UV Spectroscopy. Samples of the substances weighing 0.3-0.4 mg were dissolved in 10 ml of sulfuric acid (density 1.835), and the solutions obtained were thermostated at 70°C for 60 min. After the cooling of the reaction mixture, the spectra measurements were made relative to sulfuric acid of the same density.

Isolation of the Product of the Reaction of Oleanolic Acid with Sulfuric Acid. About 2 g of oleanolic acid was dissolved in 20 ml of H_2SO_4 (density 1.835), the solution was thermostated at 70°C for 60 min and it was then cooled and poured onto 200 mg of crushed ice. The precipitate was separated off, washed with water, dried, and crystallized from ethanol.

The product of the reaction of β-amyrin with sulfuric acid was obtained similarly.

Condensation of 3-ketooleanolic Acid with Benzaldehyde. A mixture of 2.8 g (0.005 mole) of 3-ketooleanolic acid and 0.5 g (0.005 mole) of benzaldehyde was condensed in ethanolic NaOH solution (25 ml of ethanol and 1 g of NaOH in 1.5 ml of water) for 5-6 h, after which the reaction mixture was acidified with 10% HCl. The precipitate was separated off, washed with water, dried, and crystallized from ethanol) mp 185-186°C, $C_{37}H_{51}O_3$.

SUMMARY

The single maximum of triterpenoids in sulfuric acid at 310 nm is due to the formation of a carbocation. Under the action of sulfuric acid oleanolic acid undergoes lactonization at the COOH group and the Δ^{12} >C=C< bond.

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USE OF STEROID GLYCOSIDES FOR THE AFFINITY CHROMATOGRAPHY OF CHOLESTEROL

I. V. Berezin, I. P. Andrianova, Ya. I. Lapuk, L. B. Alekseeva, P. K. Kintya, and N. E. Mashchenko

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A number of sorbents for the sorption of cholesterol has been synthesized from eight saponins of the spiro- and furostanol series immobilized on amino-Silochrome. The most effective have proved to be a sorbent containing capsicosin. Amino-Silochrome, and also sorbents containing digitonin, F-gitonin, and purpureagitoside cause hemolysis. The sorption capacity of the sorbents obtained falls on passing from saponins of the spirostanol series to saponins of the furostanol series, and also with a decrease in the length of the oligosaccharide moiety of the saponins.

The synthesis of an affinity sorbent of cholesterol using as the affinate digitonin oxidized with sodium periodate and immobilized on amino-Silochrome has been reported previously. In order to study the possibility of the use of other saponins for this purpose, we have investigated a series of steroid glycosides containing various aglycones and monosaccharide residues. Thus, we have considered saponins of the spiro- and furostanol series containing

M. V. Lomonosov Moscow State University. 2nd Moscow State Medical Institute, Institute of Chemistry, Academy of Sciences of the Moldavian SSR, Kishenev. Translated from Khimiya Prirodnykh Soedinenii, No. 5, pp. 652-655, September-October, 1980. Original article submitted April 7, 1980.

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TABLE 1. Sorption of Hemolytic Properties of Affinity Sorbents for Cholesterol Obtained by the Immobilization of Various Saponins on Amino-Silochrome

Immobilized steroid glycoside	Aglycone	Number of sugars	Type of glycoside	Sorption of cholesterol, %†	Hemolysis
Blank experiment*	-	-	-	0	4
Digitonin	Digitogenin	5	Spiro	40	8
Capsicosin	Gitogenin	5	Spiro	38.4	1
Capsicoside	(25R)-5α-Furostan-2α-3β, 22α,26-tetraol	6	Furo	23	1
F-Gitonin	Gitogenin	4	Spiro	20	15
Purpureagitoside	(25R)-5α-Furostan-2α-3β, 22α,26-tetraol	5	Furo	30	3
TFI	(25S)-5α-Furostan-3β,22α, 26-triol	4	Furo	15	1
Funkioside-F	Diosgenin	4	Spiro	20	1
ε-Asparagosides	Sarsasapogenin and (25R)-5α-furostan-3β-22α,26-triol	2-4	Spiro Furo	16	1

*Amino-Silochrome.
†The amound of cholesterol in the blood serum without sorption was taken as 100%. The amount of hemoglobin in the blood plasma without sorption was taken as the unit of hemolysis.

from 2 to 6 monosaccharide residues: capsicosin and capsicoside from the seeds of *Capsicum annum* [1], F-gitonin [2], and purpureagitoside [3] from the leaves of *Digitalis purpurea* L. (purple foxglove), TFI from the seeds of *Lycopersicon esculentum* (tomato) [4], funkioside-F from the leaves of *Funkia ovata spr.* (*Hosta caerulea*; blue plantain-lily) [5], and the sum of the ε-asparagosides from the epigeal part of *Asparagus officinalis* L. (garden asparagus) [6].

In the selection of the steroid glycosides we were guided by the consideration that they should be more accessible than digitonin and, just like it, should contain a large number of monose residues in the oligosaccharide part of the molecule.

The steroid glycosides were first oxidized with sodium periodate in 50% aqueous dioxane solution at a ratio of the steroid glycoside to oxidizing agent of 1:2 (by weight). Then a 20-fold amount of amino-Silochrome in relation to the weight of the steroid was added to the oxidation mixture. The sorbent was washed with water, dried in the air, and its capacity for binding cholesterol from blood serum was studied. For this purpose a weighed sample of sorbent was stirred with porcine blood serum for 30 min at room temperature, and cholesterol was determined in the supernatant liquid by Webster's method [7]. The amount of cholesterol in the blood serum without sorption was taken as 100%.

As was found, the most effective in relation to the binding of cholesterol was a sorbent with immobilized capsicoside; it was comparable in its specific capacity with digitonin (Table 1), and just like digitonin, contained an aglycone of the spirostanol type and five monose residues.

With a decrease in the number of monose residues in a steroid glycoside of the spirostanol type from 5 to 4 (F-gitonin and funkioside-F) the capacity of the sorbent for binding cholesterol halved (20%). Thus, the first rule for glycosides of the spirostanol series appeared: the larger the number of monoses the better is the sorption of cholesterol. The same law is observed for steroid glycosides of the furostanol series: for purpureagitoside (5 monose residues) the sorption of cholesterol amounted to 30%, and for TFI (4 monose residues) to 15%. With a further decrease in the number of monoses (steroid glycoside consisting of a mixture of ε-asparagosides with 2-4 monosaccharide residues) an equally low percentage binding, 16% was observed.

With a rise in the number of monosaccharide residues to six in a glycoside of the furostanol type (capsicoside), the sorption of cholesterol by the corresponding sorbent did not

increase. The presence of five monosaccharide residues in the saponins is probably the optimum condition for the formation of the most effective sorbent.

On a further comparison of the various types of glycosides (spiro- and furo-) with the same number of monosaccharide residues a second rule appeared: sorbents based on spirostanol glycosides sorb cholesterol better than sorbents prepared with the use of furostanol glycosides. Thus, sorbents with immobilized digitonin and capsicoside (five sugar residues, spiro-) the same number of monosaccharide residues absorbed only 30%. The same rule was retained in a comparison of spiro- and furostanol glycosides with four sugar residues: for F-gitonin and funkioside F (spiro-) the binding of cholesterol was 20%, and for TSI (furo-) it was somewhat lower, 15%. This rule confirms literature information that the capacity for forming an insoluble complex with cholesterol is an order of magnitude lower for furostanol glycosides than for spirostanol glycosides [8].

Thus, it may be concluded that the efficiency of binding of cholesterol with immobilized steroid glycosides depends on the type of aglycone and on the number of monoses in the oligosaccharide moiety of the molecule.

In addition to the finding of the most effective sorbent for the binding of cholesterol, we studied the hemolysis caused by the sorbent (a sorbent synthesized previously with immobilized digitonin caused considerable hemolysis). With this aim, porcine blood stabilized with heparin was placed in a flask with the sorbent, the mixture was stirred for 30 min, and after the separation of the plasma by centrifuging the level of hemoglobin was determined by the Derviz—Byalko method in which we changed the order of addition of the reagents. As the unit of hemolysis we took the amount of hemoglobin in the blood plasma without sorption (see Table 1).

It was established that hemolysis is caused by: the amino-Silochrome matrix and sorbents with immobilized F-digitonin, purpureagitoside, and, as mentioned above, gitonin. The remaining sorbents, including that with the highest specific activity for cholesterol (containing capsicoside) caused no hemolysis. This is shown by the fact that the amount of hemoglobin in the blood plasma after sorption on these sorbents remained the same as in the control, i.e., in the blood plasma without sorption (see Table 1). It was found that after the immobilization of a steroid glycoside on the matrix, which in itself causes hemolysis, some sorbents containing saponins ceased to exhibit a hemolyzing action. These steroid glycosides immobilized on the matrix probably exert a protective and screening influence and prevent hemolysis.

EXPERIMENTAL

Amination of Silochrome. A mixture of 100 g of Silochrome, 500 ml of toluene, and 20 ml of γ-aminopropyltriethoxysilane was heated at the boiling point of toluene for 12 h. The solid was washed on the filter with 1 liter of toluene and was dried at 45-50°C for 12 h and in a vacuum desiccator for 12 h. This gave 98 g of aminated Silochrome with a specific activity of 0.56 meq/g.

Steroid Glycosides. Digitonin was obtained from the experimental station of the I. G. Kutateladze Institute of Pharmacological Chemistry of the Academy of Sciences of the Georgian SSR. Capsicosin, capsicoside, F-gitonin, purpureagitoside, TFI, funkioside F, and a mixture of ε-asparagosides were obtained by known methods [1-6] in the Institute of Chemistry of the Academy of Sciences of the Moldavian SSR and were identified by means of their physicochemical constants in the presence of markers.

Oxidation of the Steroid Glycosides and their Immobilization. To 0.05 g of a steroid glycoside was added 0.1 g of sodium periodate dissolved in 5 ml of 50% aqueous dioxane, the mixture was stirred in the dark for 1 h, and then 1 g of amino-Silochrome was added and the mixture was stirred for another 6 h and was then left overnight at room temperature. The supernatant solution was poured off and the solvent was washed with 20 ml of water and was dried in the air. It was stored in the refrigerator.

Determination of the Specific Capacities of the Sorbents with Respect to the Binding of Cholesterol from Porcine Blood Serum. To 0.2 g of sorbent was added 2 ml of porcine blood serum, the mixture was stirred at room temperature for 30 min, 0.02 ml of the supernatant liquid was taken, and to it was added 2 ml of a 0.1% solution of ferric chloride in glacial acetic acid. Immediately after this, 1.5 ml of concentrated sulfuric acid was added, and the

mixture was kept in the dark for 30 min, after which the optical density of the resulting colored solution was measured at 570 nm (E). The analysis of a sample of blood serum without the addition of the solvent was carried out similarly (E_0). The ratio E/E_0 as a percentage corresponds to the sorption of the cholesterol in relation to its initial content in the blood serum.

Hemolysis of Blood by the Sorbents. To 0.2 g of a sorbent (one of Nos. 2-9) and 0.2 g of amino-Silochrome was added 2 ml of porcine blood stabilized with heparin. The mixture was shaken for 30 min. The blood was separated off by decantation and it was then centrifuged at 1500 rpm for 10 min and taken off and its hemoglobin content was determined. As control we used the same porcine blood stabilized with heparin: the blood plasma was separated by centrifugation and its hemoglobin content was also determined, being taken as unity.

Determination of Hemoglobin in Blood Plasma by the Derviz–Byalko Method. To 4 ml of acetate buffer, 0.2 N, pH 6, were added 2 ml of a 0.1% solution of benzidine hydrochloride in acetate buffer, 0.01 ml of the plasma under investigation, and 2 ml of 0.3% hydrogen peroxide solution and the mixture was stirred and was left for 3 min at room temperature, and then its optical density at 620 nm was determined (see Table 1).

SUMMARY

1. Sorbents for the affinity chromatography of cholesterol have been synthesized from a number of steroid saponins.

2. The hemolytic properties of the sorbents synthesized have been studied.

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