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Sterols as Complex-forming Species

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The formation of complexes of sterols with different compounds determines the biological properties of both sterols and various natural substances such as saponins and polyene antibiotics. Complex formation by sterols with phospholipids, steroid saponins, and polyene antibiotics is determined by the same characteristic features of the structure of the sterol molecule. The principal role in complex formation is played by the hydrophobic reaction of the cyclopentanoperhydrophenanthrene ring. The formation of a hydrogen bond between the hydroxyl group of the sterol and a proton acceptor, which is assumed in most complexes, has been proved only in the complexes of sterols with water and acids.
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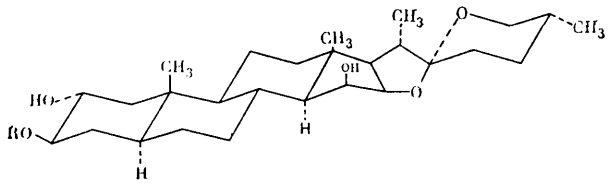
I. INTRODUCTION

The ability of sterols in general and cholesterol in particular to form complexes† with steroid saponins was detected much earlier than the determination of the structural formula of cholesterol.[2] Since then, a large number of studies have shown that sterols form complexes with a number of organic compounds: alcohols, phenols, acids and their derivatives, amines, carbohydrates, various heterocyclic compounds, and recently proteins, and also with inorganic acids and salts. Complex formation with saponins has found extensive practical application in the preparative and analytical chemistry of sterols,[3,4] and complex formation with salts and alcohols is used to separate and purify sterols.[5,6] The formation of complexes of sterols with saponins forms the basis of the use of the latter as hypocholesterolemic agents,[7,8] and in recent years it has been used to develop sorbents which selectively extract the cholesterol of blood plasma.[9-11] However, it is not so much these applied aspects that are attracting interest in the complexes of sterols as the fact that complex formation determines a large part of the biological functions of sterols. Thus it was established at an early date that the biological action of polyene antibiotics is determined by the fact that they form complexes with membrane sterols. The same or a similar reaction explains the action of various toxins, for example tetrodotoxin and saxitoxin,[12] and also the membranotropic action of triterpene glycosides.[13] The incorporation of sterols into biological membranes takes place as a result of the formation of complexes with other components of the membranes, primarily phospholipids. Finally, in recent years there has been considerable interest in the reaction of sterols with proteins in lipoproteins, lipid–protein complexes which brind about the transport of lipids in living organisms.

The aim of the present work was to systematise individual scattered data on complex formation by sterols with various compounds, and to try to determine which intermolecular interactions are responsible for the high complex-forming power of sterols and which structural functions of their molecules are involved in these interactions. It should be stated at once that there are few published data on the quantitative study of sterol complexes. The published papers generally give no information on the stability constants of the complexes, their energies of formation, etc. Thus the present review is also based chiefly on qualitative relationships between the structure of sterols and their ability to form complexes.

II. COMPLEXES WITH STEROID SAPONINS

In 1909, Windaus[2] found that cholesterol forms a sparingly soluble complex with digitonin, a steroid saponin (Ia). Complexes of this kind, which are called digitonides and which contain equimolar quantities of digitonin and a sterol, have been obtained for a whole series of sterols, and the solubility of the digitonides of different sterols depends on the nature of the latter These differences in the stability of the complexes have been widely used to separate sterols with similar structures, particularly epimers.



a) R = Xyl—Glc—Gal—
Gal—Glc

b) R = Xyl—Glc—Gal—
Gal

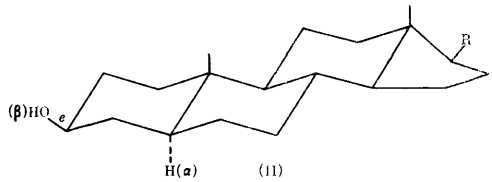
c) R = Glc—Gal—

Xyl = xylose, Glc = glucose, Gal = galactose

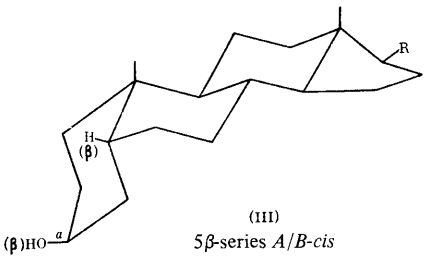
In the course of these studies, data were accumulated, relating the structure of a sterol to its ability to form a complex with digitonin. It should be emphasised, however,

†According to Ref.1, the term "complex" signifies a molecular unit formed by the association of two compounds without change in the nature of the bonds within each part.

that the classification into sterols precipitated by digitonin and sterols not precipitated, frequently encountered in the literature, is extremely relative. The formation of a complex with digitonin is not determined entirely by the structure of the sterol; the dissociation of the complex is determined to a large extent by the reaction conditions, and in particular by the solvent and temperature. Thus digitonides have the minimum solubility in ethanol, in which they are prepared, but dissociate completely in such solvents as pyridine, dimethylformamide, and dimethyl sulphoxide.[14] The complexes dissociate when studied by chromatography on silica gel[15] and with increase in temperature; when cholesterol digitonide is heated in a vacuum to 240°, the cholesterol distils off practically completely.[16] Thus generalisations relating the stability of digitonides to the structure of sterols must be based on experiments carried out under rigorously comparable conditions, and there have been few such studies; the aim of most published work has been the preparative separation of isomers. Only a few authors[17–19] have determined quantitative characteristics of the stability of the digitonides of different sterols, namely the solubility product S. It is suggested that the lower the solubility and the value of S, the greater the ability of the sterol to form a complex. The data in Table 1 show that the lowest solubilities correspond to the digitonides of 3β-hydroxyl derivatives of the 5α-gonane series (II), that is compounds with *trans*-connection of the *A* and *B* rings, and also the corresponding Δ^5-derivatives. All these compounds have a more planar molecule, compared with the 5β-conformers (III) and the 3β-configuration of the hydroxyl group:



(II)
5α-series *A/B-trans*



(III)
5β-series *A/B-cis*

R=H	androstane
R=C_nH_5	pregnane
R=CH (CH_3) $(CH_2)_2CH_3$	cholane
R=CH (CH_3) $(CH_2)_3CH$ $(CH_3)_2$	cholestane

a—OH-group in the axial position, *e*—OH-group in the equatorial position.

The 3β-hydroxyl group plays a decisive role in the formation of the complexes; its acylation leads to a sharp increase in the dissociation of the complex, and this forms the basis of one method for separating sterols from digitonides. Not only acylation, elimination (separation or reduction), or oxidation at the keto-group, however, but even epimerisation of the 3β-hydroxyl group has a marked influence on the stability of the complexes. All 3α-hydroxy-derivatives of the 5α-series form complexes with S > 1100 × 10^{-8} mole^2 litre^{-2}, that is they are much more soluble than the 3β-derivatives.

Table 1. Influence of the structure of the sterol on the solubility of the digitonide.[17–19]

Compound	Configuration of the 3-OH-group		10^8 S, mole^{2}litre^{-2}
5α-Cholestan-3β-ol	β	*e*	4
5α-Cholestan-3α-ol (epicholestanol)	α	*a*	2200 (900)
3β-Acetoxy-5α-cholestane	β	*e*	1100
Cholest-5-en-3β-ol (cholesterol)	β	*e*	4
3β-Acetoxycholest-5-ene	β	*e*	>1100
5β-Cholestan-3β-ol (coprostanol)	β	*a*	70
5β-Cholestan-3α-ol (epicoprostanol)	α	*e*	2200
Stigmasta-7,22-dien-3β-ol (stigmasterol)	β	*e*	4
5α-Ergost-7,22-dien-3β-ol (ergosterol)	β	*e*	4
Methyl 3β-hydroxy-5α-androstan-17-carboxylate	β	*e*	8
Methyl 3β-hydroxy-5β-androstan-17-carboxylate	β	*a*	>2200
5α-Pregnan-3β, 20β-diol	β	*e*	140
5β-Pregnan-3β, 20β-diol	β	*a*	>1100
5α-Pregnan-20-keto-3β-ol	β	*e*	4
5β-Pregnan-20-keto-3β-ol	β	*a*	1100
Pregn-5-en-20-keto-3β-ol	β	*e*	4
5α-Androstan-3β-ol	β	*e*	8
5-Androsten-3β-ol	β	*e*	35
5α-Androstan-17-keto-3β-ol	β	*e*	140
5α-Androstan-17-keto-3α-ol	α	*a*	2200
5β-Androstan-17-keto-3β-ol	β	*a*	1100
3α-Methyl-5α-androstan-3β-ol	β	*e*	35
3β-Methyl-5α-androstan-3α-ol	α	*a*	1100
5α, 22-Spirostan-3β-ol (tigogenin)	β	*e*	70 (16)
5β, 22-Spirostan-3β-ol (sarsapogenin)	β	*a*	280 (970)
Cholest-4-en-3-one			>2200

The difference in the reactivities of epimers of sterols with different conformations of the 3-hydroxyl group is well known. Equatorial hydroxyl groups are less shielded than axial groups, so that they undergo certain reactions more readily. For example, they are more readily acylated, and the corresponding esters are more readily hydrolysed. Epimers with an equatorial hydroxyl group are more strongly sorbed on various carriers, indicating that they form more stable complexes,[20,21] possibly by hydrogen bonding.[22] It is logical to assume that the differences in the ability of epimeric sterols to form digitonides is also related to the equatorial or axial arrangement of the 3-hydroxyl groups. It has been suggested, however, that in complex formation with digitonin, the decisive role is played not by the equatorial or axial arrangement of the 3-hydroxyl groups but by their α- or β-arrangement relative to the cyclic system.[3,23] This generalisation appears unjustified. It can be seen from Table 1 that among epimeric compounds, the lower solubility is shown by the equatorial 3-isomer, irrespective of the position, α or β, occupied by the 3-hydroxyl group in the axial epimer#. An exception is provided by coprostanol and epicoprostanol, for which the opposite relationship is observed. It was on the basis of this pair of compounds that the suggestion that the α- or β-arrangement of the hydroxyl group plays a decisive role in the formation of digitonides was first put forward, and it was subsequently repeated in the literature, in spite of the large number of exceptions. It should be emphasised that for most of these exceptions, that is when the compound with the 3β-hydroxyl group does not form a digitonide, the hydroxyl group occupies the axial position (lumisterol, the methyl esters of 3β,5β,14β-trihydroxyandrostan-17β-carboxylic acid and its 17α-epimer, methyl 3β-hydroxy-11α-acetoxy-

#In the 5α-gonane series, the 3β-hydroxyl group is equatorial, and the 3α-group is axial, whereas in the 5β-gonane series, the 3β-hydroxyl group is axial, and the 3α-group is equatorial.

5β-androstan-17β-carboxylate, and the cardenolide aglycones gitoxigenin, digitoxigenin, sarmentogenin, and strophanthidin).[4,24] Moreover, in individual cases it has been shown that the corresponding 3α-epimer, whose hydroxyl group is equatorial, forms a digitonide, in contrast to the 3β-axial epimer (epilumisterol).[25] All this indicates that the influence of the 3-hydroxyl group in the formation of complexes with digitonin is determined not by its α- or β-arrangement but by its equatorial or axial arrangement, and is related to the same steric factors as the above differences in reactivity. This suggestion naturally requires confirmation using a larger amount of experimental material than is available at present.

The lower complex-forming power of the axial epimers is not the only feature illustrating the influence of steric factors on the formation of digitonides. Thus the introduction of an additional methyl group into a sterol in position 3 makes the formation of the digitonide more difficult, while the relationship between the stabilities of the equatorial and axial epimers is preserved (see Table 1). The introduction of additional methyl groups in position 4 of the molecule on going from cholesterol to lanosterol also leads to higher solubility of the digitonide.[26] Esters of 3β-hydroxyandrostan-17-carboxylic acid, containing the bulky diphenylmethane group in the alcohol fraction of the molecule, also do not form digitonides, in contrast to the esters with simple alcohols (methanol, ethanol).[24]

The role of the aliphatic chain on the C(17) atom in the formation of sterol digitonides has been less extensively studied than the role of the hydroxyl group. The data in Table 1 show that the double bonds in the aliphatic chain of ergosterol and stigmasterol have no influence on the stability of the complex, whereas in the series of identical derivatives of cholestane, cholane, pregnane, and androstane, decrease in the length of the chain leads to a slight but regular decrease in the stability of the digitonide. The competitive formation of digitonides by cholesterol and its synthetic analogues containing unbranched aliphatic chains of different length on the C(17) atom has been studied.[27] Gradual increase in the length of the chain increases the stability of the complex, which became equal to the stability of cholesterol digitonide for the compounds containing 8–10 carbon atoms in the side chain. Further increase in the length of the aliphatic chain to C_{11}–C_{13} gave digitonides with an even higher stability, but at C_{15} the stability of the complex decreased sharply.

In addition to the dependence of complex formation on the structure of the sterol, some data have been published on the influence of the structure of the second component of the complex, that is the saponin, on this process. Complexes with sterols are formed not only by digitonin but also by a whole series of steroid saponins, and also various steroid alkaloids[20,28] and triterpene glycosides.[13] Comparison of the stability of the complex with the saponin aglycone structure indicates that spirostanol saponins containing six rings form less soluble complexes than furostanol saponins, which contain one fewer ring.[9,29,30] The structure of the glucoside residue of the saponin has a marked influence on complex formation. The optimum number of monose residues for complex formation is 4–5, and decrease in this number leads to an increase in the solubility of the complexes.[9,10,30] A quantitative study has shown that desglucosodigitonin (*Ib*), which contains four monose residues, has practically the same complex-forming power as digitonin (*Ia*), whereas glucosogalactosodigitonin (*Ic*), obtained by removing three monoses from natural digitonin, does not form a complex with cholesterol under the conditions studied.[27]

The above regular features relating the stability of the complex to the structures of both the sterol and the saponin have made it possible to put forward suggestions about the structure of the complex itself.[27] It is suggested that the digitonide is a clathrate, in which the saponin is the host, and the sterol the guest. The digitonin molecules are oriented to form a hydrophobic cabity capable of accommodating a guest molecule. This model assumes that the aglycone fraction of the saponin undergoes a hydrophobic interaction with the sterol molecule. The rigid cyclic systems of the two components ensure that the interaction is equimolar. A mobile aliphatic side chain on the C(17) atom of the guest molecule, capable of changing its position in space and undergoing an additional hydrophobic interaction with the host molecule, should, for a definite length, ensure stability of the complex. In a development of this hypothesis it may be assumed that the part of the host cavity which is formed by the glucoside residue and which is hydrophilic in character leads to stabilisation of the complex as a result of the formation of hydrogen bonds between the hydroxyl groups of the sugars and the 3-hydroxyl group of the sterol. This last suggestion agrees with the stability of the digitonides of steroids which contain in position 3 not a hydroxyl group but certain other groups capable of acting as proton donors or acceptors in hydrogen bond formation, such as amino- or dimethylamino-groups,[4,31] but not the thiol group,[32] which is a less effective partner in hydrogen bond formation. The hypothesis is supported by other factors. The ability of cholic acids to act as the host in clathrates with various compounds, including cholesterol, is well known.[33] The common features of the structures of saponins and cholic acids include not only the common hydrophobic cyclopentanoperhydrophenanthrene framework, but also the necessary presence of hydrophilic groups—the carboxyl group in cholic acid, and the hydroxyl groups of the sugars in the saponins. The blocking or elimination of these groups by esterification of the acid or the removal of monoses makes the compounds unable to form complexes.

Steroid saponins have a marked haemolytic and antimycosal action. The dependence of these properties on the chemical structure of the saponins shows regular features analogous to those of the above relationships between the complex-forming power and the structure. For example, the maximum haemolytic activity is shown by spirostanol saponins containing 4–5 monose residues.[34,35] This parallel suggests that the haemolytic action of saponins and other glycosides is due to complex formation with membrane sterols.[36,37,38] and this increases the interest in the complexes of sterols with saponins.

III. STEROL CRYSTALLINE SOLVATES

Only a few papers have been published on these sterol complexes. The crystalline hydrates of cholesterol, formed by crystallising the latter from aqueous solvents (alcohol, acetone), gradually lose water of crystallisation at ordinary temperatures, and are completely dehydrated at 80°.[39,40] The crystalline hydrates of other sterols such as β-sitosterol and ergosterol behave similarly. Sterols also form complexes with alcohols[41] and inorganic and organic acids, the highest stability being shown by the complexes with the strong acids perchloric,[42] trichloroacetic,[43] and oxalic.[44]

It is logical to assume that in complex formation by a sterol with small polar molecules, the hydrophobic inter-

action between them plays an insignificant role, and that the principal contribution is made by forces associated with the hydroxyl group. Thus only sterols with a free 3β-hydroxyl group, and not sterol esters, form both crystalline hydrates and complexes with acids. Similarly, the corresponding complexes are not formed by compounds which do not contain hydroxyl groups, such as cholestenes and cholestadienes. It has been shown qualitatively that, as in the formation of digitonides, a decisive role in complex formation with perchloric and oxalic acids is played by not only the presence but also the configuration of the 3-hydroxyl group. Thus unlike 5α-cholestan-3β-ol and cholesterol, the corresponding 3α-epimers do not form oxalates or perchlorates.[42,44]

In contrast to digitonides, where hydrogen bonding has only been assumed, in the case of the crystalline solvates various data have been published on the nature of the hydrogen bond between the hydroxyl group of the sterol and the second component of the complex.

In the crystal of cholesterol[45] and its esters containing a polar group in position 3, such as the hydrogen sulphate[46] and phosphate,[47] the molecules are packed in structures in which the rigid system of rings, the substituent on the C(3) atom, and the aliphatic side chain are arranged in different positions in the molecular layer. Within the layer, the molecules are arranged in two series with their planar rear α-sides turned towards one another, favouring the maximum hydrophobic interaction. On the other hand, neighbouring double bonds make contact through their frontal β-sides. The double layers are displaced slightly (~0.85 Å) relative to one another in such a way that the angular methyl groups projecting above the surface are entangled. The maximum hydrophobic interaction is achieved when the long axes of the molecules are parallel, but at the same time this parallel arrangement leads to an increase in the distance between the oxygen atoms of the two hydroxyl groups of two molecules of the bilayer, to a value >3.0 Å, preventing the formation of a hydrogen bond between them. On the other hand, when the two hydroxyl groups approach one another to a distance <2.5 Å to give a strong hydrogen bond, the parallel arrangement of the molecules breaks down, and the hydrophobic interaction decreases. Thus in the cholesterol crystal there is a compromise between these two requirements: the parallel arrangement of the axes of the molecules is slightly disturbed, and the O...O distance is 2.87 Å, which is at the upper limit for possible hydrogen bond formation. In cholesterol monohydrate, however, hydrogen bond formation takes place not between two cholesterol hydroxyl groups but through water molecules acting as bridges.[48,49] This makes it possible for two cholesterol molecules to be joined by a hydrogen bond while preserving the parallel arrangement of the axes of the molecules. Thus in the crystal of cholesterol monohydrate there is a greater interaction between the individual parts of the two molecules than in the crystal of the anhydrous substance, that is the inclusion of water in the polar fraction of the molecules also increases the strength of the hydrophobic interaction.

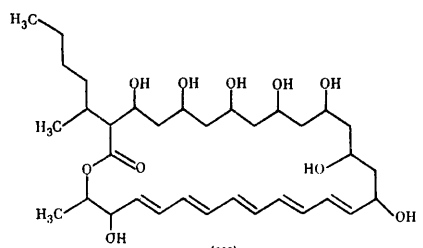
Complex formation by cholesterol with other ligands, for example acids, apparently takes place similarly. Infrared spectroscopy has shown that the reaction of cholesterol with trichloroacetic acid in carbon tetrachloride solution involves the formation between them of a hydrogen bond, in which the acid acts as proton donor, and cholesterol as proton acceptor.[50] Increase in the concentration of both components leads to aggregation of the binary particles as a result of the formation of new hydrogen bonds between them. In these aggregates, the hydroxyl group of cholesterol acts simultaneously not only as a proton acceptor, but also as a proton donor, which accepts either the hydroxyl group or the carbonyl group of the acid. In the solid complex, the role of acceptor is apparently played only by the carbonyl group of the acid; in this case the bridge formed is longer, and creates more favourable conditions for the interaction of two sterol molecules in the crystal.

The proposed model requires that not only cholesterol but also the second component of the complex should be not only a proton acceptor but also a proton donor in hydrogen bond formation. Thus cholesterol forms solid complexes with alcohols, but not with esters, and only with those amides which contain NH_2 or NHR groups.[50]

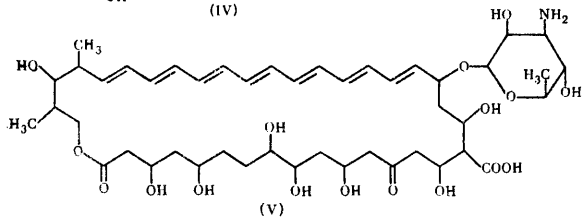
Cholesterol and other sterols form complexes with various salts, for example chlorides.[6,51] Published data on these complexes are few and often contradictory, but the hydroxyl group plays an important role in their formation. Thus only compounds with a free 3β-hydroxyl group, and not sterol esters or 3α-epicholesterol, form complexes with calcium chloride.[52] The rate of acetylation of the hydroxyl group of cholesterol in its complexes with calcium and magnesium chlorides is higher than the rate of acetylation of the sterol itself.[53] The complexes with salts also apparently contain water molecules, as confirmed in a number of cases by analysis.[6,52] The conditions of preparation of other complexes with salts also indicate that they contain water molecules, but unfortunately, no analytical data are available.

IV. COMPLEXES WITH POLYENE ANTIBIOTICS

The biological action of polyene antibiotics is due to their complex formation with the sterols of cell membranes. The proof of this mechanism and the investigation of the relationship between the structure and action of antibiotics have been the subject of a large number of publications, including a number of reviews,[54-58] so that the present review will only list briefly the characteristic features of the structure of polyene antibiotics, for example filipin (IV) and amphotericin B (V). Polyene antibiotics are characterised by the presence of a macrolide ring, formed by the closure of a lactone ring. One side of the ring consists of a system of 4–7 conjugated double bonds. The total number of carbon atoms in the ring is $4n + 4$, where n is the number of double bonds of the polyene chain. A second characteristic feature is the large number of hydroxyl groups, from 6 to 14, situated on the opposite side of the ring. Finally, most polyene antibiotics contain a mycosamine residue.



(IV)



(V)

The simultaneous presence in the molecule of a polar region, formed by a number of hydroxyl groups, and a rigid hydrophobic system of conjugated double bonds is responsible for the amphipathic properties of polyene antibiotics, for example their ability to form micellar solutions. It follows from the above material that this amphipathic character may be responsible for the ability to form complexes with sterols. Unlike the crystalline complexes described above, the complexes of sterols with antibiotics have not been isolated in the crystalline state, and their formation has been demonstrated by various physicochemical methods, primarily spectroscopic methods. The addition of sterols to solutions of polyene antibiotics produces a characteristic change in the ultraviolet spectra of the latter, namely a change in the ratio of the absorptivities of different maxima, indicating that the antibiotic reacts with the sterol. Complex formation is reversible, and depends on the solvent and temperature. The change in the spectra of the antibiotics, measured by the ratio of the absorption at 320 nm and 356 nm (the so-called 1:3 peak ratio), takes place only in the reaction with the sterol. The data in Table 2 show that the addition of other compounds such as cetyl alcohol or lecithin to a filipin solution produces no change in the spectrum; the changes brought about by secosteroids are also very slight. The ability to form a complex with an antibiotic is also influenced by the presence of a 5α-gonane system, an aliphatic chain on the C(17) atom, and a 3-hydroxyl group on the C(3) atom, although there are also unexplained exceptions, namely the 3-ketoderivatives. More definite information can be obtained by comparing the changes produced in the spectrum of filipin by its reaction with not a free sterol but a sterol in a sterol–lecithin liposome. In the case of liposomes containing 16–50% sterol, the optimum interaction is observed for sterols containing a 5α-gonane or Δ^5-gonene ring and a aliphatic chain on C(17). In this case, complex formation depends on the substituent on C(3). The maximum interaction is shown by compounds with a free 3β-hydroxyl group, and its acetylation, oxidation, or epimerisation leads to a sharp decrease in the stability of the complex. According to Ref.61, the dissociation constant of the epicholesterol–filipin complex is 15 times the corresponding constant of the cholesterol–filipin complex. Similar regular features are observed for sterols in not liposomes but natural membranes. A culture of Acholeplasma Laidlawii does not contain sterols in the cell membranes, but is capable of including them in the membrane when grown in sterol-containing media. This is widely used in the study of the influence of sterols on the properties of biological membranes.[62] It follows from the data in Table 2 that the reaction of filipin with this membrane also leads to characteristic changes in its ultraviolet spectrum. Maximum complex formation takes place in the case of membranes containing sterols of the 5α- or Δ^5-series with a 3β-hydroxyl group; the spectral changes are much less marked on going to the 5β-series (coprosterol) and to the 3α-epimers (epicholesterol).

The dependence of complex formation with an antibiotic on the structure of the sterol is confirmed by not only ultraviolet but also fluorescence spectroscopy.[63] The formation of complexes with sterols leads to quenching of the fluorescence of the antibiotics. Data on the quenching of the fluorescence of antibiotics brought about by the formation of complexes with sterols show that complex formation with filipin, amphotericin, and pimaricin is a maximum for sterols containing a 3β-hydroxyl group, and that practically no complex formation takes place when this group is acylated or epimerised.[64] Similar results have been obtained in spectroscopic studies of the reactions of sterols with other polyene antibiotics. All these data indicate that the stability of the complexes of sterols with antibiotics is determined by the same characteristic features of the sterol structure as the stability of the complexes with saponins. The following model of the complex of an antibiotic with a sterol has been proposed by a number of authors.[65,66] When the antibiotic enters the membrane its hydrophobic polyene chain reacts with the hydrophobic cyclic system of the sterol to form a 1:1 complex. Several such complexes (eight in the case of amphotericin B) join together to form a so-called semi-pore, whose inner part, containing a large number of hydroxyl groups, acts as a hydrophilic canal. Two semi-pores form a pore penetrating the membrane. The hydroxyl group of the sterol and the polar amino- and carboxyl groups of amphotericin B are on the membrane surface facing water. It is assumed that hydrogen bond formation between these groups is possible.

Table 2. Influence of the structure of the sterol on complex formation with philipine (3:7 peak ratio in the ultraviolet spectrum).

Compound	In aqueous solution (Ref.59)	In liposomes (Ref.59)*	In natural membranes (Ref.60)
Original solution	0.72	— —	—
5α-Cholestan-3β-ol	2.17	1.35; 1.94	2.99
5α-Cholestan-3α-ol	—	— —	1.16
Cholest-5-en-3β-ol	2.12	1.40; 1.95	2.18
Cholest-5-en-3α-ol	1.63	0.85; 1.04	1.47
3β-Palmitoylcholest-5-ene	1.78	— —	—
5β-Cholestan-3β-ol	1.56	0.88 —	1.35
Stigmasterol	1.70	1.71; 2.23	1.56
Ergosterol	1.88	1.48; 1.70	2.01
5α-Androstan-3β-ol	1.52	0.94; —	—
5α-Androstan-3α-ol	1.50	— —	—
Cholest-5-en-3-one	2.50	— —	—
5α-Cholestan-3-one	2.27	0.90; 0.96	—
Dihydrotachysterol	0.92	— —	—
Vitamin D_2	0.84	— —	—
Lecithin	0.88	— —	—
Cetyl alcohol	0.78	0.87; —	—

*First figure—16% sterol, second figure—50% sterol.

The change in the penetrability of natural membranes brought about by the addition of antibiotics is determined by the nature of the sterol present in the membrane.[54] The maximum change is observed when cholestanol and cholesterol are present, whereas their epimers undergo practically no reaction with antibiotics. The need for the presence of a 3β-hydroxyl group for the reaction with an antibiotic was also proved in a study of the conductivity of bimolecular lipid membranes; the addition of levorin increased the conductivity of membranes containing cholesterol by a factor of 10^5, but increased the conductivity of membranes containing esterified cholesterol and cholest-5-en-4-one by a factor of only 10.[67]

The proposed model of the sterol–antibiotic complex explains satisfactorily the role of the sterol framework. The planar polyene chain of the antibiotic molecule complements to a maximum extent the planar cyclic system of the sterol. Breakdown of the planarity of the polyene chain by the introduction of one simple bond (nystatin) or by the complete reduction of all the double bonds (hydrogenated amphotericin) reduces the complementarity of the interacting molecules.[68] An even greater influence on the

stability of the complex is shown by breakdown of the planarity of the cyclic system in secosteroids (see Table 2) or by the steric hindrance produced by geminal methyl groups in such sterols as tetrahymanol,[64] eburicol, and lichesterol.[69] On the other hand, an increase in the planarity of the sterol molecule facilitates complex formation (Table 2). This has been confirmed by studies of the conductivity of bilayer lipid membranes; the increase in the conductivity brought about by the addition of levorin is proportional to the number of double bonds in the sterol framework.[70,71] According to Ref.72, the complete aromatisation of the ring *A* of the sterol also favours complex formation with antibiotics.

Although it explains the role of the sterol framework in the hydrophobic interaction, the proposed model does not explain the importance of the aliphatic chain on C(17). The data in Table 2 show that androstane derivatives have a much lower complex-forming power than cholestane derivatives. A gradual decrease in the length of the side chain in synthetic homologues of chlolesterol also leads to a decrease in their ability to form complexes with antibiotics.[73] Another factor is the degree of branching of the side chain, as can be seen by comparing cholesterol, β-cytosterol, and stigmasterol. The introduction of double bonds not only into the rings but also into the aliphatic chain favours complex formation with antibiotics.[70,71] It should be noted, however, that the role of double bonds is different for complex formation with different antibiotics. Thus filipin forms a more stable complex with cholesterol than with ergosterol, but amphotericin, pimaricin, and nystatin form more stable complexes with ergosterol than with cholesterol.[74,78] The authors cited relate this difference to the presence of mycosamine in the structure of the last three antibiotics, but they leave open the question of how mycosamine can react with the additional double bonds in the ergosterol molecule. At the same time, this question is of not only theoretical but also applied importance; its solution facilitates the choice of particular antibiotics for treating different illnesses. The role of the 3β-hydroxyl group also remains unexplained. Although the formation of a hydrogen bond between the hydroxyl group of a sterol and the functional groups of antibiotics has been postulated repeatedly on the basis of the observed dependence of complex formation on the sterol structure, the existence of this bond has not been proved. A leading role in the formation of the sterol–antibiotic complex is apparently played by the hydrophobic interaction, particularly in solutions, where the solvent prevents hydrogen bond formation. The decisive role of the hydrophobic interaction in solutions is confirmed by the fact that complex formation is influenced by the presence of detergents and organic solvents, and is not influenced by the addition of salts and organic compounds.[78,79] In the reaction with a sterol in a membrane, the possibilities for hydrogen bond formation are greater, and this is revealed by the clearly defined influence of the nature and the sterochemistry of the substituent on C(3) on complex formation. Even in a membrane, however, the sterol–antibiotic interaction is weaker than the sterol–saponin interaction, as proved by the fact that digitonin blocks the action of antibiotics on membranes.[54] On the other hand, the fact that the antibiotic enters the membrane indicates that the interaction of the sterol with the antibiotic is stronger than the interaction of the sterol with other components of the membrane. It may also be noted that the reaction of cholesterol with levorin and amphotericin B inhibits the enzymatic esterification of cholesterol by lecithin–cholesterol–acetyltransferase.[80]

V. STEROLS IN MEMBRANES

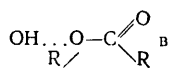
The role of cholesterol and other sterols in the structure of natural membranes and their models has been examined in detail in a number of reviews and monographs (see for example Refs.81–83). The sterol molecules are introduced between the hydrocarbon chains of phospholipids, and the intermolecular interaction between the sterol and the phospholipid is greater than the interaction between identical molecules of both phospholipids and sterols. The main structural function of cholesterol in the membrane is to stabilise the hydrophobic part of the bilayer by decreasing the mobility of the hydrocarbon chains at temperatures above the phase transformation and increasing their mobility at temperatures below the phase transformation. Both the condensing and the decondensing ("liquefying") effects of sterols have been proved by various physical methods in natural and model membranes. This influence is determined to a large extent by the structure of the sterol.

Only compounds containing a 3β-hydroxyl group influence the properties of lecithin monolayers.[84,85] The 3-keto-steroids cholestan-3-one and its Δ^4-, Δ^5-, and $\Delta^{4,6}$-derivatives have a much weaker condensing effect and have practically no influence on the permeability of liposomes for glucose, glycerol, and the Rb^+ cation. The influence of the 3-hydroxyl group is determined by its conformation; the presence of 3α-epicholesterol has no influence on the permeability of the membranes of Acholeplasma Laidlawii for glycerol and erythritol or on the energy of the phase transformation in these membranes.[86] As in the case of the complexes of sterols with saponins and antibiotics, the specific role of the 3β-hydroxyl group has been explained by assuming that a hydrogen bond is formed between this group and some acceptor group of the phospholipid. It was first suggested that the phosphate group acts as proton acceptor, but this suggestion was rejected on the basis of ^{31}P NMR data.[87,88] At almost the same time it was shown that the sterol is arranged in the lipid bilayer in such a way that its hydroxyl group is located close to the glycerol residue of the phospholipid.[89,90]§ This led to the appearance of several models of the hydrogen bond between the hydroxyl group of the sterol and the carbonyl group of the aliphatic acid of the phospholipid. The first of these postulates the existence of "hydrogen belts" in the membrane, including hydrogen bonds between hydrated cholesterol and the carbonyl group.[92] An examination of models of cholesterol and the phospholipid showed that the formation of a bond of this kind should lead to a stronger hydrophobic interaction with the hydrocarbon chain not only of the planar α-surface of the sterol but also of its β-surface, where the projecting angular methyl groups on C(10) and C(13) are inserted into a hydrophobic "loop" formed by the unsaturated aliphatic acid.[93] Although some studies by the authors of these hypotheses[94] indicated an OH...O=C bond, more detailed studies did not confirm its existence. It was shown that the influence of epicholesterol on the chemical shift of the signals in the NMR spectrum of dipalmitoyl-lecithin containing a ^{13}CO-group is much greater than the corresponding influence of cholesterol.[95] At the same time it was suggested that it is cholesterol, and not its epimer, that forms a hydrogen bond with the carbonyl group. An infrared and Raman spectroscopic study of the carbonyl

§To counterbalance this, it has recently been shown[91] that the hydroxyl group of cholesterol is situated close to the phosphate group of lecithin.

absorption of the same phosphatidylcholine in the presence of cholesterol showed that there is no hydrogen bond between the hydroxyl and carbonyl groups, at least in the anhydrous specimens.[96] Some spectral changes in the carbonyl region of hydrated dipalmitoylphosphatidylcholine are observed, however, when cholesterol is added.[97] The Raman spectrum of anhydrous crystalline dipalmitoylphosphatidylcholine shows a carbonyl group doublet, corresponding to two different acyl groups in different conformations. Hydration of dipalmitoylphosphatidylcholine, which converts it into a gel phase, transforms this doublet into a single band, as a result of the formation of the conformationally uniform phospholipid. The addition of cholesterol to the hydrated phospholipid, however, splits the carbonyl band again, and it becomes similar to the doublet in the spectrum of the crystalline specimen. The two conformations of the acyl chains of phosphatidylcholine in the gel phase in the presence of cholesterol were also demonstrated by ^{2}H and ^{13}C NMR spectroscopy.[98,99] Compounds which cannot act as proton donors, such as the methyl ester of cholesterol, do not influence the conformation of the aliphatic acid chains. Thus the influence of cholesterol on the conformation of the acyl chains of the phospholipid indicates indirectly that a hydrogen bond is formed, but cannot prove the existence of the OH...O=C bond.

An attempt was recently made to resolve this contradiction. From a detailed examination of molecular models, it was suggested[100] that a hydrogen bond is formed between the hydroxyl group of cholesterol and the ester oxygen and not the carbonyl oxygen, of the alkoxycarbonyl group. Although the ester oxygen is a weaker proton acceptor than the carbonyl oxygen, it can fulfil this role when the steric arrangement is favourable.[101,102] Examination of molecular models shows that for the maximum hydrophobic interaction between the α-side of the sterol ring and the hydrocarbon chain of lecithin, the hydroxyl group of cholesterol is arranged in such a way that it can form a linear hydrogen bond with the ester oxygen, but not with the carbonyl oxygen. In the case of the α-conformation of the hydroxyl group (epicholesterol), however, this bond is impossible. The proposed hypothesis makes it possible to explain both the difference in the conformations of the two acyl chains in the presence of cholesterol and the difference in the influences of the cholesterol epimers on the properties of the lipid layers. It should be noted, however, that no experimental proof of the formation of an



bond was obtained in this case.

The question of hydrogen bond formation was investigated by another method, by studying the influence of cholesterol on the properties of model membranes constructed from different lipids. In one case the membranes consisted of lipids containing ester or ether groups capable of acting as proton acceptors in hydrogen bonding, and in the other case these groups were replaced by hydrocarbon chains incapable of acting as proton acceptors.[103-107] It was found that the addition of cholesterol to the last membranes, where hydrogen bond formation is impossible in principle, brings about the same condensing effect as in membranes containing ester and ether groups. It is true that it was shown for the first time that there is a slight difference in the changes in the permeability of membranes of different types under the influence of cholesterol,[104] but this difference was not confirmed in subsequent studies.[105-107]

Irrespective of whether a hydrogen bond is present or absent, the localisation of the hydroxyl group close to the glycerol residue of the phospholipid, confirmed by recent studies,[108,109] is responsible for the definite arrangement of cholesterol in the membrane. The gonane ring is fixed between the C(2) and C(8) atoms of the aliphatic acid, and it is the interaction in this section that determines the stability of the complex. As in the complexes with saponins and polyene antibiotics, the stability is determined primarily by the planar α-surface of the sterol. It was shown in Refs.84 and 85 that cholestanol, which is a planar sterol of the α-gonane series, has a more marked condensing effect and a stronger influence on the permeability of membranes, compared with coprostanol. An increase in the planarity of the cyclic system by the introduction of double bonds into the *B* ring (cholesterol, dehydrocholesterol) or by the replacement of the six-membered ring by a five-membered ring (*B*-norcholesterol) increases this influence. On the other hand, the introduction of methyl groups, which break down the planarity of the α-surface of the sterol, decreases its influence on the properties of membranes. The decrease is proportional to the number of these groups: cholesterol < 4α-methylcholesterol < 4,4-dimethylcholesterol < 4,4-diethylcholesterol ≪ 4,4,14α-trimethylcholesterol (lanosterol).[110] The influence of the three-dimensional structure of the sterol on other properties of the membrane, for example the micro-viscosity, is completely analogous.[111,112] An interesting feature is that the above sequence corresponds to the step wise demethylation of the precursors of cholesterol during its biosynthesis. The biological demethylation is highly selective, and only the α-surface of the sterol is affected. This creates a planar surface, capable of maximum interaction with the aliphatic chain of the phospholipid. At the same time, practically all natural sterols contain β-methyl groups on the C(18) and C(19) atoms. Apparently, elimination of these groups does not take place because this would not lead to an increase in the strength of the contact between the sterol and the phospholipid. Moreover, according to the hypothesis of Ref.93, the β-methyl groups are necessary for more complete contact with the unsaturated aliphatic chain.

The interaction between the aliphatic chain of the phospholipid and various sterols differing in the planarity of the α-surface was recently studied by the spin probe method, using stearic acid containing nitroxyl groups on the C(5), C(7), C(12), and C(17) carbon atoms.[113] As suggested, methyl groups situated on the α-surface bring about considerable perturbation of the EPR spectrum, and this perturbation is determined by the relative arrangement of the methyl groups of the sterol and the nitroxyl groups of the probe. The methyl group of 3α-methylcholesterol brings about considerable perturbation in the region of the C(5) atom of stearic acid and less perturbation in the region of C(7) and C(12). Lanosterol, on the other hand, brings about perturbation chiefly in the region of C(12), from its C(14) methyl group, and brings about less perturbation in the region of C(5) and C(7). All this indicates that there is considerable contact between the sterol molecules and the section of the aliphatic acid from C(2) to C(9), which is broken down by the projecting axial methyl groups. The compounds studied bring about practically no perturbation of the signal in the region of the C(17) carbon atom, indicating that there is little interaction between the isooctyl chain of the sterol and the aliphatic chain of the acid.

The side chain of the sterol plays a lesser role in complex formation with a phospholipid, compared with the polycyclic system. The aliphatic chain promotes the solubility of the sterol in the membrane. Decrease in the length of the chain, and the introduction of double bonds into it,

decrease this solubility. At low sterol concentrations (up to 20%), the ordering action of different analogues of cholesterol with shortened chains is practically identical, but increase in the sterol concentration increases this effect only for cholesterol and its closest analogue with an isoheptyl chain, and compounds with a shorter chain at high concentrations no longer dissolve in the bilayer, but form a separate phase.[114,115] At the same time, increase in the length of the side chain to ten carbon atoms alters sharply the properties of the bilayer. This compound, penetrating into the membrane with its long aliphatic "tail", apparently breaks down the neighbouring layer of the bilayer.

Thus the suggestion, put forward as early as 1967,[116] that the sterol in a membrane acts as a "filler" of the space between the hydrocarbon chains of the phospholipid has now been supplemented by a number of requirements which must be satisfied by the sterol structure if it is to be the optimum structure from the viewpoint of the formation of the sterol–phospholipid complex.

In complex formation by sterols with the various groups of chemical substances examined in the present review, a decisive role is played by the planar tetracyclic system. This system, produced in the course of biochemical evolution, is the most favourable for hydrophobic interaction with the aliphatic chains of phospholipids, required for the fulfilment of the normal function of sterols in biological membranes. The two other important structural units of the sterol molecule, namely the 3-hydroxyl group and the iso-octyl chain on C(17), play a lesser role in complex formation. Only complete elimination of the aliphatic chain destroys the ability of the molecule to dissolve in a membrane, but the formation of a hydrogen bond involving the hydroxyl group has not yet been proved. The same polycyclic system interacts even more effectively with the more planar, compared with the chain of the aliphatic acid, rigid alkene system of polyene antibiotics and the planar polycyclic system of saponins. Although the formation of a hydrogen bond with the hydroxyl group of the sterol has not been proved in these cases either, the undoubted requirement of a hydrophilic region in the second component of the complex makes it more probable. Finally, in complexes with simple molecules such as water and acids, the existence of a hydrogen bond has been proved. Here also, however, the hydrogen bond plays a secondary role, orienting the molecules to give the maximum interaction of the gonane rings. The hydrophobic interaction determines complex formation in all the cases examined. This should apparently be taken into account in the study of those sterol complexes about whose structure little is as yet known, in particular lipoproteins.

As already noted, the synthesis of compounds which sorb cholesterol selectively by forming complexes with it is now an important practical problem. Up to the present, success in the preparation of these compounds has been achieved by using derivatives of the natural complex-forming saponins.[10] Synthetic compounds forming complexes of the host–guest type with hydrophobic molecules have proved to be ineffective in the case of cholesterol.[117] These compounds, however, were designed to include as guest molecules small molecules such as benzene, and molecules not containing polar groups.

The material of the present review shows that the possibility of complex formation with sterols is determined by the definite structure of the second component of the complex, in particular its amphipathic nature. This property should apparently be made the basis of the synthesis of compounds capable of forming stable complexes with sterols.

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While the present review was being prepared for publication, several new papers dealing with the formation of hydrogen bonds by sterols appeared. In Refs.118 and 119 it was shown that this bond in carbon tetrachloride solution is identical to the hydrogen bond formed by other alcohols. Papers by the authors of the model of "hydrogen belts" in membranes give some indirect evidence in support of this model.[120,121] The most important paper[122] described the influence of cholesterol ethers (3β-alkoxycholest-5-enes) on the properties of the membranes. The methyl ether of cholesterol alters the permeability of model membranes to almost the same extent as cholesterol, disproving the suggestion that cholesterol acts as a proton donor in hydrogen bond formation with phospholipids. At the same time, the authors cited showed that the influence of cholesterol derivatives on the properties of membranes is due to the presence of the oxygen-containing substituent in position 3 of the sterol, and is determined by the size of this substituent. It was suggested that oxygen may play a role as hydrogen bond acceptor, and that a bond is formed between the oxygen of the sterol and the carbonyl group of the phospholipid via a water molecule.

REFERENCES

1. "Glossary of terms used in physical organic chemistry", Zhur.Org.Khim., 1983, **19**, 15.
2. A.Windaus, Chem.Ber., 1909, **42**, 238.
3. I.N.Nazarov and L.D.Bergel'son, "Khimiya Steroidnykh Gormonov" (The Chemistry of Steroid Hormones), Izd.Akad.Nauk SSSR, Moscow, 1955, p.258.
4. P.Bladon, in "Cholesterol" (Edited by R.P.Cook), Academic Press, New York, 1958, p.80.
5. BRD P.2529521 (1977); Chem.Abstr., 1977, **86**, 145916.
6. S.S.Geras'kina and M.V.Mukhina, Khim.Farm. Zhur., 1979, **13**(12), 67.
7. J.R.Sabine, "Cholesterol", Dekker, New York, 1977, p.24.
8. D.V.Ioffe, Khim.Prirodn.Soed., 1984, 275.
9. I.V.Berezin, I.P.Andrianov, Ya.I.Lapuk, L.B.Alekseeva, P.K.Kintya, and N.E.Mashchenko, 1980, 652.
10. I.V.Berezin, Yu.M.Lopukhin, I.P.Andrianova, Ya.I.Lapuk, L.B.Alekseeva, V.I.Sergeenko, E.A.Borodin, E.M.Khaliov, and A.I.Archakov, Vopr.Med.Khim., 1980, **26**, 843.
11. G.I.Klebanov, G.I.Sazhenkin, M.P.Sherstnev, I.P.Andrianova, V.I.Sergeenko, P.V.Struchkov, and Yu.A.Vladimirov, Vopr.Med.Khim., 1982, **28**, 131.
12. P.G.Bogach, M.D.Kurskii, N.E.Kucherenko, and V.K.Rybal'chenko, "Stroenie i Funktsiya Biologicheskikh Membran" (The Structure and Function of Biological Membranes), Kiev, 1981, p.45.
13. M.M.Anisimov and V.Ya.Chirva, Uspekhi Sovr. Biologii, 1980, **90**, 351.
14. C.H.Issidorides, I.Kitagawa, and E.Mosettig, J.Org.Chem., 1962, **27**, 4693.
15. F.X.Garneau, J.Cote, O.Harvey, and J.L.Simard, J.Chromatogr., 1980, **188**, 445.
16. A.Christiani and M.Pailer, Microchim.Acta, 1937, 1, 26.
17. R.M.Haslam and W.Klyne, Biochem.J., 1953, **55**, 340.
18. C.R.Noller, J.Amer.Chem.Soc., 1939, **61**, 2717.
19. G.A.D.Haslewood, Biochem.J., 1947, **41**, 639.

20. L.F.Fieser and M.Fieser, "Steroids" (Translated into Russian), Izd.Mir, Moscow, 1965, p.280.
21. G.F.Gibbons, K.A.Mitropoulos, and N.B.Myant, "Biochemistry of Cholesterol", Elsevier, New York, 1982, p.63.
22. I.M.Hais and K.Macek, "Papirova Chromatografie" (Translated into Russian), Izd.Inostr.Lit., Moscow, 1962, p.336.
23. L.F.Fieser and M.Fieser, "Reagents for Organic Synthesis" (Translated into Russian), Vol.5, Izd. Mir, Moscow, 1971, p.107.
24. L.F.Fieser and M.Fieser, "Natural Products Related to Phenanthrene" (Translated into Russian), GKhI, Moscow, 1953, p.617.
25. J.Barnett, I.M.Heilbron, E.R.H.Jones, and K.J.Verrill, J.Chem.Soc., 1940, 1390.
26. R.J.Genedella, Lipids, 1982, **17**, 443.
27. S.Tagaki, H.Otsuka, T.Akiyama, and U.Sankawa, Chem.Pharm.Bull., 1982, **30**, 3485.
28. J.G.Roddick, Phytochemistry, 1979, **18**, 1467.
29. R.Tschesche and G.Wulff, Fortschritte Chem.Org. Naturstoffe, 1973, **30**, 461.
30. G.V.Lazau'evskii, P.K.Kintya, E.U.Pukhal'skaya, and Z.P.Sof'ina, Khim.Farm.Zhur., 1977, **11**(6), 19.
31. D.P.Dodgson and R.D.Haworth, J.Chem.Soc., 1952, 67.
32. J.Strating and H.J.Backer, Rec.Trav.Chim., 1950, **69**, 909.
33. S.Candeloro De Sanctis, E.Giglio, V.Pavel, and C.Quagliata, Acta Cryst., 1972, **28B**, 3656.
34. E.Schloesser and G.Wulff, Z.Naturforsch., 1969, **24**, 1284.
35. T.Akiyama, S.Tagaki, U.Sankawa, S.Inari, and H.Saito, Tennen Yuki, 1979, 19; Chem.Abs., 1980, **93**, 109345.
36. T.Nakamura, K.Inoue, S.Nojima, U.Sankawa, J.Shoji, T.Kawasaki, and S.Shibata, J.Pharm.Dyn., 1979, 2, 374.
37. A.M.Popov, I.G.Agafonova, and M.M.Anisimov, in collected papers "Bisloinye Lipidnye Membrany" (Bilayer Lipid Membranes), Izd.Dal'nevost.Nauchn. Tsentra, Vladivostock, 1983, p.137.
38. G.E.Dekanosidze, V.Ya.Chirva, and T.V.Sergeenko, "Biologicheskaya Rol', Rasprostranenie i Khimicheskoe Stroenie Triterpenovykh Glikozidov" (The Biological Role, Occurrence, and Chemical Structure of Triterpene Glycosides), Izd.Metsniereba, Tbilisi, 1984, p.8.
39. C.R.Loomis, G.G.Shipley, and D.M.Small, J.Lipid Res., 1979, **20**, 525.
40. N.Garti, L.Karpuj, and S.Sarig, J.Lipid Res., 1981, **22**, 785.
41. F.Kloetzer, Z.Kryst., 1936, **95**, 338.
42. W.Lange, R.G.Folzenlogen, and D.G.Kolp, J.Amer. Chem.Soc., 1949, **71**, 1733.
43. G.Deniges, Compt.rend., 1933, **196**, 1504.
44. K.Miescher and H.Kaegi, Helv.Chim.Acta, 1941, **24**, 986.
45. H.S.Shieh, L.G.Noard, and C.E.Nordman, Acta Cryst., 1981, **37B**, 1538.
46. I.Pascher and S.Sundell, Acta Chim.Scand., 1977, **31A**, 767.
47. I.Pascher and S.Sundell, Chem.Phys.Lipids, 1982, **31**, 129.
48. B.M.Craven, Nature, 1976, **260**, 727.
49. B.M.Craven, Acta Cryst., 1979, **35B**, 1135.
50. D.V.Ioffe and I.M.Ginzburg, Khim.Prirodn.Soed., 1983, 49.
51. E.I.Garlinskaya, Zhur.Prikl.Khim., 1955, **28**, 80.
52. P.Marfey, R.van Meter, and M.E.Bartlet, Z.Naturforsch., 1975, **30C**, 718.
53. P.Marfey and C.Chessin, Biochim.Biophys.Acta, 1974, **337**, 136.
54. A.W.Norman, A.M.Spielvogel, and R.G.Wong, in "Advances in Lipid Research", Vol.14, New York, 1976, p.127.
55. A.Kh.Kasumov, Uspekhi Sovr.Biologii, 1977, **83**, 23.
56. A.Kh.Kasumov, Antibiotiki, 1981, **26**, 143.
57. A.N.Klimov and V.N.Litvinova, Antibiotiki, 1968, **13**, 88.
58. A.M.Landau, "Molekulyarnye Mekhanizmy Deistviya Fiziologicheski Aktivnykh Soedinenii" (The Molecular Mechanisms of the Action of Physiologically Active Compounds), Izd.Nauka, Moscow, 1981, p.126.
59. A.W.Norman, R.A.Demel, B.De Kruyff, and L.L.M.Van Deenen, J.Biol.Chem., 1972, **247**, 1918.
60. B.De Kruyff, W.J.Gerritsen, A.Oerlemans, R.A.Demel, and L.L.M.Van Deenen, Biochim.Biophys.Acta, 1974, **339**, 30.
61. R.Bittman, W.C.Chen, and L.Blau, Biochemistry, 1974, **13**, 1374.
62. S.Razin, in "Progress in Surface and Membrane Science", Vol.9, Academic Press, 1975, p.257.
63. F.Schroeder, J.F.Holland, and L.L.Bieber, Biochemistry, 1972, **11**, 3105.
64. R.Bittman, W.C.Chen, and O.R.Anderson, Biochemistry, 1974, **13**, 1364.
65. B.De Kruyff and R.A.Demel, Biochim.Biophys. Acta, 1974, **339**, 57.
66. T.E.Andreoli, Ann.New York Acad.Sci., 1974, **235**, 448.
67. Kh.M.Kasumov and E.A.Liberman, Biofizika, 1974, **19**, 71.
68. M.P.Borisov, L.N.Ermishkin, and A.Ya.Zil'bershtein, Biofizika, 1978, **23**, 910.
69. D.Johnson and R.Subden, Canad J.Microbiol., 1977, **23**, 113.
70. A.M.Feigin, I.I.Belousova, R.I.Yakhimovich, V.N.Vasilevskii, and I.M.Tereshin, Biofizika, 1979, **24**, 330.
71. A.M.Feigin, T.A.Gianin, V.I.Pasechnik, M.N.Flerov, A.A.Bogoslovskii, G.E.Litvinova, I.I.Belousova, and I.M.Tereshin, Antibiotiki, 1981, **26**, 572.
72. S.S.Yan and H.Wang, Chin.J.Microbiol., 1976, **9**, 19; Chem.Abs., 1977, **86**, 8564.
73. T.Nakamura, M.Nischikawa, K.Inoue, S.Nojima, T.Akiyama, and U.Sankawa, Chem.Phys.Lipids, 1980, **26**, 101.
74. A.Vertut-Croquin, J.Bolard, M.Chabbert, and C.M.Gary-Bobo, Biochemistry, 1983, **22**, 2939.
75. D.B.Archer, Biochim.Biophys.Acta, 1976, **436**, 68.
76. J.D.Readio and R.Bittman, Biochim.Biophys.Acta, 1982, **685**, 219.
77. Y.Kitojima, T.Sekija, and Y.Nozawa, Biochim. Biophys.Acta, 1976, **445**, 452.
78. J.M.Patterson, M.R.Olinger, J.F.Holland, and L.L.Bieber, J.Antibiotic, 1979, **32**, 1193.
79. I.Gruda, P.Nadeau, J.Brajtburg, and G.Medoff, Biochim.Biophys.Acta, 1980, **602**, 260.
80. A.N.Klimov, A.A.Nikiforova, and A.M.Chistyakova, Biochim.Biophys.Acta, 1975, **380**, 76.
81. R.A.Demel and B.De Kruyff, Biochim.Biophys. Acta, 1976, **457**, 109.

82. V.G.Ivkov and G.N.Berestovskii, "Dinamicheskaya Struktura Lipidnogo Bisloya" The Dynamic Structure of the Lipid Bilayer), Izd.Nauka, Moscow, 1981, p.180.
83. V.G.Ivkov and G.N.Berestovskii, "Lipidnyi Bisloi Biologicheskikh Membran" (The Lipd Bilayer of Biological Membranes), Izd.Nauka, Moscow, 1982, p.56.
84. R.A.Demel, K.R.Bruckdorfer, and L.L.M.Van Deenan, Biochim.Biophys.Acta, 1972, **255**, 311.
85. R.A.Demel, K.R.Bruckdorfer, and L.L.M.Van Deenen, Biochim.Biophys.Acta, 1972, **255**, 321.
86. B.De Kruyff, R.A.Demel, and L.L.M.Van Deenen, Biochim.Biophys.Acta, 1972, **255**, 331.
87. B.De Kruyff, P.R.Cullis, and G.K.Radda, Biochim. Biophys.Acta, 1975, **406**, 6.
88. P.Yeagle, L.Hutton, C.H.Huang, and R.B.Martin, Proc.Natl.Acad.Sci.USA, 1975, **72**, 3477.
89. N.P.Franks, J.Mol.Biol., 1976, **100**, 345.
90. D.L.Worcester and N.P. Franks, J.Mol.Biol., 1976, **100**, 359.
91. A.N.Ushakov, E.E.Golubeva, V.V.Rozynov, and M.L.Tsirenina, Bioorganich.Khim., 1983, **9**, 697.
92. Brockerhoff, Lipids, 1974, **9**, 645.
93. C.H.Huang, Lipids, 1977, **12**, 348.
94. N.Chatterjie and H.Brockerhoff, Biochim.Biophys. Acta, 1978, **511**, 116.
95. A.M.Lancee-Hermkens and B.De Kruyff, Biochim. Biophys.Acta, 1972, **470**, 141.
96. S.F.Bush, H.Levin, and I.W.Levin, Chem.Phys. Lipids, 1980, **27**, 101.
97. E.Bicknell-Brown and K.G.Brown, Biochim.Biophys.Res.Comm., 1980, **94**, 638.
98. E.Oldfield, M.D.Meadows, D.M.Rice, and R.E.Jacobs, Biochemistry, 1978, **17**, 2727.
99. A.Blume and R.G.Griffin, Biochemistry, 1982, **21**, 6230.
100. F.T.Presti, R.J.Pace, and S.I.Chan, Biochemistry, 1982, **21**, 3831.
101. N.Mori, Y.Asano, and Y.Tsuzuki, Bull.Chem.Soc. Japan, 1968, **41**, 1871.
102. M.A.Abramovich, I.M.Ginzburg, and D.V.Ioffe, Teor.Eksper.Khim., 1971, **7**, 225.
103. J.W.Fong, L.J.Tirri, D.S.Deshmukh, and H.Brockerhoff, Lipids, 1977, **12**, 857.
104. L.J.Tirri, P.C.Schmidt, R.K.Pullarkat, and H.Brockerhoff, Lipids, 1977, **12**, 863.
105. S.Clejan, R.Bittman, P.W.Deroo, Y.A.Isaacson, and A.F.Rosenthal, Biochemistry, 1979, **18**, 2118.
106. R.Bittman, S.Clejan, M.K.Jain, P.W.Deroo, and A.F.Rosenthal, Biochemistry, 1981, **20**, 2790.
107. L.C.Bartholow and R.P.Geyer, Biochemistry, 1982, **21**, 1271.
108. J.Rogers, A.G.Lee, and D.C.Wilton, Biochim. Biophys.Acta, 1979, **552**, 23.
109. E.N.Lapshin, G.E.Dobretsov, D.V.Ioffe, and A.S.Kuznetsov, Biofizika, 1984, **29**, 989.
110. K.E.Bloch, in R.J.Straub (Editor), "Cell Membrane Receptors for Medicines and Hormones" (Translated into Russian), Izd.Meditsina, Moscow, 1983, p.10.
111. C.E.Dahl, J.S.Dahl, and K.E.Bloch, Biochemistry, 1980, **19**, 1462.
112. C.E.Dahl, J.S.Dahl, and K.E.Bloch, Biochim. Biophys.Res.Comm., 1980, **92**, 221.
113. C.E.Dahl, Biochemistry, 1981, **20**, 7158.
114. K.E.Suckling and G.S.Boyd, Biochim.Biophys. Acta, 1976, **436**, 295.
115. K.E.Suckling, H.A.F.Blair, G.S.Boyd, I.F.Craig, and B.R.Malcolm, Biochim.Biophys.Acta, 1979, **551**, 10.
116. D.O.Shah and J.H.Schulman, J.Lipid Res., 1967, **8**, 215.
117. F.M.Menger and M.Takeshita, J.Amer.Chem.Soc., 1981, **103**, 5938.
118. P.Mercler, C.Sandorfy, and D.Vocelle, J.Phys.Chem., 1983, **87**, 3670.
119. M.Costas and D.D.Patterson, J.Chem.Soc., Trans. Faraday Soc.I, 1985, **81**, 655.
120. L.S.Ramsammy, H.Volwerk, L.C.Lipton, and H.Brockerhoff, Chem.Phys.Lipids, 1983, **32**, 83.
121. L.S.Ramsammy, P.A.Merz, and H.Brockerhoff, Chem.Phys.Lipids, 1984, **34**, 127.
122. R.A.Demel, A.K.Lala, S.Nauda, and L.L.van Deenen, Biochim.Biophys.Acta, 1984, **771**, 142.

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