Acetylation of Nucleosides by N-Acetoxy-N-arylacetamides: Dependence on Base, Aryl Group, and Buffer Composition

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Nucleosides treated with the carcinogen N,O-diacetyl-4-stilbenylhydroxylamine (1) in water and acetone are alkylated on the bases, and only alkylation products have been detected so far. In phosphate buffer at the same pH, adenosine and guanosine are acetylated on ribose, as determined by gas chromatography–mass spectrometry, in about the same yield as the total alkylation products. This acetylation does not take place in salt solution of the same pH and ionic strength. No alkylation or acetylation of adenosine or guanosine could be detected upon treatment with N,O-diacetyl-1-naphthylhydroxylamine (6), which solvolyzes at a rate comparable to that of 1, but cytidine was converted in low yield to N^4-acetylcytidine. The results with 1 point out that apparently innocuous changes in medium may greatly alter the reactivity characteristics of some ultimate carcinogens, while the results with 6 indicate that steric hindrance in the ultimate carcinogen itself may completely alter its mechanism of decomposition from that expected. The biological consequences of these acylation reactions are unknown, although 6 has been tested for both mutagenic and carcinogenic activity and found inactive.

N,O-Diacetyl-N-arylhydroxylamines have been used as models for reactive metabolites synthesized in vivo from carcinogenic N-arylacetamides.[1] These compounds react at various rates and with various target specificities, through the loss of acetate to generate delocalized N-acetyl-N-arylnitrenium ions, which attack nucleophilic positions on nucleic acid bases. The rates of N–O cleavage of these N-acetoxyamides have been found to vary over a range of at least 10^3, but it has been proposed that C–O cleavage rates are relatively uniform.[2] This proposal arose from the observation that those acetoxyamides which lose acetate ion slowly can acetylate nucleosides. There has been little evidence that the acylation pathway competes with nitrenium ion formation for rapidly reacting acetoxyamides, with the exception of acetylation of amino groups in protein by N-acetoxy-2-acetamidofluorene.[3] We now report two instances of relatively rapid nucleoside acylation by N,O-diacetyl-N-arylhydroxylamines, one case facilitated by buffer and the second probably an example of steric acceleration.

N,O-Diacetyl-4-stilbenylhydroxylamine (1; Figure 1) is a local sarcomagen in rats[4] and initiates skin tumorigenesis in mice.[5] It has previously been shown to alkylate guanosine, adenosine, and cytidine in acetone/water reactions,[6] giving hydroxylated acetamidobibenzyl derivatives, and to alkylate methionine,[7] giving both acetamidobibenzyl and acetamidostilbene derivatives. When such reactions with adenosine and guanosine were run in phosphate buffer, not only were the alkylation products observed but also nucleosides having UV spectra similar to those of the starting material. Following trimethylsilylation, gas chromatography–mass spectrometry showed two products each from adenosine (2, 3; Figure 2) and guanosine (4, 5; Figure 2) whose mass spectra indicated unmodified bases (2, 3, m/z 208; 4, 5, m/z 296), and monoacylation in the sugar (m/z 319). Acylation at O-5′ is excluded by the prominent m/z 103 ions, while substitution at O-2′ and -3′ is shown by the characteristic[8] ribose cleavages indicated above. The intensity ratios m/z 230 > 187 for 2 and 4, and m/z 187 > 230 for 3 and 5, are empirically correlated with the analogous peak intensity ratios (m/z 230, 172) in the mass spectra of silyl derivatives of 2′- and 3′-O-methyladenosine (2%, 75%, and 55%, 2.5%, respectively). All assignments shown for 2–5 were confirmed by measurement of exact mass. The mixture of O-2′ and -3′ isomers obtained by reaction of adenosine and guanosine with 1 is a result of facile acetyl migration,[9] producing relative quantities of O-2′ and -3′ isomers qualitatively similar to that reported for monoacetyl uridines.[9] As a result it can be concluded that acylation has occurred on either or both sites, but not at O-5′.

The total yield of alkylation plus acylation products represents about 20% of adenosine and 8% of guanosine. While the yield of alkylation products from cytidine is comparable to that obtained from guanosine, no acylation of cytidine was observed in phosphate buffer. We have not yet undertaken any studies which give a clear idea of the mechanism of the phosphate catalysis. A mechanism can be envisaged in which phosphate displaces hydroxamate from the N-acetoxyamide (Figure 3), to give the anhydride of acetic acid and phosphoric acid. The role of the purine in facilitating the acetylation of the ribose may lie in catalyzing either formation of the anhydride or its reaction with ribose. If the anhydride intermediate is indeed the mechanism by which phosphate influences the process, then facilitation of the final reaction seems to be the more likely function for the purine. This model was tested by replacing N-acetoxy-N-arylacetamide with acetyl phosphate at the same concentration in the (unbuffered) reactions described. As with 1, acetylation of ribose was observed, on adenosine and guanosine. The yields were lower than observed in the reactions of 1, however, suggesting that the arylamide group confers some selectivity

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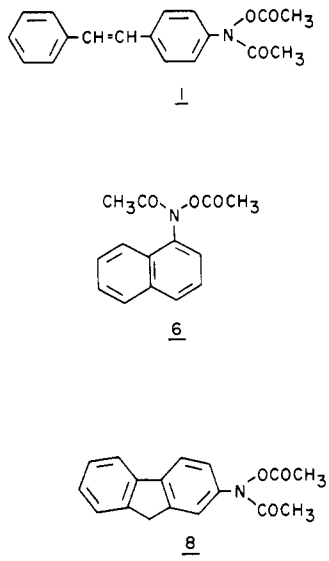

Figure 1.

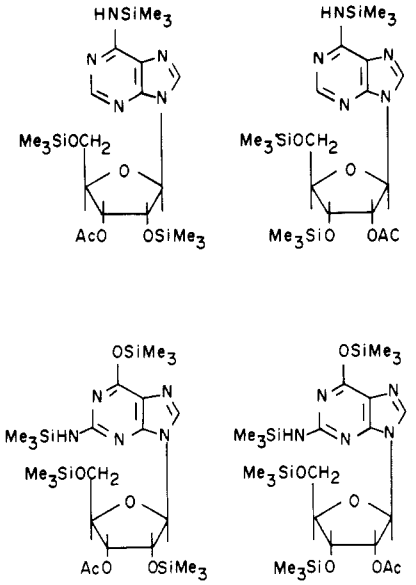

Figure 2.

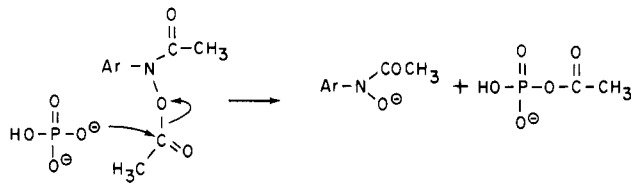

Figure 3.

on the reaction. N-Acetylation, particularly of cytidine, was not observed.

N,O-Diacetyl-1-naphthylhydroxylamine (**6**; Figure 1) acetylates the amino group of cytidine as shown by mass spectrometry of the trimethylsilyl derivative[8,10] (Figure 4). This reaction has not been reported for any other *N*-acetoxyamide. This compound is solvolyzed at a rate comparable to that of **1** or *N,O*-diacetyl-2-fluorenylhydroxylamine[1] (**8**; Figure 1), substances which fail to acetylate nucleosides, except in the presence of phosphate (data regarding the ractions of **8** are incomplete). Previous work has assumed without direct evidence that this rate

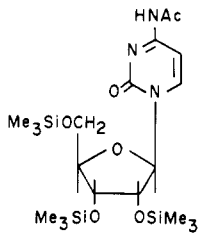

Figure 4.

of solvolysis was due to nitrenium ion generation. The acetylating capability of **6** appears to be due to steric acceleration, since the CO–N–O plane is forced to be essentially perpendicular to the plane of the naphthalene ring (CPK space-filling models). Direct acetylation by **6** at a significant rate seems simply to require a stronger nucleophile than hydroxyl, thus involving the exocyclic amino group of cytidine. The fact that we did not observe comparable acetylation of the amino groups of adenosine and guanosine agrees with the earlier observation that the rate of hydroxymethylation of deoxycytidine by formaldehyde is about 10 times faster than reaction of formaldehyde with deoxyadenosine or deoxyguanosine.[11] Such a difference in acetylation rates would be sufficient to explain our results.

The biological significance of such acetylation reactions is unknown. It is also not known whether these reactions take place in tissues treated with these compounds. It is known that, while **1** and **8** are local sarcomagens,[4] initiators of mouse skin tumorigenesis,[5] and mutagens in bacteria,[12] **6** lacks both tumorigenic activity and mutagenic activity.[12,13] There have been reports of carcinogenic acylating agents,[14] but these are extremely weak agents, given at doses about 100 times higher than those used for testing the activity of *N*-acetoxyamides.[4,5] Barry and Gutmann[15] have proposed that acetylation by compounds such as *N*-acetoxy-3-acetamidofluorene may explain their carcinogenic activity. However, the demonstration of acetylating activity in **6**, an inactive compound, makes this less likely.

Experimental Section

Compounds **1** and **6** were synthesized as described previously[1,6b] and were characterized for purity and identity by infrared spectrophotometry and homogeneity on thin-layer chromatography. Acetyl phosphate and nucleosides were obtained from Sigma Chemical Co., with the exception of 2′- and 3′-*O*-methyladenosine, which were previously prepared.[16] Reagents for trimethylsilylation were purchased from Regis Chemical Co. All other reagents were reagent grade and were used without further purification.

Mass Spectrometry. Reaction products were converted to volatile trimethylsilyl derivatives prior to mass spectrometry by heating with *N,O*-bis(trimethylsilyl)trifluoroacetamide–trimethylchlorosilane–pyridine (100:1:6) at 80 °C for 30 min in a screw-capped vial. Mass spectra of **2–5** were acquired with an LKB 9000S instrument, 70-eV ionizing energy, 270 °C ion source and separator temperatures. Compounds **2–5** were introduced by gas chromatograph (3 ft, 1% SE-30, temperature programmed at 6 °C/min from 220 °C). Exact molecular masses of the isomeric

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mixtures 2,3 and 4,5 were measured (Varian MAT 731) after direct probe introduction. Spectra of derivative 7 and the free material were recorded with a Varian 731 instrument, 70 eV, source 250 °C, with direct probe sample introduction, at low resolution (EI and FD), and high resolution with photographic recording (EI).

Acetylation of Adenosine. Compound 1 (4 mg) in 0.2 mL of acetone was added to adenosine (2 mg) in 0.2 mL of water (adjusted to pH 7.4 with dilute NaOH) or in 0.2 mL of 0.1 M phosphate buffer, pH 7.4. After reaction overnight at 37 °C, the total mixture was applied to a preparative thin-layer chromatography plate (Analtech 2-mm silica gel GF). This was developed first in ethyl acetate to remove solvolysis products of 1 and then in ethyl acetate–methanol–acetic acid (89:7:4). Under these conditions, in either reaction 8% of the adenosine was converted to products identical in the two systems. These adducts have been previously reported.[6b] In phosphate buffer, however, an additional 12% was converted to material which did not leave the origin on TLC in ethyl acetate, but was the most mobile material in the secondary eluant. Two closely spaced bands appeared which, upon isolation, each gave rise to two bands on rechromatography, in addition to a trace of underivatized nucleoside. Each band had an ultraviolet spectrum like that of adenosine, with the same pK. The latter observation implies that the base was not altered or that any derivatization was immediately labile to acid and alkali. Gas chromatography–mass spectrometry of the silylated product from each TLC fraction showed a small amount of adenosine followed by two principal components having retention times 1.09 and 1.20 relative to adenosine,[17] areas 1.8:1, assigned to structures 2 and 3, respectively. Calcd exact mass for $C_{21}H_{29}N_5O_5Si_3$: 525.2258. Found for 2 + 3: 525.2264.

Acetylation of Guanosine. Compound 1 (13.5 mg) in 1 mL of acetone was added to 0.7 mg of guanosine in 1 mL of water or phosphate buffer. After reaction overnight at 37 °C, the reaction mixture was chromatographed on a preparative cellulose thin-layer plate (Analtech 1 mm Avicel F) in 1-butanol–acetic acid–water (50:11:25). In each reaction, 4% of the guanosine was converted to previously reported alkylation products,[6b] while in buffer another 4% was converted to new compound recognized as product running just ahead of guanosine on the plate. Like the adenosine product, it was a mixture of interconvertible materials with ultraviolet spectrum and pK like that of starting material. Gas chromatography–mass spectrometry of the silylated bands recovered from TLC produced a chromatographic pattern very similar to that of the adenosine product, consisting of guanosine and two major components; retention times relative to guanosine[17] 1.25, 1.37, areas 2.2:1, corresponding to 4 and 5, respectively. Calcd exact mass for $C_{24}H_{47}N_5O_6Si$: 613.2603. Found for 4 + 5: 613.2590.

Acetylation of Cytidine. Compound 6 (25 mg) in 0.5 mL of acetone was added to 2.5 mg of cytidine in 0.5 mL of water. After reaction overnight at 37 °C, the mixture was chromatographed in the same way as the adenosine–1 reaction mixture. A new compound was identified in about 6% yield, running just ahead of cytidine. This product had ultraviolet absorbance maxima (95% ethanol) at 247 and 299 nm (247/299 = 2.1), with no pK value between pH 2 and 10. In stronger base it was converted to a compound with UV characteristics of cytidine. Mass spectrometry of its trimethylsilyl derivative showed a component with a molecular weight of 501. Presence of three silyl groups was established from the mass spectrum of the corresponding $Si(CD_3)_3$ derivative. The assignment 7 was corroborated by the corresponding high-resolution mass spectrum (calcd exact mass for $C_{20}H_{39}N_3O_6Si_3$ 501.2146, found 501.2143), and field-desorption (FD) spectrum (MH^+ = 286). The FD mass spectrum also exhibited peaks consistent with the presence of lesser amounts of di- and triacetylcytidine (m/z 328, 370), which were not represented in the spectrum of 7, but were also found in the direct-probe EI spectrum of the underivatized cytidine product. Reinspection of the sample by analytical TLC (Analtech Uniplate, 0.25 mm SiO_2 GHLF; ethyl acetate–methanol–acetic acid, 89:7:4) revealed total detectable impurities to be less than 5% of the principal product, in agreement with the EI spectrum of the silylated compound.

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Registry No. 1, 26594-44-5; 2, 82064-45-7; 3, 82064-46-8; 4, 82064-47-9; 5, 82064-48-0; 6, 38105-25-8; 7, 82064-49-1; adenosine, 58-61-7; guanosine, 118-00-3; cytidine, 65-46-3.

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A Photochemical Ring Contraction of an Imino Lactam

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The photochemical rearrangement of three tetrahydro-2-pyrazinones in aqueous medium to imidazol-5-ones is described. 1,2,5,6-Tetrahydro-3,5,5-trimethyl-2-pyrazinone (4) gives 1,2,2,4-tetramethyl-3-imidazolin-5-one (7), 1,2,5,6-tetrahydro-3,6,6-trimethyl-2-pyrazinone (5) gives 4-methyl-5-imidazolidinone (9), and 1,2,5,6-tetrahydro-1,3,5,5-tetramethyl-2-pyrazinone (6) gives 1,4-dimethyl-5-imidazolidinone (8). A mechanism involving enediimine and isoimidazole intermediates is proposed in analogy with the mechanism for rearrangement of 5,6-dihydropyrazines to imidazoles.

Earlier we reported that an imino lactone, 5,6-dihydro-3,5,5-trimethyl-1,4-oxazin-2-one (1) undergoes photoreductive dimerization in 2-propanol solvent to give meso and *dl* dimers 2 and 3.[1] Because these dimers possess the interesting property of reacting as mild one- and two-electron reducing agents,[2,3] we have been synthesizing analogues as part of a structure reactivity study. We now report on an unusual photochemical ring contraction of the imino lactam analogue, 1,2,5,6-tetrahydro-3,5,5-trimethyl-2-pyrazinone (4), which occurs to the exclusion of

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