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A novel synthesis of 14α,15α-methylene estradiol (J 824)

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A novel approach to the synthesis of the orally active estrogen 14α,15α-methylene estradiol (8, J 824) is described, starting with 3-methoxy-estra-1,3,5(10),8,14-pentaen-17α-ol (5). The 14α,15α-methylene bridge was sonochemically introduced by regioselective and stereoselective Simmons-Smith methylenation of the 14-double bond. Birch reduction of the 8-double bond provided the desired 8β-H, 9α-H steroid, whereas ionic hydrogenation afforded the 8β-H, 9β-H isomer, together with an epimerization of the 17α-hydroxy group. Oxidation of the Birch product yielded the corresponding 17-oxo steroid, which gave the title compound by diborane reduction. For radioimmunoassay development the 6-(O-carboxymethyl)-oximino derivative of 8 was prepared as hapten and the 2-hydroxy derivative of 8 was synthesized as a potential metabolite of 8, and 8 was tritium labeled as well. (*Steroids* **60**:308–315, 1995)

Keywords: estrogens; 14α,15α-methylene estradiol; steroid synthesis; hapten; tritium labeling

Introduction

Very recently we have claimed esters of 14α,15α-methylene estradiol (J 824, **8**), which, when tested versus ethynyl estradiol, in vivo displayed a higher uterotropic activity and a clearly improved pharmacokinetic behavior.[1] To obtain more information on the biological profile of these new compounds, in particular with regard to hepatic functions and the cardiovascular system, larger amounts of the key substance **8** were needed.

Ponsold and co-workers[2] described the synthesis of the 3-methylether of **8** (**4**), starting with 14-dehydro-17α-estradiol 3-methylether (**1**) (Scheme 1). The 17α-hydroxy group of **1** is essential for a stereoselective carben addition at the 14-double bond from the rear side of the molecule. On the other hand, the approach to compound **1** is rather lengthy.[3,4] In particular the reduction of the precursor 14-dehydroestrone 3-methylether suffers from only 10% yield of **1** upon chromatography.[5] The synthesis of compound **8** via intermediate **1** is thus not attractive for large-scale preparations. We describe here a novel improved approach to the synthesis of title compound **8** from 17α-hydroxy steroid **5**.[6,7] In addition the preparation of the hapten **19**, the ^{3}H-labeled compound **22**, and the catechol derivative **26** as a potential metabolite of **8** is reported.

Experimental

General methods

^{1}H-NMR spectra were recorded on a Varian Gemini 300 (300 MHz). Unless otherwise stated, deuteriochloroform was used as solvent, chemical shifts are reported as δ values in ppm downfield from tetramethylsilane as internal standard, *J* values are given in Hz. Melting points were measured with a Boetius equipment. Ultraviolet (UV) spectra were taken with a Zeiss Specord M 40 in methanolic solutions, λ_{max} in nm (log ε). Optical rotations were measured with the Polamat A (Carl Zeiss Jena), solvent chloroform (unless otherwise provided), concentration = 1 g per 100 mL, temperature = 20°C. Chromatography means flash chromatography,[8] which was performed on silica (Kieselgel 60, Merck A.G. Darmstadt, 0.04–0.063 mm). All reactions were run under a nitrogen or argon atmosphere. Usual work-up of the extract included: the organic phase was washed with saturated aqueous sodium hydrogen carbonate solution and water, dried over anhydrous sodium sulfate or magnesium sulfate, and rotary evaporated to dryness. Sonification was performed in a Sonorex cleaning bath at a frequency of 35 kHz.

3-Methoxy-14α,15α-methylenestra-1,3,5(10),8-tetraen-17α-ol (**6**)

5, prepared according to the literature,[7] (50 g; 0.18 mol), dissolved in absolute tetrahydrofuran (150 mL), and zinc dust (225 g;

Steroids Part 27. Part 26 in this series: Schwarz S, Ring S, Weber G, Teichmüller G, Palme H-J, Pfeiffer C, Undeutsch B, Erhart B, Grawe D (1994). Synthesis of 13-Ethyl-11-methylene-18,19-dinor-17α-pregn-4-en-20-yn-17-ol (Desogestrel) and its Main Metabolite 3-Oxo Desogestrel. Tetrahedron **50**:10709–10720.

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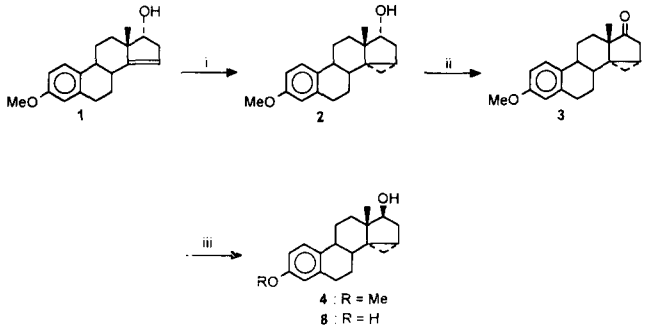


Scheme 1 Synthesis of 3-methoxy-14α,15α-methylenestra-1,3,5(10)-trien-17β-ol **(4)** from 3-methoxy-1,3,5(10),14-tetraen-17α-ol **(1)**. (i) Zn-Cu, CH_2J_2; (ii) CrO_3, H_2O, H_2SO_4, acetone; (iii) B_2H_6, THF.

3.44 g-atom) were heated to 70°C in a sonificator. Sonification was switched on and diiodomethane (60 mL; 0.37 mol) added within a period of 90 min. Sonication was continued for another 30 min. Thereafter the reaction was quenched by addition of aqueous ammonium chloride solution (20%; 1.2L) with cooling. Excess zinc was filtered off and the filtrate was extracted with ethyl acetate. The combined organic phases were washed in turn with aqueous ammonium chloride solution (20%), aqueous sodium thiosulfate solution (5%), and water until neutral reaction. To remove the excess iodo methane, a steam distillation was performed, in the course of which compound **6** precipitated. The crystals were collected by filtration and recrystallized from methanol to give **6** (42 g; 80%): m.p. 164–168°C; $[\alpha]_D$ −80°; UV 284 (4.3); ^{1}H NMR 0.47 (m, 1H, 14α,15α-CH_2), 0.92 (s, 3H, 18-H), 1.28 (m, 1H, 14α,15α-CH_2), 3.79 (s, 3H, 3-OCH_3), 3.90 (d, J = 6.1, 1H, 17β-H), 6.68 (d, J = 2.7, 1H, 4-H), 6.71 (dd, J = 8.3, 2.7, 1H, 2-H), 7.13 (d, J = 8.3, 1H, 1-H); $C_{20}H_{24}O_2$ (296.41) calculated C 81.04 H 8.16 found C 80.95 H 8.16.

3-Methoxy-14α,15α-methylenestra-1,3,5(10)-trien-17α-ol (2)

A solution of **6** (15 g; 50 mmol) in a mixture of tetrahydrofuran (300 mL) and aniline (18 mL; 0.195 mol) was added with stirring to liquid ammonia (510 mL) at −68°C to −70°C. Lithium (2.21 g; 0.318 g-atom) was then added in small portions carefully keeping the temperature in the same range and avoiding the blue color of the reaction mixture to disappear. After a reaction time of 90 min, ammonium chloride (10 g) was added and the mixture was allowed to warm up to room temperature. The solution was then diluted with water (40 mL), the tetrahydrofuran phase was separated and rotary evaporated. The resulting product was dissolved in ethyl acetate (100 mL), the solution was washed with hydrochloric acid (20%) and worked up as usual. The product was crystallized from methanol providing **2** (9.06 g; 60%): m.p. 146–147°C; $[\alpha]_D$ + 111°; UV 278 (3.31), 287 (3.29); ^{1}H NMR 0.38 (dd, J = 8.1, 3.8, 1H, 14α,15α-CH_2), 0.80 (t, J = 3.8, 1H, 14α,15α-CH_2), 0.99 (s, 3H, 18-H), 3.78 (s, 3H, 3-OCH_3), 3.84 (d, J = 6.1, 1H, 17β-H), 6.62 (d, J = 2.7, 1H, 4-H), 6.73 (dd, J = 8.8, 2.7, 1H, 2-H), 7.26 (d, J = 8.8, 1H, 1-H). $C_{20}H_{26}O_2$ (298.43) calculated C 80.50 H 8.78 found C 80.60 H 8.67. The mother liquor obtained by crystallization of **2** was subjected to chromatography (eluent: cyclohexane/ethyl acetate 70:30, v/v) yielding oily **7** (1.0 g; 6.6%) which crystallized from methanol: m.p. 82–84°C; $[\alpha]_D$ + 84°; UV 278 (4.25); ^{1}H NMR 0.96 (s, 3H, 18-H); 0.98 (d, J = 6.9, 3H, 15α-CH_3), 3.80 (s, 3H, 3-OCH_3), 3.83 (dd, J = 8.7, 8.2, 1H, 17β-H), 6.70 (d, J = 2.5, 1H, 4-H), 6.74 (dd, J = 8.2, 2.5, 1H, 2-H), 7.17 (d, J = 8.2, 1H, 1-H); $C_{20}H_{26}O_2$ (298.43) calculated C 80.50 H 8.78 found C 80.23 H 8.78.

3-Methoxy-14α,15α-methylenestra-1,3,5(10)-trien-17-one (3)

To a cooled (10°C) solution of **2** (20 g; 67 mmol) in acetone (500 mL) was added dropwise with stirring 37 mL of a chromic acid solution comprising chromic acid anhydride (15 g) dissolved in water (37.5 mL) and sulfuric acid (7.5 mL). Stirring was continued for another 15 min at 10–15°C. Propan-2-ol (15 mL) was then added to reduce the excess chromic acid, followed by addition of water (500 mL). The resulting solution was extracted with ethyl acetate. Usual work-up of the combined organic phases gave a residue (18.25 g) which on crystallization from methanol provided **3** (15.30 g; 77%): m.p. 115–117°C; $[\alpha]_D$ +99°; UV 278 (3.32), 287 (3.29); ^{1}H NMR −0.29 (dd, J = 6.0, 2.7, 1H, 14α,15α-CH_2), 0.80 (dt, J = 2.7, 6.0, 1H, 14α,15α-CH_2), 1.19 (s, 3H, 18-H), 3.79 (s, 3H, 3-OCH_3), 6.64 (d, J = 2.7, 1H, 4-H), 6.74 (dd, J = 8.5, 2.7, 1H, 2-H), 7.24 (d, J = 8.5, 1H, 1-H); $C_{20}H_{24}O_2$ (296.41) calculated C 81.04 H 8.16 found C 80.70 H 8.16.

3-Methoxy-14α,15α-methylenestra-1,3,5(10)-trien-17β-ol (4)

(a) To a suspension of sodium borohydride (10 g; 0.264 mol) in absolute tetrahydrofuran (435 mL) was added with stirring boron trifluoride-diethylether (34.8 mL; 0.276 mol) within 30 min at 0°C to 5°C. After having stirred the mixture for another 30 min, the salts were filtered off. A part of this diborane solution (360 mL) was now added within 30 min to a stirred solution of **3** (21 g; 71 mmol) in absolute tetrahydrofuran (90 mL), keeping the reaction temperature at 0–5°C. The temperature was maintained for another 2.5 h, water (1.5 L) was then added, and the precipitate filtered off. The dried product (20.63 g; 85% **4** and 15% **2**) was subjected to chromatography (eluent: cyclohexane/ethyl acetate 7:3 v/v) to give **2** (2.12 g; 10.3%) and **4** (16.35 g; 79.2%). **4:** m.p. 124–127°C (methanol); $[\alpha]_D$ + 127°; UV 278 (3.29), 287 nm (3.27); ^{1}H NMR 0.28 (m, 2H, 14α,15α-CH_2), 0.99 (s, 3H, 18-H), 3.55 (t, J = 8.0, 1H, 17α-H), 3.78 (s, 3H, 3-OCH_3), 6.62 (d, J = 2.4, 1H, 4-H), 6.73 (dd, J = 8.5, 2.4, 1H, 2-H), 7.25 (d, J = 8.5, 1H, 1-H); $C_{20}H_{26}O_2$ (298.43) calculated C 80.50 H 8.78 found C 80.38 H 8.69. (b) To liquid ammonia (50 mL) was added a solution of **10** (3 g; 10.1 mmol) in a mixture of tetrahydrofuran (30 mL) and aniline (3 mL; 32.5 mmol) at −68°C to −70°C with stirring. Lithium (0.744 g; 0.11 g-atom) was then introduced into the reaction mixture in small portions, keeping the temperature at the same range and avoiding the blue color of the solution to disappear. After 90 min the reaction was quenched by addition of ammonium chloride (2 g) and the temperature of the mixture was allowed to warm up to room temperature. The residue was diluted with water (10 mL) and the organic layer separated. The aqueous phase was extracted additionally with tetrahydrofuran and the combined organic solutions were rotary evaporated to give a product which was dissolved in ethyl acetate. This solution was washed with hydrochloric acid (20%) and worked up as usual to afford a residue, which was crystallized from methanol yielding **4** (1.51 g; 50%), identical in all respects with the compound obtained according to the protocol (a).

14α,15α-Methylenestra-1,3,5(10)-triene-3,17β-diol (8)

To **4** (20 g; 67 mmol), which was suspended in toluene (50 mL), was added diisobutyl aluminum hydride (30% solution in toluene, 65.01 g; 0.457 mol). This mixture was refluxed for 5 h and then

cooled. Ethanol (96%; 65 mL) and aqueous ethanol (50%; 65 mL) were added in turn at +20°C with stirring. Thereafter diluted hydrochloric acid was added (pH 2) and the phases were separated. The aqueous solution was extracted several times with ethyl acetate. The combined organic phases were washed with aqueous sodium acetate solution (10%) and water. Steam distillation gave crystals which were recrystallized from methanol to yield **8** (17.20 g; 90.2%): m.p. 213–214°C; $[\alpha]_D$ + 130°; UV 281 (3.29); ^{1}H NMR (pyridine-d_5) 0.25 (dd, J = 7.9, 5.0, 1H, 14α,15α-CH_2), 0.36 (dd, J = 5.0, 2.8, 1H, 14α,15α-CH_2), 1.26 (s, 3H, 18-H), 3.87 (t, J = 7.9, 1H, 17α-H), 7.01 (d, J = 2.1, 1H, 4-H), 7.13 (dd, J = 8.4, 2.1, 1H, 2-H), 7.38 (d, J = 8.4, 1H, 1-H); $C_{19}H_{24}O_2$ (284.40) calculated C 80.24 H 8.51 found C 80.21 H 8.52.

3-Methoxy-14α,15α-methylenestra-1,3,5(10),8-tetraen-17-one (**9**)

To a solution of dimethyl sulfoxide (60 mL; 0.844 mol), triethylamine (66.5 mL; 0.48 mol) and sulfur trioxide pyridine complex (29 g; 0.182 mol) was added **6** (10 g; 33 mmol) with cooling. The reaction mixture was stirred for 5 h at +5°C and was then poured into a mixture of ice water (1 L) and hydrochloric acid (18%; 36 mL). The precipitate was filtered off, washed with water, and dried. Recrystallization from chloroform/methanol provided **9** (7.65 g; 77%): m.p. 141–143°C; $[\alpha]_D$ −108°; UV 284 (4.26); ^{1}H NMR 0.15 (dd, J = 6.0, 2.5, 1H, 14α,15α-CH_2), 0.86 (t, J = 6.0, 1H, 14α,15α-CH_2), 1.17 (s, 3H, 18-H), 3.82 (s, 3H, 3-OCH_3), 6.70 (d, J = 2.5, 1H, 4-H), 6.75 (dd, J = 8.0, 2.5, 1H, 2-H), 7.13 (d, J = 8.0 1H, 1-H); $C_{20}H_{22}O_2$ (294.39) calculated C 81.60 H 7.53 found C 81.50 H 7.52.

3-Methoxy-14α,15α-methylenestra-1,3,5(10),8-tetraen-17β-ol (**10**)

9 (7.5 g; 25.4 mmol) dissolved in absolute tetrahydrofuran (30 mL) was treated with a diborane-tetrahydrofuran solution (145 mL) according to the reduction of **3.** The product (7.2 g) consisted of compounds **10** and **6** which were separated by chromatography (eluent: cyclohexane/ethyl acetate 7:3 v/v) to give **10** (5.13 g; 68%) on crystallization from methanol: m.p. 135–137°C; $[\alpha]_D$ −31°; UV 284 (4.32); ^{1}H NMR 0.37 (dd, J = 7.8, 5.1, 1H, 14α,15α-CH_2), 0.64 (dd, J = 5.2, 3.1, 1H, 14α,15α-CH_2), 0.95 (s, 3H, 18-H), 3.60 (dd, J = 8.8, 7.0, 1H, 17α-H), 3.79 (s, 3H, 3-OCH_3), 6.68 (d, J = 2.7, 1H, 4-H), 6.72 (dd, J = 8.5, 2.7, 1H, 2-H), 7.13 (d, J = 8.5, 1H, 1-H); $C_{20}H_{24}O_2$ (296.41) calculated C 81.04 H 8.16 found C 80.96 H 8.14.

3-Methoxy-14α,15α-methylen-9β-estra-1,3,5(10)-trien-17β-yl trifluoroacetate (**11**)

Triethylsilane (12.8 mL; 80.3 mmol) was added to a solution of **6** (10 g; 33 mmol) in dichloromethane (50 mL) with stirring at 20–25°C. Into this mixture trifluoroacetic acid (30 mL; 0.39 mol) was dropped in keeping the temperature in the same range. After having stirred for 16 h aqueous sodium carbonate solution (20%) was added to neutralize the reaction mixture. The organic phase was separated and worked up as usual. The residue was crystallized from methanol to give **11** (10.04 g; 75%): m.p. 102–105°C; $[\alpha]_D$ +95%; UV 278 (3.31), 287 (3.29); ^{1}H NMR 0.25 (dd, J = 6.2, 3.7, 1H, 14α,15α-CH_2), 0.69 (dd, J = 8.0, 6.2, 1H, 14α,15α-CH_2), 1.11 (s, 3H, 18-H), 3.78 (s, 3H, 3-OCH_3), 4.72 (dd, J = 9.2, 7.7, 1H, 17α-H), 6.62 (d, J = 2.8, 1H, 4-H), 6.74 (dd, J = 8.7, 2.8, 1H, 2-H), 7.25 (d, J = 8.7, 1H, 1-H); $C_{22}H_{25}F_3O_3$ (394.43) calculated C 66.99 H 6.39 found C 66.91 H 6.41.

3-Methoxy-14α,15α-methylen-9β-estra-1,3,5(10)-trien-17β-ol (**12**)

A solution of **11** (8.0 g; 20.2 mmol) in methanol (300 mL) was mixed with aqueous potassium hydroxide solution (2N; 57 mL) and the mixture was allowed to react for 2 h at room temperature. Water was then added and the precipitated crystals filtered off. Recrystallization from methanol provided **12** (5.44 g; 90%): m.p. 118–122°C; $[\alpha]_D$ +102°; UV 278 (3.33), 287 (3.27); ^{1}H NMR 0.17 (dd, J = 5.8, 3.9, 1H, 14α,15α-CH_2), 0.56 (dd, J = 8.4, 5.8, 1H, 14α,15α-CH_2), 1.07 (s, 3H, 18-H), 3.55 (dd, J = 16.0, 6.9, 1H, 17α-H), 3.78 (s, 3H, 3-OCH_3), 6.62 (d, J = 2.8, 1H, 4-H), 6.73 (dd, J = 8.5, 2.8, 1H, 2-H), 7.26 (d, J = 8.5, 1H, 1-H); $C_{20}H_{26}O_2$ (298.43) calculated C 80.50 H 8.78 found C 80.55 H 8.75.

3-Methoxy-14α,15α-methylen-9β-estra-1,3,5(10)-trien-17-one (**13**)

(a) Into a solution of **12** (5 g; 16.75 mmol) in acetone (120 mL) was dropped chromic acid solution (9 mL), as described earlier, with stirring at 10°C. After 30 min, propan-2-ol (5 mL) was added and water (150 mL). The mixture was then extracted with ethyl acetate. The extract was worked up as usual and the residue crystallized from methanol to give **13** (4.31 g; 87%): m.p. 166–167°C; $[\alpha]_D$ +263°; UV 278 (3.32), 287 (3.30); ^{1}H NMR −0.25 (dd, J = 6.0, 3.8, 2H, 14α,15α-CH_2), 1.08 (s, 3H, 18-H), 3.78 (s, 3H, 3-OCH_3), 6.63 (d, J = 2.9, 1H, 4-H), 6.74 (dd, J = 8.8, 2.9, 1H, 2-H), 7.24 (d, J = 8.8, 1H, 1-H); $C_{20}H_{24}O_2$ (296.41) calculated C 81.04 H 8.16 found C 81.00 H 8.05. (b) To a solution of **9** (3.5 g; 11.8 mmol) in dichloromethane (20 mL) and triethylsilane (4.5 mL; 28.2 mmol) was added trifluoroacetic acid (8.7 mL; 0.113 mol) at 20°C to 25°C with stirring. Stirring was continued for another 10 h followed by neutralization of the reaction mixture with aqueous sodium carbonate solution (20%). The organic phase was separated and worked up as usual to give a residue which was purified by chromatography (eluent: cyclohexane/ethyl acetate 7:3 v/v). Crystallization from methanol afforded **13** (2.64 g; 75%), identical in all respects with the compound obtained by protocol (a).

3-Methoxy-14α,15α-methylen-9β-estra-1,3,5(10)-trien-17β-ol (**12**) *and 3-methoxy-14α,15α-methylen-9β-estra-1,3,5(10)-trien-17α-ol* (**14**)

Sodium borohydride (2.19 g; 55.32 mmol) was added to a solution of **13** (8.2 g; 27.66 mmol) in a mixture of methanol and tetrahydrofuran (250 mL each) within 15 min with stirring. Stirring was continued for another 45 min. The solution was then acidified with acetic acid and concentrated in vacuo to a volume of 100 mL. Dilution with water and extraction with dichloromethane gave an extract which was worked up as usual. The product was subjected to chromatography (eluent: cyclohexane/ethyl acetate 7:3 v/v) to give **14** and **12** in that order. **14** (1.22 g; 14.8%): m.p. 139–143°C (methanol); $[\alpha]_D$ +118°; UV 278 (3.31), 287 (3.29); ^{1}H NMR 0.68 (m, 2H, 14α,15α-CH_2), 1.08 (s, 3H, 18-H), 3.68 (dd, J = 6.0, 1.8, 1H, 17β-H), 3.78 (s, 3H, 3-OCH_3), 6.62 (d, J = 2.6, 1H, 4-H), 6.73 (dd, J = 8.8, 2.6, 1H, 2-H), 7.24 (d, J = 8.8, 1H, 1-H); $C_{20}H_{26}O_2$ (298.43) calculated C 80.50 H 8.78 found C 80.68 H 8.75. **12** (5 g; 60.6%): The compound was identical in all respects with **12** prepared from **6** via **11.**

14α,15α-Methylenestra-1,3,5(10)-triene-3,17β-diyl diacetate (**15**)

8 (7.9 g; 28 mmol), dissolved in toluene (18 mL), was acetylated with acetic acid anhydride (14 mL; 0.145 mol) in the presence of

4-dimethylamino pyridine (0.143 g; 1.2 mmol) for 5 h at 50°C. The reaction was quenched by cooling to room temperature and addition of aqueous ethanol (90%; 14 mL). Water was then added and the solution was extracted with toluene. The combined extracts were washed with water and dried over anhydrous sodium sulfate. Rotary evaporation and crystallization of the residue from methanol gave **15** (8.72 g; 85%): m.p. 96–99°C; $[\alpha]_D$ +71°; UV 268 (2.89), 275 (2.89); ^{1}H NMR (pyridine-d_5) 0.22 (m, 1H, 14α,15α-CH_2), 0.29 (m, 1H, 14α,15α-CH_2), 1.02 (s, 3H, 18-H), 2.05 (s, 3H, 17β-OCOCH_3), 2.27 (s, 3H, 3-OCOCH_3), 4.75 (t, J = 8.2, 1H, 17α-H), 6.93 (d, J = 2.2, 1H, 4-H), 7.12 (dd, J = 8.2, 2.2, 1H, 2-H), 7.34 (d, J = 8.2, 1H, 1-H); $C_{23}H_{28}O_4$ (368.47) calculated C 74.97 H 7.66 found C 75.01 H 7.66.

14α,15α-Methylen-6-oxoestra-1,3,5(10)-triene-3,17β-diyl diacetate (16) *and 9ζ-hydroxy-14α,15α-methylen-6-oxoestra-1,3,5(10)-triene-3,17β-diyl diacetate* (17)

A mixture of chromium trioxide (56.1 g; 0.56 mol) and 3,5-dimethyl pyrazole (53.9 g; 0.56 mol) was stirred in dichloromethane (475 mL) for 1 h at −20°C. A solution of **15** (10 g; 27 mmol) in dichloromethane (60 mL) was then added within 10 min and the mixture was stirred for another 20 h, keeping the temperature between −10°C and −15°C during this time. Thereafter aqueous sodium hydroxide solution (5N; 230 mL) was added and the mixture was stirred for 1 h at 0°C. Dilution with water and extraction with dichloromethane gave an extract which was washed with diluted hydrochloric acid. Usual work-up gave a residue which was subjected to chromatography (eluent: tetrachloromethane/ethyl acetate 8:2 v/v) yielding **15** (0.58 g; 5.8%), **16** (3.31 g; 32%), and **17** (0.74 g; 6.8%) in that order. **16:** m.p. 163–167°C (methanol); $[\alpha]_D$ +16°; UV 247 (4.02), 299 (3.33); ^{1}H NMR 0.45 (m, 2H, 14α,15α-CH_2), 1.02 (s, 3H, 18-H), 2.05 (s, 3H, 17β-OCOCH_3), 2.31 (s, 3H, 3-OCOCH_3), 4.59 (dd, J = 9.7, 7.1, 1H, 17α-H), 7.26 (dd, J = 8.8, 2.8, 1H, 2-H), 7.48 (d, J = 8.8, 1H, 1-H), 7.73 (d, J = 2.8, 1H, 4-H); $C_{23}H_{26}O_5$ (382.46) calculated C 72.23 H 6.85 found C 72.39 H 6.82. **17:** m.p. 202–206°C (*tert*-butyl-methyl ether); $[\alpha]_D$ −10°; UV 246 (4.01), 295 (3.23); ^{1}H NMR 0.62 (m, 2H, 14α,15α-CH_2), 1.05 (s, 3H, 18-H), 2.06 (s, 3H, 17β-OCOCH_3), 2.32 (s, 3H, 3-OCOCH_3), 4.61 (dd, J = 9.5, 7.2, 1H, 17α-H), 7.33 (dd, J = 8.9, 2.5, 1H, 2-H), 7.68 (d, J = 8.9, 1H, 1-H), 7.76 (d, J = 2.5, 1H, 4-H); $C_{23}H_{26}O_6$ (398.46) calculated C 69.33 H 6.58 found C 69.25 H 6.61.

3,17β-Dihydroxy-14α,15α-methylenestra-1,3,5(10)-trien-6-one (18)

16 (3.17 g; 8.3 mmol), dissolved in 1,4-dioxane (64 mL) and methanol (128 mL), was saponified by stirring with aqueous potassium hydroxide solution (2N, 28 mL) for 3 h at room temperature. The reaction mixture was then diluted with water, acidified with hydrochloric acid and extracted with ethyl acetate. The combined organic phases were worked up as usual. The residue was crystallized from ethyl acetate to give **18:** m.p. 275–278°C; $[\alpha]_D$ +89° (1,4-dioxane); UV 255 (3.95), 325 (3.49); ^{1}H NMR (pyridine-d_5): 0.33 (m, 1H, 14α,15α-CH_2), 0.41 (m, 1H, 14α,15α-CH_2), 1.21 (s, 3H, 18-H), 3.86 (t, J = 7.9, 1H, 17α-H), 7.46 (s, 2H, 1-H and 2-H), 8.11 (s, 1H, 4-H); $C_{19}H_{22}O_3$ (298.38) calculated C 76.48 H 7.43 found C 76.39 H 7.49.

6-(O-Carboxymethyl)oximino-14α,15α-methylenestra-1,3,5(10)-triene-3,17β-diol (19)

To a solution of **18** (2 g; 6.7 mmol) in ethanol (96%; 90 mL) which was heated to 50°C, were added in turn aqueous sodium hydroxide solution (2N; 17 mL) and (O-carboxymethyl)-hydroxylamine hemihydrochloride (4.54 g; 25 mmol) with stirring. Stirring was continued for 3 h at room temperature. The solution was then concentrated in vacuo to a volume of approximately 40 mL, diluted with water (500 mL), adjusted to pH 8.5, extracted with ethyl acetate (2 × 100 mL each), and acidified with hydrochloric acid (18%). The precipitate was extracted with ethyl acetate and the combined organic solutions were worked up as usual. Crystallization of the residue from methanol provided **19** (1.76 g; 70.7%): m.p. 244–253°C (dec.); $[\alpha]_D$ + 36° (pyridine); UV 262 (4.04), 311 (3.57); ^{1}H NMR (pyridine-d_5): 0.34 (m, 2H, 14α,15α-CH_2), 1.16 (s, 3H, 18-H), 3.83 (t, J = 8.1, 1H, 17α-H), 5.17 (s, 2H, -O-CH_2-COOH), 7.30 (dd, J = 8.5, 2.2, 1H, 2-H), 7.41 (d, J = 8.5, 1H, 1-H), 8.27 (d, J = 2.2, 1H, 4-H); $C_{21}H_{25}NO_5$ (371.44) calculated C 67.91 H 6.78 N 3.77 found C 67.73 H 6.73 N 3.94.

14α,15α-Methylenestra-1,3,5(10)-triene-3,6α,17β-triol (20)

To a solution of **18** (2.5 g; 8.4 mmol) in methanol (250 mL) was added sodium borohydride (0.635 g; 16 mmol) in portions at a temperature ranging from 5–10°C. The reaction mixture was stirred for 1 h, acidified with acetic acid, and concentrated in vacuo to about 50 mL. That solution was diluted with water and extracted with ethyl acetate. Usual work-up of the combined extracts and crystallization of the residue from aqueous methanol gave **20** (2 g; 79.4%): m.p. 236–239°C; $[\alpha]_D$ +82° (pyridine); UV 282 nm (3.32); ^{1}H NMR (DMSO-d_6): 0.15 (m, 1H, 14α,15α-CH_2), 0.26 (m, 1H, 14α,15α-CH_2), 0.88 (s, 3H, 18-H), 3.39 (dd, J = 9.2, 6.3, 1H, 17α-H), 4.52 (dd, J = 10.1, 5.6, 1H, 6β-H), 6.57 (d, J = 8.2, 1H, 2-H), 6.94 (s, 1H, 4-H), 7.06 (d, J = 8.2, 1H, 1-H); $C_{19}H_{24}O_3$ (300.39) calculated C 75.97 H 8.05 found C 75.74 H 8.06.

14α,15α-Methylenestra-1,3,5(10),6-tetraene-3,17β-diol (21)

A solution of **20** (1 g; 3.3 mmol) in dimethyl sulfoxide (25 mL) was kept at 150°C for 3 h. After cooling to room temperature, water was added and the mixture extracted with ethyl acetate. The combined organic phases were washed with water, dried over anhydrous sodium sulfate, and rotary evaporated. The residue was crystallized from methanol yielding **21** (0.676 g; 72%): m.p. 223–226°C; $[\alpha]_D$ −67° (pyridine); UV 264 (3.83), 305 (3.39); ^{1}H NMR 0.35 (m, 1H, 14α,15α-CH_2), 0.43 (m, 1H, 14α,15α-CH_2), 0.99 (s, 3H, 18-H), 3.56 (dd, J = 8.8, 7.0, 1H, 17α-H), 5.35 (d, J = 9.7, 1H, 6-H), 6.35 (dd, J = 9.7, 2.8, 1H, 7-H), 6.54 (d, J = 2.8, 1H, 4-H), 6.66 (dd, J = 8.1, 2.8, 1H, 2-H), 7.13 (d, J = 8.1, 1H, 1-H); $C_{19}H_{22}O_2$ (282.38) calculated C 80.82 H 7.85 found C 80.70 H 7.89.

[2,4,6,7(n)-^{3}H]14α,15α-Methylenestra-1,3,5(10)-triene-3,17β-diol (22)*

21 (8 mg; 0.028 mmol) was dissolved in ethanol (1 mL), transferred to a tritiation flask containing palladium (10%) on charcoal. The mixture was stirred under an atmosphere of tritium gas (5 Ci) for 1 h. The catalyst was removed by filtration and the solution rotary evaporated to dryness. Labile tritium was removed using ethanol (3 × 10 mL) and the residue dissolved in the same solvent (20 mL). Crude yield: 792 mCi; analysis by silica TLC gave 90% radiochemical purity. This crude material was rotary evaporated to a small volume and applied to silica plates. The plates were eluted with carbon tetrachloride/ethyl acetate 3:1 v/v. After drying the plate, the band lining up with the marker, as visualized by UV, was taken and the silica extracted with ethanol (20 mL). The silica

was removed by filtration. Yield: 222 mCi. 55 mCi of the active solution was rotary evaporated to 1 mL and bromine in glacial acetic acid (100 μL in 5 mL) added until a persistent yellow color was obtained. The reaction was stirred for 1 h at room temperature. The solution was rotary evaporated to dryness and the residue dissolved in ethanol (0.75 mL). The solution was transferred to a tritiation flask containing palladium (10%) on charcoal (30 mg) and diisopropylethylamine (50 μL). The mixture was stirred under an atmosphere of tritium gas (3 Ci) for 2 h. The catalyst was removed by filtration and the solution rotary evaporated to dryness. Labile tritium was removed using ethanol (3 × 10 mL) and the residue dissolved in the same solvent (9 mL). Crude yield: 70 mCi; analysis by reverse phase TLC gave 70% radiochemical purity. The solution was rotary evaporated to dryness, redissolved in acetonitrile (300 μL), water (300 μL) was added, and the solution purified by reverse HPLC under the following conditions: column: hypersil ODS (250 × 4.6 mm); eluent: acetonitrile/water 35:65 v/v; flow: 1 mL/min; UV: 230 nm. The UV active peak having the same retention time as authentic **8** (retention time 38–46 min) was taken. The solution was rotary evaporated to dryness and redissolved in ethanol (7 mL). Yield of **22:** 45 mCi; radiochemical purity: 97%; specific activity as determined by mass spectrometry gave 87 Ci/mmol.

2-Acetyl-3-hydroxy-15α-methylestra-1,3,5(10),8-tetraen-17β-yl acetate (23)

Dry aluminum chloride (1.23 g; 9.2 mmol) was suspended in chlorobenzene (7.5 mL) and treated with acetyl chloride (0.77 mL; 10.8 mmol) in chlorobenzene (7.5 mL) at 0°C. **8** (0.5 g; 1.8 mmol) was added to the solution. After stirring for 3 h at 0°C to 5°C the mixture was poured into cold diluted hydrochloric acid (200 mL). The solution was extracted with ethyl acetate. The combined extracts were worked up as usual, thus yielding an oil (1.21 g) which was purified by chromatography (eluent: *n*-hexane/ethyl acetate 9:1 v/v) to afford the 15α-methyl compound **23** (0.37 g; 56%) as crystals. An analytical sample was obtained by recrystallization from methanol: m.p. 138–140°C; $[\alpha]_D$ +140° (dioxan); UV 250 (4.69); ^{1}H NMR 0.91 (s, 3H, 18-H), 0.96 (d, J = 7.0, 3H, 15α-CH_3), 2.08 (s, 3H, 17β-$OCOCH_3$), 2.63 (s, 3H, 2-$COCH_3$), 4.97 (m, 1H, 17α-H), 6.76 (s, 1H, 4-H), 7.49 (s, 1H, 1-H); $C_{23}H_{28}O_4$ (368.47) calculated C 74.97 H 7.66 found C 74.76 H 7.65.

2-Acetyl-3-methoxy-14α,15α-methylenestra-1,3,5(10)-trien-17β-yl acetate (24)

Boron trifluoride ethyl ether (10 mL; 79.6 mmol) was dropped to acetic anhydride (7.5 mL; 79.3 mmol) at 0–5°C. After stirring for 0.5 h the solution was added to a solution of **4** (2.0 g; 6.7 mmol) in dichloromethane (13 mL) at 0°C. The reaction mixture was stirred for 2 h at 0–5°C and then poured into cold aqueous sodium acetate solution (10%). Extraction with dichloromethane and usual work-up of the combined extracts afforded a semicrystalline residue (2.4 g) which was purified by chromatography (eluent *n*-hexane/ethyl acetate 9:1 v/v). **24** was obtained as crystals (1.6 g; 63%) and used for the next step without recrystallization: m.p. 188–193°C; ^{1}H NMR 0.34 (m, 2H, 14α,15α-CH_2), 1.02 (s, 3H, 18-H), 2.04 (s, 3H, 17β-$OCOCH_3$), 2.59 (s, 3H, 2-$COCH_3$), 3.87 (s, 3H, 3-OCH_3), 4.56 (dd, J = 9.0, 7.0, 1H, 17α-H), 6.65 (s, 1H, 2-H), 7.73 (s, 1H, 1-H). As a less polar by-product the 4-acetyl isomer (150 mg; 6%) was separated: m.p. 188–196°C; ^{1}H NMR 0.33 (m, 2H, 14α,15α-CH_2), 1.01 (s, 3H, 18-H), 2.04 (s, 3H, 17β-$OCOCH_3$), 2.46 (s, 3H, 2-$COCH_3$), 3.80 (s, 3H, 3-OCH_3), 4.56 (dd, J = 9.0, 7.0, 1H, 17α-H), 6.76 (d, J = 8.5, 1H, 2-H), 7.29 (d, J = 8.5, 1H, 1-H).

3-Methoxy-14α,15α-methylenestra-1,3,5(10)-triene-2,17β-diyl diacetate (25)

The 2-acetyl compound **24** (1.5 g; 3.9 mmol) was dissolved in dichloromethane (150 mL) and *m*-chloroperbenzoic acid (70%; 15 g; 60.8 mmol) was added to the solution in portions. After stirring for 5 h at room temperature, the mixture was treated with aqueous sodium hydroxide solution (5%; 150 mL) and water (100 mL). The organic layer was then separated and washed with aqueous sodium hydroxide solution (5%; 50 mL) and water (3 × 100 mL). Drying with anhydrous magnesium sulfate and evaporation in vacuo gave a foam (1.43 g) which was purified by chromatography (eluent: toluene/ethyl acetate 97:3 v/v). The crystalline 2-acetoxy compound **25** (0.83 g; 54%) was used in the next step without recrystallization: ^{1}H NMR 0.33 (m, 2H, 14α,15α-CH_2), 1.01 (s, 3H, 18-H), 2.04 (s, 3H, 17β-$OCOCH_3$), 2.30 (s, 3H, 2-$OCOCH_3$), 3.79 (s, 3H, 3-OCH_3), 4.56 (dd, J = 9.0, 7.0, 1H, 17α-H), 6.65 (s, 1H, 2-H), 6.96 (s, 1H, 1-H).

14α,15α-Methylenestra-1,3,5(10)-triene-2,3,17β-triol (26)

25 (0.79 g; 2.0 mmol) was dissolved in toluene (4 mL). Diisobutyl aluminiumhydride (4.9 mL; 26.9 mmol) in toluene (10 mL) was dropped to the solution. After refluxing for 15 h, the mixture was cooled to −10°C and treated with ethanol (96%; 5 mL) that was added dropwise while the temperature was being raised to 10°C. Subsequent dropwise addition of aqueous ethanol (50%; 5 mL) and hydrochloric acid (3N, 15 mL), stirring for 0.5 h, addition of ethyl acetate, separation of the organic layer, and usual work-up afforded crystalline product (0.51 g) which was purified by chromatography (eluent: dichloromethane/methanol 98:2 to 95:5 v/v) and crystallized from acetone/n-hexane to give **26** (0.306 g; 50%): m.p. 250–252°C; $[\alpha]_D$ +42° (dioxan); UV 289 (3.67); ^{1}H NMR (DMSO-d_6) 0.19 (m, 2H, 14α,15α-CH_2), 0.88 (s, 3H, 18-H), 4.37 (d, J = 5.0, 1H, 17β-OH), 6.38 (s, 1H, 2-H), 6.65 (s, 1H, 1-H), 8.51 (s, 1H, 2- or 3-OH), 8.52 (s, 1H, 3- or 2-OH); $C_{19}H_{24}O_3$ (300.40) calculated C 75.97 H 8.05 found C 75.31 H 8.02.

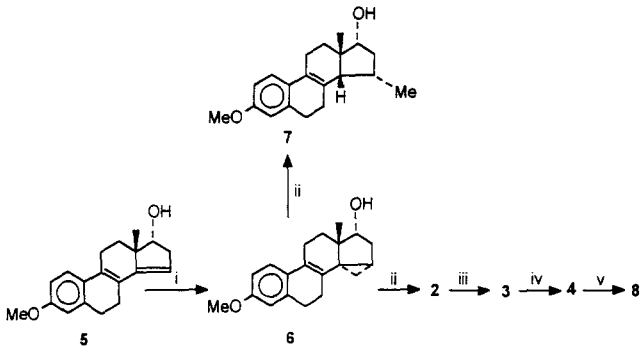
Results and Discussion

14α,15α-Methylenestra-1,3,5(10)-triene-3,17β-diol (8)

17α-Hydroxysteroid **5** was specifically chosen as starting material in an attempt to meet the conditions for a stereoselective carbene addition and to circumvent the ineffective reduction of a 17-oxo precursor as well (Scheme 2).[9]

Sonochemical reaction of **5** with zinc and diiodomethane[10,11] regioselectively and stereoselectively gave the 14α,15α-methylene compound **6.** By the use of ultrasound the reaction was considerably accelerated. No cyclopropanation at the 8-double bond and no dimers[12] was observed. Lithium–liquid ammonia reduction of **6** in the presence of aniline as proton source[13] led to compound **2.** The yield of the desired 8β-*H*,9α-*H* compound **2** was strongly dependent on the metal used and the mode of metal addition (Table 1).

Thus the addition of metal to the liquid ammonia solution of **6** always gave better results than the addition of **6** to the solution of metal in liquid ammonia. Lithium proved the metal of choice, while sodium and calcium gave unsatisfactory yields. Mother liquors, obtained from scale-up crystallizations of **2,** allowed the chromatographic isolation of



Scheme 2 Synthesis of 14α,15α-methylen-estradiol (8) from 3-methoxyestra-1,3,5(10),8,14-pentaen-17α-ol **(5)**. (i) Zn, CH_2J_2 sonification; (ii) Li, $NH_{3\ liqu.}$, $PhNH_2$; (iii) CrO_3, H_2O, H_2SO_4, acetone; (iv) B_2H_6, THF; (v) DIBAH, PhMe, 110°C.

the 15α-methyl derivative **7** (approximately 7%). Evidently, reductive cyclopropane cleavage occurs to a minor degree, when **6** reacts with lithium in liquid ammonia.

Oxidation of alcohol **2** by Jones reagent[14] provided ketone **3.** The stereoselective reduction of the 17-oxo group was best effected with diborane[2] at 0–5°C, affording **4** and **2** in the proportion of 85:15. Chromatography of the mixture yielded 17β-alcohol **4** identical in all respects with the compound prepared from **1.** The chromatographically separated 17α-alcohol **2** was recycled to **3** by Jones oxidation. Finally compound **4,** when treated with diisobutyl aluminiumhydride,[15] gave title compound **8.**

Hydrogenation of the 8-double bond and inversion of the 17α-hydroxy group in reversed order was also successful (Scheme 3).

Thus oxidation of compound **6** by the Parikh-Doering reagent[16] provided 17-ketone **9,** which afforded **10** on diborane reduction at 0–5°C and chromatography. In the following **10** was treated with lithium in liquid ammonia with addition of aniline to give compound **4.** Although vinyl cyclopropane systems similar to **9** have been reported to react readily with diborane,[17] neither double bond hydrogenation nor cyclopropane ring opening occurred during oxo group reduction of **9.** This is likely to be due to the electronic deficiency of the 8-double bond, which is in conjugation with the aromatic A-ring.

In addition to the reduction of **6** under Birch conditions attempts were made to reduce the 8-double bond of **6** by ionic hydrogenation.[18]

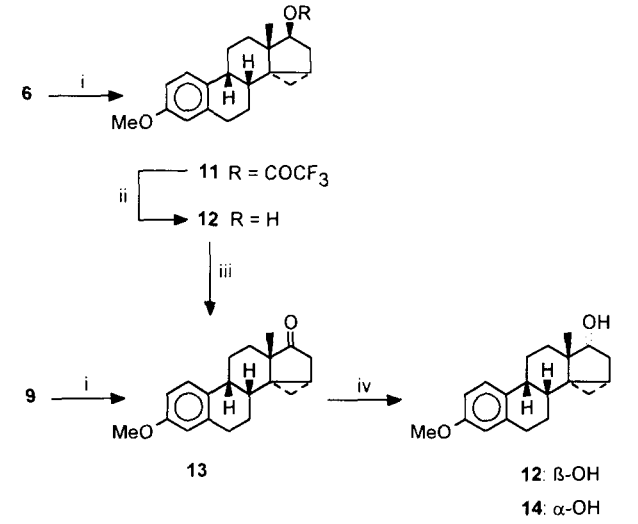
Table 1 Formation of **2** by metal-liquid ammonia reduction of **6**; dependence on the metal used and the mode of metal addition

	Formation of **2** (%)	
Metal	Metal added to the NH_3/THF/**6** solution	**6**/THF solution added to the metal/NH_3 solution
Li	72–84	20
Na	47	11
Ca	25	7

Scheme 3 Alternative synthesis of 3-methoxy-14α,15α-methylenestra-1,3,5(10)-trien-17β-ol **(4)** from 3-methoxy-14α,15α-methylenestra-1,3,5(10),8-tetraen-17α-ol **(6).** (i) DMSO, pyridine-SO_3, NEt_3, CH_2Cl_2; (ii) B_2H_6, THF; (iii) Li, $NH_{3\ liqu.}$, $PhNH_2$.

This method was shown to give high yields of 8β-*H*, 9α-*H* compounds on reduction of estra-1,3,5(10),8-tetraenes with silane as a hydride donor in trifluoroacetic acid.[19] However, when **6** was allowed to react with triethylsilane in trifluoroacetic acid, predominantly *cis* hydrogenation of the 8-double bond occurred, together with epimerization at C-17, and compound **12** was obtained upon hydrolysis of the primarily formed trifluoroacetate **11** (Scheme 4). The 8β-*H*, 9β-*H* configuration of **12** and the β-position of the 17-hydroxy group followed from NMR data. The β-position of the 17-hydroxy group was confirmed by oxidation of **12** to yield oxo steroid **13,** sodium borohydride reduction of which gave **12** and the α-alcohol **14. 13** was also formed by ionic hydrogenation of **9.**

The probable driving force of the surprising configurational change at C-17 is disclosed by a molecular model of **6:** the α-oriented 17-hydroxy and 14,15-methylene groups give rise to a considerable steric crowding at the rear site of ring D, which disappears on transformation of **6** into **12.** The mechanism of the epimerization is unclear as yet. Treatment of the acetate of **6** with triethylsilane/trifluoroacetic acid gave the acetate of **12,** demonstrating that in situ elimination of water or trifluoroacetic acid must be excluded. Probably the 17α-ester group is involved in an S_N1 mechanism or this effect may be due to participation of the cyclopropyl group.[20]



Scheme 4 Ionic hydrogenation of 3-methoxy-14α,15α-methylenestra-1,3,5(10),8-tetraen-17α-ol **(6).** (i) CF_3COOH, Et_3SiH; (ii) KOH, MeOH; (iii) CrO_3, H_2O, H_2SO_4, acetone; (iv) $NaBH_4$, MeOH/THF.

6-(O-Carboxymethyl)oximino-14α,15α-methylen-estra-1,3,5(10)-triene-3,17β-diol (19)

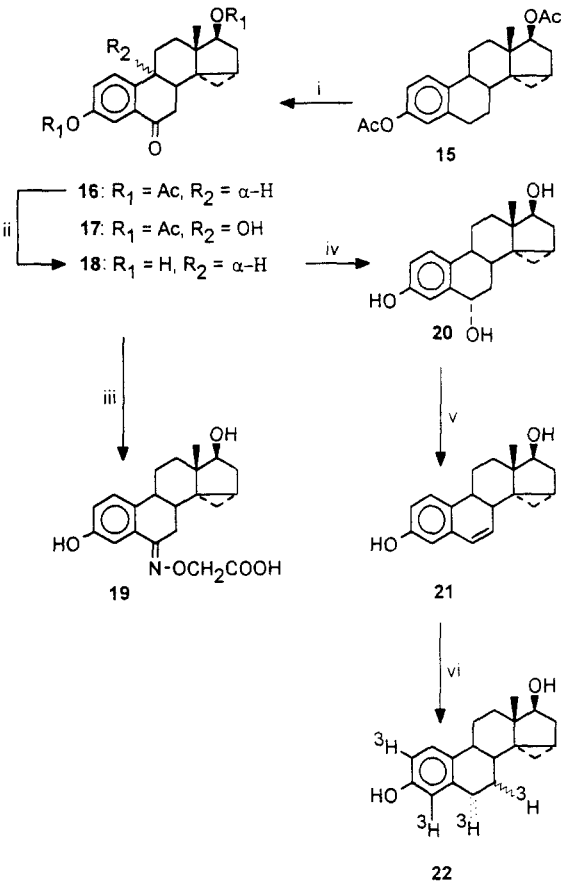
A well-established method for developing an estrogen radioimmunoassay is based on protein coupling with the steroid nucleus at position 6.[21,22] 6-(O-Carboxymethyl)oximes have been proven as haptens in this context.[23] We therefore set out to prepare the oxime derivative **19** from **8** via the 6-oxo compound **18** (Scheme 5).

Various methods have been described for the oxidation of estrogens at C-6 using chromium(VI) reagents. By oxidation with chromium(VI) oxide in acetic acid or aqueous sulfuric acid the corresponding 6-oxo derivatives were the only neutral products isolated, when an acetoxy group was substituted at C-3. However, 9-hydroxy-11-ketones and 9-hydroxy-6,11-diketones have been found as the major neutral products in case of an electron donating methoxy group at C-3.[24,25] Later on it was found that increased yields of 6-oxo-estra-1,3,5(10)-trien-3-yl acetates were obtained, when the oxidation was run with the in situ generated chromium trioxide-3,5-dimethylpyrazole complex (CrO_3-DMP).[26,27]

Considering these previous results, we treated the diacetate **15** with CrO_3-DMP in dichloromethane at −10–−15°C, which resulted in the formation of 6-oxo derivative **16.** Hydrolysis of **16** gave diol **18,** which was converted into the hapten **19** by an usual method. During the oxidation of diacetate **15** at C-6, oxyfunctionalization at C-9 occurred, affording 9-hydroxy-6-oxo compound **17** as a by-product.

9-Hydroxy-11-oxo estrogens were suggested to be formed from 3-methoxy-estra-1,3,5(10)–trienes by initial oxyfunctionalization at C-9 with chromic acid. Dehydration of the 9-hydroxy group would then afford a 9(11)-unsaturated intermediate, further attacked by oxidation.[28] However in case of **15** the weak acidic CrO_3-DMP reagent seems to prevent dehydration and thus oxidation at ring C does not occur. Nevertheless, the oxidation of the acetate **15** at C-9 is noticeable in the light of previous publications.[24,25]

Very recently, selective oxidation of methylene units adjacent to cyclopropane rings by CrO_3-DMP has been reported.[29] However, not unexpectedly, none of the 16-oxo derivatives in question have been detected as oxidation products of diacetate **15,** since methylene groups, located within five-membered rings or located in the vicinity of an acetoxy moiety, seem to be deactivated against oxidation.[29]



Scheme 5 Synthesis of the hapten 6-(O-carboxymethyl)oximino-14α,15α-methylene-3,17β-diol **(19)** and ^3H-labeled 14α,15α-methylene estradiol **(22).** (i) CrO_3-DMP, CH_2Cl_2, −15°C; (ii) NaOH, MeOH/dioxan; (iii) $(HOOC\text{-}CH_2\text{-}O\text{-}NH_2)_2$.HCl, NaOH, $EtOH/H_2O$; (iv) $NaBH_4$, MeOH; (v) DMSO, 150°C; (vi) (1) $^3H_2$, Pd-C, TLC (2) Br_2, AcOH (3) $^3H_2$, Pd-C (4) HPLC.

[2,4,6,7(n)-^{3}H]14α,15α-Methylenestra-1,3,5(10)-triene-3,17β-diol (22)

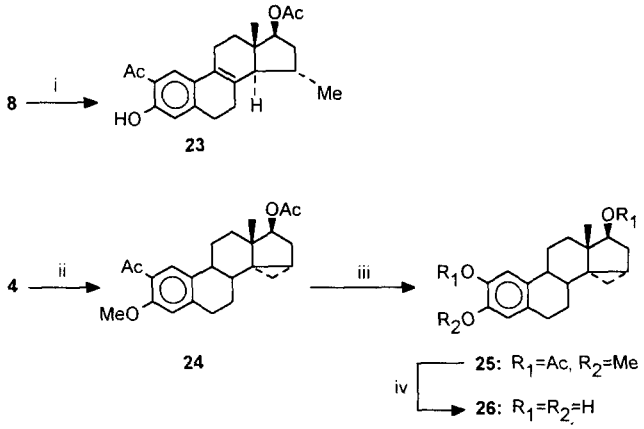
The cold precursor **21** was obtained from **18** (Scheme 5). Sodium borohydride reduction gave 6α-hydroxy derivative **20.**† Heating **20** in dimethyl sulfoxide for 3 h at 150°C[30] gave rise to complete water elimination thus affording 6-dehydro compound **21.** Tritiation of the 6-double bond occurred readily over palladium on charcoal to give the [6,7(n)-^{3}H] species. After purification by preparative TLC this compound was brominated at positions 2 and 4. Thereupon the bromide was treated with tritium in the presence of palladium on charcoal affording the title compound **22.** The product, purified by reverse phase HPLC, had a specific activity of 87 Ci/mmol and a radiochemical purity of 97%.

14α,15α-Methylenestra-1,3,5(10)-triene-2,3,17β-triol (26)

According to Xie et al.,[31] introduction of the 2-hydroxy group into estradiol derivatives is possible via Friedel Crafts acylation with acetyl chloride-aluminium chloride followed by Dakin oxidation and saponification.

In the case of 14α,15α-methylene estradiol **8,** however, acetyl chloride/aluminium chloride additionally caused quantitative cleavage of the cyclopropane ring with isomerization to the 15α-methyl-8-dehydro derivative **23.** Obviously, the ring cleavage reaction was due to hydrochloric acid formation and should be avoidable by using conditions not producing such strong acids. Indeed boron trifluoride-acetic anhydride[32,33] did not cleave the cyclopropane moiety but the resulting electrophile proved to too weak for C-acetylation of a phenol, yielding the diacetate **15** only.

In order to increase the activity towards electrophilic attack, we switched to the methylether **4.** Reaction with boron trifluoride-acetic anhydride in dichloromethane at 5°C afforded the 2-acetyl compound **24** within 2 h, accom-



Scheme 6 Synthesis of 14α,15α-methylenestra-1,3,5(10)-triene-2,3,17β-triol **(26)**. (i) AcCl, $AlCl_3$, PhCl; (ii) Ac_2O, BF_3-OEt_2, CH_2Cl_2; (iii) m-Cl PBA, CH_2Cl_2; (iv) DIBAH, PhMe, 110°C.

panied by small amounts of the 4-acetyl isomer, which was separated easily by flash chromatography. Baeyer-Villiger oxidation of **24** with *m*-chloroperbenzoic acid provided the 2-acetoxy species **25,** which was treated with diisobutyl aluminium hydride to give **26.** Pure catechol steroid **26** was obtained upon flash chromatography on silica gel (Scheme 6).

In conclusion the present study shows that regioselective and stereoselective Simmons-Smith methylenation of the 17α-hydroxy steroid **5** and subsequent Birch reduction of the resulting 14α,15α-methylene steroid **6** provide scope for an efficient route to the title compound **8,** the hapten **19,** the 2-hydroxy derivative of **8,** and the ^{3}H-labeled species **22** as well. Application of this novel synthesis serves a multigram preparation of 14α,15α-methylene estradiol **(8)** and esters thereof for clinical investigations.

Notes

*The radiochemical synthesis was performed by Jones, M.C. and Chappelle, M.R. (Amersham, U.K.).
†In the reaction product approx. 10% of the corresponding 6β-alcohol was detected by NMR-spectroscopy.

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