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Syntheses of Methoxyestrogen Glucuronide Acetate-Methyl Esters[1]

Toshio Nambara and Yoshihiko Kawarada

Pharmaceutical Institute, Tohoku University[2]

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In connection with the studies on the metabolism of female hormone the title compounds having the 16,17-ketol and -glycol structures were prepared as new and potential metabolites. These compounds were satisfactorily obtained from catechol estrogen 2- and 3-methyl ethers by Koenigs-Knorr reaction with methyl acetobromoglucuronate employing cadmium carbonate as a catalyst.

Since the first discovery of 2-methoxyestrone in human urine by Gallagher and his coworker,[3] considerable attentions have been drawn to the physiological significance of catechol O-methylation in the metabolism of female hormone. In addition the occurrence of the isomeric 3-methyl ethers of catechol estrogen in rat bile[4] and human pregnancy urine[5] has been recently clarified by several investigators. The current studies in these laboratories on the biliary metabolites strongly implied that estriol administered to the rat would undergo hydroxylation at C-2, followed by glucuronidation and O-methylation in ring A resulting in formation of methoxyestrogen 2- and 3-monoglucuronides as principal metabolites.[6] The complete structure of the steroid glucuronide isolated from the biological specimen can be usually determined by leading to the acetate-methyl ester. The present paper deals with the preparation of the title compounds having the 16,17-ketol and -glycol structures as new and potential metabolites.

An initial effort was focused on the syntheses of the catechol 3-methyl ether derivatives. Reduction of 2-benzyloxy-3-methoxy-16α-hydroxyestratrien-17-one[7] with sodium borohydride and subsequent purification by preparative thin-layer chromatography (TLC) afforded the 16α,17β-glycol (Ia), which on usual acetylation was led to the diacetate (Ib). Elimination of the protecting group at C-2 was attained with ease by hydrogenolysis over palladium-on-charcoal to provide 2-hydroxyestriol 3-methyl ether 16,17-diacetate (Ic). Recently Bernstein and his coworker proposed the use of cadmium carbonate as a more suitable catalyst for preparation of the aryl glucuronide by Koenigs-Knorr reaction.[8] In actuality condensation of Ic with 1-bromo-1-deoxy-2,3,4-tri-O-acetyl-α-D-glucopyranuronate in the presence of cadmium carbonate proceeded readily yielding the desired methyl (3-methoxy-16α,17β-diacetoxyestra-1,3,5(10)-trien-2-yl-2,3,4-tri-O-acetyl-β-D-glucopyranosid)uronate (Id) in 45% yield.

1) Part CVIII of "Studies on Steroids" by T. Nambara; Part CVII: H. Hosoda, K. Yamashita, and T. Nambara, *Chem. Pharm. Bull.* (Tokyo), **24**, 380 (1976). Following trivial names are used: estrone, 3-hydroxyestra-1,3,5(10)-trien-17-one; estriol, estra-1,3,5(10)-triene-3,16α,17β-triol; 16-epiestriol, estra-1,3,5(10)-triene-3,16β,17β-triol.
2) Location: *Aobayama, Sendai.*
3) S. Kraychy and T.F. Gallagher, *J. Am. Chem. Soc.*, **79**, 754 (1957); *idem*, *J. Biol. Chem.*, **229**, 519 (1957).
4) A. Bartke, R.E. Steele, J.G. Williams, and K.I.H. Williams, *Steroids*, **18**, 303 (1971); S. Honma and T. Nambara, *Chem. Pharm. Bull.* (Tokyo), **22**, 687 (1974); T. Nambara, J. Ishiguro, Y. Kawarada, and H. Tajima, *ibid.*, **22**, 889 (1974); S. Honma and T. Nambara, *ibid.*, **23**, 787 (1975).
5) R. Knuppen, O. Haupt, and H. Breuer, *Biochem. J.*, **128**, 1369 (1972).
6) T. Nambara and Y. Kawarada, *Chem. Pharm. Bull.* (Tokyo), **23**, 698 (1975).
7) T. Nambara, Y. Kawarada, M. Asama, S. Akiyama, M. Nokubo, and S. Honma, *Chem. Pharm. Bull.* (Tokyo), **21**, 2725 (1973).
8) R.B. Conrow and S. Bernstein, *J. Org. Chem.*, **36**, 863 (1971).

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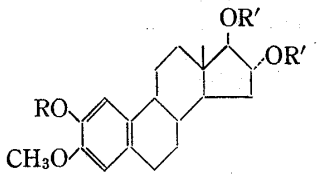
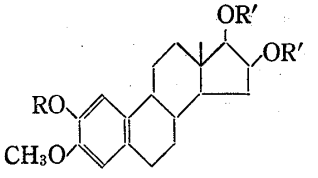
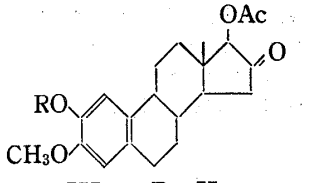
The preparation of the 16β,17β-glycol was then undertaken in a similar fashion. First, treatment of 2-benzyloxy-3-methoxyestra-1,3,5(10),16-tetraen-17-ol acetate[7] with lead tetraacetate in acetic acid furnished the 16β-acetoxy-17-ketone (IVa), which in turn was solely transformed into the 16β,17β-diol (IIa) by sodium borohydride reduction, followed by complete hydrolysis with potassium carbonate. Hydrogenolysis of the 16,17-diacetate (IIb) over palladium-on-charcoal provided 2-hydroxy-16-epiestriol 3-methyl ether 16,17-diacetate (IIc) in a satisfactory yield. When IIc and methyl acetobromoglucuronate were stirred in dry toluene with cadmium carbonate, methyl (3-methoxy-16β,17β-diacetoxyestra-1,3,5(10)-trien-2-yl-2,3,4-tri-O-acetyl-β-D-glucopyranosid)uronate (IId) was afforded in 28% yield.



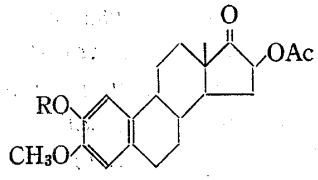
Ia : R=$C_6H_5CH_2$, R′=H
Ib : R=$C_6H_5CH_2$, R′=Ac
Ic : R=H, R′=Ac
Id : R=G′, R′=Ac



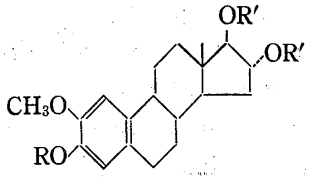
IIa : R=$C_6H_5CH_2$, R′=H
IIb : R=$C_6H_5CH_2$, R′=Ac
IIc : R=H, R′=Ac
IId : R=G′, R′=Ac



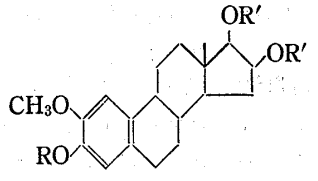
IIIa : R=H
IIIb : R=G′



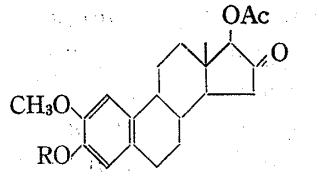
IVa : R=$C_6H_5CH_2$
IVb : R=H
IVc : R=G′



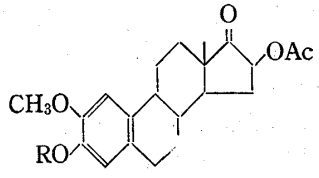
Va : R=$C_6H_5CH_2$, R′=H
Vb : R=$C_6H_5CH_2$, R′=Ac
Vc : R=H, R′=Ac
Vd : R=G′, R′=Ac



VIa : R=$C_6H_5CH_2$, R′=H
VIb : R=$C_6H_5CH_2$, R′=Ac
VIc : R=H, R′=Ac
VId : R=G′, R′=Ac



VIIa : R=H
VIIb : R=G′



VIIIa : R=$C_6H_5CH_2$
VIIIb : R=H
VIIIc : R=G′

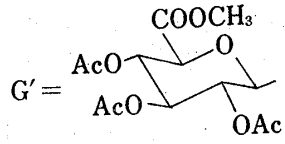


Chart 1

The next project was directed to the syntheses of the 2-methoxyestrogen 3-glucuronide derivatives. Reduction of 2-methoxy-3-benzyloxy-16α-hydroxyestratrien-17-one, derivable from 2-methoxyestrone benzyl ether in three steps,[7] with sodium borohydride yielded the 16α,17β-glycol (Va). Usual acetylation, followed by catalytic hydrogenation furnished 2-methoxyestriol 16,17-diacetate (Vc). Introduction of the glucuronyl moiety was similarly effected by Koenigs-Knorr reaction employing cadmium carbonate as a catalyst to give 2-methoxyestriol 3-glucuronide acetate-methyl ester (Vd) in a reasonable yield. The preparation of the 2-methoxy-16-epiestriol derivatives was then carried out starting from 2-methoxy-16β-acetoxyestrone 3-benzyl ether (VIIIa) which was easily obtainable from the Δ^{16}-enol acetate by lead tetraacetate oxidation. Treatment with sodium borohydride provided the corresponding 16β,17β-glycol (VIa), which on usual acetylation was converted to the diacetate (VIb). Transformation of the 3-benzyl ether into the 3-glucuronide derivative was attained in the manner as described above and in consequence the desired 2-methoxy-16-epiestriol 3-glucuronide acetate-methyl ester (VId) was satisfactorily obtained.

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As the third program the 2- and 3-monoglucuronides of methoxyestrogen with the 16,17-ketol structure were also synthesized. Condensation of the catechol 2- and 3-monomethyl ethers (IIIa, IVb, VIIa, VIIIb) with acetobromosugar in the presence of cadmium salt proceeded without affecting the ketol structure in ring D to form the glucuronide acetate-methyl esters (IIIb, IVc, VIIb, VIIIc), respectively.

The nuclear magnetic resonance (NMR) spectra of the glucuronide acetate-methyl esters verified the formation of a β-glucopyranoside linkage. The anomeric proton of the sugar moiety appeared at 4.9—5.0 ppm as a doublet (J=6—8 Hz) indicating a *trans*-diaxial relationship to the vicinal 2'-proton. The structural assignment of the above-mentioned compounds is unequivocal, since the stereochemistry of ring D in 14α-steroids has been sufficiently substantiated. It is hoped that these synthetic specimens may serve as references for characterization of the metabolites excreted in the biological fluid.

Experimental[9]

2-Benzyloxy-3-methoxyestra-1,3,5(10)-triene-16α,17β-diol (Ia)——To a solution of 2-benzyloxy-3-methoxy-16α-hydroxyestra-1,3,5(10)-trien-17-one[7] (100 mg) in MeOH (3 ml) was added $NaBH_4$ (30 mg) in MeOH (0.5 ml)–H_2O (0.2 ml) and stirred at room temperature for 1 hr. After addition of AcOH to decompose the excess reagent the resulting solution was extracted with AcOEt. The organic layer was washed with 5% $NaHCO_3$ and H_2O, dried over anhydrous Na_2SO_4, and evaporated. The crude product obtained was purified by preparative TLC using AcOEt–hexane–EtOH (80: 15: 5) as developing solvent. Recrystallization of the eluate from isopropyl ether gave Ia (74 mg) as colorless plates. mp 145—146°. $[\alpha]_D^{21}$ +72.7° (c=0.11). *Anal.* Calcd. for $C_{26}H_{32}O_4$: C, 76.44; H, 7.90. Found: C, 76.02; H, 7.70. NMR ($CDCl_3$ solution) δ: 0.78 (3H, s, 18–CH_3), 3.55 (1H, d, J=6 Hz, 17α-H), 3.82 (3H, s, 3–OCH_3), 4.13 (1H, m, 16β–H), 5.06 (2H, s, 2–$OCH_2C_6H_5$), 6.57 (1H, s, 4–H), 6.82 (1H, s, 1–H).

2-Benzyloxy-3-methoxyestra-1,3,5(10)-triene-16α,17β-diol Diacetate (Ib)——Treatment of Ia (44 mg) with Ac_2O and pyridine in the usual manner, followed by recrystallization from acetone–hexane gave Ib (27 mg) as colorless needles. mp 115—117°. $[\alpha]_D^{18}$ +4.7° (c=0.11). *Anal.* Calcd. for $C_{30}H_{36}O_6$: C, 73.14; H, 7.37. Found: C, 73.42; H, 7.33. NMR ($CDCl_3$ solution) δ: 0.83 (3H, s, 18–CH_3), 2.05, 2.08 (6H, s, 16α-,17β–OCO–CH_3), 3.82 (3H, s, 3–OCH_3), 4.95 (1H, d, J=6 Hz, 17α–H), 5.07 (2H, s, 2–$OCH_2C_6H_5$), 5.10 (1H, m, 16β–H), 6.59 (1H, s, 4–H), 6.82 (1H, s, 1–H).

3-Methoxyestra-1,3,5(10)-triene-2,16α,17β-triol 16,17-Diacetate (Ic)——A solution of Ib (24 mg) in EtOH (10 ml) was shaken with 5% Pd/C (30 mg) overnight under a stream of H_2 gas at room temperature. After removal of the catalyst by filtration the filtrate was evaporated *in vacuo*. Recrystallization of the residue from MeOH gave Ic (10 mg) as colorless needles. mp 184—185°. $[\alpha]_D^{14}$ −9.3° (c=0.05). *Anal.* Calcd. for $C_{23}H_{30}O_6$: C, 68.63; H, 7.51. Found: C, 68.40; H, 7.44. NMR ($CDCl_3$ solution) δ: 0.84 (3H, s, 18–CH_3), 2.04, 2.08 (6H, s, 16α-,17β–$OCOCH_3$), 3.82 (3H, s, 3–OCH_3), 4.96 (1H, d, J=6 Hz, 17α–H), 5.18 (1H, m, 16β–H), 6.54 (1H, s, 4–H), 6.82 (1H, s, 1–H).

Methyl (3-Methoxy-16α,17β-diacetoxyestra-1,3,5(10)-trien-2-yl-2,3,4-tri-O-acetyl-β-D-glucopyranosid)-uronate (Id)——To a solution of Ic (10 mg) in anhydrous toluene (2 ml) was added freshly prepared $CdCO_3$ (10 mg) and concentrated to 1 ml by slow distillation over a period of 1 hr to remove the moisture. To this solution was added methyl 1-bromo-1-deoxy-2,3,4-tri-O-acetyl-α-D-glucopyranuronate (20 mg) in anhydrous toluene (1 ml) and refluxed for 1 hr. Additional amounts of acetobromosugar (10 mg) and $CdCO_3$ (10 mg) were added and refluxed for 4 hr. The precipitate was removed by filtration and washed with toluene. The filtrate and washings were combined and evaporated. An oily residue was submitted to preparative TLC using $CHCl_3$–MeOH (60: 1) as developing solvent. Elution of the adsorbent corresponding to the spot (Rf 0.28) with AcOEt and recrystallization of the eluate from acetone–hexane gave Id (8 mg) as colorless needles. mp 188—190°. $[\alpha]_D^{11}$ −28.3° (c=0.09). *Anal.* Calcd. for $C_{36}H_{46}O_{15}$: C, 60.16; H, 6.45. Found: C, 60.01; H, 6.42. NMR ($CDCl_3$ solution) δ: 0.84 (3H, s, 18–CH_3), 2.04—2.08 (15H, s, 16α-,17β–$OCOCH_3$, pyranose–$OCOCH_3$), 3.72 (3H, s, pyranose–$COOCH_3$), 3.75 (3H, s, 3–OCH_3), 4.04 (1H, m, pyranose–5–H), 4.98 (2H, d, J=6 Hz, 17α–H, pyranose–1–H), 5.08—5.40 (4H, m, 16β–H, pyranose–CH–OAc), 6.57 (1H, s, 4–H), 7.07 (1H, s, 1–H).

2-Benzyloxy-3-methoxyestra-1,3,5(10)-triene-16β,17β-diol (IIa)——To a solution of IVa (200 mg) in MeOH (80 ml) was added $NaBH_4$ (50 mg) in MeOH (1 ml)–H_2O (0.4 ml) and stirred at room temperature for

9) All melting points were taken on a micro hot-stage apparatus and are uncorrected. Optical rotations were measured in $CHCl_3$. NMR spectra were recorded on a Hitachi Model R-20A spectrometer at 60 MHz or a JEOL Model PS-100 spectrometer at 100 MHz using tetramethylsilane as an internal standard. bbreviation used s=singlet, d=doublet, and m=multiplet. For preparative TLC silica gel HF_{254} (E. erck AG, Darmstadt) was used as an adsorbent.

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1 hr. The resulting solution was diluted with 10% K_2CO_3 (10 ml) and refluxed for 30 min. After removal of MeOH by evaporation the solution was extracted with AcOEt. The organic layer was washed with 5% HCl, 5% $NaHCO_3$, and H_2O successively dried over anhydrous Na_2SO_4, and evaporated. The crude product obtained was recrystallized from acetone–hexane to give IIa (172 mg) as colorless needles, mp 130—132°. $[\alpha]_D^{27}$ +63.2° (c=0.13). *Anal.* Calcd. for $C_{26}H_{32}O_4$: C, 76.44; H, 7.90. Found: C, 76.16; H, 7.93. NMR ($CDCl_3$ solution) δ: 0.85 (3H, s, 18–CH_3), 3.42 (1H, d, J=7 Hz, 17α–H), 3.84 (3H, s, 3–OCH_3), 4.16 (1H, m, 16α–H), 5.08 (2H, s, 2–$OCH_2C_6H_5$), 6.57 (1H, s, 4–H), 6.81 (1H, s, 1–H).

2-Benzyloxy-3-methoxyestra-1,3,5(10)-triene-16β,17β-diol Diacetate (IIb)——Treatment of IIa (44 mg) with Ac_2O and pyridine in the usual manner, followed by recrystallization from acetone–hexane gave IIb (45 mg) as colorless needles. mp 160—162°. $[\alpha]_D^{18}$ +52.5° (c=0.10). *Anal.* Calcd. for $C_{30}H_{36}O_6$: C, 73.14; H, 7.37. Found: C, 73.10; H, 7.36. NMR ($CDCl_3$ solution) δ: 0.92 (3H, s, 18–CH_3), 2.02, 2.04 (6H, s, 16β–, 17β–$OCOCH_3$), 3.82 (3H, s, 3–OCH_3), 4.58 (1H, d, J=7 Hz, 17α–H), 5.07 (2H, s, 2–$OCH_2C_6H_5$), 5.28 (1H, m, 16α–H), 6.58 (1H, s, 4–H), 6.81 (1H, s, 1–H).

3-Methoxyestra-1,3,5(10)-triene-2,16β,17β-triol 16,17-Diacetate (IIc)——A solution of IIb (42 mg) in EtOH (10 ml) was shaken with 5% Pd/C (40 mg) overnight under a stream of H_2 gas at room temperature. After removal of the catalyst by filtration the filtrate was evaporated *in vacuo*. Recrystallization of the residue from MeOH gave IIc (10 mg) as colorless needles. mp 243—245°. $[\alpha]_D^{15}$ +64.8° (c=0.05). *Anal.* Calcd. for $C_{23}H_{30}O_6$: C, 68.63; H, 7.51. Found: C, 68.43; H, 7.47. NMR ($CDCl_3$ solution) δ: 0.93 (3H, s, 18–CH_3), 2.02, 2.04 (6H, s, 16β–,17β–$OCOCH_3$), 3.83 (3H, s, 3–OCH_3), 4.59 (1H, d, J=7 Hz, 17α–H), 5.20 (1H, m, 16α–H), 6.55 (1H, s, 4-H), 6.83 (1H, s, 1-H).

Methyl (3-Methoxy-16β,17β-diacetoxyestra-1,3,5(10)-trien-2-yl-2,3,4-tri-O-acetyl-β-D-glucopyranosid)-uronate (IId)——Prepared from IIc (10 mg) employing Koenigs-Knorr reaction in the manner as described in Id. The crude product was submitted to preparative TLC using $CHCl_3$–MeOH (60: 1) as developing solvent. Elution of the adsorbent corresponding to the spot (*Rf* 0.33) with AcOEt gave IId (5 mg) as colorless oil. NMR ($CDCl_3$ solution) δ: 0.93 (3H, s, 18–CH_3), 2.04, 2.07 (15H, s, 16β–,17β–$OCOCH_3$, pyranose–$OCOCH_3$), 3.72 (3H, s, pyranose–$COOCH_3$), 3.75 (3H, s, 3–OCH_3), 4.05 (1H, m, pyranose–5–H), 4.60 (1H, d, J=7 Hz, 17α–H), 4.95 (1H, d, J=8 Hz, pyranose–1–H), 5.16—5.40 (4H, m, 16α–H, pyranose–CH–OAc), 6.57 (1H, s, 4-H), 7.07 (1H, s, 1-H).

Methyl (3-Methoxy-16-oxo-17β-acetoxyestra-1,3,5(10)-trien-2-yl-2,3,4-tri-O-acetyl-β-D-glucopyranosid)-uronate (IIIb)——Prepared from 2,17β-dihydroxy-3-methoxyestra-1,3,5(10)-trien-16-one 17-acetate (IIIa)[7] (24 mg) employing Koenigs-Knorr reaction in the manner as described in Id. The crude product was submitted to preparative TLC using $CHCl_3$–MeOH (80: 1) as developing solvent. Elution of the adsorbent corresponding to the spot (*Rf* 0.32) and recrystallization of the eluate from acetone–hexane gave IIIb (18 mg) as colorless leaflets. mp 122—124°. $[\alpha]_D^{16}$ −29.3° (c=0.09). *Anal.* Calcd. for $C_{34}H_{42}O_{14}$: C, 60.52; H, 6.28. Found: C, 60.17; H, 6.12. NMR ($CDCl_3$ solution) δ: 0.87 (3H, s, 18–CH_3), 2.05, 2.09 (9H, s, pyranose–$OCOCH_3$), 2.19 (3H, s, 17β–$OCOCH_3$), 3.74 (3H, s, pyranose–$COOCH_3$), 3.78 (3H, s, 3–OCH_3), 4.10 (1H, m, pyranose-5-H), 5.09 (1H, s, 17α-H), 4.90—5.40 (4H, m, pyranose–CH–OAc, -1-H) 6.61 (1H, s, 4-H), 7.11 (1H, s, 1-H).

2-Benzyloxy-3-methoxy-16β-hydroxyestra-1,3,5(10)-trien-17-one Acetate (IVa)——To a solution of 2-benzyloxy-3-methoxyestra-1,3,5(10),16-tetraen-17-ol acetate[7] (3.1 g) in AcOH (100 ml)-Ac_2O (1 ml) was added $Pb(OAc)_4$ (4 g) and stirred at room temperature for 3 hr. The resulting solution was concentrated *in vacuo* below 50° and extracted with ether. The organic layer was washed with 5% $NaHCO_3$ and H_2O, dried over anhydrous Na_2SO_4, and evaporated. The crude product obtained was chromatographed on silica gel. Elution with benzene and recrystallization of the eluate from MeOH gave IVa (780 mg) as colorless needles. mp 199—200°. $[\alpha]_D^{26}$ +109.6° (c=0.10). *Anal.* Calcd. for $C_{28}H_{32}O_5$: C, 74.97; H, 7.19. Found: C, 75.24; H, 7.32. NMR ($CDCl_3$ solution) δ: 0.99 (3H, s, 18–CH_3), 2.12 (3H, s, 16β–$OCOCH_3$), 3.83 (3H, s, 3–OCH_3), 5.04 (1H, m, 16α–H), 5.07 (2H, s, 2–$OCH_2C_6H_5$), 6.59 (1H, s, 4–H), 6.81 (1H, s, 1-H).

2,16β-Dihydroxy-3-methoxyestra-1,3,5(10)-trien-17-one 16-Acetate (IVb)——A solution of IVa (500 mg) in EtOH (190 ml) was shaken with 5% Pd/C (500 mg) under a stream of H_2 gas for 3 hr at room temperature. After removal of the catalyst by filtration the filtrate was evaporated *in vacuo*. Recrystallization of the residue from MeOH gave IVb (295 mg) as colorless needles. mp 180—182°. $[\alpha]_D^{27}$ +155.6° (c=0.10). *Anal.* Calcd. for $C_{21}H_{26}O_5 \cdot 1/4H_2O$: C, 69.49; H, 7.36. Found: C, 69.34; H, 7.55. NMR ($CDCl_3$ solution) δ: 0.99 (3H, s, 18-CH_3), 2.12 (3H, s, 16β-$OCOCH_3$), 3.83 (3H, s, 3-OCH_3), 5.04 (1H, m, 16α-H), 6.54 (1H, s, 4-H), 6.82 (1H, s, 1-H).

Methyl (3-Methoxy-16β-acetoxy-17-oxoestra-1,3,5(10)-trien-2-yl-2,3,4-tri-O-acetyl-β-D-glucopyranosid)-uronate (IVc)——Prepared from IVb (70 mg) employing Koenigs-Knorr reaction in the manner as described in Id. The crude product was submitted to preparative TLC using $CHCl_3$–MeOH (80: 1) as developing solvent. Elution of the adsorbent corresponding to the spot (*Rf* 0.30) with AcOEt and recrystallization of the eluate from acetone–hexane gave IVc (28 mg) as colorless needles. mp 186—188°. $[\alpha]_D^{16}$ +95.0° (c=0.11). *Anal.* Calcd. for $C_{34}H_{42}O_{14}$: C, 60.52; H, 6.28. Found: C, 60.32; H, 6.21. NMR ($CDCl_3$ solution) δ: 1.01 (3H, s, 18–CH_3), 2.03, 2.06 (9H, s, pyranose-$OCOCH_3$), 2.11 (3H, s, 16β-$OCOCH_3$), 3.73 (3H, s, pyranose-$COOCH_3$), 3.76 (3H, s, 3-OCH_3), 4.10 (1H, m, pyranose-5-H), 4.90—5.40 (5H, m, 16α-H, pyranose-CH-OAc, -1-H), 6.61 (1H, s, 4-H), 7.10 (1H, s, 1-H).

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2-Methoxy-3-benzyloxyestra-1,3,5(10)-triene-16α,17β-diol (Va)——To a solution of 2-methoxy-3-benzyloxy-16α-hydroxyestra-1,3,5(10)-trien-17-one[7] (90 mg) in MeOH (3 ml) was added $NaBH_4$ (20 mg) in MeOH (0.5 ml)-H_2O (0.2 ml) and stirred at room temperature for 1 hr. After addition of AcOH to decompose the excess reagent the resulting solution was extracted with AcOEt. The organic layer was washed with 5% $NaHCO_3$ and H_2O, dried over anhydrous Na_2SO_4, and evaporated. The crude product obtained was purified by preparative TLC using AcOEt–hexane–EtOH (80:15:5) as developing solvent. Recrystallization of the eluate from MeOH gave Va (76 mg) as colorless needles. mp 102—104°. *Anal.* Calcd. for $C_{26}H_{32}O_4$: C, 76.44; H, 7.90. Found: C, 76.10; H, 7.94. NMR ($CDCl_3$ solution) δ: 0.79 (3H, s, 18-CH_3), 3.55 (1H, d, J=6 Hz, 17α-H), 3.82 (3H, s, 2-OCH_3), 4.13 (1H, m, 16β-H), 5.06 (2H, s, 3-$OC\underline{H}_2C_6H_5$), 6.59 (1H, s, 4-H), 6.80 (1H, s, 1-H).

2-Methoxy-3-benzyloxyestra-1,3,5(10)-triene-16α,17β-diol Diacetate (Vb)——Treatment of Va (46 mg) with Ac_2O and pyridine in the usual manner gave Vb (40 mg) as colorless oil. NMR ($CDCl_3$ solution) δ: 0.85 (3H, s, 18-$C\underline{H}_3$), 2.05, 2.09 (6H, s, 16α-,17β-$OCOCH_3$), 3.84 (3H, s, 2-OCH_3), 4.98 (1H, d, J=6 Hz, 17α-H), 5.08 (2H, s, 3-$OC\underline{H}_2C_6H_5$), 5.20 (1H, m, 16β-H), 6.62 (1H, s, 4-H), 6.82 (1H, s, 1-H).

2-Methoxyestra-1,3,5(10)-triene-3,16α,17β-triol 16,17-Diacetate (Vc)——A solution of Vb (40 mg) in EtOH (10 ml) was shaken with 5% Pd/C (40 mg) for 6 hr under a stream of H_2 gas at room temperature. After removal of the catalyst by filtration the filtrate was evaporated *in vacuo* to give Vc (30 mg) as colorless oil. NMR ($CDCl_3$ solution) δ: 0.84 (3H, s, 18-CH_3), 2.03, 2.07 (6H, s, 16α-,17β-$OCOCH_3$), 3.82 (3H, s, 2-OCH_3), 4.96 (1H, d, J=6 Hz, 17α-H), 5.18 (1H, m, 16β-H), 6.60 (1H, s, 4-H), 6.73 (1H, s, 1-H).

Methyl (2-Methoxy-16α,17β-diacetoxyestra-1,3,5(10)-trien-3-yl-2,3,4-tri-O-acetyl-β-D-glucopyranosid)-uronate (Vd)——Prepared from Vc (20 mg) employing Koenigs-Knorr reaction in the manner as described in Id. The crude product was submitted to preparative TLC using $CHCl_3$–MeOH (60:1) as developing solvent. Elution of the adsorbent corresponding to the spot (*Rf* 0.30) with AcOEt gave Vd (5 mg) as colorless oil. NMR ($CDCl_3$ solution) δ: 0.84 (3H, s, 18-CH_3), 2.02—2.08 (15H, s, 16α-,17β-$OCOCH_3$, pyranose-$OCOCH_3$), 3.72 (3H, s, pyranose-$COOCH_3$), 3.76 (3H, s, 2-OCH_3), 4.07 (1H, m, pyranose-5-H), 4.97 (2H, d, J=7 Hz, 17α-H, pyranose-1-H), 5.06—5.40 (4H, m, 16β-H, pyranose-C$\underline{H}$-OAc), 6.77 (1H, s, 4-H), 6.80 (1H, s, 1-H).

2-Methoxy-3-benzyloxyestra-1,3,5(10)-triene-16β,17β-diol (VIa)——To a solution of VIIIa (100 mg) in MeOH (40 ml) was added $NaBH_4$ (25 mg) in MeOH (0.5 ml)–H_2O (0.2 ml) and stirred at room temperature for 1 hr. The resulting solution was diluted with 10% K_2CO_3 (5 ml) and refluxed for 30 min. After removal of MeOH by evaporation the solution was extracted with AcOEt. The organic layer was washed with 5% HCl, 5% $NaHCO_3$, and H_2O successively, dried over anhydrous Na_2SO_4, and evaporated. Recrystallization of the residue from acetone–hexane gave VIa (72 mg) as colorless needles. mp 179—180°. $[\alpha]_D^{26}$ +67.2° (c=0.10). *Anal.* Calcd. for $C_{26}H_{32}O_4$: C, 76.44; H, 7.90. Found: C, 76.27; H, 7.76. NMR ($CDCl_3$ solution) δ: 0.85 (3H, s, 18-CH_3), 3.43 (1H, d, J=7 Hz, 17α-H), 3.84 (3H, s, 2-OCH_3), 4.18 (1H, m, 16α-H), 5.08 (2H, s, 3-$OC\underline{H}_2C_6H_5$), 6.62 (1H, s, 4-H), 6.85 (1H, s, 1-H).

2-Methoxy-3-benzyloxyestra-1,3,5(10)-triene-16β,17β-diol Diacetate (VIb)——Treatment of VIa (50 mg) with pyridine and Ac_2O in the usual manner gave VIb (40 mg) as colorless oil. NMR ($CDCl_3$ solution) δ: 0.93 (3H, s, 18-CH_3), 2.01, 2.04 (6H, s, 16β-,17β-$OCOCH_3$), 3.82 (3H, s, 2-OCH_3), 4.58 (1H, d, J=7 Hz, 17α-H), 5.05 (2H, s, 3-$OC\underline{H}_2C_6H_5$), 5.28 (1H, m, 16α-H), 6.60 (1H, s, 4-H), 6.80 (1H, s, 1-H).

2-Methoxyestra-1,3,5(10)-triene-3,16β,17β-triol 16,17-Diacetate (VIc)——A solution of VIb (40 mg) in EtOH (10 ml) was shaken with 5% Pd/C (40 mg) overnight under a stream of H_2 gas at room temperature. After removal of the catalyst by filtration the filtrate was evaporated *in vacuo*. Recrystallization of the residue from MeOH gave VIc (20 mg) as colorless needles. mp 265—267°. $[\alpha]_D^{14}$ +91.9° (c=0.10). *Anal.* Calcd. for $C_{23}H_{30}O_6$: C, 68.63; H, 7.51. Found: C, 68.34; H, 7.43. NMR ($CDCl_3$ solution) δ: 0.93 (3H, s, 18-CH_3), 2.02, 2.04 (6H, s, 16β-,17β-$OCOCH_3$), 3.83 (3H, s, 2-OCH_3), 4.60 (1H, d, J=7 Hz, 17α-H), 5.20 (1H, m, 16α-H), 6.61 (1H, s, 4-H), 6.74 (1H, s, 1-H).

Methyl (2-Methoxy-16β,17β-diacetoxyestra-1,3,5(10)-trien-3-yl-2,3,4-tri-O-acetyl-β-D-glucopyranosid)-uronate (VId)——Prepared from VIc (20 mg) employing Koenigs-Knorr reaction in the manner as described in Id. The crude product was submitted to preparative TLC using $CHCl_3$–MeOH (60:1) as developing solvent. Elution of the adsorbent corresponding to the spot (*Rf* 0.35) with AcOEt gave VId (10 mg) as colorless oil. NMR ($CDCl_3$ solution) δ: 0.92 (3H, s, 18-CH_3), 2.03—2.06 (15H, s, 16β-,17β-$OCOCH_3$, pyranose-$OCOCH_3$), 3.72 (3H, s, pyranose-$COOCH_3$), 3.76 (3H, s, 2-OCH_3), 4.07 (1H, m, pyranose-5-H), 4.61 (1H, d, J=7 Hz, 17α-H), 4.98 (1H, d, J=7 Hz, pyranose-1-H), 5.14—5.40 (4H, m, 16α-H, pyranose-C$\underline{H}$-OAc), 6.81 (2H, s, 4-,1-H).

Methyl (2-Methoxy-16-oxo-17β-acetoxyestra-1,3,5(10)-trien-3-yl-2,3,4-tri-O-acetyl-β-D-glucopyranosid)-uronate (VIIb)——Prepared from 2-methoxy-3,17β-dihydroxyestra-1,3,5(10)-trien-16-one 17-acetate (VIIa)[7] (50 mg) employing Koenigs-Knorr reaction in the manner as described in Id. The crude product was submitted to preparative TLC using $CHCl_3$–MeOH (80:1) as developing solvent. Elution of the adsorbent corresponding to the spot (*Rf* 0.30) with AcOEt gave VIIb (30 mg) as colorless oil. NMR ($CDCl_3$ solution) δ: 0.87 (3H, s, 18-CH_3), 2.03, 2.06 (9H, s, pyranose-$OCOCH_3$), 2.18 (3H, s, 17β-$OCOCH_3$), 3.74 (3H, s, pyranose-$COOCH_3$), 3.78 (3H, s, 2-OCH_3), 4.10 (1H, m, pyranose-5-H), 5.10 (1H, s, 17α-H), 4.90—5.40 (4H, m, pyranose-C$\underline{H}$-OAc, -1-H), 6.87 (2H, s, 4-,1-H).

2-Methoxy-3-benzyloxy-16β-hydroxyestra-1,3,5(10)-trien-17-one Acetate (VIIIa)——To a solution of 2-methoxy-3-benzyloxyestra-1,3,5(10),16-tetraen-17-ol acetate[7] (500 mg) in AcOH (20 ml)–Ac_2O (0.2 ml)

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was added $Pb(OAc)_4$ (700 mg) and stirred at room temperature for 4 hr. The resulting solution was concentrated *in vacuo* below 50° and extracted with ether. The organic layer was washed with 5% $NaHCO_3$ and H_2O, dried over anhydrous Na_2SO_4, and evaporated. The crude product obtained was chromatographed on silica gel. Elution with benzene and recrystallization of the eluate from MeOH gave VIIIa (240 mg) as colorless needles. mp 161.5—163°. $[\alpha]_D^{25}$ +121.4° (c=0.10). *Anal.* Calcd. for $C_{28}H_{32}O_5$: C, 74.97; H, 7.19. Found: C, 74.65; H, 7.07. NMR ($CDCl_3$ solution) δ: 1.01 (3H, s, 18-CH_3), 2.11 (3H, s, 16β-OCOCH_3), 3.83 (3H, s, 2-OCH_3), 5.02 (1H, m, 16α-H), 5.07 (2H, s, 3-$OCH_2C_6H_5$), 6.62 (1H, s, 4-H), 6.79 (1H, s, 1-H).

2-Methoxy-3,16β-dihydroxyestra-1,3,5(10)-trien-17-one 16-Acetate (VIIIb)——A solution of VIIIa (400 mg) in EtOH (150 ml) was shaken with 5% Pd/C (400 mg) under a stream of H_2 gas for 3 hr at room temperature. After removal of the catalyst by filtration the filtrate was evaporated *in vacuo*. Recrystallization of the residue from EtOH gave VIIIb (227 mg) as colorless leaflets. mp 211—213°. $[\alpha]_D^{25.5}$ +171.9° (c=0.10). *Anal.* Calcd. for $C_{21}H_{26}O_5$: C, 70.37; H, 7.31. Found: C, 70.10; H, 7.60. NMR ($CDCl_3$ solution) δ: 1.02 (3H, s, 18-CH_3), 2.12 (3H, s, 16β-OCOCH_3), 3.84 (3H, s, 2-OCH_3), 5.02 (1H, m, 16α-H), 6.63 (1H, s, 4-H), 6.75 (1H, s, 1-H).

Methyl (2-Methoxy-16β-acetoxy-17-oxoestra-1,3,5(10)-trien-3-yl-2,3,4-tri-O-acetyl-β-D-glucopyranosid)-uronate (VIIIc)——Prepared from VIIIb (70 mg) employing Koenigs-Knorr reaction in the manner as described in Id. The crude product was submitted to preparative TLC using $CHCl_3$–MeOH (80: 1) as developing solvent. Elution of the adsorbent corresponding to the spot (*Rf* 0.34) gave VIIIc (40 mg) as colorless oil. NMR ($CDCl_3$ solution) δ: 1.02 (3H, s, 18-CH_3), 2.05, 2.08 (9H, s, pyranose-OCOCH_3), 2.13 (3H, s, 16β-OCO-CH_3), 3.75 (3H, s, pyranose-COOCH_3), 3.80 (3H, s, 2-OCH_3), 4.10 (1H, m, pyranose-5-H), 4.90—5.40 (5H, m, 16α-H, pyranose-CH-OAc, -1-H), 6.85 (1H, s, 4-H), 6.89 (1H, s, 1-H).

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