N²-Guanyl and N⁶-Adenyl Arylation of Chicken Erythrocyte DNA by the Ultimate Carcinogen of 4-Nitroquinoline 1-Oxide¹


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ABSTRACT

Great quantities of chicken erythrocyte DNA with high levels of modification were obtained in vitro by reaction with 4-acetoxyaminoquinoline 1-oxide, a model ultimate carcinogen of 4-nitroquinoline 1-oxide. After enzymatic hydrolysis of the modified DNA, the three main adducts were separated and isolated by semipreparative high performance liquid chromatography. These three adducts were already characterized in vivo and in vitro in our previous work (S. Galiègue-Zouitina et al., Cancer Res., 41: 4559-4565, 1981). In this paper we have identified as N-(deoxyguanosin-8-yl)-4-aminoquinoline 1-oxide (B. Bailleul et al., Cancer Res., 41: 4559-4565, 1981). In this paper we have identified as deoxyadenosin-N⁶-(4-hydroxyaminoquinoline 1-oxide) and 2-deoxyadenosin-N⁶-(4-aminoquinoline 1-oxide), respectively.

INTRODUCTION

It is clear that during the tumorigenesis process induced by environmental chemicals, DNA constitutes a critical target (1) and the knowledge of the adduct structures seems to be helpful in understanding the mechanisms involved. The heterocyclic aromatic amine 4-NQO is a potent carcinogen (2) the action of which is mediated by covalent binding of its ultimate metabolite to DNA (3, 4). We have demonstrated in our laboratory that Ac-4-HAQO constitutes a very adequate model for the study of carcinogenesis by 4-NQO (5-7). The conversion of Ac-4-HAQO to a quinoline nitrogen ion and/or a quinoline carboxylate during its reaction with DNA was postulated by us (6) and by Kawazoe (8), and strong support for this mechanism was obtained from chemical studies (9). We recently characterized the main lesions induced in DNA in vivo and in vitro by this carcinogen as two guanine adducts and an adenine adduct (7). One of the guanine adducts was previously identified in our laboratory as dGuo-C8-AQO and was postulated to be formed through the nitrogen ion (6). This adduct is DNA structure dependent; it accounts for 30 and 70% of the total DNA modification for native and denatured DNA, respectively (10).

This paper is devoted to the identification of the chemical structures of the other guanine adduct, dGuo-2-AQO, and of the adenine adduct, dAdo-AQO, which were isolated after enzymatic hydrolysis of large quantities of Ac-4-HAQO-modified DNA. These two have been shown in other works to be major products of the reaction of Ac-4-HAQO with DNA. This is the first investigation of the adenine adduct formed after hydrolysis of Ac-4-HAQO-modified DNA. Chicken erythrocyte DNA (420 mg; 1.5 mg of hydrolyzed DNA from chicken erythrocytes was the same as already used (7)) was prepared using the same procedure, in order to perform the partitioning experiments.

Preparation of the dGuo-2-AQO and dAdo-AQO Adducts from in Vitro Ac-4-HAQO-modified DNA. Chicken erythrocyte DNA (420 mg; a solution of 0.75 mg/ml in 2 mm sodium citrate buffer, pH 7) was modified by the ultimate carcinogen Ac-4-HAQO (4% of modified bases) and enzymatically hydrolyzed to nucleosides as described previously (7). The hydrolysate (total volume, 280 ml; 1.5 mg of hydrolyzed material per ml) was applied directly to a semipreparative HPLC column (C18, 10 µM Bondapak) with successive chromatographies of 2 ml of hydrolysate. The HPLC chromatographic conditions were a nonlinear gradient methanol-phosphate-EDTA buffer (1.5 mM NaH₂PO₄-0.4 mM EDTA, pH 6), from 15% during 12 min to 30% methanol. Absorbance was detected at 254 and 365 nm; this latter wavelength is characteristic of the quinoline ring. For each chromatograph the two adducts were collected and the solvent was immediately evaporated under vacuum. The purity of the two compounds was checked by analytical HPLC chromatography on a C18, 10 µM Bondapak column, under the methanol-water conditions used previously (5, 7). The buffer salt was then eliminated from the total preparation by a single semipreparative chromatograph under the same conditions. Determination of the amount of each adduct was performed by measurement of the absorbance at 365 nm of each compound, dissolved in water. (The absorbance values were about 7500 and 8000 for dGuo-2-AQO and dAdo-AQO, respectively). We prepared about 550 µg of dGuo-2-AQO and about 250 µg of dAdo-AQO.

Small quantities of ³H-labeled dGuo-2-AQO and dAdo-AQO were prepared using the same procedure, in order to perform the partitioning experiments which were carried out according to the method of Moore and Koreeda (13).

Instrumentation. HPLC separations were carried out on a Waters system consisting of a U6K injector, two 6000 A pumps, a Model 660 solvent programmer, and a Model 440 dual-wavelength detector (254 and 365 nm). The determinations of radioactivity for the partitioning experiments were made using a Beckman LS 2800 scintillator counter. The absorption spectra (normal and differential) were carried out using a Cary 219 recording spectrophotometer, in the 500 to 220 nm range. ¹H-NMR spectra were resolved in DMSO-d₆ at 60 ± 1°C (SD) and at 20 ± 1°C, using a Bruker WM 500 spectrophotometer, in the Fourier transform mode. In the case of dGuo-2-AQO resolution enhancement was further achieved by multiplication of the original FIDs by an unsifted sine-bell window. Spectra were calculated in the absolute value mode. Chemical shifts are given in ppm using the solvent signal (DMSO-d₆, 2.5 ppm) as reference.
CHEMICAL STRUCTURES OF DNA-NQO ADDUCTS

52Cy PDMS was previously used in the field of nucleic acids (14) and their molecular modifications by physical or chemical agents (15, 16). The 52Cy PDMS apparatus we used has been described in a previous publication (14). The geometrical arrangement has been slightly modified and the californium source is now located behind the thin Mylar target film on which the molecules are deposited. Fission fragments from the source pass through the film and produce ions at its front surface. The positive (or negative) ions are accelerated by a 7- to 10-kV accelerator voltage and travel down a flight tube to the microchannel electron multiplier detector. The length of the flight tube is adjustable from 23 to 100 cm. The times of flight spectra are measured by standard nuclear electronics which include two timing discriminators, a one-start-multistop module (17), coupled with a multichannel analyzer and to an IBM computer so that the spectra can be stored on disc for subsequent analysis. Mass calibration is achieved by using peaks of H+, Na+, and K+ present in the lower part of the mass spectra, and according to the expression

\[
\text{Time} = \sqrt{m} + b
\]

Mass spectra presented here correspond to a 60-min accumulation time recording, without background subtraction, and with a 93-cm flight tube length. In one case, a 53-cm length allowed the dimer of the molecular ion to be observed. From 5 to 10 µg of the purified samples were dissolved in 100 µl of methanol and the solution was evaporated on a thin aluminized Mylar foil. The major ionization mode in this technique appears to be a proton transfer and an ion-dipole attachment that give, respectively, (M + H)+ and (M + X)+, where X is an alkali ion. Because the most naturally abundant ion is sodium, ions such as (M + Na)+ and (M + 2Na-H)+ are often observed and are characteristic of these spectra (18, 19).

RESULTS

Isolation and Preparation of the Two DNA-NQO Adducts, dGuo-2-AQO and dAdo-AQO. After enzymatic hydrolysis to nucleosides of the Ac-4-HAQO-modified DNA, fractions of 2 ml of the total hydrolysate were applied to the semipreparative HPLC column. Fig. 1 shows the elution profile obtained at 365 nm. The three major well separated peaks corresponded to the three main adducts. Comparative HPLC analytical chromatography of the three compounds under conditions in which the three main adducts were already characterized (7) allowed us to assign the first major peak as the dGuo-2-AQO, the second as the dAdo-AQO, and the third as the dGuo-C8-AQO. We also recovered on this chromatographic profile the 4-aminoquinoline 1-oxide, 4-AQO, which was recovered in our previous study (7). Another peak, which we called incompletely hydrolyzed material, was assumed to be a mixture of di- and/or trinucleotides and oligonucleotides containing the carcinogen. Indeed it is known that the presence of the carcinogen hinders the enzymatic hydrolysis of modified DNA. Here we used a DNA sample with a high level of modification (4%). We also studied DNA samples with only 1% of modified bases and in this case the relative intensity of this incompletely hydrolyzed material peak was very decreased, indicating that it effectively corresponds to an incomplete enzymatic hydrolysis. The peak at R<sub>t</sub> = 21.30 was attributed to a compound which was recovered from modified DNA and poly(dG-dC)-poly(dG-dC); nothing is known about this compound (7).

With regard to the respective amounts of the three main adducts the elution profile indicated that the dGuo-2-AQO was the major adduct, as already observed (7). Due to the instability of the guanine adduct during enzymatic hydrolysis, part of it was degraded during its isolation. Indeed, we studied by analytical HPLC the stability of the isolated dGuo-2-AQO under the conditions of enzymatic hydrolysis; it appeared that after 20 h of incubation, 20% of the adduct was decomposed. After their purity was verified by analytical HPLC (see "Materials and Methods") the two adducts were dried under vacuum and submitted to chemical and spectroscopic analysis.

Identification of dGuo-2-AQO as 3-(Deoxyguanosin-N2-yl)-4-aminoquinoline 1-Oxide (dGuo-N2-AQO). A preliminary rapid investigation on this adduct was performed by absorption spectroscopy, in the quinoline ring band. Fig. 2 shows the recording of the absorption spectra of the compound under acidic (0.1 n HCl, pH 1.5), neutral (2 mm sodium citrate buffer, pH 7), and basic (0.1 n NaOH, pH 12.6) conditions, in the 500–300 nm range. These spectra were characteristic of the bound 4-AQO. Indeed the maximum of the band which was centered at 360 nm under neutral and basic conditions was totally shifted at

![](image-url)
330 nm, with a shoulder at 350 nm. This is characteristic of the 4-AQO form and was described previously by Sugimura et al. (20) and Hoshino et al. (21).

Solvent partitioning between aqueous buffers and butanol-1 at different pHs (from 1 to 11) was carried out and our results (Fig. 3) indicate that there were two pK\textsubscript{s} corresponding to acidic and alkaline conditions, indicating the lack of substitution on either the N-1 or the O\textsuperscript{6} atoms of guanine residue. (We verified by HPLC that the adduct was stable under the extraction conditions.) By differential absorption experiments we observed that for acidic pHs the ionizable species corresponded to the single ionization of the 4-amino group of the quinoline moiety.

The mass spectrum of dGuo-2-AQO (Fig. 4a) was obtained using \textsuperscript{252}Cf plasma desorption mass spectrometry. Table 1 gives the assignments of the peaks. Despite the extremely low stability of the adduct, molecular weight is reflected in the positive ion mass spectrum through the formation of ions of m/z = 426, 448, 470 and m/z = 424 in the negative ionization mode (the negative spectrum is not shown). The major fragment ions are provided by the breakage of the N-glycosidic bond and loss of deoxyribose (m/z = 332, 354, 376). Close to each of these ions, one can observe peaks that are 16 u.ma below. Such is the case for peaks m/z = 294, 316, and 338 in the positive mode and m/z = 292 in the negative mode. They correspond to the loss of the oxygen atom of 4-AQO, a phenomenon which has been also observed in the \textsuperscript{252}Cf PDMS spectrum of another adduct, dGuo-C8-AQO. The guanine moiety is also observed (m/z = 174, 196, 218). Three fragment ions (m/z = 137, 159, 181) can be assigned to the deaminated guanine moiety. Such a breakage, which has been already observed with a different desorption technique in the case of other N\textsuperscript{2}-guanyl adducts (22, 23), indicates that guanine was substituted on the exocyclic 2-amino group. It should be noted that such a deamination is not observed in the PDMS spectrum of free guanosine. The fact that the presence of complementary ions such as (4-AQO + NH + 2H\textsuperscript{+}) or 4-AQO alone is not observed is probably due to rearrangements or ultimate degradation. A further study, carried out with a modified \textsuperscript{252}Cf-PDMS technique in which both ion and neutral species can be detected in coincidence, gave additional evidence on the attachment of the quinoline moiety to the NH group of guanine.

Complete structural characterization was obtained from the \textsuperscript{1}H-NMR study (Fig. 5a). Assignments were based on comparisons with NMR spectra of 4-AQO (24) and dGuo (22) in DMSO-d\textsubscript{6}. The loss of two signals was observed: (a) the N\textsuperscript{2}-amino group protons of deoxyguanosine (expected at 6.37 ppm); and (b) the C-3 proton of quinoline which is normally coupled in 4-AQO with the C-2 proton. The NMR spectrum contains the resonances of all the protons of the molecule and their assignments are given in Table 2. The 3\textsuperscript{\textsuperscript{\textsuperscript{-}}}-OH and 5\textsuperscript{\textsuperscript{\textsuperscript{-}}}-OH protons were observed in the spectra recorded at 20°C at 5.0 and 5.3 ppm (data not shown).

These results and the comments added in Table 2 strongly support the characterization of dGuo-2-AQO as 3-(deoxyguanosin-N\textsuperscript{2}-yl)-4-aminoquinoline-1-oxide.

Identification of dAdo-AQO as 3-(Deoxyadenosin-N\textsuperscript{6}-yl)-4-aminoquinoline-1-Oxide (dAdo-N6-AQO). In the same manner as for dGuo-N2-AQO, the recording of the absorption spectra of the adenine adduct, in the 500–300 nm range under acidic, neutral, and basic conditions allowed us to observe the 4-AQO form bound to the adenosine moiety (spectra not shown).

From the solvent partitioning of dAdo-AQO at different pHs (from 1 to 11) the lack of an ionizable species at alkaline pH was observed (Fig. 3), with the presence of an ionizable function at acidic pH (We verified by HPLC that the adduct was stable in the acidic extraction conditions.). The recording of the differential absorption spectra as a function of pH (from 0.3 to 7) for deoxyadenosine, 4-AQO, and dAdo-AQO allowed us to distinguish the ionization of the 4-amino group of the quinoline moiety and the protonation of the N-7 of adenine (spectra not shown).

\begin{table}[h]
  \centering
  \begin{tabular}{|c|c|}
    \hline
    dGuo-2-AQO & dAdo-AQO \\
    \hline
    Quasimolecular ions & \\
    (M – H)\textsuperscript{-} & 424 \\
    (M + H)* & 426 \\
    (M + Na)* & 448 \\
    (M + 2Na – H)* & 470 \\
    (2M + 3Na – 2H)* & 917* \\
    Loss of oxygen & \\
    (M – 16 – H)* & 408 \\
    (M – 16 + H)* & 410 \\
    (M – 16 + Na)* & 432 \\
    (M – 16 + 2Na – H)* & 454 \\
    (2M – 16 + 3Na – 2H)* & 885* \\
    (2M – 16 + 3Na – 2H)* & 901* \\
    Loss of dRib.\textsuperscript{+} & \\
    (M – dRib.\textsuperscript{+}) & 308 \\
    (M – dRib. + H + Na)* & 332 \\
    (M – dRib. + 2Na)* & 354 \\
    (M – dRib. + 3Na – 2H)* & 376 \\
    (M – dRib. – 16)* & 292 \\
    (M – dRib. – 16 + 2H)* & 294 \\
    (M – dRib. – 16 + H + Na)* & 316 \\
    (M – dRib. – 16 + 2Na)* & 338 \\
    (M – dRib. – 16 + 3Na – H)* & 344 \\
    Base moiety & \\
    (B + 2H + Na)* & 174 \\
    (B + H + 2Na)* & 196 \\
    (B + 3Na)* & 218 \\
    Deaminated base & \\
    (B – NH + 3H)* & 137 \\
    (B – NH + 2H + Na)* & 159 \\
    (B – NH + H + 2Na)* & 181 \\
    \hline
  \end{tabular}
  \caption{\textsuperscript{252}Cf plasma desorption mass spectrometry data for dGuo-2-AQO and dAdo-AQO.}
  \end{table}

* S. Delia Negra et al., submitted for publication.
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Fig. 4. $^{252}$Cf time of flight mass spectra in the positive ionization mode of (a) dGuo-2-AQO and (b) dAdo-AQO. In the upper part of this spectrum, the scale is 6 times higher. The presence of each adduct is evidenced by observation of molecular ions and characteristic fragment ions (see Table 1). In this technique, mass is determined by the time of flight of the ions to reach an appropriate detector (see "Materials and Methods"). Time, which is recorded on the x coordinates, is proportional to the square root of mass. The y coordinates measure the number of charged species detected at a given mass.

Fig. 5. NMR spectra (500 MHz) of dGuo-2-AQO (a) and dAdo-AQO (b) in DMSO-d$_6$. Concentrations were 550 µg in 0.3 ml solvent for dGuo-2-AQO and 100 µg in 0.3 ml solvent for dAdo-AQO. The chemical shifts are measured in ppm downfield from DMSO (resonance at 2.5 ppm). In the case of dGuo-2-AQO, a resolution enhancement calculation was used (see "Materials and Methods"). Assignments are given in Table 2 for dGuo-2-AQO and in Table 3 for dAdo-AQO.

The mass spectrum in the positive mode of dAdo-AQO is shown in Fig. 4b and assignments are listed in Table 1. They provide evidence of the presence of this adduct. Molecular weight is clearly observed through the formation of (M + Na)$^+$ ion in the positive mode ($m/z^+ = 432$). As in the case of dGuo-N2-AQO, the major fragment ions come from the breakage of the N-glycosidic bond ($m/z^+ = 292, 316, and 338$). The loss of the oxygen atom of 4-AQO is observed on these fragment ions, in the positive ($m/z^+ = 278, 300, 322, and 344$) and in the negative ionization mode ($m/z^- = 276$). Adenosine and adenine moieties are not seen.

Structural identification was achieved from the proton NMR study (Fig. 5b). Assignments were based on comparisons with NMR spectra of 4-AQO (24), adenosine (25), and QAII (for the quinoline protons only) which was an (N-1 or N6) adenine adduct previously characterized by Kawazoe et al. (11); the solvent was DMSO-d$_6$. The loss of two signals was observed: (a) the N$^\alpha$-amino group protons of deoxyadenosine (expected...
of guanine, gives undoubtedly the site of substitution as the exocyclic amino group. Exchangeable protons observed here. This assignment, in addition to the absence of the MI^2 signal overlapping of the resonances of two protons, and we observed that one of them was exchangeable. We think it is the guanyl N2 proton since the cyclic Nl-H shifted downfield to 8.83 ppm because of the presence of the guanyl substituent. The signal is broad due to the quadrupolar effect of N2.

The peak, expected near 10-11 ppm (29), is generally broader than the resonance for example the Q-C8H resonance at 8.49 ppm). This signal is in fact an adenine adducts of 2-naphthylamine (28) and A-methyl-4-aminehydrocarbons (30). As has been proposed previously for the N*-adenine adducts (26-29) and some polycyclic adducts were also recovered from in vivo-modified DNA (7). The formation of N*-adenine adducts was shown previously to be formed through a reaction of Ac-4-HAQO with DNA (6, 8, 9). Another adduct (dGuo-C8-AQO) was shown previously to be formed by attachment of the nucleoside moiety to C-3 of the quinoline ring and this is in good agreement with the hypothesis of the formation of a C-3-quinoline carboxation as a reactive intermediate during the reaction of Ac-4-HAQO with DNA (6, 8, 9). Other adduct (dGuo-C8-AQO) was shown previously to be formed through a nitrenium ion (6). As we have shown recently, these three main adducts were also recovered from in vivo-modified DNA (7).

The formation of N*-adenyl adduct in DNA has been reported for different aromatic amines, (26-29) and some polycyclic hydrocarbons (30). As has been proposed previously for the N*-adenine adducts of 2-naphthylamine (28) and N-methyl-4-ami-noazobenzene (29), the formation of this lesion could constitute a promutagenic event due to the possible tautomeric equilibriu

Table 2 NMR study of dGuo-N2-AQO

<table>
<thead>
<tr>
<th>Chemical shifts (ppm)</th>
<th>Assignments</th>
<th>Multiplicity</th>
</tr>
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<tbody>
<tr>
<td>8.83</td>
<td>Q-C2H^t</td>
<td>s</td>
</tr>
<tr>
<td>8.49</td>
<td>Q-C8H^s</td>
<td>m</td>
</tr>
<tr>
<td>8.34</td>
<td>Q-C5H^m + G-NH^f</td>
<td>m</td>
</tr>
<tr>
<td>7.82</td>
<td>Q-C8H^s</td>
<td>s</td>
</tr>
<tr>
<td>7.73</td>
<td>Q-C7H^m</td>
<td>m</td>
</tr>
<tr>
<td>7.61</td>
<td>Q-C6H^m</td>
<td>m</td>
</tr>
<tr>
<td>6.72</td>
<td>Q-NH^s</td>
<td>s</td>
</tr>
<tr>
<td>6.06</td>
<td>H1'</td>
<td>m</td>
</tr>
<tr>
<td>4.44</td>
<td>H3'</td>
<td>m</td>
</tr>
<tr>
<td>3.69</td>
<td>H4'</td>
<td>m</td>
</tr>
<tr>
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<td>m</td>
</tr>
<tr>
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<td>H5* or 5'</td>
<td>m</td>
</tr>
<tr>
<td>2.80</td>
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<td>m</td>
</tr>
<tr>
<td>2.15</td>
<td>H2' or 2'</td>
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Table 3 NMR study of dAdo-N6-AQO

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<th>Assignments</th>
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<td>8.59</td>
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<tr>
<td>2.77</td>
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</table>

* Nonexchangeable proton. It is expected at 8.12 ppm in 4-AQO (16) but it is shifted downfield to 8.83 ppm because of the presence of the guanyl substituent. The signal is broad due to the quadrupolar effect of N2.

º Q, quinoline; G, guanine; s, singlet; m, multiplet.

º The chemical shift of this quinoline proton is very near to those of 4-AQO (16). These assignments indicate that the substitution occurs at N^6 of adenine. We compared the chemical shift of the C2H proton (8.25 ppm) with the values obtained for this proton in the case of 1-methyldeoxyadenosine (8.7 ppm), 3-methyladenosine (7.9 ppm), and N^6-methyladenosine (8.2 ppm), as it was made by Phillips et al. (42) for the study of another N^6-adenyl adduct. Moreover N-1- or N-3-substituted adenine should lead to an imino group on C-6 of adenine (tautomeric forms) and the chemical shifts of such a group are known to be found near 9.5 to 11 ppm. These two comparisons led us to assign the signal at 8.32 ppm to an NH2.

º This proton is expected at 8.12 ppm in 4-AQO (16) but it is shifted downfield to 8.59 ppm due to the presence of the adenyl substituent. The same shift was observed for QAII (15).

º Q, quinoline; A, adenine; s, singlet; m, multiplet; u, unresolved.

º The chemical shift of this quinoline proton is very near to those of 4-AQO (16) and the same as in QAII (15).

º The same chemical shift is observed for the dAdo C-8 proton (36).

º These assignments indicate that the substitution occurs at N^6 of adenine. We compared the chemical shift of the C2H proton (8.25 ppm) with the values obtained for this proton in the case of 1-methyldeoxyadenosine (8.7 ppm), 3-methyladenosine (7.9 ppm), and N^6-methyladenosine (8.2 ppm), as it was made by Phillips et al. (42) for the study of another N^6-adenyl adduct. Moreover N-1- or N-3-substituted adenine should lead to an imino group on C-6 of adenine (tautomeric forms) and the chemical shifts of such a group are known to be found near 9.5 to 11 ppm. These two comparisons led us to assign the signal at 8.32 ppm to an NH2.

º The intensity of this band is about 2-fold that expected for one proton (see for example the Q-C8H resonance at 8.49 ppm). This signal is in fact an overlapping of the resonances of two protons, and we observed that one of them was exchangeable. We think it is the guanyl N2 proton since the cyclic Nl-H peak, expected near 10-11 ppm (29), is generally broader than the resonance observed here. This assignment, in addition to the absence of the 2-NH2 signal of guanine, gives undoubtedly the site of substitution as the exocyclic amino group.

º Exchangeable protons.

º The same chemical shift is observed for the dGuo-C8 proton (29).

at 7.28 ppm); and (b) the C-3 proton of quinoline with the loss of coupling to the Q C-2 proton. The NMR spectrum contained the resonances of all the protons of the molecule and their assignments are given in Table 3.

Taken together, our data and the comments added in Table 3 strongly support the characterization of dAdo-AQO as 3-(deoxyadenosin-N^6-y)-4-aminoquinoline 1-oxide.

**DISCUSSION**

This paper reports the structural identification of two main DNA adducts of the carcinogen 4-NQO, the N^2-guanyl adduct (dGuo-N2-AQO) and the N^6-adenyl adduct (dAdo-N6-AQO). These two compounds were formed by attachment of the nucleoside moiety to C-3 of the quinoline ring and this is in good agreement with the hypothesis of the formation of a C-3-quinoline carboxation as a reactive intermediate during the reaction of Ac-4-HAQO with DNA (6, 8, 9). Another adduct (dGuo-C8-AQO) was shown previously to be formed through a nitrenium ion (6). As we have shown recently, these three main adducts were also recovered from in vivo-modified DNA (7).

The formation of N*-adenyl adduct in DNA has been reported for different aromatic amines, (26-29) and some polycyclic hydrocarbons (30). As has been proposed previously for the N*-adenine adducts of 2-naphthylamine (28) and N-methyl-4-aminoazobenzene (29), the formation of this lesion could constitute a promutagenic event due to the possible tautomeric equilibriu
CHEMICAL STRUCTURES OF DNA-NQO ADDUCTS

In the case of 4-NQO the mutations have been shown to be largely G:C → A:T transitions with also a few transversion mutations (37, 38). Scriber et al. (39) predicted the blockade of the polymerase by NQ adducts during the replication of modified DNA. Very recently Yoshida et al. (40) showed that the NQO-guanine adducts blocked the Escherichia coli DNA polymerase elongation in vitro in three different manners (one nucleotide before the lesion, at position opposite, and at one base beyond guanine). Any correlation with the structure of the guanine lesions was not described by these authors.

In conclusion we have shown that the main lesion induced by 4-NQO in vivo and in vitro is a N2-guanyl adduct (50%), the others being a C8-guanyl adduct (30%), an N6-adenyl adduct (10%) and some minor compounds (10%). Physicochemical investigations have begun in our laboratory to determine the modification of DNA induced by each of the adducts. For example, we recently demonstrated that the N2-guanyl adduct impaired the conversion from the B form to the Z form of a polymerase elongation in vitro in three different manners (one base beyond guanine). Any correlation with the structure of the modification of DNA induced by each of the adducts. For investigations have begun in our laboratory to determine the others being a C*-guanyl adduct (~30%), a A^-adenyl adduct.

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