# Reactions of Estradiol-2,3-quinone with Deoxyribonucleosides: Possible Insights in the Reactivity of Estrogen Quinones with DNA

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Estrogen 2,3- and 3,4-quinones are reactive species toward nucleophiles and Michael acceptors. As such, they can bind to DNA and induce cellular damages. As an alkylation model, reactions of estradiol-2,3-quinone with deoxyribonucleosides were previously studied by mass spectrometry. In this work, estrogen-deoxyribonucleoside adducts were synthesized by reaction of 17$\beta$-estradiol-2,3-quinone with deoxyguanosine or deoxyadenosine and analyzed by NMR and LC-MS$^n$ in order to determine the structure and the stereochemistry of the resulting covalent adducts. Although estradiol- and estrone-2,3-quinones were previously thought to give mainly stable adducts, identification of depurinating adducts with both nucleosides, i.e., 2-OHE$_2$-6($\alpha$,$\beta$)-N7Gua and 2-OHE$_2$-6($\alpha$,$\beta$)-N7Ade, was unambiguously obtained. This is of particular interest since depurinating adducts are generated from DNA, and therefore, their amount should be correlated to the parallel formation of apurinic sites, which might play an important role in the cancer initiation process. Besides, a byproduct, i.e., 2-hydroxy-11-oxo-estradiol, corresponding to an unstable alkylation product of 2-hydroxyestradiol has been unambiguously identified and is indicative of a plausible addition process at the C9 position of catechol estrogens. The synthetic adducts will be useful as reference compounds to further elucidate the structure of adducts formed by reaction of estrogen metabolites with DNA or oligonucleotides.

## Introduction

It is now established that estrogens are involved in carcinogenesis either as hormones or as reactive species after oxidation (*1*−*4*). Although the hormonal mechanisms are known to mediate cell proliferation, growing attention has been given in the past few years to tumor initiation in relation to the modification of macrobiomolecules, particularly DNA. Estrogens can undergo several enzymatic or chemical oxidation processes, which may lead to formation of highly electrophilic species, i.e. *o*-quinones substrates. If these *o*-quinones are not deactivated by conjugation to glutathione via glutathione-*S*-transferases, they can bind covalently to DNA and so induce the critical initiation step of chemical carcinogenesis. All the work on this topic has been recently reviewed (*5*−*7*). A particular emphasis was given on reactivity of 4-hydroxy-catechol vs 2-hydroxy-catechol estrogens since the first species have been reported to be carcinogenic in male Syrian golden hamsters whereas the latter are not (*8*, *9*). These results were corroborated by those obtained more recently by the group of Cavalieri using in vivo experiments carried out from mammary tissues of rats treated with 4-hydroxy-estradiol (*10*, *11*). These authors have postulated that 4-hydroxy-catechol estrogens can give rise to depurinating adducts whereas 2-hydroxy-catechol estrogens lead only to stable adducts. Therefore, this difference could explain the difference in carcinogenic potency of these two types of catechol estrogens. It was also shown that different types of covalent adducts could be obtained according to the reaction conditions and to the *o*-quinones reactivity. The structures established for these adducts are consistent with a DNA alkylation process involving a Michael addition mechanism as predicted from the chemical model studies. Thus, these authors have determined by NMR that the reaction of estrone-2,3-quinone (2-OHE$_1$-Q)[1] on deoxyguanosine dG essentially consists of a regioselective attack of the exocyclic N$^2$dG atom on the prochiral C6 position of the estrogen skeleton (*10*) and

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[1] Abbreviations: dA, deoxyadenosine; dG, deoxyguanosine; dC, deoxycytidine; 2-OHE$_2$, 2-hydroxyestradiol; 2-OHE$_1$, 2-hydroxyestrone; 2-OHE$_2$-Q, estradiol-2,3-quinone; 2-OHE$_1$-Q, estrone-2,3-quinone; 2-OHE$_2$-QM, 2-hydroxyestradiol quinone methide; 2-OHE$_1$-QM, 2-hydroxyestrone quinone methide; DMSO, dimethyl sulfoxide; NOE, nuclear Overhauser effect; TOCSY, total correlation spectroscopy; HMQC, heteronuclear multiple quantum coherence; HMBC, heteronuclear multiple bond correlation; CAD, collisionally activated decomposition; LC-MS$^2$, LC-MS/MS, liquid chromatography−tandem mass spectrometry; LC-MS$^3$, LC-MS/MS/MS, liquid chromatography−mass spectrometry "to the third".

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gives rise to a mixture of diastereomer adducts, 2-$OHE_1$-6($\alpha$,$\beta$)-$N^2$dG.

In previous works, we showed by using LC-MS (*12*, *13*) that the 2-catechol estrogen quinone, estradiol-2,3-quinone (2-$OHE_2$-Q), reacts with deoxyribonucleosides dG, dA, and dC to give rise to different covalent adducts. Liquid chromatography−tandem mass spectrometry (LC-$MS^2$) as well as liquid chromatography−mass spectrometry "to the third" (LC-$MS^3$) experiments carried out in an ion trap mass spectrometer allowed us to analyze the fragmentation patterns and discriminate the different isomeric adducts. The present work concerns MS and NMR characterizations of reaction products obtained by reaction of 2-$OHE_2$-Q with dG and dA to support the previous propositions and to determine the stereochemistry of all the adducts formed. The goal is to provide insights into the possible modes of covalent binding for the catechol estrogens to DNA.

## Materials and Methods

***Caution:*** *Catechol estrogen quinones are hazardous chemicals and should be handled carefully in accordance with statutory procedures and health guidelines.*

**Chemicals.** All chemicals were purchased from Aldrich (Saint Quentin Fallavier, France). LC-MS analyses used HPLC-grade solvents from Scharlau (Barcelona, Spain) and water from a Milli-Q system (Millipore, Saint Quentin en Yvelines, France).

**Reactions of Estradiol-2,3-quinone with Deoxyribonucleosides.** Syntheses have been carried out according to previously published procedures. 2-Hydroxyestradiol (2-$OHE_2$) was prepared by the method described by Gelbke et al. (*14*) and 2-$OHE_2$-Q was obtained by oxidation of the corresponding catechol with activated manganese dioxide as described by Stack et al. (*10*), according to Abul-Hajj's procedure (*15*). Addition reactions between 2-$OHE_2$-Q and deoxynucleosides, i.e., deoxyguanosine (dG), and deoxyadenosine (dA), were performed as described previously (*10*). The atom numbering for the different adducts corresponds to the one used in previous studies (*10*).

**Semipreparative HPLC.** Adducts obtained from the reaction between 2-$OHE_2$-Q and dG or dA were quantitatively purified by using semipreparative chromatographies. HPLC were performed on a PU4100 gradient pump equipped with a Rheodyne 7500 injector and connected to a PU4110 UV detector set at 280 nm. The reaction products were purified on a C18 Ultrabase (250 × 7.5 mm, 5 $\mu$m) column from SFCC (Saint Antoine, France) at a flow rate of 2 mL/min. The mobile phases consisted in mixtures of methanol:water:acetic acid in the following proportions: 10:90:0.2 for the solvent A and 90:10:0.2 for the solvent B. Analyses were achieved at 35 °C using the following gradient: a linear gradient was used with an increase from 0 to 25% B on the first 5 min, 25 to 50% B from 5 to 35 min, then 50 to 70% B from 45 to 60 min, and 70 to 100% B from 60 to 61 min, and finally an isocratic step at 100% B was maintained from 61 to 75 min. Concerning the yield of adducts, UV recording at 280 nm was used to quantify the amount purified in semipreparative conditions, making assumptions that molar extinction coefficients are rather comparable between them but also to this of 2-$OHE_2$.

**Liquid Chromatography−Electrospray Mass Spectrometry.** Tandem mass spectrometry experiments were carried out on a Finnigan LCQ (Thermo Quest, Les Ulis, France) quadrupole ion trap mass spectrometer equipped with electrospray ionization source. Ionization was achieved by using a typical spray needle voltage of 5.2 kV, toward the heated transfer capillary held at 220 °C and 5 V as the counter electrode. Helium was used as damping and relaxation gas as well as collision target gas for sequential $MS^n$ experiments. $MS^2$ experiments were carried out by isolation and subsequent excitation of the selected parent ion into the ion trap device. Sequential $MS^3$ experiments were achieved by isolation of a fragment ion from an $MS^2$ experiment into the trap and excitation of this ion in an MS "to the third" step. All MS and $MS^n$ analyses were performed in the normal scan mode under automatic gain control conditions. The mass spectrometer was interfaced to a Thermo Separation P4000 gradient HPLC system equipped with a Rheodyne 7725i injector and an UV1000 UV detector set at 280 nm. The samples were analyzed on a C18 Ultrabase (250 × 2 mm) column from Life Sciences International (Eragny, France) at a flow rate of 0.2 mL/min. The mobile phases are the same as described above to perform semipreparative HPLC. A linear gradient was used with an increase from 0 to 25% B from 0 to 5 min, 25 to 50% B from 5 to 30 min, and 50 to 100% B from 30 to 40 min, then an isocratic step at 100% B was used from 40 to 60 min.

**NMR.** NMR spectra were recorded on Bruker AM500 or Avance DMX500 spectrometers, operating at a $^1$H resonance frequency of 500 MHz. Samples were dissolved in 500 $\mu$L of DMSO-$d_6$ or $CD_3OD$, at concentrations which may vary from 0.2 to 1 mM according to the amount collected by HPLC. Chemical shifts are given in ppm/TMS by using the residual solvent signals as internal references: 2.5 ppm for $^1$H and 39.5 ppm for $^{13}$C in DMSO, 3.31 ppm for $^1$H and 49.0 ppm for $^{13}$C in $CD_3OD$. Unless otherwise indicated, all $^1$H two-dimensional experiments were acquired in the phase-sensitive mode with the time-proportional phase incrementation of the initial pulse (*16*). A relaxation delay of 1.2 to 2 s was used. 256−512 $t_1$ increments and 2048 complex data points in $t_2$ were recorded for a spectral width of 5000 Hz in the two dimensions. Prior to Fourier transform, the signal was multiplied by a shifted square sine-bell window function. For TOCSY experiments (*17*), a MLEV17 mixing scheme of different lengths (from 10 to 80 ms) with a 10 kHz spin-locking field strength was used in order to define direct and long-range correlations. In $CD_3OD$ solutions, signal OH suppression was achieved by selective irradiation of this signal during the relaxation delay. 2D heteronuclear experiments were performed by using the standard Bruker software. In $^{13}$C-$^1$H HMQC and HMBC (*18*, *19*) sequences, delays were optimized for coupling constants around 140 and 10 Hz, respectively. One-dimensional NOE experiments were recorded in difference mode by subtracting one spectrum with irradiation on resonance and another one with irradiation off resonance; in this experiment, a relaxation delay of 3 s was used.

## Results

**Reaction of 2-$OHE_2$-Q with dG.** LC-MS analysis has revealed the presence of several adducts in the reaction mixtures of 2-$OHE_2$-Q with dG (Figure 1). Three stable adducts $G_3$, $G_4$, and $G_5$ (adducts with deoxyribose) displaying $MH^+$ ions at *m*/*z* 554 and three depurinating adducts $G_1$, $G_2$, and $G_6$ (adducts without deoxyribose) at *m*/*z* 438 were detected. From the fragmentation patterns observed in LC-$MS^2$ and LC-$MS^3$ experiments, it was deduced that the C6 position of the catechol estrogen was involved in the linkage with dG for adducts $G_1$, $G_2$, $G_3$, and $G_4$ as described in Scheme 1. Furthermore, the diagnostic charge-remote fragmentation process occurring for adducts $G_5$ and $G_6$ suggested a base addition on the aromatic ring of the catechol estrogen (C1 or C4 position).

However, to assess the chemical reactivity of 2-$OHE_2$-Q toward deoxyribonucleosides, it was necessary to elucidate by NMR the structures proposed in previous studies and to determine their stereochemistry. Therefore, large-scale preparations of authentic adducts were performed and repeated purifications on semipreparative reversed phase high-performance liquid chromatography were carried out on reaction mixtures to collect fractions in a sufficient amount for structural analyses.

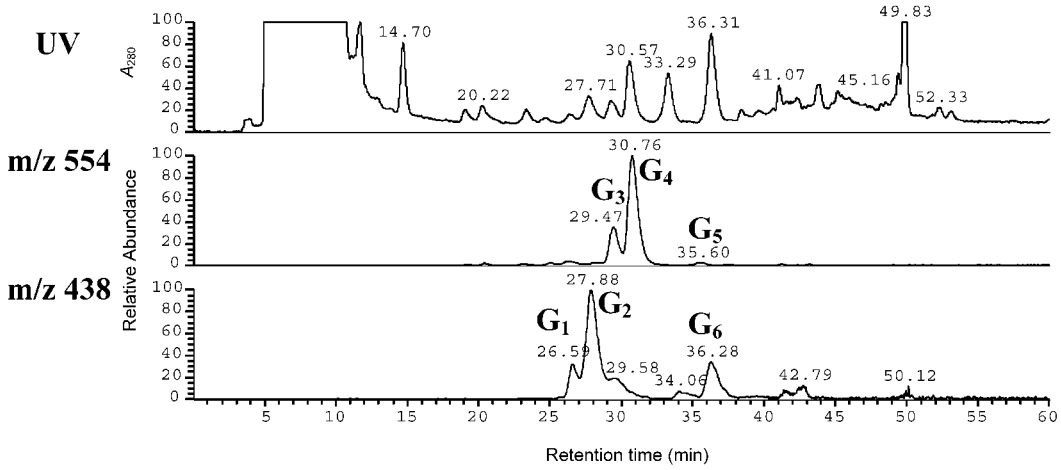
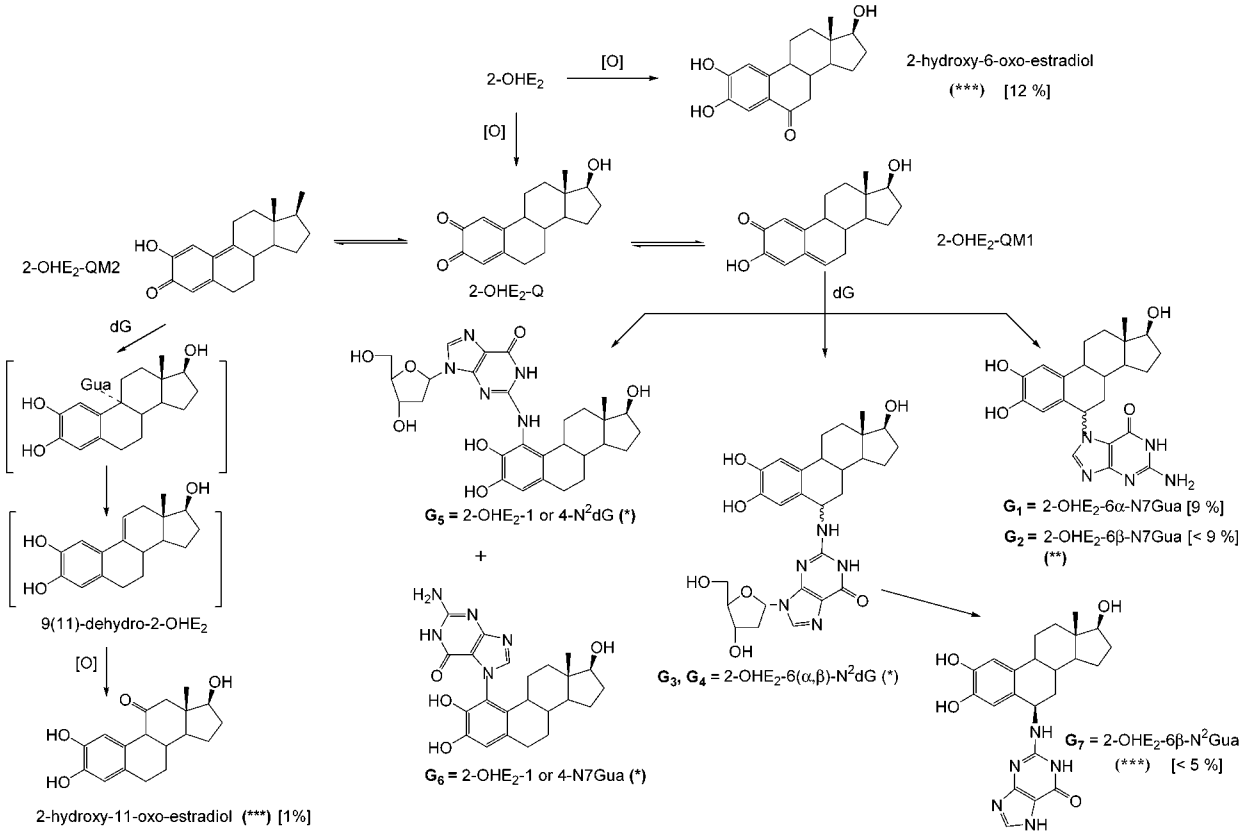



**Figure 1.** LC-MS$^2$ analysis of the reaction mixture of 2-OHE$_2$-Q with dG. Recording of ions at *m/z* 554 and *m/z* 438 reveals chromatographic separation of complete and deglycosylated adducts, respectively.

**Scheme 1. Products Identified in the Reaction Mixture of 2-OHE$_2$-Q with DG by LC-MS$^n$ (*), by LC-MS$^n$, and NMR (**), or by NMR (***)[a]**




[a] Amounts of the different adducts purified in semi-preparative conditions are given in brackets.

Semipreparative HPLC purification of the reaction mixture of 2-OHE$_2$-Q with dG allows isolation of five fractions, F$_1$ to F$_5$ (Figure 2), which were submitted to NMR analyses. Complete $^1$H and $^{13}$C NMR studies were achieved on the F$_1$, F$_4$, and F$_5$ fractions isolated as pure samples. $^1$H NMR analysis was conducted on the F$_2$ and F$_3$ fractions, which are mixtures of two and three main components, respectively. However, we were not able to isolate any fraction containing sufficient amount of adducts G$_5$ or G$_6$ to perform NMR studies; consequently they were characterized only by LC-MS$^n$ from their characteristic fragmentation pattern. Adducts G$_3$ and G$_4$ were characterized only by LC-MS$^n$, but the structure of a structurally related compound, which was purified in the fraction F$_3$ by semipreparative HPLC (G$_7$), has been achieved by NMR.

**2-OHE$_2$-6α-N7Gua (G$_1$) and 2-OHE$_2$-6*β*-N7Gua (G$_2$).** The F1 fraction, which accounts for 9% of the initial amount of 2-OHE$_2$-Q used in the reaction, was identified by LC-MS as the G$_1$ adduct (Figure 1). Its $^1$H NMR spectrum in CD$_3$OD makes evident the absence of signals in the region from 4.6 to 3.5 ppm and consequently a loss of the deoxyribose moiety. This observation is consistent with the mass spectrometric results which provided an MH$^+$ ion observed at *m/z* 438 for this adduct corresponding to a species in which the deoxyribose moiety is absent. This allows us to confirm a structure of depurinating adduct for G$_1$, corresponding to the addition of the base

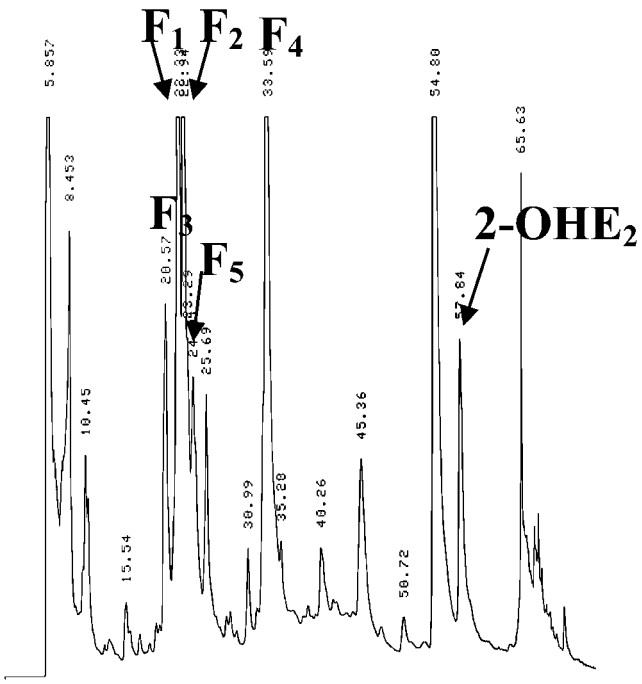



**Figure 2.** Semipreparative purification of the reaction mixture of 2-$OHE_2$-Q with dG. Yield of adducts was obtained from peak area integration with UV recording at 280 nm assuming that molar extinction coefficients of the different adducts are similar.

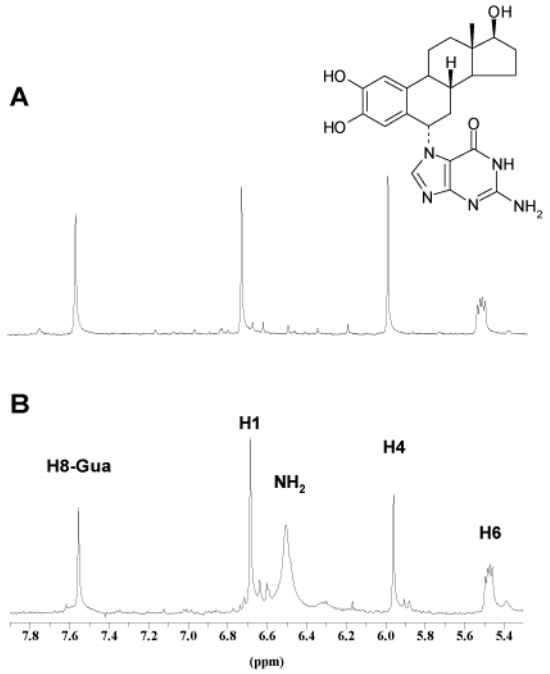



**Figure 3.** Regions 8−5 ppm of $^1$H NMR spectra of the 2-$OHE_2$-6α-N7Gua depurinating adduct (**$G_1$**) in $CD_3OD$ (A) and in DMSO (B).

moiety of deoxyguanosine to one molecule of 2-$OHE_2$. Taking into account the presence of one aromatic proton (H8-Gua) in the guanine ring, the observation of three singlets at 6.10, 6.80, and 7.58 ppm in the $^1$H NMR spectrum (Figure 3A) rules out the possibility of a reaction of nucleoside at one aromatic ring site (C1 or C4) of the steroid skeleton. Furthermore, this spectrum shows at 5.68 ppm one proton correlated in the HMQC spectrum to a methine group at 51.9 ppm. The low-field values of these $^1$H and $^{13}$C chemical shifts suggest a base-steroid coupling at a benzylic position (C6 or C9), and the nature of this carbon provides evidence for determining the reaction site as the C6 position. Therefore, the proton at 5.68 ppm (doublet of doublets with coupling constants of 11.5 and 6.1 Hz) is assigned to a H6 proton. Effectively, it presents characteristic TOCSY correlations with aliphatic protons corresponding to H7, H8, H9, H14, H15, and H16 protons of the steroid skeleton (Table 1). Most of the $^{13}$C signals of this adduct (Table 1) are very similar to the corresponding ones in the base or in the catechol estrogen 2-$OHE_2$, except for the carbons C6 and C7 that are involved in the base addition.

Since the best way to assign unequivocally the structures derives from studies of nuclear Overhauser effects between various neighboring protons (*20*), NOE experiments were conducted to establish the site of linkage to the guanine ring, as previously used in the structure determination of similar thiol or glutathione conjugates (*21*, *22*). Characteristic NOEs are found between the proton H6 at 5.68 ppm and the two protons at 6.10 and 7.58 ppm in one part, between the proton at 6.80 ppm and one aliphatic proton at 2.30 ppm in another part. These observations permitted assignment of the proton at 2.30 ppm to the H11α proton and the three aromatic protons as follows: H1 proton at 6.80 ppm, H4 proton at 6.10 ppm and H8-Gua at 7.58 ppm. Furthermore, the occurrence of a NOE between the H6 and H8-Gua protons indicates a spatial closeness for these protons and consequently implicates a base linkage at the N7 atom of dG that results in the loss of the deoxyribose due to a destabilization of the glycosidic bond (*23*, *24*). This proposal is consistent with the absence of modification in the H6 signal multiplicity when the spectrum is recorded in DMSO (Figure 3B) instead of $CD_3OD$. Besides, the occurrence in the spectrum recorded in DMSO of a broad signal at 6.50 ppm corresponding to two protons excludes the possibility of a steroid-base coupling at the $NH_2$ exocyclic site of the dG base. Therefore, all these results are consistent with the 2-$OHE_2$-6-N7Gua structure for the $G_1$ depurinating adduct. Since the protons located at C6 are prochiral, an attack of the dG nucleophile at this position gives rise to the formation of two diastereomers. The analysis of the pattern of coupling constants for the H6 signals in both the diastereomer adducts should allow us to assign their respective stereochemistries as shown below.

The $F_2$ fraction represented the same amount of reaction products as $F_1$ (9%). Its $^1$H NMR spectrum recorded in $CD_3OD$ (Figure 4A) shows a clear splitting of signals in the aromatic region (at least six signals from 8 to 6 ppm), in the H6 region (5.68 and 5.55 ppm) and in the methyl region (0.82 and 0.71 ppm). From the relative intensities of the two sets of peaks, it is possible to assign the proportion of the two species present in this fraction as 20/80. The minor signals correspond exactly to those of the $G_1$ adduct. Taking into account the analogies in TOCSY correlations for both signals at 5.68 and 5.55 ppm, it is assumed that the two $G_1$ and $G_2$ diastereomer adducts are present in the $F_2$ fraction. This is consistent with mass spectra, which display $MH^+$ ions at *m*/*z* 438 and current HPLC retention times of 26.6 and 27.9 min corresponding to the $G_1$ and $G_2$ adducts. Therefore, the signal at 5.55 ppm appearing as a doublet (4.5 Hz) of large signals is assigned to the proton H6 of the $G_2$ adduct. As above, the multiplicity of this signal does not change in DMSO (Figure 4B). Despite the chemical instability of this fraction, a comparison with the $F_1$

**Table 1. $^{13}C$ and $^{1}H$ Chemical Shifts of the $G_1$ Adduct (2-OHE$_2$-6α-N7Gua), of the $G_2$ Adduct (2-OHE$_2$-6*β*-N7Gua) and of the $G_7$ Adduct (2-OHE$_2$-6*β*-N$^2$Gua) Identified in the Reaction Mixture of 2-OHE$_2$-Q with dG[a]**

| $\delta$ $^{13}C$ (ppm) | $G_1$ DMSO | $\delta$ $^{1}H$ (ppm) | $G_1$ DMSO | $G_2$ DMSO | $G_1$ $CD_3OD$ | $G_2$ $CD_3OD$ | $G_7$ $CD_3OD$ |
|---|---|---|---|---|---|---|---|
| $C_1$ | 112.3 | $H_1$ | 6.68 | 6.80 | 6.80 | 6.88 | 6.88 |
| $C_2$ | 143.2 | | | | | | |
| $C_3$ | 144.4 | | | | | | |
| $C_4$ | 112.8 | $H_4$ | 5.96 | 6.15 | 6.10 | 6.43 | 6.55 |
| $C_5$ | 125.6 | | | | | | |
| $C_6$ | 51.9 | $H_{6\alpha}/H_{6\beta}$ | − /5.47 | 5.41/ − | − /5.68 | 5.55/ − | 5.86/ − |
| $C_7$ | 34.5 | $H_{7\alpha}/H_{7\beta}$ | 1.85/2.05 | 1.72/1.92 | 1.82/2.24 | 1.75/2.10 | 1.80/2.16 |
| $C_8$ | 38.4 | $H_{8\beta}$ | 1.55 | 1.49 | 1.68 | 1.45 | 1.34 |
| $C_9$ | 43.0 | $H_{9\alpha}$ | 2.35 | 2.12 | 2.43 | 2.18 | |
| $C_{10}$ | 131.1 | | | | | | |
| $C_{11}$ | 25.5 | $H_{11\alpha}/H_{11\beta}$ | 2.17/1.35 | 2.22/1.52 | 2.30/1.50 | 2.34/1.57 | 2.34/1.54 |
| $C_{12}$ | 36.1 | $H_{12\alpha}/H_{12\beta}$ | 1.20/1.88 | 1.22/1.90 | 1.30/2.00 | 1.32/1.97 | |
| $C_{13}$ | 42.0 | | | | | | |
| $C_{14}$ | 48.5 | $H_{14\alpha}$ | 1.20 | 1.19 | 1.32 | 1.23 | 1.25 |
| $C_{15}$ | 22.2 | $H_{15\alpha}/H_{15\beta}$ | 1.55/1.22 | 1.45/0.96 | 1.68/1.35 | 1.55/1.05 | 1.67/1.36 |
| $C_{16}$ | 29.5 | $H_{16\alpha}$-$H_{16\beta}$ | 1.88/1.38 | 1.85/1.35 | 2.03/1.50 | 1.95/1.42 | 2.03/1.52 |
| $C_{17}$ | 79.5 | $H_{17\alpha}$ | 3.55 | 3.50 | 3.65 | 3.63 | 3.67 |
| $C_{18}$ | 11.1 | $CH_3$ | 0.69 | 0.62 | 0.82 | 0.71 | 0.67 |
| $C_8$-Gua | 135.6 | $H_8$-Gua | 7.55 | 7.00 | 7.58 | 7.03 | 7.12 |

[a] $^{13}C$ and $^{1}H$ chemical shifts are given in ppm/TMS by using the residual solvent signal as internal reference: 2.5 and 39.5 ppm, respectively, in DMSO, 3.31 and 49.0 ppm, respectively, in $CD_3OD$.

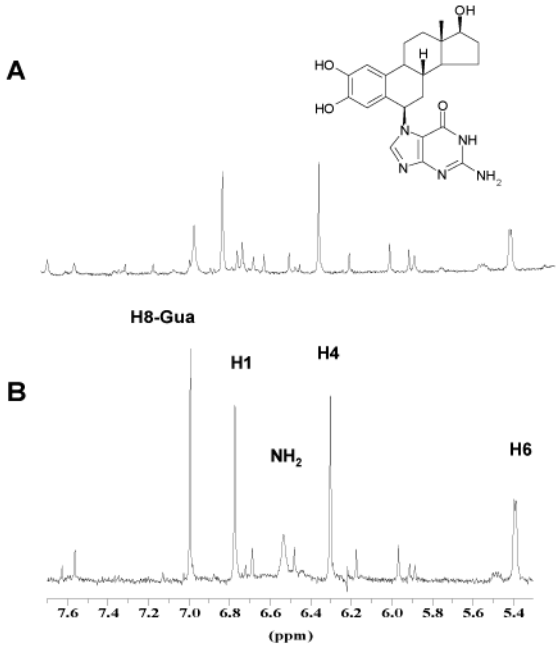



**Figure 4.** Regions 8−5 ppm of $^{1}H$ NMR spectra of the fraction F2 in $CD_3OD$ (A) and in DMSO (B): main components are 20% of 2-OHE$_2$-6α-N7Gua (**G$_1$**) and 80% of 2-OHE$_2$-6*β*-N7Gua (**G$_2$**) depurinating adducts.

fraction allowed us to assign all the protons of this $G_2$ adduct in DMSO and in $CD_3OD$ (Table 1).

In these compounds, a half-chair conformation is usually assumed for the B ring (*25*) of the steroid skeleton, and the coupling constants of an H6 proton with the two H7 protons are largely dependent on its position, α or *β* (*26*). Given the coupling constants values observed for the H6 proton, 11.5 and 6.1 Hz for $G_1$, 4.5 and less than 1 Hz for $G_2$, it is possible to determine the stereochemistry of these adducts corresponding to a base-steroid linkage by the N7dG atom on the face α of the steroid for the $G_1$ adduct, 2-OHE$_2$-6α-N7Gua and on the face *β* of the steroid for the $G_2$ adduct, 2-OHE$_2$-6*β*-N7Gua (Scheme 1).

At this stage, the NMR analysis of the $F_1$ and $F_2$ fractions provides a clear confirmation of the structures previously proposed from mass spectrometry results and allows us to determine the stereochemistry of these depurinating adducts. It is worth noting the existence of large differences in chemical shifts of the aromatic, the H6 protons and the 18-methyl groups observed between the two diastereomers, due to ring current effects from the base and, probably, conformational change in the B cycle. The chemical shift values complement coupling constants as an argument to give the stereochemistry of new adducts.

**2-OHE$_2$-6(α,*β*)-N$^2$dG (G$_3$/G$_4$).** Several attempts have been made for the purification of $G_3$ and $G_4$ by semi-preparative HPLC. Unfortunately, these adducts could never been obtained in sufficient purified amounts for NMR analysis, very likely owing to degradation occurring during the purification process, leading in particular to the related $G_7$ adduct (see further in the text). Thus, the only structural information available was generated by mass spectrometry from freshly prepared adduct mixtures. As indicated in Figure 1, both $G_3$ and $G_4$ display $MH^+$ ions at *m/z* 554, indicating that they are stable adducts. As previously reported (*12*), the MS/MS analysis of both the $MH^+$ ions of $G_3$ and $G_4$ resulted in identical product ion spectra, displaying *m/z* 438, *m/z* 287, and *m/z* 268 as the most abundant product ions. The *m/z* 438 is formed by loss of deoxyribose with proton transfer from the sugar to the guanine moiety of the adduct, whereas the *m/z* 287 and *m/z* 268 ions are the result of the cleavage of the steroid-base bond. The CAD mass spectra obtained from $MS^3$ on the *m/z* 438 ion isolated from the decomposition of the previous *m/z* 554 $MH^+$ ion also displayed two complementary ions (*m/z* 287 and 152) arising from the cleavage of the steroid-base linkage. These fragmentation patterns are known to characterize adducts with a base attachment on the C6 atom of the steroid (*10*−*13*). In absence of NMR information, and as the CAD spectra obtained from $MS^2$ and $MS^3$ experiments were identical for $G_3$ and $G_4$, it may be concluded that they correspond to diastereoisomeric adducts in which dG is linked to the C6 atom of the steroid via its $N^2$ exocyclic atom. In this case, the nucleophilic attack does not induce the destabilization of the glycosidic bond of the nucleoside. Thus, 2-OHE$_2$-6α-N$^2$dG and 2-OHE$_2$-

**Table 2. $^{13}C$ and $^{1}H$ Chemical Shifts in $CD_3OD$ of the Products $F_4$ (2-Hydroxy-6-oxo-estradiol) and $F_5$ (2-Hydroxy-11-oxo-estradiol) in Comparison to the Catechol Estrogen 2-$OHE_2$[a]**

| $\delta$ $^{13}C$ (ppm) | 2-$OHE_2$ | $F_4$ | $F_5$ | $\delta$ $^{1}H$ (ppm) | 2-$OHE_2$ | $F_4$ | $F_5$ |
|---|---|---|---|---|---|---|---|
| $C_1$ | 113.4 | 112.7 | 114.9 | $H_1$ | 6.70 | 6.84 | 6.30 |
| $C_2$ | 143.8 | 153.2 | 146.9 | | | | |
| $C_3$ | 143.8 | 145.1 | 145.0 | | | | |
| $C_4$ | 116.5 | 114.3 | 116.8 | $H_4$ | 6.40 | 7.38 | 6.60 |
| $C_5$ | 128.9 | 126.0 | 126.4 | | | | |
| $C_6$ | 29.9 | 199.8 | 24.6 | $H_{6\alpha}/H_{6\beta}$ | 2.70/2.70 | | 2.60/2.80 |
| $C_7$ | 28.6 | 44.6 | 21.0 | $H_{7\alpha}/H_{7\beta}$ | 1.18/1.78 | 2.19/2.55 | 1.75/2.18 |
| $C_8$ | 40.4 | 41.9 | 43.5 | $H_{8\beta}$ | 1.28 | 1.87 | 2.05 |
| $C_9$ | 45.4 | 44.2 | 43.5 | $H_{9\alpha}$ | 2.02 | 2.44 | 2.18 |
| $C_{10}$ | 128.9 | 143.3 | 129.2 | | | | |
| $C_{11}$ | 27.7 | 26.7 | 213.7 | $H_{11\alpha}/H_{11\beta}$ | 2.15/1.35 | 2.32/1.57 | |
| $C_{12}$ | 38.0 | 37.5 | 51.3 | $H_{12\alpha}/H_{12\beta}$ | 1.18/1.88 | 1.35/2.01 | 2.18/2.42 |
| $C_{13}$ | 44.3 | - | 48.0 | | | | |
| $C_{14}$ | 51.3 | 51.1 | 43.4 | $H_{14\alpha}$ | 1.10 | 1.35 | 1.90 |
| $C_{15}$ | 24.0 | 23.7 | 23.9 | $H_{15\alpha}/H_{15\beta}$ | 1.60/1.28 | 1.68/1.35 | 1.75/1.43 |
| $C_{16}$ | 30.7 | 30.6 | 30.8 | $H_{16\alpha}/H_{16\beta}$ | 1.95/1.45 | 2.06/1.57 | 2.10/1.55 |
| $C_{17}$ | 82.5 | 82.2 | 80.4 | $H_{17\alpha}$ | 3.65 | 3.68 | 3.66 |
| $C_{18}$ | 11.7 | 11.4 | 12.0 | $CH_3$ | 0.77 | 0.78 | 0.75 |

[a] $^{13}C$ and $^{1}H$ chemical shifts are given in ppm/TMS by using the residual solvent signal at 3.31 and 49.0 ppm, respectively, as internal reference.

6$\beta$-$N^2$dG are proposed as structures of the adducts $G_3$ and $G_4$.

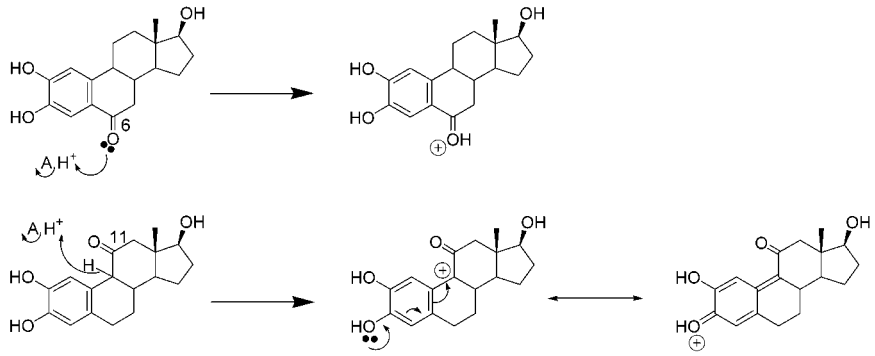
**2-$OHE_2$-6$\beta$-$N^2$Gua ($G_7$).** The yield of adduct in the F3 fraction represented nearly 5% of the amount of 2-$OHE_2$-Q used to perform the reaction. Despite the complexity of the $^{1}H$ NMR spectrum seen for this fraction, a comparison of the two regions 8.0−5.0 and 1.0−0.6 ppm with the corresponding ones for the $F_1$ and $F_2$ fractions makes evident the presence of at least three components, namely the $G_1$, $G_2$ adducts and a third compound. Taking into account the values of the $^{1}H$ chemical shifts and the mass spectra displaying $MH^+$ ions at *m/z* 438, a depurinating adduct may be proposed for this new product, called $G_7$. Its NMR characteristics are three aromatic protons, a doublet (4.0 Hz) of large signals at 5.86 ppm presenting TOCSY correlations observed for a H6 proton and a methyl signal at 0.67 ppm (Table 1). On the basis of the H6 proton multiplicity, it is suggested that this additional adduct $G_7$ might correspond to a base attachment on the $\beta$ face of the steroid by the 2-$NH_2$ exocyclic group. Surprisingly, although it represents about 5% of the amount of 2-$OHE_2$-Q used in the reaction, this depurinating adduct, 2-$OHE_2$-6$\beta$-$N^2$Gua, was not detected by LC-$MS^n$ at a retention time that should correspond to the $F_3$ peak one. Therefore, it would be secondary formed by a process involving the deoxyribose hydrolysis of the corresponding stable adducts $G_3$ or $G_4$ unambiguously characterized by LC-$MS^n$ only. This hydrolytic step might be due probably to the presence of acetic acid in the HPLC solvents used in the semipreparative HPLC procedures.

**2-Hydroxy-6-oxo-estradiol ($F_4$) and 2-Hydroxy-11-oxo-estradiol ($F_5$).** The fractions $F_4$ and $F_5$, accounting respectively for 12 and 1% of the amount of 2-$OHE_2$-Q used in the synthesis of adducts, correspond to pure compounds presenting only two aromatic protons in the $^{1}H$ NMR spectra. Furthermore, the comparison of their $^{13}C$ NMR spectra with those of the starting material 2-$OHE_2$ (*m/z* 289) makes evident the presence of additional signals at 199.8 and 213.7 ppm for $F_4$ and $F_5$, respectively, as well as the disappearance of a $CH_2$ group in the region 20−30 ppm. These observations suggest that in both cases a methylene group in 2-$OHE_2$ has been chemically modified into a carbonyl group. The position of this function on the steroid skeleton has been deduced from a complete analysis by 2D homo- or heteronuclear spectra (Table 2). In particular, the long-range correlations observed in the HMBC spectra between the CO signal at 199.8 ppm and the H4, H7 protons for $F_4$, between the CO signal at 213.7 ppm and the H11 protons for $F_5$ have allowed us to characterize these compounds as the 2-hydroxy-6-oxo-estradiol for the $F_4$ fraction and the 2-hydroxy-11-oxo-estradiol for the $F_5$ one. On the basis of the observed coupling constants, the stereochemistry is assumed to be preserved in the C8, C9, and C14 positions of the steroid skeleton for both compounds.

The positive electrospray mass spectra of $F_4$ and $F_5$ display respectively a peak at *m/z* 303 for $[M + H]^+$ and a $[M - H]^+$ peak at *m/z* 301 (data not shown). As expected for $F_4$, the direct protonation of the carbonyl group takes place at C6 (Scheme 2). Conversely, in the case of $F_5$, $[M - H]^+$ ions observed at *m/z* 301 constitute the main species representing the sample molecules. This can be explained considering the loss of hydride from both the benzylic and enolic sites during the $H^+$ approach as reported in Scheme 2, the resulting $[M - H]^+$ species being stabilized by conjugation.

**2-$OHE_2$-1(4)-$N^2$dG ($G_5$).** This adduct displaying $MH^+$ ions at *m/z* 554 (Figure 1) results from the addition of dG on the A ring of 2-$OHE_2$ without loss of deoxyribose. Accordingly, the linkage of dG must occur via its exocyclic $N^2$ atom, the only nucleophilic site of dG that does not induce destabilization of the sugar-base bond during the addition process on the steroid. The CAD spectrum of the *m/z* 554 $MH^+$ ion displays a unique product ion at *m/z* 438 (data not shown), formed by the loss of the sugar moiety of the molecule as previously described (*12*). Sequential $MS^3$ experiments carried out on the *m/z* 438 fragment ion from $G_5$ leads to the CAD spectrum presented in Figure 5. This spectrum displays major characteristic fragment ions at *m/z* 324, 312, 298, 286, and 272. The formation of these fragment ions can be attributed to charge-remote fragmentation processes, occurring from species with a highly stable charged site and giving rise to thermal decompositions in which the positive charge is not involved (*27*−*29*). These processes are frequently observed and have been extensively studied on various molecules including steroids and bile

**Scheme 2. Proposed Mechanisms for the Formation of $MH^+$ Ions for $F_4$ and $[M - H]^+$ Ions for $F_5$ Using Positive Electrospray Ionization**



acids (*30*, *31*). The formation of the *m*/*z* 324 ion is initiated by the steroid C ring opening due to a hydrogen migration from the C9 to the C12 atom of the steroid and followed by the cleavage of the C11−C12 bond with hydrogen rearrangement resulting in a methyl-naphthalene like structure as indicated in Scheme 3A. The *m*/*z* 312 ion originates in the charge-remote cleavages of the C9−C11 and C8−C14 bonds (Scheme 3B) according to a concerted mechanism as previously described (*10*). The *m*/*z* 298 ion can be produced by a hydrogen migration from the C9 to the C12 position of the steroid, leading to the C ring opening, followed by cleavage of the C6−C7 and C8−C9 bonds as represented in Scheme 3C. This constitutes an alternative to the mechanism previously proposed for the formation of this ion from 4-$OHE_1$-1-N7Gua (*10*). The *m*/*z* 286 ion from 2-$OHE_2$-1(4)-N7Gua very likely possesses the same structure as described for 4-$OHE_1$-1-N7Gua (*10*): this confirms that this ion is not related to a radical cation form of the steroid. A mechanism for the formation of this ion involves the cleavage of the C7−C8 and C9−C10 bonds of the steroid B ring and is accompanied by hydrogen migration as presented in Scheme 3D. Finally, the formation of the *m*/*z* 272 ion can be obtained from a hydrogen migration from the C8 to the C9 position of 2-$OHE_2$ resulting in the cleavage of the C9−C10 and C6−C7 bonds (Scheme 3E). Note that in this case the hydrogen migration process should affect the charged site location.

The occurrence of the *m*/*z* 152 fragment ion (Figure 5) confirms the attachment of Gua on the steroid A ring, and its formation can be explained considering the formation of an ion−dipole complex intermediate. The cleavage of the steroid base bond can occur by hydrogen transfer from the C11 to the C1 carbon atom of the steroid, leading to an ion−neutral complex species (in brackets in Scheme 4), which gives rise to the liberation of the *m*/*z* 152 ion after proton transfer from the charged steroid to the neutral base moiety of the intermediate.

Despite the lack of NMR information, this adduct could be clearly characterized as 2-$OHE_2$-1(4)-$N^2$dG on the basis of its CAD mass spectrum and the well documented decomposition schemes described for A ring substituted catechol estrogens (*10*−*13*).

**2-$OHE_2$-1(4)-N7Gua ($G_6$).** The $G_6$ adduct produces an $MH^+$ ion at *m*/*z* 438, indicative of the loss of the deoxyribose moiety. Accordingly, the nucleophilic site of dG involved in this adduct is the N7 nitrogen atom. Submitted to collisional excitation in the ion trap, the *m*/*z* 438 parent ion of $G_6$ decomposes into the same fragment ions as observed for the *m*/*z* 438 $MH^+$ ion from adduct $G_5$ (data not shown). The fragmentation pattern observed on the CAD spectrum of $G_6$ is not influenced by the steroid-base linkage on the A ring, since adducts $G_5$ and $G_6$ both give rise to highly stable charged sites which lead to the same charge remote fragmentation processes. On the basis of these data and previously reported results (*10*−*12*), the linkage site of dG could be located on the A ring of the steroid and $G_6$ was identified as 2-$OHE_2$-1(4)-N7Gua.

**Reaction of 2-$OHE_2$-Q with dA.** A similar study was carried out with deoxyadenosine (dA). LC-MS experiments have allowed us to characterize two stable adducts $A_1$ ($t_R$ = 36.7 min) and $A_3$ ($t_R$ = 39.3 min) by their $MH^+$ ions at *m*/*z* 538 and two depurinating adducts $A_2$ ($t_R$ = 38.2 min) and $A_4$ ($t_R$ = 38.7 min) at *m*/*z* 422 (not shown). As above, successive purifications of the reaction mixture have been carried out using semipreparative HPLC. Two fractions $F_1'$ and $F_2'$, which accounted respectively for 44 and 6% of the amount of 2-$OHE_2$-Q used in the reaction, were isolated as pure compounds but the fraction $F_3'$ (<1%) contains several products (data not shown).

**2-$OHE_2$-6α-$N^6$dA ($A_1$ or $A_3$).** A $^1$H NMR spectrum of the $F_1'$ fraction was recorded in DMSO (not shown) but it was not possible to realize 2D experiments since the solution decomposed overnight. Of particular impor-

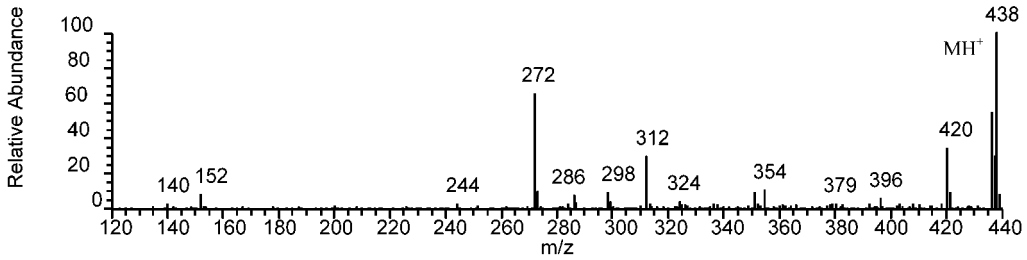
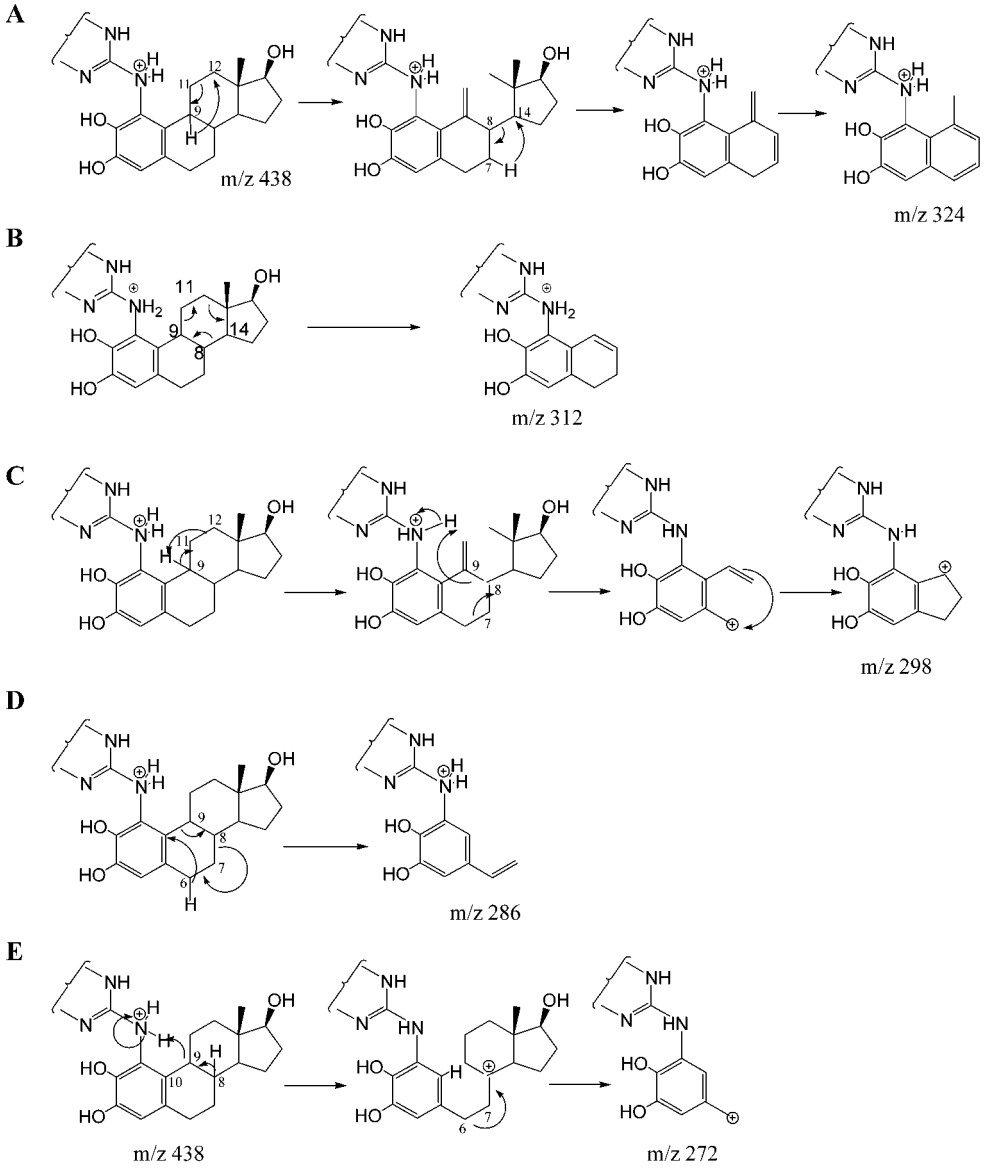
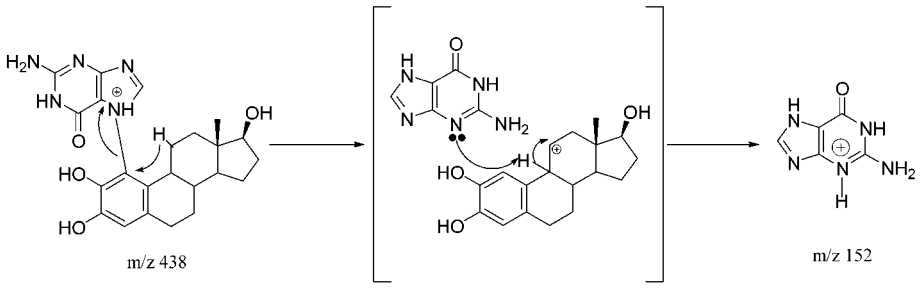



**Figure 5.** CAD mass spectrum obtained from daughter ion at *m*/*z* 438 isolated from $MH^+$ ion of $G_5$ (*m*/*z* 554). Interpretation of the main fragments is given in Schemes 3 and 4.

**Scheme 3. Proposed Decomposition Mechanisms of the *m/z* 438 Parent Ion Corresponding to an Adduct on A Ring Leading to Ions at *m/z* 324 (A), 312 (B), 298 (C), 286 (D), and 272 (E)**




**Scheme 4. Proposed Mechanism for the Formation of Ions at *m/z* 152 from the Parent Ion at *m/z* 438 Corresponding to an Adduct on A Ring**




tance is the observation of characteristic sugar signals: a triplet at 6.36 ppm for the H1′ proton and four multiplets between 4.6 and 3.5 ppm for the H4′, H3′ and H5′, H5″ protons. The spectrum is very similar to the one described by Stack et al. (*10*) as the adduct 2-$OHE_1$-6($\alpha$,$\beta$)-$N^6$dA in the reaction of dA with 2-$OHE_1$-Q. The presence of a NH doublet at 7.93 ppm suggests a reaction of the base at the C6 position by the $N^6$dA atom for this stable adduct displaying $MH^+$ ions at *m/z* 538. The NMR analysis was restricted to the assignment of protons involved in the base addition process, namely the H6, the aromatic, the NH or $NH_2$ protons as well as the methyl groups (Table 3). Taking into account the line width of the multiplet at 5.62 ppm assigned to the H6 proton, it is possible to propose for this adduct a base linkage on the $\alpha$ face of the steroid, 2-$OHE_2$-6$\alpha$-$N^6$dA. However, due to the rapid degradation, it was not possible to identify by LC-MS this compound as the $A_1$ or $A_3$ adduct.

**Table 3. $^1$H Chemical Shifts of Adducts Identified in the Reaction Mixture of 2-OHE$_2$-Q with dA[a]**

| adducts | solvent | $H_6$ | $H_1/H_4$ | $H_2/H_8$-dA | $CH_3$ | NH/NH$_2$ |
|---|---|---|---|---|---|---|
| 2-OHE$_1$−6(α,*β*)-N$^6$dA (*10*) | DMSO | 5.58 | 6.68/6.56 | 8.31/8.27 | 0.81 | 7.94/ − |
| 2-OHE$_2$−6α-N$^6$dA | DMSO | 5.62 | 6.63/6.52 | 8.32/8.22 | 0.70 | 7.93/ − |
| 2-OHE$_2$−6*β*-N7Ade | DMSO | 5.65 | 6.80/6.30 | 8.17/7.46 | 0.61 | − /7.21 |
| 2-OHE$_2$−6*β*-N7Ade | CD$_3$OD | 5.73 | 6.92/6.44 | 8.26/7.45 | 0.70 | |
| 2-OHE$_2$−6α-N7Ade | CD$_3$OD | 5.87 | 6.82/6.00 | 8.21/8.08 | 0.83 | |

[a] As a reference, the first line of this table corresponds to the stable adduct 2-OHE$_1$-6(α,*β*)-N$^6$dA obtained by Stack et al. (*10*). Chemical shifts are given in ppm/TMS by using the residual solvent signal as internal reference at 2.5 ppm for DMSO and 3.31 ppm for CD$_3$OD.

**2-OHE$_2$-6*β*-N7Ade and 2-OHE$_2$-6α-N7Ade (A$_2$, A$_4$).** In the $^1$H NMR spectrum of the F$_2$′ fraction in DMSO (not shown), the characteristic sugar signals are absent. So, in agreement with the mass spectrometry results (MH$^+$ ion observed at *m*/*z* 422), the F$_2$′ compound should correspond to a depurinating adduct. Beside a broad signal corresponding to two protons at 7.21 ppm, the spectrum shows a methyl signal at 0.61 ppm, four aromatic protons, and one doublet at 5.64 ppm with TOCSY correlations characteristic of a H6 proton (Table 3). No modification is observed for this H6 proton when the spectrum is recorded in CD$_3$OD. These observations are consistent with a reaction of the base at the C6 position by the N7dA atom, leading to a loss of the deoxyribose moiety. Given the above results, the multiplicity of the H6 proton (doublet of two large signals) allows us to propose the following structure, 2-OHE$_2$-6*β*-N7Ade for this adduct, which should correspond to the adduct A$_2$ or A$_4$ detected by LC-MS$^2$ at *m*/*z* 422.

The $^1$H NMR spectrum of the F$_3$′ fraction in CD$_3$OD is rather complex. However, on the basis of the signal integrals, this mixture should contain in addition to free sugar and free base, a very small amount of an adduct characterized by a doublet of doublets at 5.87 ppm, four singlets in the aromatic region and a methyl signal at 0.83 ppm. In this fraction, from the values of these chemical shifts and from the multiplicity of the H6 proton at 5.87 ppm, and by comparison with the dG case, it is possible to assume the presence of the diastereomer depurinating adduct 2-OHE$_2$-6α-N7Ade.

## Discussion

The *o*-quinones formed from oxidation of the catechols are considered as strong electrophiles and can react in vitro or in vivo with nucleophilic residues (*10*, *11*). Using the chemical conditions optimized by Stack et al. (*10*), covalent adducts between 2-OHE$_2$ and the deoxyribonucleosides dG, dA, and dC were obtained in minute quantities and could be characterized only by electrospray ionization mass spectrometry (*12*, *13*). The reaction between catechol estrogen quinones and deoxyribonucleosides gives rise to more or less stable covalent adducts, resulting from an attack of the base on specific electrophilic sites of the estrogen skeleton. These sites are the aromatic positions C1 or C4 (ring A) and the benzylic positions C6 or C9 (ring B) since it is known that the 2-OHE$_2$-*o*-quinone can form two quinone methides, 2-OHE$_2$-QM$_1$ and 2-OHE$_2$-QM$_2$ (*32*) (Scheme 1).

The above results demonstrate that the nucleophilic attack of the deoxyribonucleoside dG principally takes place at the C6 atom of the estradiol-2,3-quinone skeleton, via both exocyclic N$^2$dG and endocyclic N7dG atoms. The G$_3$ and G$_4$ stable adducts, 2-OHE$_2$-6(α,*β*)-N$^2$dG (Figure 1), corresponding to attack at the C6 position by the N$^2$dG atom, have been detected by LC-MS (MH$^+$ at *m*/*z* 554) (*12*, *13*), but they could not be purified for a subsequent NMR analysis. However, the structure of the depurinating adduct G$_7$, which very likely results from the hydrolysis of one of these stable adducts in the purification step, has been obtained by NMR. We know that N-glycosidic bonds are susceptible to pH variations. The acid-catalyzed hydrolysis of the base-sugar linkage has already been described from guanine (*33*) or adenine (*34*) nucleosides. Loss of a base from DNA by hydrolysis of the N-glycosidic bond is known to be one of the most frequent types of spontaneous alterations of the cell genome (*35*). If we consider that G$_7$ is resulting from G$_3$ or G$_4$, then yields we have obtained for stable adducts (nearly 5%) are not very different from those given by Stack et al. (*10*).

The two diastereomer depurinating adducts G$_1$ (2-OHE$_2$-6α-N7Gua) and G$_2$ (2-OHE$_2$-6*β*-N7Gua) have been analyzed by LC-MS and NMR and the stereochemistry of these two adducts has been determined by NMR. According to the UV profile and the reconstructed ion chromatograms of the *m*/*z* 438 diagnostic ion (Figure 1), the abundance ratio [G$_1$]/[G$_2$] of these adducts in the crude reaction mixture could be estimated to be 20/80. This result might be explained by a higher stability of the G$_2$ adduct in the acidic conditions of the reaction. Furthermore, the presence of the 2-hydroxy-11-oxo-estradiol in most fractions of the reaction mixtures might indicate the parallel formation of unstable adducts at the C9 position. Adducts G$_5$ and G$_6$ detected in LC-MS experiments as respectively stable or depurinating adduct (*12*, *13*) are formed via a base addition on the C1 or C4 aromatic position of the steroid skeleton (Figure 1). Unfortunately, they could not be isolated for a NMR characterization and therefore seem to be formed as minor adducts under the reaction conditions we have used.

Two new keto steroids were isolated as byproducts at the same time as deoxynucleosides adducts. The formation of the F$_4$ compound, 2-hydroxy-6-oxo-estradiol, may be explained by a simple oxidation of the starting steroid 2-OHE$_2$ since it is also found when the reaction was carried out without any deoxynucleoside addition in the reaction medium. On the other hand, the F$_5$ compound, 2-hydroxy-11-oxo-estradiol, might derive from a precursor adduct corresponding to reaction of the base at the C9 position as shown in Scheme 1, since this compound was not detected when the reaction was conducted without dG or dA. Such a C9 adduct was proposed in the reaction between 2- or 4-OHE$_1$ and glutathione as a result of the isomerization of these catechol estrogen quinones to highly electrophilic *p*-quinone methides 2- or 4-OHE$_1$-QM$_2$ (*36*). These adducts at the C9 position were described as unstable and decomposed into 9(11)-dehydro-hydroxyestrones (*5*). In the present case, the degradation product 9(11)-dehydro-2-OHE$_2$ might be further oxidized to give the F$_5$ compound. As support for this proposal, it

is worth noting that a "transient" adduct was detected by $^1$H NMR with no signal in the sugar region but one sharp and two broad signals in the aromatic region. These observations might indicate a steric compression in this region as expected between the H8-Gua and H1 protons for an adduct at the C9 position, as observed by Stack et al. (*10*) for the adduct at the C1 position. The possibility of addition at the C9 position is in good agreement with recent mass spectrometry results obtained in similar reactions with catechol estrogens deuterated at the C6 and C7 positions.[2] Attempts at isolation and further spectral characterization of this "transient" adduct were not successful.

The NMR data indicate that the nucleophilic attack of the deoxyribonucleoside dA takes place on the C6 atom of the estradiol-2,3-quinone, via both N$^6$dA and N7dA atoms and gives rise to stable and depurinating adducts. Amount of stable adducts, 2-OHE$_2$-6(α,*β*)-N$^6$dA, correspond to about half of the yield published previously (*10*).

In conclusion, all the results show clearly that the reaction of the quinones of 2-hydroxy-catechol estrogens with the deoxyribonucleosides dG and dA may lead to depurinating covalent adducts as well as stable adducts. At this stage, it is worth noting that the estrogen-2,3-quinones were previously (*10*) expected to form mainly stable adducts, whereas the estrogen-3,4-quinones were shown to give rise to formation of depurinating adducts. Furthermore, analysis of the NMR coupling constants allows us to establish the stereochemistry of the adduct linkages. The apurinic adducts, i.e., 2-OHE$_2$-6(α,*β*)-N7Gua, 2-OHE$_2$-6*β*-N$^2$Gua, and 2-OHE$_2$-1-N7Gua or 2-OHE$_2$-4-N7Gua, and a degradation product, 2-hydroxy-11-oxo-estradiol, generated from transient adducts have been also identified. These compounds could be considered as a signature of the formation of unstable adducts on DNA, particularly at the C9 position of catechols, and also of a C6/C9 competition for the attack of the nucleophile. Besides, such compounds or their metabolic derivatives should be considered as complementary biomarkers of adducts recently described for the 4-hydroxyestrogens in cancer initiation risk assessment (*37*, *38*).

Formation of depurinating adducts from 2-OHE$_2$ may constitute an important argument concerning the suspected incidence of the resulting apurinic sites in DNA in cancer initiation process as hypothesized for 4-hydroxylated estrogens (*7*, *11*). These sites might be responsible for mistakes in DNA replication and play a crucial role in cancer initiation process as shown for mouse skin c-H-*ras* mutations induced by dibenzo[*a*,*l*]-pyrene adduction to DNA (*39*, *40*) or for the human p53 gene mutation in codon 249 found in hepatocarcinomas (*41*, *42*) that could be attributed to the formation of the aflatoxin B1-N7Gua adduct released from DNA (*43*). Interestingly, when considering estrogen-3,4-quinones, Chakravarti et al. (*44*) were able to measure significant amounts of 4-hydroxyestradiol-1-N3-Ade and 4-hydroxyestradiol-1-N7-Gua adducts only 1 h after treatment of mouse skin with estradiol-3,4-quinone, and they noticed that the H-*ras* oncogene displayed abundant A to G mutations 6 h after treatment. Our results concerning the ability of 2-hydroxyestrogens to generate abasic sites to a significant level support the recent evidence obtained on female CD-1 mice neonates treated with 2- and 4-hydroxyestradiol that both 2- and 4-hydroxylated estradiol are carcinogenic metabolites (*45*). Furthermore, the present results might provide reference data to elucidate the structure of the different stable estrogen adducts found with oligodeoxynucleotides. They may, therefore, help us to understand at the molecular level how they could potentially disrupt cellular functions irreversibly (*46*). Work is now in progress to characterize structure of adducts resulting from alkylation of catechol estrogens to DNA sequence models.

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