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# Syntheses of 4-Hydroxyestriol Monoglucuronides and Monosulfates[1)]

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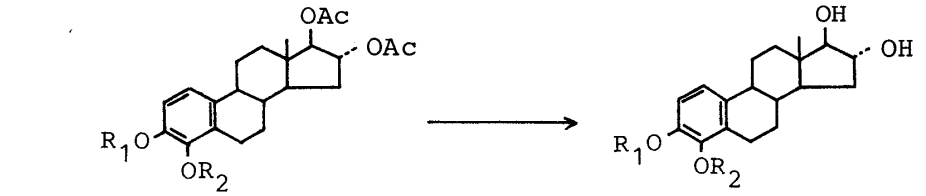
The A-ring monoglucuronides and monosulfates of 4-hydroxyestriol were synthesized from 4-hydroxyestriol 16,17-diacetate by means of the Koenigs–Knorr reaction with methyl α-acetobromoglucuronate and sulfation with sulfur trioxide–pyridine complex, respectively. The conjugated positions of these compounds were unequivocally elucidated by leading the products to guaiacol estrogens. The D-ring monoglucuronides and monosulfates of 4-hydroxyestriol were also obtained from 4-hydroxyestriol 3,4-dibenzyl ether by glucuronidation and sulfation followed by hydrogenolysis, respectively. The conjugated positions were established on the basis of spectral data of derivatives. The preparation of 4-hydroxyestradiol 17-conjugates is also described.

**Keywords**——catechol estrogen; 4-hydroxyestriol monoglucuronide; 4-hydroxyestriol monosulfate; 4-hydroxyestriol monomethyl ether; 4-hydroxyestradiol 17-conjugate; Koenigs–Knorr reaction; sulfur trioxide–pyridine complex; dicyclohexylcarbodiimide-mediated sulfation

Since the occurrence of 4-hydroxyestrogens as well as 2-hydroxyestrogens in pregnancy urine was disclosed by three groups,[2-4)] considerable attention has been directed to establishing metabolic fate of catechol estrogens in connection with their potent physiological activities. In the previous studies, we investigated the *in vitro* bioconversion of 4-hydroxyestrone,[5,6)] employing synthetic monoglucuronides and monosulfates of 4-hydroxyestrone and 4-hydroxyestradiol[7)] as reference compounds. Species difference and pH dependency were observed in the conjugated position of catechol estrogens.[6,8)] These results prompted us to clarify the metabolic fate of 4-hydroxyestriol, one of the principal catechol estrogens in human urine.[4)] Authentic specimens are required for the unambiguous characterization of metabolites formed from 4-hydroxyestriol. The present paper deals with the syntheses of 4-hydroxyestriol monoglucuronides and monosulfates together with 4-hydroxyestradiol 17-conjugates.[9,10)]

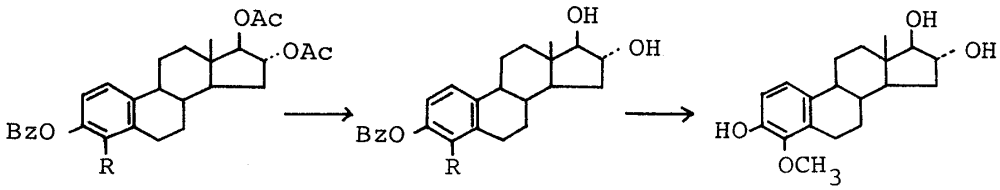
Our initial effort was focused on the preparation of 4-hydroxyestriol A-ring monoglucuronides and monosulfates (Chart 1). Introduction of a glucuronyl residue into 4-hydroxyestriol 16,17-diacetate (**1**)[11,12)] was undertaken by means of the Koenigs–Knorr reaction using cadmium carbonate as a catalyst.[13)] Condensation of **1** with methyl 2,3,4-tri-*O*-acetyl-1-bromo-1-deoxy-α-D-glucopyranuronate (methyl α-acetobromoglucuronate) in anhydrous benzene proceeded readily to afford two positional isomers, methyl 2,3,4-tri-*O*-acetyl-1-*O*-(16α,17β-diacetoxy-4-hydroxy-1,3,5(10)-estratrien-3-yl)-β-D-glucopyranosiduronate (**2**) and methyl 2,3,4,-tri-*O*-acetyl-1-*O*-(16α,17β-diacetoxy-3-hydroxy-1,3,5(10)-estratrien-4-yl)-β-D-glucopyranosiduronate (**3**), in a ratio of 4 to 1. Recrystallization of the crude product provided **2** in the pure state. Compound **3** was efficiently separated from the mother liquor by high-performance liquid chromatography (HPLC). Removal of the protecting groups in the D-ring and sugar moieties in **2** and **3** by alkaline hydrolysis under mild conditions yielded the desired 3-glucuronide (**4**) and 4-glucuronide (**5**). Inspection of the proton nuclear magnetic resonance ($^1$H-NMR) spectra of the glucuronides and their acetate-methyl esters revealed the

formation of a β-glucuronoside linkage. The anomeric proton of the sugar moiety appeared at *ca.* 5 ppm as a doublet ($J=6$—$7$ Hz), indicating a *trans*-diaxial relationship to the vicinal 2′-proton. Further evidence for the β-glucuronoside structure in these monoglucuronides was obtained by characterizing 4-hydroxyestriol liberated on incubation with β-glucuronidase.



**1**: $R_1=R_2=H$
**2**: $R_1=G'$, $R_2=H$
**3**: $R_1=H$, $R_2=G'$
**11**: $R_1=CH_3$, $R_2=H$
**12**: $R_1=H$, $R_2=CH_3$
**13**: $R_1=R_2=CH_3$
**16**: $R_1=SO_3H$, $R_2=H$
**17**: $R_1=H$, $R_2=SO_3H$

**4**: $R_1=G$, $R_2=H$
**5**: $R_1=H$, $R_2=G$
**10**: $R_1=H$, $R_2=CH_3$
**14**: $R_1=CH_3$, $R_2=H$
**15**: $R_1=R_2=CH_3$
**18**: $R_1=SO_3Na$, $R_2=H$
**19**: $R_1=H$, $R_2=SO_3Na$



**6**: $R=NO_2$
**7**: $R=NH_2$

**8**: $R=OH$
**9**: $R=OCH_3$

**10**

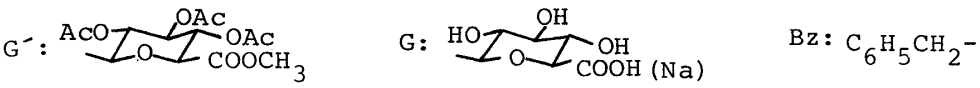
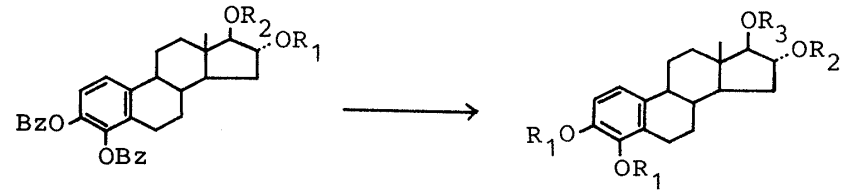


Chart 1

In order to establish the position of the glucuronyl residue in **2** and **3**, syntheses of 4-hydroxyestriol 4-methyl ether (**10**) and the 3-methyl ether (**14**) as authentic specimens were undertaken. Reduction of 4-nitroestriol 16,17-diacetate 3-benzyl ether (**6**) with zinc in acetic acid gave 4-aminoestriol 16,17-diacetate 3-benzyl ether (**7**). The 4-amino compound was converted into 4-hydroxyestriol 3-benzyl ether (**8**) by diazotization and subsequent acid hydrolysis in the manner reported by Williams *et al.*[14] Treatment of **8** with methyl iodide furnished 4-methoxyestriol 3-benzyl ether (**9**), which on hydrogenolysis over palladium-on-charcoal was led to 4-hydroxyestriol 4-methyl ether (**10**). For the preparation of the isomeric 4-hydroxyestriol 3-methyl ether (**14**), an alternative synthetic route was employed. Methylation of **1** with methyl iodide provided a mixture of methyl ethers (**11**—**13**), which was treated with methanolic sodium hydroxide and separated by preparative thin-layer chromatography (TLC) to give the desired 4-hydroxyestriol 3-methyl ether (**14**) together with the 4-monomethyl ether (**10**) and 3,4-dimethyl ether (**15**). The positions of the glucuronyl residue in **2** and **3** were unequivocally established by transforming these compounds into 4-hydroxyestriol 4- and 3-methyl ethers (**10**, **14**), respectively.[7]

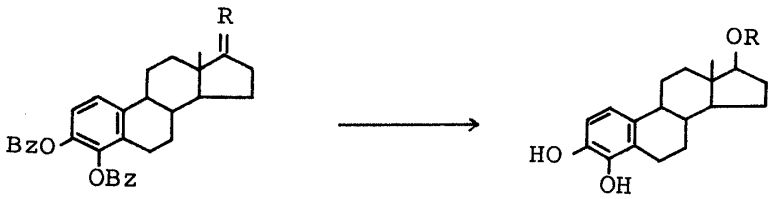
4-Hydroxyestriol A-ring monosulfates were also prepared by employing **1** as the starting material. Treatment with freshly prepared sulfur trioxide–pyridine complex furnished a mixture of 4-hydroxyestriol 16,17-diacetate 3-sulfate (**16**) and 4-sulfate (**17**) in a ratio of 10

to 1, which was estimated by reversed-phase HPLC. Alkaline hydrolysis of the mixture under mild conditions followed by recrystallization of the crude product afforded 4-hydroxyestriol 3-sulfate (**18**) as colorless needles. The mother liquor was purified by HPLC to give the isomeric 4-sulfate (**19**) as colorless needles. These monosulfates were characterized by methylation with diazomethane followed by solvolysis in the usual manner,[15] leading to the authentic 4-hydroxyestriol monomethyl ethers (**10**, **14**).



**20**: $R_1=R_2=Ac$
**21**: $R_1=R_2=H$
**22**: $R_1=G'$, $R_2=H$
**23**: $R_1=H$, $R_2=G'$
**26**: $R_1=G'$, $R_2=Ac$
**27**: $R_1=Ac$, $R_2=G'$
**28**: $R_1=SO_3H$, $R_2=H$
**29**: $R_1=H$, $R_2=SO_3H$

**24**: $R_1=R_3=H$, $R_2=G$
**25**: $R_1=R_2=H$, $R_3=G$
**30**: $R_1=R_3=H$, $R_2=SO_3H$
**31**: $R_1=R_2=H$, $R_3=SO_3H$
**32**: $R_1=R_3=Ac$, $R_2=SO_3H$
**33**: $R_1=R_2=Ac$, $R_3=SO_3H$



**34**: R=O
**35**: R= 
**36**: R= 
**38**: R= 

**37**: R=G
**39**: $R=SO_3Na$

Chart 2

Next, we attempted to synthesize 4-hydroxyestriol D-ring monoglucuronides and monosulfates (Chart 2). The Koenigs–Knorr reaction with 4-hydroxyestriol 3,4-dibenzyl ether (**21**) occurred readily to give the glucuronide acetate-methyl esters (**22**, **23**) in a ratio of 5 to 3. These positional isomers were efficiently separated by HPLC on a normal-phase column. Upon hydrolysis with methanolic alkali and subsequent hydrogenolysis over palladium-on-charcoal, **22** and **23** were transformed into the desired 16- and 17-glucuronides (**24**, **25**) in satisfactory yield. Compound **22** was also obtained from **21** as a sole product by the Koenigs–Knorr reaction using silver carbonate as a catalyst. It is of interest that the conjugated position is dependent on the catalyst, although no plausible explanation for this is at present available. This finding may make feasible the selective preparation of **24**.

The conjugated position and configuration of the glucuronoside linkage in **22** and **23** were established on the basis of $^1$H-NMR spectral data. On usual acetylation, **22** was transformed into the 17-acetate (**26**), in which the 17$\alpha$-proton signal appeared at 4.99 ppm as a doublet. On the other hand, the 16$\beta$-proton of the 16-acetate (**27**) derived from **23** appeared at 5.10 ppm as a multiplet. These data permitted us to assign the structures 16- and 17-

glucuronide acetate-methyl esters to **22** and **23**, respectively. The anomeric protons of these conjugates appeared at *ca.* 5.0 ppm as a doublet ($J=6$—7 Hz), indicating the formation of the β-glucuronoside.

Syntheses of D-ring monosulfates from **21** were then carried out. Reaction with sulfur trioxide–pyridine complex gave the disulfate as a main product. Accordingly, mild dicyclohexylcarbodiimide (DCC)-mediated sulfation[16] was employed for this purpose. On treatment with DCC and sulfuric acid in dimethylformamide (DMF), **21** was converted into a mixture of the 16- and 17-sulfates (**28**, **29**) which, on hydrogenolysis over palladium-on-charcoal followed by HPLC separation, were led to the desired 4-hydroxyestriol 16- and 17-sulfates (**30**, **31**) in a ratio of 5 to 1. These compounds exhibited characteristic infrared (IR) absorption bands (*ca.* 1250 and 1050 $cm^{-1}$) due to a sulfate group and gave 4-hydroxyestriol on usual solvolysis. In order to elucidate the sulfated position in **30** and **31**, these positional isomers were transformed into the triacetates (**32**, **33**) by the usual method. Upon acetylation the chemical shifts of the 17α-proton in **30** and the 16β-proton in **31** were moved downfield *ca.* 1 ppm in the $^1$H-NMR spectra. These data permitted us to assign the structures 16-sulfate and 17-sulfate to **30** and **31**, respectively.

In addition, 4-hydroxyestradiol 17-conjugates were also prepared. 4-Hydroxyestradiol 3,4-dibenzyl ether (**35**) was condensed with methyl α-acetobromoglucuronate in the presence of cadmium carbonate, providing the glucuronide acetate-methyl ester (**36**). Subsequent hydrolysis with methanolic alkali and hydrogenolysis yielded the desired 17-glucuronide (**37**). 4-Hydroxyestradiol 17-sulfate (**39**) was obtained from **35** by sulfation with freshly prepared sulfur trioxide–pyridine complex followed by hydrogenolysis.

The availability of these authentic specimens should assist the characterization and determination of catechol estrogen conjugates in biological fluids. Studies on the metabolism of 4-hydroxyestriol in humans are being conducted in these laboratories, and the details will be reported elsewhere.

## Experimental

All melting points were taken on a micro hot-stage apparatus and are uncorrected. Optical rotations were measured with a JASCO DIP-4 automatic polarimeter. $^1$H-NMR spectra were recorded on a JEOL FX-100 spectrometer at 100 MHz using tetramethylsilane as an internal standard. Abbreviations used are s=singlet, d=doublet, t=triplet, and m=multiplet. Mass spectra (MS) were obtained on a Hitachi M-52 mass spectrometer. High-resolution MS (high MS) spectra were obtained on a JEOL JMS-01SG-2 equipped with a JMA-2000 computer. IR spectral measurements were run on a JASCO IRA-1S spectrometer. The apparatus used for HPLC was a Waters ALC/GPC 202 high-performance liquid chromatograph equipped with a 440 UV detector (Waters Assoc., Milford, MA) monitoring the absorbance at 254 nm. HPLC was carried out on TSKgel ODS-120A (5 μm: 25 cm × 0.4 cm i.d.) (Toyo Soda Co., Tokyo), Hitachi 3053 (5 μm: 25 cm × 0.4 cm i.d.) (Hitachi Co., Tokyo), μBondapak $C_{18}$ (8—10 μm: 30 cm × 0.39 cm i.d.) (Waters Assoc.), and μPorasil (8—10 μm: 30 cm × 0.39 cm i.d.) (Waters Assoc.) columns under ambient conditions at a flow rate of 1 ml/min unless otherwise stated. For column chromatography and preparative TLC, Silica gel 60 and Silica gel $HF_{254}$ (E. Merck AG, Darmstadt) were used, respectively.

**General Procedure for the Preparation of Catechol Estrogen β-D-Glucopyranuronoside**——Freshly prepared $CdCO_3$[13] (300 mg) was added to a solution of catechol estrogen (0.5 mmol) in anhydrous benzene (15 ml), and the suspension was concentrated to *ca.* 10 ml by slow distillation over a period of 30 min to remove the moisture. After addition of methyl α-acetobromoglucuronate (200 mg), the whole was refluxed for 5 h. Additional amounts of methyl α-acetobromoglucuronate (100 mg) and $CdCO_3$ (150 mg) were added, and the mixture was refluxed for a further 5 h. The precipitate was removed by filtration and washed with benzene and $CH_2Cl_2$. The filtrate and washings were combined and evaporated down. Purification of the oily residue by means of preparative TLC or HPLC followed by recrystallization of the desired fraction gave the glucuronide acetate-methyl ester.

**16α,17β-Diacetoxy-1,3,5(10)-estratriene-3,4-diol (1)**[11,12]——Zn dust (10 g) was added to a solution of 4-nitroestriol 16,17-diacetate (1.2 g) in glacial AcOH (120 ml), and the whole was stirred for 25 min. The reaction mixture was then filtered, and the precipitate was washed with AcOH (90 ml × 2). The filtrate and washings were combined, and added within 3 min to a vigorously stirred solution of $NaIO_4$ (10 g) in 0.1 N HCl (700 ml) at room temperature. The mixture was stirred for 3 min, then extracted twice with $CHCl_3$. The organic layer was washed with $H_2O$ and after

addition of AcOH (100 ml) and KI (3 g) in $H_2O$ (0.5 ml), the mixture was stirred for 3 min. $I_2$ formed was reduced with 5% $NaHSO_3$ (100 ml). The organic layer was washed with $H_2O$ and dried over anhydrous $Na_2SO_4$. After addition of AcOH (2 ml), the solvent was evaporated off below 40 °C. The crude product was subjected to column chromatography on silica gel (30 cm × 2 cm i.d.) with benzene–AcOEt (50 : 1). The eluate was dried and evaporated, and the residue was recrystallized from MeOH–$CH_2Cl_2$ to give **1** (800 mg) as pale yellow needles, mp 221—223 °C. $[\alpha]_D^{21}$ −14.1° ($c$=0.14, $CHCl_3$). *Anal.* Calcd for $C_{22}H_{28}O_6 \cdot 1/2H_2O$: C, 66.43; H, 7.30. Found: C, 66.75; H, 7.36. High MS *m/z*: 388.1860 $[M]^+$, (Calcd for $C_{22}H_{28}O_6$: 388.1885). $^1$H-NMR ($CDCl_3$) $\delta$: 0.84 (3H, s, 18-$CH_3$), 2.04 (3H, s, $OCOCH_3$), 2.08 (3H, s, $OCOCH_3$), 4.98 (1H, d, $J$=6 Hz, 17α-H), 5.15 (1H, m, 16β-H), 6.65 (2H, s, 1- and 2-H).

**Methyl 2,3,4-Tri-*O*-acetyl-1-*O*-(16α,17β-diacetoxy-4-hydroxy-1,3,5(10)-estratrien-3-yl)-β-D-glucopyranosiduronate (2), Methyl 2,3,4-Tri-*O*-acetyl-1-*O*-(16α,17β-diacetoxy-3-hydroxy-1,3,5(10)-estratrien-4-yl)-β-D-glucopyranosiduronate (3)**——Compound **1** (100 mg) was subjected to glucuronidation in the manner described above. The oily residue obtained was subjected to preparative TLC using benzene–AcOEt (10 : 3) as a developing solvent. Elution of the adsorbent corresponding to the desired spot (*Rf* 0.48) with AcOEt and recrystallization of the product from MeOH gave **2** (60 mg) as colorless needles, mp 215—216 °C. $[\alpha]_D^{16}$ −28.6° ($c$=0.28, $CHCl_3$). *Anal.* Calcd for $C_{35}H_{44}O_{15}$: C, 59.65; H, 6.29. Found: C, 59.44; H, 6.11. $^1$H-NMR ($CDCl_3$) $\delta$: 0.83 (3H, s, 18-$CH_3$), 2.04 (9H, s, 3 × $OCOCH_3$), 2.09 (3H, s, $OCOCH_3$), 2.12 (3H, s, $OCOCH_3$), 3.76 (3H, s, $COOCH_3$), 4.13 (1H, m, 5′-H), 4.96 (1H, d, $J$=6 Hz, 17α-H), 5.02 (1H, d, $J$=6 Hz, 1′-H), 5.11 (1H, m, 16β-H), 6.75 (2H, s, 1- and 2-H). The mother liquor of **2** was subjected to HPLC on μPorasil using hexane–AcOEt (20 : 11) as a mobile phase. The fraction corresponding to the desired compound ($t_R$ 19.8 min) was collected and the solvent was evaporated off. Recrystallization of the residue from MeOH gave **3** (16 mg) as colorless needles, mp 140—142 °C. $[\alpha]_D^{13}$ −74.5° ($c$=0.21, $CHCl_3$). *Anal.* Calcd for $C_{35}H_{44}O_{15}$: C, 59.65; H, 6.29. Found: C, 59.61; H, 6.54. $^1$H-NMR ($CDCl_3$) $\delta$: 0.84 (3H, s, 18-$CH_3$), 2.04 (9H, s, 3 × $OCOCH_3$), 2.09 (3H, s, $OCOCH_3$), 2.12 (3H, s, $OCOCH_3$), 3.76 (3H, s, $COOCH_3$), 4.02 (1H, m, 5′-H), 4.78 (1H, d, $J$=7 Hz, 1′-H), 4.97 (1H, d, $J$=6 Hz, 17α-H), 5.10 (1H, m, 16β-H), 6.76 (1H, d, $J$=10 Hz, 1- or 2-H), 6.99 (1H, d, $J$=10 Hz, 2- or 1-H).

**Sodium 1-*O*-(4,16α,17β-Trihydroxy-1,3,5(10)-estratrien-3-yl)-β-D-glucopyranosiduronate (4)**——A solution of **2** (50 mg) in MeOH (5 ml) was treated with 1 N NaOH (5 ml), and the resulting solution was allowed to stand at room temperature for 12 h. The reaction mixture was then diluted with $H_2O$ (50 ml) and the organic solvent was evaporated off. The aq. solution was percolated through a column packed with Amberlite XAD-2 resin (Rohm and Haas Co., Philadelphia, PA) (25 cm × 1 cm i.d.). The column was washed with $H_2O$ (200 ml) and the desired substance was eluted with MeOH. An aq. solution of the dried eluate was applied to a column packed with Dowex 50W-X8 ($Na^+$ form) resin (3 cm × 0.6 cm i.d.) and eluted with $H_2O$ (5 ml). The effluent was evaporated down below 60 °C and the residue was reprecipitated from MeOH to give **4** (30 mg) as a colorless amorphous substance, mp 222 °C (dec.). $[\alpha]_D^{21}$ −36.8° ($c$=0.07, MeOH). *Anal.* Calcd for $C_{24}H_{31}NaO_{10} \cdot 3/2H_2O$: C, 54.44; H, 6.43. Found: C, 54.46; H, 6.16. $^1$H-NMR ($CD_3OD$) $\delta$: 0.77 (3H, s, 18-$CH_3$), 4.04 (1H, m, 5′-H), 4.60 (1H, d, $J$=6 Hz, 1′-H), 6.69 (1H, d, $J$=10 Hz, 1- or 2-H), 7.02 (1H, d, $J$=10 Hz, 2- or 1-H).

**Sodium 1-*O*-(3,16α,17β-Trihydroxy-1,3,5(10)-estratrien-4-yl)-β-D-glucopyranosiduronate (5)**——This compound was prepared from **3** (30 mg) as described for **4**. The crude product was reprecipitated from MeOH to give **5** (16 mg) as a colorless amorphous substance, mp 265 °C (dec.). $[\alpha]_D^{21}$ −20.6° ($c$=0.10, MeOH). *Anal.* Calcd for $C_{24}H_{31}NaO_{10} \cdot 1/2H_2O$: C, 56.36; H, 6.26. Found: C, 56.54; H, 6.26. $^1$H-NMR ($CD_3OD$) $\delta$: 0.77 (3H, s, 18-$CH_3$), 3.50 (1H, d, $J$=6 Hz, 17α-H), 6.62 (1H, d, $J$=10 Hz, 1- or 2-H), 6.74 (1H, d, $J$=10 Hz, 2- or 1-H).

**3-Benzyloxy-4-nitro-1,3,5(10)-estratriene-16α,17β-diol Diacetate (6)**——Anhydrous $K_2CO_3$ (100 mg) and benzyl chloride (0.1 ml) were added to a solution of 4-nitroestriol 16,17-diacetate (200 mg) in anhydrous EtOH (8 ml) and the reaction mixture was refluxed for 5 h. The precipitate was removed by filtration and washed with $CH_2Cl_2$. The filtrate and washings were combined and evaporated down. Recrystallization of the crude product from MeOH gave **6** (200 mg) as pale yellow needles, mp 170—171.5 °C. $[\alpha]_D^{16}$ −53.7° ($c$=0.38, $CHCl_3$). *Anal.* Calcd for $C_{29}H_{33}NO_7$: C, 68.82; H, 6.55; N, 2.76. Found: C, 68.39; H, 6.50; N, 2.62. $^1$H-NMR ($CDCl_3$) $\delta$: 0.83 (3H, s, 18-$CH_3$), 2.00 (3H, s, $OCOCH_3$), 2.25 (3H, s, $OCOCH_3$), 5.01 (1H, d, $J$=6 Hz, 17α-H), 5.11 (2H, s, $OCH_2Ph$), 5.15 (1H, m, 16β-H), 6.87 (1H, d, $J$=10 Hz, 1- or 2-H), 7.20 (1H, d, $J$=10 Hz, 2- or 1-H), 7.23—7.50 (5H, m, $OCH_2C_6\underline{H}_5$).

**3-Benzyloxy-1,3,5(10)-estratriene-4,16α,17β-triol (8)**——Zn dust (1.7 g) was added to a solution of **6** (200 mg) in glacial AcOH (10 ml), and the reaction mixture was stirred for 25 min. After removal of the precipitate by filtration, the filtrate was diluted with $H_2O$ and then extracted with AcOEt. The organic layer was washed with $H_2O$, dried over anhydrous $Na_2SO_4$ and evaporated down to give 4-aminoestriol 3-benzyl ether (**7**). The oily residue obtained was suspended in 22% (w/v) $H_2SO_4$ (2.6 ml) and treated with $NaNO_2$ (35 mg) in $H_2O$ (0.4 ml) in the manner described by Williams *et al.*[14] The residue was subjected to preparative TLC using benzene–AcOEt (1 : 1) as a developing solvent. Elution of the adsorbent corresponding to the spot (*Rf* 0.15) with AcOEt and recrystallization of the crude product from MeOH gave **8** (20 mg) as colorless needles, mp 87—88 °C. $[\alpha]_D^{22}$ +60.3° ($c$=0.03, MeOH). High MS *m/z*: 394.2136 $[M]^+$, (Calcd for $C_{25}H_{30}O_4$: 394.2142). $^1$H-NMR ($CDCl_3$) $\delta$: 0.80 (3H, s, 18-$CH_3$), 3.56 (1H, d, $J$=6 Hz, 17α-H), 4.17 (1H, m, 16β-H), 5.07 (2H, s, $OCH_2Ph$), 6.74 (2H, s, 1- and 2-H), 7.30—7.50 (5H, m, $OCH_2C_6\underline{H}_5$).

**3-Benzyloxy-4-methoxy-1,3,5(10)-estratriene-16α,17β-diol (9)**——A mixture of **8** (10 mg), anhydrous $K_2CO_3$ (7 mg) and methyl iodide (0.18 ml) in DMF (0.5 ml) was heated at 60 °C for 12 h. The reaction mixture was diluted

with $H_2O$ (50 ml) and then extracted with AcOEt. The organic layer was washed with 5% HCl and $H_2O$, dried over anhydrous $Na_2SO_4$ and evaporated down. Recrystallization of the crude product from MeOH gave **9** (10 mg) as colorless needles, mp 86—88 °C. $[\alpha]_D^{15}$ +117.6° ($c$=0.02, MeOH). High MS $m/z$: 408.2330 $[M]^+$, (Calcd for $C_{26}H_{32}O_4$: 408.2300). $^1$H-NMR ($CDCl_3$) $\delta$: 0.79 (3H, s, 18-$CH_3$), 3.58 (1H, d, $J$=6 Hz, 17α-H), 3.88 (3H, s, $OCH_3$), 4.18 (1H, m, 16β-H), 5.08 (2H, s, $OCH_2Ph$), 6.76 (1H, d, $J$=10 Hz, 1- or 2-H), 6.92 (1H, d, $J$=10 Hz, 2- or 1-H), 7.04—7.52 (5H, m, $OCH_2C_6\underline{H}_5$).

**4-Methoxy-1,3,5(10)-estratriene-3,16α,17β-triol (10)**——A solution of **9** (10 mg) in MeOH (5 ml) was shaken with 5% Pd–C (5 mg) under a hydrogen gas stream for 8 h. The catalyst was removed by filtration and the filtrate was evaporated down. The residue was recrystallized from MeOH to give **10** (6 mg) as colorless prisms, mp 259—262 °C. $[\alpha]_D^{28}$ +88.5° ($c$=0.03, MeOH). High MS $m/z$: 318.1816 $[M]^+$, (Calcd for $C_{19}H_{26}O_4$: 318.1829). $^1$H-NMR ($CD_3OD$) $\delta$: 0.78 (3H, s, 18-$CH_3$), 3.46 (1H, d, $J$=6 Hz, 17α-H), 3.71 (3H, s, $OCH_3$), 4.00 (1H, m, 16β-H), 6.60 (1H, d, $J$=10 Hz, 1- or 2-H), 6.86 (1H, d, $J$=10 Hz, 2- or 1-H).

**Methylation of 1 with Methyl Iodide**——A solution of **1** (100 mg) in DMF (10 ml) was heated with methyl iodide (0.5 ml) in the presence of $K_2CO_3$ (180 mg) at 60 °C for 12 h. The reaction mixture was treated with 1 N NaOH (5 ml) at room temperature for 2 h, diluted with $H_2O$ (50 ml) and extracted with AcOEt. The organic layer was washed with 5% HCl and $H_2O$, dried over anhydrous $Na_2SO_4$ and evaporated down. The residue obtained was subjected to preparative TLC using benzene–AcOEt (5:2) as a developing solvent and developed five times. The fraction corresponding to the spot ($^5Rf$ 0.22) was eluted with AcOEt and the product was recrystallized from MeOH to give **10** (10 mg), which proved to be identical with an authentic sample. Elution of the fraction corresponding to the spot ($^5Rf$ 0.18) with AcOEt and recrystallization of the product from MeOH gave **14** (6 mg) as colorless prisms, mp 215—216 °C. $[\alpha]_D^{26}$ +70.2° ($c$=0.06, MeOH). High MS $m/z$: 318.1839 $[M]^+$, (Calcd for $C_{19}H_{26}O_4$: 318.1831). $^1$H-NMR ($CD_3OD$) $\delta$: 0.78 (3H, s, 18-$CH_3$), 3.44 (1H, d, $J$=6 Hz, 17α-H), 3.79 (3H, s, $OCH_3$), 4.04 (1H, m, 16β-H), 6.69 (2H, s, 1- and 2-H). The fraction corresponding to the spot ($^5Rf$ 0.26) was eluted with AcOEt and the product was recrystallized from MeOH to give **15** (30 mg) as colorless needles, mp 184—185 °C. $[\alpha]_D^{28}$ +35.0° ($c$=0.07, $CHCl_3$). High MS $m/z$: 332.2889 $[M]^+$, (Calcd for $C_{20}H_{28}O_4$: 332.1887). $^1$H-NMR ($CD_3OD$) $\delta$: 0.78 (3H, s, 18-$CH_3$), 3.46 (1H, d, $J$=6 Hz, 17α-H), 3.72 (3H, s, $OCH_3$), 3.79 (3H, s, $OCH_3$), 4.04 (1H, m, 16β-H), 6.76 (1H, d, $J$=10 Hz, 1- or 2-H), 6.98 (1H, d, $J$=10 Hz, 2- or 1-H).

**Sodium 1,3,5(10)-Estratriene-3,4,16α,17β-tetraol 3-Sulfate (18), Sodium 1,3,5(10)-Estratriene-3,4,16α,17β-tetraol 4-Sulfate (19)**——Freshly prepared sulfur trioxide–pyridine complex (200 mg) was added to a solution of **1** (200 mg) in pyridine (2 ml), and the reaction mixture was stirred under ice-cooling for 2 h and then at room temperature for 6 h. Excess reagent was decomposed by addition of 5% $NaHCO_3$. The resulting mixture of **16** and **17** was treated with 1 N NaOH (10 ml) for 12 h at room temperature. The reaction mixture was diluted with $H_2O$ (150 ml) and applied to an Amberlite XAD-2 column (25 cm × 1 cm i.d.) as described for **4**. The crude product was recrystallized from MeOH several times to give pale yellow needles. An aq. solution of the product was applied to a Dowex 50W-X8 ($Na^+$ form) column (10 cm × 0.6 cm i.d.). The product was recrystallized from aq. MeOH to give **18** (140 mg) as colorless needles, mp 220—224 °C. $[\alpha]_D^{22}$ +34.2° ($c$=0.16, MeOH). *Anal.* Calcd for $C_{18}H_{23}NaO_7S \cdot 3H_2O$: C, 46.91; H, 6.30. Found: C, 46.92; H, 6.04. IR $\nu_{max}^{KBr}$ $cm^{-1}$: 1260, 1040 ($SO_3Na$). $^1$H-NMR ($CD_3OD$) $\delta$: 0.77 (3H, s, 18-$CH_3$), 3.44 (1H, d, $J$=6 Hz, 17α-H), 4.03 (1H, m, 16β-H), 6.73 (1H, d, $J$=10 Hz, 1- or 2-H), 7.00 (1H, d, $J$=10 Hz, 2- or 1-H). The mother liquor of **18** was subjected to HPLC on μBondapak $C_{18}$ using 0.5% $NH_4H_2PO_4$–$CH_3CN$ (9:2) as a mobile phase. The desired fraction ($t_R$ 34 min) was collected and evaporated down below 60 °C. The desired compound was obtained by using an Amberlite XAD-2 column (10 cm × 1 cm i.d.) in the manner described above. An aq. solution of the product obtained was applied to a Dowex 50W-X8 ($Na^+$ form) column (2 cm × 0.6 cm i.d.). The crude product was recrystallized from aq. MeOH to give **19** (12 mg) as colorless needles, mp 232—234 °C. $[\alpha]_D^{22}$ +15.9° ($c$=0.22, MeOH). *Anal.* Calcd for $C_{18}H_{23}NaO_7S \cdot 5/4H_2O$: C, 50.36; H, 5.95. Found: C, 50.31; H, 5.72. IR $\nu_{max}^{KBr}$ $cm^{-1}$: 1240, 1038 ($SO_3Na$). $^1$H-NMR ($CD_3OD$) $\delta$: 0.78 (3H, s, 18-$CH_3$), 3.44 (1H, d, $J$=6 Hz, 17α-H), 4.04 (1H, m, 16β-H), 6.64 (1H, d, $J$=10 Hz, 1- or 2-H), 6.98 (1H, d, $J$=10 Hz, 2- or 1-H).

**Elucidation of the Conjugated Position in Compounds 2, 3, 16 and 17**——A solution of **2**, **3**, **16**, or **17** (each 5 mg) in MeOH–ether (2:5) was treated with ethereal diazomethane at room temperature for 12 h. The resulting solution was evaporated down and the residue obtained was treated with 1 N NaOH (1 ml) for 2 h. The reaction mixture was diluted with $H_2O$ (10 ml) and applied to an Amberlite XAD-2 column (5 cm × 0.6 cm i.d.) in the manner described above. A solution of the residue obtained from **2** or **3** in 0.1 M acetate buffer (pH 5.2, 2 ml) was treated with the acetone powder (1 mg) of digestive juice from *Helix pomatia* at 37 °C for 24 h. The reaction mixture was extracted with AcOEt, and the organic layer was washed with $H_2O$, dried over anhydrous $Na_2SO_4$ and evaporated down. The products obtained proved to be identical with **10** and **14**, respectively, as judged by TLC (benzene–AcOEt (5:2)), HPLC (μBondapak $C_{18}$: $CH_3CN$–$H_2O$ (3:1)) and MS data.

The product obtained from **16** or **17** in the Amberlite XAD-2 step was subjected to solvolysis in the usual manner.[15] The solvolyzates proved to be identical with **10** and **14**.

**3,4-Dibenzyloxy-1,3,5(10)-estratriene-16α,17β-diol Diacetate (20)**——Anhydrous $K_2CO_3$ (270 mg) and benzyl chloride (0.1 ml) were added to a solution of **1** (300 mg) in anhydrous EtOH (20 ml), and the suspension was refluxed for 12 h. Additional amounts of anhydrous $K_2CO_3$ (100 mg) and benzyl chloride (0.1 ml) were added, and the mixture

was refluxed for a further 12 h. The precipitate was removed by filtration and washed with $CH_2Cl_2$. The filtrate and washings were combined and evaporated down. The oily residue was subjected to preparative TLC using benzene–AcOEt (30 : 1) as a developing solvent. Elution of the adsorbent corresponding to the spot ($^3Rf$ 0.55) with AcOEt and recrystallization of the product from MeOH gave **20** (300 mg) as colorless leaflets, mp 115—117 °C. $[\alpha]_D^{12}$ −9.9° ($c$ = 0.25, $CHCl_3$). *Anal.* Calcd for $C_{36}H_{40}O_6$: C, 76.03; H, 7.09. Found: C, 75.69; H, 7.42. $^1$H-NMR ($CDCl_3$) $\delta$: 0.84 (3H, s, 18-$CH_3$), 2.05 (3H, s, $OCOCH_3$), 2.08 (3H, s, $OCOCH_3$), 4.99 (2H, s, $OCH_2Ph$), 5.08 (2H, s, $OCH_2Ph$), 6.77 (1H, d, $J$ = 10 Hz, 1- or 2-H), 6.92 (1H, d, $J$ = 10 Hz, 2- or 1-H), 7.10—7.48 (10H, m, 2 × $OCH_2C_6\underline{H}_5$).

**3,4-Dibenzyloxy-1,3,5(10)-estratriene-16α,17β-diol (21)**——A solution of **20** (300 mg) in MeOH (20 ml) was treated with 1 N NaOH (10 ml), and the resulting solution was allowed to stand at room temperature for 2 h. After dilution with $H_2O$ (50 ml) the desired compound was extracted with AcOEt as described for **9**. Recrystallization of the crude product from MeOH gave **21** (250 mg) as colorless needles, mp 78—80 °C. $[\alpha]_D^{15}$ +30.5° ($c$ = 0.10, MeOH). *Anal.* Calcd for $C_{32}H_{36}O_4 \cdot 4/3H_2O$: C, 75.63; H, 7.41. Found: C, 75.97; H, 7.23. High MS $m/z$: 484.2611 $[M]^+$, (Calcd for $C_{32}H_{36}O_4$: 484.2611). $^1$H-NMR ($CDCl_3$) $\delta$: 0.79 (3H, s, 18-$CH_3$), 3.56 (1H, d, $J$ = 6 Hz, 17α-H), 4.01 (1H, m, 16β-H), 4.99 (2H, s, $OCH_2Ph$), 5.09 (2H, s, $OCH_2Ph$), 6.80 (1H, d, $J$ = 10 Hz, 1-or 2-H), 6.96 (1H, d, $J$ = 10 Hz, 2- or 1-H), 7.12—7.50 (10H, m, 2 × $OCH_2C_6\underline{H}_5$).

**Methyl 2,3,4-Tri-*O*-acetyl-1-*O*-(3,4-dibenzyloxy-17β-hydroxy-1,3,5(10)-estratrien-16α-yl)-β-D-glucopyranosiduronate (22), Methyl 2,3,4-Tri-*O*-acetyl-1-*O*-(3,4-dibenzyloxy-16α-hydroxy-1,3,5(10)-estratrien-17β-yl)-β-D-glucopyranosiduronate (23)**——Compound **21** (250 mg) was subjected to glucuronidation in the manner described above. The oily residue obtained was subjected to preparative TLC using benzene–AcOEt (10 : 3) as a developing solvent. Elution of the adsorbent corresponding to the spot ($Rf$ 0.41) with AcOEt and recrystallization of the product from MeOH gave **22** (100 mg) as colorless needles, mp 204—207 °C. $[\alpha]_D^{22}$ +15.2° ($c$ = 0.29, $CHCl_3$). *Anal.* Calcd for $C_{45}H_{52}O_{13}$: C, 67.48; H, 6.54. Found: C, 67.13; H, 6.74. $^1$H-NMR ($CDCl_3$) $\delta$: 0.80 (3H, s, 18-$CH_3$), 2.02 (3H, s, $OCOCH_3$), 2.04 (3H, s, $OCOCH_3$), 2.06 (3H, s, $OCOCH_3$), 3.60—4.00 (2H, m, 16β- and 17α-H), 3.76 (3H, s, $COOCH_3$), 4.05 (1H, m, 5′-H), 4.58 (1H, d, $J$ = 7 Hz, 1′-H), 4.99 (2H, s, $OCH_2Ph$), 5.09 (2H, s, $OCH_2Ph$), 6.80 (1H, d, $J$ = 10 Hz, 1- or 2-H), 6.98 (1H, d, $J$ = 10 Hz, 2- or 1-H), 7.20—7.51 (10H, m, 2 × $OCH_2C_6\underline{H}_5$). The mother liquor of **22** was subjected to HPLC on μPorasil using cyclohexane–tetrahydrofuran (10 : 3) as a mobile phase (flow rate 1.5 ml/min). The fraction corresponding to **23** ($t_R$ 3.5 min) was collected and evaporated down. Recrystallization of the residue from MeOH gave **23** (60 mg) as colorless prisms, mp 188—190 °C. $[\alpha]_D^{17}$ +0.4° ($c$ = 0.23, $CHCl_3$). *Anal.* Calcd for $C_{45}H_{52}O_{13}$: C, 67.48; H, 6.54. Found: C, 67.22; H, 6.29. $^1$H-NMR ($CDCl_3$) $\delta$: 0.76 (3H, s, 18-$CH_3$), 2.03 (6H, s, 2 × $OCOCH_3$), 2.07 (3H, s, $OCOCH_3$), 3.32 (1H, d, $J$ = 6 Hz, 17α-H), 3.76 (3H, s, $COOCH_3$), 4.08 (1H, m, 5′-H), 4.15 (1H, m, 16β-H), 4.56 (1H, d, $J$ = 6 Hz, 1′-H), 4.98 (2H, s, $OCH_2Ph$), 5.08 (2H, s, $OCH_2Ph$), 6.79 (1H, d, $J$ = 10 Hz, 1- or 2-H), 6.92 (1H, d, $J$ = 10 Hz, 2- or 1-H), 7.16—7.48 (10H, m, 2 × $OCH_2C_6\underline{H}_5$).

Compound **23** (150 mg) was prepared from **21** (250 mg) by glucuronidation in the manner described above except for the use of $Ag_2CO_3$ as a catalyst. Its purity was confirmed by HPLC and $^1$H-NMR spectral data.

**Sodium 1-*O*-(3,4,17β-Trihydroxy-1,3,5(10)-estratrien-16α-yl)-β-D-glucopyranosiduronate (24)**——A solution of **22** (50 mg) in MeOH (20 ml) was treated with 1 N NaOH (10 ml) as described for **4**. A solution of the crude product (30 mg) in MeOH (10 ml) was shaken with 5% Pd–C (15 mg) under a hydrogen gas stream for 20 h. The catalyst was removed by filtration and the filtrate was evaporated down. The residue was purified by HPLC on TSK gel ODS-120A using 0.5% $NH_4H_2PO_4$–$CH_3CN$ (5 : 1) as a mobile phase. The fraction corresponding to **24** ($t_R$ 9.5 min) was collected and evaporated down. The desired compound was obtained by using an Amberlite XAD-2 column (10 cm × 1 cm i.d.) and converted into the Na salt as described for **4**. The crude product was reprecipitated from MeOH to give **24** (25 mg) as a colorless amorphous substance, mp 235—241 °C (dec.). $[\alpha]_D^{28}$ +141.2° ($c$ = 0.04, MeOH). *Anal.* Calcd for $C_{24}H_{31}NaO_{10} \cdot 2H_2O$: C, 53.52; H, 6.55. Found: C, 53.79; H, 6.36. $^1$H-NMR ($CD_3OD$) $\delta$: 0.80 (3H, s, 18-$CH_3$), 3.64 (1H, d, $J$ = 6 Hz, 17α-H), 4.02 (1H, m, 5′-H), 4.28 (1H, d, $J$ = 7 Hz, 1′-H), 6.57 (2H, s, 1- and 2-H).

**Sodium 1-*O*-(3,4,16α-Trihydroxy-1,3,5(10)-estratrien-17β-yl)-β-D-glucopyranosiduronate (25)**——Compound **25** was prepared from **23** (50 mg) in the manner described above. The crude product was reprecipitated from MeOH to give **25** (25 mg) as a colorless amorphous substance, mp 206—216 °C (dec.). $[\alpha]_D^{28}$ +48.4° ($c$ = 0.07, MeOH). *Anal.* Calcd for $C_{24}H_{31}NaO_{10} \cdot 7/2H_2O$: C, 50.97; H, 6.77. Found: C, 50.65; H, 6.21. $^1$H-NMR ($CD_3OD$) $\delta$: 0.86 (3H, s, 18-$CH_3$), 4.35 (1H, d, $J$ = 7 Hz, 1′-H), 6.57 (2H, s, 1- and 2-H).

**Methyl 1-*O*-(17β-Acetoxy-3,4-dibenzyloxy-1,3,5(10)-estratrien-16α-yl)-2,3,4-tri-*O*-acetyl-β-D-glucopyranosiduronate (26)**——Acetic anhydride (0.5 ml) was added to a solution of **22** (30 mg) in pyridine (0.5 ml), and the resulting solution was allowed to stand at 60 °C for 1 h. The reaction mixture was diluted with $H_2O$ (50 ml) and extracted with AcOEt. The organic layer was washed successively with 5% HCl, 5% $NaHCO_3$ and $H_2O$, dried over anhydrous $Na_2SO_4$ and evaporated down. Recrystallization of the crude product from $CH_2Cl_2$–MeOH gave **26** (30 mg) as colorless needles, mp 134—136 °C. $[\alpha]_D^{18}$ −21.9° ($c$ = 0.21, $CHCl_3$). *Anal.* Calcd for $C_{47}H_{54}O_{14}$: C, 66.97; H, 6.46. Found: C, 66.85; H, 6.42. $^1$H-NMR ($CDCl_3$) $\delta$: 0.76 (3H, s, 18-$CH_3$), 2.00 (6H, s, 2 × $OCOCH_3$), 2.04 (6H, s, 2 × $OCOCH_3$), 3.76 (3H, s, $COOCH_3$), 3.97 (1H, m, 5′-H), 4.15 (1H, m, 16β-H), 4.52 (1H, d, $J$ = 7 Hz, 1′-H), 4.98 (2H, s, $OCH_2Ph$), 4.99 (1H, d, $J$ = 6 Hz, 17α-H), 5.08 (2H, s, $OCH_2Ph$), 6.76 (1H, d, $J$ = 10 Hz, 1- or 2-H), 6.94 (1H, d, $J$ = 10 Hz, 2- or 1-H), 7.20—7.48 (10H, m, 2 × $OCH_2C_6\underline{H}_5$).

**Methyl 1-*O*-(16α-Acetoxy-3,4-dibenzyloxy-1,3,5(10)-estratrien-17β-yl)-2,3,4-tri-*O*-acetyl-β-D-glucopyranosiduronate (27)**——Compound **27** was prepared from **23** (30 mg) in the manner described above. Recrystallization of the crude product from MeOH gave **27** (30 mg) as colorless needles, mp 165—166.5 °C. $[\alpha]_D^{28}$ −25.1° ($c$=0.38, $CHCl_3$). *Anal.* Calcd for $C_{47}H_{54}O_{14}$: C, 66.97; H, 6.46. Found: C, 66.69; H, 6.45. $^1$H-NMR ($CDCl_3$) $\delta$: 0.79 (3H, s, 18-$CH_3$), 2.00 (6H, s, 2×$OCOCH_3$), 2.01 (3H, s, $OCOCH_3$), 2.06 (3H, s, $OCOCH_3$), 3.68 (1H, d, $J$=6 Hz, 17α-H), 3.74 (3H, s, $COOCH_3$), 3.98 (1H, m, 5′-H), 4.58 (1H, d, $J$=6 Hz, 1′-H), 4.97 (2H, s, $OCH_2Ph$), 5.09 (2H, s, $OCH_2Ph$), 5.10 (1H, m, 16β-H), 6.78 (1H, d, $J$=10 Hz, 1- or 2-H), 6.94 (1H, d, $J$=10 Hz, 2- or 1-H), 7.20—7.48 (10H, m, 2×$OCH_2C_6\underline{H}_5$).

**1,3,5(10)-Estratriene-3,4,16α,17β-tetraol 16-Sulfate (30), 1,3,5(10)-Estratriene-3,4,16α,17β-tetraol 17-Sulfate (31)**——A solution of $H_2SO_4$ (147 mg) in DMF (12 ml) was added to **21** (484 mg) and DCC (1 g) in DMF (28 ml), and the whole was stirred under ice-cooling for 15 min. After centrifugation, the supernatant was separated and diluted with $H_2O$. The desired products were obtained by using an Amberlite XAD-4 (Rohm and Haas Co.) column (25 cm × 1 cm i.d.) as described for **4**. The residue obtained was subjected to preparative TLC using $CHCl_3$–MeOH–$H_2O$ (80 : 20 : 2.5) as a developing solvent. Elution of the adsorbent corresponding to the spot ($Rf$ 0.29) with $CHCl_3$–MeOH–$H_2O$ (70 : 30 : 6) gave a mixture of 4-hydroxyestriol 3,4-dibenzyl ether 16- and 17-sulfates (**28**, **29**) as an oily substance. IR $\nu_{max}^{KBr}$ $cm^{-1}$: 1210, 1080 ($SO_3H$). $^1$H-NMR ($CD_3OD$) $\delta$: 0.80 (2.5H, s, 18-$CH_3$), 0.81 (0.5H, s, 18-$CH_3$), 3.47 (0.8H, d, $J$=6 Hz, 17α-H), 4.09 (0.8H, m, 16β-H), 4.91 (2H, s, $OCH_2Ph$), 5.03 (2H, s, $OCH_2Ph$), 6.84 (1H, d, $J$=10 Hz, 1- or 2-H), 6.98 (1H, d, $J$=10 Hz, 2- or 1-H), 7.10—7.45 (10H, m, 2×$OCH_2C_6\underline{H}_5$). Unfortunately various efforts to separate the mixture by TLC and HPLC ended in failure. The product was therefore subjected to hydrogenolysis without further purification as described for **24**. The product obtained was separated by HPLC on Hitachi 3053 using 0.5% $NH_4H_2PO_4$–MeOH (3 : 1) as a mobile phase. The fractions corresponding to **30** ($t_R$ 24.5 min) and **31** ($t_R$ 21 min) were collected and treated as described for **24** to give **30** and **31** as oily substances. Compound **30**: $^1$H-NMR ($CD_3OD$) $\delta$: 0.80 (3H, s, 18-$CH_3$), 3.66 (1H, d, $J$=6 Hz, 17α-H), 4.05 (1H, m, 16β-H), 6.56 (2H, s, 1- and 2-H). Compound **31**; $^1$H-NMR ($CD_3OD$) $\delta$: 0.85 (3H, s, 18-$CH_3$), 3.56 (1H, d, $J$=6 Hz, 17α-H), 4.15 (1H, m, 16β-H), 6.53 (2H, s, 1- and 2-H). These compounds were very unstable and could not be further purified.

**3,4,17β-Triacetoxy-1,3,5(10)-estratrien-16α-ol 16-Sulfate (32), 3,4,16α-Triacetoxy-1,3,5(10)-estratrien-17β-ol 17-Sulfate (33)**——Compound **30** (*ca.* 25 mg) was treated with acetic anhydride (0.2 ml) and pyridine (1 ml) as described for **26**. The reaction mixture was diluted with $H_2O$ (100 ml) and then applied to an Amberlite XAD-4 column (15 cm × 1 cm i.d.) as described for **4**. The crude product was reprecipitated from ether to give **32** (20 mg) as a pale yellow amorphous substance, mp 244 °C (dec.). $[\alpha]_D^{15}$ +10.6° ($c$=0.28, MeOH). *Anal.* Calcd for $C_{24}H_{30}O_{10}S \cdot 2H_2O$: C, 52.74; H, 6.27; S, 5.87. Found: C, 53.00; H, 6.29; S, 5.90. IR $\nu_{max}^{KBr}$ $cm^{-1}$: 1210, 1041 ($SO_3H$). $^1$H-NMR ($CD_3OD$) $\delta$: 0.85 (3H, s, 18-$CH_3$), 2.06 (3H, s, $OCOCH_3$), 2.22 (3H, s, $OCOCH_3$), 2.27 (3H, s, $OCOCH_3$), 4.20 (1H, m, 16β-H), 4.98 (1H, d, $J$=6 Hz, 17α-H), 6.92 (1H, d, $J$=10 Hz, 1- or 2-H), 7.18 (1H, d, $J$=10 Hz, 2- or 1-H). Compound **33** was prepared from **31** (*ca.* 5 mg) in the manner described above. The crude product was reprecipitated from ether to give **33** (3 mg) as a pale yellow amorphous substance, mp 215 °C (dec.). IR $\nu_{max}^{KBr}$ $cm^{-1}$: 1210, 1080 ($SO_3H$). $^1$H-NMR ($CD_3OD$) $\delta$: 0.90 (3H, s, 18-$CH_3$), 2.01 (3H, s, $OCOCH_3$), 2.22 (3H, s, $OCOCH_3$), 2.27 (3H, s, $OCOCH_3$), 5.12 (1H, m, 16β-H), 6.92 (1H, d, $J$=10 Hz, 1- or 2-H), 7.22 (1H, d, $J$=10 Hz, 2- or 1-H).

**3,4-Dibenzyloxy-1,3,5(10)-estratrien-17-one (34)**——Compound **34** was prepared from 4-hydroxyestrone[12] (300 mg) as described for **20**. The oily residue obtained was subjected to column chromatography on silica gel (15 cm × 1 cm i.d.). Elution with benzene–AcOEt (50 : 1) and recrystallization of the product from ether gave **34** (300 mg) as colorless needles, mp 111.5—113 °C. $[\alpha]_D^{22.5}$ +102.4° ($c$=0.41, $CHCl_3$). *Anal.* Calcd for $C_{32}H_{34}O_3$: C, 82.37; H, 7.35. Found: C, 82.09; H, 7.48. $^1$H-NMR ($CDCl_3$) $\delta$: 0.90 (3H, s, 18-$CH_3$), 4.97 (2H, s, $OCH_2Ph$), 5.06 (2H, s, $OCH_2Ph$), 6.79 (1H, d, $J$=10 Hz, 1- or 2-H), 7.00 (1H, d, $J$=10 Hz, 2- or 1-H), 7.10—7.53 (10H, m, 2×$OCH_2C_6\underline{H}_5$).

**3,4-Dibenzyloxy-1,3,5(10)-estratrien-17β-ol (35)**——A solution of **34** (100 mg) in MeOH (10 ml) was treated with $NaBH_4$ (100 mg) under ice-cooling. The resulting solution was allowed to stand at room temperature for 1 h and extracted with AcOEt. The organic layer was washed with 5% HCl and $H_2O$, dried over anhydrous $Na_2SO_4$ and then evaporated down. Recrystallization of the residue from MeOH gave **35** (100 mg) as colorless needles, mp 79—81 °C. $[\alpha]_D^{22.5}$ +34.5° ($c$=0.30, $CHCl_3$). *Anal.* Calcd for $C_{32}H_{36}O_3 \cdot 2/3H_2O$: C, 79.90; H, 7.77. Found: C, 79.83; H, 7.93. High MS $m/z$: 468.2636 $[M]^+$, (Calcd for $C_{32}H_{36}O_3$: 468.2663). $^1$H-NMR ($CDCl_3$) $\delta$: 0.73 (3H, s, 18-$CH_3$), 3.60 (1H, t, $J$=9 Hz, 17α-H), 4.97 (2H, s, $OCH_2Ph$), 5.04 (2H, s, $OCH_2Ph$), 6.75 (1H, d, $J$=10 Hz, 1- or 2-H), 7.00 (1H, d, $J$=10 Hz, 2- or 1-H), 7.10—7.60 (10H, m, 2×$OCH_2C_6\underline{H}_5$).

**Methyl 2,3,4-Tri-*O*-acetyl-1-*O*-(3,4-dibenzyloxy-1,3,5(10)-estratrien-17β-yl)-β-D-glucopyranosiduronate (36)**——Compound **35** (100 mg) was subjected to glucuronidation in the manner described above. The oily residue obtained was purified by column chromatography on silica gel (20 cm × 1 cm i.d.). Elution with benzene–AcOEt (10 : 1) and recrystallization of the product from MeOH gave **36** (85 mg) as colorless needles, mp 171—173 °C. $[\alpha]_D^{28}$ −19.2° ($c$=0.10, $CHCl_3$). *Anal.* Calcd for $C_{45}H_{52}O_{12}$: C, 68.86; H, 6.68. Found: C, 68.97; H, 6.77. $^1$H-NMR ($CDCl_3$) $\delta$: 0.76 (3H, s, 18-$CH_3$), 2.02 (6H, s, 2×$OCOCH_3$), 2.06 (3H, s, $OCOCH_3$), 3.76 (3H, s, $COOCH_3$), 4.02 (1H, m, 5′-H), 4.61 (1H, d, $J$=7 Hz, 1′-H), 4.99 (2H, s, $OCH_2Ph$), 5.11 (2H, s, $OCH_2Ph$), 6.80 (1H, d, $J$=10 Hz, 1- or 2-H), 6.98 (1H, d, $J$=10 Hz, 2- or 1-H), 7.24—7.52 (10H, m, 2×$OCH_2C_6\underline{H}_5$).

**1-*O*-(3,4-Dihydroxy-1,3,5(10)-estratrien-17β-yl)-β-D-glucopyranosiduronic Acid (37)**——Compound **37** was pre-

pared from **36** (50 mg) as described for **24**. The product was purified by HPLC and reprecipitated from aq. MeOH to give **37** (20 mg) as a colorless amorphous substance, mp 204—210 °C (dec.). $[\alpha]_D^{28}$ +23.3° ($c$=0.11, MeOH). *Anal.* Calcd for $C_{24}H_{32}O_9 \cdot 2H_2O$: C, 57.59; H, 7.25. Found: C, 57.69; H, 7.01. $^1$H-NMR ($CD_3OD$) $\delta$: 0.86 (3H, s, 18-$CH_3$), 4.55 (1H, d, $J$=7 Hz, 1′-H), 6.56 (2H, s, 1- and 2-H).

**3,4-Dibenzyloxy-1,3,5(10)-estratrien-17β-ol 17-Sulfate (38)**——A solution of **35** (80 mg) in pyridine (2 ml) was subjected to sulfation with freshly prepared sulfur trioxide–pyridine complex (80 mg) as described for **18**. The reaction mixture was diluted with $H_2O$ (150 ml) and applied to an Amberlite XAD-2 column (15 cm × 1 cm i.d.) as described for **4**. The crude product obtained was reprecipitated from AcOEt to give **38** (80 mg) as a pale yellow amorphous substance, mp 165—168 °C (dec.). $[\alpha]_D^{25}$ +15.6° ($c$=0.13, MeOH). $^1$H-NMR ($CDCl_3$) $\delta$: 0.84 (3H, s, 18-$CH_3$), 4.99 (2H, s, $OCH_2Ph$), 5.14 (2H, s, $OCH_2Ph$), 6.84 (1H, d, $J$=10 Hz, 1- or 2-H), 7.01 (1H, d, $J$=10 Hz, 2- or 1-H). 7.20—7.62 (10H, m, 2 × $OCH_2C_6H_5$).

**Sodium 1,3,5(10)-Estratriene-3,4,17β-triol 17-Sulfate (39)**——This compound was obtained from **38** (50 mg) as described for **24**. The product was reprecipitated from aq. MeOH to give **39** (40 mg) as a colorless amorphous substance, mp 186—187.5 °C (dec.). $[\alpha]_D^{28}$ +46.8° ($c$=0.09, MeOH). *Anal.* Calcd for $C_{18}H_{23}NaO_6S \cdot 5/2H_2O$: C, 49.65; H, 6.44. Found: C, 49.54; H, 6.50. IR $\nu_{max}^{KBr}$ cm$^{-1}$: 1250, 1045 ($SO_3Na$). $^1$H-NMR ($CD_3OD$) $\delta$: 0.76 (3H, s, 18-$CH_3$), 4.27 (1H, t, $J$=9 Hz, 17α-H), 6.56 (2H, s, 1- and 2-H).

**Enzymic Hydrolysis of Catechol Estrogen Monoglucuronides**——Catechol estrogen monoglucuronides (**4**, **5**, **24**, **25**, **37**) (*ca.* 2 mg) were subjected to enzymic hydrolysis as described for the elucidation of the conjugated position of compounds **2**, **3**, **16** and **17**. The hydrolyzates proved to be identical with 4-hydroxyestriol and 4-hydroxyestradiol, respectively, on the basis of TLC behavior with benzene–AcOEt (10 : 1) as a developing solvent and MS data.

**Solvolysis of Catechol Estrogen Monosulfates**——Catechol estrogen monosulfates (**18**, **19**, **30**, **31**, **39**) (*ca.* 2 mg) were subjected to usual solvolysis.[15] The solvolyzates proved to be identical with 4-hydroxyestriol and 4-hydroxyestradiol, respectively, on the basis of TLC behavior and MS data.

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