for 60 hr. Evaporation of the extract yielded a partially methylated product (410 mg.) which was dissolved in methyl iodide (50 ml.) containing silver oxide (5 g.) and Drierite. After boiling under reflux for 10 hr., the solution was recovered by filtration and evaporated to dryness. This treatment was repeated four times when an infrared spectrum of the sirup (250 mg.) indicated the absence of any hydroxyl groups.

Anal. Calcd. for $C_{18}H_{34}O_{10}$: OMe, 52.9. Found: OMe, 51.9.

Sirupy methyl 2-*O*-(2,3,4,6-tetra-*O*-methyl-β-D-glucosyl)-3,4-di-*O*-methyl-α,β-D-xyloside was boiled under reflux with 0.5 *N* sulfuric acid (50 ml.) for 10 hr. After recovery of the sirupy mixture in the usual way, paper chromatography (solvent C) indicated the presence of a tetra-*O*-methylglucose and a di-*O*-methylxylose in approximately equal amounts.

Identification of 2,3,4,6-Tetra-*O*-methyl-D-glucose.—The sugar mixture obtained after hydrolysis was resolved on strips of Whatman No. 1 filter paper (system C) and the excised sections were eluted five times with ethanol. After treatment with Darco G-60 charcoal and Amberlite IR-120 (H) exchange resin, evaporation yielded chromatographically pure tetra-*O*-methylglucose (68 mg.) and di-*O*-methylxylose (15 mg.).

The 2,3,4,6-tetra-*O*-methyl-D-glucose was crystallized from petroleum ether (b.p. 60–80°), m.p. 87.5–90.5°, $[\alpha]^{21}D$ +82° (*c* 1.7 in water).

Anal. Calcd. for $C_{10}H_{20}O_6$: OMe, 52.5. Found: OMe, 51.3.

The 2,3,4,6-tetra-*O*-methyl-*N*-phenyl-D-glucosylamine, on recrystallization from petroleum ether, had m.p. and mixed m.p. 135–136.5°, $[\alpha]^{22}D$ +225° (*c* 1.1 in acetone).[33]

(33) J. C. Irvine and A. M. Moodie, *J. Chem. Soc.*, 95 (1908).

Characterization of 3,4-Di-*O*-methyl-D-xylose.—The 3,4-*O*-methyl-D-xylose had an electrophoretic mobility in 0.05 *M* borate solution which was identical to that of an authentic specimen.[34] The remaining sirup was converted to the aniline derivative[35] which, however, failed to crystallize. The infrared spectrum of the 3,4-di-*O*-methyl-*N*-phenyl-D-xylosylamine, $[\alpha]^{22}D$ +137° (*c* 0.5 in ethyl acetate), was indistinguishable from that of an authentic specimen.

Preparation of Methyl 2-*O*-[Methyl (2,3,4-tri-*O*-acetyl-β-D-glucosyl)-uronate]-3,4-di-*O*-acetyl-α,β-D-xyloside.—Methyl 2-*O*-[methyl (β-D-glucopyranosyl)-uronate]-α,β-D-xylopyranoside (200 mg.) was dissolved in anhydrous pyridine (15 ml.) to which freshly distilled acetic anhydride (5 ml.) was added. After 24 hr. at room temperature, the reaction mixture was poured into ice-water to form a solution which was extracted three times with chloroform. The extract was purified in the usual way and concentrated to yield a yellow sirup (238 mg.), $[\alpha]^{21}D$ +28.4° (*c* 1.6 in chloroform), which could not be induced to crystallize in ethanol, ethyl ether or aqueous mixtures of these two solvents.

Anal. Calcd. for $C_{23}H_{32}O_{16}$: OMe, 11.0; ester (as acetyl), 45.8. Found: OMe, 10.3; ester, 42.1.

Acknowledgment.—The authors are thankful to the National Research Council of Canada for financial assistance. W. D. S. B. also wishes to express his gratitude to the National Research Council of Canada for two Studentships and to the Allied Chemical Corporation for a Fellowship.

(34) H. Bouveng and B. Lindberg, *Acta Chem. Scand.*, **10**, 1283 (1956).

(35) L. Hough and J. K. N. Jones, *J. Chem. Soc.*, 4349 (1952).

MONTREAL, QUE., CANADA

[CONTRIBUTION FROM THE DEPARTMENT OF PHYSIOLOGY AND BIOCHEMISTRY, SOUTHWEST FOUNDATION FOR RESEARCH AND EDUCATION]

Synthesis of 2β-Hydroxytestosterone[1]

BY P. NARASIMHA RAO AND LEONARD R. AXELROD

RECEIVED SEPTEMBER 24, 1959

The synthesis of 2β-hydroxytestosterone (III) has been described. It has been shown that 2β-hydroxytestosterone 2,17-diacetate (I) could be selectively hydrolyzed at the C-2 position to give 2β-hydroxytestosterone 17-acetate (V) without isomerization of the 2β-hydroxyl group. Hydrolysis of I and VI by refluxing with potassium bicarbonate or potassium carbonate in aqueous methanol yielded 2,17β-dihydroxyandrosta-1,4-dien-3-one (IV).

2β-Hydroxy steroids have thus far been prepared only by biological hydroxylation.[2] By the usual synthetic methods the 2β-hydroxy group in a Δ^4-3-, keto steroid is rather an inaccessible function because it tends to isomerize to the more stable (equatorial) α-form. However, it is of great interest to develop a synthetic route to 2β-hydroxy steroids, and the present paper is concerned with the successful synthesis of 2β-hydroxytestosterone (III).

Steroids having an α- and β-acetoxy group at the C-2 position have been previously synthesized by two methods. The first method consists of acetoxylation of a Δ^4-3-keto steroid with lead tetraacetate[3] and the second one consists of acetolysis of a 6-bromo-Δ^4-3-ketone with potassium acetate[3d,3e,3f,4]

It was claimed previously[3d,3e] that both the 2α- and 2β-acetoxy compounds when hydrolyzed under mild conditions gave the more stable 2α-hydroxy compound. That is, even under seemingly mild hydrolytic conditions the 2β-hydroxy group was isomerized to the 2α-form. However, by the methods worked out in our laboratories the free compound 2β-hydroxytestosterone (III) was definitively synthesized.

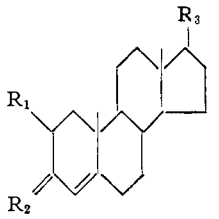
Compound I was prepared by acetolysis of 6-bromotestosterone 17-acetate with potassium acetate as described in the literature[3d,3f] with one ex-

(1) This investigation has been supported in part by a Grant from the Wyeth Institute for Medical Research, Philadelphia 1, Pa., and Grant No. A1078 of the National Institute of Arthritis of the National Institutes of Health, Bethesda, Md.

(2) (a) L. R. Axelrod, L. L. Miller and F. Herling, *J. Biol. Chem.*, **219**, 455 (1956); (b) R. M. Dodson, Arthur H. Goldhamp and R. D. Muir, THIS JOURNAL, **79**, 3921 (1957); (c) H. L. Herzog, M. J. Gentles, E. B. Hershberg, F. Carriajal, D. Sutter, W. Charney and C. P. Schaffner, *ibid.*, **79**, 3921 (1957); (d) G. Greenspan, C. P. Schaffner, W. Charney, H. L. Herzog and E. B. Hershberg, *ibid.*, **79**, 3922 (1957).

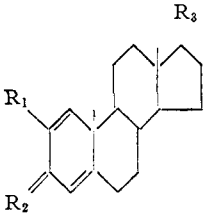
(3) (a) G. Ehrhart, H. Ruschig and W. Amuller, *Angew. Chem.*, **52**, 363 (1939); (b) T. Reichstein and C. Montigel, *Helv. Chim. Acta*, **22**, 1212 (1939); (c) E. Seebeck and T. Reichstein, *ibid.*, **27**, 948 (1944); (d) F. Sondheimer, S. Kaufmann, J. Romo, H. Martinez and G. Rosenkranz, THIS JOURNAL, **75**, 4712 (1953); (e) G. Rosenkranz, O. Mancera and F. Sondheimer, *ibid.*, **77**, 145 (1955); (f) R. L. Clarke, K. Dobriner, A. Mooradian and C. M. Martini, *ibid.*, **77**, 661 (1955).

(4) (a) D. E. A. Rivett and E. S. Wallis, *J. Org. Chem.*, **15**, 35 (1950); (b) L. F. Fieser and M. A. Romero, THIS JOURNAL, **75**, 4716 (1953); (c) J. Herran, G. Rosenkranz and F. Sondheimer, *ibid.*, **76**, 5531 (1954); (d) J. S. Baran, *ibid.*, **80**, 1687 (1958).



I, R_1 = β-OAc; R_2 = O, R_3 = β-OAc
IIa, R_1 = β-OH; R_2 = α-H, β-OH; R_3 = β-OH
IIb, R_1 = β-OH; R_2 = β-H, α-OH; R_3 = β-OH
III, R_1 = β-OH; R_2 = O; R_3 = β-OH
V, R_1 = β-OH; R_2 = O; R_3 = β-OAc
VI, R_1 = α-OAc; R_2 = O; R_3 = βOAc
VII, R_1 = α-OH; R_2 = O; R_3 = β-OH

IV, R_1 = -OH; R_2 = O; R_3 = β-OH
VIII, R_1 = -OAc; R_2 = O; R_3 = β-OAc



ception, *i.e.*, after conducting a series of time reactions it was found that a 10–12 minute reaction with potassium acetate yielded optimum condition for the highest yields of 2β-isomer. It was observed that by increasing the reaction time, compound I, which was formed initially, was then isomerized to the more stable form VI.

Since the usual hydrolytic procedures isomerize I to the α-form at the C-2 position it was decided to reduce I with lithium aluminum hydride, whereby the 2β-configuration at the C-2 position is not affected; and then to selectively oxidize the 3-hydroxyl function with manganese dioxide to give 2β-hydroxytestosterone (III). In fact, lithium aluminum hydride reduction of I gave a mixture of the 2β,3β-, and the 2β,3α-isomers IIa and IIb, of which the 2β,3β-isomer IIa predominated, and was selectively purified. The equatorial 3β-configuration in IIa, while not proved, is assumed from the known direction of hydride reduction of a Δ^4-3-ketone which yields a preponderance of 3β-alcohol. However, the mixture of isomers was oxidized with manganese dioxide at −15° to give III in approximately 20% yield based on ultraviolet data. It is interesting to note that when the oxidation was carried out at room temperature (20–25°) or even at 0° the 2-hydroxyl function was also oxidized and compound IV with a maximum in the ultraviolet at 254 mμ was obtained. Acetylation of IV with acetic anhydride and pyridine gave VIII ($\lambda_{max}^{methanol}$ 247 mμ).

The identity of compounds IV and VIII was established by comparison with authentic samples kindly provided by Dr. Baran. Either increase in reaction time or addition of more catalyst did not improve the yield of III and it was separated from the unreacted mixture of triols IIa and IIb on a silicic acid column. 2β-Hydroxytestosterone (III) exists in two different polymorphic forms, one with a m.p. 87–89° (prisms) and the other with a m.p. 163–164° (needles) and their infrared spectra are identical in all respects. If the lower melting form was dissolved in acetone–petroleum ether (boiling range 40–60°) and seeded with a small crystal of higher melting form, only the high melting form resulted.

The contribution of the hydroxyl at C-2 to the molecular rotation of III (ΔM_D − 667) strongly supports the fact that it did not isomerize in the synthetic procedure and the hydroxyl group at C-2 retained its β-configuration. It was shown in a number of instances previously that the 2β-hydroxyl group contributes more strongly to the levorotation in Δ^4-3-keto steroids.[2b,2c,2d]

It was claimed that when compounds I and VI were hydrolyzed by refluxing with potassium bicarbonate[3d] or with potassium carbonate[3e] in methanol, 2α-hydroxytestosterone (VII) was obtained. However, repetition of the above experiments failed to yield VII with a maximum in the ultraviolet at 240 mμ, and instead IV was obtained which exhibited a shift in the ultraviolet absorption maximum from 240 to 254 mμ.

The same compound (IV) was also obtained by manganese dioxide oxidation of the mixture of triols IIa and IIb at room temperature. Apparently, under the hydrolytic conditions employed, I and VI were hydrolyzed to the free hydroxyl functions and further autoxidation had taken place in the alkaline media to give 2,3-diketone IV. Similar autoxidations of α-hydroxy-keto compounds in alkaline or acidic solution to diketones have been observed by Barton, *et al.*,[5a] and Lavie, *et al.*[5b]

It was observed that compound I could be selectively hydrolyzed under controlled conditions (see Experimental) at the C-2 position with potassium hydroxide in methanol at room temperature to give 2β-hydroxytestosterone 17-acetate (V) *without isomerization to the 2α-form.* The correctness of the structure of V was supported by the fact that the molecular rotatory difference of V is more strongly levorotatory (ΔM_D − 673.8),[2b,2c,2d] and on reacetylation of V gave the *same diacetate I* and it was identified by the infrared spectrum and mixture melting point.

Experimental[6]

2β-Hydroxytestosterone 2,17-diacetate (I) was prepared essentially by the same procedure described by Clarke, *et al.*,[3f] by acetolysis of 6-bromotestosterone-17-acetate[7] with potassium acetate in glacial acetic acid with one variation. The reaction mixture was heated under reflux for 12 minutes, instead of 4 hours, whereby the 2β-hydroxytestosterone 2,-17-diacetate (I) was obtained in 40–45% yield in contrast to the 21% yield described. Compound I so obtained had m.p. 201–202°, $[\alpha]^{20}D$ −60° ($CHCl_3$), $\lambda_{max}^{methanol}$ 243 mμ (ε 15,140); ν_{max}^{KBr} 1752, 1729, 1686 and 1614 cm.$^{-1}$.

Anal. Calcd. for $C_{23}H_{32}O_5$ (388.49): C, 71.10; H, 8.30. Found: C, 71.08; H, 8.36.

Lithium Aluminum Hydride Reduction of I.—A solution of 400 mg. of 2β-hydroxytestosterone 2,17-diacetate (I) in 25 ml. of dry tetrahydrofuran was added with stirring to 470 mg. of lithium aluminum hydride in 30 ml. of tetrahydrofuran over a period of 15 minutes, and the mixture was then heated under reflux for 1 hour. The excess reagent was decomposed by addition of ethyl acetate. A saturated solution of sodium sulfate was then added until the precipitate began to adhere to the sides of the flask. Finally, 5 g. of solid sodium sulfate was added and the solution was filtered

(5) (a) D. H. R. Barton and J. F. Eastham, *J. Chem. Soc.*, 424 (1953); (b) D. Lavie and Y. Shvo, THIS JOURNAL, **81**, 3058 (1959).

(6) Analyses were performed by Micro-Tech Laboratories, Skokie, Ill. All melting points are uncorrected.

(7) (a) C. Meystre and A. Wettstein, *Experientia*, **2**, 408 (1946); (b) C. Djerassi, G. Rosenkranz, J. Romo, S. Kaufmann and J. Pataki, THIS JOURNAL, **72**, 4534 (1950).

TABLE I

MOLECULAR ROTATION DATA OF 2β-HYDROXY-Δ⁴-3-KETO STEROIDS

	M_D	M_D 2β-hydroxy	Contribution of 2β-hydroxy group
Testosterone	+314[3c]	−353.2[a]	−667.2
Testosterone acetate	+317[3c]	−356.8[a]	−673.8
Δ⁴-Androstone-3,17-dione	+566[3c]	−111.3[2b]	−677.3
Reichstein's substance S	+381[3c]	−210.4[2c]	−591.4
Reichstein's substance S 21-acetate	+554[3c]	− 97[2c]	−651

[a] This paper.

from the salts. Evaporation of filtrate under a stream of nitrogen gave 365 mg. of a mixture of 4-androstene-2β-3β,17β-triol (IIa) and 4-androstene-2β,3α,17β-triol (IIb) as a solid with a m.p. 167–171°. Fractional crystallization of this mixture from acetone ultimately gave 190 mg. of 4-androstene-2β,3β,17β-triol (IIa), m.p. 186–187°, $[\alpha]^{25}D$ −74.2° (ethanol), ν_{max}^{KBr} 3365 cm.$^{-1}$.

Anal. Calcd. for $C_{19}H_{30}O_3$ (306.4): C, 74.47; H, 9.87. Found: C, 74.70; H, 9.92.

Manganese Dioxide Oxidation of the Mixture of Triols IIa and IIb. (a) **At Room Temperature.**—To a solution of 365 mg. of the mixture of triols IIa and IIb in 75 ml. of tetrahydrofuran 3.5 g. of manganese dioxide[8] was added and stirred at room temperature (25°). At every 5 minutes a known amount of sample was withdrawn, filtered from the manganese dioxide, and after evaporating the solvent the ultraviolet spectrum in methanol was determined. At the end of 1 hour the maximum at 254 mμ remained almost constant without any further change. The solution was then filtered from the catalyst and after evaporating the solvent the residue was crystallized from acetone–petroleum ether (boiling range 40–60°) to give 264 mg. of 2,17β-dihydroxyandrosta-1,4-dien-3-one (IV), m.p. 190–192°. Two additional crystallizations from acetone–petroleum ether (boiling range 40–60°) gave analytically pure product, m.p. 204–205° $[\alpha]^{25}D$ −12.0° ($CHCl_3$), $\lambda_{max}^{methanol}$ 254 mμ (ε 15,140), ν_{max}^{KBr} 3450 and 1635 cm.$^{-1}$ (Baran[4d] gave m.p. 207–209°, $[\alpha]D$ −20.1° ($CHCl_3$), λ_{max} 254 mμ (ε 15,400), λ_{max}^{KBr} 2.87 and 6.12 μ). The infrared spectra of an authentic sample (kindly provided by Dr. Baran) and IV were found to be identical in all respects and their mixed melting point showed no depression. Acetylation of IV with pyridine and acetic anhydride in the usual manner gave 2,17β-dihydroxyandrosta-1,4-dien-3-one diacetate (VIII), m.p. 204–205°, and its identity has been established by a mixture melting point determination and comparison of an infrared spectrum with that of an authentic sample.[4d]

(b) **At 0°.**—The oxidation of the mixture of triols IIa IIb with manganese dioxide was carried out in an ice-bath at 0° and the reaction was followed spectrophotometrically as described earlier. Within the first 10 minutes the mixture showed λ_{max} 242 mμ which gradually shifted to 254 mμ as the reaction progessed and attained a constant value after one hour. After the usual working up, crystallization furnished 2,17β-hydroxyandrosta-1,4-dien-3-one (IV), m.p. 203–204°, $\lambda_{max}^{methanol}$ 254 mμ (ε 15,100). Identity was established by mixture melting point determination and comparison of infrared spectra.

(c) **At −15°.**—A solution of 1.6 g. of the mixture of triols IIa and IIb in 200 ml. of tetrahydrofuran was stirred and cooled in an ice–salt-bath. When the temperature of the reaction mixture dropped to −15°, 13 g. of manganese dioxide was introduced and the reaction was followed spectrophotometrically as described above.

As the reaction progessed a maximum at 242 mμ was observed and it attained constant value after 30 minutes of reaction indicating an approximately 20% conversion to Δ⁴-3-ketone. The solution was then filtered from the manganese dioxide and after evaporating the solvent the residue, 1.5 g., was chromatographed on a column of 45 g. of silicic acid[9] (length of silicic acid column 7.5 cm.). The column was eluted with benzene–ethanol mixtures with a gradual increase in ethanol content in the mixture. A solvent mixture of benzene–ethanol (98.5:1.5) eluted 270 mg. of 2β-hydroxytestosterone (III). After three crystallizations from acetone–petroleum ether (boiling range 40–60°) the analytical product was obtained, m.p. 163–164°, $[\alpha]^{24}D$ −108° ($CHCl_3$), $\lambda_{max}^{methanol}$ 242 mμ (ε 14,790); ν_{max}^{KBr} 3468, 1675 and 1612 cm.$^{-1}$.

Anal. Calcd. for $C_{19}H_{28}O_3$ (304.4): C, 74.96; H, 9.27. Found: C, 74.90; H, 9.58.

The second form of III had a m.p. 87–89°, $[\alpha]^{24}D$ −116° ($CHCl_3$), $\lambda_{max}^{methanol}$ 242 mμ (ε 14,450); ν_{max}^{KBr} 3468, 1675 and 1612 cm.$^{-1}$. When the compound with m.p. 87–89° was dissolved in acetone–petroleum ether (boiling range 40–60°) and seeded with a small crystal of compound of m.p. 163–164°, the higher melting form resulted. Compound III gave a positive "blue tetrazolium" test.

Acetylation of III with acetic anhydride and pyridine at 80° for 20 minutes yielded I (identified by infrared spectrum and mixture melting point) in almost quantitative yield.

2β-Hydroxytestosterone 17-Acetate (V).—Six grams of 2β-hydroxy-testosterone diacetate (I) was taken in 400 ml. of absolute methanol and the contents were stirred in an atmosphere of nitrogen at 30°. Compound I was not completely soluble in methanol under these conditions and to this mixture was added 15 ml. of 1 *M* potassium hydroxide in absolute methanol and the stirring continued. The solution gradually turned yellow and at the end of 10 minutes all the solid was in solution. Then 1.5 ml. of water was added and the contents stirred for 2 more minutes and then acidified with 25 ml. of 1 *N* acetic acid. Most of the solvent was removed under reduced pressure at room temperature and then diluted with water. The precipitated solid was then filtered and washed thoroughly with water. The solid was then crystallized from acetone to give 5 g. of 2β-hydroxytestosterone 17-acetate (IV), m.p. 195–196°, $[\alpha]^{24}D$ −103° ($CHCl_3$), $\lambda_{max}^{methanol}$ 242 mμ (ε 15,850); ν_{max}^{KBr} 3540, 1725, 1670 and 1612 cm.$^{-1}$.

Anal. Calcd. for $C_{21}H_{30}O_4$ (346.45): C, 72.80; H, 8.73. Found: C, 72.46; H, 8.8.

Acetylation of V with acetic anhydride and pyridine at 80° for 20 minutes gave I (identified by mixture melting point and infrared spectrum) in quantitative yield.

Saponification of 2β-Hydroxytestosterone Diacetate (I). (a) **With Potassium Bicarbonate.**[3d]—To a solution of 300 mg. of I in 15 ml. of methanol 300 mg. of potassium bicarbonate in 3 ml. of water was added and refluxed under nitrogen for 4 hours. It was then acidified with acetic acid, most of the solvent was removed under reduced pressure, diluted with water and then chilled. The precipitated product was filtered (200 mg.) and an ultraviolet spectrum of the crude product (in methanol) was determined. It exhibited a maximum at 253 mμ. Further purification of the product by crystallization from acetone–petroleum ether (boiling range 40–60°) gave IV, m.p. 204–205°. Acetylation of IV with acetic anhydride and pyridine gave VIII, m.p. 202–203°. These products were found to be identical (mixture melting point and infrared spectrum) with the products obtained by oxidation of mixture of triols IIa and IIb with manganese dioxide at room temperature.

(b) **With Potassium Carbonate.**[3c]—Saponification of I with potassium carbonate as described above gave IV, m.p. 203–205°, in similar yield.

Saponification of 2α-Hydroxytestosterone Diacetate (IV). (a) **With Potassium Bicarbonate**[3d] and (b) **with Potassium Carbonate.**—2α-Hydroxytestosterone diacetate (VI), m.p. 209–211°, 300 mg. was subjected to saponification (a) with potassium bicarbonate and (b) with potassium carbonate as described under the 2β-isomer. From both the experiments (a) and (b) compound IV was obtained in similar yields and its identity was established by mixture melting point determination and infrared spectrum.

Acknowledgment.—We wish to thank Dr. J. S. Baran of G. D. Searle Co. for providing us with authentic samples IV and VIII for comparison.

SAN ANTONIO 6, TEX.

(8) O. Mancera, G. Rosenkranz and F. Sondheimer, *J. Chem. Soc.*, 2189 (1953).

(9) The silicic acid used in these experiments is the Analytical Reagent grade supplied by Mallinckrodt Chemical Works with the label "Suitable for Chromatographic Analysis by the Method of Ramsey and Patterson." Before use this product was further activated by heating at 300° for 2 hours.