ETHYNYLATION OF 17-OXO STEROIDS LABELLED AT POSITION 16. SYNTHESIS OF LYNESTRENOL-16-^{3}H AT HIGH SPECIFIC ACTIVITY

A.I.A. Broess, J.S. Favier, N.P. van Vliet and D.C. Warrell*
R & D Laboratories, Organon Scientific Development Group,
Oss, The Netherlands.
Received on November 19, 1974.

SUMMARY

The reaction of estr-4-en-17-one-16-$^2H_2$ and 11α-hydroxy-estr-4-en-17-one-16-$^2H_2$ with potassium t-butoxide and acetylene in tetrahydrofuran gave the 17α-ethynyl-17β-ols with nearly complete loss of the label. Ethynylation with ethynyl magnesium bromide in tetrahydrofuran, however, proceeded with retention of the label. Application of the latter method to estrenone-16-^3H (specific activity 14.0 Ci/mmol) gave lynestrenol-16-^3H with a specific activity of 13.3 Ci/mmol.

INTRODUCTION

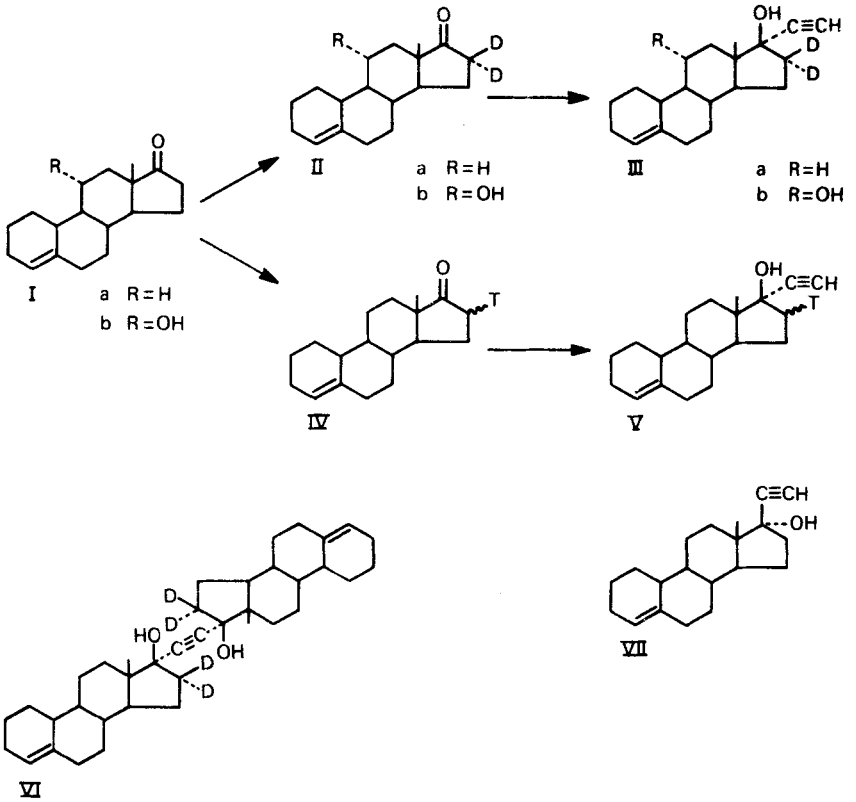
Recently the synthesis of the tritiated contraceptive progestin lynestrenol (17α-ethynyl-estr-4-en-17β-ol-^{3}H, V) has been described.[1] Estrenone (estr-4-en-17-one, Ia) was tritiated by an exchange procedure with tritiated water in dimethylformamide giving estrenone-16-^{3}H (IV) with a specific activity of 12 Ci/mmol. During the subsequent ethynylation with acetylene and potassium t-butoxide in tetrahydrofuran a considerable loss of tritium occurred, resulting in lynestrenol-^{3}H (V) with a specific activity of 800 mCi/mmol. As receptor binding studies required tritiated lynestrenol of higher specific activity we reinvestigated the ethynylation of estr-4-en-17-ones labelled at position 16.

RESULTS

Cold model experiments were carried out with estrenone (Ia) and with 11α-hydroxy-estrenone (Ib), an intermediate for the preparation of 11-substit-

*Organic Department, The Radiochemical Centre, Amersham, England.

uted lynestrenol analogues.[2] In both 17-oxo steroids the hydrogen atoms at C_{16} were exchanged for deuterium by treatment with a solution of sodium in deuteromethanol and deuterium oxide[3], giving 92.9% d_2 in IIa and 93.1% d_2 in IIb. In the IR spectra the weak band at 1410 cm^{-1}, characteristic for the 16-methylene group in 17-oxo steroids[4], disappeared on deuteration.



Subsequent ethynylation with acetylene and potassium t-butoxide in tetrahydrofuran gave the corresponding 17α-ethynyl-17β-ols IIIa and IIIb, using an excess of reagents to achieve complete conversion. In IIIa the mass spectrum indicated 7.5% d_1 and 1.6% d_2 and in IIIb a complete loss of the label.

These results are in accordance with those reported by van Kordelaar, Favier and Kitcher[1] for the ethynylation of estrenone-16-^{3}H via the same procedure.

No significant change in deuterium content occurred when the ethynylation was carried out with an excess of ethynyl magnesium bromide in tetrahydrofuran.[5] Column chromatography of the ethynylation product of IIa gave successively a 68% yield of lynestrenol-16-$^2H_2$ (IIIa, 96.8% d_2) and a 22% yield of deuterated di-(17β-hydroxy-estr-4-en-17α-yl)-acetylene VI, obviously formed via $BrMgC{\equiv}CMgBr$. In the same way IIb gave on chromatography a 62% yield of 11α-hydroxy-lynestrenol-16-$^2H_2$ (IIIb, 91.6% d_2). As loss of deuterium was negligible it seems safe to assume that in the compounds IIIa and IIIb, prepared with ethynyl magnesium bromide, the label is still at position 16.

The favourable results with the latter ethynylation procedure were confirmed with estrenone-16-^3H (IV, specific activity 14.0 Ci/mmol), prepared as previously described[1], giving lynestrenol-16-^3H (V, specific activity 13.3 Ci/mmol). These results and those previously reported[1] show a close analogy between the fate of the label of tritiated and 16-deuterated estrenone (IV and IIa respectively) in the two ethynylation reactions. On the basis of this analogy the 16-position of the tritium label in IV and V seems highly probable; the more so as randomisation of the label under the conditions of the Grignard reaction is very unlikely.

For reference purposes a minute amount of the lynestrenol-isomer 17β-ethynyl-estr-4-en-17α-ol (VII) was isolated from other sources. Physical constants of this compound are reported. On TLC it could not be detected as an impurity in the purified lynestrenol-16-^3H.

SYNTHESIS OF LYNESTRENOL-16-$^2H_2$ AND 11α-HYDROXY-LYNESTRENOL-16-$^2H_2$

Melting points are uncorrected. Optical rotations were determined for 1% solutions in chloroform. The elemental analysis was carried out with a Perkin-Elmer model 240 elemental analyser. NMR spectra were recorded with a Varian A60D spectrometer in deuterated chloroform with TMS ($\delta = 0$) as internal standard. IR spectra were obtained using a Perkin-Elmer 357 spectrophotometer. Mass spectra were recorded using a Varian MAT CH7 mass spectrometer, operating under the following conditions: electron energy 70 eV; ionising current 100 μA; ion source temperature approx. 130°C. The samples were admitted via the direct insertion probe. The deuterium content of the compounds was determined by repetitive scanning of the mass spectra over

the molecular peak region. The percentages of d_0, d_1 and d_2 were calculated from the peak intensities after correction for the natural abundance isotope peaks.

The isotope sources were deuterium oxide (99.9 atom % D) and monodeuteromethanol (99 atom % D). Commercial grade potassium t-butoxide was used without further purification. Acetylene was purified by passing it through a cold trap (-80°C), concentrated sulphuric acid, water and potassium hydroxide pellets. Tetrahydrofuran was stored over molecular sieve 4A for 2 days, decanted and then distilled. All other reagents were of analytical grade.

Analytical chromatography was performed on 0.30 mm silica gel plates unless otherwise stated. Spots were visualised by spraying with 2% sulphuric acid in ethanol, followed by heating at 110°C for 10 minutes.

Estrenone-16-$^2H_2$ (IIa)

Estrenone (Ia, 1.50 g) was added to a solution of sodium (1.50 g) in deuteromethanol (66.0 ml) and deuterium oxide (9.6 ml) and heated under reflux for 6 hours. The mixture was then concentrated under reduced pressure at approx. 30°C. Deuterium oxide (6.0 ml) was added and the precipitate was filtered off and washed with distilled water until neutral. Drying in vacuum gave 1.49 g of IIa with m.p. 111 - 112.5°C, $[\alpha]_D$ + 150°. Deuterium content: 2.1% d_0, 4.7% d_1, 92.9% d_2. IR (CS_2) 2221 (C-D), 1745 (C=O), 1672 cm^{-1} (C=C). NMR δ 5.45 (4-H,m), 0.91 (13-CH_3,s). TLC: hexane/acetone 8 : 2 (v/v) $R_f \sim 0.7$.

11α-Hydroxy-estrenone-16-$^2H_2$ (IIb)

11α-Hydroxy-estrenone (Ib, 500 mg) was deuterated in the way described for Ia giving 498 mg of IIb with m.p. 126 - 128°C, $[\alpha]_D$ + 86°. Deuterium content: 2.9% d_o, 4.0% d_1, 93.1% d_2 *. IR (CCl_4) 3617 (O-H), 3040 (=C-H), 2220 (C-D), 1741 cm^{-1} (C=O). NMR δ 5.48 (4-H,m), 3.85 (11β-H,m), 0.90 (13-CH_3,s). TLC: hexane/acetone 7 : 3 (v/v) $R_f \sim 0.6$.

*) When a solid sample was admitted to the mass spectrometer, a third deuterium atom was found. It was identified as the deuterium atom of the 11α-hydroxyl group, indicated by the IR spectrum (2669 cm^{-1}; O-D). Obviously it rapidly exchanges with atmospheric moisture during the normal procedure when a solution of the sample is evaporated prior to analysis.

Lynestrenol-16-$^2H_2$ (IIIa)

Method A (potassium t-butoxide and acetylene)

A stream of purified acetylene was passed at 0°C through a suspension of potassium t-butoxide (450 mg) in dry tetrahydrofuran (10 ml) for 1 hour. Estrenone-16-$^2H_2$ (IIa, 300 mg) was added and stirring was continued for 1 1/2 hour at 0°C, acetylene being passed through the reaction mixture during the whole period. The mixture was poured into ice-water and neutralised with 2N hydrochloric acid. Extraction with methylene chloride and recrystallisation from ether/hexane gave 260 mg of IIIa with m.p. 158 - 160°C, $[\alpha]_D$ -13°. Deuterium content: 90.9% d_0, 7.5% d_1, 1.6% d_2. TLC: hexane/acetone 8 : 2 (v/v) $R_f \sim 0.45$.

Method B (ethynyl magnesium bromide)

A solution of ethyl magnesium bromide was prepared from magnesium turnings (1.8 g), ethyl bromide (9.5 g) and tetrahydrofuran (45 ml). A fast stream of acetylene ($\sim$ 1 l/min) was then passed through for 20 minutes, keeping the temperature below 30°C by cooling in an ice-bath.[5] After standing at room temperature for 1 hour aliquots were titrated against standard acid.

The solution of ethynyl magnesium bromide (11.7 ml, 1.23 M) was then added, at room temperature and under nitrogen, to estrenone-16-$^2H_2$ (IIa, 880 mg) in tetrahydrofuran (2 ml). The mixture was stirred for 3 hours and then poured into an ice-cold solution of ammonium chloride (10.0 g) in water (75 ml). After stirring for 30 minutes, the precipitate was filtered off, washed with water and dried in vacuum. Column chromatography over silica gel (Woelm, activity grade I) using a toluene/ethyl acetate mixture 95 : 5 (v/v) as the eluent, followed by recrystallisation of the selected fractions from ether/hexane, gave 660 mg of IIIa with m.p. 159 - 160.5°C, $[\alpha]_D$ -12°. Deuterium content: 0.4% d_0, 2.6% d_1, 96.8% d_2. IR (CCl_4) 3622 (O-H), 3318 (≡C-H), 3044 (=C-H), 2235, 2214, 2187, 2144 and 2116 (C-D), 1665 (C=C), 1023 cm^{-1} (C-O). NMR δ 5.42 (4-H,m), 2.53 (≡C-H,s), 0.87 (13-CH_3,s). TLC (Merck 0.25 mm silica gel pre-coated plates): cyclohexane/ethyl acetate 9 : 1 (v/v) $R_f \sim 0.35$.

Further fractions, eluted with toluene/ethyl acetate 95 : 5 (v/v) gave, after crystallisation from acetone, 210 mg of VI, m.p. 237 - 239°C, $[\alpha]_D$ -42°.

Found: C, 84.2; "H", 10.1; O, 5.6. $C_{38}H_{54}O_2$ requires: C, 84.08; H, 10.03; O, 5.90%. Deuterium content: 3.3% d_2, 4.0% d_3, 81.4% d_4, 4.0% d_5, 6.9% d_6 and 0.3% d_7. IR (CH_2Cl_2) 3600 (O-H), 2233 and 2143 (C-D), 1667 (C=C), 1024 cm^{-1} (C-O). NMR δ 5.40 (4-H,m), 0.87 (13-CH_3,s). TLC (Merck 0.25 mm silica gel pre-coated plates): cyclohexane/ethyl acetate 9 : 1 (v/v) $R_f \sim 0.1$.

11α-Hydroxy-lynestrenol-16-$^2H_2$ (IIIb)

Method A (potassium t-butoxide and acetylene)

11α-Hydroxy-estrenone-16-$^2H_2$ (IIb, 200 mg) was ethynylated with potassium t-butoxide (400 mg) and acetylene in the way described for estrenone-16-$^2H_2$, yielding 220 mg of IIIb, m.p. 128 - 130°C, $[\alpha]_D$ -62°. Deuterium content: ~100% d_0, ~0% d_1 and d_2.

Method B (ethynyl magnesium bromide)

11α-Hydroxy-estrenone-16-$^2H_2$ (IIb, 144 mg) was ethynylated with ethynyl magnesium bromide in tetrahydrofuran (2.1 ml, 1.23 M) in the way described for estrenone-16-$^2H_2$. Column chromatography of the reaction product over silica gel (Woelm, activity grade I), using a hexane/acetone mixture 7 : 3 (v/v) as the eluent gave successively 25 mg of crude starting material IIb (deuterium content: 16.3% d_0, 22.5% d_1 and 61.2% d_2) and 98 mg of IIIb, m.p. 129 - 131°C, $[\alpha]_D$ -61°. Deuterium content: 3.7% d_0, 4.7% d_1 and 91.6% d_2. IR (CH_2Cl_2) 3618 (O-H), 3318 (≡C-H), 2233, 2213, 2185, 2144 and 2116 (C-D), 1668 (C=C), 1025 cm^{-1} (C-O). NMR δ 5.46 (4-H,m), 3.84 (11β-H,m), 2.52 (≡C-H,s), 0.86 (13-CH_3,s). TLC: hexane/acetone 7 : 3 (v/v) $R_f \sim 0.5$.

17β-Ethynyl-estr-4-en-17α-ol (VII)

This compound was isolated from mother liquors of the preparation of lynestrenol from estrenone with acetylene and potassium t-butoxide.

Repeated column chromatography over silica gel (Woelm, activity grade I), using a cyclohexane/acetone mixture 95 : 5 (v/v) as the eluent, followed by recrystallisation of the selected fractions from ether/hexane, gave pure VII. The yield was approx. 0.5% by weight, calculated on the weight of the

mother liquors. M.p. 100 - 102°C, $[\alpha]_D$ + 87°. IR (CCl_4) 3620 (O-H), 3318 (≡C-H), 3043 (=C-H), 1665 (C=C), 1043 cm^{-1} (C-O). NMR δ 5.41 (4-H,m), 2.46 (≡C-H,s), 0.90 (13-CH_3,s). Mass spectrum m/e 284 (molecular ion peak). TLC (Merck 0.25 mm silica gel pre-coated plates): cyclohexane/ethyl acetate 9 : 1 (v/v) $R_f \sim 0.45$.

SYNTHESIS OF LYNESTRENOL-16-^{3}H

This synthesis was performed at the Radiochemical Centre, Amersham. Experimental details of materials, radioactivity measurements and thin layer chromatography have been described previously.[1] The following solvent systems were used to analyse the tritiated steroids:

A. Benzene/ethanol 99 : 1 (v/v)

B. Cyclohexane/ethyl acetate 9 : 1 (v/v)

C. Cyclohexane/acetone 9 : 1 (v/v)

Estrenone-16-^{3}H (IV)

Estrenone (200 mg) was dissolved in a mixture of purified dimethylformamide (1 ml) and tritiated water (0.3 ml, 200 Ci). The mixture was sealed in an ampoule under vacuum and heated at 140°C for 64 hours. After cooling the solvent was recovered by distillation under vacuum. Labile tritium was removed by repeated distillation under vacuum of ethanol from the product (3 x 10 ml). The residue was taken up in chloroform and purified by TLC on four 0.75 mm silica gel plates, using solvent system A. The purified estrenone-16-^{3}H was extracted from the silica gel with benzene/ethanol 9 : 1 (v/v) and weighed after lyophilisation of the solution. The sample was redissolved in benzene/ethanol (200 ml, 9 : 1, v/v), the radioactivity measured and the specific activity of the compound calculated. The solution of estrenone-16-^{3}H was analysed by TLC in systems A, B and C.

Yield:	Estrenone-16-^{3}H	4.5 Ci (83 mg)
	Specific activity	14.0 Ci/mmol
	Radiochemical purity	98% in systems A, B and C.

Lynestrenol-16-^{3}H (V)

A solution of ethyl magnesium bromide was prepared from magnesium turnings (1.65 g), ethyl bromide (8.7 g) and tetrahydrofuran (67 ml). A fast

stream of acetylene ($\sim$ 400 ml/min) was then passed through for 60 min, keeping the temperature of the mixture below 30° by cooling in an ice-bath.[5] After standing at room temperature for 1 hour aliquots were titrated against standard acid.

The freshly prepared solution of ethynyl magnesium bromide in tetrahydrofuran (2.1 ml, 0.78 M) was then added at room temperature to estrenone-16-^{3}H (83 mg, 4.5 Ci) in tetrahydrofuran (10 ml) and the mixture was stirred under nitrogen for 2 1/2 hours. The reaction mixture was poured into an ice-cold solution of ammonium chloride (3 g) in water (20 ml) and stirred for 1 hour. The solution was extracted with methylene chloride (4 x 25 ml) and the organic phase was washed with water (25 ml) and dried over anhydrous sodium sulphate. The residue, after evaporation of the solvent, was purified by TLC on four 0.75 mm silica gel plates using solvent system B, followed by purification on four 0.75 mm silica gel plates using solvent system C. The weight of purified lynestrenol-16-^{3}H was determined after lyophilisation of the benzene/ethanol 9 : 1 (v/v) solution obtained on extraction of the silica gel. The sample was redissolved in benzene (500 ml), the radioactivity measured (3.58 mCi/ml) and the specific activity of the product calculated. The solution of lynestrenol-16-^{3}H was analysed by TLC in systems A, B and C.

Yield:	Lynestrenol-16-^{3}H	1.79 Ci (38.3 mg)
	Specific activity	13.3 Ci/mmol
	Radiochemical purity	98% in systems A, B and C.

Storage and stability of lynestrenol-16-^{3}H

The rate of decomposition of lynestrenol-16-^{3}H by self-radiolysis is approx. 2% per month when stored in benzene (concentration 3.58 mCi/ml) at room temperature.

DISCUSSION

It is known that in the ethynylation of carbonyl compounds which have a proton in the α-position, enolisation competes with the desired reaction.[6] Enolisation of 17-oxo steroids will lead to loss of label from position 16. It follows from our experiments that under the conditions of the reaction with potassium t-butoxide and acetylene this side-reaction leads to serious loss of

the label, whereas in the case of the Grignard reagent enolisation is obviously less important.

We feel that the ethynyl magnesium bromide procedure is of general use for the synthesis of 17α-ethynyl-17β-hydroxy steroids with high specific activity from suitable 16-tritiated-17-oxo steroids.

ACKNOWLEDGEMENT

The authors are indebted to Dr. F.J. Zeelen (Organon, Oss) for his valuable suggestions and to Mr. H.R.P. Williams (The Radiochemical Centre) for technical assistance.

REFERENCES

1. Kordelaar, J.M.G. van, Favier, J.S. and Kitcher, J.P., J. Lab. Comp., 9: 635 (1973)
2. U.S. pat. 3,767,685 (Akzona Inc.)
3. Djerassi, C., Wilson, J.M., Budzikiewicz, H. and Chamberlin, J.W., J. Am. Chem. Soc., 84: 4544 (1962)
4. Norman Jones, R. and Herling, F., J. Org. Chem., 19: 1252 (1954)
5. Brandsma, L., Preparative Acetylenic Chemistry, p. 26, Elsevier Publishing Co., Amsterdam, 1971
6. Ziegenbein, W., Chemistry of Acetylenes, pp. 208 - 212, Editor, Viehe, H.G., Marcel Dekker, New York, 1969