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SYNTHESIS OF TRITIUM-LABELED (3-O-(3-HYDROXYPROPYL)-17α-ESTRADIOL CHROMIUM TRICARBONYL: THE FIRST RADIOACTIVE TRANSITION METAL CARBONYL STEROID HORMONE.

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SUMMARY

3-O-(3-hydroxypropyl)-17β-estradiol chromium tricarbonyl **1** has been labeled in the 17α position with deuterium or tritium. These complexes were prepared by reduction of the corresponding estrone by $NaBD_4$ or $NaB[^3H]_4$. The radioactive hormone was obtained in a solid state with a specific activity of 4.1 Ci/mmole , which is sufficiently high for hormone receptor binding studies.

KEYWORDS: organometallic steroid hormone, estradiol receptor, deuteration,tritium labeling.

INTRODUCTION

There are now a sizeable number of reports which clearly indicate that the cytosolic estrogen receptor (ER) content of primary breast tumors is an important predictor of survival (1) with a more favorable course in ER- rich cases. The use of tritiated-hormone (for example [6,7]^{3}H-17β-estradiol) is a universal and efficient method of detection of estradiol receptors in very low concentrations (2). However the drawbacks associated with the use of radioisotope labels such as health hazards, high costs, limited variety of useable isotopes and biochemical instability have prompted the development of a plethora of new strategies for the introduction of cold bioprobes for several types of bioassays (3).

We have previously demonstrated on the specific example of estradiol receptors that Fourier Transform Infrared Spectroscopy (IR-FT) is a possible alternative analytical method of general applicability (5) since it circumvents the usual limitations of non-isotopic procedures, namely: sensitivity and precision.This new technique uses the metal carbonyl fragments to label the hormone and the method consists of the detection of the intense ν(CO) peaks in the 2150-1800cm^{-1} area , a region where proteins do not absorb. Compound **1** (Scheme 1) with a $Cr(CO)_3$ attached on the α face of the A ring of the steroid, maintains a good receptor binding affinity for the estradiol receptor (RBA = 28%) (5). To our knowledge

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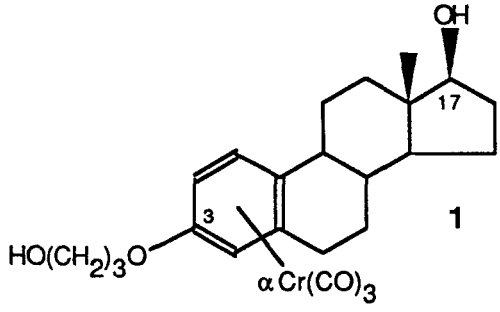
this hormone is the first non-isotopic compound that might be used to detect the receptor by an infrared spectroscopic method. In theory, this approach can be extended to any binding assay (e.g. : immunology).

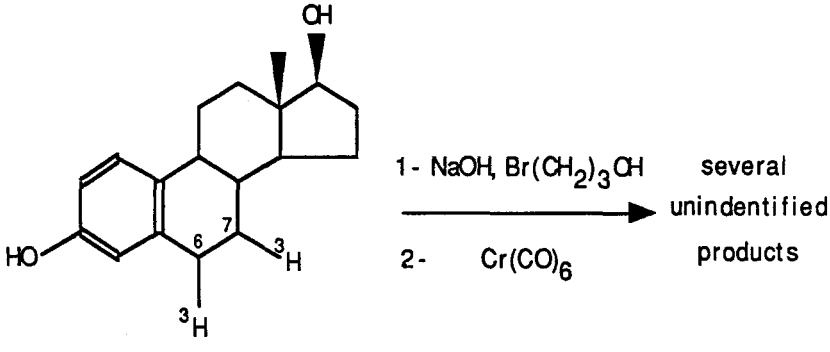


SCHEME 1

The chemical modification of any hormone leads to changes in its behavior towards its specific receptor : change in affinity (6) of physicochemical properties (7) and of hormonal specificities (8). To demonstrate the validity of our new approach, it is then necessary to study the interactions of the organometallic hormone **1** with the receptor of estradiol and check that the modified hormone does not bind excessively to the low affinity, high capacity binding proteins found in serum and tissues (7) . This can only be accomplished using a radioactive hormone. We described herein the preparation of the organometallic hormone **1** deuteriated or tritiated at the 17α position. Crystallised compound [H]-**1** is obtained in high specific activity tritium labeled form (4.1 Ci/mmole) suitable for further studies of receptor interactions.

RESULTS AND DISCUSSION

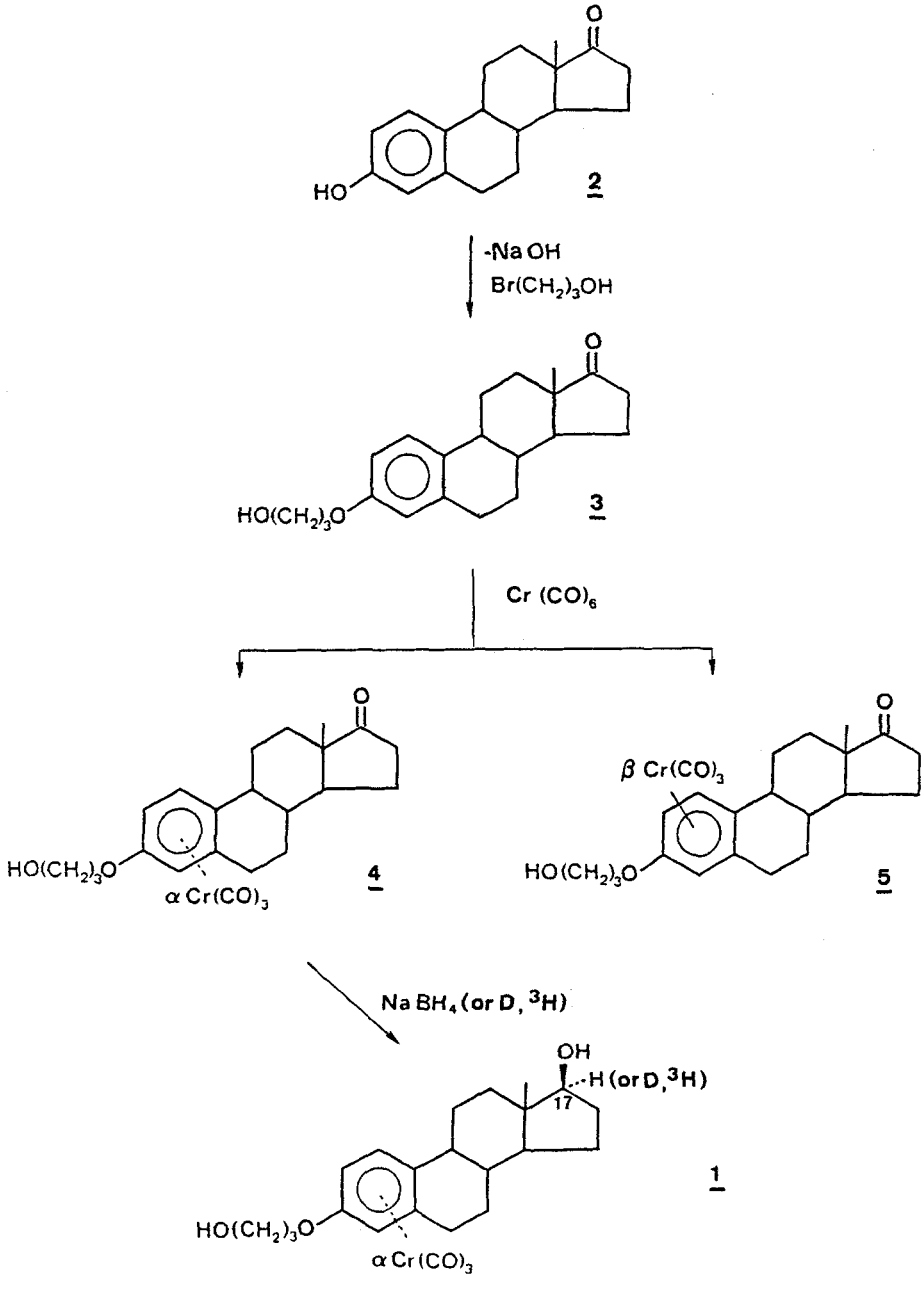
At the outset, we tried to synthesize the [6,7-^{3}H] **1**, starting from [6,7-^{3}H]-17β-estradiol using the method that we have already described for the non-radioactive product



SCHEME 2

(5). However after treatment of [6,7-^{3}H]-17β-estradiol with NaOH and 3-bromo-1-propanol, followed by refluxing with $Cr(CO)_6$ as indicated on scheme 2, we found unidentified radioactive products . This is probably due to the vigourous conditions of complexation (high temperature).

The radioactive product [17α-^{3}H]-1 was successfully prepared by the following procedure indicated on scheme 3.



SCHEME 3

Estrone **2** was heated with NaOH in acetone followed by addition of 3-bromo-1-propanol in accordance with the method described by Brewster and Putman (9). The 3-O-(3-hydroxypropyl)estrone **3** obtained was used without purification for the complexation reaction with $Cr(CO)_6$. After solvent removal the 3-O-(3-hydroxypropyl)estrone complex **4** and its diastereomer **5** were isolated by chromatography in 24% overall yield (respectively 12% and 12%).

The reduction of estrone by $NaBH_4$ is known to be a stereoselective reaction and furnishes essentially the 17β-estradiol (10). This stereoselectivity was verified by the reduction of complex **4** : the reaction of **4** with $NaBH_4$ and $NaBD_4$ in isopropanol at room temperature gave only the expected compounds [^{1}H]-**1** and D-**1**. By comparison with an authentic sample of [^{1}H]-**1** prepared by an alternative route (4,5), this compound was identified as the 3-O-(3-hydroxy- propyl) -17β-estradiol complex with $Cr(CO)_3$ on the α face of the A ring of the steroid. This reaction also permitted the identification of the corresponding estrone complexes, **4** with an α-$Cr(CO)_3$ unit and **5** with a β-$Cr(CO)_3$ moiety.

In place of $NaBH_4$, $NaBD_4$ was also used to determine the isotopic effect on the reduction reaction . We found that compound D-**1** was obtained in the same yield and under the same conditions as for [^{1}H]-**1** proving that the reduction reaction was not affected by changing hydrogen by deuterium.

Finally, the reduction by $NaB[^3H]_4$ (100 mCi; S.A. 25 Ci/mmol) of the ketone **4** gave a tritium labelled compound : [17α-^{3}H]-**1**. In this case the reaction mixture needed to be warmed at 50°C during 24 hours, but a good yield (92%) was obtained. The stability of the organometallic complex studied by U.V. spectroscopy showed that there is no decomposition of a stock solution ($1x10^{-3}$M) kept at -20°C in the dark over a period of at least one week. The specific activity of the product was determined by measuring the radioactivity present in an aliquot of the material after the content of[^{3}H]-**1** was ascertained from absorbance measurement at 319 nm. Numerous measurements were taken and the specific activity was found to be 4.1 Ci/mmol which is sufficently high to enable, for the first time, examination of the direct interaction of a high-affinity organometallic hormone with the estrogen receptor .

EXPERIMENTAL

Materials Chemicals were obtained from the following sources:Janssen ($NaBH_4$, $NaBD_4$, dibutyl ether , isopropanol); C.E.A. (France) ($NaB[H]_4$); Sigma (estrone); Amersham (England) ([6,7]^{3}H-17β-estradiol); S.D.S. (France) (ether, pentane, acetone); Merck (silica gel 7731, silica gel plates 5735).

Methods Analytical thin layer chromatography was performed using 0.2 mm silica gel plates Merck 5735 and spots were visualized by 254 nm ultraviolet light. Preparative thin layer chromatography was carried out using 1 mm silica gel plates Merck 7731 or 0.2 mm silica gel plates Merck 5735.

Dibutyl ether was dried by distillation from benzophenone-sodium; acetone, isopropanol and chromatographic solvents were used without purification.

Melting points were determined on a Kofler apparatus and were uncorrected.

Infrared spectra were determined as KBr pellets using a Bruker IF45 instrument. ^{1}H NMR spectra were taken on Bruker spectrometers, Model AM 250 and WM 500. Mass spectra were recorded on a Nermag spectrometer. The $[\alpha]_D$ values were determined on a Perkin Elmer 241 apparatus.

Radioactivity solutions were prepared as follows: 400-450 µg of the solid radioactive compound (weighed on a Mettler M8 balance) were dissolved in absolute ethanol to obtain a $1x10^{-3}$M stock solution. The concentration and the stability of the solution are checked by ultraviolet spectroscopy (measurement at 319 nm). Radioactivity was measured using A.C.S. (Amersham, England) as scintillation fluid in a LKB 1211 Rackbeta liquid scintillation counter. Specific activity was determined by measuring the radioactivity of an aliquot (50µl) of the $1x10^{-6}$M and $1x10^{-7}$ M solutions.

Synthesis **1- Attempt to prepare[6,7-^{3}H]-(3-O-(3-hydroxypropyl)estradiol chromium tricarbonyl from [6,7-^{3}H]-17β-estradiol.**

Following the method of Brewster and Putman (9), 0.005 mg ($2x10^{-8}$ mol) of [6,7]-^{3}H-17β-estradiol (S.A. 52 Ci/ mmol), previously obtained by solvent removal, was heated with 1 mg ($2x10^{-7}$ mol) of NaOH in 1ml of acetone. After 6 hours of reflux, 15 mg ($1.1x\ 10^{-4}$ mol) of 3-bromo-1-propanol was added and 10 ml of dichloromethane was added to extract the product.

The solvent was then removed, 5 mg ($2x10^{-5}$ mol) of $Cr(CO)_6$ and 1 ml of dibutyl ether were added. The mixture was heated at reflux for 4 hours. After filtration and solvent removal, the crude product was chromatographed on a silica gel plate with eluent THF/pentane : 3/2. After development in the solvent system the chromatogram was cut into 0.5 cm strips, which were then placed in vials with 0.5 ml of methanol and 5 ml of O.C.S. (Amersham, England) and the radioactivity was counted. Radioactivity was found everywhere on the chromatogram .

2- Preparation of 3-O-(3-hydroxypropyl) estrone chromium tricarbonyl α and β (complexes 4 and 5)

Estrone 2 (0.81g, $3x10^{-3}$ mol) was heated with NaOH (0.24 g, $6\ x10^{-3}$ mol) in 50 ml of acetone for 2 hours. Then 1-bromo-3-propanol (0.84 g, $6\ x10^{-3}$ mol) was added and the reflux was maintained during two days. After filtration and solvent removal, the crude product obtained was stirred with dichloromethane in order to extract the main products. 0.56 g of colorless oil was then obtained after filtration and solvent removal. $Cr(CO)_6$ (1.32 g, 6 $x10^{-3}$ mol) and dibutyl ether (130 ml) were added to the oil and the mixture was heated at reflux (140°C) for 4.5 hours. The cooled reaction mixture was then filtered and the solvent was removed, then the crude product obtained was chromatographed on silica gel plates using ether/heptane: 10/1 as eluent.

The first fraction was identified as the α-complex 4 (170 mg, yield 12% , yellow solid). Then recrystallization from ether/pentane gave a pure sample, mp 97°. ^{1}H- NMR (acetone-d_6) : H_1 : 6.12 d ; H_2 : 5.38 ; H_4 : 5.38 ; Me-13 : 0.90 s ; HO- $\underline{C}H_2$-CH_2-$\underline{C}H_2$-O- : 3.68, 4.08 t. Mass spectrum (70 eV) m/e: 464 (M^+), 380 (M^+-3CO), 328 (M^+- $Cr(CO)_3$). Anal. Calcd. for $C_{24}H_{28}O_6Cr$: C, 62.06 ; H, 6.07 ; Cr, 11.19. Found : C, 61.66 ; H, 6.29 ; Cr, 11.08.

The second fraction was identified as the β-complex **5**, 170 mg, yield 12%, yellow solid. The recrystallization from ether/pentane give a pure sample, mp 94°C, ^{1}H-NMR (acetone d_6) : H_1 : 5.97d ; H_2 : 5.26 dd ; H_4 : 5.39 d, Me-13 : 0.92 s ; HO-$\underline{C}H_2$-CH_2-$\underline{C}H_2$-O- : 3.69 m , 4.08t. Mass spectrum m/e: 464 (M^+), 380 (M^+- 3CO), 328 (M^+- $Cr(CO)_3$). Anal. Calcd for $C_{24}H_{28}O_6Cr$: C, 62.06 ; H, 6.07 . Found : C, 61.20 ; H, 6.67.

3- Preparation of 3-O-(3-Hydroxypropyl)estradiol chromium tricarbonyl : [^{1}H]-1

2 ml of isopropanol were added to the mixture of the α-complex **4** (24 mg, $5x10^{-5}$ mol), $NaBH_4$ (1.9 mg, 0.05 mmol) and NaOH (2mg, $5x10^{-5}$ mol). The mixture was heated at 50°C during 2min to dissolve the complex **4** and then stirred at room temperature during 4 hours. The mixture was poured into water and the compound was extracted with ether. After solvent removal, the residue was purified by TLC, eluent : ether, 13 mg of compound [^{1}H]-**1** were obtained (yield 58%). ^{1}H NMR (acetone d_6) : H_1 : 6.10 ; H_2 : 5.41dd ; H_4 : 5.35d ; $H_{6\alpha,\beta}$: 2.97m , 2.78m ; H_{17} : 6.77t ; Me-13 : 0.77s ; HO-$\underline{C}H_2$-$\underline{C}H_2$-$\underline{C}H_2$-O- : 4.02m, 1.91q , 3.67t ; OH : 3.67 and 4.02.

4- Preparation of [17α-D]-3-O-(3-hydroxypropyl)-estradiol chromium tricarbonyl: D-1

Following the same method used to prepare the complex [^{1}H]-**1**, α-complex **4** (11.6 mg, $2.5x10^{-5}$ mol) and $NaBD_4$ 98%D (1mg, $2.5x10^{-5}$mol) were stirred at room temperature during 4 hours. After hydrolysis, extraction with ether and solvent removal, the residue was purified by preparative TLC, eluent : ether. 7 mg of compound D-**1** were obtained, yield 58%. It was identified by mass spectrum m/e : 467 (M^+), 383 (M^+-3CO), 331(M^+-$Cr(CO)_3$).

5-Preparation of [17α-^{3}H]-3-O-(3-hydroxypropyl)-estradiol chromium tricarbonyl: [^{3}H]-1.

α- Complex **4** (9.4 mg, $2.5x10^{-5}$ mol) was dissolved in 1 ml of isopropanol and 0.2 ml of NaOH N/10 was added. This solution was then injected into the tube that contained $4x10^{-6}$ mol of Na[^{3}H]$_4$ (100 mCi, S.A. 25 Ci/ mmol) The mixture was stirred at 50°C during 24 hours. 2 ml of water were then added to the cooled solution and the product was extracted with ether. After solvent removal, purification by TLC (5735 Merck plate, eluent ether), 7 mg of yellow solid were obtained, yield 94%. The recrystallization from ether/pentane gave a radioactive pure sample , mp 130°C, . Mass spectrum m/e: 468 and 466 (M^+), 384 and 382 (M^+-3CO), 332 and 330 (M^+ -$Cr(CO)_3$).IR (KBr pellet): . The spectra of [^{1}H]-**1** and [^{3}H]-**1** were identical except for two very weak peaks at 1600 cm-1. Specific Activity (S.A.) 4.1 Ci/mmol +0.2 .

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