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Regioselective deuterium labeling of estrone and catechol estrogen metabolites

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ABSTRACT

Increased exposure to estrogens and estrogen metabolites is linked with increased rates of breast, ovarian and other human cancers. Metabolism of estrogen can led to formation of electrophilic *o*-quinones capable of binding to DNA. In order to gain insight into the mechanism of estrogen-induced DNA damage, estrone and catechol estrogens derived from estrone, have been regioselectively labeled with deuterium at the 1-position. Estrone-1-*d*, estrone-1,2,4-d_3, 4-hydroxyestrone-1-*d* and 2-hydroxyestrone-1-*d* have been synthesized with or without deuteriums at the 16-position. The key labeling step involves deuterated trifluoroacetic acid exchange catalyzed by *t*-butyl alcohol. This economical, straightforward labeling technique makes available a range of estrone compounds containing deuterium at the 1-position.

1. Introduction

Estrogen and its oxidative metabolites constitute an important class of steroids. Increased levels of life-long estrogen exposure have been linked to breast, ovarian and other human cancers [1,2]. One predominant metabolic pathway is A ring hydroxylation to form catechol estrogens (CE) (Fig. 1). In the liver and in normal breast tissue, hydroxylation at the 2-position to form 2-hydroxyestrogens (2-OHE) occurs in preference to hydroxylation at the 4-position which forms 4-hydroxyestrogens (4-OHE) [3,4]. In tissues near breast tumors or breast tumors themselves, an increased level of the 4-OHE metabolite is observed [5,6]. The 4-OHE metabolite has been shown to cause renal tumors in animal models where the 2-OHE metabolite does not [7–9]. These observations have led to increased interest in the chemistry 4-OHE and its downstream *o*-quinone metabolite, estrogen-3,4-quinone (E-3,4-Q), which can bind to various nucleophilic sites in the DNA [10].

The chemistry of the two isomeric quinones, E-3,4-Q and estrogen-2,3-quinone (E-2,3-Q) display significant differences [11]. E-3,4-Q is the more reactive electrophile [12] and binds regioselectively to guanine and adenine at the 1-position of the estrogen A ring [11,13]. Reaction at the N7-position of guanine and the N3-position of adenine generate DNA adducts that undergo glycosidic bond cleavage causing the purine rings to detach from the DNA polymer. These adducts are referred to as *depurinating adducts* (Fig. 1). These depurinating adducts and their upstream metabolic sources have been detected at elevated levels in both breast cancer patients and in women with a high risk of breast cancer [14].

Reaction of E-2,3-Q with nitrogen nucleophiles occurs at the 6-position most likely through a quinone methine tautomer [11]. The nucleophilic site reacting with E-2,3-Q in both guanine and adenine is the exocyclic amino group. Covalent binding at these exocyclic amino groups does not cause glycosidic bond cleavage and these adducts remain on the DNA and are referred to as *stable adducts*.

We have investigated the reaction of 2′-deoxyguanosine (dG) with E-3,4-Q to determine the mechanistic sequence of bond formation, re-aromatization and glycosidic bond cleavage started by the Michael addition of the dG to E-3,4-Q [15]. The structure of a reaction intermediate, still containing the ribose moiety, was characterized by LC/MS (Fig. 2). The intermediate is thermally labile (half-life of 40 min at rt) due to loss of the ribose ring to form the depurinated estrogen-DNA adduct. This thermal instability makes structural identification via NMR difficult. Based on UV/Vis data, we proposed **1** as a likely structure for this intermediate. Groopman has provided chemical evidence the reaction intermediate is consistent with **2** [16]. Access to E-3,4-Q labeled selectively at the 1-position would assist in establishing the structure of this intermediate since tautomers **1** and **2** would differ in mass if labeled with deuterium at the 1-position (Fig. 2). Since E-3,4-Q is

Abbreviations: 2-OHE, 2-hydroxyestrogens; 4-OHE, 4-hydroxyestrogens; CE, catechol estrogens; dG, 2′-deoxyguanosine; EAS, electrophilic aromatic substitution; E-2,3-Q, estrogen-2,3-quinone; E-3,4-Q, estrogen-3,4-Q; IBX, *o*-iodoxybenzoic acid.

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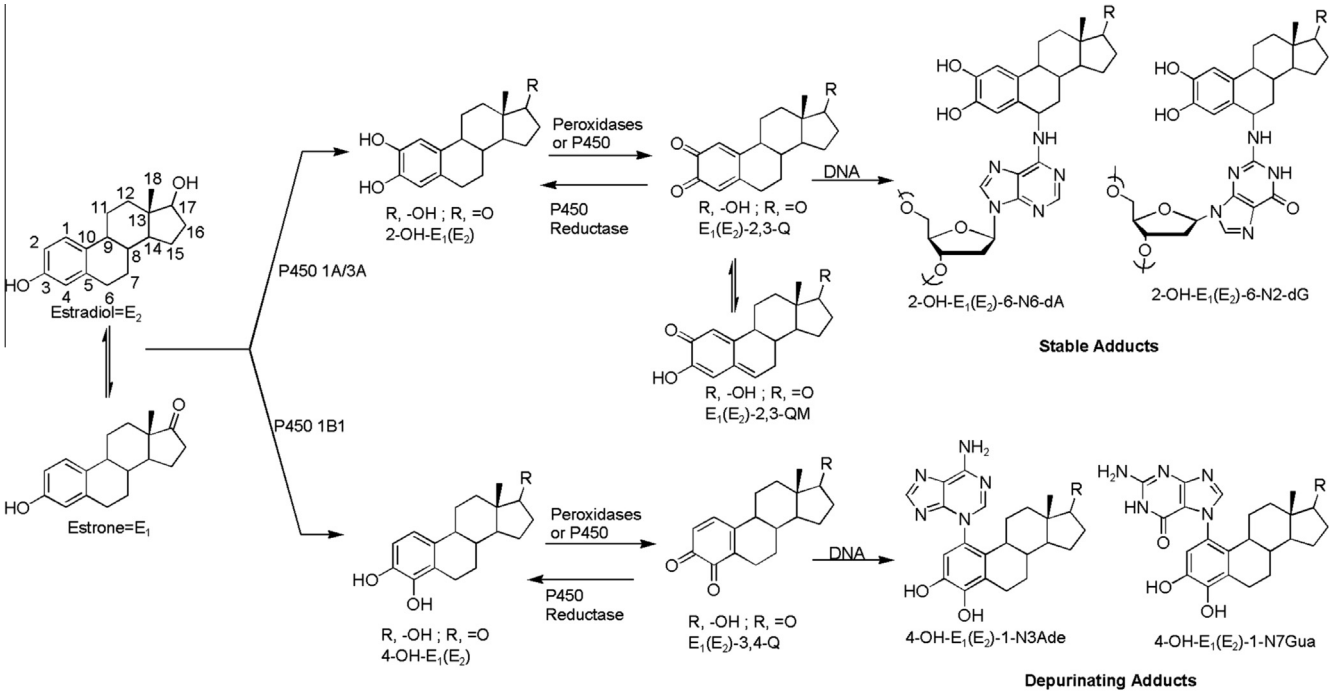

Fig. 1. Oxidative metabolism of β-estradiol and estrone to *o*-quinones and subsequent reaction with DNA.

made chemically from 4-OHE by manganese dioxide oxidation, this required the synthesis of 4-hydroxycatechol estrogens-1-*d*. Herein we report not only the synthesis of this new deuterium labeled estrogen metabolite, but also the facile synthesis of estrone-1-*d*, estrone-1,16α,16β-d_3, estrone-1,2,3,16α,16β-d_5 and 2-hydroxyestrone-1-*d*, new compounds that can be employed in mechanistic studies of estrogen metabolism.

2. Experimental

2.1. Chemicals

Estrone was purchased from Steraloids, Inc. (Newport, RI). IBX was synthesized by the method of Frigerio [17], CAUTION! IBX is explosive under impact if heated to more than 200 °C. Deuterated water was purchased from Cambridge Isotope (Andover, MA). All other chemicals and solvents were purchased from Fisher Scientific Co. (Fair Lawn, NJ.) or Aldrich Chemical Co. (Milwaukee, WI) and used as received.

2.2. Instrumentation

NMR spectra were obtained on either a Bruker AC 200 MHz spectrometer or a Bruker 400 MHz Avance III spectrometer. High resolution mass spectra were obtained at Nebraska Center for Mass Spectrometry, University of Nebraska-Lincoln. Flash column purification of compounds was conducted using a Biotage Flash40i separations module using 40 g or 90 g silica cartridges.

2.3. Synthesis of 4-OHE$_1$ and 2-OHE$_1$

4-OHE$_1$ and 2-OHE$_1$ were synthesize by Pezzella's *o*-iodoxybenzoic acid (IBX) oxidation of estrone as described previously [18] except reactions were run on a 1 g scale of estrone. The catechols were separated and purified on a Biotage Flash40i separations module (Biotage, Charlottesville, VA) using a 90 g silica gel cartridge employing a solvent pair of hexane and ethyl acetate, 75:25, respectively, containing 1% acetic acid.

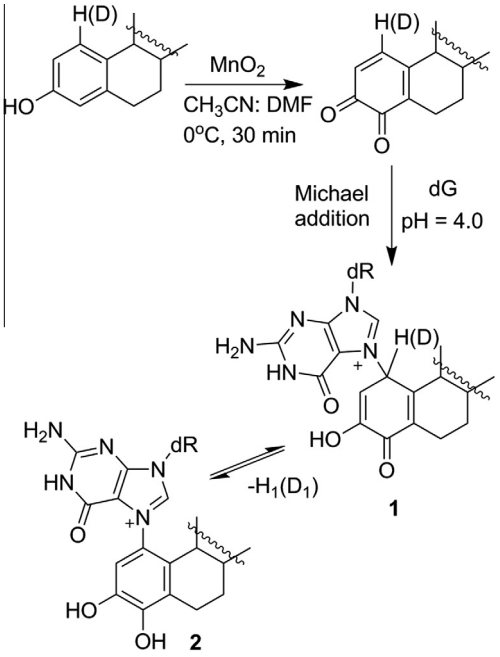

Fig. 2. Oxidation of 4-OHE to E-3,4-Q and reaction with dG.

2.4. Synthesis of estrone-1,2,3,16α,16β-d_5 (***10***) from 2-t-butylestrone (***3***)

Into an oven dried 50 mL round bottom flask equipped with an anhydrous magnesium sulfate drying tube was placed trifluoroacetic anhydride (19.4 g, 92.5 mmol). The flask was cooled to 0 °C and then carefully charged with D_2O dropwise (3.70 g, 185 mmol).