Novel and Efficient Synthesis of Estriol and its 16-Glucuronide *via* 2,4,16α-Tribromoestrone †

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A novel synthesis of estra-1,3,5(10)-triene-3,16α,17β-triol (5), sodium 3,17β-dihydroxyestra-1,3,5(10)-trien-16α-yl-β-D-glucopyranosuronate (12b) and sodium 3-hydroxyestra-1,3,5(10)-trien-17β-yl-β-D-glucopyranosuronate (10b) is described. 2,4,16α-Tribromo-3-hydroxyestra-1,3,5(10)-trien-17-one (2a) was efficiently synthesized in one step with quantitative yield by bromination of 3-hydroxyestra-1,3,5(10)-trien-17-one (1) with cupric bromide. Treatment of (2a) with NaOH in aqueous pyridine under the controlled conditions gave the 16α-hydroxy-17-ketone (4a) without ketol rearrangement. The ketol (4a) was converted in quantitative yield into the triol (5) *via* a sodium borohydride reduction in the presence of palladium chloride. Reaction of (4a) with methyl 1-bromo-1-deoxy-2,3,4-tri-*O*-acetyl-α-D-glucopyranosuronate using silver carbonate as a catalyst yielded the 16-monoglucuronide acetate methyl ester (11). The reductive removal of the bromines of (11) with sodium borohydride followed by NaOH hydrolysis gave the glucuronide (12b). A direct glucuronidation of 2,4-dibromoestra-1,3,5(10)-triene-3,17β-diol (8) and a subsequent hydrolysis of the 17-glucuronide (9) gave the glucuronide (10b).

Hydroxylation at the 16α-position, one of the two major biotransformation pathways of estradiol ‡ in man,[1] leads to estriol (5) which is now recognized as a potent uterotropic agent equivalent to estradiol [2] and is also used in estrogenic hormone therapy. Most of estriol formed during pregnancy is synthesized *via* 16α-hydroxylated C_{19} steroids,[3] a different pathway from that taken in the non-pregnant state. Since estriol is mainly present as its 16-glucuronide in biological fluids,[4] estriol 16-glucuronide (12b) is now considered in clinical practice to be the most suitable estrogen metabolite for the purpose of monitoring fetal well-being.[4]

Leeds *et al.*[5] reported the synthesis of estriol (5) from estrone (1) which involves three steps with epoxidation of the 17-enol acetate of estrone as a key reaction. This method has an overall yield of approximately 31%. On the other hand, the 16-glucuronide (12b) is not readily available, primarily because of the difficulty involved in its synthesis. Elce *et al.*[6] and Nambara and Imai [7] reported the chemical synthesis of the glucuronide. These methods use many steps from estrone with the glucuronidation of the 16α-hydroxy-17-oxo-derivative as a key step, resulting in low yields. A direct glucuronidation of estriol 3-benzoate or 3-benzyl ether, which results in the formation of 16- and 17-monoglucuronides, has also been reported.[8] Since the 16-glucuronide is separated from the 17-isomer by fractional crystallization, this method produced a low yield together with a contamination of the 17-isomer.

We recently discovered the controlled stereoselective alkaline hydrolysis of 16-bromo-17-oxo-androgens [9] and also developed the hydrolytic method for the synthesis of several 16α-hydroxy-17-oxo-androgens [10] and estrogens [11] in high yields. The present paper describes a short-step efficient synthesis of estriol (5) and its 16-glucuronide (12b) from estrone (1) with high overall yields using the controlled alkaline hydrolysis of 2,4,16α-tribromoestrone (2a), and also describes an alternative synthesis of 16-epiestriol (7) and estradiol 17-glucuronide (10b).

Results and Discussion

The bromination of estrone with cupric bromide or bromine was initially explored in order to obtain 2,4,16α-tribromoestrone (2a), which is a key intermediate for the synthesis of estriol (5) and its 16-glucuronide (12b) *via* the controlled alkaline hydrolysis in high yield. As shown in Table 1, when estrone (1) was heated in methanol under reflux for 6.5 h with 11 mol equivalents of cupric bromide, 2,4,16α-tribromide (2a) was stereoselectively produced in quantitative yield. The i.r. and ^{1}H n.m.r. spectra supported the structure of the tribromide (2a). When the longer reaction time was employed (condition D), formation of by-product, 2,4,16,16-tetrabromoestrone,§ was detected according to t.l.c. and ^{1}H n.m.r. analysis. 4,16α-Dibromoestrone (3) was isolated from the brominated products obtained under condition A using 6 mol equivalents of cupric bromide. On the other hand, bromination of estrone (1) with 3 mol equivalents of bromine in chloroform–acetic acid gave the isomeric 2,4,16-tribromoestrones (2a) and (2b) in a good yield. The ^{1}H n.m.r. spectra of the bromides (2) showed that, in accord with a previous report,[12] they were obtained as a *ca.* 3 : 1 mixture [(2a) : (2b)].

Alkaline hydrolysis of the 16α-bromo-ketone (2a) with sodium hydroxide in aqueous pyridine was examined (Table 2) in order to determine whether the corresponding 16α-hydroxy-17-ketone (4a) could be isolated in a high yield without formation of the other ketols as reported previously in a similar case.[9–11] Treatment of the bromo-ketone (2a) with 2.5 equivalents of sodium hydroxide at room temperature for 3 h (condition H) or 2.0 equivalents of alkali for 6 h (condition

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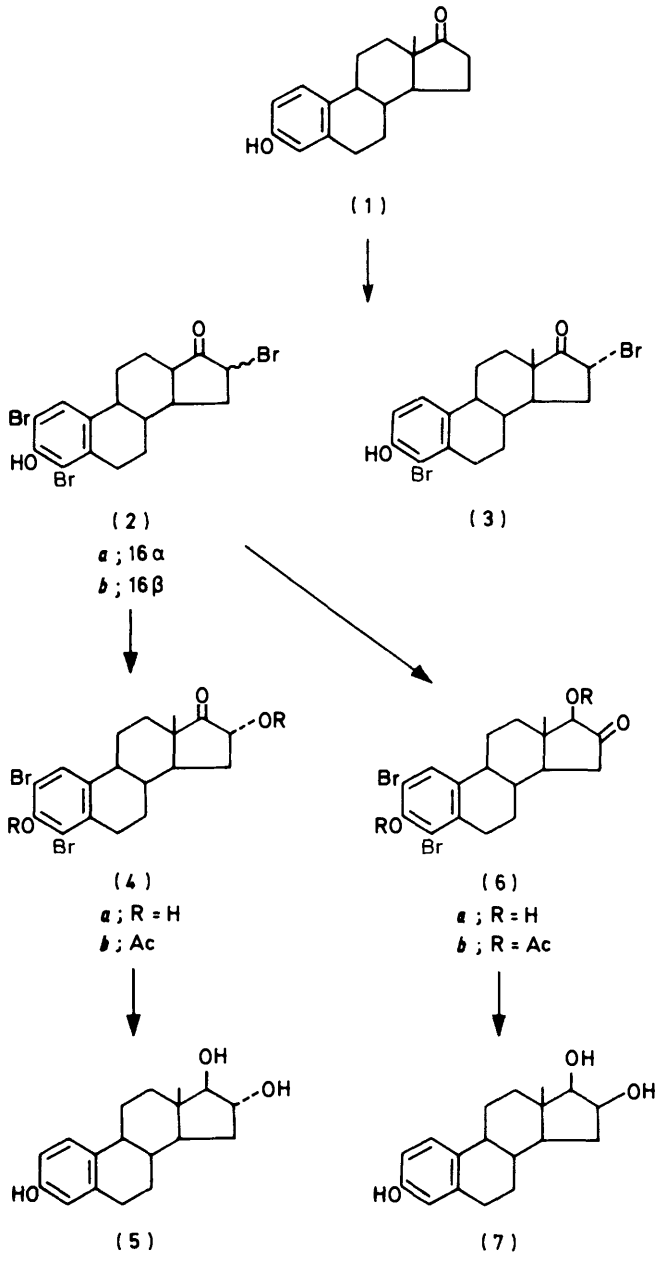
‡ The following trivial names have been used in this paper: estrone = 3-hydroxyestra-1,3,5(10)-trien-17-one, estradiol = estra-1,3,5(10)-triene-3,17β-diol, estriol = estra-1,3,5(10)-triene-3,16α,-17β-triol, 16-epiestriol = estra-1,3,5(10)-triene-3,16β,17β-triol, 4,16α-dibromoestrone = 4,16α-dibromo-3-hydroxyestra-1,3,5(10)-trien-17-one, 2,4,16α-tribromoestrone = 2,4,16α-tribromo-3-hydroxyestra-1,3,5(10)-trien-17-one, 2,4,16,16-tetrabromoestrone = 2,4,16,16-tetrabromo-3-hydroxyestra-1,3,5(10)-trien-17-one, 2,4-dibromoestradiol = 2,4-dibromoestra-1,3,5(10)-triene-3,17β-diol.

§ 2,4,16,16-Tetrabromoestrone could not be isolated as a pure solid because of its instability during isolation by chromatography and crystallization. The structure, however, was assigned on the basis of its ^{1}H n.m.r. spectrum [δ($CDCl_3$) 1.10 (s, 3 H, 18-Me), 7.26 (s, 1 H, 1-H)].



Scheme 1

G) gave, stereoselectively, the desired compound (4a) in quantitative yield. The ketol (4a) must be formed by a nucleophilic displacement of the 16β-bromine substituent of the bromide (2b) by hydroxide ion analogously with that reported for C_{18} and C_{19} steroids.[9,10] On the other hand, when 4 equivalents of sodium hydroxide and a 12 h reaction time (condition I) were employed in the same reaction, the rearranged product, the 17β-hydroxy-16-ketone (6a), was obtained as the sole product. The structures of the ketols (4a) and (6a) were identified by i.r. and ^{1}H n.m.r. spectroscopy. The bromides (2a), (4a), and (6a) were readily isolated in a pure form by crystallization from acetone in very high yields.

Reduction of the 17-carbonyl group and removal of the 2- and 4-bromine atoms of the ketol (4a) were achieved simultaneously with sodium borohydride in the presence of a transition metal,[13] palladium chloride, to give the estriol (5) in quantitative yield. [The structure of the triol (5) was assigned

Table 1. Bromination of estrone (1) with cupric bromide

	Conditions		Relative amount of products [a]			
	$CuBr_2$ or Br_2 ($CuBr_2$) (mol equiv.)	Time (h)	(1)	(3) and 2,16-Dibromide	(2)	2,4,16,16-Tetrabromide
A	6	32	5	30	65	0
B	9	24	5	10	80	5
C	11	6.5	0	0	>99	<1
D	11	24	0	0	90	10
E	3 (Br_2)	26	0	≃2	95	≃3

[a] Relative amount of product was obtained by integrating the peak areas corresponding to C-18 angular methyl of ^{1}H n.m.r. spectra of the reaction mixtures.

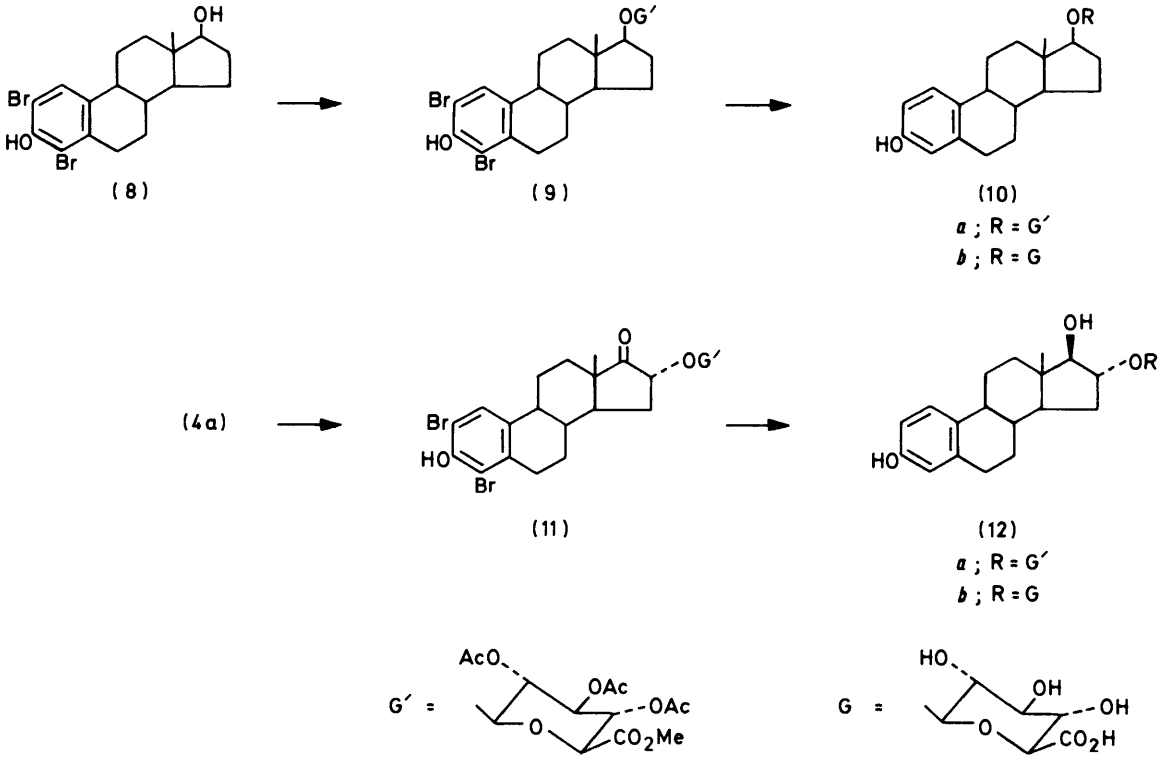
Table 2. Hydrolysis of 2,4,16α-tribromoestrone (2) with sodium hydroxide in aqueous pyridine

	Conditions		Relative amount of product [a]		
	NaOH (mol equiv.)	Time (h)	(2)	(4a)	(6a)
F	1.2	90	15	85	0
G	2	6	<1	>99	0
H	2.5	3	<1	>99	0
I	4	24	0	0	100

[a] Relative amount of product was obtained by ^{1}H n.m.r. analysis of the reaction mixtures without isolation.

on the basis of i.r. and ^{1}H n.m.r. spectral results.] The overall yield of the estriol (5) from the starting estrone (1) is more than 85%, using the intermediates (2a) and (4a) obtained in the above reactions without isolation. Together with the much improved yield, this synthesis has advantages over the previous one [5] in simplicity of handling, shorter reaction path, no need of chromatographic isolation of products, and in the use of less expensive reagents. The isomeric ketol (6a) was similarly converted into 16-epiestriol (7) in good yield.

In order to determine whether the bulky substituents in the ketol (4a), bromine atoms at C-2 and 4-positions of the ring A would protect the 3-hydroxy-group from glucuronidation with bulky 1-bromo-1-deoxy-2,3,4-tri-*O*-acetyl-α-D-glucopyrosuronate which would be an advantage in the synthesis of D-ring glucuronide, we employed 2,4-dibromoestradiol (8) [14] as a model substrate for the reaction. When the dibromide (8) was treated with the bromo-sugar and silver carbonate in dry benzene, the desired product, the 17-monoglucuronide triacetate methyl ester (9), was regioselectively obtained as the sole product in good yield. Estradiol 17-glucuronide (10b) was obtained by removal of the 2- and 4-bromine atoms of compound (9) as described above followed by subsequent hydrolysis of compound (10a) with sodium hydroxide in aqueous methanol. An Amberlite XAD-2 resin was effective in isolation of the glucuronide (10b) in high yield. Treatment of the ketol (4a) with the bromo-sugar and silver carbonate in dry benzene similarly gave the 16-monoglucuronide acetate methyl ester (11) in 85% yield. Compound (11) was similarly converted into estriol 16-glucuronide acetate methyl ester (12a) by debromination and then, finally, estriol 16-glucuronide (12b) was obtained by hydrolysis of the acetate methyl ester (12a). The overall yield of estriol 16-glucuronide (12b) from the starting estrone (1) was 65%. The yield is much better than those reported previously.[6-8] The i.r. and ^{1}H n.m.r.



Scheme 2

spectra of compounds (9), (10a), (11), and (12a) supported the structural assignments. Compounds (10b) and (12b) were identical with authentic samples in every respect and β-glucuronidase hydrolysis gave estradiol and estriol (5) as aglycones, respectively.

Deuterium-labelled compounds are essential as internal standards for a quantitative g.c.–mass spectral analysis of steroid hormones in biological fluids. We previously reported a stereoselective deuterium incorporation at the 16β-position of 16α-hydroxy-17-ketones by the controlled hydrolysis of the corresponding 16-bromo-ketones using a medium containing deuterium oxide.[9] It has also been demonstrated that the isotope is regioselectively labelled at the 2- and 4-positions of estrogens, when deuteriated reagent and solvent are used in the reductive removal of halogen from the 2- and 4-positions of estrogens.[13b,c] Thus, in addition to its simplicity and high yield, this synthesis offers the advantage of permitting the regio- and stereo-selective introduction of hydrogen isotopes into the 2-,4-,16β-, and 17α-positions of estrogens and their conjugates.

Experimental

M.p.s were measured on Yanagimoto melting-point apparatus and are uncorrected. I.r. spectra were recorded on a Shimadzu IR 400 spectrometer as KBr pellets. ^{1}H N.m.r. spectra were obtained with a JEOL PMX 60 spectrometer at 60 MHz. Chemical shifts are reported in p.p.m. (δ) relative to $SiMe_4$. Mass spectra were taken on a Hitachi RMU-7L instrument. U.v. spectra were recorded on a Shimadzu UV 300 spectrophotometer. Where analyses are indicated with only symbols of the elements, analytical results obtained for these elements were within ±0.3% of the theoretical values. The detailed analyses have been treated as a Supplementary publication SUP No. 23434 (2 pages).* T.l.c. was performed on a plate coated with a layer (0.5 mm thick) of silica gel HF (E. Merck AG) in the solvent system n-hexane–AcOEt (2 : 1, v/v).

Bromination of 3-*Hydroxyestra*-1,3,5(10)-*trien*-17-*one* (1).—(A) $CuBr_2$ *Method.* A solution of (1) (2 g, 7.4 mol) and $CuBr_2$ (6, 9, and 11 mol equiv.) in dry MeOH (400 ml) was heated under reflux for an appropriate time (Table 1). The reaction mixture was poured into ice–water (1 l) and then extracted with $CHCl_3$ (500 ml × 2). The organic layer was dried (Na_2SO_4) and evaporated to give the crude brominated products (3.3—3.8 g).

(B) Br_2 *Method.* To a solution of (1) (2 g, 7.4 mmol) in $CHCl_3$ (50 ml) and AcOH (250 ml) was added dropwise a 1M-solution of bromine (22 ml; 3 mol equiv. of Br_2) under stirring at room temperature. The mixture was allowed to stand at room temperature for 2 h and then at 50 °C for 1 h. After this time, the mixture was poured into ice–water (1 l) and extracted with $CHCl_3$ (600 ml × 2). The organic layer was washed with 5% $NaHCO_3$ and water and dried (Na_2SO_4). After evaporation of the solvent an oily substance (3.9 g) was obtained.

2,4,16α-*Tribromo*-3-*hydroxyestra*-1,3,5(10)-*trien*-17-*one* (2a). —The crude product obtained by use of 11 mol equiv. of $CuBr_2$ and a 6.5 h reaction time (a quantitative yield of crude product sufficiently pure for the next step) was recrystallized from acetone to give (2a) (3.57 g, 95%) as colourless plates, m.p. 191—193 °C, $\lambda_{max.}$ (EtOH) 283 (ε2 700) and 290 nm (2 800); $\nu_{max.}$ (KBr) 3 450 (OH), and 1 740 cm^{-1} (CO); δ($CDCl_3$) 0.93 (s, 3H, 18-Me), 4.58 (m, 1 H, 16β-H), and 7.40 (s, 1 H, 1-H). Anal. ($C_{18}H_{19}Br_3O_2$) C, H, Br.

2,4,16β-*Tribromo*-3-*hydroxyestra*-1,3,5(10)-*trien*-17-*one* (2b). —A portion (100 mg) of the oily substance obtained by the

* For details of the Supplementary publications scheme, see Notice to Authors No. 7, *J. Chem. Soc., Perkin Trans. 1*, 1981, Index issue.

bromination of (1) with Br_2 was subjected to preparative t.l.c. Areas corresponding to (2b) (R_F 0.49) and (2a) (R_F 0.51) were scraped off and eluted with AcOEt, separately. Evaporation of the organic solvent gave a solid residue. Crystallization of the solid products from acetone gave the pure 16α-isomer (2a) (51 mg, 35%), which was identical with (2a) obtained above, and pure 16β-isomer (2b) (18 mg, 12%) as colourless needles, m.p. 209—212 °C; $\lambda_{max.}$ (EtOH) 283 (ε2 700), and 290 nm (2 800); $\nu_{max.}$ (KBr) 3 450 (OH) and 1 750 cm^{-1} (CO); δ($CDCl_3$) 1.10 (s, 3 H, 18-Me), 4.15 (m, 1 H, 16α-H), and 7.37 (s, 1 H, 1-H). Anal. ($C_{18}H_{19}Br_3O_2$) C, H, Br.

4,16α-*Dibromo*-3-*hydroxyestra*-1,3,5(10)*trien*-17-*one* (3).—A portion (200 mg) of the brominated product obtained by using 6 mol equiv. of $CuBr_2$ was subjected to preparative t.l.c. A solid (55 mg) was obtained from the area corresponding to the dibromo-compound (R_F 0.30) and then recrystallized from acetone to give (3) (26 mg, 14%) as colourless needles, m.p. 186—188 °C; $\lambda_{max.}$ (EtOH) 281 (ε2 000) and 289 nm (1 900); $\nu_{max.}$ (KBr) 3 350 (OH) and 1 740 cm^{-1} (CO); δ ($CDCl_3$) 0.93 (s, 3 H, 18-Me), 4.60 (m, 1 H, 16β-H), 6.86 (d, *J* 10.0 Hz, 1 H, 2-H), and 7.20 (d, *J* 10.0 Hz, 1 H, 1-H). Anal. ($C_{18}H_{20}Br_2O_2$) C, H, Br.

The isomer of (3), 2,16β-dibromoestrone was detected in the mother liquor of the crystallization but it could not be isolated in a pure form. The relative amount of (3) to the 2,16β-dibromo-isomer was approximately 4 : 1 according to the ^1H n.m.r. spectral results for the dibromide fraction.

Hydrolysis of the Bromo-ketone (2a) *with* NaOH *in Aqueous Pyridine*.—To a solution of (2a) (150 mg, 0.29 mmol) in 75% aqueous pyridine (8 ml) was added aqueous sodium hydroxide (0.48 ml); the mixture was then set aside at room temperature for an appropriate time (Table 2). The mixture was poured into 1% HCl solution and then extracted with AcOEt (20 ml × 2). The organic layer was washed with water and dried (Na_2SO_4). After evaporation of the solvent the residue obtained (125—140 mg) was submitted to ^1H n.m.r. analysis.

2,4-*Dibromo*-3,16α-*dihydroxyestra*-1,3,5(10)-*trien*-17-*one* (4a).—The crude (2a) (1 g, 1.96 mmol) obtained under conditions C and the oily substance (2) (1 g, 1.96 mmol) obtained under conditions E were hydrolysed under conditions H to yield solid products (850 and 820 mg, respectively). Crystallization from acetone afforded (3a) (790 mg, 90% and 680 mg, 78%, respectively) as colourless needles, m.p. 205—206 °C; $\lambda_{max.}$ (EtOH) 285 nm (ε 2 600) and 292 (2 400); $\nu_{max.}$ (KBr) 3 300 and 3 500 (OH) and 1 728 cm^{-1} (CO); δ($CDCl_3$) 0.97 (s, 3 H, 18-Me), 4.40 (m, 1 H, 16β-H), and 7.36 (s, 1 H, 1-H) Anal. ($C_{18}H_{20}Br_2O_3$) C, H, Br.

3,16α-*Diacetoxy*-2,4-*dibromoestra*-1,3,5(10)-*trien*-17-*one* (4b).—The acetate (4b) was prepared by acetylation of (4a) with pyridine–Ac_2O to give a 91% yield of product (colourless needles from acetone–Et_2O), m.p. 218—220 °C; $\nu_{max.}$ 1 780, 1 760, and 1 740 cm^{-1} (CO); δ($CDCl_3$) 1.00 (s, 3 H, 18-Me), 2.10 (s, 3 H, 17-OAc), 2.40 (s, 3 H, 3-OAc), 5.45 (m, 1 H, 16β-H), and 7.50 (s, 1 H, 1-H) Anal. ($C_{20}H_{24}Br_2O_5$) C, H, Br.

2,4-*Dibromo*-3,17β-*dihydroxyestra*-1,3,5(10)-*trien*-16-*one* (6a).—The hydrolysed residue of (2a) (150 mg, 0.29 mol) obtained under conditions I (Table 2) was recrystallized from acetone to give (5a) (120 mg, 92%) as colourless plates, m.p. 220—223 °C; $\lambda_{max.}$ (EtOH) 285 (ε2 500) and 290 nm (2 700); $\nu_{max.}$ (KBr) 3 400 and 3 500 (OH) and 1 738 cm^{-1} (CO); δ($CDCl_3$) 0.77 (s, 3 H, 18-Me), 3.79 (3, 1 H, 17α-H), and 7.32 (s, 1 H, 1-H) Anal. ($C_{18}H_{20}O_3Br_2$) C, H, Br.

2,4-*Dibromo*-3,17β-*diacetoxyestra*-1,3,5(10)-*trien*-16-*one* (6b).—The ketol (6a) was acetylated with pyridine–Ac_2O to yield (6b) as colourless needles (88% yield), m.p. 188—189 °C, $\nu_{max.}$ (KBr) 1 780, 1 760, and 1 738 cm^{-1} (CO); δ($CDCl_3$) 0.82 (s, 3 H, 18-Me), 2.12 (s, 3 H, 17-OAc), 2.33 (s, 3 H, 3-OAc), 5.02 (s, 1 H, 17α-H), and 7.38 (s, 1 H, 1-H) Anal. ($C_{20}H_{24}Br_2O_5$) C, H, Br.

Estra-1,3,5(10)-*triene*-3,16α,17β-*triol* (5).—To a solution of (4a) (200 mg, 0.45 mmol) in MeOH (13 ml) were added $PdCl_2$ (170 mg, 0.64 mmol) and $NaBH_4$ (70 mg, 1.89 mmol); the mixture was then stirred under nitrogen at 0 °C for 2 h. After this time, the reaction mixture was filtered and the filtrate was poured into 4% AcOH solution (75 ml). After extraction with AcOEt (50 ml × 3), the organic layer was washed with 5% aqueous sodium hydrogen carbonate and water, dried (Na_2SO_4), and evaporated to give a solid residue. Crystallization of the residue from MeOH–AcOEt gave (5) (125 mg, 96%) as colourless needles, m.p. 277—282 °C (lit.,[5] m.p. 278.5—284 °C); δ(CD_3OD) 0.80 (s, 3 H, 18-Me), 3.50 (d, *J* 6 Hz, 1 H, 17α-H), 4.03 (m, 1 H, 16β-H), and 6.6—7.36 (m, 3 H, aromatic). Compound (6) was identical with an authentic sample of estriol in every respect.

Estra-1,3,5(10)-*triene*-3,16β,17β-*triol* (7).—Crude compound (7) (120 mg) obtained from (6a) (200 mg, 0.45 mmol) by the same treatment as described above was recrystallized twice from ether to give pure (7) (85 mg, 66%) as colourless needles, m.p. 279—285 °C (lit.,[15] m.p. 281—289 °C); δ($CDCl_3$–CD_3OD, 5 : 1) 0.85 (s, 3 H, 18-Me), 3.43 (d, *J* 5 Hz, 1 H, 17α-H), 4.10 (m, 1 H, 16α-H), and 6.73—7.23 (m, 3 H, aromatic). Compound (7) was identical with an authentic sample of epiestriol in every respect.

Methyl 2,4-*Dibromo*-3-*hydroxyestra*-1,3,5(10)-*trien*-17β-*yl*-2,3,4-*tri*-O-*acetyl*-β-D-*glucopyranosuronate* (9).—A solution of methyl 1-bromo-1-deoxy-2,3,4-tri-*O*-acetyl-α-D-glucopyranosuronate (570 mg, 1.44 mmol) in dry benzene (10 ml) was added dropwise into a stirred solution of (7) (273 mg, 0.63 mmol) in dry benzene containing anhydrous Ag_2CO_3(700 mg, 2.54 mmol). The mixture was stirred at room temperature for 48 h, additional bromo-sugar (300 mg, 0.76 mmol) and catalyst (280 mg, 1 mmol) being added to the mixture after a period of 24 h. The precipitates were filtered off and washed with benzene, and the combined filtrate was evaporated to give a viscous amber gum. The gummy substance was chromatographed on a silica gel column (60 g) with n-hexane–AcOEt to give a solid product (410 mg). Crystallization of the solid from acetone gave (9) (335 mg, 72%) as colourless needles, m.p. 172—174 °C, $\lambda_{max.}$ (EtOH) 284 (ε2 600) and 291 nm (2 700); $\nu_{max.}$ (KBr) 3 400 (OH), 1 780, 1 760, and 1 740 cm^{-1} (CO); δ($CDCl_3$) 0.73 (s, 3H, 18-Me), 2.03, 2.06, and 2.09 (s, 3 H, pyranose-OAc), 3.76 (s, 3 H, CO_2Me), 4.53 (d, *J* 5 Hz, 1 H, pyranose-anomeric), and 7.36 (s, 1 H, 1-H). Anal. ($C_{31}H_{38}Br_2O_{11}$) C, H, Br.

Methyl 3-*Hydroxyestra*-1,3,5(10)-*trien*-17β-*yl*-2,3,4-*tri*-O-*acetyl*-β-D-*glucopyranosuronate* (10a).—Reductive removal of the bromo-substituents of (9) (300 mg, 0.41 mmol) with $NaBH_4$ (67 mg, 1.8 mmol) in the presence of $PdCl_2$ (170 mg, 0.64 mmol) as described above gave a solid product (225 mg). Crystallization of the solid from acetone gave (10a) (195 mg, 83%) as colourless needles, m.p. 112—116 °C (lit.,[16] 113—114 °C); δ($CDCl_3$) 0.76 (s, 3 H, 18-Me), 2.00, 2.03, and 2.05 (s, 3 H, pyranose-OAc), 3.76 (s, 3 H, CO_2Me), 4.67 (d, *J* 6 Hz, pyranose anomeric), and 6.50–7.26 (m, 3 H, aromatic).

Sodium 3-*Hydroxyestra*-1,3,5(10)-*trien*-17β-*yl*-β-D-*glucopyranosuronate* (10b).—The above acetate methyl ester (10a) (200 mg, 0.34 mmol) was dissolved in MeOH (50 ml), and 2M-aqueous NaOH (4 ml) was added. The reaction mixture was then set aside at room temperature overnight. Subsequently the solution was concentrated under reduced pressure at <50 °C to 10 ml and poured into ice-cold water (100 ml). The solution was then passed through a column of Amberlite XAD-2 (3 × 50 cm). After a washing with water the adsorbed glucuronide was eluted with 50% aqueous MeOH. The steroid fraction was condensed to give a solid product (71 mg). The solid was recrystallized from aqueous MeOH to give (10b) (55 mg, 44%) as colourless needles, m.p. 246—250 °C (lit.,[6] 246—249 °C).

Methyl 2,4-*Dibromo*-3-*hydroxy*-17-*oxoestra*-1,3,5(10)-*trien*-16α-*yl*-2,3,4-*tri*-O-*acetyl*-β-D-*glucopyranosuronate* (11).—The dibromo-compound (4a) (1.0 g, 2.35 mmol) in benzene (250 ml) was treated with bromo-sugar (5.5 g, 14 mmol) as described above (reaction time 72 h). The gummy product was purified by silica-gel column chromatography with n-hexane–AcOEt as eluant to yield crude (11a) (1.6 g). Crystallization of the crude product from acetone–n-hexane gave pure (11) (1.3 g, 85%) as colourless needles, m.p. 250 °C; $\lambda_{max.}$ 283 (ε2 300) and 290 (2 500); $\nu_{max.}$ (KBr) 3 400 (OH) and 1 740 (CO); δ 0.92 (s, 3 H, 18-Me), 2.02 (s, 6 H, pyranose-OAc), 2.07 (s, 3 H, pyranose-OAc), 3.73 (s, 3 H, pyranose-CO_2Me), 4.10 (dd, *J* 4 Hz, 1 H, 16-H), and 7.36 (s, 1 H, 1-H). Anal. ($C_{31}H_{36}Br_2O_{12}$) C, H, Br.

Methyl 3,17β-*Dihydroxyestra*-1,3,5(10)-*trien*-16α-*yl*-2,3,4-*tri*-O-*acetyl*-β-D-*glucopyranosuronate* (12a).—Compound (11) (1 g, 1.31 mmol) was treated with $NaBH_4$ in the presence of $PdCl_2$ as described for the reaction of (4a). The product (775 mg) was crystallized from MeOH to give (12a) (745 mg, 95%), m.p. 229—231 °C (decomp.) (lit.,[7] 228—230 °C); $\nu_{max.}$ (KBr) 3 400 (OH), 1 760, 1 750, and 1 740 cm^{-1} (CO); δ($CDCl_3$–CD_3OD, 5 : 1) 0.93 (s, 3 H, 18-Me), 2.03 (s, 6 H, pyranose-OAc), 2.06 (s, 3 H, pyranose-OAc), 3.73 (s, 3 H, pyranose-CO_2Me), 4.50 (d, *J* 6 Hz, 1 H, pyranose anomeric), and 6.63—7.10 (m, 3 H, aromatic).

Sodium 3,17β-*Dihydroxyestra*-1,3,5(10)-*trien*-16α-*yl*-β-D-*glucopyranosuronate* (12b).—The above acetate methyl ester (12a) (400 mg, 0.67 mmol) was hydrolysed with 2M-aqueous NaOH in MeOH in the same manner described for (10a). The glucuronide (11b) was isolated by means of an Amberlite XAD-2 resin column, and recrystallized from aqueous MeOH to give a pure (12b) (211 mg, 65%) as colourless needles, m.p. 245—248 °C (lit.,[6] 246—249 °C).

Hydrolysis of the Glucuronides (10b) *and* (12b) *with* β-*Glucuronidase*.—A solution of the glucuronide (10b) or (12b) (10 mg) and beef-liver β-glucuronidase (Tokyo Zoki Co., Ltd.) (12 000 Fishman unit) in 0.1M-acetate buffer (pH 4.6, 20 ml) was incubated at 38 °C for 24 h. The incubated mixture was extracted with AcOEt (20 ml × 3), washed with water, dried (Na_2SO_4), and evaporated to give a solid aglycone. The solid was triturated with ether and then crystallized from aqueous MeOH to give estradiol (3 mg) as colourless needles, m.p. 173—176 °C, from (10b) digestion and estriol (5) (2 mg) as colourless plates, m.p. 278—283 °C, from (12b) digestion, respectively. The products were identical with authentic samples in every respect.

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