dried, to give 4.57 g of 3,17β-dihydroxyestra-1,3,5(10)-trien-16-one (VI) with mp 229-231°C. After recrystallization from ethanol, mp 234-235°C; according to the literature [11]: mp 236-238°C. IR spectrum, ν, cm^{-1}: 3420, 3320, 1735, 1610, 1500. PMR spectrum (in C_5D_5N; ppm): 0.94 (3 H, singlet, CH_3); 4.05 (1 H, singlet, C_{17}—H).

SUMMARY

The direct bromination of estrone acetate gives predominantly a mixture of 16α- and 16β-bromo derivatives, and also 16,16-dibromoestrone acetate. Under the action of an aqueous methanolic solution of potash, the mixture of epimeric acetates is converted into 16α-hydroxyestrone which isomerizes on heating into 16-ketoestradiol.

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TRANSFORMATION OF THE AGLYCONES IN STEROID GLYCOSIDES. 1. SYNTHESIS OF 20β-ACETOXY-16β,23-EPOXY-21,24-DINOR-5α-CHOLANE-3β,17α-DIOL 3-O-[O-β-D-GLUCOPYRANOSYL-(1 → 2)-O-β-D-GLUCOPYRANOSYL-(1 → 4)-β-D-GALACTOPYRANOSIDE]

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The pathways of the chemical transformation of aglycones and their glycosides that do not affect the glycosidic chain are considered. Starting from 3β-hydroxy-5α-pregn-16-en-20-one 3-O-[O-β-D-glucopyranosyl-(1 → 2)-O-β-D-glucopyranosyl-(1 → 4)-β-D-galactopyranoside] the corresponding 16α(H),17α(OH)-dihydropyranone glycoside has been obtained. The latter has been converted into the polyacetate of a glycoside with a 17α,20β-dihydroxytetrahydropyran ring E. The structure and stereochemistry of the final compound have been shown from the results of the ^1H and ^{13}C NMR spectra and mass spectra.

In spite of the fact that the structure and biological action of steroid and triterpenoid glycosides has long been the object of broad studies [1-3] clarity has so far been lacking on what roles the glycoside and aglycone moieties of the molecule play here. The answer to this question to a considerable degree comes up against the difficulty or impossibility of obtaining from natural sources systematic sets of compounds having, for example, the same glycosidic chain with homologous or analogous aglycones. Apparently the production of such a set

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of compounds for the purposes of biological testing can be considered only by synthesis. One of the variants of such synthesis is the introduction of a glycosidic chain into modified aglycones [4]. However, the actual synthesis, even of monosides, is fairly complicated and it is difficult to hope for a serious simplification of it. In view of this, we have begun the development of methods for the chemical transformation of aglycones in their glycosides that do not affect the glycosidic chain. Of course, the latter condition imposes serious limitations on the choice of possible reactions and of conditions for their application only in alkaline, neutral, or weakly acidic media.

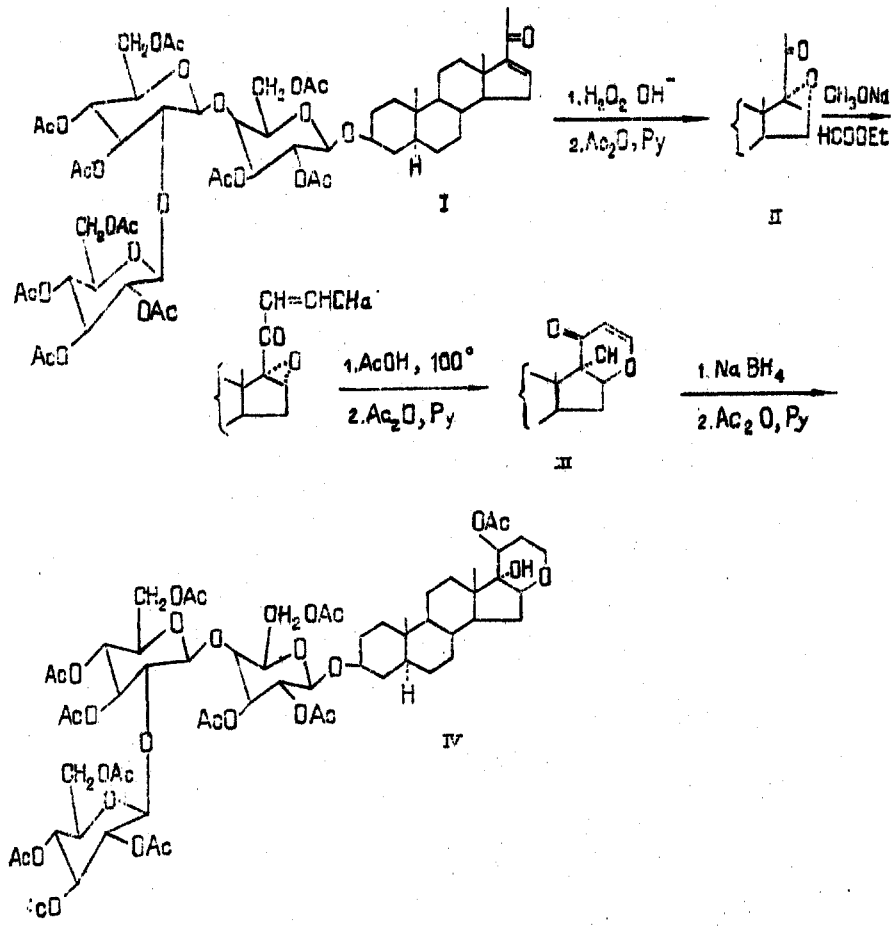
As the starting compound we selected the readily avaiable 3β-hydroxy-5α-pregn-16-en-20-one 3-O-[O-β-D-glucopyranosyl-(1 → 2)-O-β-D-glucopyranosyl-(1 → 4)-β-D-galactopyranoside] (I) [5]. The presence of the reactive Δ^{16}-20-keto group makes it possible to pass to the 16,17-pyran derivatives of steroids [6], which have revealed interesting and unusual biological properties [7].

To arrive at a 1,4-dihydropyranone derivative we used a scheme developed previously for the case of steroids of the pregnene series [8], including the conversion of the Δ^{16}-20-keto grouping into a 16α,17α-epoxide. In actual fact, the polyacetate of the pregnane glycoside (I) is quantitatively converted by epoxidation with alkaline hydrogen peroxide followed by reacetylation into the 16α,17α-epoxide (II). Its physicochemical characteristics and the features of its IR and PMR spectra are similar to those of 3β-hydroxy-16α,17α-epoxypregn-5-en-20-one 3-acetate. The presence of three multiplets from 19-OCH and OCH_2- protons in the PMR spectrum confirms the retention of the carbohydrate moiety of the molecule. On the basis of information concerning the ready heterocyclization of 23-O-substituted 16α,17α-epoxy-20-ketosteroids into 23 → 16-pyran derivatives [8], the glycosidic epoxide (II) was converted by condensation with ethyl formate in the presence of sodium methanolate into the sodium salt of an enol which, without additional purification, was heated at 100°C for 1 h. The 16α(H), 17α(OH)-dihydropyranone formed as the result of intramolecular heterocyclization (by analogy with 16α,17α-epoxysteroids, apparently taking place selectively with cleavage of the C_{16}—O bond) was reacetylated and purified by chromatography on a column of silica gel. The presence of the newly formed dihydropyranone ring E in the glycoside polyacetate (III) was confirmed by the characteristic absorption (1610, 1660 cm^{-1}) in the IR spectrum and at λ_{max} 270 nm (ε 7174) in the UV spectrum. In the PMR spectrum there are two one-proton doublets at δ 7.24 and 5.24 ppm with SSCCs of 7 Hz from the C(22), C(23) vinyl protons and three groups of multiplets from twenty-three (OCH) protons in the 5.10, 4.42, and 3.70 ppm regions. It is interesting that the resonance signal from the 18-CH_3 group (δ 0.8 ppm) is shifted upfield in comparison with the same signal of the epoxide (II) (δ 1.0 ppm).

The dihydropyranone steroid glycoside (III) was reduced with sodium tetrahydroborate. The product was reacetylated, and chromatographic purification led to the polyacetate (IV) with a saturated ring E. The presence of a 17-hydroxy group in it was shown by an absorption band in the IR spectrum at 3600 cm^{-1}. The PMR spectra had the resonance signals from 18-CH_3 and 19-CH_3 angular methyl groups (1.03 and 0.79 ppm), respectively, and from eleven acetate groupings in the 2.0-2.15 ppm region and three groups of multiplets from twenty-four OCH protons in the 3.4-3.9, 4.0-4.64, and 4.9-5.4 ppm regions, among which it is possible to isolate only the signals of the 23-H(a) atom with δ 3.43 ppm and the 23-H(e) atom with δ 4.00 ppm. In the ^{13}C spectra there are the signals of the steroid fragments (ppm): 37.0, t, C^1; 28.7, t, C^2; 34.4, t, C^4; 44.8, d, C^5; 29.0, t, C^6; 31.9, t, C^7; 34.7, d, C^8; 54.2, d, C^9; 35.6, s, C^{10}, 32.3, t, C^{12}; 47.6, s, C^{13}; 47.9, d, C^{14}; 32.9, tr, C^{15}; 86.1, d, C^{16}; 80.2, s, C^{17}; 14.3, q, C^{18}; 12.2, q, C^{19}; 81.9, d, C^{20}; 30.3, t, C^{22}; 64.3, t, C^{23}; 173.1, s, 20-CO; and from the carbohydrate moiety: 100.0, 100.8, and 101.2, s, C^1, 61.9, 63.1, and 63.2, t, OCH_2; 68.6, 69.7, 69.8, 71.3, 71.6, 72.3, 73.3., 73.4, 73.5, 74.7, 76.8, 79.5, d, OCH 168.6, 169.1, 169.3, 169.5, 170.0, 170.2, 170.4, 170.5, 170.6, 170.9, s, C=O and 20.27, 20.39, 20.45, 20.55, 20.60, 20.66, 20.72, 20.77, and 21.04, q, CH_2.

Thus, the characteristics of the steroid fragment show that ring E has the "chair" conformation [9], which contains a 17α-OH and a 20β-OAc group. Of the other signals, the C^3 doublet is located in the region of the OCH signals of the carbohydrate moiety and the signal of C^{11} falls into the general groups of signals of methyl carbon atoms. The presence in the spectrum of the carbohydrate moiety of three C^1 signals and three OCH_2 signals, and also of ten signals of acetate C=O groups indicates the retention of the lycotriose residue.

The mass spectrum of (IV) contains the peaks of the ions characteristic for fragments corresponding to the ejection of glucose and of the whole glycosidic moiety.



Thus, in the case of the steroid glycoside (III), the reduction of the conjugated ketone in ring E by sodium tetrahydroborate takes place in the manner of a 1,4-addition just as has been observed previously [9] for steroid aglycones.

EXPERIMENTAL

IR spectra were taken on a UR-20 instrument in KBr or $CHCl_3$; UV spectra on a Unicam SP-700 instrument in EtOH; and PMR spectra on Tesla BS497 HA-100 and Bruker 250 MHz spectrometers in $CDCl_3$ with TMS as internal standard. ^{13}C spectra were obtained on a Bruker M-250 spectrometer with a working frequency of 62.89 MHz in $CDCl_3$, and mass spectra on a Varian MAT-44 spectrometer with direct introduction of the sample into the ion source.

Mixtures were separated on columns of SiO_2 (40/100 μm) under a pressure of nitrogen. Plates with SiO_2 (5/40 μm) + 13% of gypsum were used for TLC. 3β-Hydroxy-5α-pregn-16-en-20-one 3-O-[O-β-D-glucopyranosyl-(1 → 2)-O-β-D-glucopyranosyl-(1 → 4)-β-D-galactopyranoside], $C_{32}H_{69}O_{17}$, was obtained from tomatoside acetate with a yield of 17%, mp 239-241°C (from CH_3OH). IR spectrum (ν, cm^{-1}): 1590, 1660, 3300-3600 (KBr). Mass spectrum (m/z): 802 (M^+). According to GLC, the ratio of D-glucose to D-galactose was 2:1 (1.0:0.55). The polyacetate (I) was obtained by acetylating the initial glycoside at 20°C for three days. R_f 0.47 [benzene—acetone (4:1)]; 0.89 ($CHCl_3$—CH_3OH—water (65:30:6)). IR spectrum, (ν, cm^{-1}): 1240-1250, 1590, 1670, 1740-1760 (KBr). PMR spectrum (δ, ppm); 0.82, 0.87 s (6 H, 18-CH_3, 19-CH_3); 2.0-2.20 (10 acetate $COCH_3$ groups); 3.7 m, 4.30 m, 5.12 m, (19-H, OCH); 6.68 m (1 H, 16-H).

Polyacetate of 3β-Hydroxy-16α,17α-epoxy-5α-pregn-20-one 3-O-[O-β-D-Glucopyranosyl-(1 → 2)-O-β-D-glucopyranosyl-(1 → 4)-β-D-galactopyranoside] (II). Simultaneously, 11.6 ml of 4 N NaOH and 18.2 ml of 30% H_2O_2 were added to a solution of 2.8 g of the glycoside acetate (I) in 72 ml of CH_3OH. The mixture was left at +4°C for 3 days and was then treated with 500 ml of water and extracted with n-butanol. The extract was evaporated, the residue was treated with ether, and the product was filtered off and dried in the air. This gave 1.8 g (96.8%) of the 16,17α-epoxide of the glycoside with R_f 0.58 ($CHCl_3$—CH_3OH—water (65:30:6)). IR spectrum (ν, cm^{-1}): 1700, 3280-3460 (KBr). Without additional purification, 1.8 g of the epoxide

was acetylated in 40 ml of pyridine with 40 ml of $(CH_3CO)_2O$ at 20°C for 3 days. The reaction mixture was treated with ice water, and the precipitate was filtered off, dried in the air, dissolved in ether, and reprecipitated with hexane. This gave 1.8 g of (II) with R_f 0.88 ($CHCl_3$–CH_3OH–water (65:30:6)); 0.62 [benzene–acetone (4:1)]. IR spectrum, ν, cm^{-1}: 1230-1260, 1705, 1740-1760 (KBr). PMR spectrum (δ, ppm): 0.8 s (3 H, 19-CH_3) 1.0 s (3 H, 18-CH_3) 1.98, 2.0, 2.05, 2.10, 2.14 m (10 acetates, $COCH_3$); 3.6 s (1 H, 16-H); 3.74 m, 4.34 m, 5.10 m (19 H, OCH protons).

Polyacetate of 3β,17α-Dihydroxy-16β,23-epoxy-21,24-dinor-5α-chol-22(23)-en-20-one 3-O-[O-β-D-Glucopyranosyl-(1 → 2)-O-β-D-glucopyranosyl-(1 → 4)-β-D-galactopyranoside] (III). To a methanol-free suspension in 30 ml of absolute benzene of the CH_3ONa obtained from 0.75 g of Na and 45 ml of absolute methanol were added 1 g of the epoxide (II) in 50 ml of absolute benzene and, after 10 min, a solution of 3.8 ml of ethyl formate in 7.5 ml of benzene. The mixture was stirred for 2 h and was left for a day at 20°C. Then the precipitate was filtered off and it was washed with benzene and ether and dried in the air. On acidification, the enol salt obtained gave an intense cherry-red coloration with $FeCl_3$. The Na enolate obtained from 1 g of the epoxide (II) was dissolved in 90 ml of glacial AcOH, and the solution was heated on the boiling water bath for 1 h. Then the reaction mixture was evaporated to dryness, and the residue was dissolved in water and extracted with $CHCl_3$. The aqueous layer was extracted with n-butanol, and evaporation of the extract gave 560 mg of the steroid glycoside. Without additional purification, it was acetylated with 10 ml of $(CH_3CO)_2O$ in 10 ml of absolute pyridine for 3 days. After working up similar to that described above, by chromatography on SiO_2 in the benzene–acetone (4:1) system, the mixture obtained (510 mg) yielded 230 mg of (III) with R_f 0.33 [benzene–acetone (4:1)]. IR spectrum (λ, cm^{-1}): 1240, 1600, 1655, 17455, 3420, 3680 (in $CHCl_3$). UV spectrum (λ_{max}, nm): 270 (ε 7174 (in CH_3OH). PMR spectrum (δ, ppm) 0.81 s (6 H, 18-CH_3, 19-CH_3), 1.99-2.10 (10 acetates); 3.28 s (1 H, OH), 3.70 m, 4.42 m, 5.10 m (20 H, OCH, OCH_2); 5.42 d (J = 7 Hz, 1 H, 22-H); 7.24 d (J = 7 Hz, 1 H, 23-H).

20-β-Acetoxy-16β,23-epoxy-21,24-dinor-5α-cholane-3β,17α-diol 3-O-[O-β-D-Glucopyranosyl-(1 → 2)-O-β-D-glucopyranosyl-(1 → 4)-β-D-galactopyranoside] (IV). At 20°C, 300 mg of $NaBH_4$ in 7 ml of water was added to a solution of 360 mg of (III) in 21 ml of DMFA. After 24 h, the reaction mixture was diluted with water (100 ml), neutralized with 10% AcOH (to pH 7), and extracted with n-butanol (4 × 50 ml). The extract was twice washed with saturated NaCl solution, the butanol was evaporated off to dryness, and the residual DMFA was evaporated off as an azeotropic mixture with heptane added five times). This gave 560 mg of a crude product (a polyhydroxysteroid glycoside), which was acetylated in 40 ml of absolute pyridine with 40 ml of $(CH_3CO)_2O$ for 4 days. Working up similar to that described above gave 240 mg of a polyacetate from which, by chromatography on a column of SiO_2 in the benzene–acetate (4:1) system, was isolated 80 mg of (IV), $C_{62}H_{88}O_{30}$, R_f 0.70 (ether–EtOH (98:2)), 0.31 [benzene–acetone (4:1)]. IR spectrum (ν, cm^{-1}): 1240-1260, 1730-1740, 3600 (in $CHCl_3$). Mass spectrum (m/z): 965 (M^+ – glucose acetate, $C_{14}H_{19}O_{10}$), 388 (aglycone). PMR spectrum (δ, ppm): 0.79 s (3 H, 19-CH_3); 1.03 s (3 H, 18-CH_3); 2.0-2.13 s (11 acetates); 3.43-3.89 m (5 H, OH, OCH); 4.0 dd (J = 11.3 Hz and 2.5 Hz, 1 H, 23-H_e); 4.08-4.63 m (9 H, OCH); 4.9-5.4 m (9 H, OCH).

SUMMARY

Starting from 3β-hydroxy-5α-pregen-16-en-20-one 3-O-[O-β-D-glucopyranosyl-(1 → 2)-O-β-D-glucopyranosyl-(1 → 4)-O-β-D-galactopyranoside], the synthesis has been performed of steroid glycosides with additional 1,4-dihydropyranone and 17α,20β-dihydroxytetrahydropyran rings E in the aglycone moiety.

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SYNTHESIS OF NEW ANTIATHEROMATOUS DRUGS.

STUDY OF THE ESTERIFICATION OF 17β-HYDROXY-5α-ANDROSTAN-3-ONE

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The acylation of dihydrotestosterone with propionyl and p-chlorophenoxyisobutyryl chlorides leads to the formation of dihydrotestosterone esters and the 3-enol acylates of the dihydrotestosterone esters. The acid hydrolysis of the 3-enol acylates converts them into the corresponding dihydrotestosterone esters.

The use of derivatives of p-chlorophenoxyisobutyric acid as antiatheromatic drugs normalizing the lipid metabolism started at the beginning of the 60's, when ethyl p-chlorophenoxyisobutyrate was tested and used [1]. The search for biologically active agents among the aroxyalkanecarboxylic acids is continuing [2, 3]. During the last 15 years, studies have appeared [4-6] showing that some androgens and their metabolites — in particular, dihydrotestosterone — possesses a hypolipoproteinemic action. However, dihydrotestosterone is inactivated and excreted from the organism in a relatively short time. Some dihydrotestosterone esters exhibiting hypolipoproteinemic properties, and 17β-hydroxy-5α-androstan-3-one p-chlorophenoxyacetate and p-chlorophenoxyisobutyrate, which prevent the development of disturbances to the lipid metabolism, have been synthesized. With these, the protective effect was expressed even after 30 days and was retained during the whole time of observation. These esters are obtained by the reaction of dihydrotestosterone with the chlorides of the acids mentioned in pyridine solution at −5-10°C.

A study of the reaction products in the production of dihydrotestosterone p-chlorophenoxyisobutyrate showed that another compound was formed in addition to the desired product.

Thin-layer chromatography showed the presence of two spots with R_f ~ 0.53 and ~0.65, the compound with R_f ~ 0.53 being dihydrotestosterone p-chlorophenoxyisobutyrate. The formation of enol acetates in the esterification with acetic anhydride of Δ^4-3-ketosteroids has been described in the literature [8-10].

Similar results were obtained in the acetylation of 17α-hydroxyprogesterone [9]. No such reactions have been described for androstan hydroxyketones having no conjugated ethylenic bond in the ring. A distinguishing feature of the process that we are introducing is acylation at a temperature of ~0°C, while in the known cases this process takes place on heating. We have suggested that the spot with R_f ~ 0.65 belongs to 5α-androstene-3,17β-diol 3,17-bis(p-chlorophenoxyisobutyrate). The reaction product that we isolated from the filtrate contained no CO absorption band at 1705 cm^{-1} that is characteristic for a carbonyl CO group at C_3. The absence of this band from the IR spectrum and the presence of bands at 1720 cm^{-1} corresponding to a carboxylic CO group, and at 1650 cm^{-1}, corresponding to Δ^3-enol, may serve as a proof of the formation of an enol acetate.

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