Synthesis and purification of Betamethasone-1,2,4-^{3}H and Betamethasone-1,2,4-^{3}H 17-Benzoate

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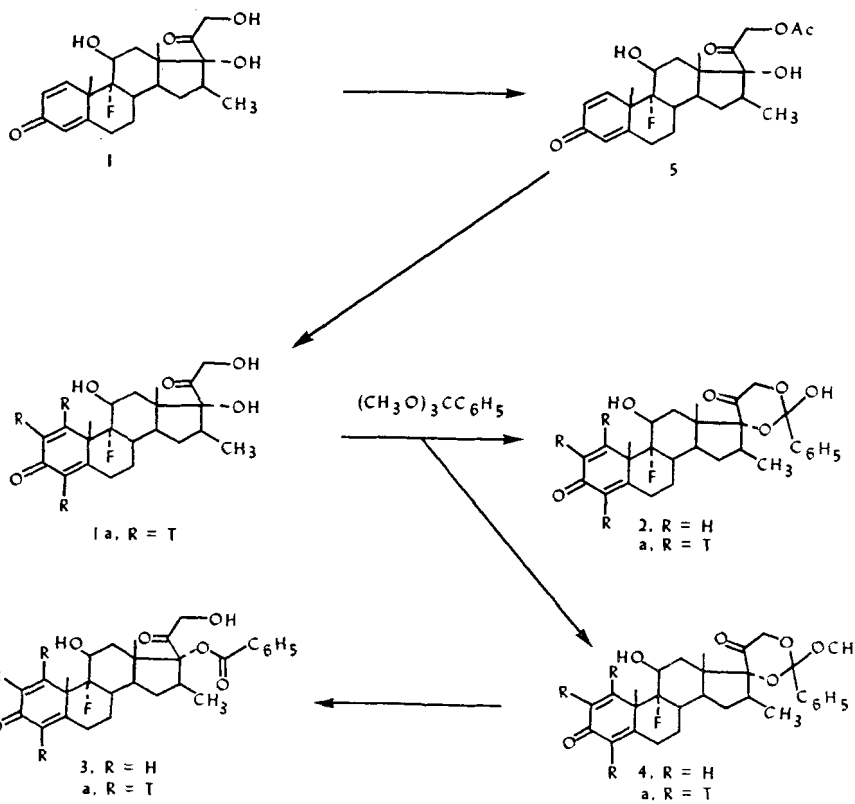
ABSTRACT

Betamethasone 21-acetate was catalytically reduced with tritium. The crude product was oxidized with 2,3-dichloro-5,6-dicyano-1,4-benzoquinone (DDQ) to betamethasone-1,2,4-^{3}H 21-acetate. Hydrolysis gave betamethasone-1,2,4-^{3}H, which, when allowed to react with trimethylorthobenzoate, gave both betamethasone-1,2,4-^{3}H 17,21-methylorthobenzoate and betamethasone-1,2,4-^{3}H 17,21-orthobenzoate. Selective hydrolysis of the betamethasone-1,2,4-^{3}H methylorthobenzoate gave the desired betamethasone-1,2,4-^{3}H 17-benzoate.

The preparation of betamethasone 17-benzoate (9α-fluoro-11β,17,21-trihydroxy-16β-methylpregna-1,4-diene-3,20-dione 17-benzoate), **3**, labeled with tritium was required in order to determine its excretion pattern and to elucidate its metabolite(s) in various animal species and man. The synthesis of **3**, as shown in Scheme I, necessitated the preparation of betamethasone 17,21-methylorthobenzoate, **4**. This reaction produced, in addition to **4**, about an equal weight of a more polar compound. The structure of this compound was shown to be betamethasone 17,21-orthobenzoate, **2**. This orthoester is of particular interest and will be reported elsewhere [2].

The synthetic scheme used to introduce the tritium was based on the work of Jerchel *et al* [3], who reported the synthesis of dexamethasone-1,2,4-^{3}H (9α-fluoro-11β,17,21-trihydroxy-16α-methylpregna-1,4-diene-3,20-dione-1,2,4-^{3}H). The ring A diene system was catalytically reduced with tritium and then restored by reoxidizing with selenium dioxide. Since **1** differs from dexamethasone only in the configuration of the C-16 methyl group, the above approach proved satisfactory in introducing a significant quantity of tritium into **1a**.

Betamethasone 21-acetate, **5**, was reduced catalytically in the presence of 8 Ci of carrier free tritium gas. The diene system was reintroduced using 2,3-dichloro-5,6-dicyano-1,4-benzoquinone (DDQ). Basic hydrolysis in a nitrogen atmosphere gave betamethasone-^{3}H, **1a**. The specific activity of this material was 9.07 Ci/mM. If one atom of tritium was introduced, the specific activity would be about 29 Ci/mM. When **1a** was allowed to react with freshly distilled trimethylorthobenzoate in dimethylformamide in the presence of 3% p-toluenesulfonic acid (p-TsOH), as described by Ercoli and Gardi [4, 5],



betamethasone-^{3}H 17,21-methylorthobenzoate, **4a**, was isolated in 28% yield. About an equal weight of **2a** was also isolated. Later experiments using the trimethylorthobenzoate as supplied by the manufacturer [6], gave both **2** and **4**, each in about 50% yield. Gas chromatography demonstrated that the trimethylorthobenzoate, as received, contained about 5% of methyl benzoate and less than 1% of methanol [7]. When methylbenzoate was substituted for the trimethylorthobenzoate, no reaction occurred.

Undoubtedly, both **2a** and **4a** are present as epimeric mixtures since Ercoli and Gardi [8] have reported the separation of the epimeric forms of several steroidal 17α, 21-ethylorthoformates.

Selective hydrolysis of **4a**, using the recommended buffer system [4], gave betamethasone-1,2,4-^{3}H 17-benzoate, **3a**, in 52% yield. The chemical and radiochemical purity of **3a** is described in the Experimental.

EXPERIMENTAL.

Melting points were determined on a Mel-Temp melting point apparatus and are uncorrected. The radioactivity was determined via liquid scintillation spectrometry in a cocktail composed of 7.0 g PPO (2,5-diphenyloxazole), 0.3 g dimethyl POPOP [1,4-bis-2-(4-methyl-5-phenyloxazolyl)benzene] and 100 g naphthalene in 1 l. 1,4-dioxane [9] using a Packard Model 3310 spectrometer equipped with automatic external standardization. The tlc plates were scanned using a Packard 7200 Radiochromatogram Scanner. Uv and ir spectra were determined with Beckman DK-1A and Perkin-Elmer 621 or Baird 4-55 spectrophotometers. Nmr results were obtained with a Varian A-60 spectrometer using tetramethylsilane as an internal standard.

Preparative tlc was done using 20 × 20 cm glass plates coated with a 250 micron thick layer of silica gel GF (Analtech, Inc.) pre-washed with methanol and reactivated by heating at 100° C for 1 hr. The solvent used for development was a mixture [10] of 80 parts benzene and 20 parts acetone; all the solvents were specially purified [11]. Typical R_f values obtained with this system for **1**, **2**, **3**, **4**, **5**, and **6** were 0.20, 0.50, 0.55, 0.45, 0.28, and 0.60, respectively. The spots were visualized under shortwave uv light or by spraying with a 50% solution of conc. sulfuric acid in methanol and then heating at 110° C for about 15 minutes. A deep blue color resulted.

Betamethasone 21-acetate (**5**).

The acylation procedure of Taub *et al.* [12] gave **5** in 91% yield. The product melted at 210-214° C and a tlc exhibited a single uv absorbing spot.

9α-Fluoro-11β,17,21-trihydroxy-16β-methylpregna-3,20-dione-1,2,4,5-^{3}H 21-acetate.

A suspension of 28.0 mg of 10% palladium on carbon in 2 ml of distilled dioxane in a 5 ml RB flask with a magnetic stirrer was pre-reduced with hydrogen. The gas was introduced into the reaction flask with an automatic Toepler pump. The excess hydrogen was removed by alternately freezing and melting the dioxane suspension in vacuo. To this suspension was added 28.0 mg (0.0635 mM) of **5**. Eight curies (0.123 mM) of carrier free tritium gas [13]

and sufficient nitrogen to increase the pressure to 1 atmosphere were introduced with the Toepler pump. Previous experiments demonstrated the need for the additional inert atmosphere to force the reduction to completion. The reaction was magnetically stirred for 18 hours. The reaction was alternately frozen and melted in vacuo, and all gases were transferred to a RB flask containing a stirred suspension of 150 mg of 10% palladium on carbon in 5 ml dioxane to adsorb any unreacted tritium gas.

The reaction was filtered and the solvent removed. A tlc showed the residue to be a mixture of the di- and tetratritiated analogs. (Under the above conditions, reductions with hydrogen gave the tetrahydro compound exclusively.) The mixture weighed 26 mg and was subjected to the DDQ oxidation without further purification.

Betamethasone-1,2,4-^3H 21-acetate (**5a**).

A solution of 26 mg of the tritiated mixture and 58.4 mg (0.34 mM) of DDQ dissolved in 2.8 ml dioxane was allowed to reflux under nitrogen for 30 hrs. This reaction time was optimum based on cold runs. The solvent was removed and the residue dissolved in 30 ml methylene chloride and washed with several 10 ml portions of a 2% aqueous sodium hydroxide solution. The organic phase was dried ($MgSO_4$), filtered and the solvent removed. The residue was dissolved in methylene chloride-methanol (7 : 1), and this solution applied equally to four 20 × 20 cm plates for preparative tlc. The appropriate radioactive areas were scraped from the plates, packed into a glass chromatography column, and eluted with 20 ml methanol. This eluate was filtered through a fine fritted glass funnel to remove any particulate matter, and the solvent removed under a stream of nitrogen at 30° C. After drying in vacuo, **5a** weighed 8 mg (29% from **5**) and was 97% radiochemically pure by tlc; the contaminant was **1a**.

Betamethasone-1,2,4-^3H (**1a**).

The 8 mg (0.0189 mM) of **5a** were hydrolyzed by allowing a solution prepared under nitrogen in 1.98 ml of 0.0092 N methanolic KOH to remain at room temperature for 1 hr. The reaction was neutralized by the addition of 0.81 ml of 0.1 N acetic acid in methanol. The solvents were removed. The residue was dissolved in 10 ml of ethyl acetate-chloroform, (1 : 1) and washed with three 10 ml portions of a saturated aqueous NaCl solution. The organic phase was dried ($MgSO_4$), filtered, and the solvent removed. The residue was dissolved in chloroform and purified by preparative tlc on two 20 × 20 cm plates. The product weighed 4.3 mg, which was 98% radiochemically pure.

The specific activity was 23.1 mCi/mg (9.07 Ci/mM) and was adjusted to 2.0 mCi/mg by diluting with 45.7 mg of **1**.

Betamethasone-1,2,4-^{3}H 17,21-methylorthobenzoate (**4a**).

To 0.53 g (2.9 mM) distilled trimethylorthobenzoate and 1.5 mg (0.0079 mM) of p-TsOH in a 5 ml RB flask with a condenser was added a solution of 50 mg (0.127 mM) of **1** in 1.5 ml of dimethylformamide. A stream of nitrogen was slowly bubbled through the solution while the reaction was heated in an oil bath at 105° C for 20 hours. The reaction was cooled to room temperature and two drops of pyridine added. Tetrachloroethylene was added to aid in the removal of the solvents in vacuo at $<35°$ C. The oily residue was dissolved in 0.7 ml chloroform and purified via preparative tlc. The crude **4a** was then dissolved in 0.35 ml peroxide free diethyl ether and percolated through a 3 g alumina column (Merck) deactivated with 3% water followed by more diethyl ether. Twenty ml of the eluate were collected and the solvent removed. The residue weighed 18 mg (28%). A tlc exhibited a single uv absorbing spot, which contained all of the radioactivity. This material was sufficiently pure and was used to prepare **3a**; ir (KBr) 3 450, 1 740-1 725, 1 665, 1 635-1 605, 1 290, 1 130-1 005, 890, 695 cm^{-1}. A sample of **4** exhibited an nmr (DMSO-d_6) δ 7.20-7.70 (m, 5), 7.25 (d, 1, $J = 10$ Hz), 6.00-6.25 (m, 2), 5.29 (d, 1, $J = 5$ Hz), 4.33 (s, 1), 4.15 (s, 2), 2.92 and 3.00 (s, 1), 1.48 (s, 3), 1.08 and 1.28 (d, 2, $J = 6$ Hz), 1.0 (s, 3); uv max (CH_3OH) 238-240 nm (a 29.7).

Betamethasone-1,2,4-^{3}H 17-benzoate (**3a**).

A solution of 18 mg (0.035 mM) of **4a** in 3.6 ml of methanol and 1.44 ml of a Sorensen citrate buffer [14] at pH 3.7 was heated in an oil bath at 50° C for 30 minutes. The solvents were removed and the residue dissolved in 25 ml of chloroform. This was washed with three 10 ml portions of distilled water, dried ($MgSO_4$), filtered, and the solvent removed. The residue was redissolved in a small amount of chloroform and purified by preparative tlc.

This gave 9.0 mg (52%) of **3a** as a white solid; uv max (CH_3OH) 233 nm (a 53.4) [15]; ir (KBr) 3 470, 3 230, 1 740-1 715, 1 670, 1 620, 1 610, 1 280, 1 105-1 060, 890, 710 cm^{-1}; nmr (DMSO-d_6) δ 7.35-8.15 (m, 5), 7.37 (d, 1, $J = 10$ Hz), 6.10-6.20 (m, 2), 5.33 (broad, 1), 5.05 (t, 1, $J = 6$ Hz), 4.36 (broad, 1), 4.05 (d, 2, $J = 6$ Hz), 1.55 (s, 3), 1.41 (d, 3, $J = 5$ Hz), 0.97 (s, 3). A tlc exhibited a single uv absorbing spot which contained greater than 99% of the radioactivity.

Betamethasone 17,21-orthobenzoate (**2**).

This material was prepared in an identical manner as described for **4a** except that the trimethylorthobenzoate was used without purification. The product of this reaction, as an oily residue, was dissolved in ethyl acetate and distributed equally on the appropriate number of 20 × 20 cm plates for

preparative tlc. The compound was eluted from the adsorbent with benzene-acetone (1 : 2). The solvent was removed from the combined eluate and the solid recrystallized from about 30 parts of ethyl acetate. Recoveries of about 70% were obtained from each of the two recrystallizations usually required to raise the melting point to 213-215° C. Additional quantities of less pure material may be obtained by adding an equal volume of skellysolve B [16] to the filtrates.

In a typical reaction, 17.7 mg (28%) of **2** was obtained from 50 mg (0.127 mM) of **1** after preparative tlc. Two recrystallizations from 30 parts ethyl acetate gave the analytical sample which melted at 213-215° C after drying in vacuo at 37° C for 20 hours. A tlc was developed after spotting 500 μg and a single uv absorbing spot was observed; uv max (CH_3OH) 232 nm (a 54.3); ir (KBr) 3 350 (broad), 1 735-1 720, 1 660, 1 640, 1 600, 1 280, 1 120-1 020, 890, 710 cm^{-1}; nmr (DMSO-d_6) δ 7.30-8.10 (m, 5), 7.34 (d, 1, J = 10 Hz), 6.00-6.25 (broad, 2), 4.98 (d, 1, J = 6 Hz), 4.42 (broad, 1), 1.49 (s, 3), 1.01 (s, 3), 0.90 (s, 3).

Anal Calcd. for $C_{29}H_{33}FO_6.C_6H_6$: C, 72.98; H, 6.82; F, 3.31.

Found : C, 73.04; H, 6.73; F, 3.26.

Prolonged heating at elevated temperatures in a attempt to remove the solvate caused decomposition.

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15. This was 97.7% of an authentic sample.
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