RADIOACTIVELY LABELLED EPOXIDES. PART III.

TRITIUM LABELLED STEROID 16α,17α-EPOXIDES

Alan J. Sparrow, Ulla Bindel and Franz Oesch
Section on Biochemical Pharmacology
Institute of Pharmacology
University of Mainz, D-6500 Mainz, West Germany

SUMMARY

Tritium labelled (17-^{3}H) 16α,17α-epoxy-androst-4-en-3-one and 16α,17α-epoxy-estra-1,3,5(10)-trien-3-ol have been prepared on a large scale (200-300 mg amounts) with efficient (i.e. activity of product exactly predictable) introduction of label. The preparative method is very inexpensive, since the tritium derives from tritiated water, the steroid starting materials are readily available, and high yields are obtained in all steps.

Key words: Δ^{16}-Steroids, 16α,17α-Epoxysteroids, Tritium, Androgens, Estrogens.

INTRODUCTION

The involvement of the microsomal enzyme epoxide hydratase in metabolism of drugs and carcinogens is under continuing investigation in our (1,2,3) and in several other laboratories (4-8). To extend study to endogenous role of the enzyme, in particular to steroid metabolism at the 16,17 bond, labelled Δ^{16}-and 16α,17α-epoxy-18 and 19 carbon steroids in substantial amounts (200-300 mg) were required. The compounds of interest, which have been shown, or supposed, to be involved in the metabolism of androgens and estrogens (9,10,11) are 1-4 (Scheme 1).

RESULTS AND DISCUSSION

We have synthesized 1-4 starting from readily available inexpensive un-

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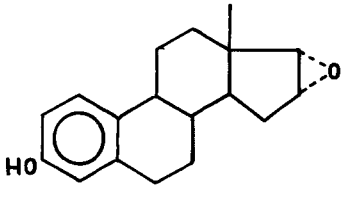
labelled steroids and tritiated water by the routes outlined in scheme 2a and b. The routes finally chosen were dictated by the experience of the authors of (12), who were unable to achieve better than 5 % yield of pure 4 by elimination reactions on testosterone or its derivatives, and by the unsatisfactory results of our attempts to apply a method, reviewed in (13), to the preparation of 3 from estrone, 5a, via its tosyl or benzenesulphonyl hydrazone with butyllithium in a variety of solvents.

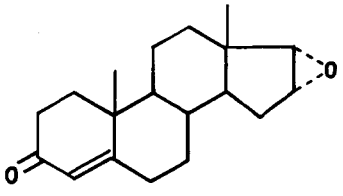
The first three steps are common to both schemes 2a and b, and were performed according to Barton et al. (14). Immediately after completion of reaction, the hydrazones 6a, 9 were isolated solid and not recrystallized (to avoid disproportionation to azine) before conversion to the vinyl iodides 7a, 10. This step is the only one in the sequence with less than 80 % yield. After a number of recrystallisations 7a and 10 could be obtained sufficiently free of by-products (ketones 5a and 8, and, probably, azines) in 50-60 % yield.

SCHEME 1



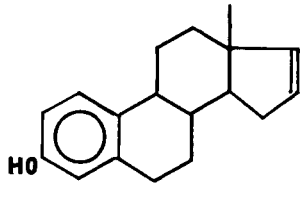
1

16α,17α Epoxyestra-1,3,5(10)trien-3-ol



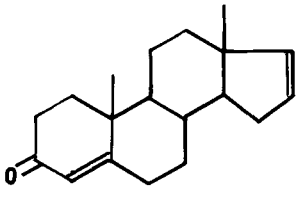
2

16α,17α Epoxyandrost-4-en-3-one



3

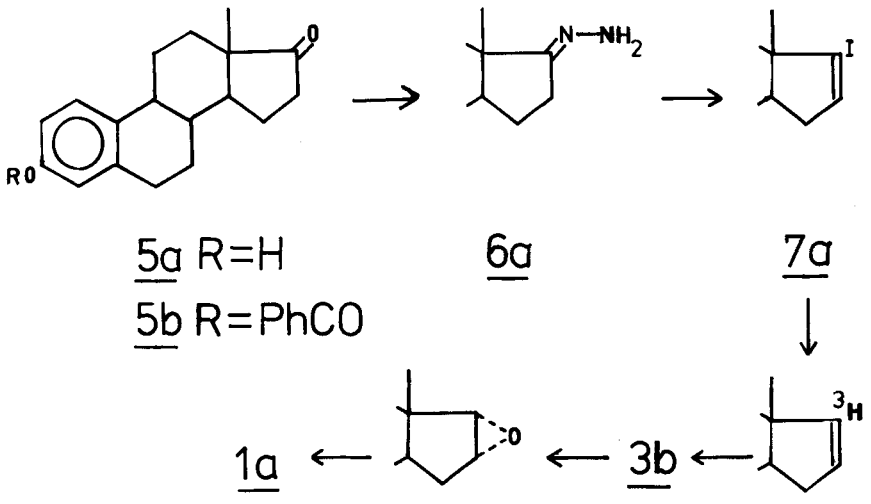
Estra-1,3,5(10),16-tetraen-3-ol



4

Androsta-4,16-dien-3-one

SCHEME 2a



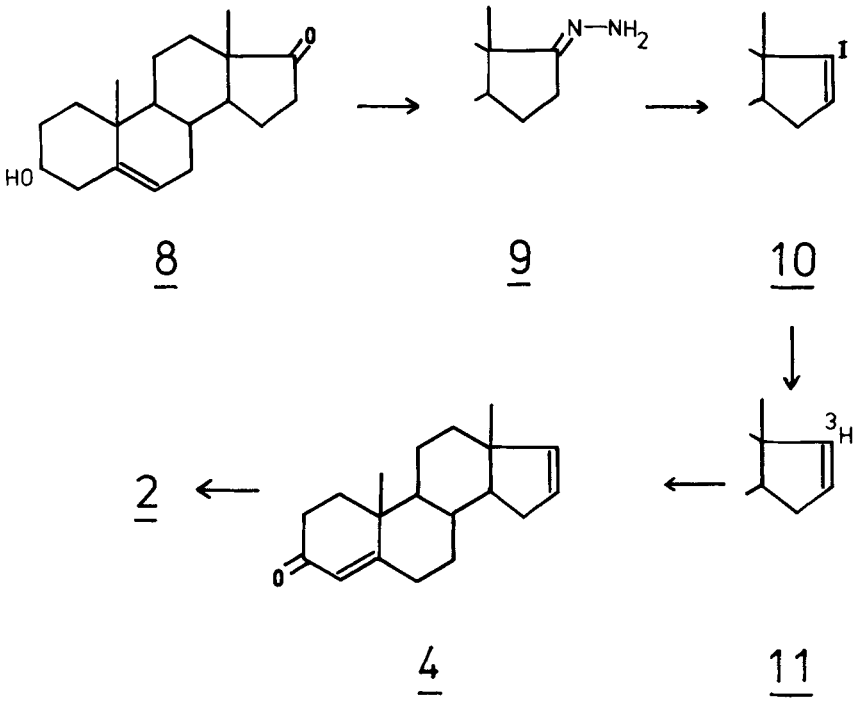
5a R=H 6a 7a

5b R=PhCO

1a 3b

1b 3a

SCHEME 2b



8 9 10

2 4 11

The label was introduced (steps 7a to 3a and 10 to 11) by treating the vinyl iodide with sodium and RO^3H (made from the alcohol and tritiated water). It was found that efficiency of introduction of the label was perfect when relatively long reaction times were used, hence the procedure described in EXPERIMENTAL. A large excess of tritiated alcohol was necessary, but the specific activity of the resulting steroid equals that of the alcohol.

After this step, the procedures diverge. 3a must be protected on the phenolic OH before epoxidation, and then deprotected afterward (15,16). 11 was oxidised by the method of Sondheimer et al. (17) and then epoxidized (18).

Overall yield of 1 and 2 starting from 5a and 8 respectively was usually close to 40 %.

EXPERIMENTAL

The nmr spectra were recorded on a Varian EM 360 spectrometer. Melting points are uncorrected. For purity determinations, a Spectra Physics SP 3500 B hplc system equipped with a Spherisorb ODS 10 µl column, 3 x 250 mm was used, applying a gradient from 30 to 75 % acetonitrile in water over 50 min with a flow rate of 0.8 ml/min.

Hydrazones 6a, 9 of Estrone and Dehydroepiandrosterone

0.5 g of 5a or 8 (Sigma Chem. Co., Steinheim, FRG) were dissolved in 3.5 ml ethanol and 0.85 ml triethylamine. Then 2.5 ml 100 % N_2H_5OH were added. The mixture was refluxed for 75 min, then cooled in ice and the crystals filtered. The filtrate was added in a slow stream to 150 ml water and the crystals filtered. Both crops of crystals were dried for 24 hr over P_2O_5 *in vacuo*. The yield was 95-100 % (slightly impure by tlc (chloroform/methanol 9:1).

Vinyl iodides 7a, 10

The yield of 6a was suspended, or of 9 dissolved, in 12 ml dry (potassium) tetrahydrofuran and 2.5ml dry (potassium hydroxide) triethylamine in a water bath at 20° under a slow stream of dry nitrogen. Then a solution of 1.2g iodine in 18 ml dry tetrahydrofuran was added over 1 hr - until the supernatant remained brown after 15 min stirring (about 12 ml of iodine solution were added). The suspension was then evaporated to dryness *in vacuo* and transferred to a separator with 50 ml ether and 20 ml water. After separation, the water was neutralised with 5 % HCl and returned to the separator. This process was repeated until after shaking with Et_2O the water phase was neutral. (In the case of 10, the ether was simply shaken with 5 % HCl). The ether was then washed with aqueous sodium sulphite, water, aqueous sodium bicarbonate and then dried over magnesium sulphate. Evaporation of ether produced an oil or a solid which was recrystallised from methanol or methanol/water until an acceptable purity (judged by mp) was reached. Yield 50-60 %. mp 7a 133 - 4° d (lit. -). mp 10 169 - 70° (lit. (14) 172 - 4°).

nmr (δ values):

7a (CD_3OD): 7.0 (d1H)H_1, 6.6(d)6.5(s)(2H)H_2H_4, 6.1 (t1H)H_{16}
3.2.-1.1(ml3H)H_6-H_{15},0.7(s3H)H_{18}.

10 ($CDCl_3/CD_3OD$): 6.1(t1H)H_{16},5.3(d1H)H_6,3.4(m1H)H_3
2.4-0.7(m23H) [H_{18} 0.8, H_{19} 1.1]

Olefins 3a, 11

1.2 mmole 7a or 10 (dried over P_2O_5 *in vacuo*) was added to 580mg powdered sodium (15) under 6 ml dry tetrahydrofuran. Then a solution of 45 µl (40 mCi) tritiated water in 0.8 ml isopropanol and 1.5 ml tetrahydrofuran was added and the mixture refluxed 5 hr. Then water was added to destroy remaining sodium and the solvents were distilled off, finally on a rotary evaporator. The residue was then transferred to a

separator with water and diethylether (25 ml of each). After separation, the water was neutralised and returned to the separator. The water was extracted once more with ether, then the combined ether phases were dried and evaporated to an oil which was crystallised from methanol/water. The yield was usually 1.0-1.1 mmole (83-92 %) of sufficient purity for the next step.

nmr ($CDCl_3$):

3a: 7.1(d1H) H_1,6.6(d)6.5(s)(2H)H_2H_4,5.9(d)5.7(d)(2H)$H_{16}H_{17}$, 3.1-1.1(m13H)H_6-H_{15},0.8(s3H)H_{18}

11: 5.8(d)5.6(d)(2H)$H_{16}H_{17}$,5.3(d1H)H_6,3.5(m1H)H_3,2.5-0.8(m24H) [H_{18}0.8, H_{19} 1.0]

The subsequent steps were performed as described in the literature for the unlabelled compounds (15-18).

Final Epoxides:

1a crystallised from methanol. mp 206-8° (lit. (16)215°). hplc showed product to be about 99 % radiochemically pure, rest olefin 3a.

nmr ($CDCl_3$/CD_3COCD_3): 7.0 (d1H)H_1,6.5(d), 6.4(s)(2H)H_2H_4,3.3(d1H)H_{16},3.1 (d1H)H_{17},2.9-1.0 (m13H)H_6-H_{15},0.7(s3H)H_{18}. Specific activity: 3·1mCi/mmol

2 crystallised from methanol/chloroform. mp 203° (lit.(18)204-5°). hplc showed product to contain small amounts of olefin 4 and androst-4-en-3,17 dione, together with an unidentified impurity having a retention time almost identical with that of 2. The product could be obtained free of these impurities, in small amounts, by chromatography on silica gel eluting with petroleum ether/ethyl acetate 8:2.

nmr. ($CDCl_3$): 5.7(s1H)H_4,3.3(d1H)H_{16},3.1(d1H)H_{17},2.6-0.6 (m23H) [H_{18} 0.8, H_{19} 1.2]. Specific activity: 2.8 mCi/mmole.

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