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Synthesis of [$^3H_2$]-21-Diazoprogesterone as a Potent Photoaffinity Labelling Reagent for the Mineralocorticoid Receptor

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Summary

[$^3H_2$]-21-Diazoprogesterone ([^{3}H]21DP), a potent photoaffinity labelling reagent, has been synthesized in four steps from progesterone. Tritium has been introduced by homogeneous tritiation with Wilkinson's catalyst.

Key Words : 21-diazoprogesterone, mineralocorticoid receptor, photoaffinity labelling, homogeneous tritiation.

Introduction

The first step in the action of aldosterone is its binding to the mineralocorticoid receptor (MR) that belongs to the superfamily of nuclear receptors (1,2). Despite recent advances in the knowledge of the MR structure (3-11), little is known about the precise mechanism by which hormones and antihormones interact with the receptor. Previous works demonstrate the crucial role of the C-18 and C-21 substituents in the ligand-MR interaction (12,13). In particular, the introduction of a polar group at the C-21 position of progesterone such as hydroxyl in glucocorticoids or a diazonium group in 21-diazoprogesterone (21DP) changes the antimineralocorticoid activity of progesterone to a full agonist activity. Radiolabelled photoaffinity labelling reagents are of particular interest to map the ligand binding domain of the receptor. [^{3}H]-Dexamethasone 21-mesylate has proved to be very efficient for labelling the glucocorticoid receptor (14). However this ligand displays no affinity for the MR. This led us to synthesize 21-diazoprogesterone (21DP) which indeed

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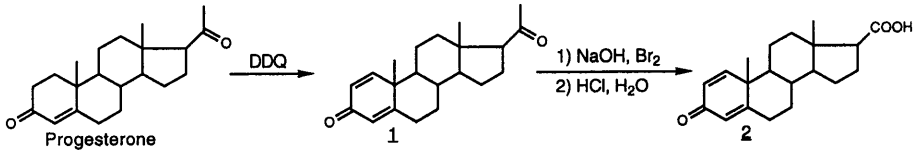
revealed a high affinity for MR and acted as a full agonist (13). Furthermore it bound irreversibly to the receptor, after UV-irradiation, with a 30-40% efficiency (15). Therefore we developed the synthesis of [^{3}H]21DP by tritiation of the 1,2 unsaturated percursor.

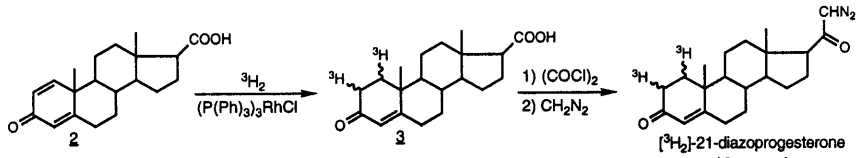
Results and discussion.

The best method to label steroids with tritium in the A ring consists of the catalytic saturation with tritium of a 1,2 unsaturated precursor. The synthesis of [1,2,15-^{3}H]-3β-hydroxyandrost-5-en-17-one by catalytic tritiation (in the presence of the wilkinson catalyst) of a 1,2-5,6-15,16 unsaturated precursor may be quoted (16) as an example of this strategy.



Scheme 1 : Synthesis of 3-oxoandrost-1,4-diene-17β-carboxylic acid 2

As presented in Scheme 1, the 1,2 double bond was generated by treatment of progesterone with 2,3-dichloro-5,6-dicyanobenzoquinone (DDQ) in refluxing anhydrous benzene for 18h (17). The 17-acetyl group was converted into the corresponding 17-carboxylic acid with sodium hypobromite in a t-butanol - water solution of sodium hydroxide (17).



Scheme 2 : Synthesis of [^{3}H$_2$]-21-diazoprogesterone ([^{3}H]21DP)

Tritiation was conducted with one equivalent of Wilkinson catalyst for 3h at room temperature under one atmosphere of tritium gas (Scheme 2). Due to the low solubility of the carboxylic acid in benzene, the reaction was performed in a 3/1 mixture of dry benzene and absolute ethanol. The resulting tritiated carboxylic acid was treated with a dilute sodium hydroxide solution and lyophilized. The carboxylate was converted into the acid chloride by action of one equivalent of oxalyl chloride in dry benzene for 3h at 0°C. After concentration and addition of an excess of an ethereal solution of diazomethane, the mixture was kept for 3h at 0°C.

[^{3}H]21DP was purified by HPLC. The specific activity, 18.7 Ci/mmol, was determined by liquid scintillation counting and UV spectrometry. ^{3}H NMR demonstrated that tritium was incorporated at the C-1 and C-2 positions as mono and bis tritiated products.

Tritiated products	1α	1β	2α	2β	1α - 2α	1β - 2β	1α - 2β
Proportion (%) #	12.9	2.2	3.0	0.4	53.3	8.5	19.7

Precision : 0.2 %

As shown in the table, the main product corresponds to a cis addition on the α face, but the 1α, 2β product represents ~ 20%.

Experimental.

Melting points (mp) were determined on a Kofler apparatus. IR spectra was recorded in $CHCl_3$ on a Perkin Elmer 1420 spectrometer. ^1H and ^{13}C NMR spectra were recorded in $CDCl_3$ on a Brucker AC 400 spectrometer. Chemical shifts are expressed in ppm relative to TMS and coupling constants in Hz.

Pregn-1,4-diene-3,20-dione (Δ1-progesterone) 1. A stirred solution of progesterone (500 mg, 1.59 mmol) in 35 ml of dry benzene was heated to reflux under an argon atmosphere with a Dean-Stark apparatus. The first 5 ml of distillate were discarded. 340 mg (1.89 mmol) of 2,3-dichloro-5,6-dicyanobenzoquinone (DDQ) were added and another 5 ml of distillate was discarded. The wine-red solution was refluxed under argon for 18 h and then filtered on celite and concentrated. The reaction mixture was diluted in ether, washed with 10% NaOH and dried ($MgSO_4$). The crude product was purified by flash chromatography (cyclohexane/AcOEt 3:1) to give 385 mg of pure 1 (77%).

mp: 141-144°C.
IR : 1690, 1650, 1620, 1600 cm^{-1}.
^1H NMR δ: 0.72 (s, 3H) and 1.25 (s, 3H) H_{18} and H_{19}, 2.14 (s, 3H, H_{21}), 6.09 (m, 1H, H_4), 6.26 (dd, 1H, J = 1.9 (H_2-H_4) and 9.8 Hz, H_2), 7.07 (d, 1H, J = 9.8 Hz, H_1).
^{13}C NMR δ: 13.40, 18.67, 22.80, 22.81, 24.53, 31.43, 32.75, 33.49, 35.46, 38.50, 43.46, 44.05, 52.18, 55.58, 63.36, 123.94, 127.60, 155.56, 168.82, 186.28, 209.11.

3-Oxoandrost-1,4-diene-17β-carboxylic acid 2. To a vigorously stirred solution of 585 mg NaOH in 3 ml H_2O, maintained at 0 °C were added dropwise first 225 µl (4.4 mmol) of bromine and then 600 µl of t-butanol. The solution was maintained at 0 °C for 10min and then 300 mg (0.96 mmol) of 1 dissolved in 5.4 ml of tertiobutanol were added over one hour. The mixture was further stirred for 6h at a temperature that did not exceed 7°C and then treated with sodium thiosulfite (50 mg) for 15 min. The mixture was concentrated and the aqueous layer was acidified with concentrated HCl, extracted with ether (3 x 90 ml) and AcOEt (90 ml). The organic layers were pooled, dried over $MgSO_4$ and concentrated. The crude product was purified by two crystallizations in ethanol to give 205 mg of pure 2 (68%).

mp > 260°C.

^{1}H NMR δ: 0.83 (s, 3H) and 1.26 (s, 3H) H_{18} and H_{19}, 6.11 (s, 1H, H_4), 6.27 (dd, 1H, J = 1.8 Hz (H_2-H_4) and 10.1 Hz, H_2), 7.09 (d, 1H, J = 10.1 Hz, H_1).

^{13}C NMR δ: 18.78, 19.10, 23.07, 23.75, 24.96, 33.17, 33.89, 36.04, 38.14, 43.91, 44.54, 52.64, 55.11, 55.37, 124.34, 127.96, 156.12, 169.30, 178.44, 186.76.

[1,2-$^3H_2$]-3-Oxoandrost-4-ene-17β-carboxylic acid 3. 5 Ml of a 3:1 mixture of anhydrous benzene and absolute ethanol were added to 18 mg of 2 (57 µmol) and 48 mg of Wilkinson's catalyst and then vigorously stirred. After complete dissolution, the solution was placed under a tritium atmosphere (60 Ci) for 3h at room temperature. The acid 3 was extracted with NaOH (1N). Then the aqueous layer was washed with CH_2Cl_2, dried over $MgSO_4$ and concentrated. Labile tritium atoms were removed by two successive evaporations with 10 ml of ethanol and the crude product was stored in absolute ethanol at -20°C.

Analytical HPLC profile (column Zorbax C8, 4.6 x 250 mm, retention time : 14min09s, elution : methanol / H_2O / TFA 60 : 40 : 0.1, 1 ml / min) revealed the presence of a major peak (88%) that comigrates with the reference.

[1,2-$^3H_2$]-21-Diazoprogesterone ([^{3}H]21DP). 490 mCi of 3 were solubilized in 0.1N NaOH and lyophilized. The sodium salt was dissolved in 250 µl of anhydrous benzene under an argon atmosphere and 30 µl of oxalyl chloride were added dropwise with vigorous stirring. After 3h the reaction mixture was concentrated at a temperature that did not exceed 10°C, then the residue was placed, in the dark, under argon at 4°C and treated with 200 µl of an ethereal solution of diazomethane. After 3h of reaction the mixture was concentrated to eliminate the excess of diazomethane and immediately purified using HPLC (column Zorbax ODS 9.8x250 mm. Eluent : acetonitrile / H_2O, 60 : 40, 1.5 ml/min) to yield pure [^{3}H]21DP (2 mCi). Radiochemical purity was checked by HPLC (98.8%, column Zorbax C8, 4.6 x 250 mm, elution : acetonitrile / H_2O, 60 : 40, 1 ml / min, retention time : 10min12s) and by thin layer chromatography (98.1%, silicagel 60 F254, eluent : cyclohexane / ethyl acetate 50 : 50, Rf = 0.46). A specific activity of 18.7 Ci / mmol was determined by liquid scintillation counting and UV spectrometry (ethanol, 242nm, ε = 8800). The location of the tritium atoms was confirmed by ^{3}H NMR (320 MHz, solvent : $CDCl_3$) and the distribution was determined by COSY ^{3}H and J-Resolv. : [1α-^{3}H]21DP : 12.9%; [1β-^{3}H]21DP : 2.2%; [2α-^{3}H]21DP : 3.0%; [2β-^{3}H]21DP : 0.4%; [1α,2α-$^3H_2$]21DP : 53.3%, J = 5.0 Hz; [1β,2β-$^3H_2$]21DP : 8.5%, J = 5.8 Hz; [1α,2β-$^3H_2$]21DP : 8.5%, J = 15.7 Hz.

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