mmol) of aniline were added to the hot methanol solution. The mixture was stirred and allowed to cool, during which time all of the sodium carbonate was consumed (about (0.5 hr). Another 0.53-g portion of Na_2CO_3 was added to completely neutralize the amine hydrobromide being formed in the reaction. The reaction mixture was stirred for another 90 min, during which time all of the sodium carbonate was consumed and most of the bicyclic product precipitated. The reaction mixture was allowed to stand in a freezer overnight and the bicyclic product was recovered by filtration. Recrystallization from hot methanol yielded from 38 to 74% (dec pt 156–157°): ν^{KBr} 1580, 1490, 1370, 1287, 1188, 1111, 745, 686 cm^{-1}; nmr (CF_3COOD) δ 4.3 (s, 4 H), 5.02 (s, 4 H), 7.67 (s, 5 H); nmr ($CDCl_3$) δ 3.87 (s, 4 H), 4.19 (s, 4 H), 6.4–6.84 and 7.09–7.34 (broad phenyl absorption, 5 H).

Anal. Calcd for $C_{12}H_{13}NO_2S$: C, 61.25; H, 5.57; N, 5 95. Found: C, 61.77; H, 5.52; N, 5.83.

5-(*p*-Methoxyphenyl)-1,3,4,6-tetrahydrothieno[3,4-*c*]pyrrole 2,2-Dioxide.—The 5-(*p*-methoxyphenyl) product was prepared by a procedure similar to that described above: yield 73–83%; dec pt 156°; ν^{KBr} 2825, 1520, 1314, 1253, 1238, 1185, 1119, 1038, 811 cm^{-1}; nmr (CF_3COOD) δ 3.95 (s, 3 H), 4.26 (s, 4 H), 4.95 (s, 4 H), 7.16 and 7.59 (AB, J = 9 Hz, 4 H); nmr (DMSO-d_6), δ 3.67 (s, 3 H), 4.07 (broad peak, 8 H), 6.41 and 6.89 (AB, J = 9 Hz, 4 H).

Anal. Calcd for $C_{13}H_{15}NO_3Si$: C, 58.85; H, 5.69; N, 5.28. Found: C, 59.36; H, 5.58; N, 5.14.

5-(*p*-Chlorophenyl)-1,3,4,6-tetrahydrothieno[3,4-*c*]pyrrole 2,2-Dioxide.—This bicyclic pyrrole was obtained by following a procedure similar to that above, with the exception that the reaction mixture was held at 55° for 1 hr. Recrystallization from hot methanol yielded 0.8 g (34%), dec pt 153° (lit.[3] 153°).

5-Methyl-1,3,4,6-tetrahydrothieno[3,4-*c*]pyrrole 2,2-Dioxide.—The dibromo sulfone (12.11 g, 40 mmol) and 130 mmol of anhydrous methylamine in 300 ml of acetonitrile were stirred at room temperature for 60 min. The reaction mixture was filtered to remove the methylamine hydrobromide which precipitated, and the filtrate was evaporated to dryness. The residue was triturated in 100 ml of ether, and the ethereal solution was reduced in volume to 25 ml and allowed to stand in a freezer overnight; 1.1 g of the bicyclic product was obtained. The residue that remained after the ether washing was dissolved in methanol and neutralized with sodium carbonate. The methanol was removed under reduced pressure and the resultant residue was also triturated with 100 ml of ether. From this ether solution 1.5 g of the bicyclic product was obtained: total yield 2.8 g (37%); mp 89–90°; ν^{KBr} 1297, 1262, 1174, 1143, 1100, 848, 792 cm^{-1}; nmr (CF_3COOD) δ 3.37 (s, 3 H), 4.10–5.17 (s, 4.22 with broad AB pattern, 8 H; nmr ($CDCl_3$) δ 2.51 (s, 3 H), 3.56 (s, 4 H), 3.75 (s, 4 H).

Anal. Calcd for $C_7H_{11}NO_2S$: C, 48.53; H, 6.39; N, 8.08. Found: C, 48.92; H, 6.07; N, 7.95.

5-Ethyl-1,3,4,6-tetrahydrothieno[3,4-*c*]pyrrole 2,2-Dioxide.—The 5-ethyl product was prepared by the same procedure as the 5-methyl product. The only deviation from the procedure is that the ethylamine hydrobromide did not precipitate: 32%; mp 98–99°; ν^{KBr} 1285, 1264, 1184, 1154, 1166, 1100, 1032, 847, 793, 770 cm^{-1}; nmr (CF_3COOD) δ 1.52 (t, J = 7 Hz, 3 H), 3.64 (q, J = 7 Hz, 2 H), 4.06–5.04 (s, 4.18 with a broad AB pattern, 8 H); nmr ($CDCl_3$) δ 1.12 (t, J = 7 Hz, 3 H), 2.75 (q, J = 7 Hz, 2 H), 3.59 (s, 4 H), 3.80 (s, 4 H).

Anal. Calcd for $C_8H_{13}NO_2S$: C, 51.35; H, 6.98; N, 7.48. Found: C, 51.08: H, 6.76; N, 7.30.

5-Benzyl-1,3,4,6-tetrahydrothieno[3,4-*c*]pyrrole 2,2-Dioxide.—The 5-benzyl product was prepared by the same procedure as the 5-methyl product: 46–60%; mp 100°; ν^{KBr} 2780, 1298, 1263, 1110, 1091, 740, 700 cm^{-1}; nmr (CF_3COOD) δ 4.16 (s, 4 H), 4.37–4.77 (s, 4.69 and broad peak, 6 H), 7.57 (s, 5 H); nmr ($CDCl_3$) δ 3.60 (s, 4 H), 3.73 (s, 4 H), 3.86 (s, 2 H), 7.31 (s, 5 H).

Anal. Calcd for $C_{13}H_{15}NO_2S$: C, 62.62; H, 6.07; N, 5.62. Found: C, 62.63; H, 5.86; N, 5.38.

Registry No.—1, 18214-57-8; 2 (R = phenyl), 35105-69-2; 2 (R = *p*-methoxyphenyl), 35105-70-5; 2 (R = *p*-chlorophenyl), 32515-66-5; 2 (R = methyl), 35105-72-7; 2 (R = ethyl), 35105-73-8; 2 (R = benzyl), 35105-74-9.

The Preparation of 17β-Hydroxyestra-4,6-dien-3-one and Its Stereospecific β-Face Reduction at Carbons 6 and 7[1]

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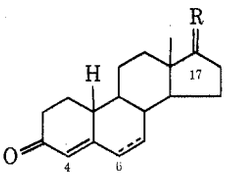
In connection with studies on the metabolism of 19-nor steroids we required estr-4-ene-3,17-dione (**1a**) labeled with a hydrogen isotope in a stable position. We decided to introduce the lable at C-7 by reducing 17β-hydroxyestra-4,6-dien-3-one (**1c**) with tris(triphenylphosphine)rhodium(I) chloride as was done in the steroidal C_{19} series.[2] Usual one-step procedures for the preparation of the diene starting material proved unsatisfactory. Reaction of chloranil (2,3,5,6-tetrachloroquinone) with estr-4-ene-3,17-dione or the corresponding 17β-hydroxy compound in refluxing *tert*-butyl alcohol gave a negligible yield of the desired $\Delta^{4,6}$-3-one compound, although the procedure gives reasonable results with C_{19} steroids.[3,4] Use of DDQ (dichlorodicyanoquinone) and acid catalysis was somewhat better, but was still unsatisfactory. Although the reaction goes to completion in 0.5 hr using a C_{19} compound,[5] only 75% of 17β-hydroxyestr-4-en-3-one (**1b**) was dehydro-



1a, R = O
b, R = 17β-OH, 17α-H
c, Δ^6-**1b**

genated in 1.5 hr and gave product mixtures that required extensive purification. However, we succeeded in obtaining complete reaction of 17β-hydroxyestr-4-en-3-one using chloranil in *tert*-butyl alcohol, ethanol, or methanol by heating to only 50° for 2–3 hr. The products were separated readily from the reagent materials by alumina column chromatography and further purification of the steroid fraction by tlc gave the pure $\Delta^{4,6}$-diene in good yield. A small amount of a mixture also was isolated and was tentatively identified as the phenolic ethyl ethers of estradiol-17β and the corresponding Δ^6 compound. This indicated that some C-1,2 dehydrogenation took place also and suggested that the same conditions, but with DDQ as oxidant, might bring about dehydrogenation at C-1 as occurs with testosterone, rather than at C-6 which occurs with 17β-hydroxyestr-4-en-3-one (19-nortestosterone).[6] How-

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ever, dehydrogenation under these conditions also occurred preferentially at C-6 as judged by gas–liquid chromatographic analysis of the products.

Preliminary experiments on the reduction of 17β-hydroxyestra-4,6-dien-3-one (**1c**) to 17β-hydroxyestr-4-en-3-one (**1b**) with the rhodium catalyst and hydrogen gas showed that complete reduction in benzene was obtained in 12 hr, while in dioxane only 3 hr was required. Dioxane was used subsequently for reductions with isotopic hydrogen. With deuterium gas, 7 hr was required for uptake of 1 mol equiv and, under the somewhat different conditions of reduction with stoichiometric amounts of tritium gas,[7] 40 hr was required for an apparent 33% uptake of tritium. Material from the latter experiment was chromatographed on silver nitrate impregnated silica gel plates to separate the 17β-hydroxyestr-4-en-3-one (**1b**) from unreacted 6-dehydro material. A portion of the product, after dilution with carrier, was refluxed with base to remove exchangeable tritium at C-6 and tlc of the 17β-hydroxyestrenone-7-^3H product gave a radiochemically pure material as judged by subsequent paper chromatography and crystallization. The 6,7-tritiated material was diluted with ^{14}C-labeled and unlabeled 17β-hydroxyestr-4-en-3-one (**1b**) and was crystallized repeatedly. There was insignificant loss of tritium, showing that reduction with the rhodium catalyst gives material readily obtainable in a radiochemically pure state. This was confirmed by the preparation of the acetate derivative. Equilibration with base caused the $^3H/^{14}C$ ratio to decrease 50% (presumably due to loss of tritium at C-6), and indicates that the label was equally distributed at C-6 and C-7 (Table I). Similar

TABLE I

DEMONSTRATION OF PURITY OF 17β-HYDROXYESTRENONE-6,7-^3H AND -7-^3H AFTER REVERSE ISOTOPE DILUTION AND CRYSTALLIZATION

17β-Hydroxyestrenone-6,7-^3H,4-^{14}C	dpm/μmol $^3H \times 10^{-3}$	dpm/μmol ^{14}C	Ratio ($^3H/^{14}C$)
$n-3$	105	2940	35.6
$n-2$	106	3010	35.1
n	106	2970	35.6
Base refluxed			18.3 (loss, 51%)
17β-Acetate, diluted and crystallized	12.2	352	34.8

results were obtained with deuterium reduction where 92% of the product was dideuterated, accompanied by an insignificant amount of trideuterated material. These are in contrast to results with heterogeneous catalysis where the distribution of the label across the double bond often is not uniform[8] or where an appreciable amount of label may be exchanged into positions α to the double bond.[9,10]

The configuration of the label at C-7 was determined by microbiological hydroxylation at C-7β, a transformation we determined occurs readily with the microorganism *B. malorum* using estrenedione as the substrate.[11] The 7β-labeled materials were converted to estrenedione by Jones oxidation. When estrenedione-7β-^3H, 4-^{14}C and estrenedione-7β-^2H, both diluted with carrier, were incubated separately with the microorganism, the hydrogen isotope label in the 7β-hydroxyestrenedione product decreased by over 97%. Since hydroxylation occurs by direct replacement of hydrogen,[12] the data show that the label at C-7β was almost exclusively β oriented. We[13] and others[14] showed that reduction with the rhodium catalyst occurs by cis addition, from which we may conclude that the label at C-6 also is essentially β oriented. Support for this assignment was obtained from nmr spectra. In estrenedione, no splitting of the 4-H signal is noted, presumably due to a broadening brought about by coupling with both the 6β and 10β hydrogens. In contrast, the 4-H signal is split when the 6β hydrogen is replaced by methyl[15] or hydroxyl,[11] due to coupling with only the C-10β proton. A similar signal for the C-4 proton is noted in the 6,7-dideuterated estrenedione, indicating that the deuterium is at C-6β as expected.

Experimental Section

Infrared spectroscopy was recorded with a Perkin-Elmer Model 137 from KBr disks; ultraviolet spectroscopy was recorded from a Perkin-Elmer Model 202 spectrophotometer, using a methanol solvent. Nmr spectra were obtained on a Varian DA-60 (60 MHz) or Varian HA100-15 (100 MHz) spectrometer; peaks are quoted in δ (parts per million) downfield from tetramethylsilane internal standard. Mass spectrometry was determined with a Varian M-66 spectrometer. Conditions for deuterium analysis have been described.[13] Radioactivity was measured by double-label scintillation counting on a Packard Tri-Carb Model 314 EX as before.[16] Melting points were taken on a Fisher–Johns hot stage. Thin layer chromatography used Merck silica gel PF-254, with preparative layers PR-254 + 366. Gas chromatography was performed with 2% QF-1 on Gas-Chrom Q (Applied Science Laboratories). 17β-Hydroxyestr-4-en-3-one was purchased from Searle Chemical Co.

17β-Hydroxyestra-4,6-dien-3-one (1c). Method A. DDQ and Acid Catalysis.—Dry HCl gas was bubbled for 1.5 hr into a stirred solution of 1 g (3.65 mmol) of 17β-hydroxyestr-4-en-3-one (**1b**) and 944 mg (3.98 mmol) of DDQ in 30 ml of purified dioxane.[17] After filtering and diluting with dichloromethane, the filtrate was washed with potassium carbonate and then with water. Analysis of a sample at this point indicated 75% conversion, as judged by the relative absorbance at 240 and 283 nm. Purification by silica gel column chromatography using ethyl acetate–benzene mixtures followed by preparative layer (5 mm) silica gel chromatography using benzene–acetone (9:1, v/v) and crystallization from benzene–hexane–diethyl ether gave 308 mg of product still contaminated with starting material. Subsequent chromatography on preparative layer silica gel–silver nitrate coated plates (30:5, v/v) in the system cyclohexane–chloroform–acetic acid (48:50:10, v/v) gave the diene free from the less polar Δ4-3 ketone. Elution with chloroform–methanol (9:1, v/v) and washing with 1% aqueous NaCl, 2% $Na_2S_2O_3$, and then water[18] gave pure material used subsequently for reduction with tritium gas: λ_{max} 283 nm [ε 26,219 (lit.[19] 26,920)]; ν 3500 (OH), 1655 (3-one), 1620, 1575 (vinylic H).

Method B. Chloranil. A solution of 5 g of 17β-hydroxyestr-4-en-3-one (**1b**) and 3.35 g of chloranil in 500 ml of ethanol was stirred at 50° for 2 hr and then was evaporated to dryness under

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reduced pressure. The residue was chromatographed on a column containing 400 g of neutral alumina (Woelm) using increasing percentages of ethyl acetate in benzene for development. The fractions containing material absorbing at 283 nm were combined to give a yield of 2.7 g of 17β-hydroxyestra-4,6-diene-3,17-dione (**1c**) based on uv analysis. This material was purified further by preparative tlc using benzene–acetone (9:1, v/v). The $\Delta^{4,6}$ product and a less polar material were eluted. The mass spectrum of the latter showed highest mass peaks of equal intensity at *m/e* 298 and 300. The infrared spectrum was similar to that of the 3-methyl ether of estradiol-17β and glc analysis indicated the presence of two compounds of approximately equal amounts. However, these could not be separated on several thin layer and paper systems and the problem was not investigated further. It appeared that they were ethyl ethers of estradiol-17β and the corresponding Δ^5 compound.

Preparation of Catalyst.—A solution of 1 g of rhodium chloride trihydrate and 6 g of triphenylphosphine in 120 ml of ethanol was refluxed for 30 min.[20] Upon cooling, the precipitated tris(triphenylphosphine)rhodium(I) chloride was collected, washed with cold ethanol and ether, and then stored at 4° in a stoppered vial.

Preparation of 6,7-Labeled 17β-Hydroxyestr-4-en-3-one (1b). A. Tritium Labeling.—A solution of 27.2 and 14 mg of tris(triphenylphosphine)rhodium(I) chloride in 2 ml of dioxane was stirred under 1 mol equiv of tritium gas. There was an apparent 3.5-Ci uptake in 7 days. After removal of tritium gas and solvent, the residue was chromatographed on silver nitrate impregnated silica gel plates as detailed above using chloroform–methanol (98:2, v/v). A radioscan of a small sample chromatographed in a similar fashion showed major radioactive peaks at the origin and in the area corresponding in mobility to 17β-hydroxyestr-4-en-3-one (**1b**). Material in this zone was eluted to give 273 mCi (7.7% yield based on tritium uptake). A portion was diluted with 4-^{14}C-labeled and unlabeled testosterone and was crystallized from benzene–hexane to constant specific activity. This was diluted further with carrier and then was acetylated with 50% acetic anhydride in pyridine at room temperature for 3 hr. After tlc and crystallization, analysis for ^{3}H and ^{14}C again was carried out on weighed crystals. Another portion of the double-labeled testosterone was refluxed with 2% KOH in methanol–water (1:1, v/v), and was analyzed by scintillation counting after purification by tlc as described previously.[4] The results are in Table I.

B. Deuterium Labeling.—A dioxane solution containing 2.5 g of 17β-hydroxyestr-4,6-dien-3-one (**1c**) and 1.25 g of the rhodium catalyst was stirred in a deuterium atmosphere 16 hr at ambient temperature and pressure. The residue from evaporation was chromatographed on 400 g of silica gel using benzene–ethyl acetate mixtures. 17β-Hydroxyestr-4-en-3-one (**1b**) came off the column with a 9:1 mixture and was purified further by preparative plate chromatography in benzene–ethyl acetate (9:1 and then 8:2, v/v) to give a material which was homogeneous on gas chromatographic analysis (QF-1): mp 172–173°; λ_{max} 241 nm; mass spectrometric analysis of the molecular ion (d_0 = 274) d_0 (3%), d_1 (4%), d_2 (93%). Oxidation with Jones reagent[21] gave estr-4-ene-3,17-dione: nmr δ 0.94 (s, 3 H, 18-methyl), 5.89 (d, J = 1.7 Hz, 1 H, 4-H).

Preparation of 7-Labeled Estrenedione.—6,7-Labeled 17β-hydroxyestr-4-en-3-one (**1b**) was refluxed with base and was purified by tlc in benzene–ethyl acetate (3:1, v/v). Oxidation with Jones reagent[21] gave 7-labeled estr-4-ene-3,17-dione (**1a**). Material which had been tritiated was diluted with estr-4-ene-3,17-dione-4-^{14}C and was crystallized repeatedly. There was an insignificant change in the ^{3}H/^{14}C ratio. Similarly, the deuterated product, after dilution with carrier (1:1), isolation, and crystallization showed one peak on gas chromatography [d_0 (51%), d_1 (3%), d_2 (47%)]. Mass spectrometric analysis of the molecular ion showed only d_0 and d_1 species [d_0 (51%)].

Incubation with *B. malorum*.—Estrenedione-7-^3H,4-^{11}C (^{14}C sp act., 2700 dpm/mg; ^{3}H/^{14}C ratio, 28.6) was incubated with respiring cultures of *B. malorum* for 20 hr and the 7β-hydroxyestrenedione product was isolated as described[11] and the acetate derivative was crystallized from benzene–hexane three times. Loss of tritium as judged by decrease in the ^{3}H/^{14}C ratio was 98%. The product from the 7β-deuterated substrate [d_1 (34%)] was analyzed by mass spectrometry directly as the alcohol, since the highest *m/e* value for the acetate was 270 ($M^+ - 60$). There was no detectable amount of deuterium on mass spectrometric analysis of the 7β-hydroxy product after three crystallizations from ethyl acetate.

Registry No.—**1a,** 13209-45-5; **1a** (7-T, 4-^{14}C), 31031-84-8; **1b** (6-T, 4-^{14}C), 35140-96-6; **1b** (7-T, 4-^{14}C) 35140-97-7; **1b** (6-D), 35140-98-8; **1b** (7-D), 35140-99-9; **1c,** 14531-84-1.

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The Use of Propionic Acid–Hydrochloric Acid Hydrolysis in Merrifield Solid-Phase Peptide Synthesis

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Merrifield solid-phase peptide synthesis[2-5] procedure is useful for the synthesis of peptides in high yield. However, several cases have been reported of incomplete couplings (for examples see ref 6–10). As work continues with the synthesis of larger peptides and proteins, the need for quick accurate analysis is becoming more important.

During the last 4 years we have been using anaerobic and aerobic propionic acid–hydrochloric acid (HCl) hydrolysis as an analytical tool. Our previous report[11] showed that blocked amino acids could be hydrolyzed easily from the resin used in Merrifield synthesis using this technique. This communication reports the results of 70 peptides hydrolyzed by these procedures, as compared to hydrogen fluoride–anisole cleavage from the resin, followed by constant boiling HCl hydrolysis.[12]

Table I gives the ratios, R, of moles of amino acids obtained from peptide resins hydrolyzed by 1:1 propionic acid–12 *N* HCl at 130° for 2 hr, and the moles of amino acids obtained from peptide resins treated with hydrogen fluoride–anisole[13,14] and then hydrolyzed by

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