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Synthesis of 3α, 11β, 17α, 21-Tetrahydroxy-[1-^{3}H]-5α-Pregnan-20-one, [1-^{3}H]-Reichstein's Compound C

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SUMMARY

In order to fully understand the metabolic transformations of adrenocortical steroids, labelled 3α,11β,17α,21-tetrahydroxy-5α-pregnan-20-one, Reichstein's Compound C was required. Starting from hydrocortisone, the synthesis of [1-^{3}H]-Reichstein's Compound C has been accomplished.

The metabolic transformations of adrenocortical steroids are reductive in nature and the ring A is reduced almost exclusively to 5β-pregnan-3α-ols with one exception [1]. This exception is 3α,11β,17α,21-tetrahydroxy-5α-pregnan-20-one, Reichstein's Compound C, a 5α-pregnan-3α-ol. The presence of 11-oxygenated-17-ketosteroids in urine represents metabolites of various corticoids. The interpretation as to their formation is currently ambiguous. It will remain so until the relationship of the individual $C_{19}O_3$-metabolites and their hormonal precursors are established. Hydrocortisone could be metabolized by two different routes, depending on whether the side chain cleavage occurs before or after ring A reduction. If the side chain cleavage occurs before the ring A reduction, the resulting product, 11β-hydroxy-4-androstene-3,17-dione is expected to metabolize primarily to the 5α-form. However, if the side chain was cleaved after the ring A reduction, the 11-oxygenated metabolites are expected to have 5β-configuration. Currently, it is not clear whether the amount of 11-oxygenated 17-ketosteroids formed from Reichstein's C affect the ratio of 5β and 5α C_{19}-metabolites present in urine. If labelled Reichstein's Compound C were made available for metabolic studies one could then determine the amount of C_{19}-metabolites derived from this compound. Accordingly, we have now synthesized [1-^{3}H]-Reichstein's Compound C.

Fukushima and Daum [2] have described earlier the preparation of Reichstein's Compound C starting from hydrocortisone (**1**). We have adopted their general procedure with some modifications that are particularly suitable for incorporation of tritium at C_1, and the steps are indicated in Figure 1. The 17,20;20,21-bis(methylenedioxy) (BMD) derivative (**2**) prepared from hydrocortisone (**1**) was stereoselectively reduced at C-5 ring junction with lithium and ammonia [3] to give the 11β-hydroxy-17,20;20,21-bis(methylenedioxy)-5α-pregnan-3-one (**3**) [4]. Bromination of (**3**) with phenyltrimethylammonium perbromide [5] gave the 2α-bromoketone (**4**). Dehydrobromination of (**4**) with calcium carbonate in refluxing dimethylacetamide [6] gave in excellent yield 11β-hydroxy-17,20;20,21-bis(methylenedioxy)-5α-pregn-1-en-3-one (**5**). Catalytic saturation of the Δ^1-double-bond in (**5**) with tritium-enriched hydrogen * using 5 % palladium on carbon in ethyl acetate gave 1,2-tritiated material. Removal of the labile tritium in the 2-position by equilibration with 1 M aqueous methanolic potassium hydroxide [7] ultimately gave 11β-hydroxy-17,20;20,21-bis(methylenedioxy)-[1-^{3}H]-5α-pregnan-3-one (**6**) with a specific radioactivity 10.78 Ci/mmole. A sample of this material was diluted with carrier to a specific radioactivity of 17.2 mCi/mmole, and subsequent synthetic

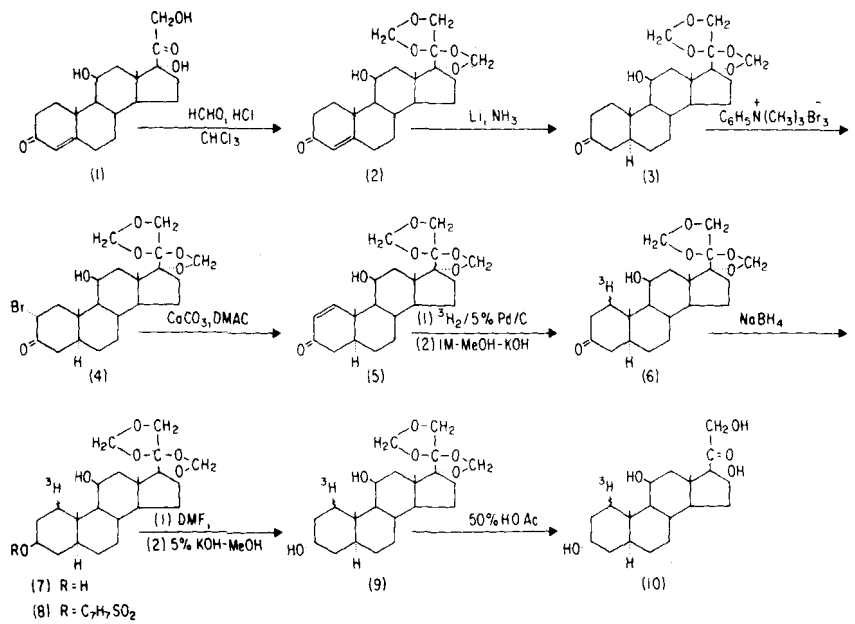

FIG. 1.

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operations were performed. The 3-keto group in (**6**) was reduced with sodium borohydride to give the 3β,11β-dihydroxy-17,20;20,21-bis(methylenedioxy)-[1-^{3}H]-5α-pregnane (**7**). Epimerization of the 3β-hydroxy group to the desired 3α-configuration and subsequent removal of the protective BMD group is then expected to yield [1-^{3}H]-Reichstein's Compound C. Treatment of the 3β-monotosylate (**8**) with N,N-dimethylformamide followed by alkaline hydrolysis and subsequent removal of the BMD group by heating with 50 % acetic acid as described by Fukushima and Daum [2] gave 3α,11β,17α,21-tetrahydroxy [1-^{3}H]-5α-pregnan-20-one (**10**), of specific radioactivity 27.3 μCi/mg. To minimize the destruction of the labelled product in the final step (removal of the BMD protective group) strict adherence to the cleanliness of reagents and glassware as recommended by Mondor [8] is extremely important.

EXPERIMENTAL

Melting points were determined on samples dried under high vacuum at 55° for 24 hr. Ultraviolet spectra were determined in methanol with a Cary recording spectrophotometer (Model 11 MS) and infrared spectra in potassium bromide discs on a Perkin-Elmer (Model 21) Spectrometer. Specific radioactivity was determined on a constant-recording Packard-Tri-Carb Liquid Scintillation Spectrometer. Light-petroleum was Mallinckrodt analytical reagent, b.p. 30-60°.

11β-Hydroxy-17,20;20,21-bis(methylenedioxy)-5α-pregnan-3-one (**3**).

To a mixture of liquid ammonia (300 ml) and lithium (0.191 g), a solution of 11β-hydroxy-17,20;20,21-bis(methylenedioxy)-4-pregnen-3-one (**2**) [2] (0.5 g) in tetrahydrofuran (70 ml) was added over a period of 15 min. The reaction mixture was stirred for an additional 45 min, and then ammonium chloride (0.25 g) was added. The deep blue color gradually disappeared and the ammonia was evaporated at room temperature. The residue was then extracted with chloroform and washed three times with brine. The organic extract was dried (Na_2SO_4) and filtered, and solvent was removed. Crystallization of the residue from acetone-light petroleum gave 11β-hydroxy-17,20;20,21-bis(methylenedioxy)-5α-pregnan-3-one (**3**) (0.4 g), m.p. 235-240°. Additional crystallization from the same solvent mixture gave the analytically pure product, m.p. 242-246°, ν_{max} 3,460, 2,740 and 1,700 cm^{-1}; lit. reported [4] m.p. 235-240° C.

2α-Bromo-11β-hydroxy-17,20;20,21-bis(methylenedioxy)-5α-pregnan-3-one (**4**).

To a cooled (5-10° C) solution of (**3**) (0.812 g) in tetrahydrofuran (25 ml), phenyltrimethylammonium perbromide (0.827 g) was added all in one lot and stirred at the same temperature for one hour. Then the reaction mixture was

diluted with water (100 ml) and extracted with ethyl acetate. The ethyl acetate extract was washed with saturated sodium bicarbonate solution and then with brine, dried (Na_2SO_4) and filtered, and the organic solvent was evaporated. The crude residue (0.928 g) was crystallized from acetone to give 2α-bromo-11β-hydroxy-17,20;20,21-bis(methylenedioxy)-5α-pregnan-3-one (**4**), m.p. 220-222° (dec.), ν_{max} 3,460, 2,740 and 1,718 cm^{-1} (Found : C, 56.82 : H, 6.80. $C_{23}H_{33}O_6$ Br requires C, 56.91; H, 6.85 %).

11β-Hydroxy-17,20;20,21-bis(methylenedioxy)-5α-pregn-1-en-3-one (**5**).

To a solution of dimethylacetamide (6 ml) and the bromoketone (**4**) (0.42 g) calcium carbonate (0.35 g) was added and the contents were refluxed under nitrogen atmosphere for 15 min. The reaction mixture was cooled, diluted with water and extracted with ethyl acetate. The ethyl acetate extract was washed with 5 % hydrochloric acid and then with brine, dried (Na_2SO_4) and filtered, and the solvent was evaporated. The residue (0.308 g) was twice crystallized from acetone to give analytically pure 11β-hydroxy-17,20;20,21-bis(methylenedioxy)-5α-pregn-1-en-3-one (**5**), m.p. 233-236°; ν_{max} 3,460, 2,740, 1,670 and 1,610 cm^{-1}, λ_{max} 230 mμ (ε, 10,330), (Found : C, 68.04; H, 7.95. $C_{23}H_{32}O_6$ requires C, 68.29; H, 7.97 %).

11β-Hydroxy-17,20;20,21-bis(methylenedioxy)-[1-^{3}H]-5α-pregnan-3-one (**6**) *.

To a solution of compound (**5**) (20 mg) in ethyl acetate (5 ml) 5 % palladium on carbon (20 mg) was added and stirred under an atmosphere of tritium-enriched hydrogen. Catalytic saturation was complete in about 30 min, and then the catalyst was removed by filtration. The solvent was evaporated, and the residue (20 mg) was equilibrated by gentle reflux for two hrs. with 1 M aqueous methanolic potassium hydroxide (10 ml). The alkaline solution was carefully neutralized with acetic acid and most of the methanol was removed under reduced pressure. The residue was then extracted with dichloromethane, washed with brine, dried (Na_2SO_4) and filtered, and the solvent was evaporated to give a crystalline residue (20 mg) with a specific radioactivity of 10.78 Ci/mmole. The tritiated compound (**6**) was found to be identical in all respects with the saturated ketone (**3**). A sample (0.642 mg) of the tritiated product (**6**) was diluted with carrier (**3**) (0.40 g) to a specific radioactivity of approximately 17.2 mCi/mmole. This diluted material was employed in the subsequent synthetic operations.

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3β,11β-Dihydroxy-17,20;20,21-bis(methylenedioxy)-[1-^{3}H]-5α-pregnane (**7**).

To a solution of compound (**6**) (0.4 g) in absolute ethanol (200 ml), sodium borohydride (0.6 g) was added and stirred at room temperature for one hr. Then acetic acid (2 ml) was added, and most of the alcohol was evaporated under vacuum. The residue was extracted with chloroform, and the chloroform extract was washed with brine, dried (Na_2SO_4) and filtered, and the solvent was evaporated. The residue was crystallized from a large volume of acetone to give pure 3β,11β-dihydroxy-17,20;20,21-bis(methylenedioxy)-[1-^{3}H]-5α-pregnane (**1**) (0.362 g) m.p. 230-235°. Lit. [2] m.p. 230-238°. The infrared spectrum of (**7**) ν_{max} 3,470, 3,420 (shoulder) and 2,780 cm^{-1} was found to be identical with that of authentic unlabelled product.

3β,11β-Dihydroxy-17,20;20,21-bis(methylenedioxy)-[1-^{3}H]-5α-pregnane-3-p-toluenesulfonate (**8**).

To a solution of compound (**7**) (0.36 g) in anhydrous pyridine (15 ml) p-toluenesulfonyl chloride (0.53 g) was added and left at room temperature for 20 hrs. Then most of the pyridine was evaporated under vacuum, and the residue was extracted with ethyl acetate. The ethyl acetate extract was washed with saturated sodium bicarbonate, and brine, dried (Na_2SO_4) and filtered, and the solvent was evaporated. The oily residue was crystallized from acetone to give light tan crystals of (**8**) (0.425 g) m.p. 170-172°, (dec.). Lit. [2] m.p. 170-172° (dec.). Infrared spectrum ν_{max} 3,560, 2,760 and 1,613 cm^{-1}, was found to be identical with authentic cold material.

3α,11β-Dihydroxy-17,20;20,21-bis(methylenedioxy)-[1-^{3}H]-5α-pregnane (**9**).

A solution of compound (**8**) (0.42 g) in N,N-dimethylformamide (18 ml) was heated at 82° for 72 hrs. The reaction mixture was then diluted with water and extracted with chloroform. The chloroform extract was washed with brine and dried (Na_2SO_4), and the solvent was evaporated. The residual oil was dissolved in 5 % aqueous methanolic potassium hydroxide (45 ml) and stirred for four hrs. Then most of the methanol was evaporated under vacuum at 35° C, and the residue was extracted with ethyl acetate. The ethyl acetate extract was washed with brine, dried (Na_2SO_4) and filtered, and solvent was evaporated. The crude residue showed three spots on thin layer chromatographic examination (Silica Gel G plate in benzene-ether, 1 : 1 system). The residue was passed through a column of neutral alumina (15 g Woelm-Activity III). Elution of the column with benzene-ether (8 : 2) gave pure 3α,11β-dihydroxy-17,20;20,21-bis(methylenedioxy)-[1-^{3}H]-5α-pregnane (**9**) (0.108 g), m.p. 229-232° (from ethyl acetate), lit. m.p. 231-234°. Infrared spectrum ν_{max} 3,440 and 2,760 cm^{-1}, identical in all respects with authentic sample.

3α,11β,17α,21-Tetrahydroxy-[1-^{3}H]-5α-pregnan-20-one (**10**).

The BMD compound (**9**) (87 mg) was suspended in 50 % aqueous acetic acid (7 ml) and heated under reflux for 40 mins. The acetic acid was evaporated under vacuum, and the residue was triturated with ether to give a crystalline product which was further purified by crystallization from ethyl acetate-ether to yield 3α,11β,17α,20-tetrahydroxy-[1-^{3}H]-5α-pregnan-20-one (**10**) (29 mg). Micro hot-stage melting point determination showed m.p. 232-238°, lit. [2] m.p. 238-242°. The infrared spectrum ν_{max} 3,600 (Shoulder), 3,460, and 1,718 cm^{-1} was identical in all respects with authentic material. Thin layer chromatography (Silica Gel G plate in chloroform-ethanol 9 : 1 system) showed the product to be pure, and the specific radioactivity was 27.3 μCi/mg or 74.5 μCi/μmole.

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