MECHANISM OF STEROIDAL ENOL ETHER FORMATION

CONFIGURATION OF TRITIUM AT C-6 OF ESTRA-1,3,5(6)-TRIEN-3-OL-17-ONE-6,7-^{3}H AS ESTABLISHED DURING THE SYNTHESIS OF 19-NOR-17α-PREGNA-3,5-DIEN-20-YN-17-OL-7-^{3}H 17-ACETATE.

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SUMMARY

The nature of the reactions involved in the synthesis of 3-cyclopentyloxy-19-nor-17α-pregna-3,5-dien-20-yn-17-ol-7-^{3}H 17-acetate, allowed the determination of the stereochemistry of the tritium atoms at C-6 in some of the precursors. One of these precursors was estra-1,3,5(6)-trien-3-ol-17-one-6,7-^{3}H in which 82% of the tritium was in the 6α position and 18% in the 6β position.

Supportive evidence for the mechanism of enol ether formation was obtained, also. Acid catalyzed enol ether formation, stereospecifically removed the triton from the 6β position. On hydrolysis of the enol ether, the proton stereospecifically entered the 6β position. Equilibration with base completed the removal of the tritium from the C-6 position.

INTRODUCTION

The preparation of a new progestational agent, 3-cyclopentyloxy-19-nor-17α-pregna-3,5-dien-20-yn-17-ol 17-acetate[1] (XI), labeled with tritium was required in order to determine its absorption and excretion rates and to investigate its biotransformation products. The synthesis, as shown in Scheme 1[2], was designed to incorporate tritium into the steroid nucleus principally at C-6 and C-7. The nature of the reactions involved, allowed the determination of the stereochemistry of the tritium atoms about C-6

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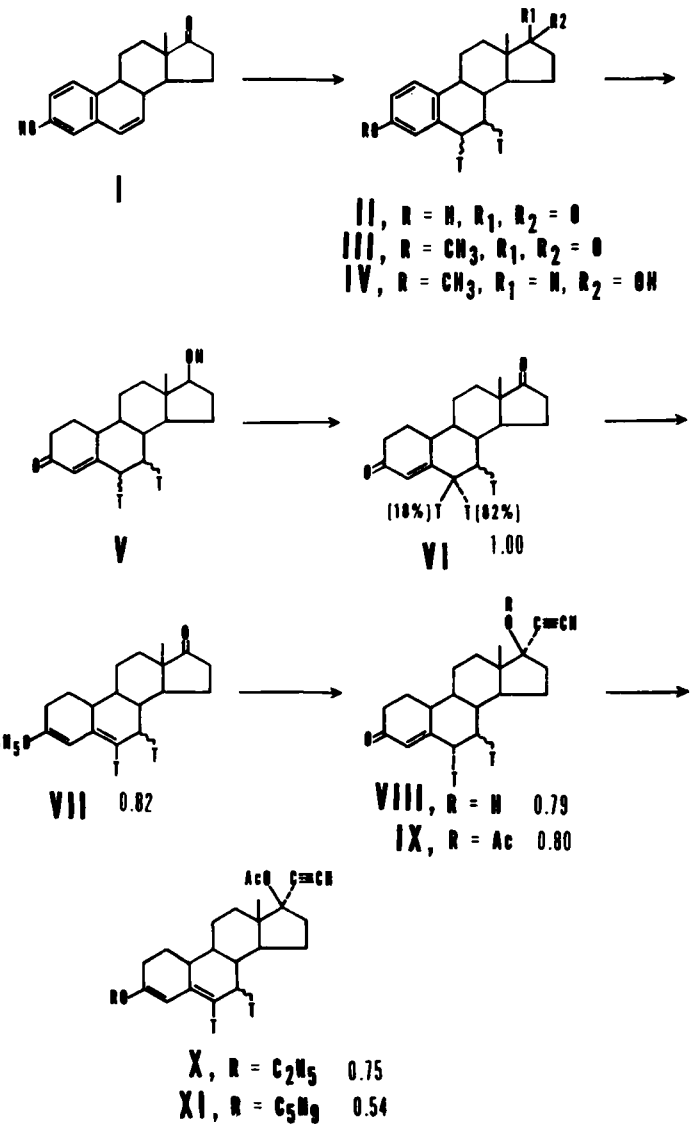
SCHEME 1


I
II, R = H, R1, R2 = O
III, R = CH3, R1, R2 = O
IV, R = CH3, R1 = H, R2 = OH
V
VI 1.00
(18%)T T(82%)
VII 0.82
VIII, R = H 0.79
IX, R = Ac 0.80
X, R = C2H5 0.75
XI, R = C5H9 0.54
XIb, R = C5H9 0.61

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in some of the precursors. In addition, supportive evidence for the mechanism of enol ether formation and hydrolysis in Δ^4-3-keto steroids[3,4,5] was obtained.

DISCUSSION

The synthetic scheme was patterned after published procedures, modified to allow the handling of small quantities of radioactive materials. The catalytic reduction of estra-1,3,5(10),6-tetraen-3-ol-17-one (6-dehydroestrone) (I) with carrier free tritium gas has been described.[6] The conversion of estrone (II-$^1H_2$) into 4-estren-3,17-dione (VI-$^1H_2$) has been reported[7] and modified[8], and has been extended to include 19-nor-17α-pregn-4-en-20-yn-17-ol-3-one[8] (VIII-$^1H_2$). The published route with slight modification, allowed the preparation of the radioactive equivalent VIII. Acetylation of VIII gave the 17-acetate (IX).

Using a known method[9], IX was converted into its ethyl enol ether (X). Treatment of X with cyclopentanol and a catalytic amount of p-toluenesulfonic acid (p-TsOH), yielded XI.

The data listed in Table I indicate the loss of 18% of the tritium when VI was converted into VII and no further statistically significant losses ($p > 0.05$ based on t-test) until the conversion of IX into X, in which a statistically significant loss ($p < 0.05$) was observed.

An additional 25% loss resulted during the final step. Thus, the over-all loss of tritium from VI was 46%.

The large loss of tritium during the synthesis of XI was unexpected, and the reaction was repeated using pyridinium tosylate[10] as the catalyst. This reaction caused a tritium loss in XIb of only 19%, or an over-all loss of 39% from VI.

In order to understand the processes involved in these reactions, IX was refluxed in 1N methanolic KOH which removed all the tritium at C-6[11], thus causing a tritium loss of 27% in the product, VIIIa. When VIIIa was converted to XIa as described above, the tritium loss for IXa, Xa, and XIa, was 0, 6, and 0% respectively, as shown in Scheme 2[2]. The 6% loss was statistically significant ($p < 0.05$).

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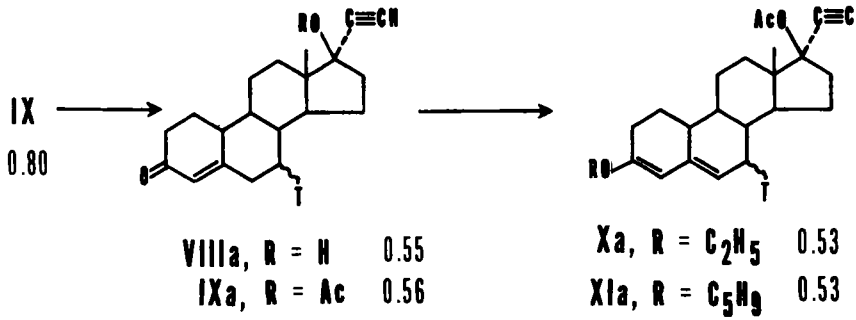
The abstraction of a C-6 proton has been postulated[12] to occur during the formation of enol ethers of Δ^4-3-keto steroids. Subsequently,[3,4,5] both the acid and base catalyzed protonation and de-protonation of Δ^4-3-keto steroids and their ethyl enol ethers were investigated and it was shown that in the formation of the enol ethers, the 6β-proton was removed preferentially, with the preference being enhanced in the absence of the C-19 methyl group.

SCHEME 2



The data summarized in Table I confirm these observations. The initial formation of VII from VI involved stereospecific loss of the 6β proton or triton. Conversely, on hydrolysis of VII, the proton stereospecifically entered the 6β position leaving tritium exclusively α at C-6. In accordance with this hypothesis, little loss of tritium occurred during the second enol ether formation of X from IX. After the remaining tritium in the 6α position was removed by base-catalyzed exchange in IX, subsequent reactions involved little or no further loss of tritium, as expected from the above hypothesis.

The transetherification of X, using p-TsOH as the catalyst, must be a reversible reaction to account for the complete loss of tritium in the 6α position. (Note that XI and VIIIa contain about the same amount of tritium.) This reaction definitely does not involve the C-7 position since no tritium loss was observed in conversion of Xa to XIa. When the more bulky pyridinium tosylate was used to catalyze the transformation of X to XIb, less tritium was lost, indicating that under these conditions, the reaction is not entirely reversible.

The loss of 6% of tritium during the conversion of IX to X was unanticipated. Originally, this slight loss was assumed to be due to a slight involvement of tritium at 6α in the conversion of IX to X. However, when IXa which was devoid of tritium at C-6, also lost 6% of the available tritium, this assumption was not tenable. Perhaps some minor mechanism for the formation of enol ethers involving the C-7 position was operative. This aspect requires further study.

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It is clear from this work that 45% of the tritium which was incorporated into I via catalytic reduction entered at C-6. Of this 18% was in the β position and 82% in the α position.

TABLE I

Specific Activities of Reactants and Products Used to Determine Tritium Retention

Reaction	Specific[a] Activity Reactant	Specific[a] Activity Product	Tritium Fraction Retained	Fraction Normalized to VI
VI → VII	788 μCi/mM	640 μCi/mM	0.824[b]	0.82
VII → VIII	10.21 μCi/mM	9.83 μCi/mM	0.962	0.79
VIII → IX	9.83 μCi/mM	9.96 μCi/mM	1.010	0.80
IX → X	68.6 μCi/mM	64.3 μCi/mM	0.936[b]	0.75
X → XI	67.8 μCi/mM	49.0 μCi/mM	0.722[b]	0.54
X → XIb	64.5 μCi/mM	52.7 μCi/mM	0.817[b]	0.61
IX → VIIIa	139 μCi/mM	94.9 μCi/mM	0.690	0.55
VIIIa → IXa	94.9 μCi/mM	95.7 μCi/mM	1.010	0.56
IXa → Xa	95.7 μCi/mM	90.5 μCi/mM	0.950	0.53
Xa → XIa	90.5 μCi/mM	90.7 μCi/mM	1.002	0.53

[a]These results are the geometric mean obtained from a logarithmic analysis of the specific activities determined either in duplicate or triplicate. The geometric mean of the coefficient of variation for these determinations was 2.4%.

[b]These results are the geometric mean obtained from a logarithmic analysis of reactions done in duplicate or triplicate. The geometric mean of the coefficient of variation for these determinations was 3.4%.

EXPERIMENTAL

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,[13] using a Packard 3310 spectrometer equipped with automatic external standardization. Uv and ir spectra were determined with Beckman DK-1a and Perkin-Elmer 621 spectrophotometers. Glpc were performed using a Warner-Chilcott Laboratories Instrument Division Model 1670 unit with a 6 ft. x 0.1 in. glass column packed with 60-100 mesh Gas-Chrom Q coated with 2% SE-30 using a helium flow rate of 50 ml/min and a temperature no higher than 213°.

The following tlc systems were employed:

System A Silica gel GF; Chloroform/Ethyl acetate (4:1)
System B Alumina GF; Cyclohexane/Ethyl acetate (1:1)
System C Alumina GF; Cyclohexane/Ethyl acetate/Pyridine (9.5:0.5:0.01)
System D Alumina GF; Cyclohexane/Ethyl acetate (7:3)
System E Alumina GF; Benzene/Acetone (9:1)
System F Alumina GF; Cyclohexane/Ethyl acetate/Pyridine (9:1:.001)
System G Silica gel GF; Cyclohexane/Ethyl acetate (1:1)

All tlc's were run on 5 x 20 cm glass plates (Analtech, Inc.) precoated with a 250 μ thick layer of the adsorbent, unless otherwise noted. Preparative tlc was done using 20 x 20 cm glass plates (Analtech, Inc.) coated with a 250 μ thick layer of silica gel GF prewashed with

methanol and reactivated by heating at 100° for 1 hour. The solvents used for chromatography and isotope dilutions were purified as previously described.[6] The compounds were visualized as blue spots on a fluorescent background when the plates were exposed to ultraviolet light, principally at 254 nm. The reported R_f values were the same as those of authentic samples. The radiochemical purity of all compounds was greater than 98% when determined by scanning the tlc plates using a Packard Radiochromatogram Scanner Model 7200.

4-Estren-3,17-dione-6,7-^{3}H[6] (VI)

Compound II was formed in quantitative yield in the catalytic reduction of 64.5 mg (0.24 mM) of I and 16 Ci of carrier-free tritium gas (New England Nuclear Corp.) and was converted without purification into III (62.5 mg, 95%, after twice recycling the recovered II). It was easier to allow a mixture of III and lithium aluminum hydride in ether to reflux for two hours rather than use a Soxhlet extractor.[7] This modification gave 60.1 mg (95%) of IV. Tlc of IV in System A gave a main spot at an R_f of about 0.52.

The product of the Birch reduction, after acid hydrolysis, was subjected to preparative tlc using System A. This gave 11.4 mg (20%) of V, which by tlc in System A exhibited one spot at an R_f of about 0.43. Sufficient non-radioactive V was added to make 19.8 mg (0.072 mM) of V which was oxidized to give 17 mg (87%) of VI. Tlc in System B exhibited one spot at an R_f of about 0.5. The specific activity was 15.6 mCi/mg (4.3 Ci/mM).

3,5(6)-Estradien-3-ol-17-one-6,7-^{3}H, 3-ethyl ether (VII)

To a solution of 17 mg of VI and 33 mg of non-radioactive VI (0.183 mM total) in 0.4 ml dioxane was added with swirling 0.1 ml triethyl orthoformate and 0.045 ml of a solution of 244 mg p-toluenesulfonic acid (p-TsOH) in 0.55 ml ethanol and 2.7 ml dioxane previously saturated with nitrogen. After 15 minutes, ten drops of pyridine and 2-3 ml benzene were added; and after swirling, the solvents were removed. The residue was dissolved in 5 ml of a mixture of 9 parts cyclohexane, 1 part ethyl acetate, and .001 parts pyridine and percolated through a 5 g Florisil column. Ten 5-ml and twenty 1-ml fractions of the eluate were collected. Fractions 10-27 were combined, filtered through a fine fritted glass funnel, and the solvents were removed. The residue gave 40 mg (73%) of VII. Tlc in System C gave a single spot at an R_f of 0.85.

19-Nor-17α-pregn-4-en-20-yn-17-ol-3-one-6,7-^{3}H (Norethindrone-6,7-^{3}H) (VIII)

The previously described ethynylation procedure[6] using a 10 molar excess of the ethylene diamine complex of lithium acetylide (Foote Mfg. Co.) was used. The crude VIII was extracted with three 10-ml portions of chloroform. The organic phases were combined, dried (K_2CO_3), filtered, and the solvent removed. The resulting oil was dissolved in 5 ml of a mixture of 7 parts cyclohexane and 3 parts ethyl acetate and percolated through a 5-g Florisil column. Three 5-ml fractions were collected. The presence of the relatively large amount of dimethyl acetamide (DMAC) which was not completely removed by this amount of Florisil, interfered with the

chromatographic separation. It was necessary to continue the separation through a second 5-g column of Florisil in order to remove the remaining DMAC completely. The above three 5-ml fractions were successively percolated through this second column followed by more of the solvent mixture. The 5-ml holdup volume was discarded and thirty 0.25-ml fractions were collected. Fractions 11-30 were combined, filtered through a fine fritted glass funnel and the solvents removed to give 22 mg (56%) of VIII. Tlc in System C exhibited a single spot at an Rf of about 0.5.

19-Nor-17α-pregn-4-en-20-yn-17-ol-3-one-6,7-^{3}H 17-Acetate (Norethindrone-6,7-^{3}H acetate) (IX)

To a solution of 22 mg (0.074 mM) of VIII in 0.5 ml dry pyridine was added 0.25 ml acetic anhydride and the resulting solution was allowed to reflux under nitrogen for 12 hr. The reaction was allowed to cool to room temperature and 10 ml ethyl acetate was added. The organic phase was washed with two 3-ml portions of a saturated NaCl solution and then 5 ml water. The aqueous phase was re-extracted with 5 ml ethyl acetate which was washed with 5 ml of the saturated NaCl solution. The organic phases were combined, dried (K_2CO_3), filtered, and the solvent removed. The residue was dissolved in 5 ml of a mixture of 8.5 parts cyclohexane, 1.5 parts ethyl acetate, and .001 parts pyridine and percolated through a 7.5-g Florisil column. One 80-ml fraction was collected and the eluate concentrated to about 0.5 ml. This solution was subjected to preparative tlc using System B. The area corresponding to an Rf between 0.75 and 0.85 was eluted successively with 20 ml of the solvent used in System B, 20 ml ethyl acetate and 35 ml methanol. The eluates were combined, filtered through a fine fritted glass funnel and the solvents were removed. The residue was dried in vacuo at 61° for 2 hrs. to give 12.4 mg (49%) of IX. This residue was dissolved in benzene, diluted with 117 mg of non-radioactive IX to give 130 mg after removal of solvent.

Quantitative tlc in System D exhibited a single spot at an R_f of about 0.5, while in System E a single spot was observed at an Rf of about 0.9; uv max. (C_2H_5OH) 239 nm (a = 49.3); ir (KBr) 3250, 1740, 1665, 1610, 1250, 1240, 1220, 1030, 1010, 890, 750 cm^{-1}. The specific activity of this material was 1.06 mCi/mg (360 mCi/mM). This material was dissolved in a mixture of 80 parts cyclohexane and 20 parts benzene and stored at 5° under nitrogen.

3-Ethoxy-19-nor-17α-pregna-3,5(6)-dien-20-yn-17-ol-6,7-^{3}H 17-Acetate (X)

To a mixture of 86.7 mg (0.254 mM) of IX in 0.51 ml triethyl orthoformate was added with swirling, 0.35 ml of a solution prepared by dissolving 18.8 mg p-TsOH in 5 ml absolute ethanol. After 1 hr. at room temperature, 0.5 ml pyridine was added to stabilize the product and the solvents were removed. The residue was dissolved in 5 ml of a mixture of 9 parts cyclohexane, 1 part ethyl acetate, and .001 part pyridine and percolated through a 10 g Florisil column. Five 5-ml fractions were collected. Fractions 2-5 were combined, filtered through a fine fritted glass funnel and the solvents were removed. The residue gave 93.1 mg (99%) of X. Tlc in System F exhibited a single spot at an Rf of about 0.85.

3-Cyclopentyloxy-19-nor-17α-pregna-3,5-dien-20-yn-17-ol-7-^{3}H 17-Acetate (XI)

To 1.54 ml of a solution prepared by dissolving 4.32 mg p-TsOH and 2.09 ml cyclopentanol in 5-ml dry benzene was added 25 ml dry benzene. This solution was distilled at atmospheric pressure and about 20 ml of distillate was collected. To the residue of this distillation was added a solution of 93.1 mg (0.246 mM) of X in 25 ml dry benzene and again the solution was distilled at atmospheric pressure. After collecting about 27 ml of distillate, the residue was cooled to room temperature and about 0.5 ml of pyridine and 4-5 ml dry benzene was added. The solvents were removed in vacuo. The residue was dissolved in about 5 ml of a mixture of 9 parts cyclohexane, 1 part ethyl acetate, and .001 parts dry pyridine and percolated through a 10-g Florisil column. Additional solvent was percolated through the column and six 5-ml fractions were collected. Fractions 2-6 were combined, filtered through a fine fritted glass funnel and the solvents removed. The residue was recrystallized three times by dissolving in about 0.5 ml methylene chloride and adding about 1.5 ml methanol containing .05% dry pyridine. The crystalline solid gave 42.3 mg (42%) of XI. To this solid was added 38.0 mg of non-radioactive XI and the mixture again recrystallized from the methylene chloride/methanol mixture to give 67.2 mg (84% recovery) of XI. The specific activity of XI was 289 mCi/mg (118 mCi/mM).

Tlc in System F exhibited a single spot at an R_f of about 0.9 which was identical with an authentic sample and contained 99+% of the radioactivity on the plate; uv max. (hexane) 242 nm (a = 49.7); ir (KBr) 3290, 1740, 1640, 1620, 1255, 1235, 1165, 1030, 845, 800 cm^{-1}.

The glpc system which was designed specifically to assay for X only (R_t of about 7 min.) in the presence of XI (R_t of about 18 min.) demonstrated the presence of <0.1% of X. This material was dissolved in a mixture of 80 parts cyclohexane, 20 parts benzene, and 0.05 parts piperidine, and was stored under nitrogen at 5°.

3-Cyclopentyloxy-19-nor-17α-pregna-3,5(6)-dien-20-yn-17-ol-6,7-^{3}H 17-acetate (XIb) (Pyridinium tosylate Method)

The conversion of 44.2 mg (65.1 μCi/mM) of X to XIb was repeated as previously described except that 0.421 mg (0.95%) of pyridinium tosylate was used as the catalyst instead of p-TsOH. About 0.021 ml of α-tocopherol was also included.

This procedure gave 27.6 mg (56%) of XIb whose specific activity was 52.8 μCi/mM. Tlc in System F exhibited a single spot at an R_f of about 0.9. Glpc demonstrated the absence of any X.

19-Nor-17α-pregn-4-en-20-yn-17-ol-3-one-7-^{3}H (Norethindrone-7-^{3}H) (VIIIa)

A solution of 150 mg (0.440 mM) of IX (S.A. = 139 μCi/mM) in 7.5 ml 0.98 N methanolic KOH was refluxed under nitrogen for 1.5 hrs., cooled and the solvent removed. One-fifth of this residue was purified via preparative tlc using System G and gave 12.5 mg (49%) of VIIIa. This material exhibited a single spot on tlc in System G at an R_f of about 0.5.

19-Nor-17α-pregn-4-en-20-yn-17-ol-7-^{3}H 17-Acetate (Norethindrone-7-^{3}H Acetate) (IXa)

The solvent was removed in vacuo from the remaining four-fifths of the above reaction and the residue of 120 mg, (0.401 mM) was acylated as previously described for IX. The product weighed 38.4 mg (28%) and exhibited a single spot at an R_f of about 0.4 on tlc in System D. The sample melted at 161.5-162.5° (authentic 163-163.5°).

3-Ethoxy-19-nor-17α-pregna-3,5(6)-dien-20-yn-17-ol-7-^{3}H 17-Acetate (Xa)

Thirty-four mg (0.1 mM) of IXa were allowed to react with triethyl orthoformate as described for X. From this reaction, 35 mg (95%) were isolated which exhibited a single spot at an R_f of 0.9 on tlc in System F.

3-Cyclopentyloxy-19-nor-17α-pregna-3,5(6)-dien-20-yn-17-ol-7-^{3}H 17-Acetate (XIa)

When 32 mg (0.085 mM) of X was allowed to react with cyclopentanol as previously described for XI, 27 mg (79%) of XIa was obtained. This material exhibited a single spot in System F at an R_f of about 0.9. Glpc demonstrated the absence of Xa.

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1. The accepted non-proprietary name for this compound is quingestanol acetate.

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