SYNTHESIS OF TRITIUM LABELED 7α-METHOXYCARBONYL-15ß,16ß-METHYLENE-3-OXO-17α-PREGN-4-ENE-21,17-CARBOLACTONE, [^{3}H]SH-D515, A HIGHLY SELECTIVE TRACER FOR THE MINERALOCORTICOID RECEPTOR

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SUMMARY

The synthesis of 7α-methoxycarbonyl-15ß,16ß-methylene-3-oxo-17α-pregn-4-ene-21,17-carbolactone (**5**, SH-D515, ZK 91587), a potent aldosterone antagonist exhibiting a strong and very selective binding to the mineralocorticoid receptor and the tritiated analog **6** is reported.

KEY WORDS: Tritium, SH-D515, aldosterone antagonist, mineralocorticoid receptor.

INTRODUCTION

The discovery and development of aldosterone antagonists with higher anti-mineralocorticoidal and reduced endocrinological properties has been the topic of intensive research efforts since the standard drug, spironolactone, was introduced into therapy some twenty years ago. In the course of our compound finding program, the antimineralocorticoid activity of some 7α-alkoxycarbonyl-15ß,16ß-methylene-spirolactones has been investigated. The compound which exhibited the highest aldosterone antagonistic activity in animals after oral treatment, SH-D-515, showed a very strong and selective affinity to the mineralocorticoid receptor. Therefore, the synthesis of a tritiated analog of SH-D515 was undertaken to investigate the potential use of this compound as a tracer for receptor binding studies.

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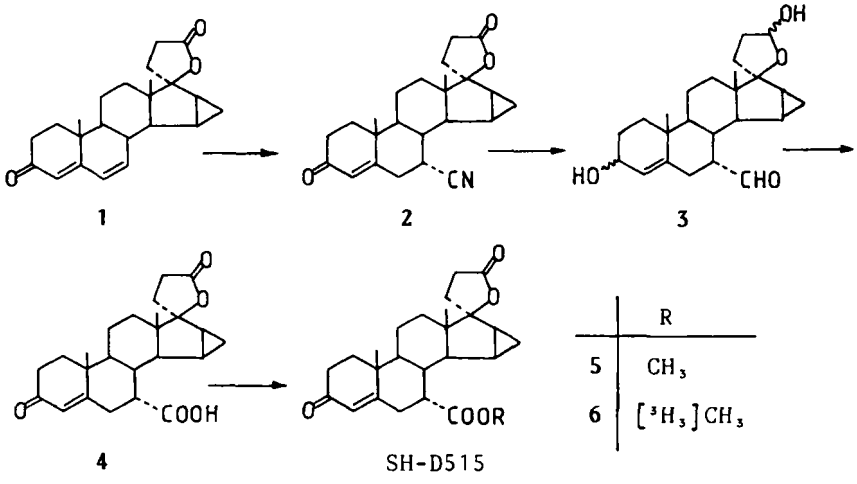
RESULTS AND DISCUSSION

It was decided to label SH-D515 in the 7α-methoxycarbonyl moiety. By doing so, it was possible to introduce the tritium in the last step of the synthesis. The five step synthesis of SH-D515 (**5**) and of the [^{3}H]-labeled analog **6** is depicted in the following scheme.



1 **2** **3**

4 SH-D515

	R
5	CH_3
6	$[^3H_3]CH_3$

7α-Alkoxycarbonyl steroids have been prepared previously (1-3). The reported methods give only low yields under drastic conditions. We therefore searched for a mild and efficient synthesis of these compounds. As starting material for our efforts we chose the double unsaturated ketone **1** (4). By reacting compound **1** with diethylaluminium cyanide (5) in tetrahydrofurane a cyano group could be introduced stereoselectively at the 7α-position of the steroid framework in 64% yield. The reduction of the cyano ketone **2** with diisobutylaluminium hydride in toluene-methylene chloride led directly to the fully reduced compound **3**. This 7α-carbaldehyde derivative was obtained as a mixture of diastereomers and used without further purification. Jones oxidation of **3** yielded the carboxylic acid **4**. The synthesis of the methyl ester **5** was accomplished in high yield by treating the carboxylic acid **4** with methyl iodide and silver oxide in dimethylformamide. The [^{3}H]-labeled title compound **6** was obtained by reacting the acid **4** with [$^3H_3$]-methyl iodide (6). The specific activity afforded 2.8 x 10^{12} Bq/mmol.

For quality and identity control the [^{3}H]-labeled SH-D515 was compared with cold material. In HPLC, HPTLC and inverse dilution analysis from ethanol the radioactive labeled SH-D515 proved to be identical with cold material. The detected radioactive impurities were <1%.

The first biochemical studies that have been performed with [^{3}H]SH-D515 showed, that contrary to [^{3}H]aldosterone, this new radioligand is stable under receptor assay conditions. The relative binding affinity of [^{3}H]SH-D515 for rat kidney mineralocorticoid receptors is four times higher compared with aldosterone, but less than one percent of the dexamethasone binding affinity to the rat liver glucocorticoid receptor (7). Therefore, we consider [^{3}H]SH-D515 to be a new specific radioligand for determination of mineralocorticoid receptors.

EXPERIMENTAL

All melting points were determined on a Büchi 510 melting point apparatus and are uncorrected. NMR spectra were taken in $CDCl_3$ on a Varian 270 MHZ spectrometer using tetramethylsilane as an internal standard. UV spectra were obtained in methanol on a Cary 14 UV spectrophotometer. Infrared spectra were obtained in KBr tablets on a Perkin-Elmer Model 621 and 580 B IR spectrophotometer. Optical rotations are specific rotations taken in chloroform (c = 0.5%). HPLC and radioactivity measurements were performed with a Perkin-Elmer liquid chromatograph, series 3 B, combined with a Berthold LB 503 and a Kratos 773 UV spectrometer.

7α-Cyano-15ß,16ß-methylene-3-oxo-17α-pregn-4-ene-21,17-carbolactone (2)

A solution of 31.8 g of 15ß,16ß-methylene-3-oxo-17α-pregna-4,6-diene-21,17-carbolactone (**1**) in 600 ml of tetrahydrofurane was treated with 180 ml of a 1.8 N solution of diethylaluminium cyanide in toluene at room temperature. After 3 hours, the reaction mixture was poured into a solution of 28 g of potassium sodium tartrate in 420 ml of ice water. The reaction product was extracted with ethyl acetate, the solution was dried and evaporated in vacuo. The crude mixture was dissolved in 200 ml of methanol, 2.59 g of potassium carbonate were added and the mixture stirred for 90 min. The solution was poured in water and extracted

with methylene chloride. The crude material was purified by column chromatography on silica gel yielding 22 g (64%) of 7α-cyano-15ß,16ß-methylene-3-oxo-17α-pregn-4-ene-21,17-carbolactone (**2**). m.p. 241 °C, $[\alpha]_D$ = +68°, UV: ε_{235} = 14400. IR (KBr): 2240, 1765, 1675, 1625 cm^{-1}. NMR ($CDCl_3$): δ = 1.05 (s, 18-CH_3), 1.21 (s, 19-CH_3), 3.27 (m, H-7), 5.83 (s, H-4) ppm.

3,5'-Dihydroxy-15ß,16ß-methylene-4-androstene[(17ß-1')-spiro-2']-perhydrofurane-7α-carbaldehyde (**3**)

6.6 g of **2** were dissolved in a mixture of 300 ml of toluene and 200 ml of dry methylene chloride and treated at -40 °C with 71 ml of a 20% solution of diisobutylaluminium hydride in toluene. The reaction mixture was stirred at -40 °C for 3 hours, then treated with 5 ml of amyl alcohol, poured into an ice cold solution of potassium sodium tartrate in water, extracted with methylene chloride and evaporated in vacuo yielding 6.5 g (97 %) of 3,5'-dihydroxy-15ß,16ß-methylene-4-androstene[(17ß-1')-spiro-2']perhydrofurane-7α-carbaldehyde (**3**).

7α-Carboxy-15ß,16ß-methylene-3-oxo-17α-pregn-4-ene-21,17-carbolactone (**4**)

A solution of 18.8 g of **3** in 750 ml of acetone was treated at -15 °C with 58.9 ml of a 8 N Jones solution. After 30 min the mixture was quenched with 10 ml of methanol, diluted with ethyl acetate and extracted with sodium hydroxide solution. The aqueous phase was washed with ethyl acetate, acidified with sulfuric acid and extracted with ethyl acetate yielding 10.1 g (52 %) of 7α-carboxy-15ß,16ß-methylene-3-oxo-17α-pregn-4-ene-21,17-carbolactone (**4**).
m.p. 263 °C, $[\alpha]_D$ = +25°, UV: ε_{242} = 15200.

7α-Methoxycarbonyl-15ß,16ß-methylene-3-oxo-17α-pregn-4-ene-21,17-carbolactone (**5**, SH-D515)

A solution of 6.6 g of **4** in 100 ml of dimethylformamide was treated with 8 g of silver oxide and 30 ml of methyl iodide and stirred for 90 min at room temperature. The reaction mixture was filtered and the excess methyl iodide was evaporated in vacuo. The dimethylformamide solution was poured into water and the

precipitate was filtered off. The crude product was isolated and purified by column chromatography on silica gel with a methylene chloride-acetone mixture followed by crystallization from acetone-diisopropyl ether yielding 3.63 g (53 %) of 7α-methoxycarbonyl-15ß,16ß-methylene-3-oxo-17α-pregn-4-ene-21,17-carbolactone (**5**). m.p. 266 °C, $[\alpha]_D$= +39.5°, UV: ε_{240}= 16600. NMR ($CDCl_3$): δ = 1.00 (18-CH_3), 1.22 (19-CH_3), 3.65 (s, 3, CH_3OCO), 5.7 (s, 1, H-4) ppm.

7α-[$^3H_3$]Methoxycarbonyl-15ß,16ß-methylene-3-oxo-17α-pregn-4-ene-21,17-carbolactone (**6**, [^{3}H]SH-D515) (8)

To a solution of 5 mg of **4** in 100 µl of dimethylformamide 5 mg of silver oxide were added. 1 mg of [$^3H_3$]methyl iodide (ca. 2.96 - 3.33 x 10^{12} Bq/mmol) was transferred to the solution in high vacuo. The mixture was stirred for 2 hours at room temperature, treated with saturated sodium chloride solution and extracted with methylene chloride. After evaporation of the solvent, the crude material was purified by semipreparative HPLC (RP_{18}; 3 µm; 240 nm; MeOH/H_2O, 65:35) to give 7α-[$^3H_3$]methoxycarbonyl-15ß,16ß-methylene-3-oxo-17α-pregn-4-ene-21,17-carbolactone (**6**), radiochemical yield 92%, specific activity 2.82 x 10^{12} Bq/mmol. For quality and identity control, HPLC (conditions above) and HPTLC were performed. HPTLC: (chloroform/acetone, 9:1; cyclohexane/ethyl acetate, 9:6). Inverse dilution analysis was done from ethanol. The radioactive impurities were shown to be < 1%.

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8. Commercially available at E.I. Du Pont de Nemours & Company Inc. 549 Albany Street, Boston, Massachusetts 02118, U.S.A. Licenced by Schering AG, Germany, under $[^3H]$SH-D515.