SYNTHESIS OF ISOMERIC 16,17-EPOXY-[17-^{3}H]-DERIVATIVES OF 3-HYDROXY AND 3-METHOXY-OESTRA-1,3,5(10)-TRIENES

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SUMMARY

Deuterium NMR spectroscopy has been used to show that a previously published synthesis of 16α,17α-epoxy-3-hydroxy-[17-^{3}H]oestra-1,3,5(10)-triene is not regiospecific but leads to additional isotope incorporation at positions -2 and/or -4. A modified synthesis is described which leads to the title compounds exclusively labelled at position -17.

Keywords: 16,17-epoxyoestrogens, tritium, regiospecific incorporation, deuterium NMR.

INTRODUCTION

In the course of our studies of the binding of steroidal and non-steroidal oestrogens and their metabolites to cellular nucleophiles (1-4) it was necessary to prepare the title compounds. For our purposes, it was imperative that the compounds were labelled exclusively in the steroidal D-ring leaving the A-ring unlabelled (2). The synthesis of Oesch *et al.* (5) demonstrated the ease with which a tritium label can be incorporated into position -17 in the oestrogen skeleton by the quenching of a Δ^{16},17-carbanion with tritiated water (Scheme 1).

The method of Oesch uses a large excess (>20 equivalents) of sodium to form

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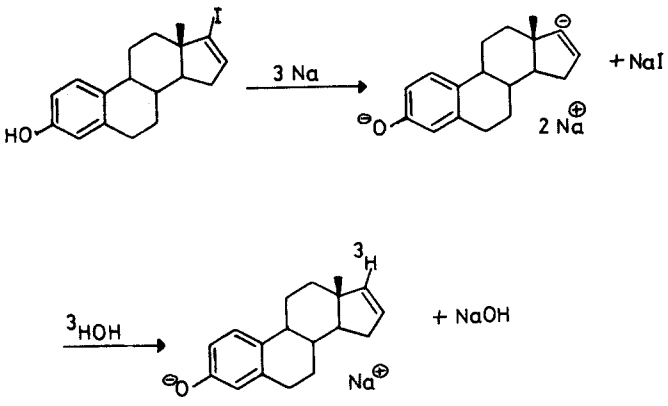


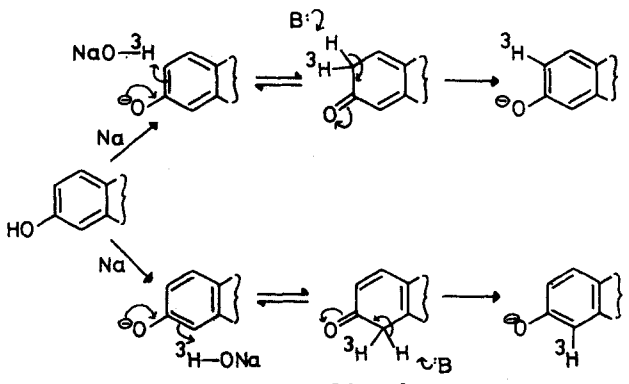
Scheme 1

the carbanion and quenches the reaction with approximately two equivalents of tritiated water dissolved in isopropanol (ca. 10 equivalents). However the presence of sodium isopropoxide and the long reaction time after the addition of the water (5h) would be expected to lead to further incorporation of label at positions -2 and/or -4 in the A-ring, via ionisation of the free 3-hydroxyl function as shown below (Scheme 2). Indeed, Oesch's observation that longer reaction times lead to a higher specific activity of product are consistent with a slow tritium/hydrogen exchange process in addition to the fast, irreversible quenching of the Δ^{16},17-carbanion.

In an attempt to confirm this analysis, we have repeated Oesch's synthesis precisely but using 99% enriched deuterated water in place of tritiated water. The product was examined by deuterium NMR spectroscopy to determine the isotopic labelling pattern. The synthesis of the title compounds has been modified to avoid this ambiguity in the labelling, and deuterium NMR has shown it to yield products with the regiospecific isotopic labelling required.

RESULTS AND DISCUSSION

Following an Oesch type synthesis using deuterated water, deuterium NMR spectroscopy of the isolated product clearly indicates that the incorporation

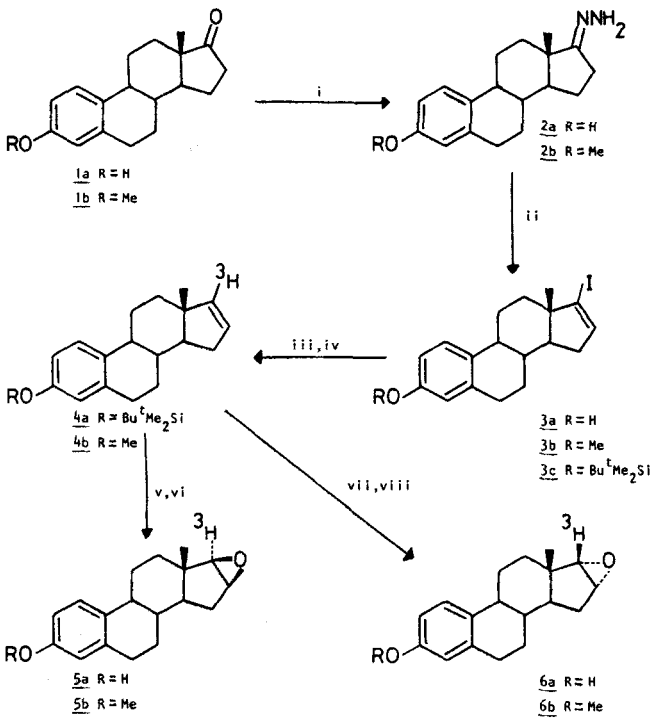


Scheme 2

of label is not exclusive to position -17. The spectrum has, as expected, a major signal at δ5.9 which, by comparison with the proton spectrum of unlabelled material, corresponds to deuterium at position -17. A second signal is observed at δ6.6, which corresponds to deuterium at position -2 and/or -4. The ^{2}H-NMR spectrum is not sufficiently well resolved to distinguish between H-2 and H-4. The ratio of incorporation at position -17 to that at position -2(4) is approximately 7:1 based on peak area.

The same synthesis was repeated with 17-iodo-3-methoxyoestra-1,3,5(10),16--tetraene (3b) and 3-t-butyldimethylsiloxy-17-iodooestra-1,3,5(10),16-tetraene (3c). It was believed that this would prevent formation of the phenolate anion and therefore inhibit the hydrogen exchange shown (Scheme 2). (It was also found experimentally easier to generate the Δ^{16},17-carbanion using two equivalents of n-butyl lithium rather than powdered sodium). Under these conditions of reaction and using deuterated water to quench the carbanion, the deuterium NMR spectrum showed the product to be labelled exclusively at position -17 (δ5.9 for both products) with no other signals observable in the spectrum. It was therefore clear that the 3-hydroxyl function must be suitably protected to ensure that no isotope is incorporated into ring-A.

The modified synthesis for the preparation of the four title compounds is shown (Scheme 3). The use of two equivalents of n-butyl lithium to generate the



i, $N_2H_4.H_2O/Et_3N/EtOH$; ii, $I_2/Et_3N/THF$; iii, n-BuLi/Et_2O/N_2; iv, $^3HOH/THF$
v, NBS/DMSO/H_2O/THF; vi, KOH/MeOH; vii, m-CPBA/CH_2Cl_2; viii, n-$Bu_4N^+F^-$/THF

Scheme 3

Δ^{16},17-carbanion was more convenient than that of twenty equivalents of powdered sodium and guarantees a more efficient use of the tritiated water used to quench the reaction. A t-butyldimethylsilyl protecting group was chosen for the phenol because of its ease of removal at the final synthetic step by a deprotecting reagent without effect on the isomeric epoxides. This protecting group was incorporated on the 0^3-position of 3a using the well-established method (6). Compounds 3b and 3c could at this stage be rigorously purified by flash chromatography on silica prior to incorporation of the tritium label. Up to this stage, all compounds had been used in the crude state since purification led only to an unnecessary loss of material. The conversion of the 17-tritiated olefins into the isomeric epoxides 5 and 6 was achieved using epoxidation methods

based on previously described reactions of unlabelled material (7). It was found that in the conversion of 4a into 5a, the t-butyldimethylsilyl protecting group was removed under the conditions used for ring closure of the intermediate bromohydrin to afford the 16β,17β-epoxide (5a) directly.

All of the reactions described (Scheme 3) were carried out initially using natural water for conversion of 3 into 4. The analytical data quoted in the experimental section was obtained from samples of non-radioactive material. In the subsequent syntheses using tritium, the identity of the labelled compounds was established by synthetic procedure and by TLC comparison with authentic unlabelled compounds.

Taken together, these experiments demonstate the radiochemical synthesis of tritiated 16α,17α-oxide of 3-hydroxyoestra-1,3,5(10)-triene previously described (5) does in fact contain up to 14% of the label in ring-A, distributed between positions -2 and -4. The modified synthesis described here leads to both α- and β-oxides of the 3-hydroxy- and 3-methoxy-oestra-1,3,5(10),--triene with deuterium or tritium labels exclusively at position -17.

EXPERIMENTAL

^{1}H-NMR spectra were recorded using a Perkin Elmer R34 spectrometer at 220 MHz in deuterated chloroform, with tetramethylsilane as both internal lock and reference. In the case of compounds containing the t-butyldimethylsilyl group, ^{1}H-NMR spectra were run unlocked, using the residual protonated chloroform signal (δ7.24) as reference. ^{2}H-NMR spectra were recorded using a Bruker AM-250 spectrometer at 38.4 MHz in acetone solution. Spectra were run unlocked with broad band proton decoupling. The natural-abundance deuterated acetone signal (δ2.04) was used as reference. Chemical shifts are reported on the δ-scale in ppm. Mass spectra were recorded using a Kratos MS25 machine in conjunction with a DS55 data station. Melting points were determined using a Kofler hot-stage microscope and are uncorrected. Flash chromatography was performed on silica gel 60 (230-400 mesh) as supplied by Merck. Preparative TLC was performed on

glass plates pre-coated with silica gel 60H as supplied by Merck. All starting materials and reagents were obtained from Aldrich Chemical Co. Ltd. or Lancaster Synthesis. Tritiated water was obtained from Amersham International plc at a batch specific activity of 140Ci/mol.

The specific activity of labelled compounds was determined by counting an aliquot of a solution whose concentration had been determined by UV spectroscopy. The extinction coefficients of the compounds were determined using unlabelled material. The results are given in Table 1. UV spectra were recorded using a Perkin Elmer 559 UV/VIS spectrometer in methanol. Radioactivity was determined using a Packard Tricarb Model 3385 liquid scintillation spectrometer. The specific activities of the compounds 4 (Table 1) did not change significantly on transformation into 5 and 6.

3-Methoxyoestra-1,3,5(10)-trien-3-one, 1b

Oestrone (1a), (1.0 g, 3.7 mmol) was treated with a solution of sodium ethoxide in ethanol (1.0 M, 10 ml), followed by dimethylsulphate (2.3 ml, 24.6 mmol). After stirring at room temperature for 1.5 h, the product was collected by filtration. The filtrate was diluted with water (100 ml) and further crystals were collected. Total yield 1.04 g (99%), m.p. 171-3°C. $^{m}/_{e}$ 284 (M^{+}, 100%). δ_H 7.2 (1H, d, H-1); 6.7 (1H, dd, H-2); 6.6 (1H, d, H-4); 3.7 (3H, s, $-OCH_3$); 0.8 (3H, s, H-18).

Steroid	λ_{max}/nm	$\lambda_{\epsilon max}$	specific activity Ci/mol
4a*	280	2057±40	76 ± 4
5a	280	2023±20	64 ± 2
6a	280	2035±20	66 ± 2
4b	278	2075±50	41 ± 4
5b	278	2012±20	48 ± 2
6b	278	2028±20	48 ± 2

Table 1: Determination of extinction coefficients and specific activities

*after removal of t-butyldimethylsilyl group to liberate the free phenol

3-Hydroxyoestra-1,3,5(10)-trien-17-hydrazone, 2a

Prepared as previously described (5). m.p. 285-290°C (dec.)

3-Methoxyoestra-1,3,5(10)-trien-17-hydrazone, 2b

1b (650 mg, 2.3 mmol) was treated with triethylamine (1.2 ml) and hydrazine hydrate (3.5 ml) as previously described (5). Yield (crude) 680 mg, m.p. 245-8°C $^m/_e$ 298 (M^+, 95%); 282 (100). δ_H 7.2 (1H, d, H-1); 6.7 (1H, dd, H-2); 6.6 (1H, d, H-4); 5.2 (2H, brs, $-NH_2$); 3.7 (3H, s, $-OCH_3$); 0.8 (3H, s, H-18).

3-Hydroxy-17-iodooestra-1,3,5(10),16-tetraene, 3a

Prepared as previously described (5). m.p. 153-4°C (dec.), (lit. (5) 133-4)

17-Iodo-3-methoxyoestra-1,3,5(10),16-tetraene, 3b

2b (630 mg, 2.1 mmol) was treated with iodine and triethylamine in dry tetrahydrofuran as previously described (5). The product was purified by flash chromatography (5% ethyl acetate in light petrol). Yield 480 mg (58% based on 1b), m.p. 143-5°C. $^m/_e$ 394 (M^+, 100%). δ_H 7.2 (1H, d, H-1); 6.7 (1H, dd, H-2); 6.6 (1H, d, H-4); 6.2 (1H, m, H-16); 3.8 (3H, s, $-OCH_3$); 0.8 (3H, s, H-18).

3-t-Butyldimethylsiloxy-17-iodooestra-1,3,5(10),16-tetraene, 3c

3a (1.07 g, 2.8 mmol) was dissolved in dry dimethylformamide (15 ml) and dry imidazole (640 mg, 3.3 mol equiv.). This solution was added to t-butyldimethylsilyl chloride (640 mg, 1.5 mol equiv.) and the mixture was stirred under anhydrous conditions for 16 h. Aqueous potassium carbonate (0.1% w/v, 30 ml) was added and the resultant precipitate was collected by filtration and purified by flash chromatography (5% ethyl acetate in light petrol). Yield 1.18 g (59% based on 1a), m.p. 124-7°C. $^m/_e$ 494 (M^+, 45%); 437 (100). δ_H 7.1 (1H, d, H-1); 6.7 (1H, dd, H-2); 6.6 (1H, d, H-4); 6.2 (1H, m, H-16); 1.0 (9H, s, $(CH_3)_3C-$); 0.8 (3H, s, H-18); 0.2 (6H, s, $(CH_3)_2Si-$).

3-Methoxy-[17-^{3}H]oestra-1,3,5(10),16-tetraene, 4b

3b (600 mg, 1.5 mmol) was dissolved in anhydrous ether under nitrogen. A solution of n-butyl lithium in hexane (1.65 M, 1.8 ml, 3.0 mmol) was added at 60°C and the mixture was then warmed to room temperature and stirred for 2 h. Tritiated water (0.6Ci, 100 μl) was dissolved in dry tetrahydrofuran (0.5 ml)

and this was added in one portion to the reaction. After a further 15 min stirring, the product was partitioned between ether (100 ml) and dilute hydrochloric acid (100 ml). The organic layer was dried ($MgSO_4$) and evaporated and the product purified by flash chromatography (5% ethyl acetate in light petrol). Yield 325 mg (80%), m.p. 66-8°C (lit. (7) 66-68). $^m/_e$ 268 (M^+, 100%). δ_H 7.2 (1H, d, H-1); 6.7 (1H, dd, H-2); 6.6 (1H, d, H-4); 5.9 (1H, d, H-17); 5.7 (1H, m, H-16); 3.8 (3H, s, $-OCH_3$); 0.8 (3H, s, H-18).

3-t Butyldimethylsiloxy-[17-^{3}H]oestra-1,3,5(10),16-tetraene, 4a

3c (600 mg, 1.2 mmol) was treated with n-butyl lithium and tritiated water (0.8Ci, 100 μl) as described above. The product was purified by flash chromatography (light petrol). Yield 355 mg (80%), m.p. 79-81.5°C. $^m/_e$ 368 (M^+, 60%); 311 (100). δ_H 7.1 (1H, d, H-1); 6.6 (1H, dd, H-2); 6.5 (1H, d, H-4); 5.9 (1H, d, H-17); 5.7 (1H, m, H-16); 1.0 (9H, s, $(CH_3)_3C-$); 0.8 (3H, s, H-18); 0.2 (6H, s, $(CH_3)_2Si-$).

3-Hydroxy-[2,4,17-$^2H_3$]oestra-1,3,5(10),16-tetraene

3a (405 mg, 1.1 mmol) was treated with powdered sodium, isopropanol and deuterated water as previously described (5), and the product was purified by flash chromatography (20% ethyl acetate in light petrol). Yield 200 mg (75%), m.p. 116-7°C. $^m/_e$ 254/255 (M^+, 100%). ^{1}H-NMR spectrum identical to that previously published (5). δ_{2_H} 6.6 (^{2}H-2/4); 5.9 (^{2}H-17).

3-Methoxy-[17-^{2}H]oestra-1,3,5(10),16-tetraene

3b (190 mg, 0.5 mmol) was treated with n-butyl lithium and deuterated water as described above. After purification, the product had a melting point and spectral characteristics identical to previous samples of 4b. δ_{2_H} 5.9 (^{2}H-17).

3-t-Butyldimethylsiloxy-[17-^{2}H]oestra-1,3,5(10),16-tetraene

3c (100 mg, 0.2 mmol) was treated with n-butyl lithium and deuterated water as described above. After purification, the product had a melting point and spectral characteristics identical to previous samples of 4a. δ_{2_H} 5.9 (^{2}H-17).

16β,17β-Epoxy-3-hydroxy-[17α-^{3}H]oestra-1,3,5(10)-triene, 5a

4a (130 mg, 0.4 mmol) was dissolved in ice cold tetrahydrofuran (4.5 ml). A cooled mixture of water (1.2 ml) and dimethylsulphoxide (4.5 ml) was added dropwise over 15 min. N-Bromosuccinimide (340 mg, 1.9 mmol) was added in portions and the mixture was stirred at room temperature for 1 h. The resultant solution was poured into water (60 ml) and extracted with chloroform. After drying ($MgSO_4$) and evaporation, the residue was dissolved in 3% methanolic KOH (2.5 ml) and the solution was heated under reflux for 1 h. After dilution with water (20 ml) and careful acidification with dilute hydrochloric acid, the reaction was extracted with ether. Drying ($MgSO_4$) and evaporation yielded a gum which was purified by preparative TLC (20% ethyl acetate in light petrol). Yield 40 mg (42%), m.p. 190-2°C. $^m/_e$ 270 (M^+, 85%); 160 (100). δ_H 7.1 (1H, d, H-1); 6.6 (1H, dd, H-2); 6.5 (1H, d, H-4); 3.6 (1H, m, H-16); 3.3 (1H, d, H-17); 0.9 (3H, s, H-18).

16β,17β-Epoxy-3-methoxy-[17α-^{3}H]oestra-1,3,5(10)-triene, 5b

4b (139 mg, 0.5 mmol) was treated with N-bromosuccinimide and methanolic KOH as described above. The product was purified by preparative TLC (5% ethyl acetate in light petrol). Yield 75 mg (51%), m.p. 110-2.5°C (lit. (7) 111-3). $^m/_e$ 284 (M^+, 100%). δ_H 7.2 (1H, d, H-1); 6.7 (1H, dd, H-2); 6.6 (1H, d, H-4); 3.8 (3H, s, $-OCH_3$); 3.5 (1H, m, H-16); 3.2 (1H, d, H-17); 0.8 (3H, s, H-18).

16α,17α-Epoxy-3-hydroxy-[17β-^{3}H]oestra-1,3,5(10)-triene, 6a

4a (130 mg, 0.4 mmol) was dissolved in ice cold dichloromethane (2.0 ml). m-Chloroperbenzoic acid (85%, 110 mg, 0.5 mmol) was dissolved in dichloromethane (2.0 ml) and this was added dropwise to the stirred steroid solution in ice. After 1.5 h, aqueous sodium sulphite (10 ml) and ether (20 ml) were added and the product was partitioned. The organic layer was dried ($MgSO_4$) and evaporated and the residue was dissolved in dry tetrahydrofuran containing 1.0 M tetra-n-butylammonium fluoride. After 45 min at room temperature, the solution was diluted with water (10 ml) and extracted with ether. The product was purified

by preparative TLC (20% ethyl acetate in light petrol). Yield 38 mg (40%), m.p. 207-9°C (lit. (5) 206-8). $^{m}/_{e}$ 270 (M^{+}, 100%). δ_H 7.1 (1H, d, H-1); 6.6 (1H, dd, H-2); 6.5 (1H, d, H-4); 3.4 (1H, d, H-16); 3.2 (1H, d, H-17); 0.8 (3H, s, H-18).

16α,17α-Epoxy-3-methoxy-[17β-^{3}H]oestra-1,3,5(10)-triene, 6b

4b (62 mg, 0.2 mmol) was treated with m-chloroperbenzoic acid as described above. The product was purified by preparative TLC (5% ethyl acetate in light petrol). Yield 35 mg (53%), m.p. 113-5°C (lit. (7) 117-8). $^{m}/_{e}$ 284 (M^{+}, 100%). δ_H 7.2 (1H, d, H-1); 6.7 (1H, dd, H-2); 6.6 (1H, d, H-4); 3.8 (3H, s, $-OCH_3$); 3.4 (1H, d, H-16); 3.2 (1H, d, H-17); 0.8 (3H, s, H-18).

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