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The Synthesis of 6-chloro-17-hydroxypregna-4,6-diene-3,20-dione-4-^{14}C acetate (Chlormadinone-4-^{14}C acetate) [*,**]

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SUMMARY

Chlormadinone-4-^{14}C acetate (V) was synthesized from 17α-hydroxyprogesterone-4-^{14}C (I) by dehydrogenation to the 4,6-diene (II) which was converted to the 6-chloro-4,6-diene (IV) via the intermediate 6α,7α-epoxy compound (III). Acetylation of (IV) gave chlormadinone-4-^{14}C acetate in 13 % overall yield. A secondary reaction product, 6-chloro-androsta-4,6-diene-3,17-dione-4-^{14}C was isolated and identified.

INTRODUCTION.

In the course of our continuing program (1–3) concerning the metabolic fate of steroidal antifertility agents it became necessary to synthesize chlormadinone-4-^{14}C acetate. The reaction sequences used (Chart 1) were dictated by the ready availability of 17α-hydroxyprogesterone-4-^{14}C (I) starting material.

RESULTS.

The practicality of the reaction sequences shown in Chart 1 was established using non-radioactive material. At each stage the reaction products were isolated and characterized. The dehydrogenation of the 4-en-3-one (I) gave

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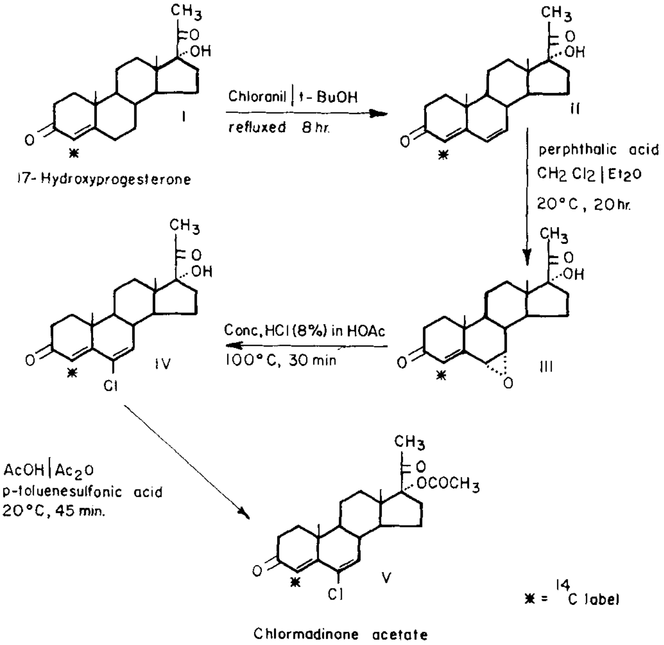
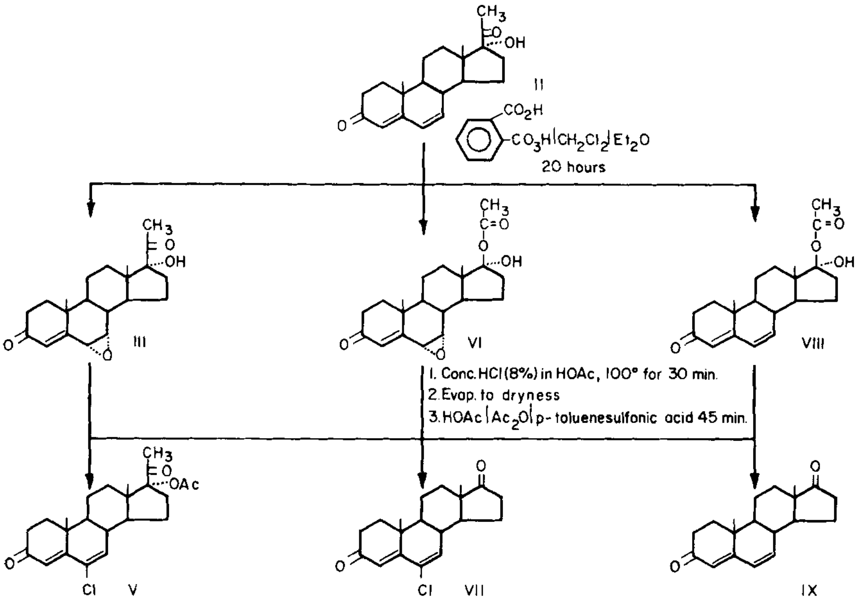
the dienone (II) in only moderate yield (50-65 %). Reaction of this compound with an ethereal solution of monoperoxyphthalic acid gave as the major product the desired 6α,7α-epoxy compound (III). The minor constituent, which became a major product when m-chloroperbenzoic acid was substituted for the monoperoxyphthalic acid, was identified as 6α,7α-epoxyandrost-4-ene-3,17-dione. Treatment of the desired (III) 6α,7α-epoxy compound with acetic acid containing hydrochloric acid at 100° C gave the 6-chloro-4,6-diene (IV). Acetylation of the reaction mixture gave chlormadinone acetate. In the final reaction sequences the total crude reaction products were used for the reactions up to the final acetylation step. At this stage preparative thin layer chromatography (ptlc) was used to separate the chlormadinone (IV) from its principal contaminant 6-chloroandrosta-4,6-diene-3,17-dione (VII Chart 2). Acetylation of the material (IV) isolated gave following further ptlc chlormadinone-4-^{14}C acetate in 12-16 % overall yield.

DISCUSSION.

The synthesis of chlormadinone-4-^{14}C acetate has been achieved for the first time although ^{3}H-labeled material has been recently reported $^{(4)}$. The overall yield while only moderate (12-16 %) gave a radiochemically and spectroscopically pure compound. The structure of the minor product following





peroxyacid treatment of the 4,6-diene (II) was of interest since in the final synthetic sequence it would not be separated until after the penultimate reaction. Following gas/liquid chromatography combined with mass spectroscopy the secondary product was shown to be 6α,7α-epoxyandrost-4-ene-3,17-dione. The identity of the compound was confirmed by direct synthesis. The compound is the result of a Baeyer-Villiger oxygen insertion between C-17 and C-20 to give a 17 hemiacetal (VI) which hydrolyzes to give the 17-ketone. In the final reaction sequence the impurity is also converted to a corresponding 6-chloro-4,6-diene (VII). The compound was isolated since it is a potential metabolite of chlormadinone acetate.

EXPERIMENTAL.

Materials and Methods. — Ultraviolet absorption (UV) spectra were recorded in neutral 95 % ethanol on a Cary 14 spectrophotometer and are presented as λ_{max} in nanometers with the log molar absorbtivity indices. Infrared spectra were measured in chloroform or acetonitrile solution, as mineral oil mulls or by the potassium bromide disc technique on a Perkin-Elmer 221 instrument, and are reported as ν_{max} in reciprocal centimeters. Mass spectra (ms) were obtained by the direct-probe technique at 15 eV on an LKB-9000 instrument. Gas-liquid chromatography and mass spectrometry (glc-ms) spectra were recorded using the LKB-9000, and high resolution mass measurements were made with the peak-matching technique on an Associated Electrical Industries model 902 mass spectrometer.

Melting points were measured in capilliary tubes using a Hoover capilliary tube melting point apparatus. Analytical thin layer chromatography (tlc) employed Silica Gel H or HF (E. Merck, Darmstadt, Germany, coated 0.2-mm thickness) glass plates using the saturated-chamber technique.

Preparative thin layer chromatography (ptlc) utilized ChromAR 1000 silica gel impregnated glass fiber paper (Mallincrodkt, Ltd., St. Louis).

Compounds on the thin layer plates or sheets were visualized by UV absorption and by spraying with a 10 % ethanolic solution of phosphomolybdic acid or a 5 % solution of ceric sulfate in 10 % (v/v) aqueous sulfuric acid. The sprayed plate was heated at 110° C for 10 minutes, at which time the separated compounds appeared as dark spots on a yellow background. Gas-liquid chromatographic studies were made with a Varian Aerograph model 2,100-2 instrument having a flame ionization detection system with 4-foot by 0.25-inch glass columns packed with either 3.8 % or 1.0 % SE-30 silicone phases on 100- to 120-mesh Chromosorb HP-AWS (Johns-Manville, New York, N. Y.). Glc-ms on the LKB-9000 used a 5-foot chromatographic column with a 3.8 % or 1 % SE-30 packing, prepared as above, and was measured at 20 eV. All separation and reaction procedures were routinely monitored by both tlc and glc, enabling immediate combination of like materials prior to further processing. All evaporations were made under reduced pressure, and all solvents were redistilled reagent grade chemicals. All compounds described had the designated elemental composition as judged by the high resolution mass spectroscopy (±15 parts per million) peak matching technique.

EXPERIMENTAL.

17-Hydroxypregna-4,6-diene-3,20-dione (II).

To a solution of 17α-hydroxyprogesterone (125 mg, 0.38 mmole) in t-butanol (10 ml) was added chloranil (227 mg, 0.93 mmole). The mixture was refluxed for 8 hours, left at room temperature overnight and evaporated under vacuum to give a pale brown residue which contained by glc 65 % of (II) (100 mg). Crystallization of the residue from dichloromethane/hexane gave needles (63 mg) of pure (II) mp 244-247°, λ_{max} 283 nm (log ε 4.45), ν_{max} 1,700 ($>C = 0$),C = 1655 ($<(C = C)_2\, C = 0$); literature(5) mp 248°, λ_{max} 284 nm(log ε 4.45).

17-Hydroxy-6α,7α-epoxypregn-4-ene-3,20-dione (III).

A solution of monoperoxyphthalic acid in diethyl ether (6.2 % w/v, 7.5 ml, 2.87 mmoles) was added to the dienone (II, 63 mg, 0.19 mmole) was dissolved in dichloromethane (12.5 ml). The resultant mixture was left at room temperature for 24 hours, then diluted with dichloromethane (12.5 ml) and shaken with a 5 % aqueous solution of sodium hydroxide (25 ml). The organic layer was washed with water, dried and evaporated to dryness to give a pale yellow residue (70 mg). Tlc and glc showed one major component (III) and a minor component (VI). Crystallization of the crude material from diethyl ether/ethanol gave (III) as white prisms (46 mg), mp 214-17°, λ_{max} 238 nm (log ε 4.17), ν_{max} 1,700 (>C = 0), 1,670 (>C = C — C = 0) cm^{-1}; literature (5) mp 227-232°, λ_{max} 241 nm (log ε 4.18).

6-Chloro-17-hydroxypregna-4,6-diene-3,20-dione (IV).

The oxide compound (46 mg, 0.13 mmole) was dissolved in a mixture of acetic acid (1.0 ml) and hydrochloric acid (12 N, 0.08 ml). The resultant solution was heated on a steam bath for 40 min and then cooled. Dichloromethane (25 ml) was added and the mixture was shaken with a 5 % aqueous solution of sodium hydroxide (25 ml). The organic layer was separated and washed with water, dried and evaporated to dryness to give a pale yellow gum (36.5 mg). This gave, following methanol recrystallizations, pale yellow microcrystalline prisms (24 mg) of (IV), mp 204-206° λ_{max} 286 nm (log ε 4.38), ν_{max} 1,700 (>C = 0) cm^{-1} 1,655 (C = C(C)C = C – C = 0) cm^{-1}; literature (5) mp 205-208°, λ_{max} 286 nm (log ε 4.37).

6-Chloro-17-hydroxypregna-4,6-diene-3,20-dione acetate (V).

A solution of the 6-chloro compound (IV, 37 mg, 0.10 mmole) in acetic anhydride (2.5 ml) and acetic acid (5.0 ml) containing p-toluenesulfonic acid (125 mg) was left at room temperature. After 40 min water (25 ml) was added and the mixture stirred for a further 20 min at which time the mixture was extracted with dichloromethane (25 ml). The methylene chloride extract was washed with 5 % aqueous sodium hydroxide solution (25 ml), water (25 ml) then dried and evaporated to dryness to give a pale yellow solid (25 mg). This gave, following recrystallization from ethanol, (95 %) colorless prisms (20 mg) of (V), mp 207-209°, λ_{max} 283 nm (log ε 4.37) ν_{max} 1,730 (—OCOCH$_3$) 1,715 (>C = O) 1,655 (C = C(Cl)C = C – C = O) cm^{-1}; literature (5) mp 208-210°, λ_{max} 284 nm (log ε 4.37).

6-Chloro-17-hydroxypregna-4,6-diene-3,20-dione-4-^{14}C acetate (V).

In order to minimize mechanical losses in the reaction sequences to give chlormadinone-4-^{14}C acetate (V) the crude reaction products were used as starting materials for the first three reaction steps. 17-Hydroxyprogesterone-4-^{14}C (1 mg, 150 µCi, New England Nuclear Corporation, Boston, Massachusetts) was mixed with 124 mg of 17-hydroxyprogesterone and subjected to the previously described reaction sequences to give (163 mg) of the 6-chloro-4,6-diene (IV). This material was subjected to ptlc on 20 × 20 cm (3) sheets of Chromar 1000 using ethyl acetate/chloroform (1 : 19) as development solvent. Three distinct zones (R_f 0.35-0.7) were visible following separation on the sheet. The separate zones were cut out, and the material was eluted. Zone 3 (IV) contained the desired 6-chloro-4,6-diene (69 mg) while zone 1 gave (38 mg) the 17-keto analogue (VII) of (IV). The material obtained from zone 3 was acetylated using acetic acid (5 ml), acetic anhydride (2.5 ml) and p-toluenesulfonic acid (125 mg) in a manner similar to that previously described to give the crude acetate as a yellow gum (65 mg). This material was subjected to ptlc in ChromAR 1000 with an ethyl acetate : chloroform (1 : 19) developing solvent to give chlormadinone-4-^{14}C acetate (19.8 mg, 14.8 μCi). The material gave only one radioactive zone R_f 0.75) tlc on an alumina plate with ethyl acetate chloroform (1 : 9) as developing solvent.

Androsta-4,6-diene-3,17-dione (IX) and 6α,7α-epoxyandrost-4-ene-3,17-dione.

To a solution of chloranil (3.5 g, 14 mmoles) in t-butanol (100 ml) was added androst-4-ene-3,17-dione (2.0 g, 1.7 mmoles). The resultant solution was heated at reflux for 8 hours, cooled and evaporated under vacuum to dryness. The brown residue was dissolved in dichloromethane (100 ml), and the solution was washed with 5 % aqueous sodium hydroxide solution and water and then dried, and a 25 % aliquot was evaporated to dryness. This gave, following crystallization from diethyl ether, pale brown microneedles of the 4,6-diene (VI) mp 165-167°, λ_{max} 282 nm (log ε 4.3) ν_{max} 1,735 ($>C = O$), 1,660 ($-(C = C)_2 C = O$) cm^{-1}. Following addition of dichloromethane (100 ml) to the above 4,6-diene solution, monoperoxyphthalic acid in diethyl ether (3.8 % w/v, 110 ml, 22.97 mmoles) was added, and resultant mixture was allowed to stand at room temperature for 22 hours. The resultant product was processed as for (III) to give a pale yellow solid (1.01 g), major absorption peak at 239 nm. Glc/ms showed the product contained 67 % of the 6α,7α-epoxy compound, the remainder was unreacted starting material.

6-Chloroandrosta-4,6-diene-3,20-dione (VII).

A solution of the crude 6α,7α-epoxy compound (300 mg, 1.0 mmole) in acetic acid (3.0 ml) and hydrochloric acid (12 N, 0.24 ml) was heated on a steam bath for 40 min, cooled and processed as for (IV) to give a yellow solid (400 mg). Glc/ms on the residue showed the 6-chloro-4,6-diene (VII) as the major component with the 4,6-diene (IX) as the secondary product and only traces of the 6α,7α-epoxy compound. Ptlc on an aliquot (20 mg) gave following development, elution and crystallization pure 6-chloro-4,6-diene (VII, 12.4 mg), mp 185-90° λ_{max} 282 nm, ν_{max} 1,740, 1,665 cm^{-1};

literature (6) mp 193-194°, λ_{max} 283 nm. This compound was identical in all respects to the secondary product isolated in the chlormadinone synthesis following the formation of compound (IV).

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