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PREPARATION OF [1,2-^{3}H]17α-METHYLTESTOSTERONE AND OF SOME UNLABELLED DERIVATIVES

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To facilitate a study of the metabolism of 17α-methyltestosterone (MeT), [1,2-^{3}H] MeT and four unlabelled derivatives of MeT were prepared. This report describes the preparation of these compounds.

[1,2-^{3}*H*]17α-*Methyltestosterone*. This was prepared by partial tritiation (at the Radiochemical Centre, Amersham, Bucks.) of 17α-methyl-17β-hydroxy-androst-1,4-dien-3-one (obtained from Ciba Laboratories, Horsham, Sussex). A hundred mg. of the steroid was dissolved in 5 ml. dioxan and shaken for 5 hr. at 22° in 12 ml. of a tritium–hydrogen mixture (5 c) in the presence of 50 mg. 10 % palladium on charcoal. Eighty mg. of steroid were recovered with an average specific activity of 18 mc/mg. [1,2-^{3}H]MeT was subsequently isolated in a 10–15 % yield by paper chromatography of portions of the tritiated material in the cyclohexane:formamide system (Savard, 1953) and then in the A system of Bush (Bush, 1952).

[1,2-^{3}H]MeT thus obtained was purified further in one of two ways.

(1) 385 mg. of unlabelled MeT were added to 1900 μc of the [1,2-^{3}H]MeT and the diluted material was recrystallized three times from methanol. The specific activity of the material isolated from the second and third recrystallization was constant and was 4·15 μc/mg; 171 mg. of the purified steroid were obtained.

(2) 2·5 mc [1,2-^{3}H]MeT were diluted with unlabelled material to a specific activity of 1·0 μc/μg. and were left overnight at 22° in acetic anhydride and pyridine in order to acetylate any impurities. After evaporation of the reagents, the diluted steroid was purified by chromatography in the Bush A system.

[1,2-^{3}H]MeT obtained by both methods appeared to be radiochemically pure when portions were rechromatographed in the Bush A or cyclohexane:formamide systems.

Unlabelled derivatives of MeT

17α-*Methyl-5*β-*androstane-3*α,17β-*diol*. This was prepared from 5 mg. 17α-methyl-17β-hydroxy-5β-androstan-3-one (obtained from Steraloids Ltd., Croydon, Surrey) by stereoselective reduction of the 3-oxo group with sodium borohydride (Barton, 1953). The product was purified by chromatography in the Bush A system (ΔR_{MR} of +0·70) and was located on paper by reaction with phosphomolybdic acid. After elution and vacuum sublimation (0·01 mm. mercury) at about 108°, the infrared = i.r. spectrum of the steroid was measured in a Perkin-Elmer 'Infracord' spectrophotometer. An absorption peak at 3400 cm.$^{-1}$ and the absence of a peak in the region 1750–1650 cm.$^{-1}$ showed that the compound contained at least one hydroxyl group and did not contain an oxo group.

17α-Methyl-17β-hydroxy-androst-4-en-3,11-dione. This was prepared by oxidation of 2·5 mg. 17α-methyl-11β,17β-dihydroxy-androst-4-en-3-one (obtained from the Upjohn Co., Kalamazoo, Michigan) with chromium trioxide in 50% aqueous acetic acid (Lieberman, Katzenellenbogen, Schneider, Studer & Dobriner, 1955). The product was purified by chromatography in system L of Gray & Shaw (1965) and was located on paper by absorption of u.v. light and by Bush soda-fluorescence (ΔR_{MR} of − 0·47). After elution and vacuum sublimation the i.r. spectrum of the steroid was measured. An absorption peak at 3400 cm.$^{-1}$ indicated the presence of an hydroxyl group and peaks at 1690 cm.$^{-1}$ and at 1600 cm.$^{-1}$ indicated that the compound contained an 11-oxo group and a Δ^4-3-oxo group.

17α-Methyl-5β-androstane-3α,11β,17β-triol. This was prepared by catalytic hydrogenation of 4 mg. 17α-methyl-11β, 17β-dihydroxy-androst-4-en-3-one by the method of Gabbard & Segaloff (1962) and by reduction of the product with sodium borohydride. The product of the hydrogenation which was purified by chromatography in the L system (ΔR_{MR} of −0·30) did not absorb u.v. light and was located on paper with alkaline *m*-dinitrobenzene. Reduction of this product with sodium borohydride yielded a compound which reacted with phosphomolybdic acid but not with alkaline *m*-dinitrobenzene and which had an R_F of 0·16 in the L system (ΔR_{MR} of +0·67). After elution, a major portion of the steroid was acetylated with acetic anhydride and pyridine at 22°, and the i.r. spectrum of the acetate was measured after vacuum sublimation at about 105°. An absorption peak at 3400 cm.$^{-1}$ indicated the presence of at least one hydroxyl group and peaks at 1740 cm.$^{-1}$, 1730 cm.$^{-1}$ and at 1250 cm.$^{-1}$ indicated that the compound contained an acetoxy group.

17α-Methyl-5β-androstane-3α,16α,17β-triol. This compound was prepared from 3·8 mg. 17α-methyl-16α,17β-dihydroxy-androst-4-en-3-one (obtained from Upjohn Co., Kalamazoo, Michigan) by the method used for the preparation of 17α-methyl-5β-androstane-3α,11β,17β-triol. The product of the hydrogenation was purified by chromatography in the L system (ΔR_{MR} of −0·40). It did not absorb u.v. light but was located with alkaline *m*-dinitrobenzene. Reduction of this product with sodium borohydride yielded a compound which reacted with phosphomolybdic acid but not with alkaline *m*-dinitrobenzene and which had an R_F of 0·40 in the Bush B5 system (Bush, 1952). The acetate of this compound was prepared by treatment with acetic anhydride and pyridine at 22° and, after vacuum sublimation at about 95°, the i.r. spectrum was measured. An absorption peak at 3400 cm.$^{-1}$ indicated that the compound contained an hydroxyl group and peaks at 1740 cm.$^{-1}$, 1725 cm.$^{-1}$ and at 1250 cm.$^{-1}$ indicated the presence of at least one acetoxy group.

Yields of purified steroids were about 50%. Losses were confined largely to those incurred in the purification procedures.

REFERENCES

Barton, D. H. R. (1953). *J. chem. Soc.* pp. 1027–1040.
Bush, I. E. (1952). *Biochem. J.* **50**, 370–378.
Gabbard, R. B. & Segaloff, A. (1962). *J. org. Chem.* **27**, 655–656.
Gray, C. H. & Shaw, D. A. (1965). *J. Endocrin.* **33**, 33–55.
Lieberman, S., Katzenellenbogen, E. R., Schneider, R., Studer, P. E. & Dobriner, K. (1955). *J. biol. Chem.* **205**, 87–91.
Savard, K. (1953). *J. biol. Chem.* **202**, 457–477.