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SYNTHESIS OF 3β-HYDROXY ANALOGUES OF STEROIDAL HORMONES, A BIOLOGICALLY ACTIVE CLASS OF COMPOUNDS

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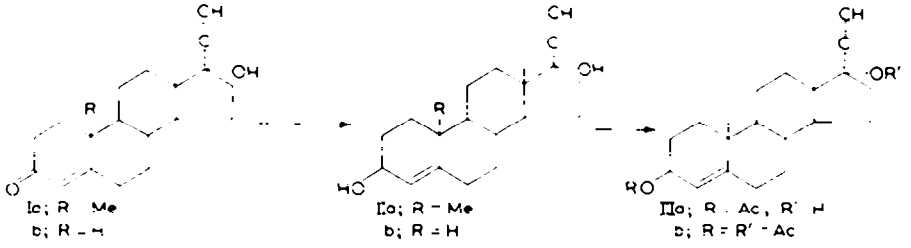
Abstract—Syntheses of 17α-ethinyl-Δ⁴-androstene-3β,17β-diol (IIa), 19-nor-17α-ethinyl-Δ⁴-androstene-3,17β-diol (IIb), Δ⁴-pregnen-3β-ol-20-one (VIa) and Δ⁴-pregnene-3β,21-diol-20-one diacetate (XIX) are described. These 3β-hydroxy analogues of steroidal hormones were found to be biologically active when assayed in animals.

BUTENANDT and Heusner[1] reported in 1938 that Δ⁴-androstene-3β,17β-diol, an analogue of testosterone in which the 3-ketone has been reduced to the 3β-hydroxy-grouping, was a potent androgenic hormone.* In this paper syntheses of the 3β-hydroxy analogues of 17α-ethinyltestosterone, 19-nor-17α-ethinyltestosterone, progesterone and desoxycorticosterone (as the 3,21-diacetate) are described, substances which were found to have high biological activity. Since starting this investigation, the progesterone analogue has also been prepared by Gut[4] who reports it to be a powerful progestational hormone.

17α-Ethinyl-Δ⁴-androstene-3β,17β-diol (IIa) can be prepared simply in 70% yield by reduction of 17α-ethinyltestosterone (Ia) with sodium borohydride in methanol. The spectral properties are in accord with the assigned structure and the retention of the double and triple bonds is confirmed through re-oxidation of (IIa) to the starting material (Ia). The β-configuration of the 3-hydroxy group is to be expected since the reduction of steroidal Δ⁴-3-ketones with sodium borohydride is

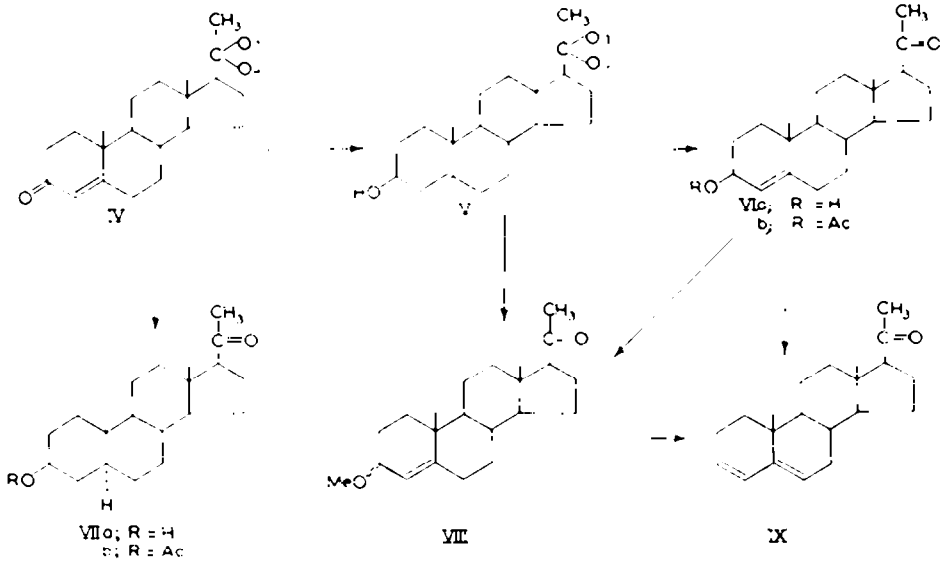


* Quite recently R. I. Dorfman found the corresponding 3,17-diacetate also to have high androgenic activity. This result was reported by S. Bernstein *et al.*[3] who considered it surprising since A. Butenandt in 1935 had stated[2] that the diol exhibited only little activity. However, in 1938 Butenandt[1] reported the result of the previous biological test to have been in error. Further, S. Bernstein *et al.*[3] found that the androgenic activity of 17α-methyl-Δ⁴-androstene-3β,17β-diol, the corresponding analogue of 17-αmethyltestosterone, was of the same order as the parent hormone.

[1] A. Butenandt and A. Heusner, *Ber.* **71**, 198 (1938).
[2] S. Bernstein, S. M. Stolar and M. Heller, *J. Org. Chem.* **22**, 472 (1957).
[3] A. Butenandt, K. Tscherning and G. Hanisch, *Ber.* **68**, 2097 (1935).
[4] M. Gut, *J. Org. Chem.* **21**, 1327 (1956).

known to yield predominantly this isomer,[5] and this assignment is confirmed by molecular rotation considerations (see below). The diol (IIa) was characterised through formation of the 3-monoacetate (IIIa) and the 3,17-diacetate (IIIb).

The analogous reduction of 19-nor-17α-ethinyltestosterone (Ib)[6] with sodium borohydride yielded a non-homogeneous product which only after repeated crystallisation gave an apparently pure substance. The spectral data and elemental analysis show this to be the hydrate of a 19-nor-17α-ethinyl-Δ^4-androstene-3,17β-diol (IIb), but it cannot be said at present whether it is the 3β- or the 3α-isomer or a mixture of the two. The stereochemical course of the metal hydride reduction of 19-nor-Δ^4-3-ketones is unknown and the molecular rotation data do not allow a definite conclusion to be made. The diol (IIb), like the corresponding 19-methyl compound (IIa), does not give an insoluble precipitate with digitonin.



The next objective was the synthesis of Δ^4-pregnen-3β-ol-20-one (VIa). Although pregnane-3,20-dione and *allo*pregnane-3,20-dione can be reduced at C-3 by means of sodium borohydride,[7] the corresponding preferential reduction of progesterone is known to proceed at C-20[8] and cannot, therefore, be used for the preparation of (VIa). The successful method proceeded from progesterone 20-*cyclo*ethylene ketal (IV), readily prepared from Δ^5-pregnen-3β-ol-20-one acetate through successive ketalisation at C-20, saponification at C-3 and Oppenauer oxidation. The details are almost identical to those subsequently described by Gut[4] and are therefore not given in the Experimental Section. The conversion of (IV) to (VIa) depends on finding such acid conditions as will cleave the ketal grouping at C-20 in (V), without causing concomitant dehydration at C-3. After considerable experimentation, it was found that reduction of (IV) with lithium aluminium hydride and

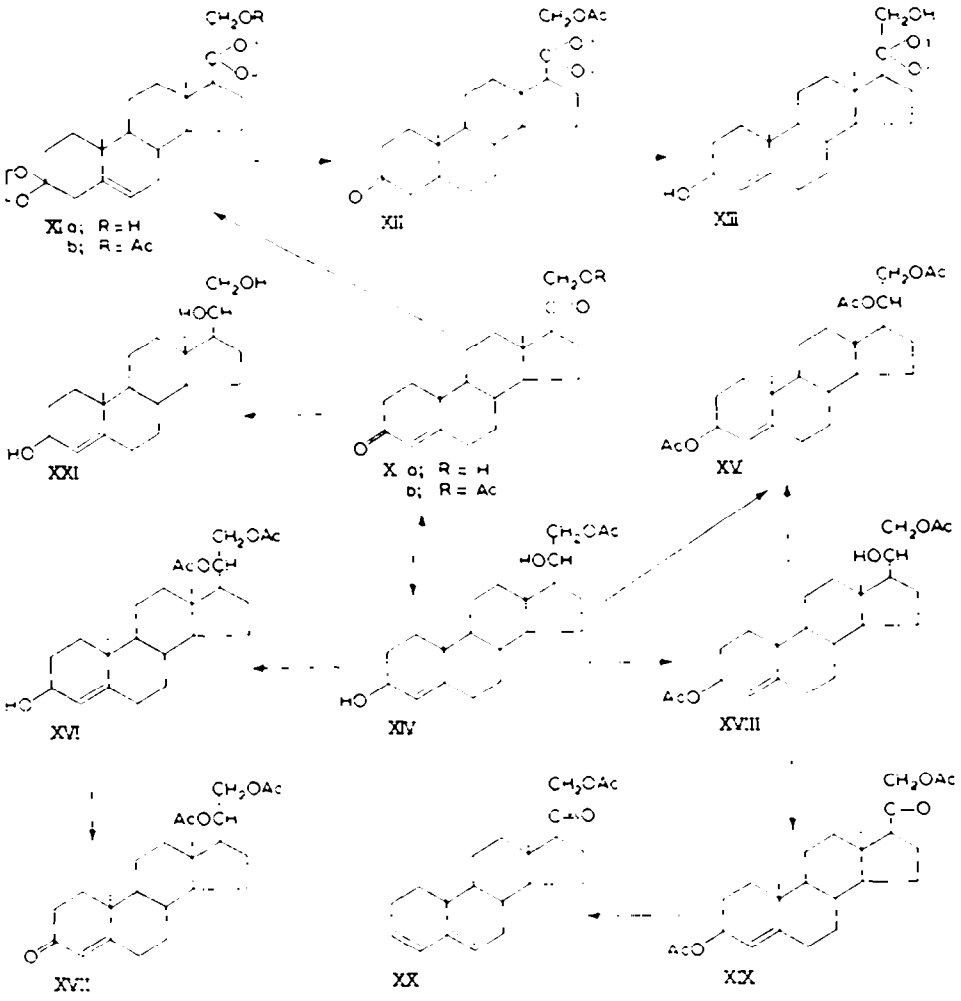
[5] *Cf.* W. G. Dauben, R. A. Micheli and J. F. Eastham, *J. Amer. Chem. Soc.* **74**, 3852 (1952); W. W. Zorbach, *J. Amer. Chem. Soc.* **75**, 6344 (1953).
[6] C. Djerassi, L. Miramontes, G. Rosenkranz and F. Sondheimer, *J. Amer. Chem. Soc.* **76**, 4092 (1954).
[7] O. Mancera, H. J. Ringold, C. Djerassi, G. Rosenkranz and F. Sondheimer, *J. Amer. Chem. Soc.* **75**, 1286 (1953); A. H. Soloway, A. S. Deutsch and T. F. Gallagher, *Ibid.* **75**, 2356 (1953).
[8] J. K. Norymberski and G. F. Woods, *J. Chem. Soc.* 3426 (1955).

subsequent chromatography gives 64% of (V), which on treatment with *p*-toluenesulphonic acid in ethanol solution at room temperature yields over 90% of (VIa). The structure of the latter was confirmed by acetylation to the acetate (VIb), dehydration to the known $\Delta^{3,5}$-pregnadien-20-one (IX)[9] and oxidation to progesterone. The hydroxy-ketone (VIa) agrees reasonably well in physical properties with the substance reported by Gut[4] by sodium borohydride reduction of the ketal (IV) and treatment of (V) with oxalic acid in ethanol. A comparative experiment showed our acid cleavage conditions to be preferable and to result in a purer product without chromatography.

Prior to the successful synthesis of the hydroxy-ketone (VIa), progesterone 20-*cyclo*ethylene ketal (IV) was reduced with sodium borohydride in aqueous ethanol and the product treated with *p*-toluenesulphonic acid in acetone. This interchange method has been found in other cases to result in the smooth conversion of *cyclo*-ethylene ketals to the parent ketones.[10,17] Three ketonic substances resulted, the first of which was $\Delta^{3,5}$-pregnadien-20-one (IX).[9] The other two substances (only one of which gave a precipitate with digitonin) apparently both possess the empirical formula $C_{21}H_{32}O_2$. It was at first thought that they were the expected 3β- and 3α-isomers of Δ^4-pregnen-3-ol-20-one, but this proved incorrect. The compound precipitated with digitonin is unaffected by manganese dioxide at room temperature,[11] (3β-hydroxy group not allylic) and was shown to be *allo*pregnan-3β-ol-20-one (VIIa) by direct comparison of its acetate with an authentic sample of *allo*pregnan-3β-ol-20-one acetate (VIIb). Sodium borohydride reduction of the Δ^4-3-ketone (IV) results, therefore, partly in the saturation of the double bond, a phenomenon which has been observed previously in another series.[12] Lithium aluminium hydride reduction of Δ^3-3-ketones is reported[12] not to cause any appreciable attack on the double bond and in fact treatment of (IV) with this reagent and subsequent treatment of the total product with *p*-toluenesulphonic acid in acetone gives a mixture of (IX) and the substance giving no precipitate with digitonin.

The last-mentioned substance appears to be a transformation product of (VIa), since it is also obtained by treatment of (VIa) with *p*-toluenesulphonic acid in acetone. It possesses a 20-keto group (infra-red), a double bond (yellow colour with tetranitromethane), and it does not contain a free 3-hydroxyl function. It is converted to (IX) by hydrochloric acid in boiling methanol. After further experimentation it was found that the substance contains a methoxyl group (Zeisel determination) and that it is obtained in increased yield when the hydroxy-ketal (V) or the hydroxy-ketone (VIa) are treated with *p*-toluenesulphonic acid in methanol. It appears to be 3-methoxy-Δ^4-pregnen-20-one (VIII), its formation presumably being due to the presence of a small amount of methanol in the acetone employed. It is of interest that the reaction of the hydroxy-ketal (V) with *p*-toluenesulphonic acid in ethanol gives no appreciable amount of the corresponding ethyl ether. The β- and what may be the α-isomer of the 3-methoxy-Δ^4-ethylene in the cholesterol series have been described recently.[13] The

[9] J. Romo, M. Romero, C. Djerassi and G. Rosenkranz, *J. Amer. Chem. Soc.* 73, 1528 (1951).
[10] *Cf.* G. Rosenkranz, J. Pataki and C. Djerassi, *J. Org. Chem.* 17, 290 (1952); G. Rosenkranz, M. Velasco and F. Sondheimer, *J. Amer. Chem. Soc.* 76, 5024 (1954).
[11] J. Attenburrow, A. F. B. Cameron, J. H. Chapman, R. M. Evans, B. A. Hems, A. B. A. Jansen and T. Walker, *J. Chem. Soc.* 1094 (1952); F. Sondheimer, C. Amendolla and G. Rosenkranz, *J. Amer. Chem. Soc.* 75, 5930 (1953).
[12] F. Sondheimer, M. Velasco, E. Batres and G. Rosenkranz, *Chem. & Ind.* 1482 (1954).
[13] C. W. Shoppee, B. D. Agashe and G. H. R. Summers, *J. Chem. Soc.* 3107 (1957).



unusually high specific rotation of (VIII) makes it unlikely that it is the pure 3β-isomer; it is probably the 3α-isomer or a molecular compound of the two, but this point has not been investigated. The easy etherification of a Δ^4-3-ol under acid conditions is comparable to the ready etherification of other steroidal allylic alcohols, e.g. of a Δ^5-7-ol (with alcoholic acetic acid)[14] or of a $\Delta^{9(11)}$-ol (with alcoholic hydrogen chloride).[15]

The preparation of Δ^4-pregnene-3β,21-diol-20-one diacetate (XIX), the 3β-acetoxy analogue of desoxycorticosterone acetate, was first attempted by application of the method successfully used in the progesterone series. Desoxycorticosterone (Xa) was converted to the di-*cyclo*ethylene ketal (XIa) and then acetylated, as described previously.[16] Treatment of the resulting ester (XIb) with *p*-toluenesulphonic acid in acetone at room temperature affected only the 3-position, as in the cortisone

[14] *Cf.* H. B. Henbest and E. R. H. Jones, *Nature, Lond.* **158**, 950 (1946); *J. Chem. Soc.* 1798 (1948).
[15] *Cf.* R. B. Turner, V. R. Mattox, L. L. Engel, B. F. McKenzie and E. C. Kendall, *J. Biol. Chem.* **162**, 571 (1946).
[16] S. Bernstein and R. H. Lenhard, *J. Amer. Chem. Soc.* **77**, 2233 (1955).

series,[17] and yielded the 20-monoketal (XII). Lithium aluminium hydride reduction of the latter gave 80% of the 3β,21-dihydroxy-20-ketal (XIII) which could be regenerated from the insoluble digitonide. Treatment of (XIII) with *p*-toluenesulphonic acid in ethanol at room temperature resulted in the removal of the protecting group at a much slower rate than in the 21-desoxy series and after 6 days only 50% of the product was ketonic, judged from the infra-red spectrum. Chromatographic purification of the resulting material, free or after acetylation, yielded no crystalline material and this method was discontinued when other acid cleavage conditions gave no more promising results.

The successful route to (XIX) involves the reduction of desoxycorticosterone acetate (Xb) with excess sodium borohydride in aqueous tetrahydrofuran at room temperature, conditions which do not affect ester groupings.[18] The product, which no longer showed appreciable ultra-violet absorption, still possessed the 21-acetoxy function (acetyl analysis) and presumably consisted mainly of Δ^4-pregnene-3β,20β,21-triol 21-monoacetate (XIV) as judged from the known predominant formation of β-alcohols by metal hydride reduction of Δ^4-3-ketones[5] and 21-hydroxy-20-ketones.[8] Partial acetylation of this material with a limited amount of acetic anhydride in pyridine yielded a mixture of products from which a triacetate and two diacetates could be obtained by chromatography. The triacetate proved to be Δ^4-pregnene-3β,20β,21-triol triacetate (XV) with properties in excellent agreement with the known compound.[8] One of the diacetates contained the free 3-hydroxy-Δ^4-system since it was oxidised to an unsaturated ketone with manganese dioxide at room temperature and a preparative oxidation with chromium trioxide in pyridine[19] yielded the known[8] Δ^4-pregnene-20β,21-diol-3-one diacetate (XVII). The triol diacetate is therefore Δ^4-pregnene-3β,20β,21-triol 20,21-diacetate (XVI) or the corresponding 3α-isomer.

The second triol diacetate is Δ^4-pregnene-3β,20β,21-triol 3,21-diacetate (XVIII) since acetylation yields the triacetate (XV) and since it is unaffected by manganese dioxide. Oxidation of this diacetate with chromium trioxide in pyridine[19] yields the required Δ^4-pregnene-3β,21-diol-20-one diacetate (XIX). The presence of the 21-acetoxy-20-keto grouping in the latter is shown by the infra-red spectrum (ν_{max} 1748 and 1730 cm^{-1})[20] as well as by the strong red colour given with triphenyltetrazolium chloride,[21] and the Δ^4-3-acetoxy grouping by acid dehydration to the $\Delta^{3,5}$-diene (XX)[9] with its high-intensity ultra-violet maximum at 228 mμ. The β-configuration of the 3-acetoxy group follows from consideration of molecular rotation (see below) and from the fact that (XIX) has been interrelated with the known triacetate (XV).

A variation of the above described route to (XIX) involves the reduction of (Xb) with an excess of lithium aluminium hydride to a mixture of triols, which is acetylated directly with 1·5 moles of acetic anhydride. Chromatography yields a diacetate fraction which on oxidation with chromium trioxide in pyridine and subsequent separation yields (XIX). This partial acetylation yields the 21-monoacetate (XIV) and the corresponding free triol (XXI). The structures of these compounds are based on the fact that both can be acetylated to the triacetate (XV), while oxidation of (XIV) with chromium trioxide in pyridine regenerates (Xb).

[17] F. Sondheimer, O. Mancera, G. Rosenkranz and C. Djerassi, *J. Amer. Chem. Soc.* **75**, 1282 (1953).
[18] H. J. Ringold, B. Löken, G. Rosenkranz and F. Sondheimer, *J. Amer. Chem. Soc.* **78**, 816 (1956).
[19] G. I. Poos, G. E. Arth, R. E. Beyler and L. H. Sarett, *J. Amer. Chem. Soc.* **75**, 422 (1953).
[20] R. N. Jones, P. Humphries, F. Herling and K. Dobriner, *J. Amer. Chem. Soc.* **74**, 2820 (1952).
[21] *Cf.* A. Zaffaroni, *Recent Progress in Hormone Research* **8**, 51 (1953).

In Table 1 the shift in molecular rotation in passing from the Δ^4-3-ketone to the Δ^4-3β-ol (Δ_1) as well as the shift in passing from the Δ^4-3-ketone to the Δ^4-3β-ol acetate (Δ_2) are recorded for the various hormone analogues described in this paper and compared with the known values in the Δ^4-cholesten-3-one and 17α-methyltestosterone series. It can be seen that in all but one case the Δ_1 value is about -200 and the Δ_2 value about 300. This is in marked contrast to the corresponding difference in molecular rotation between the Δ^4-3-ketone and the Δ^4-3α-ol or Δ^4-3α-ol acetate, which is highly positive (Table 1, last line). The one exception is in the 19-nor-17α-ethinyltestosterone series where the Δ_1 value lies about half-way between that expected for the 3β- and for the 3α-ol. It is not known whether this

TABLE 1. MOLECULAR ROTATION DATA OF 3β-HYDROXY- AND 3β-ACETOXY-Δ^4-STEROIDS[a]

Compound	M_D Ketone	M_D 3β-OH	M_D 3β-OAc	Δ_1	Δ_2
Δ^4-Cholesten-3-one	−340[b]	−178[13]	−39[13]	162	301
17α-Methyltestosterone	−248[c]	−43[3,d]	59[3]	205	307
17α-Ethinyltestosterone	−122[c,e]	69[d]		160	191	282
Progesterone	−640[f]	−439	−340	201	300
Desoxycorticosterone acetate	−692[f]		−408	–	284
19-Nor-17α-ethinyltestosterone	75[g]	124[g]		49	

	M_D Ketone	M_D 3α-OH	M_D 3α-OAc	Δ_1	Δ_2
Δ^4-Cholesten-3-one	−340[b]	−444[13]	−758[13]	+104	+418

[a] All rotations were determined in chloroform solution unless specified otherwise.
[b] A. Butenandt and A. Wolff, *Ber.* **68**, 2091 (1935).
[c] A. Sandoval, G. H. Thomas, C. Djerassi, G. Rosenkranz and F. Sondheimer, *J. Amer. Chem. Soc.* **77**, 148 (1955).
[d] Determined in methanol.
[e] Determined in chloroform containing 10% of pyridine.
[f] F. Sondheimer, S. Kaufmann, J. Romo, H. Martinez and G. Rosenkranz, *J. Amer. Chem. Soc.* **75**, 4712 (1953).
[g] Determined in ethanol.

anomaly is due to the diol (IIb) actually being an equimolar complex of the two possible isomers as has been observed in the cholesterol series[22] or whether removal of the 19-methyl group changes the Δ_1 value.

Bioassays.[*] In the Clauberg assay for oral progestational activity in the rabbit, (IIa) was found to have the same order of activity as 17α-ethinyltestosterone. In the same assay, (IIb) showed the same activity as the potent oral progestational hormone 19-nor-17α-ethinyltestosterone.

On being tested for sodium retention in adrenalectomised male rats, (XIX) showed about the same activity at the 100 and 25 μg level as desoxycorticosterone acetate did at the 25 and 6 μg level, respectively. The analogue (XIX) therefore exhibits ca. 25% of the activity of desoxycorticosterone acetate.

[*] The biological tests were carried out by the Endocrine Laboratories, Madison 1, Wisconsin, U.S.A.
[22] R. Schoenheimer and E. A. Evans, *J. Biol. Chem.* **114**, 567 (1936).

It still remains to be established whether the Δ^4-3-hydroxy hormone analogues are biologically active *per se* or because of their conversion to the corresponding Δ^4-3-ketones in the organism.

EXPERIMENTAL*

17α-*Ethinyl*-Δ^4-*androstene*-3β,17β-*diol* (IIa)

Sodium borohydride (2·5 g) was added in small portions during 4 hr to a stirred suspension of 17α-ethinyltestosterone (Ia) (5 g) in methanol (200 cc) containing water (0·2 cc). The mixture was allowed to stand overnight at room temperature and was then diluted with water and extracted with chloroform. The organic layer was washed with water, dried and evaporated. Crystallisation from acetone-hexane furnished 3·5 g (70%) of the diol (IIa) with m.p. 207–211°. The analytical sample showed m.p. 211–214°, $[\alpha]_D$ −22° (MeOH), ν_{max} 3300 cm^{-1} (acetylenic hydrogen) and free hydroxyl band, no carbonyl bands, no high intensity absorption in the ultra-violet.

(Found: C, 80·29; H, 9·72; Calc. for $C_{21}H_{30}O_2$: C, 80·21; H, 9·62%.)

The diol gave a yellow colour with tetranitromethane. No precipitate was formed with digitonin in aqueous ethanol. On oxidation with manganese dioxide in dioxane (22°, 24 hr) 17α-ethinyltestosterone m.p. 264–266° was regenerated.

The 3-*monoacetate* (IIIa) was prepared by means of acetic anhydride and pyridine at room temperature. After crystallisation from acetone-hexane it showed m.p. 156–158°, $[\alpha]_D$ −45°, ν_{max} 3300 and 1720 cm^{-1} and free hydroxyl band.

(Found: C, 77·11; H, 8·95; Calc. for $C_{23}H_{32}O_3$: C, 77·49; H, 9·05%.)

The *diacetate* (IIIb) was prepared by boiling the diol (IIa) (315 mg) in pyridine (8 cc) with acetic anhydride (4 cc) for 20 hr. Chromatography of the product followed by crystallisation from hexane gave 290 mg of (IIIb) with m.p. 181–182°, $[\alpha]_D$ −60°, ν_{max} 3300 and 1720 cm^{-1}, no free hydroxyl band.

(Found: C, 75·17; H, 8·84; Calc. for $C_{25}H_{34}O_4$: C, 75·34; H, 8·60%.)

19-*Nor*-17α-*ethinyl*-Δ^4-*androstene*-3,17β-*diol* (IIb)

19-Nor-17α-ethinyltestosterone (1 g)[6] was reduced with sodium borohydride (0·5 g) as described in the preceding experiment. Chromatography of the product on alumina gave 0·81 g of material with m.p. 110–145° which after repeated crystallisation from ether yielded the diol (IIb) as a hydrate with m.p. 147–149°, $[\alpha]_D$ −39° (EtOH), ν_{max}^{KBr} 3300 cm^{-1} and free hydroxyl band, no carbonyl bands, no high-intensity absorption in the ultra-violet. The substance gave no precipitate with digitonin in aqueous ethanol.

(Found: C, 75·26; H, 9·61; Calc. for $C_{20}H_{28}O_2 \cdot H_2O$: C, 75·43; H, 9·50%.)

Δ^4-*Pregnen*-3β-*ol*-20-*one* (VIa)

p-Toluenesulphonic acid hydrate (36 mg) was added to a solution of 400 mg of the pure 3β-hydroxy-ketal (V) (m.p. 168–170°, prepared by lithium aluminium hydride

* Melting points are uncorrected. All chromatograms were carried out with Merck "acid-washed" alumina except for those marked "Alcoa" which were carried out with "Alcoa" activated alumina, grade F-20 (Aluminum Co. of America, Pittsburgh, Pa.). Rotations were determined at room temperature in chloroform solution, unless specified otherwise. Ultra-violet spectra were measured in 95% ethanol solution on a Unicam model S.P. 500 spectrophotometer and infra-red spectra in chloroform solution (unless otherwise stated) on a Baird double beam recording spectrophotometer with sodium chloride optics. Analyses were carried out in our microanalytical laboratory under the direction of Mr. Erich Meier.

reduction of progesterone 20-*cyclo*ethylene ketal (IV) as described below) in 20 cc of absolute ethanol. The solution was allowed to stand at 23° for 90 min. Sodium bicarbonate solution was then added and the product extracted by means of chloroform. Direct crystallisation from acetone-hexene yielded 320 mg (91%) of pure (VIa), m.p. 159–161°, $[\alpha]_D$ +139°, ν_{max} 1704 cm^{-1} and free hydroxyl band, no high-intensity absorption in the ultra-violet, yellow colour with tetranitromethane, heavy precipitate with digitonin in aqueous ethanol; reported[4] m.p. 155–161° $[\alpha]_D$ +135°.

(Found: C, 79·80; H, 10·02; Calc. for $C_{21}H_{32}O_2$: C, 79·70; H, 10·19%.)

Oxidation of (VIa) with manganese dioxide in chloroform (48 hr at 23°) produced progesterone in over 80% yield. Dehydration of (VIa) with boiling methanol containing a few drops of hydrochloric acid yielded the $\Delta^{3,5}$-diene (IX).

The *acetate* (VIb) (acetic anhydride-pyridine, 16 hr at 22°) crystallised from acetone-hexane, m.p. 125–126°, $[\alpha]_D$ +95°, ν_{max} 1732 and 1704 cm^{-1}, no hydroxyl band.

(Found: C, 77·22; H, 9·47; Calc. for $C_{23}H_{34}O_3$: C, 77·05; H, 9·56%.)

Reduction of progesterone 20-cyclo*ethylene ketal* (IV) *with sodium borohydride followed by treatment with* p-*toluenesulphonic acid in acetone*

Sodium borohydride (2·0 g) was added to a solution of progesterone 20-*cyclo*ethylene ketal (m.p. 190–191°) (2·0 g) in aqueous ethanol (80 cc, 90%). After standing at 23° for 16 hr, the solution was diluted with just sufficient acetic acid to decompose the excess reagent. Water was then added and the product isolated with chloroform. The resultin gmaterial (2·0 g), m.p. 120–150°, has no high-intensity absorption in the ultra-violet, no carbonyl bands in the infra-red. This material was dissolved in dry redistilled acetone (100 cc, commercial grade), *p*-toluenesulphonic acid hydrate (150 mg) added and the solution allowed to stand at 24° for 1½ hr. Sodium bicarbonate solution was then added and the product, extracted by means of ether, chromatographed on 100 g alumina (Alcoa). Elution with benzene-ethyl acetate (99 : 1) and subsequent crystallisation from methanol gave 210 mg (13%) of $\Delta^{3,5}$-pregnadien-20-one (IX), m.p. 138–140°, λ_{max} 228 and 235 mμ, ε = 19,100 and 20,400, respectively; reported:[9] m.p. 139–142°, λ_{max} 228 and 234 mμ, ε = 18,600 and 20,000, respectively. Further elution with benzene-ethyl acetate (99 : 1) and crystallisation from hexane gave 390 mg (21%) of (VIII) with m.p. 147–149°, $[\alpha]_D$ +222°, ν_{max} 1702 cm^{-1}, no appreciable hydroxyl band, no high-intensity absorption in the ultra-violet yellow colour with tetranitromethane, no precipitate with digitionin in aqueous ethanol. (Found: C, 79·80; H, 10·18; OMe, 9·63; act. H, 0·0. Calc. for $C_{22}H_{34}O_2$: C, 79·95; H, 10·37; OMe, 9·39%.)

The substance was recovered unchanged on attempted acetylation with acetic anhydride and pyridine at room temperature. Boiling the ether (VIII, 14 mg) with methanol (1 cc) and conc. hydrochloric acid (2 drops) for 4 hr yielded the diene (IX, 8 mg), m.p. 136–138°, identified by mixed m.p. and the ultra-violet spectrum.

Finally elution with benzene-ethyl acetate (3:1) and crystallisation from acetone-hexane yielded *allo*pregnan-3β-ol-20-one (VIIa, 320 mg, 18%), m.p. 190–192°, $[\alpha]_D$ + 91° (EtOH), no colour produced with tetranitromethane, immediate precipitate with digitonin in aqueous ethanol; reported:[23] m.p. 194·5°, $[\alpha]_D$ +91°

[23] A. Butenandt and L. Mamoli, *Ber.* **68**, 1847 (1935).

(EtOH). The acetate (VIIb) showed m.p. 143–144°, $[\alpha]_D$ +74° and proved to be identical (mixed m.p., infra-red comparison) with an authentic sample (m.p. 142–144°, $[\alpha]_D$ +76°).

Reduction of (IV) *with lithium aluminium hydride followed by treatment with* p-*toluenesulphonic acid in acetone*

A solution of progesterone 20-*cyclo*ethylene ketal (m.p. 190–191°) (2·5 g) in tetrahydrofuran (30 cc) and ether (30 cc) was added during 15 min to a stirred ice-cooled suspension of lithium aluminium hydride (0·8 g) in ether (80 cc) under nitrogen. The mixture was refluxed for 1 hr, the excess reagent decomposed by means of ethyl acetate, and a saturated sodium sulphate solution added until the precipitate began to adhere to the sides of the flask. Solid sodium sulphate was added, the salts removed by filtration and washed well with ether. Evaporation of solvents yielded the crude ketal (V, 2·5 g) showing no high-intensity absorption in the ultra-violet. A 500 mg sample on chromatography on alumina (25 g), followed by elution with benzene-ethyl acetate (9:1) and crystallisation from acetone-hexane, yielded 320 mg (64%) pure 3β-hydroxy-ketal (V), m.p. 168–170°; reported:[4] m.p. 170–173°.

The crude ketal V (2·0 g) in acetone (100 cc) was treated for 1 hr with 150 mg of *p*-toluenesulphonic acid, exactly as described in the preceding experiment. Chromatographic purification of the product yielded first 240 mg (14%) of (IX), m.p. 137–140° and then 580 mg (31%) of (VIII), m.p. 146–148°. No *allo*pregnan-3β-ol-20-one (VIIa) could be isolated.

Treatment of the pure 3β-hydroxy-ketal (V) (m.p. 168–170°) under the same conditions gave 18% of the diene (IX) and 32% of (VIII).

3-*Methoxy*-Δ^4-*pregnen*-20-*one* (VIII) *from* (VIa)

(a) *With* p-*toluenesulphonic acid in acetone.* A solution containing 150 mg of the keto-alcohol (VIa) and *p*-toluenesulphonic acid hydrate (13 mg) in dry redistilled acetone (8 cc, commercial grade) was allowed to stand at 22° for 90 min. The total product, isolated with ether, no longer gave any precipitate with digitonin in aqueous ethanol. Chromatographic purification on alumina (Alcoa) gave 22 mg of the $\Delta^{3,5}$-diene (IX) and then 41 mg of the 3-methoxy-compound (VIII). Both compounds were identified by comparison with the previously described samples.

(b) *With* p-*toluenesulphonic acid in methanol.* The experiment was carried out as above, except that methanol was used instead of acetone and the reaction was allowed to proceed for 8 hr (after 90 min the reaction was incomplete). Chromatographic purification of the product on alumina (Alcoa, 7·5 g) gave (VIII) (91 mg, 58%), m.p. 146–148°, undepressed on admixture with the previously described material.

20-*Ethylenedioxy*-Δ^4-*pregnen*-21-*ol*-3-*one acetate* (*desoxycorticosterone acetate* 20-cyclo*ethylene ketal*) (XII)

A solution containing 120 mg of (XIb) (m.p. 159–161°)[16] and *p*-toluenesulphonic acid hydrate (12 mg) in dry acetone (3 cc) was allowed to stand at 22° for 14 hr. Sodium bicarbonate solution and ether were added, the organic layer was washed with water, dried and evaporated. Crystallisation of the residue from hexane produced

81 mg (75%) of the 20-monoketal (XII), m.p. 140–141°, ν_{max} 1730 and 1660 cm^{-1}, λ_{max} 241 mμ, $\varepsilon = 16{,}600$.

(Found: C, 72·30; H, 8·80; Calc. for $C_{25}H_{36}O_5$ C, 72·08; H, 8·71%.)

20-Ethylenedioxy-Δ^4-pregnene-3β,21-diol (XIII)

A solution of 450 mg of the ketal (XII) in tetrahydrofuran (12 cc) and ether (12 cc) was added slowly under nitrogen to an ice-cooled stirred solution of lithium aluminium hydride (200 mg) in dry ether (30 cc). The mixture was stirred at room temperature for 2 hr, the excess of reagent destroyed by the careful addition of ethyl acetate and saturated sodium sulphate solution added until the precipitate began to adhere to the sides of the flask. Solid sodium sulphate was added, the salts removed by filtration and washed well with ether. The filtrate was evaporated and the residue crystallised from acetone-hexane. This procedure yielded 350 mg (86%) of the 3β,21-diol (XIII), m.p. 199–201°, $[\alpha]_D$ +94° (EtOH), strong hydroxyl but no carbonyl bands in the infra-red, no high-intensity absorption in the ultra-violet.

(Found: C, 73·30; H, 9·93; Calc. for $C_{23}H_{36}O_4$: C, 73·36; H, 9·64%.)

The diol gave a heavy precipitate with digitonin in aqueous ethanol. On regeneration from the digitonide, the diol showed unchanged physical properties.

Reduction of (Xb) *with sodium borohydride followed by partial acetylation*

A solution of sodium borohydride (0·5 g) in water (4 cc) was added to desoxycorticosterone acetate (2·5 g) dissolved in tetrahydrofuran (100 cc). After being allowed to stand at room temperature for 70 hr, the solution was treated with a little acetic acid, evaporated to small volume and diluted with water. Isolation with chloroform gave 2·5 g of the crude triol 21-monoacetate (XIV) as a viscous oil, ν_{max} 1732 cm^{-1} (acetate) and strong hydroxyl band, no band at ca. 1660 cm^{-1}, no high-intensity absorption in the ultra-violet.

(Found: acetyl, 11·24. Calc. for $C_{23}H_{36}O_4$: acetyl, 11·44%.)

The total crude 21-monoacetate (XIV) in dry pyridine (25 cc) was acetylated with acetic anhydride (1·36 g) for 24 hr at room temperature. The product was isolated with ether and chromatographed on alumina (125 g). The first crystalline fractions, eluted with benzene, on crystallisation from acetone-hexane yielded 0·65 g of the triacetate (XV) as needles, m.p. 129–131°, $[\alpha]_D$ +41°, ν_{max} 1732 cm^{-1}, no hydroxyl band; reported:[8] m.p. 128–131°, $[\alpha]_D$ +40°.

(Found: C, 70·56; H, 8·93; acetyl, 29·19; Calc. for $C_{27}H_{40}O_6$: C, 70·40; H, 8·75; acetyl, 28·04%.)

Elution of the column with benzene-chloroform (2:1) gave 1:1 g of a mixture of the diacetates (XVI) and (XVIII). These were separated by a combination of chromatography and fractional crystallisation from acetone-hexane. The required 3,21-diacetate (XVIII) showed m.p. 142–144°, $[\alpha]_D$ +20°, ν_{max} 1732 cm^{-1} and free hydroxyl band.

(Found: C, 71·52; H, 9·20; acetyl, 21·21; Calc. for $C_{25}H_{38}O_5$: C, 71·74; H, 9·15; acetyl, 20·57%.)

The substance in chloroform was unaffected by treatment with manganese dioxide at room temperature for 24 hr. Acetylation with acetic anhydride and pyridine at room temperature quantitatively yielded (XV).

The 20,21-diacetate (XVI) showed m.p. 145–146°, ν_{max} 1734 cm^{-1} and free hydroxyl band, large m.p. depression on mixing with (XVIII).

(Found: C, 71·44; H, 9·36; acetyl, 20·75; Calc. for $C_{25}H_{38}O_5$: C, 71·74; H, 9·15; acetyl, 20·57%.)

The substance contained an allylic alcohol grouping, since treatment with manganese dioxide in chloroform at room temperature for 24 hr resulted in the formation of an αβ-unsaturated ketone (λ_{max} 240 mμ, $\varepsilon = 14{,}000$).

Δ^4-Pregnene-20β,21-diol-3-one diacetate (XVII)

The 20,21-diacetate (XVI, 80 mg) in pyridine (1 cc) was oxidised with chromium trioxide (100 mg) in pryidine (2 cc) at room temperature for 24 hr. Water was added and the product isolated with ether. Crystallisation from acetone-hexane gave 62 mg of Δ^4-pregnene-20β,21-diol-3-one diacetate (XVII), m.p. 154–155°, $[\alpha]_D$ +125°, ν_{max} 1736 and 1666 cm^{-1}, no hydroxyl band, λ_{max} 241 mμ, $\varepsilon = 16{,}000$; reported:[8] m.p. 155–156°, $[\alpha]_D$ +123°.

Δ^4-Pregnene-3β,21-diol-20-one diacetate (XIX)

A solution of 160 mg of the 3,21-diacetate (XVIII) in dry pyridine (2 cc) was added to a mixture of chromium trioxide (200 mg) and pyridine (4 cc). The mixture was allowed to stand at room temperature for 24 hr, diluted with water and extracted with ether. The product was chromatographed on alumina (8 g). Elution with benzene and crystallisation from pentane gave 62 mg of (XIX), m.p. 126–128°, $[\alpha]_D$ +98°, ν_{max} 1748 and 1730 cm^{-1}, no hydroxyl band, no high-intensity absorption in the ultraviolet, strong red colour with triphenyltetrazolium chloride.[21]

(Found: C, 72·16; H, 8·80; Calc. for $C_{25}H_{36}O_5$: C, 72·08; H, 8·71%.)

The presence of the Δ^4-bond was confirmed by the yellow colour produced with tetranitromethane and by the fact that (XIX) (3 mg) on treatment with *p*-toluenesulphonic acid (2 mg) in acetone (1 cc) for 1 hr at 60° was dehydrated to the $\Delta^{3,5}$-diene (XX) (λ_{max} 228 mμ, $\varepsilon = 17{,}000$).

Reduction of (Xb) *with lithium aluminium hydride followed by partial acetylation*

A solution of 4 g of (Xb) in tetrahydrofuran (100 cc) and ether (100 cc) was added dropwise in nitrogen to a stirred solution of lithium aluminium hydride (1·6 g) in ether (300 cc) with ice-cooling. The mixture was stirred for 90 min at room temperature and then for 20 min under reflux. The excess reagent was decomposed by the careful addition of ethyl acetate, and concentrated aqueous sodium sulphate added until the precipitate began to adhere to the sides of the flask. Solid sodium sulphate was added, the salts removed by filtration and washed well with tetrahydrofuran. Evaporation of solvent under reduced pressure yielded the crude triol (XXI, 3·6 g) showing a hydroxyl band but no carbonyl bands in the infra-red, no high-intensity absorption in the ultra-violet.

The above triol in dry pyridine (68 cc) was acetylated with acetic anhydride (1·65 g, 1·5 equivalents) at −10° for 18 hr. The product was isolated with ether in the usual way and chromatographed on alumina (200 g). Elution with benzene-chloroform (2:1) gave a mixture of diacetates which on direct oxidation with the chromium trioxide-pyridine complex as above, followed by two chromatographic purifications and crystallisation from pentane, yielded 120 mg of (XIX), m.p. 125–127°. The m.p. was not depressed on mixing with the above-described material, and the infra-red spectra were identical.

Further elution of the column with benzene-chloroform (5:3) gave a monoacetate fraction which on crystallisation from acetone-hexane yielded 1·06 g of the 21-monoacetate (XIV), m.p. 97 99°, $[\alpha]_D$ −46°, ν_{max} 1730 cm $^{-1}$ and free hydroxyl band.

(Found: C, 73·01; H, 9·84; acetyl, 11·38; Calc. for $C_{23}H_{36}O_4$: C, 73·36; H, 9·64; acetyl, 11·44%.)

Acetylation with excess acetic anhydride in pyridine at room temperature quantitatively produced the triacetate (XV), m.p. 129–131°. Oxidation of (XIV) with chromium trioxide-pyridine, as above, and crystallisation of the product from acetone-hexane gave desoxycorticosterone acetate (68%), m.p. 156–158°, λ_{max} 240 mμ, ε 15,800, ν_{max} 1748, 1736 and 1664 cm $^{-1}$. The m.p. was not depressed on mixing with an authentic specimen (m.p. 156–158°) and the infra-red spectra were identical.

Finally elution of the column with chloroform-methanol (19:1) produced the thiol (XXI, 0·84 g), needles from acetone, m.p. 162–163°, strong hydroxyl but no carbonyl bands in the infra-red (KBr).

(Found: C, 75·07; H, 10·57; Calc. for $C_{21}H_{34}O_3$: C, 75·40; H, 10·25%.)

The triol gave an immediate precipitate with digitonin in aqueous ethanol. On acetylation, the triacetate (XV) with m.p. and mixed m.p. 129-131° was produced in quantitative yield.

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