PREPARATION OF A PREGNANETRIOL-3(α), 17, 20

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It has been shown that urinary androsterone and etiocholanolone may arise from an extragonadal source which is believed to be the adrenal cortex (1–3). The adrenal precursor or precursors of these 17-ketosteroids, however, have not yet been determined. Hypotheses have been advanced postulating that these excretion products are derived from certain pregnane compounds carrying a hydroxyl group at C₁₇ (4, 5). With a view to testing such concepts experimentally we have begun to prepare compounds of this type for metabolic studies.

In the present paper the preparation of a pregnanetriol-3(α), 17, 20 from pregnanediol-3(α), 20(α) is described. The steps used in this conversion are shown in the accompanying diagram ((I) to (V)). Pregnanediol (I) was acetylated at position 3 to prevent the removal of this hydroxyl group during the subsequent dehydration. While pregnanediol monoacetate-3 (II) has not been described previously, a reaction mixture containing this substance has already been prepared by Marker, Kamm, and Wittle (6), who used it for a synthesis of pregnanol-3(α)-one-20. This mixture was obtained by treating pregnanediol with boiling acetic acid containing the equivalent of somewhat more than 2 moles of acetic anhydride. In the present work pregnanediol was acetylated with glacial acetic acid. After the removal of some unchanged starting material, which is only sparingly soluble in petroleum ether (7), the reaction mixture was fractionated with the aid of a column of alumina. In addition to pregnanediol diacetate two substances with the composition of a monoacetate
of pregnanediol were obtained. They melted\(^1\) at 132.5\(^\circ\) and 175.5\(^\circ\) respectively. The higher melting of these is undoubtedly identical with the pregnanediol monoacetate (m.p. 170.5\(^\circ\), uncorrected) of Butenandt and Schmidt (7), who obtained it by partial hydrolysis of the diacetate and identified it as the 20-monoacetate. The structure of the monoacetate-3 must therefore be assigned to our lower melting isomer. The stepwise conversion of this substance into etiocholanol-3(α)-one-17 (VI) affords further evidence that the unesterified hydroxyl group is attached to C\(_{20}\).

Pregnanediol monoacetate-3 was converted into Δ\(_{17}\)-pregnenol-3(α) (IV) by way of the toluenesulfonate-20 (III), which readily split off toluenesulfonic acid on treatment with boiling pyridine. The location of the double bond of the pregnenol was deduced from the structure of the pregnanetriol that was obtained by hydroxylation according to the method of Criegee (8). One of the hydroxyl groups of this glycol failed to react with acetic anhydride and was therefore considered to be tertiary. The most probable structure of a triol containing a tertiary hydroxyl group and derived from pregnanediol by these steps is represented by Formula V. Moreover, degradation with periodic acid yielded the known etiocholanol-3(α)-one-17 (VI). This proved conclusively that the triol possesses the structure of a pregnanetriol-3(α),17,20 and that it had been derived from a Δ\(_{17}\)-pregnenol-3(α).

The pregnanetriol 3(α),17,20 obtained by this procedure is not identical with the pregnanetriol-3(α),17,20 which Butler and Marrian (9) isolated from the urine of patients with adreno-genital syndrome and which they regard as a precursor of urinary etiocholanolone. The difference between the two triols must be attributed to a different spatial arrangement at either C\(_{17}\) or C\(_{20}\) or both. Our product is likewise isomeric with the four allopregnanetriols-3(β),17,20 that have recently been synthesized by Reichstein and his collaborators (10). Since their series comprises two triols that have been isolated from the adrenal cortex (11), a stereochemical correlation between the allopregnanetriols-3(β),17,20 and the pregnanetriols-3(α),17,20 may be of considerable interest. Three of Reichstein's triols were obtained by hydroxylation of Δ\(_{17}\)-allopregnenol-3(β) according to Criegee's

\(^1\) All melting points reported are corrected.
method. As we have used the same procedure, it might be inferred that our triol and the adrenal compound J, the main hydroxylation product of Reichstein, possess the same configuration at C_{17} and C_{20}. However, the starting materials for these reactions (Δ_{17}-pregnenol-3(α) and Δ_{17}-allopregnenol-3(β)) were prepared by different routes and since the location of the double bond permits the existence of a cis-trans isomerism one cannot be certain that the configuration at the double bond is the same in both cases. Additional information seems therefore necessary to establish a stereochemical relationship between our triol and its analogue in the allopregnane series.

**EXPERIMENTAL**

*Acetylation of Pregnanediol*—922 mg. of pregnanediol-3(α),20(α) and 28 cc. of glacial acetic acid (99.5 per cent) were refluxed under anhydrous conditions for 2 hours. The solution when cold was distributed between benzene and water. The benzene layer was washed free of acid and evaporated under reduced pressure. The dry product was leached with five portions of petroleum ether (totaling 130 cc.). The insoluble residue (361 mg. melting at 220–229°) was treated with 11 cc. of boiling glacial acetic acid for 2 hours and worked up as described above. The pregnanediol fraction (material insoluble in petroleum ether) weighed 112 mg. (12 per cent) and melted at 217–231°. The petroleum ether extracts were combined (180 cc.) and passed through a column (180 × 13 mm.) of Brockmann’s aluminum oxide. The column was eluted with petroleum ether to which increasing amounts of benzene had been added, and finally with pure benzene. The volumes and compositions of the eluants and the weights and melting points of the eluates are summarized in Table I.

Fractions 4 to 6 were recrystallized from 85 per cent methanol and yielded 280 mg. of rectangular plates. The melting points of these preparations (131.5–132.5°) could not be raised by further recrystallization. The compound retained solvent even when dried at 100° in vacuo. An analytical specimen free of solvent was obtained by sublimation in a high vacuum (about 10^{-4} mm. of Hg) at 110–120°.

*Analysis*—C_{29}H_{48}O_{5}. Calculated. C 76.19, H 10.57

Found. “ 76.32, “ 10.64
Fractions 2 and 7 proved to be mixtures. Fraction 3 did not yield a product with a sharp melting point on recrystallization. These fractions as well as the mother liquor of Fractions 4 to 6 were therefore subjected to another chromatographic analysis. In addition to 52 mg. of pregnanediol diacetate and 33 mg. of pregnanediol monoacetate-20, 129 mg. of pregnanediol monoacetate-3 were obtained. The total yield for this substance was 409 mg. (39 per cent).

Fraction 1 was recrystallized from methanol. 197 mg. of pregnanediol diacetate melting at 180.5–181.5° were obtained. There was no depression of the melting point on admixture with an authentic specimen. The resolidified product melted at 166°. This allotropism of pregnanediol diacetate has been described previously (3). The yield was 249 mg. (21 per cent).

Fractions 8 to 10 were recrystallized from dilute acetone. The final product melted at 174–175.5°.

<table>
<thead>
<tr>
<th>Fraction No.</th>
<th>Eluant (petroleum ether)</th>
<th>Crude Weight mg.</th>
<th>Once recrystallized M.p. °C.</th>
<th>Main component</th>
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<tr>
<td>1</td>
<td>330</td>
<td>0</td>
<td>209</td>
<td>197</td>
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<td>2</td>
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<td>10</td>
<td>122</td>
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<td>3</td>
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<td>10</td>
<td>84</td>
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<td>4</td>
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<td>6</td>
<td>240</td>
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<td>7</td>
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The total yield of pregnanediol monoacetate was 177 mg. (17 per cent).

When the acetylation was carried out for a single period of 3 hours instead of the two periods of 2 hours as described above, the yields of diacetate (28 per cent) and of unchanged starting material (20 per cent) were appreciably higher, while those of the monoacetates were correspondingly lower.

Pregnanediol Acetate-3 p-Toluenesulfonate-20—246 mg. of pregnanediol monoacetate-3 and 251 mg. of p-toluenesulfonyl chloride were dissolved in 2.5 cc. of pyridine and kept at room temperature for 24 hours. The solution was chilled and treated with 0.4 cc. of cold water which was added in portions. The mixture was distributed between benzene and water. The organic phase was washed with dilute hydrochloric acid, sodium carbonate, and water. It yielded 360 mg. of a colorless oil that crystallized upon the addition of methanol. The material was very soluble in acetone and was recrystallized from methanol. Since the compound is not stable in hot alcoholic solutions, recrystallizations were carried out as rapidly as possible. The analytical specimen which had been recrystallized three times melted at 112–115° with decomposition. The melting point varied somewhat with the rate of heating.

Analysis—C$_{20}$H$_{24}$O$_3$. Calculated, S 6.20; found, S 5.94

Upon storage at room temperature the crystals (hair-like needles) were slowly converted into a brown oil.

Δ$_{17}$-Pregnenol-3(α)—280 mg. of pregnanediol acetate-3 toluenesulfonate-20, 300 mg. of dry powdered calcium carbonate, and 6 cc. of pyridine were refluxed for 2 hours under anhydrous conditions. The reaction mixture was taken up in ether and extracted repeatedly with dilute hydrochloric acid, sodium carbonate solution, and

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* After an ethanolic solution of the toluenesulfonate was refluxed in the presence of dry calcium carbonate for 3 hours, no starting material could be recovered. The main reaction product was a pregnenol, the structure of which is being investigated.

* The addition of calcium carbonate was suggested by the work of Hückel and Tappe (12), who used it for the prevention of secondary reactions caused by the toluenesulfonic acid.
water. The ether residue failed to crystallize. It was dissolved with gentle heating in a mixture of 180 mg. of sodium hydroxide, 28 cc. of methanol, and 2 cc. of water. The solution was kept for 17 hours at room temperature and then concentrated under reduced pressure. After the addition of ether the mixture was washed with water and taken to dryness. The residue was recrystallized three times from dilute acetone. Hair-like needles melting at 118–120° were obtained. The yield of pregnenol was 87 per cent.

**Analysis**—C_{21}H_{34}O. Calculated. C 83.38, H 11.33

Found. " 82.93, " 11.08

**Hydroxylation of Δ_{17}-Pregnenol-3(α); Pregnanetriol-3(α),17,20 Diacetate**—126 mg. of Δ_{17}-pregnenol-3(α) were dissolved in 2 cc. of freshly distilled anhydrous ether. A solution of 125 mg. of osmium tetroxide in 1.3 cc. of ether was added, and the mixture kept at room temperature for 39 hours and then taken to dryness. The residue was dissolved in 10 cc. of ethanol, mixed with a solution of 630 mg. of sodium sulfite in 30 cc. of water, refluxed for 2 hours, and filtered while still hot. The precipitate was thoroughly washed with hot 95 per cent alcohol. The washings were combined with the filtrate and concentrated under reduced pressure until the alcohol had been removed. The aqueous residue was thoroughly extracted with ether. The ether phase was washed with water and taken to dryness. Material obtained by this procedure can be purified by recrystallization to yield the pregnanetriol described below. To test the homogeneity of the crude product, however, it was acetylated and subjected to chromatographic analysis. The ether residue (141 mg.) was dissolved in 2 cc. of pyridine and treated with 1 cc. of acetic anhydride at room temperature for 16 hours. Then the excess of anhydride was hydrolyzed, and the reaction mixture distributed between ether and water. The ether layer was washed with dilute hydrochloric acid, sodium carbonate, and water and yielded upon evaporation 171 mg. of crystalline material. The dry residue was dissolved with warming in 85 cc. of petroleum ether and passed through a column (125 × 13 mm.) of Brockmann's aluminum oxide. Elution with 165 cc. of petroleum ether and 500 cc. of petroleum ether containing 10 per cent of benzene yielded 1 mg. of residue. The
column was then washed twice with 200 cc. of petroleum ether containing 20 per cent of benzene, four times with 200 cc., and once with 300 cc. of petroleum ether containing 30 per cent of benzene. The eluates (Fractions a to g) weighed 3.9, 29.0, 49.0, 27.2, 16.0, 8.0, and 5.1 mg. respectively. Elution with petroleum ether containing 60 per cent of benzene (400 cc.) and with benzene (200 cc.) yielded 3.1 mg. Continued washing with ether (270 cc.) and acetone (200 cc.) eluted 3 and 19 mg. respectively (Fractions h and i). Further elution (200 cc. of acetone) yielded only 1.2 mg.

Fractions c to g all melted above 185°. Upon recrystallization from methanol and from a mixture of benzene and petroleum ether 78.3 mg. of tetragonal prisms melting at 193–196° were obtained. Fractions a and b melted over a wide range (below 155°) and yielded 7.4 mg. of the same product (m.p. 194°) on repeated recrystallization.

Analysis—C_{25}H_{46}O_{5}. Calculated. C 71.39, H 9.59

Rotation—[\alpha]_D^\circ = +71° (1% in ethanol)

Fractions h and i failed to crystallize and were hydrolyzed with sodium hydroxide in dilute methanol. The reaction product (18.8 mg.) was extracted with a small volume of acetone. The insoluble residue (2.1 mg.) was recrystallized from methanol. 0.6 mg. of colorless prisms melting at 248–252° were obtained. The acetone-soluble fraction upon recrystallization yielded 4 mg. of crystals melting at 215°. The melting point was not depressed by admixture with pregnanetriol-3(\alpha),17,20.

Pregnanetriol-3(\alpha),17,20—40.8 mg. of pregnanetriol diacetate were dissolved in 6.5 cc. of methanol and treated with 1 cc. of 3 per cent aqueous sodium hydroxide for 20 hours. The mixture was concentrated, diluted with ether, and washed with water. The residue weighed 32.5 mg. The triol crystallized from dilute alcohol in plates that retained solvent when dried in vacuo at room temperature, but became opaque upon drying at 110°. This was accompanied by a weight loss equivalent to 1 mole of ethanol. Recrystallization from acetone yielded multifaceted prisms that were found to be free of solvent. The dry compound melted at 215–218°. The yield was 91 per cent.

Analysis—C_{25}H_{46}O_{5}. Calculated. C 74.95, H 10.78

The triol did not precipitate with digitonin in 80 per cent ethanol.
Degradation of Pregnanetriol-3(α),17,20; Etiocholanol-3(α)-One-
17—0.3 cc. of a 10 per cent aqueous periodic acid (HIO₄·2H₂O)
solution was added to a solution of 12.6 mg. of pregnanetriol in
1.4 cc. of methanol. The reaction mixture was kept at room
temperature for 18 hours and was then distributed between ether
and water. The ether phase was washed with sodium carbonate
and water and evaporated. The residue (10.8 mg.) was recrystal-
lized from dilute methanol. 8.8 mg. of colorless needles were
obtained that showed a point of incomplete fusion (followed by
resolidification) at 139.5° and melted completely at 152°. Upon a
second recrystallization the transition point was raised to 142°
and the melting point to 151.5–152.5°. This behavior is in ac-
cordance with observations of Callow (13) who first described the
dimorphism of etiocholanolone. Upon admixture with a specimen
of etiocholanol-3(α)-one-17 isolated from urine (3) there was no
depression of the melting point. The final product (7.7 mg.) was
benzoylated in the usual manner (3). The benzoate melted at
165–166°. The identity of this product was confirmed by a mixed
melting point determination with the benzoate of natural etio-
cholanolone.

Analysis—C₂₅H₃₄O₃. Calculated. C 79.14, H 8.69
Found. " 78.85, " 8.74

SUMMARY

Treatment of pregnanediol with acetic acid has yielded a mixture
of acetates from which pregnanediol monoacetate-3 has been
obtained. It has been converted into Δ₁₇-pregnenol-3(α) and a
pregnanetriol-3(α),17,20.

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BIBLIOGRAPHY