precipitate was filtered and dried in a vacuum at 100°; recovered 60.0 mg. of digitonide, calcd. 62.9 mg.

N-Nitrosotomatidine.—To 56 mg. of tomatidine dissolved in 1 cc. of ethanol and 0.1 cc. of acetic acid was added 28.5 mg. of sodium nitrite dissolved in a few drops of water. After standing for a short time, addition of several drops of water and scratching induced the formation of hexagonally shaped crystals. The compound gradually changed to flat rods before melting at 234–237°. When the substance was heated beyond its melting point, evolution of gas was observed; λ_{max} 233 mμ, log ϵ 3.87; λ_{max} 360, log ϵ 1.83 (ethanol).

Anal. Calcd. for $C_{27}H_{44}N_2O_3$: N, 6.30. Found: N, 6.36.

N,O-Diacetyltomatidine.—This derivative was prepared in the usual manner with acetic anhydride and pyridine. After recrystallization from acetone and ether, the material melted unsharply at 192° (sintering at 185°), lit. 193–194°, evac. tube,[5] 192–194° uncor.[3b] Chromatographic purification (benzene–petroleum ether) did not improve the melting point.

Anal. Calcd. for $C_{31}H_{49}NO_4$: C, 74.50; H, 9.88. $C_{31}H_{47}NO_4$: C, 74.81; H, 9.52. Found: C, 74.66; H, 9.92.

Compound A.—A mixture of 616 mg. of tomatidine and 12 cc. of acetic anhydride was refluxed vigorously in an oil-bath for two hours. The solvent was removed *in vacuo*, and the oily residue dissolved in ethanol. Water was added to the point of slight turbidity and the compound allowed to crystallize. It formed shiny plates melting at 97–105°. After one recrystallization from ethanol–water 450 mg. of a compound melting at 103.5–107° was obtained. An additional 50 mg. was recovered upon concentration. For analysis a sample was recrystallized twice, m.p. 105–107°, $[\alpha]^{20}D$ −11.6° (*c* 1.25, chloroform).

Anal. Calcd. for $C_{33}H_{51}NO_5$: C, 73.16; H, 9.49; N, 2.59; $COCH_3$ (three), 23.8. Found: C, 73.12; H, 9.78; N, 2.66; $COCH_3$, 21.8 (alkaline hydrolysis).

Catalytic Hydrogenation of Compound A.—A mixture of 50.5 mg. of A and 51 mg. of platinum oxide (prereduced) in 8.0 cc. of glacial acetic acid absorbed in approximately 2 hours a slight excess above the calculated amount (1 mole) of hydrogen, when the absorption ceased. After filtration of the catalyst, the solvent was evaporated *in vacuo*, the residue dissolved in ethanol and water added. The reaction product seemed to be contaminated slightly with starting material, but after several recrystallizations from ethanol–water flat rods of melting point 119–122° were obtained. No hydrogenation took place in an ethanolic solution of A.

Anal. Calcd. for $C_{33}H_{53}NO_5$: C, 72.89; H, 9.83. Found: C, 72.98; H, 9.82.

Chromic Acid Oxidation of Compound A.—To a solution of 0.600 g. of A in 20 cc. of glacial acetic acid was added dropwise with stirring 0.500 g. of chromic acid anhydride in 10 cc. of 80% acetic acid. The reaction mixture was kept at *ca.* 10° by cooling in ice-water. After standing at room temperature (20–23°) for 1.5 hours, the dark-brown solution was poured into ice-water and extracted with ether. The ethereal solution was washed with water, dilute sodium carbonate solution and water again, dried over sodium sulfate and evaporated. The resulting oily residue was dissolved in 20 cc. of 2% ethanolic potassium hydroxide and refluxed on the steam-bath for 30 minutes. The reaction mixture was poured into ice-water and the flocculent precipitate extracted with ether. After removal of solvents a semi-crystalline product (155 mg.) was obtained and recrystallized from dilute methanol. Another recrystallization from the same solvent gave a compound with the constant m.p. 207–208°. The mixture with an authentic sample of Δ^{16}-allopregnen-3(β)-ol-20-one of m.p. 209–209.5° melted at 208–208.5°, $[\alpha]^{20}D$ +50.4° (*c* 0.91, ethanol), lit. $[\alpha]^{17}D$ +50.2° ± 4.2° (*c*, 1.4, ethanol).[13]

Anal. Calcd. for $C_{21}H_{32}O$: C, 79.70; H, 10.19. Found: C, 79.55; H, 10.30.

A mixture of 33 mg. of the above alcohol and 1.5 cc. of acetic anhydride was gently refluxed for 20 minutes. After removing the solvent *in vacuo*, the residue was recrystallized from dilute methanol: glistening, hexagonally shaped platelets; m.p. 165–168°. After sublimation in a high vacuum and recrystallization, the compound melted at 167–169°; λ_{max} 240 mμ, log ϵ 3.98; λ_{max} 320 mμ, log ϵ 1.86; $[\alpha]^{20}D$ +36.7° (*c*, 1.04, $CHCl_3$).[14]

Anal. Calcd. for $C_{23}H_{34}O_3$: C, 77.05; H, 9.56. Found: C, 77.02; H, 9.67.

The melting points of mixtures with two authentic samples[11] gave no depression. The infrared spectra of the samples compared were identical.

(13) Klyne, Schachter and Marrian, *Biochem. J.*, **43**, 231 (1948).

(14) Klyne and Marrian[13] report: m.p. 165–167°; λ_{max} 240 mμ, log ϵ 3.93; λ_{max} 320 mμ, log ϵ 1.92; $[\alpha]^{22}D$ +36.3° ± 0.7° (*c* 1.4, $CHCl_3$). Plattner, *et al.*, *Helv. Chim. Acta*, **30**, 385 (1947), report: m.p. 166–167°; λ_{max} 240 mμ, log ϵ 4.2; λ_{max} 320 mμ, log ϵ 2.1, $[\alpha]^{20}D$ +42.2° (*c* 1.42, $CHCl_3$).

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

A Partial Synthesis of Δ^5-Pregnenetriol-3β,16α, 20α and of Other 16α-Hydroxysteroids[1]

BY H. HIRSCHMANN, FRIEDA B. HIRSCHMANN AND MARGARET A. DAUS

A procedure for the introduction of the 16α-hydroxy group into the steroid molecule is described. 16α-Benzyloxy-Δ^5-pregnenol-3β-one-20 acetate prepared from $\Delta^{5,16}$-pregnadienol-3β-one-20 and benzyl alcohol gave on successive reductions with lithium aluminum hydride and with sodium and alcohol two isomeric triols. One of these was identical with the Δ^5-pregnenetriol-3β,16α,20α isolated from adrenal tumor urine, while the other, Δ^5-pregnenetriol-3β,16α,20β, upon reduction of its acetate gave the triacetate of allopregnanetriol-3β,16α,20β of the urine of pregnant mares. The latter was epimerized at C-3 by treating the 3-tosylate 16,20-diacetate with sodium acetate to give allopregnanetriol-3α,16α,20β triacetate.

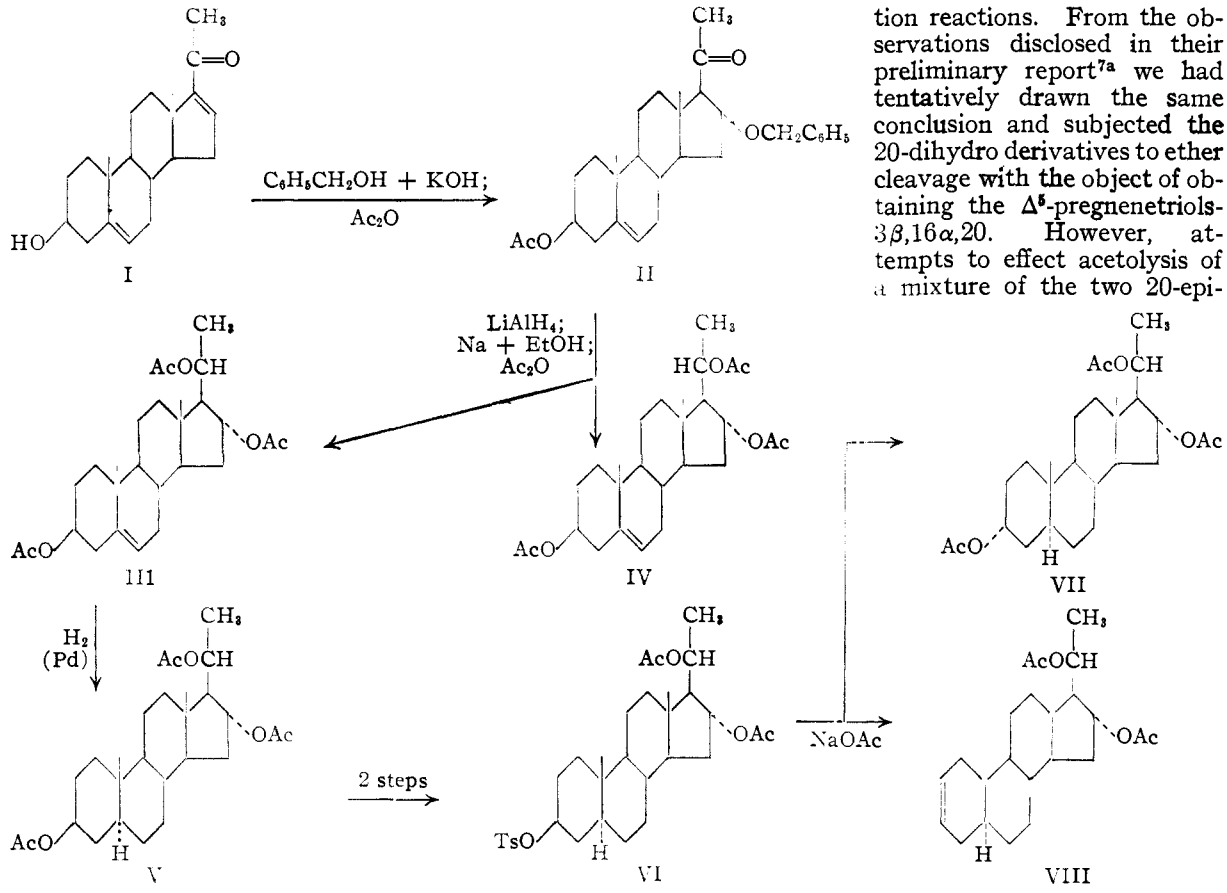
Several years ago a new steroid was isolated[2a] from the urine of a boy with an adrenal tumor and characterized[2b] as Δ^5-pregnenetriol-3β,16α,20α. This observation demonstrated that pregnane derivatives oxygenated in the 16 position can be derived from adrenal steroids, but failed to disclose whether the oxygen atom is introduced into the molecule by adrenal tissue or subsequently while the adrenal steroid is metabolized by other organs of the body. In order to investigate this question and to test the effect of 16-oxygenation on the biological activity of potent adrenal steroids methods for the synthesis of 16α-hydroxysteroids were required. 16-Hydroxysteroids with a configuration[3] opposite to that found in urinary metabolites are readily available from sapogenins[4] but their inversion to

(1) This investigation was supported by grants from the Hanna Research Fund and from the American Cancer Society on the recommendation of the Committee on Growth.

(2) (a) H. Hirschmann and F. B. Hirschmann, *J. Biol. Chem.*, **157**, 601 (1945); (b) *ibid.*, **184**, 259 (1950).

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

(4) R. E. Marker, D. L. Turner, R. B. Wagner, P. R. Ulshafer, H. M. Crooks, Jr., and E. L. Wittle, THIS JOURNAL, **63**, 774 (1941); R. E. Marker, D. L. Turner, R. B. Wagner and P. R. Ulshafer, *ibid.*, **63**, 772 (1941). The reactions described in these papers were also applied to other sapogenins.



tion reactions. From the observations disclosed in their preliminary report[7a] we had tentatively drawn the same conclusion and subjected the 20-dihydro derivatives to ether cleavage with the object of obtaining the Δ^5-pregnenetriols-$3\beta,16\alpha,20$. However, attempts to effect acetolysis of a mixture of the two 20-epi-

16α-hydroxysteroids has not been accomplished. Only 16β-hydroxysteroids or their derivatives were obtained when 16-ketosteroids devoid of a hydroxy group at C-17 were reduced under various conditions.[3,5] Inversion of 16β-tosyloxy compounds by bimolecular substitution reactions also seemed to offer little hope of success in view of the *trans* relationship of such a substituent to a hydrogen at an adjacent tertiary carbon atom, an arrangement which should greatly favor elimination reactions.

A more promising approach to the synthesis of 16α-hydroxysteroids was afforded by a reaction between Δ^{16}-20-ketosteroids and alcoholic alkali. This reaction was discovered by Marker[6] and reinterpreted by Fukushima and Gallagher[7] who were able to show that $\Delta^{5,16}$-pregnadienol-3β-one-20 acetate in the presence of potassium hydroxide adds methanol or ethanol reversibly to form 16-alkoxy derivatives. These were assigned[7b] the α-configurations on the basis of rotation data and by invoking the principle of steric hindrance to addi-

meric 16-methoxy-Δ^5-pregnenediol-$3\beta,20$ diacetates with acetic anhydride in the presence of toluenesulfonic acid either under the conditions of Huffman and Lott[8] or at higher temperatures were ineffective and treatment with acetic anhydride and boron trifluoride[9] also failed to give the desired triacetates. In a search for a more labile ether the reaction between $\Delta^{5,16}$-pregnadienol-3β-one-20 (I) and benzyl alcohol was investigated. Even under conditions which tend to minimize the formation of benzaldehyde a substance with an absorption maximum at 295 mμ was formed which is suspected[10] to be 21-benzylidene-16-benzyloxy-Δ^5-pregnenol-3β-one-20. This impurity clung tenaciously to the main product during chromatography of the acetates on alumina but could be separated on magnesium silicate–celite columns. The ultraviolet absorption spectrum of the final product (II) gave evidence of a benzenoid ring and of a non-conjugated keto group and demonstrated the absence of the α,β-unsaturated ketone grouping of the starting compound. An infrared absorption peak at 5.86 μ confirmed the presence of a non-conjugated 20-keto group.[11] In analogy with the reactions of the simple aliphatic alcohols[7] the substance therefore is to be formulated as 16-benzyloxy-Δ^5-preg-

(5) R. E. Marker and E. Rohrmann, THIS JOURNAL, **61**, 1285, 2072 (1939); **62**, 76 (1940); R. E. Marker, D. L. Turner and P. R. Ulshafer, *ibid.*, **63**, 763 (1941); R. E. Marker and A. C. Shabica, *ibid.*, **64**, 721 (1942); R. E. Marker, R. B. Wagner, P. R. Ulshafer, E. L. Wittbecker, D. P. J. Goldsmith and C. H. Ruof, *ibid.*, **69**, 2198–2200 (1947); S. Kaufmann and G. Rosenkranz, *ibid.*, **70**, 3502 (1948). Evidence which permits the assignment of the β-configuration at C-16 in these reduction products is discussed in reference 3. This paper also reports an unsuccessful attempt to reduce allopregnanediol-$3\beta,20\beta$-one-16 diacetate by the method of Huffman and Lott (*ibid.*, **71**, 719 (1949)) which converted 17β-hydroxy-16-ketosteroids to *both* 16-epimers of the corresponding glycols.

(6) R. E. Marker, *ibid.*, **71**, 4149 (1949).

(7) (a) D. K. Fukushima and T. F. Gallagher, *ibid.*, **72**, 2306 (1950); (b) **73**, 196 (1951).

(8) M. N. Huffman and M. H. Lott, *J. Biol. Chem.*, **172**, 789 (1948).

(9) H. Meerwein and H. Maier-Hueser, *J. prakt. Chem.*, N. F., **134**, 51 (1932); L. Ruzicka, W. Baumgartner and V. Prelog, *Helv. Chim. Acta*, **32**, 2069 (1949).

(10) W. P. Long, C. W. Marshall and T. F. Gallagher, *J. Biol. Chem.*, **165**, 197 (1946).

(11) R. N. Jones, V. Z. Williams, M. J. Whalen and K. Dobriner, THIS JOURNAL, **70**, 2024 (1948).

nenol-3β-one-20 acetate. Its molecular levorotation is even greater than those of the corresponding 16-methoxy and ethoxy compounds. In the region between 13 and 15 μ where none of the non-aromatic steroids investigated in this Laboratory have shown strong absorption, compound II exhibited two intense maxima at 13.66 and 14.37 μ which remained unchanged upon reduction with lithium aluminum hydride and acetylation. In view of the similar and more extended observations with trityl ethers[2b] these bands probably can be ascribed to the benzyloxy group. The intensity of absorption at 14.37 μ was therefore used as a measure of the success of ether cleavage reactions on the 20-dihydro products which were obtained with lithium aluminum hydride. Again the ether linkage was quite stable toward acid catalyzed acetolysis but could be broken with sodium and ethanol. Since this reaction is a hydrogenolysis it does not attack the bond between C-16 and oxygen and therefore cannot alter the configuration at this site.[12] This expectation is also supported by observations[13] in the sugar group where this reaction was first applied. The resulting reaction mixture was fractionated after acetylation and furnished two isomeric triacetates (III and IV). The main product (III) upon reduction with a palladium catalyst yielded a saturated triacetate (V) identical with that of allopregnanetriol-3β,16α,20β of the urine of pregnant mares. This identity shows that compound III is Δ^5-pregnenetriol-3β,16α,20β triacetate. It establishes further the 16α configuration for compound II and therefore confirms the steric course of the addition reaction as deduced from less direct evidence by Fukushima and Gallagher. Conversely this synthesis of the mare's triol demonstrates the 3β-configuration of this compound in a simpler manner than has hitherto been possible.[3] The reductions of other 20-ketosteroids with lithium aluminum hydride studied in this Laboratory[2b,14] have always given both diastereoisomers of the corresponding 20-hydroxysteroids though in varying proportions. Hence the second triacetate (IV) must be the 20-epimer of compound III and possess the structure of Δ^5-pregnenetriol-3β,16α,20α triacetate. It proved to be identical with the triacetate obtained from the tumor urine. Since the position of the double bond in the natural product had been established by exclusion of other positions rather than by an identity, this synthesis supplements the proof of structure previously given. The difference in the molecular rotations of the triacetates III and IV (average 32°) was found to be even smaller than that reported for the saturated analogs[2b] and considerably less than $\Delta[M]_D^{OAc(20\beta - 20\alpha)}$ in compounds without vicinal substituents (average 102°)[2b] or with 16β-acetoxy groups (97°).[2b] This is of interest since an interaction between 20α- and 16α-acetoxy groups has been postulated previously on chemical grounds.[2b] Like the mare's triol but contrary to the behavior of either Δ^5-pregnenetriol-3β,16α,20α or Δ^5-pregnenediol-3β,20β,Δ^5-pregnenetriol-3β,16α,20β failed to precipitate with digitonin. This suggests the possibility of using digitonin for separating the 20-epimers of Δ^5-pregnenetriol-3β,16α,20 as an alternative to the chromatographic procedure used here.

Earlier attempts[3] to convert allopregnanediol-16α,20β-one-3 diacetate to allopregnanetriol-3α,-16α,20β were unsuccessful on the scale on which they were attempted since reduction in an acid medium furnished, contrary to the Auwers–Skita rule, predominantly the 3β-isomer. The 3α-triol has now been prepared by treating allopregnanetriol-3β,16α,20β 16,20 diacetate[3] with tosyl chloride and by inverting the tosylate (VI) with sodium acetate. The resulting triacetate (VII) was accompanied by large amounts of an unsaturated diacetate (VIII) believed to be mainly or entirely Δ^2-allopregnenediol-16α,20β diacetate. The structures assigned to compounds VII and VIII follow from that of the starting material and are supported by their analyses, by their molecular rotations[15] and by the analogous behavior of methyl 3β-tosyloxyetioallocholanate.[16] Allopregnanetriol-3α,16α,20β and Δ^5-pregnenetriol-3β,16α,20β are of interest as both are likely to be present in the urine of pregnant mares.

Experimental[17]

16α-Benzyloxy-Δ^5-pregnenol-3β-one-20 Acetate (II).—Powdered potassium hydroxide (270 mg.) was dissolved at room temperature under nitrogen in 9.2 cc. of benzyl alcohol and added to 306.9 mg. of $\Delta^{5,16}$-pregnadienol-3β-one-20 (I). The resulting solution was kept under nitrogen at 23° for 3.5 hours and then distributed between ether and water. The organic phase after distillation of the ether was taken to dryness *in vacuo*. The residue (431 mg.) in 4 cc. of pyridine was treated with 2 cc. of acetic anhydride for 16 hours at room temperature. The acetates were isolated in the usual manner[2b] and chromatographed on 11 g. of magnesium silicate–celite mixture (1:1). Elution with benzene–petroleum ether and with benzene gave

(12) Sodium ethoxide can cause epimerization of a hydroxyl group by oxidation to and reduction of the corresponding ketone. However, this process requires higher temperatures and could not have operated at C-16 as it would involve at the keto stage a substance very susceptible to dehydration (*cf.* R. E. Marker and E. L. Wittle, THIS JOURNAL, **61**, 855 (1939); R. E. Marker and D. L. Turner, *ibid.*, **62**, 2540 (1940)). The isolation of triols rather than diols therefore precludes such a possibility. Finally the reduction of a 16-ketone would be expected to yield the 16β and not the 16α isomers which were actually obtained.

(13) K. Freudenberg and E. Plankenhorn, *Ann.*, **536**, 257 (1938); D. J. Bell and J. Lorber, *J. Chem. Soc.*, 453 (1940); J. S. D. Bacon, D. J. Bell and J. Lorber, *ibid.*, 1147 (1940).

(14) H. Hirschmann and F. B. Hirschmann, *J. Biol. Chem.*, **187**, 137 (1950); H. Hirschmann, M. A. Daus and F. B. Hirschmann, *ibid.*, **192**, 115 (1951).

(15) $\Delta[M]_D^{VII-VI} = -119°$ may be compared with the difference $[M]_D$ of androsterone acetate (+289°; A. Butenandt and H. Dannenbaum, *Z. physiol. Chem.*, **229**, 192 (1934); S. Lieberman, K. Dobriner, B. R. Hill, L. F. Fieser and C. P. Rhoads, *J. Biol. Chem.*, **172**, 263 (1948)) minus $[M]_D$ of Δ^{17}-androstenone-17 (+406°; H. Hirschmann, *ibid.*, **136**, 483 (1940); V. Prelog, L. Ruzicka, P. Meister and P. Wieland, *Helv. Chim. Acta*, **28**, 618 (1945); S. Lieberman, *et al.*, see above) = −117° in alcohol. Since the rotations of Δ^1-cholestene and of Δ^4-cholestene in chloroform are virtually identical (A. Fuerst and P. A. Plattner, *Helv. Chim. Acta*, **32**, 275 (1949); G. Lardelli and O. Jeger, *ibid.*, **32**, 1817 (1949)) the presence of Δ^2-isomers in either Δ^{17}-androstenone-17 or in compound VIII ought not to affect this comparison. Quite generally, however, the 2–3 position appears to be the preferred location of the double bond regardless of the reaction mechanism if a substituent at C-3 is eliminated from a 5-allosteroid. $\Delta[M]_D^{OAc(3\beta - 3\alpha)}$ in alcohol has been reviewed recently.[2a]

(16) P. A. Plattner and A. Fuerst, *ibid.*, **26**, 2266 (1943).

(17) All melting points are corrected. Acetates were dried for analysis, rotation and spectroscopy at 80°, triols at 110° *in vacuo* unless when noted otherwise. Rotations and ultraviolet spectra were measured on solutions in 95% ethanol, infrared spectra in carbon disulfide (0.8-mm. cell; 1% solutions; Perkin-Elmer spectrometer, Model B). Absorption peaks that are relatively broad and low are listed in parentheses.

in succession the acetate of the starting compound and 16α-benzyloxy-Δ⁵-pregnenol-3β-one-20 acetate (II). This was recrystallized from methanol; yield 128 mg.; m.p. of analytical sample 137–138°; $[\alpha]^{26}D$ −31° (*c* 0.6); *Anal.* Calcd. for $C_{30}H_{40}O_4$: C, 77.55; H, 8.68. Found: C, 77.58; H, 8.67. An alcoholic solution (1.07 mg./1 cc.) gave the following molecular extinction coefficients: maxima 67 at 287 (keto group), 184 at 264, 227 at 258 and 198 at 252.5 mμ; minima, 59 at 276, 174 at 262, 184 at 254.5 and 123 at 236 mμ. There were no shoulders near 239 or 295 mμ.

Reduction and Cleavage of 16α-Benzyloxy-Δ⁵-pregnenol-3β-one-20 Acetate (II).—A solution of 158 mg. of this compound in 14 cc. of dry ether was added to 151 mg. of lithium aluminum hydride in 6 cc. of ether. The mixture was stirred under anhydrous conditions for 30 minutes and worked up as described before.[14] The reaction product (the two 16α-benzyloxy-Δ⁵-pregnenediols-3β,20, 144 mg.) in 1.6 cc. of absolute ethanol was heated under a reflux while 745 mg. of sodium was added in small pieces (5 hours). Throughout, the solution must be maintained as concentrated as possible and only sufficient alcohol was added to prevent excessive precipitation of sodium ethoxide. The chilled reaction mixture was distributed between ethyl acetate and water and the residue of the organic phase acetylated (3 cc. of pyridine, 1.5 cc. of acetic anhydride for 17 hours at room temperature). As the product still showed some absorption at 14.37 μ the sodium reduction and acetylation were repeated. The resulting acetates (158 mg.) were passed through a column (99 × 8 mm.) of acid washed alumina. Elution with petroleum ether–benzene (2:1 and 1:1) and with benzene gave in succession an oily residue (22 mg.) and compounds III and IV. Only those triacetate fractions which crystallized spontaneously on evaporation of the eluant were recrystallized while the remainder was rechromatographed "in sequence."[2b]

Δ⁵-Pregnenetriol-3β,16α,20β triacetate (III) crystallized from methanol as platelets; yield 63 mg.; m.p. of analytical sample 167.5–168.5°; $[\alpha]^{28}D$ −93° (*c* 0.8); *Anal.* Calcd. for $C_{27}H_{40}O_6$: C, 70.40; H, 8.75. Found: C, 70.42; H, 8.82. Absorption maxima in the accessible finger print region of infrared radiation were at (13.70), 12.45, 12.29, 11.97, 11.07, 10.92, 10.82, 10.68, 10.53, 10.44, 10.32, 10.15, 9.79, 9.66, 9.55, 9.26, 9.12, 9.01, 8.85, 8.78, 8.56, 8.39, 8.33 and 8.03 (ester) μ.

Δ⁵-Pregnenetriol-3β,16α,20α triacetate (IV) crystallized from methanol in needles; yield 18.5 mg. The analytical sample (m.p. 178.5–180°) gave no depression on admixture of isolated material. The infrared spectra[2b] of both preparations were in close accord; $[\alpha]^{28}D$ −99° (*c* 0.5); *Anal.* Calcd. for $C_{27}H_{40}O_6$: C, 70.40; H, 8.75. Found: C, 70.54; H, 8.77. Reported for the natural product[2b]: m.p. 178.5–180° and $[\alpha]D$ −101°.

Δ⁵-Pregnenetriol-3β,16α,20β.—A solution of 16 mg. of triacetate III and of 40 mg. of sodium hydroxide in 5 cc. of 80% ethanol was refluxed for 100 minutes. The free triol was isolated by ether extraction and dissolved in benzene–methanol. The solution was concentrated to incipient crystallization and diluted with 10% petroleum ether; m.p. 294–298° with decomposition. *Anal.* Calcd. for $C_{21}H_{34}O_3$: C, 75.40; H, 10.25. Found: C, 75.02; H, 10.29. A solution of 0.5 mg. of the triol in 0.13 cc. of 80% ethanol gave no precipitate with an equal volume of digitonin (1% in the same solvent) (48 hours test).

Δ⁵-Pregnenetriol-3β,16α,20α.—Triacetate IV (4.6 mg.) was hydrolyzed like compound III. The triol was recrystallized from 95% alcohol. Its m.p. (245–247° with decomposition) was not depressed by admixture of isolated Δ⁵-pregnenetriol-3β,16α,20α.

Allopregnanetriol-3β,16α,20β Triacetate (V).—Δ⁵-Pregnenetriol-3β,16α,20β triacetate (III) (19 mg.), 192 mg. of prereduced palladium–calcium carbonate catalyst[18] and 10 cc. of 95% alcohol were shaken in a hydrogen atmosphere. Gas uptake ceased after 10 minutes. The reduced triacetate was isolated as described before[2b] and recrystallized from methanol to yield 17.6 mg. of plates (m.p. 167.5–169.5°). The final product showed no depression of its m.p. (168.5–170.5°) when mixed with a sample of the triacetate V (m.p. 169.5–171°) that had been isolated from urine. Identity was confirmed by comparison of the infrared spectra[3]; $[\alpha]^{27}D$ −49° (*c* 0.58) (previous observations[3] −47 ± 2°). *Anal.* Calcd. for $C_{27}H_{42}O_6$: C, 70.10; H, 9.15. Found: C, 69.92; H, 9.22.

Allopregnanetriol-3β,16α,20β 3-Tosylate 16,20-Diacetate (VI).—A solution of 97 mg. of allopregnanetriol-3β,16α,20β 16,20-diacetate[3] and of 234 mg. of tosyl chloride in 1.5 cc. of pyridine was kept at room temperature for 48 hours and then treated with water. The residue of an ether extract (washed free of pyridine and of acids) was recrystallized from dilute acetone and from acetone to yield 110 mg. of tosylate VI. The analytical sample (m.p. 167–169.5°, with decomposition) was dried at room temperature. *Anal.* Calcd. for $C_{32}H_{46}O_7S$: C, 66.87; H, 8.07. Found: C, 67.04; H, 8.18.

Allopregnanetriol-3α,16α,20β Triacetate (VII).—A solution of 110 mg. of tosylate VI and of 249 mg. of anhydrous sodium acetate in 4 cc. of glacial acetic acid was refluxed for 1 hour. The reaction product (obtained by ether extraction) was chromatographed on alumina. The early eluates (with petroleum ether) afforded 24 mg. of Δ[2?]-allopregnenediol-16α,20β diacetate (VIII) upon recrystallization from petroleum ether, rods or plates, m.p. 117.5–118.5° $[\alpha]^{26}D$ −13° (*c* 0.78). *Anal.* Calcd. for $C_{25}H_{38}O_4$: C, 74.59; H, 9.52. Found: C, 74.68; H, 9.62.

The later eluates obtained with mixtures of petroleum ether and benzene yielded 23 mg. of allopregnanetriol-3α,16α,20β triacetate. Compound VII crystallized from methanol in needles; m.p. 126.5–129.5°; $[\alpha]^{28}D$ −37° (*c* 0.58). *Anal.* Calcd. for $C_{27}H_{42}O_6$: C, 70.10; H, 9.15. Found: C, 70.08; H, 9.15. Absorption maxima in the accessible finger print region: (12.57), 11.19, 10.80, 10.69 10.53, 10.36, 10.23, 10.17, 10.01, 9.82, 9.65, 9.54, 9.25. (9.01, 8.88, 8.82), 8.59, 8.45, 8.32, 8.25 and 8.05 (ester) μ.

Allopregnanetriol-3α,16α,20β.—The hydrolysis of 14.3 mg. of triacetate VII and the purification of the resulting triol were carried out as described for compound III; m.p. 235–238° with decomposition. *Anal.* Calcd. for $C_{21}H_{36}O_3$: C, 74.95; H, 10.78. Found: C, 74.67; H, 10.75.

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