BIOSYNTHESIS OF STEROLS IN INSECTS

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INTRODUCTION

Insects cannot synthesize sterols de novo, so they typically require a dietary source. Cholesterol is the dominant sterol in most insects, but because plants contain only small amounts of cholesterol, plant-feeding insects generate most of their cholesterol by metabolizing plant sterols. Plants almost always contain mixtures of different sterols, but some are not readily metabolized to cholesterol. Since they are unable to biosynthesize sterols, many phytophagous and omnivorous insects satisfy their cholesterol requirement by side chain dealkylation of the C-24 alkyl group of dietary C_{28} and C_{29} phytosterols. However, not all insects that can dealkylate the phytosterol side chain produce cholesterol. In addition, certain insects,e.g., some Hymenoptera, Hemiptera, and Diptera, are unable to dealkylate the sterol side chain. Although C_{27} ecdysteroids (molting hormones), which are biosynthesized from cholesterol, are the major ecdysteroids in most insects, many of those species that are unable to dealkylate phytosterols utilize campesterol as a precursor for the C_{28} ecdysteroid makisterone A. The considerable diversity of steroid utilization between certain insect species makes it difficult to generalize about insect steroid biochemistry. The ability to disrupt certain unique aspects of steroid utilization and metabolism in insects might be exploited for developing new insect control technology.

Cholesterol is an essential component of cell membranes, a precursor for molting hormone ecdysone and a component in signal transduction pathways in insects. Since Insects are not able to synthesize sterols and have to ingest exogenous sterols from food. Phytophagous insects obtain C-24 alkylated phytosterols, such as sitosterol, campesterol, and/or stigmasterol from their host plant. These C28 and C29 sterols are converted into cholesterols (C27 sterol) in the insects. Blood-sucking insects can directly ingest cholesterol from their host animals.

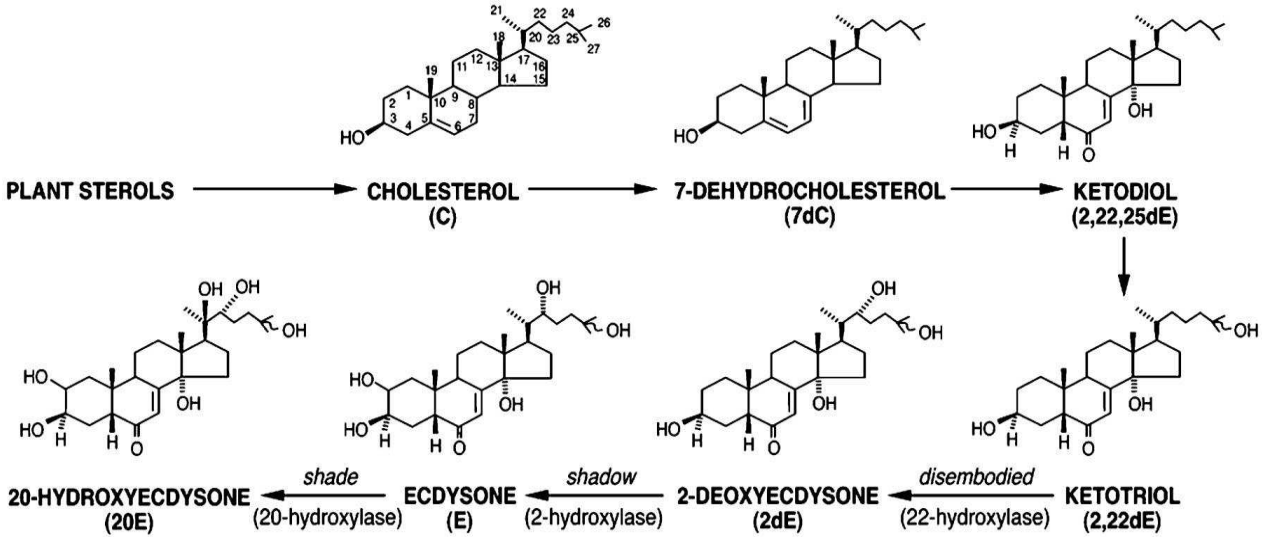


Fig1 Synthesis of Insect hormone from Plant Sterols.

Some insects have another sterol source, i.e. their associated yeasts or fungi. The leaf-cutting ant Acromyrmex octospinosus possesses 5,7-sterols, such as ergosterol, which originate in its fungal symbiotes . The ambrosia beetle Xyleborus ferrugineus requires ergosterol, which is presumably supplied by the associated fungus . Intracellular eukaryotic symbiotes in insects also supply sterols to the host insects. The anobiid beetles, Lasioderma serricorne (the cigarette beetle) and Stegobium paniceum (the drug store beetle), possess yeast like symbiotes (YLS), Symbiotaphrina kochii and S. buchneri, in the mycetome on the alimentary canal. They are ascomycetous fungi which propagate by budding in the beetles. YLS of the beetles synthesize ergosterol, which occupied four fifths of the total volume of sterols in the YLS. The beetles metabolize YLS sterols into 7-dehydrocholesterol and cholesterol . Sterols are also synthesized in YLS of planthoppers. The planthopper YLS, which are also ascomycetes (Euascomycetes: Hypocreales: Clavicipitaceae) , live in the fat body cells of the host planthopper. YLS of Laodelphax striatellus (the small brown planthopper) and Nilaparvata lugens (the brown planthopper) synthesize ergosta-5,7,24(28)-trienol, which is the only dominant sterol in the YLS directly isolated from planthoppers . On the other hand, reported that cultivated YLS in artificial medium synthesize ergosterol. Interpreted this inconsistency in the final sterol products in the YLS as follows:the YLS synthesized ergosta-5,7,24(28)-trienol under anaerobic conditions (in the planthopper) and ergosterol under aerobic conditions (in the culture medium). Ergosterol (methylcholesta-5,7,22-trienol, ergosta-5,7,22-trienol) is a common sterol synthesized by most yeasts and fungi . The ergosterol biosynthetic pathway in baker's yeast Saccharomyces cerevisiae has been well investigated , and enzymes in the ergosterol biosynthetic pathway of the yeast are all cloned and characterized .Ergosta-5,7,24(28)-trienol, which is produced by YLS of the planthopper, is an intermediate in the late pathway of ergosterol biosynthesis in yeasts and fungi (Fryberg (. The YLS are unable to metabolize ergosta-5,7,24(28)-trienol in the planthoppers and this sterol accumulates as a final product in the sterol biosynthetic pathway. This trienol is apparently converted into 24-methylene cholesterol and thereafter into cholesterol . Ergosta-5,7,24(28)-trienol in S. cerevisiae is metabolized into ergosta-5,7,22,24(28)-tetraenol by a P450 enzyme ERG5p (C22-sterol desaturase, CYP61), introducing a C22(23) double bond in the sterol side chain. Ergosta- 5,7,22,24(28)-tetraenol is then metabolized into ergosterol by ERG4p (C24=28 methylene reductase), which works during the final step in the ergosterol biosynthetic pathway .These two enzymes do not apparently work in the YLS in the internal milieu of planthoppers.

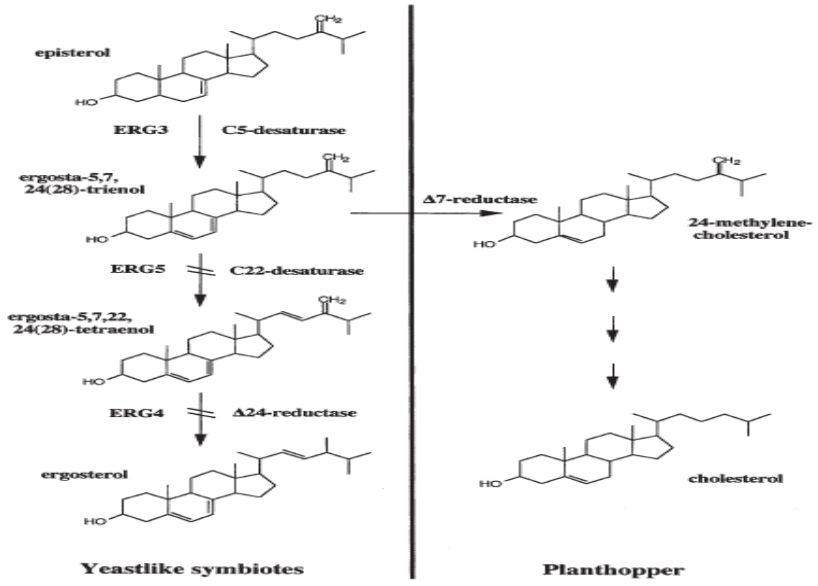


Fig. 2. Simplified late stages of the ergosterol biosynthetic pathway of planthopper YLS (based on the pathway by Parks and Casey (1995))and sterol metabolism in planthopper.

Sterols normally ingested by insects are characterized by three domains: a 3_-hydroxyl group (A), a tetracyclic ring system (B), and a side chain of 8–10 carbon atoms (C). The polar and non-polar elements, consisting of domains A versus B and C, give rise to the amphipathic nature of the molecule. This feature, when coupled with the side chain oriented to the ‘‘right’’, provides a suitable fit of the sterol into the lipid leaflet of membranes. The sterol may possess methyl groups at C-4 and they are defined accordingly as C-4 dimethyl sterol, C-4 monomethyl sterol and C-4 desmethyl (no methyls) sterols. The degree of C-4 methylation affects the hydrogen bonding ability of the C-3 hydroxyl group as evidenced in the thin-layer chromatography of these compounds. Alternatively, the sterol may possess a C-24 methyl or ethyl group, which may possess stereochemistry as either alpha- (in front) or beta- (in back) oriented. The stereochemical nomenclature for the side chain C-24 alkyl groups can be affected by neighbouring substituents when the R/S-nomenclature is used and that the configuration assignments can be different for groups in the side chain relative to the nucleus. The prefix _ in_-sitosterol is dropped in common usage for chemical reasons. The structure assignments for sterol used typically by phytosterol biochemists is different from natural product chemists, who follow the recently revised numbering of sterols by the International Union for Pure and Applied Chemistry (IUPAC) and International Union for Biochemistry (IUB).

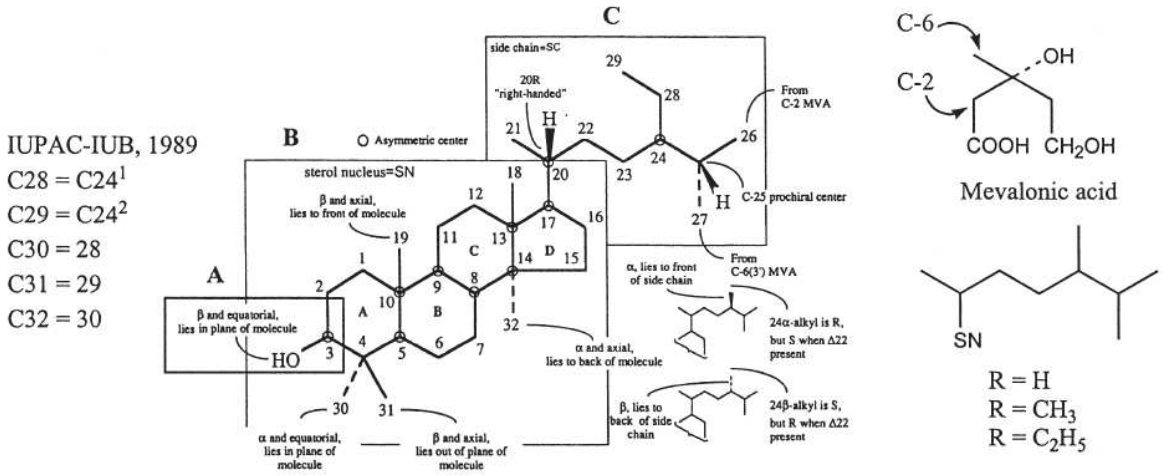


Fig3. Steroid numbering system A,B and C represent structural domains

INSECT STEROL METABOLIC PATHWAYS

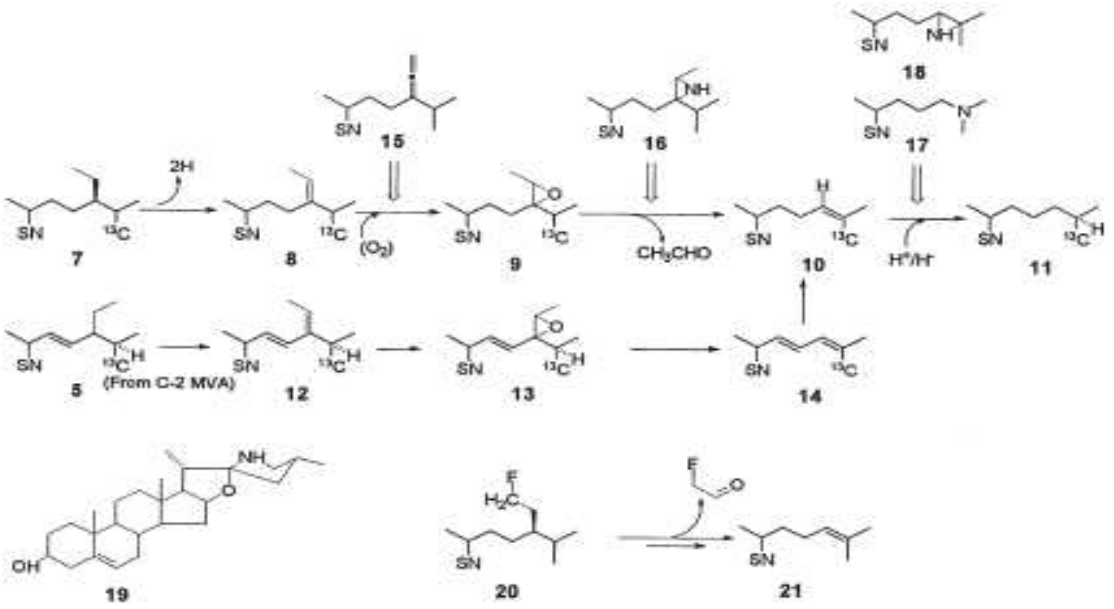
Insects operate the classic acetate-mevalonate isoprenoid pathway to an unidentified step that forms before synthesis of the 30-carbon olefin, squalene oxide. The post-lanosterol pathway normally operational in other animals is interrupted in insects. For instance, [2-3H]-lanosterol fed to animals is actively converted to cholesterol whereas in the tobacco hornworm, M. sexta, the labelled sterol is absorbed by the gut and metabolized to a sole product, 24,25-dihydrolanosterol, using a reductase-type enzyme. This enzyme, 24, 25-reductase, is also found in the cycloartenolsitosterol pathway of plants and has been studied in a cell-free system of the tobacco hornworm.

In plants 24,25-reductase normally recognizes 24-methyl and 24-ethyl desmosterol as substrate to generate campesterol and sitosterol, respectively, whereas the 24,25-reductase of insects recognizes lanosterol or desmosterol as substrates. The 24,25-reductase enzyme is a critical slow step in the insectconversion of phytosterol to ecdysteroid. As a result, the first inhibitors of sterol metabolism were targeted to inhibit the activity of this enzyme.Insects, in contrast to more derived animals, have many enzymes in the sterol pathway that can be considered as reverse

enzymes that are complementary toFIG. 2 Isoprenoid–sterol pathway. HMGR, hydroxymethyl gluturayl CoA-reductase; DXS, 1-deoxy-D-xylulose 5-phosphate synthase; SMT, sterol methyl transferase those synthesized by plants. For instance, the normal route for conversion of phytosterols involves nucleus changes as follows: the 9,19-cyclopropane group isomerizes to an 8,9-bond, which is then rearranged to the 7-bond. Next α 5- bond is introduced and the 7-bond is reduced to form the final 5-structure characteristic of phytosterol end-products. In contrast, insects can introduce the 7-bond or remove the 5-bond from the sterol nucleus, so that after feeding on plant leaves, the insect will have a sterol composition of 7- or 0-sterols . For side chain transformations, plants have an enzyme that adds α 22-bond to the sterol side chain, while insects have an enzyme to remove the 22-bond. Likewise, plants convert the 24(25)-bond to form a 24(28)-exomethylene bond while insects convert the 24(28)-bond to a 24,25-double bond. It appears that the catalysis involved with the reverse reactions operated by insects is not simply that of an equilibrium reaction, but rather unique metabolases evolved to carry out the individual reaction. Key enzymes in the post-lanosterol and post-cycloartenol pathway such as a C-4 demethylase, 8,9-isomerase and _23(24)-reductase (in corn) are also absent from insects. On the basis of present understanding of metabolic relationships, the aliphatic isooctane side chain of cholesterol occupies a position as a key branch point structure of steroid metabolism since it serves as the precursor to ecdysteroids and provides suitable three-dimensional and amphipathic characteristics for sterol to interdigitate in the membrane. Conversion of phytosterol to cholesterol was hypothesized to proceed through a series of side chain metabolisms as outlined in which the 24-alkyl group is ultimately removed and the resulting 24,25-double bond is saturated to produce the isooctane structure. Sitosterol (7) and stigmasterol (5), the major 24-ethyl cholesterols found in plants, are converted to cholesterol in insects by essentially the same side chain dealkylation pathway. Cell-free systems, originating from both the tobacco hornworm and the silkworm, B. mori, have been used to study C-24 dealkylase (lyase enzyme) and 24,25-reductase but unfortunately no detailed characterization or purification of these enzymes has been reported. Therefore, nothing significant is known regarding kinetics, except for reaction mechanisms.

Four steps occur in the 24-dealkylation pathway that converts sitosterol to fucosterol, and the first is catalysed by a single dehydrogenase enzyme that lacks stereospecificity. The next step is the stereospecific conversion of fucosterol to the (24R,28R) epoxide of fucosterol. The penultimate step is catalysed by the fucosterol epoxide lyase involving the elimination of acetaldehyde and migration of H-25 to C-24 to form the 24,25-double bond.

Finally, in the conversion of desmosterol to cholesterol, a reductase-typeenzyme is involved. In related work it was demonstrated that [2-13C]-mevalonic acid is converted to (25S)-[26-13C]-sitosterol (7) and, using a cell-free preparation from corn, [27-13C]-lanosterol was converted to 22 (25R)-[2713C]-24(28)-methylenelanosterol . This suggested a general process, with the biosynthesis of 24-ethyl phytosterols passing through three steps: (i) successive methylation at C-24, (ii) isomerization of the 24(28)-bond to the 24,25-bond, and (iii) reduction of the 24,25-double bond with net retention in configuration at C-25 in the final chiral product. In the case of the biosynthesis of cholesterol via the 24-dealkylation pathway in insects, there is also a net retention in configuration in the final chiral product (Scheme below). It can be seen that C-2 of mevalonate incorporated into C-26 (a rotation at C-25 is shown to accommodate nomenclature, cf., is the pro-S-methyl group whereas in cholesterol generated in animals, the pro-R-methyl is derived from C-2 of mevalonate. In the reduction of the 24(25)-double bond, regardless of the system engaged, both hydrogen atoms were added to the si-face of the double bond equivalent to the cis-addition of the hydride ion to C-25 from a pyridine nucleotide and of a proton to C-24.



SCHEME 1 Pathway of sterol dealkylation by insects and disruption by rationally designed inhibitors targeted for key enzymes in the pathway. The sterol nucleus (SN) in each case is cholesterol based. Structure 7 corresponds to the sitosterol side chain and structure 5 corresponds to the stigmasterol side chain. Structures 10 and 11 correspond to the desmosterol and cholesterol side chains, respectively. Structure 19 is solasodine, a common steroidal alkaloid.

A more precise understanding of the 24-dealkylation pathway was elucidated by the use of structural analogues targeted to disrupt each step. All the inhibitors shown in Scheme 2 block steps along the 24-dealkylation pathway as shown by the accumulation of substrate for the target enzyme. They are also SCHEME 2 Pathway of sterol dealkylation by insects and disruption by rationally designed inhibitors targeted for key enzymes in the pathway. The sterol nucleus (SN) in each case is cholesterol based. Structure 7 corresponds to the sitosterol side chain and structure 5 corresponds to the stigmasterol side chain. Structures 10 and 11 correspond to the desmosterol and cholesterol side chains, respectively. Structure 19 is solasodine, a common steroidal alkaloid. highly effective inhibitors of insect growth . The mechanism for enzyme inhibition by the ammonium-containing compounds (e.g., 16, 17 and 18) is probably different from the allenes (e.g., 15). It seems that the N-steroids may serve as a highenergy intermediate analogue of the cationic intermediate generated during the catalytic reaction. In contrast, the allenes may serve as a mechanism-based inactivator, much like related compounds prepared and tested with the sterol methyl transferase from yeast. Natural products, including steroidal alkaloids such as solasodine (19) that contain nitrogen in the side chain, might inhibit the 24,25-reductase as they can inhibit the sterol methyl transferase and inhibit growth of algae by inducing an accumulation of 24-desmethyl sterols with a 24,25-double bond. However, these N-steroids fail to affect the growth of the tobacco hornworm , showing important differences between plants and insects in their response to steroidal alkaloids. have studied several side chain modified monofluorinated cholesterols and sitosterols and found that 29-fluorositosterol (20) and 29-fluorostigmasterol were highly toxic to the tobacco hornworm. Abnormal development caused by the 29-fluorosterols is similar to that seen in larvae fed on fluoroacetate. Dealkylation of these fluorosterols produces a lethal product, fluoroacetate, and the inhibitor has no direct effect on phytosterol metabolism.

PHYTANIC ACID A-OXIDATION REACTIONS

Phytanic acid is a fatty acid present in the tissues of ruminants and in dairy products and is, therefore, an important dietary component of fatty acid intake. Because phytanic acid is methylated, it cannot act as a substrate for the first enzyme of the mitochondrial β-oxidation pathway (acyl-CoA dehydrogenase). Phytanic acid is first converted to its CoA-ester and then phytanoyl-CoA serves as a substrate in an α-oxidation process. The α-oxidation reaction (as well as the remainder of the reactions of phytanic acid oxidation) occurs within the peroxisomes and requires a specific α-hydroxylase (specifically phytanoyl-CoA hydroxylase, PhyH), which adds a hydroxyl group to the α-carbon of phytanic acid generating the 19-carbon homologue, pristanic acid. Pristanic acid then serves as a substrate for the remainder of the normal process of β-oxidation. Because the first step in phytanic acid oxidation involves an α-oxidation step, the process is termed α-oxidation. For more details on peroxisome function see the Refsum disease page.

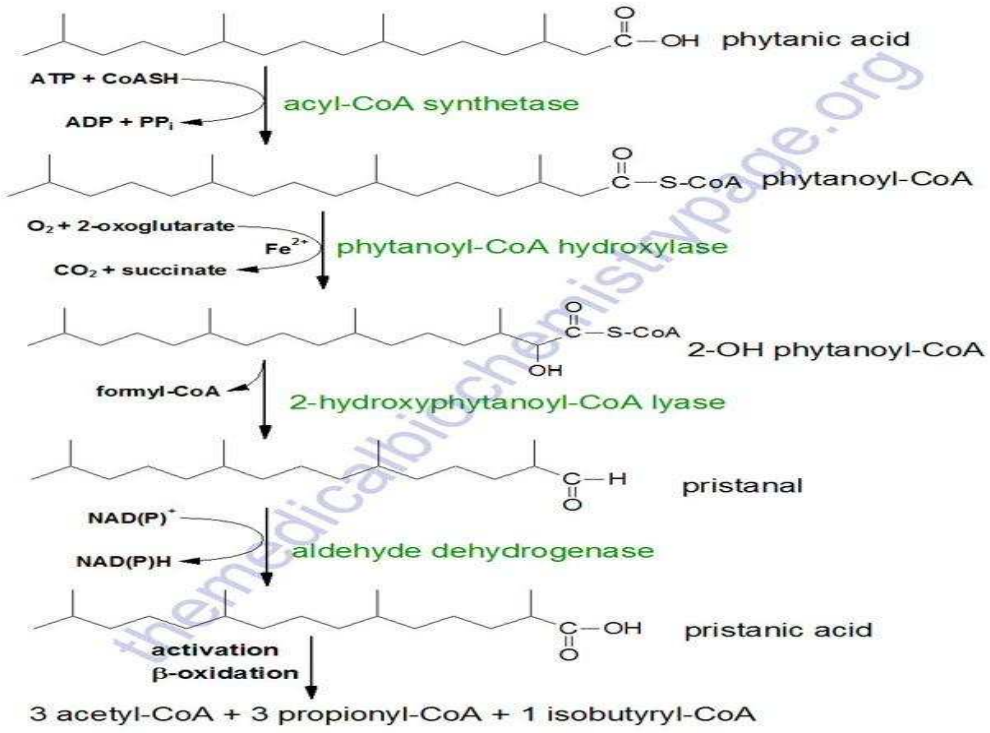


Fig 4. **Biosynthesis of Phytanic acid**

MITOCHONDRIAL (BETA) β-OXIDATION REACTIONS

Oxidation of fatty acids occurs in the mitochondria and the peroxisomes. Fatty acids of between 4–8 and between 6–12 carbon atoms in length, referred to as short- and medium-chain fatty acids (SCFAs and MCFAs, respectively), are oxidized exclusively in the mitochondria. Long-chain fatty acids (LCFAs: 10–16 carbons long) are oxidized in both the mitochondria and the peroxisomes with the peroxisomes exhibiting preference for 14-carbon and longer LCFAs. Very-long-chain fatty acids (VLCFAs: C17–C26) are preferentially oxidized in the peroxisomes.

Fatty acids must be activated in the cytoplasm before being oxidized in the mitochondria. Activation is catalyzed by fatty acyl-CoA synthetases (also called acyl-CoA ligases or thiokinases). The net result of this activation process is the consumption of 2 molar equivalents of ATP.

Fatty acid + ATP + CoA ——> Acyl-CoA + PP_i + AMP

The transport of fatty acyl-CoA into the mitochondria is accomplished via an acyl-carnitine intermediate, which itself is generated by the action of carnitine palmitoyltransferase 1 (CPT-1 or CPT-I) an enzyme that resides in the outer mitochondrial membrane. There are three CPT-1 genes in humans identified as CPT-1A, CPT-1B, and CPT-1C. Expression of CPT-1A predominates in the liver and is thus, referred to as the liver isoform. CPT-1B expression predominates in skeletal muscle and is thus, referred to as the muscle isoform. CPT-1C expression is exclusive to the brain and testes. The CPT-1A gene (symbol = CPT1A) is located on chromosome 11q13.3 and consists of 20 exons spanning 60 kb encoding a 773 amino acid protein. The CPT-1B gene (symbol = CPT1B) is located on chromosome 22q13.33 and consists of 21 exons spanning 10 kb. The CPT-1C gene (symbol = CPT1C) is located on chromosome 19q13.3 and consists of 20 exons spanning 23 kb. The activity of CPT-1C is distinct from those of CPT-1A and CPT-1B in that it does not act on the same types of fatty acyl-CoAs that are substrates for the latter two enzymes. However, CPT-1C does exhibit high-affinity malonyl-CoA binding.

Following carnitine acyl-carnitine-mediated transfer of the CPT-1-generated fatty acyl-carnitines across the inner mitochondrial membrane, the fatty acyl-carnitine molecules are acted on by the inner mitochondria membrane carnitine palmitoyltransferase 2 (CPT-2 or CPT-II) regenerating the fatty acyl-CoA molecules. The CPT-2 gene (symbol = CPT2) is located on chromosome 1p32.3 and consists of 5 exons that span 20 kb.

Each round of β-oxidation produces one mole of $FADH_2$, one mole of NADH, and one mole of acetyl-CoA. The acetyl-CoA, the end product of each round of β-oxidation, then enters the TCA cycle, where it is further oxidized to CO_2 with the concomitant generation of three moles of NADH, one mole of $FADH_2$ and one mole of ATP. The NADH and $FADH_2$ generated during the fat oxidation and acetyl-CoA oxidation in the TCA cycle then can enter the respiratory pathway for the production of ATP via oxidative phosphorylation.

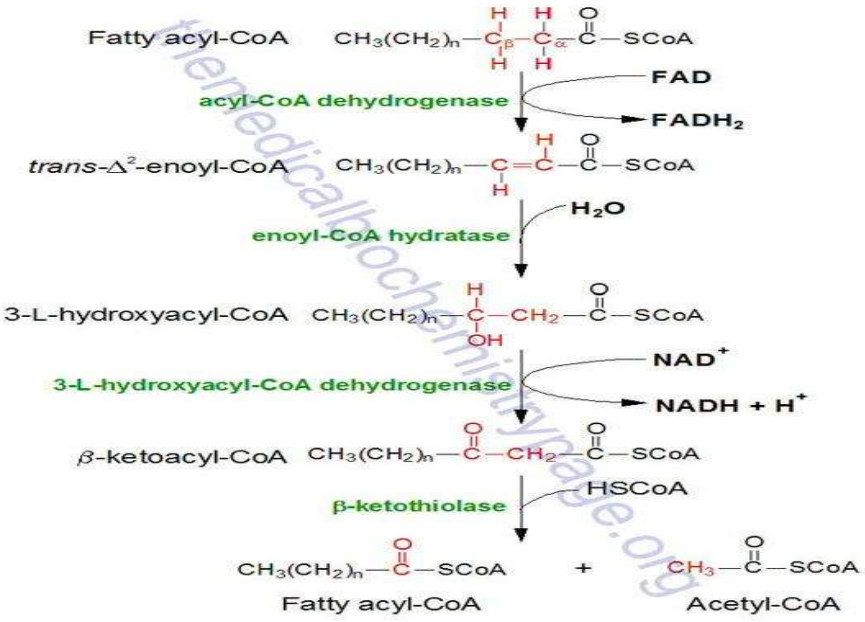


Fig 5. **Mitochondria β-oxidation**

PEROXISOMAL (BETA) B-OXIDATION REACTIONS

In addition to mitochondrial oxidation of fatty acids, the peroxisomes also play an important role in overall fatty acid metabolism. Very-long-chain fatty acids (VLCFAs: C17–C26) are preferentially oxidized in the peroxisomes with cerotic acid (a 26:0 fatty acid) being solely oxidized in this organelle. The peroxisomes also metabolize di– and trihydroxycholestanoic acids (bile acid intermediates); long-chain dicarboxylic acids that are produced by ω-oxidation of long-chain monocarboxylic acids; pristanic acid via the α-oxidation pathway (see below); certain polyunsaturated fatty acids (PUFAs) such as tetracosahexaenoic acid (24:6), which by β-oxidation yields the important PUFA docosahexaenoic acid (DHA); and certain prostaglandins and leukotrienes.

The enzymatic processes of peroxisomal β-oxidation are very similar to those of mitochondrial β-oxidation with one major difference. During mitochondrial oxidation the first oxidation step, catalzyed by various acyl-CoA dehydrogenases, results in the reduced electron carrier $FADH_2$ that then delivers its' electrons directly to the electron transport chain for synthesis of ATP. In the peroxisome the first oxidation step is catalyzed by acyl-CoA oxidases which is coupled to the reduction of O_2 to hydrogen peroxide (H_2O_2). Thus, the reaction is not coupled to energy production but instead yields a significant reactive oxygen species (ROS). Peroxisomes contain the enzyme catalase that degrades the hydrogen peroxide back to O_2.

Humans contain three peroxisomal acyl-CoA oxidases, ACOX1, ACOX2 and ACOX3. Human and rodent ACOX1 (also referred to as palmitoyl-CoA oxidase) is responsible for the oxidation of straight-chain mono- and dicarboxylic fatty acids, very long-chain fatty acids, prostaglandins, and xenobiotics. In humans, in contrast to rodents, 2-methyl branched fatty acids (primarily pristanoic acid) and the bile acid intermediates di- and tri-hydroxycoprostanic acids are desaturated in the peroxisomes by a single enzyme ACOX2 (also called branched-chain acyl-CoA oxidase). The human genome contains an ACOX3 gene but expression from the gene is detected in normal tissue only at extremely low levels. Rodent ACOX3 (also referred to as pristanoyl-CoA oxidase) is the oxidase responsible for oxidation of 2-methy branched chain fatty acids in these animals.

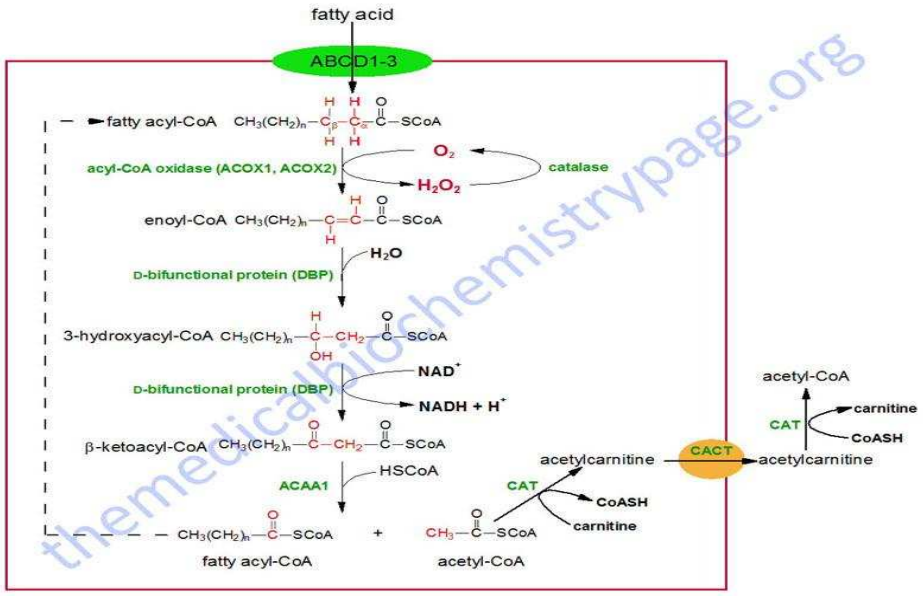


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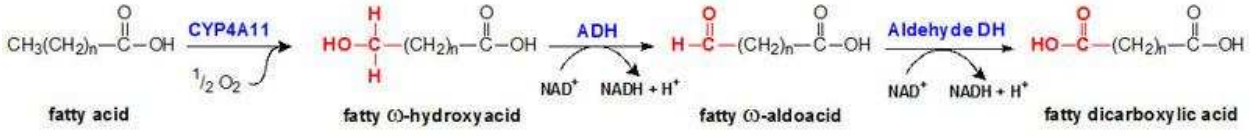
Fig 6. **Peroxisomal β-oxidation**

The hydration step and second oxidation step in peroxisomal β-oxidation is carried out by a single bifunctional enzyme as opposed to two separate enzymes as is the case in the mitochondria. There are two distinct bifunctional enzymes identified as L-bifunctional protein (LBP) and D-bifunctional protein (DBP). LBP is specific for L-3-hydroxyacyl-CoAs and DBP is specific for D-3-hydroxyacyl-CoAs. These bifunctional enzymes are also referred to as multifunctional proteins 1 and 2 (MFP-1 and -2) or L- and D-peroxisomal bifunctional enzymes (L-PBE and D-PBE). DBP is the primary, if not exclusive enzyme involved in the oxidation of VLCFAs, pristanic acid, and di- and trihydroxycholestanoic acids. The precise role of LBP in human peroxisomal lipid oxidation is unclear. Human peroxisomes contain the thiolase acetyl-CoA C-acyltransferase 1 (ACAA1) that catalyzes the terminal step in the peroxisoaml β-oxidation pathway.

The clinical significance of the activity of the acyl-CoA oxidases of peroxisomal β-oxidation is related to tissue specific oxidation processes. In the pancreatic β-cell there is little, if any, catalase expressed so that peroxisomal oxidation of VLCFAs results in an increased release of ROS that can damage the β-cell contributing to the progressive insulin deficiency seen in obesity.

MICROSOMAL (OMEGA) Ω-OXIDATION REACTIONS

The microsomal (endoplasmic reticulum, ER) pathway of fatty acid ω-oxidation represents a minor pathway of overall fatty acid oxidation. However, in certain pathophysiological states, such as diabetes, chronic alcohol consumption, and starvation, the ω-oxidation pathway may provide an effective means for the elimination of toxic levels of free fatty acids. The pathway refers to the fact that fatty acids first undergo a hydroxylation step at the terminal (omega, ω) carbon. Human ω-hydroxylases are all members of the cytochrome P450 family (CYP) of enzymes. These enzymes are abundant in the liver and kidneys. Specifically, it is members of the CYP4A and CYP4F families that preferentially hydroxylate the terminal methyl group of C10–C26 length fatty acids. CYP4A11 is the human homolog of the rat liver CYP4A1 gene whose encoded enzyme was the first ω-hydroxylase characterized. CYP4A11 utilizes NADPH and O_2 to introduce an alcohol to ω-CH_3– of several fatty acids including lauric (12:0), myristic (14:0), palmitic (16:0), oleic (18:1) and arachidonic acid (20:4). Following addition of the ω-hydroxyl the fatty acid is a substrate for alcohol dehydrogenase (ADH) which generates an oxo-fatty acid, followed by generation of the corresponding dicarboxylic acid via the action of aldehyde dehydrogenases (ALDH). Further metabolism then takes place via the β-oxidation pathway in peroxisomes.



Another human CYP4A subfamily member has been identified and designated CYP4A22. This protein is highly homologous with CYP4A11 and has been shown to exhibit lauric acid ω-hydroxylase activity. Expression of CYP4A22 is low in all tissue in which it is found. The CYP4A subfamily is not the only CYP4 family of proteins that have been found to possess ω-hydroxylase activity. The CYP4F family enzyme CYP4F3A, which is expressed in leukocytes, is necessary for the ω-hydroxylation and subsequent degradation of leukotriene B_4 (LTB_4). LTB_4 plays an important role in the modulation of inflammatory processes. The CYP4F3 gene is subject to alternative promoter usage and tissue-specific gene splicing, which results in two different proteins

being produced. These two enzymes are designated CYP4F3A and CYP4F3B, with the latter enzyme being expressed in the liver. CYP4F3B has higher affinity for arachidonic acid.

Another CYP4F family member, identified as CYP4F2, has been identified that also has LTB_4-hydroxylating activity. This CYP4F2 protein has a high degree of homology to the CYP4F3B protein and is expressed in the liver and kidneys. CYP4F2 has been shown to be the major arachidonic acid ω-hydroxylase in human liver and kidney. Indeed, the substrate specificity of CYP4F2 for arachidonic acid is much higher than that of CYP4A11 which was originally described as a signficant arachidonic acid β-hydroxylase. The formation of ω-hydroxylated arachidonic acid (20-hydroxyeicosatetraenoic acid, 20-HETE) by CYP4A11 plays an important role in the regulation of the cardiovascular system because 20-HETE is a known vasoconstrictor. Polymorphisms in the CYP4A11 gene are associated with hypertension in certain population, particular Asian populations. In addition to ω-hydroxylation of arachidonic acid and LTB_4, CYP4F2 has been shown to be responsible for the ω-hydroxylation of the phytyl tail of the tocopherols and tocotrienols (collectively known as vitamin E). Metabolism of vitamin E requires an initial ω-hydroxylation step followed by subsequent β-oxidation.

Additional members of the CYP4F subfamily have been identified in humans. These genes are designated CYP4F8, CYP4F11, and CYP4F12. CYP4F8 is present in epithelial linings and catalyzes the (ω-1)-hydroxylation of prostaglandin H_2 (PGH_2). CYP4F11 is primarily expressed in liver, but also found in kidney, heart, brain and skeletal muscle. The primary endogenous substrates for CYP4F11 are long-chain 3-hydroxydicarboxylic acids (3-OHDCAs) and the enzyme is also very active at hydroxylating various xenobiotics. CYP4F12 is expressed liver, heart, gastrointestinal and urogenital epithelia and its primary substrates are eicosanoids and xenobiotics.

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