An Estrogen−Nucleic Acid Adduct. Electroreductive Intermolecular Coupling of 3,4-Estrone-*o*-quinone and Adenine

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Estrogens have been shown to induce mammary, pituitary, cervical, and uterine tumors in rats, mice, and guinea pigs.[1] Estradiol and other estrogens induce renal carcinoma in 80−100% of Syrian hamsters within 6−8 months.[2] 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.[3] It has been proposed that the steroid estrogens may generate reactive intermediates, particularly arene oxides[4] and quinones/semiquinones, during their metabolism in analogy to the metabolism of aromatic polycyclic hydrocarbons which are known to be implicated in carcinogenesis.[5] Thus, the estrogen *o*-quinones/semiquinones produced by the oxidation of catechol estrogens by phenol oxidase,[6] prostaglandin H synthetase,[7] and cytochrome P-450 oxidase[8] have the potential to be cytotoxic and genotoxic. Studies in our laboratories have shown that 3,4-estronequinone (3,4-EQ), which can "redox-cycle" leading to the formation of hydrogen peroxide, the hydroxyl radical, and the semiquinone of 3,4-EQ,[9b] was capable of inducing exclusively single strand DNA breaks/alkali-labile sites in a human breast cancer cell line.[9a] Although hydroxyl radical production was found to correlate with 3,4-EQ-induced DNA damage,[9c] the production of hydrogen peroxide and the hydroxyl radical may only be an indicator of the metabolism of 3,4-EQ to a DNA-damaging species. Thus, two potential mechanisms may be involved in the carcinogenicity/toxicity of estrogen quinones: arylation of macromolecules including proteins, DNA and RNA, and generation of reactive oxygen species (ROS). While Michael addition products from reaction of amino acid nucleophiles are well documented,[10] many previous attempts at formation of nucleoside or DNA adducts with estrogen quinones have not been successful.[10g] The recent observations of DNA damage in our studies[9] and the reported formation of DNA adducts by estrogen quinones using ^{32}P-postlabeling techniques[11] suggested that a reactive estrogen radical intermediate may be involved in DNA adduct formation. We report here the synthesis and characterization of the first estrogen nucleic acid adduct obtained by intermolecular coupling of electrochemically reduced 3,4-EQ and adenine.

Cyclic voltammogram studies on 3,4-EQ were determined to establish the conditions for carrying out the coupling of 3,4-EQ with adenine.[12] A cyclic voltammogram of 3,4-EQ showed two peaks at −0.48 and −1.0 vs SCE, respectively. The first peak corresponds to the formation of the radical anion, or semiquinone, while the second peak is due to formation of the dianion of 3,4-EQ. After addition of excess adenine the potential of the first peak shifted anodically and oxidation of the counter peak disappeared, indicating reaction of adenine with semiquinone (Figure 1). In a typical preparative experiment, 3,4-EQ (60 mg) and adenine (100 mg) were added to the cathodic compartment of the cell filled with an argon-degassed solution of DMF−0.1 M $LiClO_4$ (30 mL).[13] The potential of the Pt-gauze cathode (3 × 5 cm) was gradually increased from −0.2 to −0.5 V vs SCE in the first 5 min of the electrolysis with initial currents of 50−80 mA. Electrolysis was terminated when the current decayed smoothly to 0.2−0.3 mA, after which the products were isolated and purified.[14] TLC and HPLC analysis indicated the formation of five reaction products. The major product **1**,[15] obtained in 14.1% yield, was found to be the coupling product between 3,4-EQ and adenine (Scheme 1). The other products were identified as the 4-hydroxyestrone, a dimeric product of estrone quinone/catechol,[10g] and two adenine−quinonimine products.[16] All the products were formed from free radical intermediates except for the quinonimines, which are most likely formed by direct reaction between the amino group of adenine and the carbonyl groups of 3,4-EQ. It is interesting to note that these products were also obtained from electrochemical reactions containing a mixture of H_2O−EtOH as the solvent. Furthermore, it is noteworthy that the product yields, as analyzed by HPLC, and the number of electrons, determined by coulometry at controlled potential, are dependent on the concentration of the starting 3,4-EQ; e.g., the number of

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(12) The cyclic voltammogram (100 mV/s) was carried out with a 1 mM solution of 3,4-EQ in a DMF−0.1 M Bu_4NClO_4 solution at a glassy carbon electrode. After addition of excess of adenine (5 equiv) the potential of the first peak shifted to the anodic side and the oxidation peak at −0.48 V vs SCE disappeared, indicating the further reaction of semiquinone with adenine. During the electrolysis an electronic integrator was used to record the quantity of electricity passed.

(13) When Bu_4NClO_4 was used in preparative experiments, the isolation reaction products was complicated by the presence of Bu_4NClO_4 in the organic extract. However, with $LiClO_4$ studies, the salt remained in the aqueous fraction, thus facilitating the separation of the reaction products.

(14) The reaction mixture was diluted with water (20 mL), extracted with CH_2Cl_2 (3 × 20 mL), dried over Na_2SO_4, and evaporated under vacuum. The residual mixture was separated by preparative TLC (silica gel) using benzene−EtOAc−MeOH (6.5:2.0:1.5) as an eluant and finally purified on a C18 reverse-phase preparative HPLC column eluted with a gradient of 80% CH_3OH−20% H_2O.

(15) Compound **1** was identified as 1-(8-adenine)-substituted 4-hydroxyestrone: mp 240 °C dec, R_f = 0.24 (benzene−EtOAc−MeOH, 6.5:2:1.5); UV (CH_2Cl_2) λ_{max} (nm) 230, 259; IR (KBr) cm^{-1} 3430, 3332, 3283, 3174, 2920, 2810, 1739, 1630, 1380; ^{1}H-NMR (DMSO-d_6) δ 9.57 (bs, 1H, OH, D_2O exchangeable), 8.62 (bs, 1H, OH, D_2O exchangeable), 8.28 (s, 1H, H-2, Ad), 8.11 (bs, 1H, NH, D_2O exchangeable), 7.31 (s, 2H, NH_2, D_2O exchangeable), 6.58 (s, 1H, H-2), 0.70 (s, 3H, 18-CH_3); ^{1}H-NMR (300 MHz) acetone-d_6 δ 8.17 (s, 1H, NH, D_2O exchangeable), 8.01 (s, 1H, H-2, Ad), 6.71 (s, 2H, NH_2, D_2O exchangeable), 6.62 (s, 1H, H-2), 2.85 (s, 2H, OH, D_2O exchangeable), 0.79 (s, 3H, 18-CH_3); FAB MS *m/z* 420.2 (M + H$^+$)$^+$, 418.2 (M−H$^+$)$^+$; EI *m/z* 419.2 (M$^+$, 3.60).

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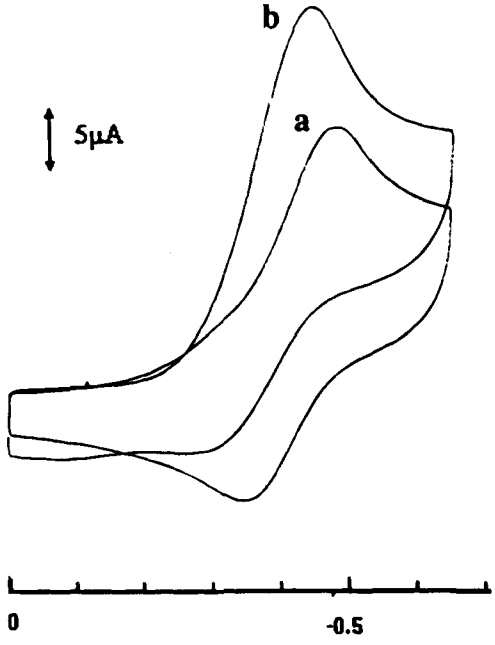
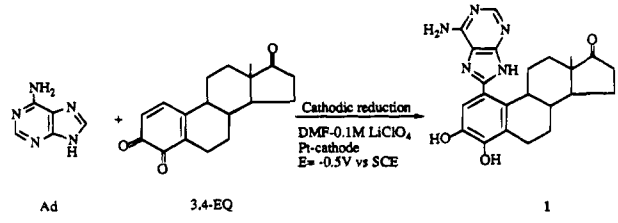


Figure 1. Cyclic voltammogram in a DMF−0.1 M Bu_4NClO_4 solution at a glassy carbon electrode ($A = 0.072$ cm^2) with scan rate 100 mV/s: (a) 3,4-EQ (1 mM), (b) 3,4-EQ plus adenine (3 mM).

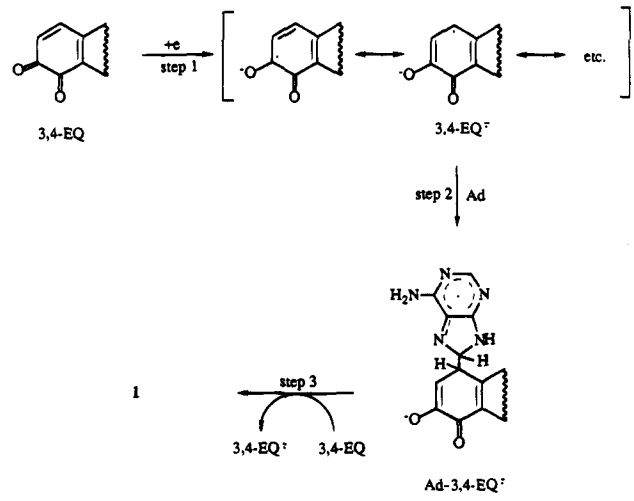
Scheme 1



electrons decreased from 0.82 F/mol (for 2.3 mM of 3,4-EQ) to 0.35 F/mol (for 7.0 mM of 3,4-EQ).

The structure of the coupling product **1** was elucidated by a combination of UV, IR, ^{1}H-NMR, and MS determinations. The mass spectrum gave signals corresponding to the assigned structure: *m/z* 419 (EI) and 420 (FAB$^+$). The presence of the adenine moiety was demonstrated by the characteristic NH_2 signals at 7.31 ppm, NH signal at 8.11 ppm, and the signal for C-2 proton at 8.28 ppm in the NMR spectrum run in DMSO-d_6. Two characteristic D_2O exchangeable OH signals at 9.57 and 8.62 ppm and a singlet at 6.58 ppm for the C-2 proton suggest a C−C coupling between the C-1 position of the catechol and the C-8 position of adenine. The assigned regiochemistry of **1** was supported by ^{1}H NOE difference experiments. Irradiation of the resonance at 6.58 ppm did not enhance the signal for protons at C-11, which is consistent with C-1-substituted 3,4-catechol estrogens.[17]

Scheme 2. Proposed Pathway for Reductive Coupling of 3,4-EQ with Adenine



The results obtained from this study allow us to formulate a hypothetical mechanism for the electroreductive coupling of 3,4-EQ and adenine (Scheme 2). The 3,4-EQ$^-$ radical anion obtained from electron transfer to 3,4-EQ (step 1) attacks adenine at C-8, which is the most common position for radical attack,[18] resulting in the formation of the Ad−3,4-EQ radical anion (step 2). The radical anion Ad−3,4-EQ$^-$ is then oxidized by 3,4-EQ to give **1** through deprotonation followed by tautomerization (step 3), resulting in the generation of 3,4-EQ$^-$ radical anion, which enters the propagation loop.[19]

Although the exact mechanism of estrogen-induced carcinogenesis is not fully understood, it appears that the carcinogenic potential of estrogens may be related to their ability to form estrogen−nucleic acid adducts via a highly reactive estrogen quinone radical species. Details of the DNA damage induced by estrogen *o*-quinones vis-à-vis radical species are under investigation.

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(16) Two quinone imine products were isolated and purified but were found to be unstable and undergo decomposition to yield adenine and 4-hydroxyestrone. These two compounds correspond to the reaction of the amino group of adenine with the C-3 and C-4 carbonyl groups of 3,4-EQ. The yields of a mixture of the quinone imine, which also contained **1** as a contaminant, ranged from 10 to 12%. Although these compounds were unstable, sufficient quantities were isolated and characterized. Both isomers gave identical UV and mass spectral data: UV (MeOH) λ_{max} (nm) 210, 262, 377, 487, 557; HRCIMS *m/z* 401.2623 (M$^+$, 2.98). The ^{1}H-NMR (acetone-d_6) spectra of one of the isomers gave signals at δ 8.22 (s, 1H, H-8, Ad), 7.08 (d, 1H, $J = 8$ Hz, H-1), 6.89 (d, 1H, $J = 8$ Hz, H-2), 6.72 (s, 1H, H-2, Ad), 6.50 (bs, 1H, NH, Ad, D_2O exchangeable), 0.91 (s, 3H, 18-CH_3). The ^{1}H-NMR (acetone-d_6) of the other quinone imine isomer gave signals at δ 7.92 (s, 1H, H-8, Ad), 6.99 (d, 1H, $J = 8$ Hz, H-1), 6.63 (d, 1H, $J = 8$ Hz, H-2), 6.52 (s, 1H, H-2, Ad), 6.46 (bs, 1H, NH, Ad, D_2O exchangeable), 0.89 (s, 3H, 18-CH_3).

(17) ^{1}H NOE difference experiments on 1-(4-methylimidazolyl)-4-hydroxyestrone (structure confirmed by X-ray crystallography) showed no enhancement of the signals for protons at C-11 when the C-2 proton at 6.85 ppm was irradiated. On the other hand, when the C-1 singlet proton at 6.96 ppm in 2-thiopropyl-4-hydroxyestrone was irradiated, a significant enhancement of the signals at 2.52 for C-11 protons was observed (manuscript submitted).

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(19) An alternative mechanism may involve reduction of the Ad−3,4-EQ$^-$ radical anion followed by protonation, tautomerization, and presumably air oxidation during the workup procedure giving rise to the coupling product **1**. This mechanism would imply the transfer of two electrons per 3,4-EQ molecule and can be ruled out as the probable mechanism since the number of electrons determined by coulometric measurement at controlled potential was found to be much lower ($n = 0.87-0.35$ F/mol). Therefore, our results suggest a chain reaction mechanism for this transformation.