REACTIONS OF PENNOGENIN AND RELATED COMPOUNDS.

III. CONVERSION OF PENNOGENIN 3,17-DIACETATE INTO 22-OXO-(20S,25R)-CHOLEST-5-ENE-3β,16α,17α,26-TETRAOL 3,16-DIACETATE IN THE REACTION WITH $BF_3 \cdot Et_2O$

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A new direction of the reaction of pennogenin diacetate with $BF_3 \cdot Et_2O$ has been found in which the splitting out of the C^{17}-α(OH) group does not take place. On the basis of an analysis of ^1H and ^{13}C NMR spectra and also high-resolution mass spectra, the structure of 22-oxo-(20S,25R)-cholest-5-ene-3b,16α,17α,26-tetraol 3,16-diacetate has been ascribed to one of the reaction products, this being the first representative of the 16,17-disubstituted steroids of the cholestane series with a modified chain. The idea of the intermediate formation of a carbocation orthoester is brought in to explain the mechanism of the new reaction direction.

In recent years, considerable interest has been aroused by steroids of the cholestane series with a modified side chain, among which powerful inhibitors of the biosynthesis of DNA and chlesterol that suppress 3-hydroxy-3-methylglutaryl-CoA reductase have been detected [1]. Compounds of this type are present in medicaments exhibiting hypocholesteremic and antiinflammatory activity [2, 3].

The stereocontrolled total synthesis of such compounds has many stages and is complicated [4]. An alternative semisynthetic route is based on the use of natural steroids satisfying the stereochemical requirements in relation to the asymmetric centers of the cyclic part of the molecule and the side chain [5, 6].

Here we report the preparation of the first representative of 16,17-disubstituted steroids of the cholestane series with a modified side chain (VIII) in the reaction of (25R)-spirost-5-ene-3β,17α-diol 3,17-diacetate (I, pennogenin diacetate) [7] with boron trifluoride etherate.

It has been found previously that, under the brief action of boron trifluoride, (I) undergoes an intramolecular conversion into a compound containing a cyclic orthoester grouping [8] for which the structure of 16-O,17-O,23,O-(1',1',1'-ethylidenetrioxy)-22,26-epoxy-(20S, 22R,25R)-cholest-5-en-3β-ol 3-acetate (IV) [9] (scheme 1) has been demonstrated. Subsequent investigation showed that (IV) was not the only product of this reaction. Together with this compound, the reaction mixture contained a series of more polar substances, one of which was 16-O,17-O,23-(1',1'-ethylidenedioxy)-22,26-epoxy-(20S,25R)-cholestt-5,22-dien-3β-ol 3-acetate (V) [9]. It was shown with the aid of TLC that the reaction mixture from the action of boron trifluoride on (I) consisted of a dynamic system the composition of which depended on the time of contact of (I) with the reactant. On brief contact, the main reaction products were (IV) and (V). An increase in the time of contact led to the conversion of products (IV) and (V) into still more polar compounds, one of which was (VIII). The proof of the structure of (VIII) was based on the analysis of the corresponding mass and ^1H and ^{13}C NMR spectra.

The values of the ^1H CSs ($CDCl_3$ and C_6D_6) of the α-H^3, H^6, and H^{19} signals indicated the structural identity of rings A/B of (VIII) with those of (I). The values of the ^1H HSs ($CDCl_3$ and C_6D_6) of the H^{27} and H^{26} protons differ considerably from the corresponding values for (I), (IV), and (V) [9] were very close to those for 22-oxo-(20S,25R)-cholest-5-ene-3β, 26-diol [5]. The doublet nature of the signal of the H^{26} protons showed the open form of the side chain of (VIII). The splitting of the H^{16} signals into a doublet of doublets indicated the absence of a proton at C^{17}, which permitted the assumption of the presence of a C^{17}–OH

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group. The proof of the location of the functional groups in ring D [as in (VIII) but not (IX)] was based on the results of ^{13}C NMR spectroscopy.

In the ^{13}C NMR ($CDCl_3$) spectrum of (VIII), the signals of 31 C atoms were recorded. In the assignment of the signals the effects of 17α-hydroxylation found in the pregnane series [12] and a comparison of the spectrum of (VIII) with those of compounds similar in structure – cryptogenin [5], 22-oxo-(20S,25R)-cholest-5-ene-3β,26-diol [5], and 3β,16β-diacetoxy-26-chlorocholest-5-en-22-one [11] – were used.

The presence of an acetoxymethine group and of a tertiary, carbonyl C-atom in ring D of (VIII) was confirmed by its ^{13}C NMR spectrum taken by the J-modulated spin echo method [10].

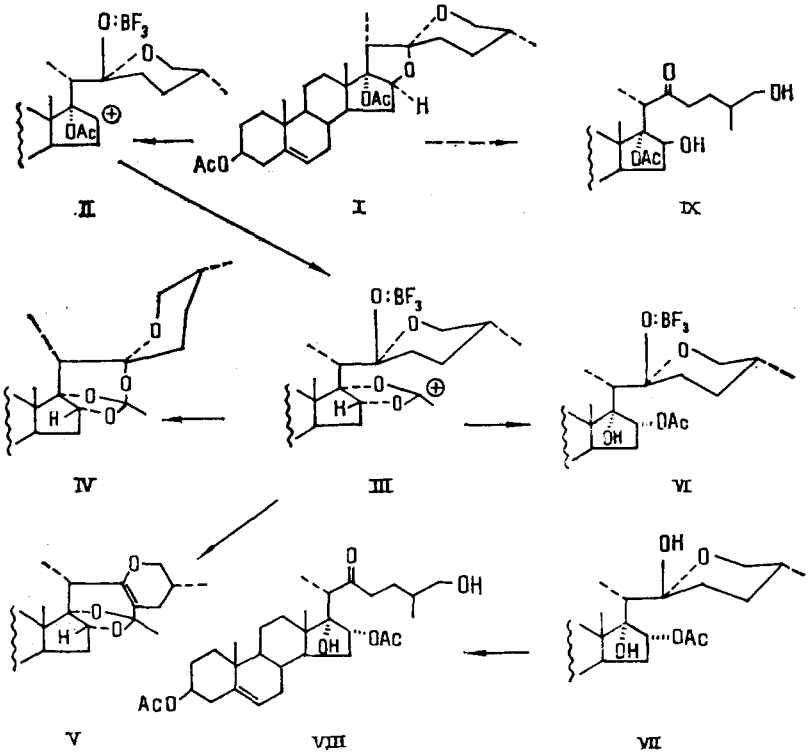
A comparison of the ^{13}C NMR spectra of (VIII) and of cryptogenin diacetate [5] confirmed the identity of the structure of the A/B rings of the two compounds. The α- and γ-effects of C^{17}-α-hydroxylation observed in the spectrum of (VIII) as compared with that of 3β,16β-diacetoxy-26-chlorocholest-5-en-22-one [11] were identical with the corresponding effects in the pregnane series: Δδ + 27 ppm and Δδ – 5.4 ppm (C^{14}), +1.2 ppm (C^{18}), and –4.4 ppm (C^{21}), respectively. The deviation of the absolute values of the β-effects from those of 17-hydroxyprogesterone were due to the use of dimethyl sulfoxide in the recording of the spectrum of the latter [12]. The descreening of the C^{22}-atom of (VIII) in comparison with that of 3β, 16β-diacetoxy-26-chlorocholest-5-en-22-one was also, apparently, due to the γ-effect. The proposed variant of the assignment of the C^{12} and C^{15} signals reflects the disappearance of the HH-interaction between α-H_a^{12} and α-H^{17} in the substitution C^{17} – αH → C^{17} – αOH (Δδ – 3.4 ppm) better than the opposite assignment. The coincidence of the ^{13}C CSs of the C^{23}–C^{27} atoms of (VIII) with those of 22-oxo-(20S,25R)-cholest-5-ene-3β,26-diol [5] showed the identity of these fragments of their structures.

The configurations of the C^{16} (β-H^{16}) and C^{17} (C^{17} – αOH) asymmetric centers was shown by a ^1H NOE experiment: the irradiation of the $C^{18}H_3$ group at δ 0.83 ppm caused a response of the protons at C^{20} (δ 2.90 ppm, quartet) and C^{16} (δ 5.12 ppm, dd).

The main directions of the fragmentation of the molecule (VIII) under electron impact are shown in Scheme 2. The peak of the M^+ ion with m/z 532 had a low intensity, but proof of its correspondence to (VIII) was obtained by the method of the metastable defocusing (MD) of the ion with m/z 514 $(M - H_2O)^+$. The elementary composition of the M^+ ion ($C_{31}H_{48}O_7$) and the nature of the distribution of the intensities of the peaks in the mass spectrum indicated the absence of a spiroketal system, because of which there was an increase in the role of cleavage at the C^{17}–C^{20} bond and the appearance of a key fragment with m/z 389 ($C_{23}H_{35}O_5$). The subsequent breakdown of the ion with m/z 389 with the liberation of two molecules of AcOH and one H_2O indicated the presence of two OAc and one OH groups in the cyclic part of the (VIII) molecule. Of the two variants of the relative positions of the OH and OAc groups in the ring D (VIII or IX), the first was considered preferable since such positions of the substituents in the side chain favor the cleavage of the C^{17}–C^{20} bond, while in structure (IX) this process would be suppressed by the initial predominant elimination of AcOH from the C^{17}–OAc group as in the case for (I) [9]. Since the ion with m/z 389 contained the OH group of the ring and, according to MD results, was formed predominantly from the $(M - H_2O)^+$ ion, the latter must have arisen by the loss of H_2O at the expense of one of the oxygen atoms of the side chain.

The instability under electron impact of structure (VIII) can be explained by the oxo-cyclo tautomerism that is characteristic of 1,5-ketols [13] if it is assumed that with a rise in temperature the tautomeric equilibrium is shifted in the direction of the hemiketal (VII). A M^+ ion of such structure regularly loses H_2O. The necessity for bringing in this mechanism is also due to the absence from the mass spectrum (VIII) of appreciable indications of breakdown at the C^{20}–C^{22} and C^{22}–C^{23} bonds which is usually well-defined for C^{22}-O-containing cholestanes [14]. Conversely, cryptogenin and (25R/S)-3β,6β-diacetoxy-25,26-dihydroxycholest-5-en-22-one possess mass-spectral characteristics similar to those of (VIII) [11].

The cyclizationof the side chain of (VIII) explains the increase in the contribution of other processes, as well. Thus, the peak of an oxonium ion with m/z 481 $(M - 2H_2O - CH_3)^+$ had a comparatively high intensity. In the cyclized variety of the ion with m/z 454 $(M - H_2O - AcOH)^+$, the C^{17}–C^{20} bond, which is in the allyl position in relation to the two π-bonds, was readily cleaved. The realization of a six-membered transition state in which H migrated from C^{17} to C^{27} explains the alternative formation in this process of the odd-electron fragments with m/z 328 and 126 (100%). The latter is characteristic for pennogenin derivatives [15]. In the absence of a C^{17}–OH group (cryptogenin), an ion with m/z 125 is formed.



Scheme 1. Scheme of the transformations of pennogenin acetate in the reaction with $BF_3 \cdot Et_2O$.

When the temperature of the experiment was raised, a pronounced redistribution of the intensities of the peaks of the ions took place in the mass spectrum of (VIII), the peak of the ion with m/z 328 becoming greatly predominant over all the others. It is possible that under these conditions the thermal decomposition of the ketal took place with the formation of 3β-acetoxyandrosta-5,15-dien-17-one.

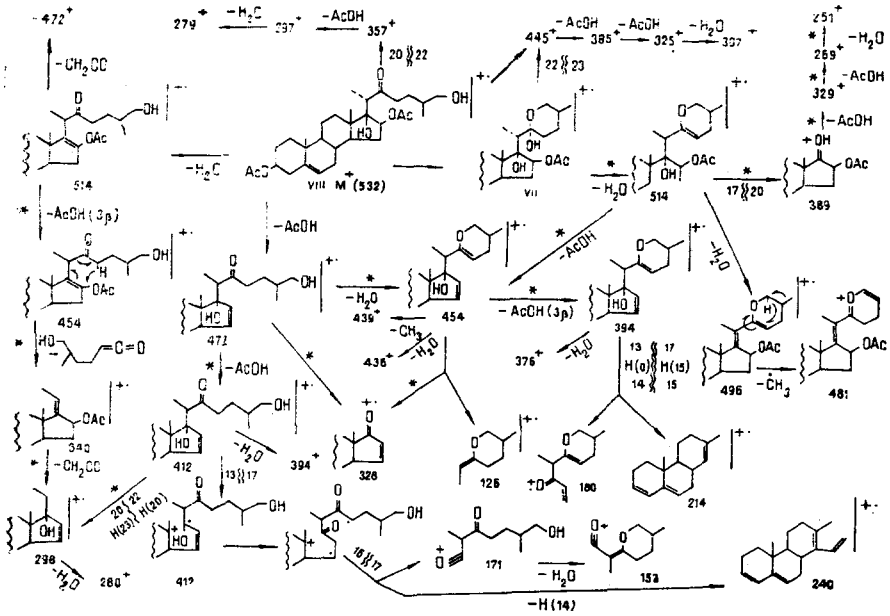
The formation of some of the ions of (VIII) can be treated by starting from the opening of the side chain, but the ions due to the simple cleavage of the C^{20}—C^{22} and C^{22}—C^{23} bonds had a low intensity. Other directions of the breakdown of the M^+ ion with an open side chain were realized through the stage of the splitting out of the C^{17}—OH or the C^{16}—OAc group, after which the C^{20}—C^{22} bond broke and ions with m/z 340, 298, and 280 arose. An ion with m/z 153 formed as the result of the cleavage of the C^{13}—C^{17} and C^{16}—C^{17} bonds is also characteristic of pennogenin derivatives. Another method for the breakdown of the steroid nucleus that is less characteristic of pennogenin derivatives is the cleavage of the C^{13}—C^{17} and C^{14}—C^{15} bonds. In addition to the usual ion for these cases with m/z 214 ($C_{16}H_{22}$) [16], the ion second in intensity in the spectrum of (VIII), m/z 180 $(C_{11}H_{16}O_2)^+$, arises at the expense of the side chain and the elements of ring D. On the migration of one H into the steroid fragment from the ion with m/z 394, another pair of mutually supplementing fragments with m/z 213 and 181 is formed.

According to its spectral characteristics, (VIII) has the structure of 22-oxo-(20S,25R)-cholest-5-ene-3β,16α,17α,26-tetraol 3,16-diacetate and is the first representative of 16,17-disubstituted steroids of the cholestane series with a modified side chain.

In view of the configuration of the C^{16} asymmetric center (β-H^{16}), it can be stated that the precursor of (VIII), like that of (IV) and (V), is the orthoester carbocation (III), (see Scheme 1). The structure of (VIII) contains two fragments responsible for the increased lability of the compound under investigation; β-hydroxyketone and 1,5-ketol systems. The investigation of the transformations of (I) and (VIII) is continuing.

EXPERIMENTAL

The ^1H and ^{13}C NMR spectra with complete decoupling from interactions with protons and by Cocq and Lallemand's method [10] were recorded on a Bruker WM-250 spectrometer at working frequencies of 250 and 69.9 MHz, respectively. The accuracy of the measurement of the CSs was



Scheme 2. Main directions of the fragmentation of (VIII) under electron impact.

better than 0.1 ppm in the ^{13}C NMR spectrum and better than 0.01 ppm in the ^{1}H NMR spectrum. The accuracy of the measurement of the SSCCs in the ^{1}H spectrum was ±0.15 Hz. The investigation was carried out on a 0.01 M solution of (VIII) at 30°C with TMS as internal standard. The INDOR technique was used for the assignment of the signals of the methyl groups in the ^{1}H NMR spectra. The overall mass spectrum, the MD spectrum, and the elementary compositions of the ions were measured on a MKh 1310 instrument with a SVP5 system for the direct introduction of the sample, the temperature of the evaporator bulb and the ionization chamber being 120°C, the ionizing voltage 50 V, and the collector current 40 μA. TLC was performed in a fixed layer of silica gel.

22-Oxo-(20S,25R)-cholest-5-ene-3β,16α,17α,26-tetraol 3,16-diacetate (VIII) from (I).

A solution of 0.5 g of (I) in 10 ml of absolute ether was treated with 10 ml of boron trifluoride etherate. After 30 min the reaction mixture was poured into 0.5 liter of 5% $NaHCO_3$, and the resulting mixture (pH 9) was stirred for 10 min. The reaction products were extracted with $CHCl_3$ and the extracts were washed with H_2O, dried with Na_2SO_4, evaporated to dryness, and chromatographed on a column of SiO_2 in the hexane–acetone system (50:0 → 50:2 gradient in 0.2 steps; 50:2–50:10 gradient in 1.0 steps; all 0.05 liter each). The fractions (20 ml) were analyzed by TLC in the hexane–ether (3:1, 2:1 and 1.5:1) and hexane–acetone (4:1) systems. The eluates containing the (VIII) were rechromatographed in the hexane–acetone system (10:0→10:2 gradient in 0.2 steps), which gave 0.02 g of chromatographically homogeneous product [R_f 0.25 in the hexane–acetone (4:1) system].

^{1}H NMR ($CDCl_3$; δ, ppm): 0.84, s, $3H^{18}$; 1.03, s, $3H^{19}$; 1.16 d, J = 7 Hz, $3H^{21}$; 0.90 d, J = 6 Hz, $3H^{27}$; 5.34 br.d, J = 5 Hz, H^{6}; 3.44 Hz, J = 6 Hz, $2H^{26}$; 4.60 m, $W_{1/2}$ = 20 Hz, α-H^{3}; 5.12 dd, J^{1} = 10Hz, J^{2} = 2 Hz, β-H^{16}; 2.54 t, J = 7.2 Hz, $2H^{23}$; 2.90 E, J = 7.5 Hz, H^{20}; 2.04 s, AcO × 2. ^{1}H NMR (C_6D_6; δ, ppm): 0.59 s, $3H^{28}$; 0.90 s, $3H^{19}$; 0.81 d, J = 6.5 Hz, $3H^{27}$; 1.05 d, J = 7 Hz, $3H^{21}$; 1.76 s, AcO – C^{16}; 1.77 s, AcO – C^{3}; 3.26 d, J = 6 Hz, $2H^{26}$; 4.82 m, α-H^{3}; 5.22 br.d, H^{6}; 5.25 t, J = 3 Hz, H^{16}; 2.65 q, J = 7 Hz, H^{20}.

^{13}C NMR ($CDCl_3$; δ, ppm): 37.0 (C^{1}), 27.9 (C^{2}), 74.0 (C^{3}), 38.2 (C^{4}), 140.0 (C^{5}), 122.3 (C^{6}), 32.0 (C^{7}), 31.9 (C^{8}), 49.6 (C^{9}), 36.7 (C^{10}), 20.4 (C^{11}), 31.7 (C^{12}), 48.3 (C^{13}), 49.0 (C^{14}), 33.4 (C^{15}), 78.5 (C^{16}), 82.6 (C^{17}), 14.5 (C^{18}), 19.3 (C^{19}), 48.6 (C^{20}), 12.7 (C^{21}), 216.3 (C^{22}), 40.9 (C^{23}), 26.2 (C^{24}), 35.1 (C^{25}), 67.6 (C^{26}), 16.5 (C^{27}), 170.5 (C^{28}), 21.4 (C^{29}), 170.3 (C^{30}), 22.3 (C^{31}).

Mass spectrum, m/z (%): 532 (M^{+}, 0.1), 514 (3), 496 (2), 481 (23), 472 (5), 454 (38), 439 (11), 436 (5), 412 (5), 394 (21), 389 (21), 385 (2), 379 (4), 376 (8), 357 (6), 340 (7), 329 (18), 328 (22), 311 (7), 298 (15), 297 (5), 280 (9), 269 (22), 268 (13), 251 (44), 240 (22), 226 ($C_{17}H_{22}$, 19), 214 (20), 213 (19), 199 (14), 197 (15), 181 (31), 180 (48), 171 (23), 153 (43), 126 (100), 115 (23), 105 (24), 97 (25), 81 (24), 69 (27), 43 (47.

SUMMARY

A new direction of the reaction of pennogenin diacetate with $BF_3 \cdot Et_2O$ has been found during which the opening of the spiroketal system is not accompanied by the splitting out of the hydroxy group at C^{17}. It has been shown that one of the products formed in this reaction is 22-oxo-(20S,25R)-cholest-5-ene-3β,16α,17α,26-tetraol 3,16-diacetate. Its fragmentation under electron impact has been studied.

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CARDIAC GLYCOSIDES OF *Acokanthera venenata*

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Six cardenolides have been isolated from the leaves of *Acokanthera venenata* G. Don: AV-1, mp 252-255°C, $[\alpha]_D^{20}$ + 39.4° (MeOH); AV-2, mp 199-208°C, $[\alpha]_D^{20}$ − 59.3° (MeOH); AV-3, mp 269-275°C/300-304°C, $[\alpha]_D^{21}$ − 69.8° (MeOH); AV-4, mp 279-289°C; AV-5, mp 222-225°C, $[\alpha]_D^{20}$ − 64.3° (MeOH); and AV-6, mp 193-196°C $[\alpha]_D^{20}$ − 23.8° (MeOH − $CHCl_3$). AV-5 has been identified as acovenoside A. AV-3 is a new cardiac glycoside: it is 1β-acetoxy-3β-(4'-O-β-D-glucosyl-3'-O-methyl-α-L-talomethylosyloxy)-14-hydroxy-5β,14β-card-20(22)-enolide (gluco-acovenoside B).

Acokanthera venenata G. Don (bushman's poison) is a South African plant containing cardiac glycosides of the cardenolide series. Its seeds which were studied in fairly great detail by Reichstein et al. [1, 2], contain acovenoside A, acovenoside B, acovenoside C, acolongifloroside K, ouabain, and a number of glycosides of unestablished structure [1].

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