THE INTRODUCTION OF DEUTERIUM INTO THE C-19 ANGULAR METHYL GROUP

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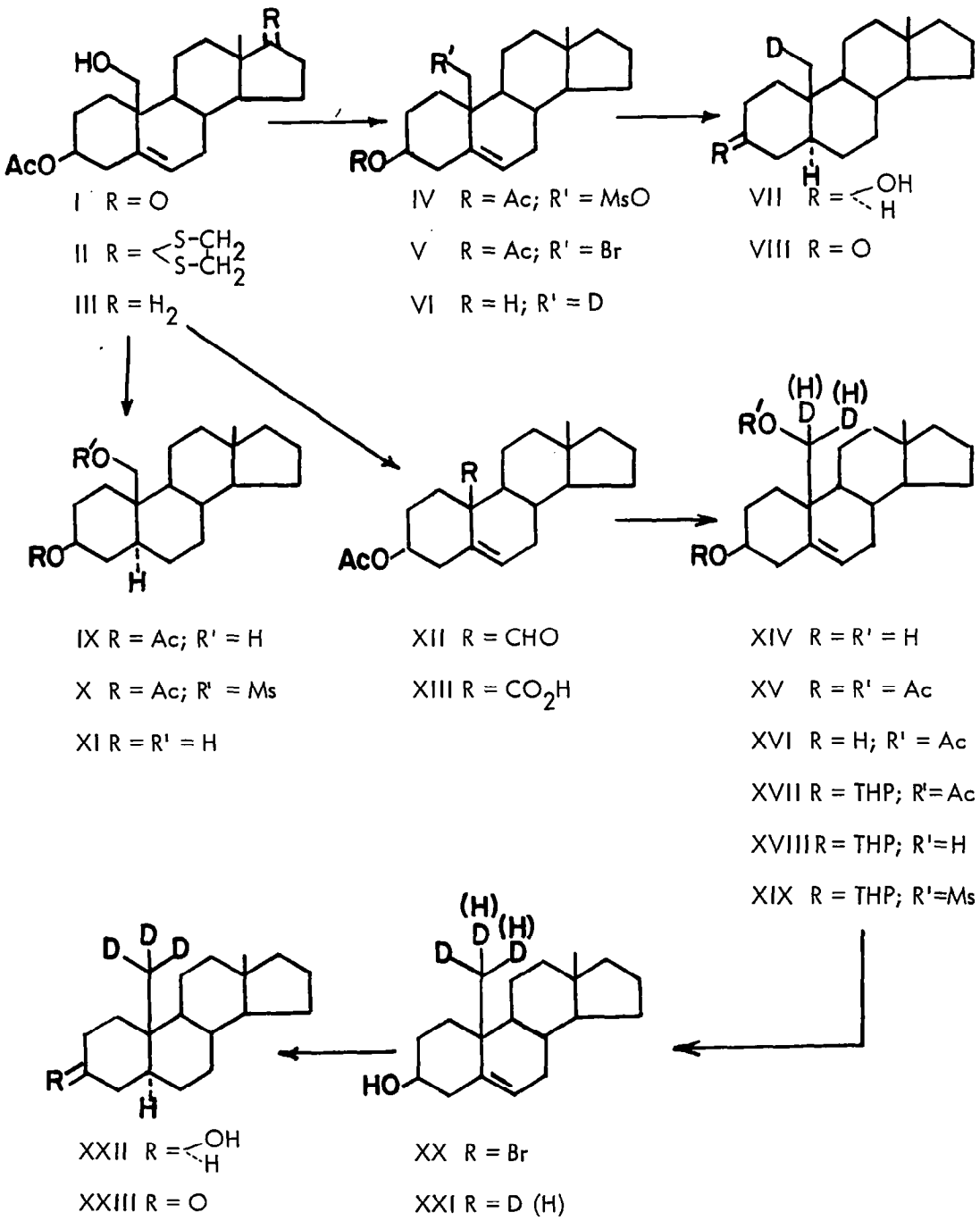
Through the use of the now readily accessible 19-hydroxy-Δ^5-steroids, methods have been developed for the introduction of one or three deuterium atoms into the C-19 angular methyl group to afford labeled substrates suitable for mass spectrometric studies. Substitution of tritiated for deuterated reagents would thus open a route to radioactive steroids labeled in the angular methyl group.

Current interest in our laboratory in a detailed knowledge of the mass spectral fragmentation behavior of keto steroids[2,3] has necessitated the synthesis of a wide variety of deuterium-labeled substrates.[4-6] In that connection, a need arose for steroids labeled selectively in the two angular methyl groups and the present article is concerned with the introduction of deuterium into the C-19 angular methyl group. These results should be of potential biochemical interest, since substitution of lithium aluminum triteride for lithium aluminum deuteride would provide steroids marked radioactively in the angular methyl group, which would be of utility in studies concerned with the biological removal of that substituent.

The starting material for all of our work was Δ^5-androstene-3β,19-diol-17-one 3-acetate (I)[8], which is readily accessible[9] from dehydroisoandrosterone. Since for our ultimate mass spectrometric studies ring D unsubstituted steroids were required, the starting ketone I was transformed[10] into the thioketal II and desulfurized with W-2 Raney nickel catalyst to afford the key intermediate Δ^5-androstene-3β,19-diol 3-acetate (III). Conversion to the 19-mesylate IV and displacement with lithium bromide in isopropyl alcohol solution provided the oily 19-bromide V, which was reduced with lithium aluminum deuteride to 19-d-Δ^5-androsten-3β-ol (VI). Catalytic hydrogenation followed by oxidation with chromium trioxide led to the required 19-d-5α-androstan-3-one (VIII).



I R = O

II R = $<^{S\text{-}CH_2}_{S\text{-}CH_2}$

III R = H_2

IV R = Ac; R' = MsO

V R = Ac; R' = Br

VI R = H; R' = D

VII R = $<^{OH}_{H}$

VIII R = O

IX R = Ac; R' = H

X R = Ac; R' = Ms

XI R = R' = H

XII R = CHO

XIII R = CO_2H

XIV R = R' = H

XV R = R' = Ac

XVI R = H; R' = Ac

XVII R = THP; R' = Ac

XVIII R = THP; R' = H

XIX R = THP; R' = Ms

XX R = Br

XXI R = D (H)

XXII R = $<^{OH}_{H}$

XXIII R = O

It is instructive to note that reversal of this reaction sequence, namely initial double bond saturation followed by displacement at C-19, failed. Thus catalytic hydrogenation of Δ^5-androstene-3β,19-diol 3-acetate (III) gave the non-crystalline 5α-androstane-3β,19-diol 3-acetate (IX), which was characterized in the form of the 19-mesylate X and the free diol XI. The latter was also produced in the lithium aluminum hydride reduction of the mesylate X. All attempts to effect displacement of the 19-mesylate function with lithium bromide proved to be abortive, in contrast to the straightforward reaction of the Δ^5-analog (IV→V). Similarly, no success was encountered in displacing the mesylate with sodium iodide, sodium ethyl mercaptide or triphenyl phosphite methiodide.[11] No attempt has been made to determine whether the beneficial effect of the 5,6-double bond is due to homoallylic conjugation[12] or simply due to the removal of the axial 6β-hydrogen atom.

For the introduction of three deuterium atoms, the following more cumbersome route had to be developed starting with the 19-carboxylic acid XIII, which was most conveniently prepared by chromium trioxide-pyridine oxidation of Δ^5-androstene-3β,19-diol 3-acetate (III) to the aldehyde XII, followed by treatment with potassium permanganate in pyridine solution. The subsequent reaction sequence and characterization of intermediates was first studied with unlabeled materials and then carried through in the deuterated series by replacing lithium aluminum hydride with lithium aluminum deuteride. Thus lithium aluminum deuteride reduction of the carboxylic acid XIII to 19-d_2-Δ^5-androstene-3β,19-diol (XIV) resulted in introduction of two deuterium atoms and it was now only necessary to protect the free 3-hydroxyl function in order to permit application of the same sequence employed earlier (IV→V→VI) to insert the remaining deuterium atom. For this purpose, the diol XIV was diacetylated (XV) and then partially saponified at C-3 to provide the 3β-hydroxy-19-acetate XVI. The latter was transformed[10] into the tetrahydropyranyl ether XVII and directly saponified to the 19-hydroxy 3β-tetrahydropyranyl ether XVIII. The

remaining steps followed well-trodden paths involving 19-mesylate formation (XIX), displacement with lithium bromide and accompanying cleavage of the now superfluous tetrahydropyranyl ether protective grouping (XX), lithium aluminum deuteride reduction (XXI) and finally catalytic hydrogenation (XXII) and oxidation at C-3 to yield 19-d_3-5α-androstan-3-one (XXIII), the isotopic composition of which was established by mass spectrometry.

The absence of a C-17 substituent was mandatory for our mass spectrometric work[13], but all of the steps should be applicable directly to the corresponding 17-ketal, which would thus preserve the C-17 functionality. This might be important if substances such as 19-t-testosterone were to be synthesized.

EXPERIMENTAL

All rotations were determined in chloroform solution and infrared spectra as potassium bromide pellets. All melting points were obtained with a Kofler block. The microanalyses are due to Messrs. E. Meier and J. Consul of the Stanford microanalytical laboratory, while mass spectra were measured by Drs. H. Budzikiewicz and J.M. Wilson. All thin-layer chromatograms (TLC) were performed with Silica Gel G (E. Merck, Darmstadt) and sprayed with ceric sulfate reagent.

Δ^5-Androstene-3β,19-diol 3-acetate (III). -- A mixture of 200 mg. of Δ^5-androstene-3β,19-diol-17-one 3-acetate (I)[8], 400 mg. of anhydrous sodium sulfate, 2 cc. of dry benzene, 200 mg. of freshly fused zinc chloride and 0.2 cc. of ethanedithiol was stirred at room temperature for 4 hr., whereupon TLC indicated the absence of starting material. Dilution with ether, thorough washing with water, drying and evaporation gave a crystalline mass (m.p. 165°) which upon recrystallization from ethyl acetate provided 165 mg. of the thioketal II, m.p. 170-172°, $[\alpha]_D$ -74° (c, 1.0).

Anal. Calcd. for $C_{23}H_{34}O_3S_2$:C, 65.38; H, 8.11; S, 15.18. Found:C, 65.28; H, 8.08; S, 15.40.

The above thioketal (220 mg.) was heated under reflux in 95% ethanol solution (40 cc.) for 8 hr. with 5 g. of W-2 Raney nickel catalyst (age, 15 days), the catalyst filtered, the filtrate concentrated and the product extracted with ether. Recrystallization from ethyl acetate-hexane provided in ca. 80% yield (average of several runs) Δ^5-androstene-3β,19-diol 3-acetate (III) as colorless crystals, m.p. 106-107°, $[\alpha]_D$ -63° (c, 1.0).

Anal. Calcd. for $C_{21}H_{32}O_3$: C, 75.86; H, 9.70. Found:C, 75.68; H, 9.78.

19-d-5α-Androstan-3-one (VIII). -- A solution of 200 mg. of Δ^5-androstene-3β,19-diol 3-acetate (III) in 1.5 cc. of pyridine and 0.2 cc. of methanesulfonyl chloride was kept at room temperature for 0.5 hr. and then processed in the usual way through ether extraction. Recrystallization of the crude product from methanol furnished 120 mg. of analytically pure (one spot on TLC developed with benzene) Δ^5-androstene-3β,19-diol 3-acetate 19-mesylate (IV), m.p. 120-121°, $[\alpha]_D$ -83° (c, 1.0).

Anal. Calcd. for $C_{22}H_{34}O_5S$: C, 64.37; H, 8.35; S, 7.81. Found:C, 64.40; H, 8.43; S, 7.82.

The mesylate IV (100 mg.), dissolved in 6 cc. of isopropyl alcohol, was heated under reflux for 2.5 hr. with 100 mg. of freshly dried lithium bromide. The inorganic precipitate was filtered, the product isolated with ether and used directly in the next step, since it resisted all attempts at crystallization. This bromide V exhibited R_f 0.5 as compared to 0.1 for the starting mesylate IV in a thin-layer chromatogram using benzene as the developing agent.

A portion (40 mg.) of the bromide V was heated under reflux for 2 hr. with a solution of 0.7 g. of lithium aluminum deuteride in 10 cc. of dry diglyme. The excess reagent was destroyed by the addition of water followed by hydrochloric

acid and the product was isolated with ether. Evaporation to dryness furnished 30 mg. of crude 19-d-Δ^5-androsten-3β-ol (VI) of m.p. 120-125°, which exhibited a negative Beilstein test and exhibited the same TLC mobility (benzene-ethyl acetate 75/25) as authentic Δ^5-androsten-3β-ol[14] prepared by Wolff-Kishner reduction of dehydroepiandrosterone. The entire crude product was dissolved in ethyl acetate and hydrogenated with 10% palladized charcoal catalyst. One recrystallization of the total hydrogenation product (m.p. 140-142°) raised the m.p. of 19-d-5α-androstan-3β-ol to 145-147°, undepressed upon admixture with an authentic specimen of 5α-androstan-3β-ol.[15] Identity was established further by TLC comparison.

A sample of 19-d-5α-androstan-3β-ol (VII) was oxidized by the Jones procedure[16] in acetone solution to yield, after recrystallization from ether, colorless needles of 19-d-5α-androstan-3-one (VIII), m.p. 98-99°, undepressed when mixed with a sample of authentic[17] 5α-androstan-3-one. The empirical composition was confirmed by mass spectrometry ($C_{19}H_{29}DO$; mol.wght., calcd. 275; found: 275) and by the n.m.r. spectrum ($CDCl_3$ solution), the area of the 1.03 ppm (19-methyl) signal corresponding to two-thirds of the area under the 0.73 ppm (18-methyl) signal.

5α-Androstane-3β,19-diol 3-acetate 19-mesylate (X). -- The catalytic hydrogenation of Δ^5-androstene-3β,19-diol 3-acetate (III) was effected in nearly quantitative yield in ethyl acetate solution at room temperature and atmospheric pressure using 10% palladized charcoal catalyst. The resulting 5α-androstane-3β,19-diol 3-acetate (IX) was non-crystalline ($[\alpha]_D$ -5°) and was, therefore, characterized by alkaline saponification to 5α-androstane-3β,19-diol (XI), which exhibited m.p. 198-200°, $[\alpha]_D$ +5° (c, 0.5) after recrystallization from ethyl acetate. The identical product (35 mg.) was obtained when 50 mg. of the 19-mesylate X was heated under reflux for 4 hr. with 0.6 g. of lithium aluminum hydride in diglyme solution.

Anal. Calcd. for $C_{19}H_{32}O_2$: C, 78.03; H, 11.03. Found:C, 77.91; H, 11.11.

A sample (185mg.) of the non-crystalline 3-acetate IX was left overnight at room temperature in pyridine solution with 0.2 cc. of methanesulfonyl chloride and the 19-mesylate X, isolated by means of ether extraction, was recrystallized from methanol; yield, 161 mg., m.p. 126-127°, $[\alpha]_D$ -14°.

Anal. Calcd. for $C_{22}H_{36}O_5S$:C, 64.05; H, 8.80; S, 7.77. Found:C, 64.14; H, 8.84;S, 8.00.

Δ^5-Androsten-3β-ol-19-al 3-acetate (XII). -- A solution of 200 mg. of Δ^5-androstene-3β,19-diol 3-acetate (III) in 2 cc. of freshly distilled pyridine was added to a slurry of 200 mg. of chromium trioxide in 2 cc. of the same solvent. After 12 hr. at room temperature, ethyl acetate (100 cc.) was added, the mixture filtered through Celite, washed with dilute hydrochloric acid, dried and evaporated. Recrystallization from methanol furnished 58-64% of the desired aldehyde, m.p. 128-129.5°, $[\alpha]_D$ -301° (c, 1.0), λmax 5.70, 5.87 and 8.0 μ.

Anal. Calcd. for $C_{21}H_{30}O_3$:C, 76.32; H, 9.15. Found:C, 76.09; H, 9.31.

3β-Acetoxy-Δ^5-androsten-19-oic acid (XIII). -- The above-described aldehyde XII (460 mg.), dissolved in 5 cc. of dry pyridine, was added to a suspension of pulverized potassium permanganate (460 mg.) in 2.5 cc. of pyridine and the mixture left at room temperature for 2.5 hr. Dilution with ether and thorough washing with dilute sulfuric acid, followed by water washing, drying and evaporation left 443 mg. of amorphous residue, TLC analysis (benzene-ethyl acetate 75/25) of which indicated the presence of three weak spots of much higher R_f value than the intense spot corresponding to the acid. Purification was effected by gradient elution chromatography on 30 g. of silicic acid (100 mesh, Malinckrodt) with benzene in the reservoir and ethyl acetate being added with stirring at such a rate as to maintain a constant level in the reservoir, the composition of the various fractions being checked by TLC analysis. In this manner there was obtained, after recrystallization from methanol, 250 mg. of the acid XIII, m.p. 186-189°, $[\alpha]_D$ -124° (c, 1.0).

Anal. Calcd. for $C_{21}H_{30}O_4$:C, 72.80; H, 8.73. Found:C, 73.04; H, 8.78.

19-d_3-5α-Androstan-3-one (XXIII). -- Reduction of 250 mg. of the acid XIII was performed in the usual manner (2.5 hr. reflux) with 2.2 g. of lithium aluminum deuteride in diglyme solution followed by recrystallization from ethanol and led to 200 mg. of 19-d_2-Δ^5-androstene-3β,19-diol (XIV) with m.p. 203-204°, undepressed upon admixture with the analytical specimen of the non-deuterated analog (m.p. 204-206°, $[\alpha]_D$ -28° (c, 1)).

Anal. Calcd. for $C_{19}H_{30}O_2$:C, 78.57; H, 10.41. Found:C, 78.69; H, 10.70.

The entire d_2-diol XIV was acetylated by keeping overnight at room temperature with acetic anhydride-pyridine and since the diacetate XV could not be crystallized, it was subjected directly to partial saponification by letting it stand for 9 hr. at room temperature with 5 cc. of 1% methanolic potassium carbonate solution. The product, isolated by extraction with ether, was purified by gradient elution chromatography on 15 g. of Merck neutral alumina (activity II) with 70 cc. of benzene in the reservoir and 50 cc. of ether being added to maintain the solvent level constant in the storage vessel. Aside from 40 mg. of completely saponified diol XIV, there was obtained after recrystallization from aqueous methanol 110 mg. of 19-d_2-Δ^5-androstene-3β,19-diol 19-acetate (XVI), m.p. 89-92°, $[\alpha]_D$ -104° (c, 1.0), the physical constants being identical with those of the analytical sample of the non-deuterated material.

Anal. Calcd. for $C_{21}H_{32}O_3$:C, 75.86; H, 9.70. Found:C, 75.29; H, 9.81.

The d_2-19-monoacetate XVI (110 mg.) was dissolved in 0.15 cc. of freshly distilled dihydropyran and kept at room temperature for 1 hr. with 2 drops of conc. hydrochloric acid. Ether extraction provided 169 mg. of oily 3β-dihydropyranyl ether 19-acetate XVII (homogeneous by TLC), which was saponified by heating under reflux for 20 min. with 10 cc. of 3% aqueous alcoholic sodium hydroxide solution. Recrystallization from ether gave 145 mg. of 19-d_2-Δ^5-androstene-

3β,19-diol 3-tetrahydropyranyl ether (XVIII), m.p. 134-136°, $[\alpha]_D$ -25° (c, 1.0), elementary analysis being performed on the non-deuterated material.

Anal. Calcd. for $C_{24}H_{38}O_3$: C, 76.96; H, 10.23. Found:C, 76.93; H, 10.21.

Mesylation of 142 mg. of the 3-tetrahydropyranyl ether XVIII was effected in pyridine solution as described above (III→IV) and the resulting 3-tetrahydropyranyl ether 19-mesylate XIX recrystallized from ethanol; yield, 150 mg., m.p. 122-123°, $[\alpha]_D$ -66° (c, 1.0). The non-deuterated analog was employed for analysis.

Anal. Calcd. for $C_{25}H_{40}O_5S$:C, 66.34; H, 8.91; S, 7.09. Found:C, 66.04; H, 9.16; S, 7.01.

A solution of the 19-d_2-mesylate XIX (149 mg.) in 16 cc. of isopropyl alcohol containing 400 mg. of lithium bromide was heated under reflux for 2 hr. No inorganic precipitate was noted and the crude bromide XX (114 mg.) isolated by ether extraction was immediately heated under reflux for 2.5 hr. in diglyme solution with 750 mg. of lithium aluminum deuteride. The resulting 19-d_3-Δ^5-androsten-3β-ol (XXI) (85 mg., m.p. 133-135°) was hydrogenated without further purification in ethyl acetate solution with 10% palladized charcoal catalyst, the catalyst filtered, the solvent evaporated to dryness and the total residue subjected to Jones oxidation[16] in acetone solution (10 min., room temperature). Recrystallization from ethyl acetate led to 18 mg. of 19-d_3-5α-androstan-3-one (XXIII), m.p. 103-103.5°, undepressed upon admixture with authentic 5α-androstan-3-one and exhibiting a mass spectral molecular ion peak at m/e 277 (Calcd., mol. wght. 277 for $C_{19}H_{27}D_3O$).

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