COMPLEXES OF CHOLESTEROL WITH CARBOXYLIC ACIDS AND THEIR AMIDES

D. V. Ioffe and I. M. Ginzburg

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It has been shown by IR spectroscopy that in dilute solutions of equimolar mixtures of cholesterol and carboxylic acids binary complexes, each with a hydrogen bond, in which the cholesterol is the acceptor and the acid the donor of a proton, are formed. With an increase in concentration, these complexes form aggregates through two types of hydrogen bond between the activated hydroxyl of the cholesterol and the oxygen atom of the C=O or the OH group of the acid. In crystalline complexes of cholesterol with carboxylic acids, only COOH...OH...O=C hydrogen bonds are realized. It has been concluded that for the formation of a crystalline complex with cholesterol the second component must be both an acceptor and a donor of a proton in a H bond. This conclusion has been confirmed by the preparation of crystalline complexes with primary and secondary amides.

In the animal organism, a considerable part of the cholesterol is present in complexes with other compounds — with phospholipids and proteins in biomembranes and lipoproteins [1-3]. It is assumed that the components in such complexes are retained by a van der Waals interaction of the hydrocarbon parts of the molecules and by a hydrogen bond of the hydroxy group of cholesterol with proton-accepting groups of the proteins or phospholipids. The formation of such an H-bond has been shown repeatedly in model systems [4, 5], but direct proofs of the formation of a hydrogen bond between the cholesterol hydroxyl and acceptor groups of proteins or of phospholipids in biological materials is lacking. On the other hand, it is known that many sterols form crystalline complexes with various classes of inorganic and organic compounds — with acids, salts, and alcohols [6, 7] — and therefore an elucidation of the nature of the bonds in complexes of cholesterol with simple compounds may be useful for understanding the structures of its complexes in biological materials. We have studied complexes of cholesterol with organic acids and their amides. The crystalline complexes that we obtained were individual chemical substances of constant composition. They all contained one molecule of cholesterol to each carboxy or amide group. The complexes with acetic and propionic acids decomposed gradually even at room temperature, losing the acid. The complexes with acids of higher molecular weight were more stable, but on heating the acids distilled off, likewise. On dissolution in inert solvents (C_6H_6, $CHCl_3$, CCl_4), the complexes partially dissociated into the initial components, and an equilibrium was set up in the solution: cholesterol + acid (amide) $\rightleftarrows$ complex. The position of this equilibrium and the nature of the intermolecular interactions as functions of the concentration and of the phase state were studied by IR spectroscopy.

In dilute solutions in CCl_4, with ordinary alcohols carboxylic acids form binary complexes having a H-bond in which the acid plays the role of donor and the alcohol the role of acceptor of a proton [8, 9]. The frequency of the stretching vibration of the hydroxy group of the alcohol ν(OH) in the IR spectra is lowered as a result of the interaction, and in the region of absorption of a carbonyl group in place of the bands of the monomers and dimers of the acid a band of a mixed complex occupying an intermediate position appears. A rise in the concentrations of the acid and the alcohol leads to aggregation of the binary complexes through the formation of H-bonds of two types between them — in one of them the acceptor of the proton of the hydroxyl of the alcohol is the carbonyl oxygen, and in the other it is the oxygen of the acid hydroxyl [9]. In concentrated solutions in CCl_4 and in equimolar mixtures, the two types of aggregates are present in comparable amounts in the

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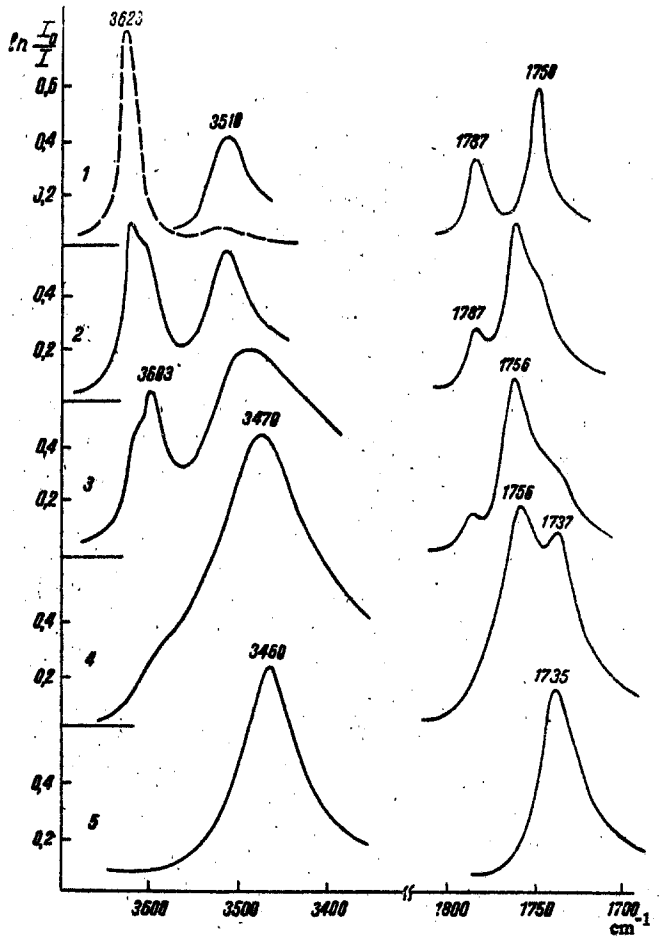
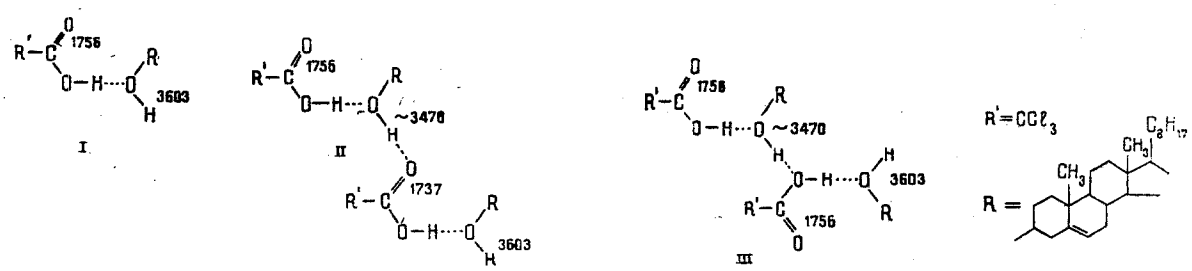


Fig. 1. IR spectra of solutions in CCl_4 of cholesterol (dashed line), of trichloroacetic acid (1), and of their complex (2-5). Concentrations, M: 1) 0.02; 2) 0.006; 3) 0.025; 4) 0.5; 5) crystal (suspension in paraffin oil).

equilibrium. These intermolecular interactions are definitely shown in the IR spectra — new bands of a bound carbonyl group and of the hydroxyl of the alcohol involved in two ...O—H... hydrogen bonds appear in them.

The IR spectra of solutions in CCl_4 with trichloroacetic acid (TCAA) shows a pattern similar to that described above (Fig. 1). In dilute solutions in the ν(OH) region it is possible to see the bands of free cholesterol molecules at 3623 cm^{-1} [10], of cholesterol in the binary complex (I) with a COOH...OH H-bond at 3603 cm^{-1}, and of TCAA monomers at 3510 cm^{-1}. In the ν(C=O) region, in addition to the band of monomers of the acid at 1787 cm^{-1} and the shoulder corresponding to a small amount of dimers, there is a carbonyl band at 1756 cm^{-1} belonging to the binary complex. With an increase in the concentration, the bands of the binary complex weaken and new bands appear at 1737 and 3470 cm^{-1} belonging to the aggregates (II).



Scheme 1

TABLE 1. Properties of Cholesterol Complexes

Second component of the complex	mp, °C	Solvent for crystallization
Acetic acid	110	Hexane
Propionic acid	110	Hexane
Oxalic acid	170	Ethyl acetate
Succinic acid	173	Ethyl acetate
Chloroacetic acid	116	Hexane
3-Chloropropionic acid	91	Hexane
Trichloroacetic acid	116	Hexane
Benzoic acid	107	Hexane
Acetamide	132	Benzene
N-Methylacetamide	109	Benzene + hexane

As can be seen from Fig. 1, curve 4, at a concentration of 0.5 M the absorption of the hydroxy group of cholesterol, ν(OH), at 3603 cm^{-1} practically disappears, while the ν(C=O) band at 1756 cm^{-1} remains dominating in the carbonyl absorption region. This indicates the existence in solution of aggregates with a free C=O group (III), as well. The spectra of solutions of the complexes of cholesterol with other carboxylic acids are qualitatively similar to the spectra given.

Thus, in carbon tetrachloride solution the complexes of cholesterol with acids are nothing other than the usual complexes with a hydrogen bond formed between alcohols and carboxylic acids in inert media. In other words, in an inert solvent cholesterol behaves in relation to an acid as an ordinary secondary alcohol and no specific features whatever are observed. Thus, the spectra of solutions of the cholesterol—TCAA complex are complately identical with the spectra of solutions of an equimolar mixture of TCAA and cyclohexanol, the latter representing a fragment of the cholesterol molecule (Fig. 2). This similarity of cholesterol to ordinary alcohols is disturbed, however, on passing to the crystalline complex. In the case of the TCAA—cyclohexanol system, as for systems with other alcohols, the passage from concentrated solutions to the pure equimolar mixture is not accompanied by any qualitative spectral changes whatever. As before, the spectrum of the mixture contains a broad band of bound OH groups of the alcohol at about 3500 cm^{-1} and a doublet in the carbonyl region (1760, 1740 cm^{-1}) corresponding to two types of H-bonds between the binary complexes (Fig. 2). In contrast to this, the spectrum of the crystalline cholesterol—TCAA complex shows substantial changes as compared with the spectra of concentrated solutions (Fig. 1, curve 5). In the first place, instead of the broad hydroxyl band at 3470 cm^{-1} a considerably narrower band appears at 3460 cm^{-1} and, in the second place, in the carbonyl region instead of the doublet at 1766, 1737 cm^{-1} a narrow isolated band can be seen at 1735 cm^{-1}. These results can be explained by assuming that in the crystal only one of the types of interaction between the binary complexes is realized. The presence of a single ν(C=O) band at 1735 cm^{-1} means that the cholesterol hydroxyl as a proton donor forms a H-bond only with the carbonyl group of the acid (II). The pronounced contraction of the ν(OH) band confirms this conclusion. The wide band in the spectra of the solutions is apparently the superposition of two close absorption bands corresponding to cholesterol hydroxy groups in the two types of hydrogen bonds described above. The realization of only one type of interaction, namely COOH...OH..O=C, in the crystalline complex also leads to a considerable contraction of the ν(OH) band.

In the crystal of cholesterol itself, there is also a hydrogen bond between hydroxy groups, and van der Waals interaction of the aliphatic parts of the molecules. However,

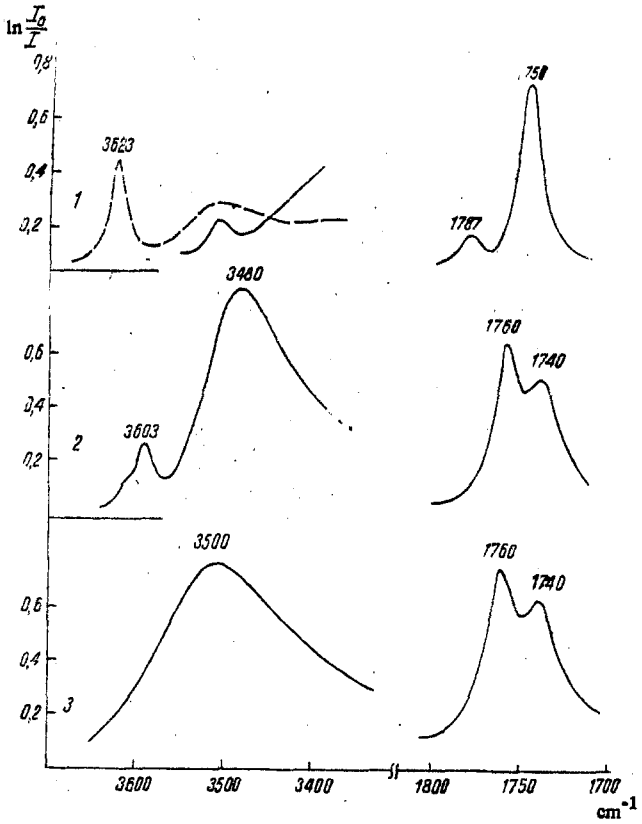


Fig. 2. IR spectra of cyclohexanol (dashed line), of trichloroacetic acid (1), and of their equimolar mixture (2 and 3): 1, 2) solutions in CCl_4, 0.1 M; 3) pure mixture.

as calculation based on a study of the results of x-ray structural investigation shows [11, 12], when the cholesterol molecules are packed in parallel, which leads to the maximum van der Waals interaction, the distance between the oxygen atoms would be too great (> 3 A) for the formation of a strong hydrogen bond. An approach of the hydroxyl to a distance permitting the formation of such a H-bond would lead to a substantial disturbance of the parallelism and to a decrease in the interaction of the aliphatic parts of the molecule. In sum, in the cholesterol crystal a certain compromise is realized between these two requirements.

In the crystals of cholesterol complexes, the formation of a hydrogen bond takes place not directly between the hydroxy groups but with the aid of an additional compound playing the role of a bridge. Such a bridge, binding the hydroxy groups of two or more molecules, must facilitate the parallel packing of their aliphatic fragments. The role of such a bridge may be played by a molecule capable of being simultaneously a donor and an acceptor of a proton in a H-bond such as, for example, a water molecule. However, the inclusion of a hydroxy group ensuring the almost parallel packing of the steroid parts of the molecules in a crystal of the monohydrate is insufficient for the formation of a strong hydrogen bond [13]. It is precisely for this reason, it may be assumed, that in the crystalline complexes of cholesterol with carboxylic acids an interaction is realized not with the hydroxy groups of the acid (III) but only with the carbonyl group (II). The greater length of the bridge in this case and its spatial structure apparently create more favorable conditions for interaction of the hydroxy groups of the cholesterol molecule with it.

The conclusion that for the formation of crystalline complexes the second component, like cholesterol itself, must possess the properties of both a donor and an acceptor of a proton in a H-bond is confirmed by the formation of such complexes with primary and secondary amines — acetamide and N-methylacetamide (NMAA). Attempts to obtain a crystalline complex of cholesterol with a tertiary amide — N,N-dimethylacetamide — under the same conditions did not give a satisfactory result, although this substance possess the same proton-accepting capacity as NMAA [14]. The complexes of cholesterol with amines that were obtained are of

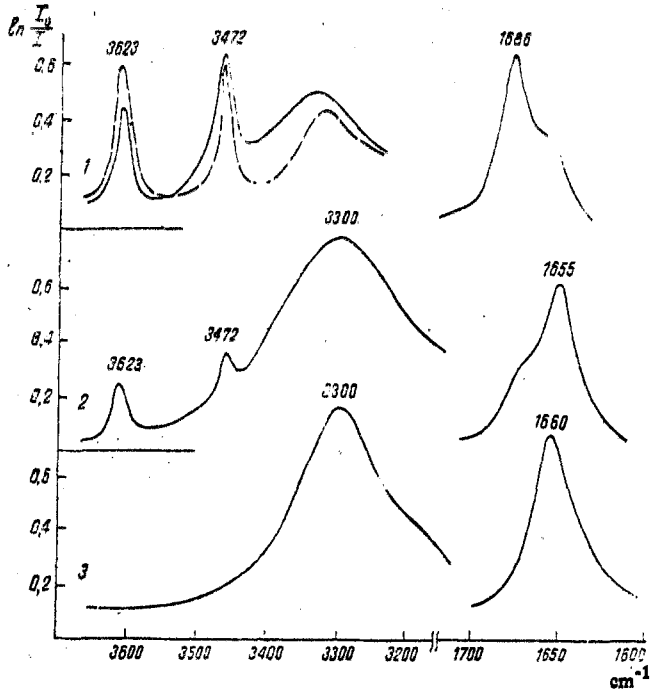


Fig. 3. IR spectra of the cholesterol—N-methylacetamide complex in solution in CCl_4 (1, 2) and in suspension in paraffin oil (3). Concentration, M: 1) 0.03; 2) 0.25; dashed line — sum of the spectra of the components at the same concentration.

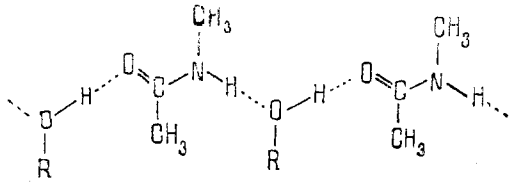
definite interest for the study of bond of cholesterol with proteins [15, 16]. The IR spectra of solutions of the complex of cholesterol with NMAA is CCl_4 are given in Fig. 3.

In dilute solutions of the complex, the absorption bands of cholesterol, ν(OH) 3623 cm^{-1}, and of the amide, ν(NH) 3472 cm^{-1} and "amide-I" 1686 cm^{-1}, and also a broad band at about 3330 cm^{-1} that is characteristic for the self-association of the amide, are observed. In addition, it can be seen that the band of the free NH groups lies on a background of additional diffuse absorption with a maximum at about 3400 cm^{-1}. This absorption can well be seen if the spectrum of the complex is compared with the combined spectra of the components (Fig. 3, dashed line). Attention is also attracted by the low intensity of the absorption bands of free OH and NH groups in the spectra of solutions of the complex as compared with the spectra of separate solutions of cholesterol and NMAA at the same concentration. With a rise in concentration, these bands disappear more rapidly in the spectra of solutions of the complex than in the spectra of solutions of the individual components. In the region of "amide-I" absorption, with an increase in the concentration of the complex almost the same pattern is observed as in the spectra of the amide itself with the only slight difference that the position of the low-frequency bands is shifted by 5-10 cm^{-1}.

The experimental results described can be interpreted in the following way. In CCl_4, the cholesterol-NMAA complex partially dissociates, and as a result an equilibrium is established in the solution between the monomers of cholesterol and of the amide, the self-associates of the amide, and cholesterol—amide complexes with a OH...O=C hydrogen bond. This hydrogen bond corresponds to the additional absorption with a maximum at about 3400 cm^{-1} appearing in the spectra of solutions of the complex. With a rise in concentration, the number of such binary complexes increases, and they begin to interaction with one another through a NH...OH hydrogen bond. In sum, chain aggregates (IV) are formed in which the NMAA molecules and the cholesterol alternate with one another, simultaneously acting as proton donors and acceptors.

Apparently, the proton-accepting capacity of the oxygen atoms of the carbonyl group of the amide and of the hydroxy group of cholesterol are fairly close, so that the absorption of NH and OH groups in the aggregate (IV) largely overlaps the bands of these groups in the chains of self-associates of the amide and of cholesterol. A similar situation also arises in the region of "amide-I" absorption. The carbonyl group linked to cholesterol in the bi-

nary complexes and in the aggregate (IV) absorbs almost in the same way as the dimers and multimers of the amide. All this creates the appearance of a simple combination of the spectra of the components of the complex at high concentrations. In other words, a spectral pattern representing the superposition of several close absorption bands does not appear so convincing as in the case of complexes of cholesterol with carboxylic acids. However, the combination of the results obtained and the very fact of the formation of crystalline complexes of cholesterol with NMAA and acetamide with a 1 : 1 composition, in our opinion, permits a model of type (IV) to be put forward. We may also note that in the spectra of solutions of mixtures of cholesterol and dimethylacetamide, which is capable of being only a proton acceptor, and, as has already been shown, does not form a crystalline complex, a pattern is observed which is typical for a OH...O=C hydrogen bond, excluding any aggregation whatever of binary H-complexes. There is a similar situation in the case of the interaction of cholesterol with carboxylic acid esters — here, likewise, only binary complexes with a hydrogen bond appear, in which the cholesterol is the donor and the ester the acceptor of the proton. No more complex aggregates are formed, and as a consequence of this no crystalline complexes are formed, either.



IV

Scheme 2

Thus, the whole sum of the experimental results obtained in the present investigation leads to the conclusion that for the formation of crystalline complexes with cholesterol the molecules of the second component must possess proton-donating and proton-accepting capacities simultaneously. By binding the cholesterol molecules with the relatively weak forces of a hydrogen bond, the second component of the complex promotes a favorable orientation of the steroid fragments, leading to the most complete van der Waals interaction.

In conclusion, the hypothesis may be put forward that in complex with proteins the cholesterol itself apparently likewise plays the role not only of a donor but also an acceptor of a proton in a hydrogen bond, in just the same way as takes place in the complexes considered in the present paper.

EXPERIMENTAL

An equimolar mixture of cholesterol and an acid or an amide was suspended in an inert solvent and the mixture was rapidly heated until dissolution was complete. The resulting solution was cooled, the precipitate was separated off, and it was crystallized from the same solvent. The melting points of the complexes are given in Table 1. The compositions of the complexes with the acids were determined by potentiometric titration of aqueous ethanolic solutions. The results of the analysis of all the compounds corresponded to the calculated figures.

IR spectra were recorded at room temperature on a Specord 75 IR spectrometer. Dried and redistilled CCl_4 and freshly recrystallized substances were used to prepare the solutions.

SUMMARY

1. In dilute solutions in CCl_4 of equimolar mixtures of cholesterol and carboxylic acids, binary complexes with a H-bond in which the cholesterol is the acceptor and the acid the donor of a proton are formed.

2. With an increase in the concentrations of the binary complexes, aggregates are formed through two types of H-bonds between the hydroxyl of the cholesterol and the oxygen atoms of the C=O and of the OH groups of the acid. In crystalline complexes, only the H-bond of the first type is realized.

3. With amides in dilute solutions, cholesterol forms 1 : 1 complexes through an OH... O=C H-bond. At high concentrations and in the crystal, the binary complexes interact with one another through NH...OH bonds.

4. A necessary condition for the formation of crystalline complexes of cholesterol is the capacity of the second component for being both an acceptor and a donor of the proton in a H-bond.

LITERATURE CITED

1. H. Brockerhoff, Lipids, 9, 645 (1974).
2. C. H. Huang, Lipids, 12, 348 (1977).
3. A. N. Klimov, in: The Biochemistry of Lipids and Their Role in Metabolism [in Russian], Moscow, (1981), p. 45.
4. V. G. Koval', Mol. Biol., 14, 18 (1976).
5. V. M. Klimovich and O. D. Kurilenko, Ukr. Khim. Zh., 42, 929 (1976).
6. L. Fieser and M. Fieser, Steroids, Reinhold, New York (1959).
7. E. N. Garlinskaya, Zh. Prikl. Khim., 28, 86 (1955).
8. J. Villepin, R. Saumagne, and M.-L. Josien, C. R. Acad. Sci. Paris, 259, 365 (1964).
9. I. M. Ginzburg, D. V. Ioffe, A. L. Smolyanskii, and I. I. Fadeeva, Zh. Obshch. Khim., 52 (1982).
10. M. Kunst, D. van Duijn, and P. Bordewijk, Z. Naturforsch., 34A, 369 (1946).
11. J. D. Bernal, D. Crowfoot, and J. Fankuchen, Philos. Trans., 239A, 135 (1946).
12. B. M. Cravan, Acta Crystallogr., 35B, 1123 (1979).
13. B. M. Craven, Nature (London), 260, 727 (1976).
14. I. G. Ginzburg and B. P. Tarasov, Zh. Obshch. Khim., 45, 2492 (1972).
15. G. V. Titova, N. N. Klyueva, K. A. Kozhevnikova, and A. N. Klimov, Biokhimiya, 45, 51 (1980).
16. A. N. Klimov, G. V. Titova, K. A. Kozhevnikova, N. N. Klyueva, and E. V. Smirnova, Biokhimiya, 46, 000 [sic] (1981).

SORPTION OF SAPONINS ON ION-EXCHANGE RESINS

P. A. Yavich

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The capacity of a number of ion-exchange resins for the saponins isolated from the seeds of the tea plant, soapwort, horse chestnut and Iberian cyclamen has been studied. IA-1 anion-exchange resin possessed the highest sorption capacity. The influence of a number of technological parameters on the capacity of the sorbent has been considered. It has been shown that increases in the diameter of the resin grain, in the rate of feed of the solution to the column, and in the concentration of alcohol in the sorbate solution lead to a fall in sorption capacity. At the same time, the type of lyophilic alcohol in the range of C_1-C_4 scarcely affects the capacity. In the case of impure extracts of the saponin-containing plants, the capacity of IA-1 anion-exchange resin fell by a factor of 3-4, but ion-exchange treatment of such extracts led to better results.

In publications on the ion-exchange purification of glycoside-containing extracts from the impurities accompanying them, nothing is ever said about the magnitude of the sorption of the desired product [1-5]. In the case of saponin-containing extracts, this is connected with the fact that sufficiently reliable rapid methods of determining them quantitatively have been developed only in recent years [6-8].

We have studied the sorption activity of ion-exchange resins marketed industrially in the sorption of the saponins obtained from the fruit of the horse chestnut (*Aesculus hippocastanum L.*), soapwort (*Saponaria officinalis* L.), the tuberous roots of the Iberian (or Caucasian) cyclamen (*Cyclamen ibericum* Stev.), and the seeds of the tea plant (*Camelia sinensis* O. Ktze). The saponins so used had physicochemical properties agreeing completely with the constants given in the literature [9-11].

I. G. Kuteteladze Institute of Pharmacochemistry, Tbilisi.

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