Preparation of Tritium-Labeled Compounds. IV. A Group of Steroids by Exposure to Tritium Gas

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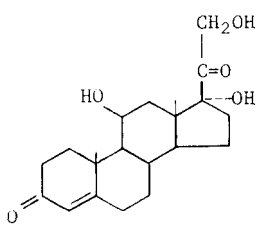
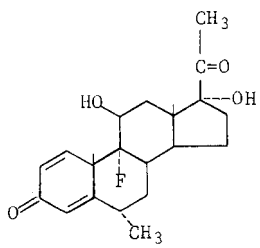
SUMMARY

Hydrocortisone, 21-desoxy-9α-fluoro-6α-methylprednisolone, and 6α-methylprednisolone, 21-acetate were labeled with tritium by the Wilzbach tritium-gas exposure method. The latter compound was also tritiated by exposure to tritium gas in the presence of a platinum-black catalyst. Although extensive purifications were required, in each case radiochemically pure material of sufficiently high specific activity for its intended use was obtained. Incorporation of stably bound tritium was nine times greater by catalytic tritiation than by the conventional Wilzbach method.

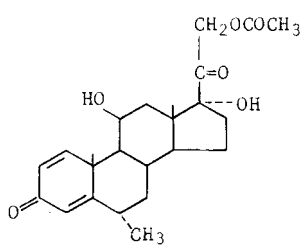
Studies of the percutaneous absorption of hydrocortisone [1] (I), 21-desoxy-9α-fluoro-6α-methylprednisolone [2] (II), and 6α-methylprednisolone, 21-acetate [3] (III) required the preparation of these steroids in radioactive forms.



I



II



III

[1] Cortef is The Upjohn Company trademark for hydrocortisone.

[2] Oxylone is The Upjohn Company trademark for 21-desoxy-9α-fluoro-6α-methylprednisolone.

[3] Medrol Acetate is The Upjohn Company trademark for 6α-methylprednisolone, 21-acetate.

Tritium labeling was chosen because of the difficulty and expense of incorporating carbon-14 into these compounds. The Wilzbach [1] tritium gas-exposure method was selected because precursors suitable for reduction with tritium to give II and III were not available.

A disadvantage of the Wilzbach method is the formation of high specific activity impurities often difficult to remove from the desired product [1-4]. This is particularly true for unsaturated compounds such as the Δ^4 and $\Delta^{1,4}$ steroids, I, II, and the free alcohol of III, which can add tritium to form high specific activity impurities very closely related to the desired product. It was felt, however, that paper and column chromatographic methods of adequate resolving power for removing these impurities were available. This proved to be true.

In the case of one of the compounds, the free alcohol of III, a comparison was made of tritium incorporation by the conventional Wilzbach method and the platinum-black modification introduced by Meshi and Takahashi [5]. As expected, tritium incorporation was greater using the platinum-black exposure method.

EXPERIMENTAL.

Radioactivity Measurements. — All counting was performed with a Tri-Carb [4] Model 314X or 314EX2A liquid scintillation spectrometer at -8° under conditions suitable for measuring tritium. Appropriate aliquots of samples were dissolved in 15 ml of diotol scintillator [6] [toluene-dioxane-methanol (350 : 350 : 210 by volume) containing 73 g naphthalene, 4.6 g of 2,5-diphenyloxazole, and 0.08 g of 1,4-bis-2-(5-phenyloxazolyl)-benzene per l]. The absolute counting efficiency for each sample was determined by recounting following addition of an internal standard of tritium-labeled toluene and results then expressed as microcuries (μc). Paper chromatograms were scanned for radioactivity with a Forro [5] 2-π or a Vanguard [6] Model 880 radiochromatogram scanner.

Paper Chromatography. — Chromatograms were developed by the descending method using 86-cm lengths of Whatman No. 2 paper (unless stated otherwise) in the following systems : (*a*) Bush B-5 [7, 8], sheet equilibrated overnight at 34° in the vapor from a mixed solvent composed of benzene-methanol-water (2 : 1 : 1 by volume) and developed in the benzene phase; (*b*) Mattox I [9], sheet saturated with methanol-formamide (1 : 1 by volume), dried 15 minutes at 37°, and developed with n-butyl acetate-formamide-water (100 : 5 : 5 by volume); (*c*) FBC [8, 10], sheet saturated with

[4] Packard Instrument Company, Downers Grove, Illinois.

[5] Forro Scientific Company, Evanston, Illinois.

[6] Vanguard Instruments, Division of Technical Measurements Corporation, North Haven, Connecticut.

methanol-formamide (1 : 1 by volume), dried 15 minutes at 37°, and developed with formamide saturated benzene-chloroform (1 : 1 by volume); (*d*) PTF [8, 10], sheet saturated with methanol-propylene glycol (1 : 1 by volume), dried 10 minutes at 37°, and developed with propylene glycol saturated toluene.

Zones absorbing ultraviolet light were located as fluorescence quenching areas when the dried chromatograms were viewed with a short wavelength ultraviolet light chromatogram scanner [11].

Tritiation and Purification of Hydrocortisone. — A 20-mg sample of hydrocortisone, deposited as a thin film on the inside of a glass tube, was exposed to 5 curies of carrier-free tritium gas at room temperature in the dark for 10 days under approximately 0.2 Atm tritium pressure. Following removal of the tritium gas, the crude sample was dissolved in ethanol and the resulting solution was evaporated to dryness *in vacuo*. Labile tritium was completely removed by repeating this procedure twice. The residue was dissolved in 4 ml of ethanol and the solution was diluted with 16 ml of water and then extracted twice with 100-ml portions of methylene chloride. The methylene chloride was removed *in vacuo* and the residue was dissolved in one ml of acetone. This solution was streaked on four 15-cm wide sheets of previously prepared chromatography paper which were then developed in the Mattox I system. The zones corresponding to hydrocortisone were cut out and the material was eluted from the paper with four 25-ml portions of acetone. Paper chromatography of a portion of this solution in the Mattox I system revealed a single radioactive zone, corresponding to hydrocortisone, but three radioactive zones, one of which corresponded to hydrocortisone, were observed following chromatography in the Bush B-5 system. The acetone solution was reduced in volume and subjected to preparative paper chromatography in the Bush B-5 system on four 15-cm wide sheets of paper. Hydrocortisone purified in this manner revealed single radioactive and ultra-violet light absorbing zones corresponding to authentic hydrocortisone when chromatographed in the Mattox I, Bush B-5, and PTF systems. The tritiated hydrocortisone was diluted with 0.095 gm of nonradioactive hydrocortisone in 30 ml of methylene chloride and applied to a 1.3 × 15 cm column of adsorbent magnesium silicate [7] (12 g) packed in methylene chloride. The following solvents were passed through the column while collecting 15-ml fractions.

45 ml methylene chloride
45 ml methylene chloride-acetone (4 : 1 by volume)
45 ml methylene chloride-acetone (2 : 1 by volume)
45 ml methylene chloride-acetone (1 : 1 by volume)
45 ml methylene chloride-acetone (1 : 4 by volume)
30 ml acetone
30 ml methanol

[7] Marketed as Florisil by the Floridin Company, Tallahassee, Florida.

The product, emerging in fractions 8 through 16, weighed 0.099 g and had a specific activity of approximately 12 μc per mg. Although this material was radiochemically pure in the 3 previously mentioned paper chromatography systems, it was only about 90% chemically pure as indicated by its ultraviolet and infrared spectra. Approximately 0.090 g of this material was diluted with 0.110 g of nonradioactive hydrocortisone and recrystallized from methanol-water to yield 0.153 g product having a specific activity of 5.8 μc per mg. Its ultraviolet and infrared spectra corresponded to those of authentic hydrocortisone.

Tritiation and Purification of 21-Desoxy-9α-fluoro-6α-methyl-prednisolone. — A 1.9-mg sample of II was exposed to 4 curies of tritium gas for 11 days as previously described. Labile tritium was removed with ethanol. The crude product was dissolved in one ml of acetone and subjected to preparative paper chromatography on four 15-cm wide sheets of Whatman No. 3 MM paper in the Bush B-5 system. The material corresponding to the product was eluted and the preparative purification procedure was repeated two additional times. Radiochemical purity, as determined by analytical paper chromatography in the Bush B-5 and FBC systems, was 70%, 90%, and100% following the first, second and third purifications, respectively. The tritiated product, eluted from the final sheets of paper, was diluted with 8 mg of nonradioactive II and extracted into methylene chloride. The specific activity of the diluted product was 110 μc per mg. Its ultraviolet and infrared spectra corresponded to those of authentic II.

Tritiation and Purification of 6α-Methylprednisolone. — A 1.0-g sample of the finely pulverized free alcohol of III was exposed to 5 curies of tritium gas for 28 days as previously described. Labile tritium was removed with ethanol. The specific activity of the crude product was 220 μc per mg. Recrystallization from ethanol-water reduced the specific activity to 86 μc per mg. This material was warmed and stirred with 1 l of methylene chloride to give a saturated solution (containing about 0.60 g of steroid). The methylene chloride was filtered and applied to a 2.4 × 28 cm column of adsorbent magnesium silicate [7] (75 g) packed in Skellysolve B. The column was eluted successively with 0.5 l portions of Skellysolve B containing 10, 20, 30, and 50% acetone, and finally with pure acetone, while collecting 100-ml fractions. The product started emerging with the 30% acetone in Skellysolve B solvent. The radiochemical purity of each fraction was determined in the Bush B-5 and PTF paper chromatography systems. Fractions showing at least 80% radiochemical purity were combined (0.422 g) and recrystallized from ethanol-water with the aid of charcoal (Darco G-60) to yield 0.249 g of product having a radiochemical purity of 90%. This material was diluted with 0.751 g of nonradioactive carrier and recrystallized from ethanol-water to give 0.747 g of product having a specific activity of 2.65 μc per mg and a radiochemical purity greater than 95%.

Acetylation of Tritiated 6α-Methylprednisolone. — The above material

was acetylated in 2.4 ml of pyridine with 0.6 ml of acetic anhydride at room temperature. The crude 21-acetate (0.684 g), obtained by dilution of the acetylating mixture with water, was recrystallized from acetone-water to give 0.642 g of 6α-methylprednisolone, 21-acetate having a specific activity of 2.47 μc per mg. Its ultraviolet and infrared spectra corresponded to those of authentic standard. The product showed single ultraviolet light absorbing and radioactive zones corresponding to standard in the Bush B-5 and the PTF paper chromatography systems.

Anal. — Calcd. for $C_{24}H_{32}O_6$: C, 69.2; H, 7.75. Found : C, 69.0; H, 7.92.

Catalytic Tritiation and Purification of 6α-Methylprednisolone. — An intimate mixture of 0.50 g of finely pulverized free alcohol of III and 0.50 g of platinum black [8] was exposed to 4 curies of tritium gas for 8 days as previously described. In this case, however, the tritium gas was entirely adsorbed on the catalyst so the pressure was approximately 10^{-5} mm. At the end of the exposure period, the sample was slurried in ethanol and filtered through diatomaceous earth [9] to remove the catalyst. The filtrate was evaporated *in vacuo* and the residue was subjected to two additional alcohol treatments to remove labile tritium. The crude product was dissolved in 0.3 l of 10% acetone in methylene chloride and applied to a 1.9 × 61 cm column of adsorbent magnesium silicate [7] (100 g) packed in Skellysolve B. The column was eluted by the gradient technique using 2 l of 30% acetone in Skellysolve B and 2 l of 70% acetone in Skellysolve B while collecting 100-ml fractions. Fractions (0.343 g total) showing 80% or greater radiochemical purity were combined and subjected once again to column chromatography as described above. The resulting product (0.297 g) still contained appreciable radiochemical impurities so it was subjected to partition chromatography. A column was prepared using 200 g of acid washed diatomaceous earth [10] as a support and 200 ml of 70% methanol in water (previously equilibrated with 2 volumes of the first mobile phase) as the stationary phase. The material from the previous column operation was mixed with 10 g of diatomaceous earth [10] and 10 ml of equilibrated stationary phase and packed on top of the column. The column was then eluted consecutively with the following mobile phases, each of which previously had been equilibrated with 0.5 volume of 70% methanol in water, while collecting 20-ml fractions.

2.5 l of 25% cyclohexane in benzene
2.0 l of benzene
2.0 l of 5% ethyl acetate in benzene.

The product emerged with the second mobile phase and was found to be radiochemically pure. This material was recrystallized from acetone-Skelly-

[8] Engelhard Industries, Inc., Newark, N. J.

[9] Marketed as Celite by the Johns-Manville Corporation, New York, N. Y.

[10] Marketed as Dicalite 4200 by the Dicalite Division of Grefco, Inc., Los Angeles, California.

solve B to yield 0.196 g of product having a specific activity of 43.4 μc per mg. Its ultraviolet and infrared spectra corresponded to those of authentic standard. The product showed single ultraviolet light absorbing and radioactive zones corresponding to standard in the Bush B-5 and Mattox I paper chromatography systems.

Acetylation of Catalytically Tritiated 6α-Methylprednisolone. — The bulk (0.150 g) of the above free alcohol of III was diluted with 0.450 g of carrier and acetylated as previously described to yield 0.597 g of crude 21-acetate. This material was recrystallized from acetone-Skellysolve B to yield a product having a specific activity of 10.5 μc per mg. A further recrystallization from acetone-water gave 0.532 g of III having a specific activity of 10.4 μc per mg. The ultraviolet and infrared spectra corresponded to those of authentic standard. The product showed single ultraviolet light absorbing and radioactive zones corresponding to standard in the Bush B-5, PTF, Mattox I, and FBC paper chromatography systems.

Anal. — Calcd. for $C_{24}H_{32}O_6$: C, 69.2; H, 7.75. Found : C, 69.3; H, 7.92.

Results and discussion.

Although rather extensive purification procedures were required, each of the steroids, tritiated by the conventional Wilzbach method, was obtained in radiochemically pure form. Paper chromatography of the three tritiated products, prior to their purifications, indicated initial radiochemical purities of only 5-10%. In the case of 6α-methylprednisolone, comparison of the specific activities of the crude and pure products also showed that the crude material was only 5% radiochemically pure. Paper chromatography indicated that reduction products were major high specific activity radiochemical impurities in the crude materials; the 4,5-dihydro product in the case of hydrocortisone and the 1,2- and 4,5-dihydro products in the case of 6α-methylprednisolone.

The major radiochemical impurities introduced by catalytic exposure tritiation of 6α-methylprednisolone were less polar (chromatographically) than both the parent compound and its 1,2- and 4,5-dihydro reduction products. This was not further investigated, however. As was the case with the conventional Wilzbach tritiation of 6α-methylprednisolone, extensive purification was required to obtain a radiochemically pure product.

The specific activities of the three steroids, tritiated by the conventional Wilzbach method, proved to be satisfactory for their intended uses. Incorporation [11] of stably bound tritium was 76 μc per curie-day exposure in the case

[11] Incorporation of tritium equals the product of the specific activity of the purified material (corrected for any dilutions) and the amount of material exposed divided by the product of the amount of tritium gas employed and the length of time of the exposure.

of 6α-methylprednisolone exposed by the conventional Wilzbach method. Although dilutions were not quantified during purification of the hydrocortisone and 21-desoxy-9α-fluoro-6α-methylprednisolone, reasonable estimates (based on 25 to 50% yields to the point of dilution) indicate that incorporation of stably bound tritium was 50-75 μc per curie-day exposure for these two steroids.

Comparable tritium incorporations have been reported for similar steroids by other workers; 30 and 140 μc per curie-day exposure for 9α-fluoro-16α-hydroxyprednisolone [12], 160 μc per curie-day exposure for 6α-methyl-17α-hydroxyprogesterone [13], 20 μc per curie-day exposure for 4-hydroxy-17α-methyltestosterone [13], 20 μc per curie-day exposure for prednisolone [5], and 70 μc per curie-day exposure for 5α-pregnan-17α,21-diol-3,11,20-trione, 21-acetate and Δ^1-5α-pregnen-17α, 21-diol-3,11-20-trione, 21-acetate [14].

Incorporation of tritium by catalytic exposure of 6α-methylprednisolone was 680 μc per curie-day, 9 times that obtained by the conventional Wilzbach method. This is in reasonable agreement with the results of Meshi and Takahashi [5] with prednisolone. In this case catalytic tritium gas exposure resulted in a tritium incorporation of approximately 120 μc per curie-day exposure, 6 times that obtained by the conventional Wilzbach method.

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