mole) of sulfur, and 13 g. (0.1 mole) of *t*-octylamine was stirred and heated at 150–175° for 2 hr. Another 13 g. (0.1 mole) of *t*-octylamine was added and the mixture was refluxed another 13 hr. The mixture was cooled, diluted with 200 ml. of heptane, and filtered. The residue was washed with heptane and dried to give 19 g. (0.61 mole) of unreacted sulfur. The combined filtrates were distilled to give 52 g. (98%) of unreacted *N*-(*t*-octyl)-6-methylthiopicolinamide (V), b.r. 135–140°/0.6 mm. Hg. There was 1 g. of residue.

Attempted quaternization of N-t-octylthiopicolinamide (I). A mixture of 25 g. (0.1 mole) of *N*-*t*-octylthiopicolinamide (I), 200 ml. of acetonitrile, and 20 g. (0.13 mole) of ethyl iodide was refluxed for 15 hr., cooled, and distilled free of ethyl iodide and acetonitrile. There remained a residue of 25 g. (0.1 mole) of unreacted *N*-*t*-octylthiopicolinamide (I).

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BRISTOL, PA.

[CONTRIBUTION FROM THE DEPARTMENT OF MEDICINE, WESTERN RESERVE UNIVERSITY AND THE UNIVERSITY HOSPITALS]

Preparation of Pregnane-3α,16α,20α-triol and of Two of Its Stereoisomers[1]

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The preparation of pregnane-3α,16α,20α-triol, of pregnane-3α,16α,20β-triol, and of pregnane-3β,16α,20β-triol is described and some of the characteristics of the infrared spectra of 16α-acetoxy steroids are pointed out.

Three 16α-hydroxysteroids with the pregnane skeleton have been isolated from urine. Two of these, allopregnane-3β,16α,20β-triol[2,3] and its 20-epimer[4,5] are found during pregnancy, while Δ⁵-pregnene-3β,16α,20α-triol[5] was encountered in a case of an adrenal tumor. The last observation suggested the possibility that the adrenal cortex might effect hydroxylations at C-16. This hypothesis was verified by Rao and Heard[6] and by Neher *et al.*[7] The Swiss group isolated 3β,-16α-dihydroxyallopregnan-20-one from an adrenal extract while the Canadian workers obtained isotopically labeled 16α-hydroxyprogesterone upon the incubation of tagged progesterone with an adrenal homogenate. If the metabolism of 16α-hydroxyprogesterone in man follows the normal pattern its chief urinary excretion product is not one of the known triols but pregnane-3α,16α,20α-triol. To facilitate the search for this compound we have carried out its synthesis from a degradation product of sapogenins, 3β-acetoxy-Δ¹⁶-pregnen-20-one.[8]

Two routes were explored. The first gave only a very low yield of the desired product, but proceeded in a stepwise manner which allowed one to deduce the structure of the final product with assurance. The 16-hydroxyl group was introduced into Ia by the benzyl alcohol method[9] which in the case of 3β-hydroxy-Δ⁵,¹⁶-pregnadien-20-one[10] and of Ib[11] has yielded hydroxy steroids with the α configuration at C-16 and the normal orientation of the side chain. The rotations of the triacetates IVb, VIIIb, and IXb corroborate these assignments also for our conversion of Ia to III. The formate group at C-3 even in the axial orientation is sufficiently reactive to allow its selective hydrolysis as was required in the conversion of III to V. The product although formulated as a 3β-hydroxysteroid failed to precipitate with digitonin. However, the structure of V follows from the disappearance of the strong formate absorption at 8.45 μ and the retention of the acetate band at 8.06 μ. The free hydroxyl group of V was oxidized with chromic acid to the acetoxydiketone VI which was reduced selectively with sodium borohydride in isopropanol[12,13] and pyr-

(1) This investigation was supported by grant C-1679 of the National Institutes of Health, U. S. Public Health Service.

(2) G. A. D. Haslewood, G. F. Marrian, and E. R. Smith, *Biochem. J.*, **28**, 1316 (1934).

(3) H. Hirschmann, F. B. Hirschmann, and M. A. Daus, *J. Biol. Chem.*, **178**, 751 (1949).

(4) S. Lieberman, B. Praetz, P. Humphries, and K. Dobriner, *J. Biol. Chem.*, **204**, 491 (1953).

(5) H. Hirschmann and F. B. Hirschmann, *J. Biol. Chem.*, **184**, 259 (1950).

(6) B. G. Rao and R. D. H. Heard, *Arch. Biochem. and Biophys.*, **66**, 504 (1957).

(7) R. Neher, P. Desaulles, E. Vischer, P. Wieland, and A. Wettstein, *Helv. Chim. Acta*, **41**, 1667 (1958).

(8) We are greatly indebted to Dr. J. J. Pfiffner of Parke, Davis and Company for a gift of this compound.

(9) H. Hirschmann, F. B. Hirschmann, and J. W. Corcoran, *J. Org. Chem.*, **20**, 572 (1955).

(10) H. Hirschmann, F. B. Hirschmann, and M. A. Daus, *J. Am. Chem. Soc.*, **74**, 539 (1952).

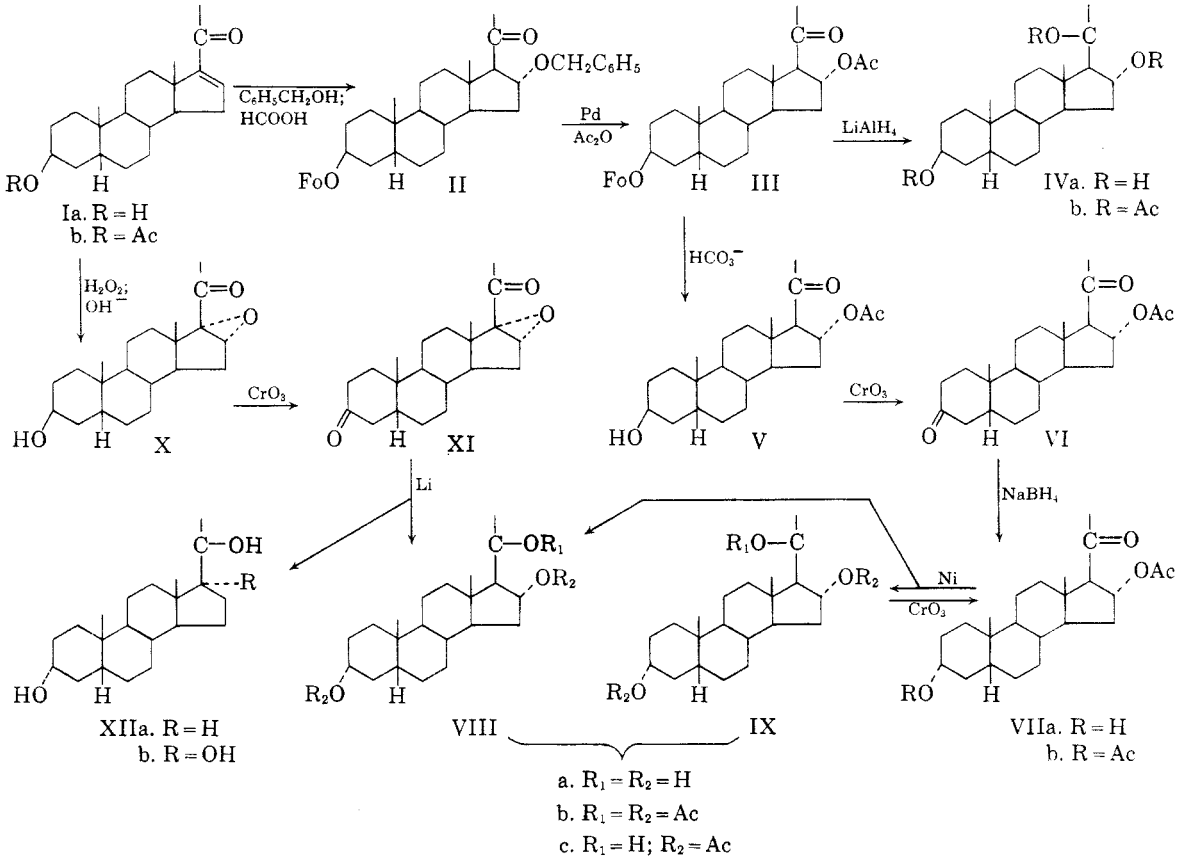
(11) H. Hirschmann and F. B. Hirschmann, *J. Am. Chem. Soc.*, **78**, 3755 (1956).

(12) H. C. Brown, E. J. Mead, and B. C. Subba Rao, *J. Am. Chem. Soc.*, **77**, 6209 (1955).

(13) The use of this solvent instead of methanol stabilized with alkali[14b] seems advantageous for the partial reduction of ketones (such as VI) which are sensitive to alkali.

idine[14] to a hydroxyacetoxyketone VIIa. The expected site for this reduction is the ketone group at C-3.[14] Evidence for the retention of the 20-keto-group was obtained by the exposure of VIIb to alkali which caused the appearance of a strong band at 239 mμ characteristic of an α-β unsaturated ketone. Compound VIIa, therefore, must have retained the 16-acetoxy-20-ketone grouping and consequently possesses its free hydroxyl group at C-3. The α configuration of this hydroxyl group is consistent with the principal course of reduction of other 5β 3-ketosteroids by sodium borohydride,[14] with the non identity of VIIa and V and with the shorter wave lengths[15,16] of the alkyl oxygen vibrations at C-3 of VIIa and b as compared to those of V and of 3β,16α-diacetoxypregnan-20-one[11] (9.78μ).

which was assigned the 20β configuration on the basis of its rotation.[17] In view of this failure to obtain the α-isomer, compound VIIb was subjected to an alternative reduction method, hydrogenation with Raney nickel,[9] which gave a mixture of the two diacetates VIIIc and IXc in which the β-isomer predominated. Again configurations were determined by comparing rotations of the di- and triacetates with the values computed for the two epimeric series. The resulting assignments were confirmed by infrared spectroscopy. Only one isomer showed hydrogen bonding between the hydroxyl group and ester carbonyl. The required close approach of these groups is possible only[18] if the hydrogen rather than the methyl at C-20 is directed towards the angular methyl group.[5,19] In agreement with this expectation, the α-isomer



Reductions with lithium aluminum hydride of 20-ketones containing either a hydroxy[9] or ether[5,10] group at the 16α position had furnished both epimers of the 20-hydroxy steroids. However, when the 16α-acetoxy-20-ketone III was reduced by the same method only one triol IVa could be isolated

was the one to show hydrogen bonding. An anomaly in R_F values probably can be explained on the same basis. In paper chromatographic separations of simple 20-epimeric alcohols the α-isomer if

(14) (a) O. Mancera, H. J. Ringold, C. Djerassi, G. Rosenkranz, and F. Sondheimer, *J. Am. Chem. Soc.*, **75**, 1286 (1953); (b) A. H. Soloway, A. S. Deutsch, and T. F. Gallagher, *J. Am. Chem. Soc.*, **75**, 2356 (1953), and references cited in these papers.

(15) J. E. Page, *J. Chem. Soc.*, 2017 (1955).

(16) R. N. Jones and F. Herling, *J. Am. Chem. Soc.*, **78**, 1152 (1956).

(17) L. H. Sarett, *J. Am. Chem. Soc.*, **71**, 1175 (1949).

(18) It is assumed that the 16α-acetoxy group is in the usual coplanar conformation of an ester group (G. W. Wheland, *Resonance in Organic Chemistry*, J. Wiley and Sons, Inc., New York, 1955, p. 235). According to L. P. Kuhn [*J. Am. Chem. Soc.*, **74**, 2492 (1952)] hydrogen bonding to oxygen is possible only if the distance of these atoms is less than 3.3 Å.

(19) J. W. Corcoran and H. Hirschmann, *J. Am. Chem. Soc.*, **78**, 2325 (1956).

separated at all traveled slower[20] suggesting a somewhat greater solubility in the hydroxylic phase. In contrast the 20-α-isomer VIIIa traveled 3 times as fast as the 20β compound IXa. This behavior is understandable if internal hydrogen bonding of VIIIa would diminish its solvation with the hydroxylic solvent and thereby reduce its solubility in the stationary phase. In agreement with this view the spectrum of crystalline VIIIa but not of IXa showed a shoulder in the hydroxyl region indicative of intramolecular hydrogen bonding.

A far shorter route to pregnane-3α,16α,20α-triol was suggested by the work of Camerino and Alberti[21] who obtained Δ^5-pregnene-3β,16α,20α-triol by reduction of 3β-hydroxy-16α,17α-epoxy-Δ^5-pregnen-20-one with sodium and alcohol. Application of this method to 16α,17α-epoxypregnane-3,20-dione (XI)[22] gave a rather large amount of oily by-products, but this could be avoided when the reduction was carried out with lithium in ethylamine.[24] The desired pregnane-3α,16α,-20α-triol was the main product, but its purification was complicated by the presence of pregnane-3α,20α-diol (XIIa) and of pregnane-3α,17α,20α-triol (XIIb) which could not be removed by recrystallization. Other impurities probably included the 20β-isomers of VIIIa, XIIa, and XIIb. Oxidation with periodic acid in the presence of sulfuric acid[25] removed the 17,20-glycols and countercurrent distribution followed by recrystallization gave pregnane-3α,16α,20α-triol identical in every respect with the sample obtained by the stepwise synthesis.

When the spectroscopic measurements obtained in the present study were compared with earlier findings[3,5,9-11] certain regularities emerged which seem to possess diagnostic significance. All 16α-acetoxy-20-ketones that could be examined in carbon disulfide[26] in sufficient concentration and with sufficient resolution to permit reliable measurement of the ketone peak showed a maximum at 5.84 μ (7 examples), while 16α-benzyloxy-20-ketones possessed the normal peak for 20-ketones (5.86 μ). Shifts in this direction have been observed for many steroid ketones with an acetoxy group at the α carbon atom.[27] Those now reported for an ester group at the β carbon are rather small if the substituents are *trans* to each other but a larger displacement (to 5.82 μ) was noted with a 16β-acyloxy-20-ketone, the oxidation product of pseudotigogenin diacetate.

As far as we are aware, the steroidal 3-formates previously studied were all of the equatorial type (3β-formoxy-Δ^5 or 3α-formoxy-5β) and had an acyl-oxygen absorption wave length of 8.48 $\pm$ 0.01μ [9,28] or longer.[29a] Two 3β-formoxy-5β compounds (II and III) both absorbed at 8.45 μ[29b] and this band in spite of the axial orientation of the ester group had a simple contour. Evidently axial acetates[16] and formates differ in this respect.

The examination of five 3α,16α,20β-triacetoxy steroids revealed the presence of 4 bands in the finger print region which were of constant wave length within rather close limits ($\pm$ 0.02 μ). These were a strong band at 9.25 or 9.26 μ, two bands of medium intensity at 10.69 $\pm$ 0.01 and 10.80 $\pm$ 0.02, and a weak one at 9.00 or 9.01 μ (1081–1080, 936–935, 928–924, 1111–1110 cm.$^{-1}$). In addition, compounds with a 5α-hydrogen or a 5–6 double bond had common peaks at 8.85 to 8.88 μ, 9.54 $\pm$ 0.01 μ, and 10.53 μ (1130–1126, 1049–1047, 950 cm.$^{-1}$) while those of the 5β-series absorbed at 8.89 $\pm$ 1, 9.41, and 10.48 or 10.49 μ (1126–1124, 1063, 954–953 cm.$^{-1}$). The common finger print bands of three 3,16α,20α-triacetoxy-steroids with either a double bond or an α or β hydrogen at C-5 were at 8.67 + 0.01, 10.50 $\pm$ 0.01 (both fairly intense) 8.89, 9.05 $\pm$ 0.02 and 10.62 $\pm$ 0.02 μ (1153–1152, 953–951, 1125, 1107–1103, 943–940 cm^{-1}). The number of characteristic frequencies is smaller than those listed[16] for simple 20α or β-acetoxy-5α-steroids without another substituent in the vicinity of C-20. As would be expected several of our bands differ from those described for the simpler structures, but a number of others including the important H and I bands appear to be but little affected by the introduction of the

(20) H. S. Bloch, B. Zimmermann, and S. L. Cohen, *J. Clin. Endocrinol.*, **13**, 1206 (1953).

(21) B. Camerino and C. G. Alberti, *Gazz. chim. ital.*, **85**, 56 (1955).

(22) The method of P. L. Julian, C. C. Cochrane, A. Magnani, and W. J. Karpel [*J. Am. Chem. Soc.*, **78**, 3153 (1956)] which was used in the preparation of X has given in other cases almost exclusively[23] the 16α17α-epoxide. The rotation of X confirms this assignment. After the preparation of X and XI was completed, Kenney, Weaver, and Wall [*J. Am. Chem. Soc.*, **80**, 5568 (1958)] described the synthesis of these compounds from Ib. The melting points agree but the rotation of our X differs widely from that of their product (wrong sign ?). XI was obtained by a different route by Mancera *et al.*[14a]

(23) B. Löken, S. Kaufmann, G. Rosenkranz, and F. Sondheimer, *J. Am. Chem. Soc.*, **78**, 1738 (1956).

(24) (a) R. A. Benkeser, R. E. Robinson, D. M. Sauve, and O. H. Thomas, *J. Am. Chem. Soc.*, **76**, 631 (1954); (b) A. S. Hallsworth and H. B. Henbest, *J. Chem. Soc.*, 4604 (1957).

(25) R. I. Cox, *Biochem. J.*, **52**, 339 (1952).

(26) Except when noted otherwise all infrared measurements and comparison data refer to solutions in carbon disulfide.

(27) R. N. Jones, P. Humphries, F. Herling, and K. Dobriner, *J. Am. Chem. Soc.*, **74**, 2820 (1952); D. H. W. Dickson and J. E. Page, *J. Chem. Soc.*, 447 (1955); G. Roberts, B. S. Gallagher, and R. N. Jones, *Infrared Absorption Spectra of Steroids*, Vol. 2, Interscience Publishers, Inc., New York, 1958, p. 22.

(28) R. N. Jones, P. Humphries, F. Herling, and K. Dobriner, *J. Am. Chem. Soc.*, **73**, 3215 (1951); E. S. Rothman and M. E. Wall, *J. Am. Chem. Soc.*, **81**, 411 (1959).

(29a) S. Archer, T. R. Lewis, C. M. Martini, and M. Jackman, *J. Am. Chem. Soc.*, **76**, 4915 (1954).

(29b) After submission of this paper we noted three examples of axial 3-formates with maxima at 8.44 and 8.45 μ. [F. C. Chang and R. T. Blickenstaff, *J. Am. Chem. Sec.*, **80**, 2906 (1958)].

16α-acetoxy group. Due to its greater variability the C-16-oxygen stretching vibration of 16α-acetates is probably not included in these listings. This peak is most likely identical with the strongest maximum in the 10 μ region in the spectra of 16α-acetoxyprogesterone[9] and 16α-acetoxypregnane-3,20-dione (9.62 and 9.67 μ, respectively). The presence of a 16-acetate band can be obscured in steroids containing a carbon-oxygen single bond at C-3 if its stretching vibration also falls into the 9.6 to 9.7 μ region. Accordingly,[16] only a single peak was seen in the spectra of 3β-acetoxy-Δ⁵ and 3β-hydroxy-5β compounds, but suggestive evidence for a second vibration was obtained from the curves of 3β-acetoxy-5α steroids in the displacement of the C-3 maximum from its normal range[16] to shorter wave lengths (9.68 to 9.70 μ). The remaining 14 spectra of 16α-acetates showed aside from the C-3 peak either another clearly resolved strong peak or at least a well defined shoulder on the C-3 stretching maximum. These observations comprised 20-hydroxysteroids of either configuration at C-20, their acetates, 20-ketones and a methyl etianate. There was no apparent correlation between band position (9.60–9.69 μ) and the nature of the side chain. In view of the constancy of the phenomenon, and the intensity of the absorption, which in several cases surpassed that of the C-3 peak, it seems probable that the band near 9.64 μ (1037 cm^{-1}) represents the C-16-oxygen stretching vibration of 16α-acetoxypregnanes.

EXPERIMENTAL[26,30]

Reports on infrared maxima are generally limited to those signifying functional groups such as hydroxyl (~2.78), 20-ketone (~5.86), acetate (~5.75 and 8.06) and formate (~5.80 and 8.45 μ) and to the carbon-oxygen bands in the 9.6 to 9.8 μ range. However, all maxima and inflections except very weak ones (strong ones underlined) are listed for the triacetates IVb, VIIIb, and IXb in the range 7.55 to 11.4 μ and for VIIIa from 7.4 to 12.5 μ.

3β-Formoxy-16α-benzyloxypregnan-20-one (II). The treatment of 500 mg. of 3β-hydroxy-Δ¹⁶-pregnen-20-one (Ia) with 15 ml. of a 3% solution of potassium hydroxide in benzyl alcohol was carried out for 3 hr. at 24° as described previously.[9,10] The crude reaction product was dissolved in 28 ml. of benzene and 14 ml. of 98–100% formic acid[9] and kept at 50–55° for 90 min. under anhydrous conditions while the pressure was reduced sufficiently to permit the distillation of 20 ml. of liquid. The reaction product (741 mg.) was fractionated on a column of 33 gm. of silica gel–Celite into the formate of (I) and into compound II by elution with mixtures of benzene and petroleum ether (1:2 to 2:1) and with benzene. The later eluates were recrystallized from acetone to give 313 mg. of 3β-formoxy-16α-benzyloxypregnan-20-one (II) melting at 153–158°. The analytical sample showed m.p. 157–159.5° and λ_{max} 5.80, 5.86, 8.45, 13.65, and 14.36 μ (last two benzyloxy[9–11]).

Anal. Calcd. for $C_{29}H_{40}O_4$: C, 76.95; H, 8.91. Found: C, 76.60; H, 8.94.

It should be noted that the more readily available 3β-acetoxy-Δ¹⁶-pregnen-20-one (Ib) could not be used in place of (Ia) as starting compound in the benzylation reaction since the alcoholysis of the acetate at C-3 was incomplete under the above conditions. We have obtained Ia from its acetate by heating 310 mg. of the latter in 122 ml. of dioxane and 48.8 ml. of 2.5% aqueous tetramethylammonium hydroxide under a reflux for 2 hr. and recrystallization of the reaction product from acetone–petroleum ether to give Ia with m.p. 183–185°.[32] After the completion of the hydrolysis experiment another procedure which minimizes addition reactions to the double bond was described.[33]

3β-Formoxy-16α-acetoxypregnan-20-one (III). A solution of 191 mg. of 3β-formoxy-16α-benzyloxypregnan-20-one (II) in 153 ml. of 95% ethanol was stirred magnetically for 2 hr. in the presence of hydrogen and of 154 mg. of prereduced and washed[9] palladium (5%) on charcoal. After the removal of catalyst and solvent the product was dissolved in 2 ml. of pyridine and acetylated with 1 ml. of acetic anhydride at 25° for 16 hr. The acetate was recrystallized from dilute methanol. The mother liquors were chromatographed on silica gel–Celite. The yield was 135 mg. 3β-Formoxy-16α-acetoxypregnan-20-one (III) melted at 102.5–105.5° and had $[\alpha]_D^{25}$ +37° (*c* 0.7) and λ_{max} 5.75, 5.81 (unresolved formate and ketone), 8.06, 8.45, 9.67 (C-16) μ.

Anal. Calcd. for $C_{24}H_{36}O_5$: C, 71.25; H, 8.97. Found: C, 71.11; H, 9.04.

3β-Hydroxy-16α-acetoxypregnan-20-one (V). A mixture of 152 mg. of 3β-formoxy-16α-acetoxypregnan-20-one (III) in 51 ml. of methanol and of 1.53 ml. of 1*N* aqueous potassium bicarbonate was swirled until the precipitate was dissolved, was kept at 23° for 6 hr. and then distributed between 600 ml. of ether and 300 ml. of water. The ether phase was washed neutral and gave 148 mg. of residue which was chromatographed on a column of 7.4 gm. of silica gel–Celite with benzene containing 10% ether as the eluant. The later eluates (91 mg.) were recrystallized from ligroin and gave 83 mg. of V, while the earlier fractions (47 mg.) were mostly starting material which on rehydrolysis furnished an additional 19 mg. of V. These two zones were separated by higher melting material from which a product with the infrared spectrum of 3β-hydroxy-Δ¹⁶-pregnen-20-one and the m.p. 179–181.5° was isolated. Another higher melting fraction (dihydroxyketone?) was obtained by elution with benzene containing 50% ether. In view of these side products the two-step hydrolysis procedure was preferred to a single longer hydrolysis period.

3β-Hydroxy-16α-acetoxypregnan-20-one showed a m.p. of 134–135.5° or a double m.p. (89° and 137°), $[\alpha]_D^{27}$ +43° (*c* = 0.7) and λ_{max} at 2.77, 5.74, 5.84, 8.06, and 9.70 μ. A solution of 0.2 mg. of compound V in 0.01 ml. of 80% ethanol when mixed with 1 or 2 volumes of 1% digitonin in the same solvent showed no precipitate.

Anal. Calcd. for $C_{23}H_{36}O_4$: C, 73.36; H, 9.64. Found: C, 73.57; H, 9.65.

16α-Acetoxypregnane-3,20-dione (VI). A solution of 149 mg. of 3β-hydroxy-16α-acetoxypregnan-20-one (V) in 14.8 ml. of acetic acid was treated with an equimolar amount of

(30) All melting points reported are corrected. Rotations were measured in 95% ethanol except when noted otherwise. The extractions and washings of 16-oxygenated 20-ketones were done rapidly in a cold room with chilled solvents and solutions. The silica gel–Celite mixture used in absorption chromatography was 2:1 and was prewashed as described.[19] The ligroin had b.p. 90–96°, the petroleum ether 60–70°. Paper chromatograms were run in the solvent system toluene, isooctane, methanol, and water (15:5:16:4)[31] and examined after reaction with a 10% solution of phosphomolybdic acid in ethanol and heating to 96° for 1 min.

(31) C. de Courcy, *J. Endocrinol.*, **14**, 164 (1956).

(32) R. E. Marker and co-workers [*J. Am. Chem. Soc.*, **62**, 3350 (1940); **64**, 210, 468 (1942)] reported a variety of melting points (188–190°, 169–172°, 180–183°), Wall *et al.*[33] gave 186–188°.

(33) M. E. Wall, H. E. Kenney, and E. S. Rothman, *J. Am. Chem. Soc.*, **77**, 5665 (1955).

chromium trioxide in 2 ml. of 90% acetic acid at 25° for 3 hr. The excess oxidant was reduced with methanol and the mixture taken up in ether and then washed with water, dilute sodium carbonate, and water. The neutral product was recrystallized from ligroin to yield 135 mg. of compound VI melting at 167–169°. The analytical sample melted at 168.5–169.5° and occasionally on reheating at 164°. $[\alpha]_D^{26}$ +51° (*c* 0.6). The spectrum showed λ_{max} 5.74, 5.83 (unresolved), 8.06, 9.67 μ, but no hydroxyl absorption.

Anal. Calcd. for $C_{23}H_{34}O_4$: C, 73.76; H, 9.15. Found: C, 74.04; H, 9.33.

3α-Hydroxy-16α-acetoxypregnan-20-one (VIIa). A solution of 109.6 mg. of 16α-acetoxypregnane-3,20-dione (VI) (0.293 mM.) in 11.7 ml. of isopropanol (distilled after a 2-hour treatment with sodium borohydride) was mixed with 2.93 ml. of a 0.05*M* solution of sodium borohydride (purified according to Brown *et al.*[12] and assayed according to Lyttle *et al.*[34]) in pyridine and kept under nitrogen for 20 min. at 25°. The mixture was chilled, acidified, and distributed between hydrochloric acid and ether. The ether layer was washed with sodium bicarbonate and with water and yielded 108 mg. of an oil which was chromatographed on silica gel–Celite with benzene containing 10 to 20% ether. The later eluates were recrystallized from ligroin to yield VIIa melting at 108–110° with infrared peaks at 2.78, 5.75, 5.84, 8.06, 9.59 (shoulder, C-3), and 9.68 μ (C-16).

Anal. Calcd. for $C_{23}H_{36}O_4$: C, 73.36; H, 9.64. Found: C, 73.46; H, 9.97.

For preparatory purposes it is advantageous to purify the reduction product after chromatography by acetylation and recrystallization. In this manner 255 mg. of VI gave 118 mg. of pure VIIb. Spectrographic examination of the earlier eluates (not acetylated) disclosed the presence of VI and of Δ^{16}-20-ketone.

3α,16α-Diacetoxypregnan-20-one (VIIb). Sixty-six mg. of 3α-hydroxy-16α-acetoxypregnan-20-one (VIIa) were acetylated with 1 ml. of pyridine and 0.5 ml. of acetic anhydride at 26° for 16 hr. The acetate was recrystallized 3 times from ligroin and gave 66 mg. of compound VIIb melting at 127–129° with λ_{max} 5.75, 5.84, 8.08, 9.61 (shoulder, C-16), and 9.73 μ (C-3). A solution of 0.16 mg. of VIIb in 1.5 ml. of 2% potassium hydroxide in *t*-butanol[33] was kept at 29° for 3.5 hr. The neutral reaction product in 10 ml. of 95% ethanol showed an absorption maximum at 239 mμ with an absorbance of 0.320 for a 1-cm. path length.

Reduction of 3α,16α-diacetoxypregnan-20-one (VIIb). A solution of 111 mg. of VIIb in 16 ml. of 95% ethanol and of 807 mg. of Raney nickel[35] were shaken with hydrogen for 9 hr. The catalyst was removed by centrifugation and the product (113 mg.) chromatographed on silica gel–Celite. The 20β isomer predominated in the earlier eluates (100 mg. with benzene containing up to 10% ether), the epimer in the later eluates (13 mg., with benzene containing 10 to 15% ether). To increase the proportion of the α isomer, the β-fractions were reoxidized with chromium trioxide in acetic acid for 2 hr. at 25° and the reaction product again reduced with nickel. The catalyst prepared according to Mozingo,[35] while less active than W-5,[36] gave a higher proportion of the α-isomer.

The main product *3α,16α-diacetoxypregnan-20β-ol* (IXc) was purified by recrystallization from petroleum ether. It showed m.p. 97–102°, $[\alpha]_D^{20}$ −43° (*c* 0.5) and λ_{max} 2.77, 5.76, 8.07, 9.60 (C-16), and 9.74 μ (C-3).

Anal. Calcd. for $C_{25}H_{40}O_5$: C, 71.39; H, 9.59. Calcd. for $C_{25}H_{40}O_5 \cdot 0.25(C_6H_{14})$: C, 71.99; H, 9.92. Found: C, 72.02; H, 9.83.

Compound IXc (18 mg.) was treated with 3 ml. of pyridine and 1.5 ml. of acetic anhydride at room temperature to yield 20 mg. of the amorphous *triacetate* (IXb) which showed $[\alpha]_D^{23}$ −19° (*c* 1.0) and λ_{max} ~8.08, 8.23, 8.37, 8.55, 8.90, 9.01, 9.26, 9.41, 9.61, 9.73, 9.87 (shoulder), 10.18, 10.30, 10.49, 10.69, 10.78, and ~11.28 μ. There was no hydroxyl peak. A mixture of 19 mg. of the triacetate, 60 mg. of sodium hydroxide, and 11 ml. of 80% ethanol were heated under a reflux for 100 min. The resulting *pregnane-3α,16α,20β-triol* (IXa) was recrystallized from acetone. Two modifications with m.p. 200–203° and 214–216° were observed. Paper chromatography showed a single spot with R_F = 0.08.

The fractions containing *3α,16α-diacetoxypregnan-20α-ol* (VIIIc) were purified by rechromatographing and recrystallizing from ligroin, dilute methanol, and from petroleum ether. The diacetate melted at 129–131° and had $[\alpha]_D^{23}$ −33° (*c* 0.3). The main hydroxyl peak was at 2.86 and a lesser one at 2.78 μ, the major acetate peak at 5.76, and the hydrogen bonded one at 5.82 μ. The ester peak at 8.07 had a shoulder at 7.96 μ. The alkyl oxygen stretching bands were at 9.74 (C-3) and 9.62 μ (shoulder, C-16). The compound was converted to the triacetate VIIIb and triol VIIIa by the procedures described for IXc. The *triacetate* again failed to crystallize. It showed $[\alpha]_D^{19}$ −27° (*c* 0.5) and λ_{max} ~8.06, 8.23, 8.39, 8.55, 8.67, 8.89, 8.94, 9.07, 9.18, 9.34, 9.65, 9.74, 9.83 (shoulder), 10.20, 10.51, 10.64 (shoulder), 10.76, 11.05, and 11.29 μ. *Pregnane-3α,16α,20α-triol* (VIIIa) was recrystallized from acetone and from dilute alcohol. It melted at 221–223° and showed in a KBr pressing λ_{max} 2.80 (shoulder), ~3.00, 7.48, 7.52 (shoulder), 7.75, 7.92, 8.03, 8.11, 8.24, 8.54, ~8.63, 8.96, 9.08, 9.18, 9.34, 9.56, 9.70, 9.81, 9.86 (shoulder), 10.01, 10.19, 10.46, 10.56, 10.71, 10.88, 11.11, 11.37, ~11.44 (shoulder), 11.72, and 11.96 μ. Paper chromatography revealed a single spot with R_F 0.26. The trichloroacetic acid fluorescence reaction[31] was negative.

Pregnane-3β,16α,20β-triol (IVa). A mixture of 79 mg. of 3β-formoxy-16α-acetoxypregnan-20-one (III), 210 mg. of lithium aluminum hydride, and 95 ml. of dry ether was made up at 0° and stirred at room temperature for 75 min. The crystalline product was acetylated and the resulting amorphous acetate chromatographed on alumina. Since the various eluates showed only minor differences in their infrared spectra they were combined, hydrolyzed, and recrystallized from mixtures of methanol and benzene to yield pregnane-3β,16α,20β-triol with m.p. 240–242° and rotation $[\alpha]_D$ −24° (*c* 0.6). On paper chromatography a single spot with extensive trailing was observed ($R_F \leqq 0.10$).

Anal. Calcd. for $C_{21}H_{36}O_3$: C, 74.95; H, 10.78. Found: C, 75.01; H, 10.79.

Reacetylation gave triacetate IVb with $[\alpha]_D$ −39° (*c* 0.9) and λ_{max} ~8.09, 8.25 (shoulder), 8.40, 8.46 (shoulder), 8.66, 8.88, 9.01, 9.26, 9.41, 9.63, 9.77, 9.87 (shoulder), 10.13, 10.28, 10.40, 10.48, 10.70, 10.78, 10.99 and 11.20 μ.

3β-Hydroxy-16α,17α-epoxypregnan-20-one (X). A solution of 591 mg. of 3β-acetoxy-Δ^{16}-pregnen-20-one (Ib) in 45 ml. of methanol was cooled to 7° and mixed with 1.1 ml. of 4*N* aqueous sodium hydroxide and 2.2 ml. of 30% hydrogen peroxide. The mixture was kept at 22° for 18 hr. and distributed between ether and water. The ether phase was washed with water, ferrous sulfate, water, acetic acid, water, sodium carbonate, and water and gave 601 mg. of residue. This material, 1 g. of potassium hydroxide and 40 ml. of methanol were heated under a reflux for 2 hr. The neutral product (537 mg.), recrystallized from dilute acetone, gave 308 mg. of 3β-hydroxy-16α,17α-epoxypregnan-20-one with m.p. 225–229°. Chromatography of the mother liquors (alumina, benzene) and recrystallization furnished an additional 90 mg. The final product had m.p. 227–229°, $[\alpha]_D^{21}$ +62° (*c* 0.87, 95% ethanol[37]) and +57° (*c* 0.7, acetone) and λ_{max} 2.77 and 5.86 μ.

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(37) This solution proved to be supersaturated. Although no crystallization could be detected during the measurement, the rotation was repeated in acetone.

Anal. Calcd. for $C_{21}H_{32}O_3$: C, 75.86; H, 9.70. Found: C, 75.25; H, 9.36.

16α,17α-Epoxypregnane-3,20-dione (XI). A solution of 149 mg. of 3β-hydroxy-16α,17α-epoxypregnan-20-one (X) in 7.3 ml. of acetic acid was oxidized with chromium trioxide as described for (V) and the product recrystallized from ligroin to yield 122 mg. of XI which melted at 171–174°. λ_{max} ~5.84 μ (unresolved).

Anal. Calcd. for $C_{21}H_{30}O_3$: C, 76.32; H, 9.15. Found: C, 75.91; H, 9.19.

Reduction of 16α,17α-epoxypregnane-3,20-dione (XI). Ethylamine (25 ml.) was distilled from barium oxide into a two-neck flask containing 56 mg. of the epoxydione XI. The receiving vessel which was equipped with a soda lime tube and a magnetic stirrer was immersed in an ice salt bath. The still was removed, 190 mg. of lithium were added, and the vessel was stoppered. The mixture was stirred in a bath maintained at 0°. After 25 min. the blue color was discharged periodically by the addition of dry ethanol (4 portions of 0.5 ml.). When all of the lithium had been dissolved and ionized, the solvent was evaporated in a current of nitrogen at room temperature. The residue was distributed between ether and hydrochloric acid. The organic phase was washed free of acid and gave 58 mg. of crystalline residue. Partition chromatography on silica gel (solvent system ethyl acetate, iso-octane, methanol, and water 150:300:135:315) followed by recrystallization gave products which had satisfactory m.p. but contained impurities with R_F 0.33 and 0.64. The reaction product was subjected to a 50 transfer countercurrent distribution between phases prepared from iso-octane, toluene, methanol, and water 125:375:360:140. Impurities with R_F 0.07 (IXa?) and 0.12 were found only in the first 2 tubes. The contents of tubes 15–21 (which contained mainly the product with R_F 0.64 (XIIa) and a little of one with R_F 0.70) were recrystallized, acetylated, and recrystallized to give pregnanediol diacetate identified by its double m.p. 180–181°, 166.5°), mixture m.p. and infrared spectrum. VIIa was found in tubes 1–10 but only the first 3 were free of an impurity identified as pregnane-3α,17α,20α-triol by its R_F (0.33), by its fluorescence reaction with trichloroacetic acid[31] and by the conversion to a 17-ketosteroid by means of periodic acid. To free VIIIa completely from XIIb and its 20-epimer (R_F 0.41), 9.8 mg. of such a mixture in 1 ml. of acetic acid were treated with 1 ml. of a 0.2*M* solution of periodic acid in 1*N* aqueous sulfuric acid for 2.5 hr. at 26°. The neutral reaction product was recrystallized from acetone. Pregnane-3α,16α,20α-triol prepared from XI melted at 223.5–224° and agreed with the sample obtained from VII in the mobility on paper, and in the infrared spectra of the triacetate and of the free triol. There was no depression of m.p. on admixture. The yield was 14 mg.

Anal. Calcd. for $C_{21}H_{36}O_3$: C, 74.95; H, 10.78. Found: C, 75.36; H, 10.47.

Reference data for rotations. Approximate values for the molecular rotations in ethanol were computed from the set of standard values given by Barton and Klyne[38] and from the molecular rotations of allopregnane-3β,16α,20β-triol[39] −54°, of its triacetate[3] −208°, of allopregnane-3β,16α,20α-triol triacetate[5] −268° (all 3 in alcohol), of allopregnane-3β,20β-diol +12° (average)[40] and of its diacetate[41] +89° (both in chloroform). The calculated figures are compared with the experimental values which are given in parentheses: Compound IVa −50° (−82°), IVb −153° (−181°) as compared to pregnane-3β,16α,20α-triol +8°, triacetate −213°, compound VIIIc −112° (−140°), IXc −170° (−180°). $\Delta[M]_D^{VIIIb-VIIIc}$ +17°, $\Delta[M]_D^{IXb-IXc}$ +91° as compared to $\Delta[M]_D^{OAc-OH}$ for the acetylation of the 20-hydroxyl group of pregnane-3α,20ξ-diol, 20α −10° and 20β +105°.[17]

The molecular rotation differences between 3β-acetoxy-Δ⁵-pregnen-20-one[42] and its 16α 17α and 16β,17β epoxide[23] are −87° and −452° (in chloroform), respectively, while the one between 3β-hydroxypregnan-20-one[43] and X equaled −115° in ethanol.

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