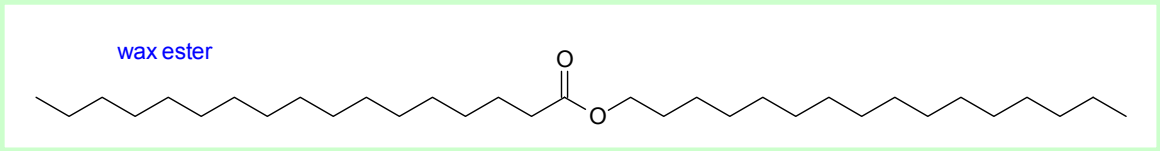
WAXES

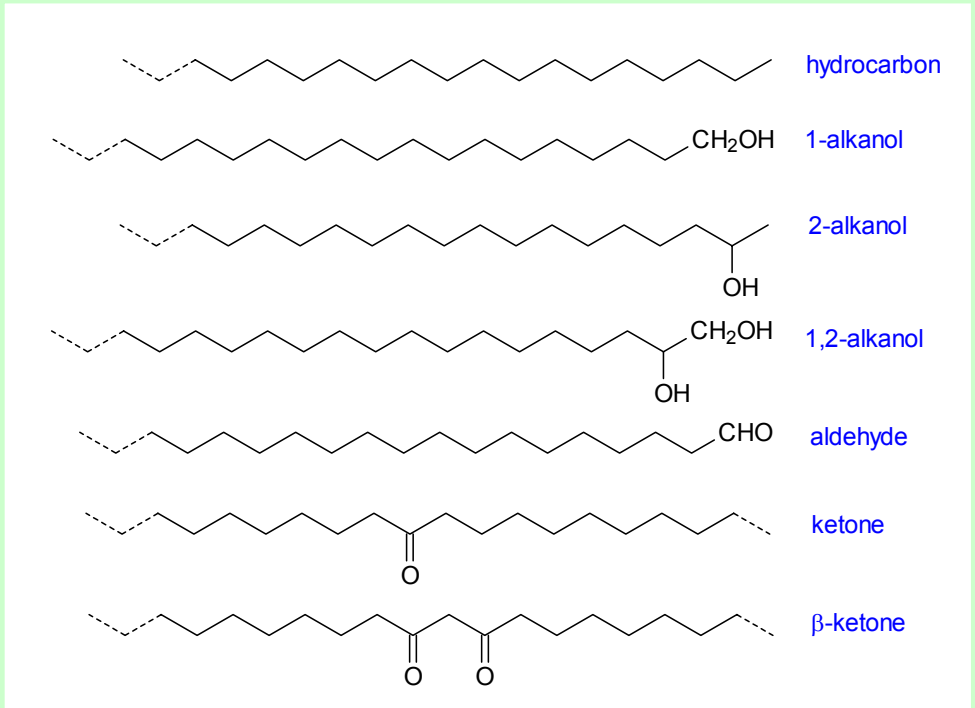
STRUCTURE, COMPOSITION, OCCURRENCE AND ANALYSIS

1. Introduction

To my knowledge, there is no satisfactory definition of the word “wax” in chemical terms. It is derived from the Anglo-Saxon word “weax” for beeswax, so a practical definition of a “**wax**“ may therefore be “**a substance similar in composition and physical properties to beeswax**”. Technologists use the term for a variety of commercial products of mineral, marine, plant and insect origin that contain fatty materials of various kinds. Biochemists link waxes with the thin layer of fatty constituents that cover the leaves of plants or provide a surface coating for insects or the skin of animals. All of these tend to contain wax esters as major components, *i.e*. esters of long-chain fatty alcohols with long-chain fatty acids.



The nature of the other lipid constituents can vary greatly with the source of the waxy material, but they include hydrocarbons, sterol esters, aliphatic aldehydes, primary and secondary alcohols, diols, ketones, β-diketones, triacylglycerols, and many more.



Also, the chain-length and degree of unsaturation and branching of the aliphatic constituents will vary with the origin of the wax, but other than in some waxes of marine origin or from some higher animals, the aliphatic moieties tend to be saturated or monoenoic.

2. Commercial Waxes

A number of waxes are produced commercially in large amounts for use in cosmetics, lubricants, polishes, surface coatings, inks and many other applications. Some of these are of mineral origin (*e.g*. montan wax from brown coal/peat deposits), and only those from living organisms are discussed here. Amongst them are -

Beeswax – Glands under the abdomen of bees secrete a wax, which they use to construct the honeycomb. The wax is recovered as a by-product when the honey is harvested and refined. It contains a high proportion of wax esters (35 to 80%). The hydrocarbon content is highly variable, and much may be "unnatural" as beekeepers may feed some to bees to improve the yield of honey. The wax esters consist of C_{40} to C_{46} molecular species, based on 16:0 and 18:0 fatty acids some with hydroxyl groups in the *omega*-2 and *omega*-3 positions. In addition, some diesters with up to 64 carbons may be present, together with triesters, hydroxypolyesters and free acids (which are different in composition and nature from the esterified acids).

Jojoba - The jojoba plant (*Simmondsia chinensis*), which grows in the semi-arid regions of Mexico and the U.S.A., is unique in producing wax esters rather than triacylglycerols in its seeds, and it has become a significant crop. It consists mainly of 18:1 (6%), 20:1 (35%) and 22:1 (7%) fatty acids linked to 20:1 (22%), 22:1 (21%) and 24:1 (4%) fatty alcohols. Therefore, it contains C_{38} to C_{44} esters with one double bond in each alkyl moiety. As methylene-interrupted double bonds are absent, the wax is relatively resistant to oxidation.

Carnauba – The leaves of the carnauba palm, *Copernicia cerifera* that grows in Brazil, have a thick coating of wax, which can be harvested from the dried leaves. It contains mainly wax esters (85%), accompanied by small amounts of free acids and alcohols, hydrocarbons and resins. The wax esters constitute C_{16} to C_{20} fatty acids linked to C_{30} to C_{34} alcohols, giving C_{46} to C_{54} molecular species.

Other vegetable "waxes" such as bayberry or Japan wax are better described as "tallows" as they consist mainly of high melting triacylglycerols.

Wool wax (lanolin) – The grease obtained from the wool of sheep during the cleaning or refining process is rich in wax esters (of 1- and 2-alkanols, and 1,2-diols), sterol esters, triterpene alcohols, and free acids and sterols. The nature of the product varies with the degree and type of processing involved, but can contain up to 50% wax esters and 33% sterol esters. A high proportion of the sterol component is lanosterol. The fatty acid components are mainly saturated and *iso*- and *anteiso*-methyl-branched-chain.

3. Plant Surface Waxes

Plant leaf surfaces are coated with a thin layer of waxy material that has a myriad of functions. This layer is microcrystalline in structure and forms the outer boundary of the cuticular membrane; it is the interface between the plant and the atmosphere. It serves many purposes, for example to limit the diffusion of water and solutes, while permitting a controlled release of volatiles that may deter pests or attract pollinating insects. It provides protection from disease and insects, and helps the plants resist drought. As plants cover much of the earth's surface, it seems likely that plant waxes are the most abundant of all natural lipids.

The range of lipid types in plant waxes is highly variable, both in nature and in composition, and Table 1 illustrates some of this diversity in some of the main components.

Table 1. The major constituents of plant leaf waxes.

Compound	Structure	
n-Alkanes	$CH_3(CH_2)_xCH_3$	21 to 35C – odd numbered
Alkyl esters	$CH_3(CH_2)_xCOO(CH_2)_yCH_3$	34 to 62C – even numbered
Fatty acids	$CH_3(CH_2)_xCOOH$	16 to 32C – even numbered
Fatty alcohols (primary)	$CH_3(CH_2)_yCH_2OH$	22 to 32C – even numbered
Fatty aldehydes	$CH_3(CH_2)_yCHO$	22 to 32C – even numbered
Ketones	$CH_3(CH_2)_xCO\ (CH_2)_yCH_3$	23 to 33C – odd numbered
Fatty alcohols (secondary)	$CH_3(CH_2)_xCHOH\ (CH_2)_yCH_3$	23 to 33C – odd numbered
β-Diketones	$CH_3(CH_2)_xCOCH_2CO\ (CH_2)_yCH_3$	27 to 33C – odd numbered
Triterpenols	Sterols, *alpha*-amyrin, *beta*-amyrin, uvaol, lupeol, erythrodiol	
Triterpenoid acids	Ursolic acid, oleanolic acid, etc	

In addition, there may be hydroxy-β-diketones, oxo-β-diketones, alkenes, branched alkanes, acids, esters, acetates and benzoates of aliphatic alcohols, methyl, phenylethyl and triterpenoid esters, and many more.

The amount of each lipid class and the nature and proportions of the various molecular species within each class vary greatly according to the plant species and the site of wax deposition (leaf, flower, fruit, *etc*.) and some data for some well-studied species are listed in Table 2.

Table 2. Relative proportions (wt %) of the common wax constituents in some plant species.

	Grape leaf	Rape leaf	Apple fruit	Rose flower	Pea leaf	Sugar cane stem
Hydrocarbons	2	33	20	58	40-50	2-8
Wax esters	6	16	18	11	5-10	6
Aldehydes	6	3	2	-	5	50
Ketones	-	20	3		-	-
Secondary alcohols	-	8	20	9	7	-
Primary alcohols	60	12	6	4	20	5-25
Acids	8	8	20	5	6	3-8

Other components present include various diol types and triterpenoid acids

4. Skin Lipids

In most animals, the main wax production is associated with the sebaceous glands of the skin. Most of these glands are associated with hair follicles, but there are also related structures on the eyelids termed Meibomian glands. Sebaceous glands secrete mainly non-polar lipids in the form of sebum onto the skin surface, where they are easily recovered for analysis. Although relatively few species have been studied in real detail, it is evident that a wide range of lipid classes are present and that these vary greatly in amount and nature between species (there may also be variation

with age). The composition of human sebum differs appreciably from that of other species, especially in the high content of triacylglycerols and in fatty acid composition. Some typical data are listed in Table 3.

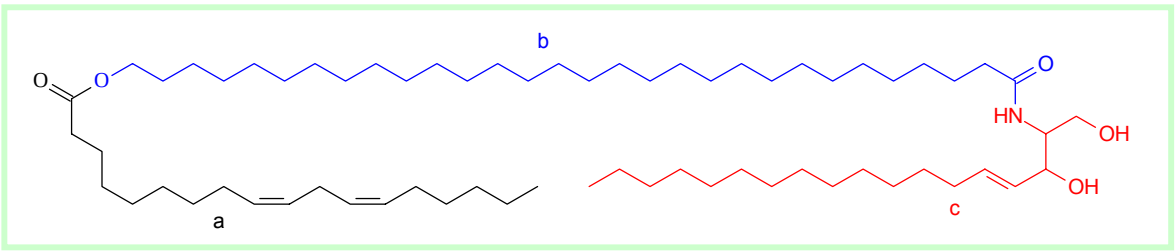
Table 3. Relative composition (wt % of the total) of the non-polar lipids from the skin surface of various species.

	Squalene	Sterols	Sterol esters	Wax esters	Diesters	Glyceryl ethers	Triacyl-glycerols	Free acids	Free alcohols
Human	12	1	3	25			41	16	
Sheep		12	46	10	21				11
Rat	1	6	27	17	21	8		1	
Mouse		13	10	5	65		6		

Adapted from Downing, D.T. Mammalian waxes. In: *Chemistry and Biochemistry of Natural Waxes*. (Ed. P.E. Kolattukudy, Elsevier, Amsterdam) (1976).

Sebaceous glands appear to be the only source of wax esters in mammalian tissues and the only tissue where squalene accumulates in significant amounts. A high proportion of the fatty acid constituents of sebum lipids can be branched chain, which are not common in other organs. Human sebum is unique in containing *cis*-6-hexadecenoic acid (6-16:1 or 'sapienic' acid), which is the single most abundant component indeed, and is accompanied by an elongation and desaturation product 5,8-octadecadienoic acid ('sebaleic' acid), also unique to human skin. Sapienic acid is formed in the sebaceous glands by a distinctive Δ6 desaturase and has powerful antibacterial properties.

Skin also contains a wide range of more polar lipids based on the ceramide backbone. They have been most studied in the skin of the pig, where a range of unusual ceramides *per se* have been identified, some of which contain linoleic acid (a) esterified to a hydroxy acid (b) that is in turn linked to a long-chain base (c). In addition, several molecular forms of glucosylceramide, based on similar structures, have been characterized.



The composition depends on the particular layer of the skin (epidermis, stratum corneum, *etc*.). Whether they should truly be called waxes is doubtful. See our web-pages on **ceramides** for further information.

5. Other Waxes

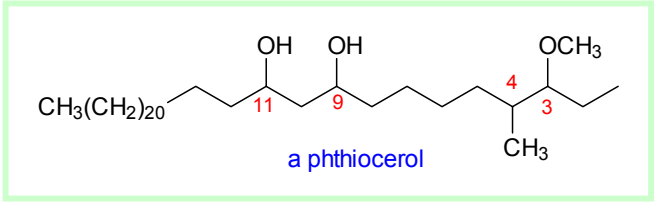
Insect waxes. The surface of insects is covered by a layer of wax that, amongst other functions, serves to restrict movement of water across the cuticle and prevent desiccation. The nature of this lipid is dependent on species, but in general a high proportion tends to be saturated alkanes (C_{23} to C_{31}) often with one or two methyl branches. In addition, wax esters, sterol esters, and free fatty alcohols and acids may be present. Some species of insect secrete triacylglycerols in their waxes

together with free sterols and other terpenoid components. The composition of beeswax is discussed above.

Marine waxes. Many marine animals from invertebrates to whales contain appreciable amounts of waxes in the form mainly of hydrocarbons and wax esters. In addition, glycerol ethers and sterols could be classified as wax components in some species. They are found in a variety of tissues from fish roe, to liver and muscle tissues. The wax esters consist of the normal range of saturated, monoenoic and polyunsaturated fatty acids typical of fish, esterified to mainly saturated and monoenoic alcohols often with the 18:1 fatty alcohol as the main component. Squalene and other terpenoid hydrocarbons are often major components of the hydrocarbon fraction, and can be accompanied by saturated (straight-chain and methyl-branched), monoenoic and polyenoic components. Waxes appear to have a variety of functions in fish, from serving as an energy source to insulation, buoyancy and even echo location. Spermaceti or sperm whale oil (wax esters, 76%; triacylglycerols, 23%) was once in great demand as a lubricant but now is proscribed.

Bird waxes. The uropygial glands of birds secrete waxes that consist largely of wax esters. The fatty alcohol components of these are usually relatively simple in nature, consisting largely of normal C_{16} and C_{18} saturated compounds, although those with branched-chains can make up an appreciable proportion in some species. However, the fatty acids can be highly complex and are often shorter chain than usual with up to four methyl branches. The main purpose of the waxes is presumed to be to give a water-proof layer to the feathers, but other functions have been suggested.

Microbial waxes. Waxes are not common in prokaryotes, but the mycobacteria produce waxes, termed 'mycoserosates', based on branched-chain alcohols or phthiocerols. These are C_{34} or C_{36} branched-chain compounds with hydroxyl groups in positions 9 and 11, or 11 and 13. These are esterified with long-chain fatty acids varying in chain length from C_{18} to C_{26} and with two to four methyl branches that may occur at the 2, 4, 6, and 8 positions. The dimycocerosate esters are major virulence factors of pathogenic mycobacteria including *Mycobacterium tuberculosis* and *M. leprae*.



a phthiocerol

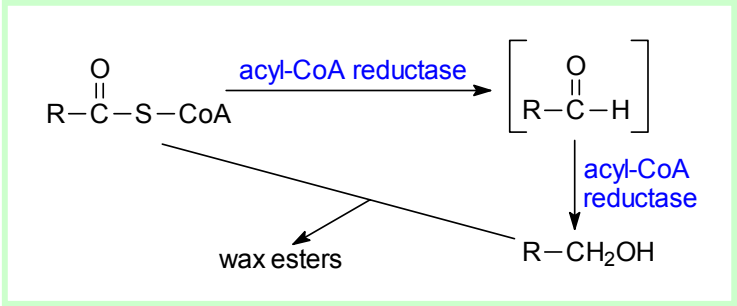
Related structures have a phenol group at the terminus of the alkyl chain, and the hydroxyl of this can be attached to carbohydrate moieties of varying complexity. Mycobacteria are unusual in a number of other ways in that their lipids can contain **triacylglycerols**, and the distinctive **mycolic acids**, for example.

6. Biosynthesis of Waxes

Because of their biochemical importance and relative ease of study, the waxes of the plant cuticle have received most study. All the aliphatic components of plant waxes are synthesised in the epidermal cells from saturated very-long-chain fatty acids (commonly C_{20} to C_{34}). 16:0 and 18:0 fatty acids are first synthesised in the stroma of plastids by the soluble enzymes forming the fatty acid synthase complex (see our web page on **fatty acid biosynthesis**). The second stage involves multiple elongation steps and is catalysed by membrane-associated multi-enzyme complexes, known as fatty acid elongases outwith the plastids. As in fatty acid synthesis de novo, each two-carbon extension of the chain involves four reactions: condensation between a CoA-esterified fatty acyl substrate and malonyl-CoA, followed by a β-keto reduction, dehydration and an

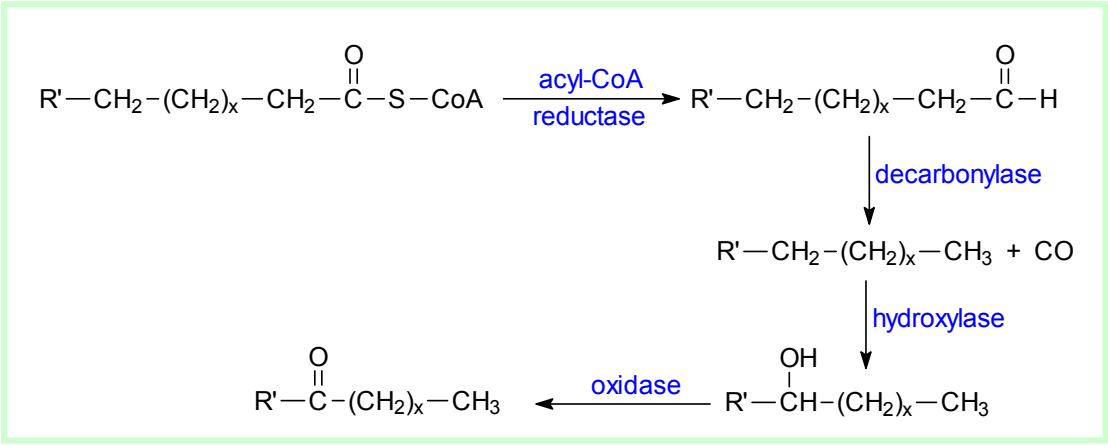
enoyl reduction. Many different forms of the elongases have been identified, and these must interact in some manner to produce the chain-length specificity observed.

There are then two main pathways for biosynthesis of wax components: an acyl reduction pathway, which yields primary alcohols and wax esters, and a decarbonylation pathway that results in synthesis of aldehydes, alkanes, secondary alcohols and ketones. In the reductive pathway, acyl-CoA esters produced by chain elongation are reduced in a two-step process via a transient aldehyde intermediate, catalysed by a single enzyme, an acyl-CoA reductase (though it was once thought that two distinct enzymes were involved).



The fatty alcohol produced can then be esterified via an acyl-CoA alcohol transacylase to form a wax ester. Similar mechanisms have been observed in studies with insects, algae and birds (uropygial glands). It seems probable that wax diols are produced by insertion of a hydroxyl group into the alkyl chain of an acyl-CoA precursor.

In the decarbonylation pathway for the synthesis of wax constituents, the first step is again the reduction of acyl-CoA ester to an aldehyde by means of an acyl-CoA reductase. Removal of the carbonyl group by an aldehyde decarbonylase yields an alkane, with one fewer carbon atom than the fatty acid precursor.



Further metabolism of the hydrocarbon is then possible, for example by insertion of a hydroxyl group into the chain via a hydroxylase or mixed-function oxidase to form a secondary alcohol. The position of the substitution depends on the species, and the specificities of the enzymes involved. Secondary alkanols can in turn be esterified to form a wax ester. Alternatively, the hydroxyl group can be oxidized with formation of a long-chain ketone. An associated pathway leads to the formation of β-diketones and 2-alkanols. Again, these processes have been studied most in plants, but similar biochemical reactions appear to occur in insects and birds.

The final step in the production of wax esters from long-chain alcohols and fatty acids involves the action of an acyl-CoA:alcohol transacylase. In animal tissues, this is an enzyme that is also required for triacylglycerol biosynthesis, i.e. an acyl CoA:diacylglycerol acyltransferase (DGAT) or

more specifically an isoform of the enzyme known as DGAT1. The same enzyme synthesises retinyl esters also. Similarly, in prokaryotes, an enzyme that is structurally distinct from that in animals is both a diacylglycerol acyltransferase and a wax ester synthase. In plants, C_{16} and C_{18} fatty acids from the fatty acid synthetase are converted to CoA esters and subjected to chain elongation prior to wax formation. An active fatty acyl CoA:fatty alcohol acyltransferase has been isolated from microsomal fractions of seeds of the jojoba plant that is responsible for production of the storage wax, but also appears to be structurally related to the wax synthases involved in the synthesis of the epicuticular waxes.

7. Analysis

Thin-layer and high-performance liquid chromatography have been used widely to isolate individual classes of waxes for more detailed analysis. On the other hand, much of the more recent published work has made use of high-temperature gas chromatography following trimethylsilylation, often in combination with mass spectrometry, so that simultaneous identification and quantification of the various molecular species can be achieved.

Further Reading

The best sources of information on waxes, from which much of the data in this web document have been culled, are the books (now out of print, unfortunately) –

- Kolattukudy, P.E. (Editor) Chemistry and Biochemistry of Natural Waxes. (Elsevier, Amsterdam) (1976).
- Hamilton, R.J. (Editor) Waxes: Chemistry, Molecular Biology and Functions. (The Oily Press, Dundee) (1995).

In addition -

- Christie, W.W. *Lipid Analysis (3rd edition)*. (Oily Press, Bridgwater) (2003).
- Kunst, L. and Samuels, A.L. Biosynthesis and secretion of plant cuticular wax. *Prog. Lipid Res.*, **42**, 51-80 (2003).
- Moldovan, Z., Jover, E. and Bayona, J.M. Systematic characterisation of long-chain aliphatic esters of wool wax by gas chromatography-electron impact ionisation mass spectrometry. *J. Chromatogr. A*, **952**, 193-204 (2002) – and references therein.
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- Tulloch, A.P. Beeswax: structure of the esters and their component hydroxy acids and diols. *Chem. Phys. Lipids*, **6**, 235-265 (1971).

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