ON STEROIDS CIII.[1]

MOLTING DEFICIENCIES PRODUCED BY SOME STEROL DERIVATIVES IN AN INSECT (PYRRHOCORIS APTERUS L.)

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ABSTRACT

Several sterol derivatives have been found to inhibit the postecdysial hardening and sclerotization of the cuticle in Pyrrhocoris. These compounds are derivatives of cholestane and 24β_F-methylcholestane containing 3β-hydroxy and 6-keto groups. It is assumed that these compounds may have ecdysone--antagonistic action.

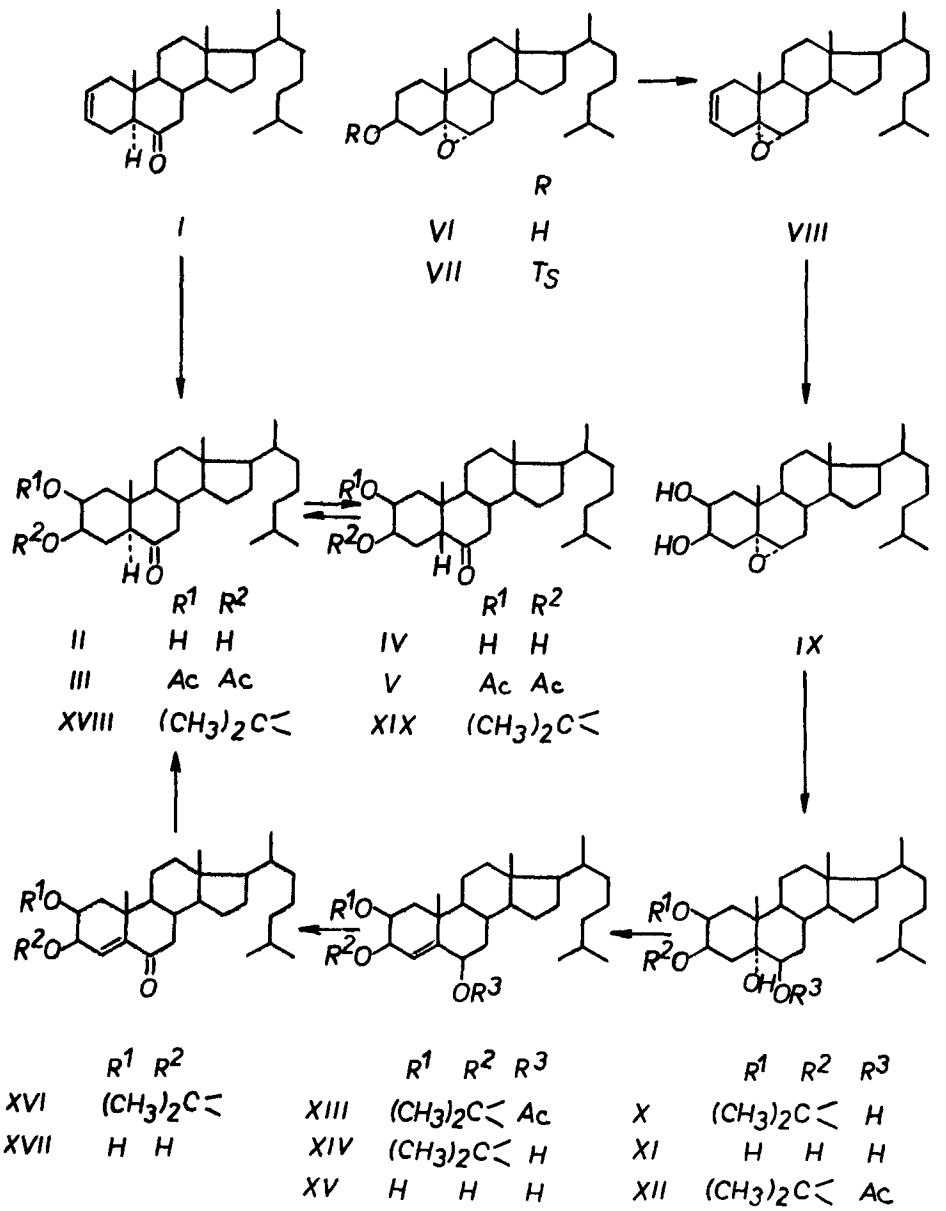
The important role which sterols play in life processes of insects appeared obvious when sterols have been recognized as essential nutritional growth factors for most insects (cf. review[2]). Cholesterol is the main sterol required for larval growth or reproduction; in certain species phytosterols and some other sterols from food sources are converted into cholesterol (cf. review[3]). In addition to the common promoting effects, sterols take part in various physiological processes

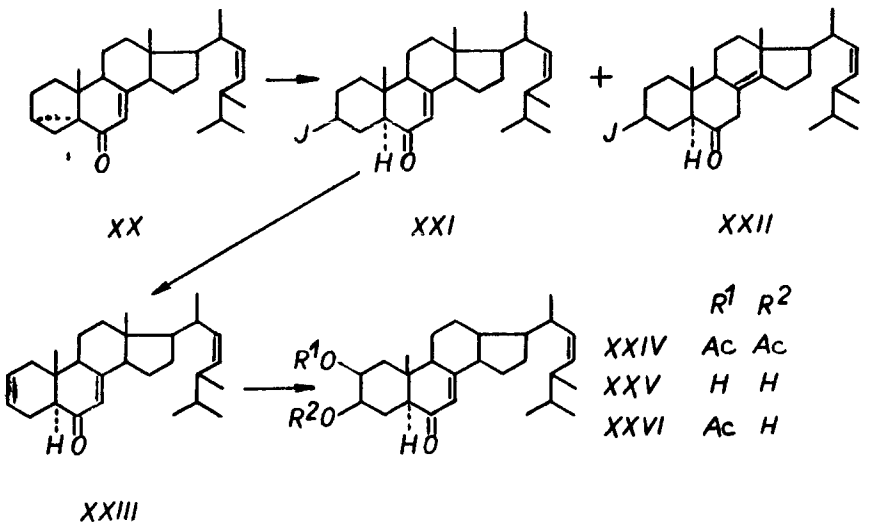
of insects, especially in tanning and cuticle sclerotization[4]. The processes of molting and cuticle sclerotization in insects are known to be stimulated by the steroid hormone ecdysone (cf. review[5]). The elucidation of its structure[6,7], confirmed by two syntheses[8-10], and isolation of closely related 20-hydroxyecdysone[11,12] raised a question to what extent structural changes in sterols will modify their physiological activity. From this point of view compounds containing some of the structural features of ecdysone appeared to be of particular interest.

For the physiological assay we selected cholestane and 24β_F-methylcholestane derivatives. For the synthesis of some substances of the first type, we set out from 5α-cholest-2--en-6-one (I)[13] which was hydroxylated by the Woodward procedure[14] and acetylated affording the diacetoxy ketone III. The effect of the 2β-hydroxyl group on the relative thermodynamic stabilities of $C_{(5)}$-epimeric 6-ketones was demonstrated by Siddall et al.[8].

In agreement with the finding of these authors, alkaline saponification of the diacetate III yielded a mixture which was separated by chromatography on silica gel to give 58% of the 5β-diol IV, characterized also as its diacetate V, and 42% of the 5α-diol II. The same result was obtained by equilibration of the 5β-isomer (60% of the 5β-and 40% of the 5α-isomer). The ORD curve of both diols exhibited a negative Cotton effect with a characteristic more negative amplitude for the 5β-isomer: a = -93 for II and a = -186 for IV in

agreement with the reported[15] values of -75 and -121 for analogous 5α- and 5β-isomers, respectively.





Another route to the cholestane derivatives started from 5,6α-epoxy-5α-cholestan-3β-ol (VI)[16,17], the tosylate of which (compound VII) was converted to the unsaturated oxide VIII in boiling aqueous dimethylformamide in the presence of lithium carbonate, i.e. by similar procedure as reported for an analogous case[18]. The resulting oxide VIII obtained in low yield was hydroxylated to the diol IX using the Woodward method. The epoxide ring in the compound IX was readily opened by a catalytic amount of perchloric acid in dioxane to yield the tetrol **XI**. The same experiment, performed in acetone solution, resulted in the formation of the acetonide X in a high yield. Acid hydrolysis of the latter compound in methanol at room temperature gave the tetrol XI. Acetylation of the acetonide X afforded the monoacetate XII which could

not be obtained in crystalline form; it was, however, homogeneous according to thin-layer chromatography. The monoacetate was therefore directly dehydrated by thionyl chloride in pyridine to give the crystalline unsaturated acetate XIII which was further characterized after alkaline hydrolysis as the hydroxy derivative XIV; acid hydrolysis of the last named compound resulted in the triol XV. Oxidation of the unsaturated alcohol XIV by chromium trioxide-pyridine complex led to the α,β-unsaturated ketone XVI which after acid hydrolysis gave the unsaturated diol ketone XVII. On hydrogenation in absolute ethanol in the presence of $Pd/CaCO_3$, the ketone XVI gave a single product, which on mild acid hydrolysis yielded the diol ketone II identical with the specimen prepared by the procedure mentioned earlier. The hydrogenation product is therefore 2β,3β-isopropylidenedioxy--5α-cholestan-6-one (XVIII). Thus, under the influence of the axial substituent in position 2, the hydrogenation takes a course different from that reported[19-21] for Δ^4-3β,6β-diols.

$24\beta_F$-Methyl-3α,5-cyclo-5α-cholesta-7,22-dien-6-one (XX)[22], which is readily accessible from ergosterol, was used as starting material for the preparation of the ergostane derivatives. This compound on treatment with hydroiodic acid yielded the 3β- iodo derivative XXI in 60% yield; analogous reaction using hydrochloric[23] and hydrobromic[13] acid had been reported for 3α,5-cyclo-5α-cholestan-6-one. Apart from the iodo derivativeXXI, an isomeric compound lacking the character of an α,β-unsaturated ketone was isolated. Its ultraviolet

absorption (log ε_{211} 4.03 and log ε_{225} 2.64; ethanol) is in conformity with the 8(14) position of the double bond[24,25]. Dehalogenation of the iodo derivative XXI with lithium carbonate in dimethylformamide led to the Δ^2-derivative XXIII. In the infrared spectrum of the compound XXIII the carbonyl band is split owing to Fermi resonance, as has been shown by comparison of spectra measured in chloroform and carbon tetrachloride solutions. The spectra differ only in positions and relative magnitudes of the split carbonyl bands. This splitting was also encountered in 5α-cholest--2,7-dien-6-one[26] but not in compounds I, XX, XXI, XXII, XXIV and XXV.

Since a model experiment with the compound XX had demonstrated considerable resistance of the conjugated and side chain double bonds towards hydroxylation, the procedure was applied to the compound XXIII which gave the substance XXIV in moderate yield. The spectral characteristics of the latter compound confirmed the presence of a Δ^7-6-keto system; the unsplit carbonyl band at 1667 cm^{-1} is in conformity with the structure XXIV.

In the preparation of the compound XXV from the diacetyl derivative XXIV, isomerization at $C_{(5)}$ could be avoided by using mild hydrolysis with potassium hydrogen carbonate at room temperature. Under these conditions, an acetate was also isolated. Assuming preferential hydrolysis of the equatorial 3β-acetoxy group, the product is formulated as 2-monoacetate XXVI.

Some of the above compounds were tested with respect to their possible ability to influence the molting process of insects. The tests were performed on larvae of Pyrrhocoris apterus.

METHOD

The larvae were fed with lime seeds and water at 25°C. The experiments were made on freshly molted last (fifth) instar larvae. Experimental specimens were immobilized by immerging them under water for 15-20 min. Then they were dried on an absorbent paper and fixed to a plastic plate by plasticine. The samples tested were implanted in solid state into a body cavity through a small incision made on the lateral part of the fourth abdominal sternite. The experimental specimens were then reared in Petri dishes at 25°C until adult ecdysis. The effects on postecdysial hardening and cutical sclerotization and on survival were recorded for several days after ecdysis. The dose administered varied from 10 to 50 γ per specimen. The weight of the last stage larva increases from 17 mg at the beginning to about 45 mg at the end. In experiments with contact application the sample was dissolved in peanut oil and applied on the uninjured cuticle.

We have observed that some of the steroids tested exert specific physiological effects on hardening of the cuticle and survival after ecdysis. The insects which have been administered these compounds grow and develop during the intermolting period; they are, however, unable to complete ecdysis. The red pigment in the epidermal cells and the black pigment of the cuticle develop normally but the whole integument remains soft and the cuticle sclerotization process is inhibited. Most of the specimens die shortly after unfinished ecdysis, those that survive remain with nonsclerotized cuticle and die after several hours. The effect of these steroids on postecdysial cuticle hardening and sclerotization

is summarized in Table I.

The results presented in Table I indicate that all the compounds possessing pronouced activity contain a keto group in position 6 and hydroxyl group in position 3. The hydroxyl group in the position 2 is not essential for activity (XXVIII). Also, the configuration at $C_{(5)}$ is not decisive (II, IV and XVII). It seems worth noting that acetylation of the hydroxyl groups (in IV) leads to loss of activity (V). Decrease of activity has been observed after introduction of Δ^7-double bond (XXVIII, XXIX) and complete loss after replacing the 6-keto group by a hydroxyl group (XVII and XV). Activity was observed both in the cholestane (II, IV, XVII) and the $24\beta_F$-cholestane (XXV, XXX) series.

The above effects were induced by administration of the compounds into the haemolymph. The following experiment was carried out in order to determine whether the above effects can be induced by topical application. Different doses of compounds II and XXVIII varying from 0.001 to 50 µg were topically administered on the larvae in acetone or peanut oil solutions. No effect was observed suggesting that the compounds do not penetrate through the uninjured insect cuticle. Next the larvae were fed with food, which had been impregnated with acetone solution of the compound II and the acetone allowed to evaporate. Even in this case the adults that emerged from these larvae were normal, their cuticle hardened and they survived.

Several cholesterol derivatives have already been

TABLE I

The Effect of Certain Cholestane and 24β_F-Methylcholestane Derivatives on the Postecdysial Survival of Pyrrhocoris Larvae

Compound	Activity*	Reference
5α-Cholest-2-en-6-one (I)	0	13
2β,3β-Dihydroxy-5α-cholestan-6-one (II)	+++	
2β,3β-Dihydroxy-5β-cholestan-6-one (IV)	+++	
2β,3β-Diacetoxy-5β-cholestan-6-one (V)	0	
5,6α-Epoxy-5α-cholestan-2β,3β-diol (IX)	0	
2β,3β-Isopropylidenedioxy-5α-cholestan-5,6β-diol (X)	0	
5α-Cholestan-2β,3β,5,6β-tetrol (XI)	+ (?)	
2β,3β-Isopropylidenedioxycholest-4-en-6β-ol (XIV)	0	
Cholest-4-en-2β,3β,6β-triol (XV)	0	
2β,3β-Dihydroxycholest-4-en-6-one (XVII)	++	
24β_F-Methyl-3α,5-cyclo-5α-cholesta-7,22-dien-6-one (XX)	0	22
24β_F-Methyl-2β,3β-dihydroxy-5α-cholesta-7,22-dien-6-one (XXV)	++	
3β-Acetoxy-5α-cholest-7-en-6-one (XXVII)	0	31
3β-Hydroxy-5α-cholestan-6-one (XXVIII)	+++	32
3β-Hydroxy-5α-cholest-7-en-6-one (XXIX)	+ (?)	33
24β_F-Methyl-3β,5-dihydroxy-5α-cholesta-7,22-dien-6-one (XXX)	++	34
24β_F-Methyl-19-norcholesta-5(10),6,8,22-tetraen-3β-ol (XXXI)	0	35

* 0 - no effect, + slight effect, imcomplete cuticle sclerotization in some specimens, ++ intermediate effect, +++ 100 per cent inhibition and mortality

reported to inhibit insect growth. It had been assumed that this action is due to a competitive inhibition of enzymes taking part in cholesterol metabolism[27]. The physiological effects reported in this paper do not seem to be similar to these growth inhibiting actions since there is little, if any, effect on the growth in Pyrrhocoris larvae. The effects described in Pyrrhocoris seem to be more specific than simple growth inhibition. They consist in inhibition of cuticle hardening and sclerotization processes which indicates that the defficiency is most probably concerned with the epidermal cells only rather than with the whole body.

The question arose whether the above mentioned effects were not due to ecdysone activity as it is known that some disproportions may arise from precocious molting induced by higher doses of ecdysone. The possibility of such an effect had to be taken in consideration since ecdysone activity was recently found in certain 2β,3β-dihydroxy-5β-cholest-7-en-6--one derivatives[28,29]. Thus the Calliphora tests[30] for ecdysone activity were performed with compounds II, IV, XXV, XXVIII and XXX and no ecdysone activity was found whereas ecdysone gave fully positive results. This indicates that the above described physiological activity is due to some other than an ecdysone effect.

Since the active compounds have a certain structural similarity to ecdysone, i.e. they contain 6-keto and 3β--hydroxyl groups, the assumption can be made that the action of these substances may in some way be antagonistic to the

processes stimulated by ecdysone.

EXPERIMENTAL

2β,3β-Diacetoxy-5α-cholestan-6-one (III). - A solution of 3.67 g of 5α-cholest-2-ene-6-one[13] in 5 cc of tetrahydrofurane and 70 cc of glacial acetic acid was treated with 3.6 g of silver acetate. The vigorously stirred suspension was treated with 2.52 g of finely powdered iodine (added in small portions) over a 0.5 hr. period at 20°C. After an additional 1 hr., 1 cc. of 20% aqueous acid was added with continuous stirring and refluxing for 3 hrs. After cooling, the solution was filtered through a column of sodium chloride, the solvent evaporated and the residue acetylated by the pyridine method to afford, after crystallization form ethanol, 1.95 g of diacetate III, m.p. 187-188°, $[\alpha]_D$ +5° (c, 2.5).

Anal. Calcd. for $C_{31}H_{50}O_5$: C, 74.06; H, 10.03.
Found: C, 73,88; H, 9.83.

Alkaline hydrolysis of 2β,3β-diacetoxy-5α-cholestan--6-one. - A solution of 3 g of diacetate III in 150 cc. of methanol was treated with 1,5 g of potassium hydroxide dissolved in 2 cc. of water and the reaction mixture allowed to stand at 20° for 75 hrs. The solvent was evaporated under reduced pressure, the residue diluted with water and the product extracted with a mixture of benzene-ethyl acetate (1:1). The solution was washed with water, dried and evaporated. The product was chromatographed on silicagel (90 g, eluted with

a mixture of chloroform-ethyl acetate 9:1), affording two separate individual fractions (1.04 g and 1.44 g). Crystallization of the first fraction from ethyl acetate-methanol--heptane afforded 836 mg of 5α-diol II, m.p. 213-216°, $[\alpha]_D$ +5° (c, 2.8) $\nu_{max}^{CHCl_3}$ 3612, 3560, 3460, 1704, 1056 cm^{-1} RD (c, 0.1; methanol, 20°); $[\phi]_{450}$ -290°; $[\phi]_{306}$ -3570°; $[\phi]_{270}$ +5710°; $[\phi]_{245}$ +5060°; $[\phi]_{230}$ +5650°. a = -93

Anal. Calcd. for $C_{27}H_{46}O_3$: C, 77.46; H, 11.08.
Found: C, 77.26; H, 11.12.

Crystallization of the second fraction from ethyl acetate--heptane yielded 1.17 g of 5β-diol IV, m.p. 179-180°, $[\alpha]_D$ -58° (c, 2.6), $\nu_{max}^{CHCl_3}$ 3600, 3570, 3430, 1698, 1425 cm^{-1} RD (c, 0.1; methanol, 20°); $[\phi]_{400}$ -1130°; $[\phi]_{310}$ -9010°; $[\phi]_{274}$ +9630°; $[\phi]_{234}$ +6440°; $[\phi]_{218}$ +7570°. a = -186

Anal. Calcd. for $C_{27}H_{46}O_3$: C, 77.46; H, 11.08.
Found: C, 77.46; H, 11.03.

Equilibration: 100 mg of 5β-diol (IV) was treated with methanolic potassium hydroxide under the conditions given above. Preparative TLC (developed with chloroform-ethyl acetate 1:1) afforded both isomers (38 mg of 5α and 57 mg of 5β, crude), which after crystallization provided pure compounds: 5α-diol II, m.p. 207-209° and 5β-diol (IV, m.p. 179-180°, both m.p.s. undepressed on admixture of specimens obtained in preceding experiments. The identities were corroborated by infrared spectra.

2β,3β-Diacetoxy-5β-cholestane-6-one (VI). - 600 mg of the 5β-diol (IV was acetylated with acetic anhydride in pyridine and afforded after crystallization from aqueous ethanol 574 mg of the diacetate V, m.p. 139-141°. Further crystallization from hexane raised the m.p. to 143-145°, $[\alpha]_D$ -45° (c, 2.1) $\nu_{max}^{CHCl_3}$ 1711, 1746, 1244, 1046 cm^{-1}.

Anal. Calcd. for $C_{31}H_{50}O_5$: C, 74.06; H, 10.03.
Found: C, 74.18; H, 10.20.

3β-Tosyloxy-5,6α-epoxy-5α-cholestane (VII). - 5,6α--Epoxy-5α-cholestane-3β-ol[16,17] (VI) (m.p. 143-144°; $[\alpha]_D$ -47°; 100 mg) was dried by distillation with benzene, dissolved in 3 cc. of pyridine and 100 mg of p-toluenesulfochloride and 0.1 cc. of triethylamine (dried over potassium hydroxide) were added. The mixture was allowed to stand at room temperature overnight, poured into water, the product which separated collected by suction, washed with water and dissolved in ether. The solution was washed with water, dried over magnesium sulfate and evaporated. The residue was dissolved in benzene, filtered through a layer of silica gel (1 g), the filtrate evaporated in vacuo and the crystalline residue (113 mg), m.p. 119-120°, crystallized from diisopropylether to yield the tosyloxy derivative VII, m.p. 119-120°, $[\alpha]_D^{22}$ -45° (c, 0.9). $\nu_{max}^{CHCl_3}$ 1600, 1598, 1363, 1177, 945 cm^{-1}.

Anal. Calcd. for $C_{34}H_{52}O_4S$: C, 73.33; H, 9.41; S, 5.75. Found: C, 73.68; H, 9.59; S, 5.89.

5,6α-Epoxy-5α-cholest-2-ene (VIII). - A mixture of 1.9 g of tosyloxy derivative VII and 2 g of lithium carbonate was stirred in a boiling mixture of 30 cc. of dimethylformamide and 0.5 cc. of water for 3 hrs. The reaction mixture was poured into water and ether, the aqueous layer extracted with ether, the combined ethereal extracts washed with water, dried over magnesium sulfate and evaporated. The remaining yellowish syrupy product (1.4 g) was dissolved in light petroleum and chromatographed on 30 g of silica gel. Fractions eluted with light petroleum gave 241 mg of colourless oil which crystallized out, m.p. 68-70°. Crystallization from ethanol yielded 147 mg of unsaturated epoxide VIII in form of microscopic needles, m.p. 75°, $[\alpha]_D^{22}$ -11° (c, 1.0); $\nu_{max}^{CHCl_3}$ 1655 cm^{-1} (double bond).

Anal. Calcd. for $C_{27}H_{44}O$: C, 84.31; 11.53.
Found: 84.11; 11.56.

5,6α-Epoxy-5α-cholestan-2β,3β-diol (IX). - A solution of 346 mg of the unsaturated oxide VIII in a mixture of 2 cc. of tetrahydrofuran and 4 cc. of glacial acetic acid was treated with 338 mg of silver acetate and to the stirred mixture 241 mg of iodine were portionwise added during 30 min. The reaction mixture was stirred for additional 45 min., 0.415 cc. of 96% acetic acid were added and the mixture stirred at 90-95° for 3 hrs. The reaction mixture was filtered through a small column of sodium chloride, washed with hot

benzene and the combined filtrates evaporated under reduced pressure and the residue evaporated three times with benzene in vacuo (removal of acetic acid). The residue was dissolved in 30 cc. of methanol, a solution of 400 mg of potassium hydroxide in 2 cc. of water added and the reaction mixture allowed to stand overnight. Methanol was evaporated in vacuo, the residue diluted with water and the product extracted with ether. The extract was washed with water, dried over magnesium sulfate and evaporated. The residue was dissolved in chloroform, 2 g of silica gel were added and the solvent removed under reduced pressure. The sorbent was put on a column prepared from 10 g of silica gel in benzene. A mixture of benzene-ether (1:1) eluted 183 mg of a product, m.p. 151-152°, which after crystallization from acetone afforded 110 mg of diol-epoxide IX as plates, m.p. 156-157°, $[\alpha]_D^{22}$ -32° (c, 1.3).

Anal.[37] Calcd. for $C_{27}H_{46}O_3$: C, 77.46; 11,08.
Found: C, 76.44; H, 11.08.

2β,3β-Isopropylidenedioxy-5α-cholestan-5,6β-diol (X). - A solution of 1.6 g of diol-epoxide IX in 125 cc. of acetone was treated with 7.5 cc. of 5% aqueous perchloric acid and allowed to stand at room temperature overnight. The reaction mixture containing crystalline product was evaporated in vacuo, the residue dissolved in ether, the extract washed with water, sodium hydrogen carbonate and water, dried over magnesium sulfate and evaporated under reduced pressure. The micro-

crystalline residue weighed 1.6 g and had m.p. 254-259°. Crystallization from acetone provided diol X, m.p. 263-265°, $[\alpha]_D^{22}$ +23° (c, 0.8). A sample for analysis was sublimed at 23°/0.6 mm Hg; m.p. 263-265°.

Anal. Calcd. for $C_{30}H_{52}O_4$: C, 75.58; H, 11.00.
Found: C, 75.55; H, 10.90.

5α-Cholestan-2β,3β,5,6β-tetrol (XI). - a. From 2β,3β--isopropylidenedioxy-5α-cholestan-5,6β-diol (X): A stirred suspension of 50 mg of diol X in 15 cc. of methanol was treated with 1 cc. of dilute hydrochloric acid (1:4). The starting material went into solution after 30 min. stirring at room temperature. After 50 min. the reaction mixture was evaporated in vacuo, the residue was dissolved in a mixture of ether-ethyl acetate, the extract washed with sodium hydrogen carbonate and water, dried over magnesium sulfate and evaporated under reduced pressure. The residue after crystallization from ethyl acetate yielded 24 mg of tetrol XI, m.p. 255-256°, $[\alpha]_D^{22}$ +14° (c, 0.8; methanol).

Anal. Calcd. for $C_{27}H_{48}O_4$: C, 74.26, H, 11.08.
Found: C, 73.68; H, 11.03.

b. From 5,6α-Epoxy-5α-cholestan-2β,3β-diol (IX):
A solution of 50 mg of diolepoxide IX in 3 cc. of dioxane was treated with 0.3 cc. of 5% aqueous perchloric acid and allowed to stand at room temperature overnight. The reaction mixture was evaporated under reduced pressure, the residue diluted

with water, the product collected by suction, washed with water and dried over potassium hydroxide in vacuo (45 mg), m.p. 140-150°. Two-fold crystallization from ethyl acetate gave 13 mg of tetrol XI, m.p. 255-256°, identical according to infrared spectrum (nujol) with the specimen prepared under a.

2β,3β-Isopropylidenedioxy-6β-acetoxycholest-4-ene (XIII). A solution of 200 mg of diol X in 6 cc. of pyridine was treated with 3 cc. of acetic anhydride and allowed to stand at room temperature overnight. The reaction mixture was poured into saturated sodium hydrogen carbonate solution and ice, the product which separated was collected by suction, washed with water and dissolved in ether. The ethereal solution was dried over magnesium sulfate and evaporated under reduced pressure. The residual crude 2β,3β-ispropylidenedioxy-6β-acetoxy-5α-cholestan-5-ol (XII) (190 mg) was dissolved in 5 cc. of dry pyridine, the solution cooled to -17° and treated with a solution of 0.5 cc. of thionyl chloride (freshly distilled) in 2 cc. of pyridine cooled to -17° as well. The reaction mixture was allowed to stand at the same temperature for 30 min. and another 30 min. at 0°. The mixture was cooled again to -17°, poured into saturated sodium hydrogen carbonate solution and ice and the product extracted with light petroleum. The combined extracts were washed thoroughly with water, dried over magnesium sulfate and evaporated under reduced pressure. Yield 166 mg of an oil

which crystallized out. Crystallization from acetone gave 57 mg of unsaturated acetate XIII, m.p. 143-144°, $[\alpha]_D^{22}$ +68° (c, 1.2).

Anal. Calcd. for $C_{32}H_{52}O_4$: C, 76.75; H, 10.47.
Found: C, 76.51; H, 10.41.

2β,3β-Isopropylidenedioxycholest-4-en-6β-ol (XIV). - a. From 2β,3β-isopropylidenedioxy-6β-acetoxycholest-4-ene (XIII): A solution of 100 mg of unsaturated acetate XIII and 50 mg of potassium hydroxide in 10 cc. of abs. methanol was refluxed for 7.5 hrs. The reaction mixture was evaporated in vacuo, the residue dissolved in ether, the solution washed with water, dried over magnesium sulfate and evaporated in vacuo. The residual oil (89 mg) crystallized out and had m.p. 160-161°. Crystallization from light petroleum afforded 60 mg of the unsaturated alcohol XIV, m.p. 162-163°, $[\alpha]_D^{22}$ +46° (c, 1.0).

Anal. Calcd. for $C_{30}H_{50}O_3$: C, 78.55; H, 10.99.
Found: C, 78.51; H, 10.93.

b. From cholest-4-en-2β,3β,6β-triol (XV): A solution of 5 mg of triol XV in 1 cc. of acetone was treated with one drop of 5% perchloric acid and allowed to stand at room temperature overnight. The reaction mixture was evaporated under reduced pressure, the residue dissolved in ether, the solution washed with sodium hydrogen carbonate solution and water, dried over magnesium sulfate and evaporated. The crude product which according to TLC contained a small quantity of

starting material was chromatographed preparatively on one 20 x 20 cm plate of silica gel (developed with benzene-ether 8:2). The eluted product (4.8 mg), m.p. 160-161°, according to infrared spectrum (chloroform) was identical with the specimen prepared under a.

Cholest-4-en-2β,3β,6β-triol (XV). - A solution of 100 mg of acetonide XIV in 5 cc. of 80% aqueous tetrahydrofuran was treated with 10 mg of p-toluenesulfonic acid and allowed to stand at room temperature for 7 days. The reaction mixture was evaporated under reduced pressure, the residue dissolved in chloroform, the solution washed with sodium hydrogen carbonate solution in water, dried over magnesium sulfate and evaporated. The product, which according to TLC still contained unreacted starting material, was chromatographed preparatively on the plate of silica gel (developed two times with ether). The eluted more polar zone afforded 37 mg of triol XV which after crystallization from chloroform (25 mg) had m.p. 221-222° and $[\alpha]_D^{22}$ -52° (c, 0.8; methanol).

Anal. Calcd. for $C_{27}H_{46}O_3$: C, 77.46; H, 11.08.
Found: C, 77.33; H, 11.05.

2β,3β-Isopropylidenedioxycholest-4-en-6-one (XVI). - A solution of 161 mg of unsaturated acetonide-alcohol XIV in 3 cc. of pyridine was oxidized with a complex prepared from 100 mg of chromium trioxide and 5 cc. of pyridine for 12 hrs. The reaction mixture was poured into saturated

sodium hydrogen carbonate solution and the product extracted with ether. The combined extracts were washed with water, dried over magnesium sulfate and evaporated. The crystalline residue (157 mg) was crystallized from methanol (containing one drop of pyridine) and yielded needles of unsaturated ketone XVI, m.p. 163-163.5°, $[\alpha]_D^{22}$ +29° (c, 1.1). $\nu_{max}^{CHCl_3}$ 1691, 1638 cm^{-1}; λ_{max}^{EtOH} 237 mμ (log ε 3.79).

Anal. Calcd. for $C_{30}H_{48}O_3$: C, 78.90; H, 10.59.
Found: C, 78.85; H, 10.67.

2β,3β-Dihydroxycholest-4-en-6-one (XVII). - A solution of 200 mg of the unsaturated acetonide-ketone XVI in ether was treated with 140 cc. of methanol and ether evaporated under reduced pressure. The fine crystalline suspension was treated with 4 cc. of dilute hydrochloric acid (1:4) and the mixture stirred at room temperature for 3 hrs. The starting material dissolved during this time and a turbid solution resulted. Methanol was evaporated under reduced pressure, the residue dissolved in ether, the solution washed with sodium hydrogen carbonate and water and dried over magnesium sulfate. Yield 190 mg of almost pure (TLC) free diol-ketone XVII. Two crystallizations from ethyl acetate afforded flat needles of m.p. 185-187°, $[\alpha]_D^{21}$ -45°, (c, 1.2). $\nu_{max}^{CHCl_3}$ 3430, 1688, 1638, 1055 cm^{-1}; λ_{max}^{EtOH} 237 mμ (log ε 3.74).

Anal.[37] Calcd. for $C_{27}H_{44}O_3$: C, 77.83; H, 10.65.
Found: C, 76.82; H, 10.59.

2β,3β-Isopropylidenedioxy-5α-cholestan-6-one (XVIII). - a. From 2β,3β-isopropylidenedioxycholest-4-en-6-one (XVI). The unsaturated ketone XVI (213 mg) in 60 cc. of abs. ethanol was hydrogenated in the presence of 500 mg of 5% palladized calcium carbonate until the hydrogen uptake ceased. The reaction mixture was filtered, the filtrate evaporated under reduced pressure and the crystalline residue crystallized from methanol (containing one drop of pyridine). Yield 150 mg of saturated 5α-ketone XVIII, m.p. 167-168°, $[\alpha]_D^{22}$ +20° (c, 1.0). $\nu_{max}^{CHCl_3}$ 1708 cm^{-1}, 1429 cm^{-1}, 1050 cm^{-1}.

RD (c, 0.1; chloroform, 20°); $[\Phi]_{450}$ 0°; $[\Phi]_{313}$ -5700°; $[\Phi]_{271}$ +8290°; $[\Phi]_{246}$ +7410°. a = -140.

Anal. Calcd. for $C_{30}H_{50}O_3$: C, 78.55; H, 10.99.
Found: C, 78.14; H, 11.01.

b. From 2β,3β-dihydroxy-5α-cholestan-6-one (II): A solution of 100 mg of dihydroxy ketone II in 10 cc. of acetone was treated with 0.5 cc. of aqueous 5% perchloric acid and allowed to stand at room temperature for 2 hrs. The reaction mixture was evaporated in vacuo, the residue dissolved in ether, the solution washed with sodium hydrogen carbonate solution and water and dried over magnesium sulfate; yield 101 mg. The acetonide was separated from unreacted starting material by means of preparative TLC (benzene-10% ether), yield 82 mg, m.p. 166-168°. Crystallization from methanol (containing one drop of pyridine) yielded compound XVIII (64 mg), m.p. 167-168°, identical according to infrared

spectrum with the specimen prepared according to a.

2β,3β-Dihydroxy-5α-cholestan-6-one (II). - A suspension of 50 mg of acetonide-ketone XVIII in 30 cc. of methanol was treated with 0.9 cc. of dilute hydrochloric acid (1:4) and the mixture stirred until all went into solution (2 hrs.) The stirring was continued for further 30 min., the solvent evaporated under reduced pressure, the residue diluted with water and the product taken into ether. The combined extracts were washed with sodium hydrogen carbonate solution and water, dried over magnesium sulfate and evaporated in vacuo. The crystalline residue (43 mg), m.p. 185-207° after two crystallizations from methanol yielded 26 mg of a product, m.p. 210-212° which according to mixed melting point and infrared spectrum was identical with the compound prepared from diacetate III.

2β,3β-Isopropylidene-5β-cholestan-6-one (XIX). - A solution of 200 mg of diolketone IV in 20 cc. of acetone was treated with 0.5 cc. of 5% aqueous perchloric acid and allowed to stand at room temperature for 2 hrs. The reaction mixture was evaporated under reduced pressure, the residue dissolved in ether, the combined extracts washed with sodium hydrogen carbonate solution and water, dried over magnesium sulfate and evaporated in vacuo. The residue (190 mg), which according to TLC contained starting material, was chromatographed on 6 g of silica gel in benzene. Fractions eluted

with a mixture of benzene-10% ether eluted uniform acetonide XIX (136 mg) which after crystallization from methanol (containing one drop of pyridine) gave needles, m.p. 143-144°, $[\alpha]_D^{22}$ -39° (c, 1.5). $\nu_{max}^{CHCl_3}$ 1702 cm^{-1}, 1051, 1032, 1246 cm^{-1}. RD (c, 0.1; chloroform, 20°); $[\Phi]_{450}$ -640°; $[\Phi]_{318}$ -9790°; $[\Phi]_{276}$ +10990°; $[\Phi]_{246}$ +8010°. a = -207.

Anal. Calcd. for $C_{30}H_{50}O_3$: C, 78.55; H, 10.99.
Found: C, 78.21; H, 10.92.

3β-Iodo-24β_F-methyl-5α-cholesta-7,22-dien-6-one (XXI). - A solution of 9 g of 24β_F-methyl-3α,5-cyclo-5α-cholesta-7,22--dien-6-one (XX)[22] in 100 cc. of dry tetrahydrofuran was treated with a solution of 15 cc. of 58% hydroiodic acid in 15 cc. of tetrahydrofuran and allowed to stand at room temperature for 20 hrs. The reaction mixture was poured onto ice, the product collected by suction, washed with water and dissolved in chloroform. The solution was washed with sodium thiosulphate solution, potassium hydrogen carbonate solution and water, dried over sodium sulfate and evaporated under reduced pressure. Repeated crystallization from ethanol yielded 3.7 g of iodoketone XXI, m.p. 156-158°, $[\alpha]_D^{20}$ -19° (c, 1.2). $\nu_{max}^{CHCl_3}$ 1668 cm^{-1}, 1621 cm^{-1}; λ_{max}^{EtOH} 246 mμ (log ε 4.19).

Anal. Calcd. for $C_{28}H_{43}OI$: C, 64.35; H, 8.29.
Found: C, 64.11; H, 8.29.

Further crop of less pure material was obtained by

extraction of aqueous filtrate after separation of crude product.

3β-Iodo-24β$_F$-methyl-5α-cholesta-8(14),22-dien-6-one (XXII). - Mother liquors after crystallization of the iodo derivative XXI were chromatographed on 150 g of silica gel. Fractions eluted with a mixture of benzene-light petroleum (1:5) yileded 3β-iodo-24β$_F$-methyl-5α-cholesta-8(14),22-dien--6-one (XXII) which after crystallization from ethamol melted at 140-142°, $[\alpha]_D^{20}$ -21° (c, 1.3). $\nu_{max}^{CHCl_3}$ 1711 cm^{-1}; λ_{max}^{EtOH} 211 mμ (log ε 4.03).

Anal. Calcd. for $C_{28}H_{43}OI$: C, 64.35; H, 8.29.
Found: C, 64.38; H, 8.51.

Subsequent fractions yielded additional 3.2 g of XXI, m.p. 156-158°, after recrystallization from ethanol.

24β$_F$-Methyl-5α-cholesta-2,7,22-trien-6-one (XXIII). - A solution of 3β-iodo-24β$_F$-methyl-5α-cholesta-7,22-dien-6-one (XXI) in 50 cc. of dimethylformamide and 1 cc. of water containing 2.5 g of lithium carbonate was refluxed under nitrogen for 90 min. The reaction mixture was diluted with a mixture benzene-light petroleum (1:2), washed with water, dried over sodium sulfate and after evaporation chromatographed on 90 g of silica gel. Fractions eluted with a mixture of light petroleum-benzene (5:1) eluted 400 mg of ergostatrienone XXIII which after crystallization from acetone had

m.p. 149-150° and $[\alpha]_D^{20}$ +29° (c, 0.1). $\nu_{max}^{CCl_4}$ 1677+1658, 1618 cm^{-1}; $\nu_{max}^{CHCl_3}$ 1668+1652, 1625 cm^{-1}; $\lambda_{max}^{heptane}$ 237 mμ (log ε 4.11), 284 mμ (log ε 2.53).

Anal. Calcd. for $C_{28}H_{42}O$: C, 85.22; H, 10.73.
Found: C, 85.28; H, 10.74.

2β,3β-Diacetoxy-24β_F-Methyl-5α-cholesta-7,22-dien-6-one (XXIV). - A solution of 1 g of iodine in 4 cc. of tetrahydrofuran was dropwise added in the course of 30 min. to a stirred solution of 1.7 g of 24β_F-methyl-5α-cholesta-2,7,22-trien-6-one (XXIII) in 25 cc. of glacial acetic acid containing 1.7 g of silver acetate. The stirring was continued for 30 min. at room temperature, a solution of 36 mg of water in glacial acetic acid was added, the temperature raised to 95° and the stirring continued for 3 hrs. The reaction mixture was filtered through a column of sodium chloride, washed with ethyl acetate and the filtrate evaporated in vacuo. The residue was dried by distillation with benzene and acetylated with 5 cc. of acetic anhydride in 15 cc. of pyridine at 37° for 18 hrs. The reaction mixture was evaporated in vacuo and the residue chromatographed on 80 g of alumina (neutral, grade III-IV). Benzene eluted 480 mg of the compound XXIV, which after crystallization from acetone had m.p. 154-157° and $[\alpha]_D^{20}$ -23° (c, 0.37). $\nu_{max}^{CHCl_3}$ 1737, 1667, 1617, 1255, 1051 cm^{-1}. RD (c, 0.3; chloroform, 20°); $[\Phi]_{589}$ -118°; $[\Phi]_{450}$ -880°; $[\Phi]_{390}$ -1220°; $[\Phi]_{374}$ 0°; $[\Phi]_{354}$ +8200°; $[\Phi]_{345}$ +6400°; $[\Phi]_{338}$ 0°; $[\Phi]_{330}$ -12800°.

Anal. Calcd. for $C_{32}H_{48}O_5$: C, 74.96; H, 9.44.
Found: C, 74.71; H, 9.20.

2β,3β-Dihydroxy-24β$_F$-methyl-5α-cholesta-7,22-dien-6-one (XXV). - A solution of 300 mg of diacetoxy derivative XXIV in 30 cc. of methanol was treated with 5 cc. of potassium hydrogen carbonate (210 mg) solution. The reaction mixture was allowed to stand at 37° for 18 hrs., taken down in vacuo, water was added and the product which separated was collected by suction, washed with water and crystallized from a mixture of heptane-ethyl acetate. M.p. 125-128°, $[\alpha]_D^{21}$ -13° (c, 0.9). $\nu_{max}^{CHCl_3}$ 3605, 3565, 3400, 1667, 1616 cm^{-1}.

Anal. Calcd. for $C_{28}H_{44}O_3$: C, 78.45; H, 10.35.
Found: C, 78.12; H, 10.31.

2β-Acetoxy-3β-hydroxy-24β$_F$-methyl-5α-cholesta-7,22-dien-6-one (XXVI). - The mother liquors after crystallization of the diol XXV were chromatographed on 9 g of silica gel and gave monoacetyl derivative XXVI, m.p. 186-188° (acetone), $[\alpha]_D^{21}$ +6° (c, 0.9). $\nu_{max}^{CHCl_3}$ 3590, 1734, 1668, 1614, 1243 cm^{-1}.

Anal. Calcd. for $C_{30}H_{46}O_4$: C, 76.55; H, 9.85.
Found: C, 76.37; H, 9.91.

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36. Melting points were determined on a Kofler block. Unless otherwise stated, optical rotations were measured in chloroform solutions. Analytical and preparative thin-layer chromatography were carried out on silica gel G (Merck).
37. The analysis gave constantly lower values for carbon.

Note. After submitting this paper to the Editor, the publication by R.Wiechert et al. HELV. CHIM. ACTA 49, 1581 (1966) appeared, where preparation of compounds II - V has been reported.