XIII. CONTRIBUTIONS TO OUR KNOWLEDGE OF THE PLANT STEROLS. PART I. THE STEROL CONTENT OF WHEAT (*TRITICUM SATIVUM*).

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IN accounts of the chemical analysis of plants, "a phytosterol" is often mentioned among the constituents. Particularly noteworthy in this connection are the researches of Power and his co-workers at the Wellcome Chemical Research Laboratories.

Descriptions of the constituents of plants or parts of plants of pharmaceutical importance have appeared during recent years in the *Journal of the Chemical Society*—and in pharmaceutical journals—and it would seem from these that phytosterol is present in seeds, roots, leaves, shoots, flowers—in latex and in bark.

In addition to this the phytosterols from special sources have been examined very carefully by various authors. The substances so described are isomeric with the animal cholesterol, in most cases having the formula $C_{27}H_{46}O$, but included in the group are certain other compounds possessing slightly different formulae which may be regarded as very nearly related. Chemically, like cholesterol, they react as unsaturated alcohols forming bromine additive compounds, and esters. They are generally optically active, and they give the cholesterol colour reactions—though sometimes in a modified form. The two chief tests of this kind are:

(1) *The Salkowski Test*—in which a few mg. of the substance are dissolved in about 2 cc. of chloroform and shaken with an equal bulk of strong sulphuric acid. The chloroform develops a blood-red or cherry-red colour which it retains for several days. On pouring this solution into a basin, the colour changes to blue, green, and then yellow, owing to absorption of water. On addition of sulphuric acid, the original colour is restored. The sulphuric acid layer is distinctly fluorescent.

(2) *The Burchardt-Liebermann Test.* A few mg. of the substance are dissolved in 2 cc. of chloroform, 20 drops of acetic anhydride added, and one drop of concentrated sulphuric acid. A violet pink colour appears. This test will detect very small traces of sterol, but it is also given by resins.

Phytosterols usually have a characteristic crystalline form. From hot alcoholic solution, bundles of glistening crystals appear on cooling, and, when slowly crystallised, these are found to be well-defined hexagonal plates.

Whereas cholesterol itself is found to be a constant constituent of animal protoplasm, in the vegetable kingdom it seems that a number of isomers exist, differing from one another to a slight extent in their properties, notably in their melting points and the melting points of their esters. These substances form mixtures which crystallise as if only a single substance were present. Mixtures of the plant sterols with cholesterol (which has not so far been found in plants) behave in the same way. Many of the substances described melt between 130° and 138° —but compounds with higher melting points have been described.

The differences in these substances are not much greater, however, than those observed in the case of the five dihydrocholesterols which have been described and which can easily be changed one into the other, as has been shown by Dorée and Gardner [1908, 1] in the case of coprosterol and ψ -coprosterol and in the case of the rest by Windaus and his co-workers [Windaus and Uibrig, 1914, 1915].

According to the researches of Windaus [1916] these bodies possess in a high degree the capacity of crystallising together so as to simulate a single compound. The various isomers in Windaus' opinion differ only in the stereoposition of H and OH groups with regard to an asymmetric carbon atom.

These varying plant sterols, as already indicated, are very widely distributed. The best known perhaps are those occurring in:

(1) *Seeds.* Hesse [1878] gave the name "phytosterol" to a substance present in Calabar beans, to which he assigned the formula $C_{26}H_{44}O + H_2O$. It melted at 132 – 133° and formed an acetate, M.P. 120° .

A similar substance had been described by earlier writers, Beneke [1862] in seed peas, Ritthausen [1863] in wheat gluten, Lindenmeyer [1863] in peas, but these authors considered it to be cholesterol. After that, a number of papers appeared describing phytosterols melting between 132° and 137° .

The formulae assigned often differ slightly from $C_{27}H_{46}O$, but the method of combustion is not entirely conclusive unless many determinations of the substance and as many esters as possible are made, since the percentage

carbon and hydrogen content would differ but little in compounds of such high molecular weight.

The phytosterols described are isolated in very small quantities, and one needs a relatively large quantity for satisfactory investigation.

Jacobson [1888] described the phytosterol of peas and beans, and in 1897 a great advance was made by Burian [1897] who isolated from wheat and rye embryos a substance which he named sitosterol, and considered to be isomeric with cholesterol. This melts at 137.5° . It is soluble in most organic solvents, crystallises from 90 % alcohol with one molecule of water of crystallisation and in ether has $[\alpha]_D = -26^{\circ}71$.

He prepared many of the derivatives of sitosterol and characterised the substance fairly completely. The acetate melted at 127° and the benzoate at $145-145.5^{\circ}$. Cholesterol benzoate shows a fine play of colour on cooling, and sitosteryl benzoate does this in a less pronounced fashion.

Ritter [1902] confirmed this work, and added to our knowledge of the phytosterol from wheat germ. After sitosterol had been thus characterised, it seemed probable that the phytosterols described as melting between 132° and 137° consisted of this substance with varying amounts of some similar compound as impurity.

Owing to the resemblance in their properties the separation of these mixtures presented unusual difficulty.

Windaus and Hauth [1907], however, were able to show that the original phytosterol of Hesse, from Calabar beans, was really a mixture of sitosterol, identical with that in wheat germ, and another alcohol, stigmasterol, m.p. 170° , having the formula $C_{30}H_{48}O$. This latter compound has two double linkages in the molecule and the acetate forms a tetrabromide. This tetrabromide settles out from glacial acetic acid on standing while the sitosteryl acetate dibromide remains in solution.

(2) *In Oils*. As already mentioned, phytosterol has been described as a constituent of many plant oils, notably by Bömer [1899] in cotton-seed oil, ground-nut oil, sesamé oil, colza oil, poppy oil, hemp-seed oil, linseed oil and castor oil. He finds that the m.p. differs slightly according to the source, but the elementary composition is the same. Sani [1903] finds a phytosterol in olive oil, and rape oil was examined first by Bömer [1899, 1901] and later by Windaus and Welsch [1909]. The latter authors showed it to be a mixture, and, by means of the bromide of the acetate, isolated two separate sterols—one melting at 148° which they named brassicasterol. This had the formula $C_{28}H_{46}O$. and like stigmasterol formed a tetrabromide of the acetate. The

other had a lower melting point, 142° , and the authors found that this was a phytosterol with the formula $C_{27}H_{46}O$.

Cocoa butter was found to contain a mixture of two phytosterols by Matthes and Rohdich [1908, 1, 2] but Heiduschka and Gloth [1908] consider that cotton seed oil contains a single substance as no tetrabromide is formed.

The capacity of mixtures of cholesterol and the plant sterols to crystallise as a single substance, is made use of in the detection of adulteration of animal oils by those of plant origin. The characteristic four-sided tablet of cholesterol is always altered by an admixture of phytosterol.

(3) *In Roots and Rhizomes.* Perhaps the earliest mention of the substance occurs in the analysis of carrots by Husemann [1861] under the name of hydrocarotol. This was later described by Euler and Nordensen [1908] under the name of daucasterol. Tutin and Clewer [1912] describe a phytosterol verosterol, from rhubarb rhizome, and Power and Salway [1914, 1] from sarsaparilla root. Rümpler [1903] gives the name betasterin to a phytosterol of M.P. 117° from beetroot.

(4) *In Stem, Leaves and Flowers.* Phytosterols have been found in the flowers of plants, in those of *Anthemis nobilis* for example, as anthesterol, by Klobb [1902] and also by Power and Browning [1914], in the flowering branches of *Clematis vitalba* by Tutin and Clewer [1914] and in the leaves and stems of *Daviesia latifolia* by Power and Salway [1914, 2]. Certain substances analogous to phytosterol have been found in olive leaves by Power and Tutin [1908]: oleasterol, $C_{30}H_{50}OH$, M.P. 174° , olestranol, $C_{25}H_{42}O_2$, M.P. $217-218^{\circ}$ and homo-olestranol, M.P. 210° .

(5) *In Latex.* Cohen [1908] has obtained mixtures of phytosterols from South African rubber, and various other bodies from latex have been described.

In addition to the isolation of phytosterol itself as a constituent of plants, Power and his co-workers made the very important discovery that it was present as a glucoside. In their analyses of plants there often occurred high-melting compounds, which seemed to be alcoholic in nature, with more than one hydroxyl in the molecule. These gave the cholesterol colour reactions, but the M.P. and the formulae served to differentiate them from true phytosterols.

As an example, ipuranol, $C_{33}H_{54}O_2(OH)_2$, M.P. $285-290^{\circ}$, occurred in the stems of *Ipomoea purpurea*, in olive bark and in nutmeg. In taraxacum root cluytianol was found, bryonol in bryony root, and similar substances in various plant organs. A list is given in the paper by Power and Salway [1913] which describes their identification as phytosterol glucosides. They were

unchanged by the usual methods of hydrolysis, but when heated with aqueous hydrochloric acid in amyl alcohol solution, dextrose and a phytosterol were formed. Different phytosterols were obtained from different glucosides and it is probable that different sugars may form part of the molecule.

These phytosterol glucosides are named phytosterolins. They may occur in the plant as well as phytosterol, but in the leaves of *Prunus serotina*, according to Power and Moore [1910], phytosterol is not present except in combination as a glucoside.

The foregoing evidence of the occurrence of a phytosterol in so many different plants and different organs of plants, suggests that in the higher plants, at least, it is a constant constituent. In the lower plants comparatively few phytosterols have been isolated, and these are described chiefly in the fungi.

The best-known representative is the ergosterol of Tanret [1889]. This was first isolated from ergot, melted at 154° , and gave an acetate melting at $159-175^{\circ}$. Later it was further examined by the same author [Tanret, 1908] and shown to be a mixture of two substances: (1) ergosterol, $C_{27}H_{42}O$, melting at 165° , forming an acetate melting at 185° , and possessing an unusually high specific rotation; and (2) fongisterol, the lower homologue, $C_{25}H_{40}O$, M.P. 144° ; this is more soluble in the different solvents, has a much lower specific rotation, and gives an acetate melting at 158.5° . These substances are further distinguished by a colour test. Ergosterol gives no colour with strong sulphuric acid at the end of one minute, while fongisterol becomes coloured ruby-red after some seconds, changing to red-violet later.

Gerard [1892, 1895] found an ergosterol present in *Penicillium glaucum*, and *Aethalium septicum*, in *Mucor mucedo*, and in *Lobaria pulmonacea*, and he divides the plant sterols into two groups: (1) the phytosterols, (2) the ergosterols. The first group occur in the higher plants, and usually have lower melting points. The second group occurs in the lower plants, and have higher melting points. The two groups are distinguished by their colour reactions. Zellner [1905, 1908, 1, 2, 1911] describes similar bodies in fat of ergot, in *Amanita muscaria* (the fly agaric), in *Trametes suaveolens* and in *Polyporus ignarius*.

A phytosterol is described in all seeds examined, and in vegetable oils derived from seeds and fruits, but no experiments have hitherto been carried out to trace the fate of this phytosterol in the subsequent development of the plant. It has been described as occurring in all the different organs, but it seemed important to ascertain whether its occurrence was limited

to any special organ, and further if it were possible from such occurrence to form any conclusion as to its probable function.

The results of the examination of the faeces of rabbits fed on a diet of cabbage, of cabbage leaves and stems, and of cabbage seeds [Ellis, 1918], led to the conclusion that while phytosterol was present in relatively large amounts in the seed, the quantity in the vegetative part was small. It seemed necessary to test this conclusion by some more systematic method of experiment, and if possible by the employment of accurate quantitative means. The brown oils giving the cholesterol colour reactions may contain appreciable amounts of a crystalline phytosterol, although it is very difficult to isolate it by the ordinary methods of solution and crystallisation.

For this purpose the wheat plant was chosen partly because the sitosterol from the germ has been so fully characterised by Burian [1897] and later by Ritter [1902], and partly because this sitosterol forms a compound with digitonin to be referred to later. Its economic importance also gives experiments on wheat a value from the utilitarian point of view.

The following experiments were carried out:

(1) The amount of phytosterol present in the whole grain was estimated, and this substance was characterised. Bran and wheat germ meal were also examined, the two latter representing the seed-coat and the germ respectively as nearly as it is possible to obtain them on a large scale.

(2) A known weight of grain was planted and the resulting wheat allowed to grow under normal conditions until well developed. The plants were then carefully collected and the phytosterol content examined.

(3) In addition to the normally developed plants, experiments were carried out with etiolated plants.

Leaves and roots were examined separately in certain of the experiments.

In every case the unsaponifiable ether extract, which would contain any sterol present, was obtained in the following way. The plant material was dried, and then ground up very finely in order to render all the tissues permeable. The dried material was then subjected to prolonged extraction with ether in a Soxhlet apparatus. The ether extract so obtained was saponified with a large excess of alcoholic sodium ethylate, according to the method of Kossel and Obermüller. In order to insure complete saponification the saponified liquid was always allowed to stand over-night. The precipitate of soap was separated by filtration, and washed with ether to remove all the ether-soluble substances. The filtrate was then shaken up with water several

times to free it from excess of alkali. Finally the washed ether extract was evaporated to dryness, the residue dried and examined.

These experiments and their results are given in detail below.

The Grain.

3.05 kilos. of grain were ground up very finely and extracted with ether. The ethereal extract was saponified, washed, and the ether evaporated to dryness. The residue consisted of crystalline matter mixed with oil. After drying it weighed 1.528 g. From this about 0.95 g. of crystalline substance was obtained, giving a yield of approximately 0.031 %.

The pure crystals resemble phytosterol and give a melting point of 137.5°. They give the cholesterol colour reactions. This appears to be the same body as that described by Burian and Ritter in the embryo. To identify it further the acetate was prepared in the way previously described. After recrystallisation from acetone, the dried crystals melted at 127°, showing that the chief phytosterol present in wheat grain is that of the embryo—sitosterol.

The Bran.

1.25 kilos. of bran were extracted, saponified, and the resulting ether extract evaporated to dryness. The residue weighed 1.267 g. It was repeatedly recrystallised from 95 % alcohol, and finally a substance was obtained melting at 142° which gave the cholesterol colour reactions and resembled phytosterol in crystalline form. This was converted into the acetate by heating with acetic anhydride and sodium acetate, and the resulting compound was recrystallised. It melted at 137°. The benzoate was prepared by dissolving the substance in dry pyridine and adding benzoyl chloride until a faint yellow colour appeared. After standing over-night this was poured into much water and filtered. The precipitate was dissolved in hot alcohol and recrystallised from that solvent. Under the microscope the crystals appeared as hexagonal plates. They melt at 133–134°. The esters on saponification yield the original substance, melting at 142°.

This phytosterol differs from sitosterol in the melting point of its esters, but for its further characterisation much more material would be necessary than was available.

The Wheat Germ Meal.

1.8 kilos. of meal were extracted. The unsaponifiable residue consisted almost entirely of crystalline matter and weighed 10.522 g. This gives a yield of about 0.5 %.

THE NORMAL WHEAT PLANTS.

500 g. of autumn wheat were planted in November, and allowed to grow until May of the following year. These plants were grown at the Chelsea Physic Garden, and the author takes this opportunity of thanking the Curator, Mr Hales, for his very kind help in superintending the plants. The plants were collected as carefully as possible and shoots and roots dried separately. The total dry weight of plants was 2250 g. When dry they were well ground up and extracted with ether.

Shoots and roots will now be described separately.

The Shoots. The dry shoots weighed 1950 g. This material was well ground up and extracted with ether. The weight of ether extract obtained was 125.69 g. It was very dark green, greasy looking, and had a curious strong odour. 48 g. of this was taken for saponification. The unsaponifiable residue weighed 5.489 g. The residue was dark orange colour, oily, with characteristic odour. It consisted of a solid mixed with a brown oil, but was not obviously crystalline. This residue was dissolved in alcohol, from which it separates in a jelly-like form, very difficult to filter. When ethyl acetate is employed as a solvent, the substance comes out in more granular form.

It was recrystallised from this solvent, but it was only obtained pure after repeated recrystallisation. The first crop of substance melted at 80°, after recrystallising at 83°. It was so difficult to separate further quantities of this solid from the oil, that a second portion, 37.73 g., of the ether extract was saponified. The unsaponifiable extract, which weighed 4.4772 g., was dissolved in ethyl acetate and boiled up with about an equal quantity of charcoal, the charcoal being subsequently repeatedly extracted with boiling ethyl acetate.

Alcohol ($C_{20}H_{42}O$). The ethyl acetate solution yielded a pure white substance after several recrystallisations. It separates from ethyl acetate and acetone in granular lumps of small crystals. The pure substance melts at 83°. The elementary composition corresponds most nearly to $C_{20}H_{42}O$.

0.1024 g.; 0.3016 g. CO_2 ; 0.13365 g. H_2O .

Calculated for $C_{20}H_{42}O$

C = 80.54

H = 14.09

Found

C = 80.32

H = 14.50.

It was converted into the acetate in the usual manner, and the resulting substance purified by recrystallisation from acetone. The acetate was very difficult to filter on account of its soft consistency. It melted at 65°. The

substance did not give the cholesterol colour reactions, and was not a phytosterol. Etard [1892] describes an alcohol, medicagol, from lucerne leaves to which he assigns the formula $C_{20}H_{42}O$. He says it forms a benzoate, but does not give figures. An interesting point about his paper is that he mentions experiments, the results of which showed that when a horse was fed with lucerne leaves, the alcohol was unaltered by passage through the digestive tract of the animal, and appeared in the faeces. This agrees with the conclusions arrived at by Dorée and Gardner [1908, 2].

Haller [1907] describes an alcohol occurring in the wax from *Raphia ruffia*, M.P. 80° , to which the formula $C_{20}H_{42}O$ is assigned, but no figures are given. A mixed melting point with the medicagol of Etard (from whom he obtained a specimen) shows that they are not the same. The acetate described by Haller seems to be the same as that obtained by the author, M.P. 65° , who concludes that the alcohol occurring in wheat leaves is the same as that in *Raphia ruffia*. It is not identical with arachyl alcohol, which melts at 71° . As it was not a phytosterol, the investigation of this substance was not carried further. The mother liquors deposited crystalline matter resembling phytosterol, but the small amount present was difficult to separate from the brown oil accompanying it in order to weigh it directly.

A method of estimation by means of the compound with digitonin was carried out. Windaus [1909] discovered that cholesterol, but not cholesterol esters, combines quantitatively with digitonin to form a highly insoluble compound, digitonin cholesteride, according to the following equation:

This compound is insoluble in water, acetone, ethyl acetate, ether and benzene.

The residue containing the sterol is dissolved by boiling in 95 % alcohol, and to this solution is added excess of digitonin in 95 % alcohol. The mixture is allowed to stand for some time, then evaporated to dryness. The precipitate is washed by decantation with ether into a previously weighed Gooch crucible until the ethereal washings give no residue on evaporation. After that it is boiled up with water, transferred to the crucible and washed with hot water until the washings no longer froth on being shaken. The compound is somewhat hygroscopic, and the crucible and contents are therefore dried for some time at 110° and weighed in a stoppered vessel, the drying being continued until the weight is constant. This method has been worked out by Gardner and the author [Fraser and Gardner, 1910] in order to estimate small amounts of cholesterol in animal tissues. The amount of digitonin available was not

sufficient to allow of the method in its application to the plant sterols being worked out completely.

It is hoped that the author may carry out that investigation at some future period.

For the purpose of this research, however, use was made of the fact that Windaus found that sitosterol and other well-known isomers of cholesterol formed analogous compounds with digitonin, with similar solubilities. A preliminary investigation with the pure substance, sitosterol, was carried out, and it was found that 0.001 g. sitosterol could be accurately measured by this means. In the case of wheat stalks the alcohol $C_{20}H_{42}O$ was removed by filtration, and washed with acetone until it no longer gave the cholesterol colour reactions. The filtrate and washings were then evaporated to dryness, dried and weighed. The weight was 1.752 g. As the amount of digitonin was limited and there was not enough to precipitate the whole of this residue and those of the other material to be presently described, the following procedure was adopted. The residue was dissolved in 20 cc. of 95 % alcohol, the solution well mixed and 2 cc. removed for the estimation, this forming a tenth part of the whole residue. The weight of compound obtained was 0.1087 g. equivalent to 0.2639 g. phytosterol from the residue from 37.73 g. This gives a total weight of 0.8802 g. phytosterol from 125.69 g., the weight of the ether extract of the shoots. On a dry weight of 1950 g. the percentage content of phytosterol is thus 0.045.

The Roots. The dried wheat roots from the normal plants weighed 300 g. The ether extract weighed 1.454 g. It consisted of a small amount of a solid apparently the same as that in the shoots and a brown oil. There was some appearance of crystalline matter. Exactly the same procedure was carried out as for the estimation of phytosterol in the shoots. The residue gave 0.113 g. of sterol, or a percentage of 0.037 on the dry weight of the roots.

ETIOLATED WHEAT PLANTS (1).

500 g. grain were planted at the Chelsea Physic Garden in November. They were covered with canvas bags to exclude the light. A few of the plants showed green in spite of this precaution, but in March signs of withering began. The plants were then carefully collected and dried, shoots and roots being separated.

The total dry weight of the plants was 203.5 g.

The Shoots. 203.5 g. dried plants gave 135.2 g. dry weight of shoots. This was extracted with ether, and saponified as before. The unsaponifiable

ether extract weighed 2.175 g. It consisted chiefly of the alcohol $C_{20}H_{42}O$ present in the normal shoots, melting at 83° , recrystallised from acetone.

From solution in acetone the mother liquors deposited a distinctly crystalline residue. The procedure adopted for the normal shoots was carried out. The amount of phytosterol present in the residue was 0.0674 g. a percentage of 0.0487 on the dry weight of shoots.

The Roots. The dry weight of the roots was 68.5 g. The unsaponifiable ether extract weighed 0.5702 g. This gave 0.0855 g. phytosterol, a percentage of 0.1252 on the dry weight of roots.

ETIOLATED WHEAT PLANTS (2).

383 g. wheat grain were grown on filter paper in an incubator in the laboratory. The plants, when grown, were completely etiolated. The dried material weighed 168 g. The unsaponifiable extract from this weighed 1.404 g. The amount of phytosterol present was 0.1175 g. or 0.07 % of the dry weight of plants.

These results are put together in the following table:

Table showing Sterol Content of Wheat grown under varying conditions.

Experiment	Weight of grain planted in g.	Dry weight of material in g.	Total dry weight of plants in g.	Amount of phytosterol in g.	Total amount of phytosterol in plants in g.	Percentage of phytosterol in roots and shoots	Total percentage of phytosterol	Amount in grain planted (0.031 %) in g.	Remarks
Normal Wheat Plants } shoots	500	1950	2250	0.8802	0.9932	0.045	0.04	0.155	Increased during growth
} roots		300		0.1130		0.037			
Etiolated Wheat Plants } shoots	500	135.2	203.5	0.0674	0.1529	0.0487	0.075	0.155	About the same as in seed
} roots		68.3		0.0855		0.1252			
Etiolated Wheat Plants }	383		168		0.1175		0.070	0.1187	About the same as in seed

SUMMARY AND DISCUSSION OF RESULTS.

(1) In order to obtain more information as to the relative amounts of phytosterol occurring in the different organs of the plant, those of the wheat plant were carefully examined. Schultze and Barbieri [1882] give some figures for the phytosterol content of the seeds and seedlings of lupin plants, but their methods are open to criticism, and they are not certain of their results. The chief phytosterol present in the grain was found to be the same

as that in the embryo—sitosterol—and as the solid part of the unsaponifiable ether extract of the grain consisted entirely of this substance, it could be separated and weighed directly. A phytosterol was present in bran, but it differed from sitosterol in its melting point, and in the melting points of its esters.

(2) In the case of the vegetative organs, other substances were present in the unsaponifiable ether extract—an alcohol $C_{20}H_{42}O$, which did not give the phytosterol colour reactions and was not precipitated by digitonin, and an oil, and it proved difficult to separate the phytosterol in order to weigh it directly. The researches of Windaus and his co-workers have shown that all the phytosterols hitherto isolated from the higher plants, which have been fully characterised, combine with digitonin to form a compound insoluble in ether, acetone, and ethyl acetate. A method of estimation based on this fact was devised, and it was found to be very accurate when applied to pure sitosterol in preliminary experiments.

(3) An estimation of the amount of phytosterol present in the normal wheat plant, grown from a known amount of grain, shows that the quantity in the adult plant is greater than in the grain, whereas in the etiolated plant the amount is very much the same as that in the grain, allowing for experimental errors. The percentage of phytosterol present in the normal plant is slightly greater than the percentage present in the grain, and in the etiolated plant it is higher still, no doubt correlated with the loss in dry weight, due to starvation.

(4) In the embryo the percentage of phytosterol present is much higher than in the plant. This points to an essential function in germination and growth. As the amount present in the grain is not appreciably altered in the starved plant, there is distinct evidence that this function is *not* a nutritive one. This seems an important point and is rather in agreement with the nature of phytosterol and the fact that it is not easily altered chemically. Its occurrence in the seed, in the seed coat, in the embryo, the root and the shoot, in the case examined, suggest a function common to all of them. While no definite conclusions can be drawn until a larger number of investigations have been made, the theory that the sterols may form an essential part of cell membranes suggests itself, but the presence of phytosterol in latex, unless it occurs there as the result of cell-destruction, and in the form of glucosides, is hardly accounted for by this theory. Many experiments will still have to be carried out before we can form any very definite conclusion as to the function of phytosterol in the plant.

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