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Bacterial Triterpenoids

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INTRODUCTION

Triterpenoids and their precursor isoprenoid hydrocarbons are among the oldest known and most ubiquitous chemicals on the earth, having been found in fossil remains from a variety of geological formations and sediments (45, 47, 81, 101, 105, 108). The reconstruction of biosynthetic pathways from such records indicates that the ability to synthesize isoprenoids such as pristane and phytane and, later, triterpenoids such as squalene and hopanes was present in non-photosynthetic bacteria before the evolution of chlorophyll biosynthesis. The occurrence of triterpenoids in bacteria, and especially in non-photosynthetic bacteria, is thus of interest from both evolutionary and functional aspects. This interest has been further stimulated by the discovery and identification of a number of unique triterpenoids in bacteria during the last decade and by conclusive proof that sterols can occur in procaryotes.

The purpose of this review is to present an overview of triterpenoid occurrence and function in bacteria. To accomplish this goal, the two major classes of known bacterial triterpenoids, i.e., acyclic and cyclic, are reviewed. Particular emphasis is focused on the acyclic triterpenoid carotenoids and the cyclic hopanoids since these two groups of compounds are rapidly emerging as major classes of bacterial natural products. This review does not address blue-green algal (cyanobacterial) triterpenoids since this subject has been addressed elsewhere (100, 101), nor are the general topics of microbial uptake and metabolism of sterols discussed except in those cases where these events involve a sterol synthesized de novo by the bacterium.

TRITERPENOID BIOSYNTHESIS

Before discussion of bacterial triterpenoids, a general description of their place in terpenoid biosynthesis is required. Figure 1 presents a general scheme of higher terpenoid formation (i.e., containing six or more isoprenoid [C_5] units and synthesized by head-to-head condensation of appropriate C_{15} or C_{20} isoprenoid precursors). Basically, two pathways are known. In the first, two molecules of farnesyl pyrophosphate (C_{15}) undergo reductive dimerization (after allylic rearrangement of one to nerolidol pyrophosphate) to form the first, acyclic, parent triterpenoid (C_{30}). Other acyclic and the cyclic triterpenoids are then derived from the initial condensation product. In all cases where the first C_{30} precursor has been identified, it has been found to be squalene.

In the second major pathway of terpenoid formation, two molecules of geranylgeranyl pyrophosphate (C_{20}) are dimerized to form the first, acyclic, parent tetraterpenoid (C_{40}), which then serves as the precursor for all other tetraterpenoids and tetraterpenoid derivatives. In all likelihood, this first acyclic tetraterpenoid is lycopersane, although questions still remain as to whether it is phytoene (32).

Of the two higher terpenoid classes, the triterpenoids contain the greatest variety of unique biosynthetic end products. These include acyclic derivatives resulting from either desaturation (triterpenoid carotenoids) or reduction (reduced squalenes) of the parent triterpenoid and cyclic derivatives such as the hopanoids and sterols. Although some exceptions do occur, such as the occurrence of the pentacyclic triterpenoid tetrahymanol in the protozoan *Tetrahymena pyriformis* (82), exclusive domains for various triterpenoids appear to exist. For example, with the exception of squalene and the immediate biosynthetic precursors of the cyclic triterpenoids (such as squalene-2,3-epoxide), the acyclic triterpenoids have been found exclusively in bacteria. Of the cyclic triterpenoids, pentacyclic compounds represent the major group found in bacteria. As discussed later, sterols, although present in some bacteria, are not a major product of bacterial triterpenoid biosynthesis. Although the natural occurrence of mono- and dicyclic triterpenoids has been reported (98, 100), neither class is yet known in bacteria.

The tetraterpenoids, although not as structurally diverse as the triterpenoids, nevertheless represent a major natural product class, ubiquitous throughout the plant and animal kingdoms. Tetraterpenoids are represented exclusively by the tetraterpenoid carotenoids (C_{40}-carotenoids) and their derivatives. Another group of tetraterpenoids has been reported, i.e., the C_{40}-ω,ω'-biphytanyl diols of thermoacidophilic bacteria (29, 35, 36, 76, 165). The biosynthesis of these compounds does not appear, however, to occur via geranylgeranyl pyrophosphate condensation, but rather by a bridging reaction between the two *O*-phytanyl side chains of a di-*O*-phytanyl glycerol molecule.

Only microbes and plants are known to synthesize the C_{40}-carotenoids. Animals (as well as microbes and plants) can, however, oxidatively degrade C_{40}-carotenoids to "apo-carotenoids" and can utilize the resulting products as essential

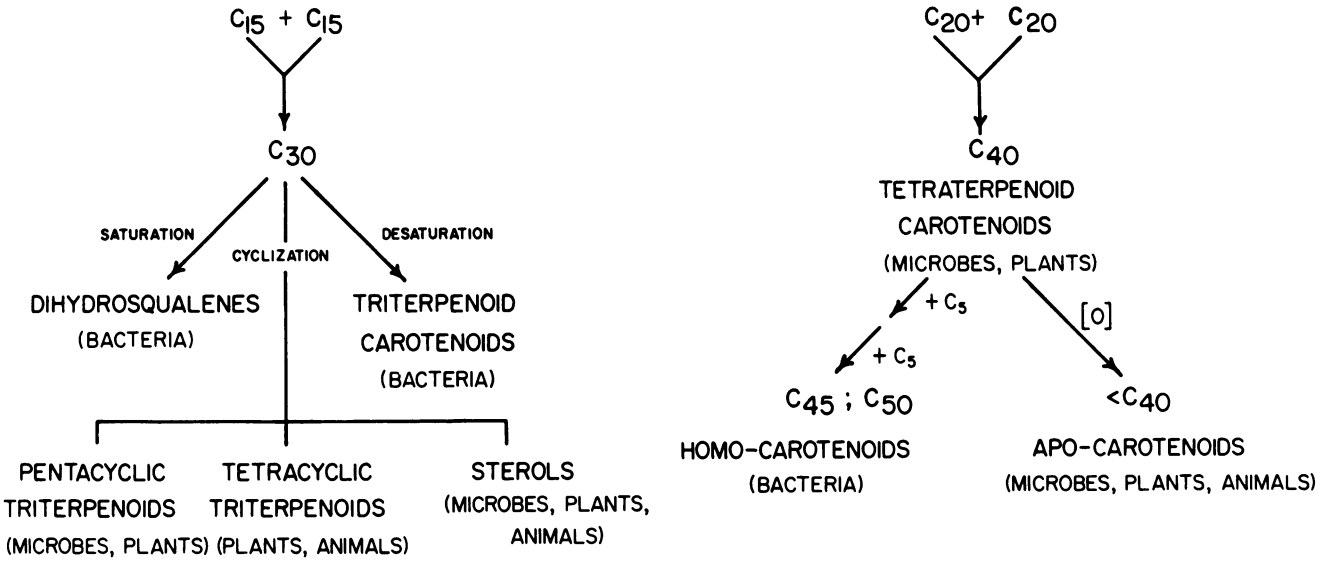


FIG. 1. End products of terpenoid biosynthesis. In general, acyclic triterpenoids other than squalene are found only in bacteria. Cyclized triterpenoids are found throughout the plant and animal kingdoms. Tetraterpenoids are represented exclusively by carotenoids and their derivatives.

nutrients, e.g., as sources of vitamin A. The biosynthesis of C_{45}- and C_{50}-carotenoids (the "homo-carotenoids") is known to occur only in non-photosynthetic bacteria (169) and involves addition of C_5-isoprenoid units onto one or both ends of a C_{40}-carotenoid. End-terminal cyclization of C_{40}-carotenoids (an event yet unknown for C_{30}-carotenoids) can be carried out by both microbes and plants to result in either mono- or bicyclic products (17). The formation of more highly cyclized tetraterpenoids is not known to occur.

BACTERIAL TRITERPENOIDS: OCCURRENCE

Table 1 summarizes the known occurrence of triterpenoids in bacteria. In using this table, however, it should be noted that in some cases the exact taxonomy of a bacterium is subject to further study and potential change. For example, *Sarcina litoralis* may be more accurately classified as a *Halococcus* sp. (22, 68) and is placed in the table accordingly. *Amoebobacter morrhuae* has been tentatively reclassified as a *Halobacterium* sp. (22), and the exact identity of *Pseudomonas rhodos* is unclear (and its guanine-plus-cytosine [G+C] content has not been reported). In addition, as with any evolving study area, additional bacterial triterpenoids will undoubtedly be discovered and potentially change the apparent trends discussed in this review.

With such limitations in mind, a number of general conclusions can be made from Table 1 and Fig. 2 concerning the occurrence of triterpenoids in bacteria. Squalene and a number of reduced squalenes have been found in widely divergent bacterial groups including chemoorganotrophs, chemolithotrophs and photoorganotrophs, with G+C contents in the bacteria ranging from 32 to 74 mol%. Thus, the ability to synthesize squalene does not appear to be unique to any specific bacterial group. Sterols and hopanoids, however, have thus far been found exclusively in aerobic or facultative anaerobic bacteria, both with G+C contents of >50 mol%. In contrast, the C_{30}-carotenoids have been found only in facultative anaerobic chemoorganotrophs with G+C contents of 30 to 40 mol%, such as *Streptococcus faecium* and *Staphylococcus aureus*. The possible exception to the latter generalization, *Pseudomonas rhodos*, requires further taxonomic characterization. No bacterium to date has been found to contain both sterols/hopanoids and C_{30}-carotenoids, an evolutionary trait which is discussed later. Only one organism, *Methylococcus capsulatus*, has been found to contain both hopanoids and sterols.

The amounts of triterpenoids occurring in bacteria vary from 0.0001 (the practical detection limit) to ≅0.6% by weight (i.e., 1 to 6,000 μg/g, dry weight). In order of lowest to highest content found to date, these are as follows: diapophytoene (0.02%), total C_{30}-carotenoids (0.05%), reduced squalenes (0.2%), sterols (0.2%), squalene (0.4%), and hopanoids (0.6%).

Preliminary claims have also been made that hopanoids exist in a variety of other bacteria including species of *Rhodopseudomonas, Methylomonas, Hyphomicrobium*, and *Nitrosomonas* (108, 119). These organisms are not listed in Table 1 since no data have yet been published identifying the hopanoids present. It is notable, however, that all of the bacteria reported by these authors as having hopanoids have G+C contents of 50 to 73 mol% and include aerobic and facultative anaerobic chemoorganotrophs, chemolithotrophs, and photoorganotrophs. Also, a number of bacteria are reported as not containing hopanoids. These include a number of bacteria with G+C contents of <50 mol%, i.e., *Streptococcus faecium, Proteus vulgaris, Bacillus subtilis, Clostridium paraputrificum*, and *Thermoplasma acidophilum*. Thus, these studies further support the conclusion that the occurrence of hopanoids/sterols or C_{30}-carotenoids in bacteria correlates directly with G+C content.

Acyclic Triterpenoids

The two major naturally occurring classes of acyclic triterpenoids, i.e., reduced squalenes and the C_{30}-carotenoids, occur almost exclusively in bacteria. One notable exception occurs, that of diapophytoene synthesis by the yeasts *Saccharomyces cerevisiae* and *Rhodotorula glutinis* (102, 148).

The discovery and characterization of the bacterial, acyclic triterpenoids is a recent event, best illustrated by the case of the C_{30}-carotenoids. Carotenoids have been long known to occur in bacteria and are noted for their structural diversity and unique biosynthetic pathways (for reviews, see

TABLE 1. Occurrence of triterpenoids and steroids in bacteria[a]

Organism[b]	Squalene	Reduced squalene(s)	Diapophytoene	Carotenoids	Hopanoids, steroids	Reference(s)
Non-photosynthetic, gram positive						
Staphylococcus aureus 209P	+ (0.003)	ND	+ (0.02)	C_{30} (0.03)	ND	32, 145–147, 156
S. aureus S41	+ (0.002)	NA	+ (0.004)	C_{30} (0.05)	NA	84
S. epidermidis	+	ND	NA	NA	NA	69
Streptococcus faecium UNH 564P	+ (0.4)	+ (0.2)	+ (0.0015)	C_{30} (0.003)	ND	150–153, 158
S. mutans	+	ND	NA	NA	NA	3
Corynebacterium xerosis	+	ND	NA	NA	NA	3
Bacterionema matruchotii	+	ND	NA	NA	NA	3
Propionibacterium acnes	+	ND	NA	NA	NA	3
Bacillus acidocaldarius	+	ND	ND	ND	Hopane, hop-22(29)ene, hop-17(21)ene; homohopanoids; glycosylated homohopanoid (0.3, total)	37, 39, 75, 112
Actinomyces bovis, *A. israelii*, *A. naeslundii*, *A. odontolyticus*, *A. viscosus*	+	ND	NA	NA	NA	3
Rothia dentocariosa	+	ND	NA	NA	NA	3
Sarcina litoralis	+	+	ND	C_{40}, C_{50} (0.05)	NA	68
Streptomyces olivaceus	NA	NA	NA	NA	Cholesterol (0.0035)	131
Cellulomonas dehydrogenans	+ (0.005)	ND	ND	C_{40}, C_{50} (0.6)	Cholesterol, β-sitosterol (0.05, total)	171
Non-photosynthetic, gram negative						
Escherichia coli	NA	NA	NA	NA	Cholesterol, campesterol, β-sitosterol, stigmasterol (0.0004, total)	132
Methanobacterium thermoautotrophicum	+ (0.26)	+ (0.35)	ND	NA	NA	166
Acetobacter rancens (*A. pasteurianum*), *A. xylinium*	NA	NA	NA	NA	Hopan-22-ol, hop-22(29)ene, homohopanoids	5, 43, 118, 121–123
Pseudomonas sp.	+	NA	NA	NA	Hopan-22-ol, hop-22(29)ene	97
Pseudomonas rhodos	NA	NA	+	C_{30}	NA	58, 59
Methylococcus capsulatus	+ (0.55)	ND	ND	ND	Hopan-22-ol, hop-22(29)ene and homohopanoids (0.3, total); 4,4-dimethyl-, 4α-methyl-, and 4-desmethylsterols (0.22, total); lanosterol, 3-epilanosterol, 24, 25-dihydrolanosterol, and others	12, 13, 15, 120
Methylbacterium organophilum	+ (0.07)	ND	ND	ND	Lanosta-8,22,24-trien-3β-ol and others (0.0003, total)	110
Azotobacter chroococcum	ND	NA	NA	NA	Lanosterol, 14-norlanosterol, 14-nordihydrolanosterol, Δ^7-ergosten-3β-ol, $\Delta^{7,22}$-ergostadien-3β-ol, ergosterol (0.01, total)	133
Halobacterium cutirubrum, *H. halobium*, *H. salinarium*	+ (0.1)	+ (0.06)	+	C_{40}, C_{50}	ND	61, 68, 72, 164
Amoebobacter morrhuae	+	+	ND	C_{40}, C_{50}	ND	68
Photosynthetic						
Rhodomicrobium vannielli	+	NA	NA	C_{40}	3β-Hydroxy-17-methylhopane, 29-hydroxy-3,17-dimethylhopane	47, 49
Rhodospirillum rubrum	+	NA	NA	C_{40}	NA	94

[a] Numbers in parentheses indicate an average, maximum content of the component(s) as percentage (dry weight) according to the studies cited. ND, Not detected during analysis (<0.0001%, by weight); NA, not analyzed.

[b] Listed in order of increasing G+C content within each group.

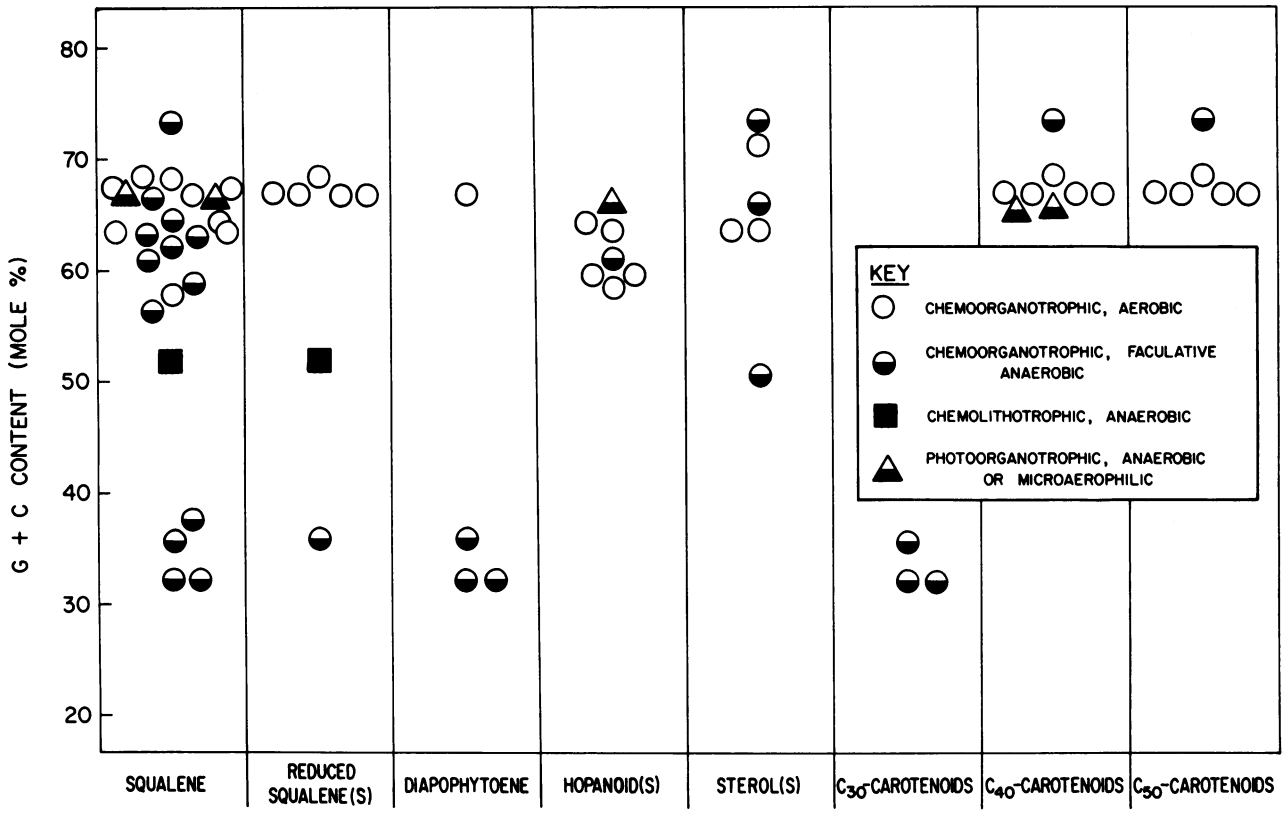


FIG. 2. Comparison of bacterial triterpenoid content and G+C content. The data presented are compiled from Table 1. G+C contents are from reference 22. Only bacteria known to contain triterpenoids are represented. If these bacteria also contain C_{40} or C_{50} carotenoids or both, it is so indicated.

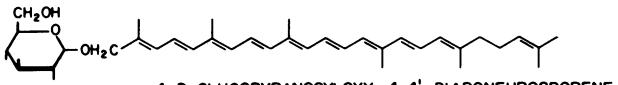
references 79, 168). It is interesting that in bacteria the final end product of carotenoid biosynthesis is usually a glycosylated carotenoid. A number of these are illustrated in Fig. 3. As discussed above, the C_{30}-carotenoids differ from the C_{40}-carotenoids by their biosynthetic route; i.e., the C_{30}-carotenoids are synthesized from C_{15}-isoprenoid precursors and the C_{40}- and C_{50}-cartenoids are synthesized from C_{20}-isoprenoid precursors.

The first C_{30}-carotenoid, the conjugated triene 4,4′-diapophytoene (also called bacterial phytoene and dehydrosqualene and given the "diapo" carotenoid nomenclature based on the number of carbon atoms in its structure), was reported in 1967, along with squalene, from *Staphylococcus aureus* 209P (145–147). Previous to these studies, it was assumed that the phytoene in *Staphylococcus aureus* was, similar to other carotenoid-producing organisms, C_{40}-phytoene (46, 143, 144), although no proper structural identification data were available to support this assumption. Later studies reported the occurrence of squalene, reduced squalenes (also called dihydrosqualenes), and diapophytoene in *Halobacterium cutirubrum* (61, 72, 164), although it was also shown, correctly, that the carotenoids present in this organism are C_{40}- and C_{50}-carotenoids (71, 72).

The first reports on the occurrence of a C_{30}-carotenoid series proceeding past diapophytoene resulted from studies on *Streptococcus faecium* UNH 564P (formerly *Enterococcus* sp. strain 564), a soil isolate (150, 151, 158). The carotenoid desaturation pathway in this organism proceeds from 15-*cis*-4,4′-diapophytoene to the conjugated nonaene, 4,4′-diaponeurosporene, and includes both a symmetrical and an unsymmetrical (with respect to chromophore) conjugated heptaene (Fig. 4). Thus, the C_{30}-carotene desaturation series in *Streptococcus faecium* directly parallels the established pathway for C_{40}-carotene desaturation (32). After synthesis of 4,4′-diaponeurosporene, a series of oxygenations and a glycosylation reaction lead to the predominant xanthophylls of the bacterium (152, 153), which vary in concentration depending on the amount of oxygen in and the nutritional state of the growth medium (154). We have also found both squalene and at least one reduced squalene (tetrahydrosqualene) in the organism (R. F. Taylor and B. H. Davies, unpublished data).

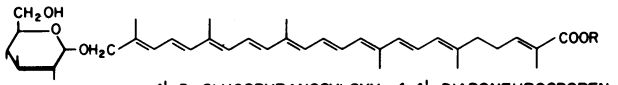
A major question concerning the C_{30}-carotenoids in *Streptococcus faecium* was whether, indeed, they did originate from C_{15}-isoprenoid precursors or, alternatively, by degradation of precursor C_{40}-carotenoids (formed, themselves, from C_{20}-isoprenoid precursors). Besides the nonpresence (based on a <0.1-μg/g [dry weight] detection limit) of C_{40}-carotenoids in the organism, other studies have provided conclusive proof that the C_{30}-carotenoids in *Streptococcus faecium* are synthesized from (C_{15}) farnesyl pyrophosphate and not (C_{20}) geranylgeranyl pyrophosphate. These include both physiochemical (mass spectrometric and nuclear magnetic resonance) and biosynthetic studies (33, 151, 155, 157). In the latter studies, comparison of radiolabeled farnesyl and geranylgeranyl pyrophosphate incorporation into the carotenoids synthesized in cell-free extracts of *Streptococcus faecium* conclusively showed that the C_{30}-carotenoids were synthesized from farnesyl pyrophosphate. These studies also showed that the glycosylation reaction to produce the end product of carotenoid biosynthesis in *Streptococcus faecium*, 4-D-glucopyranosyloxy-4,4′-diaponeurosporene

C_{30} CAROTENOIDS



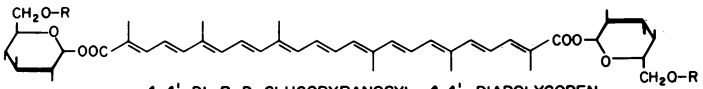
4-D-GLUCOPYRANOSYLOXY-4,4'-DIAPONEUROSPORENE

Streptococcus faecium



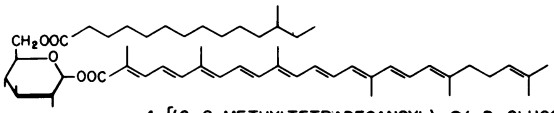
4'-D-GLUCOPYRANOSYLOXY-4,4'-DIAPONEUROSPOREN-4-OIC ACID ESTER

Staphylococcus aureus 209P



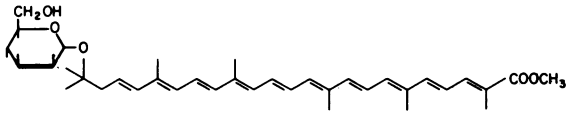
4,4'-DI-β-D-GLUCOPYRANOSYL-4,4'-DIAPOLYCOPEN-4,4'-DIOIC ACID DIESTER

Pseudomonas rhodos



4-[(6-O-METHYLTETRADECANOYL)-α-D-GLUCOPYRANOSYL]-4,4'-DIAPONEUROSPOREN-4-OIC ACID

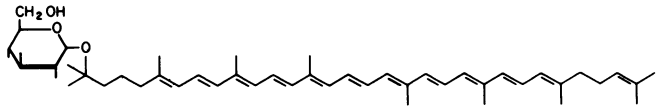
Staphylococcus aureus S41



1-D-MANNOSYLOXY-3,4-DIDENHYDRO-1,2-DIHYDRO-8'-APOLYCOPEN-8'-OIC ACID METHYL ESTER

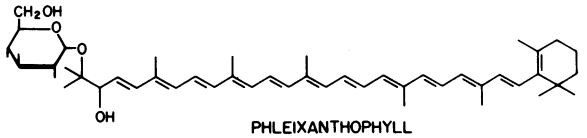
Halophilic coccus, strain SE 20-4

C_{40} CAROTENOIDS



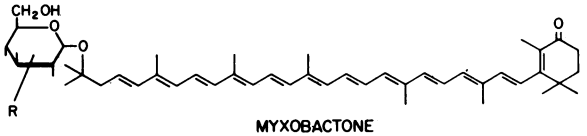
RHODOPIN GLUCOSIDE

Rhodopseudomonas acidophila



PHLEIXANTHOPHYLL

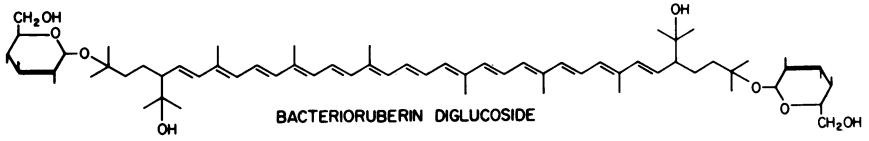
Mycobacterium phlei



MYXOBACTONE

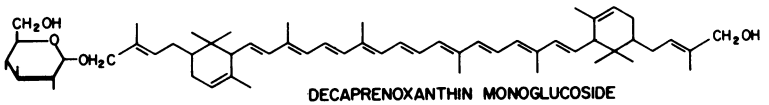
Myxococcus fulvus; Stigmatella aurantiaca

C_{50} CAROTENOIDS



BACTERIORUBERIN DIGLUCOSIDE

Halobacterium sp.



DECAPRENOXANTHIN MONOGLUCOSIDE

Corynebacterium sp.; *Arthrobacter* sp.

FIG. 3. Examples of end product bacterial carotenoids (R = fatty acyl group). The C_{30}-carotenoids have been found, to date, exclusively in non-photosynthetic bacteria. The structures of representative C_{40}- and C_{50}-carotenoids are shown for comparison purposes (1, 7, 48, 57, 59, 78, 84, 129, 152, 156, 170).

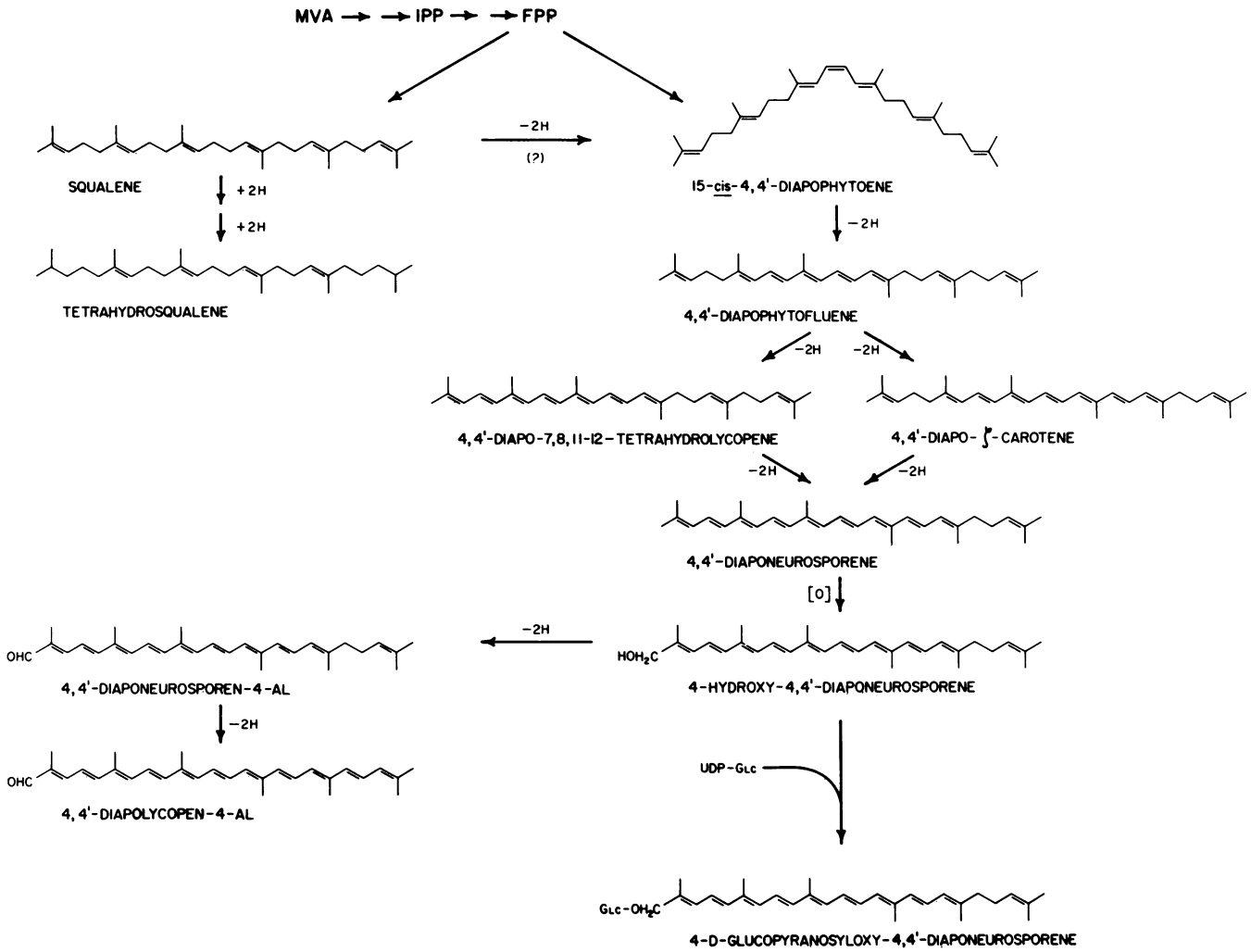


FIG. 4. Postulated biosynthesis of acyclic triterpenoids in *Streptococcus faecium* UNH 564P. Initially, mevalonic acid (MVA) is used to form isopentenyl pyrophosphate (IPP) which is polymerized to farnesyl pyrophosphate (FPP), the C_{15}-precursor to triterpenoids.

(Fig. 4), utilizes UDP-glucose. This was the first reported proof that glycosylated carotenoids are biosynthesized by the UDP-glycoside synthetic pathway which is common to a variety of other bacterial glycolipids such as lipopolysaccharides, teichoic acids, and diglucosyl diglycerides.

After our studies with *Streptococcus faecium*, we isolated and identified the carotenoids from *Staphylococcus aureus* 209P (32, 156). Once again, all of the carotenoids present in this organism are C_{30}-carotenoids (Fig. 5), differing from the *Streptococcus faecium* carotenoids primarily in the final end product xanthophylls. Of historical interest, the major xanthophyll found in *Staphylococcus aureus* under the growth conditions used, 4,4'-diaponeurosporen-4-oic acid, has spectral characteristics very similar to rubixanthin, the C_{40}-carotenoid claimed (incorrectly) by other workers to be the major carotenoid in the bacterium (46, 143, 144).

Since our characterization of the C_{30}-carotenoids in *Streptococcus faecium* and *Staphylococcus aureus*, at least two other studies have reported similar C_{30}-carotenoids in bacteria. In one, our studies with *Staphylococcus aureus* 209P were confirmed in *Staphylococcus aureus* S41, (84, 85), although the end product xanthophyll in strain S41 may be different (Fig. 3). In another study, a series of C_{30}-carotenoids has been found in both the wild type and mutants of *Pseudomonas rhodos* (58, 59). The *Pseudomonas rhodos* C_{30}-carotenoids differ from the *Streptococcus faecium* and *Staphylococcus aureus* pigments in that the carotene desaturation sequence proceeds completely to the conjugated undecaene, 4,4'-diapolycopene, before oxygenation and subsequent glycosylations occur (Fig. 6). The end product xanthophylls in this organism may be acylated with fatty acids at the glucose moieties.

The reduced squalenes represent the second group of acyclic bacterial triterpenoids and have been studied only on a limited basis to date. The occurrence of these compounds in bacteria appears, in most cases, limited to the di- and tetrahydro compounds. The notable exception is the methanogen *Methanobacterium thermoautotrophicum*, which contains all six possible reduced squalenes including the fully reduced squalene (squalane) (166).

Hopanoids and Sterols

The two primary types of cyclic triterpenoids or their derivatives occurring in bacteria are the hopanoids and sterols. In terms of amounts, the sterols occur at lower levels (Table 1) and may include complex mixtures of many different sterol types. The relatively low concentrations of sterols and hopanoids in bacteria together with problems associated with their extraction and identification were most likely responsible for the very firm conclusions accepted by

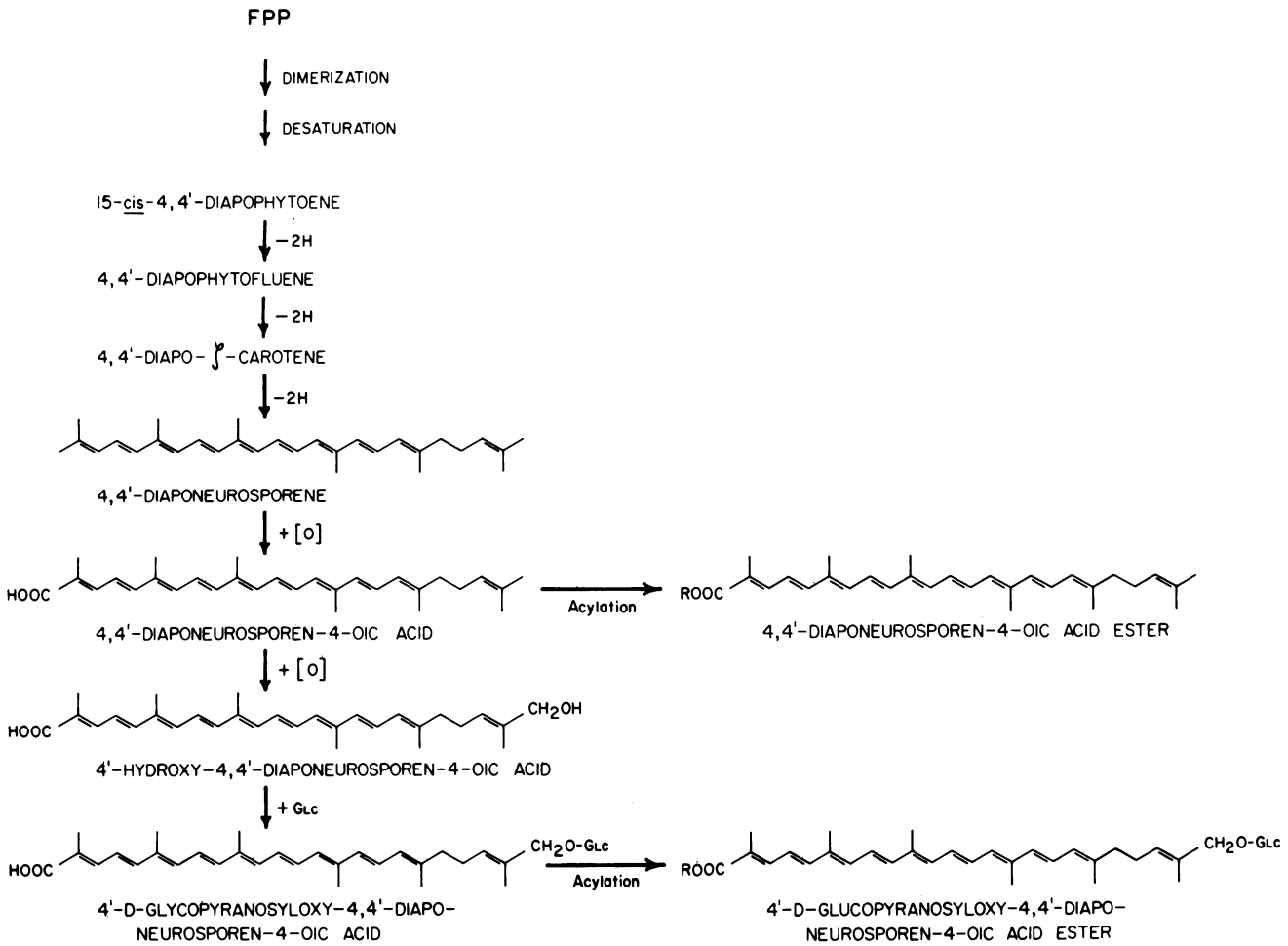


FIG. 5. Postulated biosynthesis of the C_{30}-carotenoids in *Staphylococcus aureus* 209P.

most authors up to approximately 15 years ago that cyclic triterpenoids did not exist in bacteria (e.g., see reference 8). More carefully controlled studies (notably in sterol-free media) and better analysis methods have, now, conclusively proved that sterols and hopanoids do occur in bacteria. Continuing studies will, in all likelihood, extend our knowledge of such occurrences.

Figure 7 presents a generalized biosynthetic scheme of cyclic triterpenoid biosynthesis in bacteria. Both the pentacyclic hopanoids and tetracyclic sterols originate from squalene. Hopanoids are synthesized by direct, nonoxidative cyclization of squalene (pathway 1, Fig. 7) without the initial formation of squalene-2,3-epoxide (pathway 3, Fig. 7). The latter compound is an intermediate in the formation of lanosterol, the first sterol derived from squalene. Most bacterial hopanoids include or are based in the first hopanoids derived from squalene (Table 1) including hopan-22-ol (diplopterol), hop-22(29)ene (diplopterie, hopene-b), and hop-17(21)ene (hopene-1). Two unique (to date) hopanoids have been found in the photosynthetic bacterium *Rhodomicrobium vanielli*, i.e., 3β-hydroxy-17-methyl-hopane and 29-hydroxy-3,17-dimethylhopane (49). It is interesting that the 3-hydroxy derivatives of hop-22(29)ene can be synthesized in cell-free extracts of *Acetobacter pasteurianum* and *Methylococcus capsulatus*, but only if exogenous squalene-2,3-epoxide is added to the systems (118, 120). Whereas squalene-2,3-epoxide is normally found in *Methylococcus capsulatus* (which also synthesizes sterols), the hydroxyhop-22(29)ene compounds are not found (i.e., are not detectable) under normal growth conditions, suggesting that increasing the concentration of the precursor compound modifies biosynthesis in the organism toward production of the two hydroxyhopanoids.

Hopanoids may also act as precursors for the homohopanoids, i.e., hopanoids containing more than 30 carbon atoms (pathway 2, Fig. 7). A number of such homohopanoids, characterized by high hydroxyl group substitution in added carbon chain end groups, have been found in bacteria (Fig. 7). It is assumed that such homohopanoids are synthesized by the addition of the C_5 or C_6 side chain to the parent hopanoid (43). Such an addition would appear to be analogous to the biosynthesis of C_{45}- and C_{50}-carotenoids from C_{40}-carotenoid precursors. However, the linear carbon chain nature of the end groups in homohopanoids indicates that their precursor(s) and mechanism of addition differ from the case of the homocarotenoids, where C_5-isoprenoid units are the end group precursors.

The homohopanoids themselves may be further modified by glycosylation and acylation. For example, an *N*-acylglucosamine derivative of a C_{35}-homohopanoid [itself derived from hop-22(29)ene] has been characterized in *Bacillus acidocaldarius* (75, 112). Figure 8 presents the postulated biosynthetic scheme for this hopanoid glycolipid, which is the most complex hopanoid reported to date and which may represent up to 25% of the glycolipid fraction of the cells (75, 77). It is interesting to compare the similarity of this glycosy-

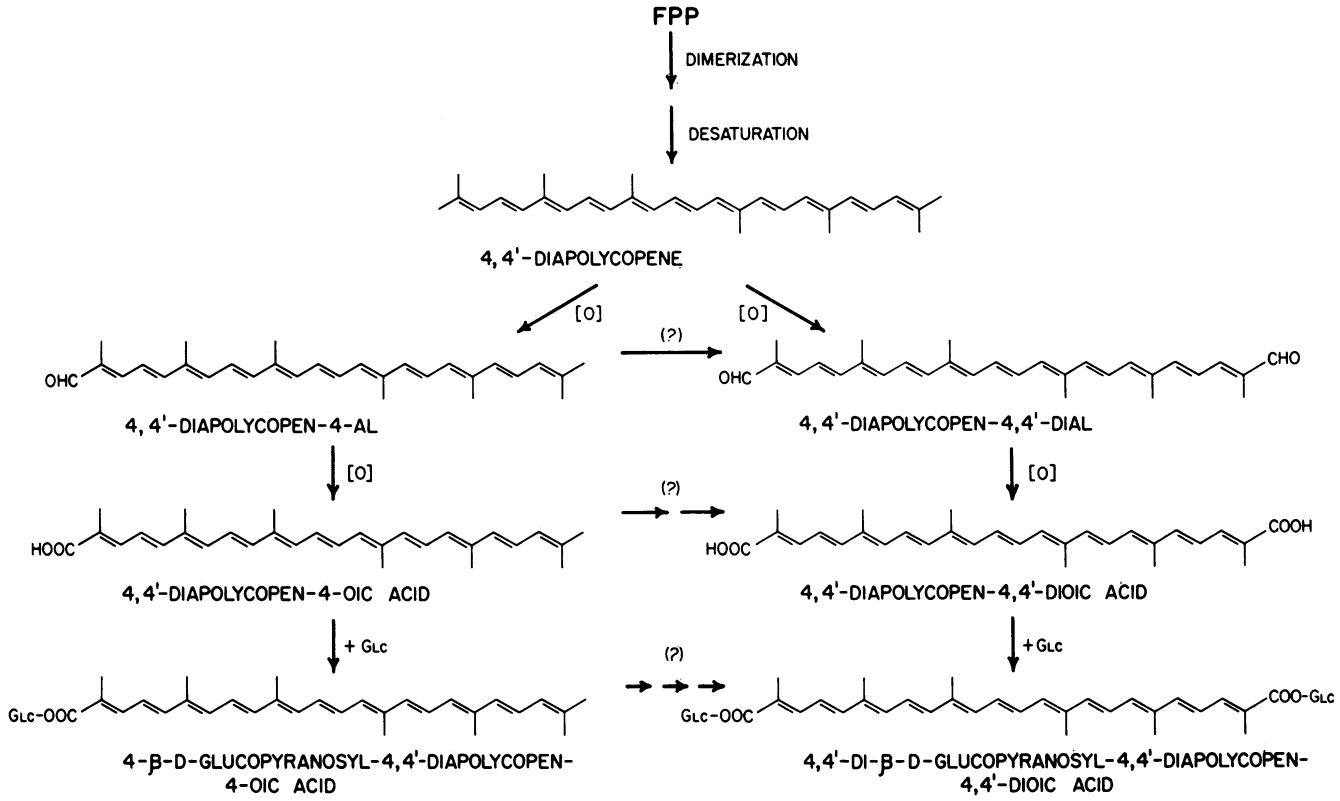


FIG. 6. Postulated biosynthesis of the C_{30}-carotenoids in *Pseudomonas rhodos*.

lated and acylated hopanoid with the end products of C_{30}-carotenoid biosynthesis such as those present in *Staphylococcus aureus* (Fig. 5) which are also glycosylated and acylated. This similarity in structure may suggest a common similarity in function (see below).

Sterol biosynthesis in bacteria is assumed to proceed via squalene-2,3-epoxide as an intermediate in a manner similar to sterol biosynthesis in eucaryotes. In most cases, the sterols found in bacteria are the same as those found in higher organisms, e.g., lanosterol, cholesterol, ergosterol, etc. In one case, however, that of *Methylococcus capsulatus*, besides the common sterols a unique series of 4α-methyl-, 4-desmethyl-, and 4,4-dimethylsterols are present (13, 15). The presence of hopanoids, unique sterols, and common sterols in *Methylococcus capsulatus*, a combination occurrence unknown in any other bacterium, may place this organism in a unique biosynthetic and evolutionary position between procaryotes and eucaryotes.

EVOLUTION AND BIOSYNTHESIS OF BACTERIAL TRITERPENOIDS

The placement of the bacterial triterpenoids into an evolutionary perspective can be considered from a combined genetic/biosynthetic viewpoint.

Reference has already been made to the apparent correlation between the type of triterpenoid in a bacterium and its G+C content (Fig. 2). It has been postulated that increasing G+C content reflects evolutionary pressures induced by environments containing increasing amounts of UV and visible light, i.e., the increasing amounts of sunlight which reached the earth's surface as its atmosphere changed from anaerobic to aerobic (135). This increased presence of light and oxygen, or a combination of the two, provided a destabilizing influence on anaerobic organisms, especially at their cell membrane (their first point of contact with the environment) and in their nucleic acids (due to the susceptibility of bases such as thymine to UV-induced dimerization). Thus, the response of primitive bacteria to such environments would be expected to include changes in their genetic material or their physical structure or both.

The evolution of bacterial triterpenoids, which are postulated as having their primary function in membranes (see below), appears to parallel the expected evolution of higher G+C contents. This is best illustrated by the evolution of cyclic triterpenoids in bacteria. For example, cyclic triterpenoids are not known to occur in bacteria with G+C contents of <50 mol%. The acyclic triterpenoids which do occur in such bacteria, e.g., the C_{30}-carotenoids, may function as membrane stabilization/protective agents, as will be discussed later.

A major evolutionary advancement in bacterial triterpenoid biosynthesis was the evolution of squalene cyclase(s). The cyclization of squalene and the biosynthetic steps leading to the Δ5-sterols (such as cholesterol) are now considered a natural, evolutionary development with final, full expression in eucaryotes (100, 101). Bacterial cyclization of squalene is, however, characterized by a more primitive, less substrate-specific enzyme(s) than that found in blue-green algae and eucaryotes. For example, when *Acetobacter pasteurianum* (G+C content, ≅58 mol%), which normally synthesizes hopanoids (Table 1), was provided, in a cell-free system, with squalene-2,3-epoxide, it produced 3-hydroxyhopanoids by cyclization of the epoxide substrate. The 3-hydroxyhopanoids and squalene-2,3-epoxide are not detectable in the bacterium under normal growth conditions (5, 118). In *Methylococcus capsulatus* (G+C content, ≅62

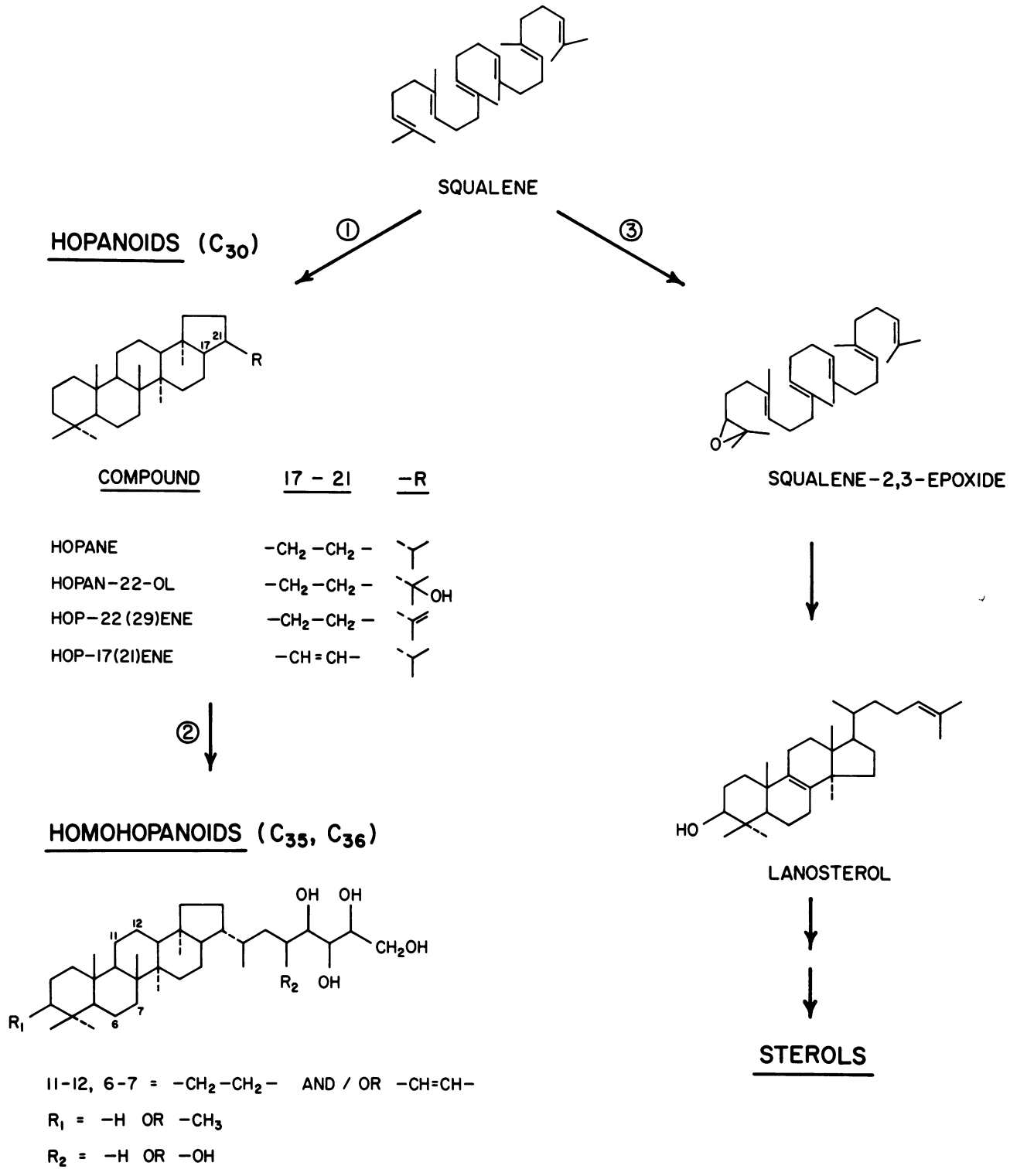


FIG. 7. Biosynthetic routes for cyclic triterpenoid formation in bacteria leading to either the pentacyclic hopanoids or tetracyclic sterols.

mol%), two squalene cyclases have been reported, one cyclizing squalene or its epoxide to hopanoids (similar to the case of *Acetobacter pasteurianum*) and the other cyclizing squalene epoxide (which is normally present in the organism) to lanosterol or 3-epilanosterol (119, 120). The synthesis of the latter lanosterol epimer together with the ability of cell-free extracts of the organism, when provided with excess squalene-2,3-epoxide, to synthesize 3-hydroxyhopanoids (not normally found in the organism) again illustrate the non-specificity of the cyclase(s) present. These examples thus contrast with the very specific squalene cyclase in eucaryotes which acts specifically on the 3(S) enantiomer of squalene epoxide and not on the 3(R) epoxide enantiomer or on squalene (9, 177).

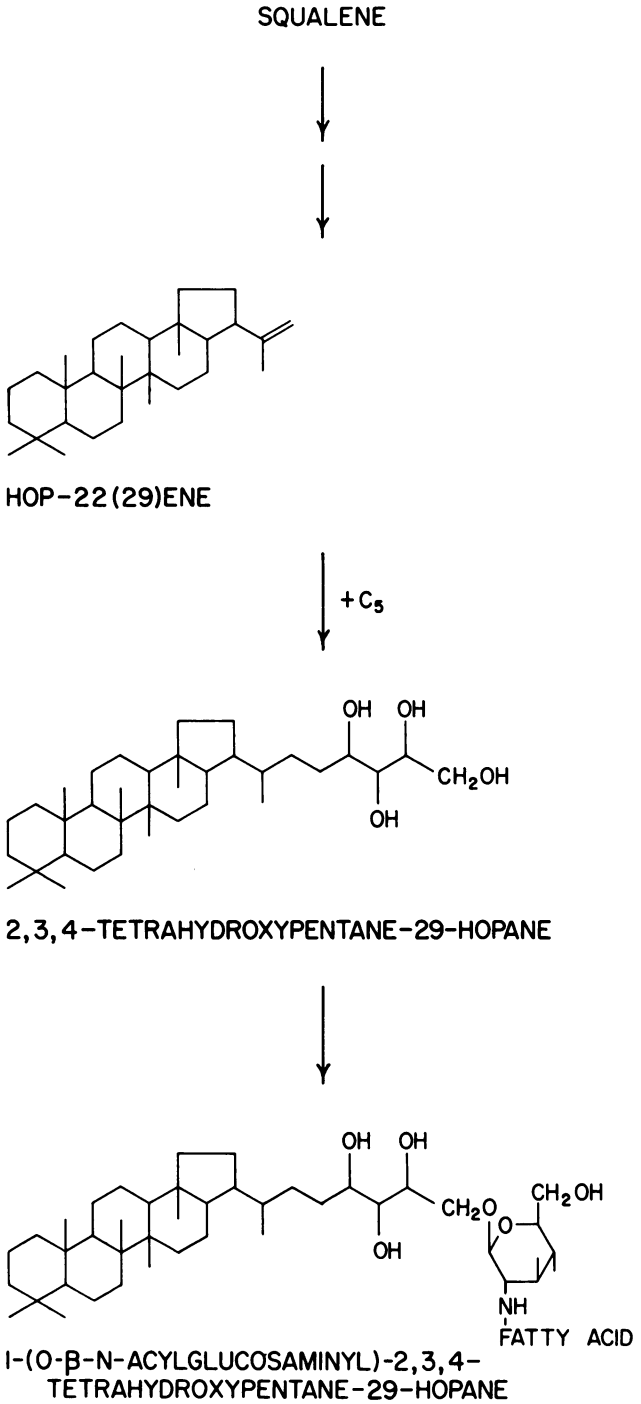


FIG. 8. Postulated biosynthesis of the hopanoid glycolipid present in *Bacillus acidocaldarius*.

Methylococcus capsulatus is also unique among bacteria examined to date in its ability to carry out sterol biosynthesis only to 4α-methylsterols, apparently unable to carry out the last demethylation leading to the biosynthesis of more highly evolved sterols. Other bacteria, however, can proceed past this step in sterol biosynthesis, including, for example, *Azotobacter chroococcum* (G+C content, ≅66 mol%), *Streptomyces olivaceus* (G+C content, ≅70 mol%), and *Cellulomonas dehydrogenans* (G+C content, ≅73 mol%). The apparent trend seen of increasing G+C content and more evolved sterol biosynthesis meets its exception in *Escherichia coli* (G+C content, ≅50 mol%). Notably, in those bacteria which can form higher sterols, the levels produced are much lower than those found in eucaryotes. Only the methylsterols of *Methylococcus capsulatus* are produced at levels comparable to sterols in eucaryotes. Thus, whereas various bacteria contain, in sum, all of the evolutionary biosynthetic steps leading to higher sterols, such pathways have not come to dominance in bacteria. This is in sharp contrast to the apparent absolute requirement in all eucaryotes for a substance represented by cholesterol or its derivatives (99). Apparently, the triterpenoids produced by bacteria satisfactorily meet their needs for such molecules in their normal cellular activities. One notable exception is the requirement for sterols by species of *Mycoplasma* (140) which is met by direct uptake from their growth medium. Questions also remain about those bacteria containing neither sterols nor triterpenoids and the identity of cellular components meeting their needs for the functions (especially in the cell membrane) apparently provided by sterols or triterpenoids.

The evolution of bacterial triterpenoid biosynthesis also correlates with the evolution of the earth's atmosphere from anaerobic to aerobic. The biosynthesis of both the acyclic triterpenoids (reduced squalenes and the C_{30}-carotenoids) and the cyclic hopanoids can proceed in the absence of molecular oxygen. This includes the introduction of oxygen-containing groups, such as hydroxyl groups, into the triterpenoids. Such reactions can proceed anaerobically, presumably by hydration reactions (17, 119). Thus, the early ancestors of modern day bacteria could well have evolved the triterpenoids known today under anaerobic or semianaerobic conditions.

FUNCTION OF BACTERIAL TRITERPENOIDS

Membrane Structure

Any discussion of triterpenoid function must include consideration of their structural and chemical properties. There is little doubt that the predominantly lipophilic bacterial triterpenoids are associated primarily with the bacterial cell membrane. This hydrophobicity is not limiting, however, since it decreases as the primary end products of bacterial triterpenoid biosynthesis are reached, i.e., C_{30}-xanthophylls and carotenoid glycosides (Fig. 3 to 6), oxygenated hopanoids and homohopanoids (Fig. 7), and the known hopanoid glycolipid (Fig. 8). It is these end products with their mixed polar/apolar domains which are the most pertinent to discussions on bacterial triterpenoid function.

The occurrence of triterpenoids and their most predominant derivatives, i.e., the sterols, in membranes is well documented and has been the subject of numerous reviews (19, 34, 95, 99, 100, 119). For example, monooxygenated sterols or their esters and glycosides have long been studied as architectural membrane components. In such a role, sterols such as cholesterol are associated with the membrane phospholipid bilayer in (primarily) a longitudinal orientation with the hydroxyl group in a latitudinal orientation. The net effect of such positioning is the suppression of bilayer phase transitions and the stabilization of the bilayer into an intermediate fluidity state between crystalline and fully fluid. This stabilization can, in turn, affect a variety of membrane functions including permeability, nutrient transport, osmotic stability, and membrane-associated enzyme activity.

A similar function of membrane stabilization has been postulated for the hopanoids based on their structural simi-

larities to sterols (74, 99, 108, 109, 112, 119). Only limited studies to date, however, have compared the effects of hopanoids and sterols on membrane state transitions (14, 53, 54, 112). In these studies, the hopanoid glycolipid (and its aglycone) from *B. acidocaldarius* was found to condense lipid monolayers, thus decreasing fluidity, in a manner similar to cholesterol.

Carotenoids have also been studied as possible membrane components (50, 68, 70, 71, 86, 88, 92, 125), although more emphasis has been placed on their photoprotective function(s) (see below). In a membrane stabilization role, carotenoids appear to function in a manner similar to hopanoids and sterols, causing bilayer condensation and increased rigidity. This is supported by both in vitro and in vivo studies. For example, when carotenoids from *Acholeplasma (Mycoplasma) laidlawii* were incorporated into spin-labeled multilamellar phosphatidylcholine vesicles, a marked decrease in fluidity of the bilayer fatty acyl groups was observed by electron paramagnetic resonance spectrometry (125). In a later study, a C_{50}-carotenoid was shown to significantly stabilize unilamellar lecithin vesicles to osmotic shock (14). Early in vivo studies assessed the effect of the carotenoid biosynthesis inhibitor diphenylamine on the growth of *Micrococcus lysodeikticus* and *Sarcina lutea* and found increased membrane instability with decreased carotenoid content (136). Diphenylamine inhibits higher carotenoid biosynthesis, resulting in the accumulation of phytoene, the first (colorless) carotenoid precursor. This study was challenged in another which utilized protoplasts derived from native *Sarcina lutea* grown in the absence or presence of diphenylamine and from a mutant of the native strain which contained only the colorless carotenoids phytoene and phytofluene (92). None of the protoplasts showed any significant change in osmotic fragility in comparison to each other. Questions have been raised, in turn, about the latter study since it did not address changes in fatty acyl composition in the protoplasts, any resultant maintenance of membrane fluidity, and consequently no changes in osmotic fragility (50). Other studies utilizing mutants of *Acholeplasma laidlawii* which do not synthesize carotenoids have shown that exogenous sterol can substitute for the carotenoids as a growth requirement (114, 138, 139, 141). If membrane synthesis in *Acholeplasma laidlawii* is altered by variation in the growth medium to contain varying amounts of carotenoids, membrane rigidity is altered with a resultant increased fluidity and lower osmotic fragility as carotenoid content decreases (50). Further, when the carotenoids were selectively removed from *Acholeplasma laidlawii* membranes by incubation with phosphatidylcholine vesicles, the carotenoid-depleted membranes showed an increase in fluidity as measured by increases in the mobility of the hydrocarbon chains of spin-labeled membrane fatty acids (125).

It thus appears that, in the majority of studies carried out to date, carotenoids can act as membrane stabilizers. In the case of *Acholesplasma laidlawii*, it should also be noted that the carotenoids in this organism have been characterized only by UV-visible spectrophotometric and chromatographic analysis methods (139, 141). The problems associated with identifying carotenoids, and especially for distinguishing C_{30}- from C_{40}-carotenoids, by using such incomplete analysis have been discussed elsewhere (149). It would be very interesting to establish whether *Acholeplasma laidlawii*, which has a G+C content of ≅32 mol% (22), contains C_{30}- rather than the reported C_{40}-carotenoids.

Based on the available data from sterol and higher carotenoid studies and on the structures of the bacterial triterpenoids, it is logical that the latter be considered as membrane components and sterol precursors. For example, the homohopanoids and the *B. acidocaldarius* hopanoid glycolipid (Fig. 7 and 8) possess characteristics well suited for membrane integration. These include not only the obvious structural similarities of the hopanoid ring structure to sterols, but also the composition and polarity of their other component parts. Based on CPK molecular models (60), the *B. acidocaldarius* glycolipid has a molecular size of ≅0.7 by 0.6 by 2.6 mm (width by depth by length). If it is assumed that the acyl group of the glycolipid is derived from either C_{17}- or C_{19}-ω-cyclohexanoic fatty acids which predominate in the bacterium (38, 74, 106), then a structure results resembling a phospholipid with a polar headgroup consisting of the glucosaminyl-tetrahydroxyl portion of the complex and a two-chain apolar region consisting of the hopanoid ring system and the fatty acyl chain. With molecular models, this complex has dimensions of ≅1.2 by 0.6 by 2.6 nm; i.e., addition of the fatty acyl group increases the width of the complex but not its length or depth. Such a complex could be inserted into one layer of a bilayer bacterial cell membrane assuming a fluid-mosaic type of membrane with a lipid bilayer ranging from 3.5 to 4.5 mm in thickness (16, 96, 136). This is in agreement with and extends preliminary theories on the integration of hopanoids into bilayer membranes (100, 109, 112, 119). Further studies are now necessary to characterize the nature of hopanoid membrane complexes to provide conclusive structural proof for their membrane integration.

A similar, albeit tentative, conclusion can be reached for a membrane-stabilizing function for the C_{30}-carotenoids. Based on available data, two possible modes of membrane integration can be postulated for these compounds. In the first, and utilizing as a model compound the acylated, glycosylated C_{30}-carotenoid 4′-D-glucopyranosyloxy-4,4′-diaponeurosporene acid ester from *Staphylococcus aureus* (Fig. 5), a carotenoid glycolipid could exist as a transmembrane component in a complex again resembling a phospholipid (Fig. 9). For example, if such a complex is based on the predominant phospholipid and fatty acid occurring in *Staphylococcus aureus*, i.e., phosphatidylglycerol and 12-methyltetradecanoate (anteiso-15:0), respectively (8, 172), then its molecular dimensions are ≅1.2 by 0.6 by 4.5 nm. The length of this complex together with its polar-apolar-polar structure would favor transmembrane integration with its polar portions extending out of either side of the bilayer. Also, the length of a fatty acid such as anteiso-15:0 is approximately half that of the carotenoid aglycone (≅1.6 versus 3 nm). Thus, integration of two such complexes, or a normal phosphatidyl-glycerol molecule containing this fatty acid, could occur in opposition in the membrane with a tail-to-tail fit of the fatty acyl chains.

The evidence for the proposed membrane integration of C_{30}-carotenoids as presented in Fig. 9 is both preliminary and indirect. Our studies with both the *Staphylococcus aureus* and *Streptococcus faecium* carotenoids have consistently found that the major xanthophylls are associated as components of larger complexes, presumably membrane associated, which often require drastic extraction methods (32, 152, 156). For example, extraction of the *Staphylococcus aureus* carotenoids with methanolic KOH results in isolation of the methyl ester of 4,4′-diaponeurosporen-4-oate as the major xanthophyll, apparently due to both transesterification of acylated carotenoids and per se methylation of the free carotenoate in the organism. Extraction of the *Staphylococcus aureus* carotenoids without the use of base results in the isolation of acylated carotenoids and the free caroten-

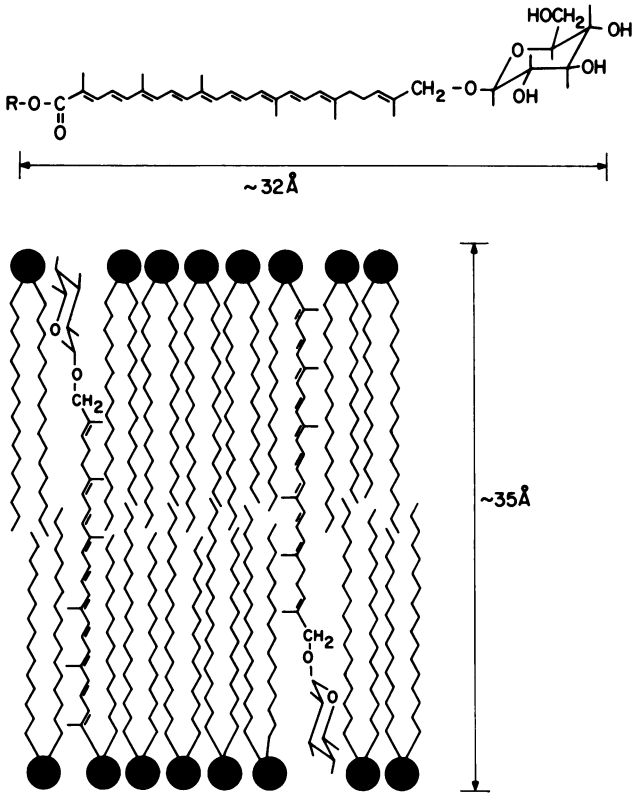


FIG. 9. Possible model for integration of the C_{30}-carotenoid glycolipid from *Staphylococcus aureus* 209P into the lipid bilayer of a bacterial membrane. (1Å = 0.1 nm.)

oate. Similar results have been found by other workers and, if saponification or base is not used in the extraction procedure, complex carotenoids structurally compatible with membrane integration are found. For example, the end product C_{30}-xanthophylls from both *Staphylococcus aureus* S41 and *Pseudomonas rhodos* are acylated (Fig. 3) (59, 84), and examples of acylated, glycosylated C_{40}-carotenoids have been found in the myxobacteria such as *Myxococcus fulvus, Stigmatella aurantiaca*, and *Chrondromyces apiculatus* (56, 57, 115) and in *Nocardia kirovani* (167). Whereas the latter carotenoid glycolipids are based on C_{40}- rather than C_{30}-carotenoids, their structures are still consistent with transmembrane integration given the variability possible in bacterial membrane architecture. Also, the C_{40}-ω,ω'-biphytanyl diols found in thermoacidophiles (see above) present another example of transmembrane isoprenoid complexes with, again, a polar-apolar-polar structure.

The transmembrane integration of C_{30}-carotenoids thus appears plausible and could result in membrane stabilization or a means to fix the carotenoid chromophore in the membrane to maximize its postulated photoprotective function or both. Since most bacterial end product carotenoids are known to be glycosides, it is probable that new studies utilizing carefully controlled extractions with or without base will reveal that most of these glycosides also exist as acylated carotenoid glycolipids.

A second possible means for integration of C_{30}-carotenoids into bacterial membranes involves association into a protein complex. Such a complex is not incompatible with the existence of the carotenoid glycolipid and could, in fact, represent an ultimate membrane unit for carotenoids in bacteria. The carotenoprotein concept is illustrated in Fig. 10, again utilizing the *Staphylococcus aureus* xanthophyll as a model compound. In this case, the glycosidic or acidic component or both of the C_{30}-carotenoid glycoside provides the functional group for protein interaction. Figure 10 represents the complex as involving a transmembrane protein, although this need not be the only type of membrane integrated or membrane-associated protein involved in such complexes. Also, direct association of the acylated carotenoid glycoside with the protein is possible and could provide a means of integrating the protein itself into the lipophilic environment of the bilayer membrane. Once again, such a complex could represent a means of maximizing carotenoid function in bacterial membranes.

The carotenoprotein model for C_{30}-carotenoid integration is, again, supported by preliminary and indirect evidence. Studies with the *Staphylococcus aureus* pigments also included preliminary attempts to isolate the carotenoids from the organism by using aqueous extraction methods (Taylor, unpublished data). Cells were disrupted and the cell fragments were extracted with 0.01 M $NaHPO_4$, pH 7, containing 1% Triton X-100. The extract was then chromatographed on Sephadex G200, using the same buffer. The cell residue from the aqueous extraction was subsequently extracted with organic solvents by the usual method for recovery of the remaining carotenoids (156). Gel permeation chromatography of the aqueous extract resulted, as expected, in a number of protein peaks, only one of which also contained color (absorbance maxima at 280 and 470 nm). Based on absorbance measurements, it was estimated that the carotenoid (unidentified but preliminary analysis indicated that it is a derivative of 4,4-diaponeurosporen-4-oic acid) in the

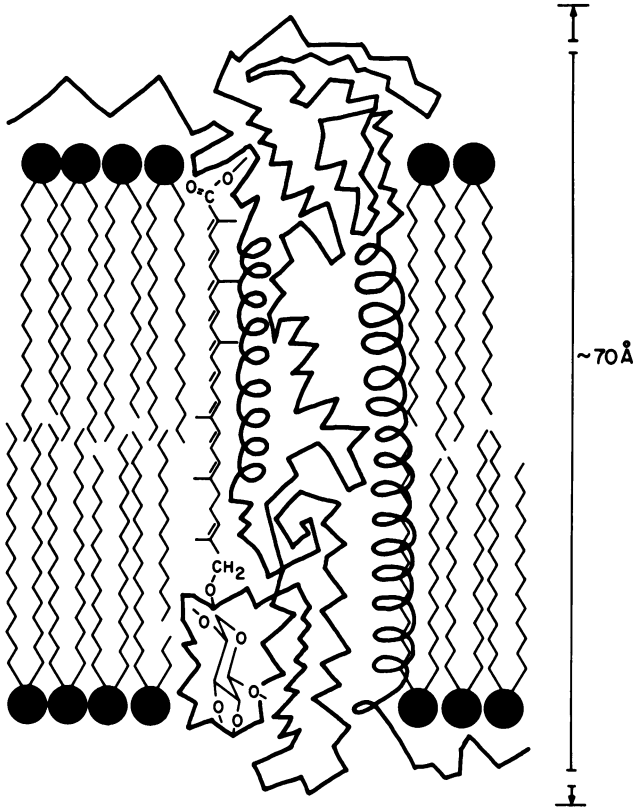


FIG. 10. Possible model for membrane integration of a protein complexed with the C_{30}-carotenoid glycolipid from *Staphylococcus aureus* 209P. (1Å = 0.1 nm.)

carotenoprotein isolated represented ≅4% of the total carotenoid present in *Staphylococcus aureus*.

Although no other studies have been reported concerning the isolation of C_{30}-carotenoproteins, such complexes are well known for C_{40}-carotenoids (for reviews, see references 18, 149, 178). In one of the first modern studies on carotenoproteins in non-photosynthetic bacteria, it was shown that the carotenoids in aqueous extracts from *Corynebacterium poinsettiae, C. michiganense, Mycobacterium phlei*, and *Micrococcus agilis* were associated with specific proteins (127). In the case of *Corynebacterium michiganense*, the water-soluble carotenoids associated with a 35S component which was not present in colorless mutants of the same organism. Other studies in bacteria have isolated C_{40}- or C_{50}-carotenoproteins or glycoproteins or both from the membranes (or chromatophores) of *Sarcina flava, Rhodospirillum rubrum, Rhodopseudomonas sphaeroides*, and a *Chromatium* sp. (21, 134, 159, 160). Bacteriorhodopsin, which contains C_{20}-retinal complexed to a protein, has been isolated from the membranes of *Halobacterium halobium* and *H. cutirubrum* (104). In each of these cases, the extraction of the carotenoproteins required aqueous detergent solutions, and further separation of the carotenoid from its protein complex required disruptive methods such as saponification or treatment with organic solvents. It is likely that new studies utilizing aqueous instead of the predominant organic solvent extraction methods for bacterial carotenoids will add significantly to the known occurrence of bacterial carotenoproteins.

Other Functions

Besides their role in membrane stabilization, no additional functions have been postulated for the hopanoids, e.g., as precursors to intracellular hormones and growth factors as is the case with the sterols. This is not the case, however, with the acyclic triterpenoids. The reduced squalenes, for example, have been postulated as functioning as both membrane components and components of a reversible hydrogen transfer system (3, 69, 164, 166). In the latter, squalene, its reduced derivatives, and possibly diapophytoene (dehydrosqualene) may accept and donate hydrogen in a reversible manner, allowing an organism to control its internal reduction potential or membrane hydrogen transfer mechanisms or both. If such a function is proven, it could provide an example of a primitive precursor of electron transfer and photosynthetic processes. It is also possible that this function for squalenes evolved as the result of changes in the environment from anaerobic to aerobic, providing a protective function against oxygen to the bacterium (166). In support of this postulate, it has been found that as *H. cutirubrum* (which contains squalene, reduced squalenes, and diapophytoene; Table 1) is cultured under increasingly more anaerobic conditions, the cellular ratios of squalene to reduced squalenes decrease proportionately; i.e., more reduced squalenes are formed with no significant changes in total squalenes present (163).

The C_{30}-carotenoids may be involved in photoprotective functions or energy transfer reactons or both similar to the C_{40}-carotenoids. The role of carotenoids as accessory light-gathering pigments in photosynthesis is well known (62, 173). A second function, most likely more primitive to their role in photosynthesis, is the ability of carotenoids to protect other molecules against light-induced destruction in the presence of molecular oxygen, apparently by quenching free radical, singlet oxygen, and triplet sensitizer reactions (for reviews, see references 24, 62–64). Such protective functions were first suggested based on studies utilizing native and (noncarotenoid-containing) mutant strains of *Rhodopseudomonas sphaeroides* which showed that, whereas both strains grew in a similar manner in an anaerobic, photosynthetic environment, the growth of the mutant strain was inhibited in the presence of light and air due to destruction of its bacteriochlorophyll (137). Later studies confirmed that bacteriochlorophyll is protected from photodestruction in the presence of oxygen by carotenoids (28, 40, 44). Numerous studies followed in carotenogenic, non-photosynthetic bacteria which showed that colorless mutant strains were more sensitive to photoinduced lethality (40, 55, 73, 86, 87, 89, 90). These studies have led to the conclusion that exogenous (e.g., dyes such as toluidine blue) or endogenous (e.g., bacteriochlorophyll, cytochromes, flavins, etc.) photosensitizers mediate photoreactions which, in the absence of normally present carotenoids, result in destructive photooxidations. The site of the damaging or lethal oxidations is presumed to be in or at the cell membrane, based on the known location of bacterial carotenoids at the membrane and other supporting studies. For example, photodynamic lethality in carotenogenic bacteria has been correlated in bacteria with increased cellular permeability, and carotenoids have been directly associated in bacteria with the photoprotection of a variety of membrane-associated molecules including succinic dehydrogenase, NADH oxidase, ATPase, quinones, and electron transport chain components (6, 23, 89, 113, 124). Also, carotenoids can protect phosphatidylcholine liposomes from photoinduced oxidative damage resulting in lipid peroxidation (4, 65). Such protection appears to be optimal for the lethal effects of visible light but not as significant for those resulting from X rays or UV light (87). This is supported by studies which have established that, to act as effective photoprotective molecules, carotenoids must contain at least nine conjugated carbon-carbon double bonds (26, 27, 91, 93). This chromophore length is also a requirement for the efficient quenching of singlet oxygen (10, 41, 42, 63, 64).

In addition to their function in transferring radiant energy to acceptor chlorophyll molecules during photosynthesis, other, non-photosynthetic energy transfer reactions have been proposed for carotenoids. Perhaps the best example of such a reaction in bacteria is the light-mediated bacteriorhodopsin system of *H. halobium* which appears similar in its mechanism of activation to the vertebrate rhodopsin system (30, 103, 107). In *H. halobium*, bacteriorhodopsin mediates light energy conversion into proton release and uptake, utilizing a cyclic photochemical reaction based in the isomerization of its retinal chromophore. Known end results of this process include ATP production, regulation of various transport processes, and protein phosphorylation (80, 116, 130, 142). Other studies indicate that retinoids and carotenoids can act as electron conductors, involved in the transfer of electrons across membranes or to membrane-associated components such as proteins (31, 52, 66, 111). This proposed function is based on the properties of extended π-electron systems such as those found in carotenoids, which may enhance electron transport through the bilayer membrane by either facilitating electron tunneling or directly accepting an electron on one side of a membrane and donating it to a suitable acceptor on the other side. Various studies have supported this theory for carotenoid function, including those showing the photoactivation of bilayer membranes (128, 161, 162) and charge transfers in model systems utilizing retinoids, carotenoids, and other polyisoprenoid

compounds (2, 11, 20, 25, 51, 67, 83, 107, 117, 128, 161, 174, 175). Although still highly speculative, the action of carotenoids as electron transfer molecules could provide a direct evolutionary link between anaerobic and aerobic respiratory processes.

The chemistry and structures of the C_{30}-carotenoids suggest that they could function in photoprotective and energy transfer reactions in a manner similar to the retinoids and C_{40}-carotenoids. For example, in those bacteria producing C_{30}-carotenoids past diapophytoene, the end product carotenes and xanthophylls all contain 9 or 11 conjugated double bonds. These chromophores meet the postulated requirements for effective photoprotection. The apparent association of C_{30}-carotenoids with cell membranes either as carotenoid glycolipids or carotenoproteins suggests their potential role in transmembrane proton pumps or electron transfer components or both. Certainly an unanswered question relative to the very existence of the C_{30}-carotenoids is why they evolved at all, especially if such evolution occurred in non-photosynthetic bacteria occupying primarily low-light environments. An alternative (or additional) function for the C_{30}-carotenoids as charge transfer molecules could address this question.

CONCLUSION

The bacterial triterpenoids present a unique and challenging problem to both the microbiologist and the natural product biochemist. They represent a bridge between a primitive, anaerobic, and low-light environment and our present oxidative environment. Although they occur, as a class, in bacteria of widely divergent physiologies, specific structural types appear exclusive to bacteria depending on G+C content. Their recent discovery and characterization during the past decade leaves many unanswered questions regarding the extent of their occurrence and, most important, their function. It appears, however, that the function(s) of the bacterial triterpenoids is similar to those of the higher terpenoids (C_{40}-carotenoids and sterols) and include functions closely involved with membrane architecture and processes. As such functions are defined for the bacterial triterpenoids they will provide new insights into the evolution of modern day procaryotes and eucaryotes from primitive microorganisms.

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