Synthesis of the 11-(Hydrogen Succinate) and 11-(β-D-Glucopyranosiduronic Acid) Derivative of Estra-1,3,5(10)-triene-3,11α,17β-triol

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3,11α-Dihydroxyestra-1,3,5(10)-trien-17-one (11α-hydroxyestrone) has been converted into the 11α-hydroxyestradiol derivatives named in the title, for radioimmunoassay purposes.

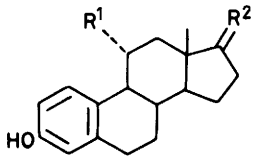
Radioimmunoassays using ^{125}I as a γ-emitting nuclide[1] have become popular for steroids and other small molecules, but in some cases lack sensitivity or specificity. For efficient and specific recognition of the substrate by an antibody, the hapten linked to albumin to obtain the antigen should be designed in such a way as not to mask the essential structural features and functional groups of the molecule to be assayed.[2] We wished to develop an assay for steroidal estrogens which could, in principle, be extended to the various patterns of functionality found in estrogen metabolites (*e.g.* 2-OH, 4-OH, 6-OH, 15α-OH, or 16α-OH), with minimal cross-reactivity. Available estrogen radioimmunoassays are based mainly upon the use of 6-carboxymethyloxime conjugates,[3] which would be expected to lessen antibody recognition of functionality in rings A and B.

The 11α-hydroxy group has been successfully used as the site of the bridge in an assay for progesterone,[4] so we chose to use 11α-hydroxyestradiol [estra-1,3,5(10)-triene-3,11α,17β-triol] (**1**), which is not a significant metabolite, as the basis for a new immunoassay.

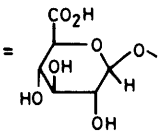
A second potential problem to be avoided is that of 'bridge recognition,' which may arise if the same bridge is used in both the radioligand and the immunogen, resulting in low sensitivity. If a different bridge is used for the radioligand the sensitivity is likely to be preserved.[5] We therefore synthesized 11α-hydroxyestradiol 11-(hydrogen succinate) for use in the immunogen and the 11-glucuronide as the basis for a radioligand.

The synthesis of 11α-hydroxyestrone (**2**) was easily achieved using the method of Gabbard and co-workers[6] [9(11)-dehydrogenation of estrone by DDQ (2,3-dichloro-5,6-dicyanobenzoquinone), protection as the ethylene acetal at C-17, hydroboration–oxidation to introduce 11α-OH, and acidic hydrolysis of the ethylene acetal]. The 3,11-bis(hydrogen succinate) was made according to Allen and Redshaw[5] (but using 4-dimethylaminopyridine catalysis), and selective hydrolysis of the hydrogen succinate group at C-3 in acidic aqueous dioxane gave the required 11α-hydroxyestrone 11-(hydrogen succinate) (**3**). Finally, reduction of the 17-oxo group with sodium borohydride gave 11α-hydroxyestradiol 11-(hydrogen succinate) (**4**), suitable for linking to albumin to produce the required antigen.

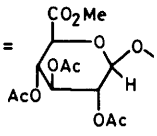
The synthesis of the corresponding 11-(β-D-glucopyranosiduronic acid) derivative ('glucuronide') (**5**) required the initial protection of the phenolic hydroxy group as the benzyl ether, followed by derivatisation of the rather hindered 11α-hydroxy group with methyl (2,3,4-tri-*O*-acetyl-1-bromo-1-deoxy-α-D-glucopyranosid)uronate and cadmium carbonate. The procedure used by Corrie[4] for the corresponding reaction with 11α-hydroxyprogesterone (refluxing toluene as solvent) was reported to give only a low yield of the required derivative. In our case there was little evidence of reaction at C-11 under Corrie's conditions, and the product was an intractable gum.



(**1**) R^1 = OH, R^2 = α-H, β-OH
(**2**) R^1 = OH, R^2 = O
(**3**) R^1 = $OCOCH_2CH_2CO_2H$, R^2 = O
(**4**) R^1 = $OCOCH_2CH_2CO_2H$, R^2 = α-H, β-OH

(**5**) R^1 = 

, R^2 = α-H, β-OH

(**6**) R^1 = 

, R^2 = O, 3-benzyl ether

After numerous trials we found that the required product (**6**) was obtained in acceptable yield (35%) if the reaction was conducted in a concentrated slurry of the bromo-sugar derivative in refluxing benzene, when reaction was complete in 2 h. Reduction of the 17-oxo group with borohydride in methanol then gave the corresponding estradiol derivative. Catalytic hydrogenolysis removed the benzyl ether protecting group, and the glucuronide was de-esterified by mild alkaline hydrolysis, followed by careful acidification to give the 11-glucuronide (**5**) of 11α-hydroxyestradiol.

Production of the antibody and development of the radioimmunoassay are being carried out by Dr. G. F. Read, at the Tenovus Institute for Cancer Research, Cardiff, and will be reported elsewhere. With minor modifications to the synthetic procedure, as required for additional functional groups in rings A, B, or D, the corresponding 11α-bridged haptens and radioligands should be readily accessible for radioimmunoassays of other estrogens.

Experimental

I.r. spectra refer to KBr discs. ^1H N.m.r. spectra were recorded at 100 MHz in $(CD_3)_2CO$, unless otherwise stated, with Me_4Si as internal standard. M.p.s were determined with a Reichert microscope and are uncorrected. High-performance liquid chromatography (h.p.l.c.) was performed with 5μ Nucleosil analytical and semipreparative columns. All solvents were redistilled before use. Oestrone was obtained from Sigma (UK), Poole, Dorset. Ether is diethyl ether.

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3-*Hydroxyestra*-1,3,5(10),9(11)*tetraen*-17*one*.—A suspension of estrone (1.1 g) in methanol (300 ml) was warmed until the substrate had dissolved. DDQ (1.0 g) was added in one portion and the resulting red solution was stirred at room temperature until it became pale yellow (10 min). Most of the solvent was then removed under reduced pressure and the residual slurry was poured into saturated aqueous sodium hydrogen carbonate (100 ml). The precipitated steroid was collected and washed with more of the same solution until the washings were colourless. After being dried at 40 °C *in vacuo* the product was a pale yellow solid (980 mg). An analytical sample was obtained from methanol, m.p. 250—252 °C (lit.,[6] 255—258 °C); $\nu_{max.}$ 3 260, 1 720, and 1 601 cm^{-1}; δ 0.92 (s, 18-H_3), 6.1 (m, 11-H), 6.56 (m, 4-H), 6.6 (m, 2-H), and 7.48 (d, *J* 8 Hz, 1-H) (Found: C, 80.5; H, 7.5. Calc. for $C_{18}H_{20}O_2$: C, 80.6; H, 7.5%).

17,17-*Ethylenedioxyestra*-1,3,5(10),9(11)-*tetraen*-3-*ol*.—The above product (980 mg) was dissolved in benzene (15 ml) with ethanediol (5 ml) and toluene-*p*-sulphonic acid (10 mg) and the mixture was heated for 2 h under reflux with azeotropic removal of water. The organic layer was washed with aqueous sodium hydrogen carbonate (10 ml), dried ($MgSO_4$), and taken to dryness. Purification by h.p.l.c. (20% ethyl acetate in hexane as mobile phase) gave the ethylene acetal (860 mg), m.p. 184—189 °C (from EtOAc–hexane) (lit.,[6] 185—189 °C); $\nu_{max.}$ 3 370 and 1 601 cm^{-1}; δ 0.84 (s, 18-H_3), 3.9 (m, OCH_2CH_2O), 6.1 (m, 11-H), 6.54 (m, 4-H), 6.6 (m, 2-H), and 7.44 (d, *J* 8 Hz, 1-H) (Found: C, 76.6; H, 7.7. Calc. for $C_{20}H_{24}O_3$: C, 76.9; H, 7.7%).

3,11α-*Dihydroxyestra*-1,3,5(10)-*trien*-17-*one* (**2**).—A solution of the foregoing ethylene acetal (800 mg) in dry tetrahydrofuran (THF) (25 ml) was cooled in an ice-bath. Diborane (10 ml; 1M in THF) was added and the mixture was stirred at room temperature for 2 h. Hydrogen peroxide (20 ml; 30% aqueous solution) and aqueous sodium hydroxide (20 ml; 5M) were added and the mixture was stirred at room temperature overnight. Water (50 ml) was then added, and the steroid was extracted into ethyl acetate (3 × 50 ml). The organic fraction was dried ($MgSO_4$) and taken to dryness under reduced pressure. The resulting oil was dissolved in methanol (10 ml), treated with toluene-*p*-sulphonic acid (10 mg), and the mixture was heated under reflux for 20 min, then poured into water (50 ml). Extraction of the steroid into ethyl acetate (3 × 50 ml) followed by drying of the combined extracts ($MgSO_4$), removal of the solvent under reduced pressure, and h.p.l.c. separation of the products (20% EtOAc in hexane as mobile phase) gave recovered 9(11)-dehydroestrone (270 mg) and 11α-hydroxyestrone (**2**) (280 mg), m.p. 261—262 °C (from acetone–hexane) (lit.,[6] 265—269 °C); $\nu_{max.}$ 3 460, 3 500, and 1 725 cm^{-1}; δ 0.82 (s, 18-H_3), 4.1 (dt, *J* 6 and 10 Hz, 11β-H), 6.56 (m, 4-H), 6.6 (m, 2-H), and 7.8 (d, *J* 8 Hz, 1-H) (Found: C, 75.5; H, 7.8. Calc. for $C_{18}H_{23}O_3$: C, 75.5; H, 7.7%).

3-*Hydroxy*-17-*oxoestra*-1,3,5(10)-*trien*-11α-*yl Hydrogen Succinate* (**3**).—A solution of 11α-hydroxyestrone (**2**) (350 mg) in dry pyridine (10 ml) with succinic anhydride (700 mg) and 4-dimethylaminopyridine (100 mg) was heated under reflux for 6 h. The mixture was then poured into ethyl acetate (50 ml) and was washed in turn with dilute HCl (3 × 50 ml) and water. The solvent was removed under reduced pressure and the resulting oil was taken up into aqueous dioxane (10 ml) with one drop of conc. hydrochloric acid, and heated under reflux for 30 min. The steroid was then extracted into ethyl acetate (50 ml) and the extract was washed with water (50 ml) and dried ($MgSO_4$). Removal of the solvents gave a semi-solid product (400 mg). Purification by h.p.l.c. (reverse-phase column, mobile phase: 44% water, 55% MeOH, 1% AcOH) gave 3-*hydroxy*-17-*oxoestra*-1,3,5(10)-*trien*-11α-*yl hydrogen succinate* (230 mg), m.p. 190 °C (decomp.) (from acetone); $\nu_{max.}$ 3 380, 1 740, and 1 720 cm^{-1}; δ 0.92 (s, 18-H_3), 2.62 (s, $OCOCH_2CH_2CO_2H$), 5.34 (dt, *J* 6 and 10 Hz, 11β-H), 6.48—6.6 (m, 2- and 4-H), and 6.96 (d, *J* 8 Hz, 1-H) (Found: C, 67.9; H, 6.75. $C_{22}H_{27}O_6$ requires C, 68.4; H, 6.7%).

3,17β-*Dihydroxyestra*-1,3,5(10)-*trien*-11α-*yl Hydrogen Succinate* (**4**).—A solution of compound (**3**) (200 mg) in methanol (5 ml) was stirred at 5 °C with sodium borohydride (50 mg) until reduction was complete (5 min; t.l.c.). Normal work-up followed by purification by h.p.l.c. as for the estrone derivative gave 3,17β-*dihydroxyestra*-1,3,5(10)-*trien*-11α-*yl hydrogen succinate* (**4**) (180 mg), m.p. 205—207 °C (from EtOAc–hexane); $\nu_{max.}$ 3 550, 3 350, 1 730, and 1 700 cm^{-1}; δ 0.80 (s, 18-H_3), 2.64 (s, $OCOCH_2CH_2CO_2H$), 3.72 (t, *J* 8 Hz, 17α-H), 5.34 (dt, *J* 6 and 10 Hz, 11β-H), 6.5—6.7 (m, 2- and 4-H), and 7.0 (d, *J* 8 Hz, 1-H) (Found: C, 67.7; H, 7.3. $C_{22}H_{29}O_6$ requires C, 67.5; H, 7.2%).

3,11α-*Dihydroxyestra*-1,3,5(10)-*trien*-17-*one* 3-*Benzyl Ether*.—A solution of 11α-hydroxyestrone (**2**) (280 mg) in ethanol (15 ml) containing sodium hydroxide (100 mg) was heated under reflux with benzyl bromide (5 ml) until reaction was complete (t.l.c.; *ca.* 1.5 h). Water was added and the steroid was extracted into ethyl acetate (50 ml); the extract was washed with water (3 × 50 ml), dried ($MgSO_4$), and evaporated under reduced pressure. Purification by h.p.l.c. (mobile phase 20% EtOAc in hexane) gave 11α-*hydroxyestrone* 3-*benzyl ether* (220 mg), m.p. 156—158 °C (from EtOAc–hexane); $\nu_{max.}$ 3 480 and 1 720 cm^{-1}; δ($CDCl_3$) 0.88 (s, 18-H_3), 3.3 (dt, *J* 6 and 10 Hz, 11β-H), 4.04 (s, OCH_2Ph), 5.76 (s, 4-H), 6.8 (d, *J* 8 Hz, 2-H), 7.3—7.7 (m, $PhCH_2$), and 7.6 (d, *J* 8 Hz, 1-H) (Found: C, 79.6; H, 7.4. $C_{25}H_{28}O_3$ requires C, 79.8; H, 7.45%).

Methyl (3-*Benzyloxy*-17-*oxoestra*-1,3,5(10)-*trien*-11α-*yl* 2,3,4-*Tri*-O-*acetyl*-β-D-*glucopyranosid*)*uronate* (**6**).—A solution of 11α-hydroxyestrone 3-benzyl ether (200 mg) in benzene (10 ml) containing cadmium carbonate (200 mg) and methyl (2,3,4-tri-*O*-acetyl-1-bromo-1-deoxy-α-D-glucopyranosid)uronate (200 mg) and concentrated by distillation to *ca.* 2 ml and then stirred and heated under reflux for 2 h. The cadmium carbonate was filtered off and washed on the filter with benzene (5 ml). The combined filtrate and washings were taken to dryness under reduced pressure, and the resulting gum was purified by h.p.l.c. (35% EtOAc in hexane) to give the *title compound* (**6**) (280 mg), m.p. 115—117 °C (from ethyl acetate–hexane); $\nu_{max.}$ 1 750 cm^{-1}; δ($CDCl_3$) 0.86 (s, 18-H_3), 1.97, 2.01, and 2.06 (each s, OAc), 3.76 (s, OMe), 4.1 (m, 5′-H), 4.2 (m, 11β-H), 4.8—5.4 (3 H, m, 3 × C*H*OAc), 5.05 (s, CH_2Ph), 6.72 (s, 4-H), 6.76 (d, *J* 8 Hz, 2-H), 7.4 (m, Ph), and 8.0 (d, *J* 8 Hz, 1-H) (Found: C, 65.9; H, 6.5. $C_{38}H_{44}O_{12}$ requires C, 66.0; H, 6.4%).

Methyl (3-*Benzyloxy*-17β-*hydroxyestra*-1,3,5(10)-*trien*-11α-*yl* 2,3,4- *Tri*-O-*acetyl*-β-D-*glucopyranosid*)*uronate*.—A solution of the foregoing compound (280 mg) in methanol (5 ml) at 5 °C was stirred with sodium borohydride (50 mg) for 5 min, and then poured into ethyl acetate and water. The organic layer was washed and dried ($MgSO_4$) and the solvent was removed under reduced pressure. Purification of the residue by h.p.l.c. (25% EtOAc in hexane with 1% MeOH) gave the 11α-*hydroxyestradiol derivative* (260 mg), m.p. 124—128 °C (from ether–hexane); $\nu_{max.}$ 3 520, 1 755, and 1 735 cm^{-1}; δ 0.92 (s, 18-H_3), 1.95, 1.96, and 2.00 (each s, OAc), 3.74 (s, OMe), 4.4 (m, 5′- and 11β-H), 4.8—5.5 (3 H, m, 3 × C*H*OAc), 5.08 (s, CH_2Ph), 6.68 (s, 4-H), 6.72 (d, *J* 8 Hz, 2-H), 7.4 (m, Ph), and 8.08 (d, *J* 8 Hz, 1-H) (Found: C, 65.8; H, 6.7. $C_{38}H_{46}O_{12}$ requires C, 65.7; H, 6.6%).

3,17β-*Dihydroxyestra*-1,3,5(10)-*trien*-11α-*yl*-β-D-*Glucopyranosiduronic Acid* (**5**).—The foregoing product (150 mg) was

dissolved in ethyl acetate (7 ml) containing one drop of conc. hydrochloric acid and was hydrogenated over a 10% palladium–charcoal catalyst for 3 h, when t.l.c. showed complete debenzylation. The catalyst was removed by filtration and washed with more ethyl acetate (20 ml). The combined filtrate and washings were taken to dryness under reduced pressure, and the resulting gum was dissolved in methanol (10 ml) and the solution was heated under reflux for 30 min with saturated aqueous sodium hydrogen carbonate (1 ml). The solution was adjusted to pH 7 with dilute hydrochloric acid, then poured onto a short column (*ca.* 2.5 cm) of Spherisorb ODS. This column was washed with water (50 ml) and then with ethanol (50 ml). The ethanol fraction gave the required *glucuronide* (**5**) (98 mg) after removal of the solvent; m.p. 215—217 °C (from acetone–hexane); $\nu_{max.}$ 3 450 and 1 720 cm^{-1}; δ 0.93 (s, 18-H_3), 4.4 (dt, *J* 6 and 10 Hz, 11β-H), 4.8 (d, *J* 8 Hz, 5′-H), 6.5 (s, 4-H), 6.54 (d, *J* 8 Hz, 2-H), and 8.06 (d, *J* 8 Hz, 1-H) (Found: C, 61.9; H, 6.8. $C_{24}H_{32}O_9$ requires C, 62.1; H, 6.9%).

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