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SYNTHESIS OF [6β,7β-^{2}H]- AND [6β,7β-^{3}H]-21-HYDROXY-19-NORPREGN-4-ENE-3,20 DIONE

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SUMMARY

The (6β,7β-^{2}H) and (6β,7β-^{3}H) labelled 19-nordeoxycorticosterone 6 was prepared by selective catalytic hydrogenation of 21-hydroxy-19-norpregna-4,6-diene-3,20-dione (5) which in turn was synthesized from 19,21-dihydroxypregna-4,6-diene-3,20-dione (1).

Key Words: 6-Dehydro-19-hydroxydeoxycorticosterone, 6-Dehydro-19-nordeoxycorticosterone, [6β,7β-^{2}H]- and [6β,7β-^{3}H]-19-nordeoxycorticosterone.

INTRODUCTION

In connection with an ongoing biochemical study, we required ^{2}H- and ^{3}H-labelled 19-nordeoxycorticosterone (6a and 6b). We envisioned that these compounds could easily be prepared by selective reduction of the 6,7 double bond of a 4,6-dien-3-one system such as 5 with $^{2}H_{2}$ or $^{3}H_{2}$. This paper describes in detail the synthesis of the precursor 5 and its conversion to the title compounds 6.

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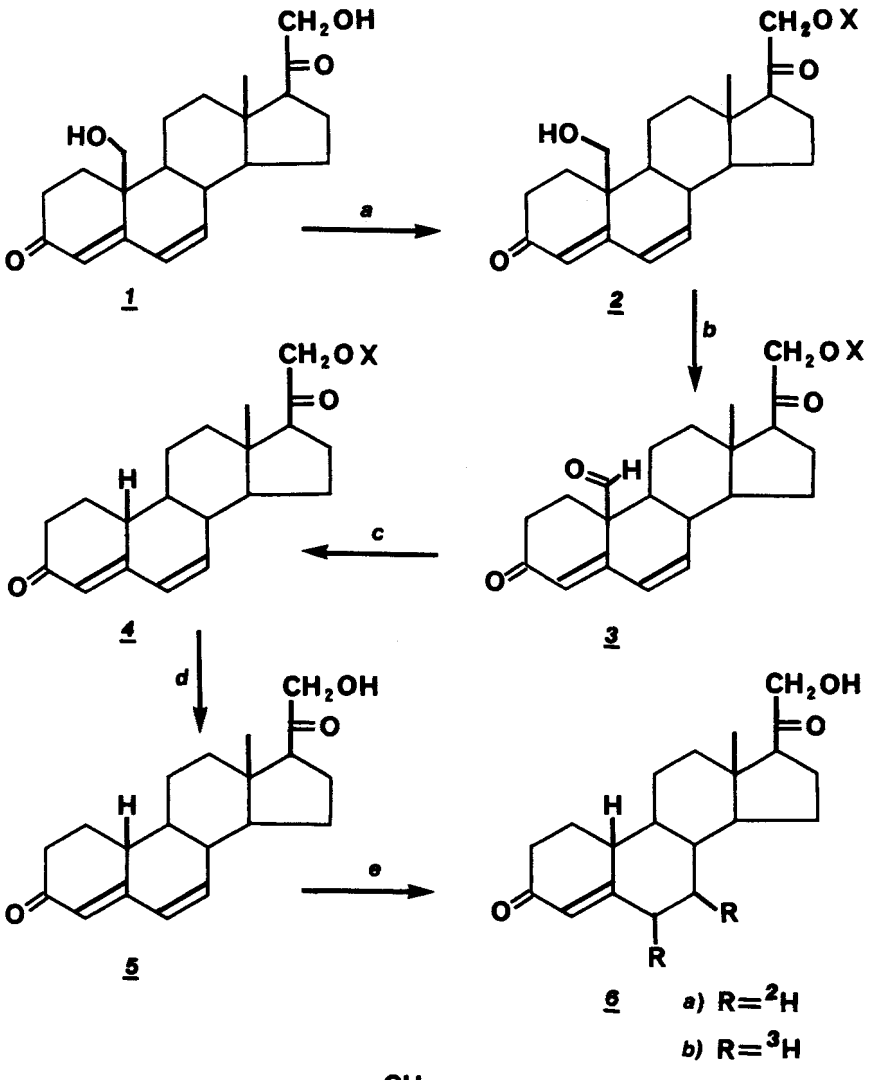

a) t-Butyldimethylsilyl Chloride/Imidazole/DMF. b) Pyridinium chlorochromate/CH_2Cl_2.
c) Aq. MeOH/KOH, RT, Argon. d) Aq. MeOH/HCl, RT. e) $(Ph_3P)_3RhCl$/Dioxane/ ^2H or ^3H.

DISCUSSION

Treatment of the 19,21-diol 1 with about 1.2 equivalent of t-butyldimethylsilyl chloride and imidazole in DMF (1) gave almost exclusively the 21-silyl derivative 2 which, on oxidation with pyridinium chlorochromate in methylene chloride (2) at room temp. for 15 hrs furnished the 19-aldehyde 3. Decarbonylation of the 19-aldehyde was achieved by stirring the aldehyde 3 in aqueous methanolic potassium hydroxide (3) under an argon atmosphere for 4 hrs. This gave the 19-nor-21-silyl ether 4 which on hydrolysis with aqueous methanolic HCl furnished the desired 19-nor-4,6-diene-3,20-dione 5. The NMR data and the UV absorption at 239 nm were consistent with structure 5. It is to be noted that during the conversion of compound 3 to 5, the intermediate 4 need not be isolated. In fact, after the base treatment of 3, the reaction mixture was acidified to pH 1 and stirred for 10-12 hrs at room temperature. This one-pot reaction directly produced the desired diene 5 in good yield.

The final step of the synthesis was the selective reduction of the 6,7 double bond. Hydrogenation of 5 in ethanol over 5% Pd/C proceeded very fast; within one hour complete saturation of diene system occurred. On a microscale synthesis it would be impractical to follow the uptake of H_2 gas, especially tritium. With the use of a soluble catalyst such as tris(triphenylphosphine)-rhodium chloride (4) in benzene, the rate of reduction was slowed down such that even after 20 hrs the yield of the desired 4-en-3-one was only 30% and the remainder was starting material. However, the same reduction with 1.4 equivalent of $^2H_2$ in dioxane using tris(triphenylphosphine)rhodium chloride (5) proceeded in 18 hrs to give the [6β,7β-^{2}H]-19-nordeoxycorticosterone (6a) in 90% yield. The assignment of the 6β,7β-stereochemistry for the ^{2}H was based on the observed β-face reduction in the corresponding androgen series (5). In a similar manner, the dien 5 was hydrogenated with tritium gas (carrier free) for 3 days, and the product purified by preparative TLC to give radiochemically pure [6β,7β-^{3}H]-19-nordeoxycorticosterone (6b) (~20% yield).

EXPERIMENTAL

Melting points were determined on a Kofler melting point apparatus and are uncorrected. The UV spectra were recorded in ethanol on a Perkin-Elmer Lamda-5 spectrophotometer. Radioassays were obtained using a Packard Tri-Carb Model 574 liquid scintillation counter. Carrier-free tritium gas was purchased from NEN Products (duPont. Boston, MA). ^1H NMR spectra were recorded on a Varian EM-390 spectrometer in $CDCl_3$ or CCl_4 as noted in the Experimental Section and the chemical shifts are reported in ppm (δ) from tetramethylsilane. NMR and UV spectra refer to nonradioactive material or reference standards. The authors gratefully acknowledge a gift of 19,21-dihydroxypregna-4,6-diene-3,17-dione (1) from Dr. J. Kalvoda, CIBA-GEIGY Corp, Basel.

19,21-Dihydroxypregna-4,6-diene-3,20-dione 21-t-Butyldimethylsilyl Ether (2).

19-Hydroxy-6-dehydrodeoxycorticosterone (1) (1 gm, 3 mM) was added to a cooled (5°C) solution of t-butyldimethylsilyl chloride (0.6 gm, 4 mM) and imidazole (0.55 gm, 8 mM) in dry dimethylformamide (20 ml). The mixture was stirred at 5°C for 20 hrs and then at 25°C for 4 hrs. The solution was diluted with water (200 ml), and the product extracted with ether. The ether extract was washed with water, dried and concentrated. The residue was dissolved in ether-hexane (1:2) and filtered through a 10 x 2.5 cm column of silica gel and washed with the same solvents. The combined filtrate, upon concentration, furnished 21-silyl ether as white fluffy material (1.13 gm, mp 79-82°C). NMR ($CDCl_3$) δ 0.07 (6H, S, $Si(CH_3)_2$), 0.73 (3H, S, 18-H_3), 0.91 (9H, S, $SiC(CH_3)_3$) 3.80 (2H, AB quartet, J_{AB} 11 Hz, $_{A-B}$ = 7.6 Hz (19-H_2), 4.01 (2H, S, 21-H_2), 5.56 (1H, S, 4-H) 6.0 (2H, S, 6 and 7-H).

Anal. Calcd. for $C_{27}H_{42}O_4Si$: C, 70.74; H, 9.17. Found: C, 70.86; H, 9.51.

19-Oxo-21-hydroxypregna-4,6-diene-3,20-dione 21-t-Butyldimethylsilyl Ether (3).

To a stirred solution of the 19-hydroxy analog 2 (0.95 gm) in dry methylene dichloride (25 ml), was added in portions pyridinium chlorochromate (3 gm). The mixture was stirred at room temperature for 15 hrs. It was then diluted

with ether (100 ml) and the whole mixture was filtered through a short column of silica gel. The column was washed with ether, and the combined filtrate was concentrated in vacuo to give a gummy residue. Purification of this product on preparative TLC on silica gel plates (20 x 40 cm, 1 mm thickness; solvent: hexane-ethyl acetate 2:1) yielded the pure 19-aldehyde 3 (548 mg (mp 72-75°C). NMR (CCl_4) 0.06 (6H, s, $Si(CH_3)_2$), 0.70 (3H, s, 18-H_3), 0.94 (9H, s, $SiC(CH_3)_3$), 4.09 (2H, s, 21-H_2), 5.77 (1H, s, 4-H), 6.10 - 6.35 (2H, multiplets 6 and 7-H), 9.63 (1H, 19-CHO); UV (ethanol) 283 nm (log ε 4.3).

Anal. Calcd. for $C_{27}H_{40}O_4Si$: C, 71.05; H, 8.77. Found: C, 70.97; H, 9.33.

21-Hydroxy-19-norpregna-4,6-diene-3,20-dione (5).

A solution of the 19-aldehyde 3 (400 mg) in aqueous methanolic potassium hydroxide solution (600 mg of KOH dissolved in 5 ml of water and 15 ml of methanol) was stirred at room temperature under argon atmosphere for 4 hrs. The solution was cooled (5-10°C) and acidified to pH 1 with 2N HCl. The mixture was stirred at room temperature overnight, and then extracted with ethyl acetate. The organic extract was washed with water, dried and concentrated in vacuo at 40°C to give a semisolid. Purification by preparative TLC on silica gel (1 mm, 20x20 cm, hexane-ethyl acetate 3:2) furnished the 19-nor-4,6-diene 5 (182 mg) (mp 169-71°) NMR($CDCl_3$): δ 0.74 (3H, s, 18-H_3), 4.2 (2H, s, 21-H_2), 5.79 (1H, s, 4-H), and 6.21 (2H, s, 6- and 7-H). UV (ethanol) 282 nm (log ε 4.2).

Anal. Calcd. for $C_{20}H_{26}O_3$: C, 76.43; H, 8.28. Found: C, 76.06; H, 8.36.

Hydrogenation of 21-hydroxy-19-norpregna-4,6-diene-3,20-dione (5).

Several conditions employing different catalysts in different solvent systems were tried: Three typical experimental methods are described below.

Method A: A solution of the 21-hydroxy-4,6-diene-3,20-dione 5 (25 mg) in absolute ethanol (5 ml) was hydrogenated with $^1H_2$ gas over 5% Pd/C (10 mg). The progress of the hydrogenation was monitored by the UV absorption at 283 and 239. After about 20 min the UV spectrum showed absorption at 239 nm

for a 4-en-3-one, and none at 283 nm (4,6-diene-3-one). Continuation of the reaction for a total of one hr gave a product which no longer showed absorption at 239 nm, indicating complete saturation of the double bonds. These results indicate that this method might be useful for large scale preparations whereby one can measure and stop the reaction after an uptake of one molar equivalent of hydrogen. However, for microscale synthesis, this method was not suitable.

Method B: A solution of the 4,6-diene-3,20-dione 5 (20 mg), tris (triphenylphosphine) rhodium chloride (20 mg) in dry benzene (4 ml) was hydrogenated with $^{1}H_2$ gas for 16 hrs. The mixture was filtered through a short column of silica gel and the column washed with ether. The combined filtrate was concentrated to give a semisolid (18 mg). The UV and NMR analysis of this product indicated a mixture of the expected 4-ene-3,20-dione 6 and the starting 4,6-diene-3,20-dione 5 in a ratio of 1:2.

Method C: A solution of the 4,6-diene 5 (16 mg, 0.05 mM) and tris (triphenylphosphine) rhodium chloride (6 mg) in dry dioxane (1 ml) was placed in a 3 ml vial with a tiny magnetic bar and sealed with a septum. Through the two syringe needles injected through the septum, the system was alternatively evacuated and filled with $^{2}H_2$ gas. After the final evacuation, the system was filled with deuterium gas and then the needles removed. The solution was stirred for 18 hrs. At this point 20 µL of the solution were withdrawn and analyzed for the absorption at 239 and 283 nm in the UV spectrum which indicated that the expected 4-ene-3,20-dione had been formed in 95% yield, and less than 5% of the starting material was left. The excess deuterium gas was removed by flushing with nitrogen, and the solvent was blown off under nitrogen. The residue was purified by preparative TLC on silica gel (0.5 mm, 20x20 cm, hexane-ethyl acetate 5:3; developed thrice). The major band was scraped off and eluted with 5% methanol in chloroform. Removal of the solvent under reduced pressure furnished (6β,7β-^{2}H)-19-nordeoxycorticosterone (6a) (11 mg). mp 129-31°C. NMR ($CDCl_3$) δ 0.71 (3H, s, 18-H_3), 4.16 (2H, s, 21-H_2) and 5.8 (1H, bs, 4-H). UV (ethanol) 239 nm (log ε 4.2). The product was identical with that of an authentic sample (6,7).

[6,7-^{3}H]-21-Hydroxy-19-norpregn-4-ene-3,20-dione (6b)

A solution of the 4,6-diene-3,20-dione 5 (16 mg), tris(triphenylphosphine)-rhodium chloride (6 mg) in dioxane (2 mL) was hydrogenated for 3 days with carrier-free tritium gas (3 Ci, 58 Ci/mmole). (The tritium gas purchased in a breakseal tube was transferred to the reaction flask by standard vacuum line techniques.) After flushing the system with nitrogen, the solvent was evaporated under a stream of nitrogen. Ethyl acetate (10 mL) followed by water (5 mL) was added to the residue and the mixture was stirred for 1 hr. The organic layer was separated, washed with water (5 ml X 2) and dried with Na_2SO_4. Removal of the solvent *in vacuo* gave a gummy residue (690 mCi) which, after purification by preparative TLC on silica gel (hexane-ethyl acetate (5:3)), furnished pure [6,7-^{3}H]-19-nordeoxycorticosterone (6b) (390 mCi). Dilution technique with the reference nonradioactive material, and the autoradiochromatogram showed that the product was radiochemically at least 95% pure.

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