Aust. J. Chem., 1983, **36**, 403–7

The Structure and Function of Oestrogens. V* Synthesis of (9,12,12-$^2H_3$)- and (11ξ,12,12-$^2H_3$)-Oestradiol

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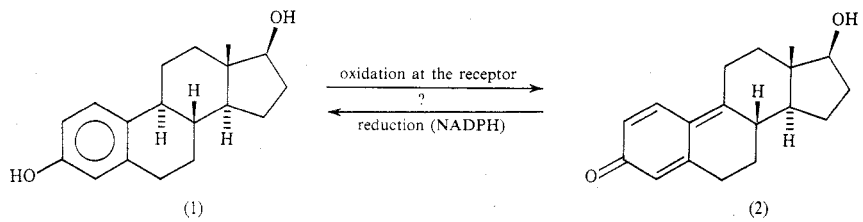
Abstract

17,17-Ethylenedioxy-3-methoxy(9,12,12-$^2H_3$)-9β-oestra-1,3,5(10)-trien-11-one (3) was reduced to the mixture of 11-epimeric alcohols (4) which upon elimination of DHO gave the (12,12-$^2H_2$)-9(11)-dehydrooestrone derivative (5b). Treatment of (5b) with ($^2H_6$)diborane followed by oxidation afforded the (9,12,12-$^2H_3$)alcohol (8a); hydride reduction of the corresponding tosylate then gave 17,17-ethylenedioxy-3-methoxy(9,12,12-$^2H_3$)oestra-1,3,5(10)-triene (7). Acid hydrolysis of (7), followed by demethylation, and reduction with sodium borohydride yielded (9,12,12-$^2H_3$)oestradiol (10). Sodium borohydride reduction of (11ξ,12,12-$^2H_3$)oestrone (6), prepared in several steps from the 9β,12,12-trideutero ketone (3), gave (11ξ,12,12-$^2H_3$)oestradiol (9). [The two trideuterated oestradiols (9) and (10) were required for biological studies.]

Introduction

In Part I of this series we outlined a new hypothesis for the mode of action of oestrogens at the molecular level.[1] The key postulate is that when oestradiol (1) reaches the receptor site in the target organs the phenolic ring A is oxidized to a quinone methide (2) which then suffers reduction by a biological source of hydride such as NADPH to regenerate oestradiol (1). While the oestradiol molecule remains



(1) (2)

at the active site participating in this rapid oxidation–reduction cycle it is possible that the high local consumption of oxidant and/or reductant provides the stimulus for the observed increase in RNA synthesis, and then protein synthesis in the target

* Part IV, *Aust. J. Chem.*, 1983, **36**, 339.

[1] Collins, D. J., and Matthews, W. A., *Aust. J. Chem.*, 1979, **32**, 1093.

0004-9425/83/020403$02.00

tissue. Attempts to obtain indirect evidence concerning the quinone methide hypothesis were inconclusive,[2,3] and it was desirable to seek direct evidence of removal of the H9 atom which is implicit in the conversion of oestradiol (1) into the corresponding quinone methide (2). One way to do this would be to administer the two different specifically trideuterated oestradiols (9) and (10) intravaginally to different sets of test animals, and, after a suitable interval, to recover the deuterated oestradiols and examine their deuterium content by g.c.–m.s. The loss of one deuterium atom from (10) but retention of the three deuterium atoms in (9) would constitute proof that the H9α atom of oestradiol is removed during residence in the target organ. This paper describes the synthesis of (11ξ,12,12-$^2H_3$)oestra-1,3,5(10)-triene-3,17β-diol (9) and (9,12,12-$^2H_3$)oestra-1,3,5(10)-triene-3,17β-diol (10).

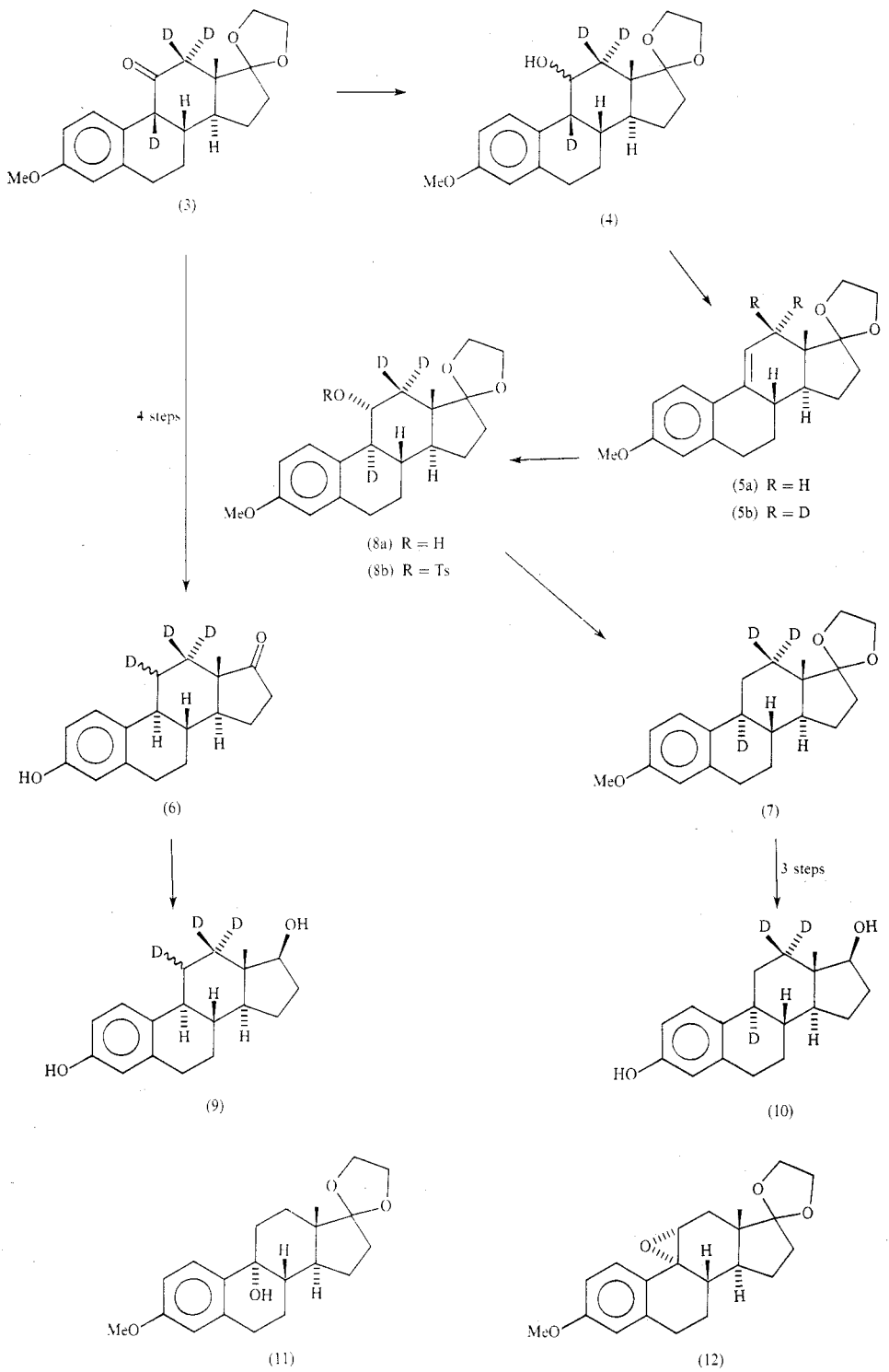
Results and Discussion

(11ξ,12,12-$^2H_3$)Oestrone (6) was prepared as described previously from 17,17-ethylenedioxy-3-methoxy(9β,12,12-$^2H_3$)oestra-1,3,5(10)-trien-11-one (3)[4] (Scheme 1). Reduction of (6) with sodium borohydride gave (11ξ,12,12-$^2H_3$)oestra-1,3,5(10)-triene-3,17β-diol (9) which had the following isotopic composition: $^2H_4$, 6·8; $^2H_3$, 83·2; $^2H_2$, 5·5; $^2H_1$, 2·9; $^2H_0$, 1·6%.

(9,12,12-$^2H_3$)Oestradiol (10) was prepared from the 9β,12,12-trideutero ketone (3) by a route analogous to that used for the synthesis of (9,11,11,12,12-$^2H_5$)oestrone.[4] The ketone (3) cannot be used directly because it has abnormal 9β-stereochemistry. In order to introduce a 9α-deuterium atom it was necessary to convert the ketone (3) into the 9(11)-dehydro compound (5b). The first step in this conversion was the hydride reduction of (3) to the 11-epimeric mixture of alcohols (4). This reaction also produced some of the undeuterated 9(11)-dehydro compound (5a) which had to be removed by column chromatography in order to avoid isotopic dilution in the formation of the 12,12-dideutero olefin (5b). The undeuterated 9(11)-olefin (5a) arises from dehydration of the 9α-alcohol (11) which in turn comes from hydride reduction of a small amount of the corresponding 9α,11α-epoxide (12) present as a contaminant in the 11-ketone which was deuterated to give (3). The extreme susceptibility of the 9α-alcohol (11) to dehydration to give (5a),[4] and the ease of removal of the latter from the 11-epimeric alcohols (4) make it practical to use the crude trideutero ketone (3). This is especially so because (3) cannot be obtained crystalline, and chromatography of a deuterated ketone is likely to result in loss of deuterium. Also, the 9βH-11-ketone is particularly susceptible to 9-hydroxylation.[4]

Dehydration of the purified 11-epimeric alcohols (4) with phosphoryl trichloride in pyridine afforded the 12,12-dideuterated 9(11)-dehydro compound (5b). Treatment of this compound with ($^2H_6$)diborane, followed by oxidation with alkaline hydrogen peroxide, yielded the trideutero 11α-alcohol (8a). Reduction of the derived tosylate (8b) with lithium aluminium hydride in ether gave compound (7), together with some of the 12,12-dideuterated olefin (5b) formed by β-elimination. Because the chromatographic separation of (5b) and (7) is difficult and wasteful, the crude mixture was treated with ($^2H_6$)diborane then oxidized with alkaline hydrogen peroxide in order to convert the contaminant (5b) into the trideutero 11α-alcohol (8a).

[2] Collins, D. J., Matthews, W. A., and Stone, G. M., *Aust. J. Chem.*, 1979, **32**, 1107.
[3] Collins, D. J., and Stone, G. M., *Aust. J. Biol. Sci.*, 1983, **36**, in press.
[4] Collins, D. J., and Sjövall, J., *Aust. J. Chem.*, 1983, **36**, 339.



Scheme 1

The last compound could be readily separated from the (9α,12,12-$^2H_3$)compound (7) by chromatography, and recycled. Acid hydrolysis of pure (7), followed by successive demethylation with hydrobromic acid in acetic acid and reduction with sodium borohydride, afforded (9,12,12-$^2H_3$)oestra-1,3,5(10)-triene-3,17β-diol (10) which had the following isotopic composition: $^2H_4$, 12·2; $^2H_3$, 75·2; $^2H_2$, 8·0; $^2H_1$, 3·0; $^2H_0$, 1·7%.

The results of biological experiments with the two trideuterated oestradiols (9) and (10) will be reported elsewhere.

Experimental

Procedural details and instrumentation were as described in Part IV.[4]

(a) Reduction of 3-Hydroxy(11ξ,12,12-$^2H_3$)oestra-1,3,5(10)-trien-17-one (6) with Sodium Borohydride

A solution of (11,12,12-$^2H_3$)oestrone (6) (64·0 mg) ($^2H_4$, 7·8; $^2H_3$, 86·2; $^2H_2$, 3·2; $^2H_1$, 1·7; $^2H_0$, 1·1%)[4] in methanol (3·2 ml) containing 20% sodium hydroxide (0·032 ml) was added to a solution of sodium borohydride (24 mg) in methanol (3·2 ml). The mixture was stirred at room temperature for 1 h, then water (5·0 ml) and 2 N hydrochloric acid (3 ml) were added, and the precipitate was collected and washed with water. Crystallization from aqueous methanol gave *(11ξ,12,12-$^2H_3$)oestra-1,3,5(10)-triene-3,17β-diol* (9) (45 mg), m.p. 176–179° (undepressed on admixture with unlabelled oestradiol). T.l.c. (silica gel, $CHCl_3$) showed only one spot with the same R_F as pure unlabelled oestradiol. The mass spectrum showed the parent ion at *m/z* 259; the isotopic content was $^2H_4$, 6·8; $^2H_3$, 83·2; $^2H_2$, 5·5; $^2H_1$, 2·9; $^2H_0$, 1·6%.

(b) 17,17-Ethylenedioxy-3-methoxy(12,12-$^2H_2$)oestra-1,3,5(10),9(11)-tetraene (5b)

A solution of 17,17-ethylenedioxy-3-methoxy(9,12,12-$^2H_3$)-9β-oestra-1,3,5(10)-trien-11-one (3) (1·45 g) ($^2H_3$, 91·6; $^2H_2$, 4·3; $^2H_1$, 2·7; $^2H_0$, 1·5%)[4] in dry ether (100 ml) was reduced with lithium aluminium hydride in the usual way, and the product was chromatographed on Merck basic alumina (grade II). Elution with benzene and benzene containing ether (4%) gave the undeuterated 9(11)-olefin (5a) (140 mg). Further elution with benzene containing ether (10–20%), and with ether and ether containing methanol (1%), afforded 17,17-ethylenedioxy-3-methoxy-(9,12,12-$^2H_3$)-9β-oestra-1,3,5(10)-trien-11ξ-ol (4) (1·1 g).

To an ice-cold solution of the trideutero 11-epimeric alcohols (4) (0·96 g) in dry pyridine (10 ml) phosphoryl trichloride (2·0 ml) was added dropwise during 10 min. The mixture was allowed to stand at room temperature for 48 h, then poured into ice-water. The mixture was kept overnight, then extracted with ether/benzene. Evaporation of the washed and dried extract, and recrystallization of the crude product (824 mg) from ethanol gave pure *17,17-ethylenedioxy-3-methoxy(12,12-$^2H_2$)-oestra-1,3,5(10),9(11)-tetraene* (5b), m.p. 152–154°.

(c) 17,17-Ethylenedioxy-3-methoxy(9,12,12-$^2H_3$)oestra-1,3,5(10)-trien-11α-ol (8a)

A solution of 17,17-ethylenedioxy-3-methoxy(12,12-$^2H_2$)oestra-1,3,5(10),9(11)-tetraene (5b) (618 mg) in tetrahydrofuran (7 ml) was reduced with ($^2H_6$)diborane, prepared from sodium borodeuteride (0·8 g) in diglyme (12 ml) and boron trifluoride (3·2 ml) in diglyme (3·0 ml), in the manner described previously for reduction of the corresponding 11,12,12-trideuterated 9(11)-dehydro compound.[4] After removal of the excess of ($^2H_6$)diborane with nitrogen, the mixture was cooled and treated carefully with 2 N sodium hydroxide (15 ml), ethanol (6 ml) and 30% hydrogen peroxide (6 ml). The mixture was heated under reflux for 1 h, then most of the solvents were removed in vacuum. The residue was dissolved in ether/benzene, washed with water, then dried (Na_2SO_4) and evaporated to give a colourless gum (650 mg). This was dissolved in benzene and adsorbed on Merck basic alumina (grade II). Elution with benzene gave a semisolid (35 mg) consisting mainly of the starting olefin. Subsequent elution with ether and ether containing methanol (1%) gave *17,17-ethylenedioxy-3-methoxy(9,12,12-$^2H_3$)oestra-1,3,5(10)-trien-11α-ol* (8a) as a colourless gum (610 mg).

(d) 17,17-Ethylenedioxy-3-methoxy(9,12,12-$^2H_3$)oestra-1,3,5(10)-triene (7)

A solution of the 9,12,12-trideutero alcohol (8a) (610 mg) in dry pyridine (7·5 ml) was treated with *p*-toluenesulfonyl chloride (344 mg), and the mixture was kept at room temperature for 10 days. It was then poured into ice-water (*c.* 70 ml) containing concentrated sulfuric acid (4·84 g), and the product was isolated carefully with ether/benzene, as described previously,[4] to give a colourless gum (766 mg).

The *p*-toluenesulfonate (8b) (766 mg) was heated under reflux with lithium aluminium hydride (500 mg) in dry ether (80 ml) for 24 h, and the usual workup gave a gum (539 mg) which was shown by g.l.c. to contain about 11% of the 12,12-dideuterated 9(11)-dehydro compound (5b). This was removed by reconversion into the 11α-alcohol (8a) by treatment of the total crude product in tetrahydrofuran (6 ml) with ($^2H_6$)diborane, prepared from sodium borodeuteride (0·5 g) in diglyme (7·5 ml) and boron trifluoride etherate (4·8 ml) in diglyme (4·6 ml), in the usual way.[4] After removal of the excess of ($^2H_6$)diborane, 2 N sodium hydroxide (17·5 ml), ethanol (7 ml) and 30% hydrogen peroxide (7 ml) were added, and the mixture was heated under reflux for 1 h. The usual workup yielded a colourless gum (500 mg) which was dissolved in benzene and adsorbed on Merck basic alumina (grade II). The fractions eluted with hexane and hexane containing benzene (2–8%) were combined and the product (311 mg) was twice recrystallized from methanol to yield pure *17,17-ethylenedioxy-3-methoxy(9,12,12-$^2H_3$)oestra-1,3,5(10)-triene* (7) (163 mg), m.p. 102–105°, which was shown by t.l.c. (silica gel/benzene containing 10% chloroform) to be homogeneous and to have the same R_F (0·32) as authentic undeuterated material.[4]

(e) (9,12,12-$^2H_3$)Oestra-1,3,5(10)-triene-3,17β-diol (10)

A solution of 17,17-ethylenedioxy-3-methoxy(9,12,12-$^2H_3$)oestra-1,3,5(10)-triene (7) (160 mg) in acetone (23 ml) containing 2 N sulfuric acid (2·3 ml) was stirred at room temperature for 20 h, then 4 N sodium hydroxide (2·0 ml) and water (10 ml) were added and most of the acetone was removed in vacuum. Extraction with ether/benzene gave 3-methoxy(9,12,12-$^2H_3$)oestra-1,3,5(10)-trien-17-one as a solid (110 mg) which was used directly in the next step.

A mixture of the ketone (110 mg) in glacial acetic acid (1·1 ml) and 48% hydrobromic acid (0·68 ml) was heated under vigorous reflux in a nitrogen atmosphere for 1 h. Addition of ice to the cooled mixture gave a pink solid which was extracted with ether/benzene and washed with water, 2% sodium bicarbonate (× 3), water (× 3), then dried (Na_2SO_4) and evaporated. The fawn-coloured solid (103 mg) was triturated with a small volume of acetone, and the residue was recrystallized from acetone to give pure *3-hydroxy(9,12,12-$^2H_3$)oestra-1,3,5(10)-trien-17-one* (60 mg), m.p. 261–262°.

Reduction of the (9,12,12-$^2H_3$)oestrone (60 mg) with sodium borohydride in the manner described in (*a*), and crystallization of the product from aqueous methanol, gave pure *(9,12,12-$^2H_3$)oestra-1,3,5(10)-triene-3,17β-diol* (10), m.p. 178–180°, undepressed on admixture with unlabelled oestradiol. T.l.c. (silica gel/chloroform containing 3% of methanol) showed only one spot, R_F 0·34, identical with that of authentic unlabelled oestradiol. The mass spectrum showed the parent ion at *m/z* 275, and g.c.–m.s. revealed the isotopic composition $^2H_4$, 12·2; $^2H_3$, 75·2; $^2H_2$, 8·0; $^2H_1$, 3·0; $^2H_0$, 1·7%.

Acknowledgments

We thank Professor B. Hogberg of the Company A. B. Leo for a gift of oestrone 3-methyl ether and Syntex S.A. Mexico (through Dr J. A. Edwards) for a gift of oestrone. G.c.–m.s. measurements were kindly made by Professor J. Sjövall and Dr M. Axelson of the Karolinska Institute, Stockholm.

Manuscript received 15 October 1982