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Alkylation of guanosine and 2′-deoxyguanosine by *o*-quinone α-(*p*-anisyl)methide in aqueous solution †

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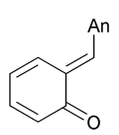
Rates of alkylation of guanosine and 2′-deoxyguanosine with *o*-quinone α-(*p*-anisyl)methide were measured by flash photolysis in a series of aqueous sodium hydroxide solutions and bicarbonate ion, *t*-butylhydrogenphosphonate ion, and biphosphate ion buffers. The data so obtained provide rate profiles for these nucleoside plus quinone methide reactions over the range pH = 7–14, which furnish guanosine and deoxyguanosine acidity constants consistent with literature information. These profiles also provide rate constants that show the reaction of *o*-quinone α-(*p*-anisyl)-methide with guanosine and deoxyguanosine to be fairly fast processes, considerably faster than the biologically wasteful reaction of the quinone methide with water, which is the ubiquitous medium in biological systems; that makes the quinone methide a potent guonosine and deoxyguanosine alkylator.

Introduction

Quinone methides, in addition to being useful synthetic intermediates[1] and playing prominent roles in wood chemistry,[2] also show pronounced biological activity.[3] They have, for example, been implicated as the ultimate cytotoxins responsible for the effects of agents that function through the alkylation of DNA, which is believed to occur predominantly on the purine residues of 2′-deoxyguanosine.[4] In order to determine how rapidly this alkylation takes place, and how it depends upon the ionization state of the nucleoside, we have measured rates of reaction of *o*-quinone α-(*p*-anisyl)methide, **1** An = *p*-$CH_3OC_6H_4$, with deoxyguanosine, **2** and also with guanosine itself, **3**.



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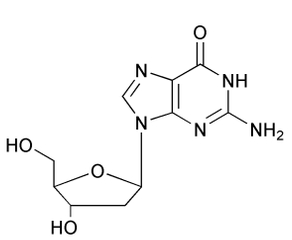

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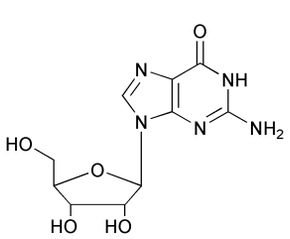

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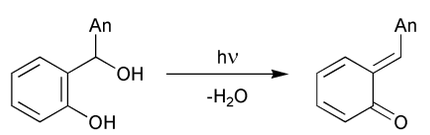
We generated *o*-quinone α-(*p*-anisyl)methide directly in our aqueous reaction solutions, as we had before,[5] by flash photolytic dehydration of *o*-hydroxy-α-(*p*-anisyl)benzyl alcohol, **4**, eqn. 1.



(1)

4 **1**

Experimental

Materials

o-Hydroxy-α-(*p*-anisyl)benzyl alcohol was a sample that had been prepared before.[5] All other materials were the best available commercial grades.

Kinetics

Rate measurements were made using a conventional (microsecond) flash photolysis system that has already been described.[6] Substrate concentrations in the reacting solutions prior to flashing were of the order of 10^{-5} M, and the temperature of these solutions was controlled at 25.0 ± 0.05 °C. Reactions were monitored by following the decay of quinone methide absorbance at λ = 400 nm. The data so obtained conformed to the firt-order rate law well, and observed first-order rate constants were obtained by least squares fitting of a single exponential function.

Results

Rates of reaction of *o*-quinone α-(*p*-anisyl)methide with guanosine and deoxyguanosine were measured in aqueous sodium hydroxide solutions and in bicarbonate ion, *tert*-butylhydrogenphosphate ion, and biphosphate ion buffers. Measurements were made in series of solutions of constant sodium hydroxide or buffer concentration, and therefore constant hydronium ion concentration, but varying guanosine or deoxyguanosine concentration. Nucleoside concentrations were always at least ten times greater than initial quinone methide concentrations but at least ten times less than sodium hydroxide or buffer concentrations.

As Fig. 1 illustrates, observed first-order quinone methide decay rate constants determined under these conditions were accurately proportional to the first power of guanosine or deoxyguanosine concentration. The data were therefore

† Electronic supplementary information (ESI) available: Table S1 of rate data. See http://www.rsc.org/suppdata/ob/b4/b400098f/

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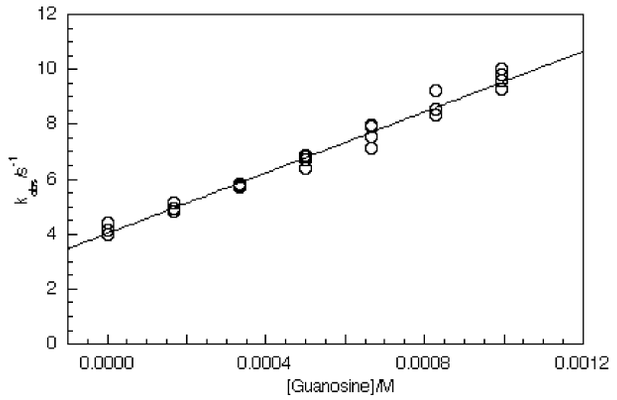


Fig. 1 Reactant dilution plot for the reaction of *o*-quinone α-(*p*-anisyl)-methide with guanosine in aqueous bicarbonate ion buffer solutions, $[HCO_3^-] = 0.016$ M $[CO_3^=] = 0.008$ M, at 25 °C.

analyzed by least squares fitting of a linear function. The zero-guanosine and deoxyguanosine concentration intercepts obtained in this way are consistent with rate constants determined before for decay of *o*-quinone α-(*p*-anisyl)methide in these solutions in the absence of guanosine or deoxyguanosine.[5] The slopes of these linear relationships, $\Delta k_{obs}/\Delta$[guanosine] or $\Delta k_{obs}/\Delta$[deoxyguanosine], are bimolecular rate constants for alkylation by *o*-quinone α-(*p*-anisyl)methide of guanosine or deoxyguanosine in whatever states of ionization are present at the various hydronium ion concentrations of these solutions. These bimolecular rate constants are listed in Table S1 (ESI †) and are displayed as the rate profiles of Fig. 2. Hydronium ion concentrations needed for the construction of Fig. 2 were obtained by calculation using literature values of the p*K*a's of the buffer acids and acidity coefficients recommended by Bates.[7]

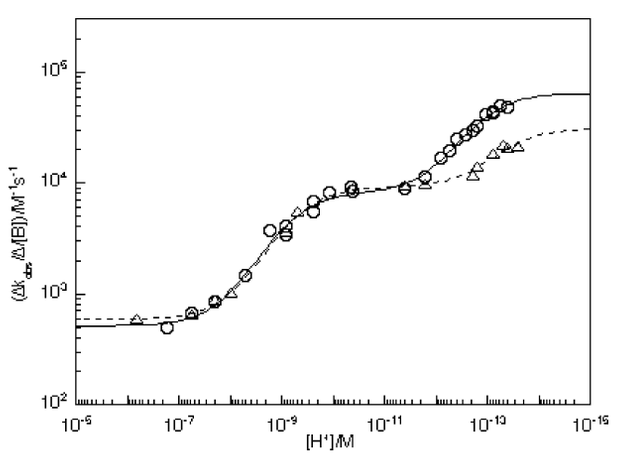


Fig. 2 Rate profiles for the reaction of *o*-quinone α-(*p*-anisyl)methide with guanosine (O) and deoxyguanosine (Δ).

Discussion

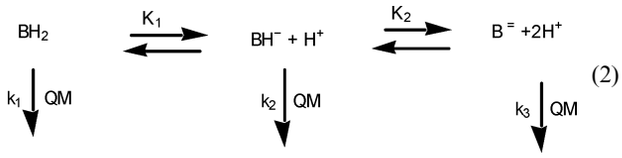
The rate profiles of Fig. 2 show that rates of reaction of *o*-quinone α-(*p*-anisyl)methide with guanosine and deoxyguanosine increase with decreasing hydronium ion concentration. This is consistent with the fact that both guanosine and deoxyguanosine possess acidic groups whose ionization converts less basic into more basic species, plus the expectation that the more basic species would be better nucleophiles and thus react with the quinone methide at faster rates. The rates of reaction, moreover, do not increase linearly with decreasing medium acidity, but they rather pass through three horizontal plateaus reminiscent of the titration curve of a dibasic acid.

This behaviour suggests that the solutions represented by Fig. 2 contain three nucleophilic species formed by two successive ionizations of guanosine or deoxyguanosine, each of which reacts with *o*-quinone α-(*p*-anisyl)methide at a different rate, as

Table 1 Equilibrium and rate constants obtained by fitting eqn. 3 for the reaction scheme of eqn. 2 with data for the reaction of *o*-quinone α-(*p*-anisyl)methide with guanosine and deoxyguanosine

Parameter	Guanosine	Deoxyguanosine
$K_1/10^{-10}$ M	7.36 ± 1.18	5.33 ± 0.74
pK_1	9.13 ± 0.07	9.27 ± 0.06
$K_2/10^{-14}$ M	13.2 ± 3.2	4.45 ± 2.13
pK_2	12.88 ± 0.11	13.35 ± 0.21
$k_1/10^2$ M^{-1} s^{-1}	5.13 ± 0.42	5.87 ± 0.32
$k_2/10^2$ M^{-1} s^{-1}	81.4 ± 0.50	91.4 ± 0.47
$k_3/10^2$ M^{-1} s^{-1}	642 ± 65	308 ± 55

shown in the reaction scheme of eqn. 2. The rate law that corresponds to this reaction scheme is shown in eqn. 3. Least squares fitting of this expression gave the rate and equilibrium constants listed in Table 1, which were used to draw the lines shown in Fig. 2.



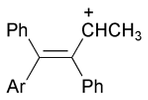
(2)

$$\frac{\Delta k_{obs}}{\Delta[B]} = \frac{k_1[H^+]^2 + k_2K_1[H^+] + k_3K_1K_2}{[H^+]^2 + K_1[H^+] + K_1K_2} \qquad (3)$$

It may be seen that this model fits the experimental data well. It gives acidity constants, moreover, that are wholly consistent with reports of these quantities in the literature. For example, pK_1 = 9.13 obtained here for guanosine agrees well with an early report of pK = 9.25,[8] as well as with a recent determination, pK = 9.22.[9] The result obtained here for the second ionization of guanosine, pK_2 = 12.88, is also consistent with pK = 12.33,[8] as well as with the report that this pK is greater than 12.[9b] It is significant as well that similar values of pK_1 were obtained here for guanonsine and deoxyguanosine, for this acidity constant is known to refer to ionization of the N-H bond of the purine amide group,[8] which has the same local environment in these two nucleosides. The pK_2 values determined here for guanosine and deoxyguanosine, on the other hand, are probably different from one another, and this is consistent with the assignment of this acidity constant to ionization of a ribose hydroxyl group.[8] In guanosine this ionization produces an alkoxide ion that is stabilized by hydrogen bonding from the second unionized ribose hydroxyl group, but in deoxyguanosine a second hydroxyl group is not present and such stabilization is not possible; hence the ribose hydroxyl group of deoxyguanosine is less acidic than that of guanosine and pK_2 (deoxyguanosine) is greater than pK_2 (guanosine).

The rate constants determined here show that the reactions of *o*-quinone α-(*p*-anisyl)methide with guanosine and deoxyguanosine are fairly fast processes, but they are not as fast as the reaction of deoxyguanosine with some carbocation alkylators such as the tamoxifen cation, **5**, Ar = $C_6H_4CH_2CH_2N(CH_3)_2$.[10] Tamoxifen, **6** is an antioestrogen used in the treatment and control of breast cancer. It is metabolized in the body to the tamoxifen cation, which then operates by alkylating DNA through reaction with its deoxyguanosine residues. A recent flash photolytic study of this system[10a] reported rate constants for the reaction of tamoxifen cation with the neutral and anionic forms of deoxyguanosine that are two orders of magnitude greater than those determined here for reaction of *o*-quinone α-(*p*-anisyl)methide with deoxyguanosine.

This greater reactivity, however, does not make tamoxifen cation a stronger deoxyguanosine alkylator than the present quinone methide. This is because these alkylators also react with water, the ubiquitous solvent in biological systems, and



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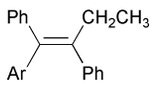

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the reaction of tamoxifen cation with water is also faster than the reaction of *o*-quinone α-(*p*-anisyl)methide with water. In fact, the rate of the water reaction of the tamoxifen cation is three orders of magnitude greater than that of the reaction of *o*-quinone α-(*p*-anisyl)methide with water, a factor that overwhelms the two orders of magnitude rate difference of the deoxyguanosine reactions. As a result, *o*-quinone α-(*p*-anisyl)-methide is actually a more potent deoxyguanosine alkylator than is tamoxifen cation.

Acknowledgements

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