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Communications

Interaction and Reactivity of Carcinogenic *N*-Acetyl-*N*-(acyloxy)-2-aminofluorene with Deoxyguanosine. An Intramolecular Approach

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Summary: Solvolysis of **3** in water–acetone mixtures yields the "adduct" **4** (65% in water) with product and rate data consistent with the hypothesis that hydrophobic guanine–fluorene stacking, similar to that which occurs when the carcinogenic aminofluorene metabolite is intercalated in DNA, is responsible for selective binding of the carcinogen at the C-8 guanine center.

Carcinogenic aromatic amides are metabolized to hydroxamic acid derivatives of the type ArN(Ac)OAc, which react with nucleic acid bases in DNA.[1] Although the fine details of the reaction pathway are under active investigation,[2,3] a generally accepted mechanism involves heterolytic cleavage of the N–O bond leading to a nitrenium ion,[3] which reacts with nucleophilic sites in DNA. The main product observed with *N*-acetoxy-*N*-acetyl-2-aminofluorene, **1**, the most studied derivative, results from reaction at the C-8 guanine center.[4] The same type of product **2** is formed, in model-reaction conditions, between deoxyguanosine and **1**.[5] However, yields are low due to the highly favored hydrolysis of the hydroxamic acid ester function (mixed anhydride) in **1**, hence a large excess of the latter must be used. This precludes a detailed mechanistic examination of the reaction between the carcinogen and the nucleoside. As a consequence, a number of questions remain to be answered. An intriguing point, for example, is the selectivity of the reaction in which the electrophilic nitrenium ion attacks the only slightly nucleophilic C-8 center of guanine and not the more nucleophilic N-7 and O-6 sites, as observed with the usual alkylating agents. In addition, reaction with DNA is more complex as additional factors intervene. Intercalation of the drug, prior to reaction, has been postulated.[6]

In order to have an insight into the reaction mechanism and to study the influence upon the reaction of ring–ring stacking interactions between the nucleic bases and the fluorene ring, as may occur in the intercalation step in DNA, we have designed model compound **3** in which the carcinogen is joined to the base by a flexible link. This approach is based upon results that we previously obtained for DNA intercalator models[7] and relies on the following hypotheses: (1) the flexible link allows intramolecular ring–ring stacking between guanine and fluorene; (2) the use of a link as part of the leaving group allows the reaction

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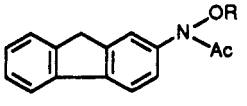
Table I. Pseudo-First-Order Rate Constants for Solvolysis of Model Compound 3 and Yields of Formation of the Substitution Product 4

Me_2CH-H_2O[a]					
		Y_{Cl}[b]	$k_{25°C}$, s^{-1}	r[c]	% formation of 4[d]
80	20	−0.80	1.71×10^{-4}	0.999	25 ± 1
50	50	1.73	2.21×10^{-3}	0.999	40 ± 1
20	80	3.77	2.7×10^{-2}	0.996	60 ± 2
10	90	4.28	3.9×10^{-2}	0.995	65 ± 2
0	100	4.57	6.9×10^{-2}	0.995	65 ± 2

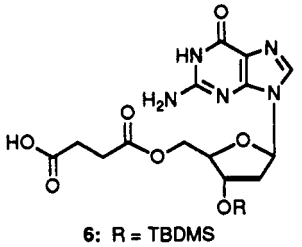
[a] Me_2CO added to aqueous phosphate buffer, pH 7. [b] Reference 11. [c] "r": correlation coefficients for a linear treatment of concentration vs time data. [d] Determined by HPLC analysis at completion of reaction.

to proceed without constraints; (3) from a preparative point of view the use of the link incorporating the deoxyribose and the succinyl moiety may lead to a reaction product 4 that is a direct precursor of the adduct 2 formed in DNA. In the past we have shown that such "heterodimeric molecules" involving an intercalator and a base exist in water predominently with folded conformations as a result of ring–ring stacking interactions.[7] We report here the synthesis and solvolytic behavior of model compound 3.

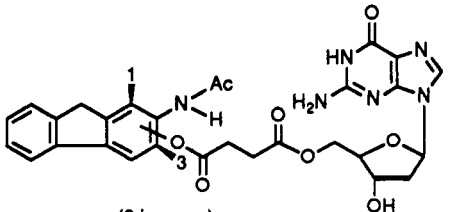
Compound 3[8] was obtained in 75% yield by coupling the hydroxamic acid 5[9] and the protected nucleoside 6 using isobutyl chloroformate in dimethylformamide in the presence of *N*-methylmorpholine at −25 °C, followed by careful deprotection of the 3′-*tert*-butyldimethylsilyl group with hydrofluoric acid–pyridine in tetrahydrofuran at 25 °C for 6 h. The nucleoside 6 was prepared in a four-step sequence (overall yield 67%) from deoxyguanosine: protection of the $OH_{5'}$ by dimethoxytritylation (conditions under which the NH_2 guanosine is also tritylated); protection of the $OH_{3'}$ by the *tert*-butyldimethylsilyl group; removal of the dimethoxytrityl protecting groups with 80% acetic acid at 20 °C; and succinylation of the $OH_{5'}$ with succinic anhydride in dichloromethane catalyzed by (dimethylamino)pyridine at 20 °C.



1: R = Ac
5: R = H



6: R = TBDMS
7: R = H

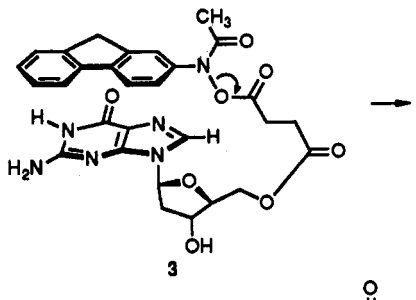


(2 isomers)

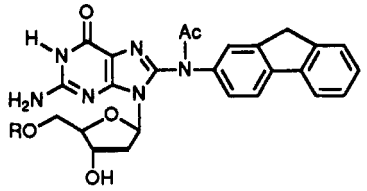
8a—1 8b—3

The rates of solvolysis of 3 were studied in a series of acetone–water mixtures at neutral pH at 25 °C. Results were obtained by using HPLC and involved the disappearance of 3 (measured to at least 90% conversion) in each solvent. Good pseudo-first-order kinetics were found (see Table I). Plotting the data according to the Grunwald–Winstein relation $\log k/k_0 = mY$,[10,11] we find that the rate of solvolysis correlates well with the ionizing power of the solvent (for Y_{Cl}, $r = 0.996$), with the m value equal to 0.5.

Scheme I



3



2: R = H
4: R = $COCH_2CH_2CO_2H$

Analysis of the solvolysis mixtures revealed the formation of three types of reaction products: (1) the nucleoside 7 and the hydroxamic acid 5[12] resulting from hydrolysis of the mixed anhydride function; (2) a mixture of isomers 8,[13] resulting from a Bamberger-type reaction; (3) the C-8 substitution product 4, which was characterized by analytical and NMR data and correlation[14] with the known "adduct" 2 formed in DNA. The relative proportion of the three types of products varied as a function of the solvolysis medium. We focused on the yield of the substitution product 4 in the different solvents. The values are given in Table I.

A number of interesting points emerge from the data shown in Table I. The rate acceleration observed when the ionizing power of the solvent is increased, following the Grunwald–Winstein equation, is good evidence that the reaction proceeds through an ionic pathway and further supports the involvement of a nitrenium ion as an intermediate. Perhaps more interesting is the observation that the proportion of substitution increases spectacularly with the amount of water and reaches a value as high as 65% in pure water. An interpretation is shown in Scheme I. When the percentage of water in the solvolysis medium increases, the model system 3 most likely adopts folded conformations in which the guanine and fluorene rings are intramolecularly stacked as a result of "hydrophobic" interactions.[15] Such intramolecular ring–ring stacking in-

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(12) The hydroxamic acid 5 decomposes partly into unidentified materials in the coarse of solvolysis.
(13) Isomers 8a and 8b are most conveniently isolated and characterized by refluxing 3 in dichloromethane for 30 h (90% yield). ^1H NMR indicates the presence of a ca. 50:50 mixture of isomers 8a and 8b.
(14) 4 is quantitatively transformed into 2 by a 15-min treatment in 0.1 M sodium hydroxide in methanol at 25 °C.

teractions in water have been amply demonstrated in the past for heterodimeric systems of type $Ar_1(CH_2)_nAr_2$, where Ar are nucleic bases and intercalators.[7] The geometry of the stacked complex is favorable for an attack on the C-8 position of guanine by the developing nitrenium ion. The arrangement of the two rings is close to the geometry of the transition state required for the electrophilic attack at C-8 by the nitrenium ion. This hypothetical scheme explains both the efficiency of the attack at the C-8 site of guanine and the dramatic effect of the solvent conditions on the yield.[16]

These observations, based upon our carefully designed molecule, suggest the importance of stacking interactions. In addition, they permit a more general comment on the process of adduct formation between DNA and polycylic aromatic amine metabolites that can intercalate in DNA. Intercalation, closely analogous to our intramolecular complexation, determines the relative position of a DNA base and a carcinogen substance. Our results suggest that in such a situation, following the generation of the reactive species, in our case a nitrenium ion, the site of attack on the base and the efficiency of this process are controlled by the stacking phenomenon. Our model provides evidence for a frequently quoted hypothesis, inadequately substantiated previously because of the complexity of the biological system.

(15) Direct experimental evidence for intramolecular ring–ring stacking in 3 could not be obtained, due to its high reactivity in water ($t_{1/2}$ = 10 min at 25 °C) combined with poor solubility in water (smaller than 10^{-3} at 25 °C). These preclude a ^1H NMR or UV study, as was achieved in other examples.[7]

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(17) Financial support from the "Association de la Recherche pour le Cancer" and the "Ligue Nationale Française pour le Cancer" are gratefully acknowledged. We thank Dr. A. Greene for his interest in our work.

Concerted Mechanism of the Aminolysis of *O*-Ethyl *S*-(2,4-Dinitrophenyl) Thiocarbonate

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Summary: The linear Brönsted-type plot with slope β = 0.56 found in the aminolysis of *O*-ethyl *S*-(2,4-dinitrophenyl) thiocarbonate indicates a concerted mechanism, which is explained through instability of the putative zwitterionic tetrahedral intermediate, caused by the EtO group. Had the mechanism been stepwise the position of the Brönsted break should have been at pK_a 9.3 (the experimental pK_a range is 6.4–11.5).

The mechanism of the aminolysis of oxyesters[1,2] and carbonates[3] has been extensively studied and the influence of the nucleofuge and nonleaving groups of the substrate on the kinetics has been assessed.[3] Since the mechanism of the aminolysis of thioesters and thiocarbonates has been less studied,[4-6] we now report on the kinetics of the reaction of *O*-ethyl *S*-(2,4-dinitrophenyl) thiocarbonate (DNPTC) with a series of secondary alicyclic amines. The object is to shed more light into the mechanism of the aminolysis of thio compounds and to analyze the influence of the nonleaving group of the substrate on the above mechanism, by comparison with the aminolysis of 2,4-dinitrophenyl thiolacetate (DNPTA).[6] We report in this paper that there is an abrupt change in mechanism from a stepwise, via a tetrahedral intermediate for the thiolacetate aminolysis, to a concerted one for the thiocarbonate reactions. That is, this remarkable change in mechanism occurs when the Me group of DNPTA is replaced by a EtO group.

DNPTC was prepared by a similar method described for analogous thiocarbonates.[7] Previously, 2,4-dinitrophenol was obtained by a modification of a reported procedure.[6,8] The purification of the amines, kinetic measurements and product studies were carried out as described.[6]

In all cases, under amine excess, pseudo-first-order rate coefficients (k_{obsd}) were obtained. The plots k_{obsd} vs free-amine concentration ([N]) at constant pH were linear with the slopes (k_N) independent of pH, except for the reactions with piperazine (PA) at low pH values, where the above slopes were pH dependent. This fact is due to the competing reactions of PA and its conjugate acid (PAH) with DNPTC; in this case the k_N values were determined as previously.[6] The experimental conditions, and k_{obsd} and k_N values are shown in Table I.

Figure 1 shows the linear Brönsted-type plot, statistically corrected,[5,9] obtained for the present reactions (correlation coefficient 0.997). The magnitude of the slope (β = 0.56 ± 0.05) is much smaller than those found for curved Brönsted plots at low pK_a values in the aminolyses of several oxyesters and thioesters and carbonates.[1-3,5,6,10-12] These curved Brönsted plots have been interpreted in terms of a tetrahedral intermediate ($T^{\pm}$) in the reaction path and a change in the rate-determining step. The large Brönsted slope at low pK_a values ($\beta \approx 0.8$–1.0) is indicative of the breakdown of $T^{\pm}$ being the rate-determining step.[2,3,5,6,10-12]

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