

Syntheses of Catechol Estrogen 16,17-Ketols, New and Potential Metabolites¹⁾TOSHIO NAMBARA, YOSHIHIKO KAWARADA, MIKIKO ASAMA, SETSUKO AKIYAMA,
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In order to characterize the biliary metabolites the catechol estrogen 16,17-ketols and their monomethyl ethers have been synthesized as reference compounds employing the method worked out by Gallagher and his co-workers. The preparation of 2-methoxy-16 α -hydroxyestrone 3-glucuronide acetate-methyl ester (Vd) has also been described.

Since the first report dealing with isolation of 2-methoxyestrone from the human urine³⁾ the occurrence of several kinds of catechol estrogens and their 2-methyl ethers was demonstrated.⁴⁾ Recently considerable attentions have been focused on the biochemical significance of catechol O-methylation involved in the metabolism of estrogen.⁵⁾ In addition, excretion of the isomeric 3-methyl ether in the rat bile⁶⁾ and human pregnancy urine⁷⁾ has also been clarified. The current works on the biliary metabolites of estrogen strongly imply the possible occurrence of catechol estrogen having the 16,17-ketol structure in the rat. The present paper describes the preparation of isomeric 2,16 α -dihydroxyestrone monomethyl ethers and their related compounds employing the method worked out by Gallagher and his co-workers.⁸⁾

An initial project was directed to the synthesis of 2-methoxy derivative employing 2-methoxyestrone 3-benzyl ether (I) as a starting material, derivable from estrone in several steps.⁹⁾ Treatment with isopropenyl acetate and a catalytic amount of sulfuric acid in the usual manner provided the Δ^{16} -enol acetate (II) in a fairly good yield. Oxidation with *m*-chloroperbenzoic acid afforded the epoxyacetate (III), which on brief exposure to sulfuric acid was converted into the 16 α -hydroxy-17-ketone (IVa) and its acetate (IVb). Removal of the benzyl group at C-3 in IVa and IVb was effected by hydrogenolysis over palladium-on-charcoal yielding 2-methoxy-16 α -hydroxyestrone (Va) and the 16-monoacetate (Vb), respectively. Being treated with sodium hydroxide under a stream of nitrogen gas, IVa underwent the ketol rearrangement with ease to provide the isomeric 17 β -hydroxy-16-ketone (VIa) in a satisfactory yield. The structural assignment was unequivocal since it is sufficiently substantiated that in the C/D-*trans* steroids the 17 β -hydroxy-16-ketone is the most

- 1) This paper constitutes Part LXVII of the series entitled "Analytical Chemical Studies on Steroids"; Part LXVI: T. Nambara, K. Shimada, S. Iwamura, M. Mori, and M. Nokubo, *Chem. Pharm. Bull.* (Tokyo), **21**, 2474 (1973). A part of this work has been presented as a preliminary report: T. Nambara, S. Honma, M. Asama, S. Akiyama, and M. Nokubo, *Chem. Pharm. Bull.* (Tokyo), **21**, 914 (1973).
- 2) Location: a) Aobayama, Sendai; b) Shimosakunobe, Kawasaki.
- 3) S. Kraychy and T.F. Gallagher, *J. Am. Chem. Soc.*, **79**, 754 (1957); *idem*, *J. Biol. Chem.*, **229**, 519 (1957).
- 4) R. Knuppen and H. Breuer, "Advances in Biosciences," Vol. 3, ed. by G. Raspé, Pergamon Press, Vieweg, 1969, pp. 81—92.
- 5) T. Nambara, S. Honma, and K. Kanayama, *Chem. Pharm. Bull.* (Tokyo), **20**, 2235 (1972) and references quoted therein.
- 6) A. Bartke, R.E. Steele, J.G. Williams, and K.I.H. Williams, *Steroids*, **18**, 303 (1971).
- 7) R. Knuppen, O. Haupt, and H. Breuer, *Biochem. J.*, **128**, 1369 (1972).
- 8) N.S. Leeds, D.K. Fukushima, and T.F. Gallagher, *J. Am. Chem. Soc.*, **76**, 2943 (1954).
- 9) T. Nambara, S. Honma, and S. Akiyama, *Chem. Pharm. Bull.* (Tokyo), **18**, 474 (1970).

stable ketol of four possible isomers.¹⁰⁾ Elimination of the protecting group at C-3 in VIa and its acetate (VIb) was similarly attained by hydrogenolysis to afford 2-methoxy-3,17 β -dihydroxyestratrien-16-one (VIIa) and the 17-monoacetate (VIIb), respectively.

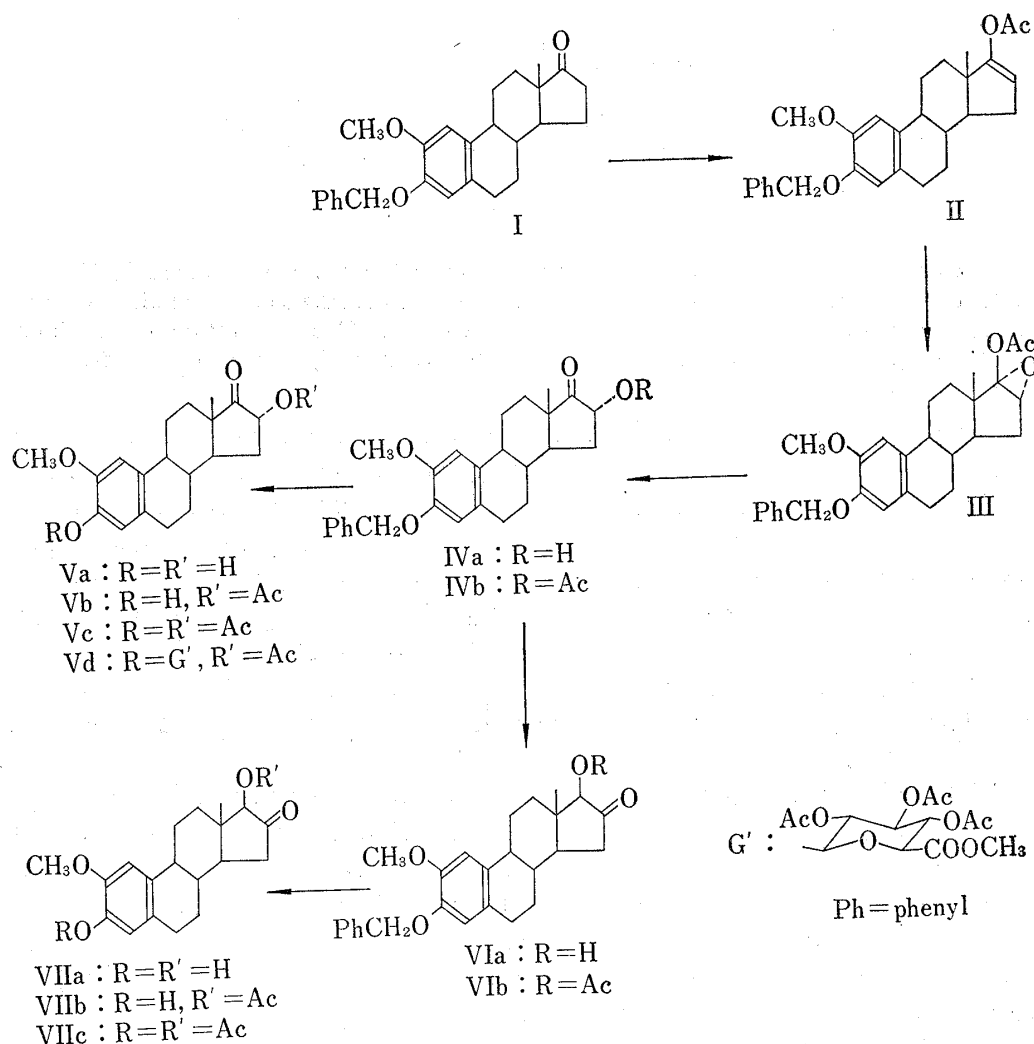


Chart 1

The possible occurrence of 2-methoxy-16 α -hydroxyestrone 3-glucuronide in the rat bile prompted us further to prepare the acetate-methyl ester derivative. Condensation of methyl acetobromoglucuronate with Vb in the presence of cadmium carbonate¹¹⁾ took place readily to provide methyl (2-methoxy-16 α -acetoxy-17-oxoestra-1,3,5(10)-trien-3-yl)-2,3,4-tri-O-acetyl- β -D-glucopyranosiduronate in 47% yield.

The preparation of the isomeric 3-methyl ethers having 16,17-ketol structure from 2-benzyloxyestrone methyl ether (IX) was then undertaken. Transformation into the enol acetate (X), followed by epoxidation gave the epoxyacetate (XI), which in turn was led to the 16 α -hydroxy-17-ketone (XIIa) and the 16-acetate (XIIb) by treatment with mineral acid in the manner as mentioned above. Subsequent debenzoylation was easily attained by hydrogenolysis to furnish the 2,16 α -dihydroxyestrone 3-methyl ether (XIIIa). When XIIa

10) J. Fishman, *J. Am. Chem. Soc.*, **82**, 6143 (1960); T. Nambara and J. Fishman, *J. Org. Chem.*, **27**, 2131 (1962); T. Nambara, H. Hosoda, and M. Usui, *Chem. Pharm. Bull. (Tokyo)*, **17**, 947 (1969).

11) R.B. Conrow and S. Bernstein, *J. Org. Chem.*, **36**, 863 (1971); T. Nambara, T. Anjyo, and S. Goya, *Chem. Pharm. Bull. (Tokyo)*, **19**, 2183 (1971).

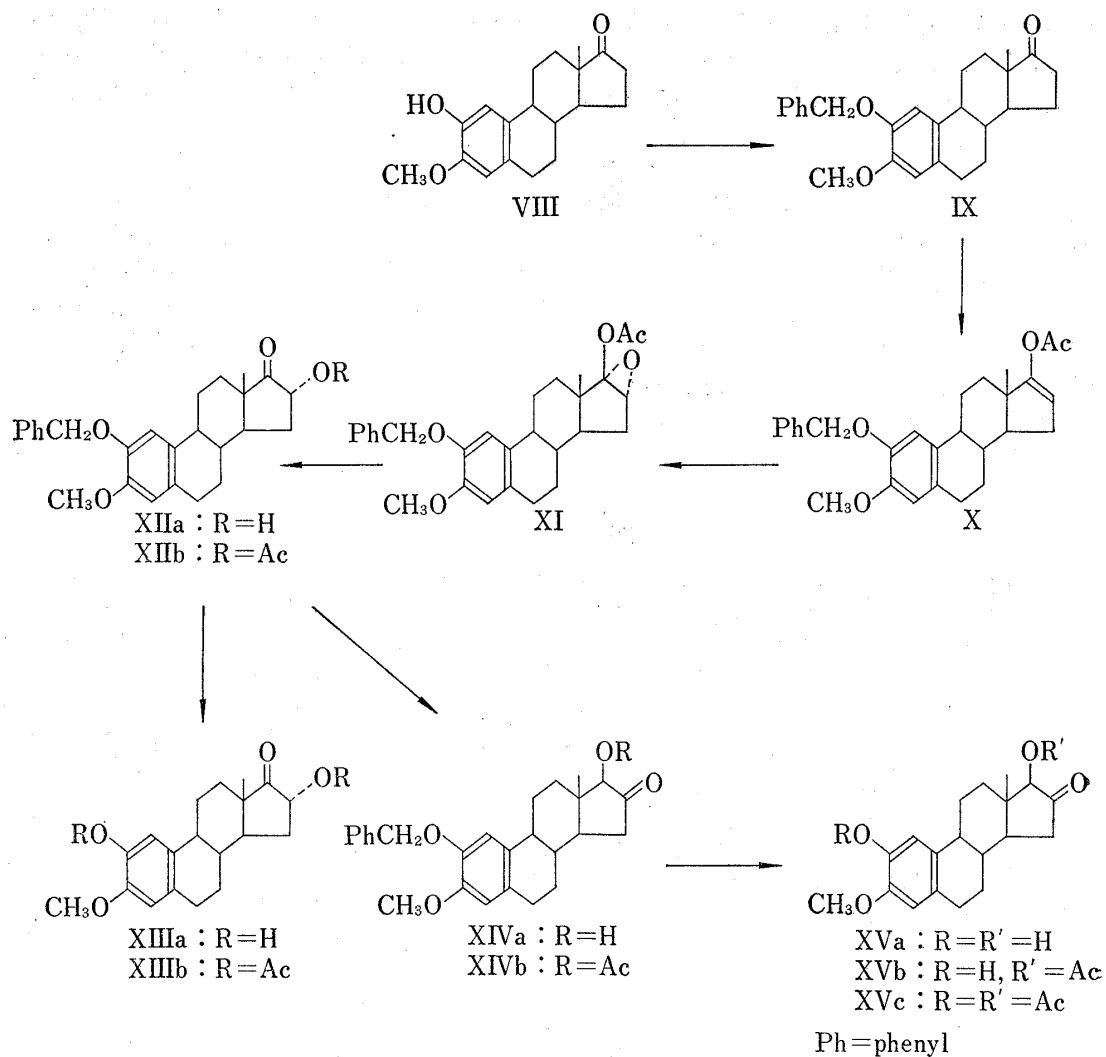


Chart 2

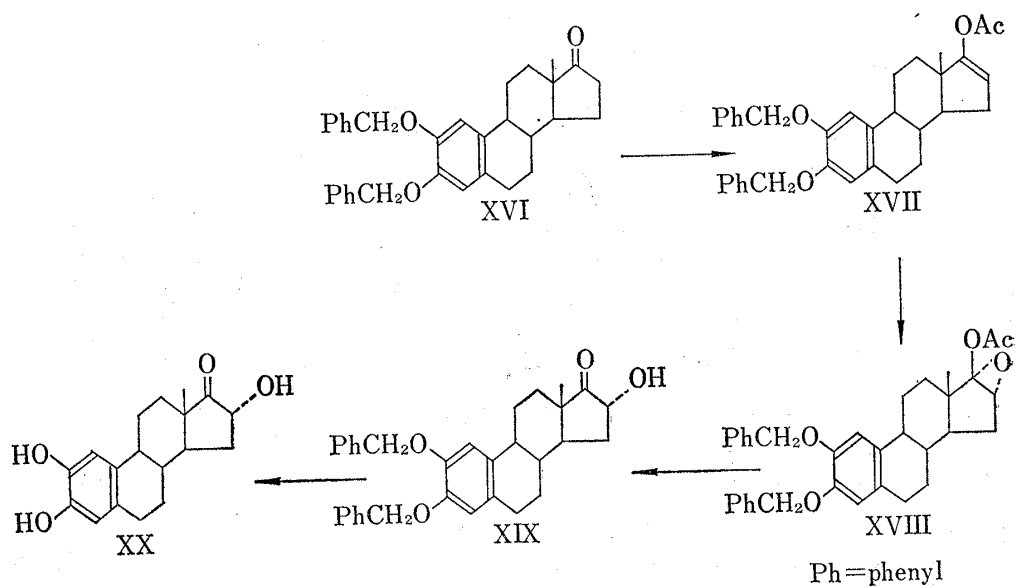


Chart 3

was treated with alkali, the facile rearrangement was effected to provide the 17 β -hydroxy-16-ketone (XIVa) in a reasonable yield. Usual acetylation and hydrogenolysis afforded 2,17 β -dihydroxy-3-methoxyestratrien-16-one (XVa), the 17-monoacetate (XVb) and 2,17-diacetate (XVc).

The synthesis of 2,16 α -dihydroxyestrone was then carried out in a similar fashion. 2-Hydroxyestrone dibenzyl ether (XVI) was transformed into the Δ^{16} -enol acetate (XVII), which on treatment with per acid was led to the epoxyacetate (XVIII). The cleavage of the 16 α ,17 α -oxido ring with sulfuric acid resulted in formation of the 16 α -hydroxy-17-ketone (XIX) in a reasonable yield. Simultaneous elimination of both benzyl groups was effected by catalytic hydrogenation yielding the desired 2,16 α -dihydroxyestrone (XX).

It is hoped that these synthetic specimens¹²⁾ may serve as references for characterization of the metabolites in the biological material.

Experimental¹⁴⁾

2-Methoxy-3-benzyloxyestra-1,3,5(10),16-tetraen-17-ol Acetate (II)—To a solution of 2-methoxy-3-benzyloxyestra-1,3,5(10)-trien-17-one (I)⁹⁾ (2.4 g) in isopropenyl acetate (30 ml) was added a catalyst solution (2 ml) (isopropenyl acetate (5 ml) and conc. H₂SO₄ (0.1 ml)) and refluxed for 2.5 hr. The solution was concentrated to one-half of its volume by slow distillation over a period of 3 hr. An additional 7 ml of isopropenyl acetate was added and the solution was again concentrated to ca. 10 ml over another 6 hr. The resulting solution was diluted with ether, washed with cold 5% NaHCO₃ and H₂O, dried over anhydrous Na₂SO₄, and evaporated. The residue was submitted to column chromatography on silica gel (60 g). Elution with benzene and recrystallization of the eluate from MeOH gave II (1.7 g) as colorless needles. mp 143.5–144.5°. [α]_D²⁵ +98.2° (*c*=0.11). *Anal.* Calcd. for C₂₈H₃₂O₄: C, 77.75; H, 7.46. Found: C, 78.05; H, 7.71. NMR (CCl₄ solution) δ : 0.90 (3H, s, 18-CH₃), 2.09 (3H, s, 17-OCOCH₃), 3.75 (3H, s, 2-OCH₃), 4.93 (2H, s, 3-OCH₂C₆H₅), 5.45 (1H, m, 16-H), 6.47 (1H, s, 4-H), 6.65 (1H, s, 1-H).

2-Methoxy-3-benzyloxy-16 α ,17 α -epoxyestra-1,3,5(10)-trien-17 β -ol Acetate (III)—To a solution of II (50 mg) in benzene (5 ml) was added *m*-chloroperbenzoic acid (23 mg) and allowed to stand at room temperature overnight. The resulting solution was diluted with ether, washed with cold 5% Na₂S₂O₃, 5% NaHCO₃, and H₂O, successively, dried over anhydrous Na₂SO₄, and evaporated. Recrystallization from MeOH gave III (50 mg) as colorless leaflets. mp 152.5–154°. [α]_D²⁵ +92.0° (*c*=0.09). *Anal.* Calcd. for C₂₈H₃₂O₅: C, 74.97; H, 7.19. Found: C, 74.99; H, 7.26. NMR (CDCl₃ solution) δ : 1.00 (3H, s, 18-CH₃), 2.11 (3H, s, 17 β -OCO-CH₃), 3.83 (3H, s, 2-OCH₃), 5.07 (2H, s, 3-OCH₂C₆H₅), 5.46 (1H, m, 16 β -H), 6.60 (1H, s, 4-H), 6.80 (1H, s, 1-H).

2-Methoxy-3-benzyloxy-16 α -hydroxyestra-1,3,5(10)-trien-17-one (IVa)—To a solution of III (50 mg) in MeOH (10 ml)–acetone (0.7 ml) was added 6*N* H₂SO₄ (2 ml) and allowed to stand at room temperature overnight. The resulting solution was diluted with AcOEt, washed with cold 5% NaHCO₃ and H₂O, dried over anhydrous Na₂SO₄, and evaporated. Recrystallization from ether gave IVa (31 mg) as colorless needles. mp 161–163°. [α]_D²⁵ +122.7° (*c*=0.11). *Anal.* Calcd. for C₂₆H₃₀O₄: C, 76.82; H, 7.44. Found: C, 76.55; H, 7.63. NMR (CDCl₃ solution) δ : 0.98 (3H, s, 18-CH₃), 3.82 (3H, s, 2-OCH₃), 4.39 (1H, t, *J*=4.5 Hz, 16 β -H), 5.08 (2H, s, 3-OCH₂C₆H₅), 6.63 (1H, s, 4-H), 6.82 (1H, s, 1-H).

2-Methoxy-3-benzyloxy-16 α -hydroxyestra-1,3,5(10)-trien-17-one Acetate (IVb)—Treatment of IVa (1.6 g) with Ac₂O (28 ml) and pyridine (28 ml) in the usual manner. The crude product was submitted to preparative TLC using hexane–AcOEt (5:1) as developing solvent. Elution of the adsorbent corresponding to the spot (*R_f* 0.28) and recrystallization of the eluate from MeOH gave IVb (0.9 g) as colorless needles. mp 138–139°. [α]_D²⁵ +14.1° (*c*=0.11). *Anal.* Calcd. for C₂₈H₃₂O₅: C, 74.97; H, 7.19. Found: C, 74.85; H, 7.33. NMR (CDCl₃ solution) δ : 1.00 (3H, s, 18-CH₃), 2.12 (3H, s, 16 α -OCOCH₃), 3.85 (3H, s, 2-OCH₃), 5.10 (2H, s, 3-OCH₂C₆H₅), 5.46 (1H, m, 16 β -H), 6.65 (1H, s, 4-H), 6.84 (1H, s, 1-H).

12) It has recently been elucidated that Va, Vd, and XIIIa occurred as biliary metabolites in the rat administered with estrone.¹³⁾

13) T. Nambara, S. Honma, M. Asama, S. Akiyama, and M. Nokubo, *Chem. Pharm. Bull.* (Tokyo), **21**, 914 (1973); H. Watanabe and J.A. Menzies, *Steroids*, **21**, 123 (1973).

14) All melting points were taken on a micro hot-stage apparatus and are uncorrected. Optical rotations were measured in CHCl₃ unless otherwise specified. Nuclear magnetic resonance (NMR) spectra were recorded on Hitachi Model R-20A spectrometer at 60 MHz using tetramethylsilane as an internal standard. Abbreviation used s=singlet, t=triplet, and m=multiplet. For preparative thin-layer chromatography (TLC) silica gel H (E. Merck AG, Darmstadt) was used as an adsorbent.

2-Methoxy-3,16 α -dihydroxyestra-1,3,5(10)-trien-17-one (Va)—A solution of IVa (30 mg) in EtOH (25 ml) was shaken with 5% Pd/C (30 mg) under a stream of H₂ gas at room temperature for 21 hr. After removal of the catalyst by filtration the filtrate was evaporated to give a crystalline product. Recrystallization from acetone–hexane gave Va (14 mg) as colorless needles. mp 201.5–204°. $[\alpha]_D^{17} + 131.9^\circ$ ($c=0.04$). *Anal.* Calcd. for C₁₉H₂₄O₄: C, 72.12; H, 7.65. Found: C, 71.93; H, 7.72. NMR (CDCl₃ solution) δ : 1.00 (3H, s, 18-CH₃), 3.82 (3H, s, 2-OCH₃), 4.41 (1H, t, $J=4.5$ Hz, 16 β -H), 6.62 (1H, s, 4-H), 6.75 (1H, s, 1-H).

2-Methoxy-3,16 α -dihydroxyestra-1,3,5(10)-trien-17-one 16-Acetate (Vb)—A solution of IVb (500 mg) in EtOH (300 ml) was shaken with 5% Pd/C (250 mg) under a stream of H₂ gas at room temperature for 10 hr. After removal of the catalyst by filtration the filtrate was evaporated to give a crystalline product. Recrystallization from benzene gave Vb (300 mg) as colorless prisms. mp 245–247°. $[\alpha]_D^{17} + 47.5^\circ$ ($c=0.10$). *Anal.* Calcd. for C₂₁H₂₆O₅: C, 70.37; H, 7.31. Found: C, 69.86; H, 7.33. NMR (CDCl₃ solution) δ : 1.00 (3H, s, 18-CH₃), 2.11 (3H, s, 16 α -OCOCH₃), 3.83 (3H, s, 2-OCH₃), 5.46 (1H, m, 16 β -H), 6.64 (1H, s, 4-H), 6.77 (1H, s, 1-H).

2-Methoxy-3,16 α -dihydroxyestra-1,3,5(10)-trien-17-one Diacetate (Vc)—Treatment of Va with Ac₂O and pyridine in the usual manner, followed by recrystallization from acetone–hexane gave Vc as colorless prisms. mp 220–222°. $[\alpha]_D^{17} + 161.5^\circ$ ($c=0.05$). *Anal.* Calcd. for C₂₃H₂₈O₆·1/2H₂O: C, 68.88; H, 6.98. Found: C, 68.78, 68.70; H, 7.16, 7.01. NMR (CDCl₃ solution) δ : 1.00 (3H, s, 18-CH₃), 2.12 (3H, s, 16 α -OCOCH₃), 2.28 (3H, s, 3-OCOCH₃), 3.78 (3H, s, 2-OCH₃), 5.43 (1H, m, 16 β -H), 6.75 (1H, s, 4-H), 6.87 (1H, s, 1-H).

2-Methoxy-3-benzyloxy-17 β -hydroxyestra-1,3,5(10)-trien-16-one (VIa)—To a solution of IVa (50 mg) in acetone (2.4 ml)–H₂O (0.8 ml) was added 1N NaOH (0.2 ml) and refluxed for 15 min. The resulting solution was diluted with H₂O and neutralized with 5% HCl. The precipitate was collected by filtration, washed with H₂O, and submitted to preparative TLC using benzene–ether (4:1) as developing solvent. Elution of the adsorbent corresponding to the spot (R_f 0.32) and recrystallization of the eluate from acetone–hexane gave VIa (32 mg) as colorless needles. mp 185–186°. $[\alpha]_D^{25} - 52.2^\circ$ ($c=0.10$). *Anal.* Calcd. for C₂₆H₃₀O₄: C, 76.42; H, 7.44. Found: C, 76.82; H, 7.48. NMR (CDCl₃ solution) δ : 0.76 (3H, s, 18-CH₃), 3.84 (4H, s, 2-OCH₃, 17 α -H), 5.08 (2H, s, 3-OCH₂C₆H₅), 6.62 (1H, s, 4-H), 6.83 (1H, s, 1-H).

2-Methoxy-3-benzyloxy-17 β -hydroxyestra-1,3,5(10)-trien-16-one Acetate (VIb)—Treatment of VIa (100 mg) with Ac₂O (1 ml) and pyridine (2 ml) in the usual manner, followed by recrystallization from MeOH gave VIb (90 mg) as colorless needles. mp 122–124°. $[\alpha]_D^{25} - 60.6^\circ$ ($c=0.12$). *Anal.* Calcd. for C₂₈H₃₂O₅: C, 74.97; H, 7.19. Found: C, 75.17; H, 7.12. NMR (CDCl₃ solution) δ : 0.86 (3H, s, 18-CH₃), 2.17 (3H, s, 17 β -OCOCH₃), 3.85 (3H, s, 2-OCH₃), 5.09 (3H, s, 17 α -H, 3-OCH₂C₆H₅), 6.65 (1H, s, 4-H), 6.83 (1H, s, 1-H).

2-Methoxy-3,17 β -dihydroxyestra-1,3,5(10)-trien-16-one (VIIa)—A solution of VIa (50 mg) in AcOEt (1 ml)–EtOH (10 ml) was shaken with 5% Pd/C (50 mg) under a stream of H₂ gas at room temperature for 4 hr. After removal of the catalyst by filtration the filtrate was evaporated to dryness. Recrystallization from acetone–hexane gave VIIa (36 mg) as colorless needles. mp 213–215°. $[\alpha]_D^{25} - 43.6^\circ$ ($c=0.11$). *Anal.* Calcd. for C₁₈H₂₄O₄: C, 72.12; H, 7.65. Found: C, 72.39; H, 7.79. NMR (CDCl₃ solution) δ : 0.78 (3H, s, 18-CH₃), 3.88 (4H, s, 2-OCH₃, 17 α -H), 6.68 (1H, s, 4-H), 6.82 (1H, s, 1-H).

2-Methoxy-3,17 β -dihydroxyestra-1,3,5(10)-trien-16-one 17-Acetate (VIIb)—A solution of VIb (80 mg) in AcOEt (1 ml)–EtOH (18 ml) was shaken with 5% Pd/C (100 mg) under a stream of H₂ gas at room temperature for 6 hr. After removal of the catalyst by filtration the filtrate was evaporated to dryness. Recrystallization from MeOH gave VIIb (50 mg) as colorless needles. mp 251–253°. $[\alpha]_D^{25} - 54.6^\circ$ ($c=0.11$). *Anal.* Calcd. for C₂₁H₂₆O₅: C, 70.37; H, 7.31. Found: C, 70.02; H, 7.15. NMR (CDCl₃ solution) δ : 0.87 (3H, s, 18-CH₃), 2.18 (3H, s, 17 β -OCOCH₃), 3.85 (3H, s, 2-OCH₃), 5.09 (1H, s, 17 α -H), 6.64 (1H, s, 4-H), 6.77 (1H, s, 1-H).

2-Methoxy-3,17 β -dihydroxyestra-1,3,5(10)-trien-16-one Diacetate (VIIc)—Treatment of VIIa (30 mg) with Ac₂O (1 ml) and pyridine (2 ml) in the usual manner, followed by recrystallization from acetone–hexane gave VIIc (28 mg) as colorless leaflets. mp 196–198°. $[\alpha]_D^{20} - 57.0^\circ$ ($c=0.12$). *Anal.* Calcd. for C₂₃H₂₈O₆·1/2H₂O: C, 68.88; H, 6.98. Found: C, 68.95, 68.59; H, 7.14, 7.03. NMR (CDCl₃ solution) δ : 0.87 (3H, s, 18-CH₃), 2.18 (3H, s, 17 β -OCOCH₃), 2.29 (3H, s, 3-OCOCH₃), 3.81 (3H, s, 2-OCH₃), 5.11 (1H, s, 17 α -H), 6.77 (1H, s, 4-H), 6.90 (1H, s, 1-H).

Methyl (2-Methoxy-16 α -acetoxy-17-oxoestra-1,3,5(10)-trien-3-yl)-2,3,4-tri-O-acetyl- β -D-glucopyranosiduronate (Vd)—To a solution of Vb (170 mg) in anhydrous toluene (5 ml) containing CdCO₃ (200 mg) was added dropwise a solution of methyl 1-bromo-1-deoxy-2,3,4-tri-O-acetyl- α -D-glucopyranuronate (500 mg) in toluene (5 ml) over a period of 4 hr. Being refluxed for 19 hr, an additional amount of acetobromo sugar (200 mg) was added over a period of 1.5 hr and then refluxed for 35 hr. After removal of the precipitate by filtration the filtrate was evaporated *in vacuo*. An oily residue was then chromatographed on silica gel (30 g). Elution with benzene–ether (10:1 to 5:1) gave a pale yellow oil, which in turn was submitted to preparative TLC using benzene–AcOEt (6:1) as developing solvent. Elution of the adsorbent corresponding to the spot (R_f 0.44) and trituration of the eluate with hexane afforded amorphous substance (150 mg). Recrystallization from MeOH gave Vd as colorless needles. mp 177–179°. $[\alpha]_D^{20} + 53.1^\circ$ ($c=0.31$, MeOH). *Anal.* Calcd. for C₃₄H₄₂O₁₄: C, 60.52; H, 6.28. Found: C, 60.58; H, 6.26. NMR (CDCl₃ solution) δ : 1.01 (3H, s, 18-CH₃), 2.03, 2.06 (9H, s, pyranose-OCOCH₃), 2.11 (3H, s, 16 α -OCOCH₃), 3.73 (3H, s, pyranose-COOCH₃),

3.77 (3H, s, 2-OCH₃), 4.10 (1H, m, pyranose-5-H), 5.22 (5H, m, 16β-H, pyranose-CH-OAc and -1-H), 6.84 (2H, s, 1- and 4-H).

2-Benzoyloxy-3-methoxyestra-1,3,5(10)-trien-17-one (IX)—To a solution of 2-hydroxy-3-methoxyestra-1,3,5(10)-trien-17-one (VIII)¹⁵⁾ (450 mg) in EtOH (30 ml) were added C₆H₅CH₂Cl (0.46 ml) and K₂CO₃ (0.9 g) and refluxed for 3 hr. After removal of the precipitate by filtration the filtrate was evaporated. The residue was dissolved in AcOEt, washed with H₂O, and dried over anhydrous Na₂SO₄. On usual work-up the crude product obtained was submitted to preparative TLC using benzene-ether (7:1) as developing solvent. Elution of the adsorbent corresponding to the spot (*R_f* 0.58) gave IX (690 mg) as colorless oil. $[\alpha]_D^{25} + 112.1^\circ$ (*c*=0.06). *Anal.* Calcd. for C₂₆H₃₀O₃: C, 79.96; H, 7.74. Found: C, 79.73; H, 7.82. NMR (CCl₄ solution) δ : 0.83 (3H, s, 18-CH₃), 3.73 (3H, s, 3-OCH₃), 4.93 (2H, s, 2-OCH₂C₆H₅), 6.45 (1H, s, 4-H), 6.70 (1H, s, 1-H).

2-Benzoyloxy-3-methoxyestra-1,3,5(10),16-tetraen-17-ol Acetate (X)—IX (223 mg) was treated with isopropenyl acetate (15 ml) and catalyst solution (1 ml) in the manner as described in III. The crude product was submitted to preparative TLC using benzene-ether (13:1) as developing solvent. Elution of the adsorbent corresponding to the spot gave X (96 mg) as pale yellow oil. NMR (CDCl₃ solution) δ : 0.87 (3H, s, 18-CH₃), 2.08 (3H, s, 17-OCOCH₃), 3.75 (3H, s, 3-OCH₃), 5.04 (2H, s, 2-OCH₂C₆H₅), 5.50 (1H, m, 16-H), 6.56 (1H, s, 4-H), 6.79 (1H, s, 1-H).

2-Benzoyloxy-3-methoxy-16 α ,17 α -epoxyestra-1,3,5(10)-trien-17 β -ol Acetate (XI)—To a solution of X (400 mg) in benzene (25 ml) was added *m*-chloroperbenzoic acid (250 mg) and allowed to stand at room temperature overnight. The resulting solution was diluted with ether, washed with cold 5% Na₂S₂O₃, 5% NaHCO₃, and H₂O, successively, dried over anhydrous Na₂SO₄, and evaporated. Recrystallization from MeOH gave XI (400 mg) as colorless needles. mp 141–143°. $[\alpha]_D^{25} + 51.4^\circ$ (*c*=0.10). *Anal.* Calcd. for C₂₈H₃₂O₅: C, 74.97; H, 7.19. Found: C, 74.82; H, 7.26. NMR (CDCl₃ solution) δ : 0.90 (3H, s, 18-CH₃), 2.13 (3H, s, 17 β -OCOCH₃), 3.80 (3H, s, 3-OCH₃), 5.05 (2H, s, 2-OCH₂C₆H₅), 5.44 (1H, m, 16 β -H), 6.57 (1H, s, 4-H), 6.79 (1H, s, 1-H).

2-Benzoyloxy-3-methoxy-16 α -hydroxyestra-1,3,5(10)-trien-17-one (XIIa)—To a solution of XI (450 mg) in MeOH (100 ml)-acetone (17 ml) was added 6*N* H₂SO₄ (20 ml) and allowed to stand at room temperature overnight. The resulting solution was diluted with AcOEt, washed with cold 5% NaHCO₃ and H₂O, dried over anhydrous Na₂SO₄, and evaporated. The crude product obtained was submitted to preparative TLC using benzene-ether (10:1). Elution of the adsorbent corresponding to the spot (*R_f* 0.38) and recrystallization of the eluate from MeOH gave XIIa (190 mg) as colorless needles. mp 139–140°. $[\alpha]_D^{25} + 76.9^\circ$ (*c*=0.10). *Anal.* Calcd. for C₂₆H₃₀O₄: C, 76.82; H, 7.44. Found: C, 76.71; H, 7.58. NMR (CDCl₃ solution) δ : 0.97 (3H, s, 18-CH₃), 3.82 (3H, s, 3-OCH₃), 4.38 (1H, t, *J*=4.5 Hz, 16 β -H), 5.08 (2H, s, 2-OCH₂C₆H₅), 6.59 (1H, s, 4-H), 6.81 (1H, s, 1-H).

2-Benzoyloxy-3-methoxy-16 α -hydroxyestra-1,3,5(10)-trien-17-one Acetate (XIIb)—Treatment of XIIa with Ac₂O and pyridine in the usual manner, followed by recrystallization from acetone-hexane gave XIIb as colorless needles. mp 147–149°. $[\alpha]_D^{25} + 109.1^\circ$ (*c*=0.11). *Anal.* Calcd. for C₂₈H₃₂O₅: C, 74.97; H, 7.19. Found: C, 74.80; H, 7.11. NMR (CDCl₃ solution) δ : 0.98 (3H, s, 18-CH₃), 2.10 (3H, s, 16 α -OCOCH₃), 3.82 (3H, s, 3-OCH₃), 5.07 (2H, s, 2-OCH₂C₆H₅), 5.43 (1H, m, 16 β -H), 6.58 (1H, s, 4-H), 6.80 (1H, s, 1-H).

2,16 α -Dihydroxy-3-methoxyestra-1,3,5(10)-trien-17-one (XIIIa)—A solution of XIIa (60 mg) in EtOH (15 ml) was shaken with 5% Pd/C (15 mg) under a stream of H₂ gas at room temperature for 7.5 hr. After removal of the catalyst by filtration the filtrate was evaporated. The crude product obtained was submitted to preparative TLC using benzene-ether (10:7) as developing solvent. Elution of the adsorbent corresponding to the spot (*R_f* 0.35) and recrystallization of the eluate from MeOH gave XIIIa (32 mg) as colorless needles. mp 195–197°. $[\alpha]_D^{25} + 182.3^\circ$ (*c*=0.10). *Anal.* Calcd. for C₁₉H₂₄O₄: C, 72.12; H, 7.65. Found: C, 72.18; H, 7.89. NMR (CDCl₃ solution) δ : 0.99 (3H, s, 18-CH₃), 3.84 (3H, s, 3-OCH₃), 4.43 (1H, m, 16 β -H), 6.58 (1H, s, 4-H), 6.86 (1H, s, 1-H).

2,16 α -Dihydroxy-3-methoxyestra-1,3,5(10)-trien-17-one Diacetate (XIIIb)—Treatment of XIIIa with Ac₂O (0.5 ml) and pyridine (1 ml) in the usual manner, followed by recrystallization from acetone-hexane gave XIIIb (15 mg) as colorless needles. mp 221–223.8°. $[\alpha]_D^{25} + 197.7^\circ$ (*c*=0.06). *Anal.* Calcd. for C₂₃H₂₈O₆·1/2H₂O: C, 68.88; H, 6.98. Found: C, 68.71, 68.88; H, 7.08, 7.05. NMR (CDCl₃ solution) δ : 0.99 (3H, s, 18-CH₃), 2.11 (3H, s, 16 α -OCOCH₃), 2.27 (3H, s, 2-OCOCH₃), 3.76 (3H, s, 3-OCH₃), 5.44 (1H, m, 16 β -H), 6.65 (1H, s, 4-H), 6.91 (1H, s, 1-H).

2-Benzoyloxy-3-methoxy-17 β -hydroxyestra-1,3,5(10)-trien-16-one (XIVa)—To a solution of XIIa (100 mg) in acetone (4.8 ml)-H₂O (1.6 ml) was added 1*N* NaOH (0.4 ml) and refluxed for 15 min under a stream of N₂ gas. The resulting solution was diluted with ether, washed with 5% HCl and H₂O, and dried over anhydrous Na₂SO₄. After evaporation of solvent an oily residue was submitted to preparative TLC using benzene-ether (4:1) as developing solvent. Elution of the adsorbent corresponding to the spot (*R_f* 0.32) and recrystallization of the eluate from acetone-hexane gave XIVa (64 mg) as colorless needles. mp 162–163°. $[\alpha]_D^{25} - 28.6^\circ$ (*c*=0.11). *Anal.* Calcd. for C₂₆H₃₀O₄: C, 76.82; H, 7.44. Found: C, 77.13; H,

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7.39. NMR (CDCl₃ solution) δ : 0.76 (3H, s, 18-CH₃), 3.81 (1H, s, 17 α -H), 3.83 (3H, s, 3-OCH₃), 5.09 (2H, s, 2-OCH₂C₆H₅), 6.62 (1H, s, 4-H), 6.85 (1H, s, 1-H).

2-Benzoyloxy-3-methoxy-17 β -hydroxyestra-1,3,5(10)-trien-16-one Acetate (XIVb)—Treatment of XIVa with Ac₂O and pyridine in the usual manner, followed by recrystallization from acetone–hexane gave XIVb as colorless needles. mp 147–149°. $[\alpha]_D^{25} +109.1^\circ$ ($c=0.11$). Anal. Calcd. for C₂₈H₃₂O₅: C, 74.97; H, 7.19. Found: C, 74.80; H, 7.11. NMR (CDCl₃ solution) δ : 0.98 (3H, s, 18-CH₃), 2.10 (3H, s, 16 α -OCOCH₃), 3.82 (3H, s, 3-OCH₃), 5.07 (2H, s, 2-OCH₂C₆H₅), 5.43 (1H, m, 16 β -H), 6.58 (1H, s, 4-H), 6.80 (1H, s, 1-H).

2,17 β -Dihydroxy-3-methoxyestra-1,3,5(10)-trien-16-one (XVa)—A solution of XIVa (150 mg) in AcOEt (1 ml)–EtOH (30 ml) was shaken with 5% Pd/C (150 mg) under a stream of H₂ gas at room temperature for 4 hr. After removal of the catalyst by filtration the filtrate was evaporated to dryness. Recrystallization from acetone–hexane gave XVa (95 mg) as colorless needles. mp 210–212°. $[\alpha]_D^{25} -75.3^\circ$ ($c=0.11$). Anal. Calcd. for C₁₉H₂₄O₄: C, 72.12; H, 7.65. Found: C, 71.95; H, 7.62. NMR (CDCl₃ solution) δ : 0.76 (3H, s, 18-CH₃), 3.84 (4H, s, 3-OCH₃, 17 α -H), 6.57 (1H, s, 4-H), 6.87 (1H, s, 1-H).

2,17 β -Dihydroxy-3-methoxyestra-1,3,5(10)-trien-16-one 17-Acetate (XVb)—A solution of XIVb (100 mg) in AcOEt (2 ml)–EtOH (20 ml) was shaken with 5% Pd/C (100 mg) under a stream of H₂ gas at room temperature for 6 hr. After removal of the catalyst by filtration the filtrate was evaporated to dryness. Recrystallization from MeOH gave XVb (57 mg) as colorless needles. mp 191–192°. $[\alpha]_D^{25} -85.0^\circ$ ($c=0.12$). Anal. Calcd. for C₂₁H₂₆O₅: C, 70.37; H, 7.31. Found: C, 70.44; H, 7.43. NMR (CDCl₃ solution) δ : 0.84 (3H, s, 18-CH₃), 2.16 (3H, s, 17 β -OCOCH₃), 3.82 (3H, s, 3-OCH₃), 5.07 (1H, s, 17 α -H), 6.54 (1H, s, 4-H), 6.82 (1H, s, 1-H).

2,17 β -Dihydroxy-3-methoxyestra-1,3,5(10)-trien-16-one Diacetate (XVc)—Treatment of XVa (48 mg) with Ac₂O (1 ml) and pyridine (2 ml) in the usual manner gave XVc (45 mg) as colorless oil. $[\alpha]_D^{25} -66.6^\circ$ ($c=0.13$). Anal. Calcd. for C₂₃H₂₈O₆·1/2H₂O: C, 68.88; H, 6.98. Found: C, 68.35; H, 7.06. NMR (CDCl₃ solution) δ : 0.85 (3H, s, 18-CH₃), 2.17 (3H, s, 17 β -OCOCH₃), 2.29 (3H, s, 2-OCOCH₃), 3.77 (3H, s, 3-OCH₃), 5.07 (1H, s, 17 α -H), 6.64 (1H, s, 4-H), 6.90 (1H, s, 1-H).

2,3-Dibenzoyloxyestra-1,3,5(10)-trien-17-one (XVI)—To a solution of 2-hydroxy-3-benzoyloxyestra-1,3,5(10)-trien-17-one⁹⁾ (60 mg) in EtOH (5 ml) containing anhydrous K₂CO₃ (100 mg) was added C₆H₅CH₂Cl (0.2 ml) and refluxed for 2 hr. After usual work-up the crude product was submitted to preparative TLC using benzene–ether (6:1) as developing solvent. Elution of the adsorbent corresponding to the spot and recrystallization of the eluate from hexane gave XVI (48 mg) as colorless needles. mp 128–129.5° (reported mp 163–163.5°).¹⁶⁾ Anal. Calcd. for C₃₂H₃₄O₃: C, 82.37; H, 7.35. Found: C, 82.44; H, 7.35. NMR (CDCl₃ solution) δ : 0.89 (3H, s, 18-CH₃), 5.10 (4H, s, 2- and 3-OCH₂C₆H₅), 6.70 (1H, s, 4-H), 6.91 (1H, s, 1-H).

2,3-Dibenzoyloxyestra-1,3,5(10),16-tetraen-17-ol Acetate (XVII)—To a solution of XVI (300 mg) in isopropenyl acetate (15 ml) was added a catalyst solution (1 ml) (isopropenyl acetate (5 ml) and conc. H₂SO₄ (0.1 ml)) and refluxed for 1.5 hr. The solution was concentrated by slow distillation in the manner as described in II. The resulting solution was diluted with ether, washed with cold 5% NaHCO₃ and H₂O, dried over anhydrous Na₂SO₄, and evaporated. The residue was submitted to preparative TLC using hexane–AcOEt (20:3) as developing solvent. Elution of the adsorbent corresponding to the spot gave XVII (140 mg) as pale yellow oil. NMR (CDCl₃ solution) δ : 0.90 (3H, s, 18-CH₃), 2.11 (3H, s, 17-OCOCH₃), 5.07 (4H, s, 2- and 3-OCH₂C₆H₅), 5.51 (1H, m, 16-H), 6.67 (1H, s, 4-H), 6.87 (1H, s, 1-H).

2,3-Dibenzoyloxy-16 α ,17 α -epoxyestra-1,3,5(10)-trien-17 β -ol Acetate (XVIII)—To a solution of XVII (115 mg) in benzene (5 ml) was added *m*-chloroperbenzoic acid (70 mg) and allowed to stand at room temperature overnight. The resulting solution was diluted with ether, washed with 5% Na₂S₂O₃, 5% NaHCO₃, and H₂O, successively, and dried over anhydrous Na₂SO₄. Evaporation of solvent gave XVIII (100 mg) as colorless amorphous substance, which in turn was submitted to further step without purification.

2,3-Dibenzoyloxy-16 α -hydroxyestra-1,3,5(10)-trien-17-one (XIX)—To a solution of XVIII (100 mg) in MeOH (24 ml)–acetone (4 ml) was added 6N H₂SO₄ (6 ml) and stirred at room temperature for 3 hr. The resulting solution was diluted with AcOEt, washed with cold 5% NaHCO₃ and H₂O, and dried over anhydrous Na₂SO₄. After usual work-up an oily product was submitted to preparative TLC using benzene–ether (7:1) as developing solvent. Elution of the adsorbent corresponding to the spot (*R_f* 0.35) and recrystallization of the eluate from MeOH gave XIX (65 mg) as colorless needles. mp 125–125.5°. $[\alpha]_D^{18} +149.9^\circ$ ($c=0.13$). Anal. Calcd. for C₃₂H₃₄O₄: C, 80.13; H, 6.93. Found: C, 80.39; H, 7.25. NMR (CDCl₃ solution) δ : 0.97 (3H, s, 18-CH₃), 4.40 (1H, m, 16 β -H), 5.09 (4H, s, 2- and 3-OCH₂C₆H₅), 6.69 (1H, s, 4-H), 6.90 (1H, s, 1-H).

2,3,16 α -Trihydroxyestra-1,3,5(10)-trien-17-one (XX)—A solution of XIX (50 mg) in AcOEt (3 ml)–EtOH (3 ml) was shaken with 5% Pd/C (20 mg) under a stream of H₂ gas at room temperature overnight. After removal of the catalyst by filtration the filtrate was evaporated. The crude product was submitted to preparative TLC using benzene–AcOEt (2:1) as developing solvent. Elution of the adsorbent corresponding to the spot and recrystallization of the eluate from AcOEt gave XX (26 mg) as colorless needles. mp 229–231°. $[\alpha]_D^{22} +153.1^\circ$ ($c=0.16$, MeOH). Anal. Calcd. for C₁₈H₂₂O₄: C, 71.50; H, 7.33. Found:

C, 71.21; H, 7.35. NMR (CD₃OD solution) δ : 0.95 (3H, s, 18-CH₃), 4.34 (1H, m, 16 β -H), 6.46 (1H, s, 4-H), 6.69 (1H, s, 1-H).

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