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Novel *spiro*-quinone formation from 3′-hydroxydiethylstilbestrol after oxidation with silver oxide

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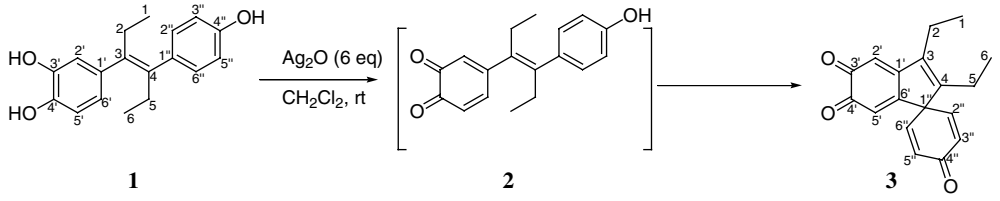
Abstract—The human carcinogen diethylstilbestrol (DES) is metabolized into 3′-hydroxydiethylstilbestrol (3′-OH-DES) (**1**). Chemical oxidation of the catechol metabolites with silver oxide in CH_2Cl_2 affords a novel *spiro*-quinone (**3**) in quantitative yield. Protection of the phenolic OH group followed by oxidation gives 4″-OCH_3-DES-3′,4′-Q (**5**) in excellent yield.

Diethylstilbestrol (DES) is a synthetic estrogen, which was used from 1940 to 1971 as a treatment to prevent spontaneous abortions in women. It is known to be a human carcinogen based on sufficient evidence of carcinogenicity in both animals[1] and humans.[2] Despite intensive investigation, the mechanistic details of carcinogenesis by this synthetic estrogen still remain obscure. In addition to being a potent estrogen, DES is extensively biotransformed in numerous mammalian species to oxidative metabolites, some of which are capable of damaging cellular macromolecules.[3] Both hormonal effects and metabolic activation are thought to act in concert in the process of DES-induced carcinogenesis.[4,5] DES, analogous to natural estrogens, is mainly metabolized to the corresponding catechol, 3′-OH-DES,[6,7] which can be further oxidized in situ to a reactive *ortho*-quinone, DES-3′,4′-Q. The electrophilic quinone can react with DNA to form depurinating adducts[8–10] that may lead to cancer initiating mutations.[11,12]

Analysis of depurinating adducts in vivo and in vitro requires the availability of synthesized depurinating adducts. In the past, we have synthesized standard depurinating adducts of natural[13,14] and synthetic estrogens[9,15] by oxidation of the catechol estrogens to the corresponding *ortho*-quinones, followed by their reaction with 2′-deoxyguanosine (dG) or adenine (Ade) via a 1,4-Michael addition. To synthesize the standard depurinating adducts of 3′-OH-DES (**1**), we initially followed the same strategy of oxidizing the catechol **1** with Ag_2O to the corresponding quinone, DES-3′,4′-Q (**2**) and subsequent reaction of the latter with dG or Ade. Quite surprisingly, we could not detect any adduct after the reaction mixture was analyzed at different time points by mass spectrometry (MS). The ^1H NMR analysis of the quinone solution formed by reacting the catechol **1** with Ag_2O in $CDCl_3$ ruled out the formation of DES-3′,4′-Q (**2**) and instead we observed the formation of a sterically crowded unreactive *spiro*-quinone (**3**) in almost quantitative yield (Scheme 1).[16] In this



Scheme 1. Synthesis of *spiro*-quinone (**3**) from 3′-OH-DES (**1**).

Keywords: Diethylstilbestrol; Catechol estrogen; Oxidation; Depurinating adducts; *spiro*-Quinone.
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communication, we report the formation of a novel *spiro*-quinone (**3**) after oxidation of **1**. This finding is potentially relevant in understanding the mechanism of tumor initiation by DES.

The structure of **3** was elucidated by detailed analyses.[17] The HR-FAB gave a molecular ion peak at *m*/*z* 280.31742, corresponding to $C_{18}H_{16}O_3$ (calculated mass 280.31784). Disappearance of characteristic d, d, and dd signals in ^{1}H NMR and appearance of two singlets at δ 6.26 and 6.12 ppm indicated a substitution at C-6′ took place. On the other hand, disappearance of one aromatic signal in ^{13}C NMR with concomitant appearance of an unusual high-field signal at δ 58.8 ppm indicated that one of the aromatic ring sp^2 carbons had been converted to an sp^3 carbon. Detailed 1- and 2-D NMR spectroscopy, including APT, HSQC, and HMBC experiments, was performed to assign the chemical shifts in ^{1}H and ^{13}C spectra. The signal at δ 58.8 ppm, in the APT spectrum of **3**, was found to be due to a quaternary carbon and was further confirmed by the absence of direct ^{1}H/^{13}C correlation in the HSQC experiment. Furthermore, the long range ^{1}H/^{13}C correlations of the carbon at δ 58.8 ppm with the protons at δ 6.12 (H-5′), 6.65 (H-2″), and 6.48 ppm (H-3″), in the HMBC experiment, indicated the attachment of C-1″ with C-6′ (Fig. 1). The remaining ^{13}C and ^{1}H signals were also assigned and confirmed with the help of APT, HSQC, and HMBC experiments (Fig. 1).

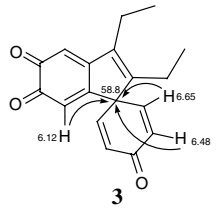
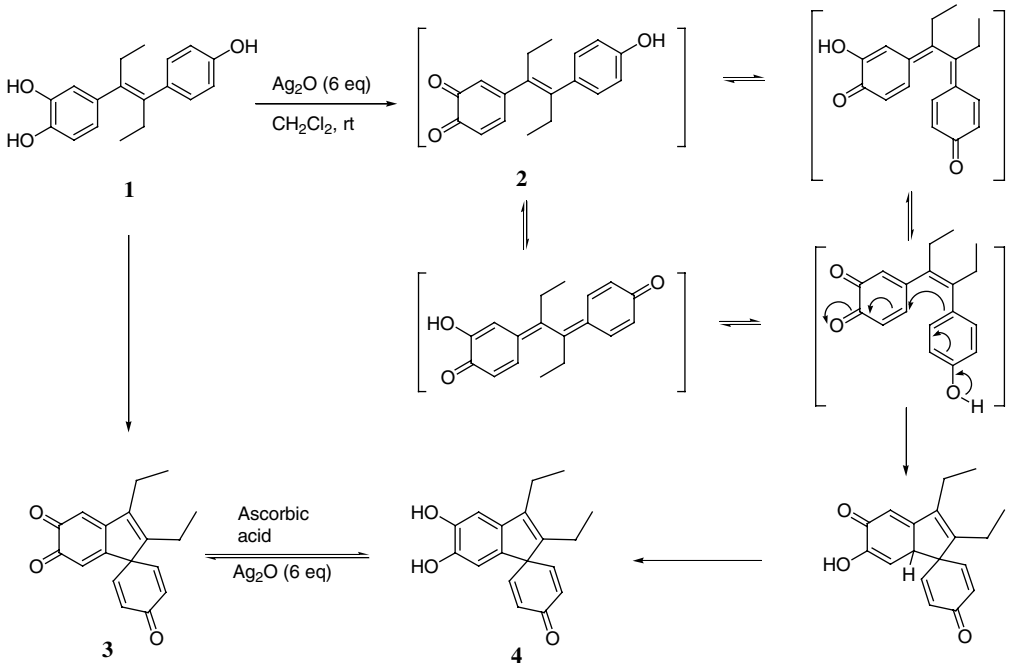


Figure 1. Important long range ^{1}H/^{13}C correlations in *spiro*-quinone (**3**).

The rationale for the production of *spiro*-quinone (**3**) entails the initially formed DES-3′,4′-Q, which in the tautomeric form 3′-OH-DES-4′,4″-Q, affords a series of rearrangements to form, initially, a *spiro*-catechol (**4**) as shown in Scheme 2. Further oxidation of this compound leads to the *spiro*-quinone (**3**). The reaction conditions were modified to try to stop the reaction at DES-3′,4′-Q, but, we were not able to block the formation of the *spiro*-quinone (**3**). Moreover, the complete formation of **3** required excess of the oxidant Ag_2O (~6 equiv). Use of 2 equiv of Ag_2O produced only a mixture of products in which *spiro*-catechol (**4**) was a major product (Table 1, Scheme 2).

Alternatively, the conversion of the quinone of DES to the *spiro*-compound can be blocked by using a catechol in which the phenolic ring is protected. Hence, oxidation of the methoxylated catechol **5** with Ag_2O yielded the yellowish green quinone, 4″-OCH$_3$-DES-3′,4′-Q (**6**) (Scheme 3). Similarly, oxidation of 3′-OH-hexestrol(HES) (**7**), in which the central double bond C3–C4 is saturated, with Ag_2O formed the corresponding *ortho*-quinone, HES-3′,4′-Q (**8**) (Scheme 3). These results suggest that the central double bond renders the *ortho*-quinone of DES, DES-3′,4′-Q, unstable. This finding is very important with respect to elucidation of the mechanism of tumor initiation by the synthetic estrogen DES. In fact, formation of the depurinating adducts at the N-3 position of adenine and N-7 position of guanine occurs only after treatment of the catechol, 3′-OH-DES, with lactoperoxidase in the presence of DNA.[18] This result suggests that the

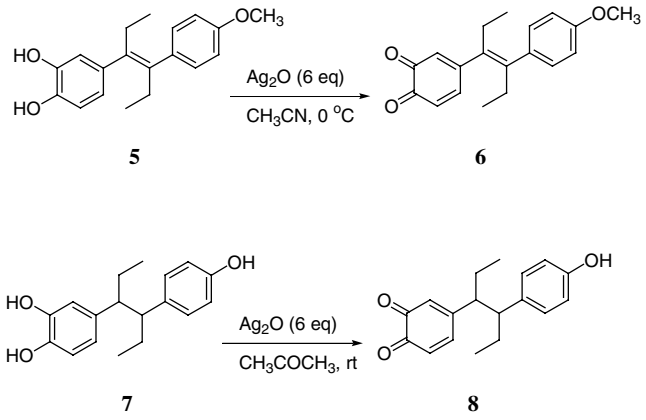


Scheme 2. Sequential conversion of DES-3′,4′-Q (**2**) to *spiro*-compounds (**3** and **4**).

Table 1. Oxidation of catechols with Ag_2O[a]

Catechol	Ag_2O (equiv)	Solvent	Product	Time (min)	Yield (%)
3′-OH-DES (**1**)	6	CH_2Cl_2	*spiro*-Quinone (**3**)	10	99
3′-OH-DES (**1**)	2	CH_2Cl_2	*spiro*-Catechol (**4**)	25	30
3′-OH-DES (**1**)	6	CH_3COCH_3	*spiro*-Quinone (**3**)	30	70
3′-OH-4″-OCH_3-DES (**5**)	6	CH_2Cl_2	4″-OCH_3-DES-3′,4′-Q (**6**)	30	40
3′-OH-4″-OCH_3-DES (**5**)	6	CH_3CN	4″-OCH_3-DES-3′,4′-Q (**6**)	20	85
3′-OH-HES (**7**)	6	CH_3COCH_3	HES-3′,4′-Q (**8**)	30	99

[a] The reactions were run at room temperature with the exception of 3′-OH-4″-OCH_3-DES (**5**), which was at 0 °C.



Scheme 3. Synthesis of *ortho*-quinones **6** and **8** from catechols **5** and **7**, respectively.

precursor 3′-OH-DES intercalates into DNA to form a physical complex. Subsequently, lactoperoxidase catalyzes the oxidation to DES-3′,4′-Q in situ, enabling reaction with DNA. In conclusion, the instability of the DES-3′,4′-Q indirectly proves the importance of the preliminary physical complex of the 3′-OH-DES in the formation of DNA adducts.

Acknowledgements

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16. 3′-OH-DES (**1**) was efficiently oxidized to *spiro*-quinone (**3**) under mild oxidation conditions. Typically, a solution of 3′-OH-DES (**1**) (1 mmol) in CH_2Cl_2 (10 mL) was stirred at room temperature and Ag_2O (6 mmol, Aldrich Chemical Co.) was added. After 10 min, TLC indicated complete consumption of **1**. The dark orange colored solution was filtered and the solvent was evaporated under a stream of nitrogen to afford almost pure **3**.
17. Spectroscopic data of **3**: ^{1}H NMR ($CDCl_3$): δ 6.65 (d, J = 10.2 Hz, 2H, H-2″,6″), 6.48 (d, J = 10.2 Hz, 2H, H-3″,5″), 6.26 (s, 1H, H-2′), 6.12 (s, 1H, H-5′), 2.47 (q, J = 7.81 Hz, 2H, CH_2), 2.17 (q, J = 7.81 Hz, 2H, CH_2), 1.19 (t, J = 7.81 Hz, 3H, CH_3), 1.08 (t, J = 7.81 Hz, 3H, CH_3); ^{13}C NMR ($CDCl_3$): δ 184.9 (C-4″), 179.3 (C-4′), 179.2 (C-3′), 159.7 (C-4), 156.6 (C-1′), 155.8 (C-6′), 146.5 (CH-2″,6″), 144.1 (C-3), 130.9 (CH-3″,5″), 124.7 (CH-5′), 115.7 (CH-2′), 58.8 (C-1″), 20.9 (CH_2), 18.8 (CH_2), 14.9 (CH_3), 13.3 (CH_3).
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