Synthesis of High Specific Activity 17α-Cyanomethyl-17ß-hydroxy-[14α,15α-^{3}H]estra-4,9-dien-3-one

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SUMMARY

An improved synthesis of the tritium labelled form of the new pharmacon DIENOGEST is described. The [14α,15α-^{3}H]DIENOGEST was obtained with a specific activity of 51 Ci/mmol and a radiochemical purity > 98%.
Key words: tritium labelling, labelled steroids, chromatographic purification

INTRODUCTION

17α-Cyanomethyl-17ß-hydroxy-estra-4,9-dien-3-one, a new progestin applied in hormonal contraception, hormonal replacement therapy, and also useful in the treatment of endometriosis, has been developed in Jena [1]. It was registered in the World Health Organisation under the name of DIENOGEST. Its metabolic behaviour was investigated [2] using the tritium labelled form of this compound .

The first synthesis of 17α-cyanomethyl-17ß-hydroxy-[14α,15α-^{3}H]estra-4,9-dien-3-one was published in 1980 [3,4]. Continuing work on this new pharmacon required a further preparation of the labelled compound. However, there was the disadvantage that the synthesis of 17α-cyanomethyl-17ß-hydroxy-[14α,15α-^{3}H]estra-4,9-dien-3-one required a radioactive multistep procedure including a BIRCH reduction at the beginning [5].

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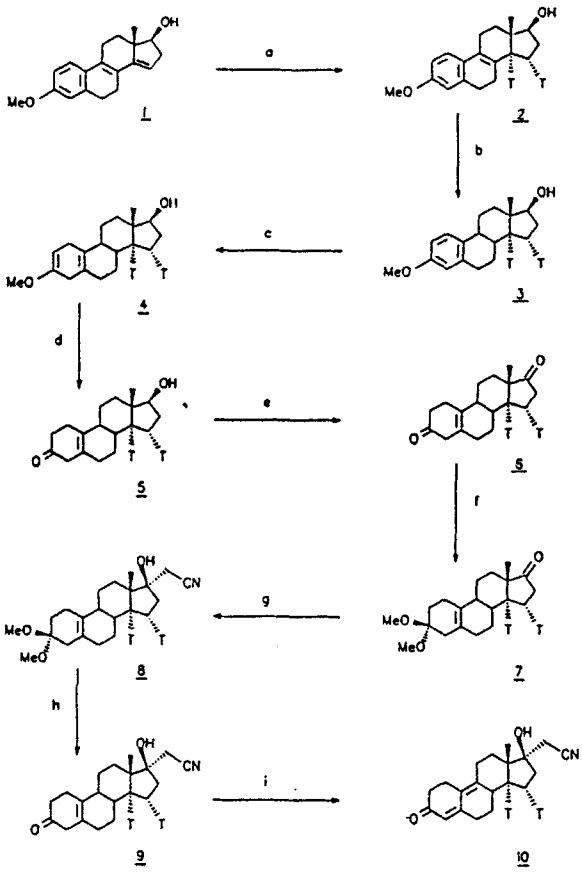
Our efforts have been directed at devising methods to shorten the synthesis time. We have been able to do this by taking advantage of the timesaving flash chromatography [6] and a new procedure for introducing the 17α-cyanomethyl group [7] into the steroid skeleton. Omitting the determination of the specific activities of the intermediates **2** - **8** we were able to carry out a rapid synthesis of the desired product which was to have a high specific activity (> 50 Ci/mmol) so that it can be used in radioimmunoassays. The necessary experience for working on a high radioactive scale was gathered in previous syntheses [8,9].

RESULTS and DISCUSSION

The synthesis described here is shown in SCHEME 1. Compared with the first synthesis [3,4] the present one is shorter by one step, and it is unusual, but necessary, to have the tritium introduced at high specific activity in the first step of what is a 9-stage synthesis. A few years ago, we reported on a similar procedure, namely the synthesis of [14α,15α-^{3}H]norgestrel [8].

As can be seen from SCHEME 1, the tritiation reaction **a** is followed by eight radioactive steps. For this reason, a sufficiently high amount of 3-methoxy-estra-1,3,5(10),8,14-pentaen-17ß-ol (**1**) was required as starting material. We used 0.5 mmol **1** and obtained 3-methoxy-[14α,15α-^{3}H]estra-1,3,5(10),8-tetraen-17ß-ol (**2**) with a tritium content of about 30 Ci. In inactive attempts, 0.5 mmol **1** dissolved in 8 ml toluene was quantitatively hydrogenated with hydrogen of 0.8 at in presence of 200 mg 5 % Pd/$BaSO_4$ within 40 min. Under the same conditions the hydrogenation reaction using T_2 needed more time. However, we stopped the reaction after 55 min in order to avoid the undesired cis-hydrogenation of the 8-double bond [10]. After usual workup, only traces of pentaenol **1** could be detected in the tetraenol **2** by using thin-layer chromatography (TLC) [3,4].

After removing most of the labile tritium, the tetraenol **2** was transferred into the apparatus for a two-step BIRCH reduction. This reduction takes place in liquid ammonia/THF with metallic lithium and specific proton sources. The inactive studies were carried out several



SCHEME 1

times before the radioactive run was performed. The 8,9-double bond of 2 was trans-hydrogenated to give 3-methoxy-[14α,15α-^{3}H]estra-1,3,5(10)-trien-17ß-ol (3) which was then dearomatized in a following reduction step resulting in 3-methoxy-[14α,15α-^{3}H]estra-2,5(10)-dien-17ß-ol (4) .

The first reduction step was run at - 33 ^{0}C using aniline as proton donator [11]. A blue colour was generated and maintained over the needed reaction time of 1.5 hr by adding metallic lithium in small portions. The end of the reaction was detected by radio TLC. Then the mixture was cooled to - 50 ^{0}C, 2-propanol as a more acidic proton source [5] was added, and lithium was added again to generate and to maintain the blue colour for 2 hr.

Because of the high activity and the known danger of liberation of residual tritium from labile positions in the case of BIRCH reductions, our specific attention was attracted to this problem. Although such multicurie-BIRCH reductions are regarded as hazardous in the literature [12], we were able to demonstrate that these reactions can be still performed without complications.

Labile tritium arises as a consequence of preliminary exchange reactions. A catalyst mediated isotope exchange may occur in the tritiation reaction **a** between T_2 and the protons of the 17ß-OH group and also with the activated protons in benzylic (C-6 and C-9) or allylic (C-7 and C-11) positions. Under the conditions of the BIRCH reductions isotope exchange may proceed also between these positions and liquid ammonia. The reductions were carried out under slight argon pressure. The argon-ammonia vapour leaving the reaction vessel as waste gas during the work up procedure was passed through three washing bottles filled with water. If any labile tritium should be contained in this gas, it would be absorbed to a great extent. This simple precaution proved successful. 300, 80, and 15 mCi of labile tritium, respectively, were found in the washing bottles .

The reactions **d**, **e**, and **f** were carried out as described in [3,4] but without purification. As shown in a radio chromatogram (FIG. 1), the 3,3-dimethoxy-[14α,15α-^{3}H]estr-5(10)-en-17-on (7) formed was accompanied by many by-products. Because the dimethyl ketal 7 was less sensitive to converting the 3-keto-5(10)-ene system to the conjugated form than for instance 4, 5, and 6, it was purified by flash chromatography [6]. Using silica gel as sorbent and cyclohexane-ethyl acetate (4:1) as eluent we obtained pure dimethyl ketal 7. The efficiency of such a simple flash chromatography can be demonstrated by the radio chromatogram shown in FIG. 1.

In previous syntheses [3,4,9] the introduction of the cyanomethyl group required two steps. Firstly, the dimethyl ketal 7 had to be converted to a 17-spiro-oxirane using trimethylsulfonium iodide and potassium tert.-butylate, and this spiro-oxiran was subsequently

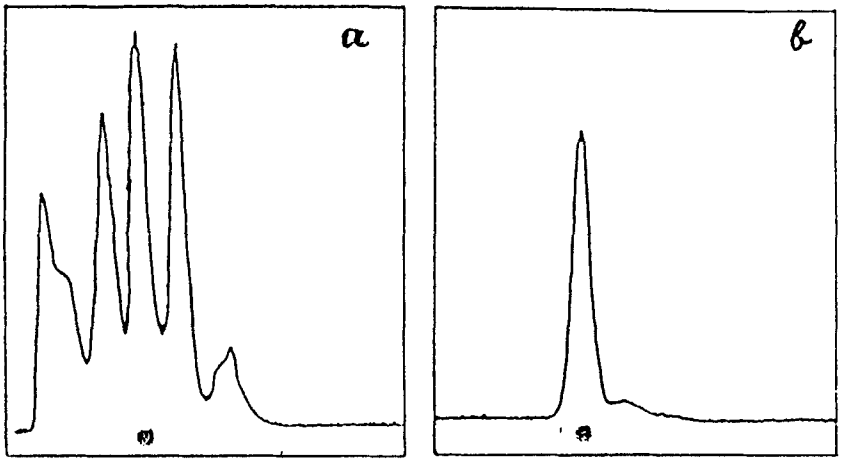


FIGURE 1

Radio-chromatograms of **a)** the crude product of 3,3-dimethoxy-[14α,15α-^{3}H]estr-5(10)-en-17-one (7) and **b)** the flash chromatographically purified one.

The by-products in **a)** are unknown, the radioactive peak of 7 is marked by the spot of inactive 3,3-dimethoxy-estr-5(10)-en-17-one. The solvent system was cyclohexane - acetone (4 : 1)

cleaved by sodium cyanide to obtain 3,3-dimethoxy-17α-cyanomethyl-[14α,15α-^{3}H]estr-5(10)-en-17ß-ol (8). In the present work we used a new cyanomethylation procedure [7] which allowed us to obtain the cyanomethyl compound 8 in one step under mild conditions and without using the toxic sodium cyanide. The required cyanomethyl lithium was synthesized from butyl lithium and acetonitrile in THF at - 50 °C. Addition of the 17-keto steroid 7 to this solution at - 50 °C gave the desired cyanomethyl steroid 8 in high yield within 0.75 hr. A radio chromatogram of 8 after flash chromatographic purification [6] is shown in FIG. 2.

Ketal cleavage of the cyanomethyl compound 8 as reported in [3,4] afforded 17α-cyanomethyl-17ß-hydroxy-[14α,15α-^{3}H]estr-5(10)-en-3-one (9) containing only traces of by-products. In accordance with the inactive synthesis this compound was to be finally converted to 17α-cyanomethyl-17ß-hydroxy-[14α,15α-^{3}H]estra-4,9-dien-3-one (10) by an exact bromination-dehydrobromination process as regards the amount of bromine and a precise temperature management. It was not possible to meet these requirements in a small scale radioactive synthesis. We used a freshly prepared solution of bromine in

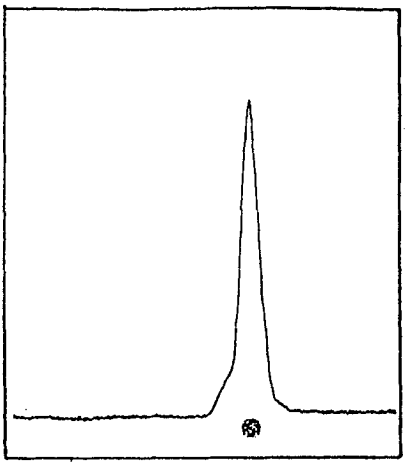


FIGURE 2

Radio-chromatogram of the flash chromatographically purified 3,3-dimethoxy-17α-cyanomethyl-[14α,15α-^{3}H]estr-5(10)-en-17ß-ol (**8**).

The spot of the inactive 3,3-dimethoxy-17α-cyanomethyl-estr-5(10)-en-17ß-ol agrees with the radioactive peak of **8** . The solvent system was trichloromethane - n-hexane - methanol (9 : 9 : 2)

trichloromethane (c. 25 mg Br_2/ml) for the bromination-dehydrobromination process which was carried out according to [13]. In spite of the mild reaction conditions the crude reaction product was a mixture containing less polar by-products mainly with the desired compound **10** being present in 30% yield only (FIG. 3). The occurence of by-products in this step is discussed in the literature [13] as a consequence of an excess of bromine and/or a temperature mismanagement.

Nevertheless, the fact that the by-products were less polar than **10** was favourable for a chromatographic purification of the final compound . Again, we used flash chromatography [6]. In a twofold process pure **10** could be isolated. Its specific activity was found to be 51 Ci/mmol, its total radioactivity 300 mCi. The high purity of the final product is demonstrated in FIG. 3. The u.v. absorption spectrum was identical with that of the inactive DIENOGEST.

The results show that the combination of multistep syntheses including sensitive intermediates

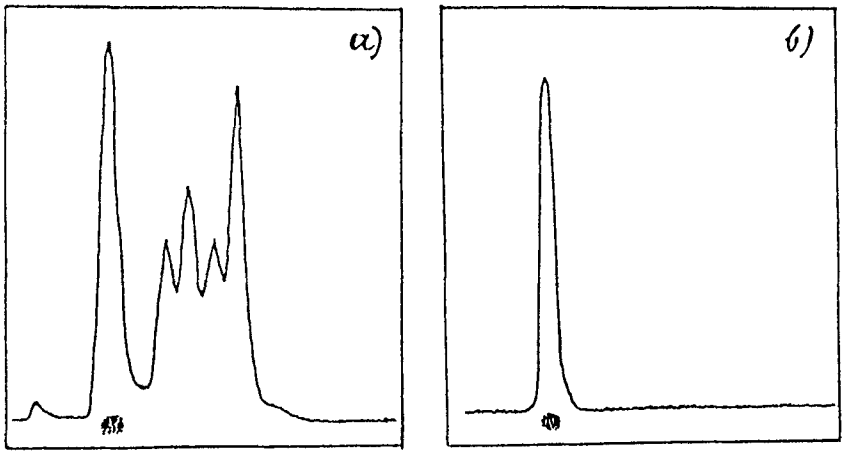


FIGURE 3

Radio-chromatograms of **a)** the crude product of 17α-cyanomethyl-17ß-hydroxy-[14α,15α-^{3}H]estra-4,9-dien-3-one (**10**) and **b)** the twofold flash chromatographically purified one.

The more polar by-products in **a)** are unknown, the radioactive peak of **10** is marked by the spot of inactive 17α-cyanomethyl-17ß-hydroxy-estra-4,9-dien-3-one. The solvent systems were toluene - ethyl acetate (6 : 4 and 1 : 1).

with properly selected flash chromatographic conditions is very useful in overcoming additional difficulties resulting from the nature of tritiated intermediates and a small preparative scale.

EXPERIMENTAL

1. Material and methods

The tritiation reaction was performed in a special apparatus used also in former labelling studies [3,4,8,9]. Carrier free tritium gas was purchased from Fa. BIOTREND, Köln (Germany).

Solvents were of analytical grade. THF was freshly distilled from $LiAlH_4$. Trichloromethane was freshly distilled from $CaCl_2$ and filtered through aluminia (activated, basic). The solution of n-butyl lithium (15% in n-hexane) was a product of Merck (Germany). Ammonia was purchased from Alphagaz Dresden (Germany) and was freshly distilled before use. TLC was carried out on silica gel plates (Merck 5554) with different solvents. The spots of inactive reference substances were visualized by spraying with vanillin in sulphuric acid and heating. The flash chromatography was carried out on silica gel for column chromatography (Merck 9385).

2. The two-step BIRCH reduction to 3-methoxy-[14α,15α-^{3}H]estra-2,5(10)-dien-17ß-ol (4)

A gentle stream of dry argon was passed for 10 min through a four-necked flask (200 ml) equipped with a dry ice condenser, thermometer, dropping funnel and magnetic stirrer. Then liquid ammonia was distilled over. When approximately 35 ml was collected, a solution of 2 (128 mg, 0.44 mmol) in THF (11 ml) containing aniline (0.1 ml, 1.1 mmol) was added. Small pieces of metallic lithium (2-4 mg/piece; 14 mg, 2.0 mmol), freshly cut under hexane, were added to the mixture at - 33 °C. Already the first piece of lithium coloured the solution blue. The next piece was added when the blue colour began to dissipate. After 1.5 hr a radio-TLC (petroleum ether-acetone 4:1) showed nearly complete conversion of 2 to 3. The mixture was cooled to - 50 °C and treated with 2-propanol (1.5 ml, 19.5 mmol). Lithium (2 x 30 mg, then 10 portions of 8 mg; 140 mg, 20.2 mmol) was added to maintain the blue colour for 2 hr. A radio TLC (petroleum ether-acetone 4:1) showed 4 as the main component, but also two polar by-products with one of them being probably unconverted 3 . The cooling bath was removed and argon gently passed through. The argon-ammonia mixture leaving the reaction vessel had to pass three washing bottles filled with water. A slow rise of temperature made it possible to remove the ammonia within 1.5 hr. The mixture was treated with THF (10 ml) and saturated aqueous solution of ammonium chloride (10 ml). Separation of the organic layer, concentration in vacuo, treatment with a saturated solution of sodium chloride (50 ml), extraction with benzene (20 ml), washing with brine and water, and lyophilisation afforded crude 4 (130 mg) which was stored overnight at - 18 °C in benzene-methanol containing a drop of 25% aqueous ammonia and processed without further purification.

3. The cyanomethylation to 3,3-dimethoxy-17α-cyanomethyl-[14α,15α-^{3}H]estr-5(10)-en-17ß-ol (8)

Dry argon was passed through a round-bottom three-necked flask (50 ml) equipped with a magnetic stirrer, dropping funnel, thermometer, and a septum. n-Butyl lithium (1 ml, 1.6 mmol) was added with a syringe and diluted with THF (1 ml) freshly distilled from $LiAlH_4$. After cooling to - 50 °C acetonitrile (0.104 ml, 1.98 mmol) was added with a syringe within 1 min . 3,3-Dimethoxy-[14α,15α-^{3}H]estr-5(10)-en-17-on (7) purified by flash chromatography (22 mg, 0.06 mmol) was dissolved in THF (1 ml), and this solution was added dropwise to the cyanomethylating reagent. The reaction mixture was stirred for 45 min at - 50 °C. Then the cooling bath was removed and water (10 ml) added. Extraction with benzene (15 ml), washing with water (3 x 2 ml) and lyophilisation afforded crude 3,3-dimethoxy-17α-cyanomethyl-[14α,15α-^{3}H]-estr-5(10)-en-17ß-ol (8) (32 mg) . Flash chromatography afforded pure 8 (20 mg, 0.055 mmol).

4. The flash chromatography

A simple graduated 10 ml pipette was used as column. A wad of cotton wool was used to support silica gel (40 ... 63 μm, 5 ml). Equilibration of the column was performed with the appropriate eluent. The slight pressure needed for dropping the column was generated by aid of a pipette ball. The substance to be purified was dissolved in a few drops of eluent and transferred to the column. Elution, radio-TLC, and lyophilisation of the fractions being of interest afforded the purified substance.

4.1 *3,3-Dimethoxy-[14α,15α-^{3}H]estr-5(10)-en-17-on (7)*

Crude 7 (55 mg) was treated with eluent (cyclohexane-ethyl acetate 4:1, containing 1 drop NEt_3; 2.5 ml) in small portions and the solution formed (an insoluble residue weighing ~ 20 mg) was transferred on the

column. The eluent was fractionated in portions at 0.5 ml. The fractions 11 - 16 contained pure **7** (20 mg, 0.068 mmol).

4.2 *3,3-dimethoxy-17α-cyanomethyl-[14α,15α-^{3}H]estr-5(10)-en-17β-ol (**8**)*

Crude **8** (32 mg) was dissolved in eluent (trichloromethane-n-hexane-methanol 9:9:2, containing 1 drop of pyridine) and was transferred on the column. Fractionating in portions at 0.5 ml gave in the fractions 8 -12 pure **8** (20 mg, 0.055 mmol).

4.3 *17α-cyanomethyl-17β-hydroxy-[14α,15α-^{3}H]estra-4,9-dien-3-one (**10**)*

Crude **10** (23 mg) was dissolved in eluent (ethyl acetate-toluene 4:6, 1 ml) and was transferred on the column. The eluent was fractionated in portions at 3 ml. The fractions 6-9 contained **10** (8.8 mg) in a still impure form. The flash chromatography was repeated using ethyl acetate-toluene 1:1. By fractionating in portions at 1 ml very pure **10** (1.9 mg) was obtained in the fractions 8 - 11 . It showed λ_{max} 304 nm and a specific activity of 51 Ci/mmol.

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