236. *Synthesis of* [19-^{14}C]*Testosterone.**

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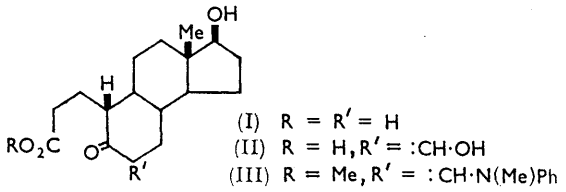
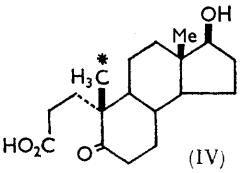
The synthesis of [19-^{14}C]testosterone is described. The keto-acid (I) obtained from 17β-acetoxy-19-nortestosterone was blocked at C-6 with an *N*-methylanilinomethylene group to give (III). Alkylation of (III) with [^{14}C]methyl iodide and subsequent hydrolysis yielded the keto-acid (IV). Treatment of (IV) with 2M-acetic anhydride–ethyl acetate–perchloric acid reagent gave the enol-lactone (V) which was then converted into [19-^{14}C]-testosterone by the conventional procedure.

The biosynthetic transformation of androgens into œstrogens is well established. Incubation of [^{14}C]testosterone with normal human ovarian tissue converted it into [^{14}C]-17β-œstradiol.[1,2] In this transformation of androgens into œstrogens, C-19 oxygenated steroids have been shown to be the immediate œstrogen precursors, in which subsequent elimination of the C-19 moiety occurs resulting in the aromatisation of ring A.[3-5]

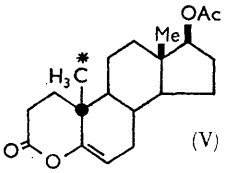
Unequivocal determination of the fate of the C-19 unit in the aromatisation requires the synthesis of steroids labelled at C-19, and we here describe the synthesis of [19-^{14}C]testosterone. Our approach involved the preparation of the keto-acid (I) from 19-nortestosterone, blocking of the C-6 position with an *N*-methylanilinomethylene group, and



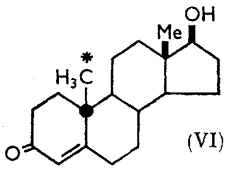
(I) R = R′ = H
(II) R = H, R′ = :CH·OH
(III) R = Me, R′ = :CH·N(Me)Ph



(IV)



(V)



(VI)

methylation of the angular position with [^{14}C]methyl iodide, to give the keto-acid (IV). It had been shown [6] that the final group forming the quaternary centre preferentially assumes the axial configuration. Consequently it was expected that the [^{14}C]methyl group in (IV) would assume the axial position to give predominantly the natural isomer. Transformation of the keto-acid (IV) into [19-^{14}C]testosterone could then be completed by the conventional procedure.[7,8]

Since the publication of our preliminary communication, Rakhit and Gut have reported [9] the synthesis of [19-^{14}C]testosterone. (Birch [10] had attempted the synthesis of testosterone

* Preliminary communication, *Chem. and Ind.*, 1963, 1838.

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[2] L. R. Axelrod and J. W. Goldzieher, *Proc. Endocrinol. Soc.*, 1961, **43**, 24; *J. Clin. Endocrinol. and Metab.*, 1962, **22**, 431.
[3] T. Morato, M. Hayano, R. I. Dorfman, and L. R. Axelrod, *Biochem. Biophys. Res. Comm.*, 1961, **6**, 334.
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[5] T. Morato, K. Raab, H. J. Brodie, M. Hayano, and R. I. Dorfman, *J. Amer. Chem. Soc.*, 1962, **84**, 3764.
[6] L. B. Barkley, W. S. Knowles, H. Raffelson, and Q. E. Thompson, *J. Amer. Chem. Soc.*, 1956, **78**, 4111.
[7] R. B. Turner, *J. Amer. Chem. Soc.*, 1950, **72**, 579.
[8] G. Fujimoto, *J. Amer. Chem. Soc.*, 1951, **73**, 1856.
[9] S. Rakhit and M. Gut, Amer. Chem. Soc. 145th Meeting, Sept. 1963, Abstracts of Papers, 104-c; *J. Amer. Chem. Soc.*, 1964, **86**, 1432.
[10] A. J. Birch, *Chem. and Ind.*, 1951, 616.

by the same route,[9] and concluded that if the C-6 position was not blocked methylation would occur primarily at C-6, owing to the *trans* B/C fusion.)

The stages involved in the present synthesis were first carried out with non-radioactive material to determine the optimum conditions. Ozonolysis of 17β-acetoxy-19-nortestosterone [11] followed by alkaline hydrolysis with N-ethanolic sodium hydroxide gave 17β-hydroxy-5-oxo-3,5-seco-4-norœstrane-3-carboxylic acid (I), which condensed with ethyl formate in the presence of sodium methoxide in a mixture of benzene and tetrahydrofuran to give the acid (II). This condensed with methylaniline in methanol solution to give the methylanilinomethylene-ketone [12] which was treated with diazomethane to give the ester (III) (85%). The C-19 methyl group was introduced into (III) by the angular methylation procedure of Birch and Robinson.[13] The compound (III) was treated with methyl iodide in t-butyl alcohol in the presence of potassium t-butoxide, and the crude product was hydrolysed first with sulphuric acid and then with sodium hydroxide to remove the methylanilinomethylene blocking group. One-step hydrolysis with alkali to remove the blocking group [12] gave inferior results. From the reaction mixture the acid (IV) crystallised with some difficulty on trituration with acetone, but a superior method of isolation was as follows. The crude product, after removal of the methylanilinomethylene group, was esterified with diazomethane, acetylated, and chromatographed on alumina. The fractions eluted with benzene were hydrolysed with 2% alcoholic sodium hydroxide, and compound (IV) was isolated without difficulty. The keto-acid (IV) was converted into testosterone through the intermediate enol-lactone (V) by the procedure of Turner [7] modified by Fujimoto,[8] except that, instead of refluxing the acid with a mixture of acetic anhydride and acetyl chloride for over 40 hours, it was treated at 20° with 2M-acetic anhydride–ethyl acetate–perchloric acid reagent [14] for exactly 2 minutes, whereby the lactone (V) was obtained in 85% yield.

In the radiosynthesis, 27 mc of [^{14}C]methyl iodide (New England Nuclear Corp., Boston, Mass.) reacted with (III) to give ultimately [19-^{14}C]testosterone (4%) with a specific activity of 3·97 μc/mg. and identical with non-radioactive testosterone.

Experimental

Melting points were determined on samples dried under a high vacuum at 55° for 24 hr. Ultraviolet spectra were determined in methanol with a Cary recording spectrophotometer (model 11 MS), and infrared spectra in potassium bromide discs on a Perkin-Elmer (model 21) spectrophotometer.

Optical rotations were measured in ethanol solution unless otherwise stated at 25° ± 3° with a Zeiss–Winkel polarimeter.

Merck reagent grade aluminium oxide labelled as suitable for chromatography was treated with ethyl acetate and activated to give Brockmann activity III.

Light petroleum was Mallinckrodt analytical reagent, b. p. 30—60°.

17β-*Hydroxy-5-oxo-3,5-seco-4-norœstrane-3-carboxylic Acid* (I).—17β-Acetoxy-19-nortestosterone (2·5 g.) was ozonised [11] to give 17β-acetoxy-5-oxo-3,5-seco-4-norœstrane-3-carboxylic acid (2 g.), m. p. 108—113°. It was dissolved in N-ethanolic sodium hydroxide (50 ml.) and heated under reflux for 3 hr. The solution was cooled, and acidified with acetic acid. Most of the ethanol was removed under a vacuum, and the residue diluted with water, extracted with ethyl acetate, and the extract dried (Na_2SO_4). The residue (1·8 g.) obtained after removal of solvent gave the *acid* (I), m. p. 162—163° (from acetone–light petroleum), $[\alpha]_D$ +9·8° (*c* 1·6), $\nu_{max.}$ 3440, 3390, 1717, and 1700 cm.$^{-1}$ (Found: C, 69·65; H, 8·85. $C_{17}H_{26}O_4$ requires C, 69·35; H, 8·9%).

[11] J. A. Hartman, A. J. Tomasewski, and A. S. Dreiding, *J. Amer. Chem. Soc.*, 1956, **78**, 5662.
[12] R. B. Woodward, F. Sondheimer, D. Taub, K. Heusler, and W. M. McLamore, *J. Amer. Chem. Soc.*, 1952, **74**, 4223.
[13] A. J. Birch and R. Robinson, *J.*, 1944, 501.
[14] (*a*) J. S. Fritz and G. H. Schenk, *Analyt. Chem.*, 1959, **31**, 1808; (*b*) patents pending on the conversion of δ-keto-acids into enol-lactones with this reagent.

17β-*Hydroxy*-6-*hydroxymethylene*-5-*oxo*-3,5-*seco*-4-*norœstrane*-3-*carboxylic Acid* (II).—To a suspension of sodium methoxide (9·1 g.) in dry benzene (350 ml.) freshly distilled ethyl formate (31 ml.) was added and stirred for 15 min. at 25° under nitrogen. Then a solution of the keto-acid (I) (8·6 g.) in dry tetrahydrofuran (35 ml.) was introduced and the mixture was stirred for 16 hr. at the same temperature. The mixture acquired an orange colour and the sodium salt was precipitated. It was filtered under an atmosphere of dry nitrogen and the precipitate was washed with benzene (100 ml.) and with light petroleum (50 ml.). The dry sodium salt was dissolved in ice–water (200 ml.), acidified with N-hydrochloric acid, the aqueous solution extracted with ethyl acetate, and the extract washed with water, dried (Na_2SO_4), filtered, and evaporated. The resulting crude gum (8·8 g.) gave the *acid* (II), m. p. 172—174° (from benzene), $[\alpha]_D$ −40° (*c* 1·3), $\lambda_{max.}$ 288 mμ (ε 9317), $\nu_{max.}$ 3420, 1720, 1705, 1625, and 1580 cm.$^{-1}$ (Found: C, 66·9; H, 8·1. $C_{18}H_{26}O_5$ requires C, 67·05; H, 8·1%). An alcoholic solution of (II) gave an intense reddish purple colour with ferric chloride.

Methyl 17β-*Hydroxy*-6-*methylanilinomethylene*-5-*oxo*-3,5-*seco*-4-*norœstrane*-3-*carboxylate* (III).—The hydroxymethylene compound (II) (8·4 g.) was dissolved in methanol (95 ml.), and methylaniline (19 ml.) was added. The yellow solution was set aside at room temperature for 24 hr. Most of the methanol was removed under a vacuum, and the residue was leached with small lots of light petroleum to remove the excess of methylaniline. The crude residue did not give the colour with ferric chloride immediately. It was esterified with excess of diazomethane in ether solution. The crude residue (9 g.) obtained after removal of the ether was chromatographed on alumina (300 g.), and the fractions eluted with benzene–ether (8 : 2) were combined to give the ester (III) (7·8 g.) as a light yellow gum which failed to crystallise, $[\alpha]_D$ +82·3° (*c* 1·1), $\nu_{max.}$ 3500, 1730, 1715, 1637, 1596, and 1497 cm.$^{-1}$.

17β-*Hydroxy*-5-*oxo*-3,5-*seco*-4-*norandrostane*-3-*carboxylic Acid* (IV).—(a) *Methylation.* To a cooled solution of potassium (0·287 g.) in t-butyl alcohol (10 ml.) under nitrogen, a solution of the methylanilinomethylene compound (III) (0·498 g.) in t-butyl alcohol (5 ml.) was added and the mixture was stirred for ½ hr. Methyl iodide (0·9 ml.) was added and the mixture heated under reflux for 2 hr. Most of the t-butyl alcohol was removed under reduced pressure, and the residue was diluted with water and extracted with ether. The crude material (0·5 g.) obtained after evaporation of the solvent was hydrolysed.

(b) *Hydrolysis.* The above residue (0·5 g.) was heated under reflux for 2 hr. with a mixture of water (12·5 ml.), ethanol (7·5 ml.), and concentrated sulphuric acid (1·9 ml.). The organic material was extracted with ethyl acetate and the residue, after removal of the solvent, was refluxed with 5% sodium hydroxide solution (25 ml.) for 4 hr. The mixture was cooled, acidified with dilute hydrochloric acid, and the liberated keto-acid extracted into ethyl acetate, dried (Na_2SO_4), filtered, and the solvent removed, to give a gum (0·355 g.).

(c) *Separation of* (IV) *from the above reaction product.* The above gum (0·355 g.) was dissolved in ether (20 ml.), and esterified with an excess of diazomethane in ether solution. The residue obtained after removal of the ether was acetylated with pyridine (2 ml.) and acetic anhydride (2 ml.) at room temperature. Most of the pyridine and excess acetic anhydride were removed under a high vacuum, and the residue was chromatographed on alumina (14 g.). The fractions eluted with benzene were combined (157 mg.) and hydrolysed by boiling with 2% alcoholic sodium hydroxide for 3 hr. The mixture was cooled, acidified with dilute hydrochloric acid, and most of the ethanol removed under a vacuum. It was extracted with ethyl acetate and the extract was washed with saturated sodium chloride solution, dried (Na_2SO_4), filtered, and the solvent removed. Recrystallisation from acetone–light petroleum gave the *acid* (IV) (36 mg.), m. p. 196—200°. Further recrystallisation gave m. p. 204—206°, $[\alpha]_D$ +37·4° (*c* 1·12), $\nu_{max.}$ 3420, 1705, 1695, and 1050 cm.$^{-1}$ (Found: C, 69·95; H, 9·4. $C_{18}H_{28}O_4$ requires C, 70·1; H, 9·15%), mixed m. p. 204—206° with a sample, prepared by ozonolysis followed by alkaline hydrolysis of 17β-acetoxytestosterone.

The above procedure was repeated with compound (III) (500 mg.) and [^{14}C]methyl iodide (0·9 ml., 27 mc), to give C-19 labelled compound (IV) (33 mg.), m. p. 202—203°.

17β-*Acetoxy*-5-*hydroxy*-3,5-*seco*-4-*norandrost*-5-*ene*-3-*carboxylic Acid* 3,5-*Lactone* (V).—The keto-acid (IV) (100 mg.) was dissolved in 2M-acetic anhydride–ethyl acetate–perchloric acid reagent [14] (2 ml.) at 20°. After exactly 2 min. the reaction was quenched by the addition of water (5 ml.), the product extracted with ethyl acetate, the extract washed with saturated sodium hydrogen carbonate solution and sodium chloride solution, dried (Na_2SO_4), and the solvent evaporated. Chromatography of the residue on silica gel (10 g.) gave the lactone (V)

(100 mg.), m. p. 129·5—130·5° (from acetone–hexane), $[\alpha]_D$ −10·6° (*c* 1·0 in $CHCl_3$), $\nu_{max.}$ 1768, 1735, 1692, and 1250 cm.$^{-1}$ (Found: C, 72·5; H, 8·45. Calc. for $C_{20}H_{28}O_4$: C, 72·25; H, 8·5%).

Lactonisation of C-19 labelled compound (IV) (31 mg.) with 2M-acetic anhydride–ethyl acetate–perchloric acid reagent (0·7 ml.) by a procedure essentially identical to the above yielded radioactive compound (V) (30 mg.), m. p. 129—130°.

[19-^{14}C]*Testosterone* (VI).—A solution of methylmagnesium iodide in ether (0·65 ml., 8 equiv.) was added under nitrogen with stirring to a solution of the [19-^{14}C]enol lactone (V) (31 mg.) in ether (4 ml.). After the addition the solution was stirred at room temperature for 20 hr., decomposed with saturated ammonium chloride solution, and extracted with ether. The extract was washed with sodium chloride solution, dried (Na_2SO_4), filtered, and the solvent evaporated to give a residue (31 mg.). The crude Grignard reaction product (31 mg.) was dissolved in methanol (15 ml.), and an aqueous solution of sodium hydroxide (0·5 g. in 2·5 ml.) was added. The solution was stirred under nitrogen at room temperature for 24 hr. The excess of alkali was neutralised with acetic acid and the methanol removed under a vacuum. The residue was taken into ether and the ether solution was washed with sodium chloride solution and dried (Na_2SO_4). The product (30 mg.) obtained after removal of the solvent was chromatographed on alumina (1·5 g.). The three fractions eluted with benzene–ether (8 : 2) (10 ml. each) were combined and crystallised from acetone–hexane to give [19-^{14}C]testosterone (11 mg.), m. p. and mixed m. p. 154—155°, with the same infrared spectrum as an authentic sample. Its specific activity was 3·97 μc/mg. as determined on a constant-recording Packard-Tri-Carb Liquid Scintillation Spectrometer.

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