SYNTHESIS OF (21-$^{3}H_{3}$)-PREGNENOLONE.

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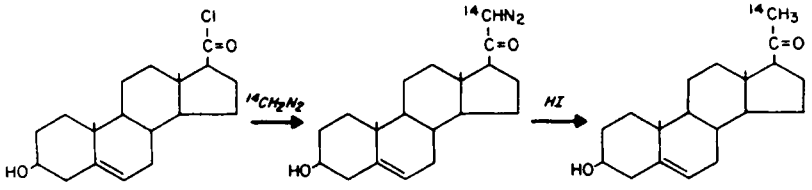
A simple procedure for the preparation of 21-^{3}H-pregnenolone using pregnenolone as starting material is described. The procedure involved removal of the C-21-methyl group of the steroid and the side-chain was reconstructed with ^{3}H-CH_3MgI in two steps: a Grignard reaction followed by oxidation of the resulting alcohol. An overall yield of 71% was achieved.

Key words: ^{3}H-Methyliodide, Pregnenolone, Grignard Reaction

INTRODUCTION

As part of an ongoing research program in steroid hormone biochemistry, we had need for pregnenolone, tritiated at C-21, of moderate specific activity. A previously reported (1, 2) synthesis of (21-^{14}C)-pregnenolone involved the preparation of the (21-^{14}C)-diazoketone 2 by reaction of etienic acid chloride 1 with $^{14}CH_2N_2$ and its subsequent reduction with HI (Scheme 1). By using tritiated N-methyl-N-nitrosourea, as a precursor for tritiated CH_2N_2 (21-^{3}H) diazoketone 2 could be prepared in the same way and subsequently reduced with HI to give (21-^{3}H)-pregnenolone. However, only one-third of the radioactivity from the tritiated urea reagent would be retained in the product. Some tritiated 21-chloromethylated side products



SCHEME 1

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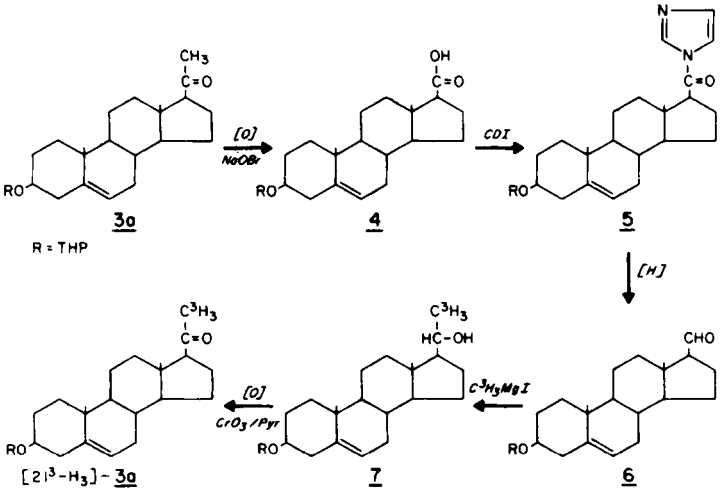
(3) also are formed when the acid chloride is reacted with diazomethane resulting in further wastage of the label. An alternate synthesis (2) of (21-^{3}H)-pregnenolone chosen to minimize the loss of label was achieved by preparation of the diazoketone 2 using non-radioactive CH_2N_2 and reducing it with tritiated hydrogen iodide. The preparation of ^{3}HI, is a tedious process and like the preparation of the tritiated diazomethane, involves radioactive gaseous products and required special care. We therefore decided to prepare (21-^{3}H)-pregnenolone by the method shown in Scheme II which was found to be efficient and safe.

RESULTS AND DISCUSSION

3-Tetrahydropyranyletienic acid 4 prepared by the oxidation of 3-tetrahydropyranyl derivative of pregnenolone (3a) was reduced to the aldehyde 6 by lithium tri-t-butoxy aluminum-hydride, via the imidazolid derivative, 5 (4). The aldehyde 6 was then reacted with tritiated methylmagnesium iodide to yield the 20-hydroxy compound 7. Compound 7 was oxidized with chromium trioxide in pyridine (5), and the tetrahydropyranyl group was removed subsequently by heating in ethanol with a catalytic amount of pyridinium p-toluene-



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sulfonate (6). Oxidation of 7 to 3a under basic conditions was necessary to avoid removal of the 3-tetrahydropyranyl group and to avoid the loss of isotope from C-21 by acid-catalyzed enolization. The choice of tetrahydropyran as a protecting group was dictated by its inertness to Grignard reagents and the mild conditions used for its removal.

EXPERIMENTAL

Dihydropyran, lithium-tert-tributoxyaluminumhydride and N,N'-carbonyl diimidazole (CDI) were purchased from Aldrich Chem. Co., (Milwaukee, WI). Tritiated methyl iodide (97 mCi/mmol) was purchased from New England Nuclear Corp. (Boston, MA.). Tetrahydrofuran and ethyl ether were distilled from $LiAlH_4$ prior to use. Mass spectrometry was done on a DuPont 21-492B mass spectrometer interfaced to a Hewlett Packard 21-094 data acquisition system. Infrared spectroscopy was performed on a Perkin Elmer 521 instrument. High pressure liquid chromatography (HPLC) was performed on a Waters Associates unit, consisting of dual M-6000A pumps, fitted with a Perkin Elmer Silica A column (0.26x25 cm). Thin layer chromatography was done on Whatman Silica gel plates (20x5 cm) using water:methanol: chloroform:toluene (1:20:60:120) to develop the chromatogram. Authentic pregnenolone had an R_f value of 0.485 under these conditions.

Preparation of 3-tetrahydropyranyletienic acid, 4:

The 3-tetrahydropyran derivative of pregnenolone was prepared quantitatively from pregnenolone and dihydropyran by the general method described by Grieco et al (6). This product was subjected to oxidation by hypobromite (7) as follows: a cold solution of sodium hypobromite, prepared by cautiously adding 2.8 ml of Br_2 to 72 ml of 3M NaOH, was added dropwise to a solution containing 6.0g (19 mmol) of the 3-THP-derivative of pregnenolone in 75% aqueous dioxane. The solution was stirred at 0°C for 2 hr. After addition of a 10% solution of sodium sulfite to destroy excess sodium hypobromite, the reaction mixture was carefully neutralized with

1N HCl. The resulting precipitate was collected, washed and dried (5.4g; m.p. 198-207°C).

Conversion of 4 to 6: Three g of 4 (8 mmol) and 3.6 g of N, N'-carbonyldiimidazole (23 mmol) were added to 40 ml of THF and the mixture heated under reflux for 45 min. After cooling to room temperature, the solution was poured into cold water and the imidazolid derivative 5 that precipitated was filtered, washed with water and dried. The yield was 3.3 g (95%), m.p. 168-175°C. Without further purification, the imidazolid (ca 8 mmol) was dissolved in 80 ml of THF (anhydrous) and lithium-tri-t-butoxyaluminum hydride (2.7 g, 12 mmol) in 40 ml anhydrous THF was added to it dropwise at room temperature with stirring. After addition of the reducing agent to the imidazolid was completed the solution was concentrated in vacuo, diluted with 150 ml of water and the resulting suspension was brought to pH 4-5 with 1N HCl. The methylene chloride extract was washed with water until neutral, dried over anhydrous Na_2SO_4 and evaporated to dryness. The resulting residue (2.5 g) was purified by column chromatography on 100 g silica gel, using benzene as eluant. The yield was 1.7 g (ca. 60%). The 3-tetrahydropyranyl-Δ5-cholenaldehyde melted at 119-123°C and had the characteristic aldehyde absorption at 1723 cm^{-1} in its infrared spectrum.

Conversion of 6 to 7: ($^3H_3$)-Methylmagnesium iodide was prepared by the reaction of ($^3H_3$)-methyl iodide (100 mCi) with magnesium turnings. The vial containing (^3H)-methyl iodide (150 mg) was fused to one end of a H-shaped joint attached to a 25 ml round bottom flask containing 39 mg of magnesium turnings and a magnetic stir bar as shown in Fig. 1.

The vial break seal was broken by a falling magnet, anhydrous ether (3 ml) immediately added through the septum and the solution distilled into the round bottom blask which was pre-cooled in a dry ice acetone bath. When all the ether solution had distilled over, the cooling bath was removed and the solution stirred at

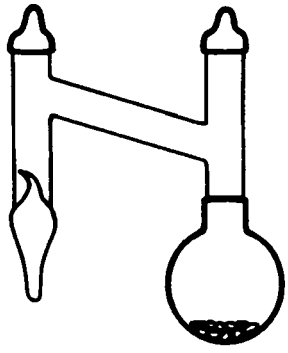

Figure 1

room temperature for 30 min. Tetrahydropyranyletienic aldehyde, (400 mg), 6 , dissolved in 4 ml of anhydrous benzene was then added through the septum to the Grignard reagent and stirred overnight. The reaction mixture was then poured into 50 ml water, extracted twice with 100 ml ethyl acetate, and the combined ethyl acetate extracts washed neutral. The crude product, (21-$^3H_3$)-3,20-dihydroxy-(3-tetrahydropyranyl)-Δ5-pregnene (436 mg), 7, obtained by evaporation of the ethyl acetate was used without purification.

Preparation and purification of (21-$^3H_3$)-pregnenolone:

The crude product 7 from above was oxidized with chromium trioxide (250 mg) in pyridine (10 ml) at room temperature for 4 h. The reaction mixture was then poured into water, extracted with ethyl acetate and the ethyl acetate evaporated in vacuo. The residue was dissolved in 15 ml absolute ethanol (6) and the solution stirred at 50°C for 2 h to effect the removal of the tetrahydropyranyl protecting group. Work-up and chromatography on silica gel (75 g) using benzene with increasing amounts of ethyl acetate, resulted in the recovery of a peak of radioactivity with 15% ethyl acetate in benzene. Thin layer chromatography indicated that 94% of the label was due to pregnenolone. A portion of the labeled material (10 mg) was further purified by high

pressure liquid chromatography using CH_2Cl_2:EtOAc (9:1) as eluant at a flow rate of 1 ml/min. The retention volume (8 ml) of the tritium labeled material was identical to that of standard (4-^{14}C)-pregnenolone chromatographed under the same conditions. The radioactive material (97 mCi/mmol) was characterized as 3β-hydroxy-Δ5-pregnen-20-one, 3, by its mass spectrum (m/e:316(M^+)), its infrared spectrum (3450 cm^{-1}, 1710 cm^{-1}) and by its mixed melting point taken with authentic pregnenolone (190-92°). The radioactive yield, based on tritiated methyl iodide was 71%.

CONCLUSION

The method of preparation of (21-$^3H_3$)-pregnenolone described above is an efficient and simple one which may be extended to the preparation of other similarly labeled C-21 steroids such as progesterone and 17-hydroxyprogesterone. The preparation of (21-^{14}C)-labeled C-21 steroids could likewise be achieved using ^{14}C-labeled methyl iodide for formation of the Grignard reagent.

REFERENCES

1. Porto A. and Grox E. A.-J. Labelled Compounds 4:276(1968)
2. Pussemier L., VandeVelde V. and Piraux M. - J. Labelled Compounds 16:651 (1979)
3. Hilgetag G. and Martini A. (eds) Preparative Organic Chemistry, 4th Ed., John Wiley and Sons, Inc. p. 948 (1972)
4. Entwistle I. D., Boehm P., Johnstone R. A. W. and Telford R. P. - J. Chem. Soc. Perkin I 35:27, (1980)
5. Poos G. I., Arth G. E., Beyler R. E. and Sarett, L. H. - J. Amer. Chem. Soc. 75:422 (1953)
6. Miyashita M., Yoshikoshi A. and Grieco P. A. - J. Org. Chem. 24:3772 (1977)
7. Staunton J. and Eisenbraun E. J. - Org. Synth. 42:4 (1962)