SYNTHESIS OF ^{13}C-LABELLED MEDROXYPROGESTERONE ACETATE WITH THREE ^{13}C ISOTOPES [1]

P. Narasimha Rao* and Kalyani M. Damodaran
Department of Organic and Biological Chemistry, Southwest Foundation for Research and Education, P. O. Box 28147, San Antonio, Texas 78284

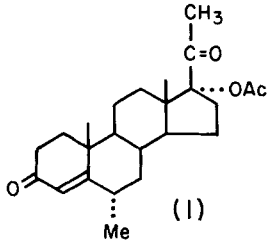
SUMMARY

17α-Hydroxyprogesterone (2) was converted to an enol-lactone 8 in which the 20-oxo group was selectively protected as an ethylene acetal. The enol-lactone 8 was subjected to Claisen condensation with phenyl acetate-1,2-$^{13}C_2$ in the presence of sodium hydride, which on subsequent treatment with a mixture of acetic acid and hydrochloric acid followed by acetylation gave 17α-acetoxyprogesterone-3,4-$^{13}C_2$ (9). The enol-ether 10 obtained from 9 was treated with tetrabromomethane-^{13}C, in pyridine-dioxane solution, to give the 6-dibromomethylene derivative 11, which was then hydrogenated to yield finally 17α-acetoxy-6α-methyl-^{13}C-pregn-4-ene-3,20-dione-3,4-$^{13}C_2$ (12).

Key Words: Long-acting contraceptive, phenyl acetate-1,2-$^{13}C_2$, tetrabromomethane-^{13}C, preparative high pressure liquid chromatography (HPLC).

INTRODUCTION

Medroxyprogesterone acetate (MPA) (1) has been in clinical use since 1962 as a contraceptive agent and is used in over 70 countries around the world, involving many millions of women. Because of its long duration of action, extensive validation of its safety is required. Studies employing radioactive MPA are not desirable because residual amounts of the radioactive material will remain in



(1)

*Author to whom all correspondence should be addressed.

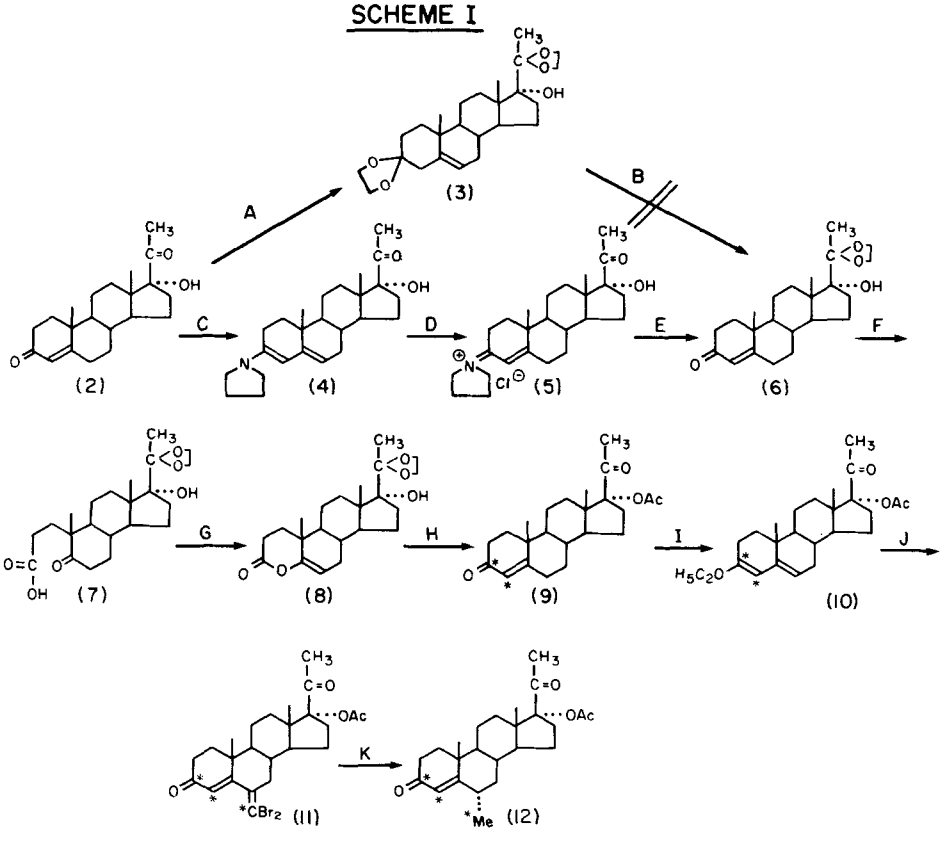
0362-4803/82/030415-10$01.00
© 1982 by John Wiley & Sons, Ltd.
Received June 30, 1981
Revised August 14, 1981

the human tissue for periods of 6 months or longer. Carbon-13 labelled MPA is considered useful for these studies and we have developed a synthetic route whereby three carbon-13 isotopes have been incorporated into MPA with an isotope enrichment amounting to greater than 90%.

DISCUSSION

The synthetic approach we adopted for incorporating three carbon-13 isotopes into medroxyprogesterone acetate consists of two stages. The first goal was to synthesize 17α-acetoxyprogesterone with two carbon-13 isotopes located at C-3 and C-4 in the A ring. After having achieved this, in the second stage we incorporated a ^{13}C-methyl group at C-6 and then isomerized the epimeric mixture of 17α-acetoxy-6ξ-methyl-^{13}C-pregn-4-ene-3,20-dione-3,4-$^{13}C_2$ to give 17α-acetoxy-6α-methyl-^{13}C-pregn-4-ene-3,20-dione-3,4-$^{13}C_2$ (medroxyprogesterone-$^{13}C_3$ acetate). For incorporating carbon isotopes at C-3 and C-4 of the steroid nucleus, Turner's classical procedure [2] is unsurpassed, and we have therefore followed this general procedure with some modifications as required in the case of 17α-hydroxyprogesterone. The initial task in this study was to convert 17α-hydroxyprogesterone (2, Scheme I) to the key enol-lactone 8 in which the 20-oxo group was selectively protected. The synthesis of 8 can be achieved starting from 17α-hydroxyprogesterone-20-cyclic acetal (6). The preparation of 6, starting from 17α-hydroxyprogesterone (2), was described in a patent [3] with few experimental details. According to this procedure the 3,20-bis acetal 3 obtained from 2 was selectively hydrolyzed with 3N perchloric acid in tetrahydrofuran to give the 20-mono acetal 6. However, when we attempted this procedure under a variety of conditions, we obtained only the 3-mono acetal, instead of the desired 20-acetal 6. We finally prepared the 20-acetal 6 by a different procedure as outlined in Scheme I. The Δ^4-3-oxo group present in 17α-hydroxyprogesterone (2) was selectively protected by reacting with pyrrolidine in methanol solution to give the enamine 4. This enamine 4 was treated with an excess of dry hydrogen chloride gas in chloroform solution to give the iminium hydrochloride 5. The iminium hydrochloride 5 was transformed to the desired 20-cyclic acetal 6 in excellent yield by reacting with ethylene glycol, triethyl orthoformate and p-toluenesulfonic acid in tetrahydrofuran solution at room temperature [4] followed by hydrolysis with dilute ammonium hydroxide. The ring-A of the ethylene acetal 6 was cleaved by a modified sodium metaperiodate-potassium permanganate oxidation [5] to give the seco-acid 7 in 60% yield. The seco-acid 7 was treated with a mixture of acetic anhydride, sodium acetate and triethylamine at 145°C for 30 minutes to give the desired enol-lactone 8 in 70% yield. We subjected the enol-lactone 8 to Claisen condensation with phenyl acetate-1,2-$^{13}C_2$ (98 atom %) in the presence of sodium hydride with some modification of Turner's procedure [2]. We observed that our enol-lactone 8 is not readily soluble in benzene, the solvent which was originally employed by Turner [2] in his studies. After some investigation we found that tetrahydrofuran can be substituted for benzene.

SCHEME I



A: Ethylene glycol, PTSA, 90°C

B: 3N $HClO_4$, THF, room temperature

C: Pyrrolidine, MeOH, Δ

D: $CHCl_3$-ether, HCl gas

E: Ethylene glycol, $HC(OEt)_3$, PTSA, THF, room temp., dil. NH_4OH

F: K_2CO_3-H_2O, CH_2Cl_2-t-BuOH, $NaIO_4$-H_2O, $KMnO_4$-H_2O, 40-45°C; 50% H_2SO_4, 0°C

G: Ac_2O-NaOAc, NEt_3, 145°C

H: NaH, THF, phenyl acetate-1,2-$^{13}C_2$, room temp., dil. HCl; HOAc-conc. HCl, 60°C; HOAc-$(CF_3CO)_2O$-PTSA, room temp.

I: $HC(OEt)_3$-EtOH-PTSA, benzene, 100°C

J: $^{13}CBr_4$, dioxane-pyridine, room temp., 100°C

K: 2% Pd-$SrCO_3$-H_2, 1 atm., methyl cellosolve, room temp.; dil. HCl

Accordingly we carried out the Claisen condensation in tetrahydrofuran at room temperature for 48 hours and the resulting condensation product was cyclized by reacting with a mixture of acetic acid and conc. hydrochloric acid (10:1) at 60°C for 45 minutes to give 17α-hydroxyprogesterone-3,4-$^{13}C_2$. The total material without additional purification was converted to its 17-acetate by treatment with a mixture of acetic acid, trifluoroacetic anhydride and p-toluenesulfonic acid at room temperature for 30 minutes. Chromatographic purification of the acetylated material gave the pure 17α-acetoxyprogesterone-3,4-$^{13}C_2$ (**9**). The proton NMR spectrum of **9** exhibited a doublet centered at 5.79 ppm with a larger coupling constant ($J_{^{13}C\text{-}H}$ = 160 Hz, 4-H) indicating the presence of carbon-13 isotope at C-4. The carbon-13 NMR spectrum of **9** exhibited a pair of doublets at 200.52, 197.93 (J = 52 Hz, ^{13}C-3) and 125.32, 122.76 (J = 52 Hz, ^{13}C-4)ppm., indicating that two carbon-13 isotopes are located adjacent to each other and confirmed one is located at C-3 and the other at C-4 position in the A-ring. Mass spectral analysis of **9** further confirmed the location of the two C-13 isotopes and indicated 96 atom % incorporation. The structure of **9** is also in accordance with the mechanism proposed by Turner [2] for the condensation of a steroid ring-A enol-lacetone with phenyl acetate. According to this mechanism, this carbonyl group of phenyl acetate will be incorporated at the 3 position and the methyl carbon will occupy the position 4 in ring A.

A ^{13}C-methyl group at C-6 was then introduced into 17α-acetoxyprogesterone-3,4-$^{13}C_2$ (**9**) by employing tetrabromomethane-^{13}C (91 atom %) by the general procedure described by Liisberg *et al.* [6]. Treatment of compound **9** with triethyl orthoformate and p-toluenesulfonic acid in refluxing benzene yielded the ethyl enol ether **10**. Reaction of the enol ether **10** with tetrabromomethane-^{13}C and pyridine in dioxane, first at room temperature for 24 hours and then at reflux for 4 hours, gave the 6-dibromomethylene derivative **11**. Hydrogenation of the 6-dibromomethylene derivative **11** over 2% palladium-strontium carbonate catalyst in methyl cellosolve at 1 atmosphere pressure and at room temperature for 10 minutes, followed by treatment with dil. hydrochloric acid for 1 hour afforded the 17α-acetoxy-6ξ-methyl-^{13}C-pregn-4-ene,3,20-dione-3,4-$^{13}C_2$ as a mixture of 6α- and 6β-methyl epimers in which the desired 6α-methyl epimer predominated. Extensive purification of this mixture of epimers by "dry column" chromatography on silica gel and preparative HPLC gave, ultimately, pure medroxyprogesterone-$^{13}C_3$ acetate. This purified material showed carbon-13 enrichment of 90 atom % based on mass spectrometry, and chemical purity of 98% based on its HPLC on two different columns and solvent systems.

EXPERIMENTAL

Most chemicals and solvents were analytical reagent grade and were used without further purification. Some reagents and solvents like pyrrolidine, acetic anhydride, triethyl orthoformate, tetrahydrofuran, pyridine, and methyl cellosolve were purified according to standard laboratory

procedures. Phenyl acetate-1,2-$^{13}C_2$, 99 atom %, and tetrabromomethane-^{13}C, 91 atom %, were purchased from KOR Isotopes, Cambridge, Massachusetts, and were used without further purification. All organic extracts were dried over anhydrous sodium sulfate, unless otherwise specified, and evaporated *in vacuo*.

Purity and identity of new compounds were established by normal spectral (IR, NMR, MS) and analytical (TLC, HPLC and chemical analysis) techniques. Melting points were determined on a Thomas-Hoover capillary melting point apparatus and were uncorrected. IR spectra were obtained in a potassium bromide disc using a Perkin-Elmer Model 467 grating spectrophotometer. U.V. spectra were measured in methanol solution using a Varian Cary 210 spectrophotometer. NMR spectra were obtained with a Varian EM-390 spectrometer for proton and a CFT-20 spectrometer for carbon-13 in deuterochloroform (unless otherwise specified) and are reported in ppm downfield from the internal standard, tetramethylsilane. Mass spectra were determined on a Finigan quadrupole mass spectrometer. "Dry column" chromatograpy was performed on Woelm silica gel in a nylon column as described by Loev and Goodman [7]. TLC analyses were done on silica gel GF (Analtech) glass plates (2.5 x 10 cm with 250 μM layer and prescored). High pressure liquid chromatography (HPLC) was carried out on Waters Associates, Inc. HPLC equipment (Model 202) with an LDC Spectromonitor III (Model 1203) employing μPorasil column (0.39 x 30 cm, Waters Associates), and Partisil column (PXS 10/25-ODS-3, 0.46 x 25 cm, Whatman). Preparative HPLC was carried out on Waters Prep LC/System 500 employing PrepPak 500/silica cartridge. Microanalyses were performed by Midwest Microlab, Ltd., Indianapolis, Indiana.

17α-Hydroxy-3-(1-pyrrolidinyl)-pregn-3,5-dien-20-one (4)

Freshly distilled pyrrolidine (11.08 g, 155.7 mmoles) was added under nitrogen atmosphere to a solution of 17α-hydroxyprogesterone (2, 50 g, 151.3 mmoles) in boiling methanol (1400 ml) and the mixture allowed to cool to room temperature (2 hr). The reaction mixture was further cooled in ice (1 hr) and the precipitated pale yellow crystals were filtered and dried *in vacuo* over potassium hydroxide pellets. Concentration of the mother liquors followed by cooling gave additional crystalline material which amounted to a total yield of 58 g.

17α-Hydroxypregn-4-en-20-one-3-(pyrrolidiniminium)chloride (5)

A solution of the enamine 4 (58 g) in dry chloroform (500 ml) was diluted with anhydrous ether (300 ml) and cooled in an ice-bath. Dry hydrogen chloride gas was bubbled into the solution for 15 min. The ice-bath was removed and more hydrogen chloride gas was passed until the crystalline precipitate which formed was redissolved giving a clear orange-colored solution. Nitrogen gas was rapidly bubbled through the reaction mixture for 1 hr to drive off excess hydrogen chloride gas. Water (50 ml) was added to the reaction mixture and the contents were shaken well. The yellow

crystalline precipitate formed was filtered and dried *in vacuo*. Traces of water were removed by azeotropic distillation of added benzene. The yield was 58 g.

17α-Hydroxypregn-4-en-3-one cyclic 20-(1,2-ethanediyl acetal) (6)

A mixture of the iminium chloride 5 (91 g), p-toluenesulfonic acid monohydrate (1.8 g), anhydrous tetrahydrofuran (45 ml), freshly distilled triethyl orthoformate (135 ml) and ethylene glycol (135 ml) was stirred at room temperature for 8 hr and transferred into a separatory funnel containing water (2000 ml) and 58% ammonium hydroxide (200 ml), and the organic product extracted with dichloromethane. The dichloromethane extract was washed with water and brine:water (1:1), and dried. The solution was filtered through a column of silica gel (1000 g) using dichloromethane as eluent. Evaporation of the solvent followed by trituration of the residue with ether gave a pale yellow crystalline solid (60 g). Recrystallization from dichloromethane-ether-petroleum ether afforded pure compound 6; mp 228-230°C (Lit. [8] mp 235-237°C); IR: ν_{max} 3560, 1658, 1610 cm^{-1}; UV: λ_{max} 241 nm (ϵ = 14,150); NMR: 0.84(s, 3H, 18-CH_3), 1.17(s, 3H, 19-CH_3), 1.38(s, 3H, 21-CH_3), 4.00(m, 4H, -OCH_2CH_2O-), 5.76(s, 1H, -CO-CH=C-)ppm; Analysis: calc'd for $C_{23}H_{34}O_4$: C, 73.76; H, 9.15; found: C, 73.90; H, 9.09.

17α-Hydroxy-4-nor-pregn-5-oxo-3,5-seco-3-oic acid cyclic 20-(1,2-ethanediyl acetal) (7)

A solution of the enone 6 (25 g, 66.75 mmoles) in dichloromethane (125 ml) was diluted with tertiary butanol (310 ml). A solution of potassium carbonate (15 g) in water (150 ml) was added and the mixture stirred vigorously. A solution (60 ml) of sodium metaperiodate (69 g) in water (500 ml) at 40°C was added to the reaction mixture followed by 0.8% potassium permanganate solution (6 ml). Periodate and permanganate solutions were alternately added so as to maintain a light permanganate color in the reaction mixture. All the periodate solution was added during a 30 min period. The reaction mixture was then warmed to 40-45°C. Additional quantity and permanganate solution was added as needed to maintain the light purple color. At the end of 2.5 hr, when TLC of the reaction mixture showed no starting material, the reaction mixture was diluted with water (2000 ml) and cooled to 0°C. The mixture was acidified with 50% sulfuric acid to pH 3-4, and the organic material extracted with ethyl acetate. The extract was washed with water, and the solvent was distilled off to give a white crystalline residue. Trituration of the residue with ether followed by recrystallization of the solid from ethyl acetate-ether afforded pure crystalline keto acid 7; mp 210-212°C. IR: ν_{max} 3450, 1740, 1705 cm^{-1}; NMR: 0.87(s, 3H, 18-CH_3), 1.11(s, 3H, 19-CH_3), 1.35(s, 3H, 21-CH_3), 3.98(m, 4H, -OCH_2CH_2O-)ppm; MS: m/e = 394 (M^+); Analysis: calc'd for $C_{22}H_{34}O_6$: C, 66.98; H, 8.69; found: C,67.19; H, 8.56.

17α-Hydroxy-4-oxa-pregn-5-en-3-one cyclic 20-(1,2-ethanediyl acetal) (8)

A mixture of keto acid 7 (20 g, 53.12 mmoles), anhydrous sodium acetate (20 g) and freshly distilled acetic anhydride (450 ml) was heated at reflux in an atmosphere of nitrogen (batch temperature, 145°C) for 20 min; freshly distilled triethyl amine (45 ml) was added to the reaction mixture and the refluxing continued for an additional 10 min. The reaction mixture was cooled, and sodium acetate filtered and the filtrate evaporated. The residue was dissolved in a mixture consisting of equal amounts of dichloromethane, ethyl acetate and toluene (250 ml). The solvent mixture was then distilled off at 90°C in order to remove the last traces of acetic anhydride present in the residue. Trituration of the residue with ether followed by recrystallization of the solid from dichloromethane-methanol containing 0.5% pyridine gave analytically pure compound 8;mp 253-255°C; IR: ν_{max} 3560, 1770, 1750, 1680 cm^{-1}; NMR: 0.82(s, 3H, 18-CH_3), 1.10(s, 3H, 19-CH_3), 1.34(s, 3H, 21-CH_3), 3.98(m, 4H, -OCH_2CH_2O-), 5.27(m, 1H, -O-C=CH-)ppm; MS: m/e = 376 (M^+); Analysis: calc'd for $C_{22}H_{32}O_5$: C, 70.19 H, 8.57; found: C, 69.93; H, 8.88.

17α-Acetoxy pregn-4-ene-3,20-dione-3,4-$^{13}C_2$ (9)

Sodium hydride-mineral oil suspension (66%, 8.986 g, 247.12 mmoles) was washed with dry petroleum ether followed by dry tetrahydrofuran. A solution of the enol-lactone acetal 8 (21.17 g, 156.23 mmoles) in tetrahydrofuran (150 ml) was added and the mixture stirred at room temperature for 1 hr in an atmosphere of nitrogen. Phenyl acetate-1,2-$^{13}C_2$ (98 atom %, 15.2 g, 138.15 mmoles) was added and the mixture stirred at room temperature for 42 hr. The reaction mixture was cooled in ice and carefully acidified with cold 1N hydrochloric acid to pH 3-4. The organic material was extracted with ethyl acetate, the extract washed with water and brine, and dried. The solvent was evaporated and the unreacted phenyl acetate and phenol were distilled off *in vacuo* at 100°C to give a dark brown foam.

The dark brown product (22.45 g) was dissolved in glacial acetic acid (500 ml), treated with conc. hydrochloric acid (50 ml) and the mixture heated at 60°C in an atmosphere of nitrogen for 45 min. The reaction mixture was poured into ice and water (2000 ml) and the product extracted with dichloromethane. The extract was washed with water, saturated sodium bicarbonate and brine:water (1:1), and dried. Evaporation of the solvent gave a dark brown residue which was dissolved in dichloromethane and filtered through a column of silica gel. The filtrate after evaporation gave a light brown crystalline product (17.3 g).

The above product (17.3 g) was combined with another batch prepared in a previous reaction (3.9 g, total 21.2 g) and reacted with a mixture of glacial acetic acid (18 ml), trifluoroacetic anhydride (17 ml), p-toluenesulfonic acid monohydrate (5.5 g) and stirred at room temperature for 30 min. The reaction product was diluted with dichloromethane (500 ml) and the solution washed with cold 5% sodium bicarbonate solution and brine:water (1:1), and dried. Evaporation of the

solvent gave a residue which was dissolved in dichloromethane, the solution was filtered through a short column of silica gel (150 g), and the filtrate evaporated. The solid residue was crystallized from a solvent mixture consisting of dichloromethane, ether, methanol and petroleum ether (10:10:1:1). The impure material from the mother liquors was chromatographed on a silica gel "dry column", using ethyl acetate:hexanes (1:1) as solvent system, and the purified product crystallized to give 17α-acetoxy pregn-4-ene-3,20-dione-3,4-$^{13}C_2$ (**9**, 8.6 g) as a white crystalline solid; mp 238-240°C; IR: ν_{max} 1730, 1715, 1685, 1615 cm^{-1}; UV: λ_{max} 241 nm (ϵ - 14,500); Proton NMR: 0.67(s, 3H, 18-CH_3), 1.20(s, 3H, 19-CH_3), 2.03(s, 3H, 21-$COCH_3$), 2.10(s, 3H, 17-$OCOCH_3$), 5.79(d, $J_{^{13}C-H}$ = 160 Hz, 1H, -^{13}CO-^{13}CH=C-)ppm; ^{13}C-NMR: 199.23(d, $J_{^{13}C-^{13}C}$ = 52 Hz, ^{13}C-3); 124.01(d, $J_{^{13}C-^{13}C}$ = 52 Hz, ^{13}C-4)ppm; MS: m/e = 374 (M^+); Analysis: calc'd for $C_{21}{}^{13}C_2H_{32}O_4$: C, 74.29; H, 8.61; found: C, 74.20; H, 8.24.

17α-Acetoxy-3-ethoxy-pregn-3,5-dien-20-one-3,4-$^{13}C_2$ (10)

17α-Acetoxy-progesterone-3,4-$^{13}C_2$ (**9**, 10.2 g, 27.23 mmoles) and p-toluenesulfonic acid (27.4 mg) were dissolved in dry benzene (137 ml). Triethyl orthoformate (4.66 ml) and absolute ethanol (4.12 ml) were added and the solution was refluxed for 3.75 hr. After cooling and addition of pyridine (3.8 ml), the solution was washed with water, dried, and the solvent evaporated to give a yellow foam (10.8 g). Recrystallization from methanol containing 0.5% pyridine gave enol ether **10** as pale yellow crystals; mp 145-148°C; IR: ν_{max} 1730, 1710 cm^{-1}; Proton NMR: 0.66(s, 3H, 18-CH_3), 0.97(s, 3H, 19-CH_3), 1.30(t, J=7.5 Hz, 3H, -CH_2CH_3), 2.03(s, 3H, 21-$COCH_3$), 2.09(s, 3H, 17-$OCOCH_3$), 3.80(q, J=7.5 Hz, 2H, -CH_2CH_3), 5.12(d, $J_{^{13}C-H}$ = 160 Hz, 1H, ^{13}C=^{13}CH-), 5.23(m, 1H, -C=CH-)ppm; ^{13}C-NMR: 154.61 (d, $J_{^{13}C-^{13}C}$ = 52 Hz, ^{13}C-3), 98.99(d, $J_{^{13}C-^{13}C}$ = 52 Hz, ^{13}C-4)ppm; MS: m/e = 402 (M^+); Analysis: calc'd for $C_{23}{}^{13}C_2H_{36}O_4$: C, 73.65 H, 9.08; found: C, 73.41; H, 9.75. The sample retains undetermined amount of solvent of crystallization.

6-Dibromomethylene-^{13}C-17α-acetoxy-pregn-4-ene-3,20-dione-3,4-$^{13}C_2$ (11)

The ethyl enol ether **10** (10.60 g, 26.34 mmoles) was dissolved in a mixture of dioxane (50 ml) and pyridine (4.4 ml, 53 mmoles). Tetrabromomethane-^{13}C (91 atom %, 20.1 g, 53 mmoles) was added, and the resulting yellow solution was let stand at room temperature for 40 hr. A crystalline precipitate consisting of an addition compound of 1 mole pyridine hydrobromide and 1 mole tetrabromomethane-^{13}C was filtered off, and the filtrate heated at 100°C for 3.75 hr. After cooling, a precipitate, mainly consisting of pyridine hydrobromide, was removed by filtration; the filtrate was diluted with ether (500 ml), washed thoroughly with water and brine, and dried. The solvent was distilled off, the residue dissolved in dichloromethane and the solution filtered through a short column of silica gel. The product was further purified by silica gel "dry column" chromatography using ethyl acetate:benzene (1:4), as solvent system and crystallized from benzene-hexanes to give

the 6-dibromomethylene derivative **11** (8.8 g). Recrystallization from benzene-hexanes afforded a white crystalline solid; mp 108-112°C; IR: ν_{max} 1730, 1715, 1640, 760 cm^{-1}; UV: λ_{max} 250 nm (ϵ = 13,080), $\lambda_{shoulder}$ 282 nm (ϵ = 7,790); Proton NMR: 0.66(s, 3H, 18-CH_3), 1.12(s, 3H, 19-CH_3), 2.02(s, 3H, 21-$COCH_3$), 2.08(s, 3H, 17-$OCOCH_3$), 5.98(d, $J_{^{13}C\text{-}H}$ = 160 Hz, 1H, -$^{13}CO^{13}CH$=C-)-ppm; ^{13}C-NMR: 198.49(d, $J_{^{13}C\text{-}^{13}C}$ = 52 Hz, ^{13}C-3); 126.91, (d of d, $J_{^{13}C\text{-}^{13}C}$ = 52 Hz, $J_{long\ range}$ $^{13}C\text{-}^{13}C$ of ^{13}C-4 and 6 dibromomethylene ^{13}C = 4 Hz); 88.55 and 88.38(d, J=3.9 Hz, C-6 = $^{13}CBr_2$)ppm; MS: m/e = 543, 547 (M^+); Analysis: calc'd for $C_{21}{}^{13}C_3H_{30}Br_2O_4$: C, 58.28; H, 5.82; Br, 25.64; found: C, 56.15, 55.60, 55.06; H, 5.97, 5.58, 5.64; Br, 28.06, 28.27. The compound retains benzene as solvent of crystalllization [cf. 6].

17α-Acetoxy-6α-methyl-^{13}C-pregn-4-ene-3,20-dione-3,4-$^{13}C_2$ (12)

2% Palladium-strontium carbonate catalyst (1.6 g) in methyl cellosolve (120 ml) was reduced with hydrogen at 1 atmosphere and at 25°C. The 6-dibromomethylene derivative **11** (4.4 g) in methyl cellosolve (210 ml) and triethyl amine (3 ml) were added and the mixture stirred well under hydrogen at 1 atmosphere and at 25°C. During a 30 min period, 510 ml of hydrogen was absorbed. The catalyst was filtered, and the filtrate acidified with 10% by volume of 1N hydrochloric acid. After stirring for 1 hr at room temperature, equal volume of water was added, and the product isolated with dichloromethane as a pale yellow foam. The product was combined with similar products obtained from other hydrogenation experiments and the combined crude material was chromatographed on silica gel "dry column" using ethyl acetate:hexanes (1:1) as the solvent system. Since the purified product still contained minor impurities, it was rechromatographed on silica gel "dry column" using benzene:ethyl acetate (4:1) as solvent system to give 6 g of material which showed a single spot on TLC (benzene:ethyl acetate, 4:1). Crystallization of this material from isopropanol gave fairly pure product (2 g).

The mother liquor material (4 g) which contained both 6α- and 6β-methyl isomers was treated with hydrogen chloride gas in dry chloroform solution in order to epimerize the 6β-methyl to 6α-methyl steroid. The impure product was purified by chromatography on silica gel "dry column" employing benzene:ethyl acetate (4:1) as solvent system, and was added to the initially crystallized pure product. The combined material still showed minor impurities when examined by HPLC. The compound was then purified by preparative HPLC using dichloromethane:ethyl acetate (88:12) as the solvent system to give material of 96% purity. A second chromatographic purification on preparative HPLC using dichloromethane:acetonitrile (93:7) solvent system followed by crystallization from isopropanol ultimately afforded medroxyprogesterone $^{13}C_3$ acetate of 98-99% purity (1.80 g); mp 196-197°C; IR: ν_{max} 1730, 1715, 1685 cm^{-1}; UV: λ_{max} 241 nm (ϵ = 15,520); Proton-NMR: 0.66(s, 3H, 18-CH_3), 1.16(s, 3H, 19-CH_3), 1.28(d of d, $J_{H\text{-}H}$ = 6 Hz, $J_{^{13}C\text{-}H}$ = 150 Hz, 3H, -$^{13}CH_3$), 2.02(s, 3H, 21-$COCH_3$), 2.08(s, 3H, 17-$OCOCH_3$), 2.38(q, J=6 Hz, 1H, C-6H), 5.82(d,

$J_{^{13}C-H}$ = 160 Hz, 1H, -^{13}CO-^{13}CH=C-)ppm; ^{13}C-NMR: 199.64(d, $J_{^{13}C-^{13}C}$ = 52 Hz, ^{13}C-3); 120.34(d of d, $J_{^{13}C-^{13}C}$ = 52 Hz, $J_{\text{long range}}$ ^{13}C-^{13}C of ^{13}C-4 and 6α-Me^{13}C=4.4 Hz) and 18.33(d, $J_{^{13}C-H}$ = 4.2 Hz, 6α-$^{13}CH_3$)ppm. MS: m/e - 389 (M^+); Analysis: calc'd for $C_{21}{}^{13}C_3H_{34}O_4$: C, 74.77; H, 8.80; found C, 74.88; H, 8.60.

ACKNOWLEDGEMENTS

This investigation was supported by a contract No. H9/181/186(K), from the World Health Organization, Geneva, Switzerland. The authors wish to thank Dr. Kenneth A. Hill of this department for carbon-13 NMR spectral data and helpful discussions, and Dr. Paul W. O'Connell, Upjohn Company, Kalamazoo, Michigan, for providing generous quantities of 17α-hydroxyprogesterone and medroxyprogesterone used in this study.

REFERENCES

1. Presented at the IUPAC 12th International Symposium on the Chemistry of Natural Products, Puerto de la Cruz, Tenerife, Canary Islands, Spain, September 21-27, 1980, Abstract No. C-35.
2. Turner, R. B. - J. Am. Chem. Soc. **72**, 579 (1950).
3. Zderic, J. A., Halpern, O., and Iriarte, J. - U.S. Patent 3,099,655 (30 July 1963); Chem. Abstr. **60**, 619e (1963).
4. Kanno, S., Haruyama, T., and Sugano, M. - Jap. Patent 7903,053 (11 Jan 1979); Chem. Abstr. **90**, 204366s (1979).
5. Lemieux, R. and von Rudloff, E. - Can. J. Chem. **33**, 1701, 1710 (1955).
6. Liisberg, S., Godtfredsen, W. O., and Vangedal, S. - Tetrahedron **9**, 149 (1960).
7. Loev, B. and Goodman, M. M. - Prog. Separ. Purif. **3**, 73 (1970).
8. Halpern, O. and Zderic, J. A. - Chem. and Ind. 1540 (1962).