SYNTHESIS OF 2-METHOXY AND 4-METHOXY EQUINE ESTROGENS*

Pemmaraju N. Rao and Chandrasiri W. Somawardhana

Department of Organic Chemistry, Southwest Foundation for Biomedical Research, P.O. Box 28147, San Antonio, TX 78284, USA

Received October 21, 1986
Revised June 8, 1987

ABSTRACT

4-Methoxyequilin and 2-methoxyequilin were synthesized from the corresponding 4-bromoequilin and 2-iodoequilin derivatives, respectively, by nucleophilic displacement of halogen with methoxide ion in the presence of copper (II) chloride and 15-crown-5-ether. 4-Bromoequilin was prepared by reacting equilin with one equivalent of N-bromoacetamide. 2-Iodoequilin was prepared by reductive dehalogenation of 2,4-diiodoequilin, which in turn was obtained by treatment of equilin with two equivalents of iodine in methanolic ammonium hydroxide solution. 4-Methoxyequilenin and 2-methoxyequilenin were prepared from the corresponding 4-iodo- and 2-iodo-7ξ,8ξ-epoxyestrone derivatives, respectively. Nucleophilic displacement of iodine with methoxide ion was carried out as described earlier with simultaneous aromatization of the B ring leading to 4- and 2-methoxyequilenin derivatives. Alternatively, 4-methoxyequilenin was obtained from 4-methoxyequilin by selenium dioxide oxidation.

INTRODUCTION

It is well known that oxidative metabolism of steroidal estrogens in man results in aromatic hydroxylation either at the C-2 or C-4 position to yield the catechol derivatives (1,2). Catechol estrogens have been implicated in the mechanism of estrogen-mediated carcinogenesis (3-5). Subsequent methylation of the catechols results in 2- and 4-methoxy metabolites. Equine estrogen preparations containing 3-hydroxy-1,3,5(10),7-estratetraen-17-one (equilin) and 3-hydroxy-1,3,5(10),6,8-estrapentaen-17-one (equilenin) have been used for many years in estrogen replacement therapy (6,7), and, surprisingly, none of the metabolic studies on these hormones have been directed toward detection of 2- or 4-methoxy derivatives. After administration of [^{3}H]equilin sulfate to postmenopausal women and normal men, Bhavnani and associates (8,9) have identified 1,3,5(10),7-estratetraene-3,17β-diol (17β-dihydroequilin), equilenin, and 1,3,5(10),6,8-estrapentaene-3,17β-diol

(17β-dihydroequilenin) as some of the metabolites. However, they have shown that the largest fraction of radioactivity (70.5%) was present in the more polar, unknown metabolites (9). It is probable that catechol derivatives are present in this fraction. Paul *et al* (10) have developed a radioenzymatic assay for catechol estrogens, and Purdy *et al* (11) using this assay have determined the relative rates of 2- and 4-hydroxylation of several estrogens. This method involves methylation of catechol metabolites in the presence of catechol-O-methyltransferase and [methyl-^{3}H]-S-adenosylmethionine, extraction of the methyl ethers so formed with heptane, and then analysis by high performance liquid chromatography (hplc). The radiochromatographic peaks were identified by comparison with authentic samples of estrogen methyl ethers. This valuable analytical procedure could be used for positive identification of catechol metabolites of equilin and equilenin if authentic reference standards were available. In order to facilitate our studies with equine estrogens, we synthesized the 2- and 4-methoxy derivatives of equilin and equilenin.

EXPERIMENTAL

All solvents and chemicals were analytical reagent grade and were used without further purification. Triethyl orthoformate and tetrahydrofuran were distilled before use. Dry column chromatography was performed on Woelm silica gel in a nylon column as described by Loev and Goodman (12) with the modifications of Hadd and Caspi (13). Samples were dried under high vacuum at room temperature for 48 h before determining their melting points on a Thomas-Hoover Model 6406 capillary melting point apparatus. Optical rotations were recorded with an Autopol II automatic polarimeter at 589 nm at 24°C. Ultraviolet spectra were recorded in methanol solution using a Varian Cary 210 spectrophotometer. Infrared spectra were obtained in potassium bromide discs using a Perkin-Elmer Model 467 grating infrared spectrophotometer. Proton nmr spectra were recorded on a Varian EM-390 spectrometer, and chemical shifts were reported in ppm downfield from the internal tetramethylsilane standard. Mass spectra were obtained from a Finnigan Model MAT212 quadrupole mass spectrometer. Thin-layer chromatography was done on silica gel GF (Analtech) glass plates. Preparative hplc was carried out on a Waters Associates Prep LC/System 500 employing PrepPak-500 silica columns. Analytical hplc was carried out on a Waters Associates chromatograph, consisting of a system controller (Model 720), sample processor (WISP Model 710B), data module (Model 730), two high pressure pumps (Models 6000A and M45), and a variable wavelength LDC Spectromonitor III detector (Model 1204) employing Rainin Hibar RT Lichrosorb diol columns. Microanalyses were performed by Galbraith Laboratories, Inc., Knoxville, Tennessee.

4-Bromo-3-hydroxy-1,3,5(10),7-estratetraen-17-one (**2**)

To a stirred solution of equilin (**1**, 2.68 g, 10 mmol) in dichloromethane (400 mL) and ethanol (20 mL), a solution of N-bromoacetamide (1.41 g, 10.2 mmol) in ethanol (15 mL) was slowly added. The yellow color of N-bromoacetamide solution disappeared instantly. Stirring was continued for another 1 h, and the solvents were evaporated under vacuum. The residue was crystallized with a mixture of acetone and hexane to give 4-bromo-3-hydroxy-1,3,5(10),7-estratetraen-17-one (**2**, 3.09 g, 89% yield); mp 204-206°C; $[\alpha]$ +276° (c = 0.09, MeOH); λ_{max} 282 nm (ε = 1990), 288 nm (ε = 1990); ν_{max} 3560, 1742 cm^{-1}; δ 0.78(s, 3H, 18-CH_3), 5.56(m, 1H, C-7 H), 6.97(d, J = 8.7 Hz, 1H, aromatic), 7.17(d, J = 8.7 Hz, 1H, aromatic); m/e = 346.2, 348.1; Anal. calc'd for $C_{18}H_{19}O_2Br$: C, 62.26; H, 5.51; Br, 23.01; Found: C, 61.62; H, 5.64; Br, 23.75.

4-Bromo-3-hydroxy-17-ethylenedioxy-1,3,5(10),7-estratetraene (**3**)

Compound **2** (2.50 g, 7.2 mmol) was suspended in dichloromethane (200 mL), and ethylene glycol (3.43 mL, 61 mmol), triethyl orthoformate (3.43 mL, 22 mmol), and *p*-toluenesulfonic acid (130 mg, 0.68 mmol) were added. The resultant mixture was magnetically stirred. A clear solution was obtained after 15 min and stirring was continued for 2 h. Dichloromethane (200 mL) was added and the solution was washed with saturated sodium bicarbonate solution (2 x 200 mL) and brine (2 x 200 mL), and dried over anhydrous sodium sulfate. The solvent was evaporated and the syrup obtained was crystallized with methanol to give 4-bromo-3-hydroxy-17-ethylenedioxy-1,3,5(10),7-estratetraene (**3**) (2.68 g, 95% yield); mp 136-138°C; $[\alpha]$ +167° (c = 0.10, $CHCl_3$); λ_{max} 237 nm (ε = 1950), 288 nm (ε = 1165); ν_{max} 3560 cm^{-1}; δ 0.78(s, 3H, 18-CH_3), 3.98(m, 4H, -OCH_2CH_2O-), 5.42(d of d, J = 1.5 Hz, J = 1.5 Hz, 1H, C-7 H), 5.77(s, 1H, 3-OH), 6.95(d, J = 8.4 Hz, 1H), 7.15(d, J = 8.4 Hz, 1H); m/e = 390.1, 392.1; Anal. calc'd for $C_{20}H_{23}O_3Br$: C, 61.39; H, 5.92; Br, 20.42; Found: C, 61.40; H, 5.78; Br, 20.30.

3-Hydroxy-4-methoxy-17-ethylenedioxy-1,3,5(10),7-estratetraene (**4**)

Compound **3** (1.68 g, 4.28 mmol) and anhydrous cupric chloride (573 mg, 4.28 mmol) were dried under vacuum for 2 h at 100°C before use. Sodium (0.99 g, 42.8 mmol) was dissolved in anhydrous methanol (100 mL) under a nitrogen atmosphere. After complete dissolution of the metal, methanol was removed under a stream of nitrogen. To the white solid obtained, anhydrous N,N-dimethylformamide (30 mL), cupric chloride, and 15-crown-5 (0.70 mL, 3.5 mmol) were added at room temperature. The solution became blue in color, and compound **3** in N,N-dimethylformamide (10 mL) was added to the flask. The reaction flask was immersed in an oil bath maintained at 100°C. Blue color changed after 5 min and a metallic copper color appeared. After 15 min at 100°C, the reaction mixture was poured into water (200 mL) at room temperature, and the aqueous solution was acidified with dilute hydrochloric acid. A white precipitate formed, and the mixture was extracted with dichloromethane (3 x 100 mL). Combined organic layers were washed with saturated sodium bicarbonate solution (2 x 100 mL) and brine (2 x 100 mL). Organic phase was dried over anhydrous sodium sulfate and evaporated to dryness under vacuum. The residue was purified on a dry silica gel column using ether:hexane:benzene (1:5:4) to yield 3-hydroxy-4-methoxy-17-ethylenedioxy-1,3,5(10),7-estratetraene (**4**) (1.05 g, 72% yield); mp 168.5-169.5°C; $[\alpha]$ +176° (c = 0.11, MeOH); λ_{max} 281 nm (ε = 1390); ν_{max} 3370 cm^{-1}; δ 0.74(s, 3H, 18-CH_3), 3.80(s, 3H, -OCH_3), 3.92(m, 4H, -OCH_2CH_2O-), 5.45(d of d, J = 1.5 Hz, J =

1.5 Hz, 1H, C-7 H), 5.68(s, 1H, 3-OH), 6.86(d, J = 8.7 Hz, 1H), 6.94(d, J = 5.7 Hz, 1H); m/e = 343.3; Anal. calc'd for $C_{21}H_{26}O_4$: C, 73.66; H, 7.65; Found: C, 73.80; H, 7.52.

3-Hydroxy-4-methoxy-1,3,5(10),7-estratetraen-17-one (**5**)

Compound **4** (232 mg, 0.68 mmol) was dissolved in tetrahydrofuran (10 mL); then glacial acetic acid (30 mL) and water (10 mL) were added successively. The solution was stirred for 7 h at room temperature. The solvents were evaporated under a stream of nitrogen, and the syrup obtained was chromatographed on a column of dry silica gel using ether:hexane (6:4) as the eluent. 3-Hydroxy-4-methoxy-1,3,5(10),7-estratetraen-17-one (**5**) (196 mg, 97% yield) was isolated from the column and crystallized from a mixture of acetone and hexane; mp 179-180°C; $[\alpha]$ +290° (c = 0.1, MeOH); λ_{max} 282 nm (ε = 1430); ν_{max} 3470, 1736 cm^{-1}; nmr data reported in Table 1; m/e = 298.2; Anal. calc'd for $C_{19}H_{22}O_3$: C, 76.48; H, 7.43; Found: C, 76.80; H, 7.31.

2,4-Diiodo-3-hydroxy-1,3,5(10),7-estratetraen-17-one (**6**)

Equilin (**1**, 5.36 g, 20 mmol) was dissolved in methanol (1000 mL) and concentrated ammonium hydroxide solution (750 mL) was added. The solution was cooled in an ice bath, and iodine (10.21 g, 40.2 mmol) in methanol (250 mL) was added dropwise over a period of 30 min. The resulting colorless solution was stirred for an additional 1 h at ice-bath temperature. Glacial acetic acid (700 mL) was added, stirring was continued for 5 min, and the solvents were evaporated under reduced pressure. The residual syrup was dissolved in ethyl acetate (1000 mL) and washed with saturated sodium bicarbonate solution (2 x 300 mL) and brine (2 x 300 mL). Ethyl acetate solution was dried over anhydrous sodium sulfate and evaporated under reduced pressure. Crude product (11.40 g) was dissolved in a minimum amount of dichloromethane and then diluted with acetone to obtain pure crystals of 2,4-diiodo-3-hydroxy-1,3,5(10),7-estratetraen-17-one (**6**) (4.60 g, 44% yield); mp 141-148°C (dec); $[\alpha]$ +204° (c = 1.12, MeOH); λ_{max} 288 nm (ε = 2451), 299 nm (ε = 2266); ν_{max} 3340, 1720 cm^{-1}; δ 0.93(s, 3H, 18-CH_3), 5.50(d of d, J = 2.1 Hz, J = 2.1 Hz, 1H, C-7 H), 5.89(s, 1H, 3-OH), 7.94(s, 1H, C-1 H); m/e = 520.0; Anal. calc'd for $C_{18}H_{18}I_2O_2$: C, 41.56; H, 3.49; I, 48.80; Found: C, 41.89; H, 3.77; I, 49.23.

3-Hydroxy-2-iodo-1,3,5(10),7-estratetraen-17-one (**7**)

Compound **6** (3.7 g, 7.1 mmol) was dissolved in N,N-dimethylformamide (20 mL), ascorbic acid (1.78 g, 10.1 mmol) and formic acid (2.09 mL, 55.4 mmol) were added, and the reaction mixture was refluxed for 1.5 h. The reaction product was cooled to room temperature and poured into cold water (300 mL). The aqueous mixture was extracted with dichloromethane (5 x 250 mL), and the combined extracts were washed with saturated sodium bicarbonate solution (2 x 250 mL) and brine (300 mL). The organic solution was dried over anhydrous sodium sulfate and evaporated under reduced pressure. The reaction product was chromatographed on a dry silica gel column with ether:hexane (1:1) to isolate 3-hydroxy-2-iodo-1,3,5(10),7-estratetraen-17-one (**7**) (2.69 g, 96% yield). Compound **7** was dissolved in a minimum amount of tetrahydrofuran and diluted with ether to obtain crystals; mp 172-176°C (dec); $[\alpha]$ +240° (c = 0.33, MeOH); λ_{max} 270 nm (ε = 3940), 286 nm (ε = 3940); ν_{max} 3410, 1742 cm^{-1}; δ 0.61(s, 3H, 18-CH_3), 5.42(m, 1H, C-7 H), 6.71(s, 1H), 7.57(s, 1H); m/e = 394; Anal. calc'd for $C_{18}H_{19}IO_2$: C, 54.84; H, 4.86; I, 32.19; Found: C, 55.11; H, 5.01; I, 32.88.

3-Hydroxy-2-iodo-17-ethylenedioxy-1,3,5(10),7-estratetraene (**8**)

Compound **7** (2.80 g, 7.1 mmol) was dissolved in a minimum amount of tetrahydrofuran and the solution was diluted with dichloromethane (60 mL). Ethylene glycol (3.03 mL, 53 mmol), triethyl orthoformate (3.03 mL, 19 mmol), and *p*-toluenesulfonic acid (130 mg, 0.68 mmol) were added, and the mixture was stirred at room temperature for 2 h, according to the procedure described for the synthesis of compound **3**. The reaction product was chromatographed on a dry silica gel column using ether:hexane (1:1) as the solvent to give 3-hydroxy-2-iodo-17-ethylenedioxy-1,3,5(10),7-estratetraene (**8**) (2.14 g, 69% yield). We found that this product decomposed rapidly, and it was therefore characterized only by its proton nmr spectrum; δ 0.72(s, 3H, 18-CH_3), 3.93(m, 4H, -OCH_2CH_2O-), 5.43(m, 1H, 7-H), 6.75(s, 1H), 7.57(s, 1H). It was immediately used for the synthesis of 3-hydroxy-2-methoxy-17-ethylenedioxy-1,3,5(10),7-estratetraene (**9**).

3-Hydroxy-2-methoxy-17-ethylenedioxy-1,3,5(10),7-estratetraene (**9**)

Compound **8** (2.14 g, 4.89 mmol) was reacted in N,N-dimethylformamide solution (60 mL) with sodium methoxide [freshly prepared from sodium (1.13 g, 4.92 mmol) and methanol] in the presence of anhydrous cupric chloride (0.66 mg, 4.92 mmol) and 15-crown-5-ether (1.44 mL, 7.26 mmol), according to the procedure described for the synthesis of compound **4**. The reaction mixture was chromatographed on a dry silica gel column using ether:hexane (4:6) to yield the pure sample of 3-hydroxy-2-methoxy-17-ethylenedioxy-1,3,5(10),7-estratetraene (**9**); mp 180-181.5°C; [α] +145° (c = 0.26, MeOH); λ_{max} 237 nm (ε = 2139); ν_{max} 3430 cm^{-1}; δ 0.73(s, 3H, 18-CH_3), 3.85(s, 3H, -OCH_3), 3.92(m, 4H, -OCH_2CH_2O-), 5.44(d of d, J = 2.1 Hz, J = 2.1 Hz, 1H, C-7 H), 5.67(s, 1H, 3-OH), 6.69(s, 1H), 6.72(s, 1H); m/e = 342.2; Anal. calc'd for $C_{21}H_{26}O_4$: C, 73.66; H, 7.65; Found: C, 73.50; H, 7.54.

3-Hydroxy-2-methoxy-1,3,5(10),7-estratetraen-17-one (**10**)

Compound **9** (1.29 g, ~3.78 mmol) was treated with a mixture of tetrahydrofuran: glacial acetic acid:water (1:3:1) (30 mL), according to the procedure described for the synthesis of compound **5**. The reaction product was purified by hplc on a Chromegabond diol column using 5% ethanol in heptane as the solvent system to give 3-hydroxy-2-methoxy-1,3,5(10),7-estratetraen-17-one (**10**); mp 189°; [α] +246° (c = 0.39, MeOH); λ_{max} 287 nm (ε = 2705); ν_{max} 3380, 1728 cm^{-1}; nmr data are reported in Table 1; m/e = 298.2; Anal. calc'd for $C_{19}H_{22}O_3$: C, 76.48; H, 7.43; Found: C, 76.38; H, 7.61.

7ξ,8ξ-Epoxy-3-hydroxy-1,3,5(10)-estratrien-17-one (**11**)

Equilin (**1**, 2.5 g, 9.3 mmol) was suspended in dichloromethane (600 mL) and 0.5 *M* sodium bicarbonate solution (500 mL) was added. *m*-Chloroperbenzoic acid (5 g) in dichloromethane (200 mL) was added slowly to the stirred steroid suspension during a period of 30 min. The mixture was stirred for an additional 1 h and the organic phase was separated and successively washed with 10% sodium bisulfite solution (2 x 150 mL), saturated sodium bicarbonate solution (2 x 150 mL), and brine (2 x 150 mL). Dichloromethane solution was dried over anhydrous sodium sulfate and evaporated to dryness under vacuum. The solid obtained was crystallized with a mixture of acetone:hexane to give 7ξ,8ξ-epoxy-3-hydroxy-1,3,5(10)-estratrien-17-one (**7**)

(2.55 g, 96% yield); mp 190-228°C; [α] +255° (c = 0.11, MeOH); λ_{max} 279 nm (ε = 1940), 285 nm (ε = 1836); ν_{max} 3340, 3010, 1730 cm^{-1}; δ 0.95(s, 3H, 18-CH_3), 5.43(s, 1H, 3-OH), 6.54(d, J = 2.4 Hz, 1H, C-4 H), 6.65(d of d, J = 7.8 Hz, J = 2.4 Hz, 1H, C-2 H), 6.93(d, J = 7.8 Hz, 1H, C-1 H); m/e = 284.2; Anal. calc'd for $C_{18}H_{20}O_3$: C, 76.03; H, 7.09; Found: C, 76.32; H, 7.18.

7ξ,8ξ-Epoxy-3-hydroxy-4-iodo-1,3,5(10)-estratrien-17-one (**12**)
and 7ξ,8ξ-Epoxy-3-hydroxy-2-iodo-1,3,5(10)-estratrien-17-one (**16**)

Compound **11** (4.03 g, 14.2 mmol) was dissolved in methanol (400 mL) and concentrated ammonium hydroxide (350 mL) was added. The solution was cooled in an ice bath and a solution of iodine (3.6 g, 14.2 mmol) in methanol (250 mL) was added during a period of 30 min. The resulting colorless solution was stirred for 1 h at room temperature; then glacial acetic acid (300 mL) was added and stirring continued for another 5 min. The solvents were evaporated under vacuum and the residual syrup was dissolved in ether (1 L) and washed with a solution of saturated sodium bicarbonate (3 x 300 mL) and brine (2 x 300 mL). The organic layer was dried over anhydrous sodium sulfate and evaporated under reduced pressure. Thin-layer chromatography using ether:hexane (9:1) as solvent showed that the residue contained 4-iodo-, 2-iodo-, 2,4-diiodo-epoxides, and unreacted starting material (**11**). The 4-iodo compound (**12**) was separated from the 2-iodo derivative (**16**) by chromatography on a dry silica gel column using ether:hexane (9:1) as the eluent. 7ξ,8ξ-Epoxy-3-hydroxy-4-iodo-1,3,5(10)-estratrien-17-one (**12**) was obtained in 30% yield (1.74 g); mp 120-126°C (decomp.); [α] +193° (c = 0.11, MeOH); λ_{max} 238 nm (ε = 4895), 282 nm (ε = 2483); ν_{max} 3250, 1720 cm^{-1}; δ 0.97(s, 3H, 18-CH_3), 5.48(s, 1H, 3-OH), 6.88(d, J = 8.7 Hz, 1H), 6.97(d, J = 8.7 Hz, 1H); m/e = 410; Anal. calc'd for $C_{18}H_{19}IO_3$: C, 52.70; H, 4.67; I, 30.93; Found: C, 52.91; H, 4.74; I, 30.78.

The 7ξ,8ξ-epoxy-3-hydroxy-2-iodo-1,3,5(10)-estratrien-17-one (**16**) separated by dry column chromatography was slightly contaminated with starting compound (**11**). It was finally purified by hplc on a silica gel column using 5% ethyl acetate in dichloromethane as the eluent to give pure 7ξ,8ξ-epoxy-3-hydroxy-2-iodo-1,3,5(10)-estratrien-17-one (**16**) in 13% yield (0.75 g); mp 160°C (decomp.); [α] +164° (c = 0.11, MeOH); λ_{max} 238 nm (ε = 4625), 285 nm (ε = 3133), 292 nm (ε = 3245); ν_{max} 3390, 1720 cm^{-1}; δ 0.93(s, 3H, 18-CH_3), 6.63(s, 1H), 7.38(s, 1H); m/e = 410; Anal. calc'd for $C_{18}H_{19}IO_3$: C, 52.70; H, 4.67; I, 30.93; Found: C, 52.91; H, 4.80; I, 30.91.

3-Hydroxy-4-methoxy-17-ethylenedioxy-1,3,5(10),6,8-estrapentaene (**14**)

Compound **12** (650 mg, 1.6 mmol) was treated with ethylene glycol (0.73 mL, 13 mmol), triethyl orthoformate (0.73 mL, 4.64 mmol), and *p*-toluenesulfonic acid (34 mg, 0.17 mmol) for 2 h, according to the procedure described for the synthesis of compound **3**. 7ξ,8ξ-Epoxy-3-hydroxy-4-iodo-17-ethylenedioxy-1,3,5(10)-estratriene (**13**) (418 mg, 58% yield) was obtained after purification by dry column chromatography on silica gel. It was characterized only by its proton nmr spectrum and used in the next step since it decomposes readily; δ 0.92(s, 3H, 18-CH_3), 3.92(m, 4H, -OCH_2CH_2O-), 6.85(d, J = 8.4 Hz, 1H), 6.95(d, J = 8.4 Hz, 1H).

Compound **13** (1.0 g, 2.2 mmol) was reacted in N,N-dimethylformamide (30 mL) with sodium methoxide [freshly prepared from sodium (578 mg, 25 mmol) and methanol] in the presence of anhydrous cupric chloride (335 mg, 2.5 mmol) and

15-crown-5-ether (0.75 mL, 3.8 mmol) according to the procedure described for the synthesis of compound **4**. The reaction product was chromatographed on a dry silica gel column using ether:hexane (1:1) as the eluent. 3-Hydroxy-4-methoxy-17-ethylenedioxy-1,3,5(10),6,8-estrapentaene (**14**) (654 mg, 87% yield) was obtained from the column; mp 137-138°C; $[\alpha]$ -45° (c = 0.08, MeOH); λ_{max} 274 nm (ε = 4582), 285 nm (ε = 5673), 295 nm (ε = 4473); ν_{max} 3420 cm^{-1}; δ 0.77(s, 3H, 18-CH_3), 3.93(m, 7H, -OCH_2CH_2O- and -OCH_3), 7.19(d, J = 9.0 Hz, 1H), 7.29(d, J = 9.0 Hz, 1H), 7.72(d, J = 9.1 Hz, 1H), 7.82(d, J = 9.2 Hz, 1H); m/e = 340.2; Anal. calc'd for $C_{21}H_{24}O_4$: C, 74.09; H, 7.11; Found: C, 73.85; H, 7.12.

3-Hydroxy-4-methoxy-1,3,5(10),6,8-estrapentaen-17-one (**15**)

Method A. 3-Hydroxy-4-methoxy-17-ethylenedioxy-1,3,5(10),6,8-estrapentaene (**14**) (600 mg, 1.8 mmol) was dissolved in tetrahydrofuran (5 mL); then glacial acetic acid (15 mL) and water (5 mL) were added. The resulting solution was refluxed for 15 min and the solvents were evaporated under a stream of nitrogen. The residue was chromatographed on a column of dry silica gel using ether:hexane (6:4) as the eluent. 3-Hydroxy-4-methoxy-1,3,5(10),6,8-estrapentaen-17-one (**15**) (489 mg, 94% yield) was separated from the column; mp 208-210°C; $[\alpha]$ +51° (c = 0.07, $CHCl_3$); λ_{max} 238 nm (ε = 53,760), 274 nm (ε = 3360), 285 nm (ε = 4480), 297 nm (ε = 3600), 329 nm (ε = 1440), 342 nm (ε = 2080); ν_{max} 3420, 1735 cm^{-1}; nmr data are reported in Table 1; m/e = 296.2; Anal. calc'd for $C_{19}H_{20}O_3$: C, 77.00; H, 6.80; Found: C, 76.91; H, 6.91.

Method B. Compound **5** (290 mg, ~1 mmol) was dissolved in *tert*-butanol (18 mL); then pyridine (0.09 mL) and selenium dioxide (128 mg, 1.2 mmol) were added. The mixture was refluxed for 1.5 h and then cooled to room temperature. The solvent was evaporated under a stream of nitrogen, and the residue was chromatographed on a column of dry silica gel using benzene:ether (1:1) as the eluent. 3-Hydroxy-4-methoxy-1,3,5(10),6,8-estrapentaen-17-one (**15**) was isolated from the column, then crystallized from acetone to give 125 mg (43% yield) of compound **15**; mp 208-210°C. The ir, uv, and nmr spectra were found to be identical with the sample prepared by Method A.

3-Hydroxy-2-methoxy-17-ethylenedioxy-1,3,5(10),6,8-estrapentaene (**18**)

Compound **16** (333 mg, 0.8 mmol) was treated with ethylene glycol (0.37 mL, 6.5 mmol), triethyl orthoformate (0.37 mL, 2.3 mmol), and *p*-toluenesulfonic acid (17 mg) for 2 h, according to the procedure described for the synthesis of compound **3**. 7ξ,8ξ-Epoxy-3-hydroxy-2-iodo-17-ethylenedioxy-1,3,5(10)-estratriene (**17**) (224 mg, 61% yield) was obtained after column chromatography on dry silica gel using ether:hexane (9:1) as the eluent. Compound **17** has a tendency to decompose easily and was therefore used in the next step immediately. It was characterized only by its proton nmr spectrum; δ 0.90(s, 3H, 18-CH_3), 3.91(m, 4H, -OCH_2CH_2O-), 6.57(s, 1H), 7.33(s, 1H). Compound **17** (218 mg, 0.48 mmol) was heated with anhydrous cupric chloride (73 mg, 0.54 mmol), sodium methoxide [freshly prepared from sodium (128 mg, 5.57 mmol) and methanol], and 15-crown-5-ether (0.16 mL, 0.8 mmol) in N,N-dimethylformamide (30 mL), according to the procedure described for the synthesis of compound **14**. 3-Hydroxy-2-methoxy-17-ethylenedioxy-1,3,5(10),6,8-estrapentaene (**18**) (116 mg, 71% yield) was isolated; mp 183-184°C; $[\alpha]$ -42° (c = 0.37, MeOH); λ_{max} 278 nm (ε = 3588); ν_{max} 3420 cm^{-1}; δ 0.77(s, 3H, 18-CH_3), 3.97(m, 7H, -OCH_2CH_2O- and

-OCH_3), 7.11(d, J = 8.4 Hz, 1H), 7.19(s, 1H), 7.25(s, 1H), 7.52(d, J = 8.4 Hz, 1H); m/e = 340.1; Anal. calc'd for $C_{21}H_{24}O_4$: C, 74.09; H, 7.11; Found: C, 74.39; H, 7.36.

3-Hydroxy-2-methoxy-1,3,5(10),6,8-estrapentaen-17-one (**19**)

Compound **18** (333 mg, 1 mmol) was heated with a mixture of tetrahydrofuran (3 mL), acetic acid (9 mL), and water (3 mL) for 15 min. Then the reaction mixture was worked up according to the Method A described for the synthesis of compound **15**. 3-Hydroxy-2-methoxy-1,3,5(10),6,8-estrapentaen-17-one (**19**) (274 mg, 95% yield) was recovered; mp 251-252°C; $[\alpha]$ +53° (c = 0.076, MeOH); λ_{max} 278 nm (ε = 4602), 289 nm (ε = 4680), 300 nm (ε = 3822), 315 nm (ε = 2340), 330 nm (ε = 3744); ν_{max} 3400, 1720 cm^{-1}; nmr data are reported in Table 1; m/e = 296.2; Anal. calc'd for $C_{19}H_{20}O_3$: C, 77.00; H, 6.80; Found: C, 77.06; H, 6.73.

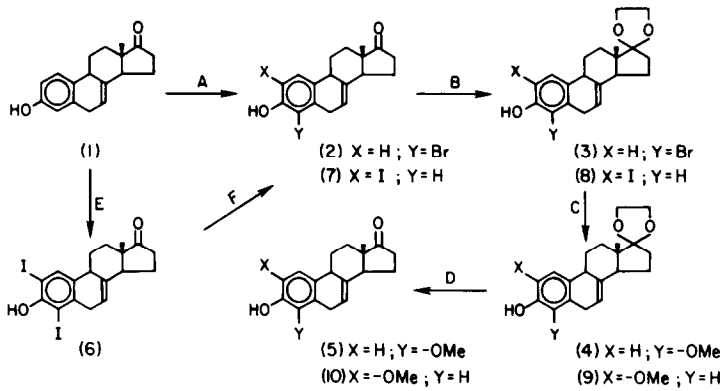
RESULTS AND DISCUSSION

We have previously reported (14) a novel, one-step procedure which involves nucleophilic displacement of the halogen atom of 2-bromoestradiol by methoxide ion under the catalysis of copper (I) iodide, to give the corresponding 2-methoxyestradiol. Following our procedure (with some modifications) Numazawa and Ogura (15) have converted 2- and 4-halogenated estrogens into 2- and 4-methoxy estrogens, and Zheng *et al* (16) have prepared 2,4-dimethoxy estrogens from the corresponding 2,4-dibromoestrogen derivatives. To synthesize the 2- and 4-methoxy derivatives of equilin and equilenin by this procedure, the corresponding 2- and 4-halogenated substrates were required. A number of methods for bromination at the C-2 and C-4 positions of steroidal estrogens have been described in the literature (17-19). When these procedures were employed for the preparation of 2- and 4-bromo derivatives of equilin and equilenin, we obtained only the 4-bromo derivatives, and none of the 2-bromo compounds were discernible. Apparently the C-4 position in equilin and equilenin is more reactive toward electrophilic halogenation than the C-2 position.

However, when equilin was reacted with two equivalents of iodine in methanolic ammonium hydroxide solution (20), we obtained 2,4-diiodoequilin. In view of the greater reactivity of the C-4 position in equilin, we reasoned that reductive dehalogenation of 2,4-diiodoequilin might give the desired 2-iodoequilin. Recently, Numazawa *et al* (21) have shown that 2,4-dihalogenated estrogens can be selectively dehalogenated to give the 2-halogenated derivative. Using this method, 2,4-diiodoequilin was reacted with a mixture of formic acid and ascorbic acid in

N,N-dimethylformamide to obtain 2-iodoequilin in good yield. It is essential to carry out the reductive dehalogenation in the presence of ascorbic acid. When formic acid alone was employed, the equilin derivative was oxidized to the equilenin derivative (20).

Scheme I. Synthesis of 2- and 4- Methoxyequilin Derivatives



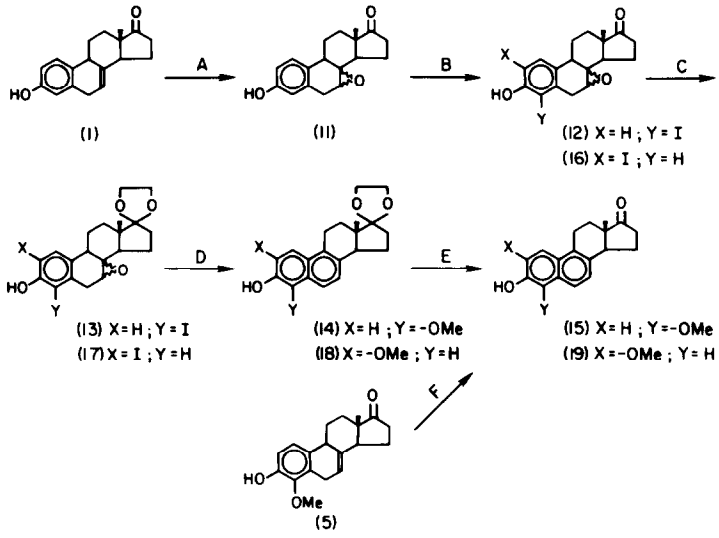
Reagents: (A) CH_2Cl_2, EtOH, N-bromoacetamide
(B) $OH(CH_2)_2OH$, $CH(OEt)_3$, tetrahydrofuran, *p*-TsOH·H_2O
(C) NaOMe, $CuCl_2$, 15-crown-5-ether, N,N-dimethylformamide
(D) AcOH, tetrahydrofuran, H_2O (3:1:1)
(E) I_2 (2 equiv), NH_4OH, MeOH
(F) HCOOH, ascorbic acid, N,N-dimethylformamide

2- and 4-Methoxyequilin derivatives

The synthetic sequence employed for the preparation of 2- and 4-methoxyequilin derivatives is presented in Scheme I. Equilin (**1**) was brominated with N-bromoacetamide to give 4-bromoequilin (**2**). The 17-oxo group in compound **2** was protected by reacting with ethylene glycol, triethyl orthoformate, and *p*-toluenesulfonic acid in tetrahydrofuran solution at room temperature to give the 17-ethylene ketal (**3**). Nucleophilic displacement of the bromine at C-4 in compound **3** by methoxide ion was carried out by phase transfer catalysis with sodium methoxide in N,N-dimethylformamide in the presence of copper (II) chloride and 15-crown-5-ether

to give the 4-methoxy derivative (**4**). The 17-ethylene ketal (**4**) was treated with dilute acetic acid in tetrahydrofuran solution to remove the protective group and yield 4-methoxyequilin (**5**). When equilin (**1**) was reacted with two equivalents of iodine in methanolic ammonium hydroxide solution, 2,4-diiodoequilin (**6**) was obtained in good yield. Treatment of the diiodo derivative (**6**) with a mixture of formic acid and ascorbic acid in N,N-dimethylformamide resulted in 2-iodoequilin (**7**). The 2-iodo compound (**7**) was then converted to 2-methoxyequilin (**10**) by the same sequence of reactions described earlier for the preparation of **5**.

Scheme II. Synthesis of 2- and 4- Methoxyequilenin Derivatives



Reagents: (A) *m*-Chloroperbenzoic acid, CH_2Cl_2
(B) I_2 (1 equiv), NH_4OH, MeOH
(C) $OH(CH_2)_2OH$,$CH(OEt)_3$, tetrahydrofuran, *p*-TsOH·H_2O
(D) NaOMe, $CuCl_2$, 15-crown-5-ether, N,N-dimethylformamide
(E) AcOH, tetrahydrofuran, H_2O (3:1:1)
(F) SeO_2, *t*-BuOH, pyridine

2- and 4-Methoxyequilenin derivatives

It has been reported (22) that equilenin can be readily prepared from equilin by a two-step reaction process which involves preparation of 7ξ,8ξ-epoxyestrone and

subsequent cleavage of the epoxide to give equilenin. This appeared very attractive and we decided to employ 7ξ,8ξ-epoxyestrone (**11**) (Scheme II) as the starting material for our projected synthesis of 2- and 4-methoxyequilenin derivatives. Since epoxidation of equilin eliminated unsaturation in the B ring, halogenation of 7ξ,8ξ-epoxyestrone (**11**) was expected to yield the desired 2- and 4-halogenated derivatives. Accordingly, when the epoxy compound (**11**) was reacted with one equivalent of iodine in methanolic ammonium hydroxide solution, a mixture of 4- and 2-iodo compounds (**12**) and (**16**) was obtained. The 4-iodo derivative (**12**), which predominated in the reaction mixture, was separated from the 2-iodo derivative (**16**) by chromatography. The 17-oxo group in compounds (**12**) and (**16**) was protected by reacting with ethylene glycol to give the 17-ethylene ketal derivatives (**13**) and (**17**). Nucleophilic displacement of the iodine in compounds **13** and **17** was carried out by phase transfer catalysis with sodium methoxide in N,N-dimethylformamide in the presence of copper (II) chloride and 15-crown-5-ether, with simultaneous aromatization of the B ring, to give 4- and 2-methoxyequilenin derivatives (**14**) and (**18**), respectively. Finally, removal of the protecting group at C-17 in these compounds was effected by treatment with dilute acetic acid in tetrahydrofuran solution, yielding the 4- and 2-methoxyequilenin derivatives (**15**) and (**19**), respectively. Alternatively, 4-methoxyequilenin (**15**) was prepared by oxidation of 4-methoxyequilin (**5**) with selenium dioxide (23). The proton nmr spectral data as reported in Table 1 support the structures assigned to the methoxy derivatives (**5**), (**10**), (**15**), and (**19**).

Compound (**5**) shows two doublets at 6.86(J = 8.7 Hz) and 6.93(J = 8.7 Hz) ppm, indicating ortho coupling of C-1 and C-2 aromatic protons. The multiplet at 5.56 and a singlet at 3.80 ppm are attributed to the olefinic proton at C-7 and the methyl protons of the 4-methoxy group, respectively. 4-Methoxyequilenin (**15**) gives four doublets at 7.29(J = 8.6 Hz), 7.36(J = 8.6 Hz), 7.76(J = 9.1 Hz), and 7.92(J = 9.1 Hz) ppm, characteristic of the ortho coupling of the two aromatic protons at C-1 and C-2 and at C-6 and C-7. A singlet at 3.97 ppm represents the methyl protons of the 4-methoxy group. In 2-methoxyequilin (**10**) the two aromatic protons at C-1 and C-4 resonate at

6.72 ppm as a singlet with no visible coupling and confirm the para-orientation of these protons. The olefinic proton at C-7 appears as a multiplet at 5.57 ppm and the methyl protons of the 2-methoxy group appear as a singlet at 3.87 ppm. 2-Methoxyequilenin (**19**) shows two doublets at 7.22 (J = 8.4 Hz) and 7.61(J = 8.4 Hz) ppm, which represent the aromatic protons of C-6 and C-7. Aromatic protons of C-1 and C-4 appear as singlets at 7.23 and 7.30 ppm, with no visible coupling. A singlet at 4.04 ppm represents the methyl protons of the 2-methoxy group.

TABLE 1. PROTON NMR DATA OF 2-METHOXY AND 4-METHOXY EQUINE ESTROGENS

	(δ) ppm Downfield from Tetramethylsilane			
Compound	18-CH_3	-OCH_3	7-H	Aromatic Ring Protons
4-Methoxyequilin (**5**)	0.77 (s)	3.80 (s)	5.56 (m)	6.86 (d, J = 8.7 Hz), 6.93 (d, J = 8.7 Hz)
2-Methoxyequilin (**10**)	0.76 (s)	3.87 (s)	5.57 (m)	6.72 (s)
4-Methoxyequilenin (**15**)	0.80 (s)	3.97 (s)		7.29 (d, J = 8.6 Hz), 7.36 (d, J = 8.6 Hz), 7.76 (d, J = 9.1 Hz), 7.92 (d, J = 9.1 Hz)
2-Methoxyequilenin (**19**)	0.76 (s)	4.04 (s)		7.22 (d, J = 8.4 Hz), 7.23 (s), 7.30 (s), 7.61 (d, J = 8.4 Hz)

We anticipate using the 2- and 4-methoxy equine estrogens as reference standards in radioenzymatic assay by hplc to study catechol estrogen formation, and the chromatographic profile is presented in Figure 1.

ACKNOWLEDGMENT

This work was supported in part by a research fellowship grant (C.W. Somawardhana) from the Ruth Chapman Cowles and Andrew G. Cowles Memorial Trust.

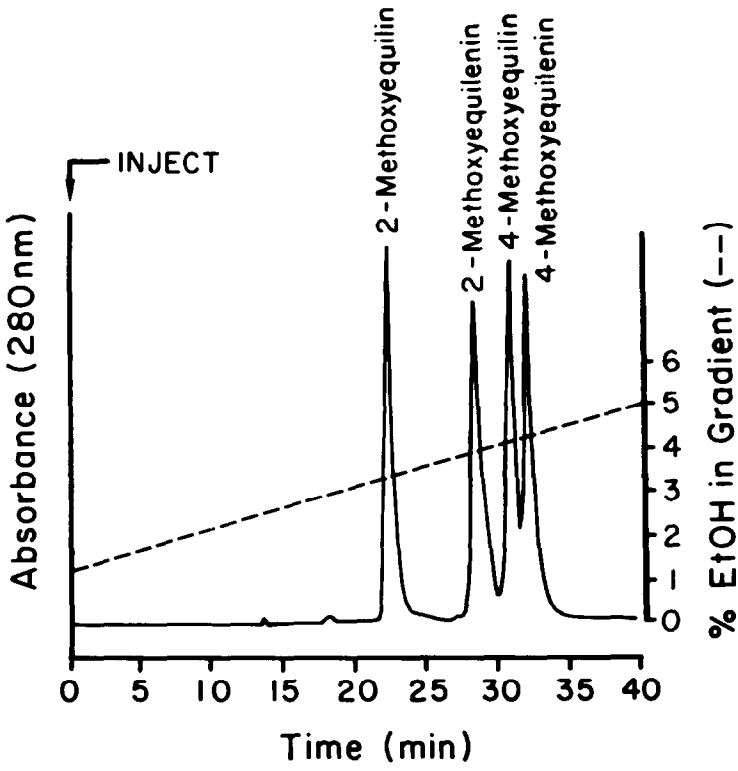


Figure 1. High performance liquid chromatographic profile of 2- and 4-methoxy derivatives of equilin and equilenin. Two Rainin Hibar RT Lichrosorb diol (5 μm, 4 mm x 25 cm) columns were used in series. A linear gradient (number 6, Waters 720 system controller) was employed in the separation, consisting of solvent A (12.5% ethanol in heptane) and solvent B (heptane) using 10 → 90% solvent A over a period of 80 min at a flow rate of 1.5 mL/min. A variable wavelength LDC Spectromonitor III at 280 nm was used for detection.

REFERENCES

1. Ball, P. and Knuppen, R., ACTA ENDOCRINOL (Suppl) 232, 1 (1980).
2. Fishman, J., NEUROENDOCRINOLOGY 22, 363 (1976).
3. Purdy, R.H., PROG. CANCER RES THER 31, 401 (1984).
4. Li, S.A., Klicka, J.K., and Li, J.J., CANCER RES 45, 181 (1985).
5. Liehr, J.G., Stancel, G.M., Chorich, L.P., Bousfield, G.R., and Ulubelen, A.A., CHEM BIOL INTERACT 59, 173 (1986).

6. Pan, C.C., Woolever, C.A., and Bhavnani, B.R., J CLIN ENDOCRINOL METAB 61, 499 (1985).
7. Bhavnani, B.R., Sarda, I.R., and Woolever, C.A., J CLIN ENDOCRINOL METAB 52, 741 (1981).
8. Bhavnani, B.R., Woolever, C.A., Benoit, H., and Wong, T., J CLIN ENDOCRINOL METAB 56, 1048 (1983).
9. Bhavnani, B.R. and Woolever, C.A., J STEROID BIOCHEM 17, 217 (1982).
10. Paul, S.M., Purdy, R.H., Hoffman, A.R., and Axelrod, J., in: *Catechol Estrogens* (Merriam, G.R. and Lipsett, M.B., Editors), Raven Press, New York (1983), pp 83-90.
11. Purdy, R.H., Moore, P.H., Jr., Williams, M.C., Goldzieher, J.W., and Paul, S.M., FEBS LETT 138, 40 (1982).
12. Loev, G. and Goodman, M.M., PROG SEPAR PURIF 3, 73 (1970).
13. Hadd, H. and Caspi, E., J CHROMATOGR 71, 353 (1972).
14. Rao, P.N. and Burdett, J.E., Jr., SYNTHESIS, No. 3, 168 (1977).
15. Numazawa, M. and Ogura, Y., J CHEM SOC, CHEM COMMUN, No. 9, 533 (1983).
16. Zheng, X.-H., Wang, W.-L., Zhong, Z.-Z, Xu, Z.-B., and Zhao, H.-M., STEROIDS 40, 121 (1982).
17. Zontova, V.N., Rzheznikov, V.M., and Pivnitsky, K.K., STEROIDS 25, 827 (1975).
18. Wilbur, D.S. and O'Brien, H.A., Jr., J ORG CHEM 47, 359 (1982).
19. Horiuchi, C.A. and Satoh, J.Y., J CHEM SOC, CHEM COMMUN, No. 12, 671 (1982).
20. Rao, P.N., Purdy, R.H., Moore, P.H., Jr., and Goldzieher, J.W., J STEROID BIOCHEM 13, 1291 (1980).
21. Numazawa, M., Kimura, K., Ogata, M., and Nagaoka, M., J ORG CHEM 50, 5421 (1985).
22. Ginsig, R., U.S. Patent 3,647,831 (March 7, 1972). CHEM ABSTR 76, Abstr No. 141192V (1972).
23. Rao, P.N., Jacob, E.J., and Axelrod, L.R., J CHEM SOC C, (17) 2855 (1971).

*Presented at the VII International Congress on Hormonal Steroids, Madrid, Spain, September 21-27, 1986.