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SYNTHESIS OF TRITIUM-LABELLED BIOLOGICALLY ACTIVE ANALOGUES OF PROGESTERONE BY SELECTIVE HYDROGENATION OF 16α,17α-CYCLOHEX-3'-EN-PREGNA-1,4-DIEN-3,20-DIONE

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Summary.

The procedure of selective hydrogenation with gaseous tritium of 16α,17α-cyclohex-3'-en-pregna-1,4-dien-3,20-dione (**St0**) has been elaborated, and isotopically labelled 16α,17α-cyclohexanopregna-1,4-dien-3,20-dione (**St1**), 16α,17α-cyclohex-3'-en-pregn-4-en-3,20-dione (**St2**), 16α,17α-cyclohexanopregn-4-en-3,20-dione (**St3**) with molar radioactivity of 41, 44, 85 Ci/mmol, respectively, obtained. By hydrogenation with gaseous hydrogen, tritium labelled (**St3**) was converted to 16α,17α-cyclohexano-5α-pregnan-3,20-dione (**5α-St4**) and 16α,17α-cyclohexano-5β-pregnan-3,20-dione (**5β-St4**).

Key words: progesterone analogues, labelled steroids, tritium

INTRODUCTION.

The selective hydrogenation of suitable precursors with gaseous tritium provides one of the main methods of production of labelled compounds [1 - 5]. However it is still necessary for the tritiation conditions to be optimised for each class of biologically active compounds. The interest in tritium labelled cycloalkanoprogesterone derivatives has been aroused by the fact [6] that they are effective progestins and provide selective biological functions, with corresponding biological activity. 16α,17α-Cyclohex-3'-en-pregna-1,4-dien-3,20-dione (**St0**) has been chosen as the starting compound. A number of biologically active tritium labelled steroids such as

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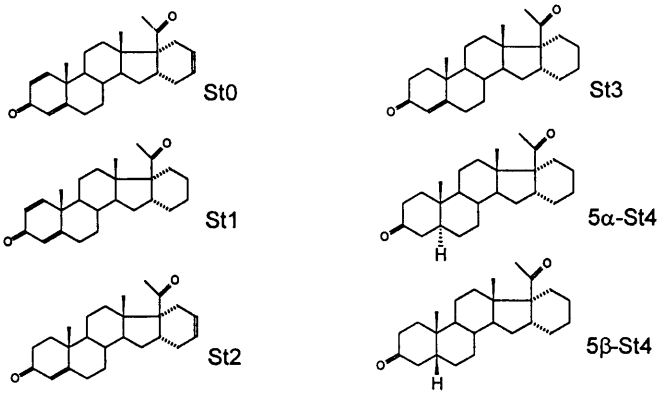
16α,17α-cyclohexanopregna-1,4-dien-3,20-dione (**St1**), 16α,17α-cyclohex-3'-en-pregn-4-en-3,20dione (**St2**), 16α,17α-cyclohexanopregn-4-en-3,20-dione (**St3**), 16α,17α-cyclohexano-5α-pregnan-3,20-dione (**5α-St4**) and 16α,17α-cyclohexano-5β-pregnan-3,20-dione (**5β-St4**) were synthesised by the hydrogenation of (**St0**) with gaseous tritium.



EXPERIMENTAL

Solvents, catalysts and other reagents were prepared and purified according to standard procedures. The unlabelled steroids (standards) were either commercial preparations or synthesised at the N.D.Zelinsky Institute of Organic Chemistry, RAS [7]. Preparative amounts of the starting compound (**St0**) were synthesised from (**St2**) according to the procedure described in [8]. Analysis and purification of the preparations was conducted by high performance liquid (HPLC) and thin layer (TLC) chromatography.

HPLC was performed using:

10x250 mm Silasorb C_{18}, 13 μm column at a flow rate of 2 ml/min,

system (I): methanol-water (95:5), 9.95 min (**St0**), 11.29 min (**St1** and **St2**), 13.94 min (**St3**);

system (II): methanol, 6.72 min (**St0**), 10.54 min (**St3**), 16.86 min (**5β-St4**), 19.57 min (**5α-St4**);

system (III): methanol-water (4:1), 9.63 min (**St1** and **St2**), 11.29 min (**St3**).

3.3x150 mm Separon SIX column, at the flow rate of 0.5 ml/min;

system (IV): hexane - iso-propanol (95:5), 9.94 min (**St2**), 13.10 min (**St1**).

3.3x150 mm Separon SGX C_{18} column, the flow rate 0.5 ml/min;

system (V): methanol-water (4:1), 5.95 min (**St0**), 9.15 min (**St1** and **St2**), 12.11 min (**St3**);

system (IV): methanol-water (85:15), 4.44 min (**St0**), 5.48 min (**St1** and **St2**), 7.02 min (**St3**).

TLC was performed on Sorbfil-UV254 plates:

system (VII): hexane-ether (1:1), three-time development, R_f (**St2**) 0.51, (**St1**) 0.39;

system (VIII): hexane - iso-propanol (95:5), R_f (**St2**) 0.64, (**St1**) 0.53;

system (IX): hexane - iso-propanol (90:10), R_f (**5α-St4**) 0.78, (**5β-St4**) 0.74.

Radioactivity was measured by liquid scintillation counting with 30% tritium registration efficiency in a dioxane scintillation cocktail; a Berthold LB506 HPLC Radioactivity Monitor was also used.

The optimum reaction conditions were found using 0.1% tritium according to the procedure described in [9]. The dependence of the molar radioactivity value on the duration of the process (0.2-2 h), on the nature of the catalyst and on the catalyst-to-compound ratio (from 1:2 to 4:1) was also studied.

METHOD OF SELECTIVE HYDROGENATION WITH GASEOUS TRITIUM

A mixture of 10 mg of steroid (**St0**) and 20 mg of (triphenylphosphine)rhodium chloride in 0.5 ml of ethyl acetate was placed in an ampoule, frozen with liquid nitrogen, evacuated and the ampoule was afterwards filled with gaseous tritium to a pressure of 400 hPa. The reaction was conducted with stirring over the course of 10-180 min. The contents of the ampoule were once again frozen in liquid nitrogen and the system evacuated. Labile tritium was removed by three-fold evaporation of the reaction mixture with ethyl acetate-methanol mixture (5:1). The reaction products were applied onto a silica gel plate and developed three times in system (VII). The developed plate was divided into three zones. In the case of 40 min reaction time the upper zone contained labelled compounds with an overall radioactivity of about 0.7 Ci, the middle zone - of about 1.1 Ci, and the lower zone - 0.57 Ci. The reaction products were extracted by ethyl acetate (5x10 ml), filtered and evaporated to dryness and dissolved in an ethyl acetate-methanol mixture (2:1).

Further analysis and preparative purification was performed by HPLC using the systems (I-VI). About 70% of the applied material could be recovered from the TLC plate. The upper

zone (2.2 mg total) contained 14% of **St2** and 86% of **St3**. The middle zone (3.1 mg total) contained 14% of **St1**, 26% of **St2**, 60% of **St3**. The lower zone - 1.6 mg (18% **St1**, 3% **St2**, 79% **St3**). Hence, the reaction mixture contained 11% (0.75 mg) of **St1**, 17% (1.15 mg) of **St2**, and 72% (4.98 mg) of **St3**.

The molar radioactivities of the labelled **St1-St3** were 41, 44, 85 Ci/mmol, respectively (the radiochemical purity was 95-97% after reversed and normal phase chromatography. The labelled steroids were stored at -10°C as ethyl acetate-methanol (2:1) solution.

After 20 min of hydrogenation on the same catalyst, the reaction mixture contained 1.5% of **St0**, 39% of (**St1+St2**), 46% of **St3**; after 90 min hydrogenation resulted in 5.5% of (**St1+St2**) and 85% of **St3**.

PROCEDURE FOR HYDROGENATION OF [^{3}H]St3 WITH GASEOUS HYDROGEN

1) Liquid-state hydrogenation:

50 mCi of [^{3}H]St3, 0.2 ml of ethanol and 5 mg of 5% Pd/$CaCO_3$ were placed in the reaction ampoule, frozen by liquid nitrogen and the system evacuated to a pressure of 0.1 Pa. The gaseous hydrogen was introduced up to a pressure of 400 hPa, and the reaction allowed to proceed for 2 h at room temperature with stirring. The contents of the ampoule were then filtered, the catalyst washed with ethanol (3x1 ml), the filtrates combined, evaporated and purified by HPLC using system (II). Analysis of the reaction mixture showed it to contain 72% of **5β-St4** and **5α-St4**. After purification by HPLC, the yield of **5β-[^{3}H]St4** was 40-50% and of **5α-[^{3}H]St4** 3-4%. The radiochemical purity of the preparations, determined by TLC using system (IX), was 95-97%.

2) Solid-state hydrogenation:

0.2 ml of an ethanol solution of 50 mCi [^{3}H]**St3** and 10 mg of 5% Pd/$BaSO_4$ were placed in a reaction ampoule and the solvent evaporated off. The dry mixture was evacuated to a pressure of 0.1 Pa, and gaseous hydrogen was introduced to a pressure of 400 hPa. The reaction was conducted over 15 min at 80°C, and the contents of the ampoule then dissolved in 2 ml of methanol and treated as described above. The analysis of the reaction mixture showed the yield to be as follows: 32% of **5β-St4**, 11% of **5α-St4**. The molar

radioactivity of the preparations synthesised by methods 1 and 2 did not practically differ from the molar radioactivity of [^{3}H]**St3**.

DISCUSSION

Selective hydrogenation of the C_1=C_2 bond was found to be unsuccessful if heterogeneous catalysts were used, although the adsorption of the **A** ring with its double bond system, conjugated with the carbonyl group, should be more effective than the **D** ring. Besides, during the hydrogenation of **St0**, even with the Lindlar catalyst, strong degradation was observed after 25 min of the reaction, and the total yield over the sum of the steroids did not exceed 10-15% of its calculated value, the ratio of the components being as follows: 2.5% of **St0**, 12.7% of (**St1+St2**), 47.0% of **St3**, 37.8% of **St4**.

Hydrogenation in the presence of homogeneous catalysts proved to be successful. The kinetic data showed that the rate of the C_1=C_2 bond hydrogenation is close to that of the C_3'=C_4' bond. So, the reaction mixture contained several hydrogenation products in any case. The optimum reaction time, that varied within 15-40 min., depended on the main desired product (Fig.1). Under our optimum conditions, the maximum amount of [^{3}H]**St1** present in the reaction mixture was estimated as 10-15%, [^{3}H]**St2** 20-25%, [^{3}H]**St3** 60-70%, but since the isolation of these labelled compounds is a multi-stage process (see below), the real yield of [^{3}H]**St1** was 5-10%, [^{3}H]**St2** 10-15%, [^{3}H]**St3** 50-60%, and the molar radioactivities were 41, 44, 85 Ci/mmol, respectively.

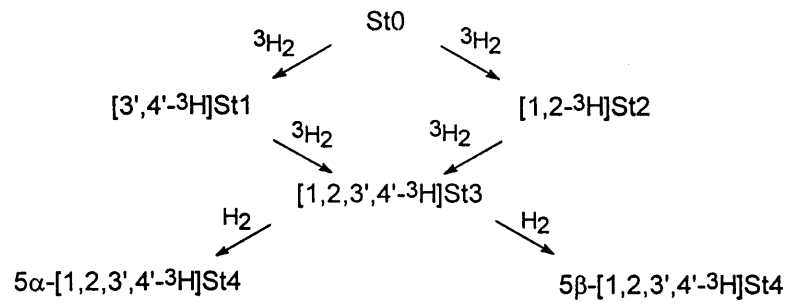


Fig.1. Synthesis of tritium-labelled biologically active analogues of progesterone by selective hydrogenation of St0 .

The production of individual [^{3}H]steroids was only possible if the radiochemical purity was greater than 95-97%. The required standards were therefore synthesised [8]. Chromatographic studies showed that under reversed phase HPLC conditions retention of **St0**, (**St1** and **St2**), **St3**, **St4** differ greatly, while **St1** and **St2** had virtually the same retention time. In normal phase HPLC, **St1** and **St2** were well separated but retention times of **St2**, **St3** and **St1**, **St0** were close. Therefore, to achieve successful separation of progesterone labelled derivatives after selective hydrogenation had been conducted, the following scheme was proposed.

TLC on silica gel plates in the system (VII) was used to separate labelled steroids from the catalyst. In addition, separation of [^{3}H]**St2** from 35-40% of [^{3}H]**St1** and 25-30% of [^{3}H]**St3** was successfully achieved (with the loss of [^{3}H]**St2** being under 3-4%). The partially purified tritium labelled steroids were re-purified by reversed phase HPLC using system (I), and the required radiochemical purity was attained by the normal phase HPLC using system (IV).

It has been mentioned that the C_4=C_5 bond in **St0** could not be reduced by using homogeneous catalysts, and that considerable decomposition of steroids was observed when using heterogeneous catalysts.

Therefore, the following scheme was proposed to synthesize 5α-[^{3}H]St4 and 5β-[^{3}H]St4 (Fig.1). **St0** was reduced to **St3** with gaseous tritium using a homogeneous catalyst, followed by heterogeneous hydrogenation of the C_4=C_5 bond with gaseous hydrogen. At this juncture, the ratio of 5α-[^{3}H]St4 and 5β-[^{3}H]St4 appeared to be (1:14) in the case of liquid-state hydrogenation and (1:3) - in the solid-state process that is a more preferable one for the production of tritium labelled **5α-St4**.

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