SYNTHESIS OF [14α,15α-^{3}H]-NORGESTREL

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SUMMARY: The preparation of the well-known gestogen norgestrel in a tritium labelled form is described. Starting material was 18-methyl-3-methoxy-estra-1,3,5(10),8,14-pentaen-17ß-ol acetate. In a four step synthesis 17α-ethinyl-17ß-hydroxy-18-methyl-[14α,15α-^{3}H]-estr-4-en-3-one was obtained with a specific activity of 52 Ci/mmol and a radiochemical purity > 99 %.

KEY WORDS: Norgestrel: 17α-ethinyl-17ß-hydroxy-18-methyl-estr-4-en-3-one, Gestogen, Tritium, Synthesis

INTRODUCTION

During our work on the synthesis and the biological effects of steroid hormones, we needed a radioimmunoassay (RIA) for the worldwide used gestogen norgestrel [1]. Taking into consideration our former studies on steroid labelling [2 - 5] we used again the tritium labelling in 14,15-position for preparing the tracer, because the necessary 18-methyl-3-methoxy-estra-1,3,5(10),8,14-pentaen-17ß-ol 1a is an available product of the pharmaceutical factory VEB Jenapharm (GDR).

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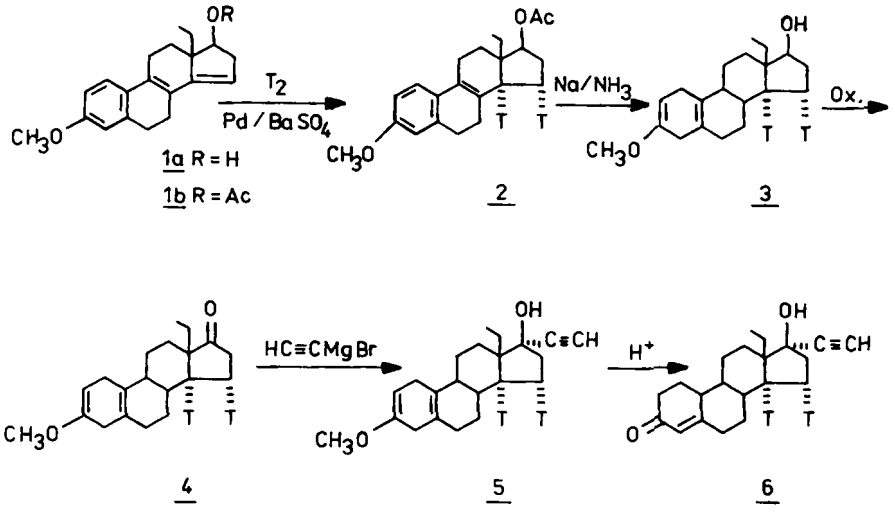
Schulz has recommended the 15,16-position for tritium labelling of norgestrel and other gestogens [6]. He used a 15,16-dehydro derivative as starting material, and prepared it in a four step synthesis including a microbial 15α-hydroxylation. In agreement with our results [4] for the 13-methyl-pentaenol derivative, the catalytic hydrogenation of the analogous 13-ethyl derivative 1a proceeded mainly to the 14α-product. However, the ratio 14α/14β was lower, due to the greater hindrance of the 13-ethyl group in comparison to the 13-methyl group. However, 18-methyl-3-methoxy-estra-1,3,5(10),8(14)-pentaen-17β-ol acetate 1b was hydrogenated in the required high ratio of 14α/14β > 90 %.

RESULTS AND DISCUSSION

Catalytic hydrogenation of 1b with tritium gas in benzene in the presence of 5 % palladium black on barium sulfate [7] yielded 18-methyl-3-methoxy-[14α,15α-^{3}H]-estra-1,3,5(10),8-tetraen-17β-ol acetate 2. A two-step reduction of 2 with sodium in liquid ammonia resulted in 18-methyl-3-methoxy-[14α,15α-^{3}H]-estra-2,5(10)-dien-17β-ol 3 under simultaneous cleavage of the 17β-acetate [8,9]. Oppenauer oxidation [10] of 3 gave 18-methyl-3-methoxy-[14α,15α-^{3}H]-estra-2,5(10)-dien-17-one 4 which was converted by ethinylation with Grignard-reagent [6] to 18-methyl-17α-ethinyl-3-methoxy-[14α,15α-^{3}H]-estra-2,5(10)-dien-17β-ol 5. Acidic cleavage yielded 18-methyl-17α-ethinyl-17β-hydroxy-[14α,15α-^{3}H]-estr-4-en-3-one 6 [11], the [14α,15α-^{3}H]-norgestrel.

During catalytic hydrogenation of 1,04 mmol 1b a consumption of 1,15 mmol tritium was measured. This higher value resulted from the uptake of the catalyst (3 µmol T_2/mg Pd) and further reduction of the 8,9-double bond at a lower rate, although the faster reduction of the 14,15-double bond was stopped



after 30 minutes, by monitoring with the measured hydrogenation curve [5]. After centrifugation of the catalyst, twofold exchange of labile tritium and freeze-drying, a specific activity of 57 Ci/mmol for 2 was found.

The labelled compound 2 was converted by a two-step Birch reduction of the 8,9-double bond (sodium/liquid ammonia) with aniline [8] to the 8ß,9α-configuration and of the aromatic A ring with isopropanol [9] to the 2,5(10)-diene-system under simultaneous cleavage of the 17ß-acetate to 3. The second reduction step did not proceed quantitatively in spite of the excess of reagent. In this way, traces of 18-methyl-3-methoxy-estradiol remained in 3. The specific activity of 3 showed the same value as for 2.

Oxidation of the 17ß-hydroxyl group to the 17-ketone is necessary for ethinylation. In order to retain the given protection of the 3-ketone as an enolether - to prevent attack in this position during ethinylation - we used the Oppenauer oxidation [10] in a neutral to weak basic medium. The reaction was quantitative and the specific activity of the compound 4 formed remained the same as 3.

The 17-ketone was ethinylated by reaction in situ to 5 with ethinylmagnesium bromide generated from ethylmagnesium bromide and acetylene. This reaction again did not proceed quantitatively in spite of excess of reagent. Nearly 10 % of 4 remained unchanged.

Consecutive treatment of crude 5 with concentrated hydrochloric acid yielded [14α,15α-^{3}H]-norgestrel 6 with two impurities: 18-methyl-17α-ethinyl-estradiol and 18-methyl-estr-4-ene-3,17-dione.

Preparative t.l.c. separation of the crude product obtained yielded 6 with a radiochemical purity >99 % and a specific activity of 52 Ci/mmol.

EXPERIMENTAL

All active experiments were made in boxes. The hydrogenation with tritium (produced in USSR) was performed in a special apparatus, which was also applied in former labelling studies [4,12,13]. For measuring the specific activity we used a Beckman LS-233 liquid scintillation spectrometer; for monitoring the radio-t.l.c.s we employed the Berthold-Frieseke scanner II. The chemicals used were p.a. grade.

The solvent system for t.l.c. on silica gel (H and PF Merck) or Silufol plates (Kavalier; ČSSR) was cyclohexane/ethyl acetate (1:1) for the reaction steps 1 - 3 and butyl acetate for steps 4 and 5 and for preparative isolation of the labelled norgestrel. After development, the analytical chromatograms were sprayed with vanillin-sulfuric acid and heated to 180 °C to reveal the spots, or were viewed under uv-light.

18-Methyl-3-methoxy-[14α,15α-^{3}H]-estra-1,3,5(10),8-tetraen-17β-ol acetate (2)

Compound 1b (351,6 mg, 1,04 mmol), catalyst (921 mg 5 % palla-

dium black on barium sulfate) and anhydrous benzene (8 ml) were put in a flask and, under stirring, hydrogenation with tritium ($p > 600$ torr) was performed at room temperature. The tritium uptake was finished after 30 minutes (uptake 1,15 mmol). The catalyst was centrifuged and washed with benzene. To the combined supernatants ethanol (20 ml) was added and the mixture stirred for two hours at 40 - 50 °C to remove labile tritium. After a two-fold freeze drying compound 2 (364,8 mg) was obtained with a specific activity of 57 Ci/mmol.

18-Methyl-3-methoxy-/14α,15α-^{3}H/-estra-2,5(10)-dien-17β-ol (3)

Compound 2 (364,8 mg) was dissolved in a mixture of tetrahydrofuran (8 ml), aniline (0,35 ml) and liquid ammonia (100 ml). Under stirring, sodium (600 mg) was added and stirring of the dark blue solution continued for 30 minutes. Then isopropanol (1,5 ml) was added and after further stirring for one hour the solution was discoloured by addition of more isopropanol. After warming up to room temperature the steroid was precipitated by addition of water and removed by filtration. After washing with water the precipitate was dissolved in benzene and freeze-dried. Compound 3 (296,4 mg) was obtained as a fine white powder with a specific acitivity of 56 Ci/mmol.

18-Methyl-3-methoxy-/14α,15α-^{3}H/estra-2,5(10)-dien-17-one (4)

Compound 3 (296,4 mg) was dissolved in toluene (12 ml) and, after addition of cyclohexanone (1,25 ml) and aluminium tert. butylate (500 mg), the mixture was heated under stirring and reflux for one hour. After cooling to 60 °C, a solution of NaK-tartrate (750 mg) in water (7,5 ml) was added and then the organic solvents were removed on a rotary evaporator.
The residue was extracted with benzene, the organic layer washed with NaCl-solution, dried with Na_2SO_4 and then

freeze-dried. Compound 4 (290 mg) was obtained with the same specific activity as 3.

18-Methyl-17α-ethinyl-3-methoxy-[14α,15α-^{3}H]-estra-2,5(10)-dien-17β-ol (5)

Tetrahydrofuran (50 ml ; anhydrous) was saturated with acetylene at -5 °C to 0 °C. Then Grignard reagent, prepared from magnesium (2,4 g) and ethylbromide (10,5 g) in tetrahydrofuran (75 ml; anhydrous), was added within 20 minutes under cooling followed by further conducting of acetylene for 30 minutes. Compound 4 (290 mg), dissolved in tetrahydrofuran (10 ml; anhydrous), was added and the mixture stirred for 2 hours while coming up to room temperature. The reaction was poured into a saturated solution of NH_4Cl in water (300 ml) and after separating the upper organic layer the aqueous solution was extracted with benzene. The combined organic layers were then freeze-dried and directly used for the last reaction step.

18-Methyl-17α-ethinyl-17β-hydroxy-[14α,15α-^{3}H]-estr-4-en-3-one (6)

The resulting raw material of 5 was dissolved in dimethylformamide (3 ml) and concentrated HCl (0,3 ml) added. After one hour the mixture was poured into saturated NaCl-solution (30 ml) and the precipitate formed was extracted with benzene. The organic layer was added with NaCl-solution until the pH value was neutral and then freeze-dried.

The resulting oily residue was purified by preparative t.l.c. on silica gel plates (2 mm; 20 x 20 cm; Merck, FRG) with butyl acetate as solvent. The final product 6 had a radiochemical purity $\geq$ 99 % and a specific activity of 52 Ci/mmol.

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