STUDIES IN SYNTHESES OF STEROID METABOLITES. PART III.[1]

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Several ketolic and glycolic metabolites of corticosterone have been synthesized. Corticosterone acetate was hydrogenated in the presence of palladium on charcoal to afford the 5β-dihydro derivative II, in addition to the 5α compound I. Tetrahydro Kendall's Compound B (VIII) was prepared by reduction of the 20-ketal derivative of tetrahydro Kendall's Compound A (VIa). Solvolysis of the tosylate IVb furnished allo-tetrahydro Compound B (Xc). In the 5β series the two tetrols XII and XIV were synthesized by reduction of the corresponding 11-keto analogs XIa and XIIIa. In the 5α series the analogous pair of tetrols (XV and XVII) was prepared by reduction of Xa and epimerization of the ditosylate XVIb, respectively.

Corticosterone is metabolized in man to afford, among others, the two ketolic compounds allo-THB (Xc) and its 5β epimer THB (VIII).[2,3,4] In addition to ketolic compounds 20,21-glycols have been found[5] to be the metabolic products of corticosterone, the relative amounts of glycols being roughly twice the amount of ketols. While the nature of the glycols was investigated[5] only by a group determination method, it may be assumed that in qualitative analogy with the metabolism of hydrocortisone 20α and 20β-hydroxy isomers, in both the 5α and 5β series, are produced. To enable identification and a quantitative determination of the different metabolites of corticosterone we have now prepared the following reference

compounds, for none of which has a synthesis been previously described: allo-THB (Xc), THB (VIII), 5β-pregnane-3α,11β,20β,21-tetrol (XII), its 20α epimer XIV, 5α-pregnane-3α,11β,20β,21-tetrol (XV) and its 20α epimer XVII. Two other compounds of interest in this connection, namely 5β-pregnane-3α,20β,21-triol-11-one (XIa) and the corresponding 20α epimer XIIIa have been previously synthesized (vide infra). The above eight compounds are the 17-desoxy analogs of allo-THF, THF, β-cortol, cortol, β-allo-cortol, allo-cortol, β-cortolone and cortolone, respectively.

In previous papers of this series it was shown[1] that palladium on charcoal hydrogenation of the 4,5 double bond in hydrocortisone and cortisone gives rise to appreciable amounts of 5β compounds. We have now extended our study to corticosterone acetate which, when hydrogenated in ethyl acetate in the presence of palladium on barium sulfate was previously reported[6] to afford the 5α-dihydro derivative I in about 70% yield. Corticosterone was found[7] to furnish 5α-dihydrocorticosterone in 61% yield when absolute alcohol and palladium black were used. In our hands hydrogenation of corticosterone acetate in ethyl acetate in the presence of palladium on charcoal afforded a 50% yield of I and a 31% yield of the 5β isomer II, separable by fractional crystallization and chromatography.

Raney nickel hydrogenation[1,8] of II yielded the known[9] 3β-alcohol IIIa. In distinction from corresponding experiments in the 17-hydroxylated series[1] the epimeric 3α alcohol could not be isolated from this reaction. In order to synthesize THB we have resorted to a scheme previously used[1] in the synthesis of THF. 5β-Pregnane-

3α,21-diol-11,20-dione-21-acetate (VIb), prepared by bromination of 5β-pregnane-3α-ol-11,20-dione in methanol[10] followed by treatment with potassium acetate, was hydrolyzed and the THA (VIa) formed was protected in the 20 position by ketal formation. The ketal VIIa was next reduced with sodium borohydride and the triol VIIb heated with dilute sulfuric acid to furnish VIII in 39% overall yield from THA. Acetylation of VIII and oxidation provided VIc, identical with the acetylation product of THA-21-acetate (VIb), thus establishing that in passing from VIb to VIII only the 11-keto group was involved. The product so obtained melted at 144-6° and had a specific rotation of +114° (methanol). It is therefore different from a substance m.p. 196-7°, $(\alpha)_D$ +88°, claimed[11] to be THB.

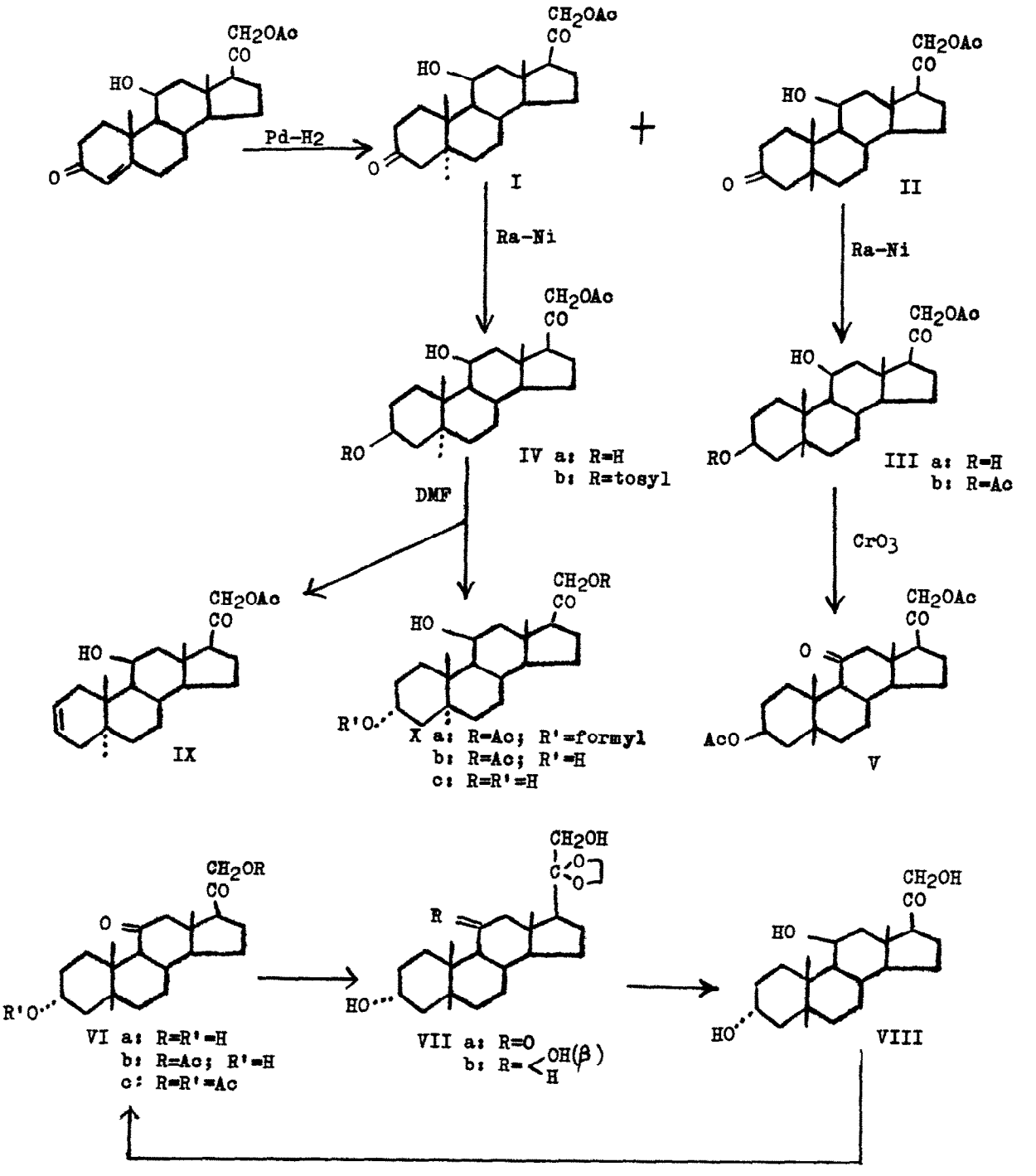
Next 5α-pregnane-3β,11β,21-triol-20-one-21-acetate (IVa), obtained[6] by Raney nickel hydrogenation of I, was used as the starting material for the synthesis of allo-THB (Xa). The tosylate IVb, prepared in 72% yield, was treated with DMF[12] to afford a mixture of three compounds which were separated by chromatography. The least polar compound, isolated in 18% yield, was the elimination product IX. It was followed by the formate Xa (36-9%) and then by the partially hydrolyzed product Xc (7.5%). Hydrolysis of Xa or Xb with potassium bicarbonate furnished allo-THB.

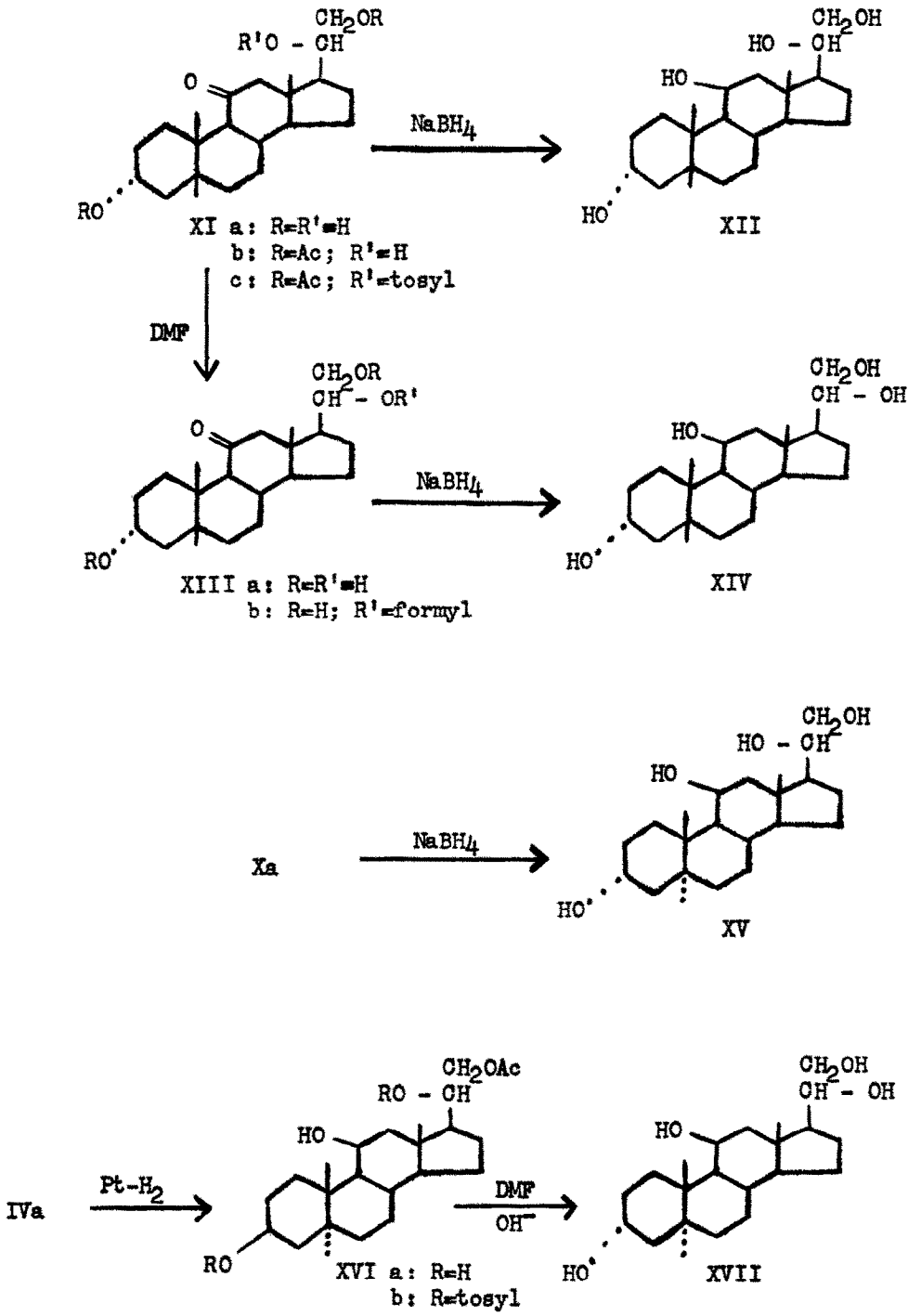
The two epimeric 20,21-glycols XIa and XIIIa were previously prepared[13] by platinum reduction of the parent ketol VIc followed by hydrolysis. The 20α isomer XIIIa was now prepared in a facile manner by epimerization of the 20β-tosylate XIc and hydrolysis. Sodium borohydride reduction of the two ketones XIb and XIIIa afforded the corresponding tetrols XII and XIV.

In the 5α series, for the synthesis of the tetrol XV we have reduced the allo-THB derivative Xa with sodium borohydride in sodium hydroxide. In addition to the predominant formation of the 20β isomer a minor amount of the 20α epimer XVII appeared to be present. The latter compound was prepared more conveniently starting with the dioldione I: hydrogenation in the presence of platinum yielded the tetrol XVIa in 58% yield, which was converted to the ditosylate XVIb in 89% yield. Inversion at the 3 and 20 carbon atoms by heating with DMF was followed by hydrolysis of the resulting diformate to afford XVII in 10% overall yield from I.

In the course of Raney nickel hydrogenations of I it was observed that with rapid agitation and reaction times in excess of three hours the yield of IVa was rapidly diminishing. This was accompanied by formation of the tetrol XVIa. No corresponding reduction of the 20-keto group in the presence of Raney nickel was observed with 17-hydroxylated analogs (e.g. 5α- and 5β-dihydrocortisol acetate)[1].

NOTE. After the preparation of this manuscript a similar synthesis of THB has been published by Lewbart and Mattox[17] who employed 12α-bromo-5β-pregnane-3α,21-diol-11,20-dione diacetate as their starting material.


CH2OAc
CO
HO
O
Pd-H2
I
II
Ra-Ni
RO
IV a: R=H
b: R=tosyl
III a: R=H
b: R=Ac
DMF
CrO3
CH2OR
R'O
X a: R=Ac; R'=formyl
b: R=Ac; R'=H
c: R=R'=H
IX
AcO
V
VI a: R=R'=H
b: R=Ac; R'=H
c: R=R'=Ac
CH2OH
R
VII a: R=O
b: R=OH(β)
VIII


CH2OR
R'O - CH
O
RO
NaBH4
CH2OH
HO - CH
HO
HO
XI a: R=R'=H
b: R=Ac; R'=H
c: R=Ac; R'=tosyl
XII
DMF
CH2OR
CH - OR'
O
RO
NaBH4
CH2OH
CH - OH
HO
HO
XIII a: R=R'=H
b: R=H; R'=formyl
XIV
Xa
NaBH4
CH2OH
HO - CH
HO
HO
XV
IVa
Pt-H2
CH2OAc
RO - CH
HO
RO
DMF
OH⁻
CH2OH
CH - OH
HO
HO
XVI a: R=H
b: R=tosyl
XVII

EXPERIMENTAL

Ultraviolet spectra were measured in 95% ethanol. Infrared spectra were taken in potassium bromide discs. Melting points are uncorrected. Optical rotations were measured in chloroform, except where stated otherwise.

Hydrogenation of corticosterone acetate[6a]. A solution of 10g of corticosterone acetate in 1 liter of ethyl acetate was vigorously agitated for 5 hrs with 5g of 5% palladium on charcoal in an atmosphere of hydrogen. Concentration of the filtered solution to 120 ml and then to 60 ml afforded a total of 4.45g of 5α-pregnane-11β-21-diol-3,20-dione-21-acetate (I), m.p. 183-8°. The pure sample melted at 189-190° (reported[6a] 190-2°).

Evaporation of the main filtrate to dryness and addition of ether afforded crude 5β-pregnane-11β,21-diol-3,20-dione-21-acetate (II), which after several fractional crystallizations from ether gave a total of 3.54g of material melting at 147-161°. The contaminant (I) (about 15%) could be best removed by chromatography to afford II of m.p. 157-9° (ether) (reported[9] 158-9°).

5β-Pregnane-3β,11β,21-triol-20-one-21-acetate (IIIa). A solution of 3.54g of the crude 5β isomer II obtained above in 900 ml of dioxane was agitated under hydrogen for 3 hrs in the presence of 8 teaspoonfuls of Raney nickel.* The product was crystallized from acetone to give 1.45g of IIIa, m.p. 194-6°. The pure sample melted at 197-8° (Kofler block 201-2°), $(\alpha)_D^{25}$ +127° (acetone), undepressed on admixture with authentic material (reported[9] 199-200°, $(\alpha)_D^{14}$ +129° (acetone)). Chromatography of the filtrates over Florisil furnished 550mg of the same material and then 280mg of IVa, m.p. 188-192°.

*Total weight approximately 25 grams.

Acetylation of IIIa gave the diacetate IIIb showing a pronounced tendency to solvate. Without further purification this was oxidized with chromic acid in acetic acid in the usual manner to afford in 75% overall yield 5β-pregnane-3β,21-diol-11,20-dione-3,21-diacetate (V), m.p. 168-9° (ether) (reported[14] 169-171°).

5β-Pregnane-3α,21-diol-11,20-dione-20-ethylene ketal (VIIa). A solution of 11g of the acetate VIa[10] in 770 ml of methanol was mixed with a solution of 11g of potassium bicarbonate in 170 ml of water and allowed to stand for 40 hrs. Removal of most of the solvent *in vacuo* at room temperature was followed by the usual chloroform extraction procedure. The product was washed with acetone to afford 8.8g of VIa, m.p. 217-223°.

A solution of 9.20g of this product and 1.3g of p-toluenesulfonic acid in 920 ml of ethylene glycol was distilled *in vacuo* at 70-5° over a 5 hr period until 50 ml remained. Aqueous bicarbonate was added and the product isolated with chloroform. Recrystallization from ethyl acetate containing a drop of pyridine afforded 5.96g of VIIa, m.p. 192-3°, $(\alpha)_D^{25}$ +67°, unchanged on further recrystallizations. Anal. Calcd. for $C_{23}H_{36}O_5$: C, 70.37; H, 9.24. Found: C, 70.09; H, 9.11.

5β-Pregnane-3α,11β,21-triol-20-one-20-ethylene ketal (VIIb). A solution of 6.14g of the ketal VIIa in 100 ml of methanol was treated with a solution of 10g of sodium borohydride in 30 ml of water and allowed to concentrate on the steam bath until a syrup remained. Another 100 ml of methanol and a solution of 10g of sodium borohydride in 30 ml of water were added and the distillation repeated. After addition of water the product was isolated with ethyl acetate and

crystallized by addition of ether to afford 5.30g of VIIb, m.p. 143-4°. The analytical sample melted at 144-5° (ethyl acetate), $(\alpha)_D^{27}$+59°.

Anal. Calcd. for $C_{23}H_{38}O_5$: C, 70.01; H, 9.71. Found: C, 69.82; H, 9.99.

THB (VIII). A solution of 7.95g of the ketal VIIb in 500 ml of methanol was refluxed for 30 minutes with 50 ml of 8% (v/v) aqueous sulfuric acid. After cooling 20g of solid sodium bicarbonate was added and the mixture distilled in vacuo at room temperature. The semisolid residue was treated with water until the salts just dissolved. Three extractions with ethyl acetate yielded a gum which crystallized on scratching with ether. Filtration and concentration of the ether filtrate afforded a second crop, bringing the total to 5.54g, m.p. 142-5°. The pure sample melted at 144-6° (ethyl acetate), $(\alpha)_D^{25}$+114° (methanol) (reported[11] 196-7°, $(\alpha)_D$+88°).

Anal. Calcd. for $C_{21}H_{34}O_4$: C, 71.96; H, 9.78. Found: C, 72.09; H, 10.00.

The diacetate was prepared in the usual manner and was directly oxidized with 5% chromic acid in acetic acid. The product was identical in all respects with 5β-pregnane-3α,21-diol-11,20-dione diacetate (VIc) prepared by acetylation of VIb and melted at 111-2° (methanol) (reported 100-110°[15]; 82-90°[15]; 95-105°[16]).

5α-Pregnane-3β,11β,21-triol-20-one-3-tosylate-21-acetate (IVb). A solution of 3.09g of IVa[6a] and 4g of p-toluenesulfonyl chloride in 25ml of pyridine was stored at 0° for 5 hrs. Addition of ice and water followed by filtration and recrystallization from methanol gave 3.1g, m.p. 164° (dec.), $(\alpha)_D^{25}$+80.5°.

Anal. Calcd. for $C_{30}H_{42}O_7S$: C, 65.91; H, 7.74. Found: C, 65.77; H, 7.81.

Treatment of IVb with DMF. A solution of 2.96g of IVb in 120 ml

of DMF was kept at 80° for 60 hrs. After removal of the solvent *in vacuo* and addition of water the gummy product was taken up in benzene and chromatographed over 60g of Florisil. With 5% ether in benzene there was obtained the unsaturated ester IX (400mg) which after two recrystallizations from heptane melted at 147-7.5°, $(\alpha)_D^{26}$ +126° (365mg).

Anal. Calcd. for $C_{23}H_{34}O_4$: C, 73.76; H, 9.15. Found: C, 73.88; H, 9.02.

Further elution with 5% and with 20% ether in benzene afforded the formate Xa (1.03g) which after recrystallization from methanol or a little acetone melted at 182-3° (0.68g). The analytical sample melted at 184-5°, $(\alpha)_D^{25}$ +111°.

Anal. Calcd. for $C_{24}H_{36}O_6$: C, 68.54; H, 8.63. Found: C, 68.29; H, 8.40.

With ether-benzene (1:1) there was obtained first 30mg of the starting tosylate, followed by Xb (210mg), which on recrystallization from ether melted at 138-142° (160mg). Further recrystallizations gave a sample m.p. 142-3°, $(\alpha)_D^{26}$ +100°.

Anal. Calcd. for $C_{23}H_{36}O_5$: C, 70.37; H, 9.24. Found: C, 70.70; H, 9.55.

When the chromatogram was carried out quickly, hydrolysis of the formate group was prevented and the yield of Xa was correspondingly higher.

Allo-THB (Xc). A solution of 432mg of the formate Xa was mixed with a solution of 430mg of potassium bicarbonate in 7 ml of water and stored for 20 hrs at room temperature. Concentration *in vacuo* at room temperature afforded a solid which was collected, water-washed and combined with an ethyl acetate extract of the filtrate. Recrystallization from ethyl acetate or acetone afforded material melting at 210-5°, which after two additional recrystallizations

melted at 221-4°, $(\alpha)_D^{25}$ +133° (reported[4] m.p. 204-6°), 235 mg. Anal. Calcd. for $C_{21}H_{34}O_4$: C, 71.96; H, 9.78. Found: C, 71.92; H, 9.55. The same product was also obtained when Xb was hydrolyzed in a similar fashion.

5β-Pregnane-3α,11β,20β,21-tetrol (XII). Reduction of 500mg of the triolone diacetate XIb[13] was performed as described for VIIb except that 5% aqueous sodium hydroxide was substituted for water as solvent for sodium borohydride. Evaporation of ethyl acetate gave a solid which was recrystallized from aqueous methanol in the form of needles, m.p. 201-4°, 289mg. Further recrystallizations gave a specimen melting at 203-4°, $(\alpha)_D^{24}$ +53° (methanol). Anal. Calcd. for $C_{21}H_{36}O_4$: C, 71.55; H, 10.30. Found: C, 71.31; H, 10.51.

5β-Pregnane-3α,20α,21-triol-11-one (XIIIa). A solution of 2.12g of the tosylate XIc[13] in 80 ml of DMF was heated at 80° for 70 hrs. After removal of the solvent in vacuo the crude formate XIIIb was refluxed with 70 ml of 5% methanolic potassium hydroxide for 1 hr. After concentration the product was isolated with ethyl acetate and crystallized from methanol-ether or acetone and weighed 280mg, m.p. 208-212°. Recrystallization gave a sample m.p. 212-3°, $(\alpha)_D^{25}$ +52° (ethanol) (reported[13] 219-221°, $(\alpha)_D$ +55°).

5β-Pregnane-3α,11β,20α,21-tetrol (XIV). Reduction of 200mg of the triolone XIIIa with sodium borohydride was carried out as described for XII. The reaction mixture was diluted with water, acidified with hydrochloric acid and the solid A filtered. The filtrate was exhaustively extracted with ethyl acetate, the extracts washed with water and evaporated. Crystallization of

the residue from acetone-petroleum ether furnished dense prisms which were combined with the solid A and recrystallized from acetone. The product melted at 246-9° and weighed 125mg. The analytical sample had the m.p. 251-2°, $(\alpha)_D^{23}$ +39° (methanol).

Anal. Calcd. for $C_{21}H_{36}O_4$: C, 71.55; H, 10.30. Found: C, 71.44; H, 10.20.

5α-Pregnane-3α,11β,20β,21-tetrol (XVa). Reduction of 393mg of the allo-THB derivative Xa was performed as above. The syrup was acidified with dilute hydrochloric acid and well extracted with ethyl acetate. Crystallization of the product from acetone afforded 133mg of XVa, m.p. 195-202°, which on further recrystallization melted at 201-3°, $(\alpha)_D^{25}$ +21° (methanol), λ_{max}^{KBr} 10.00 μ (5α,3α-ol).

Anal. Calcd. for $C_{21}H_{36}O_4$: C, 71.55; H, 10.30. Found: C, 71.60; H, 10.19.

Concentration of the original acetone filtrate to a small volume yielded a total of 56mg of a solid melting at 163-202°, the infrared spectrum of which strongly suggested that it was a mixture of XV and its 20α epimer XVII.

5α-Pregnane-3β,11β,20β,21-tetrol-21-acetate (XVIa). A solution of 11g of IVa in 250 ml of acetic acid was hydrogenated at 3 atm. pressure in the presence of 3g of platinum oxide. After 4 hrs the absorption ceased, the mixture was filtered and the solids washed with acetic acid. On dissolving in 1 liter of hot ethanol, filtration and concentration to 200 and then 80 ml, there was obtained a total of 6.39g of the monoacetate XVIa, m.p. 230-4°. The pure sample (ethanol) melted at 239-241°.

Anal. Calcd. for $C_{23}H_{38}O_5$: C, 70.01; H, 9.71. Found: C, 70.14; H, 9.63.

5α-Pregnane-3β,11β,20β,21-tetrol-3,20-ditosylate (XVIb).

A solution of 4.36g of XVIa and 9g of p-toluenesulfonyl chloride in 45 ml of pyridine was stored at 4° for 40 hrs. Addition of ice-water precipitated a crude product which was filtered, washed with methanol and recrystallized from ethyl acetate. The substance weighed 6.97g and melted at 152-4°. The analytical sample melted at 157° (dec.).

Anal. Calcd. for $C_{37}H_{50}O_9S_2$: C, 63.23; H, 7.17. Found: C, 62.93; H, 7.02.

5α-Pregnane-3α,11β,20α,21-tetrol (XVII). Treatment of 6.97g of the ditosylate XVIb with 280 ml of DMF and hydrolysis were carried out as described above. The product was isolated by extraction with ethyl acetate and crystallization initiated by scratching with a few drops of benzene. The tetrol was recrystallized from acetone to afford 0.68g, m.p. 212-5°. The pure sample melted at 218-220°, $(\alpha)_D^{25}$ +13° (methanol), λ_{max}^{KBr} 10.05 μ.

Anal. Calcd. for $C_{21}H_{36}O_4$: C, 71.55; H, 10.30. Found: C, 71.59; H, 10.25.

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