CHOLESTEROL ANALOGUES WITH OXA- AND OXA-AZA SIDE CHAIN A NOVEL CLASS OF INHIBITORS OF CHOLESTEROL BIOSYNTHESIS

Klaus Irmscher, Josef M. Kraemer and Herbert Halpaap

Research Laboratories of E. Merck AG, Darmstadt

Revision received May 4, 1966

The synthesis of neutral and basic monoethers of pregn-5-en-3ß,20α-diol, androst-5-en-3ß,17ß-diol, androst-5-en-3ß,16ß-diol, and of 20-aza-24-oxacholesterol is described. Saturated and chlorosubstituted derivatives of some of these compounds were prepared. 22-Oxa-25-azacholesterol and 20-oxa-21-nor-25-azacholesterol are potent inhibitors of cholesterol synthesis.

The cholesterol synthesis in the liver of men and mammals is known to be inhibited by exogenous cholesterol in a feedback mechanism. This gave rise to the idea of synthesizing compounds simulating the presence of cholesterol in the blood stream. The cholesterol analogues prepared by Counsell and coworkers [1-3], where one or two C-atoms of the side chain in the 17-position are replaced by nitrogen, actually showed hypocholesterolemic activity. In addition to the desired inhibition of 3-hydroxy-3-methyl-glutaryl-coenzyme A reductase, which leads to a decrease in cholesterol synthesis, it was also found that the mono- and diazacholesterols prepared by the Searle group possess an inhibiting effect on desmosterol reductase [3-5].

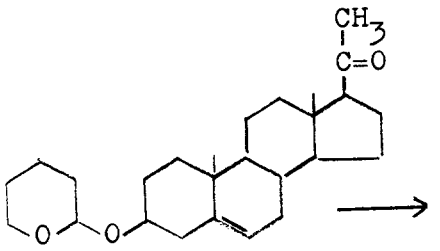
For this reason we wanted to investigate a number of cholesterol analogues where one side chain C-atom is replaced by oxygen and, if desired, another one by nitrogen. It was uncertain whether in this case also the hydrogenation of the 24-double bond would be inhibited.

Starting material of the synthesis of 22-oxa compounds was the 3ß-hydroxypregn-5-en-20-one 3-tetrahydropyranyl ether (I) described by Ott and coworkers [6]. Reduction with sodium/ethanol produced a mixture of the alcohols epimeric in C-20 which were separated by chromatography. In analogy to the corresponding 3-monoacetates the desired 20α-hydroxy compound II was found to be the more polar one of the epimers.

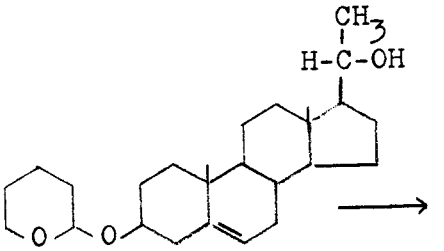
Etherification of II was achieved by reaction of the corresponding alcoholate accessible by the action of potassium amide in liquid ammonia with 2-chloroethyl-dimethylamine in boiling dioxane. After cleavage of the tetrahydropyranyl ether IIIa in aqueous-alcoholic hydrochloric acid 22-oxa-25-azacholesterol (IVa) was obtained, the hydroxy group of which could be substituted by chlorine (IVb) with thionyl chloride.

Recently Cross and coworkers [8] have described the synthesis of a 22-oxa-25-azacholesterol. In this product the ether side chain is in the 20ß-position, while the isohexyl residue of the cholesterol side chain is known to correspond sterically to a residue of a pregnane derivative [9] in the 20α-position. The product of the Syntex group therefore is to be considered as a 20-iso-22-oxa-25-azacholesterol, while our compound IVa, which differs in its physical data from that of Cross, corresponds to the natural stereochemistry of cholesterol in C-20 and therefore is the actual 22-oxa-25-azacholesterol.

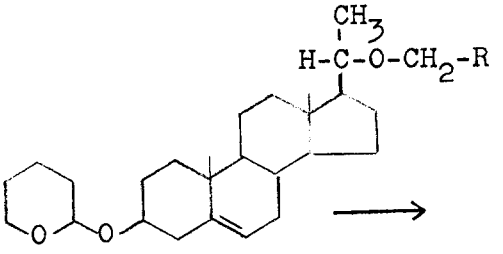
The neutral ethers IIIb and c were obtained by reaction of II with isoamyl bromide or isobutyl bromide in the presence of sodium hydride. Addition of triethylamine proved to be useful. Acid hydrolysis produced 22-oxacholesterol IVc, or 22-oxa-24-norcholesterol IVd, respectively.



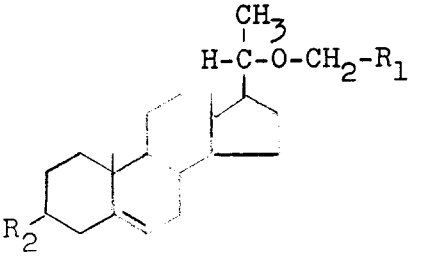
I



II



III

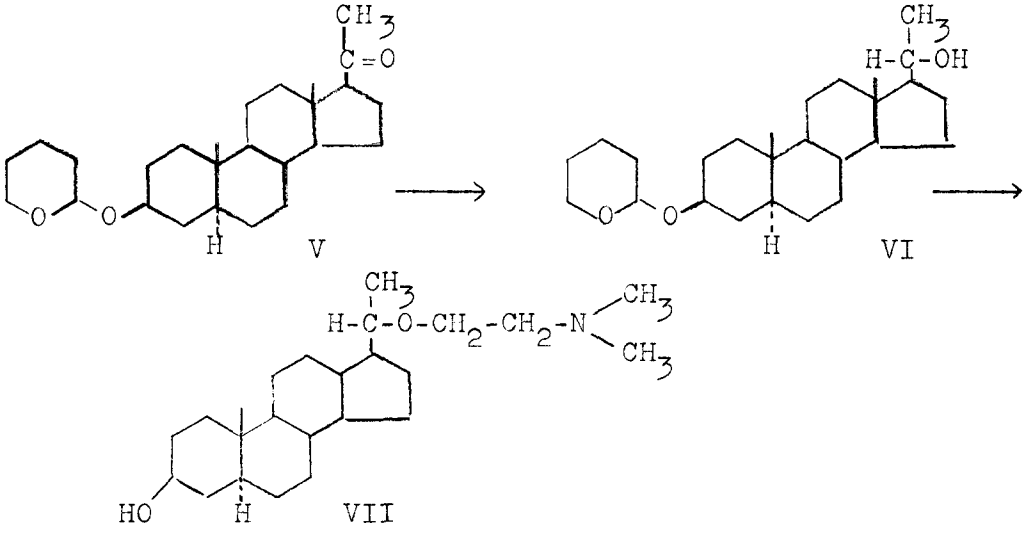


IV

a) R = $-CH_2-N(CH_3)_2$
b) R = $-CH_2-CH(CH_3)_2$
c) R = $-CH(CH_3)_2$

a) R_1 = $-CH_2-N(CH_3)_2$, R_2 = OH
b) R_1 = $-CH_2-N(CH_3)_2$, R_2 = Cl
c) R_1 = $-CH_2-CH(CH_3)_2$, R_2 = OH
d) R_1 = $-CH(CH_3)_2$, R_2 = OH

Similarly 3ß-hydroxy-5α-pregnan-20-one 3-tetrahydropyranyl ether (V) [10] was reduced in 20-position. The 20α-hydroxy product VI separated by chromatography differed in its physical data from the reduction product with a 20ß-hydroxy group obtained by Sondheimer and coworkers [10] from V by reduction with sodium borohydride. Reaction of VI with 2-chloroethyl-dimethylamine and subsequent splitting of the tetrahydropyranyl ether produced 22-oxa-25-azacholestan-3ß-ol VII.

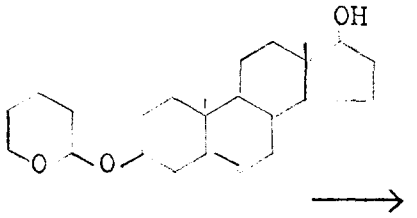


The alkali resistent tetrahydropyranyl ether was used as a protective group for the 3-hydroxy group also for the preparation of the 20-oxa-21-norcholesterol derivatives. Catalytical reduction of the 17-keto group of the dehydroepiandrosterone derivative [6] in the presence of Raney-nickel with hydrogen yielded the known [6] 3-tetrahydropyranyl ether of androst-5-en-3ß,17ß-diol (VIII). The etherification with 3-bromopropyl-dimethylamine was accomplished by reacting its hydrobromide [11] with VIII in the presence of an excess of sodium hydride. After cleavage of the tetrahydropyranyl ether 20-oxa-21-nor-25-azacholesterol (IXa) resulted.

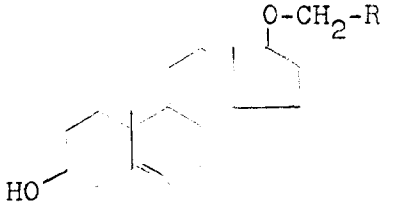
Several N-alkylated derivatives of the 20-oxa-21-nor-24-aza series were prepared. Thus, the reaction of VIII, which

in these cases was converted into the alcoholate by the potassium amide process, with 2-chloroethyl-methyl-isopropylamine [12] yielded the tetrahydropyranyl ether of 20-oxa-21-nor-24-methyl-24-azacholesterol, while the use of 2-chloroethyl-ethyl-isopropylamine [13] yielded the corresponding N-ethyl derivative. Hydrolysis produced IXb or IXc, respectively. Similarly 20-oxa-21,27-bisnor-24-ethyl-24-azacholesterol (IXd) was produced from VIII with 2-chlorotriethylamine [14].

Derivatives with abbreviated side chain were synthesized by reaction of VIII with 2-chloroethyl-dimethylamine, isoamyl bromide or isobutyl bromide. After hydrolysis 20-oxa-21,24-bisnor-25-azacholesterol (IXe), 20-oxa-21,24-bisnorcholesterol (IXf) or 20-oxa-21,23,24-trisnorcholesterol (IXg) resulted.



VIII



IX

a) R = $-CH_2-CH_2-N(CH_3)_2$

b) R = $-CH_2-N(CH_3)-CH(CH_3)_2$

c) R = $-CH_2-N(C_2H_5)-CH(CH_3)_2$

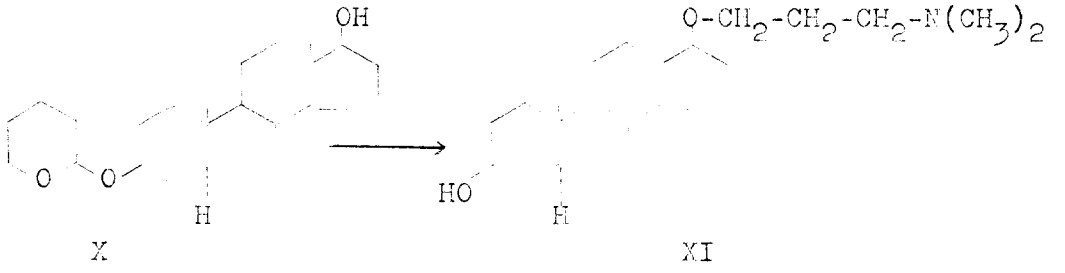
d) R = $-CH_2-N(C_2H_5)_2$

e) R = $-CH_2-N(CH_3)_2$

f) R = $-CH_2-CH(CH_3)_2$

g) R = $-CH(CH_3)_2$

Androstan-3ß,17ß-diol 3-tetrahydropyranyl ether (X) [15] prepared by catalytic hydrogenation from the corresponding 17-ketone, was reacted with 3-bromopropyl-dimethylamine to produce a corresponding cholestane analogue, 20-oxa-21-nor-25-azacholestan-3ß-ol (XI), after hydrolysis.



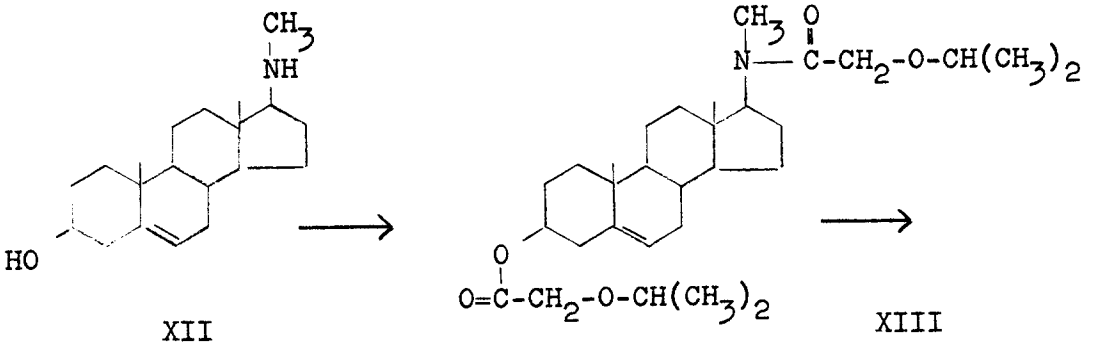
X XI

20-Aza-24-oxacholesterol was synthesized starting from 17ß-methylaminoandrost-5-en-3ß-ol (XII). The latter compound, described by Counsell and coworkers [2], was readily prepared according to the process used by Ciba [16] in the pregnane series by reaction of 3ß-hydroxyandrost-5-en-17-one acetate with methylamine in a sealed tube and reduction of the imine with sodium borohydride. Esterification of XII with isopropyloxyacetyl chloride [17] produced the diester XIII the reduction of which with lithium aluminium hydride gave 20-aza-24-oxacholesterol (XIVa). The 3-hydroxy group could be substituted by chlorine (XIVb) with thionyl chloride. XIVa was hydrogenated under pressure to 20-aza-24-oxacholestan-3ß-ol (XVa), which was converted into the 3-chloro compound XVb by thionyl chloride [x].

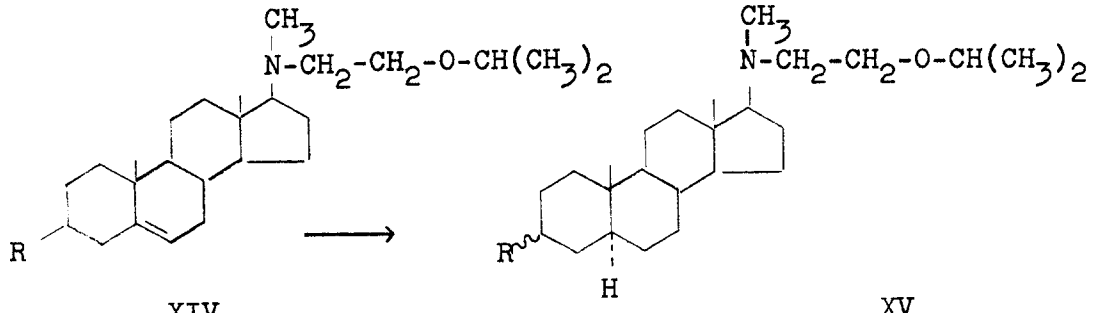
It was of interest to determine whether the sterically similar cholesterol analogues bearing the side chain at C-16 instead of C-17 would exhibit similar biological properties. For the synthesis of these compounds 3ß-hydroxyandrost-5-en-16-one [18, 19] (XVIa) was protected as the tetrahydropyranyl ether (XVIb), the keto group reduced with sodium borohydride,

---

x) Judging from the position of the hydrogen atom at C-3 the chlorine probably possesses the 3α-configuration. For comparison see: N.S. Bhacca and D.H. Williams, Applications of NMR Spectroscopy in Organic Chemistry, Holden-Day, San Francisco, 1964, p. 74, table 4-2.

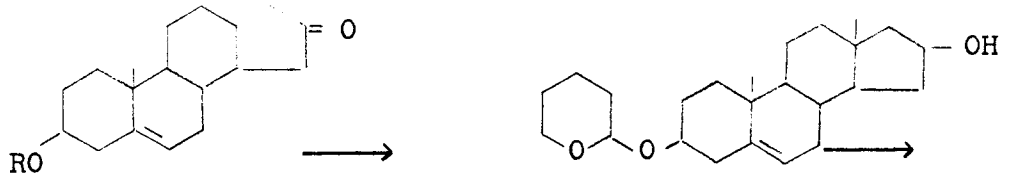


XII XIII



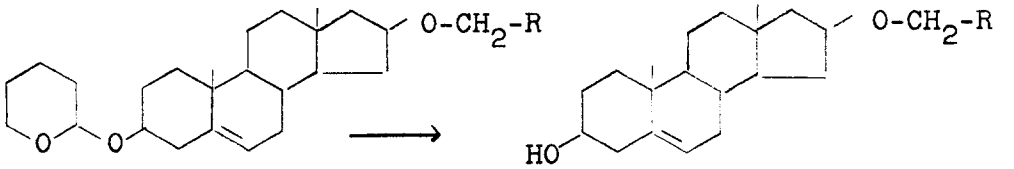
XIV XV

a.) R = ß-OH
b.) R = α-Cl



XVI a) R = H
b) R =

XVII



XVIII XIX

a) R = $-CH_2-N(CH_3)_2$

b) R = $-CH_2-CH_2-N(CH_3)_2$

and XVII etherified with 2-chloroethyl-dimethylamine or 3-chloro-propyl-dimethylamine. Splitting of the resulting tetrahydro-pyranyl ethers XVIIIa and b gave the basic ethers XIXa and XIXb.

The synthesized compounds were investigated during a 10-day assay in normal rats and rats fed with 6-propylthiouracil at a dose of 10 mg daily for their effect of changing the serum cholesterol level. Determination was made

a) by photometric measurement of the color reaction according to Liebermann-Burchard of the chloroform-methanol extract obtained from the serum as compared to cholesterol.

b) By gas-chromatographic determination of the relation of cholesterol to desmosterol after previous saponification of the serum with alkali and isolation of the total sterol by preparative layer chromatography[20-21] on silica gel.

c) By thin layer chromatographic evaluation of the total sterol after previous saponification of the serum with alkali on silica gel layers impregnated with silver nitrate.

In all preparations investigated, including 20,25-diaza-cholesterol, there was a parallilism between the photometri lowering of the serum sterol level determined photometrically according to a) and the desmosterol portion found by gas chromatography. The oxa-compounds where C-25 was replaced by nitrogen, in particular compounds IVa, IXa, and XI, showed a desmosterol portion of more than 50 per cent of the serum sterols. In the 20-oxa-21-nor-24-aza derivatives, especially compounds IXd and IXe, the desmosterol portion was slightly lower, but still considerable. These findings are parallel to those obtained in the mono-aza-cholesterol series [3,22]. Of these, the 25- and 24-aza-cholesterol also are the most active compounds. Thus, our results demonstrate that 25- and 24-aza-cholesterols and related compounds are highly potent hypocholesterolemic agents, regardless whether C-20 or

C-22 are present as carbon atoms or whether they are replaced by oxygen or nitrogen. These substances therefore produce an absolute reduction of cholesterol, while the total sterol level remains largely constant. The presumable lowering of total sterols in the Liebermann-Burchard reaction is mainly due to the remission values of desmosterol which are 60 per cent less compared to cholesterol.

The animal experiments were conducted by Dr. Voege and Dr. Freisberg, Medical Research Laboratories of E. Merck AG, the gas-chromatographic determinations by Dr. Ripphahn, Chemical Research Laboratories of E. Merck AG, Darmstadt.

EXPERIMENTAL [23]

3ß-Hydroxyandrost-5-en-16-one tetrahydropyranyl ether (XVIb). - A solution of 1.8 g. of 3ß-hydroxyandrost-5-en-16-one (XVIa) in 26 ml. of dihydropyran was treated with a drop of concentrated hydrochloric acid and refluxed for 90 min. After addition of a potassium hydroxide pellet to the reaction mixture the solvent was evaporated in vacuo, the residue dissolved in ether, and filtered. Upon concentration 1.2 g. of the tetrahydropyranyl ether (XVIb) crystallized, m.p. 177-178°.

Anal. Calcd. for $C_{24}H_{36}O_3$ (372.6): C, 77.3; H, 9.7. Found: C, 77.1; H, 9.8.

Pregn-5-en-3ß,20α-diol 3-tetrahydropyranyl ether (II). - To a solution of 43.8 g. of 3ß-hydroxypregn-5-en-20-one 3-tetrahydropyranyl ether (I) in 4.4 l. of boiling absolute ethanol 220 g. of sodium were added within 1.5 hr. in portions which kept the solution refluxing. Then 1.5 l. of ethanol was distilled off, the remaining part cooled, and poured into 8 l. of iced water. The crude product obtained by following the usual work-up with chloroform was combined with that of a second run of equal size and chromatographed with petroleum ether/benzene (3:7) on 1.42 kg. of alumina. Elution with benzene and benzene/chloroform (9:1) yielded 25.3 g. of a mixture of I with the 20ß-isomer of II and little II. Benzene/chloroform (1:1) eluted 37.1 g. of II containing about 5 per cent of the 20ß-isomer and finally 11.3 g. of II together with more polar products (3-hydroxy compounds). Recrystallization of the main fraction from acetone gave 20.2 g. of pure II (23 %), m.p. 143-147°; $[\alpha]_D^{24}$ - 46°; IR-band at 3500/cm (OH); no CO-band.

Anal. Calcd. for $C_{26}H_{42}O_3$ (402,6): C, 77.6; H, 10.5. Found: C, 77.5; H, 10.4.

Another 5.9 g., m.p. 113-115°, crystallized from the mother liquor.

Using the same procedure 3ß-hydroxy-5α-pregnan-20-one 3-tetrahydropyranyl ether (V) was reduced to 5α-pregnan-3ß,20α-diol 3-tetrahydropyranyl ether (VI), m.p. 135-147°. The yield after one recrystallization of the chromatographically purified fraction from acetone was 22 %. An analytical sample melted at 152-159°; $[\alpha]_D^{20}$ + 29; IR-band 3450/cm (OH); no CO-band; NMR: 4.7 (-O-CH-O-); 1.25 and 1.15 (21); 0.8 (19); 0.65 (18).

Anal. Calcd. for $C_{26}H_{44}O_3$ (404,6): C, 77.2; H, 11.0. Found: C, 76.5; H, 10.7.

Androst-5-en-3ß,17ß-diol 3-tetrahydropyranyl ether (VIII). 3ß-Hydroxyandrost-5-en-17-one 3-tetrahydropyranyl ether (72.5 g.) was hydrogenated in 9 l. of methanol with 65 g. of methanol wet Raney-nickel until the reaction came to a standstill. After 42 hr. 5.24 l. of hydrogen were consumed. After removal of the catalyst and concentration of the filtrate 67 g. (92 %) of VIII crystallized, m.p. 160-162°. An analytical sample melted at 162-164°. (Ott and coworkers [6] obtained the product after reduction with lithium aluminium hydride with a melting point of 161-162°, corr.). The mother liquor yielded additional 2.7 g., m.p. 150-152°.

Accordingly 3ß-hydroxyandrostan-17-one 3-tetrahydropyranyl ether was reduced to 5α-androstan-3ß,17ß-diol 3-tetrahydropyranyl ether (X) with a yield of 78 %, m.p. 164-166°. A pure sample melted at 175-177°. (Marquet and coworkers [15] found 168-169.5°, corr., after reduction with potassium borohydride.)

Androst-5-en-3ß,16ß-diol 3-tetrahydropyranyl ether (XVII). The 16-keto compound XVIb (1.4 g.) was dissolved in 100 ml. of dioxane, treated with sodium borohydride (1.4 g.) and ethylenediaminetetraacetic acid (0.4 g.; Titriplex II of E. Merck AG, Darmstadt) and stirred for 45 min. at room temperature. The reaction mixture was filtered, diluted with water, adjusted to pH 7.2 with potassium sodium tartrate, and extracted with ether. The extract yielded the crude 3.16-diol 3-tetrahydropyranyl ether which crystallized from n-hexane with a melting point of 131-133° (yield 1.1 g.).

22-Oxa-25-azacholesterol 3-tetrahydropyranyl ether (IIIa). - Liquid ammonia (100 ml.) was dried with metallic potassium, then treated with a trace of ferric nitrate, and 1.6 g. of finely cut potassium within 1 hr. After stirring at - 70° for 1 hr. a solution of 16.1 g. of pregn-5-en-3ß,20α-diol 3-tetrahydropyranyl ether (II) in 50 ml. of absolute tetrahydrofurane was added dropwise in 20 min. After stirring at - 10° for another hour the ammonia was evaporated by a stream of nitrogen

through a drying tube. The remaining solvent was removed in vacuo at room temperature. To the residue dissolved in 50 ml. of absolute dioxane a solution of 8.5 g. freshly distilled 2-chloroethyl-dimethylamine in 10 ml. of absolute dioxane was added dropwise and refluxed for 2 hr. After the addition of another 4.3 g. of 2-chloroethyl-dimethylamine in 5 ml. of absolute dioxane the reaction mixture was refluxed for 3 hr., then cooled, the potassium chloride filtered off, and the solvent evaporated. Chromatography on 160 g. of silica gel gave 9.65 g. of the starting material with benzene/chloroform (1:1) as eluting agents; with chloroform/methanol/triethylamine (100:10:1) 7.55 g. of IIIa were obtained. One recrystallization from acetone yielded 4.94 g. of pure IIIa, m.p. 104°. The analytical sample melted at 106-108°. The IR-spectrum showed no OH-band. NMR: 5.44 (6); 4.77 (-O-CH-O-); 6 protons next to O between 4.2 and 3.1; 2.49 (triplet, CH_2-N); 2.29 ($-N(CH_3)_2$); 1.23 and 1.13 (21); 1.03 (19); 0.67 (18).

Anal. Calcd. for $C_{30}H_{51}NO_3$ (473,7): C, 76.1; H, 10.9; N, 3.0. Found: C, 75.6; H, 10.9; N, 2.8.

Accordingly were prepared :

22-Oxa-25-azacholestan-3ß-ol 3-tetrahydropyranyl ether by reacting VI with 2-chloroethyl-dimethylamine, m.p. 83-85° (from acetone); $[\alpha]_D^{20}$ + 16°; NMR: 4.65 (-O-CH-O-), 6 protons next to O between 4.2 and 3.0; 2.62 (triplet, CH_2-N); 2.39 ($-N(CH_3)_2$); 1.19 and 1.09 (21); 0.78 (19); 0.62 (18).

Anal. Calcd. for $C_{30}H_{53}NO_3$ (475,8): C, 75.7; H, 11.2; N, 2.9; O, 10.1. Found: C, 75.2; H, 11.4; N, 3.3; O, 10.7.

20-Oxa-21-nor-24-methyl-24-azacholesterol 3-tetrahydropyranyl ether by reacting VIII with 2-chloroethyl-methyl-isopropylamine, m.p. 131-133° (from acetone); $[\alpha]_D^{20}$ - 36°; NMR: 5.4 (6); 4.77 (-O-CH-O-); 2.58 (triplet, CH_2-N); 2.27 ($N-CH_3$); 1.06 and 0.95 ($-CH(CH_3)_2$); 1.02 (19); 0.77 (18).

Anal. Calcd. for $C_{30}H_{51}NO_3$ (473.7): C, 76.1; H, 10.9; N, 3.0. Found: C, 76.2; H, 10.9; N, 2.9; 3.3.

20-Oxa-21-nor-24-ethyl-24-azacholesterol 3-tetrahydropyranyl ether by reacting VIII with 2-chloroethyl-ethyl-isopropylamine, m.p. 104-107° (from acetone); $[\alpha]_D^{20}$ - 34°; NMR: 5.4 (6); 4.78 (-O-CH-O-); 1.06 and 0.94 ($-CH(CH_3)_2$); 1.03 (19 and triplet $-CH_2-CH_3$); 0.77 (18).

Anal. Calcd. for $C_{31}H_{53}NO_3$ (487.8): C, 76.3; H, 11.0; N, 2.9. Found: C, 76.3; H, 11.0; N, 3.2.

20-Oxa-21,27-bisnor-24-ethyl-24-azacholesterol 3-tetrahydropyranyl ether by reacting VIII with 2-chlorotriethylamine, m.p. 100-103° (from acetone); $[\alpha]_D^{22}$ - 29°. NMR: 5.42 (6); 4.74 (-O-CH-O-); 1.02 (19 and triplet $-CH_2-CH_3$); 0.75 (18).

Anal. Calcd. for $C_{30}H_{51}NO_3$ (473,7): C, 76.1; H, 10.9; N, 3.0. Found: C, 76.6; H, 10.9; N, 2.6.

20-Oxa-21.24-bisnor-25-azacholesterol 3-tetrahydropyranyl ether by reacting VIII with 2-chloroethyl-dimethylamine, m.p. 98-102° (from acetone); $[\alpha]_D^{24}$ - 31°; NMR: 5.4 (6); 4.72 (-O-CH-O-); 2.47 (triplet, CH_2-N); 2.27 ($-N(CH_3)_2$); 1.01 (19); 0.76 (18).

Anal. Calcd. for $C_{28}H_{47}NO_3$ (445,7): C, 75.5; H, 10.6; N, 3.1. Found: C, 75.0; H, 10.6; N, 3.3.

20-Oxa-21-nor-25-azacholesterol 3-tetrahydropyranyl ether. -
A dispersion of sodium hydride (73 g.; 20 % in mineral oil) diluted with 900 ml. of absolute xylene was added to a solution of 35 g. of VIII in 1.1 l. of absolute xylene, and refluxed for 90 min. The cooled mixture was treated with 75 g. of 3-bromopropyl-dimethylammoniumbromide, and refluxed for 4 hr. Then 73 g. of a sodium hydride dispersion diluted with 250 ml. of absolute xylene was added, and refluxing continued for another period of 4 hr. After decomposing with iced water the reaction mixture was worked up with chloroform and water as usually. The crude product was chromatographed in benzene on 350 g. of silica gel. Elution with benzene/chloroform (8:2) gave an unpolar by-product and starting material, with chloroform/methanol/triethylamine (100:1:1) 38.5 g. of the desired compound. A sample was recrystallized from acetone, m.p. 68-71°; $[\alpha]_D^{24}$ - 38°; NMR: 5.43 (6); 4.76 (-O-CH-O-); 2.24 ($-N(CH_3)_2$); 1.03 (19); 0.76 (18).

Anal. Calcd. for $C_{29}H_{49}NO_3$ (459,7): C, 75.8; H, 10.7; N, 3.1. Found: C, 75.7; H, 10.7; N, 2.9.

Accordingly were prepared

20-Oxa-21-nor-25-azacholestan-3ß-ol 3-tetrahydropyranyl ether from X. M.p. after recrystallization from acetone 63-65°; $[\alpha]_D^{20}$ + 4°; NMR: 4.67 (-O-CH-O-); 2.33 ($-N(CH_3)_2$); 0.81 (19); 0.73 (18).

Anal. Calcd. for $C_{29}H_{51}NO_3$ (461.7): C, 75.4; H, 11.2; N, 3.0. Found: C, 75.7; H, 11.4; N, 2.9; 3.1.

Compound XVII was reacted in the same way. The obtained tetrahydropyranyl ethers XVIIIa (with 2-chloroethyl-dimethylamine) and XVIIIb (with 3-chloropropyl-dimethylamine) were purified chromatographically on basic alumina but not isolated crystalline. Refluxing with 5 % aqueous alcoholic hydrochloric acid resulted in the crystalline hydrochlorides XIXa and XIXb, respectively.

16ß-(2'-Dimethylaminoethoxy)-androst-5-en-3ß-ol hydrochloride, m.p. 265-268° (from methanol/acetone).

Anal. Calcd. for $C_{24}H_{42}ClNO_2$ (412,1): C, 69.4; H, 10.1; Cl, 8.9; N, 3.5. Found: C, 69.1; H, 10.3; Cl, 8.6; N, 3.9.

16ß-(3'-Dimethylaminopropoxy)-androst-5-en-3ß-ol hydrochloride, m.p. 258-260° dec. (from acetone/chloroform).

Anal. Calcd. for $C_{25}H_{44}ClNO_2$ (426,1): C, 69.6; H, 10.3; Cl, 8.6; N, 3.4. Found: C, 69.5; H, 10.1; Cl, 9.0; N, 3.9.

22-Oxa-cholesterol (IVc). -
A solution of 6.0 g. of pregn-5-en-3ß,20α-diol 3-tetrahydropyranyl ether (II) in 300 ml. of xylene was given into 35 g. of a 20 % sodium hydride dispersion in mineral oil and 160 ml. of xylene and refluxed in a nitrogen atmosphere for 2 hr. Then a solution of 45 g. of isoamylbromide and 32 ml. of triethylamine in 240 ml. of xylene was added, refluxed for another hour, and poured into 1 l. of water. The organic phase was separated, the aqueous layer extracted 3 times with 200 ml. portions of chloroform, and the combined organic phases washed twice with a little water and dried over sodium sulfate. After evaporation of the solvent the residue was dissolved in 20 ml. of chloroform, applied as a band on 2 glass plates (1 m x 20 cm) covered with a 2 mm layer of silica gel (E. Merck AG, $PF_{254+366}$), and developed twice in chloroform and in a mixture of petroleum ether/chloroform (40:60). Three bands were visible in the UV light of 366 mμ by brightening of the blue fluorescence. The upper zone was identified as 22-oxacholesterol (IIIb), the middle as a byproduct, 3ß-hydroxy-pregn-5-en-20-one 3-tetrahydropyranyl ether presumably, and the lower zone as nonreacted starting material II. The upper and the lower band was eluted with a mixture of chloroform/methanol (90:10) to give 3.2 g. of IIIb and 2.0 g. of II. The crude 22-oxacholesterol 3-tetrahydropyranyl ether (3.2 g., IIIb) was dissolved in 32 ml. of ether, treated with 0.25 ml. of concentrated hydrochloric acid in 32 ml. of methanol, and left 1 hr. at room temperature. After addition of 320 ml. of ether the ethereal solution was washed neutral with several portions of water, dried over sodium sulfate, and evaporated. The residue was crystallized from methanol to give 1.9 g. of 22-oxacholesterol (IVc) with a m.p. at 140° after another recrystallization from methanol.
NMR: 5.33 (6); 4 hydrogens next to O between 3.9 and 2.9; 1.18 and 1.08 (21); 1.00 (19); 0.94 and 0.84 (26, 27); 0.66 (18).

Anal. Calcd. for $C_{26}H_{44}O_2$ (388,6): C, 80.4; H, 11.4. Found: C, 80.2; H, 11.3.

22-Oxa-24-norcholesterol (IVd). -
The reactions were carried out under the conditions described for the synthesis of 22-oxacholesterol (IVc) with 12.3 g. of pregn-5-en-3ß,20α-diol 3-tetrahydropyranyl ether (II) and 86 g. of isobutyl bromide as starting materials. After preparative layer chromatography 3 bands could be scraped off the upper of which contained 22-oxa-24-norcholesterol 3-tetrahydropyranyl ether (2.7 g., IIIc), the middle a byproduct, 3ß-hydroxypregn-5-en-20-one 3-tetrahydropyranyl ether presumably, and the lower nonreacted starting material II

(6.0 g.). Cleavage of the tetrahydropyranyl ether (IIIc) and crystallization of the crude product from methanol yielded 1.1 g. of 22-oxa-24-norcholesterol (IVd). The melting point was at 122° after a further recrystallization. NMR: 5.3 (6); 4 hydrogens next to O between 3.8 and 2.7; 1.18 and 1.08 (21); 1.00 (19); 0.94 and 0.83 (26, 27); 0.67 (18).

Anal. Calcd. for $C_{25}H_{42}O_2$ (374,6): C, 80.2; H, 11.3. Found: C, 80.2; H, 11.5.

20-Oxa-21,24-bisnorcholesterol (IXf). - Androst-5-en-3ß,17ß-diol 3-tetrahydropyranyl ether (15.0 g., VIII) was reacted with 120 g. of isoamyl bromide according to the procedure described for the preparation of IVc. Preparative layer chromatography separated 3 compounds. After elution 20-oxa-21,24-bisnorcholesterol 3-tetrahydropyranyl ether (9.7 g.) was isolated from the upper band, 3ß-hydroxy= androst-5-en-17-one 3-tetrahydropyranyl ether from the middle identified by comparison of its melting point, TLC, and IR-spectrum with an authentic specimen, and nonreacted starting material (3.5 g., VIII) from the lower band. Removal of the tetrahydropyranyl rest and crystallization of the crude material from methanol gave 4.7 g. of 20-oxa-21,24-bisnorcholesterol (IXf) having a m.p. at 128° after another recrystallization from the same solvent.
NMR: 5.36 (6); 3.56, 3.45, and 3.34 (22); 1.02 (19); 0.94 and 0.84 (26, 27); 0.76 (18).

Anal. Calcd. for $C_{24}H_{40}O_2$ (360,6): C, 79.9; H, 11.2. Found: C, 80.1; H, 11.2.

20-Oxa-21,23,24-trisnorcholesterol (IXg). - Reaction of 15.0 g. of androst-5-en-3ß,17ß-diol 3-tetrahydro= pyranyl ether (VIII) with 112 g. of isobutyl bromide and work-up as described for compound IVc yielded 20-oxa-21,23,24-trisnorcholesterol 3-tetrahydropyranyl ether (3.0 g.), 3ß-hydroxyandrost-5-en-17-one tetrahydropyranyl ether, and starting material (11.0 g., VIII). 20-Oxa-21,23,24-trisnor= cholesterol (1.7 g., IXg) was obtained by cleaving its tetrahydropyranyl ether and by two crystallizations from methanol with a m.p. at 131°.
NMR: 5.3 (6); 3.22 (22); 1.02 (19); 0.93 and 0.83 (26, 27); 0.76 (18).

Anal. Calcd. for $C_{23}H_{38}O_2$ (346,6): C, 79.7; H, 11.1. Found: C, 79.6; H, 11.1.

22-Oxa-25-azacholesterol (IVa). - IIIa (3.35 g.) was refluxed in ethanolic hydrochloric acid (5 %, 67 ml.) for 3 hr., poured into water, treated with 3 ml. of a 7 % solution of sodium bicarbonate, and worked up with chloroform. The crude product (2.7 g.) was crystallized from acetone to give 2.35 g. IVa, m.p. 158-161°. An analytical sample recrystallized from methanol/acetone

melted at 161-162°; $[\alpha]_D^{24}$ - 30°; NMR: 5.45 (6); 4 hydrogens next to O between 4.0 and 2.9; 2.53 (triplet, CH_2-N); 2.32 ($-N(CH_3)_2$); 1.23 and 1.13 (21); 1.03 (19); 0.69 (18).

Anal. Calcd. for $C_{25}H_{43}NO_2$ (389,6): C, 77.1; H, 11.1; N, 3.6. Found: C, 76.6; H, 10.8; N, 3.9; 3.0.

Accordingly were obtained from the corresponding tetrahydropyranyl ethers :

22-Oxa-25-azacholestan-3ß-ol (VII), double m.p. 135-136° and 144-145° (from acetone); $[\alpha]_D^{20}$ + 34°; NMR: 2.56 (triplet, CH_2-N); 2.32 ($-N(CH_3)_2$); 1.19 and 1.09 (21); 0.79 (19); 0.63 (18).

Anal. Calcd. for $C_{25}H_{45}NO_2$ (391,7): C, 76.7; H, 11.6; N, 3.6; O, 8.2. Found: C, 76.1; H, 11.7; N, 4.3; O, 8.6.

20-Oxa-21-nor-25-azacholesterol (IXa), m.p. 153-154° (from acetone); $[\alpha]_D^{24}$ - 48°; NMR: 5.4 (6); 3.50 (triplet, O-CH_2); 2.24 ($-N(CH_3)_2$); 1.03 (19); 0.66 (18).

Anal. Calcd. for $C_{24}H_{41}NO_2$ (375,6): C, 76.8; H, 11.0; N, 3.7. Found: C, 76.2; H, 10.8; 11.2; N, 3.6; 3.8.

Hydrochloride, m.p. 282-285° (from methanol/acetone).

20-Oxa-21-nor-24-methyl-24-azacholesterol (IXb), m.p. 117-119° (from acetone), $[\alpha]_D^{20}$ - 51°; NMR: 5.4 (6); 2.61 (triplet, CH_2-N); 2.29 (-N-CH_3); 1.0 and 0.98 (26, 27); 1.03 (19); 0.77 (18).

Anal. Calcd. for $C_{25}H_{43}NO_2$ (389,6): C, 77.1; H, 11.1; N, 3.6. Found: C, 76.9; H, 11.3; N, 3.8.

20-Oxa-21-nor-24-ethyl-24-azacholesterol (IXc), m.p. 87-89° (from acetone); $[\alpha]_D^{20}$ - 45°.

Anal. Calcd. for $C_{26}H_{45}NO_2$ (403,7): C, 77.4; H, 11.2; N, 3.5. Found: C, 77.0; H, 11.3; N, 3.8.

20-Oxa-21,27-bisnor-24-ethyl-24-azacholesterol (IXd), m.p. 100-103° (from acetone); $[\alpha]_D^{20}$ - 51°; NMR: 5.37 (6); 4 hydrogens next to O between 3.9 and 3.1; 1.03 (19 and triplet -CH_2-CH_3); 0.76 (18).

Anal. Calcd. for $C_{25}H_{43}NO_2$ (389.6): C, 77.1; H, 11.1; N, 3.6. Found: C, 76.5; H, 11.2; N, 3.6.

20-Oxa-21,24-bisnor-25-azacholesterol (IXe), m.p. 158-161° (from acetone); $[\alpha]_D^{24}$ - 52°; NMR: 5.38 (6); 3.57 (triplet, O-CH_2); 2.49 (triplet, CH_2-N); 2.27 ($-N(CH_3)_2$); 1.02 (19); 0.77 (18).

Anal. Calcd. for $C_{23}H_{39}NO_2$ (361,6): C, 76.4; H, 10.9; N, 3.9. Found: C, 76.3; H, 11.2; N, 4.0; 3.7.

20-Oxa-21-nor-25-azacholestan-3ß-ol (XI), m.p. 137-138° (from acetone); $[\alpha]_D^{20}$ + 8°; NMR: 3.43 (triplet, O-CH_2); 2.33 (triplet, CH_2-N); 2.18 (-N(CH_3)$_2$); 0.80 (19); 0.73 (18).

Anal. Calcd. for $C_{24}H_{43}NO_2$ (377,6): C, 76.4; H, 11.5; N, 3.7. Found: C, 76.4; H, 11.7; N, 3.5.

17ß-Methyliminoandrost-5-en-3ß-ol. - A solution of sodium methoxide prepared from 1.5 g. of sodium and 25 ml. of absolute methanol was added to the suspension of 3ß-acetoxyandrost-5-en-17-one (5 g.) in 50 ml. of absolute methanol whereby the substance dissolved. After addition of methylamine (7 ml.) at - 50° the solution was heated in a sealed tube at 90° for 7 hr. The reaction mixture was poured into water and extracted with chloroform. The washed and dried extract was evaporated to dryness in vacuo and the residue crystallized from ether to yield 4.5 g. of 17ß-methyl=iminoandrost-5-en-3ß-ol, m.p. 221-223°.

Anal. Calcd. for $C_{20}H_{31}NO$ (301,5): C, 79.6; H, 10.3; N, 4.7. Found: C, 79.3; H, 10.1; N, 4.6.

17ß-Methylaminoandrost-5-en-3ß-ol (XII). - To a suspension of 5 g. of the foregoing imine in 150 ml. of absolute ether 4 g. of sodium borohydride in 100 ml. of dry tetrahydrofuran was added dropwise under stirring in 15 min. After 1 hr. reflux methanol, water, and potassium sodium tartrate were added and the mixture was well shaken. The organic layer was separated and the aqueous phase extracted with ether. The combined organic phases were washed neutral, dried, and concentrated. 17ß-Methylaminoandrost-5-en-3ß-ol crystallized from ether in a 95 % yield, m.p. 203-204°; $[\alpha]_D^{22}$ - 59.0°.

Anal. Calcd. for $C_{20}H_{33}NO$ (303,5): C, 79.2; H, 10.9; N, 4.6. Found: C, 79.2; H, 10.9; N, 4.5.

17ß-Methylaminoandrost-5-en-3ß-ol 3,17-di-(isopropyl=oxyacetate) (XIII). - XII (14.45 g.) was dissolved in 120 ml. of absolute benzene, cooled with ice, then after addition of 12.2 g. of triethyl=amine treated dropwise with a solution of 32.6 g. of isopropyl=oxyacetyl chloride in 30 ml. of absolute benzene. The reaction mixture was refluxed under exclusion of moisture, then cooled, poured into 1.5 l. of a 5 % sodium bicarbonate solution, and worked up with benzene and water as usually. The crude product was chromatographed in benzene on 450 g. of silica gel. A mixture of benzene/chloroform (8:2) eluted 0.3 g. of 3ß-chloro-17ß-methyl=aminoandrost-5-ene N-isopropyloxyacetate, m.p. 115-117° (from ether); IR: 1650/cm (N-C=O); NMR: 5.38 (6); 4.5 (broad, 3); 4.18

(CO-CH_2-O); 2.95 (N-CH_3); 1.26 and 1.16 (> $C(CH_3)_2$); 1.04 (19); 0.76 (18).

Anal. Calcd. for $C_{25}H_{39}ClNO_2$ (421,1): Cl, 8.4; N, 3.3. Found: Cl, 8.2; N, 3.1.

It was followed by a mixture (5 g.) of this substance with 17ß-methylaminoandrost-5-en-3ß-ol 3-chloroacetate-N-isopropyloxyacetate, then by 1.5 g. of the pure foregoing compound, m.p. 201-203° (from acetone); IR: 1750 ($ClCH_2$-C=O); 1660 and 1630/cm (N-C=O); NMR: 5.4 (6); 4.7 (broad, 2); 4.19 and 4.04 (CO-CH_2); 2.94 (N-CH_3); 1.26 and 1.15 (> $C(CH_3)_2$); 1.03 (19); 0.76 (18).

Anal. Calcd. for $C_{27}H_{41}ClNO_4$ (479.1): C, 67.7; H, 8.6; Cl, 7.4; N, 2.9; O, 13.4. Found: C, 67.5; H, 9.0; Cl, 7.4; N, 2.9; O, 13.9.

The next eluted fraction (3 g.) contained the mixed ester and the desired compound XIII, finally 9.7 g. of pure XIII. Crystallization from ether yielded 6.9 g. of XIII, m.p. 120-122°. A further recrystallization raised the m.p. to 124-126°; $[\alpha]_D^{24}$ - 102°.

Anal. Calcd. for $C_{30}H_{49}NO_5$ (503,7): C, 71.5; H, 9.8; N, 2.8; O, 15.9. Found: C, 71.3; H, 9.8; N, 2.7; O, 15.6.

20-Aza-24-oxacholesterol (XIVa). - To a cooled suspension of lithium aluminium hydride (1.56 g.) in 70 ml. of absolute ether a solution of XIII (6.7 g.) in absolute ether (100 ml.) was added dropwise. After 5 hr. reflux the mixture was decomposed with water (20 ml.) and worked up with ether. Concentration of the dried ethereal phase deposited 0.38 g. of a substance, m.p. 180-186°, raised to 198-201° by recrystallization. The structure of 17ß-methylaminoandrost-5-en-3ß-ol N-isopropyloxyacetate was derived from the spectral data. IR: 3400 (OH); 1640/cm (N-C=O); NMR: 5.37 (6); 4.15 (CO-CH_2-O); 2.93 (N-CH_3); 1.24 and 1.14 (> $C(CH_3)_2$); 1.01 (19); 0.75 (18).

The mother liquor was chromatographed in petroleum ether/ether (3:2) on 150 g. of neutral alumina to give 3.6 g. of XIVa recrystallized from petroleum ether (3.2 g.), m.p. 87-89°; $[\alpha]_D^{22}$ - 59°. The IR-spectrum showed no CO-band. NMR: 5.37 (6); 4 hydrogens next to O between 4.0 and 3.0; 2.29 (N-CH_3); 1.19 and 1.09 (26, 27); 1.00 (19); 0.79 (18).

Anal. Calcd. for $C_{25}H_{43}NO_2$ (389.6): C, 77.1; H, 11.1; N, 3.6. Found: C, 77.1; H, 11.1; N, 3.6.

20-Aza-24-oxacholestan-3ß-ol (XVa). - XIVa (1.5 g.) was hydrogenated in glacial acetic acid (80 ml.) with platinum dioxide (0.5 g.) at 7 kg/cm^2 and 62° until no more hydrogen was taken up. The catalyst was filtered and the solvent distilled off. Basic alumina (5 g.) was added to the crude material dissolved in chloroform. Evaporation of the solvent

left the adsorbate which was placed in petroleum ether on the top of a column prepared from 45 g. of basic alumina. Elution with petroleum ether yielded 270 mg. of XVa-3-acetate, m.p. 65-69° (from acetone). IR-bands at 1730, 1270, and 1245/cm, no OH-band; NMR: 4.75 (3); 3 hydrogens next to O between 4.0 and 3.1; 2.30 ($N-CH_3$); 2.05 ($COCH_3$); 1.21 and 1.11 (26, 27); 0.82 (19); 0.78 (18).

Further elution with mixtures of petroleum ether/ether (4:1 to 2:3) produced 970 mg. of XVa, m.p. 103-105° (from petroleum ether). NMR: 4 hydrogens next to O between 4.1 and 3.1; 2.27 ($N-CH_3$); 1.19 and 1.09 (26, 27); 0.78 (18, 19).

3ß-Chloro-22-oxa-25-azacholest-5-ene (IVb). - A mixture of 2.35 g. of IVa and 2.35 g. of dry calcium carbonate in 95 ml. of absolute ether and 47 ml. of absolute tetrahydrofuran was treated dropwise with 47 ml. of thionyl-chloride at 15°, then stirred at room temperature for 4 hr., and filtered. The residue was washed with ether and the combined filtrates were evaporated in vacuo to dryness. The remainder was taken up in chloroform, the solution washed with aqueous sodium bicarbonate and water, dried, filtered, and concentrated. The crude product was purified by chromatography on 110 g. of basic alumina with petroleum ether/chloroform (2:1) to give 1.8 g. of IVb. After recrystallization from acetone the melting point was at 97-98°; $[\alpha]_D^{20}$ - 22°; IR-spectrum without OH-band; NMR: 5.38 (6); 2.47 (CH_2-N) (triplet); 2.27 ($-N(CH_3)_2$); 1.22 and 1.11 (21); 1.03 (19); 0.66 (18).

Anal. Calcd. for $C_{25}H_{42}ClNO$ (408,1): C, 73.6; H, 10.4; Cl, 8.4; N, 3.4. Found: C, 73.7; 73.4; H, 10.6; Cl, 8.4; N, 4.1.

Accordingly were prepared

3α-Chloro-20-aza-24-oxacholestane (XVb) from XVa, m.p. 65-67° (from acetone); $[\alpha]_D^{20}$ + 19°; NMR: 4.47 (3); 2.27 ($N-CH_3$); 1.18 and 1.09 (26, 27); 0.77 (18, 19).

Anal. Calcd. for $C_{25}H_{44}ClNO$ (410,1): C, 73.2; H, 10.8; Cl, 8.7; N, 3.4. Found: C, 73.7; H, 10.8; Cl, 8.4; N, 3.3.

3ß-Chloro-20-aza-24-oxacholest-5-ene (XIVb) from XIVa. The chromatographically purified product was converted into its hydrochloride by adding ethereal hydrochloric acid to the ethereal solution of XIVb. M.p. after recrystallization from chloroform/ether was at 239-240°; $[\alpha]_D^{20}$ - 33°.

Anal. Calcd. for $C_{25}H_{43}Cl_2NO$ (444.6): C, 67.6; H, 9.8; Cl, 16.0; N, 3.2. Found: C, 67.5; H, 9.9; Cl, 16.0; N, 3.1.

REFERENCES

1. Counsell, R.E., Klimstra, P.D., Ranney, R.E., and Cook, D.L., J.MED.PHARM.CHEM., 5, 720 (1962).
2. Counsell, R.E., Klimstra, P.D., and Ranney, R.E., IBID, 5, 1224 (1962).
3. Counsell, R.E., Klimstra, P.D., Nysted, L.N., and Ranney, R.E., J.MED.CHEM., 8, 45 (1965).
4. Sachs, B.A. and Wolfman, L., CIRCULATION, 26, 669 (1962).
5. Thompson, M.J., Dupont, J., and Robbins, W.E., STEROIDS, 2, 99 (1963).
6. Ott, A.C., Murray, M.F., and Pederson, R.L., J.AMER. CHEM.SOC., 74, 1239 (1952).
7. Hirschmann, H., Daus, M.A., and Hirschmann, F.B., J.BIOL.CHEM., 192, 115 (1951).
8. Cross, A.D., Denot, E., Acevedo, R., and Crabbé, P., STEROIDS, 5, 585 (1965).
9. See Fieser, L.F. and Fieser, M., Steroide, Verlag Chemie, Weinheim/Bergstrasse, 1961, p. 372-373.
10. Sondheimer, F., Velasco, M., and Rosenkranz, G., J.AMER.CHEM.SOC., 77, 5673 (1955).
11. Gibbs, C.F., Littmann, E.R., and Marvel, C.S., J.AMER. CHEM.SOC., 55, 753 (1933).
12. Biel, J.H., J.AMER.CHEM.SOC., 71, 1306 (1949).
13. Wright, J.B., Lincoln, E.H., Heinzelmann, R.V., and Hunter, J.H., J.AMER.CHEM.SOC., 72, 3536 (1950).
14. Slotta, K.H. and Behnisch, R., BER.DTSCH.CHEM.GES., 68, 754 (1935).
15. Marquet, A., Kagan, H.B., Dvolaitzky, M., Lematre, J., and Jacques, J., BULL.SOC.CHIM.FRANCE 1960, 539.
16. Ciba S.A., Basel, Belg.Pat. No. 586621 (1960).
17. Rothstein, B., BULL.SOC.CHIM.FRANCE IV, 51, 838 (1932).
18. Fajkoš, J. and Šorm, F., CHEM.LISTY, 47, 1207 (1953); COLLECTION CZECHOSLOV.CHEM.COMMUN., 19, 349 (1954); C.A. 49, 358c (1955).
19. Huffman, M.N., Lott, M.H., and Tillotson, A., J.BIOL.CHEM., 218, 565 (1956).
20. Halpaap, H., CHEMIE-ING.-TECHN., 35, 488 (1963).
21. Halpaap, H., CHEMIKER-ZTG., 89, 835 (1965).

22. Dutch Published Application of E. Merck AG No. 64.01648, Derwent Compl. Specific. Farm. Doc., Basic No. 13896, Vol. 277, 337 (1964), Ger. Prior. March 21, 1963; Dutch Published Application of E. Merck AG No. 64.02671, Derwent Compl. Specific. Farm. Doc., Basic No. 14014, Vol. 281, 273 (1964), Ger. Prior. April 1, 1963.

23. Melting points are uncorrected. Rotations are for chloroform solutions. Infrared spectra were determined in potassium bromide platelets on the Leitz Infrarotspektrograph. The NMR measurements were performed in deuteriochloroform with tetramethylsilane as internal standard on the Varian A 60 instrument. Peak positions are reposted in ppm downfield from tetramethylsilane. Analyses were carried out by Dr. M. Hochenegger, Analytical Laboratory of the E. Merck AG, Darmstadt.