)-isopentenyl-3,4-dehydrorhodopin **11** was synthesized in five steps (yield 47%), and the third end group **26** which is characteristic for 3,4,3',4'-tetrahydrobisanhydrobacterioruberin **10** was formed in 56% yield (4 steps) **10**, **11**.

From the C₁₅-building blocks **24** - **26** the corresponding phosphonium salts **27** - **29** have been prepared (yields: 65%, 50%, 56%). By Wittig reactions of these phosphonium salts with the C₂₀-dialdehyde **30** (crocetindialdehyde) and the C₃₀-aldehyde **31** respectively the following carotenoids have been synthesized up to now **12**, **13**: (*all-E*, 2*S*,2'*S*)-bacterioruberin **6**, (*all-E*, 2*S*,2'*S*)-monoanhydrobacterioruberin **7**, (*all-E*, 2*S*,2'*S*)-bisanhydrobacterioruberin **8**, (*all-E*, *S*)-trisanhydrobacterioruberin **9** and (*S*)-2-isopentenyl-3,4-dehydrorhodopin **11**. By comparison of the chiroptical data of the natural and the synthetic compounds, the (2*S*)- and (2'*S*)-configuration of the natural products was established.

2.4.2. Cyclic C₅₀-carotenoids

For the synthesis of the C₂₀-end groups of cyclic C₅₀-carotenoids (-)-α-pinene (**32**) was chosen as starting material which was transformed to (-)-trans-verbenol (**33**) (yield 72%), (Figure 7). The pyrolysis gave the compound **34** with the desired stereochemistry at C(2) (yield 32%). Compound **35** which is the common precursor for all these end groups was received from **34** in five steps (yield 61%). In a total of 16 steps compound **34** was transformed via **36** → **37** to the desired building block **38** (total yield 12%), which gave the corresponding phosphonium salt **39** (38% yield). For the synthesis of the β- and γ-end group

which contain two asymmetric carbon atoms the epoxidation according to Corey ¹⁴ was selected, which has been previously used also in carotenoid syntheses ¹⁵. Epoxidation of **35** gave **40** (yield 88%). Opening of this epoxide with LiHNCH₂CH₂NH₂ gave **41**, a compound with the ε-end group (74%), whereas the reaction with LDA/EtMgBr gave **46** with the exocyclic end group. The further syntheses of **45** and **50** were carried out identically. Oxidation gave the ketone **42** and **47** respectively which were transformed to the epoxides **43** and **48**. Afterwards the epoxides were opened to the aldehydes **44** and **49**. From **44** the desired C₂₀-phosphonium salt **45** was synthesized (6 steps, yield 11%).

By Wittig reactions of the C₁₀-dialdehyde **51** with **39** and **45** respectively (all-*E*, 2*R*,2'*R*)-C.p. 450 **15** and (all-*E*, 2*R*,6*R*,2'*R*,6'*R*)-decaprenoxanthin **16** have been synthesized ¹⁶, ¹⁷. Comparison of the chiroptical data prove that the natural and synthetic compounds are identical. The total synthesis of optically active sarcinaxanthin **17** is in progress.

3. ACKNOWLEDGEMENTS

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