Synthesis of 4- and 16α-hydroxylated equine estrogens

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4-Hydroxyequilin, 4-hydroxyequilenin, and 16α-hydroxyequilenin were synthesized as authentic specimens for the metabolic studies of equine estrogens. The synthetic route leading to the 4-hydroxylated compounds was started from o-vanillin, which was transformed into the β-ketosulfoxide ***(2b)*** *by sequential multistep reactions. This was converted to the α,β-unsaturated ketone* ***(3)*** *as Michael acceptor. Condensation of* ***3*** *with 2-methylcyclopentane-1,3-dione, followed by ring closure with methanesulfonic acid provided the cyclized estrapentaene* ***(5)****. Several oxidoreduction reactions were then performed to give the desired compounds. Preparation of 16α-hydroxyequilenin was attained by reductive cleavage of the 16α,17α-epoxide formed from equilenin.* (*Steroids* **55**:250–255, 1990)

Keywords: steroids; 4-hydroxyequilin; 4-hydroxyequilenin; 16α-hydroxyequilenin; equine estrogens; equilin; equilenin

Introduction

Equine estrogens (equilin and equilenin) are steroids produced by the fetoplacental unit of the pregnant mare.[1–9] These estrogens are used in estrogen replacement therapy for postmenopausal and estrogen-deficient women.[10,11] However, the metabolic fate of these hormones has not yet been thoroughly investigated. Recently, Bhavnani and associates have shown that normal postmenopausal women, and men given [^{3}H]equilin and its sulfate, largely excreted equilin in the urine as unknown polar metabolites.[12,13] By analogy with the metabolism of classic estrogens such as estrone and estradiol,[14] this result implies the possible hydroxylation either at the ortho positions to the C-3 phenolic hydroxyl group or at the aliphatic 16α-position, leading to the formation of the catecholic or α-ketol structure. To assist in the identification of the metabolites, reference steroids were required. Rao and Somawardhana reported the synthesis of 2- and 4-methoxy equine estrogens for unambiguous identification of catechol metabolites.[15] We also have recently synthesized 2-hydroxyequilin and 2-hydroxyequilenin.[16] Our metabolic studies of equine estrogens required 4- and 16α-hydroxylated equine estrogens, the syntheses of which are described herein.

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Experimental

All melting points (mp) were determined with a micro hot-stage apparatus (Mitamura Co., Japan) and are uncorrected. Optical rotations were measured with a model 201 polarimeter (Union Giken Co., Japan). Ultraviolet (UV) spectra were recorded with a UV-200 spectrometer (Shimadzu Co., Japan). ^{1}H Nuclear magnetic resonance (NMR) spectra were recorded with a R-40 spectrometer (90 MHz) (Hitachi Co., Japan) in $CDCl_3$. Chemical shifts are given as the δ value with tetramethylsilane as the internal standard. The abbreviations used are s, singlet; d, doublet; dd, doublet of doublets; m, multiplet; and t, triplet. Infrared spectra were obtained using an IR A-102 spectrometer (Japan Spectroscopic Co., Japan) and are expressed in cm^{-1}. Low and high mass spectral (MS) measurements were made on an LKB 9000 spectrometer (Shimadzu Co.) with the ionizing voltage at 20 eV and a JMS-DX303 (Japan Electron Optic Laboratory Co., Japan) with the ionizing voltage at 70 eV, respectively. Column chromatography was performed with Kiesel gel 60 (70-230 mesh, E. Merck). All organic solvent extracts were dried over anhydrous Na_2SO_4.

Methyl 4-(2-benzyloxy-3-methoxyphenyl)butyrate ***(1)***

o-Vanillin was converted to **1** by a sequential multistep reaction via benzylation, Knoevenagel reaction with malonic acid, esterification with EtOH, catalytic hydrogenation, lithium aluminum hydride reduction,

chlorination, nucleophilic substitution with potassium cyanide, alkaline hydrolysis, and esterification with MeOH in about 30% overall yield. Boiling point (bp): 178 to 181° C (0.5 mm Hg). IR (neat): 1,730 (CO), 1,595 and 1,580 (aromatic). ^{1}H NMR: 1.90 (2H, m), 2.30 (2H, t, J = 7 Hz), 3.61 (3H, s, $COOCH_3$), 3.88 (3H, s, OCH_3), 5.03 (2H, s, $OCH_2C_6H_5$), 6.75 to 7.15 (3H, m, aromatic), 7.3 to 7.6 (5H, m, $OCH_2C_6H_5$).

5-(2-Benzyloxy-3-methoxyphenyl)-1-methylsulfinylpentan-2-one ***(2a)***

A solution of **1** (28 g) in tetrahydrofuran (THF) (25 ml) was added dropwise to a stirred solution of NaH (5 g) and dimethyl sulfoxide (80 ml) at 0° C under N_2. The reaction mixture was stirred at room temperature for 40 minutes, 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 eluant to give **2a** (26.6 g, 95.5%) as an oil. IR (neat): 1,705 (C = O), 1,580 (aromatic), 1,050 (SO). ^{1}H NMR: 1.80 (2H, m), 2.48 (4H, m), 2.50 (3H, s, SCH_3), 3.50 (2H, s), 3.80 (3H, s, OCH_3), 4.90 (2H, s, $OCH_2C_6H_5$), 6.80 (3H, m, aromatic), 7.30 (5H, m, $OCH_2C_6H_5$).

6-(2-Benzyloxy-3-methoxyphenyl)-2-methylsulfinylhexan-3-one ***(2b)***

A solution of **2a** (11.6 g) in THF (20 ml) was added dropwise to a stirred suspension of KH (1.6 g) in THF (35 ml) at 0° C under Ar, and the reaction mixture was stirred for 30 minutes. CH_3I (4.8 g) in THF (10 ml) was then added to this solution, and the whole mixture was stirred at 0° C for 30 minutes. After evaporation of the solvent, this 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 eluant to give **2b** (10.2 g, 85%) as an oil. IR (neat): 1,700 (CO), 1,580 (aromatic), 1,050 (SO). ^{1}H NMR: 1.20 (1.6H, d, J = 7 Hz), 1.30 (1.4H, d, J = 7 Hz), 1.85 (2H, m), 2.33 (1.6H, s), 2.35 (1.4H, s), 2.50 (4H, m), 3.64 (1H, m), 3.80 (3H, s, OCH_3), 4.96 (2H, s, $OCH_2C_6H_5$), 6.7 (3H, aromatic), 7.30 (5H, m, $OCH_2C_6H_5$).

6-(2-Benzyloxy-3-methoxyphenyl)-1-hexen-3-one ***(3)***

A solution of **2b** (8.6 g) in xylene (40 ml) was refluxed for 3 hours under N_2. Evaporation of the solvent gave an oil, which was then column-chromatographed using *n*-hexane/AcOEt (10 : 1) as the eluant to give **3** (4.4 g, 62%) as an oil. IR (neat): 1,675 (CO), 1,610, 1,580 (aromatic). ^{1}H NMR: 1.85 (2H, m), 2.55 (4H, m), 3.80 (3H, s, OCH_3), 4.90 (2H, s, $OCH_2C_6H_5$), 5.61 (1H, dd, J = 3 and 7 Hz, olefine), 5.96 (1H, dd, J = 3 and 18 Hz), 6.24 (1H, dd, J = 7 and 18 Hz), 6.80 (2H, m), 7.30 (5H, m, $OCH_2C_6H_5$). MS m/z (%): 310 (M^+, 2), 219 (3), 137 (10), 91 (100).

(±)-2-[6-(2-Benzyloxy-3-methoxyphenyl)-3-oxohexyl]-2-methylcyclopentane-1,3-dione ***(4)***

A mixture of **3** (2.9 g) and 2-methylcyclopentane-1,3-dione (1.4 g) in AcOEt (6 ml) containing triethylamine (0.2 ml) was stirred at room temperature for 36 hours. After evaporation of the solvent in vacuo, the residue was column-chromatographed with *n*-hexane/AcOEt (3 : 1) as the eluant to give **4** (3.7 g, 94%) as an oil. IR (neat): 1,760 and 1,720 (CO), 1,580 (aromatic). ^{1}H NMR: 1.03 (3H, s, CH_3), 3.80 (3H, s, OCH_3), 4.90 (2H, s, $OCH_2C_6H_5$), 6.60 to 7.00 (3H, aromatic), 7.30 (5H, m, $OCH_2C_6H_5$). MS m/z (%): 422 (M^+, 2), 240 (27), 219 (9), 149 (21), 137 (11), 125 (14), 113 (15), 91 (100).

(±)-4-Benzyloxy-3-methoxyestra-1,3,5(10),8,14-pentaen-17-one ***(5)***

Methanesulfonic acid (2 ml) was added to a solution of **4** (2.4 g) in dry CH_2Cl_2 (5 ml) at 0° C, and the reaction mixture was stirred for 15 minutes. 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 **5** (1.6 g, 75%). Pale yellow needles, mp 103 to 105° C. IR (Nujol): 1,740 (CO), 1,590 (aromatic). ^{1}H NMR: 1.12 (3H, s, 18-CH_3), 3.83 (3H, s, OCH_3), 4.92 (2H, s, $OCH_2C_6H_5$), 5.80 (1H, t, J = 2 Hz), 7.30 (5H, m, $OCH_2C_6H_5$). MS m/z (%): 386 (M^+, 100), 358 (34), 239 (12). UV λ_{max}nm(ε): 327 (20,200), 312 (27,000), 307 (22,000). Anal. calculated for $C_{26}H_{26}O_3$: C, 80.80; H, 6.78. Found: C, 80.62, H, 6.82.

(±)-4-Benzyloxy-3-methoxyestra-1,3,5(10),6,8,14-hexaen-17-one ***(6)***

A solution of **5** (386 mg) and 2,3-dichloro-5,6-dicyanobenzoquinone (238 mg) in benzene (10 ml) was refluxed for 4 hours. After removal of the insoluble material by filtration, the filtrate was evaporated in vacuo. Recrystallization of the crude product from MeOH gave **6** (96 mg, 25%). Colorless needles, mp 169 to 170.5° C. IR (Nujol): 1,740 (CO), 1,590 and 1,520 (aromatic). ^{1}H NMR: 1.18 (3H, s, 18-CH_3), 3.95 (3H, s, OCH_3), 5.13 (2H, s, $OCH_2C_6H_5$), 6.25 (1H, m, 15-H), 7.2 to 7.6 (7H, m, aromatic), 7.6 (1H, d, J = 8 Hz, 2-H), 7.97 (1H, d, J = 8 Hz, 1-H). MS m/z (%): 384 (M^+, 44), 293 (100), 265 (17), 91 (39). UV λ_{max}nm(ε): 314 (11,200), 305 (13,400), 266 (41,300). Anal. calculated for $C_{26}H_{24}O_3$: C, 81.22; H, 6.29. Found: C, 81.40; H, 6.36.

(±)-4-Hydroxyequilenin 3-methyl ether ***(7a)***

A solution of **6** (192 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 **7** (98 mg, 66%). Colorless prisms, mp 239 to 241° C. IR (Nujol): 3,400 (OH), 1,730 (CO), 1,625 and 1,600 (aromatic). ^{1}H NMR: 0.81 (3H, s, 18-CH_3),

4.00 (3H, s, OCH_3), 6.00 (1H, m), 7.28 (2H, d, J = 8 Hz), 7.50 (1H, d, J = 8 Hz), 8.08 (1H, d, J = 8 Hz). MS m/z (%): 296 (M^+, 100), 281 (16), 253 (12). UV λ_{max}nm(ε): 346 (3,300), 337 (3,800), 305 (4,450), 294 (4,570), 254 (3,960). Anal. calculated for $C_{19}H_{20}O_3$: C, 77.00; H, 6.80. Found: C, 76.99; H, 6.81.

(±)-4-Hydroxyequilenin ***(7b)***

NaI (500 mg) and trimethylchlorosilane (380 mg) was added to a solution of **7a** (56 mg), and the reaction mixture was heated at 70° C under Ar. The reaction mixture was diluted with AcOEt, washed with 5% $Na_2S_2O_3$ and H_2O, and dried. After evaporation of the solvent in vacuo, the crude product was recrystallized from MeOH containing a trace amount of AcOH to give **7b** (31 mg, 58%) as slightly colored prisms, mp 220° C (decomp). IR (Nujol): 3,500 and 3,380 (OH), 1,720 (CO), 1,640 and 1,600 (aromatic). MS m/z (%): 282 (M^+, 100), 266 (29), 239 (20), 226 (19), 225 (18), 165 (18). Anal. calculated for $C_{18}H_{18}O_3$: C, 76.57; H, 6.43. Found: C, 76.70; H, 6.21.

(±)-4-Benzyloxy-3-methoxyestra-1,3,5(10),8-tetraen-17-one ***(8)***

A mixture of **5** (200 mg) and 10% Pd/C (40 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 **8** (141 mg, 70%). Colorless plates, mp 149 to 151° C. IR (Nujol): 1,735 (CO). ^{1}H NMR: 0.96 (3H, s, 18-CH_3), 3.82 (3H, s, OCH_3), 4.90 (2H, s, $OCH_2C_6H_5$), 6.66 and 6.88 (each 1H, d, J = 8 Hz, 1- and 2-H), 7.30 (5H, m, $OCH_2C_6H_5$). MS m/z (%): 388 (M^+, 10), 298 (22), 150 (11), 91 (100). UV λ_{max}nm(ε): 279 (15,500), 272 (15,000). Anal. calculated for $C_{26}H_{28}O_3$: C, 80.38; H, 7.28. Found: C, 80.38; H, 7.27.

(±)-4-Benzyloxy-8α-hydroxy-3-methoxyestra-1,3,5(10),9(11)-tetraen-17-one ***(9)***

m-Chloroperbenzoic acid (207 mg) was added to a solution of **8** (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 at room temperature for 20 minutes. After the addition of 5% $NaHSO_3$ to decompose the excess reagent, the resulting solution was washed with H_2O, dried, and evaporated in vacuo. The residue was dissolved in $CHCl_3$ (10 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 **9** (246 mg, 61%). Colorless prisms, mp 139 to 140° C. IR (Nujol): 3,350 (OH), 1,720 (CO), 1,595 (aromatic). ^{1}H NMR: 0.88 (3H, s, 18-CH_3), 3.82 (3H, s, OCH_3), 4.95 (2H, s, $OCH_2C_6H_5$), 5.93 (1H, t, J = 5 Hz, 11-H), 6.75 and 7.24 (each 1H, d, J = 9 Hz, 1- and 2-H), 7.32 (5H, m, $OCH_2C_6H_5$). MS m/z (%): 404 (M^+, 3), 386 (7), 295 (10), 267 (3), 237 (4), 209 (5), 91 (100). UV λ_{max}nm(ε): 303 (7,200), 260 (16,200). Anal. calculated for $C_{26}H_{28}O_4$: C, 77.20; H, 6.98. Found: C, 77.17; H, 6.99.

(±)-4,8α-Dihydroxy-3-methoxy-9α-estra-1,3,5(10)-trien-17-one ***(10)***

Catalytic reduction of **9** (186 mg) was carried out with 10% Pd/C (100 mg) in EtOH (20 ml) under H_2. After the usual work-up, the crude product was recrystallized from MeOH to give **10** (118 mg, 81%). Colorless prisms, mp 199 to 201° C. IR (Nujol): 3,560 and 3,450 (OH), 1,730 (CO), 1,595 (aromatic). ^{1}H NMR: 0.98 (3H, s, 18-CH_3), 3.80 (3H, s, OCH_3), 6.58 and 6.72 (1H, d, J = 9 Hz, 1- and 2-H). MS m/z (%): 316 (M^+, 100), 298 (85), 270 (13), 255 (13), 241 (12), 204 (24), 200 (14), 192 (18), 177 (20). Anal. calculated for $C_{19}H_{24}O_4$: C, 72.12; H, 7.65. Found: C, 72.00; H, 7.37.

(±)-4-Hydroxyequilin 3-methyl ether ***(11a)***

A mixture of **10** (61 mg) and thionylchloride (0.2 ml) in dry pyridine (4 ml) was heated at 80° C for 2.5 hours. After the 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 with benzene/AcOEt (6 : 1) as the eluant to give **11a** (40 mg, 69%). Colorless prisms, mp 217 to 219°C. IR (Nujol): 3,410 (OH), 1,740 (CO), 1,620 and 1,585 (aromatic). ^{1}H NMR: 0.78 (3H, s, 18-CH_3), 3.35 (2H, m), 3.82 (3H, s, OCH_3), 5.53 (1H, m, 7-H), 6.62 and 6.73 (each 1H, d, J = 9 Hz, 1- and 2-H). MS m/z (%): 298 (M^+, 100), 255 (11), 241 (10), 150 (12). UV λ_{max}nm(ε): 278 (4,900). Anal. calculated for $C_{19}H_{22}O_3$: C, 76.48; H, 7.43. Found: C, 76.32; H, 7.18.

(±)-4-Hydroxyequilin ***(11b)***

Demethylation of **11a** (80 mg) with trimethylchlorosilane (500 mg) in acetonitrile was carried out in the same manner as described for **7b.** The crude product was chromatographed on QAE-Sephadex LH 20 using MeOH as the eluant to give **11b** (46 mg, 58%) as colorless granules, mp 202 to 204° C. IR (Nujol) cm^{-1}: 3,550 and 3,400 (OH), 1,720 (CO), 1,620 and 1,590 (aromatic). MS m/z (%): 284 (M^+, 100), 242 (15), 229 (15). UV λ_{max}nm(ε): 275 (8,500).

3,17-Diacetoxyestra-1,3,5(10),6,8,16-hexaene ***(13)***

A solution of equilenin (200 mg) and pyridinium *p*-toluenesulfonate (80 mg) in isopropenylacetate (6 ml) was refluxed for 1 hour. The solution was concentrated to one half of its volume by slow distillation over a period of 4 hours. The resulting solution was diluted with AcOEt, then washed with 5% $NaHCO_3$ and H_2O, dried, and evaporated in vacuo. The crude product was column-chromatographed with *n*-hexane/AcOEt (10 : 1) as the eluant to give **13** (128 mg, 49%).

Colorless needles (MeOH), mp 117.5 to 118° C. $[\alpha]_D$ + 123.0 (c = 0.24, $CHCl_3$). ^1H NMR: 0.73 (3H, s, 18-CH_3), 2.18 (3H, s, 17-$OCOCH_3$), 2.31 (3H, s, 3-$OCOCH_3$), 5.61 (1H, m, 16-H), 7.13 (1H, d, J = 3 and 9Hz, 2-H), 7.43 (1H, d, J = 3 Hz, 4-H), 7.16, 7.55, and 7.86 (each 1H, d, J = 9 Hz, 1-, 6- and 7-H). Anal. calculated for $C_{22}H_{22}O_4$: C, 75.41; H, 6.33. Found: C, 75.31; H, 6.28.

16α,17α-Epoxy-3,17-diacetoxyestra-1,3,5(10),6,8-pentaene (14)

A solution of **13** (110 mg) and *m*-chloroperbenzoic acid (130 mg) in $CHCl_3$ (2 ml) was stirred at room temperature for 3 hours. The reaction mixture was diluted with AcOEt, then washed with 5% $NaHSO_3$, 5% $NaHCO_3$, and H_2O, dried, and evaporated in vacuo. Recrystallization of the crude product from MeOH gave **14** (110 mg, 95%) as colorless needles, mp 145 to 146° C. $[\alpha]_D$ + 57.4 (c = 0.42). ^1H NMR: 0.73 (3H, s, 18-CH_3), 2.11 and 2.31 (each 3H, s, 3- and 17-$OCOCH_3$), 4.07 (1H, s, 16β-H), 7.18 (1H, dd, J = 3 and 9 Hz, 2-H), 7.46 (1H, d, J = 3 Hz, 4-H), 7.06, 7.66, and 7.90 (each 1H, d, J = 9 Hz, 1-, 6-, and 7-H). Anal. calculated for $C_{22}H_{22}O_5$: C, 72.11; H, 6.05. Found: C, 71.93; H, 6.02.

16α-Hydroxyequilenin (15)

To a solution of **14** (40 mg) in MeOH (3 ml) was added 12 M H_2SO_4 (0.3 ml), and the reaction mixture was heated at 50° C for 1.5 hours. The resulting solution was diluted with AcOEt, washed with 5% $NaHCO_3$ and H_2O, dried, and evaporated in vacuo. The crude product was column-chromatographed with *n*-hexane/AcOEt (1 : 1) as the eluant to give **15** (20 mg, 70%). Colorless leaflets (MeOH), mp 214 to 216° C. $[\alpha]_D$ +96.2 (c = 0.26). ^1H NMR: 0.83 (3H, s, 18-CH_3), 4.50 (1H, m, 16β-H), 7.00 to 7.16 (3H, unresolved 2-, 4- and 6-H), 7.45 and 7.73 (each 1H, d, J = 9 Hz, 1- and 7-H). High MS m/z: 288.1252 (calculated for $C_{18}H_{18}O_3$: 288.1292).

Results and discussion

Synthesis of 4-hydroxy equine estrogens

Preparation of 4-hydroxyequilenin has been performed by oxidation of equilenin with Fremy's salt.[17] Another method of C-4 hydroxylation, which involves reductive amination via nitration with sodium nitrate, has been developed for the synthesis of catechol estrogens.[18] However, these methods appeared to be unsuitable for application to both equilin and equilenin because these compounds are unstable under such conditions. We have recently developed a low-cost, efficient method for preparation of 2-hydroxylated equine estrogens using vanillin and isovanillin.[16] Therefore, the synthetic route to obtain 4-hydroxyequilin and 4-hydroxyequilenin followed the same reaction scheme by using *o*-vanillin as the starting material (Scheme 1).

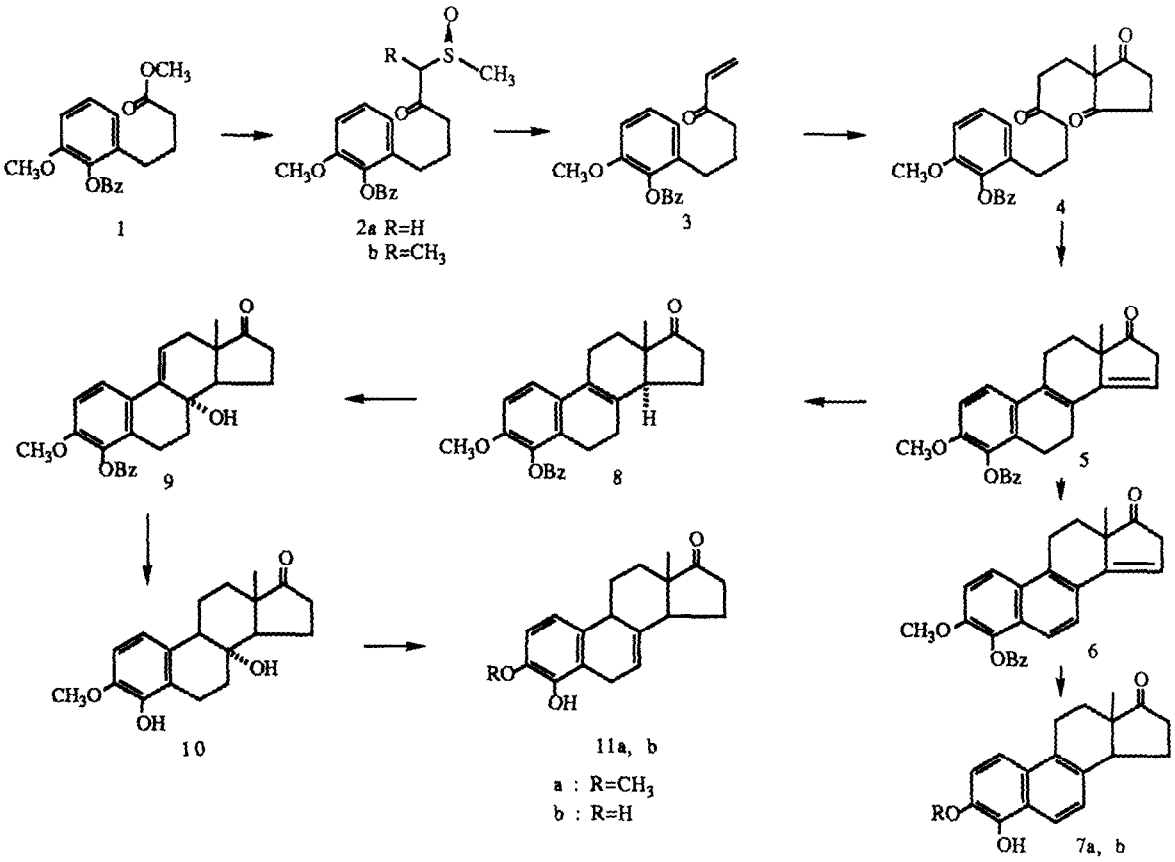
Methyl 4-(2-benzyloxy-4-methoxyphenyl)butyrate **(1)**, prepared from *o*-vanillin by the conventional method[19,20] was converted into the β-ketosulfoxide **(2a)** by treatment with dimsyl anion. When **2a** was methylated with methyliodide, the α-methylated β-ketosulfoxide **(2b)** was obtained as a mixture of diastereomers due to the asymmetry at the α carbon and the sulfur atoms. Without separation of these stereoisomers, thermal elimination of the methyl sulfinyl group was performed in refluxing xylene to give the corresponding enone **(3)** as the sole product. This product was then condensed with 2-methylcyclopentane-1,3-dione for construction of the D ring to furnish the Michael adduct **(4)**. Ring closure of the triketone **(4)** with methanesulfonic acid proceeded by dehydration to yield the cyclized estrahexaene **(5)** as a key compound.

Transformation of **5** into the equilenin type was effected by dehydrogenation with 2,3-dichloro-5,6-dicyanobenzoquinone to provide the estrapentaene **(6)**. Catalytic hydrogenation of **6** with 10% palladium on carbon was performed to generate the 14α-H adduct, yielding 4-hydroxyequilenin 3-methyl ether **(7a)**. The 14α configuration was confirmed by the ^1H NMR signal due to the C_{18} proton at δ 0.81, similar to those found for analogous 14α-steroidal compounds.[21]

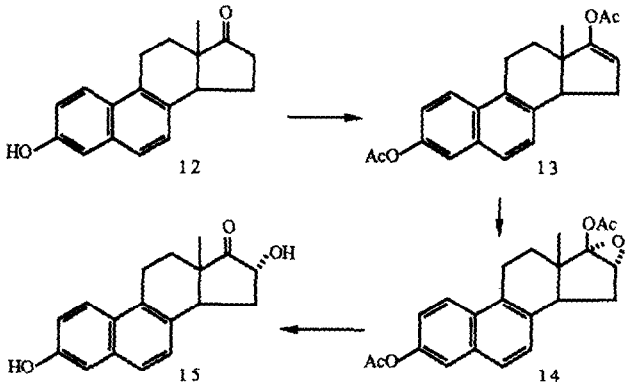
On the other hand, conversion of the hexaene **(5)** into the equilin type was performed by multistep reactions as follows. The Δ^{14}-double bond in **5** was first hydrogenated by the method described above to generate the 14α-H adduct. The resulting tetraene **(8)** was subjected to successive epoxidation with *m*-chloroperbenzoic acid and ring opening with benzoic acid, affording the estratetraen-8α-ol **(9)**. The styrenic bond in **9** was hydrogenated by the usual catalytic reduction to give the estratrien-8α-ol **(10)**. The orientation of the hydrogen at C-9 was presumed to be α because the hydrogen should preferentially attack from the α side in the molecule. Actually, the C_{18} proton signal in the ^1H NMR spectrum was shifted to δ 0.98, revealing a B/C-*cis* configurational assignment.[22] Dehydration of the 8α-ol **(10)** with thionyl chloride in pyridine provided the desired 4-hydroxyequilin 3-methyl ether **(11a)**. Finally, demethylation of the methyl ethers (**7a** and **11a**) was achieved on brief exposure to trimethylsilyliodide to give 4-hydroxyequilenin **(7b)** and 4-hydroxyequilin **(11b)**, respectively.

Synthesis of 16α-Hydroxyequilenin

Initially, selective 16α-bromination of equilin and equilenin with cupric bromide was attempted to prepare the 16α-bromo derivatives, which could be transformed into the 16α-hydroxy-17-ketosteroid by controlled alkaline hydrolysis.[23] Difficulties were encountered in the preparation of the brominated product, yielding only a complex mixture. It has been reported that 16α-hydroxyestrone can be synthesized by the ring opening of the 16α,17α-epoxy derivative, readily obtainable from estrone in two steps.[24] Using this procedure (Scheme 2), we prepared the Δ^{16}-enol-



Scheme 1



Scheme 2

acetate **(13)**, which was then oxidized with *m*-chloroperbenzoic acid to afford the epoxyacetate **(14)**. This material exhibited the signal for the 16β-proton at δ 4.07 in the ^{1}H NMR spectrum. On treatment with sulfuric acid in heated methanol, ring opening of the epoxide **(14)** was accomplished to yield 16α-hydroxyequilenin **(15)**. Unfortunately, this approach to obtain the equilin derivative caused dehydrogenation in the B ring, yielding an equilenin derivative. These synthetic samples, together with the 2-hydroxylated derivatives, may be helpful for structural elucidation in metabolic studies, and the details will be reported in the near future.

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equilenin. We also thank the staff of the Center for Instrumental Analysis of Hokkaido University for elemental analyses.

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