[Chem. Pharm. Bull.
30(12)4417—4421(1982)]

Syntheses of 4-Hydroxyestrogen Monoglucuronides and Monosulfates[1]

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(Received May 10, 1982)

4-Hydroxyestrone 3- and 4-monoglucuronides were synthesized from catechol estrogens by means of the Koenigs–Knorr reaction with methyl α-acetobromoglucuronate. The position of the glucuronyl residue introduced was unequivocally elucidated by leading the products to the known 4-hydroxyestrone monomethyl ethers. Sulfation of 4-hydroxyestrone with sulfur trioxide–pyridine complex followed by fractional crystallization provided 4-hydroxyestrone 3-sulfate. The isomeric 4-sulfate was prepared from 4-hydroxyestrone 3-benzyl ether by sulfation and subsequent hydrogenolysis. 4-Hydroxyestradiol monoglucuronides and monosulfates were also obtained from the corresponding 17-ketones by borohydride reduction. The preparation of guaiacol estrogen monoglucuronides and monosulfates is also described.

Keywords——catechol estrogen; 4-hydroxyestrone monoglucuronide; 4-hydroxyestrone monosulfate; 4-hydroxyestradiol monoglucuronide; 4-hydroxyestradiol monosulfate; guaiacol estrogen conjugate; Koenigs–Knorr reaction; sulfur trioxide–pyridine complex

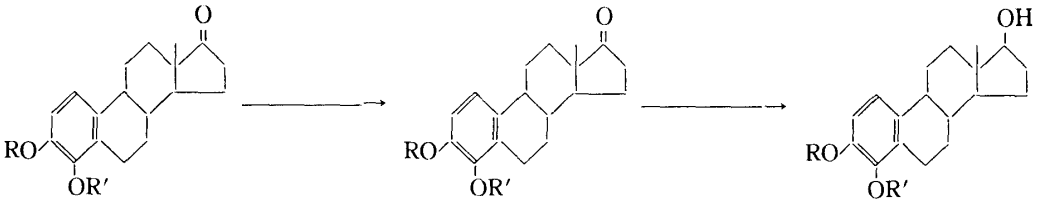
The occurrence of 4-hydroxyestrogens as well as the well-known 2-hydroxyestrogens in pregnacy urine has recently been demonstrated.[2] It is of interest to clarify the metabolic pathway of these compounds in view of their possible physiological significance. These catechols are excreted in the urine principally as glucuronides and sulfates, but their nature still remains unclear. Authentic specimens, therefore, are required for the characterization of these conjugates. The present paper deals with the syntheses of 4-hydroxyestrogen monoglucuronides and monosulfates.

Our initial effort was focused on the preparation of 4-hydroxyestrone monoglucuronides. Introduction of a glucuronyl residue into 4-hydroxyestrone (**1**)[3] was undertaken by means of the Koenigs–Knorr reaction using cadmium carbonate as a catalyst.[4] Condensation of **1** with methyl 1-bromo-1-deoxy-2,3,4-tri-*O*-acetyl-α-D-glucopyranuronate in anhydrous benzene proceeded readily to afford methyl (4-hydroxy-17-oxo-1,3,5(10)-estratrien-3-yl-2,3,4-tri-*O*-acetyl-β-D-glucopyranosid)uronate (**2**) and methyl (3-hydroxy-17-oxo-1,3,5(10)-estratrien-4-yl-2,3,4-tri-*O*-acetyl-β-D-glucopyranosid)uronate (**4**) in a ratio of 4 to 1. These products were efficiently separated by preparative thin-layer chromatography (TLC). Hydrolysis with methanolic sodium hydroxide under mild conditions removed the protecting groups of the sugar moiety in **2** and **4**, yielding the desired 3-glucuronide (**3**) and 4-glucuronide (**5**), respectively. 4-Hydroxyestradiol 3-glucuronide (**6**) and 4-glucuronide (**7**) were prepared from the corresponding 4-hydroxyestrone monoglucuronides (**3**, **5**) by reduction with sodium borohydride.[5]

The proton nuclear magnetic resonance (^{1}H-NMR) spectra of the glucuronide acetate-methyl esters indicated 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. The position of the glucuronyl residue in **2** and **4** was unequivocally established by transforming these products into 4-hydroxyestrone monomethyl ethers (**12** and **13**,[6] respectively). Methylation of **2** with diazomethane in the usual manner and subsequent alkaline hydrolysis gave 4-methoxyestrone 3-glucuronide (**9**). Enzymic hydrolysis of **9** with β-glucuronidase (*Helix pomatia*) afforded 4-methoxyestrone (**12**) which was identified by TLC and mass spectral (MS) analyses. In a similar fashion, **4** was unambi-

guously characterized by leading it to the known 4-hydroxyestrone 3-methyl ether (**13**). Methyl (3-methoxy-17-oxo-1,3,5(10)-estratrien-4-yl-2,3,4-tri-*O*-acetyl-β-D-glucopyranosid)uronate (**10**) obtained from **13** by means of the Koenigs–Knorr reaction proved to be identical with the 3-methyl ether derived from **4** by usual methylation with diazomethane.

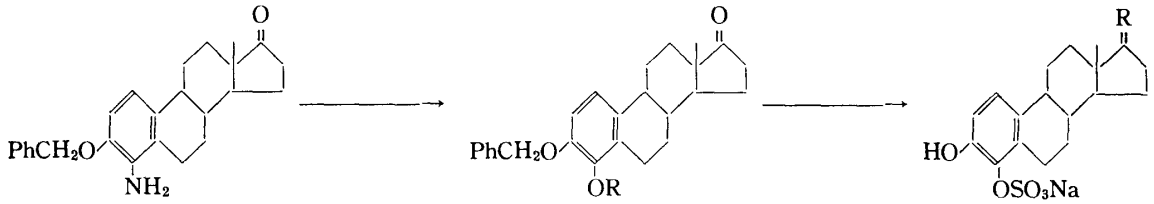
It has been reported that the Koenigs–Knorr reaction of 2-hydroxyestradiol 17-acetate takes place preferentially at the C-2 hydroxyl group, providing the 2-glucuronide as a sole product.[7] It is of interest that a distinct difference in reactivity exists between the isomeric 2,3- and 3,4-catechols, although we cannot explain this result at present. The synthetic route involving direct Koenigs–Knorr reaction of the free 3,4-catechol with methyl α-acetobromoglucuronate is favorable for simultaneous preparation of both isomeric monoglucuronides.



1: R=R′=H
12: R=H, R′=CH_3
13: R=CH_3, R′=H

2: R=G′, R′=H
3: R=G, R′=H
4: R=H, R′=G′
5: R=H, R′=G
8: R=G′, R′=CH_3
9: R=G, R′=CH_3
10: R=CH_3, R′=G′
11: R=CH_3, R′=G
14: R=SO_3Na, R′=H
21: R=SO_3Na, R′=CH_3
22: R=CH_3, R′=SO_3Na

6: R=G, R′=H
7: R=H, R′=G
15: R=SO_3Na, R′=H



16

17: R=H
18: R=SO_3Na

19: R=O
20: R= 

Chart 1

Next, we attempted to synthesize 4-hydroxyestrogen monosulfates. Treatment of 4-hydroxyestrone (**1**) with freshly prepared sulfur trioxide–pyridine complex furnished a mixture of 4-hydroxyestrone 3-sulfate (**14**) and 4-sulfate (**19**) in a ratio of 4 to 1. The product ratio was determined by high-performance liquid chromatography (HPLC) on a reversed-phase column with electrochemical detection. Recrystallization of the crude product gave 4-hydroxyestrone 3-sulfate (**14**) as colorless needles. Unfortunately, the isomeric 4-hydroxyestrone 4-sulfate (**19**) could not be obtained as a pure compound. Therefore, an alternative synthetic route (**17**) was chosen. On treatment with nitrous acid followed by hydrolysis with hot acid,[8] 4-aminoestrone 3-benzyl ether (**16**)[6] was converted into 4-hydroxyestrone 3-benzyl ether (**17**)

in a fairly good yield. Sulfation with sulfur trioxide–pyridine complex gave the 3-benzyl ether 4-sulfate (**18**) which, on hydrogenolysis over palladium-on-charcoal, was led to 4-hydroxyestrone 4-sulfate (**19**).

4-Hydroxyestradiol 3-sulfate (**15**) and 4-sulfate (**20**) were prepared from 4-hydroxyestrone monosulfates (**14**, **19**) by borohydride reduction. In addition, guaiacol estrogen monosulfates (**21**, **22**) were synthesized from **12** and **13**, respectively, by sulfation with sulfur trioxide–pyridine complex. These compounds were also obtained from 4-hydroxyestrone monosulfates (**14**, **19**) by usual methylation with diazomethane.

It is hoped that the availability of these catechol estrogen conjugates may serve to clarify the metabolic fate of 4-hydroxyestrogens. The metabolic activation of female hormones and its physiological significance in living animals should be a fertile field for study.

Experimental

All melting points were taken on a micro hot-stage apparatus and are uncorrected. Optical rotations were measured on a JASCO DIP-4 automatic polarimeter. NMR spectra were redorded on a JEOL FX-100 spectrometer at 100 MHz using tetramethylsilane as an internal standard. Abbreviations used are s=singlet, d=doublet, and m=multiplet. MS were obtained on a Hitachi M-52 mass spectrometer under the following conditions: ionization current 100 μA; ionization voltage 25 eV; ion source temperature 180°C. Infrared (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 Yanagimoto VMD-101 electrochemical detector. The potential of the detector was set at +0.9 V *vs.* an Ag/AgCl reference electrode. HPLC was carried out on a TSK GEL LS-410 ODS-SIL (5 μm) column (25 cm×0.4 cm) (Toyo Soda Co.) with acetonitrile–0.5% $NH_4H_2PO_4$ (1: 3) under ambient conditions. For column chromatography and preparative TLC, Silica gel 60 and Silica gel HF_{254} (E. Merck AG, Darmstadt) were used, respectively.

Methyl (4-Hydroxy-17-oxo-1,3,5(10)-estratrien-3-yl-2,3,4-tri-*O*-acetyl-β-D-glucopyranosid)uronate (2), Methyl (3-Hydroxy-17-oxo-1,3,5(10)-estratrien-4-yl-2,3,4-tri-*O*-acetyl-β-D-glucopyranosid)uronate (4)——Freshly prepared $CdCO_3$[4] (1 g) was added to a solution of 3,4-dihydroxy-1,3,5(10)-estratrien-17-one (**1**)[3] (1 g) in anhydrous benzene (20 ml), and the suspension was concentrated to *ca.* 15 ml by slow distillation over a period of 30 min to remove moisture. Then methyl 1-bromo-1-deoxy-2,3,4-tri-*O*-acetyl-α-D-glucopyranuronate (500 mg) was added, and the whole was refluxed for 3 h. Additional amounts of acetobromosugar (500 mg) and $CdCO_3$ (1 g) were added, and the mixture was refluxed for a further 7 h. The precipitate was removed by filtration and washed with benzene and CH_2Cl_2. The filtrate and washings were combined and evaporated down. The oily residue was subjected to preparative TLC using benzene–AcOEt (5: 2) as a developing solvent. Elution of the adsorbent corresponding to the spot (*Rf* 0.30) with AcOEt and recrystallization of the product from MeOH gave **2** (650 mg) as colorless leaflets, mp 176—177.5°C. $[\alpha]_D^{20}$ +5.3° (*c*=0.53, $CHCl_3$). *Anal.* Calcd for $C_{31}H_{38}O_{12}\cdot 3/2H_2O$: C, 59.14; H, 6.50. Found: C, 59.38; H, 6.29. ^{1}H-NMR ($CDCl_3$) δ: 0.90 (3H, s, 18-H_3), 2.04 (9H, s, $OCOCH_3$), 3.76 (3H, s, $COOCH_3$), 4.10 (1H, m, 5′-H), 5.05 (1H, d, *J*=6 Hz, 1′-H), 6.80 (2H, s, 1-, 2-H). Elution of the adsorbent corresponding to the spot (*Rf* 0.24) with AcOEt and recrystallization of the product from MeOH gave **4** (160 mg) as colorless needles, mp 117—119°C. $[\alpha]_D^{20}$ +66.5° (*c*=0.07, MeOH). *Anal.* Calcd for $C_{31}H_{38}O_{12}\cdot 3/2H_2O$: C, 59.14; H, 6.50. Found: C, 59.18; H, 6.20. ^{1}H-NMR ($CDCl_3$) δ: 0.90 (3H, s, 18-H_3), 2.04 (9H, s, $OCOCH_3$), 3.79 (3H, s, $COOCH_3$), 4.10 (1H, m, 5′-H), 4.85 (1H, d, *J*=7 Hz, 1′-H), 6.85 (1H, d, *J*=10 Hz, 1- or 2-H), 7.10 (1H, d, *J*=10 Hz, 1- or 2-H).

Sodium (4-Hydroxy-17-oxo-1,3,5(10)-estratrien-3-yl-β-D-glucopyranosid)uronate (3)——A solution of **2** (100 mg) in MeOH (2 ml) was treated with 1 N NaOH (1 ml), and the resulting solution was allowed to stand at room temperature for 12 h. The reaction mixture was then diluted with H_2O (200 ml) and percolated through a column packed with Amberlite XAD-2 resin (Rohm and Haas Co., Philadelphia, Pa.) (15 cm×2 cm). The column was washed with H_2O (200 ml) and the desired substance was eluted with MeOH. An aq. solution of the eluate was applied to a Dowex 50W-X8 (Na^+ form) resin column (5 cm×1 cm) and the desired compound was eluted with water (5 ml). The effluent was evaporated down under reduced pressure at below 60°C and the residue was reprecipitated from MeOH–ether to give **3** (86 mg) as a colorless amorphous substance, mp 238°C (dec.). $[\alpha]_D^{20}$ +22.7° (*c*=0.09, MeOH). *Anal.* Calcd for $C_{24}H_{29}NaO_9\cdot 2H_2O$: C, 55.38; H, 6.39. Found: C, 55.21; H, 6.14.

Sodium (3-Hydroxy-17-oxo-1,3,5(10)-estratrien-4-yl-β-D-glucopyranosid)uronate (5)——This compound was prepared from **4** (100 mg) in the same manner as described for **3**. The crude product was reprecipitated from MeOH–ether to give **5** (90 mg) as a colorless amorphous substance, mp 241°C (dec.). $[\alpha]_D^{20}$ +58.5° (*c*=0.51, MeOH). *Anal.* Calcd for $C_{24}H_{29}NaO_9\cdot 3/2H_2O$: C, 56.46; H, 6.12. Found: C, 56.07; H, 6.34.

Sodium (4,17β-Dihydroxy-1,3,5(10)-estratrien-3-yl-β-D-glucopyranosid)uronate (6)——A solution of **3** (100 mg) in MeOH (4 ml) was treated with $NaBH_4$ (50 mg) under ice-cooling, and the resulting solution was

allowed to stand at room temperature for 1 h. The reaction mixture was diluted with H_2O (50 ml) and percolated through a column packed with Amberlite XAD-2 resin (15 cm × 1 cm). The column was washed with H_2O (100 ml) and the desired substance was eluted with MeOH. An aq. solution of the eluate was applied to a Dowex 50W-X8 (Na^+ form) resin column (5 cm × 1 cm). The product was reprecipitated from MeOH–ether to give **6** (92 mg) as a colorless amorphous substance, mp 218°C (dec.). $[\alpha]_D^{20}$ +0.0° (c=0.08, MeOH). *Anal.* Calcd for $C_{24}H_{31}NaO_9 \cdot 3/2H_2O$: C, 56.13; H, 6.67. Found: C, 56.00; H, 6.80.

Sodium (3,17β-Dihydroxy-1,3,5(10)-estratrien-4-yl-β-D-glucopyranosid)uronate (7)——This compound was prepared from **5** (100 mg) in the same manner as described for **6**. The crude product was reprecipitated from MeOH–ether to give **7** (89 mg) as a colorless amorphous substance, mp 242°C (dec.). $[\alpha]_D^{20}$ −2.6° (c=0.19, MeOH). *Anal.* Calcd for $C_{24}H_{31}NaO_9 \cdot 5/2H_2O$: C, 54.23; H, 6.82. Found: C, 53.88; H, 6.80.

Methyl (4-Methoxy-17-oxo-1,3,5(10)-estratrien-3-yl-2,3,4-tri-*O*-acetyl-β-D-glucopyranosid)uronate (8)——A solution of **2** (50 mg) in ether–$CHCl_3$ (10: 1) was treated with ethereal diazomethane, and the resulting solution was kept at room temperature for 12 h. After evaporation of the organic solvent, the residue was recrystallized from MeOH to give **8** (36 mg) as colorless needles, mp 232—234°C. $[\alpha]_D^{20}$ +89.4° (c=0.04, $CHCl_3$). *Anal.* Calcd for $C_{32}H_{40}O_{12}$: C, 62.33; H, 6.54. Found: C, 62.16; H, 6.55. ^{1}H-NMR ($CDCl_3$) δ: 0.92 (3H, s, 18-H_3), 2.02 (3H, s, $OCOCH_3$), 2.05 (3H, s, $OCOCH_3$), 2.08 (3H, s, $OCOCH_3$), 3.72 (3H, s, OCH_3 or $COOCH_3$), 3.74 (3H, s, OCH_3 or $COOCH_3$), 4.10 (1H, m, 5′-H), 5.02 (1H, d, J=7 Hz, 1′-H), 6.93 (2H, s, 1-, 2-H).

Sodium (4-Methoxy-17-oxo-1,3,5(10)-estratrien-3-yl-β-D-glucopyranosid)uronate (9)——This compound was prepared from **8** (30 mg) in the manner as described for **3**. The crude product was reprecipitated from MeOH–ether to give **9** (12 mg) as colorless amorphous substance, mp 198°C (dec.). $[\alpha]_D^{20}$ +37.9° (c=0.16, MeOH). *Anal.* Calcd for $C_{25}H_{31}NaO_9 \cdot 2H_2O$: C, 56.18; H, 6.60. Found: C, 55.95; H, 6.27.

Methyl (3-Methoxy-17-oxo-1,3,5(10)-estratrien-4-yl-2,3,4-tri-*O*-acetyl-β-D-glucopyranosid)uronate (10)——This compound was prepared from 3-methoxy-4-hydroxy-1,3,5(10)-estratrien-17-one (**13**)[6] (200 mg) in the manner as described for **2** and **4**. The crude product was subjected to preparative TLC using hexane–AcOEt (2: 1) as a developing solvent. Elution of the adsorbent corresponding to the spot (*Rf* 0.13) with AcOEt and recrystallization of the product from MeOH gave **10** (81 mg) as colorless needles, mp 118—119°C. $[\alpha]_D^{20}$ +76.3° (c=0.12, $CHCl_3$). *Anal.* Calcd for $C_{32}H_{40}O_{12} \cdot H_2O$: C, 60.56; H, 6.67. Found: C, 60.75; H, 6.66. ^{1}H-NMR ($CDCl_3$) δ: 0.92 (3H, s, 18-H_3), 2.02 (3H, s, $OCOCH_3$), 2.05 (3H, s, $OCOCH_3$), 2.08 (3H, s, $OCOCH_3$), 3.68 (3H, s, OCH_3 or $COOCH_3$), 3.80 (3H, s, OCH_3 or $COOCH_3$), 6.70 (1H, d, J=10 Hz, 1- or 2-H), 7.10 (1H, d, J=10 Hz, 1- or 2-H). This compound was also prepared from **4** by methylation with diazomethane. The two products were indistinguishable.

Sodium (3-Methoxy-17-oxo-1,3,5(10)-estratrien-4-yl-β-D-glucopyranosid)uronate (11)——This compound was prepared from **10** (50 mg) in the manner as described for **3**. The crude product obtained was reprecipitated from MeOH–ether to give **11** (19 mg) as a colorless amorphous substance, mp 208°C (dec.). $[\alpha]_D^{20}$ +87.9° (c=0.06, MeOH). *Anal.* Calcd for $C_{25}H_{31}NaO_9 \cdot 7/2H_2O$: C, 53.47; H, 6.82. Found: C, 53.11; H, 6.32.

Enzymic Hydrolysis of Guaiacol Estrogen Monoglucuronides with β-Glucuronidase——A mixture of an aq. solution (4 ml) of guaiacol estrogen monoglucuronide (**9**, **11**) (*ca.* 2 mg), 0.1 M acetate buffer (pH 4.7, 20 ml) and acetone powder of *Helix pomatia* digestive juice (3 mg) was incubated at 37°C for 24 h, then extracted with AcOEt. The organic phase was washed with H_2O, dried over anhydrous Na_2SO_4, and evaporated down. The products obtained were proved to be identical with **12** and **13**, respectively, as judged by TLC with benzene–AcOEt (10: 1) as a developing solvent and by MS analyses [m/z 300 (M^+)].

Sodium 3,4-Dihydroxy-1,3,5(10)-estratrien-17-one 3-Sulfate (14)——Freshly prepared sulfur trioxide-pyridine complex (70 mg) was added to a solution of **1** (100 mg) in pyridine (2 ml), and the reaction mixture was stirred under ice-cooling for 12 h. Excess reagent was decomposed by addition of 5% $NaHCO_3$, and the resulting solution was extracted with ether. The aq. layer was percolated through a column packed with Amberlite XAD-2 resin (15 cm × 2 cm). The column was washed with H_2O and the desired compound was eluted with MeOH. An aq. solution of the eluate was applied to a Dowex 50W-X8 (Na^+ form) (5 cm × 1 cm) column. HPLC of the eluate gave two peaks in a ratio of 4 to 1, corresponding to **14** and **19**, respectively. The product was recrystallized from MeOH–ether several times to give **14** (78 mg) as colorless needles, mp 181°C (dec.). $[\alpha]_D^{20}$ +118.7° (c=0.64, MeOH). *Anal.* Calcd for $C_{18}H_{21}NaO_6S \cdot H_2O$: C, 52.29; H, 5.60. Found: C, 52.36; H, 5.66. IR ν_{max}^{KBr} cm^{-1}: 1035, 1255 (SO_3Na). ^{1}H-NMR ($CDCl_3/CD_3OD$) δ: 0.92 (3H, s, 18-H_3), 6.80 (1H, d, J=10 Hz, 1- or 2-H), 7.10 (1H, d, J=10 Hz, 1- or 2-H).

3-Benzyloxy-4-hydroxy-1,3,5(10)-estratrien-17-one (17)——A suspension of 4-amino-3-benzyloxy-1,3,5(10)-estratrien-17-one (**16**)[6] (550 mg) in 22%(w/v) H_2SO_4 (10 ml) was treated with $NaNO_2$ (150 mg) in H_2O (1 ml), and the resulting suspension was stirred under ice-cooling for 1 h. Excess reagent was decomposed by addition of urea, and the reaction mixture was refluxed for 40 min. The resulting solution was extracted with AcOEt, and the organic layer was washed with H_2O, dried over anhydrous Na_2SO_4, and evaporated down. The residue was subjected to column chromatography using hexane–AcOEt (5: 1) as a developing solvent. The eluate was recrystallized from MeOH to give **17** (380 mg) as colorless needles, mp 150.5—152.5°C. $[\alpha]_D^{20}$ +116.4° (c=0.30, $CHCl_3$). *Anal.* Calcd for $C_{25}H_{28}O_3$: C, 79.76; H, 7.50. Found: C, 79.55; H, 7.56. ^{1}H-NMR ($CDCl_3$) δ: 0.91 (3H, s, 18-H_3), 5.07 (2H, s, OCH_2Ph), 6.74 (2H, s, 1-, 2-H), 7.36 (5H, s,

$OCH_2C_6H_5$).

Sodium 3,4-Dihydroxy-1,3,5(10)-estratrien-17-one 4-Sulfate (19)——Sodium 3-benzyloxy-4-hydroxy-1,3,5(10)-estratrien-17-one 4-sulfate (**18**) was prepared from **17** (100 mg) in the same manner as described for **14**. A solution of the crude product (48 mg) in MeOH (20 ml) was shaken with 5% Pd-C (20 mg) under a hydrogen gas stream for 20 h. The catalyst was removed by filtration and the filtrate was evaporated down. An aq. solution of the residue was applied to a Dowex 50W-X8 (Na^+ form) resin column (5 cm × 1 cm). The product was recrystallized from MeOH–ether to give **19** (36 mg) as colorless needles, mp 159°C (dec.). $[\alpha]_D^{20}$ +99.7° (c=0.07, MeOH). *Anal.* Calcd for $C_{18}H_{21}NaO_6S \cdot 1/2H_2O$: C, 53.46; H, 5.48. Found: C, 53.71; H, 5.69. IR ν_{max}^{KBr} cm^{-1}: 1030, 1250 (SO_3Na). ^{1}H-NMR ($CDCl_3/CD_3OD$) δ: 0.92 (3H, s, 18-H_3), 6.70 (1H, d, J=10 Hz, 1- or 2-H), 7.00 (1H, d, J=10 Hz, 1- or 2-H).

Sodium 1,3,5(10)-Estratriene-3,4,17β-triol 3-Sulfate (15)——This compound was prepared from **14** (50 mg) in the same manner as described for **6**. The crude product was reprecipitated from MeOH–ether to give **15** (40 mg) as a colorless amorphous substance, mp 215—217°C. $[\alpha]_D^{20}$ +50.3° (c=0.37, MeOH). *Anal.* Calcd for $C_{18}H_{23}NaO_6S \cdot H_2O$: C, 52.93; H, 6.17. Found: C, 52.53; H, 6.20. IR ν_{max}^{KBr} cm^{-1}:1050, 1250 (SO_3Na).

Sodium 1,3,5(10)-Estratriene-3,4,17β-triol 4-Sulfate (20)——This compound was prepared from **19** (50 mg) in the same manner as described for **6**. The crude product was reprecipitated from MeOH–ether to give **20** (42 mg) as a colorless amorphous substance, mp 239—241°C. $[\alpha]_D^{20}$ +42.2° (c=0.11, MeOH). *Anal.* Calcd for $C_{18}H_{23}NaO_6S \cdot H_2O$: C, 52.93; H, 6.17. Found: C, 53.09; H, 5.83. IR ν_{max}^{KBr} cm^{-1}: 1045, 1250 (SO_3Na).

Sodium 3-Hydroxy-4-methoxy-1,3,5(10)-estratrien-17-one 3-Sulfate (21)——This compound was prepared from **12** (100 mg) in the same manner as described for **14**. The crude product was reprecipitated from MeOH–ether to give **21** (46 mg) as a colorless amorphous substance, mp 208—210°C. $[\alpha]_D^{20}$ +9.7° (c=0.78, MeOH). *Anal.* Calcd for $C_{19}H_{23}NaO_6S \cdot 1/2H_2O$: C, 55.46; H, 5.88. Found: C, 55.76; H, 6.41. This compound was also prepared from **14** by methylation with diazomethane. The two products were indistinguishable.

Sodium 4-Hydroxy-3-methoxy-1,3,5(10)-estratrien-17-one 4-Sulfate (22)——This compound was prepared from **13** (100 mg) in the same manner as described for **14**. The crude product was reprecipitated from MeOH–ether to give **22** (42 mg) as a colorless amorphous substance, mp 221—223°C. $[\alpha]_D^{20}$ +127.1° (c=0.19, MeOH). *Anal.* Calcd for $C_{19}H_{23}NaO_6S \cdot 2H_2O$: C, 52.04; H, 6.21. Found: C, 52.38; H, 5.73. This compound was also prepared from **19** by methylation with diazomethane. The two products were indistinguishable.

Acknowledgement The authors are indebted to the staff of the central analytical laboratory of this Institute for elemental analyses and spectral measurements. This work was supported in part by a grant from the Ministry of Education, Science and Culture, which is gratefully acknowledged.

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