Reactions of 3,4-Estrone Quinone with Mimics of Amino Acid Side Chains

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Reaction of 3,4-estrone *o*-quinone (3,4-EQ) with several amino acid side chain mimics, including 4-ethylphenol, 4-methylimidazole, acetic acid, and propanethiol, gave a mixture of several products including the catechol, Michael addition products, and dimeric products of the catechol. On the other hand, several other amino acid side chain mimics, including ethanol, acetamide, 1-ethylguanidine, and 3-methylindole, did not result in any addition products or catechol formation. Michael additions to 3,4-EQ with 4-methylimidazole, acetate, and 4-ethyl phenoxide resulted in 1,4-addition, leading to C-1 adducts while reaction with propanethiol gave the C-2 addition product.

Introduction

Most chemical carcinogens exert their activity either through covalent interaction of a reactive metabolite with DNA in the target organ (*1*) or by modulating one or more of a variety of biochemical and biological steps related to the process of tumor formation. Carcinogenic hormones are widely believed to belong to the latter group of chemicals because of their proliferative effect on target cells. Estrogens have been shown to induce mammary, pituitary, cervical, and uterine tumors in rats, mice, and guinea pigs (*2*). Estradiol and other estrogens induce renal carcinoma in 80−100% of Syrian hamsters within 6−8 months (*3*). Although the exact mechanism for carcinogenesis induced by estrogenic compounds is not fully understood, it is generally believed that metabolic activation of estradiol leading to the formation of catechol estrogens is a prerequisite for its genotoxic activity (*4*). It has been proposed that the steroid estrogens may generate reactive intermediates, particularly arene oxides (*5*−*8*) and quinones/semiquinones, during their metabolism, in analogy to the metabolism of aromatic polycyclic hydrocarbons which are known to be implicated in carcinogenesis (*9*).

The estrogen *o*-quinones produced by the oxidation of catechol estrogens by phenol oxidase (*10*), prostaglandin H synthase (*11*), and cytochrome P-450 oxidase (*12*) have the potential to be cytotoxic and genotoxic. These compounds can undergo one-electron redox cycling resulting in the formation of semiquinone, superoxide anion, and hydroxyl radical. Studies in our laboratories have shown that 3,4-EQ[1] was capable of inducing specific DNA damage in a human breast cancer cell line (*13*). These studies showed that 3,4-EQ is unique among *o*-quinones in that it causes exclusively single strand DNA breaks/alkali-labile sites. Furthermore, studies by Nutter *et al.* (*14*) provided conclusive evidence documenting the production of hydrogen peroxide, the hydroxyl radical, and the semiquinone of 3,4-EQ. Although production of reactive oxygen species (ROS) through redox cycling has been implicated in damage to macromolecules, including DNA, RNA, and proteins (*15*), the assignment of the actual ROS by 3,4-EQ-induced DNA damage awaits further study.

Another potential mechanism which may be involved in the carcinogenicity/toxicity of estrogens involves adduct formation with DNA and/or proteins. Estrogen *o*-quinones/semiquinones and estrogen 1,2-epoxide have been proposed to be responsible for estradiol's genotoxic activities. Studies in our laboratory showed that the major pathway for irreversible binding of estrogens to protein involves the estrogen *o*-quinones/semiquinones and not the estrogen arene oxides (*16*). Thus, it is quite possible that the carcinogenicity/toxicity of estrogens may be in part due to estrogen *o*-quinones/semiquinones which are capable of arylation of protein nucleophiles. Although our knowledge of conjugation of estrogen *o*-quinones with thiols (*17*−*19*) and primary amines (*20, 21*) is well documented, very little is known about the reactions of amino acid side chain nucleophiles with estrogen quinones. Considering the many different cellular proteins, nucleophilic side chains, and reactive estrogen metabolites which potentially contribute to total covalent binding of estrogens, it seems quite likely that some other nucleophilic centers may be involved in protein arylation which could change protein structure and cellular function, resulting in a toxic response. This paper reports on the reactions of estrogen *o*-quinones with amino acid side chain mimics to provide us with an understanding of the chemistry of conjugate addition of various nucleophiles to estrogen quinones.

Experimental Procedures

Chemicals. Estrone was purchased from Steraloids (Wilton, NH). 4-Ethylphenol, 4-methylimidazole, 1-propanethiol, 3-methylindole, and 1-ethylguanidine sulfate were obtained from Aldrich Chemical Co. (Milwaukee, WI). Acetamide was obtained from Sargent-Welch Scientific Co. (Skokie, IL). Deuterated solvents were purchased from Aldrich Chemical Co. The synthesis of the catechol estrogens was carried out as described by Stubenrauch and Knuppen (*22*). The estrogen *o*-quinones

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[1] Abbreviations: 3,4-estrone quinone, 3,4-EQ; reactive oxygen species, ROS; 4-hydroxyestrone, 4-OHE_1; triethylamine, Et_3N.

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Table 1. Crystal Data for Compound 3

parameter	compound
empirical formula	$C_{26}H_{34}O_4N_2$
formula wt (amu)	438.57
crystal color and habit	colorless needle
cryst dimensions (mm)	0.50 × 0.10 × 0.10
cryst system	orthorhombic
a (Å)	10.386(2)
b (Å)	22.577(3)
c (Å)	10.177(2)
V (Å^3)	2386.5(6)
Z	4
space group	$P2_12_12_1$ (#19)
calcd density (g/cm^3)	1.220
F_{000}	944
μ(Cu Kα) (cm^{-1})	6.23
no. of measured reflections	
total	5053
unique	4398
no. of observed refl. ($I > 3.00\sigma(I)$)	2011
R_{int}	0.047
R	0.048
wR	0.051
max e$^-$ in final difference map	+0.21 e$^-$/Å^3
min e$^-$ in final difference map	−0.26 e$^-$/Å^3

were synthesized by the oxidation of the catechol estrogens by activated MnO_2 as described previously in our laboratory (*23*). **Caution:** *The estrogen o-quinones are potentially hazardous and were handled in accordance with NIH Guidelines for the Laboratory Use of Chemical Carcinogens* (*24*).

Characterization of Compounds. All of the compounds were characterized by nuclear magnetic resonance ([^{1}H]-NMR), ultraviolet (UV), infrared (IR), and mass spectrometry (MS). Melting points (uncorrected) were taken on a Fisher-Johns apparatus. [^{1}H]-NMR spectra were obtained with a GE-300 MHz NMR spectrometer, and the chemical shift data are reported in parts per million (δ) downfield from tetramethylsilane as an internal standard. Nuclear Overhauser effect (NOE) difference spectra were recorded by applying a presaturation pulse with a decoupler on resonance and subtracting the trace from the corresponding reference spectra recorded under identical conditions but with the decoupler off-resonance. Typical spectra were obtained from 64 transients. The UV spectra were obtained using a Beckman DU-70. The IR spectra were obtained on a Nicolet 5 DXC FT-IR spectrometer. Mass spectral data were determined on an AEI MS-30, a VG 7070 E-HF, and a Finnigan MAT 95.

X-ray Crystallography. Colorless crystals of **3** were grown in tetrahydrofuran. All measurements were made on a Rigaku AFC6S X-ray diffractometer with graphite monochromated Cu Kα radiation ($\lambda = 1.54178$ Å) using the $\omega - 2\theta$ scan mode. The crystal temperature was maintained at 173(1) K by using a Molecular Structure Corp. low temperature device. Intensities were corrected for Lorentz and polarization effects. Equivalent reflections were merged and absorption effects were corrected using the ψ scan technique (transmission factors: 0.94−1.00) as described by North *et al.* (*25*). The structure was solved by direct methods using SHELXS86 (*26*) and refined and finished using the TEXSAN structure analysis package (*27*). All nonhydrogen atoms were refined anisotropically. Hydrogens bonded to carbon atoms were placed in calculated positions (0.95 Å) and were not refined. Hydrogens bonded to heteroatoms were located in difference Fourier maps, and their positional parameters were refined. Crystal data are given in Table 1.

Synthesis of Catechol Estrogens and Estrogen Quinones. Synthesis of the 4-OHE_1 was carried out as described by Stubenrauch and Knuppen (*22*) and the quinone as described in our laboratory (*23*). 3,4-EQ (**1**): ^{1}H-NMR ($CDCl_3$) δ 0.85 (s, 3H, 18-CH_3), 6.21 (d, 1H, H-2), 7.16 (d, 1H, H-1). 4-OHE_1 (**2**): ^{1}H-NMR ($CDCl_3$) δ 0.89 (s, 3H, 18-CH_3), 6.71−6.75 (dd, 2H, H-1 and H-2).

Reaction of 3,4-Estrogen *o*-Quinone (1) with 4-Methylimidazole. 4-Methylimidazole (28.90 mg, 0.35 mmol) in CH_2Cl_2 (15 mL) was added to quinone (**1**, 100 mg, 0.35 mmol) in CH_2Cl_2 (10 mL) and stirred at room temperature. After 30 min there was no quinone left (checked by TLC), and product **3** precipitated as a white solid. The solid was filtered off, washed with CH_2Cl_2, and recrystallized from THF: mp 288−290 °C dec; R_f 0.04 in 25% EtOAc in benzene and $R_f = 0.17$ in 25% benzene in EtOAc; UV (CH_2Cl_2) λ_{max} (nm) 241, 286; IR (KBr) cm^{-1} 3400, 2935, 2927, 2890, 1736, 1617, 1617, 1507, 1405, 1384; ^{1}H-NMR ($CDCl_3$) δ 7.93 (s, 1H, H-2 imidazole), 6.85 (s, 1H, H-2), 6.65 (s, 1H, H-5 imidazole), 5.30 (s, 2H, OH, D_2O exchangeable), 2.28 (s, 3H, CH_3 imid.), 0.85 (s, 3H, 18-CH_3); EI MS *m*/*z* 366.2 (M$^+$, 100), 351 (2.55), 325 (22.6), 310 (10.8). These data are in agreement with the proposed structure of **3**. The mother liquid was evaporated to dryness and applied to a silica gel column. Elution of the column with 25% EtOAc in benzene gave 4-hydroxyestrone **2**: mp 263−265 °C [lit. mp 260−265 °C (*22*)], $R_f = 0.56$ in 25% EtOAc in benzene. Further elution of the column gave trace amounts of an orange-yellow solid **4**: mp 242−245 °C dec; $R_f = 0.34$ in 25% EtOAc in benzene; UV (CH_2Cl_2) λ_{max} (nm) 229, 466; IR (KBr) cm^{-1} 3440, 2962, 2926, 1740, 1735, 1660, 1364, 1261, 1097, 1023, 902; ^{1}H-NMR ($CDCl_3$) δ 7.39 (s, 1H, H-2′), 7.32 (d, 1H, H-2, $J = 8$ Hz), 7.22 (d, 1H, H-1, $J = 8$ Hz), 5.32 (bs, 1H, OH), 3.00−1.42 (30 H, m, methylene and methine protons), 1.22 (s, 3H, 18′-CH_3), 0.92 (s, 3H, 18-CH_3); high resolution EI MS *m*/*z* (obsd) 568.2836 (M$^+$, 9), 286 (100), 284 (83.6), 269 (31), 267 (11), 226 (19.3); (calcd) 568.7160.

Reaction of 3,4-Estrogen *o*-Quinone (1) with 1-Propanethiol. 1-Propanethiol (31.8 μL, 0.35 mmol) was added to a solution of quinone (**1**, 100 mg, 0.35 mmol) in CH_2Cl_2 (20 mL) which resulted in decoloration of the solution in 20 min. Evaporation of the CH_2Cl_2 left a solid that showed the presence of three spots on TLC which were separated using preparative TLC (25% EtOAc in benzene). The spot having the lower R_f value was identified as 4-OHE_1. The spot with the higher R_f value gave a light yellow solid of **5**: mp 102−103 °C dec; ^{1}H-NMR ($CDCl_3$) δ 6.96 (s, 1H, H-1), 6.42 (s, 1H, OH, D_2O exchangeable), 5.38 (s, 1H, OH, D_2O exchangeable), 2.63 (t, 2H, -SCH_2-), 1.56 (q, 2H, -CH_2CH_3), 1.00 (t, 3H, CH_3), 0.91 (s, 3H, 18-CH_3); EI MS *m*/*z* 360.2 (100), 155 (4), 97(3.7).

Reaction of 3,4-Estrogen *o*-Quinone (1) with 4-Ethylphenol. To a solution of 4-ethylphenol (43.01 mg, 0.35 mmol) in CH_2Cl_2 (30 mL) was added three drops of $(Et)_3N$ and 3,4-estrogen *o*-quinone (**1**, 100 mg, 0.35 mmol), and the reaction mixture was stirred at room temperature. The quinone was totally consumed within 30 min (checked by TLC). Evaporation of CH_2Cl_2 left a solid that showed several spots on TLC that were separated on a silica gel column. Elution of the column with 25% EtOAc in benzene yielded red-orange product **6**, 36 mg: mp 223 °C dec; $R_f = 0.53$ in 25% EtOAc in benzene; UV (CH_2Cl_2) λ_{max} (nm) 233, 270; IR (KBr) cm^{-1} 3400, 2940, 2926, 2840, 1739, 1505, 1363, 1211; ^{1}H-NMR ($CDCl_3$) δ 7.28 (d, 2H, arom.), 7.25 (s, 1H, H-2), 6.97 (d, 2H, arom.), 5.40 (s, 2H, OH, D_2O exchangeable), 2.69 (q, 2H, CH_2), 1.24 (t, 3H, CH_3), 0.97 (s, 3H, 18-CH_3); EI MS *m*/*z* 406 (M$^+$, 100), 321 (12), 282 (18), 107 (96). The data are in agreement with the structure of the proposed compound. Further elution of the column gave **2** followed by compound **4**.

Reaction of 3,4-Estrogen *o*-Quinone (1) with Acetic Acid. To a solution of quinone (**1,** 100 mg, 0.35 mmol) in CH_2Cl_2 (10 mL) and acetic acid (105 μL, 1.75 mmol) was added five drops of $(Et)_3N$ in CH_2Cl_2 (10 mL), and the mixture was stirred at room temperature. After 45 min, the reaction was complete (absence of quinone checked by TLC). The methylene chloride solution was evaporated to dryness, which showed several spots on TLC that were separated on a silica gel column. Elution of the column with 25% EtOAc in benzene gave compound **2**. Further elution of the column yielded a dark-red product **7**: mp 138−140 °C dec; $R_f = 0.38$ in 25% EtOAc in benzene; UV (CH_2Cl_2) λ_{max} (nm) 232, 274; IR (KBr) cm^{-1} 3420, 2928, 2810, 1739, 1734, 1653, 1190; ^{1}H-NMR ($CDCl_3$) δ 7.28 (s, 1H, H-2), 5.02 (bs, 2H, OH, D_2O exchangeable) 2.53 (s, 3H, -$COCH_3$), 0.93 (s, 3H, 18-CH_3); high resolution EI MS *m*/*z* (observed) 344.16 31 (M$^+$, 19.5), 303 (M$^+$ + 2H$^+$ - CH_3CO, 19), 302 (M$^+$ + H$^+$ -

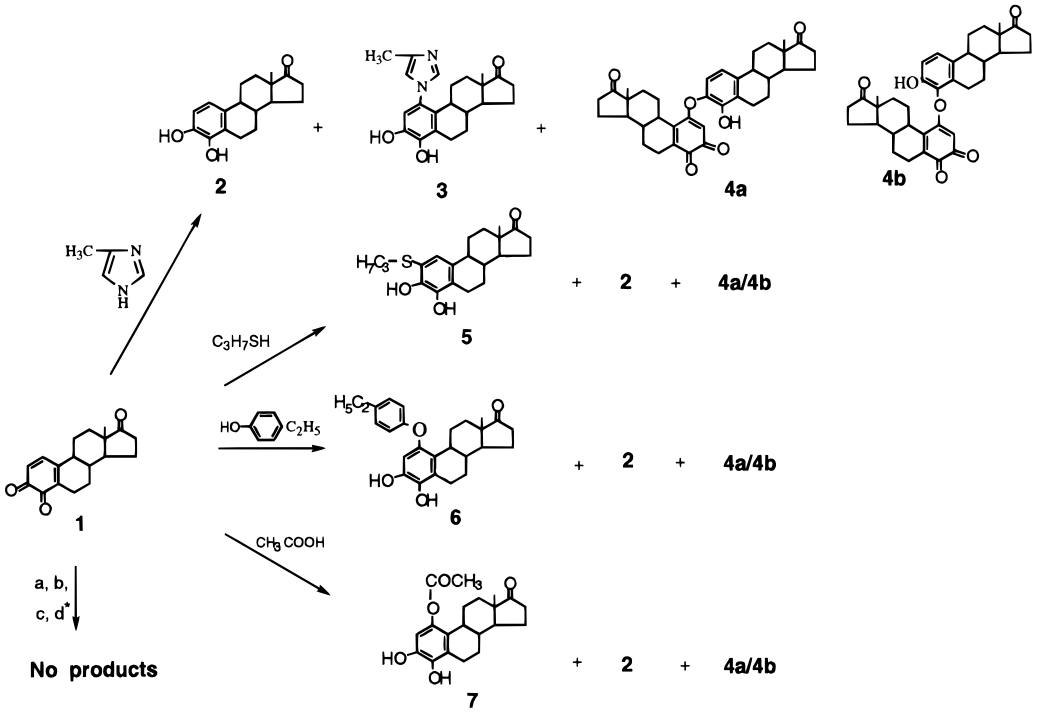

Figure 1. Products from the reaction of 3,4-estrone-*o*-quinone with 4-methylimidazole, 4-ethylphenol, 1-propylthiol, and acetic acid. *Reagents: a = ethanol; b = acetamide; c = 1-ethylguanidine; d = 3-methylindole.

CH_2CO, 100), 286 (M^+ - CH_3COO, 21); (calcd) 344.4120. Continued elution of the column gave a dimeric product of 3,4-estrogen *o*-quinone, which gave ^{1}H-NMR and EI MS data consistent with the structure of **4**.

Results and Discussion

The reactions of 3,4-EQ with several amino acid side chain mimics gave different levels of complexities in the spectrum of reaction products. All reactions were normally complete within 1 h as determined by loss of quinone. The reaction products were isolated and purified by a combination of TLC and column chromatography and characterized by UV, IR, NMR, and mass spectral data.

The reaction of 3,4-EQ with 4-methylimidazole was complete within 1 h and resulted in the formation of one major compound identified as the Michael addition product **3** (Figure 1). Trace amounts of compound **2** were isolated and found to be identical to an authentic sample of 4-hydroxyestrone, and its formation may involve reduction of 3,4-EQ either by the catechol adduct **3** or by the catechol precursors to **4** and its isomers. Compound **3** was formed as a white precipitate, and its purification involved recrystallization from tetrahydrofuran and full characterization by UV, IR, NMR, and MS. The proton NMR spectrum of **3** indicates the presence of a hydroquinone adduct which showed a singlet peak in the aromatic region at 6.85 ppm, two OH protons, D_2O exchangeable, as well as two other peaks at 7.93 and 6.65 ppm characteristic of the imidazole group. The appearance of a singlet at 6.85 ppm could not differentiate between the C-1 and C-2 protons, since the chemical shifts in $CDCl_3$ for both C-1 and C-2 protons in 4-hydroxyestrone (**2**) are observed at 6.71−6.75 ppm. Thus, the downfield shift that is observed following addition at either the C-1 or C-2 position would be difficult to interpret. Conclusive evidence for the structure of the imidazolo adduct was obtained from X-ray crystallo-

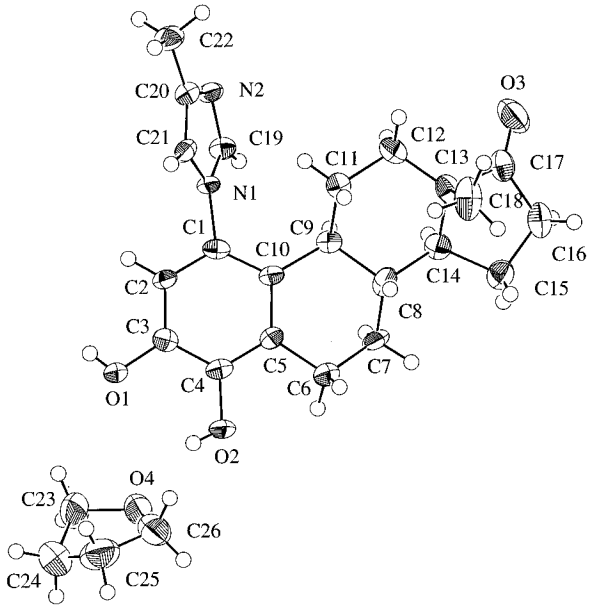

Figure 2. ORTEP drawing of **3** with crystallographic numbering system. Ellipsoids representing the non-hydrogen atoms are shown at the 50% probability level.

graphic analysis performed on a single crystal of **3**. Figure 2 is an ORTEP drawing of **3** which clearly shows the result of a 1,4-Michael addition in which the N-1 nitrogen of 4-methylimidazole attacks the C-1 position of 3,4-EQ. The X-ray structural data show that this product crystallizes as a tetrahydrofuran solvate. Crystallographic data for **3** are summarized in Table 1.

Compound **4a/4b** was found to be dimeric, and its structure was deduced from UV, MS, and NMR data. This compound was found to be identical to that obtained previously from reactions of 3,4-EQ with lysine (*21*). Products of oxidative coupling can be formed through either a C−C or C−O bond formation as described previously (*28*). Confirmation of the presence of a C−O

bond comes from the NMR spectrum showing one singlet at 6.06 ppm, which can be attributed to one proton of the quinone moiety, and two other singlets at 6.13 and 6.32 ppm which are attributed to two aromatic protons of the catechol moiety. On the basis of the NMR spectra, it is not possible to discriminate between **4a** and **4b** which could arise through C−O coupling reactions. It is interesting to note that **4** was formed in all reactions in which addition products and catechol are formed. However, compound **4** was not obtained following reaction of 3,4-EQ with ethanol, acetamide, ethylguanidine, and 3-methylindole in which no reaction products were observed. While the exact reasons for this are not fully understood, it is quite likely that a small amount of reduction of 3,4-EQ by any of the Michael adduct catechols would give **2**, which under the reaction conditions would partly Michael add to 3,4-EQ to give the catechol precursors to **4**, which in turn would be oxidized to **4** by 3,4-EQ, thereby regenerating **2**. This redox interchange chemistry is consistent with the effect of heteroatom substituents on the quinone/catechol redox potential. Alternatively, a one-electron transfer from the reactant via a charge-transfer complex will result in oxidative phenolic coupling through a C−C or C−O bond formation (*29*). Studies by Murty and Penning (*30*) on reactions of 1,2-naphthoquinone with cysteine showed that none of their products corresponded to either the bis-thiol adducts or coupled phenol products, although they indicated that it is possible that they were formed in minor quantities. Therefore, it is quite conceivable that the differences in these results may be related to the structural differences and/or the reactivities of the estrogen *o*-quinones and the *o*-naphthoquinone.

In the reaction of 3,4-EQ with propanethiol, one major product **5** and two minor products **2** and **4** were isolated and identified using UV, IR, NMR, and MS data. Compound **5** was isolated, purified, and recrystallized to give a light yellow solid. The proton NMR spectrum of **5** showed the presence of the propyl group, two OH protons at 6.42 and 5.38 ppm, D_2O exchangeable, and a singlet at 6.96 for the C-1 proton which shifted to a lower magnetic field due to the presence of an adjacent thiol group. Support for 1,6-Michael addition of thiols to 3,4-EQ is based on earlier studies by Jellinck (*19*) and us (*17*) in which thiols were found unequivocally to add at C-2 using specifically labeled [1-^{3}H]- and [2-^{3}H]-4-hydroxyestrogens.

Reaction of 3,4-EQ with 4-ethylphenol was extremely slow, and only traces of products were observed. Since the phenolic group in tyrosine may be in the ionized form in biological systems, reactions of 3,4-EQ with 4-ethylphenol were carried out in the presence of triethylamine and were complete within 30 min. Three products, **6**, **2**, and **4a/4b**, were isolated and characterized. Proton NMR of **6** showed two AB doublets at 7.28 and 6.97 ppm assigned to the aromatic phenol, a triplet at 1.24 ppm corresponding to the methyl group in the ethyl side chain and a singlet at 7.25 ppm attributed to the C-2 proton of the steroid. Support for 1,4-Michael addition of the phenoxide is obtained from NOE studies (see next paragraph). Mass spectra showed a molecular ion (100%) at m/z 406 which supports the structure proposed for **6**. Reactions with 3,4-EQ and acetic acid were also conducted in a manner identical to that described for 4-ethylphenol in which triethylamine was used in the reaction mixture. As before, a number of reaction products including **2**, **7**, and **4a/4b** were isolated and found in high enough purity to permit further characterization. Spectral data obtained for the red-brown adduct **7** are consistent with a 1,4-Michael addition of the acetate nucleophile. Proton NMR showed the presence of a singlet at 7.28 ppm attributed to the C-2 proton (supported by NOE studies as discussed in the next paragraph), a broad singlet at 5.02 for OH, D_2O exchangeable, and a singlet at 2.53 ppm corresponding to the carbonyl methyl.

In order to identify the regiochemistry of adduction to 3,4-EQ by acetate and phenoxide, we carried out detailed NOE difference spectra on **3**, **5**, **6**, and **7**. Since the structures of **3** and **5** are unequivocally established, NOE studies on these two compounds were found extremely helpful in assigning the regiochemistry of acetate and phenoxide addition to 3,4-EQ. Irradiation of the resonance at 6.85 ppm in **3** assigned to the C-2 proton in ring A did not enhance the signal for protons at C-11, which is consistent with C-1 substituted 3,4-catechol estrogens. On the other hand, when the singlet at 6.96 ppm in **5** was irradiated, a significant enhancement of the signals at 2.37 ppm for C-11 protons was observed. These studies support C-2 addition of thiols and are consistent with our previous results using tritium labeling experiments (*17*). When the singlet resonances at 7.25 and 7.28 ppm in **6** and **7**, respectively, were irradiated, no enhancement of the C-11 protons was observed. These results suggest that the acetate and phenoxide react with 3,4-EQ by 1,4-Michael addition, resulting in the formation of the C-1 adducts. While the exact reasons for the differences in addition of various nucleophiles are not yet fully understood, the results obtained from this investigation showed that "hard" nucleophiles add to C-1 of 3,4-EQ while "soft" nucleophiles add to the C-2 position of 3,4-EQ. These results are supported by our preliminary molecular orbital calculation studies, which showed that charge density data predict that C-1 is a "hard" electrophile while C-2 is a "soft" electrophile. These observations are essentially in agreement with other reports on the addition of nucleophiles to *o*-quinones (*31*−*33*).

It is interesting to note that while reactions of 3,4-EQ with phenol, acetate, thiol, and imidazole lead to addition products, several other amino acid side chain mimics, including ethanol, acetamide, ethylguanidine, and methylindole, did not result in any addition products or catechol formation (Figure 1) which may be explained by the decreased nucleophilicity of these groups.

It is now well established that reactive intermediates play an important role in the cytotoxicity of chemicals and drugs. The fact that estrogens are metabolized to catechols (*34*), which are in turn oxidized to the *o*-quinones/semiquinones (*35*), may provide an explanation for their toxicity/carcinogenicity by a number of mechanisms including redox cycling and the generation of ROS as well as arylation of macromolecules (*36*). Studies in our laboratory are continuing to investigate the importance of redox cycling in the carcinogenicity of 3,4-EQ (*13, 14, 37*). Covalent binding of estrogen quinones to proteins may inhibit essential functions of the enzyme(s) or regulatory protein(s), which may ultimately lead to their toxic effect. Preliminary studies in our laboratory showed that estrogen quinones react with bovine serum albumin by covalent binding to possibly several nucleophilic centers. Previous studies have shown that the primary sites of covalent modification of proteins by alkylating agents include cysteine SH, histidine N (of the imidazole), and the N-terminal NH_2 group and the

ϵ-amino group of lysine (*38*). Whether the estrogen quinones can also modify proteins at these sites as well as other sites remains to be elucidated.

The results obtained from this investigation as well as earlier studies show that model compounds representing the amino acid side chains of cysteine, lysine, histidine, aspartic acid, glutamic acid, and tyrosine have been found to react with estrogen *o*-quinones, leading to the formation of catechol estrogen adducts. On the other hand, amino acid side chain mimics of arginine, serine, tryptophan, asparagine, and glutamine were found not to be involved in adduct formation. Since it is quite possible that the cytotoxicity of the estrogens could depend to a large degree on metabolic conversion to the reactive *o*-quinone/semiquinone intermediates, the reactions of amino acid side chain mimics described in the present *in vitro* study might well occur as well *in vivo*. Studies with model proteins containing a representative array of amino acids are currently being conducted.

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