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Active Nonaromatic Intermediates in the Conversion of Steroidal Estrogens into Catechol Estrogens†

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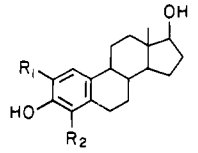
ABSTRACT: A mechanism is proposed for mixed-function oxidase-catalyzed formation of the catechol estrogens 2-hydroxy- and 4-hydroxyestradiol from estradiol. This mechanism involves nonaromatic epoxyenones as intermediates. The isomeric 1α,2α-epoxy-17β-hydroxyestr-4-en-3-one and 1β,2β-epoxy-17β-hydroxyestr-4-en-3-one (the latter as its 17-acetate) were synthesized from 17β-hydroxy-5α-estran-3-one. The isomeric 4α,5α-epoxy-17β-hydroxyestr-1-en-3-one and 4β,5β-epoxy-17β-hydroxyestr-1-en-3-one were prepared from 19-nortestosterone. From incubations of [6,7-^{3}H]estradiol with microsomes from MCF-7 human breast cancer cells, which principally catalyze the formation of 2-hydroxyestradiol from estradiol, we were able to isolate a ^{3}H-labeled product with the chromatographic properties of 1β,2β-epoxy-17β-hydroxyestr-4-en-3-one (as its 17-acetate). The soluble protein fraction of homogenates of rat liver, which is devoid of estrogen 2-/4-hydroxylase activity, has been shown to catalyze the formation of 2- and 4-hydroxyestradiol from the 1α,2α-epoxide and from the 4α,5α- and 4β,5β-epoxides, respectively. We suggest that these results taken together strongly support a role for epoxyenones as intermediates in the formation of catechol estrogens.

The formation of catechol estrogens from natural and synthetic steroidal estrogens is the major pathway of metabolism of these hormones (Ball & Knuppen, 1980; MacLusky et al., 1981; Fishman, 1983). In vivo, the endogenous female sex hormone estradiol (**1**) is metabolized in large part to 2-hydroxyestradiol (**2**) and in a much smaller amount to 4-hydroxyestradiol (**3**). The formation of catechol estrogens has been demonstrated to occur in a wide variety of normal and neoplastic tissues in experimental animals and humans (Poth et al., 1983). It is now apparent that catechol estrogens cannot be regarded merely as weak estrogens, since they bind to estrogen receptors and have biological effects as agonists or antagonists of the parent hormone (MacLusky et al., 1983; Schneider et al., 1984).



1 $R_1 = R_2 = H$
2 $R_1 = OH$, $R_2 = H$
3 $R_1 = H$, $R_2 = OH$

The formation of catechol estrogens is catalyzed by P-450 mixed-function oxidase (estrogen 2-/4-hydroxylases) found in numerous tissues, with highest activity in the mammalian liver (Poth et al., 1983). Estrogen 2-hydroxylase activity from mouse liver represents the contribution of at least four, and from rat liver of five, separable forms of mixed-function oxidases (Lang & Nebert, 1981; Ryan et al., 1982). However,

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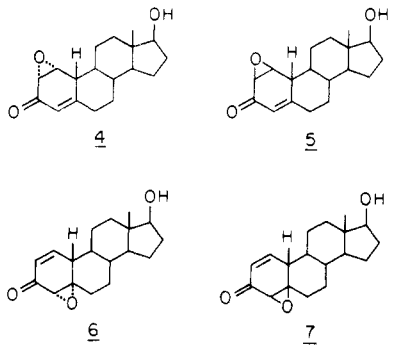
such purified reconstituted monooxygenases have so far been used principally to study the mechanism of the oxidation of nonsteroidal aromatic substrates such as benzo[*a*]pyrene. Catechol estrogen formation catalyzed by liver estrogen 2-hydroxylases is known to proceed without migration of hydrogen in an overt NIH shift (Nambara et al., 1971) and not to inolve an isotope effect (Fishman et al., 1970; Numazawa et al., 1977), but other mechanistic details are obscure. Consequently, the exact nature and the biological activities of putative intermediates (Soloway & Le Quesne, 1980) have remained conjectural.

In contrast to tumor formation induced by benzo[*a*]pyrene, the occurrence of estrogen-induced carcinogenesis is still the subject of considerable debate. The synthetic estrogen diethylstilbestrol has been classified as a carcinogen by the International Agency for Research on Cancer on the basis of a positive association between exposure and the occurrence of various cancers in humans, squirrel monkeys, and rodents (Tomatis et al., 1978). The continuous administration of estradiol or other estrogens produces a wide variety of cancers in experimental animals (Jull, 1976). In humans, the estrogen-responsive breast and endometrium are not believed to develop cancer unless they are stimulated by estrogens, which also promote the growth of these tissues (Lipsett, 1979). It is therefore not surprising that estrogens are usually referred to as tumor promoters (Upton et al., 1984) rather than as inducers of cellular transformation. In 1980, Le Quesne et al. proposed that estradiol is converted into catechol estrogens via ring A dihydrophenolic epoxides, which can be stabilized as their enone epoxide tautomers (**4**–**7**). A comparative study



of the ability of **4**, **6**, and **7** to induce the neoplastic transformation of the A-31-1-13 subclone of Balb/c 3T3 cells showed that **6** was the most active inducer of the neoplastic transformation of these fibroblasts (Purdy et al., 1983). Evidence from this well-established in vitro genotoxicological system (Kakunaga, 1973) demonstrated that **6** was more potent than estradiol as a transforming agent. This focuses attention on whether or not **4**–**7** are genuine, if transient, intermediates in the formation of catechol estrogens.

We presented preliminary results of the purification of ^{3}H-labeled products from the incubation of [6,7-^{3}H]estradiol with microsomes from human breast cancer cells (Purdy et al., 1983). These products, after acetylation, were chromatographically similar by HPLC[1] to the 17-acetate of either **4** or **5**. In this paper, we provide data supporting the intermediacy of **5** in the biosynthesis of 2-hydroxyestradiol, together with details of the syntheses of relevant steroidal epoxyenones.

[1] Abbreviations: HEPES, 4-(2-hydroxyethyl)-1-piperazineethanesulfonic acid; HPLC, high-performance liquid chromatography; ^{1}H NMR, proton nuclear magnetic resonance; IR, infrared spectroscopy; S-9, supernatant from liver homogenate centrifuged at 9000*g* for 20 min; S-100, supernatant from S-9 fraction centrifuged at 105000*g* for 1 h.

Since estrogen 2-/4-hydroxylase activity of washed rat liver microsomes was found to be increased after addition of the S-100 fraction of liver homogenates, we investigated the catalytic conversion of **4** to 2-hydroxyestradiol (**2**) and of **6** and **7** to 4-hydroxyestradiol (**3**) by this soluble protein fraction. These results are also consistent with epoxyenones as intermediates in the formation of catechol estrogens.

Experimental Procedures

Materials

Solvents were reagent-grade. Tetrahydrofuran, 1,4-dioxane, and diethyl ether were distilled from benzophenone ketyl. The following chemicals were obtained from Aldrich Chemical Co. (Milwaukee, WI): trimethylsilyl chloride, phenylselenyl chloride, benzeneseleninic anhydride, selenium dioxide, and *m*-(chloroperoxy)benzoic acid. 19-Nortestosterone was purchased from Searle Chemicals, Inc. (Chicago, IL), or from Sigma Chemical Co. (St. Louis, MO). Lithium metal and lithium tri-*tert*-butoxyaluminum hydride were purchased from Alfa Products (Danvers, MA). Hydrogen peroxide and 2-methyl-2-propanol were obtained from J. T. Baker Chemical Co. (Phillipsburg, NJ).

Analytical Procedures

Infrared spectra were taken on a Perkin-Elmer Model X99 spectrophotometer, and the absorptions are reported in wave numbers (cm^{-1}). The ^{1}H NMR spectra were obtained on a Varian T-60 spectrometer, and the chemical shift data are reported in parts per million (δ) downfield from tetramethylsilane used as an internal standard. Ultraviolet spectra were obtained with a Cary 14 spectrophotometer. Optical rotations were obtained with a Perkin-Elmer Model 241 polarimeter, and the concentrations are expressed in grams per 100 mL. Mass spectra were obtained on a Nuclide 12-90-G instrument. Melting points were determined on a Thomas-Hoover capillary melting point apparatus and are uncorrected. Elemental analyses were performed by Spang Microanalytical Laboratories (Eagle Harbor, MI). All evaporations and removals of solvents were performed, unless otherwise stated, under water-aspirator vacuum on a rotary evaporator below 45 °C.

*Synthesis of 1α,2α-Epoxy-17β-hydroxyestr-4-en-3-one (**4**)*

17β-Hydroxy-5α-estr-1-en-3-one. Phenylselenyl chloride (1.08 g, 6 mmol) was added to a stirred solution of 17β-hydroxy-5α-estran-3-one (Bowers et al., 1958; 1.14 g, 4.15 mmol) in dimethylformamide (20 mL). The brown color of the solution faded to pale yellow in 40 min. After another 20 min, the yellow solution was evaporated to dryness and the residue dissolved in tetrahydrofuran (15 mL) and treated with 30% H_2O_2 (5 mL). During the addition of H_2O_2, the temperature was kept below 45 °C. After being stirred for 1 h, the pale yellow solution was extracted with ether. The ethereal layer was washed with dilute $NaHCO_3$ solution and water, dried over anhydrous Na_2SO_4, and evaporated to yield a yellow oil. Flash chromatography on silica gel (1:3 ethyl acetate/hexane) and crystallization from this solvent mixture gave 615 mg (54%) of the product. In the initial reaction with either acetonitrile or ethyl acetate as solvent instead of dimethylformamide, the yields were 559 mg (49%) and 456 mg (40%), respectively: mp 144–145 °C; $[\alpha]^{25}_D$ +121° (*c* 0.92, CH_3OH); IR (KBr) 3410, 1660, 1255, 1025 cm^{-1}; NMR ($CDCl_3$) δ 0.78 (s, 3 H, 18-CH_3), 3.65 (br t, 1 H, H-17), 5.93 (d, J = 10 Hz, 1 H, H-2), 7.03 (d, J = 10 Hz, 1 H, H-1); mass spectrum, m/z 274 (M^+). Anal. Calcd for $C_{18}H_{24}O_3$: C, 74.97; H, 8.39. Found: C, 74.91; H, 8.42.

1α,2α-Epoxy-17β-hydroxy-5α-estran-3-one. α-Epoxidation of the above enone was accomplished by dissolving it (1 g, 3.6 mmol) in methanol (100 mL), cooling the solution to 0 °C, and adding a mixture of 4 N NaOH (5.5 mL) and 30% H_2O_2 (5.5 mL). After 15 min, the reaction was worked up via ether extraction and washing of the ether extract with dilute HCl and $NaHCO_3$ solutions. The ether layer was dried with Na_2SO_4 and concentrated to give 820 mg (77%) of the alcohol. This compound was characterized as its 17-acetate, prepared in the usual way with acetic anhydride and pyridine, and crystallized from hexane: mp 151 °C; $[\alpha]^{25}_D$ +106° (*c* 1.1, CH_3OH); IR (KBr) 2935, 2855, 1735, 1440, 1255 cm^{-1}; NMR ($CDCl_3$) δ 0.83 (s, 3 H, 18-CH_3), 2.03 (s, 3 H, 17-$OCOCH_3$), 3.21 (d, *J* = 4 Hz, 1 H, H-2), 3.62 (d, *J* = 4 Hz, 1 H, H-1), 4.61 (br t, 1 H, H-17); mass spectrum, *m/z* 332 (M^+). Anal. Calcd for $C_{20}H_{28}O_4$: C, 72.24; H, 8.50. Found: C, 72.26; H, 8.49.

3,17β-Bis[(trimethylsilyl)oxy]-1α,2α-epoxy-5α-estr-3-ene. A mixture of the above epoxide (334 mg, 1 mmol) and bis(trimethylsilyl)trifluoroacetamide (5 mL) was refluxed under N_2 for 14 h. Evaporation of volatile material gave the oily labile product. The yield was over 85% as estimated by inspection of the NMR spectrum: IR (film) 3035, 2970, 2780, 1662, 1450, 1255 cm^{-1}; NMR ($CDCl_3$ without TMS) δ 0.07 [s, 9 H, 17-$Si(CH_3)_3$], 0.23 [s, 9 H, 3-$Si(CH_3)_3$], 0.75 (s, 3 H, 18-CH_3), 3.16 (m, 1 H, H-2), 3.57 (overlapping br t, d due to H-1, br t due to H-17), 4.67 (br s, 1 H, H-4); mass spectrum, *m/z* 434 (M^+).

1α,2α-Epoxy-17β-hydroxyestr-4-en-3-one (**4**). To a solution of the above bis(trimethylsilyl) ether (475 mg, 1.1 mmol) in ethyl acetate (10 mL) was added phenylselenyl chloride (651 mg, 3.4 mmol). The red color of the phenylselenyl chloride disappeared after 2-h stirring at room temperature. Tetra-*n*-butylammonium fluoride (1 M solution in tetrahydrofuran, 2 mL) and saturated NH_4Cl solution (5 mL) were added, and stirring was continued for 30 min at 25 °C. The aqueous layer was extracted with ethyl acetate. The combined ethyl acetate layers were washed with water and dried over anhydrous Na_2SO_4. Evaporation of the solvent afforded a dark yellow oil to which was added tetrahydrofuran (15 mL) and 30% H_2O_2 (4 mL). The temperature was maintained between 25 and 45 °C with an ice bath. After the reaction mixture had been stirred for 1 h, ether (50 mL) was added. The ethereal layer was washed with dilute $NaHCO_3$ and water and dried over anhydrous Na_2SO_4. Evaporation of the solvent yielded a yellow oil that was purified by flash chromatography on silica gel (1:3 ethyl acetate/hexane) and crystallized from this solvent mixture to yield 68 mg (20%) of the desired epoxyenone: mp 123–124 °C; $[\alpha]^{25}_D$ +112° (*c* 0.96, CH_3OH); IR (film) 3450, 3020, 2935, 2870, 1670, 1625, 1220 cm^{-1}; NMR ($CDCl_3$) δ 0.83 (s, 3 H, 18-CH_3), 3.39 (dd, *J* = 5 and 2 Hz, 1 H, H-2), 3.71 (overlapping br t, dd due to H-1, br t due to H-17), 5.72 (br s, 1 H, H-4); mass spectrum, *m/z* 288 (M^+). Anal. Calcd for $C_{18}H_{24}O_3$: C, 74.97; H, 8.39. Found: C, 74.91; H, 8.42.

Synthesis of 1β,2β-Epoxy-17β-hydroxyestr-4-en-3-one (5) 17-Acetate

17β-Hydroxy-5α-estr-1-en-3-one 17-Acetate. 17β-Hydroxy-5α-estr-1-en-3-one (1 g, 3.6 mmol) was acetylated by means of acetic anhydride (1 mL) and pyridine (2 mL) in the usual way. After recrystallization of the product from hexane/acetone, 1.05 g (91%) of colorless crystals was obtained: mp 141 °C; $[\alpha]^{25}_D$ +114° (*c* 1, EtOH); IR (film) 3035, 2915, 1730, 1675, 1600, 1250, 1050, 1030 cm^{-1}; NMR ($CDCl_3$) δ 0.80 (s, 3 H, 18-CH_3), 2.03 (s, 3 H, 17-$OCOCH_3$), 4.67 (br t, 1 H, H-17), 5.97 (d, *J* = 10 Hz, 1 H, H-2), 7.07 (d, *J* = 10 Hz, 1 H, H-1).

3β,17β-Dihydroxy-5α-estr-1-ene 17-Acetate. A solution of the above 17-acetate (3.0 g, 9.5 mmol) in dry tetrahydrofuran (12 mL) was added with stirring to a cold (−70 °C) solution of lithium tri-*tert*-butoxyaluminum hydride (11.0 g, 43 mmol) in 150 mL of the same solvent. The reaction mixture was stirred at −70 °C for 2.5 h and then held at 0 °C for 16 h. The reaction mixture was poured into excess cold 10% acetic acid and the product extracted with ethyl acetate. The organic extract was washed with saturated Na_2SO_4 solution and water and dried over anhydrous Na_2SO_4. Removal of solvent gave a crude product that was purified by flash chromatography on silica gel (1:3 ethyl acetate/hexane) to give 2.0 g (66%) of the product after crystallization from acetone: mp 95–96 °C; $[\alpha]^{25}_D$ +80° (*c* 0.7, EtOH); IR (film) 3470, 3030, 1730, 1640, 1250 cm^{-1}; NMR ($CDCl_3$) δ 0.83 (s, 3 H, 18-CH_3), 2.03 (s, 3 H, 17-$OCOCH_3$), 2.70 (1 H, OH), 4.3–4.6 (2 H, overlapping triplets, H-1 and H-3), 5.73 (m, 2 H, H-2 and H-2); mass spectrum, *m/z* 318 (M^+), 300 (M − H_2O). Anal. Calcd for $C_{20}H_{30}O_3 \cdot {}^1/_2C_3H_6O$: C, 73.36; H, 9.56. Found: C, 73.86; H, 9.39.

3β,17β-Dihydroxy-1β,2β-epoxy-5α-estrane 17-Acetate. The above alcohol (2.0 g, 5.3 mmol) was dissolved in dry benzene (40 mL), and *m*-(chloroperoxy)benzoic acid (1.3 g, 7.9 mmol) was added. The mixture was stirred at room temperature for 2 h and worked up via addition of saturated Na_2SO_3 solution, extraction with benzene, washing the organic layer with saturated $NaHCO_3$ solution and water, and drying (Na_2SO_4). Removal of solvent gave the product, which was recrystallized from petroleum ether/ethyl acetate: mp 166 °C; $[\alpha]^{25}_D$ +47° (*c* 0.8, EtOH); IR (film) 3450, 2960, 1730, 1260, 1030, 800 cm^{-1}; NMR ($CDCl_3$) δ 0.80 (s, 3 H, 18-CH_3), 2.0 (s, 3 H, 17-$OCOCH_3$), 3.2 (br s, 2 H, H-1 and H-2), 3.96 (br t, 1 H, H-3), 4.6 (br t, 1 H, H-17); mass spectrum, *m/z* 334 (M^+), 316 (M − H_2O). Anal. Calcd for $C_{20}H_{30}O_4$: C, 71.82; H, 9.04. Found: C, 71.78; H, 8.97.

1β,2β-Epoxy-17β-hydroxy-5α-estran-3-one 17-Acetate. The above alcohol (1 g, 3 mmol) was dissolved in dichloromethane (5 mL) and added to a stirred suspension of chromium trioxide (1.8 g, 18 mmol) in dichloromethane (75 mL) and pyridine (2.8 g, 36 mmol). The resulting black suspension was stirred for an additional 30 min. Workup involved filtration, washing the precipitate with ether, and washing the combined organic extracts with 10% NaOH, 10% HCl, 10% $NaHCO_3$, and water. After being dried (anhydrous Na_2SO_4), the ethereal layer was evaporated to give a crude product that was crystallized from hexane/acetone to yield 750 mg (75%) of the desired ketone: mp 149 °C; $[\alpha]^{25}_D$ +60° (*c* 0.95, CH_3OH); IR (KBr) 2920, 2840, 1730, 1718 1450, 1255 cm^{-1}; NMR ($CDCl_3$) δ 0.83 (s, 3 H, 18-CH_3), 2.03 (s, 3 H, 17-$OCOCH_3$), 3.05 (d, *J* = 3.9 Hz, 1 H, H-2), 3.48 (d, *J* = 3.9, 1 H, H-1), 4.61 (br t, 1 H, H-17); mass spectrum, *m/z* 332 (M^+). Anal. Calcd for $C_{20}H_{28}O_4$: C, 72.24; H, 8.50. Found: C, 72.20, H, 8.56.

1β,2β-Epoxy-17β-hydroxyestr-4-en-3-one (**5**) *17-Acetate.* The above ketone (131 mg, 0.4 mmol) was heated under reflux in chlorobenzene (2 mL) with benzeneseleninic anhydride (142 mg, 0.4 mmol) for 4 h. Removal of solvent under reduced pressure gave a dark residue. Purification of this product by preparative thin-layer chromatography (silica gel, 1:3 ethyl acetate/hexane) and crystallization from this solvent mixture yielded 29 mg (22%) of the desired epoxyenone: mp 161 °C; $[\alpha]^{25}_D$ −191° (*c* 0.9, CH_3OH); IR (film) 3010, 2930, 2845, 1725, 1665, 1625, 1260, 1045 cm^{-1}; NMR ($CDCl_3$) δ 0.87 (s,

3 H, 18-CH_3), 2.03 (s, 3 H, 17-$OCOCH_3$), 3.37 (br s, 1 H, H-2), 3.75 (d, J = 4 Hz, 1 H, H-1), 4.63 (br t, 1 H, H-17), 5.73 (br s, 1 H, H-4); mass spectrum, m/z 330 (M^+), 270 (M − CH_3COOH).

Synthesis of 4α,5α-Epoxy-17β-hydroxyestr-1-en-3-one (6)

17β-Hydroxy-3β-[(trimethylsilyl)oxy]estr-4-ene 17-Acetate. To a solution of 3β,17β-dihydroxyestr-4-ene 17-acetate (Hanson & Wilkins, 1974; 4.3 g, 13.5 mmol) imidazole (1.8 g, 27 mmol) in dry dimethylformamide (30 mL) was added trimethylsilyl chloride (3 mL). The mixture was stirred at room temperature for 1 h and triturated with ether (30 mL). The resulting ethereal layer was washed sequentially with cold 10% aqueous HCl, 10% aqueous $NaHCO_3$ and water and dried over anhydrous Na_2SO_4. Evaporation of the organic solvent afforded 5.0 g (96%) of the crude ether. Recrystallization from hexane yielded colorless crystals of the desired intermediate: mp 85–87 °C; IR (film) 2930, 1730, 1250, 1065, 1045, 890, 840 cm^{-1}; NMR ($CDCl_3$) δ 0.1 (s, 9 H, $SiMe_3$), 0.80 (s, 3 H, 18-CH_3), 2.0 (s, 3 H, 17-$OCOCH_3$), 4.17 (br t, 1 H, H-3), 4.6 (br t, 1 H, H-17), 5.3 (s, 1 H, H-4); mass spectrum, m/z 390 (M^+), 300 (M − $HOSiMe_3$). Anal. Calcd for $C_{23}H_{38}O_3Si$: C, 70.72; H, 9.80; Si, 7.19. Found: C, 70.78; H, 9.65; Si, 6.94.

3β,17β-Dihydroxy-4α,5α-epoxyestrane 17-Acetate. To a solution of the above silyl ether (5.0 g, 12.8 mmol) in dry benzene (60 mL) was added 85% *m*-chloroperbenzoic acid (3.4 g, 20 mmol), and the mixture stirred for 2 h at 25 °C. The excess *m*-chloroperbenzoic acid was destroyed by the addition of saturated Na_2SO_3 solution (28 mL). The benzene layer was separated, washed with 10% $NaHCO_3$ solution and water, and dried over anhydrous Na_2SO_4. Evaporation of the solvent gave a white solid, which was dissolved in tetra-*n*-hydrofuran (100 mL). Saturated aqueous NH_4Cl (25 mL) and tetra-*n*-butylammonium fluoride (5 mL) were then added, and the reaction mixture was stirred at room temperature for 1 h. After evaporation of the tetrahydrofuran, cold water (100 mL) was added and the mixture extracted with ether. The ether layer was dried over anhydrous Na_2SO_4. Evaporation of the organic solvent gave 3.4 g (85%) of the desired product: mp 159 °C; $[\alpha]^{25}_D$ +7.6° (*c* 1, EtOH); IR (film) 3450, 1730, 1245, 1045, 1015 cm^{-1}; NMR ($CDCl_3$) δ 0.83 (s, 3 H, 18-CH_3), 2.03 (s, 3 H, 17-$OCOCH_3$); mass spectrum, m/z 334 (M^+), 316 (M − H_2O). Anal. Calcd for $C_{20}H_{30}O_4$: C, 71.83; H, 9.04. Found: C, 71.85; H, 9.00.

4α,5α-Epoxy-17β-hydroxyestran-3-one 17-Acetate. Oxidation of the above alcohol was carried out as follows: chromium trioxide (6.1 g, 60.7 mmol) was added to a mixture of pyridine (10 mL) and methylene chloride (250 mL). The resulting mixture was stirred at 25 °C under N_2 for 15 min. A solution of 3β,17β-dihydroxy-4α,5α-epoxyestrane 17-acetate (3.4 g, 10.2 mmol) in dichloromethane (15 mL) was added. Stirring was continued for 30 min at room temperature, ether (50 mL) was added, and the black mixture was filtered. The residue was washed 3 times with ether, and the combined organic extracts were washed with aqueous 10% NaOH, 10% HCl, 10% $NaHCO_3$, and water. The organic layer was dried over Na_2SO_4, and the solvent evaporated to give the crude ketone. On crystallization from petroleum ether/acetone, 2.6 g (76%) of colorless plates was isolated: mp 149–150 °C; $[\alpha]^{25}_D$ −76° (*c* 0.76, CH_3OH); IR (KBr) 2915, 2885, 1732, 1715, 1435, 1250 cm^{-1}; NMR ($CDCl_3$) δ 0.83 (s, 3 H, 18-CH_3), 2.02 (s, 3 H, 17-$OCOCH_3$), 2.98 (s, 1 H, H-4), 4.63 (br t, 1 H, H-17); mass spectrum, m/z 332 (M^+). Anal. Calcd for $C_{20}H_{28}O_4$: C, 72.26; H, 8.43. Found: C, 72.15; H, 8.36.

4α,5α-Epoxy-17β-hydroxyestran-3-one. A solution of the above 17-acetate (700 mg, 2.1 mmol) in a mixture of dioxane, water, and methanol (6:1:1), 80 mL) was stirred with $Ba(OH)_2 \cdot 8H_2O$ (3.5 g, 11.1 mmol) for 16 h at room temperature. The solvent was removed under reduced pressure. Water (50 mL) was added to the residue, the water layer was extracted with ether 4 times, and the combined ethereal layers were washed with water 3 times and dried over anhydrous Na_2SO_4. Evaporation of the ether afforded a colorless oil, which crystallized from methanol to give the desired ketol (500 mg, 82%): mp 156 °C; $[\alpha]^{25}_D$ −33° (*c*, 1.2, CH_3OH); IR (KBr) 3420, 2909, 1710, 1450, 1255 cm^{-1}; NMR ($CDCl_3$) δ 0.78 (s, 3 H, 18-CH_3), 2.98 (s, 1 H, H-4), 3.65 (br t, 1 H, H-17); mass spectrum, m/z 190 (M^+). Anal. Calcd for $C_{18}H_{26}O_3$: C, 74.48; H, 8.97. Found: C, 74.59; H, 8.98.

4α,5α-Epoxy-17β-hydroxyestr-1-en-3-one (**6**). A solution of the above epoxide (900 mg, 3.1 mmol), selenium dioxide (690 mg, 6.2 mmol), and acetic acid (2 mL) in 2-methyl-2-propanol (180 mL) was heated under reflux for 16 h under N_2. Precipitated selenium was removed by filtration through celite. Evaporation of the solvent gave a dark brown residue, which was triturated with ethyl acetate. The ethyl acetate solution was washed 5 times with $NaHCO_3$ solution and 3 times with water and then dried over anhydrous Na_2SO_4. After removal of solvent, the product was purified by flash chromatography on silica gel (1:3 ethyl acetate/hexane) and crystallized from this solvent mixture to give 396 mg (44%) of the desired epoxyenone: mp 98–100 °C; $[\alpha]^{25}_D$ +212° (*c* 3.05, CH_3OH); IR (KBr) 3440, 3040, 2940, 1680, 1615, 1450, 1264 cm^{-1}; NMR ($CDCl_3$) δ 0.83 (s, 3 H, 18-CH_3), 3.27 (d, J = 2.1 Hz, 1 H, H-4), 3.68 (br t, 1 H, H-17), 5.99 (ddd, J = 10.8, 2.1, and 2.1 Hz, 1 H, H-2), 6.72 (dd, J = 10.8 and 2.1 Hz, 1 H, H-1); mass spectrum, m/z (M^+). Anal. Calcd for $C_{18}H_{24}O_3$: C, 74.97; H, 8.39. Found: C, 74.89; H, 8.37.

Synthesis of 4β,5β-Epoxy-17β-hydroxyestr-1-en-3-one (7).

A solution of the known 4β,5β-epoxy-17β-hydroxyestran-3-one (Mihailovic et al., 1977; 600 mg, 2.1 mmol), selenium dioxide (460 mg, 4.1 mmol), and acetic acid (2 mL) in 2-methyl-2-propanol (150 mL) was refluxed for 14 h under N_2. The reaction was worked up and the product purified by flash chromatography as described above and crystallized from ethyl acetate/hexane to give 500 mg (83%) of the desired epoxyenone: mp 125–126 °C; $[\alpha]^{25}_D$ +265° (*c* 1.4, MeOH); IR (KBr) 3440, 3040, 2940, 1680, 1612, 1445, 1260 cm^{-1}; NMR ($CDCl_3$) δ 0.83 (s, 3 H, 18-CH_3), 3.25 (dd, J = 2.0 and 1.2 Hz, 2 H, H-4), 3.68 (br t, 1 H, H-17), 5.98 (ddd, J = 10.7, 2.0, and 1.2 Hz, 1 H, H-2), 6.74 (dd, J = 10.7 and 5.3 Hz, 1 H, H-1); mass spectrum, m/z 288 (M^+). Anal. Calcd for $C_{18}H_{24}O_3$: C, 74.97; H, 8.39. Found: C, 74.57; H, 8.25.

Metabolism Studies

Isolation Experiment with MCF-7 Cell Microsomes. [6,7-^{3}H]Estradiol (New England Nuclear, Boston, MA) was diluted with unlabeled estradiol and purified by HPLC on a 0.46 × 50 cm Chromegabond Diol column (E. S. Industries, Marlton, NJ) developed with a linear gradient of 2.5–17.5% 2-propanol in heptane over a 1-h period at a flow rate of 0.5 mL/min.

The preparation of microsomes from MCF-7 cells was performed as described by Hoffman et al. (1979). Incubations were carried out with 3 mg of microsomal protein, 5 μM [6,7-^{3}H]estradiol (5.61×10^6 dpm), 0.5 mM NADPH, and 5 mM $MgCl_2$ in a total volume of 1 mL of 10 mM HEPES buffer (pH 7.4). The incubations included 1 mM **4**, **6**, and

7 to trap any reactive intermediates that might be formed. The epoxide hydrolase inhibitor 1,2-epoxy-3,3,3-trichloropropane (1 mM) was used to retard further metabolism of the epoxyenones (White, 1980). Incubations were carried out for 10 min at 0, 24, 37, and 50 °C and for a control at 50 °C with boiled microsomes, followed by cooling to 0 °C. The reaction mixtures were extracted twice with 5 mL of cold ethyl acetate. The combined organic phases were washed 3 times with 1 mL of water, dried over Na_2SO_4, and evaporated under N_2, and the residues were partially purified by HPLC on a 0.46 × 50 cm Chromegabond CN column (E. S. Industries) developed with 50% chloroform in dichloromethane at a flow rate of 0.5 mL/min, collecting 1-min fractions with a MultiRac Model 2111 collector (LKB, Rockville, MD). The radioactivity in 10% aliquots of the eluate fractions was determined after evaporation of the aliquot, addition of 8 mL of Liquifluor/toluene (New England Nuclear), and counting in a Beckman LS7500 microprocessor-controlled scintillation counter (Beckman Instruments, Palo Alto, CA). HPLC was performed on a Waters Model 720 system controller (Waters Assoc., Milford, MA), sample processor (Wisp Model 710B), and a 254-nm UV detector (Model UV III, Laboratory Data Control, Riviera Beach, FL) to monitor the elution of **4**, **6**, **7**, and **1**. The eluate fractions containing **4** were combined and rechromatographed in the same system to remove small amounts of **1**. The purified fractions containing **4** were combined, evaporated under N_2, and acetylated for 16 h in 0.2 mL of pyridine/acetic anhydride (1:1 v/v). Unlabeled **5** 17-acetate (150 μg) was added to the residues after acetylation, and the mixture was chromatographed on a 1 × 25 cm Partisil PXS silica column (Whatman, Inc., Clifton, NJ) in sequence with a 1 × 25 cm micro-Porasil silica column (Waters Assoc.) The solvent system was 5% ethyl acetate in dichloromethane at a flow rate of 0.5 mL/min. The radioactive fractions containing **5** 17-acetate were combined and rechromatographed in the same system. The resulting fractions containing **5** 17-acetate were rechromatographed on two Rac Partisil 10 silica columns (Whatman, Inc.) in tandem, with the solvent 30% ethyl acetate in heptane at a flow rate of 0.5 mL/min. The radioactive fractions containing **5** 17-acetate were combined and evaporated under N_2; a 50% aliquot was dissolved in 0.2 mL of 0.05 M potassium *tert*-butoxide in 2-methyl-2-propanol for 1 min at 25 °C, followed by the addition of 1 mL of 5% aqueous acetic acid and extraction with 1 mL of ethyl acetate. The ethyl acetate extracts were dried over Na_2SO_4, evaporated under N_2, and chromatographed in the above system containing 1% acetic acid in the 30% ethyl acetate/70% heptane solvent. The flow rate was 1 mL/min, and the presence of 2-hydroxyestradiol 17-acetate (Rao & Axelrod, 1960) in the eluate was monitored at 280 nm.

S-100-Catalyzed Isomerization of **4** *to* **2** *and of* **6** *and* **7** *to* **3**. The S-100 fraction from homogenates of rat liver from Sprague-Dawley rats treated with 2-(acetylamino)fluorene (22.3 mg/kg ip in corn oil 24 h prior to sacrifice) was prepared as described by Purdy and Marshall (1984). Incubations of 50 μM **4**, **6**, or **7** were performed with 0 and 10–40 μg of soluble protein in 500 μL of 50 mM HEPES buffer (pH 7.0) containing 0.75 μM *S*-(*methyl*-^{3}H]adenosyl-L-methionine (7.5 μCi), 100 Sigma units of catechol *O*-methyltransferase, 0.33 mM L-ascorbic acid, and 40 mM MgCl. After incubation for 1–5 min at 37 °C, 1 mL of 0.05 M borate buffer (pH 10) containing 20 μg of recovery standards of 2-methoxyestradiol and 4-methoxyestradiol was added, and the radioactive monomethyl ethers were extracted with 6 mL of *n*-heptane. The radioactivity in 0.5-mL aliquots of the heptane extracts was determined, the remainder of the extracts was evaporated under N_2, and the products were identified by HPLC (Purdy et al., 1982). The picomoles of **2** or **3** formed in the reactions was calculated from the recovered dpm of the monomethyl ethers after subtraction of the dpm formed nonenzymatically in the absence of the S-100 fraction.

Results

Synthesis. Both compound **4** and the 17-acetate of compound **5** were prepared from 17β-hydroxy-5α-estran-3-one, which was readily obtained by Birch reduction of 19-nortestosterone (Bowers et al., 1958). In accord with the known preference of 5α-3-ones to enolize toward the 2-position, treatment of 17β-hydroxy-5α-estran-3-one with phenylselenyl chloride followed by hydrogen peroxide gave 17β-hydroxy-5α-estr-1-en-3-one. The structure of this compound was established by the ^{1}H NMR spectrum, which showed the olefinic H-1 and H-2 as doublets (J = 10 Hz) at δ 7.03 and 5.93, respectively.

Treatment of 17β-hydroxy-5α-estr-1-en-3-one with methanolic alkaline hydrogen peroxide gave 1α,2α-epoxy-17β-hydroxy-5α-estran-3-one. The stereochemistry of this epoxide is predicted on stereoelectronic grounds (Corey, 1954) and is consistent with the ^{1}H NMR spectral data (see below). Treatment of this alcoholic epoxy ketone with excess bis(trimethylsilyl)trifluoroacetamide gave the labile silyl enol ether 3,17β-bis[(trimethylsilyl)oxy]-1α,2α-epoxy-5α-estr-3-ene, which without rigorous purification was converted by means of phenylselenyl chloride, tetra-*n*-butylammonium fluoride, and hydrogen peroxide into 1α,2α-epoxy-17β-hydroxyestr-4-en-3-one (**4**).

Much difficulty was experienced in preparing the analogous 1β,2β-epoxy-17β-hydroxyestr-4-en-3-one (**5**). This was apparently due to the instability of the β-oriented epoxide ring in the presence of dehydrogenating reagents, which led to low and variable yields of products. This problem was apparently reduced by protection of the 17β-hydroxyl group. Accordingly, 17β-hydroxy-5α-estr-1-en-3-one was acetylated and then reduced with lithium tri-*tert*-butoxyaluminum hydride in tetrahydrofuran at −70 °C to give exclusively the 3β-allylic 17β-acetoxy alcohol. This compound was treated with *m*-(chloroperoxy)benzoic acid in benzene to provide 3β,17β-dihydroxy-1β,2β-epoxy-5α-estrane 17-acetate, which was then oxidized to the 3-keto derivative with chromium trioxide/pyridine. Refluxing this ketone with benzeneseleninic anhydride in chlorobenzene as solvent gave the desired 17-acetate of **2** in low (22%) but reproducible yield. The 1β,2β stereochemistry for this epoxide would be predicted from the stereochemistry and mechanisms of the reaction used for its synthesis. Furthermore, Dreiding models of the 17-acetates of **4** and **5** reveal a dihedral angle of 30° between H-1 and H-10 in **4** and of 90° in **5**. Even though the values of $J_{H\text{-}1\text{-}H\text{-}10}$ obtained for **4** and **5** cannot be exactly predicted on the basis of the Karplus relationship for cyclohexanes (in epoxides the J values are generally smaller; Tori et al., 1964), they are in general accord with the prediction that $J_{H\text{-}1\text{-}H\text{-}10}$ would be greater in **4** than in **5**.

The 4α,5α-epoxy-17β-hydroxyestr-1-en-3-one (**6**) was prepared starting with the known 3β,17β-dihydroxyestr-4-ene 17-acetate, which was converted by means of trimethylsilyl chloride/imidazole into the 3β-(trimethylsilyl) ether. The steric hindrance afforded by this protecting group enabled epoxidation with *m*-(chloroperoxy)benzoic acid to proceed exclusively from the α face of the molecule. The 3β alcohol was regenerated with aqueous tetra-*n*-butylammonium fluoride/ammonium chloride and oxidized to the 3-ketone with

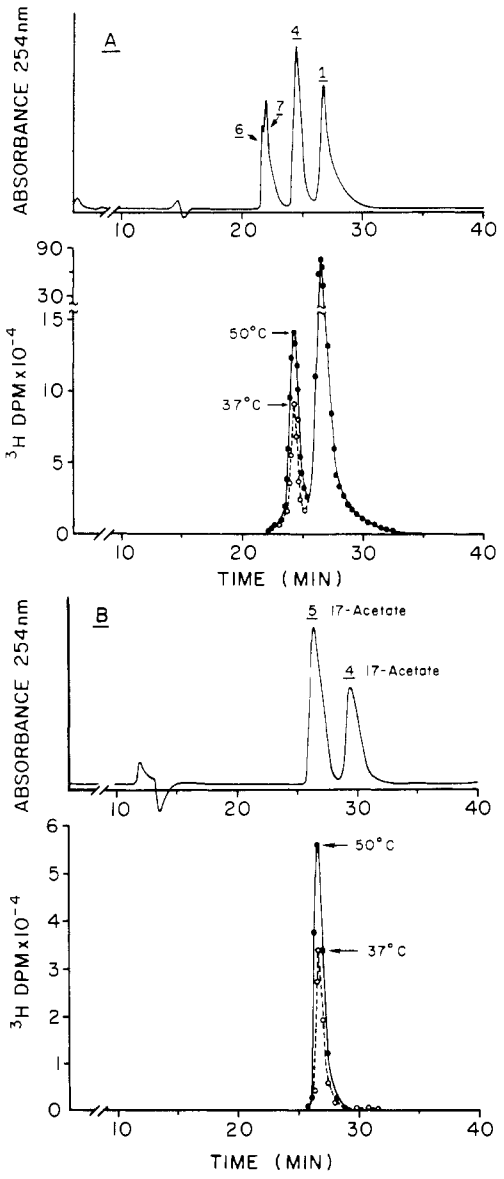


FIGURE 1: Separation by HPLC of the ^{3}H-labeled metabolites obtained after incubation of [6,7-^{3}H]estradiol with MCF-7 cell microsomes on a (A) 0.46 × 50 cm Chromegabond CN column developed with 50% chloroform in dichloromethane at 0.5 mL/min or (B) after acetylation on two RAC Partisil 10 silica columns in tandem developed with 30% ethyl acetate in heptane at 0.5 mL/min. The eluate fractions (1 min) of the radioactive products are shown below for the products obtained from 10-min incubations at 37 (○) and 50 °C (●). The elution of the chromatographic standards of estradiol (**1**) and the epoxyenones (**4**, **6**, and **7**) are shown above the eluate fractions.

chromium trioxide/pyridine. The 17-acetate group was then hydrolyzed cleanly in the presence of the 4α,5α-epoxide group with barium hydroxide in dioxane–water–methanol. The final dehydrogenation to the desired epoxyenone **6** was effected with selenium dioxide in 2-methyl-2-propanol. Finally, 4β,5β-epoxy-17β-hydroxyestr-1-en-3-one (**7**) was prepared from 19-nortestosterone by epoxidation with hydrogen peroxide to give the 4,5-epoxide of known β configuration (Mihailovic et al., 1977), followed by dehydrogenation with selenium dioxide in 2-methyl-2-propanol.

Isolation Experiment. The data in Figure 1A show the separation by HPLC of the ^{3}H-labeled products obtained after incubation of [6,7-^{3}H]estradiol with microsomes from human breast cancer cells in the presence of 1 mM **4**, **6**, and **7** to trap any of these possible intermediates that might be formed from estradiol. The ^{3}H-labeled catechols **2** and **3** are too polar to be eluted from the column under these conditions. The formation of **2** and **3** from **1** by MCF-7 cell microsomes has been demonstrated previously (Purdy et al., 1982, 1983) and was found to be a ratio of about 9:1 of **2**:**3**, respectively, with the microsomes that were used in this isolation experiment. It was previously shown by White (1980) that the 1 mM concentration of 1,2-epoxy-3,3,3-trichloropropane used in this study blocks conversion of the 4β,5β-epoxide of norethindrone to its dihydrodiol in the presence of rat liver microsomes. In Figure 1A radioactivity was recovered in the fractions containing **4** but not **6** or **7**. After acetylation and further purification by HPLC, the final chromatogram (Figure 1B) showed that a radioactive metabolite was obtained with the retention time of **5** 17-acetate in this system, although this metabolite was contaminated by about 150 μg of nonradioactive material not absorbing at 254 nm, which presumably came from the microsomal lipid extract and/or solvent impurities. The retention time of estradiol diacetate is 17 min in this system, and we have been unable to find another acetate of a metabolite of estradiol that has the retention of **5** 17-acetate in this system. The data for the isolation of **5** 17-acetate are summarized in Table I. A maximum yield of 5% from estradiol is calculated for the microsomal incubation performed at 50 °C.

Table I: Summary of Results of Duplicate Isolation Experiments

incubation temp (°C)	av recovered ^{3}H dpm with retention time of **5** 17-acetate by HPLC[a]: final HPLC[b]	cor for losses[c]	% recovered[d]
0	<300		
24	18 500	48 700	0.9
37	46 200	143 000	2.6
50	73 050	278 000	5.0
50 (boiled microsomes)	880	2 880	<0.1

[a] A total of 150 μg of unlabeled carrier added after acetylation of radioactive fractions obtained from second HPLC (see Experimental Procedures). [b] Shown in Figure 1B for 37 and 50 °C incubations. [c] Calculated from recovery of unlabeled carrier in final HPLC and the amounts used for the determination of radioactivity. [d] Calculated from [(amount in column 3)/(5.61 × 10^6 dpm)] × 100%.

Treating a 50-μg amount of **5** 17-acetate with 50 mM potassium *tert*-butoxide in 2-methyl-2-propanol for 1 min at 25 °C converted this epoxide to authentic 2-hydroxyestradiol 17-acetate (Rao & Axelrod, 1960) in about 95% yield after extraction and separation by HPLC. When we used these conditions to react the radioactive material isolated from the HPLC shown in Figure 1B, we were unable to obtain more than 10% of the resulting radioactivity as ^{3}H-labeled 2-hydroxyestradiol 17-acetate. There was also a correspondingly low yield of unlabeled 2-hydroxyestradiol 17-acetate product from unlabeled **5** 17-acetate in these radioactive fractions.

Enzymatic Isomerization of Epoxyenones to Catechol Estrogens. These reactions were performed in 50 mM HEPES buffer at pH 7.00 to minimize nonenzymatic formation of **2** and **3** by general acid–base catalysis. The results shown in Figure 2 were obtained for the isomerization of **4** and **6** and **7** to **2** and **3**, respectively, as a function of increasing amounts of soluble protein from rat liver homogenates. No significant isomerization above that of the buffer control was obtained with 40 μg of S-100 protein heated at 100 °C for 3 min. The presence of 50 μM 4-phenylchalcone oxide (Mullin & Hammock, 1982) did not alter the S-100-catalyzed formation of **2** from **4** or of **3** from **6** and **7**.

Discussion

The discovery of the NIH shift has shed light on the mechanism of many aromatic hydroxylation reactions in bi-

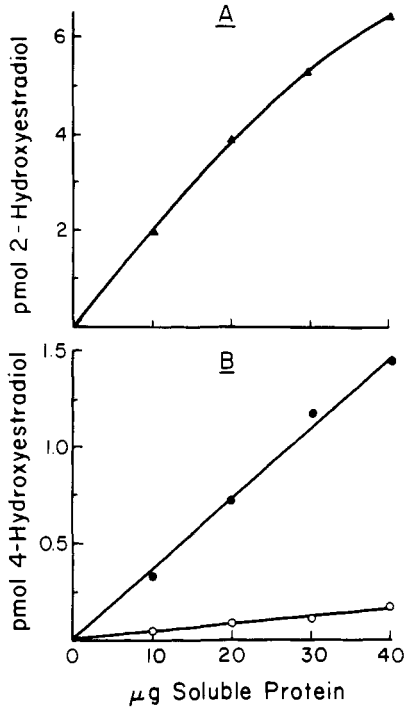


FIGURE 2: Isomerization of **4** (▲) to 2-hydroxyestradiol in panel A and of **6** (●) and **7** (○) to 4-hydroxyestradiol in panel B by the soluble protein (S-100) fraction of rat liver homogenates. The radioenzymatic procedure of Purdy et al. (1982) was used to determine product formed per 5 min by the designated micrograms of protein S-100. Reactions were performed for 5 min at 37 °C in 50 mM HEPES buffer (pH 7.0).

ological systems (Daly et al., 1972). The diagnostic feature of this mechanism, involving arene oxide intermediates, is the retention and ortho migration of hydrogen from the position of hydroxylation. In the case of phenolic substrates, such hydrogens are not retained in ortho hydroxylations because exchange can occur from a ketonic tautomer of the intermediate (Daly et al., 1972). The formation of an 1,2-epoxide intermediate, **4** or **5**, in 2-hydroxyestradiol biosynthesis is in accord with the data of Numazawa and Nambara (1977) for the higher ratio of the glutathione 1- to 4-thioethers of 2-hydroxyestradiol obtained from estradiol vs. the ratio of these thioethers obtained from 2-hydroxyestradiol. In order to test the intermediacy of **4** or **5** in the formation of 2-hydroxyestradiol, we used microsomes from MCF-7 cells, whose growth is known to be stimulated by catechol estrogens such as 2-hydroxyestrone (Schneider et al., 1984).

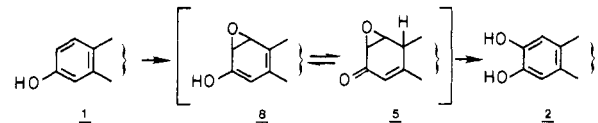
Separation by HPLC of the extracts of the ^{3}H-labeled metabolites obtained after incubation of MCF-7 cell microsomes with [6,7-^{3}H]estradiol and NADPH in the presence of 1 mM unlabeled epoxyenones **4**, **6**, and **7** for 10 min demonstrated that there was an increasing amount of a less polar radioactive metabolite with the chromatographic mobility of **4** as the temperature of incubation increased from 0 and 24 °C (not shown) to 37 and 50 °C (Figure 1A). Control experiments with boiled microsomes incubated at 50 °C or microsomes incubated at 50 °C in the absence of the above epoxyenones failed to yield such a radioactive metabolite in a greater amount than 10^{3} dpm. These results are similar to those reported by Yamamoto and Bloch (1970), who found that microsomes previously heated in 50 °C for 5 min accumulate ^{14}C-labeled 2,3-oxidosqualine on incubation with [^{14}C]squalene. Such heat treatment was also employed by Selkirk et al. (1971) to inhibit microsomal epoxide hydrolase and allow their isolation of 5,6-epoxydibenz[*a*,*h*]anthracene as a metabolic intermediate.

Since we are able to synthesize **5** only as its 17-acetate, the identification of the above less polar radioactive metabolite (Figure 1A) was carried out after acetylation of this metabolite under mild conditions, where the epoxyenone groups of **4**–**7** are unaffected. In an analytical HPLC system where the 17-acetates of **4** and **5** are completely separated (Figure 1B), the acetate of this less polar metabolite was indistinguishable in mobility from **5** 17-acetate. The low yield in the base-catalyzed isomerization of both radioactive and unlabeled **5** 17-acetate to 2-hydroxyestradiol 17-acetate is believed to be due to the presence of unlabeled impurities in this rigorously purified radioactive metabolite fraction, which probably reacted with the radioactive product during the course of the aromatization of **5** 17-acetate. The loss of substantial radioactivity from the epoxyenone may additionally be explained by base-catalyzed exchange at C-6, which is a vinylogous α position to the 3-keto group (Chaudhuri & Gut, 1969).

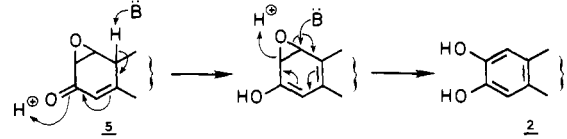
The 5% yield of ^{3}H-labeled **5** 17-acetate obtained under optimal conditions in the isolation experiment (Table I) represents a material yield of only about 80 ng of the acetate of this putative intermediate. Rigorous structural and stereochemical confirmation of identity of this product would require at least 1000-fold more material than was obtained in this experiment if **5** could have been isolated in the absence of the addition of unlabeled carrier.

It was previously found that the rate of formation of (2-hydroxyethinyl)estradiol from ethinylestradiol was 60% greater with an S-9 liver microsomal preparation from 2-(acetylamino)fluorene-treated rats than was obtained from an equivalent amount of washed microsomes from this S-9 preparation (Purdy & Marshall, 1984). This apparently increased estrogen 2-hydroxylase activity of the S-9 preparation compared to the activity in the microsomes themselves was not due to 2-hydroxylase activity in the microsomal supernatant fraction (S-100) of the liver homogenate fraction. We therefore used this S-100 fraction to determine if it would catalyze the formation of **2** from **4** and of **3** from **6** and **7**. The results demonstrated that the S-100 fraction catalyzed the aromatization of the epoxyenones **6** and **7** to 4-hydroxyestradiol and **4** to 2-hydroxyestradiol. 4-Phenylchalcone oxide, a potent inhibitor of soluble epoxide hydrolase activity from liver tissue (Mullin & Hammock, 1982), did not affect the aromatization of these epoxyenones by the S-100 fraction buffered at pH 7.0. Studies are in progress to characterize this isomerization process, which, unlike epoxide hydrolysis, does not involve the net addition of water.

The epoxyenone **5** is the stable form of the dienol **8**, which we proposed (Le Quesne et al., 1980) as the most likely initial intermediate in the biological oxidation of estradiol (**1**) to 2-hydroxyestradiol (**2**). Dienols are known to be stabilized

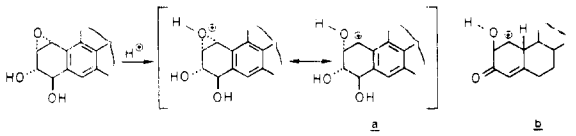


by tautomerism to the derived enones, and this stabilization may offset to some degree the loss of aromatic stabilization attendant upon conversion of **8** to **5**. Aromatic stabilization is restored in the conversion of **8** and/or **5** to **2**. This does



not imply knowledge of the microscopic sequence of proton

shifts but simply indicates a possible order of such shifts. We speculate that the initial cytochrome P-450 mediated attack on the aromatic ring of estradiol (**1**), leading to **8**, may be slower than the rearomatization of **8** to **2**. But if **8** tautomerizes to **5**, this compound is stable enough to dissociate from the active site of hydroxylation and exert independent biological effects. The mechanism responsible for the genotoxic activity of an epoxyenone such as **5** may be different from that occurring for polycyclic arometic hydrocarbon-derived dihydrodiol epoxides, where the carbocationic resonance contributor a is stabilized by the highly conjugated remainder of the molecule and thus would have sufficient lifetime for covalent bond formation with macromolecular nucleophiles. An



a b

analogous steroidal intermediate b would be relatively unstabilized, and we are therefore reluctant to invoke it or a canonical equivalent as a key biological reactant at this time. If steroidal epoxyenones such as **5** exert their genotoxic effects through reactions with cellular nucleophiles, S_N2-like reactions may be involved. Alternatively, cytochrome P-450 mediated oxidation of 2-hydroxyestrogens to semiquinone radical anions or *o*-quinones may be involved in the formation of such estrogen–nucleophile adducts (Nelson et al., 1976; Ball & Knuppen, 1980). In order to determine whether epoxyenones such as **5** or further oxidation products of 2-hydroxyestrogens are the key biological electrophiles in the genotoxicity of estrogens, it will be necessary to determine unequivocally the structures of these adducts formed in cellular systems where the parent estrogen is carcinogenic.

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Registry No. **1**, 50-28-2; **2**, 362-05-0; **3**, 5976-61-4; **4**, 100649-20-5; **4** (dihydro, 17-acetate), 74049-76-6; **5** (17-acetate), 100897-55-0; **5** (3β,5α-tetrahydro, 17-acetate), 100814-70-8; **5** (5α-dihydro, 17-acetate), 4201-74-5; **6**, 100814-69-5; **6** (3β-tetrahydro, 17-acetate), 73465-44-8; **6** (dihydro, 17-acetate), 100896-60-4; **6** (dihydro), 3704-17-4; **7**, 85382-36-1; PhSeCl, 5707-04-0; Bu_4NF, 429-41-4; $LiAlH(OBu\text{-}t)_3$, 17476-04-9; CrO_3, 1333-82-0; $(PhSeO)_2O$, 17697-12-0; Me_3SiCl, 75-77-4; *m*-$ClC_6H_4CO_3H$, 937-14-4; SeO_2, 7446-08-4; $(Me_3Si)_2NC(O)CF_3$, 21149-38-2; 17β-hydroxy-5α-estran-3-one, 1434-85-1; 19-nortestosterone, 434-22-0; mixed function oxidase, 9040-60-2; 17β-hydroxy-5α-estr-1-en-3-one, 73991-16-9; 3,17β-bis[(trimethylsilyl)oxy]-1α,2α-epoxy-5α-estr-3-ene, 100896-59-1; 17β-hydroxy-5α-estr-1-en-3-one 17-acetate, 15019-21-3; 3β,17β-dihydroxy-5α-estr-1-ene 17-acetate, 74049-78-8; 17β-hydroxy-3β-[(trimethylsilyl)oxy]estr-4-ene 17-acetate, 100814-71-9; 3β,17β-dihydroxyestr-4-ene 17-acetate, 25485-46-5.

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