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HYDROGEN BONDS OF 5α-CHOLESTANOLS AND THEIR ETHERS

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It has been shown by IR spectroscopy that the equatorial oxygen atoms of cholestanol and its methyl ether possess a greater capacity for forming H bonds as proton acceptors than the axial atoms of the corresponding epimeric compounds. The constants of the equilibrium phenol + ether $\rightleftarrows$ H-complex (1:1) in CCl_4 at room temperature are 13 and 7 liter/mole, respectively, for the methyl esters of cholestanol and of epicholestanol.

The determining role of the orientation of the 3-hydroxy group in the complex-formation by sterols is well known [1]. Sterols containing an equatorial 3-hydroxy group possess a greater tendency to form complexes than the epimeric compounds the hydroxy group of which is present in the axial position. This is shown in the formation of crystal hydrates and of complexes with acids, saponins, polyenic antibiotics, and phospholipids. An explanation of the observed differences in complex-formation is based on differences in the possibility of the formation of a hydrogen bond between the hydroxy group of the sterol and some acceptor of the proton of the second component of the complex, although the existence of a hydrogen bond has been shown experimentally only in sterol hydrates [2, 3].

In complexes with polyenic antibiotics and phospholipids there has been no proof of the formation of hydrogen bonds, but their existence is one of the working hypotheses in considering the structure of the complexes [4, 5]. The possibility of the formation of a hydrogen bond between the hydroxyl of a sterol and the ester group of a phospholipid has been confirmed by a consideration of molecular models [6-8]. Here, because of the hydrophobic interaction of the rigid cyclopentanoperhydrophenanthrene system and the fatty-acid chains of the phospholipid, the sterol molecule is fixed in relation to the phospholipid in such a way that only an equatorial, but not an axial, hydroxyl can form a hydrogen bond with the ester group of the phospholipid.

Thus, in a consideration of the possibility of the appearance of a hydrogen bond with the participation of an equatorial or an axial hydroxyl, it is assumed that the difference arises only because of the rigid fixation of the sterol molecules relative to the second component of the complex, and, consequently, because of the different positions of the hydroxyl in relation to the proton-acceptor. It is assumed that in general the proton-donating capacity of the 3-hydroxy group for the formation of a hydrogen bond does not depend on its orientation in the sterol molecule. The validity of this hypothesis has been confirmed by Kunst et al. [9] — in CCl_4 solution the thermodynamic parameters of the hydrogen bonds of the two epimeric 5α-cholestanols with tetrahydrofuran as proton acceptor are equal and the parameters of the hydrogen bond for dimerization are practically the same.

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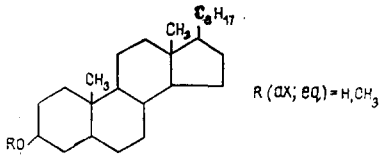
Nevertheless, in a number of papers [10, 11] the hypothesis has been expressed that in complexes of sterols with phospholipids the formation of hydrogen bonds in which the sterols are the proton donors is unlikely. In place of this, a hypothesis has been put forward of a difference in the hydration of the equatorial and axial hydroxy groups of sterols and the formation of a bond between the sterol and the phospholipid through a water "bridge." This emphasizes the role of the oxygen atoms of sterols as proton-acceptors in hydrogen bonds [12].

Quantitative differences in the electron-donating properties of the 3-hydroxy groups of sterols due to their orientation are known [13]. Because of great steric hindrance, an hydroxy group in the axial position is esterified with greater difficulty than an equatorial one [14]. For the same reason, an equatorial ether is hydrolyzed more readily [15]. Both these reactions include an attack on the lone electron pair of the oxygen atom of the sterol which is less spatially accessible in the axial conformation. A difference in the hydrogen bonds of the two conformers of a sterol should therefore not be expected in those cases where the sterols act as proton-donors sterically unhindered in both conformers (as was the case in [9]) but it should be when they act as proton-acceptors in hydrogen bonds.

With this aim we have made a comparative study of the proton-accepting capacity of cholestanol (5 α-cholestan-3β-ol and of epicholestanol (5α-cholestan-3β-ol) the 3-hydroxy groups of which occupied the equatorial and axial positions, respectively, and also of the methyl ethers of these sterols:



As a partner in the interaction with the sterols we took trichloroacetic acid (TCAA). It is known that in dilute solutions in inert solvents equimolar mixtures of trihaloacetic acids and alcohols form binary associates in which the acid participates as donor and the alcohol as acceptor of a proton, COOH...OH. A similar hydrogen bond arises between molecules of trichloroacetic acid and cholesterol in CCl_4 [16], causing a low-frequency shift of the ν(OH) band of the free hydroxy group of cholesterol in the IR spectrum by ~20 cm^{-1}. An additional confirmation of the formation of such a mixed H-complex is the disappearance from the IR spectrum of the carbonyl bands ν(C=O) of the monomer and of the cyclic dimer of TCAA and the appearance of a new band occupying an intermediate position and characteristic for any complexes with hydrogen bonds of the $CX_3COOH...B$ type (where X is a halogen).

In the IR spectra of dilute solutions in CCl_4 of equimolar TCAA–cholestanol (eq) and TCAA–epicholestanol (ax) systems a pattern similar to that described above is observed. It can be seen in Fig. 1 that in the spectrum of both mixtures there is a lowering of the intensity of the ν(OH) bands of the free equatorial and axial hydroxy groups and the bands of the hydroxy groups of the cholestanols bound to the oxygen atom to molecules of the acid appear shifted by ~20 cm^{-1}. Changes in the spectra typical for this type of interaction are also observed in the region of absorption of the carbonyl group. However, attention is attracted by the fact that the relative intensities of the free and bound hydroxy groups in the spectra of the two systems recorded under identical conditions differ appreciably. Furthermore, it can be seen that the ν(OH) band of the free hydroxyl of cholestanol (Fig. 1a) decreases to a greater extent than the corresponding band of epicholestanol (Fig. 1b). In other words, the proportion of free equatorial hydroxy groups (cholestanol) proves to be somewhat smaller than of axial hydroxy groups (epicholestanol). This qualitative result is in harmony with ideas on the smaller accessibility of the lone electron pairs of an oxygen atom in the axial position as compared with the equatorial position.

For a quantitative comparison of the proton-accepting capacity of equatorial and axial oxygen atoms, we investigated the methyl ethers of the sterols in systems with phenol and with pentachlorophenol (PCP) as proton donors. The quantitative investigation of such systems with the sterols themselves is difficult because of the pronounced overlapping of the ν(OH) bands of the phenols and the cholestanols and the necessity for always using an excess of the latter. We used the classical standard scheme when a dilute solution in CCl_4 of the phenol (proton donor, AH) is treated with an excess of the ether (proton acceptor, B) and the constant of the equilibrum $AH + B \rightleftarrows AH...B$ is calculated from the decrease in the IR band of free hydroxy

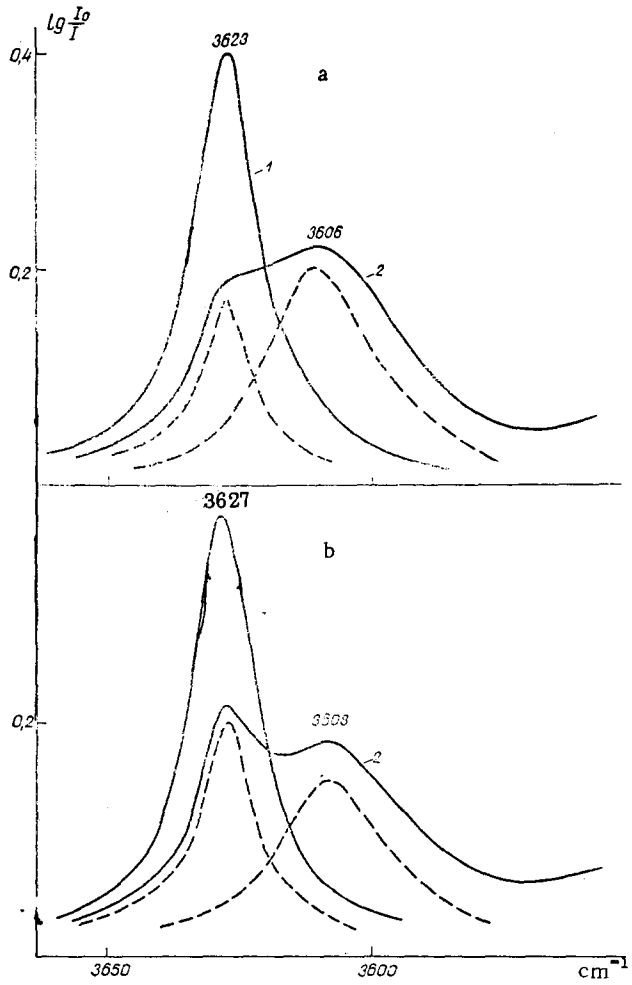


Fig. 1. IR spectra of solutions in CCl_4 of: a) cholestanol (1) and its equimolar mixture with TCAA (2); b) epicholestanol (1) and its equimolar mixture with TCAA (2). Concentration 0.006 M; layer thickness 1 cm. The bands have been separated graphically (dashed lines).

groups of the phenol, ν(OH). Figure 2 shows that a greater decrease in the intensity of the ν(OH) band of the phenol takes place in the case of the cholestanol ether (equatorial oxygen atom, dashed line) than in the case of the epicholestanol ether (axial oxygen atom, full line). Correspondingly, the broad band of a bound hydroxyl, which is shifted in the low-frequency direction by almost 300 cm^{-1} is stronger for the complex with the cholestanol ether than for the complex with the epicholestanol ether. In the case of the ethers of cholestanol and of epicholestanol at room temperature, the values of the constant of the equilibrium phenol + ether $\rightleftarrows$ complex (1:1), as found from the ν(OH) band at 3610 cm^{-1}, have values, with an error of 15-20%, of 13 and 7 liter/mole, respectively, which confirms the greater tendency of an equatorial oxygen atom to be a proton-acceptor in a hydrogen bond than an axial oxygen atom.

Similar results were also obtained for the systems with PCP. Although PCP is a very strong proton donor in hydrogen bonds (the low-frequency shift of ν(OH) that we have observed amounted to 350 cm^{-1}), the equilibrium constants in systems with the ethers studied proved to be far smaller than in the systems with phenol considered above, which can serve only as a qualitative confirmation of the conclusion concerning the relative proton-accepting capacities of equatorial and axial oxygen atoms of sterols.

The results obtained are of interest for the study of complexes of sterols in general. It may be assumed that the difference in the electron-donating capacities of equatorial and axial hydroxy groups of sterols forms the basis of the observed difference in the complex-forming capacities of the sterols.

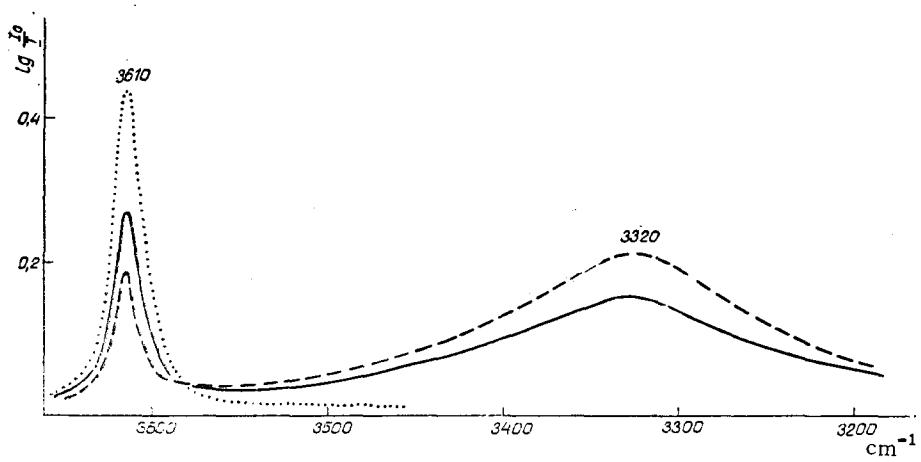


Fig. 2. IR spectra of solutions in CCl_4 of phenol (dotted line) and its mixtures with the methyl ether of epicholestanol (full line) and of cholestanol (dashed line). Concentrations (M): phenol, 0.01; cholestanol and epicholestanol ethers, 0.1. Layer thickness 0.2 cm.

EXPERIMENTAL

Cholestanol was obtained by the hydrogenation of cholesterol [17], and epicholestanol by the method of Chang and Blickenstaff [18]. The methyl ethers were obtained by methylating the sterols with diazomethane [19]. All the products were purified chromatographically and had melting points and $[\alpha]_D^{20}$ values corresponding to those given in the literature.

IR spectra were recorded on a Specord 75IR spectrometer at room temperature. Zeolite-dried and freshly distilled CCl_4 was used as solvent. Dismountable liquid cells with CaF_2 windows were used.

SUMMARY

Equatorial oxygen atoms of sterols (cholestanol and its methyl ether) possess a higher capacity for forming hydrogen bonds as proton-acceptors than axial oxygen atoms (epicholestanol and its methyl ether). The constants of the equilibrium phenol + ether $\rightleftarrows$ H-complex (1:1) in CCl_4 at room temperature amounted to 13 and 7 liter/mole, respectively, for the methyl ethers of cholestanol and of epicholestanol.

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STEROID GLYCOSIDES OF THE ROOTS OF *Capsicum annuum*

I. THE STRUCTURE OF CAPSICOSIDES A_1, B_1, AND C_1

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Three new steroid glycosides of the spirostan series — capsicosides A_1, B_1, and C_1 — have been isolated from a methanolic extract of the roots of red pepper. In an investigation of the products of complete acid hydrolysis of these glycosides, a single aglycon — gitogenin — was identified. The complete chemical structure of each of the capsicosides has been shown with the aid of complete and partial acid hydrolysis, methylation and methanolysis, and periodate oxidation, and also by physicochemical methods of investigation.

Pepper seeds contain steroid glycosides of the furostan series. The structure of one of them has been established [1].

In the roots of red pepper of the variety Podarok Moldovy we have detected glycosides belonging to the spirostan and series [2, 3].

In the present paper we give the results of the isolation and a proof of the chemical structures of three new glycosides of gitogenin which we have called capsicosides A_1 (I), B_1 (II), and C_1 (III).

By chromatography on a silica gel column of the total substances of a methanolic extract of the roots of the plant collected in the flowering and fruit-bearing phase we isolated three (1, 2, 3) chromatographically individual glycoside fractions which were numbered in order of increasing polarity in a thin layer of silica gel and gave positive reactions with the Sannie reagent [4] and negative reactions with Ehrlich's reagent [5], which showed their spirostanol nature.

For each fraction of the glycosides the IR spectrum showed characteristic absorption bands with λ_{max} in KBr (cm^{-1}) of 3500-3400 (OH), 987, 920, 900, 850 (900 > 920) of a spiroketal chain of the (25R) series [6].

To determine the nature of the aglycon, each of the fractions was subjected to complete acid hydrolysis with 2.5% sulfuric acid. Three aglycons were detected in each fraction. After their separation on silica gel impregnated with 2% silver nitrate, three compounds were isolated which were identified as gitogenin, tigogenin, and diosgenin.

These results permitted the assumption that each fraction of glycosides consisted of a three-component mixture of glycosides of close structures and had as the aglycons gitogenin, diosgenin, and tigogenin.

We did not succeed in separating and isolating individual glycosides from the fractions obtained. We first acetylated fractions 1-3, after which the acetylated glycosides were separated on a column of silica gel.

By the acetylation and chromatographic separation of the peracetates followed by their saponification we obtained only capsicosides A_1, B_1, and C_1 in the individual state.

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