[Chem. Pharm. Bull. 36( 8 )2993—2999(1988)]

# Synthesis of C-2 Catecholic Equilin and Equilenin Derivatives for Use in Metabolic Studies

SHIGEO IKEGAWA, TAKAO KUROSAWA, and MASAHIKO TOHMA*

*Faculty of Pharmaceutical Sciences, Higashi-Nippon-Gakuen University, Ishikari-Tobetsu, Hokkaido 061–02, Japan*

(Received January 14, 1988)

In order to clarify the metabolic fate of equine estrogens, 2-hydroxyequilin, 2-hydroxyequilenin and their isomeric monomethyl ethers were synthesized as authentic specimens. Vanillin and isovanillin were employed as starting materials leading to the desired β-ketosulfoxides (**2a**, **b**). Condensation of the α,β-unsaturated ketones (**4a**, **b**) obtained by thermal elimination of **3a** and **3b** with 2-methylcyclopentane-1,3-dione provided the triketones (**5a**, **b**), which were cyclized to the estrapentaens (**6a**, **b**). Several oxido-reduction reactions were then performed to give the title compounds (**8**, **13**).

**Keywords**——equine estrogen metabolite; equilin; equilenin; 2-hydroxyequilin; 2-hydroxyequilenin; 2-methoxyequilin; 2-methoxyequilenin; 2-hydroxyequilin 3-methyl ether; 2-hydroxyequilenin 3-methyl ether

Following the isolation of the unsaturated estrogens in ring B (equilin and equilenin) from urine of the pregnant mare by Girard *et al.* in 1932,[1] these equine estrogens have been frequently prescribed for replacement therapy in both postmenopausal women and women deficient in estrogens. The *in vivo* metabolism of these estrogens has not yet been investigated in any species, although the biosynthetic pathway has been clarified to some extent.[2] It is well documented that the metabolism of steroid hormones in local target tissues can play an important role in the appearance of their hormonal activity. It is necessary therefore to clarify their metabolic fate. In order to facilitate metabolic studies of these estrogens, authentic samples are required. Based on the fact that classical estrogens can undergo extensive metabolism, either conversion to catechol (C-2 or C-4 hydroxylated) estrogens or modification of the D ring,[3] C-2 catecholic equilin and equilenin derivatives were synthesized.

Numerous methods of C-2 hydroxylation involving Fries rearrangement,[4] Friedel Crafts acylation,[5] reductive amination *via* nitration using sodium nitrate[6] and nucleophilic replacement with sodium methoxide,[7] have been developed for the synthesis of catechol estrogens. However, these methods appeared to be unsuitable for application to equilin and equilenin because of the chemically unstable nature of these compounds under such conditions. In previous studies,[8] we developed an efficient method for the synthesis of aromatic steroids involving thermal elimination of the β-ketosulfoxides. This synthetic route was therefore applied to the synthesis of the title compounds starting from vanillin and isovanillin, which possess a suitable catechol structure in the molecule.

Initially, methyl 4-(3-benzyloxy-4-methoxyphenyl)butyrate (**1a**) and methyl 4-(4-benzyloxy-3-methoxyphenyl)butyrate (**1b**) were prepared from vanillin and isovanillin by the application of conventional methods.[9,10] On treatment with dimsyl anion obtained from sodium hydride (NaH) and dimethyl sulfoxide (DMSO), these substances were quantitatively converted into the β-ketosulfoxides (**2a**, **b**). Subsequent methylation with methyl iodide in the presence of potassium hydride provided the enone precursors (**3a**, **b**). The proton nuclear

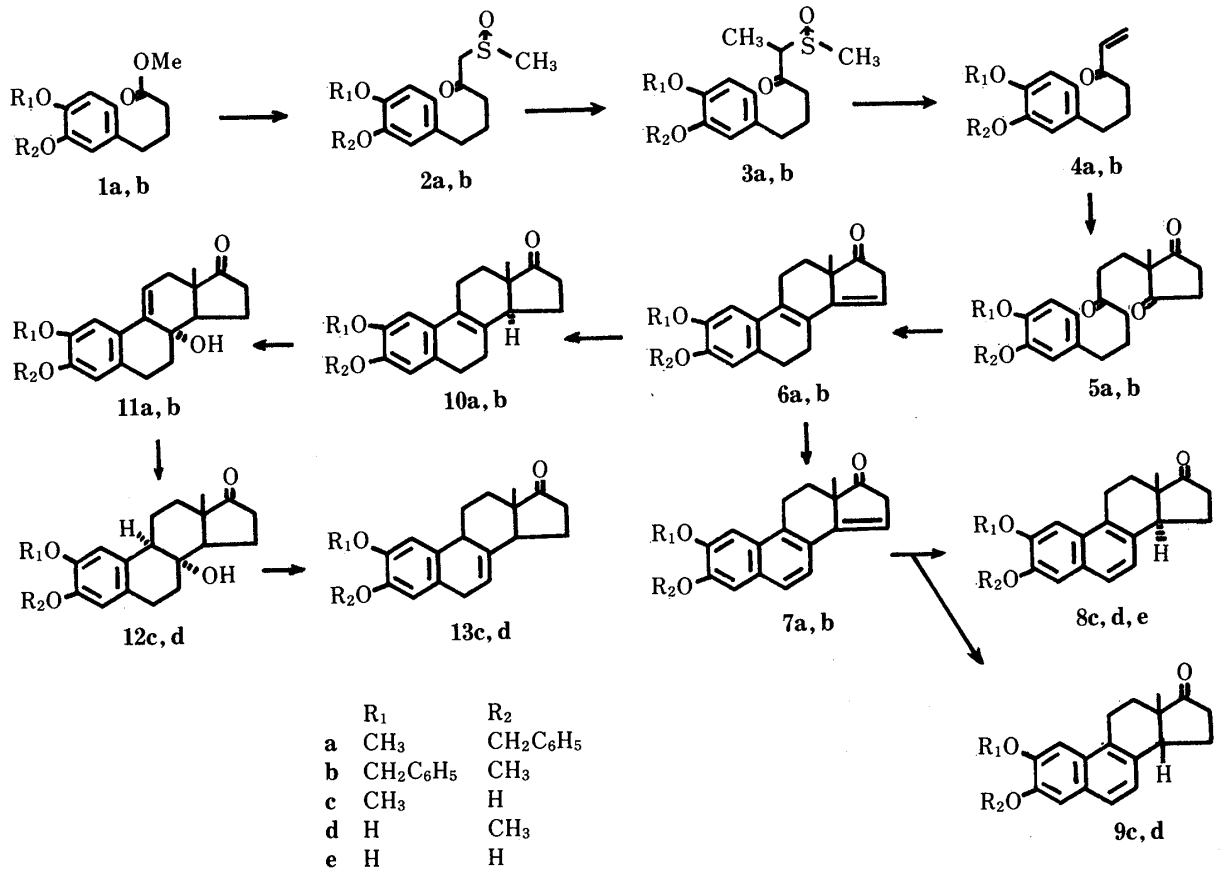


Chart 1

magnetic resonance ($^1$H-NMR) spectra of these products revealed methine signals at 3.68 and 3.72 ppm as a pair of quartets in **3a** and at 3.78 ppm as a multiplet in **3b**, indicating a diastereo mixture due to the asymmetry at the C-2 carbon and the sulfur atoms. Without separation of these enantiomers, thermal elimination of the methylsulfinyl group was performed in refluxing xylene to give the corresponding enones (**4a, b**) as the sole product. These enones were then condensed with 2-methylcyclopentane-1,3-dione in the presence of triethylamine for construction of the D ring in the steroid to furnish the Michael adducts (**5a, b**) in satisfactory yields. On treatment with methanesulfonic acid in methylene chloride at 0 °C, ring closure of **5a** and **5b** proceeded with dehydration to give the cyclized estrapentaenes (**6a, b**) as key intermediates.

Transformation of these pentaenes into equilenin derivatives was then carried out. To introduce the 6,7-double bond in the B ring, **6a** and **6b** were dehydrogenated with 2,3-dichloro-5,6-dicyanobenzoquinone (DDQ) in benzene to yield the respective estrahexaenes (**7a, b**) whose structures were characterized by inspection of their $^1$H-NMR and ultraviolet (UV) spectra. Catalytic hydrogenation of **7a** and **7b** with 10% palladized charcoal afforded the desired 2-methoxyequilenin (**8c**) and 2-hydroxyequilenin 3-methyl ether (**8d**), respectively, accompanied with a small amount of their corresponding 14$\beta$-isomers (**9c, d**): these C-14 epimers could be efficiently separated by column chromatography on silica gel. The 14$\alpha$ configuration toward **8c** and **8d** was unequivocally established from the spectral data. The $^1$H-NMR signals due to the $C_{18}$-protons at 0.80 and 0.81 ppm in **8c** and **8d** were shifted downfield to 1.16 and 1.15 ppm in **9c** and **9d**, respectively, comparably to those of analogous 14$\alpha$- and 14$\beta$-steroidal compounds,[11] confirming these configurational assignments. The absorption maxima of both **8c** and **8d** in the UV spectra were bathochromically shifted (4—8 nm) compared to those of **9c** and **9d**, similary to those of the above equilenin isomers.[8,12] *O*-

Demethylation of **8c** on brief exposure to trimethylsilyliodide afforded the desired 2-hydroxyequilenin (**8e**) in good yield.

Our next efforts were focused on the preparation of equilin derivatives. For this purpose, **6a** and **6b** were hydrogenated with an equimolar amount of hydrogen over 10% palladized charcoal in benzene to give mainly the corresponding 14α-estratetraenes (**10a, b**). The stereochemistry at C-14 of these products was confirmed by inspection of their $^1$H-NMR spectra as well as by a comparison with those of the related estra-1,3,5(10),8-tetraenes.[8]

Transposition of the olefinic bond at the C-8 position to the unconjugated C-7 has been reported, where successive oxidation and reduction resulted in the formation of an estra-1,3,5(10)-trien-8-ol which would be capable of undergoing dehydration to an estra-1,3,5(10),7-tetraene.[13] Employing the procedure described, epoxidation of **10a** and **10b** with *m*-chloroperbenzoic acid in a mixture of 5% sodium bicarbonate and methylene chloride at 0°C and subsequent ring opening with benzoic acid in chloroform were then undertaken to afford the respective tetraenols (**11a, b**) in about 70% yields. Catalytic hydrogenation of the styrenic bond in these products by the method described above resulted in the formation of the trienols (**12c, d**) in about 80% yields. The orientation of the hydrogen at C-9 was presumed to be α because the reagent should preferentially attack from the less hindered α-side in the molecule.[14] The downfield shift to 0.97 ppm of the $C_{18}$ proton signals in the $^1$H-NMR spectra justified a B/C-*cis* configurational assignment.[13] Dehydration of these 8α-alcohols with thionyl chloride in pyridine proceeded in the expected direction to furnish the required 2-hydroxyequilin monomethyl ethers (**13c, d**), respectively. Their structures were confirmed from their $^1$H-NMR spectra in which the characteristic $C_7$ proton signals were observed at 5.47 ppm. *O*-Demethylation of **13c** and **13d** to provide the desired 2-hydroxyequilin was attempted with demethylating agents such as trimethylsilyliodide and boron tribromide. However, the product could not be isolated in the pure form, although its formation was clearly demonstrated on thin layer chromatography (TLC). The exceptional lability of the desired structure precluded its use in metabolic studies, but considerable information could be obtained by employing the methyl ethers (**13c, d**) under demethylating conditions in which the desired compound was transiently generated.

The availability of these synthetic samples may be helpful for structural elucidation in metabolic studies. In fact, we have identified these catecholic equine estrogen metabolites from the bile of rats administered with equilin. The details will be reported in the near future.

## Experimental

All melting points were determined with a Mitamura micro hot-stage apparatus, and are uncorrected. UV spectra were recorded with a Shimadzu UV-200 spectrometer in ethanol. Infrared (IR) spectra were obtained using a JASCO IR A-102 spectrometer and expressed in $cm^{-1}$. $^1$H-NMR spectra were recorded with a Hitachi R-40 spectrometer (90 MHz) in $CDCl_3$ unless otherwise stated. Chemical shifts are given as the δ value with tetramethylsilane as the internal standard (s, singlet; d, doublet; dd, doublet of doublets; t, triplet; q, quartet; m, multiplet). Mass spectral (MS) measurements were made on a Shimadzu-LKB 9000 spectrometer with the ionizing voltage at 20 eV. Column chromatography was performed with Kiesel gel 60 (70—230 mesh, E. Merck). All organic extracts were dried over anhydrous $Na_2SO_4$.

**Preparation of Methyl 4-(3-Benzyloxy-4-methoxyphenyl)butyrate (1a) and Methyl 4-(4-Benzyloxy-3-methoxyphenyl)butyrate (1b)**——Vanillin and isovanillin were converted to **1a** and **1b** by a sequential multistep reaction *via* benzylation, the Knoevenagel reaction with malonic acid,[9] esterification with EtOH, catalytic hydrogenation, lithium aluminum hydride reduction, chlorination, nucleophilic substitution with potassium cyanide,[10] alkaline hydrolysis, and esterification with MeOH in about 40% overall yields. **1a**: bp 180—187 °C (1 mmHg). IR (neat): 1725, 1600, 1580. $^1$H-NMR: 1.90 (2H, m), 2.25 (2H, t, $J$=7 Hz), 2.53, (2H, t, $J$=7 Hz), 3.60 (3H, s, $COOCH_3$), 3.80 (3H, s, $OCH_3$), 5.09 (2H, s, $OC\underline{H}_2C_6H_5$), 6.80 (3H, m, aromatic), 7.30 (5H, m, aromatic). **1b**: bp 194–198 °C (1 mmHg). IR (neat): 1730, 1605, 1580. $^1$H-NMR: 1.90 (2H, m), 2.32 (2H, t, $J$=7 Hz), 2.56 (2H, t, $J$=7 Hz), 3.64 (3H, s, $COOCH_3$), 3.88 (3H, s, $OCH_3$), 5.12 (2H, s, $OC\underline{H}_2C_6H_5$), 6.75 (3H, m, aromatic), 7.35 (5H, m, aromatic).

**5-(3-Benzyloxy-4-methoxyphenyl)-1-methylsulfinylpentan-2-one (2a)**——A solution of **1a** (31.4 g) in tetrahydro-

furan (THF) (50 ml) was added dropwise to a stirred solution of NaH (6.1 g) and DMSO (120 ml) in THF (100 ml) at 0 °C under Ar. The reaction mixture was stirred at room temperature for 40 min and then poured into ice-water. The resulting solution was acidified with 2 M HCl and extracted with $CHCl_3$ three times. The combined extract was washed with $H_2O$, dried, and evaporated *in vacuo*. The crude product was column-chromatographed with AcOEt–MeOH (99 : 1) as the eluent to give **2a** (33 g). Colorless needles (*n*-hexane–AcOEt), mp 83–84 °C. IR (Nujol): 1710 (CO), 1040 (SO). $^1$H-NMR: 1.85 (2H, m), 2.5 (4H, m), 2.60 (3H, s, $SCH_3$), 3.62 (2H, m), 3.81 (3H, s, $OCH_3$), 5.06 (2H, s, $OC\underline{H}_2C_6H_5$), 6.70 (3H, m, aromatic), 7.35 (5H, m, $OCH_2C_6\underline{H}_5$). *Anal.* Calcd for $C_{20}H_{24}O_4S$: C, 66.75; H, 6.71. Found: C, 66.40; H, 6.88.

**5-(4-Benzyloxy-3-methoxyphenyl)-1-methylsulfinylpentan-2-one (2b)**——The ester (**1b**, 17 g) was treated with dimsyl anion prepared from NaH (3.3 g) and DMSO (65 ml) in THF (90 ml), as described for **2a**. The crude product was column-chromatographed using AcOEt–MeOH (99 : 1) as the eluent to give **2b** (18.5 g). Colorless needles (*n*-hexane–AcOEt), mp 94—95 °C. IR (Nujol): 1710 (CO), 1030 (SO). $^1$H-NMR: 1.80 (2H, m), 2.55 (4H, m), 2.58 (3H, s, $SCH_3$), 3.66 (2H, d, $J$=13 Hz), 3.85 (3H, s, $OCH_3$), 5.18 (2H, s, $OC\underline{H}_2C_6H_5$), 6.65 (3H, m, aromatic), 7.35 (5H, m, $OCH_2C_6\underline{H}_5$). *Anal.* Calcd for $C_{20}H_{24}O_4S$: C, 66.65; H, 6.71. Found: C, 66.40; H, 6.89.

**6-(3-Benzyloxy-4-methoxyphenyl)-2-methylsulfinylhexan-3-one (3a)**——A solution of **2a** (14.3 g) in THF (20 ml) was added dropwise to a stirred suspension of KH (1.6 g) in THF (50 ml) at 0°C under Ar and the mixture was stirred for 30 min. $CH_3I$ (6.2 g) in THF (10 ml) was then added to this solution and the whole mixture was stirred at 0 °C for 20 min. After evaporation of the solvent, the residue was dissolved in $CHCl_3$, and this solution was washed with $H_2O$, dried, and evaporated *in vacuo*. The crude product was column-chromatographed using AcOEt as the eluent to give **3a** (13.2 g). Colorless needles (*n*-hexane–AcOEt), mp 92—93.5 °C. IR (Nujol): 1700 (CO), 1035 (SO). $^1$H-NMR: 1.21, 1.34 (each 1.5H, respectively, d, $J$=7 Hz, $CH_3$), 1.85 (2H, m), 2.38, 2.41 (each 1.5H, respectively, s, $SCH_3$), 2.50 (4H, m), 3.68, 3.72 (each 0.5H, respectively, q, $J$=7 Hz, methine), 3.80 (3H, s, $OCH_3$), 5.06 (2H, s, $OC\underline{H}_2C_6H_5$), 6.80 (3H, m, aromatic), 7.35 (5H, m, $OCH_2C_6\underline{H}_5$). *Anal.* Calcd for $C_{21}H_{26}O_4S$: C, 67.36; H, 7.00. Found: C, 67.32; H, 7.04.

**6-(4-Benzyloxy-3-methoxyphenyl)-2-methylsulfinylhexan-3-one (3b)**——Methylation of **2b** (12 g) was performed with KH (1.34 g) and $CH_3I$ (5.2 g) in THF (70 ml) as described for **3a**. The crude product was column-chromatographed using AcOEt as the eluent to give **3b** (11.2 g). Colorless needles (*n*-hexane–AcOEt), mp 104.5—105.5 °C. IR (Nujol): 1700 (CO), 1030 (SO). $^1$H-NMR: 1.24, 1.36 (1.8H, 1.2H, respectively, d, $J$=7 Hz, $CH_3$), 1.80 (2H, m), 2.42, 2.44 (1.8H, 1.2H, respectively, each s, $SCH_3$), 2.60 (4H, m), 3.78 (1H, m, methine), 3.82 (3H, s, $OCH_3$), 5.06 (2H, s, $OC\underline{H}_2C_6H_5$), 6.66 (3H, m, aromatic), 7.33 (5H, m, $OCH_2C_6\underline{H}_5$). *Anal.* Calcd for $C_{21}H_{26}O_4S$: C, 67.36; H, 7.00. Found: C, 67.30; H, 7.09.

**6-(3-Benzyloxy-4-methoxyphenyl)-1-hexen-3-one (4a)**——A solution of **3a** (5.8 g) in xylene (20 ml) was refluxed for 3 h under Ar. Evaporation of the solvent gave an oil, which was then column-chromatographed using *n*-hexane–AcOEt (10 : 1) as the eluent to give **4a** (3.2 g) as a pale yellow oil. IR (neat): 1690 (CO). $^1$H-NMR: 1.90 (2H, m), 2.50 (4H, m), 3.79 (3H, s, $OCH_3$), 5.06 (2H, s, $OC\underline{H}_2C_6H_5$), 5.71 (1H, dd, $J$=3, 9 Hz, olefinic), 6.17 (1H, dd, $J$=3, 18 Hz, olefinic), 6.31 (1H, dd, $J$=9, 18 Hz, olefinic), 6.71 (3H, m, aromatic), 7.30 (5H, m, $OCH_2C_6\underline{H}_5$). MS *m/z* (%): 310 ($M^+$, 6), 149 (30), 91 (100).

**6-(4-Benzyloxy-3-methoxyphenyl)-1-hexen-3-one (4b)**——Thermal elimination of **3b** (4.9 g) in xylene (20 ml) and subsequent purification of the crude product were performed as described for **4a** to give **4b** (2.8 g) as a colorless oil. IR (neat): 1680 (CO). $^1$H-NMR: 1.90 (2H, m), 2.60 (4H, m), 3.82 (3H, s, $OCH_3$), 5.02 (2H, s, $OC\underline{H}_2C_6H_5$), 5.72 (1H, dd, $J$=3, 9 Hz, olefinic), 6.08 (1H, dd, $J$=3, 18 Hz, olefinic), 6.32 (1H, dd, $J$=9, 18 Hz, olefinic), 6.73 (3H, m, aromatic), 7.30 (5H, m, $OCH_2C_6\underline{H}_5$). MS *m/z* (%): 310 ($M^+$, 8), 149 (30), 91 (100).

**(±)-2-[6-(3-Benzyloxy-4-methoxyphenyl)-3-oxohexyl]-2-methylcyclopentane-1,3-dione (5a)**——A mixture of **4a** (2.0 g) and 2-methylcyclopentane-1,3-dione (1.0 g) in AcOEt (4 ml) containing triethylamine (0.2 ml) was stirred at room temperature for 36 h. After evaporation of the solvent *in vacuo*, the residue was column-chromatographed using *n*-hexane–AcOEt (2 : 1) as the eluent to give **5a** (2.4 g). Colorless needles (*n*-hexane–AcOEt), mp 71—72 °C. IR (Nujol): 1760 (CO), 1720 (CO). $^1$H-NMR: 1.03 (3H, s, $CH_3$), 3.78 (3H, s, $OCH_3$), 5.07 (2H, s, $OC\underline{H}_2C_6H_5$), 6.70 (3H, m, aromatic), 7.30 (5H, m, $OCH_2C_6\underline{H}_5$). MS *m/z* (%): 422 ($M^+$, 12), 219 (9), 137 (11), 91 (100). *Anal.* Calcd for $C_{26}H_{30}O_5$: C, 73.91; H, 7.16. Found: C, 73.98; H, 7.19.

**(±)-2-[6-(4-Benzyloxy-3-methoxyphenyl)-3-oxohexyl]-2-methylcyclopentane-1,3-dione (5b)**——Condensation of **4b** (3.1 g) with 2-methylcyclopentane-1,3-dione (1.5 g) in AcOEt (6 ml) containing triethylamine (0.3 ml) and subsequent purification of the crude product were performed as described for **5a** to give **5b** (3.8 g). Colorless needles (*n*-hexane–AcOEt), mp 103—103.5 °C. IR (Nujol): 1760 (CO), 1720 (CO). $^1$H-NMR: 1.06 (3H, s, $CH_3$), 3.80 (3H, s, $OCH_3$), 5.05 (2H, s, $OC\underline{H}_2C_6H_5$), 6.7 (3H, m, aromatic), 7.30 (5H, m, $OCH_2C_6\underline{H}_5$). MS *m/z* (%): 422 ($M^+$, 3), 219 (5), 137 (11), 91 (100). *Anal.* Calcd for $C_{26}H_{30}O_5$: C, 73.91; H, 7.16. Found: C, 73.84; H, 7.22.

**(±)-3-Benzyloxy-2-methoxyestra-1,3,5(10),8,14-pentaen-17-one (6a)**——Methanesulfonic acid (1 ml) was added to a solution of **5a** (1.00 g) in dry $CH_2Cl_2$ (3 ml) at 0 °C and the reaction mixture was stirred for 15 min. The resulting mixture was diluted with $CH_2Cl_2$, washed with $H_2O$, dried, and evaporated *in vacuo*. Recrystallization of the crude product from MeOH gave **6a** (0.81 g). Colorless plates, mp 160—162 °C. IR (Nujol): 1745 (CO). $^1$H-NMR: 1.12 (3H, s, 18-$CH_3$), 3.85 (3H, s, $OCH_3$), 5.10 (2H, s, $OC\underline{H}_2C_6H_5$), 5.80 (1H, m, 15-H), 6.67 (1H, s, 4-H), 6.83 (1H, s, 1-H),

7.30 (5H, m, $OCH_2C_6\underline{H}_5$). MS *m/z* (%): 386 ($M^+$, 84), 295 (75), 267 (100). UV $\lambda_{max}$ nm (ε): 327 (22000), 305 sh. (16000). *Anal.* Calcd for $C_{26}H_{26}O_3$: C, 80.80; H, 6.78. Found: C, 80.79; H, 6.79.

**(±)-2-Benzyloxy-3-methoxyestra-1,3,5(10),8,14-pentaen-17-one (6b)**——Treatment of **5b** (2.2 g) with methanesulfonic acid (2 ml) was performed as described for **6a**. Recrystallization of the crude product from MeOH gave **6b** (1.8 g). Colorless needles, mp 153–153.5 °C. IR (Nujol): 1750 (CO). $^1$H-NMR: 1.10 (3H, s, 18-$CH_3$), 3.82 (3H, s, $OCH_3$), 5.60 (2H, s, $OC\underline{H}_2C_6H_5$), 5.77 (1H, t, $J$=3 Hz, 15-H), 6.63 (1H, s, 4-H), 6.83 (1H, s, 1-H), 7.30 (5H, m, $OCH_2C_6\underline{H}_5$). MS *m/z* (%): 386 ($M^+$, 100), 295 (26), 239 (21). UV $\lambda_{max}$ nm (ε): 327 (22000), 305 sh (16000). *Anal.* Calcd for $C_{26}H_{26}O_3$: C, 80.80; H, 6.78. Found: C, 80.54; H, 6.79.

**(±)-3-Benzyloxy-2-methoxyestra-1,3,5(10),6,8,14-hexaen-17-one (7a)**——A solution of **6a** (386 mg) and DDQ (238 mg) in benzene (10 ml) was stirred at room temperature for 10 min. After removal of the insoluble material by filtration, the filtrate was evaporated *in vacuo*. Recrystallization of the crude product from MeOH gave **7a** (361 mg). Colorless needles, mp 213—215 °C. IR (Nujol): 1740 (CO). $^1$H-NMR: 1.17 (3H, s, 18-$CH_3$), 3.96 (3H, s, $OCH_3$), 5.22 (2H, s, $OC\underline{H}_2C_6H_5$), 6.20 (1H, m, 15-H), 7.1—7.5 (9H, aromatic). MS *m/z* (%): 384 ($M^+$, 100), 265 (48), 91 (11). UV $\lambda_{max}$ nm (ε): 307 (16700), 296 (19400), 285 (16500), 267 (49000). *Anal.* Calcd for $C_{26}H_{24}O_3$: C, 81.22; H, 6.29. Found: C, 81.21; H, 6.17.

**(±)-2-Benzyloxy-3-methoxyestra-1,3,5(10),6,8,14-hexaen-17-one (7b)**——Treatment of **6b** (386 mg) with DDQ (238 mg) in benzene (10 ml) was performed as described for **7a**. Recrystallization of the crude product from MeOH gave **7b** (357 mg). Colorless prisms, mp 173–174.5 °C. IR (Nujol): 1740 (CO). $^1$H-NMR: 1.13 (3H, s, 18-$CH_3$), 3.95 (3H, s, $OCH_3$), 5.20 (2H, s, $OC\underline{H}_2C_6H_5$), 6.15 (1H, m, 15-H), 7.0—7.4 (9H, m, aromatic). MS*m/z* (%): 384 ($M^+$, 100), 293 (8), 91 (20). UV $\lambda_{max}$ nm (ε): 307 (16700), 295 (18900), 285 (16400), 265 (44700). *Anal.* Calcd for $C_{26}H_{24}O_3$: C, 81.22; H, 6.29. Found: C, 81.44; H, 6.29.

**(±)-2-Methoxyequilenin (8c)**——A solution of **7a** (210 mg) in a mixture of EtOH (15 ml) and benzene (3 ml) was stirred with 10% Pd–C (100 mg) at room temperature under $H_2$. After removal of the catalyst by filtration, the solvent was evaporated off *in vacuo*. Recrystallization of the crude product from MeOH gave **8c** (118 mg). Colorless prisms, mp 258—260 °C. IR (Nujol): 3250 (OH), 1730 (CO). $^1$H-NMR: 0.81 (3H, s, 18-$CH_3$), 4.03 (3H, s, $OCH_3$), 7.16 (1H, d, $J$=8 Hz, 6-H), 7.17 (1H, s, 4-H), 7.25 (1H, s, 1-H), 7.56 (1H, d, $J$=8 Hz, 7-H). MS *m/z*: 296 ($M^+$, 1). UV $\lambda_{max}$ nm (ε):332 (4200), 317 (2800), 300 (3700), 288 (5000), 278 (4800), 254 (4100). *Anal.* Calcd for $C_{19}H_{20}O_3$: C, 77.00; H, 6.80. Found: C, 76.84; H, 6.82. The mother liquor was column-chromatographed using $CHCl_3$–MeOH (100 : 1.5) as the eluent to give (±)-2-methoxy-14β-equilenin (**9c**, 10 mg). Colorless prisms (MeOH), mp 201—202.5 °C. IR (Nujol): 3300 (OH), 1730 (CO). $^1$H-NMR: 1.16 (3H, s, 18-$CH_3$), 4.01 (3H, s, $OCH_3$), 7.10 (1H, s, 4-H), 7.16 (1H, d, $J$=8 Hz, 6-H), 7.20 (1H, s, 1-H), 7.46 (1H, d, $J$=8Hz, 7-H). UV $\lambda_{max}$ nm (ε): 326 (4100), 312 (2500), 292 (3400), 281 (5300), 272 (5300). *Anal.* Calcd for $C_{19}H_{20}O_3$: C, 77.00; H, 6.80. Found: C, 76.86; H, 6.83.

**(±)-2-Hydroxyequilenin 3-Methyl Ether (8d)**——Catalytic reduction of **7b** (192 mg) with 10% Pd–C (100 mg) in a mixture of EtOH (15 ml) and benzene (3 ml) was performed as described for **8c**. Recrystallization of the crude product from MeOH gave **8d** (101 mg). Colorless prisms, mp 283—285°C. IR (Nujol): 3400 (OH), 1730 (CO). $^1$H-NMR: 0.80 (3H, s, 18-$CH_3$), 4.00 (3H, s, $OCH_3$), 7.10 (1H, s, 4-H), 7.15 (1H, d, $J$=8 Hz, 6-H), 7.37 (1H, s, 1-H), 7.57 (1H, d, $J$=8 Hz, 7-H). MS *m/z* (%): 296 ($M^+$, 100). UV $\lambda_{max}$ nm (ε) : 331 (4560), 323 (2200), 317 (2850), 304 (3800), 290 (4900), 280 (4600). 253 (3600). *Anal.* Calcd for $C_{19}H_{20}O_3$: C, 77.00; H, 6.80. Found: C, 76.87; H, 6.81. The mother liquor was column-chromatographed using $CHCl_3$–MeOH (100 : 1) as the eluent to give (±)-2-hydroxy-14β-equilenin 3-methyl ether (9d) (9 mg). Colorless leaflets (MeOH), mp 186—189 °C. IR (Nujol): 3250 (OH), 1735 (CO). $^1$H-NMR: 1.15 (3H, s, 18-$CH_3$), 4.00 (3H, s, $OCH_3$), 7.10 (1H, s, 4-H), 7.15 (1H, d, $J$=8 Hz, 6-H), 7.38 (1H, s, 1-H), 7.55 (1H, d, $J$=8 Hz, 7-H). UV $\lambda_{max}$ nm (ε): 327 (4300), 319 (1960), 312 (2600), 293 (4100), 282 (5300), 273 (5200). *Anal.* Calcd for $C_{19}H_{20}O_3$: C, 77.00; H, 6.80. Found: C, 76.77; H, 6.74.

**(±)-2-Hydroxyequilenin (8e)**——Trimethylchlorosilane (400 mg) and NaI (550 mg) were added to a solution of **8c** (60 mg) in $CH_3CN$ (5 ml) and the reaction mixture was heated at 60°C for 3 h under Ar. After evaporation of the solvent *in vacuo*, the resulting solution was diluted with AcOEt, washed with 5% $Na_2SO_3$ and $H_2O$, dried, and evaporated *in vacuo*. The crude product was subjected to chromatography on a QAE Sephadex borate form column using 0.005 M AcOH in MeOH as the eluent to give **8e** (51 mg). Colorless prisms (MeOH), mp 259—261.5 °C. IR (Nujol): 3500, 3200 (OH), 1735 (CO). $^1$H-NMR ($CD_3OD$–$CDCl_3$ = 1 : 5): 0.72 (3H, s, 18-$CH_3$), 6.99 (1H, d, $J$=8.5 Hz, 6-H), 7.06 (1H, s, 4-H), 7.18 (1H, s, 1-H), 7.38 (1H, d, $J$=8.5 Hz, 7-H). MS *m/z* (%): 282 ($M^+$, 100), 226 (24), 225 (25), 213 (14). UV $\lambda_{max}$ nm (ε): 333 (4670), 326 (2570), 318 (3100), 303 (4000), 298 (4950), 282 (4670), 256 (3500). *Anal.* Calcd for $C_{18}H_{18}O_3$: C, 76.57; H, 6.43. Found: C, 76.47; H, 6.49.

**(±)-3-Benzyloxy-2-methoxyestra-1,3,5(10),8-tetraen-17-one (10a)**——A mixture of **6a** (386 mg) and 10% Pd–C (50 mg) in benzene (15 ml) was stirred at room temperature under $H_2$ until 1 mol of the gas had been taken up. After removal of the catalyst by filtration, the solvent was evaporated *in vacuo*. Recrystallization of the crude product from MeOH gave **10a** (303 mg). Colorless needles, mp 153.5—155.5 °C. IR (Nujol): 1735 (CO). $^1$H-NMR: 0.89 (3H, s, 18-$CH_3$), 3.85 (3H, s, $OCH_3$), 5.08 (2H, s, $OC\underline{H}_2C_6H_5$), 6.65 (1H, s, 4-H), 6.76 (1H, s, 1-H), 7.30 (5H, m, $OCH_2C_6\underline{H}_5$). MS *m/z* (%): 388 ($M^+$, 7), 279 (29), 91 (100). UV $\lambda_{max}$ nm (ε): 304 (10000), 284 (12000). *Anal.* Calcd for $C_{26}H_{28}O_3$: C, 80.38; H, 7.27. Found: C, 80.19; H, 7.22.

**(±)-2-Benzyloxy-3-methoxyestra-1,3,5(10),8-tetraen-17-one (10b)**——Hydrogenation of **6b** (350 mg) with 10%

Pd–C (50 mg) in benzene (15 ml) was performed as described for **10a**. Recrystallization of the crude product from MeOH gave **10b** (267 mg). Colorless prisms, mp 105—106 °C. IR (Nujol): 1730 (CO). $^1$H-NMR: 0.85 (3H, s, 18-$CH_3$), 3.82 (3H, s, $OCH_3$), 5.05 (2H, s, $OC\underline{H}_2C_6H_5$), 6.62 (1H, s, 4-H), 6.75 (1H, s, 1-H), 7.30 (5H, m, $OCH_2C_6\underline{H}_5$). MS *m/z* (%): 388 ($M^+$, 100), 297 (23), 91 (61). UV $\lambda_{max}$ nm ($\varepsilon$): 304 (9500), 285 (12000). *Anal.* Calcd for $C_{26}H_{28}O_3$: C, 80.38; H, 7.27. Found: C, 80.21; H, 7.24.

**(±)-3-Benzyloxy-8α-hydroxy-2-methoxyestra-1,3,5(10),9(11)-tetraen-17-one (11a)**——*m*-Chloroperbenzoic acid (207 mg) was added to a solution of **10a** (388 mg) in a mixture of $CH_2Cl_2$ (10 ml) and 5% $NaHCO_3$ (10 ml) at 0 °C, and the reaction mixture was stirred for 20 min. After addition of 5% $NaHSO_3$ to decompose the excess reagent, the resulting solution was extracted with $CHCl_3$. The organic layer was washed with 5% $NaHCO_3$ and $H_2O$, dried, and evaporated *in vacuo*. The residue was dissolved in $CHCl_3$ (5 ml), and benzoic acid (400 mg) was added to this solution. The mixture was allowed to stand at room temperature overnight. The resulting solution was washed with 5% $NaHCO_3$ and $H_2O$, dried, and evaporated *in vacuo*. Recrystallization of the crude product from MeOH gave **11a** (295 mg). Colorless prisms, mp 163—165 °C. IR (Nujol): 3550 (OH), 1730 (CO). $^1$H-NMR: 0.92 (3H, s, 18-$CH_3$), 3.82 (3H, s, $OCH_3$), 5.08 (2H, s, $OC\underline{H}_2C_6H_5$), 5.97 (1H, t, $J$=5 Hz, 11-H), 6.60 (1H, s, 4-H), 6.92 (1H, s, 1-H), 7.32 (5H, m, $OCH_2C_6\underline{H}_5$). MS *m/z* (%): 404 ($M^+$, 3), 386 (12), 259 (27), 91 (100). UV $\lambda_{max}$ nm ($\varepsilon$): 260 (16200), 302 (7280). *Anal.* Calcd for $C_{26}H_{28}O_4$: C, 77.20; H, 6.98. Found: C, 77.08; H, 6.98.

**(±)-2-Benzyloxy-8α-hydroxy-3-methoxyestra-1,3,5(10),9(11)-tetraen-17-one (11b)**——Epoxidation of **10b** (388 mg) with *m*-chloroperbenzoic acid (207 mg) and subsequent treatment with benzoic acid (400 mg) was performed as described for **11a**. Recrystallization of the crude product from MeOH gave **11b** (291 mg). Colorless needles, mp 170—172.5 °C. $^1$H-NMR: 0.90 (3H, s, 18-$CH_3$), 3.81 (3H, s, $OCH_3$), 5.06 (2H, s, $OC\underline{H}_2C_6H_5$), 5.80 (1H, t, $J$=5 Hz, 11-H), 6.65 (1H, s, 4-H), 6.90 (1H, s, 1-H), 7.30 (5H, m, $OCH_2C_6\underline{H}_5$). UV $\lambda_{max}$ nm ($\varepsilon$): 302 (6500), 260 (15500). MS *m/z* (%): 404 ($M^+$, 57), 386 (77), 295 (74), 267 (28), 91 (100). *Anal.* Calcd for $C_{26}H_{28}O_4$: C, 77.20; H, 6.98. Found: C, 77.12; H, 7.01.

**(±)-3,8α-Dihydroxy-2-methoxy-9α-estra-1,3,5(10)-trien-17-one (12c)**——Catalytic reduction of **11a** (200 mg) was carried out with 10% Pd–C (100 mg) in MeOH (30 ml) under $H_2$. After the usual work-up, the crude product was recrystallized from *n*-hexane–AcOEt to give **12c** (128 mg). Colorless needles, mp 153—154 °C. IR (Nujol): 3550 (OH), 3420 (OH), 1730 (CO). $^1$H-NMR: 0.97 (3H, s, 18-$CH_3$), 3.80 (3H, s, $OCH_3$), 6.50 (1H, s, 4-H), 6.58 (1H, s, 1-H). MS *m/z* (%): 316 ($M^+$, 93), 298 (100). *Anal.* Calcd for $C_{19}H_{24}O_4$: C, 72.12; H, 7.65. Found: C, 72.03; H, 7.52.

**(±)-2,8α-Dihydroxy-3-methoxy-9α-estra-1,3,5(10)-trien-17-one(12d)**——Catalytic reduction of **11b** (98 mg) with 10% Pd–C (50 mg) in EtOH (10 ml) under $H_2$ was carried out as described for **12c**. Recrystallization of the crude product from benzene–MeOH gave **12d** (62 mg). Colorless prisms, mp 222—224.5 °C. IR (Nujol): 3480 (OH), 3350 (OH), 1725 (CO). $^1$H-NMR: 0.97 (3H, s, 18-$CH_3$), 3.85 (3H, s, $OCH_3$), 6.60 (1H, s, 4-H), 6.70 (1H, s, 1-H). MS *m/z* (%): 316 ($M^+$, 57), 298 (100). *Anal.* Calcd for $C_{19}H_{24}O_4$: C, 72.12; H, 7.65. Found: C, 71.96; H, 7.44.

**(±)-2-Methoxyequilin (13c)**——A mixture of **12c** (56 mg) and thionylchloride (0.2 ml) in dry pyridine (4 ml) was heated at 50 °C for 2.5 h. After addition of $H_2O$, the resulting solution was extracted with $CH_2Cl_2$. The organic layer was washed with 2 M HCl and $H_2O$, dried, and evaporated *in vacuo*. The crude product was column-chromatographed using benzene–AcOEt (6 : 1) as the eluent to give **13c** (39 mg). Colorless plates (MeOH), mp 218—220 °C. IR (Nujol): 3330 (OH), 1735 (CO). $^1$H-NMR: 0.77 (3H, s, 18-$CH_3$), 3.82 (3H, s, $OCH_3$), 5.47 (1H, m, 7-H), 6.62 (2H, s, 1- and 4-H). MS *m/z* (%): 298 ($M^+$, 100). UV $\lambda_{max}$ nm ($\varepsilon$): 283 (5000). *Anal.* Calcd for $C_{19}H_{22}O_3$: C, 76.48; H, 7.43. Found: C, 76.29; H, 7.54.

**(±)-2-Hydroxyequilin 3-Methyl Ether (13d)**——Dehydration of **12d** (86 mg) with thionylchloride (0.2 ml) in pyridine (4 ml) was carried out as described for **13c**. The crude product was column-chromatographed using benzene–AcOEt (7 : 1) as the eluent. Recrystallization of the eluate from MeOH gave **13d** (46 mg). Colorless plates, mp 277—279.5 °C. IR (Nujol): 3400 (OH), 1725 (CO). $^1$H-NMR: 0.78 (3H, s, 18-$CH_3$), 3.84 (3H, s, $OCH_3$), 5.47 (1H, m, 7-H), 6.59 (1H, s, 4-H), 6.77 (1H, s, 1-H). *Anal.* Calcd for $C_{19}H_{22}O_3$: C, 76.48; H, 7.43. Found: C, 76.28; H, 7.29.

**Acknowledgements** This work was supported by a grant from the Ministry of Education, Science and Culture, Japan. The authors are indebted to the staff of the Center for Instrumental Analysis of Hokkaido University for the elemental analyses.

## References

1) A. Girard, G. Sandulesco, A. Fridenson, and J. J. Rutgers, *C. R. Acad. Sci.*, **194**, 909 (1932); *idem, ibid.*, **195**, 981 (1932).
2) R. D. H. Heard and M. M. Hoffman, *J. Biol. Chem.*, **135**, 801 (1940); H. A. Salhanick and D. L. Berliner, *ibid.*, **227**, 583 (1957); L. Stárka, H. Breuer, and L. Cedard, *J. Endocr.*, **34**, 447 (1958); L. Stárka and H. Breuer, *Z. Physiol. Chem.*, **344**, 124 (1966); B. R. Bhavnani, R. V. Short, and S. Solomon, *Endocrinology*, **85**, 1172 (1969); *idem, ibid.*, **89**, 1152 (1971); B. R. Bhavnani and R. V. Short, *ibid.*, **92**, 657 (1973); *idem, ibid.*, **92**, 1397 (1973); B. R. Bhavnani, L. J. Martin, and R. D. Baker, *ibid.*, **96**, 1009 (1975); B. R. Bhavnani and C. A. Woolever, *ibid.*, **103**, 2291 (1978); B. R. Bhavnani, R. D. Baker, and C. A. Woolever, *J. Steroid Biochem.*, **10**, 701 (1979).

3) P. Ball and R. Knuppen, *Acta Endocrinol. Suppl.*, **232**, 1 (1980); G. R. Meriam and M. B. Lipsett, "Catechol Estrogens," Raven Press, New York, 1983.
4) T. Nambara, S. Honma, and S. Akiyama, *Chem. Pharm. Bull.*, **18**, 474 (1970).
5) J. A. Menzies, P. Toft, and H. Watanabe, *J. Label. Comp.*, **8**, 505 (1972).
6) H. P. Gelbke, O. Haupt, and R. Knuppen, *Steroids*, **21**, 205 (1973); H. P. Gelbke, P. Ball, O. Haupt, and R. Knuppen, *ibid.*, **22**, 151 (1973); G. Stubenrauch and R. Knuppen, *ibid.*, **28**, 733 (1976).
7) P. N. Rao and J. E. Burdett, Jr., *Synthesis*, **1977**, 169.
8) T. Kurosawa, M. Tohma, Y. Oikawa, and O. Yonemitsu, *Chem. Pharm. Bull.*, **26**, 1533 (1978); *idem, ibid.*, **29**, 2101 (1981); T. Kurosawa, U. Niitsu, and M. Tohma, *ibid.*, **35**, 585 (1987).
9) J. Koo, M. S. Fish, G. N. Walker, and J. Blake, "Organic Syntheses," Coll. Vol., IV, ed. by John Wiley and Sons, Inc., New York, 1963, pp. 327—329.
10) F. L. Cook, C. W. Bowers, and C. L. Liotta, *J. Org. Chem.*, **39**, 3416 (1974).
11) N. S. Bhacca and D. H. Williams, "Applications of Nuclear Magnetic Resonance Spectroscopy in Organic Chemistry," Holden-Day Inc., San Francisco, 1964, p. 16.
12) W. E. Bachmann, W. Cole, and A. L. Wilds, *J. Am. Chem. Soc.*, **62**, 824 (1940); W. S. Johnson, J. W. Petersen, and C. D. Gutsche, *ibid.*, **69**, 2942 (1947); T. Miki, K. Hiraga, and T. Asako, *Chem. Pharm. Bull.*, **13**, 1285 (1965).
13) R. P. Stein, G. C. Buzby, Jr., and H. Smith, *Tetrahedron*, **26**, 1917 (1970).
14) D. N. Kirk and M. P. Hartshorn, "Steroid Reaction Mechanisms," Elsevier Publ. Co., Amsterdam, 1968, pp. 81—88.