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New Synthesis of 2-Hydroxyestrogen 2-Monoglucuronides[1)]

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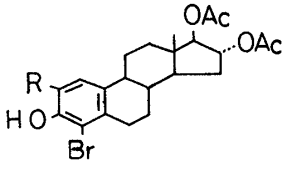
New synthetic routes leading to catechol estrogen 2-monoglucuronides are described. Selective introduction of a glucuronyl residue into the C-2 hydroxyl group was undertaken by utilizing a steric interaction of the 3-hydroxyl group with a bulky substituent at C-4. For this purpose, 4-bromo-2-hydroxyestriol 16,17-diacetate was used as a key intermediate. The Koenigs–Knorr reaction of this catechol with methyl α-acetobromoglucuronate in the presence of cadmium carbonate proceeded preferentially toward the C-2 hydroxyl group. Subsequent reductive dehalogenation followed by alkaline hydrolysis provided the desired 2-hydroxyestriol 2-glucuronide. In a similar fashion, 2-hydroxyestradiol and 2-hydroxyestrone 2-glucuronides were also prepared.

Keywords——catechol estrogen; active metabolite; 2-hydroxyestriol 2-glucuronide; 4-bromo-2-hydroxyestrogen; Koenigs–Knorr reaction; selective glucuronidation

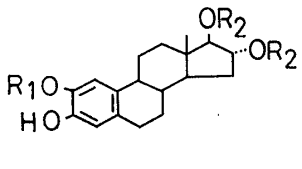
In recent years, considerable attention has been focused on the physiological significance of the catechol formation of estrogens in living animals.[2,3)] The metabolism, in particular glucuronidation, of catechol estrogens in humans is an attractive subject for investigation.[4)] The synthesis of 2-hydroxyestrogen ring A monoglucuronides has previously been developed by several groups.[5,6)] Although Röhle and Breuer[5)] reported the selective glucuronidation of the catechol at C-2, it is sufficiently substantiated that the chemical properties of the two phenolic groups are virtually indistinguishable. In actuality, direct Koenigs–Knorr reaction of the catechol provides a mixture of two isomeric monoglucuronides whose separation is somewhat tedious. Other methods are also unsatisfactory with respect to simplicity and versatility for introducing a glucuronyl moiety at the C-2 hydroxyl group. The present paper deals with the selective synthesis of 2-monoglucuronides of 2-hydroxylated estriol, estradiol and estrone.

An initial project was directed to the preparation of 2-hydroxyestriol 2-glucuronide by selective glucuronidation of the hydroxyl function at C-2. For this purpose, the use of 4-bromo-2-hydroxyestriol 16,17-diacetate as a key intermediate was examined. It was supposed that the Koenigs–Knorr reaction would proceed preferentially toward C-2 due to the steric interaction of the 3-hydroxyl group with the bulky bromine atom at C-4. Treatment of estriol 16,17-diacetate[7)] with a bromine solution in glacial acetic acid provided the 2,4-dibromo compound (**1**). Subsequent reaction with nitrous acid in acetic acid did take place regioselectively at C-2[8)] to afford solely the 4-bromo-2-nitro derivative (**2**) in a reasonable yield. The structural assignment was justified on the basis of elemental analysis and proton nuclear magnetic resonance (^{1}H-NMR) spectral data. Compound **2** was then reduced to the corresponding amine (**3**) by catalytic hydrogenation over palladium-on-charcoal without disturbing the C-4 substituent. Periodate oxidation of **3** in the usual manner furnished the desired 4-bromo-2-hydroxyestriol 16,17-diacetate (**4**).

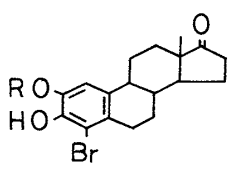
Introduction of a glucuronyl residue into the 2,3-catechol was carried out by means of the Koenigs–Knorr reaction using freshly prepared cadmium carbonate as a catalyst. As was expected, condensation of **4** with methyl 2,3,4-tri-*O*-acetyl-1-bromo-1-deoxy-α-D-gluco-



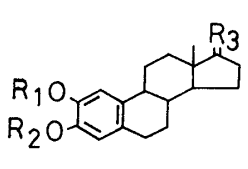
1 : R=Br
2 : R=NO_2
3 : R=NH_2
4 : R=OH
5 : R=OG′



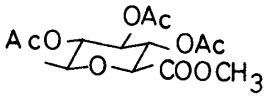
6 : R_1=G′, R_2=Ac
7 : R_1=G, R_2=H



8 : R=H
9 : R=G′



10 : R_1=G′, R_2=H, R_3=O
11 : R_1=G′, R_2=H, R_3= 
12 : R_1=G′, R_2=Ac, R_3=
13 : R_1=G, R_2=H, R_3=O
14 : R_1=G, R_2=H, R_3= 

G′ : 

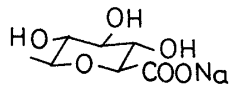
G : 

Chart 1

pyranuronate (methyl α-acetobromoglucuronate) in anhydrous toluene proceeded preferentially toward C-2 to afford methyl (4-bromo-16α,17β-diacetoxy-3-hydroxy-1,3,5(10)-estratrien-2-yl 2,3,4-tri-*O*-acetyl-β-D-glucopyranosid)uronate (**5**) together with a trace amount of its positional isomer. Recrystallization of the crude product provided **5** in the pure state. Reductive dehalogenation was effected, yielding 2-hydroxyestriol 16,17-diacetate 2-glucuronide acetate-methyl ester (**6**), when **5** was subjected to catalytic hydrogenation over palladium-on-charcoal. Removal of the protecting groups in ring D and the sugar moiety by alkaline hydrolysis under mild conditions provided the desired 2-hydroxyestriol 2-glucuronide (**7**) in a satisfactory yield.

Selective introduction of a glucuronyl moiety into the catechol was similarly achieved for 4-bromo-2-hydroxyestrone (**8**). The Koenigs–Knorr reaction with methyl α-acetobromoglucuronate in the presence of cadmium carbonate took place exclusively at C-2 to furnish the 2-glucuronide acetate-methyl ester (**9**). Elimination of bromine at C-4 was readily achieved by hydrogenolysis using palladium-on-charcoal as a catalyst to provide 2-hydroxyestrone 2-glucuronide acetate-methyl ester (**10**) in a reasonable yield. When palladium chloride and sodium borohydride were employed alternatively, simultaneous reduction occurred at both the C-17 and C-4 positions, yielding 2-hydroxyestradiol 2-glucuronide acetate-methyl ester (**11**). On usual acetylation with acetic anhydride and pyridine, **11** was converted to the 3,17-diacetate (**12**). Subsequent elimination of the protecting groups in **10** and **11** with methanolic sodium hydroxide provided the desired 2-hydroxyestrone 2-glucuronide (**13**) and 2-hydroxyestradiol 2-glucuronide (**14**), respectively.

Inspection of the ^{1}H-NMR spectrum of estriol 2-glucuronide acetate-methyl ester revealed the formation of a β-glucuronide linkage. The anomeric proton signal of the glucuronyl moiety appeared at 4.94 ppm as a doublet (J=8 Hz). Further evidence for the structural assignment of the 2-monoglucuronides was obtained by characterizing the 2-hydroxyestrogen 3-methyl ethers liberated on incubation with a β-glucuronidase preparation on the basis of usual criteria.[6,9]

The facile availability of these authentic specimens should assist the determination of catechol estrogens in biological fluids in connection with their metabolism and physiological significance.

Experimental

Melting points were taken on a Yanagimoto hot stage apparatus and are uncorrected. Optical rotations were measured with a JASCO DIP-4 automatic polarimeter. ^{1}H-NMR spectra were recorded on a JEOL FX-100 spectrometer at 100 MHz using tetramethylsilane as an internal standard. Abbreviations used are s=singlet,

d=doublet, m=multiplet and br=broad. Mass spectra (MS) were obtained on Hitachi M-52 and JEOL JMS-01SG-2 spectrometers. For column chromatography and preparative thin layer chromatography (TLC), Silica gel 60 and Silica gel HF_{254} (E. Merck AG, Darmstadt) were used, respectively.

2,4-Dibromo-1,3,5(10)-estratriene-3,16α,17β-triol 16,17-Diacetate (1)——A 5% Br_2 solution in glacial AcOH was added dropwise to a stirred solution of estriol 16,17-diacetate (1.29 g) in glacial AcOH (200 ml) at room temperature. The bromine solution was added until the reaction mixture was no longer decolorized. After stirring for an additional 1 h, the reaction mixture was poured into ice-water. The precipitate was collected by filtration, washed with water and recrystallized from EtOH to give **1** (1.1 g) as colorless plates, mp 185—188 °C. $[\alpha]_D^{20}$ −52.5° (c=0.1, $CHCl_3$). *Anal.* Calcd $C_{22}H_{26}Br_2O_5$: C, 49.83; H, 4.94; Br, 30.13. Found: C, 50.04; H, 5.05; Br, 30.36. ^{1}H-NMR ($CDCl_3$) δ: 0.83 (3H, s, 18-CH_3), 2.04 (3H, s, $OCOCH_3$), 2.08 (3H, s, $OCOCH_3$), 4.95 (1H, d, J=6 Hz, 17α-H), 5.15 (1H, m, 16β-H), 5.81 (1H, s, 3-OH), 7.33 (1H, s, 1-H).

4-Bromo-2-nitro-1,3,5(10)-estratriene-3,16α,17β-triol 16,17-Diacetate (2)——A solution of **1** (2 g) in AcOH (150 ml) was treated with 10% $NaNO_2$ (10 ml), and the whole was stirred at room temperature for 30 min. The reaction mixture was poured into water, and the resulting precipitate was collected by filtration and washed with water. The crude product was recrystallized from acetone to give **2** (1.62 g) as pale yellow plates, mp 208—211 °C. $[\alpha]_D^{20}$ −35.6° (c=0.1, $CHCl_3$). *Anal.* Calcd for $C_{22}H_{26}BrNO_7$: C, 53.23; H, 5.27; N, 2.82; Br, 16.10. Found: C, 53.19; H, 5.18; N, 2.59; Br, 16.23. ^{1}H-NMR ($CDCl_3$) δ: 0.84 (3H, s, 18-CH_3), 2.05 (3H, s, $OCOCH_3$), 2.09 (3H, s, $OCOCH_3$), 4.95 (1H, d, J=6 Hz, 17α-H), 5.17 (1H, m, 16β-H), 7.97 (1H, s, 1-H).

2-Amino-4-bromo-1,3,5(10)-estratriene-3,16α,17β-triol 16,17-Diacetate (3)——A solution of **2** (876 mg) in EtOH–$CHCl_3$ (5:1) (100 ml) was shaken with 5% Pd/C (900 mg) under H_2 gas at room temperature and atmospheric pressure for 30 min. After removal of the catalyst by filtration, the filtrate was evaporated down under reduced pressure. The crude product was chromatographed on silica gel with hexane–AcOEt (4:1). The dried eluate was recrystallized from ether–hexane to give **3** (781 mg) as colorless plates, mp 120—125 °C. $[\alpha]_D^{20}$ −93.7° (c=0.03, $CHCl_3$). *Anal.* Calcd for $C_{22}H_{28}BrNO_5$: C, 56.65; H, 6.05; N, 3.00; Br, 17.13. Found: C, 57.03; H, 6.07; N, 2.88; Br, 17.01. High MS m/z: 465.1198 $(M)^+$ (Calcd for $C_{22}H_{28}BrNO_5$: 465.1151). ^{1}H-NMR ($CDCl_3$) δ: 0.82 (3H, s, 18-CH_3), 2.04 (3H, s, $OCOCH_3$), 2.07 (3H, s, $OCOCH_3$), 4.94 (1H, d, J=6 Hz, 17α-H), 5.16 (1H, m, 16β-H), 6.61 (1H, s, 1-H).

4-Bromo-1,3,5(10)-estratriene-2,3,16α,17β-tetraol 16,17-Diacetate (4)——A solution of **3** (500 mg) in AcOH (150 ml) was added to a vigorously stirred solution of sodium metaperiodate (5 g) in 0.1 N HCl (350 ml) at room temperature, and the whole was stirred for 5 min. The reaction mixture was extracted with $CHCl_3$. The organic layer was washed with water and, after addition of AcOH (10 ml) and KI (1.5 g), was stirred for 15 min. The iodine formed was reduced with 5% $NaHSO_3$ (100 ml). The organic layer was washed with water, fortified with AcOH (2 ml) and dried over anhydrous Na_2SO_4. After evaporation of the solvent, the residue was chromatographed on silica gel with hexane–AcOEt (10:3). Recrystallization of the eluate from acetone–ether gave **4** (240 mg) as colorless plates, mp 223—225 °C (dec.). $[\alpha]_D^{20}$ −15.5° (c=0.1, $CHCl_3$). *Anal.* Calcd for $C_{22}H_{27}BrO_6$: C, 56.54; H, 5.82; Br, 17.10. Found: C, 56.84; H, 5.76; Br, 17.56. High MS m/z: 466.1000 $(M)^+$ (Calcd for $C_{22}H_{27}BrO_6$: 466.0993). ^{1}H-NMR ($CDCl_3$) δ: 0.82 (3H, s, 18-CH_3), 2.04 (3H, s, $OCOCH_3$), 2.08 (3H, s, $OCOCH_3$), 4.95 (1H, d, J=6 Hz, 17α-H), 5.15 (1H, m, 16β-H), 5.47 (2H, br s, 2- and 3-OH), 6.82 (1H, s, 1-H).

Methyl (4-Bromo-16α,17β-diacetoxy-3-hydroxy-1,3,5(10)-estratrien-2-yl 2,3,4-tri-*O*-acetyl-β-D-glucopyranosid)uronate (5)——Freshly prepared $CdCO_3$ (200 mg) and methyl α-acetobromoglucuronate (200 mg) were added to a solution of **4** (100 mg) in dry toluene (30 ml). The whole was concentrated to *ca.* 25 ml by distillation to remove the moisture and then refluxed for 24 h, additional methyl α-acetobromoglucuronate (50 mg) and $CdCO_3$ (50 mg) being added to the mixture after a period of 12 h. The precipitate was filtered off and washed with toluene and AcOEt. The filtrate and washings were combined and evaporated down under reduced pressure. The crude product was chromatographed on silica gel with hexane–AcOEt (10:3). Recrystallization of the eluate from MeOH gave **5** (103 mg) as colorless needles, mp 165—168 °C. $[\alpha]_D^{20}$ −74.0° (c=0.06, $CHCl_3$). *Anal.* Calcd for $C_{35}H_{43}BrO_{15}\cdot 3/2H_2O$: C, 51.85; H, 5.71; Br, 9.85. Found: C, 51.86; H, 5.22; Br, 9.62. ^{1}H-NMR ($CDCl_3$) δ: 0.84 (3H, s, 18-CH_3), 2.02 (3H, s, $OCOCH_3$), 2.04 (6H, s, $OCOCH_3$), 2.07 (3H, s, $OCOCH_3$), 2.09 (3H, s, $OCOCH_3$), 3.74 (3H, s, $COOCH_3$), 4.11 (1H, m, 5′-H), 4.96 (2H, m, 1′-H, 17α-H), 5.10—5.40 (4H, m, 16β-H, 2′-, 3′-, 4′-H), 6.93 (1H, s, 1-H).

Methyl (16α,17β-Diacetoxy-3-hydroxy-1,3,5(10)-estratrien-2-yl 2,3,4-tri-*O*-acetyl-β-D-glucopyranosid)uronate (6)——A solution of **5** (60 mg) in EtOH (20 ml) was shaken with 5% Pd/C (60 mg) under H_2 gas at room temperature and atmospheric pressure for 30 min. After removal of the catalyst by filtration, the filtrate was evaporated down under reduced pressure. The residue was purified by preparative TLC using hexane–AcOEt (1:1) as the developing solvent. Recrystallization of the crude product from ether–hexane gave **6** (43 mg) as colorless needles, mp 115—120 °C. $[\alpha]_D^{20}$ −46.1° (c=0.01, $CHCl_3$). *Anal.* Calcd for $C_{35}H_{44}O_{15}\cdot H_2O$: C, 58.17; H, 6.41. Found: C, 58.56; H, 6.13. ^{1}H-NMR ($CDCl_3$) δ: 0.85 (3H, s, 18-CH_3), 2.05 (3H, s, $OCOCH_3$), 2.07 (6H, s, $OCOCH_3$), 2.11 (3H, s, $OCOCH_3$), 2.13 (3H, s, $OCOCH_3$), 3.76 (3H, s, $COOCH_3$), 4.12 (1H, m, 5′-H), 4.94 (1H, d, J=8 Hz, 1′-H), 4.99 (1H, d, J=6 Hz, 17α-H), 5.19 (1H, m, 16β-H), 5.25 (1H, m, 3′-H), 5.32 (2H, m, 2′-, 4′-H), 6.67 (1H, s, 4-H), 6.87 (1H, s, 1-H).

Sodium (3,16α,17β-Trihydroxy-1,3,5(10)-estratrien-2-yl β-D-glucopyranosid)uronate (7)——A solution of **6** (10 mg) in MeOH (10 ml) was treated with 2 N NaOH (0.3 ml), and the whole was set aside at room temperature for 12 h. The resulting solution was then concentrated under reduced pressure below 50 °C to *ca.* 2 ml and poured into

ice-water. The solution was then passed through an Amberlite XAD-2 column (20 cm × 1 cm i.d.). After thorough washing with water, the desired glucuronide was eluted with MeOH. Recrystallization of the dried eluate from MeOH gave **7** (3.2 mg) as colorless needles, mp >300 °C. *Anal.* Calcd for $C_{24}H_{31}NaO_{10}\cdot 2H_2O$: C, 53.53; H, 6.55. Found: C, 53.03; H, 6.13. MS (FAB) *m/z*: 501 $(M)^+$.

4-Bromo-2,3-dihydroxy-1,3,5(10)-estratrien-17-one (8)——Treatment of 2-amino-4-bromoestrone[8] (270 mg) with sodium metaperiodate followed by chromatographic purification was carried out in the manner described for **4**. Recrystallization of the eluate from benzene gave **8** (175 mg) as colorless needles, mp 200—204 °C (dec.). $[\alpha]_D^{20}$ −62.8° (c=0.05, $CHCl_3$). *Anal.* Calcd for $C_{18}H_{21}BrO_3$: C, 59.18; H, 5.79; Br, 21.87. Found: C, 59.10; H, 5.53; Br, 21.65. ^{1}H-NMR ($CDCl_3$) δ: 0.90 (3H, s, 18-CH_3), 5.32 (1H, s, 2- or 3-OH), 5.46 (1H, s, 3- or 2-OH), 6.86 (1H, s, 1-H). MS *m/z*: 364 $(M)^+$.

Methyl (4-Bromo-3-hydroxy-17-oxo-1,3,5(10)-estratrien-2-yl 2,3,4-tri-*O*-acetyl-β-D-glucopyranosid)uronate (9)——Treatment of **8** (100 mg) with methyl α-acetobromoglucuronate and $CdCO_3$ followed by chromatographic purification was carried out in the manner described for **5**. Recrystallization of the eluate from MeOH gave **9** (79 mg) as colorless needles, mp 233—235 °C (dec.). $[\alpha]_D^{20}$ −38.9° (c=0.06, $CHCl_3$). *Anal.* Calcd for $C_{31}H_{37}BrO_{12}$: C, 54.63; H, 5.47; Br, 11.72. Found: C, 54.44; H, 5.15; Br, 11.59. ^{1}H-NMR ($CDCl_3$) δ: 0.91 (3H, s, 18-CH_3), 2.03 (3H, s, $OCOCH_3$), 2.05 (3H, s, $OCOCH_3$), 2.11 (3H, s, $OCOCH_3$), 3.76 (3H, s, $COOCH_3$), 4.14 (1H, m, 5′-H), 4.96 (1H, m, 1′-H), 5.28 (3H, m, 2′-, 3′-, 4′-H), 6.97 (1H, s, 1-H).

Methyl (3-Hydroxy-17-oxo-1,3,5(10)-estratrien-2-yl 2,3,4-tri-*O*-acetyl-β-D-glucopyranosid)uronate (10)——Catalytic hydrogenation of **9** (50 mg) over Pd/C followed by TLC purification was carried out in the manner described for **6**. Recrystallization of the crude product from MeOH gave **10** (38 mg) as colorless needles, mp 205—210 °C (lit. mp 210—213 °C).[10] ^{1}H-NMR ($CDCl_3$) δ: 0.90 (3H, s, 18-CH_3), 2.05 (6H, s, $OCOCH_3$), 2.12 (3H, s, $OCOCH_3$), 3.76 (3H, s, $OCOCH_3$), 4.12 (1H, m, 5′-H), 4.98 (1H, m, 1′-H), 5.34 (3H, m, 2′-, 3′-, 4′-H), 6.70 (1H, s, 4-H), 6.92 (1H, s, 1-H).

Methyl (3,17β-Dihydroxy-1,3,5(10)-estratrien-2-yl 2,3,4-tri-*O*-acetyl-β-D-glucopyranosid)uronate (11)——$PdCl_2$ (70 mg) and $NaBH_4$ (70 mg) were added to a solution of **9** (71 mg) in MeOH (40 ml), and the whole was stirred under N_2 gas at 0 °C for 30 min. The reaction mixture was filtered and the filtrate was concentrated to *ca.* 10 ml and then poured into 4% AcOH. The precipitate was extracted with AcOEt, and the extract was washed with water and dried over anhydrous Na_2SO_4. After evaporation of the solvent, the crude product was purified by preparative TLC using hexane–AcOEt (1 : 1) as the developing solvent. Elution of the adsorbent corresponding to the spot with AcOEt and recrystallization of the product from ether gave **11** (25 mg) as colorless needles, mp 227—232 °C. $[\alpha]_D^{20}$ +45.8° (c=0.03, $CHCl_3$). *Anal.* Calcd for $C_{31}H_{40}O_{12}\cdot 1/2H_2O$: C, 60.67; H, 6.73. Found: C, 60.30; H, 6.38. ^{1}H-NMR ($CDCl_3$) δ: 0.78 (3H, s, 18-CH_3), 2.04 (6H, s, $OCOCH_3$), 2.13 (3H, s, $OCOCH_3$), 3.64 (1H, br s, 17α-H), 3.75 (3H, s, $COOCH_3$), 4.11 (1H, m, 5′-H), 4.93 (1H, m, 1′-H), 5.28 (3H, m, 2′-, 3′-, 4′-H), 6.64 (1H, s, 4-H), 6.87 (1H, s, 1-H).

Methyl (3,17β-Diacetoxy-1,3,5(10)-estratrien-2-yl 2,3,4-tri-*O*-acetyl-β-D-glucopyranosid)uronate (12)——Treatment of **11** with pyridine and Ac_2O in the usual manner followed by preparative TLC gave **12** as a colorless amorphous substance. ^{1}H-NMR ($CDCl_3$) δ: 0.82 (3-H, s, 18-CH_3), 2.03 (6H, s, $OCOCH_3$), 2.06 (6H, s, $OCOCH_3$), 2.24 (3H, s, 3-$OCOCH_3$), 3.74 (3H, s, $COOCH_3$), 4.17 (1H, m, 5′-H), 4.67 (1H, m, 17α-H), 5.06 (1H, m, 1′-H), 5.28 (3H, m, 2′-, 3′-, 4′-H), 6.73 (1H, s, 4-H), 7.01 (1H, s, 1-H).

Sodium (3-Hydroxy-17-oxo-1,3,5(10)-estratrien-2-yl β-D-glucopyranosid)uronate (13)——Compound **10** (10 mg) was treated with methanolic NaOH in the manner described for **7**. Purification of the crude product by Amberlite XAD-2 chromatography gave **13** (4.5 mg). The eluate proved to be identical with an authentic sample.[10]

Sodium (3,17β-Dihydroxy-1,3,5(10)-estratrien-2-yl β-D-glucopyranosid)uronate (14)——Compound **11** (10 mg) was treated with methanolic NaOH in the manner described for **7**. Purification of the crude product by chromatography on Amberlite XAD-2 resin followed by recrystallization from MeOH gave **14** (4.5 mg) as colorless plates, mp >300 °C. *Anal.* Calcd for $C_{24}H_{31}NaO_9\cdot 3/2H_2O$: C, 56.13; H, 6.67. Found: C, 55.82; H, 6.71. MS (FAB) *m/z*: 485 $(M)^+$.

Elucidation of the Conjugated Position in 7, 13 and 14——A solution of **7**, **13**, or **14** (5 mg) in MeOH–ether (2 : 5) (10 ml) was treated with ethereal diazomethane at room temperature for 12 h. The resulting solution was evaporated down under reduced pressure. The residue was dissolved in 0.1 M acetate buffer (pH 4.0, 20 ml) and incubated with limpet (*Patella vulgata*) β-glucuronidase (Sigma Chemical Co.) (5000 units) at 37 °C for 24 h. The incubated mixture was extracted with AcOEt. The organic layer was washed with water, dried over anhydrous Na_2SO_4 and evaporated down under reduced pressure. The residue was purified by preparative TLC using hexane–AcOEt (2 : 1) as the developing solvent. Elution of the adsorbent corresponding to the spot (*Rf* 0.3—0.5) with AcOEt gave 2-hydroxyestrogen 3-methyl ether. The products obtained from **7**, **13** and **14** proved to be identical with the corresponding authentic samples.

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References and Notes

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