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ON THE MECHANISM OF ARYLAMINE-DNA ADDUCT FORMATION

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Abstract: 7-Methoxy-9-benzylguanine was converted to a mixture of 8-keto-9-benzylguanine and 8-methoxy-9-benzylguanine upon treatment with aqueous base. This model system highlights the reactivity of a heteroatom-N7 guanyl species (implicated as an intermediate in the mechanism of formation of arylamine-guanine adducts) toward nucleophilic attack at C8.

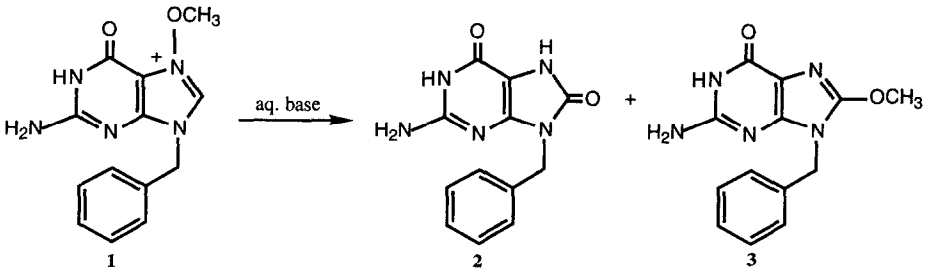
The formation of guanine C8 adducts by amidoaryl carcinogens is unique among organic agents known to covalently modify DNA. Unlike most carcinogenic molecules, DNA adduct formation by arylamines does not appear to fit the classical DNA nucleophile-carcinogen electrophile relationship. The sulfate ester of an N-acetyl hydroxylamine or a nitrenium ion derived by ionization of this species is believed to be the ultimate genotoxin,[1] and the chemistry of C8 amidoarylation appears to involve guanine nucleophilic attack on an electrophilic nitrogen site consistent with S_N2 behavior.[2] However, the C8 atom of guanosine is not particularly nucleophilic compared to the N7, O6, and N2 sites of this base,[3] where the majority of electrophiles react.

Among possible mechanisms which could rationalize the unusual ability of arylamines to preferentially produce guanosine C8 adducts are direct nucleophilic attack by the guanine C8 position on the arylamide electrophile or nucleophilic attack by another position of the guanine base (such as the N7 site), followed by migration of the arylamide moiety to C8. Evidence has been obtained in favor of both mechanisms. In support of the direct attack mechanism, N-acetyl-N-(acyloxy) aminofluorene tethered to deoxyguanosine formed higher percentages of C8 adducts in 90% and 100% water compared to acetone/water mixtures.[4] The authors concluded that pi stacking increased the yields of C8 adduct in this study. They further suggested that the site of attack by the DNA base upon the arylamine carcinogen and the efficiency of this reaction could be decided by hydrophobic stacking *in vivo*. In a different report, N-acetoxyaminofluorene generated *in situ* was reacted with guanine derivatives possessing substituents at the C8 position to produce nucleoside-arylamine adducts consistent with initial reaction at the N7 atom.[5] An N7-C8 rearrangement could then occur after deprotonation at C8 via an ylid intermediate.

To further study the mechanism of arylamine adduct formation in DNA, we wished to synthesize a guanine analog possessing a substituent at N7 that could mimic the reactivity of the proposed N7 guanyl-arylamine intermediate. Our choice of a mechanistic probe was dictated by synthetic accessibility and stability of the

analog. 9-Benzylguanine-7-oxide[6] was methylated with trimethyloxonium tetrafluoroborate in CH_3NO_2 to give 7-methoxy-9-benzylguanine **1**, our model for the N7 guanyl-arylamine adduct. Confirmation of the position of methylation of 9-benzylguanine-7-oxide was sought by the conversion of **1** to 8-keto-9-benzylguanine **2** in refluxing sodium hydroxide, conditions that were previously observed to produce **2** from 9-benzylguanine-7-oxide and to give 8-keto hypoxanthine from hypoxanthine 7-oxide.[7] Consistent with methylation of the N7 oxygen by Meerwein's salt was the formation of **2** as the major reaction product under these conditions, but 8-methoxy-9-benzylguanine **3** was found to be a minor product (Figure 1). Since the presence of base could promote N7-C8 rearrangement by abstraction of the C8 proton and subsequent ylid formation, a more detailed investigation of the reaction was undertaken. The ratio of **3** to **2** observed for the reaction of **1** with aqueous hydroxide was dependent on base concentration, counterion and temperature, with optimum conditions (aq. KOH [2M], 0° C) giving a **3/2** ratio of 2:3 as determined by ^1H NMR integration of the reaction mixture. Lower base concentrations and elevated reaction temperatures led to the formation of higher percentages of **2**. 8-Methoxy-9-benzylguanine **3** did not convert to 8-keto-9-benzylguanine **2** in aqueous base. Authentic standards synthesized by different routes were used to confirm the structures of compounds **2** and **3**.[7,8] 7-Methoxy-9-benzylguanine **1** was stable in DMSO at room temperature and did not exchange the C8 proton upon addition of D_2O, but was not soluble in unbuffered water. **1** gave a complex mixture of products upon treatment with triethylamine in acetonitrile and produced primarily 8-ethoxy-9-benzylguanine upon treatment with sodium ethoxide in DMSO.

In order to determine whether the formation of **3** proceeded through an intramolecular or intermolecular process, CD_3OD (0.1 mL, 2.46 mmol) was mixed with aqueous KOH (2N, 1.4 mL) at 0° C and **1** was added (23.5 mg, 86.7 μmol). The product mixture consisted of 8-(d_3-methoxy)-9-benzylguanine (55%), 8-methoxy-9-benzylguanine (26%), and 8-keto-9-benzylguanine (19%), as determined from the ^1H NMR spectrum of the reaction mixture. 8-Keto-9-benzylguanine **2** was not produced from **3** in aqueous base and exchange of the methyl ether moiety in **3** with (d_4)-methanol did not occur under the conditions of the reaction. The determination of methyl ether exchange for 7-methoxy-9-benzylguanine **1** could not be adequately addressed



1 2 3

Figure 1. Reaction of 7-methoxy-9-benzylguanine in aqueous base.

due to the decomposition of **1** under these conditions.

An N7-C8 rearrangement seems unlikely as a major reaction pathway in these experiments based on the preference for deuterated methoxide incorporation at the C8 position, indicating that an intramolecular cage process is not required for production of the 8-methoxy compound. The deuterated 8-methoxy compound probably forms through an intermolecular addition-elimination mechanism, which is also the likely pathway for formation of **2** in this system. The deuterated methoxide experiment shows that the presence of methoxide ion preferentially leads to production of **3** in the presence of a much greater concentration of hydroxide ion. This result is surprising because the experimentally determined nucleophilicity of methoxide ion is similar to that of hydroxide ion.[9] A possible explanation for this result is that the intermediate present in methoxide attack at the C8 position has one potential site of deprotonation, the C8 proton, whose removal would lead to product formation. Upon hydroxide attack at C8, a base could also remove a proton from C8 and lead to the addition-elimination product. A proton is also available for abstraction from the newly added hydroxyl group, a pathway which would not lead to the addition-elimination product directly (see Figure 2). Production of the 8-methoxy compound could therefore be favored kinetically over 8-keto production.

The reactivity of 7-methoxy-9-benzylguanine in aqueous base serves to reinforce previous studies where the introduction of a positive charge at the N7 imidazole ring enhances the electrophilicity of the C8 carbon.[7,10-12] Thus, this study lends support to the hypothesis that N7 aminoalkylation, followed by nucleophilic attack at C8 by water, may be responsible for the production of 8-ketodeoxyguanosine upon the reaction of DNA with 4-hydroxyaminoquinoline-1-oxide[13] (This species, often called 8-hydroxydeoxyguanosine, has been shown to

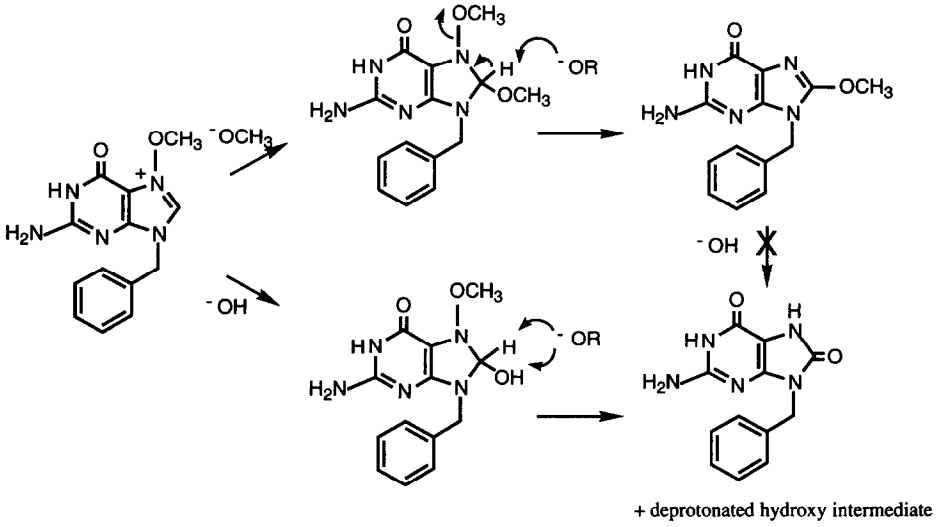


Figure 2. Mechanistic rationale for the high level of 8-methoxy production.

exist predominantly as the 8-keto isomer[14]). A similar reaction has been described for the O-acylated 3-oxides of guanine and xanthine, which are attacked by nucleophiles in aqueous solution to give 8-substituted purines.[15]

The ultimate carcinogen 4-acyloxyaminoquinoline 1-oxide displays a broad spectrum of arylamine-guanine reactivity. In addition to C8 adducts[16] and 8-keto residues,[13] an N7 guanyl adduct derived from the treatment of RNA with activated 4-hydroxyaminoquinoline-1-oxide has been reported.[17] Initial N7 attack could be responsible for all of the observed guanyl species, or C8 and N7 attack could occur separately. The present study demonstrates the electrophilicity of the C8 position of guanine after heteroatom modification at N7 and suggests, along with data obtained from the study of 4-acyloxyaminoquinoline-1-oxide, that 8-keto-deoxyguanosine could be a major lesion in DNA treated with activated arylamines. This work also suggests that 8-ketodeoxyguanosine should be the major species resulting from an initial N7-arylamine adduct, although unique electronic properties of each adduct could contribute to the particular reaction pathway.

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