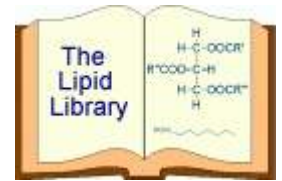


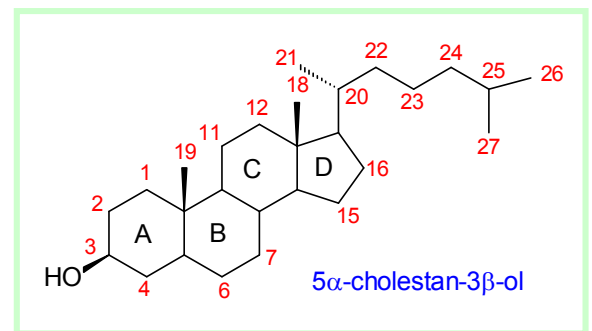
# STEROLS 3. PLANT STEROLS AND RELATED LIPIDS



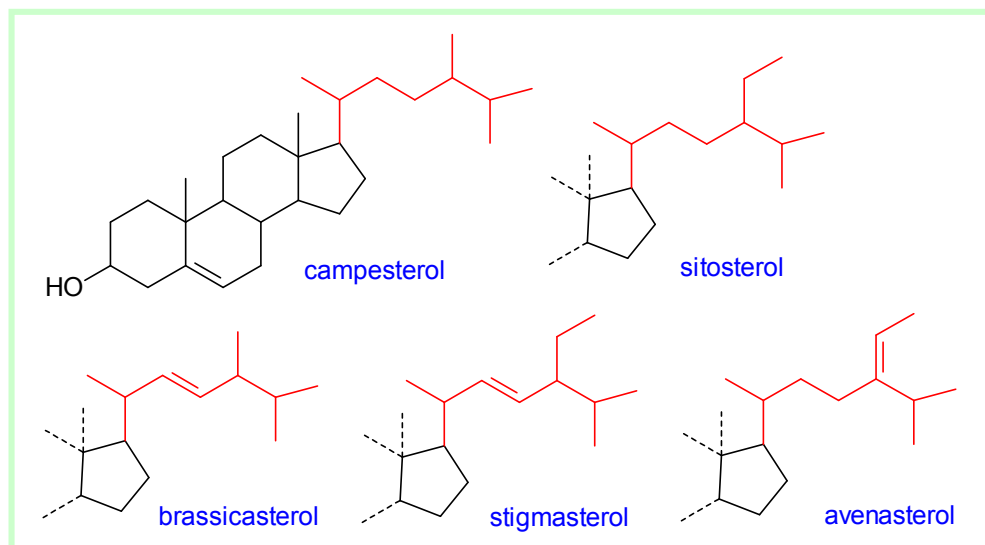
## STRUCTURE, OCCURRENCE, BIOCHEMISTRY AND ANALYSIS

### 1. Plant Sterols

Plants contain a rather different range of sterols from animals. Like **cholesterol**, to which they are related structurally and biosynthetically, plant sterols form a group of triterpenes with a tetracyclic cyclopenta[*a*]phenanthrene structure and a side chain at carbon 17. The four rings (A, B, C, D) have *trans* ring junctions, and the side chain and two methyl groups (C-18 and C-19) are at an angle to the rings above the plane with  $\beta$  stereochemistry (as for the hydroxyl group on C-3 also). The basic sterol from which other sterol structures are defined is 5 $\alpha$ -cholesten-3 $\beta$ -ol.



The phytosterols (as opposed to zoosterols) include campesterol,  $\beta$ -sitosterol, stigmasterol and  $\Delta^5$ -avenasterol, some of which are illustrated below. These more common plant sterols have a double bond in position 5, and a definitive feature – a one- or two-carbon substituent with variable stereochemistry in the side chain at C-24, which is preserved during subsequent metabolism. Occasionally, there is a double bond in this chain that can be of the *cis* or *trans* configuration. Phytosterols can be further classified on a structural or biosynthetic basis as 4-desmethyl sterols (i.e. with no substituent on carbon-4), 4 $\alpha$ -methyl sterols and 4,4-dimethyl sterols. In addition, the 4-desmethyl sterols may be subdivided into  $\Delta^5$ -sterols,  $\Delta^7$ -sterols and  $\Delta^5,7$ -sterols depending on the position of the double bonds in the B ring. As the name suggests, brassicasterols are found mainly in the brassica family of plants. Phytosterols (fully saturated) are normally present at trace levels only in plants, but they are relatively abundant in cereal grains.



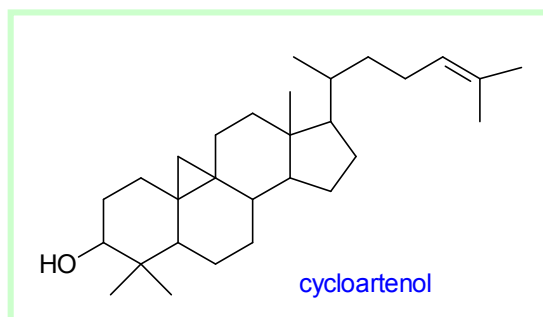
Many different sterols may be present in plant species (over 200 have been characterized), and the amounts and relative proportions of all are dependent on the plant species. As a rough generality, a typical plant sterol mixture would be 70% sitosterol, 20% stigmasterol and 5% campesterol. Table 1 contains data from some representative commercial seed oils. Cholesterol is usually a minor component only of plant sterols, but it is unwise to generalize too much as it can be the main sterol of red algae and of some families of higher plants (Solanaceae, Liliaceae, Scrophylariaceae), for example. It can also be a significant constituent sterol of chloroplasts, shoots, pollen and leaf surface lipids in other plant families. Some tissues can contain unique sterols; thus pollen may contain pollinastanol. Yeasts and fungi tend to contain ergosterol as their main sterol component (see below).

**Table 1. Sterol composition in some seed oils of commercial importance (mg/Kg).**

	Corn oil	cottonseed	olive	palm	rapeseed	safflower	soybean	sunflower
cholesterol	-	-	-	26	-	-	-	-
campesterol	2691	170	28	358	1530	452	720	313
stigmasterol	702	42	14	204	-	313	720	313
$\beta$ -sitosterol	7722	3961	1310	1894	3549	1809	1908	2352
$\Delta$ 5-avenasterol	468	85	29	51	122	35	108	156
$\Delta$ 7-stigmastenol	117	-	58	25	306	696	108	588
$\Delta$ 7-avenasterol	-	-	-	-	-	104	36	156
brassicasterol	-	-	-	-	612	-	-	-
other	-	-	-	-	-	69	-	39

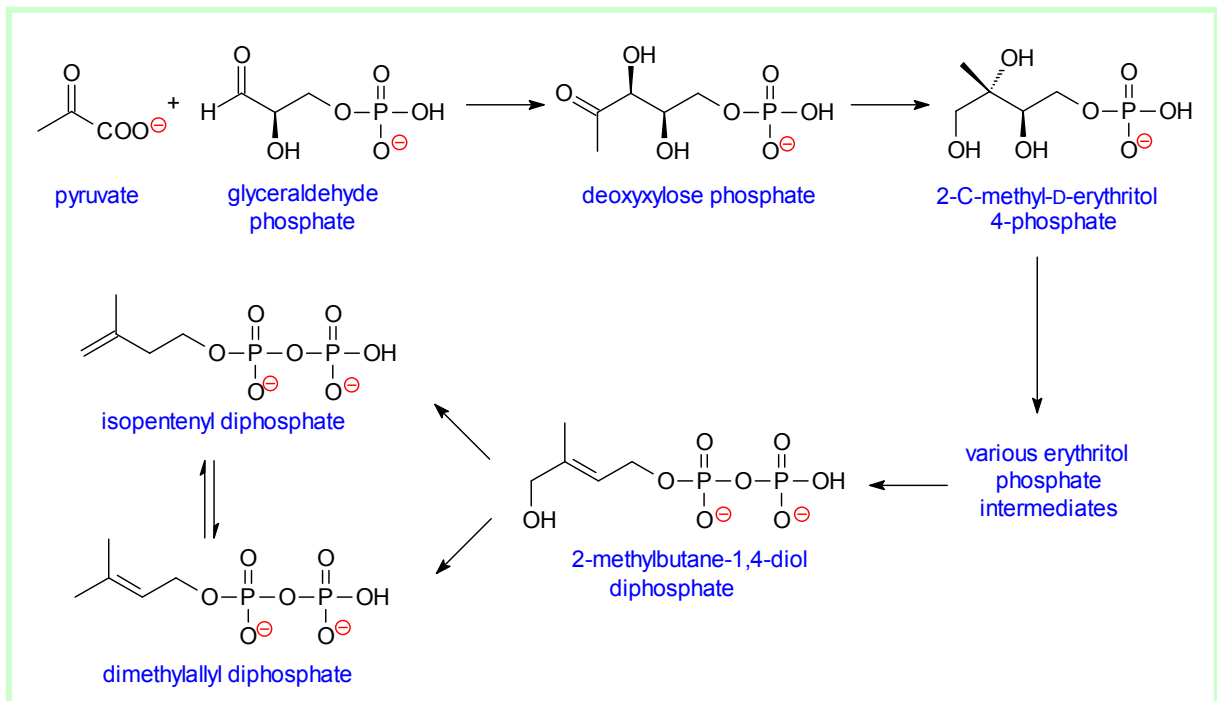
Data from Gunstone, F.D., Harwood, J.L. and Padley, F.B. *The Lipid Handbook (Second Edition)* (Chapman & Hall, London) (1994).

The biosynthetic route to plant sterols resembles that to cholesterol in many aspects in that it follows an isoprenoid biosynthetic pathway with isopentenyl pyrophosphate, derived primarily from mevalonate, as the key building block in the cytoplasm (but not plastids) at least. However, in photosynthetic organisms (as opposed to yeast and fungi), it differs in that the important intermediate in the route from squalene is **cycloartenol** rather than lanosterol. Then, the enzyme sterol methyltransferase 1 is of special importance in that it converts cycloartenol to 24-methylene cycloartenol, as the first step in introducing the methyl group onto C-24. There are more than thirty enzyme-catalysed steps in the overall process, each associated with membranes. The 4,4-dimethyl- and 4 $\alpha$ -methylsterols are part of the biosynthetic pathway, but are only minor if ubiquitous sterol components of plants.



In addition, an alternative pathway for the biosynthesis of isopentenyl pyrophosphate and dimethylallyl pyrophosphate, the isoprene units, which does not use mevalonic acid as a precursor has been found in plant chloroplasts, algae, cyanobacteria, eubacteria, and some parasites, but not in animals. This route is variously termed the 'non-mevalonate', '1-deoxy-D-xylulose-5-phosphate' (DOXP) or better the '2C-methyl-D-erythritol 4-phosphate (MEP)' pathway. In the first step, pyruvate and glyceraldehyde phosphate are combined to form deoxyxylose phosphate, which is in turn converted to 2C-methyl-D-erythritol 4-phosphate. The pathway then proceeds via various erythritol intermediates until isopentenyl pyrophosphate and dimethylallyl pyrophosphate are

formed, when sterol biosynthesis thereafter continues via squalene and cycloartenol. There is evidence that some of the isoprene units are exchanged between the cytoplasm and plastids.



Like cholesterol, plant sterols are amphiphilic and are vital constituents of all membranes, and especially of the plasma membrane, the mitochondrial outer membrane and the endoplasmic reticulum. The three-dimensional structure of the plant sterols is such that there are planar surfaces at both the top and the bottom of the molecules, which permit multiple hydrophobic interactions between the rigid sterol and the other components of membranes. Indeed, they must govern the physical properties of membranes to an appreciable extent. It is believed that sitosterol and 24-methylcholesterol are able to regulate membrane fluidity and permeability in plant membranes by restricting the mobility of fatty acyl chains in a similar manner to cholesterol in mammalian cells. They may be involved in how plant membranes adapt to changes in temperature. Stigmasterol has much less effect on lipid ordering and no effect on the permeability of membranes. In the plasma membrane, plant sterols associate with the glycosphingolipids such as glucosylceramide in **raft**-like subdomains, analogous to those in animal cells.

Plant sterols can modulate the activity of membrane-bound enzymes. Thus, stigmasterol and cholesterol regulate the activity of the  $\text{Na}^+/\text{K}^+$ -ATPase in plant cells, probably in a manner analogous to that of cholesterol in animal cells. Stigmasterol may be required specifically for cell differentiation and proliferation. Certain sterols, such as campesterol in *Arabidopsis thaliana*, in minute amounts are precursors of oxidized steroids that act as growth hormones and are collectively named **brassinosteroids**, which have crucial importance for growth and development. Cholesterol is a precursor for the biosynthesis of some steroidal saponins and alkaloids, as well as of other sterols including the ecdysteroids (insect moulting hormones).

Substantial amounts of phytosterols are available as by-products of the refining of vegetable oils and of tall oil from the wood pulp industry. There is increasing interest in such commercial sources of plant sterols to be added as “nutraceuticals” to margarines and other foods, as it appears that they can inhibit the uptake of cholesterol from the diet and thereby reduce the levels of this in the plasma low-density lipoproteins. Hydrogenated phytosterols or “stanols” are also used for this purpose. In addition, dietary supplements of plant sterols have been reported to have anti-cancer effects. The actual absorption of dietary plant sterols and stanols in humans is low (0.02-3.5%) compared to cholesterol (35-70%). In some rare cases, increased levels of plant sterols in plasma serve as markers for an inherited lipid storage disease (phytosterolemia). There is evidence that

while plant sterols can substitute for cholesterol in maintaining membrane function in mammalian cells, they can exert harmful effects by disrupting cholesterol homeostasis.

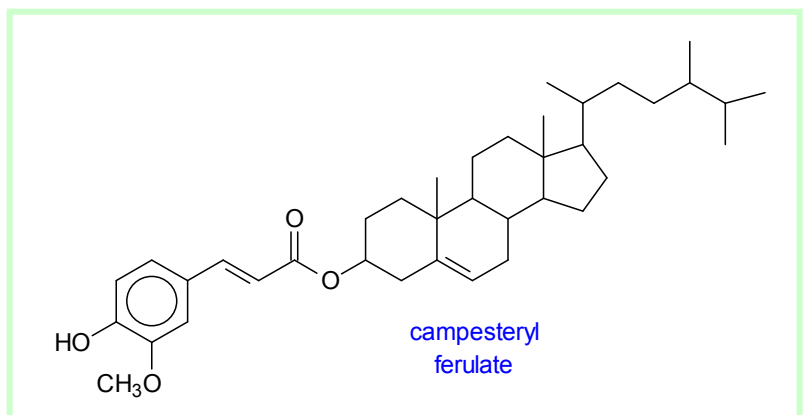
Phytosterols can be subjected to autoxidation in a similar manner to that of cholesterol in animals, resulting in ring products such as hydroxy-, keto-, epoxy- and triol-derivatives. In addition, enzymic reactions can oxidize the side chain. These oxy-phytosterols can enter the food chain and, although they are not efficiently absorbed, they have been detected in human plasma and have the potential to exert a variety of biological effects.

## 2. Sterol Esters

**Sterol esters** are present in plant tissues, but as minor components relative to the free sterols other than in waxes. Usually the sterol components of sterol esters are similar to the free sterols, although there may be relatively less of stigmasterol. The fatty acid components tend to resemble those of the other plant tissue lipids, but there can be significant differences on occasion. Sterol esters are presumed to serve as inert storage forms of sterols, as they are often enriched in the intermediates of sterol biosynthesis and can accumulate in lipid droplets within the cells. However, they have been found in membranes, especially in microsomes and mitochondrial preparations although their function here is uncertain. They may also have a role in transport within cells and between tissues, as they have been found in the form of soluble lipoprotein complexes.

Biosynthesis of sterol esters in *Arabidopsis thaliana* is known to occur in the endoplasmic reticulum and involves transfer of a fatty acyl group to the sterol from position *sn*-2 of phosphatidylethanolamine. However, other enzymes may exist but have yet to be characterized. In yeasts, two sterol ester synthases have been identified that utilize CoA esters of fatty acids as substrate. Little appears to be known of the catabolism of sterol esters in plants, but specific sterol ester hydrolases have been characterized from yeasts.

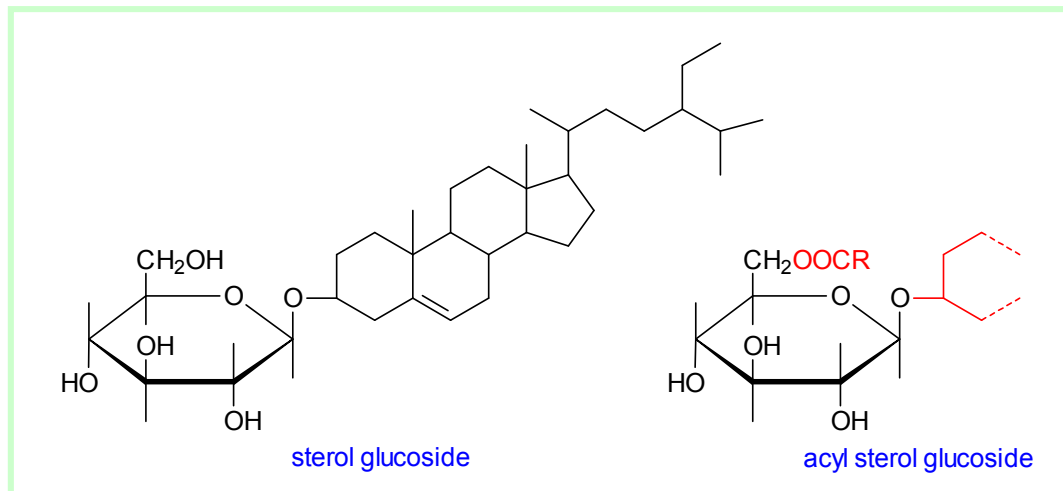
Certain distinctive phytosterol esters occur in the aleurone cells of cereal grains, including *trans*-hydroxycinnamate, ferulate (4-hydroxy-3-methoxy cinnamate) and *p*-coumarate esters. Similarly, rice bran oil is a rich source of esters of ferulic acid and a mixture of sterols and triterpenols, termed ' $\gamma$ -orizanol', and an example of one of these compounds is illustrated. This is sold as a health food supplement, because of claimed beneficial effects, including cholesterol-lowering and antioxidant activities. It is also reputed to enhance muscle growth and sports performance. However, none of these effects have been confirmed by rigorous clinical testing.



## 3. Sterol Glycosides

Leaf and other tissues in plants contain a range of **sterol glycosides** and **acyl sterol glycosides**, and typical examples (glucosides of  $\beta$ -sitosterol) are illustrated. Most of the common plant sterols occur in this form, and the carbohydrate moieties (glucose, xylose, arabinose) can vary also with the plant species. The carbohydrate moiety can sometimes be quite complex with up to five hexose units linked in a linear fashion. Similarly, the nature of the fatty acid component in the acyl

sterol glycosides can vary as well as the hydroxyl group to which they are linked, although it is usually position 6. In potato tubers, for example, the 6'-palmitoyl- $\beta$ -D-glucoside of  $\beta$ -sitosterol is the major species, while the corresponding linoleate derivative predominates in soybeans. Usually, the acyl sterol glycosides are present at concentrations that are two to ten fold greater than those of the non-acylated forms.



Plant, animal, fungal and most bacterial sterol glycosides have a  $\beta$ -glycosidic linkage, but in a few bacterial species there is an  $\alpha$ -linkage.

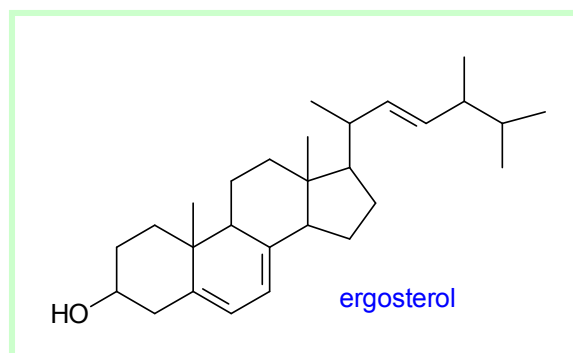
Sterol glycosides and acyl sterol glycosides may be involved in the adaptation of plant membranes to low temperatures. Biosynthesis involves reaction of free sterols with uridine diphosphoglucose (UDP-glucose) in the presence of a UDP-glucose:sterol glucosyltransferase in plant membranes, probably the plasma membrane. Sitosterol- $\beta$ -D-glucoside in the plasma membrane is the primer molecule for cellulose synthesis in plants and it is required for the initiation of glucan polymerization. The sterol is eventually removed from the polymer by a specific cellulase enzyme.

Sterol glycosides have only rarely been reported from organisms other than plants, although cholesteryl glucoside has been found in a few animal tissues. The gram-negative bacterium *Helicobacter pylori*, one of the most common human pathogens, is an important exception in that it contains appreciable amounts of unique cholesterol glucosides, including cholesteryl-6-O-acyl- $\alpha$ -D-glucopyranoside, in its cell walls. *Borrelia burgdorferi*, the causative agent of Lyme disease, and related organisms produce 6-O-acylated cholesteryl  $\beta$ -D-galactopyranosides, which may act as immunogens. It is known that the cholesterol of these lipids is obtained from an animal host and is not synthesised by bacteria.

In addition, a number of species of monocotyledons contain complex steroidal **saponins**, which consist of a furostanol- or spirostanol-based aglycone and an oligosaccharide chain of two to five hexose or pentose moieties attached to the 3-hydroxyl group of the sterol. Further conjugates of this type include other triterpenes with saccharide units linked at various positions.

#### 4. Sterols in Yeasts and Fungi

Yeasts and fungi, together with microalgae and protozoa, can contain an enormous range of different sterols. **Ergosterol** ((22E)-ergosta-5,7,22-trien-3 $\beta$ -ol) is the main sterol in fungi and yeasts, and is accompanied by other sterols not normally abundant in higher plants including zymosterol (5 $\alpha$ -cholesta-8,24-dien-3 $\beta$ -ol). Like cholesterol and in contrast to the plant sterols, it is synthesised via lanosterol as the key intermediate. Some antifungal drugs are targeted against ergosterol biosynthesis. Under some conditions, especially those that retard growth, a high proportion of the sterols in yeasts can be in esterified form.



Many mutants defective in ergosterol biosynthesis have been isolated, and these have yielded a great deal of information on the features of the sterol molecule required for its structural role in membranes of yeast and fungi. Ergosterol stabilizes the liquid-ordered phase in the same manner as cholesterol, and also forms rafts with sphingolipids, whereas lanosterol does not. It is also evident that ergosterol has a multiplicity of functions in the regulation of yeast growth.

Ergosterol esters are synthesised in yeast by enzymes (ARE1 and ARE2) related to ACAT1 and ACAT2 that perform this function in animals.

Protozoans also synthesise many different sterols. For example, some species of *Acanthamoeba* and *Naegleria* produce both lanosterol and cycloartenol, but only latter is used for synthesis of other sterols, especially ergosterol. In some other protozoan species, sterol biosynthesis occurs via lanosterol.

## 5. Bacterial Sterols

It has long been recognised that some bacteria take up cholesterol and other sterols from host animals for use as membrane constituents. Indeed, an external source of sterols is required for growth in species of *Mycoplasma*. In addition, there have been a number of reports of biosynthesis of sterols by various bacterial species, although a high proportion of these appear now to have been discounted because of fungal contamination. In particular, the possibility of sterol biosynthesis in cyanobacteria has been controversial, and molecular biology studies have yet to detect the presence of the required enzyme squalene epoxide cyclase.

That said, there is good evidence that a few species of prokaryotes at least have the capacity to synthesise sterols *de novo*. Among the eubacteria certain methylotrophs (*Methylobacterium* and *Methylosphaera* species) produce mono- and dimethyl sterols, including lanosterol. Similarly, some soil bacteria produce 4-desmethylsterols. It has now been established from gene sequence studies that certain bacteria contain enzymes of the sterol biosynthesis pathway, but as these have no obvious evolutionary link it seems probable that they were acquired via lateral transfer from eukaryotes. Of course, **hopanoids** take the place of sterols in many more species of bacteria.

## 6. Analysis

In the analysis of animal and plant sterols, a sterol fraction is first isolated from lipid extracts by thin-layer or column chromatography, following hydrolysis if necessary. Individual components can then be determined by gas chromatography in the presence of an internal standard (e.g. epicoprostanol or betulin), often after conversion to trimethylsilyl ether derivatives to give sharper peaks. Mass spectrometry may be required for identification of individual components. Analysis of the minor oxysterols that may be found in plasma or foods is a rather specialized task, because

they tend to be present at rather low levels and there is a danger of further oxidation or side reactions during the analytical process. Rigorous attention to detail is necessary for meaningful results.

Sterol esters are trans-methylated for GC analysis of the fatty acid components, although the reaction may again be much slower than with glycerolipids. Intact sterol esters are best analysed by reversed-phase HPLC. Analysis of sterol glycosides is a more specialized endeavour that can be more concerned with carbohydrate than with lipid chemistry.

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