Constitution of sterols and their connection with other substances occurring in nature

Nobel Lecture, December 12, 1928

The exceptional distinction which the Royal Academy of Sciences has accorded to my work "on the sterols and their relationship to other natural products" places upon me the honour and duty of giving an account of my research to this assembly.

The sterols are nitrogen-free secondary alcohols of high molecular weight which contain in their molecules a number of alicyclic systems. A sharp distinction between the sterols and other naturally occurring hydroaromatic alcohols is not always possible.

The sterols are widely distributed in the animal, vegetable, and fungal kingdoms. The best-known sterol is cholesterol, which was first discovered in human gall-stones, and received the name cholesterol because of its presence in bile. It is a mono-unsaturated alcohol, with the formula \( C_{27}H_{46}O \), found in all the higher animals, partly as free alcohol, and partly as fatty acid esters; it is present in particularly large quantities in the brain and the adrenal cortex and as a pathological deposit in gall-stones, sclerotic aorta, and other organs affected by fatty degeneration. The fluctuations of cholesterol content to which human blood serum is subject are very surprising; during pregnancy the quantity is considerably increased, while during most infectious diseases it is noticeably reduced.

In the lower animals, the insects, the echinodermata, and the sponges a number of sterols occur which are collectively known, together with cholesterol, as zoosterols. The majority have the same formula as cholesterol and are very similar to it; only the spongosterol, discovered by Henze in Suberites domuncul, appears not to be an unsaturated compound, and is more clearly differentiated from cholesterol than the other zoosterols.

In the vegetable kingdom, too, wherever they have been sought, sterols have always been found. They are grouped together as phytosterols and occur in plants partly as free alcohols and partly as glucosides. The most widely distributed are phytosterols (sitosterols) which have the same formula as cholesterol; there are, however, also phytosterols which contain not one, but
two double bonds, and saturated phytosterols are also frequently mixed in small quantities with unsaturated ones. Somewhat apart from the typical sterols are sterol-like alcohols, which are differentiated from sitosterols by the number of carbon atoms, and, finally, those which contain more than one hydroxyl group. The variety of the phytosterols is therefore great, and it is noticeable that, despite this great variety, animal cholesterol has never been discovered among the numerous plant sterols.

Specific sterols are also found in fungi and are classified as mycosterols. Ergosterol was first isolated from ergot by Tanret, and it is also found in numerous other fungi and especially in yeast. It has the formula C_{27}H_{42}O and contains, in contrast to cholesterol and sitosterol, not merely one, but three double bonds. It is particularly significant that this ergosterol is mixed in very small quantities (about 1/10%) with all zoosterols and phytosterols. In addition to ergosterol still other sterols are found in fungi - zymosterol in yeast, and fungisterol in ergot. There are certainly many more.

It is surprising that in bacteria, as far as they have been examined, no sterols occur. Panzer noted their absence from tuberculous and diphtheria bacilli, and I can confirm this result for the tubercule bacilli, as I have worked on several kilogrammes of these. I have shown by physiological means that ergosterol does not occur even in traces in tubercle wax.

Chemically by far the most thoroughly examined is the sterol of the higher animals, cholesterol. The laborious and protracted investigation of its constitution has shown that it probably possesses the structure (I). According to Wieland's research this formula may be resolved into the complete structural formula (II). This formula is very complicated and has no similarity to the formulae of sugar, fatty acids, or the amino acids which occur in protein.

The synthesis of such a substance appears to the chemist particularly diffi-
cult, and up till now I have not dared to attempt it, as success is extremely improbable. Furthermore, the majority of physiologists have not been inclined to believe the animal organism capable of such a synthesis, for it is known that other, seemingly simpler, syntheses - e.g. that of tyrosine and tryptophan - have not succeeded in the animal organism.

If the human and animal organism were really not in a position to produce cholesterol from other constituents, then the cholesterol of the higher animals, the carnivores and herbivores, would necessarily originate in their food. But as the herbivore does not receive cholesterol but phytosterol in its food, it must possess the ability to absorb the phytosterol of its vegetable nourishment and to convert it to cholesterol.

To test this assumption Schonheimer carried out experiments on rabbits in the Institute of Pathology in Freiburg. He found 96.9% of the ingested sitosterol (0.2 g per day) in the excrement, and concluded from this that sitosterol is not absorbed by the intestinal canal of the rabbit. There can therefore be no question of the herbivore using the phytosterol in its food to form the cholesterol of its body substance. On the contrary, it must be in the position to form cholesterol from other components of its nourishment.

Whether or not mycosterols behave like phytosterols still remains to be accurately established. On the other hand, it is known that cholesterol, which is so extremely similar to sitosterol in its physical and chemical properties, behaves physiologically in quite a different manner. It is absorbed by both herbivores and carnivores and particularly easily when bile salts are available in abundance. Schonheimer found only 50% of the ingested cholesterol (0.2 g per day) in the excrement of rabbits. Furthermore, the cholesterol content of the blood is greatly increased after feeding with cholesterol, and it is noticeable that after long periods of feeding with cholesterol symptoms of disease
appear in the animal. Mice, rats, and cats accumulate cholesterol, which is introduced with the food, principally in the liver, so that the cholesterol content of this organ can increase to more than five times normal, and a severe anisotropic fatty degeneration of the liver becomes apparent. If small doses of cholesterol are fed to rabbits deposits occur at first only in the intima of the blood vessel, which is the organ most sensitive to cholesterol in the rabbit. This observation is of very great interest, for according to Aschoff a disease pattern arises at this point which is identical with that of atherosclerosis in man. The hypothesis of a genetic connection between ingested cholesterol and atherosclerosis in man thus cannot be dismissed. Since phytosterols, which cannot be absorbed, bring about no such symptoms, it should be investigated whether or not true atherosclerosis presents itself in societies which are truly vegetarian (abstaining also from milk and eggs).

Although the animal thus is in a position to absorb cholesterol from its food with the aid of bile salts, nevertheless not even the carnivore is dependent on this cholesterol introduced from outside. As Beumer has demonstrated, puppies fed on a diet deficient in cholesterol exhibit after four weeks an increase of cholesterol which is twenty times greater than the amount given in the food. Particularly painstaking have been the investigations of Randles and Knudson into the question of a cholesterol synthesis. They have succeeded in maintaining white rats on a diet completely free of sterols, on the basis of the discovery that pulverized alfalfa, on extraction with cold ether, relinquishes its sterols completely, but retains enough vitamins for the maintenance of rats. The scientists mentioned reached the incontestable conclusion that the cholesterol content of fully-grown rats fed on a diet free of cholesterol is many times greater than the cholesterol content of new-born rats. The only possible explanation for this is that the organism of the rat is capable of forming cholesterol from substances which are different from the sterols and other substances soluble in ether.

There is thus in the animal organism a cholesterol synthesis, and the synthetic capabilities of the animal have been gravely underestimated here, as in many other cases.

In the animal organism some substances are found which according to their formulae could be related to cholesterol. These are isocholesterol, coprosterol, the bile acids, and bufotoxins.

About isocholesterol, which occurs in the lanolin of sheep, only very little is known.

Coprosterol is found in the faeces of carnivores; it must be formed from
cholesterol through the action of intestinal bacteria. However, it has remarkably not been possible outside the intestinal canal to change cholesterol into coprosterol by the use of putrefactive bacteria.

Coprosterol is according to its formula a dihydrosterol, but it is not identical with the normal dihydrocholesterol which, according to Willstatter's research, arises from the catalytic hydrogenation of cholesterol, and which is likewise found in small quantities in faeces.

According to the long-continued research which I have been engaged on,

\[
\text{Cholesterol} ightarrow \text{Coprosterol}
\]

...
of cholesterol from animal matter, this transformation would certainly take place.

Using coprosterol as a starting-point, an important conversion from the sterols to bile acids has also been discovered. The familiar bile acids (formulae (V)) derive from an acid \( \text{C}_{24}\text{H}_{40}\text{O}_{2} \), Wieland's cholanic acid; that is, they contain 3 carbon atoms less than cholesterol and coprosterol. These three carbon atoms may be separated out of coprosterol as acetone via the corresponding hydrocarbon, coprostane, by using an oxidizing agent; and in this way

\[
\text{CH}_3
\]

\[
\text{C}_1\text{H}_2\text{O}\text{H}_2\text{CH}_2\text{CH}_2
\]

Wieland's cholanic acid is arrived at, as formula (VI) indicates. Moreover, some time ago Wieland carried out the appropriate synthesis, and succeeded in returning from cholanic acid to coprostanol.

The chemical connection between cholesterol and bile acids is completely clarified by this, and it is interesting that bile acids also derive not from normal cholesterol, or rather dihydrocholesterol, but allocholesterol, or coprosterol.

Does this close chemical relationship correspond to a biological connection? That is, are the bile acids produced through the degradation of cholesterol within the organism as well? Upon this question Thannhauser and his colleagues have recently been engaged, and have produced important results. First they demonstrated on a dog with a gall-bladder fistula that the cholesterol ingested with the food is by no means sufficient as a source for the bile
It is, moreover, never transformed into bile acid in the animal organism even in the case of intravenous injection; on the contrary, isomeric allocholesterol and coprosterol bring about an increase of the bile acids corresponding to the quantity injected. This discovery is of very great significance; it shows first of all that the animal organism is not capable of forming allocholesterol from cholesterol. It does, however, make it probable that allocholesterol plays an important part in the intermediate metabolism. Perhaps things are so placed that the organism synthesizes, not the cholesterol, but the allocholesterol, and that this represents the parent substance of coprosterol as well as of the bile acids. Perhaps, the sterol secreted in the intestinal canal, which
is hydrogenated to coprosterol, is not cholesterol at all, but the unstable allocholesterol, and perhaps the formation of gall-stones in bile depends on the fact that the easily soluble allocholesterol is prematurely transformed into the difficultly soluble cholesterol.

At any rate the attempt to prepare coprosterol with putrefactive bacteria outside the intestinal canal should be repeated on allocholesterol.

In place of the bile acids of the type of oxycholanic acid, there is found in the bile of sharks a scymnol sulphuric acid, which is an acid ester of scymnol, a polyvalent alcohol of the formula $C_{27}H_{46}O_5$. The assumption suggests itself that this scymnol is a multiply hydroxylated cholesterol, but that has not yet been established; and likewise it is very probable that the basic component of bufotoxin, bufotalan, is an oxylactone of the formula $Ca_{24}H_{38}O_3$, closely related to the bile acids.

What, then, is the biological role of cholesterol itself? Many attempts have been made to solve this question. A number of scientists place in the foreground the physical - especially the colloid-chemical - properties of cholesterol, and they point to the ability of cholesterol to emulsify fat, and to its importance for the permeability of the cell. The conditions prevailing here still badly need explanation.
We are well informed about one biological property of cholesterol, but it is uncertain whether this is of any significance for the organism. It concerns the ability of cholesterol to detoxicate a series of haemolytic poisons, and to increase the resistance of the red blood corpuscles.

It was Ransom who first discovered that blood serum is in a position to detoxicate haemolytic poisons like, for example, saponin, and that the cholesterol present in the blood serum is the effective factor in this. Later, it was found that cholesterol forms easily characterized and easily crystallized additive compounds with a number of saponins, especially with the digitalis-saponins, and that these additive compounds are not toxic. The detoxication thus depends on the formation of complex compounds. Some of the complex compounds are so difficultly soluble that they make an exact evaluation of cholesterol possible; a separation of the cholesterol ester from cholesterol may be carried out also with the help of the digitonin method.

In recent years a fact which has come to excite the interest of chemists and physiologists above all is the connection between the sterols and the antirachitic vitamin. The antirachitic vitamin is present in comparatively large quantities in the unsaponifiable portion of fish-liver oils, which is an effective remedy for rickets. The German physician Huldschinsky was as far as I know the first to discover that as well as fish-liver oils, irradiation with ultraviolet light constitutes a remedy for rickets. Two American scientists, Hess and Steenbock, then discovered independently of each other that it is not even necessary to irradiate the diseased organism, but that it is sufficient to irradiate the food with which it is to be nourished. These scientists, and at about the same time two English scientists, Rosenheim and Webster in London, next established that the substance which can be activated is situated in the unsaponifiable part of the nutriment, and is identical with the sterols.

It was at first believed that all sterols - animal, vegetable, and fungal sterols - would be activated by ultraviolet light; but it then became apparent from physical measurements (Heilbron, Pohl) and from biological experiments (Rosenheim and Webster, Hess and Windaus), that cholesterol and sitosterol contain in traces an admixture which renders activation possible, and that cholesterol may comparatively easily be freed from this contamination. Proof was then successfully given that this admixture is identical with the ergosterol of fungi, or, to put it more circumspectly, manifests exactly the same absorption spectrum and exactly the same physiological behaviour as ergosterol.

The biological effect of ergosterol is extremely surprising. A fortnight's admixture of 1/20,000 mg daily of irradiated ergosterol to "rachitogenic"
nutriment is sufficient to protect a rat from rickets. Irradiated ergosterol is more than 100,000 times more effective than good fish-liver oil. All the scientists who have tested this result are in a position to confirm its accuracy. Opinions are still divided only about whether ergosterol is the only substance which is transformed into antirachitic vitamin under irradiation, or whether there are other precursors.

Jendrassik and Kemenyffr in Budapest take the view that the capacity of cholesterol for being activated should be attributed for by far the greater part to an admixture of ergosterol, but that cholesterol itself is self-activating, however small this effect may be. And Bills and his co-workers have likewise reported that by painstaking refinement the susceptibility of cholesterol to activation can indeed be depressed to a thirtieth part, but there would remain constant. The view that cholesterol itself may be activated does not hold good. It is certainly true that when cholesterol is refined with blood-charcoal or by way of dibromide a certain susceptibility remains; but this depends on the

![Fig. 1. Absorption coefficient. Ergosterol 0.04% in ether.](image)
exceptional difficulty of separating—or rather, destroying—the last remnants of ergosterol. By repeated purification of cholesterol via crystallized allocholesterol, a cholesterol was successfully obtained the activity of which is at least 300,000 times less than that of ergosterol; and Kon and Steenbock have by repeated oxidation of cholesterol with potassium permanganate similarly obtained a completely inactive cholesterol. Other substances which are similar in structure to ergosterol—zymosterol, choleatrienic acid methyl ester, and digitaligenin—cannot be activated. The assertion that digitaligenin might be activated was propounded by Holtz and myself.

Thus up till now the only precursor of vitamin D in existence is ergosterol.

For the transformation of ergosterol into antirachitic vitamin, ultraviolet light of a wavelength of between 253 and 302 m$\mu$ is suitable; light of a longer wavelength than 313 m$\mu$ or shorter than 248 m$\mu$ has no definite effect. This result is easily understood if we consider the absorption spectrum of ergosterol (Fig. I).

Activation was still obtained at -183$^\circ$. Some 700-1000 erg are necessary to produce the quantity of vitamin D which has an immediate physiological effect (about 1/50,000 mg). If irradiation continues too long the vitamin D is destroyed.

Besides ultraviolet light, cathode rays are also suitable for the activation of ergosterol. Knudson and Coolidge obtained the transformation when they caused high-tension cathode rays of 100,000 to 350,000 volts to act upon the sterol for 30 seconds. After 900 seconds, however, the vitamin had disintegrated. Whether a-rays are effective appears not yet to have been attempted, but it is indeed very probable. With so-called dark electrical discharge or with X-rays we have obtained no activation of ergosterol.

We also attempted, with sensitizers, to make ergosterol sensitive to long-wave light. In this certain light reactions were successfully produced; but they are principally of a nature different from that of the ultraviolet reaction, and do not lead to vitamin D.

If ergosterol and eosin are irradiated with visible light, a stoichiometrical reaction takes place between the ergosterol and the eosin, by which eosin hydrogenates and ergosterol is dehydrated under the formation of a pinacol, C$_{54}$H$_{82}$O$_2$. If all the eosin is hydrogenated the reaction is brought to a standstill. If ergosterol and eosin are treated with visible light in the presence of oxygen, the eosin acts as a catalyst and straight away a crystallized ergosterol-peroxide arises. But both products are ineffective against rickets; thus, no vitamin formation has been observed using visible light.
Equally small has been our success so far in obtaining the transformation of ergosterol into vitamin D by chemical means. It is, however, not apparent why this method should not still be successful. On the contrary, a whole series of scientists believe that vitamin D can be produced in the vital functions even without ultraviolet light. Thus Volt, indicated that grass seedlings cultivated in darkness, derived from vitamin-free seeds of Lolium perenne, contained a small quantity of antirachitic protective material. These experiments need re-examination. Opinions are also divided over whether the abundance of vitamin D in cod-liver oil originates in the nutriment of the cod, which is rich in vitamins, or whether the vitamin is formed synthetically within the organism.

What, then, takes place when ergosterol is irradiated? Of course there is a chemical alteration along with the physiological activation; this announces its presence in that, when irradiated, ergosterol, which, like all sterols, yields with digitonin an insoluble additive compound, gradually loses its precipitability through digitonin. The course of the chemical transformation is even easier to follow in the alteration of optical rotation. In the usual solvents ergosterol manifests a strong laevorotation which under irradiation slowly diminishes and finally is transformed into a weak dextrorotation. As an example the curve for ligroin may be given (Fig. 2).

![Figure 2](image-url)

Fig. 2. Irradiation of ergosterol in normal benzine (Kahlbaum) 0.35% solution. Period of irradiation in hours.
At the same time as the alteration of the optical rotation, an alteration of the ultraviolet absorption spectrum and a remarkable increase in solubility ensue. In order to obtain from irradiation values which may be easily reproduced, it is necessary not only to exclude oxygen completely, which is very difficult, but also to irradiate all parts of the solution as far as possible uniformly. First of all we attempted the activation by putting a quartz-spiral around the mercury lamp and sending through it a 0.25 % alcoholic solution of ergosterol under nitrogen pressure (Figs. 3 and 4).

Later we heated the etherized solution of ergosterol to boiling point in a double-walled quartz vessel, and led through it pure nitrogen, while within the quartz vessel a magnesium spark gap was generated (Fig. 5). We then worked up the irradiation products so obtained in an air-sealed container, freed them of unprocessed ergosterol, and investigated their rotation.
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sorption spectrum, physiological effect, and analytical composition. While the physiological effect was entirely good, we made the surprising observation that our irradiation products always contained between 2% and 4%, too little carbon; moreover, the numerous nitrogen-containing esters which we have examined did indeed yield a correct atomic relation between C and N, but always in too low percentages. The deficit can only be based on an absorption of oxygen by the activated ergosterol. We therefore proceeded to refine our nitrogen with even greater care, and for that purpose we made use of Kautsky's apparatus. Here we had success in so far as the deficit in carbon decreased to between 0.5% and 1.5%, depending on the period of irradiation. But even so, we never obtained entirely correct analytical composition. We first obtained incontestable values when we irradiated solutions of ergosterol in highly evacuated, sealed quartz vessels, with constant agitation. Under these conditions irradiation products arise which not only give an analytical composition exactly of the formula $\text{C}_{27}\text{H}_{42}\text{O}$, but also yield a number of crystallized derivatives of correct analytical composition.

There can, then, remain no doubt that ergosterol does not change its analytical composition under irradiation. The only remaining possibility is that ultraviolet light has either a polymerizing or an isomerizing effect upon ergosterol. In order to test experimentally the possibility of a polymerization, we undertook to ascertain the molecular weight of irradiated ergosterol, and found values which fall within the margin of error for the simple molecular weight. Thus a demonstrable polymerization does not take place during irradiation.

There are a number of possibilities for the process of isomerization. First, it might be assumed that a displacement of the secondary alcohol group to a double-bond took place, by which the unsaturated alcohol group (VII) would be transformed into a saturated ketone group (VIII).

\[
\text{CH}_3 - \text{CH} - \text{CH} \quad \rightarrow \quad \text{CH}_2 - \text{CH} - \text{CH}_2
\]

In this process the hydroxyl group would thus disappear. We therefore tested whether the hydroxyl content of the irradiation product of ergosterol is the same as of ergosterol itself, and for this we made use of Zerewitinoff's method. Unirradiated ergosterol gave in a series of determinations a mean value of 1.02 active hydrogen atoms; irradiated ergosterol gave a mean value of 1.1 active hydrogen atoms per molecule. The determinations thus demon-
strate that the hydroxyl group does not disappear during irradiation, and that therefore the first of the possibilities under discussion for isomerization collapses. Moreover, we have prepared the isomer in question here, ergostadie-none, and established that it is ineffective antirachitically.

As a second possibility for an isomerization, it must be taken into consideration that during irradiation a double bond rearranges itself as a ring bond. Stobbe discovered a similar reaction to light (see (IX)) in the phenylfulgids, which are transformed into dihydro-naphthalene derivatives.

\[
\begin{align*}
\text{1-Phenyl-1,4-dihyronaphthalene-2,3-} & \quad \leftrightarrow \\
\text{dicarboxylic acid anhydride} & \quad \text{Diphenyl-} \\
\text{fulgid} & \quad \rightarrow \\
\text{1-Phenyl-1,2-dihyronaphthalene-2,3-} & \quad \text{dicarboxylic acid anhydride}
\end{align*}
\]

In the photoisomerization of carvone (X) even two double bonds would disappear (XI).

In order to test whether the number of double bonds alters during the ultraviolet irradiation of ergosterol, we employed titration with perbenzoic acid and quantitative catalytic hydrogenation. The measurements gave the result that, like the hydroxyl group, the number of double bonds remains unaltered with irradiation, and even with excessive irradiation.

\[
\begin{align*}
\text{CH} & \quad \rightarrow \\
\text{H}_2\text{C} & \quad \text{CH}_3 \\
\text{C} & \quad \text{CH}_2 \\
\text{HC} & \quad \text{CO} \\
\text{CH}_3 & \\
\text{C}_{\text{carvone}} & \quad \rightarrow \\
\text{H}_2\text{C} & \quad \text{CH}_3 \\
\text{C} & \quad \text{CH}_2 \\
\text{HC} & \quad \text{CO} \\
\text{CH}_3 & \\
\text{C}_{\text{carvone-camphor (?)}}
\end{align*}
\]

The isomerization therefore is based on a displacement of double bonds or a steric rearrangement here or at another position in the molecule.
The nature of these displacements or rearrangements has not yet been determined; for as yet too little is known about the more detailed structure of ergosterol. It is, however, important that also the saturated perhydration products of ergosterol, and of its irradiation product, which both have the formula C_{27}H_{48}O, are different.