[Chem. Pharm. Bull. 23(7)1613—1616(1975)] UDC 547.92.057 : 615.357.011.5

Syntheses of 16β-Oxygenated Catechol Estrogen Methyl Ethers, New and Potential Metabolites[1]

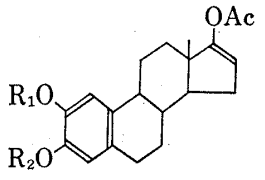
Toshio Nambara and Yoshihiko Kawarada

Pharmaceutical Institute, Tohoku University[2]

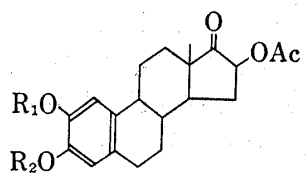
(Received October 28, 1974)

In order to characterize the biliary metabolites formed from estriol the isomeric monomethyl ethers of 2,16β-dihydroxy-estrone (IIIa, IIIb) and -estradiol (IVb, IVd) have been synthesized as the reference compounds.

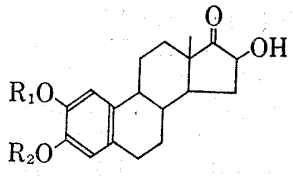
2-Methoxyestrone, one of the principal metabolites of female hormone in man, was first isolated from human pregnancy urine by Gallagher and his co-worker.[3] Since this report the *in vitro* and *in vivo* formation of several kinds of 2-methoxyestrogens was also demonstrated.[4] In addition, excretion of the isomeric 3-methyl ethers of catechol estrogen in rat bile[5,6] and human pregnancy urine[7] has recently been clarified. The current works on the biliary metabolites of estriol strongly suggest the possible occurrence of the catechol estrogens



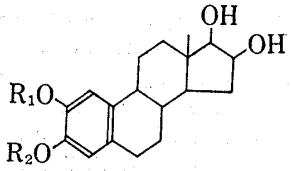
Ia : $R_1=CH_3$, $R_2=C_6H_5CH_2$
Ib : $R_1=C_6H_5CH_2$, $R_2=CH_3$



IIa : $R_1=CH_3$, $R_2=C_6H_5CH_2$
IIb : $R_1=CH_3$, $R_2=H$
IIc : $R_1=C_6H_5CH_2$, $R_2=CH_3$
IId : $R_1=H$, $R_2=CH_3$



IIIa : $R_1=CH_3$, $R_2=H$
IIIb : $R_1=H$, $R_2=CH_3$



IVa : $R_1=CH_3$, $R_2=C_6H_5CH_2$
IVb : $R_1=CH_3$, $R_2=H$
IVc : $R_1=C_6H_5CH_2$, $R_2=CH_3$
IVd : $R_1=H$, $R_2=CH_3$

Chart 1

1) This paper consitutes Part LXXIX of the series entitled "Analytical Chemical Studies on Steroids"; Part LXXVIII: S. Honma and T. Nambara, *Chem. Pharm. Bull.* (Tokyo), **23**, 787 (1975). Following trivial names are used: estrone, 3-hydroxyestra-1,3,5(10)-trien-17-one; estradiol, estra-1,3,5(10)-triene-3,17β-diol; estriol, estra-1,3,5(10)-triene-3,16α,17β-triol.
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) R. Knuppen and H. Breuer, "Advances in Biosciences," Vol. 3, ed. by G. Raspé, Pergamon Press, Vieweg, 1969, pp. 81—92.
5) A. Bartke, R.E. Steele, J.G. Williams, and K.I.H. Williams, *Steroids*, **18**, 303 (1971).
6) T. Nambara, S. Honma, M. Asama, S. Akiyama, and M. Nokubo, *Chem. Pharm. Bull.* (Tokyo), **21**, 914 (1973); S. Honma and T. Nambara, *ibid.*, **22**, 687 (1974); T. Nambara, J. Ishiguro, Y. Kawarada, and H. Tajima, *ibid.*, **22**, 889 (1974).
7) R. Knuppen, O. Haupt, and H. Breuer, *Biochem. J.*, **128**, 1369 (1972).

having the 16β,17β-glycol and 16β-hydroxy-17-ketone structures in the rat. This paper describes the preparation of the isomeric monomethyl ethers of 2,16β-dihydroxy-estrone and -estradiol for obtaining the authentic samples.

An initial effort was directed to the syntheses of the catechol 2-methyl ether derivatives. The Δ^{16}-enol acetate (Ia), obtainable from 2-methoxyestrone 3-benzyl ether,[8] was chosen as a starting compound. Treatment with lead tetraacetate in acetic acid provided the 16β-acetoxy-17-ketone (IIa) in a satisfactory yield. The stereochemistry of the substituent at C-16 was unambiguously assigned to be β, since it is well established that the reaction with lead tetraacetate proceeds sterically opposite to the other reactions at that center.[9] Removal of the benzyl group at C-3 was effected by catalytic hydrogenation over palladium-on-charcoal yielding 2-methoxy-16β-acetoxyestrone (IIb). Subsequent hydrolysis with potassium bicarbonate under the mild conditions formed the desired 2-methoxy-16β-hydroxyestrone (IIIa), which was obviously distinguishable from the isomeric 16,17-ketols.[10]

Alternatively reduction of IIa with sodium borohydride, followed by alkaline hydrolysis furnished the 16β,17β-diol (IVa). Elimination of the protecting group at C-3 by hydrogenolysis afforded the 2-methoxy-16β-hydroxyestradiol (IVb) in a reasonable yield.

The preparation of the isomeric 3-methyl ethers was then undertaken in a similar fashion employing the Δ^{16}-enol acetate (Ib), derivable from 2-benzyloxyestrone 3-methyl ether,[8] as a starting material. Oxidation of Ib with lead tetraacetate formed the 16β-acetoxy-17-ketone (IIc). Debenzylation was readily effected by catalytic hydrogenation to give the catechol 3-monomethyl ether (IId), which on treatment with bicarbonate was led to 2,16β-dihydroxy-estrone 3-methyl ether (IIIb). Reduction of IIc with sodium borohydride and subsequent hydrolysis yielded the 16β,17β-glycol (IVc), which in turn was converted by hydrogenolysis into the desired 2,16β-dihydroxyestradiol 3-methyl ether (IVd).

The authentic specimens thus obtained will be helpful for characterization of the metabolites in the biological material.

Experimental[11]

2-Methoxy-3-benzyloxy-16β-hydroxyestra-1,3,5(10)-trien-17-one Acetate (IIa)——To a solution of 2-methoxy-3-benzyloxyestra-1,3,5(10),16-tetraen-17-ol acetate (Ia) (500 mg) in AcOH (20 ml)–Ac_2O (5 drops) was added $Pb(OAc)_4$ (700 mg) and stirred at room temperature for 4 hr. The resulting solution was diluted with ether, washed with 5% $NaHCO_3$, 10% $Na_2S_2O_3$, and H_2O, successively and dried over anhydrous Na_2SO_4. On usual work-up an oily residue was chromatographed on silica gel (10 g). Elution with benzene and recrystallization of the eluate from MeOH gave IIa (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 (5% solution in $CDCl_3$) 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, -$OC\underline{H}_2C_6H_5$), 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 (IIb)——A solution of IIa (400 mg) in EtOH (150 ml) was shaken with 5% Pd/C (400 mg) under a stream of H_2 gas at room temperature for 3 hr. After removal of the catalyst by filtration the filtrate was evaporated *in vacuo*. Recrystallization from EtOH gave IIb (227 mg) as colorless leaflets. mp 211—213°. $[\alpha]_D^{25.5}$ +171.9° (c=0.10). *Anal.* Calcd. for $C_{21}H_{26}O_5$:

8) T. Nambara, Y. Kawarada, M. Asama, S. Akiyama, M. Nokubo, and S. Honma, *Chem. Pharm. Bull.* (Tokyo), **21**, 2725 (1974).

9) W.S. Johnson, B. Gastambide, and R. Pappo, *J. Am. Chem. Soc.*, **79**, 1991 (1957); T. Nambara and J. Fishman, *J. Org. Chem.*, **27**, 2131 (1962).

10) It is sufficiently substantiated that in the C/D-*trans* steroids the stability sequence of four isomeric ketols is as follows: 17β-OH, 16=O>17α-OH, 16=O>16α-OH, 17=O>16β-OH, 17=O (J. Fishman, *J. Am. Chem. Soc.*, **82**, 6143 (1960)).

11) All melting points were taken on a micro hot-stage apparatus and are uncorrected. Optical rotations were measured in $CHCl_3$ unless otherwise specified. Nuclear magnetic resonance (NMR) spectra were recorded on a Hitachi Model R-20A spectrometer at 60 MHz using tetramethylsilane as an internal standard. Abbreviation used s=singlet, d=doublet, and m=multiplet. For preparative thin-layer chromatography (TLC) silica gel HF_{254} (E. Merck AG, Darmstadt) was used as an adsorbent.

C, 70.37; H, 7.31. Found: C, 70.10; H, 7.60. NMR (5% solution in $CDCl_3$) 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).

2-Methoxy-3,16β-dihydroxyestra-1,3,5(10)-trien-17-one (IIIa)——To a solution of IIb (50 mg) in acetone (5 ml)–MeOH (5 ml) was added $KHCO_3$ (50 mg) dissolved in MeOH (10 ml)–H_2O (2 ml) and stirred at room temperature for 24 hr. The resulting solution was neutralized with 5% HCl and extracted with ether. The organic phase was washed with 5% $NaHCO_3$ and H_2O and dried over anhydrous Na_2SO_4. On usual work-up an oily residue was submitted to the preparative TLC employing $CHCl_3$–EtOH (95: 5) as developing solvent. Elution of the adsorbent corresponding to the spot (*Rf* 0.40) with AcOEt and recrystallization of the eluate from MeOH gave IIIa (17 mg) as colorless plates. mp 185—188°. $[\alpha]_D^{26.5}$ +185.4° (c=0.09). *Anal.* Calcd. for $C_{19}H_{24}O_4$: C, 72.12; H, 7.65. Found: C, 72.04; H, 7.77. NMR (5% solution in $CDCl_3$) δ: 1.00 (3H, s, 18-CH_3), 3.85 (3H, s, 2-OCH_3), 4.05 (1H, m, 16α-H), 6.66 (1H, s, 4-H), 6.79 (1H, s, 1-H).

2-Methoxy-3-benzyloxyestra-1,3,5(10)-triene-16β,17β-diol (IVa)——To a solution of IIa (100 mg) in MeOH (40 ml) was added $NaBH_4$ (25 mg) dissolved in MeOH (0.5 ml)–H_2O (0.2 ml) and stirred at room temperature for 1 hr. To the resulting solution was added 10% K_2CO_3 (5 ml) and refluxed for 30 min. After removal of MeOH by evaporation the residue was extracted with AcOEt. The organic phase was washed with 5% HCl, 5% $NaHCO_3$, and H_2O, successively and dried over anhydrous Na_2SO_4. On usual work-up the crude product was recrystallized from acetone–hexane to give IVa (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 (5% solution in $CDCl_3$) 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, -$OCH_2C_6H_5$), 6.62 (1H, s, 4-H), 6.85 (1H, s, 1-H).

2-Methoxyestra-1,3,5(10)-triene-3,16β,17β-triol (IVb)——A solution of IVa (150 mg) in EtOH (100 ml) was shaken with 5% Pd/C (150 mg) under a stream of H_2 gas at room temperature for 6 hr. After removal of the catalyst by filtration the filtrate was evaporated *in vacuo*. Recrystallization from MeOH gave IVb (28 mg) as colorless plates. mp 239—241°. $[\alpha]_D^{26}$ +121.4° (c=0.12 in MeOH). *Anal.* Calcd. for $C_{19}H_{26}O_4$: C, 71.67; H, 8.23. Found: C, 71.61; H, 8.45. NMR (1.25% solution in $CDCl_3$) δ: 0.84 (3H, s, 18-CH_3), 3.49 (1H, d, J=7 Hz, 17α-H), 3.81 (3H, s, 2-OCH_3), 4.18 (1H, m, 16α-H), 6.55 (1H, s, 4-H), 6.69 (1H, s, 1-H).

2-Benzyloxy-3-methoxy-16β-hydroxyestra-1,3,5(10)-trien-17-one Acetate (IIc)——To a solution of 2-benzyloxy-3-methoxyestra-1,3,5(10),16-tetraen-17-ol acetate (Ib) (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 diluted with ether, washed with 5% $NaHCO_3$, 10% $Na_2S_2O_3$, and H_2O, successively and dried over anhydrous Na_2SO_4. On usual work-up an oily residue was chromatographed on silica gel (40 g). Elution with benzene and recrystallization of the eluate from MeOH gave IIc (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 (5% solution in $CDCl_3$) 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, -$OCH_2C_6H_5$), 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 (IId)——A solution of IIc (500 mg) in EtOH (190 ml) was shaken with 5% Pd/C (500 mg) under a stream of H_2 gas at room temperature for 3 hr. After removal of the catalyst by filtration the filtrate was evaporated *in vacuo*. Recrystallization from MeOH gave IId (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 (5% solution in $CDCl_3$) 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).

2,16β-Dihydroxy-3-methoxyestra-1,3,5(10)-trien-17-one (IIIb)——To a solution of IId (100 mg) in acetone (10 ml)–MeOH (10 ml) was added $KHCO_3$ (50 mg) dissolved in MeOH (10 ml)–H_2O (2 ml) and stirred at room temperature for 24 hr. The resulting solution was neutralized with 5% HCl and extracted with ether. The organic phase was washed with 5% $NaHCO_3$ and H_2O and dried over anhydrous Na_2SO_4. On usual work-up an oily residue was submitted to the preparative TLC employing $CHCl_3$–EtOH (95: 5) as developing solvent. Elution of the adsorbent corresponding to the spot (*Rf* 0.37) with AcOEt and recrystallization of the eluate from acetone–hexane gave IIIb (35 mg) as colorless plates. mp 228—230°. $[\alpha]_D^{25}$ +96.3° (c=0.13). *Anal.* Calcd. for $C_{19}H_{24}O_4 \cdot 1/4H_2O$: C, 71.11; H, 7.70. Found: C, 71.42; H, 7.88. NMR (1.25% solution in $CDCl_3$) δ: 1.01 (3H, s, 18-CH_3), 3.85 (3H, s, 3-OCH_3), 4.00 (1H, m, 16α-H), 6.53 (1H, s, 4-H), 6.80 (1H, s, 1-H).

2-Benzyloxy-3-methoxyestra-1,3,5(10)-triene-16β,17β-diol (IVc)——To a solution of IIc (200 mg) in MeOH (80 ml) was added $NaBH_4$ (50 mg) dissolved in MeOH (1 ml)–H_2O (0.4 ml) and stirred at room temperature for 1 hr. To the resulting solution was added 10% K_2CO_3 (10 ml) and refluxed for 30 min. After removal of MeOH by evaporation the residue was extracted with AcOEt. The organic phase was washed with 5% HCl, 5% $NaHCO_3$, and H_2O, successively and dried over anhydrous Na_2SO_4. On usual work-up the crude product was recrystallized from acetone-hexane to give IVc (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 (5% solution in $CDCl_3$) 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, -$OCH_2C_6H_5$), 6.57 (1H, s, 4-H), 6.81 (1H, s, 1-H).

3-Methoxyestra-1,3,5(10)-triene-2,16β,17β-triol (IVd)——A solution of IVc (80 mg) in EtOH (50 ml) was shaken with 5% Pd/C (80 mg) under a stream of H_2 gas at room temperature for 24 hr. After removal of the catalyst by filtration the filtrate was evaporated *in vacuo*. Recrystallization from acetone-hexane

gave IVd (52 mg) as colorless needles. mp 204—205°. $[\alpha]_D^{26.5}$ +90.1° (c=0.16 in MeOH). *Anal.* Calcd. for $C_{19}H_{26}O_4$: C, 71.67; H, 8.23. Found: C, 71.75; H, 8.53. NMR (1% solution in $CDCl_3$) δ: 0.85 (3H, s, 18-CH_3), 3.49 (1H, d, J=7 Hz, 17α-H), 3.82 (3H, s, 3-OCH_3), 4.18 (1H, m, 16α-H), 6.52 (1H, s, 4-H), 6.81 (1H, s, 1-H).

Acknowledgement The authors are indebted to all the staffs of central analytical laboratory of this Institute for elemental analyses and spectral measurements. This work was supported in part by a Grant-in-Aid from the Naito Foundation, which is gratefully acknowledged.

[Chem. Pharm. Bull. 23(7)1616—1619(1975)]

UDC 547.92.057 : 615.356.011.5

Synthesis of 2β-Hydroxycholecalciferol [2β-OH-D_3]

CHIKARA KANEKO, SACHIKO YAMADA, AKIKO SUGIMOTO and MASAYUKI ISHIKAWA

Division of Chemistry, Research Institute for Medical and Dental Engineering, Tokyo Medical and Dental University[1]

(Received December 11, 1974)

As part of a general exploration of the structure/activity relationships of the vitamin D system, the 2β-hydroxylated analogue of cholecalciferol (vitamin D_3) has been prepared from 2β-hydroxy-7-dehydrocholesterol obtained in our previous work as starting material *via* (i) photochemical conrotatory opening (2 N_f pericyclic reaction) of the B-ring and (ii) thermal 1,7-antarafacial hydrogen shift (3 N_f pericyclic reaction).

Importance of 1α-hydroxy function of cholecalciferol (vitamin D_3) to induce either intestinal calcium transport or bone calcium mobilization activity has been demonstrated by the studies on 1α,25-dihydroxycholecalciferol [1α,25-$(OH)_2$-D_3][2-4] and 1α-hydroxycholecalciferol [1α-OH-D_3].[5-8] The increased clinical significance[9-16] of these two hydroxylated derivatives of vitamin D has led recently to synthesis and biological testing of various derivatives, hy-

1) Location: *2-3-10, Kanda, Surugadai, Chiyoda-ku, Tokyo, 101, Japan.*
2) M.F. Holick, H.K. Schnoes, H.F. DeLuca, T. Suda and R.J. Cousins, *Biochemistry*, **10**, 2799 (1971).
3) D.E.M. Lawson, D.R. Fraser, E. Kodicek, H.R. Morris and D.H. Williams, *Nature*, **230**, 228 (1971).
4) A.W. Norman, J.F. Midgett, H.G. Nowicki, V. Williams and G. Popjak, *Science*, **173**, 51 (1971).
5) M.F. Holick, E.J. Semmler, H.K. Schnoes, and H.F. DeLuca, *Science*, **180**, 190 (1973).
6) D.H.R. Barton, R.H. Hesse, M.M. Pechet, and E. Rizzardo, *J. Am. Chem. Soc.*, **95**, 2748 (1973).
7) C. Kaneko, S. Yamada, A. Sugimoto, Y. Eguchi, M. Ishikawa, T. Suda, M. Suzuki, S. Kakuta, and S. Sasaki, *Steroids*, **23**, 75 (1974).
8) The analogues of vitamin D having a hydroxy group in a *pseudo* 1α-position also show a similar biological activity though in far less efficiency: 5,6-*trans*-D_3; M.F. Holick, M. Garabedian and H.F. DeLuca, *Biochemistry*, **14**, 2715 (1972), dihydrotachysterol; *b*) S. Sagar, R.L. Estrada, and M. Kaye, *Arch. Intern. Med.*, **130**, 768 (1972).
9) A.S. Brickman, J.W. Coburn, and A.W. Norman, *New. Eng. J. Med.*, **287**, 891 (1972).
10) J.M. Omdahl and H.F. DeLuca, *Physiological Reviews*, **53**, 352 (1973).
11) Y. Tanaka, H. Frank, and H.F. DeLuca, *Endoclinology*, **92**, 417 (1973).
12) Y. Tanaka, *Farumashia*, **10**, 319 (1974).
13) T.M. Chalmers, M.W. Davie, J.O. Hunter, K.F. Szaz, B. Pelc, and E. Kodicek, *The Lancet*, **1973**, 696.
14) M. Peacock, J.C. Gallagler, and B.E. Nordin, *The Lancet*, **1974**, 385.
15) H. Wako, S. Taniguchi, Z. Suzuki, Y. Hirooka, M. Hirota, T. Sasaki, C. Kaneko, M. Ishikawa, S. Sasaki, T. Suda, and M. Abe, The 8th Kotsu Taisha Kenkyukai, abstract of papers, p. 16, Tokyo (1974).
16) Y. Hirooka, T. Sasaki, M. Hirota, C. Kaneko, M. Ishikawa, S. Sasaki, T. Suda, and H. Wako, The 8th Kotsu Taisha Kenkyukai, abstract of papers, Tokyo 1974, p. 17.