EVOLUTIONARY PERSPECTIVES OF ISOPRENOID CHEMISTRY

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ABSTRACT

Precambrian isoprenoid chemistry has been discerned from the information available on the distribution profile of isoprenoid structural types present in contemporary organisms. The taxonomic groupings known from the phylogenetic schema based on molecular data and the endosymbiotic origins of eukaryote organelles are taken into account in the discussion.

INTRODUCTION

ISOPRENE, the five-carbon hydrocarbon is a building block for several important natural products. The studies on the biochemistry of isoprenoids received impetus in 1956[1], when it was shown that mevalonic acid acts as a growth factor for *Lactobacillus acidophilus*. Since then, the intermediacy of mevalonic acid in the biosynthesis of numerous terpenoids and sterols has been established. To date, the distribution of isoprenoid structural types in a wide variety of organisms is known. However, this information is insufficient for deriving phylogeny although it can be effectively used as a taxonomic indicator. Consequently, the evolution of isoprenoid chemistry is best understood in the context of taxonomic groupings based on other criteria.

More recently, the evolutionary development of mevalonate pathway based on the comparative biochemistry has been outlined by Chapman and Ragan[2]. In our review and discussions, we have discussed the Precambrian isoprenoid chemistry against the background of the phylogenetic schema made on the sequences of proteins and nucleic acids[3,4]. We have also discussed the diversity spectrum of carotenoids in plastids taking into account both the algal phylogeny[5] and the endosymbiotic origins of plastids. A schematic representation of the taxonomic groupings relevant to our discussions along with the various probable symbiotic events are shown in figure 1. Our basic assumption is that the phylogenetic relationships of organisms shown in figure 1 is essentially correct and that the isoprenoid structural types of the Precambrian ancestors were not unlike those of their modern descendants.

ISOPRENOID STRUCTURAL TYPES IN PRIMITIVE PROKARYOTES

The first group of organisms that diverge from the prokaryote stem in figure 1 includes clostridia (fermentative obligate anaerobes), and *Chromatium* and *Chlorobium* (anaerobic photoautotrophs). It is generally believed that fermentative obligate anaerobes were the first organisms to appear on the earth[6]. Recent microfossil evidence indicating the presence of stromatolites in the 3400–3500 million year (m.y.) old Pilbara Block of Western Australia[7] suggests that anaerobic photoautotrophs arose early in prokaryote evolution.

It is of interest to note that squalene (C_{30}) has been identified in *Clostridium*[8]. In addition, menaquinone (isoprenoid quinone) is also present in some clostridial species[9]. Similarly, different isoprenoid types are also present in *Chromatium* (purple sulphur bacteria) and *Chlorobium* (green sulphur bacteria). The photosynthetic organisms possess bacteriochlorophyll (Bchl) and carotenoids (C_{40}) to harvest light and transfer the excitation energy to a reaction centre from which electrons flow through photosystem I either in a cyclic path or in a noncyclic path. During this process, ATP and reducing equivalents which are needed for biosynthetic reactions are generated. The purple sulphur bacteria contain two acylic carotenoids namely spirilloxanthin and rhodopinal along with an aromatic carotenoid, okenone[10]. Bacteriochlorophyll a which contains a phytyl

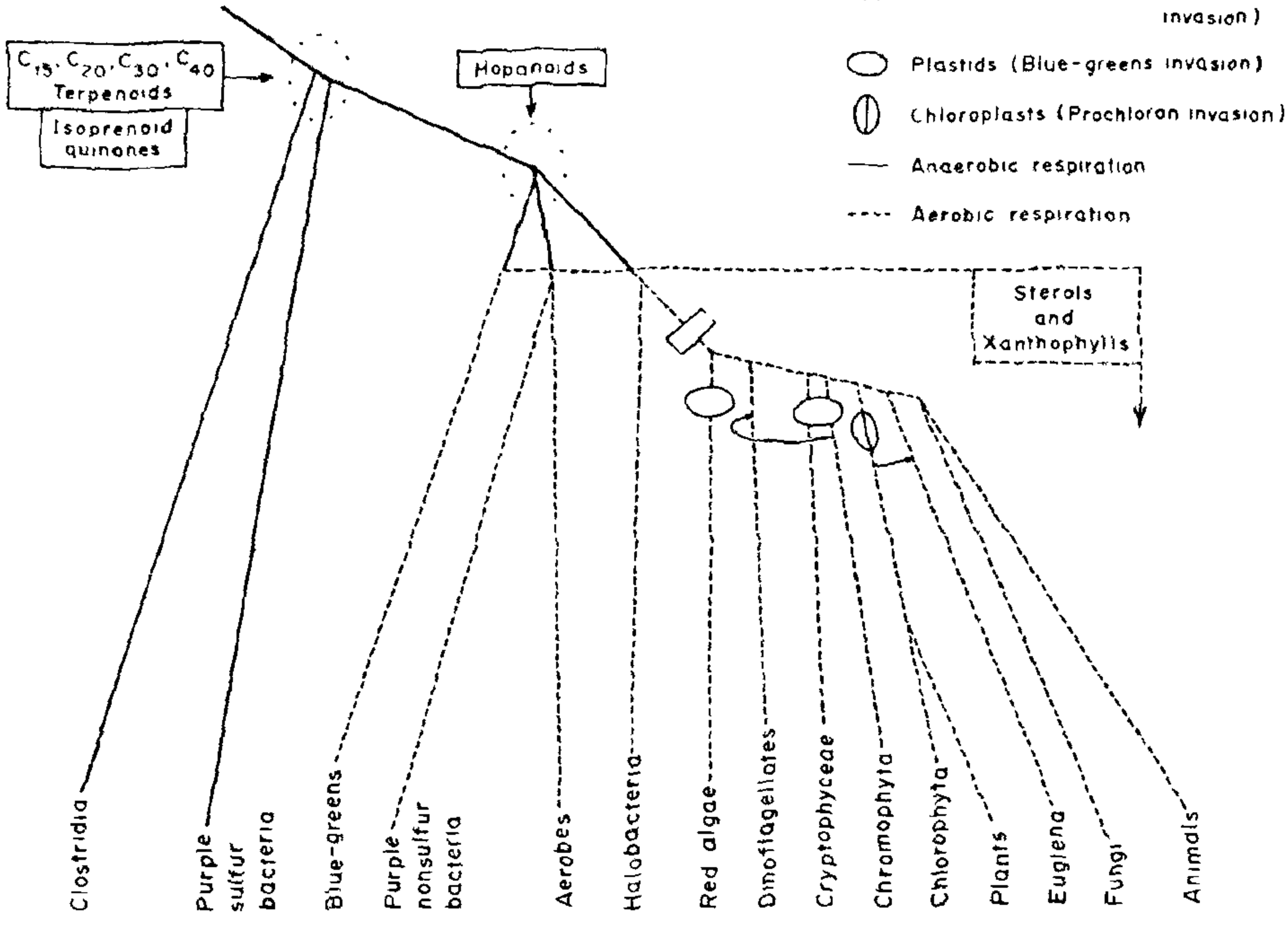


Figure 1. A schematic representation of taxonomic groupings including symbiotic associations in prokaryotes and protists based on molecular information (Ref. 3–5, 33–38).

(C_{20}) side chain is present in this group. On the other hand, green sulphur bacteria contain two aromatic carotenoids namely chlorobactene and *isorenieratene*[10], *and the* Bchl *c and* Bchl d present in this group contain farnesyl (C_{15}) side chains[5]. These gram-negative obligate anaerobes possess either menaquinone or ubiquinone as redox-carriers[11].

The obvious inference is that the mevalonate pathway leading to triterpenes and tetraterpenes was present in the first prokaryotes.

Mevalonic acid possesses six carbon atoms and it follows that one carbon of each mevalonic acid must be lost during its incorporation into triterpenes and tetraterpenes. The formation of mevalonic acid from acetate, the derivation of Δ^3-isopentenyl pyrophosphate (Δ^3-IPP) from mevalonic acid, the isomerisation of Δ^3-IPP to dimethylallyl pyrophosphate (DMAPP) and the subsequent formation of farnesyl pyrophosphate (FPP) *via* geranyl pyrophosphate (GPP) are now well known. The chemistry of these reactions has some interesting features. The condensation of Δ^3-IPP with DMAPP proceeds via the elimination of pyrophosphate grouping followed by the formation of *trans* GPP. The head-to-tail condensation which amounts to alkylation of olefin proceeds with complete inversion at the centre during the formation of a new C–C bond. This reaction has two stages: a *trans* addition followed by a *trans* elimination[12,13]. The trans GPP condenses with another molecule of Δ^3-IPP to give *trans* FPP (figure 2).

Squalene is known to arise by tail-to-tail condensation of two molecules of FPP. A fascinating feature of this condensation[14,15] is that squalene synthetase recognises the prochiral protons of tail and the coupling process is attended

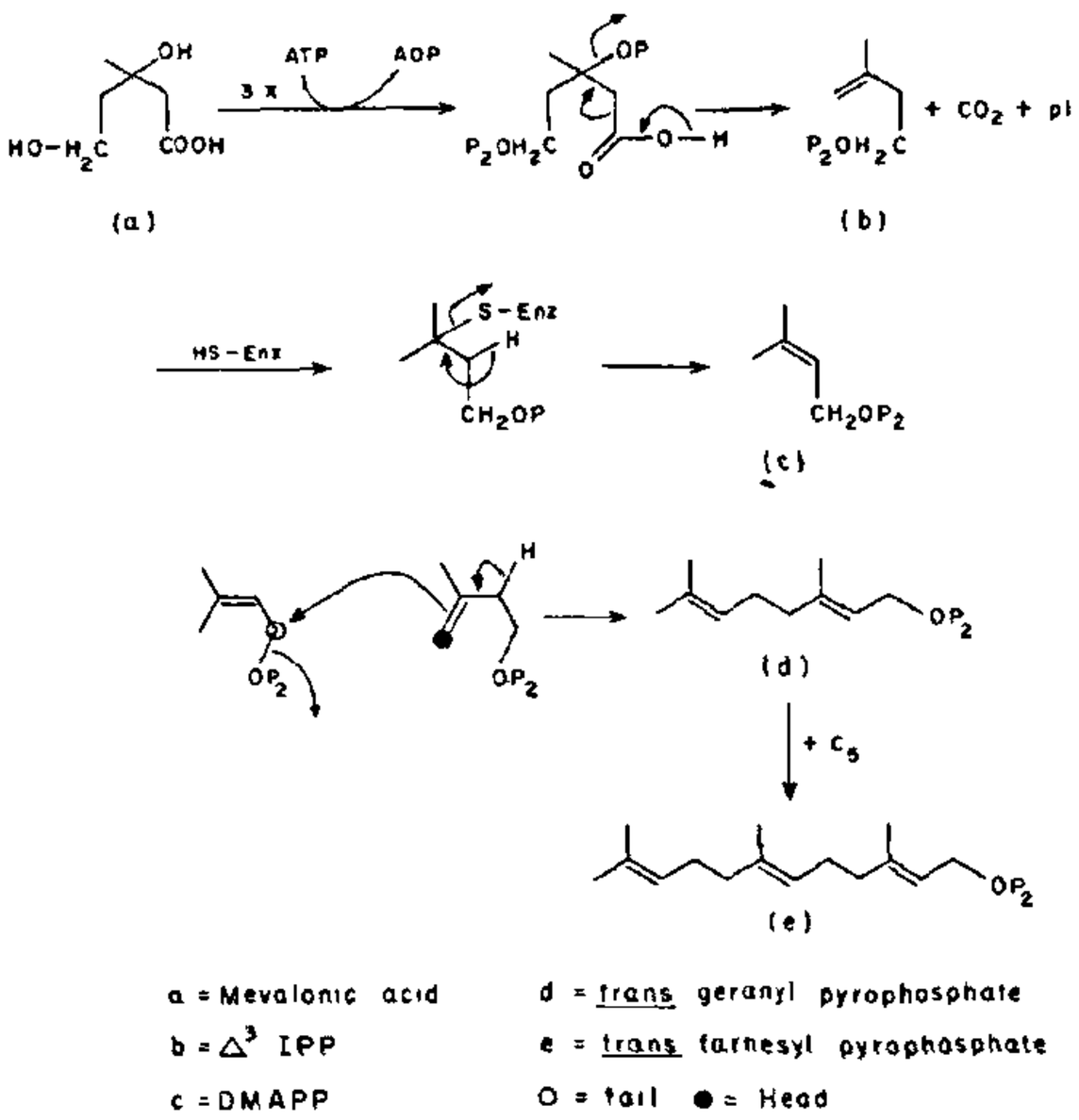


Figure 2. Basic steps in the biosynthesis of Δ^3-IPP and *trans* FPP.

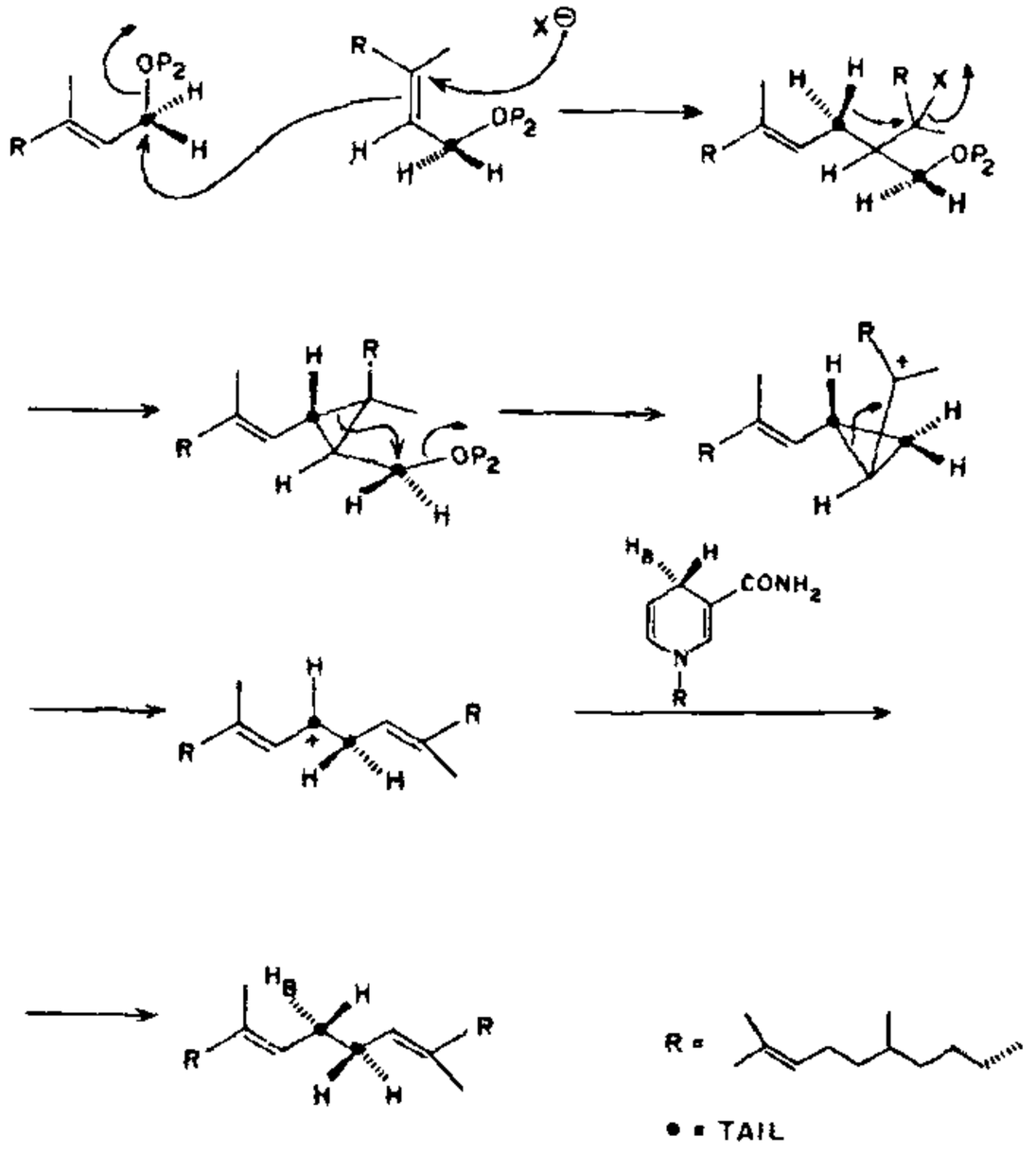


Figure 3. Features of tail-to-tail condensation of two molecules of FPP yielding squalene.

with the retention of the stereochemistry of the one unit while bringing about the highly specific inversion of the other (figure 3). In all six units of Δ^3-IPP are assembled with the loss of pyrophosphate groups to yield squalene, the C_{30} hydrocarbon.

The first step in the biosynthesis of carotenoids is the generation of C_{40} hydrocarbon phytoene which is formed by the reductive dimerisation (tail-tail) of geranyl-geranyl pyrophosphate (C_{20}). In this reaction, cyclopropylcarbinyl pyrophosphate which is an intermediate loses pyrophosphate group to form cyclopropylcarbinyl cation. The stereospecific loss of R-proton and S-proton from the cyclopropylcarbinyl cation yield all-*trans* phytoene and 15-*cis* phytoene respectively[16] (figure 4).

The all-*trans* phytoene is converted into lycopene by a series of desaturation reactions each of which forms a new double bond and brings the previously isolated double bonds into conjugation. The intermediates in the formation of lycopene are phytofluene, tetrahydrolycopene and neurosporene[16].

Lycopene can undergo hydration at C-1, 2. It can also undergo cyclization by the addition of the C-5, 6, double bond to the C-4, 2, double

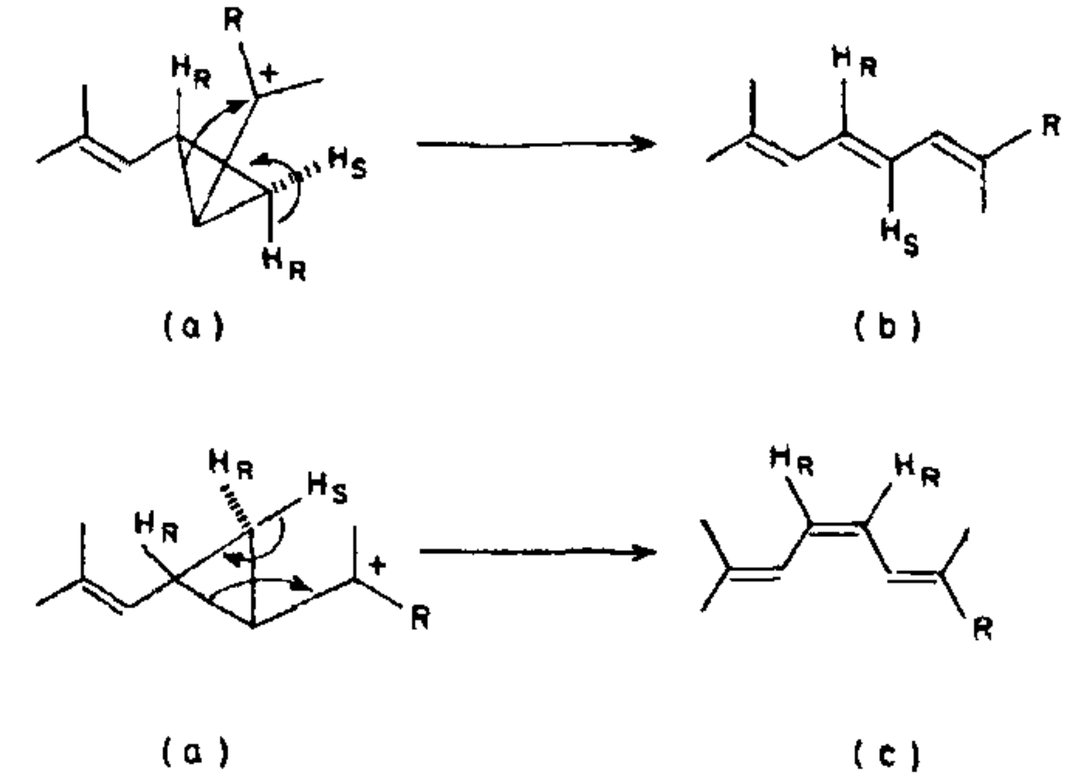


Figure 4. Mechanism of stabilisation of cyclopropylcarbinyl cation.

bond when a carbonium ion is formed which gets stabilised leading to different ring systems, β, γ, and ε. These alicyclic cartenoids can further undergo aromatisation. In this way, lycopene can give rise to alicyclic and aromatic carotenoids.

Menaquinone and ubiquinone which are derivatives of naphthaquinone and benzoquinone respectively are derived in part from the shikimate

pathway. In the biosynthesis of menaquinone[17], the seven carbon atoms of naphthaquinone are derived from shikimate, and the remaining three carbon atoms are derived from 2-ketoglutarate. The isoprenoid side chain is derived from Δ^3-IPP whereas the methyl group which gets added on subsequently is derived from S-adenosylmethionine. In the ubiquinone biosynthesis[18,19], shikimate gives rise to *p*-hydroxybenzoate which subsequently undergoes prenylation to give rise to 2-decaprenylphenol. This intermediate undergoes a series of oxidative reactions following which methyl groups from S-adenosyl methionine are introduced.

The distribution data of these isoprenoids indicate that members of gram-positive taxa in general have menaquinones whereas members of gram-negative family have either menaquinones or ubiquinones or both. However the majority of gram-negative obligate aerobes have ubiquinones[20].

HOPANOIDS ORIGINATED IN ANAEROBES

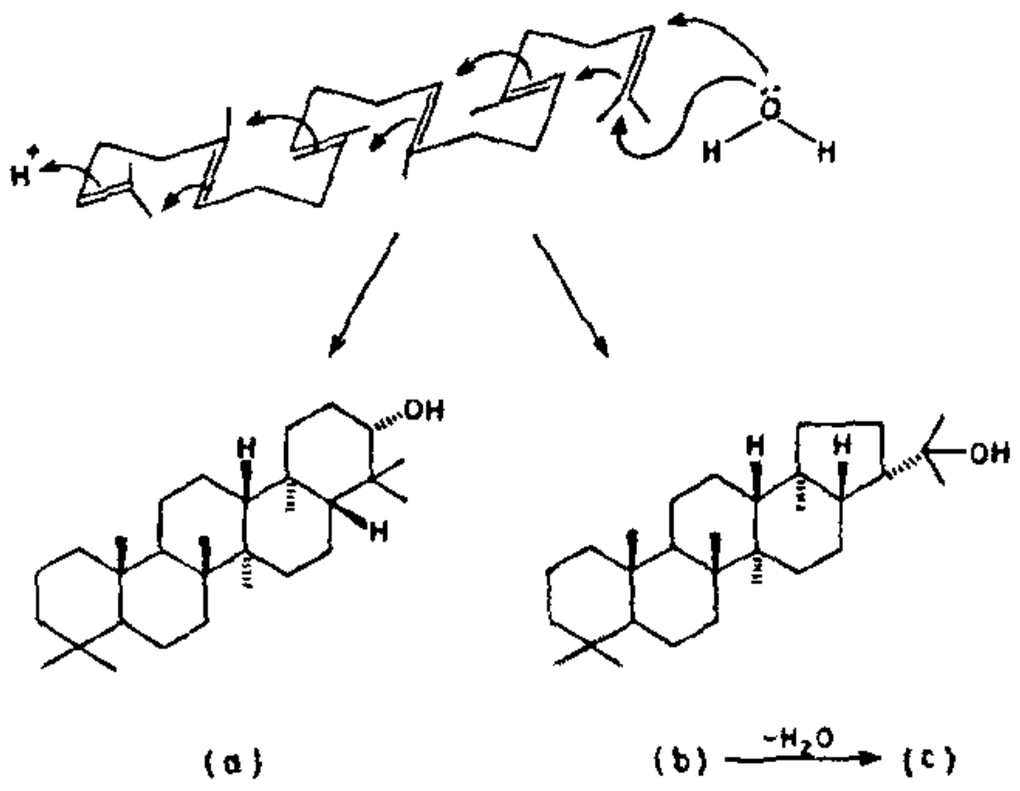
In figure 1, blue green alga (cyanobacteria) appears around the time of divergence of two major branches: one which gives rise to aerobes (*Rhodospirillaceae* and others) and the other referred to as 'eukaryote host'. Interestingly, the 'eukaryote host' also contains halobacteria, a member of a unique group of organisms called archaebacteria.

The appearance of blue-green algae on the primitive earth was a major event because of the fact that the atmosphere became oxygenic only due to the oxygen-releasing photosynthetic activity of the blue-greens. Knoll[21] suggests that the blue-greens arose around 2800–2900 m.y. ago; but these organisms began to proliferate later in the Proterozoic when the available area for their colonization greatly increased due to terminal cratonization. Initially, oxygen was used up by available oxygen sinks; and when all the available sinks were saturated, free oxygen began to accumulate in the atmosphere[21]. The geological, geochemical and paleobiological evidences suggest that the atmosphere became oxygenic around 2000 m.y. ago[22].

The point that is relevant here is that although the blue-greens are aerobes, their ancestors were anaerobes which eventually acquired aerobic respiration due to their own oxygen-releasing photosynthesis. This would suggest that the branch that gave rise to *Rhodospirillaceae* (purple non-sulphur bacteria) as well as the 'eukaryotic host' branch were initially anaerobes which eventually acquired aerobic respiration. It is possible that hopanoids originated in the ancestors of these three branches when sufficient oxygen was not present in the atmosphere to support respiration. This view is supported by the hopanoid distribution data[23] which show cyanobacteria and purple nonsulphur bacteria contain hopanoids while none of the obligate anaerobes do. Moreover, a number of aerobes (including some eukaryotes) contain hopanoids.

Squalene by virtue of its being able to take up various chair and boat conformations act as precursors of polycyclic systems. In the biosynthesis of hopanoids, the cyclization of squalene takes place in the absence of oxygen in its least constrained all chair conformation as shown in figure 5.

In their speculations on the molecular evolution of biomembranes, Rohmer *et al*[23] suggest



a = TETRAHYMENOL ; b = DIPLOPTEROL , c = DIPLOPTENE

Figure 5. Representative hopanoids in prokaryotes.

that the biosynthesis of hopanoids being more primitive than that of sterols, could have evolved towards the latter in oxygenic environment. They further suggest that hopanoids may play the same role in membranes of prokaryotes as sterols do in eukaryotes.

OXYGEN-RESPIRATION MODIFIED ISOPRENOID CHEMISTRY

Once oxygen-respiration became possible, it paved the way for the biosynthesis of oxygenated derivates of terpenoids. Squalene underwent cyclization giving rise to squalene-2,3-oxide which in turn gave rise to lanosterol and cycloartenol, the precursors of cholesterol and phytosterols (sterols of plants and algae) respectively. Similarly, the carotenoids gave rise to their oxygenated products, the xanthophylls.

The oxygen-releasing photosynthesis in blue-greens involves two photosystems which utilise chlorophyll *a*, phycobiliproteins and carotenoids as light-harvesting pigments, and water as an electron donor. The carotenoids present in the blue-greens are β-carotene, zeaxanthin, echinenone, myxoxanthophyll and oscillaxanthin[24]. The sterols in this group include cholesterol, 22-dehydrocholesterol, campesterol, sitosterol and stigmasterol[5]. The anaerobic photosynthesis of purple non-sulphur bacteria involves photosystem I which is similar to that in purple sulphur bacteria. Consequently, both these groups have spirilloxanthin and rhodopinal. However, purple nonsulphur bacteria, being aerobes have in addition, spheroidene and its oxygenated derivatives spheroidenone and hydroxyspheroidenone[10]. A notable feature is that sterols and carotenoids present in blue-green algae and purple nonsulphur bacteria are relatively simple.

It may be mentioned here that members of halophiles contain diphytanylglycerol diether[25], which together with diphytanyldiglycerol tetraethers constitute the unique archaebacterial lipids. The $C_4{}^0$-biphytane is derived *via* head to head linkage of two phytanes. In addition, halobacteria also contain normal C_{30} and C_{40} terpenoids as well as an unique bacteriorhodopsin[25].

Once aerobic-respiration had fully become established, complex cell types evolved eventually giving rise to eukaryotes. The microfossil evidence from the Becksprings Dolomite of Southern California suggests that the first eukaryotes arose around 1300 m.y.[26] ago. Similarly, the Bittersprings microflora from central Australia suggests that the primitive eukaryotes had become fully established by 900 m.y. ago[6]. With the appearance of obligate aerobes and eukaryotes, the sterols and xanthophylls also underwent further modifications giving rise to a wide variety of oxygenated derivatives. In general, the various steps in the biosynthesis of sterols from cycloartenol[27] include: (i) opening of the cyclopropane ring; (ii) demethylation at C-4 and C-14; (iii) conversion of a Δ^8-sterol into a Δ^7- or $\Delta^{5,7}$- or Δ^5-sterols; (iv) alkylation at C-24 and (v) insertion of a side chain double bond at C-22. Similarly, the derivations of xanthophylls include, among others, alicyclic xanthophylls, acetylenic xanthophylls and allenic xanthophylls.

DIVERSITY SPECTRUM OF TRITERPENES AND TETRATERPENES IN EUKARYOTES

There have been two views to explain the origin of eukaryotes from prokaryotes. One is that the eukaryotic organelles arose due to compartmentalization of DNA within a single line of prokaryotes[28] and the other is that they arose due to serial endosymbiosis among different prokaryotes[29]. Figure 1 supports the endosymbiotic theory and further indicates that the 'eukaryotic host' engulfed purple non-sulphur bacteria and blue-green alga which eventually became mitochondria and plastids respectively[3,4].

The biochemical phylogeny of algae and other protists[5] shows that red algae (*Rhodophyceae*) and cryptophycean algae (*Cryptophyceae*) arose early in eukaryote evolution (figure 1). We favour the view that dinoflagellates (*Dinophyceae*) also arose from the primitive eukaryotic stem. This view is primarily based on the fact that dino-

flagellates do not have the conventional nucleosome structure in their chromatin[30] and that they show primitive feature in their DNA in having 5-hydroxymethyl uracil[31].

A noteworthy event in the evolutionary history of eukaryotes is the divergence of two major lines: the chromophyte line (Chromophyta) and the chlorophyte line (Chlorophyta). The former is generally characterised by chlorophyll (Chl) a, Chl c_1 and Chl c_2 and the latter by Chl a and Chl b. In the chromophyte line, *Haptophyceae*, *Bacillariophyceae*, *Eustigmatophyceae*, *Xanthophyceae*, *Rhaphidophyceae*, *Pheophyceae* and *Crysophyceae* separate in that order. In the chlorophyte line, the order of separation is *Prasinophyceae*, *Chlorophyceae* and the higher plants. Euglenoids (*Euglenophyceae*), ciliates, animals and fungi arose after the divergence of chromophyte-chlorophyte series[5].

The red algae contain campasterol, sitosterol and stigmasterol along with cycloartenol and cholesterol. The cryptophycean algae contain stigmasterol and ergosterol[5]. These two groups contain relatively simple sterols but the sterol profile in dinoflagellates is unique. There is a dominance of 4-methyl sterols in this group and the characteristic derivative is dinosterol (4, 23, 24-trimethyl-5αH-cholest-22-en-β-ol[32]. However, most of the groups of chromophyte and chlorophyte lines contain, among other sterols, cycloartenol and cholesterol but lanosterol is not found in them. On the other hand, protozoans, fungi and animals contain, among others, cholesterol and lanosterol but cycloartenol is not present in these groups. Clearly, lanosterol and cycloartenol have followed distinct evolutionary paths in eukaryotes. Interestingly, *Euglena* is unique in having both cycloartenol and lanosterol[5].

The carotenoid distribution data in eukaryotic algae[24] shows taxonomically interesting patterns. The red algae contain α and β carotenes together with xanthophylls namely lutein and zeaxanthin. In cryptophycean algae, α-carotene predominates over β-carotene and an acetylenic xanthophyll alloxanthin is also present. Dinoflagellates contain two allenic xanthophylls namely peridinin and fucoxanthin. The former xanthophyll is characteristic of this group and is usually associated with small amounts of diadinoxanthin. In chromophyte line, in addition to β-carotene, two acetylenic xanthophylls namely diatoxanthin and diadinoxanthin are generally present. The characteristic pigment in *Chrysophyceae*, *Haptophyceae* and *Phaeophyceae* is fucoxanthin; and in *Xanthophyceae* and *Eustigmatophyceae*, the characteristic pigment is vaucheriaxanthin (allenic xanthophyll). In contrast, the chlorophyte line contains α-carotene, β-carotene, lutein epoxide, zeaxanthin, violoxanthin, and neoxanthin (allenic xanthophyll). *Euglena* is again unique in that although it resembles chlorophyte line in having Chl a and Chl b, it carries an acetylenic xanthophyll (diadinoxanthin) which is unknown in the chlorophyte line.

In figure 6, we have shown structures of representative carotenoids.

The diversity spectrum of carotenoids in eukaryotic algae can be discerned if we accept that

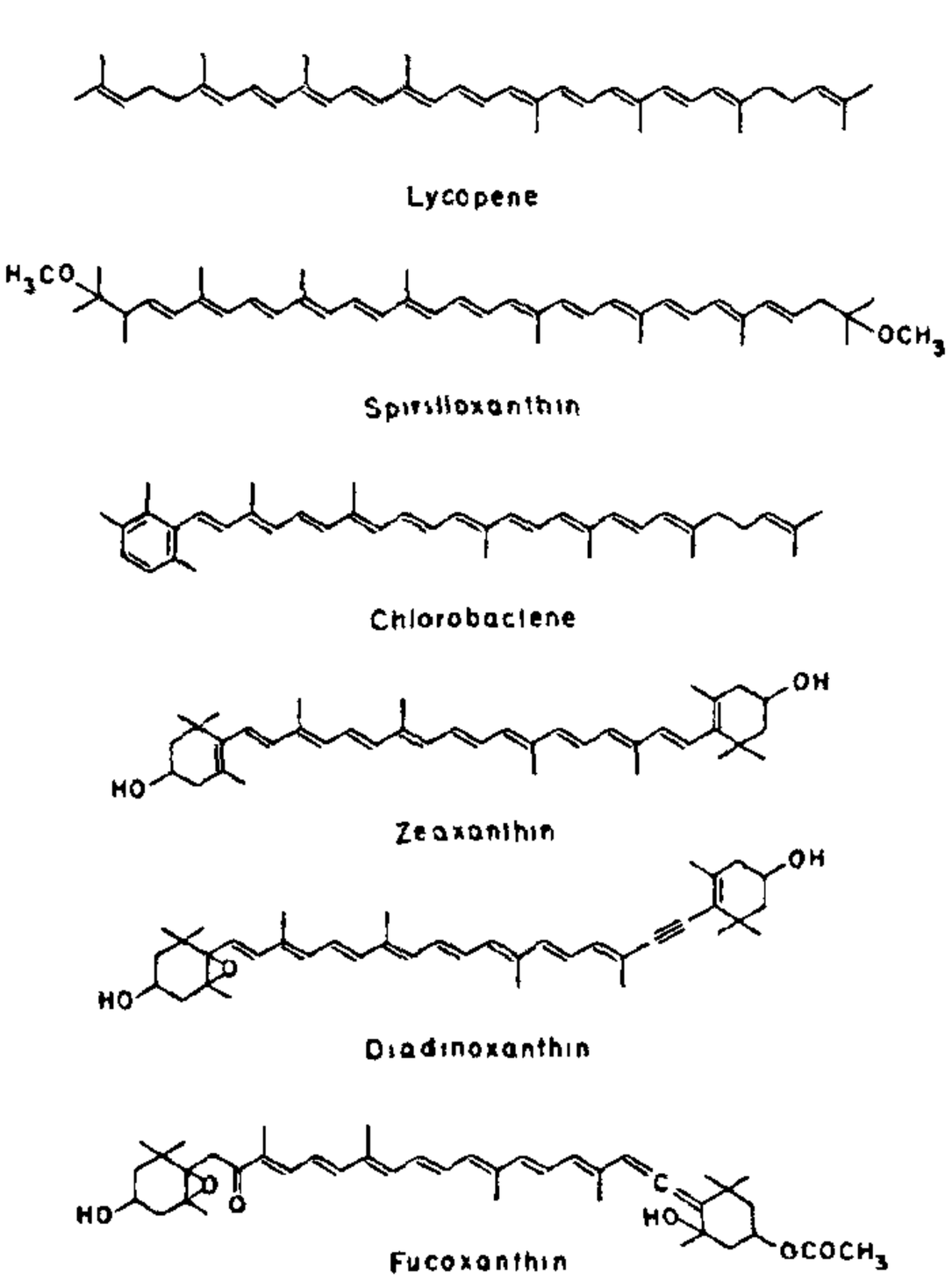


Figure 6. Representative carotenoids showing acyclic, aromatic, alicyclic, acetylenic and allenic structures.

plastids arose more than once in different algal lines. The amino acid sequence data of phycobiliproteins from blue-green algae, red algae and cryptophycean algae supports this view and further suggests that the plastids of red algae probably arose from the blue greens prior to those of cryptophycean algae[33]. Similarly, a comparative study of T_1-oligonucleotides of 16S rRNA from blue-greens and photosynthetic eukaryotes[34] suggests that plastids arose polyphyletically. A similar conclusion has also been drawn from the phylogenetic tree based on cycochrome C_6 sequences[35]. It is of interest to note that *Prochloron*, an oxygen-releasing photosynthetic prokaryote, contains Chl a and Chl b similar to those in green algae and higher plants. Based on the pigment composition, Stanier and Cohen-Bazire[36] suggest that *Prochloron* gave rise to the chloroplasts of green algae and higher plants.

The plastids of red algae contain xanthophylls similar to those in the blue-greens. The plastids of cryptophycean algae contain in addition an acetylenic xanthophyll. The chromophyte line which probably obtained its plastids from the blue greens has the capability to form both acetylenic and allenic xanthophylls. It has been suggested that the dinoflagellates obtained their plastids by the capture of a chromophycean alga[37]. This would explain the presence of acetylenic and allenic xanthophylls in dinoflagellates. It has also been suggested that the chloroplasts of euglenoids arose due to symbiosis between a green alga and a mastigophorean[38]. Here, it may be reasonable to assume that the endosymbiont was an ancestral green alga which possessed the ability to form acetylenic xanthophyll. This property has been lost in the present-day green algae although euglenoids have retained it.

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ANNOUNCEMENT

RAMESHWARDASJI BIRLA SMARAK KOSH AWARD

(vide *Curr. Sci.* 1984, p. 1282)

Mr. Shriyans Prasad Jain, Chairman of the Trust arranged a function on Monday the 28th January 1985 for the presentation of the Award at Bombay. Dr Raja Ramanna, Chairman of the Atomic Energy Commission, who was the chief guest, presented the award to Prof. G. N. Ramachandran. Dr B. B. Gaitonde, a member of the Award Jury, said Prof. Ramachandran belonged to that class of scientists whose contributions blazed new trials in science.

On the occasion, Prof. Ramachandran stressed the need to include biophysics as a separate discipline to every teaching and research institution at the master's degree level. Regretting that the progress to establishing full-fledged courses in biophysics was slow, he hoped the state of affairs would change in the next five years. He pointed out that during the last few years scientists had succeeded in imitating nature in the production of new living organisms. Biotechnology and bioengineering had turned into highly specialised experimental fields, covering aspects of biology that were almost unthought of 20 years ago.