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REACTION OF THIOL NUCLEOPHILES WITH 1,2-EPOXY- AND 4,5-EPOXY-ESTRENE-3-ONE-17β-OLS

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Summary—Four ring A steroidal epoxyenones as probable intermediate in the formation of catechol estrogens were synthesized. The isomeric 1α,2α-epoxy-17β-hydroxyestr-4-en-3-one (**9**) and 1β,2β-epoxy-17β-hydroxyestr-4-en-3-one (**8**) were synthesized from 17β-hydroxy-5α-estra-3-one. The isomeric 4α,5α-epoxy-17β-hydroxyestr-1-en-3-one (**11**) and 4β,5β-epoxy-17β-hydroxyestr-1-en-3-one (**10**) were prepared from 19-nortestosterone. The reaction of **9** and **10** with sodium/ethanethiol resulted in the formation of three types of reactions leading to multiple products: 1,4-addition, opening of epoxide, and epoxide opening followed by dehydration. Reaction of **8** with ethanethiol gave only one compound identified as 2-ethanethio-1,4-estradien-17β-ol-3-one, while reaction of **9** with ethanethiol gave an unusual product identified as 4-estren-1α,17β-diol-3-one. Unlike reaction of ethanethiol with **9** and **10**, reaction with *N*-acetylecysteine or glutathione results in epoxide opening followed by dehydration leading to the formation of estradiol-4-thioethers.

INTRODUCTION

The formation of catechol estrogens via 2- and 4-hydroxylation of estradiol is now considered a major pathway of estrogen metabolism in humans and animals [1, 2]. Further metabolism of catechol estrogens leads to their *O*-methylated derivatives which are biologically inactive [3], or oxidation to the ortho-semiquinones and quinones [4, 5] which are strong electrophiles and therefore can bind irreversibly to nucleophilic centers. Recently, it has been proposed by several investigators that an estrogen arene oxide [6–8] is the intermediate that may be involved in the genotoxic activity of estrogens.

A recent report that certain steroid estrogens can transform mouse fibroblasts in culture [9], and the findings that ethinylestradiol can induce hepatic tumors in hamsters in the presence of 7,8-benzoflavone [10] and that appropriate chemical modification can reduce its carcinogenicity without affecting its estrogenicity [11] are strong arguments that metabolic activation may play a role in the mechanism of carcinogenicity of estrogens. Thus, it is now generally accepted that the oncogenic potential of an estrogen is related to its relative rate of catechol estrogen formation rather than to its activity as a hormone.

Earlier studies in our laboratory [12] suggested that the major pathway for irreversible binding of estrogens to macromolecules involves estrogen-*O*-quinones/semiquinones and not estrogen 1,2-epoxides. To substantiate these results, studies involving the synthesis of estrogen-*O*-quinones [13] and their reaction with glutathione [14, 15] gave compounds that were similar to those obtained from the urine of animals treated with ^{14}C-labeled estradiol.

In order to explore further whether the estrogen–epoxide intermediates may be involved in estrogen's genotoxic activity, we carried out this investigation on the synthesis of the epoxyestrenolones (ketotautomers of estrogen–epoxides) and their reaction with thiols.

EXPERIMENTAL PROCEDURES

Melting points (uncorrected) were taken on a Fisher–Johns apparatus. The ^{1}H-NMR spectra were obtained with a JEOL-90Q 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 Beckman DU-70. Mass spectra were obtained on a AEI MS-30. Solvents were reagent grade. Tetrahydrofuran was distilled from benzophenone ketyl. The following chemicals were obtained from Aldrich Chemical Co. (Milwaukee, Wis.); trimethylsilyl chloride, phenylselenyl chloride, selenium

dioxide, *m*-(chloroperoxy)benzoic acid, ethanethiol, ethylene glycol, and palladium on charcoal (10%). 19-Nortestoestrone was purchased from Steraloids, Wilton, N.H.

Synthesis of 1α,2α-epoxy-17β-hydroxyestr-4-en-3-one (9)

17β-Hydroxy-5α-estr-1-en-3-one (1). Phenylselenyl chloride (2.872 g, 15 mmol) was added to a stirred solution of 17β-hydroxy-5α-estran-3-one (prepared according to the method of Liston and Howarth [17], 3.92 g, 14.2 mmol) in ethylacetate (125 ml). The resulting red–orange solution was stirred until it had turned pale yellow (1 h). At this point H_2O (25 ml) was added to the reaction mixture. After the aqueous phase has been drawn off, 55 ml of THF was added. The H_2O_2 (30%, 3.5 ml, 40.6 mmol) was added dropwise, keeping the temperature below 35°; stirring was continued for 1 h. The reaction mixture was washed with $NaHCO_3$ (5%), water, dried over magnesium sulfate and concentrated to yield a yellow oil. Flash chromatography on silica gel (1:3 ethylacetate/hexane) and crystallization with the same solvent mixture gave 2.14 g (55%) of the product (**1**); m.p. 146–147 (reported 144–145°C) NMR ($CDCl_3$) δ 0.76 (s, 3H, C_{18}-C<u>H</u>$_3$), 3.64 (brt, 1 H, C_{17}-<u>H</u>), 5.94 (d, J = 10 Hz, 1H, C_2-<u>H</u>), 7.06 (d, J = 10 Hz, 1H, C_1-<u>H</u>).

1β,2β-Epoxy-17β-hydroxy-5α-estran-3-one (2) and 1α,2α-epoxy-17β-hydroxy-5α-estran-3-one (3). The epoxidation of enone **1** was carried out by dissolving it (1 g, 3.7 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 mixture was made neutral by the addition of acetic acid. The methanol was removed on rotatory evaporator, water added and extracted with ethyl acetate. The organic layer was washed with water, dried over anhydrous magnesium sulfate and concentrated. The residue obtained was chromatographed over a column of silica gel and eluted with 20% ethyl acetate:hexane. The first compound eluted was 350 mg (32% yield), characterized as its 1β-2β-epoxy-17-acetate, prepared in the usual way with acetic anhydride and pyridine, and crystallized from hexane: m.p. 163–64°C (reported 149°C) ^{1}H-NMR ($CDCl_3$) δ 0.79 (s, 3H, C_{18}-C<u>H</u>$_3$), 2.0 (s, 3H, 17-O-COC<u>H</u>$_3$), 3.16 (d, J = 4 Hz, 1H, C_2<u>H</u>), 3.34 (m, 1H, C1-<u>H</u>), 4.60 (brt, 1H, C_{17}-<u>H</u>). The second compound eluted was in 42% yield (450 mg). It was characterized as its 1α,2α-epoxy-17-acetate and crystallized from hexane: m.p. 157–158°C (reported 151°C). ^{1}H-NMR ($CDCl_3$) δ 0.72 (s, 3H, C_{18}-C<u>H</u>$_3$), 2.0 (s, 3H, 17-OCOC<u>H</u>$_3$), 3.19 (d, 1H, J = 4 Hz, C_2-<u>H</u>), 3.61 (d, J = 4 Hz, 1H, C_1-<u>H</u>), 4.61 (brt, 1H, C_{17}-<u>H</u>).

3,17β-Bis[(trimethylsilyl)oxy]-1β,2β-epoxy-5α-estr-3-ene (4). A mixture of the 1β,2β-epoxide (**2**; 800 mg, 2.8 mmol) and bis(trimethylsilyl)-trifluoroacetamide (4 ml) was stirred at 140°C under N_2 for 2 h. The mixture was cooled to 100°C, triethylamine (0.2 ml) was added and stirring was continued at 110–115°C for 20 h. The yield was over (1.1 g) 85% as estimated by TLC and NMR spectrum. ^{1}H-NMR ($CDCl_3$ without TMS) δ 0.06 [s, 9H, 17-OSi(C<u>H</u>$_3$)$_3$], 0.14 [s, 9H, C_3-OSi(C<u>H</u>$_3$)$_3$], 0.67 (s, 3H, C_{18}-C<u>H</u>$_3$), 2.99 (d, J = 2–3 Hz, 1H, C_1-<u>H</u>), 3.19 (m, 1H, C_2-<u>H</u>), 3.5 (brt, 1H, C_{17}-<u>H</u>), 5.16 (d, H = 4 Hz, 1H, C_4-<u>H</u>).

1β,2β-Epoxy-4β-phenylselenyl-17β-hydroxy-5α-estr-3-one (6). To a solution of the above bis(trimethylsilyl)ether (**4**; 1.1 g 2.55 mmol) in ethylacetate (20 ml) was added phenylselenyl chloride (570 mg) and stirring was continued for 1.5 h at room temperature. Tetrabutylammonium fluoride (1 M solution in THF, 4 ml) was added and stirring was continued for 30 min at room temperature. The aqueous layer was extracted with ethylacetate. The combined organic layer was washed with water and dried over anhydrous magnesium sulfate. The residue obtained was chromatographed over a column of silica gel and eluted with 30% ethylacetate/hexane to give 500 mg (54%) of the product. m.p. 85–87°C ^{1}H-NMR ($CDCl)_3$ δ 0.78 (s, 3H, C_{18}-C<u>H</u>$_3$), 3.3 (m, 3H, C_1 4-<u>H</u>), 3.67 (brt, 1H, C_{17}-<u>H</u>), 7.3 (m, 3H, Arom. <u>H</u>), 7.54 (m, 2H, Arom. <u>H</u>).

1β,2β-Epoxy-17β-hydroxyestr-4-en-3-one (8). The above phenylselenyl derivative (**6**; 0.12 g, 0.28 mmol) was dissolved in THF (20 ml). $NaIO_4$ (0.197 g, 0.58 mmol) was dissolved in MeOH:H_2O (7:5, 4 ml) and added dropwise over a period of 5 min at room temperature. Stirring was continued for 30 h. Solvent was evaporated under vacuum, water was added and extracted with dichloromethane. The residue obtained was chromatographed over a column of silica gel. First the substrate was eluted in 25% EtOAc:hexane. Further elution with 30% EtOAc:hexane gave epoxy enone 42 mg (52% yield). The yield was calculated on the basis of the substrate consumed, m.p. 128–130°C, ^{1}H-NMR ($CDCl_3$) δ 0.68 (s,3H, C_{18}-C<u>H</u>$_3$), 3.42

(br, s, 1H, C_2-H), 3.77 (d, 1H, J = 4 Hz, C_1-H), 3.66 (brt, 1H, C_{17}-H), 5.89 (s, 1H, C_4-H).

3,17β-Bis[(trimethylsilyl)oxy]-1α,2α-epoxy-5α-estr-3-one (5). 1α,2α-Epoxide (3; 1.28 g, 4.4 mmol) was treated similarly with bis(trimethylsilyl)trifluoroacetamide. The product obtained was over 85% yield as estimated by TLC and NMR (1.615 g): ^{1}H-NMR (without TMS) 3.1 (m, 1H, H-1), 3.5 (m, 2H, C_{17}, 2-H), 4.6 (br, s, 1H, C_4-H). 0.07 [s, 9H, 17-Si$(CH_3)_3$], 0.22 [s, 9H, 3-Si$(CH_3)_3$]. δ 0.66 (s, 3H, C_{18}-CH$_3$).

1α,2α-Epoxy-4β-phenylselenyl-17β-hydroxy-5α-estr-3-one (7). In the same manner the above bis(trimethylsilyl)ether (5; 1.615 g, 3.7 mmol) was treated with phenylselenyl chloride (1 g). The residue obtained was purified over a column of silica gel and eluted with 30% EtOAc:hexane to give 800 mg (60%) of 7, m.p. 82–84°C. ^{1}H-NMR ($CDCl_3$) δ 0.78 (s, 3H, C_{18}-CH$_3$), 3.35 (d, J = 4 Hz, C_4-H), 3.50 (s, 1H, C_2-H), 3.68 (overlapping brt, d due to H-1, brt due to C_{17}-H), 7.31 (m, 3H, Arom. H), 7.52 (m, 2H, Arom. H).

1α,2α-Epoxy-17β-hydroxyestr-4-en-3-one (9). Similarly the above phenyl-selenyl derivative (7; 100 mg; 0.23 mmol) was treated with $NaIO_4$ (98 mg; 0.46 mmol). The compound was eluted from a column of silica gel in 30% EtOAc:hexane to give 35 mg (53% yield; on the basis of the substrate consumed) of 9; m.p. 122–123°C (reported 123–124°C). ^{1}H-NMR ($CDCl_3$) δ 0.84 (s, 3H, C_{18}–CH$_3$), 3.45 (m, 1H, C_2-H), 3.78 (m, 2H, C_{17}, 1-H), 5.79 (brt, 1H, C_4-H).

Reaction of ethanethiol with 4β,5β-epoxy-17β-hydroxyestr-1-en-3-one

(A) Sodium metal (9.2 mg, 0.4 mmol) was added to a solution of 4β,5β-epoxyenone (10; 54 mg; 0.19 mmol) in ethanethiol (2 ml) with stirring under nitrogen. Stirring was continued for 2 h at room temperature. Ethanethiol was evaporated under vacuum, water was added, acidified with AcOH and extracted with ethylacetate. The residue obtained was applied over a column of silica gel. The compound which eluted in 15% ethylacetate:hexane gave 35 mg (56% yield), m.p. 84–86°C which was characterized as 4-ethylthio-estradiol (14). ^{1}H-NMR ($CDCl_3$) δ 0.77 (s, 3H, C_{18}-CH$_3$), 1.21 (t, 3H, s, CH_2CH_3, 2.64 (q, 2H, S-CH$_2$), 3.73 (brt, 1H, C_{17}-H), 6.84 (d, J = 9 Hz, 1H, C_2-H), 7.24 (d, J = 9 Hz, 1H, C_1-H). u.v. 260, 286 nm.

(B) Sodium metal (7 mg; 0.3 mmol) was added in ethanethiol (2 ml). When the sodium completely dissolved, 4β,5β-epoxyenone (10) was added as a solid and stirring was continued at room temperature for 45 min. Water was added, acidified with acetic acid and extracted with ethylacetate. The residue was purified over a column of silica gel. 4-Ethanethiol-estradiol (14) was obtained in 7% yield. Further elutions with 25% EtOAc:hexane gave 24 mg (43% yield), of 1,4 bis(ethanethiol)-17β-hydroxyestr-4-en-3-one (15), m.p. 151–153°C, ^{1}H-NMR ($CDCl_3$) δ 0.81 (s, 3H, C_{18}-CH$_3$), 2.74 (m, 4H, 2 × S CH$_2$), 3.71 (m, 2H, C, 17-H); mass spectrum, *m/e* 394 (M^+), λ_{max} at 248 nm.

(C) Ethanethiol (0.5 ml) was dissolved in THF (dry) and small pieces of sodium metal (6 mg, 0.25 mmol) was added. When the sodium completely dissolved, the above epoxy enone (32 mg, 0.11 mmol) in THF was added dropwise at room temperature with vigorous stirring. Stirring was continued for 45 min. After usual workup and flash chromatography, the following three compounds were isolated: 4-Ethanethiol-estradiol (14; 36% yield). 1β,4α-Bis-(ethanethiol)-5,17β-dihydroxy-5β-estran-3-one (16) (20%), m.p. 162–64°C; ^{1}H-NMR ($CDCl_3$) δ 0.8 (s, 3H, C_{18}-CH$_3$), 2.48 (m, 4H, 2 × S CH$_2$), 3.0 (d, J = 10 Hz, 1H, C_{10}-H), 3.73–3.54 (m, 3H, C_1, C_4, 17-H); mass spectrum *m/e* 412 (M^+). 4α-Ethanethiol-5,17β-dihydroxy-5β-estr-1-en-3-one (17) (5% yield), m.p. 88–91°C; ^{1}H-NMR ($CDCl_3$) δ 0.78 (s, 3H, C_{18}-CH$_3$), 2.6 (m, 2H, SCH$_2$), 3.11 (s, 1H, C_{10}-H), 3.73 (m, 2H, C_4, 17-H), 6.11 (d, J = 9 Hz, 1H, C_2-H) and 6.77 (d, J = 9 Hz, 1H, C_1-H); mass spectrum *m/e* 350 (M^+); λ_{max} 240 nm.

Reaction of ethanethiol with 4α,5α-epoxy-17β-hydroxyestr-1-en-3-one

4α,5α-Epoxyenone (11; 32 mg; 0.11 mmol) was dissolved in ethanethiol (1 ml). Sodium (5.75 mg; 0.25 mmol) was added in small pieces and stirring continued for 1 h. After usual workup and column chromatography over silica gel, the following compounds were isolated. 4-Ethanethiol-estradiol (14; 8% yield), TLC, m.p., NMR, u.v. were identical to the compound obtained from 4β,5β-epoxyenones. 1β,4β-Bis(ethanethiol)-5,17β-dihydroxy-5α-estran-3-one (18) 35% yield) m.p. 155–57°C; ^{1}H-NMR ($CDCl_3$) δ 0.78 (s, 3H, C_{18}=CH$_3$), 2.47 (m, 4H, 2 × SCH$_2$), 3.0 (d, J = 10 Hz, 1H, C_{10}-H), δ 3.53–3.7 (m, 3H, C_1, C_4, 17-H); mass spectrum *m/e* 412 (M^+). 1β-Ethanthiol-5,17β-dihydroxy-5α-estran-3-one (19) (15% yield), m.p. 97–100°C; ^{1}H-NMR ($CDCl_3$) δ 0.79 (s, 3H,

C_{18}-C<u>H</u>$_3$), 2.47 (m, 2H, S-C<u>H</u>$_2$), 3.5–3.7 (m, 2H, C_1, 17-<u>H</u>); mass spectrum *m/e* 352 (M^+).

Reaction of ethanethiol with 1α,2α-epoxyenone (9)

Sodium metal (8 mg; 0.36 mmol) was added to ethanethiol (1 ml). When the sodium completely dissolved, compound **9** (35 mg) dissolved in THF (1 ml) was added and stirring continued for 45 min. After usual workup, the residue was purified on a column of silica gel and eluted in 45% EtOAc:hexane to give 21 mg (60% yield) of **12**, m.p. 203–205°C, λ_{max} 240 nm; ^{1}H-NMR ($CDCl_3$) δ 0.8 (s, 3H, C_{18}-C<u>H</u>$_3$), 3.67 (brt, 1H, C_{17}-<u>H</u>), 4.46 (brt, 1H, C_1-<u>H</u>), 5.89 (s, 1H, C_4-<u>H</u>), mass spectrum *m/e* 290 (M^+).

Reaction of ethanethiol with 1β,2β-epoxyenone (8)

Similarly **8** was treated with ethanethiol in the presence of sodium metal to result in formation of compound **13** in 30% yield, m.p. 138–140°C; λ_{max} 252 nm; ^{1}H-NMR ($CDCl_3$) 0.8 (s, 3H, C_{18}-CH_3), 3.76 (m, 1H, C_{17}-<u>H</u>), 6.19 (s, 1H, C4-<u>H</u>), 6.92 (s, 1H, C_1-<u>H</u>); IR (KBr) 3395 (C-17 alcohol), 2931 (aliphatic CH), 2868, 1645 (C-3 carbonyl), 1595 (C=C) cm^{-1}. Mass spectrum *m/e* 332 (M^+).

Reaction of 4β,5β-epoxy-17β-hydroxyestr-1-en-3-one with N-acetyl cysteine

The reaction mixture contained epoxyenone (**18**; 100 mg; 0.35 mmol), *N*-acetyl-L-cysteine (57 mg; 0.35 mmol), methanol (4 ml) and 4 ml Tris buffer (0.1 M, pH = 9.2). The reaction was carried out at 55°C for 48 h. The methanol was removed under vacuum and the unreacted substrate was removed by extraction with CH_2Cl_2. The aqueous layer was passed over a column of XAD-2. Elution with water gave no compounds. Further elution was continued with methanol (200 ml). Fractions of 20 ml were collected and u.v. of each fraction was checked. Methanol was removed to give a residue of 70 mg (46%) which melts at 150–155°C. u.v. = 282 nm; mass spectrum shows *m/e* 433. The compound was injected over reverse phase (C18) semipreparative column in MeOH:H_2O (50:50; v/v) which showed a single peak. The retention time was 12 min. ^{1}H-NMR ($CDCl_3$:DMSO-d_6; 3:1) δ 0.75 (s, 3H, C_{18}-CH_3), 1.87 (s, 3H, COC<u>H</u>$_3$), 6.78 (d, J = 10 Hz, 1H, C_1-<u>H</u>), 7.12 (d, J = 10 Hz, 1H, C_2-<u>H</u>).

Reaction of 4β,5β-epoxy-17β-hydroxyestr-1-en-3-one with glutathione

Under similar reaction conditions the epoxyenone (**10**) was treated with glutathione. After usual workup, the residue obtained was purified over HPLC (C18). The compound was eluted in MeOH:H_2O (45:55). It melts at 190–195°C, λ_{max} = 259,292 nm. ^{1}H-NMR (DMSO-d_6) 0.64 (s, 3H, C_{18}-C<u>H</u>$_3$), 6.77 (d, J = 10 Hz, 1H, C_1-H), 7.06 (d, J = 10 Hz, 1H, C_2-<u>H</u>). FAB mass spectrometry gave *m/e* 576 (FAB^-), 578 (FAB^+).

RESULTS AND DISCUSSION

The synthesis of the 4α,5α- (**11**) and 4β,5β-epoxy-17β-hydroxyestr-1-ene-3-one (**10**) was carried out in essentially the same way as described by LeQuesne *et al.* [16]. However, attempts to synthesize the 1,2-epoxyenones (**8,9**) using LeQuesne's procedure gave very low yields. Thus a modified procedure was used in this investigation. Both compounds **8** and **9** were prepared from 17β-hydroxy-5α-estrane-3-one, which was readily obtained from 19-nortestosterone as described by Liston and Howarth [17]. The structure of 17β-hydroxy-5α-estrane-3-one was established by comparing its spectral data with those reported earlier in the literature [18]. Treatment of 17β-hydroxy-5α-estrane-3-one with phenylselenylchloride followed by H_2O_2 treatment gave 5α-enone (**1**). Treatment of **1** with methanolic alkaline hydrogen peroxide gave a mixture of the 1α,2α- and 1β,2β-epoxyones (**2,3**) which were separated by column chromatography using silica gel. The structures of these compounds were characterized using ^{1}H-NMR spectroscopy and compared to those reported earlier [16]. Treatment of compound **2** with excess bis(trimethylsilyl)-trifluoroacetamide in the presence of triethylamine gave the labile silyl enol ether (**4**), which without further purification was converted to the 4-selenophenylsilyl ethers by treatment with phenylselenyl chloride. Tetra-n-butylammonium fluoride treatment of the selenyosilyl ethers gave the 4-selenophenyl derivative **6** which when oxidized with $NaIO_4$ gave the desired epoxyenone **8**. The synthesis of epoxyenone **9** was obtained in essentially the same way from **3** as described above.

In order to determine the stereochemical and regiospecific reaction products obtained from treating the epoxyenones with either cysteine

or glutathione, initial studies were carried out using ethanethiol as the nucleophile. It was found that several reaction products were obtained and depended on the method of generating the thiolate anion as well as on the length of time of the reaction. Thus, treatment of 4β,5β-epoxyenone (**10**) with ethanethiol in the presence of sodium metal resulted in three types of reactions: (a) 1,4-addition of ethanethiol, (b) opening of epoxide, and (c) epoxide opening followed by dehydration. One can rationalize the formation of each of these compounds by nucleophilic attack of the thiolate anion at the 4-position resulting in the formation of compound **17**. Compound **17** may either undergo dehydration to form **14** or be transformed to **16** by 1,4-addition of the thiolate anion at the 1β-position. Dehydration of **16** results in the formation of compound **15**. It is interesting to note that when compound **16** was treated with HCl, de-ethylthiolation rather than the usual dehydration reaction product was observed. Indeed the product was identified using m.p., NMR, u.v. and MS as compound **17** and was identical to that obtained by direct reaction of epoxyenone with thiolate anion.

When 4α,5α-epoxyenone (**11**) was treated with ethanethiolate, analogous reaction products were observed in which both 1,4- addition, and epoxide opening followed by dehydration were observed. In addition, an unusual product was obtained which was characterized as the β-hydroxyenone (**19**). It is interesting to note that when reaction conditions were prolonged that the major product obtained from treatment of thiolate with either the 4α,5α- or 4β,5β-epoxyenones results in the formation of the 4-thioethyl aromatized product (**14**).

Unlike the 4,5-epoxyenones, treatment of the 1,2-epoxyenones (**8,9**) with thiolate anion results in the formation of only one product. Thus, when compound **8** was treated with ethanethiol only compound **13** was obtained which was found to exist in the ketotautomeric form as evidenced by data obtained from spectroscopic studies. ^{1}H-NMR spectra showed two singlets at δ 6.19 and 6.92. The u.v. spectrum showed the presence of an absorption peak at 252 nm which is characteristic of an α-mono-substituted 1,4-dienone system. Definitive proof for the keto-tautomeric form was obtained from infrared spectra of **13** which showed the presence of two characteristic bands at 1645 and 1595 cm^{-1} indicating the presence of an α,β-unsaturated ketone. Although literature reports indicate that ketoautomers of phenols are exceedingly unstable and tautomerize to the phenols even at very low temperatures [19] the results obtained from this study support the ketotautomeric form of compound **13**.

Reaction of thiolate with the 1α,2α-epoxyenone (**9**) gave only one compound which was identified as the β-hydroxyenone **12**. This compound showed the presence of an absorption maximum at 240 nm indicating the presence of a β,β-disubstituted α,β-unsaturated system. Furthermore, ^{1}H-NMR showed the presence of a singlet at δ 5.89, which is characteristic of the C4-H. Furthermore, the NMR spectra showed the absence of the ethanethiol peaks. Mass spectral data showed the presence of one hydroxyl group which is most likely at the C-1α position.

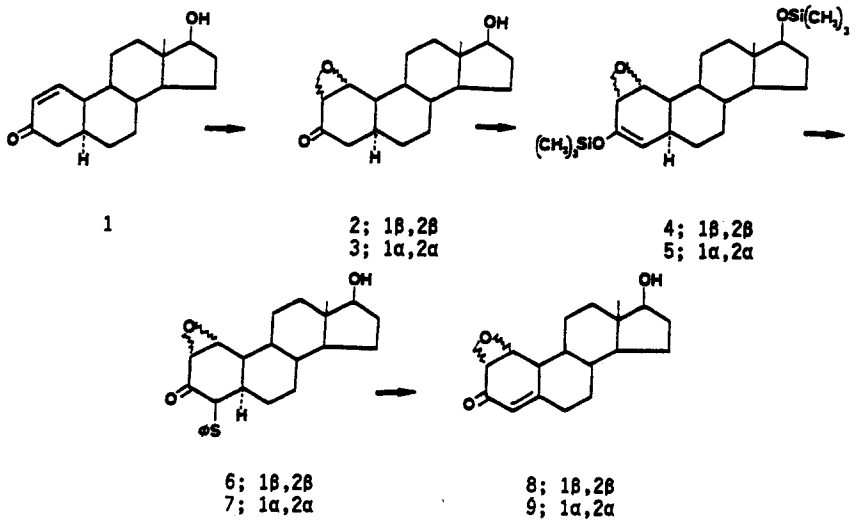


Fig. 1. Pathway for synthesis of 1,2-epoxy-17β-hydroxyestr-4-ene-3-ones.

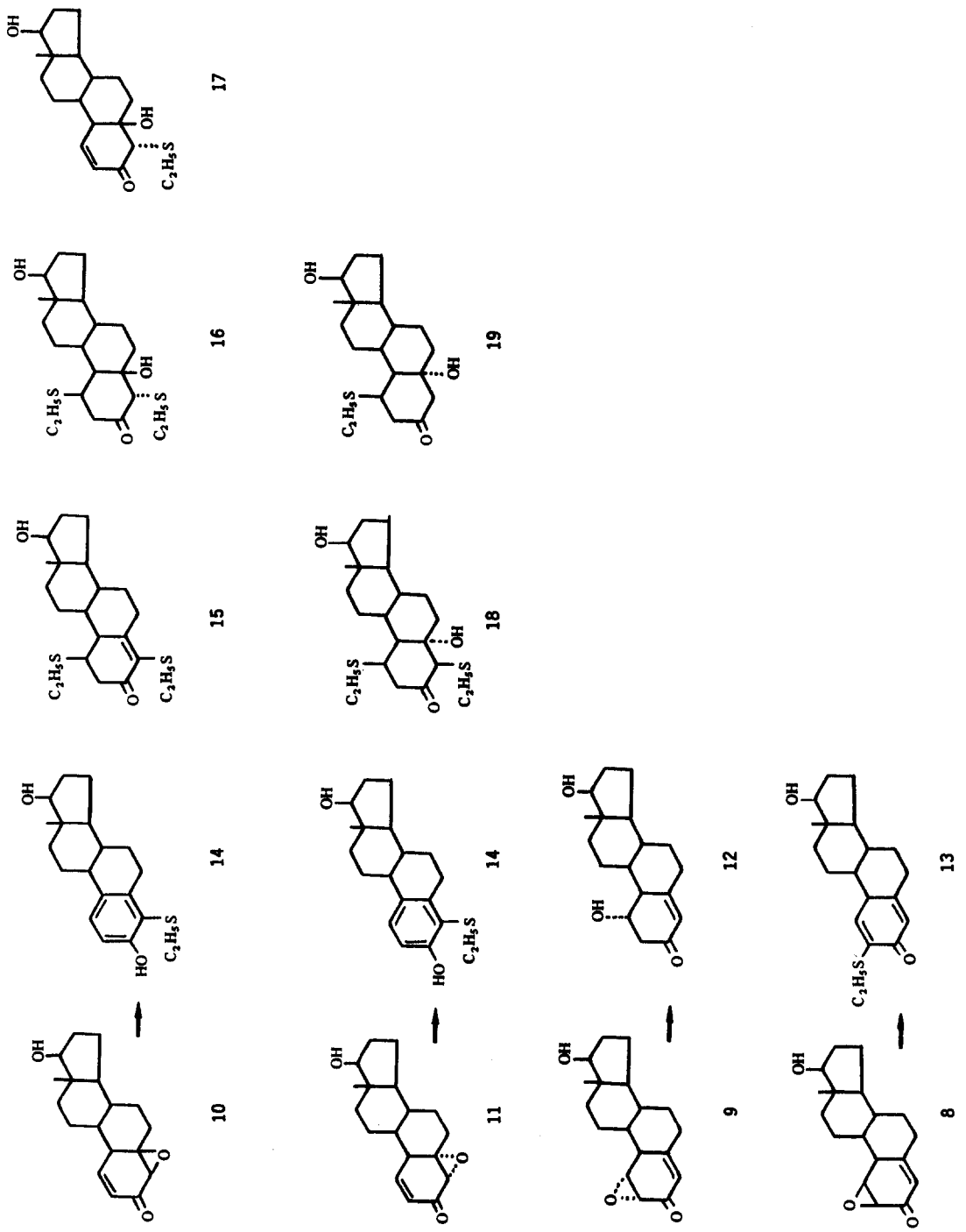


Fig. 2. Reaction of ethanethiol with expoxyenones.

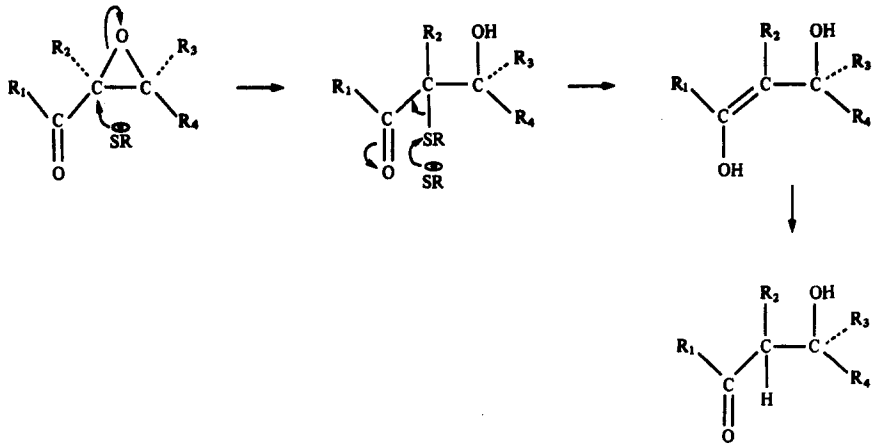
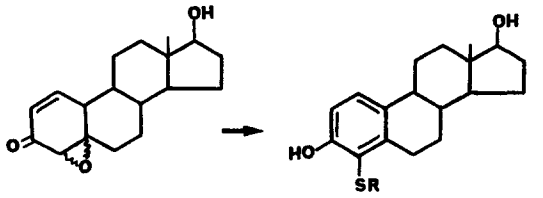


Fig. 3. Proposed mechanism for formation of **12** and **19**.



10; 11

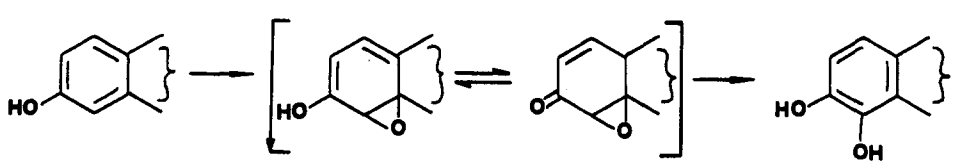
20; R = N-acetylcysteine
21; R = glutathione

Fig. 4. Reaction of 4,5-epoxyenones with glutathione and *N*-acetylcysteine.

The formation of all the reaction products obtained from this study can be rationalized by one or combination of 1,4-addition, epoxide opening and dehydration. However, two unique compounds, the β-hydroxyenones **12** and **19** are not quite obvious. However, one can envision the formation of these products if one considers the following proposed mechanism for the formation of **12** and **19** (Fig. 3). The organo-sulfur-mediated reduction of the epoxyketones may involve an initial alkylthio attack at the α-carbon followed by nucleophilic attack of a second thiolate anion on the thiol leading to the formation of the β-hydroxyenone.

Since the epoxyenones have been proposed as the ketotautomers of estradiol epoxides which are presumed to be formed during metabolism of estradiol to catechol estrogens [16], we were interested in the reaction products obtained from treating the epoxyenones with glutathione and *N*-acetylcysteine. Indeed when both compounds **10** and **11** were reacted in a buffer system with both glutathione and *N*-acetylcysteine, only one compound was isolated, namely, the 4-thio ether derivatives (**20,21**) of estradiol (Fig. 4). It is interesting to note that only one water soluble reaction product was observed (confirmed by HPLC) as compared to several products when ethanethiol was used as the nucleophile. While the exact reasons for the observed differences in these results are not fully understood, it is quite possible that reaction conditions carried out in a buffer system will result in a preferential attack of the nucleophile at the 4-position followed by dehydration resulting in the aromatized product (**20,21**). However, reactions in an organic solvent leads to multiple reaction products as shown in Fig. 2.

The epoxyenone, such as **10**, is the stable form of the dienol **23**, which is presumably the most likely intermediate formed in the oxidation of estradiol (**22**) to 4-hydroxyestradiol (**24**). Thus, if the dienol **23** tautomerizers to **10**, it may be stable enough to dissociate from the enzyme and exert independent biological effects. If indeed this is the case then one would expect that the epoxyenones (**8–11**), which were found in this



22 23 10 24

Scheme 1

study to be quite reactive, may exert their genotoxic effects through reactions with cellular nucleophiles. Studies are currently underway to determine whether the structures of the glutathione and cysteine adducts prepared in this study using *in vitro* experiments are also the same from *in vivo* studies.

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